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Supplementary Materials for

SLAMF7 engagement superactivates macrophages in acute and chronic inflammation

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The PDF file includes:

Figs. S1 to S17
Tables S1 and S2

Other Supplementary Material for this manuscript includes the following:

Data files S1 to S4

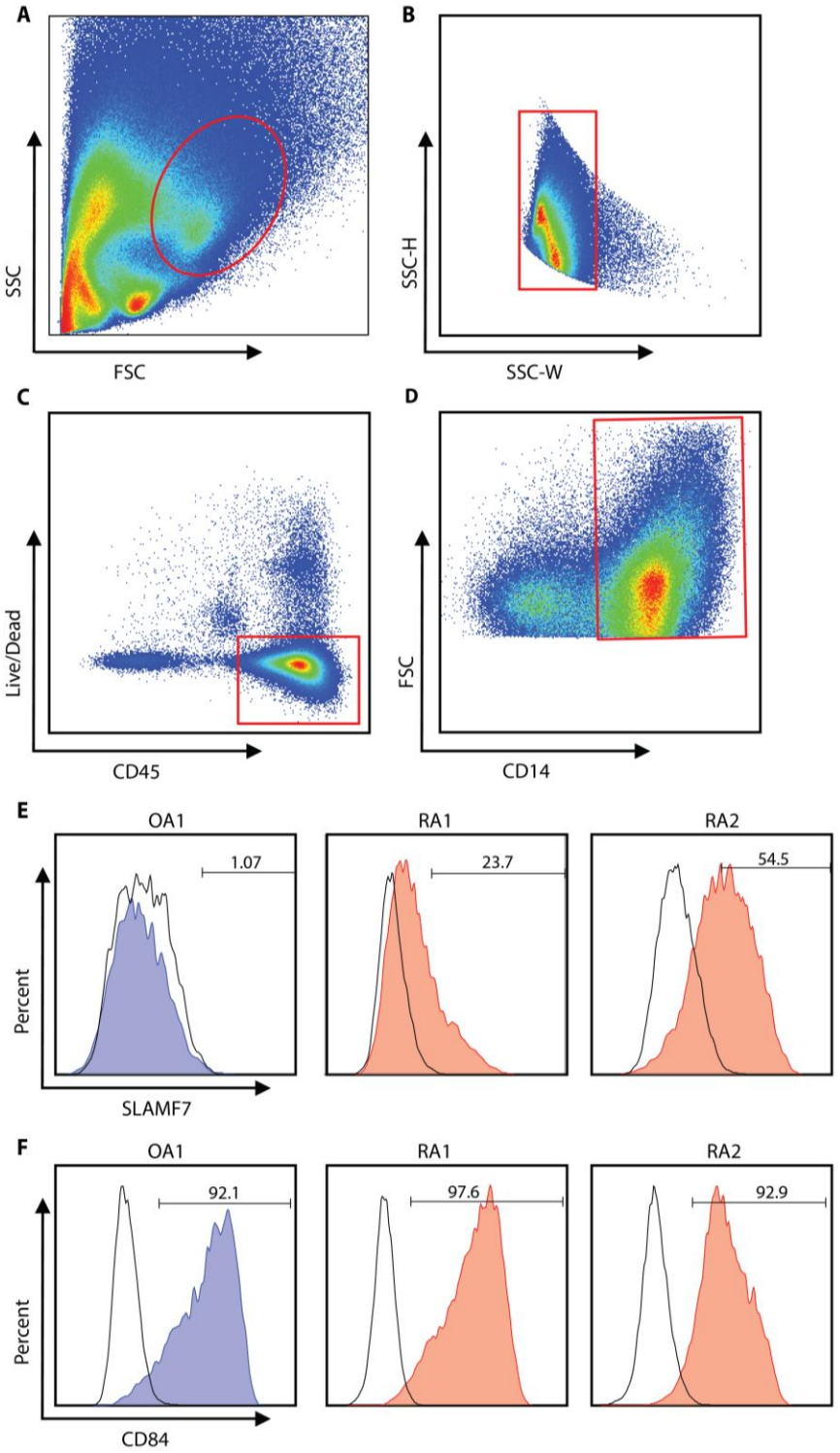

Figure S1. Gating for flow cytometric analysis of macrophages. A) Myeloid cells were selected based on forward and side scatter. B) Doublets were excluded with side scatter height and width. C) CD45+ cells were selected with exclusion of live/dead dye. D) CD14+ cells were selected for further analysis. Representative histograms with gating for E) SLAMF7 and F) CD84 expression are shown in CD14+ cells from patients with OA or RA. Black line, isotype control staining. Filled red or blue, specific antibody staining.

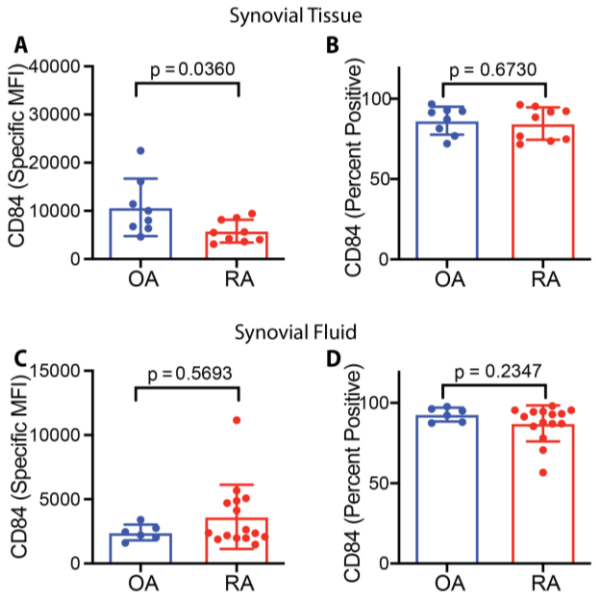

Figure S2. CD84 expression on synovial macrophages. A) Specific MFI for CD84, and B) percent of macrophages expressing CD84 in synovial tissue from patients with OA (n=8) or RA (n=9). C) Specific MFI for CD84 , and D) percent of macrophages expressing CD84 in synovial fluid from patients with OA (n=6) or RA (n=15). Data represent mean ± SD. The Mann-Whitney test was used for statistical comparisons.

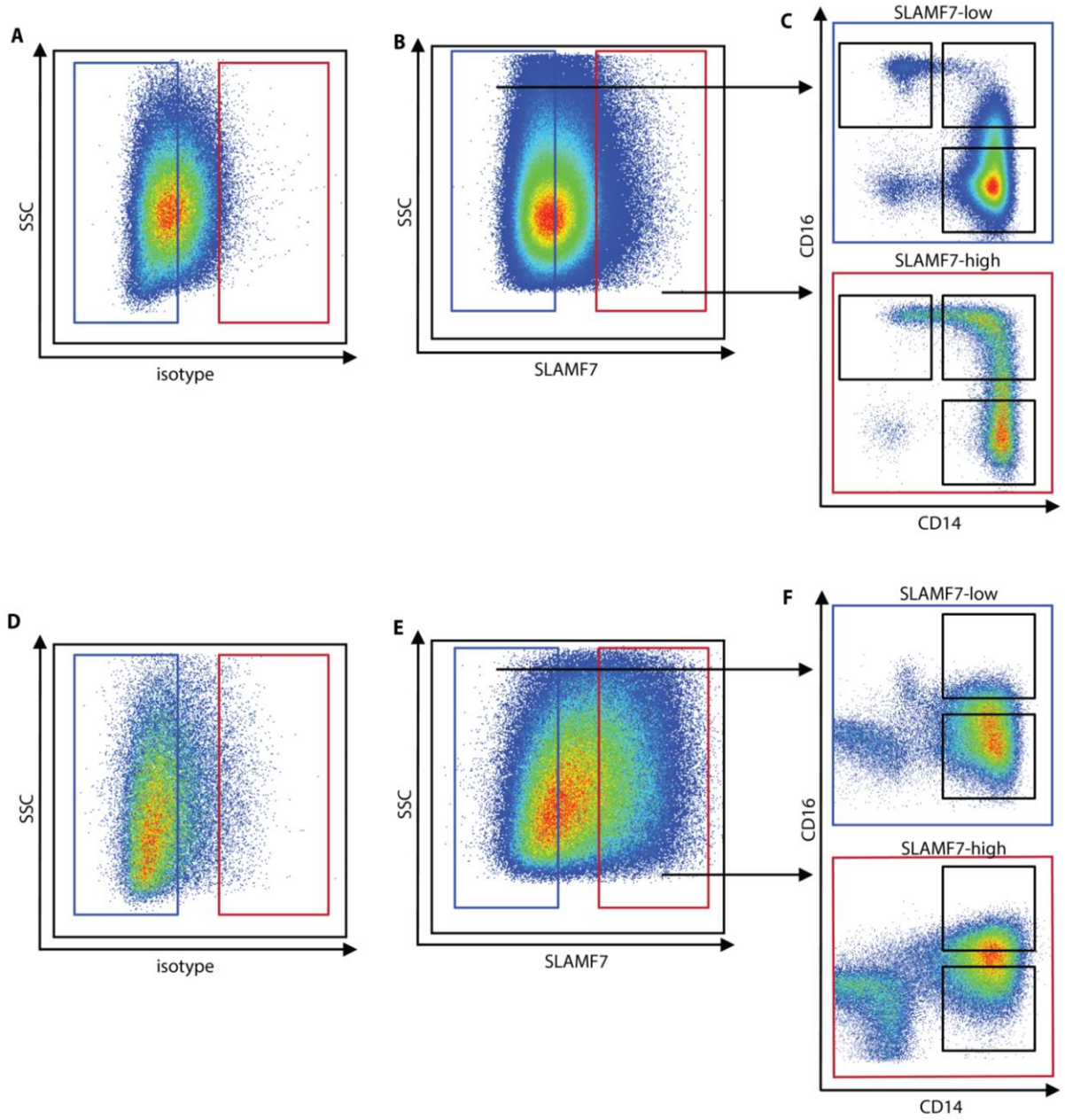

Figure S3. Gating strategy for cell sorting. Myeloid cells were selected by gating on live CD45+ cells as in Fig. S1, with exclusion of lymphocytes positive for CD3, CD19 or CD56. A) An isotype control was used to define the threshold for SLAMF7-positive cells from peripheral blood. B) Gating to sort SLAMF7-low and SLAMF7-high cells. C) Populations of monocytes defined by CD14 and CD16 expression were sorted from SLAMF7-low (outlined in blue) and SLAMF7-high (outlined in red) cells from peripheral blood. D) An isotype control was used to define the threshold for SLAMF7-positive cells from synovial fluid. E) Gating to sort SLAMF7-low and SLAMF7-high cells. F) Macrophage populations defined by CD14 and CD16 expression were sorted from SLAMF7-low (outlined in blue) and SLAMF7-high (outlined in red) cells from synovial fluid.

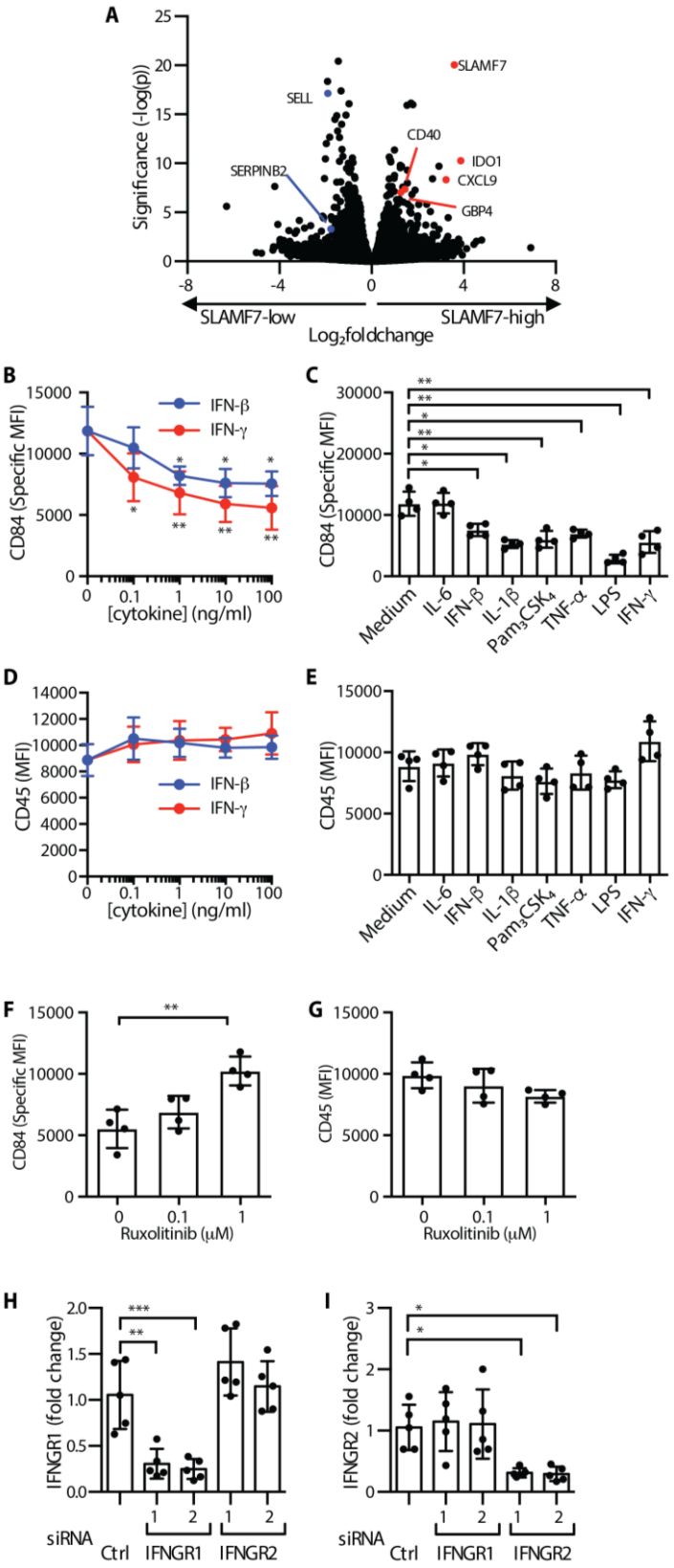

Figure S4. Interferon regulation of surface marker expression on macrophages. A) Differential gene expression for SLAMF7-high compared to SLAMF7-low CD14+CD16- cells from synovial fluid (n=4 donors). B) Specific MFI for CD84 on macrophages incubated with different doses of IFN-γ or IFN-β. C) Specific MFI for CD84 on macrophages incubated with 100 ng/ml of cytokines. IFN-β and IFN-γ results are the same as the

100 ng/ml dose in panel B. D) MFI for CD45 on macrophages incubated with different doses of IFN-γ or IFN-β. E) MFI for CD45 expression on macrophages incubated with 100 ng/ml of cytokines. IFN-β and IFN-γ results are the same as the 100 ng/ml dose in panel D. F-G) Macrophages were incubated with ruxolitinib or DMSO prior to IFN-γ treatment (10 ng/ml). Specific MFI for F) CD84 and MFI for G) CD45 was measured after 16h. Data represent mean ± SD of 4 donors. H-I) Monocytes were treated with siRNA control or siRNA targeting *IFNGR1* or *IFNGR2*, then potentiated with IFN-γ (10 ng/ml) for 24h. H) *IFNGR1* and I) *IFNGR2* were quantified by RT-PCR relative to macrophages treated with control siRNA alone. Data represent mean ± SD of 5 donors. Statistics were calculated using the one-way ANOVA with Dunnett's multiple comparisons test. Ctrl, control siRNA; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

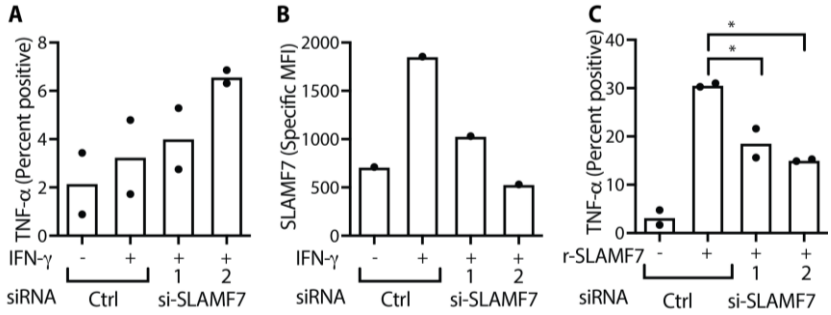

Figure S5. SLAMF7 is necessary for macrophage activation by r-SLAMF7. Macrophages were treated with siRNA control or siRNA targeting *SLAMF7*. They were then potentiated with IFN-γ (10 ng/ml) for 24 hours. A) TNF-α expression was quantified with intracellular staining or B) SLAMF7 expression was quantified with surface staining. C) After potentiation with IFN-γ, macrophages were stimulated with r-SLAMF7 (100 ng/ml) for 2.5h, and TNF-α was quantified with intracellular staining. Data in A and C represent mean of duplicate samples, and data in B represent specific MFI of samples from an experiment representative of at least 2 independent experiments. Statistics were calculated using the one-way ANOVA with Dunnett's multiple comparisons test. r-SLAMF7, recombinant SLAMF7 protein; *, $p < 0.05$.

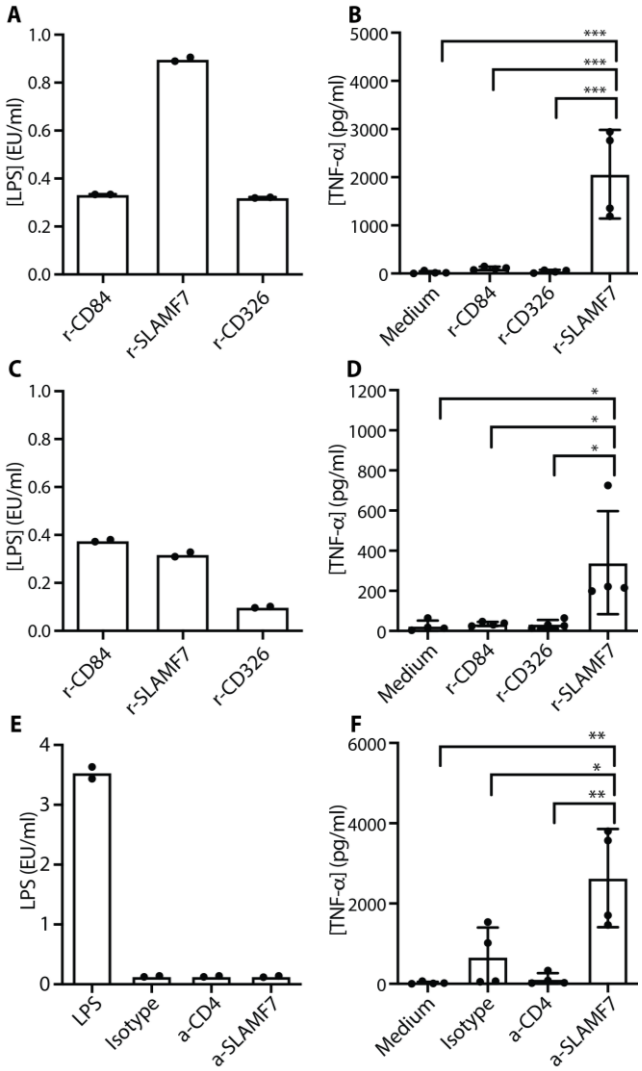

Figure S6. Validation of reagents used for SLAMF7 activation. A) Endotoxin levels were quantified in different protein preparations. B) Monocytes were potentiated with IFN-γ (10 ng/ml) for 24 hours, then stimulated with recombinant proteins for 3 hours. Secreted TNF-α was measured by ELISA. C) Endotoxin levels were quantified in different protein preparations after treatment with an endotoxin depletion column. D) Monocytes were potentiated with IFN-γ (10 ng/ml) for 24 hours, then stimulated with endotoxin-depleted recombinant proteins for 3 hours. Secreted TNF-α was measured by ELISA. E) Endotoxin levels were quantified in different antibodies or LPS as a control. F) Monocytes were potentiated with IFN-γ (10 ng/ml) for 24 hours, then stimulated with antibodies for 3 hours. Secreted TNF-α was measured by ELISA. Data in A, C, and E represent mean of duplicate samples. Data in B, D, and F represent mean ± SD of 4 donors. Statistics were calculated using the one-way ANOVA with Dunnett's multiple comparisons test. r-SLAMF7, recombinant SLAMF7 protein; r-CD84, recombinant CD84 protein; r-CD326, recombinant CD326 protein; isotype, mouse IgG2b, isotype antibody; a-CD4, anti-CD4 antibody; a-SLAMF7, anti-SLAMF7 antibody; EU, endotoxin unit; *, $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$.

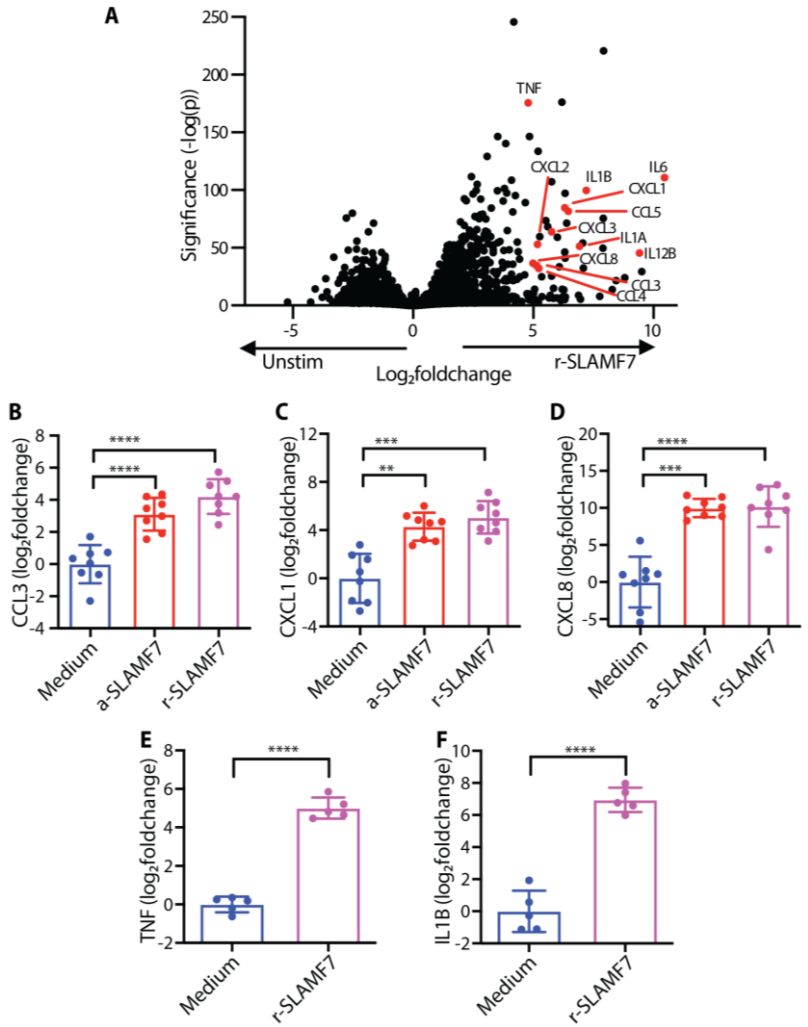

Figure S7. Inflammatory cascade driven by SLAMF7 engagement. Macrophages were potentiated with IFN-γ (10 ng/ml) for 24 hours prior to subsequent treatment with a-SLAMF7 (10 µg/ml) or r-SLAMF7 (1 µg/ml) for 4h. A) Differential gene expression for macrophages incubated with r-SLAMF7 for 4h (n=4 donors) compared to unstimulated macrophages (n=4 donors). B-D) RT-PCR was used to quantify expression of B) *CCL3*, C) *CXCL1*, and D) *CXCL8* relative to unstimulated macrophages. Data represent mean ± SD of 8 donors. E-F) Macrophages purified from synovial fluid of patients with RA were potentiated with IFN-γ (10 ng/ml) for 24 hours then stimulated with r-SLAMF7 (100 ng/ml) for 3h. RT-PCR was used to quantify expression of E) *TNF* and F) *IL1B* relative to unstimulated macrophages. Data represent mean ± SD of 5 donors. Statistics were calculated using the one-way ANOVA with Dunnett's multiple comparisons test. **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$; unstim, unstimulated; a-SLAMF7, anti-SLAMF7 antibody; r-SLAMF7, recombinant SLAMF7 protein.

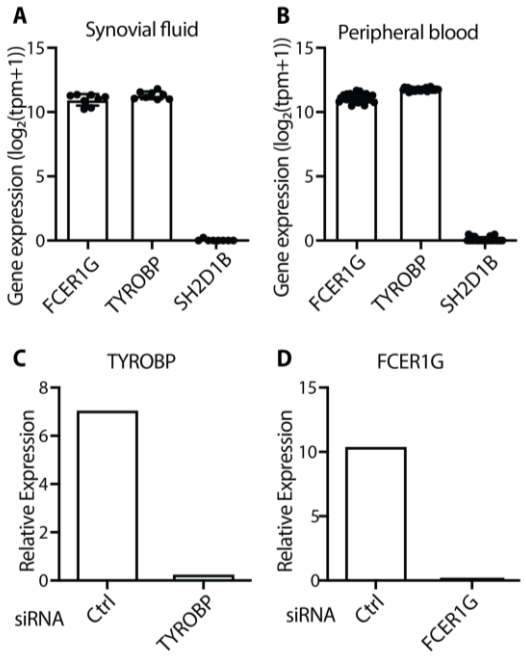

Figure S8. *FCER1G* and *TYROBP* are highly expressed by macrophages. A-B) Expression of *FCER1G*, *TYROBP*, and *SH2D1B* in CD14+CD16- macrophages from A) synovial fluid (n=8 samples) and B) peripheral blood (n=24 samples) in RNA-seq data from Fig. 2. Data represent mean ± SD. C) Macrophages were treated with a control siRNA or one targeting *FCER1G*. *FCER1G* relative to *GAPDH* using RT-PCR. D) Macrophages were treated with a control siRNA or one targeting *TYROBP*. *TYROBP* relative to *GAPDH* using RT-PCR. Data in C-D represent mean of duplicate samples. Tpm, transcripts per million.

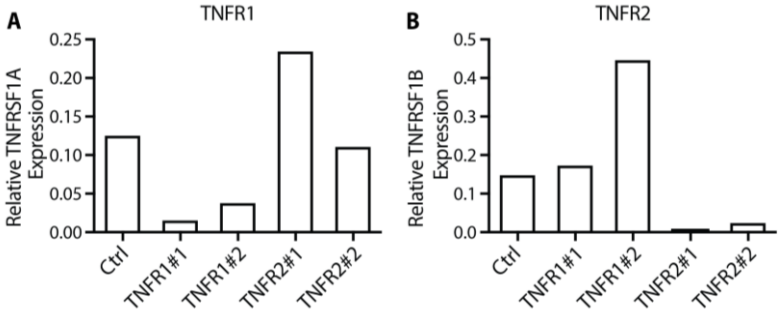

Figure S9. Silencing of *TNFRSF1A* and *TNFRSF1B* with siRNA. Gene expression of A) *TNFRSF1A* and B) *TNFRSF1B* relative to *GAPDH* in macrophages treated with control siRNA, or two different siRNAs targeting either *TNFRSF1A* or *TNFRSF1B*. Data represent the mean of duplicate samples.

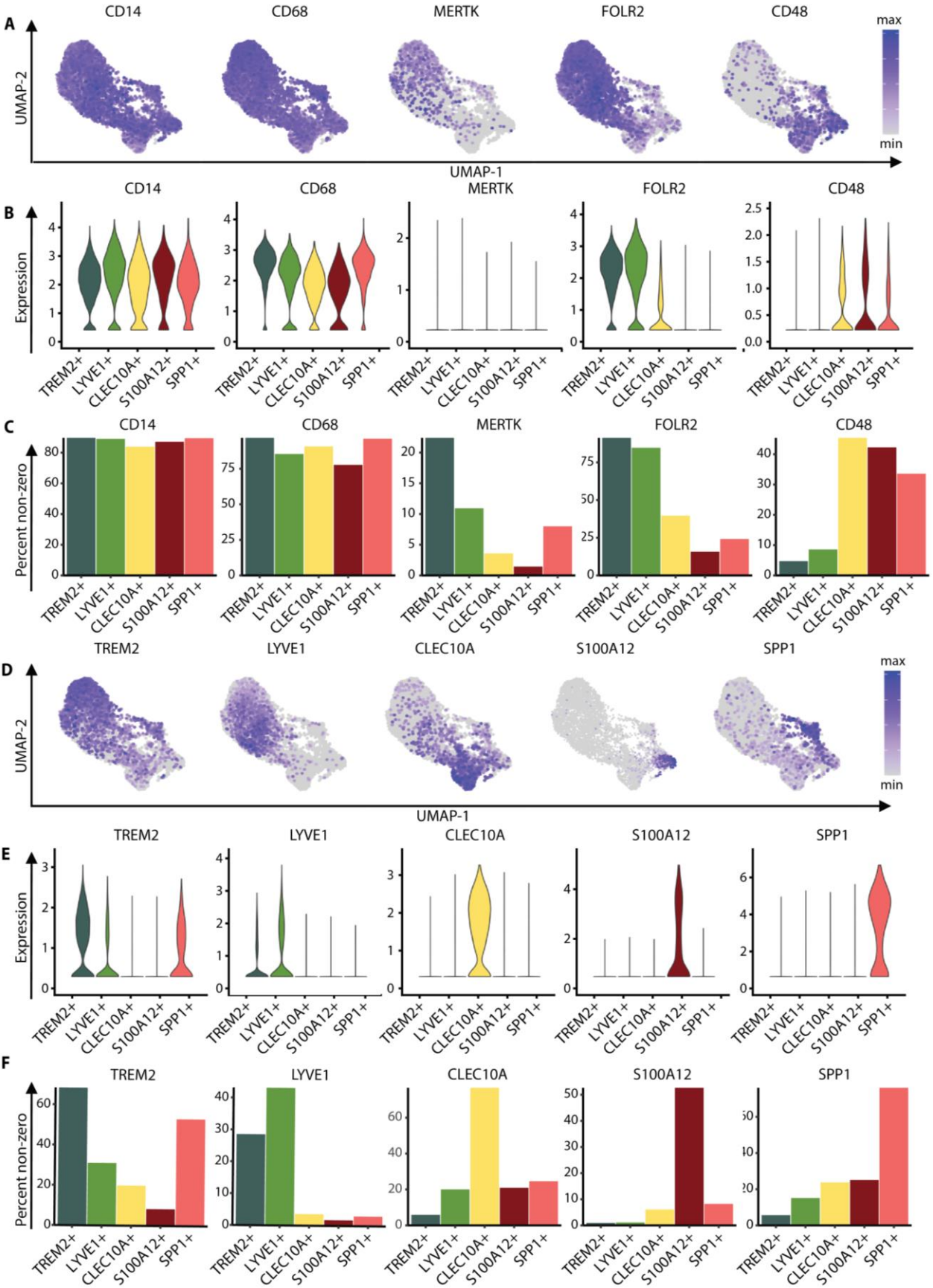

Figure S10. Macrophage populations in synovial tissue from healthy controls or patients with untreated rheumatoid arthritis. A) UMAP plots from Fig. 5D showing gene expression values in synovial macrophage

populations. B) Violin plots of gene expression in macrophage clusters from Fig. 5D. C) Percent of cells with expression of genes in macrophage clusters from Fig. 5D. D) UMAP plots from Fig. 5D showing gene expression values in synovial macrophage populations. E) Violin plots of gene expression in macrophage clusters from Fig. 5D. F) Percent of cells with expression of genes in macrophage clusters from Fig. 5D.

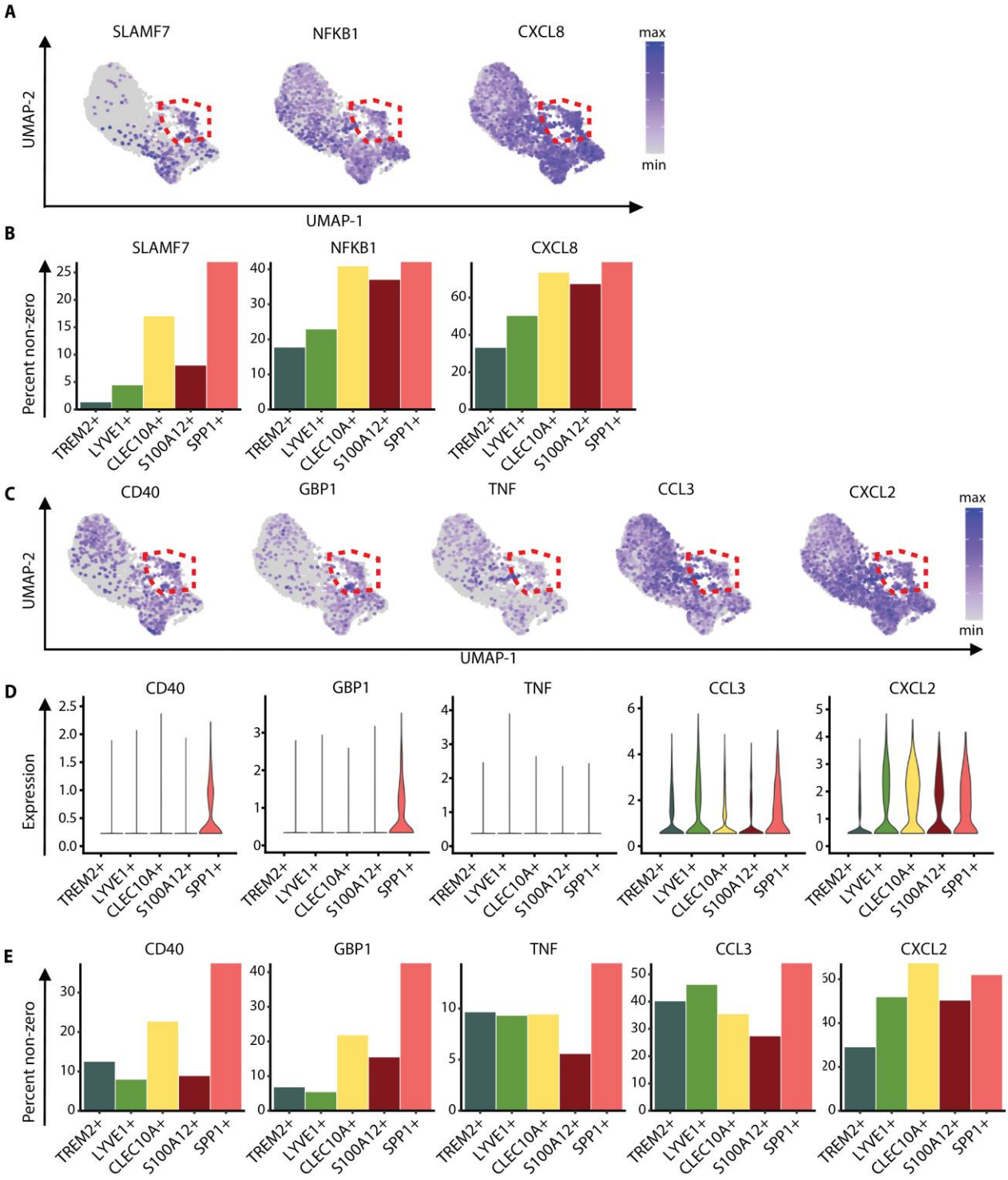

Figure S11. Evidence for SLAMF7 activation in macrophages from patients with untreated rheumatoid arthritis. A) UMAP plots from Fig. 5D showing gene expression values in synovial macrophage populations. B) Percent of cells with expression of genes in macrophage clusters from Fig. 5D. C) UMAP plots from Fig. 5D showing gene expression values in synovial macrophage populations. D) Violin plots of gene expression in macrophage clusters from Fig. 5D. E) Percent of cells with expression of genes in macrophage clusters from Fig. 5D.

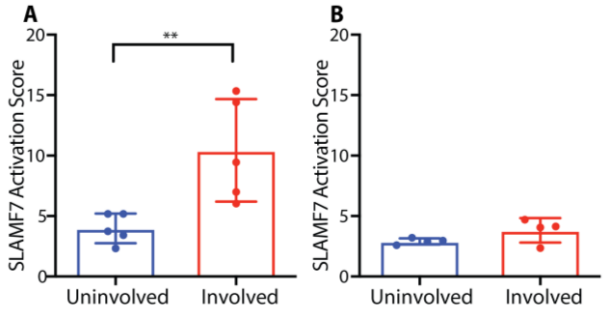

Figure S12. High SLAMF7 activation in ileal macrophages from samples with high GIMATS module intensity scores. A) SLAMF7 activation score for donors reported to have a high GIMATS module intensity score (n=5). B) SLAMF7 activation score for donors reported to have a low GIMATS module intensity score (n=4). Data represent mean ± SD. The paired t-test was used for statistical comparisons. **, $p \leq 0.01$.

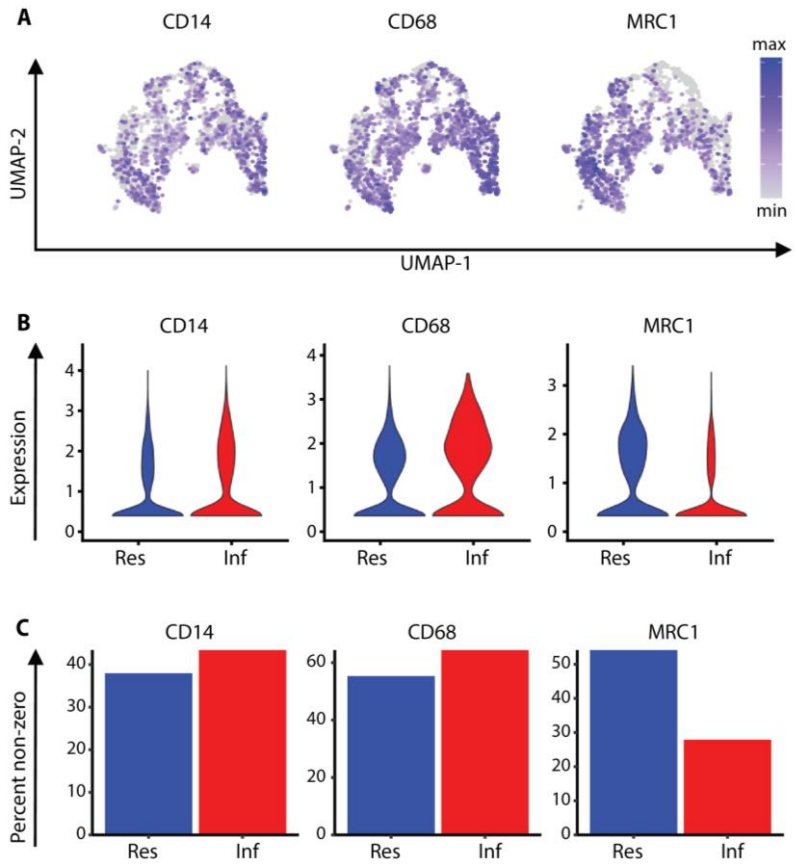

Figure S13. Macrophage populations in ileal tissue from patients with inflammatory bowel disease. A) UMAP plot from Fig. 6D showing gene expression values in ileal macrophage populations. B) Violin plots of gene expression in macrophage clusters from Fig. 6D. C) Percent of cells with expression of genes in macrophage clusters from Fig. 6D. Res, resident macrophages; Inf, Inflammatory macrophages.

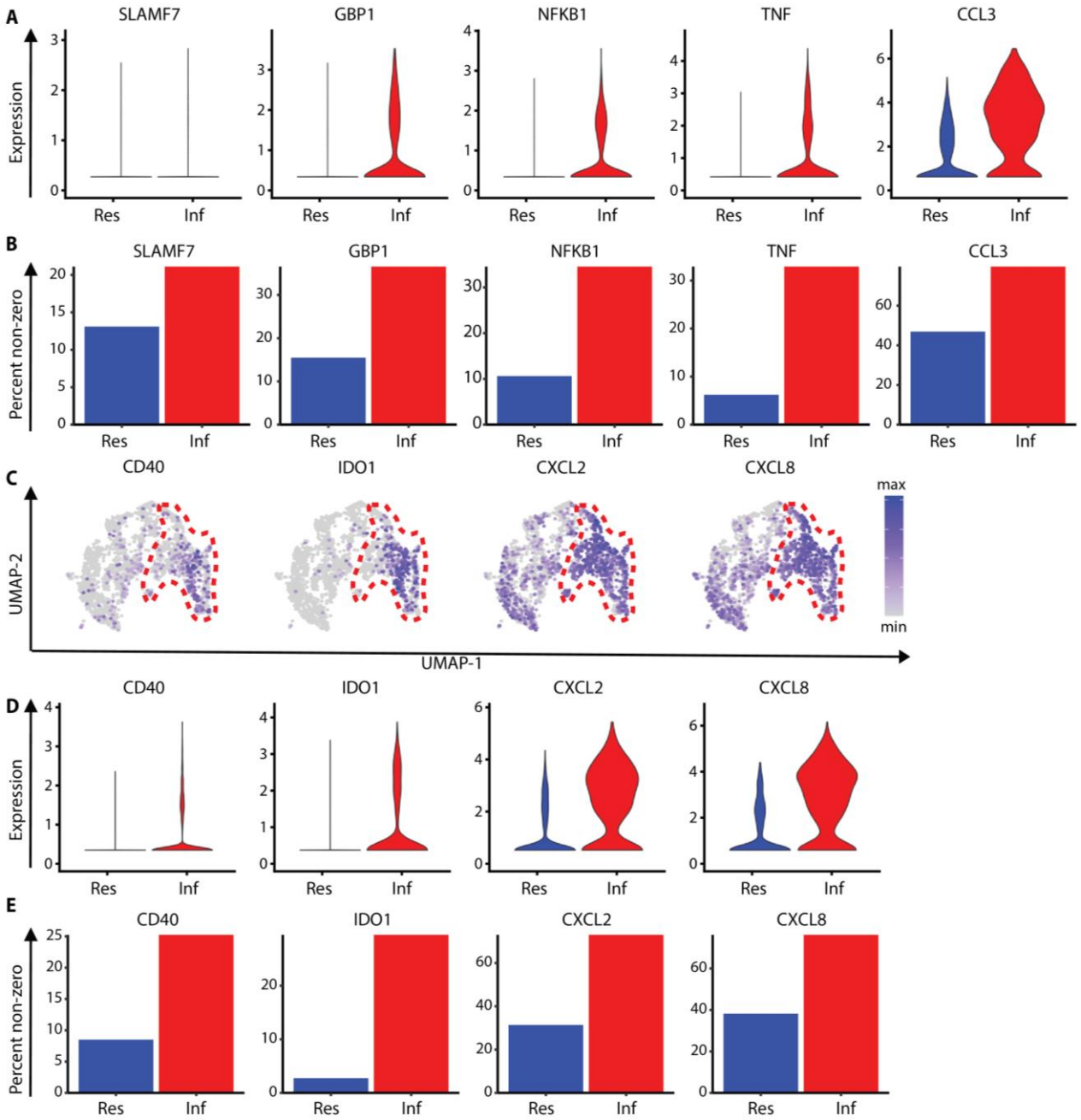

Figure S14. Evidence for SLAMF7 activation in macrophages from patients with inflammatory bowel disease. A) Violin plots of gene expression in macrophage clusters from Fig. 6D. B) Percent of cells with expression of genes in macrophage clusters from Fig. 6D. Res, resident macrophages; Inf, Inflammatory macrophages. C) UMAP plot from Fig. 6D showing gene expression values in ileal macrophage populations. D) Violin plots of gene expression in macrophage clusters from Fig. 6D. E) Percent of cells with expression of genes in macrophage clusters from Fig. 6D. Res, resident macrophages; Inf, Inflammatory macrophages.

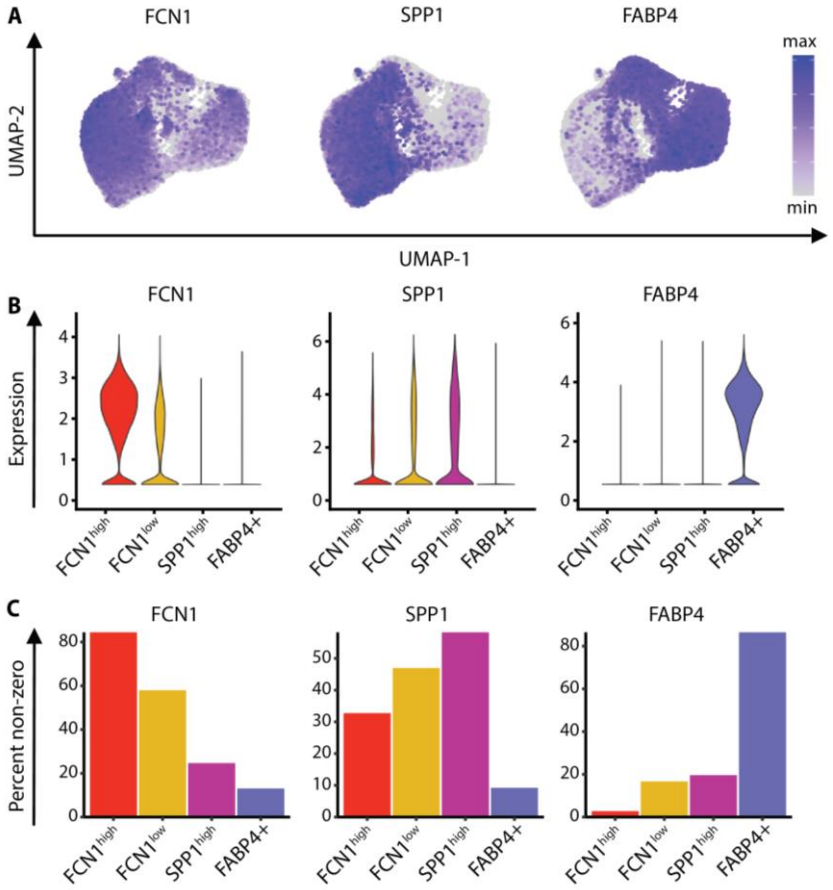

Figure S15. Macrophage populations in bronchoalveolar lavage from patients with COVID-19. A) UMAP plot from Fig. 6G showing gene expression in lung macrophage populations. B) Violin plots of gene expression in macrophage clusters from Fig. 6G. C) Percent of cells with expression of genes in macrophage clusters from Fig. 6G.

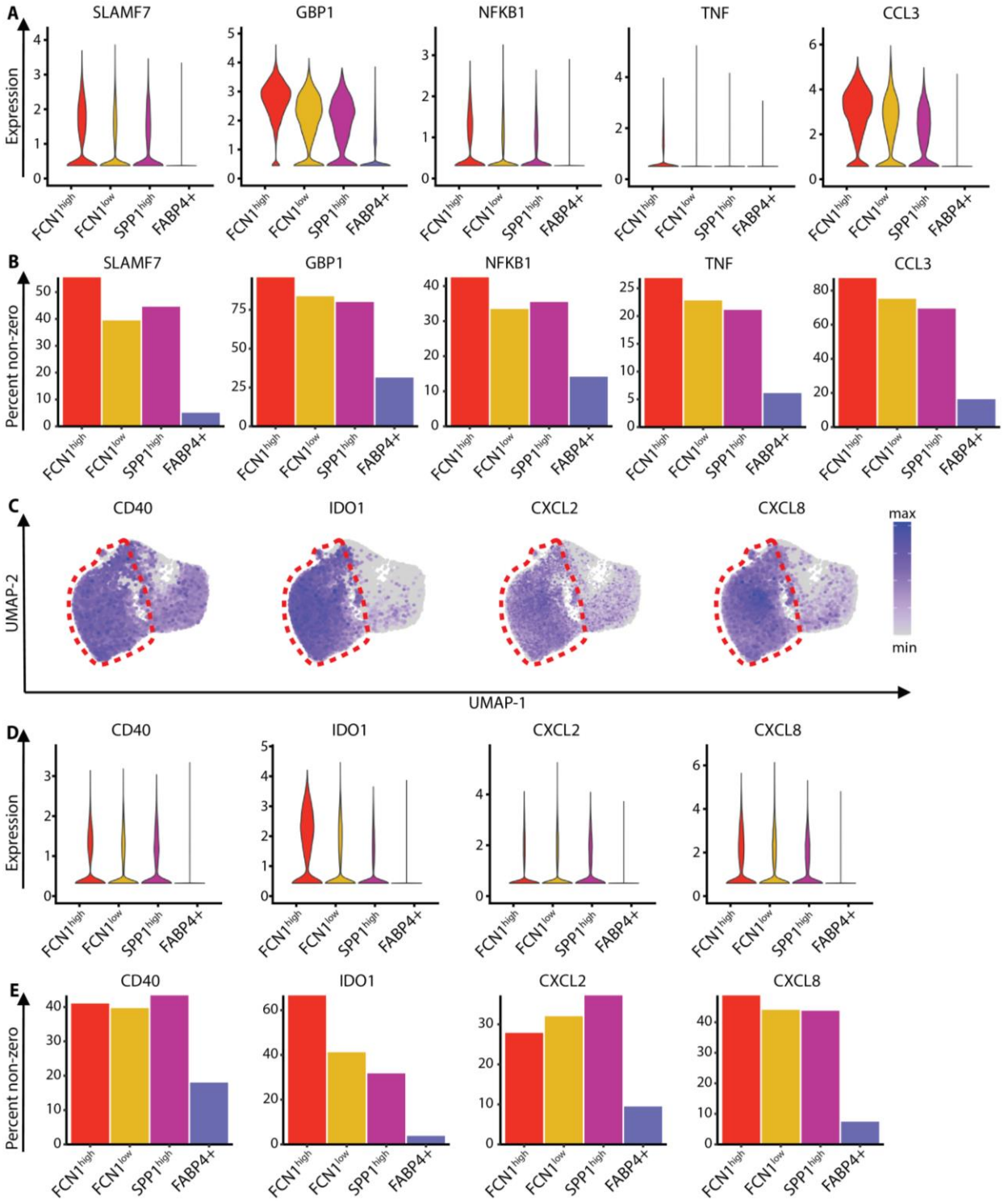

Figure S16. Evidence for SLAMF7 activation in macrophages from patients with COVID-19 infection. A) Violin plots of gene expression in macrophage clusters from Fig. 6G. B) Percent of cells with expression of genes in macrophage clusters from Fig. 6G. C) UMAP plot from Fig. 6G showing gene expression in lung macrophage populations. D) Violin plots of gene expression in macrophage clusters from Fig. 6G. E) Percent of cells with expression of genes in macrophage clusters from Fig. 6G.

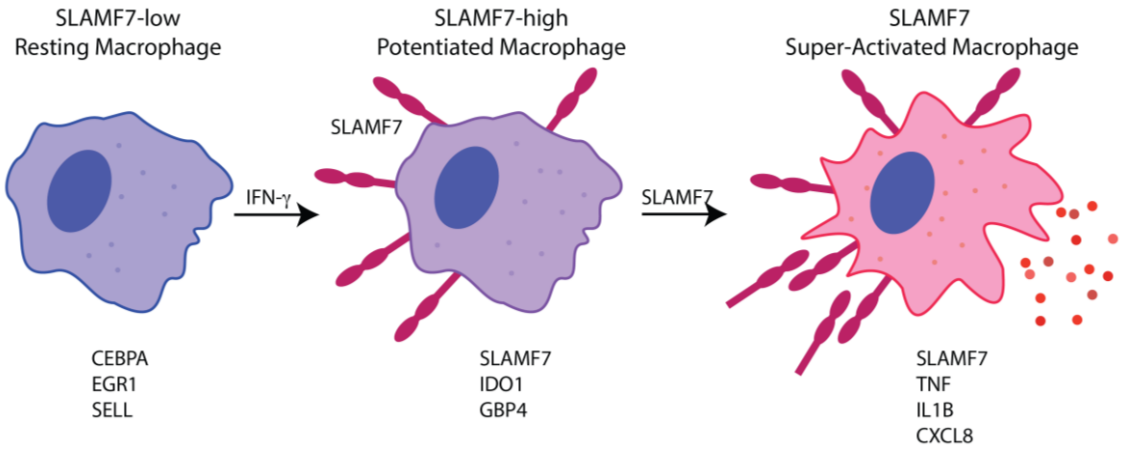

Figure S17. Model for SLAMF7 super-activation of macrophages. Resting macrophages express anti-inflammatory transcription factors, but low levels of SLAMF7. After potentiation with IFN-γ, these cells are reprogrammed to a state with high levels of *SLAMF7* and other IFN-induced genes. Subsequent engagement of the SLAMF7 receptor on these IFN-γ potentiated macrophages drives profound activation of inflammatory pathways and production of cytokines. An autocrine TNF-α signaling loop further amplifies this inflammatory pathway, resulting in exuberant cytokine release characteristic of the SLAMF7 superactivated macrophage.

Table S1: Demographic information for donors

ID	Age	Sex	Disease	Specimen Type	Figure	Platform	Analysis
OA1	73	F	Osteoarthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
OA2	61	F	Osteoarthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
OA3	64	M	Osteoarthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
OA4	60	F	Osteoarthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
OA5	70	F	Osteoarthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
OA6	58	F	Osteoarthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
OA7	85	F	Osteoarthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
OA8	66	F	Osteoarthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA1	59	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA2	68	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA3	71	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA4	67	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA5	68	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA6	36	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA7	86	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA8	28	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
RA9	76	F	Rheumatoid arthritis	Synovial tissue	Fig. 1	Flow cytometry	Validation of SLAMF7 protein expression
Donor 1	67	M	Rheumatoid arthritis	Synovial fluid, peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 2	45	F	Rheumatoid arthritis	Synovial fluid, peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 3	42	M	Rheumatoid arthritis	Synovial fluid, peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 4	60	F	Rheumatoid arthritis	Synovial fluid	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 6	59	F	Rheumatoid arthritis	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 7	59	F	Rheumatoid arthritis	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 8	37	M	Rheumatoid arthritis	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 9	67	F	Rheumatoid arthritis	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7

Donor 10	67	M	Healthy control	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 11	45	F	Osteoarthritis	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 12	55	F	Healthy control	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 13	57	F	Healthy control	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor 14	74	F	Healthy control	Peripheral blood	Fig. 2	RNA-seq	Analysis of sorted cells with high or low SLAMF7
Donor A	22	F	Healthy control	Peripheral blood	Fig. 3	RNA-seq	Analysis of cells stimulated with SLAMF7 *in vitro*
Donor B	22	F	Healthy control	Peripheral blood	Fig. 3	RNA-seq	Analysis of cells stimulated with SLAMF7 *in vitro*
Donor C	23	F	Healthy control	Peripheral blood	Fig. 3	RNA-seq	Analysis of cells stimulated with SLAMF7 *in vitro*
Donor D	70	F	Osteoarthritis	Peripheral blood	Fig. 3	RNA-seq	Analysis of cells stimulated with SLAMF7 *in vitro*

Table S2: Primer List

Primer	Sequence	
CCL3	Forward:	CGGCAGATTCCACAGAATTTC
	Reverse:	AGGTCGCTGACATATTTCTGG
CXCL1	Forward:	AACCGAAGTCATAGCCACAC
	Reverse:	CCTCCCTTCTGGTCAGTTG
CXCL8	Forward:	ATACTCCAAACCTTTCCACCC
	Reverse:	TCTGCACCCAGTTTTCCTTG
FCER1G	Forward:	TCGACTGAAGATCCAAGTGC
	Reverse:	ACCGCATCTATTCTAAAGCTACTG
GAPDH	Forward:	AATCCCATCACCATCTTCCAG
	Reverse:	AAATGAGCCCCAGCCTTC
IFNGR1	Forward:	CGATTATGATCCCGAAACTACCTG
	Reverse:	GGATACTGGAATCGCTAACTGG
IFNGR2	Forward:	CATTTTCGTTGCTGTCGGTG
	Reverse:	CTCTAAGATGGGCTGAGTTGG
IL1B	Forward:	ATGCACCTGTACGATCACTG
	Reverse:	ACAAAGGACATGGAGAACACC
SLAMF7	Forward:	AAACTTCTCAAGCCCCATCC
	Reverse:	CAGAAACCAAAGAAATAGCCCC
TNF	Forward:	ACTTTGGAGTGATCGGCC
	Reverse:	GCTTGAGGGTTTGCTACAAC
TNFSFR1A	Forward:	TGCCAGGAGAAACAGAACAC
	Reverse:	TCCTCAGTGCCCTTAACATTC
TNFSFR1B	Forward:	GTCCACACGATCCCAACAC
	Reverse:	TGTCACACCCACAATCAGTC
TYROBP	Forward:	GACCCGGAAACAGCGTATC
	Reverse:	TCGGGCTCATTTGTAATACGG