Article

Cell Metabolism

Obesity-Induced Cellular Senescence Drives Anxiety and Impairs Neurogenesis

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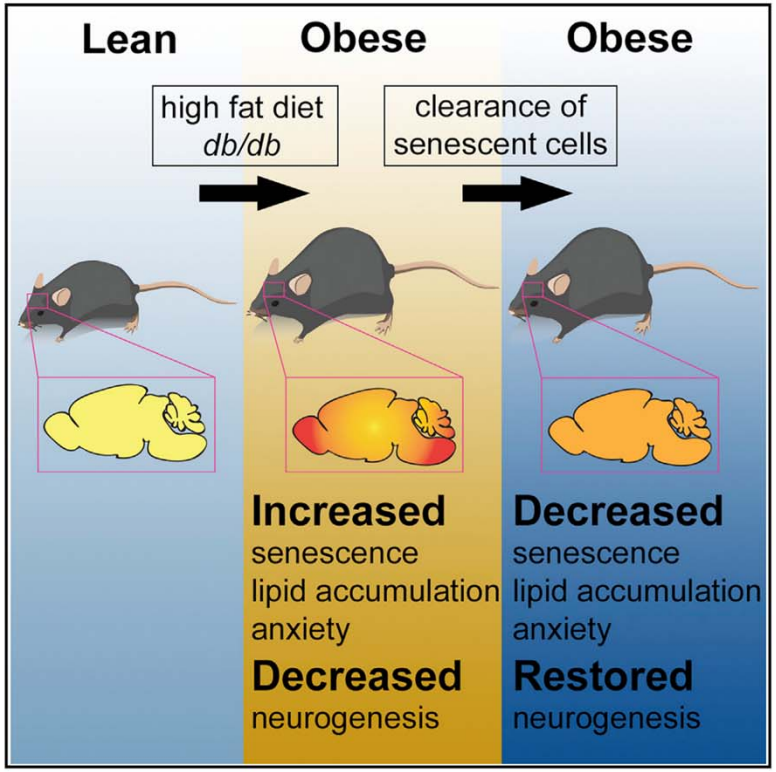


Research aim & interest

- Investigate the correlation between obesity, senescence, and anxiety-like behavior

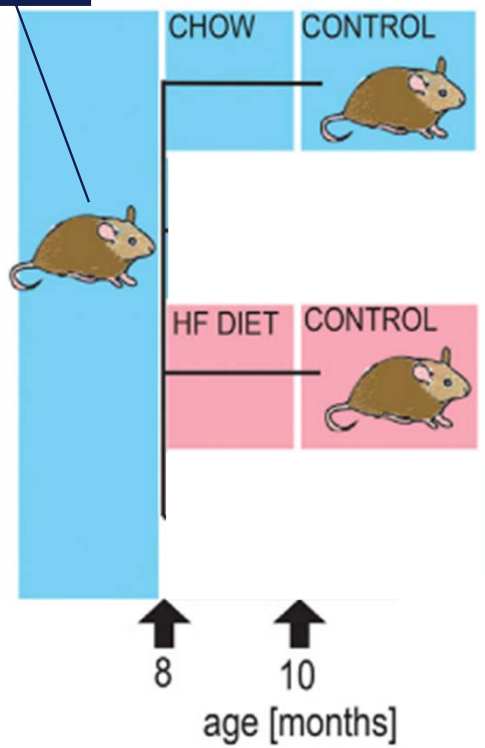
Other studies:

- Removal of senescent cells improves phenotypes in mouse models of
  - Parkinson‘s disease *(Chinta et al., 2018)*
  - Tau-dependent neurodegenerative dieases *(Musi et al. 2018; Bussian et al., 2018)*

Obese mice show increased anxiety-like behavior not related to body mass

Methods

C57Bl/6

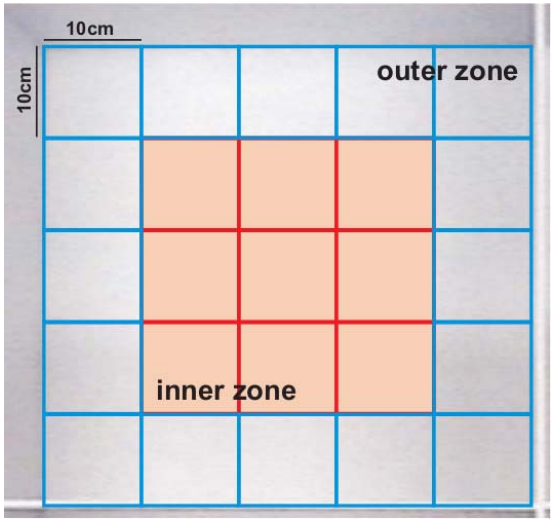


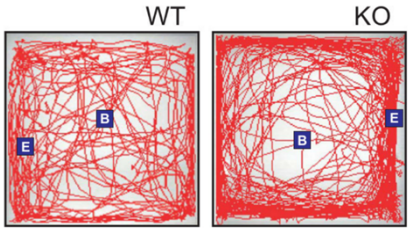
To measure anxiety-like behavior

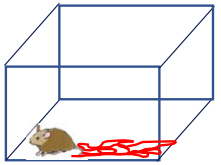
Open-field test (OF)

- Evaluates the tendendency to remain close to walls & avoid open spaces (central zone)

→ Increased anxiety = decreased time spent in central zone

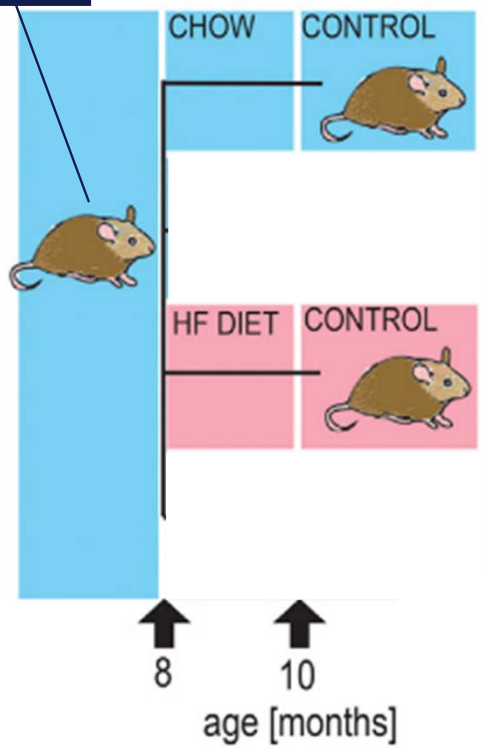






Methods

C57Bl/6

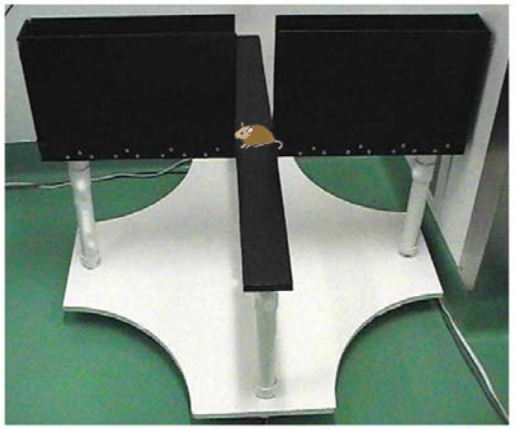


To measure anxiety-like behavior

Elevated plus maze (EPM)

- Based on animals‘ natural fear of heights & open spaces

→ Increased anxiety = decrease in # of head-pokes & entries into the open arms



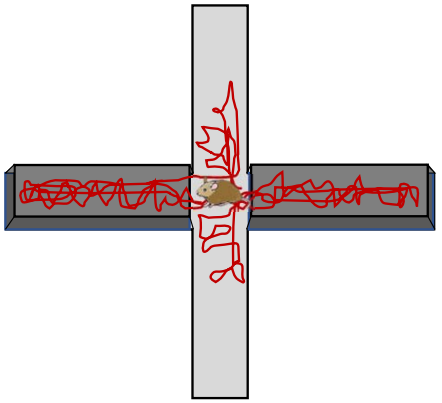
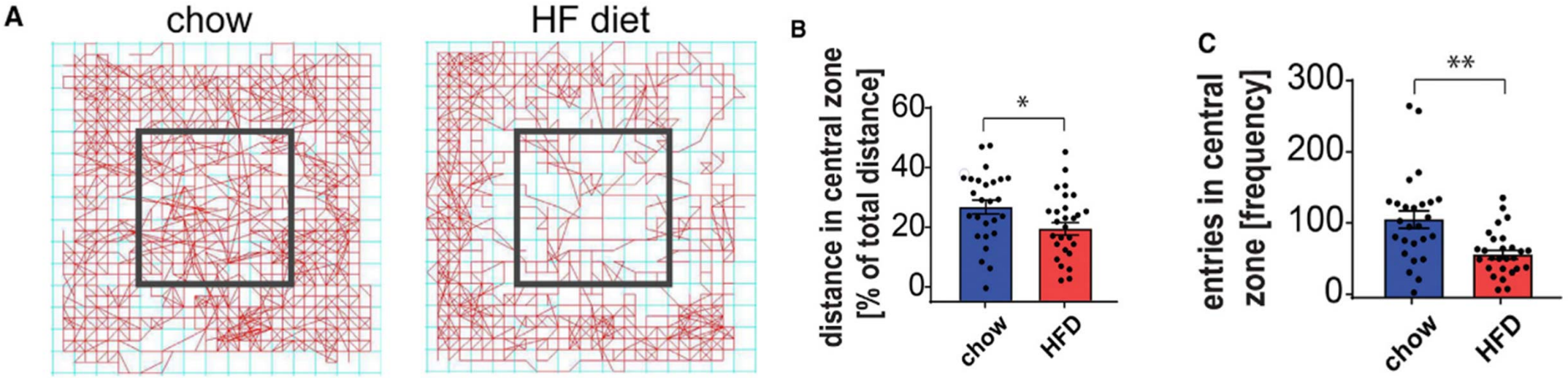


Fig. 1 Obese mice exhibit anxiety-like behavior



HF diet fed mice

- Less inclined to explore the central area
- Total distance covered significantly decreased in HFD animals

! Anxiety measurements were analyzed as a function of the total distance traveled during experimental testing !

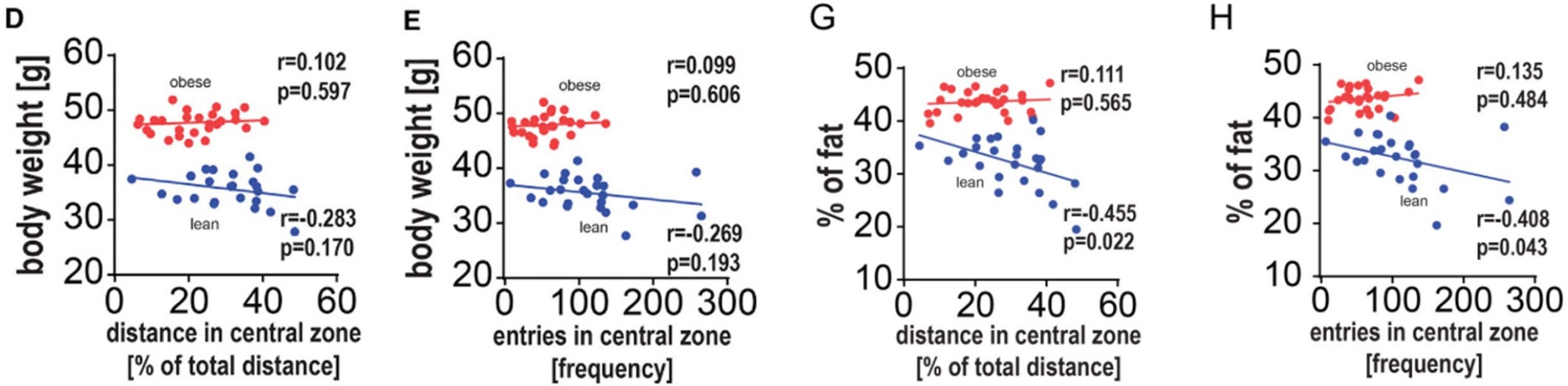
Fig. 1 Obese mice exhibit anxiety-like behavior – not directly associated to increased body mass

Previously shown:

- Obesity impacts activity & exploratory behavior → thereby contributing to anxiety-like behavior
  (Friend et al. 2017; Guillemot-Legris and Muccioli, 2017)

Investigation of body weight & body composition in the context of anxiety-like behavior

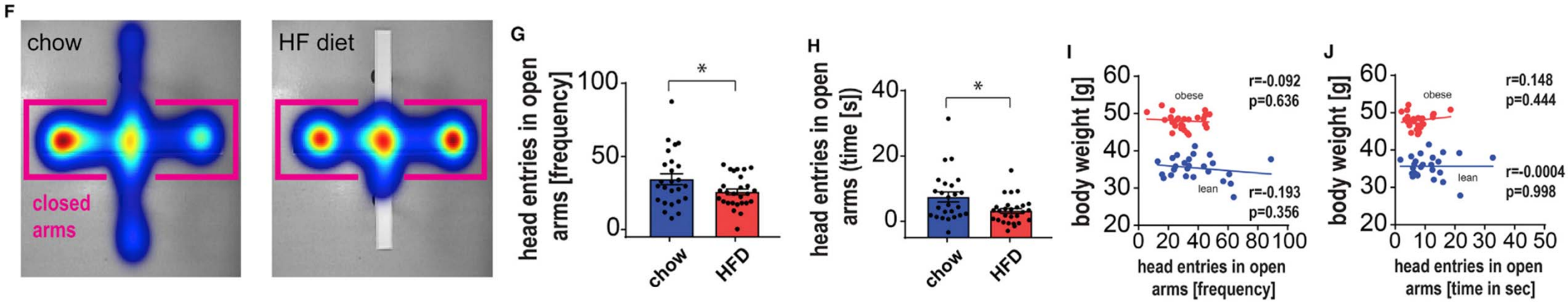
Fig. 1 Obese mice exhibit anxiety-like behavior – not directly associated to increased body mass

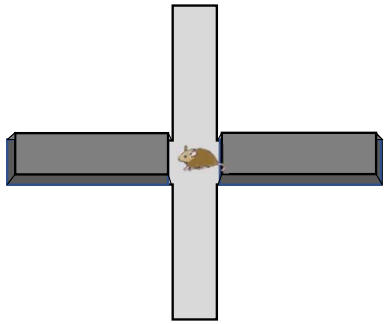


HF diet fed mice

- No significant correlation between body weight or percentage of fat mass with anxiety-like behavior

Fig. 1 Obese mice exhibit anxiety-like behavior – not directly associated to increased body mass





HF diet fed mice

- Decreased entries into open arms of EPM (frequency & time)
- No significant correlations between body mass & fat mass and anxiety parameters

Fig. 1 Obese mice exhibit anxiety-like behavior – not directly associated to increased body mass

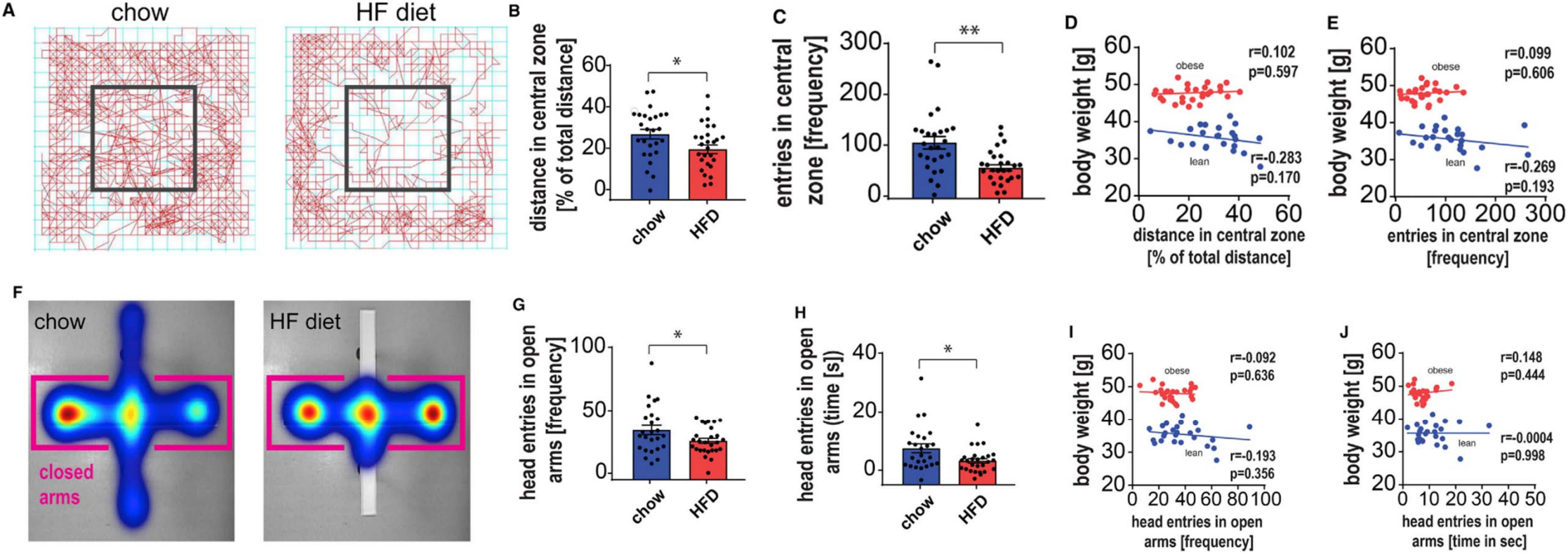
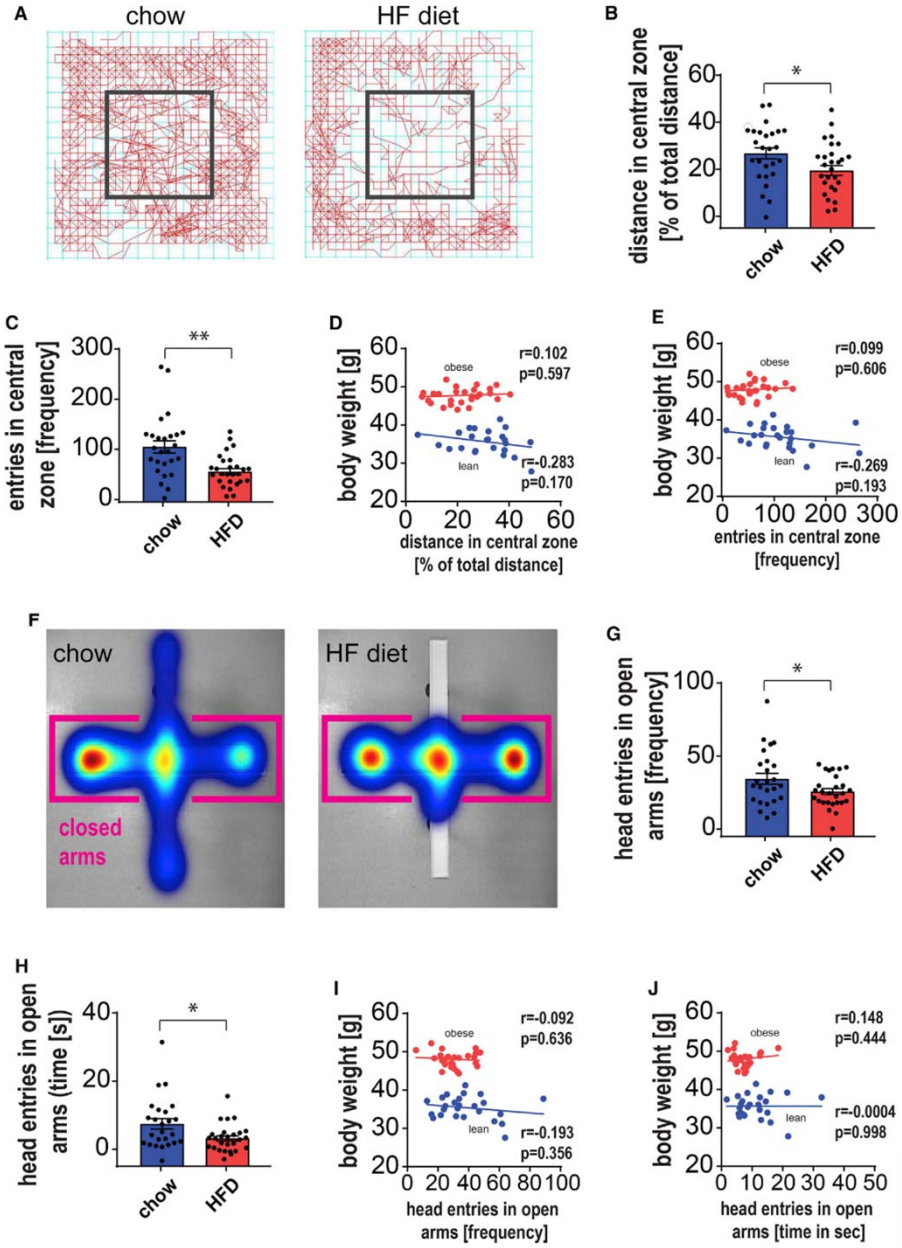
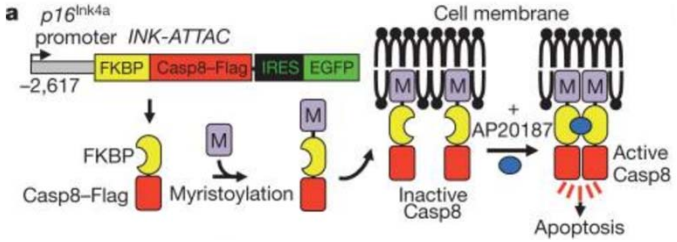


Fig. 1 Obese r ke behavior – not directly d body mass



Pharmacogenetic and Pharmacologic clearance of senescent cells alleviates obesity-related behavioral changes

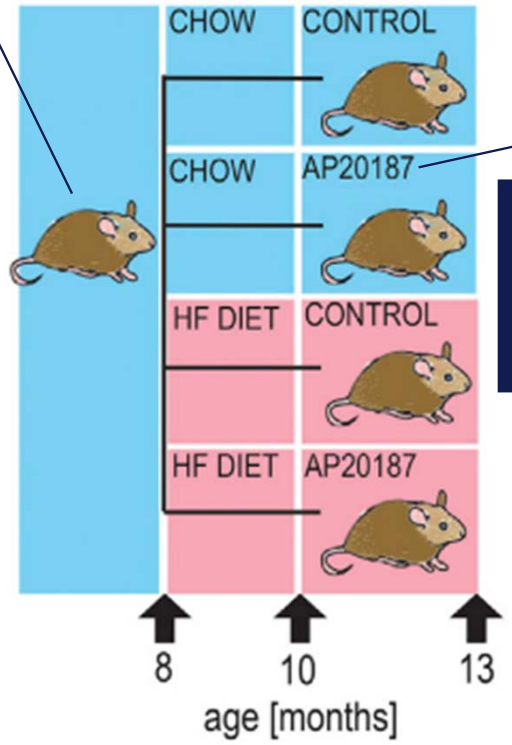
Methods



Adapted from Baker et al. 2011, Nature

- $p16^{Ink4a}$ = marker for senescent cells
- promoter of $p16^{Ink4a}$ was replaced with one that is transcriptionally active exclusively in senescent cells

C57Bl/$6^{INK\text{-}ATTAC}$



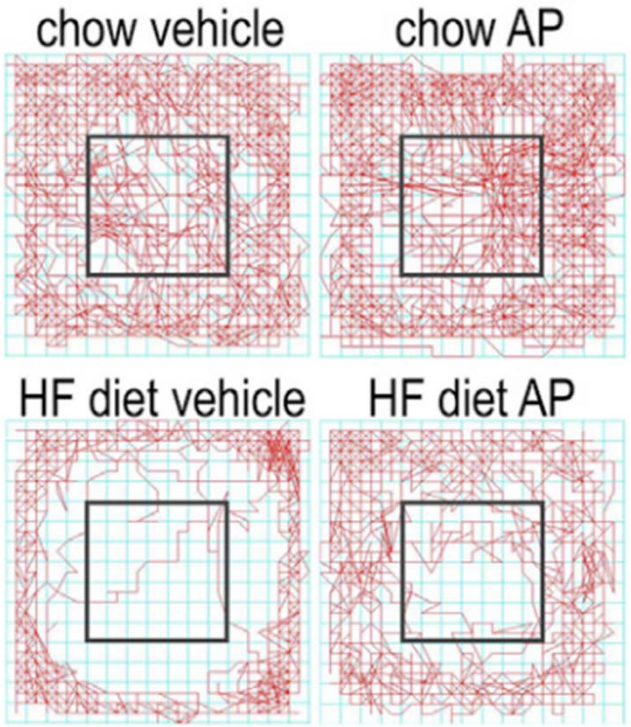
AP020187 = synthetic drug

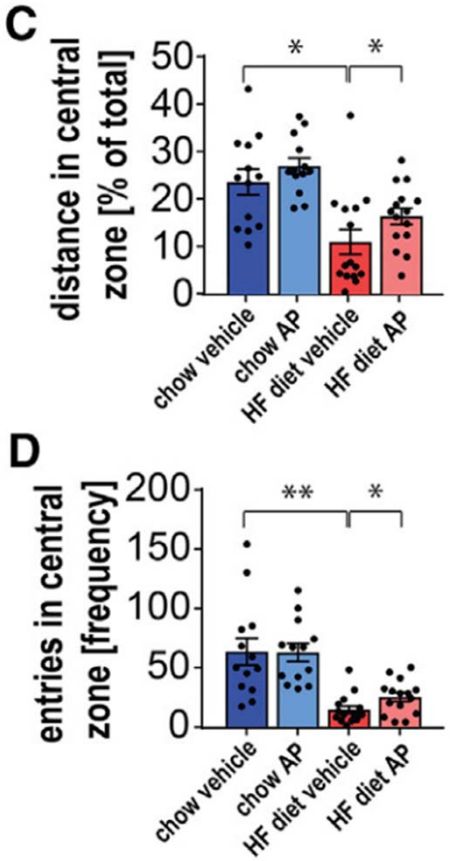
- Cross-links the ATTAC fusion protein (FKBP-Casp8)
  → caspase 8 activation → apoptosis

To measure anxiety-like behavior

- Open-field test (OF)
- Elevated plus maze (EPM)

Fig. 2 Clearance of senescent cells from obese mice alleviates obesity-related behavioral changes





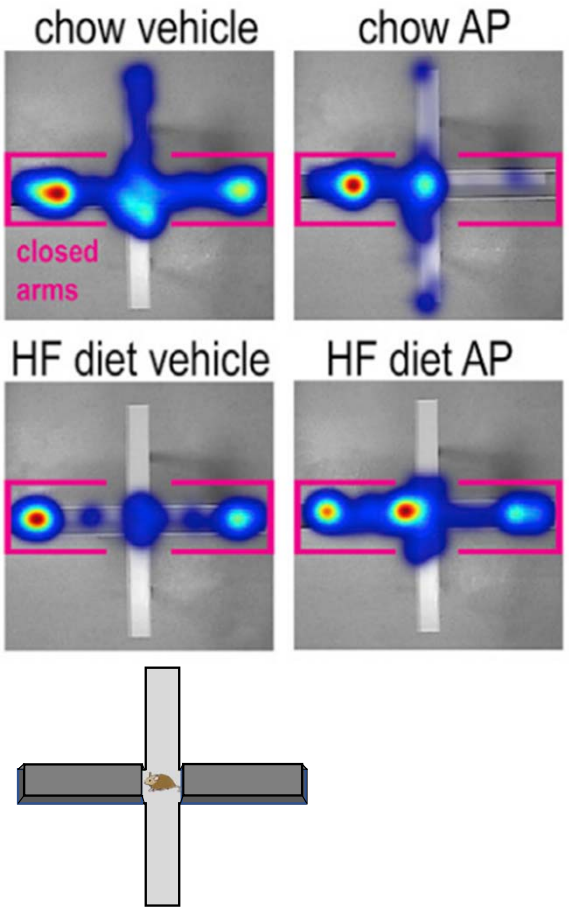
HF diet fed mice

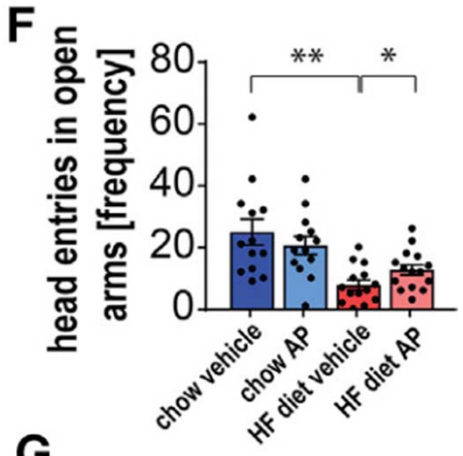
- Less inclined to explore the central zone

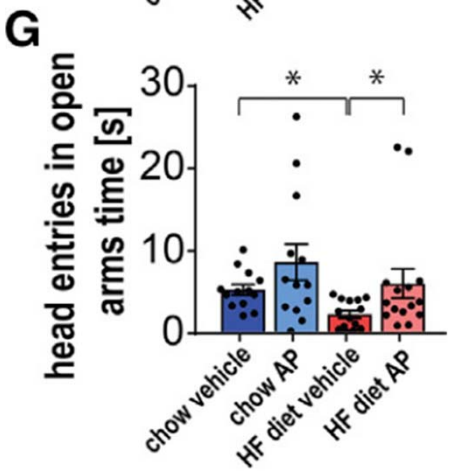
AP treatment in HF diet fed mice

- reduced HFD-induced anxiety-like behavior
- Increased distance covered in the central zone
- Increased entries into the central zone

Fig. 2 Clearance of senescent cells from obese mice alleviates obesity-related behavioral changes







HF diet fed mice

- Avoid entries into the open arms

AP treatment in HF diet fed mice

- Increased frequency of head entries into open arms

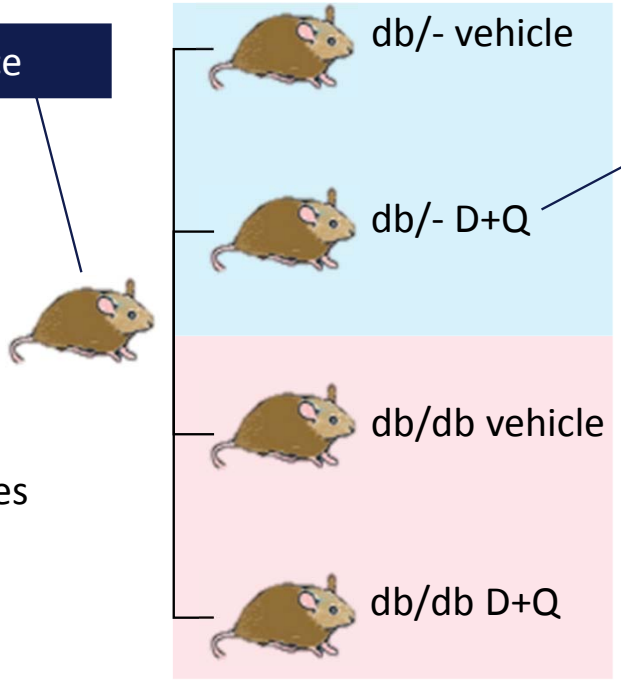
First conclusions

Specific elimination of p16^{Ink4a+}-senescent cells from obese INK-ATTAC mice reduces anxiety-like behavior
(but has no effect on memory performance)

Methods

db/db mice

- Obesity caused by point mutation in leptin receptor gene *lepr*
  → leading to spontaneous Diabetes Mellitus Type II



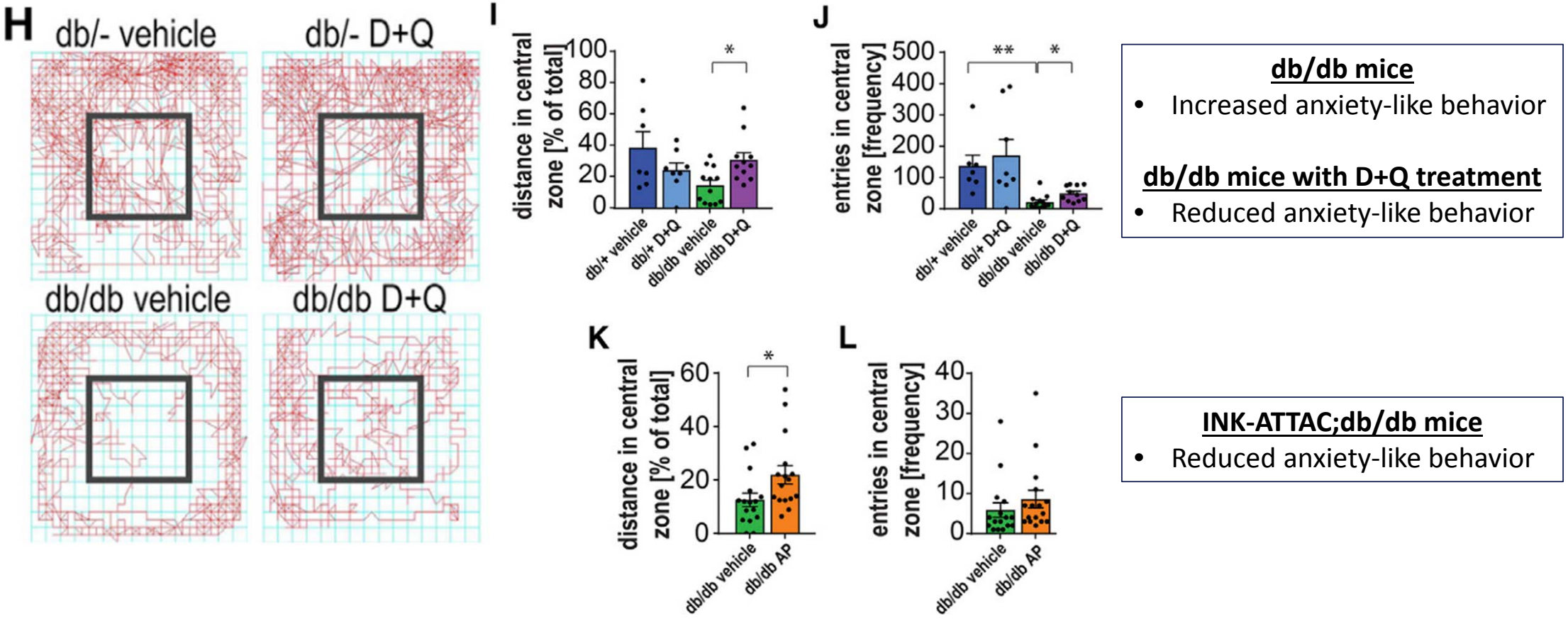
D + Q
= senolytic cocktail of Dasatinib + Quercetin

- D + Q treatment: 5 days every 2 weeks for 8 weeks

To measure anxiety-like behavior

- Open-field test (OF)
- Elevated plus maze (EPM)

Fig. 2 Clearance of senescent cells from obese mice alleviates obesity-related behavioral changes



db/db mice

- Increased anxiety-like behavior

db/db mice with D+Q treatment

- Reduced anxiety-like behavior

INK-ATTAC;db/db mice

- Reduced anxiety-like behavior

Conclusive remarks

Pharmacological or pharmacogenetic clearance of senescent cells in two different mouse models of obesity significantly alleviates anxiety-like bheavior

Pharmacological and pharamacogenetic senolytic approaches reduce senescent cell burden and alleviate systemic inflammation

Methods

SA-β-Gal

- Senescence-associated beta-galactosidase activity
- Most widely used biomarker for senescent cells

p16

- Cyclin dependent kinase inhibitor
- Invovled in cell cycle arrest
- p16+ cells accumulate in age-dependent manner in multiple tissues

TAF

- Telomere-associated DNA damage foci
- Used to detect senescent cells and quantify tissue aging

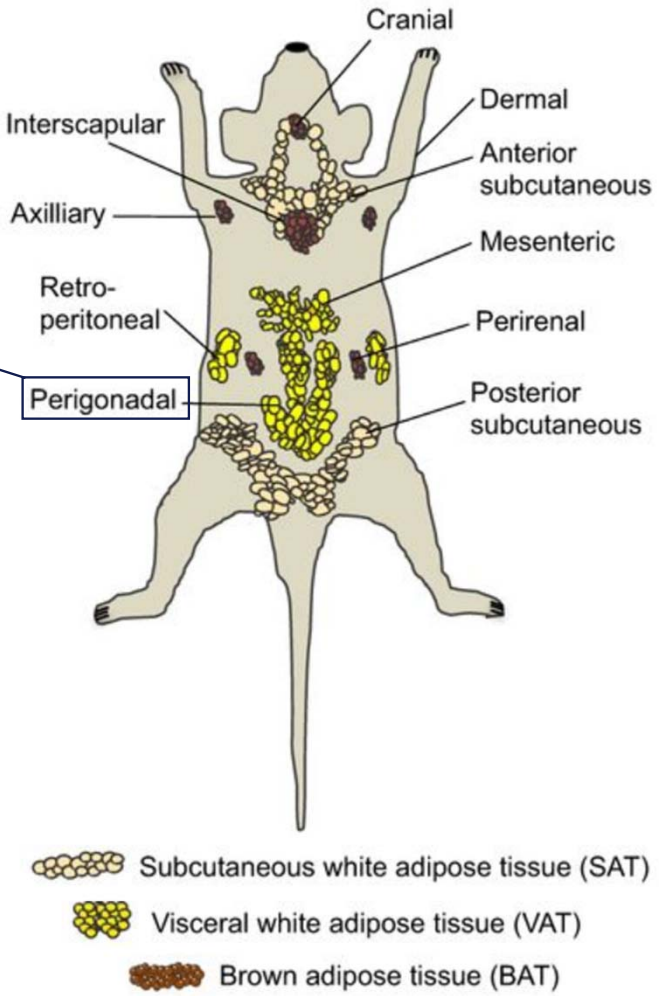
p21

- Cyclin dependent kinase inhibitor
- Involved in cell cylce arrest
- Maintains viability of DANN damage-induced senescent cells

γ-H2A.X

- Marker for DNA damage

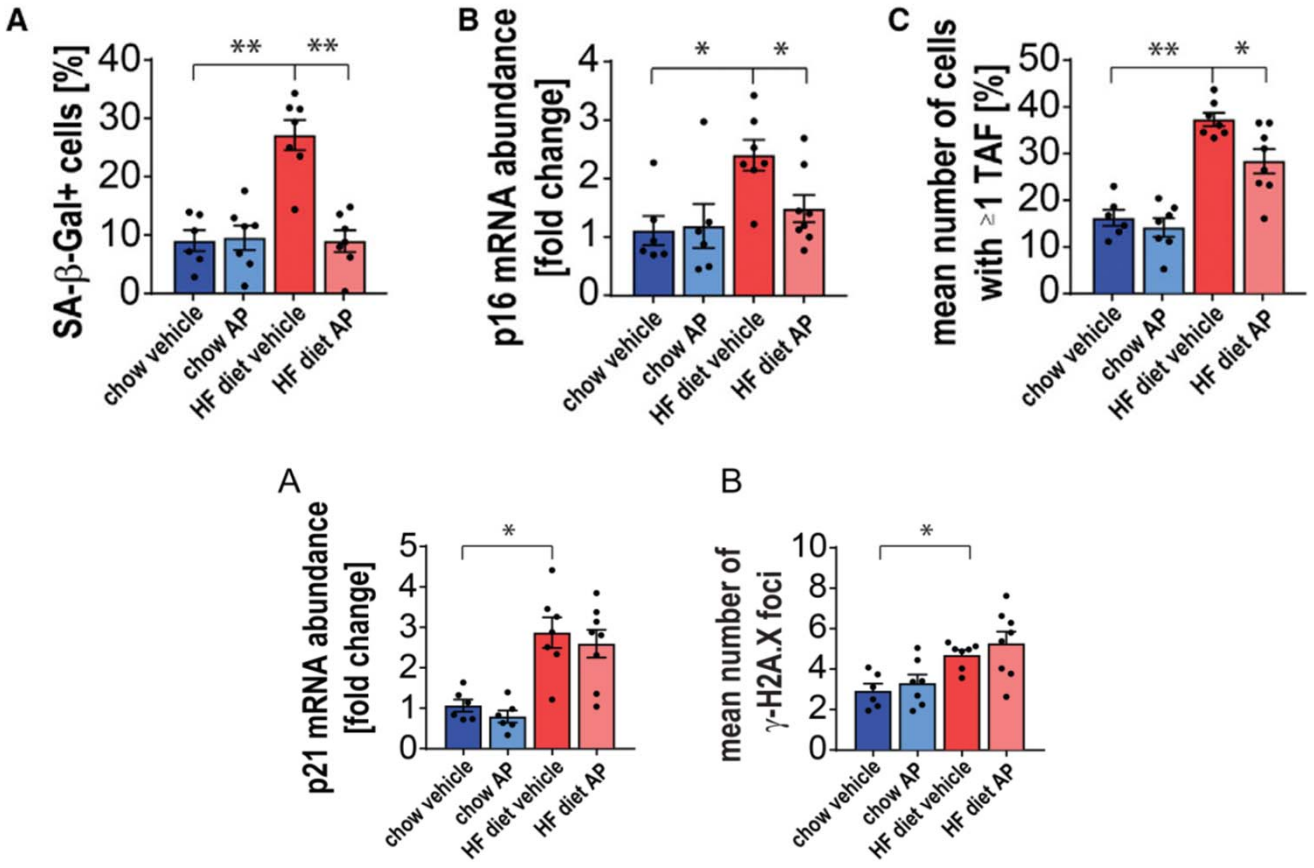
Measurement of senescence markers



Adapted from Schoettl et al. 2018, Journal of Experimental Biology

Wang et al. 2018, Front Genet.; Liu et al. 2019, PNAS; Yosef et al. 2017, EMBO J.

Fig. 3 clearance of senescent cells from obese animals reduces circulating cytokine levels



INK-ATTAC mice on HFD

- Increased senescence markers SA-β-Gal, p16, TAF

INK-ATTAC mice on HFD + AP treatment

- Significant reduction of senescence markers

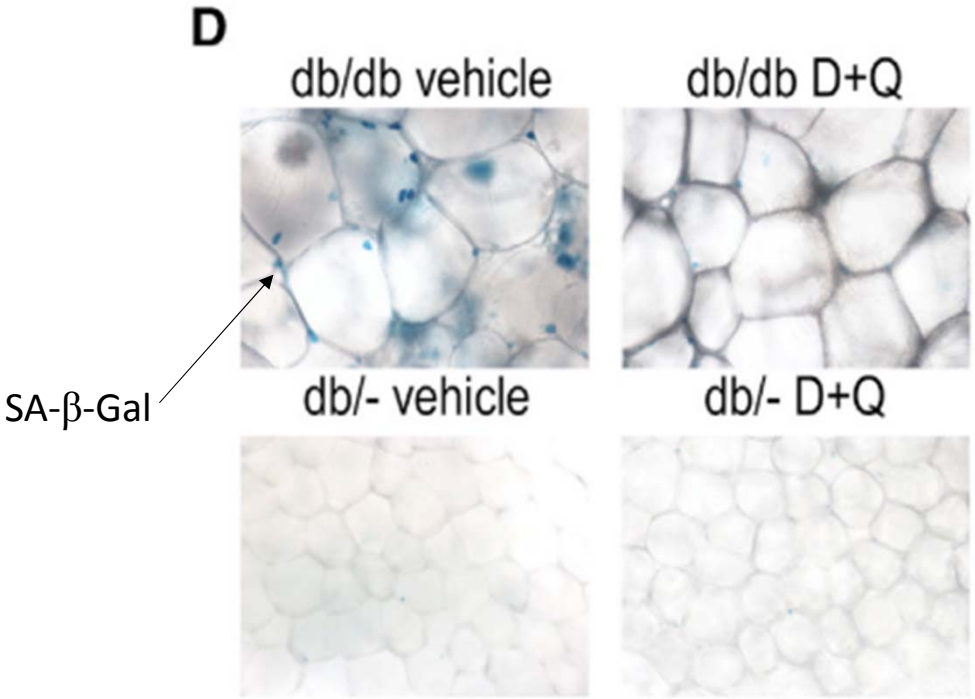
INK-ATTAC mice on HFD

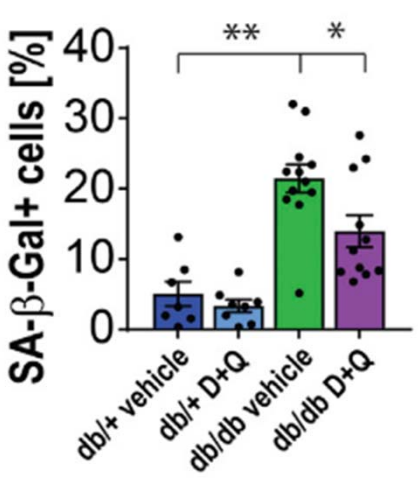
- Increased senescence markers p21 mRNA, γ-H2A.X

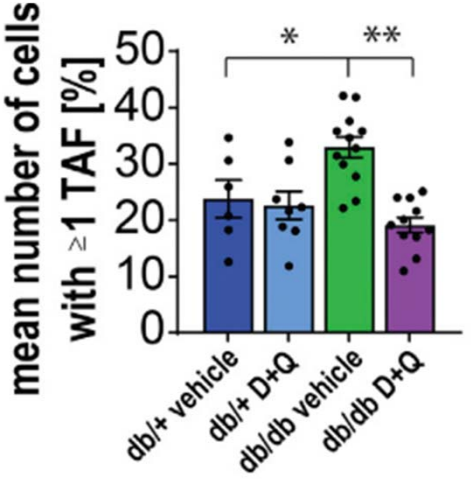
INK-ATTAC mice on HFD + AP treatment

- No significant reduction of senescence markers

Fig. 3 clearance of senescent cells from obese animals reduces circulating cytokine levels







db/db mice

- Increased senescence markers SA-β-Gal, TAF

db/db mice + D+Q treatment

- Significant reduction of senescence markers

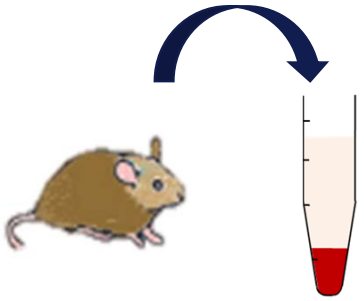
Methods

Hypothesis

- Systemic effect of applied senolytics reduce pro-inflammatory SASP factors capable of penetrating the blood-brain barrier
  → impact on the brain

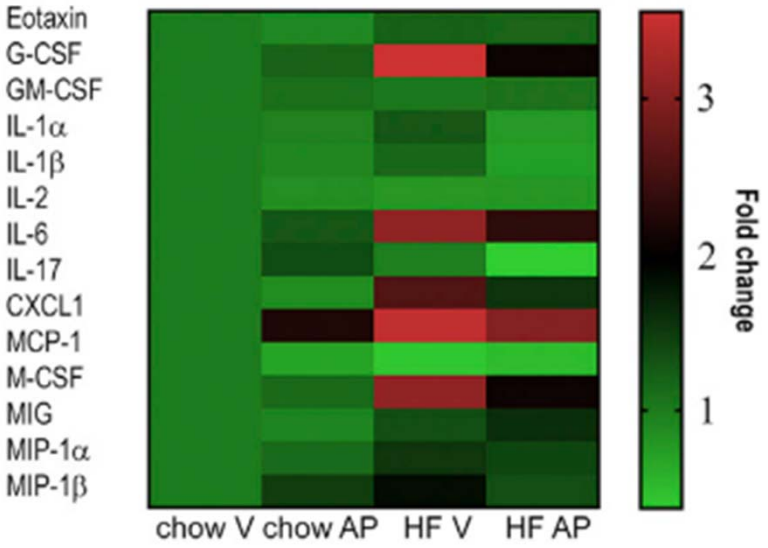
SASP

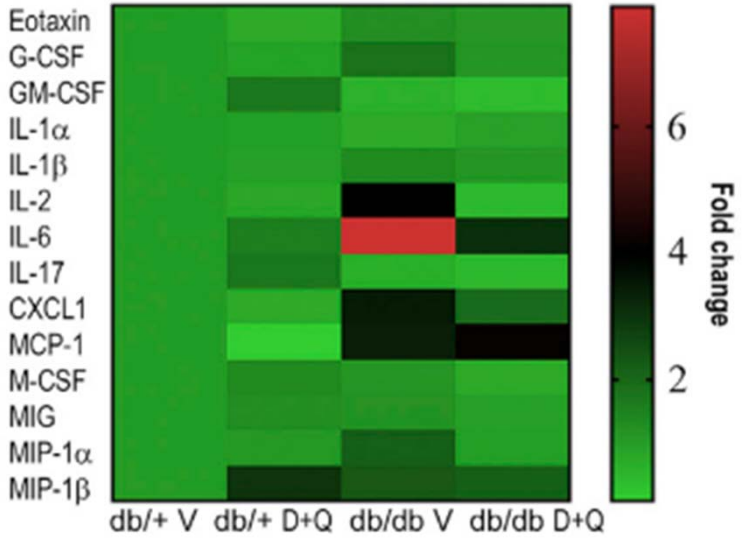
- senescence associated secretory phenotype
- „communication tool“ with immune system
  → orchestrate senescent cell clearance
  → stimulation of progenitor cells to repair tissue
- Chronic exposure to SASP → tissue damage contributing to tissue dysfunction during aging and age-related diseases



Analysis of blood plasma for SASP factors

Fig. 3 clearance of senescent cells from obese animals reduces circulating cytokine levels





HFD INK-ATTAC mice

- Up-regulation of SASP factors
  G-Csf, IL-1α, IL-1β, Kc/Cycl1, Mcp-1, Mig, Mip, TNF-α

HFD INK-ATTAC mice + AP treatment

- Reduced up-regulation of SASP factors upon AP treatment

db/db mice

- Up-regulation of SASP factors
  G-Csf, IL-2, IL-6, CXCL1, Mcp-1, Mip

db/db mice + D+Q treatment

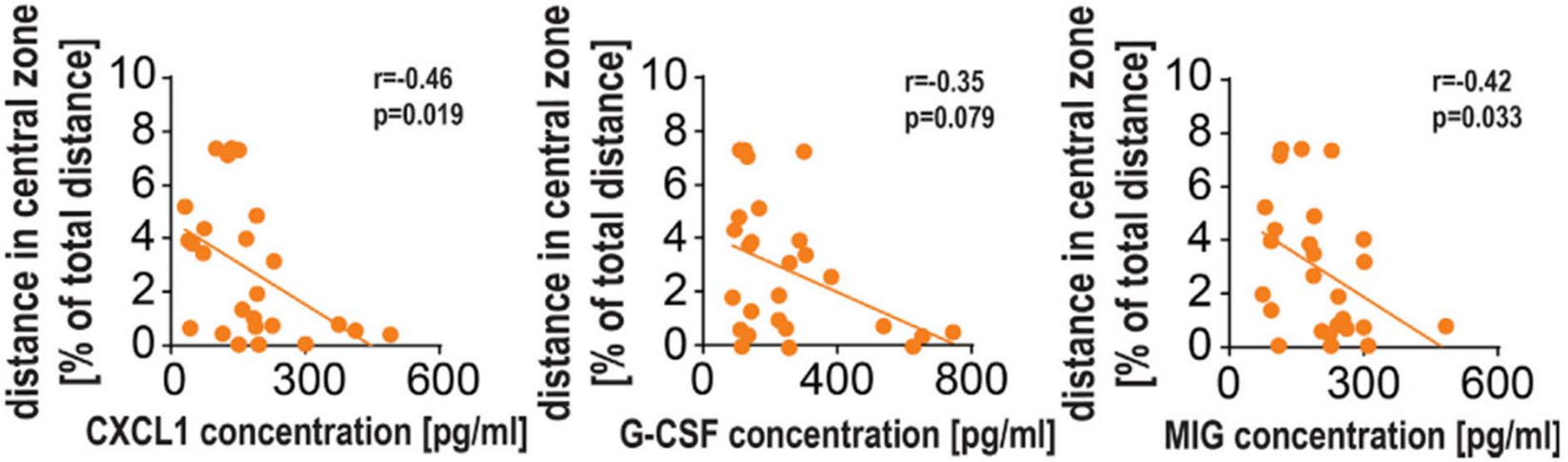
- reduced up-regulation of SASP factors upon D+Q treatment

Fig. 3 clearance of senescent cells from obese animals reduces circulating cytokine levels

Peripherally derived cytokines

- inhibit neurogenesis
- drive anxiety & depression

→ *Correlation of cytokine expression in bloodstream with parameters of anxiety-like behavior ?*



<u>AP-treated HFD mice & D+Q treated db/db mice</u>

- Significant negative correlation to plasma levels of Cxcl1, G-Csf, Mig
- No correlation to TNF-α, IL-6 and Mcp-1

Fig. S3 senescent cells reduce physical function

Transplanted senescent cells result in

- Physical dysfunction
  - *Rotarod performance*
  - *Grip strength*
  - *Endurance*
- long-lasting systemic effects in tissues distantly located to injection site

Xu et al. 2018, Nature Medicine

Transplantation of senescent / non-senescent cells into lean mice

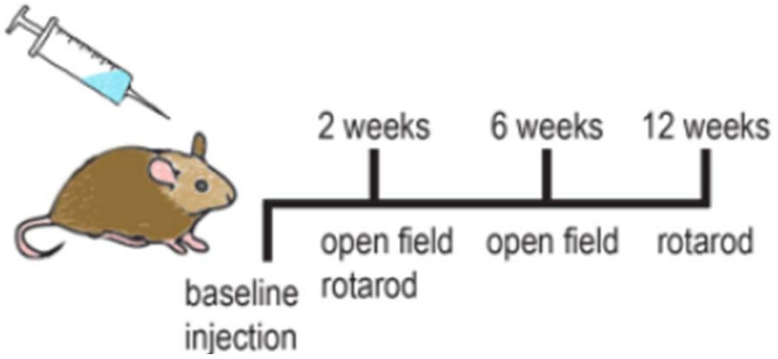
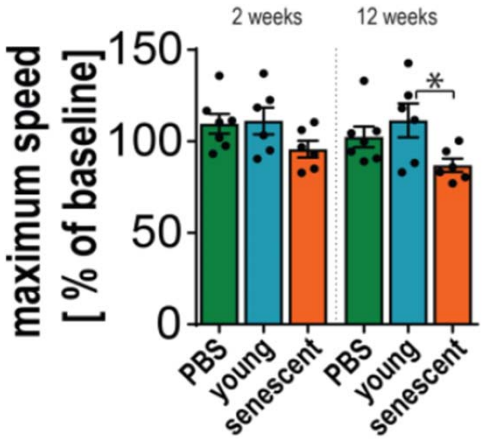
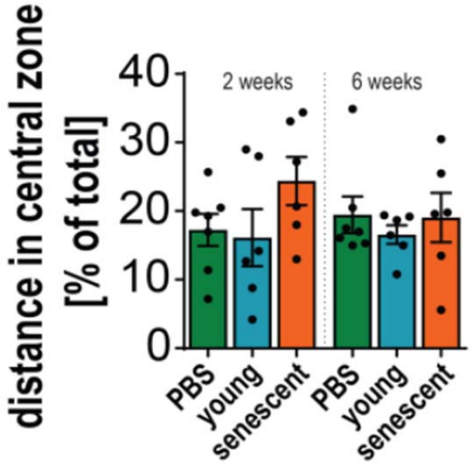
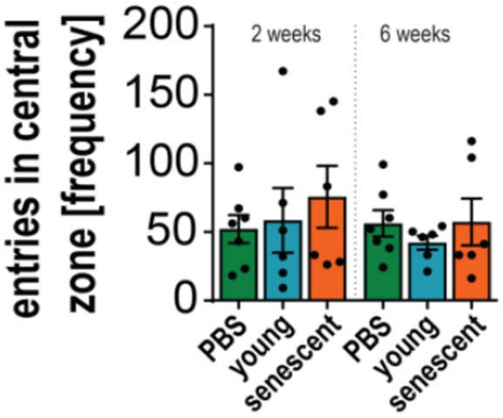


Fig. S3 senescent cells reduce physical function



Rotarod
Reduced physical function





OF test
No effect on anxiety-like behavior

Conclusive remarks

The presence of senescent cells elsewhere in the body is not sufficient to induce an anxiety-like phenotype

Senolytic treatment reduces the frequency of senescent cells in the amygdala and hypothalamus

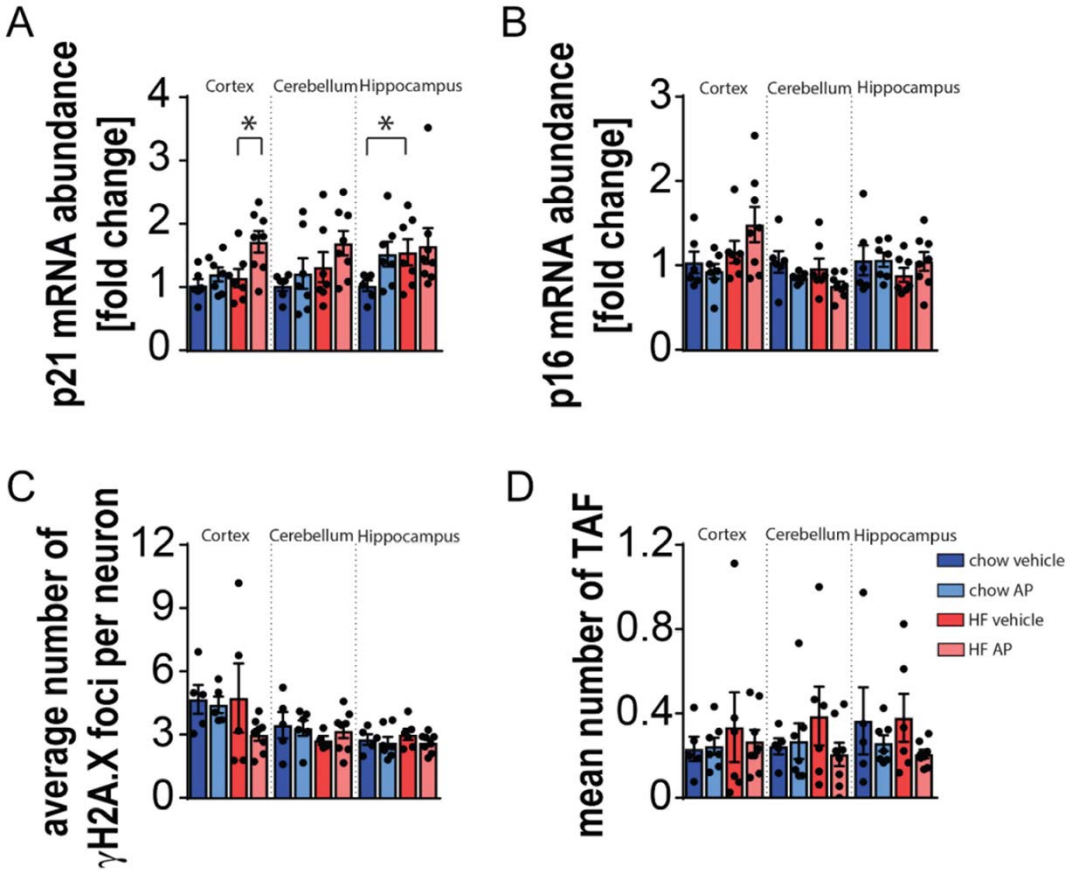
But not in other regions of the brain

Markers of senescence in the brain

Hypothesis

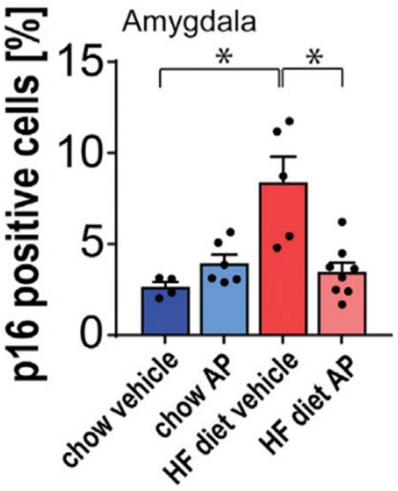
- Obesity could induce senescence specifically in the brain
  → contributing to anxiety-like phenotype

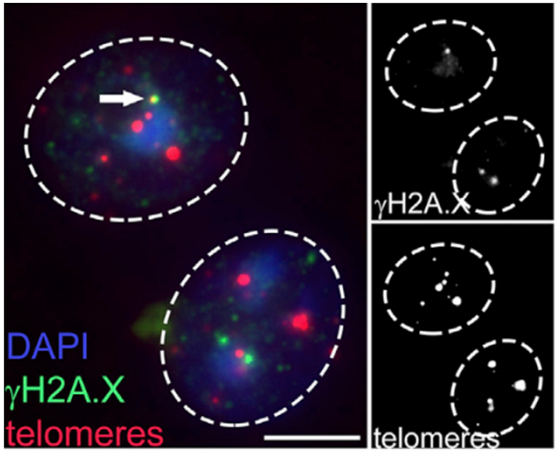
Fig. S4 Markers of senescence in the brain

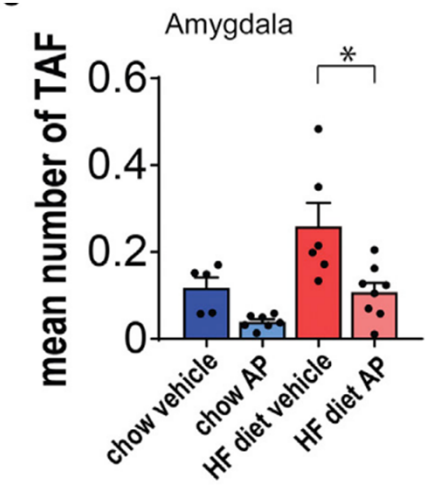


- No difference in senescence markers among all experimental groups *(p21, p16, H2A.X, TAF)*
- Consistent with absence of differences in memory and learning *(assessed by Stone's maze)*

Fig. 4 Markers of senescence in the amygdala are reduced after treatment with AP20187



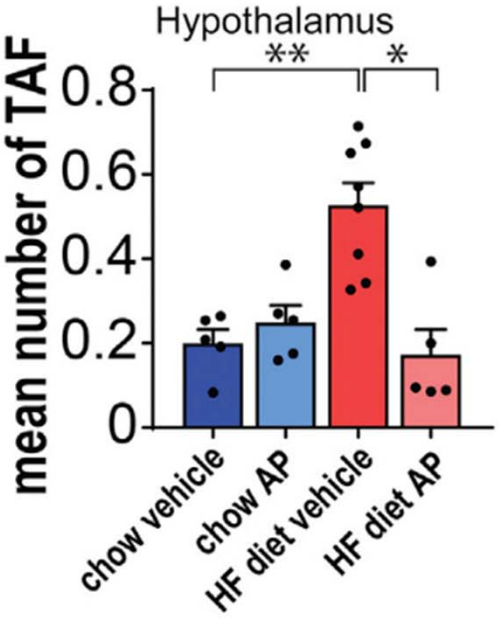


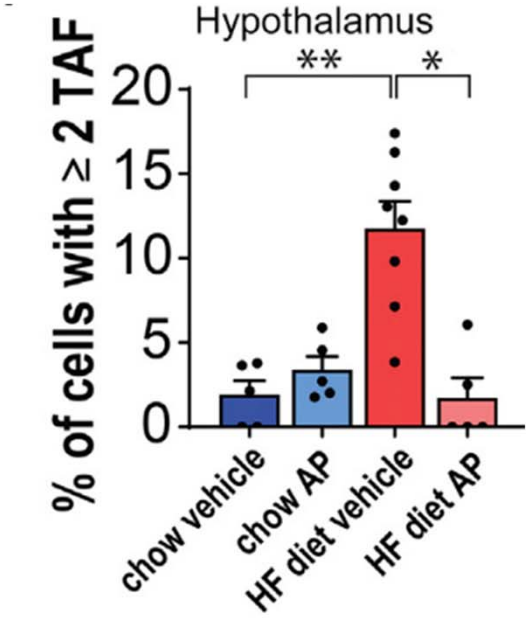


Amygdala

- Significant increase in p16^{+} cells in HFD-mice
- Significant increase in TAF^{+} neurons in HFD mice
- Significant reduction of p16^{+} and TAF^{+} cells upon AP treatment

Fig. 4 Markers of senescence in the amygdala are reduced after treatment with AP20187





Hypothalamus

- Significant increase in TAF$^+$ cells in HFD mice
- Significant reduction of TAF$^+$ cells upon AP treatment

Conclusive remarks

HFD does not induce senescence in regions of the brain implicated in learning, memory or motor neuron control (cortex, cerebellum, hippocampus)

HFD induces senescence in the hypothalamus and amygdala which may contribute to anxiety-like behavior

Treatment with AP reduced senescent cell abundance

Clearance of senescent cells decreases periventricular acc. of lipid-laden glia in obese animals

Senescent cells induce lipid accumulation in the brain

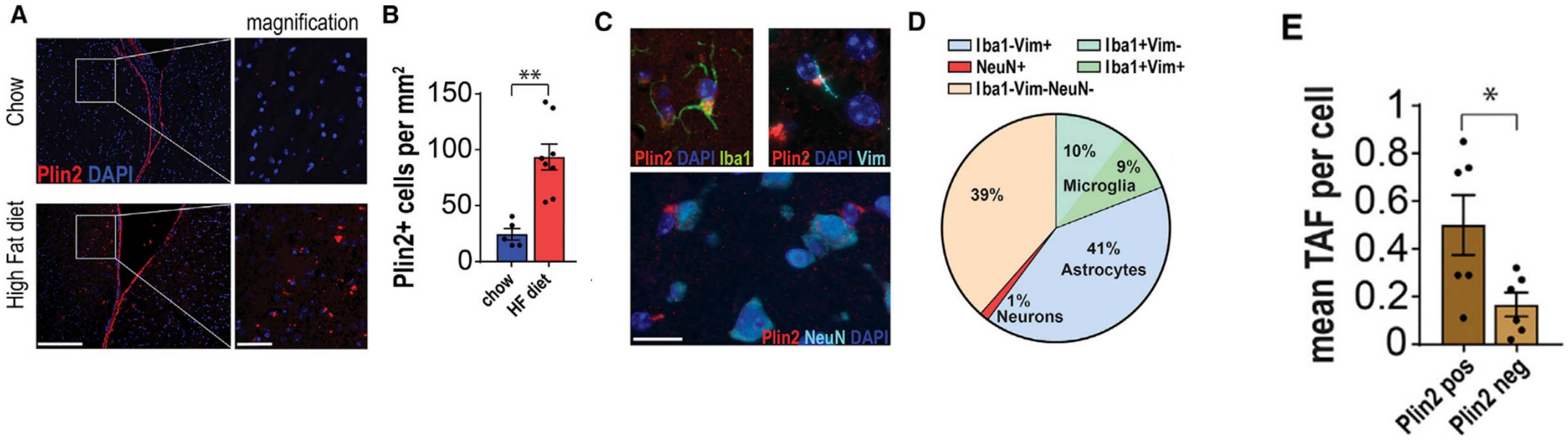
- Senescent cells accumulate in obesity → prevalence related to accumulation of ectopic fat
- Cells accumulating lipid droplets in the brain occur in close proximity to ventricles
  - # of cells increases with age in Alzeheimer‘s disease & respective mouse models

Ogrodnik et al. 2017, Nat. Commun. ; Shimabukuro et al. 2016, Sci. Rep. ; Hamilton et al. 2015, Cell Stem Cell

Analysis of perilipin 2 (Plin2) in the brain

- Plin2 = protein surrounding lipid droplets

Fig. 5 Obesity-related acc. of lipid droplets in senescent periventricular glia is reduced upon senescent cell clearance

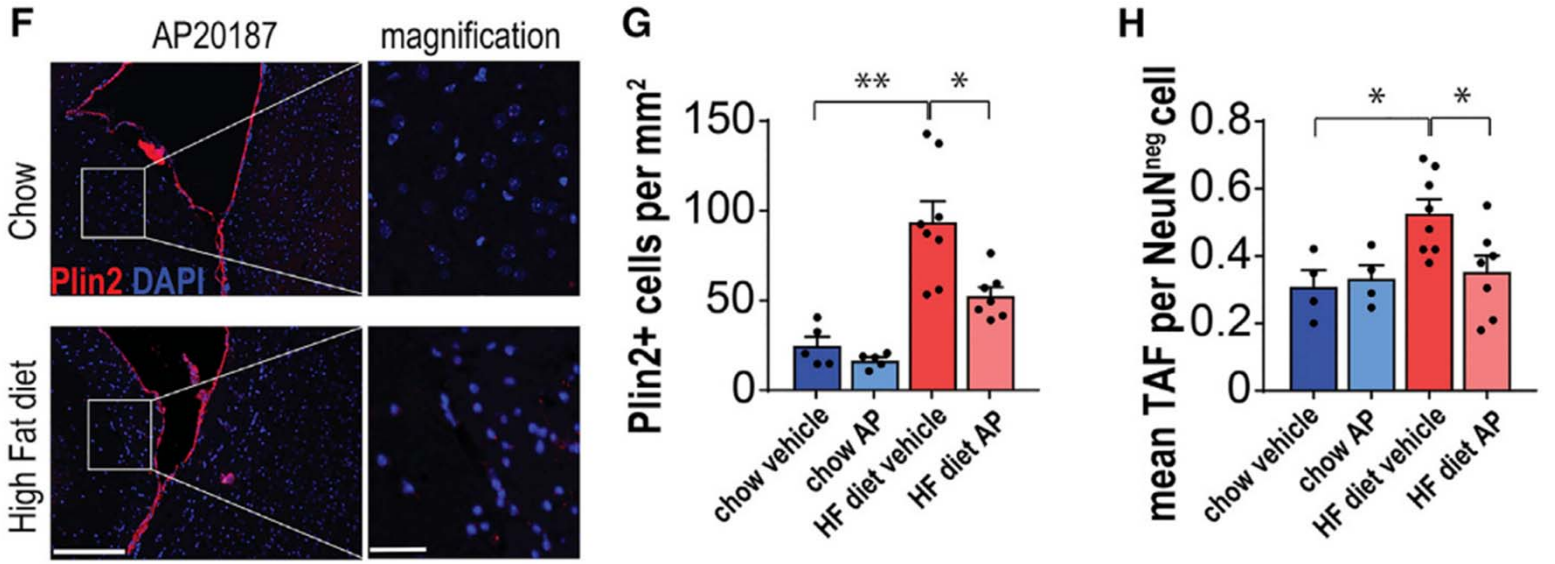


Significant increase in Plin2⁺ cells in HFD mice

Plin2⁺ cells are mostly astrocytes and microglia

Higher frequencies of TAF in Plin2⁺ cells

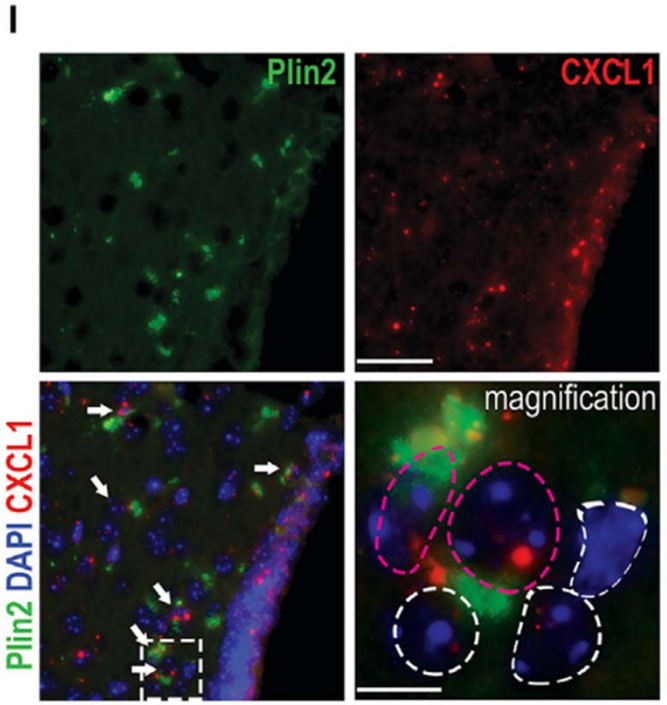
Fig. 5 Obesity-related acc. of lipid droplets in senescent periventricular glia is reduced upon senescent cell clearance

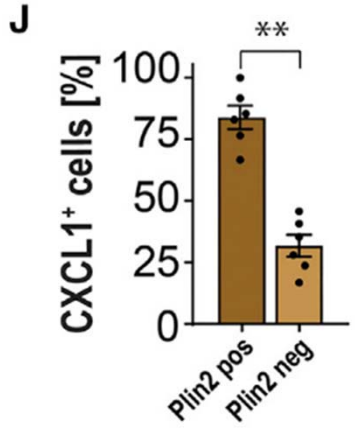


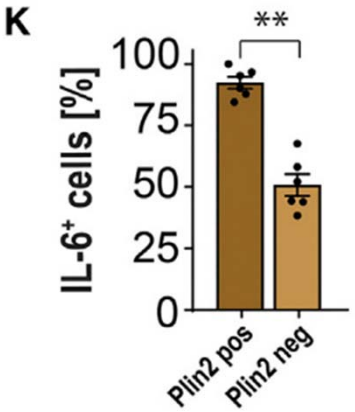
HFD mice + AP treatment

- Significant reduction of Plin2^{+} cells
- Significant reduction in TAF frequencies

Fig. 5 Obesity-related acc. of lipid droplets in senescent periventricular glia is reduced upon senescent cell clearance



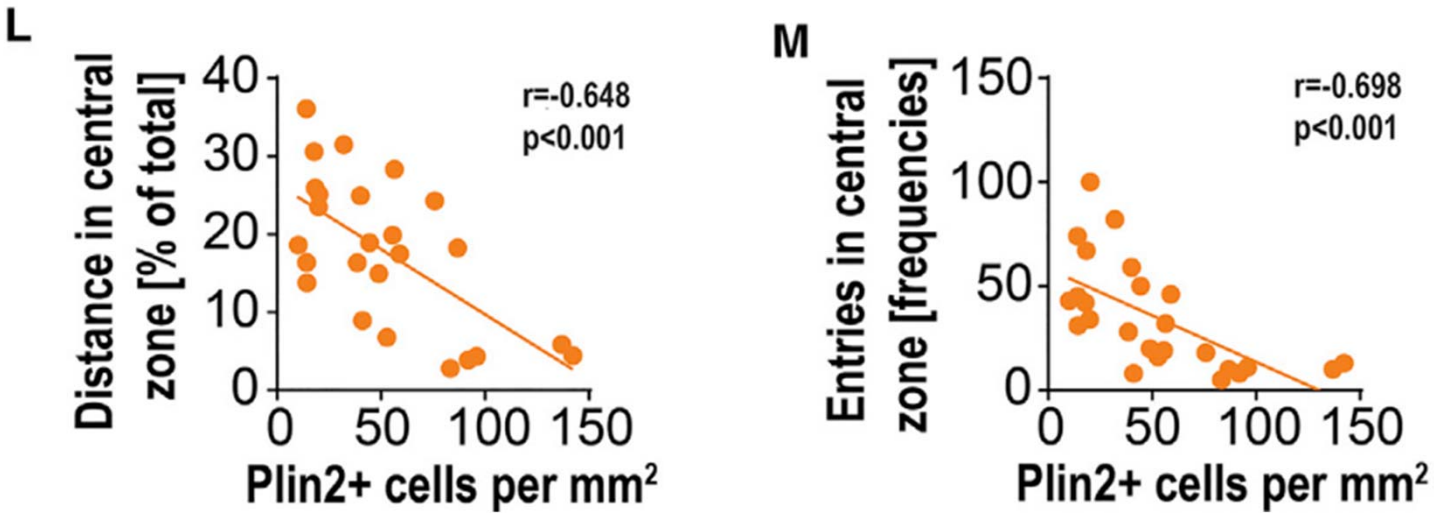




Assessment of neuroinflammation by RNA ISH

- More than 80% of Plin2$^+$ cells are also Cxcl1 and IL-6 positive in lateral ventricle of HFD mice

Fig. 5 Obesity-related acc. of lipid droplets in senescent periventricular glia is reduced upon senescent cell clearance



Anxiety markers in HFD mice

- Strong negative correlation with abundance of Plin2⁺ cells in LV

Conclusive remarks

Data suggest correlation between the accumulation of lipid-laden secescent glial cells in obese animals and anxiety-like behavior

Suppression of the ALISE phenotype reduces acc. of cytosolic chromatin fragments and the SASP

ALISE = accumulation of lipids in senescence

Methods

Induction of senescence by X-ray irradiation

Normal FBS

Lipid-deprived FBS

Mouse adult fibroblast culture (MAF)

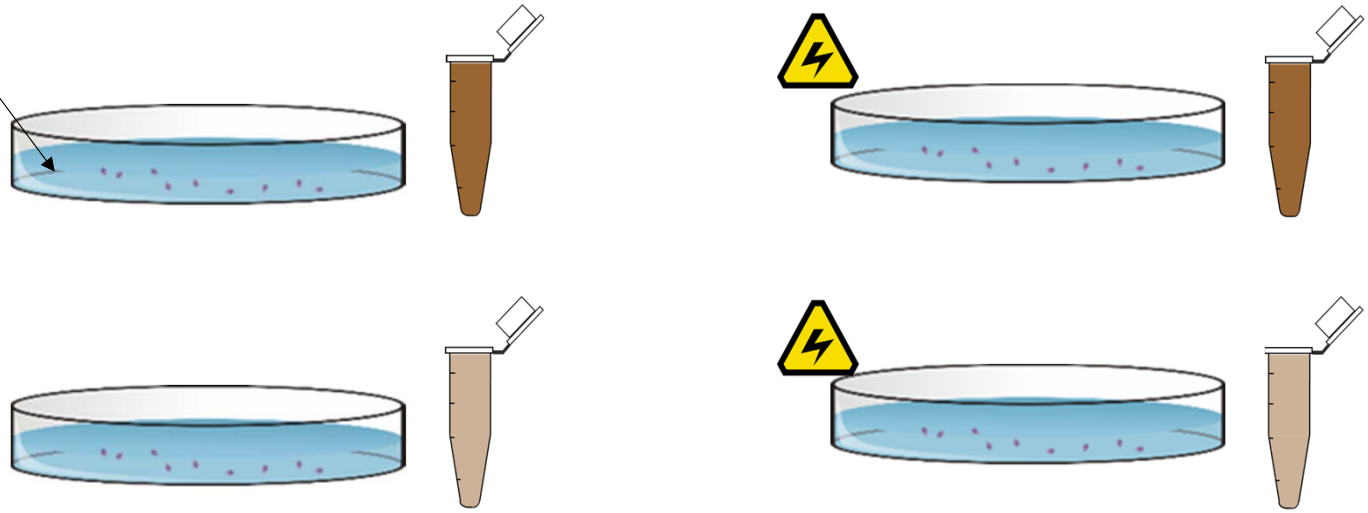
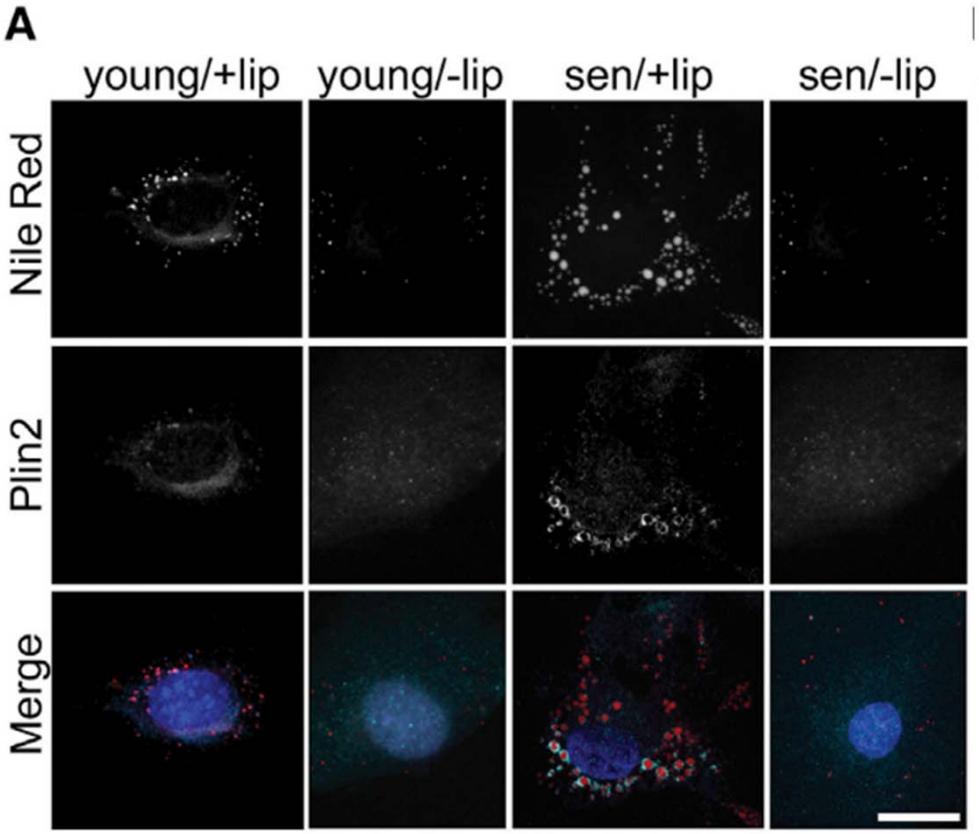
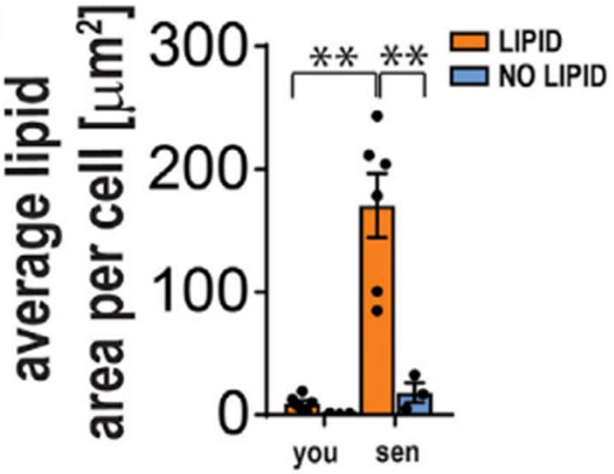


Fig. 6 ALISE phenotype drives CCF accumulation and the SASP



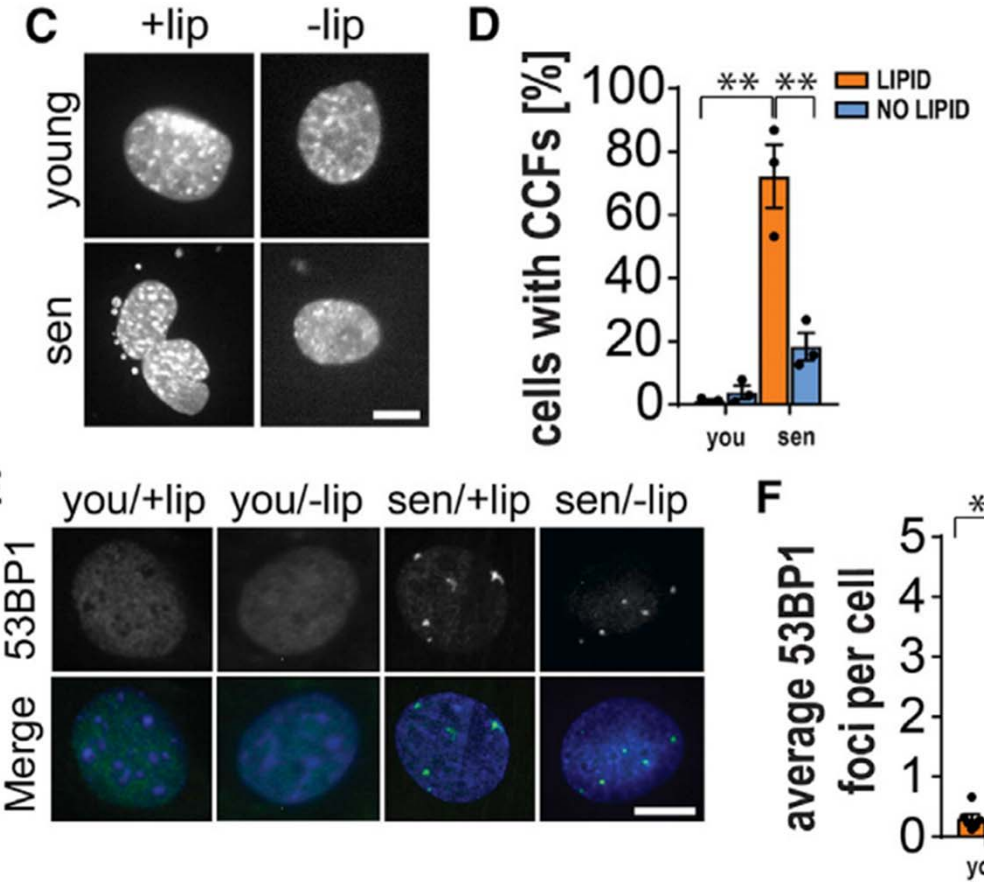
Nile red: lipophilic dye



<u>In absence of extracellular lipids</u>

Decreased number of cells displaying ALISE phenotype

Fig. 6 ALISE phenotype drives CCF accumulation and the SASP



- Senescent cells contain cytoplasmic chromatin fragments (CCFs)
  - Activate DANN-sensing cGAS-STING pathway (which is a major driver of the SASP)

Dou et al. 2017, Nature; Ivanov et al. 2013, J. Cell Biol.

Abrogation of ALISE phenotype

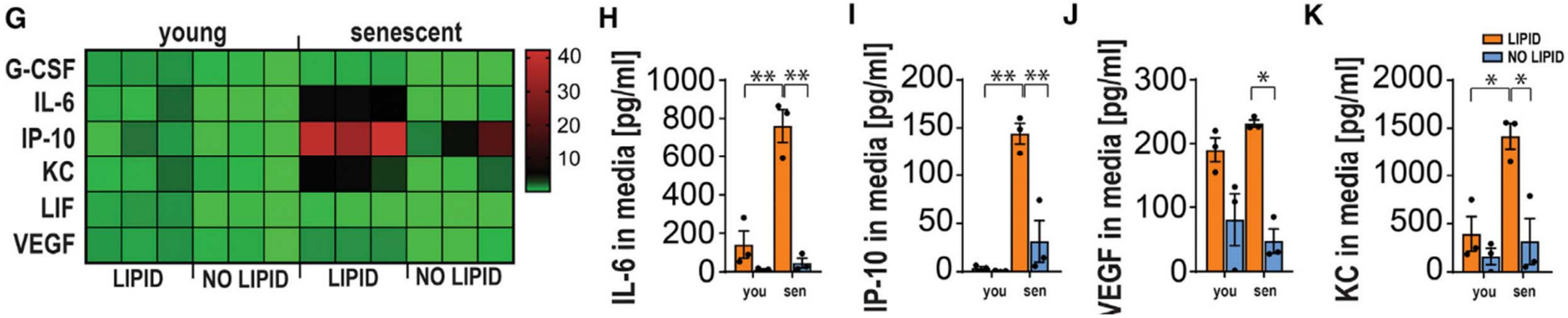
- Significantly reduces CCF in senescent cells
- No difference in average number of DNA damage foci

Fig. 6 ALISE phenotype drives CCF accumulation and the SASP

Hypothesis

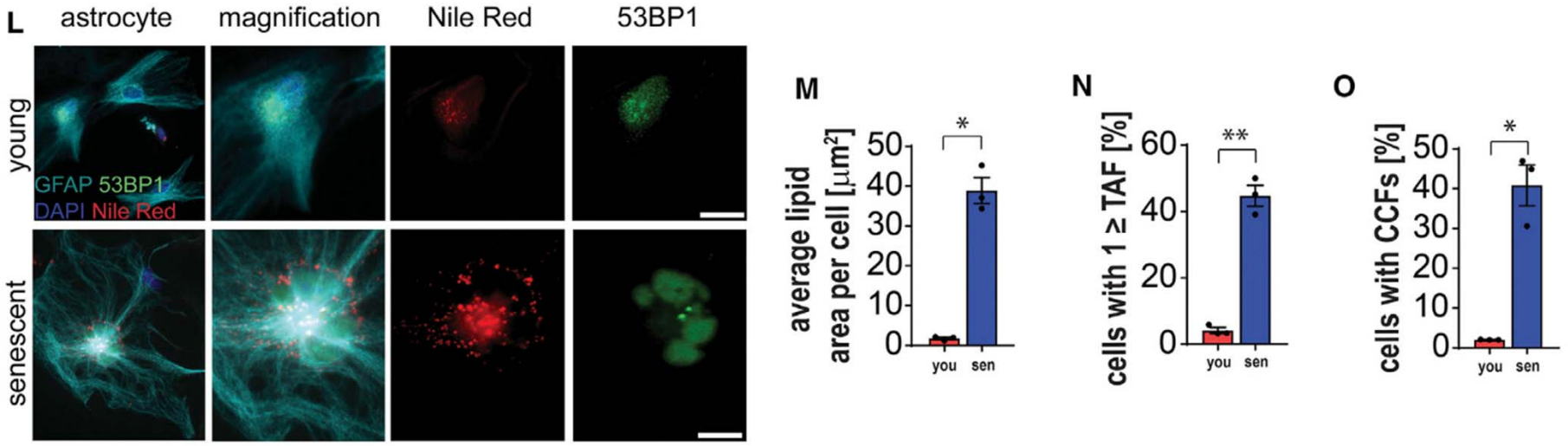
- Enhanced lipid deposition impacts CCF and SASP

Fig. 6 ALISE phenotype drives CCF accumulation and the SASP



Significant reduction of several key components of SASP
(IL-6, Kc (CxCl-1), Ip-10 (Cxcl-10) and Lix (Cxcl-5)

Fig. 6 ALISE phenotype drives CCF accumulation and the SASP



Primary mouse astrocytes (as confirmation for MAF)

- Increased fat buildup
- Increased TAF
- Increased # of CCF in senescent astrocytes

Conclusive remarks

Data suggest that excessive ALISE may be a contributor of genomic instability

→ resulting in the release of chromatin fragments and activation of SASP

Impaired neurogenesis in HFD animals is rescued by clearance of senescent cells

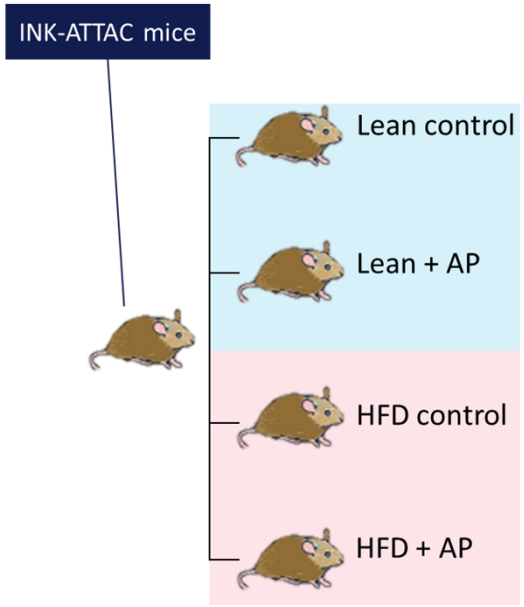
- Ectopic buildup of lipid droplets in AD brains
  - Induces dysfunction of neuronal stem cells within the subventricular zone (SVZ)
  - Suppresses adult neurogenesis
  - Causes cognitive impairment

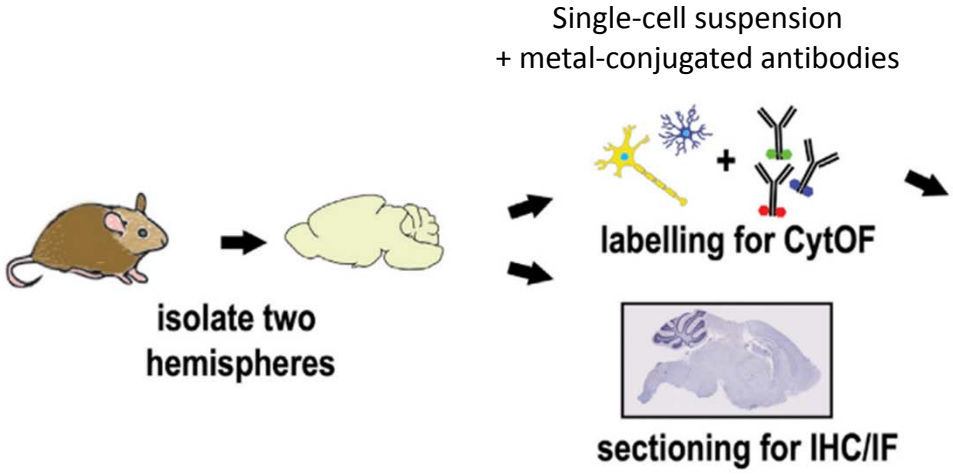
Hamilton et al. 2015, Cell Stem Cell

Hypothesis

- Presence of ALISE glial cells could impair adult neurogenesis in the SVZ

Methods





- Spanning-tree progression analysis of density-normalized events (SPADE)
- Heatmap shows intensity of antibody signal
- Size of spot determined by number of cells within this population
- Allows mapping & discriminating of different brain cells
  - *Astrocytes*
  - *Oligodendrocytes*
  - *Microglia*
  - *Neurons*
  - *Ependymal cells*
  - *Pericytes*
  - *Endothelial cells*

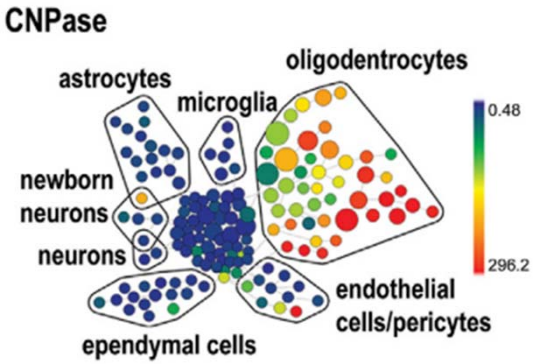
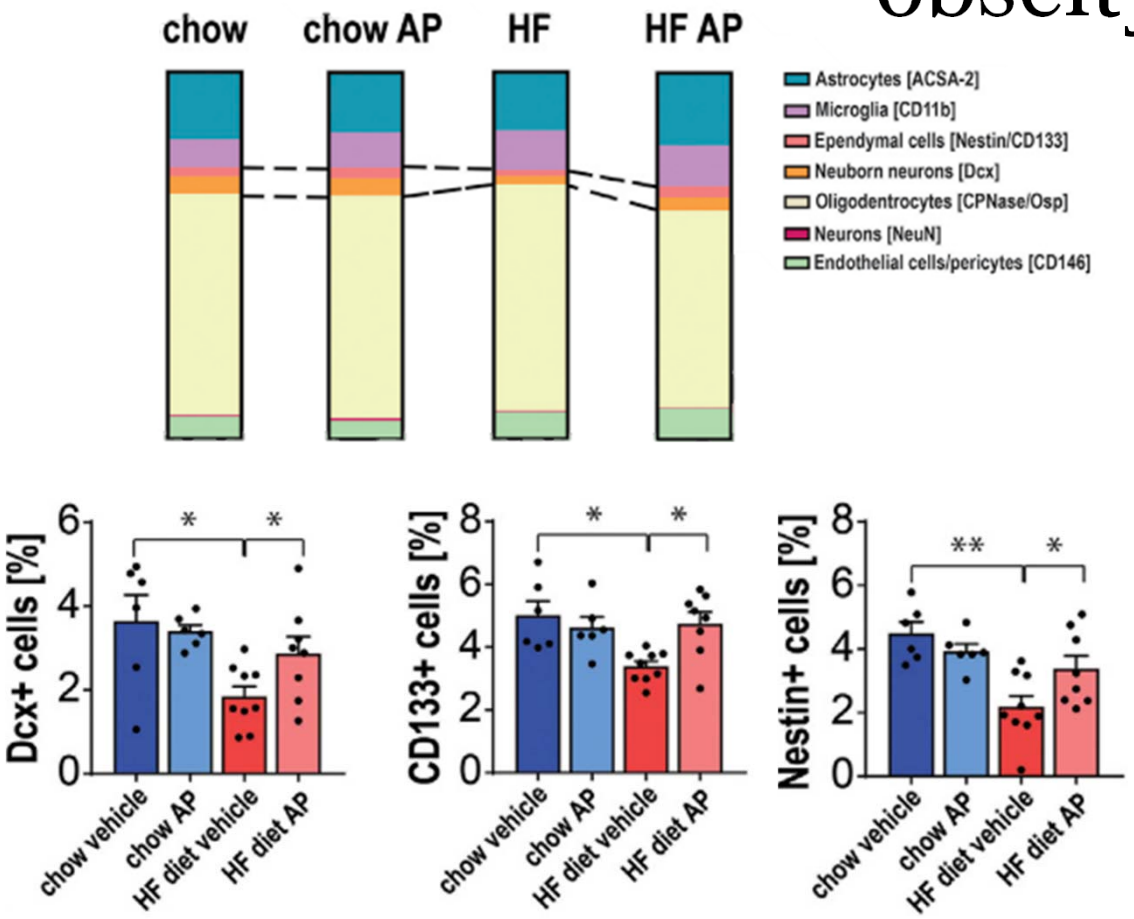


Fig. 7 Clearance of senescent cells partially reverses the neural progenitor cell depletion induced by obseity



HF diet mice

Significant decrease of

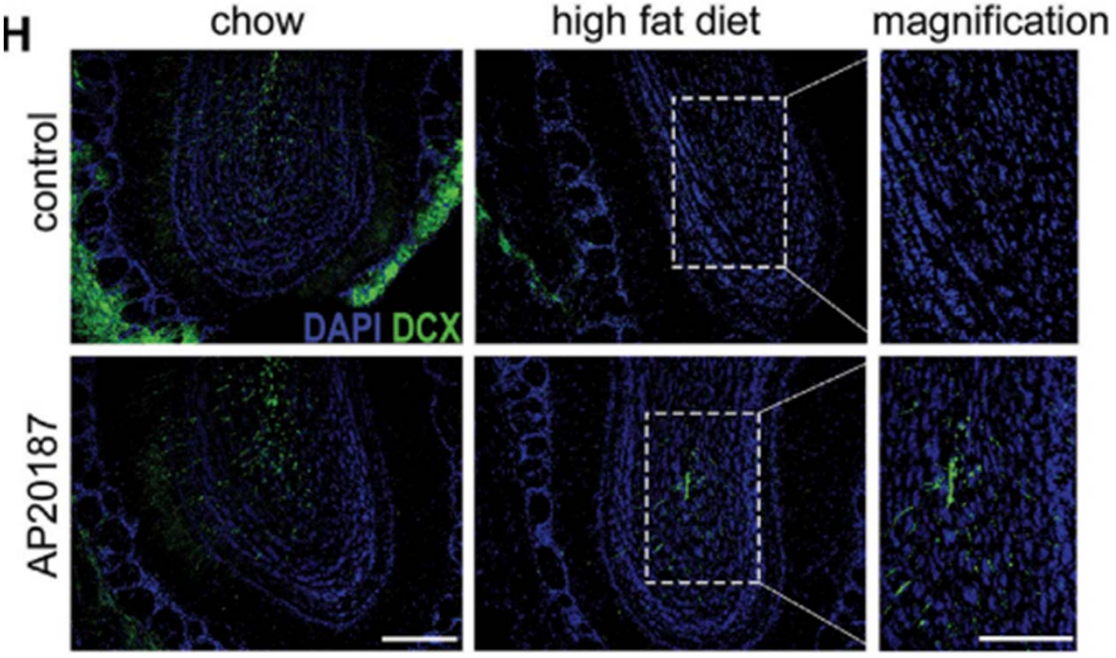
- Neuronal precursor cells *(Nestin)*
- Immature neurons *(Dcx)*
- Ependymal cells *(CD133)*

HF diet mice + AP treatment

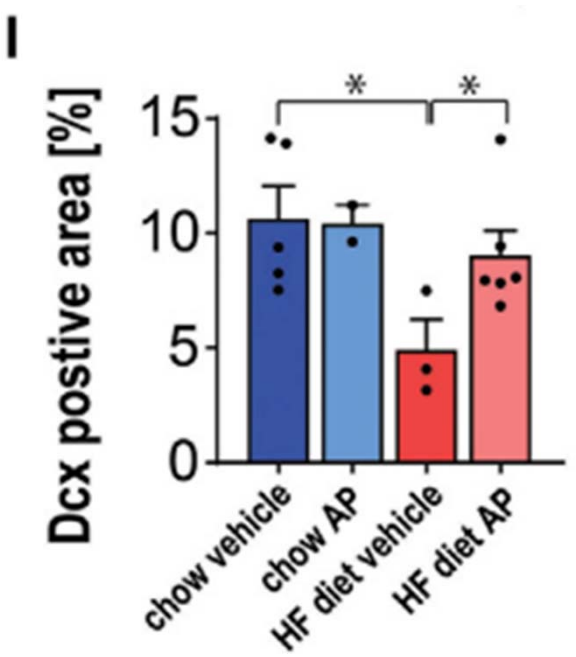
Significant increase of

- Neuronal precursor cells
- Immature neurons
- Ependymal cells

Fig. 7 Clearance of senescent cells partially reverses the neural progenitor cell depletion induced by obseity



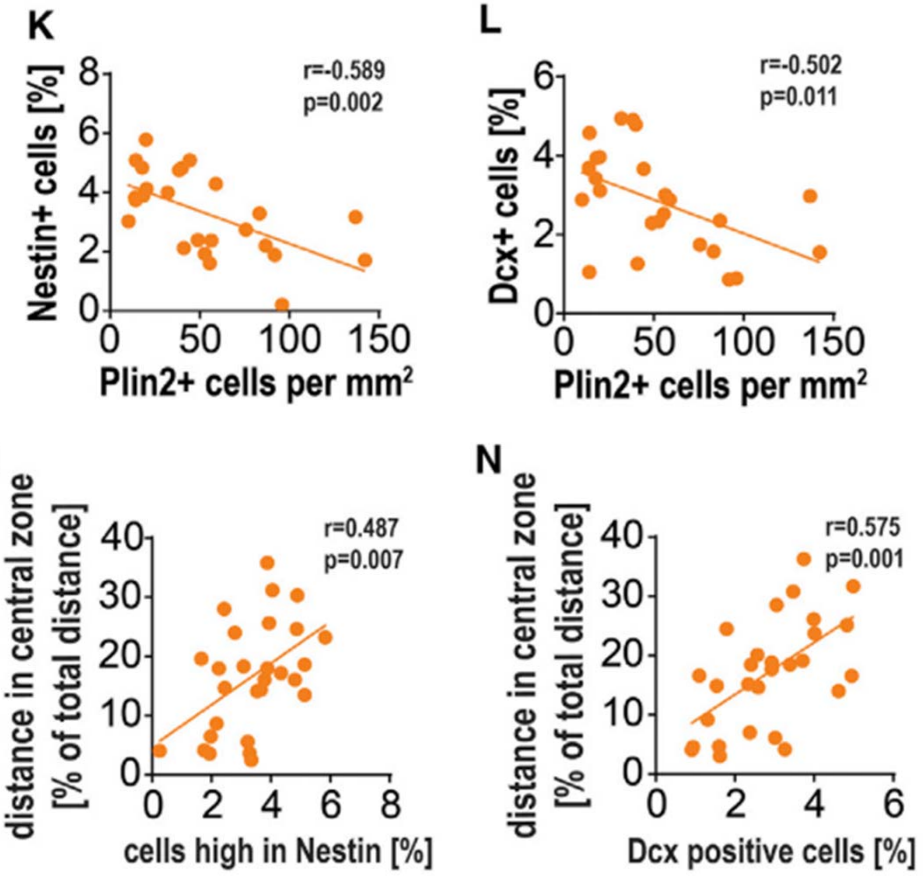
Dcx staining in olfactory bulb



HF diet mice

AP treatment partially „rescued“ loss of Dcx^{+} immature neurons

Fig. 7 Clearance of senescent cells partially reverses the neural progenitor cell depletion induced by obseity



Negative correlation of Plin2^{+} cells and adult neurogenesis markers
(Nestin = neuronal precursor cells, Dcx = immature neurons)

Positive correlation of distance travelled in the central zone and frequencies of detected neurogenesis marker positive cells
(Nestin = neuronal precursor cells, Dcx = immature neurons)

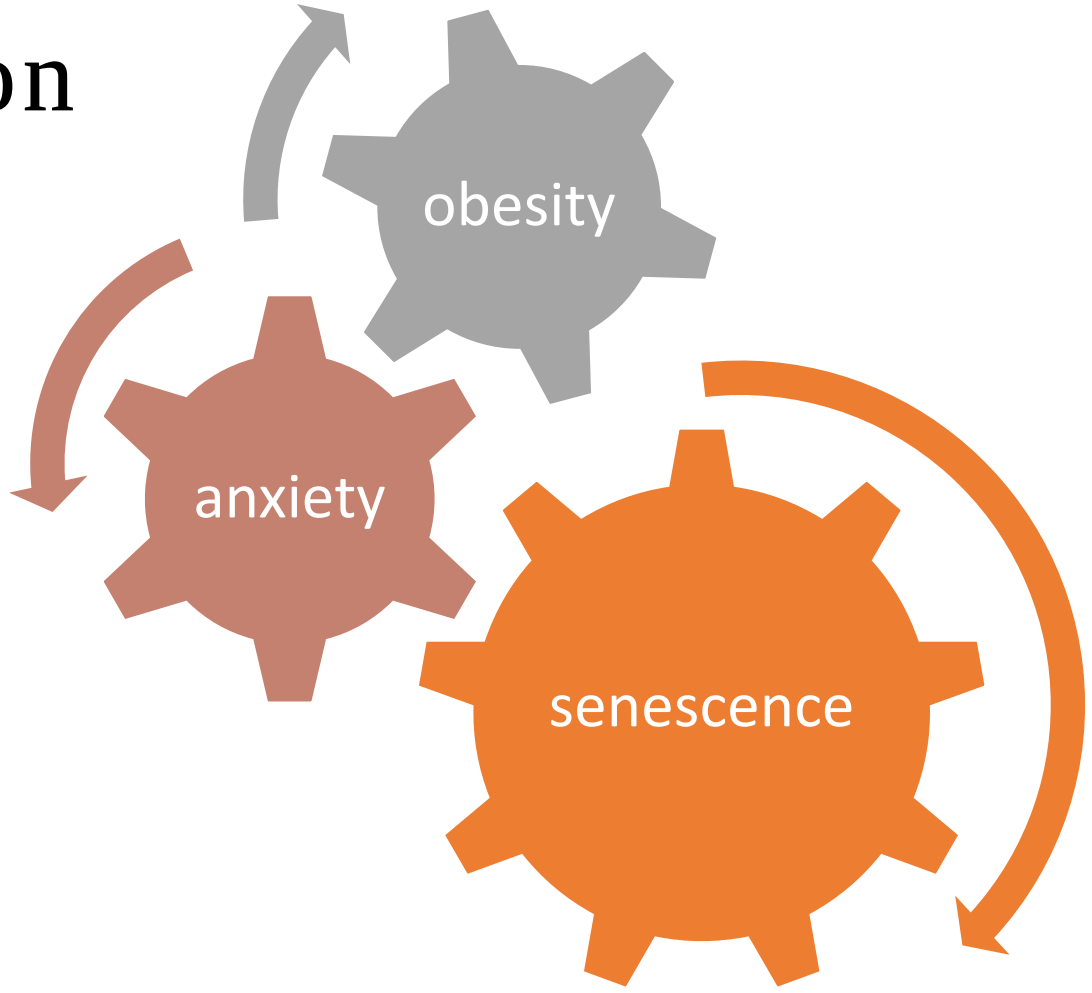
Conclusive remarks

Data indicate a causal role for senescent cells in obesity induced neurogenesis

targeting senescent cells in obese mice alleviates obesity-related anxiety-like behaviour by clearance of periventricular fat accumulation and restoration of adult neurogenesis

Discussion

Discussion



Discussion

- Anxiety-like behavior is not simply caused by increased body mass

  Canetti et al. 2016, Cunningham et al. 2012, Fisher et al. 2017, Kouidrat et al. 2017, Matini et al. 2014

- HFD induces senescence in multiple organs

  Schafer et al. 2016, Tchkonia et al. 2010, Xu et al. 2015, Ogrodnik et al. 2017

- Senescent glial cells involved in neurodegenerative diseases (tau-dependent pathology, Parkinson's disease)

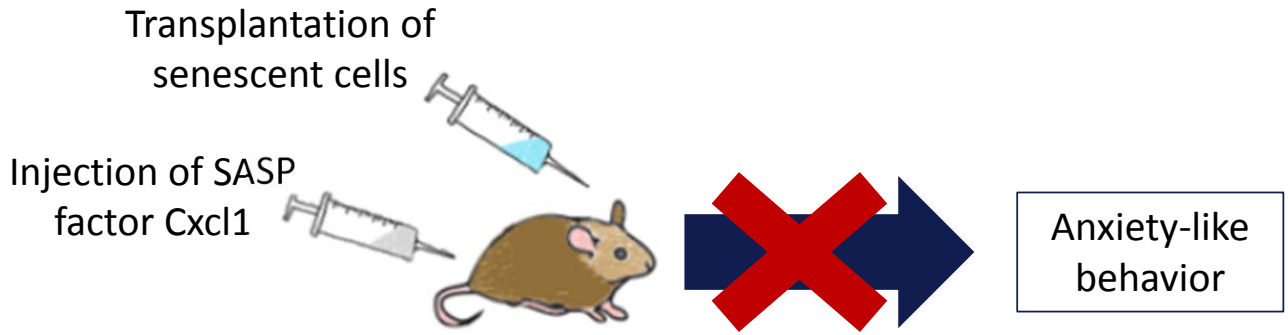
  Bussian at al 2018, Chinta et al 2018, Musi et al. 2018

Discussion

- Senescent, pro-inflammatory glial cells frequently found in close proximity to brain areas expressing markers of neuronal precursor cells & immature neurons
  → negative effect of senescent cells on stem cells

- Increased SASP factors in blood plasma and brain during obesity
  - Reduced upon pharmacogenetic & pharmacologic clearance

Discussion



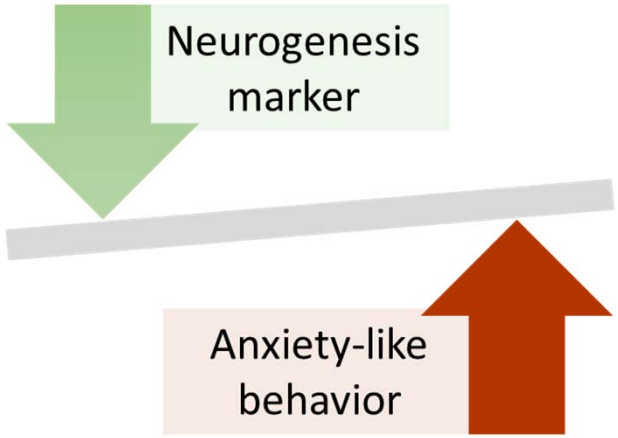
?

Anxiety-like phenotype driven by obesity may be a result of senescence occurring in specific regions of the brain

Discussion

- Clearance of senescent cells in obese mice partially restores the neural stem cell pool

- Neg. correlation of neurogenesis marker and anxiety-like behaviour

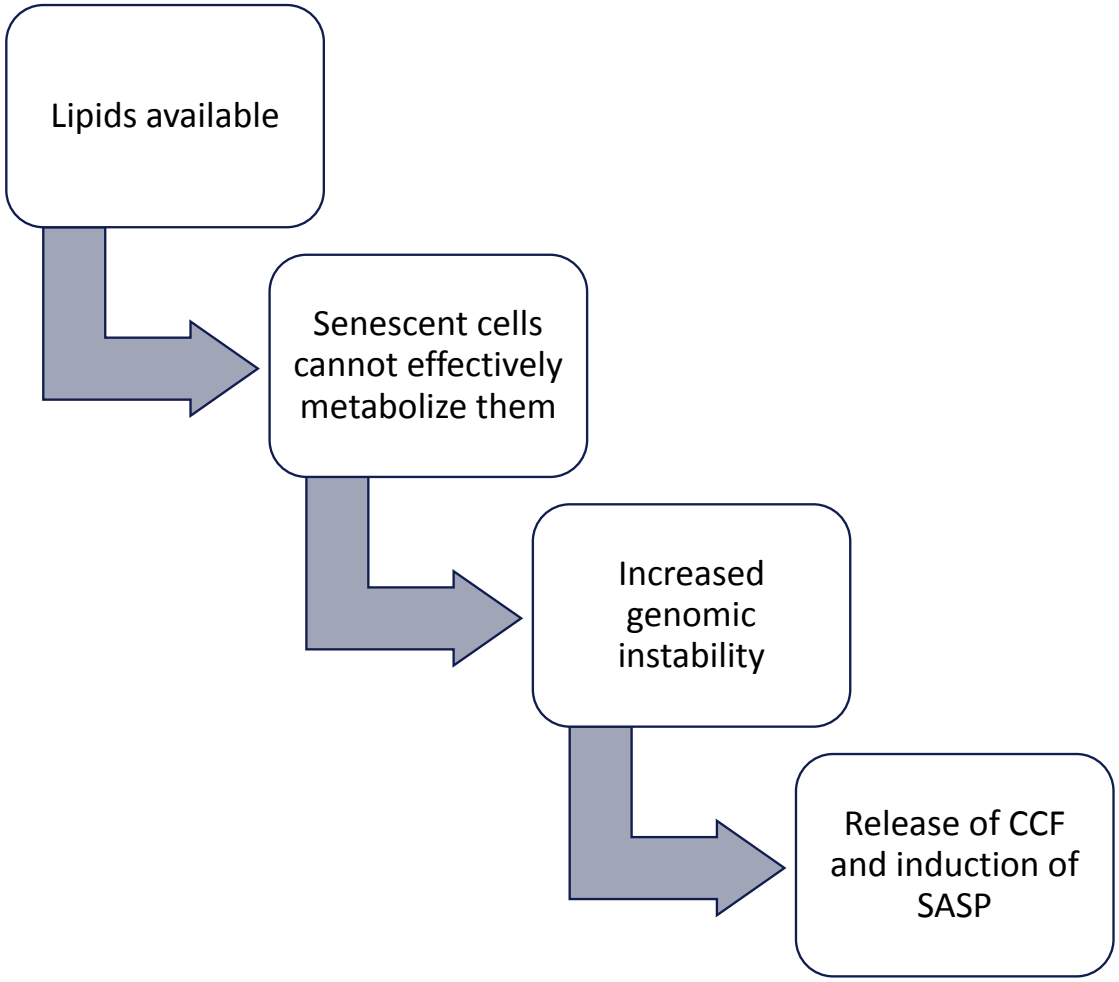


! Anxiety itself may impair neurogenesis

Discussion

- Senescent glial cells accumulate lipids
  - Phenotype: ALISE
  - *Accumulation of lipid droplets in SVZ occurs in mouse models of Alzheimer's Disease & human Alzheimer's patients*
    Hamilton et al. 2015
- Presence of lipid-containing senescent cells contributes to impaired neurogenesis
  (rather than accumulation of lipids per se)
- Elimination of senescent cells improves neurogenesis & alleviates mouse anxiety-like behavior

Main conclusion



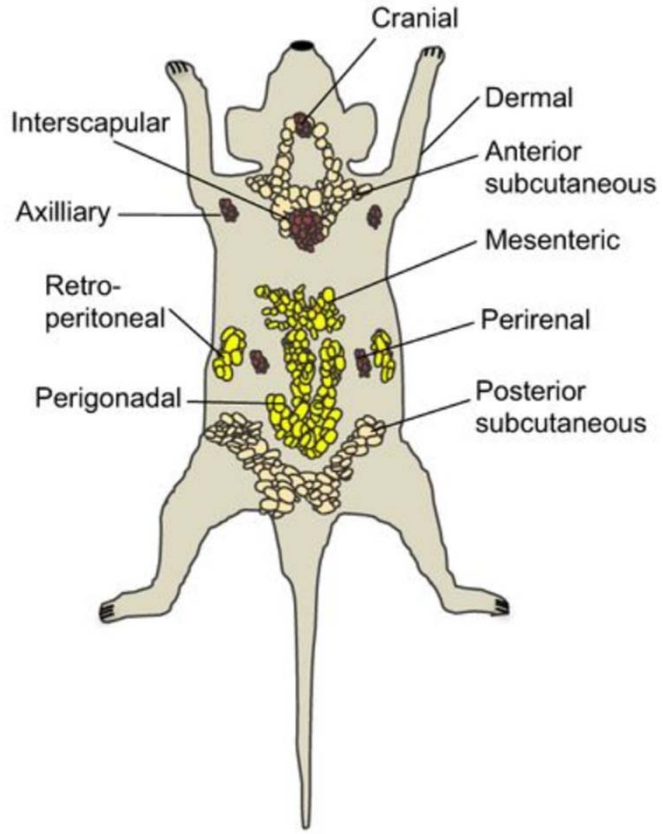
General remarks

- Some conclusions are based on data that is not shown
- Some sentences/explanations don‘t make sense
  e.g. *„Interestingly, clearance of senescent cells led to a significant increase in the population of astrocytes in obese animals, whereas no differences between lean and obese animals were detected.”*
- Figure description is relatively sparse
- Insane amount of supplementary figures without any explanation or description
- Experimental study doesn‘t distinguish which senescent cell type is responsible for inducing anxiety-like behavior

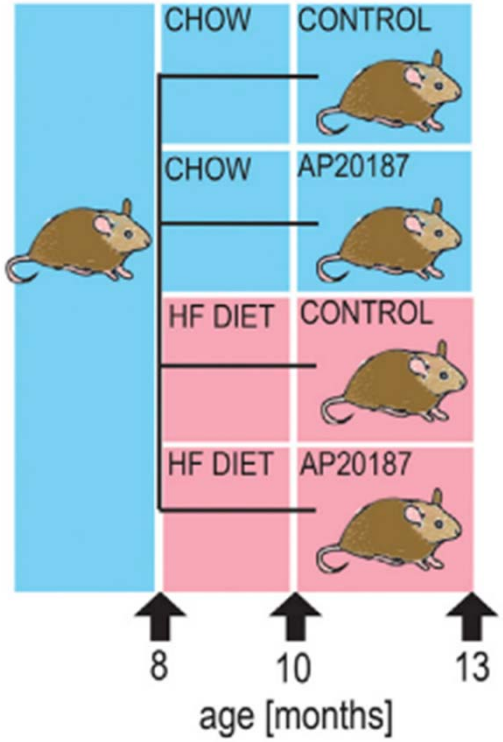
Subcutaneous white adipose tissue (SAT)

Visceral white adipose tissue (VAT)

Brown adipose tissue (BAT)



Adapted from Schoettl et al. 2018, Journal of Experimental Biology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