



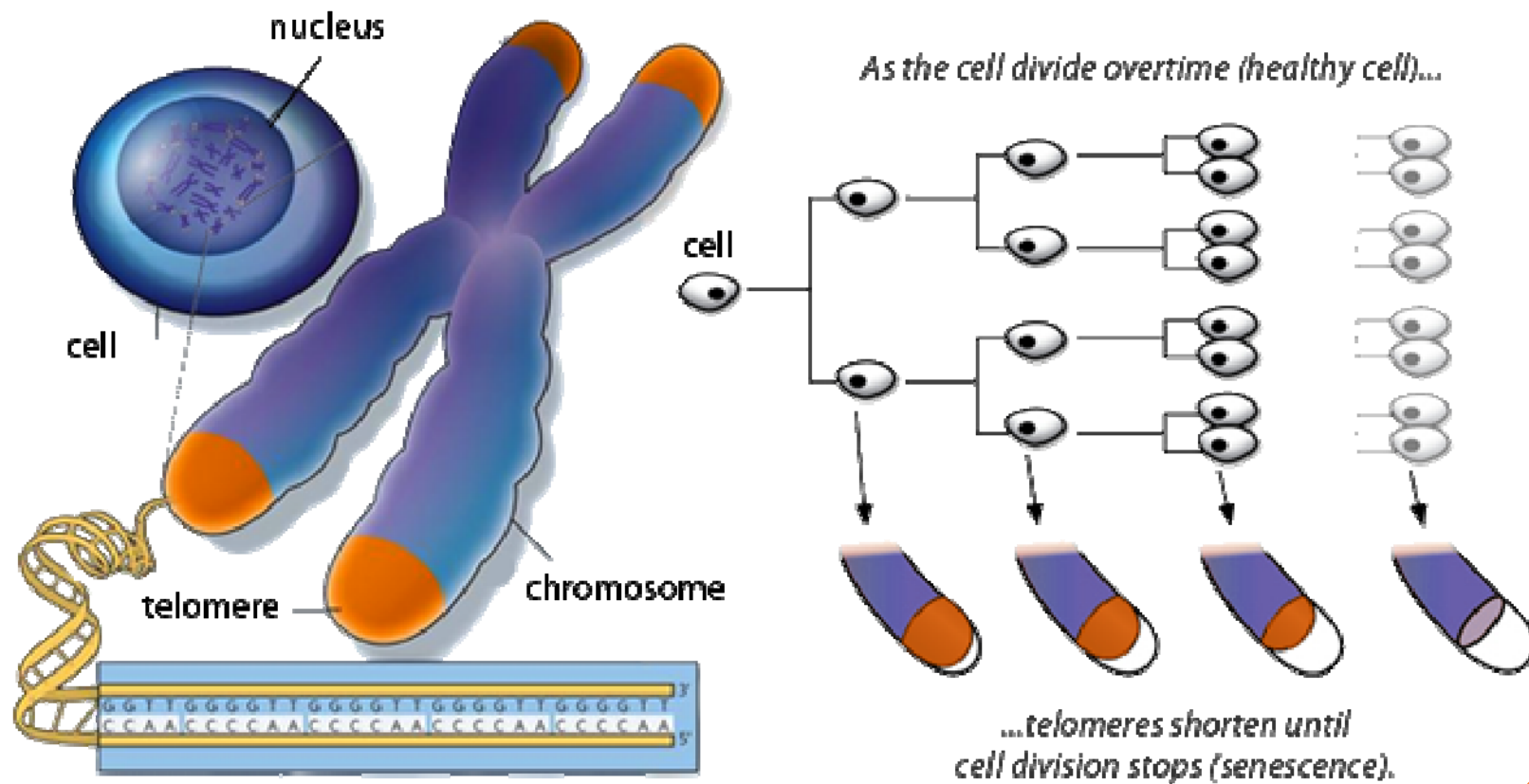
**Ciências
ULisboa** Faculdade
de Ciências
da Universidade
de Lisboa



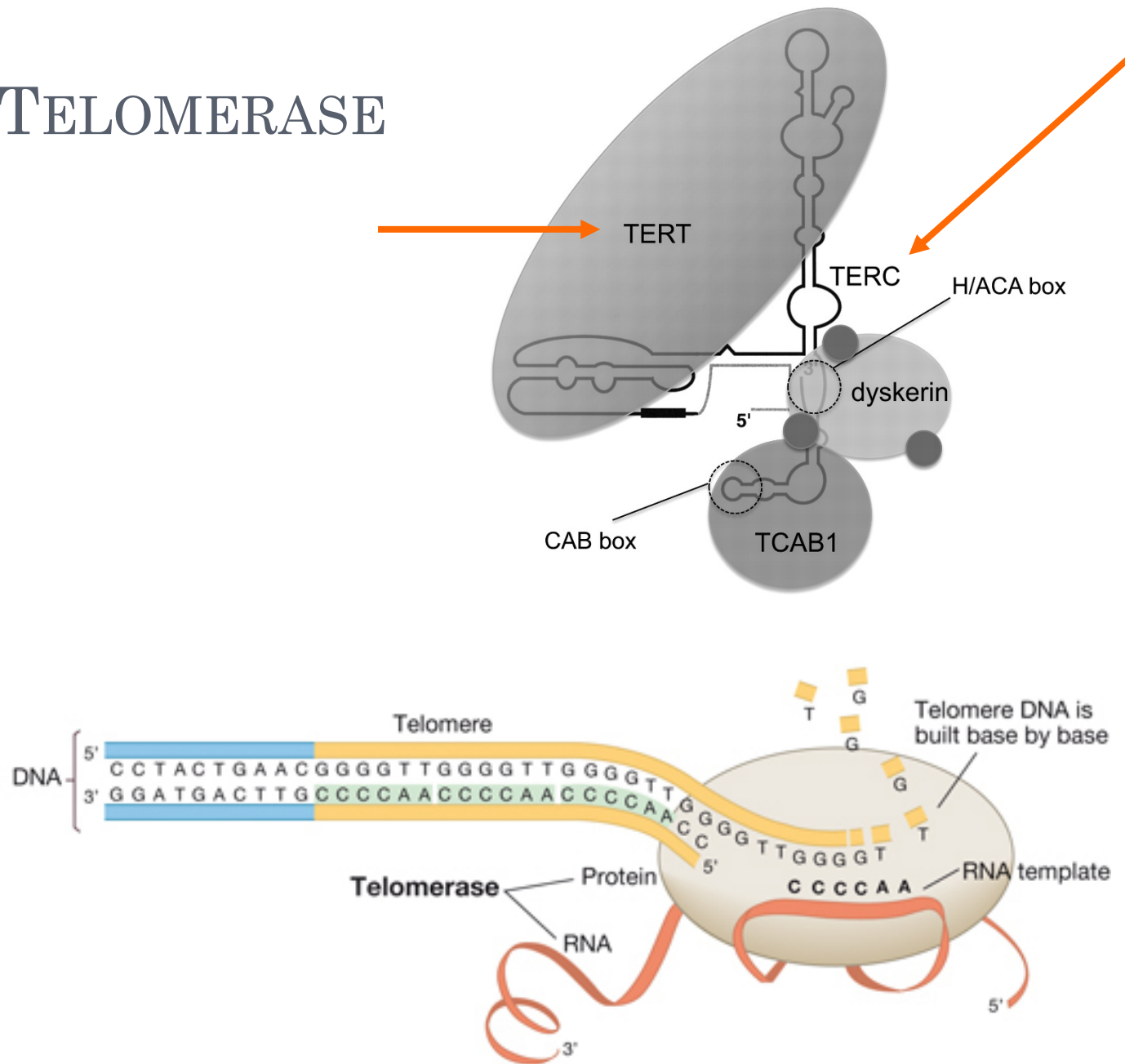
TELOMERASE REACTIVATION REVERSES TISSUE DEGENERATION IN AGED TELOMERASE-DEFICIENT MICE (JASKELIOFF ET AL., 2011)

Andreia Henriques
Nuno Domingues

CHROMOSOMES AND TELOMERES



TELOMERASE



RELEVANCE

Ageing

Loss of telomere function

Loss of genome integrity

Widespread p53 activation
Apoptosis

Marked tissue atrophy and
several diseases

Ataxia-telangiectasia



Premature ageing syndromes



Liver Cirrhosis



OBJECTIVES

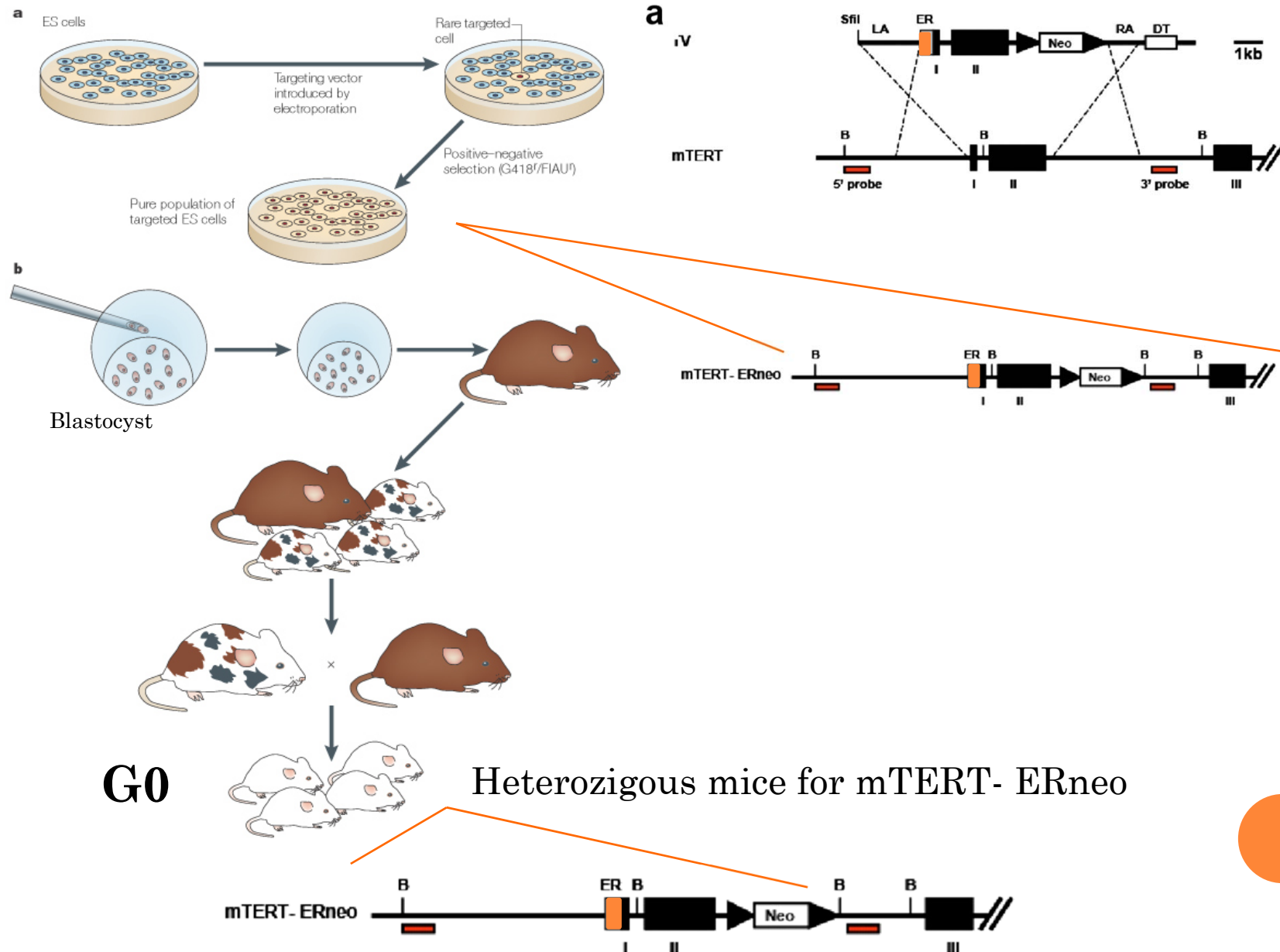
Multi-system degeneration in adult mice with severe telomere dysfunction



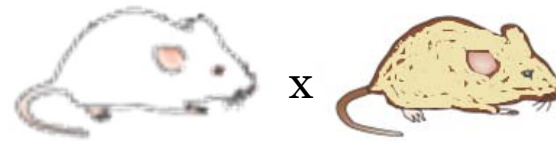
Halted/ Reversed by reactivation of telomerase?



METHODS – GENERATION OF TERT-ER MICE



Heterozygous mice
for mTERT- ERneo



CREIIA mice



Cre Recombinase
activity in embryo
tissues



Inbreeding of
mTERT-ER mice

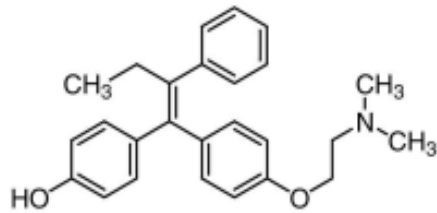
G4



Homozygous mice for mTERT-ER



METHODS – ASSESSMENT OF TELOMERASE ACTIVITY



4-OHT

4-hidroxitamoxifen activates the expression of TERT-ER



Isolated cells
from G0 and G4 mice

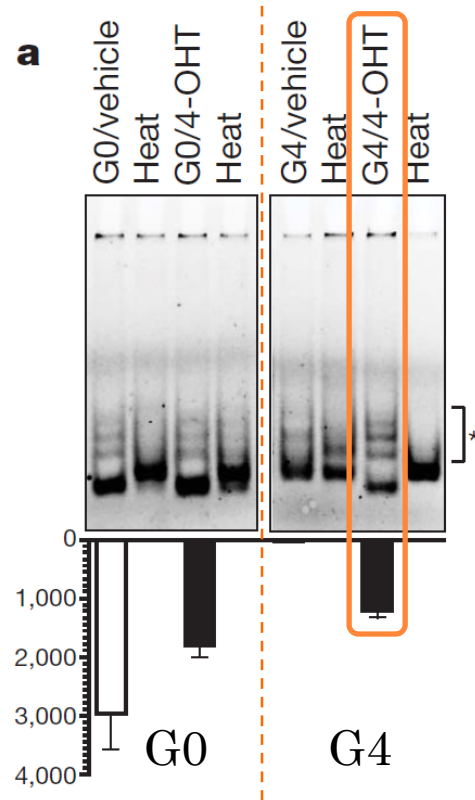


- ✓Telomerase activity
- ✓Telomere integrity
- ✓Proliferation capacity

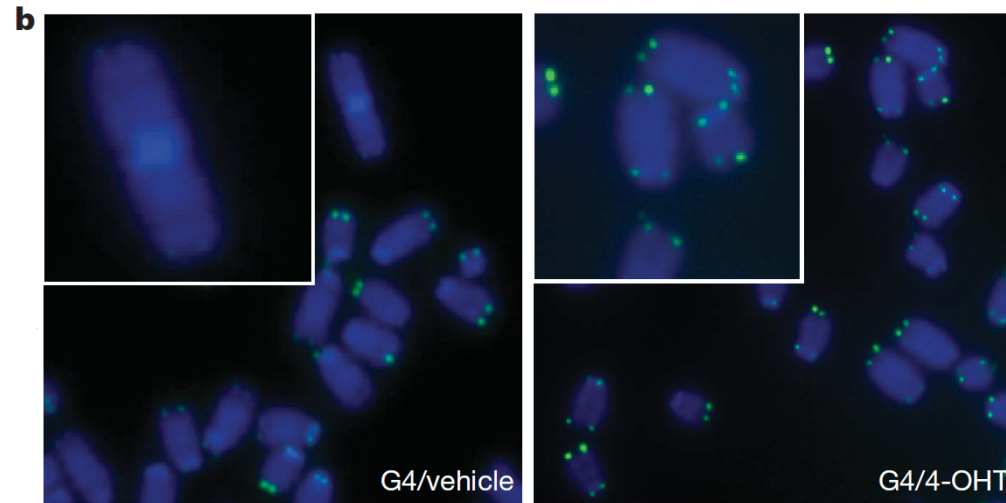


RESULTS – MODEL VALIDATION

In primary cell cultures of G0 and G4 mice:



Telomere-specific FISH in splenocytes



G0 vehicle Regular telomerase

G0 4-OHT Regular and induced telomerase

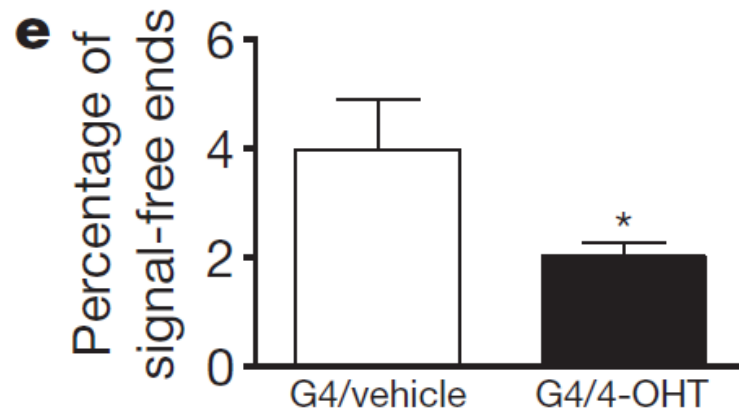
G4 vehicle No telomerase

G4 4-OHT Induced telomerase

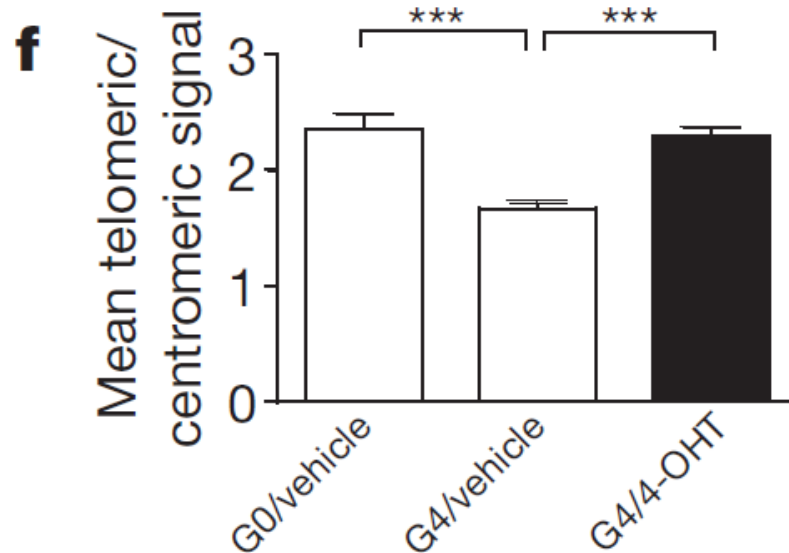
4-OHT restores Telomerase activity in TERT-ER cells



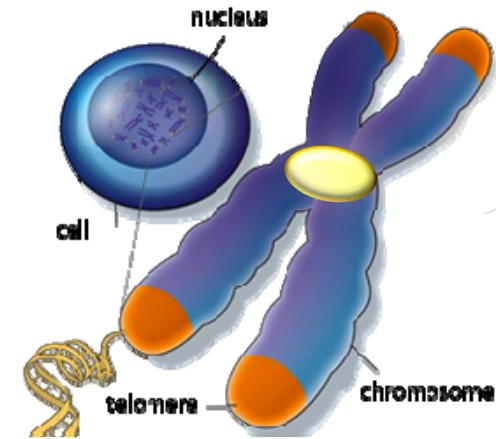
RESULTS



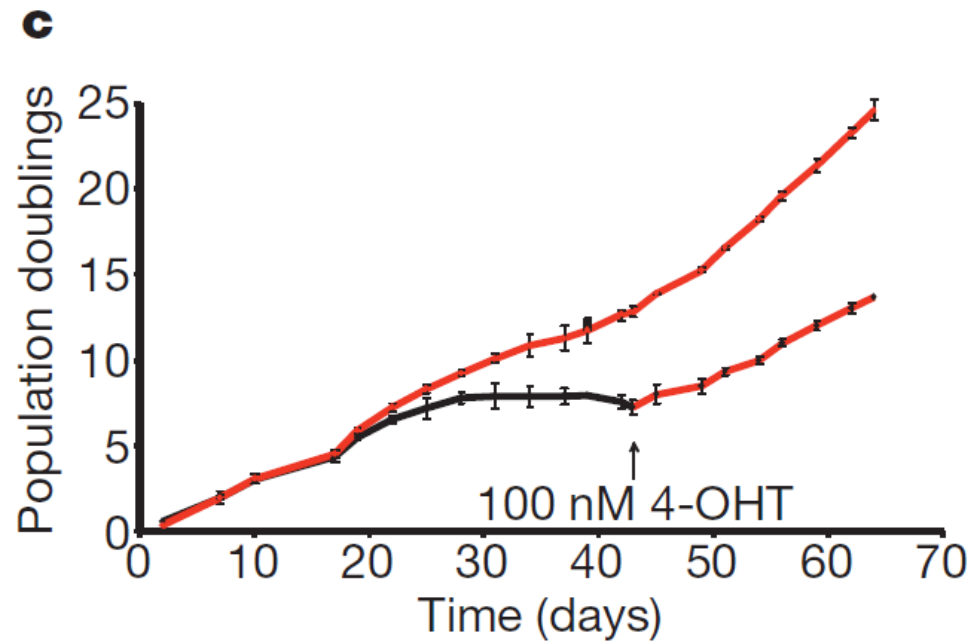
Signal free ends decrease with 4 telomerase reactivation



Telomere/Centromere Ratio is reestablished by telomerase reactivation

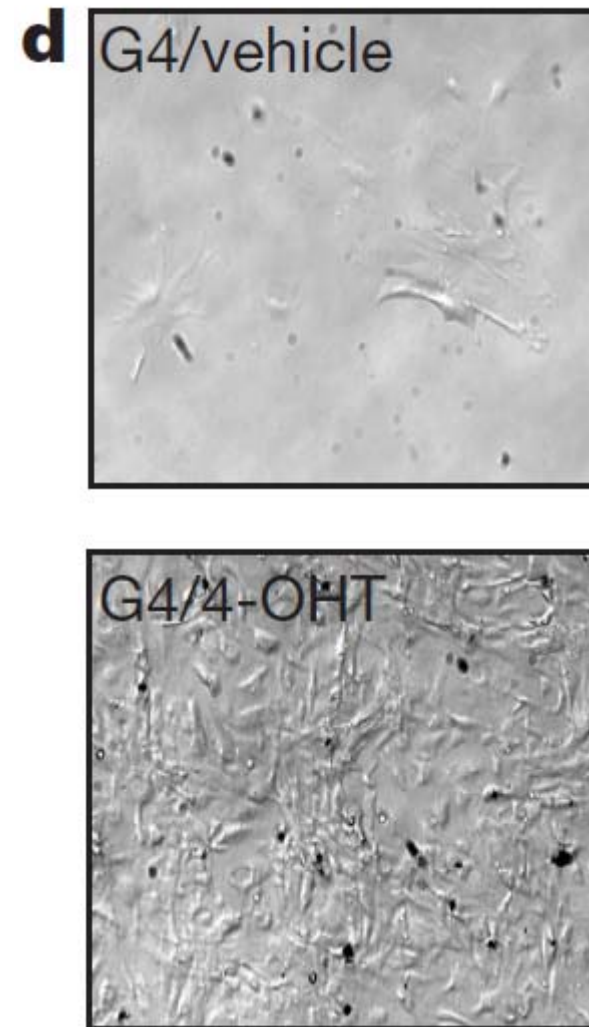


RESULTS

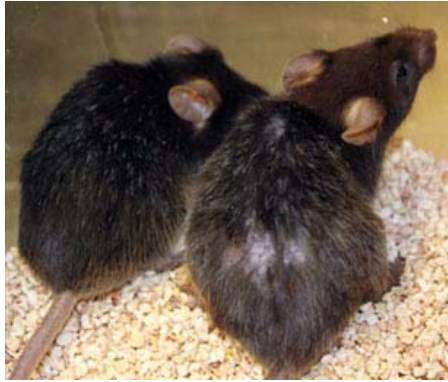


Telomerase activity in TERT-ER cells restores division hability

Fibroblasts



RESULTS



Loss of telomere function



Loss of genome integrity



Widespread p53 activation
Apoptosis



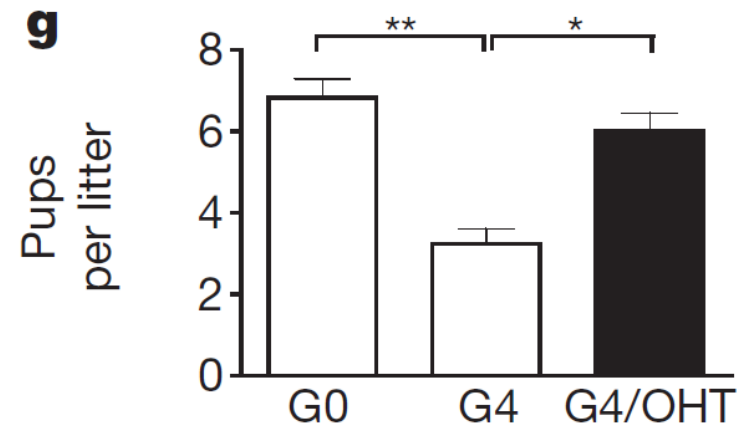
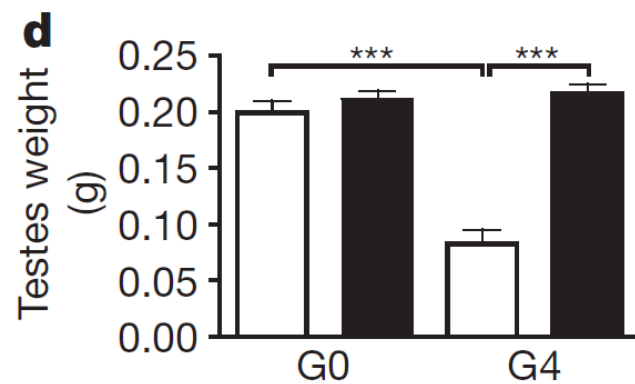
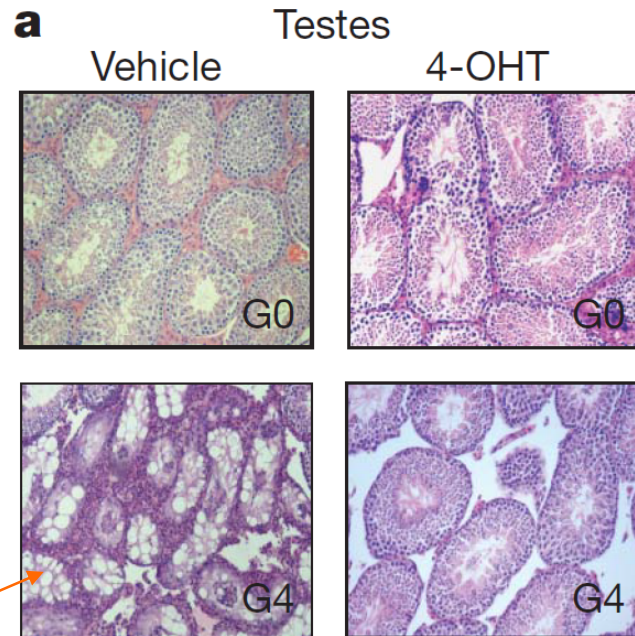
Entrenched tissue
degeneration

Testes, spleen and intestinal crypts

Highly proliferative organs



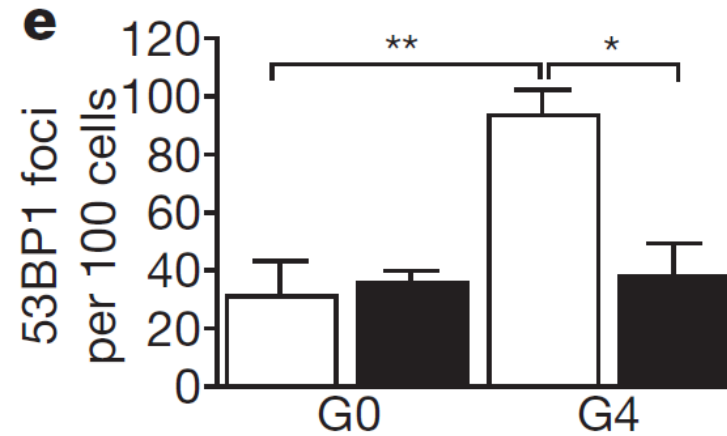
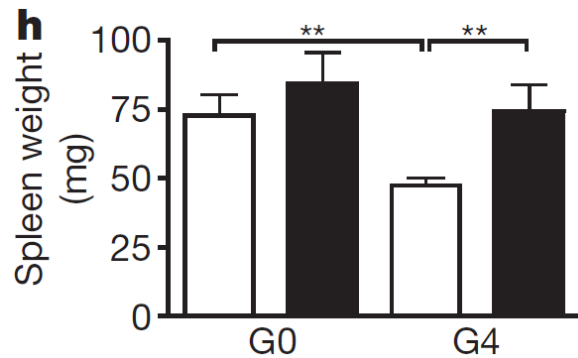
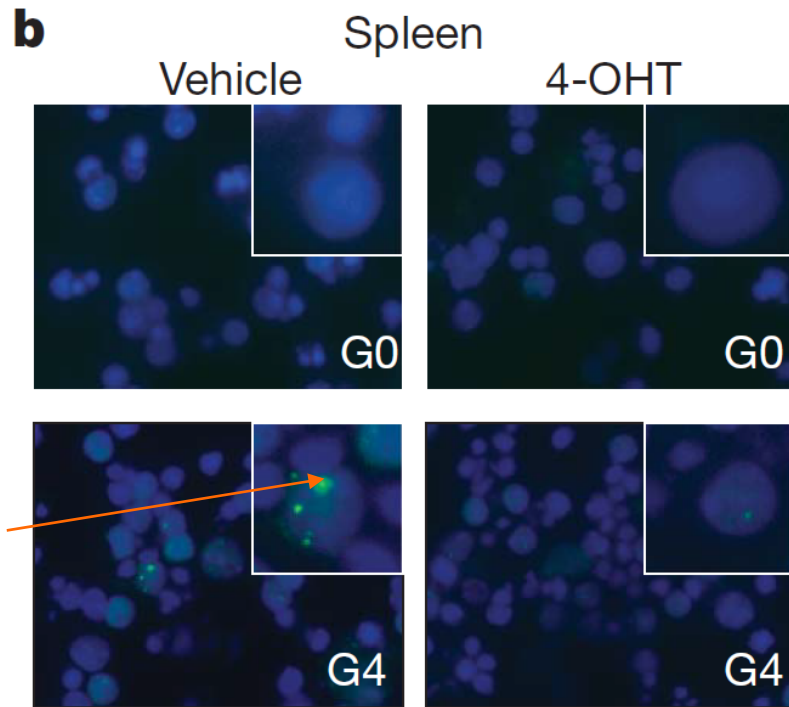
RESULTS -



4 weeks of continuous 4-OHT exposure
Testes and Fecundity recover



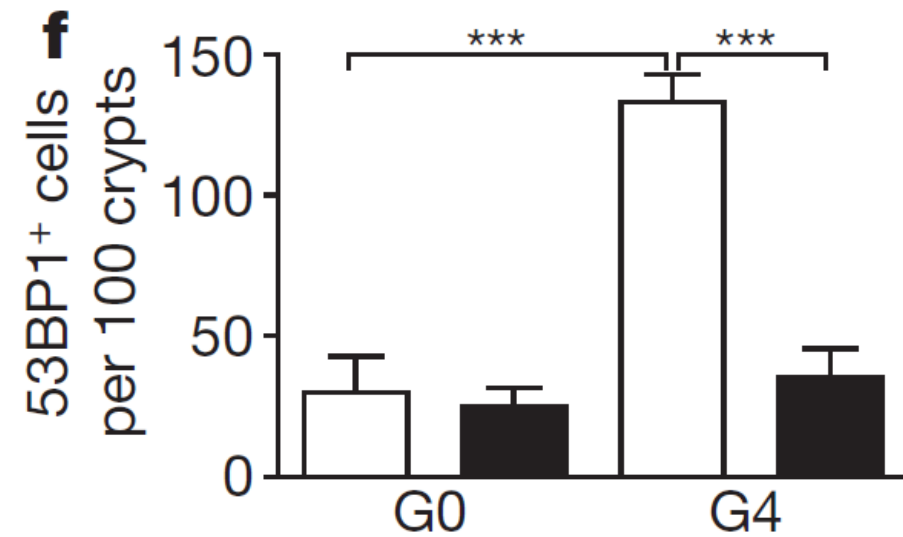
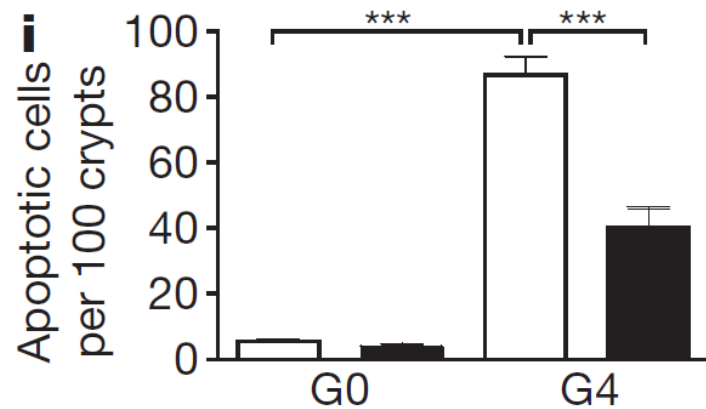
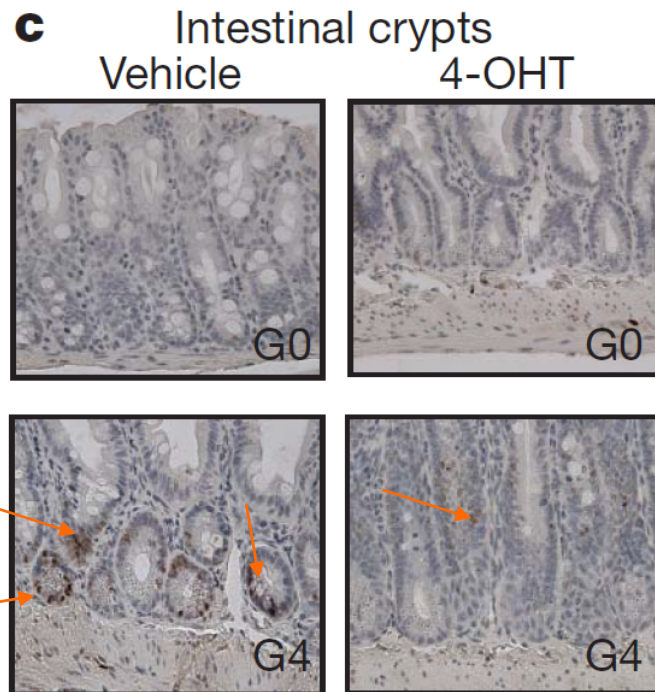
RESULTS



53BP1
p53-mediated DNA damage
signalling

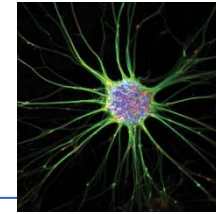


RESULTS



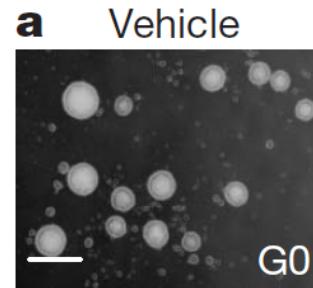
RESULTS — NSC FUNCTION AND POTENCY

In primary cell cultures of G0 and G4 mice:



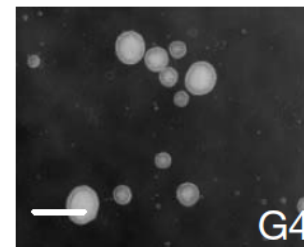
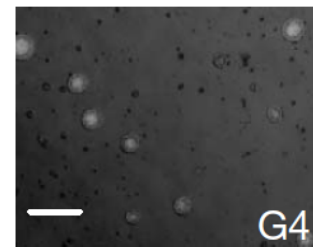
Neurospheres

Regular
telomerase



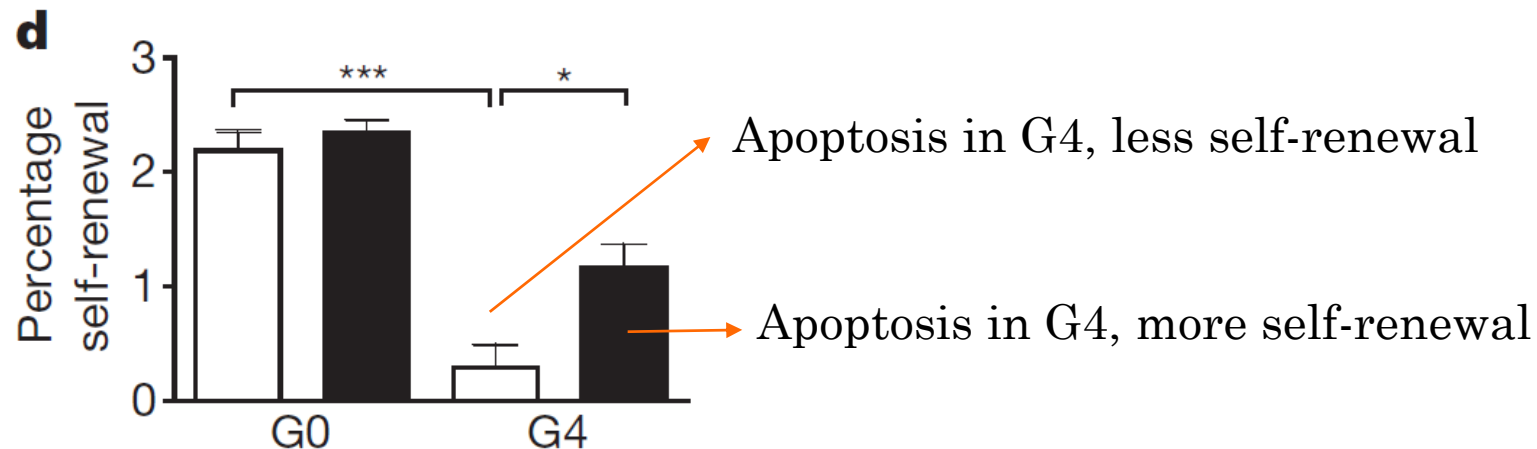
Regular
telomerase

↓ SELF-RENEWAL



↑ SELF-RENEWAL

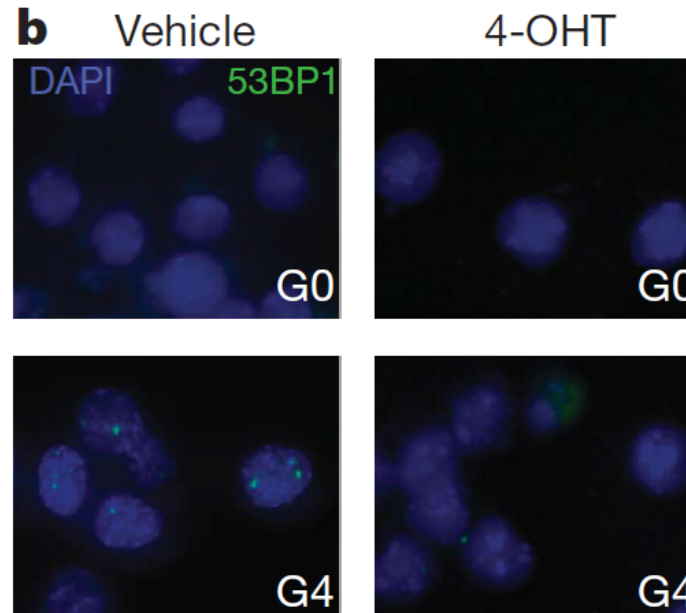
NSCs (Neural stem cells)



RESULTS

53BP1
p53

NSCs (Neural stem cells)

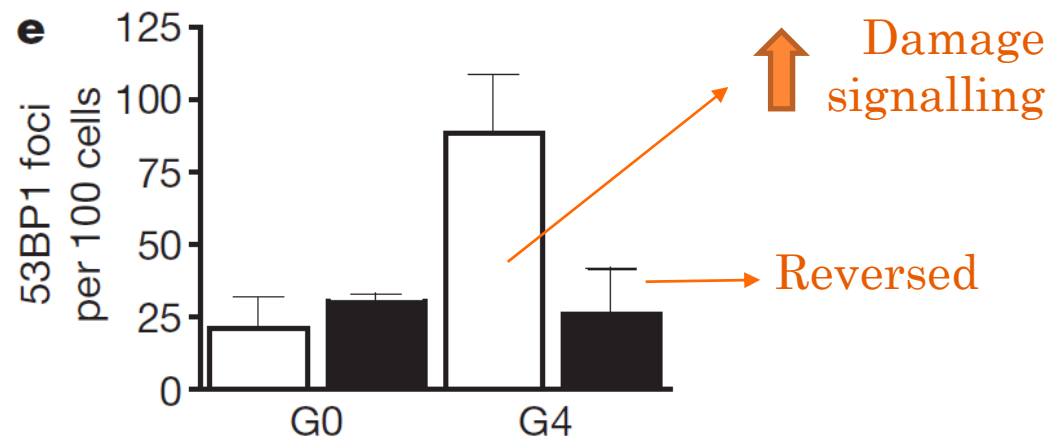


Regular
telomerase

Regular
telomerase

↑
Damage
signalling

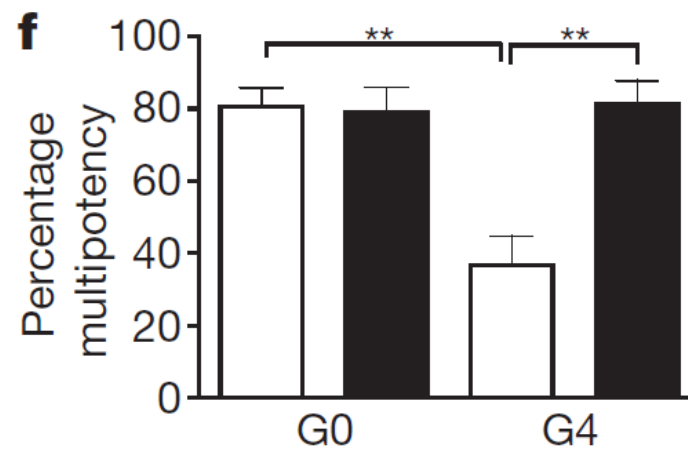
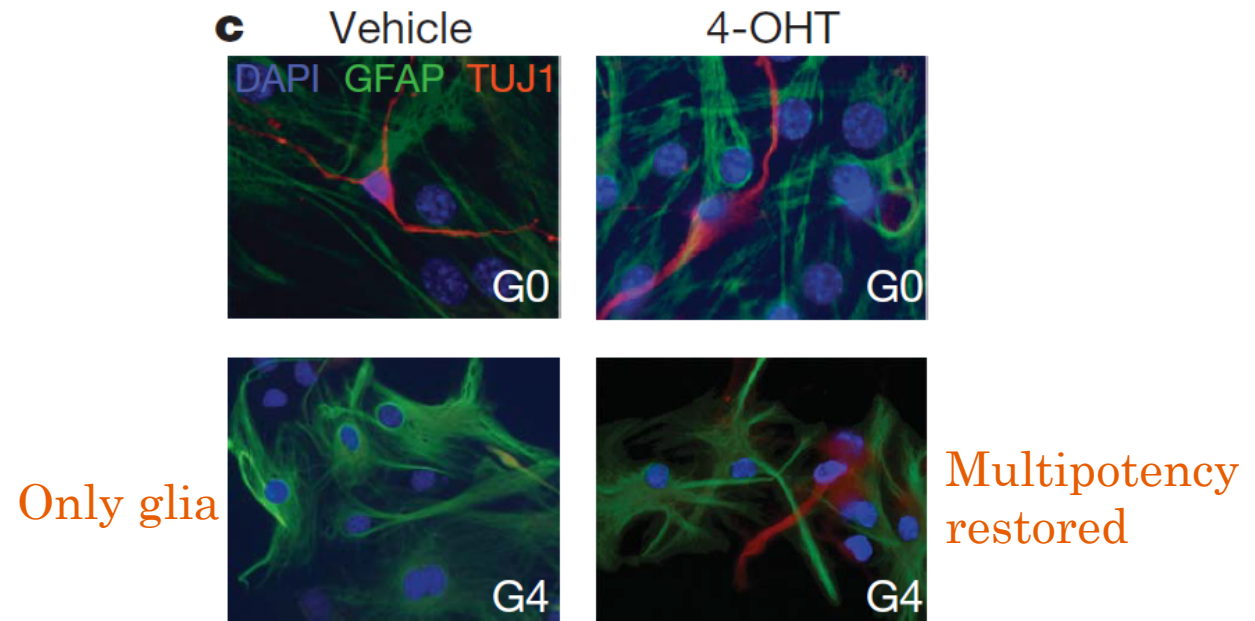
↓
Damage
signalling



RESULTS

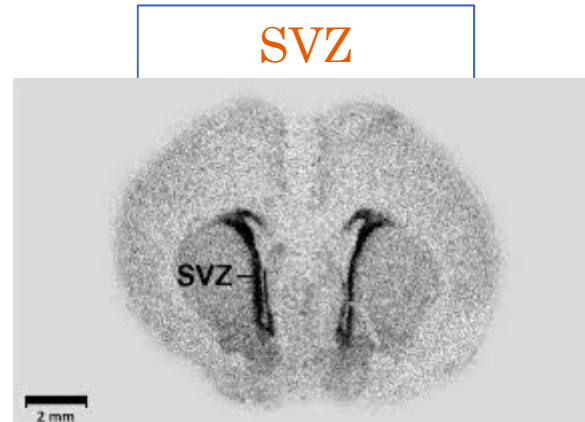
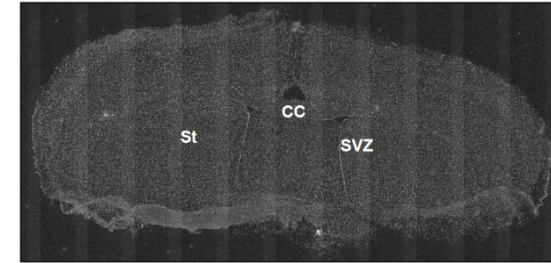
TUJ1
Neuronal cells

GFAP
Glial cells



RESULTS

PROLIFERATION AND DIFFERENTIATION OF NSCs FOLLOWING TELOMERASE REACTIVATION



SVZ

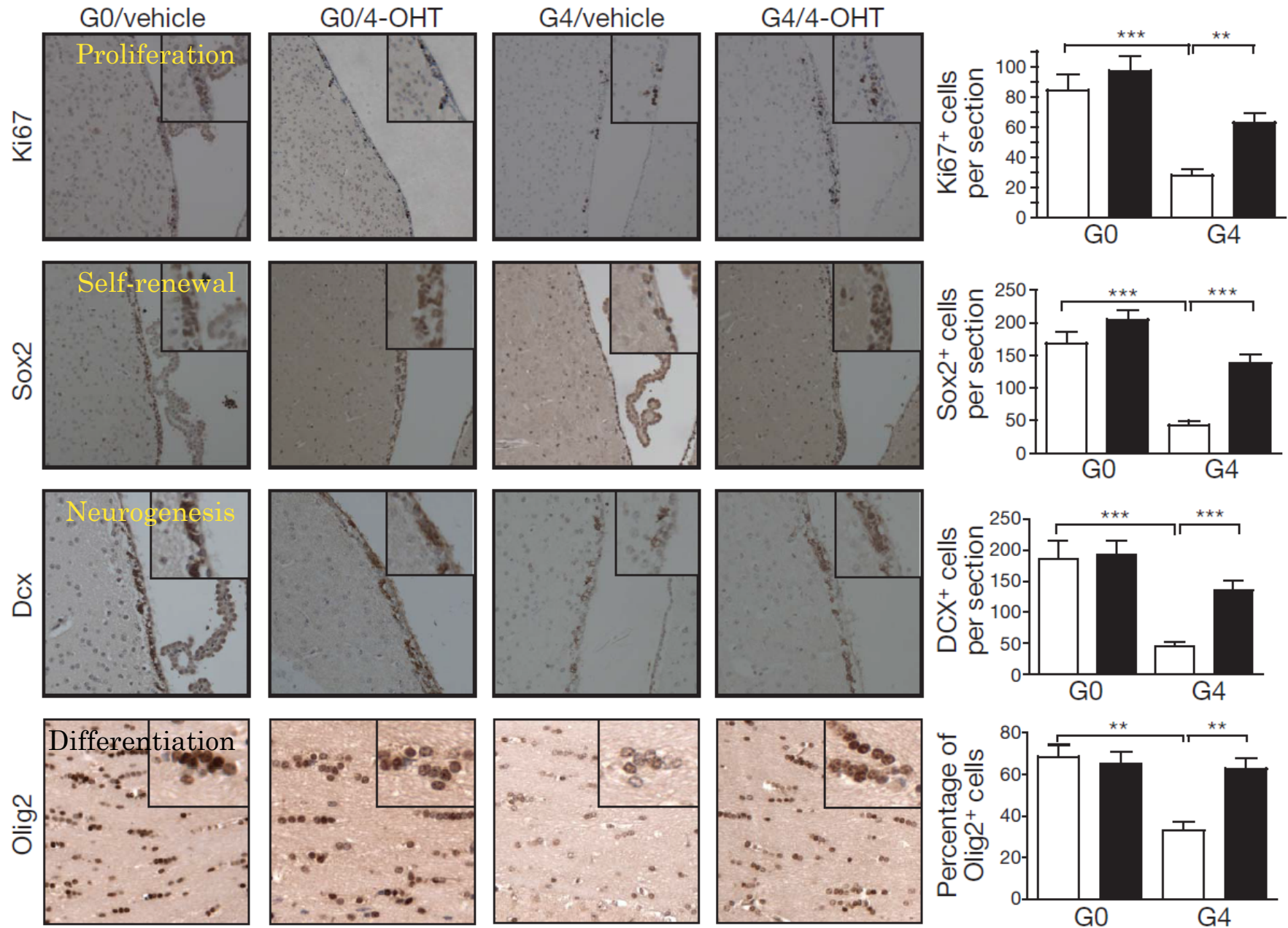
Neuronal stem cell zone

Self-renewal	Sox2	– maintenance of pluripotency, self-renewal in NSCs
Neurogenesis	Dcx	– microtubule associated protein in progenitor NCs
Differentiation	Olig2	– bHLH anti-neurigenic and neurigenic – oligodendrocyte differentiation
Proliferation	Ki67	– Cell proliferation, synthesis of rRNA

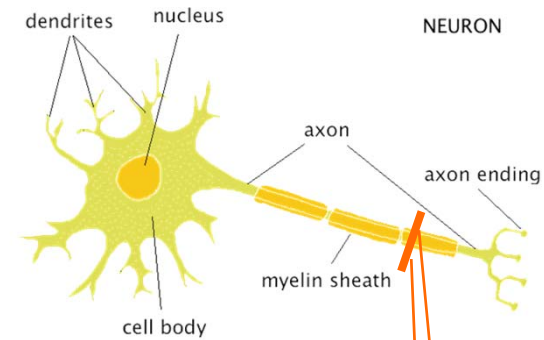


RESULTS

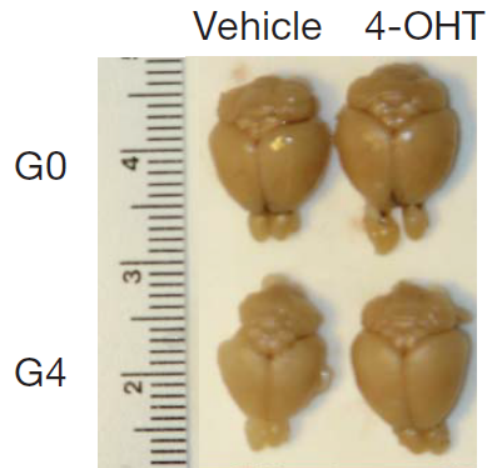
SVZ, in vivo



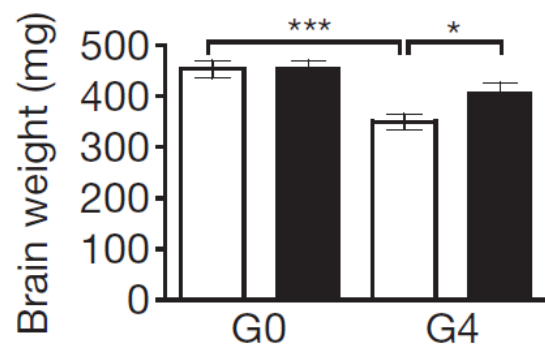
RESULTS — BRAIN SIZE AND MYELINATION



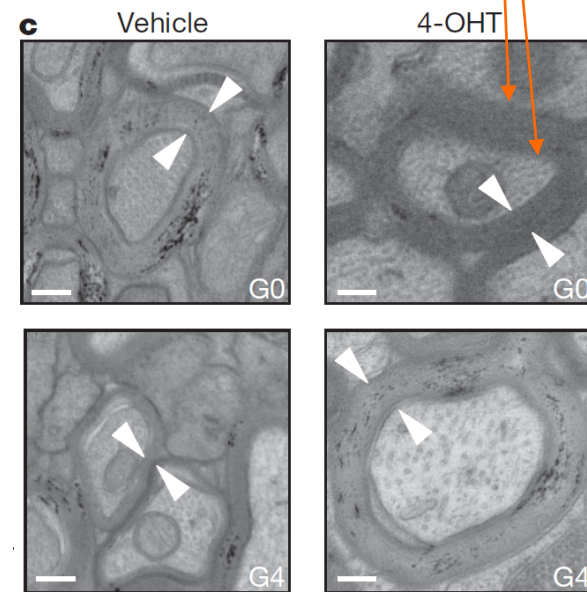
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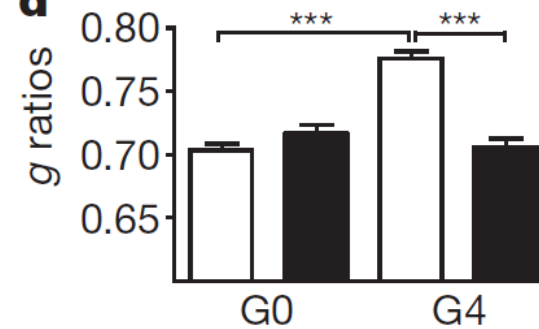
b



c



d

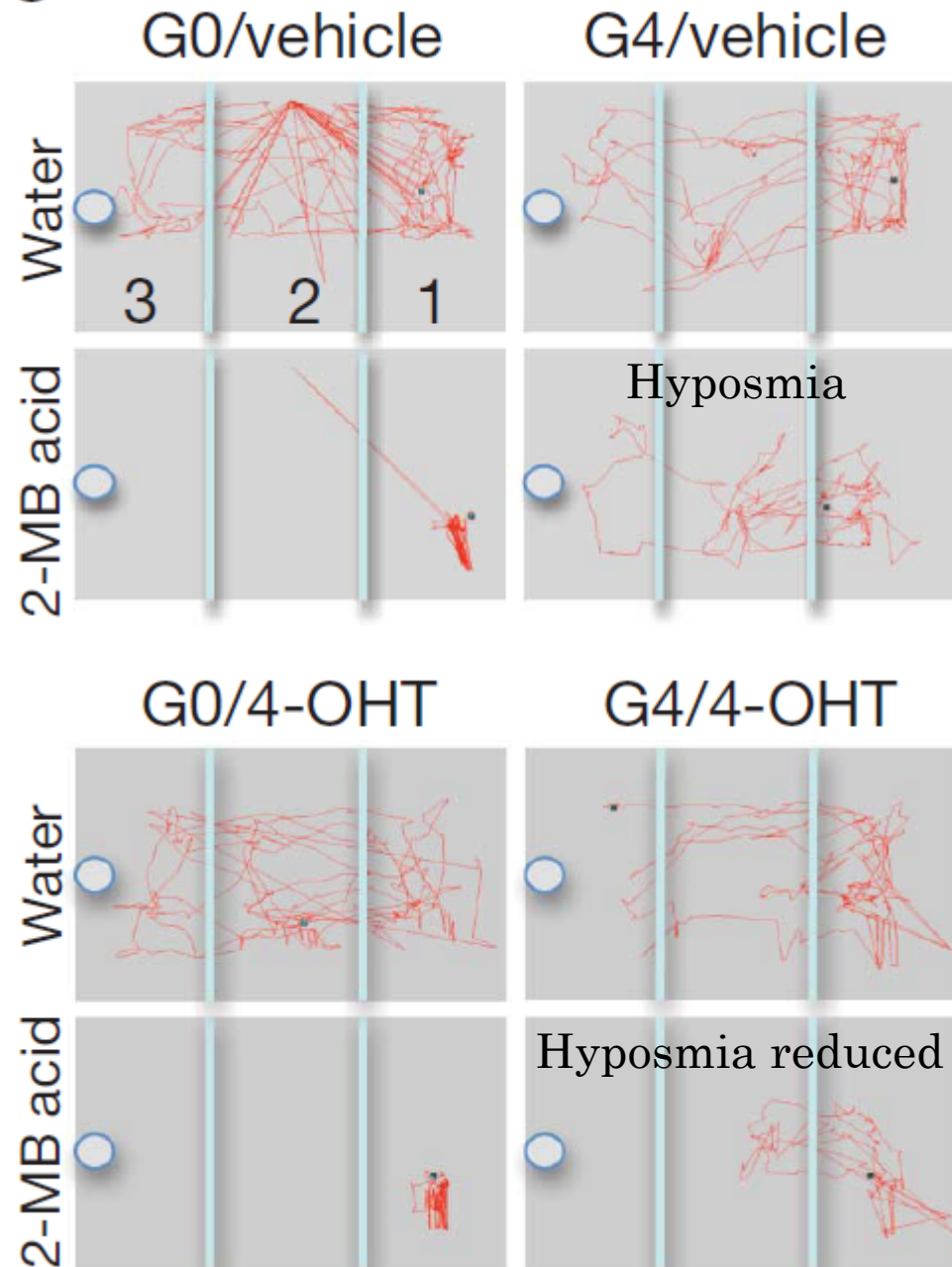


RESULTS

OLFACTORY FUNCTION

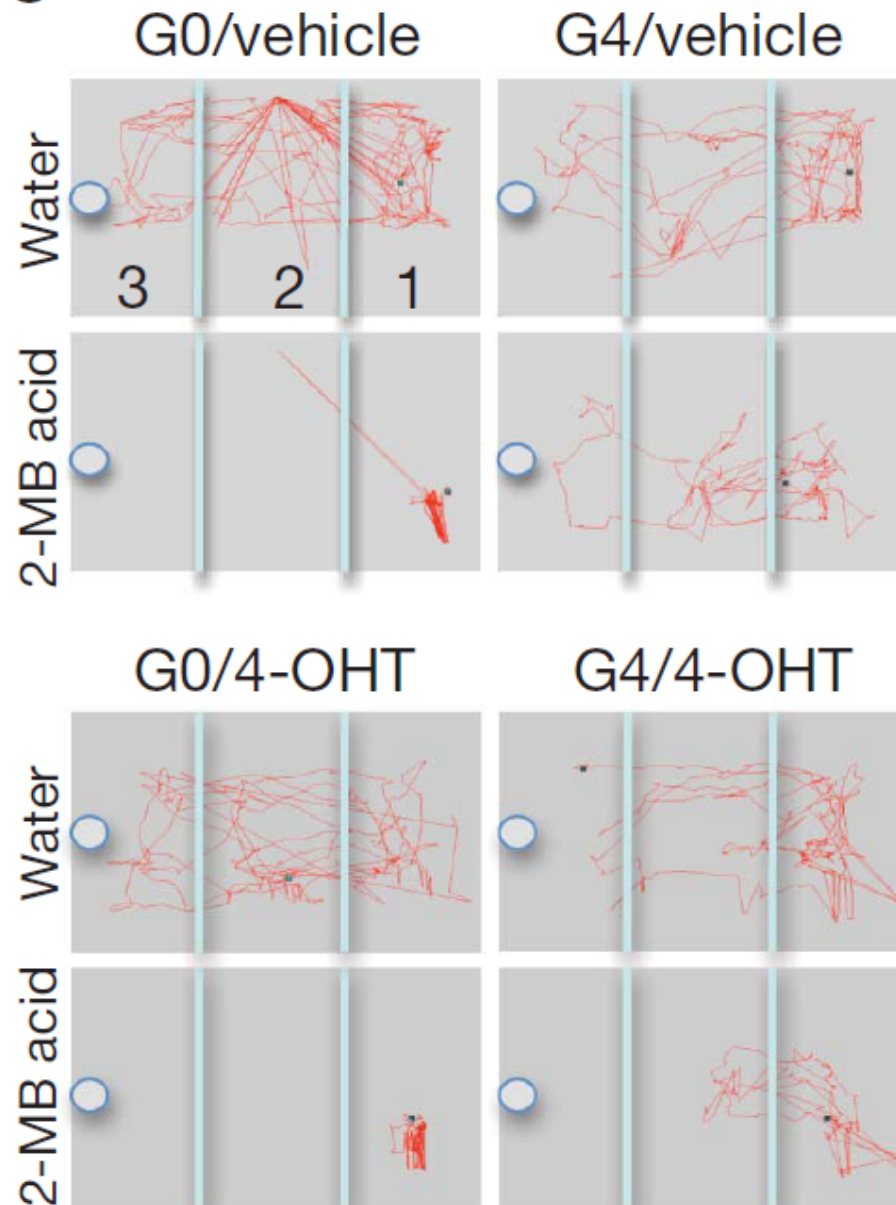


①

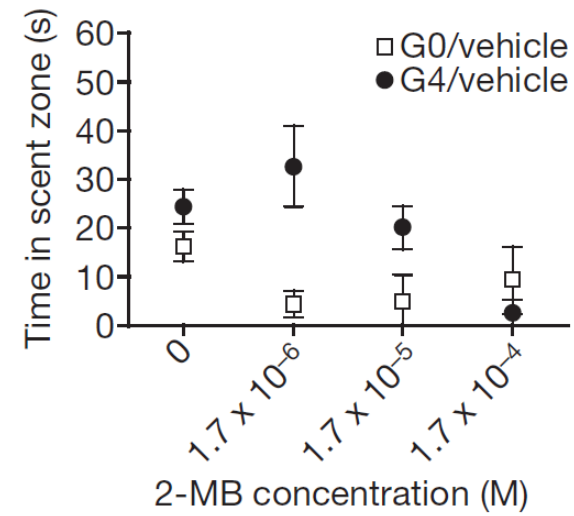


RESULTS

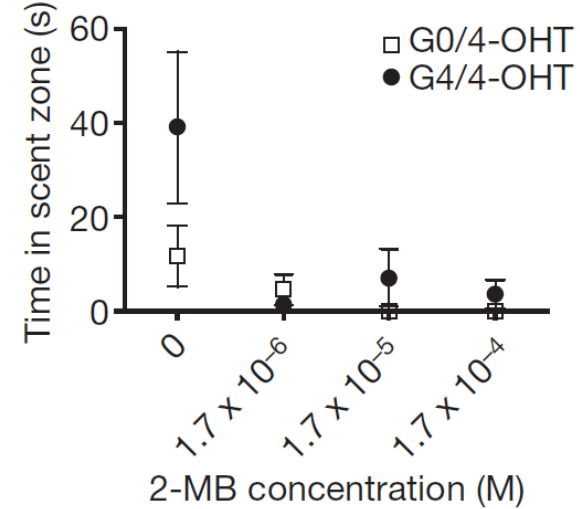
e



f



g



CONCLUSIONS

Short-term reactivation of telomerase reverses entrenched multi-system degeneration:

- Enhanced proliferation and differentiation of affected cells/tissues

But... Can promote cancer if:

- Done at a later period in life
- Done for longer periods of time



THANK YOU FOR THE ATTENTION!!

