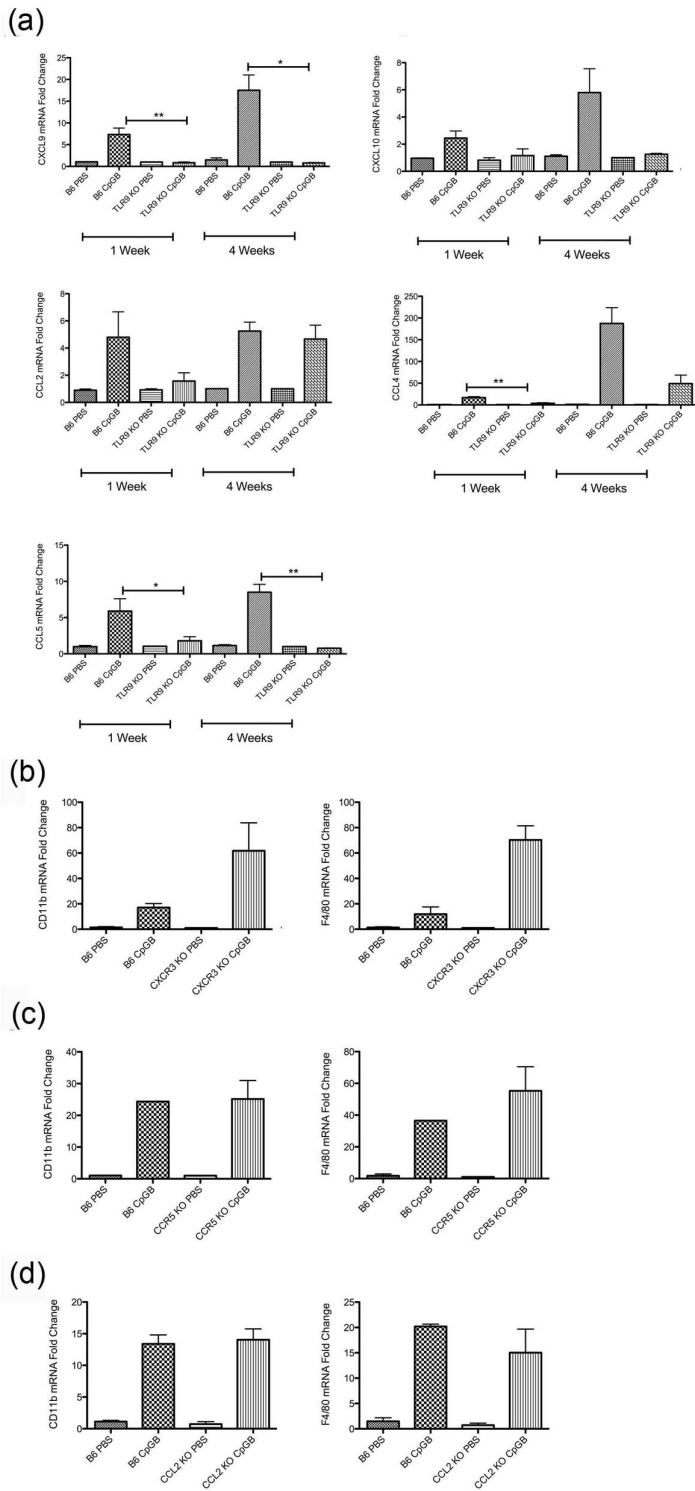
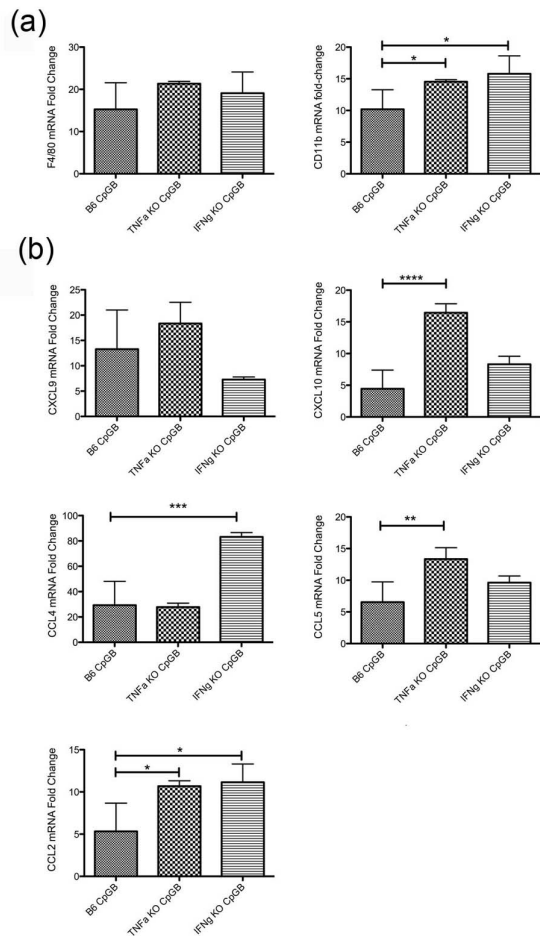


S. Figure 1: TLR9-dependent TNF α and IFN γ induction, no type I interferon response to CpGB DNA treatment, and specific recruitment of macrophages to CpGB DNA treated skin. (A) IFN γ and TNF α gene expression analysis, comparing wildtype (B6) and TLR9-deficient (TLR9 KO) mice. (B) Gene expression analysis for type I interferon-inducible genes: MX-2 and IFIT1. (C) Gene expression analysis for markers of dendritic cells (CD11c), B cells (CD19), T cells (CD3) and neutrophils (Ly6G). (D) Gene expression analysis for macrophage markers, F4/80 and CD11b. (A, D) B6 PBS 1 week: n=4; B6 CpGB 1 week: n=5; TLR9 KO PBS 1 week: n=3; TLR9 KO CpGB 1 week: n=5; B6 PBS 4 week: n=3; B6 CpGB 4 week: n=5; TLR9 KO PBS 4 week: n=1; TLR9 KO CpGB 4 week: n=2. (B, C) PBS 1 week: n=8; CpGB 1 week: n=11; PBS 4 week: n=3; CpGB 4 week: n=5. Gene expression is normalized to GAPDH and compared to the respective PBS treated mouse. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. (E, F) CD3-PE staining; (G, H) Alexa Fluor 488-conjugated IgG2b isotype control; (E, G) 1 week PBS-treated B6 skin; (F, H) 1 week CpGB DNA-treated B6 skin. All skin counterstained with DAPI nuclear staining. All images taken at 10X magnification. (I) Nanostring gene expression analysis for macrophage markers ARG1, CD163, and NOS at 1 week in B6 mice; PBS n=3, CpG n=3. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001.

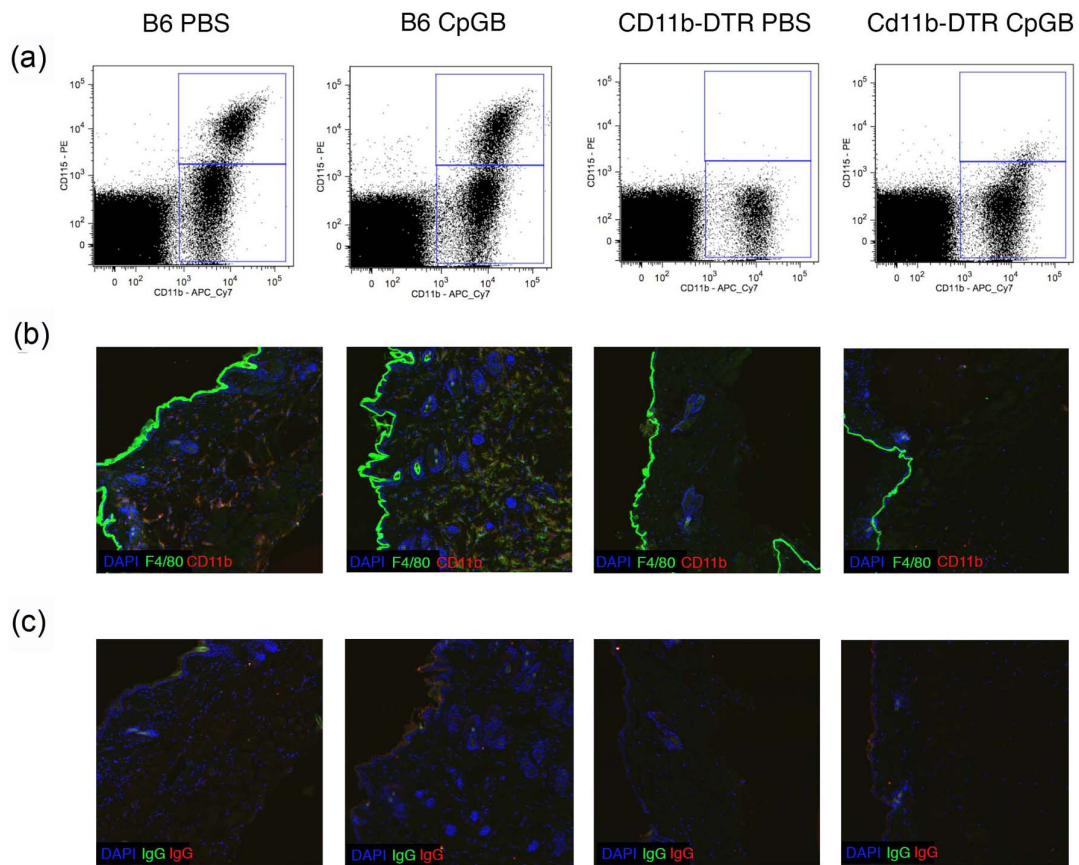


S. Figure 2: Chemokine expression after CpGB DNA treatment is TLR9-dependent, and cell recruitment is not dependent on CXCR3, CCR5, or CCL2 signaling. (A) Gene expression analysis for chemokines, CXCL2, CCL2, CCL4, CCL5, CXCL9, and CXCL10, comparing wildtype (B6) and TLR9-deficient (TLR9 KO) mice. B6 PBS 1 week: n=4; B6 CpGB 1 week: n=5; TLR9 KO PBS 1 week: n=3; TLR9 KO CpGB 1 week: n=5; B6 PBS 4 week: n=3; B6 CpGB 4 week: n=5; TLR9 KO PBS 4 week: n=1; TLR9 KO CpGB 4 week: n=2. (B-D) Gene expression analysis for macrophage markers, F4/80 and CD11b, comparing 1 week PBS and CpGB DNA treated wildtype (B6) mice to (B) CXCR3-deficient (CXCR3 KO) mice, (C) CCR5-deficient (CCR5 KO) mice, and (D) CCL2-deficient (CCL2 KO) mice. (B) B6 PBS: n=2; B6 CpGB: n=3; CXCR3 KO PBS: n=1; CXCR3 KO CpGB: n=3 (C) B6 PBS: n=2; B6 CpGB: n=1; CCR5 KO PBS: n=1; CCR5 KO CpGB: n=3 (D) B6 PBS: n=2; B6 CpGB: n=3; CCL2 KO PBS: n=2; CCL2 KO CpGB: n=2. Gene expression is normalized to GAPDH and compared to the respective PBS treated mouse. * $p < 0.05$; ** $p < 0.01$.

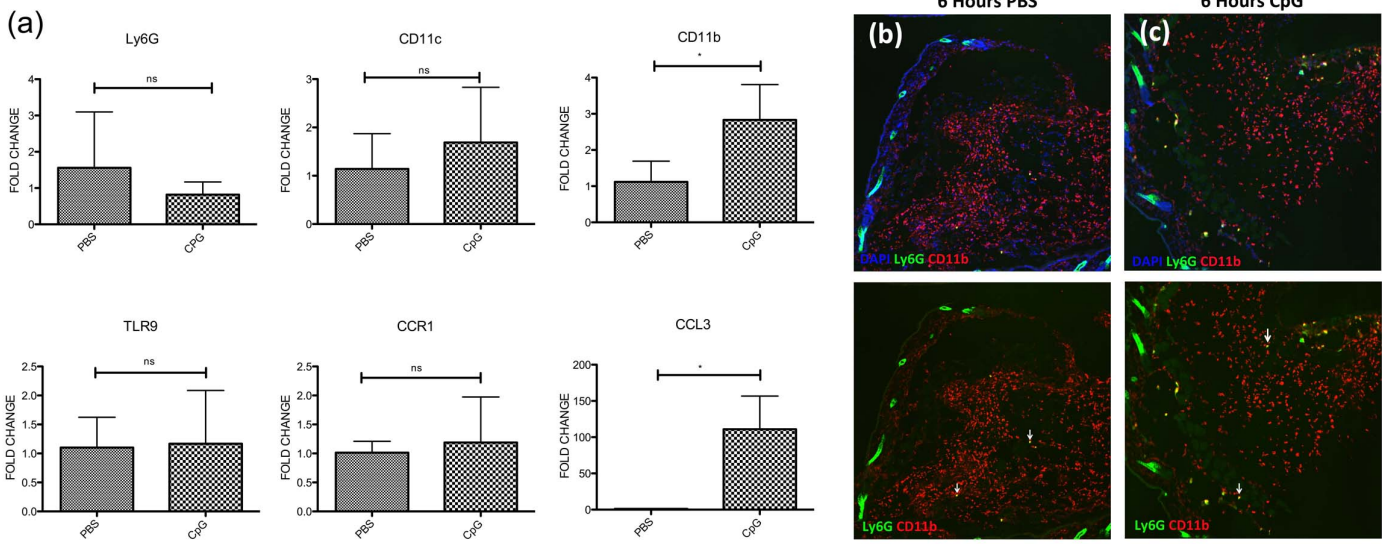


S. Figure 3: CpGB DNA-induced chemokine expression and cell recruitment are not dependent on IFN γ or TNF α signaling.

(A) CD11b and F4/80 gene expression comparisons between wildtype (B6), TNF α -deficient, and IFN γ -deficient mice after treatment with CpGB DNA for 1 week. (B) Gene expression comparisons of chemokines: CXCL9, CXCL10, CCL2, CCL4, and CCL5, between wildtype (B6), TNF α -deficient, and IFN γ -deficient mice after treatment with CpGB DNA for 1 week. For B6 CpGB DNA-treated mice, n=11 and for all IFN γ and TNF α -deficient CpGB DNA-treated mice, n=3. Data collected from 2 experiments. Gene expression is normalized GAPDH and compared to the respective PBS-treated mouse. * p<0.05; ** p<0.01; *** p<0.001, ****p<0.0001



S. Figure 4: Initial CpGB DNA-induced skin inflammation is not dependent on granulocytes.
 (A) Ly6G, CD11c, CD11b, TLR9, CCR1, and CCL3 gene expression comparisons from skin of wildtype (B6) mice 6 hours following after subcutaneous injection with CpGB n=3 and PBS n=3. Gene expression is normalized GAPDH and compared to the respective PBS-treated mouse. * p<0.05; ** p<0.01; *** p<0.001, ****p<0.0001 (B) Immunofluorescence staining for CD11b and Ly6G in B6 skin 6 hours following subcutaneous injection with PBS. (B) Immunofluorescence staining for CD11b and Ly6G in B6 skin 6 hours following subcutaneous injection with CpG.



S. Figure 5: CD11b depletion by diphtheria toxin inducible mouse model.

(A) Flow cytometry analysis of PBMCs in B6 and CD11b-DTR mice after DT i.p. injection and 2 days of treatment with PBS or CpGB DNA, using CD11b-APC_Cy7, CD115-PE, Ly6C-Pacific Blue, and AmCyan Live/Dead cell staining. Data is gated on all live single cells. Data shown is a representative image. (B-C) Immunofluorescence from skin of B6 and CD11b-DTR mice after DT i.p. injection and 2 days of treatment with PBS or CpGB DNA. All sections are counterstained with DAPI. All images are taken at 20X magnification. (B) Colocalization staining of F4/80-Alexa Fluor 488 and CD11b-PE (C) Isotype Controls: IgG2b-Alexa Fluor 488 and IgG2b-PE.

Gene Name	Abbreviation	mRNA Fold Change	Standard Deviation
Angiotensin II receptor-like 1	Agtr1	0.40	0.49
Brain-derived neurotrophic factor	Bdnf	0.59	0.20
Burkitt lymphoma receptor 1	Blr1	1.00	0.15
Bone morphogenetic protein 10	BMP10	0.73	0.29
Bone morphogenetic protein 15	BMP15	0.43	0.24
Bone morphogenetic protein 6	BMP6	0.51	0.31
Chemokine binding protein 2	Ccbp2	0.54	0.32
Chemokine (C-C motif) ligand 1	CCL1	0.58	0.13
Chemokine (C-C motif) ligand 11	CCL11	0.35	0.38
Chemokine (C-C motif) ligand 12	CCL12	1.02	0.72
Chemokine (C-C motif) ligand 17	CCL17	0.43	0.14
Chemokine (C-C motif) ligand 19	CCL19	0.77	0.13
Chemokine (C-C motif) ligand 2	CCL2	3.41	3.13
Chemokine (C-C motif) ligand 20	CCL20	0.68	0.11
Chemokine (C-C motif) ligand 4	CCL4	19.54	7.78
Chemokine (C-C motif) ligand 5	CCL5	7.21	6.31
Chemokine (C-C motif) ligand 6	CCL6	0.19	0.14
Chemokine (C-C motif) ligand 7	CCL7	3.09	2.16
Chemokine (C-C motif) ligand 8	CCL8	0.08	0.06
Chemokine (C-C motif) ligand 9	CCL9	0.60	0.22
Chemokine (C-C motif) receptor 1	CCR1	0.41	0.45
Chemokine (C-C motif) receptor 10	CCR10	0.51	0.19
Chemokine (C-C motif) receptor 1-like 1	CCR1L1	0.91	0.22
Chemokine (C-C motif) receptor 2	CCR2	0.40	0.44
Chemokine (C-C motif) receptor 3	CCR3	1.21	1.22
Chemokine (C-C motif) receptor 4	CCR4	0.54	0.22
Chemokine (C-C motif) receptor 5	CCR5	2.16	1.73
Chemokine (C-C motif) receptor 6	CCR6	0.85	0.15
Chemokine (C-C motif) receptor 7	CCR7	1.32	1.30
Chemokine (C-C motif) receptor 8	CCR8	0.59	0.16
Chemokine (C-C motif) receptor 9	CCR9	0.95	0.72
Chemokine (C-C motif) receptor-like 1	CCRL1	0.50	0.20
Chemokine (C-C motif) receptor-like 2	CCRL2	1.64	0.35
Chemokine-like receptor 1	Cmklr1	0.41	0.35
CKLF-like MARVEL transmembrane domain containing 2a	Cmtm2a	0.55	0.32
CKLF-like MARVEL transmembrane domain containing 3	Cmtm3	1.14	0.42
CKLF-like MARVEL transmembrane domain containing 4	Cmtm4	0.68	0.30
CKLF-like MARVEL transmembrane domain containing 5	Cmtm5	0.60	0.11

Table 1: Chemokine array for CpGB DNA-treated skin.

All 84-gene expression analysis of chemokine and chemokine receptors on the RT2 Profiler™ PCR Array: Mouse Chemokines & Receptors (SA Biosciences). Data is given as an average mRNA fold change in comparison to PBS-treated mice in addition to standard deviation. n=4.