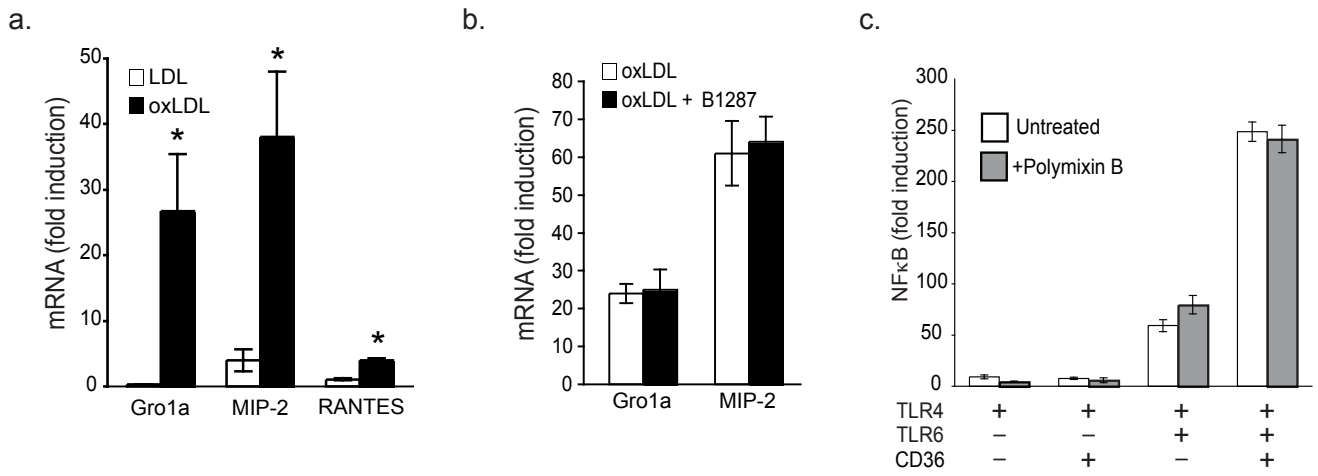
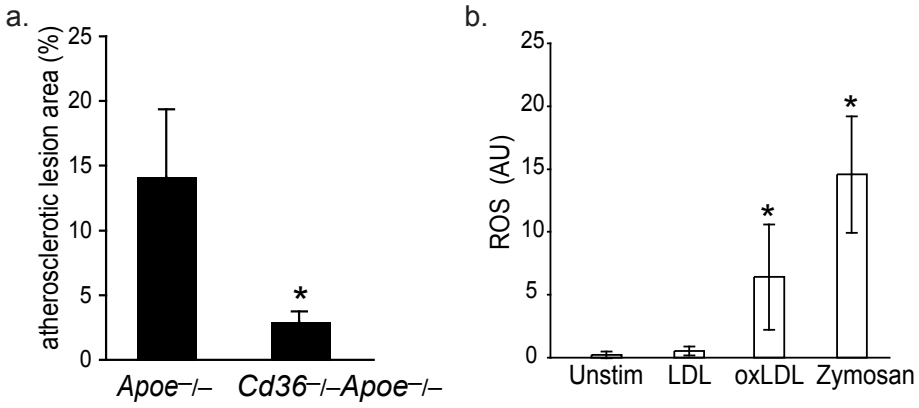
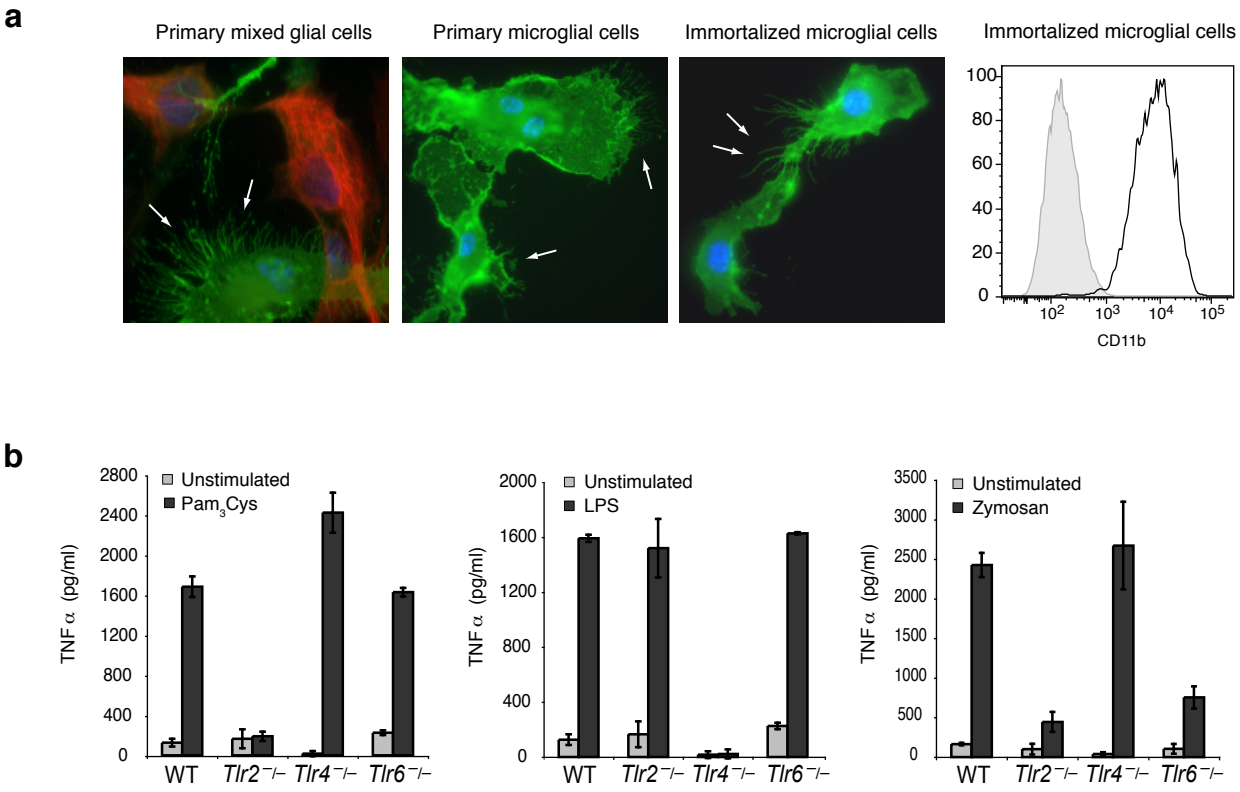
Stewart et al.
CD36 ligands promote sterile inflammation through assembly of a TLR 4 and 6 heterodimer



Supplementary Figure 1. OxLDL specifically induces macrophage chemokine expression.
(a) Macrophages were stimulated with LDL and oxLDL (50 ug/ml, 6 h) and chemokine mRNA expression was analyzed by QRT-PCR. Data shows fold induction compared to unstimulated cells.
(b) Macrophages were stimulated with oxLDL (50 ug/ml, 6 h) in the presence and absence of the MD-2 inhibitor B1287 (10 ug/ml). Chemokine mRNA expression was measured as in panel a. (c) HEK293 cells expressing the indicated TLRs in combination with CD36 and an NF-κB luciferase reporter gene were stimulated with oxLDL (50 ug/ml, 5 h) in the presence or absence of polymixin B to block the biological effects of LPS. Luciferase activity was measured. Data are mean ± s.d. triplicate samples in a single experiment and are representative of an experimental group of 3. *P<0.005



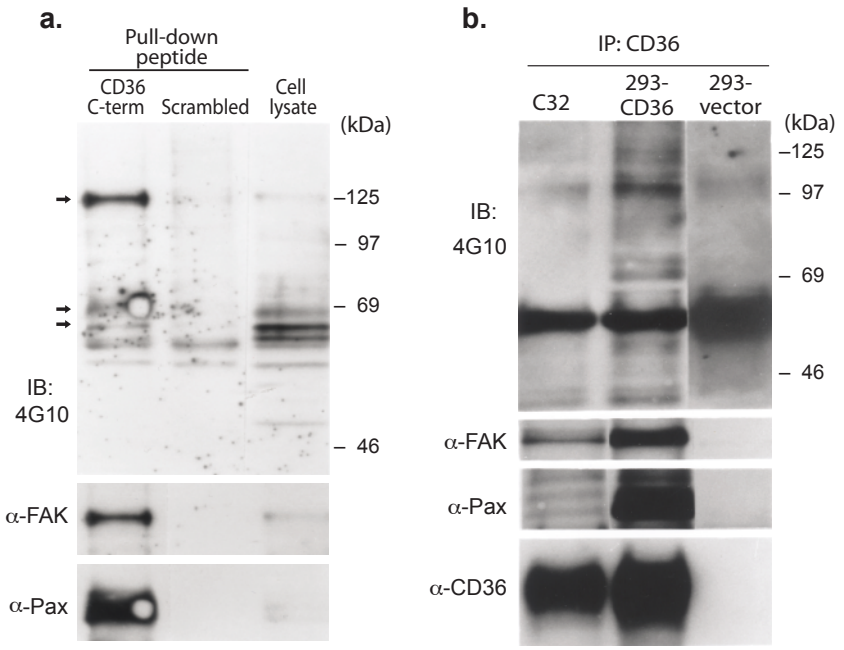
Supplementary Figure 2. Targeted deletion of CD36 reduces atherosclerosis in *Apoe*–/– mice. (a) Morphometric analysis of lesion area in the aorta of female *Apoe*$^{-/-}$ and *Cd36*$^{-/-}$*Apoe*$^{-/-}$ mice fed a western diet (21% [w/w] fat; 0.15% [w/w] cholesterol) for 12 weeks (n=10/group). Aortas were pinned open and lesion area of the aorta en face was quantified using IPLab Spectrum software. Lesion area is expressed as a percent of the total aortic area ± s.d. *P<0.05. (b) Reactive oxygen species production in macrophages stimulated with LDL or oxLDL (50 μg/ml) or zymosan (1 μg/ml) for 45 min. Data are the mean ± s.d. of triplicate samples. *P<0.005



Supplementary Figure 3. Immortalized mouse microglial cells retain their morphological and functional characteristics.

(a) Immortalized mouse microglial cells show 100 % purity, as indicated by staining with an antibody to CD11b (green), and retain the typical morphology of primary microglia. These pure immortalized microglial cultures were derived from mixed primary glial cultures that contained microglia and astrocytes, as shown by staining with antibodies against CD11b (green) and the astrocytic marker GFAP (red). Note the morphological similarity between resting primary and immortalized microglia with typical fine processes (arrows). Immortalized microglial cultures contained only CD11b+ microglia as quantified by flow cytometry.

(b) Immortalized WT, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$ and *Tlr6*$^{-/-}$ microglia were stimulated with Pam3Cys (TLR2-1 ligand; 100nM), LPS (TLR4 ligand; 100 ng/ml) or zymosan (TLR2-6 ligand; 1 μg/ml) for 18 h and TNFα was quantified by ELISA. Data presented are the mean of triplicate samples ± s.d., and are representative of three separate experiments. * P<0.05.



Supplementary Figure 4. The CD36 C-terminus binds tyrosine phosphorylated proteins, including FAK and Paxillin.

(a) To identify signaling molecules that bind to CD36, a peptide corresponding to the cytoplasmic C-terminus of CD36 (460-472) or a scrambled peptide was incubated with 1mg of HUVEC lysate. Bound proteins were analyzed by immunoblotting for phosphotyrosine (4G10 Ab). Unprecipitated cell lysate (10 μg) was included as a positive control. Three phosphoproteins that specifically bound the CD36 C-terminal peptide were identified at approximately 58, 70 and 125 kDa (arrows). Reprobing of the blot identified the 70 and 125 kDa proteins as Paxillin (Pax) and focal adhesion kinase (FAK). **(b)** To confirm these interactions CD36 was immunoprecipitated from cell lysates of C32 melanoma cells, CD36- or vector-transfected HEK293 cells and precipitated proteins were analyzed by immunoblotting for phosphotyrosine (4G10 Ab), FAK, Paxillin and CD36. Data are representative of three separate experiments.

Supplementary Table

oxLDL-induced chemokine mRNA expression

C-C Chemokines	*P* value	Fold change	C-X-C Chemokines	*P* value	Fold change
Ccl1	0.508	1.19	*Cxcl1* (KC/Gro1)	0.083	2.68
Ccl2 (MCP-1/JE)	0.087	-2.66	*Cxcl2* (MIP-2)	0.031	5.60
Ccl4 (MIP-1b)	0.492	-1.24	*Cxcl4*	0.492	1.16
Ccl5 (RANTES)	0.002	18.21	*Cxcl5* (ENA-78)	0.876	-1.01
Ccl6 (MRP1)	0.249	1.28	*Cxcl9* (CMK)	0.352	6.90
Ccl7 (MCP-3)	0.491	1.73	*Cxcl10* (IP-10)	0.002	4.77
Ccl8 (MCP-2)	0.199	1.61	*Cxcl11* (I-TAC)	0.404	4.45
Ccl9 (MIP-1g/MRP-2)	0.165	1.98	*Cxcl12* (PBSF)	0.580	1.43
Ccl11 (eotaxin)	0.466	1.22	*Cxcl13* (Angie)	0.241	-5.84
Ccl12 (MCP-5)	0.561	1.22	*Cxcl15* (lungkine)	0.544	2.87
Ccl17 (ABCD-2)	0.588	3.53			
Ccl19 (MIP-3b, ELC)	0.466	1.22	*Cx3Cl1* (fractalkine)	0.381	5.87
Ccl20 (CKb4/LARC)	0.276	6.29			

Primary murine macrophages were stimulated with 50 ug/ml oxLDL for 12 h. Chemokine expression was measured by QRT-PCR using RT2 Profiler Arrays for mouse chemokines. Data are expressed as fold change over mock-treated cells (n=3/group). Statistical analysis was performed by Student's t-test.