

Supplemental Figure S1

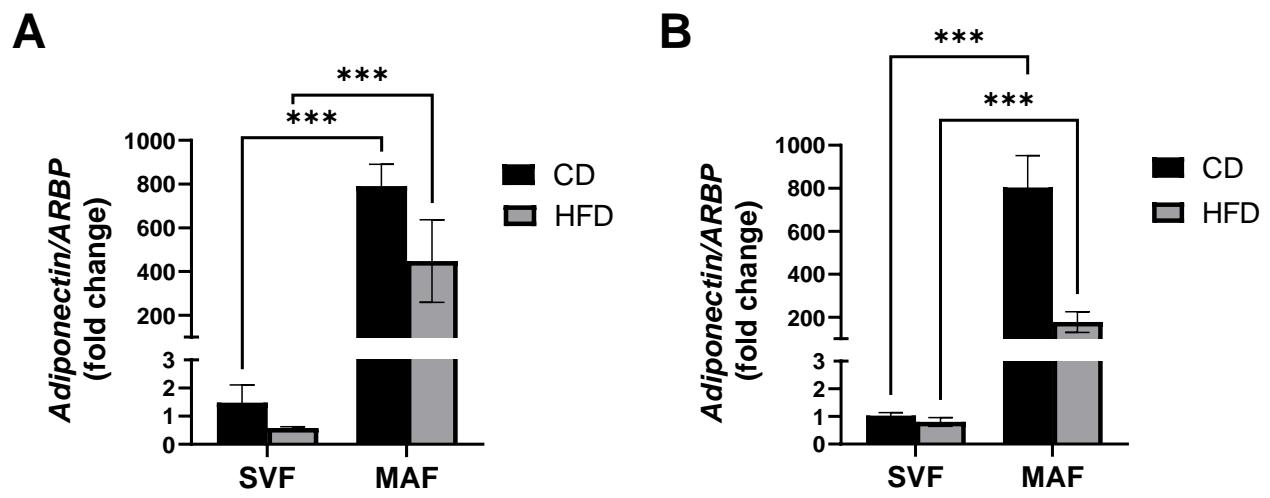


Figure S1. Validation of adipose fractionation. *Adiponectin* mRNA expression in SVF and MAF from SQ (**A**) and VF (**B**) of female WT mice either on CD or HFD (n=5-6). Data represent mean \pm SEM. ***p<0.001. SQ, subcutaneous adipose tissue; VF, visceral adipose tissue; CD, chow diet; HFD, high fat diet; SVF, stromovascular fraction; MAF, mature adipocyte fraction.

Supplemental Figure S2

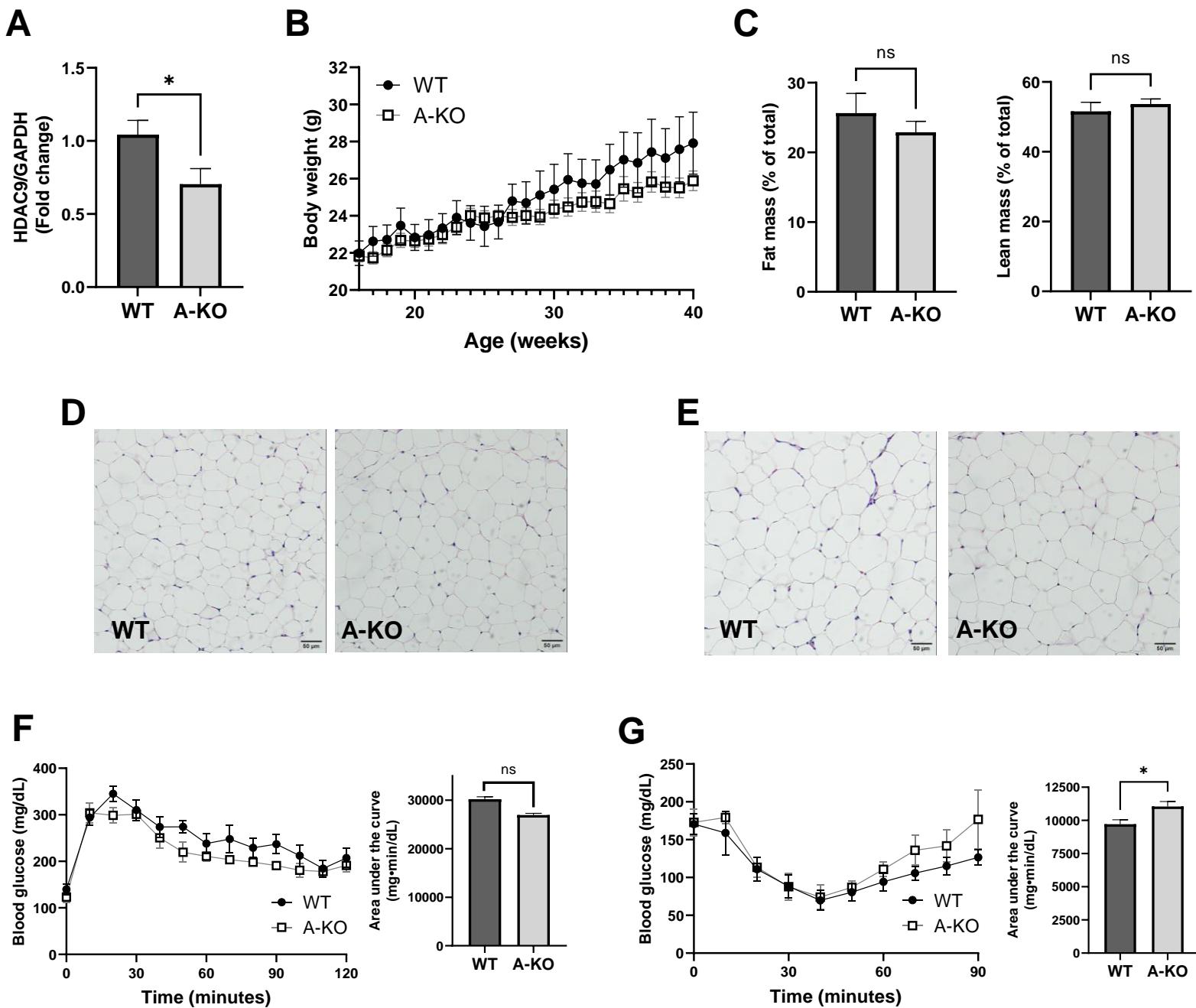


Figure S2. A-KO does not improve metabolic health in CD-fed female mice. **(A)** HDAC9 mRNA expression level in SQ from CD-fed female mice ($n=8-9$). **(B)** Growth curves of CD-fed female WT and A-KO mice ($n=8-9$). **(C)** Fat mass and lean mass measured by whole body composition nuclear magnetic resonance ($n=8-9$). Representative H&E images of SQ **(D)** and VF **(E)** from CD-fed female WT and A-KO mice. Glucose tolerance **(F)** and insulin tolerance test **(G)** quantified by area under the curve ($n=4$). Data represent mean \pm SEM. * $p<0.05$. SQ, subcutaneous adipose tissue; VF, visceral adipose tissue.

Supplemental Figure S3

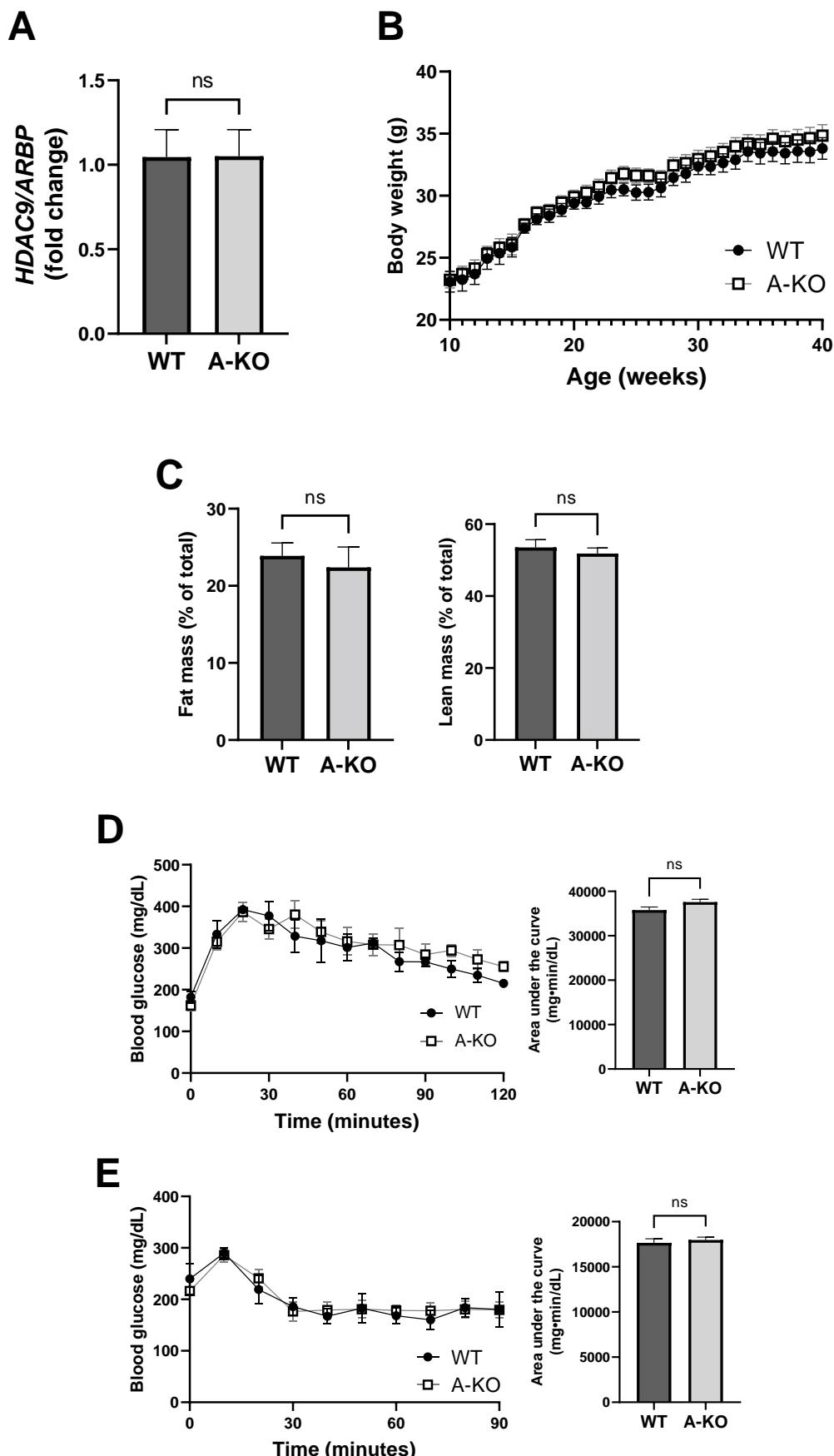
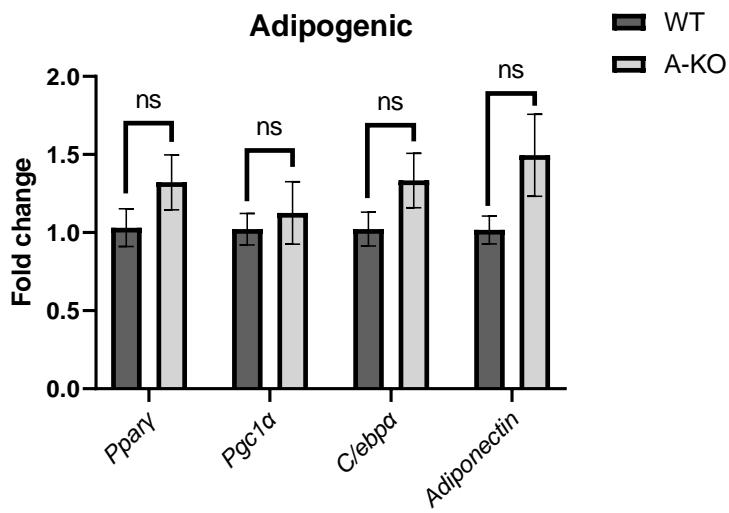


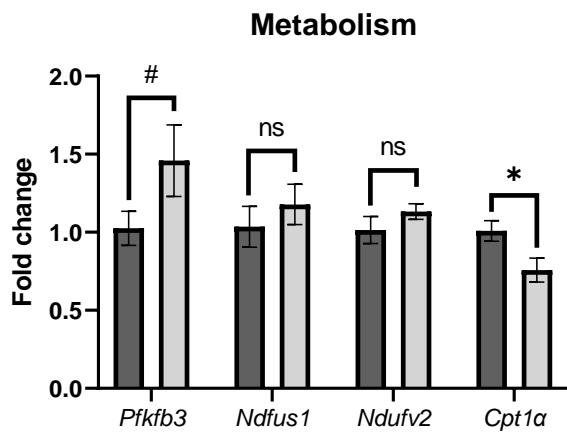
Figure S3. A-KO does not improve metabolic health in CD-fed male mice. **(A)** Expression of *HDAC9* mRNA in SQ CD-fed male mice determine by qPCR ($n=3-4$). **(B)** Growth curves of CD-fed male WT and A-KO mice ($n=10-15$). **(C)** Fat mass and lean mass measured by whole body composition nuclear magnetic resonance ($n=7-10$). Glucose tolerance **(D)** and insulin tolerance test **(E)** quantified by area under the curve ($n=4$). Data represent mean \pm SEM.

Supplemental Figure S4

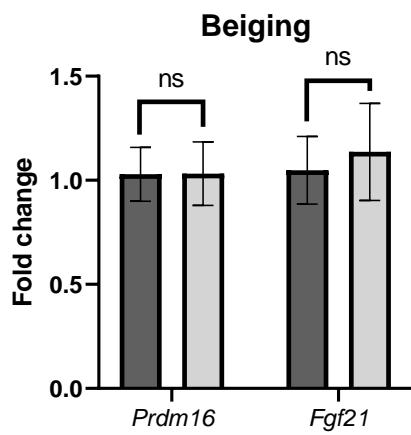
A



B



C



D

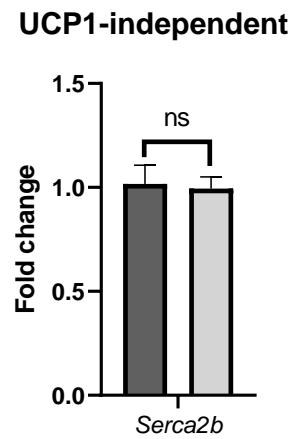


Figure S4. Adipocyte-specific *HDAC9* gene deletion does not significantly alter gene expression in VF from HFD-fed female mice. mRNA expression of **(A)** adipogenic, **(B)** metabolic genes **(C)** classic beiging and **(D)** UCP1-independent beiging genes in SQ from HFD-fed female mice (n=5-6). Data represent mean \pm SEM. #p<0.1; *p<0.05. VF, visceral adipose tissue; PPAR γ , peroxisome proliferator-activated receptor gamma; Pgc1 α , PPAR γ coactivator 1-alpha; C/ebpa, CCAAT/enhancer-binding protein alpha; Pfkfb3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; Ndfus1, NADH-ubiquinone oxidoreductase; Ndufv2, NADH-ubiquinone oxidoreductase flavoprotein 2; Cpt1 α , carnitine palmitoyltransferase 1a; Prdm16, PR/SET domain 16; Fgf21, fibroblast growth factor 21; Serca2b, sarco/endoplasmic reticulum Ca $^{2+}$ ATPase 2b.

Supplemental Table S1

Table S1: Demographic data of human subjects.

BMI (kg/m ²)	Age (years)	Gender	Diabetes Mellitus
21.4	55	M	Yes
23.3	50	M	No
24.0	68	M	No
24.0	43	M	Yes
24.5	64	M	No
25.0	62	M	No
27.1	65	M	No
27.8	60	M	No
28.7	82	M	No
34.9	53	F	No
35.3	44	M	No
42.2	64	F	Yes

Supplemental Table S2

Table S2: Primer Sequences.

Gene Name	Species	Forward	Reverse
<i>Adiponectin</i>	mouse	GCAC TGGCAAGTTCTACTGCAACA	AGAGAACGGCCTTGTCC TTGTTGA
<i>Arbp</i>	mouse	AGCTGAAGCAAAGGAAGAGTCGA	ACTGGTTGCTTGGCGGGATTAG
<i>Aromatase</i>	mouse	GCTGAACCCC ATGCAGTATAA	AGCCAAAAGGCTGAAAGTACC
<i>Atp5a1</i>	mouse	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
<i>C/ebpa</i>	mouse	AGAAGTCGGTGGACAAGAACAGCA	GCGTTGTTGGCTTATCTCGGCT
<i>Cpt1a</i>	mouse	CATGATTGCAAAGATCAATCGG	CTTGACATGCGGCCAGTG
<i>Fabp4</i>	mouse	ATGAAATCACCGCAGACGACAGGA	TGTGGTCGACTTCCATCCCAC TT
<i>Fgf21</i>	mouse	CTCCACATGAGAGCTAGAACAC	CTGATATCCAGGGCGAAGTAAG
<i>Gatm</i>	mouse	CAGACACAAATTGGCCGCTC	CCCAGGTAGTTGTAACCTGGC
<i>Hdac9</i>	mouse	AGGATGATGATGCCTGTGGTGGAT	GAGTTGTGCTTGATGCTGCCTTGT
<i>Ldha</i>	mouse	ACATTGTCAAGTACAGTCCACAC	TTCCAATTACTCGGTTTGGGA
<i>Leptin</i>	mouse	CAAGCAGTGCCTATCCAGA	AAGCCCAGGAATGAAGTCCA
<i>Ndfus1</i>	mouse	AACC ACTGTTCTGCAGGCTT	AGCGACAACCTTGGAGCTT
<i>Ndfuv2</i>	mouse	GCAAGGAATTGCATAAGACAGC	TAGCCATCCATTCTGCCTTTG
<i>Pfkfb3</i>	mouse	TACGAAGCTCACACGCTATCT	GCCTCTCGTCGATACTGACCTA
<i>Pgc1a</i>	mouse	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCTGTTTC
<i>Ppar</i>	mouse	ACATAAAGTCCTCCGCTGACCA	AAATCGGATGGCCACCTTTGC
<i>Prdm16</i>	mouse	AGATGAACCAGGCATCCACTCGAA	ACTTCCGGCTAAGCTGT CATCAT
<i>Ryr2</i>	mouse	ATGGCTTAAGGCACAGCG	ATGGCTTAAGGCACAGCG
<i>Serca2b</i>	mouse	ACCTTGCCGCTCATTTCAG	AGGCTGCACACACTCTTACC
<i>Tst</i>	mouse	CCTGCTGTAGGTTCACCTTTAGG	GGAGGCACCAAGAGCAATTCTAAA
<i>Ucp1</i>	mouse	AAGCTGTGCGATGTCCATGT	AAGCCACAAACCCTTGAAAA
<i>Gapdh</i>	human	CGACTTCAACAGCAACTCCC ACTCTTCC	TGGGTGGTCCAGGGTTCTTACTCC TT
<i>Hdac9</i>	human	TACTCAGGCGGAAGGATGGA	CCAGTTGGCCCATTGTTGG