

Supplemental information

**ADH5-mediated NO bioactivity maintains
metabolic homeostasis in brown adipose tissue**

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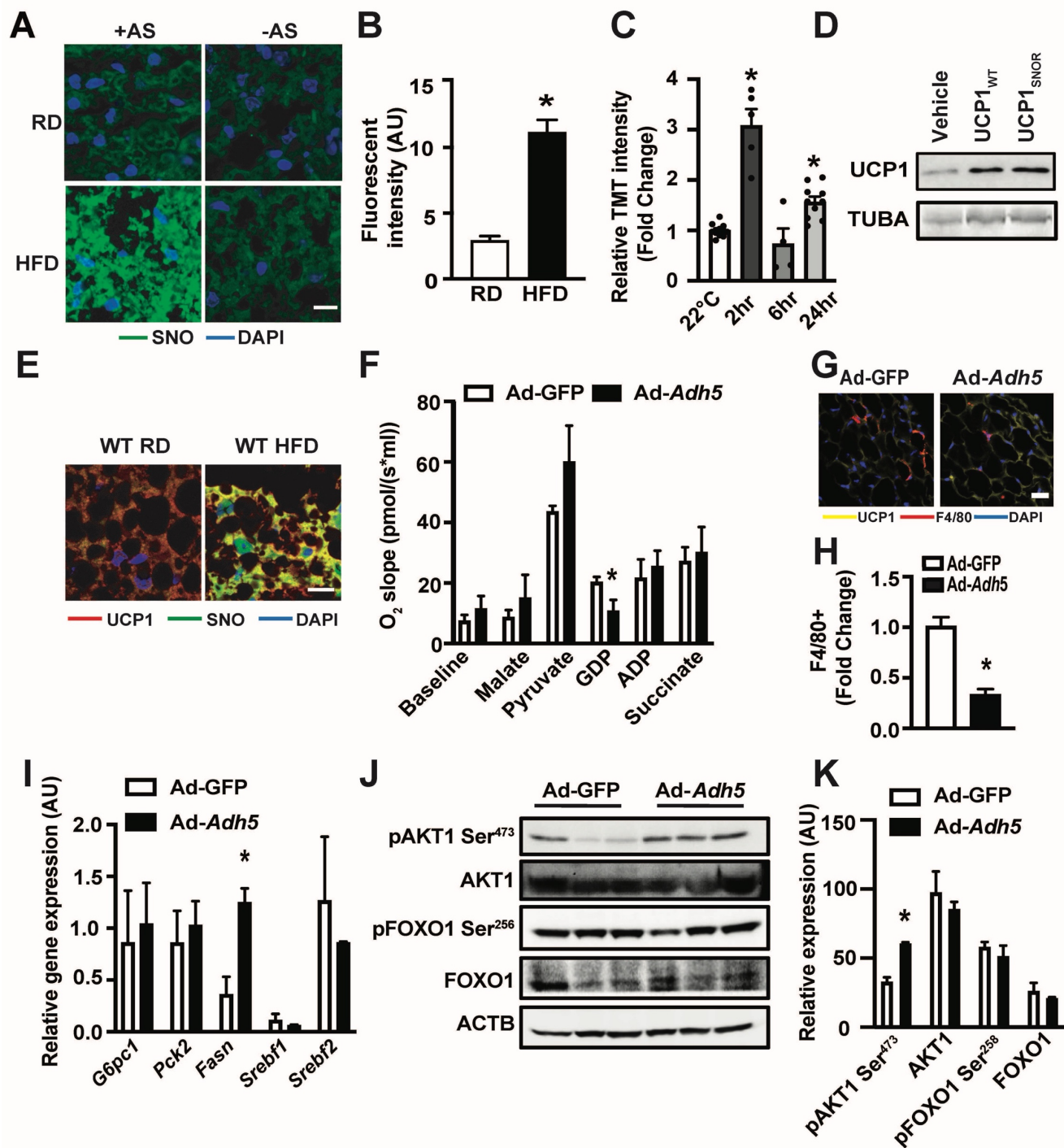


Figure S1. BAT ADH5 protects against obesity-associated metabolic dysfunction. Related to Figure 1 and Figure 2.
A&B. Representative images (63x) and quantification of protein S-nitrosylation (SNO) staining in interscapular BAT (iBAT) from mice fed a regular diet (RD) or high-fat diet (HFD) for 10 weeks. Scale bar: 10 μ m. Data are shown as Pearson's correlation coefficient (n=3 mice/group). **C.** Quantification of protein S-nitrosylation (SNO) in interscapular BAT (iBAT) from WT mice exposed to RT or 4°C for 2, 6 or 24 h. n = 4-7 mice/group. Data were normalized to ACTB input. **D.** Representative western blot of UCP1 expression 24-48 hrs in human BAT cells (hTERT A41hBAT-SVF) transiently transfected with 2.5 μ g UCP1_{WT} or UCP1_{SNOR}. TUBA (TUBULIN) is loading control. **E.** Representative images (63X) of immunohistochemistry for S-nitrosylated UCP1 in BAT from mice fed a RD or HFD for 8-10 weeks. Scale bar: 10 μ m. **F.** O_2 consumption rates in freshly isolated BAT from mice on a HFD for 8 weeks followed by interscapular transduction of

Adeno-GFP or Adeno-*Adh5* (2.5×10^9 pfu/mouse) for additional 2 weeks (n=5 mice/group). **G&H.** Representative images (20X) and quantification of F4/80 staining in BAT from mice in **F**. n=5-7 mice/group, 3-5 fields/replicate. Scale bar: 20 μ m. **I.** qPCR analysis measuring levels of mRNAs encoding the indicated genes in the liver from mice in **F** (n=3 mice/group). Expression was normalized to *Gapdh*. **J.** Representative western blots and densitometric analysis **K.** of expression of tested proteins in the liver from mice in **F** (n=3 mice/group). Data were normalized to ACTB. All data are presented as means \pm SEM. *indicates statistical significance as determined by Student's *t* test in **B**, **F**, **G**, **H**, **I** and **K**. For **C**, *indicates statistical significance compared to the 22°C group as determined by one-way ANOVA; $p < 0.05$.

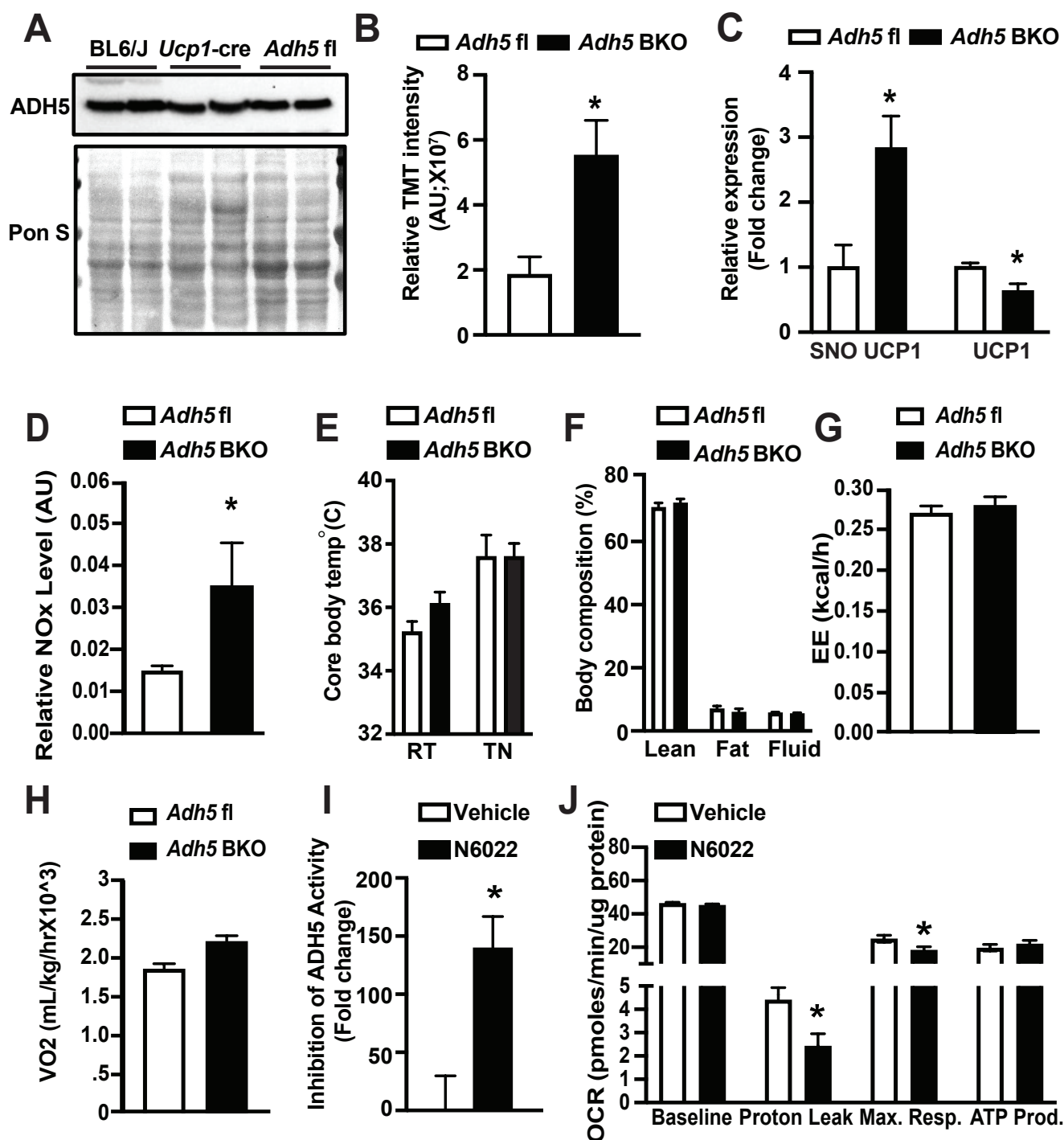


Figure S2. Metabolic and biochemical characterization of BAT-specific *Adh5* KO mice. Related to Figure 3. A.

Representative western blot expression of ADH5 in BAT from C57BL/6J, *Adh5^{fl}* and *Ucp1-cre* mice. Each lane represents a sample from an individual mouse. **B.** Quantification of protein SNO in BAT of *Adh5^{fl}* or *Adh5^{BKO}* mice on a RD for 8 weeks. *n* = 3 mice. Values were normalized to ACTB. **C.** Quantification of SNO-UCP1 and total UCP1 in BAT from **B.** Values were normalized to ACTB input. **D.** Nitrite/nitrate (NOx) release from BAT explants from *Adh5^{fl}* or *Adh5^{BKO}* mice on a RD for 8 weeks. *n* = 5-6 mice. Values were normalized to total RNA content. **E.** Core body temperature measured in *Adh5^{fl}* or *Adh5^{BKO}* mice housed at RT (22°C) or TN (30°C) for 3 weeks. *n* = 5-11 mice/group. **F.** Body composition of *Adh5^{fl}* or *Adh5^{BKO}* mice on RD for 8 weeks. *n* = 5-6 mice/group. **G.** Energy expenditure and **H.** whole body VO₂ were measured in *Adh5^{fl}* or *Adh5^{BKO}*

mice housed in CLAMs at RT, n= 3-4 mice/group. **I.** ADH5 activity in differentiated human BAT cells (hTERT A41hBAT-SVF) in the presence or absence of 25 nM of N6022. Activity was normalized to protein content. n=3 experimental replicates. **J.** O₂ consumption rates in cells in **I.** Rates were normalized to protein content. n=3 experimental replicates. All data are presented as means \pm SEM; *indicates statistical significance as determined by Student's *t* test; p<0.05.

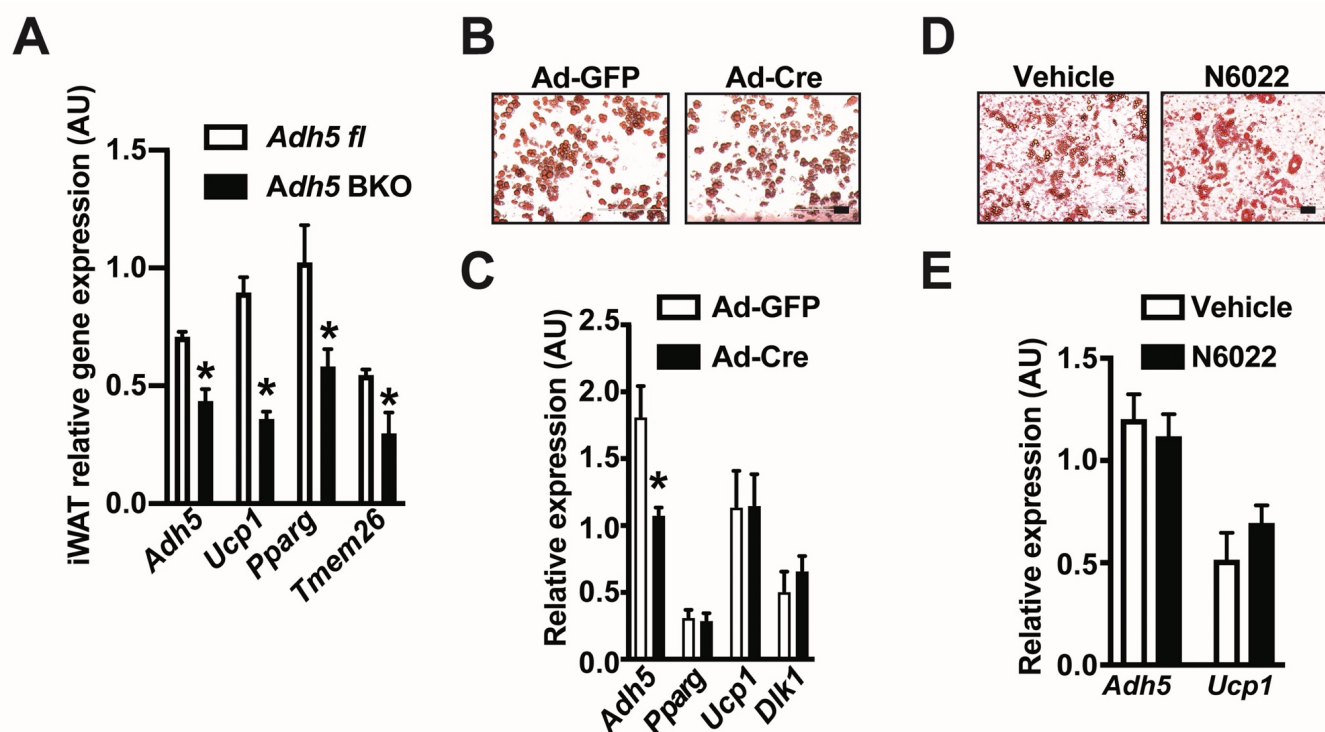


Figure S3. The role of ADH5 in beige-like marker expression and BAT differentiation. Related to Figure 4. **A.** qPCR analysis measuring levels of mRNAs encoding the indicated genes from inguinal white adipose tissue (iWAT) from *Adh5*^{fl} and *Adh5*^{BKO} mice housed at 30°C following cold exposure to 4°C for 24 h. n=4-5 mice/group. Expression is normalized to *Gapdh*. **B.** Representative Oil Red O staining images (10X), and **C.** qPCR analysis measuring levels of mRNAs encoding the indicated genes in brown adipocytes (BAs) differentiated from SVF of *Adh5*^{fl} mice. Pre-adipocytes were transduced with Adeno-GFP or Adeno-Cre (1X10¹² pfu/mouse) at day 5 of differentiation. Scale bar: 10µm. Data were normalized to *Gapdh* (n = 3, biological replicates). **D.** Representative Oil Red O staining images (10X, Scale bar: 10µm.), and **E.** qPCR analysis measuring levels of mRNAs encoding the indicated genes in differentiated human BAT cells (hTERT A41hBAT-SVF) in the presence or absence of 25 nM of N6022. Gene expressions were normalized to *Rna18s*. n=3 experimental replicates. All data are presented as means ± SEM; *indicates statistical significance as determined by Student's *t* test; p<0.05.

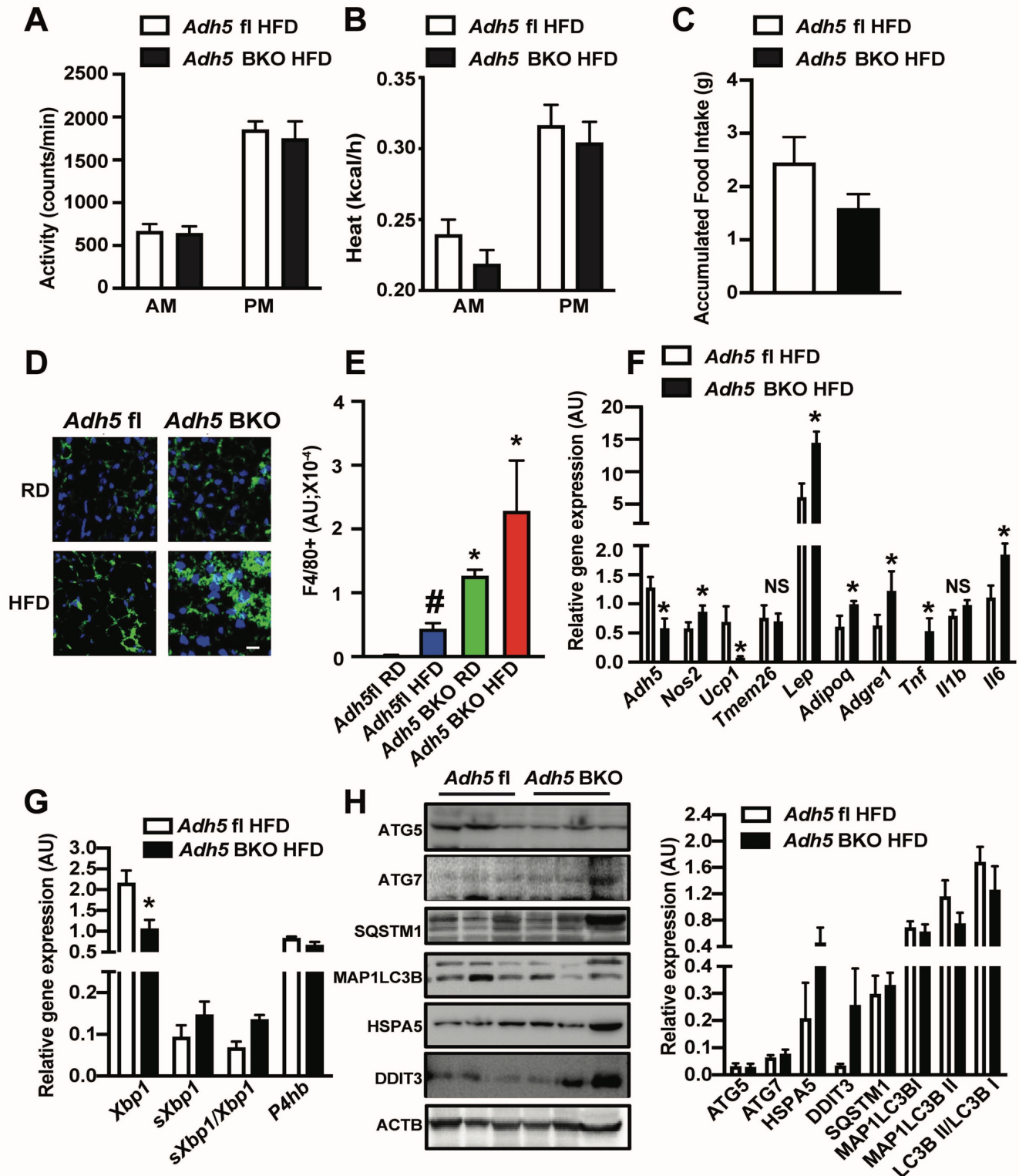
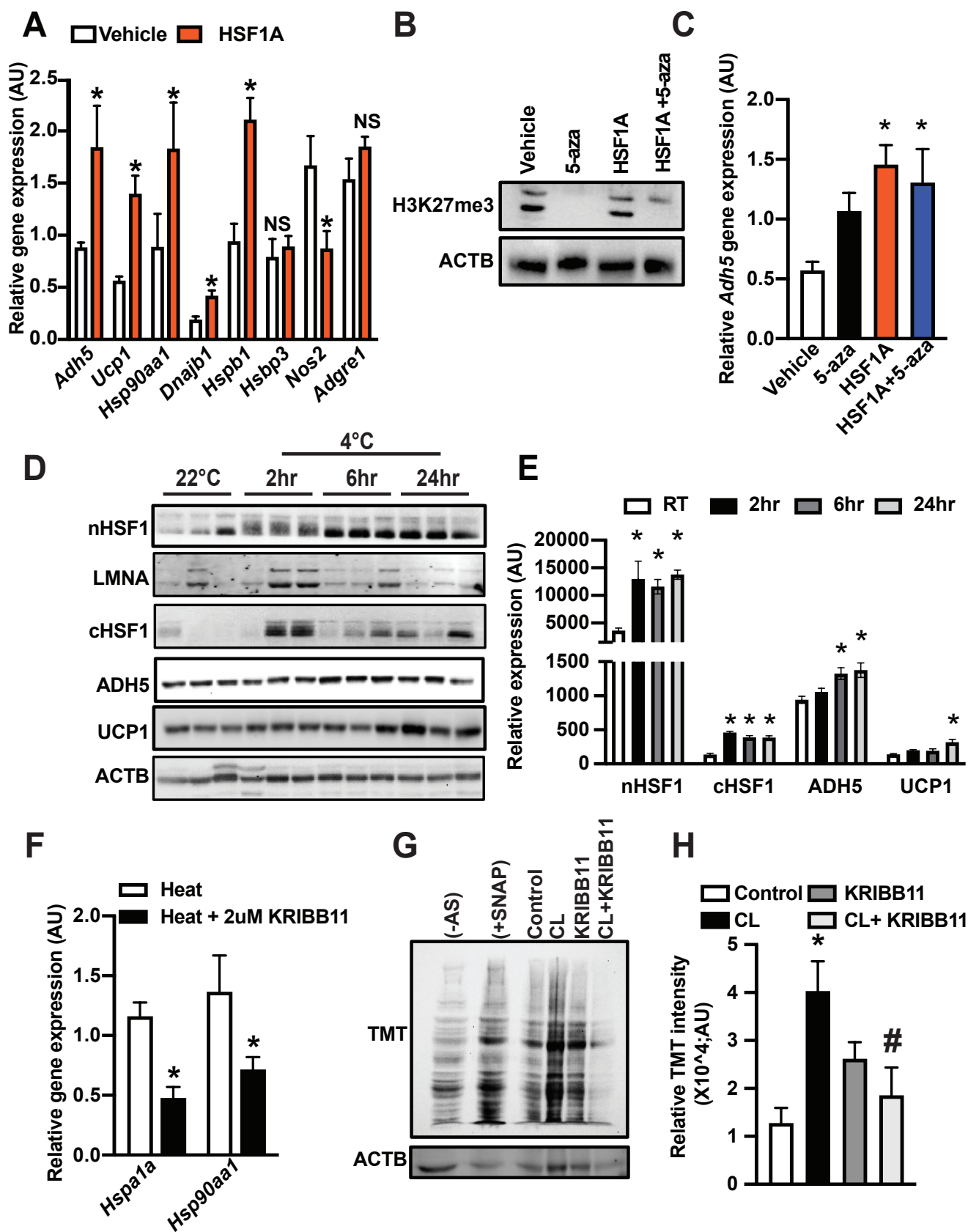


Figure S4. Inflammatory and metabolic profiles in BAT with loss or gain of *Adh5* function in the context of obesity. Related to Figure 5. A-C. Activity, heat and food intake were measured in HFD-fed (8 weeks) *Adh5*^{fl} or *Adh5*^{BKO} mice housed in CLAMs at RT, n=4-5 mice/group. D-E. Representative immunofluorescent (20X) staining images and quantification of F4/80 in BAT from *Adh5*^{fl} (n=3-7 mice/group) and *Adh5*^{BKO} (n=3-5 mice/group) mice fed a RD or HFD for 16 weeks. Scale bar: 10µm. Quantification includes 3-5 fields/replicate. F. qPCR analysis measuring levels of mRNAs

encoding the indicated genes in BAT from mice in **A** (n=3-5 mice/group). Gene expressions were normalized to *Gapdh*. **G.** qPCR analysis measuring levels of mRNAs encoding the indicated genes in BAT from **A**. Gene expressions were normalized to *Gapdh*; n = 3-5 mice/group. **H.** Representative western blots and quantification of BAT from **A**. Data were normalized to ACTB, n =3 mice/group. All data are presented as means \pm SEM; *indicates genetic effects in mice with same diet, # indicates dietary effects in same type of mice. Statistical significance was determined by Student's *t* test in **A-C**, **F&G&H**; and by one-way ANOVA in **E**. $p < 0.05$.



Supplemental Figure 5. HSF1 is involved in DIO-mediated and thermogenic BAT SNO and *Adh5* activation. Related to Figure 5. **A.** qPCR analysis measuring levels of mRNAs encoding the indicated genes from mice fed a HFD for 10 weeks followed by interscapular treatment with vehicle (DMSO; n=4 mice/group) or an HSF1A activator (1 mM; n=7 mice/group) every other day for 2 weeks. Gene expressions were normalized to *Gapdh*. **B.** Representative western blots and **C.** qPCR analysis in BAT from HFD-fed mice (16 weeks on the diet) treated with or without 2 μ M 5-aza in the presence or absence of 20 μ M HSF1A for 72 h. n = 3 independent experiments. Loading control for **B** was ACTB; for **C** gene expression was normalized to *Gapdh*. **D&E.** Representative western blots and quantification of the expression of nuclear and cytosolic HSF1 (nHSF1 and cHSF1, respectively), ADH5 and UCP1 in BAT of WT mice exposed to RT or 4°C for 2, 6 or 24 h. Data were normalized to LMNA for nuclear, and ACTB for cytosolic fractions. n= 5-10/group. **F.** qPCR analysis measuring levels of mRNAs encoding the indicated genes in BAT explants of mice treated with or without 2 μ M KRIBB11 for 2 h prior to 30 min of heat (43°C, n = 6 mice/group). Gene expressions were normalized to *Gapdh*. **G&H.** Representative protein SNO and quantification in control or CL316,243 (1 μ M, 2 h) treated BAT explants with or without 2 μ M KRIBB11 for 2 h (n = 6 mice). All data are presented as mean \pm SEM. * indicates statistical significance compared to the vehicle (A), RT (E), heat (F), and control (H) groups; #indicates KRIBB11 effects in **H**. Statistical significance was determined by Student's t-test in **A&F**, and by ANOVA in **C**, **E&H**. p<0.05.

Table S1. Oligonucleotide information, related to STAR methods.

GFP F	GAAGCCAACGCCTGCAAAATC
GFP R	CCAACGGGTATGAGCTATTCC
Human <i>18S</i> F	GTAACCCGTTGAACCCCAT
Human <i>18S</i> R	CCATCCAATCGGTAGTAGCG
Human <i>ADH5</i> F	TCATGGAGATGACCGATGGA
Human <i>ADH5</i> R	GAATGGACGAGTGGCAATTT
Mouse <i>Adgre1</i> F	AGGACTGGAAGCCCATAGCCAA
Mouse <i>Adgre1</i> R	GCATCTAGCAATGGACAGCTG
Mouse <i>Adh5</i> F	GGCAACGTGAAGGTCATGAGA
Mouse <i>Adh5</i> R	GCTACTCCCACTACCACACTGACA
Mouse <i>Adipoq</i> F	GCACTGGCAAGTTCTACTGCAA
Mouse <i>Adipoq</i> R	GTAGGTGAAGAGAACGGCCTTGT
Mouse <i>Dlk1</i> F	GACCCACCCTGTGACCCC
Mouse <i>Dlk1</i> R	CAGGCAGCTCGTGCACCCC
Mouse <i>Dnajb1</i> F	TTCGACCGCTATGGAGAGGAA
Mouse <i>Dnajb1</i> R	CACCGAAGAACTCAGCAAACA
Mouse <i>Fasn</i> F	AGAGATCCCGAGACGCTTCT
Mouse <i>Fasn</i> R	GCCTGGTAGGCATTCTGTAGT
Mouse <i>G6pc1</i> F	CGACTCGCTATCTCCAAGTGA
Mouse <i>G6pc1</i> R	GTTGAACCAGTCTCCGACCA
Mouse <i>Gapdh</i> F	TGTGTCCGTCGTGATCTGA
Mouse <i>Gapdh</i> R	CCTGCTTCACCACCTTCTTGAT
Mouse <i>Hsf1</i> F	ACAGTGTACCCCGCTGTTG
Mouse <i>Hsf1</i> R	GACTGCACCAGTGAGATGAGGAA
Mouse <i>Hsf2a</i> F	GCAGTGTGTTC AACATGTGTCAG
Mouse <i>Hsf2a</i> R	AGTTCCCATCCAGGAATGCAAG
Mouse <i>Hsp90aa1</i> F	ATGGCAGCAAAGAAACAC
Mouse <i>Hsp90aa1</i> R	GTATC ATCAGCAGTAGGGTCA
Mouse <i>Hspal a</i> F	GAGGAGTTCAAGAGGAAG
Mouse <i>Hspal a</i> R	TGATGGATGTGTAGAAGTC
Mouse <i>Hsp27</i> F	AAGGAAGGCGTGGTGGAGAT
Mouse <i>Hsp27</i> R	TTCGTCCTGCCTTTCTTCGT
Mouse <i>Lep</i> F	GAGACCCCTGTGTCGGTTC
Mouse <i>Lep</i> R	CTGCGTGTGTGAAATGTCAATG
Mouse <i>Nos2</i> F	GTTCTCAGCCCAACAATACAAGA
Mouse <i>Nos2</i> R	GTGGACGGGTCGATGTCAC
Mouse <i>Pck1</i> F	CTGCATAACGGTCTGGACTTC
Mouse <i>Pck1</i> R	CTGCATAACGGTCTGGACTTC
Mouse <i>Ph4b</i> F	CCAGATCAAGCCCCACCTGAT
Mouse <i>Ph4b</i> R	AGTTGCGCCCAACCAGTACTT
Mouse <i>Ppargc1a</i> sF	TATGGAGTGACATAGAGTGTGCT
Mouse <i>Ppargc1a</i> R	CCACTTCAATCCACCCAGAAAG
Mouse <i>Srebfl</i> F	CTGCATAACGGTCTGGACTTC
Mouse <i>Srebfl</i> F	GGCCCGGGAAGTCACTGT
Mouse <i>Srebfl</i> 2 F	GCGTTCTGGAGACCATGGA
Mouse <i>Srebfl</i> 2 R	ACAAAGTTGCTCTGAAAACAAATCA
Mouse <i>sXbp1</i> F	GGTCTGCTGAGTCCGCAGCAGG

Mouse <i>sXbp1</i> R	AGGCTTGGTGTATACATGG
Mouse <i>Tmem26</i> F	ACCCTGTCATCCCACAGAG
Mouse <i>Tmem26</i> R	TGTTTGGTGGAGTCCTAAGGTC
Mouse <i>Tnf</i> F	CCCTCACACTCAGATCATCTTCT
Mouse <i>Tnf</i> R	GCTACGACGTGGGCTACAG
Mouse <i>Ucp1</i> F	ACTGCCACACCTCCAGTCATT
Mouse <i>Ucp1</i> R	CTTTGCCTCACTCAGGATTGG
Mouse <i>Xbp1</i> F	ACGGCCTTGTGGTTGAGAAC
Mouse <i>Xbp1</i> R	TGTCCATTCCCAAGCGTGTT
Cloning: Mouse <i>Ucp1</i> F	CTGGCTAGCGTTTAAACTTAGCCACCATGGTG AACCCGACAAC TTC
Cloning: Mouse <i>Ucp1</i> R	AGCGGGTTTAAACGGGCCCTTTACGCATAATC CGGCACATCATACGGATACGCATAATCCGGCA CATCATACGGATATGTGGTACAATCCACT GTC
Cloning: Mouse <i>Ucp1</i> mutant F	GACAGTGGATGCAACCACATATCCGTATG
Cloning: Mouse <i>Ucp1</i> mutant R	TGTCTGGACTTCATCAGC