









We gratefully acknowledge support of the
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DFG

PROGRAM AND ABSTRACTS OF THE

# 2ND COLOGNE AGEING CONFERENCE 2016

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# WELCOME

We are very happy to welcome you to the **2nd Cologne Ageing Conference**. The conference is jointly organised by the CECAD Cluster of Excellence for Ageing Research, the Max Planck Institutes for Biology of Ageing and Metabolism Research and the Center of Molecular Medicine Cologne (CMMC).

The sessions of the conference will contain the following topics:

- Stem cells, senescence, and regeneration
- Protein homeostasis and proteotoxic diseases
- Nuclear organization and genome stability
- Metabolism

### The scientific organising board

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Joseph-Stelzmann-Straße 9b, D-50931 Cologne





# CONFERENCE INFORMATION

**Venue:**
MTI building, Main Lecture Hall of the Centers for Biochemistry and Physiology,
Medical Faculty, University of Cologne
Joseph-Stelzmann-Str. 9, Building 44b, D-50931 Cologne
(access via "Studentenweg" - connecting Robert-Koch-Str. 21 and Joseph-Stelzmann-Str. 26)

**Date:**
Sunday, April 3 to Tuesday, April 5, 2016

**Posters:**
Posters will be displayed during the meeting in 2 sessions in the MTI building.

You will find the number of your poster in this abstract volume. According to this number, you may mount your poster in the exhibition area. Material (glue strips) to fix the posters will be provided.

Posters scheduled for **Poster Session I** (posters with even numbers) will be presented on Sunday, April 3, 2016. Posters scheduled for **Poster Session II** (posters with odd numbers) will be presented on Monday, April 4, 2016. The presenters will be available at their posters during the main poster session slots outlined in the program.
The posters should be **set up** on Sunday from 12:00 to 14:00 and **removed** on Tuesday, April 5, 2016 from 9:00 to 13:00.

A **prize** will be awarded to the best poster on Tuesday, April 5, 2016.

**Presentations:**
For oral presentations we will provide Mac as well as PC computers which allow PowerPoint presentations only.

**Internet:**
Internet access via Wireless LAN is free of charge in the MTI building at the SSID "eduroam". You will find further information in your conference bag.

**All abstracts should not be cited in bibliographies. All material contained herein have to be treated as personal communication and should be cited as such only with the consent of the author!**

# PROGRAM

## 3 APRIL 2015

**Session I: Stem cells, senescence and regeneration**
*Chair: David Vilchez*

14.00 – 14.15 Welcome **Björn Schumacher**

14.15 – 14.20 Introduction to session **David Vilchez**

14.20 – 14.50 **Aziz Aboobaker** University of Oxford, Department of Zoology, Oxford, UK
"The molecular and cellular basis of stem cell underpinning of an immortal life history"

14.50 – 15.20 **Jan van Deursen** Mayo Clinic, Rochester, MN, US
"How senescent cells drive aging and desease"

15.20 – 15.32 **Marco Demaria** ERIBA, Groningen, NL
"Cellular senescence promotes adverse reactions to chemotherapy"

15.32 – 15.44 **Laura Greaves** Newcastle University, Newcastle, UK
"Age-associated mitochondrial dysfunction promotes intestinal tumour development"

15.44 – 15.56 **Lida Katsimpardi** Harvard University, Cambridge, MA, US
"Young blood as a therapy for age-related neurodegenerative diseases"

15.56 – 16.30 *Coffee break*

*Chair: Elena Rugarli*

16.30 – 17.00 **Joan Mannick** Novartis Institutes for BioMedical Research, Cambridge, MA, US
"mTOR inhibition improves immune function in elderly humans"

17.00 – 17.30 **Thomas A. Rando** Stanford Dep. of Neurology, Stanford University, Stanford, CA, US
"Epigenetic mechanisms of stem cell aging and rejuvenation"

17.30 – 17.42 **Olena Kucheryavenko** Institute for Ageing and Institute for Cell and Molecular Biosciences, Newcastle, UK
"Interventions to limit senescence-induced bystander effect"

17.42 – 17.54 **Helen Tauc** Institut für Biochemie & Molekulare Biologie, Universität Ulm, DE
"Nipped-A/TRRAP maintains midgut homeostasis during aging in Drosophila by regulating intestinal stem cell proliferation"

17.54 – 18.06 **Yosef Reut** Weizmann Institute of Science, Rehovot, IL
"Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL"

18.06 – 21.00 *Poster session I / Kölsch, Snacks*



## 4 APRIL 2015

**Session II: Protein homeostasis and proteotoxic diseases**
*Chair: Thorsten Hoppe*

09.00 – 09.05 Introduction to session Thorsten Hoppe

09.05 – 09.35 **Anne Bertolotti** MRC Laboratory of Molecular Biology, Biomedical Campus Cambridge, Cambridge, UK
"Preventing protein quality control failure and neurodegenerative diseases"

09.35 – 10.05 **Monique Breteler** German Center for Neurodegenerative diseases (DZNE), Bonn, DE; Harvard T.H. Chan School of Public Health Department of Epidemiology, Boston, MA, US

10.05 – 10.17 **Jonathan Byrne** Institute of Molecular Biology (IMB), Mainz, DE
"Antagonistic Pleiotropy in the autophagic machinery modulates lifespan conversely over age in C. elegans"

10.17 – 10.29 **Ilias Gkikas** Institute of Molecular Biology and Biotechnology, Heraklion, GR
"An alternative hypoxia response mechanism independent of HIF-1 involves mitochondrial metabolic adaptation"

10.29 – 10.41 **Michael Lammers** CECAD, Cologne, DE
"Lysine-acetylation in cellular regulation, ageing and disease"

10.41 – 11.10 *Coffee break*

*Chair: Adam Antebi*

11.10 – 11.40 **Ivan Dikic** IBC II, Goethe University, Frankfurt, DE
"Ubiquitin networks in regulation of proteostasis and autophagy"

11.40 – 12.10 **Stefan Jentsch** Max Planck Institute of Biochemistry, Dept. of Molecular Cell Biology, Martinsried, DE
"Splicing fidelity controlled by the ubiquintin relative hub1"

12.10 – 12.22 **Marie Lechler** DZNE, Tübingen, DE
"Investigating stress granule insolubility with age"

12.22 – 12.34 **Hyun Kate Lee** Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, DE
"Age-dependent aberrant transition of membraneless organelles into pathological aggregates"

12.34 – 12.46 **Michal Schweiger** University Hospital of Cologne, Functional Epigenomics, CCG, Cologne, DE
"Splicing during heat shock is regulated by the bromodomain protein BRD4 and the heat shock transcription factor 1"

12.46 – 14.50 *Poster session II, Lunch*

**Session III: Nuclear Organization and Genome Stability**
*Chair: Björn Schumacher*

14.50 – 14.55 Introduction to session **Björn Schumacher**

14.55 – 15.25 **Eric Gilson** IRCAN, Nice, FR
"The double life of telomeric proteins"

15.25 – 15.55 **Jacqueline Jacobs** NKI, Amsterdam, NL
"Mechanisms underlying genome instability upon DNA repair at telomeres"

15.55 – 16.07 **Nils de Wind** Dep. of Human Genetics, Leiden University Medical Center, Leiden, NL
"Endogenous DNA damage, replication stress, and ageing"

16.07 – 16.19 **Nard Kubben** National Cancer Institute (NCI), Bethesda, Maryland, US
"Repression of the antioxidant NRF2 pathway in premature aging"

16.19 – 16.50 *Coffee break*

*Chair: Nils-Göran Larsson*

16.50 – 17.20 **Andrei Seluanov** University of Rochester, Department of Biology, Rochester, NY, US
"SIRT6 is the new guardian of the genome"

17.20 – 17.50 **Manuel Serrano** Centro Nacional de Investigaciones Oncológicas, Madrid, ES
"Tissue repair: an integrated view of senescence and reprogramming"

17.50 – 18.02 **Argyris Papantonis** CMMC, Cologne, DE
"Common determinants of global 3D chromatin reorganization upon senescence of different primary human cells: a link to the SASP

18.02 – 18.14 **Lene Rasmussen** Center for Healthy Aging, Copenhagen, DK
"Rev1-deficiency induces age-related disorders via PARP1 activation and impaired mitochondrial homeostasis"

18.14 – 18.26 **Gisela Slaats** CECAD, Cologne, DE
"DNA replication sress underlies renal phenotypes in CEP290-associated joubert syndrome"

19.00 *Dinner (only invited guests)*





## 5 APRIL 2016

**Session IV: Metabolism**
*Chair: Jens Brüning*

09.00 – 09.05 Introduction to session **Jens Brüning**

09.05 – 09.35 **Michael Hall** Biozentrum University of Basel, Basel, CH
"mTOR signaling in growth and metabolism"

09.35 – 10.05 **Brian Luke** Institute for Molecular Biology, Mainz, DE
"The regulation of TORC1 activity has important influences on the DNA damage checkpoint response"

10.05 – 10.17 **Cornelis Calkhoven** ERIBA, Groningen, NL
"mTORC1-C/EBPβ regulation of healthspan and lifespan"

10.17 – 10.29 **Alessandro Cellerino** Scuola Normale Superiore, BIO@SNS, Pisa, I
"MicroRNA-29 controls a compensatory response to limit neuronal iron accumulation during aging"

10.29 – 10.41 **Collin Ewald** ETH Zürich, Zürich, CH
"Reduced insulin/IGF-1-signalling implicates extracellular matrix remodelling in longevity"

10.41 – 11.10 *Coffee break*

*Chair: Linda Partridge*

11.10 – 11.40 **David Sabatini** Whitehead Institute for Biomedical Research, MIT, Cambridge, MA, US
"Control of growth and metabolism by mTOR"

11.40 – 12.10 **Aurelio Teleman** DKFZ Heidelberg, DE
"Physiological regulation by stearic acid"

12.10 – 12.40 **Marian Walhout** University of Massachusetts, MA, US
"Nutritional regulatory networks"

12.40 – 12.52 **Dario Valenzano** Max Planck Institute for Biology of Ageing, Cologne, DE
"Changes in the intestinal microbiota affect ageing in the short-lived African turquoise killifish"

12.52 – 13.15 Poster prize

13.15 – 14.00 *Lunch*

# SPEAKER ABSTRACTS

**LT1**

**Notes**

## The molecular and cellular basis of of stem cell underpinning of an immortal life history

*Aziz Aboobaker*

*University of Oxford, Department of Zoology, Oxford UK*

A few animals can claim to have evolved an immortal somatic lineage, and this is particularly true of those species that obligately asexual and use fission as reproductive mode. Studying these species may reveal how highly conserved molecular and cellular processes are can be adapted to allow an immortal life history, in contrast to most animals that age. One focus of our group are the pluripotent adult stem cells in planarian flatworms. We have begun to understand how these cells underpin an immortal life history and have uncovered that these cells are a sensitive system to uncover novel mechanisms that control the balance between effective stem cell mediated homeostasis and both hypo- and hyper-function. Some of these mechanisms involve genes that are conserved but as yet uncharacterised in mammals/humans but have been implicated in human disease processes, particularly cancer.

**LT2**

## How senescent sells drive aging and disease

*Jan van Deursen*

*Mayo Clinic, Rochester, MN, US*

Cellular senescence has emerged as a potentially important contributor to aging and age-related disease and as an attractive target for therapeutic exploitation. Direct evidence for the deleterious effects of senescence in aging originates from BubR1-progeroid mice in which inactivation of the p16Ink4a senescence pathway or the elimination of p16Ink4a-positive senescent cells dramatically attenuates aging. Using transgenic mouse models that selectively kill p16Ink4a positive cells, we have investigated the role of senescence in health and life span of normal mice, as well as its role in common age-related diseases. The implications of these studies for the design and effectiveness of senotherapies to extend healthy lifespan will be discussed.



**Notes**

## ST1

## Cellular senescence promotes adverse reactions to chemotherapy

*Marco Demaria[1,2,*] Monique O'Leary[1], Jianhui Chang[3], Lijian Shao[3], Kristin Koenig[1], Catherine Le[1], Emmeline Academia[1], Sumner Klimarx[1], Alexis Valdovinos[1], Fatouma Alimirah[1], Brian K. Kennedy[1], Simon Melov[1], Daohong Zhou[3], and Judith Campisi[1,4]*

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*2 European Institute for the Biology of Aging, University Medical Center Groningen, Groningen, Netherlands*
*3 Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR, USA*
*4 Lawrence Berkeley National Laboratory, Life Sciences Division, Berkeley, CA, USA*
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### Introduction

Cellular senescence is a complex stress response whereby cells lose irreversibly their capacity to proliferate (Campisi, 2013). Senescent cells develop the senescence-associated secretory phenotype (SASP), characterized by the expression and secretion of inflammatory cytokines, chemokines, growth factors and proteases (Childs et al., 2015; Coppe et al., 2008).Despite a main role for cellular senescence to act as a tumor suppressive mechanism, mounting evidence indicates that senescent cells participate in multiple physiological and pathological stages, including aging and tissue repair (Baker et al., 2011; Demaria et al., 2015; Demaria et al., 2014). Chemotherapy is a vastly used anti-cancer therapeutic approach based on impairing mitosis and targeting highly proliferative cells (Singal and Iliskovic, 1998). The non-specificity of chemotherapy often leads to several inflammation-based side effects, including immunosuppression, fatigue, organ damage, cognitive impairment and secondary neoplasms, often recognized as age-related diseases. Several chemotherapy drugs induce cells in the tumor microenvironment to diverse cellular states, including senescence (Schmitt, 2003). Therapy-induced senescence (TIS) has been considered an attractive avenue to stimulate the immune system, but also as a source of chronic inflammation and drug resistance (Ewald et al., 2010).

### Results

To more precisely assess the physiological role of TIS in vivo, we used a recently generated mouse model (p16-3MR) in which p16INK4a-positive senescent cells can be detected in living animals, isolated from tissues, and eliminated upon treatment with an the otherwise ineffective drug Ganciclovir (GCV) (Demaria et al., 2014). Here, we show that mouse and human primary fibroblasts and cells in vivo are induced to senescence and develop a SASP upon treatment with the standard chemotherapeutic agents Doxorubicin and Paclitaxel. The TIS cells persist for several weeks in mouse tissues and promote local and systemic inflammation. The burden of inflammation is reduced upon elimination of senescent cells, and correlates with a faster and more efficient recovery from the chemotherapy-induced bone marrow suppression. The removal of senescent cells from p16-3MR mice is also associated with reduced fatigue and improved strength, resulting in increased spontaneous physical activity. Strikingly, treatment with GCV prevents mice from developing cardiomyopathy, a major side effect of Doxorubicin.
Moreover, we show that TIS cells promote cancer relapse and the formation of metastasis in a mouse model of breast cancer.

### Perspectives

Together, our data suggest that senescent cells generated upon chemotherapy treatment in normal tissues can contribute to adverse reactions and to accelerated aging through cell-non-autonomous effects. The elimination of TIS cells might represent a novel therapeutic approach to reduce the short- and long-term toxicity of standard ant-cancer interventions, increase healthspan and promote survival.

References

[1] Baker, D.J., Wijshake, T., Tchkonia, T., LeBrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L., and van Deursen, J.M. (2011). Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479, 232-236.
[2] Campisi, J. (2013). Aging, cellular senescence, and cancer. Annual review of physiology 75, 685-705.
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[4] Coppe, J.P., Patil, C.K., Rodier, F., Sun, Y., Munoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y., and Campisi, J. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6, 2853-2868.
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[6] Demaria, M., Ohtani, N., Youssef, S.A., Rodier, F., Toussaint, W., Mitchell, J.R., Laberge, R.M., Vijg, J., Van Steeg, H., Dolle, M.E., et al. (2014). An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Developmental cell 31, 722-733.
[7] Ewald, J.A., Desotelle, J.A., Wilding, G., and Jarrard, D.F. (2010). Therapy-induced senescence in cancer. J Natl Cancer Inst 102, 1536-1546.
[8] Schmitt, C.A. (2003). Senescence, apoptosis and therapy--cutting the lifelines of cancer. Nat Rev Cancer 3, 286-295.
[9] Singal, P.K., and Iliskovic, N. (1998). Doxorubicin-induced cardiomyopathy. The New England journal of medicine 339, 900-905.

## Notes

**ST2**

**Notes**

## Age-associated mitochondrial dysfunction promotes intestinal tumour development

*Laura C Greaves[1,2] , Anna LM Smith[1,2], James N Sampson[1], Craig Stamp[1,2], Angela Baker[1], Ghazaleh Alimohammadiha[1,2], Claire A Richardson[3], Bhavana Gupta[1], John C Mathers[2,4], Robert W Taylor1, Doug M Turnbull[1,2]*

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*3 Institute of Neuroscience, Newcastle University,*
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### Introduction

Stem cells with mitochondrial oxidative phosphorylation (OXPHOS) defects caused by somatic mitochondrial DNA (mtDNA) mutations are commonly detected in the colorectal epithelium of older individuals (Taylor et al., 2003). In addition, cells with mtDNA mutations are also well documented in age-related colorectal cancers. However, it is unknown whether the mtDNA mutations detected in normal tissue affect stem cell biology or play a role in age-related colorectal cancer development. Here we have directly investigated the effect of mtDNA mutations and OXPHOS defects on stem cell division kinetics by examining intestinal stem cells in a mouse model which accumulates high levels of mtDNA mutations over time (PolgA+/mut (Trifunovic et al., 2004)). These mice accumulate colonic crypts with mitochondrial OXPHOS dysfunction with age in a similar manner to ageing humans (Baines et al., 2014). We then induced adenoma formation (Barker et al., 2009) in these animals to investigate the effects of mitochondrial dysfunction on early stage tumour growth.

### Results

Multiple thymidine analogue labelling of colonic stem cells revealed that those stem cells with mitochondrial complex I deficiency re-enter the cell cycle, on average, 30% more often than their wild-type counterparts. Complex IV dysfunction did not affect the stem cell re-entry time in the same way, suggesting that complex I deficiency specifically, is speeding up the frequency of stem cell divisions. Next we knocked out the tumour suppressor APC in stem cells in mice with mitochondrial dysfunction and normal controls. Those mice with mitochondrial dysfunction had a significantly shortened lifespan due to accelerated tumour growth compared with controls, suggesting that mitochondrial dysfunction enhances the progression of intestinal tumours.

### Perspectives

Our results show that age-related mtDNA mutations resulting in complex I deficiency can increase stem cell cycle re-entry rate, which is exacerbated when these cells are transformed. Currently the mechanism by which this occurs is unknown. Elucidating these pathways is vital as this may reveal novel therapeutic options utilising mitochondrial pathways for cancer treatment. In addition, development of strategies to reduce the accumulation of mtDNA mutations in intestinal stem cells could diminish the age-related colorectal cancer burden

References

Baines, H.L., et al. Mech Ageing Dev 139c, 22-30.
Barker, N., et al. Nature 457, 608-611.
Taylor, R.W.et al, J Clin Invest 112, 1351-1360.
Trifunovic, A.et al, Nature 429, 417-423.

**ST3**

**Notes**

## Young blood as a therapy for age-related neurodegenerative diseases

*Lida Katsimpardi[1,2], Pierre-Marie Lledo[2], Lee Rubin[1]*

*1 Harvard University, 7 Divinity Ave, Cambridge, MA, USA*
*2 Institut Pasteur, 25 rue du Dr Roux, Paris, France*

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### Introduction

Aging is an irreversible process of functional deterioration that affects all tissues at the molecular, cellular and organismal levels. In the brain, the decline of neurogenesis together with an age-related vascular deterioration lead to cognitive degeneration with a disastrous health impact on the individual. Cerebrovascular dysfunction is a major cause of cognitive impairment in the elderly but is also involved in age-related neurodegenerative diseases1. Finding ways and molecules that reverse this process and ameliorate age- and disease-related cognitive impairment by targeting vascular deterioration would be of great therapeutic value. In this work we show that factors circulating in the young blood can rejuvenate the aged brain by increasing neurogenesis and olfactory capacity, as well as by restoring the vasculature and the cerebral blood flow to youthful levels. Moreover, we identified a youthful systemic factor, GDF11, that can recapitulate the beneficial effects of young blood2.

### Results

We found that young blood increases stem and progenitor cell numbers in the aged subventricular zone and induces a striking remodeling of the aged vasculature resulting in increased blood flow. Furthermore, we observed an increase in new neurons in the olfactory bulb, which results in an overall improvement in olfactory behavior. We performed microarray analysis to unravel specific molecular pathways that are involved in the changes due to young blood. Additionally, we identified GDF11 as a systemic factor that recapitulates the beneficial effects of young blood on neurogenesis and vascular remodeling in the aged mouse. Extending our studies of GDF11 in the brain we discovered its target cell populations, as well as specific molecular pathways that are involved within its mode of action.

### Perspectives

Increasing neurogenesis by systemic factors is extremely important in the context of age-related neurodegenerative diseases3. In the case of Alzheimer's disease (AD), where the plaques and neurofibrillary tangles exist in both SVZ and hippocampus neurogenic areas, compromised neurogenesis is associated with impairments in learning and memory4. This suggests that reversing the age-related neurogenic decline by exposure of young systemic factors could be also used as a therapeutic tool in other models of neurodegenerative diseases. Moreover, since the etiology of neurodegenerative diseases like AD shows an increasingly prominent role for cerebral blood flow and vascular abnormalities as major disease factors, we believe that our findings could open new possibilities for treatment, especially by using a single youthful factor like GDF11.

References

[1] Farkas E, Luiten PG. Cerebral microvascular pathology in aging and alzheimer's disease. Prog Neurobiol. 2001;64:575-611
[2] Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, Chen JW, Lee RT, Wagers AJ, Rubin LL. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science. 2014;344:630-634
[3] Brinton RD, Wang JM. Therapeutic potential of neurogenesis for prevention and recovery from alzheimer's disease: Allopregnanolone as a proof of concept neurogenic agent. Curr Alzheimer Res. 2006;3:185-190
[4] Ashe KH. Learning and memory in transgenic mice modeling alzheimer's disease. Learn Mem. 2001;8:301-308

**LT3**

## mTOR inhibition improves immune function in elderly humans

*Joan Mannick*

*Novartis Institutes for BioMedical Research, Massachusetts, US*

Inhibition of the mTOR pathway extends lifespan in all species studied to date, and in mice delays the onset of age-related diseases and co-morbidities. However, it is unknown if mTOR inhibition affects aging or its consequences in humans. To begin to assess the effects of mTOR inhibition on human aging-related conditions, we evaluated whether the mTOR inhibitor RAD001 ameliorated immunosenescence (the decline in immune function during aging) in elderly human volunteers, as assessed by response to influenza vaccination. RAD001 enhanced the response to influenza vaccine at doses that were relatively well tolerated. RAD001 also reduced the percentage of programmed death (PD)-1-positive CD4 and CD8 T lymphocytes that accumulate with age and have a diminished response to antigenic stimulation. The reduction in PD-1-positive T lymphocytes may underlie RAD001-mediated immune enhancement. The effects of mTOR inhibitors on additional biomarkers in elderly subjects will also be discussed. These results suggest that mTOR inhibition has beneficial effects on immunosenescence in elderly subjects. It remains to be determined if mTOR inhibitors improve other aging-related conditions in humans.



**Notes**

**LT4**

## Epigenetic mechanisms of stem cell aging and rejuvenation

*Thomas A. Rando*

*Stanford Dep. of Neurology, Stanford University, CA, US*

The decline of tissue regenerative potential with age correlates with impaired stem cell function. However, the mechanisms accounting for many aging stem cell phenotypes remain largely unknown. Using skeletal muscle stem cells as a model system, we identify cell death by mitotic catastrophe as a cause of impaired stem cell proliferative expansion in aged animals. The mitotic cell death is driven by a deficiency in p53, which is found to be under microenvironmental regulation by Notch signaling. Ligand-dependent stimulation of Notch activates p53 in muscle stem cells via indirect inhibition of Mdm2 expression through Hey transcription factors. Pharmacologic activation of p53 promotes the expansion of p53-deficient aged muscle stem cells in vivo. Taken together, these findings illuminate a Notch-p53 signaling axis that plays an important role in age-dependent cell survival and tissue regeneration.



**Notes**

**ST4**

**Notes**

## Interventions to limit senescence-induced bystander effect

*Kucheryavenko O., Wordsworth J., von Zglinicki T., Nelson G.*

*Institute for Cell and Molecular Biosciences and Newcastle University Institute for Ageing, Campus for Ageing and Vitality, Edwardson Building, Newcastle upon Tyne, NE4 5PL, UK*

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### Introduction

Senescence can be triggered by activation of oncogenes or in response to persistent DNA damage due to internal or external stressors. Independent of the causative trigger, senescent cells exit from cell cycle, undergo changes in chromatin confirmation [1], produce increased levels of reactive oxygen species (ROS) [2] and start to secrete a wide range of pro-inflammatory molecules, commonly termed as a senescence-associated secretory phenotype (SASP) [3]. SASP and ROS functions range from recruitment of immune cells, re-enforcement of senescence in the cell itself to induction of senescence in surrounding cells, bystanders [4]. Here, we compare bystander affects of oncogene-induced (OIS) and replicative (RepSen) modes of senescence on proliferating cells in direct 2D co-cultures.

### Results

We identified that oncogene-induced and replicatively senescent cells rely on different mechanisms to induce DNA damage response (DDR) in bystander cells and we tested intreventions that effectively alleviate the senescence-like DDR in bystander cells. We found that in replicatively senescent cells inhibition of NF-kB only mildly supressed the bystander effect, despite reducing secretion of soluble pro-inflammatory molecules. On the contrary, treatment with an NF-kB inhibitor significantly reduced OIS triggered DDR in bystander cells, whilst reducing levels of secreted IL-1a (although not levels of IL-6 and IL-8). We conclude that the bystander effect imposed by RepSen cells is mainly mediated by short-lived ROS species, while OIS cells rely on pro-inflammatory molecules for transmission of bystander effect.

### Perspectives

This work suggests that senescent cells selectively utilize components of their secretory program to communicate with their proximate microenvironment, and that treatments to mitigate affects of senescent cells in vivo require targetting different pathways for replicatively senescent cells compared to oncogene induced senescence.

References
[1] H. Chen et al. Mol. Cell, vol. 59, no. 5, pp. 719–31, Aug. 2015.
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[3] J.-P. Coppé et al. PLoS One, vol. 5, no. 2, p. e9188, Jan. 2010.
[4] G. Nelson et al. Aging Cell, vol. 11, no. 2, pp. 345–9, Apr. 2012.

**ST5**

**Notes**

## Nipped-A/TRRAP maintains midgut homeostasis during aging in Drosophila by regulating intestinal stem cell proliferation

*Helen M Tauc[1], Alpaslan Tasdogan[2], Patrick Meyer[3] and Petra Pandur[1]*

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### Introduction

Adult stem cells uphold a delicate balance between quiescent and active states, a deregulation of which leads to age-associated diseases such as cancer. In the Drosophila midgut, the rate of intestinal stem cell (ISC) proliferation is vital in maintaining tissue homeostasis and can have profound effects on lifespan [1]. In young flies, the ISCs divide to generate a self-renewed ISC and a post-mitotic progenitor called the enteroblast (EB). During aging, the homeostasis of the intestinal epithelium deteriorates as a result of excessive ISC proliferation and misdifferentiation of EBs [2]. A large effort has focused on defining signaling pathways that regulate ISC proliferation, however, less is known about transcriptional changes that occur within aging ISCs and their impact on ISC function.

### Results

Here, using RNA sequencing, we screened the transcriptome of young and old ISCs to uncover differentially regulated genes that are potentially important in maintaining ISC integrity and function throughout aging. Nipped-A, the homolog of mammalian Transcription/Translation Associated Protein (TRRAP), an important subunit of histone acetyltransferase (HAT) complexes, was significantly upregulated in aged ISCs. Our analyses demonstrated that knocking down Nipped-A in the ISCs and EBs results in a dramatic reduction of ISC proliferation and a progressive loss of ISCs and EBs during aging. Knocking down Nipped-A also inhibits the proliferative ISC response after tissue damage and markedly reduces tumor growth after overexpression of oncogenic Ras. These findings led us to conclude that Nipped-A is an essential factor in regulating ISC proliferation, and thereby midgut homeostasis, under various conditions. Furthermore, our results indicate that Nipped-A functions, at least partly, by regulating dMyc expression in ISCs/EBs and by modulating the chromatin landscape of ISCs/EBs by regulating specific histone acetylation marks.

### Perspectives

With Nipped-A we introduce a novel factor that plays a crucial role in maintaining intestinal tissue homeostasis shedding light onto the regulation of the proliferative activity of ISCs during aging. In mammals, TRRAP has been shown to hold important roles in regulating both normal and cancer stem cells. Our findings will spur research on investigating the interplay between TRRAP, c-Myc and histone modification during aging in vertebrates.

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**ST6**

## Directed Elimination of Senescent Cells by Inhibition of BCL-W and BCL-XL

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### Introduction

Cellular senescence limits tumorigenesis and tissue damage in a cell autonomous manner. While short-term induction of cellular senescence can be beneficial in various settings, long-term persistence of senescent cells appears to be deleterious to the organism. These cells commonly secrete pro-inflammatory factors that facilitate their removal by the immune system. The same factors can promote local inflammation, tissue aging, tissue destruction, and potentially tumorigenesis in a cell non-autonomous manner (1-3). Resistance of senescent cells to apoptotic stimuli may contribute to their accumulation, yet the molecular mechanisms allowing their prolonged viability within tissues are poorly characterized.

### Results

Here we show that senescent cells upregulate the anti-apoptotic proteins BCL-W and BCL-XL through a combination of increased transcription and cap-independent translation. Joint inhibition of BCL-W and BCL-XL by siRNAs or the small molecule ABT-737 induced apoptosis specifically in senescent cells induced to senesce by DNA damage, replicative exhaustion or expression of activated Ras oncogene. Notably, treatment of mice with ABT-737 efficiently eliminated senescent cells induced by DNA damage in the lungs as well as senescent cells formed in the epidermis by activation of p53 through transgenic p14ARF. Elimination of senescent cells from the epidermis led to an increase in hair follicle stem cell proliferation.

### Perspectives

Our findings reveal that BCL protein family activation is a central molecular mechanism by which senescent cells acquire increased resistance to apoptosis, and which supports their retention within tissues. Our results suggest that clearance of senescent cells by ABT-737 might lead to tissue renewal and improved tissue fitness. The finding that senescent cells can be eliminated pharmacologically opens the way to new strategies for the treatment of age-related pathologies characterized by the presence of senescent cells.

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**Notes**

**LT5**

## Preventing protein quality control failure and neurodegenerative diseases

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The deposition of misfolded proteins is a defining feature of many age-dependent human diseases, including the increasingly prevalent neurodegenerative diseases. Why aggregation-prone proteins accumulate in aged cells remains largely unclear. Cells normally strive to ensure that proteins get correctly folded and have powerful and sophisticated mechanisms to maintain homeostasis under adverse conditions. However, with age, the cellular defence systems against misfolded proteins gradually fail, leading to the accumulation of misfolded proteins with devastating consequences for cells and organism.
In principle, improving the cells' ability to deal with misfolded proteins should represent a generic approach to reduce the pathology in diverse protein misfolding diseases. My lab has identified powerful strategies to improve the cells' ability to deal with misfolded proteins and implemented one of such strategy in mice to safely prevent two unrelated neurodegenerative disease. Our work demonstrates that generic approaches aimed at helping cells to survive protein quality control failures can be useful to prevent protein misfolding diseases, including the devastating neurodegenerative diseases.



**Notes**

## LT6

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## Notes

**ST7**

## Antagonistic Pleiotropy in the autophagic machinery modulates lifespan conversely over age in C. elegans

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The process of autophagy is a crucial component of organismal survival, ensuring nutrient availability in times of stress and ensuring the degradation of damaged or dysfunctional organelles [1]. Autophagy is generally considered to be beneficial to an organism and the lifespan extension by numerous longevity pathways have already been shown to be dependent on autophagy [2]. However, accumulating evidence suggests that in certain contexts autophagy can be detrimental to health [3,4].

In an RNAi screen for genes which behave in an antagonistic fashion in lifespan regulation, the transcription factor PHA-4/FOXA was identified as a top candidate. Inhibition of pha-4 early in life is known to shorten lifespan but we show that inhibition of pha-4 and its downstream target bec-1 can extend the lifespan of C. elegans (25% and 60% respectively) from mid-life onwards. This modulation of lifespan is highly time dependent and provides evidence for a genetic pathway behaving according to the Antagonistic Pleiotropy theory of aging [5].

Both pha-4 and bec-1 are key members of the autophagy pathway and similarly we find that inhibition of the other genes involved in the process of autophagesome formation also extend lifespan strongly in the post-reproductive worm. This extension was specific to inhibition of the early stages of autophagesome formation as inhibition of downstream genes such as atg-7 or atg-12 had no positive effects on lifespan.

Our data suggests that, during aging, a combination of over-activation and dysregulation of autophagy has negative effects on organismal lifespan and that these detrimental effects are linked to the formation of the autophagesome.

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**Notes**

**ST8**

**Notes**

## An alternative hypoxia response mechanism independent of HIF-1 involves mitochondrial metabolic adaptation

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### Introduction

Hypoxia has been implicated in the pathophysiology of several common and devastating disorders including stroke, ischemic heart disease and cancer. Survival under hypoxia at the cellular, tissue, and organismal level, requires activation of various hypoxia-responsive genes, involved in mitochondrial function, glucose metabolism, glycolysis, autophagy, the unfolded protein response (UPR) and apoptosis. Although, hypoxia-inducible factor-1 (HIF-1) is an essential transcription factor coordinating many of these transcriptional responses to hypoxia, it is becoming apparent that HIF-1-independent hypoxia-responses can also occur. The complex regulatory network activated upon hypoxia, independently of HIF-1, is not fully understood [1, 2].

### Results

We uncovered an alternative hypoxia response mechanism independent of HIF-1 which requires mitochondrial metabolic adaptation. Specifically, we found that expression of T09A5.7/TRIAP-1, the C. elegans homolog of the mammalian TRIAP1/p53CSV, is associated with hypoxia and its activity is HIF-1-independent. Importantly, TRIAP-1 promotes organismal survival under conditions of prolonged hypoxia in the absence of HIF-1. In addition, TRIAP-1 mediates mitochondrial metabolic adaptation upon hypoxia. We further demonstrated that TRIAP-1 regulates various stress response pathways including autophagy, UPR and the intrinsic apoptotic pathway. Last, we showed that TRIAP-1 can regulate C. elegans lifespan when oxygen is abundant. Based on these findings, we propose a novel role of this gene in organismal adaptation to hypoxia independent of HIF-1.

### Perspectives

Tumor cells are often challenged by extreme oxygen deprivation. It is therefore essential for tumor survival to acquire hypoxia adaptation in response to low oxygen concentration. TRIAP-1 has been associated with various types of cancers and stress responses. Our findings suggest a novel, HIF-1-independent, role for TRIAP-1 in hypoxia adaptation, which could modulate hypoxia-induced resistance to anticancer drugs.

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**ST9**

## Lysine-acetylation in cellular regulation, ageing and disease

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Lysine-acetylation was first discovered already in 1964 by Vincent Allfrey to occur on histones. 20 years afterwards α-tubulin was identified as the first cytosolic protein to be acetylated followed by the tumor suppressor protein p53 and the HIV transcriptional regulator Tat. The sirtuins (Sir; silent information regulator) were discovered in a yeast genetic screen to be involved in gene silencing. It turned out that the yeast enzyme Sir2, which is homologous to mammalian Sirt1, has an NAD+-dependent deacetylase activity. Sirtuins have been shown later to be involved in lifespan regulation in yeast, flies and worms. Furthermore, they are implicated in healthy aging and do play protective roles in the development of severe diseases such as cardiovascular and neurodegenerative diseases as well as cancer. Several thousand lysine-acetylation sites have been identified in the proteome of diverse organisms by quantitative mass-spectrometry. The identification of differently charged or uncharged lysine acylations puts another level of complexity on this post-translational modification. Notably, less than 1% of these lysine-acylation sites were functionally characterized so far. One of the major challenges in the acylation research field is to distinguish between biologically relevant and irrelevant sites. We use a combined synthetic biological, biophysical and cell biological approach to structurally and functionally investigate how protein function is regulated by site-specific lysine-acetylation. Our recent data show that proteins of the Ras-superfamily, regulators and effector proteins thereof are dynamically regulated by lysine-acetylation using diverse molecular mechanisms. Tackling the lysine-acylation machinery might allow the development of novel therapeutic strategies to treat age-related diseases.



**Notes**

**LT7**

## Ubiquitin networks in regulation of proteostasis and autophagy

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An increasing number of distinct functions have been assigned to different types of ubiquitin modifications (mono ubiquitin, Lysine-linked and Met1-linked ubiquitin chains). In these processes Ub acts as a signalling component able to trigger molecular events in cells. Ubiquitin-dependent degradation of multiple proteins depends on specific Ub receptors on the proteasome including Rpn10 and Rpn13. These receptors recognize preferentially 48-linked Ub chains. We have also shown that other Ub chains have different role in regulating protein stability by regulation of specific proteases. For example linear ubiquitination controls the NF-κB and apoptotic pathways downstream of TNF receptors. Mice and humans deficient in this pathway develop skin disorders, secondary inflammation in many organs and non-functional immune responses.
Removal of harmful protein aggregates, damaged organelles and microbes is mediated by autophagy, a process by which the cell sequesters cytosolic cargo and delivers it for degradation by the lysosome. Several experimental evidence will be presented about the molecular machinery underlying selective autophagy pathways and how their deregulation can case human diseases including neurodegeneration and cancer



**Notes**

**LT8**

**Notes**

## Fidelity controlled by the Ubiquitin Relative Hub1

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Alternative splicing of pre-mRNAs is a highly effective measure to greatly amplify the protein repertoire of cells. It is textbook knowledge that alternative splicing is guided by trans-acting factors of the SR-protein family, which bind to specific sites in pre-mRNAs, thereby defining exons or introns. However, we recently discovered a strikingly different control mechanism for alternative splicing, which involves the non-covalent incorporation of the ubiquitin-family protein Hub1 into the spliceosome. We found that Hub1 binds both to the U1/U2 snRNP and to the (U4/U6.5) tri-snRNP and that it promotes splicing through activation of a DEAD-box ATPase. In vivo, yeast Hub1 promotes alternative splicing via non-canonical splice sites of HEH1, which encodes an ageing-preventing rDNA tether of the nuclear periphery. On the other hand, it may also cause undesired splicing events via cryptic splice sites leading to aberrant transcripts. Indeed, overexpression of Hub1 is highly toxic in yeast, but only when the mRNA surveillance (decay) machinery is functionally compromised. Intriguingly, unrestrained Hub1-mediated splicing activation is prevented by a sensible mechanism involving the limitation of the spliceosomal DEAD box protein through Hub1-promoted unproductive splicing of its pre-mRNA. Since Hub1 is transcriptionally activated by cadmium and oxidative stress, Hub1-mediated splicing may also constitute a novel cellular stress response.

**ST10**

## Investigating stress granule insolubility with age

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For all organisms promoting protein homeostasis is a high priority in order to optimize cellular functions and resources. However, there is accumulating evidence that aging leads to a collapse in protein homeostasis and widespread non-disease protein aggregation [1].
Our recent work reveals that RNA granule components become highly insoluble with age in C. elegans. Several of the RNA-binding proteins identified to aggregate with age contain low-complexity sequences with "prion-like" domains (Alberti algorithm). These sequences are required for the formation of RNA granules. One of these RNA-binding proteins with a "prion-like" domain is the stress granule marker, polyadenylate-binding protein PAB-1. We find that PAB-1 accumulates with age in insoluble cytoplasmic puncta in wild-type C. elegans. We show that another stress granule marker, TIAR-2 (homolog of TIA-1), also aggregates with age. Both PAB-1 and TIAR-2 co-localize in stress granule-like puncta with age. Delaying aging through dietary restriction or reducing insulin/IGF-1 signaling prevents RNA granule protein insolubility with age. The delay in age-related aggregation in animals with reduced insulin/IGF-1 signaling is dependent on the transcription factor HSF-1. Investigating chaperones downstream of HSF-1 reveals promising candidates playing a role in preventing RNA-binding protein aggregation with age. RNA granules are normally highly dynamic structures yet our results suggest that aging is a sufficient stress to cause the irreversible aggregation of RNA granule proteins, which could impair the function of RNA granules. As stress granule proteins are also found in pathological aggregates associated to neurodegenerative diseases, the formation of RNA granule protein aggregates with age may provide a seed for disease aggregation.

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**Notes**

**ST11**

**Notes**

## Age-dependent aberrant transition of membraneless organelles into pathological aggregates

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In this study, we use biophysical concepts within cell biology and protein biochemistry to study subcellular organization and how it is influenced by aging. Specifically, we investigate how changes in the dynamics of subcellular membraneless organelles lead to age-related diseases.

Many subcellular organelles are not bound by membranes. Membraneless organelles consist largely of RNA and RNA-binding proteins1 and several were shown to possess the material properties of a liquid that dynamically rearranges its components2,3. However, many components of membraneless organelles are also found in non-dissolvable aggregates in neurodegenerative diseases4, raising the possibility that physiological organelles convert from a liquid to a solid state in disease. To test this hypothesis, we examined FUS, a protein that are found as aggregates in Amyotropic Lateral Sclerosis (ALS) neurons. In healthy cells, FUS forms various membraneless organelles: paraspeckles and DNA damage repair sites in the nucleus, and stress granules in the cytoplasm. We examined the biophysical properties of these organelles using previous established criteria2: 1) organelle shape, 2) dynamic rearrangement of molecules within organelles as well as with the surrounding, and 3) organelle fusion. We find that all of these organelles posess the properties of liquids that are 10x as viscous as water. Interestingly, FUS protein by itself formed liquid droplets in vitro that become more viscous with time, eventually hardening into solid aggregates (Figure 1A)5.

Our finding indicates that pathological aggregates might arise from aberrant liquid to solid transition of membraneless organelles. We show that a mutation in a highly disordered domain of FUS accelerate the liquid-solid transition5. Such domains are commonly found in many aggregating proteins as well as not yet disease-linked RNA binding proteins. Thus liquid-solid transition may represent a common mechanism by which protein aggregates form in diseases. Our finding also suggests that active cellular processes are required to prevent aggregate formation in vivo, and that these processes become less effective as cells age. Understanding these mechanisms will have significant impact in not only understanding age-related diseases, but also cellular aging in general.

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## ST12

# Splicing during heat shock is regulated by the bromodomain protein BRD4 and the heat shock transcription factor 1

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### Introduction

In recent years the bromodomain protein BRD4 has gained extensive attention – mainly due to its involvement in tumor growth and its widespread success as therapeutic target in leukemia, lung cancer, melanoma and diverse other tumor entities. As partner of the positive transcription elongation complex pTEFb BRD4 regulates RNA Polymerase II and transcriptional elongation and, by binding to acetylated histones, it links the transcription process to epigenetic patterns. Recently we have shown that the bromodomain protein BRD4 is an integral member of the oxidative stress response by regulating KEAP1 [1]. There are several lines of evidence implicating BRD4 in the splicing process. We thus wondered whether BRD4 might be involved in the transfer from post-transcriptional splicing towards co-transcriptional splicing under stress conditions.

### Results

Using genome-wide splicing analyses of RNA-Seq experiments generated under different stressors we found that BRD4 is indeed involved in the splicing process. This is not the case under normal, unstressed conditions, also not under oxidative stress, but under heat shock (HS). Under HS we found that a BRD4 knock down leads to an increased intron retention rate. We further found that BRD4 interacts with HSF1, co-localizes in nuclear stress bodies and, besides mRNA processing, regulates SatIII RNA expression in an HSF1 dependent manner. This finding might also partly explain why Shalgi and colleagues find a functional co-transcriptional splicing process whereas post-transcriptional splicing is severely impaired under HS [2]. The co-transcriptional process under HS may be protected from splicing factor depletion by recruitment of BRD4 to nSB leading to the maintenance of regular splicing of primary stress response genes.
Taken together, our findings connect BRD4 to the splicing process, but also show that BRD4 acts as a partner of HSF1 in the heat stress response.

### Perspectives

Genome sequencing projects have uncovered widespread splicing defects in cancer. Dvinge et al showed in a pan-cancer study that abnormal RNA splicing and in particular intron retention is a common characteristic of many cancers even in the absence of splicing factor mutations [3]. Since the HS response utilizes many factors required for the proteotoxic stress response in cancer, BRD4 might as well join the splicing complex in the nSB and transmit here the stress response and promote pre-mRNA splicing. We are now investigating the splicing profiles in different cancer entities and their relation to BRD4 inhibitor response.



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**Notes**

**LT9**

## The double life of telomeric proteins

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Telomeric proteins bind telomeric DNA to protect chromosome ends from degradation and inappropriate DNA damage response activation. Interestingly, they are also able to localize outside telomeric regions, where they can regulate the transcription of genes involved in metabolism, immunity, and differentiation as well as preventing replicative damages in heterochromatin regions. We will discuss recent data suggesting that this multifunctionality of telomeric proteins is not simply a meaningless side effect of evolution but instead delineates an important mechanism of telomere signaling by which telomere changes control the ability of their associated factors to regulate genome-wide transcriptional programs and genome stability. This mechanism is expected to enable a greater diversity of cellular responses adapted to specific cell types and telomeric changes, and may therefore represent a pivotal aspect of development, aging, and telomere-mediated diseases.



**Notes**

**LT10**

**Notes**

## Mechanisms underlying genome instability upon DNA repair at telomeres

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Telomeres are specialized nucleoprotein structures that help maintain genome integrity by protecting natural chromosome ends from being recognized and handled as broken DNA. Telomeres in human somatic cells progressively shorten with every cell division, eventually causing telomere dysfunction. Loss of telomere protection activates a DNA damage-like response that initiates cell death or senescence, thereby contributing to aging while preventing outgrowth of potentially cancerous cells. However, deprotected chromosome ends are also processed by DNA repair factors, causing chromosome end-to-end fusions by non-homologous end-joining. If cells with chromosomes fused at their telomeres escape from apoptosis and senescence due to insufficient DNA damage checkpoint activity, continuation of cell division results in missegregation of chromosomes and unbalanced chromosomal rearrangements. This compromises cell viability but can also promote cancer development by causing genetic diversification and selective outgrowth of variant cell clones. Similarly, the inaccurate or inappropriate repair of DNA lesions can contribute to aging or tumorigenesis by fueling genetic alteration. The mechanisms underlying the control of DNA damage responses and repair activities are not completely understood. Therefore, our laboratory aims to identify the genes and activities that play important roles in the telomere damage response and telomere-driven genomic instability. Through functional genetic screening in mammalian cells we identified several factors, without a previously recognized role at telomeres, that promote ligation of telomere-deprotected chromosome ends by non-homologous end-joining and thereby contribute to genomic instability upon telomere uncapping. These factors are known to participate in diverse activities, including posttranslational modification by ubiquitylation, histone methylation and DNA damage tolerance. Through mechanistic characterization of the activities of these factors at telomeres and at DNA DSBs we aim for a better understanding of how cells respond to telomere dysfunction and to DNA damage.

**ST13**

## Endogenous DNA damage, replication stress, and ageing

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### Introduction
The accumulation of genomic and mitochondrial DNA damage and mutations have both been associated with ageing. We wanted to investigate these possibilities. To this aim we generated mice deficient for the DNA translesion synthesis gene Rev1, essential for the mutagenic replication of helix-distorting DNA lesions, including lipid-peroxidation-derived nucleotide adducts [1].

### Results
Rev1-deficient mice are born at sub-Mendelian ratios, display growth retardation, and progressively develop ageing-related phenotypes. Furthermore, Rev1 mice develop a variety of ageing-associated tumors. Remarkably, Rev1 mice also become obese and develop insulin-resistant diabetes. Rev1-deficient hematopoietic stem cells display proliferative defects, indicative of an important role of translesion synthesis in the stem cell maintenance. To further investigate the causal role of replication stress at helix-distorting DNA lesions we intercrossed Rev1 mice with Xpc (nucleotide excision repair-deficient) mice. Rev1Xpc mice develop severe progeroid features. Although the hematopoietic stem cell population is significantly reduced in Rev1Xpc mice, the hematopoietic compartment initially develops normally but collapses at the age of 4 months, displaying hallmarks of oxidative and replication stress, mitochondrial dysfunction, genome instability, senescence and apoptosis. Thus, nucleotide excision repair and translesion synthesis protect stem and proliferating cells against the deleterious consequences of replication stress at endogenous helix-distorting oxidative DNA lesions, thereby preventing features of ageing and ageing-associated pathologies.

### Perspectives
Our data provide strong evidence for a key role of replication stress at endogenous DNA lesions in ageing and in the development of ageing-associated cancer. Moreover, our data suggest that replication stress at endogenous helix-distorting DNA lesions is causally related with the development of diabetes type 2. We envisage that Rev1 and Rev1Xpc mice will be important tools to generate mechanistic insights in ageing and associated pathologies and, ultimately, in the development of preventive strategies.

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**Notes**

**ST14**

**Notes**

## Repression of the antioxidant NRF2 pathway in premature aging

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### Introduction

Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare, invariably fatal premature aging disorder. The disease is caused by constitutive production of progerin, a mutant form of the nuclear architectural protein lamin A, leading through unknown mechanisms to extensive morphological, epigenetic and genomic damage and to mesenchymal stem cell (MSC) attrition in vivo [1-3]. We set out to identify primary HGPS disease mechanisms underlying these defects.

### Results

Using a high-throughput high-content imaging-based siRNA screen we identify the longevity-promoting NRF2 antioxidant pathway as a driver mechanism in HGPS. Progerin sequesters NRF2 and thereby causes its subnuclear mislocalization, resulting in impaired NRF2-mediated transcriptional activation of antioxidants and consequently chronic oxidative stress. Suppressed NRF2 activity or increased oxidative stress are sufficient to recapitulate HGPS aging defects and re-activation of NRF2 activity in HGPS patient cells reverses progerin-associated nuclear aging defects and restores in vivo viability of MSCs in an animal model.

### Perspectives

These findings establish repression of the NRF2-mediated antioxidative response as a key contributor to the premature aging phenotype, and suggest NRF2 activating compounds as a promising novel therapeutic strategy in HGPS and aging diseases.

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**LT11**

## SIRT6 is the new guardian of the genome

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Genomic instability is on of the critical hallmark of the aging process. Activation of the transposons and accumulation of the Double Strand Brakes (DSB) contribute to age-related genomic instability. SIRT6 is histone deacetylase and mono-ADP-ribosylase enzyme involved in multiple processes that are tightly link with longevity. I will discuss our recent results on the role of SIRT6 as a central regulator of double strand break repair and repression of retrotransposable elements. I will also present data that long-lived species of mammals evolve more efficient SIRT6 protein.



**Notes**

**LT12**

## Tissue repair: an integrated view of senescence and reprogramming

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Reprogramming of differentiated cells into pluripotent cells can occur in vivo, but essentially nothing is known about the mechanisms, processes, and mediators involved. We have generated mice where we can induce ubiquitous expression of the four Yamanaka reprogramming factors. These factors, when expressed continuously during 1 week, produce widespread de-differentiation in multiple tissues. Upon switching off the reprogramming factors, de-differentiated tissues re-differentiate and homeostasis is restored. We have found that senescence participates in the process of in vivo reprogramming. Senescence is a cellular response to damage characterized by an abundant production of cytokines and other extracellular factors, which recruit inflammatory cells and can orchestrate tissue remodeling. I will present an integrated view of tissue repair whereby tissue injury, through senescence, primes surviving cells to undergo partial reprogramming and initiate tissue repair.



**Notes**

**ST15**

## Common determinants of global 3D chromatin reorganization upon senescence of different primary human cells: a link to the SASP

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### Introduction

While the human genome encodes genetic information in its linear sequence, precise spatio-temporal execution of gene expression programs is heavily influenced by its 3D organization [1]. Replicative senescence is the physiological outcome of primary cell expansion, both in vitro and in vivo, and is accompanied by marked gene expression changes [2,3]. To date focus has mostly been on replicative arrest, but we argue that several gene expression features underscore the link between senescence and 3D chromatin reorganization. However, still the structure-to-function relationship of chromatin in senescence remains poorly characterized.

### Results

First, we drove human primary endothelial, lung fibroblast, and mesenchymal stem cells into replicative senescence, and performed ultra-deep RNA sequencing to assess transcriptional changes therein. We used the resulting lists to identify a "common backbone" for the three cell type-specific transcriptional programs, with an emphasis on differentially-regulated factors that associate with chromatin. We saw genes coding for proteins involved in chromatin conformation being suppressed, and the levels of transcriptional repressors being induced. Next, we used the whole-genome variant of the chromosome conformation capture technology, "Hi-C" [4], to identify global changes in 3D chromatin organization. We generated and analyzed >5 billion reads from proliferating and senescent cells, leading to interaction maps of <20-kbp resolution. Using these we identified marked reorganization at the multi-Mbp domain scale, a feature underlied by the ubiquitous increase of the nuclear volume in senescent cells. The gain and loss of spatial interactions and the shift in topological domain boundaries correlates with non-histone chromatin constituents extruded from the nucleus into extracellular space, thus contributing to the senescence-associated secretory phenotype (SASP) [5].

### Perspectives

Our work is the first to identify a link between the SASP and the reorganization of 3D nuclear architecture. In its outlook this work points to particular "checkpoint" in-nucleus events that prepare the cell's response in the face of senescent arrest.

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**Notes**

**ST16**

## Rev1-deficiency induces age-related disorders via PARP1 activation and impaired mitochondrial homeostasis

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### Introduction

Aging is defined as the gradual decline of cellular, tissue and organismal homeostasis resulting in cellular senescence, organismal dysfunction and, ultimately, death. Mitochondrial function plays an important role in aging as well as onset of multiple human age-related diseases such as cognitive decline, neurological abnormalities, and cancer. Using various model systems, we have shown that mitochondrial dysfunction results in complex genomic instability, which involves both nucleotide metabolism as well as multiple major DNA repair and DNA lesion synthesis/bypass pathways (1-7). DNA lesions that escape repair will arrest the replication fork, which can lead to replication stress, DNA breaks, and genome instability. To ensure the continuation of replication at damaged DNA templates, translesion synthesis polymerases (TLS) can transiently displace the replicative polymerases and replicate across the lesion. Since TLS polymerases frequently introduce the incorrect nucleotide opposite the lesion, TLS is a mutagenic process. The Rev1 protein plays a central role in TLS at helix-distorting DNA lesions.

### Results

To investigate the biological functions of the TLS protein Rev1, with special focus on mutagenic translesion synthesis in mitochondria, we used Rev1-deficient (Rev1 KO) MEFs and mice. These mice display mild progeroid symptoms suggesting a role for TLS in preventing premature aging. We investigated the molecular mechanisms underlying progeria in these mice and found that the absence of a functional Rev1 protein causes multifactorial mitochondrial dysfunction including abnormal mitochondrial morphology. This phenotype is particularly evident during cellular stress. The mitochondrial abnormalities appear to be caused by NAD+ depletion triggered by activation of the DNA damage sensor PARP-1.

### Perspectives

The results will contribute to basic understanding of DNA repair, aging, nucleotide metabolism, and mitochondrial function. They could have implications for understanding many aspects of human aging and age-associated human diseases. In the long term, the proposed studies could also stimulate development of novel diagnostic and therapeutic agents for treating human disease.

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**Notes**

**ST17**

## DNA Replication Sress Underlies Renal Phenotypes in CEP290-Associated Joubert Syndrome

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**Background**
Juvenile ciliopathy syndromes that are associated with renal cysts and premature renal failure are commonly the result of mutations in the gene encoding centrosomal protein CEP290. In addition to centrosomes and the transition zone at the base of the primary cilium, CEP290 also localizes to the nucleus; however, the nuclear function of CEP290 is unknown. Recent data suggest a role for DNA damage signalling in renal ciliopathies.

**Methods**
We set out to extend this correlation to a broader clinical base and investigated the role of CEP290 loss in DNA damage signaling and replication stress. We used primary cells isolated from kidneys of Cep290LacZ/LacZ mice with Joubert syndrome symptoms and their wild-type littermates to investigate DNA damage signaling and the replication stress response. In addition, primary cells from Joubert syndrome patients and zebrafish embryos depleted for CEP290 have been examined.

**Results**
We demonstrate that reduction of cellular CEP290 in primary human and mouse kidney cells as well as in zebrafish embryos leads to enhanced DNA damage signaling and accumulation of DNA breaks ex vivo and in vivo. Compared with those from wild-type mice, primary kidney cells from Cep290-deficient mice exhibited supernumerary centrioles, decreased replication fork velocity, fork asymmetry, and increased levels of cyclin-dependent kinases (CDKs). Treatment of Cep290-deficient cells with CDK inhibitors rescued DNA damage and centriole number. Moreover, the loss of primary cilia that results from CEP290 dysfunction was rescued in 3D cell culture spheroids of primary murine kidney cells after exposure to CDK inhibitors.

**Conclusion**
Together, our results provide a link between CEP290 and DNA replication stress and suggest CDK inhibition as a potential treatment strategy for a wide range of ciliopathy syndromes. Our findings support the overall hypothesis that renal ciliopathies are initially caused by DNA damage and replication stress during early stages of development.



**Notes**

**LT13**

## mTOR signaling in growth and metabolism

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TOR (target of rapamycin) is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, cellular energy, and stress. TOR was originally discovered in yeast but is conserved in all eukaryotes including plants, worms, flies, and mammals. The discovery of TOR led to a fundamental change in how one thinks of cell growth. It is not a spontaneous process that just happens when building blocks (nutrients) are available, but rather a highly regulated, plastic process controlled by TOR-dependent signaling pathways. TOR is found in two structurally and functionally distinct multiprotein complexes, TORC1 and TORC2. The two TOR complexes, like TOR itself, are highly conserved. Thus, the two TOR complexes constitute an ancestral signaling network conserved throughout eukaryotic evolution to control the fundamental process of cell growth. As a central controller of cell growth, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.
While the role of TOR in controlling growth of single cells is relatively well understood, the challenge now is to understand the role of TOR signaling in disease and in coordinating and integrating overall body growth and metabolism in multicellular organisms. This will require elucidating the role of TOR signaling in individual tissues. Data on the role of mammalian TORC1 (mTORC1) and mTORC2 in controlling cellular processes and in specific tissues will be presented.



**Notes**

**LT14**

## The regulation of TORC1 activity has important influences on the DNA damage checkpoint response

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The DNA damage checkpoint plays a critical role in ensuring genome integrity by preventing cell division in the presence of genotoxic stress. When the checkpoint fails, unrepaired/damaged DNA gets passed on to daughter cells, which can lead to genome instability and aneuploidy, both of which are hallmarks of cancer and aging. One source of checkpoint inactivation is referred to as adaptation, whereby the checkpoint is actively extinguished despite the persistence of non-repaired DNA. Although the prevention of checkpoint adaptation may be critical in ensuring genome stability, its regulation and the downstream consequences remain poorly characterized. Using S. cerevisiae as a model organism, we demonstrate that TORC1 signaling can provoke inactivation of the DNA damage checkpoint in the presence of persistent (irreparable) DNA damage. This is achieved, at least in part, by keeping levels of the polo-like kinase, Cdc5, high. Indeed, rapamycin treatment reduces Cdc5 levels and hence prevents checkpoint adaptation. We demonstrate that in repair- proficient cells the prevention of checkpoint adaptation increases cell viability following the exposure of cells to chronic DNA damage. Strikingly, by preventing adaptation in repair- deficient cells, either with rapamycin or through introduction of an adaptation-defective CDC5 allele, we were able to hyper-sensitize cells to camptothecin (CPT). Importantly, whereas CPT resistance and aneuploidy developed in >80% of repair-defective cells, this was reduced to <25% when adaptation was prevented. Finally, we demonstrate that rapamycin-mediated lifespan extension is dependent on the DNA damage checkpoint. Together, our results suggest that TOR-mediated checkpoint regulation plays an important role in genome stability during aging and following treatment with genotoxic drugs.



**Notes**

**ST18**

**Notes**

## mTORC1-C/EBPβ regulation of healthspan and lifespan

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The mammalian target of rapamycin complex 1 (mTORC1) is a central regulator of physiological adaptations in response to changes in nutrient supply. Inhibition of mTORC1 signaling increases health- and lifespan in many species. Major downstream targets of mTORC1-signaling are the mRNA-translation regulators p70 ribosomal protein S6 kinase 1 (S6K1p70) and the 4E-binding proteins (4E-BPs). However, little is known about vertebrate mRNAs that are specifically controlled by mTORC1 at the level of translation and convert mTORC1 signaling into physiologic responses. Here we show that translation of the CCAAT/Enhancer Binding Protein β (C/EBPβ mRNA into the C/EBPβ-LIP isoform is suppressed in response to reduced mTORC1 signaling in vitro and in vivo caused by pharmacological inhibition, caloric restriction or genetic elimination of 4E-BPs. Intriguingly, mice lacking the cis-regulatory uORF in the C/EBPβ-mRNA that is required for C/EBPβ-LIP induction through mTORC1 signaling recapitulate beneficial phenotypes that are similar to those induced by CR and reduced mTORC1 signaling, although caloric intake is not reduced. These phenotypes include leanness with increased fat metabolism, insulin sensitivity and glucose tolerance and increased physical activity with elevated energy expenditure [1]. In addition, motor coordination is enhanced and preserved during ageing in the uORF-deficient mice. A lifespan determination experiment revealed for female uORF-deficient mice an extended median survival of 20% and a maximal survival of 10% that is mainly due to reduced cancer incidence specifically in female mice. Thus, our data suggest that translational adjustment of C/EBPβ-isoform expression is one of the decisive processes in mTORC1-mediated determination of healthspan and lifespan.

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**ST19**

## MicroRNA-29 controls a compensatory response to limit neuronal iron accumulation during aging

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### Introduction

A large number of transcriptional changes were described during aging. In many instances, it is remains unclear whether all of these changes are detrimental and contribute to age-dependent deteriorations or some represent a compensatory response that limits damage.Iron is known to accumulate with age in the human brain (1) and iron overload can promote aging (2). Here, we investigated the relationship between age-dependent iron overload and microRNA expression using as model organism the short-lived fish N. furzeri, a paricularly convenient model to study aging in vertebates (3).

### Results

1.We used miRNA-seq to investigate age-dependent miRNA expression in the brain, liver, skin and heart of the short-lived fish N. furzeri. We detected only two miRNAs that were commonly regulated, and one of these is miR-29. Analysis of correlation between targets and microRNAs in the brain independently identified miR-29 as a regulator of age-dependent gene regulation.
2.Brain iron content increases with age over 10-fold in N.furzeri.
3.MiR-29 is expressed in neurons and its expression is induced by iron overload in cultured mouse neurons and in the N. furzeri brain in vivo.
4.MiR-29 negatively regulates the expression of iron responsive protein 2 (IRP2), the master protein that controls iron homeostasis in neurons by inducing iron intake and repressing iron discharge
5.We generated transgenic fish with neuronal-specific mir-29 deficiency. These transgenic fish show a chronic up-regulation of IRP2 and of its target transferrin receptor 1a (TFR1A) resulting in increased age-dependent iron accumulation
6.Transcriptome profiling and histological analysis revealed an increased expression of aging-related phenotypes upon miR-29 deficiency. In particular, an accumulated accumulation of lipofuscin (a product of iron-mediated lipid peroxidation) reveals that some of these progetic phenotypes may be directly caused by iron overload
These data demonstrate that up-regulation of miR-29 during aging is a compensatory response to limit the cellular damage induced by iron accumulation.

### Perspectives

One of the most deregulated pathways upon miR-29 deficiency is oxidative phosphorylation. a link between miR-29 and mitochondrial dysfunction is a priority for future investigations..

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**Notes**

**ST20**

**Notes**

## Reduced insulin/IGF-1-signalling implicates extracellular matrix remodelling in longevity

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### Introduction

Interventions that delay ageing mobilize mechanisms that protect and repair cellular components, but it is unknown how these interventions might slow the functional decline of extracellular matrices, which are also damaged during ageing. Reduced insulin/IGF-1 signalling (rIIS) extends lifespan across the evolutionary spectrum.

### Results

Here we show that rIIS can promote C. elegans longevity through a program that requires the Nrf (NF-E2-related factor) orthologue SKN-1 acting in parallel to DAF-16. SKN-1 is inhibited by IIS and has been broadly implicated in longevity. When IIS is decreased, SKN-1 most prominently increases expression of collagens and other extracellular matrix genes. Diverse genetic, nutritional, and pharmacological pro-longevity interventions delay an age-related decline in collagen expression. These collagens mediate adulthood extracellular matrix remodelling, and are needed for ageing to be delayed by interventions.

### Perspectives

The importance of collagen production in diverse anti-ageing interventions implies that extracellular matrix remodelling is a generally essential signature of longevity assurance, and that agents promoting extracellular matrix youthfulness may have systemic benefit.

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**LT15**

## Control of growth and metabolism by mTOR

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mTOR is the target of the immunosuppressive drug rapamycin and the central component of a nutrient- and hormone-sensitive signaling pathway that regulates cell growth and proliferation. We now appreciate that this pathway becomes deregulated in many human cancers and has an important role in the control of metabolism and aging. We have identified two distinct mTOR-containing proteins complexes, one of which regulates growth through S6K and another that regulates cell survival through Akt. These complexes, mTORC1 and mTORC2, define both rapamycin-sensitive and insensitive branches of the mTOR pathway. I will discuss new results from our lab on the regulation and functions of the mTORC1 and mTORC2 pathways.



**Notes**

**LT16**

## Physiological regulation by stearic acid

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Mitochondrial dysfunction has been linked to aging in both physiological and pathophysiological conditions. In particular, impaired mitochondrial function has been causally linked to development of neurodegenerative disease such as Parkinson's. I will present recent findings that a small lipid metabolite, stearic acid, acts as a signaling molecule to regulate mitochondrial function via a new molecular signaling route. Interestingly, dietary supplementation with stearic acid can delay the occurrence of neurodegenerative phenotypes in Drosophila models of Parkinson's disease, suggesting that dietary stearic acid plays a role in promoting healthspan.



**Notes**

**LT17**

## Nutritional regulatory networks

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Gene regulation and metabolism lie at the heart of most biological processes. Both are accomplished by complex networks harboring hundreds of nodes and thousands of edges. We study these networks and the interactions between them mainly in the nematode C. elegans, because it is amenable to high-throughput, large-scale genetics and genomics. In addition, we study interspecies network interactions between C. elegans and bacteria, that may help illuminate connections between mammalian intestinal cells and the gut microbiota.



**Notes**

**ST21**

## Changes in the intestinal microbiota affect ageing in the short-lived African turquoise killifish

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Vertebrates are equipped with a vast and complex intestinal microbial community, which plays a fundamental role in host metabolism. Growing evidences indicate that specific intestinal microbial communities are associated with a healthy physiological status. Microbial imbalance, or dysbiosis, is associated with several severe conditions, including inflammation and cancer. Although intestinal microbial transplants can positively impact acute intestinal infections and improve insulin sensitivity, there is no direct evidence that manipulating the intestinal microbiota can positively affect the host ageing process. To address this question, we used the short-lived African turquoise killifish as a vertebrate model organism. Sequencing the microbial 16S rRNA gene in young and old fish we found that young fish had higher microbial species diversity and overall higher microbial count. Specifically, young fish had higher representation of Firmicutes, Bacteroidites and Actinobacteria, while old fish had higher representation of Vibrionales, Proteobacteria and Gammaproteobacteria. Additionally, young fish intestines were enriched for bacterial genes involved in amino acid and glucose metabolism, while old intestines were enriched with bacteria associated with generalised response to pathogenicity.
We then asked whether transplanting young fish intestinal content to old fish could affect the ageing process. After antibiotic treatment, we recolonized middle-age fish intestines with different microbial donor pellets; when we transplanted young-fish microbial pellets to middle age fish, we observed a significant increase in lifespan compared to control groups treated with old-fish intestinal content. We tested whether fish receiving a younger microbiota would also be affected in more generalized ageing biomarkers – such as spontaneous locomotor activity – and observed a significant increase in overall motility in old fish treated with young microbial pellets, compared to old fish that received same age-group microbial pellets.
Overall, our results show that the intestinal microbial community can play a fundamental role during vertebrate ageing and that targeted microbial manipulations can slow down ageing and have a significant impact on individual survival. Future studies will reveal what mechanisms underlie the positive contribution of a healthy microbial community on the host metabolism and ageing process.



**Notes**

# POSTER ABSTRACTS

**P1**

## Detection of DNA damage response RNAs (DDRNAs) at dysfunctional telomeres

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**Introduction**
Our lab has recently shown that small non-coding RNAs are novel components of the DNA damage response (DDR) machinery [1]. These small RNAs, named DDRNAs, are involved in the control of the DNA damage response (DDR) activation, have the sequence of the damaged locus and their biogenesis depends on Dicer and Drosha endoribonucleases [1,2]. Upon TRF2 inactivation telomeres are uncapped and a DDR is triggered [3]. Different approaches are being undertaken to detect the presence of DDRNAs upon telomere uncapping.

**Results and Perspectives**
In order to detect telomeric DDRNAs, a qRT-PCR method designed to quantify miRNAs was employed. Since DDRNAs have sequences corresponding to the damaged sites, the two potential sources of DDRNAs in this system are the G-rich and the C-rich strands of the telomere. The results obtained showed an up regulation of telomeric DDRNAs for both strands upon telomere uncapping. Moreover, DDRNA quantification was measured in telomere-damaged cells upon Dicer and Drosha KDs showing an expected decrease compared to uncapped cells. It is a reasonable hypothesis that a longer precursor is needed for the generation of DDRNAs. This can be tested by qPCR [4]. We will report on our progresses in our attempts to monitor telomeric precursor transcripts' accumulation.

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## P2

### Disturbed protein homeostasis in Cockayne syndrome- a circulus vitiosus may cause premature aging

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Cockayne syndrome (CS) is a progeria characterized by childhood onset of degenerative symptoms reminiscent of the aging body as loss of subcutaneous fat, alopecia, cataracts, neurological degeneration and cachexia, accompanied with developmental delay resulting in a severe phenotype ("cachectic dwarfs") witch can lead to childhood death. It is a model disease of "accelerated" aging and its exploration should foster our understanding of the "normal" aging process. CS can be caused by the recessive mutation of 5-6 genes that are all involved in a branch of Nucleotide-Excision Repair (NER), thus explaining the elevated UV-sensitivity of the patients, however, total loss of NER is not necessarily followed by premature aging suggesting that a loss of alternative functions of the CS-proteins may cause premature aging. One common alternative function of at least 5 CS-proteins is transcription of the ribosomal RNA by RNA polymerase I. Here we show that a disturbed RNA polymerase I transcription is followed by a decreased translational fidelity at the ribosomes and oxidised proteins initiating endoplasmic stress that elicits a unfolded protein response that in turn represses RNA polymerase I transcription. Oxidative hypersensitivity- a hallmark of CS cells and the pathophysiologic difference to cells with the mild UV-sensitive syndrome, which can also be caused by mutations in some CS proteins, can be overcome by chemical chaperones. Moreover, chemical chaperones can break the circulus vitiosus and restore RNA polymerase I transcription and growth of CS-cells. As these chaperones are approved by the FDA for the treatment of neurodegenerative diseases, our findings imply a possible treatment for a devastating childhood disorder and may have impact to our understanding of the aging process itself.





## P3

### Dissecting the functional role of Ercc1 NER factor in pancreatic cells

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Aging in eukaryotes is accompanied by stochastic DNA damage accumulation, improper folding of proteins and as a result - a progressive atrophy of tissues and organs. Accumulating evidence suggests that loss of genomic maintenance may causally contribute to aging. Premature aging (progeroid) syndromes with underlying mutations in nucleotide excision repair (NER) factors exemplify the role of DNA damage in aging. However, the exact mechanisms that lead to tissue failure during aging or upon DNA damage accumulaton are unknown. Our study focuses on unraveling the role of the NER factor ERCC1 in pancreatic cells in Ercc1 knockout mice that die postnatally and exhibit premature aging symptoms as well as developmental defects[1]. We investigate the physiological and morphological changes in Ercc1 knockout pancreatic cells and, more specifically, association of ERCC1 deficiency with endoplasmic reticulum stress.

Transmission electron microscopy studies showed ER lumen dilation in the in Ercc1-/- pancreas, with no apparent changes in ER lumen in the liver and kidney. Also, we observed significant changes in the expression levels of ER stress-related genes and increased DDR signalling detected by immuofluoresense in Ercc1-/- pancreatic cells. Those findings suggest that Ercc1 deficiency triggers DNA damage response associated with perturbation of proper ER function (i.e. accumulation of unfolded protein aggregates), thereby causing ER stress. We hypothesize that DDR-induced senescence triggers ER stress, as indicated by the increased percentage of HMGB1-associated positively stained nuclei in Ercc1-/- pancreatic cells and from upregulation of several SASP-associated genes according to microarray data from Ercc1Δ pancreas.

Ercc1-/- acinar cell phenotype such as ER disorganisation/dilation, "blocked" ducts and extensive collagenization and its similarity to pancreatitis phenotype[2] made us hypothesize that pancreatitis is a downstream consequence of irreparable DNA damage and ER stress that result from ERCC1 deficiency. Our goal is to establish a functional relationship between DNA damage, DDR, ER stress and senescense[3] in wildtype and Ercc1-/- pancreatic cells in vitro and in vivo using immunofluoresence, mRNA expression analysis and immunoprecipitation assays.

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## P4

### Loss of mitochondrial aspartyl-tRNA synthetase (DARS2) leads to progressive neurodegeneration

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**Introduction**
Mitochondrial tRNA synthetases (ARS2) are enzymes which are helping the initial step of mitochondrial translation – covalent attachment of an amino acid to its cognate tRNA. Defects in different ARS2 lead to versatile phenotypes, which are tissue-specific. Defects of DARS2 result in leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) [1]. DARS2 whole-body knock out mice are embryonic lethal, which explains the importance of this group of enzymes for mitochondria well-being. We wanted to analyze a conditional knockout with DARS2 depletion in forebrain neurons, since LBSL affects nervous system. This was achieved by CaMKIIα-Cre excision of exon 3 of the Dars2 gene, using Cre-loxp technology.

**Results**
The signs of neurodegenerations were measured using different visualization techniques, electron microscopy, immunohistochemistry and histological stainings. Mitochondrial OXPHOS was examined by Western Blot, BN-PAGE and In organello translation rate.
The signs of neurodegeneration started at 20 weeks of age when knockout mice showed apoptosis in the cortex, which was followed by progressive atrophy of the cortex and hippocampus and abnormal behavior at 29 weeks of age. Furthermore, loss of neurons led to an increased immune response and severe diffuse reactive astrogliosis. Affected neurons presented OXPHOS deficiency and defective mitochondrial structures.

**Perspectives**
Mitochondrial dysfunction, mediated by DARS2 deficiency, triggers late-onset progressive neurodegeneration with a high specificity to different brain regions in an age-dependent manner. Earlier timepoints should be investigated in depth in order to follow up the onset of the disease phenotype.

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## P5

### A role for Drosophila CerS schlank homeodomain in the regulation of lipid and energy homeostasis, independent of its catalytic activity

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**Introduction**
Ceramide Synthases (CerS) are key enzymes of the sphingolipid metabolism. All CerS studied to date contain a catalytically active Lag1p motif and many CerS contain in addition a homeodomain of unknown function [3]. Many pathogenic changes are associated with changes in ceramide levels.
We have characterized the single Drosophila CerS schlank and we showed its requirement for both (dihydro)ceramide synthesis and the regulation of triacylglycerol (TAG) metabolism [1].
Now we found that the schlank homeodomain is involved in regulating lipid metabolism independent of the catalytic lag1p motif [2].
Here we show our approach of versatile modifications of schlank by "genomic engineering" to analyze the role of the homeodomain in regulating lipid metabolism in vivo. Genomic engineering allowed us to generate a founder knockout line (KO) by deleting the schlank - gene and replacing it with an integration site of ΦC31. Integration of schlank variants carrying distinct mutations in the homeodomain by ΦC31 is used to reintroduce modified schlank-gene DNA into the native locus in the KO.

**Results**
We successfully generated a schlank KO that phenotypically resembles the described P-element mutant alleles [1]. Furthermore, we reintroduced wildtype schlank DNA and rescued the phenotype.
In addition, preliminary data of two reintegrated schlank - variants with mutations within putative nuclear localization sequences (NLS-1, NLS-2) within the homeodomain exist. Whereas knock in lines with mutations in NLS-1 (schlank KI NLS1) had no obvious phenotype, mutations in NLS-2 (schlank KI NLS2) lead to developmentally delayed animals appearing much slimmer, which indicated loss of body fat. Finally, adult animals show severe motoric defects. Therefore, we wanted to investigate whether the mutation of the NLS-2 site within the homeodomain affects fat and energy metabolism.
To test this we measured parameters such as triacylglycerol (TAG), ATP, and AMP-activated protein kinase (AMPK). Here we show that TAG levels and ATP levels are reduced in schlank KI NLS2 mutants as compared to control. Previous studies found that reduced ATP leads to more phosphorylated AMPK (p-AMPK), which is considered to serve as energy sensor [4]. We hypothesized that reduced ATP in schlank KI NLS2 mutants will also result in increased p-AMPK. Immunoblotting results demonstrate that p-AMPK is indeed significantly upregulated in schlank KI NLS2 mutants. Thus, this suggests that the TOR pathway (target of rapamycin complex) is downregulated resulting in smaller animals.

**Perspectives**
Our data provide evidence that the homeodomain of CerS schlank might be involved in the regulation of energy homeostasis via modulation of TOR signaling pathway.

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## P6

### Autophagy is pro-senescent in Proximal Tubular S3 Segments upon Acute Kidney Injury

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**Introduction**
Autophagy mediates degradation and turnover of misfolded proteins and damaged organelles [1]. It is also upregulated during Ischemia-Reperfusion (I/R) injury of the kidney [2] which is known to induce cellular senescence and fibrotic injury in renal epithelial cells. Although autophagic activity has been considered reno-protective during acute kidney injury (AKI) [3], the functional role of autophagy in long term renal repair, onset of tubular cellular senescence and development of chronic kidney disease (CKD) is not known.

**Results**
Mice were generated with Tamoxifen inducible Atg5 gene deletion in proximal tubular S3 segment (Atg5 Δflox/Δflox), which is the most damaged part of the nephron during I/R injury. After 30 days of renal I/R, Atg5 Δflox/Δflox kidneys displayed significantly reduced tubular senescence, reduced expression of pro-senescent cell cycle regulators p16INK4a and p19ARF. Further, there was diminished interstitial fibrosis and decreased intra-renal immune infiltration. FITC-Sinistrin based GFR measurements indicated improved GFR recovery in Atg5 Δflox/Δflox animals. To correlate this long term outcome with early ischemic damage, kidneys were analysed 2 hours and 3 days after reperfusion. Interestingly, the Atg5 Δflox/Δflox displayed accelerated cell death at 2 hours in comparison to control kidneys but presented with reduced tubular damage and inflammation at day 3.

**Perspectives**
In summary, our results show that lack of autophagy compromises early survival mechanisms in severely damaged tubular cells within the S3 segment. However, the persistence of such damaged cells contributes to maladaptive repair and pro-inflammatory changes, leading to development of a senescent phenotype and CKD. These observations are pivotal while considering non-selective autophagy enhancement as a novel therapy for treatment of AKI.

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## P7

### X chromosome instability phenotypes in Azheimer's Disease in women: is it a aging problem?

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**Introduction**
X chromosome instability has been a long established feature in Alzheimer's Disease (AD), but also of aging in women. Changes in the X chromosome has been related to aneuploidy, replication asynchrony, to cohesion related alterations named premature centromere division (PCD) and to X chromosome skewing. PCD of chromosome X has been found in peripheral blood lymphocytes and neuronal tissue in female AD patients. These results raised a question: ''Is the X chromosome inactivation pattern, PCD, aneuploidy distributed in peripheral blood lymphocytes in women affected by AD and/or how are these instabilities affected by the aging process?"

To address this question we used fluorescent in situ hybridisation (FISH) for centromere region of X chromosome to determine aneuploidy and possible correlation with PCD, X in lymphocytes of AD females and age-matched controls. We also used the androgen receptor on the X chromosome in order to explore the methylation status by using q PCR. This method would show us any deviation from the 50:50% X inactivation status in peripheral blood lymphocytes of 10 AD women compared to age matched controls.

**Results and Conclusion**
In AD patients our results showed a marked and significant increase in the frequency of the X chromosome aneuploidy comparing with age matched controls ($p<0.001$). Also, a significant difference was detected in the PCD,X frequency between AD
females when compared with age matched controls ($p<0.001$). In addition, a strong and significant ($p<0.001$) correlation between the frequency of aneuploidy and PCD of chromosome X was found in AD group, as well as in control group ($p<0.01$). Our results showed skewed inactivation patterns (>90%)in AD women but not in age matched controls.

**Perspectives**
These findings suggest that an epigenetic alteration on the inactivation centers in the region of the centromere results in a array of instability phenotypes and that X chromosome instability in our view relates not only to aging, by might be a novel property that could account for the higher incidence of AD in women and may have a biomarker potential.

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## P8

### Adaptive immunodeficiency accelerates intestinal aging of telomere-dysfunctional mice

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**Introduction**
Mice with dysfunctional telomeres suffer from accelerated aging phenotypes. The accumulation of DNA damage triggers checkpoint responses, which compromise stem cell function and prevent genomic instability. However, loss of stem cell function contributes to aging pathologies. Recently, several studies provided evidence that oncogene induced stress and DNA damage both engage the immune system preventing tumour formation and the survival of damaged cells 1. Little is known about a crosstalk between aging associated DNA damage responses and the immune system. To this end we set up studies comparing aging wild type mice with Rag2 knockout mice, and the respective cohorts in a telomerase (Terc) knockout background.

**Results**
Late generation Terc-/- mice develop age related pathologies in highly proliferative tissues like the hematopoietic system and the intestine characterized by stem cell dysfunction, impaired tissue regeneration, accumulating DNA damage, gastrointestinal crypt atrophy and reduced B and T cell proliferation 2. Our preliminary data show improved survival of aging late generation Terc-/- mice as compared with double knockout mice (Rag2-/- Terc-/-). The histologic analysis reveals aggravated crypt atrophy, fibrosis, a higher incidence of precancerous microadenoma, and more DNA damage in the gastrointestinal epithelium of Rag2-/- Terc-/- mice compared to Terc-/- mice. FACS analysis revealed increased macrophages infiltration in Rag2 Terc (DKO) lamina propria of intestine.

**Perspectives**
To our knowledge increase macrophages and absence of T cells in Rag2 Terc (DKO) are responsible for wasting colonic phenotype. Innate and adaptive cytokines miscommunication in gut also contributes to pathology. Further experiments are ongoing to confirm these phenotypes mainly by initiation of damage (extrinsic (IR)) and resulting immune cells infiltration in gut lamina propria, detailed sub-phenotyping of infiltrating cells by FACS analysis, at RNA and protein level.

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## P9

### Porphyromonas gingivalis induces senescence in endothelial cells

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**Introduction**
Chronic oral inflammatory diseases like periodontitis have been shown to be linked to adverse cardiovascular outcomes[1-2]. The underlying mechanism of this correlation is largely unknown. Senescence refers to a phenotype of permanent irreversible growth arrest described in mammalian cells in culture. Several lines of evidence point towards an important role of senescence in aging processes in vivo. The hypothesis is that senescence exhausts the reserves of somatic cells that are involved in cell-division and thereby cell-renewal– including repair and regeneration[3-4]. The association of advanced age with poor oral health [5] leads us to hypothesize that Porphyromonas gingivalis generally associated with diseases like periodontitis induce cellular senescence in endothelial cells. We are studying the induction of senescence by oral bacteria or bacterial-challenged cells in vitro using human umbilical cord endothelial cells (HUVEC).

**Results**
HUVEC were incubated with different MOI of P.gingivalis, RA was extracted and the expression of p16INK4A and p21CIP1WAF were determined by Real Time rtPCR. For the detection of an additional marker of cellular senescence, fixed cells were stained for Senescence-associated β-Galacosidase (SA-β-Gal).
We could demonstrate that repeated incubation (2x 24h) of HUVEC with P. gingivalis (MOI: 1, 10, 25, 75, 100) leads to a significant upregulation of p16INK4A and p21CIP1WAF mRNA and protein and to a significant increase in SA-ß Gal-positive cells.

**Perspectives**
To better understand the interplay between periodontitis, senescence induction, and cardiovascular outcomes, we will measure inflammatory mediators in the supernatant of P.gingivalis -challenged HUVEC, and we will treat ApoE mice with P.gingivalis. To prove the concept that knockout of senescence pathways in endothelial cells is able to protect from inflammatory-derived vascular degeneration, we will cross ApoE KO mice with mice possessing an inducible endothelial-specific knockout of p16INK4a (p16INK4a EC KO).

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## P10

### Inhibition of TORC1 promotes cell viability following DNA damage

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**Introduction**
In response to DNA damage, cells activate the DNA damage checkpoint, leading to cell cycle arrest. The arrest ensures that damage is not propagated to daughter cells, while at the same time providing time for repair events. When damage is irreparable, cells remain arrested for an extended period, but eventually extinguish the checkpoint and continue to proliferate despite persistent damage - a process referred to as checkpoint adaptation. Checkpoint adaptation frequently leads to increased genome instability and severely compromised viability.

**Results**
Inhibition of TORC1 signalling via rapamycin treatment or nutrient starvation is able to prevent checkpoint adaptation in yeast and stably sustain a G2 arrested state. The prolonged G2 arrest has the effect of dramatically improving viability following the repair of damaged DNA. Importantly, we found that the protective effects of rapamycin were dependent on both an intact DNA damage checkpoint as well as functional DNA repair pathways. We have recently elucidated relevant downstream regulators involved in the rapamycin-mediated protective effect, including autophagy, the kinase Sch9 and the yeast Polo-like kinase 1 homologue, Cdc5. In order to better understand how checkpoint adaptation is prevented under TORC1- inhibitory conditions, we analysed the effects of rapamycin treatment on Cdc5 protein levels. Strikingly, we found that Cdc5 protein levels are decreased when TORC1 is inhibited. However, neither Cdc5 protein degradation nor protein half-life are significantly affected by rapamycin treatment indicating that the regulation of Cdc5 takes place at the transcriptional level.

**Perspectives**
We would like to further investigate the link between TORC1 inhibition and the prevention of checkpoint adaptation and thereby focus on Cdc5 transcription levels. Furthermore, we are investigating the role of other TORC1 downstream effectors such as Tap42 and their role in regulating checkpoint adaptation. Taken together, our results will provide insights on how nutritional cues via the TORC1 signalling pathway can modulate the DNA damage response and thereby influence genome stability.





## P11

### Senescence-Induced oxidative stress causes endothelial dysfunction

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**Introduction**
Endothelial dysfunction is an early pathophysiological hallmark in the development of cardiovascular disease (CVD) [1]. Senescence, which is considered as the cellular equivalent of aging, was proposed to be involved in endothelial dysfunction [2]. Aging has striking effect on heart and arterial system, leading to an increase in risk of cardiovascular disease (CVD) [3]. Endothelial dysfunction is one of the first pathophysiological hallmarks in the development of atherosclerosis and is also detected in aging arteries. However, the functional data showing the causal relationship is poorly established.

**Results**
Endothelium function (vasodilatory responses) in aortic vessels of young WT, aged WT and of telomere deficient (Terc-/-) mice of inbred generation 3 (G3) was observed. The endothelial function was equally impaired in both, aged WT and Terc-/- G3 mice; the later also accompanied by significant telomere shortening in aortic endothelium. In addition to their elevated p16INK4a and p19ARF gene expression, significant increase in number of γH2AX foci and p21CIP1/WAF1 protein expression was observed. This suggested a strong association between the presence of stress-induced senescence and impaired endothelial function. The elevated reactive oxygen species (ROS) levels in these animals, explained their dysfunctional state. Antioxidant treatment restored the vasodilatory function in aged and Terc-/- G3 mice aortas, which highlighted the functional link between the presence of senescence and oxidative stress.

**Perspective**
Our data suggest that cellular senescence is an important early mechanistic cornerstone of vascular degeneration that occurs as part of human aging. We provided functional evidence that goes beyond available in vitro data, showing endothelial impairment in the presence of senescent endothelial cells. Our results suggest that oxidative stress is the mediator of senescence-induced endothelial dysfunction. Even though vascular aging is a complex process and it is difficult to reflect its complexity in model systems, we were able to identify cellular senescence pathways leading to the development of endothelial dysfunction. This finding holds the potential of identifying new targets to prevent CVD.

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## P12

### The role of XPF in mammalian development & disease

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Nucleotide Excision Repair (NER) pathway is a conserved pathway that removes bulky helix-distorting DNA lesions [1]. Whereas defective NER of damaged DNA has been established as the underlying cause of mutations leading to skin cancer, the links between NER defects and the developmental and metabolic abnormalities seen in NER disorders remain obscure. Besides DNA repair, earlier studies have shown that distinct NER factors play also a role in the regulation of gene expression [2], the transcriptional reprogramming of pluripotent stem cells [3], or the fine-tuning of growth hormones during mammalian development [4]. However, the functional contribution of NER to the complex NER developmental disorders remains elusive, primarily due to current difficulties in dissecting the multiple roles of NER proteins in an intact organism.
Here, we report the generation of a new series of mice carrying a biotin-tagged version of the NER factor XPF (i.e. bioXPF animals). BioXPF animals were then crossed with mice expressing the BirA transgene; BirA specifically recognizes and biotinylates the short tag, thus creating a very high affinity "handle" for isolating XPF-bound proteins partners by binding to streptavidin. We then identified the proteomic components of XPF-containing protein complexes by recovering proteins from streptavidin-bound nuclear extracts from UV-irradiated Mefs expressing biotin-tagged XPF and analyzing these complexes by mass spectrometry. Next, through biotin-tagged chromatin immunoprecipitation (ChIP) experiments coupled to next-generation sequencing, we identified the genomic targets of XPF in transcriptionally induced with trans-retinoic acid (tRA) and UV- irradiated MEFs.

The identification of novel protein partners of XPF and its genomic targets will aid in the understanding of XPF role in processes beyond NER during mouse development and disease.

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## P13

### Age-dependent changes in the expressed immunoglobulin repertoire of a short-lived teleost

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Ageing individuals exhibit a pervasive decline in B-cell function, including decreased naïve B-cell production [1] and reduced antibody affinity [2]. These changes contribute to an overall immunosenescent phenotype, with important implications for health and lifespan. The diversity of the antibody repertoire is also widely thought to decrease with age [3]; however, the relatively long lifespans of most conventional vertebrate model organisms have prevented a thorough investigation of this phenomenon. The advent of the short-lived killifish Nothobranchius furzeri [4] provides an exciting opportunity to investigate age-related changes to the antibody repertoire in much greater detail than previously possible.

Using high-throughput sequencing of long-insert clones from the killifish genome BAC library, I have sequenced and assembled the killifish immunoglobulin heavy chain locus to a high degree of accuracy; sequence characterisation is underway. I will then use the characterised sequence to investigate the effect of ageing on the adaptive immune system in this model organism, by comparing the expressed antibody repertoires [5] of young and old fish cohorts. Additionally, I will characterize changes in expressed antibody repertoires following lifespan-enhancing manipulations, such as dietary restriction [6] and gut microbial transfer. Further experiments will investigate strategies to improve killifish lifespan by targeting humoral immunity. The results of these experiments may have important implications for the broader understanding of humoral immunosenescence in clinical and other applied contexts.

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## P14

### A new role of p53 in maintaining the hematopoietic stem cell pool

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**Introduction**
The tumor suppressor p53 has major functions in DNA damage response pathways by triggering cell cycle arrest and apoptosis. Hence, p53 mutations are associated with large numbers of hematological malignancies, including AML, CLL and MDS. In hematopoietic stem cells (HSCs) p53 has been proven crucial for maintenance of HSC quiescence as well as self-renewal and senescence. Recently we identified a novel mechanism ensuring genomic integrity of HSCs: As a response to DNA damage, HSCs do not arrest at the G1/S boundary, unlike differentiated cells, but enter the cell cycle in conjunction with massive apoptosis [1]. With p53 being involved in DNA damage pathways, our aim was to study its role in this previously unknown mechanism in detail.

**Results**
Here we report an unexpected new role of p53 in the intrinsic regulation of the HSC pool. As a response to DNA damage, HSCs from p53 knockout mice do not enter the cell cycle and undergo apoptosis as seen in HSCs from wild type mice but preserve their quiescent state protecting them from cell death. Whereas wildtype mice lose almost all their HSCs of the bone marrow shortly after irradiation, the number of HSCs in p53-/- mice remains unaffected. This effect is primarily stem cell specific but can be also observed in a lesser extent in early progenitors. However, it is not present in less primitive progenitors, such as Lin-ckit+ cells. Importantly, transplanted p53-/- HSCs show extensive engraftment abilities upon sublethal irradiation with the corresponding mice developing symptoms of leukaemia such as high number of blast cells in bone marrow and lack of mature blood cells. Together with our previously published data we believe that we have discovered a novel p53-dependent stem cell specific checkpoint which is responsible for driving DNA-damaged HSCs out of quiescence, removing them from the stem cell pool. We suggest that this proposed mechanism may play an important role in preventing leukemic transformation of HSCs.

**Perspectives**
We plan to further scrutinize our proposed mechanism: One the one hand we want to identify its key players, on the other hand we want to determine the consequences of "checkpoint failure". To this end, we will apply single-HSC DNA sequencing and single-HSC RNA-Seq to detect mutations, mutation frequencies, chromosomal abnormalities and changes of gene expression in wildtype and p53-/- HSCs isolated from transplanted mice under various stress conditions.

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## P15

### The phosphorylation of APP at T668 mitigates γ-secretase processing of the precursor and reduces nuclear spheres formation

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**Introduction**
The distinctive pathological features of Alzheimer's disease (AD) are the progressive deposition of the extracellular amyloid plaques and intracellular neurofibrillary tangles. In the amyloidogenic processing of amyloid precursor protein (APP), cleavage of beta C-terminal fragment (β-CTF, produced after β-secretase cleavage), by γ-secretase engenders the amyloid β peptide (Aβ) and the APP intracellular domain (AICD). [1] The AICD interacts with FE65 protein, is translocated to the nucleus and by further interacting with TIP60 and BLM proteins, generates nuclear spheres. These spheres have been proposed to be involved in neuronal cell cycle re-entry, gene expression and apoptosis. [2]
Previously, we were able to show that APP phosphorylation at T668 (pT668) resulted in the ablation of nuclear spheres. Furthermore, we were able to show immunohistochemically that control brain showed less nuclear spheres positive cells as compared to AD brain samples. This suggested pT668 to be neuroprotective since it ablates the production of nuclear spheres, which have already been proposed to be toxic. [3]

**Results**
1) The β-secretase cleavage of pT668 resulted in the enrichment of phosphorylated CTFs (pCTFs), indicating that pCTFs escape γ-secretase cleavage activity.
2) The inhibition of γ-secretase cleavage resulted in the elevation of pCTFs. This implies that endogenous CTFs can also be phosphorylated by endogenous kinases.
3) We also found that CTF 99 (i.e.C99) has better membrane tethering as compared to CTF57 (i.e. AICD57) which has subsequently better tethering as compared to CTF50 (i.e. AICD50). Consequently, C99 has better potential to be phosphorylated at T668 than AICD57, which has better potential to be phosphorylated than AICD50.

**Perspectives**
Taken together, our findings propose pT668 to be an intrinsic mechanism crucial for nuclear sphere generation, which might play a significant role in neurodegeneration.

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## P16

### Multiple Rad52-mediated homology-directed repair mechanisms are required to prevent telomere attrition-induced senescence in Saccharomyces cerevisiae

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Most human somatic cells express insufficient levels of telomerase, resulting in telomere shortening and eventual senescence, both of which are hallmarks of ageing. Homology-directed repair (HDR) is important for maintaining proper telomere function in yeast and mammals. In Saccharomyces cerevisiae, Rad52 is required for almost all HDR mechanisms and telomerase-null cells senesce faster in the absence of Rad52. However, it has been unclear how Rad52 delays senescence. In this study, we made use of rad52 separation-of-function mutants to find that multiple Rad52-mediated HDR mechanisms are required to delay senescence, including both Rad51-dependent and Rad51-independent break-induced replication, as well as sister chromatid recombination. In addition, we show that misregulation of histone 3 lysine 56 acetylation, which is known to be defective in sister chromatid recombination, also causes accelerated senescence. We propose a model where Rad52 is needed to repair telomere attrition-induced DNA replication stress at both subtelomeric and telomeric regions.





## P17

### Rad21 is Essential for the balancing of Hematopoietic Stem Cell Self-renewal and Differentiation

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**Introduction**
Cohesin mediates sister chromatid cohesion, which is important for faithful chromosome segregation and post-replicative DNA repair. In addition to the canonical role, cohesin also regulates gene expression by forming long-range chromosomal cis-interactions. Rad21 is an integral subunit of the cohesin complex. A recent study showed by thymocyte-specific deletion of Rad21 that cohesin-mediated enhancer-promoter interaction regulates T cell differentiation via controlling transcription, Rag reconbinase recruitment, histone modification and T cell receptor rearrangement. However, the role of cohesin in hematopoietic stem cells (HSCs) is largely unknown.

**Results**
To address this, we utilized shRNA knockdown of the key cohesin component gene Rad21 in HSCs and showed that knockdown of Rad21 impairs HSC differentiation during in vivo repopulation and in vitro culture, while simultaneously accumulating HSCs. Ki67 staining showed a significant reduction in the proliferative index of Rad21 knockdown HSCs relative to control HSCs. Knockdown of Rad21 led to defective HSC repopulation ability in vivo and declined single-cell colony forming potential. Furthermore, Rad21 knockdown HSCs exhibited hypersensitivity to γ-irradiation. Although it is known that defective sister chromatid cohesion can induce aneuploidy, there was no difference in aneuploidy between Rad21 knockdown HSCs and control HSCs, which indicates that the defects of Rad21 knockdonw HSCs result from impaired non-canonical cohesin functions instead of defective sister chromatid cohesion.

**Perspectives**
Taken together, these data suggest that Rad21 is an important regulator of HSC and prompt a non-canonical role of cohesin in regulating HSC self-renewal and differentiation.

## P18

### Identifying genomic signatures of repeated adaptation to annualism in African Killifishes

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**Introduction**
African killifishes in the family Nothobranchiidae evolved adaptations to ephemeral waters by adopting an annual life cycle [1]. Annualism is a complex phenotype that is characterized by diapausing embryos, rapid maturation to adulthood and short adult lifespan (<1yr) [2]. Phylogenetic reconstruction suggests this complex trait to have evolved independently at least 3 times within the family [3]. To characterise the genomic changes that underlie the convergent evolution of the phenotypes associated to annualism, we ask 1) How does a compressed life cycle affect genome charateristics? 2) How many genomic regions are under positive selection before the radiation of each annual clade? 3) Since genes affecting longer lifespan are expected to no longer contribute to fitness in annual fishes, do we observe an increase in genes under relaxed purifying selection in annual species? What are these genes? 4) Is the repeated evolution of annualism associated with a fixed set of genes? Combining the recently published genomes of Nothobranchius furzeri [4, 5] with additional whole genome high-throughput sequencing in other African killifishes, we will answer these questions by applying population genetic statistics to scan for signatures of selection during key transitional points in the Nothobranchiidae clade.

**Results**
We obtained de novo genome assemblies for 4 killifish species, representing both annual and non-annual clades. In addition, we performed whole genome resequencing of 39 species at low coverage. Preliminary results show an expansion of repetitive elements within the annual clade Nothobranchius. There is evidence of pervasive relaxed purifying selection in the Nothobranchius genome, but not the Callopanchax genome, suggesting distinct effects of selection in the two annual clades. Genes involved in several ageing-related pathways show reduced selective constraints in annual species.

**Perspectives**
We will functionally validate a subset of the identified candidate genes by CRISPR Cas-9. With our approach, we aim to identify and validate the key genes that are associated with the repeated evolution of annualism and that are potentially important in regulating ageing and lifespan in other vertebrates, including mammals.

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## P19

### Deciphering age-related dysfunction in mouse liver

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**Introduction**
Aging influences multi-organ homeostasis and rejuvenation [1-4]. Liver, despite possessing a remarkable ability of regeneration, has been reported to show signs of diminished regenerative capacity due to aging. However, mechanism of age-related liver dysfunctions remains poorly defined [5]. Specifically, the effect of aging on hepatocyte functions remains to be investigated in detail. To address this, we examined hepatocyte proliferation and characteristic functions in young and aged hepatocytes.

**Results**
Our results revealed that aged hepatocytes exhibit impaired hepatic functions such as cytochromes P450 activity, albumin and urea secretion and proliferation capacity.

**Perspectives**
We are currently investigating age-related dysfunctions in vivo and aim to identify signaling molecules that possess the ability to reverse age-related dysfunctions in vivo. Taken together, identification of key regulators of aging may lead to the successful therapy for liver diseases that are frequently augmented due to aging.

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## P20

### Ethical and Philasiphical Codes of Ageing Research

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**Introduction**
Attempting to explain the conduct of a professional so that the major targets and values of a specific profession be served is the definition of a code of ethics.
Many noticeable developments in the field of ageing have occurred during few past decades, which create a sense of excitement, optimism, a degree of unease, and hesitation in some aspects. Questions and comments from public and philosophers regarding the science of ageing indicate that some present are wondering, uneasily 'What do scientists really wish to achieve? In the current presentation, I will discuss the main goals of ageing science and related ethical and philosophical implications.

**Results**
Adequate ethical and philosophical platform clearly facilitates recruitment of old people to participate in research. At present, the fundamental ethical codes are subjected to widespread discussion by relevant parties to guarantee successful research on human aging, protection of elderly people from unpleasant interference, and supply advantages e.g. innovations of research, therapy, and health care.

**Perspectives**
Clarification of ethical and philosophical global codes would facilitate ageing research and more determined international cooperation and commitment for achieving scientific goals.

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## P21

### Zebrafish cardiomyocytes regenerate despite experiencing DNA damage

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**Introduction**
Zebrafish efficiently regenerate their hearts upon various kinds of injury, while mammals lack this ability. During the course of zebrafish heart regeneration replacement of the lost myocardium is known to occur by re-entry of spared cardiomyocytes (CMs) at the wound border into the cell cycle followed by proliferation. A thorough understanding of the processes that regulate regenerative cardiomyocyte proliferation could be of immense value in the treatment of myocardial infarction in humans. This study aims to understand the molecular events that regulate CM cell cycle during regeneration in zebrafish

**Results**
We performed an unbiased high-throughput gene expression analysis of regenerating hearts. Microarray analysis revealed DNA damage repair (DDR) related genes to be significantly upregulated at 7 days post injury (dpi) which was also confirmed by quantitative PCR. To ascertain whether CMs harbor DNA damage we performed immunostaining for γh2a.x (marker for DNA damage) which revealed that a high percentage of CMs at the wound border showed DNA damage which was evident at 3 dpi and peaked at 7 dpi while gradually reducing at 14 dpi and returning to baseline levels at 30 and 60 dpi. This temporal profile was similar to CM proliferation indicators like PCNA immunostaining and EdU incorporation which revealed a peak in cell cycle re-entry at 7dpi and gradually reduced at later timepoints. Inhibition of DNA repair using inhibitors for critical DNA damage response components like Dna-pkcs and Atm reduced CM proliferation, suggesting that an efficient DNA repair response is necessary for CM proliferation. We also observe that BMP signaling, which we have recently found to be essential for regenerative cardiomyocyte proliferation, reduces γh2a.x levels.

**Perspectives**
In mammals, DNA damage is associated with aging and loss of regenerative abilities. Thus it is surprising that we found prominent DNA damage and activation of the DDR in young fish which regenerate the heart efficiently. The temporal profile of γh2a.x in CMs is similar to proliferation indices which suggest that CMs might experience replicative stress. We propose that zebrafish cardiomyocytes can efficiently overcome DNA damage, which might be a pre-requisite for their regenerative proliferation. We speculate that a differential ability to cope with replicative stress could be part of the reason why zebrafish, but not mammalian adult hearts can regenerate.

## P22

### Role of DAF-16 in promoting DNA damage tolerance and stress resistance in C.elegans.

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**Introduction**
Aging is characterized by the declining functioning of tissues and organs and the steadily increased risk of succumbing to aging-associated diseases. The importance of genome maintenance for withstanding the aging process has become particularly evident in a variety of genetic disorders that are caused by heritable mutations in DNA repair genes and are manifested in premature aging in a multitude of tissues [1]. Despite the presence of highly specialized DNA repair systems that maintain genome stability [2], a fraction of DNA damage might persist and lead to increased senescence or cell death with advancing age. Recent work in mice and C. elegans has shed new light on the mechanisms through which developing and aging animals respond to persistent DNA damage.

**Results**
We are using the nematode C. elegans as a model organism to study how DNA damage accumulation with ageing impinges on pathways involved in longevity assurance. Insulin/IGF-1-mediated signaling (IIS) comprises a conserved longevity assurance pathway and attenuation of IIS activity leads to activation of the FOXO transcription factor DAF-16 resulting in lifespan extension, elevated stress resistance, and enhanced pathogen defense. Previous studies have proposed that DAF-16 functions as a switch, as in the presence of a stress insult, DAF-16 translocates to the nucleus to delay reproduction and growth while increasing stress resistance and longevity. The activity of DAF-16 is controlled by distinct phosphorylation events that can either activate of inactive DAF-16. To investigate the function of DAF-16 in the DNA damage response, we employ a transgenic strain defective of two key DNA damage repair genes (xpc-1 and csb-1, components of the Nucleotide Excision Repair pathway) and expressing DAF-16::GFP. Consistent with DNA damage-induced IIS attenuation, we observed a nuclear localization of DAF-16::GFP following UV-induced DNA lesions. Gene expression and functional analysis suggests that DAF-16 regulates distinct target genes when responding to DNA damage or when responding to other environmental stress factors such as starvation. To understand the regulatory events leading to specific DAF-16 activation in the DNA damage response, we performed a whole proteome analysis using Mass Spectrometry coupled in parallel with a phosphopeptide enrichment using the TiO2 protocol adapted for label free quantitative proteomics to map the specific post-translational modification (PTM) DAF-16 undergoes.

**Perspectives**
We are currently undertaking biochemical and genetic approaches to investigate signaling pathways that regulate DAF-16 activity and to identify co-factors that determine the distinct DAF-16 target gene regulation in the DNA damage response.

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## P23

### Epigenetic age predictions with buccal swabs are more precise in combination with cell-type-specific DNA methylation patterns

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**Introduction**
Age-estimation of persons with (allegedly) unknown age is highly relevant in legal medicine – today more than ever: it is decisive for the legal status of young refugees in asylum procedure and for the degree of penalty for young offenders. Aging is reflected by highly reproducible DNA methylation changes that can be utilized for epigenetic age estimation [1-3]. DNA can be harvested non-invasively from cells at the inside of a person´s cheek by buccal swabs – but these specimens comprise heterogeneous mixtures of buccal epithelial cells and leukocytes, which have very different epigenetic makeup.

**Results**
In this study, we have trained an age-predictor based on three age-associated CpG sites (associated with the genes PDE4C, ASPA, and ITGA2B) for buccal swab samples to reach a mean absolute deviation (MAD) of 4.3 years in a training set and 8 years in a validation set. The composition of buccal epithelial cells versus leukocytes was estimated by two additional CpGs that are generally methylated in one or the other cell type. Estimations by this "Buccal-Cell-Signature" correlated with cell counts in cytological stains (R2 = 0.94). Combination of cell type-specific and age-associated CpGs into one multivariate model enabled age-predictions with MADs of 4.6 years and 6.3 years in test and validation sets, respectively.

**Perspectives**
These results demonstrate that the cellular composition in buccal swab samples can be assessed by two cell-type-specific CpGs to improve the precision of epigenetic age predictions.

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## P24

### Role of VHL-1 in extracellular matrix formation in C. elegans

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Germline mutations of von-Hippel-Lindau (VHL) gene are the cause of an autosomal dominant multi-tumor syndrome, which is characterized by the formation of hemangioblastomas, pheochromocytomas and renal cell carcinoma. Even more importantly, mutations in this gene are found in more than 80% of all sporadic clear-cell renal cell carcinomas – the most common malignant renal tumor – making it the most prominent renal tumor suppressor gene. Tumor formation upon loss of pVHL is partly explained by highly increased hypoxia-inducible factor Signaling (HIF), which is normally repressed by the role of pVHL as part of an E3-ubiquitin ligase. However, pVHL fulfills other functions such as promotion of ciliogenesis, which are less well understood.
Interestingly, pVHL has also been linked to the regulation of extracellular matrix formation, a process that has a strong impact on the tumor microenvironment influencing tumor growth, vascularization and metastasis. However, how pVHL exerts this function on the molecular level is incompletely understood. We thus set out to examine this role of pVHL using Caenorhabditis elegans as a model.





## P25

### CRISPR/Cas9 based forward genetic screen for mediators of ER stress protection in human cells

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**Introduction**
Accumulation of molecular damages along with decline in repair capacities are prominent hallmarks of aging. Particularly, disruption of proteostasis is among most pronounced age-related changes [1]. Our group previously found that activation of systemic protein quality control by external stimuli confers stress tolerance (a feature frequently linked to extended longevity and health span) in the nematode C. elegans [2]. Our data is in line with observations by other groups linking improved protein quality control and life span extension. Outlined findings highlight the importance of healthy proteostasis for age-related organ maintenance and indicate proteostasis enhancers as potential mediators of healthy aging. To date genome wide forward genetic screens for genes implicated in protein quality control were almost exclusively performed in model organisms such as nematodes and flies. Findings obtained in these models require validation in the mammalian system.

**Results**
We used CRISPR/Cas9 genome editing for high throughput gene inactivation in mammalian cells. This approach allows us to perform forward genetic screens for mediators of stress tolerance directly in mammals. We will present first data from the pilot screen for genomic changes conferring protection against protein folding stress in the endoplasmic reticulum (ER) in BJ human fibroblasts transduced with pooled lentiviral CRISPR library composed of 122,411 unique gRNAs targeting 19,050 human protein coding genes and 1,864 miRNA precursors.

**Perspectives**
We aim to identify novel mammalian factors promoting cell survival under proteotoxic stress.

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## P26

### Rev1-deficiency induces age related disorders via PARP1 activation and impaired mitochondrial homeostasis

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Endogenous and exogenous agents that damage the DNA constantly challenge genomes of every organism. Unrepaired lesions within the genome act as obstacles for DNA replication and transcription machineries, which can cause replication fork arrest. The stalled replication can generate ssDNA breaks and gaps as well as DSBs, which left unrepaired, lead to genomic instability. Translesion synthesis (TLS) is activated in response to replication stress and fork arrest and carried out by the Y family of DNA polymerases that can replicate the damaged DNA templates. Rev1 is one of the key enzymes in this process that mediate the assembly of TLS machinery on PCNA and has deoxycytidyl transferase activity. Mice lacking Rev1 protein show premature aging phenotypes, which resemble physiological disorders related to aging such as liver degeneration, type 2 diabetes, and obesity. Our data show that mitochondrial functions are impaired in MEF cells from these mice. They have lower reserve capacity and are more sensitive to reactive oxygen species compared to wild type animals. The expression and activity of PARP1 in cells from Rev1–/¬– mice is increased. Rev1–/¬– cells have higher mitochondrial DNA (mtDNA) that suggest increased mitochondrial fusion/decreased mitochondrial fission, and decreased mitophagy. Rev1–/¬– cells have higher mitochondrial membrane potential and reactive oxygen species. The data from electron microscopy and western blot show that the mitochondrial dynamics is altered and hepatocytes in Rev1–/– mice. Hepatocytes of Rev1–/– mice posses smaller and in some cases aggregated mitochondria. In concordance with these results, there is an increase in phosphorylated form DRP1 in hepatocytes. Our results suggest that activation of PARP1 in Rev1–/¬– cells disrupt mitochondrial homeostasis, which could be mediated through increased consumption of NAD+. This is followed by a decrease in the activity of the other NAD+ dependent enzymes such as Sirt1 that are required for the intact mitochondrial function and homeostasis.



## P27

### Target and biomarker identification platform to design new drugs against aging and age-related diseases

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Our scientific team develops omics data analysis methods to identify potential targets for therapeutic intervention against age-related diseases and aging.
Since modern omics data is high dimensional, i.e. the number of features in it is much higher than the number of measurements, the use of traditional machine learning methods is impossible due to the emerging problem of overfitting. Therefore, it is necessary to develop new mathematical methods to analyze this type of data.
In our work we use the models of statistical physics to analyze gene networks stability and predict their dynamics over time. The proposed models allow us to link gene network stability with mortality. The models we developed were validated on omics data of different types, such as transcriptome, proteome and metabolome measured in different tissues of various organisms.
Our techniques open new opportunities to identify targets to develop new therapy candidates against aging.

## P28

### Epigenetic senescence markers demonstrate heterogeneity in cellular aging within mesenchymal stem cells

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**Introduction**
Extensive ex vivo expansion of mesenchymal stem cells (MSCs) is a necessary prerequisite for their application in cell-based therapies. This is however a major drawback as long-term culture will finally drive MSCs into replicative senescence, which hampers the therapeutic function of MSCs and has to be carefully considered in terms of quality control. To this end we developed an Epigenetic-Senescence-Signature, based on six CpG sites, that facilitates estimation of passage numbers or cumulative population doublings [1,2]. In this study we implemented deep sequencing of barcoded, bisulfite converted PCR amplicons to investigate DNA methylation levels of the signature's CpG sites. We utilized this high-throughput approach to compare the state of cellular aging in different subpopulations of MSCs [3].

**Results**
Initially, we analyzed the Epigenetic-Senescence-Signature in various passages of MSCs and human umbilical vein endothelial cells (HUVECs). Overall, senescence-associated DNA methylation changes were very similar in both cell types. The correlation between predicted and real passage numbers was R = 0.67 and R = 0.97 for MSCs and HUVECs, respectively. The lower correlation in MSCs might be partly attributed to the heterogeneous composition of these cell preparations which consist of several subpopulations [4]. We therefore isolated single-cell derived clones by limiting dilutions for MSCs of early and late passages. The newly implemented high-throughput approach was compared to our former pyrosequencing analysis and showed very similar results. Individual MSC subsets revealed drastic differences in their state of cellular aging according to our Epigenetic-Senescence-Signature. Neither the adipogenic nor the osteogenic differentiation potential of single-cell-derived clones correlated with their senescence prediction. Furthermore, DNA methylation levels of neighboring CpG sites showed huge differences within MSC subpopulations while they were rather constant in whole cell preparations.

**Perspectives**
High-throughput analysis is a time and cost effective way to determine DNA methylation levels at several CpG sites in a big sample cohort. Using this approach we found that the epigenetic state of cellular aging is not homogeneous within MSCs. It rather seems that gradual changes in senescence-associated CpG sites reflect changes in the composition of cellular subsets.

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## P29

### Specific signature for acute and chronic senescent mesenchymal stromal cells

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**Introduction**
In depth understanding of mechanisms that induce cellular senescence is still lacking since we do not have a clear-cut definition of what a senescent cell is. Several features are used to identify senescent cells, such as enlarged and flattened morphology, senescence-associated β-galactosidase activity, senescence-associated heterochromatin foci, altered gene expression, telomere-dysfunction-induced foci, DNA segments with chromatin alterations reinforcing senescence (DNA-Scars), senescence-associated secretory phenotype (SASP). Nevertheless, many of these markers are not senescent cell specific.
To further clarify the classification of senescent cells, hints may be derived by the study of cellular metabolism, autophagy, proteasome activity and SASP in senescence. In this scenario, we decided to study these aspects of senescence in Mesenchymal Stromal Cells (MSC). These cells contain a subpopulation of stem cells that are able to differentiate in mesodermal derivatives (adipocytes, chondrocytes, osteocytes). In addition, they can also contribute to the homeostatic maintenance of many organs. Moreover, MSC are under scrutiny in cellular therapy aiming at treatment of several human diseases.

**Results**
We induced MSC senescence by oxidative stress, doxorubicin treatment, X-ray irradiation and replicative exhaustion. The first three are considered inducers of acute senescence while extensive proliferation triggers replicative senescence also named as chronic senescence. In all conditions, but replicative and high IR dose senescence, we detected a reduction of the autophagic flux, while proteasome activity was impaired in peroxide-treated and irradiated cells. Differences were observed also in metabolic status. In general, all senescent cells evidenced metabolic inflexibility and prefer to use glucose as energy fuel. Irradiated cells with low dose of X-ray and replicative senescent cells show a residual capacity to use fatty acids and glutamine as alternative fuels, respectively. SASP evaluation by LC/MS/MS followed by gene ontology and network analysis evidenced that in all senescent conditions there is a core o protein pathways associate with i) extracellular matrix and cellular junction remodeling; ii) oxy/redoxy functions iii) regulation of gene expression.

**Perspectives**
The differences in metabolic needs and in the activity of proteasome and autophagic vacuoles among the several forms of senescent MSC we analyzed allow identification of a specific algorithm for every phenotype. our finding may pave the way to carry out similar investigation on other cell types and with other stressors; this is to have a complete perspective of senescence.

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## P30

### The role of mitochondria in biological aging of the lung

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**Introduction**
Physiological aging of the lung could lead to development of pulmonary emphysema resulting in deterioration of lung function (3). It has been suggested that mitochondrial dysfunction and release of mitochondrial reactive oxygen species (ROS) play a crucial role in these processes. Different proteins are implicated in the production of ROS by mitochondria (2). The adaptor protein p66shc induces mitochondrial ROS generation and translates oxidative signals into apoptosis (4). Cyclophilin D (CypD) is a regulatory component of the mitochondrial permeability transition pore participating in ROS release and apoptosis (1). The aim of this study was to elucidate the role of p66shc and CypD in development of emphysema during lung aging.

**Methods**
The mean linear intercept (MLI), which serves as parameter for development of emphysema, and pulmonary vascular muscularization were investigated in lungs from mice of different ages (2, 12 and 24 months) by histology. Lungs from mice deficient for CypD (CypD-/-) or p66shc (p66shc-/-) were compared to lungs from wildtype (WT) mice.

**Results**
Histological analysis did not reveal any significant differences in MLI and the degree of pulmonary vascular muscularization in lungs from wild type (WT) mice of different ages. However, MLI and pulmonary vascular muscularization was increased in p66shc-/- lungs from 24 month old mice, and from 12 and 24 month old mice, respectively, compared to WT lungs of the respective age. In CypD-/- lungs only MLI, but not pulmonary vascular muscularization was increased in 24 month old mice compared to WT mice.

**Conclusion**
The mitochondrial proteins p66shc and CypD regulate development of pulmonary vascular remodeling and/or emphysema during aging of the lung. Further studies to determine functional parameters in these lungs, as well as decipher underlying mechanisms are warranted.

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## P31

### Modulation of Treg/Th17 and γδ T cell plasticity by the PPARγ-axis in skin inflammation during aging

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**Introduction**
In human inflammatory skin diseases the pivotal role of the cytokine interleukin 17 (IL-17) is impressively reflected by the high efficacy of modern drugs targeting the IL-23/IL-17 axis. However, little is known about the age-dependent regulation of T cells plasticity leading to increased IL-17 production and the onset of skin inflammation. By means of global gene expressions profiling in T cells from inflamed vs. healthy murine skin and by immunofluorescent analysis of human and murine skin samples, we identified the Peroxisome proliferator-activated receptor gamma (PPARγ) – previously implicated in negative regulation of Th17 differentiation in other autoimmune models – as well as co-regulators of PPARγ and antioxidant enzymes to be significantly down-regulated during skin inflammation and with age.

**Results**
To investigate how age dependent changes in T cell plasticity impact skin inflammation we first systematically analyzed biobank material from human psoriasis patients and healthy subjects of different age groups and secondary lymphoid organs from CD18$^{hypo}$ PL/J mice spontaneously developing a psoriasiform dermatitis with age. Immunofluorescent analyses of skin samples of human psoriasis patients and of inflamed skin from CD18$^{hypo}$ PL/J mice both showed a predominance CD3$^+$IL-17$^+$ T cells and presence of Foxp3$^+$IL-17$^+$ double-positive T cells in correlation with decreases in PPARγ expression in T cells with age. In addition, we found age-dependent increases in memory-type IL-17$^+$ γδ T cells in local lymph nodes of CD18$^{hypo}$ PL/J mice.
In global gene expression analyses of CD90.1+ T cells isolated from inflamed vs. healthy CD18hypo PL/J skin, we then found significant downregulation of PPARγ and its co-regulators as well as antioxidant enzymes during skin inflammation. Whereas mRNA expression levels of PPARγ and antioxidant enzymes, including glutathion-peroxidases (Gpx2, -4, -18) and superoxide-dismutases (SOD1, SOD3) responded well to treatment with redox-modulator dimethylfumarate (DMF), other regulators such as Hif-1α, Nrf2 and Hemoxygenase were not significantly altered in T cells after 14 days of DMF treatment. The functional role of the PPARγ axis in Treg/Th17 and γδ T cell plasticity was further evaluated in lentiviral overexpression studies in murine T cells, and in knockout mouse models and confirmed in human skin samples at the expression level. Furthermore, binding of PPARγ co-regulators to the RORc promoter was detectable in chromatin immunoprecipitation (ChIP) assays, substantiating the potential role of these transcriptional co-regulators in Th17 differentiation.

**Perspectives**
In conclusion, T cell plasticity and the Foxp3/RORγt balance in skin αβ and γδ T cells during aging in vivo and in vitro are at least in part dependent on the PPARγ-axis. Further experiments will now focus on the role of endogenous and applied PPARγ activators and antioxidant enzymes on T cell plasticity and skin inflammation during aging.

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## P32

### Silent Substitutions in HTRA1 Impair the Unfolded Protein Response in Retinal Pigment Epithelial Cells

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**Introduction**
High Temperature Requirement A1 (HTRA1) harbors high frequency, high-risk single nucleotide polymorphisms (SNPs) in patients with Age-Related Macular Degeneration (AMD) [1]. Recently, we and others demonstrated that two such SNPs, rs1049331 and rs2293870, are synonymous but nonetheless exert deleterious consequences on the HtrA1 protein product's stability and conformation [2,3]. Our objective is to understand the role of HtrA1 in the retinal pigment epithelia (RPE), a site of early and progressive lesions in AMD.

**Results**
Collapse of proteostasis is one of the long-standing components of aging and age-associated disorders. We mimicked conditions of proteotoxicity in cultured human RPE (ARPE19) by preventing the glycosylation and ER-to-Golgi translocation of nascent peptides. We found that intracellular HtrA1 protein levels accumulated in a tunicamycin dose- and time–dependent manner. During chronic proteotoxicity, HtrA1 knockdown cells failed to resolve ER stress and died, even in the face of upregulated Grp78/Grp94 chaperones. This is consistent with impairment of the adaptive unfolded protein response (UPR) mediated by IRE1, PERK, and ATF6. Restoration of WT HtrA1 cDNA rescued knockdown cells from apoptosis, but a cDNA encoding the misfolding-prone HtrA1 that harbors two AMD-linked silent substitutions showed reduced efficacy in reversing ER stress-associated death. Moreover, even though the CMV-driven re-expression of this variant exceeded the level of HtrA1 in parental and/or GFP shRNA control cells, double SNP ("dSNP") HtrA1 re-expression lowered RPE's threshold of tolerance for subtoxic doses of tunicamycin.

**Perspectives**
We conclude that HtrA1 is part of RPE's toolkit for unfolded protein response, and is essential for survival during ER stress. RPE of AMD patients who harbor the silent substitutions in HtrAl, rs1049331 and rs2293870, are predicted to have enhanced sensitivity to ER stress and diminished ability for adaptive UPR.

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## P33

### An alternative hypoxia response mechanism independent of HIF-1 involves mitochondrial metabolic adaptation

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**Introduction**
Hypoxia has been implicated in the pathophysiology of several common and devastating disorders including stroke, ischemic heart disease and cancer. Survival under hypoxia at the cellular, tissue, and organismal level, requires activation of various hypoxia-responsive genes, involved in mitochondrial function, glucose metabolism, glycolysis, autophagy, the unfolded protein response (UPR) and apoptosis. Although, hypoxia-inducible factor-1 (HIF-1) is an essential transcription factor coordinating many of these transcriptional responses to hypoxia, it is becoming apparent that HIF-1-independent hypoxia-responses can also occur. The complex regulatory network activated upon hypoxia, independently of HIF-1, is not fully understood [1, 2].

**Results**
We uncovered an alternative hypoxia response mechanism independent of HIF-1 which requires mitochondrial metabolic adaptation. Specifically, we found that expression of T09A5.7/TRIAP-1, the C. elegans homolog of the mammalian TRIAP1/p53CSV, is associated with hypoxia and its activity is HIF-1-independent. Importantly, TRIAP-1 promotes organismal survival under conditions of prolonged hypoxia in the absence of HIF-1. In addition, TRIAP-1 mediates mitochondrial metabolic adaptation upon hypoxia. We further demonstrated that TRIAP-1 regulates various stress response pathways including autophagy, UPR and the intrinsic apoptotic pathway. Last, we showed that TRIAP-1 can regulate C. elegans lifespan when oxygen is abundant. Based on these findings, we propose a novel role of this gene in organismal adaptation to hypoxia independent of HIF-1.

**Perspectives**
Tumor cells are often challenged by extreme oxygen deprivation. It is therefore essential for tumor survival to acquire hypoxia adaptation in response to low oxygen concentration. TRIAP-1 has been associated with various types of cancers and stress responses. Our findings suggest a novel, HIF-1-independent, role for TRIAP-1 in hypoxia adaptation, which could modulate hypoxia-induced resistance to anticancer drugs.

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## P34

### Modelling of human Nonalcoholic Fatty Liver Disease with hepatocyte like cells derived from pluripotent stem cells

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**Introduction**
Nonalcoholic fatty liver disease (NAFLD) is an increasingly common diagnosis in the Western Hemisphere. It is defined by an accumulation of lipid droplets in more than 5% of hepatocytes. In the beginning the disease is rather benign, but later on patients develop steatohepatitis, cirrhosis and up to 27% of these patients end up with hepatocellular carcinoma. The molecular reasons of this disease are still questioned, but it is well-recognised that NAFLD is strongly associated with obesity and insulin resistance. In this metabolism-based field of research, results obtained from rodent model systems cannot be easily extended to humans as both organisms differ in their metabolisms. Unfortunately, liver cells from steatosis patients are very rarely available and not suitable for longer experiments as hepatocytes rapidly dedifferentiate in culture.

**Results**
We have established a human model system for NAFLD based on hepatocyte like cells (HLCs) generated from pluripotent stem cells. We are able to induce the accumulation of lipid droplets (LDs) in these cells by adding oleic acid (OA) into the medium. LD formation has been documented by staining with Oil Red O or BODIPY. After fat induction with OA the expression of PLIN2, a protein covering LDs, was consistently up-regulated. As PLIN2 knockout mice are protected against the development of steatosis, we selected PLIN2 expression as a molecular marker for the successful induction of steatosis. We thoroughly investigated the consequences of LD accumulation on the level of gene expression. We found that many GO categories related to lipid, glucose and sterol metabolism were up-regulated in HLCs after OA induction. Interestingly, many members of the Peroxisome proliferator-activated receptor (PPAR) pathway, which is important for the regulation of lipid metabolism, were up-regulated after fat induction. Modelling PPARα in HLCs with small molecules resulted in profound gene expression changes. Inhibition of PPARα with GW6471 resulted in down-regulation of genes involved in lipid catabolism, while activation via Fenofibrate reduced expression of AGPAT2 and HMGCR, which are involved in biosynthesis of phospholipids and cholesterol, respectively. Also insulin signalling was affected by PPARα modulation.

**Perspectives**
Although obesity and NAFLD are increasing health problems worldwide, there is no specific treatment for NAFLD at the moment. Our HLC-based NAFLD model can be used in the future to screen for drugs that might interfere with LD accumulation.





## P35

### Age-associated mitochondrial dysfunction promotes intestinal tumour development

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**Introduction**
Stem cells with mitochondrial oxidative phosphorylation (OXPHOS) defects caused by somatic mitochondrial DNA (mtDNA) mutations are commonly detected in the colorectal epithelium of older individuals (Taylor et al., 2003). In addition, cells with mtDNA mutations are also well documented in age-related colorectal cancers. However, it is unknown whether the mtDNA mutations detected in normal tissue affect stem cell biology or play a role in age-related colorectal cancer development. Here we have directly investigated the effect of mtDNA mutations and OXPHOS defects on stem cell division kinetics by examining intestinal stem cells in a mouse model which accumulates high levels of mtDNA mutations over time (PolgA+/mut (Trifunovic et al., 2004)). These mice accumulate colonic crypts with mitochondrial OXPHOS dysfunction with age in a similar manner to ageing humans (Baines et al., 2014). We then induced adenoma formation (Barker et al., 2009) in these animals to investigate the effects of mitochondrial dysfunction on early stage tumour growth.

**Results**
Multiple thymidine analogue labelling of colonic stem cells revealed that those stem cells with mitochondrial complex I deficiency re-enter the cell cycle, on average, 30% more often than their wild-type counterparts. Complex IV dysfunction did not affect the stem cell re-entry time in the same way, suggesting that complex I deficiency specifically, is speeding up the frequency of stem cell divisions. Next we knocked out the tumour suppressor APC in stem cells in mice with mitochondrial dysfunction and normal controls. Those mice with mitochondrial dysfunction had a significantly shortened lifespan due to accelerated tumour growth compared with controls, suggesting that mitochondrial dysfunction enhances the progression of intestinal tumours.

**Perspectives**
Our results show that age-related mtDNA mutations resulting in complex I deficiency can increase stem cell cycle re-entry rate, which is exacerbated when these cells are transformed. Currently the mechanism by which this occurs is unknown. Elucidating these pathways is vital as this may reveal novel therapeutic options utilising mitochondrial pathways for cancer treatment. In addition, development of strategies to reduce the accumulation of mtDNA mutations in intestinal stem cells could diminish the age-related colorectal cancer burden

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## P36

## LaminAC in HSC Ageing

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**Introduction**
The small RhoGTPase Cdc42 regulates cytoskeletal protein tubulin polarity as well as intranuclear polarity of H4K16ac in young HSCs. Its elevated activity is linked to HSC ageing and correlates with loss of polarity in aged HSCs1. This could imply that extra- and intracellular stimuli are transmitted to the nucleus through cytoskeleton re-organisation, which is regulated by Cdc42, resulting in alteration of HSC nuclear architecture. The transmission of the forces between the cytoskeleton and nucleus is facilitated by the LINC complex and lamins, especially laminAC, which is required for organized nuclear architecture2,3.

**Results**
Our preliminary data show that LMNA gene is down-regulated with ageing as it was shown before4, but it is up-regulated again with Cdc42 activity inhibition.

**Perspectives**
We would like to understand the role of LaminAC in re-organization of HSCs nuclear architecture upon aging, anticipating that information on changes of nuclear structure over time will be instrumental to investigate stem cell aging and aging-associated diseases.

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## P37

## Identifying age-dependent heterologous seeds for amyloid-β aggregation

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Aging is the most important risk factor for neurodegenerative diseases. Previously we identified in C. elegans several hundred proteins that consistently become more insoluble during normal aging and in the absence of disease aggregating proteins [1]. Although aging is such an important player, it remains unknown which changes are relevant for disease initiation. Our hypothesis is that some proteins in the aggregating proteome build heterologous seeds with age and drive the pathological aggregation of Aβ in Alzheimer's disease.
We found that detergent-insoluble proteins from aged wild-type C. elegans seed the aggregation of Aβ in vitro. To evaluate at which age the animals form these heterologous seeds, we collected synchronized C. elegans populations at four different time points. Our data reveal that significant seeding activity appears only with advanced age. Proteomic analysis of these four ages identified 79 proteins which only start to aggregate at day 10 or day 14. We are currently testing the seeding activity of these aggregation-prone proteins. Our preliminary results demonstrate that two aggregation-prone proteins, KIN-19 and arginyl(R) amino-acyl tRNA Synthetase, are found in Aβ aggregates in C. elegans.
Importantly, we show that detergent-insoluble proteins from aged mouse brains have the potential to seed Aβ in vitro. In addition, we will present preliminary results from our ongoing in vivo study with mice expressing human APP containing the Swedish mutation [2].
Overall, these results highlight that physiological protein aggregation with age could constitute a heterologous seed for disease aggregation. Identifying the key seeding proteins would have a major impact in advancing our understanding of disease aggregation with age and how to influence it.

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## P38

### Planarian, a novel model system for studying ageing

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An amazing 25% of the planarian parenchyma consists of adult stem cells. This remarkable characteristic confers them great plasticity: they can regenerate a new full planarian from very tiny fragments of their bodies and they can stand long periods of starvation while maintaining their stem cell population stable. Historically, many planarian species have been considered immortal. According to Dalyell (Dalyell 1814), planarians can be called almost immortal under the edge of a knife. In addition, planarian stem cells do not show any signs of ageing since they seem to be indefinitely able to divide and differentiate into any cell type in the planarian body to maintain normal homeostasis and during regeneration. There are several studies that suggest that the process of regeneration and starvation, which are normal occurrences in the life of any planarian, are two rejuvenating processes. So regeneration and starvation should be able to rejuvenate the planarian stem cell pool and confer them the infinite capability of division and differentiation into any cell type. So far, how these processes may be able to rejuvenate the stem cell pool is not fully understood.

Our lab uses the asexual strain of the planarian species Schmidtea mediterranea to address these questions. The S. mediterranea genome is sequenced and it is amenable to most of the molecular techniques, which are available in any other model systems including high-throughput RNAi screenings, proteomics and transcriptomics. We have so far focused on the process of starvation. Although both fasting and caloric restriction have been shown to extend life span in all the organisms where it has been tested, not much is yet known about how they regulate stem cells. We have previously demonstrated that indeed planarians maintain their stem cells numbers during starvation and that starved planarians are able to respond stronger to amputation than well-fed ones. Here we will show you the outlines of the three lines of research we are undertaking together with preliminary results.

**Keywords**
Planarian, stem cell, starvation, regeneration





## P39

### From ageing to cancer: shifts in non-coding RNAome and epigenome as common basis of the both diseases

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**Introduction**
Impaired balance between DNA methylation and demethylation, deregulation of chromatin remodeling, genomic instability and activity of transposable elements (TEs) become apparent in aging cells and are typical of cancer cells. This research aims to identify in what way the shifts in expression of non-coding RNAs, esp. miRNAs, can lead to these abnormalities and contribute to carcinogenesis. MiRNA targets within gene transcripts were predicted in silico using the TargetScan software.

**Results**
Transcripts of genes encoding histone deacetylases HDAC1/2/4/6/7/8/9 and SIRT1/3/5/7, de novo DNA methyltransferases DNMT3A/B/L as well as many histone methyltransferases carry targets for at least one of miRNAs miR-155, miR-23a/b, miR-375, miR-21, miR-29, miR-206, miR-19, miR-221/222, miR-181 and miR-18, hyperexpression of which is essential for abnormal proliferation and surviving of tumour cells [1]. In addition, these miRNAs can silence around 60% of genes involved in DNA repair. Down-regulation of other miRNAs (usually, miR-15a/16, miR-17-5p, miR-122, miR-31, miR-143, miR-145 and miR-320) allows overexpression of genes encoding histone acetyltransferases, histone demethylases and components of chromatin remodeling complexes [1]. Described shifts in miRNAome can cause the increase of overall level of chromatin acetylation and expression and, therefore, make possible the reactivation of silent oncogenes and TEs, which takes place against the background of DNA repair impairment at that.
At the same time, age-related shifts in DNA methylation and chromatin remodeling resemble these shifts during carcinogenesis. As is known, expression of genes encoding the de novo methyltransferases DNMT1 and DNMT3a as well as the histone deacetylase SIRT1 declines with age, whereas expression of genes encoding histone demethylases KDM5B and KDM6B increases. In addition, after the loss of piRNAs and endo-siRNAs in early embryogenesis and after silencing of some miRNA genes during differentiation, TE sequences cannot be methylated de novo. As a result, with age the increasing number of both stem and differentiated cells can reach a threshold of critical DNA demethylation and following reactivation of dormant TEs. Subsequent apoptosis of the majority of these cells defends organism from possible cancer appearance, but causes ageing as biological phenomenon [2]. Nevertheless, sooner or later, some cells avoid apoptosis and undergo tumour transformation; thereby, TE derepression leads to the both diseases - ageing and cancer.

**Perspectives**
Overall, ageing and cancer can be delayed or even prevented by total knockout or removing of the TEs from genome. Another way is to suppress the reactivation of TEs from latency.

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## P40

### MicroRNA-29 acts on Contractility in the Heart of Fish

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**Introduction**
Cardiovascular diseases are major cause for death worldwide and aging is the most important risk factor for cardiovascular disease [1] Aged hearts show specific molecular alterations compared to that of younger, including different gene transcription pattern [2]. Among a variety of epigenetic effectors, microRNAs (miRNAs) play a central role in regulation of cardiac pathophysiology during aging [3]. The turquoise killifish Nothobranchius furzeri (NF) is a novel model organism for aging studies [4] in which a number of studies evidenced a clear role for miRNAs during NF aging [5]. Indeed, the expression of several miRNAs changes during aging in the brain of NFs as well as in skin and liver [5], but no information is currently available about miRNAs in the heart. In the present study, we investigate changes of miRNAs during NF's cardiac aging.

**Results**
1. miRNA-Seq technology has been used to investigate age-dependent miRNA expression in hearts of the short-lived fish NF. Three different time points were analyzed; 26 up-regulated miRNAs and 31 down-regulated were identified.
2. miR-29 turned out among the most up-regulated miRNAs in the heart of NFs. To study the role of miR-29 during cardiac aging, we generated a transgenic zebrafish in which miR-29 expression was down-modulated by a miR-29 sponge. In this transgenic animals, miR-29 deficiency determined morphological and histological alterations including hypertrophy, calcification and chronic inflammation.
3. Evaluation of cardiac function by echocardiography revealed a significant reduction in ejection fraction area (FAC).
These data suggest that up-regulation of miR-29 is a compensatory response to limit the cellular damage.

**Perspectives**
miR-29 targets are mainly structural proteins and enzymes associated to DNA methylation process. The investigation of the role of miR-29 during heart aging may clarify molecular mechanisms of the aging-associated cardiac pathophysiology.

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## P41

### The long noncoding RNA H19 controls endothelial cell ageing and inflammation

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**Introduction**
Long noncoding RNAs (lncRNAs) are endogenously expressed noncoding RNAs with a length of more than 200 nucleotides, which can regulate gene expression through various mechanisms. Ageing is the main risk factor for cardiovascular disease. We hypothesize that detrimental factors like ageing and inflammation regulate the expression of lncRNAs that in turn regulate endothelial cell (EC) functions.

**Results**
Using next generation sequencing, we identified differentially regulated lncRNAs in several tissues of young and aged mice. The most profoundly regulated lncRNA was H19, a known conserved lncRNA with unknown functions in the endothelium. Microarray profiling of lung ECs from 2 and 18 months old mice revealed a downregulation of H19 with ageing. qPCR analysis of the same tissue from Ku80-/- progeria mice showed similar effects compared to WT littermates. In the aortic intima of 20 months old C57BL/6J mice, H19 was also downregulated.

SiRNA- and LNA GapmeR-mediated knockdown of H19 in human umbilical vein ECs (HUVECs) reduced proliferation and increased the senescence marker p21, as well as senescence associated β-Galactosidase activity. Since senescence is associated with inflammatory EC activation, we determined the inflammatory activation of ECs after silencing of H19. The cell surface adhesion molecules ICAM-1 and VCAM-1 both were upregulated after H19 depletion. Gain of function experiments confirmed the loss of function data, showing that lentiviral overexpression of H19 led to a downregulation of p21, ICAM-1 and VCAM-1.

An ex vivo aortic ring assay with young and aged mice showed an impaired sprouting capacity of ECs with ageing. Depletion of H19 in aortic rings from young animals inhibited sprouting to levels comparable with those of old animals, while overexpression of H19 in aortic rings from aged animals rescued the impaired sprouting capacity of aged ECs.

A high throughput luciferase pathway reporter assay after siRNA-mediated silencing and lentiviral overexpression of H19 revealed an involvement of H19 in the JAK2/STAT3 pathway. These findings were confirmed by Western blot, showing that H19 reduced the phosphorylation of STAT3. Furthermore, exon array analysis, after depletion of H19 confirmed that H19 regulates STAT3 controlled genes.

**Perspectives**
In summary, aging reduced endothelial H19 expression, likely in a KLF2 dependent manner. Functionally, H19 knockdown induces a senescent-like phenotype and increases the inflammatory activity of ECs, most likely through modulation of STAT3 signaling, which was recently shown to induce senescence in vivo. Together, these results identify H19 as an aging-regulated lncRNA that controls pivotal endothelial cell functions.

## P42

### Telomerase reverse transcriptase (TERT) and shelterin complex therapy in delaying ageing

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Telomeres are the tandem repeats of the TTAGGG sequence and these are bounded by a complex of protein called Shelterin.Telomere protection targets six proteins, TRF1, TRF2, TIN2, RAP1, POT1, and TPP1, the shelterin complex protects the ends of telomeres from genomic rearrangement and binds to telomeres, and protects telomeres by repressing DNA damage response (DDR) at telomeres and preventing chromosomes fusions. Loss of shelterin proteins derepresses DDR and allow non homologous end joining (NHEJ) of chromosomes ends, resulting in chromosome end-to-end fusions and genomic instability. Telomerase confirms limitless proliferation potential to most human cells. Dysfunction of telomerase can lead to either cancer or ageing pathogenesis. Anti-ageing activity of telomerase has been demonstrated in mice and it is found that over expression of TERT genetically engineered causes enhanced expression of the p53, p16, p19ARF tumor suppressors. This enhance the fitness of various physiological barriers accompanied by systemic delays in ageing.
By Shamshul Huda





## P43

### The role of MMS19 in transcription and Nucleotide Excision Repair

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**Introduction**
MMS19 is involved in Nucleotide Excision Repair (NER), transcription and chromosomal segregation in mammalian cells. The human MMS19 protein is capable of physical interactions with the XPB and XPD helicases, subunits of the TFIIH complex [1]. Most recently, it was shown that MMS19 assembles iron-sulfur proteins required for DNA metabolism and genomic integrity [2, 3]. Yeast mms19 (MET18) deletion mutant cells are moderately sensitive to DNA-damaging agents and are temperature-sensitive for growth [4]. In MET18 mutant cells, both transcription-coupled and global genomic NER are deficient [5]. Currently, despite the iron-sulfur methabolism, any mechanistic understanding of how MMS19 functions in transcription and DNA repair during development or with advancing age remains elusive.

**Results**
We have recently generated a Mmms19-/- mice to dissect the functions of the protein in an in vivo model. Unexpectedly the total depletion of MMS19 results to be embryonically lethal.

**Perspectives**
Using the recently established in vivo biotinylation tagging approach, we are generating a mouse model carrying a biotin tagged version of the MMS19, allowing to define the MMS19-bound proteome and MMS19-bound gene targets, during early stages of embryogenesis; the biotin tagged MMS19 knock-in allele is also flanked by LoxP sites, allowing us to generate tissue-specific Mms19-/- animals, when crossed with the appropriate Cre transgenic mouse lines. Liver-specific knock-out animals will be the first to be generated by crossing MSS19fl/- with mice that carry the Cre transgene, whose expression is driven by the albumin promoter.
We will validate the sub-complex associated partners with pull-down and Western Blot assays.
Finally we will combine Chip-seq and RNA-seq data to dissect the role of MMS19 in transcription regulation.

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## P44

### The role of tenascin N in continuously erupting rodent incisors

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**Introduction**
Even though tenascins were first discovered over 30 years ago, their in vivo function is still poorly understood. In mammals the tenascin protein family consists of 4 members: 1. tenascin C, which is the best studied tenascin; 2. tenascin X, mutations lead to a subtype of Ehler-Danlos-Syndrome1; 3. tenascin R, a brain specific extracellular matrix (ECM) protein; and 4. tenascin N or W, which is the least known tenascin. Tenascin N is found in the periosteum and in the periodontal ligament as well as in the pulp mesenchyme of adolescent mice. In adult humans tenascin N expression is restricted to the periosteum and the heart. However, in most solid tumours, including melanoma, glioblastoma, and breast and lung cancer, tenascin N is strongly expressed in the perivascular ECM. Until now, the in vivo function of tenascin N is completely unknown.

**Results**
To elucidate the function of tenascin N we have generated a knockout mouse. Tenascin N knockout animals are vital and fertile, however their lifespan is limited to the age of 6 to 12 month and their body weight is significantly reduced. Histological analysis of the teeth revealed a wider periodontal space as well as an obliteration of the pulp in the lower incisor. By in situ hybridization we observed that fgf-10 and axin-2 expression is decreased in the region of the neurovascular bundle. Interestingly, the incisor neurovascular bundle was recently identified as an origin for mesenchymal stem cells2. Similar phenotypes were described in integrin alpha-113 and periostin deficient mice4, both proteins are mainly found in the periodontal ligament. This leads to the question whether the primary defect is in the periodontal ligament or in the stem cell niche, respectively. To address this question we plan to use a periodontal ligament specific conditional knockout mouse model.

**Perspectives**
The aim of this project is to define the in vivo function of tenascin N. In a broader context this study should reveal whether tenascin N is needed for mesenchymal stem cell maintenance or whether tenascin N plays a role in healthy ageing.

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## P45

### Genome instability during ageing in the short-lived turquoise killifish

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**Introduction**
Ageing is characterized by increased genomic instability. Specific somatic alterations are significantly associated with cancer, autism and epilepsy. We developed a method to identify and test the phenotypic effect of recurrent somatic alterations associated with normal ageing, which combines transcriptome sequencing in different somatic tissues with transgenesis.

**Results**
We used the short-lived turquoise killifish as model organism, due to its short lifespan in captivity (16 weeks of median lifespan) and its recently sequenced and assembled genome. We tested genetic markers associated with DNA damage response and genome instability and found them to be significantly increased in brain and muscle of aged fish. We further tested expression levels of the genes in DNA repair pathways and observed decreased expression in brain and muscle. We recently found that key genes involved in DNA damage response are under strong positive selection in the turquoise killifish. Taken together, these results suggest that the probability for DNA lesions is increased in aged fish and that DNA repair mechanisms play key roles in the evolution of this species. We next set out to identify somatic variants in total transcripts with ageing. We found several hundred genetic variants enriched in aged fish compared to young fish. One third of the total variants were incorporated in genes, while the rest occurred in intergenic regions. Some of the variants were recurrently identified in independent biological samples. Interestingly, the pathways associated with neuronal regulation were statistically overrepresented among genes carrying somatic variants in the aged brain. Furthermore, to test the biological effect of DNA damage and repair on ageing symptoms, age-associated disease or lifespan, we generated 6 transgenic fish lines that have enhanced DNA repair capacity.

**Perspectives**
Altogether, our work will help reveal whether improving DNA repair mechanisms has beneficial effects on lifespan and on the ageing process.

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## P46

### Asymmetric segregation of plasmid DNA in mammalian cells

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**Introduction**
What happens to foreign DNA in mammalian cells during divisions? Generally non-centromeric DNA molecules are mitotically instable in eukaryotes. A result of this is their absence in an ever increasing proportion of the progeny of an originally affected cell (e.g. (1)). Exogenous sources can be DNA of pathogens or DNA, typically plasmids, artificially introduced into cells. For the latter it is known since decades that plasmid-born protein expression is transient, persisting only for a few cell cycles (2). This is consistent with the notion that plasmid DNA is somehow eliminated during subsequent divisions. Yet, how this is achieved is unclear.

**Results**
Here, we show that dividing human and canine cells segregate transfected plasmid DNA asymmetrically, preferentially into the daughter cell harboring the young centrosome. Independently of how they entered the cell, most plasmids clustered in the cytoplasm. Throughout mitosis, these clusters remained relatively immobile, confined by the endoplasmic reticulum (ER) and physically associated to membranes reminiscent of the ER, as revealed by live cell and electron microscopy imaging. At mitosis entry, most clusters localized near the centrosomes. As the two centrosomes split to assemble the bipolar spindle, predominantly the old centrosome migrated away, promoting a biased co-segregation of the young centrosome and plasmid cluster together. Downregulation of the centrosomal protein Ninein abolished this bias, indicating that it is a controlled process. Thus, we suggest that DNA clustering, relative cluster immobility through association to ER membrane, initial proximity between the cluster and the centrosomes, and subsequent differential behavior of the two centrosomes, together bias the segregation of plasmid DNA during mitosis. This process leads to their progressive elimination from the proliferating population.

**Perspectives**
The discovered mechanism might apply to any kind of foreign DNA molecules in mammalian cells possibly preventing thereby genetic instability. Furthermore, the results provide a functional framework for understanding and studying the differential behavior of centrosomes during mammalian mitoses, as reported in stem cells, for example. We suggest that this differential behavior might drive the asymmetric segregation of many more cellular compounds in many cell types.

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## P47

### Repression of the antioxidant NRF2 pathway in premature aging

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**Introduction**
Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare, invariably fatal premature aging disorder. The disease is caused by constitutive production of progerin, a mutant form of the nuclear architectural protein lamin A, leading through unknown mechanisms to extensive morphological, epigenetic and genomic damage and to mesenchymal stem cell (MSC) attrition in vivo [1-3]. We set out to identify primary HGPS disease mechanisms underlying these defects.

**Results**
Using a high-throughput high-content imaging-based siRNA screen we identify the longevity-promoting NRF2 antioxidant pathway as a driver mechanism in HGPS. Progerin sequesters NRF2 and thereby causes its subnuclear mislocalization, resulting in impaired NRF2-mediated transcriptional activation of antioxidants and consequently chronic oxidative stress. Suppressed NRF2 activity or increased oxidative stress are sufficient to recapitulate HGPS aging defects and re-activation of NRF2 activity in HGPS patient cells reverses progerin-associated nuclear aging defects and restores in vivo viability of MSCs in an animal model.

**Perspectives**
These findings establish repression of the NRF2-mediated antioxidative response as a key contributor to the premature aging phenotype, and suggest NRF2 activating compounds as a promising novel therapeutic strategy in HGPS and aging diseases.

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## P48

### Interventions to limit senescence-induced bystander effect

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**Introduction**
Senescence can be triggered by activation of oncogenes or in response to persistent DNA damage due to internal or external stressors. Independent of the causative trigger, senescent cells exit from cell cycle, undergo changes in chromatin confirmation [1], produce increased levels of reactive oxygen species (ROS) [2] and start to secrete a wide range of pro-inflammatory molecules, commonly termed as a senescence-associated secretory phenotype (SASP) [3]. SASP and ROS functions range from recruitment of immune cells, re-enforcement of senescence in the cell itself to induction of senescence in surrounding cells, bystanders [4]. Here, we compare bystander affects of oncogene-induced (OIS) and replicative (RepSen) modes of senescence on proliferating cells in direct 2D co-cultures.

**Results**
We identified that oncogene-induced and replicatively senescent cells rely on different mechanisms to induce DNA damage response (DDR) in bystander cells and we tested intreventions that effectively alleviate the senescence-like DDR in bystander cells. We found that in replicatively senescent cells inhibition of NF-kB only mildly supressed the bystander effect, despite reducing secretion of soluble pro-inflammatory molecules. On the contrary, treatment with an NF-kB inhibitor significantly reduced OIS triggered DDR in bystander cells, whilst reducing levels of secreted IL-1a (although not levels of IL-6 and IL-8). We conclude that the bystander effect imposed by RepSen cells is mainly mediated by short-lived ROS species, while OIS cells rely on pro-inflammatory molecules for transmission of bystander effect.

**Perspectives**
This work suggests that senescent cells selectively utilize components of their secretory program to communicate with their proximate microenvironment, and that treatments to mitigate affects of senescent cells in vivo require targetting different pathways for replicatively senescent cells compared to oncogene induced senescence.

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## P49

### Cross-strand binding of TFAM to a single mtDNA molecule forms the mitochondrial nucleoid

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Mammalian mtDNA is packaged by mitochondrial transcription factor A (TFAM) into mitochondrial nucleoids that are of key importance in controlling the transmission and expression of mtDNA. Nucleoid ultrastructure is poorly defined and therefore we used a combination of biochemistry, super-resolution microscopy, and electron microscopy to show that mitochondrial nucleoids have an irregular, ellipsoidal shape and contain typically a single copy of mtDNA. Rotary shadowing electron microscopy revealed that nucleoid formation in vitro is a multi-step process initiated by TFAM aggregation and cross-strand binding. Super-resolution microscopy of cultivated cells showed that increased mtDNA copy number increases nucleoid numbers without altering their sizes. Electron cryo-tomography visualized nucleoids at high resolution in isolated mammalian mitochondria and confirmed the sizes observed by superresolution microscopy of cell lines. We conclude that the fundamental organizational unit of the mitochondrial nucleoid is a single copy of mtDNA compacted by TFAM, and we suggest a packaging mechanism.

## P50

### Investigating the role of neutrophil infiltrations in DNA-damage induced senescence

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Neutrophils have been shown to be key players in the recognition and elimination of pathogens, however, recent data has revealed that neutrophils may play other roles in disease, notably the development of cancer. Senescence, the state of irreversible arrest observed in somatic cells is characterised by a secretory phenotype (SASP) which includes pro-inflammatory cytokines, chemokines and extracellular matrix proteases. The SASP is believed to play a role in the recruitment and activation of immune cells. However, the relationship between neutrophil recruitment and senescence has not been completely investigated. Our data show that neutrophil infiltrations correlate with cells positive for senescent markers in NF-κb1-/- mice tissues, a mouse model of chronic inflammation and accelerated ageing. However, it is still not known whether neutrophils are recruited by senescent cells or are inducers of senescence in tissues. Chemotaxis assays show that secreted factors produced by senescent cells lead to increased neutrophil migration, suggesting a role for senescence in neutrophil recruitment. Additionally, in vitro co-culture experiments reveal that neutrophils induce a DNA damage response (DDR), a central mediator of cellular senescence, in surrounding cells in a paracrine fashion. This effect was observed both in mouse hepatocytes and human fibroblasts co-cultured with neutrophils. Mechanistically, we observed that neutrophil-induced DDR is ROS dependent, since treatment with catalase is able to suppress the DDR increase. Altogether, our data suggest that accumulation of senescent cells with age may contribute to the recruitment of neutrophils via the SASP and that neutrophils may in turn induce more DNA damage in tissues.



## P51

### Lysine-acetylation in cellular regulation, ageing and disease

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Lysine-acetylation was first discovered already in 1964 by Vincent Allfrey to occur on histones. 20 years afterwards α-tubulin was identified as the first cytosolic protein to be acetylated followed by the tumor suppressor protein p53 and the HIV transcriptional regulator Tat. The sirtuins (Sir; silent information regulator) were discovered in a yeast genetic screen to be involved in gene silencing. It turned out that the yeast enzyme Sir2, which is homologous to mammalian Sirt1, has an NAD+-dependent deacetylase activity. Sirtuins have been shown later to be involved in lifespan regulation in yeast, flies and worms. Furthermore, they are implicated in healthy aging and do play protective roles in the development of severe diseases such as cardiovascular and neurodegenerative diseases as well as cancer. Several thousand lysine-acetylation sites have been identified in the proteome of diverse organisms by quantitative mass-spectrometry. The identification of differently charged or uncharged lysine acylations puts another level of complexity on this post-translational modification. Notably, less than 1% of these lysine-acylation sites were functionally characterized so far. One of the major challenges in the acylation research field is to distinguish between biologically relevant and irrelevant sites. We use a combined synthetic biological, biophysical and cell biological approach to structurally and functionally investigate how protein function is regulated by site-specific lysine-acetylation. Our recent data show that proteins of the Ras-superfamily, regulators and effector proteins thereof are dynamically regulated by lysine-acetylation using diverse molecular mechanisms. Tackling the lysine-acylation machinery might allow the development of novel therapeutic strategies to treat age-related diseases.

## P52

### MicroRNA-15b regulates mitochondrial homeostasis and the senescence-associated secretory phenotype through sirtuin 4/SIRT4

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**Introduction**
Mammalian sirtuins are involved in the control of metabolism and life-span regulation. Here, we link the mitochondrial sirtuin SIRT4 and its regulation by miR-15b with mitochondrial dysfunction, cellular senescence, and skin aging.

**Results**
SIRT4 expression significantly increased in human dermal fibroblasts undergoing replicative or stress-induced senescence triggered by UVB or gamma-irradiation. In-vivo, SIRT4 mRNA levels were upregulated in photoaged vs. non-photoaged human skin. Interestingly, in all models of cellular senescence as well as in photoaged skin, upregulation of SIRT4 expression was associated with decreased levels of miR-15b. The latter was causally linked to increased SIRT4 expression because miR-15b targets a functional binding site in the SIRT4 gene and transfection of oligonucleotides mimicking miR-15b function prevented SIRT4 upregulation in senescent cells. Importantly, increased SIRT4 negatively impacted on mitochondrial functions and contributed to the development of a senescent phenotype. Accordingly, we observed that inhibition of miR-15b, in a SIRT4-dependent manner, increased generation of mitochondrial reactive oxygen species (mtROS) that could be prevented by co-treatment with the mtROS scavenger mitoQ, decreased mitochondrial membrane potential, and inhibited the expression of nuclear encoded, mitochondrial regulatory genes, including CytC, TFAM, and NRF1. Moreover, increased mtROS was linked to elevated DNA double strand breaks accompanied by increased p21WAF cell cycle inhibitor levels and a decrease in proliferating BrdU+ cells. Lastly, miR-15b modulated the SASP, given that inhibition of miR-15b reduced, via upregation of SIRT4, the expression of IL-1α, IL-1β, IL-6, and IL-8 by up to 75%. Taken together, miR-15b is a negative regulator of stress-induced SIRT4 expression thereby counteracting senescence associated mitochondrial dysfunction and regulating the SASP and possibly organ aging, such as photoaging of human skin.

**Perspectives**
Further studies employing conditional mouse models deficient in SIRT4 expression and/or function in the skin are necessary to define more closely SIRT4-dependent and -independent pathways downstream of miR-15b



## P53

### Analysis of changes in gene expression in individual aged mice provides a unique perspective of Hematopoietic Stem Cell Aging

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**Introduction**
Hematopoietic stem cell (HSC) aging is associated with functional decline characterised by well-established aging phenotypes, yet the molecular changes contributing to this are still unclear. Analysis of gene expression in HSCs is hindered by their rarity and unusually low RNA content. Previous studies analysed the transcriptome of young and old HSCs pooled from numerous mice. These studies reported hundreds or thousands of differentially expressed genes1,2,3,4. Nevertheless there is little consensus among them (3 genes). As we have shown previously that stem cell frequency and functionality varies dramatically in aged mice, we wished to explore gene expression changes in HSCs isolated from individual mice. In this study, we used low-input total RNA sequencing and R package edgeR to analyse gene expression in long-term HSCs from 4 6-month and 5 24-month month old C57BL/6 mice.

**Results**
Aged HSC show an increase in RNA content per individual stem cell which is positively correlated with HSC pool size (r2=0.8). Our data does not confirm massive changes in gene expression upon aging. Differentially expressed genes included those encoding for histones and cell cycle related genes. Interestingly, only one gene was common between our study and those previously reported. This gene, a cell surface receptor, has been shown to regulate cell-fate decisions in embryonic stem cells but it has not been investigated in HSCs. We have confirmed its upregulation in aged HSCs at protein level and demonstrated the effect of its stimulation on HSC function, including downstream transcriptional activation and migration of HSCs.

**Perspectives**
Taken together, our study provides a unique perspective of transcriptome modulation with HSC aging in individual mice. We identified a promising candidate as a potential regulatory factor which may contribute to HSC aging.

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## P54

### Mechanical regulation of transcription controls Polycomb-mediated gene silencing during lineage commitment

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Maintenance of tissue homeostasis requires a highly control of stem cell differentiation and self-renewal. Recent studies suggest that mechanical interactions between cell and extracellular matrix as well as neighboring cells play a vital role in regulation the fate decisions of stem cells. However, there is a limit in understanding the mechanism of how mechanical forces adjust gene expression and so to influence cell fate. Here, we found that those forces promote global transcriptional repression and Polycomb-Repressive-Complex-2 (PRC2) accumulation through regulation of nuclear actin availability. The level of nuclear actin is limited by local actin polymerization coupling with the enrichment of non-muscle myosin IIA (NMIIA) and a nuclear membrane protein Emerin (Emd) at the outer nuclear membrane (ONM), which serve as a mechanosensor complex. The retaining of Emd at ONM leads to a switch from H3K9me2,3 to H3K27me3 at lamin-associated domains and constitutive heterochromatin, hence induces the rearrangement of chromatin. Restoration of nuclear actin by depletion of its transporter Exportin-6 enhances transcription and counteracts PRC2-mediated gene silencing. Thus, our results reveal a novel mechanism of how extrinsic mechanical forces regulate chromatin organization, transcription and gene silencing during lineage progression.





## P55

### Investigating stress granule insolubility with age

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For all organisms promoting protein homeostasis is a high priority in order to optimize cellular functions and resources. However, there is accumulating evidence that aging leads to a collapse in protein homeostasis and widespread non-disease protein aggregation [1].
Our recent work reveals that RNA granule components become highly insoluble with age in C. elegans. Several of the RNA-binding proteins identified to aggregate with age contain low-complexity sequences with "prion-like" domains (Alberti algorithm). These sequences are required for the formation of RNA granules. One of these RNA-binding proteins with a "prion-like" domain is the stress granule marker, polyadenylate-binding protein PAB-1. We find that PAB-1 accumulates with age in insoluble cytoplasmic puncta in wild-type C. elegans. We show that another stress granule marker, TIAR-2 (homolog of TIA-1), also aggregates with age. Both PAB-1 and TIAR-2 co-localize in stress granule-like puncta with age. Delaying aging through dietary restriction or reducing insulin/IGF-1 signaling prevents RNA granule protein insolubility with age. The delay in age-related aggregation in animals with reduced insulin/IGF-1 signaling is dependent on the transcription factor HSF-1. Investigating chaperones downstream of HSF-1 reveals promising candidates playing a role in preventing RNA-binding protein aggregation with age. RNA granules are normally highly dynamic structures yet our results suggest that aging is a sufficient stress to cause the irreversible aggregation of RNA granule proteins, which could impair the function of RNA granules. As stress granule proteins are also found in pathological aggregates associated to neurodegenerative diseases, the formation of RNA granule protein aggregates with age may provide a seed for disease aggregation.

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## P56

### UV-B-induced nuclear DNA damage in Caenorhabditis elegans: Impact on mitochondrial biogenesis and neuronal network

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**Introduction**
Nucleotide excision repair (NER) is an essential DNA damage response (DDR) mechanism for keeping DNA intact, and is highly conserved across different species. Recently, C. elegans has been employed to study NER during development and in the context of a whole organism. The present study aims to determine the effects of UV-B-induced DDR on mitochondrial physiology in NER deficient backgrounds and use a NER deficient Caenorhabidits elegans model to further study NER associated neurodegeneration in humans.

We hypothesise that there is a nucleo-mitochondrial communication in response to UV-B-induced DNA damage, leading to a change in mitochondrial physiology, which could be the cause of neurodegeneration seen in patients with deficiencies in NER.

**Results**
UV-B irradiation was used to induce specific DNA lesions (e.g. transcription-blocking lesions), which resulted in a reduction of oxygen consumption in NER deficient nematodes and a reduction in mitochondrial activity as measured by TMRE staining of active mitochondria. We did not detect an induction of mitochondrial unfolded protein response (UPRmt), however an increased induction of mitochondrial targeted GFP in intestinal and muscle tissues was observed. In parallel, our work aims at establishing a C. elegans model for NER neurodegeneration leading to the classification and quantification of neuronal defects.

**Perspectives**
Generating a neurodegenerative model for NER could improve diagnosis and treatment not only of NER derived disorders, but also other neurodegenerative diseases such as mitochondrial disorders. With an ageing population and increasing incidences of skin cancer, further studies to enhance our understanding of DNA damage responses and NER mechanisms have become of uttermost importance.





## P57

### Molecular characterization and functional analysis of human young and elderly hematopoietic stem and progenitor cells

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**Introduction**
The advancement in the health science field along with development of new technologies has remarkably extended human lifespan. Our understanding of ageing is scarce, in particular the ageing of the hematopoietic stem and progenitor cell (HSPC) compartment. Our aim is to perform a phenotypic, functional and molecular characterization of HSCs to unravel the molecules involved in ageing in order to rejuvenate elderly HSCs.

**Results**
Elderly and young human CD133+ HSPCs underwent a general cell surface expression screening, functional assays and microRNA microarray analyses. Our results showed that human elderly CD133+ cells have an increased frequency of HSCs and decreased frequency of common lymphoid progenitors (CLPs) compared to young donors as previously reported [1, 2]. Additionally, we found that the frequency of common myeloid progenitors (CMPs) was also decreased in elderly compared to young donors. The functional data revealed no differences in the proliferation potential, however, we observed differences in the expression of some adhesion and homing receptors after 7 days of in-vitro culture in addition to different potential in the colony-forming unit assay. Moreover, microRNA analysis using an Agilent microarray was performed. During our molecular analysis we identified several microRNAs that were differentially expressed between young and elderly donors. Using Real Time PCR we have validated these candidates and currently we are investigating their rejuvenating potential through overexpression or inhibition of the microRNA candidates .

**Perspectives**
Altogether, our efforts to unravel aging of the hematopoietic system revealed that there are little differences in the cell surface expression of screened markers between young and elderly CD133+. Nevertheless, after 7 days of in-vitro culture of CD133+ cells, some surface molecules are lower expressed in elderly donors. These results shed light into the different functional potential of elderly HSCs. Finally, our molecular analysis has resulted in microRNA candidates which are currently under thorough investigation for its rejuvenation potential.

**Acknowledgments**
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## P58

### Changes in telomere protein composition induced by tumorigenic conversion of normal human fibroblasts

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**Introduction**
Telomeres - special chromatin structures that cap and protect the ends of human chromosomes - have been widely implicated in the cellular processes of ageing and cancer. Several lines of evidence suggest that cancer development might be accompanied by reorganization of telomeric chromatin. However, a systematic, comprehensive study of the tumorigenesis-associated changes in telomere protein composition is still missing.

**Results**
We have applied the Quantitative telomeric chromatin isolation protocol (QTIP) [1] to compare telomeric states in isogenic cell lines representing several stages of the transformation process. Using the approach developed by Hahn. et al. [2, 3], human embryonic lung fibroblasts were converted into tumorigenic cells in a step-by-step fashion using serial introduction of genes encoding the hTERT subunit of telomerase, the SV40 large T and small T antigens, and the H-RasV12 oncogene.
Pairwise comparison of the four cell lines has revealed transformation-induced alterations in abundance and/or telomere occupancy of multiple proteins, including some unexpected protein networks.
Currently, we are investigating the biological relevance of these findings. Besides, several novel telomeric proteins have been identified and are being validated.

**Perspectives**
All in all, this project may open up novel avenues for investigating the roles of telomeres in cancer, which in turn could facilitate the development of telomere-based prognostic, diagnostic and/or therapeutic strategies for oncology patients.

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## P59

### Is the essential function of Dna2, a conserved nuclease/helicase, in telomere biology?

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**Introduction**
DNA2 encodes a conserved and essential helicase/nuclease which is involved in DNA replication, Okazaki fragment maturation and DNA resection during double strand break (DSB) repair. In mammalian cells DNA2 preferentially associates with telomeres during replication and has a role in G-quadruplex DNA cleavage. Expression of DNA2 is significantly increased in human cancers [1]. In yeast, Dna2 has a role in the DNA replication checkpoint and is one of three DNA damage signal transducers (Ddc1, Dna2, Dpb11) which activate the central yeast kinase Mec1 (ATR in humans). In yeast, DNA2 deletion is lethal, but three mechanisms are known to suppress the lethality of dna2Δ: overexpression of RAD27ScFEN1, a nuclease involved in Okazaki fragment processing, deletion of PIF1, a helicase involved in long flap formation during DNA replication, or deletion of RAD9, a DNA damage checkpoint protein [2].

**Results**
We now report that, in contrast to published data, null mutations of other checkpoint genes, DDC1, RAD17 and CHK1, suppress the lethality of dna2Δ mutants at the level similar to that of rad9Δ mutation. Double mutants initially grow poorly but get fitter with time. Such a pattern of growth (initial sickness and recovery over time) is also observed in strains with defects at telomeres. Indeed, a Southern blot analysis of dna2Δ cells confirms telomeres are longer in such cells.

**Perspectives**
It has been proposed that the essential function of Dna2 is in Okazaki fragment processing and lagging strand synthesis. We propose an alternative hypothesis, based on the observation that checkpoint gene deletions suppress dna2Δ, whereas they exacerbate defects in other core DNA replication proteins [3]. Our hypothesis is that the essential function of Dna2 specifically is in telomere maintenance, rather than in general chromosome replication. Our model fits with published data showing that DNA2 binds and is important at telomeres in mammalian cells, and is specifically required for maturation of telomeric DNA replication intermediates in yeast. Our results suggest that Dna2, like the CST complex, might be a telomere-focused DNA replication protein.

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## P60

### Methylation of Glutamine 105 on Histone H2A; Epigenetic Regulation of Ribosome Biogenesis

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**Introduction**
Ribosome biogenesis is tightly coupled to cell growth and division, and is therefore a highly regulated process that is responsive to many endogenous and exogenous stimuli. Regulation occurs at all stages of ribosome assembly; from the transcription of ribosomal DNA (rDNA) in the nucleolus, to the co-transcriptional processing of the ribosomal RNA (rRNA), and through to the final stages of quality control of mature ribosomes. During ageing, and in many diseases including cancer, ribosome biogenesis is deregulated [1]. In order to be able to treat such diseases and shed light onto the mechanisms leading to ageing, it is of critical importance for us to further our understanding of how ribosome biogenesis is regulated. Recently, the methylation of glutamine 105 on histone H2A (H2AQ105me) in Saccharomyce cerevisiae (Q104 in mammals) was identified as an rDNA-specific histone modification [2]. Initial studies showed H2AQ105me to increase rDNA transcription by depleting the rDNA of nucleosomes2, thus implicating this histone modification in the regulation of rDNA transcription.
**Results**
Now, using SILAC labelling of cell lysates followed by pull-downs and Mass Spectrometry [3], we have identify components of the rRNA processing machinery as preferentially binding methylated H2AQ105, suggesting that glutamine methylation also regulates the co-transcriptional processing of rRNA. Furthermore, we have identified the acetylation of lysine 56 on histone H3 (H3K56ac), as being required for the methylation of H2AQ105. We show that both epigenetic marks fluctuate during the cell cycle and are at their most abundant in S-phase, when the chromatin at the rDNA is at its most compact.

**Perspectives**
We are now working to dissect the molecular mechanisms of the processes highlighted above.

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## P61

### Investigating the contribution of individual tissues to organismal ageing using chimeric turquoise killifish

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During vertebrate ageing, the relative importance of cell autonomous versus systemic mechanisms is still unclear. To investigate the impact of different tissues on whole organism ageing, I plan to separately modulate the ageing rate in individual tissues and characterize the effect on the whole organism. I am generating tissue-specific chimeras that combine different organisms that drastically differ in lifespan, using one tissue from a long-lived donor species, and all the others from a short-lived acceptor one (and vice versa). Fish of the genus Nothobranchius are particularly well suited for this goal, as different species show a wide range of maximum life spans – ranging from 4 to 20 months – with other phenotypes being very similar. I am generating an acceptor strain, where only the tissue to be replaced expresses a suicide gene, and a donor strain, which combines a ubiquitous knock-in and a tissue specific knock-out of the suicide gene, to eliminate all tissues but the one to be donated. I expect chimera creation to be feasible for different Nothobranchius species.

**Perspectives**
Short-lived / long-lived Nothobranchius chimeras could answer the question of to what extent lifespan and ageing are caused by tissue-tissue interactions or by cell intrinsic processes. This approach will answer whether there are particular cell type(s) that drive the ageing process in vertebrates. This strategy will help to narrow down the "proximal cause" of ageing, and identify the best candidates for cell-based therapies.

## P62

### Nuclear spheres in neurodegeneration

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Nuclear spheres are protein aggregates consisting of FE65, TIP60, BLM and other yet unknown proteins. Generation of these structures in the cellular nucleus is putatively modulated by the amyloid precursor protein (APP), either by its cleavage or its phosphorylation. Nuclear spheres were preferentially studied in cell culture models and their existence in the human brain was unknown so far. In the present study we were able to show that nuclear spheres are existent in the aged human brain to a low extent. However, the comparison of human frontal cortex brain samples from Alzheimer disease (AD) patients to age-matched controls revealed a dramatically and highly significant enrichment of nuclear spheres in the AD brain. Co-staining demonstrated that neurons are distinctly affected by nuclear spheres but never astrocytes. Nuclear spheres were predominantly found in neurons that were negative for APP T668 phosphorylation. Cell culture experiments revealed that JNK3 mediated APP phosphorylation reduces the amount of sphere positive cells, which could be partly restored by the APP T668A mutation. The study suggests that nuclear spheres are a new APP-derived central hallmark of the disease and might be of crucial relevance for the molecular mechanisms in neurodegeneration.





## P63

### The use of urinary peptidomics in the assessment of suitability of mouse models of ageing

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**Introduction**
Ageing is a complex process characterised by a systemic and progressive deterioration of biological functions. As ageing is associated with an increased prevalence of age-related chronic conditions, understanding molecular mechanisms involved in ageing can pave a way for therapeutic interventions and managing complications. Animal models like mice are commonly used in ageing research as they have a shorter lifespan in comparison to humans and are also genetically close to humans. To assess the translatability of mouse ageing to human ageing, the urinary proteome in 89 wild-type (C57/BL6) mice aged between 8-96 weeks was investigated using electrophoresis coupled to mass spectrometry (CE-MS)

**Results**
Using age as a continuous variable, 163 peptides significantly correlated with age in mice were identified. To investigate the relevance of using mouse models in human ageing studies, a comparison was performed with a previous correlation analysis using 1227 healthy subjects [1]. In mice and humans, a decreased urinary excretion of fibrillar collagens and increased of uromodulin fragments was observed with advanced age. Of the 163 peptides correlating with age, 54 peptides had a strong homology to human ageing. These ortholog peptides including several collagen fragments (N= 48) and uromodulin (N= 6) were used to generate an ageing classifier that was able to discriminate the age among both wild-type mice and healthy subjects. Additionally, the ageing classifier depicted that telomerase knock-out mice were older than their chronological age.

**Perspectives**
In conclusion, we have demonstrated that mice can be good models to study human ageing. One major advantage of using urinary peptidomics to study ageing is the ability to obtain a readout representative of human ageing using mouse models. Hence, with a focus on ortholog urinary peptides mouse models can serve as readout of interventions in the management ageing-associated complications towards humans.

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## P64

### The role of ageing in the development of rheumatoid arthritis

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**Introduction**
Rheumatoid arthritis is a chronic inflammatory condition characterized by inflammation of the joints leading to cartilage destruction and bone erosion. The mesenchymal-origin synovial fibroblasts (SFs) have a crucial role in the initiation and development of the pathology [1]. SFs of hTNF-overexpressing transgenic mice [hTNFTg (Tg197)] that develop spontaneous arthritis are characterized by a specific arthritogenic phenotype [1,2]. Mutations in ERCC1 protein (Nucleotide-Excision-Repair family) cause severe progeroid syndrome in humans and mice, as well as inflammatory responses in specific tissues, such as adipocytes [3]. As adipocytes are of mesenchymal origin like SFs, we investigate how Ercc1 defect affects inflammatory responses and consequently interferes with arthritis pathology.

**Results**
To evaluate the effect of defective NER in the development of arthritis, we used either progeroid Ercc1 mutant (Ercc1-/Δ) or conditional Ercc1KO mice with tissue specificity for mesenchymal-origin cells (ColVICre/Ercc1f/-). ColVICre/Ercc1f/- mice show reduced body weight compared to controls (ColVICre/Ercc1f/+) indicating possible defects in mesenchymal-origin cells. In vivo arthritis scoring and histopathological evaluation of the ankle joints of both lines, showed no signs of arthritis pathology including inflammation, bone erosion or cartilage destruction. In vitro assays of isolated SFs were performed to investigate their arthritogenic phenotype, thus the cells' ability to adhere and migrate towards specific ECMs, to proliferate and to secrete proinflammatory cytokines. Ercc1-/Δ and ColVICre/Ercc1f/- SFs showed significantly lower adhesive and migratory ability and cell proliferation capacity than arthritogenic Tg197 SFs and comparable to those of WT cells. However, in later time points (3.5 and 4.5 months of age) SFs of Ercc1-/Δ mice showed increased proliferation, similar to that of Tg197 SFs. LPS stimulation of Ercc1-/Δ SFs resulted in an increase of secreted TNF and IL-6 levels compared to WT. Ercc1 deletion in mesenchymal-origin cells was indicated to cause partial amelioration of arthritis when the Collagen-Antibody-Induced-Arthritis (CAIA) model was used in ColVICre/Ercc1f/- mice.

**Perspectives**
Overall our results show that Ercc1 defect does not lead to the development of spontaneous arthritis pathology. On the contrary, induction of arthritis in MSC specific deletion of Ercc1 leads to amelioration of induced arthritis pathology, coming to agreement with the in vitro observed non-arthritogenic phenotype of both Ercc1-/Δ and ColVICre/Ercc1f/- SFs. We continue investigating possible interactions of the systemic and tissue-specific Ercc1 deletion with the spontaneous arthritis phenotype of Tg197 mice.

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## P65

### Adult Neurogenesis in the Hippocampus and Olfactory bulb of Cat, Pangolin, and Rat; A Comparative Study

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**Introduction**
Adult neurogenesis was investigated in the hippocampus and olfactory bulb of three mammalian species namely cat, pangolin, and rat. This is with a view to evaluate the comparative cellular neuromorphology, proliferation of new neurons, acetylcholinesterase and glial fibrillary acidic protein (GFAP) activities in the hippocampus and olfactory bulb of cat, pangolin and rat.

Twenty cat, pangolin, and rat each were obtained from local sources in Osun State, Nigeria. The animals were sacrificed under ketamine anesthesia. Followed by transcardial perfusion with phosphate buffered saline (PBS). Five animals each were further cardiac perfused with sodium sulphide solution, and their brains were harvested and fixed in 10% neutral buffered formalin for immunohistochemical studies. The brains of another set of five animals each were harvested following PBS perfusion and fixed in 10% NBF for histological and histochemical studies. 5 µm paraffin sections in the sagittal planes were obtained and processed. These sections were stained with Hematoxylin and eosin (H&E), cresyl fast violet (CFV), Ki-67, and glial fibrillary acidic protein (GFAP). Following perfusion with PBS, the brains of the remaining ten animals were harvested and homogenized (10 % w/v) in PBS for biochemical estimation of the activities of acetylcholinesterase (AChE) in the hippocampus and olfactory bulb. Stained sections were viewed under a Leica DM3000 digital light microscope and digital photomicrographs were taken. Image J (NIH public domain software) was used for neuronal and glial cell count. One way ANOVA was used to analyse data obtained, followed by student Newman-kuels (SNK) test for multiple comparison, with $p<0.05$ accepted as significant value.

**Results**
Results showed that the activities of acetylcholinesterase (AChE) in the hippocampus was reduced from cat, to rat, to pangolin (761.04 ± 2.11 IU/L, 758.31 ± 2.96 IU/L, 597.01 ± 7.72 IU/L respectively), the differences between them were statistically significant $p<0.001$. Also in the olfactory bulb, the same pattern was observed in cat, rat, and pangolin (574.27 ± 7.41 IU/L, 571.92 ± 5.29 IU/L, and, 452.61 ± 3.26 IU/L respectively) with statistically significant difference $p<0.001$. Similar pattern was observed for neuronal count in the hippocampus of cat, rat, and pangolin (79 ± 9.29 µm2, 75.12 ± 6.31 µm2, and, 63.10 ± 2.19 µm2 respectively) and that of the olfactory bulb of the cat, rat, and pangolin (54.21 ± 12.64 µm2, 53.21 ± 12.63 µm2, and, 50.26 ± 12.91 µm2 respectively). The differences in both groups were statistically significant. $P<0.001$ in both. Ki-67 positive neurons were noted in the hippocampus and olfactory bulb in pangolin and rat but not in cat. Glial fibrillary acidic protein (GFAP) positive astrocytes were noted in the subventricular zone of the dentate gyrus of the hippocampus and olfactory bulb of all the animals except the olfactory bulb of pangolin where it was sparse.

**Perspective**
Data from this study suggest that there was evidence of adult neurogenesis in the hippocampi and olfactory bulbs of pangolin and rat but not in the cat, and there was no relationship between cholinergic activities and adult neurogenesis in the hippocampi and olfactory bulbs of the studied animals.

## P66

### Constitutive mTORC1 signalling distorts DNA replication programme and sensitises TSC deficient cells to genotoxic stress

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**Introduction**
Tuberous Sclerosis manifests as non-malignant apoptotic neoplasia. Exorbitant mTORC1 signalling can sensitise TSC-/- cells to stress-induced death. Seminal reports identified that TSC-/- cells are susceptible to mild genotoxic stress, in part by augmented p53 function. Studies thereafter unveiling energetic shortfall due to unmet anabolic demand, refined our understanding of the stress-sensitive phenotype. However, the role and extent of cell cycle alterations involved in TSC-/- cells' hypersensitivity to genotoxic stress is poorly understood.

**Results**
We report cell cycle alterations and futile checkpoint responses in TSC1-/- fibroblasts exposed to low-dose genotoxins accompanied by elevated nucleotide incorporation rates despite only modest origin over-firing. Strikingly, an increased propensity for asymmetric fork progression and profuse chromosomal aberrations under external genotoxic stress suggests that constitutive mTORC1 activity proves detrimental to stress adaptation.

**Perspectives**
We conclude that fundamentally slower DNA synthesis at individual forks and low stress tolerance imposes negative selection on genomic instability and could detain TSC-mutant tumours benign.

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## P67

### DNA damage-induced necrotic neurodegeneration & ageing

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**Introduction**
DNA damage is a major contributing factor in ageing and has been implicated in neurodegeneration. A critical question that emerges is whether intrinsic neuronal stress response pathways engage to protect against DNA damage-triggered neurodegeneration. Moreover, although it is well-established that DNA damage induces apoptosis, the contribution of necrotic cell death to DNA damage-related pathology remains largely elusive. To detect spontaneous necrotic cell death during ageing UV-hypersensitive ercc-1 C. elegans mutants were used, which are defective in the nucleotide excision repair pathway (NER) [1]. We next generated neuronal reporter strains carrying the ercc-1 mutation and observed decreased neuron viability and increased susceptibility to neurodegeneration as animals aged. Given the interplay between ERCC1 and the DNA damage and oxidative stress response pathways, we examined ercc-1 mutant stress sensitivity and responses. We are currently dissecting the crosstalk between DNA damage-induced necrosis and neurodegeneration, aiming to identify evolutionarily conserved molecular mechanisms interfacing these processes.

**Results**
During physiological ageing DNA damage repair-deficient animals exhibit increased dopaminergic neuron loss as well as spontaneous necrotic cell death. Moreover, under stress conditions such as UV-C irradiation and oxidative stress which trigger DNA damage increased dopaminergic (necrotic) neurodegeneration is observed.

**Perspectives**
Future plans involve tracking of other neuronal populations in DNA damage repair-deficient worms. Moreover, assessment of neuronal cell death after acute and chronic stress will be peformed as well rescue of the mutant phenotype by re-introduction of the gene.

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## P68

### Functional changes in the interaction between the gut microbiota and the immune system during ageing

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**Introduction**
The composition of intestinal microbiota becomes significantly altered with age in humans. Changes in the composition of gut bacterial communities have also been linked to several, often age-related diseases like obesity, inflammatory conditions, frailty and other co-morbidities. Understanding whether changes in the gut microbiota are correlative or causative for these conditions is an open question. However, the long-lived nature of most vertebrate experimental model organisms makes this question hard to address. The turquoise killifish (Nothobranchius furzeri) is the shortest-lived vertebrate reproduced in captivity and is characterized by a medium lifespan of 4 months and by rapid ageing. My goal is to profile age-related changes in the intestinal microbiota of the turquoise killifish by using 16s sequencing in a longitudinal study. Since our group recently showed that a single gut microbiota transfer from young fish prolongs lifespan in old recipient fish, we will also conduct a serial transfer of young gut microbiota as a follow-up experiment. To test the role of a functioning adaptive immune system in the maintenance of a "healthy" gut microbiota, I am developing two transgenic fish lines. One uses the endogenous fish Rag2 promoter driving GFP to identify developing B- and T-Lymphocytes and their differentiation niches throughout ageing in the turquoise killifish. A Rag2-knockdown fish line – generated by CRISPR/Cas9 – will be instead used to test the effects of a missing adaptive immune system on the microbiota composition, lifespan and on the structure of immune organs such as the thymus and the intestine. Understanding the mechanisms by which a young microbiota can improve health and slow down ageing in complex vertebrate model organisms can have direct applications in the clinic and could significantly impact modern medicine.

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## P69

### DNA dModulation of longevity and stress response pathways by SUMOylation in C. elegans

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**Introduction**
The nematode Caenorhabditis elegans is an ideal model organism for studying the biology of ageing; the pathways modulating the ageing process are well-characterized and conserved. Amongst these signaling pathways, insulin/IGF-1 has a major role in determining the lifespan of animals, mainly through the DAF-16/FOXO transcription factor and the stress response-related transcription factor SKN-1/NRF2 [1]. Interestingly, these two key transcription factors contain putative SUMOylation sites. SUMOylation, the attachment of SUMO (small ubiquitin-related modifier) to a protein, is a posttranslational modification implicated in the regulation of diverse cellular processes, including the DNA damage response, sub-cellular protein localization and protein-protein interactions, among others [2]. Protein SUMOylation levels increase progressively during ageing [3]. However, whether elevated SUMOylation is only an unrelated consequence of the ageing process or it serves a causative, regulatory role in senescent decline is not understood.

**Results**
The C. elegans genome contains a single gene encoding SUMO (smo-1), rendering the nematode a convenient model in which to genetically dissect the role of SUMOylation in organismal physiology and ageing. Deletion of smo-1 causes embryonic lethality. Nevertheless, we find that RNAi knockdown of smo-1 initiated at the L4 stage shortens the lifespan of both wild type and long-lived animals. Neuron-specific knockdown of smo-1 does not alter median lifespan. Notably, knockdown of the SUMO protease gene (ulp-1), extents the lifespan of long-lived mutants (clk-1, daf-2, ife-2), but not wild type animals. In addition, we observed that manipulation of SUMOylation levels by either knockdown of smo-1 or ulp-1 influences the activity of DAF-16 and SKN-1, as well as, stress resistance and energy metabolism, in a genetic background- and age-dependent manner.

**Perspectives**
We are currently investigating the possible tissue-specific and cell non-autonomous mechanisms by which SUMOylation modulates the activity of key, stress response transcription regulators, and how these mechanisms interface with main signalling pathways that impinge on longevity.

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## P70

### The role of microRNAs in proteostasis and aging

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Eukaryotic cells provide two proteolytic strategies to maintain protein homeostasis (proteostasis) by elimination of damaged proteins: The autophagy-lysosome pathway and the ubiquitin-proteasome system (UPS). For a long time, both pathways were considered to function independently by handling different substrates with specific turnover rates. Nowadays, however, accumulating evidence suggests that both pathways communicate with each other because impairment of one system affects the activity of the other. Moreover, selective autophagy receptors including p62 were shown to bind to both ubiquitinated substrates and the autophagosomal membrane, thereby ensuring the degradation of the cargo in mature autolysosomes [1]. However, the field is still lacking mechanistic insights how this crosstalk is regulated, especially under conditions of proteotoxic stress and aging.
Our ultimate goal is to unravel the so far unknown crosstalk mechanisms of both proteolytic systems and their adaption to aging dependent environmental changes. Here, we will focus on the role of microRNAs (miRNAs) as so far poorly investigated in the context of proteostasis. miRNAs are short non-coding RNAs that negatively regulate the translation of a target mRNA by binding to the 3' UTR. We propose that stress, jeopardizing one or both proteolytic systems and thereby proteostasis in general, results in the up- or downregulation of certain miRNAs. This response might be regulatory and probably induces the UPS and/or autophagy to re-establish proteostasis. For identification of these key miRNAs, we performed an RNA-sequencing approach in Caenorhabditis elegans. Worms were treated with specific proteotoxic reagents that induce or inhibit either proteolytic system. Bioinformatical sequence analysis identified several regulated (up- or down-regulated) miRNAs. Current efforts focus on screening candidate miRNA-deletion mutants for protein degradation defects and identifying relevant target mRNAs. Intriguingly, several candidate miRNAs are also important to support longevity.
Uncovering the molecular mechanism of the proteolytic network is a central challenge that addresses the aging society. Long-term damage of the proteome, often occurring during aging, leads to neurodegenerative diseases like Alzheimer's or Parkinson's disease. Novel insights into the regulation of proteolytic networks may contribute to therapies for aging-related diseases.

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## P71

### DNA methylation changes in plasticity genes accompany the formation and maintenance of memory

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The ability to form memories is a prerequisite for an organism's behavioral adaptation to environmental changes. At the molecular level, the acquisition and maintenance of memory requires changes in chromatin modifications. In an effort to unravel the epigenetic network underlying both short- and long-term memory, we examined chromatin modification changes in two distinct mouse brain regions, two cell types and three time points before and after contextual learning. We found that histone modifications predominantly changed during memory acquisition and correlated surprisingly little with changes in gene expression. Although long-lasting changes were almost exclusive to neurons, learning-related histone modification and DNA methylation changes also occurred in non-neuronal cell types, suggesting a functional role for non-neuronal cells in epigenetic learning. Finally, our data provide evidence for a molecular framework of memory acquisition and maintenance, wherein DNA methylation could alter the expression and splicing of genes involved in functional plasticity and synaptic wiring.

## P72

### Investigations on cell- and tissue-specific activity of DNA repair upon UVB irradiation in development and ageing of C. elegans

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**Introduction**
DNA damage and genome instability are hallmarks of ageing. In humans congenital defects in DNA repair mechanisms such as nucleotide excision repair (NER) lead to decreased repair capacity of UV-induced lesions, subsequently increasing the risk of cancer. Additionally, patients with NER deficiency display accelerated ageing including neurodegeneration and developmental growth defects. NER activity is highly conserved in C. elegans and NER deficient animals are incapable of repairing DNA damage inflicted by UV irradiation, resulting in growth arrest and decreased life span.

**Results**
We investigate the tissue-specific and age-dependent activity of NER in maintaining genome stability in C. elegans. To this end, we established an in vivo imaging system to monitor NER activity in C. elegans: We generated transgenic animals expressing the central NER factors XPA-1::GFP, CSB-1::GFP or CSA-1::GFP. In vivo expression analysis reveals a progressive decline of XPA-1::GFP during ageing in all tissues, except the neuronal system. Further, we are performing tissue-specific rescue studies of the highly UVB sensitive xpa-1 mutant animals by constitutively driving expression of XPA-1::GFP in muscles, intestine, cuticle, and the neuronal system. Neuronal or intestinal XPA-1 expression, respectively, is sufficient to rescue UVB induced developmental arrest and restores life span to control levels.
In parallel, we apply a UVC laser system for precise single-cell DNA damage induction in cuticle tissue, and observe rapid recruitment of XPA-1::GFP to the site of irradiation. This system allows investigating cell type-specific dynamics of DNA damage response factors during ageing and in the context of a whole organism. To further understand tissue-specific relevance of DNA repair, we created transgenic animals that ectopically express the A. thaliana photolyase PHR1, which specifically repairs cyclobutane pyrimidine dimers (CPDs), which comprise the main lesion type induced by UV. Ubiquitous and constitutive expression of PHR1::GFP enhances UV resistance of wild type and NER deficient animals.

**Perspectives**
Decrypting tissue-specific DNA damage responses and genome maintenance in C. elegans will lead to a better understanding of the complex human disorders that are caused by DNA repair defects and allow the development of intervention strategies to counteract age-related tissue-decline, cancer development and promote longevity.





## P73

### Deletions of members of the PAF1 complex reveal interactions with the ESCRT-II complex as well as a neighbouring gene effect

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The PAF1 complex (Cdc73, Paf1, Ctr9, Leo1 and Rtf1) affects RNA levels by affecting transcription, histone modifications and post-transcriptional RNA processing and has been reported to play a role in telomere biology. For example, deletion of CDC73, PAF1 or CTR9 cause an 80% decrease in TLC1 RNA levels. TLC1 is needed for proper telomere extension and therefore cdc73Δ, paf1Δ and ctr9Δ have short telomere length.
In this study we used telomere defective yeast strains to understand the function of the PAF1 complex at telomeres and potentially in cancer (since the CDC73 human orthologue is a tumour suppressor gene). We found that for a similar effect over TLC1 RNA, telomere defective cells can better tolerate lack of CDC73 than lack of PAF1 or CTR9. To better understand what other telomere related pathways might be differently affected in paf1Δ/ctr9Δ and cdc73Δ cells, we analysed CST component expression and TERRA levels in cells deleted for the PAF1c members. We also examined the interplay between the PAF1 complex and Vps36, the gene located next to CDC73. Vps36, part of the ESCRT-II complex, is involved in sorting proteins for vacuolar degradation.
We found that PAF1 and CTR9, but not CDC73, are needed for controlled low levels of Ten1 and TERRA. These results place the PAF1 complex as an important regulator of telomere health. Additionally, we saw a neighbouring gene effect between CDC73 and VPS36 and that VPS36 transcription is regulated by the PAF1 complex.

**Keywords**
PAF1 complex, neighbouring gene effect, VPS36, telomeres.

## P74

### Radiation-induced aging and genetic instability of mesenchymal stem cells : An issue for late health effects?

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Mesenchymal stem cells (MSCs) are a source of adult multipotent cells important in tissue regeneration. The aim of this study is to analyse the interaction of non-physiological high oxygen and low-dose gamma-irradiation onto growth, senescence and DNA damage in murine MSC.
There has not been much interest in the past to study the response of adult stem cells such as MSCs to radiation exposure, probably because for connective tissue tumors (sarcomas, derived from mesenchymal cells) the radiation-associated excess relative risk (ERR) among the A-bomb survivors was much lower than for carcinoma or for leukaemia (Preston et al 2003). This picture looks much different, however, when patients irradiated with higher radiation doses for therapeutic purposes are studied: external beam radiotherapy confers a high risk for the induction of therapy-associated secondary tumours, in particular sarcomas derived from mesenchymal stem cells.
In the following chapters I will try to explain why adult stem cells, in particular MSCs should be considered an important target for radiation-associated disease. This is also relevant in view of an increasing number of clinically approved MSC based therapies. These involve the collection of MSCs from various anatomical sites of a patient (including sites that might have
been exposed to diagnostic, therapeutic or occupational ionizing radiation), followed by forced in-vitro expansion of the cells and autologous re-implantation.
We show the influence of environmental stress such as unphysiological high oxygen, radiation-induced genotoxic stress onto stemness, potency and genetic stability of MSCs.
The influence of genetic defects such as p53 mutational status or Rb1 deficiency interacts with genotoxic and cellular stress imposed on MSCs in a different manner than in cells with only limitted self renewing capacity.



## P75

### Epigenetic transition and immortalization of human adult stem cells by epigenetic agents

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**Introduction**
Waddington´s famous epigenetic landscape metaphor simplyifying illustrates canalization of stem cell development. This is a result of genetic and epigenetic determinants wherebyat genetic and exogenous influences can be tolerated below a distinct threshold, without affecting the pre-determined developmentaling path.
Our hitherto studies provide evidence for that epigenetic agents can serve as exogenous effectors to force adult somatic stem cells into a related canal while altering their stem cell state and differentiation options. This is our central hypothesis.

**Results**
We have shown that unrestricted somatic stem cells from umbilical cord blood can be altered by epigenetic agents to transit into a new stem cell type with extended differentiation potential. Furthermore they become immortal, due to an acquired ALT („alternative lengthening of telomeres“) mechanism, which is known to be of fundamental importance for the initial stages of embryogenesis but also for a variety of tumors.

**Perspectives**
Thus our epigenetically established immortal SpheeUSSC stem cells may serve as a unique model to dissect the mechanisms underlying ALT. mechanism. In addition, a variety of different distinct adult stem cell types will be treated by different protocols containing epigenetic agents, e.g. 5´ Aza-2-deoxycytidin, Trichostatin A, in order to evaluate their transition potential into new stem cell types and if applicable the differentiation potential of them.

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Foued Ghanjati and Simeon Santourlidis
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## P76

### Autophagosomal acyl-CoA synthetases activate fatty acids required for autophagy

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**Introduction**
Autophagy is a central homeostasis and stress response pathway conserved in all eukaryotes of removing damaged proteins, protein aggregates or dysfunctional organelles. Defects in autophagy are linked to aging related diseases like neurodegeneration, tumor biogenesis or obesity. A unique feature of autophagy is the de novo formation of double-membrane vesicles, so-called autophagosomes (AP), mediated by hierarchical assembly and function of a conserved machinery driving nucleation, expansion, and closure of AP. Cells carefully control number, size, and cargo of AP to respond adequately to stress conditions, but regulatory mechanisms underlying AP biogenesis are poorly understood.

**Results**
Analyzing the core autophagy machinery by proteomics, we identified two novel physical interactors, Faa1 and Faa4, acyl-CoA synthetases (ACS), which activate free fatty acids by thio-esterification with coenzyme A. Interestingly, activated acyl-CoA can be utilized for lipid synthesis and editing, thus affecting membrane biogenesis of the cell.
Cytological analysis demonstrated an enrichment of Faa1 and Faa4 on growing and mature AP suggesting a function downstream of AP nucleation. Importantly, our analysis showed that autophagy depends on acyl-CoA synthesis, either by ACSs or the redundant fatty acid synthase (FAS) complex, highlighting the impact of these redundant pathways.

**Perspectives**
Employing a multidisciplinary approach, we are addressing the questions of how ACSs are recruited to APs. Furthermore, we are investigating the specific function of ACSs for formation, turnover and regulation of APs.





## P77

### Splicing during heat shock is regulated by the bromodomain protein BRD4 and the heat shock transcription factor 1

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**Introduction**
In recent years the bromodomain protein BRD4 has gained extensive attention – mainly due to its involvement in tumor growth and its widespread success as therapeutic target in leukemia, lung cancer, melanoma and diverse other tumor entities. As partner of the positive transcription elongation complex pTEFb BRD4 regulates RNA Polymerase II and transcriptional elongation and, by binding to acetylated histones, it links the transcription process to epigenetic patterns. Recently we have shown that the bromodomain protein BRD4 is an integral member of the oxidative stress response by regulating KEAP1 [1]. There are several lines of evidence implicating BRD4 in the splicing process. We thus wondered whether BRD4 might be involved in the transfer from post-transcriptional splicing towards co-transcriptional splicing under stress conditions.

**Results**
Using genome-wide splicing analyses of RNA-Seq experiments generated under different stressors we found that BRD4 is indeed involved in the splicing process. This is not the case under normal, unstressed conditions, also not under oxidative stress, but under heat shock (HS). Under HS we found that a BRD4 knock down leads to an increased intron retention rate. We further found that BRD4 interacts with HSF1, co-localizes in nuclear stress bodies and, besides mRNA processing, regulates SatIII RNA expression in an HSF1 dependent manner. This finding might also partly explain why Shalgi and colleagues find a functional co-transcriptional splicing process whereas post-transcriptional splicing is severely impaired under HS [2]. The co-transcriptional process under HS may be protected from splicing factor depletion by recruitment of BRD4 to nSB leading to the maintenance of regular splicing of primary stress response genes. Taken together, our findings connect BRD4 to the splicing process, but also show that BRD4 acts as a partner of HSF1 in the heat stress response.

**Perspectives**
Genome sequencing projects have uncovered widespread splicing defects in cancer. Dvinge et al showed in a pan-cancer study that abnormal RNA splicing and in particular intron retention is a common characteristic of many cancers even in the absence of splicing factor mutations [3]. Since the HS response utilizes many factors required for the proteotoxic stress response in cancer, BRD4 might as well join the splicing complex in the nSB and transmit here the stress response and promote pre-mRNA splicing. We are now investigating the splicing profiles in different cancer entities and their relation to BRD4 inhibitor response.

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## P78

### Fly longevity upon decreased insulin signaling depends on tissue-specific regulation of proteostasis and metabolism

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**Introduction**
Drosophila insulin-like peptides (DILPs) are upstream regulators of the IIS pathway in flies. Down-regulation of this pathway is known to increase life-span across many model-organisms [1]. In the fly this phenotype is dependent on the central transcription factor dFOXO. Of seven DILPs, four are produced in median neuro-secretory cells (MNCs) in the brain. We investigated the effect of MNC ablation on dFOXO knockout and wild type (wDah) flies.

**Results**
Label-free shotgun proteomics [2] was carried out for samples from four distinct tissues: brain, gut, fat body, and thorax. Using a two-factor model of differential expression and incorporating prior information via network propagation [3,4], proteins that responded to MNC ablation in a foxo-dependent or -independent manner were identified [5]. Functional analysis revealed that reduced insulin signaling acts tissue-specifically on proteostasis and metabolism to increase life-span.

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## P79

### Age-related morphological and immunological changes in the intestine of African turquoise killifish

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The human intestine harbours trillions of microbes that are essential for human health and the composition of this gut microbiota is significantly altered in the elderly. Whether the age-related changes in the community structure of intestinal microbes are a cause or a consequence of organismal ageing remains elusive, and experimental studies on chronological ageing are often hampered by the long-lived nature of common vertebrate model organisms.

Sequencing of the gut microbiota of the short-lived African turquoise killifish (Nothobranchius furzeri) has recently revealed (1) that the complexity of the intestinal flora of these fish is similar to that of mice and zebrafish, and (2) that the gut microbiota of old fish exhibits decreased diversity and indicates an inflammation-associated state, similar to the aged human gut. Predictive metagenome analysis functionally associates the microbiota of young fish with increased carbohydrate and glycosylation metabolism, processes that are essential for the establishment of the first line of defense of the intestinal barrier, the mucus layer.

After analysis of young and old intestines for general morphological changes, including hyperproliferation and inflammation, I aim to unravel age-associate dynamics in the abundance of intestinal T-lymphocytes by flow cytometry. To elucidate the role of the mucus layer in intestinal ageing, I will (1) determine the number of mucus-secreting goblet cells, and (2) characterise age-related changes in mucus composition. Finally, I will apply a combination of fluorescence in situ hybridization and immunofluorescence to explore the spatial segregation of bacteria in the aged gut.

Investigation of chronological ageing in the fish intestine is key for the establishment of the African turquoise killifish as a model for microbiota research and might provide crucial insights for future studies on ageing processes at the host-microbe interface.

## P80

### Genome editing of tert and p53 in the short-lived killifish Nothobranchius furzeri

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**Introduction**
The tumor suppressor p53 and telomerase play critical roles in ensuring genomic stability and are known to be involved in the aging process. To further study their effects on aging and related diseases, Nothobranchius furzeri is a promising model organism. Its maximum lifespan is up to approximately one year, a fact can help circumvent the longevity problems that are often obstructive in aging research. Additionally, the genome of N. furzeri has been recently assembled at our institute [1] as well as at Stanford University [2], providing further insight into the biology of this killifish that shows typical signs of (mammalian) aging.

**Results**
We have established TALEN and CRISPR/Cas technology in this organism to specifically address the effects of deleting p53 and tert, the latter encoding the enzymatic part of the telomerase.
Knockouts of tert could be successfully generated in N. furzeri by applying TALEN technology. By injection of the necessary components into the one-cell stage of N. furzeri, germline-transmittable frameshift mutations could be created.
Complete loss-of-function of telomerase was shown in homozygous tert knockouts by the TRAP assay, which measures telomerase activity in vitro. Interestingly, neither mRNA levels of tert, nor telomere length (mean length: 4.5 – 6.7 kb in skin and muscle tissue) or regenerative capacity of the fin seem to be dramatically affected. In contrast, the viability of the offspring of subfertile homozygous tert knock-out pairs is drastically reduced by about 50% in the first 4 weeks post hatching.
CRISPR/Cas technology was used to generate p53 knockout mutants. Quantitative real-time analysis showed an upregulation of p53 and its subsequent target p21 in wildtype N. furzeri embryos upon irradiation with 10Gy, which was lost in p53 knockout mutants.
These results indicate that both TALEN as well as CRISPR/Cas technology can be applied to induce double-strand breaks and subsequent mutations at specific loci in N. furzeri.

**Perspectives**
The influence of both p53 and tert knockout mutations shall be investigated considering lifespan and general health status (e.g. tumorigenesis) in Nothobranchius furzeri.
Another main focus will be the creation of double knockout mutants containing both p53 and tert mutations. Research on combinatory effects promises new insights in the aging process.

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## P81

### DNA Replication Sress Underlies Renal Phenotypes in CEP290-Associated Joubert Syndrome

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**Background**
Juvenile ciliopathy syndromes that are associated with renal cysts and premature renal failure are commonly the result of mutations in the gene encoding centrosomal protein CEP290. In addition to centrosomes and the transition zone at the base of the primary cilium, CEP290 also localizes to the nucleus; however, the nuclear function of CEP290 is unknown. Recent data suggest a role for DNA damage signalling in renal ciliopathies.

**Methods**
We set out to extend this correlation to a broader clinical base and investigated the role of CEP290 loss in DNA damage signaling and replication stress. We used primary cells isolated from kidneys of Cep290LacZ/LacZ mice with Joubert syndrome symptoms and their wild-type littermates to investigate DNA damage signaling and the replication stress response. In addition, primary cells from Joubert syndrome patients and zebrafish embryos depleted for CEP290 have been examined.

**Results**
We demonstrate that reduction of cellular CEP290 in primary human and mouse kidney cells as well as in zebrafish embryos leads to enhanced DNA damage signaling and accumulation of DNA breaks ex vivo and in vivo. Compared with those from wild-type mice, primary kidney cells from Cep290-deficient mice exhibited supernumerary centrioles, decreased replication fork velocity, fork asymmetry, and increased levels of cyclin-dependent kinases (CDKs). Treatment of Cep290-deficient cells with CDK inhibitors rescued DNA damage and centriole number. Moreover, the loss of primary cilia that results from CEP290 dysfunction was rescued in 3D cell culture spheroids of primary murine kidney cells after exposure to CDK inhibitors.

**Conclusion**
Together, our results provide a link between CEP290 and DNA replication stress and suggest CDK inhibition as a potential treatment strategy for a wide range of ciliopathy syndromes. Our findings support the overall hypothesis that renal ciliopathies are initially caused by DNA damage and replication stress during early stages of development.

## P82

### Exome-matching of essential amino acids in the diet predicts anabolic efficiency in flies and mice

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**Introduction**
Dietary restriction (DR) extends lifespan across species. In flies, dietary protein is critical for DR-induced lifespan-extension, and individual essential amino acids (EAAs) are key to longevity and fecundity. Restriction of tryptophan or methionine alone also extends lifespan in mice and rats. However, a dietary excess or limitation of single EAAs can also be detrimental to health and life history traits, but the EAA requirements of an organism are difficult to define as they are affected by numerous factors. As the number of exons transcribed and translated at any given time is relatively high, a possible general predictor of the EAA requirement of an organism could be its exome, potentially allowing a rational design for a balanced dietary EAA ratio.

**Results**
In Drosophila, we have found that development and fecundity are improved in a predictable manner by matching dietary AAs to the exome obtained from in silico translation of the genome. Stimulation of anabolic traits was achieved at lower levels of the exome-matched diet, compared to exome-mismatched AAs, yet promotion of anabolism had no negative effects upon lifespan. In mice too, compared to exome-mismatched AA sources exome-matching increased body growth rates according to the same exome-based predictive model.

**Perspectives**
The amino acid profile of a food source is an important factor when considering the usage of dietary nitrogen, anabolic traits, and lifespan. The exome composition of an organism may provide a suitable template for its AA requirements. Our results show that dietary AA balance, as defined by the exome, is critical for the modulation of key life history traits, including growth, reproduction, and ageing, in both vertebrates and invertebrates. Because this model is driven by the genome sequence of the organism in question, the principle can be applied to any organism whose genome sequence is known. Therefore our findings carry possible implications for interventional applications in human nutrition.

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## P83

### Rejuvenation of mesenchymal stem cells derived from iPS cells originating from aged individuals

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**Introduction**
The in vitro expansion and application potential of human bone marrow derived mesenchymal stem cells (hBM-MSCs) obtained from aged donors are limited by their short life span in culture and restricted differentiation potential. Recent studies have reported the derivation of hMSCs (iMSCs) from induced pluripotent stem cells (iPSCs) as a possible solution. However, little is known whether the age-associated phenotype is reverted to a rejuvenated state when iPSCs derived from hBM-MSCs of aged donors are re-differentiated into iMSCs.

**Results**
To obtain new insights into the potential roles of age in deriving iMSCs from iPSCs we induced pluripotency in hBM-MSCs from fetal femur (55 days post conception) and aged donors (60-70 years) and subsequently differentiated them into iMSCs. Higher levels of ROS, phosphorylated γH2AX and slower proliferation rates were detected in hBM-MSCs from aged individuals. Normal Karyotypes were detected in BM-MSCs of both age groups. Fetal hBM-MSCs could be reprogrammed more efficiently and faster compared to hBM-MSCs from aged donors using either retroviral or episomal based reprogramming. hBM-MSCs and their corresponding iMSCs both fetal and aged expressed a typical MSC surface marker pattern and multipotency. iMSCs from aged parental hBM-MSCs acquired morphologies, senescent phenotypes and transcriptomes similar to that of fetal hBM-MSCs and iMSCs. Additionally, hBM-MSCs and their corresponding (iMSCs) both fetal and aged shared similar (PDGF-AA, MCP-1, MIF, Serpin E1) secretome profiles. Furthermore iMSCs derived from fetal MSC-iPSCs show a similar immunophenotype as well as trilineage differentiation potential as the parental cells. These similarities were also observed in iMSCs derived from dermal fibroblasts and the ES line H1. iMSCs acquired a rejuvenation signature but were not pluripotent.

In summary we have demonstrated that (a) the efficiency of inducing pluripotency in hBM-MSCs is dependent on donor age. (b) The transcriptomes of iPS cells derived from both fetal and aged BM-MSCs are more similar to that of hESCs than the parental cells. (c) iMSCs irrespective of donor age re-acquire features typical of BM-MSCs.

**Perspectives**
In conclusion, derivation of iMSCs by-passes the shortfalls associated with the expansion of native MSCs and these cells have tremendous potential in regenerative medicine. To investigate this, we transplant these cells into partial hepatectomized Gunn rats and in bone defect models of mini pigs to evaluate their regenerative potential. Furthermore the iMSCs will be implemented into protocols for deriving liver buds.

## P84

### Proteomic characterization of neurons within the substantia nigra in the context of ageing

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**Introduction**
Ageing, a progressive and irreversible process, ultimately leading to death, is assumed to be the main risk factor for neurodegenerative disorders. Within neurodegenerative disorders specific neuronal subpopulations are degenerating, for example dopaminergic neurons located in the substantia nigra during Parkinson's disease [1, 2]. Reasons for selective neuronal vulnerability as well as factors preventing neurodegeneration during ageing are still an open issue [3]. By proteomic analysis of different ageing states, we aim at gaining a better understanding of molecular changes occurring in the substantia nigra during the growing old. With that we hope to get a deeper understanding of healthy and diseased age-related changes.

**Results**
So far we established a laser microdissection based proteomic analysis of single neurons within the substantia nigra [4]. While methods in the past had the big disadvantage that it was not possible to analyze specific cell populations of a complex tissue, laser microdissection overcomes this problem and can reveal deeper insights into the proteome of single neurons.

**Perspectives**
We hope that the results of our ageing study could give a deeper understanding of the molecular processes underlying ageing and could be used as scale for comparison in other studies. Furthermore, it might give hints which mechanisms could have an impact in protecting specific cells from neurodegeneration.

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## P85

### Investigating selective pressures on mitochondrial DNA mutations in stem cell populations

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**Introduction**
Mitochondrial DNA (mtDNA) mutations can be inherited or may occur somatically during ageing. A number of ageing stem cell populations e.g. colon, stomach, and blood show an accumulation of acquired pathogenic somatic mtDNA mutations with age [1], with no evidence of selective pressures on these mutations [2]. In contrast, in patients with inherited mtDNA mutations resulting in mitochondrial disease, the mutation load decreases over time in blood [3], indicating selection against pathogenic mtDNA mutations in this tissue. However, it is unknown whether this selection is a common feature of all mitotic cells with inherited mtDNA mutations, and why this selection does not occur with somatic mtDNA mutations. Here we have investigated the levels of the inherited m.3243A>G mutation and mitochondrial OXPHOS function in various mitotic and post-mitotic cells. In addition, we have investigated whether such differing selective pressures are also detected in mouse models of acquired (PolgA+/mut) and inherited (tRNAAla C5024T) mtDNA mutations.

**Results**
Mitochondrial OXPHOS subunit protein levels were normal in the gut epithelial tissues of patients with the inherited m.3243 A>G mutation, whereas there was evidence of OXPHOS deficiency in the gut smooth muscle in these patients. This was associated with higher levels of heteroplasmy in the smooth muscle than the epithelium, suggesting selective loss of the m.3243A>G mutation from mitotic tissues over time. Similarly, in a mouse model with the inherited m.5024C>T mutation, there is progressive loss of the mutation from epithelial tissues over time, whereas the mutation load in the smooth muscle, heart, skeletal muscle and tail remains high throughout life. These data are in contrast to both normal ageing humans and the PolgA+/mut mouse, where mitotic tissues accumulate somatic mtDNA mutations over time with no such selective processes occurring.

**Perspectives**
We have confirmed that there is selective loss of inherited pathogenic mtDNA mutations in mitotic epithelial tissues over time in both human and mouse models of mtDNA disease, but not in normal ageing tissues. Future work will involve characterising the point at which these selective processes occur and investigation of the underlying molecular mechanisms. Elucidating such mechanisms may enable the removal of cells with mitochondrial dysfunction from ageing tissues to promote healthy ageing.

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## P86

### Nipped-A/TRRAP maintains midgut homeostasis during aging in Drosophila by regulating intestinal stem cell proliferation

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**Introduction**
Adult stem cells uphold a delicate balance between quiescent and active states, a deregulation of which leads to age-associated diseases such as cancer. In the Drosophila midgut, the rate of intestinal stem cell (ISC) proliferation is vital in maintaining tissue homeostasis and can have profound effects on lifespan [1]. In young flies, the ISCs divide to generate a self-renewed ISC and a post-mitotic progenitor called the enteroblast (EB). During aging, the homeostasis of the intestinal epithelium deteriorates as a result of excessive ISC proliferation and misdifferentiation of EBs [2]. A large effort has focused on defining signaling pathways that regulate ISC proliferation, however, less is known about transcriptional changes that occur within aging ISCs and their impact on ISC function.

**Results**
Here, using RNA sequencing, we screened the transcriptome of young and old ISCs to uncover differentially regulated genes that are potentially important in maintaining ISC integrity and function throughout aging. Nipped-A, the homolog of mammalian Transcription/Translation Associated Protein (TRRAP), an important subunit of histone acetyltransferase (HAT) complexes, was significantly upregulated in aged ISCs. Our analyses demonstrated that knocking down Nipped-A in the ISCs and EBs results in a dramatic reduction of ISC proliferation and a progressive loss of ISCs and EBs during aging. Knocking down Nipped-A also inhibits the proliferative ISC response after tissue damage and markedly reduces tumor growth after overexpression of oncogenic Ras. These findings led us to conclude that Nipped-A is an essential factor in regulating ISC proliferation, and thereby midgut homeostasis, under various conditions. Furthermore, our results indicate that Nipped-A functions, at least partly, by regulating dMyc expression in ISCs/EBs and by modulating the chromatin landscape of ISCs/EBs by regulating specific histone acetylation marks.

**Perspectives**
With Nipped-A we introduce a novel factor that plays a crucial role in maintaining intestinal tissue homeostasis shedding light onto the regulation of the proliferative activity of ISCs during aging. In mammals, TRRAP has been shown to hold important roles in regulating both normal and cancer stem cells. Our findings will spur research on investigating the interplay between TRRAP, c-Myc and histone modification during aging in vertebrates.

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## P87

### Computational analyses of gene expression and ChiP-seq data from progeroids, age-related diseased and healthy aged mammals

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Genes related to aging have been identified using data mining techniques in several model organisms[1]. However, specific gene sets allowing to separate from each other three distinct groups that are all associated with aging have not been described so far. These three groups are progeriods (due to genetic background), age-related diseases (pathological) and normal aged. As a progeroid group, we considered Hutschinson-Gilford progeria, Werner syndrome and Cockayne syndrome. In the age-related diseased group were individuals with cardiovascular disease, arthritis, dementia, cataract, Alzeheimer's and Parkinson's disease, while the healthy aged group were medically fit. Next generation sequencing techniques have become an important tool to make new discoveries in biological research thereby replacing array-based approaches.

We constructed queries for mining data types of interest from the NCBI databases (GEO[2] and SRA[3]). These datasets include data obtained with three different technologies — microarrays, RNA-seq and ChIP-seq. They comprise all three groups mentioned above for either human or the mouse model organism. We analysed all the different datasets, microarray, RNA-seq and Chip-seq data. For every type, each experiment was analysed separately and the statistically significant gene sets or annotated peaks were then combined and used for the downstream analyses such as gene set enrichment tests using hypergeometric testing (GOterms and pathway information, KEGG).

Initially, we identified differentially expressed genes (DEG) among the three groups, the transcription factors involved, and inferred network regulatory relationships (modules of co-regulated genes, transcriptional regulators and conditions that influence regulations). We then also identified interacting proteins using gene sets with similar expression profiles. Finally, we developed a method to identify genomic biomarkers related to chronic DNA damage pathways.

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## P88

### A C.elegans RNAi screen identifies novel genes with antagonistic pleiotropic effects

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**Introduction**
In the year 1957, George C. Williams formulated the antagonistic pleiotropy hypothesis of aging [1], which was later mathematically supported by William D. Hamilton [2]. This theory predicts that some genes mediate beneficial effects early in life when natural selection is strong, but are detrimental late in life when natural selection is weak. Natural selection favors these beneficial effects on fitness early in life at the expense of enriching harmful effects late in life; hence leading to the evolution of aging. We screened 800 chromatin regulators and transcription factors in order to identify novel genes that exhibit antagonistic pleiotropy in C.elegans. Genes were inactivated in the post-reproductive phase by the use of RNAi from day nine of adulthood onwards.

**Results**
The screen led to the identification of several genes that significantly extend lifespan upon late-life inactivation, while shortening lifespan when inactivated early in life. The forkhead box (FOX) A transcription factor pha-4 represents a top candidate of our screen. Late-life inactivation of pha-4 resulted in a strong lifespan extension of 33%. In contrast, inactivation of pha-4 from the first day of adulthood reduced C.elegans lifespan. This is of particular interest, as pha-4 has been shown to mediate diet-restriction and germline-less induced longevity [3, 4]. Our findings indicate that, depending on the age, pha-4 is either needed for normal lifespan and longevity or exhibits detrimental effects.

**Perspectives**
We are currently aiming to identify and to characterize the biological processes underlying the observed negative effects of pha-4 late in life. Generally, genes that behave according to the antagonistic pleiotropy hypothesis may represent promising drug targets to fight age-associated diseases. Our findings emphasize that timing needs to be addressed to fully understand how genes and gene networks impinge on the aging process.

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## P89

### Deciphering the molecular signature of natural selection in wild population of the short-lived African turquoise Killifish

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**Introduction**
Living in extreme habitats is often associated with high selection pressure. Adaptations to harsh environments are favored by positive selection and leave a distinct molecular signature in the genome. Novel beneficial mutations rapidly reach maximum frequency in populations (fixation) and are maintained at high frequencies via purifying selection, which removes detrimental mutations. These adaptive genomic events can be detected by applying statistical analyses developed in the field of population genetics to results derived from high-throughput sequencing in individuals from wild populations. We study the turquoise killifish (Nothobranchius furzeri), an annual teleost fish that inhabits ephemeral ponds in the African savanna and dwells in an extreme environment characterized by seasonal water availability and periodic desiccation of its habitat. The turquoise killifish life cycle is characterized by an arrested developmental state called diapause, which enables it to survive through the dry season, and by an explosive maturation, achieving sexual maturation in 3-4 weeks. In the brief rainy season, turquoise killifish hatch, reach sexual maturation and reproduce. I hypothesize that the extreme conditions in which the turquoise killifish lives constitute a strong selective pressure that left a detectable genomic signature. For this reason, I sequenced the genome of four distinct turquoise killifish wild populations and performed a genome-wide statistical analysis aimed at identifying regions associated to adaptive events. This study will provide important insights into the evolution of extreme life history traits such as short lifespan and embryonic diapause.

**Results**
By performing genome-wide statistical analysis in populations living in different habitats, we identified putative regions that underwent recent adaptations. Furthermore, we were able to estimate population size and inter-population genetic variation.

**Perspectives**
In future studies we will overlap the candidate genomic regions exhibiting signatures of positive selection with the regions already identified to be associated – in experimental crosses – with survival, color and sex determination [1, 2]. Furthermore, we will functionally test the identified regions using established genome-editing transgenesis techniques (CRISPR/cas9, TOL2).

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**P90**

## Converting C/EBPβ-translation into a reporter system to search for calorie restriction mimetics

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**Introduction**
Aging is the major risk factor for cancer formation and metabolic diseases and deregulation of mRNA translation plays a major role in their etiology. However, drugs that target aberrant translation are scarce. C/EBPβ is a transcriptional regulator of genes related to glucose and fat metabolism. We have shown previously that the N-terminally truncated C/EBPβ-LIP protein isoform is generated by translation re-initiation at a downstream AUG codon after initial translation of a cis-regulatory upstream open reading frame (uORF) in the 5'UTR of its mRNA. In a recent study (Zidek et al, 2015) We show that inhibition of mTORC1/4EBP/eIF4E signaling pathway by rapamycin strongly reduces translation re-initiation, resulting in reduced translation into the C/EBPβ-LIP isoform. Intriguingly, mice lacking the cis-regulator uORF in the C/EBPβ mRNA, which is required for mTORC1-stimulated translation into LIP, display improved metabolic phenotype with features also found under calorie restriction, including reduced tumor incidence, leanness and extended life span.

**Results**
We have now developed a C/EBPβ-uORF controlled dual-luciferase reporter system to screen for compounds that decrease the translation re-initiation index and therefore may have calorie restriction mimetic properties. The system was validated using pharmacological inhibition of mTORC1 (rapamycin and PP242), genetic mutations in the mTORC1 pathway (TSC1/TSC2), overexpression of WT and mutated translation initiation factors (eIF2α and eIF4E) and a specific regulator of translation re-initiation (DENR). Then in collaboration with ScreeningPort, we screened a library of 780 FDA approved drugs. Our preliminary data show that candidate compounds which decrease the translation re-initiation index also repress C/EBPβ-LIP expression.

**Perspectives**
With the translation initiation/re-initiation reporter we present a reliable system suitable for high throughput screening to search for novel compounds against cancer and other age-related diseases in which translationally controlled genes are causally implicated.

References
Zidek, L.M. et al. Deficiency in mTORC1-controlled C/EBPβ-mRNA translation improves metabolic health in mice. EMBO reports 16, 1022-1036 (2015)



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