

Supplemental information

**Transplantation of committed
pre-adipocytes from brown adipose tissue improves
whole-body glucose homeostasis**

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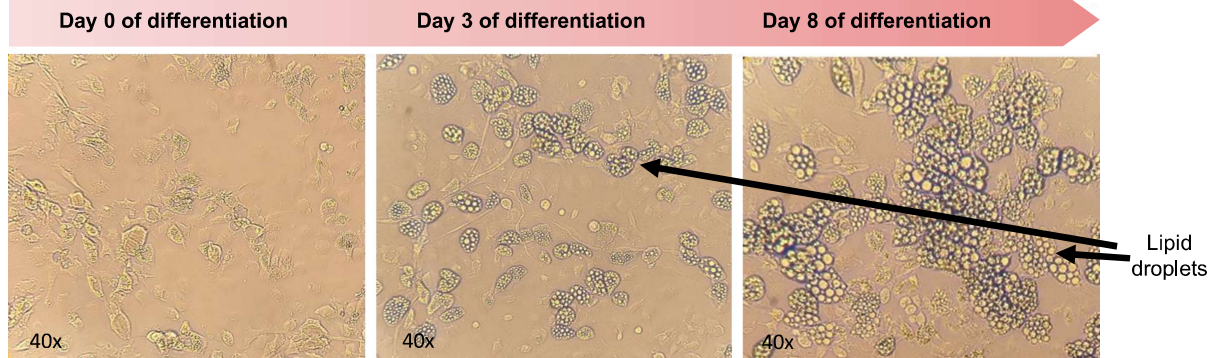
Supplemental Figures

Figure S1: Adipogenic differentiation of pre-adipocytes from BAT SVF and tracing of transplanted committed pre-adipocytes. Related to Figures 1-7

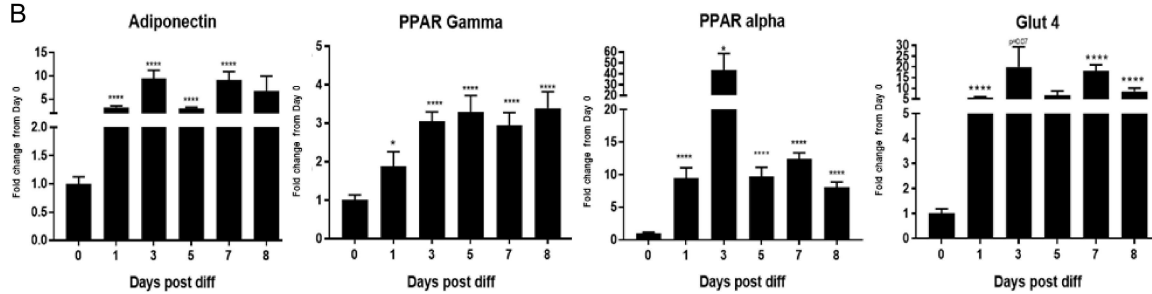
(A) 40x magnification of pre-adipocytes from BAT SVF showing the cells over days 0, 3 and 8 of differentiation. (B) Expression of genes involved in adipogenesis in pre-adipocytes from BAT SVF over days 0, 1, 3, 5, 7 and 8 of differentiation (n=4-16 per group; *p<0.05, **p<0.01, ***p<0.001, ****p<0.001 vs Day 0 of differentiation) (C) IVIS Bioluminescent imaging of 1 million committed pre-adipocytes from Luciferase transgenic mice (in 2mmx4mm cylindrical silk scaffolds) using 50 μ L of Luciferin; 3 min exposures; open filter; 1-week and 3-weeks post-transplant. (D, E) Representative images from IVIS Bioluminescent imaging of C57BL/6 Male mice transplanted with 1 million committed pre-adipocytes from Luciferase transgenic mice (Committed pre-adipocytes) or empty scaffolds (Sham) using 100 μ L of Luciferin; 3 min exposures; open filter). (F) Scaffold location *in vitro* 4 weeks post-transplant. (G) 40X magnification of committed pre-adipocytes from extracted scaffold, 4 weeks post-transplant. (H) IVIS Bioluminescent imaging of cells isolated from scaffolds 4 weeks post-transplant and control cells isolated from scWAT of a Luciferase Tg mouse; using 50 μ L of Luciferin; 3 min exposures; open filter. All data are represented as mean \pm SEM.

Figure S1

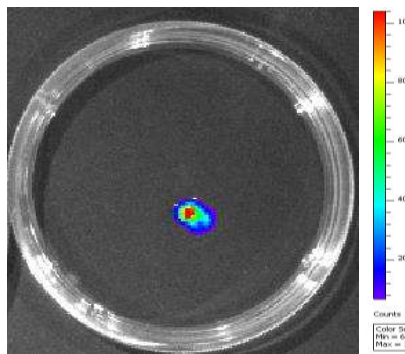
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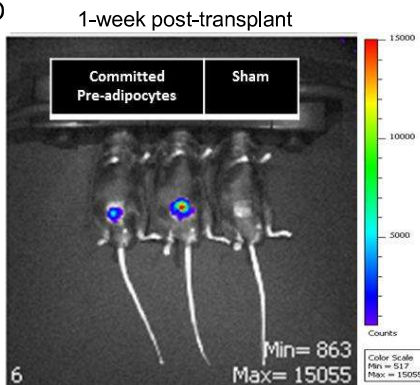
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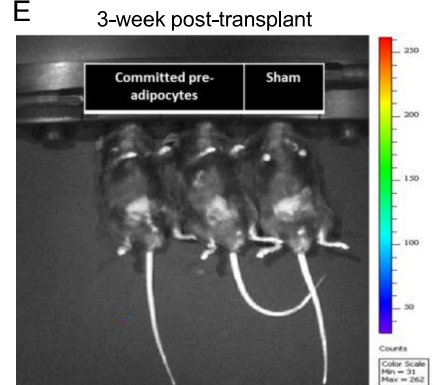
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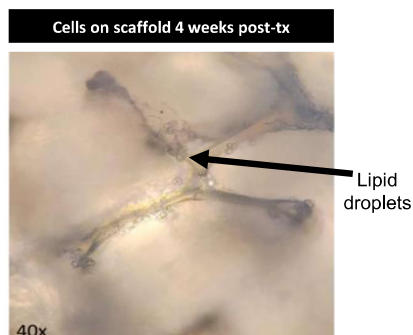
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G



H

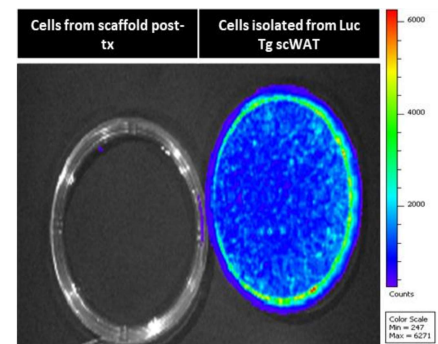


Figure S2: Transplantation of WT committed pre-adipocytes affects metabolic health and AKT signaling in WT recipients. Related to Figures 1-2.

(A) Weekly body weight (n=11-24 per group; *p<0.05 vs Sham-WT at 12 weeks post-transplant; two-way-ANOVA). (B) GTT AUC 4- and 8-weeks post-transplant in (n=5-46 per group; *p<0.05 vs Sham-WT at 4 weeks post-transplant, **p<0.01 vs Sham-WT at 8 weeks post-transplant, @@@p<0.001 vs 4 weeks post-transplant; two-tailed t-test). (C) Western blotting protein expression for pAKT/AKT and blots for pAKT and AKT in scWAT. (D-F) Western blotting protein expression for pAMPK/AMPK and blots for pAMPK, AMPK and GAPDH in TA (D), iBAT (E), and Liver (F) (n=3-5 per group; insulin-stimulated conditions depicted using 'i'; *p<0.05, **p<0.01 as described [*= insulin effect; #= group effect]; two-tailed t-test). All data are represented as mean ± SEM.

Figure S2

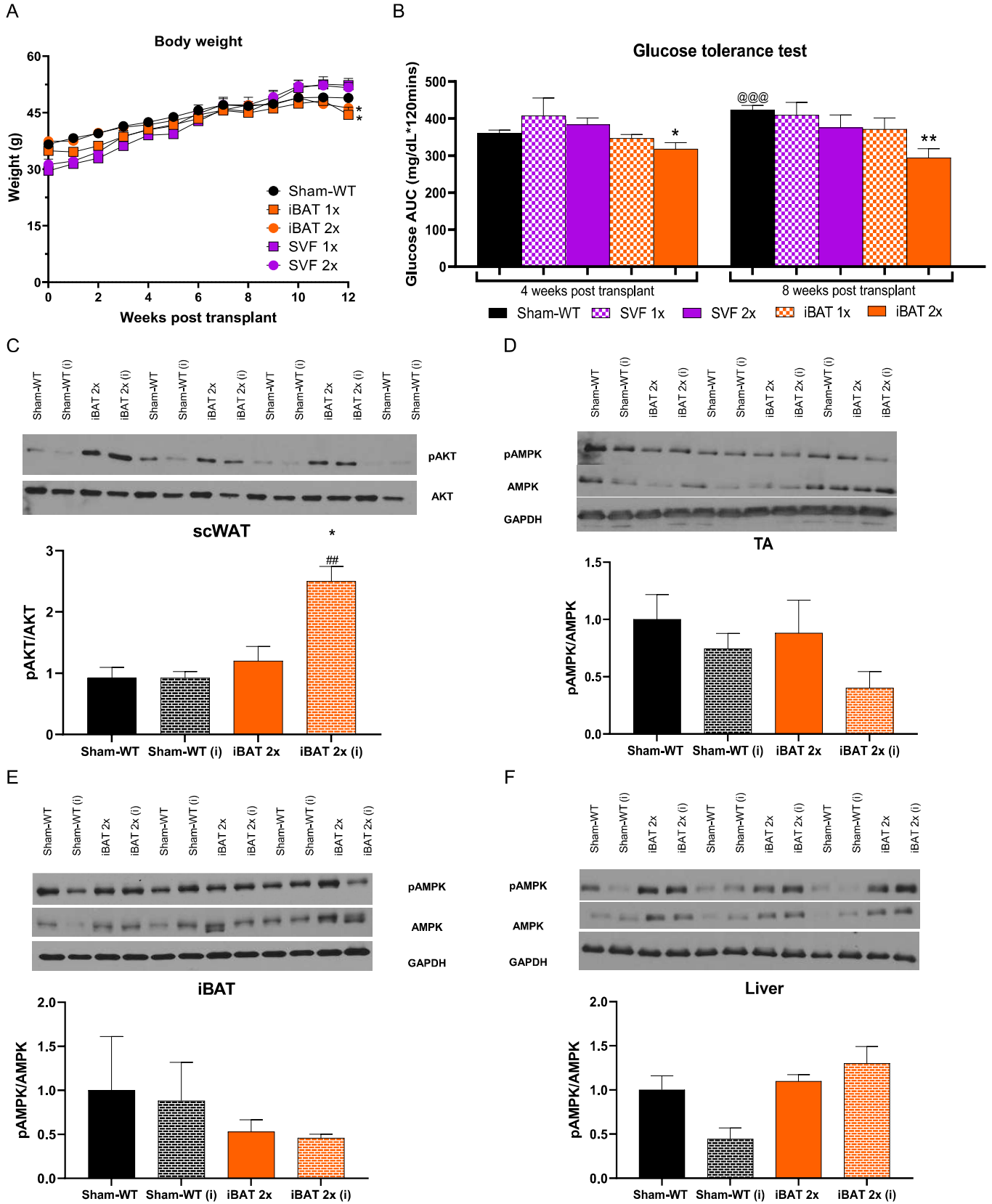


Figure S3: Transplantation of committed pre-adipocytes from WT donors alters gene expression in WT recipients. Related to Figures 1-2.

(A-E) Gene expression analysis of TA (A), Liver (B), iBAT (C), scWAT (D) and pgWAT (E), in Sham-WT and iBAT 2x mice, 12 weeks post-transplant (n=3-9 per group; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs Sham-WT; two-tailed t-test). All data are represented as mean \pm SEM.

Figure S3

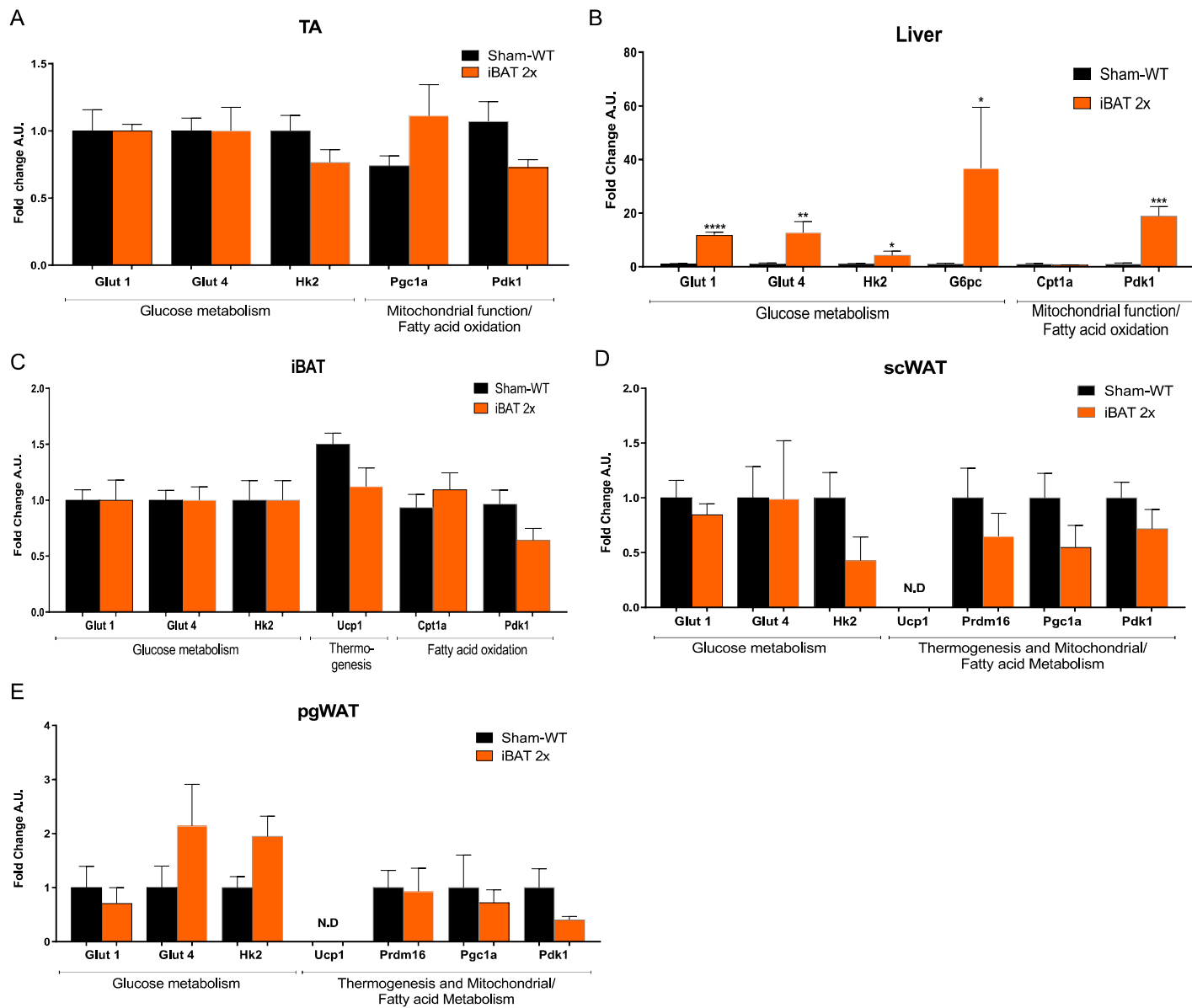


Figure S4: Transplantation of WT committed pre-adipocytes alters pathways associated with glucose, insulin, and lipid metabolism in livers of WT recipient mice. Related to Figure 3.

(A) Heat-map showing top 75 differentially expressed genes (DEGs) in iBAT 2x mice compared to Sham-WT mice. (n=7-9 per group; significance threshold: 1-fold-change, $p < 0.01$). Color scale indicates scaled \log_2 normalized expression. (B, C) Significantly upregulated (B) or downregulated (C) Gene ontology (GO) biological process (BP) terms based on the Over-Representation Analysis of iBAT 2x livers versus Sham-WT livers (n=7-9 per group). All data are represented as mean \pm SEM.

Figure S4

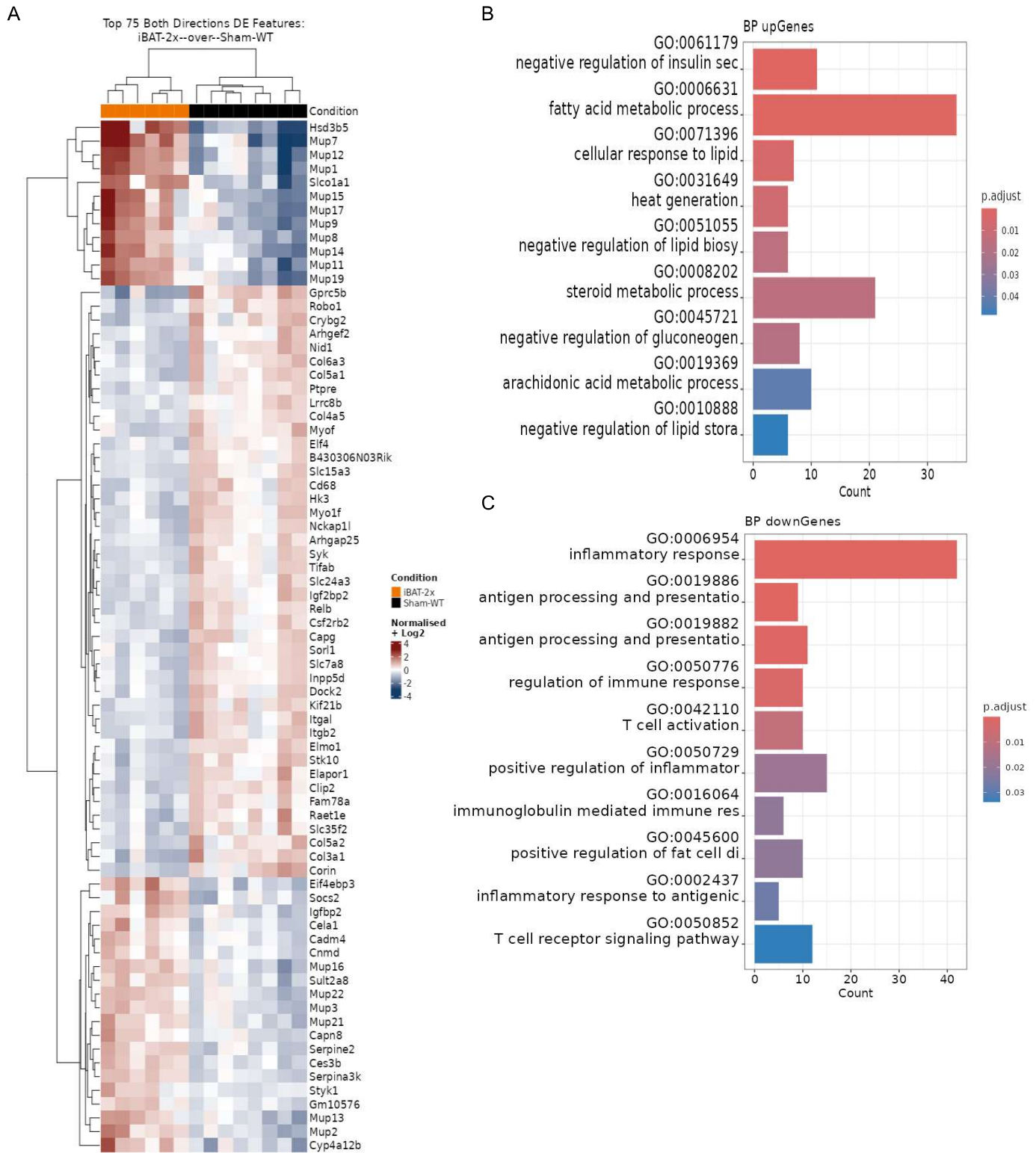
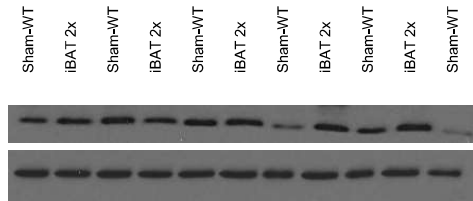


Figure S5: Transplantation of WT committed pre-adipocytes affects protein expression and plasma FGF21 in WT recipient mice. Related to Figure 3.

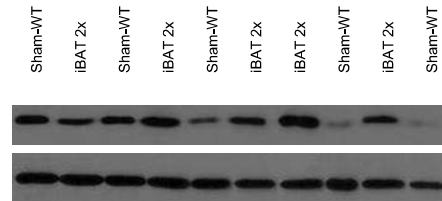
(A-C) Western blotting protein blots (Sham-WT and iBAT 2x groups) for MUP1 (A), MUP11 (B), and MUP12 (C) in the Liver, 12 weeks post-transplant (n=4-6 per group). (D, E) Plasma Adiponectin (ng/mL) (D) and Plasma FGF21 (ng/mL) (E) after 12-hour fast, 12 weeks post-transplant (n=3-12 per group). All data are represented as mean \pm SEM.

Figure S5

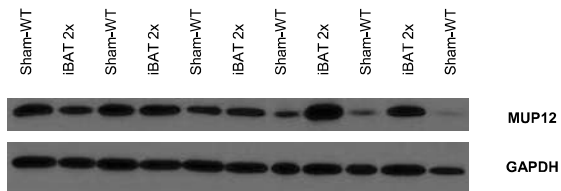
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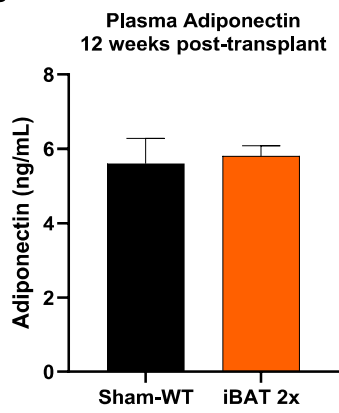
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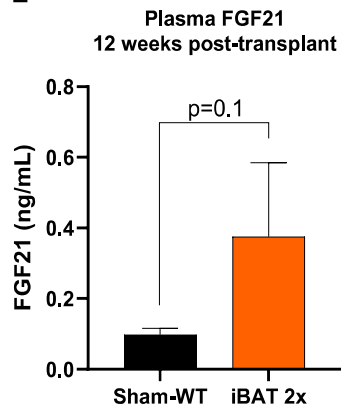


Figure S6: Transplantation of committed pre-adipocytes from WT donors affects AKT but not AMPK signaling in FGF21^{-/-} recipient mice. Related to Figures 4-5.

(A) Western blotting protein expression for pAKT/AKT and blots for pAKT and AKT in scWAT. (B-E) Western blotting protein expression for pAMPK/AMPK and blots for pAMPK, AMPK and GAPDH in TA (B), iBAT (C), scWAT (D) and Liver (E). (F) Western blotting protein expression for pAKT/AKT in Sham-WT, Sham-KO and WT-to-KO recipients in TA (n=2-3 per group; insulin-stimulated conditions depicted using 'i'; *p<0.05, **p<0.01 as described [*= insulin effect; #= group effect]; two-tailed t-test). All data are represented as mean \pm SEM.

Figure S6

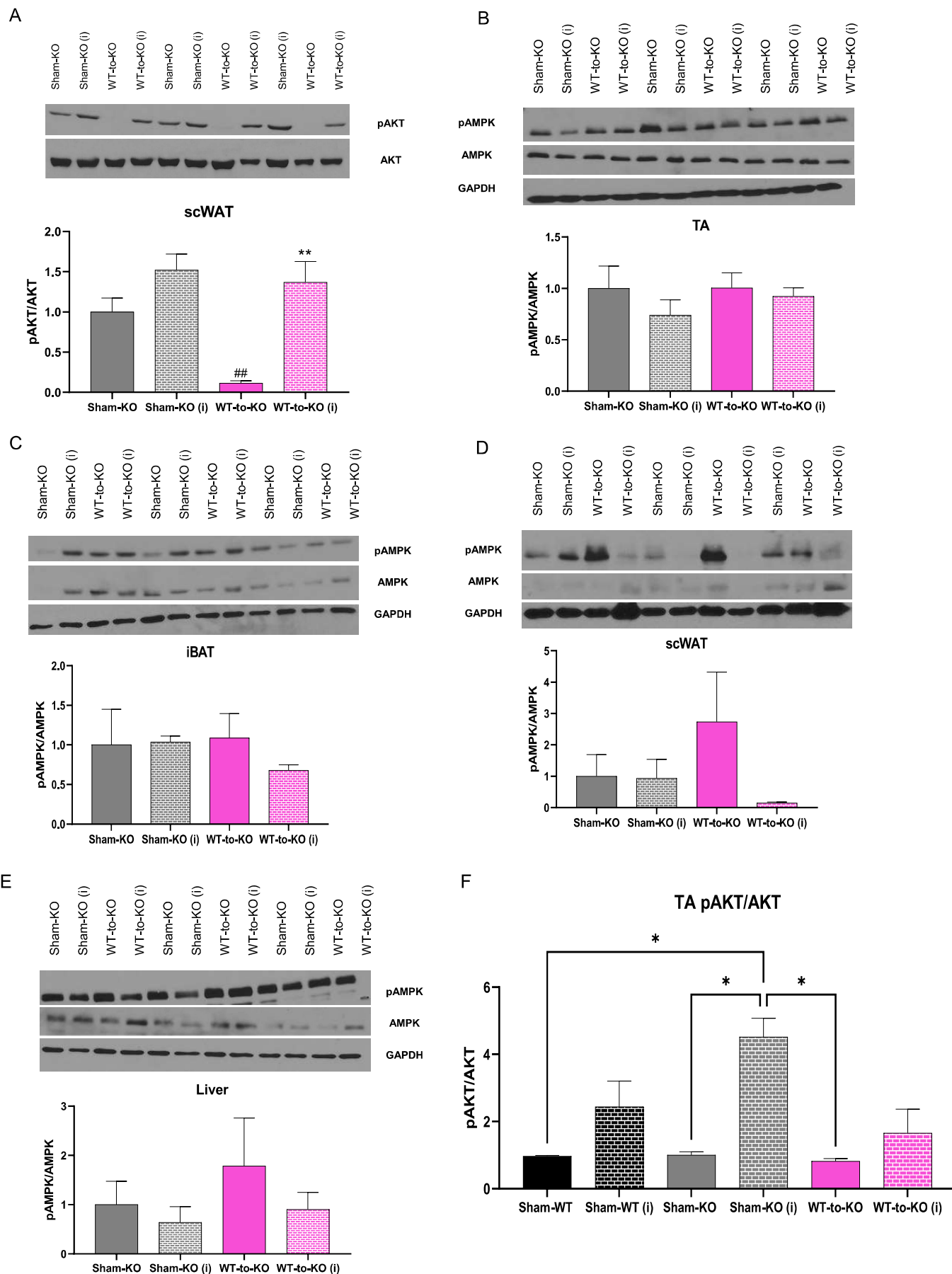


Figure S7: Transplantation of committed pre-adipocytes from WT donors alters gene expression in the liver of FGF21^{-/-} recipient mice. Related to Figures 4-5.

(A-B) Gene expression analysis in Liver (A) and pgWAT (B) in Sham-KO and WT-to-KO mice, 12 weeks post-transplant (n=3-6 per group; **p<0.01 vs Sham-KO; two-tailed t-test). (C-E) Western blotting protein blots (Sham-KO and WT-to-KO groups) for MUP1 (C), MUP11 (D), and MUP12 (E) in the Liver (n=4-6 per group). All data are represented as mean \pm SEM.

Figure S7

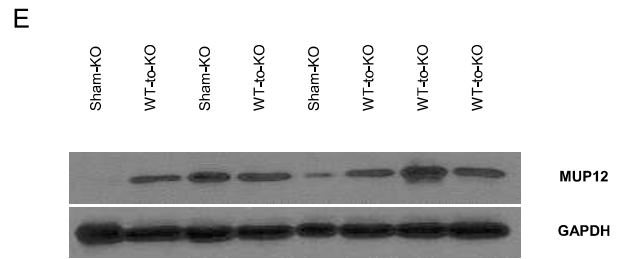
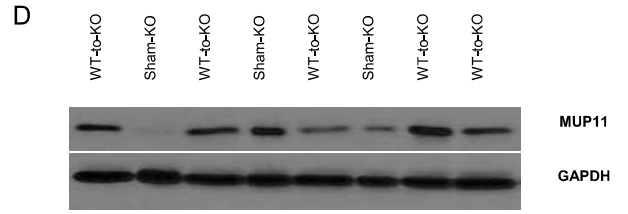
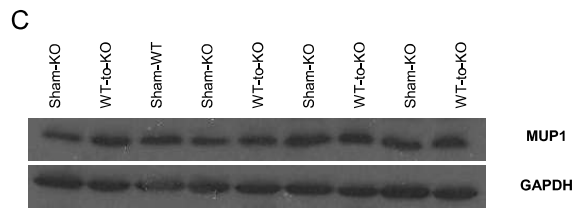
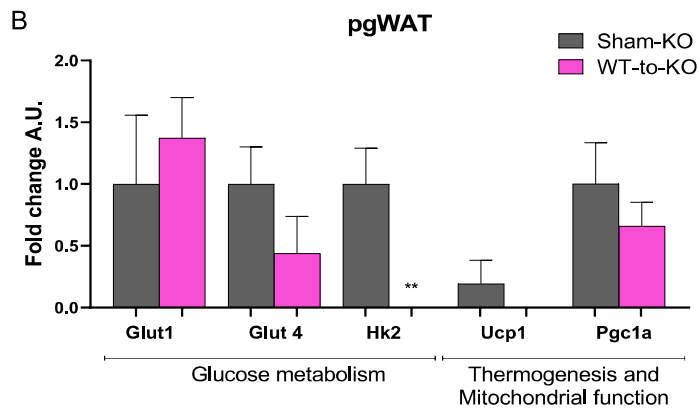
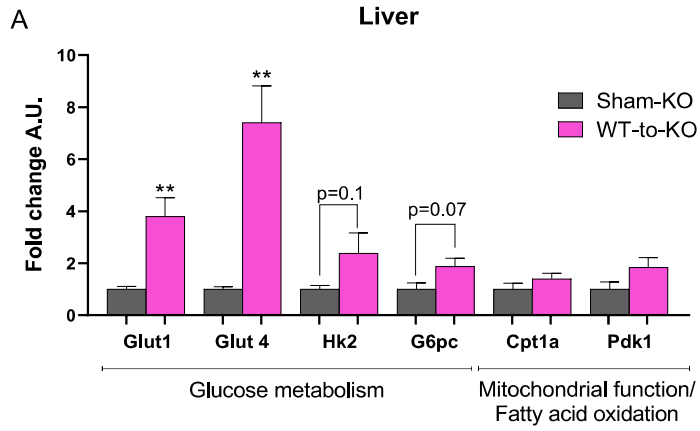
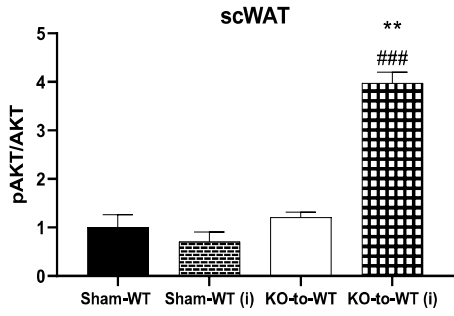
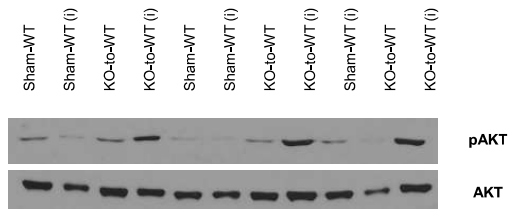


Figure S8: Transplantation of committed pre-adipocytes from FGF21^{-/-} donors affects AKT but not AMPK signaling in WT recipient mice. Related to Figures 6-7.

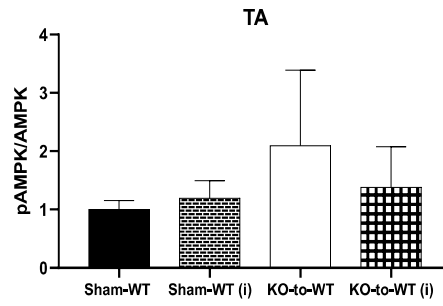
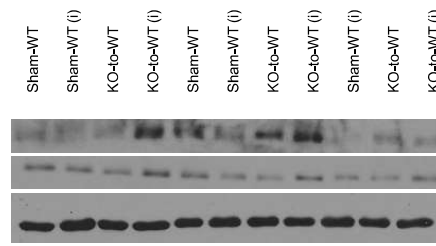
(A) Western blotting protein expression for pAKT/AKT and blots for pAKT and AKT in scWAT. (B-E) Western blotting protein expression for pAMPK/AMPK and blots for pAMPK, AMPK and GAPDH in TA (B), iBAT (C), scWAT (D) and Liver (E). (n=2-3 per group; insulin-stimulated conditions depicted using 'i'; *p<0.05, **p<0.01 as described [*= insulin effect; #= group effect]; two-tailed t-test). All data are represented as mean \pm SEM.

Figure S8

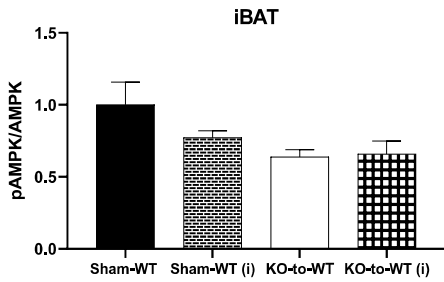
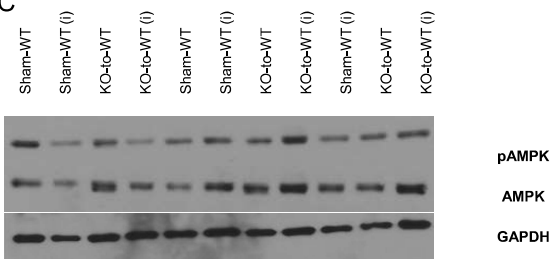
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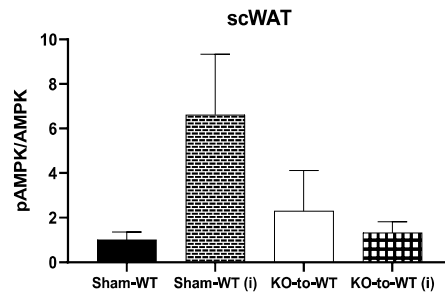
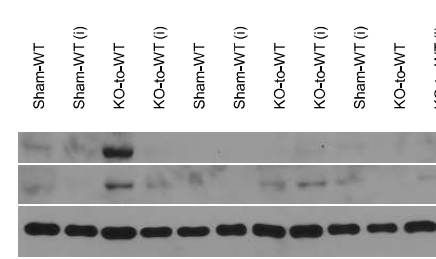
B



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D



E

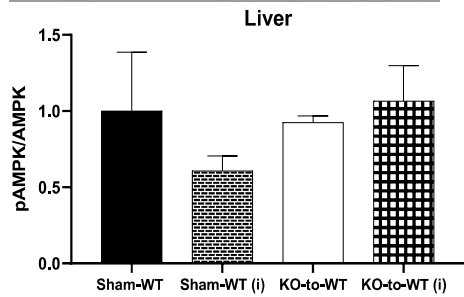
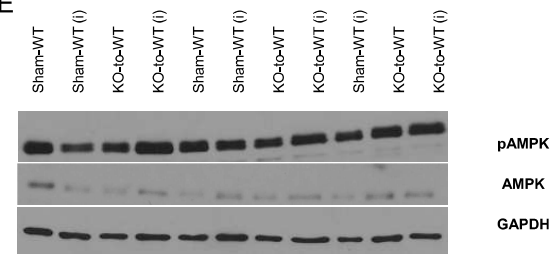


Figure S9: Transplantation of committed pre-adipocytes from FGF21^{-/-} donors affects AKT but not AMPK signaling in FGF21^{-/-} recipient mice. Related to Figures 6-7.

(A) Protein expression for pAKT/AKT and blots for pAKT and AKT in scWAT. (B-E) Western blotting protein expression for pAMPK/AMPK and blots for pAMPK, AMPK and GAPDH (B) TA, (C) iBAT, (D) scWAT and (E) Liver. (F) Western blotting protein expression for pAKT/AKT in Sham-WT, Sham-KO and KO-to-KO recipients in Liver (n=2-3 per group; insulin-stimulated conditions depicted using 'i'; *p<0.05, **p<0.01 as described [*= insulin effect; #= group effect]; two-tailed t-test). All data are represented as mean ± SEM.

Figure S9

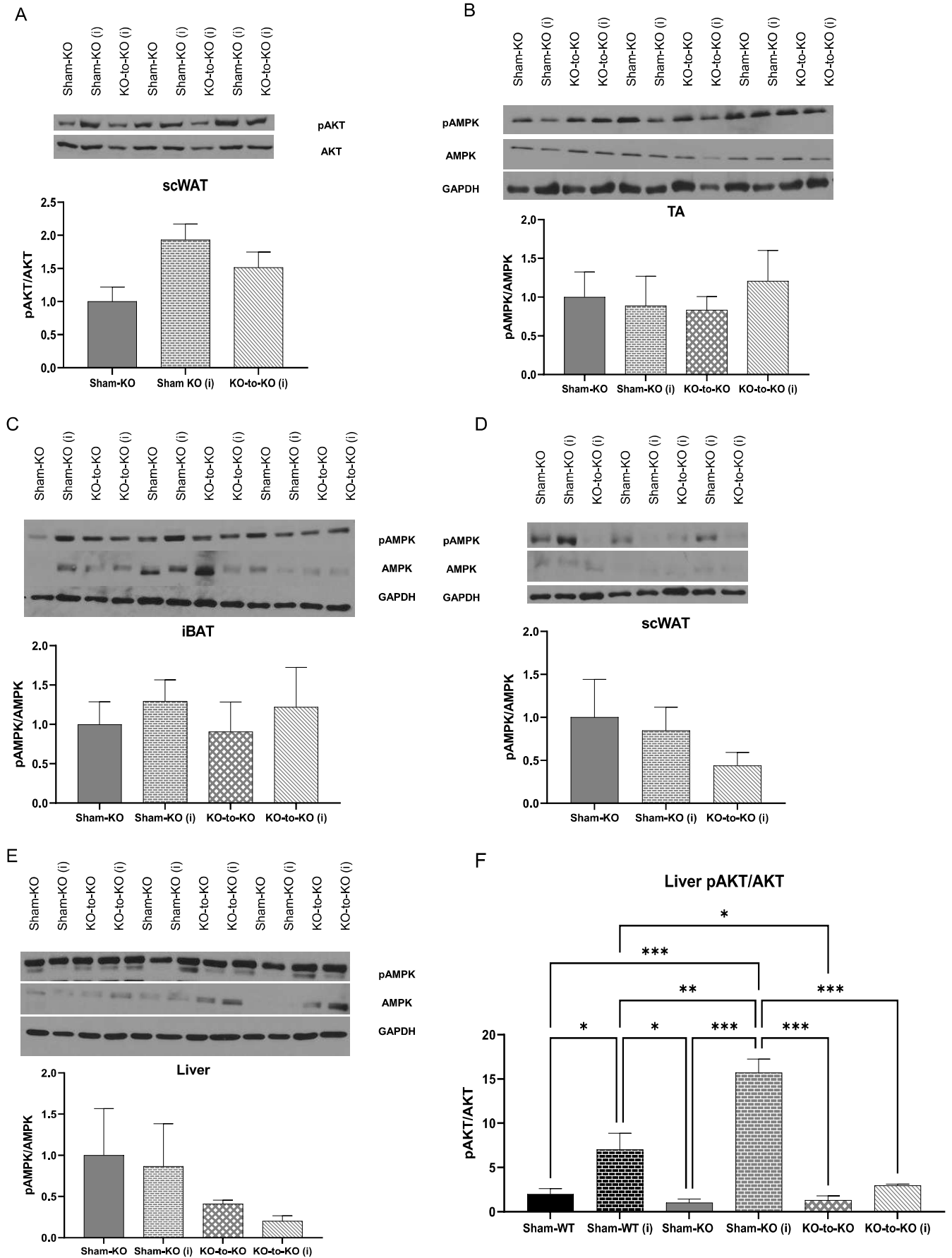


Figure S10: Transplantation of committed pre-adipocytes from FGF21^{-/-} donors alters gene expression in the liver of WT and FGF21^{-/-} recipient mice. Related to Figures 6-7.

(A, B) Gene expression analysis in Liver (A) and pgWAT (B) in Sham-WT and KO-to-WT mice, 12 weeks post-transplant (n=3-6 per group; *p<0.05, **p<0.01, ***p<0.001 vs Sham-WT; two-tailed t-test). (C, D) Gene expression analysis in Liver (C) and pgWAT (D) in Sham-KO and KO-to-KO mice, 12 weeks post-transplant (n=3-6 per group; *p<0.05, **p<0.01 vs Sham-KO; two-tailed t-test). (E, F) Gene (E) and protein (F) expression analysis in Liver of Sham-WT and KO-to-WT mice, 12 weeks post-transplant (n= 2-5 per group). (G, H) Gene (G) and protein (H) expression analysis in Liver of Sham-KO and KO-to-KO (F) mice, 12 weeks post-transplant (n=2-6 per group). All data are represented as mean ± SEM.

Figure S10

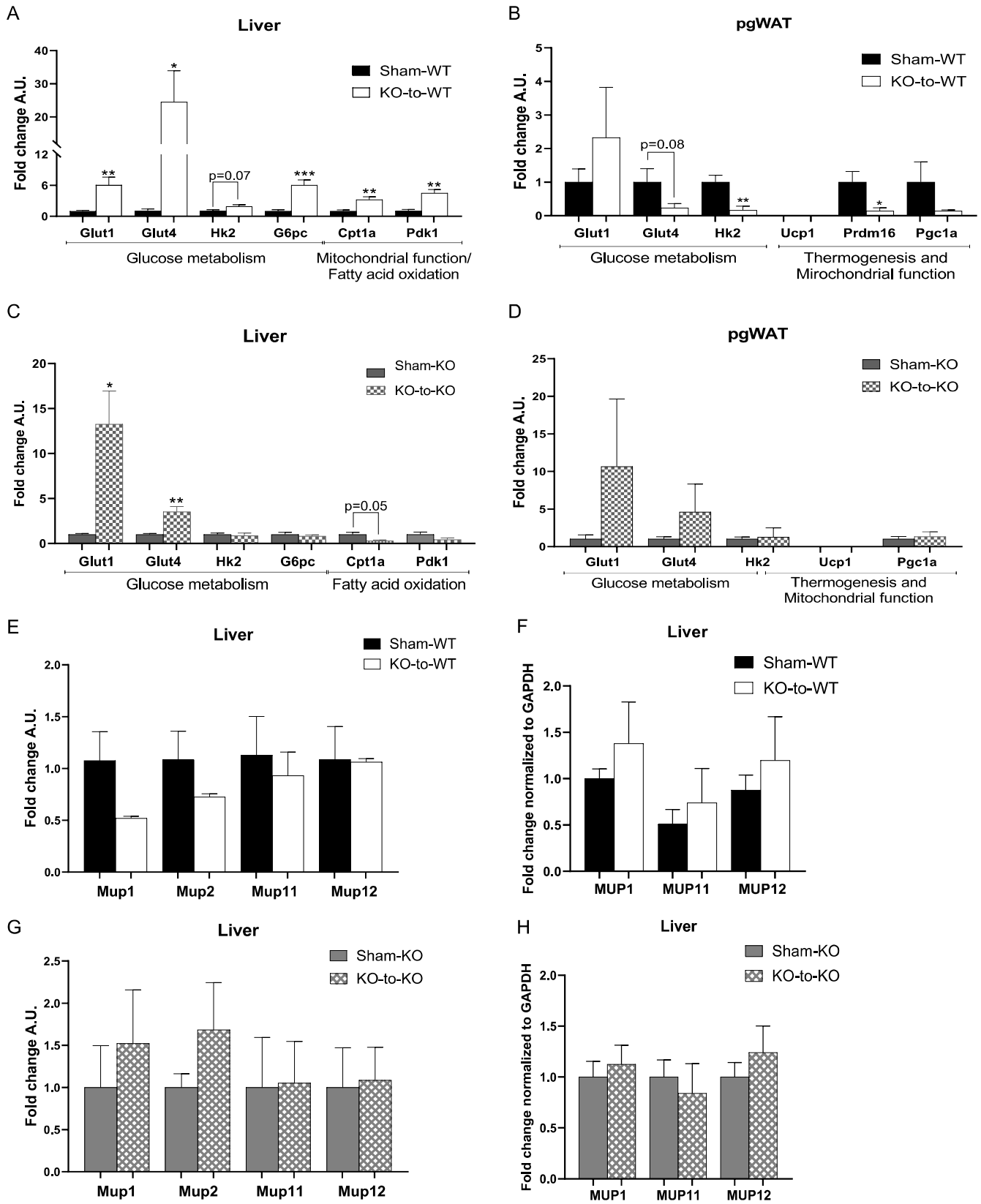
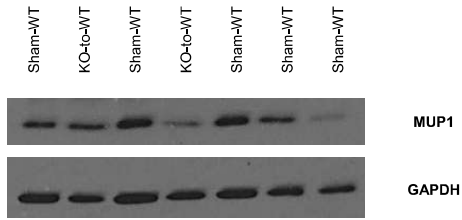


Figure S11: Transplantation of committed pre-adipocytes from FGF21^{-/-} donors does not affect MUP protein expression in the liver of WT and FGF21^{-/-} recipient mice. Related to Figures 6-7.

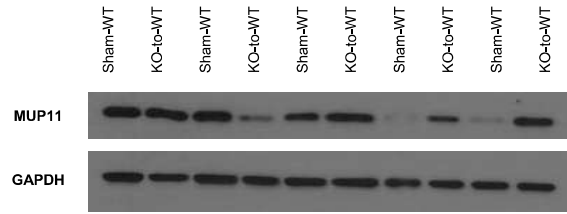
(A-C) Western blotting protein blots (Sham-WT and KO-to-WT groups) for MUP1 (A), MUP11 (B), and MUP12 (C) in the Liver (n=2-6 per group). (D-F) Western blotting protein blots (Sham-KO and KO-to-KO groups) for MUP1 (D), MUP11 (E), and MUP12 (F) in the Liver (n=2-6 per group). All data are represented as mean \pm SEM.

Figure S11

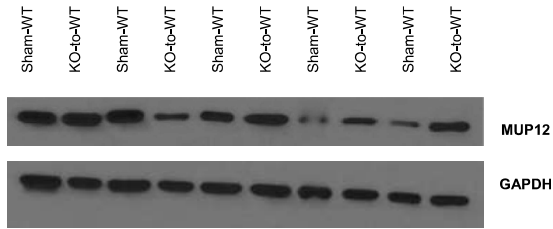
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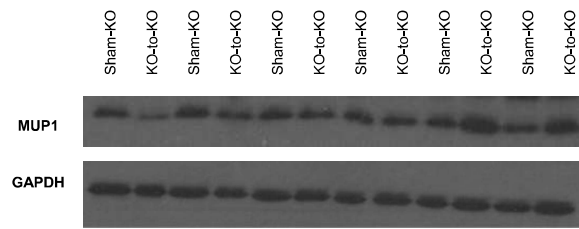
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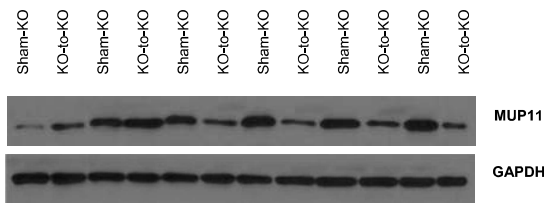
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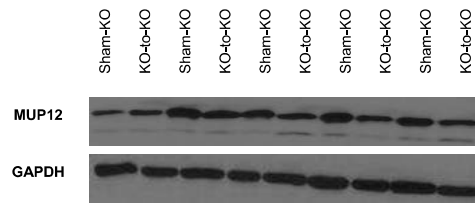


Figure S12: Adipogenic differentiation of human brown adipocytes from AgeX-NP88 cell line and tracing of transplanted hBAs. Related to Figure 8.

(A) 40x magnification of pre-adipocytes from BAT SVF showing the cells over days 3 and 8 of differentiation. (B) Expression of genes involved in adipogenesis in differentiating brown adipocytes over days 0, 1, 3, 5, 7 and 10 of differentiation (n=3 per group; *p<0.05, **p<0.01, ***p<0.001, ****p<0.001 vs Day 0 of differentiation). (C-H) Gene expression analysis in TA (C), Liver (D, E), iBAT (F), scWAT (G) and pgWAT (H) in Sham, HBA 2x and HBA 4x mice, 20 weeks post-transplant (n=4-5 per group; *p<0.05, **p<0.01, ***p<0.001 vs Sham; two-tailed t-test). All data are represented as mean \pm SEM.

Figure S12

A

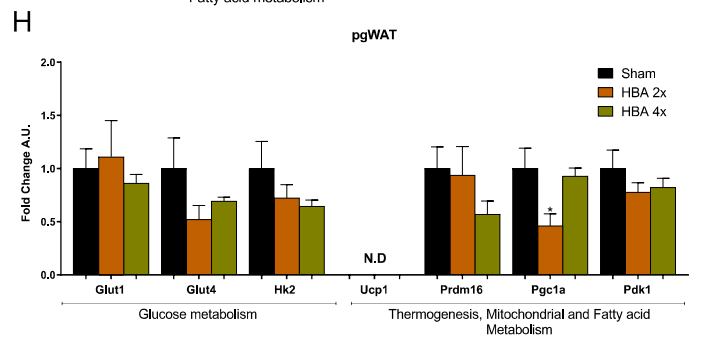
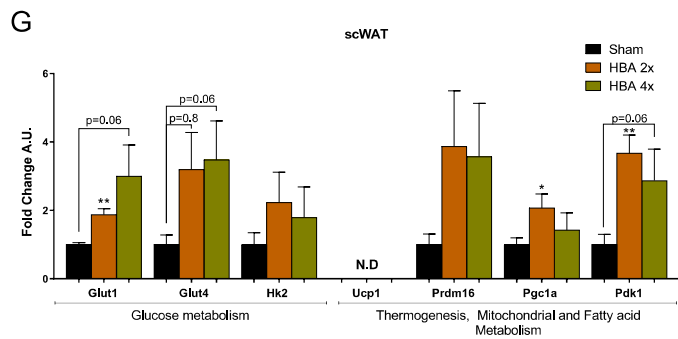
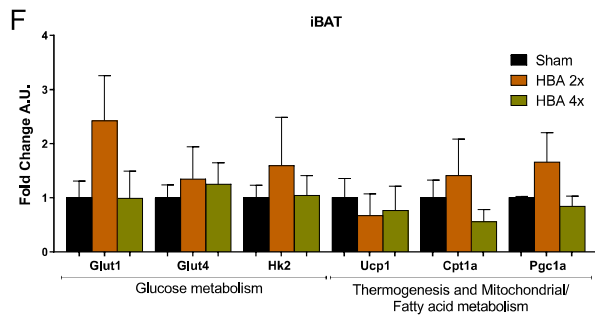
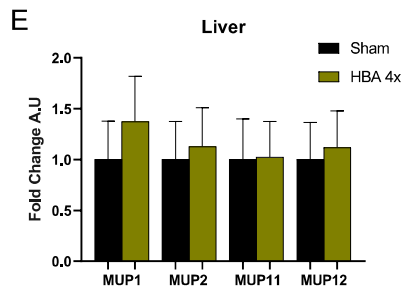
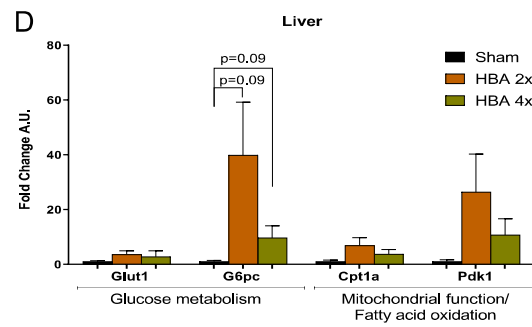
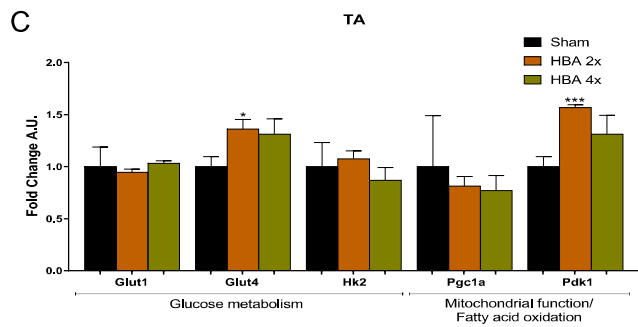
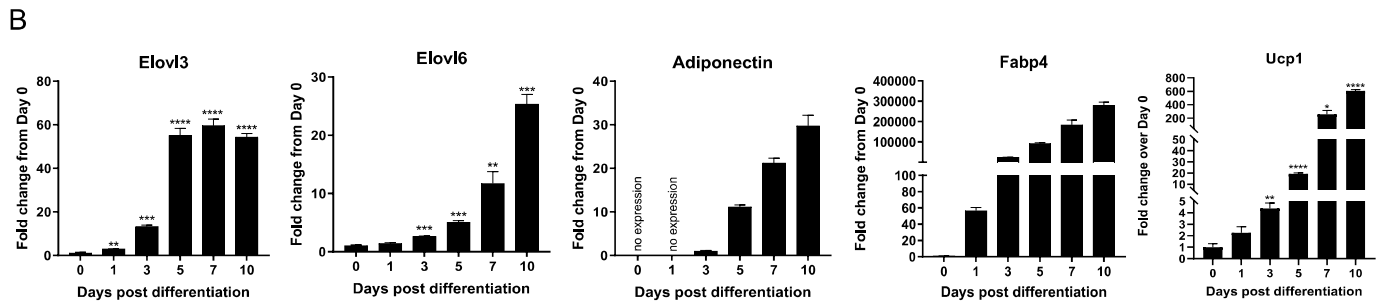
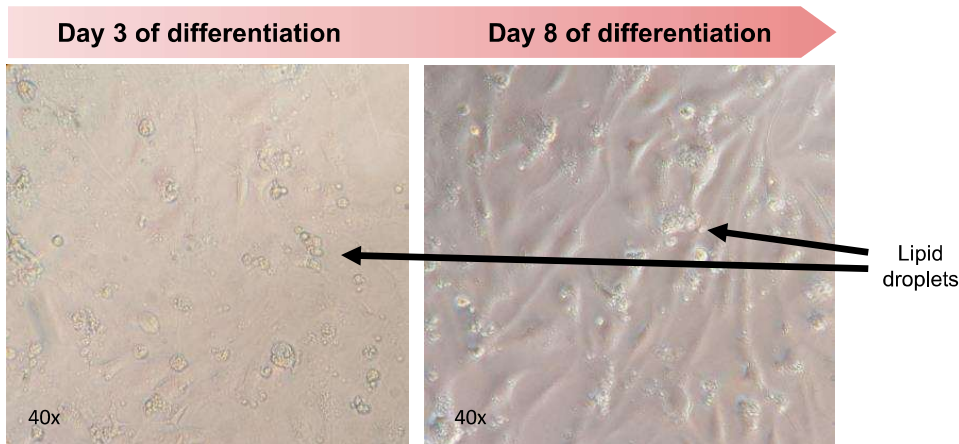


Table S1. qPCR forward and reverse primer sequences for the genes measured. Related to Figures S1 and S12.

Mouse Primers		
Gene	Forward Sequence	Reverse Sequence
<i>Adipoq</i>	AGATGGCACTCCTGGAGAGAAG	ACATAAGCGGCTTCTCCAGGCT
<i>Pparg</i>	GTACTGTTCGGTTTCAGAAGTGCC	ATCTCCGCCAACAGCTTCTCCT
<i>Ppara</i>	ACCACTACGGAGTTCACGCATG	GAATCTTGCAGCTCCGATCACAC
<i>Glut 4</i>	ATCATCCGGAACCTGGAGG	CGGTCAGGCGCTTTAGACTC
<i>Glut 1</i>	GCTTCTCCAACCTGGACCTCAAAC	ACGAGGAGCACCGTGAAGATGA
<i>Hk2</i>	AGAGAACAAGGGCGAGGAG	GGAAGCGGACATCACAATC
<i>G6pc</i>	AGGTCGTGGCTGGAGTCTTGTC	GTAGCAGGTAGAATCCAAGCGC
<i>Prdm16</i>	ATCCACAGCACGGGTGAAGCCAT	ACATCTGCCACAGTCCTTGCA
<i>Ucp1</i>	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
<i>Pgc1a</i>	GAATCAAGCCACTACAGACACCG	CATCCCTCTTGAGCCTTTCTGTG
<i>Cpt1a</i>	AAAGATCAATCGGACCCTAGACA	CAGCGAGTAGCGCATAGTCA
<i>Pdk1</i>	GGCGGCTTTGTGATTTGTAT	ACCTGAATCGGGGATAAAC
<i>Mup1</i>	ATGAAGCTGCTGCTGTGT	TCATTCTCGGGCCTTGAG

<i>Mup2</i>	ATGAAGCTGCTGCTGCTGT	TCATTCTCGGGCCTGGAG
<i>Mup11</i>	ATGAAGATGCTGTTGCTG	TCATTCTCGGGCCTGGAG
<i>Mup12</i>	ATGAAGATGCTGCTGCTG	TCATTCTCGGGCCTGGAG (Same for <i>Mup2</i> , <i>Mup11</i> and <i>Mup12</i>)
<i>Beta-actin</i>	CATTGCTGACAGGATGCAGAAGG	TGCTGGAAGGTGGACAGTGAGG
Human Primers		
Gene	Forward Sequence	Reverse Sequence
<i>Elovl3</i>	CCACACCACGATGGAACACT	CCTTGGGCCTGATGTAGGTC
<i>Elovl6</i>	AAGCAGACAGGAGAACACTCG	TAGGTGCCGACCACCGAATA
<i>Adipoq</i>	GGCCATCTCCTCCTCACTTC	ATCCTGCCCTCTGGTATG
<i>Fabp4</i>	GACCTGGACTGAAGTTCGCA	ACTTGCTTGCTAAATCAGGGA
<i>Ucp1</i>	AGGCGTGAAGAGCAAGGAAA	CCCCATCTTCACTCAGAGACTG

Table S2. Donors, Recipients, Cell number transplanted, and Abbreviations used for different transplantation models. Related to Figures 1-8, Figures S2 - S12.

Donor Mice	Recipient mice	Cell number transplanted	ID/ Abbreviation used
WT (C57BL/6) BAT SVF	WT (C57BL/6)	1 million	SVF 1x
WT (C57BL/6) BAT SVF	WT (C57BL/6)	2 million	SVF 2x
WT (C57BL/6) BAT committed pre-adipocytes	WT (C57BL/6)	1 million	iBAT 1x
WT (C57BL/6) BAT committed pre-adipocytes	WT (C57BL/6)	2 million	iBAT 2x
WT (C57BL/6) BAT committed pre-adipocytes	FGF21 ^{-/-}	2 million	WT-to-KO
FGF21 ^{-/-} BAT committed pre-adipocytes	WT (C57BL/6)	2 million	KO-to-WT
FGF21 ^{-/-} BAT committed pre-adipocytes	FGF21 ^{-/-}	2 million	KO-to-KO
N/A	WT	Sham/ Empty Scaffolds	Sham-WT

N/A	FGF21 ^{-/-}	Sham/ Empty Scaffolds	Sham-KO
N/A	NSG	Sham/Empty Scaffolds	Sham
AgeX-NP88 cell line	NSG	2 million	HBA 2x
AgeX-NP88 cell line	NSG	4 million	HBA 4x