1   **Adiponectin Preserves Metabolic Fitness During Aging**

2   Na Li[1,3], Zhuzhen Zhang[1], Shangang Zhao[1], Yi Zhu[1, #], Christy M. Gliniak[1], Lavanya

3   Vishvanath[1], Yu A. An[1], May-yun Wang[1], Yingfeng Deng[1], Qingzhang Zhu[1], Toshiharu

4   Onodera[1], Orhan K Oz[2], Ruth Gordillo[1], Rana K. Gupta[1], Ming Liu[3], Tamas L. Horvath[4],

5   Vishwa Deep Dixit[4, 5] and Philipp E. Scherer[1, 6, *]

6

7   [1] Touchstone Diabetes Center, Department of Internal Medicine, The University of Texas

8   Southwestern Medical Center, Dallas, TX USA.

9   [2] Department of Radiology, University of Texas Southwestern Medical Center, Dallas, TX

10   USA.

11   [3] Department of Endocrinology and Metabolism, Tianjin Medical University General

12   Hospital, Tianjin 300052, China.

13   [4] Department of Comparative Medicine and Immunobiology, Yale School of Medicine, New

14   Haven, Connecticut 06520, USA.

15   [5] Yale Center for Research on Aging, Yale School of Medicine, New Haven, Connecticut

16   06520, USA.

17   [6] Department of Cell Biology, The University of Texas Southwestern Medical Center, Dallas,

18   TX USA.

19   [#] Current address: Children's Nutrition Research Center, Department of Pediatric, Baylor

20   College of Medicine, Houston, TX, USA.

21

22   Running title: *Adiponectin and Aging*

23   *To whom correspondence should be addressed:

24   Philipp E. Scherer, Ph.D.

25   Departments of Internal Medicine

26   University of Texas Southwestern Medical Center

27   Address: L5.210, 5323 Harry Hines Boulevard, Dallas, TX 75390-8549

28   Tel: (+1)214-648-8715; Email: Philipp.Scherer@UTSouthwestern.edu

29 **Abstract:**

30 Adiponectin is essential for the regulation of tissue substrate utilization and systemic

31 insulin sensitivity. Clinical studies have suggested a positive association of circulating

32 adiponectin with healthspan and lifespan. However, the direct effects of adiponectin on

33 promoting healthspan and lifespan remain unexplored. Here, we are using an adiponectin

34 null mouse and a transgenic adiponectin overexpression model. We directly assessed

35 the effects of circulating adiponectin on the aging process and found that adiponectin null

36 mice display exacerbated age-related glucose and lipid metabolism disorders. Moreover,

37 adiponectin null mice have a significantly shortened lifespan on both chow and high-fat

38 diet (HFD). In contrast, a transgenic mouse model with elevated circulating adiponectin

39 levels has a dramatically improved systemic insulin sensitivity, reduced age-related tissue

40 inflammation and fibrosis, and a prolonged healthspan and median lifespan. These

41 results support a role of adiponectin as an essential regulator for healthspan and lifespan.

Introduction

Healthspan and lifespan are intimately linked. Improving healthspan should help enhance the overall quality of life for an aging population, and possibly even extend lifespan (Crimmins, 2015; Piskovatska et al, 2019). According to current estimates, by 2050, the number of older adults in US, above the age 65 years are expected to double, rising from 40.2 million to approx. 88 million (https://www.cdc.gov/nchs/products/databriefs/db106.htm). In the U.S., the average lifespan is around 79.3 years, while the estimated healthspan is only 67.3 years, indicating that the individuals will on average live up to 20% of their lives in an unhealthy state (Olshansky, 2018). Moreover, 35-40% of adults aged 65 and above are obese. Given both aging and obesity are independent risk factors for chronic diseases, it is important to further determine how the confluence of adiposity and aging impacts healthspan and lifespan. The primary health problems associated with elderly individuals are obesity and associated metabolic disorders, including insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease, hypertension, cardiovascular disease, and many types of cancers. These diseases are global public health problems, significantly accelerating the aging process, and severely decreasing the quality of life and overall life expectancy (Jura & Kozak, 2016). Thus, increasing healthspan by prolonging a disease-free period of elderly individuals may be equally important as increasing lifespan. Simple strategies, such as caloric restriction, or pharmacological interventions, such as metformin or rapamycin treatment, can promote both healthspan and lifespan in mice (Bhullar & Hubbard, 2015; Bitto et al, 2016; Martin-Montalvo et al, 2013; Minor et al, 2010). However, the effectiveness of such an approach in humans still awaits confirmation. The

4

65   search for novel and effective strategies to extend these processes is still one of the major

66   goals of geroscience research.

67   Adiponectin was one of the earliest adipokines described (Scherer *et al*, 1995). Since its

68   discovery, significant efforts have been made to study its regulation, biogenesis, and

69   physiological effects. As an excellent biomarker for mature adipocytes, circulating

70   adiponectin levels are inversely correlated with fat mass, distinguishing it from most of

71   the other adipokines, including leptin (Hu *et al*, 1996). Adiponectin exerts pleiotropic

72   effects, including improving glucose tolerance, increasing insulin sensitivity, enhancing

73   lipid clearance, and reducing systemic inflammation and tissue fibrosis (Scherer, 2006).

74   Our previous studies have indicated that a lack of adiponectin in mice leads to glucose

75   intolerance and hyperlipidemia (Nawrocki *et al*, 2006; Xia *et al*, 2018). Conversely,

76   increasing adiponectin levels in an adiponectin transgenic mouse model, greatly improves

77   metabolic homeostasis and produces a metabolically healthy obese phenotype (Combs

78   *et al*, 2004; Kim *et al*, 2007). Similarly, chronic administration of adiponectin ameliorates

79   glucose intolerance and enhances insulin sensitivity in both type 1 and 2 diabetic mice

80   (Berg *et al*, 2001). These observations fully support the favorable effects of adiponectin

81   in promoting metabolic health.

82   Most of the previous published literature focuses on beneficial effects of adiponectin in

83   younger mice or diet-induced obese mice within less than 20 weeks of an HFD challenge.

84   Whether similar beneficial effects could be observed in aging mice (older than 100 weeks)

85   remains unexplored. Beyond its possible role in healthspan, some human genetics

86   studies have implicated adiponectin as a longevity gene (Atzmon *et al*, 2008). One

5

87   potential mechanism of particular interest, with robust effects on elevating circulating

88   adiponectin levels, is the starvation hormone fibroblast growth factor-21 (FGF21). It

89   extends lifespan in both male and female mice (Holland *et al*, 2013). Similarly,

90   thiazolidinediones (TZDs), agonists of the peroxisome proliferator-activated receptor γ

91   (PPARγ), also significantly increase circulating adiponectin levels, and ameliorate aged-

92   related tissue function decline (Viljoen & Sinclair, 2009; Yu *et al*, 2002). In addition, female

93   mice harbor higher circulating adiponectin levels and live longer compared to male mice

94   (Gehrand *et al*, 2016). All these observations point to a positive correlation between high

95   circulating adiponectin and longevity and implicate adiponectin as a novel circulating

96   hormone that may directly promote both healthspan and lifespan in mice. To test this

97   hypothesis, we used our established mouse models of adiponectin overexpression and

98   complete absence of adiponectin and assessed the effect of circulating adiponectin on

99   the aging process. Our results reveal that adiponectin null mice have a significantly

100   reduced healthspan and lifespan, while adiponectin transgenic mice have a significantly

101   prolonged healthspan.

102   **Methods**

103   **Animals experiments**

104   Adiponectin knockout mice (APN-KO) (Nawrocki *et al.*, 2006) and adiponectin transgenic

105   mice (Combs *et al.*, 2004) with wild-type controls are on a pure C57BL6J background. All

106   of the animal experimental protocols have been approved by the Institutional Animal Care

107   and Use Committee of University of Texas Southwestern Medical Center at Dallas. The

108   mice were housed under standard laboratory conditions (12 h on/off; lights on at 7:00

109   a.m.) and temperature-controlled environment with food and water available *ad libitum*.

110   Mice were fed a standard chow-diet (number 5058, LabDiet, St. Louis, MO) or high-fat

111   diet (60% energy from fat, D12492, Research Diets) for various periods as indicated in

112   the Figures. All experiments were initiated at approximately 8 weeks of age, unless

113   indicated otherwise. Mouse phenotyping studies were performed with controls and a

114   minimum of two independent cohorts with more than 5 mice in each group.

115   **Systemic tests**

116   Systemic tests were previously described (Zhao *et al*, 2014; Zhu *et al*, 2017). In brief, oral

117   glucose tolerance tested were performed on overnight fasted mice. The mice orally

118   received 1.25g or 2 g of glucose per kg body weight dissolved in phosphate buffered

119   saline (Cat. 806552, Sigma-Aldrich). Injection volume was calculated based on 10 µl/g

120   body weight. Blood glucose concentrations were measured by glucose meters (Contour)

121   at the indicated time points. For ITTs, mice were fasted for 6 h in the morning, and chow-

122   fed animals were intraperitoneally injected with insulin at a dose of 0.5 U per kg body

123   weight, while HFD-fed animals were injected with a dose of 0.75 U per kg body weight.

7

124   Blood glucose concentrations were measured by glucose meter at the indicated time

125   points; For T.G. clearance, mice were fasted (16 h), then gavaged 15 ul g^{-1} bodyweight

126   of 20% intralipid (Fresenius Kabi Clyton, L.P.). Blood was collected at timed intervals then

127   assayed for T.G. levels (Infinity; Thermo Fisher Scientific) and FFA levels (NEFA-HR);

128   Wako Pure Chemical Industries). For some of the experiments, area under curve (AUC)

129   was calculated.

130   **Blood parameters**

131   Blood was taken from fed animals in the morning and was centrifuged at 8000 g for 5 min,

132   and then the supernatants were collected for multiple analyses. Adiponectin was

133   measured using an ELISA kit from Invitrogen (Catalog number: EZMADP-60K). Serum

134   insulin levels were measured using ALPCO Mouse Insulin ELISA Jumbo kit (Cat. Number:

135   80-INSMS-E10). Mercodia Developing Diagnostic). Serum IGF-1 levels were measured

136   by Mouse/Rat IGF-1 Quantikine ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA).

137   Serum parameters were measured and calculated with a VITROS analyzer (Ortho Clinical

138   Diagnostics) at UTSW metabolic core.

139   **RT-qPCR and Analysis**

140   RNA was extracted from fresh or frozen tissues by homogenization in TRIzol reagent

141   (Invitrogen) as previously described (Zhu *et al*, 2016). We used 1 µg RNA to transcribe

142   cDNA with a reverse transcription kit (Bio-Rad). Most of RT-qPCR primers were from the

143   Harvard Primer Bank (https://pga.mgh.harvard.edu/primerbank/). The relative expression

144   levels were calculated using the comparative threshold cycle method, normalized to the

145   housekeeping gene *Gapdh*.

146   **Histological Analyses**

147   For all histological analyses, four sections from at least three mice per group were stained

148   and the examiner, typically a pathologist, was blinded to the genotype and/or treatment

149   condition, as previously described (Zhao *et al*, 2020). In brief, for immunohistochemistry

150   (IHC), tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5

151   μm) were deparaffinized, heat retrieved (buffer with 10 mM Tris, 1.0 mM EDTA, PH=8.0,

152   94–96 °C for 30min, cool naturally), perforated (0.2% Triton × 100, 10 min), blocked in 3%

153   BSA (Sigma, A9418) and then incubated with Mac2 (1:500 dilution, Cat#: 125401,

154   BioLegend) primary antibodies. IHC and Hematoxylin (Vector, H3401) and Eosin Y

155   (Thermo, 6766007) staining (HE staining) were performed using standard protocols or

156   under the manufacturer's instructions. Detection of IHC signal was performed with

157   Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and DAB substrate kit for

158   peroxidase (Vector Laboratories) followed by hematoxylin counterstaining (Vector

159   Laboratories). For immunofluorescence of perilipin (1:500 dilution NB100-60554,

160   Novus),Mac2, insulin (1:500, Dako #A0564) and glucagon (1:500, Invitrogen #18-0064),

161   after incubation with primary antibody, slides were washed and incubated with Secondary

162   antibodies (1:250 dilution) used were Alexa Fluor 488 or 594 donkey anti-rabbit IgG

163   (HCL) ,Alexa Fluor 488 or 594 donkey anti-goat IgG (HCL) (Invitrogen) or Alexa Fluor

164   488 or 594 donkey anti- guinea pig IgG (HCL)at room temperature for 1 hour, then

9

165   washed and sealed with Prolong Gold antifade reagent with DAPI (Life technology

166   P36941).

167   **Metabolic Cage Experiments**

168   Metabolic cage studies were conducted using a PhenoMaster System (TSE systems) at

169   USTW Metabolic Phenotyping Core as previously described (Zhao *et al*, 2019) . Mice

170   were acclimated in temporary holding cages for 5 days before recording. Food intake,

171   movement, and CO_2 and O_2 levels were measured at various intervals (determined by

172   collectively how many cages were running concurrently) for the indicated period shown

173   on Figures.

174   **Statistics**

175   All values are expressed as the mean ± SEM. The significance between the mean

176   values for each study was evaluated by Student t tests for comparisons of two groups.

177   One way or two-way ANOVA was used for comparisons of more than two groups. The

178   box-and-whisker analysis was performed to exclude potential outliner data accordingly.

179   *P* ≤ 0.05 is regarded as statistically significant. For lifespan analysis, data were calculated

180   using the GraphPad Prism 7 and OASIS 2 software. Log-rank (Mantel–Cox) tests were

181   used to analyze Kaplan–Meier curves.

182

183

184

185

186

Results

Altered adiponectin levels in adiponectin null (APN-KO**) and adiponectin overexpressing transgenic mice (ΔGly)**

Male adiponectin null mice (APN-KO) (Nawrocki *et al.*, 2006) and adiponectin transgenic (ΔGly) mice (Combs *et al.*, 2004) were used for this study. The initial number of mice for each group in the study and a detailed scheme of the phenotypic assessments performed is outlined in (**Fig. S1A-B**). APN-KO were challenged with chow (NCD) or high-fat diet (HFD). ΔGly mice were challenged with chow diet (NCD). Consistent with expectations, serum adiponectin was absent in APN-KO mice (**Fig. S1C**). For ΔGly transgenic mice, circulating adiponectin levels were increased by 50% (**Fig. S1C**). All these observations indicate that our loss and gain of function mouse models indeed alter circulating adiponectin levels effectively as expected.

Deletion of adiponectin in aged mice shortens lifespan on HFD.

Given that the loss of adiponectin leads to impaired glucose tolerance and lipid clearance, we wanted to test whether these mice have a shortened lifespan. A cohort of APN-KO and WT mice was used to measure the lifespan. The survival curves for APN-KO reveal a statistically significant shortened lifespan compared to WT control both in the chow diet cohort (**Fig. 1A**) and in the HFD cohort (**Fig. 1B**). Thus, loss of adiponectin in mice accelerates the aging process and shortens lifespan.

Loss of adiponectin impairs glucose and lipid homeostasis during aging.

11

207   Glucose intolerance is a hallmark of the aging process (DeFronzo, 1981). Compared to

208   WT mice, APN-KO mice did not show any striking difference in body weight at middle-

209   and advanced-aged, both on chow diet and on HFD (**Fig. 2A-B**). We examined glucose

210   homeostasis in aged mice (100 weeks for with the HFD cohort and 140 weeks for the

211   chow diet cohort). In accordance with previous metabolic studies of young adiponectin

212   null mice, differences in glucose tolerance were marginal in mice fed standard chow diet

213   (**Fig. 2C**). APN-KO mice fed HFD, in contrast, exhibited significantly higher glucose

214   excursions during an OGTT (**Fig. 2D**) reflecting impaired glucose tolerance. However, no

215   significant difference in plasma insulin level was observed during the OGTT at the

216   different time points (**Fig. S2A**). This indicates that APN-KO mice are more susceptible

217   to diet-induced insulin resistance.

218   To elucidate the effects of adiponectin on lipid metabolism of aged mice, we performed a

219   triglyceride (TG) clearance test by gavaging the WT and APN-KO mice with 20% intralipid.

220   Triacylglycerol levels in both NCD and HFD-fed APN-KO mice peaked at higher levels

221   and showed a slower clearance of lipids from plasma (**Fig. 2E-F**). This highlights a

222   prevailing impaired lipid clearance in APN-KO mice. Furthermore, although APN-KO and

223   WT mice consume comparable amounts of diet (**Fig. 2G**), indirect calorimetry studies

224   show that APN-KO mice had a significantly higher respiratory exchange ratio (**Fig. 2H**),

225   indicative of carbohydrate being a more predominant fuel source in the absence of

226   adiponectin. Combined, these results suggest adiponectin is necessary to maintain

227   proper lipid homeostasis. Lack of adiponectin prompts glucose metabolism to be more

228   prevalent.

229   **Deletion of adiponectin in aged mice exacerbates tissue functional decline.**

230   The aging process is associated with gradual decline and deterioration of functional

231   properties at the tissue level. In aging adipose tissue, this is manifest as expansion of B

232   cells in fat-associated lymphoid clusters(Camell *et al*, 2019), enrichment of senescent-

233   like pro-inflammatory macrophages and loss of tissue protective macrophage subsets

234   that drive inflammaging and compromises glucose and lipid metabolism(Camell *et al*,

235   2017; Lumeng *et al*, 2011; Tchkonia *et al*, 2010). In the liver and kidney, dysfunction is

236   usually apparent as overexpression of extracellular matrix (ECM) protein constituents,

237   such as collagen and the resulting increased fibrosis (Kim *et al*, 2016). We examined

238   whether the deletion of APN will affect the function of these major organs. We collected

239   adipose tissue, kidney, and liver from separate aging cohorts of young (20 weeks) and

240   old (100 weeks for HFD cohort and 140weeks for chow diet cohort) mice. Compared to

241   WT mice, APN-KO mice did not show significant morphological differences in adipocytes

242   in both young and aged mice. However, the epididymal fat of APN-KO mice fed either

243   HFD or chow diet show increased pro-inflammatory-like macrophages in the aged mice,

244   as demonstrated by a prominent signal for the macrophage marker Mac2 (**Fig. 3A-B**).

245   This demonstrates that the loss of adiponectin accelerates adipose tissue inflammation,

246   a characteristic marker of the increased aging process. We do not know whether these

247   macrophages originate from bone marrow-derived monocytes that infiltrate the tissue or

248   whether the lack of adiponectin enhances differentiation of proliferating tissue resident

249   monocytes into macrophages.

250   We also examined the age-related decline of health parameters in two other vital organs,

251   kidney and liver. Even during normal aging, the kidney develops age-related structural

252   changes and displays functional declines, including nephrosclerosis, loss of renal mass

13

253   or compensatory hypertrophy of the remaining nephrons, with a corresponding decrease

254   in glomerular filtration rate (GFR) and renal blood flow RBF (Weinstein & Anderson, 2010).

255   Clinical studies have demonstrated that adiponectin is elevated in patients with chronic

256   kidney disease, suggesting a possible compensatory upregulation to alleviate further

257   renal injury (Christou & Kiortsis, 2014). Morphologically, APN-KO mice fed either the HFD

258   or the chow diet show more severe interstitial and periglomerular fibrosis. Compared to

259   aged WT mice, the glomeruli in aged APN-KO mice have collapsed tufts, accompanied

260   by hypertrophic Bowman's capsules (**Fig. 3C**). Meanwhile, aged APN-KO mice exhibited

261   a significant increase in kidney weight as compared with aged WT mice (**Fig. S2C**). To

262   determine the cause of this severe glomerular and tubulointerstitial damage in APN-KO

263   mice, we investigated the glomerular infiltration with macrophages. Immunohistochemical

264   staining with Mac2 antibodies reveals a significant increase in Mac-2 positive

265   intraglomerular signal in the old mice which is vastly more abundant in the APN-KO mice

266   fed the HFD (**Fig. 3D**).

267   Aging increases the susceptibility of various liver diseases as well, responsible for a

268   deteriorated quality of life in the elderly and increasing mortality rate. Several studies

269   suggest that hypoadiponectinemia predicts liver fibrosis and accelerates hepatic tumor

270   formation (Park *et al*, 2015). Thus, we explored whether the lack of adiponectin may

271   exacerbate age-induced dysfunction and dysmorphology of the liver. Unlike other diet-

272   induced obese mouse models, we did not find any enhanced lipid droplet accumulation

273   in the livers of APN-KO mice compared to WT mice upon short term and long-term

274   exposure to HFD treatment (**Fig. 3E**). However, we found many inflammatory infiltrates

275   in the livers of APN-KO mice on HFD diet. The expression of inflammatory markers is

14

276 significantly increased in aged APN-KO mice fed on HFD and chow diet (**Fig. 3G, Fig.**

277 **S2B**), indicative of increased inflammation in the liver. Moreover, Trichrome staining

278 highlighting the ECM reveals increased hepatic fibrosis in old APN-KO mice on the chow

279 diet that was even more evident under HFD conditions (**Fig. 3F**). Mirroring these

280 histological findings, the expression levels of liver fibrosis markers, such as Col1α1 and

281 αSMA, are strikingly increased in older HFD and chow diet fed APN-KO mice (**Fig. 3G,**

282 **Fig. S2B**). Liver damage was further confirmed by elevated serum AST and ALT levels

283 in HFD fed APN-KO mice compared with control mice (**Fig. 3H**). All of these observations

284 support that adiponectin plays an essential role in maintaining normal liver function during

285 the aging process.

286 Upon comparing young WT vs APN-KO mice (20 weeks) that were exposed for 8 weeks

287 to HFD, no genotype-specific differences were observed in the kidney and the liver. This

288 therefore indicates that the pathological changes in older APN-KO mice genuinely reflect

289 age-related chronic changes rather than simple developmental differences that would be

290 apparent in the young mice as well. These findings clearly indicate that the lack of

291 adiponectin during aging exacerbates liver and renal damage, at least in part through

292 proinflammatory mechanisms.

293 **Increasing adiponectin protects mice from aged induced metabolic dysfunction**

294 Clinically, adiponectin levels are significantly higher in centenarians and in some of their

295 offspring, suggesting that adiponectin may be a key driver to promote healthspan and

296 lifespan. As the elimination of adiponectin shortens healthspan and lifespan, we

297 wondered whether increasing adiponectin by our previously established transgenic

298 mouse model (that we refer to as the "ΔGly mouse") could promote both healthspan and

299   lifespan. A large cohort of WT and ΔGly mice were placed on chow diet to assess their

300   lifespan. After calculation, a median lifespan in Control mice was around 117 weeks, while

301   this value in ΔGly mouse has been extended to 128 weeks (9% extension), indicating that

302   increasing circulating adiponectin prolongs median lifespan. However, the maximum

303   lifespan is comparable in Control and ΔGly mice, as the overall survival curves were not

304   different by log rank test (**Fig. 4A**).

305   Besides its positive effects in prolonging median lifespan, we determined if increasing

306   adiponectin levels may have beneficial effects in extending healthspan. Previous studies

307   indicated that increasing adiponectin levels results in improved glucose and lipid profiles

308   in younger mice (Berg *et al.*, 2001). However, whether these beneficial effects of

309   adiponectin carry to older age has not been assessed. When fed with a chow diet, ΔGly

310   mice show a similar body weight during lifespan, compared to littermate controls (**Fig.**

311   **4B**). Then we measured fasting glycemia, insulin, and IGF-1(**Fig. 4C**). Under 16hr fasted

312   conditions, ΔGly mice have a significantly lower fasting glycemia, accompanied by a

313   robust reduction in plasma insulin. Moreover, a reduction in circulating IGF-1 levels is

314   observed in ΔGly mice. Lower IGF-1 levels are thought to play a key role as a mediator

315   of health- and lifespan extension (Bartke *et al*, 2003). To test whether the improvements

316   in systemic insulin sensitivity are also associated with improvements at the level of the

317   pancreatic β cell, we performed H&E staining on pancreatic sections. Consistent with the

318   reduced demand on islets to produce and release insulin in a more insulin-sensitive

319   environment, the average islet size was considerably reduced by adiponectin

320   overexpression,   with   islet   structural   integrity   fully   preserved   (**Fig.   4D**).

321   Immunohistochemical analysis of islets exhibits a normal composition with α cells

16

322   (glucagon) and β cells (insulin) in ΔGly mice. During an oral glucose tolerance test, ΔGly

323   mice displayed a much lower glucose excursion than littermates (**Fig. 4E**). In addition,

324   insulin levels in ΔGly mice were significantly lower in response to the glucose challenge,

325   which further supports improved insulin sensitivity (**Fig. 4F**). To confirm this, we

326   performed insulin tolerance tests. ΔGly mice show a significant increase in insulin

327   sensitivity (**Fig. 4G**), which is consistent with our results for the young mice. Moreover,

328   when orally challenged with triglycerides, ΔGly mice display enhanced lipid clearance (**Fig.**

329   **4H-I**), with correspondingly lower FFA values (**Fig. 4J**). These data demonstrate that

330   increasing adiponectin levels significantly promotes metabolic fitness in aged mice.

331   **Increasing adiponectin levels improves the age-related functional decline in**

332   **tissues of aged mice.**

333   To probe tissue functional declines that might contribute to metabolic syndrome in the

334   elderly, we evaluated the function of fat and liver in aged mice. Aging is associated with

335   a redistribution of fat from the periphery to central fat deposition(Kuk *et al*, 2009). The

336   redistribution and ectopic fat deposition with aging appear to accelerate onset of multiple

337   age-related diseases. A histological examination of adipose tissue showed that ΔGly mice

338   harbor much smaller adipocytes in subcutaneous and gonadal fat (**Fig. 5A**) compared to

339   controls at the age of 140 weeks. In agreement with the epididymal adipocyte size and

340   fat mass, inflammation is potently suppressed in visceral fat tissues of ΔGly mice, as

341   demonstrated by a significantly reduced Mac-2 staining (**Fig. 5B**). Moreover, it was quite

342   apparent that visceral fat pad weight was reduced in ΔGly mice with a slightly increase in

343   subcutaneous adipose tissue (**Fig. 5C**). Aged WT mice revealed an unclear boundary in

344   the hepatic lobule with lose cellular cytoplasm, while ΔGly mice entirely prevented lipid

17

345 droplet accumulation and age-related deterioration of the morphology of the liver (**Fig.**

346 **5D**). Furthermore, gene expression of inflammation and fibrosis markers in the livers were

347 dramatically reduced in ΔGly mice compared with their littermates (**Fig. 5E**). Combined,

348 these findings strongly support that adiponectin promotes metabolic fitness, by

349 maintaining a proper fat distribution, and reducing adipose tissue inflammation, along with

350 reducing inflammation and fibrosis in liver.

Discussion

Based on data from clinical correlations as well as ample preclinical results, we appreciate that elevated levels of adiponectin are generally associated with an improved overall metabolic phenotype. Here, we systematically assessed the impact of adiponectin in the context of aging. Using adiponectin-null and adiponectin overexpressing mouse models, we have made the following observations:1) The lack of adiponectin in mice curtails healthspan by impairing glucose and lipid homeostasis, and accelerating fibrogenesis in multiple tissues, resulting in reduced healthspan; 2) The lack of adiponectin in mice shortens lifespan both on chow and HFD. 3) Increasing adiponectin levels in aged adiponectin overexpressing mice produces a healthy metabolic phenotype, with greatly increased glucose tolerance and insulin sensitivity, enhanced lipid clearance, lowered visceral fat and potent protection from inflammation and fibrosis; 4) Adiponectin overexpressing mice on a chow diet show a 9% increase in median lifespan. All these observations support that adiponectin is a vastly underestimated player in healthspan and lifespan.

With the extension of life expectancy, larger segments of the elderly population suffer from various chronic diseases. The normal aging process is associated with chronic inflammation and thereby increases susceptibility to these chronic morbidities (Goldberg & Dixit, 2015). Indeed, we observed an exacerbated pro-inflammatory state in aged WT mice compared with younger WT animals. In order to combat these age-related inflammatory changes, we need effective anti-inflammatory interventions. But these interventions should not negatively impact desired innate and adaptive immune responses. Circulating adiponectin levels are negatively correlated with inflammatory

19

374 markers in diabetic patients (Krakoff *et al*, 2003; Mantzoros *et al*, 2005) and in non-

375 diabetic subjects (Choi *et al*, 2007). Among different adipokines, adiponectin is

376 recognized as a major adipokine regulating inflammation in a number of cell types. Our

377 previous studies indicated acute APN depletion leads to an upregulation of inflammatory

378 genes (Xia *et al.*, 2018). Adipose tissues are also susceptible to fibrosis. Chronic

379 inflammation frequently results in fibrosis, which leads to functional declines in tissues.

380 During normal aging, fibrosis occurs in small steps. Due to gradual deposition of collagens,

381 organs become rigid and dysfunctional. Eventually, this causes the health to decline. We

382 found indeed that aged WT mice develop more severe fibrosis compared to young WT

383 mice.

384 Adiponectin has potent anti-fibrotic effects in the liver by activating peroxisome

385 proliferator-activated receptor-gamma pathways (Shafiei *et al*, 2011), which in turn

386 diminishes the expression of pro-fibrotic genes. Despite having reduced levels of

387 triglyceride accumulation in the liver, the chronic lack of adiponectin dramatically

388 exacerbates age-related liver fibrosis in parallel with disruption of liver function. In contrast,

389 ΔGly mice are completely protected from diet- and aging- induced steatohepatitis and

390 fibrosis, indicating of a crucial role of adiponectin in regulating liver inflammatory reactions

391 and fibrosis. Hence, the anti-inflammatory impact and potent anti-fibrotic actions of

392 adiponectin make it a potent novel regulator enhancing health span.

393

394 Obesity-associated chronic inflammation and insulin resistance are regarded as a pivotal

395 risk factors for the development of several age-related pathological sequelae (Huffman &

396 Barzilai, 2009; Kanneganti & Dixit, 2012).Improved metabolic homeostasis is positively

20

397   associated with lifespan in humans and mice. Prolongevity intervention, caloric restriction

398   and long-lived Ames dwarf mice have increased adiponectin expression (Hill *et al*, 2016).

399   We found adiponectin mimics to some extent the impact of caloric restriction on reduction

400   in inflammation and improved metabolic homeostasis. Reduction in adiposity is

401   considered to be a hallmark of caloric restriction, which is an important component of its

402   beneficial effects on metabolism. After long-term caloric restriction, aged mice display

403   lower adiposity, smaller adipocytes and improved insulin sensitivity (Miller *et al*, 2017).

404   Strikingly, increases in adiponectin expression are detected in these smaller adipocytes

405   after caloric restriction. Adipocyte hypertrophy is associated with cellular stress and

406   obesity-associated metabolic complications. Due to the limited capacity to expand

407   subcutaneous adipose tissue in aged populations, adipocyte hypertrophy also occurs in

408   visceral fat, which is associated with lipid spillover in multiple tissues in aging and ectopic

409   fat accumulation(Tchkonia *et al*, 2013). Hypertrophic adipocytes and impaired

410   redistribution of lipids exert a negative impact on insulin responsiveness, contributing to

411   many metabolic diseases frequently observed in the elderly. Thus, metabolic disorders

412   frequently go hand in hand with aging. Clinical studies have identified an strong inverse

413   relationship between circulating adiponectin and insulin resistance in obese individuals

414   (Turer *et al*, 2011). Our previous data suggested that adiponectin strongly suppresses

415   hepatic gluconeogenesis and enhances fatty acid oxidation, thereby strongly contributing

416   to an overall beneficial metabolic regulation (Wang & Scherer, 2016). Our aged

417   adiponectin transgenic mice still have dramatically improved insulin sensitivity in parallel

418   with reduced plasma insulin. All of this happens primarily due to an increase in adipocyte

419   numbers in subcutaneous fat of ΔGly mice. In the absence of the protective effects of

420 adiponectin, aged APN-KO mice exacerbated diet-and aging-induced glucose intolerance

421 and lipid disorders. Moreover, ΔGly mice show improved insulin sensitivity in parallel with

422 lower insulin and IGF-1 levels, and higher IGF-1 in APN-KO mice. Attenuated activation

423 of the growth hormone –insulin-like growth factor I (IGF-I) axis is also an integral

424 component of the beneficial effects of caloric restriction leading to prolonged healthspan

425 and lifespan in rodents. Interestingly, elevated adiponectin is detected in all long-live mice:

426 This includes the adipocyte-specific insulin receptor knockout mice (FIRKO), the Ames

427 dwarfs (df/df) and GHRKO mice (Blüher *et al*, 2002; Masternak *et al*, 2012; Wang *et al*,

428 2006). These similarities across all these models suggest that an increase in adiponectin

429 levels may be the common denominator driving longevity in all these models.

430

431 Combined, our studies and along with previous reports, demonstrate that adipose tissue

432 plays a vital role in the aging process. In aging, dysfunctional fat tissue leads to ectopic

433 fat deposition, lipodystrophic adipocytes, and subcutaneous fat loss, thereby contributing

434 to increased systemic inflammation, metabolic disturbances, and functional declines in

435 other organs. However, healthy fat pads have characteristic features not only in terms of

436 the quantity, but more importantly, by the quality of adipose tissue (Kusminski *et al*, 2012).

437 Adiponectin is a key player maintaining glucose and lipid homeostasis on the basis of its

438 lipid storing capacity and its ability to communicate with other organs. Thus,

439 overexpressing adiponectin results in the healthy expansion of subcutaneous adipose

440 tissue, a reduction of visceral fat and improvement of inflammation and fibrosis in the liver,

441 all of which greatly alleviates metabolic disturbances and protects against tissue

442 functional decline during the aging process. In contrast, adiponectin deficiency increases

22

443   susceptibility to metabolic diseases in the elderly. Impaired glucose tolerance and lipid

444   clearance, severe inflammation accompanied by dysfunctional liver and kidney all reduce

445   the quality of life and lifespan in the elderly. Thus, the ability to prolong health span by

446   maintaining adiponectin levels provides a promising therapy for aged- related disorders

447   and improving quality of life in older individuals.

448

449   **Study approval**

450   The Institutional Animal Care and Use Committee of the University of Texas

451   Southwestern Medical Center approved all animal experiments (APN:2015-101207G).

452

453   **Author contributions**

454   NL performed most of the experiments. ZZ, SZ, YAA, YZ, LV and MW conducted some

455   of the experiments. ZZ, YD and TO helped with the breeding of mouse models. RG

456   performed the metabolite measurement experiment. QZ, RKG, ML and VDD gave useful

457   suggestions to this study. NL and PES wrote the manuscript. SZ, YZ, CG and VDD

458   revised the manuscript. PES, TLH and VDD were involved in experimental design,

459   experiments, data analysis, and the interpretation of data.

460

466   **Competing interests**: None of the authors declare any conflicts of interest.

467

468

References:

Atzmon G, Pollin TI, Crandall J, Tanner K, Schechter CB, Scherer PE, Rincon M, Siegel G, Katz M, Lipton RB *et al* (2008) Adiponectin levels and genotype: a potential regulator of life span in humans. *J Gerontol A Biol Sci Med Sci* 63: 447-453

Bartke A, Chandrashekar V, Dominici F, Turyn D, Kinney B, Steger R, Kopchick JJ (2003) Insulin-like growth factor 1 (IGF-1) and aging: controversies and new insights. *Biogerontology* 4: 1-8

Berg AH, Combs TP, Du X, Brownlee M, Scherer PE (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7: 947-953

Bhullar KS, Hubbard BP (2015) Lifespan and healthspan extension by resveratrol. *Biochim Biophys Acta* 1852: 1209-1218

Bitto A, Ito TK, Pineda VV, LeTexier NJ, Huang HZ, Sutlief E, Tung H, Vizzini N, Chen B, Smith K *et al* (2016) Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. *Elife* 5

Blüher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, Kahn CR (2002) Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev Cell* 3: 25-38

Camell CD, Günther P, Lee A, Goldberg EL, Spadaro O, Youm YH, Bartke A, Hubbard GB, Ikeno Y, Ruddle NH *et al* (2019) Aging Induces an Nlrp3 Inflammasome-Dependent Expansion of Adipose B Cells That Impairs Metabolic Homeostasis. *Cell metabolism* 30: 1024-1039.e1026

490   Camell CD, Sander J, Spadaro O, Lee A, Nguyen KY, Wing A, Goldberg EL, Youm YH,

491   Brown CW, Elsworth J *et al* (2017) Inflammasome-driven catecholamine catabolism

492   in macrophages blunts lipolysis during ageing. *Nature* 550: 119-123

493   Choi KM, Ryu OH, Lee KW, Kim HY, Seo JA, Kim SG, Kim NH, Choi DS, Baik SH (2007)

494   Serum adiponectin, interleukin-10 levels and inflammatory markers in the metabolic

495   syndrome. *Diabetes Res Clin Pract* 75: 235-240

496   Christou GA, Kiortsis DN (2014) The role of adiponectin in renal physiology and

497   development of albuminuria. *J Endocrinol* 221: R49-61

498   Combs TP, Pajvani UB, Berg AH, Lin Y, Jelicks LA, Laplante M, Nawrocki AR, Rajala

499   MW, Parlow AF, Cheeseboro L *et al* (2004) A transgenic mouse with a deletion in the

500   collagenous domain of adiponectin displays elevated circulating adiponectin and

501   improved insulin sensitivity. *Endocrinology* 145: 367-383

502   Crimmins EM (2015) Lifespan and Healthspan: Past, Present, and Promise.

503   *Gerontologist* 55: 901-911

504   DeFronzo RA (1981) Glucose Intolerance and Aging. *Diabetes Care* 4: 493-501

505   Gehrand AL, Hoeynck B, Jablonski M, Leonovicz C, Ye R, Scherer PE, Raff H (2016) Sex

506   differences in adult rat insulin and glucose responses to arginine: programming effects

507   of neonatal separation, hypoxia, and hypothermia. *Physiol Rep* 4

508   Goldberg EL, Dixit VD (2015) Drivers of age-related inflammation and strategies for

509   healthspan extension. *Immunological reviews* 265: 63-74

510   Hill CM, Fang Y, Miquet JG, Sun LY, Masternak MM, Bartke A (2016) Long-lived

511   hypopituitary Ames dwarf mice are resistant to the detrimental effects of high-fat diet

512   on metabolic function and energy expenditure. *Aging cell* 15: 509-521

513 Holland WL, Adams AC, Brozinick JT, Bui HH, Miyauchi Y, Kusminski CM, Bauer SM,

514   Wade M, Singhal E, Cheng CC *et al* (2013) An FGF21-adiponectin-ceramide axis

515   controls energy expenditure and insulin action in mice. *Cell Metab* 17: 790-797

516 Hu E, Liang P, Spiegelman BM (1996) AdipoQ is a novel adipose-specific gene

517   dysregulated in obesity. *J Biol Chem* 271: 10697-10703

518 Huffman DM, Barzilai N (2009) Role of visceral adipose tissue in aging. *Biochimica et*

519   *biophysica acta* 1790: 1117-1123

520 Jura M, Kozak LP (2016) Obesity and related consequences to ageing. *Age (Dordr)* 38:

521   23-23

522 Kanneganti TD, Dixit VD (2012) Immunological complications of obesity. *Nature*

523   *immunology* 13: 707-712

524 Kim IH, Xu J, Liu X, Koyama Y, Ma HY, Diggle K, You YH, Schilling JM, Jeste D, Sharma

525   K *et al* (2016) Aging increases the susceptibility of hepatic inflammation, liver fibrosis

526   and aging in response to high-fat diet in mice. *Age (Dordr)* 38: 291-302

527 Kim J-Y, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T,

528   Durand JL, Li H, Li G *et al* (2007) Obesity-associated improvements in metabolic

529   profile through expansion of adipose tissue. *The Journal of Clinical Investigation* 117:

530   2621-2637

531 Krakoff J, Funahashi T, Stehouwer CD, Schalkwijk CG, Tanaka S, Matsuzawa Y, Kobes

532   S, Tataranni PA, Hanson RL, Knowler WC *et al* (2003) Inflammatory markers,

533   adiponectin, and risk of type 2 diabetes in the Pima Indian. *Diabetes Care* 26: 1745-

534   1751

27

535 Kuk JL, Saunders TJ, Davidson LE, Ross R (2009) Age-related changes in total and

536   regional fat distribution. *Ageing research reviews* 8: 339-348

537 Kusminski CM, Holland WL, Sun K, Park J, Spurgin SB, Lin Y, Askew GR, Simcox JA,

538   McClain DA, Li C *et al* (2012) MitoNEET-driven alterations in adipocyte mitochondrial

539   activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity.

540   *Nature medicine* 18: 1539-1549

541 Lumeng CN, Liu J, Geletka L, Delaney C, Delproposto J, Desai A, Oatmen K, Martinez-

542   Santibanez G, Julius A, Garg S *et al* (2011) Aging is associated with an increase in T

543   cells and inflammatory macrophages in visceral adipose tissue. *Journal of*

544   *immunology (Baltimore, Md : 1950)* 187: 6208-6216

545 Mantzoros CS, Li T, Manson JE, Meigs JB, Hu FB (2005) Circulating adiponectin levels

546   are associated with better glycemic control, more favorable lipid profile, and reduced

547   inflammation in women with type 2 diabetes. *J Clin Endocrinol Metab* 90: 4542-4548

548 Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen

549   M, Gomes AP, Ward TM, Minor RK, Blouin MJ *et al* (2013) Metformin improves

550   healthspan and lifespan in mice. *Nat Commun* 4: 2192

551 Masternak MM, Bartke A, Wang F, Spong A, Gesing A, Fang Y, Salmon AB, Hughes LF,

552   Liberati T, Boparai R *et al* (2012) Metabolic effects of intra-abdominal fat in GHRKO

553   mice. *Aging Cell* 11: 73-81

554 Miller KN, Burhans MS, Clark JP, Howell PR, Polewski MA, DeMuth TM, Eliceiri KW,

555   Lindstrom MJ, Ntambi JM, Anderson RM (2017) Aging and caloric restriction impact

556   adipose tissue, adiponectin, and circulating lipids. *Aging Cell* 16: 497-507

28

557   Minor RK, Allard JS, Younts CM, Ward TM, de Cabo R (2010) Dietary interventions to

558   extend life span and health span based on calorie restriction. *J Gerontol A Biol Sci*

559   *Med Sci* 65: 695-703

560   Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, Pang Z, Chen

561   AS, Ruderman NB, Chen H *et al* (2006) Mice lacking adiponectin show decreased

562   hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-

563   activated receptor gamma agonists. *J Biol Chem* 281: 2654-2660

564   Olshansky SJ (2018) From Lifespan to Healthspan. *JAMA* 320: 1323-1324

565   Park P-H, Sanz-Garcia C, Nagy LE (2015) Adiponectin as an anti-fibrotic and anti-

566   inflammatory adipokine in the liver. *Curr Pathobiol Rep* 3: 243-252

567   Piskovatska V, Strilbytska O, Koliada A, Vaiserman A, Lushchak O (2019) Health Benefits

568   of Anti-aging Drugs. *Subcell Biochem* 91: 339-392

569   Scherer PE (2006) Adipose tissue: from lipid storage compartment to endocrine organ.

570   *Diabetes* 55: 1537-1545

571   Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF (1995) A novel serum protein

572   similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270: 26746-26749

573   Shafiei MS, Shetty S, Scherer PE, Rockey DC (2011) Adiponectin regulation of stellate

574   cell activation via PPARγ-dependent and -independent mechanisms. *Am J Pathol* 178:

575   2690-2699

576   Tchkonia T, Morbeck DE, Von Zglinicki T, Van Deursen J, Lustgarten J, Scrable H, Khosla

577   S, Jensen MD, Kirkland JL (2010) Fat tissue, aging, and cellular senescence. *Aging*

578   *Cell* 9: 667-684

579 Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, Kirkland JL

580 (2013) Mechanisms and metabolic implications of regional differences among fat

581 depots. *Cell metabolism* 17: 644-656

582 Turer AT, Khera A, Ayers CR, Turer CB, Grundy SM, Vega GL, Scherer PE (2011)

583 Adipose tissue mass and location affect circulating adiponectin levels. *Diabetologia*

584 54: 2515-2524

585 Viljoen A, Sinclair A (2009) Safety and efficacy of rosiglitazone in the elderly diabetic

586 patient. *Vasc Health Risk Manag* 5: 389-395

587 Wang Z, Al-Regaiey KA, Masternak MM, Bartke A (2006) Adipocytokines and lipid levels

588 in Ames dwarf and calorie-restricted mice. *J Gerontol A Biol Sci Med Sci* 61: 323-331

589 Wang ZV, Scherer PE (2016) Adiponectin, the past two decades. *Journal of molecular*

590 *cell biology* 8: 93-100

591 Weinstein JR, Anderson S (2010) The aging kidney: physiological changes. *Adv Chronic*

592 *Kidney Dis* 17: 302-307

593 Xia JY, Sun K, Hepler C, Ghaben AL, Gupta RK, An YA, Holland WL, Morley TS, Adams

594 AC, Gordillo R *et al* (2018) Acute loss of adipose tissue-derived adiponectin triggers

595 immediate metabolic deterioration in mice. *Diabetologia* 61: 932-941

596 Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, Olefsky JM (2002)

597 The Effect of Thiazolidinediones on Plasma Adiponectin Levels in Normal, Obese, and

598 Type 2 Diabetic Subjects. *Diabetes* 51: 2968-2974

599 Zhao S, Li N, Zhu Y, Straub L, Zhang Z, Wang MY, Zhu Q, Kusminski CM, Elmquist JK,

600 Scherer PE (2020) Partial leptin deficiency confers resistance to diet-induced obesity

601 in mice. *Mol Metab*: 100995

602   Zhao S, Mugabo Y, Iglesias J, Xie L, Delghingaro-Augusto V, Lussier R, Peyot ML, Joly

603   E, Taib B, Davis MA *et al* (2014) alpha/beta-Hydrolase domain-6-accessible

604   monoacylglycerol controls glucose-stimulated insulin secretion. *Cell Metab* 19: 993-

605   1007

606   Zhao S, Zhu Y, Schultz RD, Li N, He Z, Zhang Z, Caron A, Zhu Q, Sun K, Xiong W *et al*

607   (2019) Partial Leptin Reduction as an Insulin Sensitization and Weight Loss Strategy.

608   *Cell Metab* 30: 706-719.e706

609   Zhu Y, Gao Y, Tao C, Shao M, Zhao S, Huang W, Yao T, Johnson JA, Liu T, Cypess AM

610   *et al* (2016) Connexin 43 Mediates White Adipose Tissue Beiging by Facilitating the

611   Propagation of Sympathetic Neuronal Signals. *Cell metabolism* 24: 420-433

612   Zhu Y, Zhao S, Deng Y, Gordillo R, Ghaben AL, Shao M, Zhang F, Xu P, Li Y, Cao H *et*

613   *al* (2017) Hepatic GALE Regulates Whole-Body Glucose Homeostasis by Modulating
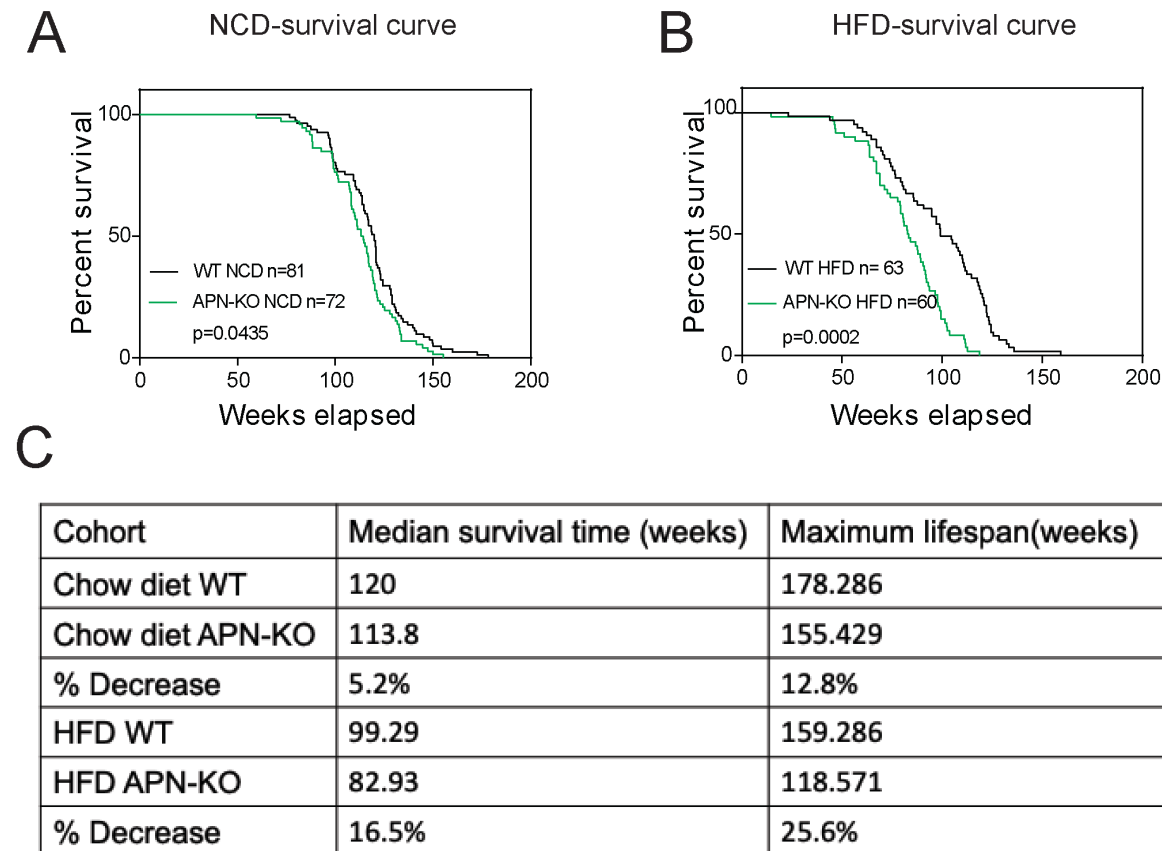
614   Tff3 Expression. *Diabetes* 66: 2789-2799

615

Figures:

Figure 1

A NCD-survival curve

B HFD-survival curve

C

Cohort	Median survival time (weeks)	Maximum lifespan(weeks)
Chow diet WT	120	178.286
Chow diet APN-KO	113.8	155.429
% Decrease	5.2%	12.8%
HFD WT	99.29	159.286
HFD APN-KO	82.93	118.571
% Decrease	16.5%	25.6%

Fig. 1: Lack of APN in aging mice shortens lifespan

A. Kaplan-Meyer survival curves for WT and APN-KO mice on chow diet.

B. Kaplan-Meyer survival curves for WT and APN-KO mice on HFD.

C. Median survival time and maximum lifespan for each cohort. *n* denotes the number of mice per group. *P* values were determined by log-rank (Mantel–Cox) test.

32

Figure 2

Fig. 2: Lack of APN in aging mice attenuates glucose and lipid

33

626 **homeostasis.**

627   A. Body-weights during aging processes for WT and APN-KO mice fed on chow

628   diet.

629   B. Body-weights during aging processes for WT and APN-KO mice fed on HFD.

630   C. An OGTT ($2g\ kg^{-1}$ bodyweight; single gavage) on chow diet-feeding WT and

631   APN-KO mice at 110-week old (n=7 per group).

632   D. An OGTT ($1.25\ g\ kg^{-1}$ bodyweight; single gavage) on HFD-feeding WT and APN-

633   KO mice at 85-week old (n=8 for WT, n=7 for APN-KO mice).

634   E. T.G. clearance test (20% intralipid; $15\ ul\ g^{-1}$ bodyweight; single gavage) in chow

635   diet-feeding WT and APN-KO mice at 110-week old ($n = 9$ for WT, n=10 for APN-

636   KO mice).

637   F. T.G. clearance test (20% intralipid; $15\ ul\ g^{-1}$ bodyweight; single gavage) in HFD-

638   feeding WT and APN-KO mice at 85-week old (n=8 per group) .

639   G. Metabolic cage analyses showing food intake for chow diet-feeding WT in APN-

640   KO mice at 110-week old (n=8 for WT, n=7 for APN-KO mice). Data are mean ±

641   SEM. Student's *t* test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for WT vs *APN-KO.*

642   H. Metabolic cage analyses showing respiratory exchange rate (RER) chow diet-

643   feeding WT and APN-KO mice at 110-week old (n=8 for WT, n=7 for APN-KO

644   mice). Data are mean ± SEM. Student's *t* test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

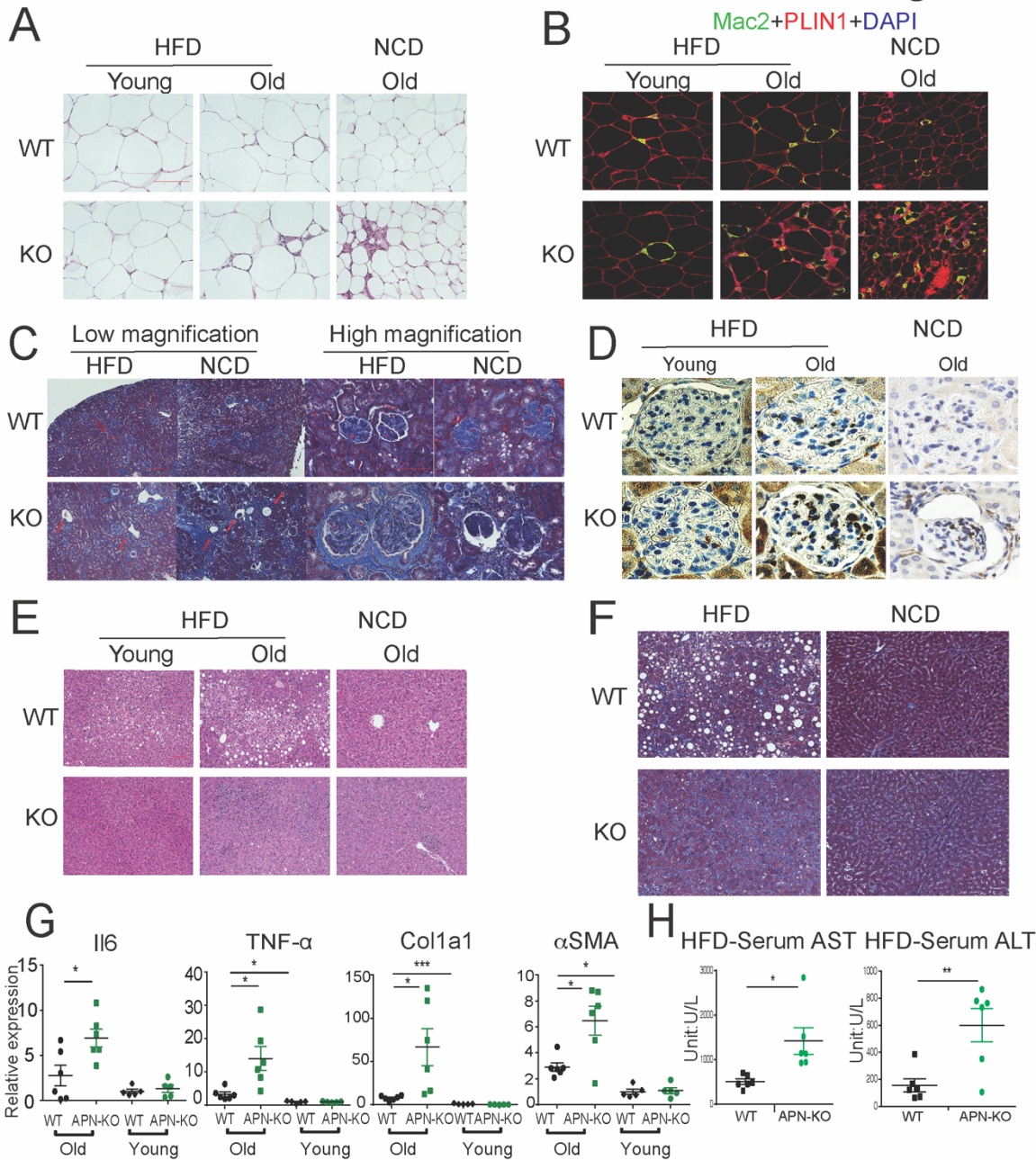645   for WT vs *APN-KO.*

646

Figure 3

Fig. 3: Deletion of APN in aged mice exacerbate functional decline.

A. H&E staining of an Epi fat depot of 20-week old and 100-week old WT and APN-KO mice fed on HFD or 140-week old WT and APN-KO mice on chow diet.

B. Mac2 staining of an Epi fat depot of 20-week old and 100-week old WT and APN-KO mice fed on HFD or 140-week old WT and APN-KO mice on chow diet.

C. Trichrome staining of kidney sections reveals severe interstitial and periglomerular fibrosis in 110-week old APN-KO mice fed on HFD and 140-week old APN-KO mice fed on chow diet. Collapsed tufts are seen inside widened Bowman's capsules forming glomerular cysts (red arrow)

D. Mac2 staining of kidney sections of 20-week old and 100-week old WT and APN-KO mice fed on HFD or chow diet.

E. H&E staining of Liver of 20-week old and 100-week old WT and APN-KO mice fed on HFD, 140-week old WT and APN-KO mice on chow diet. Note the extensive inflammatory cell infiltrates in the liver of the aged APN-KO mice fed on HFD.

F. Trichrome stains of liver sections from 20-week old and 100-week old WT and APN-KO mice fed on HFD or 140-week old WT and APN-KO mice on chow diet, examine liver fibrosis.

G. Expression of inflammatory and fibrosis markers in liver tissues of 20-week old and 100-week old WT and APN-KO mice fed on HFD or chow diet (n=5 per groups of young cohorts, n=6 per groups of aged cohorts).

H. Serum AST and ALT activities in 100-week old WT and APN-KO mice fed on HFD (n=6 per group). Bar, 100µm.Data are mean ± SEM. Student's t test: $*p <$ 0.05, $**p < 0.01$, $***p < 0.001$ for WT vs *APN-KO.*
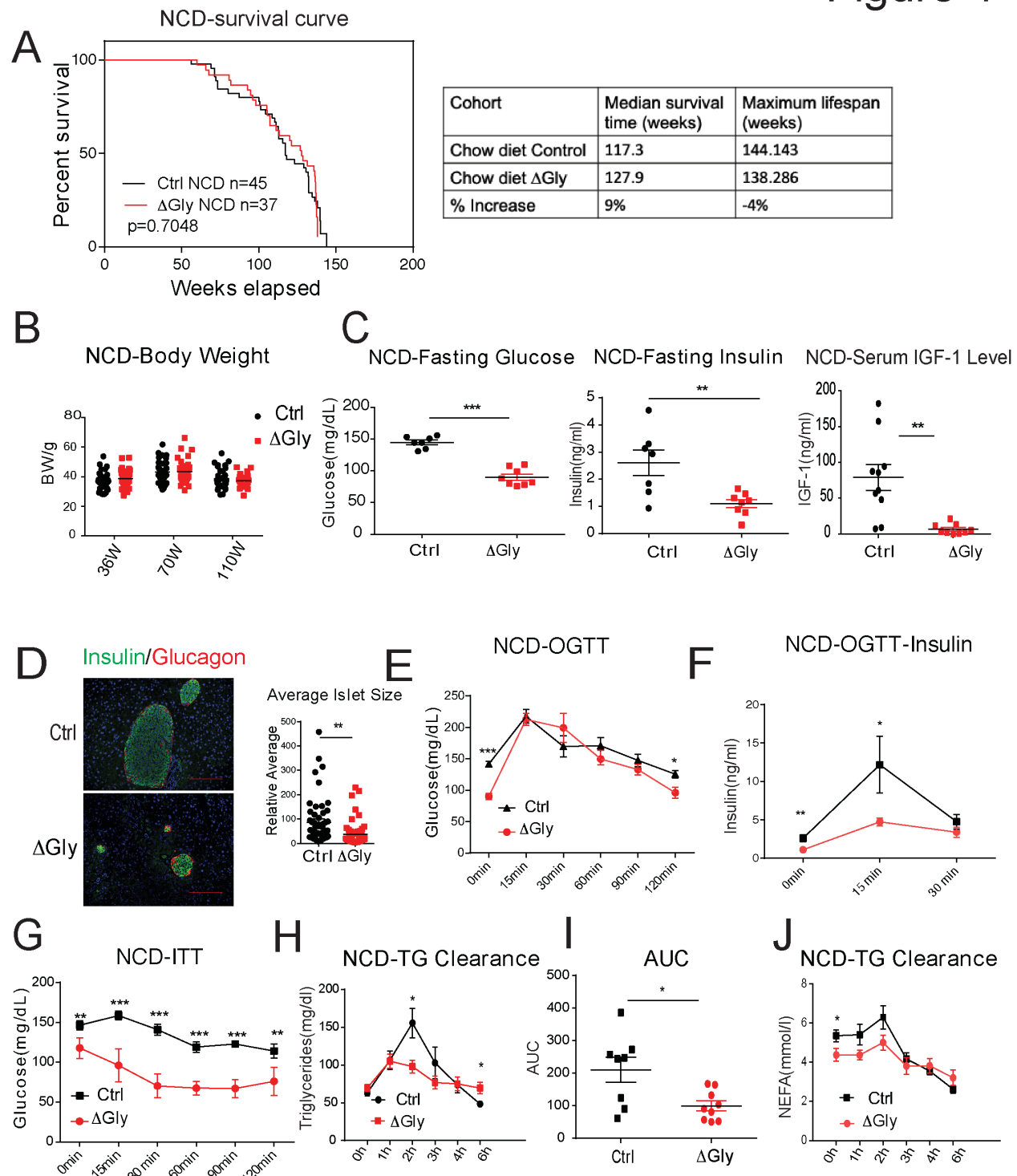
Figure 4

672

Fig. 4: Increasing adiponectin protect against aging- induced metabolic

disturbance.

675 A. Kaplan-Meyer survival curves for WT and ΔGly mice on chow diet. Median

676 survival time and maximum lifespan for each cohort. *n* denotes the number of

677 mice per group. *P* values were determined by log-rank (Mantel–Cox) test.

678 B. Body-weights during aging processes for controls and ΔGly mice fed on chow

679 diet.

680 C. Systemic glucose, insulin and IGF-1 levels in 50-week old controls and ΔGly

681 mice after fasting 16h.

682 D. Insulin and glucagon IF staining of pancreases from controls and ΔGly mice at

683 140-week old (left). Right: Relative average islet size.

684 E. An OGTT (2 g kg^{-1} bodyweight; single gavage) revealed marginally improved

685 glucose tolerance in 50-week ΔGly compared with controls (n=8 per group).

686 F. Serum insulin levels during glucose tolerance test performed in panel C (n=8 per

687 group).

688 G. ITT in controls and ΔGly mice at 50-week old. (n=8 per group)

689 H. T.G. clearance test in controls and ΔGly mice at 50-week old (n=8 for WT, n=9

690 for ΔGly mice)

691 I. AUC calculated based on H.

692 J. Circulating FFA levels in controls and ΔGly mice at 50-week old during T.G.

693 clearance performed in panel I. (n=8 for WT, n=9 for ΔGly mice).Bar,100μm.Data

694 are mean ± SEM. Student's *t* test: *p < 0.05, **p < 0.01, ***p < 0.001 for controls
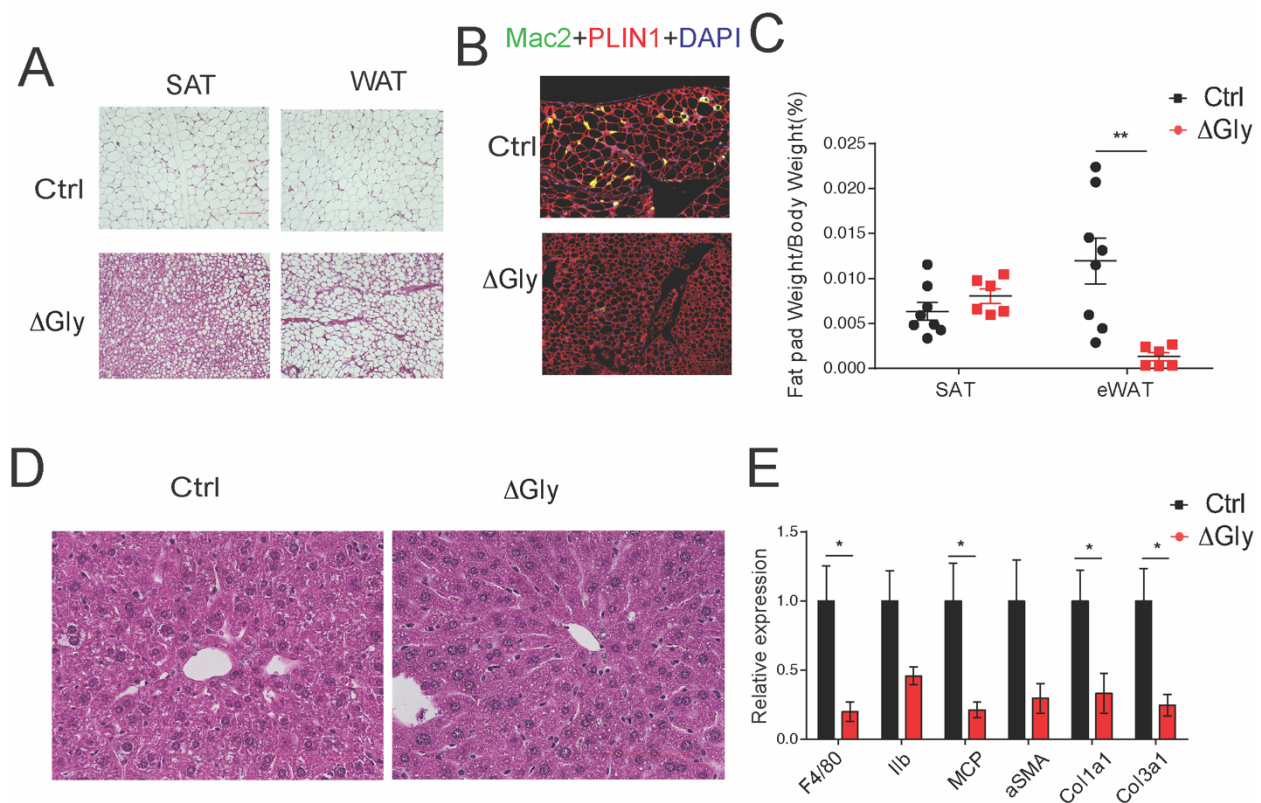
695 vs ΔGly.

696

Figure 5

697

Fig. 5: Old adiponectin overexpressing mice exhibit improved glucose and lipid homeostasis.

A. H&E staining of SubQ fat depot and Epi fat depot of 140-week old controls and ΔGly mice fed on chow diet.

B. Mac2 staining of Epididymal fat sections in 140-week old controls and ΔGly mice.

C. Relative subcutaneous and visceral fat pad weights of 140-week old controls and ΔGly mice fed on chow diet (n=8 for controls, n=6 for ΔGly mice).

D. H&E staining of Liver from 140-week old controls and ΔGly mice fed on chow diet.

707   E. Expression of inflammatory and fibrosis markers in liver of 140-week old controls

708   and ΔGly mice fed on chow diet (n=8 for controls, n=6 for ΔGly mice). Bar,

709   100µm.Data are mean ± SEM. Student's *t* test: *p < 0.05, **p < 0.01, ***p < 0.001
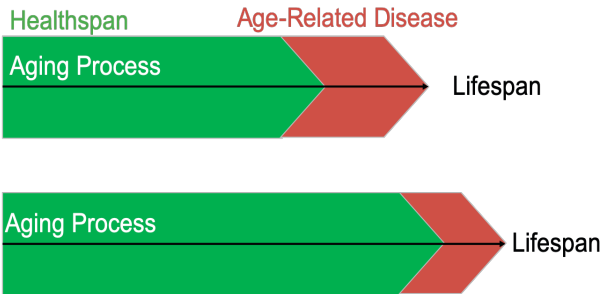
710   for WT vs ΔGly.

711 **Supplemental Figure Legends:**

Sup Figure 1

A

	Chow diet		HFD		Chow diet	
Genotype	WT	APN-KO	WT	APN-KO	Ctrl	ΔGly
Cohort	81 male	72 male	63 male	60 male	45 male	37 male

B
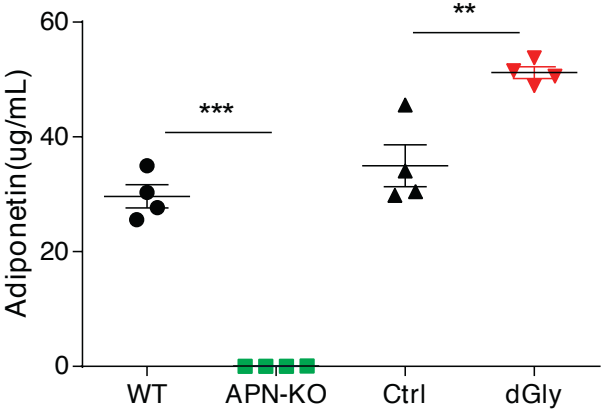

C

Serum Adiponectin Concentration

712

41

713 **Fig. S1: Mouse Models used for longevity studies: APN-KO Mice and ΔGly Mice**

714 **S1A.** Experimental strategy for longevity experiments.

715 S1B. Diagram of the aging process. Lifespan and healthspan are always strongly

716 coupled.

717 S1C. Circulating adiponectin levels measured in 50-week old APN-KO and ΔGly mice

718 with their controls fed on chow diet respectively (n=4 per group).
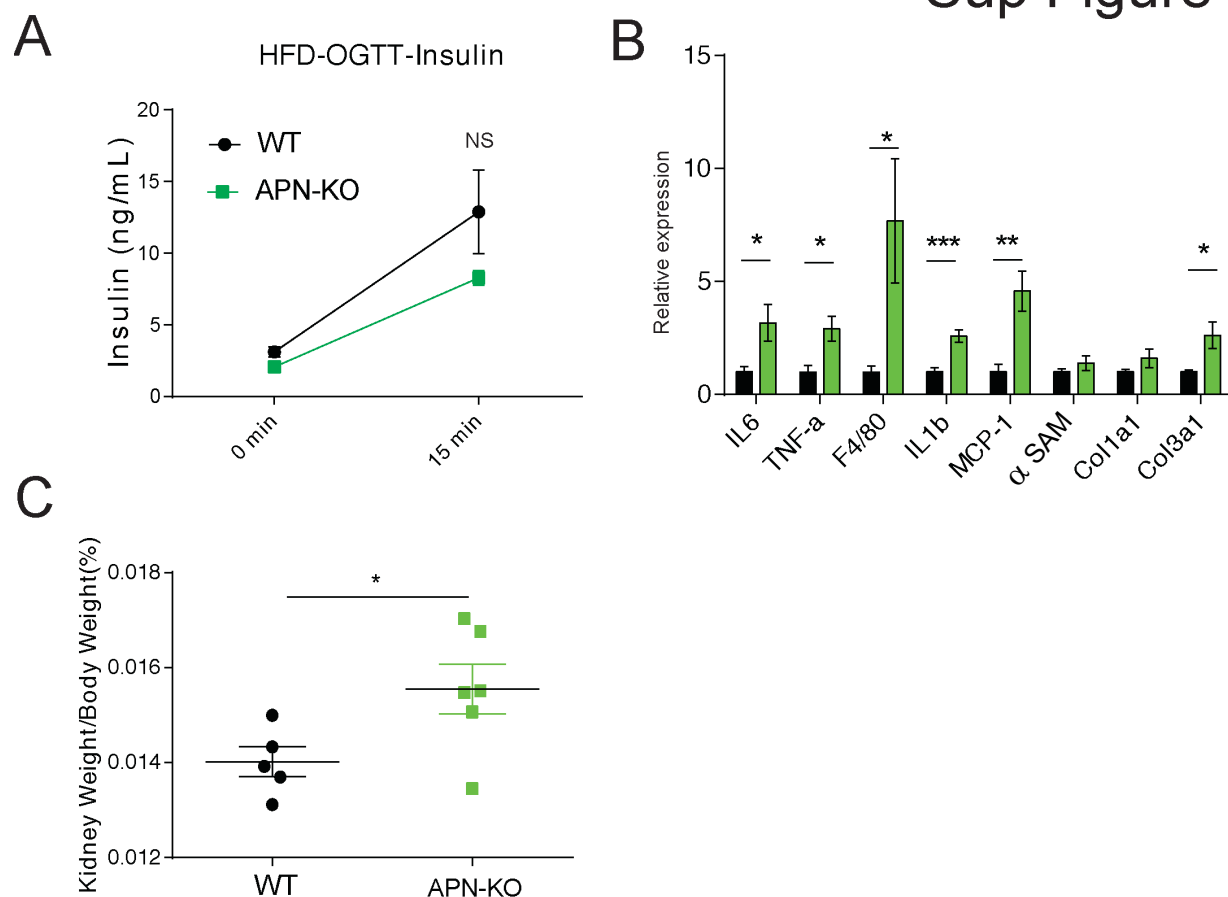

719

720 **Fig. S2: Insulin levels in APN-KO mice during OGTTs.**

721   S2A. No difference in insulin levels during OGTTs in aged APN-KO mice on HFD. And

722   chow diet fed aged APN-KO mice do not improve glucose tolerance. Serum insulin

723   levels during glucose tolerance test performed in Fig.3D. (n=8 for WT, n=7 for APN-KO

724   mice.

725   S2B. Expression of inflammatory and fibrosis markers in liver of 140-week old WT and

726   APN-KO mice fed on chow diet (n=7 for WT, n=7 for APN-KO mice).

727   S2C. The relative wet kidney weight with respect to body weight of 140-week old WT

728   and APN-KO mice fed on chow diet (n=5 for WT, n=6 for APN-KO mice). Bar,

729   100μm.Data are mean ± SEM. Student's t test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for

730   WT vs APN-KO.