Supplementary Figures

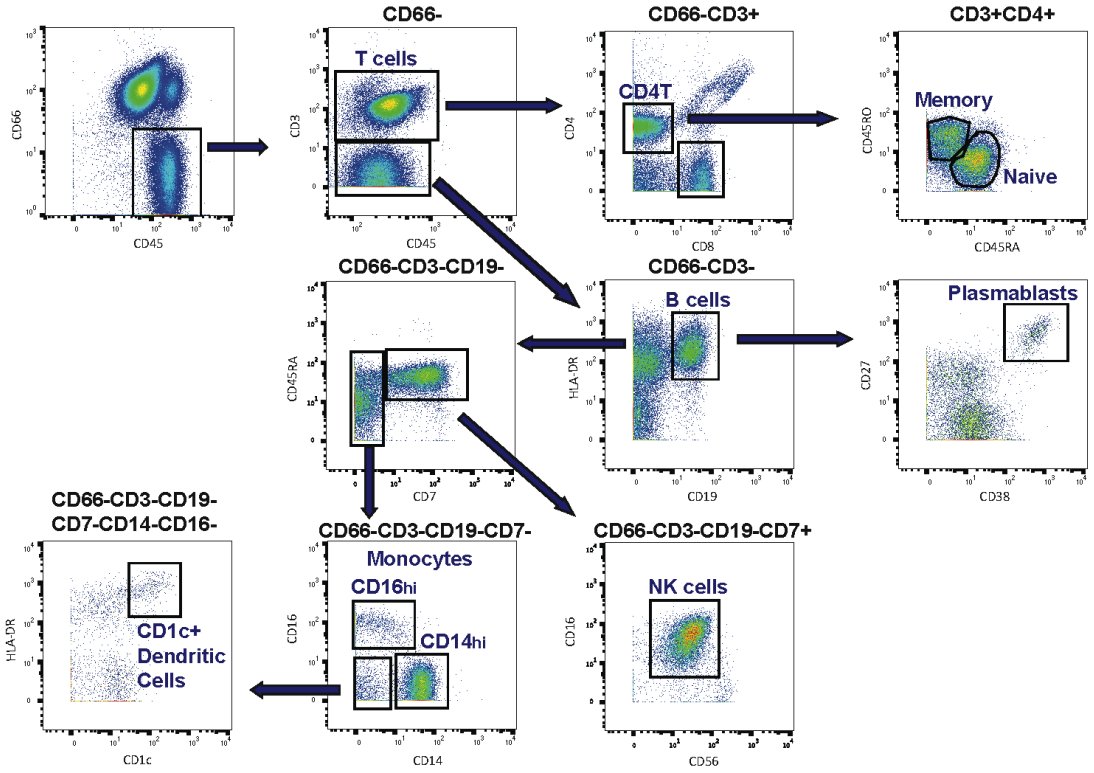

Figure S1. Gating strategy for peripheral blood leukocyte cell subpopulations. Whole blood was processed and analyzed via mass cytometry as in **Figure 1**. CD4, CD8, CD45RA, CD45RO were used to subset T cells. CD19, HLADR, CD27, and CD38 were used to subset B cells. CD16, CD56, and CD7 were used to subset NK cells. CD16 and CD14 were used to subset monocytes. CD1c was used to subset dendritic cells. Representative data from one donor are shown.

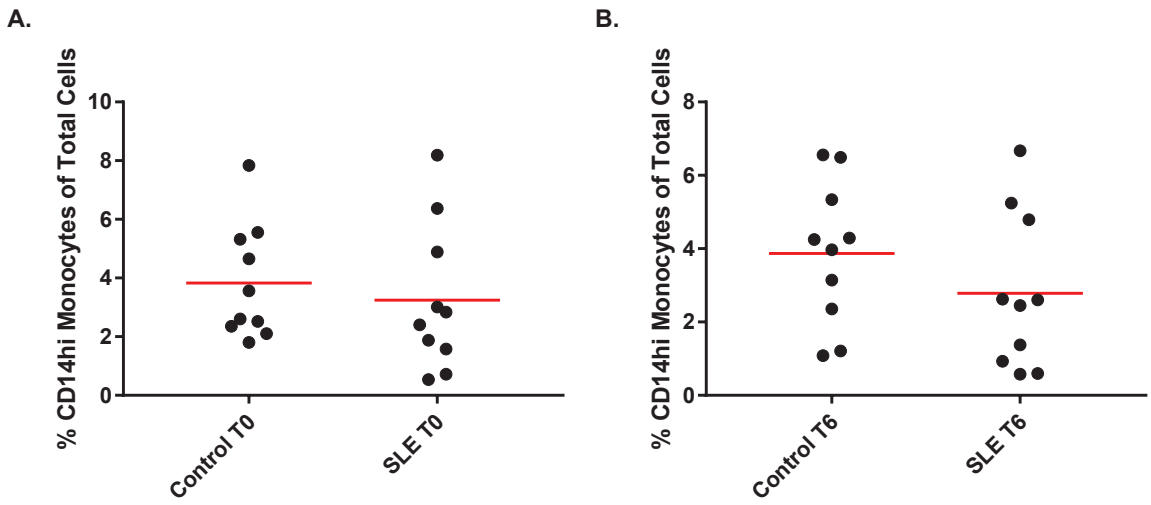

Figure S2. Variance in frequency of $CD14^{hi}$ monocytes in SLE patients and controls peripheral blood samples. Whole blood from SLE patients and controls was processed and analyzed via mass cytometry as in **Figure 1**, at T0 and T6 timepoints. $CD14^{hi}$ population frequency is calculated as the percent of $CD66^{-}CD3^{-}CD19^{-}CD7^{-}CD33^{+}CD11c^{+}HLADR^{+}CD14^{hi}CD16^{lo}CD4^{+}$ cells out of CD45+ total cells analyzed via mass cytometry, for each specific sample. Each dot represents a patient or control sample, at either T0 (**A**) or T6 (**B**), and the red line represents the average within the group. Average for 10 patients and 10 controls are shown. Mann-Whitney calculation yielded p-value=0.48 for T0 (**A**) and p=0.28 for T6 (**B**).

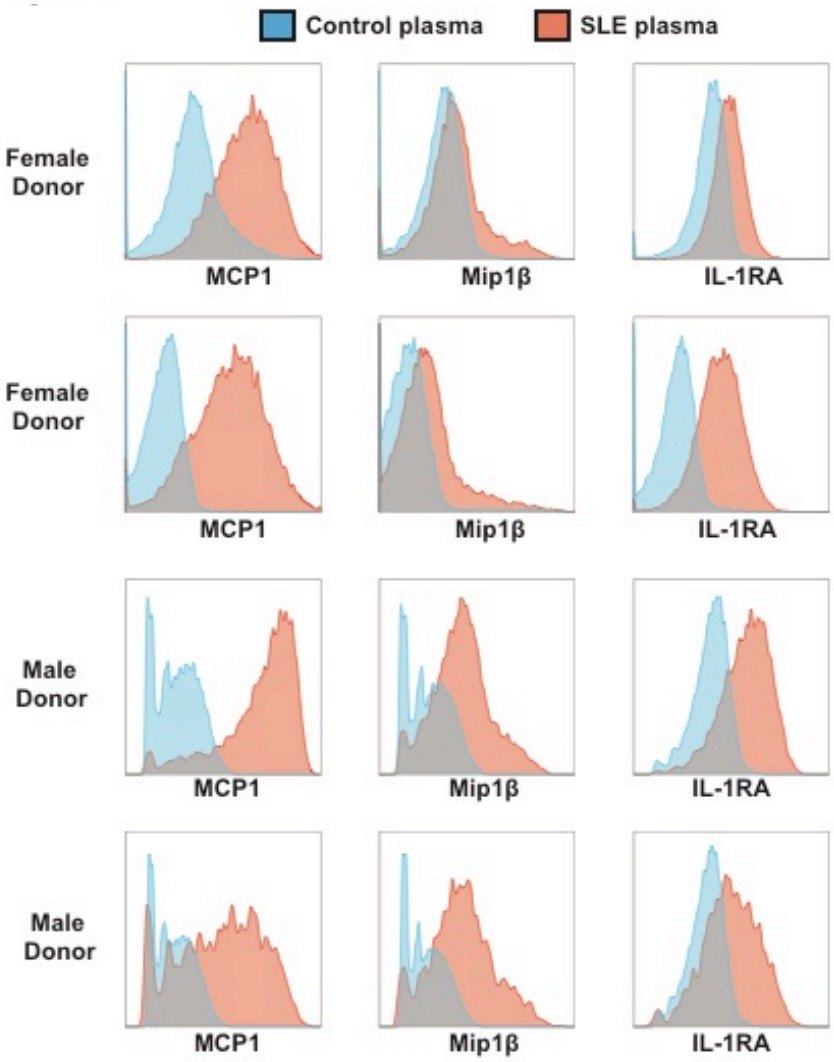

Figure S3. Effect of healthy donor gender in the induction of the monocyte MCP1/Mip1β/IL-1RA signature by SLE plasma. Plasma from one pair of female SLE patient and healthy matched control were separately incubated with two different female and two different male healthy donor blood samples. Blood samples were processed and analyzed by mass cytometry as in **Figure 1**. Hand-gated on $CD14^{hi}$ monocytes, histograms for MCP1, Mip1β, and IL-1RA cytokines are shown, induced either by SLE plasma (red) or control plasma (blue).

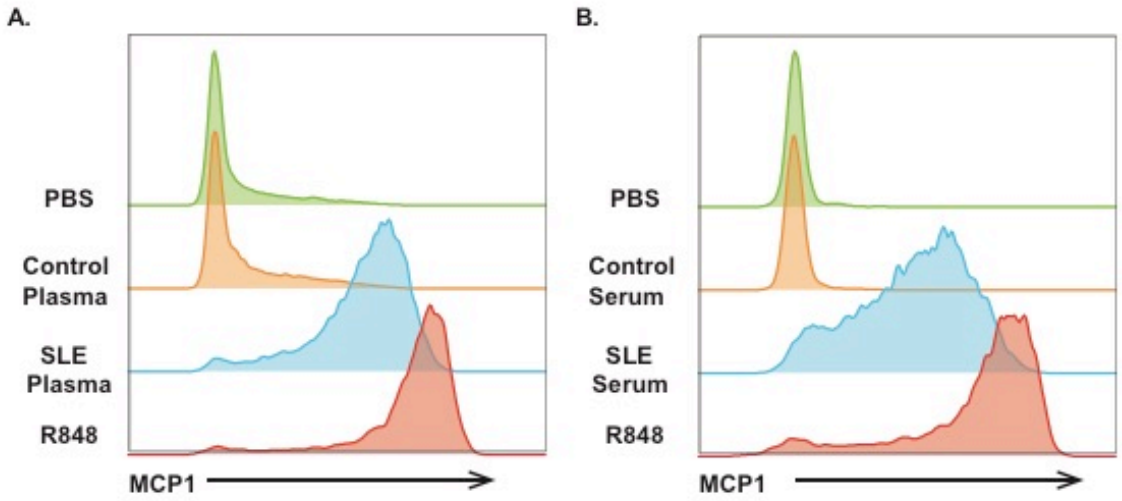

Figure S4. Effect of plasma vs. serum in the induction of the monocyte MCP1/Mip1β/IL-1RA signature. Plasma and serum from one pair of female SLE patient and healthy matched control were separately incubated with one healthy donor peripheral blood sample, then processed and analyzed by mass cytometry as in **Figure 1**. Hand-gated on $CD14^{hi}$ monocytes, histograms for MCP1 are shown, induced either by SLE plasma (**A**) or serum (**B**). R848 stimulation of healthy donor peripheral blood was included as positive technical control. One representative data set shown.

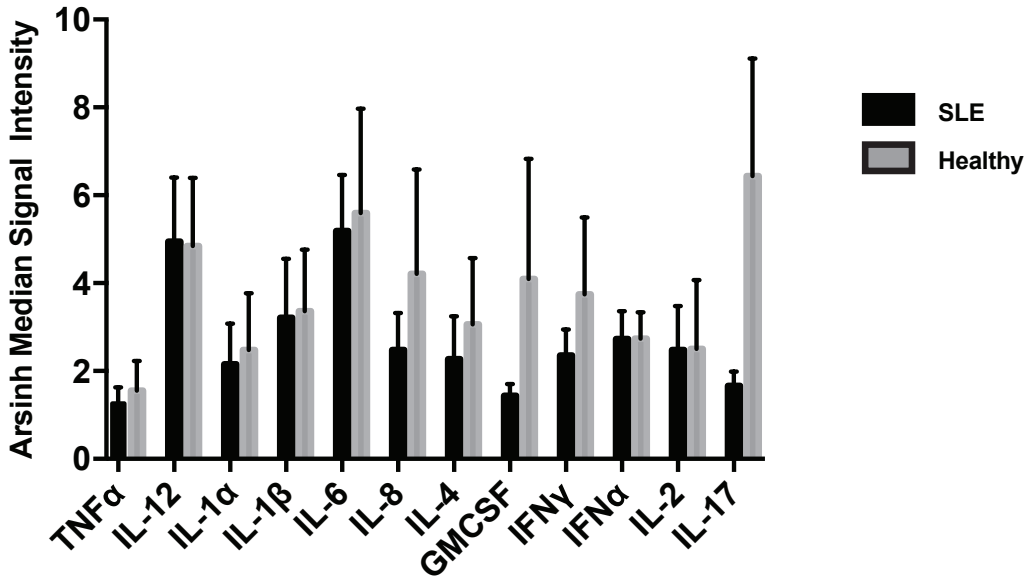

Figure S5. Variance in CD14^{hi} monocytes cytokine induction in healthy donor blood, by serum from SLE patients (black) and controls (grey). Following incubation of serum samples with healthy donor peripheral whole blood, cells were processed and analyzed by mass cytometry as described in **Figure 1**. CD14^{hi} monocyte population was defined as in **Figure 3C**. Median count values from healthy donor blood incubated with or without serum samples were subtracted (Control PT6-Healthy T0, SLE PT6-Healthy T0) to create a response index (median signal intensity). Average responses from 18 SLE patients sera, and 18 control sera with standard deviation are shown.

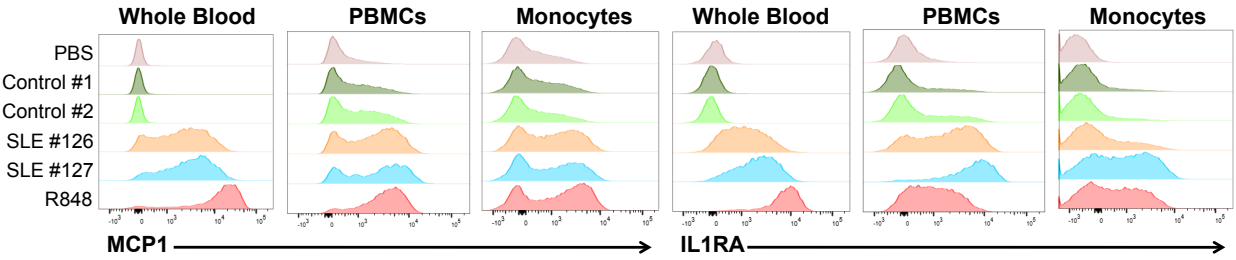

Figure S6. MCP1 and IL1RA are induced by plasma from SLE patients and R848 when incubated with whole blood, PBMCs, and CD14^{hi} monocytes. Plasma from SLE patients (SLE #126, SLE #127), healthy participants (Control #1, Control #2), and R848 were incubated for 6 hours at 37C with a protein transport inhibitor with whole blood, PBMCs, or CD14^{hi} monocytes isolated from one healthy donor (different from healthy participants' plasma). Processed cells were stained with fluorescently labeled antibodies (**Table S5**) and analyzed by flow cytometry. Hand-gated on CD14^{hi} monocytes, histograms for MCP1 and IL1RA are shown. MCP1 and IL1RA were induced by plasma from SLE patients and R848, in all conditions (whole blood, PBMCs, and CD14^{hi} monocytes), with a bimodal distribution in the CD14^{hi} monocytes, likely related to the cellular isolation process.

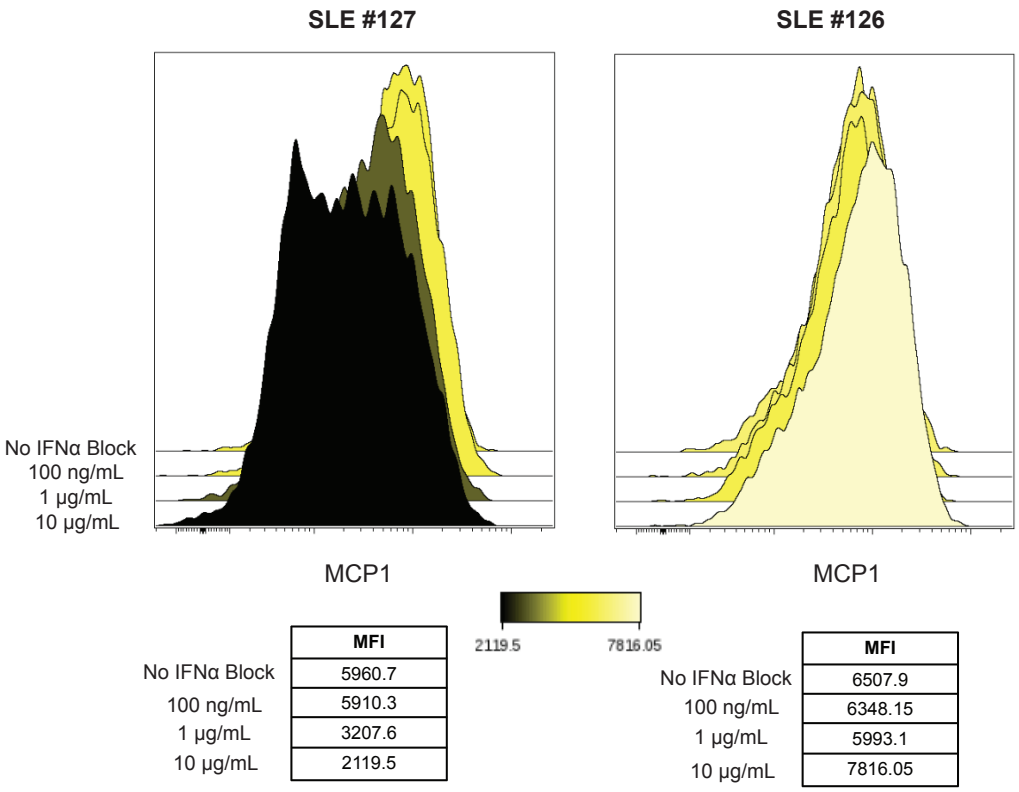

Figure S7. Effect of anti-IFNα on SLE-plasma induced MCP1 production. Plasma samples from SLE patients were incubated for 1 hour at 37C with anti-IFNα IgA antibody at several increasing concentrations separately (indicated above). Following this anti-IFNα treatment, these plasma samples were incubated with healthy donor peripheral whole blood, and cells were lysed and fixed as described in Figure 1. Processed cells were stained with fluorescently labeled antibodies (Table S5) and analyzed by flow cytometry. Hand-gated on CD14hi monocytes, histograms for MCP1 are shown. MCP1 is induced by SLE plasma samples (no IFNα block). In SLE #127, MCP1 production is decreased with increasing concentrations of anti-IFNα that were pre-incubated with SLE plasma. However, in SLE#126, MCP1 production remains grossly unchanged with increasing concentrations of anti-IFNα.

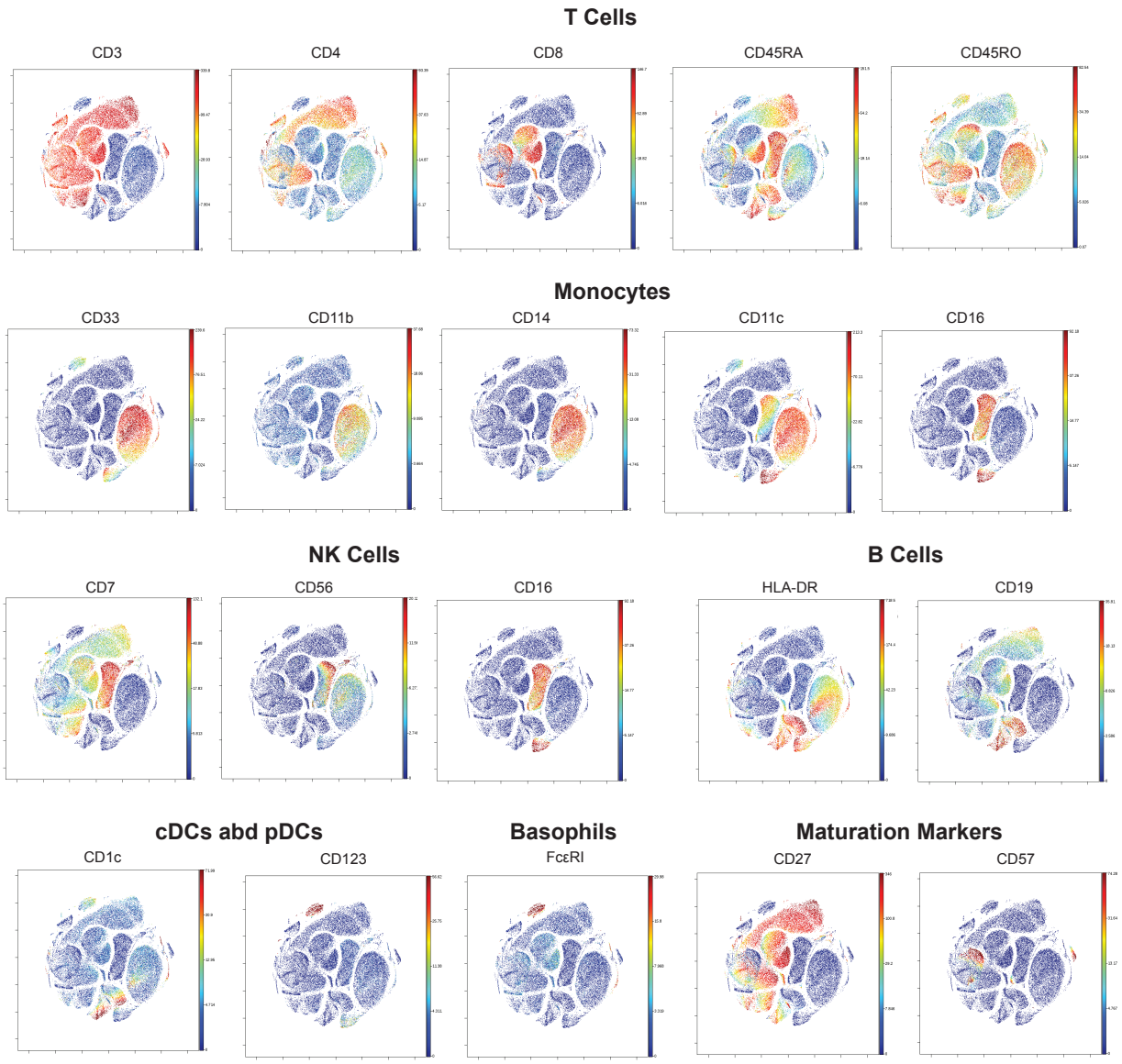

Figure S8. viSNE Maps Show Distinct Clusters Representing Different Cell Types. t-SNE analysis was performed with 20 surface markers used in our experiments (**Figure 1, Table S5**). Each dot in the viSNE map represents an individual cell. In all panels, the same viSNE map is shown, colored sequentially by the labeling intensity of each surface marker indicated at the top of the visNE map. visNE maps are grouped by cell types defined based on surface marker expression.

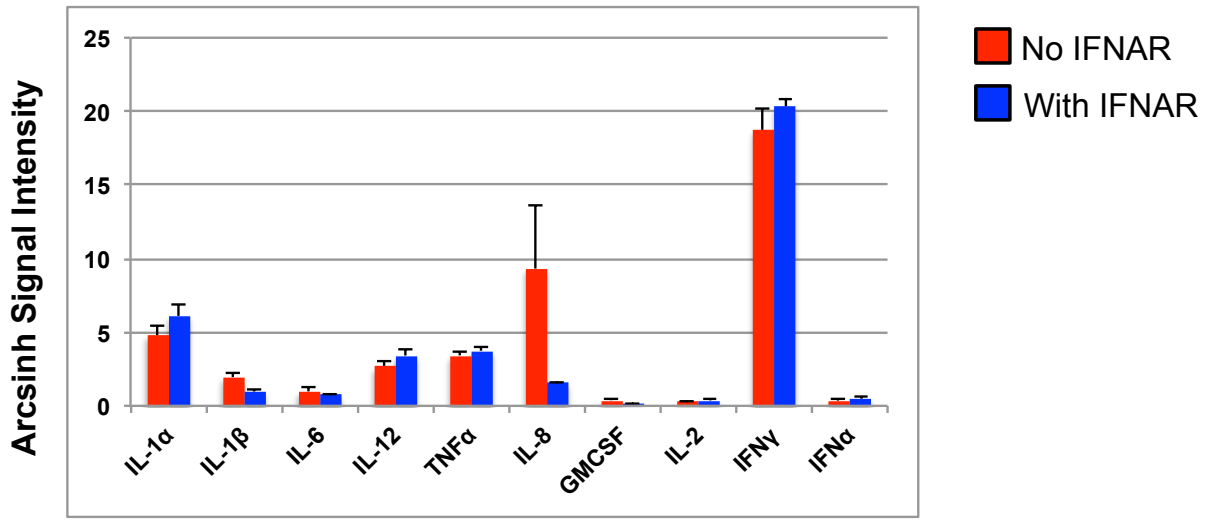

Figure S9. Variance in CD14^{hi} monocytes cytokine induction in healthy donor blood by plasma from SLE patients, with or without IFNAR pre-treatment. Following incubation of serum samples with healthy donor peripheral whole blood pre-treated or not with IFNAR, cells were processed and analyzed by mass cytometry as described in **Figure 1**. CD14^{hi} monocyte population was defined as in **Figure 3C**. Median count values from healthy donor blood pre-treated or not with IFNAR before incubation with serum samples were subtracted (SLE PT6 without ruxolitinib-SLE PT6 with IFNAR) to create a response index (median signal intensity). Average responses from 4 SLE patient plasma samples + SD are shown.

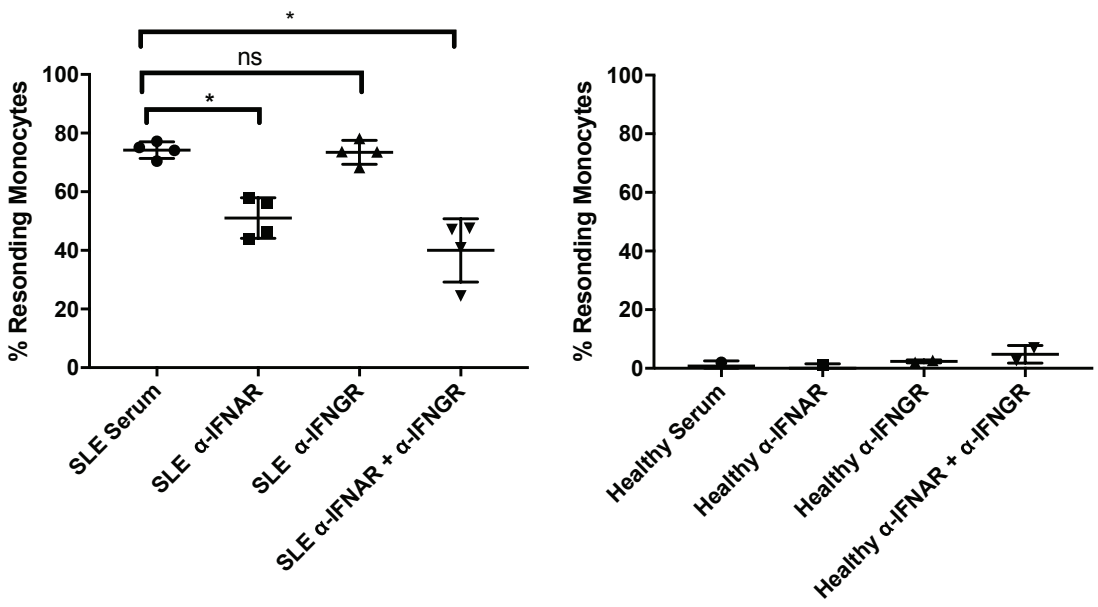

Figure S10. Effect of IFNGR blockade on SLE-plasma induced MCP1 production. Plasma from four clinically active SLE patients (left) and healthy participants (right) were incubated with the same healthy donor blood and lysed/fixed as described in **Figure 1**. Healthy donor blood was either untreated (serum), or pre-treated for 90 minutes at 37C with anti-IFNAR antibody (α-IFNAR), anti-IFNGR antibody (α-IFNGR), or anti-IFNAR+anti-IFNGR antibody together (α-IFNAR + α-IFNGR), prior to incubation with the plasma samples. Processed cells were stained with fluorescently labeled antibodies (**Table S5**) and analyzed by flow cytometry. Hand-gated on CD14hi monocytes, percent responding monocytes were those that demonstrated MCP1 cytokine levels higher than the 95th percentile of unstimulated sample (healthy donor blood only). Averages, minimum and maximum values are shown. Statistical significance (*) was calculated using paired two-tailed t-tests for the conditions indicated (serum vs. serum + IFNAR blockade; serum vs. serum + IFNGR blockade; serum vs. serum + IFNAR/IFNGR blockade).

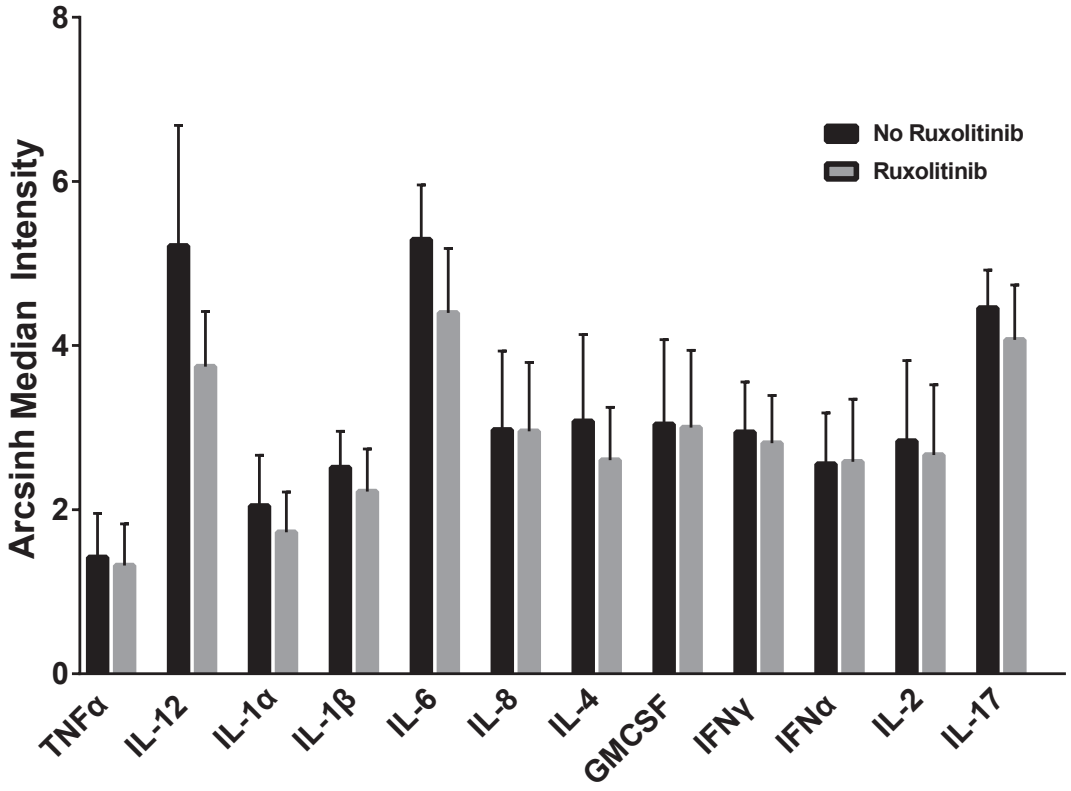

Figure S11. Variance in CD14^{hi} monocytes cytokine induction in healthy donor blood by serum from SLE patients, with or without ruxolitinib pre-treatment. Following incubation of serum samples with healthy donor peripheral whole blood pre-treated or not with ruxolitinib, cells were processed and analyzed by mass cytometry as described in **Figure 1**. CD14^{hi} monocyte population was defined as in **Figure 3C**. Median count values from healthy donor blood pre-treated or not with ruxolitinib before incubation with serum samples were subtracted (SLE PT6 without ruxolitinib-SLE PT6 with ruxolitinib) to create a response index (median signal intensity). Average responses from 25 SLE patient sera samples + SD are shown.

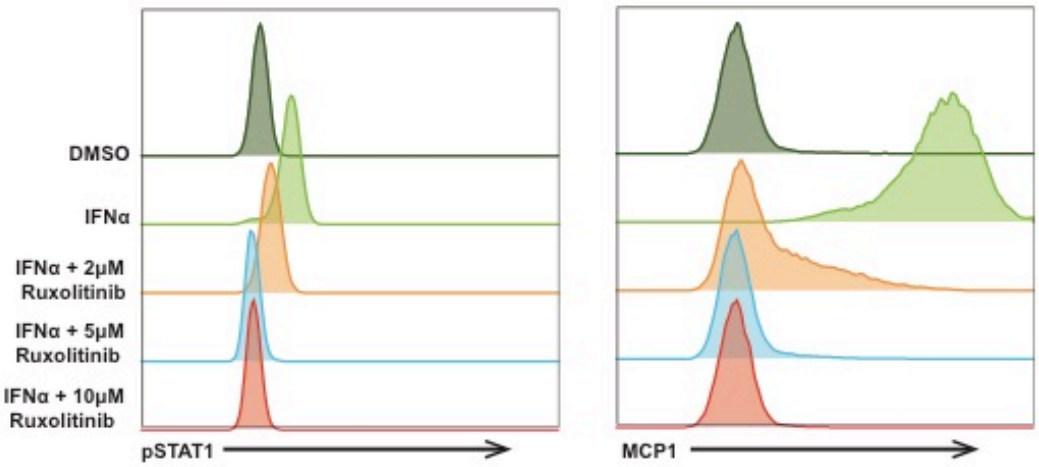

Figure S12. Ruxolitinib dose titration to evaluate effect of JAK1/2 inhibition on the MCP1/Mip1β/IL-1RA signature. Following incubation of IFNα with healthy donor peripheral whole blood pre-treated with ruxolitinib at different concentrations, cells were lysed and fixed as described in **Figure 1**. Processed cells were stained with fluorescently labeled antibodies (**Table S5**), and analyzed by flow cytometry. Hand-gated on $CD14^{hi}$ monocytes, histograms for pSTAT1 and MCP1 are shown. One representative experiment is shown.

Supplementary Tables

Table S1. Summary of demographic data for control and SLE patients. "As needed" medications were non-prescription medications used sporadically by study participants. For inclusion/exclusion criteria, see Methods, Study Participants section.

Characteristic	Healthy Controls (N=10)	SLE Patients (N=10)
Age-- yrs (Range)	15 (12-17)	13 (9-16)
Sex, no. (%)		
Male	3 (30)	3 (30)
Female	7 (70)	7 (70)
Medical history-- no. (%)		
Allergic rhinitis	1 (10)	1 (10)
Acid reflux	1 (10)	0 (0)
Asthma	2 (20)	1 (10)
Migraines	1 (10)	0 (0)
"As needed" medication usage-- no. (%)		
Beta agonists	2 (20)	1 (10)
Antihistamines	2 (20)	0 (0)
Non steroidal anti-inflammatory	1 (10)	3 (30)
Inhaled corticosteroids	1 (10)	1 (10)

Table S2. Conversion of arcsinh difference to absolute median fold change induction. For all of the mass cytometry analysis, cytokine induction was calculated using the median scaled arcsinh values of T6 samples minus the median scaled arcsinh value of T0 condition. Calculations for this fold change converion was based on the supplemental material from the Bendall and Simonds et al., 2011 publication.

Arcsinh Difference	Absolute Fold Change
0.6	2
1.4	5
2.1	10
3.7	50
4.4	100

Table S3. Summary of laboratory and clinical information for 18 newly diagnosed untreated SLE patients, at 4 different disease timpoints. S: sex/gender. Mos Dx: Months since Diagnosis. Flare: as defined per ACR criteria and clinical assessment (17, 71). Abbreviations: ESR, erythrocyte sedimentation rate; C3/C4, complement components 3 & 4; HCQ, hydroxychloroquine; MMF, mycophenylate mofetil; ASA, aspirin; CSA, cyclosporine; NSAIDs, LFTs, liver function tests; CTX, Cytoxan.

SLE Pt	Age (yrs)	S	Mos Dx	SLEDAI	Flare	C3	C4	ds DNA	Other Auto Abs	Malar Rash	Heme	Active Renal	Other	Treatment
#31	11	F	0	4	Yes	86	6.4	80	Yes	Yes	Yes	No	Oral ulcers, Arthritis, Elevated LFTs	Start High dose steroids
			4	10	No	113	10.9	20	Yes	Yes	Yes	No	Elevated LFTs	Low dose steroids, HCQ
			10	2	No	126	21.6	0	No	No	No	No		Low dose steroids, HCQ
			16	0	No	134	24.4	0	No	No	No	No		Low dose steroids, HCQ
#43	12	F	0	22	Yes	35	2.7	160	Yes	No	No	Yes	Arthritis, Seizures, Encephalopathy	Start Solumedrol, CTX
			72	2	No	108	11.1	0	No	No	No	No		Low dose steroids, MMF, HCQ, baby ASA
			78	2	No	114	13.3	0	No	No	No	No		Low dose sterpoids, MMF, HCQ, baby ASA
			80	16	**Yes**	121	11.8	10	No	Yes	No	Yes	Hyperthyroid	Increase steroids, HCQ, baby ASA
#51	15	F	0	10	Yes	42	3.1	5120	Yes	Yes	No	No		Start Steroids
			6	4	No	86	5	160	Yes	Yes	No	No		Start HCQ, baby ASA
			12	6	No	89	<5	>1000	Yes		No	No		MTX, Low dose steroids
			18	6	**Yes**	65	<5	>1000	Yes	Yes	No	Yes	Oral ulcers, Arthritis, Hair loss	Steroid pulse
#53	12	F	0	10	Yes	110	26.9	0	No	Yes	Yes	Yes		Start HCQ
			3	2	No	117	28.3	0	No	No	No	No		Low dose steroids, HCQ
			9	0	No	105	24.4	0	No	No	No	No		Low dose steroids, HCQ, baby ASA
			12	0	No	102	21.8	0	No	No	No	No		Low dose steroids, HCQ, baby ASA
#66	17	M	0	18	Yes	53	11	1280	Yes	Yes	Yes	No	Arthritis, Left opthalmoplegia	Start High dose steroids
			3	2	No	80	17	160	Yes	No	No	No		Low dose steroids, HCQ, baby ASA
			18	2	No	78	12	160	No	No	No	No		Low dose steroids, baby ASA

			42	2	No	94	15	80	No	No	No	No		Low dose steroids, HCQ, baby ASA
#71	14	F	0	30	Yes	28	2.2	5120	Yes	Yes	Yes	Yes	Oral ulcers, Serositis	Start High dose steroids, CTX
			3	0	No	116	22.3	0	No	No	No	No		CTX, High dose steroids, HCQ
			38	0	No	100	22	0	No	No	No	No		MMF, Low dose steroids, HCQ
				0	No	120	21	0	No	No	No	No		Low dose steroids, HCQ
#72	7	F	0	10	Yes	87	9.5	0	Yes	Yes	Yes	No	Oral ulcers, Arthritis	Start High dose steroids, HCQ
			3	2	No	101	11	0	Yes	Yes	No	No		Low dose steroids, HCQ, NSAIDs
			13	2	No	96	15.3	0	No	No	No	No		Low dose steroids, HCQ, Imuran, NSAIDs
			20	8	**Yes**	73	8.2	20	Yes	Yes	No	Yes	Arthritis	Start MMF, Tacrolimus
#74	9	F	0	17	Yes	68	13.3	160	Yes	Yes	Yes	No	Oral ulcers, Arthritis	Start High dose steroids, HCQ
			24	4	No	91	11	320	Yes	No	No	No		Low dose steroids, HCQ, MTX, NSAIDs
			30	4	No	81	7.8	320	Yes	No	No	No		Low dose steroids, HCQ, Imuran, NSAIDs
			33	8	**Yes**	79	7.1	1280	Yes	No	Yes	No	Severe Arthritis	Increase steroids, HCQ, Imuran, NSAIDs
#85	16	F	0	4	Yes	115	32.2	0	No	Yes	Yes	No	Photosensitivity	Start High dose steroids
			5	0	No	139	42.6	0	No	No	No	No		Low dose steroids, HCQ
			12	0	No	140	45	0	No	No	No	No		HCQ
			18	0	No	120	40	0	No	No	No	No		HCQ
#86	12	F	0	28	Yes	<40	<8	2560	Yes	Yes	Yes	Yes	Arthritis	Start Steroid pulse, CTX
			12	2	No	92	8.1	0	Yes	Yes	Yes	No	Non compliant with therapy	HCQ, low dose steroids,

													Imuran, baby ASA
			52	4	No	79	3.6	45	Yes	No	No	No	Non compliant with therapy	HCQ, low dose steroids, Imuran, baby ASA
			60	20	**Yes**	39	<2.9	796	Yes	Yes	Yes	Yes		Start Steroid pulse
#90	14	F	0	22	Yes	25	4.7	640	Yes	Yes	Yes	Yes	Serositis	Start Steroid pulse, CTX
			3	10	No	111	16.2	0	Yes	Yes	Yes	Yes		CTX, Low dose steroids, HCQ
			42	0	No	130	30.6	0	No	No	No	No		MMF, HCQ, Low dose steroids
				0	No	120	25	0	No	No	No	No		Low dose steroids, HCQ
#100	14	F	0	18	Yes	59	5.8	320	Yes	Yes	Yes	Yes	Arthritis, Serositis	Start Steroids pulse, CTX
			12	8	No	126	24.3	40	Yes	No	No	No		MMF, Low dose steroids, HCQ
			15	20	**Yes**	117	19.9	40	Yes	No	No	No	Changes in memory, Vasculitis, Chest pain	MMF, increase steroids, HCQ
			30	5	No	104	16.2	0	No	No	No	No		MMF, low dose steroids
#102	13	F	0	24	Yes	27	3	>1280	Yes	No	Yes	Yes	Serositis	Start Steroid pulse, CTX, HCQ
			3	18	No	106	16	2560	Yes	No	Yes	Yes	Pleuritis	Low dose steroids, HCQ, CTX
			9	12	No	121	16.6	1280	Yes	Yes	No	Yes		High dose steroids, MMF, HCQ, received rituximab and steroids pulse
			12	18	No	103	17.5	640	No	No	No	Yes		Mid dose steroids, MMF, HCQ
#108	13	M	0	18	Yes	74	14	10	Yes	Yes	No	Yes		Start steroid pulse, CTX
			6	2	No	111	16.1	0	No	No	No	No		CSA, MMF, Low dose steroids, HCQ
			9	2	No	107	17.5	0	No	No	No	No		CSA, MMF, Low dose steroids, HCQ
			12	5	No	107	15.9	0	No	No	No	No		CSA, MMF, Low dose steroids, HCQ

			0	29	Yes	44	6.3	>1280	Yes	Yes	Yes	Yes	Oral ulcers, Serositis	Start Steroid pulse, CTX
			3	4	No	114	20.6	40				No	Hair loss	MMF, HCQ, tapering steroids
#111	14	F	19	0	No	141	22.1	0				Yes	Concern for compliance	MMF, HCQ,low dose steroids
			33	9	**Yes**	49	1.9	>1280				Yes		Non compliant, s/p ritux
			0	23	Yes	31	6.8	>1280	Yes	No	Yes	Yes	Serositis	Start Steroid pulse, CTX, HCQ
#113	11	F	3	6	No	130	38.8	40	No	No	No	Yes	Proteinuria, hematuria still	CTX, Low dose steroids, HCQ
			12	2	No	128	29.3	320	No	No	No	No		CTX, Low dose steroids, HCQ
			24	6	No	129	28	20	No	Yes	No	No		Low dose steroids, HCQ, MMF
			0	4	Yes	60	8.3	320	Yes	No	Yes	No	Arthritis	Start high dose steroids, HCQ, baby ASA
			3	4	No	73	7.7	20	No	No	No	No	Eating disorder	Imuran, HCQ, baby ASA, steroids taper
#116	13	F	12	4	No	90	13.1	10	No	No	No	No		Imuran, HCQ, baby ASA, Low dose steroids
			18	4	No	83	14.8	80	No	No	No	No		Imuran, HCQ, baby ASA, Low dose steroids, NSAIDs
			0	22	Yes	39	4	320	Yes	Yes	Yes	Yes	Serositis, Cerebritis	Start Steroid pulse, CTX
			3	14	No	163	45	0	Yes	Yes	Yes	Yes	Aspiration pneumonia	Rituximab, MMF, CTX, High dose steroids
#117	10	F	18	4	No	151	48.8	0	No	No	No	Yes	On dialysis	Imuran, HCQ, baby ASA, Low dose steroids
			82	4	No	108	19.6	0	No	No	No	Yes		Imuran, HCQ, baby ASA, Low dose steroids

Table S4. Box plot values from generalized estimating equation model applied to the longitudinal analysis of monocyte cytokine signature and SLE patients' disease activity. Normalized signal intensity values for MCP1, Mip1β and IL-1RA indicating the minimum, 1st quartile, median, mean, 3rd quartile, and maximum for every box plot shown in **Figure 4B, 4C**.

Clinically Active (Dx + Flare)	MCP1	Mip1β	IL-1RA
Minimum	1.3	1.27	2.81
1st Quartile	3.76	2.28	6.33
Median	17.04	4.04	6.98
Mean	35.9	5.291	15.5
3rd Quartile	41.75	6.76	19.29
Maximum	168.84	16.49	90.11

Clinically Inactive (PreFlare + Remission)	MCP1	Mip1β	IL-1RA
Minimum	1.18	1.15	2.8
1st Quartile	2.36	1.72	3.28
Median	3.12	2.62	4.52
Mean	4.817	3.171	5.588
3rd Quartile	5.62	3.59	6.9
Maximum	24.56	20.46	21.3

Dx	MCP1	Mip1β	IL-1RA
Minimum	1.3	1.27	2.81
1st Quartile	2.82	2.28	4.6
Median	5.7	4.63	6.76
Mean	38.28	5.334	16.62
3rd Quartile	34.43	6.76	12.46
Maximum	168.84	15.06	90.11

Flare	MCP1	Mip1β	IL-1RA
Minimum	2.25	1.76	3
1st Quartile	10.59	2.732	6.428
Median	21.93	4.02	7.775
Mean	31.74	5.216	13.542
3rd Quartile	42.04	6.44	19.608
Maximum	131.33	16.49	39.77

Pre-Flare	MCP1	Mip1β	IL-1RA
Minimum	2.28	1.19	2.97
1st Quartile	2.692	1.775	3.51

Median	3.665	3.36	5.245
Mean	6.125	3.529	6.576
3rd Quartile	4.742	4.155	6.705
Maximum	24.56	9.58	21.3

Remission	**MCP1**	**Mip1β**	**IL-1RA**
Minimum	1.18	1.15	2.8
1st Quartile	2.27	1.715	3.26
Median	3.02	2.58	4.52
Mean	4.482	3.079	5.334
3rd Quartile	5.655	3.145	7.175
Maximum	16.11	20.46	9.87

Table S5. Summary of antibodies used for mass and fluorescence-based cytometry analysis. Antibody information includes clone and manufacturer company.

Antigen	Clone	Manufacturer	Mass/ Fluorescence
Surface markers			
CD1c	L161	Biolegend	161
CD3	UCHT1	BD	144
CD4	SK3	Biolegend	155
CD7	M-T701	BD	149
CD8	SK1	Biolegend	142
CD11b	ICRF44	Biolegend	146
CD11c	B-ly6	BD/BD	152/PECy7
CD14	M5E2	Biolegend	154/Brilliant Violet 421
CD16	B73.1	eBioscience	153
CD19	SJ25C1	Santa Cruz	163
CD20	H1	BD	156
CD27	L128	BD	162
CD33	P67.6	Santa Cruz	167
CD45	HI30	Biolegend	139
CD45RA	HI100	Biolegend	173
CD45RO	UCHL1	Biolegend	145
CD56	MY31	BD	168
CD57	HCD57	Biolegend	113
CD66	B1.1/CD66	BD/Biolegend	176/Brilliant Violet 605
CD123	9F5	BD	150
HLADR	L243	Biolegend	174/Alexa Flour 700
FcεRI	CRA1	Biolegend	143
Cytokines			
IL-1α	364-3B3-14	Biolegend	147
IL-1β	H1b-98	Biolegend	169
IL-1RA	AS17	Santa Cruz	157
IL-2	MQ1-17H12	eBioscience	172
IL-4	8D4-8	BD	151
IL-6	MQ2-13A5	Biolegend	164
IL-8	E8N1	BD	160
IL-12/IL-23 p40	C8.6	Biolegend	171
IL-17A	BL168	Biolegend	148
Perforin	B-D48	Abcam	141
GMCSF	BVD2-21C11	BD	159
Mip1β	D21-1351	BD	158
TNFα	Mab11	Biolegend	166
IFNγ	4S.B3	Biolegend	165
IFNα	LT27:295	Miltenyi	175
MCP1	5D3-F7	BD	170/APC