Who Wants To Live Forever?

Killifish as a short lived vertebrate model for aging and disease.

2nd TJC – Johanna Schaffenrath

An aging population

Investigating aging and age related diseases

Supercentenarians

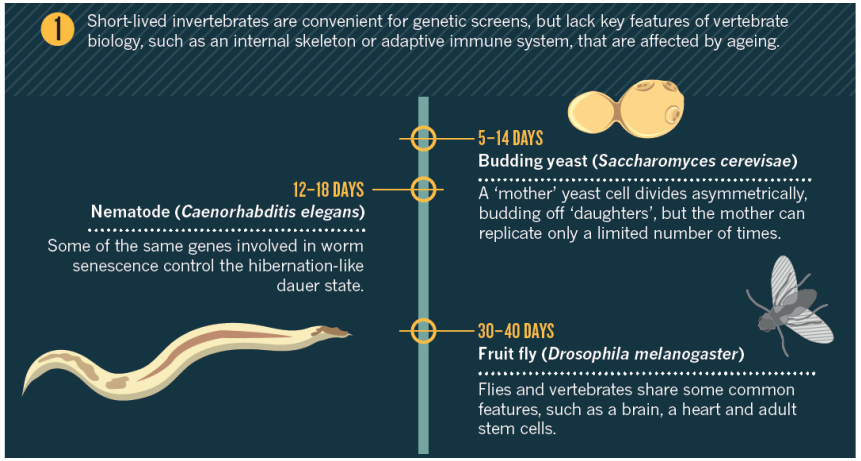
Why aging?

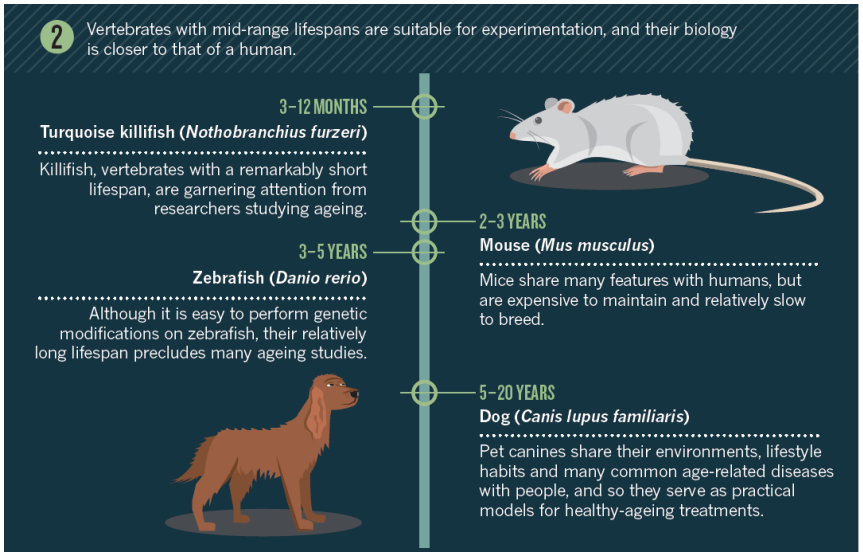
- Mutation Accumulation
- Antagonistic Pleiotrophy
- Programmed Death
- Molecular Clock

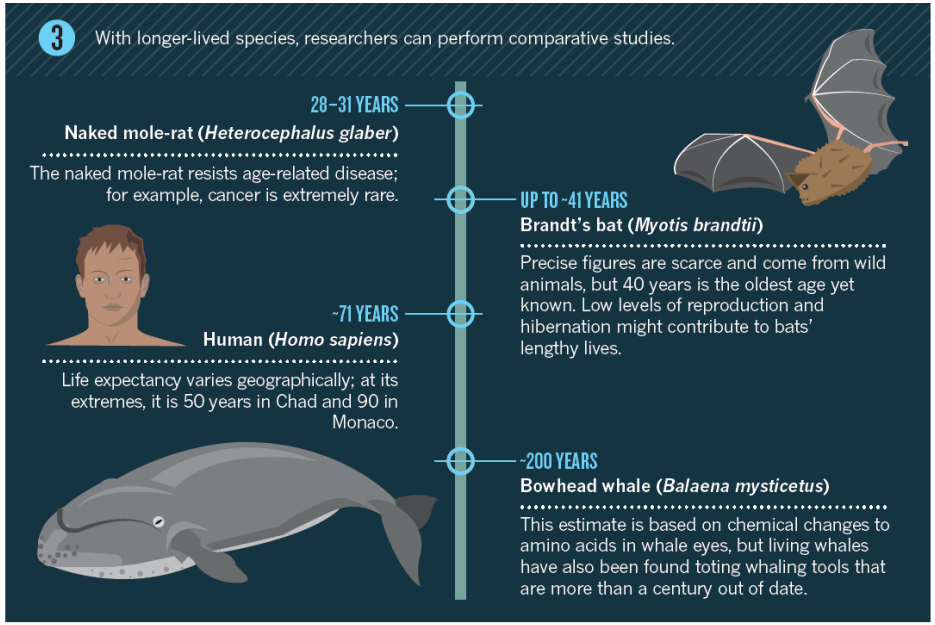
Investigating age related changes

- Neurodegeneration
- Cancer
- Loss of abilities
- Cardiovascular Diseases

Aging models



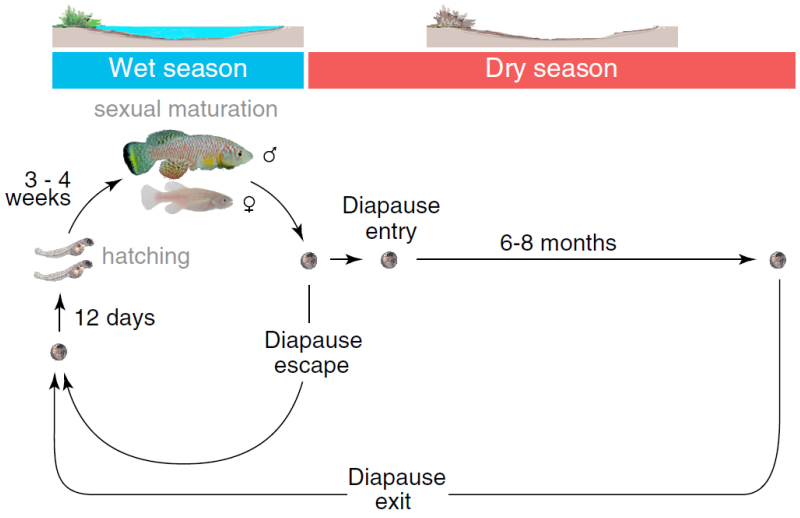




Nature vol. 535, 2016

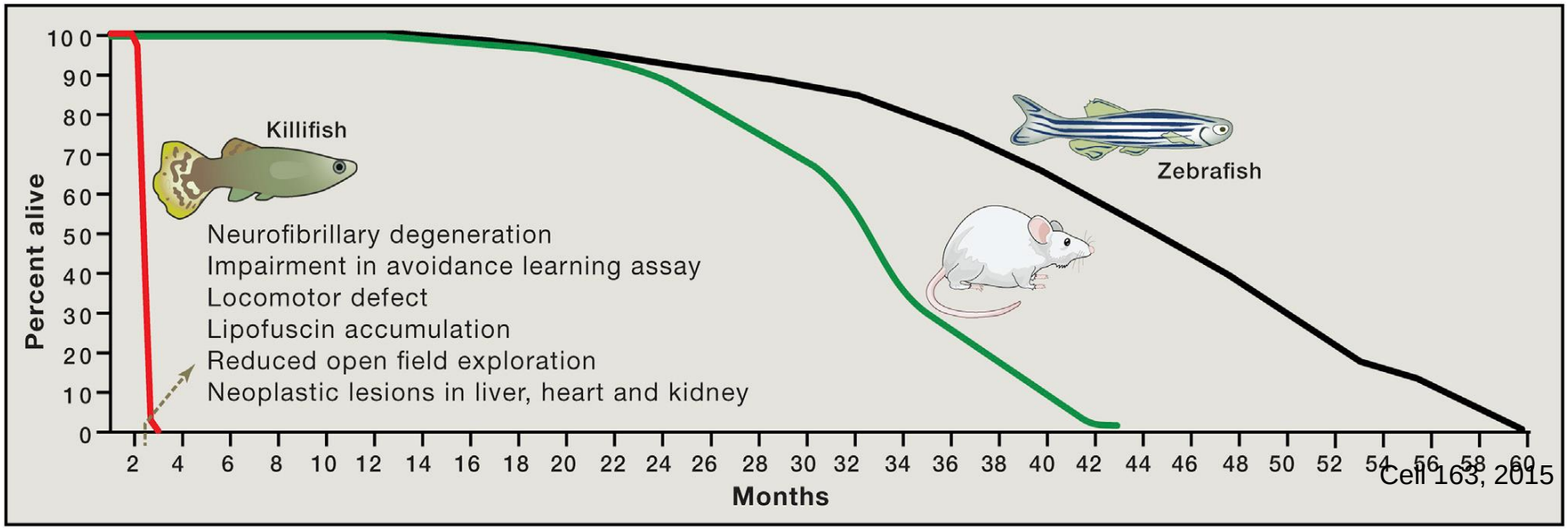
The Killifish as a model for aging

- End of 20th century → first Killifish studies
- Development in transient mud pools during wet season - dormant state in dry season - hatching in wet season
- Many different killifish with different lifespans
- Reference genome & CRISPR/Cas9 protocol (2015)



Valenzano et al., 2015, Cell *163*

Do Killifish age in a similar way to humans?



Cell 163, 2015

GFAP upregulation	Increased apoptosis	Mitochondrial impairments
Lipofuscin accumulation	Spinal curvature	Decreased fin regeneration
Degeneration of neurons	Decreased fecundity	Age-dependent gene expression
Amyloid aggregation	Telomer shortening	
Decreased learning	Neoplastic lesions	

Baumgart et al., 2015

Advantages & Disadvantages

Pros	Cons
Short lifespan	Many parameters influencing lifespan
Vertebrates	Requires good surveillance
High reproductivity	Special facilities required
Fish with range of lifespans	Common techniques not always possible
Similarities with human aging	
Orthologues for genes involved in human dysfunctions	
Similar telomere length	

Disease Models & Mechanisms 2016, 9

Resource

Cell

A Platform for Rapid Exploration of Aging and Diseases in a Naturally Short-Lived Vertebrate

Itamar Harel,[1] Bérénice A. Benayoun,[1] Ben Machado,[1] Param Priya Singh,[1] Chi-Kuo Hu,[1] Matthew F. Pech,[2,3] Dario Riccardo Valenzano,[1,5] Elisa Zhang,[1] Sabrina C. Sharp,[1] Steven E. Artandi,[2,3,4] and Anne Brunet[1,4,*]

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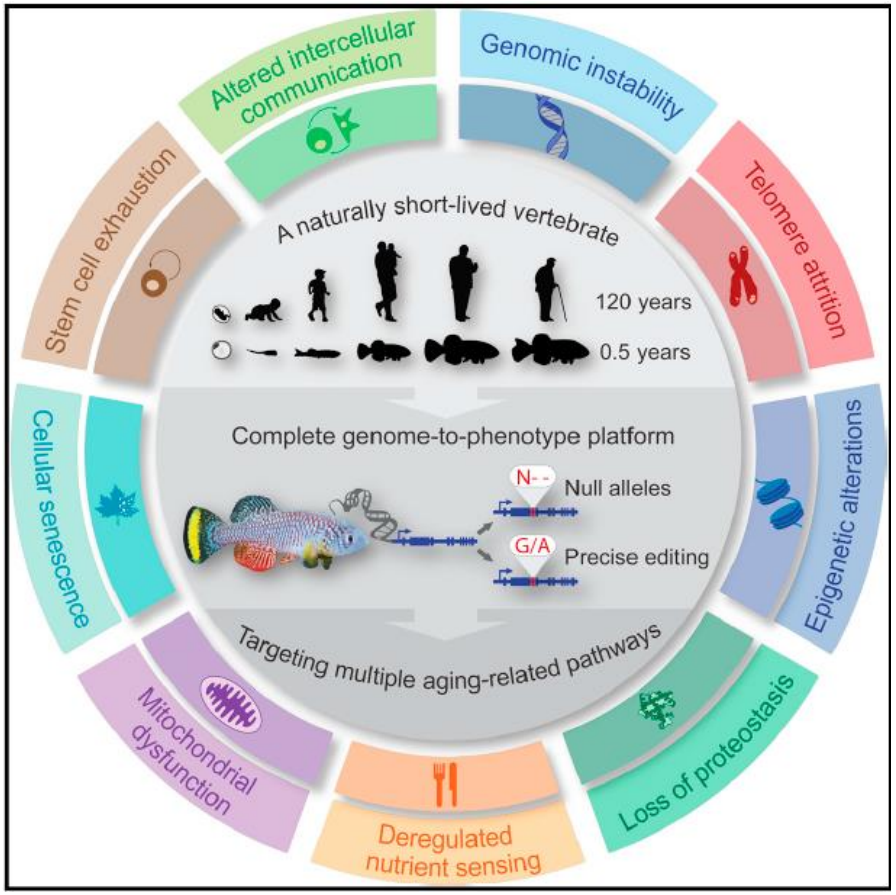
Cell Systems
Article

Longitudinal RNA-Seq Analysis of Vertebrate Aging Identifies Mitochondrial Complex I as a Small-Molecule-Sensitive Modifier of Lifespan

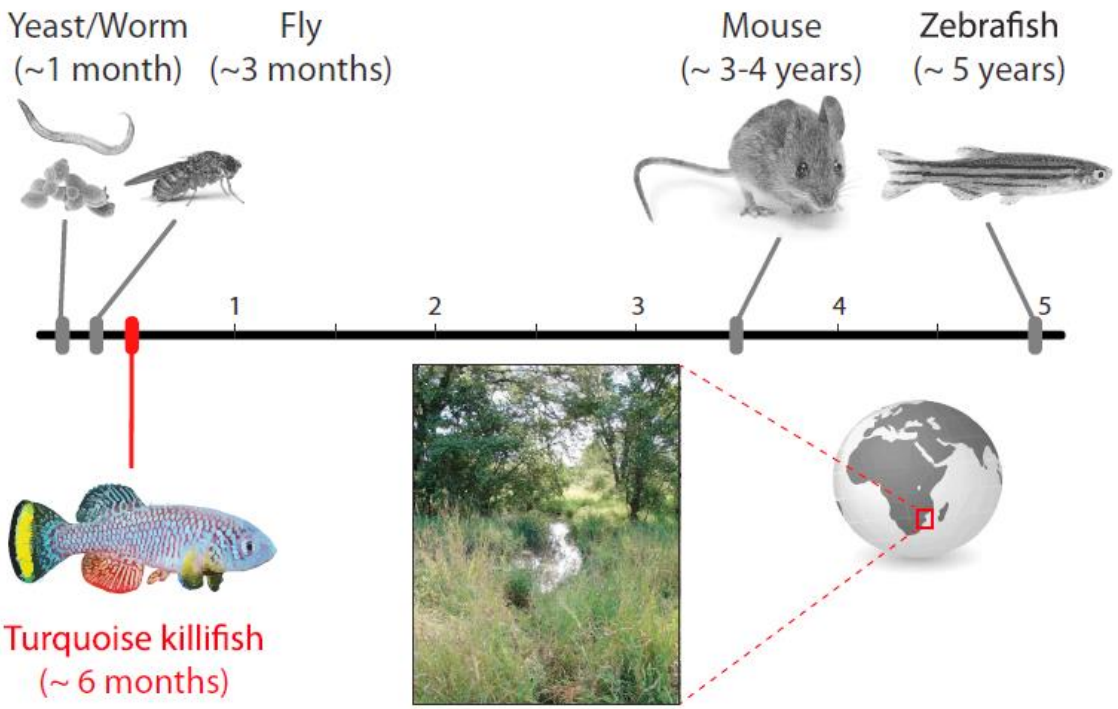
Mario Baumgart,[1,6] Steffen Priebe,[2,6] Marco Groth,[1,6] Nils Hartmann,[1,6] Uwe Menzel,[2] Luca Pandolfini,[3,7] Philipp Koch,[1] Marius Felder,[1] Michael Ristow,[4] Christoph Englert,[1,5] Reinhard Guthke,[2] Matthias Platzer,[1] and Alessandro Cellerino[1,3,*]

A Platform for Rapid Exploration of Aging and Diseases in a Naturally Short-Lived Vertebrate

- Integrative genomic and genome-editing toolkit
  - De novo assembled genome
  - CRISPR/Cas9
- Mutation of many genes encompassing the hallmarks of aging
  - Establishing stable lines
- Tools to further investigate candidates arising from human genome-wide studies

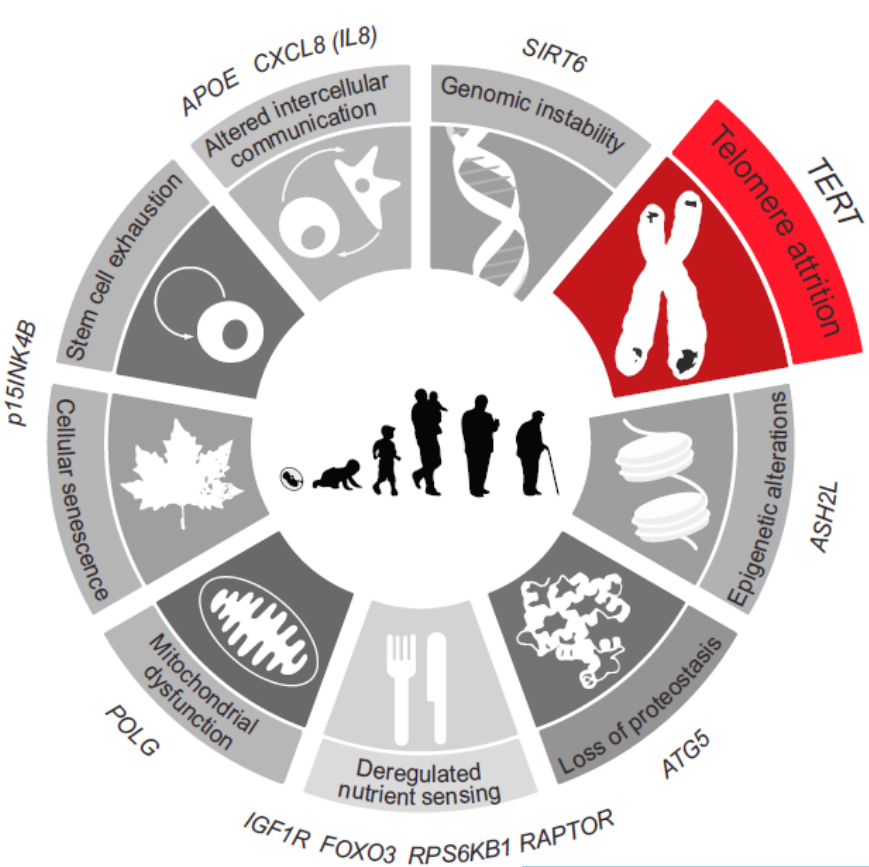


Lifespan of non-vertebrate and vertebrate aging model systems



- Compressed life cycle due to brief rainy season in Zimbabwe and Mozambique
- 30-40 d from egg to egg laying adult
- 4-6 mth lifespan in lab conditions

Genes encompassing the 9 hallmarks of vertebrate aging

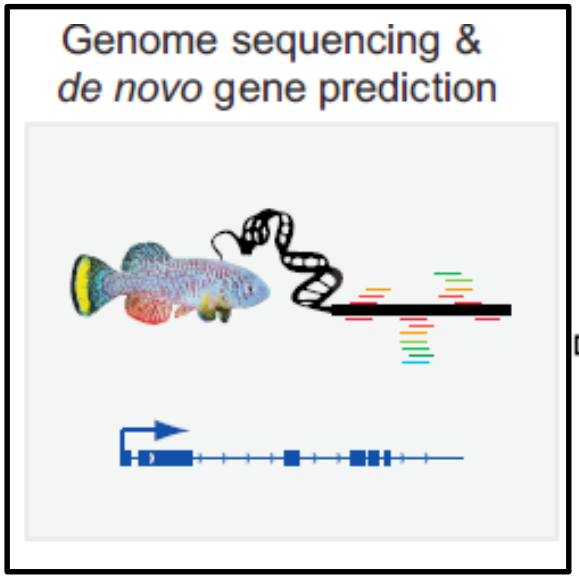


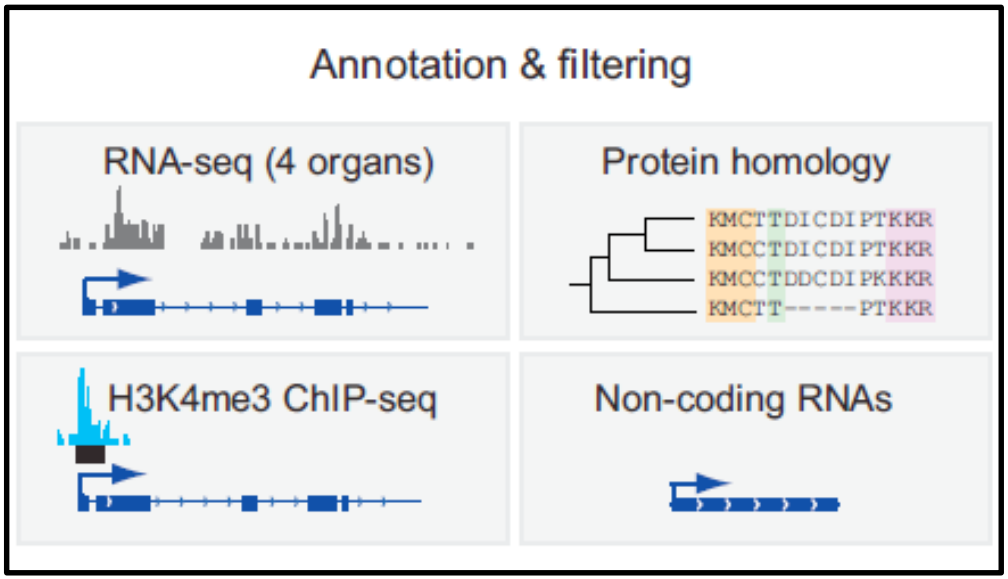
Protein subunit of telomerase

S6 kinase

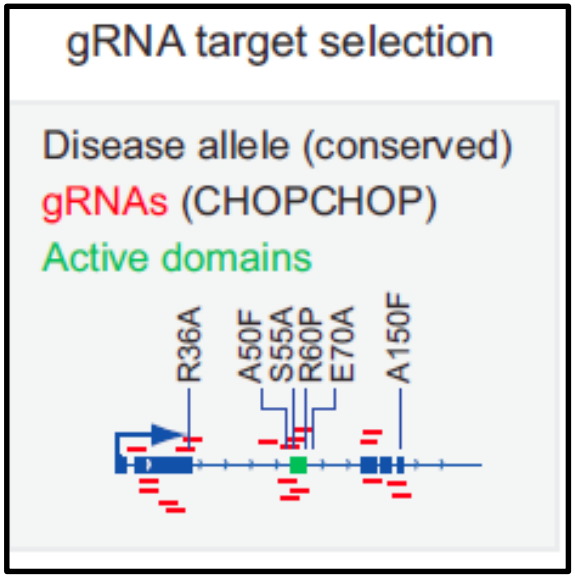
Insulin-like growth factor receptor 1

CRISPR/Cas9 gRNAs in a new model organism

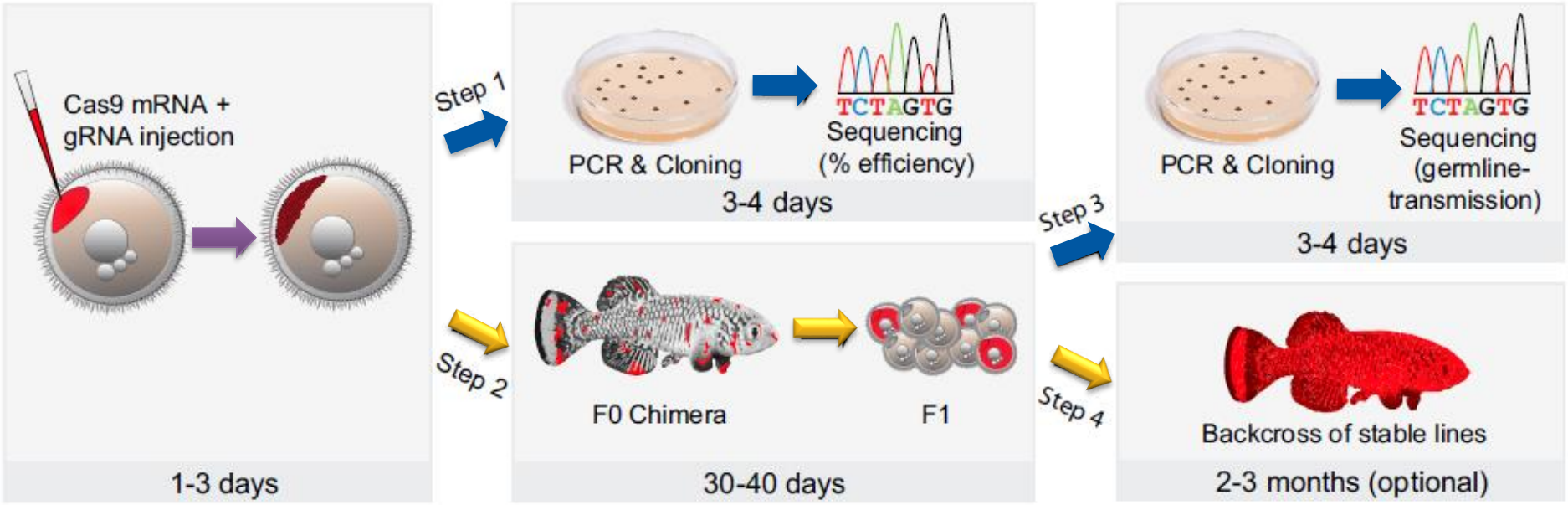




1) Identification of genes & building models
2) RNA seq analysis, ChIPseq to define TSS
3) Support annotation esp. for nc-RNA
4) Additional support for coding RNA annotation with protein homology
5) Design guide RNA (gRNA) for gene editing



CRISPR/Cas9 genome-editing strategy



Generation of 2-5 independent gRNA sequences.

Microinjection into fertilized eggs at single cell stage.

Successful editing used for F0

F0 crossed with WT to generate F1

For QC – cloning and sequencing after 72 hours.

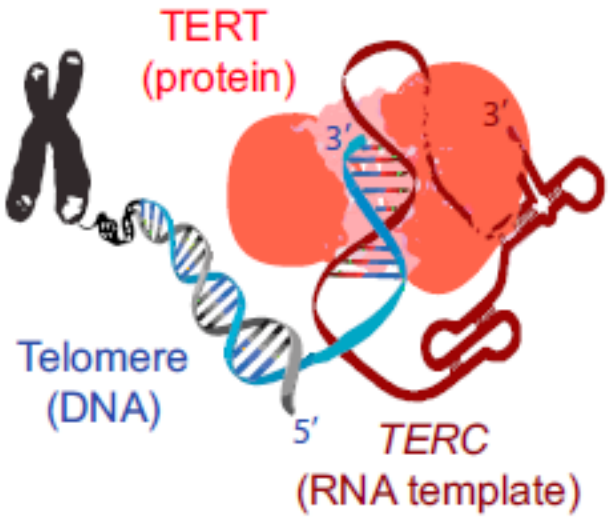
Successful F1 as stable lines

Backcrossed to minimize potential off target editing

Rapid genome editing of TERT

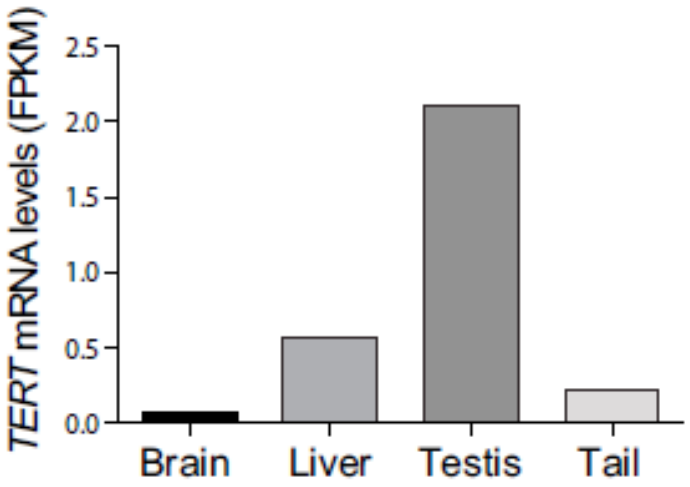
For humans: mutations in TERT cause tissue homeostasis failure like Dyskeratosis congenita

- Premature aging
- Bone marrow failure
- Pulmonaly fibrosis
- Reduced fertility
- Cancers



Prediction of TERT sequence in gene model suggests conserved telomerase components in turquoise killifish

TERT expression in different tissues

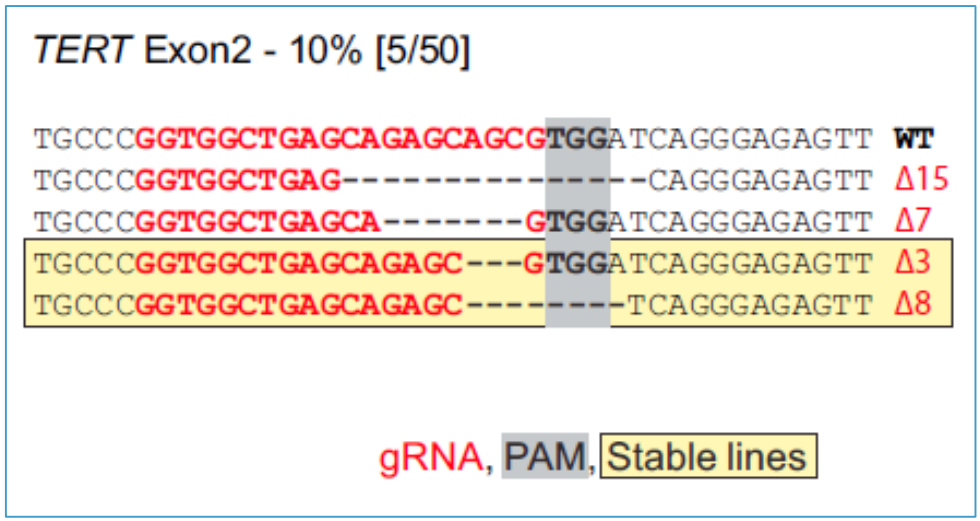


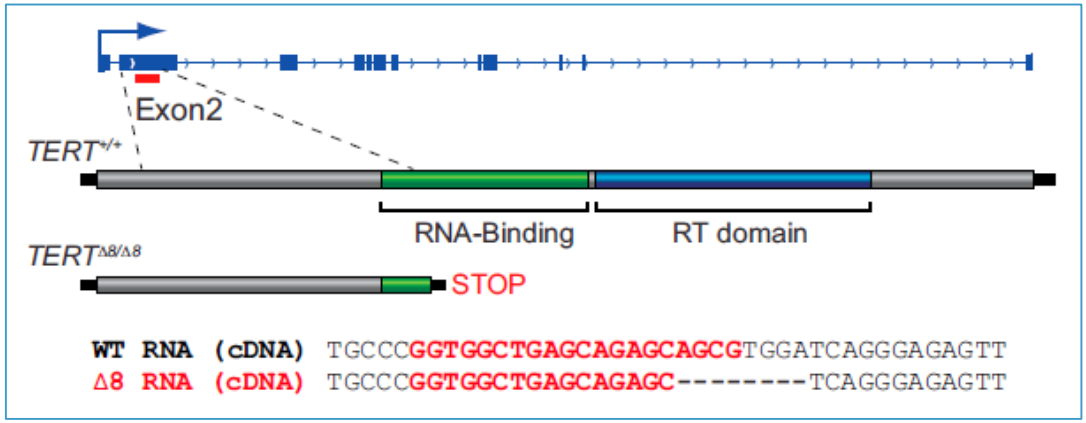
RNA seq analysis of different tissues:

mRNA expression enriched in testis

Similar to human DKC patients

Successful gene editing



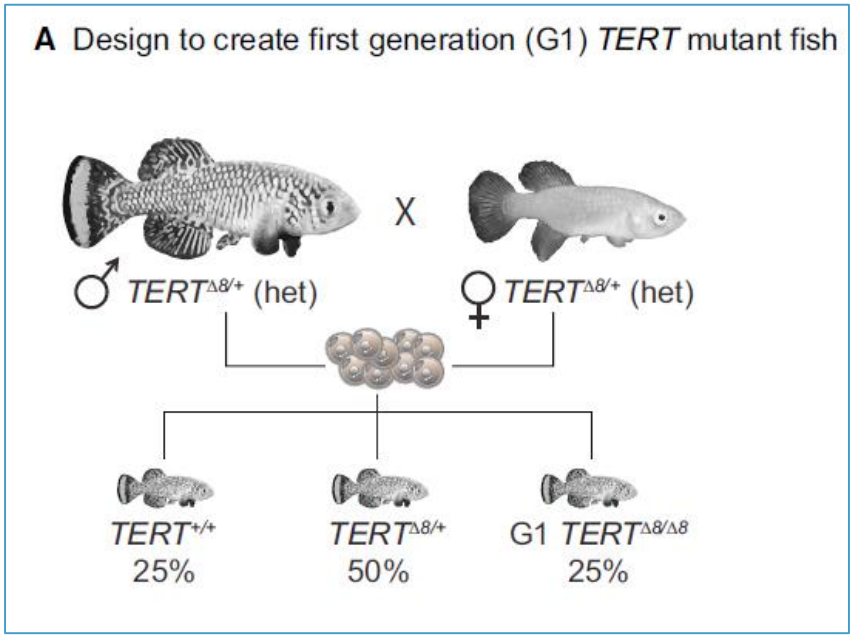


2 gRNAs targeting regions in TERT exon2:

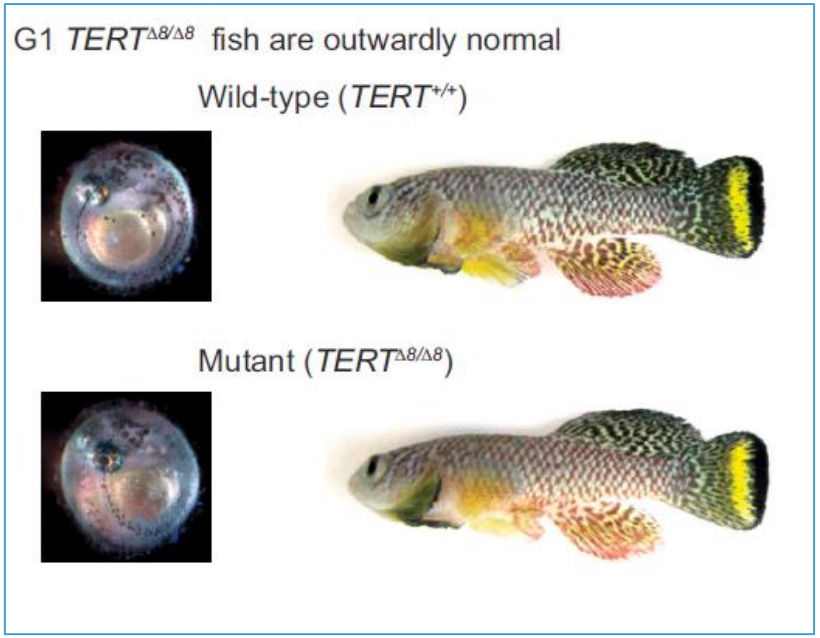
- Injection in embryos
- Raised to sexual maturity
- Crossed with WT
- Stable lines with 2 deletions
- TERT premature stopped after 3 or 8 bp

TERT Δ8/Δ8 sucessfully introduced within 2 months

Generation of stable mutant lines

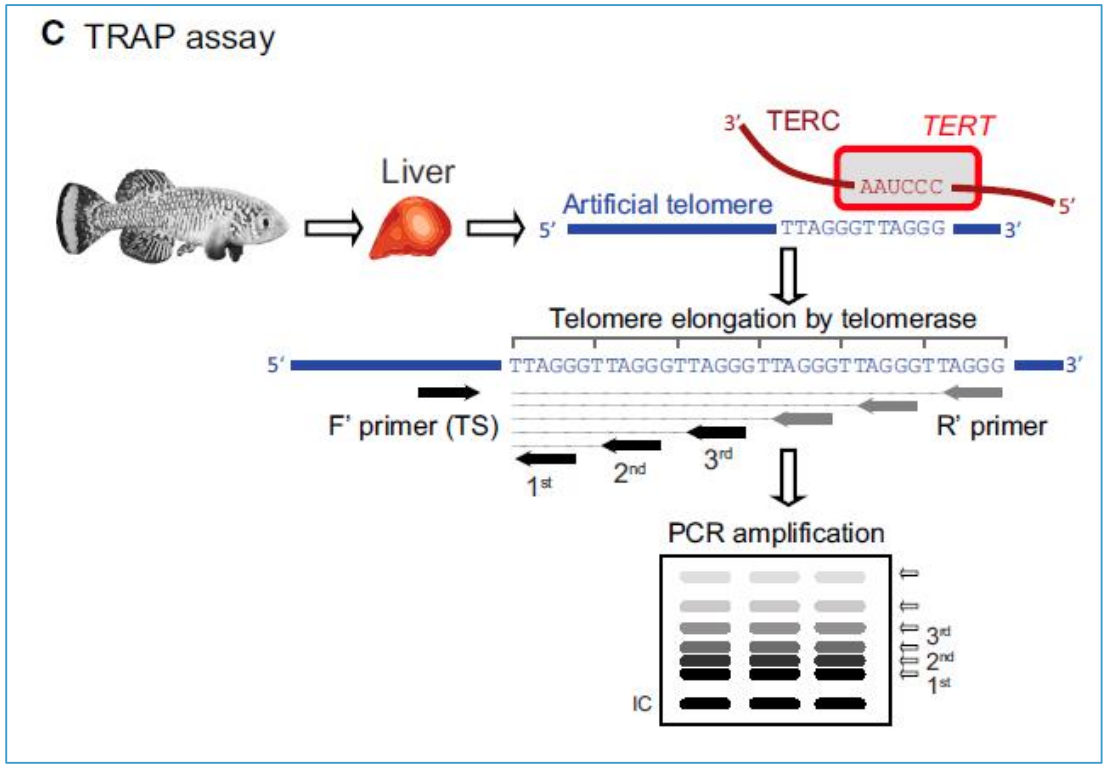


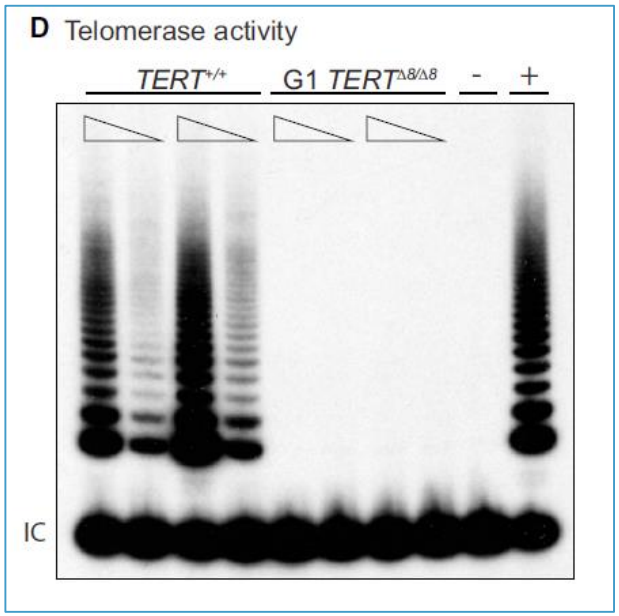
Normal Mendelian distributaion suggests no embryonic lethality



Back-crossing 3 generations to prevent off target mutations
&
crossing heterozygous fish to generate again homozygous individuals

Telomerase activity loss of TERT $^{\Delta 8/\Delta 8}$



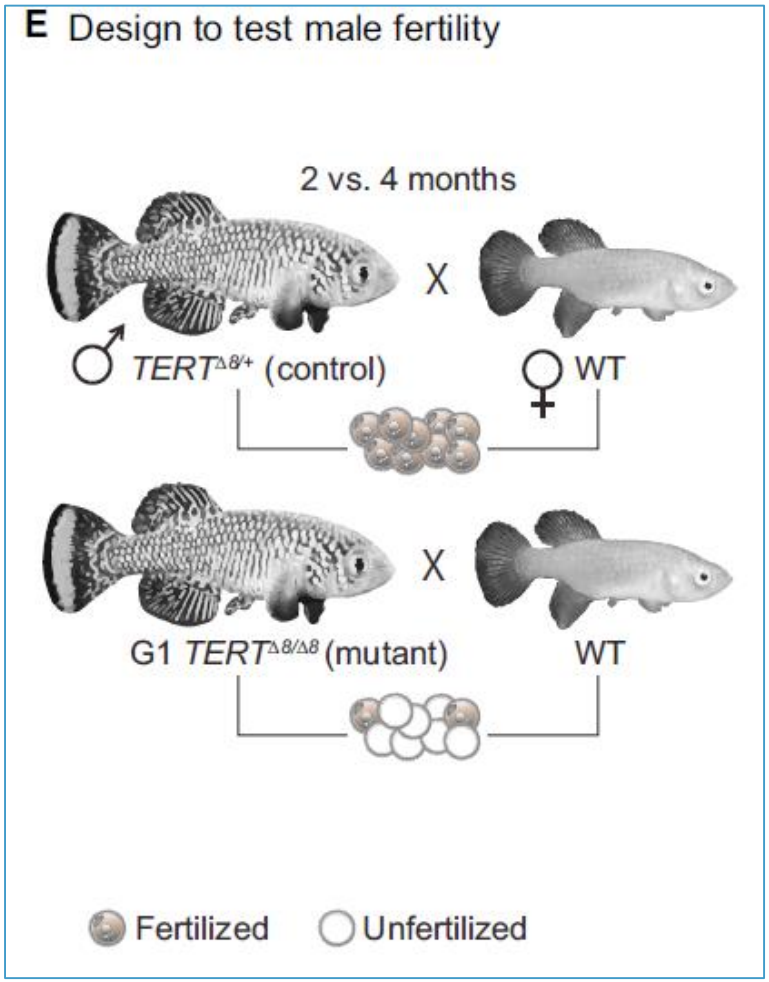


Enzymatic activity of telomerase

TRAP assay – to check for true loss of function:

Radiolabelled NTs used in PCR amplification to get autoradiographic products.

Investigation of male fertility in TERT $^{\Delta 8/\Delta 8}$ G1

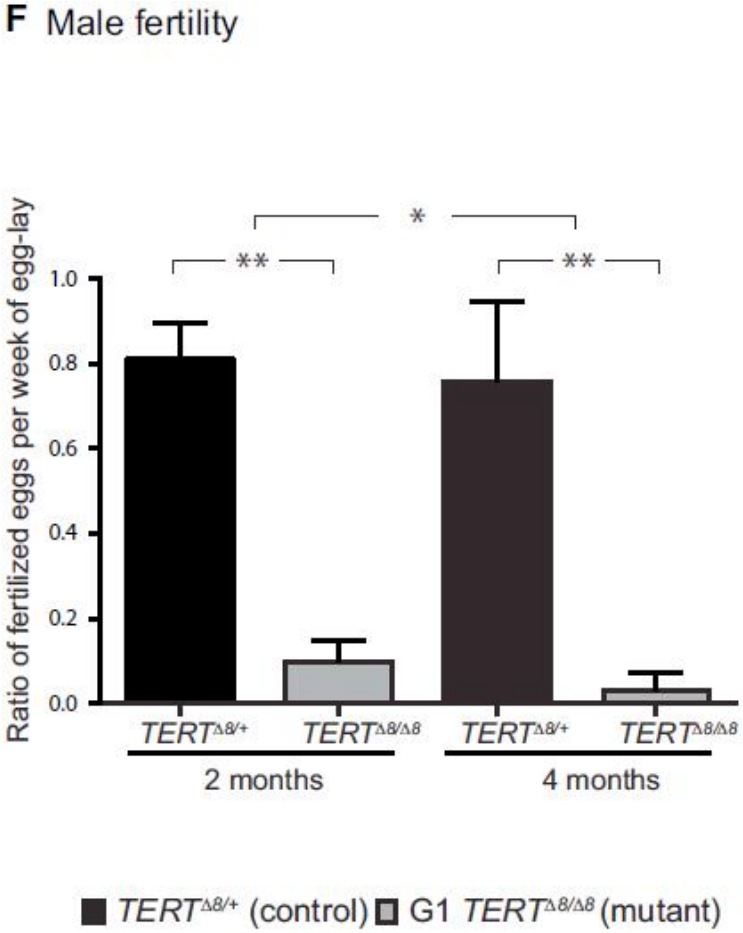


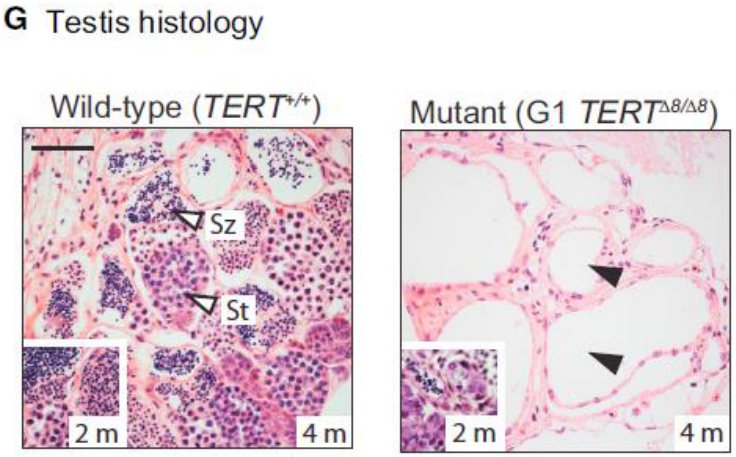
humans with haploinsufficency for telomerase show failure in Highly proliferative tissue

- Blood
- Skin
- Intestine
- Germline (high TERT)

Fertility test of TERT $^{\Delta 8/\Delta 8}$ G1 males compared to TERT $^{\Delta 8/+}$ siblings by crossing to young WT females

Investigation of male fertility in TERT $^{\Delta 8/\Delta 8}$ G1



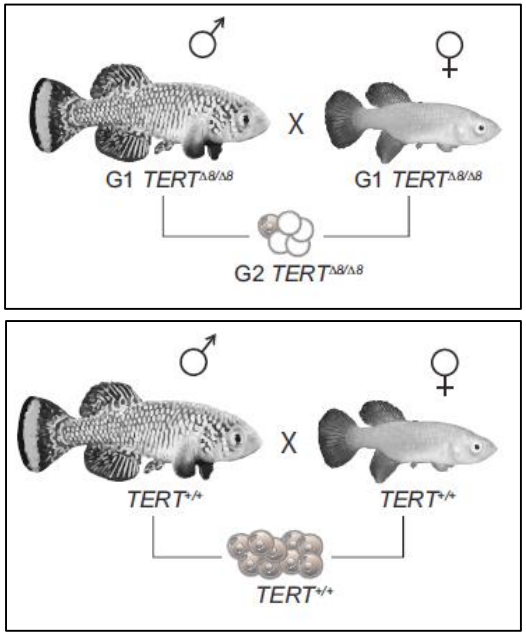


Fertilizing ability strongly decreased in TERT $^{\Delta 8/\Delta 8}$ males

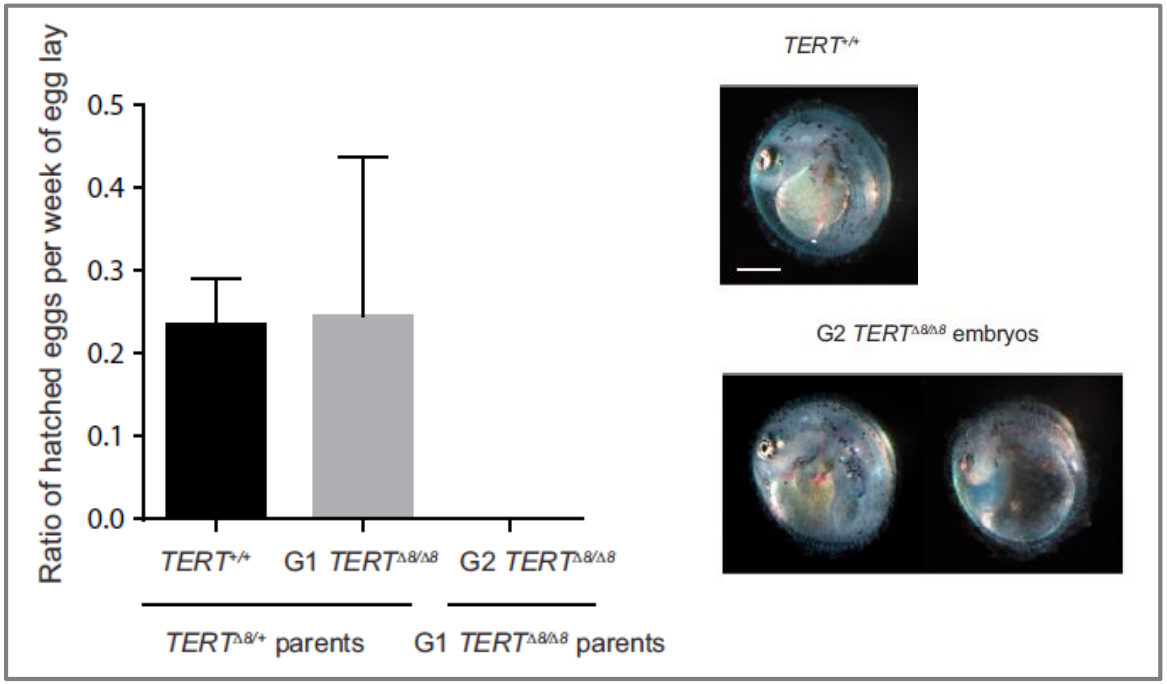
Testes showed atrophy and loss of germ cells (also females affected)

Low proliferative tissue (heart, muscle, liver, kidney) showed no significant defects

Analysis of genetic anticipation



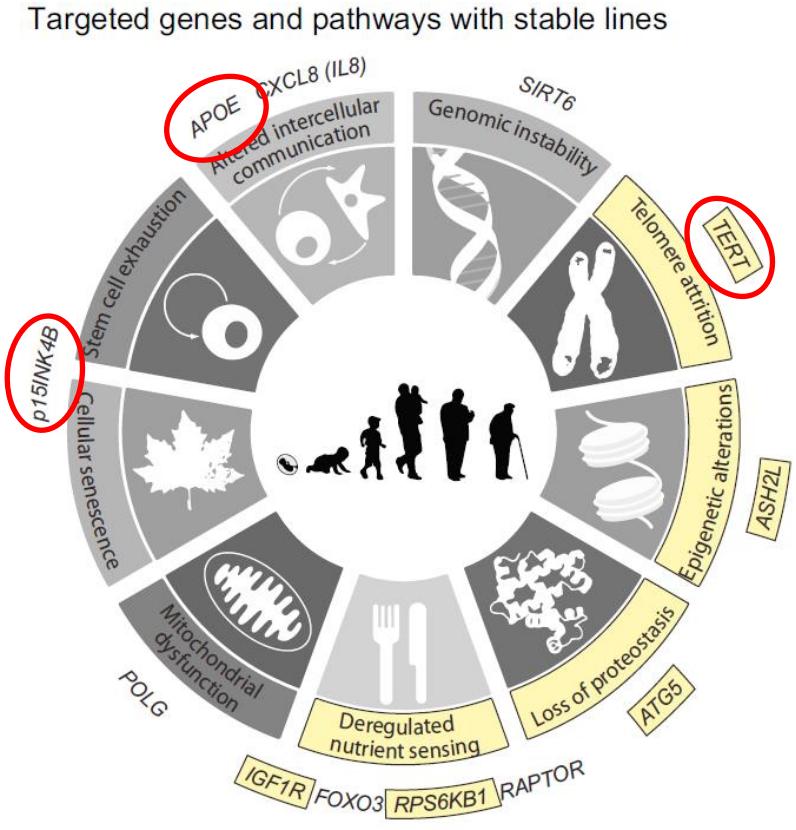
Crossing
G1 TERT $^{\Delta 8/\Delta 8}$ to
generate
G2 TERT $^{\Delta 8/\Delta 8}$
embryos

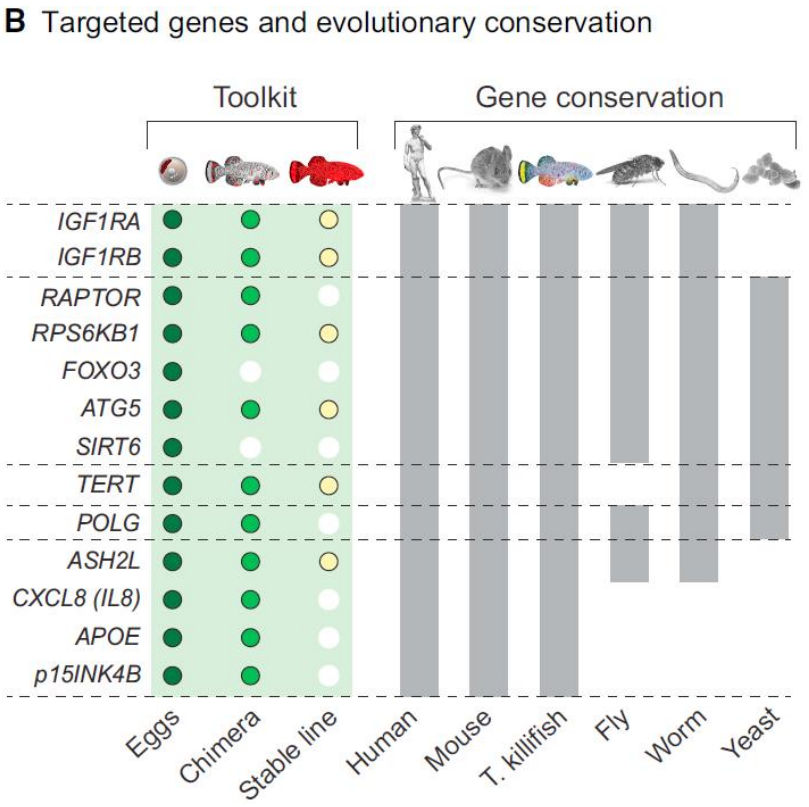


G2 embryos showed gross abnormalities and died prior to hatching

Genetic anticipation = cumulative germline damage; also occurring in human DKC patients

Toolbox of mutants encompassing the hallmarks of aging





Construction of 5 gRNA for each gene of interest → identification of at least 1 successful gRNA → generation of stable mutatnt lines → online platform

Conclusions

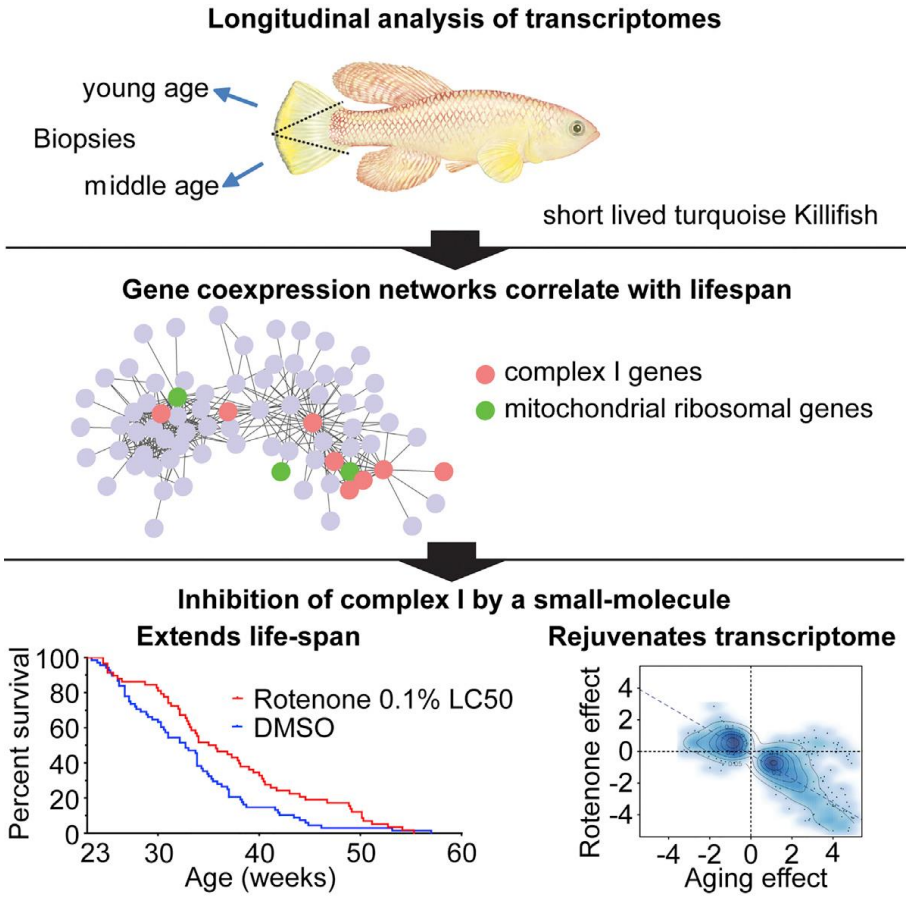
- Turquoise killifish with
  - Short / compressed lifespan,
  - well characterization &
  - low costs

  is highly suited for aging reasearch

- Good for exploration of human longevity candidate genes

Longitudinal RNA-Seq Analysis of Vertebrate Aging Identifies Mitochondrial Complex I as a Small-Molecule-Sensitive Modifier of Lifespan

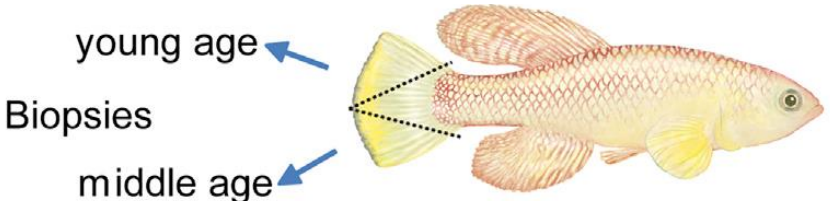
- Longitudinal transcriptomics
- Transcriptomics of shorter and longer lived individuals
- Identification of lifespan modulators
- Rejuvenation of the transcriptome



Baumgart et al., 2016, Cell Systems 2

Basic experimental setup

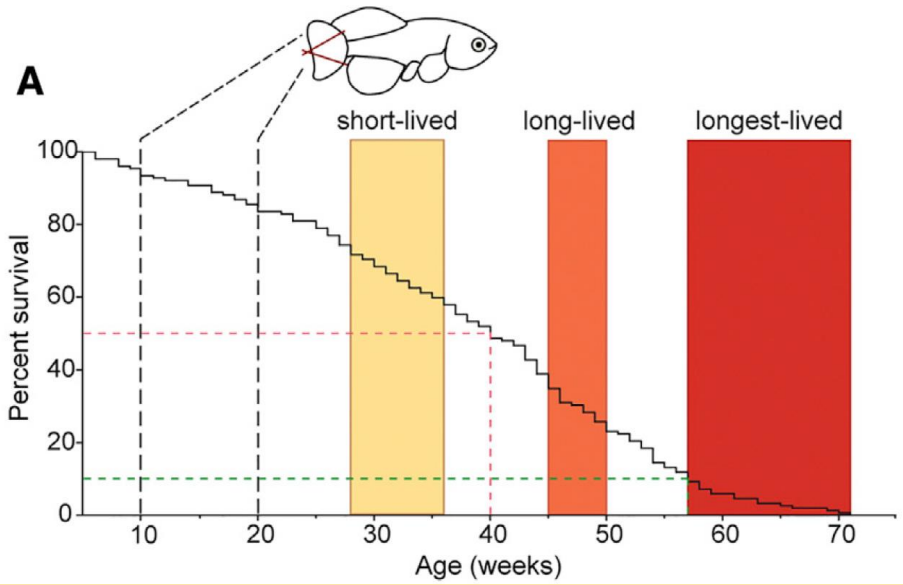
- Different Killifish models available (3-12 month lifespan)
  - Highly inbred strains (e.g. GRZ) with shorter lifespans
  - Strains collected more recently (e.g. MZM0410) with longer lifespans



- Taking 2 fin biopsies

  at two time points during early adult stage
  - RNA seq analysis
  - Weighted gene co-expression network analysis (WGCNA)

Lifespan analysis of MZM-0410



MZM-0410 shows 42 times more variations than highly inbred strains

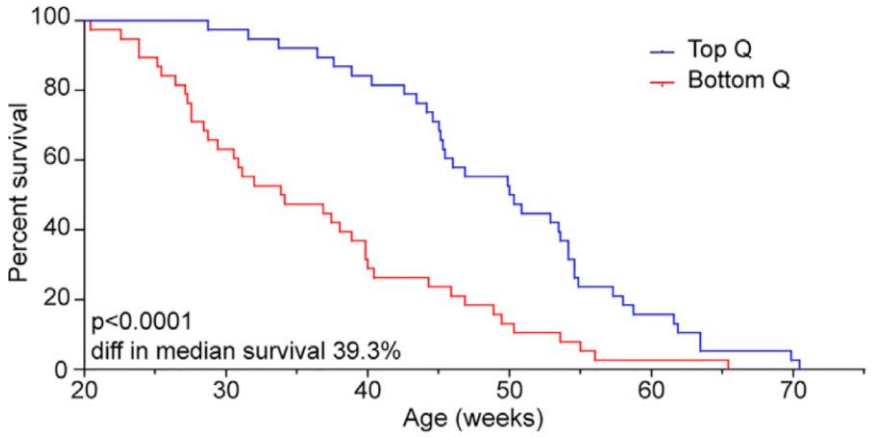
Biopsies at 10 & 20 weeks of age

Median lifespan 40 weeks

10% survivorship after 58 weeks

Differences in survival between first and last quartile of increase in body weight

45 individuals for:
Short-lived group (28-36 weeks)
Long-lived group (45-50 weeks)
Longest-lived group (57-71 weeks)



Analysis of 10 & 20 weeks biopsy

Recording of:
- Age at sexual maturity
- Weight
- Length
- At 10 & 20 weeks each
- Calculated growth rate between biopsies

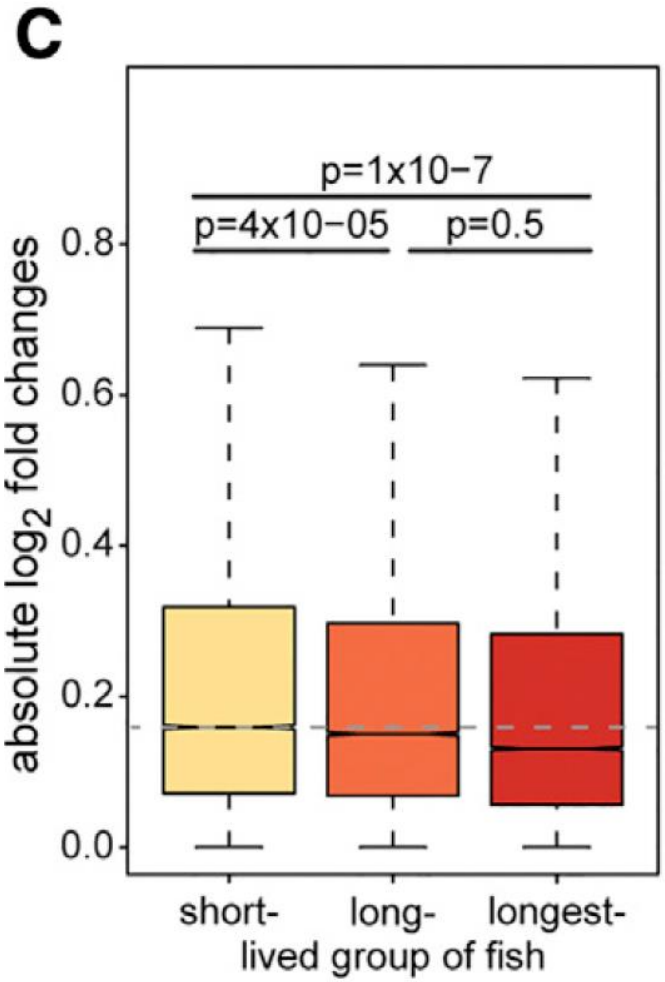
RNA seq analysis of 23 546 genes

Compared to the N. furzeri reference genome

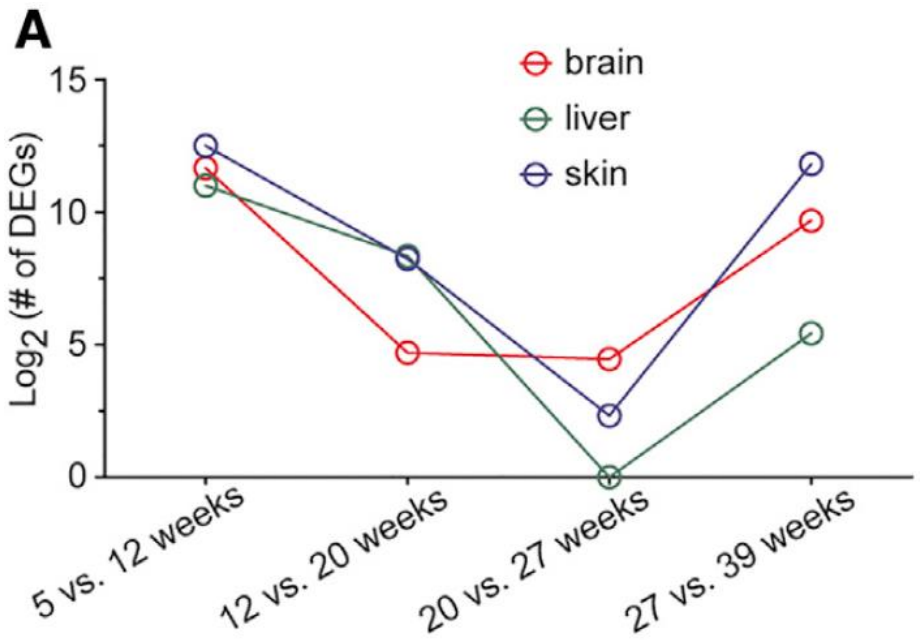
Comparison between both biopsies of the same fish:

- Largest difference in short-lived fish

- Smallest modulations in longest-lived fish



Charakterization of age dependent gene expression

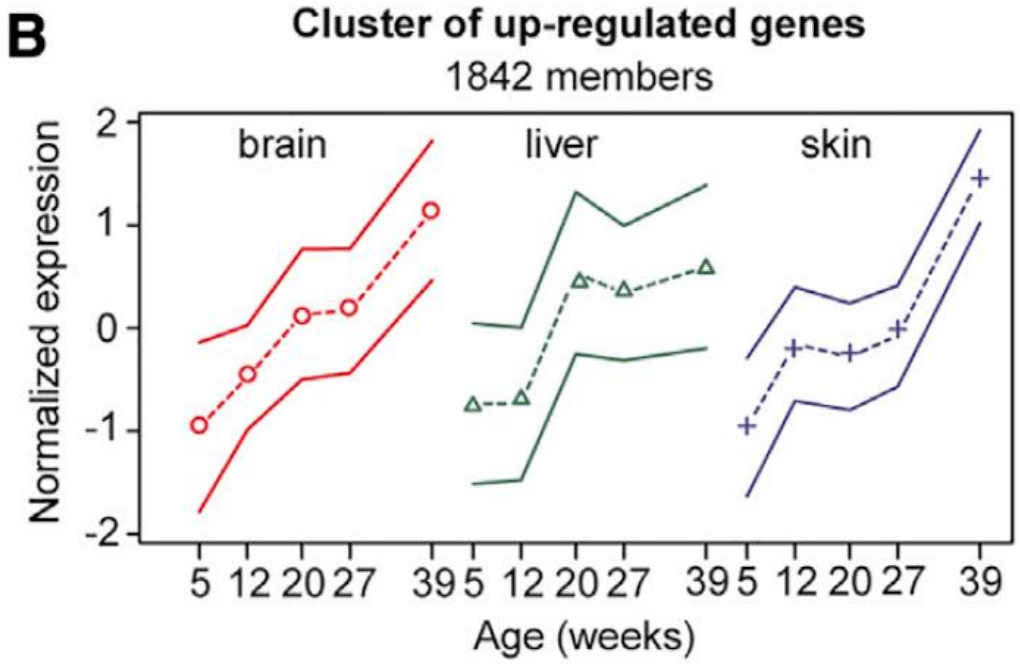


Non genetic factors contributing to age of death are present in early life

Analysis of RNAseq data from 5 age groups of differentially expressed genes between first and last age group

Pairwise comparison of age groups regarding differentially expressed genes

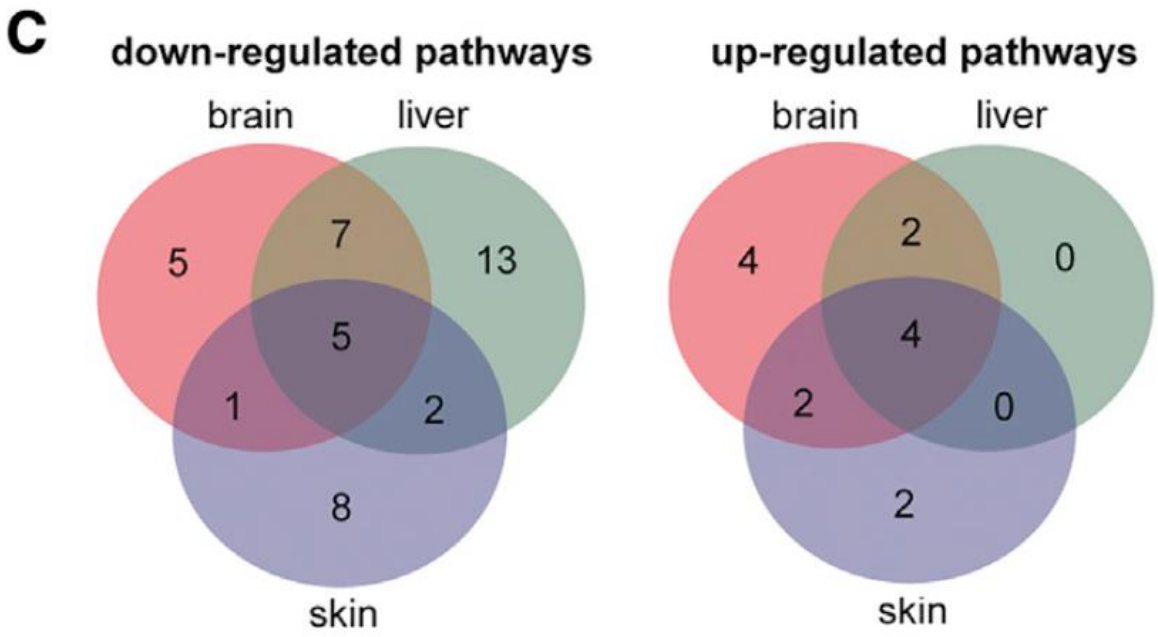
Age dependent regulation across tissues



Analysis of genes differentially expressed in at least 2 tissue types

Age dependent regulation seems to be similar across the 3 tissues

Different KEGG pathways expression in different tissues



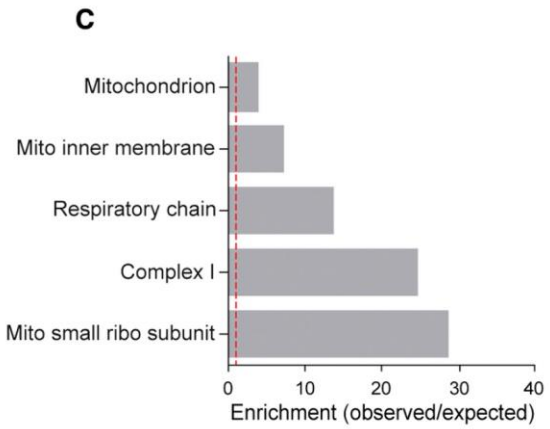
down-regulated pathways
dre04110 Cell cycle
dre03030 DNA replication
dre00020 Citrate cycle (TCA cycle)
dre03013 RNA transport
dre00310 Lysine degradation

up-regulated pathways
dre03010 Ribosome
dre04060 Cytokine-cytokine receptor interaction
dre04620 Toll-like receptor signaling pathway
dre04630 Jak-STAT signalling pathway

Up- and Down-regulated age associated pathways in different tissues

9 of the KEGG pathways are regulated in all three tissues

Activity of Respiratory Chain Complex I affects lifespan



WGCNA analysis of 936 genes correlated with age of death

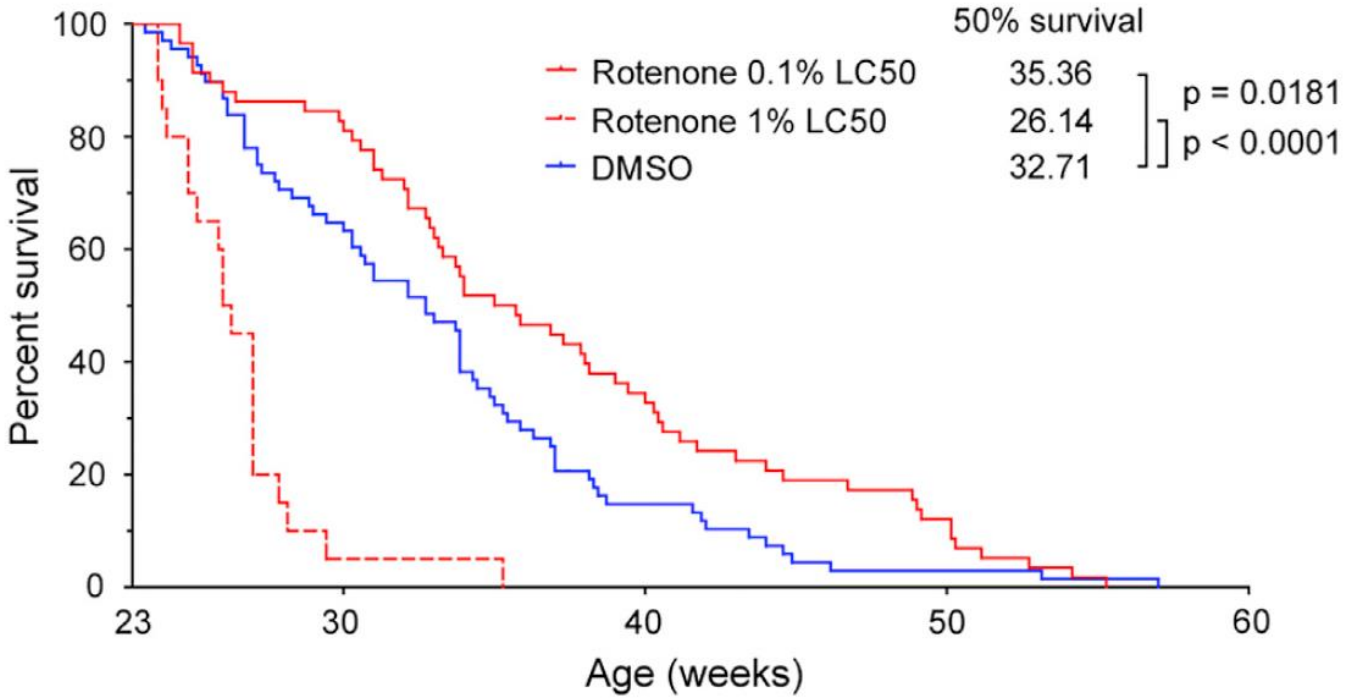
Top enrichment for complex I of respiratory chain and genes coding for parts of complex I

Network analysis showed genes coding for NDUFs & MRPs are highly co-regulated → Consistent with mouse studies

2 experiments for confirmation:

Comparison of skin RNAseq data from highly inbred GRZ strain with MZM-0410 (12 weeks) → higher complex I gene expression in GRZ

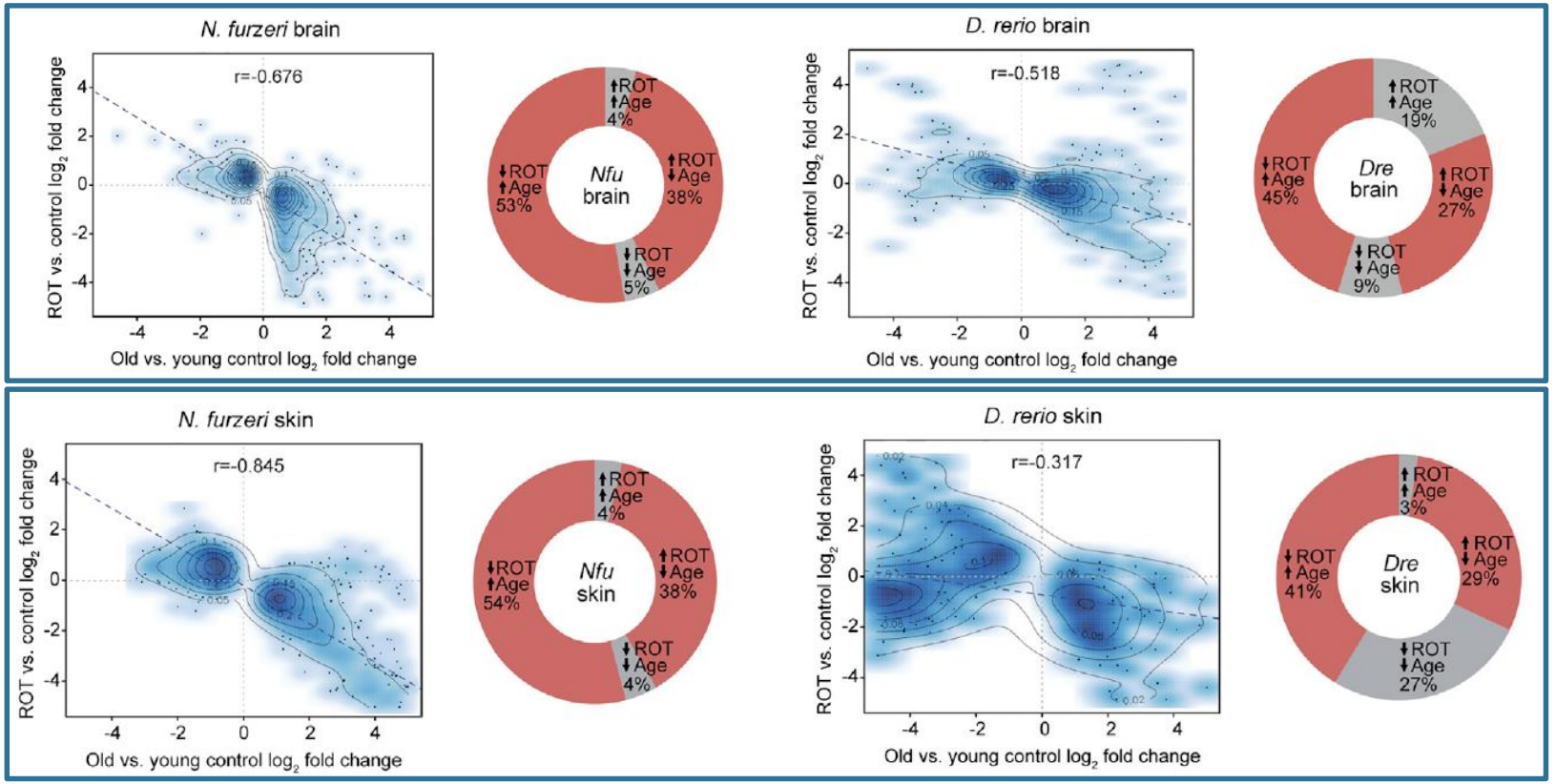
Relevance of Complex I for lifespan determination



Inhibition of Complex I by rotenone treatment from 23 weeks:

- Lower concentration → lifespan extension
- Higher concentration → lifespan shortening

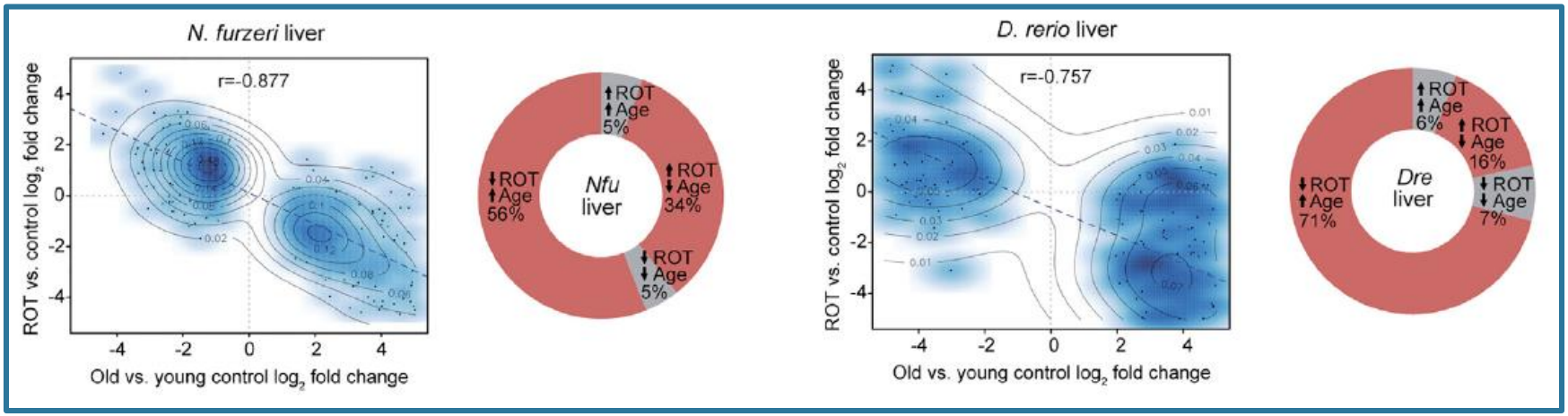
RNAseq analysis of different tissue after ROT treatment



4 weeks treatment with 15pM ROT & vehicle of old (23 w) and young (5 w) killifish

8 weeks treatment with 3.75nM ROT & vehicle of old (36m) and young (12m) zebrafish

RNAseq analysis of different tissue after ROT treatment



In brain, skin & liver the vast majority (~90%) of upregulated genes during aging were downregulated by ROT and vice versa

→ Resulting in a highly significant negative regression

Conclusions

- Data obtained with N. furzeri in short time could be confirmed with D. rerio

- Data suggest complex I of respiratory chain as potential target for prevention of age-related dysfunctions

Thank You For
Your Attention