

GeroScience (2020) 42:715–725

<https://doi.org/10.1007/s11357-019-00151-6>

ORIGINAL ARTICLE



Check for updates

Blockade of the NLRP3 inflammasome improves metabolic health and lifespan in obese mice

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Received: 30 August 2019 / Accepted: 27 December 2019 / Published online: 23 January 2020

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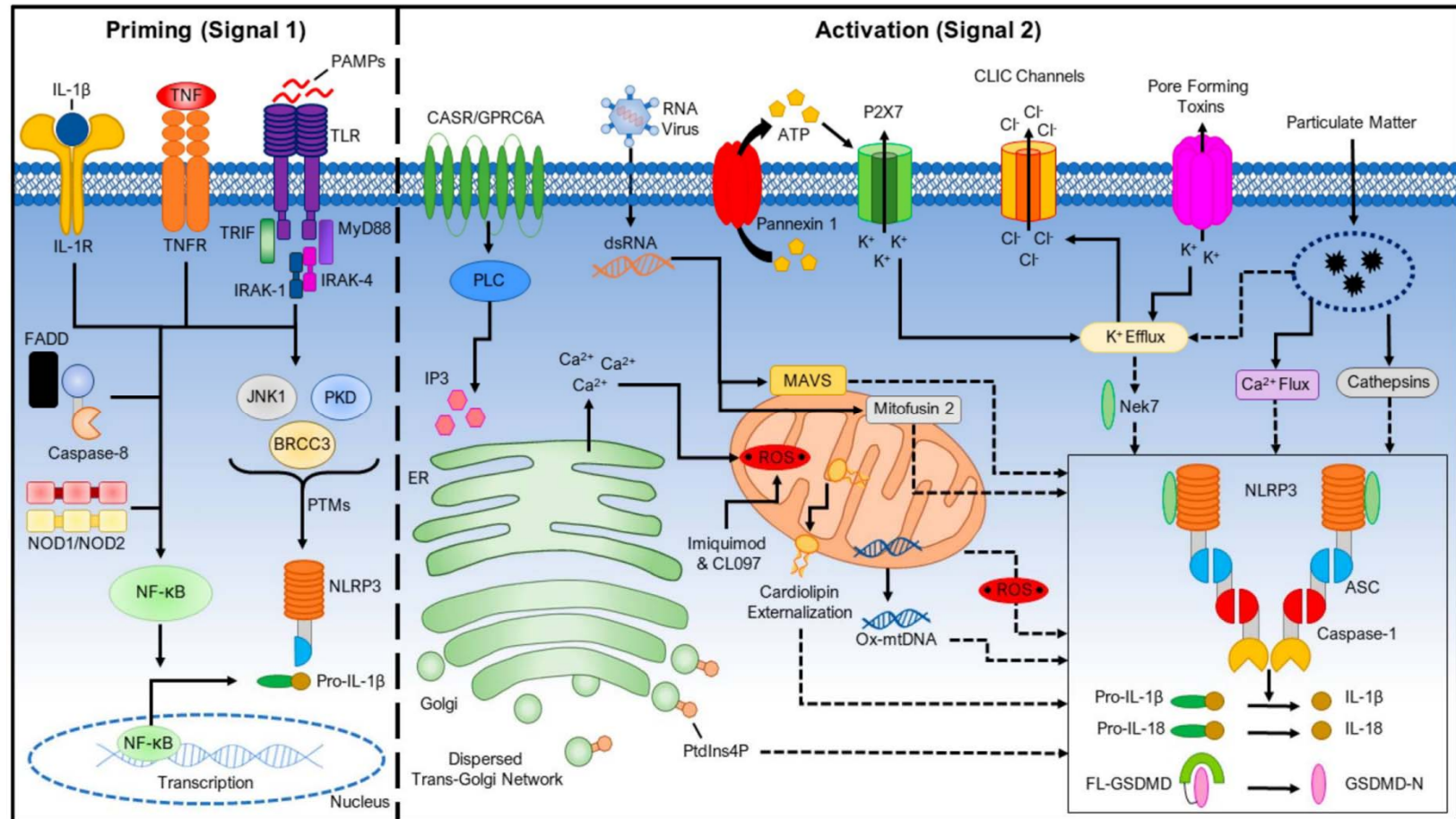
NLRP3 inflammaosme

- Pattern-Recognition Receptors that form inflammasomes
 - Leucine-rich repeat-containing proteins (NLR) family member
- Critical component in innate immunity
- Mediates
 - caspase-1 activation
 - Secretion of pro-inflammatory cytokines (IL-1 β , IL-18) upon microbial infection & cellular damage
- dysfunctional activation linked to inflammatory disorders
(*cryopyrin-assoc. periodic syndromes, Alzheimer's, diabetes, autoinflammatory diseases, atherosclerosis,...*)

Kelley et al. 2019 PMID: 31284572

Two-signal Model for NLRP3 inflammasome activation

- **priming signal** provided by microbial components / endogenous cytokines
- Activation of TF NF-kappaB & subsequent upregulation of NLRP3, pro-IL-1 β
- Caspase-8 & FAS-mediated death domain protein, NOD1/2 => priming by regulating NF-kB
- Post-translational modifications of NLRP3
- **Activation signal** provided by various stimuli (extracell. ATP, pore-forming toxins, RNA viruses, ...)
- Ionic flux, mitochondrial dysfunction, ROS generation lysosomal damage activate NLRP3



Kelley et al. 2019 PMID: 31284572

NLRP3

- NLRP3^{-/-} mice:
 - resistant to development of obesity upon HFD
 - Protected from obesity-induced insulin resistance
 - Protected from cardiac damage
- NLRP3 upregulated after MI, atherosclerosis, ischemic heart disease, diabetic cardiomyopathy, chronic heart failure, hypertension
- Genetic deletion attenuates age-related degenerative changes
(*glycemic control, bone loss, cognitive function, motor performance*)

Aim

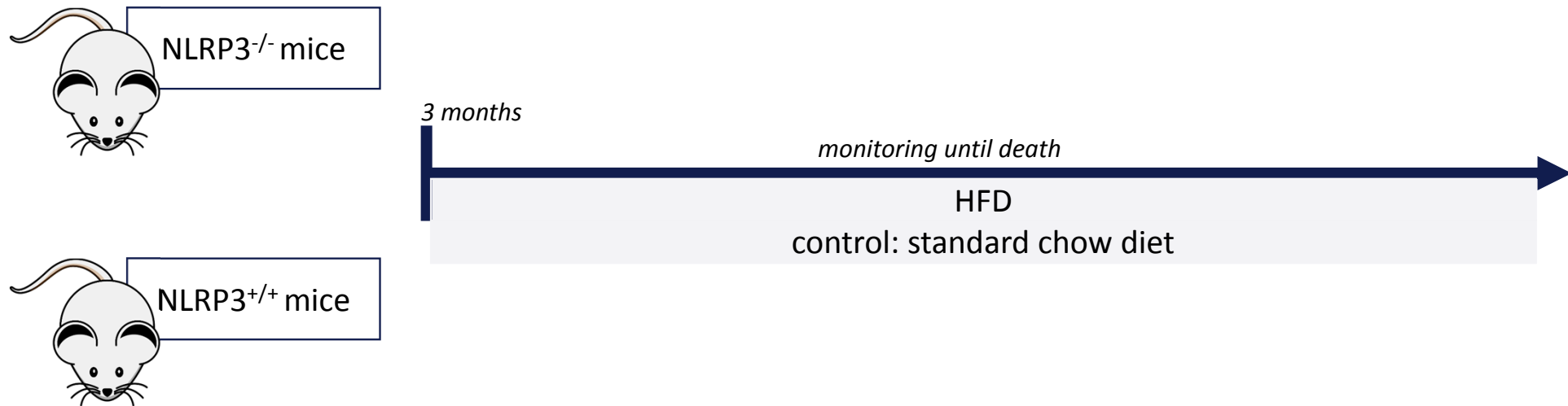
- Determine whether the genetic deletion of NLRP3 has an effect on lifespan
- Can genetic deletion of NLRP3 prevent metabolic aging in mice fed with high fat diet (HFD) ?

Methods

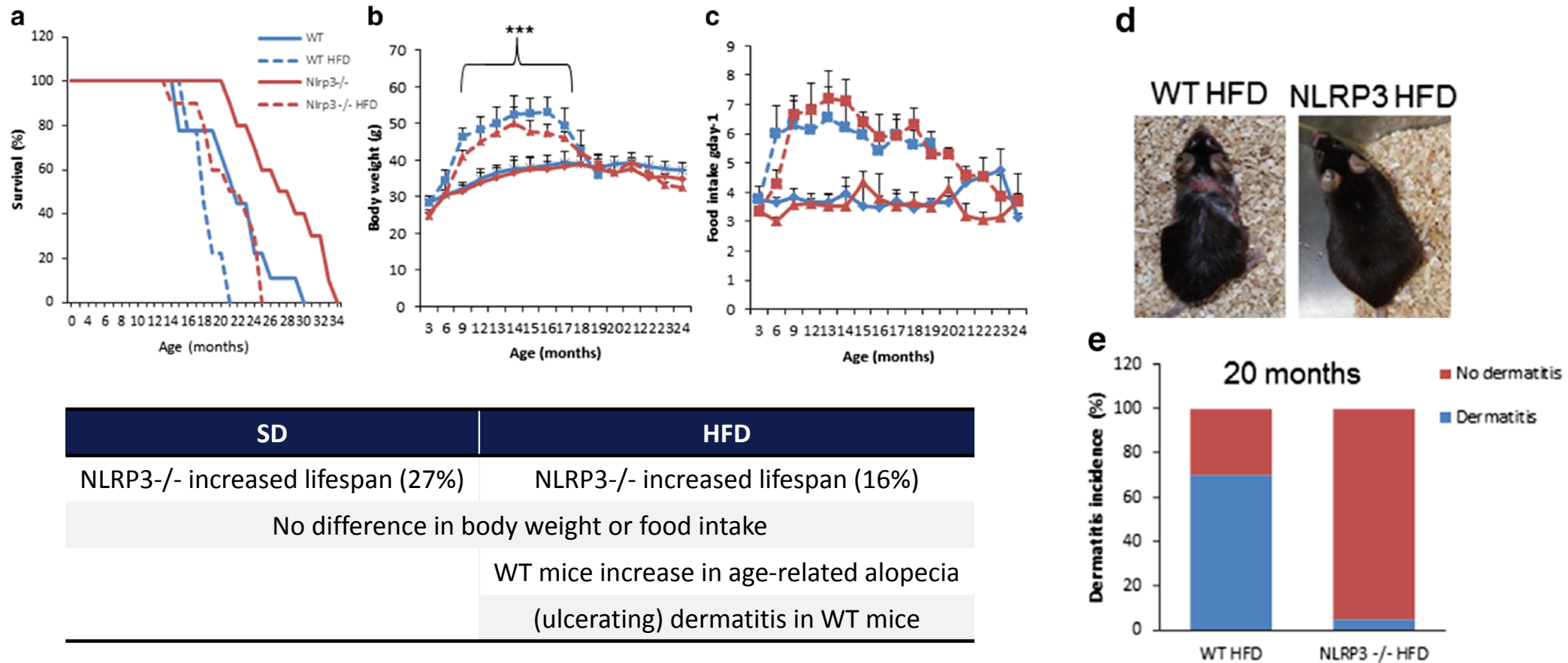
- Male NLRP3^{-/-} mice (C57BL/6 background)
 - young (3months) vs. old (20months)
 - Two nutritional groups:
 - a) regular chow diet/standard diet
 - b) high fat diet (HFD)
- Survival assessment
- Monitoring of body weight, food intake
- Glucose and insulin tolerance test

Results

NLRP3 deficiency & metabolic impairment



NLRP3 deficiency in aged and obese mice

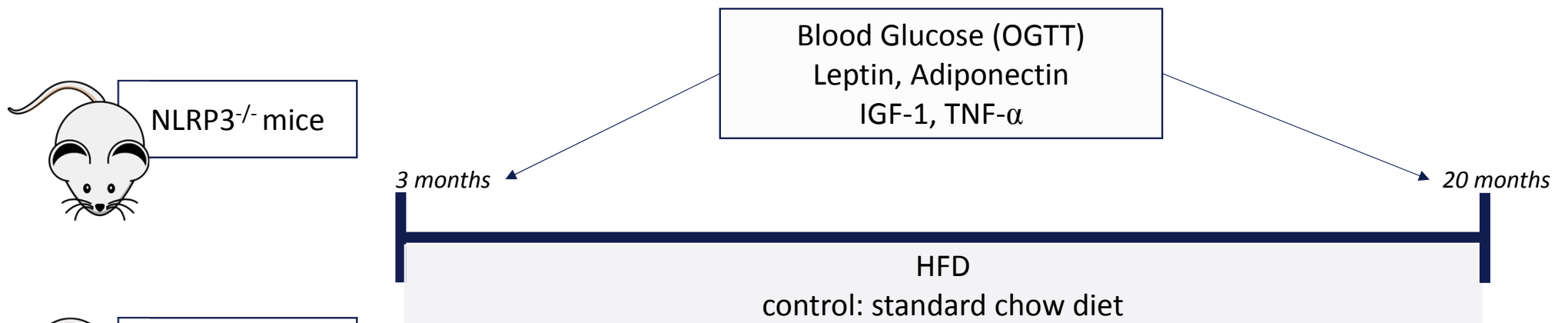


NLRP3 deficiency in aged and obese mice

NLRP3 deficiency improved lifespan in aged obese mice

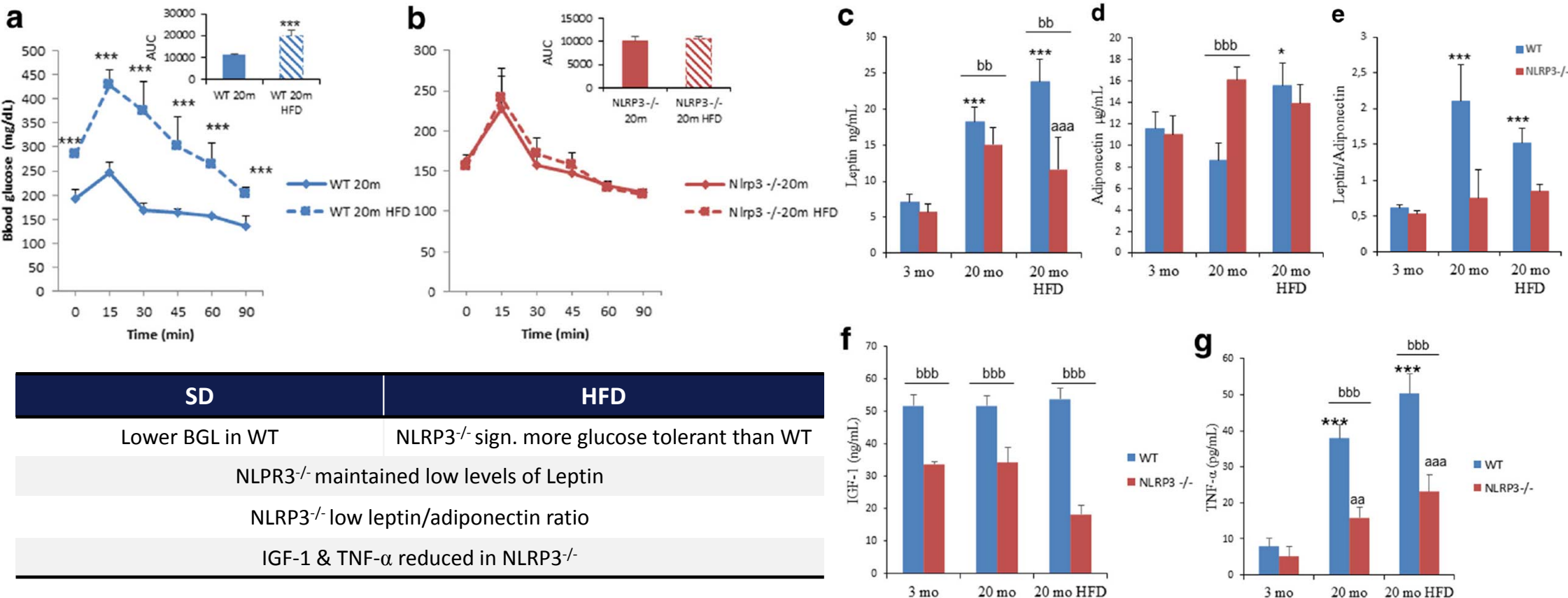
NLRP3 ablation protects against inflammation and HFD-induced skin lesions associated with inflammation

NLRP3 deficiency in aged and obese mice



- **Leptin** = regulator of body weight
- Dysregulation of **leptin/adiponectin ratio** associated with cardiovascular disease, metabolic syndrome, non-alcoholic fatty liver disease
- **Fasting BGL levels & IGF-1** = predictors of diabetes & short lifespan
 - Reduced BGL levels & IGF-1 associated with stress resistance & anti-aging effect
- **TNF-α** involved in maintenance & homeostasis of immune system, inflammation & host defense; but dysregulation associated with chronic inflammatory diseases

NLRP3 deficiency & metabolic impairment



NLRP3 deficiency & metabolic impairment

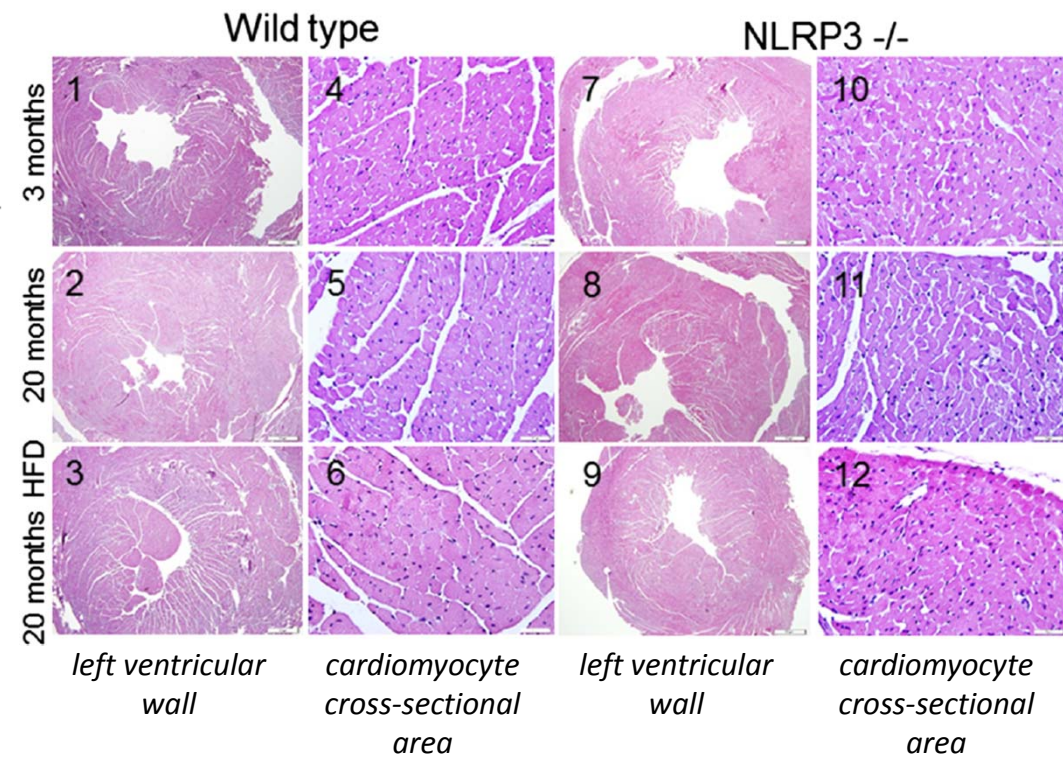
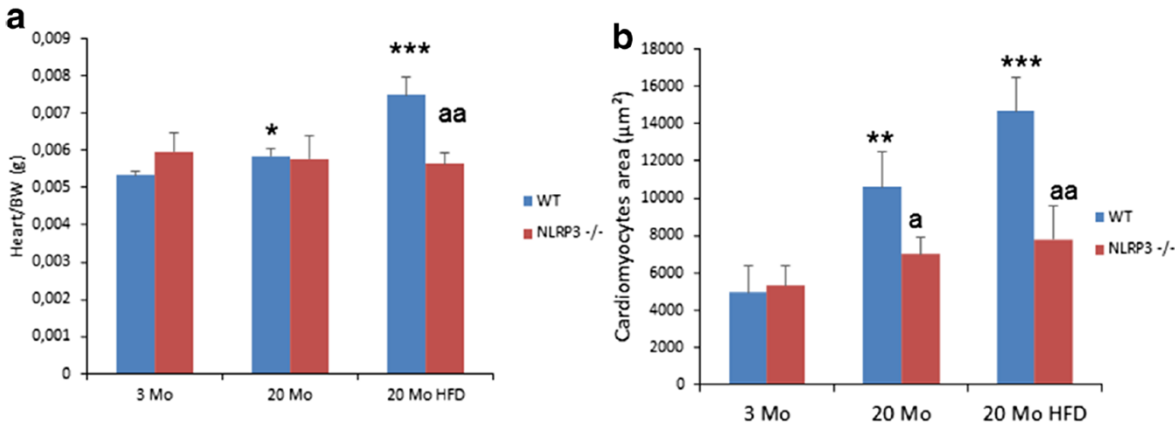
NLRP3 deficiency diminished metabolic impairment induced by HFD during aging

Absence of NLRP3 improved metabolic homeostasis in obese mice during aging

NLRP3 deficiency in cardiac & liver integrity

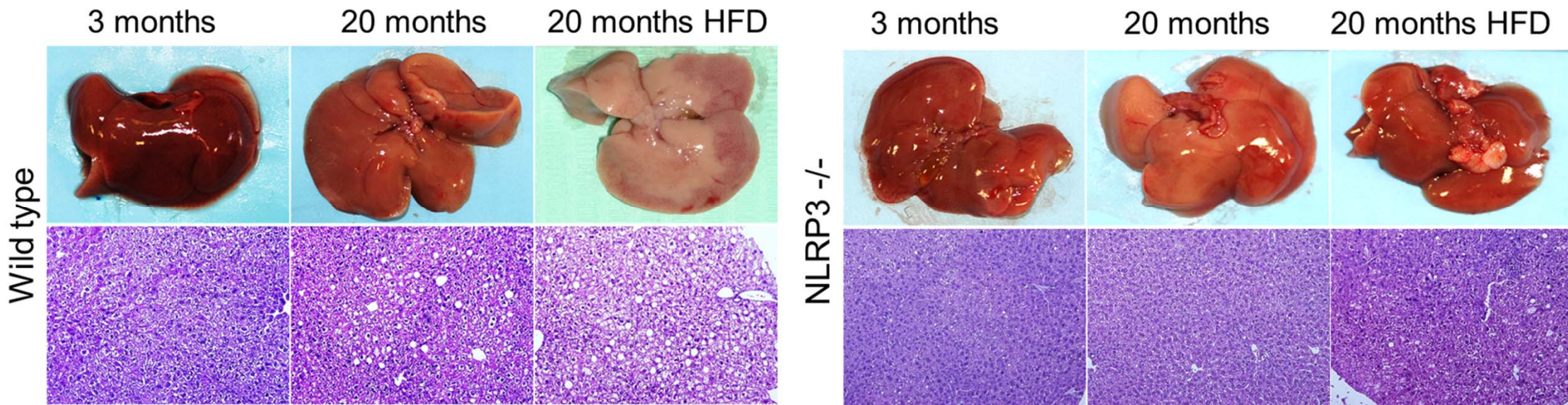
- Analysis of
 - heart weight (normalized to body weight)
 - Cardiomyocyte area
 - Left ventricular wall thickness
- Typical pathophysiological feature of cardiac aging, cardiac hypertrophy → thickness of left ventricular wall & cardiomyocyte cross-sectional area

NLRP3 deficiency in cardiac & liver integrity



- Increased heart weight in old WT HFD mice
- Left ventricular thickness increased in old WT and old WT HFD mice (1-3, 7-8)
- Increased cardiomyocyte cross-sectional area in old WT HFD (4-6, 10-12)

NLRP3 deficiency in cardiac & liver integrity



- Old WT HFD mice showed characteristic pale color
- Lipid accumulation & steatosis in old WT HFD mice

- NLRP3^{-/-} mice show normal liver coloration

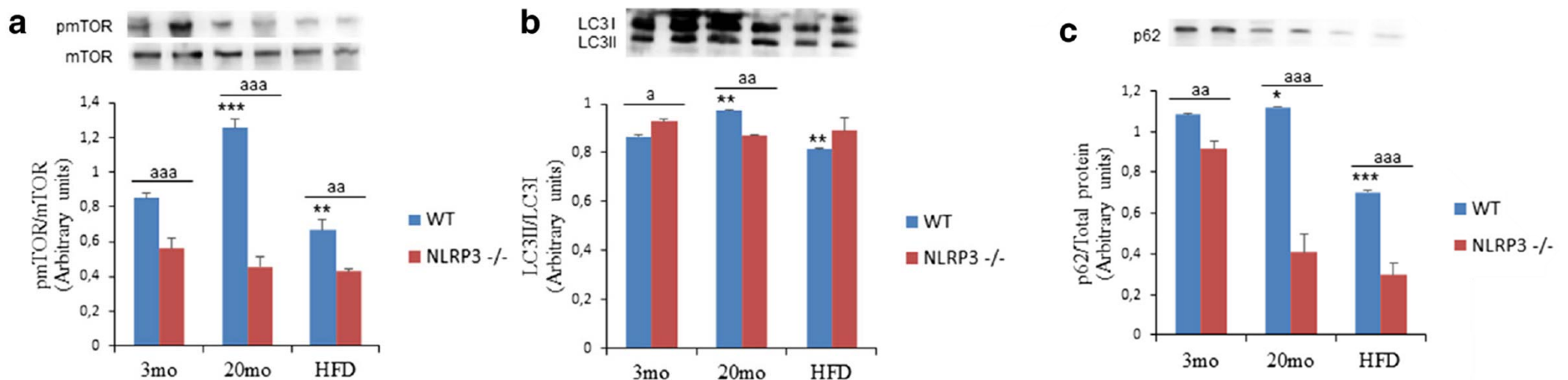
NLRP3 deficiency in cardiac & liver integrity

NLRP3 deletion preserves cardiac and liver integrity

NLRP3 in age-associated metabolic changes

- Analysis of signalling pathways of mTOR and autophagy in the heart
- mTOR
 - involved in healthspan
 - associated with autophagy (*therefore, indirectly with cell homeostasis via protein degradation & removal of damaged intracellular organelles*)
- Autophagic dysfunction linked to aging and obesity (*blocked autophagic flux, accumulation of non-degraded substrates in form of autophagosome*)

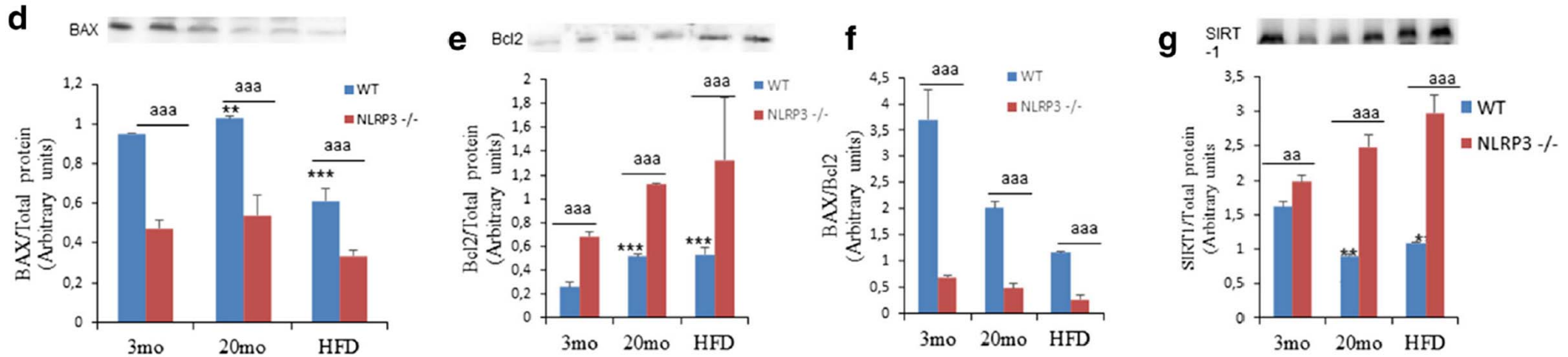
NLRP3 in age-associated metabolic changes



- mTOR phosphorylation decreased in old NLRP3 deficient HFD mice
- Normal levels of LC3II protein expression and reduction of p62/SQSTM1 in old NLRP3 deficient HFD mice
→ high quality of autophagy

- *LC3II critical player in autophagy*
- *P62/SQSTM1 (ubiquitin- & LC3-binding protein) is increased when autophagy is impaired*

NLRP3 in age-associated metabolic changes



	cardiac	Pro-apoptotic BAX ¹	anti-apoptotic Bcl2 ¹	BAX/Bcl2 proportion ²	SIRT-1 ¹
WT	old	↑	~↑	↑	↓
	old HFD	↓	~↑	↑	↓
NLRP3 ^{-/-}	old	↓	↑		↑
	old HFD	↓	↑		↑

SIRT-1 involved in heart protection & metabolic improvement during aging (by mTOR inhibitor & autophagy induction)

Discussion

Discussion

- Main molecular pathways impaired during aging
 - Glucose metabolism
 - Insulin response
 - Dysregulation of mTOR
 - SIRT1
 - Inflammation
- Age-dependent changes highly associated with lifestyle may be exacerbated by hypercaloric nutrition

Discussion

NLRP3 associated with damage induced by HFD during aging

→ improving lifespan & healthspan

NLRP3^{-/-} increases longevity & healthspan despite HFD

NLRP3 deficient mice: no weight gain despite HFD

potential explanation: activation of metabolic parameters (AMPK) >> counteracting diabetes, obesity, aging

NLRP3 deficiency reduced levels of IGF-1, mTOR vs. Increased SIRT-1 & improved autophagy proteins

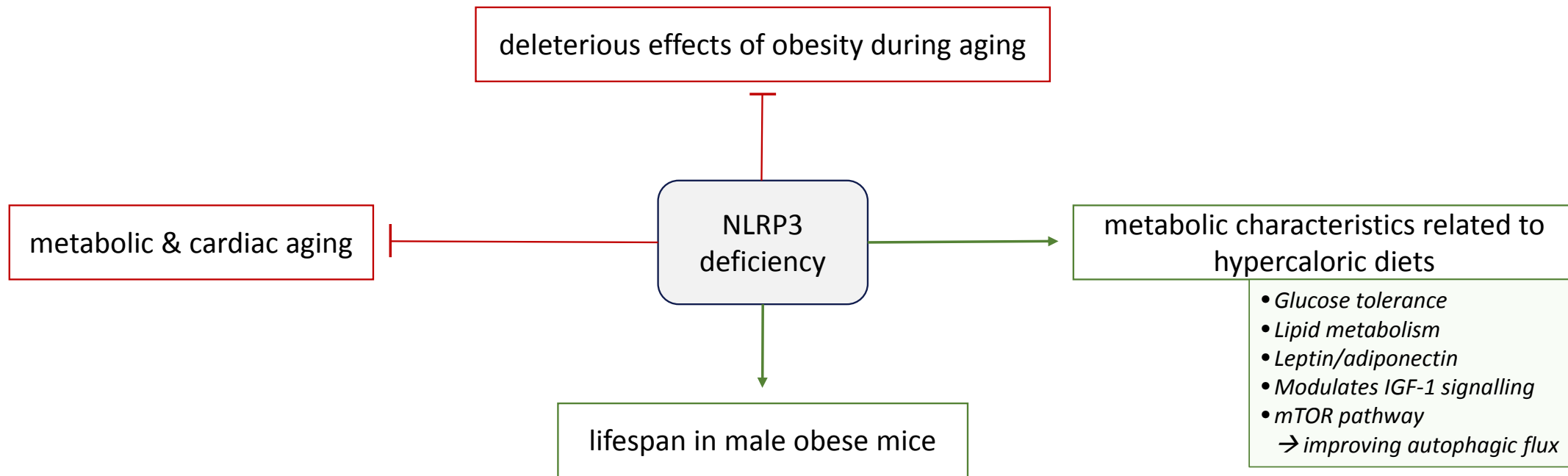
→ improving lifespan, high quality autophagy in NLRP3^{-/-} mice

NLRP3 ablation reduced p62/SQSTM1 & increased autophagic flux in cardiac tissue in old HFD mice

NLRP3^{-/-} mice on SD: similar weight gain as WT

→ protective effect of NLRP3 not related to obesity during aging but w/ downstream effectors arising from HFD & inducing IL-1 β , IL-18

Conclusion



Limitations & comments

- Only male mice → gender differences ?
- Scale on graphs not uniform (e.g. BGL WT: 500mg/dl, NLRP3^{-/-} 300mg/dl)
- Western Blot band description ??