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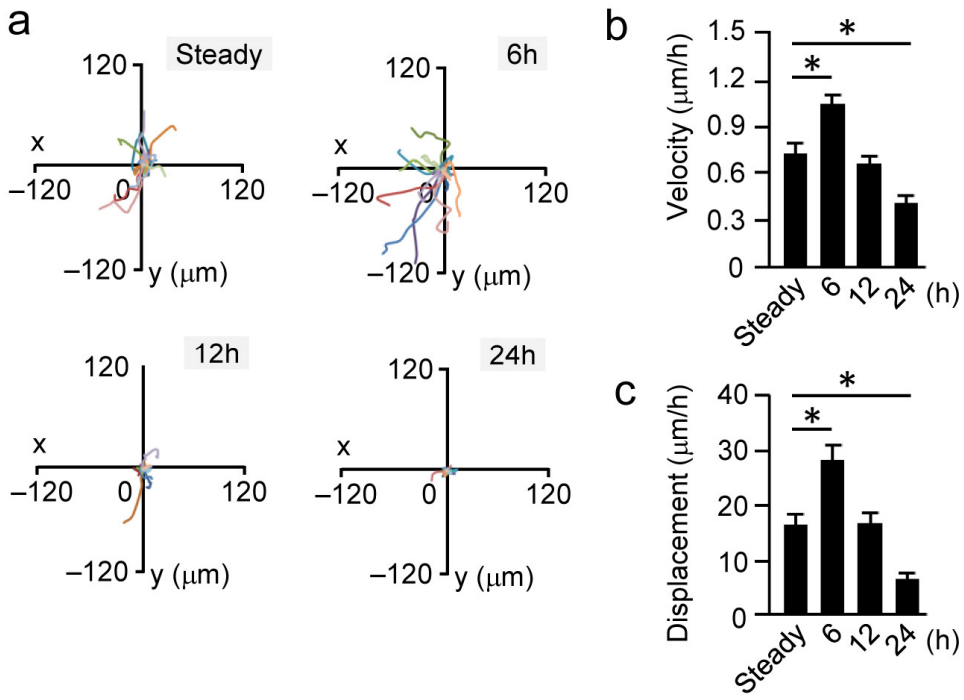
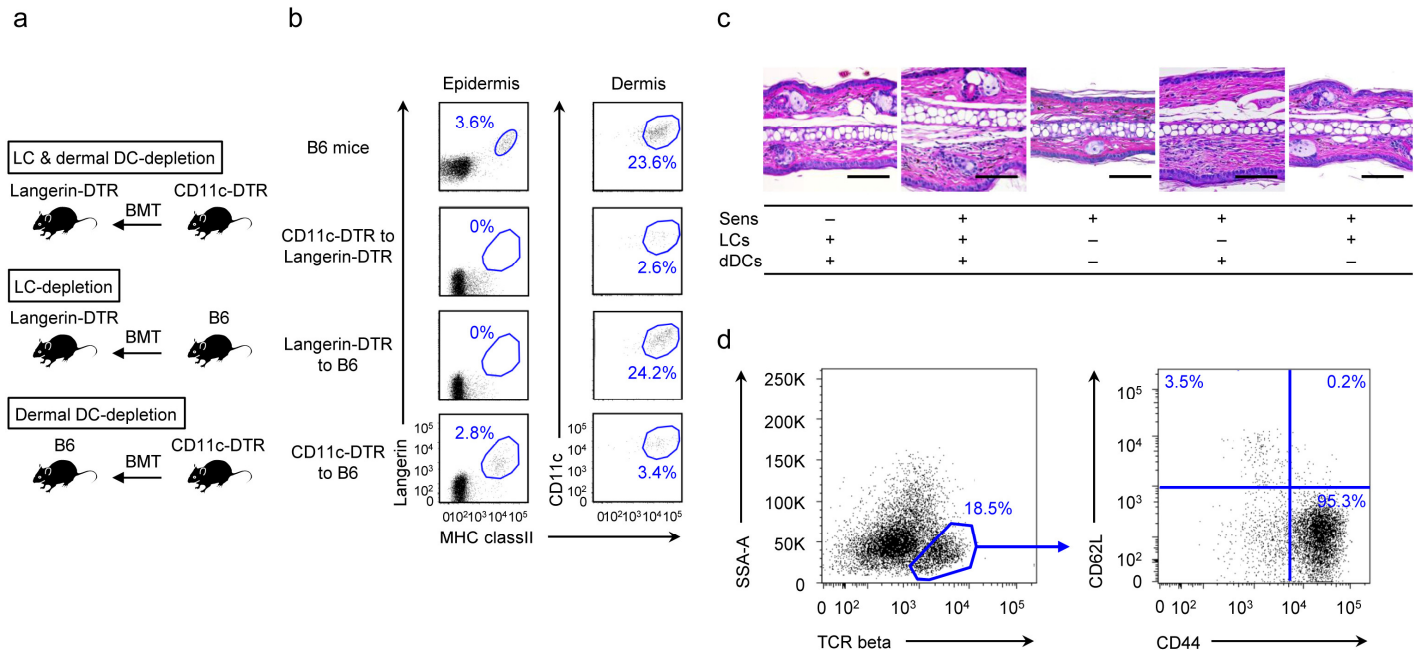


Supplementary Figure 1

DC motility in the elicitation phase of CHS.

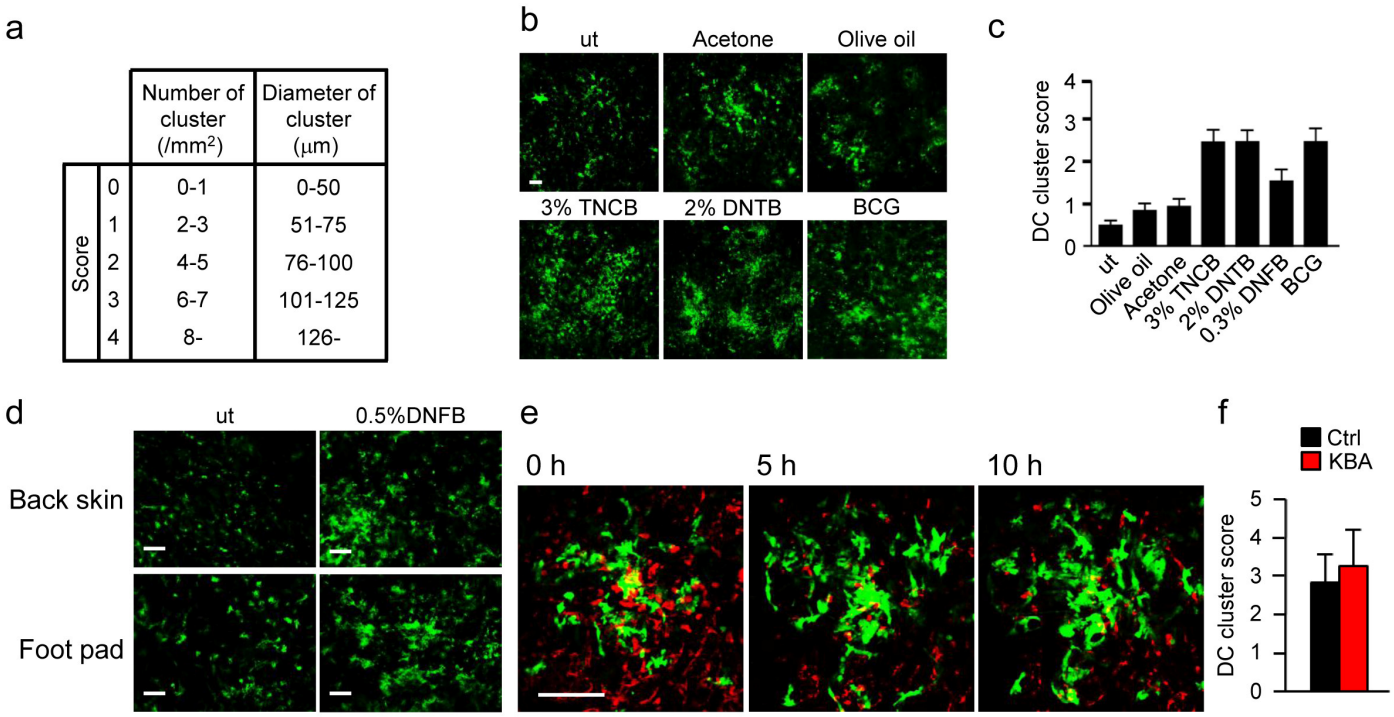
(a) Superimposed 30-min tracks of 30 randomly selected dermal DCs in the x–y plane, setting the starting coordinates to the origin. Tracks of a steady state, 6, 12, and 24 h after the elicitation with DNFB are shown. (b and c) Velocity (b) and displacement (c) of dDCs at each time point (n=30). Each bar represents the mean + SD. *, $P < 0.05$.



Supplementary Figure 2

Subset-specific depletion of cutaneous DCs.

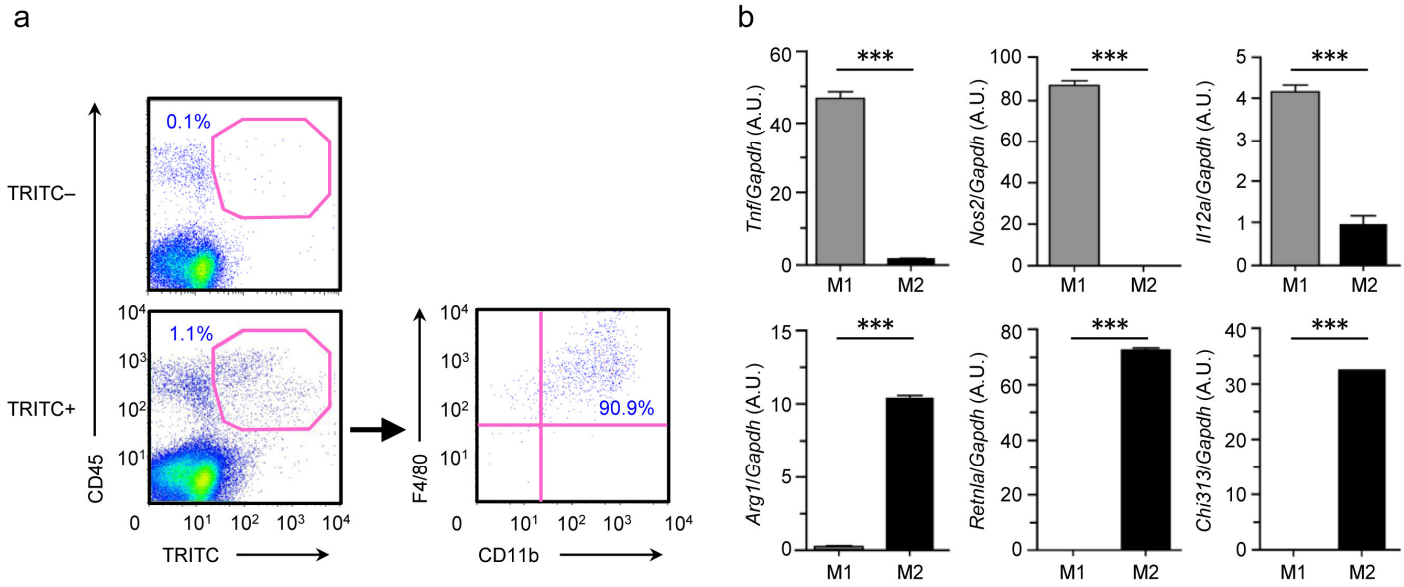
(a) A schematic representation of our strategy to generate subset-specific cutaneous DC depletion models. To deplete all cutaneous DC subsets, Langerin-DTR mice were transferred with BM cells from CD11c-DTR mice, and DT was injected. To selectively deplete LCs, Langerin-DTR mice were transferred with BM cells from C57BL/6 mice, and DT was injected. To selectively deplete dDCs, C57BL/6 mice were transferred with BM cells from CD11c-DTR mice, and DT was injected. BMT; BM transplantation. (b) FACS plots of each group of mice after DT treatment. In dermis, the percentages in CD45+ cells were indicated. (c) Histological findings of the ear skin after CHS. HE staining of the ears of mice 24 h after challenge with DNFB. Mice were pretreated with or without sensitization, depleted of LCs and/or dDCs, and challenged with DFNB. Scale bar = 100 μm. (d) CHS response was induced on the ear skin, and skin-infiltrating cells were stained and analyzed with TCR beta, CD44, and CD62L antibodies by flow cytometry.



Supplementary Figure 3

dDC clusters are formed in response to various stimuli.

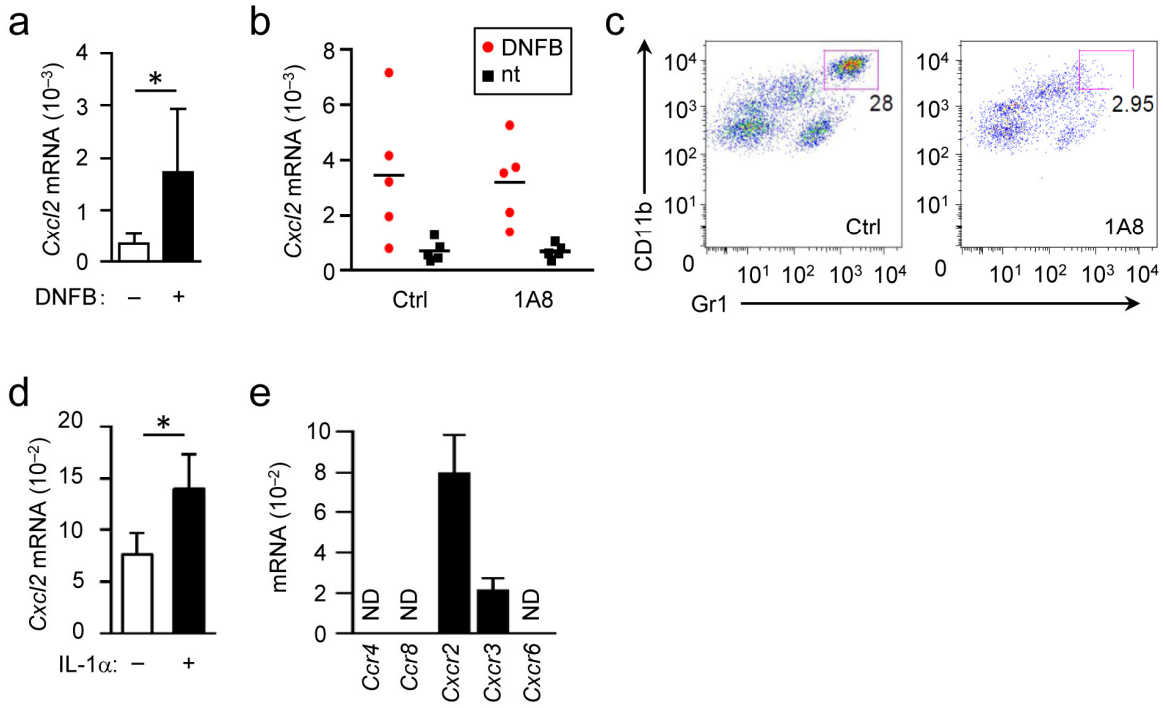
(a) The scoring criteria for DC clusters by numbers and diameters of clusters. (b) DC (green) cluster formation 24 h after topical application without (NT) or with acetone, olive oil, 3% TNCB, 2% DNTB, 0.3% DNFB, or *Mycobacterium bovis BCG*-inoculation (n=4, each). (c) Scores of DC cluster numbers of each group 24 h after each stimuli. (d) DC (green) cluster formation 24 h after topical application without (NT) or with 0.5% DNFB on the back skin and footpad. Scale bar = 100 μm. (e) Mobility of DCs and T cells of the cluster by treatment with anti-LFA-1 treatment. Anti-LFA-1 neutralizing antibody, KBA, was injected intravenously 14 h after elicitation. T cell (red) clustering was dissolved but DC (green) clustering persisted 10 h after KBA-treatment. Scale bar = 100 μm. (f) Score of DC cluster number 24 h after DNFB application with KBA (red) or control IgG (black) treatment (n=5, each).



Supplementary Figure 4

Analysis of M1 and M2 macrophage markers.

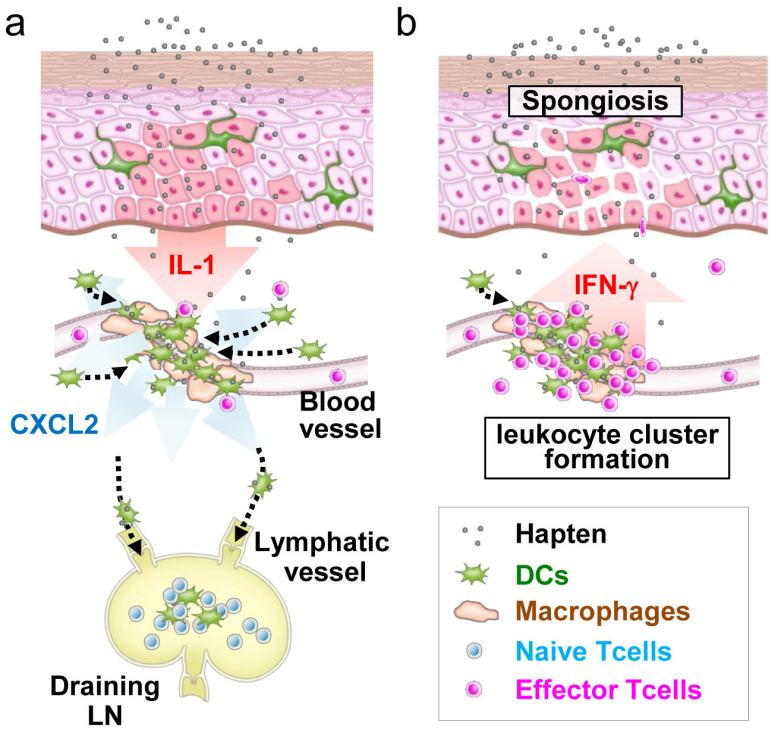
(a) TRITC-conjugated dextran was injected and dermal suspension was prepared 24 h later. CD45+ and TRITC+ cells were further analyzed with CD11b and F4/80 antibodies by flow cytometry. (b) M1 macrophage markers, such as *TNF-a*, *Nos2*, and *IL-12a*, and M2 macrophage markers, such as *arginase (Arg)-1*, *Retnla*, and *Chi313*, were examined in BM-derived M1 and M2 macrophages. Each bar represents the mean + SD (n=3). A.U., arbitrary units. *, $P < 0.05$. ***$P < 0.0001$.



Supplementary Figure 5

Neutrophils are not essential for CXCL2 expression in DNFB-painted skin.

(a) Relative amount of *Il1r1* 24 h after with or without DNFB-sensitization (n=5). (b) Relative amount of *Cxcl2* in DNFB-painted skin in 1A8- or control IgG-treated mice (n=5, each). (c) FACS plot of DNFB-painted skin prepared from 1A8- or control IgG-treated mice. CD11b+ Gr-1+ neutrophils were significantly depleted with 1A8-treatment. (d) Relative amount of *Il1r1* from dermal macrophages cultured with or without IL-1α (n=4, each). (e) RT-PCR analysis of chemokine receptor mRNA expression in BM-derived DCs.



Supplementary Figure 6

A schema of immunological events in CHS response.

Sensitization phase. Epidermal contact with antigens triggers release of IL-1 in the skin, which activates macrophages that subsequently attract dDCs to perivascular area via CXCL2 to form clusters. In the absence of antigen-specific effector/memory T cells, DC clustering is a transient event, and hapten-carrying DCs migrate into draining LNs to establish sensitization. (b) Elicitation phase. In the presence of antigen-specific effector/memory T cells, the antigen is recognized efficiently in the DC clusters by antigen-specific effector T cells to form clusters, and inflammation is induced promptly via activation and proliferation of antigen-specific effector T cells.

Supplementary Table 1: Chemokine expression profiles in M1- vs M2-phenotype macrophages with or without IL-1α treatment by means of microarray analysis.

ratio (log2)

Gene Description	Gene Symbol	M2/M1	M2_IL-1α /M1_IL-1α	M1_IL-1α /M1	M2_IL-1α /M2
chemokine (C motif) ligand 1	*Xcl1*	0.079755	-0.11996	0.029899	-0.16982
chemokine (C-C motif) ligand 1	*Ccl1*	0.001259	-0.24313	0.237145	-0.00725
chemokine (C-C motif) ligand 2	*Ccl2*	-0.44104	-0.33997	0.00156	0.10263
chemokine (C-C motif) ligand 3	*Ccl3*	-0.21153	-0.07617	0.08055	0.21591
chemokine (C-C motif) ligand 4	*Ccl4*	-0.56782	-0.30699	0.253582	0.514407
chemokine (C-C motif) ligand 5	*Ccl5*	-5.72304	-4.22995	-0.01614	1.476948
chemokine (C-C motif) ligand 6	*Ccl6*	1.88874	2.10452	-0.24231	-0.02653
chemokine (C-C motif) ligand 7	*Ccl7*	-0.2329	-0.32698	0.10564	0.01156
chemokine (C-C motif) ligand 8	*Ccl8*	-1.61746	-1.40666	-0.05775	0.153052
chemokine (C-C motif) ligand 9	*Ccl9*	0.44612	0.50154	-0.03128	0.02414
chemokine (C-C motif) ligand 11	*Ccl11*	0.077222	0.340821	-0.10453	0.159072
chemokine (C-C motif) ligand 12	*Ccl12*	-3.17708	-2.41643	-0.25347	0.507182
chemokine (C-C motif) ligand 17	*Ccl17*	1.713942	3.668465	-0.06557	1.888951
chemokine (C-C motif) ligand 20	*Ccl20*	0.160738	-0.42807	0.24176	-0.34705
chemokine (C-C motif) ligand 21a	*Ccl21a*	-0.09737	-0.12556	-0.03861	-0.0668
chemokine (C-C motif) ligand 22	*Ccl22*	-0.02726	1.771884	-0.12263	1.676516
chemokine (C-C motif) ligand 24	*Ccl24*	4.180073	4.708531	0.077052	0.60551
chemokine (C-C motif) ligand 25	*Ccl25*	-0.2785	-0.32217	0.142979	0.099304
chemokine (C-C motif) ligand 26	*Ccl26*	0.133507	-0.12029	0.103554	-0.15024
chemokine (C-C motif) ligand 27a	*Ccl27a*	0.127154	0.115419	0.007782	-0.00395
chemokine (C-C motif) ligand 27b	*Ccl27b*	0.246656	0.148537	0.048522	-0.0496

chemokine (C-C motif) ligand 28	*Ccl28*	1.03498	1.441795	-0.18907	0.217748
chemokine (C-X-C motif) ligand 1	*Cxcl1*	-0.04569	-0.02674	0.007147	0.026103
chemokine (C-X-C motif) ligand 2	*Cxcl2*	-1.61789	1.432005	0.130248	3.180143
chemokine (C-X-C motif) ligand 3	*Cxcl3*	0.185853	0.371034	-0.14298	0.042196
chemokine (C-X-C motif) ligand 5	*Cxcl5*	0.150911	0.063672	0.178769	0.09153
chemokine (C-X-C motif) ligand 9	*Cxcl9*	-7.44194	-6.83237	-0.00444	0.605132
chemokine (C-X-C motif) ligand 10	*Cxcl10*	-6.8282	-5.0165	-0.16726	1.644438
chemokine (C-X-C motif) ligand 11	*Cxcl11*	-4.88792	-5.05843	0.11235	-0.05816
chemokine (C-X-C motif) ligand 12	*Cxcl12*	0.455115	0.009638	0.245324	-0.20015
chemokine (C-X-C motif) ligand 13	*Cxcl13*	-0.20062	-0.25052	-0.02902	-0.07892
chemokine (C-X-C motif) ligand 14	*Cxcl14*	0.389156	0.354584	0.107933	0.073361
chemokine (C-X-C motif) ligand 15	*Cxcl15*	-0.16601	-0.05923	-0.05137	0.055403
chemokine (C-X-C motif) ligand 16	*Cxcl16*	-2.73158	-1.55457	0.0482	1.225208
chemokine (C-X-C motif) ligand 17	*Cxcl17*	0.019214	0.148531	0.002397	0.131714
chemokine (C-X3-C motif) ligand 1	*Cx3cl1*	0.228177	0.266712	-0.0622	-0.02366

Supplementary Table 2: Primer sets used in this study.

Gene	Forward	reverse
Il1r1	ATGAGTTACCCGAGGTCCAGTG	TACTCGTGTGACCGGATATTGC
CxCl2	CAAACCGAAGTCATAGCCAC	TCTGGTCAGTTGGATTTGCC
Ccr4	GAAGAGCAAGGCAGCTCAAC	GACCTCCCCAAATGCCTTGA
Ccr8	ATAATTGGTCTTCCTGCCTCGAT	CTGAGGAGGAACTCTGCGTC
Cxcr2	ACTACTGCAGGATTAAGTTTACCTC	TCTCTGAGTGGCATGGGACA
Cxcr3	GCCATGTACCTTGAGGTTAGTGA	ATCGTAGGGAGAGGTGCTGT
Cxcr6	ACTGGGCTTCTCTTCTGATGC	AAGCGTTTGTTCTCCTGGCT
Tnf	CCCCAAAGGGATGAGAAGTT	CACTTGGTGGTTTGCTACGA
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Il12a	CTGTGCCTTGGTAGCATCTATG	GCAGAGTCTCGCCATTATGATTC
Arg1	ACCATAAGCCAGGGACTGAC	AGGAGAAGGCGTTTGCTTAG
Retnla	CCAATCCAGCTAACTATCCCTCC	ACCCAGTAGCAGTCATCCCA
Chi313	AGAAGGGAGTTTCAAACCTGGT	GTCTTGCTCATGTGTGTAAGTGA
Gapdh	GGCCTCACCCCATTTGATGT	CATGTTCCAGTATGACTCCACTC