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Supplemental Information

Functional Inactivation of Mast Cells Enhances Subcutaneous Adipose Tissue Browning in Mice

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Supplementary Materials

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Supplementary Table 1. Primers for quantitative real-time PCR (related to Star Methods).

Gene	Forward primers (5' to 3')	Reverse primers (5' to 3')
Ucp1	CACTCAGGATTGGCCTCTACG	GGGGTTTGATCCCATGCAGA
Prdm16	CCACCAGACTTCGAGCTACG	ACACCTCTGTATCCGTCAGCA
Pgc1α	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Cidea	TGACATTCATGGGATTGCAGAC	GGCCAGTTGTGATGACTAAGAC
Elovl3	TTCTCACGCGGGTTAAAAATGG	GAGCAACAGATAGACGACCAC
Ndufv2	GCAAGGAATTTGCATAAGACAGC	TAGCCATCCATTCTGCCTTTG
Ndufb5	CAAGAGACTGTTTGTCGTCAAGC	TGTTCACCAGTGTTATGCCAAT
Tmem26	ACCCTGTCATCCCACAGAG	TGTTTGGTGGAGTCCTAAGGTC
CD137	CGTGCAGAACTCCTGTGATAAC	GTCCACCTATGCTGGAGAAGG
Tbx1	TGTGCCCGTAGATGACAAGC	GTACTCGGCCAGGTGTAGC
Cited1	ACTAGCTCCTCTGGATCGACA	GACCCAGTTTTGCATGGGC
Shox2	TATCCAGACGCTTTCATGCG	GGCTCCTATAAGGACACCTTTGT
Pdgfrα	AGCAGGCAGGGCTTCAACGG	ACACAGTCTGGCGTGCGTCC
AEBP1	GTACCCACACACTTCAGTCAG	CTCCGCTTGTTCATTCCAGC
GATA2	GCCGGGAGTGTGTCAACTG	AGGTGGTGGTTGTCGTCTGA
Tph1	TGACGCTGCCGATTCTCCAG	GCATGTTGCAACTCGCCAGC
β-actin	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA

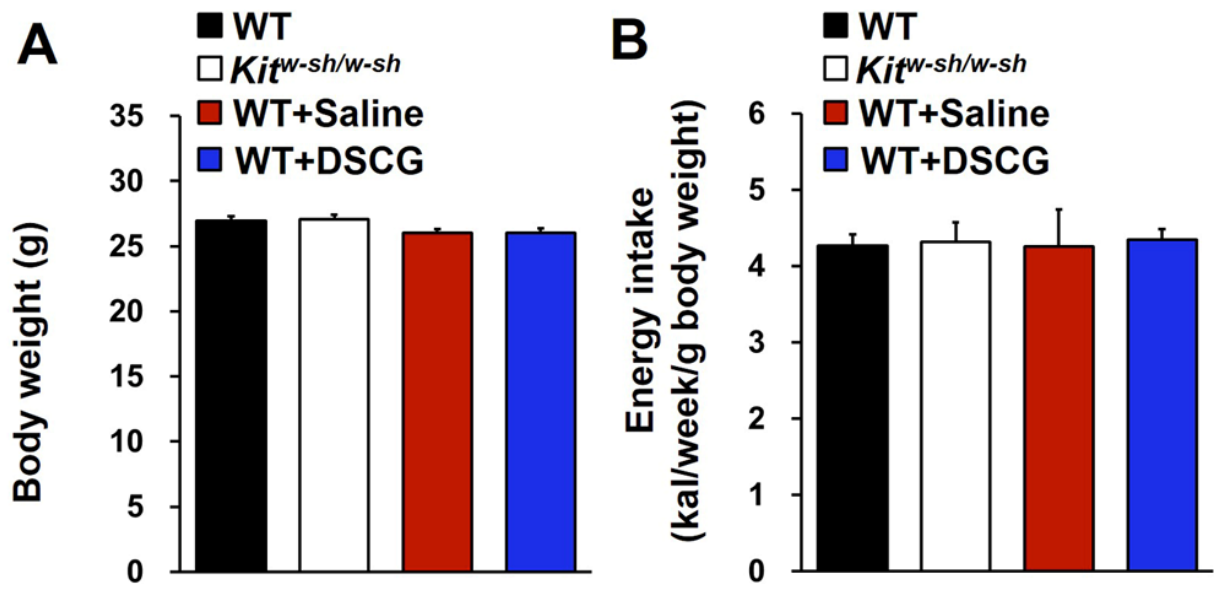

Figure S1. Bodyweight and energy intake from mice on a chow diet (related to Figure 1). (**A**) Bodyweight of 12-week-old WT mice (n=13), *Kit*$^{w\text{-}sh/w\text{-}sh}$ mice (n=13), and WT mice receiving saline (n=13) or DSCG (n=16) treatment. (**B**) Energy intake in the indicated groups (n=13-16 per group). Data are mean±SEM.

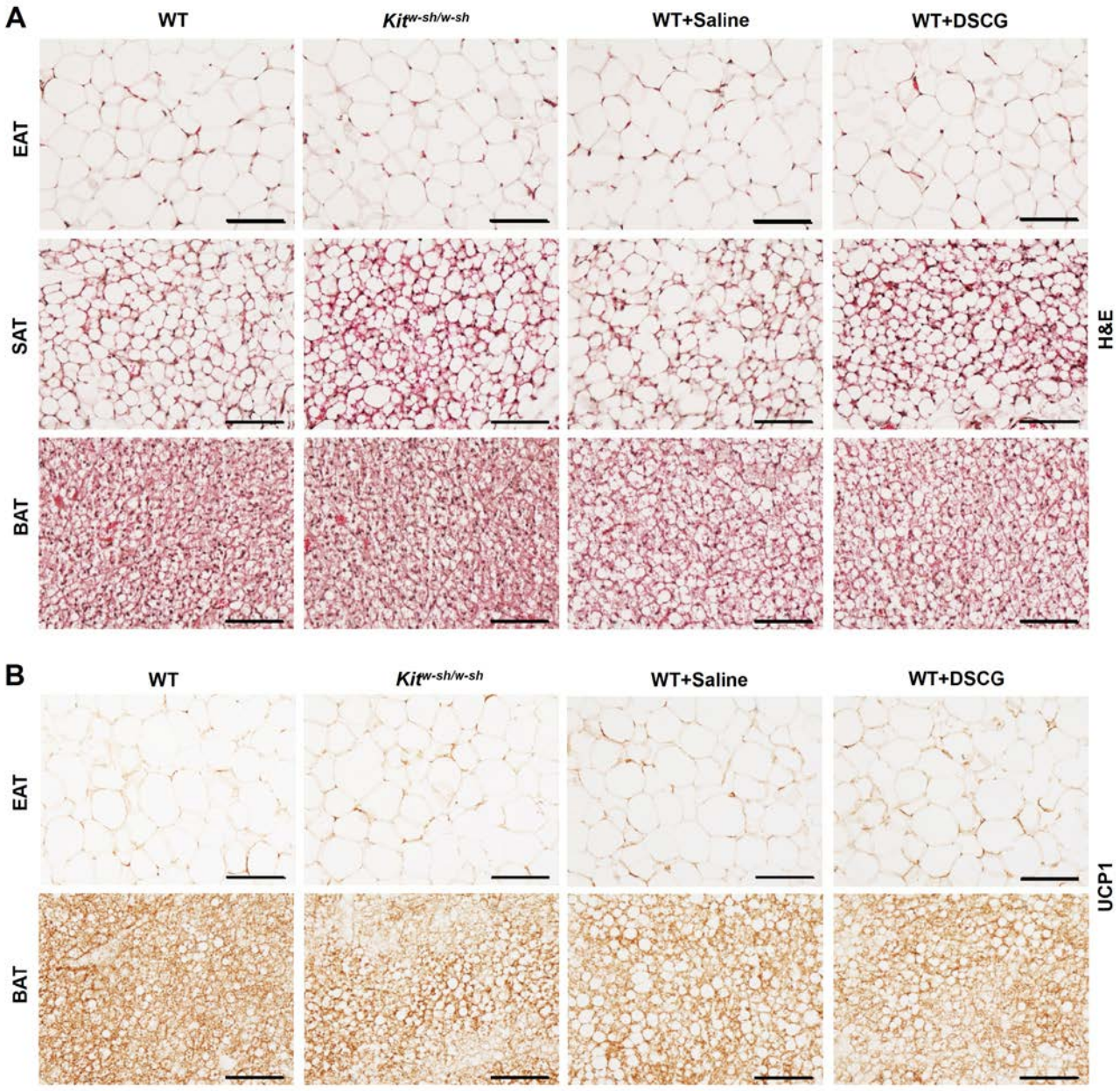

Figure S2. Morphology and UCP1 expression in mouse adipose tissues (related to Figure 1). (**A**) Representative haematoxylin and eosin staining of EAT, SAT, and BAT sections from indicated groups of mice on a chow diet (n=8 per group); scale bar: 100 μm. (**B**) Representative UCP1 staining of EAT and BAT sections from indicated groups of mice on a chow diet (n=8 per group); scale bar: 100 μm.

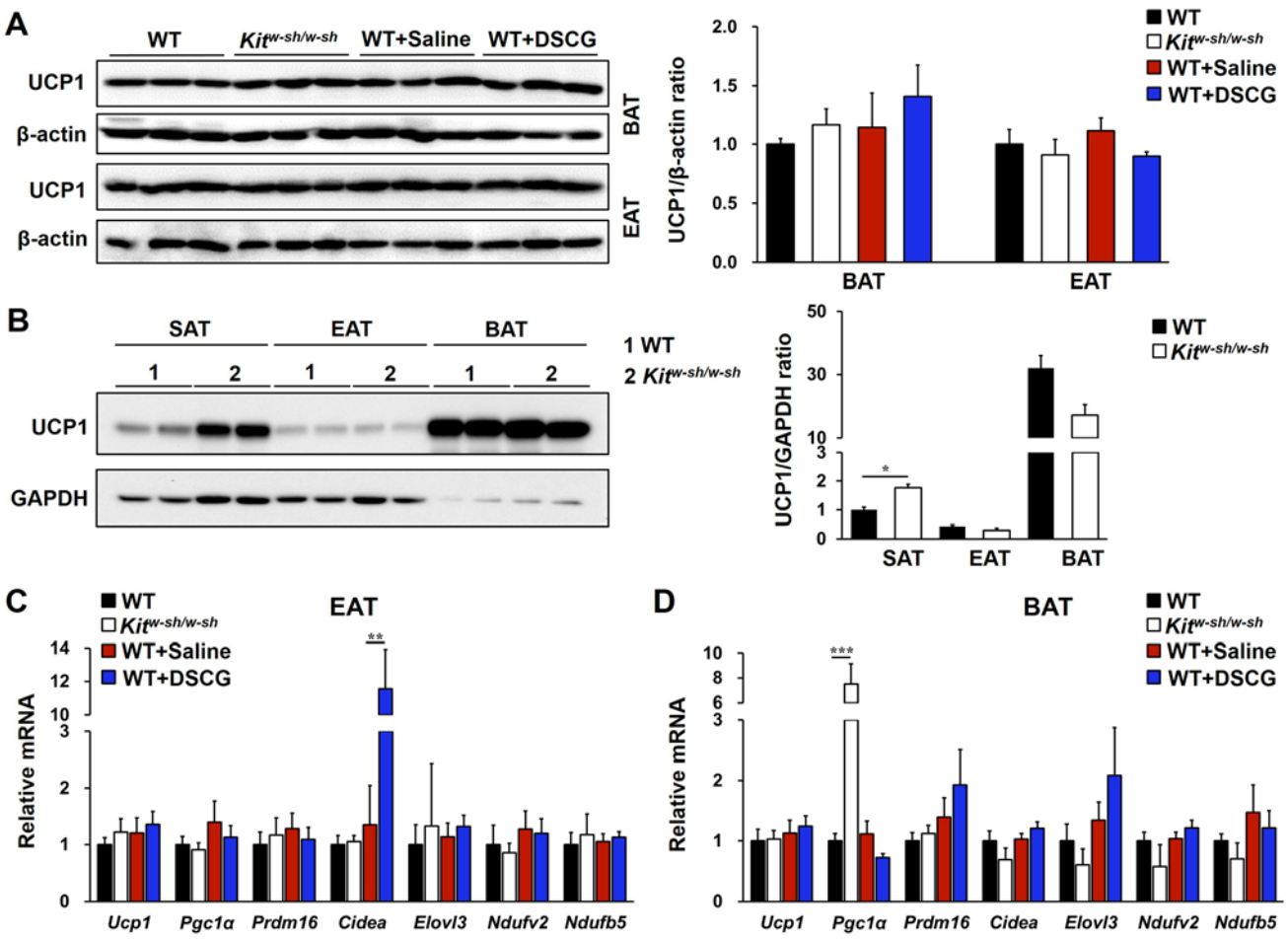

Figure S3. Thermogenic program in mouse adipose tissues (related to Figure 1).
(**A**) Immunoblot analysis of UCP1 and quantification relative to β-actin in EAT and BAT (n=6 per group). Representative immunoblots are shown to the left. (**B**) Immunoblot analysis of UCP1 and quantification relative to GAPDH in SAT, EAT and BAT on the same immunoblot. Representative immunoblots are shown to the left. **C/D.** RT-PCR analysis of thermogenic and mitochondrial genes in EAT (**C**) and BAT (**D**) from different groups of mice as indicated (n=12 per group). Data are mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001.

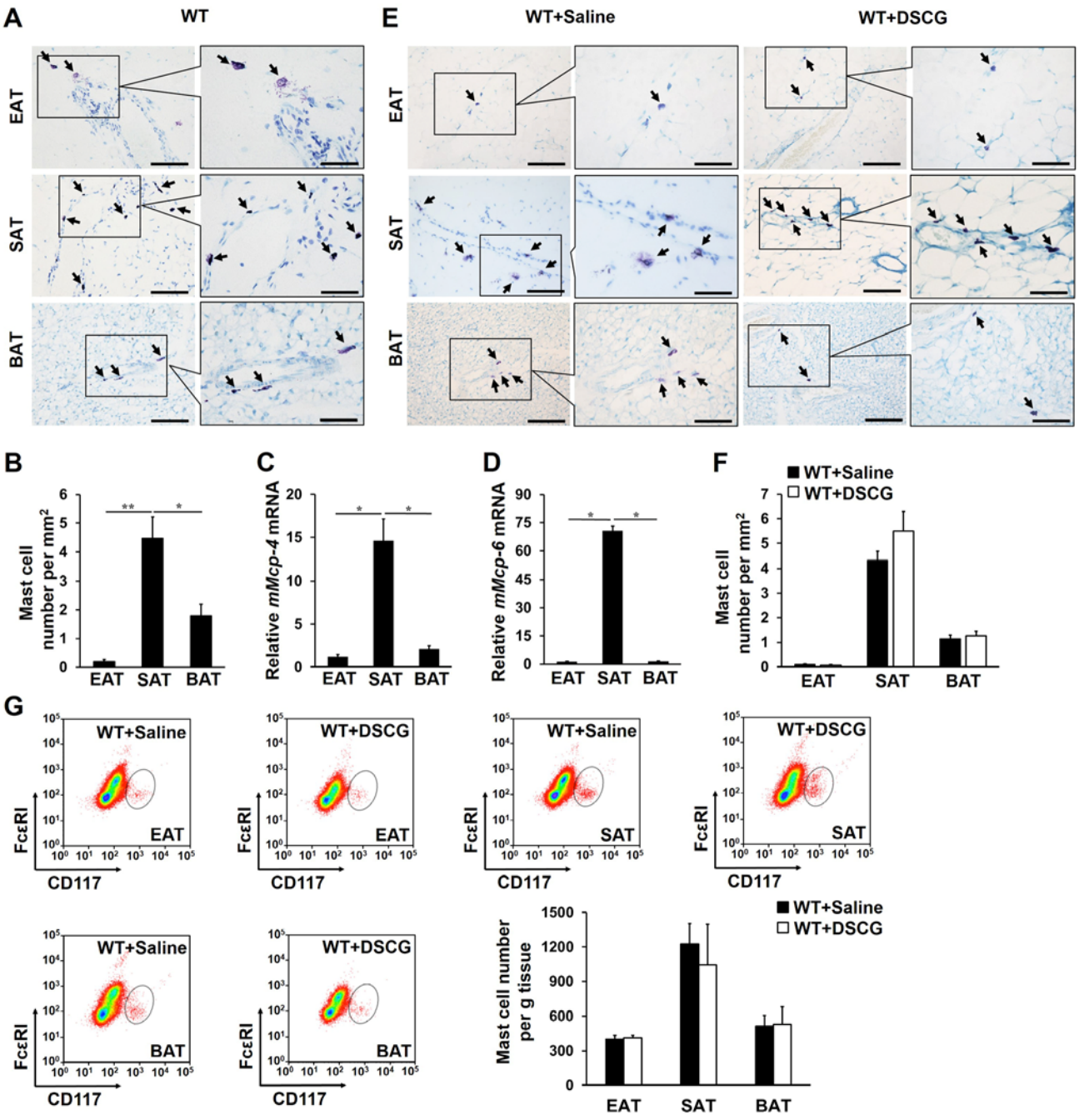

Figure S4. MC numbers and MC marker mRNA levels in different adipose tissues from WT mice treated with saline or DSCG (related to Figure 2).
(**A**)/(**B**) Toluidine blue staining for MCs of representative adipose tissue (EAT, SAT, and BAT) sections (**A**) and MC number quantification (**B**) from WT mice (n=6 per group). (**C**)/(**D**) RT-PCR analysis of MC markers *mMcp-4* (**C**) and *mMcp-6* (**D**) in different adipose tissues (n=6 per group). (**E**)/(**F**) Toluidine blue staining for MCs of representative EAT, SAT, and BAT sections (**E**) and MC number quantification (**F**) in saline- (n=8) and DSCG-treated (n=10) WT mice. Arrows indicate MCs; scale bar: 100 μm, inset scale bar: 50 μm. (**G**) FACS analysis for MCs number in EAT, SAT, and BAT and MC number quantification in saline- (n=8) and DSCG-treated (n=8) WT mice. **P*<0.05, ***P*<0.01.

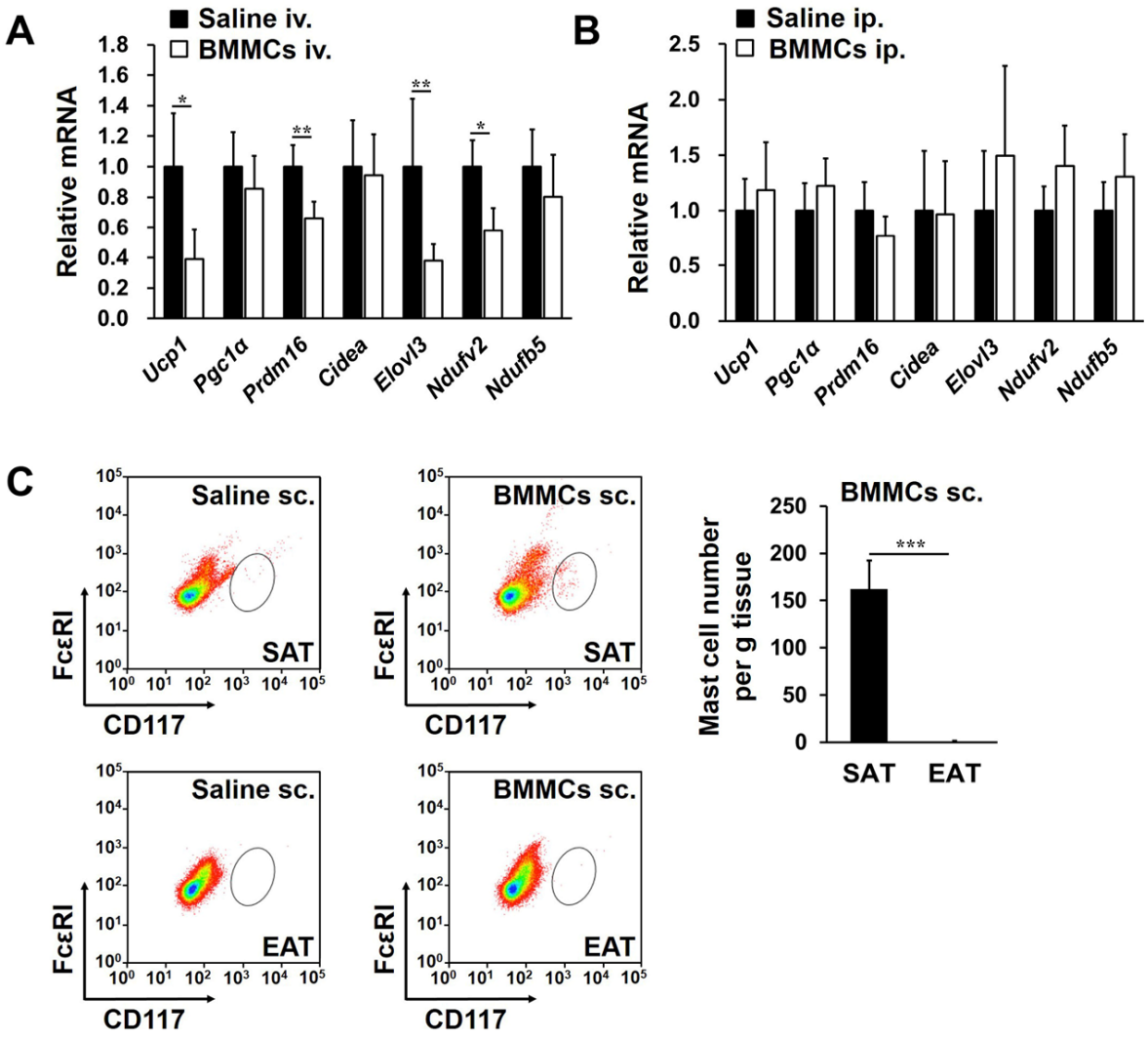

Figure S5. MC number quantification in adipose tissues from *Kit*$^{w\text{-}sh/w\text{-}sh}$ mice receiving saline or BMMCs (related to Figure 2).
(**A**)/(**B**) Real-time PCR analysis of thermogenic and mitochondrial genes in SAT from *Kit*$^{w\text{-}sh/w\text{-}sh}$ mice received saline or BMMC adoptive transfer via intravenous injection (iv.) (**A**) (n=9-10 per group), or intraperitoneal injection (ip.) (**B**) (n=7-9 per group). **(C)** FACS analysis for MCs number in EAT and SAT and MC number quantification in *Kit*$^{w\text{-}sh/w\text{-}sh}$ mice received subcutaneous (sc.) saline (n=8) or BMMC (n=8) adoptive transfers. Data are mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001.

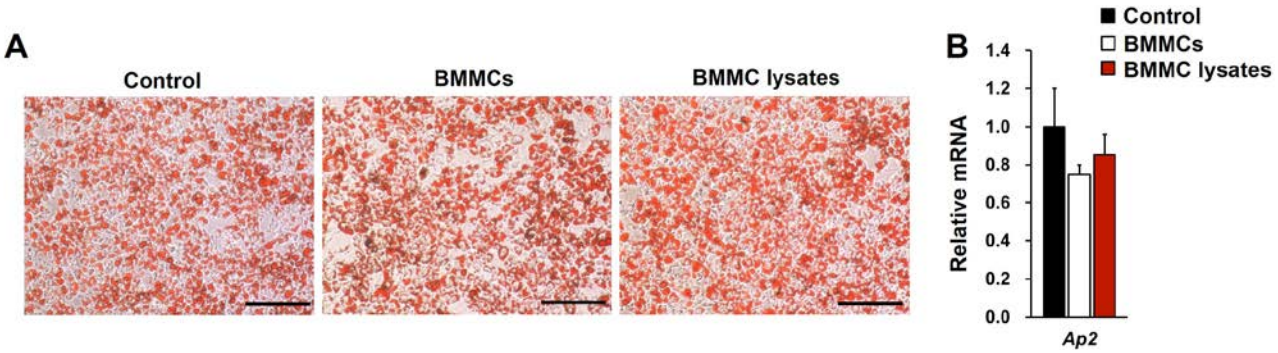

Figure S6. The effects of live BMMCs and BMMC lysates on SAT preadipocyte adipogenesis (related to Figure 4).
(**A**) Oil-red O staining for browning-differentiated SAT adipocytes in the absence (Control) or presence of live BMMCs or BMMC lysates (n=4 per group); scale bar: 200 μm. (**B**) Real-time PCR analysis of adipogenic gene *Ap2* (fatty acid binding protein-4) in adipocytes from different treatments (n=4 per group). Data are mean±SEM.

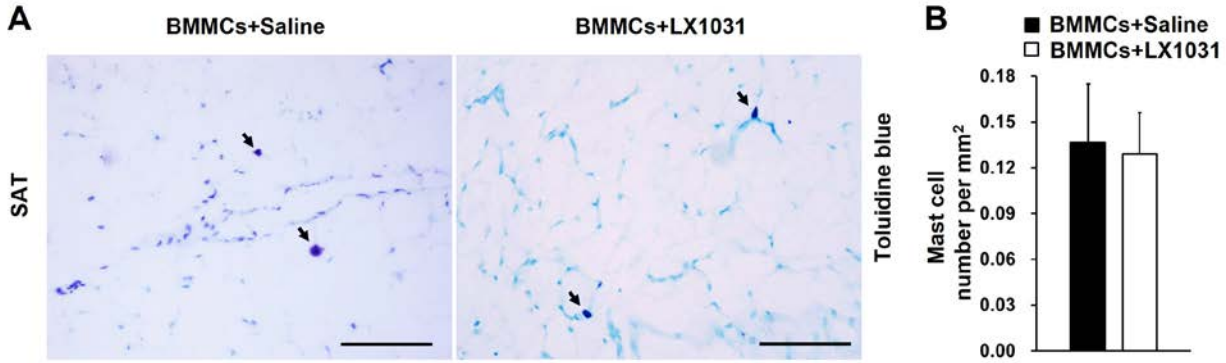

Figure S7. (related to Figure 5) **(A)/(B)** Toluidine blue staining of MCs in SAT sections and MC number quantification from *Kit*$^{w\text{-}sh/w\text{-}sh}$ mice that received subcutaneous BMMC reconstitution and treated by subcutaneous injection of saline or LX1031 (n=8 per group); scale bar: 100 μm. Data are mean±SEM.

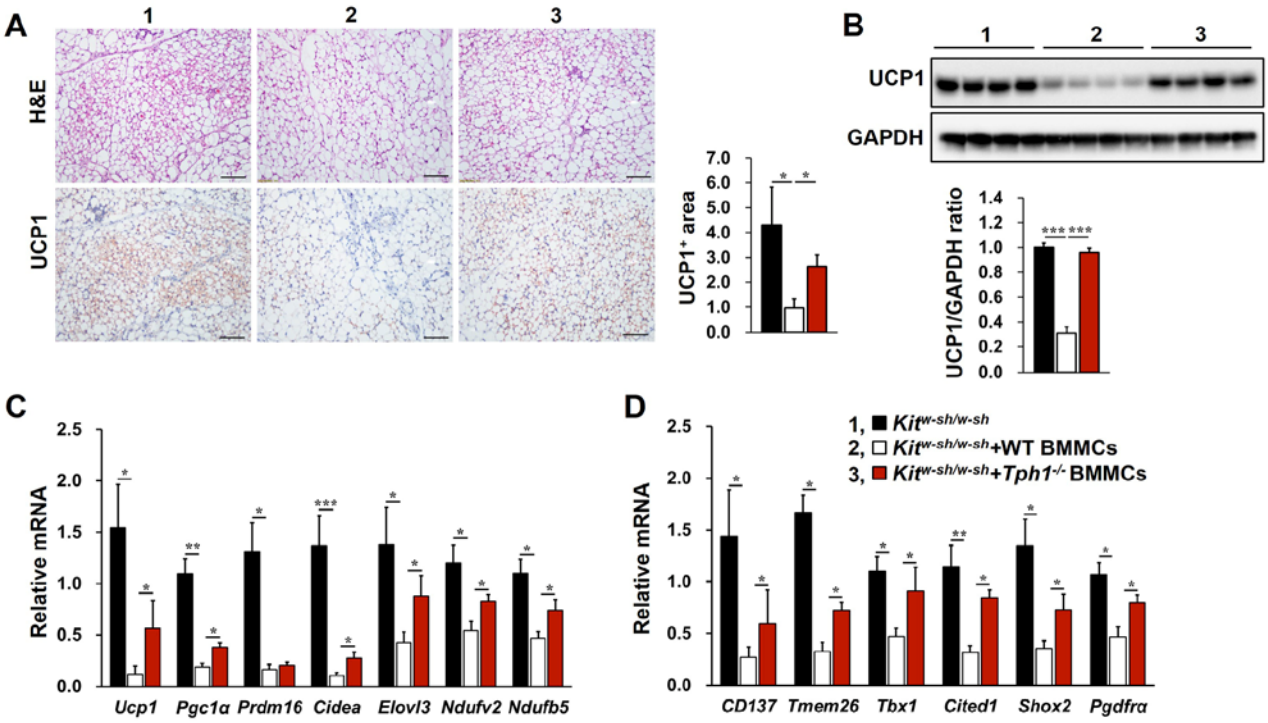

Figure S8. *Kit*$^{w\text{-}sh/w\text{-}sh}$ mouse SAT browning after receiving without and with subcutaneous adoptive transfer of BMMCs from WT and *Tph1*$^{-/-}$ mice (related to Figure 6).
(A) Haematoxylin and eosin staining, UCP1 immunostaining and quantification of SAT sections from indicated groups of mice (n=8 per group). Representative images are shown to the left. (**B)** Immunoblot analysis of SAT UCP1 and quantification relative to GAPDH from indicated mice (n=6 per group). **(C)/(D)** Real time-PCR analysis of thermogenic and mitochondrial genes **(C)** and beige cell markers **(D)** in SAT from from different mice as indicated (n=8 per group). Data are mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001. Scale bar: 100 μm.