

Supplementary Materials for

**Withaferin A promotes white adipose browning and prevents obesity
through sympathetic nerve-activated Prdm16-FATP1 axis**

Supplementary Figure 1 – 6

Supplementary table 1

Supplementary Figure 1. A 3-week dose-ranging study of WA. (A-D) Mice were housed at 22°C, fed a HFD, and treated with WA at doses ranging from 0 to 200 µg/kg for 7 days (DMSO, n = 10; WA 0.2 µg/kg, n = 5; WA 2 µg/kg, n = 10; WA 20 µg/kg, n = 8; WA 200 µg/kg, n = 5). **(A)** Schematic illustration of experiments. Mice received DMSO for four days as acclimation, then they were treated with WA or DMSO for 7 consecutive days. **(B)** Body weight. **(C)** Adipose tissue weight. **(D)** Food intake. **(E-H)** Mice were housed at 22°C, fed a HFD, and treated with WA at doses ranging from 0 to 200 µg/kg for 14 days (DMSO, n = 9; WA 0.2 µg/kg, n = 5; WA 2 µg/kg, n = 10; WA 20 µg/kg, n = 8; WA 200 µg/kg, n = 5). **(E)** Schematic illustration of experiments. Mice received DMSO for four days as acclimation, then they were treated with WA or DMSO for 14 consecutive days. **(F)** Body weight. **(G)** Adipose tissue weight. **(H)** Food intake. **(I-L)** Mice were housed at 22°C, fed a HFD, and treated with WA at doses ranging from 0 to 200 µg/kg for 21 days (DMSO, n = 10; WA 0.2 µg/kg, n = 5; WA 2 µg/kg, n = 9; WA 20 µg/kg, n = 9; WA 200 µg/kg, n = 5). **(I)** Schematic illustration of experiments. Mice received DMSO for four days as acclimation, then they were treated with WA or DMSO for 21 consecutive days. **(J)** Body weight. **(K)** Adipose tissue weight. **(L)** Food intake. **(M-O)** Western blot with quantification of Ucp-1 and PGC1α in iWAT of mice treated with WA for 7 days **(M)**, for 14 days **(N)**, and for 21 days **(O)**. Values are mean ± SEM. Significance was determined by one-way ANOVA with Dunnett multiple comparisons. *p < 0.05, **p < 0.01, and ***p < 0.001.

Supplementary Figure 2 (related to figure 1). Analysis of VO₂, VCO₂ and EE in mice treated with WA. (A) EE of WA or DMSO-treated mice (n = 5). EE per whole

animal is plotted against body weight. In all panels, lines show fitted regressions. **(B)** Indirect calorimetry was performed to quantify the motor activity of WA or DMSO-treated mice during complete 24 hr light-dark cycles, the arrow indicates the time of WA or DMSO injection ($n = 5$). **(C, D)** Oxygen consumption (VO_2) ($n = 5$). VO_2 per whole animal is plotted against body weight. In all panels, lines show fitted regressions. **(E, F)** Carbon dioxide production (VCO_2) ($n = 5$). VCO_2 per whole animal is plotted against body weight. In all panels, lines show fitted regressions. Values are mean \pm SEM. Significance was determined by Student's t test. $^{**}p < 0.01$.

Supplementary Figure 3 (related to figure 3). Analysis of VO_2 , VCO_2 and EE in denervated or sham-operated mice treated with WA. **(A)** EE of denervated or sham-operated mice treated with WA or DMSO ($n = 5$). EE per whole animal is plotted against body weight. In all panels, lines show fitted regressions. **(B)** Indirect calorimetry was performed to quantify the motor activity of denervated or sham-operated mice treated with WA or DMSO during complete 24 hr light-dark cycles, the arrow indicates the time of WA or DMSO injection ($n = 5$). **(C, D)** VO_2 of denervated or sham-operated mice treated with WA or DMSO ($n = 5$). VO_2 per whole animal is plotted against body weight. In all panels, lines show fitted regressions. **(E, F)** VCO_2 of denervated or sham-operated mice treated with WA or DMSO ($n = 5$). VCO_2 per whole animal is plotted against body weight. In all panels, lines show fitted regressions. Values are mean \pm SEM. Significance was determined by one-way ANOVA with Bonferroni test. $^*p < 0.05$; $^{**}p < 0.01$.

Supplementary Figure 4 (related to figure 5). Analysis of VO_2 , VCO_2 and EE in

shGFP, shPrdm16 or shFATP1-injected mice treated with WA. (A) EE of shGFP, shPrdm16 or shFATP1-injected mice treated with WA or DMSO (n = 5). EE per whole animal is plotted against body weight. In all panels, lines show fitted regressions. **(B)** Indirect calorimetry was performed to quantify the motor activity of shGFP, shPrdm16 or shFATP1-injected mice treated with WA or DMSO during complete 24 hr light-dark cycles, the arrow indicates the time of WA or DMSO injection (n = 5). **(C, D)** VO₂ of shGFP, shPrdm16 or shFATP1-injected mice treated with WA or DMSO (n = 5). VO₂ per whole animal is plotted against body weight. In all panels, lines show fitted regressions. **(E, F)** VCO₂ of shGFP, shPrdm16 or shFATP1-injected mice treated with WA or DMSO (n = 5). VCO₂ per whole animal is plotted against body weight. In all panels, lines show fitted regressions. Values are mean ± SEM. Significance was determined by one-way ANOVA with Tukey post hoc test. *p < 0.05; **p < 0.01.

Supplementary Figure 5. Correlation analysis between Prdm16, FATP1, Pgc1α and DβH in human adipose tissue. ⁵³ Correlation analysis between Prdm16, FATP1, Pgc1α and DβH in human subcutaneous **(A-D)** and visceral **(E-H)** adipose tissues (using GTExv5 databases).

Supplementary Figure 6. Original full western blot images.

Supplementary table 1: Primers used in the present study

Primer	Forward Primer 5'-3'	Reverse Primer 5'-3'
<i>Ucp1</i>	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
<i>Prdm16</i>	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
<i>FATP1</i>	AGCCTGGTCAAGTTCTGTTCTGGA	AGAAGAGTCGATCATCCATGCCCT

<i>Pgc1α</i>	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
<i>Ppara</i>	GGGTACCACTACGGAGTTCACG	CAGACAGGCACTTGTGAAAACG
<i>Pparγ</i>	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
<i>Cox7α1</i>	CAGCGTCATGGTCAGTCTGT	AGAAAACCGTGTGGCAGAGA
<i>Cox8β</i>	GAACCATGAAGCCAACGACT	GCGAAGTTCACAGTGGTTCC
<i>Cited</i>	ACTAGCTCCTCTGGATCGACA	GACCCAGTTTTGCATGGGC
<i>Elovl3</i>	TTCTCACGCGGGTTAAAAATGG	GAGCAACAGCTAGACGACCAC
<i>Cpt1</i>	TGGCATCATCACTGGTGTGTT	GTCTAGGGTCCGATTGATCTTTG
<i>Cidea</i>	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
<i>Mcad</i>	ATGACGGAGCAGCCAATGAT	TCGTCACCCTTCTTCTCTGCTT
<i>Metrn1</i>	CTGGAGCAGGGAGGCTTATTT	GGACAACAAAGTCACTGGTACAG
<i>HSP70</i>	TGGTGCTGACGAAGATGAAG	AGGTCGAAGATGAGCACGTT
<i>Nrf1</i>	CAGCAACCCTGATGGCACCGTGTC	GGCCTCTGATGCTTGCGTCGTCTG
<i>Dio2</i>	AGAGTGGAGGCGCATGCT	GGCATCTAGGAGGAAGCTGTTC
<i>Adrb3</i>	TTGTCCTGGTGTGGATCGTG	TTGGAGGCAAAGGAACAGCA
<i>LepR</i>	TGTTTTGGGACGATGTTCCA	AAAGATGCTCAAATGTTTCAGGC
<i>JAK2</i>	AGAAAGGGCGGAATAAGGGC	CTGCTCCAACTCACGAATCCT
<i>Stat3</i>	CACCTTGATTGAGAGTCAAGAC	AGGAATCGGCTATATTGCTGGT
<i>Stat5</i>	CAGATGCAAGTGTTGTATGGGC	GCTGGCTCTCGATCCACTG
<i>PI3K</i>	CGAGAGTGTCGTCACAGTGTC	TGTTGCTTCCACAAACACAG
<i>AKT</i>	ATGAACGACGTAGCCATTGTG	TTGTAGCCAATAAAGGTGCCAT

<i>mTOR</i>	CAGTTCGCCAGTGGACTGAAG	GCTGGTCATAGAAGCGAGTAGAC
<i>S6K1</i>	CTGAGAAGGCCGATCCATCC	TGACCTTGCGTACCAGGAAGA
<i>PDE3b</i>	AAAGCGCAGCCGGTTACTAT	CCATATTGCGAGCTTCATTTAGC
<i>AMPK</i>	TCTGAGGGGCACCAAGAAAC	GTGGGTGTTGACGGAGAAGAG
<i>FoxO1</i>	CAATCTGTCCCTTCACAGCA	CTCCCTCTGGATTGAGCATC
<i>Mchr1</i>	ATGGATCTGCAAGCCTCGTTG	CCCGCCAATGTGAAATTATCCTG
<i>Pomc</i>	ATGCCGAGATTCTGCTACAGT	TCCAGCGAGAGGTGAGTTT
<i>Agrp</i>	ATGCTGACTGCAATGTTGCTG	CAGACTTAGACCTGGGAACTCT
<i>Npy</i>	ATGCTAGGTAACAAGCGAATGG	TGTCGCAGAGCGGAGTAGTAT
<i>ROCK1</i>	GACTGGGGACAGTTTTGAGAC	ATCCAAATCATAAACCAGGGCAT
<i>TRPC</i>	TTCACTGAGACCTTTTTGACCG	TGGCACTCAACGAAGTCACTG
<i>Sitr1</i>	GCTGACGACTTCGACGACG	TCGGTCAACAGGAGGTTGTCT
<i>MAGEL</i>	AATGCCGCATGTTCCCATTAC	ATGGATCATCACGACACCAGG
<i>MFN2</i>	AGAACTGGACCCGGTTACCA	CACTTCGCTGATACCCCTGA
<i>BDNF</i>	TCATACTTCGGTTGCATGAAGG	AGACCTCTCGAACCTGCCC
<i>Socs3</i>	GAGTACCCCCAAGAGAGCTTACTA	CTCCTTAAAGTGGAGCATCATACTG
<i>Gpr17</i>	GCTTACTCTGAGCAATGCGGA	GTGATAAACCAACCGGGTAGG
<i>PTEN</i>	TGGATTCGACTTAGACTTGACCT	GCGGTGTCATAATGTCTCTCAG
<i>GSK3b</i>	ATGGCAGCAAGGTAACCACAG	TCTCGGTTCTTAAATCGCTTGTC
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA