Supplementary information

Natural killer T cells in adipose tissue prevent insulin resistance

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Supplementary figure 1

(a) Histogram represents the percentage AT-derived iNKT cells (CD1d/αGC-loaded tetramer) of the AT-resident T cells (TCRβ+) in WT mice injected with an isotype or αNK1.1 antibody. Bar graph represents absolute numbers of AT-resident iNKT cells in the WT mice injected with an isotype or αNK1.1 antibody. N=10 mice per group, total 20 mice. **(b)** H&E and **(c)** Oil-red-O staining of representative liver sections of the WT and CD1d-null mice fed LFD or HFD for 19 weeks (scale bars indicate 100 μm). **(d-i)** N=10 mice per group, total 40 mice. **(d)** Liver weight as percentage of total body weight. **(e)** Liver triglyceride (TG) content. **(f)** Plasma aspartate aminotransferase (AST) levels. **(g)** Plasma alanine aminotransferase (ALT) levels. **(h)** Changes in gene expression of selected inflammatory genes as determined by quantitative RT-PCR. Mean expression in WT LFD mice was set at 1. **(i)** Plasma free fatty acid (FFA) levels. **(i)** Plasma TG levels. **(j)** Plasma FPLC lipoprotein profiling. For each group of mice, 10μl of pooled plasma was used.

Supplementary figure 2

(a) SLAMf1 (CD150) expression on AT-derived iNKT cells. The histogram displays the gating of SLAMf1, i.e. the SLAMf1 expression on TCRβ- cells versus iNKT cells. Bar graphs represent SLAMf1 expression on iNKTs derived from SCAT, VAT and spleen. N=10 WT mice per group, total 20 mice. **(b)** Intracellular cytokine staining of spleen and visceral AT-extracted iNKTs from 4 WT mice. Shown are both representative histograms and averages in bar graphs. **(c)** Intra-peritoneal glucose tolerance test of WT mice fed LFD for 18 weeks injected with vehicle (PBS) or αGalCer (5 μg). Shown are plasma glucose concentrations and the AUC for the two groups. N=10 per group, total 20 mice.

Supplementary figure 3

(a) Macrophage gating (F4/80+) and macrophage CD11c expression on AT-derived stromal vascular cells (SVC). **(b)** Macrophage numbers, macrophage polarization, CD4+CD25+ numbers and NK cell numbers in SCAT and VAT derived from WT and CD1d-null mice on a HFD. **(c)** Histogram represents the percentage AT-derived CD25+ cells (of TCRβ+CD4+ cells) of the CD1d-null mice injected with an isotype or αCD25 antibody. Bar graph represents absolute numbers of AT-resident CD4+CD25+ cells in the WT mice injected with an isotype or αCD25 antibody. N=10 mice per group, total 20 mice. **(d)** Intra-peritoneal glucose tolerance test of control (Isotype antibody) and Treg-depleted (αCD25 antibody) WT mice on a LFD. Shown are plasma glucose and insulin concentrations, together with the AUC for the two groups. N=10 mice per group, total 20 mice.

Supplementary figure 4

(a) Microarray based fold change versus fold change scatter plot comparing gene expression profiles between WT HFD group (x axis) and CD1d-/- HFD group (y axis). Genes of interest encoding classical inflammatory markers or adipokines are highlighted in red (upregulated) or blue (downregulated). Fold changes represent the mean of 4-6 mice per experimental group. **(b)** H&E staining of VAT from WT and CD1d-null mice after 19 weeks of HFD feeding. Scale bars indicate 100μm. VAT adipocyte sizes (area per adipocyte, μm^2) in HFD-fed WT and CD1d-null mice are presented in a histogram and boxplot. Boxplots show the median area per adipocyte for both groups, and 10^{th} to 90^{th} percentiles. N=4 mice per group (random), total 8 mice. **(c)** Adipocyte numbers per microscopic field. At least 3 microscopic fields from 10 different animals were analyzed. Presented are averages ± SEM. **(d)** Fat mass in WT and CD1d-null mice under LFD conditions. **(e)** Quantitative RT-PCR of selected lipogenic (FAS,

SCD), lipid droplet (Perilipin, Lipin, PPARγ) and thermogenic (UCP1, PPARα) genes in adipose tissue. Mean expression in WT LFD mice was set at 1. Fold inductions were normalized for housekeeping gene expression (36B4). N=9 mice per group, total 18 mice. **(f)** Plasma glycerol levels in WT and CD1d-null mice under LFD conditions.

Supplementary figure 5

(a) Chemokine receptor and CD62L gating on human AT-derived and blood iNKT cells. **(b)** sh RNA knockdown of human CD1d. The quantitative RT-PCR (left panel) shows the knockdown of human CD1d in mature SGBS adipocytes. The Western blot (right panel) shows the effectiveness of the shRNA on a protein level, by knockdown of human CD1d in HeLa cells stably expressing human CD1d. Na^+K^+-ATPase is presented as a loading control. **(c)** Upper panel, Oil-Red-O staining of differentiated mature SGBS adipocytes transduced with scrambled sh RNA or sh RNA for human CD1d. Lower left panel, quantitative RT-PCRs for a few adipoycte differentiation genes are shown. Mean expression in the scrambled sh RNA transduced cells was set at 1. Fold inductions were normalized for housekeeping gene expression (36B4). Lower right panel, adiponectin levels in the supernatant of the adipocytes are shown, after 24hr of incubation.

Supplementary figure 6

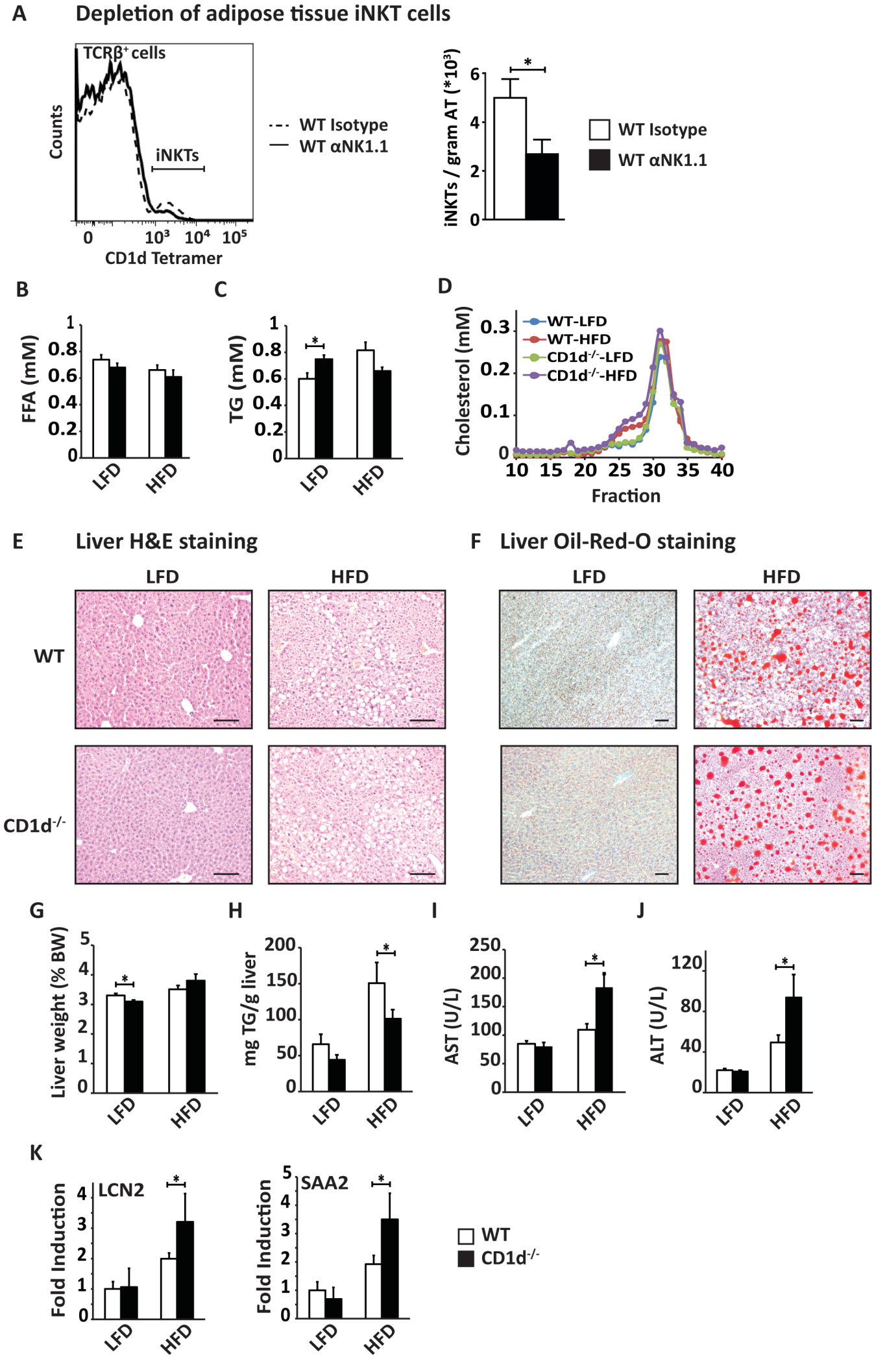
(a) Quantitative RT-PCR (left panel) showing the overexpression of human CD1d in undifferentiated SGBS pre-adipocytes. Western blot (right panel) shows the overexpression on a protein level, again in undifferentiated SGBS pre-adipoyctes. Na^+K^+-ATPase is presented as a loading control. **(b)** Intracellular IL-4, IL-13 and IFN-γ staining of iNKT cells alone, and cocultured for 18hr with undifferentiated SGBS pre-adipocytes and mature adipocytes, with

and without prior loading of the (pre)adipocytes with the CD1d-restricted iNKT cell ligand α-Galactosyl Ceramide (αGalCer, αGC). CD1d blocking and knockdown in the adipocytes depleted intracellular cytokine staining in the co-cultured iNKT cells. Bars represent the mean results of 5 different iNKT cell lines cocultured with the (pre)adipocytes. **(c)** IL-4, IL-13 and IFN-γ levels in the supernatants of iNKT cells alone, and undifferentiated SGBS pre-adipocytes and mature adipocytes alone, with and without loading of the (pre)adipocytes with αGalCer. No cytokines were detected, except for low levels of IL-13 and IFN-γ in the iNKT cell alone supernatant.

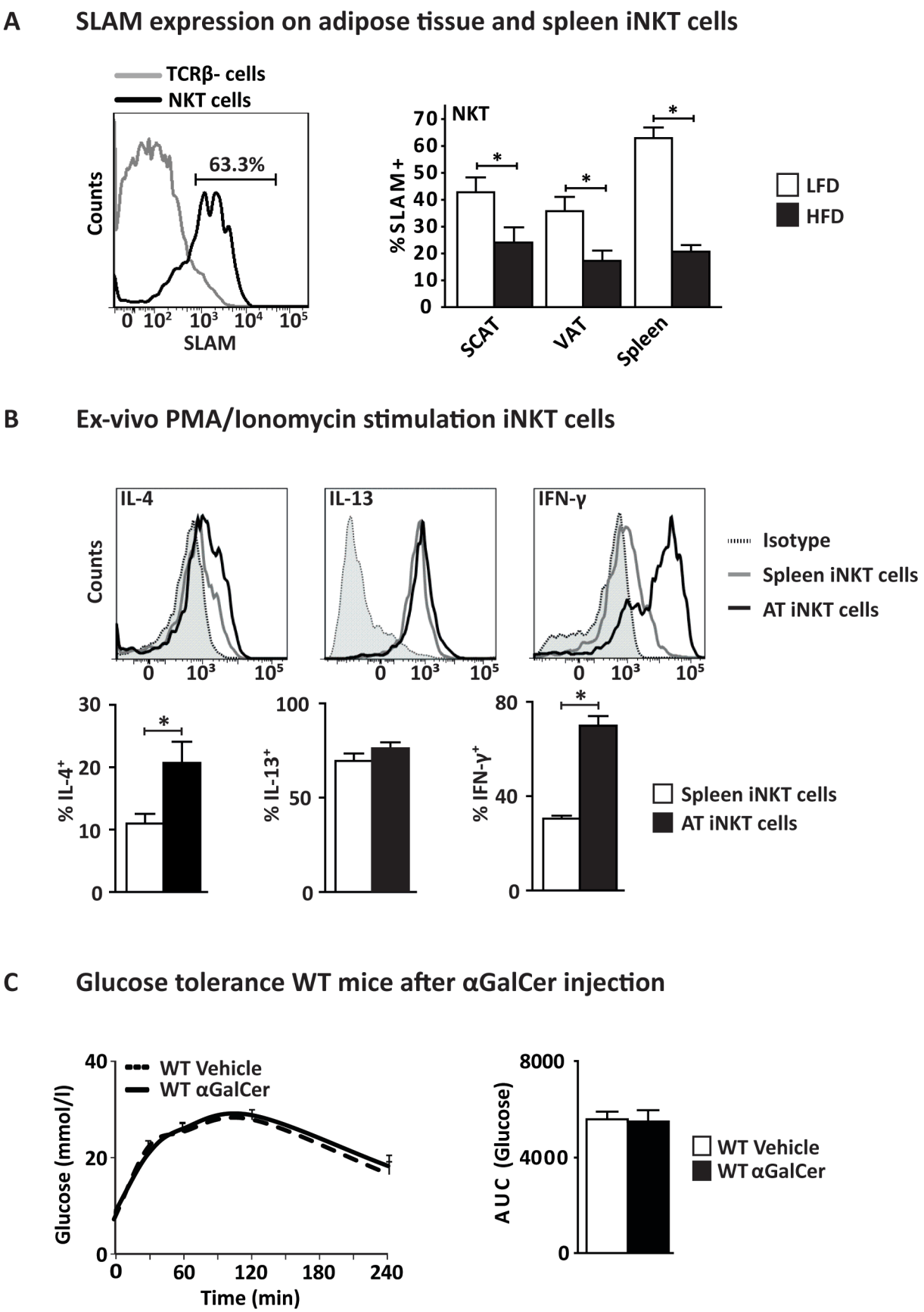
Supplementary table 1 **Primer sequences for quantitative RT-PCRs**

Gene	Forward primer	Reverse primer
mLep (Leptin)	AGAAGATCCCAGGGAGGAAA	TGATGAGGGTTTTGGTGTCA
mAdipoq((Adiponectin)	GCAGAGATGGCACTCCTGGA	CCCTTCAGCTCCTGTCATTCC
mIl4	CCCCAGCTAGTTGTCATCCTG	CGCATCCGTGGATATGGCTC
mIl13	CCTGGCTCTTGCTTGCCTT	GGTCTTGTGTGATGTTGCTCA
mIfng	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
mLcn2	GGGAAATATGCACAGGTATCCTC	GCCACTTGCACATTGTAGCTC
mSaa2	TGGCTGGAAAGATGGAGACAA	AAAGCTCTCTCTTGCATCACTG
mTnfa	CAACCTCCTCTCTGCCGTCAA	TGACTCCAAAGTAGACCTGCCC
mIl6	CTTCCATCCAGTTGCCTTCTTG	AATTAAGCCTCCGACTTGTGAAG
mAdam8	AGTTCCTGTTTATGCCCCAAAG	AAAGGTTGGCTTGACCTGCT
mCcl2	CCCAATGAGTAGGCTGGAGA	TCTGGACCCATTCCTTCTTG
mF4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
mCd68	CATCCCCACCTGTCTCTCTC	CCATGAATGTCCACTGTGCT
mCd11c	TCAACCAGCACCAGACAGAG	AAACATCCTGTAATGGCTTGTG
mFas	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
mScd1	TTCTTGCGATACACTCTGGTGC	CGGGATTGAATGTTCTTGTCGT
mPlin1	CAAGCACCTCTGACAAGGTTC	GTTGGCGGCATATTCTGCTG
mLipin 1	CGCCAAAGAATAACCTGGAA	TGAAGACTCGCTGTGAATGG
mPparg	CGCTGATGCACTGCCTATGA	AGAGGTCCACAGAGCTGATTCC
mUcp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
mPpara	CACGCATGTGAAGGCTGTAA	CAGCTCCGATCACACTTGTC
m36B4	AGCGCGTCCTGGCATTGTGTGG	GGGCAGCAGTGGTGGCAGCAGC
hNPC2	CAGTGAAAAGCGAATATCCCTCTA	TTTGGTTTTTGTCATCCTGAAGT
hCD1d	GTGGCCTCCTTGAGTCA	ACAGGCTTTGGGTAGAATC
hProsaposin	GCCAGAACACAGAGACAGCA	GCTGTGGTTTCTGCCAAGAT
hGLA	TGGAAAATTTGGCAGATGGT	AAAGAGGCCACTCACAGGAG
hHLA-B	CTACCCTGCGGAGATCAC	TAGGACAGCCAGGCCAGCAACA
hPPARG	CCTATTGACCCAGAAAGCGATT	CATTACGGAGAGATCCACGGA
hFABP4	CCTTTAAAAATACTGAGATTTCCTTCA	GGACACCCCCATCTAAGGTT
hLPL	ATGTGGCCCGGTTTATCA	CTGTATCCCAAGAGATGGACATT
hADIPOQ(Adiponectin)	CCTGGTGAGAAGGGTGAGAA	CACCGATGTCTCCCTTAGGA
hB2M	TTCTGGCCTGGAGGCTATC	TCAGGAAATTTGACTTTCCATTC
hBeta-Actin	GATCGGCGGCTCCATCCTG	GACTCGTCATACTCCTGCTTGC

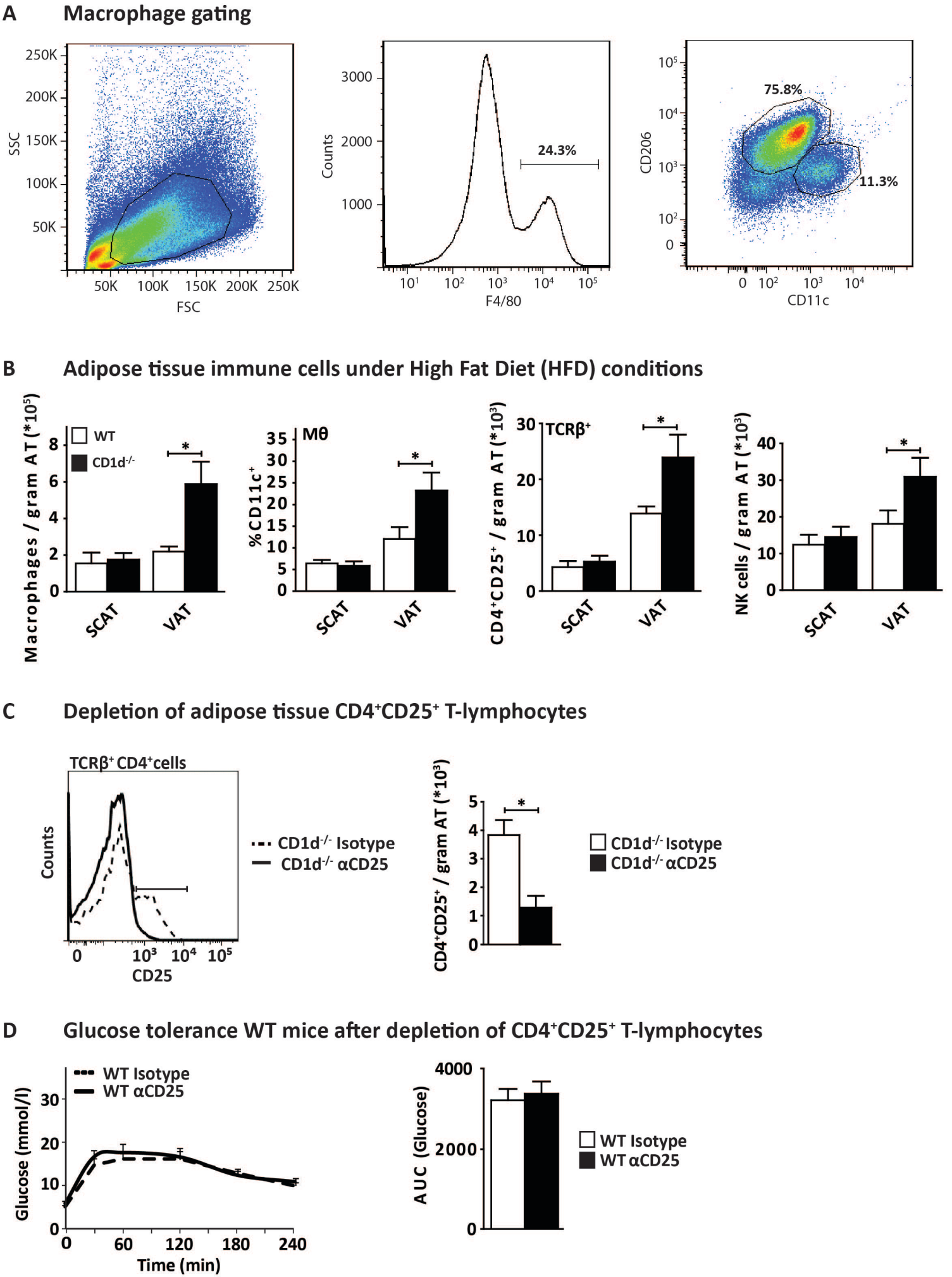
Supplemental figure 1



Supplemental figure 2

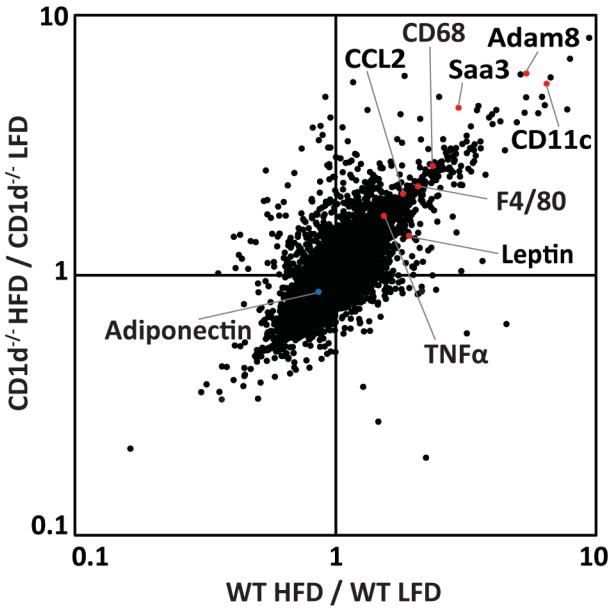


Supplemental figure 3

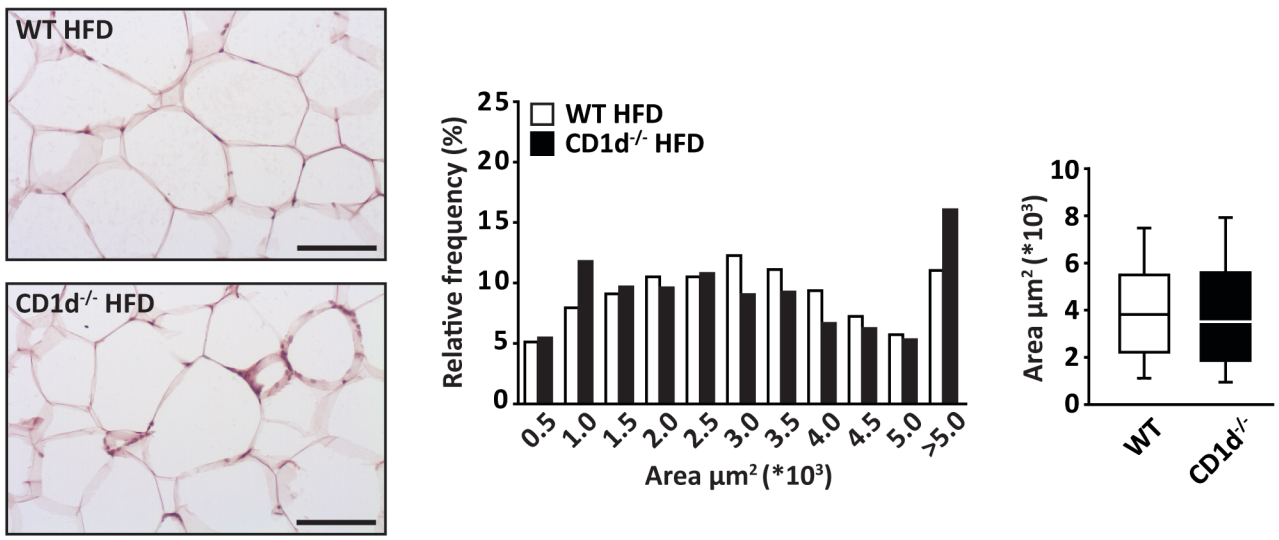


Supplemental figure 4

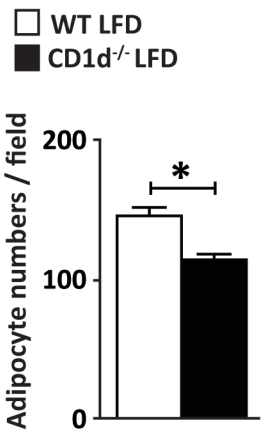
A Micro-array data HFD / LFD



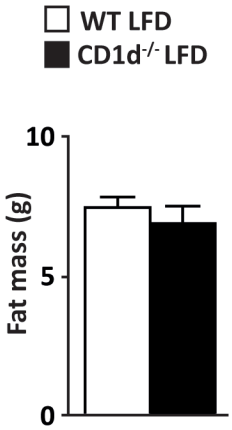
B Adipocyte size under high fat diet (HFD) conditions



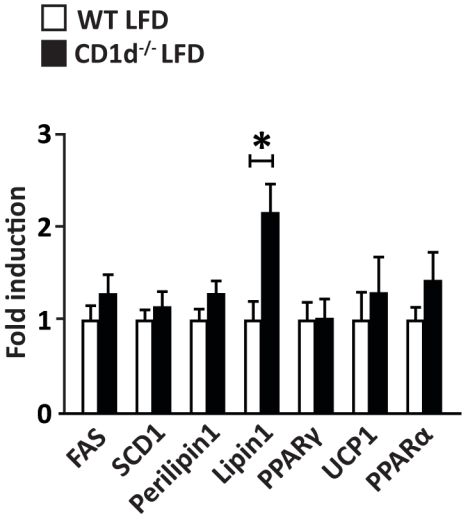
C Adipocyte numbers



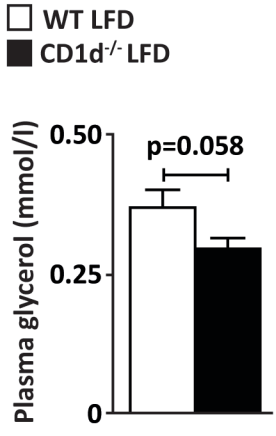
D Fat mass



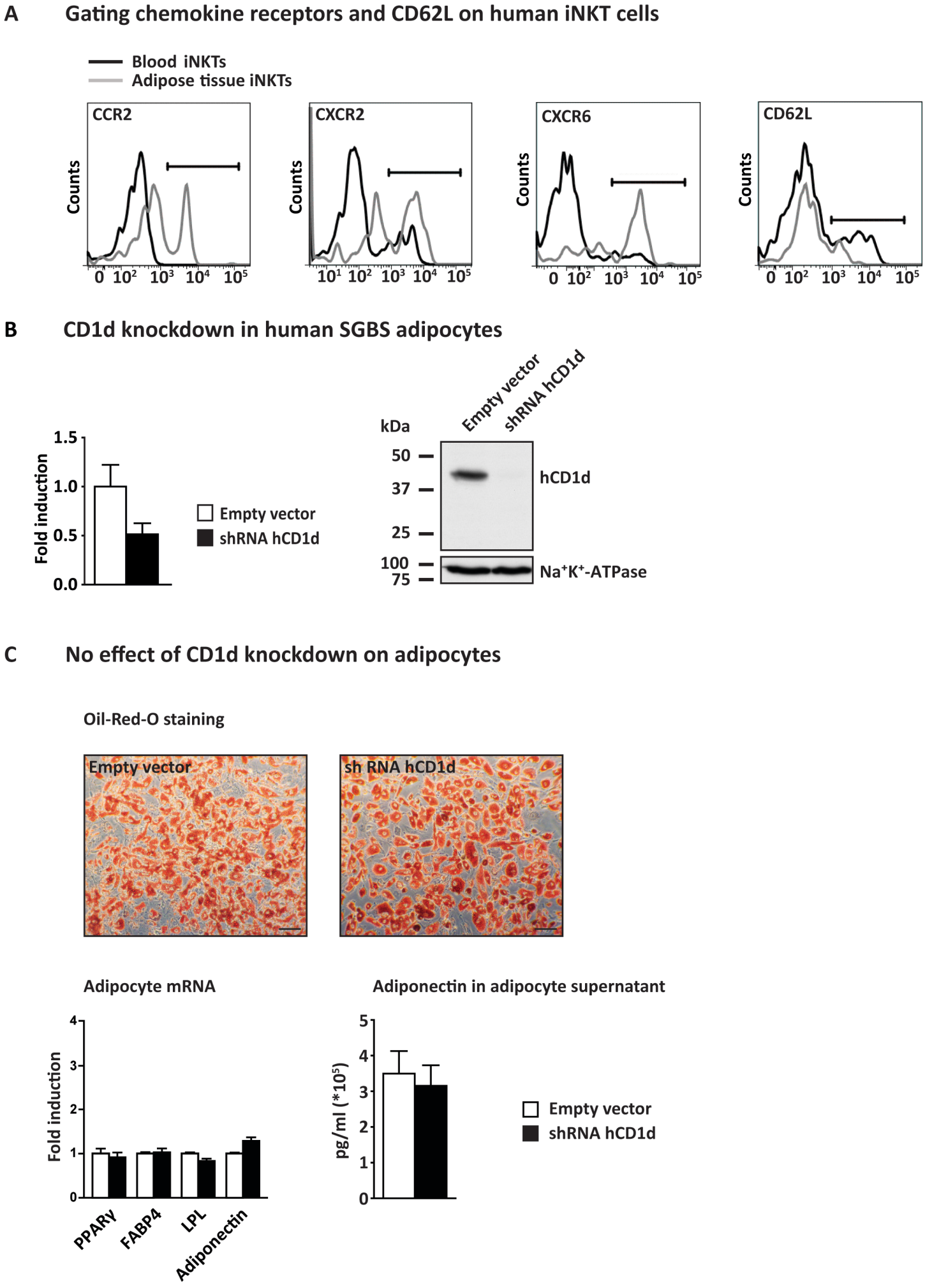
E Gene expression in AT



F Plasma glycerol



Supplemental figure 5



Supplemental figure 6