Expanded Materials and Methods

Isolation of AT-derived SVCs and flow cytometry

One gram of peri-epididymal fat from lean and obese C57BL/6J mice (15 weeks of LF or HF diet, respectively) was minced in PBS containing 2% bovine serum albumin (BSA) and 250 U/ml of collagenase type II (Worthington) and incubated at 37°C for 1 h. The digested tissue was passed through a 70-μm cell strainer (BD Biosciences) and the flow-through centrifuged. After aspirating the supernatant, red blood cells were lysed with ACK lysing buffer (Gibco). The remaining cells were washed with DMEM supplemented with 10% FCS, counted and labeled with conjugated antibodies or their respective isotype controls before acquisition by a FACScan.

Analysis of inflammatory cells in AT by immunohistochemistry

Peri-epididymal AT (from mice on LF or HF diet for 21 weeks) was fixed in periodate-lysine-paraformaldehyde fixative as described previously (see reference in the text), and embedded in paraffin. Five-micron sections were stained for rat anti-mouse CD45, Mac-3, I-A^b (BD Pharmingen) and CD3 (Abcam), and then incubated with appropriate biotinylated secondary antibodies followed by incubation with avidin-biotin complex (Vector). Next, the reaction was visualized with 3-amino-9-ethyl carbazole (DAKO). Sections were counterstained with Gill's hematoxylin solution (Sigma). Positive cells were counted in 10 consecutive visual fields at the same magnification.

Culture and differentiation of 3T3-L1 cells

Murine 3T3-L1 pre-adipocytes were cultivated in DMEM high glucose supplemented with 10% FCS. After reaching confluency (day 0), cells were stimulated with DMEM containing 10% FCS, 0.5 mmol/L 3-Isobutyl-1-methylxanthine (Sigma), 1 µmol/L dexamethasone (Sigma), and 10 µg/ml of porcine insulin (Sigma), to induce differentiation. At day 2, media were replaced by DMEM with 10% FCS and 10 µg/ml of insulin, and changed every 48 h. At day 11, cells were stimulated with recombinant mouse IFNγ (Chemicon) at 10, 50, or 100 U/ml, and harvested 24 h after stimulation.

Culture and activation of T cells *in vitro*

Splenic CD4+ T cells were positively selected from C57BL/6J male mice and cultured *in vitro* with 2 µg/ml of anti-CD28 (Bioexpress) and 10 ng/ml of recombinant mouse IL-12 (R&D Systems) in a plate coated with 5 µg/ml of anti-CD3 (BD Pharmingen). After 48 h of incubation at 37°C, cells were transferred to fresh plates and incubated with 10 U/ml of recombinant mouse IL-2 (R&D Systems) for 72 h. Cells were then washed and again incubated in a plate coated with anti-CD3. After 48 h, conditioned media were used to stimulate differentiated 3T3-L1 cells for 24 h in the presence or absence of a neutralizing anti-IFNγ antibody at 10 µg/ml.

Sequences of mouse primers

TNFα, 5’-CTGTAGCCCACGTCGTAGC-3’ and 5’-TTGAGATCCATGCCGTTG-3’; CD68, 5’-CTCTCTAAGGCTACAGGCTGCT-3’ and 5’-TCACGGTTGCAAGAGAAACA-3’; MCP-1, 5’-GGCTGGAGAGCTACAAGAGG-3’ and 5’-TCTTGAGCTTGGTGACAAAAAC-3’; RANTES, 5’-AGCAGCAAGTGCTCCAATC-3’ and 5’-GGGAAGCGTATACAGGGTC-3’; IL-10, 5’-ACTGCACCCACTTCCCAGT-3’ and 5’-TGTCCAGCTGGTCCTTTGTT-3’; STAT-1, 5’-TGAGATGTCCCGGATAGTGG-3’ and 5’-CGCCAGAGAGAAATTCGTGT-3’; IFNγ, 5’-TCTGGAGGAACTGGCAAAAG-3’ and 5’-TTCAAGACTTCAAAGAGTCTGAGG-3’; IP-10, 5’-GCTGCCGTCATTTTCTGC-3’ and 5’-TCTCACTGGCCCGTCATC-3’; MIG, 5’-CTTTTCCTTTTGGGCATCAT-3’ and 5’-GCATCGTGCATTCCTTATCA-3’; CXCR3, 5’-GCCAAGCCATGTACCTTGAG-3’ and 5’-GGAGAGGTGCTGTTTTCCAG-3’; I-A^{b}, 5’-GTGGTGCTGATGGTGCTG-3’ and 5’-CCATGAACTGGTACACGAAATG-3’; CD3, 5’-TCCCAACCCAGACTATGAGC-3’ and 5’-GCGATGTCTCTCCTATCTGTCA-3’; GAPDH, 5’-TGGGTGTGAACCATGAGAAG-3’ and 5’-GCTAAGCAGTTGGTGGTGC-3’.

Supplementary figure 1. Plasma levels of adiponectin, leptin, and total cholesterol.

Values representing the animals individually and the average in each group are plotted for each measurement. WT/LF, wild type mice/low fat diet; IFN$\gamma^{-/-}$LF, IFNγ-deficient mice/low fat diet; WT/HF, wild type mice/high fat diet; IFN$\gamma^{-/-}$HF, IFNγ-deficient mice/high fat diet. §p<0.05 relative to WT/LF; #p<0.05; n=5-6 in each group.

Supplementary figure 2. IFNγ deficiency limits the number of crown-like formations in obese visceral AT.

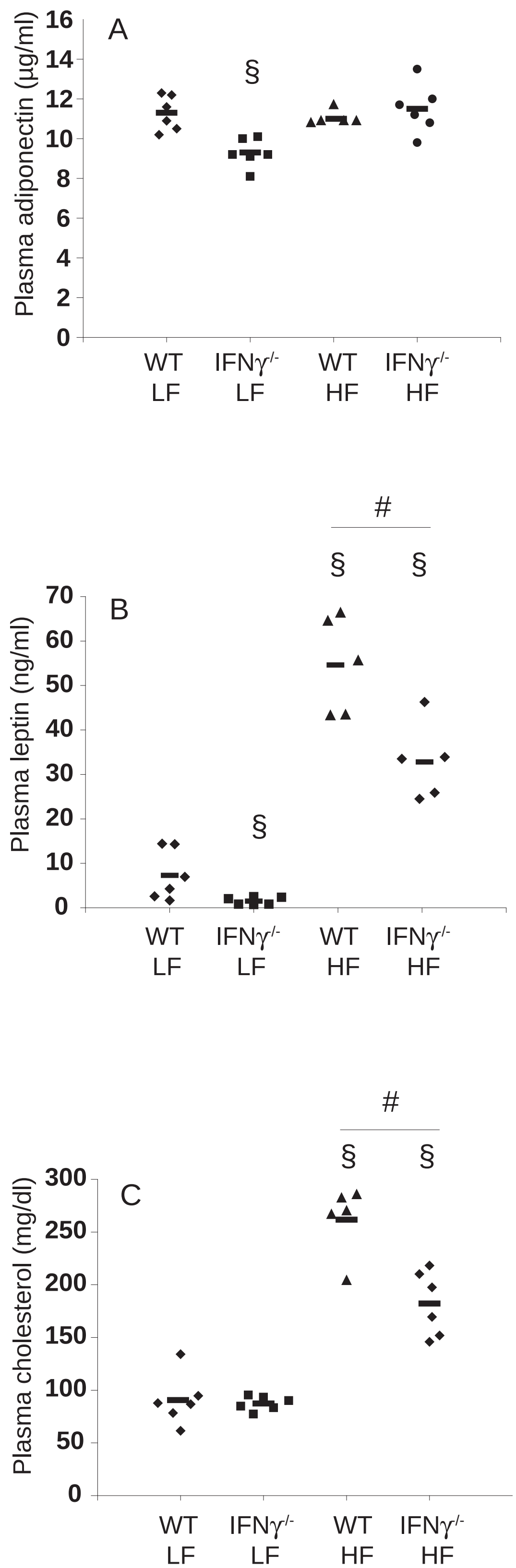
Peri-epididymal AT was fixed and paraffin-embedded. Sections were stained with anti-CD45 antibody, and "crowns" were counted in 10 consecutive fields in each slide. One crown is the result of positive cells around one single adipocyte. A representative picture from each group is shown (A-D). Numbers from each group were plotted in the graph (E). Differences were calculated by Student`s t test. WT/LF, wild type mice/low fat diet; IFNγ$^{-/-}$LF, IFNγ-deficient mice/low fat diet; WT/HF, wild type mice/high fat diet; IFNγ$^{-/-}$HF, IFNγ-deficient mice/high fat diet; *p<0.05; n=5-6 in each group.

Supplementary figure 3. Plasma levels of total cholesterol, triglycerides, and glucose in ApoE$^{-/-}$ and IFNγR$^{-/-}$ApoE$^{-/-}$

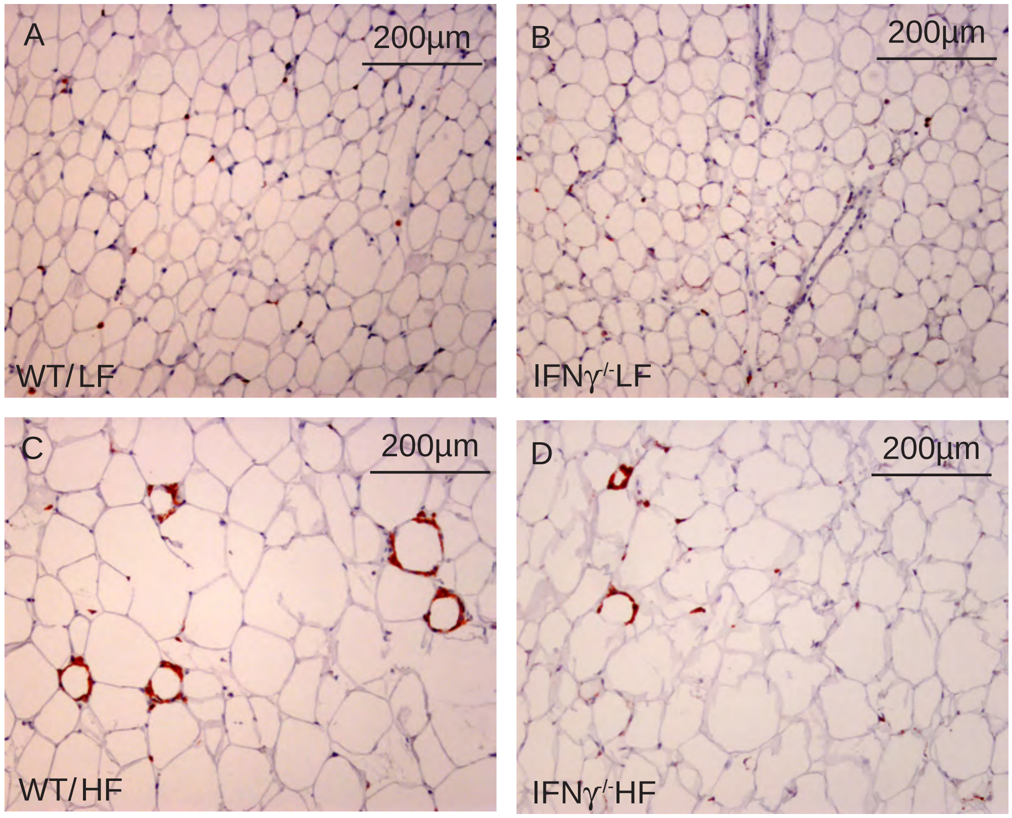
Values representing the animals individually and the average in each group are plotted for each measurement. ApoE$^{-/-}$, apolipoprotein E-deficient mice; IFNγR$^{-/-}$ApoE$^{-/-}$, IFNγ-receptor-deficient and ApoE-deficient mice. *p<0.01 vs ApoE$^{-/-}$; n=9 in each group.

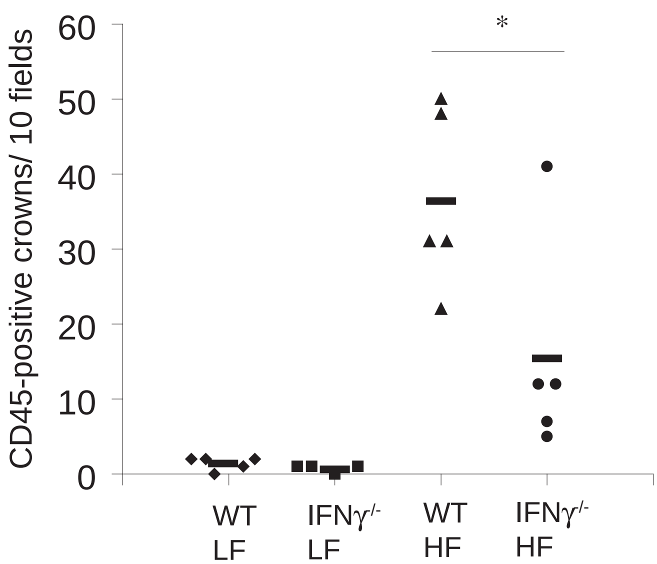
Supplementary table 1. Transcription profiling study
Differentiated 3T3-L1 cells were stimulated with 100 U/ml of recombinant mouse IFNγ or left untreated (controls). After 4 and 24 h, control and treated cells were harvested and mRNA was extracted and used in a microarray screening. The table shows the CC and CXC chemokines and receptors that significantly changed compared to controls, ranked by their p value at 24h. n=5 for each group at 4 h, and n=6 for each group at 24 h.

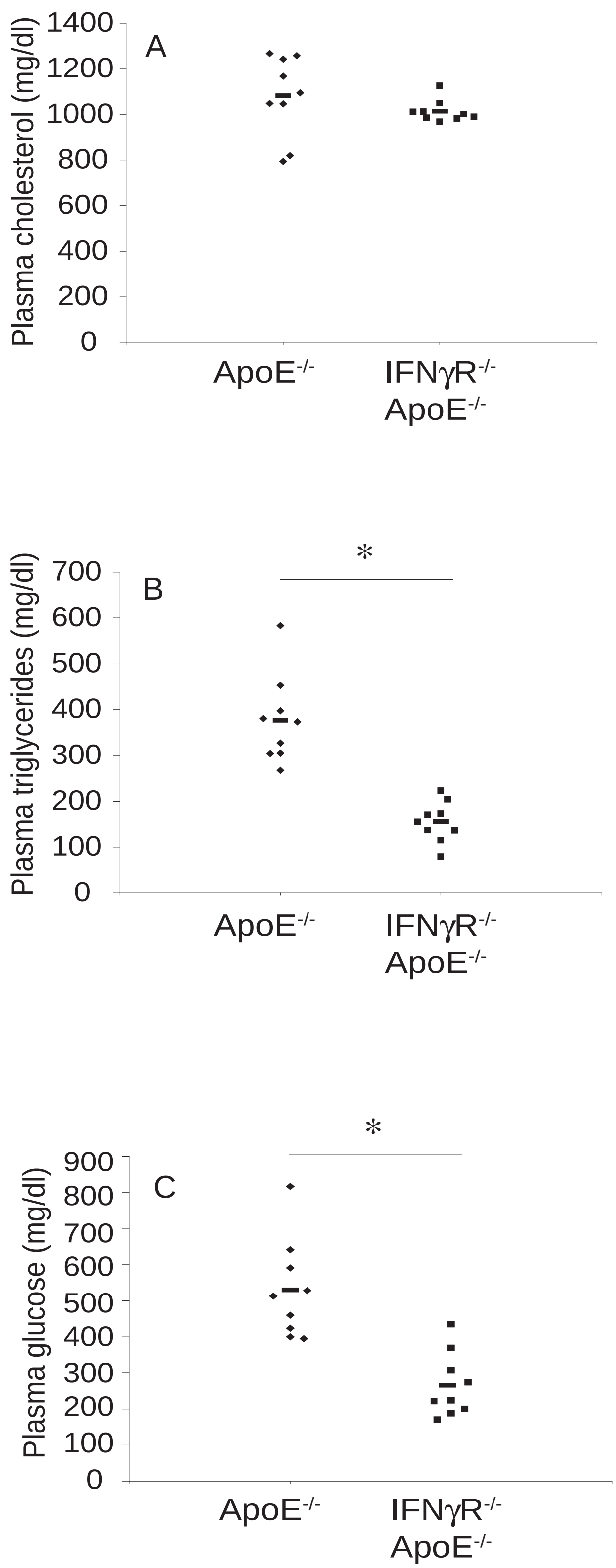
Gene	Other denominations	4h paired t statistic	4h paired p value	24h paired t statistic	24h paired p value
Chemokine (C-X-C motif) ligand 9	Cxcl9, Mig	5.159	0.006703	31.630	0.000001
Chemokine (C-X-C motif) ligand 10	Cxcl10, IP-10	5.316	0.006020	20.312	0.000005
Chemokine (C-C motif) ligand 5	Ccl5, RANTES	4.797	0.008668	16.707	0.000014
Chemokine (C-C motif) ligand 8	Ccl8, MCP-2	9.577	0.000664	16.511	0.000015
Chemokine (C-X-C motif) ligand 11	Cxcl11, I-TAC	3.984	0.016351	8.116	0.000461
Chemokine (C-C motif) ligand 2	Ccl2, MCP-1	3.645	0.021865	6.508	0.001279
Chemokine (C-C motif) ligand 7	Ccl7, MCP-3	3.851	0.018286	5.907	0.001980
Chemokine (C-X-C motif) ligand 13	Cxcl13, BLC, BCA-1	4.013	0.015961	5.281	0.003243
Chemokine (C-C motif) receptor-like 1	Ccrl1	0.195	NS	5.151	0.003613
Chemokine (C-X-C motif) ligand 2	Cxcl2, GRO-β, MGSA-β	12.422	NS	4.976	0.004190
Chemokine (C-X-C motif) ligand 12	Cxcl12, SDF-1α/β	14.831	NS	4.007	0.010249
Chemokine (C-X-C motif) receptor 3	Cxcr3	14.784	NS	3.999	0.010336
Chemokine (C-C motif) ligand 25	Ccl25, TECK	3.378	0.027842	3.934	0.011023
Chemokine (C-X-C motif) ligand 16	Cxcl16	3.317	0.029452	3.858	0.011907
Chemokine (C-X-C motif) ligand 7	Cxcl7, NAP-2	0.515	NS	3.734	0.013510
Chemokine (C-C motif) receptor 1	Ccr1	2.744	NS	3.619	0.015239
Chemokine (C-X-C motif) ligand 1	Cxcl1, GRO-α, MGSA-α	14.008	NS	3.403	0.019192
Chemokine (C-X-C motif) ligand 15	Cxcl15	1.027	NS	3.016	0.029567
Chemokine (C-C motif) ligand 28	Ccl28, MEC	27.063	NS	2.771	0.039325
Chemokine (C-C Motif) Ligand 12	Ccl12	8.944	0.000864	22.227	NS
Chemokine (C-X3-C Motif) Ligand 1	Cx3cl1, Fractalkine	6.665	0.002632	11.542	NS
Chemokine (C-C Motif) Ligand 4	Ccl4, MIP-1β	4.243	0.013233	12.488	NS
Chemokine (C-X3-C) receptor 1	Cx3cr1	3.384	0.027684	10.979	NS


A
Plasma adiponectin (µg/ml)
16
14
12
10
8
6
4
2
0
§
WT LF
IFNγ-/- LF
WT HF
IFNγ-/- HF
B
Plasma leptin (ng/ml)
70
60
50
40
30
20
10
0
#
§
§
§
WT LF
IFNγ-/- LF
WT HF
IFNγ-/- HF
C
Plasma cholesterol (mg/dl)
300
250
200
150
100
50
0
#
§
§
WT LF
IFNγ-/- LF
WT HF
IFNγ-/- HF

Supplementary figure 2






A
Plasma cholesterol (mg/dl)
1400
1200
1000
800
600
400
200
0
ApoE-/-
IFNγR-/-
ApoE-/-
B
*
Plasma triglycerides (mg/dl)
700
600
500
400
300
200
100
0
ApoE-/-
IFNγR-/-
ApoE-/-
C
*
Plasma glucose (mg/dl)
900
800
700
600
500
400
300
200
100
0
ApoE-/-
IFNγR-/-
ApoE-/-