

The Roles of SIRT6 in genome stability and longevity

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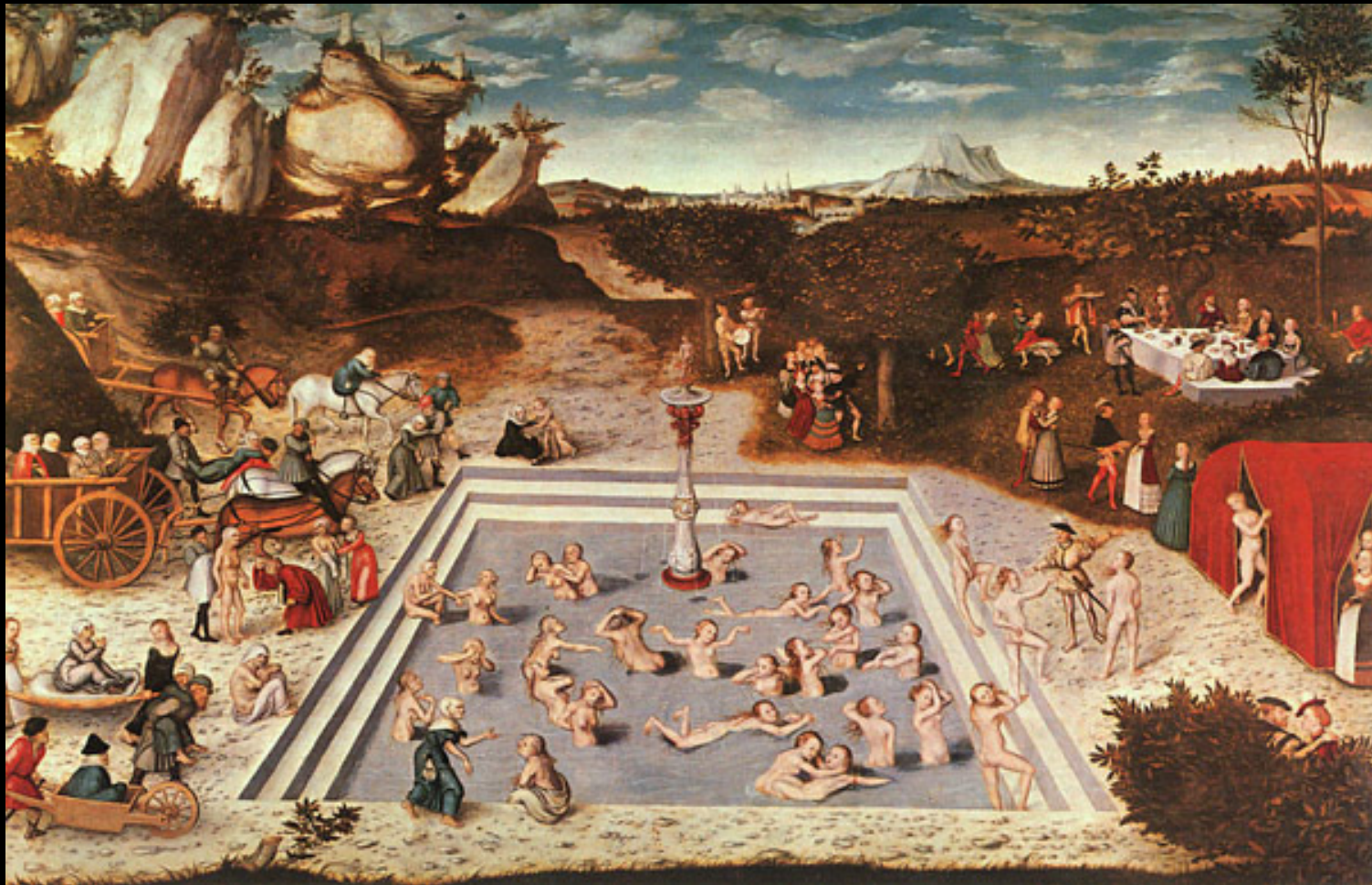


NIA, Baltimore
December 17, 2019

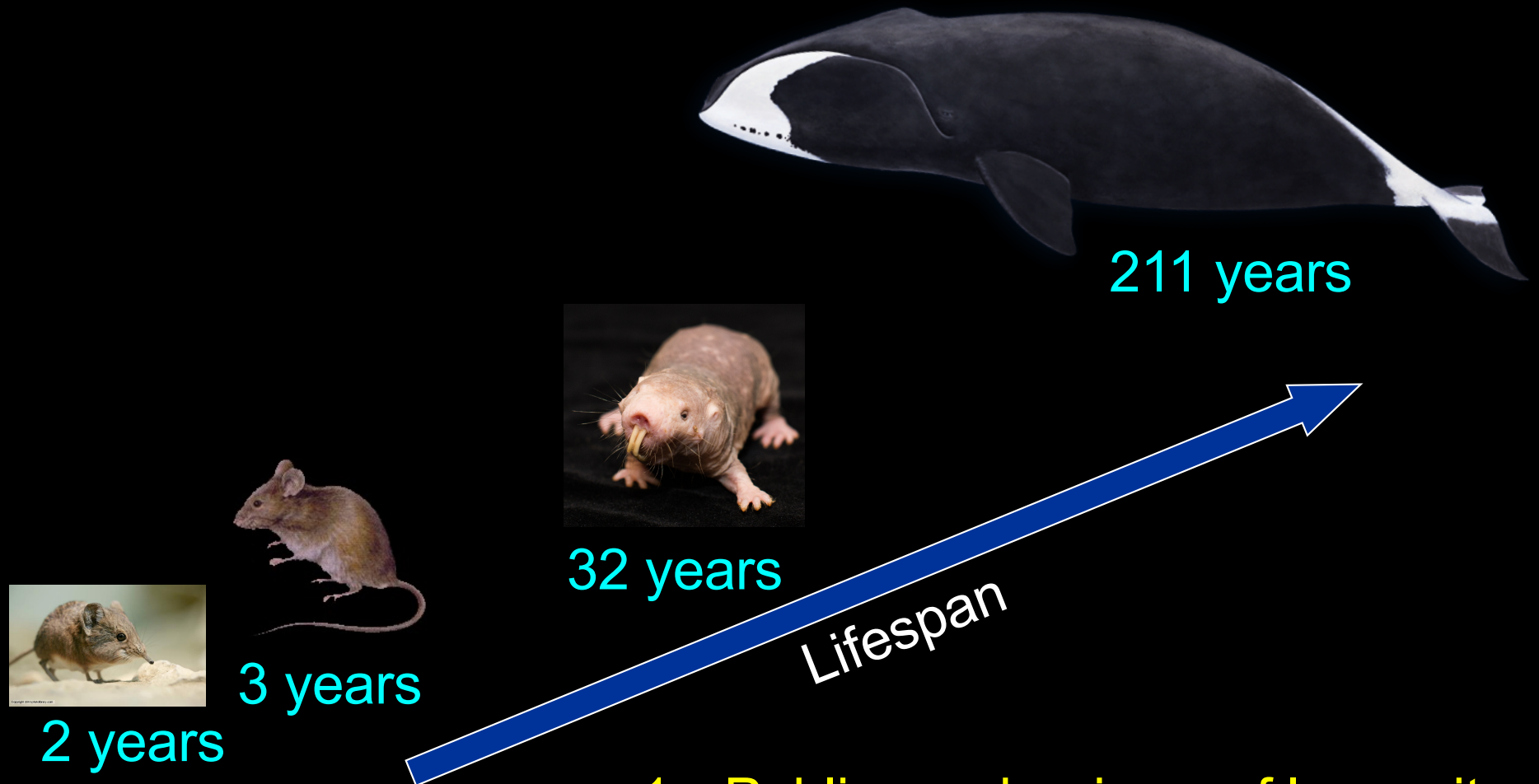


The Fountain of Youth (1546)

Lucas Cranach

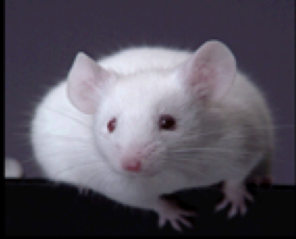


Mammals differ up to 100-fold in their lifespan and cancer susceptibility



1. Public mechanisms of longevity
2. Private mechanisms of longevity

Natural variation: Rodents present a wide variety of body sizes and lifespans



Mouse
30 g / 3yrs



Rat
400 g / 4 yrs



Hamster
105 g / 4 yrs



Gerbil
53 g / 6 yrs



Deer mouse
20 g / 8 yrs



Red squirrel
200g / 10 yrs



Chipmunk
96 g / 10 yrs



Guinea pig
728 g / 12 yrs



Woodchuck
5000 g / 14 yrs



Blind mole rat
160 g / >21 yrs



Capybara
55000 g / 15 yrs



Paca
9000 g / 16 yrs



Fox squirrel
800g / 16 yrs



Chinchilla
642 g / 17 yrs



Porcupine
8600 g / 23 yrs



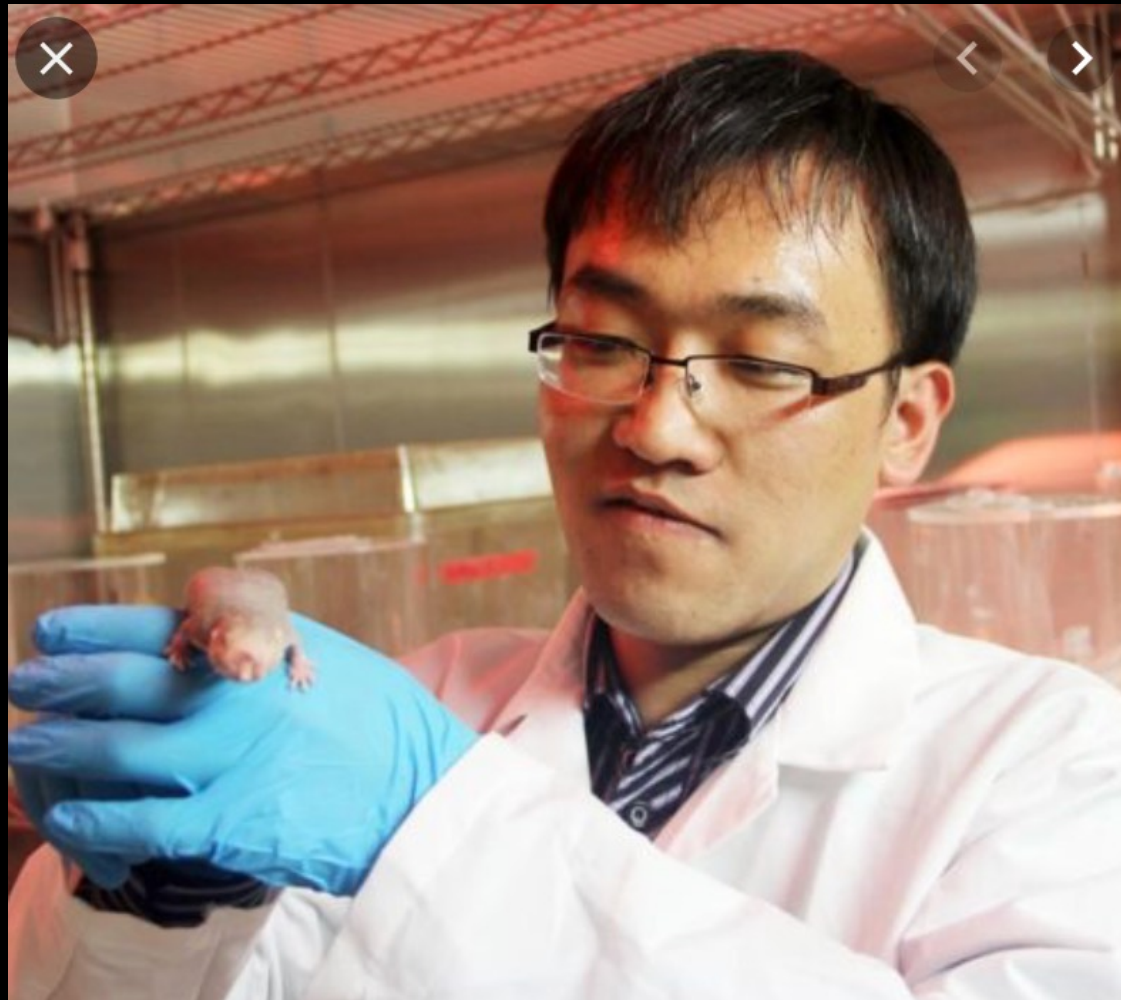
Beaver
20250 g / 24 yrs



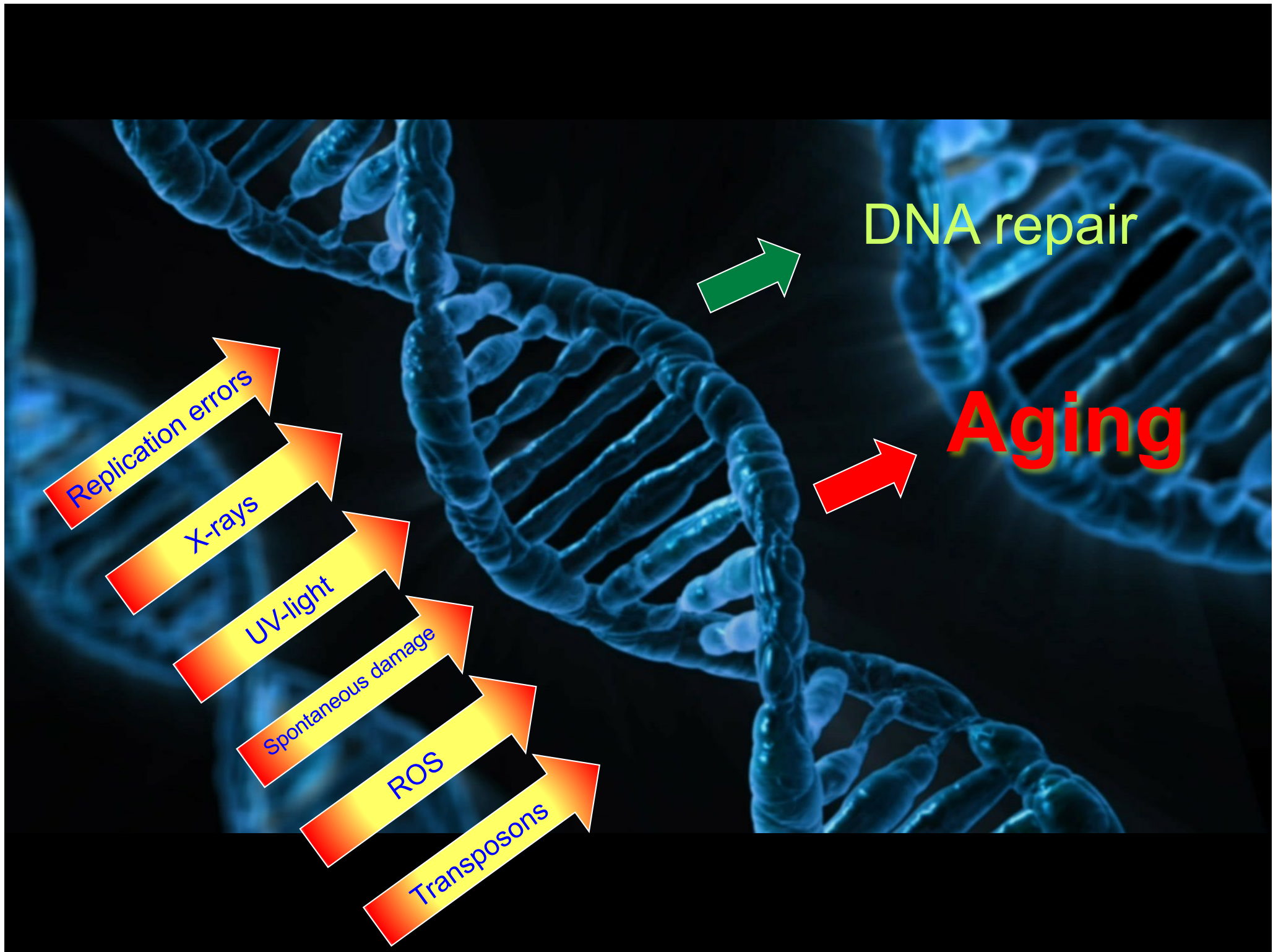
Gray Squirrel
530 g / 24 yrs



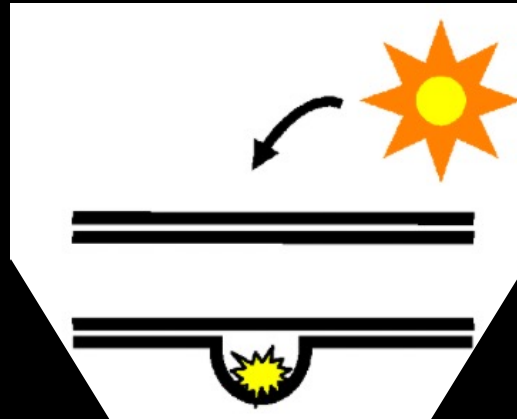
Naked Mole Rat
35 g / >32 yrs



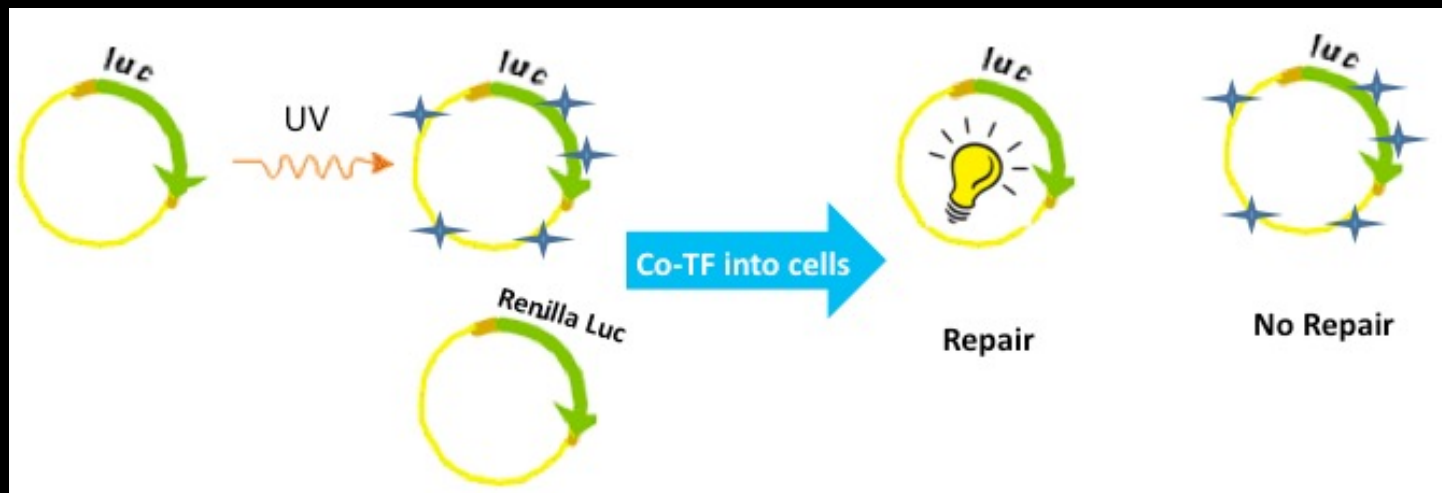
Xiao Tian



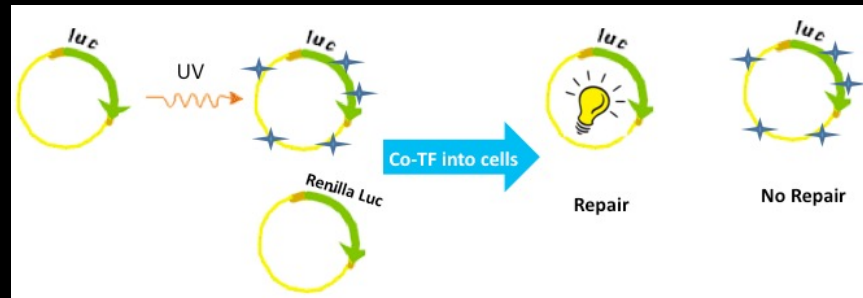
Nucleotide excision repair



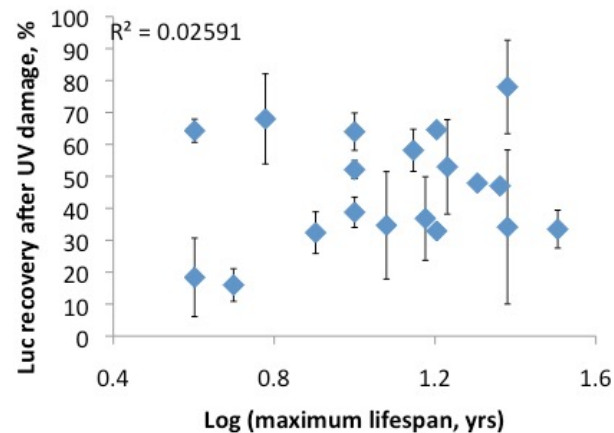
Plasmid reactivation assay



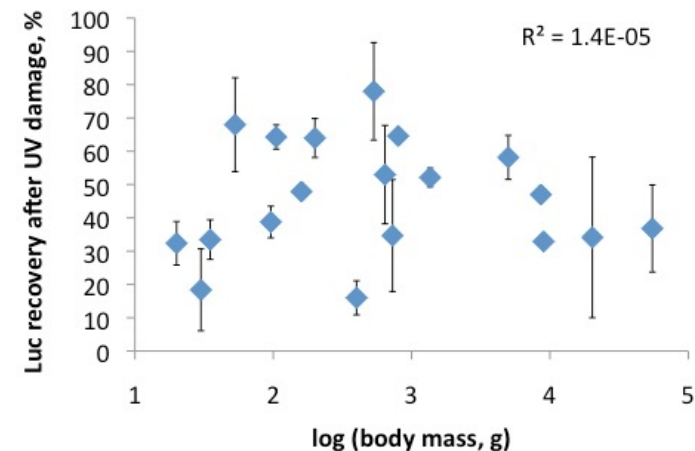
Nucleotide excision repair efficiency does not correlate with longevity



NER efficiency is not correlated with maximum lifespan

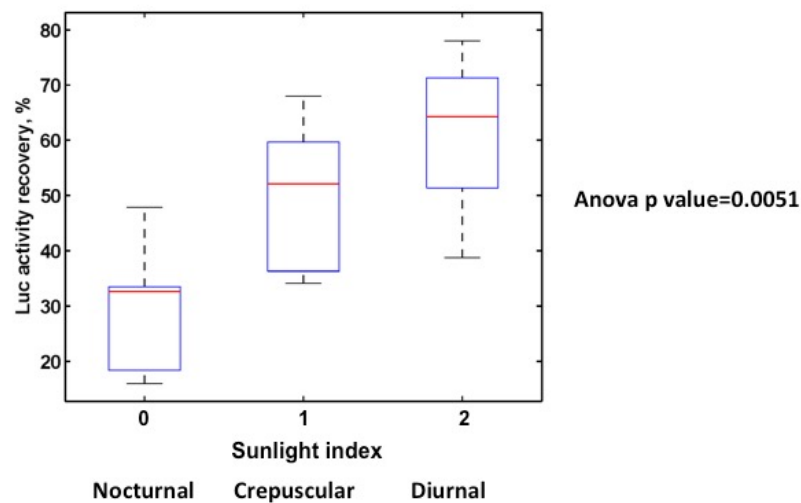


NER efficiency is not correlated with body mass

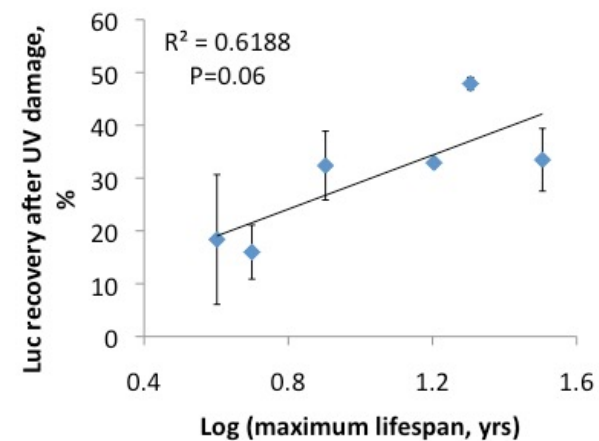


Nucleotide excision repair efficiency correlates strongly with sunlight exposure

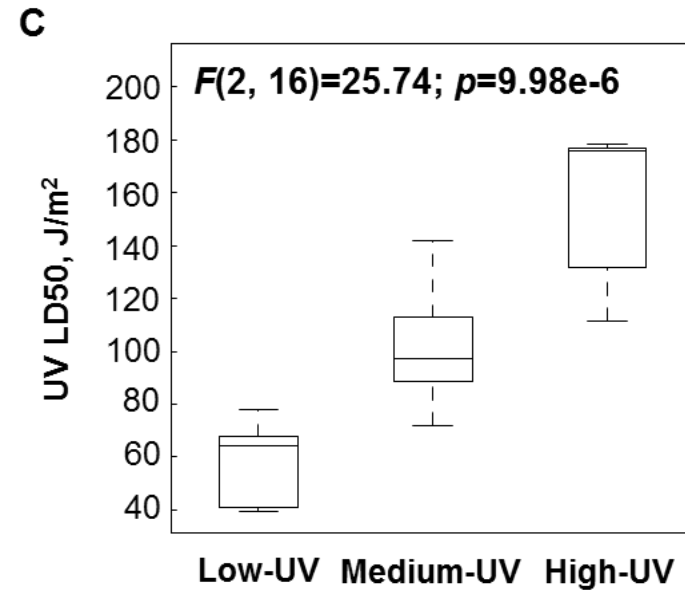
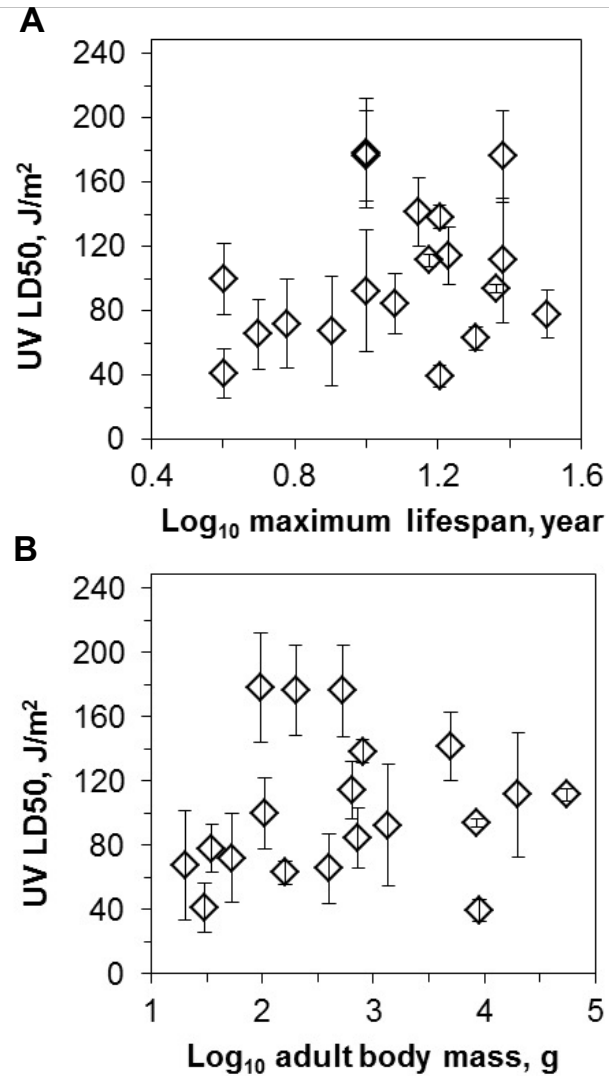
NER is correlated with sunlight exposure



NER is correlated with maximum lifespan in nocturnal
species (removing the sunlight effect)

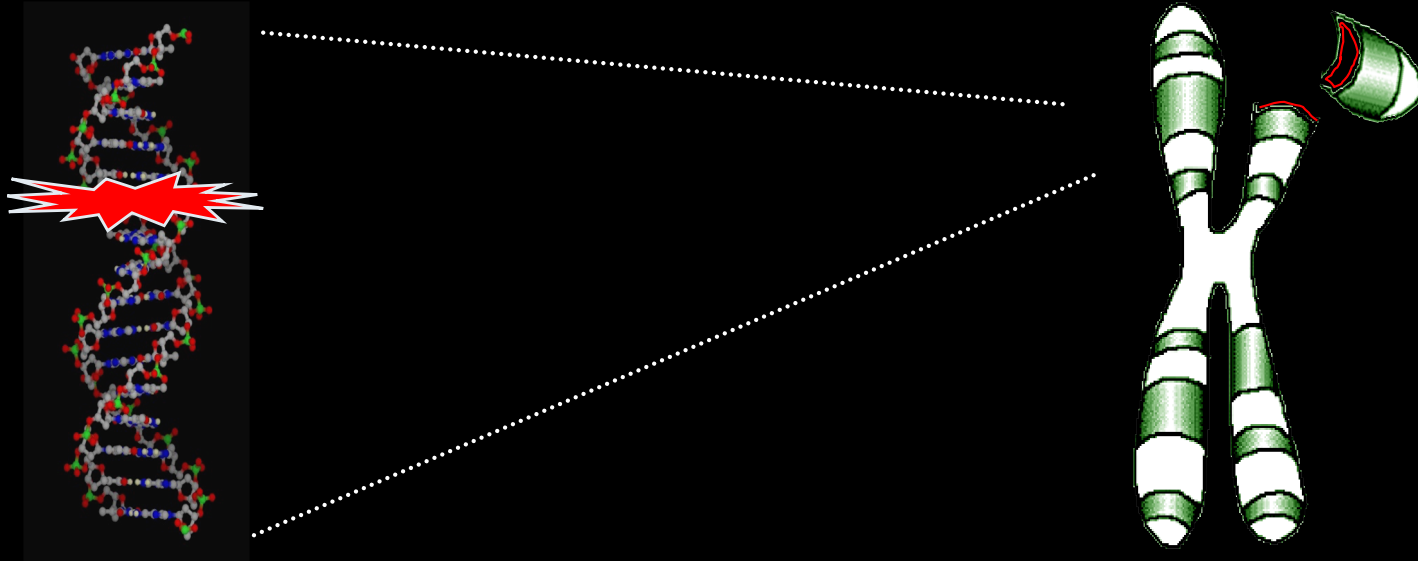


UV LD50 correlates with sunlight exposure



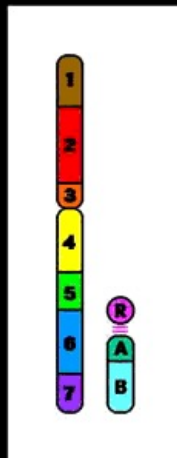
- NER does not correlate with lifespan
- Sun exposure is the main driving force of NER evolution
- Lifespan may play a role if the effect of sunlight is removed

DNA double-strand break

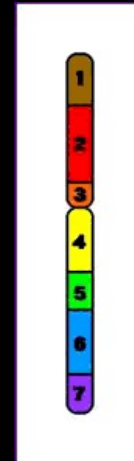


Can lead to genomic rearrangements such as :

Translocation

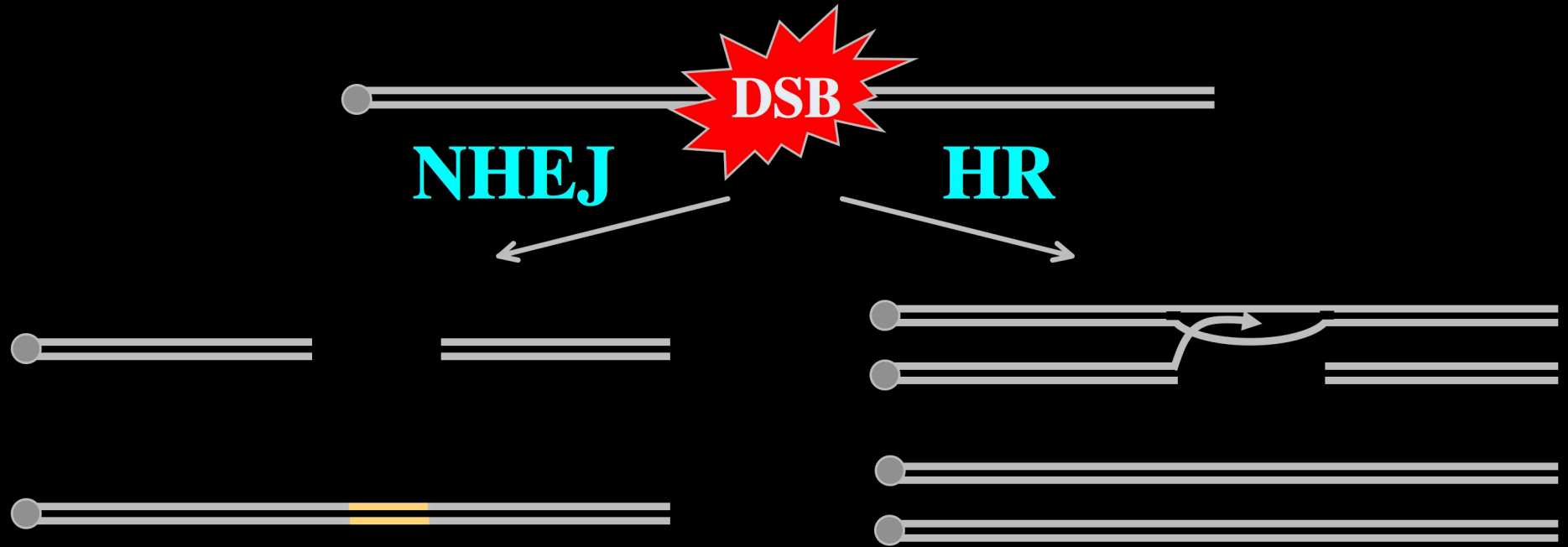


Inversion

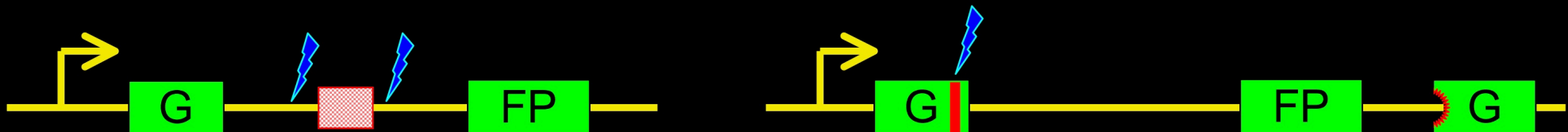


etc.

DNA double-strand break repair



Reporter cassettes for detection of
NHEJ and HR

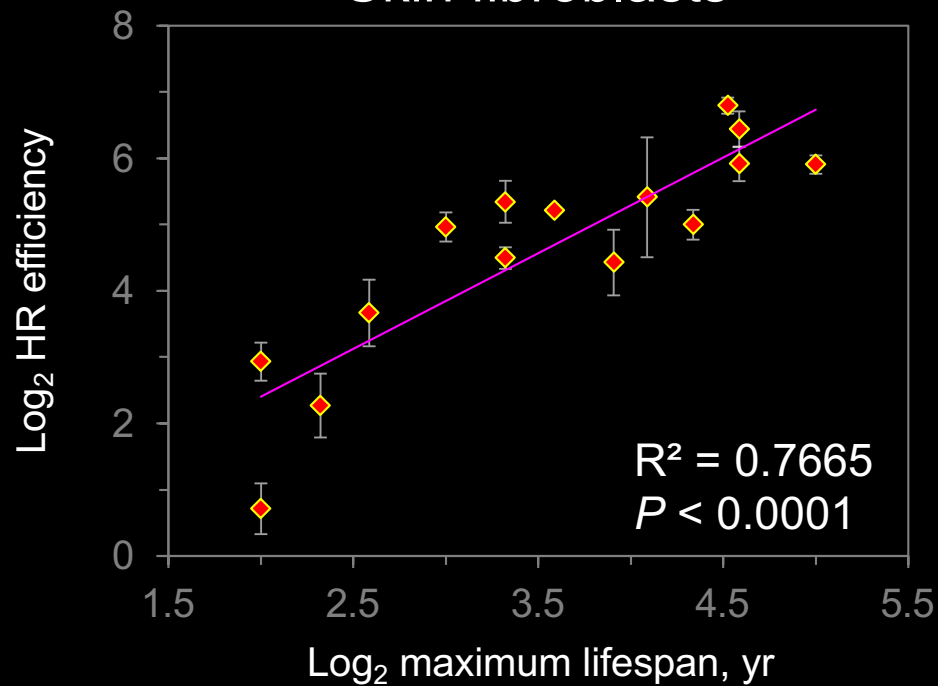




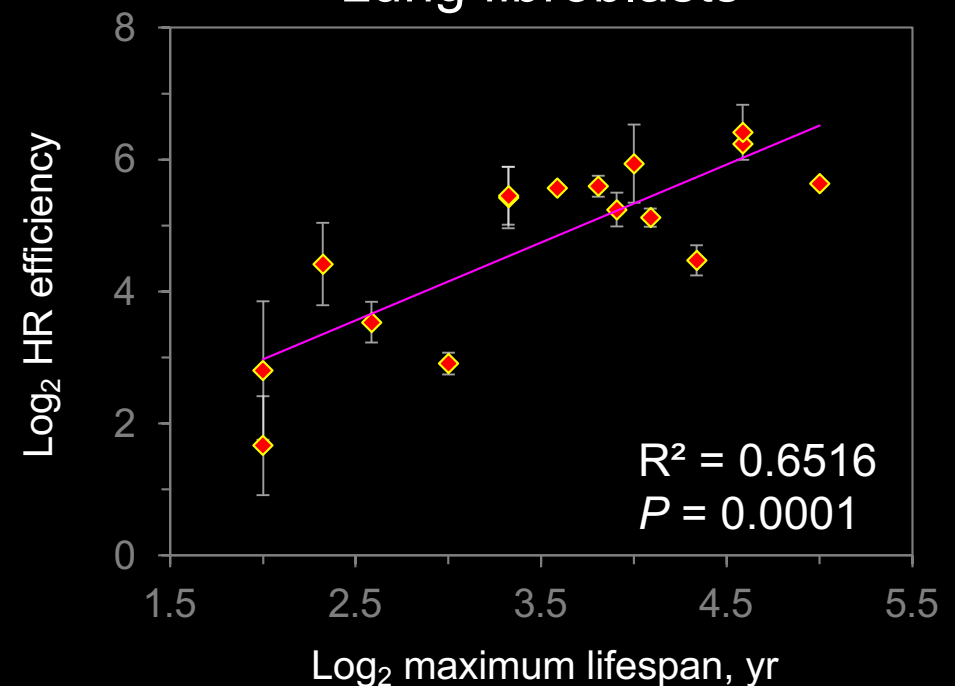
Xiao Tian

HR efficiency correlates with maximum lifespan

Skin fibroblasts

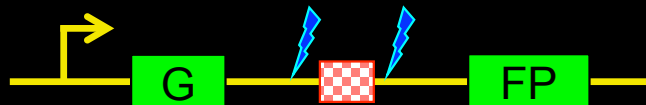
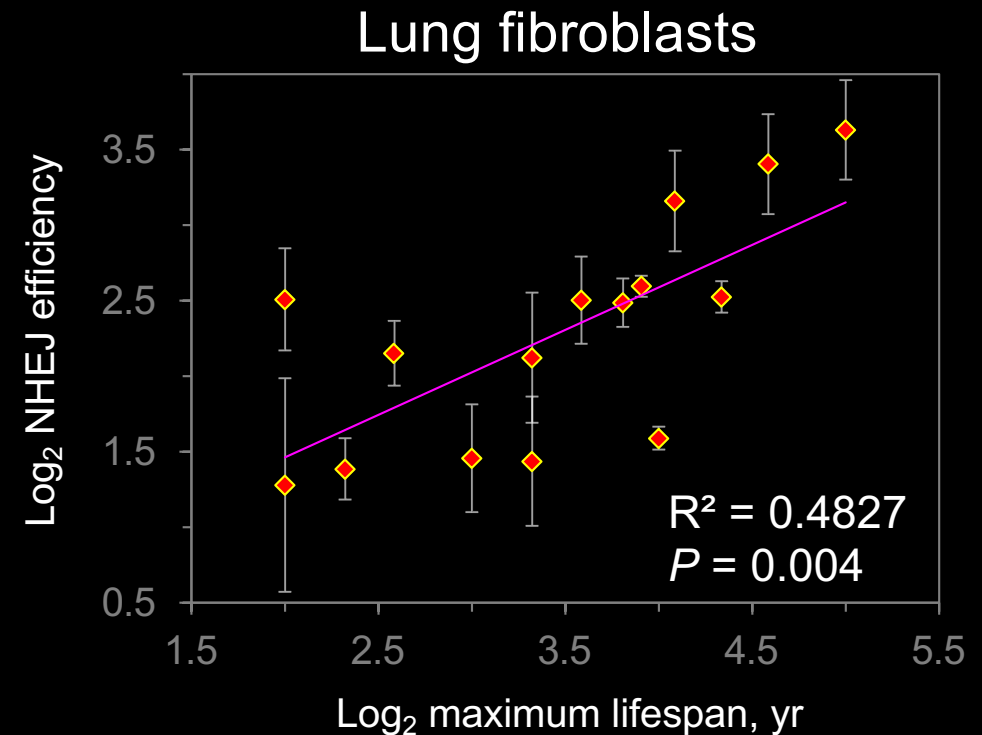
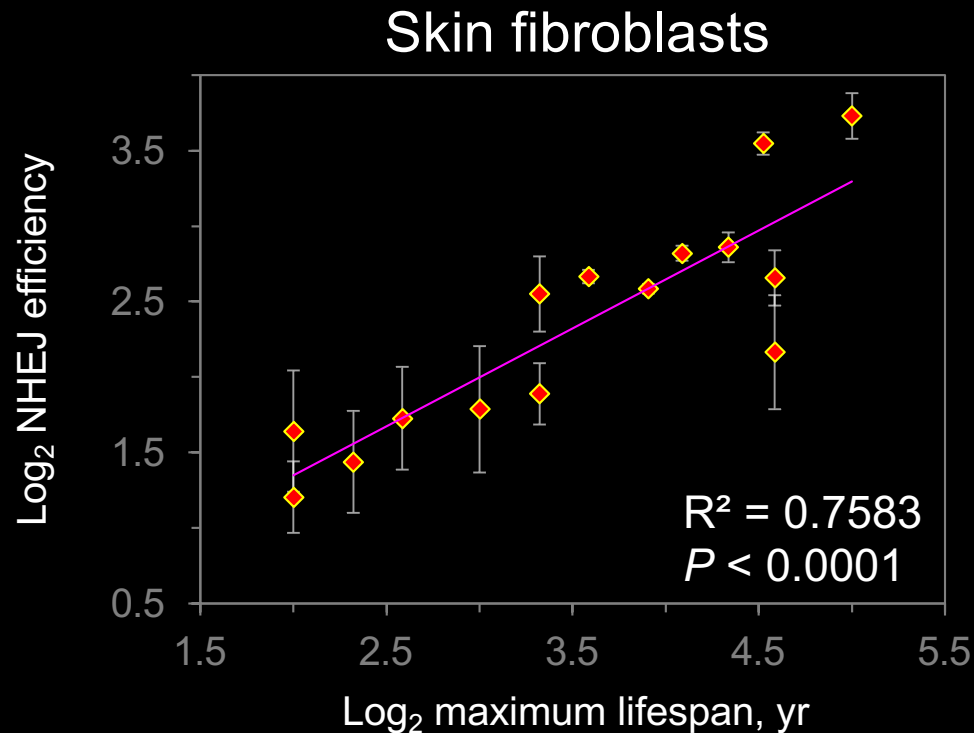


Lung fibroblasts



Tian et al, Cell, 2019

NHEJ efficiency correlates with maximum lifespan



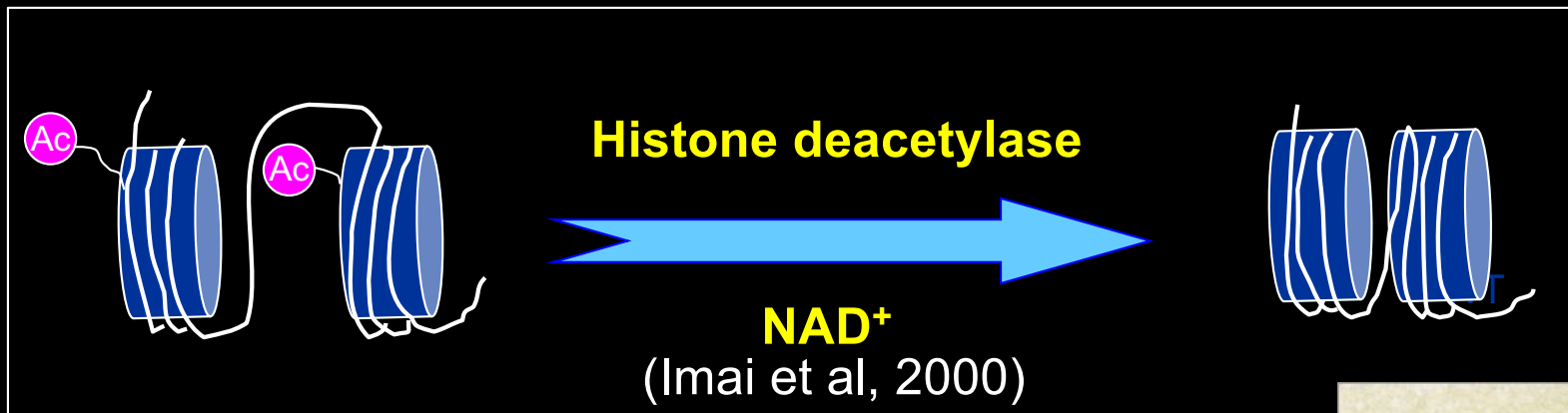
Long-lived species have more efficient DNA repair

- What is the mechanism?



Long-lived species have more efficient DNA repair What is the mechanism?

- **SIRT6** -



- **KO mice exhibit signs of premature aging**
 - **Genomic instability** (Mostoslavsky et al, 2006)
 - **Hypoglycemia** (Zhong et al, 2010)

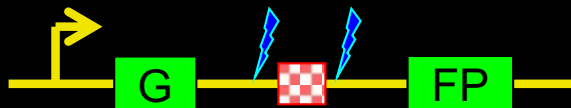
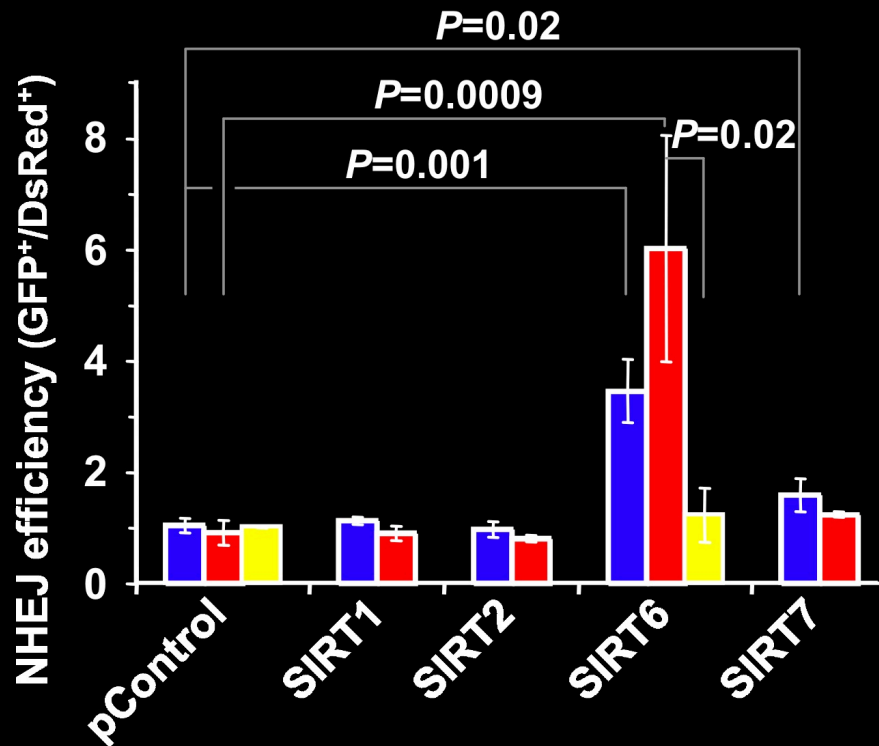


- **Overexpression extends lifespan in mice —**
- MOSES mice** (Kanfi et al, 2012)

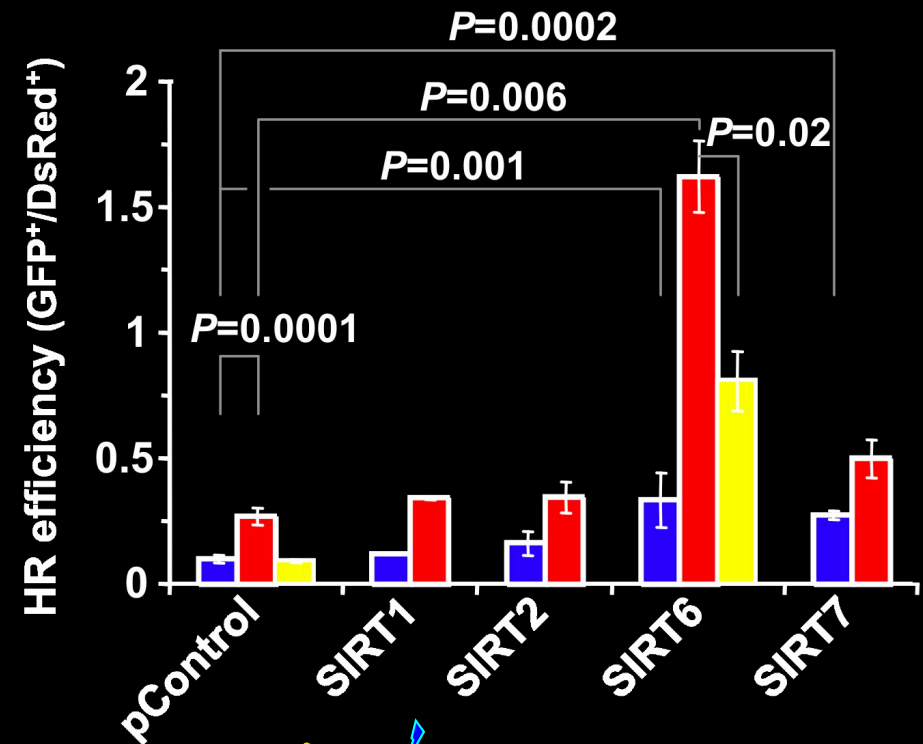


SIRT6 stimulates DNA double strand break repair

Nonhomologous end joining



Homologous recombination



■ Untreated

■ Paraquat

■ Paraquat + NAM

SIRT6 in DSB repair

Oxidative stress



JNK

P

S10

SIRT6

ADP

SNF2H

Mostoslavsky Lab

K521
PARP1

PAR

DNA Repair

HR
NHEJ
BER
NER

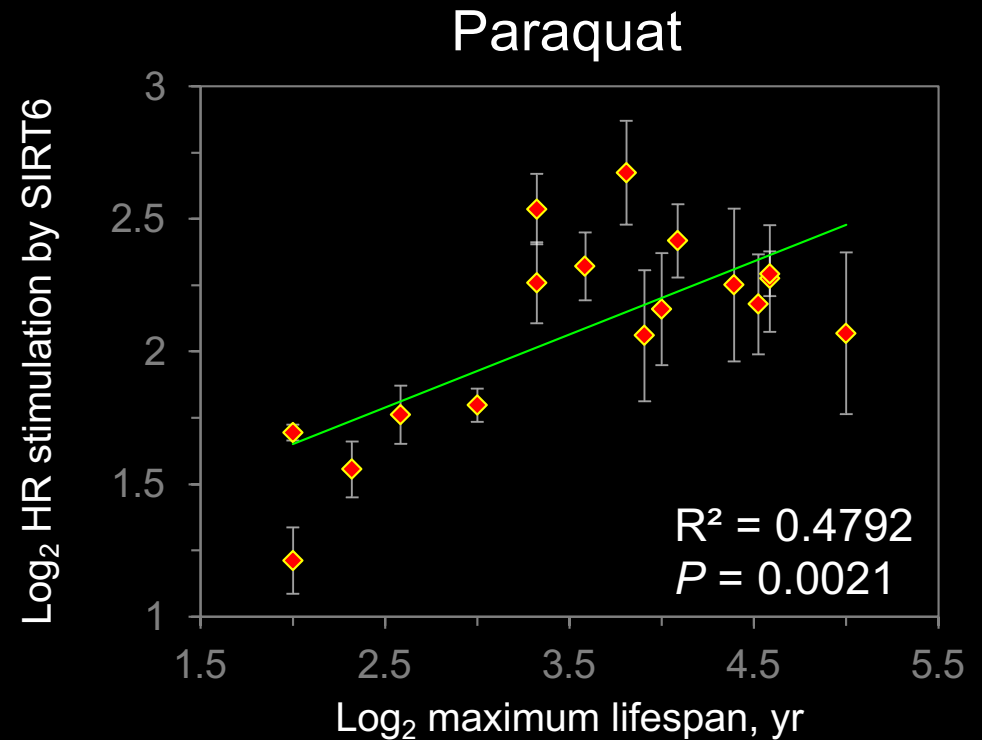
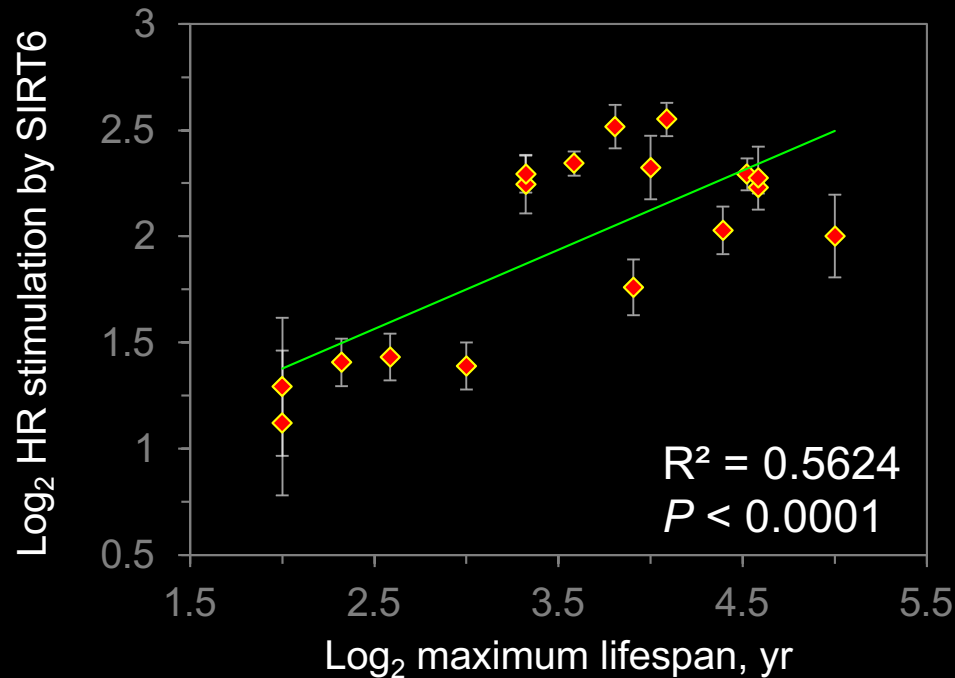


Mao et al. Science, 2011

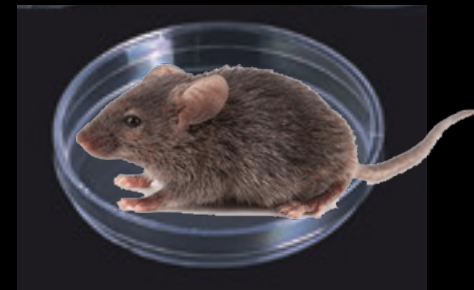
Mao et al. PNAS, 2012

Van Meter et al. Cell Rep., 2016

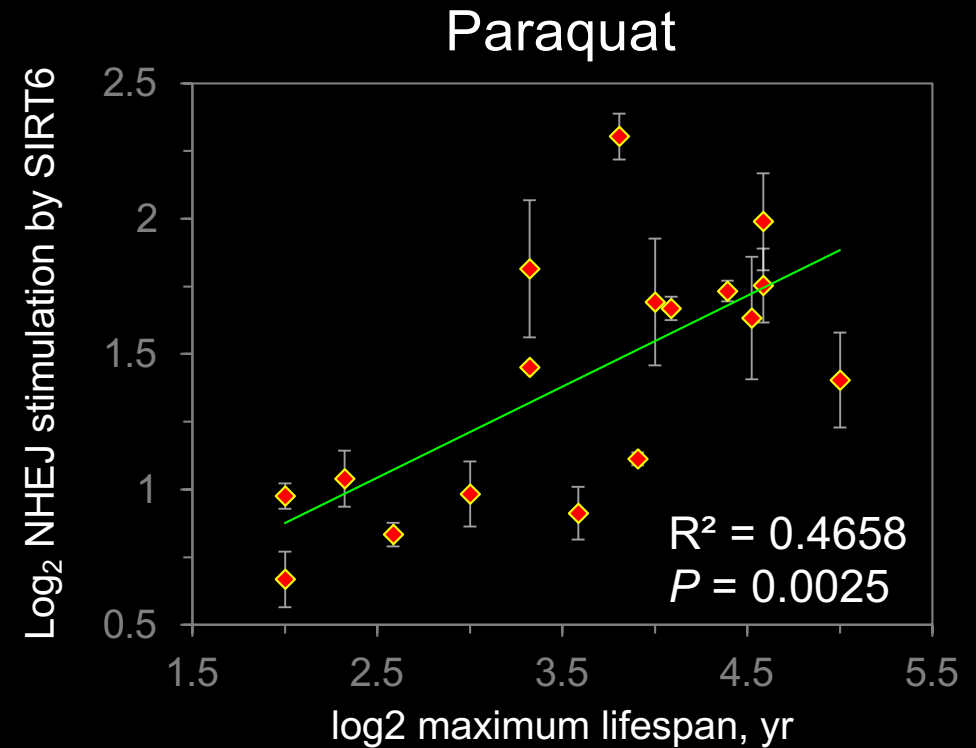
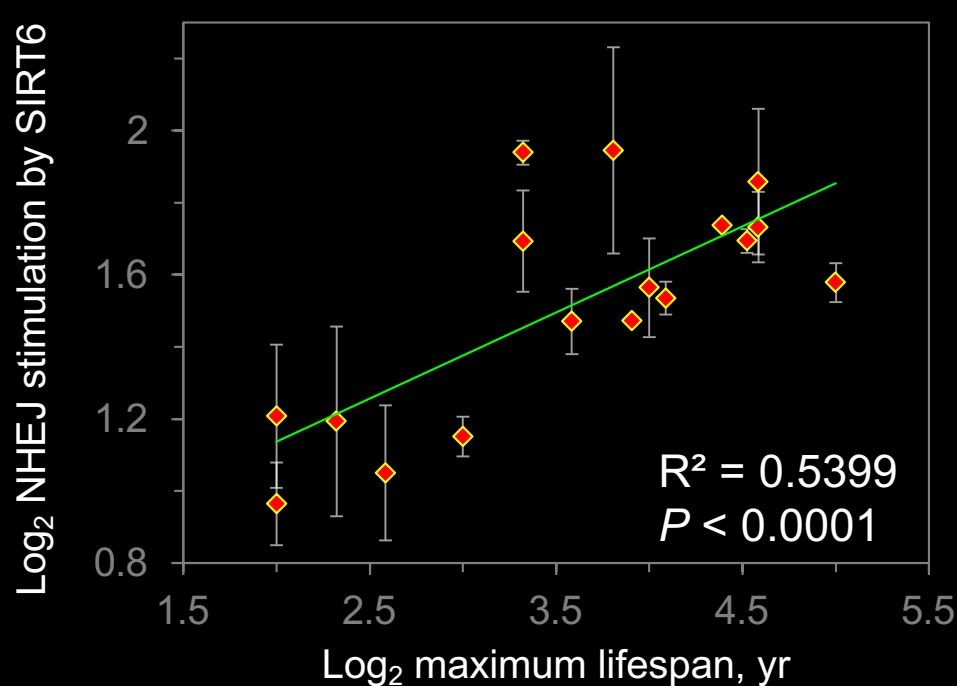
SIRT6 from long-lived species is better at stimulating HR in mouse cells



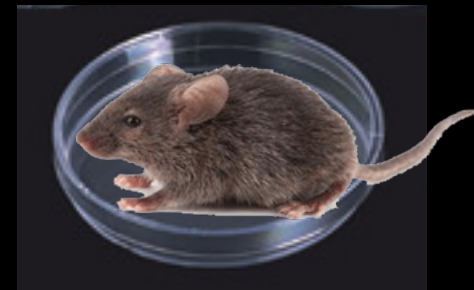
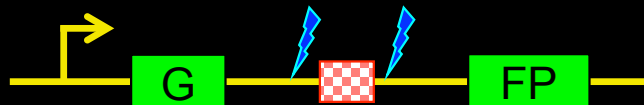
Tested in mouse cells
with integrated HR reporter



SIRT6 from long-lived species is better at stimulating NHEJ in mouse cells



Tested in mouse cells
with integrated NHEJ reporter



Two extremes



MLS: 4 years

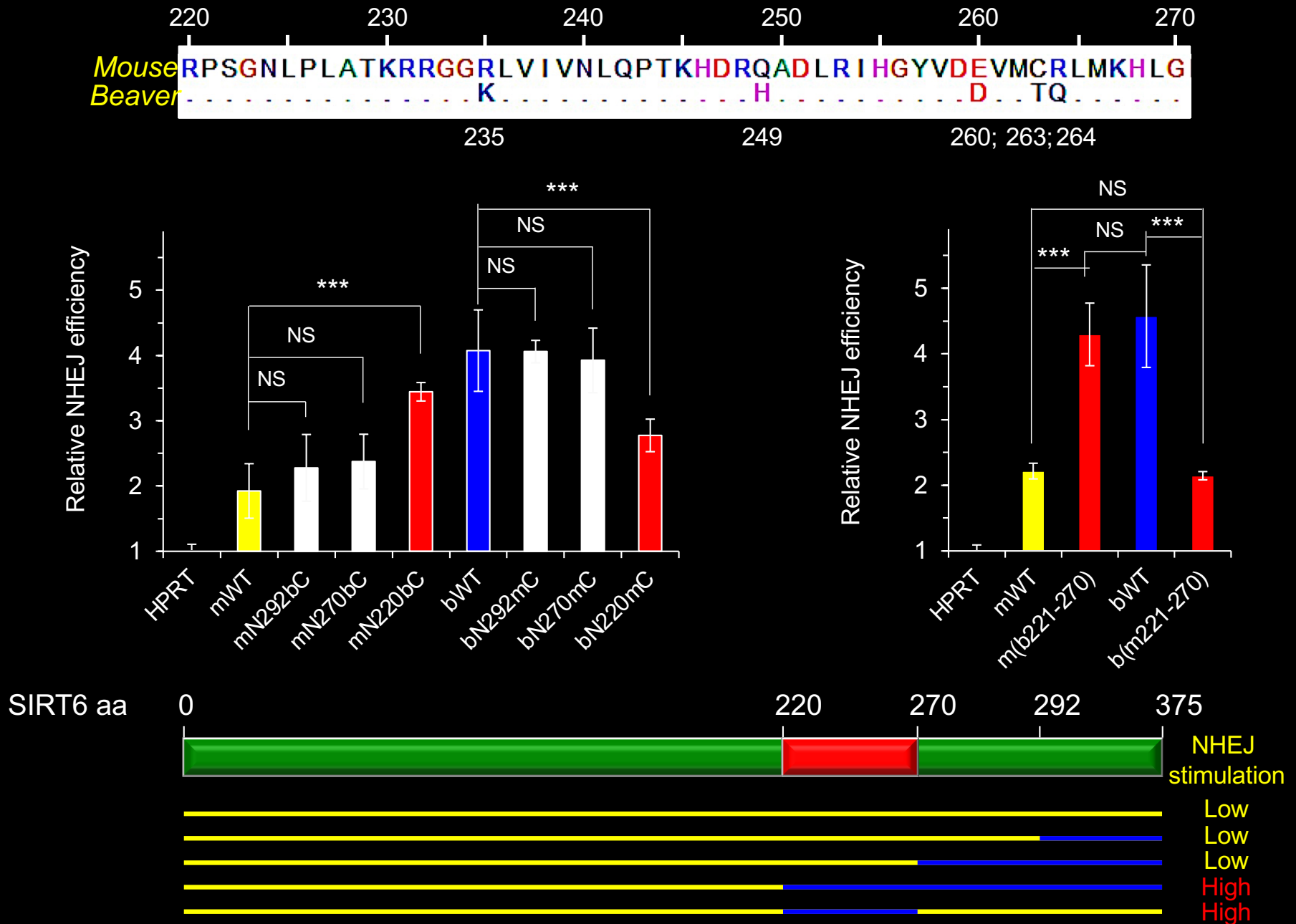
Worst SIRT6



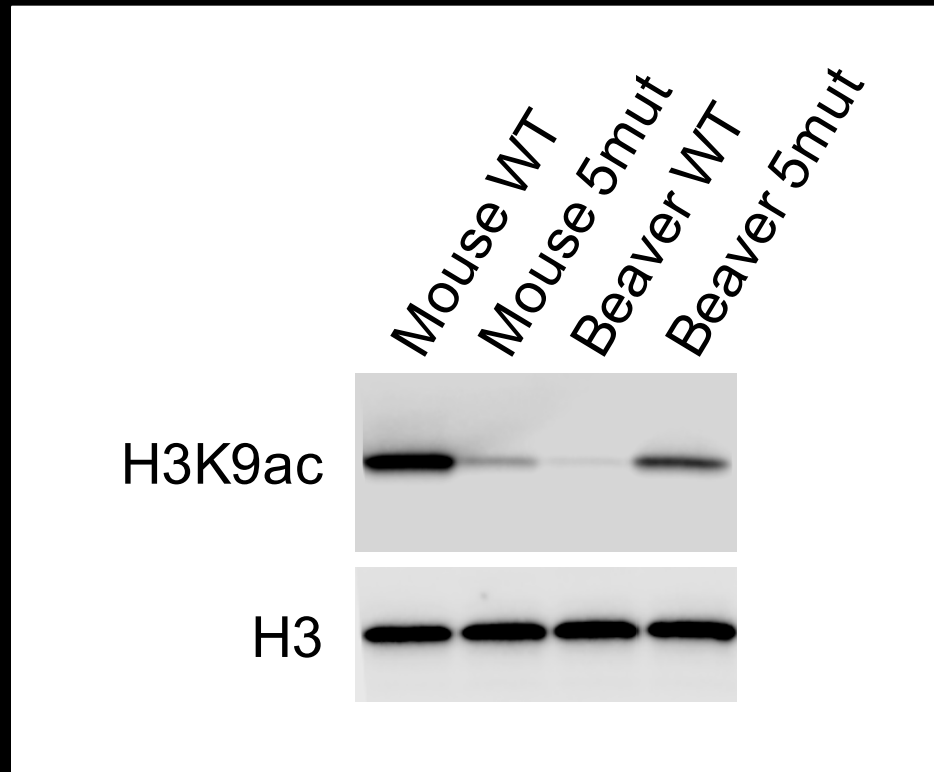
MLS: 24 years

One of the best SIRT6

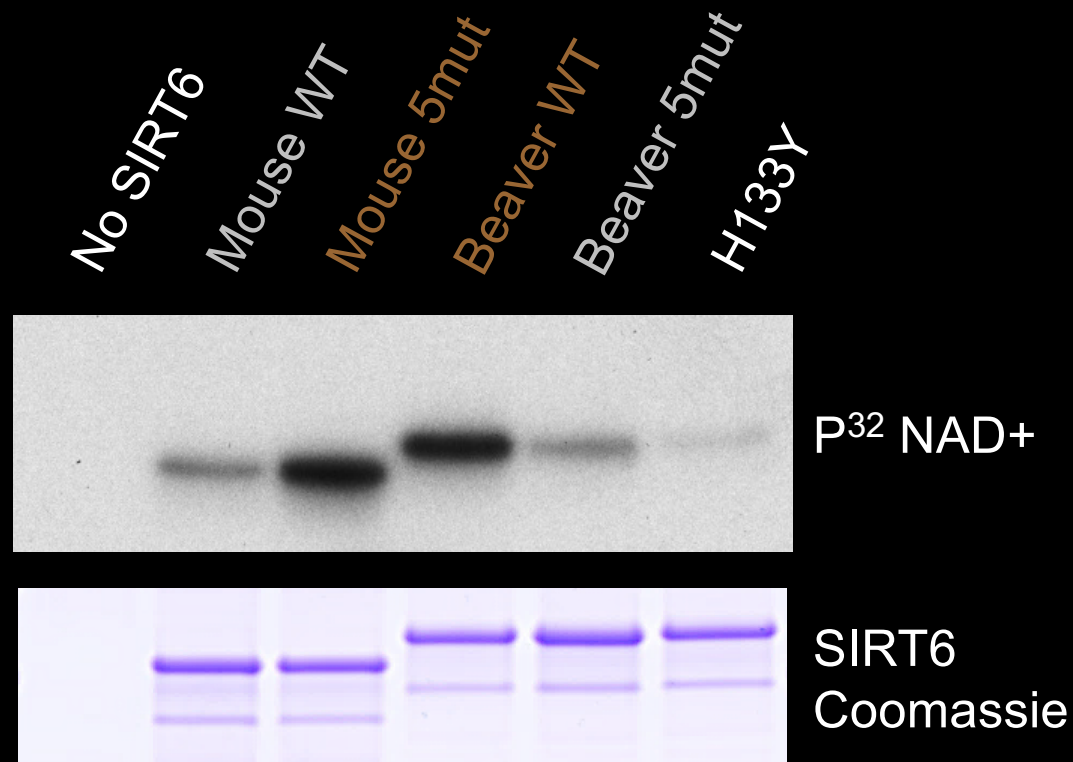
SIRT6 region 220-270 is responsible for the difference in DNA repair between mouse and beaver



Deacetylation activity of mouse and beaver SIRT6

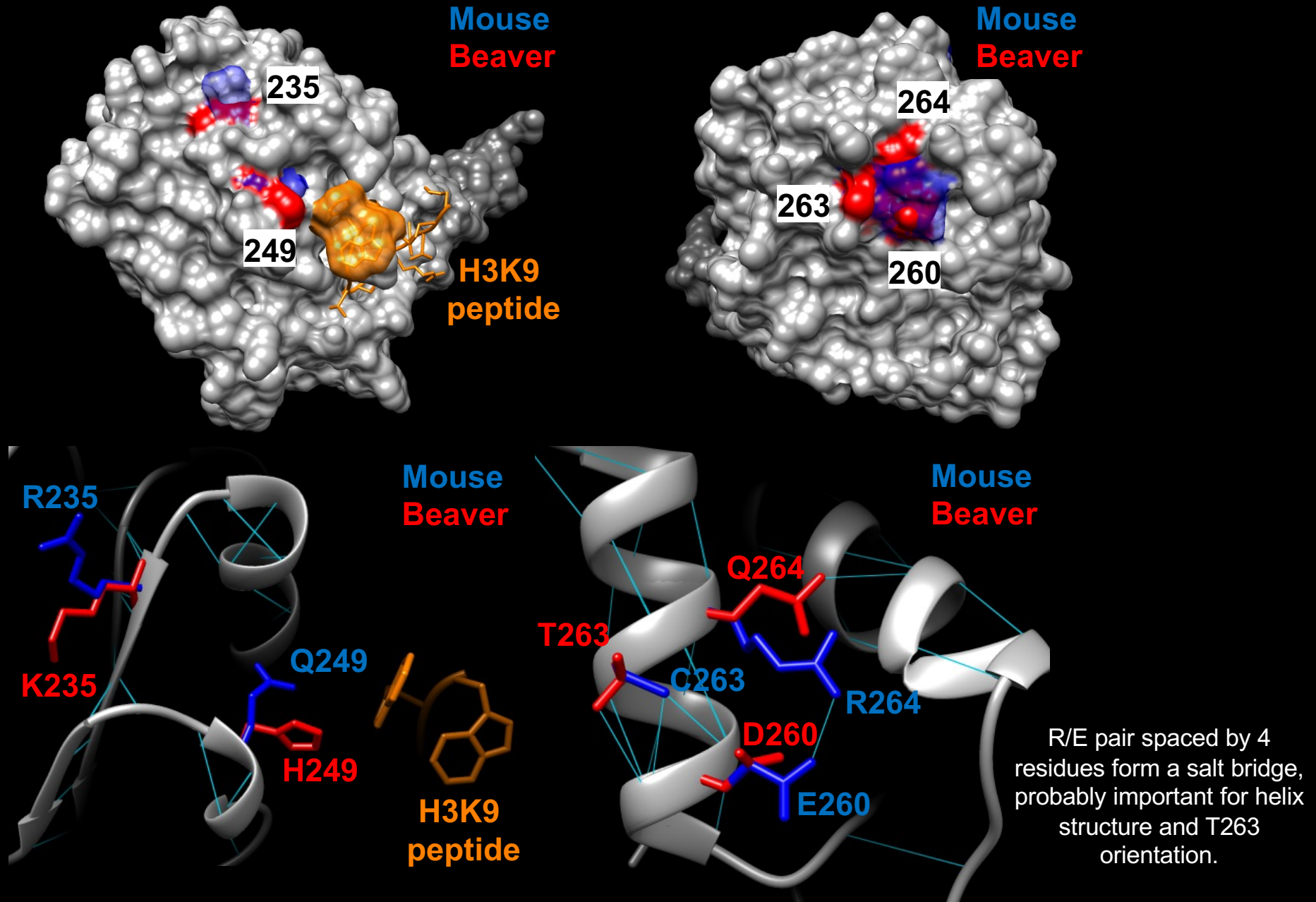


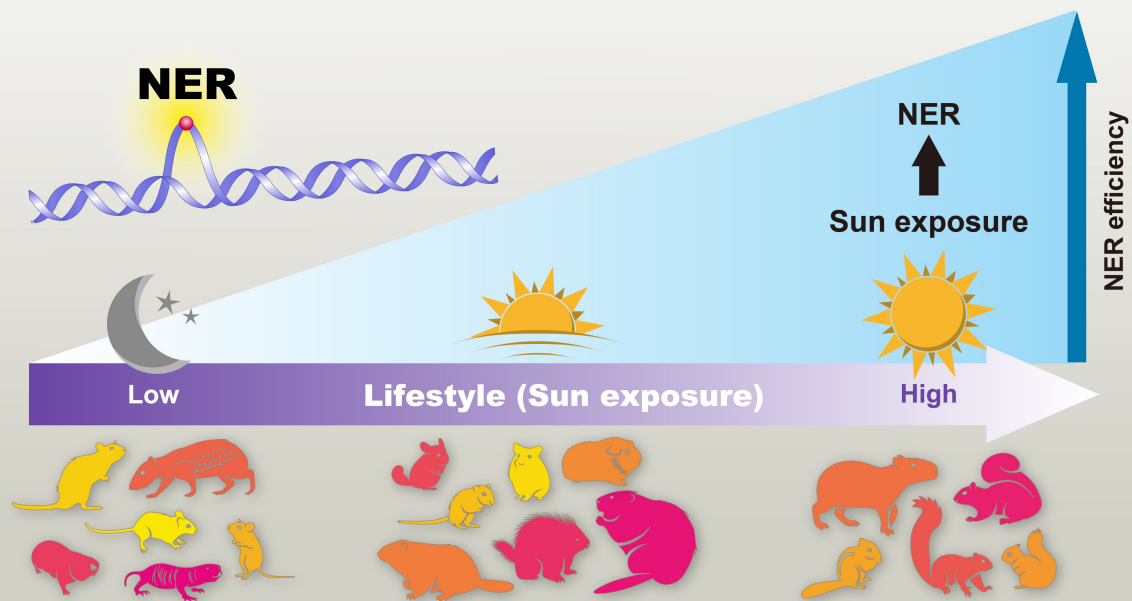
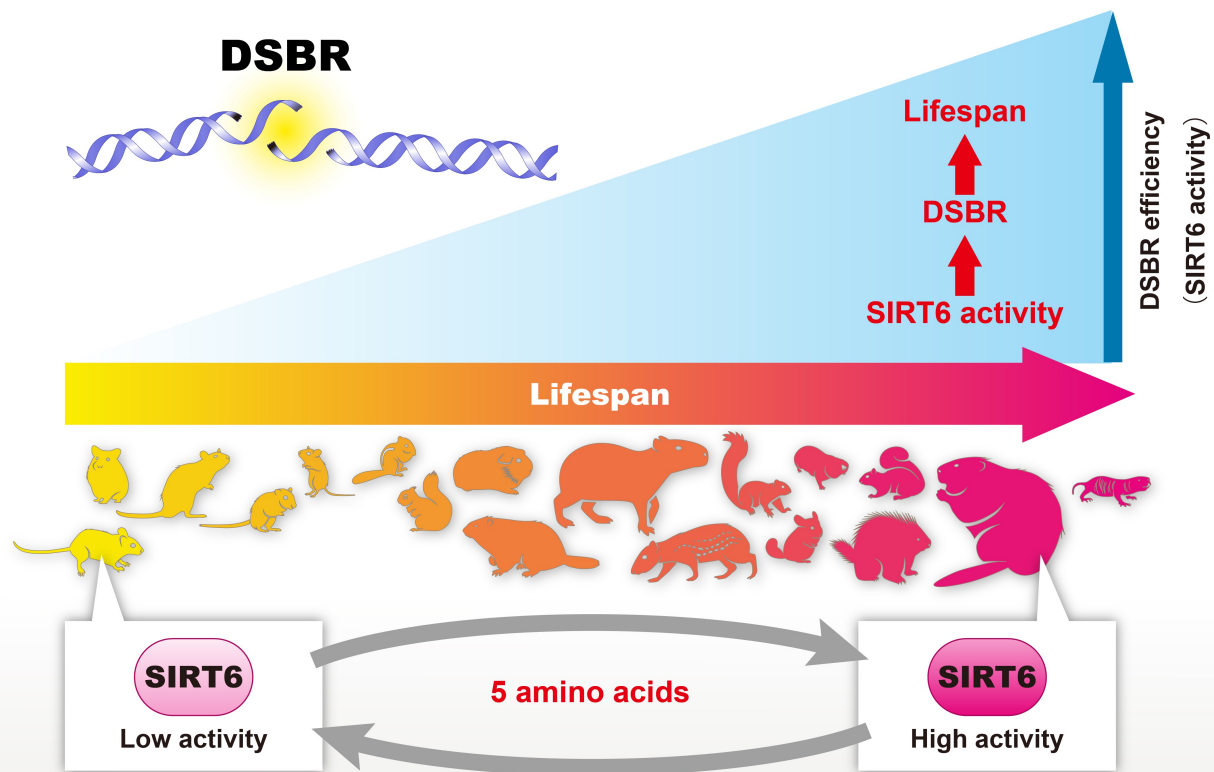
Five beaver aa increase mono-ADP-ribosylation activity of SIRT6



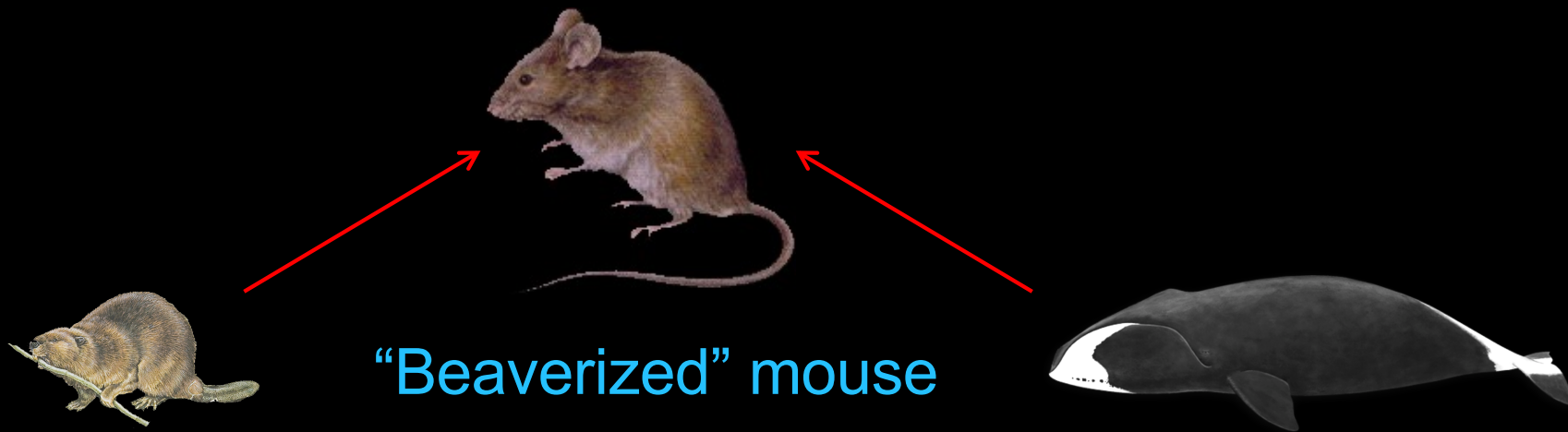
Tian et al, Cell, under review

The five aa responsible for DNA repair difference are surface exposed in SIRT6 structure





- Long-lived species evolve more efficient DNA DSB repair
- SIRT6 is primarily responsible for the differences in DNA DSB repair
- Five aa determine the difference in SIRT6 activity between mouse and beaver
- Can improved DNA repair extend lifespan?



bleed
trim

Cell

Cell

Volume 123
Number 4

Month x, year

www.cell.com



Volume 123 Number 4 Pages xxx-xxx • Month Day, Year



Matt Simon

Human Centenarian SIRT6 Allele

N-terminus

Catalytic Core Domain

C-terminus

355aa



Asparagine to Lysine (N308K)

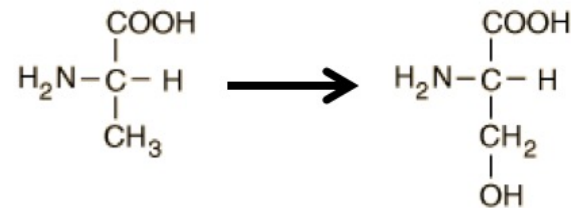


Polar $\rightarrow$ Charged(+)

EESPTR**ING**SIP**AG**PKQEP WT

EESPTR**K**GSIP**S**GPKQEP Centenarian

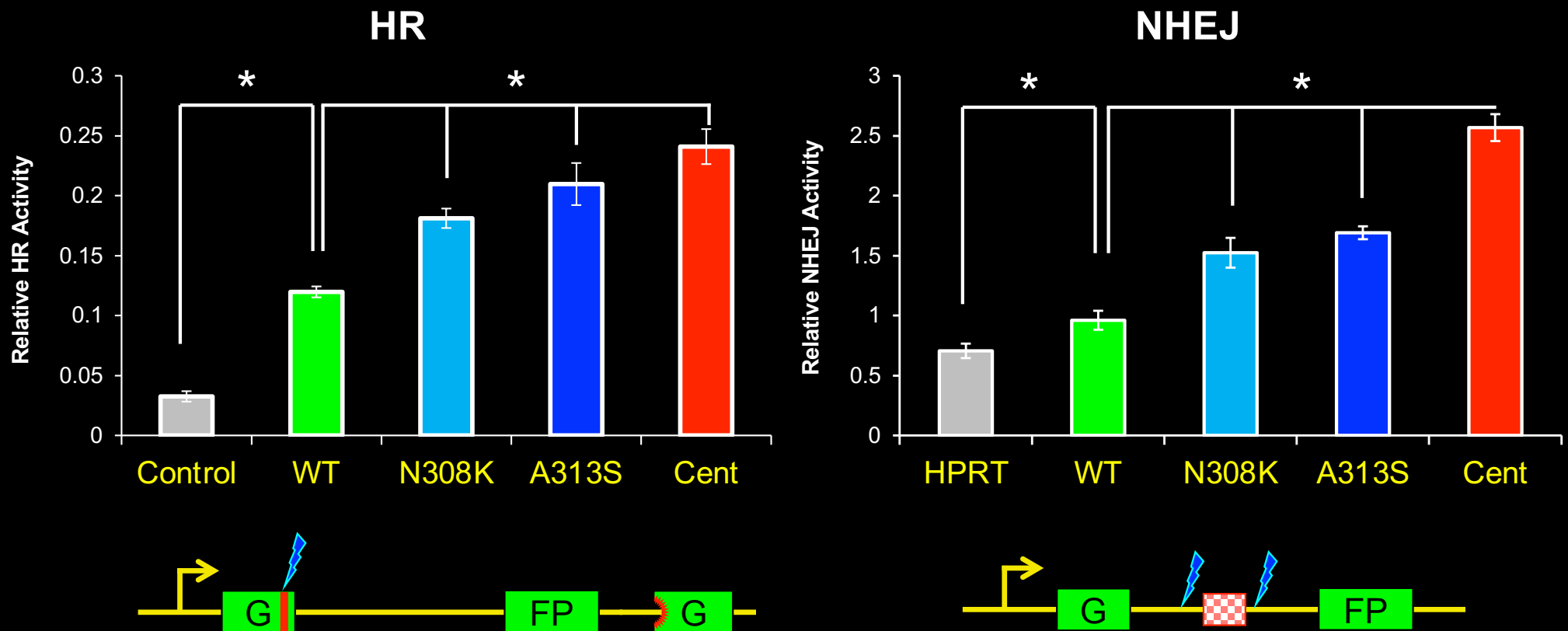
Alanine to Serine (A313S)



Hydrophobic $\rightarrow$ Polar

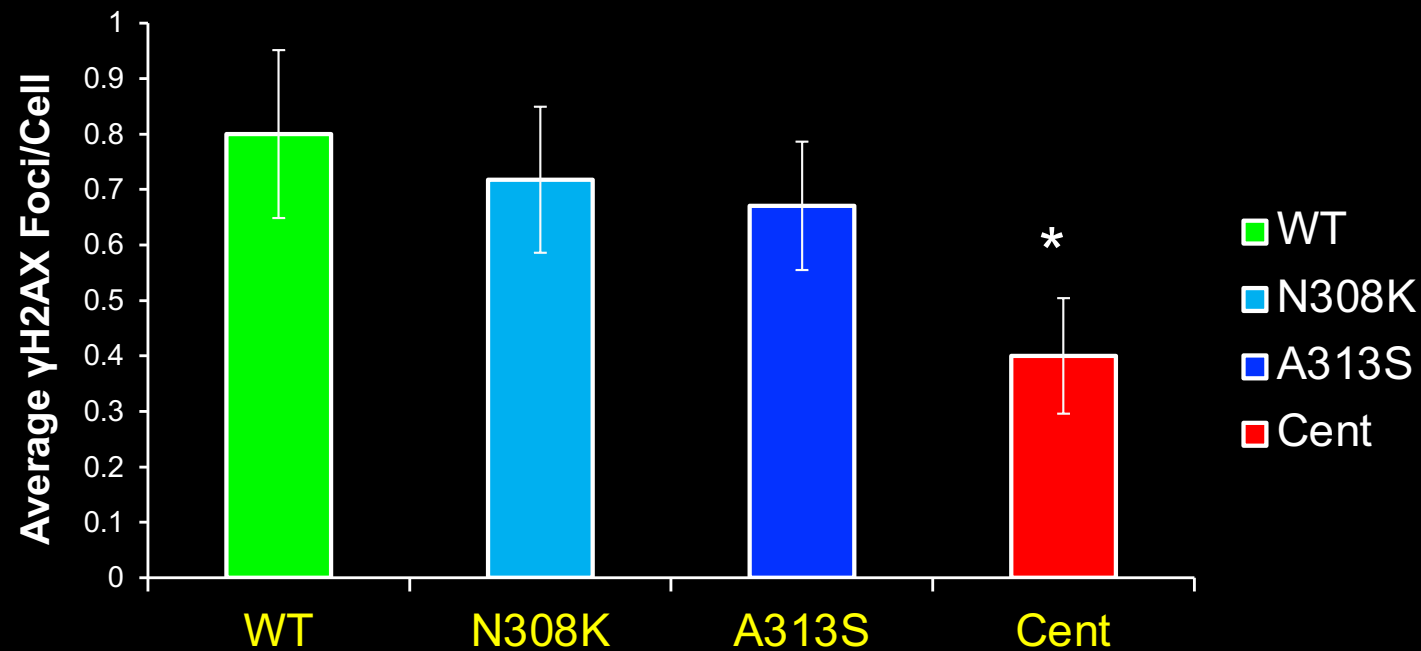
With Yousin Suh

DSB repair Efficiency is Elevated in Centenarian SIRT6 Allele



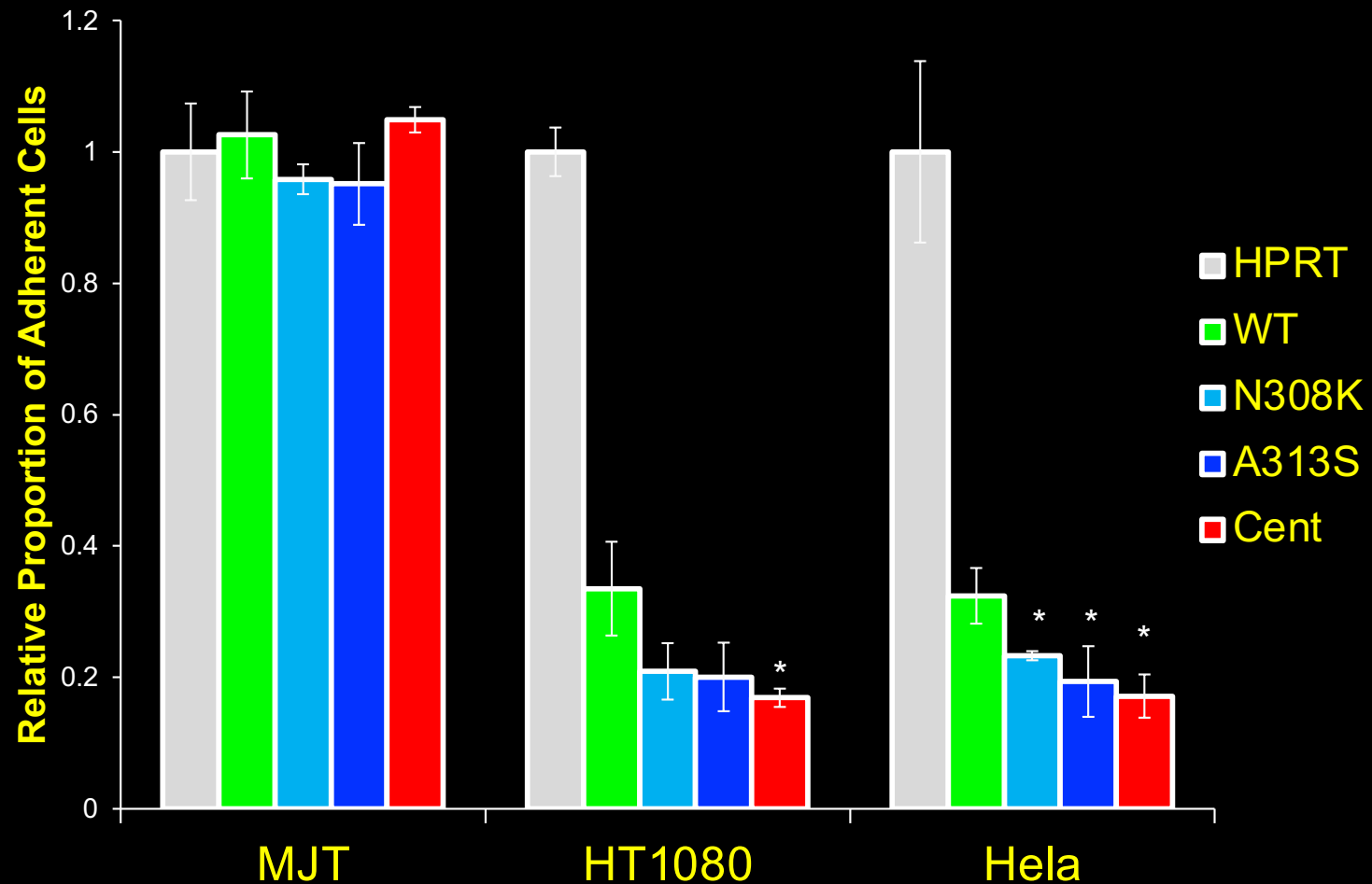
Data compiled from 5 separate transfections over 2 separate experiments

Basal γ H2AX foci are reduced MJT KO cells expressing centenarian SIRT6 variants



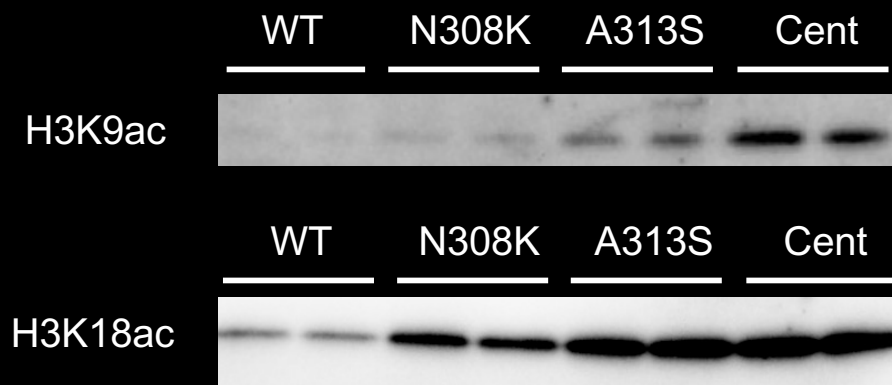
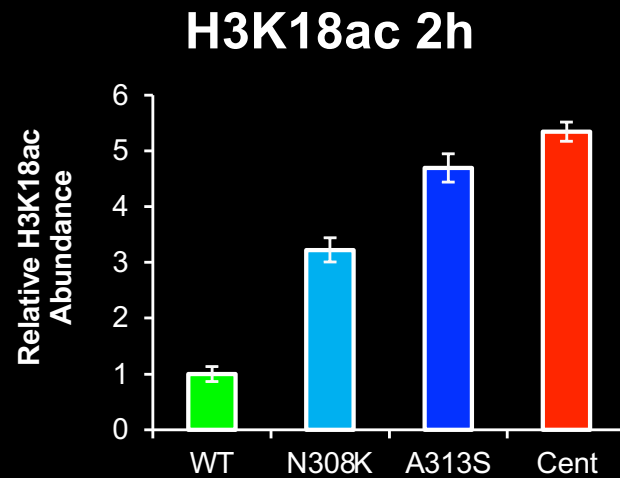
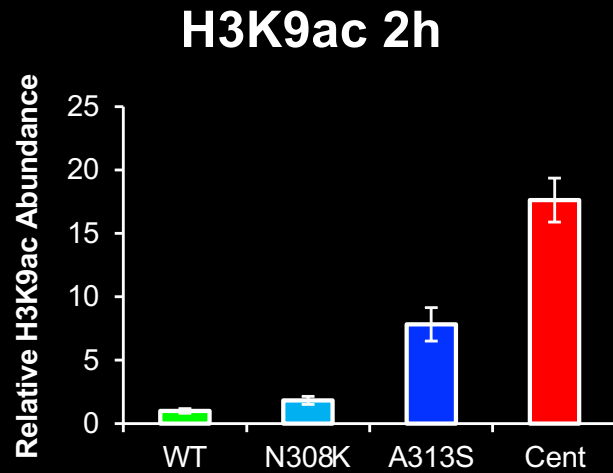
80+ cells assayed per cell type, cumate dosage tailored for equivalent SIRT6 expression

Centenarian allele enhances SIRT6-mediated cell death in cancer cells

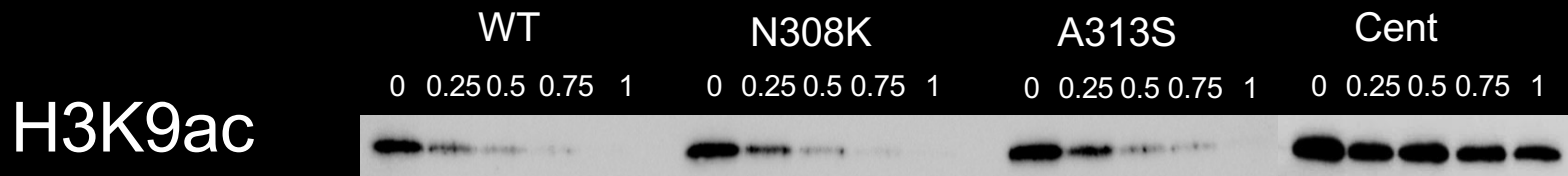
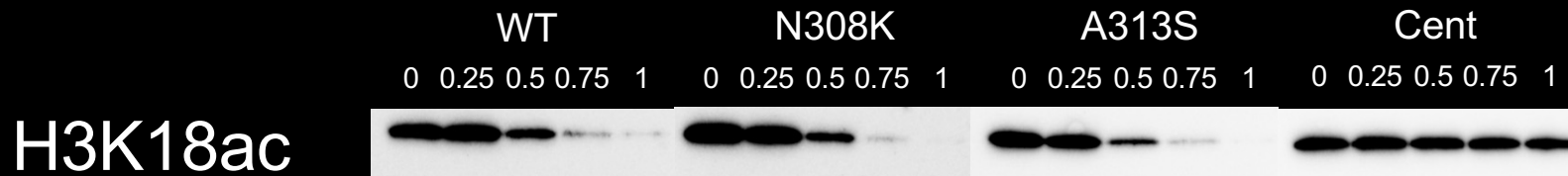
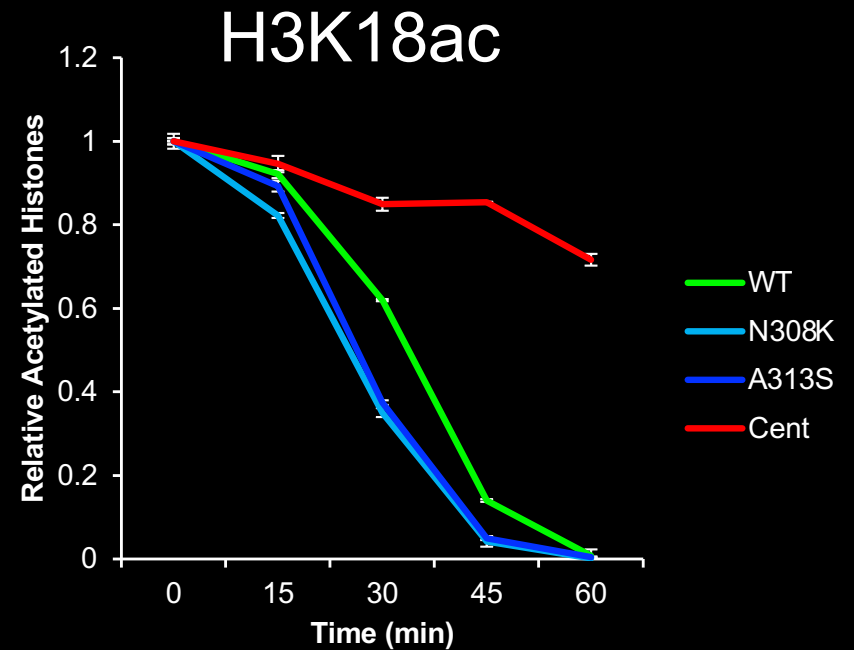
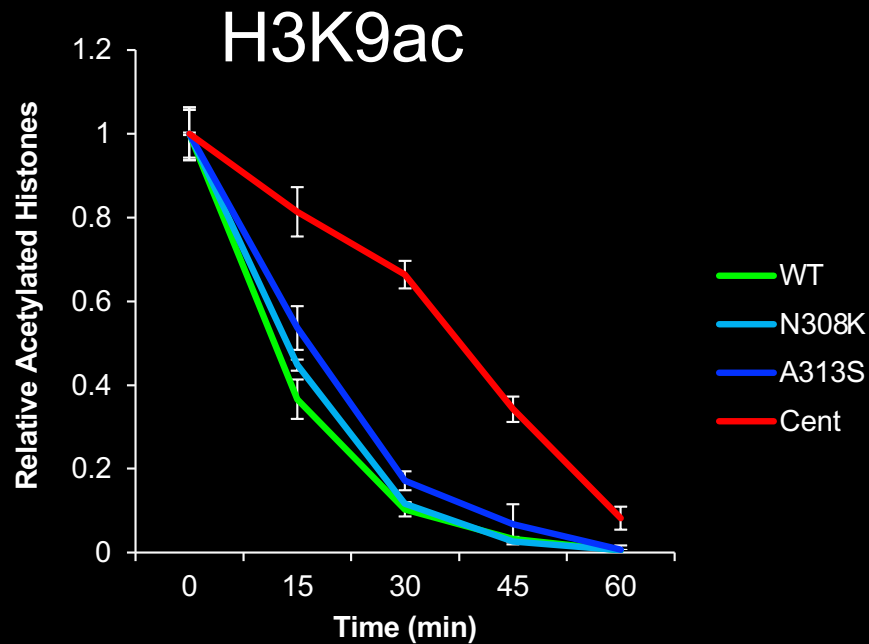


3 wells per trial (n), error bars = SD, significance calculated compared to WT

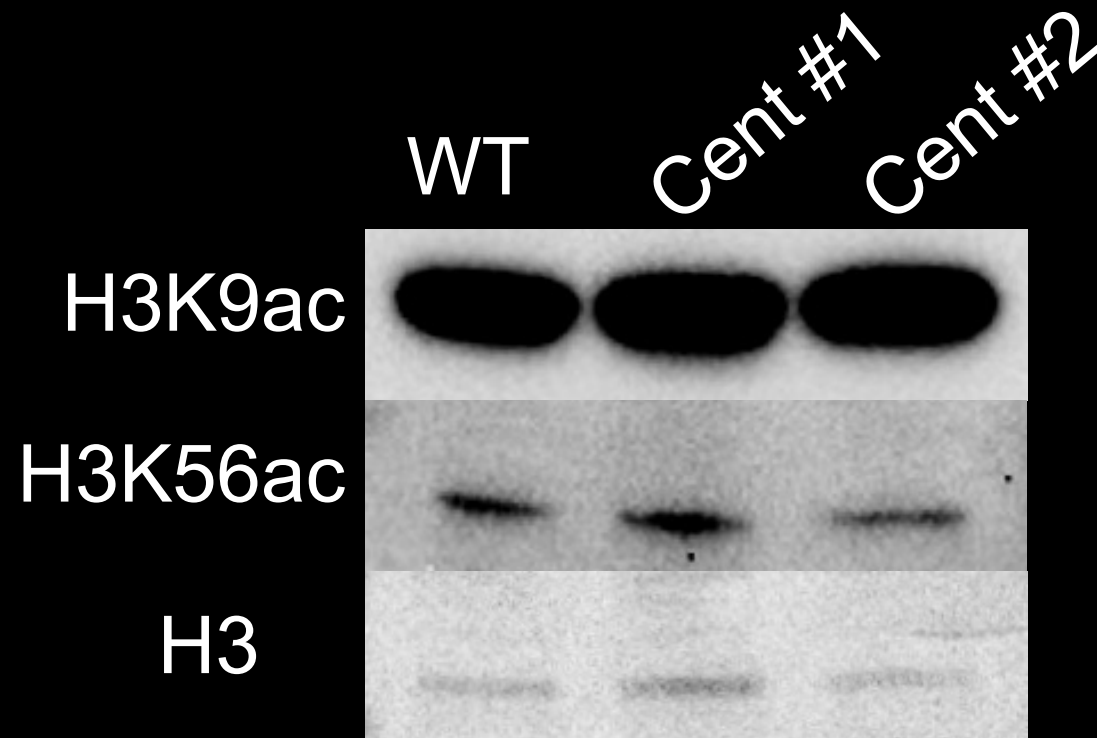
Centenarian SIRT6 shows reduced deacetylase activity



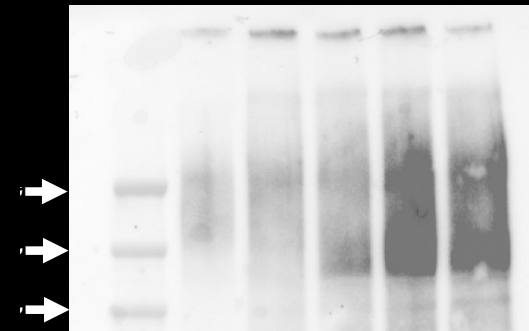
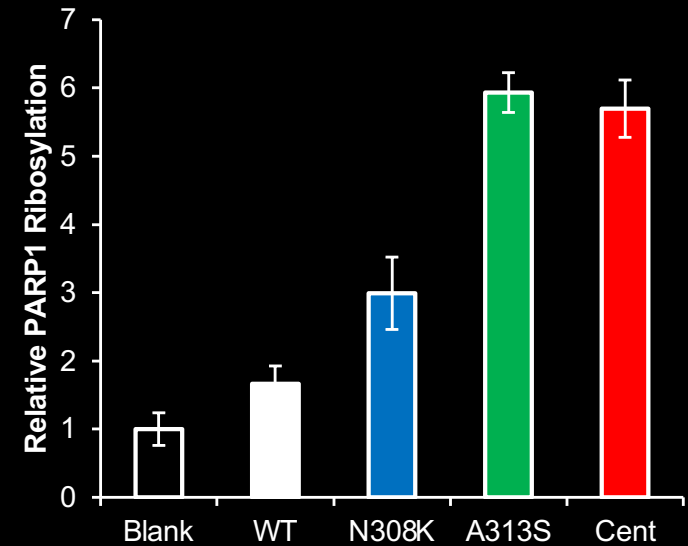
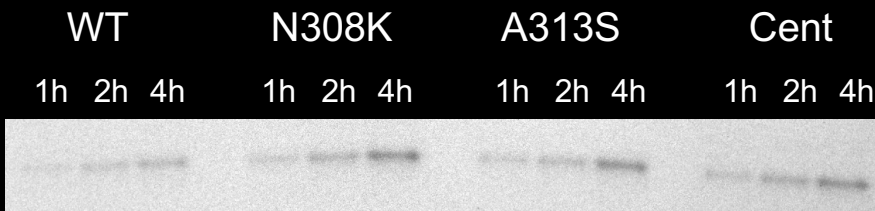
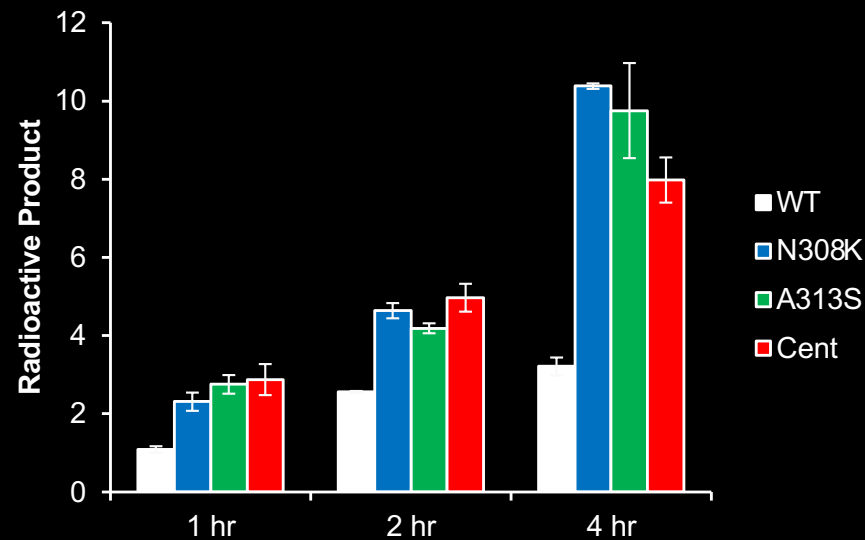
Centenarian SIRT6 shows reduced deacetylation rate



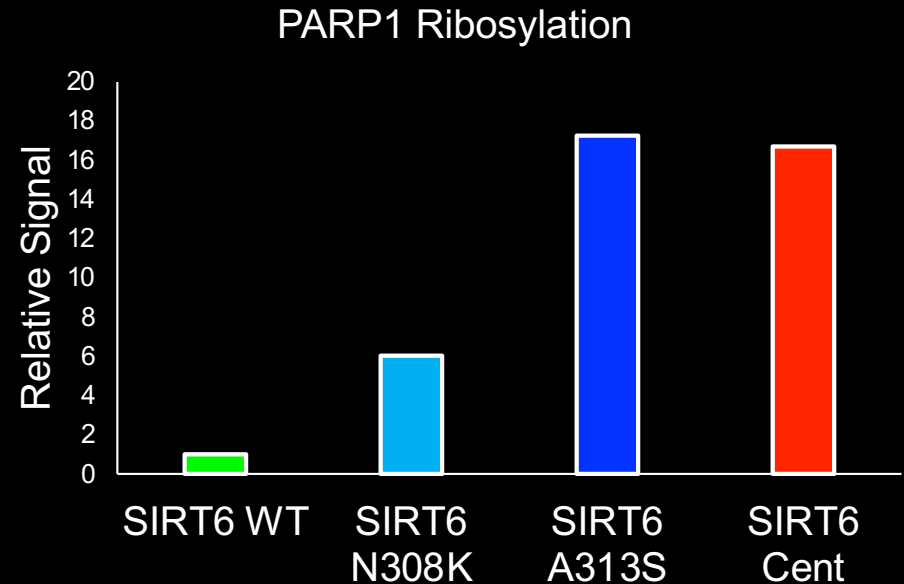
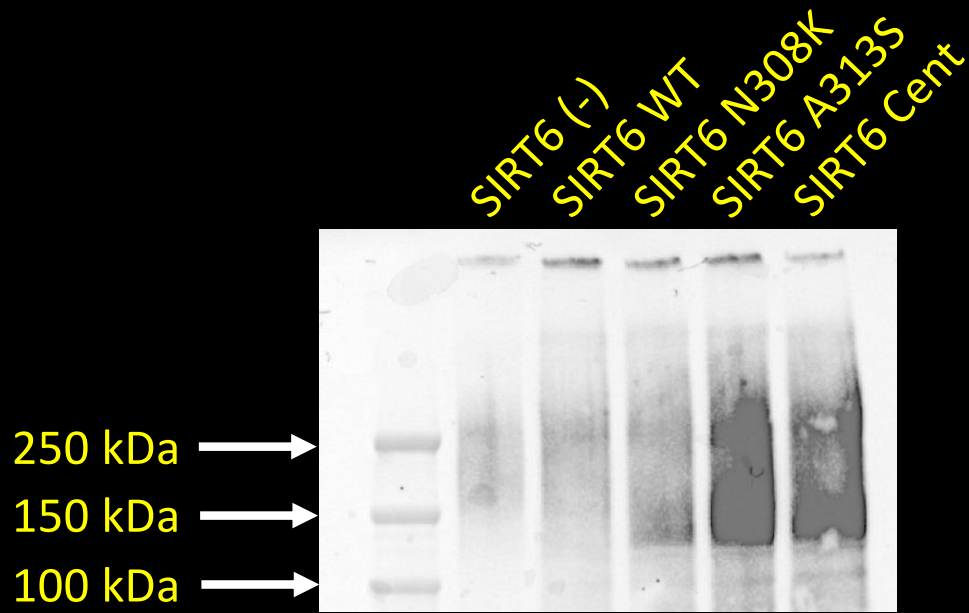
H3 acetylation in centSIRT6 MSC cell lines



Centenarian SIRT6 alleles have higher auto-ribosylation activity



Centenarian SIRT6 has higher PARP1 ribosylation activity

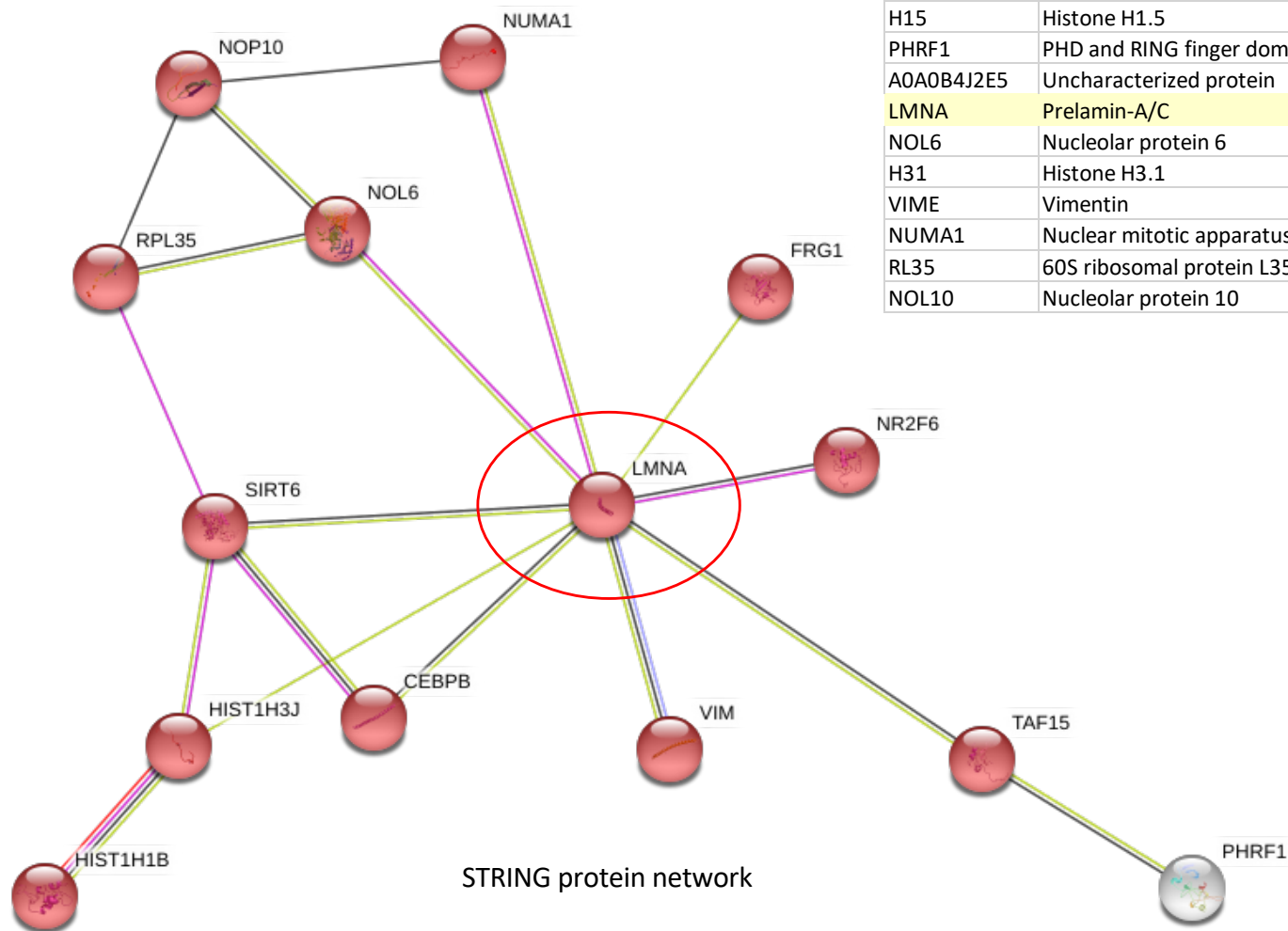


Antibody is against poly(ADP-ribose)

SIRT6 CENT allele interacts more strongly with LMNA under basal conditions

(Pull-down with anti-SIRT6; Quantitative MS with Tandem Mass Tags)

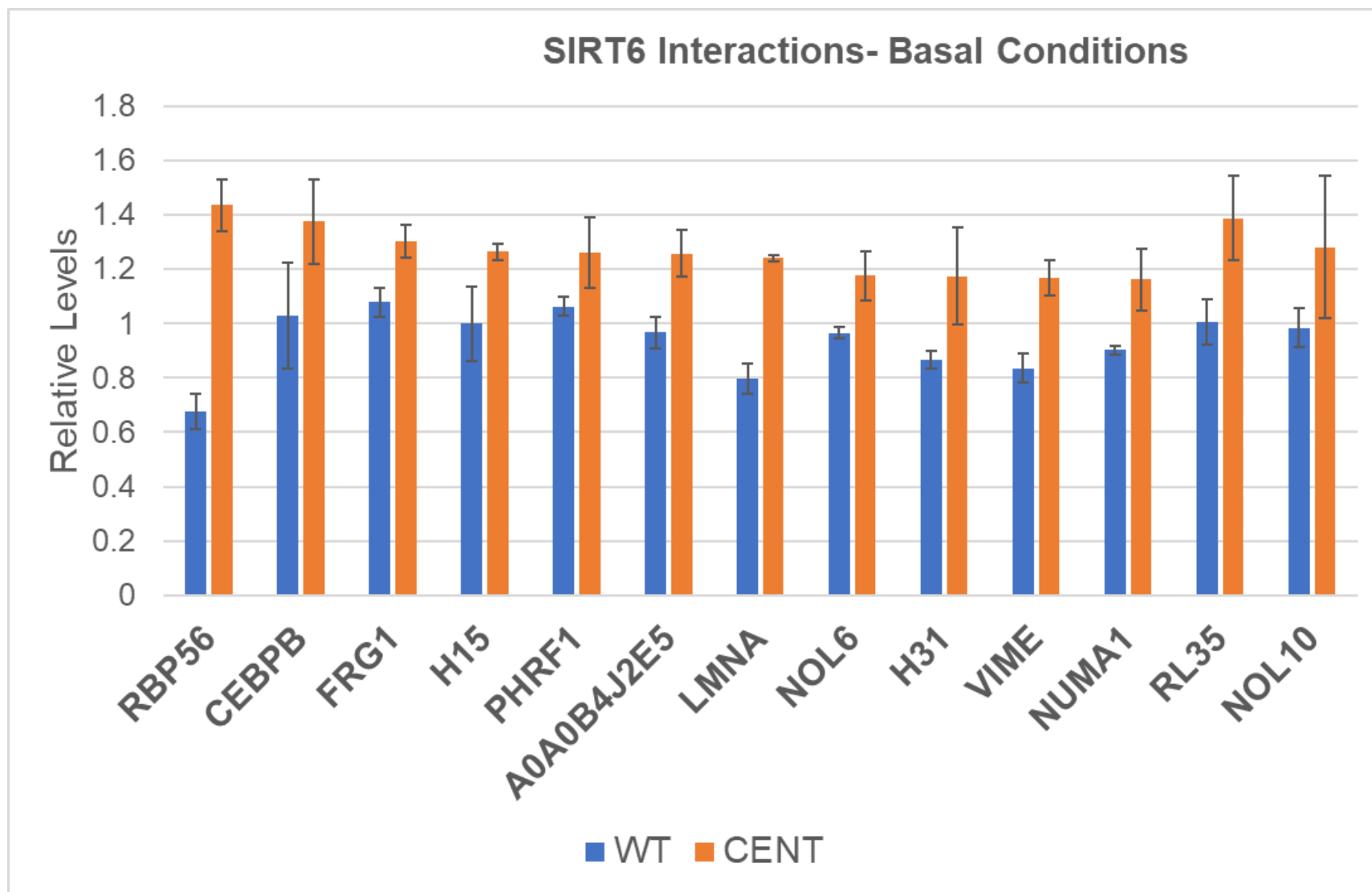
Name	Description	# Peptides	p (CENT vs WT)
RBP56	TATA-binding protein-associated factor 2N	5	0.0002
CEBPB	CCAAT/enhancer-binding protein beta	1	0.0119
FRG1	Protein FRG1	2	0.0336
H15	Histone H1.5	11	0.0516
PHRF1	PHD and RING finger domain-containing protein 1	3	0.0445
A0A0B4J2E5	Uncharacterized protein	3	0.0354
LMNA	Prelamin-A/C	38	0.0025
NOL6	Nucleolar protein 6	2	0.0250
H31	Histone H3.1	3	0.0534
VIME	Vimentin	40	0.0188
NUMA1	Nuclear mitotic apparatus protein 1	2	0.0227
RL35	60S ribosomal protein L35	4	0.0056
NOL10	Nucleolar protein 10	1	0.0581



Cellular Component (GO)			
GO-term	description	count in gene set	false discovery rate
GO:0031981	nuclear lumen	13 of 4030	2.17e-06

SIRT6 CENT allele interacts more strongly with LMNA and several other proteins under basal conditions

(Pull-down with anti-SIRT6; Quantitative MS with Tandem Mass Tags)

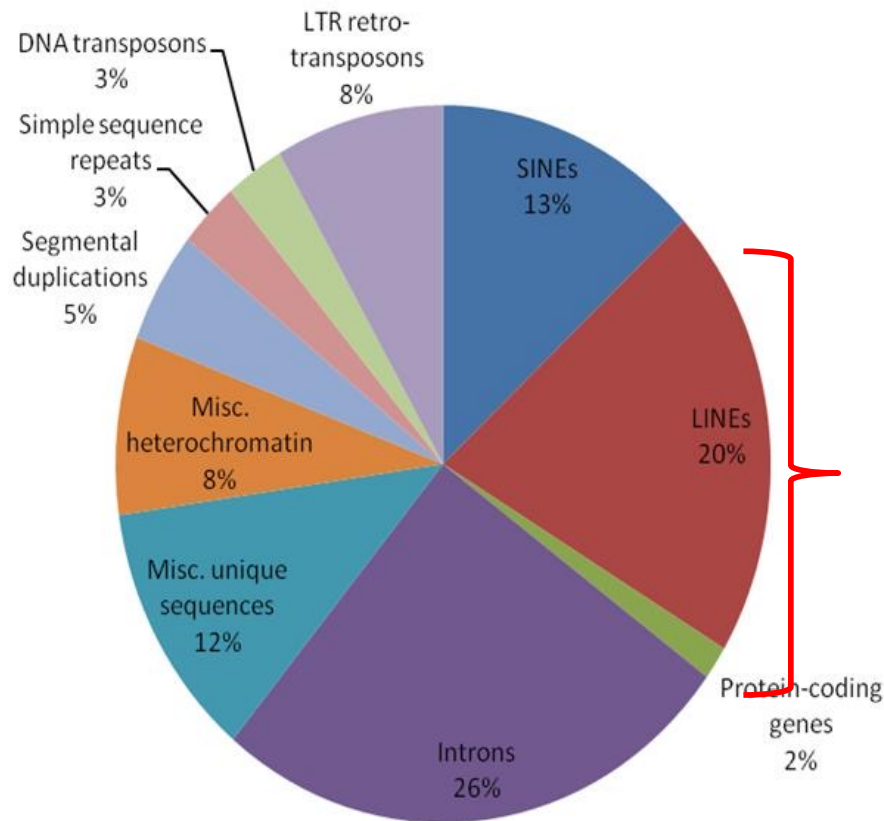


p-values are shown on prev slide Table- all are <0.05

CentSIRT6 summary

- centSIRT6 provides higher stimulation of DNA repair
- centSIRT6 has lower deacetylation activity in vitro, but no significant difference in vivo
- centSIRT6 shows enhanced mono-ADP ribosylation activity and PARP1 stimulation
- Interacts more strongly with Lamin A

L1 Transposons



Components of the Human Genome

- **Non-LTR retrotransposon**
 - Constitutes large portion of mammalian genomes
 - Active, partially active and inert copies
- **Encodes 2 proteins: ORF1 and ORF2**
 - ORF1 = RNP factor
 - ORF2 = Reverse transcriptase, endonuclease

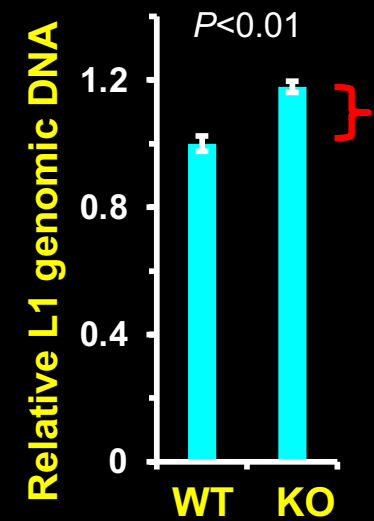
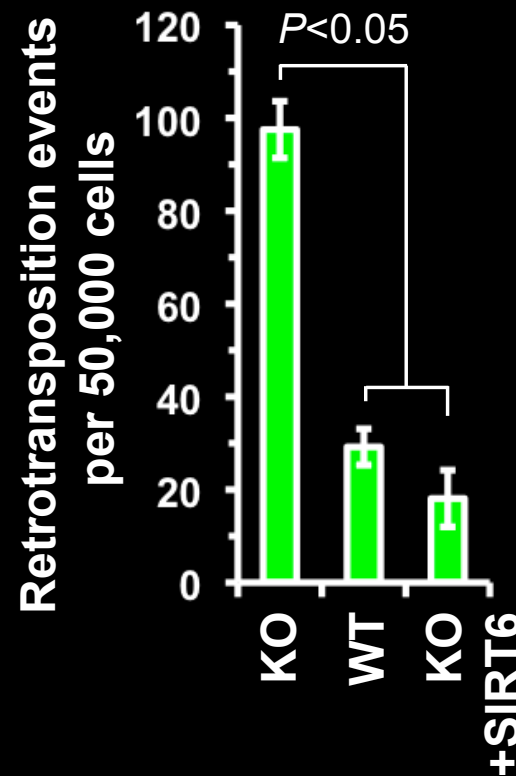
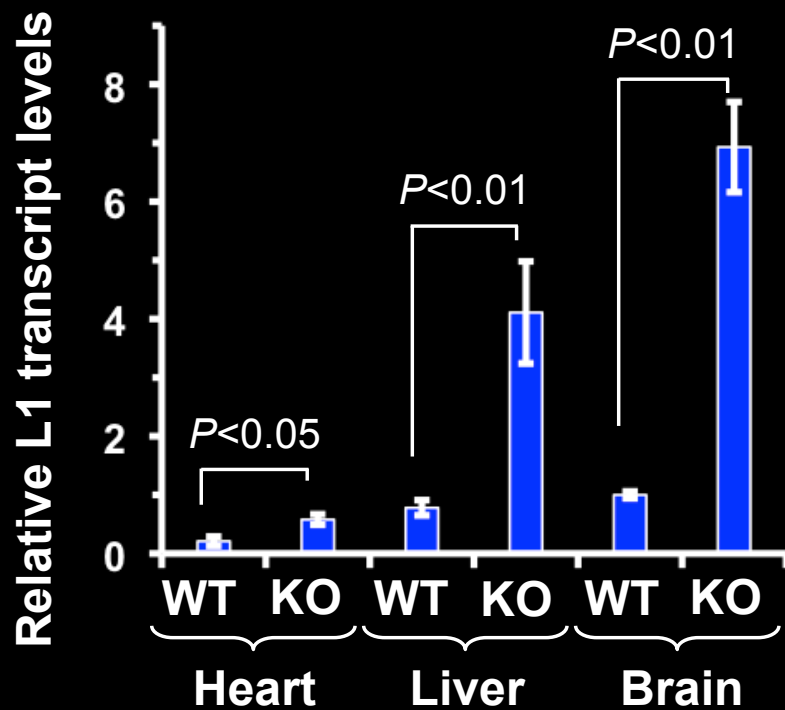


Michael van Meter

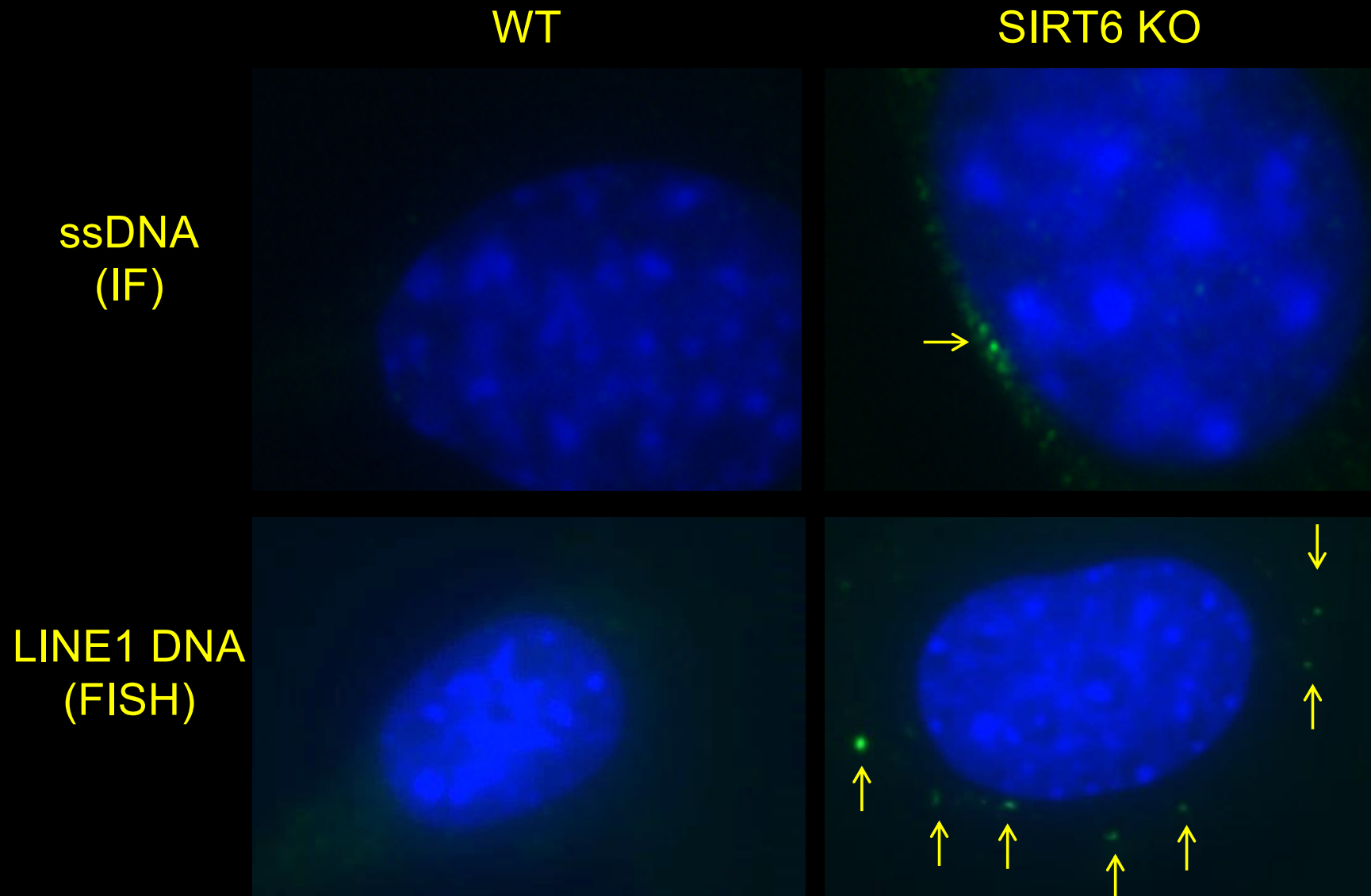
SIRT6 represses LINE1s



SIRT6^{-/-}

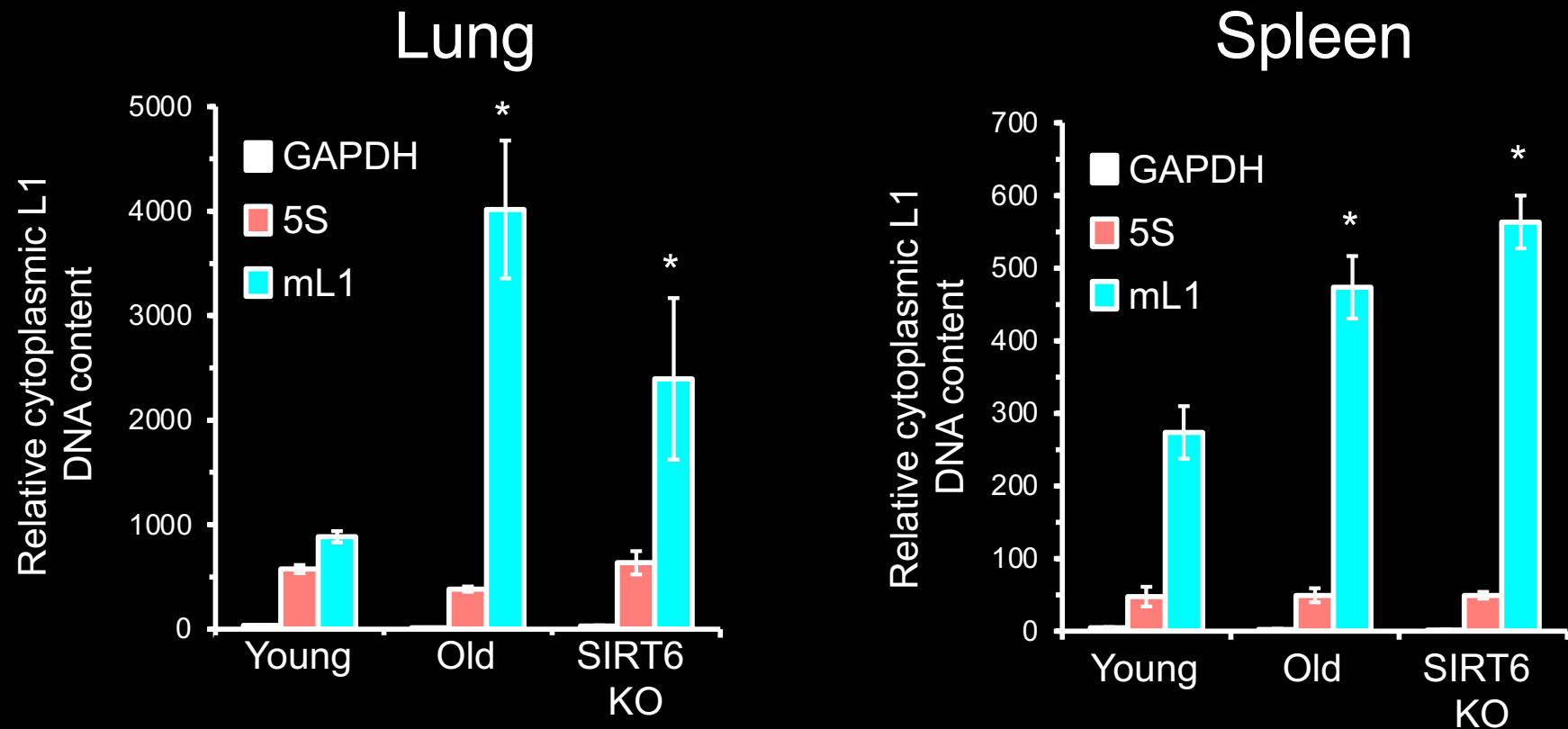


Cytoplasmic LINE1 DNA is enriched in SIRT6 KO cells

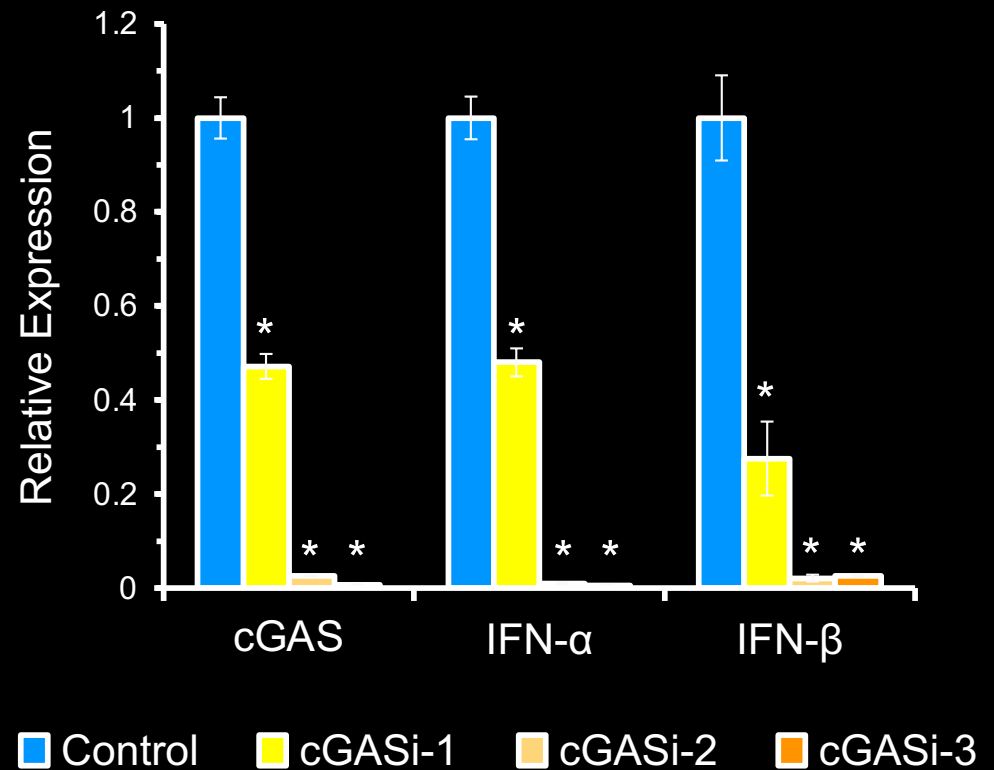
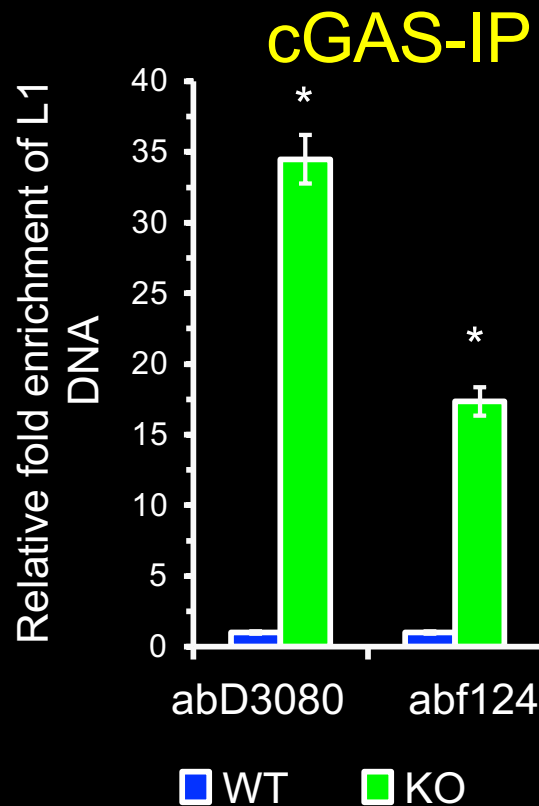
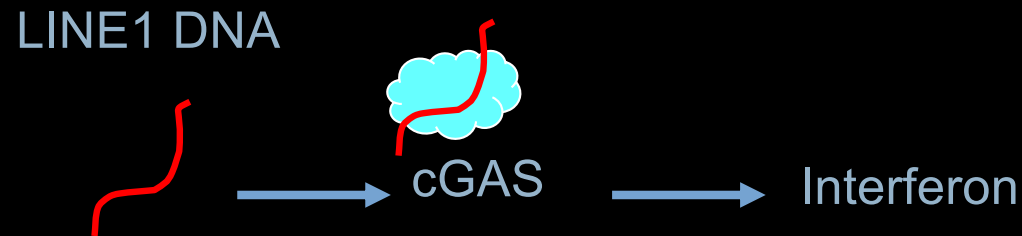


Cytoplasmic LINE1 DNAs are elevated in the tissues of aged (24 month old) and SIRT6 KO mice

Cell fractionation



Cytosolic L1 DNA drives IFN response via cGAS signaling



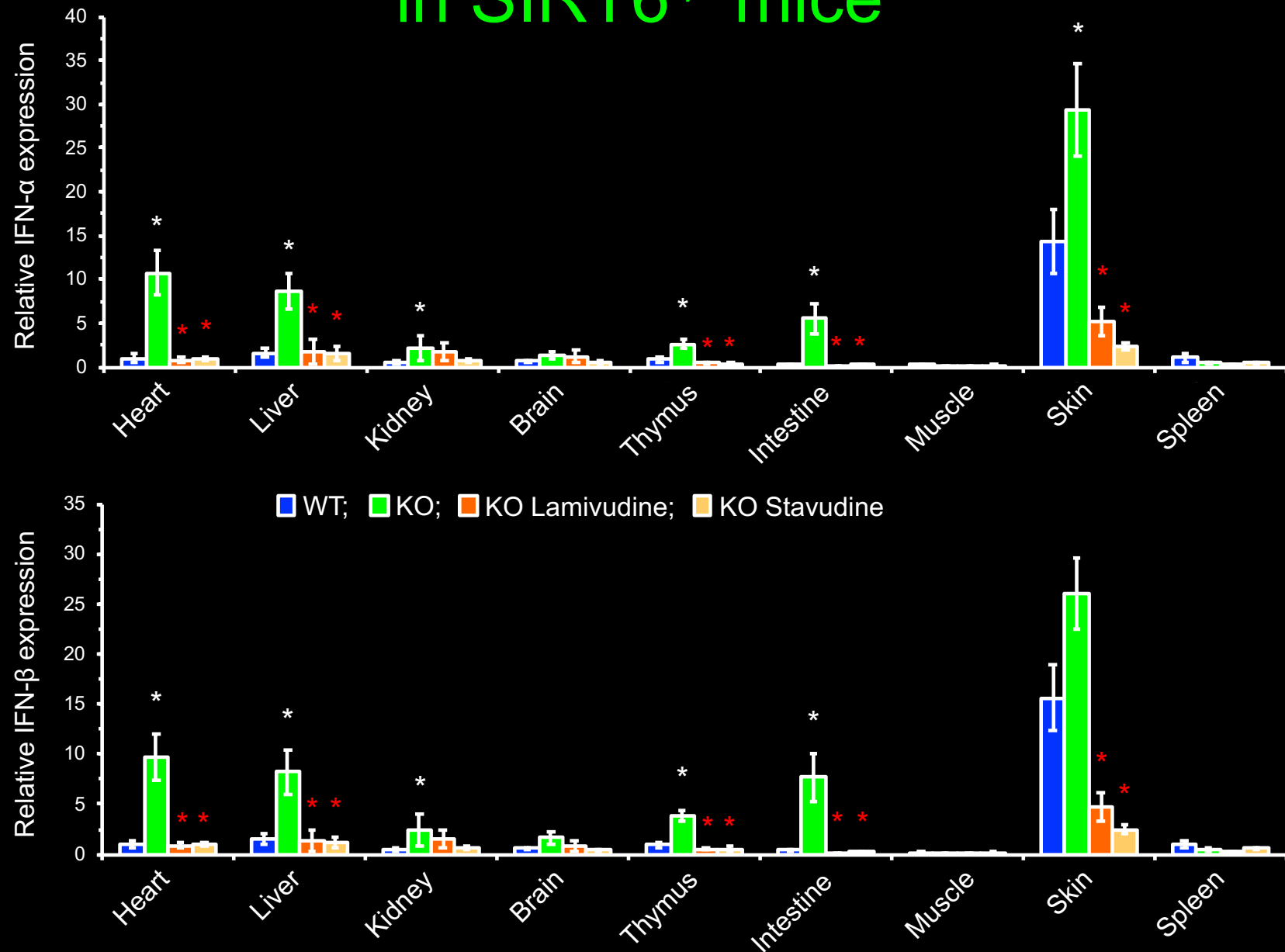
If we exterminate LINE1s will the mice live longer?



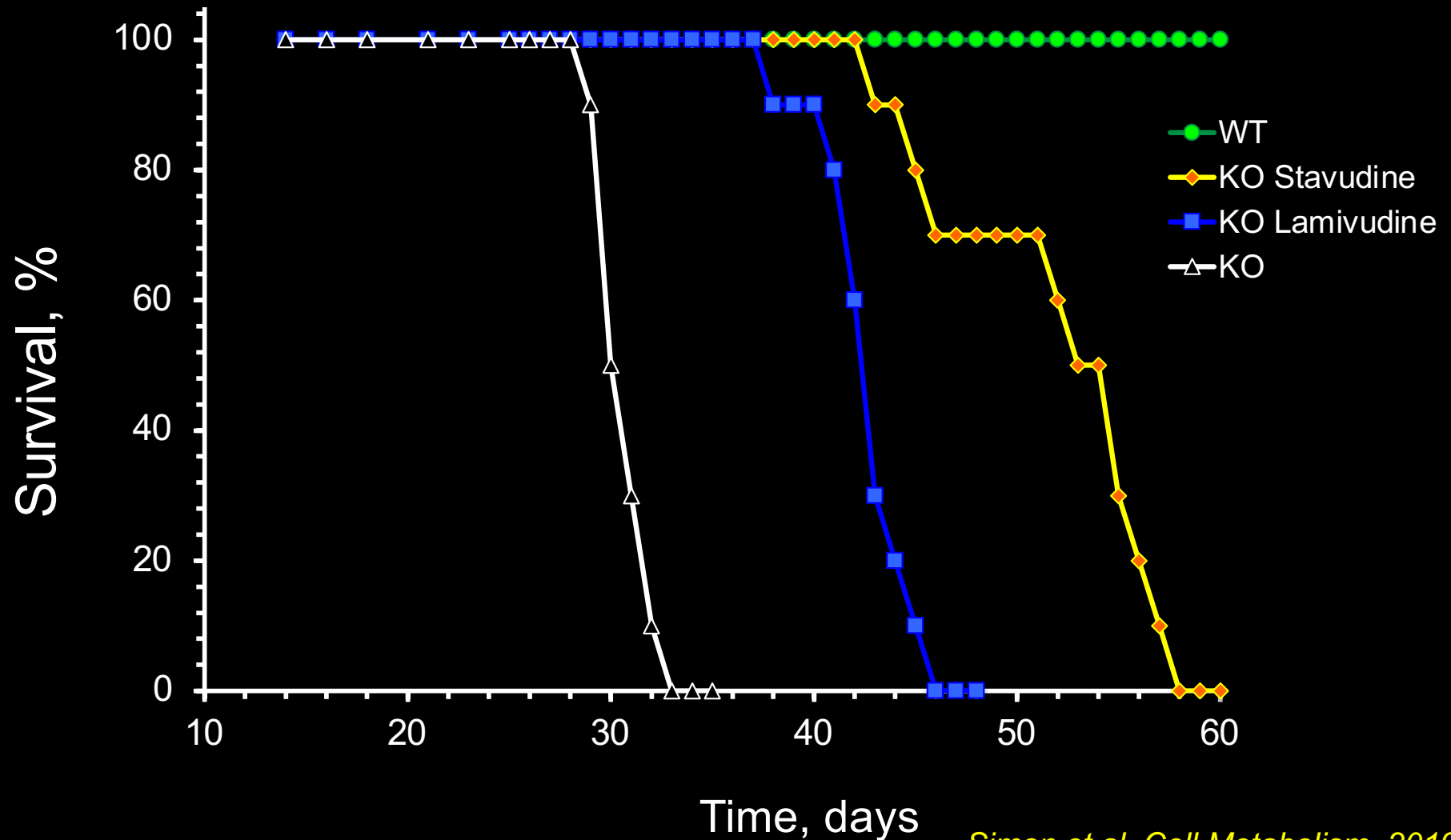
Nucleoside reverse transcriptase inhibitors (NRTIs)

Stavudue
Lamivudine

NRTI treatment rescues interferon response in SIRT6^{-/-} mice

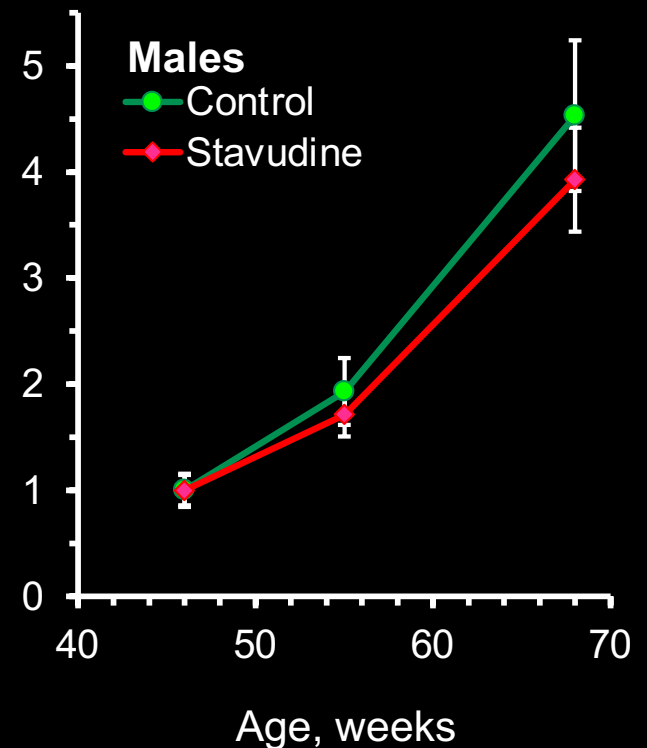
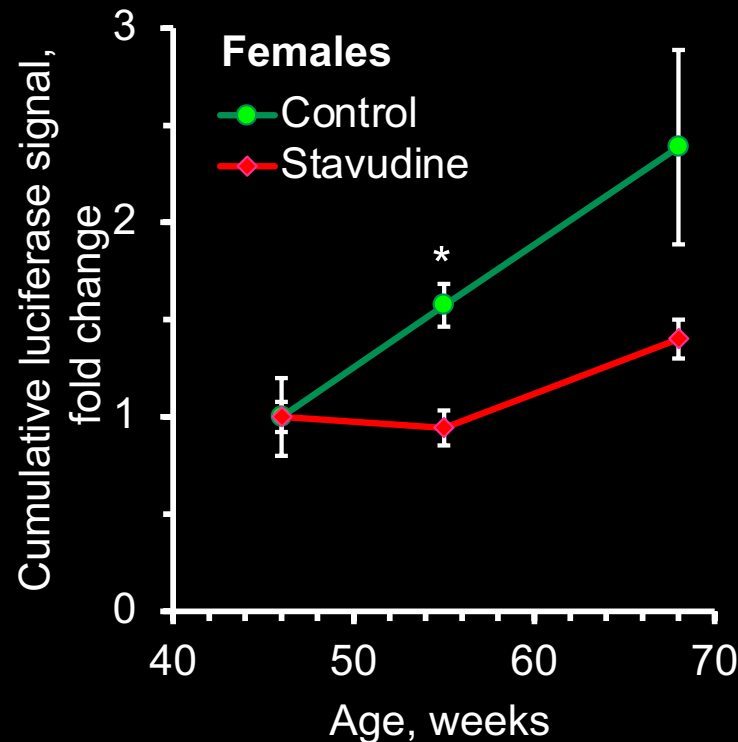
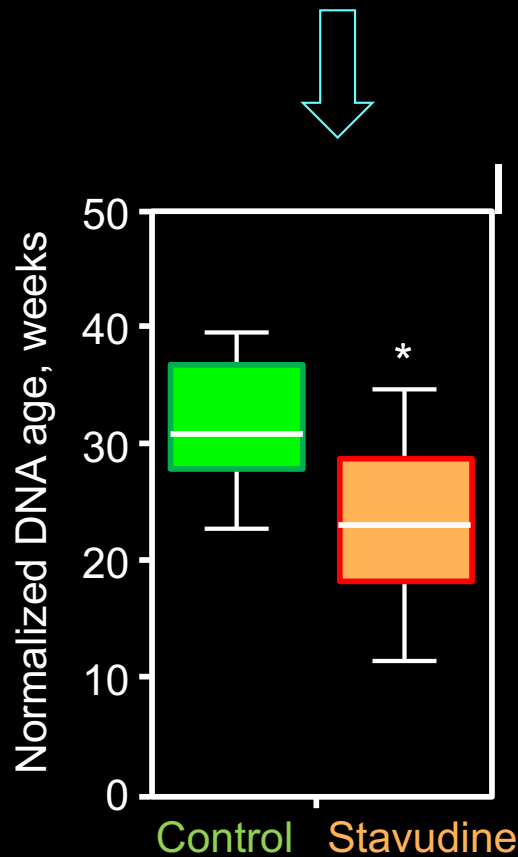


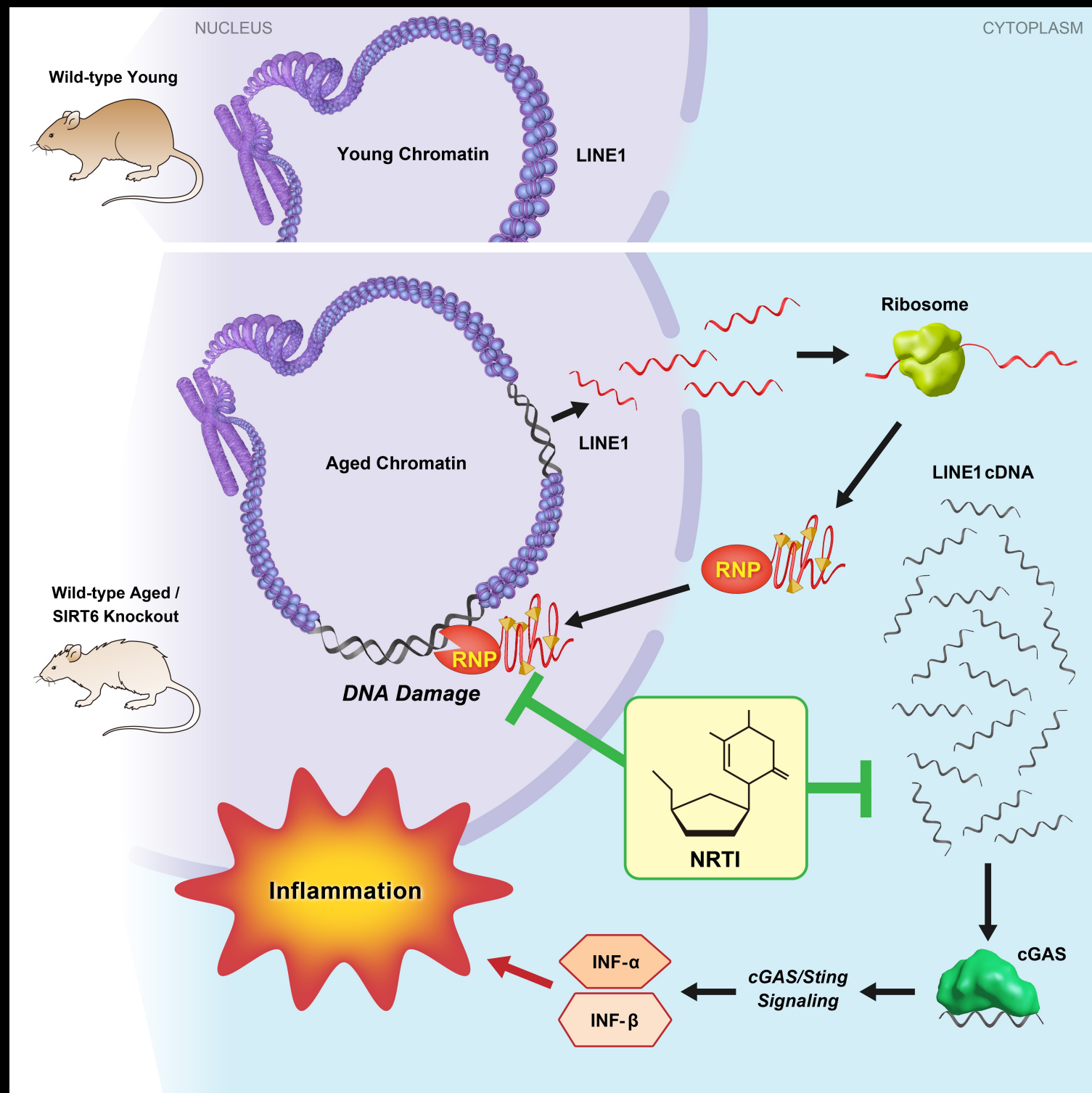
Reverse transcriptase inhibitors extend lifespan of Sirt6^{-/-} mice



NRTI treatment reduces methylation age and p16 expression in WT mice

p16-LUC mice (Sharpless lab)





Cell Metabolism

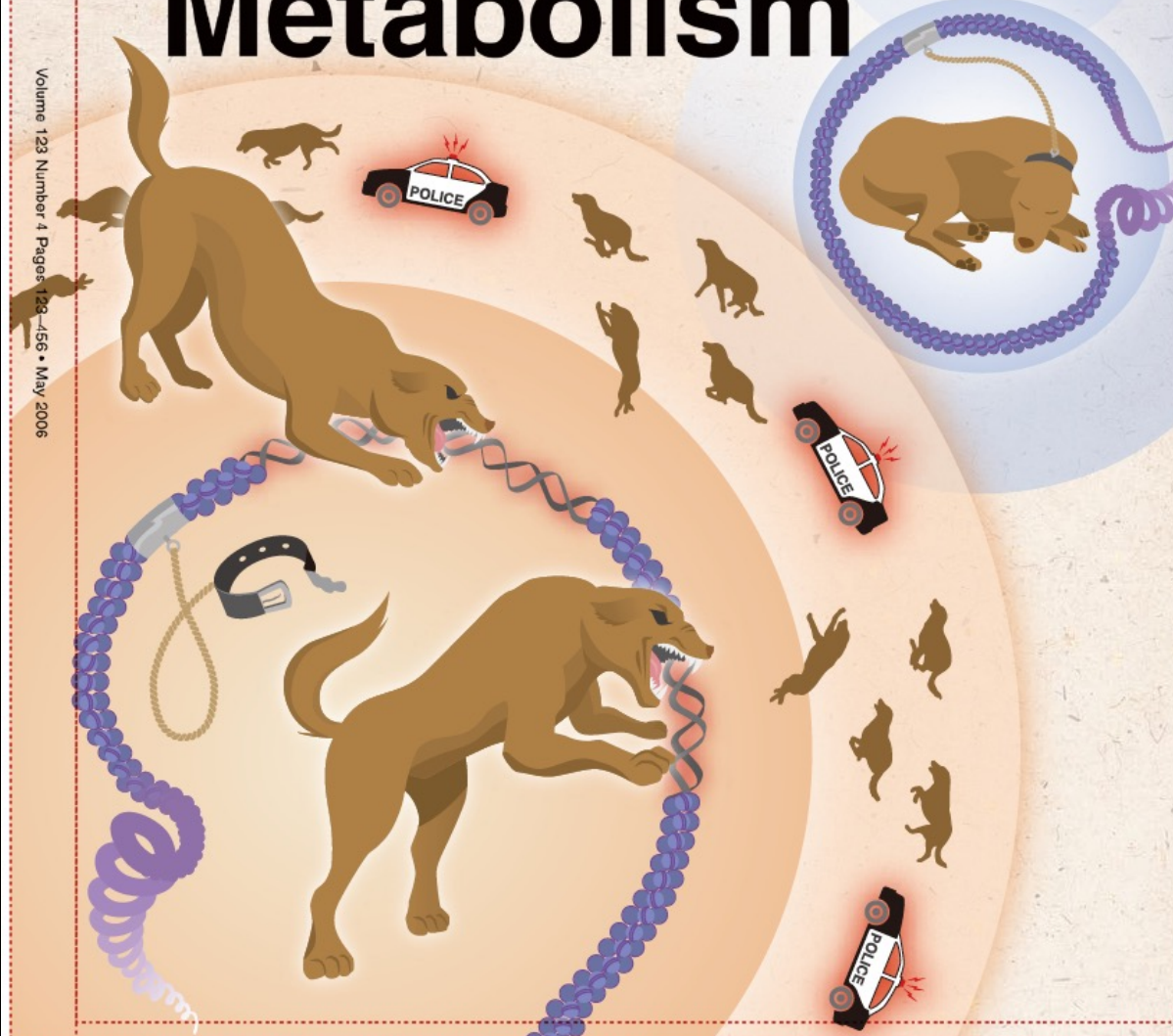
Volume 123 Number 4 Pages 128-156 • May 2006

Cell Metabolism

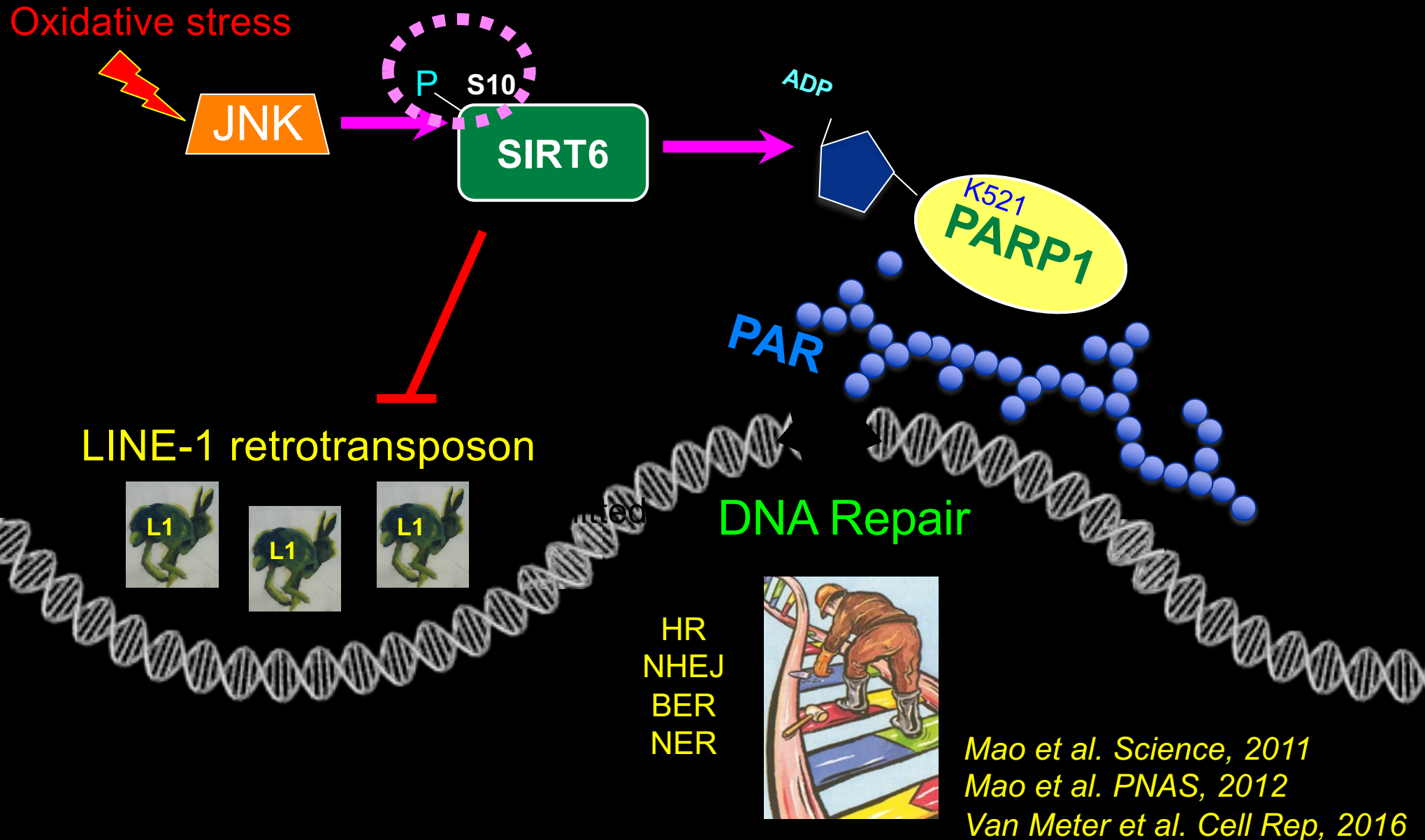
Volume 123
Number 4

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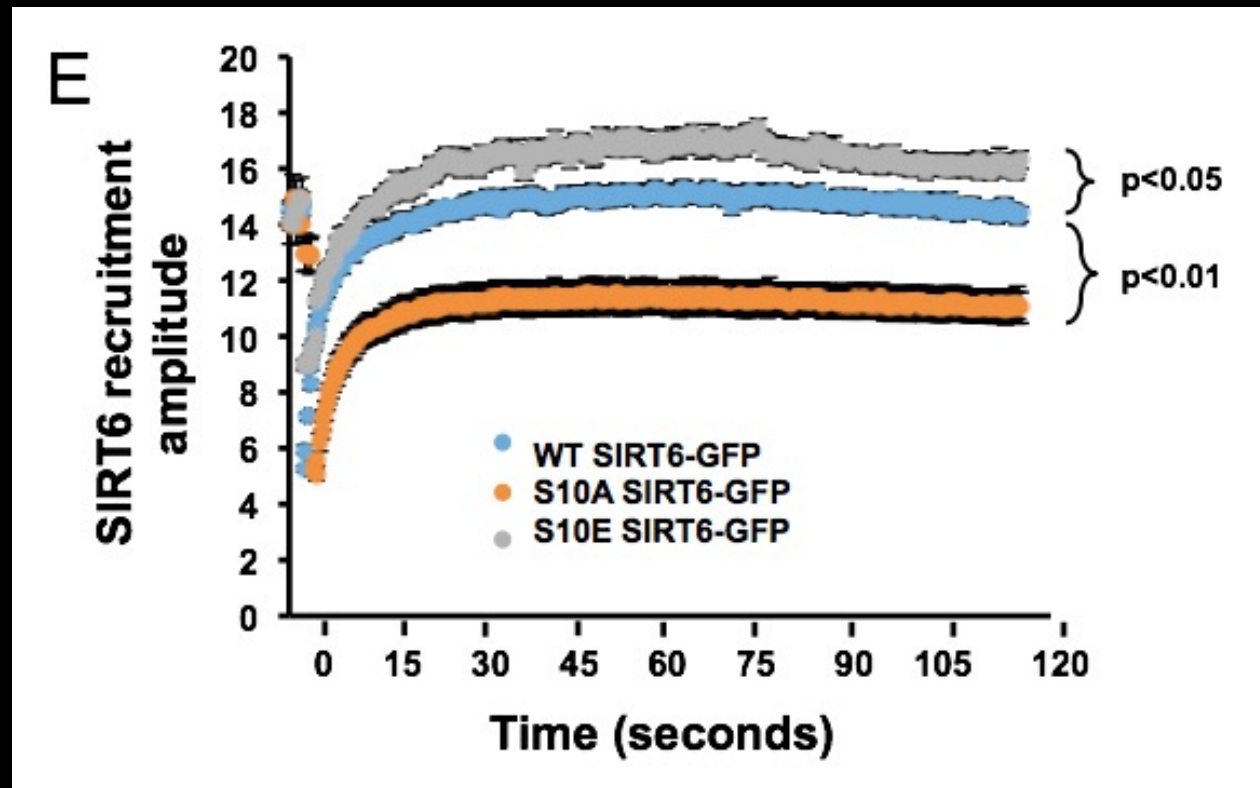
www.cellpress.com



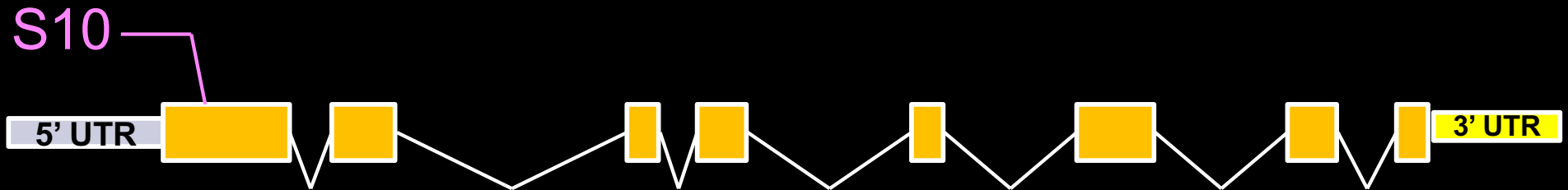
SIRT6 is responsible for induction of multiple DNA repair pathways in response to stress



S10 phosphorylation regulates SIRT6 recruitment to DNA breaks

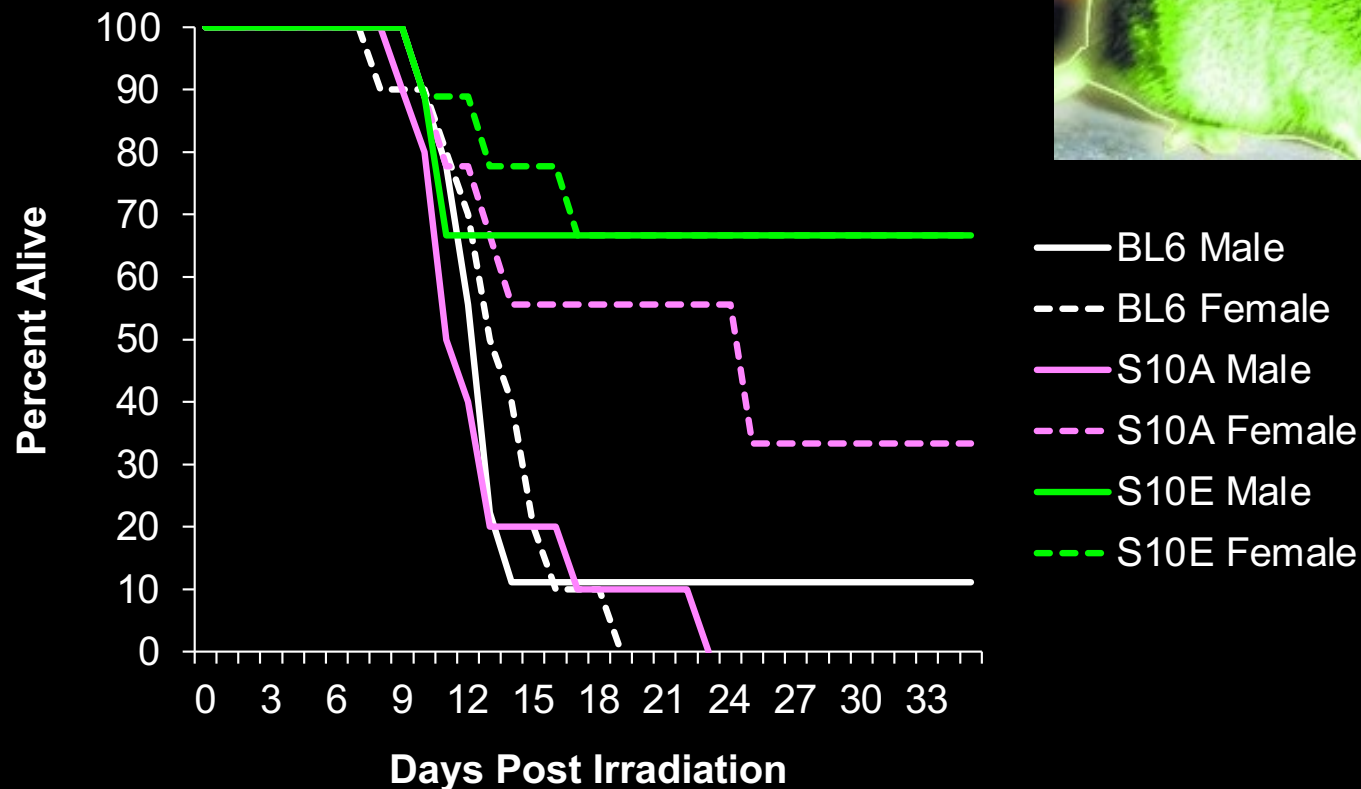


SIRT6 CRISPR Mice



- **S10**: JNK phosphorylation site
 - **S10A**: Unable to be phosphorylated
 - **S10E**: Mimics constitutive phosphorylation

SIRT6 S10E Mice Demonstrate Extreme Resistance to Radiation



N=9-10 mice/sex/genotype. Each cohort was exposed to 9 Gy (LD75/15)
All animals ~4m old



DNA repair



LINE1 silencing
(Chromatin maintenance)



SIRT6
NAD⁺

Longevity



Mechanisms of longevity and cancer resistance

Species-specific

Conserved



Hyaluronan



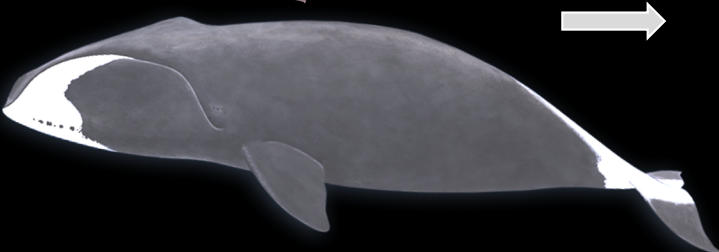
***Concerted
cell death-
transposons***



FGF
dependence



Resistance
to inflammation



?

...

Insulin/IGF1

mTOR

***DNA
DSB repair***

SIRT6

...



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- | | |
|---------------------|------------------|
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