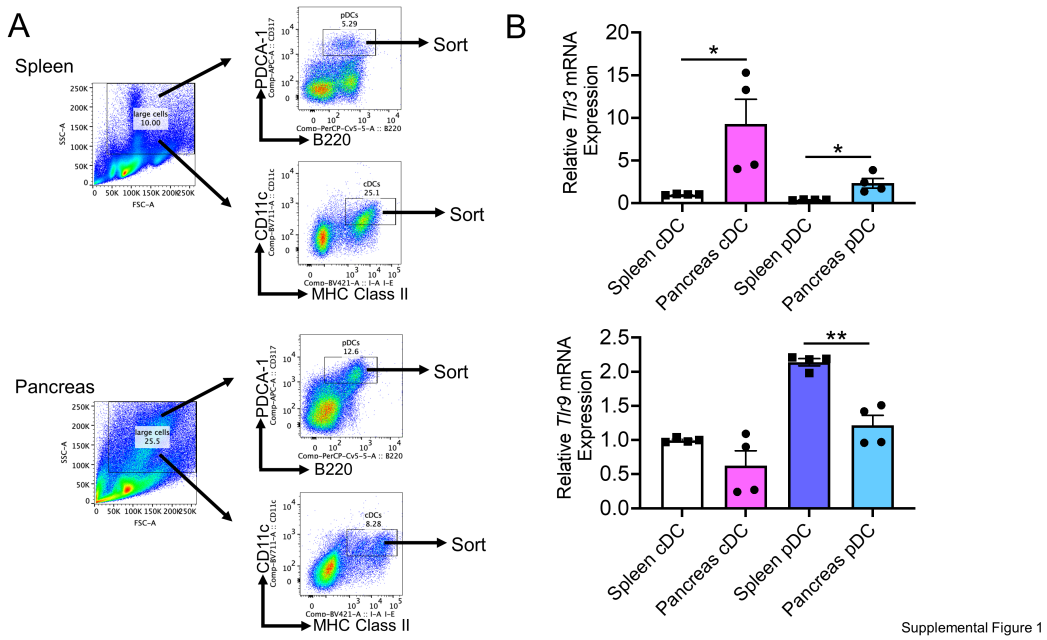
Supplemental Material

A Positive Cytokine/Chemokine Feedback Loop Establishes Plasmacytoid Dendritic Cell-Driven Autoimmune Pancreatitis in IgG4-Related Disease

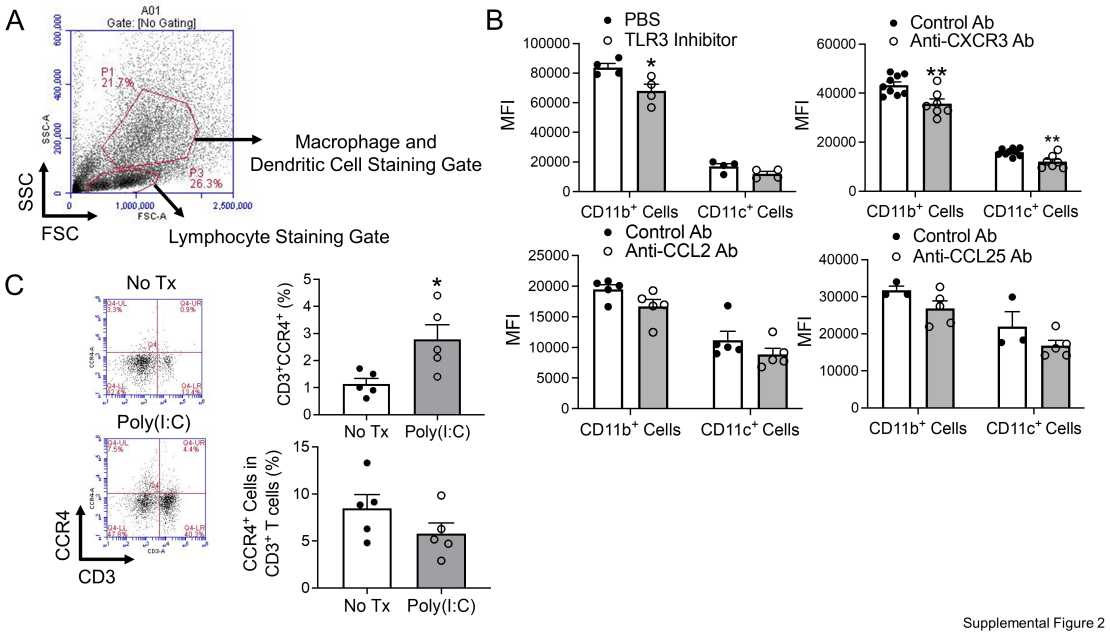
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Supplemental Figure 1. mRNA expression of *Tlr3* and *Tlr9* in conventional and plasmacytoid dendritic cells.

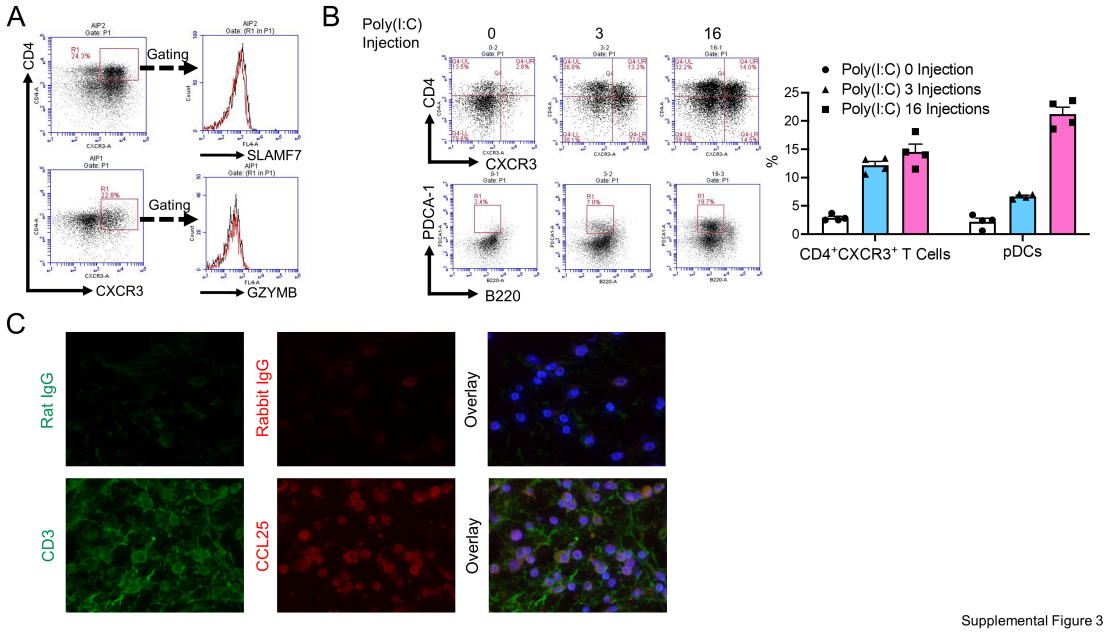
(A) FACS-sorting gating strategies used for isolation of plasmacytoid dendritic cells (pDCs) and conventional DCs from pancreases and spleens of untreated MRL/MpJ mice (n=4). **(B)** Expression of *Tlr3* and *Tlr9* mRNA in each DC subset determined by quantitative reverse transcription polymerase chain reaction. Each dot corresponds to the value in an individual mouse. Results shown are combined data of two independent experiments and are expressed as means + standard error. Statistical analyses were performed using unpaired two-tailed Student's t test. *P<0.05, **P<0.01.



Supplemental Figure 2

Supplemental Figure 2. Percentages of CD3$^+$C-C chemokine receptor 4$^+$ T cells in the pancreas.

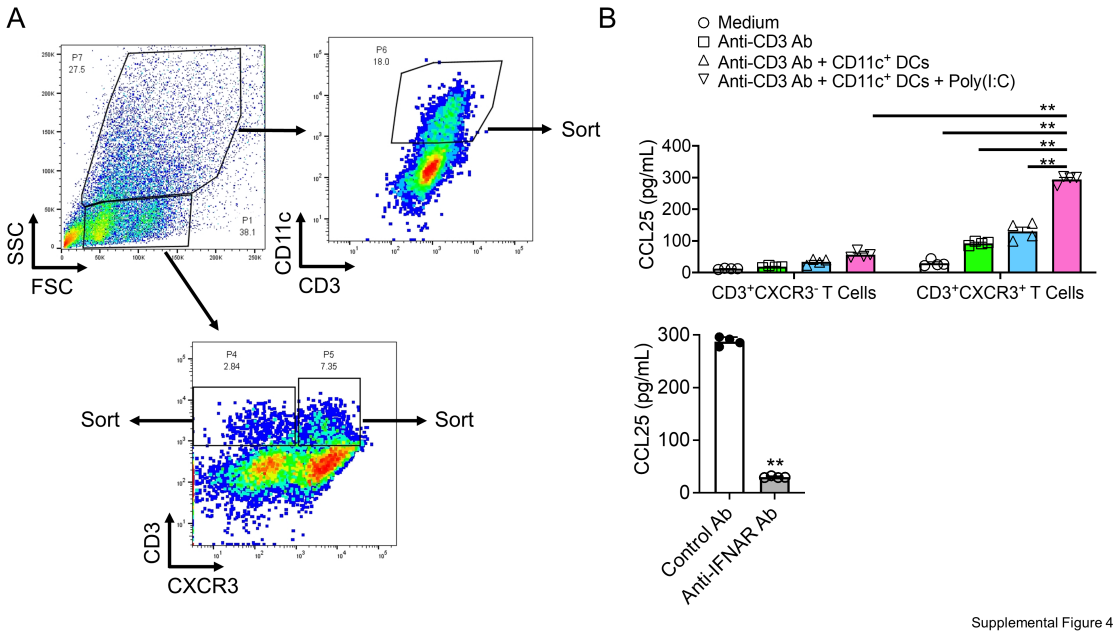
Groups of MRL/MpJ mice were treated as described in Figures 1, 3, 4, 6, and 7. After sacrifice pancreases were processed for acquisition of pancreatic mononuclear cells (PMNCs). **(A)** Flowcytometry gating for analysis of stained lymphocytes, or macrophages and dendritic cells among PMNCs. **(B)** Mean fluorescence intensity (MFI) in CD11b$^+$ or CD11c$^+$ cells. **(C)** Groups of MRL/MpJ mice were treated as described in Figure 3. After sacrifice pancreases were processed for acquisition of PMNCs. The percentages of pancreatic CD3$^+$C-C chemokine receptor 4 (CCR4)$^+$ T cells within PMNCs and the percentages of CCR4$^+$ cells within CD3$^+$ T cells; left panel: representative flowcytometric analyses; right panel: bar graphs of cumulative data from individual mice. Each dot in (B) and (C) corresponds to the value in individual mice. Statistical analyses were performed using unpaired two-tailed Student's t test. Results are expressed as means + standard error. *P<0.05, **P<0.01.



Supplemental Figure 3. Early and late accumulation of CD4$^+$C-X-C motif chemokine receptor 3$^+$ T cells and plasmacytoid dendritic cells in the pancreas during autoimmune pancreatitis development.

(A) MRL/MpJ mice were treated as described in Figure 3. After sacrifice pancreases were processed for acquisition of pancreatic mononuclear cells (PMNCs). PMNCs were stained with Alexa Fluor 488-conjugated anti-CD4 Ab, PE-conjugated anti-C-X-C motif chemokine receptor 3 (CXCR3) Ab, in combination with APC-conjugated anti-SLAMF7 Ab or APC-conjugated anti-granzyme B (GZYMB) Ab. Cells were then subjected to gated flowcytometric analysis as indicated to determine expression of cell-surface SLAMF7 and intracellular GZYMB (right panels; control Ab, black lines, anti-SLAMF7 Ab or anti-GZYMB Ab, red lines). **(B)** Groups of MRL/MpJ mice were treated as described in Figure 3, but in this case PMNCs were extracted from polyinosinic-polycytidylic acid (poly(I:C))-treated mice for a total of three times (n=4) or 16 times (n=4); in addition, PMNCs were extracted from untreated MRL/MpJ mice (n=4) to obtain base line data. PMNCs were stained with PE-conjugated anti-PDCA-1 Ab and FITC-conjugated anti-B220 Ab and were stained with PE-conjugated anti-CXCR3 Ab and Alexa Fluor 488-conjugated anti-CD4 Ab to evaluate accumulation of pDCs and CD4$^+$CXCR3$^+$ T cells, respectively. Left panel: representative flowcytometric analyses; right panel: bar graphs of cumulative data from individual mice. Each dot corresponds to the value in one mouse. Results are expressed as means + standard error. **(C)** MRL/MpJ mice

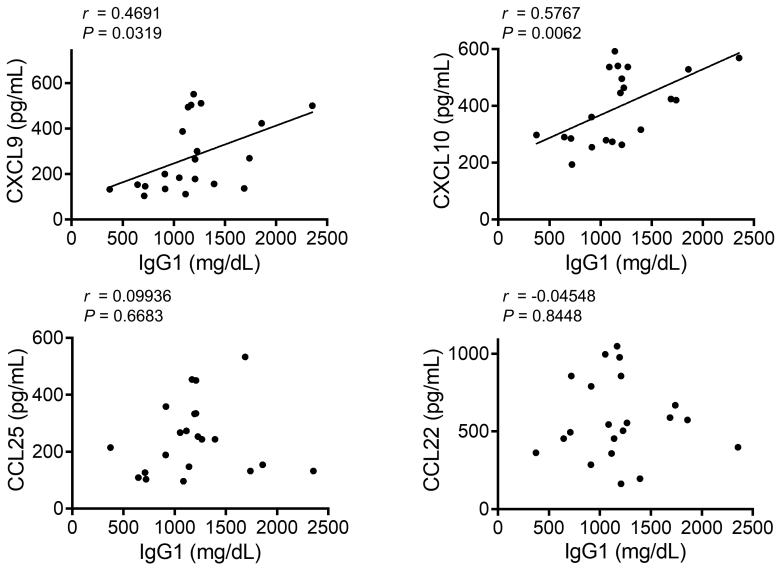
were treated as described in Figure 3. After sacrifice mouse pancreases were processed for tissue fixation and staining with anti-C-C motif chemokine ligand 25 (CCL25) Ab (red fluorescence) and anti-CD3 Ab (green fluorescence); rat IgG and rabbit IgG were used as isotype-matched control Abs. Results shown are the representative images in three independent experiments. Magnification X1200.



Supplemental Figure 4

Supplemental Figure 4. $CD3^+$C-X-C motif chemokine receptor 3^+ T cells produce C-C motif chemokine ligand 25 in response to IFN-α secreted by $CD11c^+$ dendritic cells.

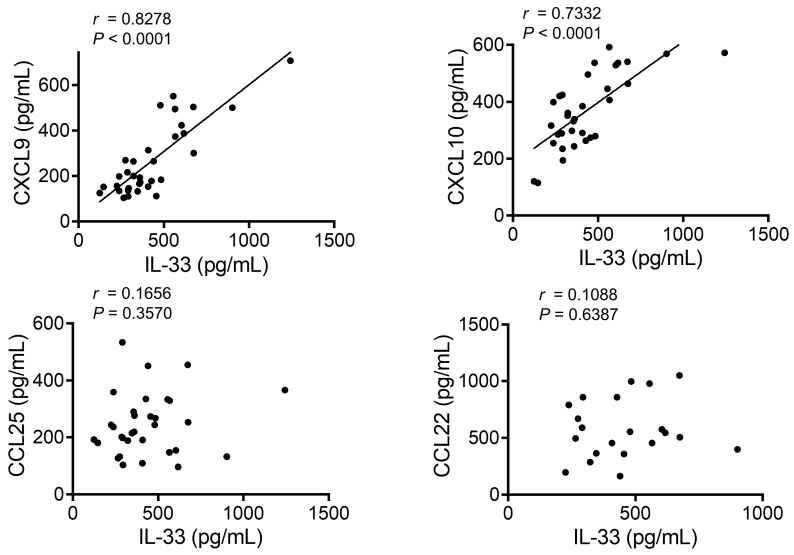
(A) FACS-sorting gating strategy for acquisition of $CD3^+$C-X-C motif chemokine receptor 3 $(CXCR3)^+$ T cells, $CD3^+CXCR3^-$ T cells, and $CD11c^+$ dendritic cells (DCs). **(B)** Pancreatic mononuclear cells (PMNCs) were isolated from pancreases of MRL/MpJ mice (n=3) administered polyinosinic-polycytidylic acid (poly(I:C)) twice a week for a total of 3 times by intraperitoneal injection. PMNCs were subjected to FACS-sorting to acquire purified $CD3^+CXCR3^+$ T cells, $CD3^+CXCR3^-$ T cells, and $CD11c^+$ DCs after which they were cultured for 48 hours (at $1X10^5$/mL) in quadruplicate as indicated in the presence of anti-CD3 Ab (5 μg/mL) alone or with poly(I:C)(25 μg/mL); in addition, in some experiments, anti-IFN αβ receptor Ab (anti-IFNAR Ab, 100 μg/mL) or control Ab was added to the culture. Cell culture supernatants obtained from each well were subjected to enzyme-linked immunosorbent assay for measurement of CCL25. Each dot represents the CCL25 concentration value derived from each well. Results shown are representative one of two independent experiments and are expressed as means + standard error. Statistical analyses were performed using Kruskal–Wallis test and Bonferroni-corrected Mann-Whitney U test. **P<0.01.



Supplemental Figure 5

Supplemental Figure 5. Co-relation between serum IgG1 levels and those of chemokines in patients with autoimmune pancreatitis and IgG4-related disease.

Serum samples of patients with autoimmune pancreatitis and IgG4-related disease (AIP/IgG4-RD) were prepared as described in Figure 11. Serum levels of IgG1, C-X-C motif chemokine ligand 9 (CXCL9), CXCL10, C-C motif chemokine ligand 25 (CCL25), and CCL22 in patients with AIP/IgG4-RD were measured by enzyme-linked immunosorbent assay. Each dot represents one patient. P-values and correlation coefficient (r) values, as determined by the Spearman's rank correlation test.



Supplemental Figure 6

Supplemental Figure 6. Co-relation between serum IL-33 levels and those of chemokines in patients with autoimmune pancreatitis and IgG4-related disease.

Serum samples of patients with autoimmune pancreatitis and IgG4-related disease (AIP/IgG4-RD) were prepared as described in Figure 11. Serum levels of IL-33, C-X-C motif chemokine ligand 9 (CXCL9), CXCL10, C-C motif chemokine ligand 25 (CCL25), and CCL22 in patients with AIP/IgG4-RD were measured by enzyme-linked immunosorbent assay. Each dot represents one patient. P-values and correlation coefficient (r) values, as determined by Spearman's rank correlation test, are shown.

Supplemental Table 1. Reagents, sources and identifier.

TLR ligands and inhibitors	**Sources**	**Identifier**
TLR3/ds RNA complex inhibitor	Calbiochem	Cat#614310
Poly(I:C)	InvivoGen	Cat#tlrl-pic
CpG	InvivoGen	Cat#tlrl-1585
PCR primers		
Target	**Sources**	**Identifier**
mouse TLR3	Qiagen	Cat#QT00122983
mouse TLR9	Qiagen	Cat#QT01043049
Abs for *in vivo* and *in vitro* studies		
Ab (Clone)	**Sources**	**Identifier**
Anti-mouse BST2 Ab (120G8)	Dendritics	Cat#DDX0390
Anti-mouse IFNAR Ab (MAR1-5A3)	BD Biosciences	Cat#561183
Anti-mouse ST2 Ab (245707)	R&D Systems	Cat#MAB10041
Anti-mouse CXCR3 Ab (CXCR3-173)	Bio X Cell	Cat#BE0249
Anti-mouse CCL2 Ab (2H5)	Bio X Cell	Cat#BE0185
Anti-mouse CCL25 Ab (Polyclonal)	R&D Systems	Cat#AF-481-NA
Hamster IgG (Isotype Control Ab)	Bio X Cell	Cat#BE0091
Rat IgG (Isotype Control Ab)	Sigma-Aldrich	Cat#14131
Mouse IgG (Isotype Control Ab)	Sigma-Aldrich	Cat#15381
Goat IgG (Isotype Control Ab)	Invitrogen	Cat#02-6202
Anti-mouse IFN-α/β receptor Ab (Polyclonal)	R&D Systems	Cat#AF3039
Goat IgG (Isotype Control Ab)	Invitrogen	Cat#02-6202
Anti-mouse CD3 Ab (145-2C11)	Invitrogen	Cat#16-0031-82
Abs for immunofluorescence and immunohistochemical studies		
Ab (Clone)	**Sources**	**Identifier**
Anti-mouse CCL25 Ab (Polyclonal)	Novus Biologicals	Cat#NBP1-40993
Anti-mouse CD3 Ab (CD3-12)	Abcam	Cat#ab11089
Alexa Fluor 546-Anti-Rabbit IgG Ab	Invitrogen	Cat#A11010
Alexa Fluor 488-Anti-Rat IgG Ab	Invitrogen	Cat#A21210
Rat IgG (Isotype Control Ab)	Sigma-Aldrich	Cat#14131
Rabbit IgG (Isotype Control Ab)	Sigma-Aldrich	Cat#12-370
MACS beads		
Anti-mouse CD11c Microbeads Ultrapure	Miltenyi Biotec	Cat#130-125-835

Supplemental Table 1. Continued.

ELISA kits		
Mouse	**Sources**	**Identifier**
CCL2	Invitrogen	Cat#88-7391-22
CCL17	R&D Systems	Cat#MCC170
CCL22	R&D Systems	Cat#MCC220
CCL25	Invitrogen	Cat#EMCCL25
CXCL9	R&D Systems	Cat#MCX900
CXCL10	R&D Systems	Cat#DY466
IFN-α	R&D Systems	Cat#42120-1
IFN-β	R&D Systems	Cat#42400-1
IFN-γ	Invitrogen	Cat#88-7314-88
IL-4	Invitrogen	Cat#88-7044-22
IL-17	Invitrogen	Cat#88-7371-22
IL-33	Invitrogen	Cat#88-7333-88
TNF-α	Invitrogen	Cat#88-7324-88
Human	**Sources**	**Identifier**
CCL22	R&D Systems	Cat#DMD00
CCL25	Invitrogen	Cat#EHCCL25
CXCL9	R&D Systems	Cat#DCX900
CXCL10	R&D Systems	Cat#DIP100
IFN-α	R&D Systems	Cat#41100-1
IL-33	R&D Systems	Cat#D3300B

Supplemental Table 1. Continued.

Ab for flowcytometric analysis and FACS sorting			
Ab (Clone)	**Fluorochrome**	**Sources**	**Identifier**
Anti-mouse B220 Ab (RA3-6B2)	FITC	Invitrogen	Cat#11-0452-82
Anti-mouse B220 Ab (RA3-6B2)	PerCP-Cy5.5	BioLegend	Cat#103235
Anti-mouse CCR2 Ab (SA203G11)	Alexa Fluor 647	BioLegend	Cat#150603
Anti-mouse CCR4 Ab (2G12)	PE	BioLegend	Cat#131203
Anti-mouse CCR7 Ab (4B12)	Alexa Fluor 647	BioLegend	Cat#120111
Anti-mouse CCR9 Ab (9B1)	Alexa Fluor 647	BioLegend	Cat#129709
Anti-mouse CD3 Ab (17A2)	FITC	BioLegend	Cat#100204
Anti-mouse CD3 Ab (145-2C11)	PE	Invitrogen	Cat#12-0031-82
Anti-mouse CD3 Ab (145-2C11)	PerCP-Cy5.5	BioLegend	Cat#100327
Anti-mouse CD4 Ab (GK1.5)	Alexa Fluor 488	BioLegend	Cat#100425
Anti-mouse CD8 Ab (53-6.7)	Alexa Fluor 488	BioLegend	Cat#100726
Anti-mouse CD11b Ab (M1/70)	FITC	Invitrogen	Cat#11-0112-82
Anti-mouse CD11c Ab (N418)	PE	Invitrogen	Cat#12-0114-81
Anti-mouse CD11c Ab (N418)	BV711	BioLegend	Cat#117349
Anti-mouse CD11c Ab (N418)	APC	BioLegend	Cat#117309
Anti-mouse CXCR3 Ab (220803)	PE	R&D Systems	Cat#FAB1685P
Anti-human/mouse Granzyme B Ab (QA18A28)	APC	BioLegend	Cat#396407
Anti-mouse I-A/I-E Ab (M5/114.15.2)	BV421	BioLegend	Cat#107632
Anti-mouse PDCA-1 Ab (eBio927)	PE	Invitrogen	Cat#12-3172-81
Anti-mouse PDCA-1 Ab (927)	APC	BioLegend	Cat#127015
Anti-mouse SLAMF7 Ab (4G2)	APC	BioLegend	Cat#152003
Rat IgG1 (Isotype Control Ab)	APC	BioLegend	Cat#400411
Rat IgG2a (Isotype Control Ab)	Alexa Fluor 647	BioLegend	Cat#400526
Rat IgG2b (Isotype Control Ab)	Alexa Fluor 647	BioLegend	Cat#400626