Supplemental Figure Legends

Figure S1. Alignment of HSP90 sequences from representative species and homologs, Related to Figure 1

The N-terminal domain amino acid sequences of HSP90 family members. T5,7 are highlighted with boxes, and the extreme N-terminus (ENT) region (a.a. 1-11), in gray.

Figure S2. p-HSP90α did not increase with aging in the lung, Related to Figure 1

HSP90α phosphorylation in lung of young (3 mo) and old (24 mo) mice (in arbitrary units) (n=5 each group). Quantification is shown on the right. All values are expressed as mean ± s.e.m.

Figure S3. DNA-PK inhibition did not increase DNA DSBs, Related to Figure 5

(A)H2AX phosphorylation (γ-H2AX) in resting muscle isolated from young (2 months old) and old (18 mo) WT and SCID mice (n=3 each group). Quantification is shown on the right. All values are expressed as mean ± s.e.m.: *, $p<0.05$. (B) H2AX phosphorylation (γ-H2AX) in resting skeletal muscle isolated from 17 months old fl/fl and muscle specific DNA-PK knockout (MDPKO) mice. Quantification is shown on the right. All values are expressed as mean ± s.e.m. (C) H2AX phosphorylation (γ-H2AX) in resting skeletal muscle isolated from WT and AMPKα2 knockout mice fed either vehicle or DNA-PKcs inhibitor. Quantification is shown on the right. All values are expressed as mean ± s.e.m. (D) H2AX phosphorylation (γ-H2AX) in resting skeletal muscle isolated from middle age (MA) mice treated with either vehicle or DNA-PKcs inhibitor. Quantification is shown on the right. All values are expressed as mean ± s.e.m.

Figure S4. DNA-PK inhibition increases skeletal muscle mitochondrial function, Related to Figure 5

(A) VO_2 of WT and SCID mice skeletal muscle mitochondria in states 2, 3, 4 and RC (n=6 per each genotype) All values are given as mean ± s.e.m. Mann-Whitney Test: *, $p<0.05$. (B) Running distance before exhaustion during three consecutive days of treadmill running for middle-age (MA) and HFD-fed (Ob, for 3-4 months) WT and SCID mice (n=5-9 for each group). All values are given as mean ± s.e.m. Mann-Whitney Test: *, $p<0.05$; **, $p<0.01$ between WT and SCID values.

Figure S5. **Metabolic function of DNA-PK is not immune-related, Related to Figure 5**

(A) Relative mRNA levels of PGC-1α and PPARδ in skeletal muscle (M), WAT (W), BAT (B) and liver (L) of HFD-fed WT and Rag1-/- mice are shown. The mRNA levels were measured using real-time PCR and are expressed in arbitrary units (A.U.) (n=3 per genotype). Results were shown as mean ± s.e.m. (B) The distance (in meters) lean (RCD) and HFD-fed (HFD, for 3-4 months) WT and Rag1-/- mice ran on the treadmill before exhaustion (n=5 per each genotype). Results were shown as mean ± s.e.m.

Figure S6. Scid mice are protected against obesity and insulin resistance, Related to Figure 6

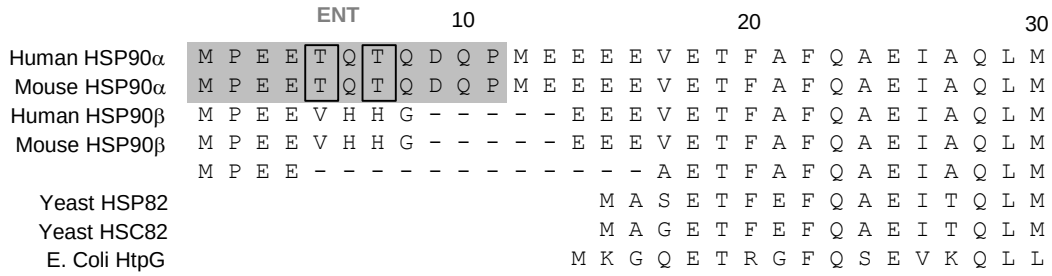
(A) Weight gain of WT (black symbols) and SCID (white symbols) littermates fed regular chow diet (RCD) as a function of the time on the diet (n=5-8 per genotype). All values are expressed as mean ± s.e.m. (B) Weight gain of WT (black symbols) and SCID (white symbols) littermates fed high fat diet (HFD, 60% fat by calories) as a function of the time on the diet (n=5-7 per genotype). All values are expressed as mean ± s.e.m. **, $p<0.01$ (Two-way repeated measures Anova) between WT and SCID mice. (C) Food intake for WT and SCID mice (n=5-7 per each genotype). All values are expressed as mean ± s.e.m. (D) fat absorption was measured by fecal analysis (n=5 per each genotype). All values are given as mean ± s.e.m. (E) Fat mass index (total fat mass/body weight) as measured by NMR spectroscopy for WT and SCID mice after 20 weeks on the respective diet (n=7-8 per genotype). All values are expressed as mean ± s.e.m. Mann-Whitney Test: **, $p<0.01$ between WT and SCID values. (F) Abdominal fat in WT and SCID fed HFD (for 3-4 months). (G) Epididymal fat (EWAT) mass of WT and SCID mice fed HFD relative to body weight (B.W.) (n=3 per genotype). All values are given as mean ± s.e.m. **, $p<0.01$ between WT and SCID values. (H) Lean mass index (lean mass/total body weight) of mice on HFD was measured by NMR spectroscopy (n=7). All values are given as mean ± s.e.m. (I) Body weight of middle-age WT and SCID mice. (n=7-8 per genotype). All values are expressed as mean ± s.e.m. Mann-Whitney Test: ***, $p<0.001$ between WT and SCID values. (J) Plasma concentrations of triacylglyceride for middle-age WT and SCID mice (6-10 per treatment group). All values are expressed as mean ± s.e.m. Mann-Whitney Test: *, $p<0.05$ between WT

and SCID values. (K) HOMA-IR for young (3 mo) and middle-age (14 mo) WT and SCID mice (n=6-10 per treatment group). All values are expressed as mean ± s.e.m. Mann-Whitney Test: **, $p<0.01$ between 3 mo and 14 mo values.

Figure S7. Hyperinsulinemic-euglycemic clamp study performed on mice fed HFD (±DNA-PK inhibitor), Related to Figure 6

(A**)** Time course of blood glucose level. (B) Time course of glucose infusion rate. (n=7-8). All values are given as mean ± s.e.m. *, $p<0.05$ (Two-way ANOVA) between the treatment groups. (C**)** Plasma concentrations of FFAs, triacylglyceride, glucose and insulin in WT and muscle specific DNA-PKcs knockout mice. (n=6-8 for each genotype). All values are given as mean ± s.e.m.

Fig. S1 (Park)


ENT
10
20
30
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Human HSP90β M P E E V H H G - - - - - E E E V E T F A F Q A E I A Q L M
Mouse HSP90β M P E E V H H G - - - - - E E E V E T F A F Q A E I A Q L M
M P E E - - - - - - - - - - - - A E T F A F Q A E I A Q L M
Yeast HSP82 M A S E T F E F Q A E I T Q L M
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E. Coli HtpG M K G Q E T R G F Q S E V K Q L L

Fig. S2 (Park)

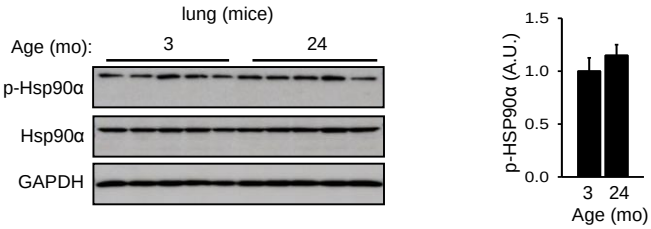
lung (mice)
Age (mo):
3
24
p-Hsp90α
Hsp90α
GAPDH
p-HSP90α (A.U.)
1.5
1.0
0.5
0.0
3
24
Age (mo)

Figure S3 (Park)

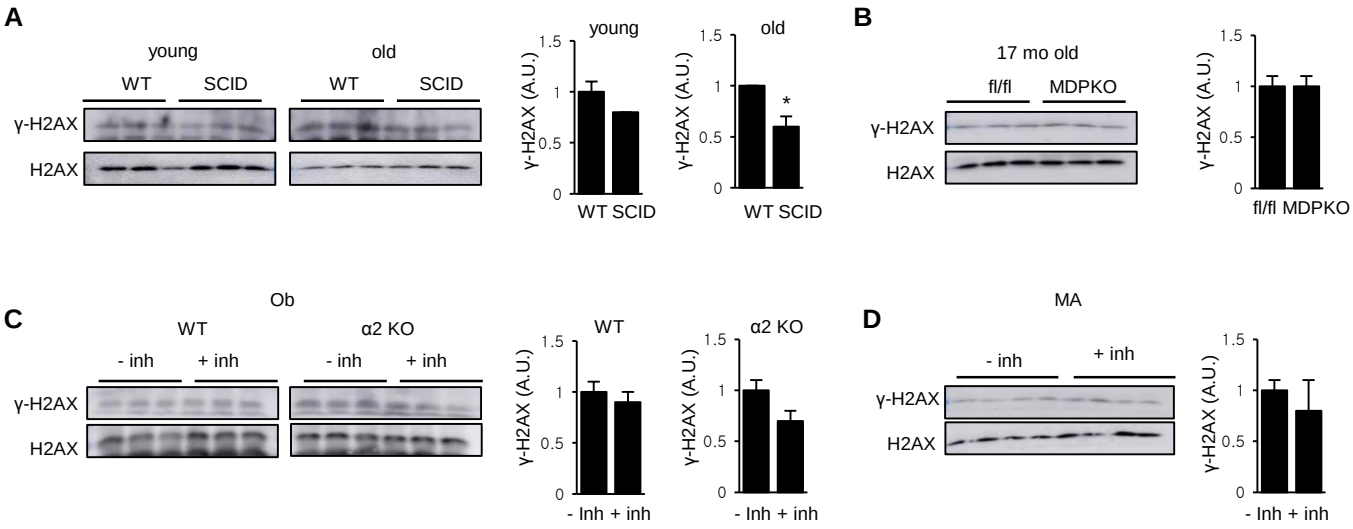

Figure S4 (Park)

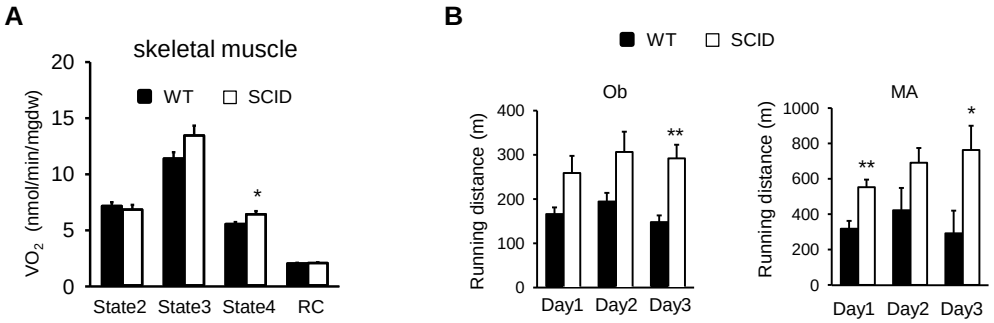

Figure S5 (Park)

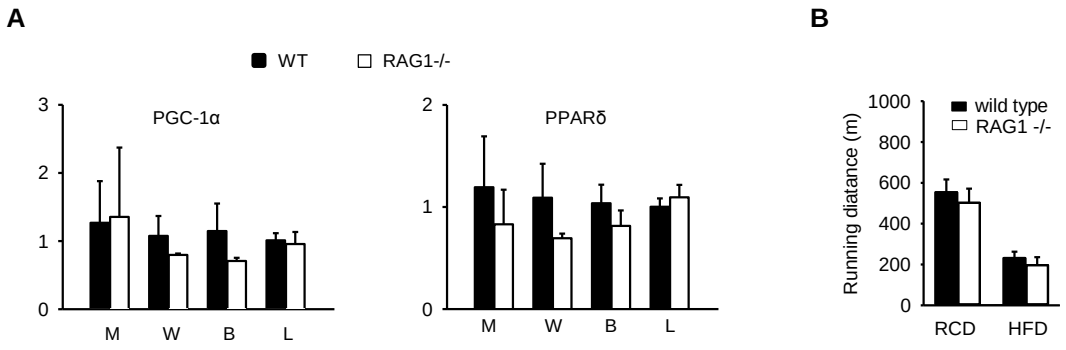

Figure S6 (Park)

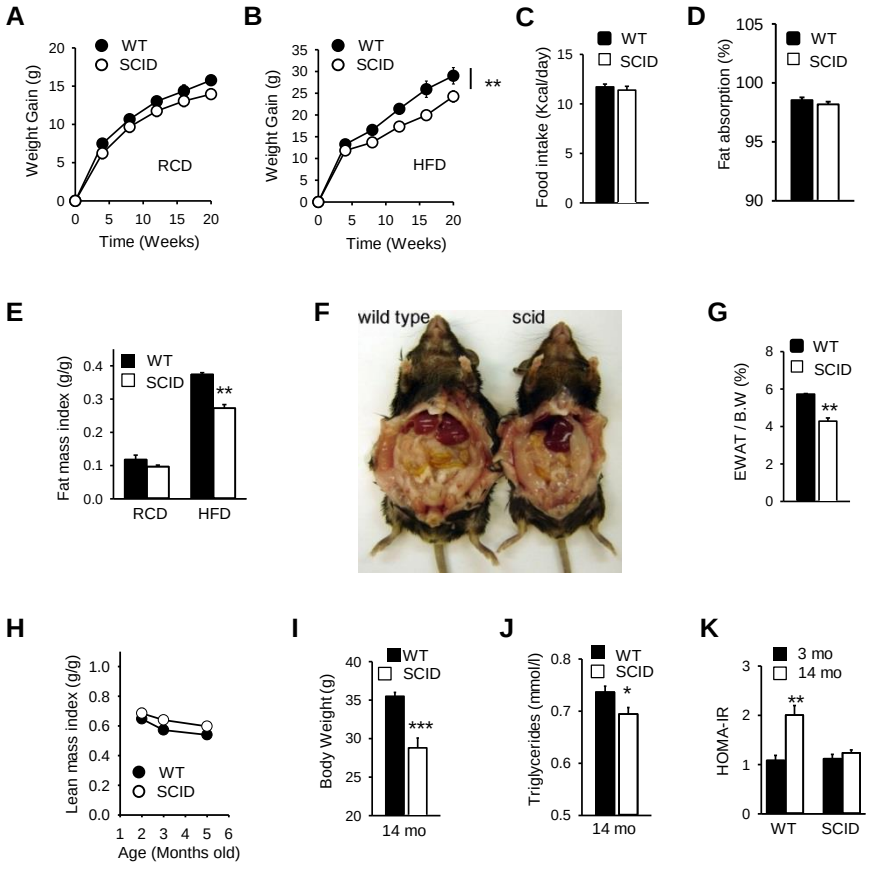

Figure S7 (Park)