Supplemental Data

Supplemental figures, tables, and legends

CCL17 signals through CCR8 to induce CCL3 expression and restrain atheroprotective Tregs

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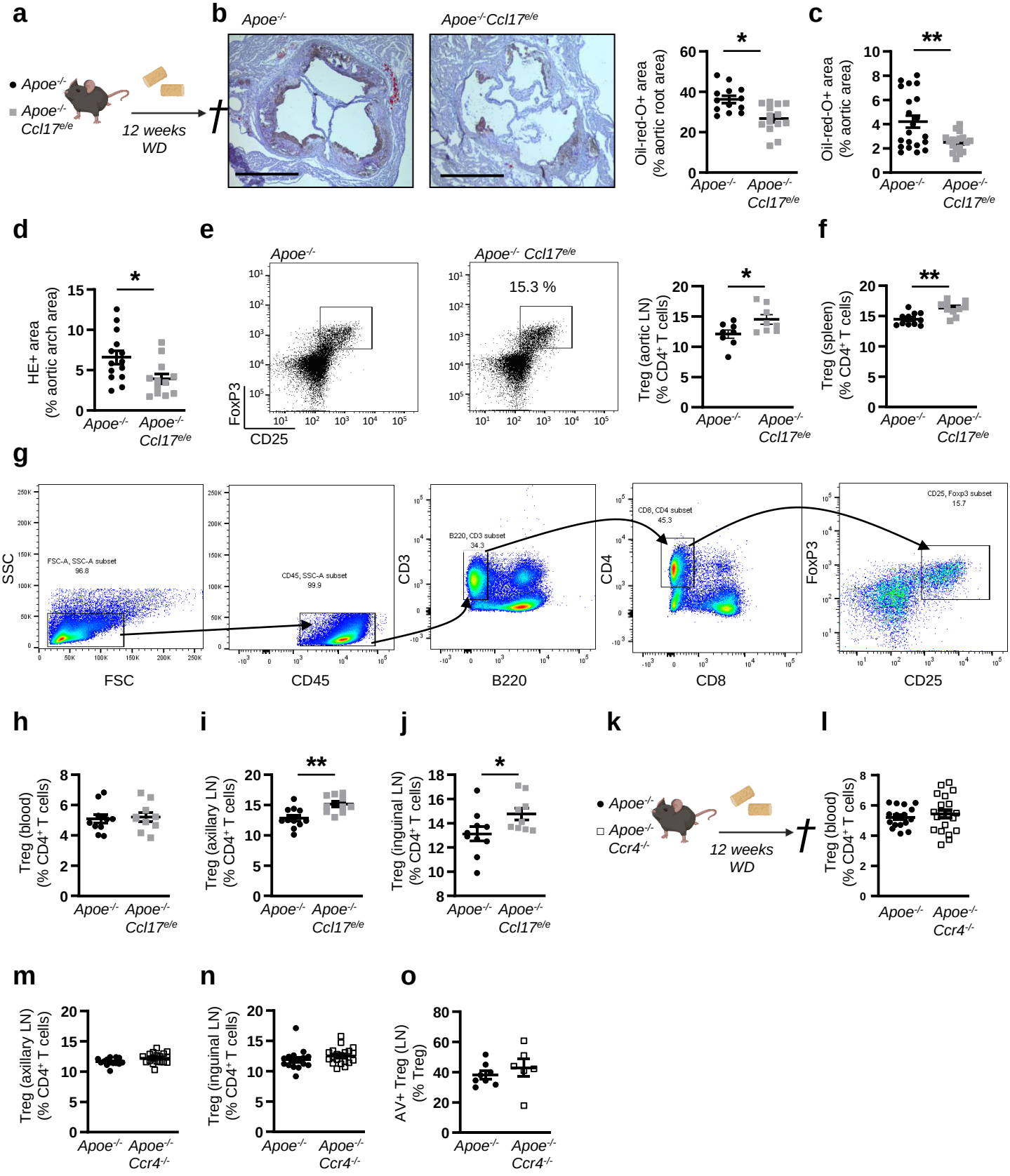
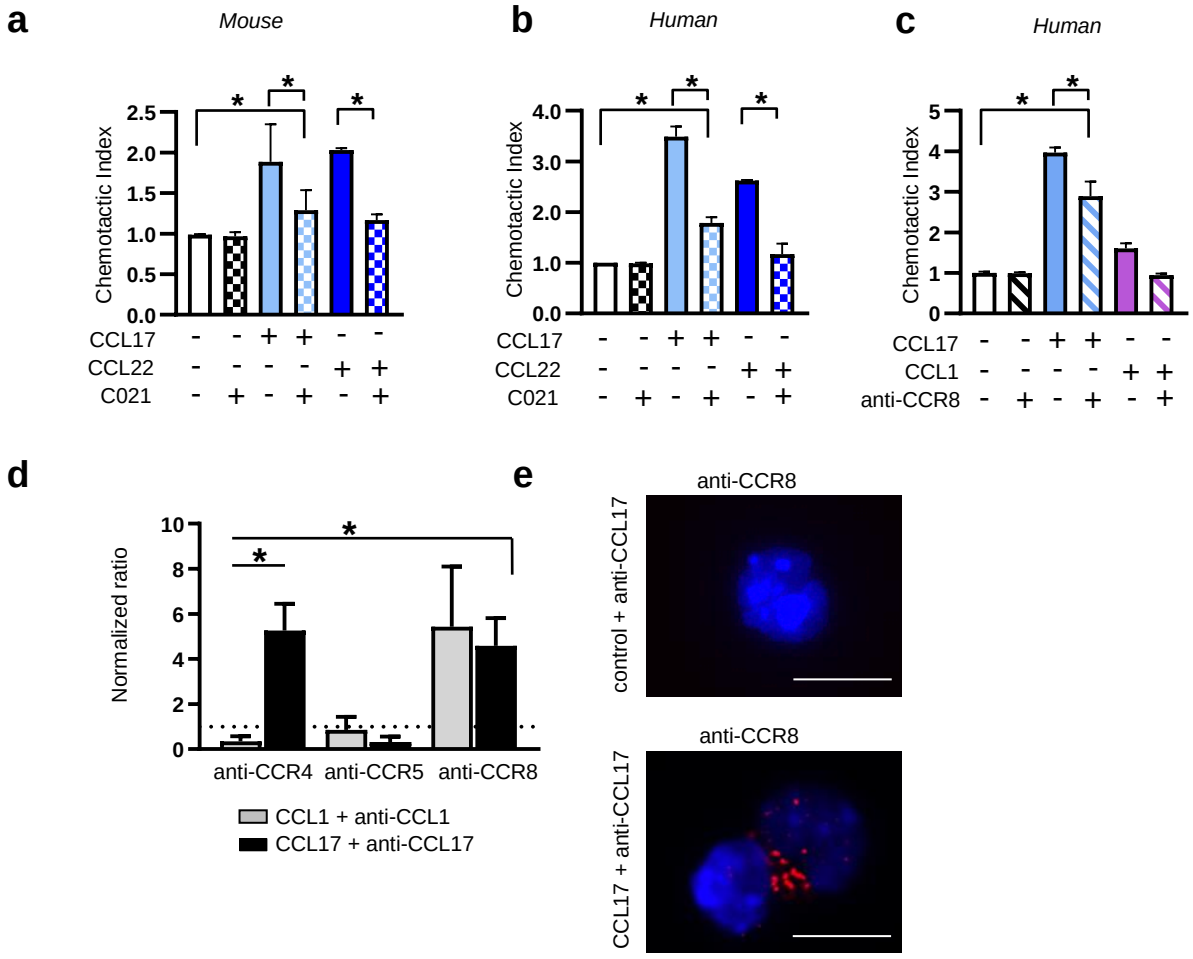


Supplemental Figure 1

Supplemental Figure 1. Effects of CCL17 and CCR4 deficiency on atherosclerosis and Treg numbers.

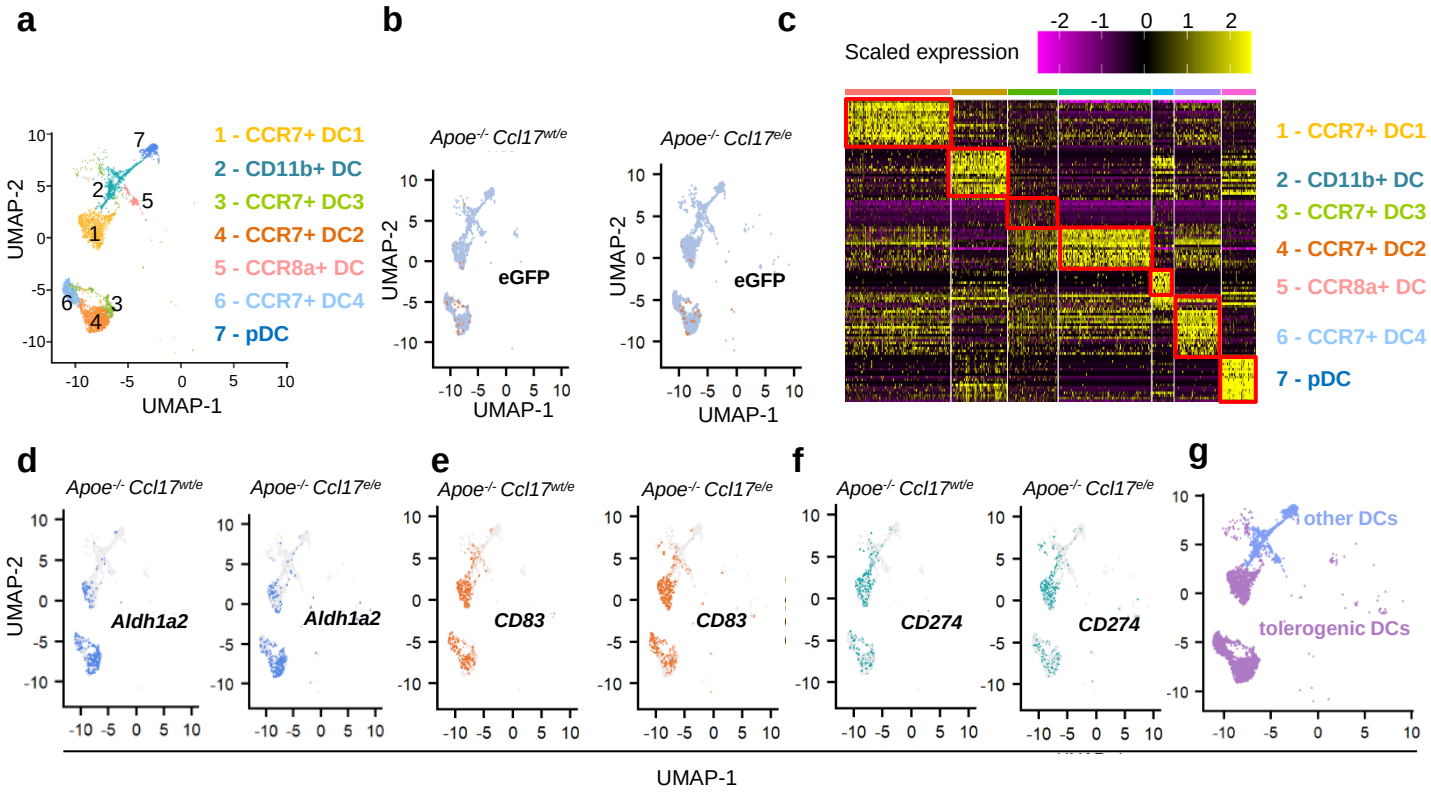
(a) Experimental scheme of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice fed a Western diet (WD) for 12 weeks. **(b)** Representative images and quantification of lesion area measured after Oil-Red-O staining for lipid deposits in the aortic root (n=13-14). Scale bar = 500 µm. **(c)** Quantification of lesion area measured after Oil-Red-O staining for lipid deposits in the thoraco-abdominal aorta (n=18-21); **(d)** Atherosclerotic lesion size in aortic arches, as quantified after H&E staining (n=12-14); **(e-i)** Representative dot plots and flow cytometric quantification of CD45^{+}CD3^{+}CD4^{+}CD25^{+}FoxP3^{+} Tregs in para-aortic LNs (**e**, n=9-11) and spleen (**f**; n=10-11); **(g)** Gating strategy for CD45^{+}CD3^{+} CD4^{+}CD25^{+}FoxP3^{+} Tregs in lymphatic organs; **(h-i)** Representative dot plots and flow cytometric quantification of CD45^{+}CD3^{+}CD4^{+}CD25^{+}FoxP3^{+} Tregs in blood (**h**; n=10-11), axilliary (**I**; n=10-11) and inguinal LNs (**j**; n=9-10). **(k)** Experimental scheme of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccr4*$^{-/-}$ mice fed WD for 12 weeks; **(l-n)** Flow cytometric quantification of CD45^{+}CD3^{+}CD4^{+}CD25^{+}FoxP3^{+}Tregs in blood (**L**; n=16-20), axilliary (**m**; n=14-18) and inguinal LNs (**n**; n=16-20) of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$ *Ccr4*$^{-/-}$ mice after 12 weeks WD. **(o)** Analysis of Annexin-V expression on Tregs (CD45^{+}CD3^{+}CD4^{+} CD25^{+}FoxP3^{+}) from isolated LNs (paraaortic, axillary and inguinal combined). **(a-o)** Data represent mean±SEM. **P*<0.05, ***P*<0.01 compared to *Apoe*$^{-/-}$, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.

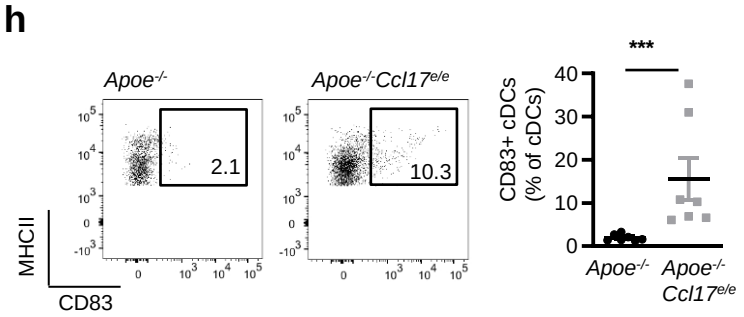


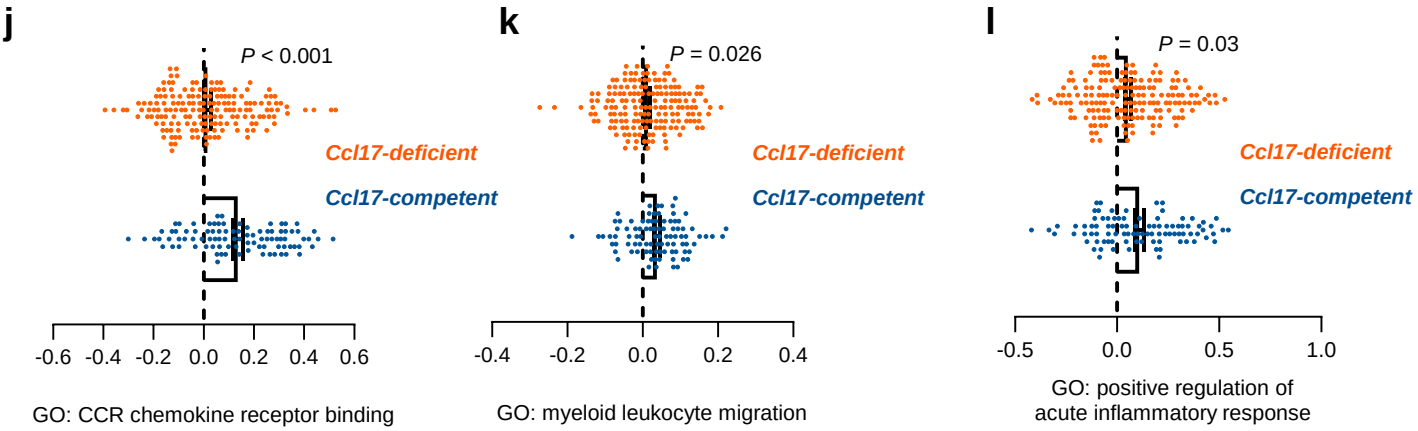
Supplemental Figure 2

Supplemental Figure 2. CCL17-induced migration via CCR4 or CCR8.

(a) Transwell migration assay with $CD4^+$ T cells (isolated from *Apoe*$^{-/-}$ mice) towards recombinant mouse CCL17 (100 ng/ml) or CCL22 (50 ng/ml) in the presence or absence of the CCR4 inhibitor C021 dihydrochloride (0.5 µM). **(b)** Transwell migration assay with $CD4^+$ T cells (isolated from human blood PBMCs) towards recombinant human CCL17 (100 ng/ml) or CCL22 (50 ng/ml), in the presence or absence of the CCR4 inhibitor C021 dihydrochloride (0.5 µM). Migrated cells were quantified by flow cytometry. **(c)** Transwell migration assay with $CD4^+$ T cells (isolated from human blood PBMCs) towards recombinant human CCL17 (100 ng/ml) or CCL1 (50 ng/ml) in the presence or absence of a blocking antibody to CCR8 (2 µg/ml). Migrated cells were quantified by flow cytometry. **(a-c)** The chemotactic index was calculated as the ratio of chemokine-stimulated and unstimulated migration (n=3-5; 4-5 replicates each); (**d, e**) Interactions between mouse CCL17 or CCL1 and CCR4, CCR5 or CCR8 were assessed on the surface of adherent conventional DCs isolated from LNs of *Apoe*$^{-/-}$ mice using the Duolink proximity ligation assay after incubation with recombinant mouse CCL17, CCL1 (100 ng/ml) or PBS vehicle (control) and respective antibodies to CCR4, CCR5 and CCR8, as indicated. Signals generated by interactions between ligands and receptors on the cDC surface were quantified and normalized to untreated controls (dotted line) (n=3). (**d**). Shown are representative images recorded with a Leica SP8 confocal microscope for anti-CCR8 and anti-CCL17 after PBS and CCL17 treatment (**e**, scale bar = 10 µm). **(a-d)** Data represent mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001; compared to control condition, as analyzed by Kruskal-Wallis with Dunn`s multiple comparison test.



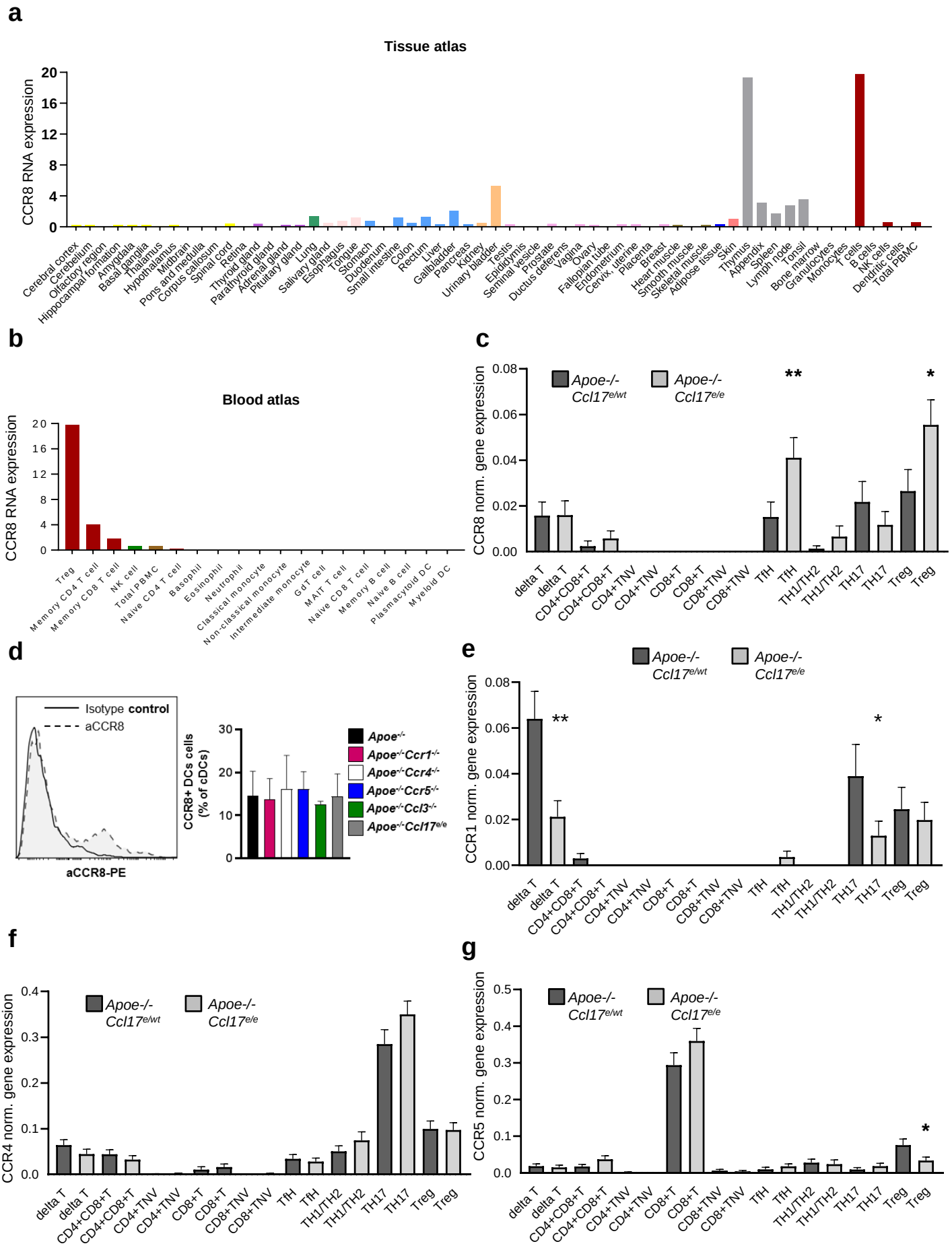




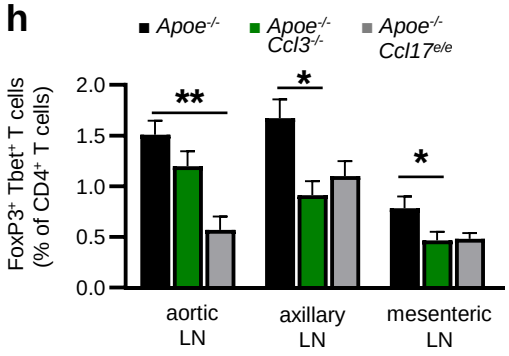
Supplemental Figure 3

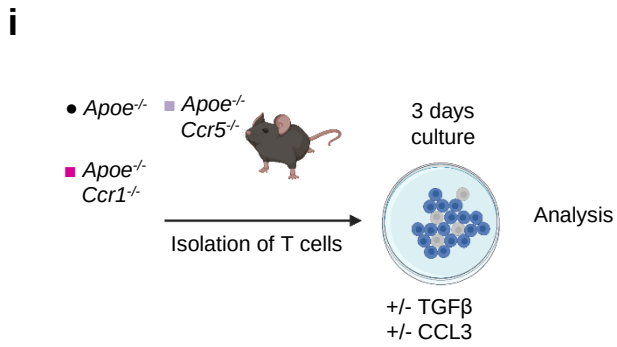
Supplemental Figure 3. Identification of tolerogenic DCs in the LNs of *Apoe*$^{-/-}$*Ccl17*$^{wt/e}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice.

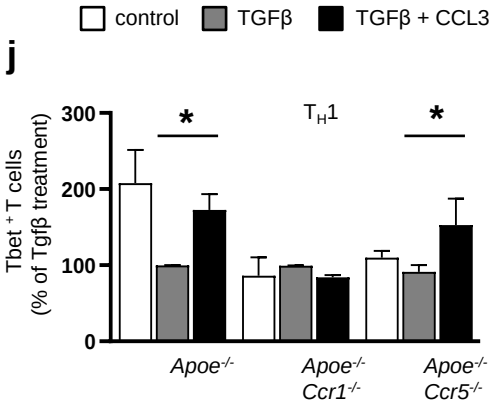
(a) UMAP projection of 4731 single cells, colored by inferred cell types, in sorted cells (viable CD45+CD3-CD11c+) from LNs of *Apoe*$^{-/-}$*Ccl17*$^{wt/e}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice. **(b)** UMAP visualization overlaid with the expression of eGFP *Apoe*$^{-/-}$*Ccl17*$^{wt/e}$ (left panels) or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ (right panel). **(c)** Heatmap of the top 20 marker genes from each cluster and cell type assignment of each cluster. **(d-f)** UMAP visualization overlaid with the expression of *Aldh1a2* (**d**), *CD83* (**e**) and *CD274* (**f**) in 7 distinct DC clusters of sorted cells (viable CD45+CD3-CD11c+) from LNs of *Apoe*$^{-/-}$*Ccl17*$^{wt/e}$ (left panel) or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ (right panel) mice (n=6-8). **(g)** UMAP projection of single cells, colored by inferred cell types including tolerogenic DCs and other DCs, in sorted cells (viable CD45+CD3-CD11c+) from LNs of *Apoe*$^{-/-}$*Ccl17*$^{wt/e}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice. **(h,i)** Representative dot plots and flow cytometric quantification of CD83^{+} (**h**) and CCR7^{+} (**i**) tolerogenic DCs among CD45^{+}CD11c^{+}MHCII^{+} cDCs in aortic LNs of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice (both n=6-7); Data represent mean±SEM. **P*<0.05, ****P*<0.001 compared to control, as analyzed Mann-Whitney test. **(j-l)** GSVA score was calculated in GO term CCR chemokine receptor binding **(j)**, myeloid leukocyte migration **(k)** and positive regulation of acute inflammatory response **(l)** in 172 eGFP-expressing CCL17-deficient cells from tolerogenic DCs of *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice or 92 CCL17-expressing cells from tolerogenic DCs of *Apoe*$^{-/-}$*Ccl17*$^{wt/e}$ mice fed on chow diet.



Supplemental Figure 4



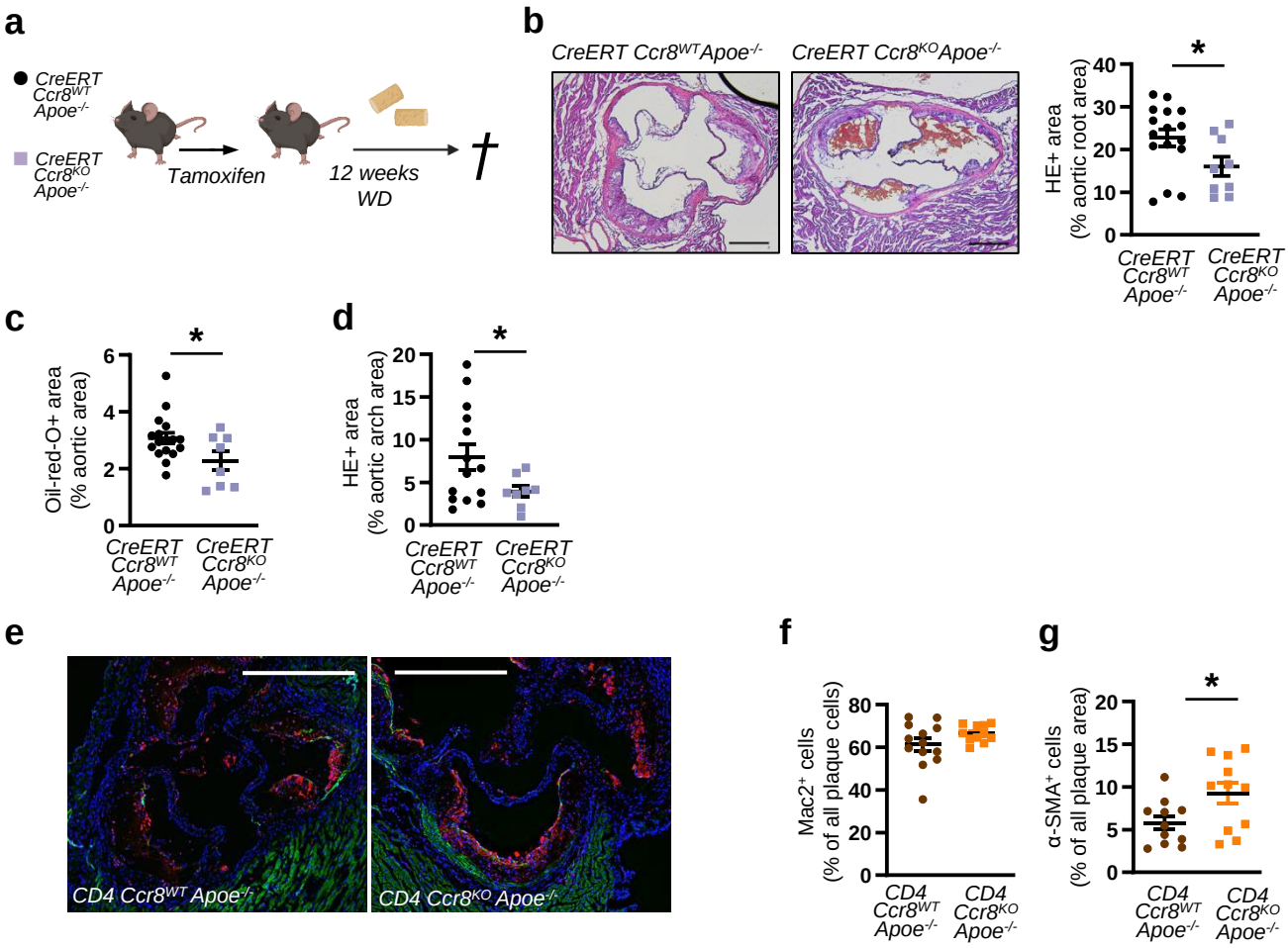




Supplemental Figure 4 continued

Supplemental Figure 4. CCR8 transcript expression across tissues and cell types and impact of CCL3 on in vitro differentiation of T helper cell subsets.

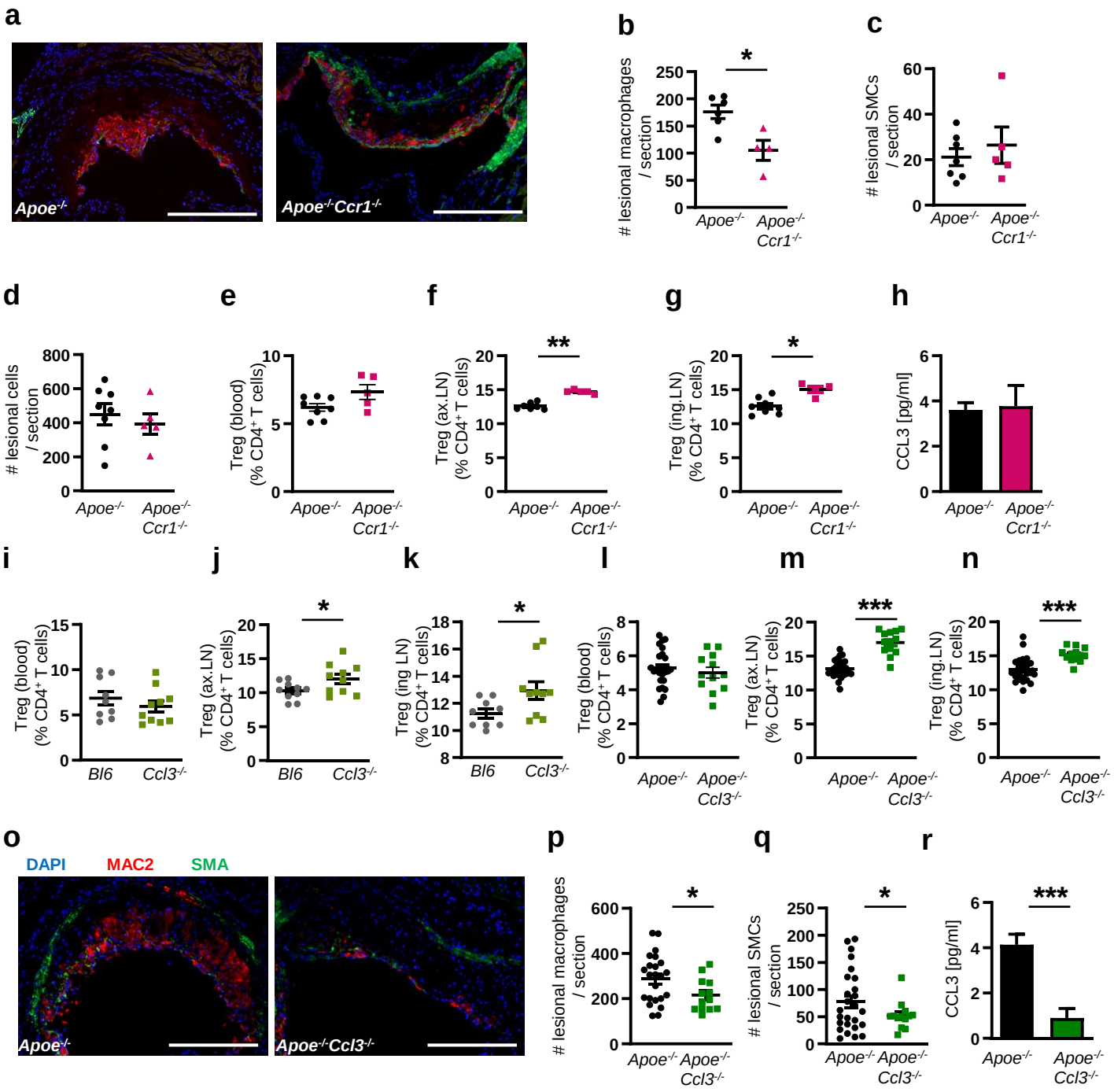
(a) *CCR8* mRNA expression in different tissues of consensus datasets from *The Human Protein Atlas*. Tissues of the same system (nervous system, endocrine system, digestion system, etc.) are depicted in the same color. **(b)** *CCR8* mRNA expression in different blood cell types of consensus datasets from *The Human Protein Atlas*. **(c)** *Ccr8* mRNA expression in different T-cell populations of the para-aortic LNs from *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ and *Apoe*$^{-/-}$*Ccl17*$^{e/wt}$ mice fed a WD for 6 weeks. **(d)** Flow cytometric analysis of CCR8 expression on CD45^{+}CD11c^{+}MHCII^{+} DCs in LN suspensions from *Apoe*$^{-/-}$, *Apoe*$^{-/-}$*Ccr1*$^{-/-}$, *Apoe*$^{-/-}$*Ccr4*$^{-/-}$, *Apoe*$^{-/-}$*Ccr5*$^{-/-}$, *Apoe*$^{-/-}$*Ccl3*$^{-/-}$ or *Apoe*$^{-/-}$ *Ccl17*$^{e/e}$ mice (n=3-4 per group); **(e-g)** *Ccr1* (**e**)*,Ccr4* (**f**) *and Ccr5* (**g**) mRNA expression in different T-cell populations of the para-aortic lymph nodes from *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ and *Apoe*$^{-/-}$*Ccl17*$^{e/wt}$ mice fed a WD for 6 weeks. **(h)** Flow cytometric quantification of CD45^{+}CD3^{+}CD4^{+}FoxP3^{+}Tbet^{+} cells in aortic, axillary and mesenteric LN of *Apoe*$^{-/-}$, *Apoe*$^{-/-}$*Ccl3*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice. **(i)** Experimental scheme where CD4^{+}CD62L^{+} T cells from spleens of *Apoe*$^{-/-}$, *Apoe*$^{-/-}$*Ccr1*$^{-/-}$or *Apoe*$^{-/-}$*Ccr5*$^{-/-}$ mice were isolated and cultured for 3 days under Treg polarizing conditions (TGFβ) in the presence or absence of recombinant mouse CCL3; **(j)** Quantification of CD4^{+}Tbet^{+} Th_1 cells (n=3), pre-gating CD45^{+}, using flow cytometry analysis. **(c-i)** Data represent mean±SEM. **P*<0.05, ***P*<0.01 compared to *Apoe*$^{-/-}$*Ccl17*$^{e/wt}$ as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate or compared to TGFβ, as analyzed by Kruskal-Wallis with Dunn's multiple comparisons test.



Supplemental Figure 5

Supplemental Figure 5. Effect of systemic and T cell-specific CCR8 deficiency on atherosclerosis.

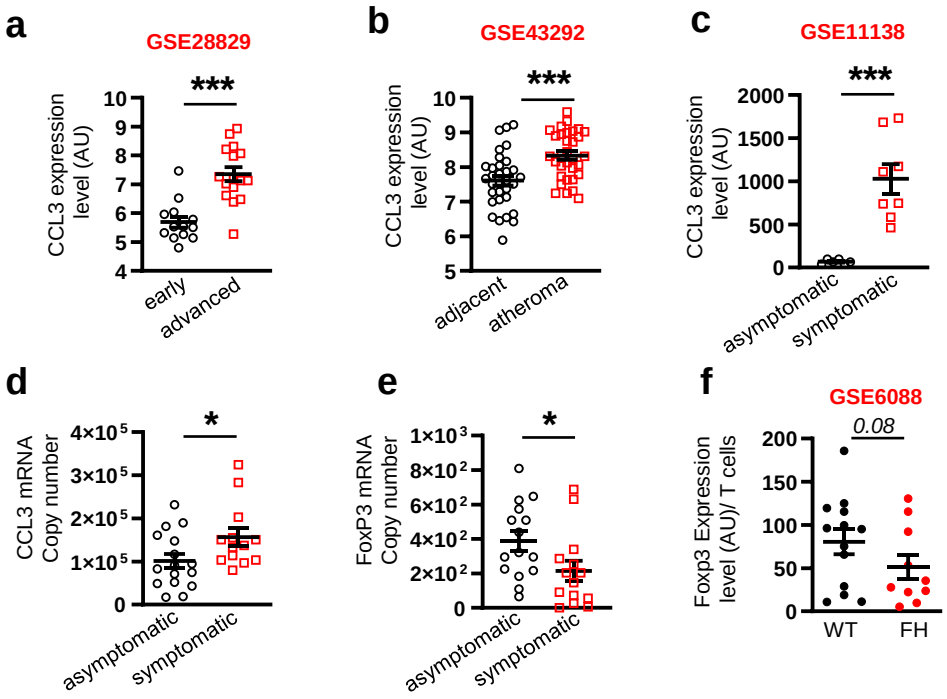
(a) Experimental scheme of *Apoe*$^{-/-}$ *Cre*$^{ERT-}$*Ccr8*^{WT}or *Apoe*$^{-/-}$ *Cre*$^{ERT+}$*Ccr8*^{KO} mice fed a Western diet (WD) for 12 weeks. **(b)** Representative images and quantification of lesion area measured after HE-staining in the aortic root (n=9-16). Scale bar = 500µm. **(c)** Quantification of lesion area measured after Oil-Red-O staining for lipid deposits in the thoraco-abdominal aorta (n=8-17). **(d)** Atherosclerotic lesion size in aortic arches, as quantified using H&E staining (n=9-17). **(e)** Representative images of staining for MAC2 (macrophages), SMA (smooth muscle a-actin) and DAPI (nuclei) staining in aortic root sections. Scale bar = 250µm. **(e-g)** Quantification of the percentage of lesional macrophages (**f**; n=11-13) and smooth muscle cells (**g**; n=11) of *Apoe*$^{-/-}$ *Cre*$^{ERT-}$ *Ccr8*^{WT}or *Apoe*$^{-/-}$ *Cre*$^{ERT+}$ *Ccr8*^{KO} mice after 12 weeks of WD. **(a-g)** Data represent mean±SEM. **P*<0.05 versus *Apoe*$^{-/-}$ *Ccr8*^{WT}, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.



Supplemental Figure 6

Supplemental Figure 6. Effect of CCR1 and CCL3 deficiency on lesional characteristics and Treg numbers.

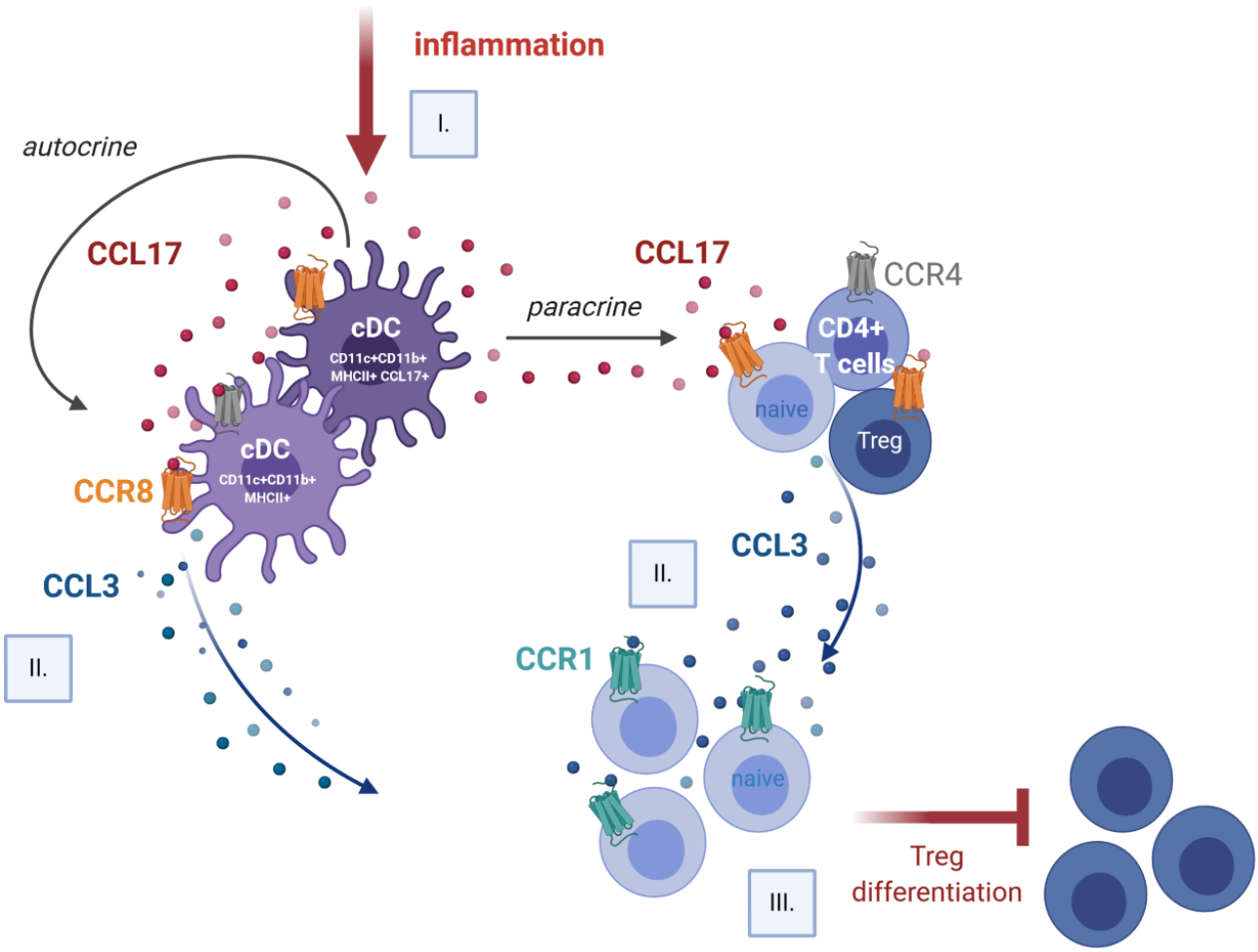
(a) Representative images of staining for Mac2 (macrophages), SMA (smooth muscle a-actin) and DAPI (nuclei) in aortic root sections of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccr1*$^{-/-}$ mice after 12 weeks of Western diet (WD). Scale bar = 250µm; **(b-d)** Quantification of the number of lesional macrophages (**b**; n=4-6), lesional smooth muscle cells (SMCs) (**c**; n=5-7) and total lesional cells (**d**; n=5-8) in *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccr1*$^{-/-}$ mice after 12 weeks WD; **(e-g)** Quantification of Tregs ($CD45^+CD3^+CD4^+CD25^+$ $FoxP3^+$) in blood (**e**; n=5-8), axillary (**f**; n=5-7) and inguinal LNs (**g**; n=4-8) of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccr1*$^{-/-}$ mice after 12 weeks of WD; **(h)** Plasma concentrations of CCL3 in *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccr1*$^{-/-}$ mice after 12 weeks of WD, as measured by ELISA (n=5-7); **(i-k)** Quantification of Tregs ($CD45^+CD3^+CD4^+CD25^+FoxP3^+$) in blood (**I**; n=9-10), axillary (**j**; n=10) and inguinal LNs (**k**; n=9-10) of *C57Bl6* or *Ccl3*$^{-/-}$ mice; **(l-n)** Quantification of Tregs ($CD45^+CD3^+CD4^+$ $CD25^+FoxP3^+$) in blood (**L**; n=12-25), axillary (M n=13-28) and inguinal LNs (**n**; n=13-29) of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl3*$^{-/-}$ mice, after 12 weeks of WD; **(o)** Representative images of staining for MAC2 (macrophages), SMA (SMCs) and DAPI (nuclei) in aortic root sections of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl3*$^{-/-}$ mice after 12 weeks of WD. Scale bar = 250µm. **(p-r)** Quantification of the number of lesional macrophages (**p**; n=13-23), lesional SMCs (**q**; n=13-26) and total lesional cells (**r**; n=13-28) in aortic root sections of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl3*$^{-/-}$ mice after 12 weeks of WD. **(a-r)** Data represent mean±SEM. **P*<0.05; ***P*<0.01; ****P*<0.001 compared to *Apoe*$^{-/-}$ or Bl6, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.



Supplemental Figure 7

Supplemental Figure 7. CCL3 and FOXP3 mRNA expression in human plaques.

(a) mRNA expression of CCL3 in advanced atherosclerotic plaques or early lesions derived from GSE28829 dataset (n=13-16); **(b)** mRNA expression of CCL3 in human atheroma plaque (atheroma) or paired distant macroscopically intact tissue (adjacent) derived from GSE43292 dataset (n=32); **(c)** CCL3 mRNA expression in symptomatic or asymptomatic patients with carotid and coronary plaques derived from GSE11138 dataset (n=6-8); **(d, e)** Quantification of CCL3 **(d)** and FoxP3 **(e)** mRNA copy numbers normalized to housekeeping mRNA (10^5 GAPDH or b-actin mRNA copies, respectively) in atherosclerotic lesions of carotid atherectomy specimens from symptomatic or asymptomatic patients using real-time PCR (n=13-16); **(f)** FoxP3 mRNA expression of freshly isolated T cells from hetero- or homozygous patients with familial hypercholesterolemia (FH) derived from GSE6088 (n=10-13). **(a-f)** Data represent mean±SEM. **P*<0.05; ***P*<0.01; ****P*<0.001 versus corresponding controls, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.



Supplemental Figure 8

Supplemental Figure 8. Synopsis of the proposed pathway.

(**I.**) Sterile inflammation triggers the activation of a subset of cDCs, which respond by releasing CCL17. (**II.**) In turn, CCL17 binds to CCR8 on cDCs (autocrine) and on $CD4^+$ T cells (paracrine) to stimulate an upregulation of CCL3 expression and release. (**III.**) Subsequently, CCL3 interacts with CCR1 on naïve T cells, thereby blocking the differentiation and expansion of Tregs.

Table S1

12 weeks WD	*Apoe*$^{-/-}$	*Apoe*$^{-/-}$ *Ccl17*$^{e/e}$	*P-value*
Leukocytes [x 10^6/ml]	2.6 ± 0.2	2.9 ± 0.3	0.397
Neutrophils [x 10^5/ml]	5.1 ± 0.4	7.2 ± 1.4	0.273
Classical Monocytes [x 10^4/ml]	6.5 ± 0.7	6.1± 0.7	0.426
Non-classical Monocytes [x 10^4/ml]	3.4 ± 0.5	3.7 ± 0.5	0.824
B cells [x 10^6/ml]	1.4 ± 0.1	1.4 ± 0.2	0.756
T cells [x 10^5/ml]	1.9 ± 0.2	1.9 ± 0.1	0.809
Thrombocytes [x 10^3/µl]	1162 ± 136	965 ± 123	0.223
Cholesterol [mg/dL]	773 ± 49	743 ± 44	0.646
Triglycerides [mg/dL]	154 ± 20	130 ± 10	0.318
Body Weight [g]	27.0 ± 1.2	26.9 ± 1.3	0.956

Table S1. Shown are total leukocyte counts, the percentage of neutrophils, classical and non-classical monocytes, B cells, T cells and thrombocytes in peripheral blood, plasma cholesterol and triglycerides levels and body weight of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice after 12 weeks of WD (n=9-11). Data represent mean±SEM, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.

Table S2

12 weeks WD	***Apoe*$^{-/-}$**	***Apoe*$^{-/-}$ *Ccr4*$^{-/-}$**	***P-value***
Leukocytes [x 10^6/ml]	4.0 ± 0.4	3.8 ± 0.3	0.969
Neutrophils [x 10^5/ml]	9.1 ± 0.9	8.1 ± 0.9	0.448
Classical Monocytes [x 10^5/ml]	2.7 ± 0.4	2.4 ± 0.2	0.844
Non-classical Monocytes [x 10^5/ml]	1.4 ± 0.1	1.8 ± 0.3	0.189
B cells [x 10^6/ml]	1.6 ± 0.2	1.5 ± 0.1	0.596
T cells [x 10^5/ml]	4.0 ± 0.3	4.4 ± 0.5	0.994
Thrombocytes [x 10^3/µl]	781 ± 79	803 ± 42	0.811
Cholesterol [mg/dL]	1231 ± 92	1265 ± 76	0.765
Triglycerides [mg/dL]	228 ± 25	223 ± 22	0.884
Body Weight [g]	25.6 ± 1.3	28.4 ± 0.8	0.003**

Table S2. Shown are total leukocyte counts, the percentage of neutrophils, classical and non-classical monocytes, B cells, T cells and thrombocytes in peripheral blood, plasma cholesterol and triglycerides levels and body weight of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$ *Ccr4*$^{-/-}$ mice after 12 weeks of WD (n=16-20). Data represent mean±SEM, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.

Table S3

Gene symbole	marker	Gene symbole	marker	Gene symbol	marker
Oas3	immunogenic	Slco5a1	tolerogenic	Hmgcs1	tolerogenic
Isg20	immunogenic	Rasal2	tolerogenic	Rab30	tolerogenic
Nt5c3	immunogenic	Galnt12	tolerogenic	Rad50	tolerogenic
Ifit2	immunogenic	Tbc1d15	tolerogenic	Tnnt2	tolerogenic
Ddx58	immunogenic	Mmp7	tolerogenic	Cyp51	tolerogenic
Ifit1	immunogenic	Mical3	tolerogenic	Sc5d	tolerogenic
Irf7	immunogenic	Rubcn	tolerogenic	Phka1	tolerogenic
Oasl2	immunogenic	Sdhaf1	tolerogenic	Cln5	tolerogenic
Pml	immunogenic	Mmp23	tolerogenic	Zfp180	tolerogenic
Slfn5	immunogenic	Hlx	tolerogenic	Ak2	tolerogenic
Eif2ak2	immunogenic	Nuak1	tolerogenic	Mylip	tolerogenic
Dhx58	immunogenic	Eno3	tolerogenic	Vcam1	tolerogenic
Oas1a	immunogenic	Pik3r3	tolerogenic	Slc7a6	tolerogenic
Rtp4	immunogenic	Josd1	tolerogenic	Rcor3	tolerogenic
Parp12	immunogenic	Vhl	tolerogenic	Zfp568	tolerogenic
Xaf1	immunogenic	Hecw2	tolerogenic	Ttc39a	tolerogenic
B4galt5	immunogenic	Kcnn1	tolerogenic	Mvd	tolerogenic
Ifi44	immunogenic	Chka	tolerogenic	Fbxl3	tolerogenic
Ifi204	immunogenic	Scube3	tolerogenic	Glt28d2	tolerogenic
Stat2	immunogenic	Zfp36l1	tolerogenic	Sox4	tolerogenic
Lgals9	immunogenic	Ift57	tolerogenic	Pyroxd1	tolerogenic
Lgals3bp	immunogenic	Etfdh	tolerogenic	Nup62	tolerogenic
Dtx3l	immunogenic	Appl1	tolerogenic	Kdm5b	tolerogenic
Zbp1	immunogenic	Dnajc22	tolerogenic	Inca1	tolerogenic
Usp18	immunogenic	Csnk1g1	tolerogenic	Prdx3	tolerogenic
Irf9	immunogenic	Cables2	tolerogenic	Mkks	tolerogenic
Ifi203	immunogenic	Chst2	tolerogenic	Vrk2	tolerogenic
Ppm1k	immunogenic	Tpm1	tolerogenic	Mios	tolerogenic
Trim25	immunogenic	Lzts2	tolerogenic	Ppcs	tolerogenic
Ifi47	immunogenic	Mpp5	tolerogenic	Cd2bp2	tolerogenic
Sp100	immunogenic	Ace2	tolerogenic	Rb1cc1	tolerogenic
		Sqle	tolerogenic	Mcemp1	tolerogenic
		Cdcp1	tolerogenic	Psmd1	tolerogenic
		Tsc1	tolerogenic		
		Fut4	tolerogenic		
		Creld1	tolerogenic		
		Kif13b	tolerogenic		
		Acp2	tolerogenic		
		Wdr91	tolerogenic		
		Nostrin	tolerogenic		
		Spats2	tolerogenic		
		B4galnt1	tolerogenic		
		Tmem8	tolerogenic		
		Gfpt1	tolerogenic		
		St8sia6	tolerogenic		
		Ccdc120	tolerogenic		
		Elk3	tolerogenic		
		Arfrp1	tolerogenic		
		Stxbp2	tolerogenic		

Table S3. Overview of immunogenic versus tolerogenic gene panel (based on ref.7 method section). 82 specifically upregulated genes were listed as tolerogenic genes, and 31 Interferon-stimulated genes (ISGs) were listed as immunogenic. The tolerogenic score in our LN DCs was calculated using the top 20 genes that distinguished tolerogenic DCs and immunogenic DCs as follows: tolerogenic score = [1 + mean (top 20 upregulated tolerogenic genes)]/[1 + mean (top 20 upregulated immunogenic genes)].

Table S4

Cytokine/ Chemokine	*Apoe*$^{-/-}$ *pg/ml*	Ccl17$^{eGFP/eGFP}$ Apoe$^{-/-}$ *pg/ml*	P-value
IL-1β	*b.t.*	*b.t.*	-
IL-2	*b.t.*	*b.t.*	-
IL-4	0.03 ± 0.03	0.89 ± 0.33	***0.0370**
IL-5	5.71 ± 1.13	9.92 ± 1.59	0.0531
IL-6	2.45 ± 1.23	8.19 ± 2.89	0.1011
IL-9	4.08 ± 1.28	3.34 ± 0.20	0.5972
IL-10	*b.t.*	*b.t.*	-
IL-12p70	0.13 ± 0.07	0.28 ± 0.09	0.1947
IL-13	1.86 ± 0.43	2.26 ± 0.92	0.9572
IL-17A	0.33 ± 0.31	0.21 ± 0.21	0.7600
IL-22	169 ± 94.4	37.6 ± 8.3	0.2239
IL-23	17.4 ± 11.1	22.1 ± 11.6	0.7761
IL-27	24.7 ± 15.5	21.7 ± 3.1	0.8572
CCL1#	3.51 ± 0.58	3.03 ± 0.53	0.8212
CCL2	64.9 ± 13.5	50.6 ± 3.3	0.3506
CCL3	6.47 ± 0.63	4.80 ± 0.41	***0.0406**
CCL4	2.91 ± 0.48	3.41 ± 0.25	0.3812
CCL5	21.3 ± 2.1	22.6 ± 2.4	0.6862
CCL7	149 ± 33.2	124 ± 14.0	0.4991
CCL11	113 ± 71.7	307 ± 110	0.1668
CXCL1	18.8 ± 3.8	29.9 ± 2.8	0.0901
CXCL2	10.4 ± 1.26	12.2 ± 0.6	0.2302
CXCL10	24.8 ± 1.81	26.6 ± 1.9	0.5084
TNFα	1.26 ± 0.64	1.59 ± 0.39	0.6766
GM-CSF	*b.t.*	*b.t.*	-
IFNy	*b.t.*	*b.t.*	-

Table S4. Shown are cytokine and chemokine concentrations (pg/ml) measured by multiplex bead array in plasma samples from *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice after 12 weeks of WD (n=9-11); *b.t.* = below threshold, #CCL1 was not included in the multiplex bead array and therefore measured by ELISA. Data represent mean±SEM, **P*<0.05 versus corresponding controls as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.

Table S5

4 weeks WD	*Apoe*$^{-/-}$ *isotype*	*Apoe*$^{-/-}$ *anti-CCR8*	*P-value*
Leukocytes [x 10^6/ml]	2.6 ± 0.4	2.4 ± 0.4	0.703
Neutrophils [x 10^5/ml]	7.9 ± 1.3	7.8 ± 1.6	0.969
Classical Monocytes [x 10^5/ml]	1.4 ± 0.2	1.5 ± 0.4	0.784
Non-classical Monocytes [x 10^4/ml]	9.8 ± 2.6	7.2 ± 1.4	0.396
B cells [x 10^6/ml]	1.1 ± 0.2	1.0 ± 0.1	0.731
T cells [x 10^5/ml]	2.9 ± 0.8	2.0 ± 0.2	0.965
Thrombocytes [x 10^3/µl]	1048 ± 77	882 ± 77	0.149
Cholesterol [mg/dL]	1041 ± 97	1061 ± 85	0.877
Triglycerides [mg/dL]	117 ± 17	107 ± 15	0.666
Body Weight [g]	33.2 ± 0.6	33.6 ± 0.5	0.641

Table S5. Shown are total leukocytes counts, the percentage of neutrophils, classical and non-classical monocytes, B cells, T cells and thrombocytes in peripheral blood, plasma cholesterol and triglycerides levels and body weight of *Apoe*$^{-/-}$ mice injected with or without anti-CCR8 antibody after 4 weeks of WD (n=8-10). Data represent mean±SEM, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.

Table S6

12 weeks WD	*Apoe*$^{-/-}$ *CD4*$^{Cre-}$*Ccr8*$^{fl/fl}$	*Apoe*$^{-/-}$ *CD4*$^{Cre+}$*Ccr8*$^{fl/fl}$	*P-value*
Leukocytes [x 10^6/ml]	2.0 ± 0.3	3.1 ± 0.7	0.375
Neutrophils [x 10^5/ml]	5.7 ± 0.6	6.8 ± 1.0	0.355
Classical Monocytes [x 10^5/ml]	2.1 ± 0.5	3.0 ± 0.6	0.265
Non-classical Monocytes [x 10^5/ml]	1.8 ± 0.6	2.8 ± 0.8	0.308
B cells [x 10^6/ml]	0.5 ± 0.1	1.1 ± 0.3	0.458
T cells [x 10^5/ml]	0.9 ± 0.2	1.6 ± 0.4	0.185
Thrombocytes [x 10^3/µl]	1326 ± 203	950 ± 231	0.241
Cholesterol [mg/dL]	1659 ± 205	1201 ± 101	0.098
Triglycerides [mg/dL]	270 ± 20	314 ± 14	0.089
Body Weight [g]	31.4 ± 1.4	28.5 ± 1.3	0.133

Table S6. Shown are total leukocytes counts, the percentage of neutrophils, classical and non-classical monocytes, B cells, T cells and thrombocytes in peripheral blood, plasma cholesterol and triglycerides levels and body weight of *Apoe*$^{-/-}$ *CD4Cre-Ccr8*$^{fl/fl}$ or *Apoe*$^{-/-}$ *CD4Cre+Ccr8*$^{fl/fl}$ mice after 12 weeks of WD (n=12-14). Data represent mean±SEM, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.

Table S7

12 weeks WD	*Apoe*$^{-/-}$	*Apoe*$^{-/-}$ *Ccr1*$^{-/-}$	*P-value*
Leukocytes [x 10^6/ml]	1.7 ± 0.1	2.0 ± 0.5	0.524
Neutrophils [x 10^5/ml]	4.9 ± 1.1	7.3 ± 1.9	0.435
Classical Monocytes [x 10^5/ml]	1.1 ± 0.1	1.3 ± 0.3	0.354
Non-classical Monocytes [x 10^4/ml]	4.7 ± 0.7	2.9 ± 1.3	0.171
B cells [x 10^5/ml]	6.1 ± 0.8	6.9 ± 1.9	0.833
T cells [x 10^4/ml]	8.4 ± 1.3	6.6 ± 2.7	0.435
Thrombocytes [x 10^3/µl]	1314 ± 121	1026 ± 45	0.202
Cholesterol [mg/dL]	1126 ± 165	1136 ± 295	0.974
Triglycerides [mg/dL]	230 ± 42	277 ± 54	0.622
Body Weight [g]	34.3 ± 0.8	34.2 ± 0.9	0.999

Table S7. Shown are total leukocytes counts, the percentage of neutrophils, classical and non-classical monocytes, B cells, T cells and thrombocytes in peripheral blood, plasma cholesterol and triglycerides levels and body weight of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccr1*$^{-/-}$ mice after 12 weeks of WD (n=5-9). Data represent mean±SEM, as analyzed by Mann-Whitney test.

Table S8

12 weeks WD	*Apoe*$^{-/-}$	*Apoe*$^{-/-}$*Ccl3*$^{-/-}$	*P-value*
Leukocytes [x 10^6/ml]	1.5 ± 0.1	1.9 ± 0.3	0.225
Neutrophils [x 10^5/ml]	4.0 ± 0.5	3.9 ± 0.6	0.982
Classical Monocytes [x 10^5/ml]	1.0 ± 0.1	1.2 ± 0.2	0.250
Non-classical Monocytes [x 10^4/ml]	3.8 ± 0.5	6.0 ± 1.1	0.094
B cells [x 10^5/ml]	6.4 ± 0.9	8.0 ± 1.2	0.305
T cells [x 10^5/ml]	1.4 ± 0.1	1.4 ± 0.2	0.939
Thrombocytes [x 10^3/µl]	1078 ± 108	963 ± 56	0.365
Cholesterol [mg/dL]	717 ± 68	704 ± 120	0.930
Triglycerides [mg/dL]	220 ± 12	150 ± 10	0.001***
Body Weight [g]	27.5 ± 0.6	26.1 ± 0.4	0.062

Table S8. Shown are total leukocytes counts, the percentage of neutrophils, classical and non-classical monocytes, B cells, T cells and thrombocytes in peripheral blood, plasma cholesterol and triglycerides levels and body weight of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl3*$^{-/-}$ mice after 12 weeks of WD (n=7-10). Data represent mean±SEM, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.

Table S9

4 weeks WD	***Apoe*$^{-/-}$**	***Apoe*$^{-/-}$ *Ccl17*$^{e/e}$**	***Apoe*$^{-/-}$ *Ccl17*$^{e/e}$ + *CCL3***	***P-value Apoe*$^{-/-}$ *vs. Apoe*$^{-/-}$ *Ccl17*$^{e/e}$**	***P-value Apoe*$^{-/-}$ *vs. Apoe*$^{-/-}$ *Ccl17*$^{e/e}$ + *CCL3***
Leukocytes [x 10^6/ml]	4.0 ± 0.4	5.4 ± 0.4	4.7 ± 0.5	0.075	0.512
Neutrophils [x 10^6/ml]	1.1 ± 0.1	1.3 ± 0.2	1.6 ± 0.5	0.999	0.999
Classical Monocytes [x 10^5/ml]	1.7 ± 0.2	2.1 ± 0.3	1.9 ± 0.2	0.321	0.762
Non-classical Monocytes [x 10^5/ml]	0.8 ± 0.1	1.2 ± 0.2	1.0 ± 0.2	0.3944	0.797
B cells [x 10^6/ml]	1.9 ± 0.2	2.4 ± 0.2	1.8 ± 0.3	0.356	0.943
T cells [x 10^5/ml]	5.1 ± 0.4	8.5 ± 0.7	7.3 ± 0.7	0.002***	0.054
Thrombocytes [x 10^3/µl]	764 ± 76	774 ± 79	819 ± 101	0.996	0.894
Cholesterol [mg/dL]	1101 ± 36	1031 ± 69	984 ± 32	0.584	0.272
Triglycerides [mg/dL]	276 ± 39	206 ± 17	171 ± 24	0.227	0.700
Body Weight [g]	26.2 ± 1.4	23.4 ± 1.4	23.7 ± 1.5	0.151	0.408

Table S9. Shown are total leukocytes counts, the percentage of neutrophils, classical and non-classical monocytes, B cells, T cells and thrombocytes in peripheral blood, plasma cholesterol and triglycerides levels and body weight of *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$*Ccl17*$^{e/e}$ mice fed a WD for 4 weeks and injected with or without recombinant mouse CCL3 3x weekly after 4 weeks of WD (n=8-10). Data represent mean±SEM, as analyzed by Student's t-test with Welsh correction or Mann-Whitney test, as appropriate.

Supplemental Materials and Methods

Major Resources Table

Antibody/Peptide/Antagonist	Vendor or Source	Concentration used
Primary antibodies for Immunohistochemistry		
Anti-Mac2	Cedarline	2.5 µg/ml
Anti-Smooth muscle actin (SMA)	Sigma	10 µg/ml
Anti-CCL17/TARC	R&D systems	4 µg/ml
Anti-CCL1	Acris	62 µg/ml
Anti-CCR4	Thermo Fisher	10 µg/ml
Anti-CCR5	Santa Cruz Biotechnology	4 µg/ml
Anti-CCR8	Abcam	20 µg/ml
Secondary antibodies for Immunohistochemistry		
Anti-Rabbit IgG	Dianova	10 µg/ml
Anti-Rat IgG	Sigma-Aldrich	2.5 µg/ml
Primary antibodies for Flow cytometry		
Anti-Mouse CD45	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CD11b	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CD115	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse GR1	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse B220	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CD3	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CD4	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CD8	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CD25	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse FoxP3	Thermo Fisher Scientific	2 µg/50 µl
Anti-Mouse CD11c	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse MHC-II	BD Biosciences	1 µg/50 µl
Anti-Mouse Tbet	Thermo Fisher Scientific	2 µg/50 µl
Anti-Mouse Gata3	Thermo Fisher Scientific	2 µg/50 µl
Anti-Mouse Rorγt	Thermo Fisher Scientific	2 µg/50 µl
Anti-Mouse CD83	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CCR7	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse IDO	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CD274	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CD86	Thermo Fisher Scientific	1 µg/50 µl
Anti-Mouse CCR8	Biolegend	1 µg/50 µl
Rat IgG2b kappa Isotype control	Biolegend	1 µg/50 µl
Anti-Human CD4	Biolegend	0.5 µg/50 µl
Anti-Human CCR4	Biolegend	0.5 µg/50 µl

Anti-Human CCR8	Biolegend	0.5 µg/50 µl
Primary antibodies for Cell culture		
Anti-CD3	Thermo Fisher Scientific	5 µg/ml
Anti-CD28	Thermo Fisher Scientific	1 µg/ml
Anti-CCR8	R&D Systems	2 µg/mL
Primary antibodies for Proximity ligation assay on Jurkat cells		
Anti-CCL1	R&D Systems	2 µg/mL
Anti-CCL17	Thermo Fisher Scientific	2 µg/mL
Anti-CCR4	Thermo Fisher Scientific/Biolegend	8 µg/mL
Anti-CCR8	Thermo Fisher Scientific	8 µg/mL
Primary antibodies for *in vivo* application		
Anti-CCR8	Biolegend	5 µg
Rat IgG2b kappa Isotype control	Biolegend	5 µg
Recombinant peptides		
Murine CCL17 (TARC)	Biolegend	100 ng/ml
Murine TGF-β	Biolegend	5 ng/ml
Murine CCL3	Peprotech	100 ng/ml (*in vitro*)
Murine CCL3	Peprotech	20 µg (*in vivo*)
Murine CCL1	Peprotech	50 ng/ml or 100 ng/ml
Murine CCL20	Peprotech	100 ng/ml
Murine CCL22	Peprotech	50 ng/mL
Human CCR8 proteoliposome	Abnova	62.5-2000 ng/ml
Human CCL17 (TARC)	Biolegend	100 ng/ml
Human CCL17-AF467 (TARC)	Self-made	100 ng/ml
Human CCL1	Peprotech	50 ng/mL
Human CCL1-AF467	ALMAC	20 nM
Human CCL22	Peprotech	50 ng/mL
Antagonist		
C 021 Dihydrochloride	Tocris	0.5 µM
Cell isolation kit		
$CD4^+$ T cells	Miltenyi Biotec	
$CD4^+CD62^+$ T cells	Miltenyi Biotec	
$CD4^+CD25^+$ T cells	Miltenyi Biotec	
Monocytes	Miltenyi Biotec	
Neutrophils	Miltenyi Biotec	
Untouched™ Human CD4 T Cells	Dynabeads™ Invitrogen	
Multiplex-bead-array and Duolink® Kits		
Cytokine/Chemokine 26-Plex Mouse ProcartaPlex™ Panel 1	Thermo Fisher Scientific	

Duolink® In Situ Red Kit Goat/Rabbit	Sigma-Aldrich	
Duolink® In Situ Probe anti-Goat/Rabbit/Mouse Kit, Duolink® flowPLA Detection Kit - FarRed	Sigma-Aldrich	

Mice

Ccr4$^{-/-}$ mice[1] were kindly provided by K. Pfeffer (Heinrich-Heine-Universität, Düsseldorf, Germany) and *Ccl17*$^{e/e}$ (GFP reporter knock-in) mice[2] were kindly provided by I. Förster (Universität Bonn, Germany). *Ccl3*$^{-/-}$ mice were purchased from the Jackson Laboratories (Bar Harbor, USA). *Ccr1*$^{-/-}$ mice and *Ccr5*$^{-/-}$ mice kindly provided by P.M. Murphy and W.A. Kuziel, respectively, have been previously characterized.[3,4] *Ccr4*$^{-/-}$, *Ccr1*$^{-/-}$, *Ccr5*$^{-/-}$, *Ccl17*$^{e/e}$ and *Ccl3*$^{-/-}$ mice were crossed with *Apoe*$^{-/-}$ mice purchased from the Jackson Laboratories. All strains were backcrossed for at least 10 generations to the C57Bl/6 background. All animals were housed in the local animal facility under specific pathogen-free conditions. Depending on the type of study, mice were either fed a normal chow diet (steady state) or a Western Type diet (WD; for atherosclerosis studies) containing 21% fat and 0.15-0.2% cholesterol (Altromin 132010, Sniff TD88137) starting at 8-10 weeks of age for 4 or 12 weeks before sacrifice. For the rescue experiment using CCL3 injections, mice were injected 3x weekly with 20 µg recombinant mouse CCL3 or PBS control by intraperitoneal injection. For the experiment using anti-CCR8 blocking antibody, mice were injected 3x weekly with 5 µg anti-CCR8 antibody or isotype control by intraperitoneal injection. For single cell RNA sequencing (scRNA-seq), male *Ccl17*$^{wt/e}$*Apoe*$^{-/-}$, and *Ccl17*$^{e/e}$*Apoe*$^{-/-}$ mice were fed a normal chow diet or 6 weeks of WD. All experimental mice were sex- and age-matched. All experiments were approved by local authorities and complied with German animal protection law (Regierung von Oberbayern, Germany). Every effort was made to minimize suffering.

Histology and immunofluorescence

Atherosclerotic lesion size was assessed by analyzing cryosections of the aortic root by staining for lipid depositions with Oil-Red-O. In brief, hearts with the aortic root were embedded in Tissue-Tek O.C.T. compound (Sakura) for cryosectioning. Oil-Red-O^{+} atherosclerotic lesions were quantified in 4 µm transverse sections and averages were calculated from 3 sections. The thoraco-abdominal aorta was fixed with 4% paraformaldehyde and opened longitudinally, mounted on glass slides and stained enface with Oil-Red-O.Aortic arches with the main branch points (brachiocephalic artery, left subclavian artery and left common carotid artery) were fixed with 4% paraformaldehyde and embedded in paraffin. Lesion size was quantified after Hematoxylin and Eosin (H&E)-staining of 4 µm transverse sections and averages were calculated from 3-4 sections.For analysis of the cellular composition or inflammation of atherosclerotic lesions, aortic root sections were stained with an antibody to Mac2 (Cedarline) and SMA (Dako). Nuclei were counter-stained by 4',6-Diamidino-2-phenylindol (DAPI). After incubation with a secondary FITC- or Cy3-conjugated antibody (Life Technologies) for 30 minutes at room temperature, sections were embedded with VectaShield Hard Set Mounting Medium (Vector laboratories) and analyzed using a Leica DM4000B LED fluorescence microscope and charge-coupled device (CCD) camera. Blinded image analysis was performed using Diskus, Leica Qwin Imaging (Leica Lt.) or Image J software. For each mouse and staining, 2-3 root sections were analyzed and the average was taken.

Laboratory blood parameters and Flow cytometry

Whole blood from the mice was collected in EDTA-buffered tubes. Thrombocyte counts were determined using a Celltac Automated Hematology Analyzer (Nihon Kohden). Afterwards, samples were subjected to red-blood-cell lysis for further analysis using flow cytometry. Spleen and lymph nodes (LNs) were mechanically crushed and passed through a 30 µm cell strainer (Cell-Trics, Partec) using Hank's Medium (Hanks' Balanced Salt Solution + 0.3 mmol/l EDTA + 0.1% BSA; Gibco by life technologies) to obtain single cell suspensions. Leukocyte subsets were analyzed using the following combination of surface markers: neutrophils ($CD45^+CD11b^+CD115^-Gr1^{high}$), classical monocytes ($CD45^+CD11b^+CD115^+GR1^{high}$), non-classical monocytes ($CD45^+CD11b^+CD115^+GR1^{low}$), B cells ($CD45^+B220^+$), T cells ($CD45^+CD3^+$). Regulatory T cells (Tregs) were classified as $CD45^+CD3^+CD4^+CD25^+Foxp3^+$ (gating strategy used to identify Treg throughout the manuscript is depicted in **Supplemental Figure 1G**) and its subpopulation as $CD45^+CD3^+CD4^+FoxP3^+Tbet^+$. Generally, Th1 cells were defined as $CD45^+CD3^+CD4^+Tbet^+$, Th2 cells as $CD45^+CD3^+CD4^+Gata3^+$, and Th17 cells as $CD45^+CD3^+CD4^+Ror\gamma t^+$. Foxp3/Tbet/Gata3/Rorγt transcription factors were stained using the Foxp3/Transcription Factor Staining Buffer Set (eBioscience). Annexin-V^+ cells were analyzed using the Dead Cell Apoptosis kit (Thermo Fisher Scientific). Cell populations and marker expression were analyzed using a FACSCanto-II, FACSDiva software (BD Biosciences) and the FlowJo analysis program (Treestar).

Plasma lipid levels

Cholesterol and triglyceride levels were analyzed using mouse EDTA-buffered plasma and quantified using enzymatic assays (c.f.a.s. cobas, Roche Diagnostics) according to the manufacturer's protocol.

Fluorescence-activated cell sorting and tolerogenic DC analysis

For the isolation of dendritic cells (DCs), LNs were mechanically crushed and passed through a 30 µm cell strainer (Cell-Trics, Partec) using Hank's Medium (Hanks' Balanced Salt Solution + 0.3 mmol/l EDTA + 0.1% BSA; Gibco by life technologies) to obtain single cell suspensions. Conventional DCs are isolated from this suspension by fluorescence-activated cell sorting (BD FACSAria), by gating for $CD45^+CD11c^+MHCII^+$ cells. Furthermore, $eGFP^+Ccl17^{wt/e}$ and $eGFP+Ccl17^{e/e}$ DCs were isolated by gating for the endogenous eGFP signal in the FITC channel (pre-gating: $CD45^+CD11c^+MHCII^+$). Flow cytometric analysis of tolerogenic DCs in aortic LNs was performed by gating pre-gating for $CD45^+CD11c^+MHCII^+$ followed by evaluation of CD83, CCR7, IDO, and CD274 on pre-gated cDCs.[5-8]

For the isolation of T and B cells, spleens were mechanically crushed and passed through a 30 µm cell strainer (Cell-Trics, Partec) using Hank's Medium (Hanks' Balanced Salt Solution + 0.3 mmol/l EDTA + 0.1% BSA; Gibco by life technologies) to obtain single cell suspensions. Cell subsets are isolated by fluorescence-activated cell sorting (BD FACSAria), by gating for $CD45^+CD3^+$ cells (T cells) or $CD45^+CD19^+$ cells (B cells). After cell sorting, all cells were cultured in 96 well round-bottom plates ($1x10^5$ cells/well) (Corning Costar by Sigma-Aldrich/Merck) in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM L-Glutamine and 1% Penicillin/Streptomycin (all Gibco by Life technologies), unless stated otherwise, and with/without specific stimuli as indicated for the individual experiments.

Immunomagnetic cell isolation

For the isolation of monocytes and neutrophils, bone marrow cells were harvested by flushing femurs with Hank's Medium (Hanks' Balanced Salt Solution + 0.3 mmol/l EDTA + 0.1% BSA) (Gibco by life technologies). Monocytes are isolated using the mouse Monocyte Isolation Kit and a LS separation column (all Miltenyi Biotec), according to the manufacturer's protocol. Neutrophils are isolated using the mouse Neutrophil Isolation Kit and a LS separation column (all Miltenyi Biotec), according to the manufacturer's protocol. After isolation, all cells were cultured in 96 well round-bottom plates ($1x10^5$ cells/well) (Corning Costar by Sigma-Aldrich/Merck) in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, 2mM L-Glutamine and 1% Penicillin/Streptomycin (All Gibco by Life technologies) unless stated otherwise, with/without specific stimuli as indicated for the individual experiments.

For the isolation of $CD4^+$, $CD4^+CD62L^+$ and $CD4^+CD25^+$ T cells, spleens were mechanically crushed and passed through a 30 µm cell strainer (Cell-Trics, Partec) using Hank's Medium (Hanks' Balanced Salt Solution + 0.3 mmol/l EDTA + 0.1% BSA; Gibco by life technologies) to obtain single cell suspensions. $CD4^+$ T cells are subsequently isolated using the mouse $CD4^+$ T cell Isolation Kit and a LS separation column (all Miltenyi Biotec), according to the manufacturer's protocol. $CD4^+CD62L^+$ T cells are subsequently isolated using the mouse $CD4^+CD62L^+$ T cell Isolation Kit II and a LC separation column (all Miltenyi Biotec), according to the manufacturer's protocol. $CD4^+CD25^+$ T cells are subsequently isolated using the mouse $CD4^+CD25^+$ T cell Isolation Kit and a LS separation column (all Miltenyi Biotec), according to the manufacturer's protocol. After isolation, cells were cultured in 96 well round-bottom plates ($1x10^5$ cells/well) (Corning Costar by Sigma-Aldrich/Merck) in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM L-Glutamine and 1% Penicillin/Streptomycin (all Gibco by Life technologies), unless stated otherwise, and with/without specific stimuli as indicated for the individual experiments.

For the isolation of human $CD4^+$ T cells, 18 ml whole blood was harvested from healthy volunteers and mixed with 2 ml citrate to avoid blood coagulation. Whole blood was then diluted with same volume of T cell isolation buffer (phosphate-buffered saline + 2 mmol/l EDTA + 0.1% BSA; Gibco by life technologies) and gently layered over 2-fold volume of Biocoll solution (1.077 g/ml; Bio&SELL), followed by centrifugation for 25 minutes at 600 g without brake. The top layer of plasma was removed and mononuclear cells in the middle layer were carefully harvested and transferred to a new tube. The mononuclear cells were washed with T cell isolation buffer twice and centrifuged for 10 minutes at 300 g without brake. The supernatants were discarded, and cell pellets were resuspended with T cell isolation buffer to reach a final density of $1×10^8$ cells/ml. Human $CD4^+$ T cells were isolated from this cell suspension with Dynabeads™ Untouched™ Human CD4 T Cells Kit (Invitrogen) according to manufacturer's protocol.

Transmigration assay

Mouse and human $CD4^+$ T cells were isolated according to manufacturer's protocols as detailed above. Transmigration assays were performed using HTS Transwell-96 well plate (3.0 µm pore size with polycarbonate membrane; Corning). Murine or human recombinant CCL17 (Biolegend) was added to bottom chambers at a concentration of 100 ng/mL in RPMI-1640 medium containing 0.5% BSA. Murine

or human recombinant CCL1 or CCL22 (Peprotech) was added to bottom chambers at a concentration of 50 ng/mL in RPMI-1640 medium containing 0.5% BSA. Mouse $CD4^+$ T cells from *Apoe*$^{-/-}$, *CCR8*^{WT}*Apoe*$^{-/-}$ or *CCR8*^{KO}*Apoe*$^{-/-}$ mice or human $CD4^+$ T cells ($1x10^5$) were added to the top chamber in the presence or absence of CCR4 receptor antagonist C 021 dihydrochloride (Tocris) at a concentration of 0.5 µM; or human $CD4^+$ T cells ($1x10^5$) were pretreated with or without anti-CCR8 antibody (R&D Systems) and added to the top chamber and allowed to migrate for 3 hours. The number of cells migrated was analyzed by flow cytometry (FACSCanto II, BD Biosciences) and FlowJo v.10 software (Tree Star Inc.). The chemotactic index was calculated as the ratio of chemokine-stimulated to unstimulated migration.

In another quantitative transmigration assay (checkerboard), HTS Transwell-96 well plates (3.0 µm pore size with polycarbonate membrane; Corning) were also used. Isolated $CD4^+$ T Cells ($1×10^5$) were added to the upper chamber of each well in a total volume of 80 µl of RPMI-1640 medium containing 0.5% BSA. Murine recombinant CCL17 (Biolegend) was used at concentrations of 1 µg/ml, 100 ng/ml, 10 ng/ml, 1 ng/ml or 0 ng/ml in RPMI-1640 medium containing 0.5% BSA in the lower, upper, or both lower and upper chambers of the Transwell to generate 'checkerboard' analysis matrix of positive, negative, and the absent gradients of CCL17, respectively. Cells were collected from the lower chamber 3 h later and counted. The number of cells migrated was analyzed by flow cytometry (FACSCanto II, BD Biosciences) and FlowJo v.10 software (Tree Star Inc.). The chemotactic index was calculated as the ratio of migrated cell counts of each well to unstimulated migration without murine recombinant CCL17 in both lower and upper chamber.

T effector polarization assay

Splenic $CD4^+CD62L^+$ T cells were obtained by immunomagnetic cell isolation as described previously. $CD4^+CD62L^+$ T cells ($1×10^5$) were cultured in 96-well tissue round bottom culture plates in the presence of anti-CD3e (pre-coated overnight, 5µg/ml), anti-CD28 (1 µg/ml) and supplemented with TGF-β (5 ng/ml) in the presence or absence of CCL17 (100 ng/mL) for 3 days. Regulatory T cells (Tregs) were classified as $CD45^+CD3^+CD4^+CD25^+Foxp3^+$, Th1 cells were defined as $CD45^+CD3^+CD4^+Tbet^+$, Th2 cells as $CD45^+CD3^+CD4^+Gata3^+$, and Th17 cells as $CD45^+CD3^+CD4^+Ror\gamma t^+$. Foxp3/Tbet/Gata3/Rorγt transcription factors were stained using the Foxp3/Transcription Factor Staining Buffer Set (eBioscience).The number of Treg, Th1, Th2, Th17 cells was analyzed by flow cytometry (FACSCanto II, BD Biosciences) and FlowJo v.10 software (Tree Star Inc.).

Co-culture experiments

DCs were isolated from cell suspension from lymph nodes by fluorescence-activated cell sorting (BD FACSAria), by gating for $CD45^+CD11c^+MHCII^+$ cells as aforementioned. Sorted DCs were subsequently co cultured for 3 days in 96-well tissue flat bottom culture plates with splenic naïve $CD4^+CD62L^+$ T cells obtained by immunomagnetic cell isolation as described previously in a DC:T cell ratio of 1:2 (in general $2.5×10^4$: $5×10^4$ cells), with/without specific stimuli as indicated for the individual experiments. The percentage of regulatory T cells ($CD45^+CD3^+CD4^+CD25^+FoxP3^+$ cells) relative to CD4+ T cells was analyzed by flow cytometry (FACSCanto II, BD Biosciences) and FlowJo v.10 software (Tree Star Inc.). Supernatants were collected for further ELISA or Multiplex-bead-array analysis.

Multiplex-bead-array

Cell culture supernatants and mouse plasma were analyzed for various cytokines using the “Cytokine & Chemokine 26-Plex Mouse ProcartaPlex™ Panel 1” (Thermo Fisher Scientific (eBioscience)), sample preparation and analysis was performed according to the manufacturer’s protocol. The kit allows the simultaneous detection and quantification of soluble murine IFNγ; IL-12p70; IL-13; IL-1β; IL-2; IL-4; IL-5; IL-6; TNFα; GM-CSF; IL-10; IL-17A; IL-18; IL-22; IL-23; IL-27; IL-9; GRO-α (CXCL1); IP-10 (CXCL10); MCP-1 (CCL2); MCP-3 (CCL7); MIP-1α (CCL3); MIP-1β (CCL4); MIP-2 (CXCL2); RANTES (CCL5); Eotaxin (CCL11). The bead-based assay follows the principles of a sandwich immunoassay. Fluorescent magnetic beads are coupled with antibodies specific to the analytes to be detected. Beads are differentiated by their sizes and distinct spectral signature (color-coded) by flow cytometry using the Luminex™ xMAP.

ELISA

CCL3 plasma (EDTA-plasma of full blood) levels or CCL3 levels in cell culture supernatants were quantified by ELISA using a commercially available kit (Ccl3 Mouse Uncoated ELISA Kit with Plates from Thermo Fisher Scientific or Mouse CCL3/MIP-1 alpha Quantikine ELISA Kit by R&D System) following the manufacturer’s protocol. The final measurement of absorbance was carried out using a plate reader set to 450 nm with a correction factor of 550 nm.

Cyclic AMP signaling

Levels of cyclic adenosine monophosphate (cAMP) were measured in confluent Flp-In system and Flp-In™ TREx-293 (HEK293) cells (Invitrogen). HEK293 cells were transfected using plasmids harboring sequences of CCR4 and CCR8 (Missouri S&T cDNA Resource Center, www.cdna.org). The sequence of the luciferase-cAMP binding site fusion protein from the pGloSensor™-20F-vector (Promega) was amplified and ligated into a bicistronic pIRESneo vector (Clontech) to obtain the reporter gene plasmid. HEK293 cells were transfected with the reporter gene vector using Eco-Transfect (OZBioscience), stable clones were selected as host cell lines for expressing receptor constructs using the Flp-In system[9] and reselected with G418 and hygromycin B. After incubation with luciferin-EF (2.5 mM, Promega) at room temperature for 2 hours, cells were stimulated with CCL17, CCL1 or CCL20 (100 ng/ml each) or left unstimulated (PBS control) and luminescence indicating the reduction of cAMP was recorded over time.

Proximity Ligation Assay

Proximity ligation was carried out using the Duolink In Situ Red Kit Goat/Rabbit (Sigma-Aldrich) on PFA fixated mouse dendritic cells cultured on collagen-coated cover slips which were pre-incubated with recombinant mouse CCL1 (Peprotech) and CCL17 (Biolegend) using primary polyclonal antibodies to mouse CCL17 (R&D systems), mouse CCL1 (Acris), mouse CCR4 (Thermo Scientific), mouse CCR5 (Santa Cruz Biotechnology) and mouse CCR8 (Abcam) according to the manufacturer’s instructions. Imaging was performed using fluorescence microscopy (Leica DM4000) after which deconvolution algorithms for wide field microscopy were applied to improve overall image quality (Huygens professional 16.10; SVI). The number of Duolink detected interactions was determined in the processed

images using the Leica LAS 4.2 analyses software. In order to more accurately resolve the interactions detected with Duolink, representative dendritic cell samples of each condition were also visualized with a Leica SP8 3X microscope using a combination of 3D confocal microscopy (DAPI) and 3D STED nanoscopy (Duolink Red). Image processing and deconvolution of the resultant 3D datasets was performed using the Leica LAS X and Huygens professional software packages.

Proximity ligation was also carried out using the Duolink In Situ Probe anti-Goat or anti-Rabbit or anti-mouse Kit (Sigma-Aldrich) on PFA fixated CCR4-transfected or CCR8-transfected Jurkat cells which were pre-incubated with or without recombinant human CCL1 (Peprotech) and CCL17 (Biolegend) using primary polyclonal antibodies against human CCL17 (Thermo Fisher Scientific), human CCL1 (R&D Systems), human CCR4 (Thermo Fisher Scientific or Biolegend) and human CCR8 (Thermo Fisher Scientific). Cells were then treated with ligase and polymerase according to the manufacturer's instructions of Duolink flowPLA Detection Far Red kit (Sigma-Aldrich). The fluorescent signal was analyzed by flow cytometry (FACSCanto II, BD Biosciences) and FlowJo v.10 software (Tree Star Inc.).

Expression, purification and labeling of CCL17

The gene encoding native CCL17 was inserted into a pET-32a(+) vector between Kpn I and Xho I restriction sites. The expression of recombinant CCL17 in E.coli One Shot BL21(DE3)Star cells in LB medium was induced by 0.1 mM IPTG when the OD600 reached 0.8-1.0. Inclusion bodies were isolated and resuspended in binding buffer (50 mM Tris, 500 mM NaCl, 4 M Gnd-HCl, 40mM Imidazole, 10mM ß-mercaptoethanol, pH 7.4). The extract was loaded on a HisTrap HP column (Cytiva Europe GmbH) equilibrated with equilibration buffer (50 mM Tris, 500 mM NaCl, 6 M Gnd-HCl, pH 7.4). After washing with 2% of elution buffer (50 mM Tris, 500 mM NaCl, 2 M Imidazole, pH 7.4), the protein was eluted using a gradient of 2-50% elution buffer, followed by dialysis against acetic acid and lyophilization. The lyophilizate was resuspended in 10 mM Tris (pH 8.0), 3 units EKMax Protease (ThermoFisher Scientific) were added, and the solution was incubated for 16h at 37°C in order to remove the tag. The cleaved product was further purified using a 3mL RESOURCE™ RPC column with an acetonitrile + 0.1% TFA gradient. After lyophilization, the protein was refolded in 50 mM Tris, 10 mM cysteine, 0.5 mM cystine (pH 8.0) for 24h at 4°C under gentle stirring, purified using a HiTrap™ Heparin HP column and HPLC. The correct mass and folding were verified by mass spectrometry and NMR, respectively.
CCL17 was labeled using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and Alexa Fluor™ 647 Cadaverine (ThermoFisher Scientific). Briefly, CCL17 was incubated in presence of 10-fold molar excess of EDC and dye in 10mM MES (pH 6.0). After 10 min the labeled product was purified using Zeba™ Spin Desalting Columns (Life Technologies GmbH, Darmstadt, Germany) and stored at 4°C.

Surface plasmon resonance (SPR)

SPR was performed on a BIAcore X100 instrument (Cytiva Europe GmbH) using neutravidin-modified C1 sensor chips[10] coated with biotinylated human recombinant CCL17 to 1300 resonance units. Sensograms were obtained by injecting different concentrations of CCR8-carrying proteoliposomes or with CCR4-carrying liposomes (positive control), mock protein-carrying or pure lipopsomes (negative controls) in running buffer (62.5-2000 ng/mL in HBS-EP + buffer). Analytes were perfused over the chip for 270 seconds (at 20 µL/min) followed by a final dissociation phase of 180 seconds. The sensor chip

was regenerated with two pulses of 60 seconds of NAS (30% acetonitrile, 100 mM NaOH, 0.1% SDS). Responses from analyte injections were overlaid with the fit of 1:1 interaction model (Langmuir) determined using BIACORE X100 evaluation 2.0 software.

Competitive chemokine receptor-ligand binding

HEK293 cells stably transfected with human CCR8 (HA-tagged to monitor expression) and mock HEK293 cells (10^5 each) were incubated in binding buffer (HBSS supplemented with 20 mM HEPES and 0.2% BSA) with increasing concentrations of unlabeled human recombinant CCL17 or CCL1. After 20 min at 4°C, recombinant human CCL17 or synthetic human CCL1 (ALMAC) labeled with Alexa-Fluor 647 at the C-terminus (20 nM final concentration each) was added and further incubated for 30 min. After washing with binding buffer and fixation in 2% PFA/PBS, fluorescence intensity was measured by flow cytometry (FACSCantoII) and analyzed using FlowJo v.10 software (Ashland, Oregon, USA). Background binding to mock HEK293 cells was subtracted and data were normalized to binding without unlabeled chemokine (control) and subjected to nonlinear fitting.

CCR8 internalization assay

For isolation of $CD4^+$ T cells from *CCR8^{WT}Apoe$^{-/-}$* or *CCR8^{KO}Apoe$^{-/-}$* mice, thymus or lymph nodes were mechanically crushed and passed through a 30 µm cell strainer (Cell-Trics, Partec) using Hank's Medium (Hanks' Balanced Salt Solution + 0.3 mmol/l EDTA + 0.1% BSA; Gibco by life technologies) to obtain single cell suspensions. Respectively. $CD4^+$ T cells are subsequently isolated using the mouse $CD4^+$ T cell Isolation Kit (Miltenyi Biotec) as described before. Cells (1×10^5) were incubated with recombinant murine CCL17 (100 ng/ml) at 37°C for 1 h and surface stained with antibodies against CD4, CCR8 and CCR4 at 4°C for 30 min. CCR8 fluorescence intensity of $CD4^+$ T cells was analyzed by flow cytometry (FACSCanto II, BD Biosciences) and FlowJo v.10 software (Tree Star Inc.). Human $CD4^+$ T cells were isolated from peripheral blood mononuclear cell with Dynabeads™ Untouched™ Human CD4 T Cells Kit (Invitrogen) according to manufacturer's protocol as aforementioned. Cells (1×10^5) were incubated with recombinant human CCL17 (100 ng/ml), CCL1 (50 ng/mL) or CCL22 (50 ng/mL, all Peprotech) at 37°C for 20 min or 40 min and surface stained with antibodies against CD4, CCR8 and CCR4 at 4°C for 30 min. Cells were then washed with PBS. CCR8 and CCR4 fluorescence intensity of $CD4^+$ T cells was analyzed by flow cytometry (FACSCanto II, BD Biosciences) and FlowJo v.10 software (Tree Star Inc.).

RNA purification and Real time PCR

Total mRNA was isolated from frozen mouse axillary lymph node with Trizol (Invitrogen). Isolated RNA was subsequently transcribed into cDNA using iScriptTM cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Real time PCR was then performed with QuantiNovaTM Probe PCR Kit (Qiagen) in QuantStudio 6 Real-Time-PCR system (Thermofisher). The threshold cycle (Ct) values of the target genes were normalized to that of the housekeeping gene (endogenous control) encoding 18S ribosomal RNA. All data were analyzed by adopting $2^{-\Delta\Delta Ct}$ method. Relative mRNA expression is shown, with the average from control samples set as 1. The TaqMan gene expression assays used in

this study were as follows: Mm00441259_g1 (CCL3) and Mm03928990_g1 (Rn18S) (All Life Technologies).

Quantification of FoxP3 mRNA and CCL3 copy numbers in human plaque specimens was performed as described[11-13] and correlated with the clinical phenotype, either defined by asymptomatic stable atherosclerosis or by symptomatic disease, as apparent by cerebral ischemic events, e.g. transient ischemic attacks or stroke. The following primers/probes were applied: FoxP3_h_fwd GCCCGGATGTGAGAAGGTCTT, FoxP3_h_rev GCCCTGCCCTTCTCATCCAG, FoxP3_h_Sonde 5'FAM-CTTCCTCAAGCACTGCCAGGCGGAC-3'TAM; hCCL3-fwd: CTGCACCATGGCTCTCTGC; hCCL3-rev: CTGAAGCAGCAGGCGGTC, hCCL3-Sonde:CTCTGCATCACTTGCTGCTGACACGC.

Microarray data acquisition and data processing of published data sets

For the CCR8 mRNA expression in various human tissues and in different blood cell types, datasets were downloaded from the The Human Protein Atlas (https://www.proteinatlas.org).

For the CCL3, CCR8 and FOXP3 mRNA expression in human plaques or T cells from patients with familial hypercholesterolemia, four microarray datasets (GSE43292[14], GSE28829[15], GSE11138[16] and GSE6088) were downloaded from GEO (http://www.ncbi.nlm.nih.gov/geo). The GSE43292 dataset contained 32 human atheroma plaque or 32 paired distant macroscopically intact tissue. The GSE28829 dataset consisted of 16 advanced atherosclerotic plaques and 13 early lesions. The GSE11138 dataset consisted of 8 symptomatic and 6 asymptomatic patients with carotid and coronary plaque. The GSE6088 dataset consisted of 10 hetero- or homozygous patients with familial hypercholesterolemia and 13 control subjects. Data preprocessing included transform gene probes into gene symbols, data consolidation, and normalization. Probes without gene symbols were deleted. Probes with maximal expression were retained for further analysis if the probes contained more than one probe.

Cell suspension preparation for scRNA-seq

For the scRNA-seq experiment of pooled lymph nodes (including mesenteric, paraaortic, inguinal, axillary, brachial and mandibular LNs), eight *Ccl17*$^{wt/e}$*Apoe*$^{-/-}$ and six *Ccl17*$^{e/e}$*Apoe*$^{-/-}$ male mice were (C57BL/6J background) fed on normal chow diet. Perfusion was performed with 5 mL PBS through left ventricular puncture until the liver yields a pale color. Pooled lymph nodes from either *Ccl17*$^{wt/e}$*Apoe*$^{-/-}$ or *Ccl17*$^{e/e}$*Apoe*$^{-/-}$ mice were mechanically crushed and passed through a 30 µm cell strainer (Cell-Trics, Partec) using Hank's Medium (Hanks' Balanced Salt Solution + 0.04% BSA; Gibco by life technologies) to obtain single cell suspensions, followed by B cell depletion using CD19 MicroBead (Miltenyi Biotec) according to the manufacturer's instructions. B cell-depleted fractions were stained with Fixable Viability Dyes eFluor™ 660, anti-CD45, anti-CD3, anti-CD19 and anti-CD11c (All from eBioscience™) at 4°C for 20 minutes. After washing in PBS for 5 min, cells were resuspended in Hank's Medium (Hanks' Balanced Salt Solution + 0.04% BSA; Gibco by life technologies) and then isolated by fluorescence-activated cell sorting (BD FACSAria), by gating for Live CD45^{+}CD11c^{+}CD3^{-}CD19^{-} cells. The cell suspension with viability > 80% was ready for subsequent single cell capture and library preparation.

For the scRNA-seq experiment of para-aortic LNs, seven *Ccl17*$^{wt/e}$*Apoe*$^{-/-}$ and ten *Ccl17*$^{e/e}$*Apoe*$^{-/-}$ male mice (C57BL/6J background) were fed on 6-week WD. Paraaortic lymph nodes of same genotype were pooled and strained as aforementioned. Cells were resuspended in Hank's Medium (Hanks' Balanced

Salt Solution + 0.04% BSA; Gibco by life technologies), stained, and isolated by fluorescence-activated cell sorting (BD FACSAria), by gating for Live $CD45^{+}CD19^{-}MHCII^{+}$ cells. The cell suspension with viability > 80% was ready for subsequent single cell capture and library preparation.

Single-cell RNA Sequencing

Cell suspension were loaded into a 10x Genomics Chromium Next GEM Chips and encapsulated with Single Cell 3' v3.1 barcoded gel bead using the 10x Genomics Chromium controller according to the manufacturer's instructions. Single-cell libraries were then constructed according to the manufacturer's instructions. Libraries from individual samples were sequenced on an Illumina NovaSeq platform. Sequencing depth was set to be around 50,000 reads per cell for pooled lymph nodes from various positions and around 65,000 reads per cell for paraaortic lymph nodes.

Analysis of scRNA-Seq data

Fastq files of sorted $CD45^{+}CD11c^{+}CD3^{-}CD19^{-}$ cells from lymph nodes of *Ccl17*$^{wt/e}$*Apoe*$^{-/-}$ and *Ccl17*$^{e/e}$*Apoe*$^{-/-}$ mice on a chow diet were aligned to the customized reference genome (eGFP were added to mm10 reference) individually using CellRanger Software (10x Genomics). Individual datasets were then aggregated using the CellRanger aggr command without subsampling normalization. The aggregated dataset was then analyzed using the R package Seurat (version 3.1.4).[17,18] The dataset was trimmed of cells expressing < 200 or > 5,000 genes for exclusion of noncell or cell aggregates. Cells containing > 10% mitochondrial genes were presumed to be of poor quality and were also discarded. A "LogNormalize" method was employed to normalize the gene expression for each cell by the total expression, the resulting expression values were then multiplied by 10,000 and log transformed. The most highly variable genes in the dataset were discovered with FindVariableFeatures function and used in principal component analysis (PCA), followed by a linear transformation ('scaling'). PCA was used for dimensionality reduction, and uniform manifold approximation and projection (U-MAP) was then used for two-dimensional visualization of the clusters. Visualization of gene expression with feature plot was generated with Seurat function FeaturePlot. Marker genes of each cluster were found by FindAllMarkers function.

Similar alignment was performed in sorted $CD45^{+}CD19^{-}MHCII^{+}$ cells from paraaortic lymph nodes of *Ccl17*$^{wt/e}$*Apoe*$^{-/-}$ and *Ccl17*$^{e/e}$*Apoe*$^{-/-}$ mice on 6-week WD individually using CellRanger Software (10x Genomics). Individual datasets were then aggregated using the CellRanger aggr command without subsampling normalization. Cells expressing < 200 or > 4,000 genes were filtered out for exclusion of noncell or cell aggregates. Cells containing > 5% mitochondrial genes were also discarded. Similar normalization, scaling, PCA, clustering and U-MAP analysis were then performed.

Tolerogenic score

XCR1+ tolerogenic DCs undergo a continuous homeostatic maturation that is essential for central tolerance and that occurs irrespective of IFN-I according to previous study.[19] 82 specifically upregulated genes during thymic and peripheral homeostatic XCR1+ DC maturation in this study were listed as tolerogenic genes. Interferon-stimulated genes (ISGs) were among the few discriminators of immunogenic and tolerogenic XCR1+ DCs. 31 ISGs from this study were listed as immunogenic genes

(Supplemental Table 3). The tolerogenic score was then calculated using the top 20 genes that distinguished tolerogenic DCs and immunogenic DCs as follows: tolerogenic score = [1 + mean (top 20 upregulated tolerogenic genes)]/[1 + mean (top 20 upregulated immunogenic genes)].

Gene Set Variation Analysis (GSVA) enrichment score

To generate GSVA enrichment score of each eGFP-expressing Ccl17-deficient cell from tolerogenic DCs of *Apoe*$^{-/-}$ *Ccl17*$^{e/e}$ mice and each Ccl17-expressing cell from tolerogenic DCs of *Apoe*$^{-/-}$ *Ccl17*$^{wt/e}$ mice fed on chow diet, the ontology gene sets v.7.1 were provided in MSigDB (https://www.gsea-msigdb.org/gsea/msigdb). The analysis was implemented using R package gsva.

Data availability

The scRNA-seq data of *Ccl17*$^{wt/e}$*Apoe*$^{-/-}$ and *Ccl17*$^{e/e}$*Apoe*$^{-/-}$ mice fed on chow diet or on a 6-week HFD are available for reproducing the results. The authors declare that all R scripts used to process data are available from the corresponding author if requested. Schematic panels in the figures were created using Biorender.com.

Statistics

All data are expressed as mean±SEM, as indicated. Statistical calculations were performed using the up-to-date version of GraphPad Prism (GraphPad Software Inc.). After verifying normal distribution via D'Agostino-Pearson omnibus normality test, unpaired Student's t-test with Welch's correction, Mann-Whitney test, One-way ANOVA or Kruskal-Wallis with Dunnett's or Dunn's multiple comparisons or two-way ANOVA with Sidak's multiple comparisons test were used, as appropriate. P-values <0.05 were considered as being statistically significant.

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