Figure S1

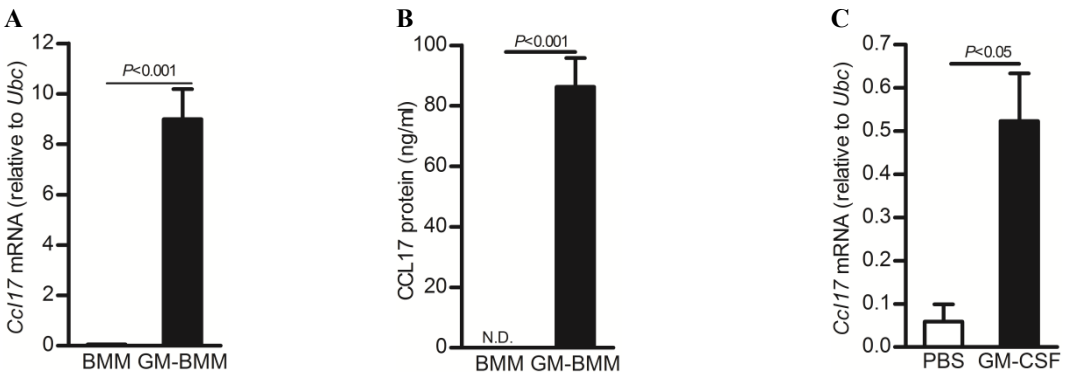

Figure S1: GM-CSF upregulates CCL17 expression in in vitro-derived and ex vivo murine macrophage populations

(A-B) Murine bone cells were cultured in either M-CSF (5,000 U/ml) or GM-CSF (20 ng/ml) for 7 days to derive BMM and GM-BMM, respectively: **(A)** *Ccl17* mRNA (qPCR) and **(B)** secreted CCL17 by ELISA. **(C)** *Ccl17* mRNA expression in resident peritoneal macrophages following treatment with either PBS (vehicle) or GM-CSF (20 ng/ml) for 24 h (n=4). Graphs are plotted as mean ± SEM. N.D., not detected. P values were obtained using a t-test.

Figure S2

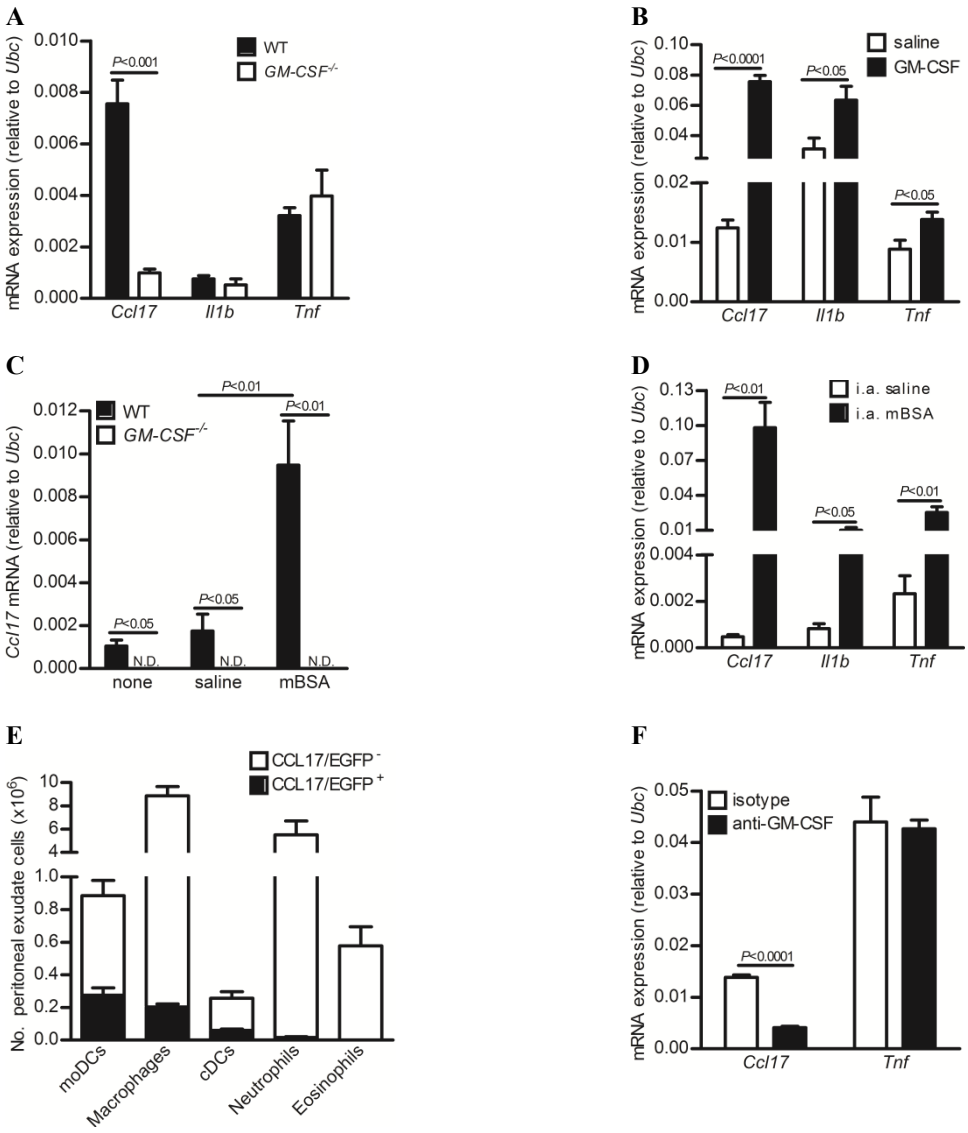

Figure S2: Dependence of CCL17 expression on GM-CSF in vivo

(A-B) *Ccl17*, *Il1b* and *Tnf* mRNA expression (qPCR) **(A)** in the naïve hind footpad of WT and *GM-CSF*$^{-/-}$ mice and **(B)** in the hind footpad of WT mice 4 h post intraplantar (i.pl.) injection of GM-CSF (20 ng). n=12 mice/group.

(C) *Ccl17* mRNA expression in non-injected naïve joints (none) or joints injected intra-articularly (i.a.) with saline or methylated BSA (mBSA) from WT and *GM-CSF*$^{-/-}$ mice. **(D)** mRNA expression in WT mice 7 days following i.a. injection of mBSA or saline (day 0) and subcutaneous (s.c.) injection of GM-CSF (500 ng) on days 0-2. n=5 mice/group. N.D., not detected.

(E-F) Antigen-induced peritonitis was induced in **(E)** *Ccl17*$^{E/+}$ mice and the number of CCL17/EGFP^{+} and CCL17/EGFP^{-} moDCs (CD115^{+}MHCII^{+}CD11c^{+}), macrophages (CD115^{+}MHCII$^{+/-}$CD11c^{-}), cDCs (CD115^{-}MHCII^{+}CD11c^{+}), neutrophils (Ly6G^{+}CD11b^{+}) and eosinophils (Ly6G^{-}CD11b^{int}SSc^{hi}) in the peritoneum (day 4) quantified. **(F)** WT mice were treated i.p. on days 1 and 2 with anti-GM-CSF mAb or isotype control following antigen-induced peritonitis induction and CCL17 and TNF mRNA expression was determined in total peritoneal exudate cells (day 4). n=5 mice/group.

Graphs were plotted as mean ± SEM. P values were obtained using a t-test.

Figure S3

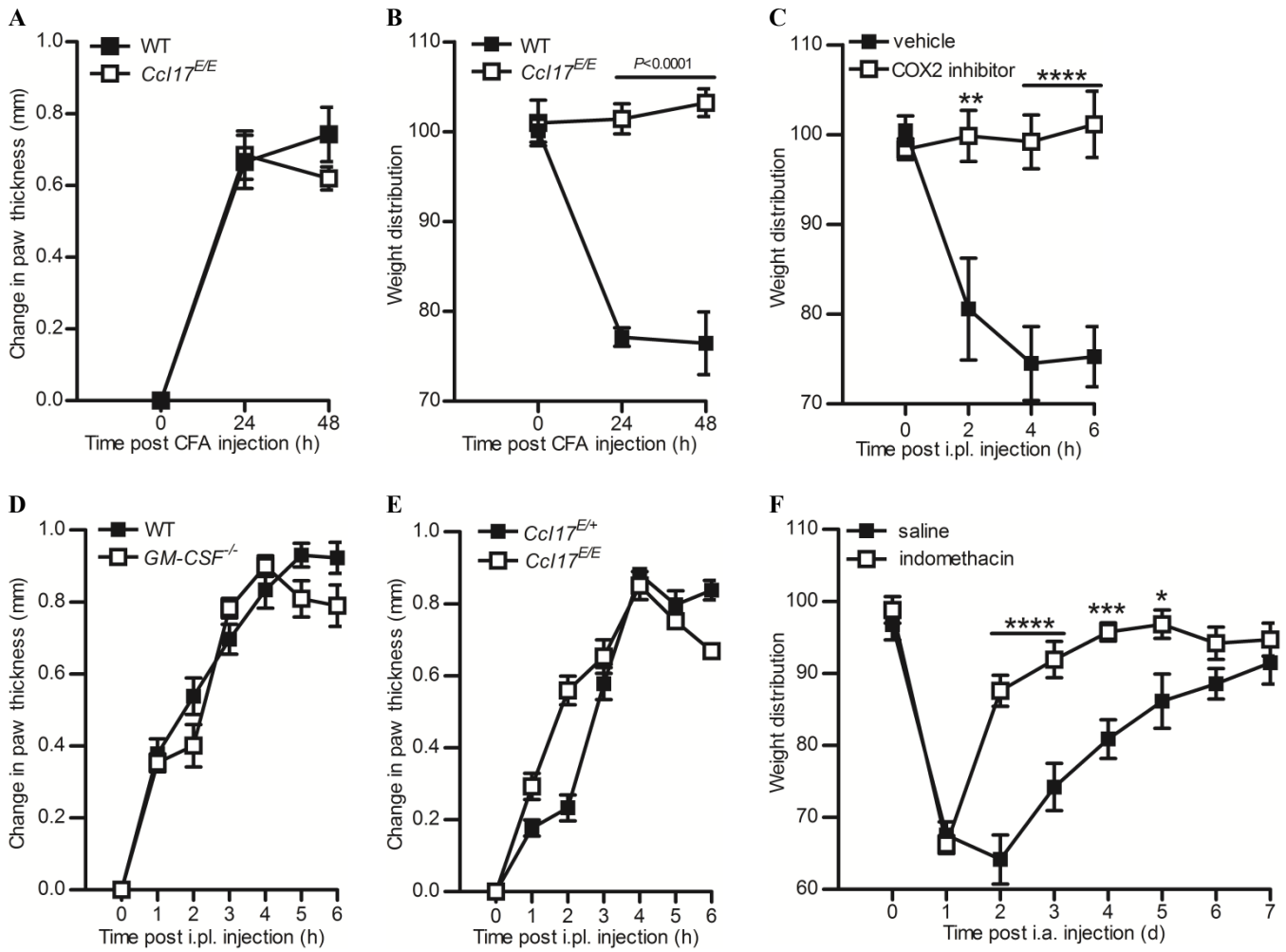

Figure S3: The requirement for CCL17 in GM-CSF-dependent inflammatory and arthritic pain

(A-B) WT and *Ccl17*$^{E/E}$ mice received an intraplantar (i.pl.) injection of Complete Freund's Adjuvant (CFA) and **(A)** pain (incapacitance meter) and **(B)** footpad swelling (callipers) were measured (n=5 per group).

(C) WT mice were pre-treated with the COX2 inhibitor, SC58125 (i.p. 5 mg/kg) or vehicle, 30 min prior to i.pl. zymosan and pain (incapacitance meter) was measured. **(D-E)** change in paw thickness (swelling) following i.pl. injection of zymosan in **(D)** WT and *GM-CSF*$^{-/-}$ mice and **(E)** *Ccl17*$^{E/+}$ and *Ccl17*$^{E/E}$ mice (n=5 per group).

(F) Zymosan-induced arthritis in WT mice; indomethacin (1 mg/kg) was given i.p. daily from day 1 and pain measured. n=6 per group.

Results are mean ± SEM. P values were obtained using a 2-way ANOVA test.

*P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001, vehicle/saline vs.COX2 inhibitor/indomethacin.

Figure S4

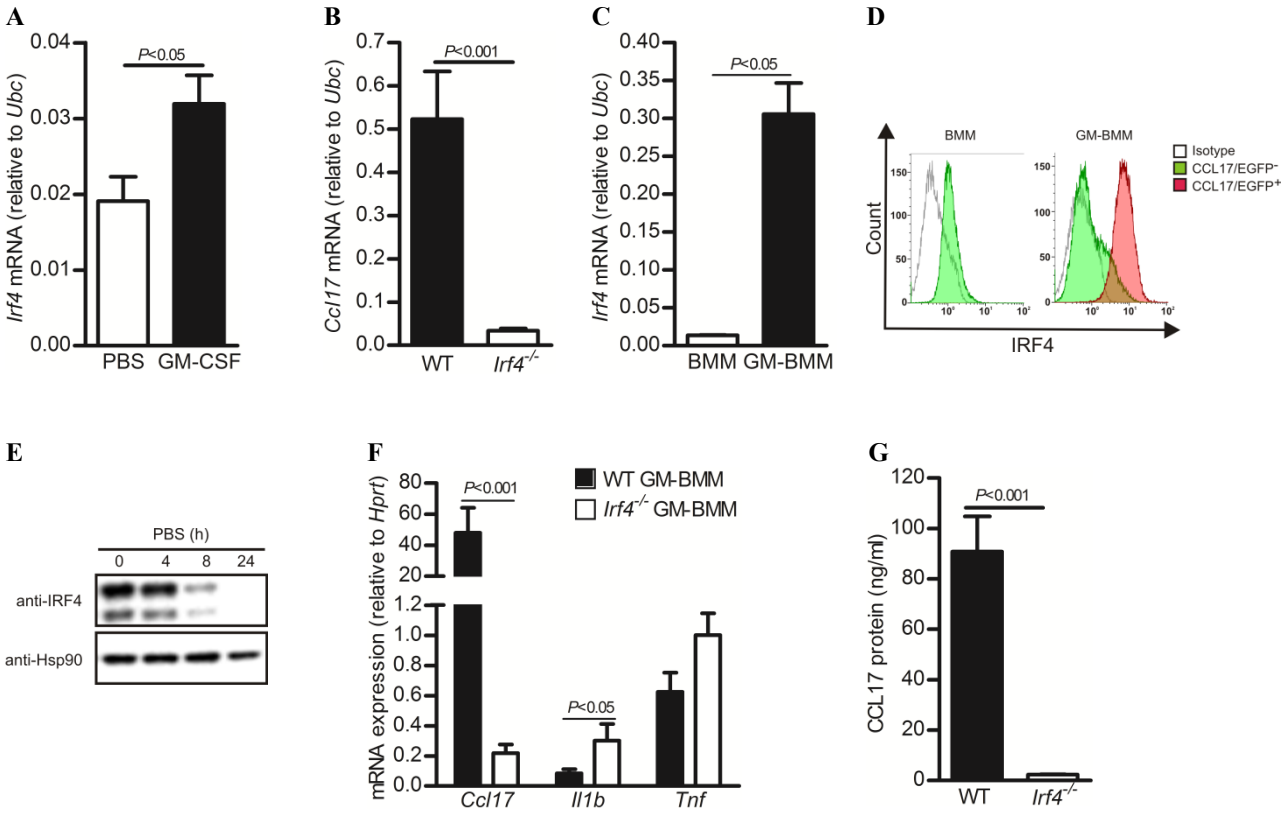

Figure S4: GM-CSF-induced CCL17 expression in ex vivo- and in vitro-derived macrophage populations is IRF4-dependent

(A-B) Resident peritoneal macrophages were treated with PBS (vehicle) and GM-CSF (20 ng/ml) for 24 h: **(A)** *Irf4* mRNA expression in WT macrophages and **(B)** *Ccl17* mRNA expression in WT and *Irf4*$^{-/-}$ macrophages (n=4).

(C-E) Murine bone marrow cells were cultured in either GM-CSF (20 ng/ml) or M-CSF (5,000 U/ml) for 7 days to derive GM-BMM and BMM, respectively: **(C)** basal *Irf4* mRNA expression (qPCR), **(D)** representative histograms of IRF4 in CCL17/GFP^{+} and CCL17/GFP^{-} populations of GM-BMM and BMM (from *Ccl17*$^{E/+}$ mice) and **(E)** whole cell lysates from GM-BMM following withdrawal of GM-CSF for indicated periods of time were subjected to Western blotting with anti-IRF4 and anti-Hsp90 antibodies (n=5). **(F-G)** Bone marrow cells from WT and *Irf4*$^{-/-}$ mice were cultured in GM-CSF (20 ng/ml) for 7 days to derive GM-BMM: **(F)** mRNA expression of cytokines and **(G)** secreted CCL17 by ELISA (n=4).

Graphs are plotted as mean ± SEM. P values were obtained using a t-test.

Figure S5

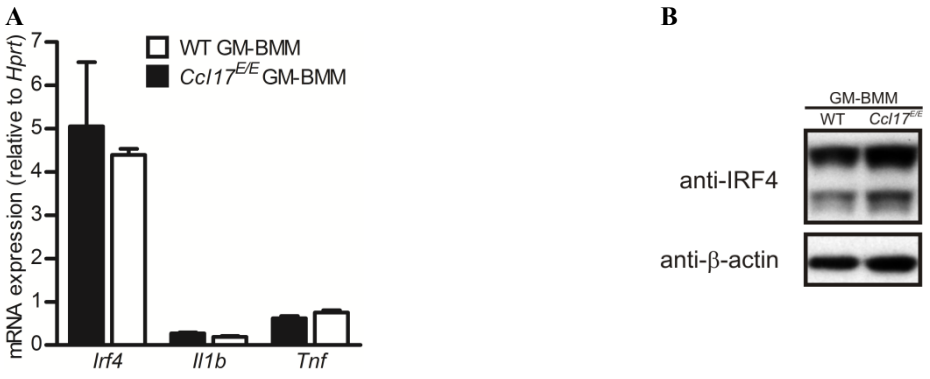

Figure S5: IRF4 is not downstream of CCL17 in murine macrophages

(A-B) Bone marrow cells from both from WT and *Ccl17*$^{E/E}$ mice were cultured in GM-CSF (20 ng/ml) for 7 days to derive GMBMM: **(A)** basal mRNA expression (qPCR) and **(B)** whole cell lysates were subjected to Western blotting with anti-IRF4 and anti-actin antibodies (n=3). Graphs are plotted as mean ± SEM.

Table S1: Cytokines/chemokines, according to their log_2 fold-change in gene expression (microarray), following GM-CSF treatment versus PBS control for 16 h ($P< 0.05$) in human monocytes (n=3 donors).

Cytokine/chemokine	**Log_2 fold-change**	***P*-value**
CCL17	5.33	3.42E-07
INHBA	4.77	5.53E-08
IL1b	4.34	3.52E-08
CXCL7	4.20	3.00E-09
CXCL5	4.14	4.60E-09
CXCL1	3.78	1.57E-06
CCL3	3.17	3.98E-05
IL19	2.94	2.15E-06
CCL24	2.45	1.90E-05
TNF	2.01	0.008
CCL4	1.86	0.013
CXCL2	1.74	0.005
CXCL3	1.69	0.018
CCL7	1.26	0.049
CCL1	1.18	0.027
CCL14	1.17	0.018
CCL26	0.93	0.045
IL9	-1.75	0.011