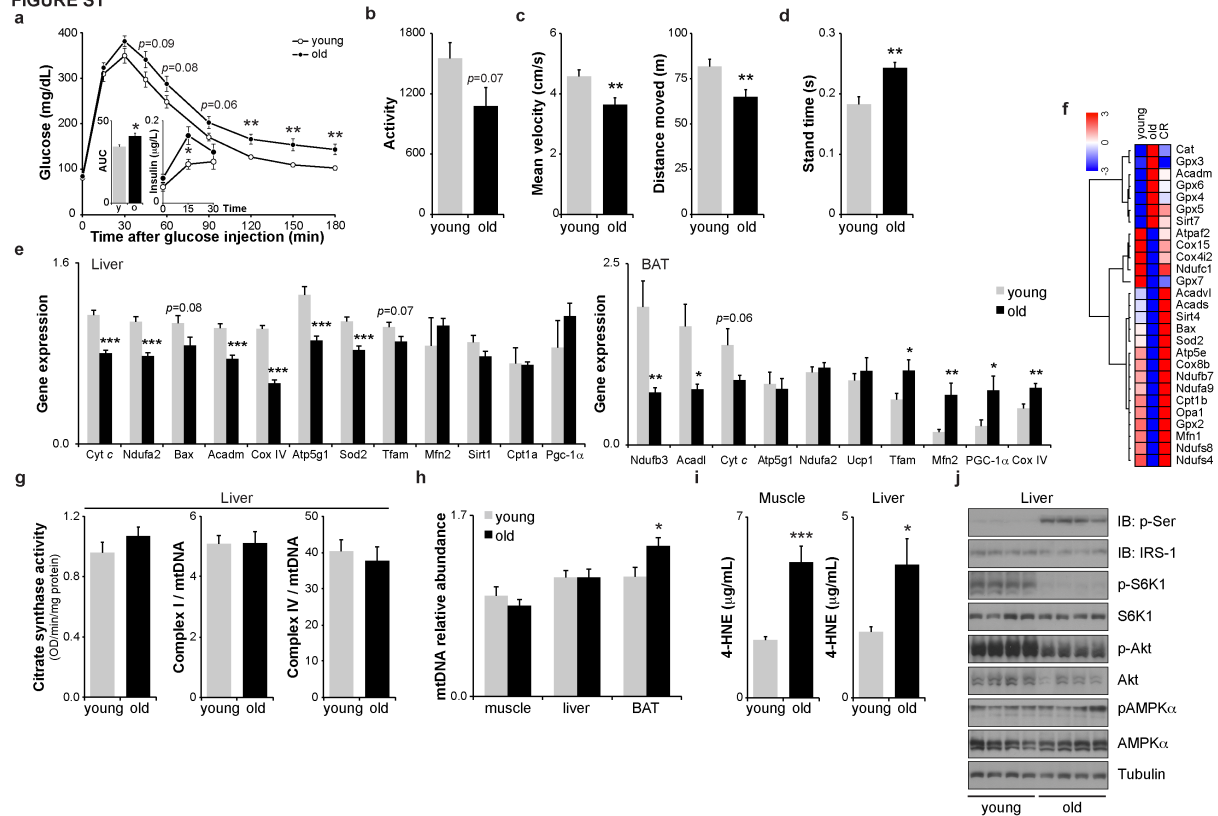# Supplementary data

The metabolic footprint of aging in mice

Riekelt H. Houtkooper[1], Carmen Argmann[2], Sander M. Houten[3], Carles Cantó[1], Ellen H. Jeninga[1], Pénélope A. Andreux[1], Charles Thomas[4], Raphaël Doenlen[4], Kristina Schoonjans[1], Johan Auwerx[1*]

FIGURE S1

Figure S1. Clinical and molecular phenotyping of aged mice. (a) Glucose tolerance after intraperitoneal glucose injection. The insets show the area under the curve (AUC) of the glucose levels (left panel) and the plasma insulin levels during the first 30 minutes of the glucose tolerance test. (b) Activity during 24h indirect calorimetry recording. (c) Velocity and total distance moved in a square open-field test. (d) Stand time —time standing on one paw— during gait analysis. (e) Gene expression of young and old mice in liver and brown adipose tissue (BAT). (f) Heat map showing gene expression differences for selected (mitochondrial) genes between young (5 months), old (25 months) and caloric restricted C57BL/6N mice from published microarray datasets (Edwards et al., 2007). (g) Enzymatic activity of citrate synthase (CS), and complex I and complex IV of oxidative phosphorylation in liver. (h) mtDNA abundance in various tissues. (i) Tissue ROS damage as determined by 4-hydroxynonenal (4-HNE). (j) Western blot analysis of relevant metabolic signaling pathways in liver. pAMPKα represents phosphorylation/activation of the α-subunit of AMPK; Immunoprecipitated IRS1 was used to measure its phosphorylation on serine residues (p-Ser); p-S6K1 reflects S6K1 phosporylation/activation. Tubulin is used as a loading control. Values are expressed as mean±SEM; n=6-10. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.
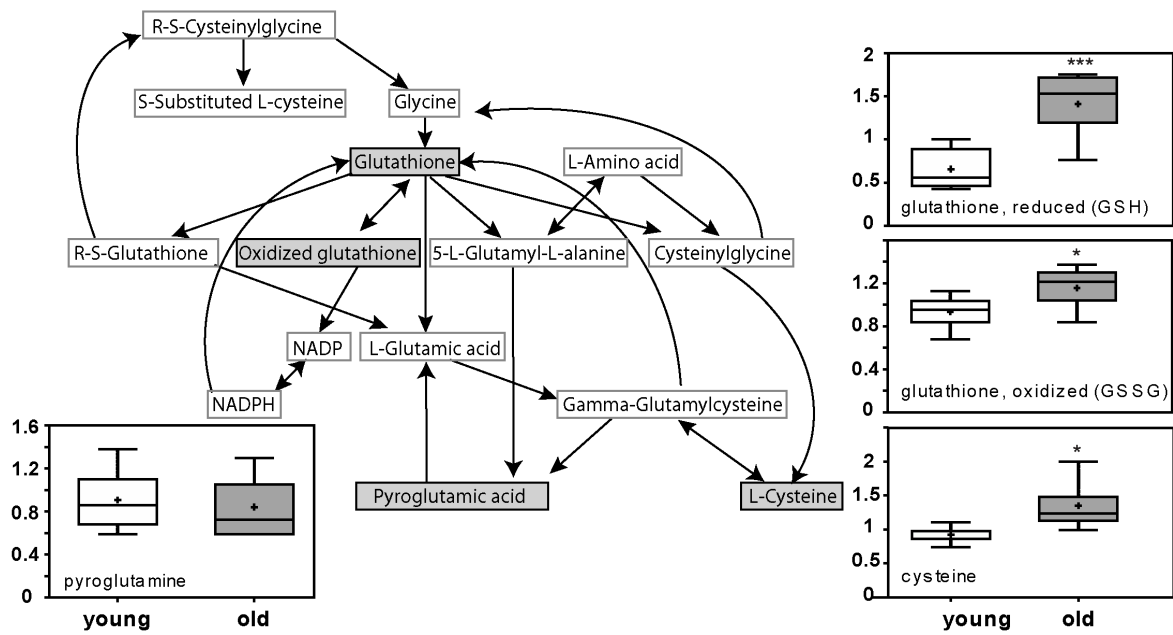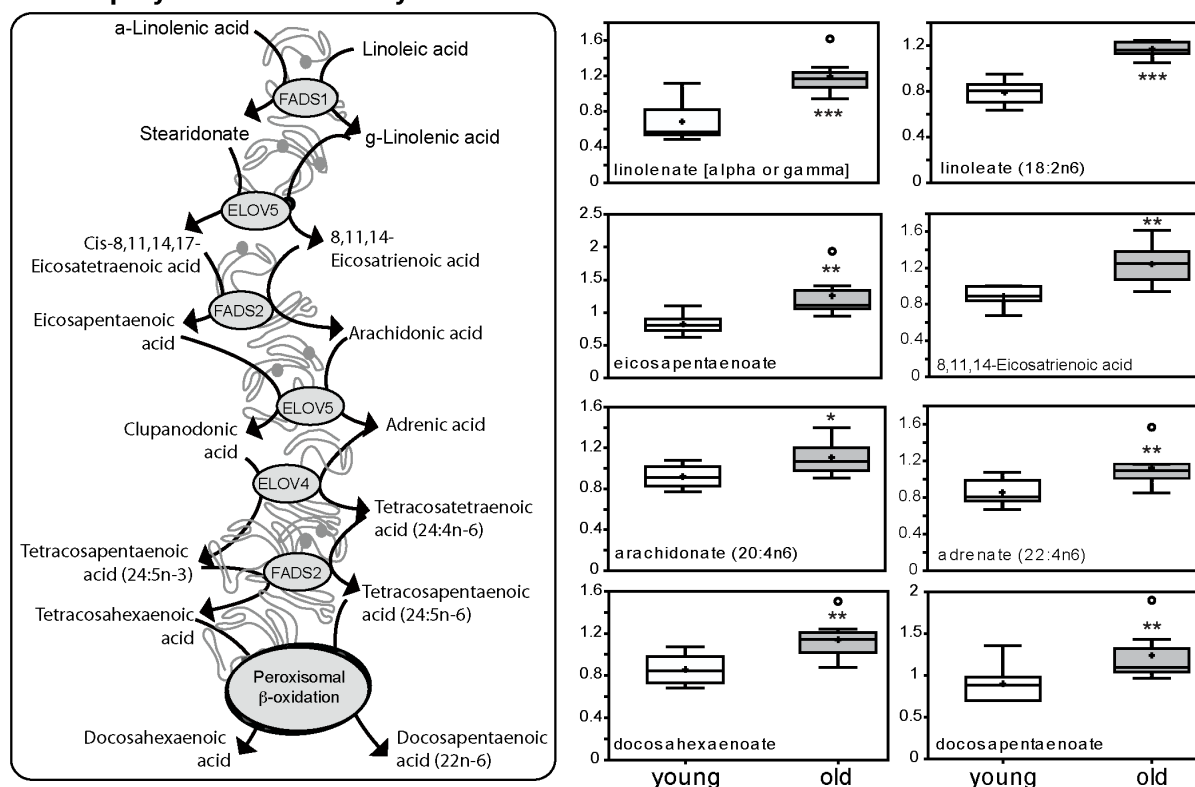
Figure S2. Glutathione pathway is disturbed in aging liver. The glutathione metabolic pathway is an example of a metabolic pathway where a key node, glutathione, has been altered in aging mice, which impacts many possible biological outcomes as demonstrated by the respective box plots of down stream metabolites.

FIGURE S3

Muscle poly-unsaturated fatty acid metabolism

Figure S3. Polyunsaturated fatty acid pathway is disturbed in aging muscle. The poly-unsaturated biosynthetic pathway is an example of a metabolic pathway where key nodes, linolenic and linoleic acid, have been altered in aging mice, which impacts many possible biological outcomes as demonstrated by the respective box plots of down stream metabolites.

Supplementary methods

Primer sequences for quantitative PCR and mtDNA analysis.

For RNA:

Gene	Forward primer	Reverse primer
Atp5g1	GCTGCTTGAGAGATGGGTTC	AGTTGGTGTGGCTGGATCA
Bax	TGGAGCTGCAGAGGATGATTG	CACGGAGGAAGTCCAGTGTC
Cox IV	TGGGAGTGTTGTGAAGAGTGA	GCAGTGAAGCCGATGAAGAAC
Cpt1a	GCACTGCAGCTCGCACATTACAA	CTCAGACAGTACCTCCTTCAGGAAA
Cpt1b	CCCATGTGCTCCTACCAGAT	CCTTGAAGAAGCGACCTTTG
Cyt c	TCCATCAGGGTATCCTCTCC	GGAGGCAAGCATAAGACTGG
Lcad	GTAGCTTATGAATGTGTGCAACTC	GTCTTGCGATCAGCTCTTTCATTA
Mcad	GGCCATTAAGACCAAAGCAGA	GTGTCGGCTTCCACAATGAAT
Mfn2	ACGTCAAAGGGTACCTGTCCA	CAATCCCAGATGGCAGAACTT
Ndufa2	GCACACATTTCCCCACACTG	CCCAACCTGCCCATTCTGAT
Ndufb3	TACCACAAACGCAGCAAACC	AAGGGACGCCATTAGAAACG
Pgc-1α	AAGTGTGGAACTCTCTGGAACTG	GGGTTATCTTGGTTGGCTTTATG
Sirt1	TGTGAAGTTACTGCAGGAGTGTAAA	GCATAGATACCGTCTCTTGATCTGAA
Sod2	CTCTGGCCAAGGGAGATGTTA	ACGGCTGTCAGCTTCTCCTTA
Tfam	AAGTGTTTTTCCAGCATGGG	GGCTGCAATTTTCCTAACCA
Ucp1	CTTTGCCTCACTCAGGATTGG	ACTGCCACACCTCCAGTCATT
Ucp3	ACTCCAGCGTCGCCATCAGGATTCT	TAAACAGGTGAGACTCCAGCAACTT

For mtDNA:

Gene	Forward primer	Reverse primer
16s	CCGCAAGGGAAAGATGAAAGAC	TCGTTTGGTTTCGGGGTTTC
Cox2	GTTGATAACCGAGTCGTTCTGC	CCTGGGATGGCATCAGTTTT
Ucp2	CTACAGATGTGGTAAAGGTCCGC	GCAATGGTCTTGTAGGCTTCG
Hk2	TCTGGCTCTGAGATCCATCTTCA	CCGGCCTCTTAACCACATTCC

References to supplementary data

Edwards, M.G., Anderson, R.M., Yuan, M., Kendziorski, C.M., Weindruch, R., and Prolla, T.A. (2007). Gene expression profiling of aging reveals activation of a p53-mediated transcriptional program. BMC Genomics *8*, 80.