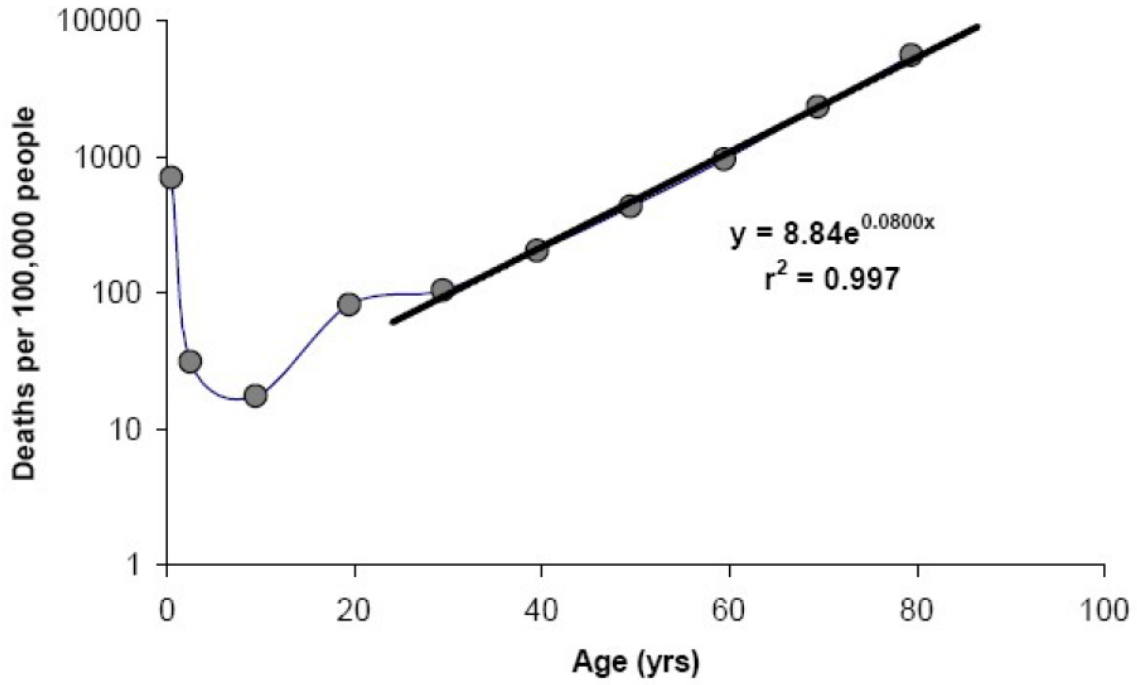
Approaching the underlying molecular mechanism of aging
-
Non-canonical model system of aging

Journal Club on Lab Animal Science

Regina Reimann

Aging / Senescence: A risk factor for death



www.senescence.info

1. Exponential increase in mortality with age
2. Physiological changes, typically leading to a functional decline with age
3. Increased susceptibility to certain diseases

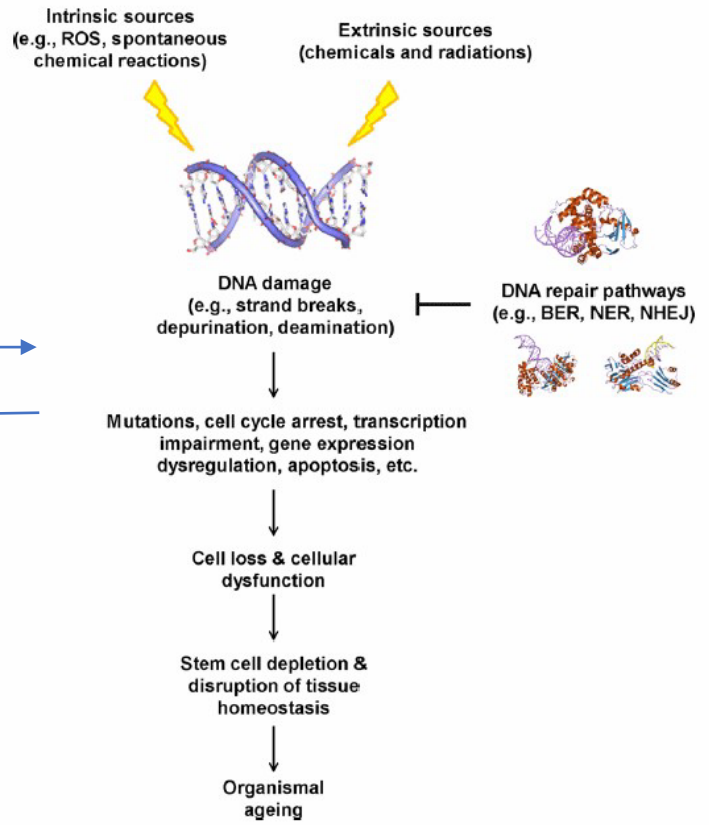
Why do we age? - Theories of aging.

1. Programmed Senescence

Birth	Reproduction	Senescence	Maximal lifespan
Humans	13.5 yrs	>40 yrs	
Monkeys	4.5 yrs	>20 yrs	
Mice	0.12 yrs	>1 yr	

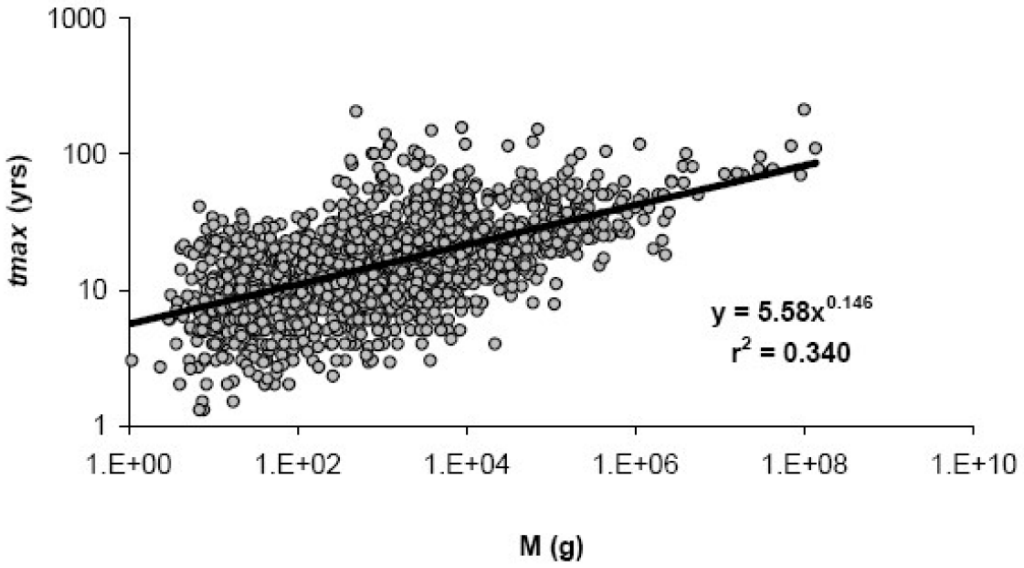
Aging occur on a fixed schedule triggered by the genetic program.

2. Damaged based theory of aging



Continuous process of damage accumulation originating in by-products metabolism.

Comparative biology of senescence

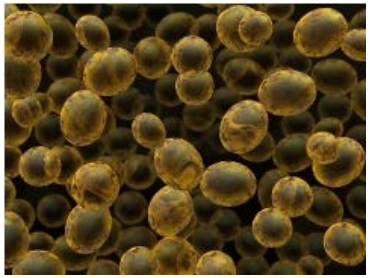


www.senescence.info

- Body size correlates with lifespan between species: larger animals live longer than smaller animals
- Brain mass correlates with tmax / in some case the size of other organs correlate
- Metabolic rate (questioned)
- Age at sexual maturation

Canonical models of senescence

Yeast



https://d3pddo38v7j30h.cloudfront.net/blog/wpcontent/uploads/2010/12/yeast-cells-web.jpg

C. elegans: 2-3 weeks

https://en.wikipedia.org/wiki/Caenorhabditis_elegans

Drosophila: 2 months

https://en.wikipedia.org/wiki/Drosophila_melanogaster

Mouse: 3 years

https://simple.wikipedia.org/wiki/Mouse

Limitation of canonical models:

- Yeast: no studies on multicellular and systemic aging.
- Nematode worms and flies: post-mitotic (no stem cell function and cancer) and lack an adaptive immune system.
- Mouse: commands considerable time and resources for lifespan studies.

Non-Canonical models of senescence

Short-lived: 4-5 month.

Turquoise killifish
Nothobranchius furzeri

- Shortest-lived vertebrate in captivity
- Wide set of aging phenotypes including cancer
- Sequenced genome
- Efficient transgenesis

Immortal

Planarian
Schmidtea mediterranea

- Potentially immortal lifespan
- Pluripotent adult stem cell
- Capable of whole body regeneration
- Can perform RNAi screens

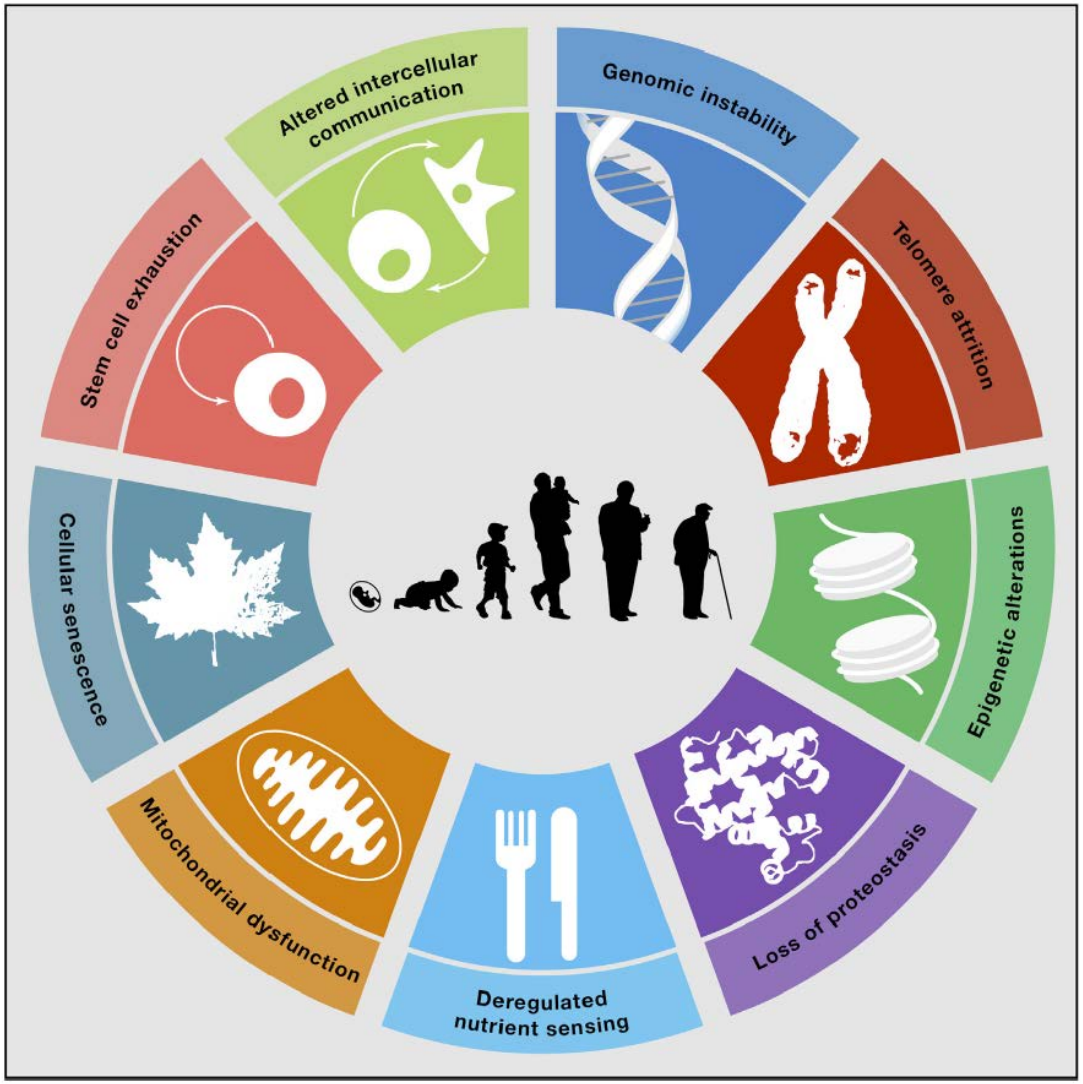
Long-lived: 31 years.

Naked mole-rat
Heterocephalus glaber

- Exceptionally long-lived
- Resistant to cancer
- Resistant to age-related diseases
- Breeds in captivity, sequenced genome

Valenzano et al, 2017

The hallmarks of aging



Lopez-Otin et al, 2013

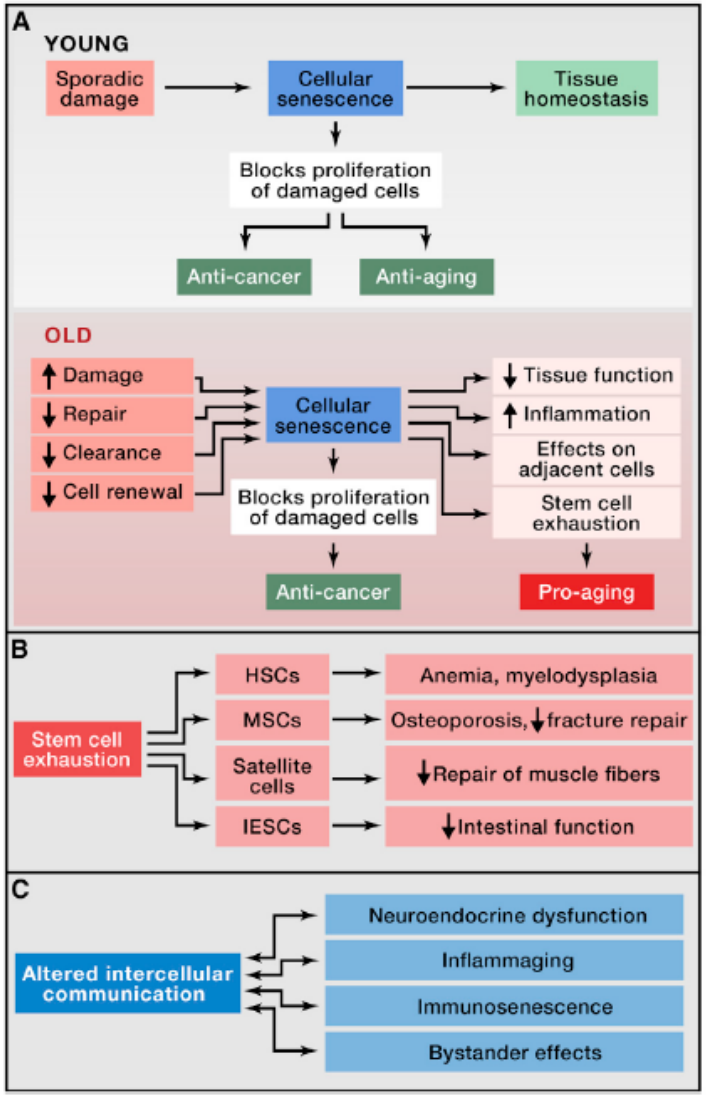
Cellular senescence

A stable arrest of the cell cycle coupled to stereotyped phenotypic changes

- Replicative senescence: telomer shortening
- Developmentally programmed senescence
- SIPS: stress-induced premature senescence
- OIS: Oncogen induced senescence

www.senescence.info

SIPS: stress-induced premature senescence



Lopez-Otin et al, 2013

Naked mole rat: secret of a long and healthy live

Valenzano et al, 2017

- Higher protein stability
- Less increase in cysteine oxidation
- Higher level of cytoprotetive NRF2 signaling

Cancer inhibition

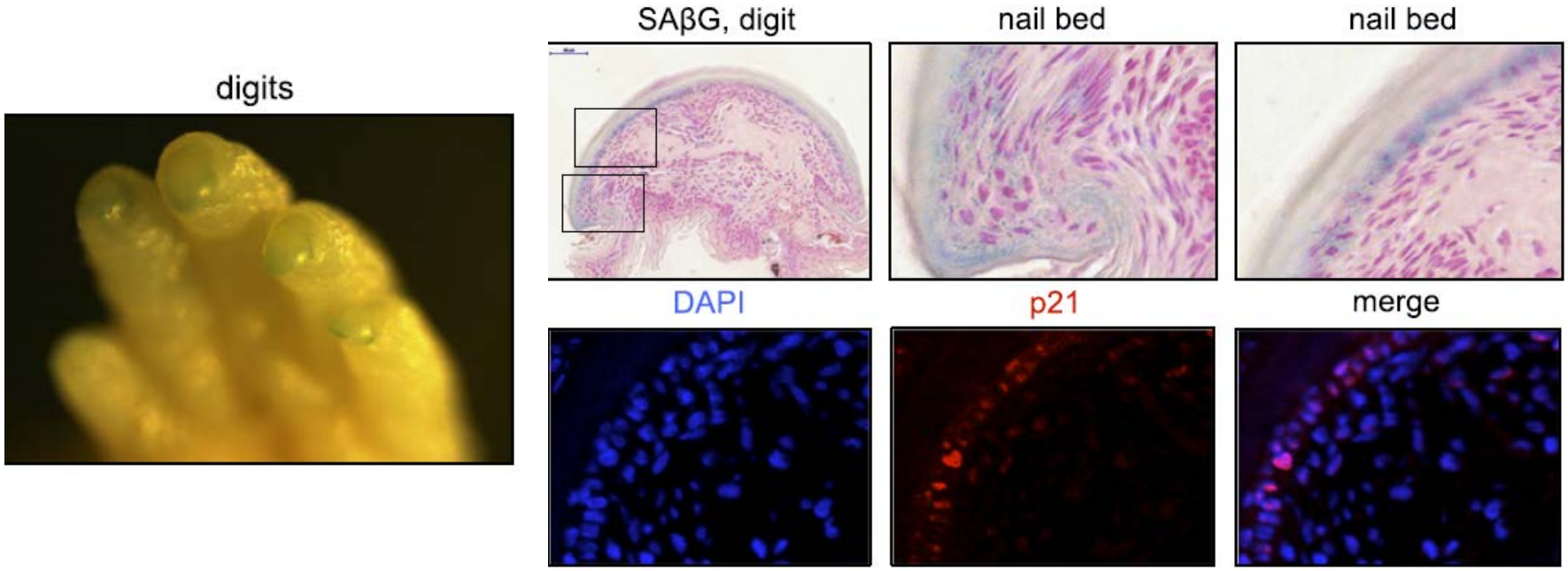
- Early contact inhibition
- p15/p16 hybrid
- High translation fidelity

Cellular senescence:

- No display of replicative senescence
- Expression of telomerase in somatic tissue

⟶ Other forms of cell senescence (SIPS, OIS, developmentally programmed senescence)?

SIPS: Developmental programmed senescence in naked mole rats (NMR)



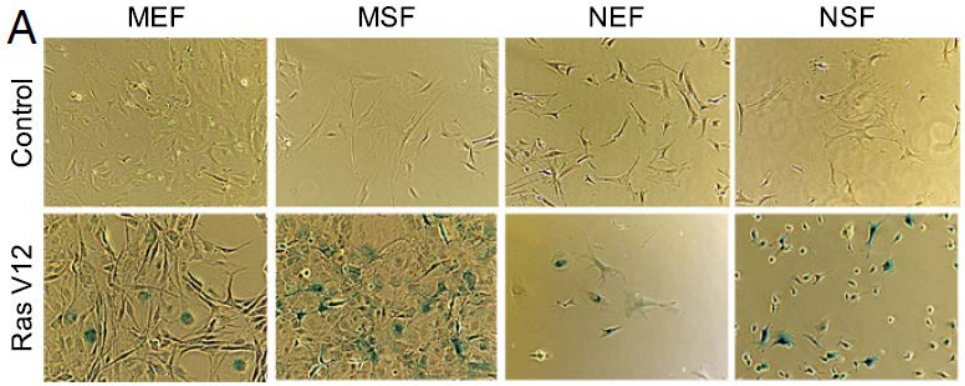
Senescence-associated β-galactosidase (SA-β-gal) staining.

β-galactosidase: Lysosomal hydrolase normally active at pH4, but it senescent cells it often happens o be activated at pH6.

P21 (CDK inhibitor): Major target of p53. Cell cycle arrest.

Y. Zhao et al., 2018

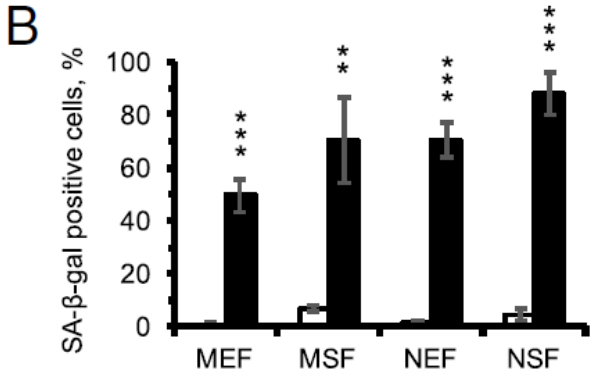
SIPS: Oncogene-Induced Senescence



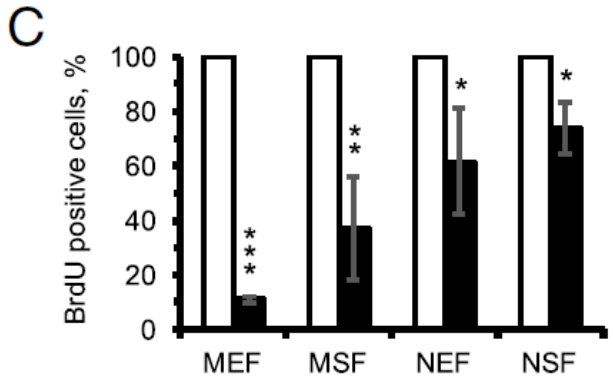
Transfection with:

- HRasV12: oncoprotein
- Control: GFP

MEF: mouse embryonic fibroblast
MSF: mouse skin fibroblast
NEF: NMR embryonic fibroblast
NSF: NMR skin fibroblast



Oncogene-induced senescence, quantified by SA-β-gal.

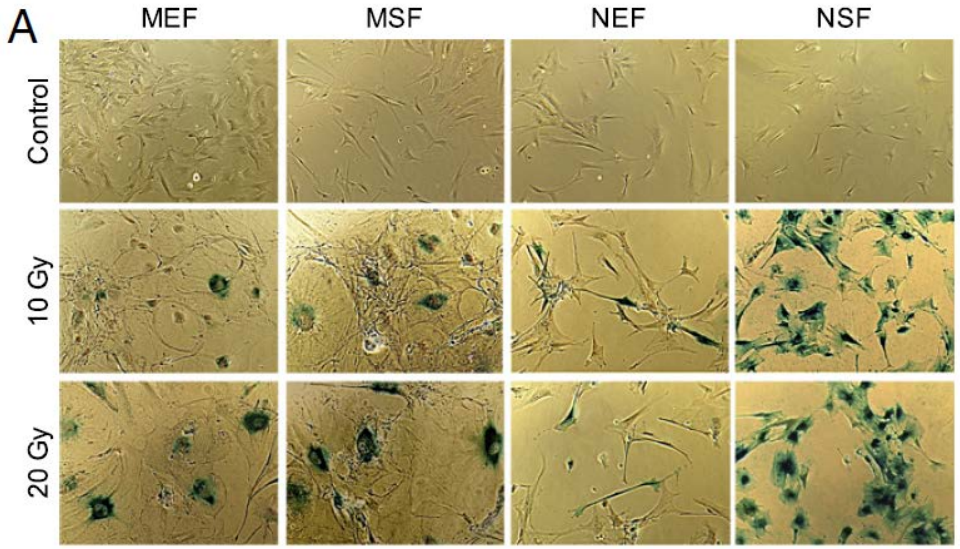


White: control
Black: HRasV12

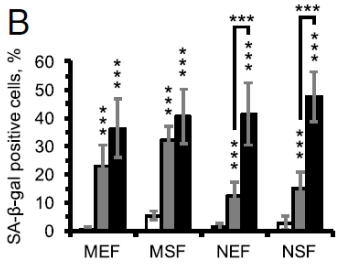
Reduction in DNA synthesis, quantified by BrdU staining.

Y. Zhao et al., 2018

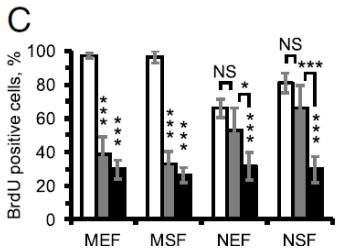
Attenuated senescence in response to γ-irradiation



MEF: mouse embryonic fibroblast
MSF: mouse skin fibroblast
NEF: NMR embryonic fibroblast
NSF: NMR skin fibroblast



γ-irradiation induced senescence, quantified by SA-β-gal.

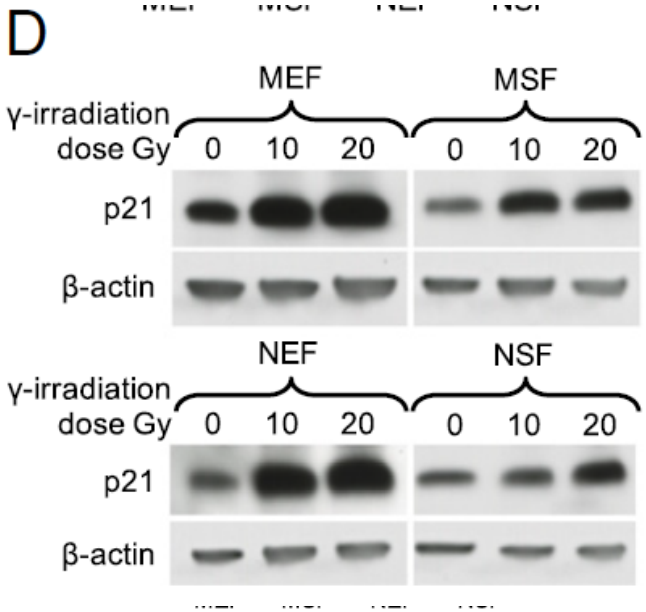


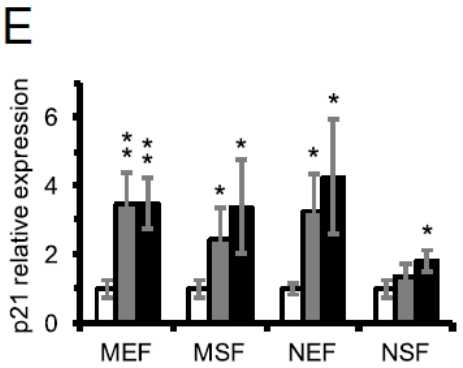
Reduction in DNA synthesis, quantified by BrdU staining.

White: control
Grey: 10 Gy
Black: 20 Gy

Y. Zhao et al., 2018

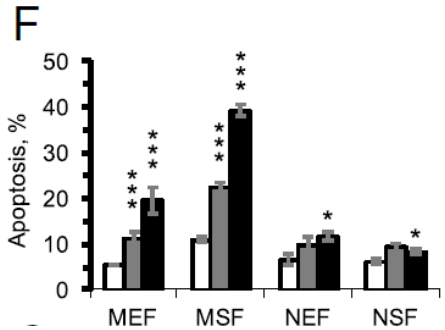
Attenuated senescence and apoptosis in response to γ-irradiation - II



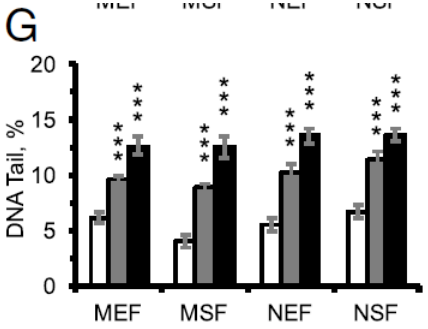


White: control
Grey: 10 Gy
Black: 20 Gy

P21: Cell cycle arrest in response to γ-irradiation.



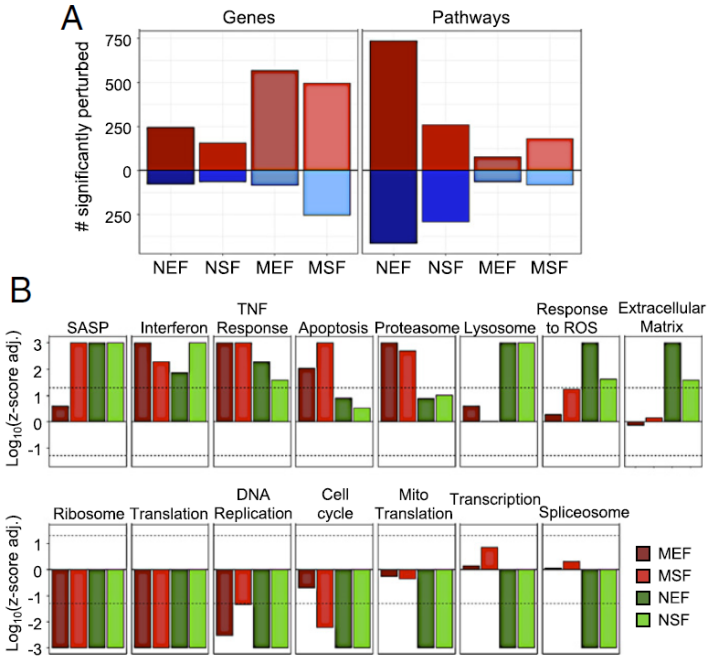
Annexin V FACS apoptosis assay



DNA damage quantified with Comet assay

Y. Zhao et al., 2018

Gene expression change in the NMR upon γ-irradiation are less drastic, but more systematic.



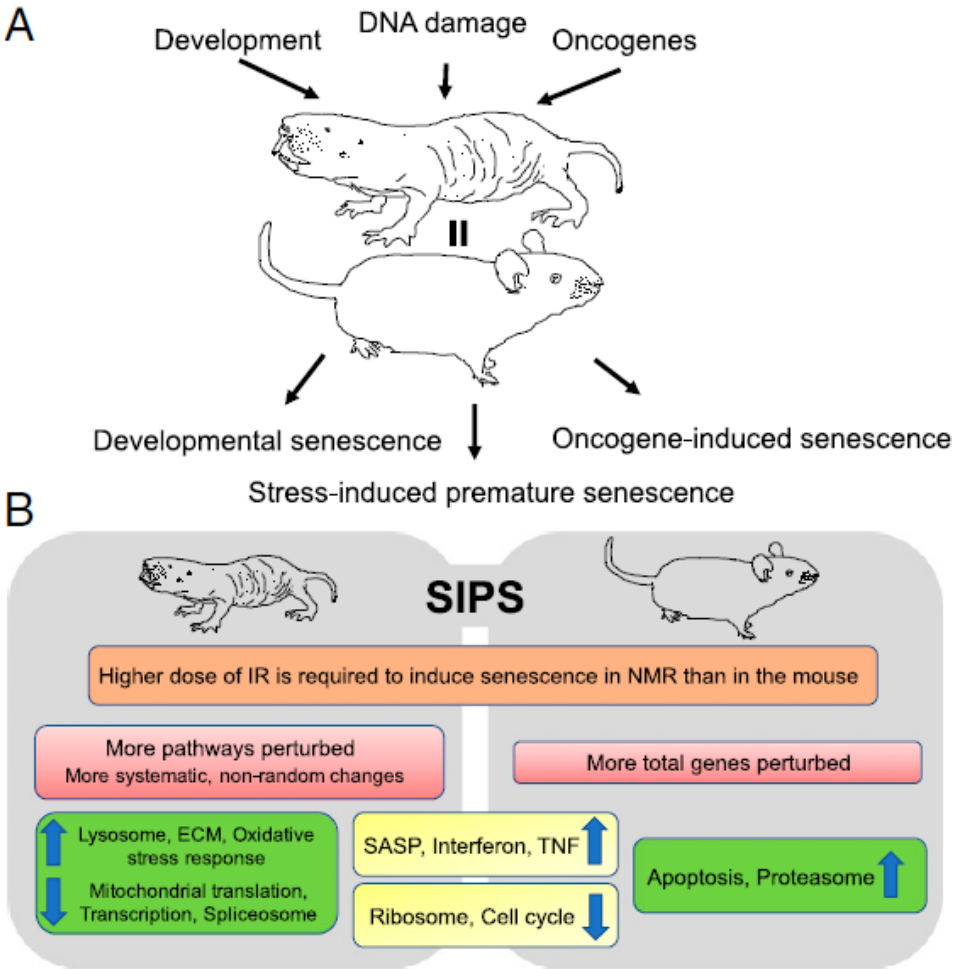
- Two times more differentially expressed genes mouse vs NMR fibroblasts
- More pathways enriched in NMR vs mouse fibroblasts
- Shared pathway alterations: immune response, cell cycle, DNA replication, translation, ribosome protein genes
- NMR unique down-regulation: transcription, spliceosome, mitochondrial translation
- NMR unique up-regulation: protein and glycoprotein metabolism, lipid metabolism, lysosomes, extracellular matrix and oxidative stress response.

Functional enrichement of changes

- 20 Gy of γ-irradiation versus untreated
- RNA collection 12 d later (all irradiated cells displayed positive SA-β-gal staining
- RNAseq, three biological replicates
- Uniform annotation of genes between species, 10959 gene coverage

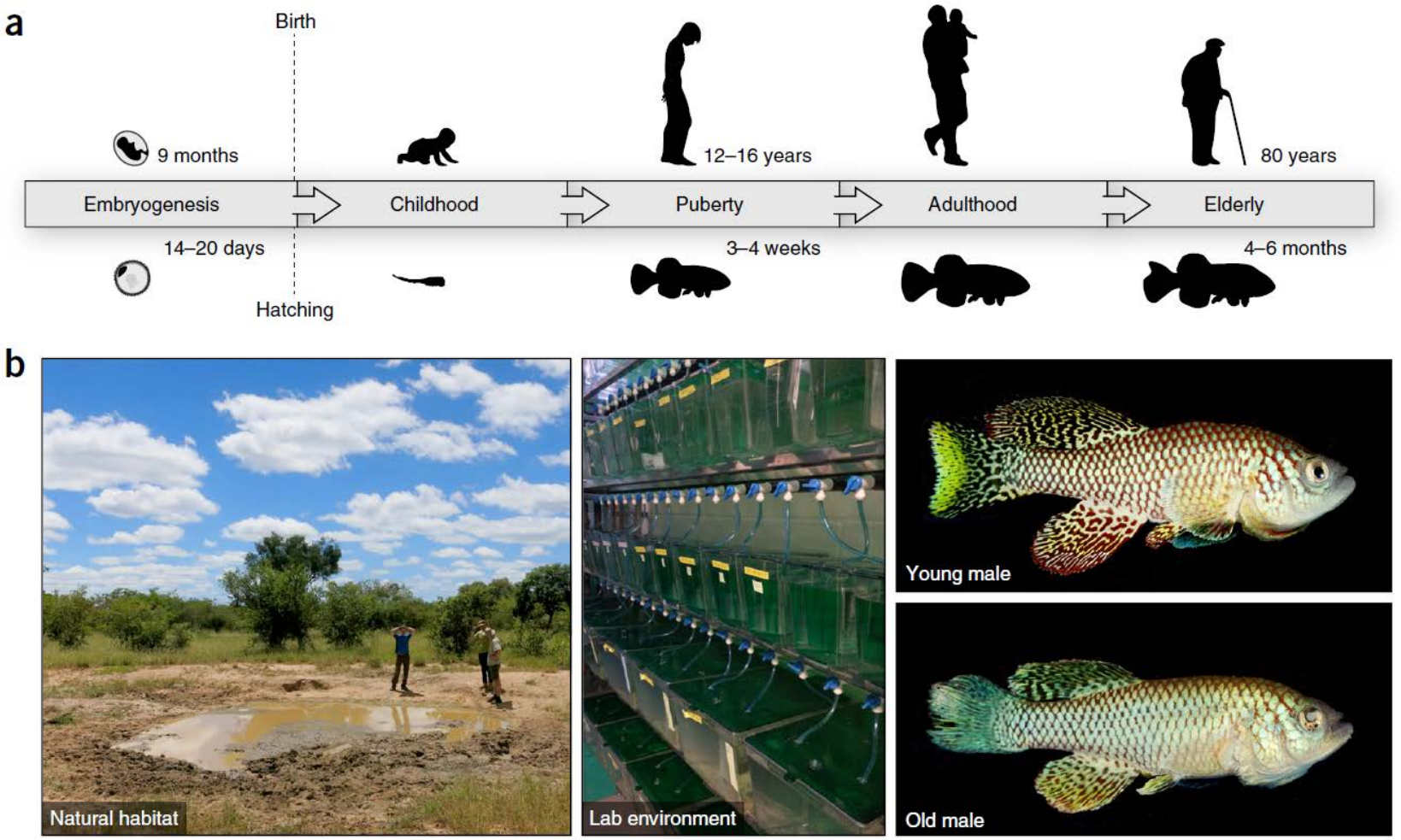
Y. Zhao et al., 2018

Conclusion from comparative analysis of senescence between mice and NMR



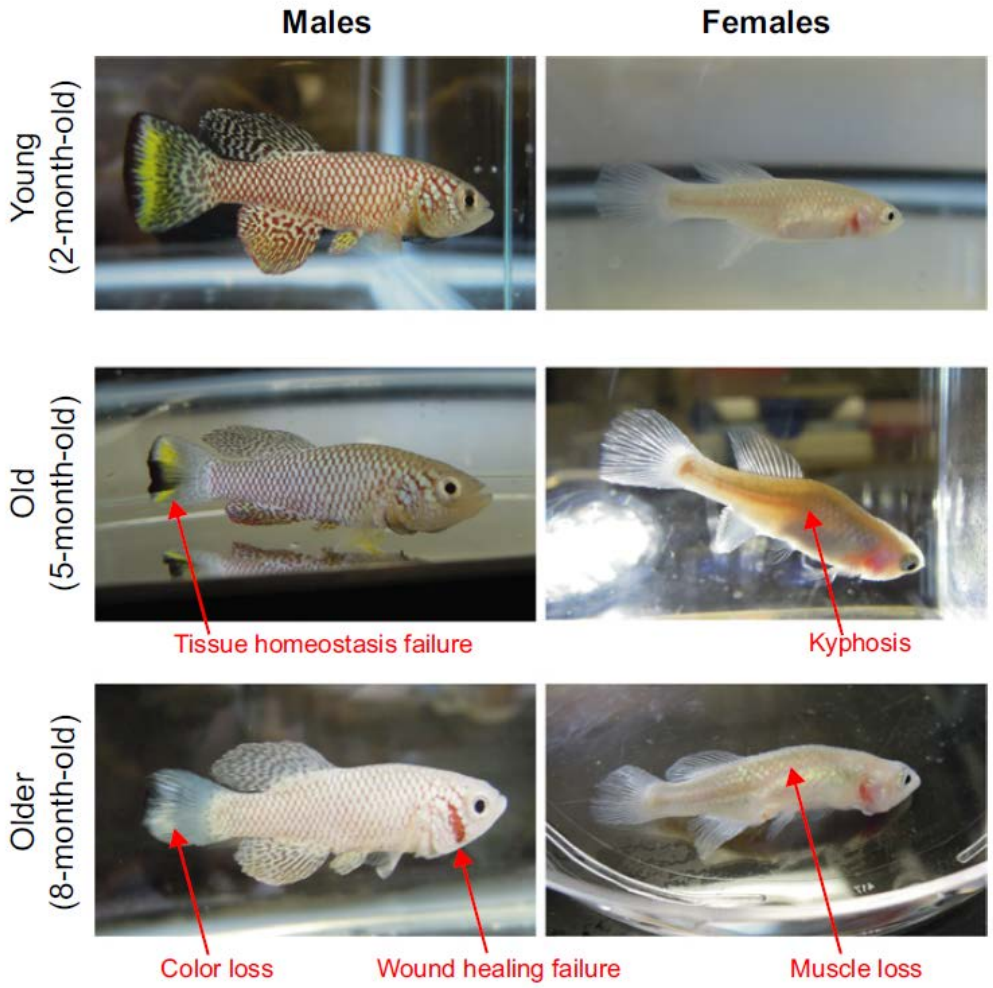
Y. Zhao et al., 2018

Turquioise killifish: live fast, die young



Valenzano et al, 2017

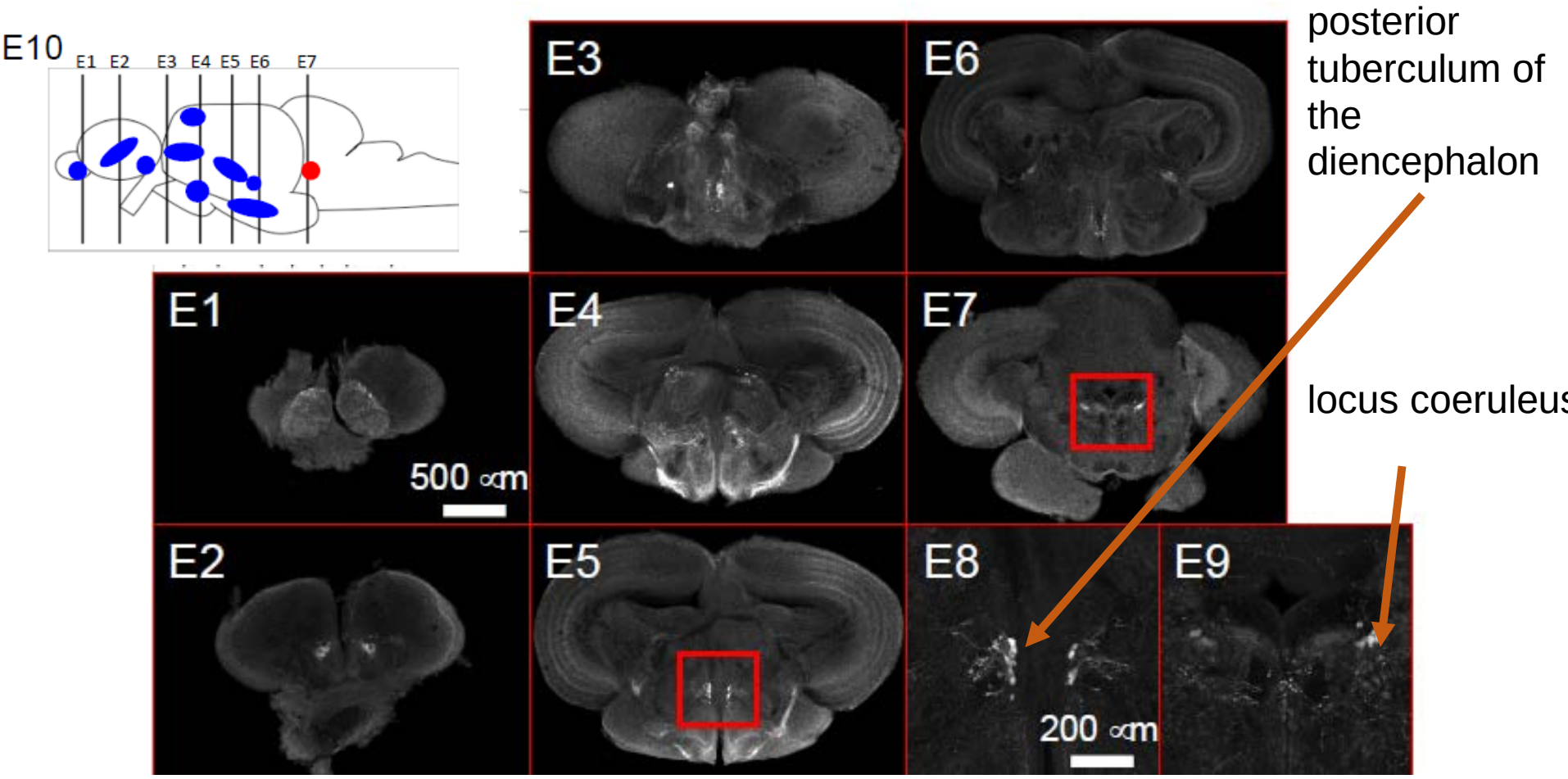
Aging phenotypes



- Decline in reproduction
- Decline in fertility
- Decline in cognition
- Decline in mobility
- Decline in regeneration and tissue homeostasis
- Neural and muscular degeneration
- Cancerous lesions
- Multiple cause of death

Hu et al, 2018

Localisation of TH+ positive neurons and fibres



Coronal sections at different rostro–caudal levels show the localization of TH+ fibers and neurons in N. furzeri (3 month-old). / TH+: Tyrosine hydroxylase (to detect dopaminergic and noradrenergic neurons)

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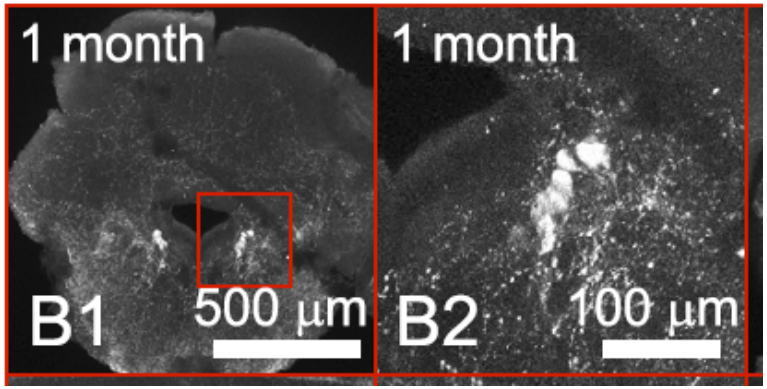
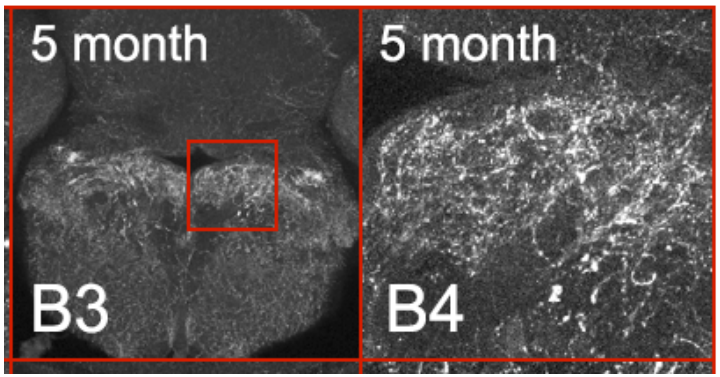
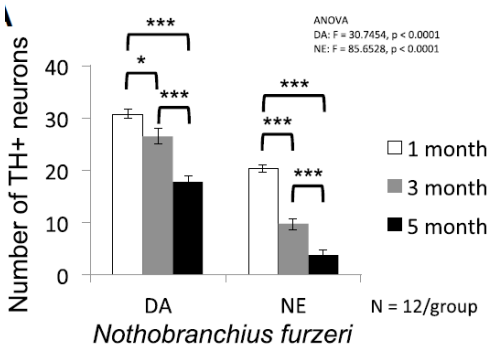
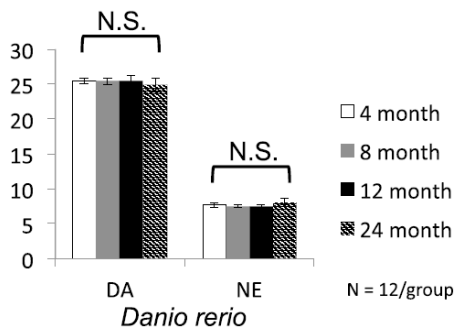
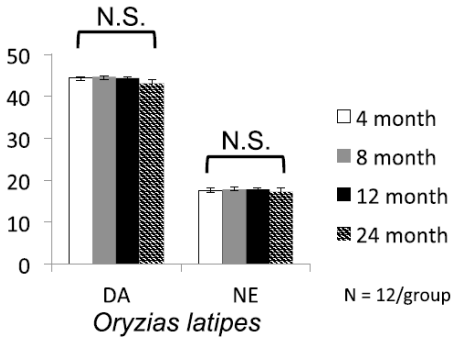
Degeneration of TH+ positive neurons

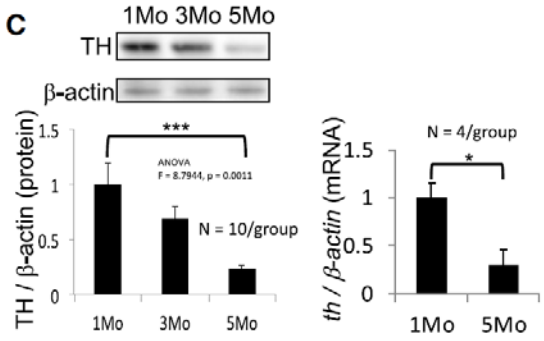


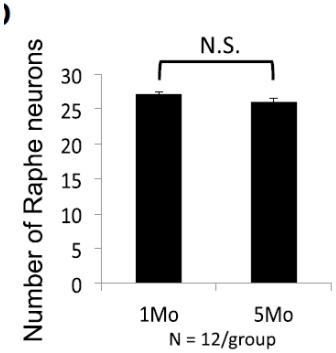












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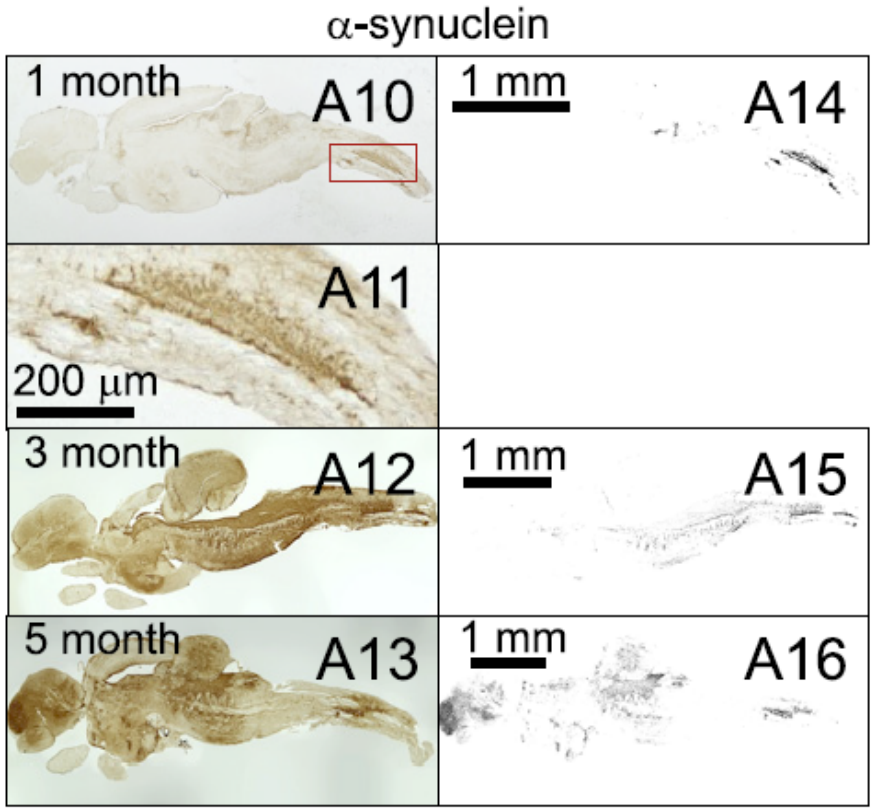
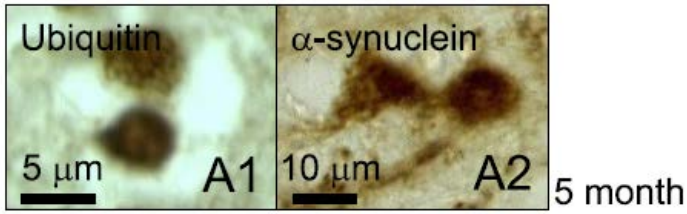
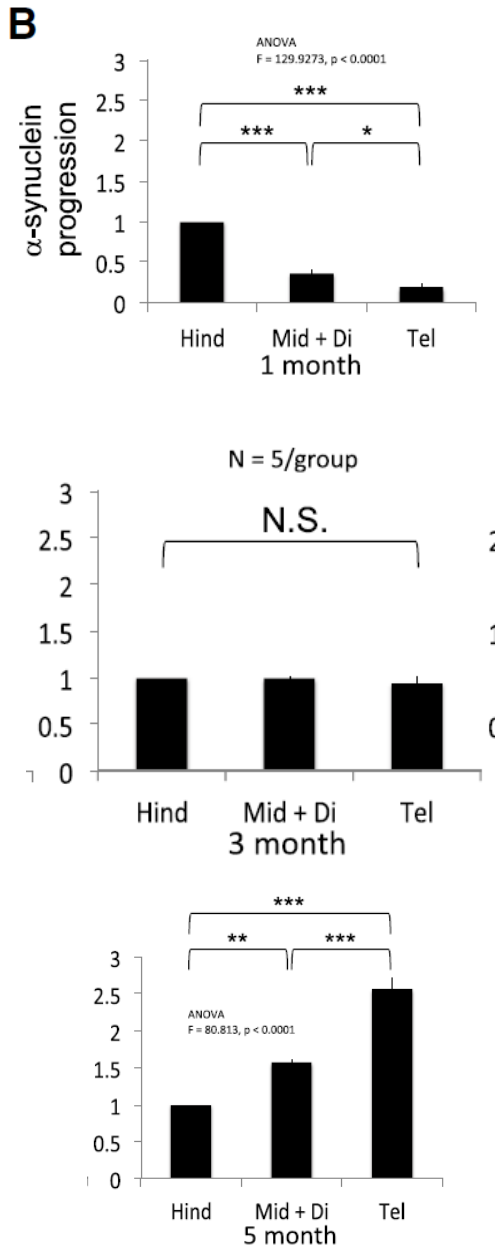
Progression of α-Synuclein pathology







N. fuzeri α-synuclein antibody: peptide / rabbit

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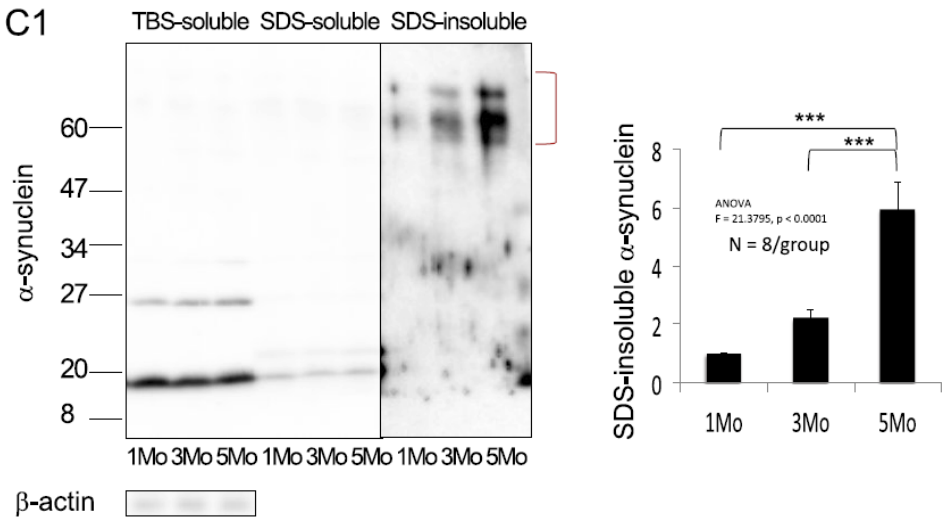
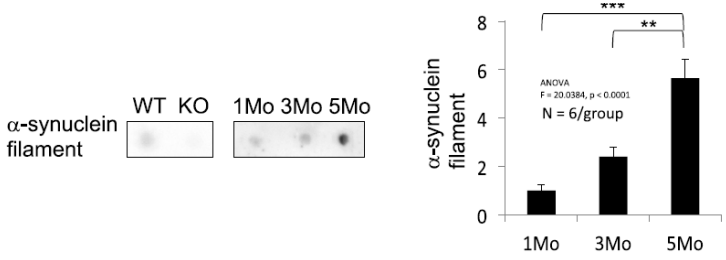
Progression of α-Synuclein pathology



Immunoblot anaylsis of SDS-insoluble fraction.



Dot-blot analysis of α-Synuclein fibrils in the brain.

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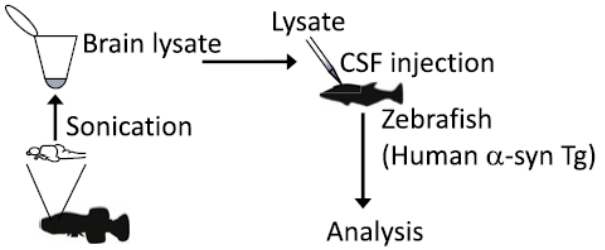
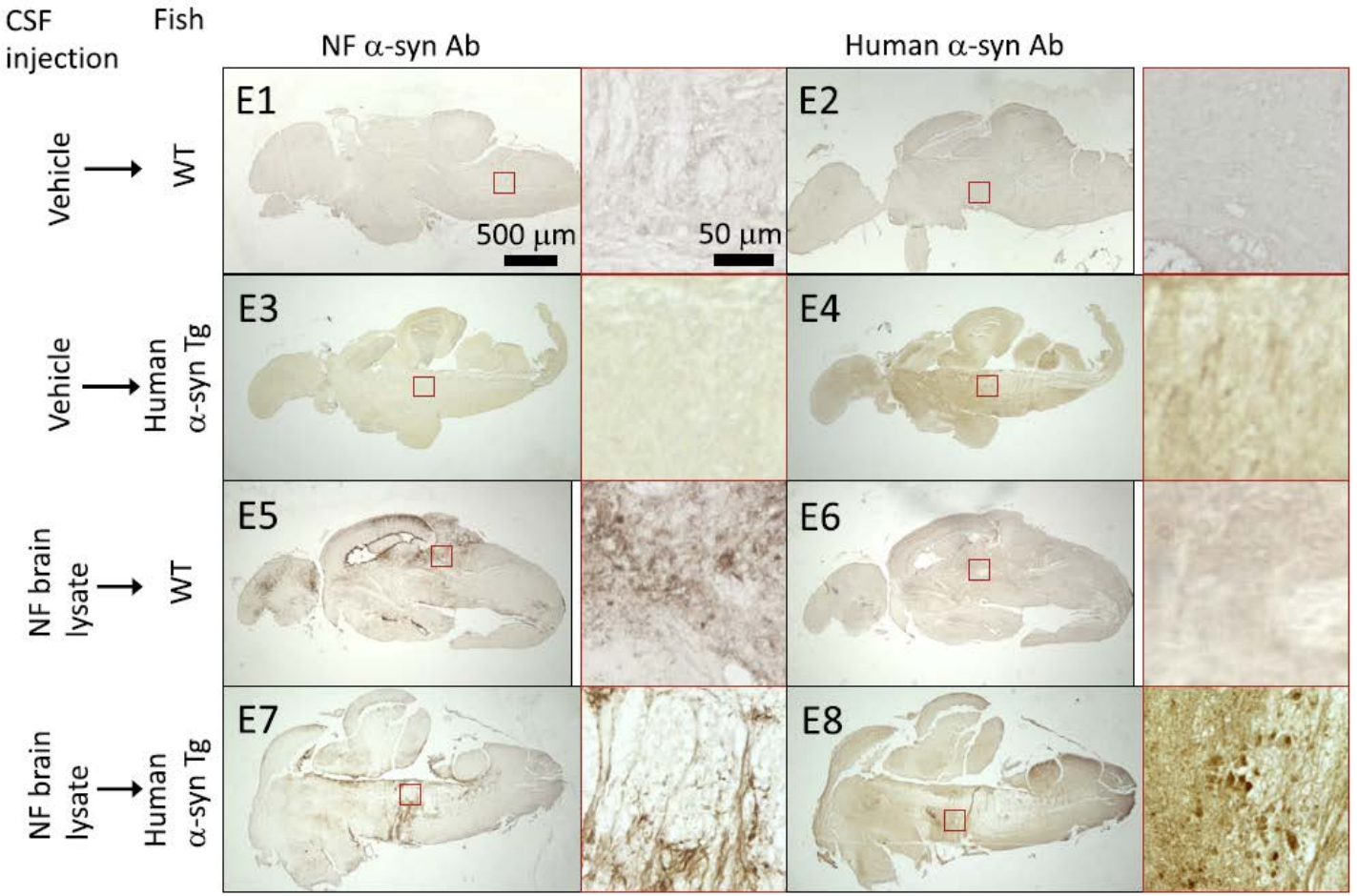
Passage of α-Synuclein seeds from Turquoise killifish to Zebrafish





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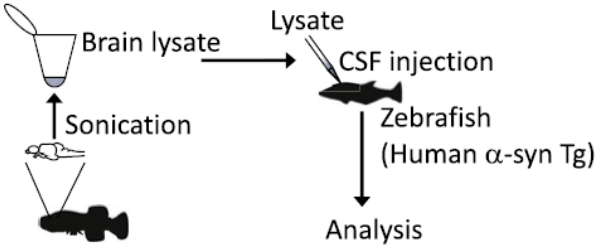

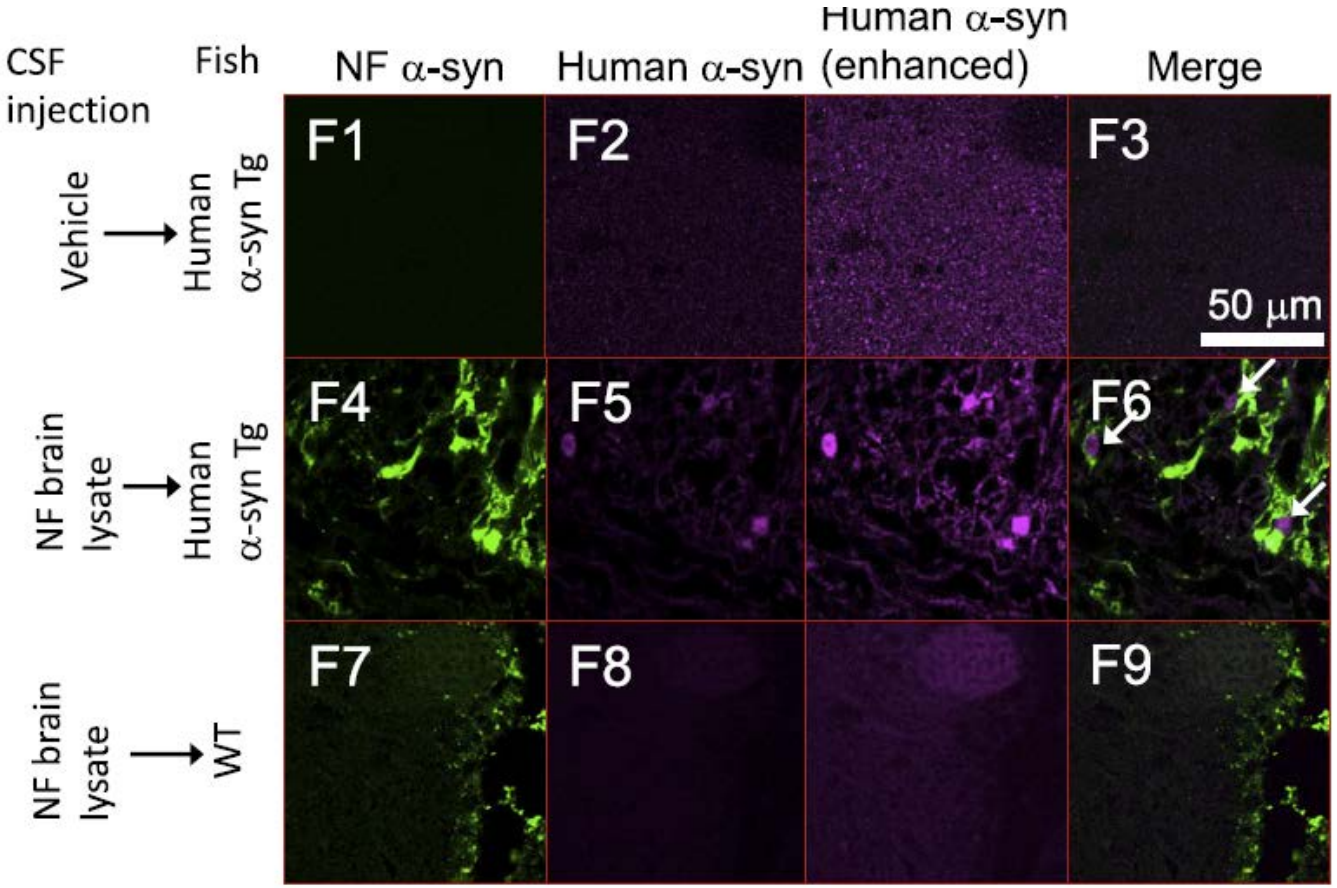

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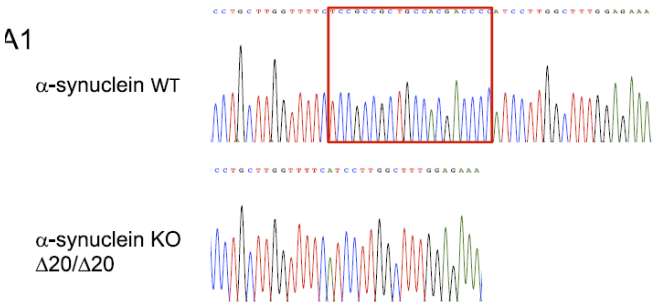
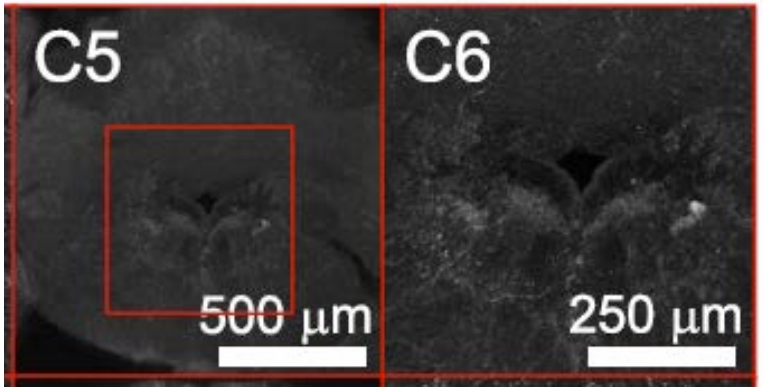
Rescue experiment-I



WT CCTGCTTGGTTTTCTCCGCCGCTGCCACGACCCCATCCTTGGCTTTGGAGAAA
Δ20 CCTGCTTGGTTTTC [TCCGCCGCTGCCACGACCCC] ATCCTTGGCTTTGGAGAAA

Generation of 20 bp deletion in the exone of α-Synuclein (CRISPR-Cas9).
Western blot / immunohistochemistry : no expression

wt



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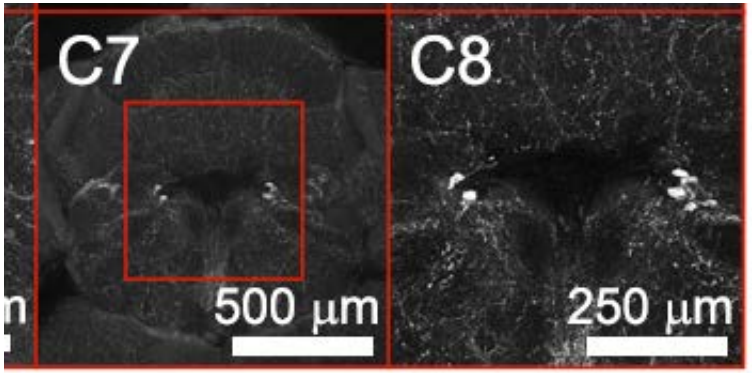

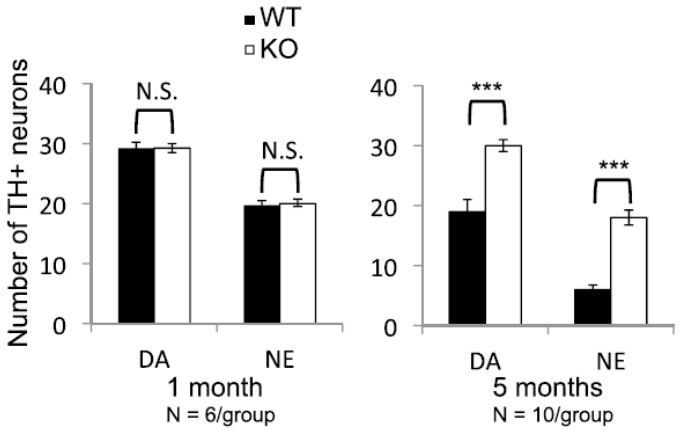

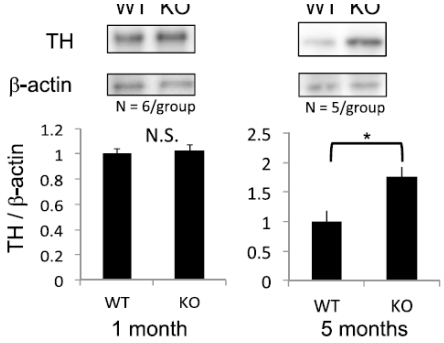

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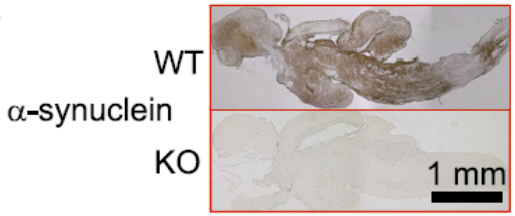
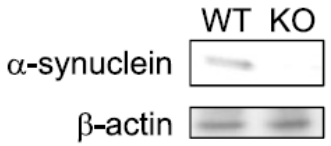
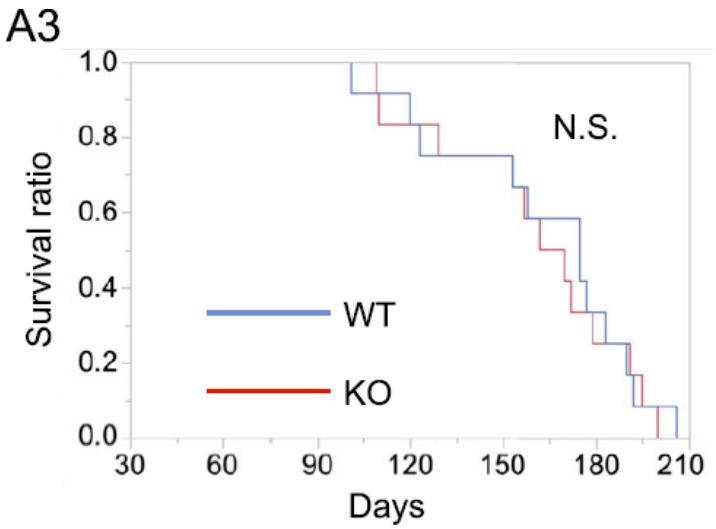
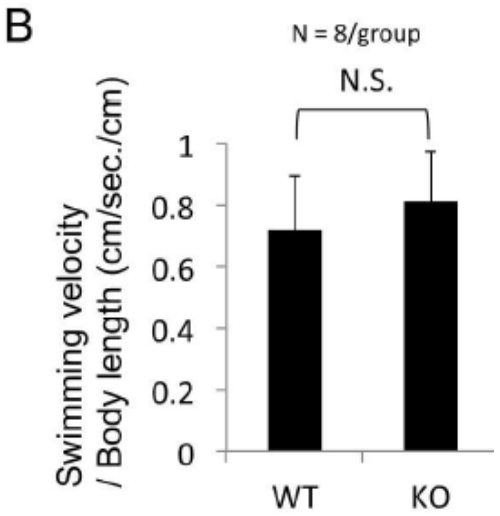


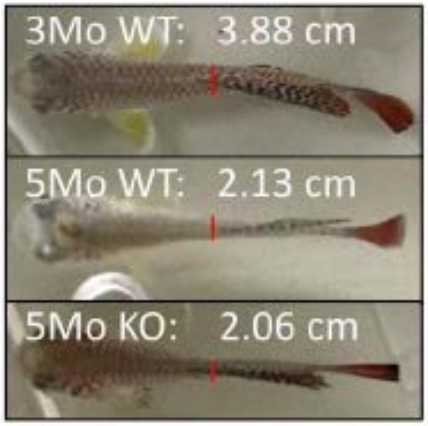


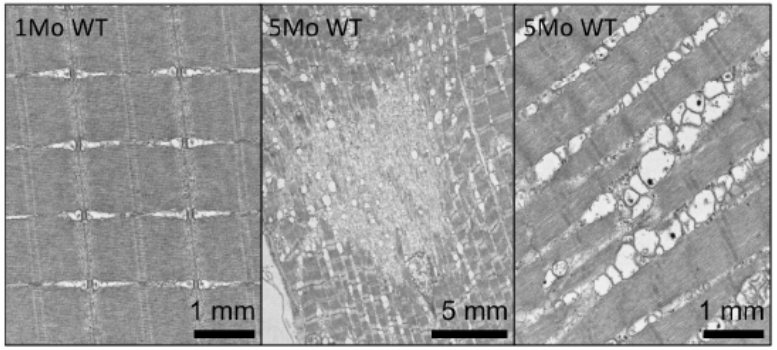
Hideaki Matsui., 2019

Rescue experiment-II



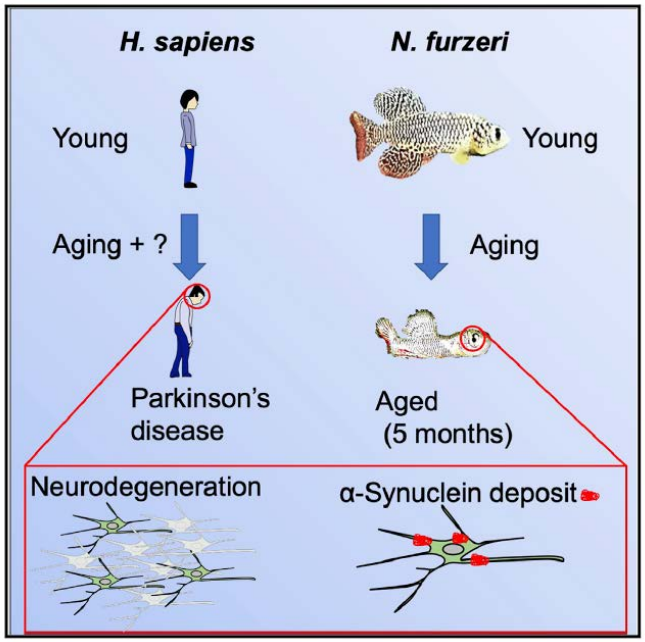






Hideaki Matsui., 2019

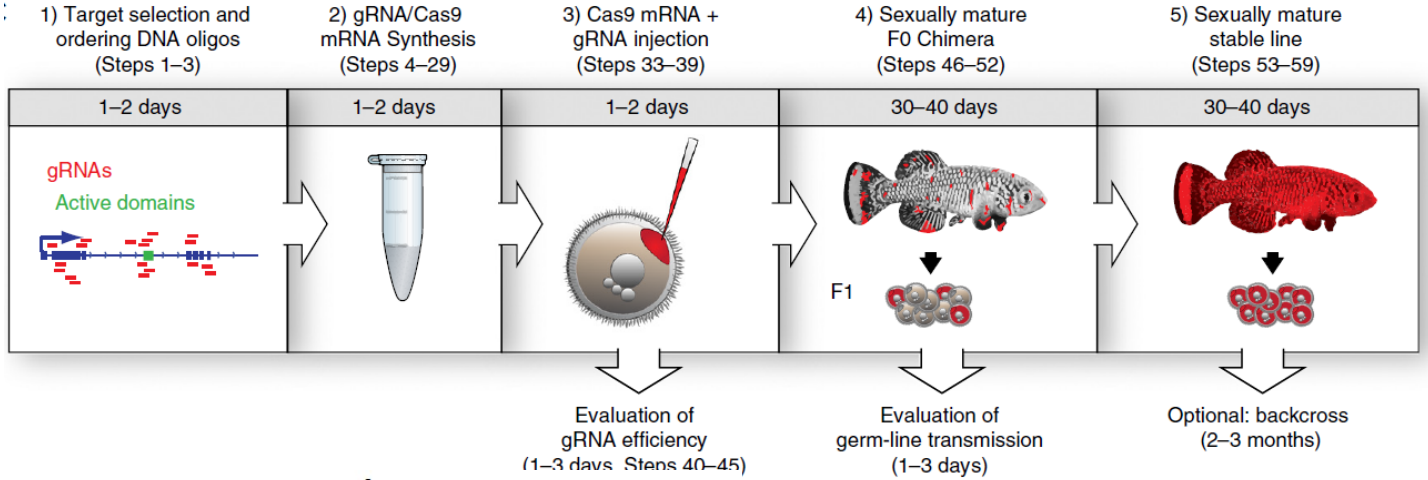
Summary: Dopaminergic degeneration in the Turquoise killifish

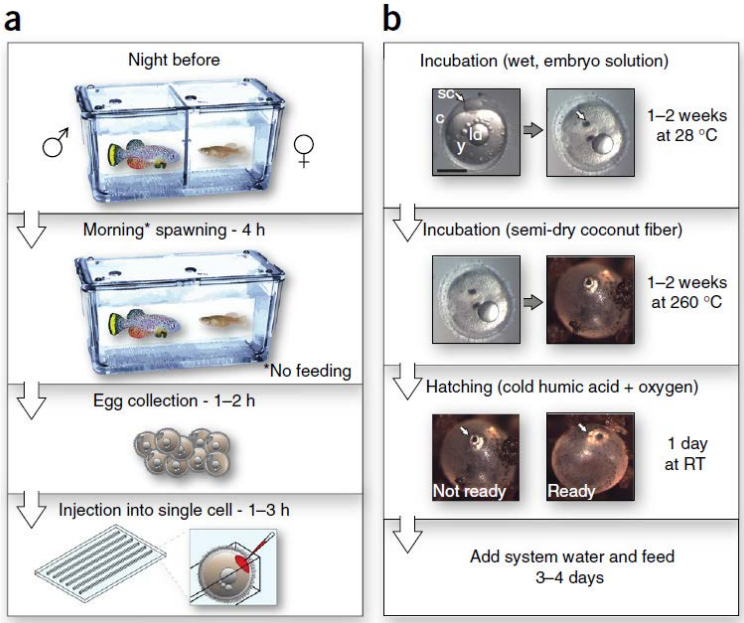


- Annual killifish reveals age-dependent degeneration of dopamine neurons
- Aged killifish shows accumulation of α-Synuclein in the brain
- Dopamine neurodegeneration is ameliorated by genetic depletion of α-Synuclein

Hideaki Matsui., 2019

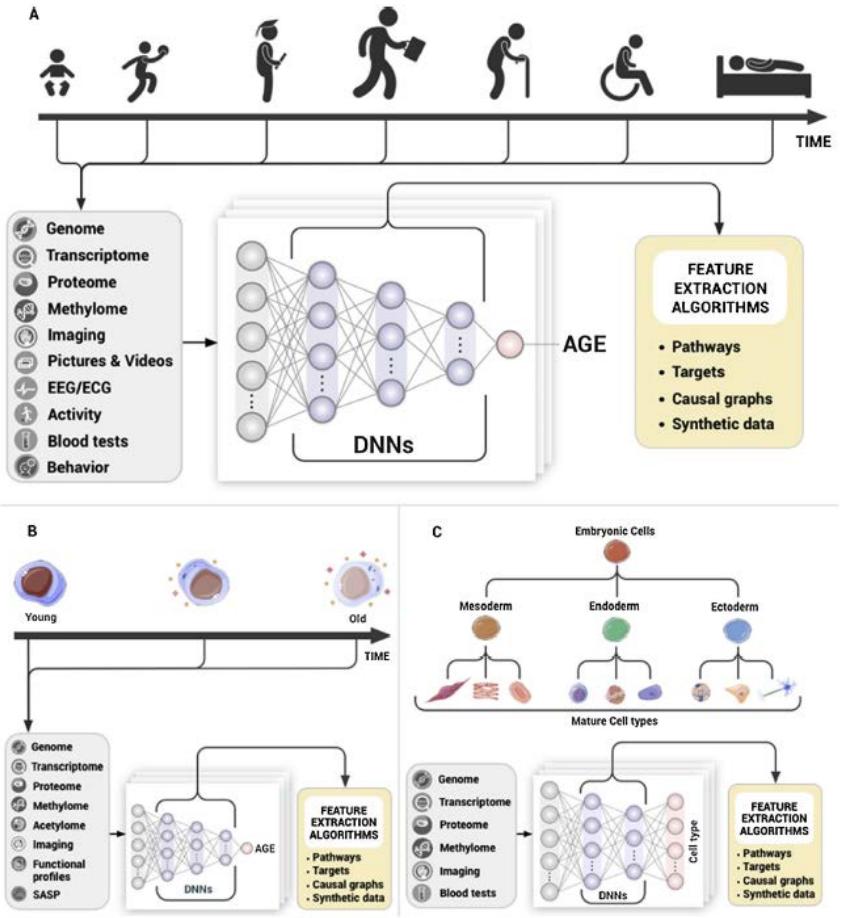
Efficient genome engineering approaches for the Turquoise kilifish





Itamar Harel et al., 2016

Artificial intelligence for aging and longevity



Application of artificial intelligence of aging for biomarker development and target identification (at different levels).

Zhavoronkov et al. 2019

Thank you for your attention!

MONITORING SHEET FOR PRION INOCULATED MICE

CHECKED BY:

DATE:

WPI:

					body weight (g)		Neurological Scoring			comment
cage #	mouse #	mouse line	inocul. date	researcher	reference	weight	A	B	Total (T)	
cage #	mouse #	mouse line	inocul. date	researcher	reference	weight	A	B	Total (T)	
cage #	mouse #	mouse line	inocul. date	researcher	reference	weight	A	B	Total (T)	
cage #	mouse #	mouse line	inocul. date	researcher	reference	weight	A	B	Total (T)	
cage #	mouse #	mouse line	inocul. date	researcher	reference	weight	A	B	Total (T)	