

## SUPPLEMENTARY MATERIAL

### SUPPLEMENTARY FIGURE LEGENDS

**Supplemental Figure 1.** *Atp6V0d1* expression changes during differentiation in 3T3-L1 cells, and adipogenesis is affected by *Atp6V0d1* inhibition. **A-D**, Fold change in expression of *Atp6V0d1*, *Pparg*, *Cebpa* and *Cebpb* genes during 3T3-L1 adipocyte differentiation. Gene expression is normalized by the geometric mean of the expression of *β-actin*, *Gapdh* and *36b4* (n = 3 per group). **E**, Representative western blot of ATP6V0D1 and PPAR-γ in 3T3-L1 adipocytes from Days 0–12 of differentiation (n = 3 per group). **F**, Representative image of lipid droplets stained by Oil Red O and fluorescent dye in 3T3-L1 adipocytes. **G**, Representative western blot of ATP6V0D1, PPAR-γ and CEBPα expression in 3T3-L1 adipocytes treated with shRNA targeting *Atp6v0d1* or control shRNA (n = 3 per group). **H-I**, Representative immunoblots and quantification of ATP6V0D1 protein levels in normal chow diet (NCD) and high fat diet (HFD) mice. A-D and I were analyzed by two-tailed unpaired Student's *t*-test. Data represent the mean ± SEM.

**Supplemental Figure 2.** Loss of adipose *Atp6v0d1* induces hepatosteatosis and lipodystrophy. **A**, Construction strategy for adipocyte-specific *Atp6v0d1* knockout mice (*Atp6v0d1<sup>AKO</sup>*). **B**, Representative gel electrophoresis image showing genomic excision of *Atp6v0d1* region in adipose tissues of *Atp6v0d1<sup>AKO</sup>*. **C**, Representative western blot of ATP6V0D1 in adipose tissues of control and *Atp6v0d1<sup>AKO</sup>*. **D**, Representative images of control and *Atp6v0d1<sup>AKO</sup>* mice. **E**, Representative images of liver, inguinal WAT (iWAT), epidermal white adipose tissue (eWAT), retroperitoneal WAT (rWAT), and brown adipose tissue (BAT). **F**, H&E staining of liver and adipose tissue sections. **G**, Plasma leptin. **H**, Plasma adiponectin. **I**, Liver weight. **J**, Hepatic cholesterol. **K**, Hepatic triglyceride. **L**, Hepatic non-esterified fatty acids. **M**, AST to ALT ratio.

**Supplemental Figure 3.** *Atp6v0d1<sup>AKO</sup>* mice aged 36 weeks exhibit fatty liver,

**lipodystrophy and insulin resistance.** **A**, Plasma cholesterol. **B**, Plasma triglycerides. **C**, Plasma non-esterified fatty acids. **D**, Intraperitoneal glucose tolerance test (IPGTT). **E**, Insulin tolerance test (ITT). **F**, Liver weight.

**Supplemental Figure 4.** **A**, Heart rate data of control and *Atp6v0d1*<sup>AKO</sup> mice in cardiac function examination by echocardiography in 8, 12, 16, 20, 24, 28 and 36 weeks. **B**, EF% and FS% of control and *Atp6v0d1*<sup>AKO</sup> mice aged 8, 12, 16, 20, 24, 28 and 36 weeks. **C**, qPCR quantification of the mRNA levels of *Nppa*, *Nppb*, *Myh6* and *Myh7* from control and *Atp6v0d1*<sup>AKO</sup> heart tissues. Data represent the mean ± SEM (n = 4 - 5 per group; two-tailed unpaired Student's t-test)

**Supplemental Figure 5.** **A**, Representative images of adult mice cardiomyocytes isolated from WT and *Atp6v0d1*<sup>AKO</sup> hearts. **B**, Representative immunoblots of individual respiratory chain complexes.

**Supplemental Figure 6.** **A**, Differentially expressed genes analyzed by volcano plots between control and *Atp6v0d1*<sup>AKO</sup> mice hearts. **B**, The top 10 (a ranking by P-value) significantly enriched GO terms in biological process group (BP), cellular component group (CC) and molecular function group (MF). **C**, The 15 most relevant Reactome pathways (<https://reactome.org>) according to the upregulated and downregulated subgroups of DEGs.

**Supplemental Figure 7. Rosiglitazone ameliorates cardiomyopathy in *Atp6v0d1*<sup>AKO</sup> mice.** **A**, Schematic illustration of Rosiglitazone treatment (50 µg per 10 g mouse weight), delivered via intragastric gavage to control and *Atp6v0d1*<sup>AKO</sup> mice. **B**, Echo measurement of left ventricular ejection fraction (EF%) and fractional shortening (FS%) of control and *Atp6v0d1*<sup>AKO</sup> mice with or without Rosiglitazone treatment. **C**, Representative images of hearts from control and *Atp6v0d1*<sup>AKO</sup> mice with or without Rosiglitazone treatment. **D-E**, Heart weight (HW)

to tibia ratio and lung weight to tibia ratio of control and *Atp6v0d1*<sup>AKO</sup> mice with or without Rosiglitazone treatment. **F-H**, Representative immunoblots and quantification of IRS1 and IRS2 protein levels. Data are normalized to GAPDH. **I**, Representative immunoblots of FOXO1, AMPK, p-AMPK and PPAR $\gamma$  protein levels for each group. **J-L**, Quantification of specified densitometric ratios. M, Representative immunoblots of CD36, PDH and p-PDH protein levels. **N-O**, Quantification of specified densitometric ratios (n = 4–6 per group). Data represent the mean  $\pm$  SEM (one-way ANOVA).

**Supplemental Figure 8.** **A**, Echocardiographic evaluation of systolic function of control and *Atp6v0d1*<sup>AKO</sup> mice after tail vein injection of AAV9-cTnt-myocardin or AAV9-vectors. n = 4-6 mice per group. Data are presented as mean  $\pm$  SEM. **B**, The myocardin protein expression in mice cardiomyocyte by immunostaining, cardiomyocyte size and fibrosis in *Atp6v0d1*<sup>AKO</sup> mice after tail vein injection of AAV9-cTnt-myocardin or AAV9-vectors. Left: Immuno-fluorescence imaging of myocardin. Middle: Representative images of WGA stain. Right: Representative transverse sections with Masson trichrome staining. **C-D**, Representative immunoblots and quantification of IRS1 protein levels in NCD and HFD mice. Data represent the mean  $\pm$  SEM (n = 4 per group; two-tailed unpaired Student's t-test). **E-F**, Representative immunoblots and quantification of IRS2 protein levels in NCD and HFD mice. Data represent the mean  $\pm$  SEM (n = 3 per group; two-tailed unpaired Student's t-test).

**Supplemental Figure 9.** Representative immunoblots and quantification of ANP protein levels in rat neonatal cardiomyocytes infected with myocardin siRNA (si-myocardin) or scrambled siRNA (si-Scramble) as a control. Data represent the mean  $\pm$  SEM (n = 7 per group; two-tailed unpaired Student's t-test)

**Supplementary Table 1. Antibodies used in the study**

Target	Species originated	Dilution	Brand	Catalog number
ATP6V0d1	Rabbit	1:2000	Abcam	ab202897
PPAR $\gamma$	Rabbit	1:1000	CST	#2435
CEBP $\alpha$	Rabbit	1:500	Proteintech	18311-1-AP
FASN	Rabbit	1:500	Proteintech	10624-2-AP
$\beta$ -tubulin	Mouse	1:5000	Proteintech	66240-1-Ig
$\beta$ -actin	Mouse	1:5000	Proteintech	60008-1-Ig
ANP	Rabbit	1:1000	Abclonal	A1609
$\alpha$ MHC	Rabbit	1:1000	ABclonal	A12964
$\beta$ MHC	Rabbit	1:1000	Santa cruz	Sc-168678
CD36	Rabbit	1:1000	Abcam	ab133625
Glut1	Rabbit	1:1000	Proteintech	21829-1-AP
Glut4	Mouse	1:1000	Proteintech	66846-1-Ig
PDH	Rabbit	1:1000	Proteintech	18068-1-AP
p-PDH	Rabbit	1:1000	Absin	Abs140331
IRS1	Rabbit	1:1000	CST	#2390
IRS2	Rabbit	1:1000	CST	#3089
FoxO1	Rabbit	1:1000	CST	#2880
PPAR $\alpha$	Rabbit	1:1000	Abcam	ab215270
PPAR $\delta$	Rabbit	1:1000	Abcam	ab178866
Pgc1 $\alpha$	Rabbit	1:1000	Abcam	ab191838
AMPK	Rabbit	1:1000	CST	#5831
p-AMPK	Rabbit	1:1000	CST	#2535
PI3K	Rabbit	1:1000	CST	#4257
p-PI3K	Rabbit	1:1000	CST	#4228
AKT	Rabbit	1:1000	CST	#9272
p-AKT-S473	Rabbit	1:1000	CST	#9271

p-AKT-T308	Rabbit	1:5000	CST	#9275
MYOCD	Rabbit	1:1000	sigma-aldrich	SAB4200539
SRF	Rabbit	1:100 (ChIP)	CST	#5147
GAPDH	Rabbit	1:5000	CST	#2118

**Supplementary Table 2. Primers used in the study**

Gene		
Atp6v0d1-m	Forward	5'-CAATGCCATTCTGGTGGAC-3'
	Reverse	5'-TTTCGGATTATCTCGATGTTCA-3'
Pparg-m	Forward	5'-GAAAGACAACGGACAAATCACC-3'
	Reverse	5'-GGGGGTGATATGTTGAACTTG-3'
Cebpa-m	Forward	5'-CAAGAACAGCAACGAGTACCG-3'
	Reverse	5'-GTCACTGGTCAACTCCAGCAC-3'
Cebpb-m	Forward	5'-CTGCGGGGTTGTTGATGT-3'
	Reverse	5'-ATGCTCGAAACGGAAAAGGT-3'
Fasn-m	Forward	5'-TGGGTTCTAGCCAGCAGAGT-3'
	Reverse	5'-ACCACCAGAGACCGTTATGC-3'
Scd1-m	Forward	5'-GCGATACACTCTGGTGCTCA-3'
	Reverse	5'-CCCAGGGAAACCAGGATATT-3'
Dgat1-m	Forward	5'-TTCCGCCTCTGGGCATT-3'
	Reverse	5'-AGAATCGGCCACAATCCA-3'
Dgat2-m	Forward	5'-GGCGCTACTCCGAGACTAC-3'
	Reverse	5'-TGGTCAGCAGGTTGTGTGTC-3'
Fabp4-m	Forward	5'-GGATGGAAAGTCGACCACAA-3'
	Reverse	5'-TGGAAGTCACGCCTTCATA-3'
Fatp1-m	Forward	5'-AGGTCAATGAGGACACGATGGAG-3'
	Reverse	5'-CTGGTACATTGAGTTAGGGTCCAAC-3'
Lipe-m	Forward	5'-GCGCTGGAGGAGTGTFFFF-3'
	Reverse	5'-CGCTCTCCAGTTGAACCAAG-3'
Lpl-m	Forward	5'-GGGAGTTGGCTCCAGAGTT-3'
	Reverse	5'-TGTGTCTTCAGGGTCCTAG-3'
Plin1-m	Forward	5'-AACGTGGTAGACACTGTGGTACA-3'
	Reverse	5'-TCTCGGAATTGCGCTCTCG-3'
Plin2-m	Forward	5'-GACCTTGTGTCCTCCGCTTAT-3'

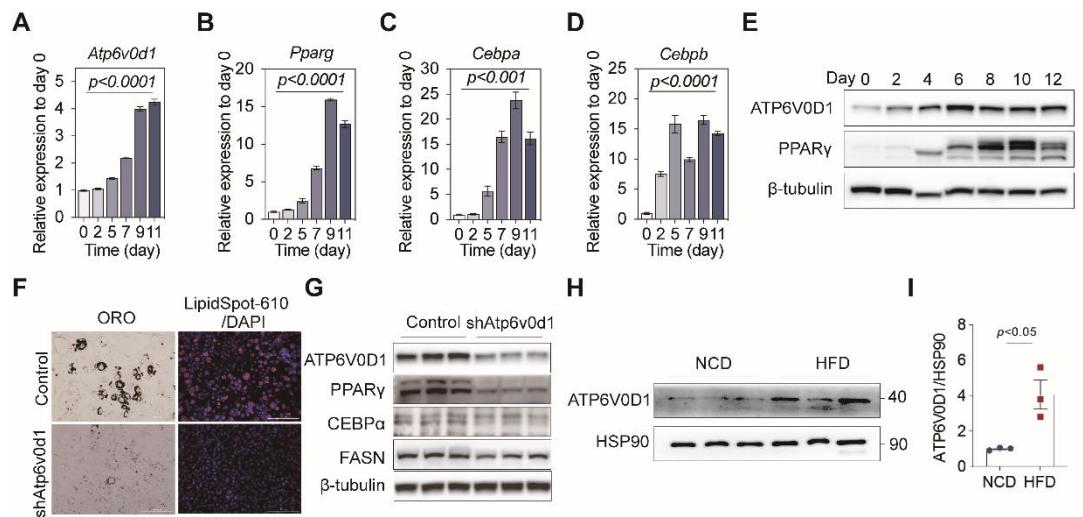
	Reverse	5'-CAACCGCAATTGTGGCTC-3'
Plin3-m	Forward	5'-GGAGGAACCTGTTGTGCAG-3'
	Reverse	5'-ACCATCCCATACTGTGGAAC-3'
Adpoq-m	Forward	5'-GGAGAGAAAGGAGATGCAGGT-3'
	Reverse	5'-CTTCCTGCCAGGGTTC-3'
Pepck-m	Forward	5'-ATGTGTGGCGATGACATT-3'
	Reverse	5'-AACCGTTTCTGGGTTGAT-3'
36b4-m	Forward	5'-ACTGGTCTAGGACCCGAGAAG-3'
	Reverse	5'-CTCCCACCTTGTCTCCAGTC-3'
$\beta$ -actin-m	Forward	5'-CTAAGGCCAACCGTGAAAAG-3'
	Reverse	5'-ACCAGAGGCATACAGGGACA-3'
Gapdh-m	Forward	5'-AGCTTGTCACTAACCGGAAG-3'
	Reverse	5'-TTGATGTTAGTGGGGTCTCG-3'
Myh6-m	Forward	5'-GCCAGTACCTCCGAAAGTC-3'
	Reverse	5'-GCCTTAACATACTCCTCCTGTC-3'
Myh7-m	Forward	5'-CGTCCAATGACGACC-3'
	Reverse	5'-CACTGCCTCCTCTACTTCTG-3'
Nppa-m	Forward	5'-GCTTCCAGGCCATATTGGAG-3'
	Reverse	5'-GGGGCATGACCTCATCTT-3'
Nppb-m	Forward	5'-GAGGTCACTCCTATCCTCTGG-3'
	Reverse	5'-GCCATTCCCTCCGACTTTCTC-3'
Cd36-m	Forward	5'-ACAGACGCAGCCTCCT-3'
	Reverse	5'-GATTCAGATCCGAACA-3'
Acadl-m	Forward	5'-TTCCTCGGAGCATGACATTTC-3'
	Reverse	5'-GCCAGCTTTCCCAGACCT-3'
Acadm-m	Forward	5'-TGATGTGGCGGCCATTAAGA-3'
	Reverse	5'-GGTTAGAACGTGCCAACAGAA-3'
Acads-m	Forward	5'-AAGTTGGATCCGCACAGCAG-3'
	Reverse	5'-CAAGCTTGGTGCCGTTGAG-3'

Cpt1a-m	Forward	5'-CTCCGCCTGAGCCATGAAG-3'
	Reverse	5'-CACCA GTGATGCCATTCT-3'
Cpt1b-m	Forward	5'-GTCGCTTCTTCAAGGTCTGG-3'
	Reverse	5'-AAGAAAGCAGCACGTTCGAT-3'
Cpt1c-m	Forward	5'-GATTTCTGTACGTCACCCCC-3'
	Reverse	5'-AGGTGGTCTTTCCACCCC-3'
Fasn-m	Forward	5'-GGAGGTGGTGATAGCCGGTAT-3'
	Reverse	5'-TGGGTAATCCATAGAGCCCAG-3'
Acsl1-m	Forward	5'-TGCCAGAGCTGATTGACATTC-3'
	Reverse	5'-GGCATACCAGAACGGTGGTGAG-3'
Acsl3-m	Forward	5'-AACCACGTATCTTCAACACCATC-3'
	Reverse	5'-AGTCCGGTTGGAACTGACAG-3'
Acsl5-m	Forward	5'-TCCTGACGTTGGAACGGC-3'
	Reverse	5'-CTCCCTCAATCCCCACAGAC-3'
Fabp3-m	Forward	5'-ACCTGGAAGCTAGTGGACAG-3'
	Reverse	5'-TGATGGTAGTAGGCTTGGTCAT-3'
Pk-m	Forward	5'-GTTCTCACGGAGTCTGGC-3'
	Reverse	5'-GAGTCACGGCAATGATAGG-3'
Pfk-m	Forward	5'-CTGTGGTCCGAGTTGGTAT-3'
	Reverse	5'-ACGTGGCCTCCCTGAT-3'
Hk1-m	Forward	5'-GGCAGATTGAGGAAACCC-3'
	Reverse	5'-GGATGCTCCGAACATAAGAA-3'
Hk2-m	Forward	5'-ATGCCTGCTTATTACG-3'
	Reverse	5'-CCATTGTCTGTCACCCTTACT-3'
Pdk1-m	Forward	5'-GATGGCTATGAGAACGC-3'
	Reverse	5'-GTATTGTCTGTCCTGGTGAT-3'
Pdk2-m	Forward	5'-CTCCATCCGAATGCTAAC-3'
	Reverse	5'-TGGGAAGGGACATAGACC-3'
Pdk3-m	Forward	5'-CTCAACTGCTCCTCGTCC-3'

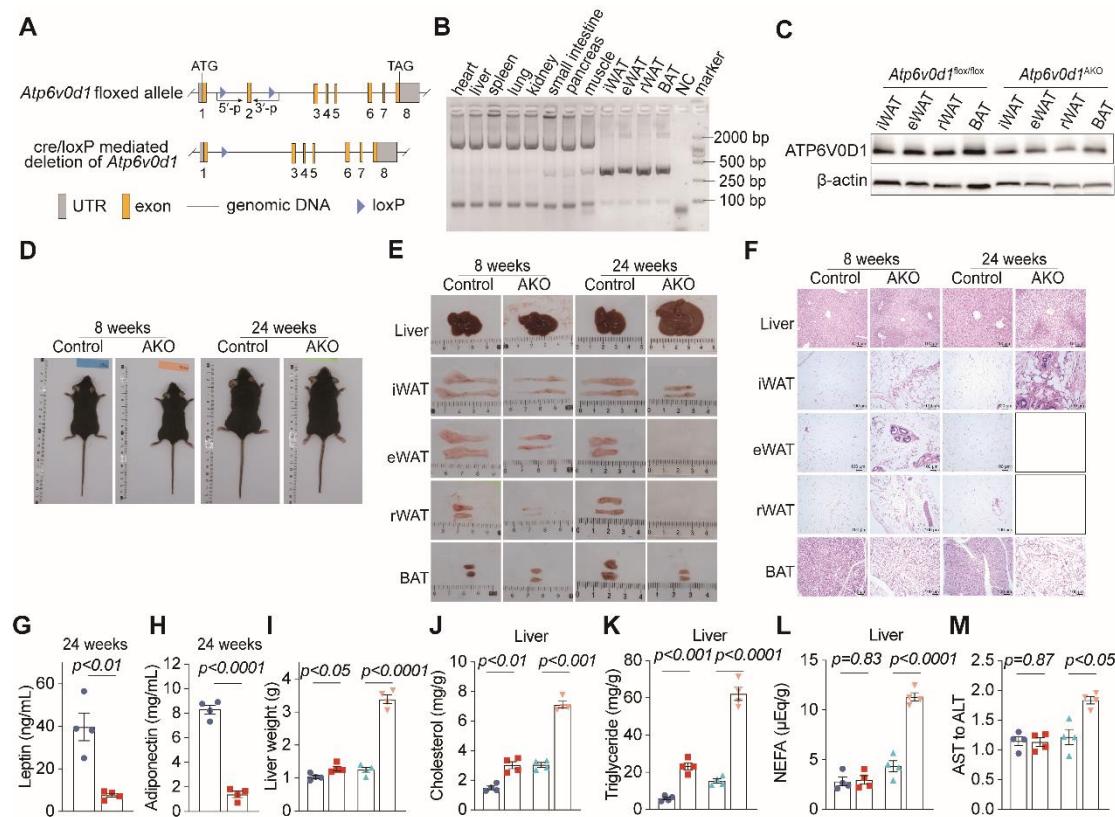
		Reverse	5'-GGCAAGCCATAACCAAAT-3'
Pdk4-m	Forward	5'-TGTGGTCCCTACAATGGC-3'	
	Reverse	5'-AATGTGGCTGGGTTCC-3'	
Cs-m	Forward	5'-AATCAGGAGGTGCTTGTCT-3'	
	Reverse	5'-AGGAATAGCGAGGGTCAG-3'	
Slc2a1-m	Forward	5'-CGTCGTTGGCATCCTTA-3'	
	Reverse	5'-CCTTCTTCTCCCGCATC-3'	
Slc2a4-m	Forward	5'-GCCTGCCCGAAAGAGT-3'	
	Reverse	5'-TGAAGACCGTATTGACCACA-3'	
Myoecd-m	Forward	5'-ACACTCCTGGGTCTGAACA-3'	
	Reverse	5'-GCGGTATTAAGCCTGGTTAGC-3'	
FoxO1-m	Forward	5'-TCAAGGATAAGGGCGACAGC-3'	
	Reverse	5'-CCTCCCTCTGGATTGAGCATHC-3'	
Irs1-m	Forward	5'-CGATGGCTTCTCAGACGTG-3'	
	Reverse	5'-CAGCCCCTTGTTGATGTTG-3'	
Irs2-m	Forward	5'-CTGCGTCCTCTCCCAAAGTG-3'	
	Reverse	5'-GGGGTCATGGCATGTAGC-3'	
Myoecd-r	Forward	5'-GATAGTAAGAACCGCCACAA-3'	
	Reverse	5'-TGAAGCAGCCGAGCAT-3'	
Irs1-r	Forward	5'-CCTGGAGTATTATGAGAACGAG-3'	
	Reverse	5'-CCGCAATGGCAAAGTGT-3'	
Irs2-r	Forward	5'-CCCCTGTGGTCGGATT-3'	
	Reverse	5'-TGAGTGATGAGGCTGGGTATG-3'	
FoxO1-r	Forward	5'-GGATAAGGGCGACAGCAACA-3'	
	Reverse	5'-TCTTGCCTCCCTCTGGATTG-3'	
ChIP	CArG	Forward	5'-TTTGATGGCAAGACGACTT-3'
region-r		Reverse	5'-TTCTGTTCCCTAACAGACACCTAT-3'
ChIP Ctrl1-r	Forward	5'-TAGCACCAACAGGACCCA-3'	
	Reverse	5'-GCATCGTGAAGAAGGCATA-3'	

ChIP Ctrl2-r	Forward	5'-AGCAACCGCAAAGGAAA-3'
	Reverse	5'-AGCAACTACCACTGGGAGAC-3'

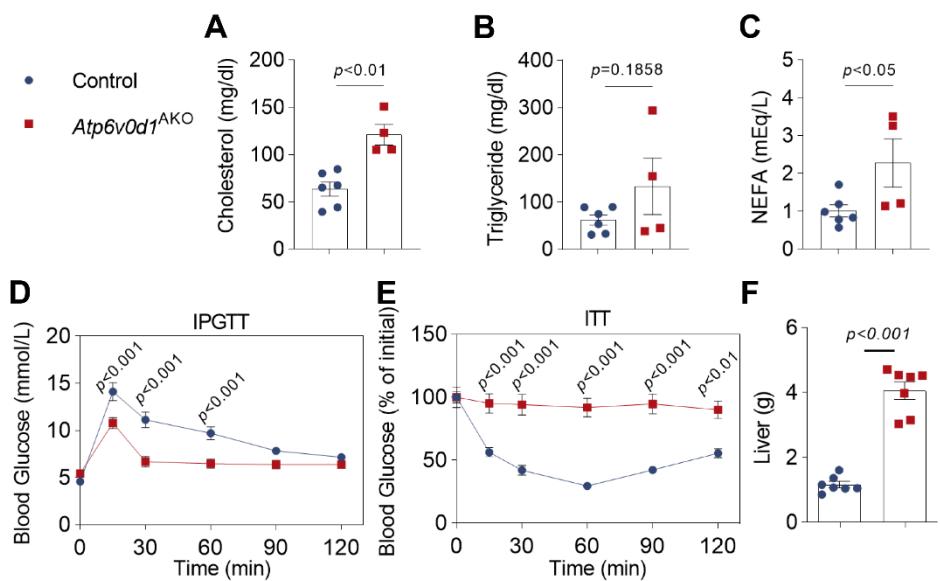
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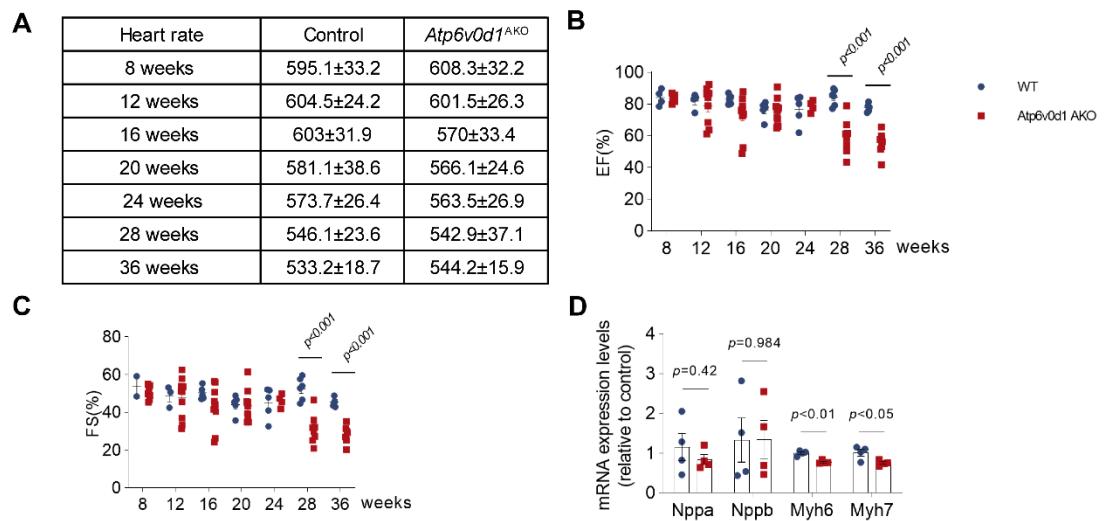
Supplementary Figure 1



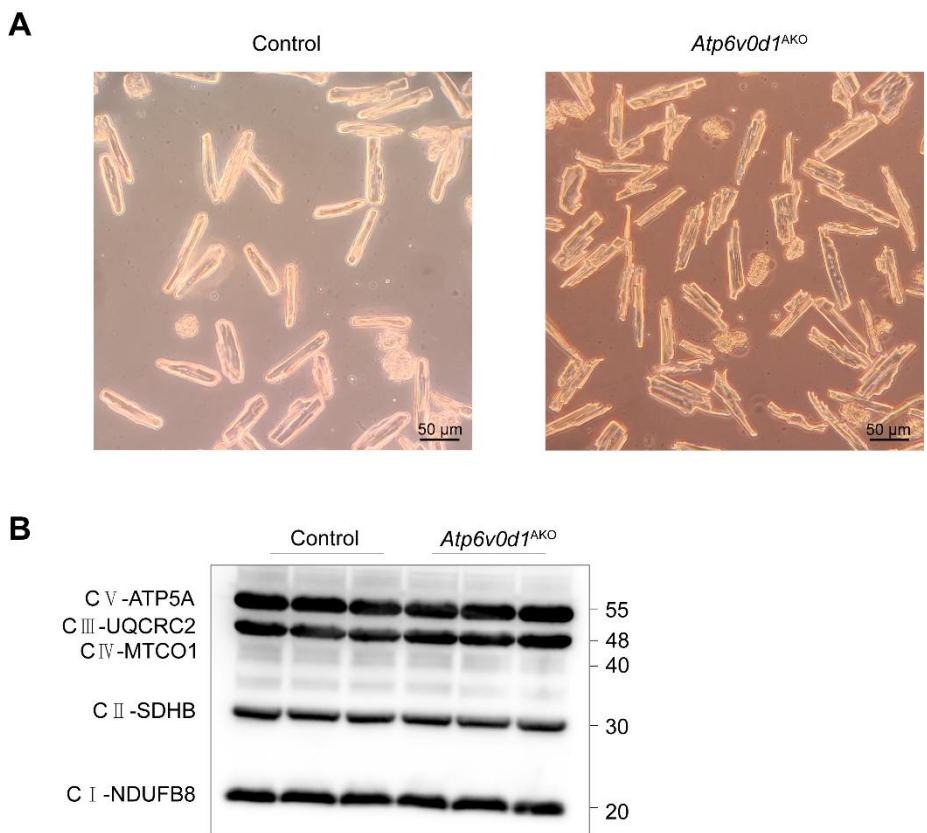
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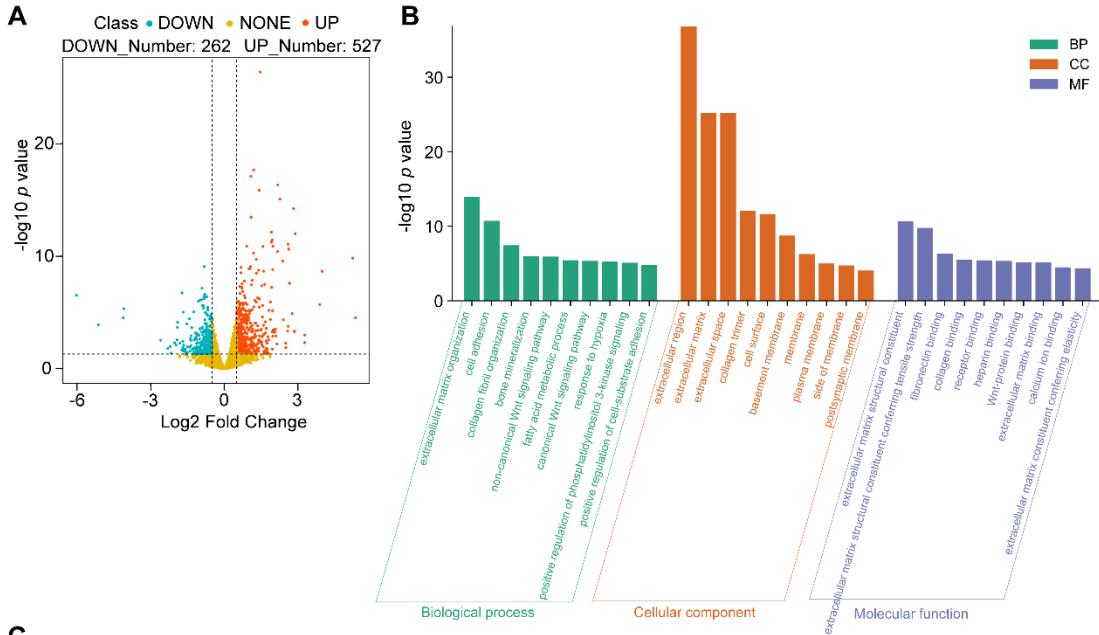


Supplementary Figure 3



Supplementary Figure 4



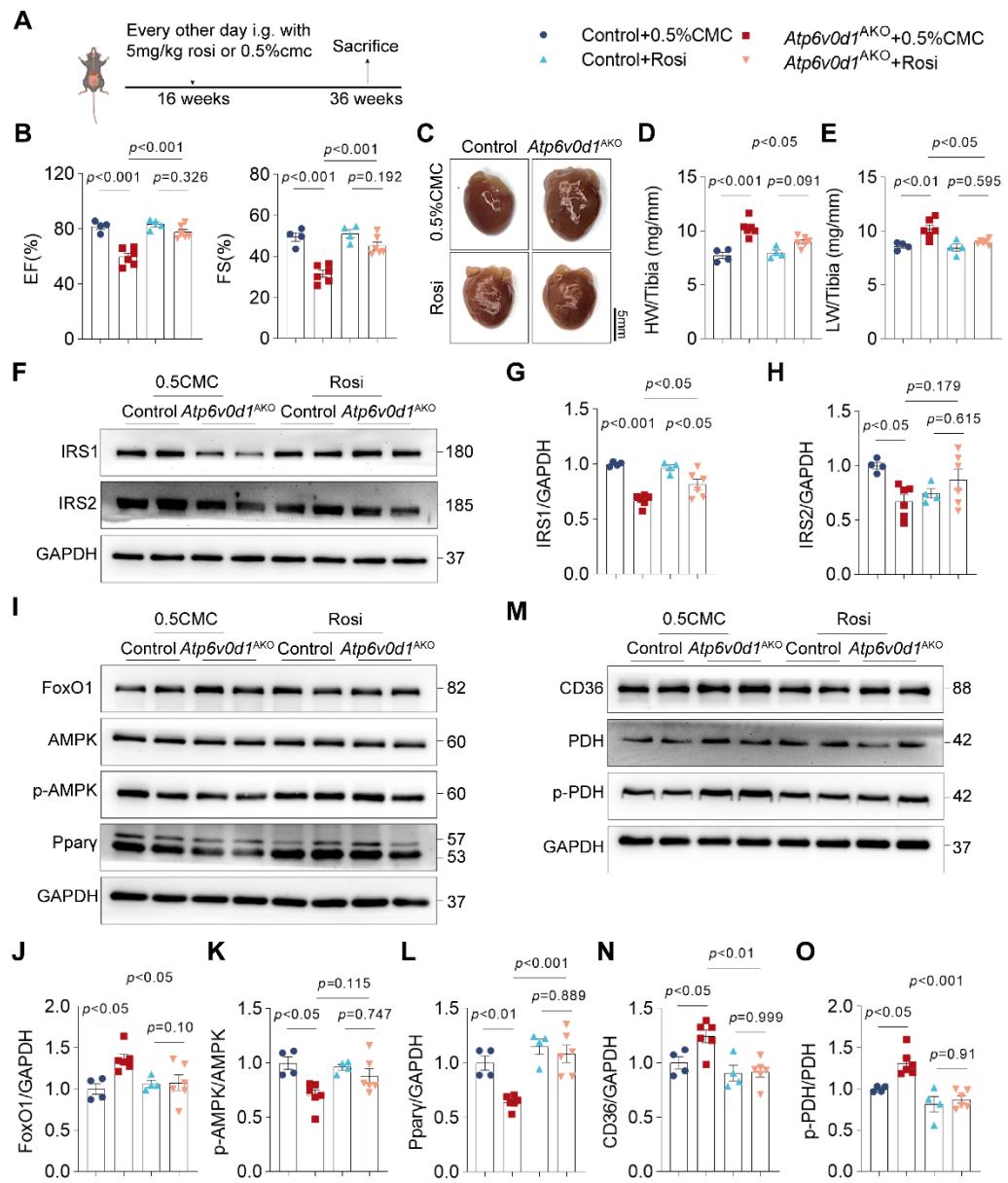


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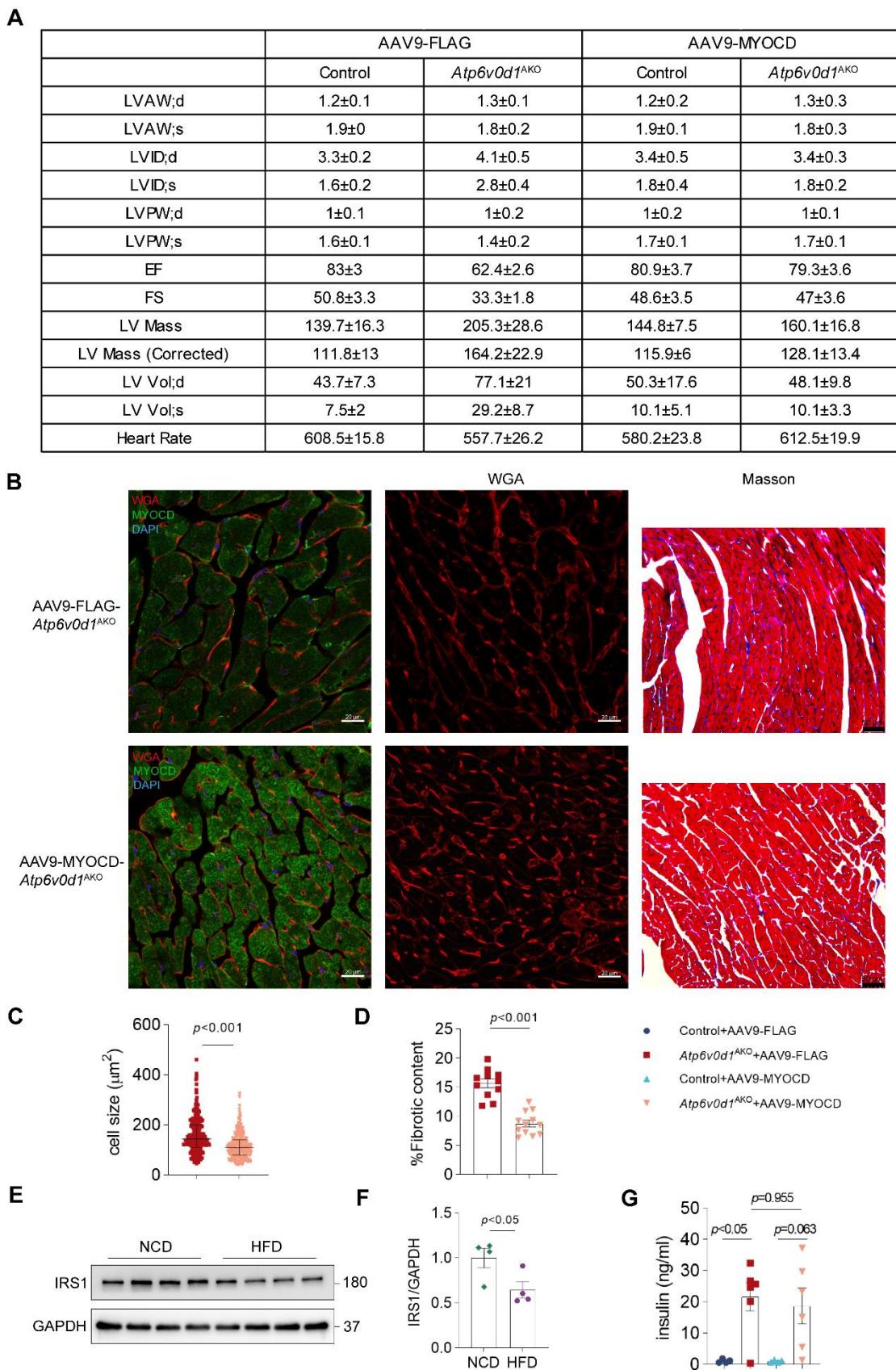
The 15 most relevant pathways sorted by *p* value using the upregulated and downregulated subgroups of DEGs.

	Term	Input Number	Background Number	P-Value	Corrected P-Value
<b>Upregulated Genes</b>					
	Extracellular matrix organization	39	263	2.81E-26	1.55E-23
	Metabolism	71	1609	2.63E-17	7.27E-15
	Degradation of the extracellular matrix	20	128	1.74E-14	3.22E-12
	Collagen formation	16	78	1.85E-13	2.57E-11
	Collagen degradation	14	55	4.78E-13	5.30E-11
	Integrin cell surface interactions	15	70	6.09E-13	5.63E-11
	Assembly of collagen fibrils and other multimeric structures	13	54	6.01E-12	4.76E-10
	Collagen biosynthesis and modifying enzymes	13	57	1.08E-11	7.50E-10
	Collagen chain trimerization	11	38	4.78E-11	2.94E-09
	Metabolism of lipids	30	585	2.39E-09	1.32E-07
	Fatty acid metabolism	16	167	5.14E-09	2.53E-07
	Signal Transduction	68	2305	5.47E-09	2.53E-07
	ECM proteoglycans	9	43	3.22E-08	1.37E-06
	Hemostasis	25	497	7.38E-08	2.92E-06
	Molecules associated with elastic fibres	8	35	1.05E-07	3.87E-06
<b>Downregulated Genes</b>					
	Metabolism	27	1609	1.98E-05	6.75E-03
	Attenuation phase	3	13	1.60E-04	2.72E-02
	Receptor-type tyrosine-protein phosphatases	3	18	3.71E-04	3.27E-02
	Neuronal System	9	314	3.85E-04	3.27E-02
	HSF1-dependent transactivation	3	22	6.29E-04	4.28E-02
	Interleukin-7 signalling	3	26	9.80E-04	4.85E-02
	Regulation of HSF1-mediated heat shock response	4	61	9.99E-04	4.85E-02
	IRS activation	2	6	1.24E-03	4.98E-02
	Protein-protein interactions at synapses	4	66	1.32E-03	4.98E-02
	Cytokine Signaling in Immune system	10	477	1.91E-03	6.06E-02
	PI3K/AKT activation	2	9	2.40E-03	6.06E-02
	Cellular response to heat stress	4	79	2.47E-03	6.06E-02
	Cooperation of PDCL (PhLP1) and TRiC/CCT in G-protein beta folding	3	37	2.51E-03	6.06E-02
	Protein folding	3	38	2.69E-03	6.06E-02
	Chaperonin-mediated protein folding	3	38	2.69E-03	6.06E-02

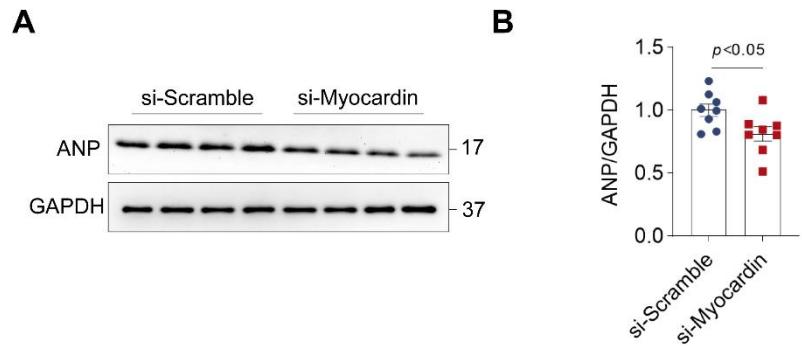
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



**Supplementary Figure 9**