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Supplemental information

An agonistic anti-signal regulatory protein α antibody for chronic inflammatory diseases

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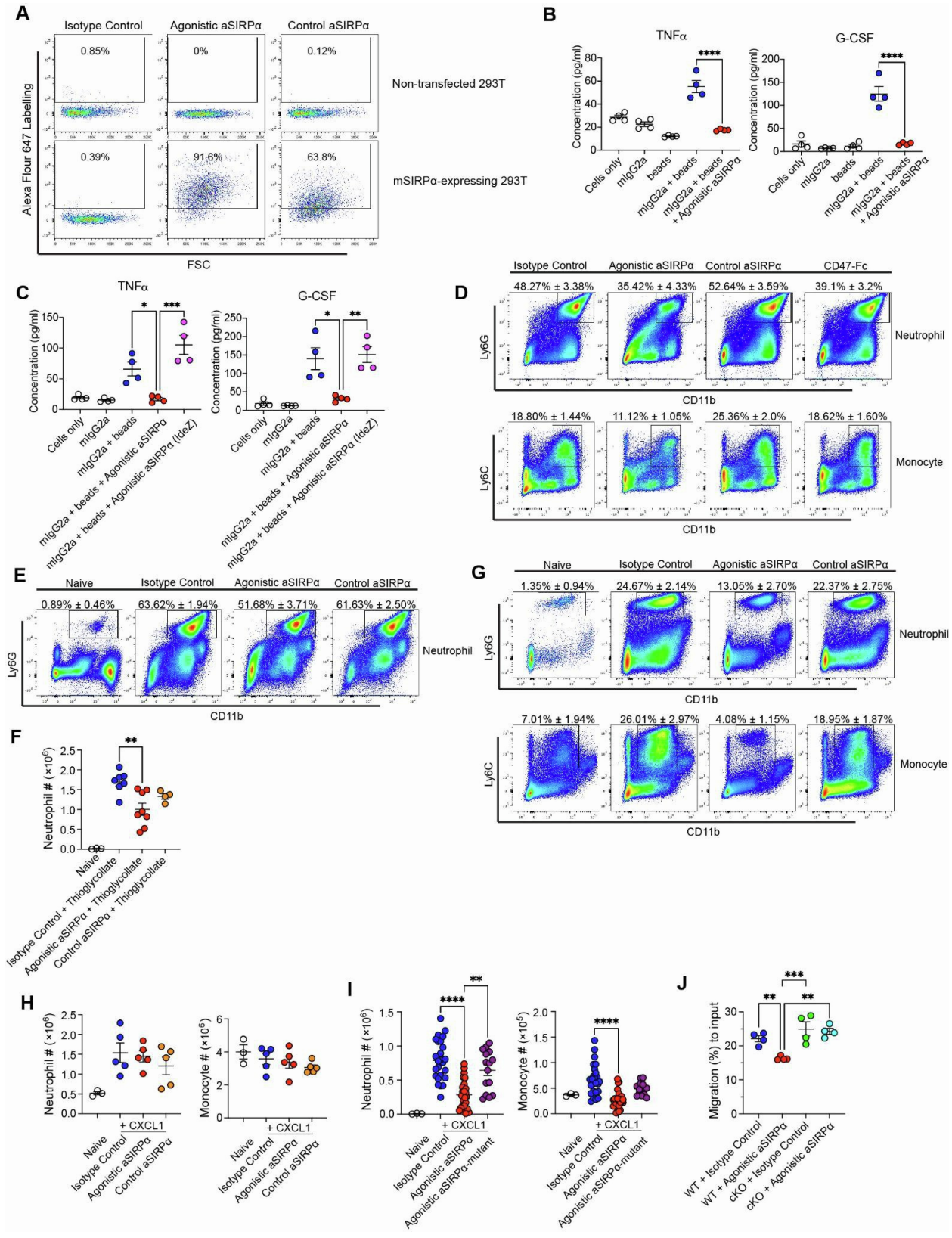

Figure S1 (related to Figure 2). Additional characterization of agonistic anti-Sirpα antibody and

representative flow cytometric plots.

(**A**) Representative dot plots of Alexa Fluor 647 labeled isotype control, the agonist anti-SIRPα and the control anti-SIRPα binding with SIRPα. The 293T cells were transfected with or without SIRPα before staining. (**B, C**) Production of inflammatory TNFa and G-CSF in the RAW 264.7 cell-mIgG2a-protein A beads immune complex system. Cells were treated with isotype control, Agonist aSIRPα, Control aSIRPα, or IdeZ protease pre-treated agonistic aSIRPα (C) at indicated concentration for one hour before protein A beads were added for another 24 hours' culture. Cell medium supernatants were collected for luminex assay for cytokine quantification. (**D**) Representative flow cytometric gating of neutrophils ($Ly6G^+GR1^+$) and monocytes ($Ly6G^-GR1^+$) from monoclonal antibody treated mice for Figure 2C. (**E, F**) Flow cytometric analysis of peritoneal neutrophils (gated $Ly6G^+CD11b^+$) from thioglycollate challenged mice. Representative dot plots (E) and average cell numbers (F) of neutrophils. (**G**) Representative dot plots of neutrophils (gated $Ly6G^+CD11b^+$) and monocytes ($Ly6C^+CD11b^+$) for Figure 2E. (**H**) Average neutrophil and monocyte cell numbers in spleen collected 4 hours after CXCL1 injection. (**I**) Average neutrophil and monocyte cell numbers in peritoneal cavity 4 hours after CXCL1 injection. Mice were treated with indicated antibodies 16hr before CXCL1 injection. (**J**) Quantification of transmigration assay with bone marrow neutrophils 4 hours after transmigration, migrating to 10 ng/ml CXCL1. Bone marrow cells were isolated from overnight Isotype control or agonistic aSIRPα treated WT or $LysMCre^+Sirpa^{Loxp/Loxp}$ mice. Data are from one representative experiment of two independent experiments with at least three biological replicates per group. Each symbol represents one mouse. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, by Ordinary One Way ANOVA with Tukey's multiple comparisons test.

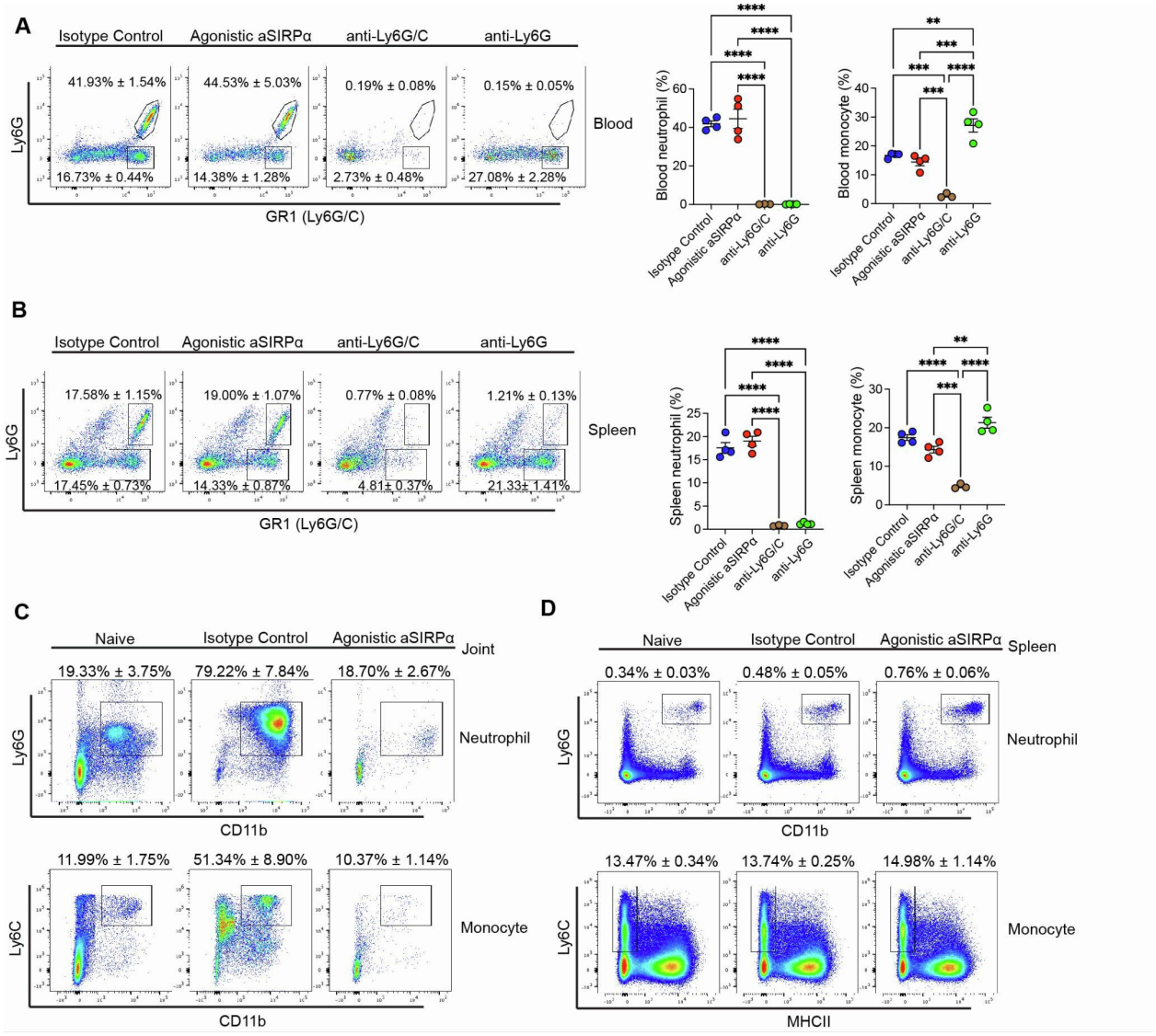

Figure S2 (related to Figure 3) Representative flow cytometry plots of blood, spleen, and joint lymphocytes from KBxN arthritis mice.

(**A, B**) Flow cytometric analysis of blood (A) and spleen (B) neutrophils ($Ly6G^+$) (Ly6G/C is GR1) and monocytes ($Ly6G^-Ly6C^+$) from mice with indicated antibody treatment. Representative dot plots (left) and average cell percentages (right) of neutrophils and monocytes. (**C**) Representative dot plots of neutrophils ($Ly6G^+CD11b^+$) and monocytes ($Ly6C^+CD11b^+$) for Figure 4E. (**D**) Representative dot plots of spleen neutrophils ($Ly6G^+CD11b^+$) and monocytes ($Ly6C^+CD11b^+MHCII^-$) for Figure 4F. Data for are from one representative experiment of two independent experiments with at least three technical replicates per group. Each symbol represents one mouse. *P <

0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, by Ordinary One Way ANOVA with Tukey's multiple comparisons test.

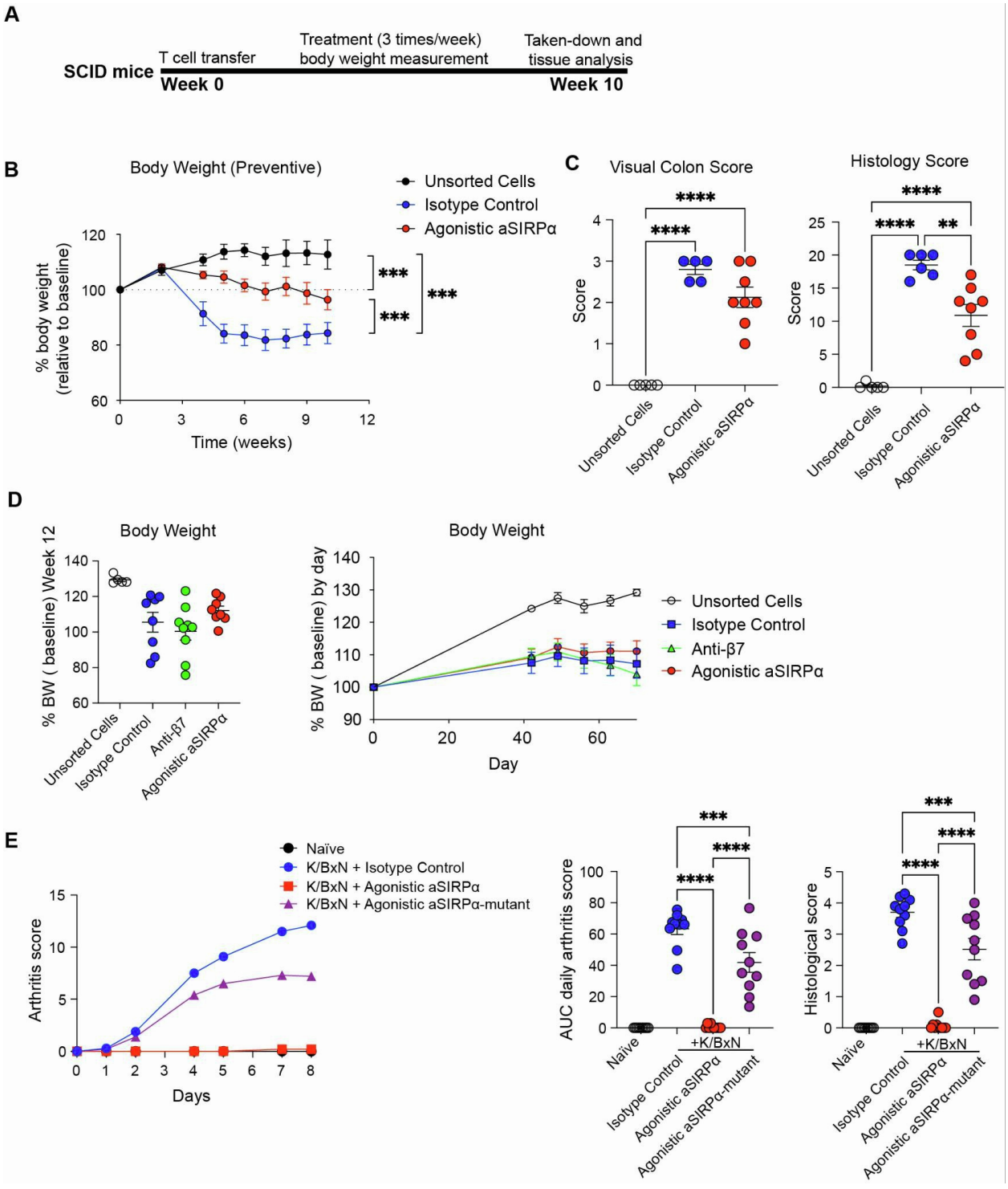

Figure S3 (related to Figure 4 and discussion). Body weight from transfer colitis mice and reduced therapeutic efficacy in agonistic anti-Sirpα DANA mutant antibody.

(**A**) Schema of the CD45RB^{high}CD4^{+} T cell transfer colitis preventive model. C.B17 SCID mice were transferred with unsorted or sorted CD45RB^{high}CD4^{+} T cells, and all mice were treated 3 times per week until the end of the

study. All mice were euthanized by Week 10. (**B, C**) Percentage of body weight to baseline of Week 0 in the $CD45RB^{high}CD4^{+}$ T cell transfer colitis model. Visual colon scores and histological scores of colons. (**D**) In the therapeutic model of Figure 4A, mouse body weight was measured at the beginning as baseline and again measured at Week 12 at the end of the experiment. (**E**) Clinical arthritis scores and paw/joint histological scores of mice received various treatments and K/BxN serum transfer. Anti-Sirpα DANA mutant antibody carries amino acid mutations to abrogate Fc binding to Fc receptors. Data for are from one representative experiment of two independent experiments with at least three technical replicates per group. Each symbol represents one mouse. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, by Ordinary One Way ANOVA with Tukey's multiple comparisons test.

Table S1 (related to Figure 1). Elevated SIRPA and neutrophil/monocyte associated genes in IBD biopsies.

	Crohn's Disease inflamed v uninflamed		Ulcerative Colitis inflamed v uninflamed	
Symbol	Log Fold Change	FDR	Log Fold Change	FDR
SIRPA	0.92525523	1.93E-05	0.64693961	8.82E-05
CSF3	0.69977411	0.00018332	0.6569778	0.00089511
CXCL8	1.50874913	3.12E-06	1.53507407	4.49E-05
S100A8	2.46943274	3.20E-06	2.67787325	3.75E-05
S100A9	1.45178803	6.94E-06	1.63951405	6.63E-05
VNN2	0.96334373	0.00204402	0.99420002	0.00298004
NCF2	1.13740821	3.80E-05	0.97764397	8.50E-05
FCGR2A	0.91239283	9.15E-05	0.61994322	0.00039825

Statistical analysis of transcriptional data of neutrophil/monocyte associated gene expression in inflamed and uninflamed UC/CD colonic biopsies. FDR is the false discovery rate. Mean fold change is represented in log format.

Table S2 (related to Figure 1). Elevated SIRPA and neutrophil/monocyte associated genes in baseline colonic biopsies of patients who failed with Vedolizumab or Infliximab.

	Biologic Non-responder vs Responder at Baseline			Vedolizumab Non-responder vs Responder at Baseline			Infliximab Non-responder vs Responder at Baseline		
Gene	Log Fold Change	P Value	FDR	Log Fold Change	P Value	FDR	Log Fold Change	P Value	FDR
SIRPA	0.333	0.020	0.288	0.334	0.056	0.333	0.020	0.288	0.334
S100A8	1.225	0.006	0.288	0.969	0.081	1.225	0.006	0.288	0.969
S100A9	0.830	0.008	0.288	0.668	0.090	0.830	0.008	0.288	0.668
VNN2	0.868	0.009	0.288	1.014	0.014	0.868	0.009	0.288	1.014
CXCL8	1.044	0.006	0.288	1.006	0.053	1.044	0.006	0.288	1.006
FCGR2A	0.769	0.018	0.288	0.940	0.032	0.769	0.018	0.288	0.940
NCF2	0.663	0.016	0.288	0.684	0.069	0.663	0.016	0.288	0.684
CSF3	0.463	0.024	0.288	0.491	0.068	0.463	0.024	0.288	0.491
NLRP3	0.603	0.004	0.288	0.663	0.045	0.603	0.004	0.288	0.663

Statistical analysis of transcriptional data of neutrophil/monocyte associated gene expression in baseline colonic biopsies of patients who responded or failed with Vedolizumab and Infliximab. FDR is the false discovery rate. Mean fold change is represented in log format.

Table S3 (related to Figure 3). Agonistic anti-SIRPα treatment reduces inflammatory genes in joint tissues.

	Isotype Control vs Naïve Mice		Agonistic aSIRPα vs Isotype Control	
Gene	Log Fold Change	FDR	Log Fold Change	FDR
Il1b	6.890	0.013	-5.164	0.054
Il6	5.120	0.009	-4.052	0.032
Ccl7	4.946	0.007	-2.810	0.011
Ccl8	4.018	0.012	-3.432	0.008
Ccl12	NA	NA	-2.082	0.048
Cxcl1	4.860	0.007	-2.961	0.007
Cxcl2	3.692	0.005	-3.292	0.018
Cxcl3	NA	NA	-2.475	0.113
Csf3	NA	NA	NA	NA
Csf3r	3.516	0.012	-2.201	0.035
Fcgr2b	2.079	0.004	-1.194	0.017
Clec4d	3.819	0.002	-3.265	0.005
Clec4e	3.780	0.009	-2.817	0.020
Cd68	6.890	0.013	-5.164	0.054
Cd80	5.120	0.009	-4.052	0.032
Ncf2	4.946	0.007	-2.810	0.011
Sirpa	4.018	0.012	-3.432	0.008

Statistical analysis of differential expression of genes related to myeloid cells and genes of cytokines and chemokines in naïve, Isotype control and Agonistic aSIRPα treated mice on Day7 after K/BxN serum transfer. FDR is the false discovery rate. Mean fold change is represented in log format.