

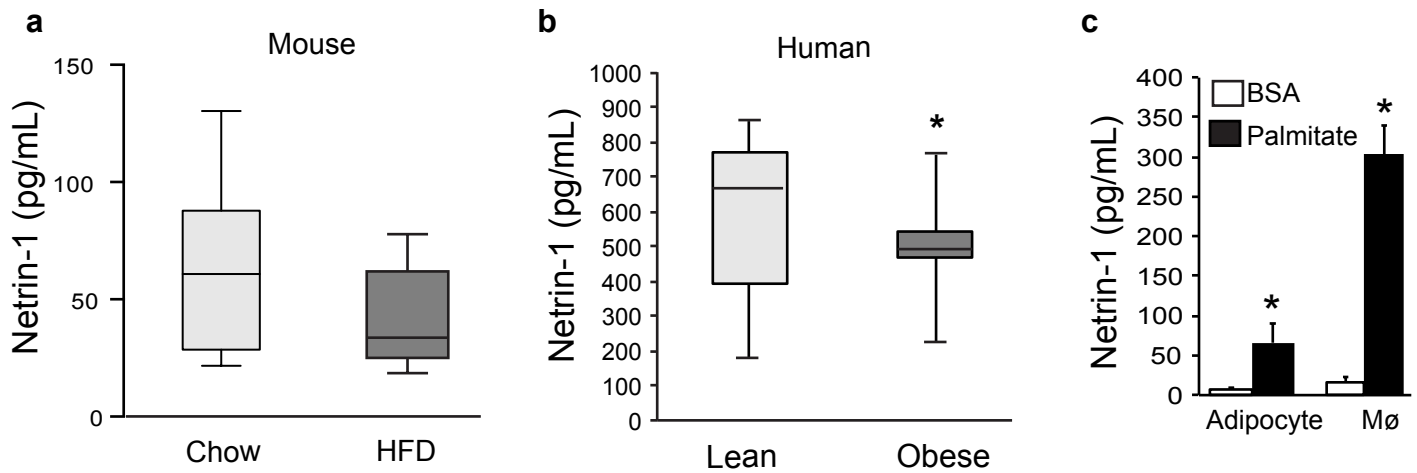
Data supplement

Netrin-1 promotes adipose tissue macrophage accumulation and insulin resistance in obesity

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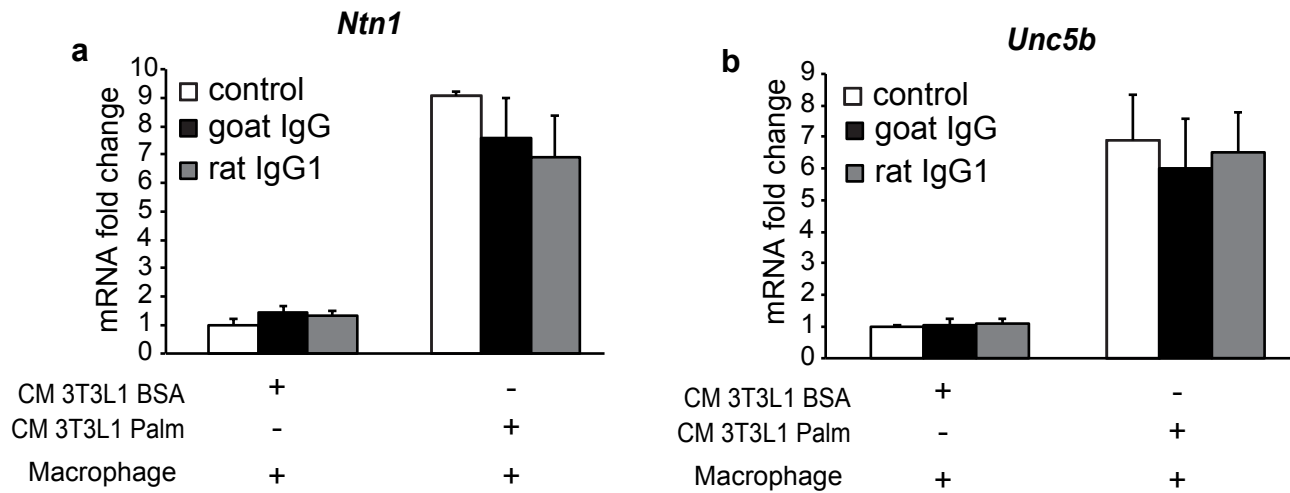
Supplemental Figure 1



Secreted concentrations of Netrin-1 in serum and conditioned media.

(a) Serum levels of netrin-1 in mice fed a chow diet (n=9) or HFD (n=10). (b) Serum levels of netrin-1 in obese (n=20) or lean (n=20) human subjects. (c) Concentration of netrin-1 in conditioned media of 3T3L1 adipocytes or BMDM treated with 250 μ M BSA or palmitate (n=4). Data are the mean \pm s.e.m. *P < 0.05.

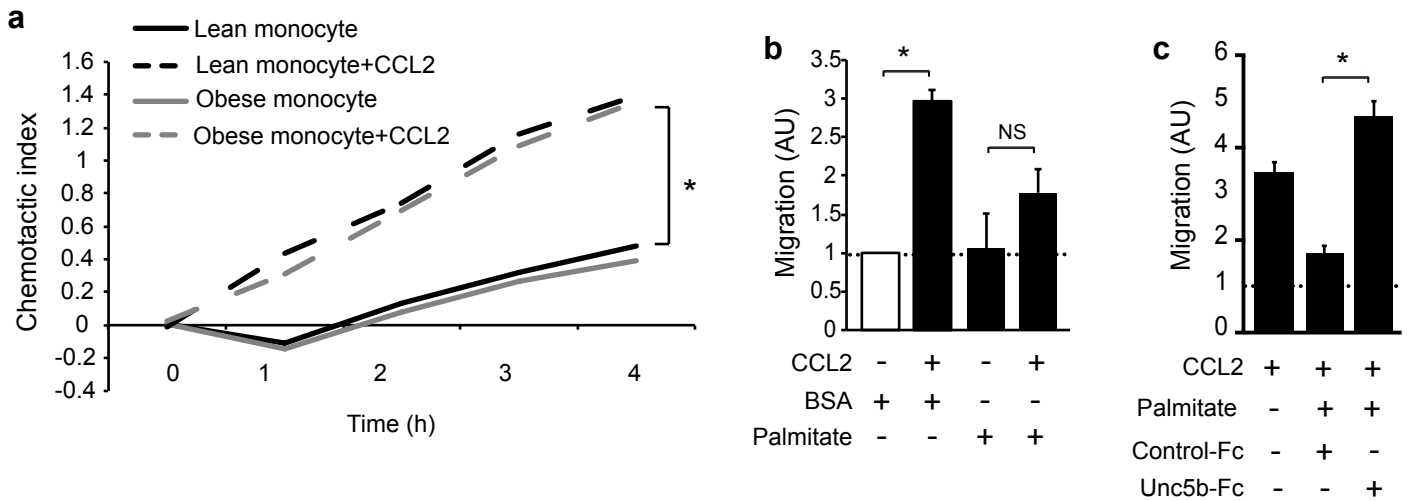
Supplemental Figure 2



Conditioned media of 3T3L1 adipocytes treated with palmitate induces the expression of *Ntn1* and *Unc5b*

(a-b) qRT-PCR analysis of mRNA for *Ntn1* (a) and *Unc5b* (b) in BMDMs treated with conditioned media from BSA or palmitate-treated 3T3L1 adipocytes in the presence of the corresponding IgG controls for anti-TNF α (goat IgG) and anti-IL-6 (rat IgG1). Data are the mean \pm s.d of triplicate samples from a single experiment.

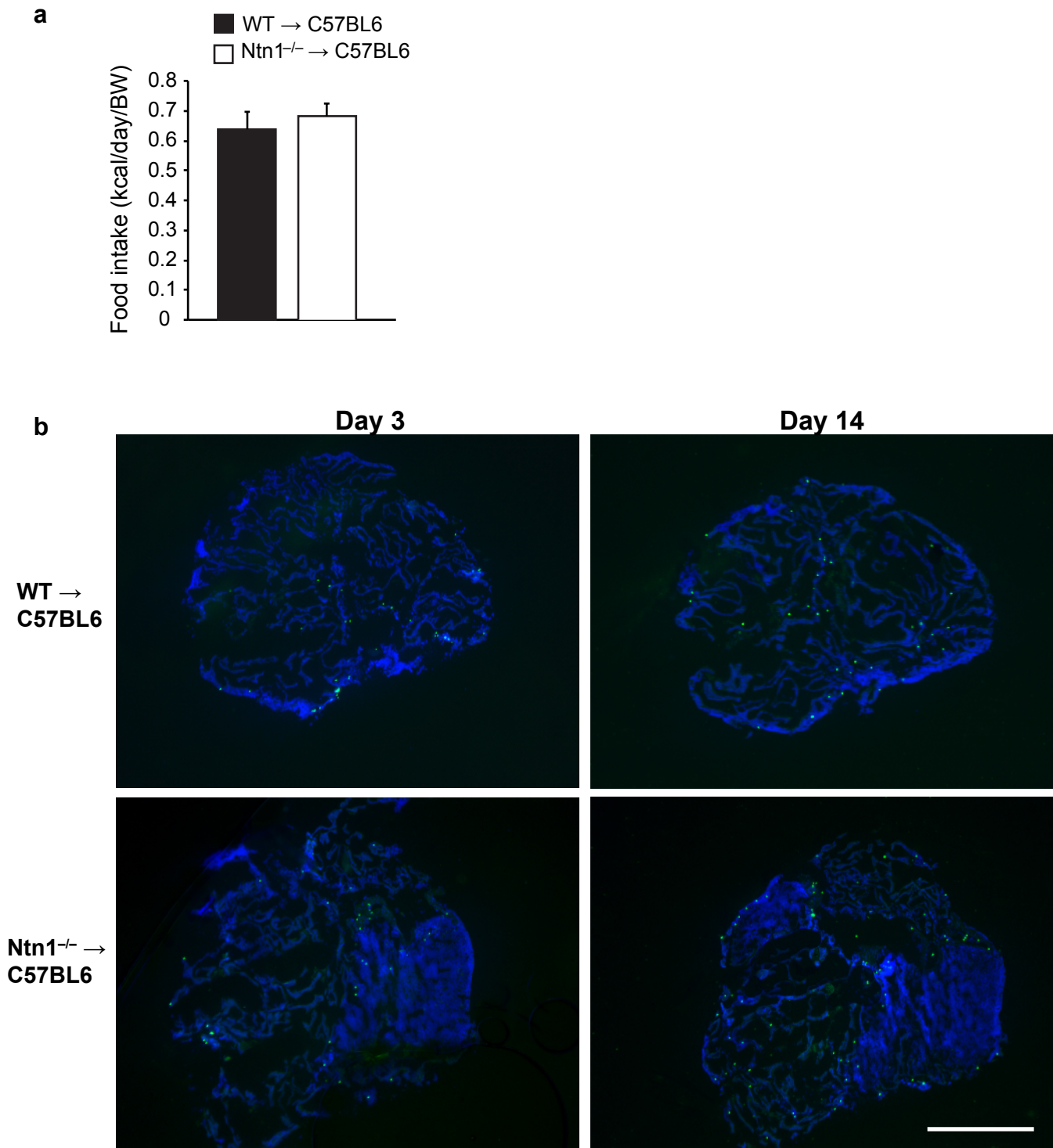
Supplemental Figure 3



CCL2 promotes the migration of blood monocytes but not of palmitate-treated BMDM

(a) Migration of monocytes isolated from chow or HFD-fed mice to CCL2. (b) Migration to CCL2 of bone marrow derived macrophages pre-treated with BSA or palmitate, and (c) in the additional presence of Unc5b-FC or control-FC. Data are the mean \pm s.d of a single experiment and are representative of 2 (a) or 3 (b-c) independent experiments. * $P < 0.05$.

Supplemental Figure 4



Netrin-1 deficiency does not affect food intake but induces the migration of macrophages to the lymph nodes.

(a) Food intake in C57BL6 mice transplanted with WT (n=10) or *Ntn1*^{-/-} (n=9) bone marrow fed HFD. Data are the mean \pm s.e.m. *p<0.05. (b) Representative images of mesenteric lymph nodes of HFD-fed WT (n=5) and *Ntn1*^{-/-} (n=5) mice showing fluorescent bead accumulation 3 and 14 days after monocyte labeling. Scale bar 500 μ m. Data are the mean number of beads per section (6 sections per lymph node).

Supplementary Table 1.**Neuronal guidance cues gene expression profiling of HFD or chow WAT.**

Expression of neuronal guidance cues of the netrin, slit, semaphorin and ephrin families and their receptors in WAT of *C57BL6* mice fed chow or HFD (n=3 mice/group).

Full Name	Refseq #	Gene Symbol	Fold Regulation HFD/Chow
Netrin 1	NM_008744	Ntn1	2.0705
Netrin 3	NM_010947	Ntn3	-1.2834
Netrin 4	NM_021320	Ntn4	-1.5052
Netrin G1	NM_030699	Ntng1	-1.2834
Netrin G2	NM_133501	Ntng2	-2.362
Deleted in colorectal carcinoma	NM_007831	Dcc	-1.7532
Neogenin	NM_008684	Neo1	-2.639
Unc-5 homolog A	NM_153131	Unc5a	-1.4439
Unc-5 homolog B	NM_029770	Unc5b	3.2944
Unc-5 homolog C	NM_009472	Unc5c	1.5157
Unc-5 homolog D	NM_153135	Unc5d	-1.2834
Adenosine A2a receptor	NM_009630	Adora2a	-6.8211
Adenosine A2b receptor	NM_007413	Adora2b	2.6027
Slit homolog 1	NM_015748	Slit1	-1.7171
Slit homolog 2	NM_178804	Slit2	4.4383
Slit homolog 3	NM_011412	Slit3	1.5052
Roundabout homolog 1	NM_019413	Robo1	1.3013
Roundabout homolog 2	NM_175549	Robo2	-1.2834
Roundabout homolog 3	XM_001476840	Robo3	-10.8528
Roundabout homolog 4	NM_028783	Robo4	-9.7811
Ephrin A1	NM_010107	Efna1	-3.7064
Ephrin A2	NM_007909	Efna2	1.1173
Ephrin A3	NM_010108	Efna3	-1.2834
Ephrin A4	NM_007910	Efna4	2.7132
Ephrin A5	NM_010109	Efna5	-2.5847
Ephrin B1	NM_010110	Efnb1	-1.6021
Ephrin B2	NM_010111	Efnb2	-1.366
Ephrin B3	NM_007911	Efnb3	-1.257
Eph receptor A1	NM_023580	Epha1	1.1251
Eph receptor A2	NM_010139	Epha2	-1.0497
Eph receptor A3	NM_010140	Epha3	14.8254
Eph receptor A4	NM_007936	Epha4	-5.4264
Eph receptor A5	NM_007937	Epha5	1.4743
Eph receptor A6	NM_007938	Epha6	-48.1679
Eph receptor A7	NM_010141	Epha7	1.0792
Eph receptor A8	NM_007939	Epha8	-1.2834
Eph receptor A10	NM_177671	Epha10	-1.2834
Eph receptor B1	NM_173447	Ephb1	-2.5669
Eph receptor B2	NM_010142	Ephb2	2.6574
Eph receptor B3	NM_010143	Ephb3	1.3195
Eph receptor B4	NM_010144	Ephb4	-3.0738
Eph receptor B6	NM_007680	Ephb6	-4.1699

Full Name	Refseq #	Gene Symbol	Fold Regulation HFD/Chow
Semaphorin 3A, secreted	NM_009152	Sema3a	-1.1408
Semaphorin 3B, secreted	NM_009153	Sema3b	1.4641
Semaphorin 3C, secreted	NM_013657	Sema3c	1.1728
Semaphorin 3D, secreted	NM_028882	Sema3d	-1.4044
Semaphorin 3E, secreted	NM_011348	Sema3e	1.979
Semaphorin 3F, secreted	NM_011349	Sema3f	-2.2038
Semaphorin 3G, secreted	NM_001025379	Sema3g	-2.514
Semaphorin 4A, transmembrane	NM_013658	Sema4a	-4.1699
Semaphorin 4B, transmembrane	NM_013659	Sema4b	-1.9185
Semaphorin 4C, transmembrane	XM_898566	Sema4c	-1.5476
Semaphorin 4D, transmembrane	NM_013660	Sema4d	1.2142
Semaphorin 4G, transmembrane	NM_011976	Sema4g	31.779
Semaphorin 5A, transmembrane	NM_009154	Sema5a	-85.6274
Semaphorin 5B, transmembrane	NM_013661	Sema5b	-1.2746
Semaphorin 6A, transmembrane	NM_018744	Sema6a	-2.1287
Semaphorin 6B, transmembrane	NM_013662	Sema6b	-2.3784
Semaphorin 6C, transmembrane	NM_011351	Sema6c	-2.2658
Semaphorin 6D, transmembrane	NM_172537	Sema6d	-1.1019
Semaphorin 7A, GPI anchor	NM_011352	Sema7a	-1.4439
Plexin A1	NM_008881	Plxna1	-2.5315
Plexin A2	NM_008882	Plxna2	-1.1251
Plexin A3	NM_008883	Plxna3	1.0792
Plexin A4	NM_175750	Plxna4	1.1892
Plexin B1	NM_172775	Plxnb1	-1.5801
Plexin B2	XM_001003435	Plxnb2	2.639
Plexin B3	NM_019587	Plxnb3	-1.3472
Plexin C1	NM_018797	Plxnc1	6.498
Plexin D1	NM_026376	Plxnd1	1.7291
Neuropilin 1	NM_008737	Nrp1	2.2038
Neuropilin 2	NM_010939	Nrp2	2.0279
Mouse 18S rRNA gene, clones 5a,6,7	K01364	18SrRNA	1.2354

Supplementary Table 2. RT-PCR primer sequences

	Forward (5'-3')
Mouse <i>Ntn1</i>	CAGCCTGATCCTTGCTCGG
Mouse <i>Unc5b</i>	TGGATCTTTCAGCTCAAGACCCAG
Mouse <i>Adora2b</i>	GCGAGAGGGATCATTGCTGTC
Mouse <i>Dcc</i>	CAAGCTGGCTTTTGTACTCTTCG
Mouse <i>Neogenin</i>	CTAGCATTGTAGTGAGCTGGAC
Mouse <i>Emr1</i>	CCCCAGTGTCCTTACAGAGTG
Mouse <i>Cd206</i>	CTCTGTTTCAGCTATTGGACGC
Mouse <i>Il10</i>	GCTCTTACTGACTGGCATGAG
Mouse <i>Pparg</i>	TCGCTGATGCACTGCCTATG
Mouse <i>Nos2</i>	GTTCTCAGCCCAACAATACAAGA
Mouse <i>Ccl2</i>	TTAAAAACCTGGATCGGAACCAA
Mouse <i>Tnfa</i>	ATGAGCACAGAAAGCATGATCCGC
Mouse <i>Il6</i>	CCAAGAGGTGAGTGCTTCCC
Mouse <i>Il4</i>	GGTCTCAACCCCCAGCTAGT
Mouse <i>28s</i>	TGGGGAATGCAGCCCAAG
Mouse <i>Gapdh</i>	TGTGAGGGAGATGCTCAGTG
Human <i>NTN1</i>	CTCACACTGTCCCTCGGCAAGAAGT
Human <i>UNC5H</i>	CAGCCTTAAGGTCAAGGTCTACAGCTC
Human <i>TNFA</i>	GAGGGCTGATTAGAGAGAGGTC
Human <i>SEMA3A</i>	GTGCCAAGGCTGAAATTATCCT
Human <i>EFNB2</i>	ACTGCTGGGGTGTTTTGATGG
Human <i>CD68</i>	GCTACATGGCGGTGGAGTACAA
Human <i>Il10</i>	GACTTTAAGGGTTACCTGGGTTG
Human <i>DCC</i>	GGTTCTTCTGCCAGTGGATTGTTGG
Human <i>SEMA3E</i>	GGTTACGCCTGTCACATAAAGA