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Supporting Information

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Hepatic suppression of mitochondrial complex II assembly drives systemic metabolic benefits

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Supplemental Figure 1

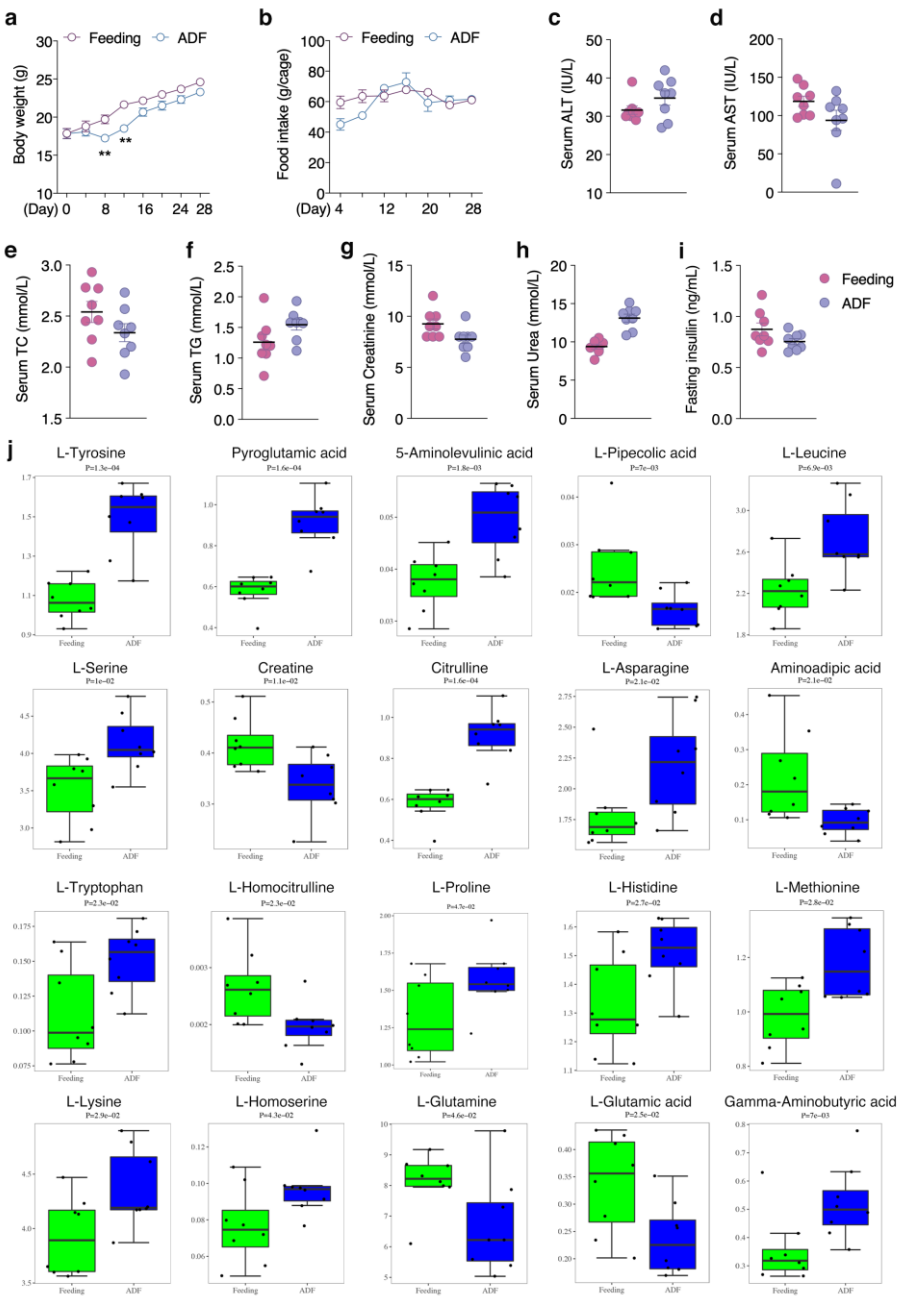

Figure S1 (Related to Figure 1). Blood analysis of mice under ADF intervention. a-b, Body weight curve (**a**) and food intake (**b**) during ADF intervention for 4 weeks, n=8. **c-i**, serum ALT, AST, total cholesterol, total glycerides, creatinine, urea and fasting insulin levels of mice after ADF intervention, n=8. **j**, Boxplots of significant amino acids metabolites differed after ADF intervention ($p < 0.05$). Values are mean ± SEM, *$p < 0.05$, **$p < 0.01$.

Supplemental Figure 2

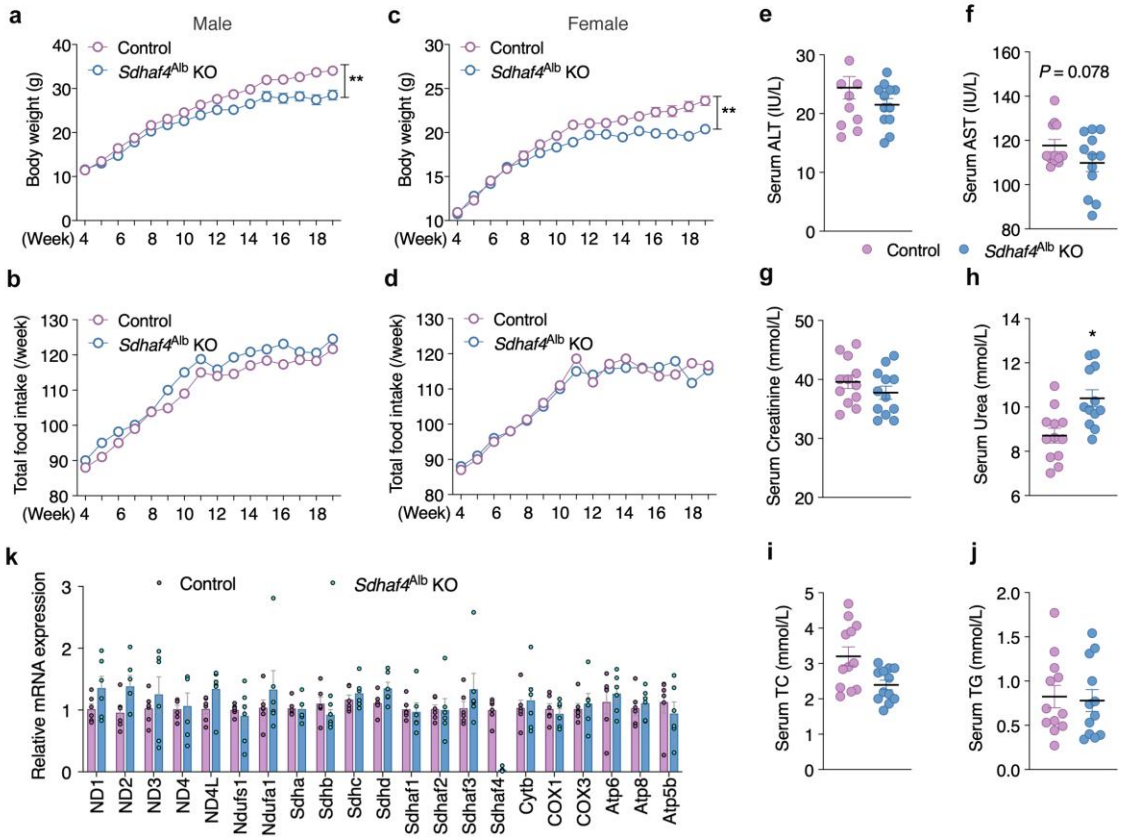

Figure S2 (Related to Figure 2). Characterization of hepatic *Sdhaf4* knockout mice. a-b, Body weight curve for both male (**a**) and female (**b**) control and *Sdhaf4*^{Alb} KO mice from age of 4 weeks to 19 weeks under normal feeding, n=9. **c-d**, Food intake curve for both male (**c**) and female (**d**) mice from age of 4 weeks to 19 weeks under normal feeding, n=9. **e-j**, Serum ALT (**e**), AST (**f**), creatinine (**g**), urea (**h**), total glycerides (**i**) and cholesterol (**j**) levels of control and *Sdhaf4*^{Alb} KO mice at the age of 8 weeks, n=9. **k**, mRNA levels of representive mitochondrial complex subunits in liver of control and *Sdhaf4*^{Alb} KO mice, n=9. Values are mean ± SEM, *p< 0.05, **p< 0.01.

Supplemental Figure 3

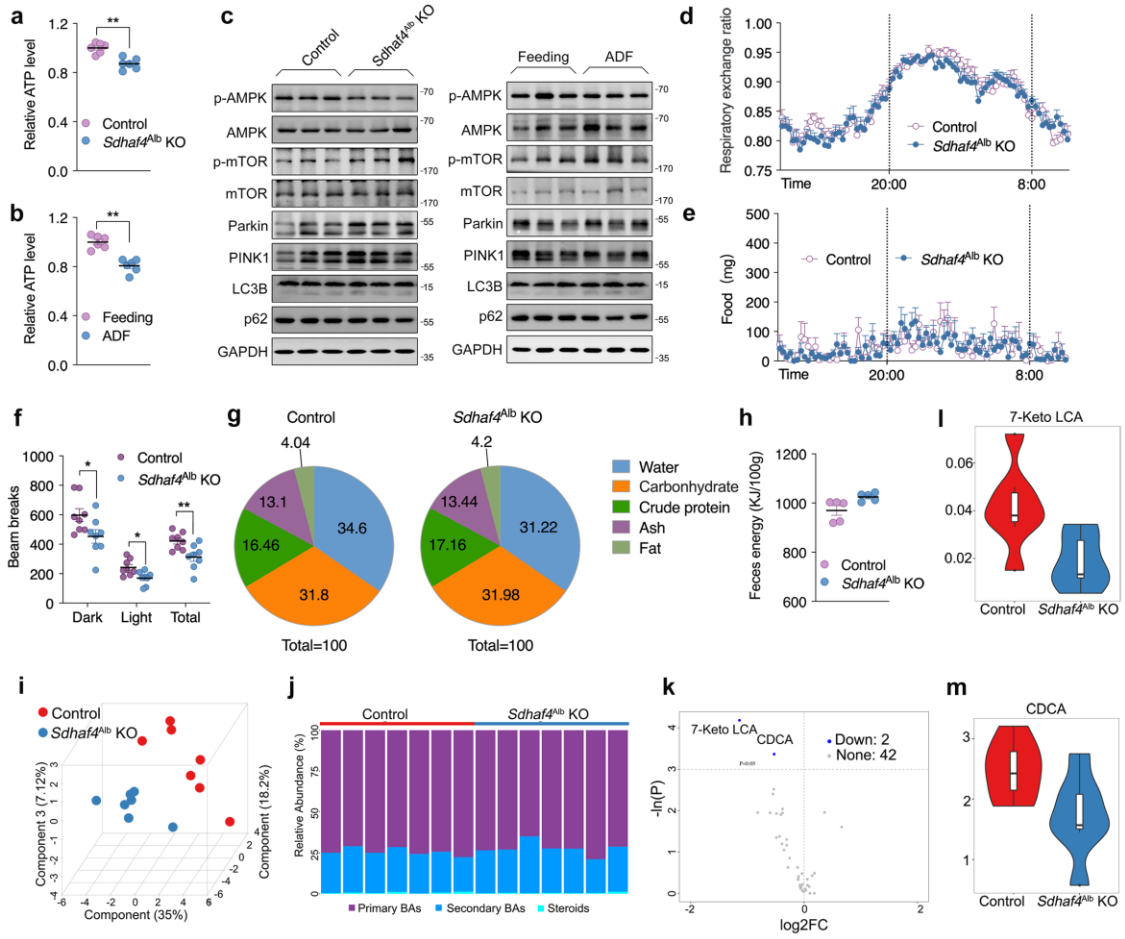

Figure S3 (Related to Figure 2). Hepatic *Sdhaf4* knockout mice present comparable changes in hepatic mitophagy, energy consumption, and bile acid metabolism. a-b, ATP level in the liver of control and *Sdhaf4*^{Alb} KO mice (**a**), regular feeding and ADF intervened mice for 4 weeks (**b**), n=6. **c**, Immunoblots analysis of mitophagy associated genes in control and *Sdhaf4*^{Alb} KO mice, regular feeding and ADF intervened mice for 4 weeks, n=3. **d-f**, Metabolic cage analysis of control and *Sdhaf4*^{Alb} KO mice at the age of 8 weeks, respiratory exchange ratio (**l**), food intake (**m**), and ambulatory activity (**n**) for 24 hours, n=8. **g-h**, Feces composition (**g**) and residue engery (**g**) in control and *Sdhaf4*^{Alb} KO mice, n=5. **i-k**, Profile analysis of bile acids in control and *Sdhaf4*^{Alb} KO mice, 3D PLS-DA scores plot (i), stacked chart figure for relative abundance of each metabolite classes (j), and the volcano plot showing the differential metabolites (k), n=7. **l-m**, Violin plot of differential metabolites 7-Keto LCA (**l**) and CDCA (**m**), n=7. Values are mean ± SEM, *p< 0.05, **p< 0.01. 7-Keto LCA, 7-keto Lithocholic Acid; CDCA, Chenodeoxycholic acid.

Supplemental Figure 4

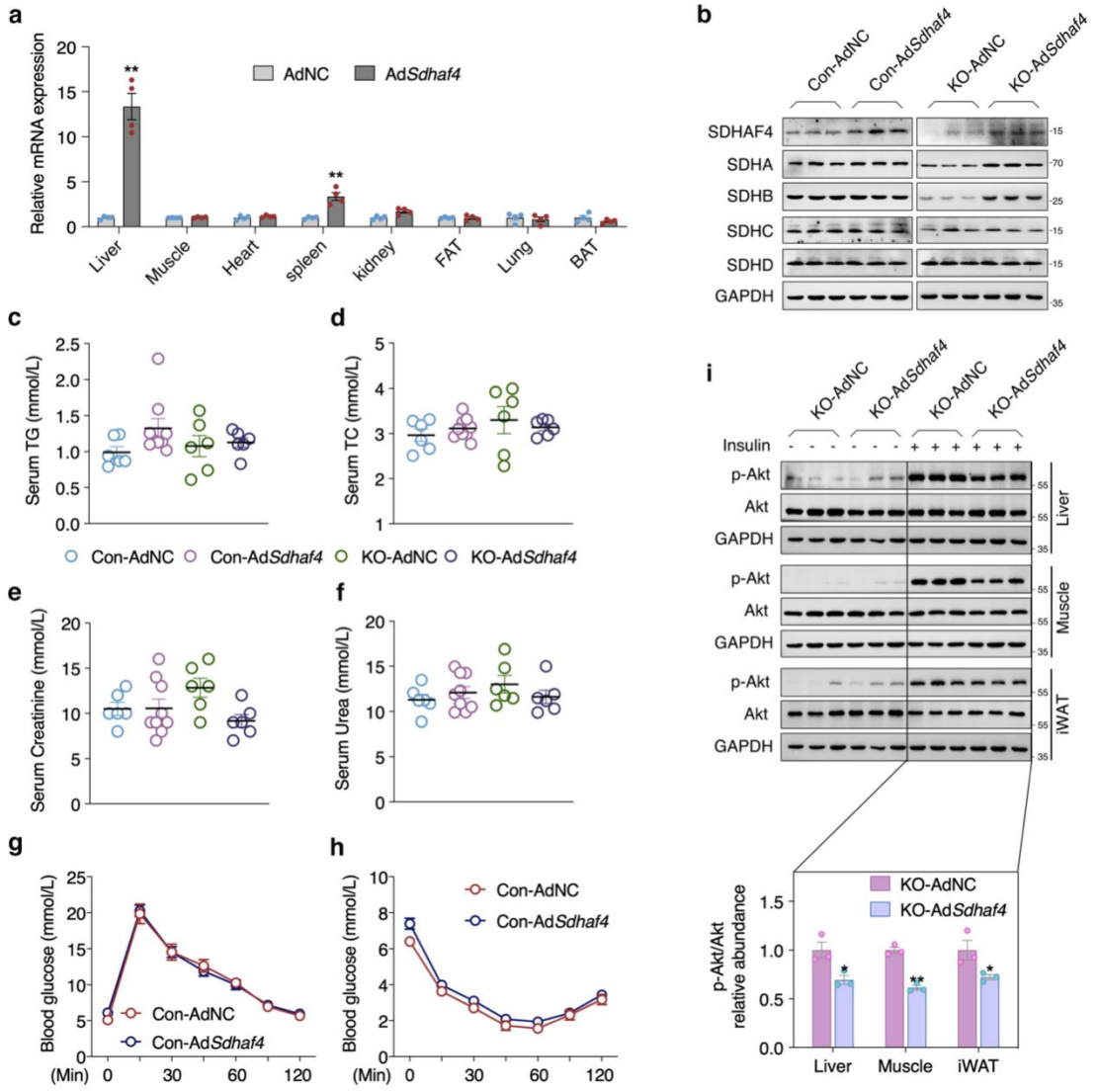

Figure S4 (Related to Figure 3). Hepatic rescue of *Sdhaf4* expression in knockout mice suppresses metabolic sensitivity. a, mRNA levels of *Sdhaf4* in tissues of control mice after adenovirus infection for 3 weeks, n=4. **b,** Immunoblots analysis of SDHAF4 and complex II subunits in control and *Sdhaf4*^{Alb} KO mice after adenovirus mediated hepatic *Sdhaf4* overexpression for 3 weeks, n=3. **c-f**, serum total glycerides (**c**), total cholesterol (**d**), creatinine (**e**), and urea (**f**) levels of control and *Sdhaf4*^{Alb} KO mice after hepatic *Sdhaf4* overexpression for 3 weeks, n=6 or 8. **g-h**, Glucose tolerance test (**g**) and insulin tolerance test (**h**) in control mice after adenovirus mediated hepatic *Sdhaf4* overexpression for 2 weeks, n=6, or 8. **i**, Immunoblots analysis of p-Akt level in iWAT, muscle, and liver tissues of control and *Sdhaf4*^{Alb} KO mice under adenovirus infection with or without insulin challenge, n=3. Values are mean ± SEM, *p< 0.05, **p< 0.01.

Supplemental Figure 5

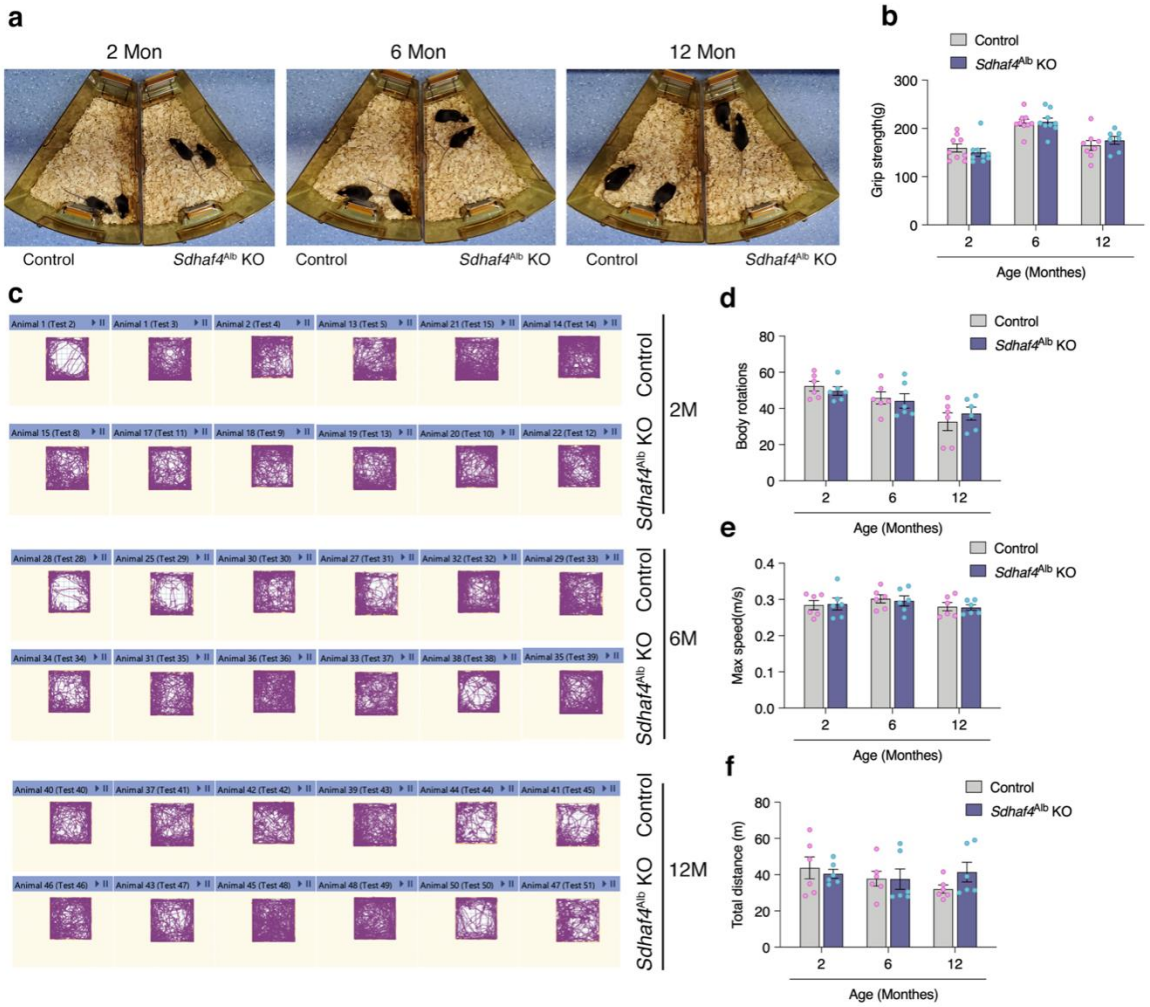

Figure S5 (Related to Figure 3). Aging performance of hepatic *Sdhaf4* knockout mice. a, Representative images of control and *Sdhaf4*^{Alb} KO mice at the age of different ages. **b**, Grip strength analysis of control and *Sdhaf4*^{Alb} KO mice at the age of different ages, n=9. **c-f**, Open filed test of control and *Sdhaf4*^{Alb} KO mice at the age of different ages, trace image of mice activity (**c**), body rotation records (**d**), max speed travelled (**e**), and total distance travelled (**f**), n=6. Values are mean ± SEM.

Supplemental Figure 6

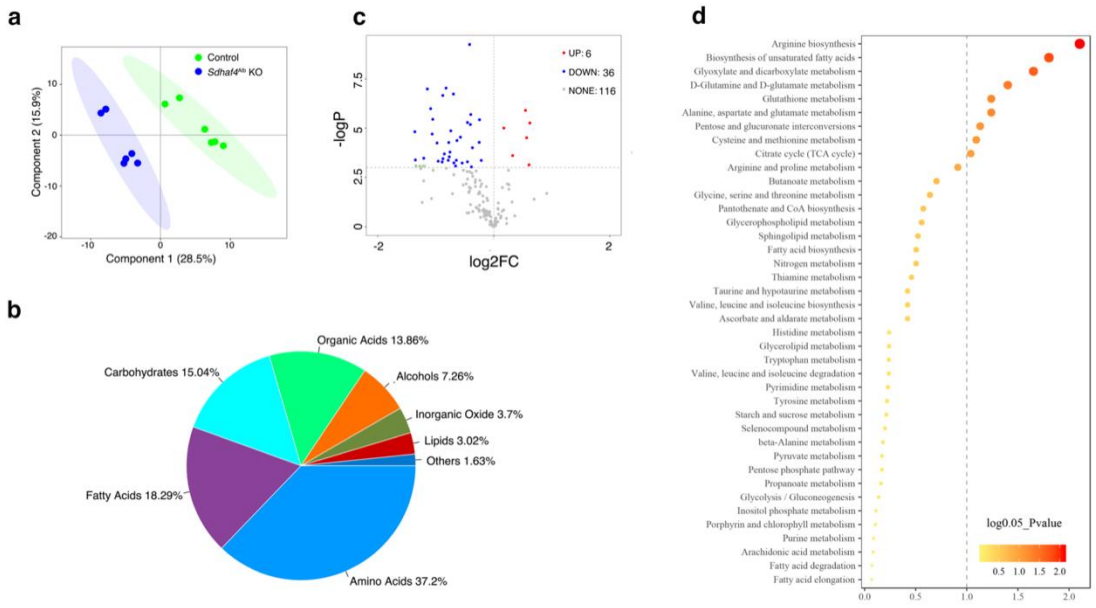

Figure S6 (Related to Figure 6). Metabolomics analysis for serums of control and *Sdhaf4*^{Alb} KO mice. a, 2D PLS-DA scores plot revealing classifications of the samples. **b**, Metabolite classes and compositions. **c**, Enhanced volcano plot showing the differential metabolites. **d**, Metabolic pathway enrichment analysis, n=6.

Supplemental Figure 7

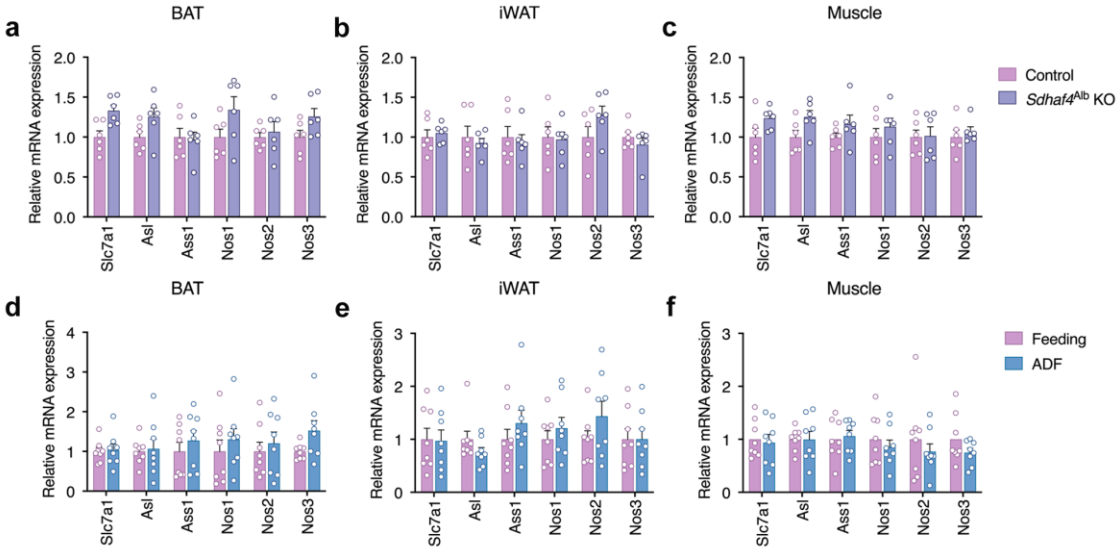

Figure S7(Related to Figure 6). NO synthesis in peripheral tissues of *Sdhaf4*^{Alb} KO mice and ADF intervened mice. a-c, mRNA levels of NO synthesis related genes in the BAT (**a**), iWAT (**b**), and muscle (**c**) tissues of control and *Sdhaf4*^{Alb} KO mice, n=6. **e-f**, mRNA levels of NO synthesis related genes in the BAT (**d**), iWAT(**e**), and muscle (**f**) tissues of mice under regular feeding or ADF intervention for 8 weeks, n=8. Values are mean ± SEM, *p< 0.05, **p< 0.01.

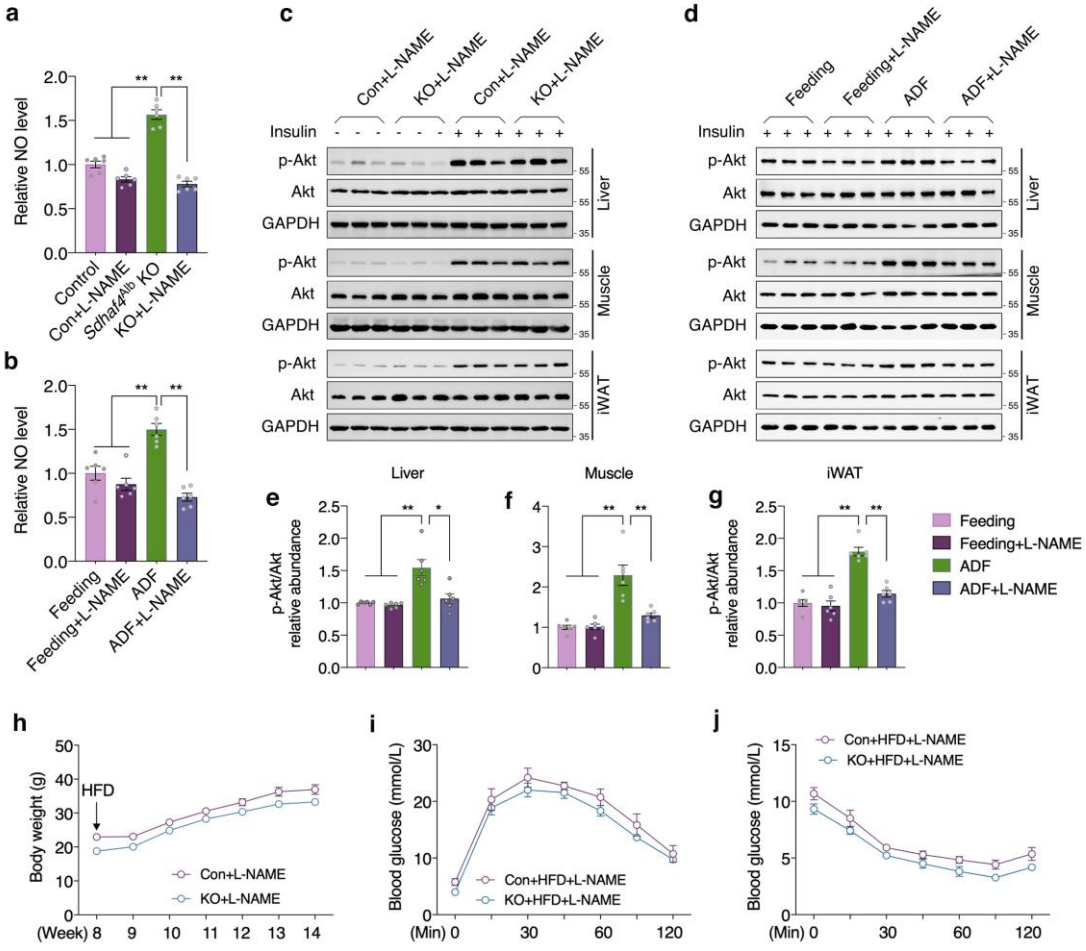

Figure S8 (Related to Figure 6). Inhibition of NO production abolishes metabolic benefits of *Sdhaf4*^{Alb} KO mice. a, Relative NO level of control and *Sdhaf4*^{Alb} KO mice under L-NAME supplement. **b,** Relative NO level of regular feeding and ADF intervened mice under L-NAME supplement. **c,** Immunoblots analysis of p-Akt level in iWAT, muscle, and liver tissues of control and *Sdhaf4*^{Alb} KO mice under L-NAME supplement with or without insulin challenge, n=3. **d-g**, Immunoblots analysis of p-Akt level in iWAT, muscle, and liver tissues of regular feeding and ADF intervened mice under L-NAME supplement with or without insulin challenge, n=6. **h**, Body weight curve of control and *Sdhaf4*^{Alb} KO mice supplement with L-NAME for 7 weeks and HFD for 6 weeks, n=8. **i-j**, Glucose tolerance test (**i**) and insulin tolerance test (**j**) for control and *Sdhaf4*^{Alb} KO mice supplement with L-NAME for 7 weeks and HFD for 6 weeks, n=8. Values are mean ± SEM, *p< 0.05, **p< 0.01.

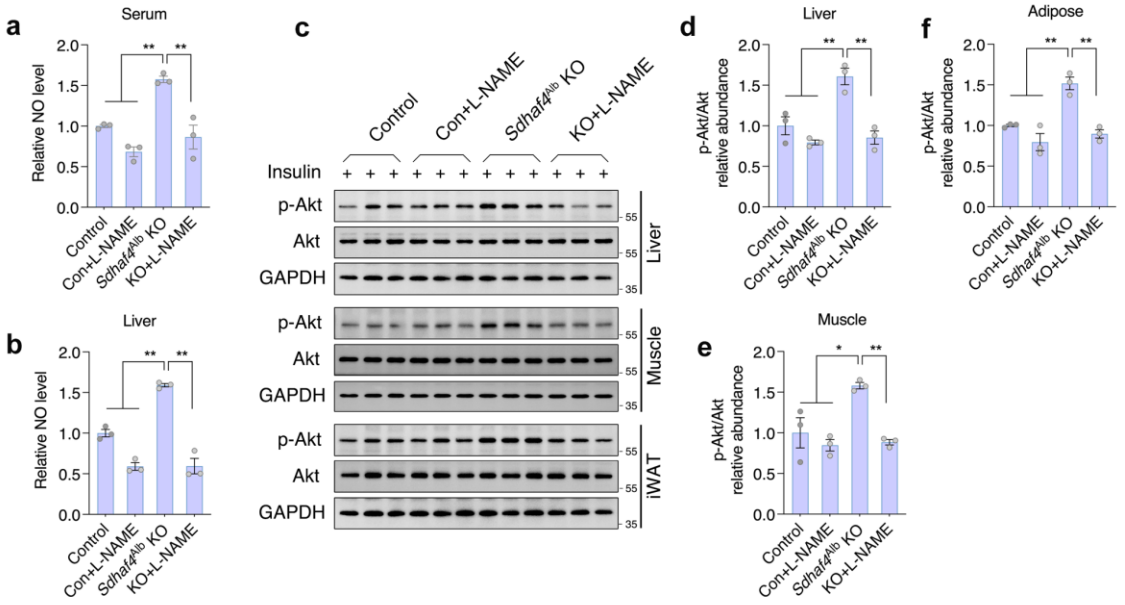

Figure S9 (Related to Figure 6). Short-term inhibition of NO production suppresses insulin signaling in target tissues. **a-b**, Serum NO (**a**) and hepatic NO (**b**) level in the control and *Sdhaf4*^{Alb} KO mice with or without L-NAME supplement for 24 h, n=3. **c-f**, Immunoblots analysis of p-Akt level in iWAT, muscle, and liver tissues of control and *Sdhaf4*^{Alb} KO mice under L-NAME supplement for 24h, followed by insulin challenge for 15 min, n=3. Values are mean ± SEM, *p< 0.05, **p< 0.01.

Supplemental Table 1

Species	Gene	Primer (5'-3')
Mouse	*Gapdh*	Forward: CTTCAACAGCAACTCCCACTCTTCC
		Reverse: GGTGGTCCAGGGTTTCTTACTCC
Mouse	*Sdhaf1*	Forward: AAGTTCTGAGCCTGTACCGC
		Reverse: TTAAACGAGCGCGGCAAAAA
Mouse	*Sdhaf2*	Forward: ACACACAGGGCAGTCATCTG
		Reverse: ATAGCACCCTTGGGCTTGAC
Mouse	*Sdhaf3*	Forward: TAAGACTGTTGGTCCTGGCG
		Reverse: GGGAGAGAGGTGCCAAAACA
Mouse	*Sdhaf4*	Forward: AGGGACGCTGCATTGACTTT
		Reverse: GACTCTGCCCTGACACACTT
Mouse	*Asl*	Forward: CTCTCAACCTGCTGTCGCTA
		Reverse: CTGTCTCTCTTTGCATTGGCA
Mouse	*Ass1*	Forward: ACCTCCGGGTCTCTACACAA
		Reverse: ATTCAGGGCTGTGCCAGAAA
Mouse	*Nos1*	Forward: CGGGTGTCGACAATCCAAGA
		Reverse: GGGAGGATCCAGTTAGGAGC
Mouse	*Nos2*	Forward: ACAGGGAGAAAGCGCAAAAC
		Reverse: TGCTGTGCTACAGTTCCGAG
Mouse	*Nos3*	Forward: CATGGGCAACTTGAAGAGTGTG
		Reverse: TAGGTGATGCTGCCCACTTC
Mouse	*Slc7a1*	Forward: GAAGGGCTCATTGTGGATCTCT
		Reverse: CCGTCACGTAGCTGTAGAGG
Mouse	*Sdha*	Forward: AGAGATACGCACCTGTTGCC
		Reverse: ACTGGGATGGGCTCCTTAGT
Mouse	*Sdhb*	Forward: TTCCACTCGTTGGCGCTTAG
		Reverse: CCTCGACAGGCCTGAAACTG
Mouse	*Sdhc*	Forward: TGCGTTCTTGCTGAGACAT
		Reverse: AGAGAGACCCCTCCACTCAA
Mouse	*Sdhd*	Forward: CCCAGCACATTCACCTGTCA
		Reverse: GTCCCCATGAACGTAGTCGG
Mouse	*ND1*	Forward: CATTCTAATCGCCATAGCCT
		Reverse: GTTGTTAAAGGGCGTATTGG
Mouse	*ND2*	Forward: AAATCCTATCACCCTTGC
		Reverse: TTTGTTGCTGCTTCAGTT
Mouse	*ND3*	Forward: CTGACTCCCCCAAATAAATCT
		Reverse: TGAATTGCTCATGGTAGTGG
Mouse	*ND4*	Forward: CCATGTAGGAACCCTAAACC

		Reverse: TTCCTCATAGGGAGAGAAGG
Mouse	*ND4L*	Forward: GGACACTTATATTTCGCTCT
		Reverse: TTGGACGTAATCTGTTCCGTA
Mouse	*ND5*	Forward: TATAACCGCATCGGAGAC
		Reverse: TGGTAGTCATGGGTGGAG
Mouse	*Ndufs1*	Forward: CTCCTCTTGCCCTTGACTGG
		Reverse: CCAGCCCTTCATTACAGGCA
Mouse	*Ndufa1*	Forward: GAGAGGTAAAGCCGGGTCAC
		Reverse: GACCAAGCACACCCCCATAA
Mouse	*Cytb*	Forward: CTGTTCGCAGTCATAGCC
		Reverse: AAGAATCGGGTCAAGGTG
Mouse	*Cox1*	Forward: GAGCGGGAATAGTAGGCACC
		Reverse: CGGCTAGAGGTGGGTAGACT
Mouse	*Cox2*	Forward: GCCGACTAAATCAAGCAA
		Reverse: TAGGACAATGGGCATAAA
Mouse	*Cox3*	Forward: CGAAACCACATAAATCAAG
		Reverse: GTCGTAGTAGGCAAACAA
Mouse	*Cox6c*	Forward: AGCGTCTGCGGGTTCATATT
		Reverse: CGCCAAACTTATAGGCAGCG
Mouse	*Cox7c*	Forward: ATTTCTTCCGCCTTCCGTGT
		Reverse: CCGCCACTTGTTTTCCACTG
Mouse	*Uqcrq*	Forward: TGAGCCACGCGTCTATCTTC
		Reverse: TTGGCTGTAGCAGTCAAGGG
Mouse	*Uqcrb*	Forward: TCTCAGGTCAAAATGGCGGG
		Reverse: GTATGGTGAGACCAGGCACA
Mouse	*Atp6*	Forward: CCTATTCCCATCCTCAAA
		Reverse: GGGTTCATGTTCGTCCTT
Mouse	*Atp8*	Forward: ACTGGCACCTTCACCAAA
		Reverse: GGTAATGAATGAGGCAAATAGA
Mouse	*Atp5pb*	Forward: TCCAGGGGTATTACAGGCAAC
		Reverse: ATTGGCTGAGCTTGAGCCTT
Mouse	*Atp5b*	Forward: GTTGGTCCTGAGACCTTGGG
		Reverse: TCCGATTTTCCCACCCTTGG