Loss of a proteostatic checkpoint in intestinal stem cells contributes to age-related epithelial dysfunction

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Background

Protein Homeostasis (=Proteostasis)

- encompasses the balance between protein synthesis, folding, re-folding and degradation

- Perturbed in aging-systems (elevated oxidative and metabolic stress, changes in protein turnover rates, decline in protein degradation machinery and changes in proteostatic control mechanisms)
  - Accumulation of misfolded and aggregated proteins (Alzheimer‘s and Parkinson‘s disease)

Proteostatic stress

„all cells“

- Heat Shock Response (HSR) or Unfolded Protein Response (UPR)
  - Upregulation of Chaperons
  - Irreversible damage: degradation by proteasome or autophagy

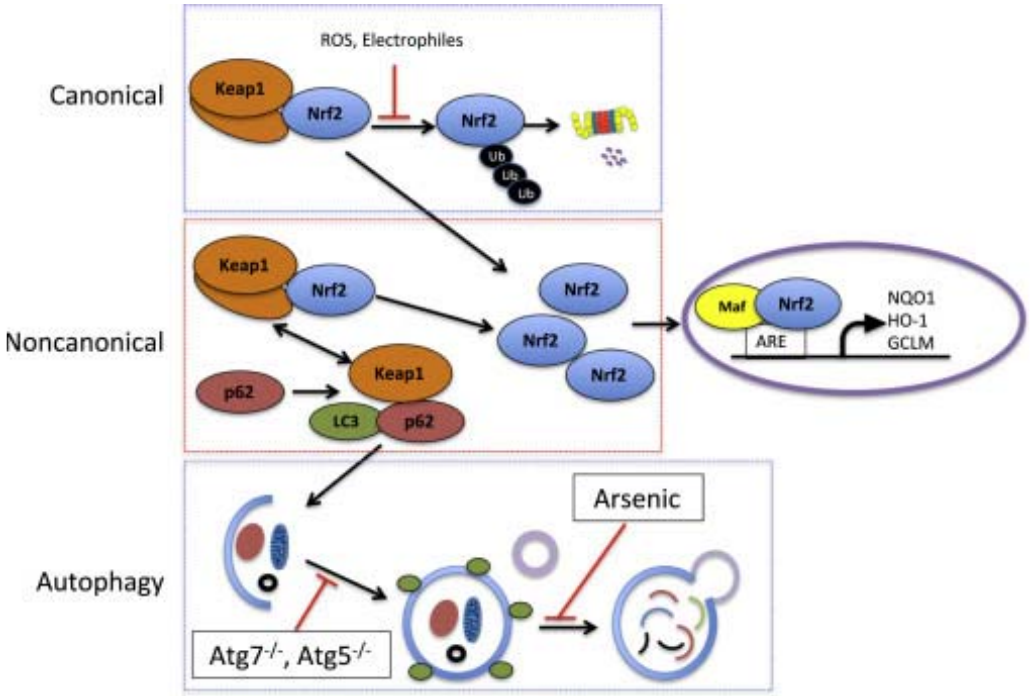
Stem-cells (SCs)

- Embryonic SCs (ECs):
  - unique pattern of chaparone expression + elevated 19S proteasome activity
  - Chaparone (e.g. HspA5, HspA8) and co-chaperone expression (e.g. Hop) shared with mesenchymal SCs (MSCs) and neuronal SCs (NSCs)
  - Elevated Autophagy shared with hematopoetic SCs (HSCs), MSCs, dermal and epidermal SCs
    - Defective Autophagy in HSCs contributes aging

Intestinal stem cells (ISCs) in Drosophila

- Vast majority of mitotically competent cells in intestinal epithelium
- Self-renewal after tissue damage due to ISCs regenerating all differentiated cell types
- Intestinal epithelium in aging flies dysfunction, exhibiting hyperplasia and mis-differentiation of ISCs and daughter cells -> inflammatory conditions –> loss of proteostatic capacity in ISCs
- Constitutive Activation of Unfolded Protein Response in ER (UPR-ER) elevated oxidative stress, constitutive activation of JNK and PERK Kinases –> Reducing PERK expression in ISCs sufficient to promote homeostasis and extend lifespan

Nrf2/CncC-mediated anti-oxidative response



Source: Jiang, T., Harder, B., Rojo de la Vega, M., Wong, P. K., Chapman, E., & Zhang, D. D. (2015). p62 links autophagy and Nrf2 signaling. Free Radical Biology and Medicine, 88, 199–204.

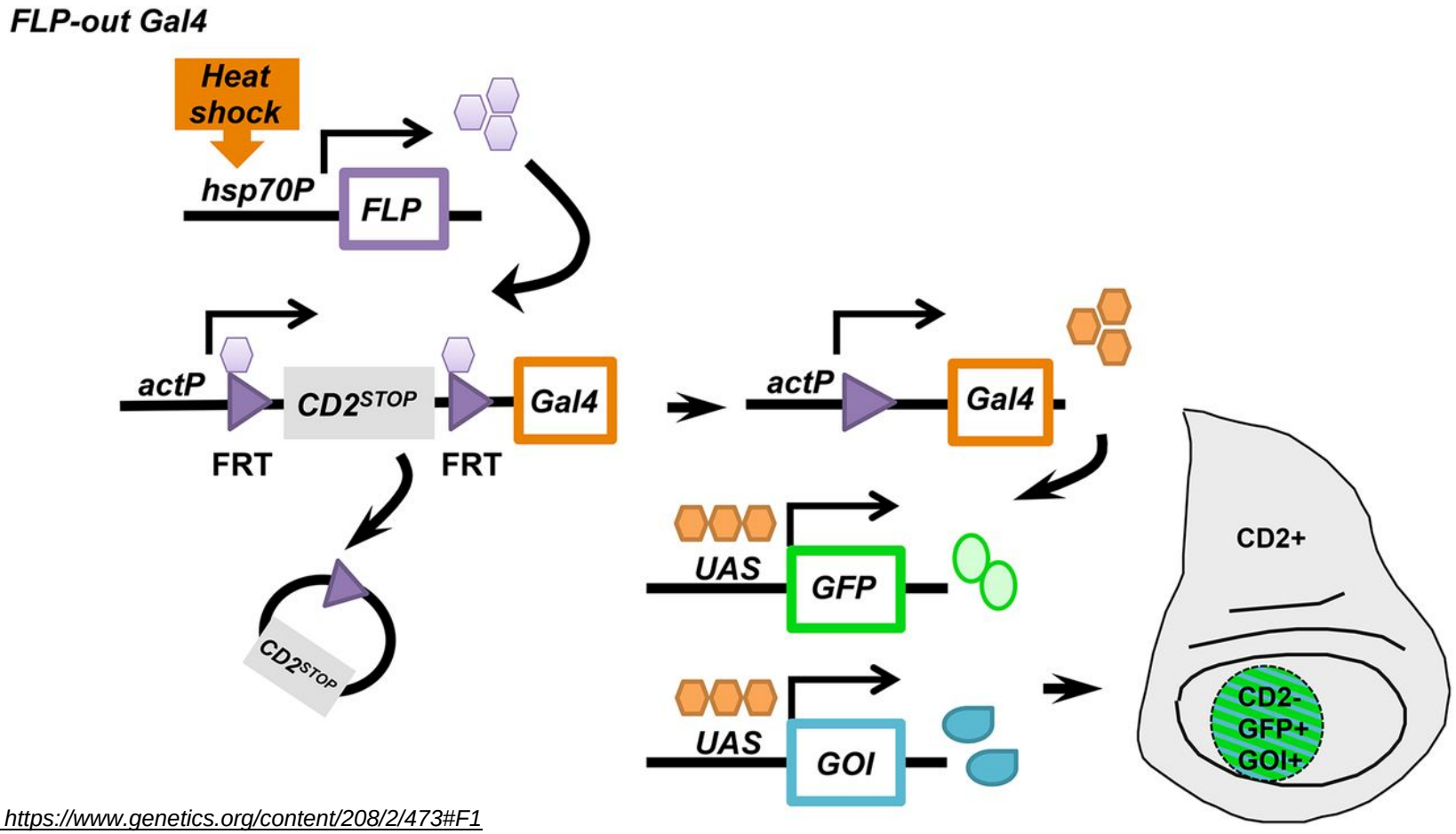
- In Drosophila ICs downregulation of CncC (Homologue of Nrf2) for Proliferation and regenerative Response
- Non-canonical Response in Drosophila:
  - Keap1 binds to Atg8a (LC3-Homologue) and Ref²p (p62-Homologue)

The Role of Nrf2/CncC

- In aging flies: Chronic inactivation of CncC (Nrf2 homologue) – regulators of antioxidant response
- In the study shown that CncC links cellcyle control with preotstatic responses in ISCs via accumulation of dacapo and transcriptional activation of genes encoding proteasis and proteasome subunits
- Transient „proteostatic checkpoint“ – celarance of protein aggregates before cell cyle activity is resumend
- Old flies: Checkpoint is impaired – reactivated with CncC activator

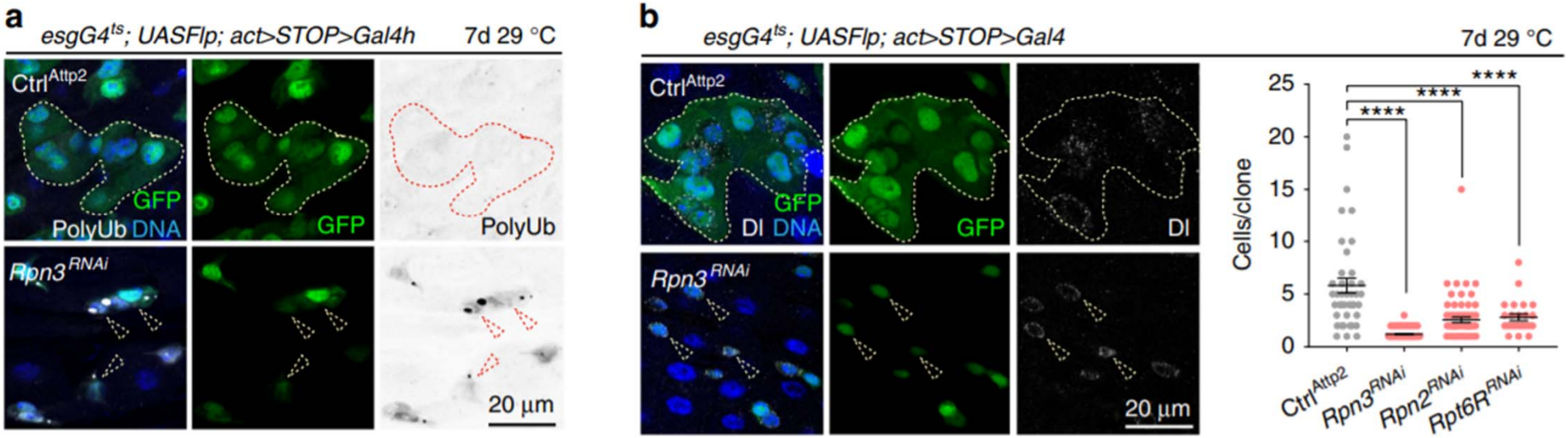
Results

Methodik



Source: https://www.genetics.org/content/208/2/473#F1

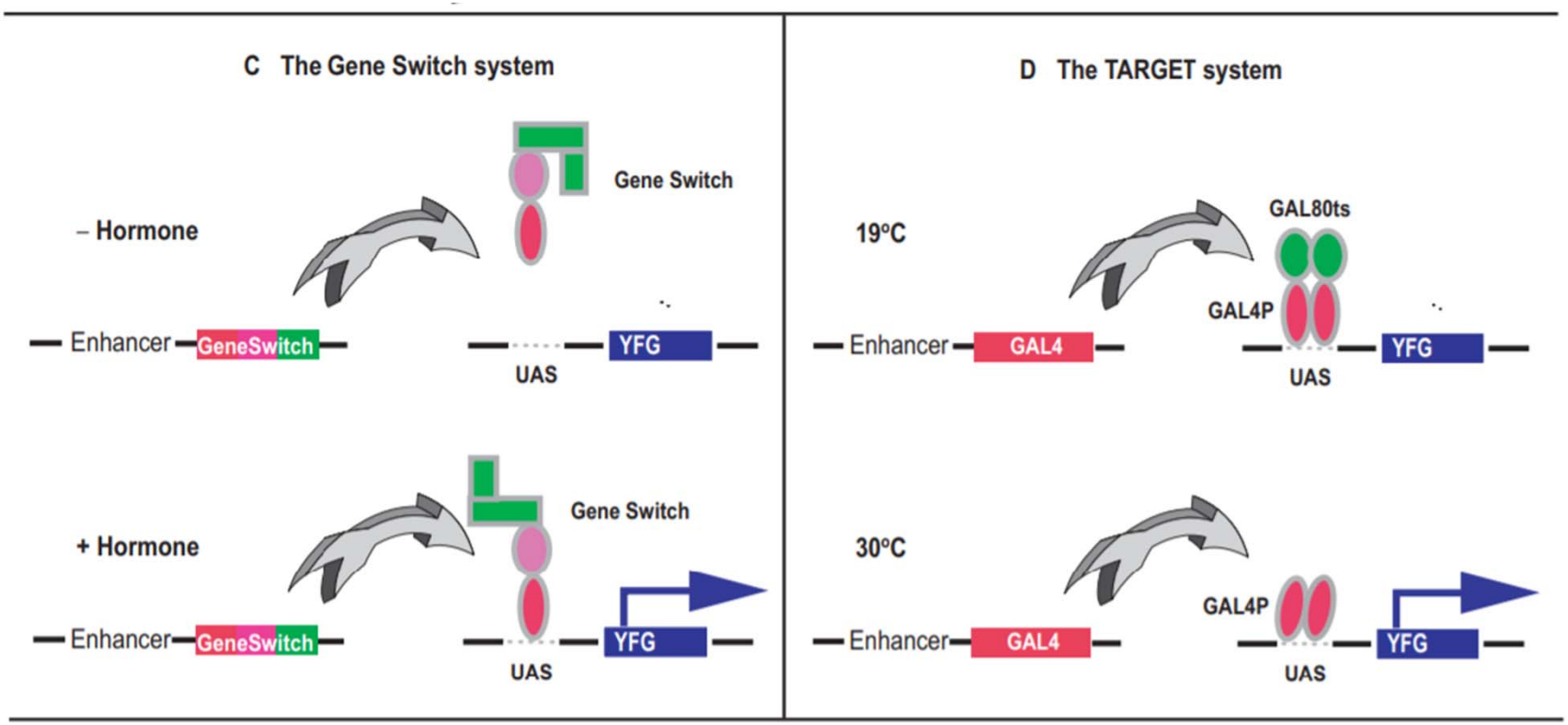
A proteostatic checkpoint in ISCs



Causes of observed effects

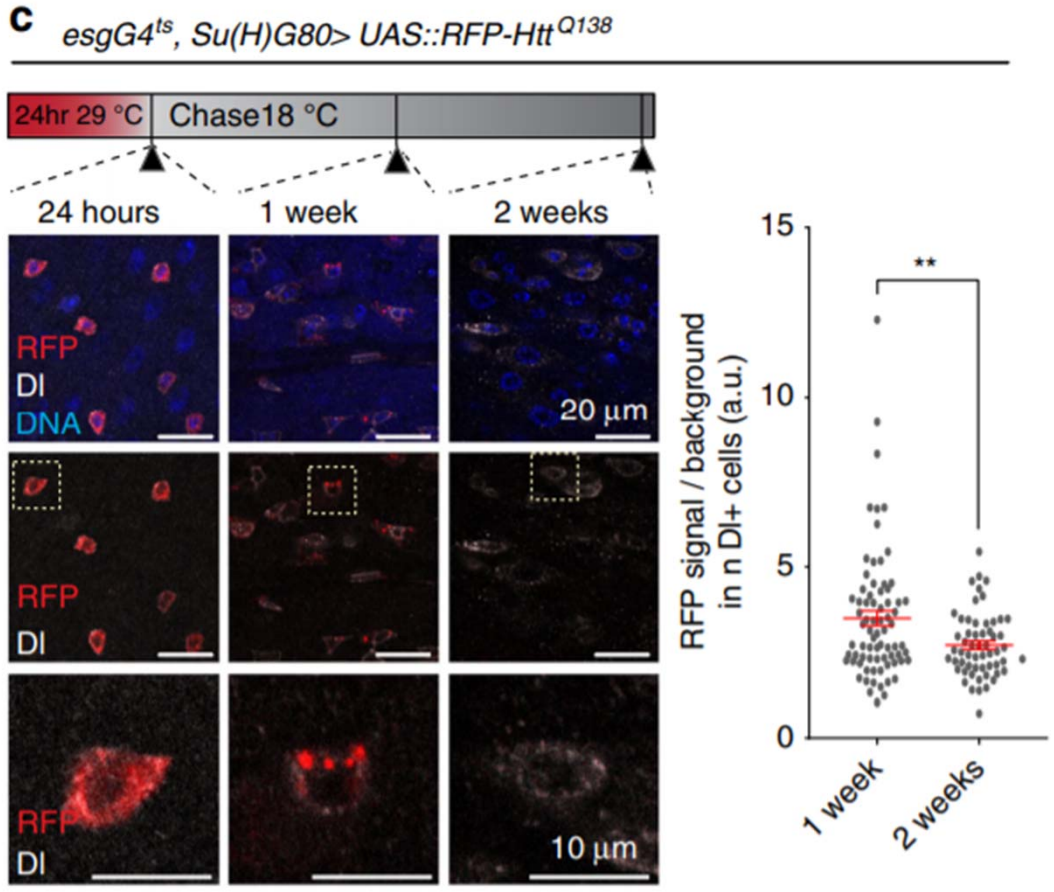
- Unspecifically impaired ISC function due to proteostatic stress
- Accumulation of cell cycle regulators due to decreased proteasome function
- Increased cell death of differentiated cells in such conditions
- A specific cell cycle arrest in ISCs due to presence of protein aggregates

TARGET-System

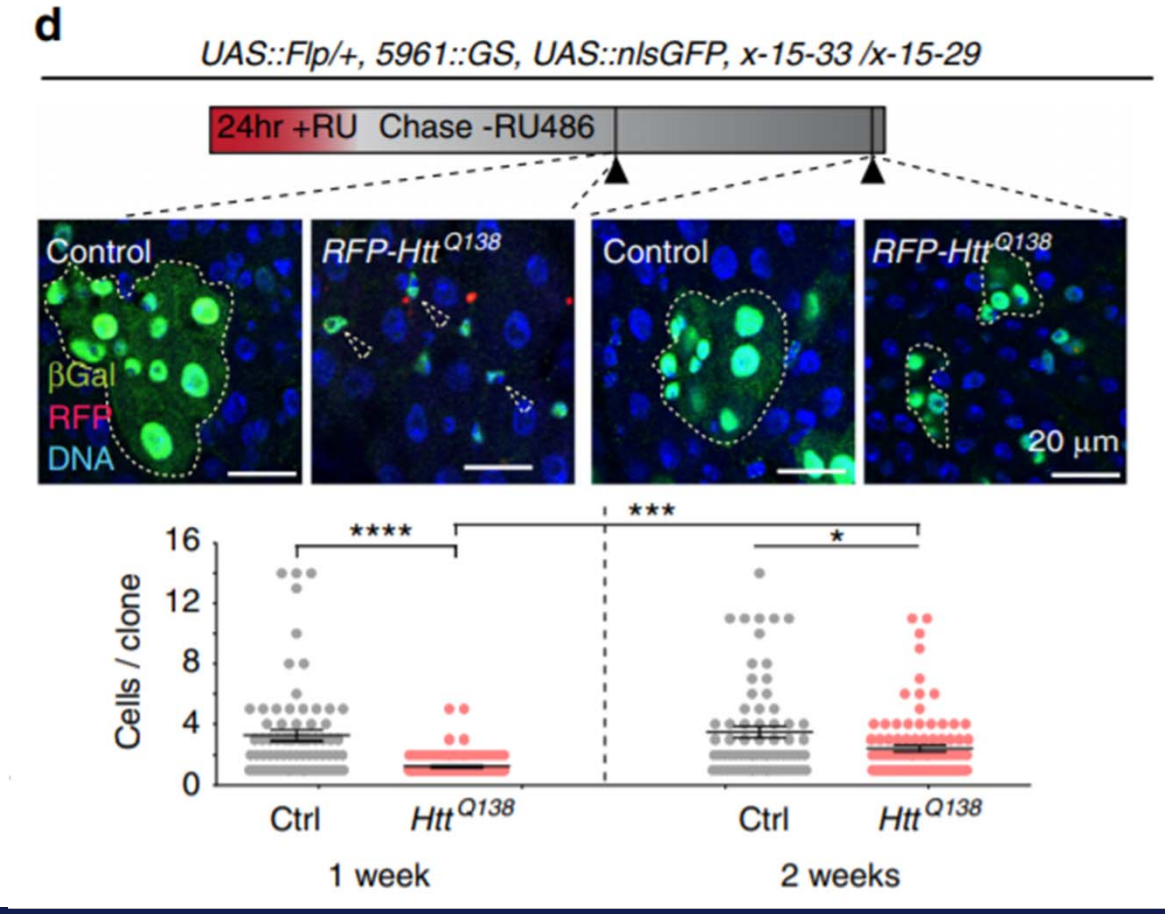


Source: S. E. McGuire, Z. Mao, R. L. Davis, Spatiotemporal gene expression targeting with the TARGET and Gene-Switch systems in Drosophila. Sci. STKE 2004, pl4 (2004).

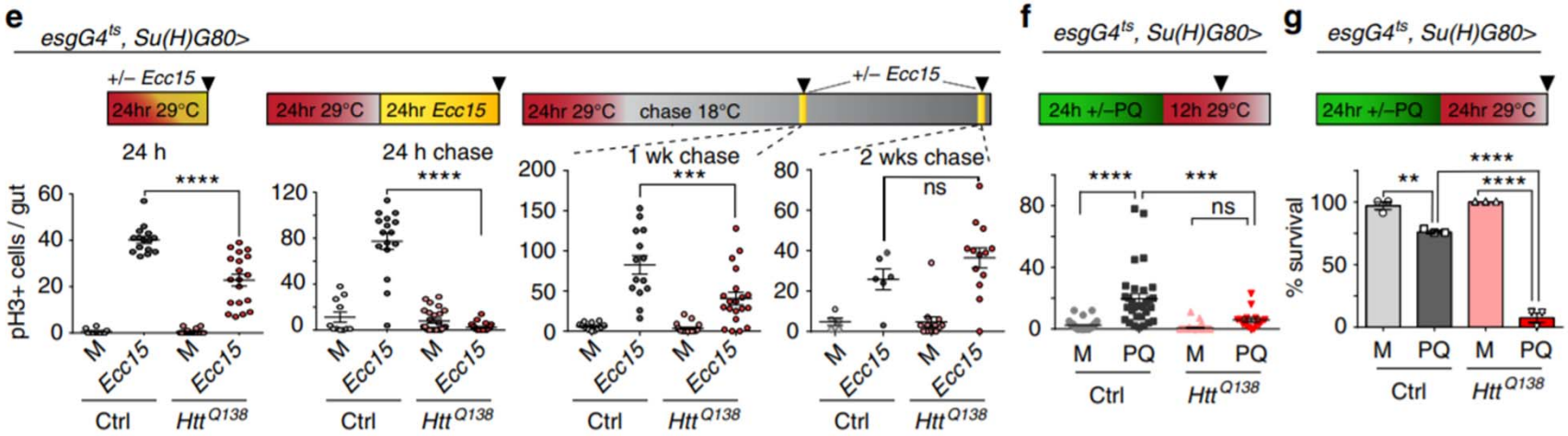
Time course – experiment with Expression of mRFP-Htt



Lineage-tracing experiments



Timing of proliferative impairment by protein aggregates in ISCs

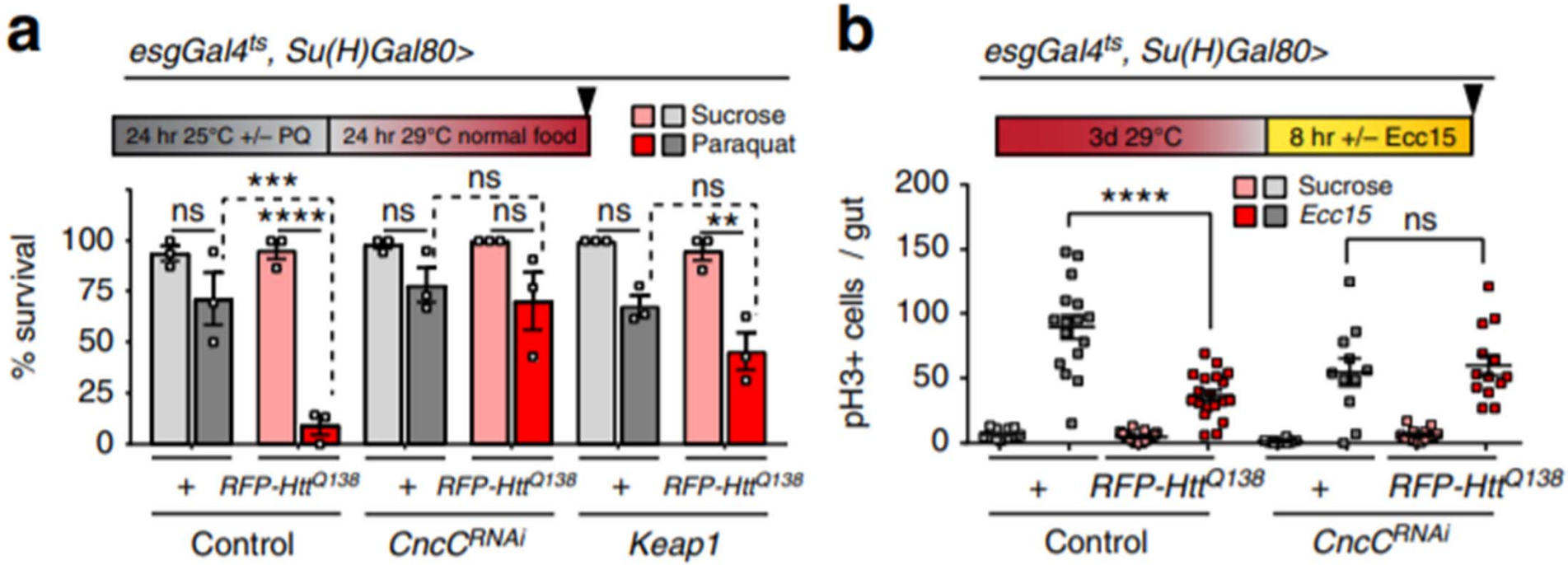


Observations

- ISCs undergo cell cycle arrest in response to protein aggregate formation
- Arrest is sustained until aggregates are cleared
- ISCs can undergo normal proliferative response after aggregate clearance

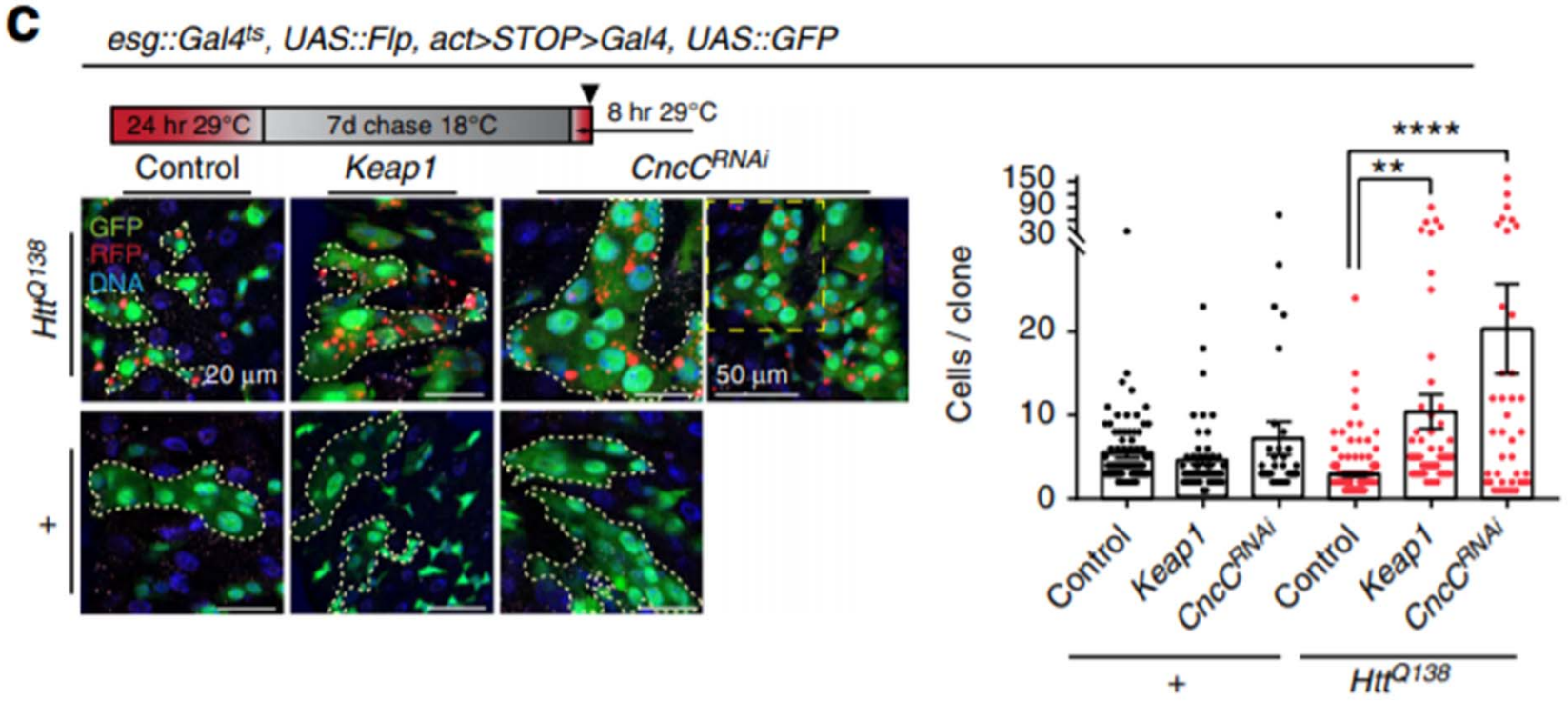
“Proteostatic Checkpoint”

Nrf2/CncC regulates the proteostatic checkpoint

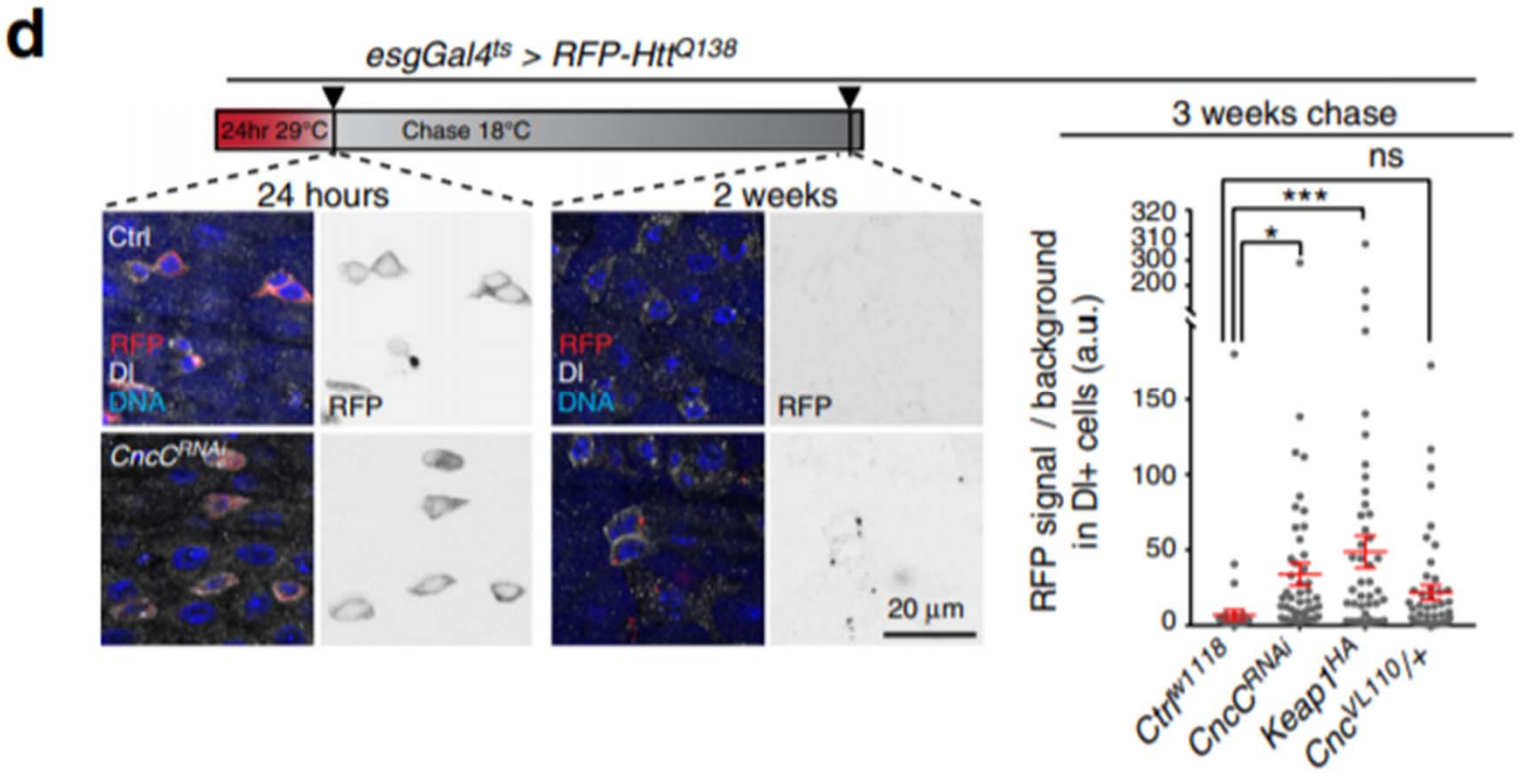


Suggestion: CncC was involved in the proteostatic checkpoint

Lineage-tracing of Keap1-overexpressing or CncC-deficient ISCs

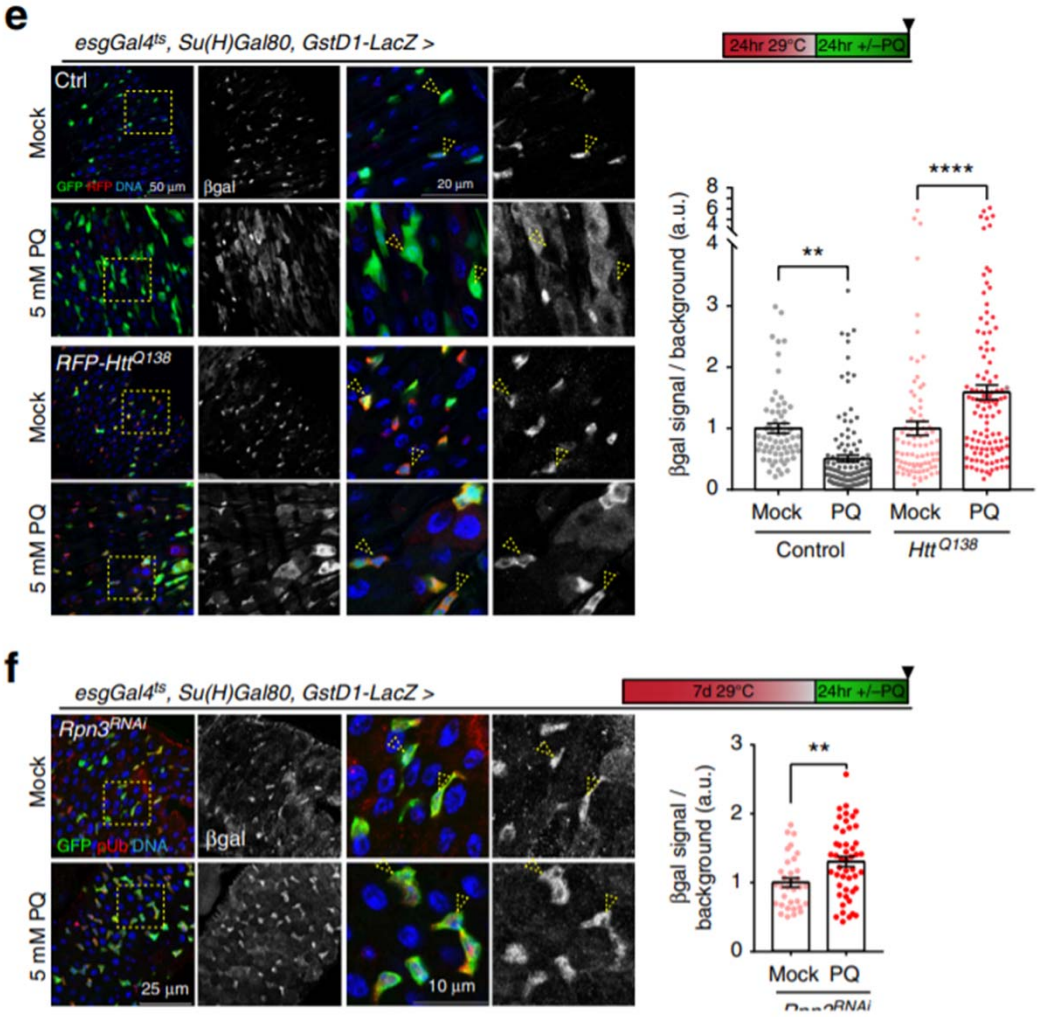


Observation: clones derived from ICs in which CncC was knocked down or in which Keap1 was overexpressed, grew at a higher rate than wild-type clones and exhibited increased levels of mRFP-Htt puncta

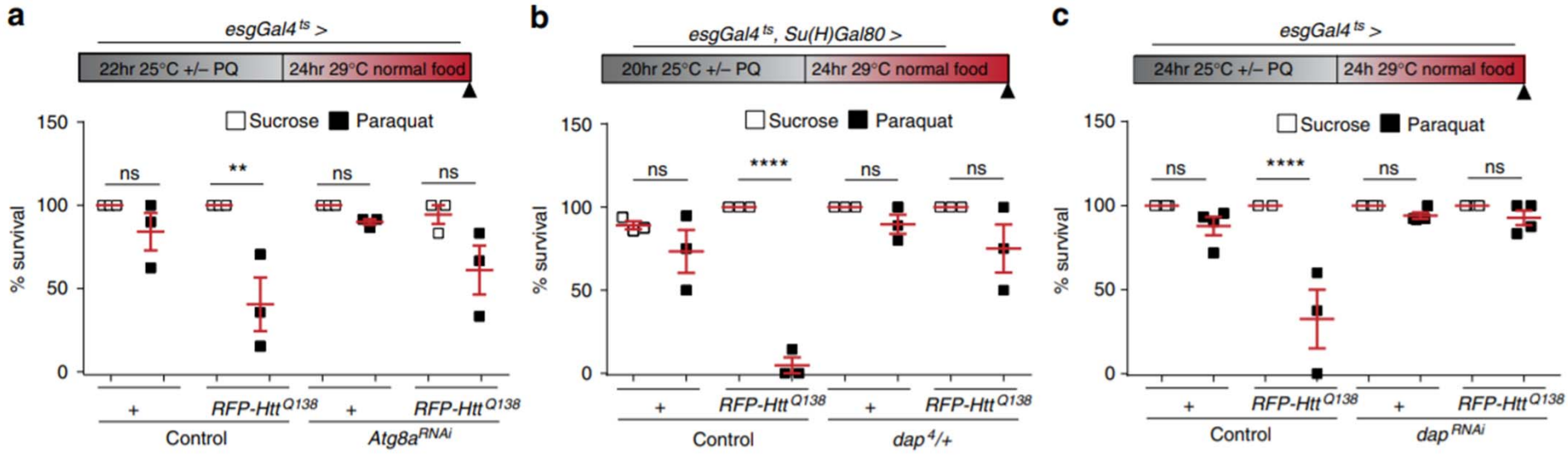

d
esgGal4ts > RFP-HttQ138
24hr 29°C
Chase 18°C
24 hours
2 weeks
Ctrl
RFP
Dl
DNA
RFP
CncCRNAi
20 µm
3 weeks chase
ns
***
*
RFP signal / background in Dl+ cells (a.u.)
320
310
300
200
150
100
50
0
Ctrlw1118
CncCRNAi
Keap1HA
CncVL110/+

Nrf2 Activity in presence of mRFP-Htt

- Increased activity of Nrf2 in presence of mRFP-Htt aggregates or when Rpn3 is knocked down
- Wildtype-ISCs exhibit high basal, CncC-dependent expression of this reporter (GstD1::LacZ), in conditions of regenerative pressure CncC is selectively inactivated (by Keap1)
- CncC-Inactivation is essential for elevation of reactive oxygen species in ISCs
- No repression of GstD1::LacZ reporter in ISCs bearing mRFP-Htt aggregates -> presence of aggregates impairs ability to inactivate CncC under regenerative pressure

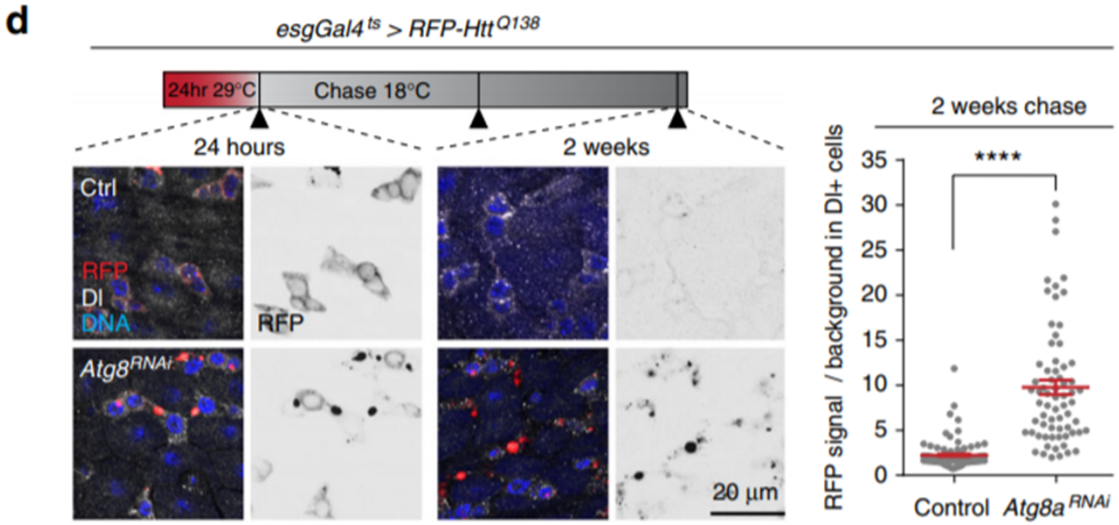


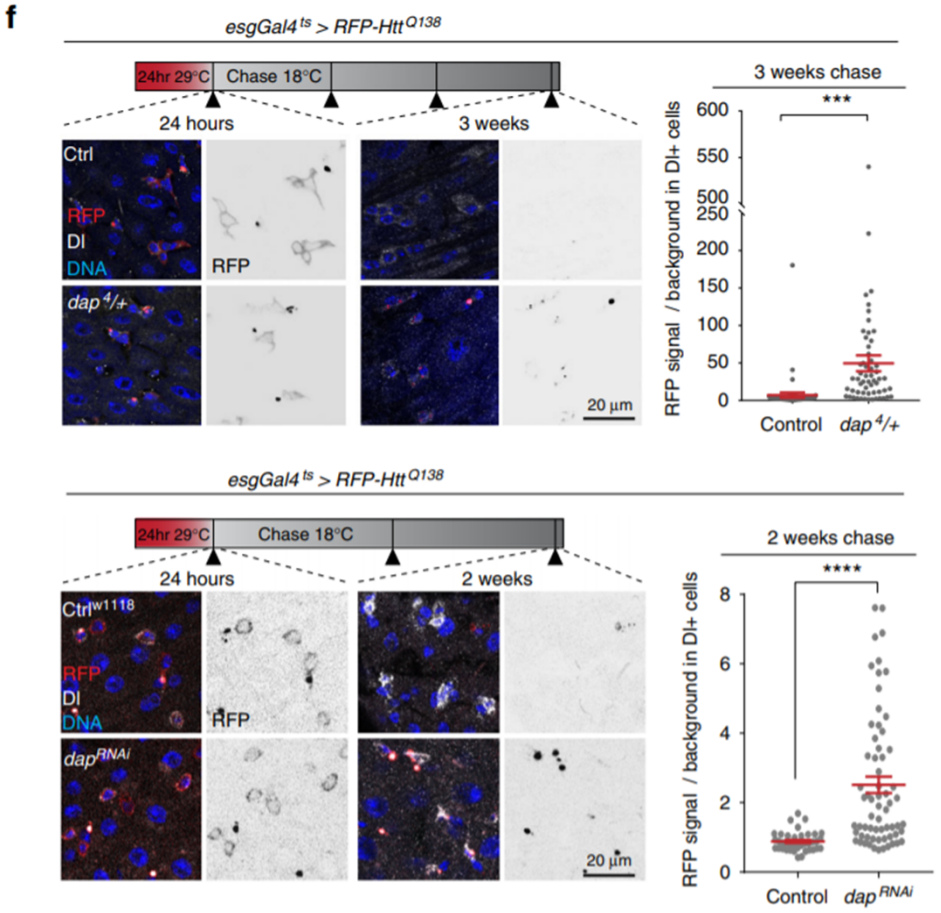
Atg8a-CncC-Dap Pathway controls the proteostatic checkpoint



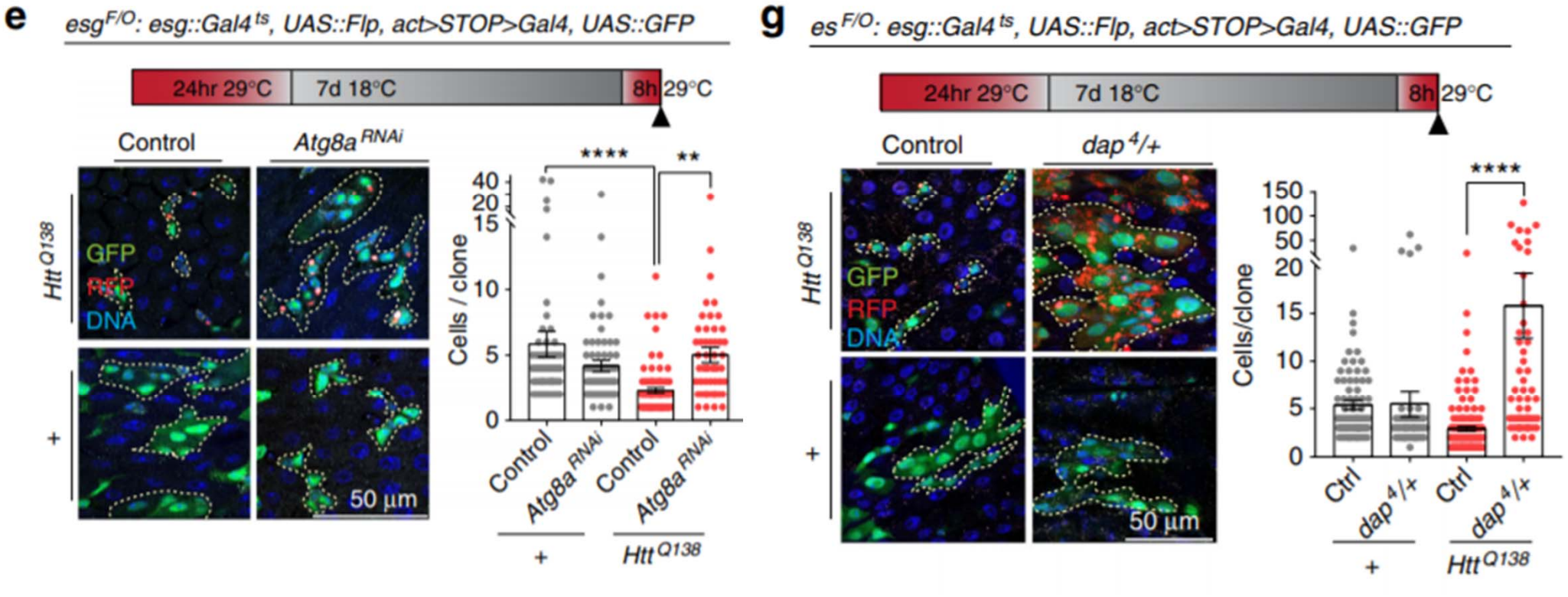
PQ-induced Mortality is reduced in flies bearing mRFP-Htt aggregates, when Atg8a and dap4 is knocked down or dap-deficient

Atg8a- and dap4-knockdown inhibit Clearance of mRFP-Htt-aggregates

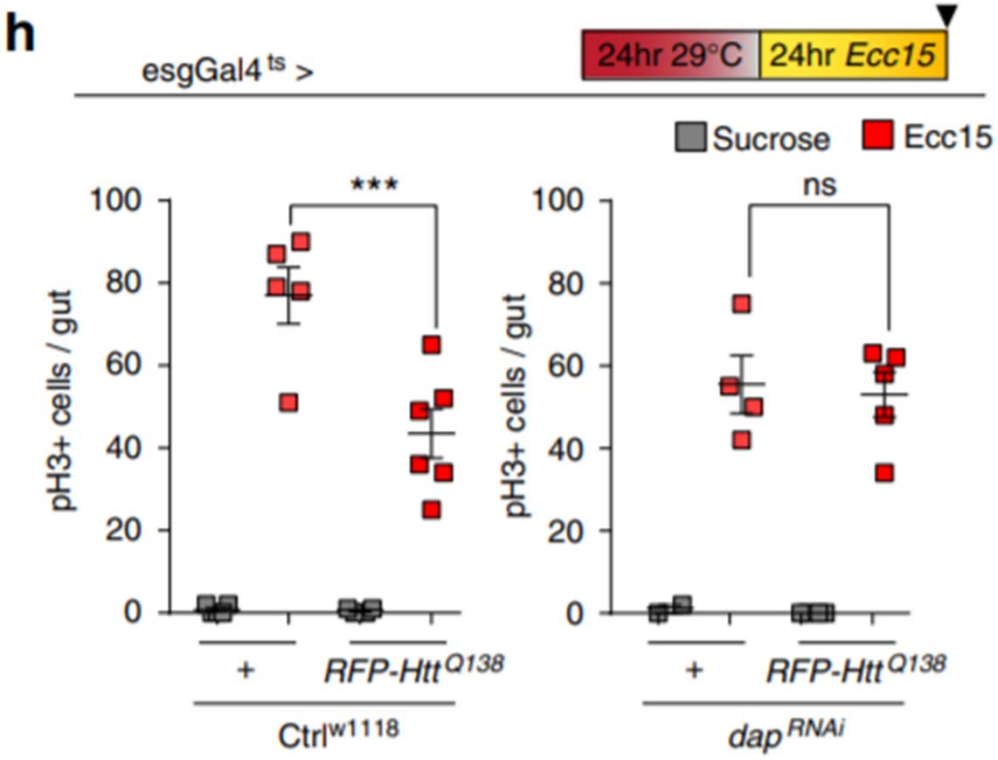




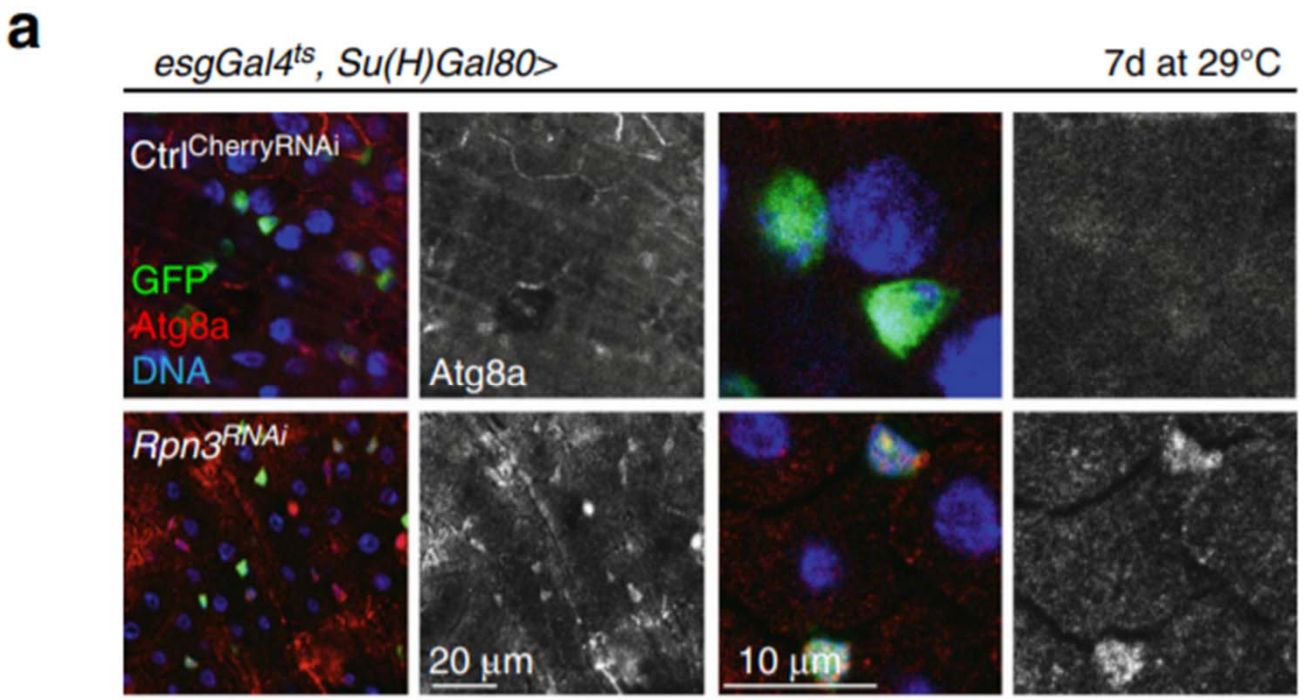
Atg8a- and dap4-knockdown rescue lineage growth



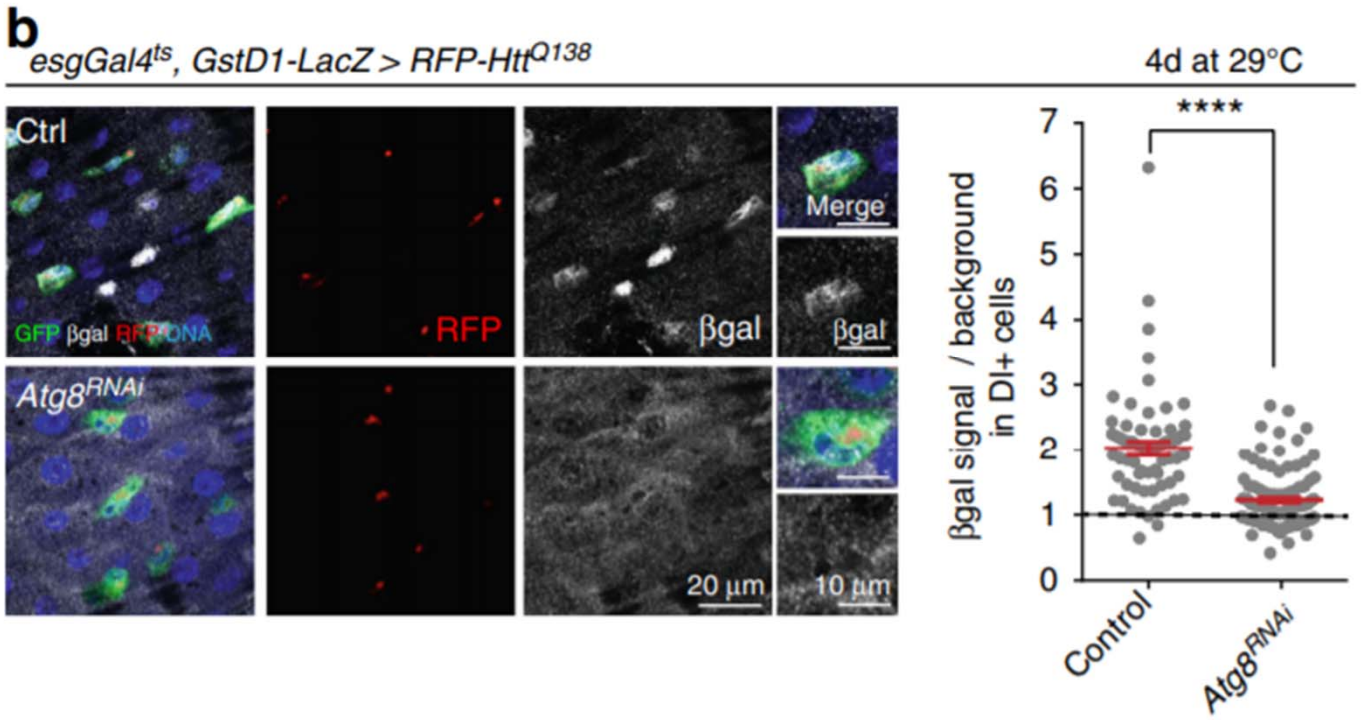
dap4-knockdown rescued mRFP-Htt-induced inhibition of proliferation



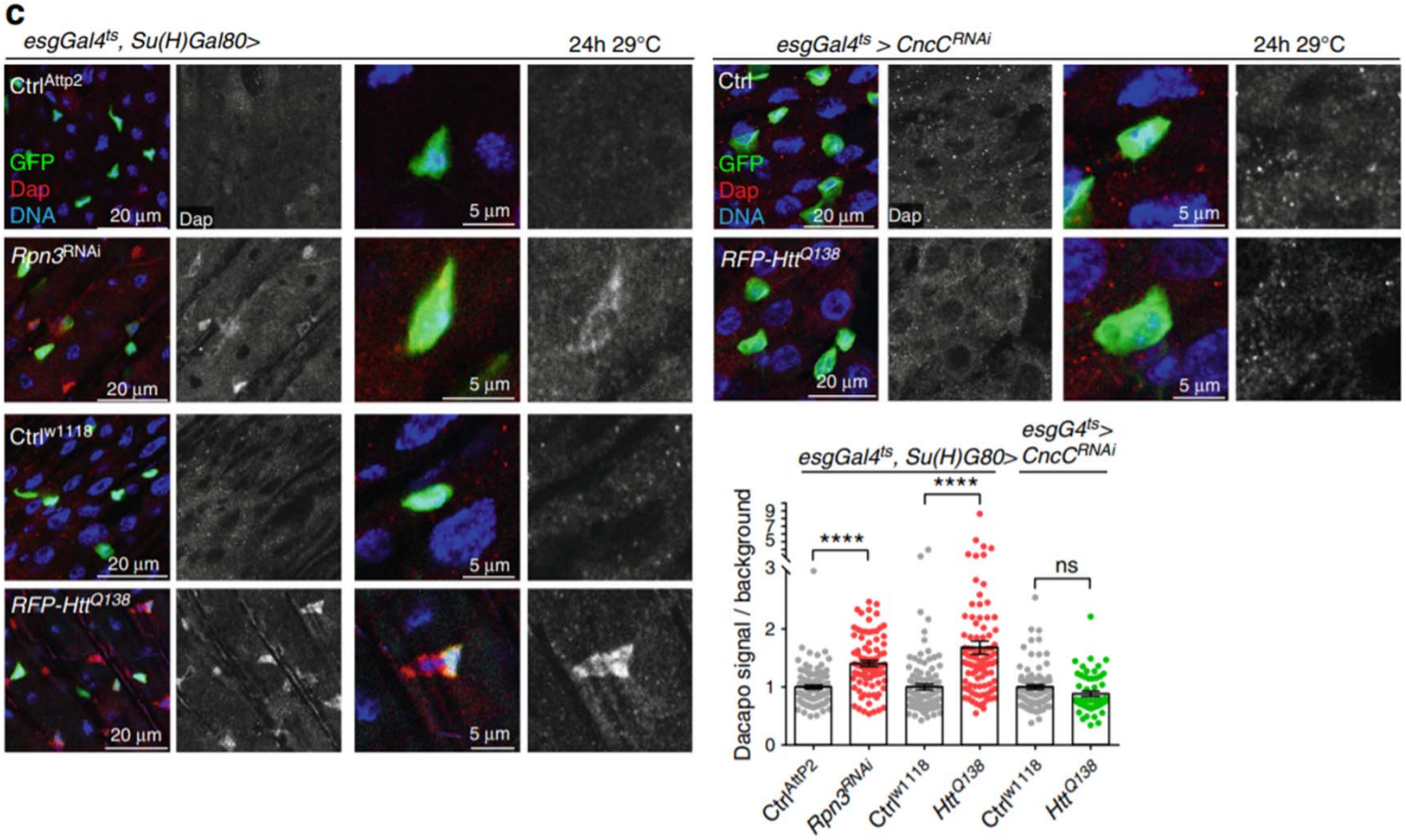
Atg8a-levels in ISCs experiencing proteostatic stress



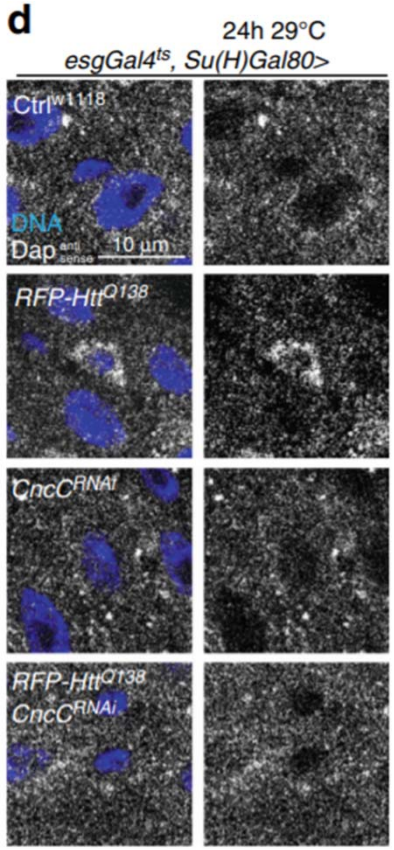
Atg8a-knockout resulted in loss of persistent Nrf2 activation



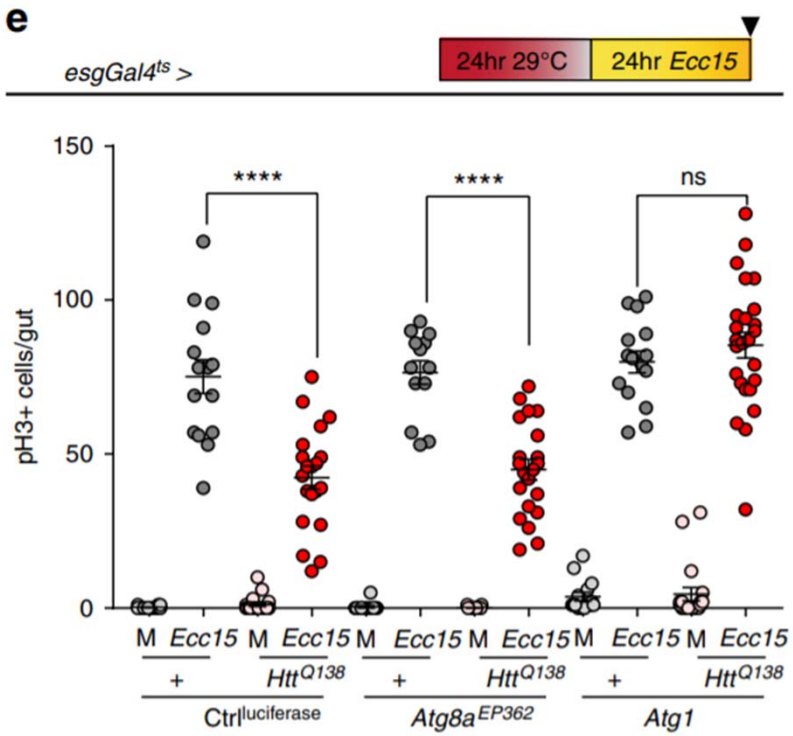
Dacapo as inhibitor of CncC activity in proteostatic checkpoint



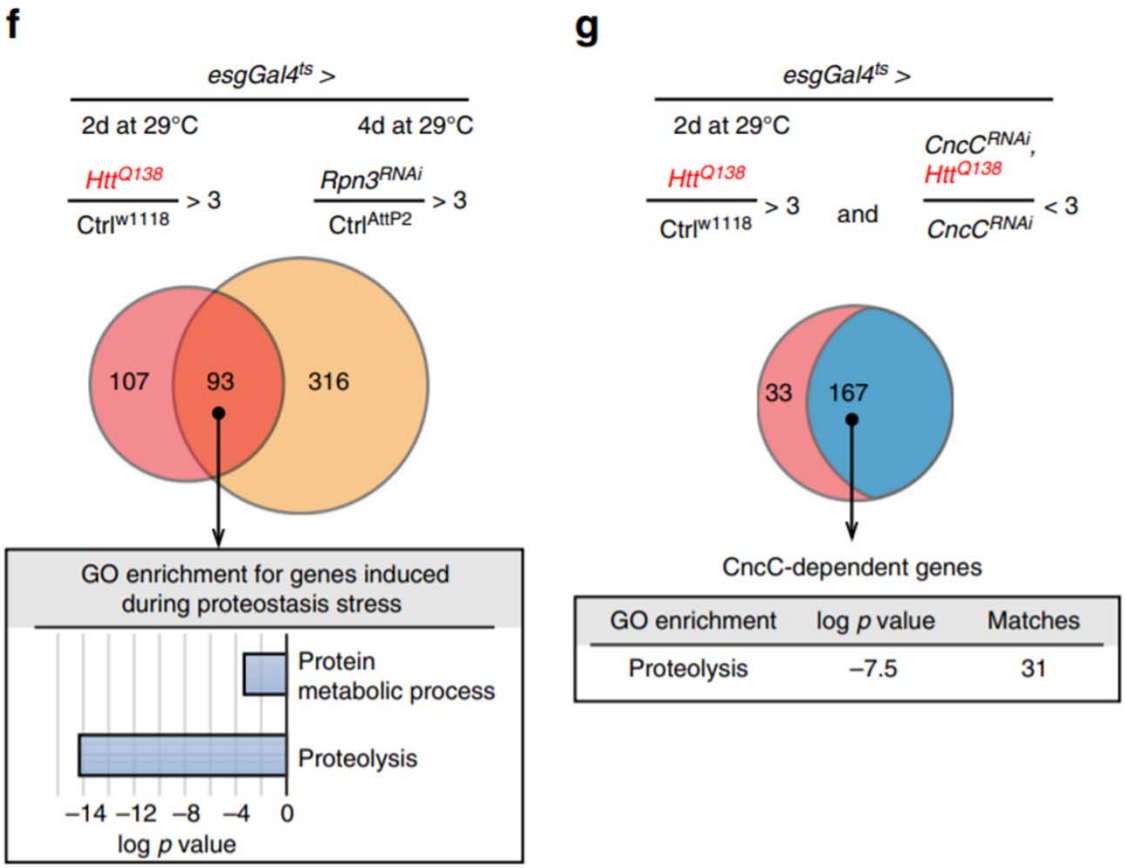
FISH (fluorescent in situ hybridization)



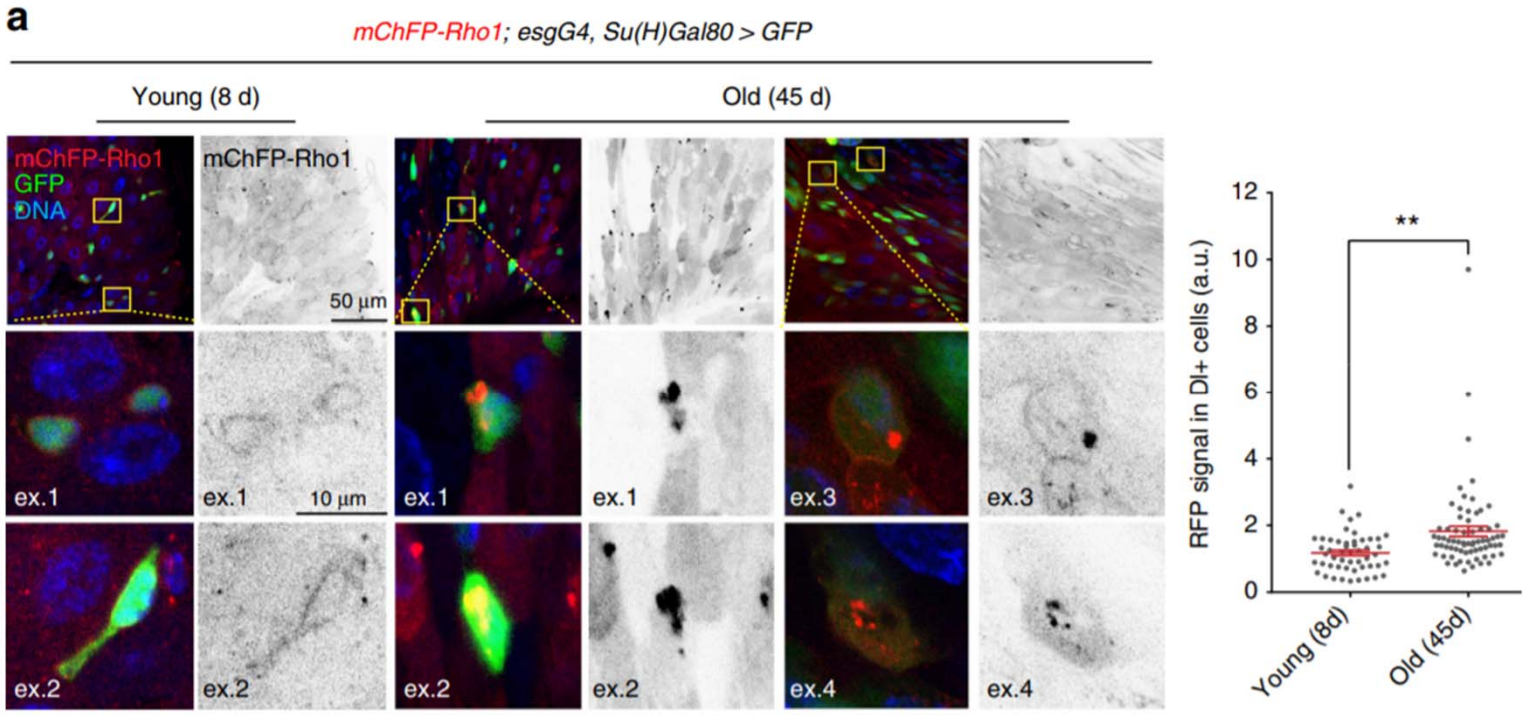
Over-expression Atg1 limits activtation of proteostatic checkpoint



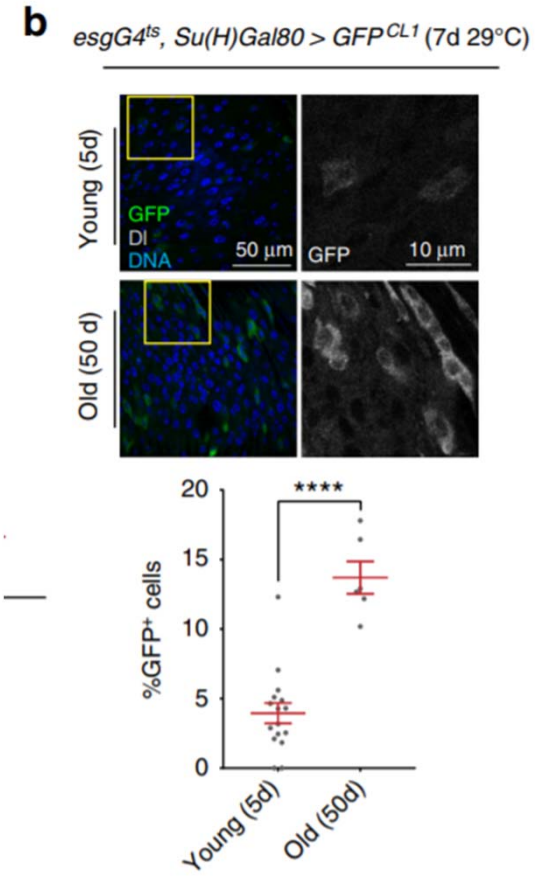
Transcriptional program downstream of CncC in ISCs



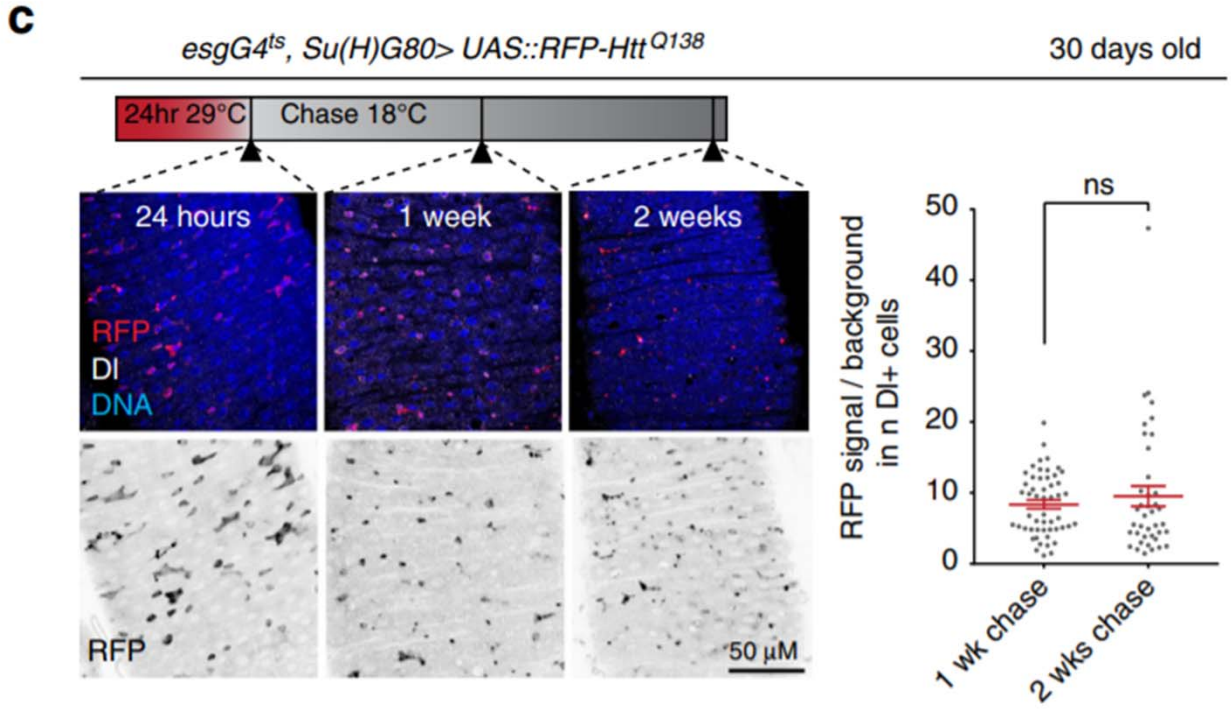
Decline of the proteostatic checkpoint in aging flies

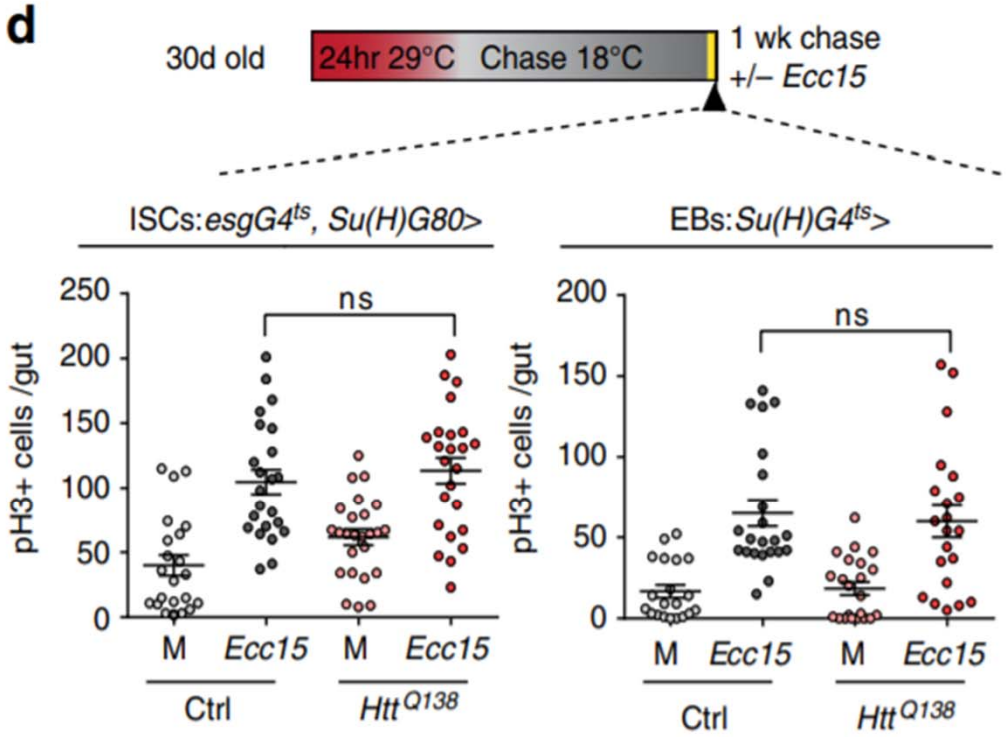


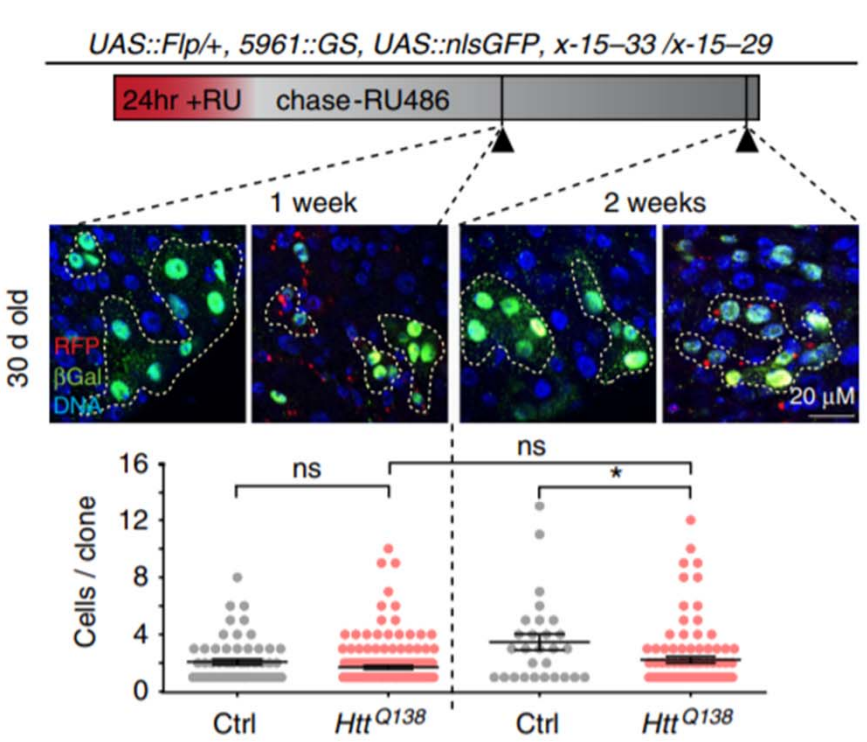
Age-related decline in proteostatic capacity



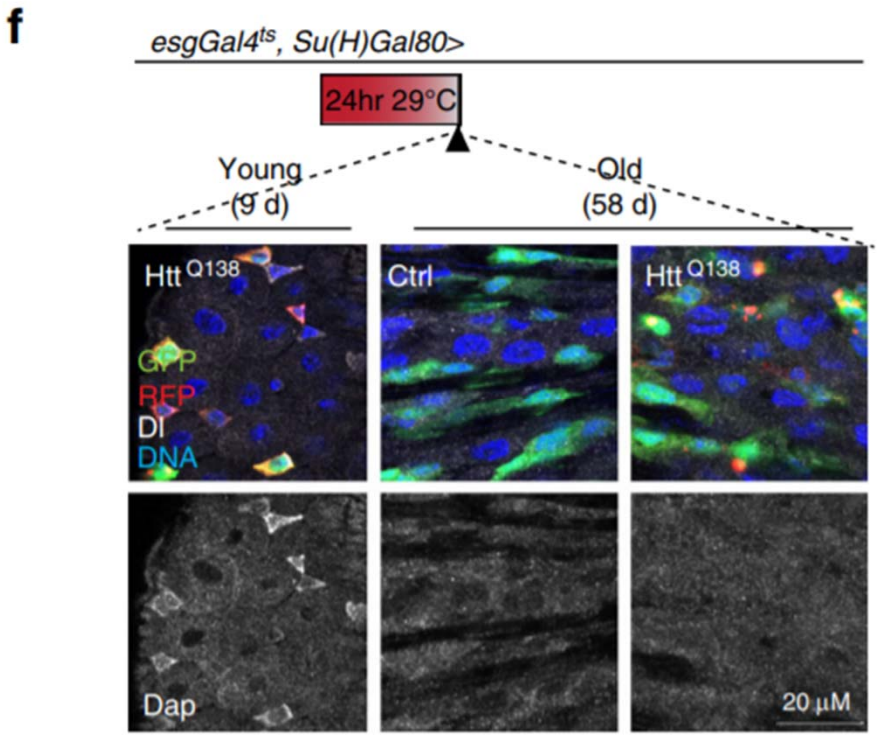
ISCs proliferating even in presence of mRFP-Htt-aggregates



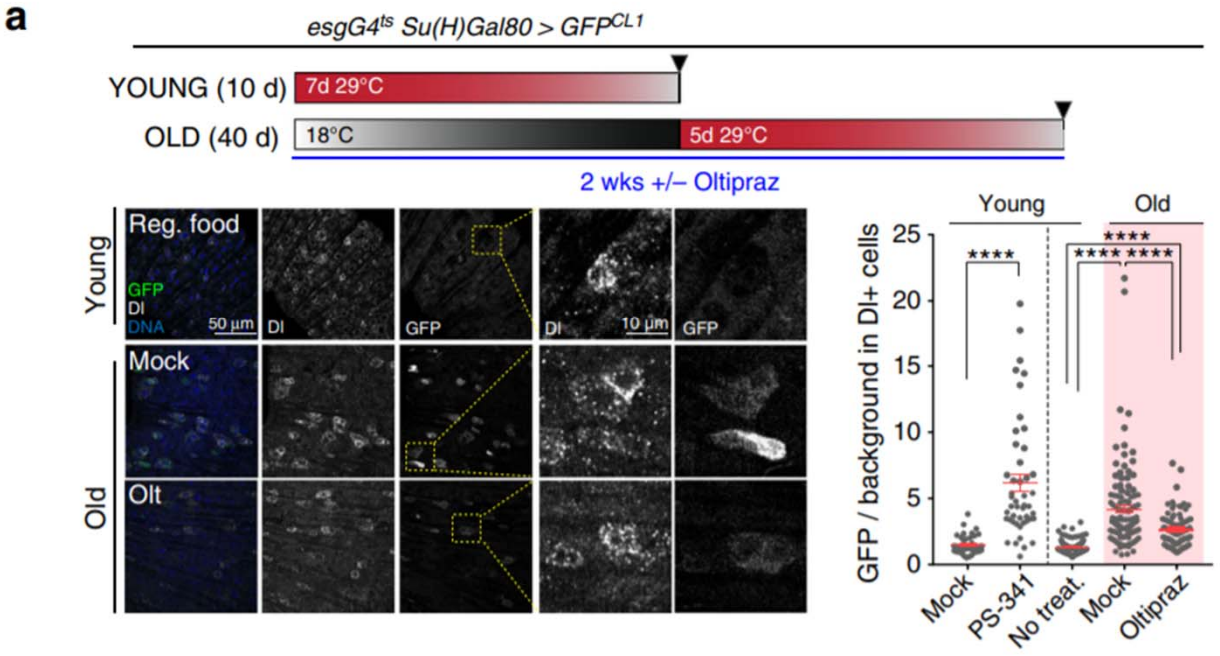

d
30d old
24hr 29°C
Chase 18°C
1 wk chase
+/– Ecc15
ISCs:esgG4ts, Su(H)G80>
EBs:Su(H)G4ts>
pH3+ cells /gut
250
200
150
100
50
0
ns
M
Ecc15
Ctrl
HttQ138


e
UAS::Flp/+, 5961::GS, UAS::nlsGFP, x-15–33 /x-15–29
24hr +RU
chase-RU486
1 week
2 weeks
30 d old
RFP
βGal
DNA
20 μM
Cells / clone
16
12
8
4
0
ns
*
Ctrl
HttQ138

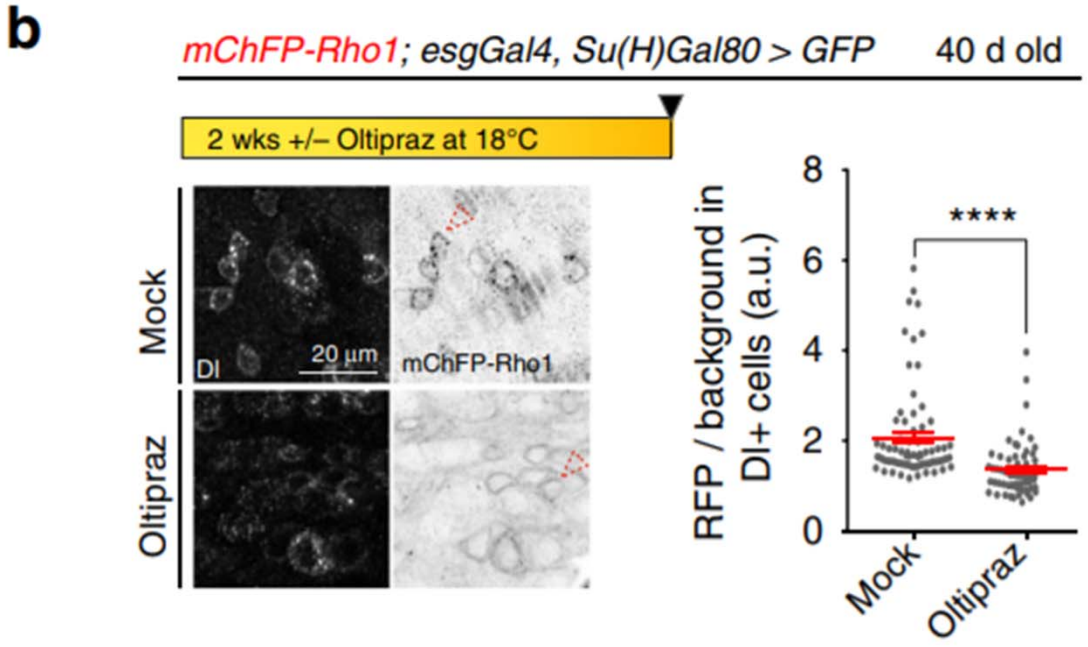
Impaired induction of dacapo expression durch Htt in old flies



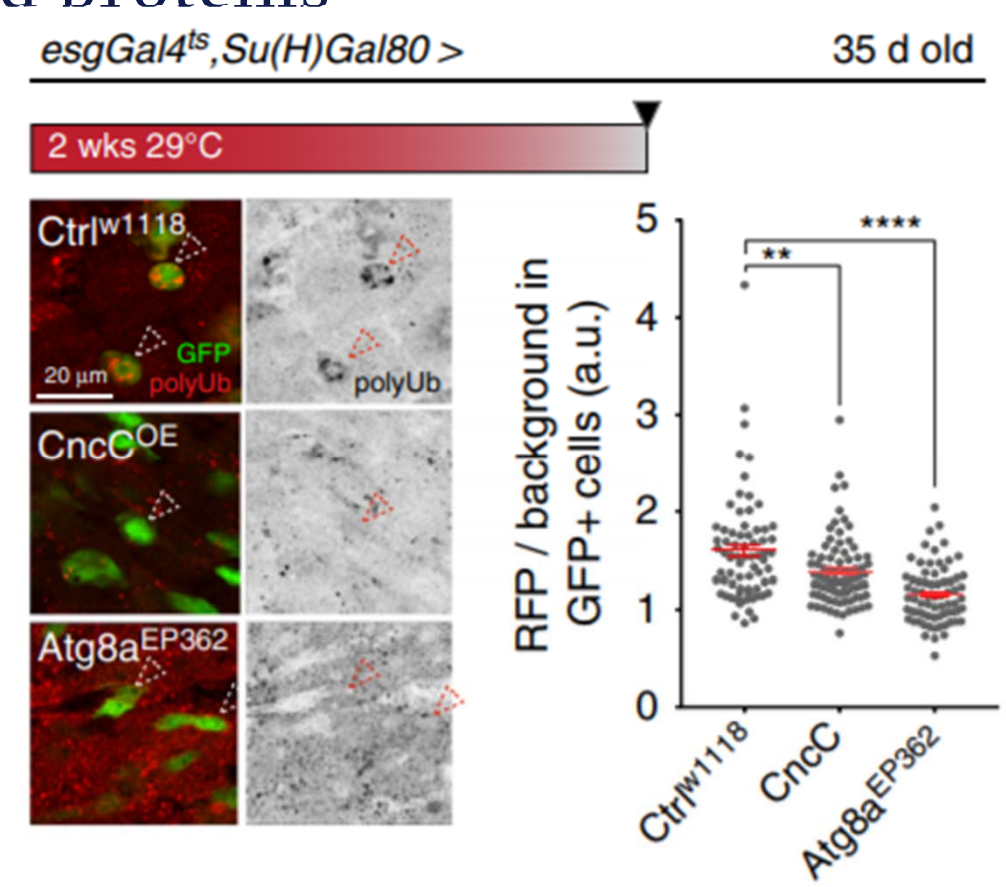
Restoration of proteostatic checkpoint with Oltipraz (Nrf2/CncC activator)



Reduction of Rho1 in Oltipraz-treated flies



CncC and Atg8a over-expression leads to reduction of poly-ubiquitined proteins



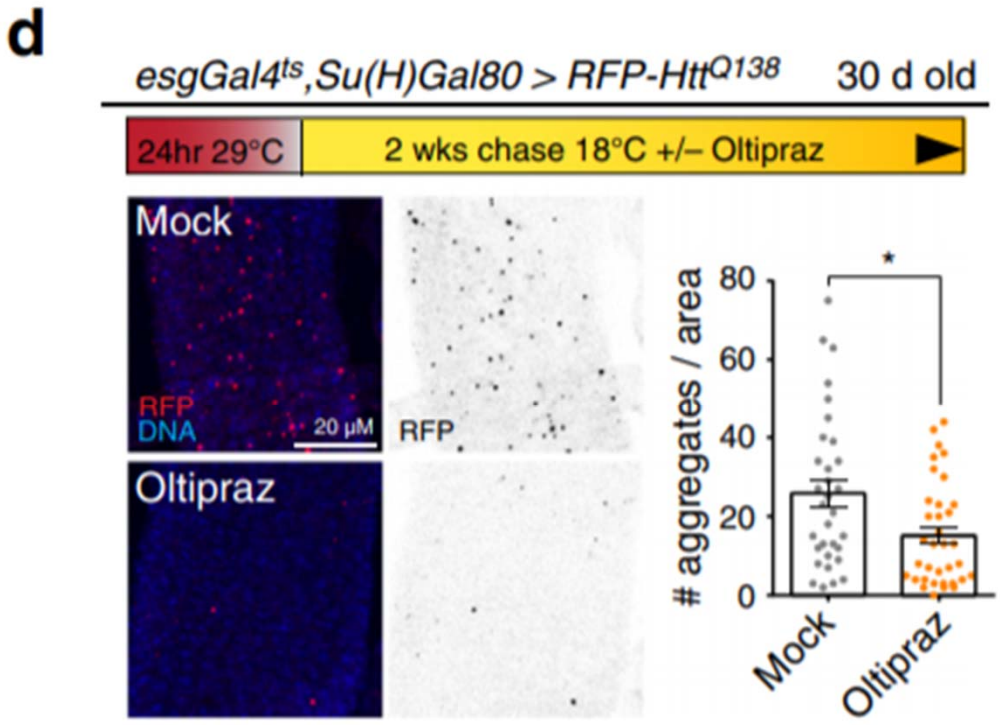
Discussion

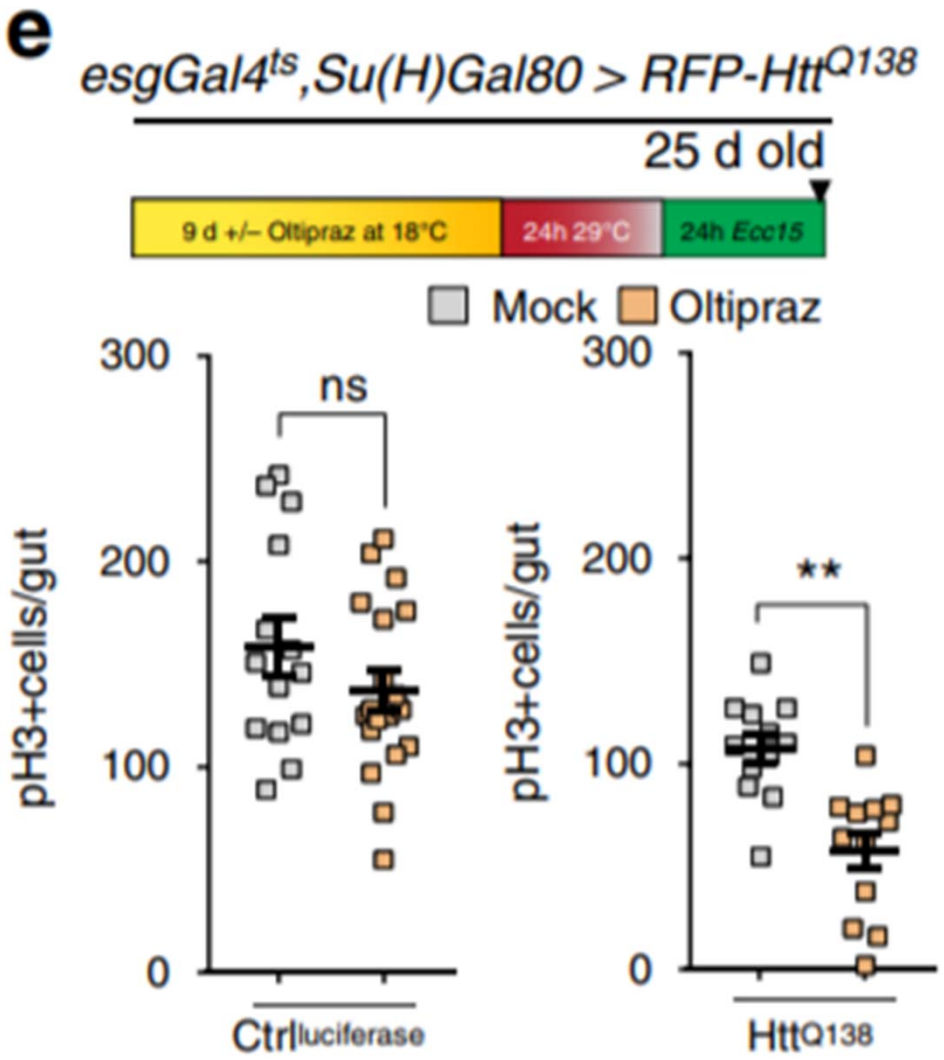
Discussion

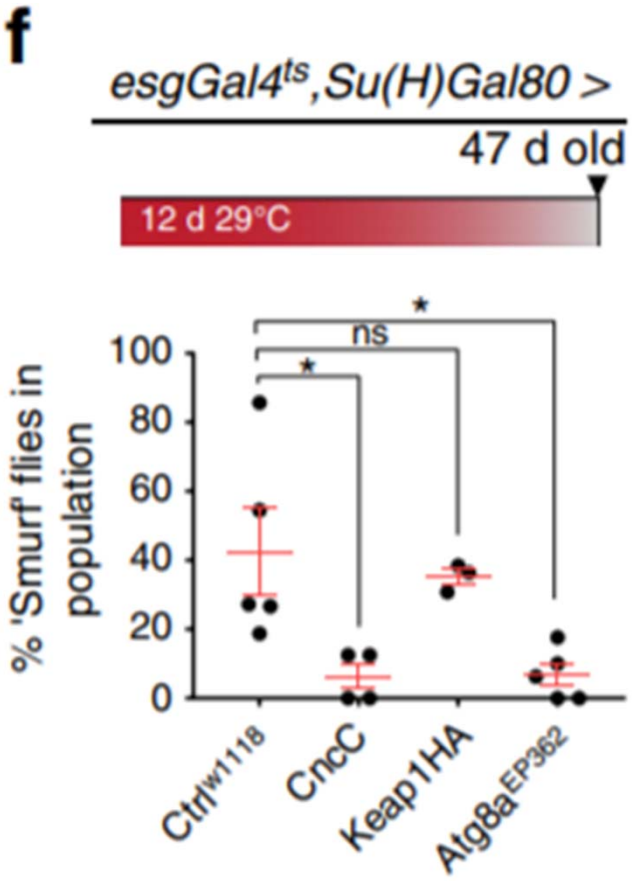
- Coordianted control of aggregate-clearance and cellcycle-progression are essential for longevity-tissue-homeostasis
- Decrease of ability to clear damaged protein aggregates in ISCs in old flies
- Important role of Nrf2/CncC in proteostatic Checkpoint (impact on proliferative activity, self-renewal and differentiaton of trachel-base-cells had already been shown)
- Unique CncC or Nrf2-mediated Inhibition of cell proliferation in ISCs
- Activtation of Cncc after zytosolic proteostatic stress is distant: unfolded protein stress in ER -> CncC is specifically inactivated by ROS/JNK signaling pathway
- Coordination of cell-cycle-arrest and aggregate-clearance by simultaneous induction of dacapo and other genes encoding for proteases and proteolysis

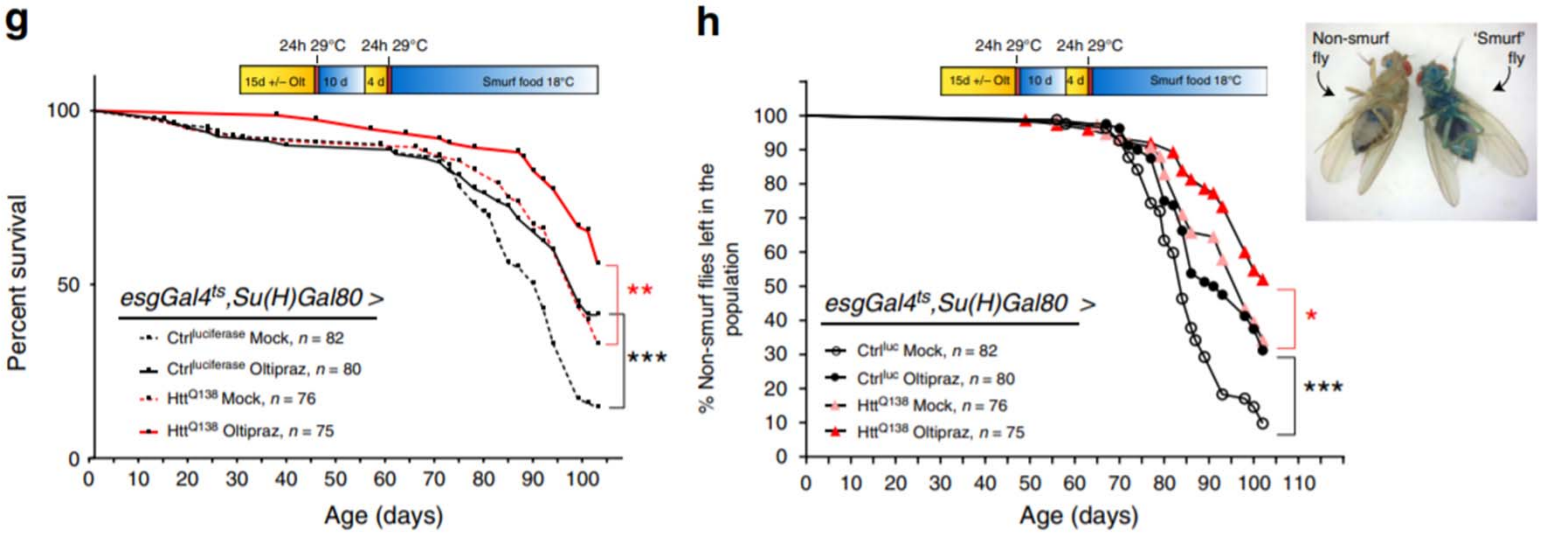
Discussion

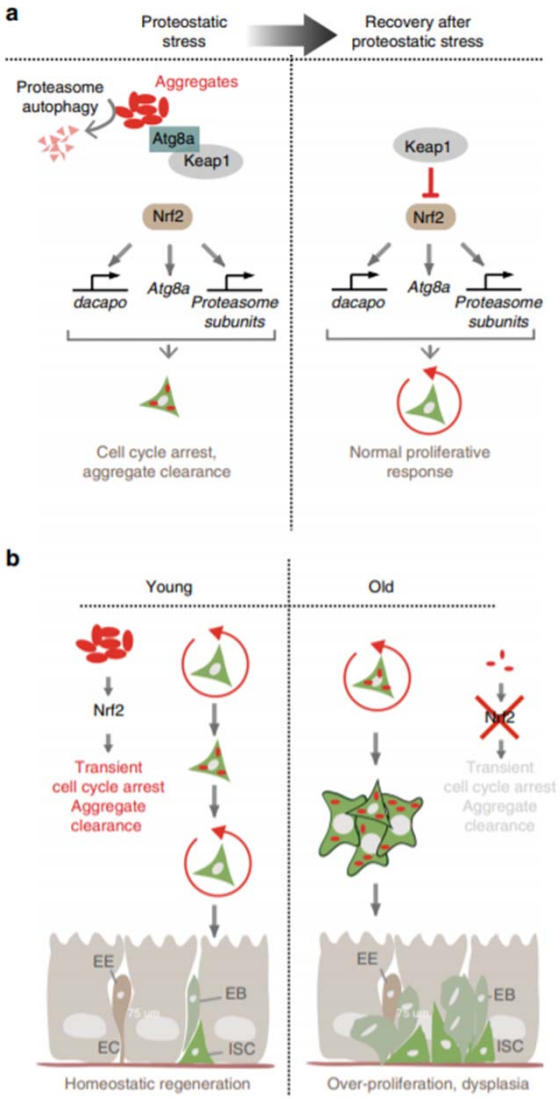
- Dap deficient ISCs clones exhibit significantly higher aggregates than wildtype ISC clones -> tightly coordinated by dacapo for effective ISC proteasis
- Atg8a induction leading to dual purpose: sustaining activation of proteostatic checkpoint and elevated autophagic flux
- Flies with otipraz treated flies have better barrier function and extand life-span


d
esgGal4ts,Su(H)Gal80 > RFP-HttQ138
30 d old
24hr 29°C
2 wks chase 18°C +/– Oltipraz
Mock
RFP
DNA
20 μM
RFP
Oltipraz
aggregates / area
80
60
40
20
0
*
Mock
Oltipraz


e
esgGal4ts,Su(H)Gal80 > RFP-HttQ138
25 d old
9 d +/– Oltipraz at 18°C
24h 29°C
24h Ecc15
Mock
Oltipraz
pH3+cells/gut
300
200
100
0
ns
**
Ctrlluciferase
HttQ138


f
esgGal4ts,Su(H)Gal80 >
47 d old
12 d 29°C
% 'Smurf' flies in population
100
80
60
40
20
0
ns
*
*
Ctrlw1118
CncC
Keap1HA
Atg8aEP362


g
24h 29°C
24h 29°C
15d +/– Olt
10 d
4 d
Smurf food 18°C
Percent survival
esgGal4ts,Su(H)Gal80 >
Ctrlluciferase Mock, n = 82
Ctrlluciferase Oltipraz, n = 80
HttQ138 Mock, n = 76
HttQ138 Oltipraz, n = 75
**
***
Age (days)
h
% Non-smurf flies left in the population
Non-smurf fly
'Smurf' fly
*
***


a
Proteostatic stress
Recovery after proteostatic stress
Proteasome autophagy
Aggregates
Atg8a
Keap1
Nrf2
dacapo
Atg8a
Proteasome subunits
Cell cycle arrest, aggregate clearance
Keap1
Nrf2
dacapo
Atg8a
Proteasome subunits
Normal proliferative response
b
Young
Old
Nrf2
Transient cell cycle arrest Aggregate clearance
Nrf2
Transient cell cycle arrest Aggregate clearance
EE
EB
EC
ISC
Homeostatic regeneration
EE
EB
ISC
Over-proliferation, dysplasia