

Supplemental Information

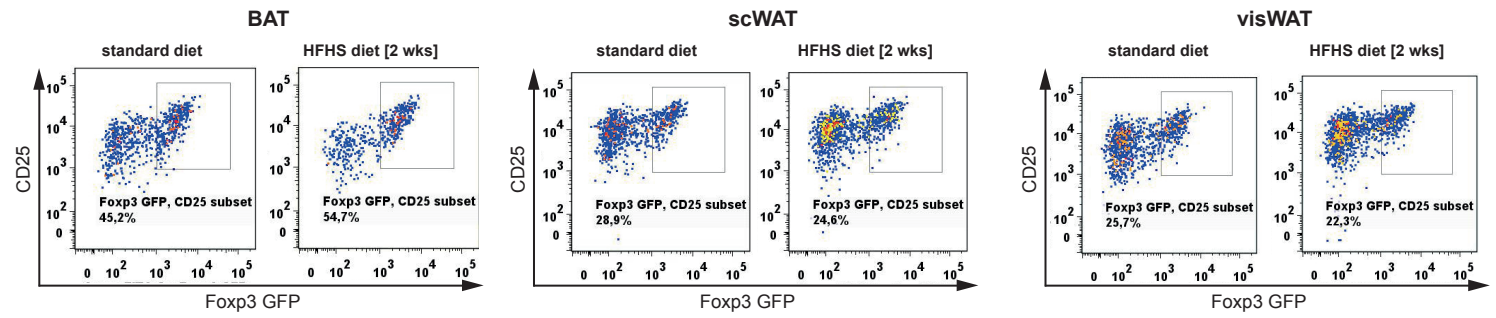
A Stat6/Pten Axis Links Regulatory

T Cells with Adipose Tissue Function

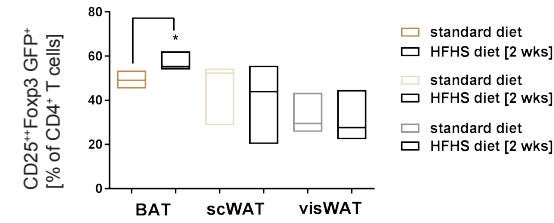
Stefanie Kälin, Maike Becker, Verena B. Ott, Isabelle Serr, Fabian Hosp, Mohammad M.H. Mollah, Susanne Keipert, Daniel Lamp, Francoise Rohner-Jeanrenaud, Victoria K. Flynn, Martin G. Scherm, Lucas F.R. Nascimento, Katharina Gerlach, Vanessa Popp, Sarah Dietzen, Tobias Bopp, Purna Krishnamurthy, Mark H. Kaplan, Manuel Serrano, Stephen C. Woods, Philipp Tripal, Ralf Palmisano, Martin Jastroch, Matthias Blüher, Christian Wolfrum, Benno Weigmann, Anette-Gabriele Ziegler, Matthias Mann, Matthias H. Tschöp, and Carolin Daniel

Figure S1

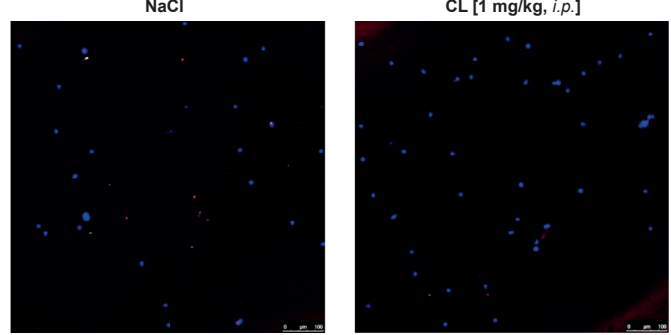
A Treg induction with naive CD4⁺T cells from fat after HFHS diet [2 wks]



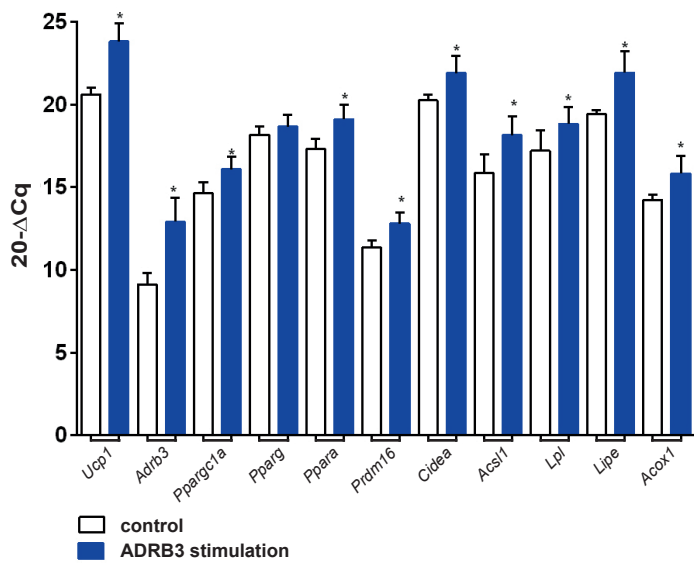
B Treg induction with naive CD4⁺T cells from fat



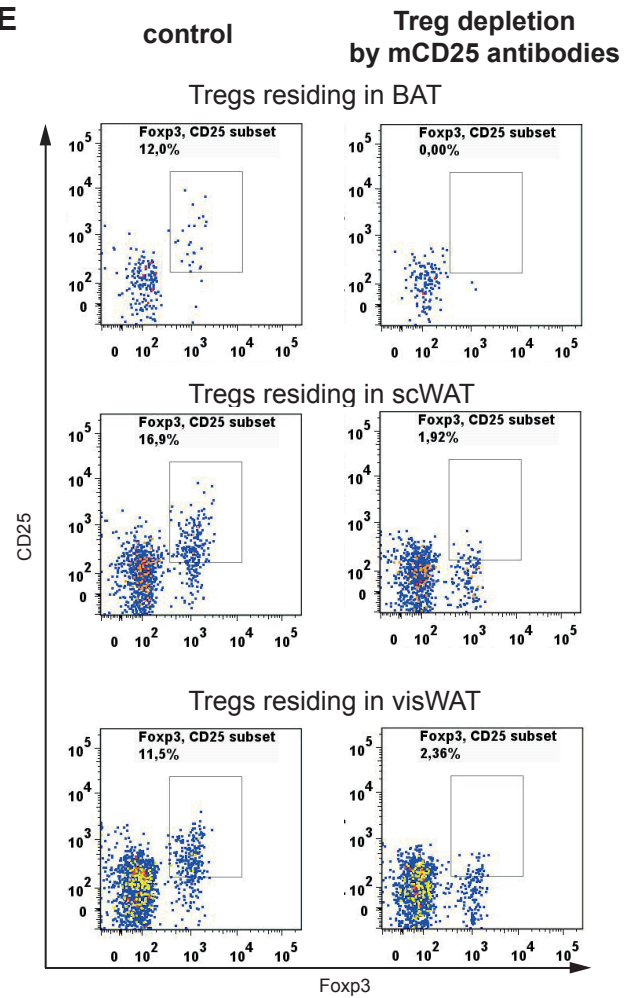
C negative control



D mRNA abundance of BAT after ADRB3 stimulation *in vivo*



E



F mRNA abundance of BAT after ADRB3 stimulation *in vivo* in the presence or absence of Tregs

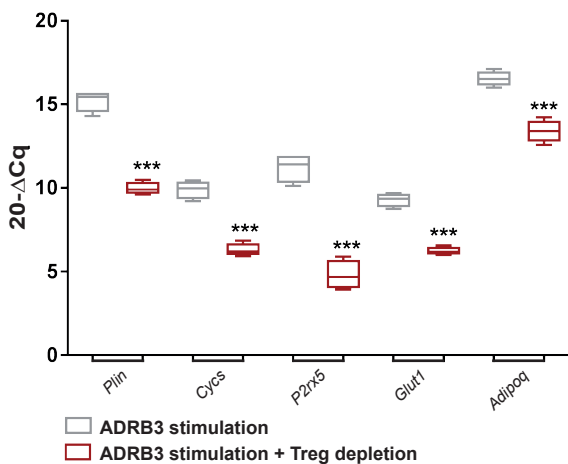


Figure S1 related to Figures 1-3: Treg enhancement by ADRB3 stimulation is required for adipose tissue function.

(A+B) (A) Representative FACS plots for *in vitro* Treg induction assays using limited TCR stimulation and naïve CD4⁺T cells purified from BAT, scWAT and visWAT of BALB/c mice on standard diet or upon 2 wk of HFHS diet. (B) Quantification of (A). n=4.

(C) Confocal microscopy images for negative control stainings of CD3 and Foxp3. Shown are stainings with secondary antibodies in the absence of primary antibodies using CD4⁺T cells from mice kept at room temperature or subjected to 3 d of 1mg/kg CL. Scale bar = 100 μ m.

(D) mRNA expression of genes involved in BAT tissue function upon treatment of BALB/c mice with CL [2 d, 1 mg/kg, *i.p.*] *in vivo*. n=6 per group.

(E) Representative FACS plots demonstrating Treg depletion efficacy in fat depots after 3 d of anti-CD25 antibody treatment.

(F) mRNA expression of genes involved in BAT function after ADRB3 stimulation (3 d, 1 mg/kg CL, *i.p.*) in the presence or absence of Tregs. Tregs were depleted using anti-CD25 antibodies. n=4 per group.

Data are presented as box-and-whisker plots with min and max values for data distribution. * = $p < 0.05$, *** = $p < 0.001$.

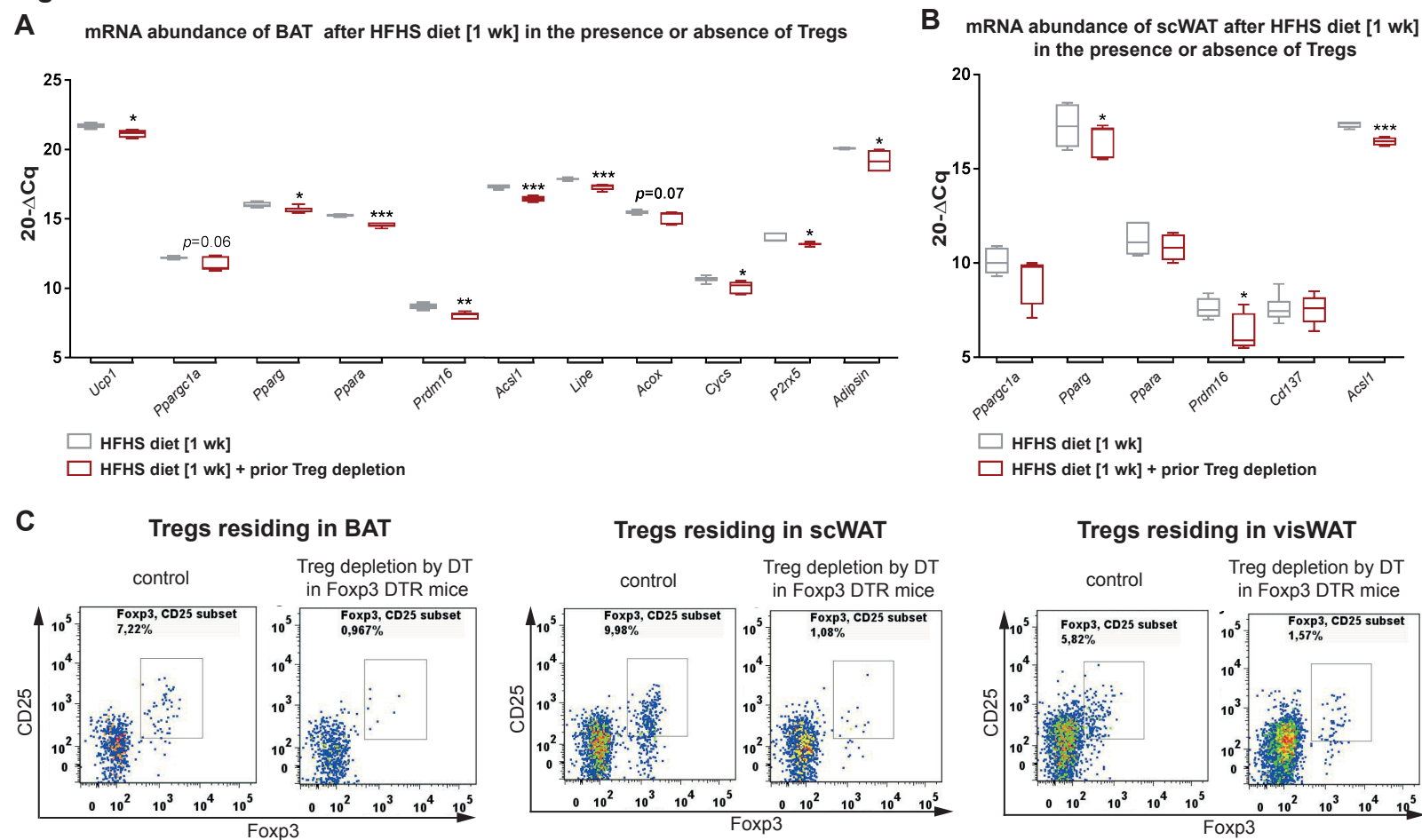
Figure S2

Figure S2 related to Figure 3: Diet-induced thermogenesis in adipose tissue is impaired in the absence of Tregs.

(A+B) Analysis of mRNA abundance of genes involved in BAT (A) and scWAT (B) function after 1 wk HFHS diet with or without additional Treg depletion. Treg depletion was achieved by using anti-CD25 antibodies. n=6 per Treg-complete group, n=4 for Treg-depleted group.

(C) Representative FACS plots demonstrating Treg depletion efficacy in BAT, scWAT and visWAT 48 h after administration of DT.

Data are presented as box-and-whisker plots with min and max values for data distribution. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Figure S3

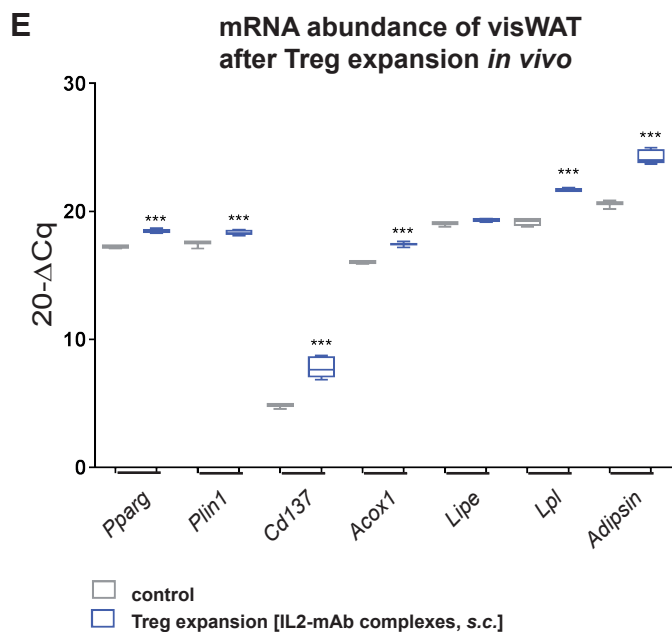
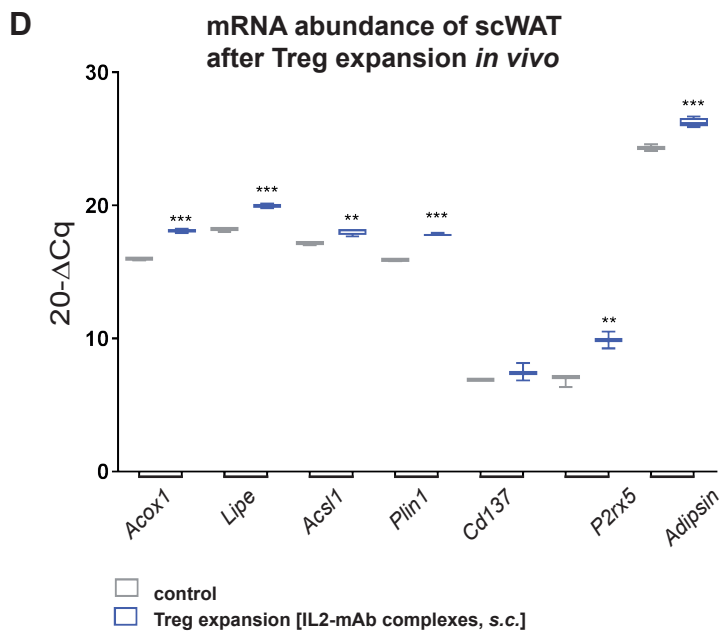
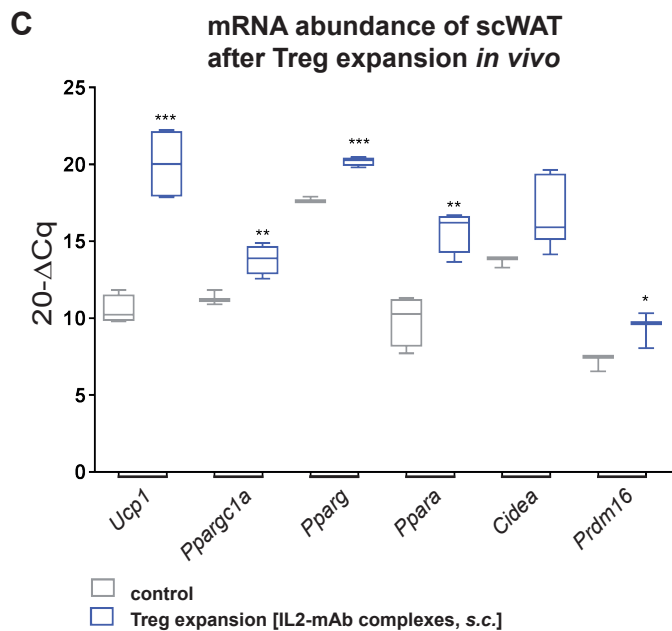
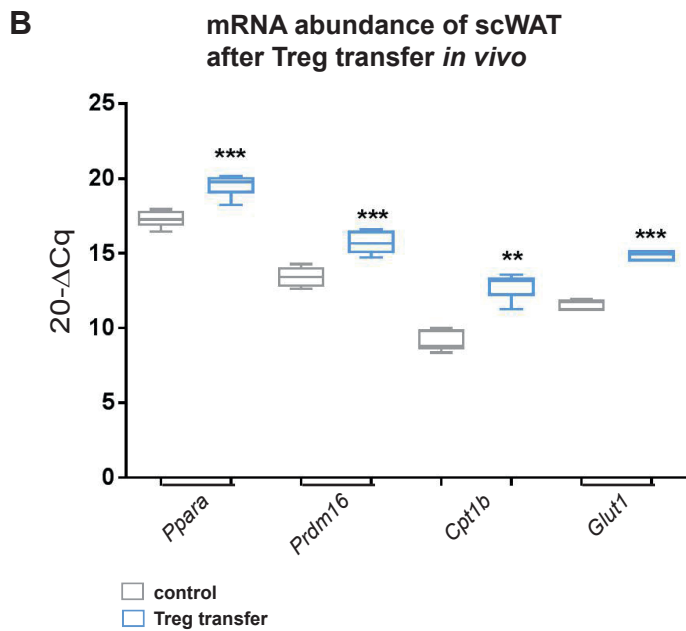
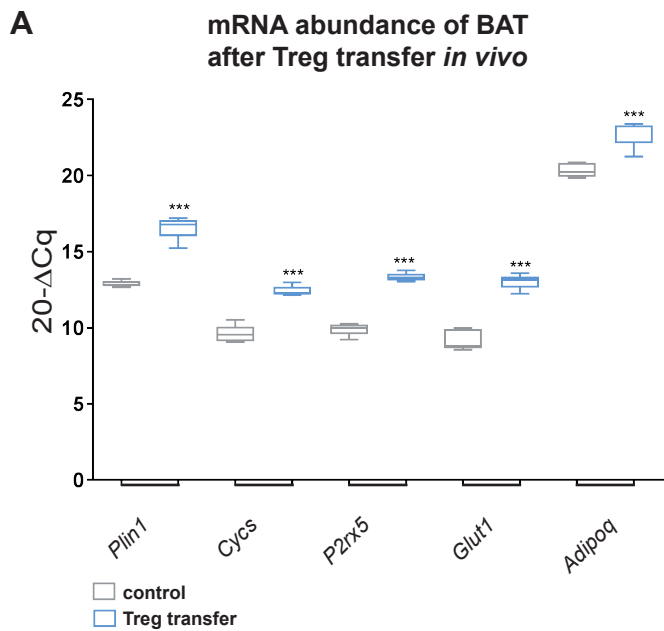


Figure S3 related to Figure 3: Role of transferred or expanded Tregs for adipose tissue function.

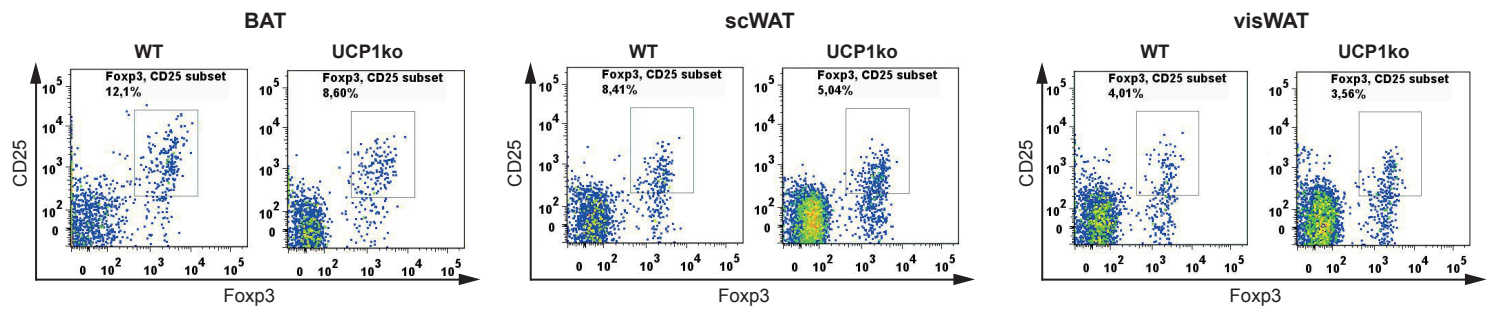
(A+B) In gain-of-function experiments, CD4⁺CD25⁺⁺Foxp3GFP⁺Tregs were adoptively transferred into congenic recipients. Analysis of BAT (A) and scWAT (B) function by RT-qPCR was performed 1 wk after transfer. n=5 per group.

(C-E) A selective expansion of Foxp3⁺Tregs was assessed using IL-2-mAb complexes [3 d, 6 µg per injection, *s.c.*]. Analysis of scWAT (C+D) and visWAT (E) function was analyzed by RT-qPCR. n=4 per group.

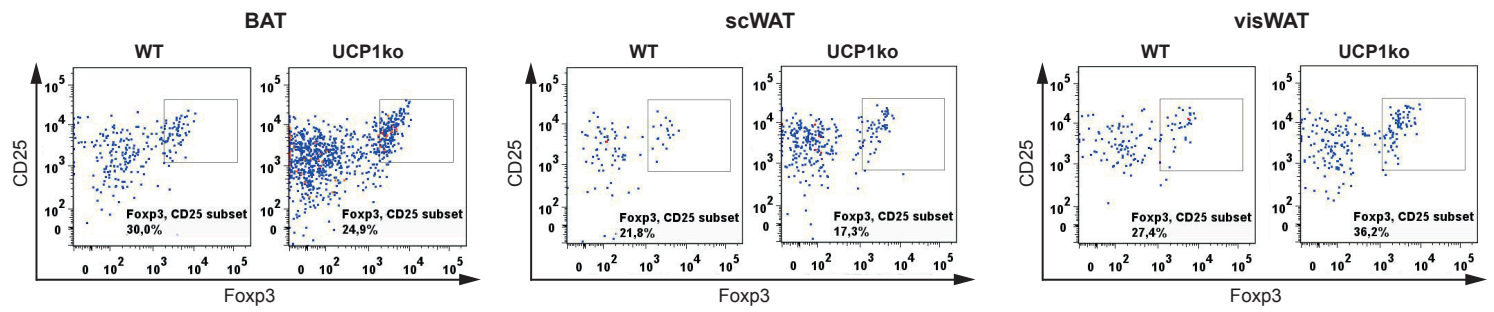
Data are presented as box-and-whisker plots with min and max values for data distribution. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Figure S4

A Fat-residing CD4⁺CD25⁺Foxp3⁺Tregs purified from



B Treg induction with naive CD4⁺CD25^{low}CD44^{low}T cells from fat



C Proliferative status of CD4⁺T cells during Treg induction with naive CD4⁺CD25^{low}CD44^{low} from fat

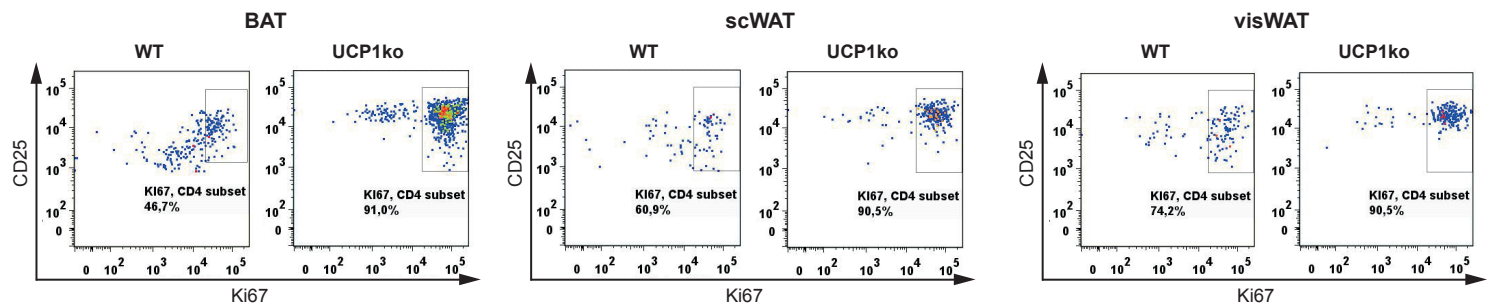


Figure S4 related to Figure 3: Impaired Treg enhancement in the absence of UCP1.

(A) Representative FACS plots for the identification of *ex vivo* CD4⁺CD25⁺⁺Foxp3⁺Tregs from fat depots of WT or UCP1ko mice.

(B) Representative FACS plots for *in vitro* Treg induction assays using limited TCR stimulation and naïve CD4⁺T cells purified from BAT, scWAT and visWAT of WT or UCP1ko animals.

(C) Representative FACS plots depicting the proliferative status of CD4⁺T cells during Treg induction assays from (B).

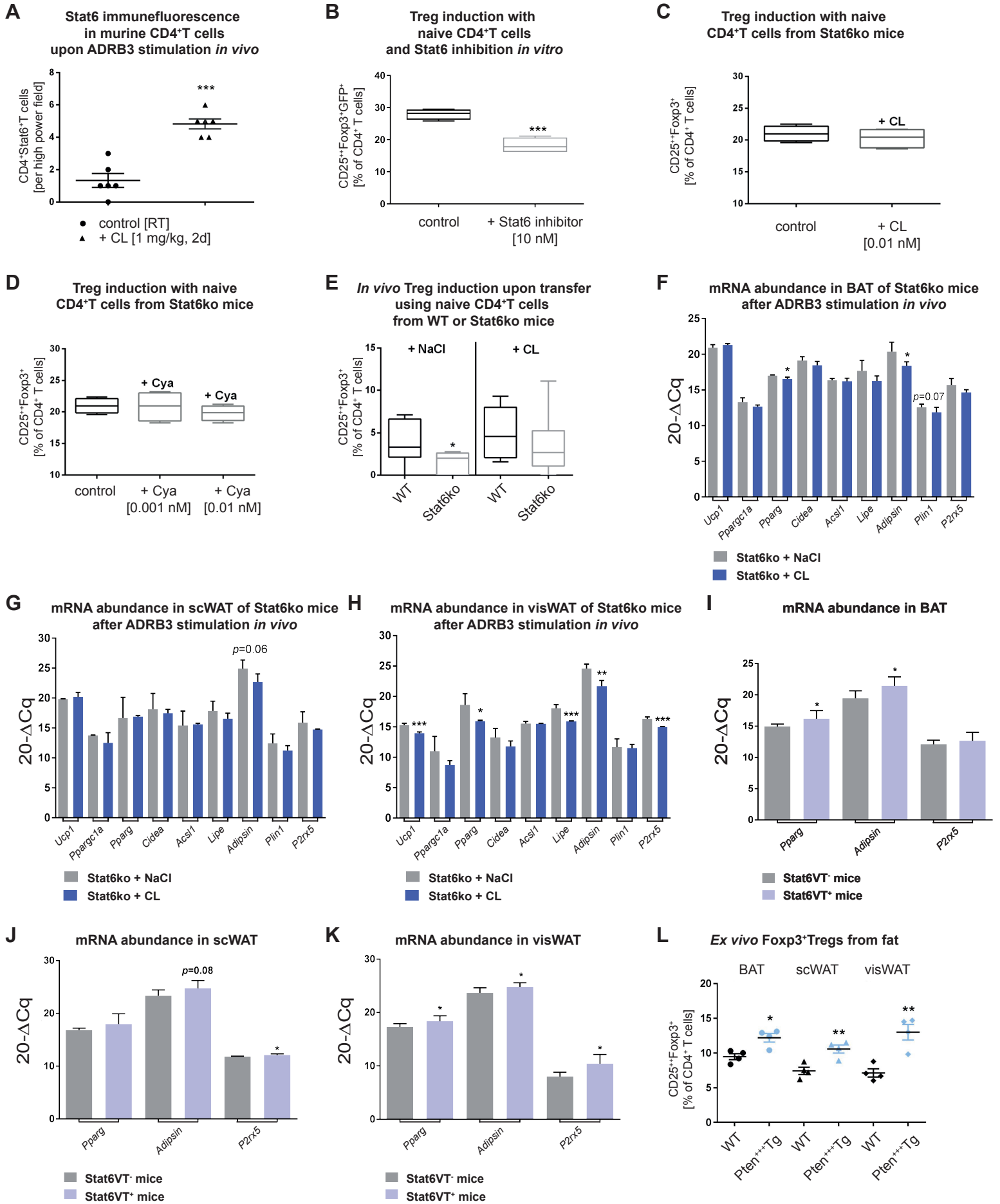
Figure S5

Figure S5 related to Figures 4+5: Role of Stat6 in Treg accumulation of fat-residing CD4⁺T cells.

(A) Summary graph for the enumeration of CD4⁺Stat6⁺T cells per high power field as assessed by immunofluorescence using CD4⁺T cells from mice treated with CL [2 d, 1 mg/kg] or NaCl. n=6 per group.

(B) Summary graph of Stat6 inhibition for *in vitro* Treg induction assays of naïve CD4⁺T cells purified from iLNs of BALB/c Foxp3 GFP reporter mice. n=4 per condition.

(C+D) *In vitro* Treg induction assay of naïve CD4⁺T cells from iLNs of Stat6ko mice using limited TCR stimulation after *in vitro* stimulation with CL [0.01 nM] (C) or a titration of Cya [0.001 nM and 0.01 nM] (D). n=4 per condition.

(E) Summary graph for analysis of CD25⁺⁺Foxp3⁺Tregs after *in vivo* Treg induction using adoptive T cell transfer of naïve CD4⁺T cells from WT or Stat6ko mice in congenic CD90.1 BALB/c recipients with or without stimulation with CL (3 d, 1 mg/kg, *i.p.*). n=8 per group.

(F-H) mRNA expression of genes involved in BAT (F), scWAT (G) and visWAT (H) function after *in vivo* ADRB3 stimulation (3 d, 1 mg/kg CL) in Stat6ko animals. n=4 per group.

(I-K) mRNA expression in BAT (I), scWAT (J) and visWAT (K) of Stat6VT⁺ vs. Stat6VT⁻ animals. n= 6 per group.

(L) Identification of *ex vivo* CD4⁺CD25⁺⁺Foxp3⁺Tregs purified from BAT, scWAT and visWAT of WT or PtenTg mice that had ~40-fold higher *Pten* expression compared to WT mice. n= 4 per group.

Data are presented as box-and-whisker plots with min and max values for data distribution or as mean±SEM. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Table S1 related to STAR Methods.

gene	forward	reverse	source
<i>Histone</i>	5' -ACTGGCTACAAAAGCCG-3'	5' -ACTTGCCTCCTGCAAAGCAC-3'	Sigma Aldrich
<i>Ucp1</i>	5' -GGCCTCTACGACTCAGTCCA-3'	5' -TAAGCCGGCTGAGATCTTGT-3'	Sigma Aldrich
<i>Adrb3</i>	5' -AACTGAAACAGCAGACAGGGAC-3'	5' -CCCCATGTACACCCTAGTT-3'	Sigma Aldrich
<i>Ppargc1a</i>	5' -GCAACATGCTCAAGCCAAAC-3'	5' -TGCAGTTCCAGAGAGTTCCA-3'	Sigma Aldrich
<i>Pparg</i>	5' -GCCCTTTGGTGACTTTATGGA-3'	5' -CAGCAGGTTGTCTTGGATG-3'	Sigma Aldrich
<i>Ppara</i>	5' -GCCTGTCTGTCGGGATGT-3'	5' -GGCTTCGTGGATTCTCTTG-3'	Sigma Aldrich
<i>Prdm16</i>	5' -CAAGTGCCATCTGTGCAACC-3'	5' -TTCGAGTGGATGCCTGGTTC-3'	Sigma Aldrich
<i>Cidea</i>	5' -TCAGACCTTAAGGGACAACACGCA-3'	5' -TTCTTTGGTTGCTTGCAGACTGGG-3'	Sigma Aldrich
<i>Lipe</i>	5' -GGCTCACAGTTACCATCTCACC-3'	5' -GAGTACCTTGCTGTCTGTCC-3'	Sigma Aldrich
<i>Lpl</i>	5' -CCCCAGTCGCCTTTCTCCTGAT-3'	5' -CTCTGGCTCTGACCTTGTTGAT-3'	Sigma Aldrich
<i>Acs11</i>	5' -ACCACCTTCTGGTATGCCAC-3'	5' -TGACATCGTCGTAGTAGTACACC-3'	Sigma Aldrich
<i>Acox1</i>	5' -CAGGAAGAGCAAGGAAGTGG-3'	5' -CCTTCTGGCTGATCCCATA-3'	Sigma Aldrich
<i>Il6</i>	5' -AGCCCACCAAGAACGATAGTC-3'	5' -GCATCAGTCCCAAGAAGGCA-3'	Sigma Aldrich
<i>Stat6</i>	5' -ACCTGTCCATTCGCTCACTG-3'	5' - ATCTGGGGCTCTGGAGTAGG-3'	Sigma Aldrich
<i>Borcs6</i>	5' -AAGCCGTGGACATGAGCATT-3'	5' -CAGCAAGCAGTTTCCACCAG-3'	Sigma Aldrich
<i>18s</i>	N/A	N/A	Quantitect Primer Assay (Qiagen)
<i>Pten</i>	N/A	N/A	Quantitect Primer Assay (Qiagen)
<i>Adipsin</i>	5' -AACCGGACAACCTGCAATCT-3'	5' -GAGTCTCCCCTGCAAGTGTC-3'	Sigma Aldrich
<i>Cd137</i>	5' -GGTGGACAGCCGAACGTGAA-3'	5' -AACCCTGCTTCGTTAGCTCC-3'	Sigma Aldrich
<i>P2rx5</i>	5' -CTGCAGCTCACCATCCTGT-3'	5' -CACTCTGCAGGGAAGTGTC-3'	Sigma Aldrich
<i>Cycs</i>	5' -GAACAAGTGTGGTTGCACCG-3'	5' -ATGCTTGCCTCCCTTTCCA-3'	Sigma Aldrich
<i>Plin1</i>	5' -AGATCCCGGCTCTTCAATACC-3'	5' -AGAACCTTGTCAGAGGTGCTT-3'	Sigma Aldrich

Primer sequences used for qPCR analyses.