

Homoharringtonine, a novel senotherapeutic, mitigates diet- and age-associated obesity and insulin resistance

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure Legends

Supplementary Figure 1. HHT shows a senotherapeutic activity in multiple human cells and murine preadipocytes. **A.** Secondary screening of 110 compounds in replicatively senescent (RS) human dermal fibroblasts (HDFs) identifies 10 candidate compounds. **B.** Senotherapeutic activity of candidate compounds in various senescent human cells. **C.** senomorphic activity of candidate compounds. **D.** Cytotoxicity of HHT and other senolytics in young (Y) HDFs and preadipocytes. Dose-dependent effect of HHT on senescence-associated β -galactosidase (SA β G) staining (**E**) and LDH activity (**F**) in senescent 3T3-L1 cells. Effect of HHT in apoptosis of human senescent preadipocytes (**G**). Effect of HHT in apoptosis (**H**) and age-associated marker expression (**I**) in senescent 3T3-L1. Scale bar, 200 μ m. Values are presented as means $\pm$ SEM. HHT, homoharringtonine; Y, young; RS, replicatively senescent. RS HDFs, replicatively senescent human dermal fibroblasts; RS HUVEC, replicatively senescent human endothelial cell; PS hrPE, PS human retinal pigment epithelium; LDH, Lactate dehydrogenase; PARP, Poly (ADP-ribose) polymerase (PARP). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0005$.

Supplementary Figure 2. Validation of *in vivo* metabolic effects of candidate compounds. After feeding HFD (60%) for 6 weeks, mice were administered with candidate compounds for 6 weeks. Body weight, food intake, and glucose tolerance (IPGTT) were measured during the treatment (n=5 to 6). **A.** Treatment: PBS, compound 223, compound 386, HHT. **B.** Treatment: PBS, compound 441, compound 584, compound 1242. **C.** Treatment: PBS, compound 1707, compound 930. Compounds 231 and 1806 were withdrawn from the study

due to the toxic effect on mice. **D.** HTT treatment effects on other organs. **E.** HHT treatment effects in young mice. IPGTT: Intraperitoneal glucose tolerance test. HF, high-fat; HHT, homoharringtonine; PBS, phosphate buffered saline; * $p < 0.05$ and ** $p < 0.01$ vs HF-PBS. Data were analyzed with two-tailed Student's t test.

Supplementary Figure 3. HHT moderately increases thermogenesis and browning in the adipose tissue of HFD-fed obese mice. After feeding HFD for 6 weeks, mice were administered with HHT or PBS for 8 weeks while they were kept on HFD. Metabolic cage analysis (A-D). **A.** VO_2 . **B.** VCO_2 . **C.** Energy expenditure. **D.** Histological analysis of brown adipose tissue after staining with H&E. Scale bar, 200 μm . **E.** Expression levels of PGC1 α and UCP1 proteins in the brown adipose tissue. Values are presented as means $\pm$ SEM. HF, high-fat; HHT, homoharringtonine; PBS, phosphate buffered saline; PGC1 α , Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; UCP1, uncoupling protein 1. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Data were analyzed with two-tailed Student's t test.

Supplementary Figure 4. Normalized expression values of the representative genes of cell clusters. **A.** Heatmap reveals top 3 specific genes enriched in each cell population. **B.** Representative gene expression of major cell populations including mature adipocyte (Cluster 5), adipocyte precursor cells (APCs), mesothelial-like cells, macrophages, T- and B-cells. **C.** Expression of *Adipoq*, *Cfd*, *Vegfa* in mature adipocytes. **D.** Histological analysis of liver after staining with H&E. Scale bar, 100 μm . **E.** Triglyceride (TG) levels in the liver. **F.** Plasma levels of alanine aminotransferase (ALT). Values are presented as means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the HF-PBS group; two-tailed Student's t test when comparing two groups.

Supplementary Figure 5. The senotherapeutic effect of HHT restores adipogenic potential of senescent 3T3-L1 preadipocytes. **A.** Senescence-associated β -galactosidase (SA β G) staining and quantification. Scale bar, 200 μm . **B.** Expression levels of p16 protein and its quantification. MDI, IBMX, dexamethasone and insulin * $p < 0.05$ and ** $p < 0.01$. Values are presented as means $\pm$ SEM.

Supplementary Figure 6. **A.** Histological analysis of epididymal WAT after staining with H&E and measurement of adipocyte size. Scale bar, 100 μm . **B.** Expression levels of IL-1 β , phosphorylated NF- κ B, and NF- κ B proteins in the epididymal WAT and their quantification (**C**). Values are presented as means $\pm$ SEM. HHT, homoharringtonine; IL-1 β : interleukin-1 β , NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells, PBS, phosphate buffered saline. * p <0.05, ** p <0.01. Data were analyzed with two-tailed Student's t test. **D.** SA β G staining in the human adipose tissue sections. Scale bar, 100 μm .

Supplementary Figure 7. **A.** 12 proteins identified by mass spectrometry. **B.** Western blotting of 5 identified proteins.

Supplementary Figure 8. Long-term treatment effect on health and hematopoiesis. GOT (**A**), GPT (**B**), urine creatin (**C**), urine urea nitrogen (**D**), serum creatin (**E**), and serum urea nitrogen (**F**). **G.** The effect of long-term HHT treatment on hematopoietic cells. Data were analyzed with Student's t test. UN: urea nitrogen.

SUPPLEMENTARY MOVIE LEGENDS

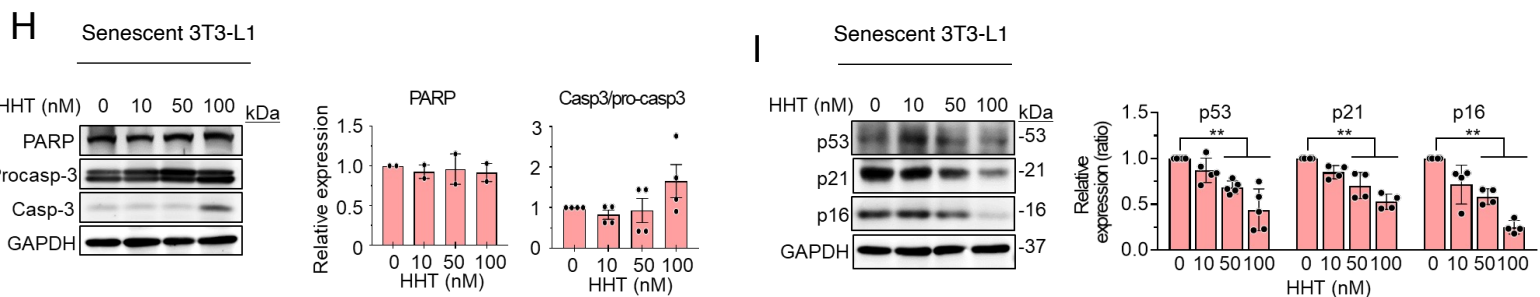
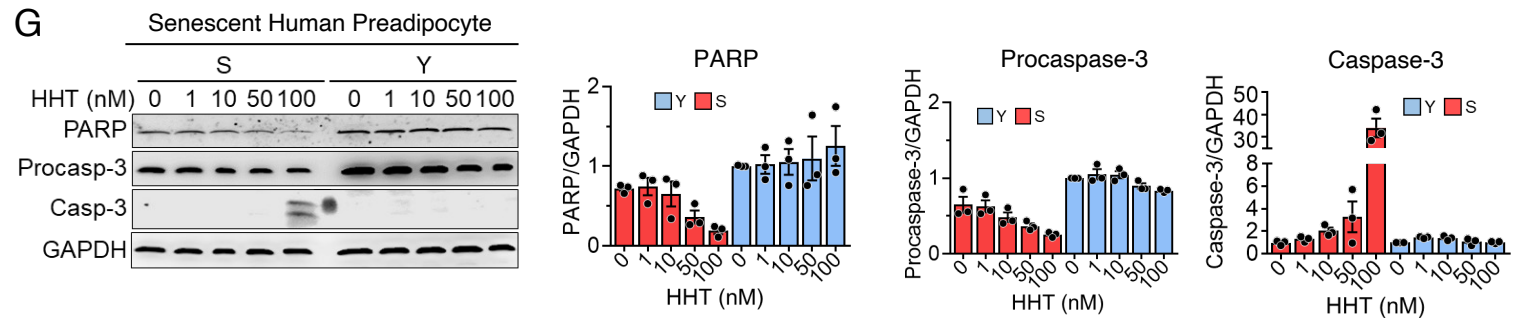
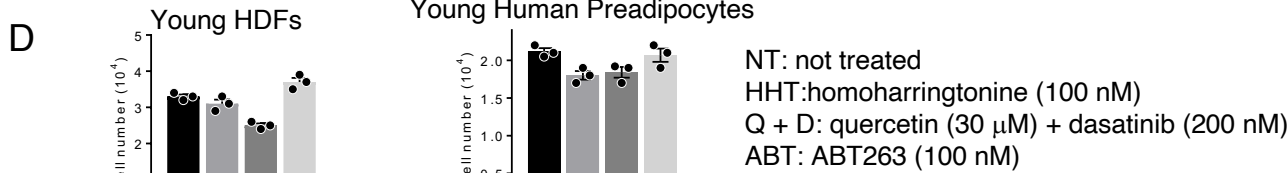
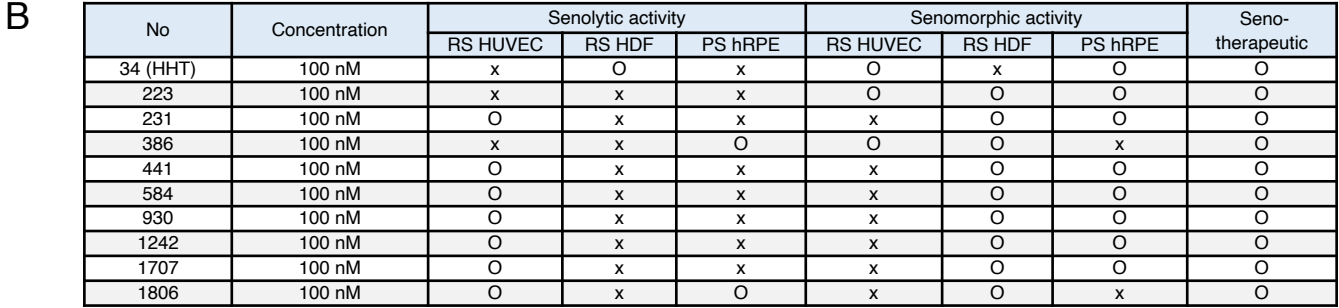
Supplementary Movie 1. HTT promotes healthspan in mouse model of aging. HHT treatment improves physical activity of aged mice.

SUPPLEMENTARY TABLE LEGENDS

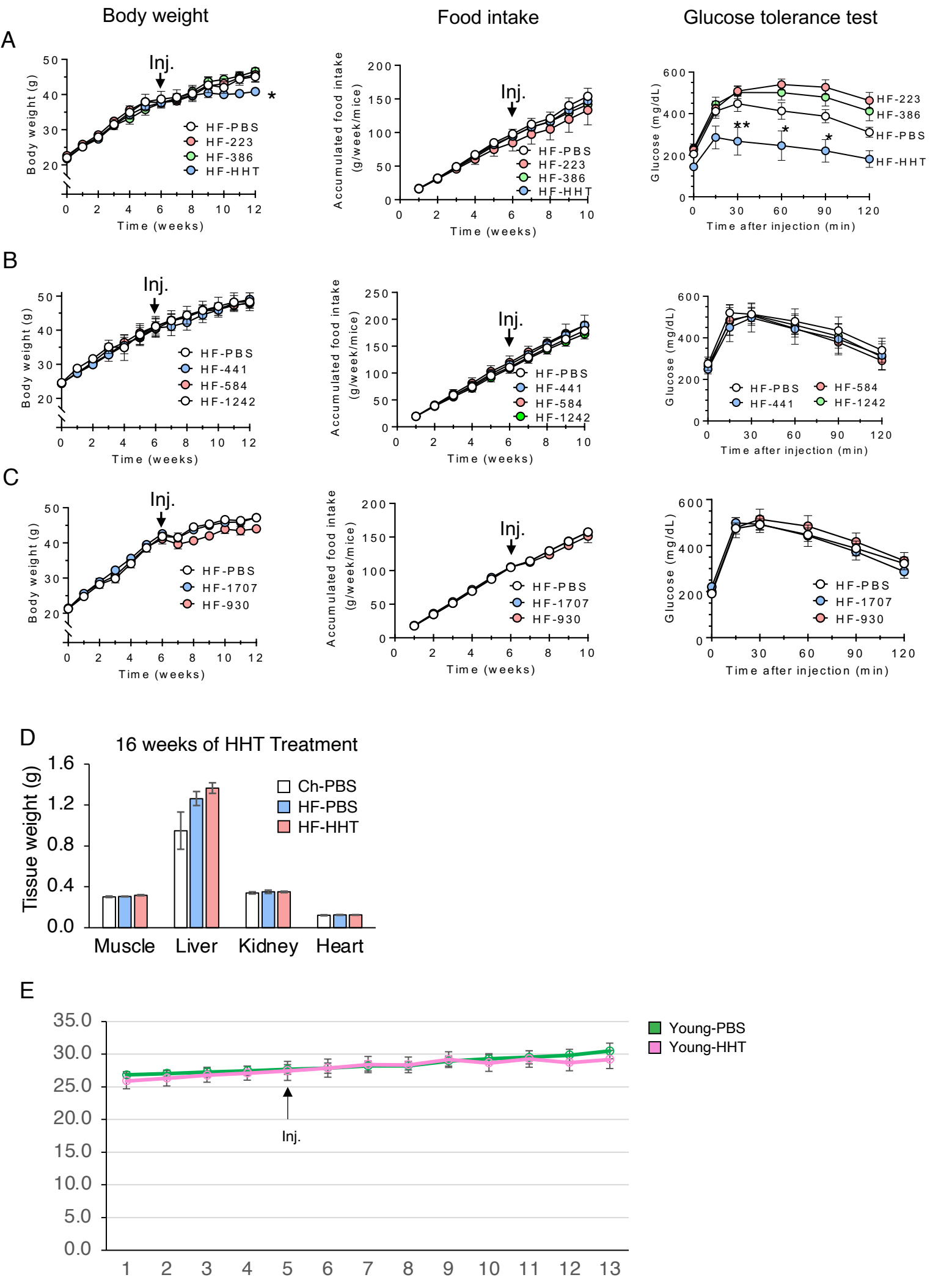
Supplementary Table 1. Primary antibodies used in the experiment

Supplementary Table 2. RT-qPCR primers used in the experiment

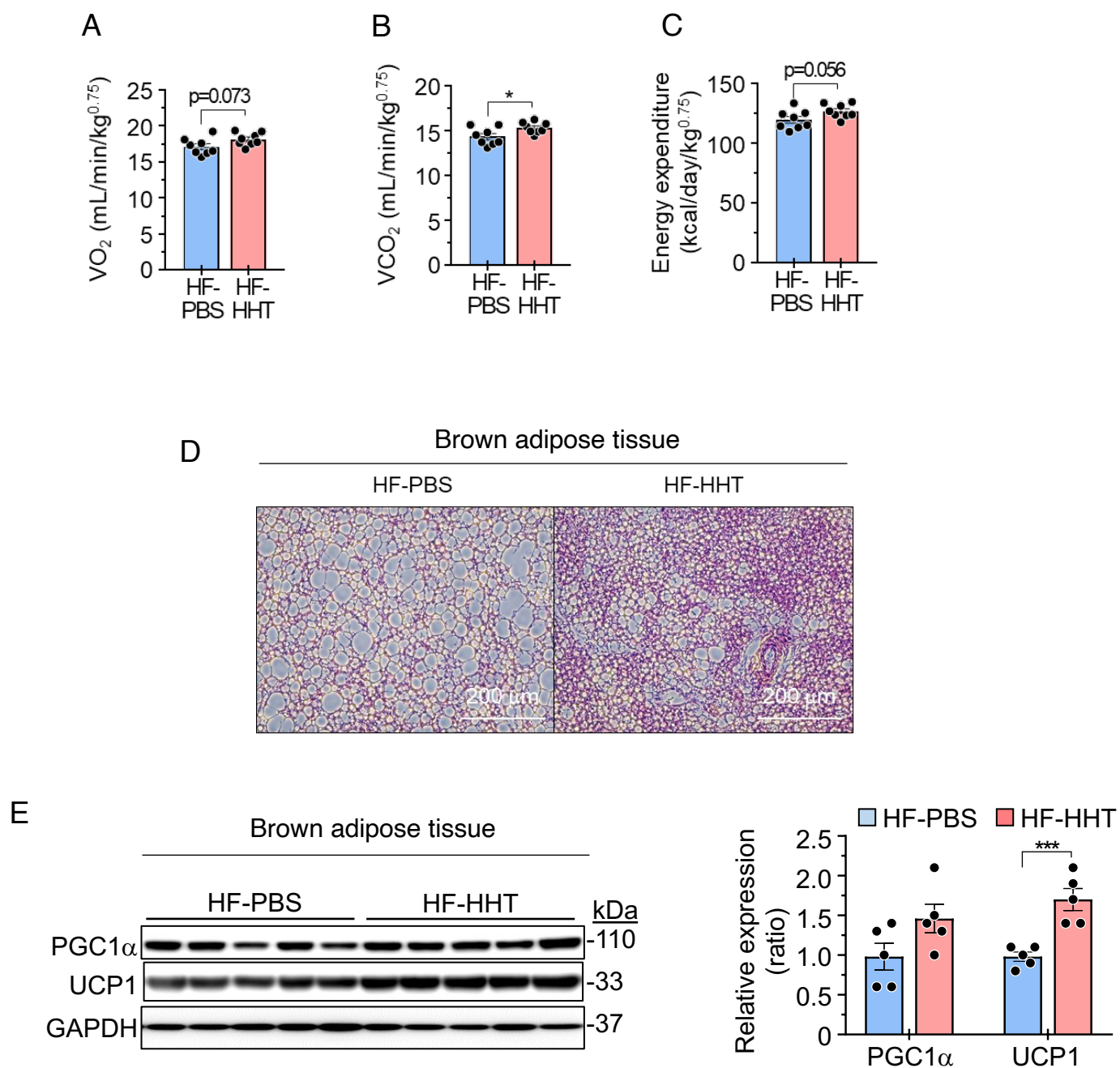
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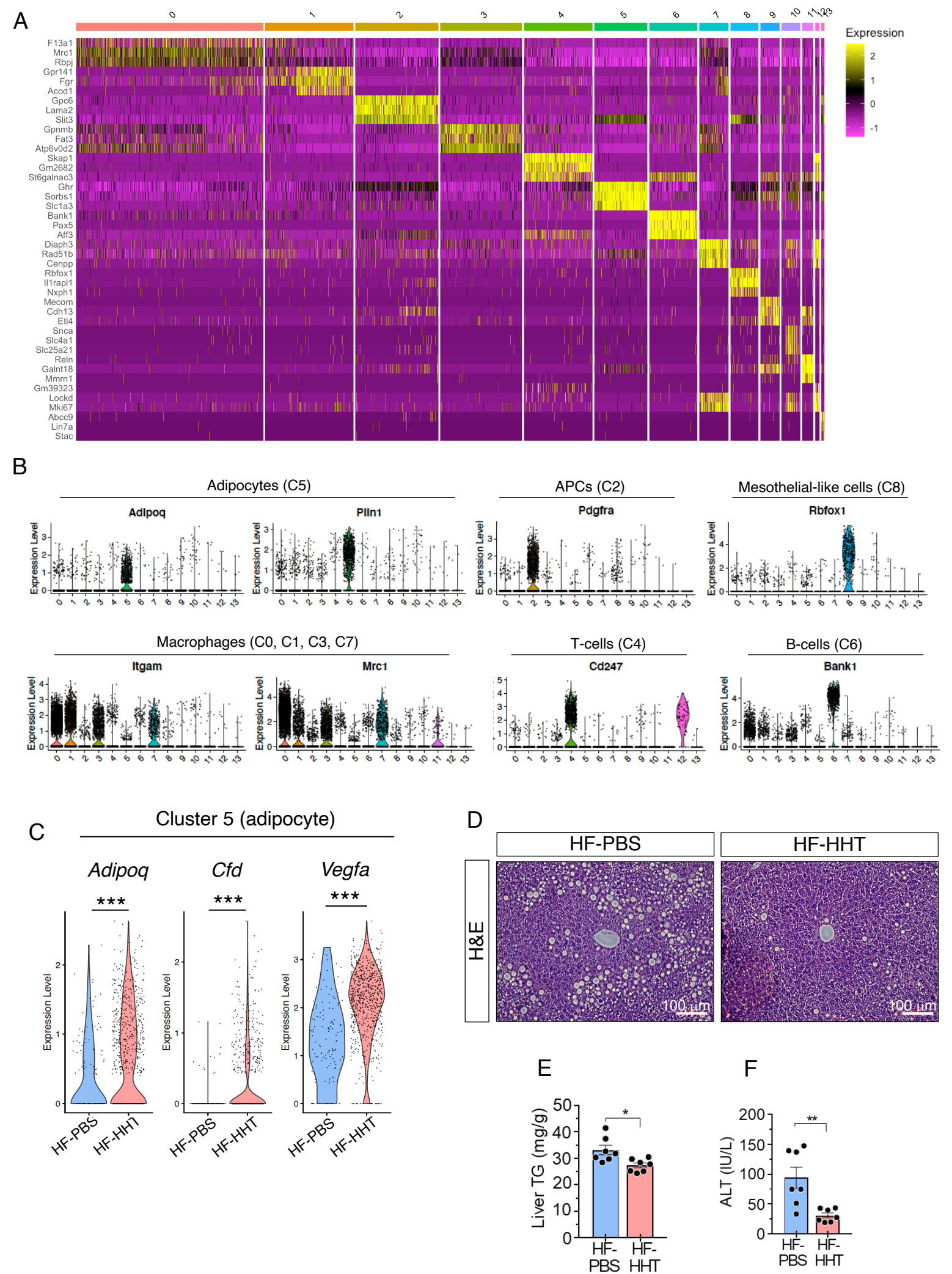
Supplementary Fig. 2 (related to Fig. 2)



Supplementary Fig. 3 (related to Fig. 3)

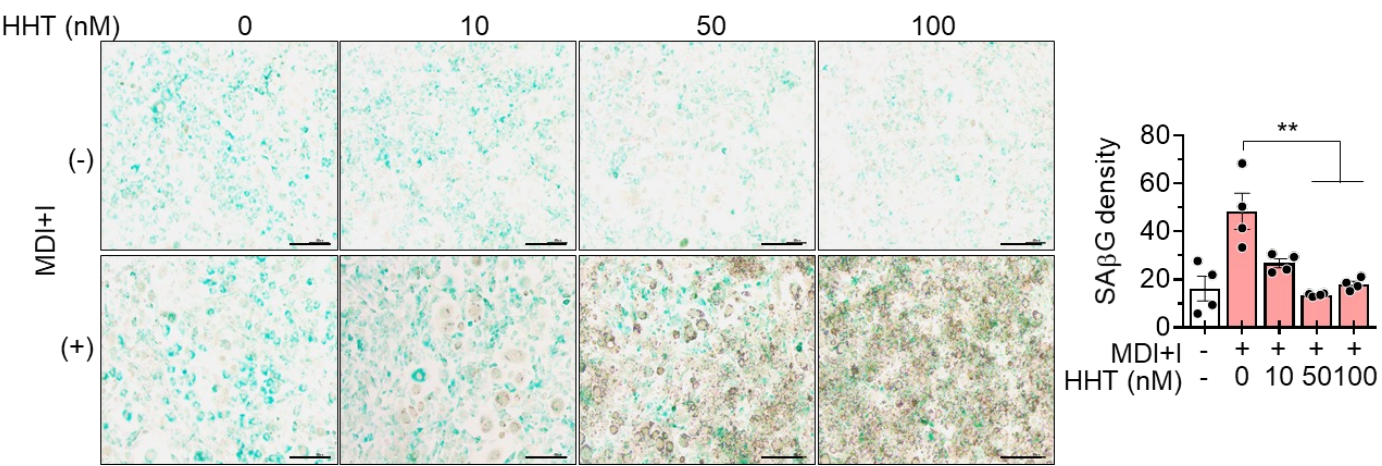


Supplementary Fig. 4 (related to Fig. 4)

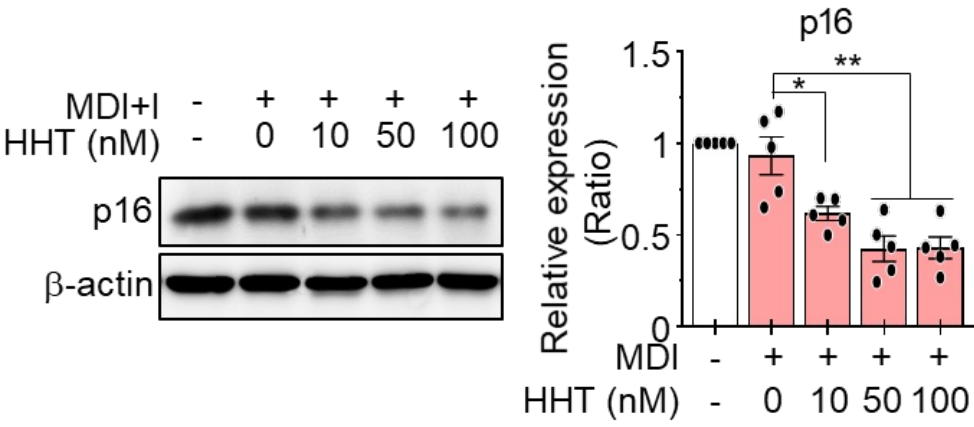


Supplementary Fig. 5 (related to Fig. 5)

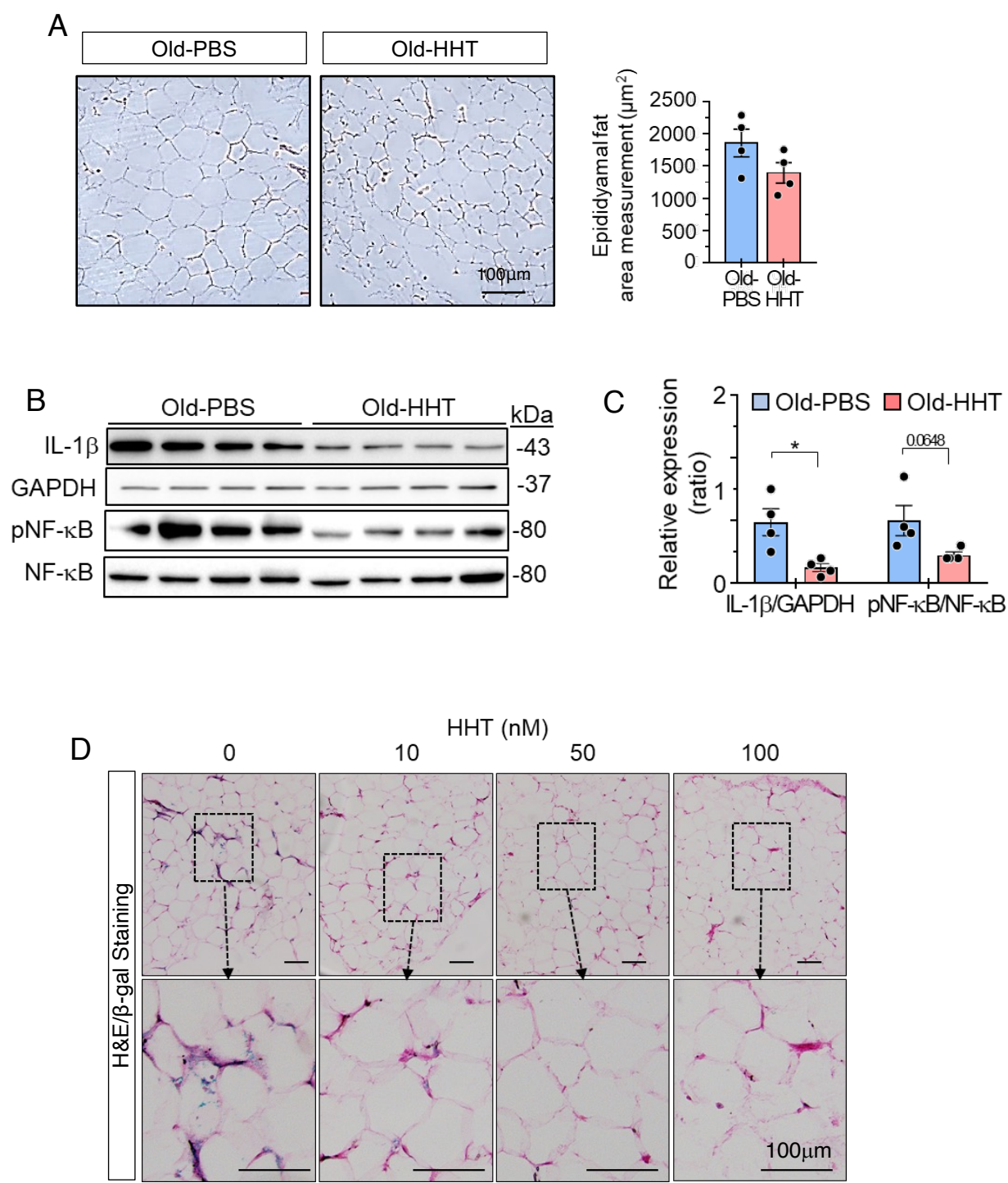
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B



Supplementary Figure 6 (related Fig. 6)

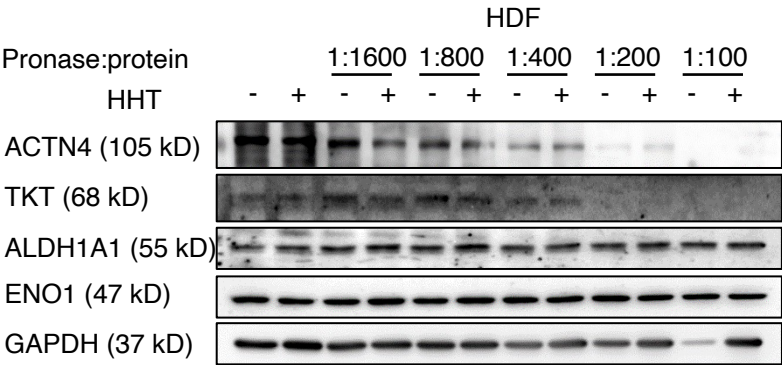


Supplementary Figure 7 (related Fig. 7)

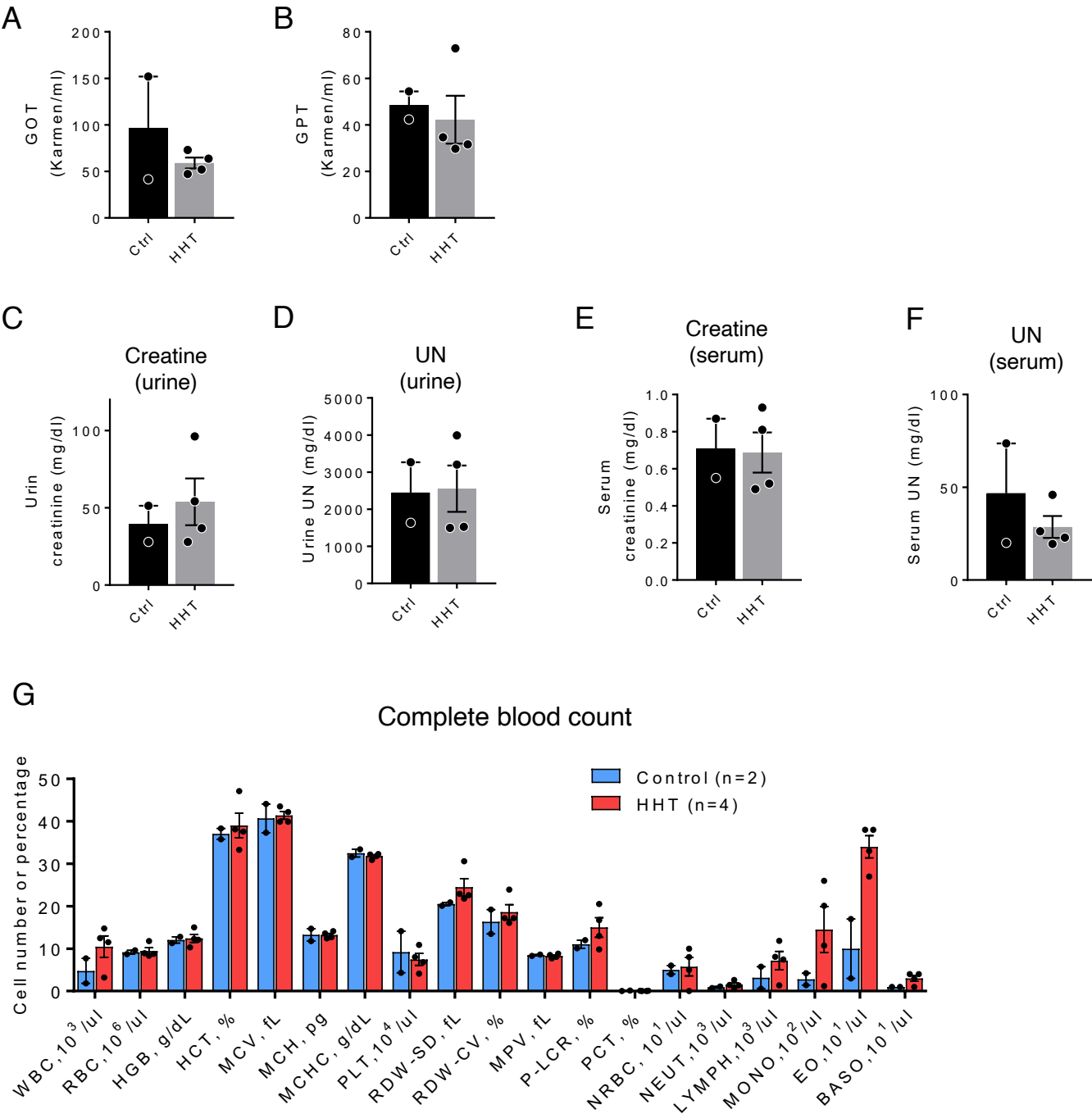
A

	HHT binding proteins	M.W.
1	Clathrin heavy chain 1 (CLTC)	180 kDa
2	Alpha-actinin-4, alpha-actinin-1	110 kDa
3	Heat shock 70 kDa protein 5 (HSPA5)	78 kDa
4	TKT protein (transketolase)	68 kDa
5	Muscle Pyruvate kinase (PKM2)	58 kDa
6	Thioredoxin reductase 1 (TXNRD1)	55 kDa
7	Retinal dehydrogenase 1 (ALDH1A1)	55 kDa
8	Enolase 1	47 kDa
9	Heat shock 70 kDa protein 8 isoform 2 (HSC54)	70 kDa
10	GAPDH	37 kDa
11	Uracil DNA glycosylase	35 and 39 kDa
12	Ribosomal protein L7	30 kDa

B



Supplementary Figure 8 (related Fig. 8)



Supplementary Table 1. Primary antibodies used in the experiment

Antibody	Technology	Catalog number
Actinin Alpha 4(ACTN4)	Abcam	Ab59468
Adipose triglyceride lipase (ATGL)	Cell Signaling Technology	#2138
Aldehyde dehydrogenase 1 family, member A1(ALDH1A1)	Thermo Fisher Scientific	PA5-34901
β-actin	Gene Tex	GT5512
Caspase-3	Cell Signaling Technology	#9662S
Enolase 1(ENO1)	Abcam	ab155102
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	Santa Cruz Biotechnology	sc-25778
Heat Shock 70kDa Protein 5(HSPA5)	LifeSpan Biosciences	LS-C312961
Heat shock 70 kDa protein 8(HSPA8)	LifeSpan Biosciences	LS-C312344
Hormone-sensitive lipase (HSL)	Cell Signaling Technology	#4107
Interleukin-1 beta (IL-1β)	Santa Cruz Biotechnology	sc-1251
Nuclear factor kappa B (NF-κB)	Cell Signaling Technology	#3034
Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1-α)	Abcam	ab54481
Peroxisome proliferator-activated receptor gamma (PPARγ)	Abcam	ab59256
phospho-NF-κB(pNF-κB)	Cell Signaling Technology	#3033
Poly (ADP-ribose) polymerase(PARP)	Santa Cruz Biotechnology	sc-7150
Pyruvate kinase M2(PKM2)	Cell Signaling Technology	#4053S
p16	Santa Cruz Biotechnology	sc-56330

p21	Santa Cruz Biotechnology	sc-817
p53	R&D systems	AF1355
Ribosomal protein L7(RPL7)	Abcam	ab72550
Thioredoxin reductase 1(TXNRD1)	Novus Biologicals	NBP2-20619
Transketolase (TKT)	Thermo Fisher Scientific	PA5-43192
Uncoupling protein 1 (UCP1)	Abcam	ab23841

Supplementary Table 2. RT-qPCR primers used in the experiment

Gene name	Primer sequence	Product size (bp)
<i>Ucp1</i>	Forward: CATCACCACCCTGGCAAAA Reverse: AGCTGATTTGCCTCTGAATGC	80
<i>Prdm16</i>	Forward: ACCTGCCACAGCAAAGAA Reverse: CCATCCAAGCAGAGAAGTAGAC	98
<i>Ppargc1a</i>	Forward: GAATCAAGCCACTACAGACACCG Reverse: CATCCCTCTTGAGCCTTTCGTG	136
<i>Tbx1</i>	Forward: GGCAGGCAGACGAATGTTC Reverse: TTGTCATCTACGGGCACAAAG	102
<i>Tnfrsf9</i>	Forward: GAGGTCAGAAGAGAAAGGGTTG Reverse: GTAGAGGACCCAGGTTTGATTC	97
<i>Cited1</i>	Forward: AACCTTGAGTGAAGGATCGC Reverse: GTAGGAGAGCCTATTGGAGATGT	128
<i>Gapdh</i>	Forward: CGTTGAATTTGCCGTGAGT Reverse: CAGTGGCAAAGTGGAGATTG	101