SUPPLEMENTAL MATERIAL

Regulatory T cells license macrophage pro-resolving functions during atherosclerosis regression

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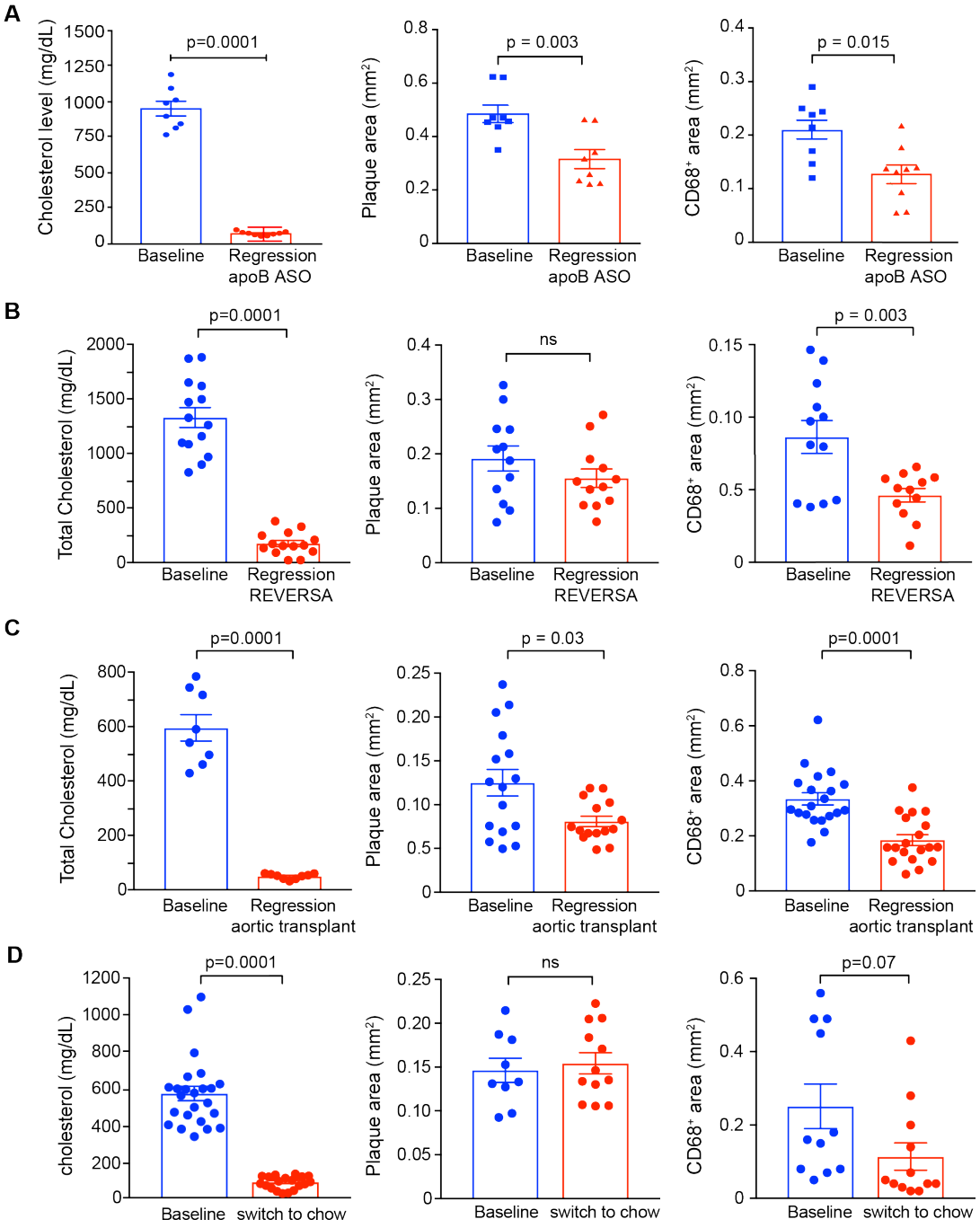
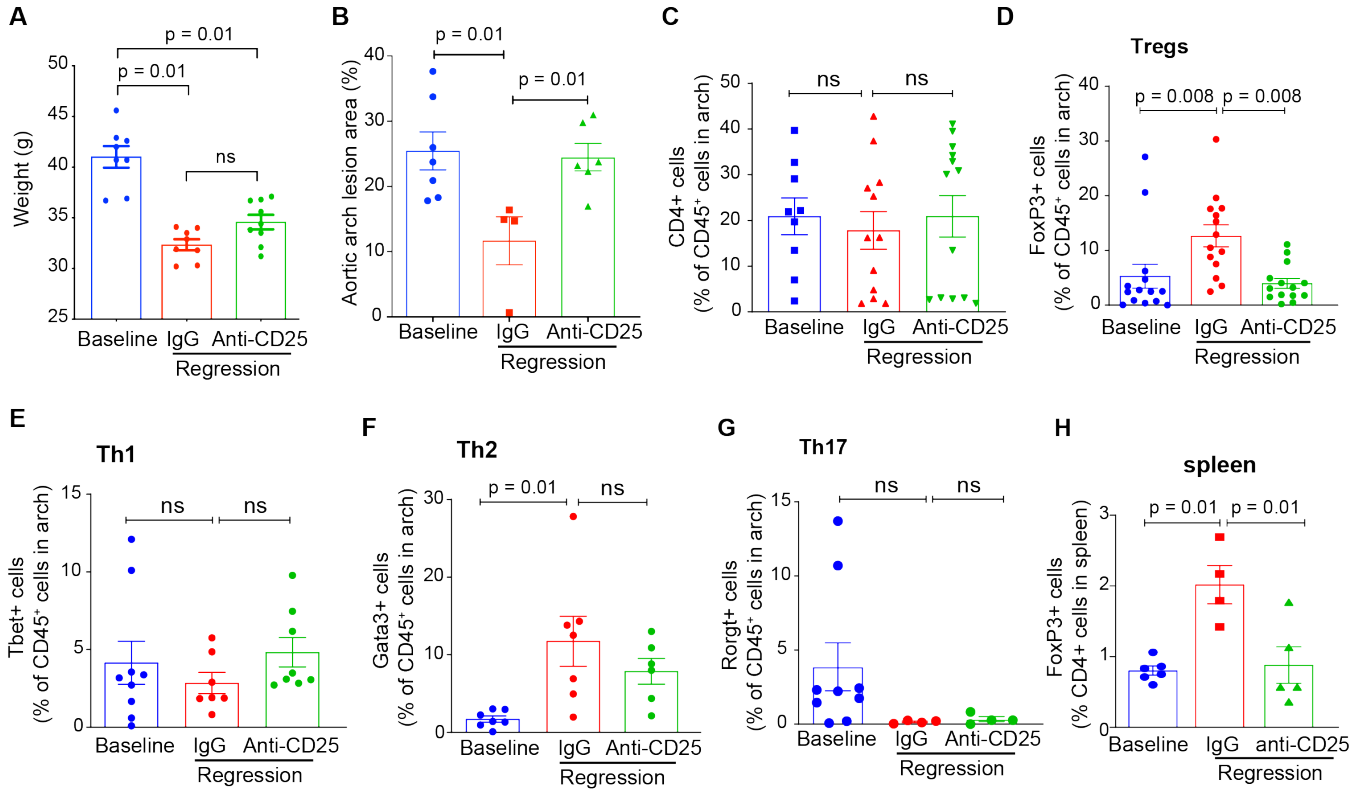
Online Figure I



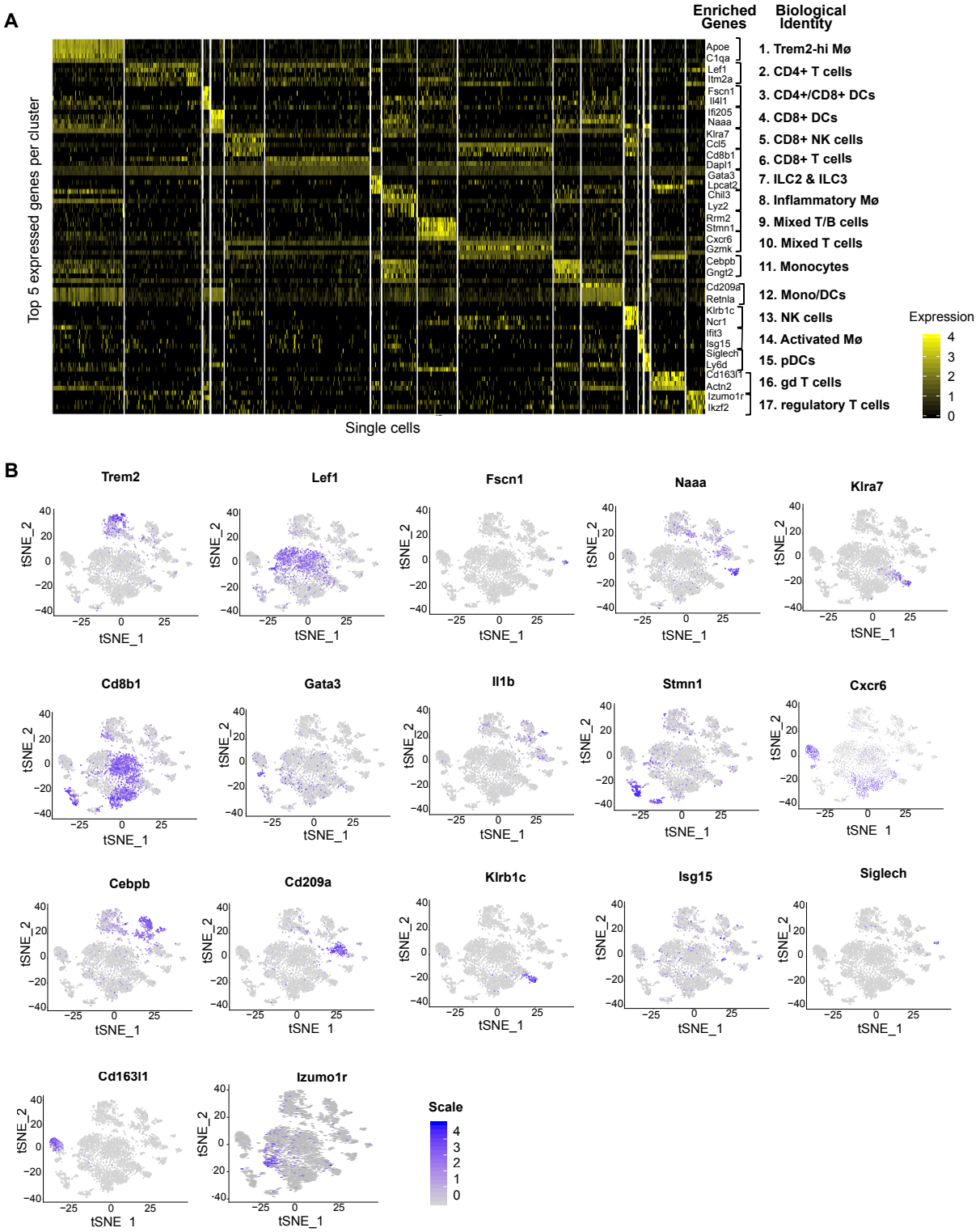
Online Figure I. Quantification of total cholesterol, lesion area and immunohistochemical staining for the macrophage marker, CD68 in cross-sections of the, **A)** aortic root of atherosclerotic C57BL/J6 mice at baseline (AAV-PCSK9 and western diet for 20 weeks) and 3 weeks after lipid lowering using apoB-ASO (regression), n=8-9 mice/group. **B)** aortic root of atherosclerotic REVERSA mice at baseline (16 weeks WD) and 4 weeks after lipid lowering by MTP deletion (regression), n=12-14 mice/group. **C)** aortic arch of atherosclerotic *Apoe*$^{-/-}$ mice (16 weeks WD) after transplant to WT (regression), n=8-10/group for cholesterol assays; n=18-20/group for lesion area and immunostaining. **D)** aortic root of atherosclerotic C57BL/J6 mice at baseline (AAV-PCSK9 and western diet for 20 weeks) and 3 weeks after switching to chow diet to halt the plaque progression. n=20-24/group for cholesterol assays; n=10-14/group for lesion area and immunostaining. Data is expressed as mean ± s.e.m. P values were determined by a Student's t-test.

Online Figure II



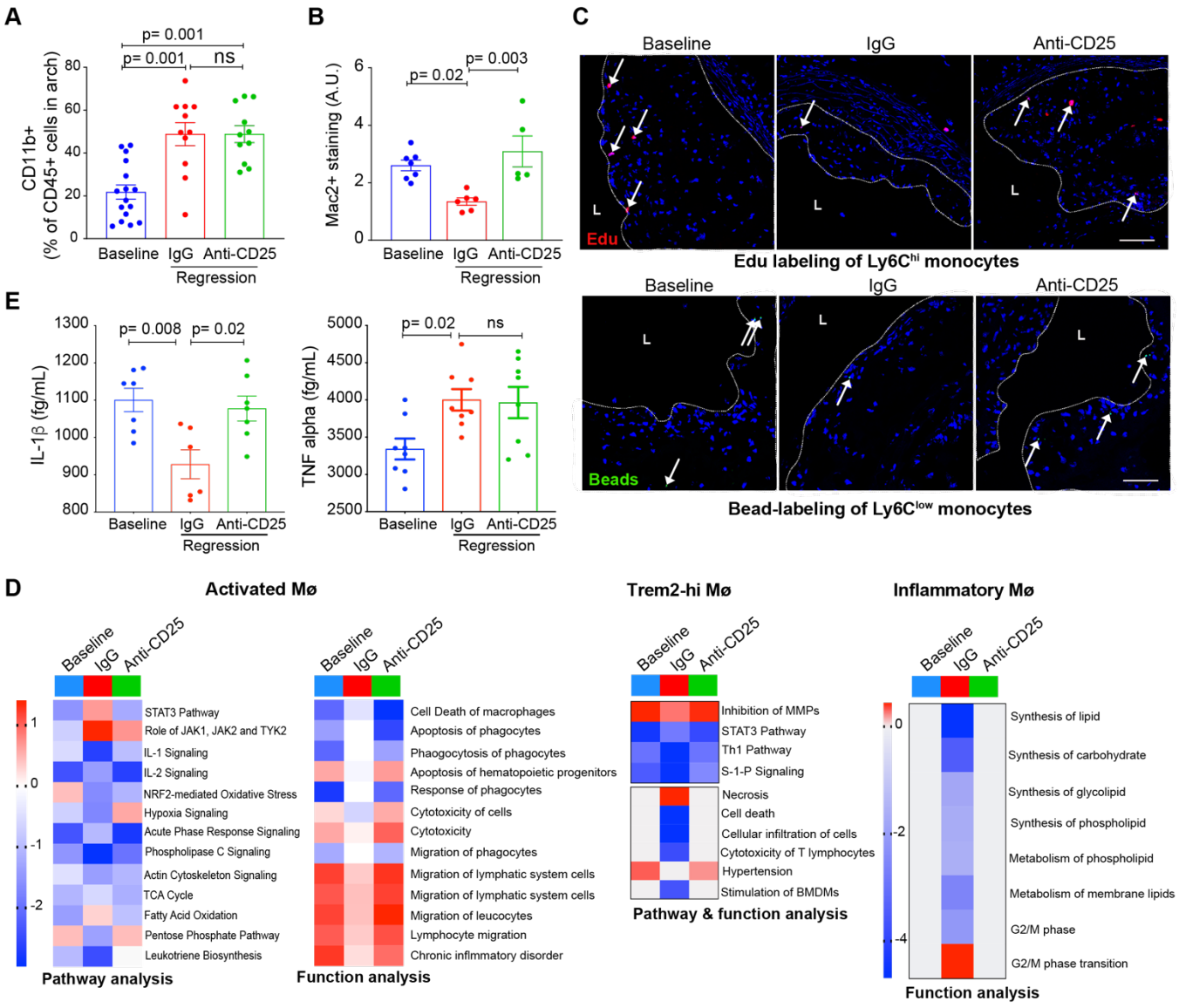
Online Figure II. A) Body weight, **B)** Quantification of lesion area of the aortic arch by *en face* analysis, and **C-G)** Quantification of $CD4^+$ T cell subsets as a % of CD45+ cells in aortic arch plaques by flow cytometric analysis in mice at baseline and after regression + IgG or anti-CD25 treatment (n=9-12 mice/group). **C)** CD4+ cells, **D)** $FoxP3^+$, **E)** Tbet+, **F)** Gata3+ and **G)** RORγδ+. **H)** Flow cytometric quantification of $FoxP3^+$ T cells in the spleen of mice at baseline and after regression + IgG or anti-CD25 treatment (n=4-6 mice/group). Data are mean ±SEM. P values were determined by a one-way ANOVA with post-hoc Sidak's test.

Online Figure III



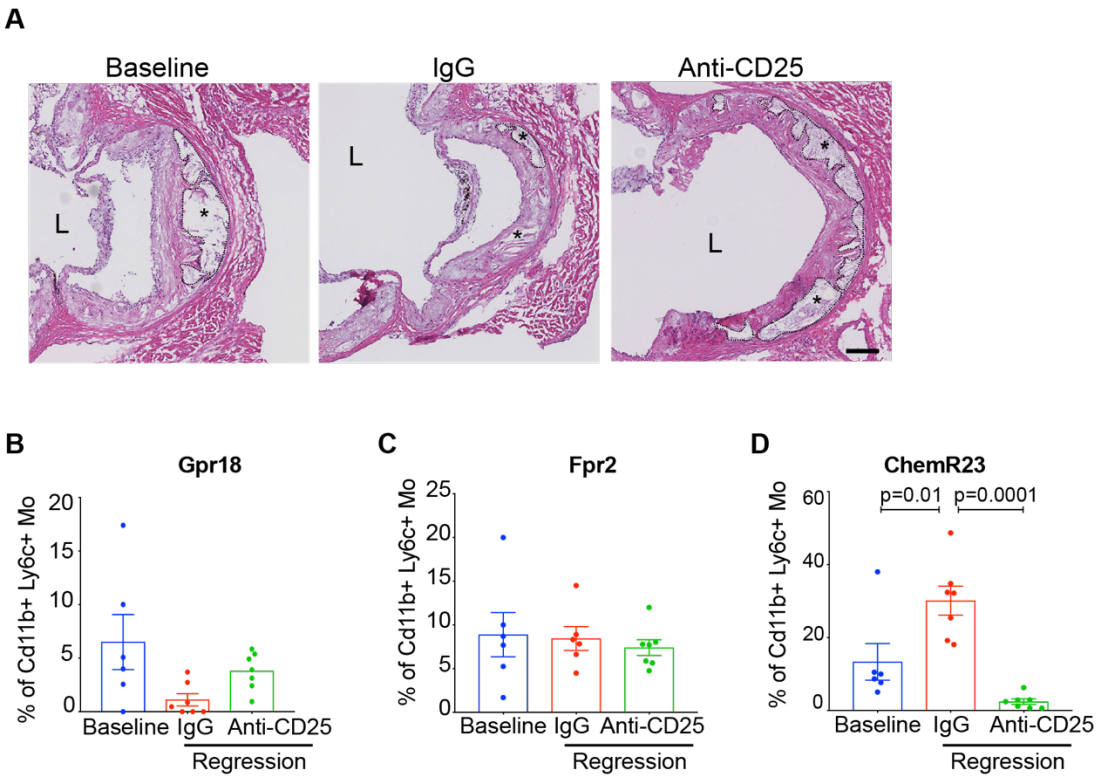
Online Figure III. A) Heatmap showing the 5 most highly expressed genes (ordered by decreasing p value) in each cluster (n=17) and top 2 selected enriched genes used for the biological identification of each cluster (scale: log2 fold change) identified from single-cell RNA-sequencing of CD45+ cells from aortic arches from baseline, IgG regression and anti-CD25 regression mice groups. Data was analyzed by Seurat. **B)** Gene expression patterns projected onto t-SNE plots of *Trem2, Lef1, Fscn1, Naa, Klra7, Cd8b1, Gata3, Il1b, Stmn1, Cxcr6, Cebpb, Cd209a, Klrb1c, Isg15, Siglech, Cd163l1* and *Izumo1r* (scale: log-transformed gene expression).

Online Figure IV



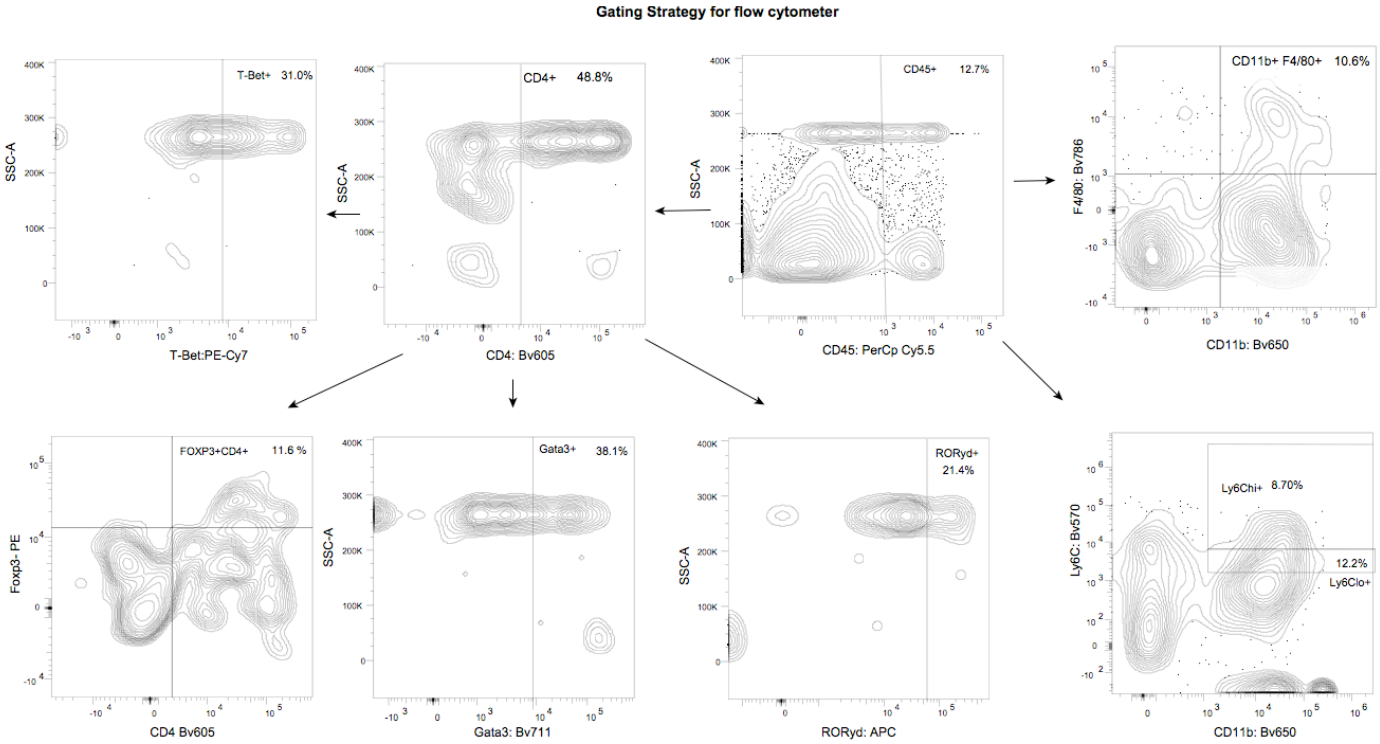
Online Figure IV. A) Flow cytometric quantification of CD11b^{+} cells as a % of CD45+ cells in aortic arch plaques in mice at baseline and after regression + IgG or anti-CD25 treatment (n=11-16 mice/group). **B)** Quantification of immunohistochemical staining for the macrophage marker Mac2 in aortic root plaques of mice at baseline and after regression + IgG or anti-CD25 treatment (n=5-7 mice/group). **C)** Representative images of Edu-labeled Ly6C^{hi} monocyte-derived macrophages and bead-labeled Ly6C^{lo} monocyte-derived macrophages in aortic root plaques 21 days after pulse-labeling. **D)** Signaling pathway and molecular function analysis of activated macrophages, Trem2-hi macrophages, and inflammatory macrophages clusters from single-cell RNA-sequencing of CD45+ cells from digested aortic arches from baseline, IgG regression and anti-CD25 regression mice groups, analyzed using IPA. **E)** Plasma IL-1β and TNF-α levels as measured by CBA assay, in mice at baseline and after regression + IgG or anti-CD25 treatment, n= 6-7 mice per group. Data are mean ±SEM. P values were determined by a one-way ANOVA with post-hoc Sidak's test).

Online Figure V



Online Figure V. A) Representative images of atherosclerotic plaques used for quantification of necrotic area. **B-C)** Quantification of **B)** Gpr18, **C)** Fpr2 and **D)** ChemR23 receptor expression in Ly6C+ monocytes in the digested aortic arches of baseline, IgG regression and anti-CD25 regression mice by flow cytometric analysis. Data are mean ±SEM. P values were determined by a one-way ANOVA with post-hoc Sidak's test.

Online Figure VI. Gating strategy for flow cytometer as shown for CD45, CD4, Foxp3, T-bet, Gata3, RORgd, CD11b+ F4/80+ and CD11b+ Ly6C+ populations performed by Flowjo analysis.



Gene	logFC	*P*-value
Lgals3	1.01	1.05E-03
Gzma	0.97	7.49E-01
Ccl4	0.86	2.13E-01
C1qc	0.83	7.07E-02
C1qb	0.74	8.67E-01
Ms4a7	0.68	2.63E-02
Rpl12	0.68	1.39E-05
Uba52	0.63	2.75E-05
Cd40lg	0.60	4.21E-03
Tyrobp	0.58	1.15E-01
Lef1	0.58	4.74E-03
H2-Eb1	0.57	5.79E-01
Ctsl	0.57	4.82E-01
Rpl39	0.56	8.98E-05
Slbp	0.54	6.94E-02
Klra3	0.54	1.58E-02
Hmgn1	0.53	2.81E-02
C1qa	0.52	3.04E-01
Cbx3	0.52	2.17E-02
Gm8730	0.50	2.09E-03
Gm10116	0.50	2.62E-02
Gm9844	0.49	3.55E-03
Gm10076	0.49	4.33E-05
Drosha	0.48	6.36E-01
Dynll1	0.48	8.04E-04
Rplp2	0.48	3.95E-05
Il4	0.47	1.64E-03
Rpl38	0.46	8.38E-05
Ptma	0.46	7.66E-04
Mif	0.46	6.79E-02
Rps26	0.46	3.37E-03
S100a4	0.45	1.44E-02
Npm1	0.44	2.38E-03
Rps12	0.44	2.77E-02
Rps28	0.44	2.38E-06
Rln3	0.44	8.14E-02
Rpl36	0.43	2.45E-04
Ran	0.42	4.80E-03
Jchain	0.42	1.24E-02
Fchsd2	0.42	7.95E-02
Bank1	0.41	1.58E-02
Tagln2	0.41	5.78E-02
Ccl3	0.41	2.28E-01
Bst2	0.41	3.99E-02
Ly6a	0.41	4.38E-02
Rps23	0.41	3.35E-05
Il7r	0.40	1.73E-01
Mmp12	0.39	2.86E-02
Cd14	0.39	5.04E-02
Rpl15	0.38	9.56E-04
Hbb-bt	0.38	1.58E-02
Gm9493	0.38	7.26E-03
Rps10	0.38	3.16E-02
Rpl35	0.38	2.56E-03
Fcer1g	0.37	8.80E-01
Itm2a	0.37	7.52E-01
Ifi30	0.37	6.15E-03
Nebl	0.37	5.09E-03
Id2	0.37	3.03E-01
Rps15a	0.36	3.56E-03
Aif1	0.36	5.04E-02
Edem2	0.36	8.07E-02
Rpl41	0.36	8.18E-05
Rps4x	0.36	4.38E-03
Rps20	0.36	1.27E-02
Cd247	0.35	7.94E-02
Parp1	0.35	7.95E-02
Rpl27a	0.35	1.17E-03
Isg15	0.35	4.29E-01
Qpct	0.35	2.99E-02
Rps7	0.35	2.11E-02
Sostdc1	0.35	4.64E-01
Rpl37a	0.35	1.20E-04
Rpl23	0.34	4.01E-03
C5ar1	0.34	2.99E-02
Gm10073	0.34	1.40E-01
Csf1r	0.34	5.06E-01
Cst3	0.34	7.56E-01
Rpl26	0.34	4.94E-03
Slc25a5	0.34	7.96E-02
Ttc39c	0.33	1.54E-02
Rps2	0.33	2.17E-02
Rpl22l1	0.33	1.05E-01
Dek	0.33	1.54E-01
Rpl37	0.33	3.99E-03
Rpl36a	0.33	3.70E-02
Gm43352	0.33	5.04E-02
Ust	0.33	1.71E-01
Rpl10	0.32	3.28E-02
Rps24	0.32	2.36E-02
Tmem176b	0.32	1.68E-01
Gusb	0.32	6.93E-02
Rbm3	0.32	3.68E-01
Lgmn	0.32	4.74E-01
Rps27	0.32	1.16E-03
Gm11808	0.31	1.24E-01
Rps29	0.31	4.86E-03
Rpl23a	0.31	4.81E-03
Rpl27	0.31	6.44E-03
Cd74	0.31	7.12E-02
Gm2000	0.31	5.16E-02
Lysmd1	0.31	2.07E-01
Lyz2	0.31	7.75E-01
Psap	0.30	5.51E-01
Cyb5a	0.30	7.43E-01
Rplp0	0.30	5.19E-02
Ctsc	0.30	4.29E-01
Pla2g7	0.30	1.58E-02
Lrrc20	0.30	1.71E-01
Cyb5r3	0.30	2.43E-01
Sccpdh	0.30	5.15E-01
Pycard	0.30	1.02E-01
Rps3a1	0.30	2.18E-03
Anp32e	0.30	8.90E-02
Tmem123	0.30	6.55E-02
Sh3rf1	0.30	1.71E-01
Ccl7	0.29	5.04E-02
Crip2	0.29	8.14E-02
Tox	0.29	2.00E-01
Cd24a	0.28	5.04E-02
Ly86	0.28	5.04E-02
Capn3	0.28	4.05E-01
S100a11	0.28	3.07E-01
Tmsb10	0.28	1.41E-02
Rps8	0.28	2.51E-02
Ly6k	0.28	3.12E-02
H2-Ab1	0.28	3.72E-01
Rpl17	0.28	1.15E-02
Retnla	0.27	9.44E-02
Hsp90ab1	0.27	9.62E-02
Rpsa	0.27	2.87E-02
Nfatc1	0.27	3.57E-01
Gmfg	0.27	5.90E-01
Gm11707	0.27	4.29E-01

Gene	logFC	*P*-value
Junb	-2.40	2.83E-15
Dusp1	-2.14	6.64E-10
Fos	-1.88	1.61E-05
Rgs1	-1.31	1.69E-03
Ppp1r15a	-1.28	2.04E-04
Jund	-1.22	1.60E-07
Jun	-1.17	8.38E-04
Cd69	-1.13	7.00E-04
Klf6	-1.13	1.82E-03
Nfkbia	-1.12	3.56E-03
Rgs2	-1.06	1.92E-02
Btg2	-1.02	3.91E-04
Pim1	-0.94	4.49E-03
Penk	-0.87	1.46E-01
Socs3	-0.86	7.05E-03
Socs1	-0.84	4.44E-03
Ccr7	-0.80	5.45E-03
Ctla4	-0.78	4.10E-02
Dapl1	-0.77	2.54E-02
Ier2	-0.77	5.58E-03
Tmcc1	-0.72	2.66E-02
Hbb-bs	-0.71	2.14E-02
Prdx5	-0.70	7.96E-03
Rasgrp1	-0.69	8.84E-03
Igtp	-0.69	2.43E-02
Atp11b	-0.64	4.09E-02
Cd83	-0.62	1.59E-01
Serpina3g	-0.62	9.27E-02
Zfp36l1	-0.62	4.19E-03
Cd28	-0.60	6.03E-03
Ctsd	-0.60	3.17E-02
Gsr	-0.58	7.58E-02
Cd27	-0.58	1.01E-02
Gadd45g	-0.56	1.46E-01
Lag3	-0.54	3.95E-02
Hspa5	-0.54	1.85E-02
B4galnt1	-0.54	2.82E-02
Zfp36l2	-0.53	1.54E-02
Bhlhe40	-0.53	2.84E-01
Itgb1	-0.52	7.61E-02
Nrp1	-0.52	4.40E-02
Ptpn6	-0.52	4.14E-02
Pmaip1	-0.51	8.39E-02
Sod2	-0.51	2.14E-01
Neat1	-0.51	7.43E-02
Sdc4	-0.50	2.39E-01
Il2rb	-0.50	1.38E-01
Serp1	-0.49	1.60E-01
Ptpn22	-0.49	1.99E-01
Klrg1	-0.49	6.80E-01
2310001H17Rik	-0.49	8.76E-02
Phlda1	-0.48	1.70E-01
Ccl6	-0.48	1.29E-01
Pou2f2	-0.47	1.61E-01
Gm8369	-0.47	1.48E-01
Btg1	-0.47	8.39E-04
Batf	-0.47	3.25E-01
Klf2	-0.47	5.98E-03
Erdr1	-0.46	1.35E-01
Ptger2	-0.46	2.58E-01
Capg	-0.46	2.55E-01
Ecm1	-0.45	3.60E-02
Malat1	-0.45	5.81E-06
Bambi	-0.45	3.12E-01
Rgs16	-0.44	1.69E-01
Cxcr6	-0.44	7.06E-01
Nrn1	-0.44	2.76E-01
Cd37	-0.43	2.73E-02
Socs2	-0.43	7.21E-02
Ralgps2	-0.43	3.60E-02
Ubc	-0.43	3.93E-03
H2-K1	-0.42	2.11E-03
Gzmk	-0.41	7.21E-02
Hexa	-0.40	4.96E-02
Pdcd4	-0.40	5.28E-02
Dnajc7	-0.40	1.84E-01
Tsc22d3	-0.39	4.62E-02
Ms4a4c	-0.39	2.17E-01
Epcam	-0.38	1.03E-01
Plk3	-0.38	8.15E-01
Tnfsf11	-0.38	7.26E-02
Gbp6.1	-0.38	1.03E-01
Hsd11b1	-0.38	4.09E-01
Cd79a	-0.37	3.39E-01
Sfi1	-0.37	1.03E-01
Gstm1	-0.37	3.39E-01
Dusp2	-0.36	7.14E-01
Polr2g	-0.36	1.39E-01
Msrb1	-0.36	4.07E-01
Pecam1	-0.35	2.85E-01
Sclt1	-0.35	4.90E-01
Sat1	-0.35	4.29E-01
Il1r2	-0.34	5.66E-01
Apoc4	-0.34	1.46E-01
Izumo1r	-0.34	3.16E-02
Nt5e	-0.34	3.91E-01
Hmgn3	-0.34	2.45E-01
Gimap3	-0.34	6.74E-02
Fam101b	-0.34	2.25E-01
Angptl2	-0.34	2.51E-01
H1f0	-0.34	1.88E-01
Hba-a1	-0.33	7.21E-02
Gem	-0.33	1.91E-01
Lck	-0.33	5.54E-03
Gm12216	-0.33	1.03E-01
Rgs10	-0.32	6.04E-01
Ngp	-0.32	2.39E-01
Limd2	-0.32	1.91E-02
Mnda	-0.32	1.03E-01
Nfkbid	-0.32	5.47E-01
Car2	-0.31	1.46E-01
BC021614	-0.31	3.03E-01
Cfp	-0.31	7.21E-02
Tmpo	-0.31	2.38E-01
Sepp1	-0.31	3.47E-01
Sh2d2a	-0.31	5.14E-01
Serpinb9	-0.30	1.46E-01
Bcl2a1b	-0.30	3.74E-01
Ccnd2	-0.30	2.22E-01
Tnfrsf4	-0.29	7.72E-01
Tuba1b	-0.29	6.82E-01
Trp53inp1	-0.28	5.22E-01
Apoe	-0.28	3.04E-01
Atp1b1	-0.28	1.86E-01
Mdfic	-0.28	2.15E-01
Ctsz	-0.28	4.71E-01
Tnfsf8	-0.27	2.62E-01

Online Table I. Differential gene expression of aortic Tregs isolated from progressing and regressing plaques. Genes with induced expression in regressing compared to progressing plaques are shown under the red header; genes with reduced expression are shown under the blue header. Data are expressed as average log fold change (FC) of regression plaques versus progressing plaques. Non-adjusted P values were calculated using the Wilcoxon rank-sum test.