Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

Supplementary Table 1			
Surface Marker	**Clone**	**Color**	**Vendor**
BDCA-1	L161	APC	Biolegend
CCR2	K036C2	APC	Biolegend
CCR3	5E8	PE	Biolegend
CCR4	L291H4	APC	Biolegend
CCR7	G043H7	BV421	Biolegend
CD3	UCHT1	FITC	Biolegend
CD4	RPA-T4	PerCP-Cy/5.5	Biolegend
CD8	HIT8a	APC-Cy7	Biolegend
CD11b	M1/70	PerCP-Cy/5.5	Biolegend
CD11b-activated	CBRM1/5	FITC	Biolegend
CD11c	3.9	APC	Biolegend
CD14	MϕP9	APC-Cy7	BD
CD15	HI98	PE-Cy7	Biolegend
CD16	3G8	PE	BD
CD32	FUN-2	APC	Biolegend
CD34	561	FITC	Biolegend
CD40	5C3	FITC	Biolegend
CD40L	24-31	BV421	Biolegend
CD45RA	HI100	BV421	Biolegend
CD56	HCD56	APC	Biolegend
CD57	HCD57	APC	Biolegend
CD62L	DREG-56	FITC	Biolegend
CD64	10.1	APC	Biolegend
CD69	FN50	BV421	Biolegend
CD80	2D10	FITC	Biolegend
CD86	IT2.2	APC	Biolegend
CD94	DX22	APC	Biolegend
CX3CR1	2A9-1	PE-Cy7	Biolegend
CXCR3	G025H7	BV421	Biolegend
HLA-DR	Tu39	BV421, FITC	Biolegend
IgM	G20-127	APC	BD
KIR2 (DL2/DL3)	DX27	APC	Biolegend
NKG2D	1D11	BV421	Biolegend
Nkp30	P30-15	APC	Biolegend
Nkp46	9E2	BV421	Biolegend
TCR α/β	IP26	BV421	Biolegend
TLR2	T2.5	FITC	Biolegend
TLR4	HTA125	APC	Biolegend
TRAIL	RIK-2	APC	Biolegend

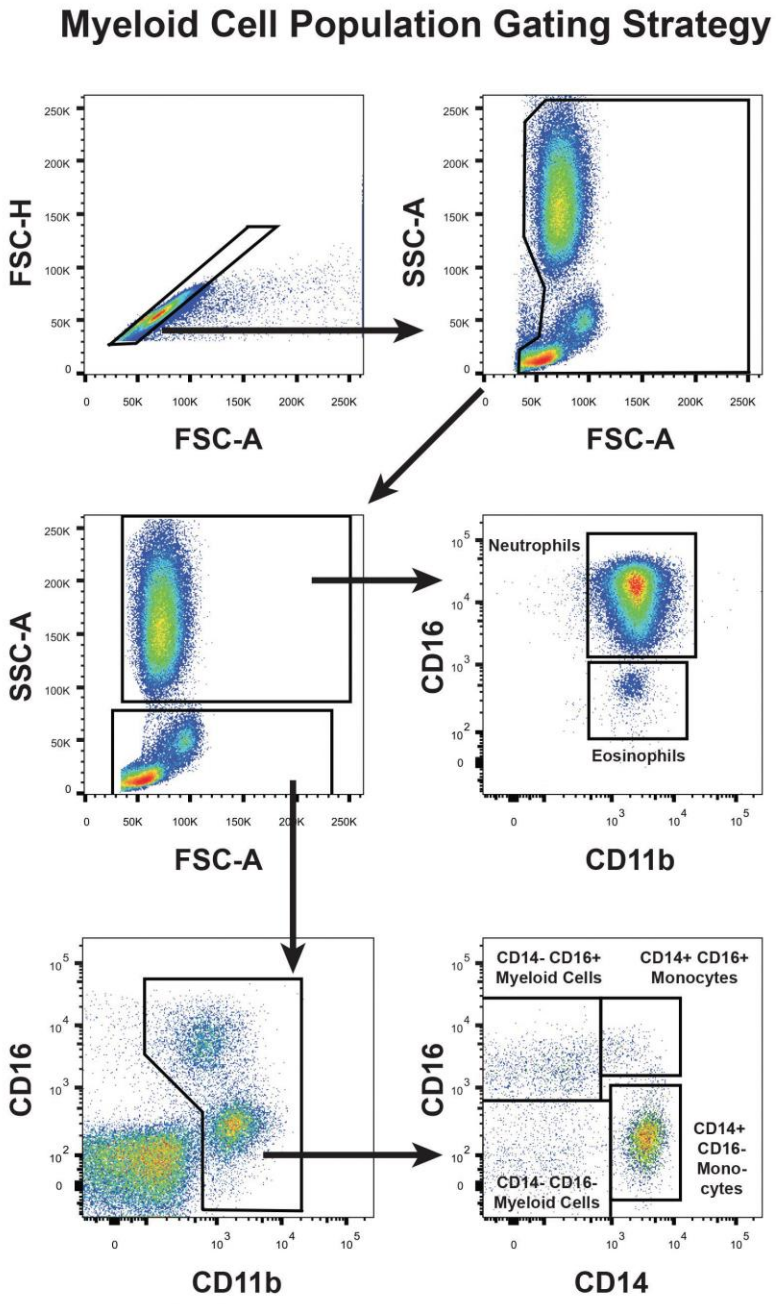
Supplementary Table 2			
Surface Marker	**Immune Role**	**Population Expression**	**Antibody Clone**
CD11c	Marker of maturity on many myeloid cells	CD14+ CD16+ CD14+ CD16- CD14- CD16+	3.9
BDCA-1	Marker on dendritic cell subpopulations	CD14- CD16-	L161
CD83	Marker on dendritic cell subpopulations	None	HB15e
CCR2	Receptor for monocyte chemoattractant protein	CD14+ CD16+ CD14+ CD16- CD14- CD16-	K036C2
CD62L	Adhesion molecule used in leukocyte trafficking	CD14+ CD16- CD14- CD16+ CD14- CD16	DREG-56
CX3CR1	Receptor for the fractalkine ligand	All	2A9-1
CD40	Myeloid activation receptor	CD14+ CD16+ CD14- CD16+ CD14- CD16-	5C3
CD80	Antigen presenting cell co-stimulation molecule	None	2D10
CD86	Antigen presenting cell co-stimulation molecule	CD14+ CD16+ CD14+ CD16- CD14- CD16+	IT2.2
$CD11b^{activated}$	Activated form of CD11b	None	CBRM1/5
HLA-DR	MHC II surface receptor used in antigen presentation	All	Tu39
TLR2	Receptor for pathogen-associated molecular patterns	All	T2.5
TLR4	Receptor for pathogen-associated molecular patterns	CD14+ CD16+ CD14+ CD16- CD14- CD16+	HTA125

Supplemental Table 3 – Regression Analysis of CD11b+ Non-Granulocyte Myeloid Cells

Surface Marker	Leukocyte Change (normalized)		ALSFRS-R Change (normalized)		Participants
CD14+ CD16+ Monocytes					
Surface Marker	**MFI/year**	**SD**	**Score per year per leukocyte change**	**P-value**	**Number**
HLADR	-0.0006	1.2994	1.1819	0.5712	24
CD11c	-0.3212	1.6508	-0.7826	0.5565	24
CD40	0.1411	0.3546	-11.1803	0.1242	24
CD62L	0.4423	0.6009	-4.8210	0.1614	24
CD86	0.2117	1.2221	2.8163	0.4454	12
CCR2	0.0750	1.0963	0.0294	0.9920	24
CX3CR1	0.5860	2.8813	4.1353	0.0310	12
TLR2	-0.0516	0.3901	-2.8284	0.5707	19
TLR4	0.0520	0.6723	3.8637	0.3139	17
CD14+ CD16- Monocytes					
Surface Marker	**MFI/year**	**SD**	**Score per year per leukocyte change**	**P-value**	**Number**
HLADR	0.0118	1.1557	1.9014	0.5578	24
CD11c	-1.2774	2.1522	0.0634	0.9788	24
CD40	0.1540	0.9866	0.5766	0.9341	24
CD62L	0.4759	1.4270	-2.5597	0.3157	24
CD86	-0.2157	0.4566	-12.9637	0.1480	12
CCR2	-0.0378	1.0705	-8.3085	0.2231	24
CX3CR1	0.4510	2.6939	6.2899	0.0639	12
TLR2	0.0197	1.1223	-3.6077	0.3692	19
TLR4	-0.4237	1.5322	3.6594	0.2625	17
CD14- CD16+ Cells					
Surface Marker	**10^4 cells/year**	**SD**	**Score per year per leukocyte change**	**P-value**	**Number**
HLADR	-0.0408	0.1244	23.7533	0.5233	23
CD11c	-0.0558	0.1601	11.0610	0.7701	23
CD40	-0.0097	0.0980	0.7804	0.9881	23
CD62L	-0.0065	0.1061	-58.5218	0.0253	23
CD86	-0.0269	0.1143	-45.9212	0.5431	12
CX3CR1	-0.0618	0.2403	-1.2713	0.9304	11
TLR2	-0.0184	0.0955	2.2583	0.9374	18
CD14- CD16- Cells					
Surface Marker	**10^4 cells/year**	**SD**	**Score per year per leukocyte change**	**P-value**	**Number**
HLADR	0.0016	0.1495	-24.3453	0.5120	23
CD11c	-0.0323	0.1879	-0.5498	0.9866	23
CD40	0.0332	0.1476	-9.3630	0.8174	23
CD62L	0.0181	0.1654	-25.2739	0.3652	23
CD86	-0.0368	0.0552	-224.2873	0.0399	11
BDCA-1	0.0153	0.1322	61.4612	0.2098	23
CCR2	-0.0195	0.0793	0.8807	0.9812	23
CX3CR1	-0.0272	0.2496	61.4058	0.0255	11
TLR2	0.0240	0.1236	-56.6426	0.1942	18

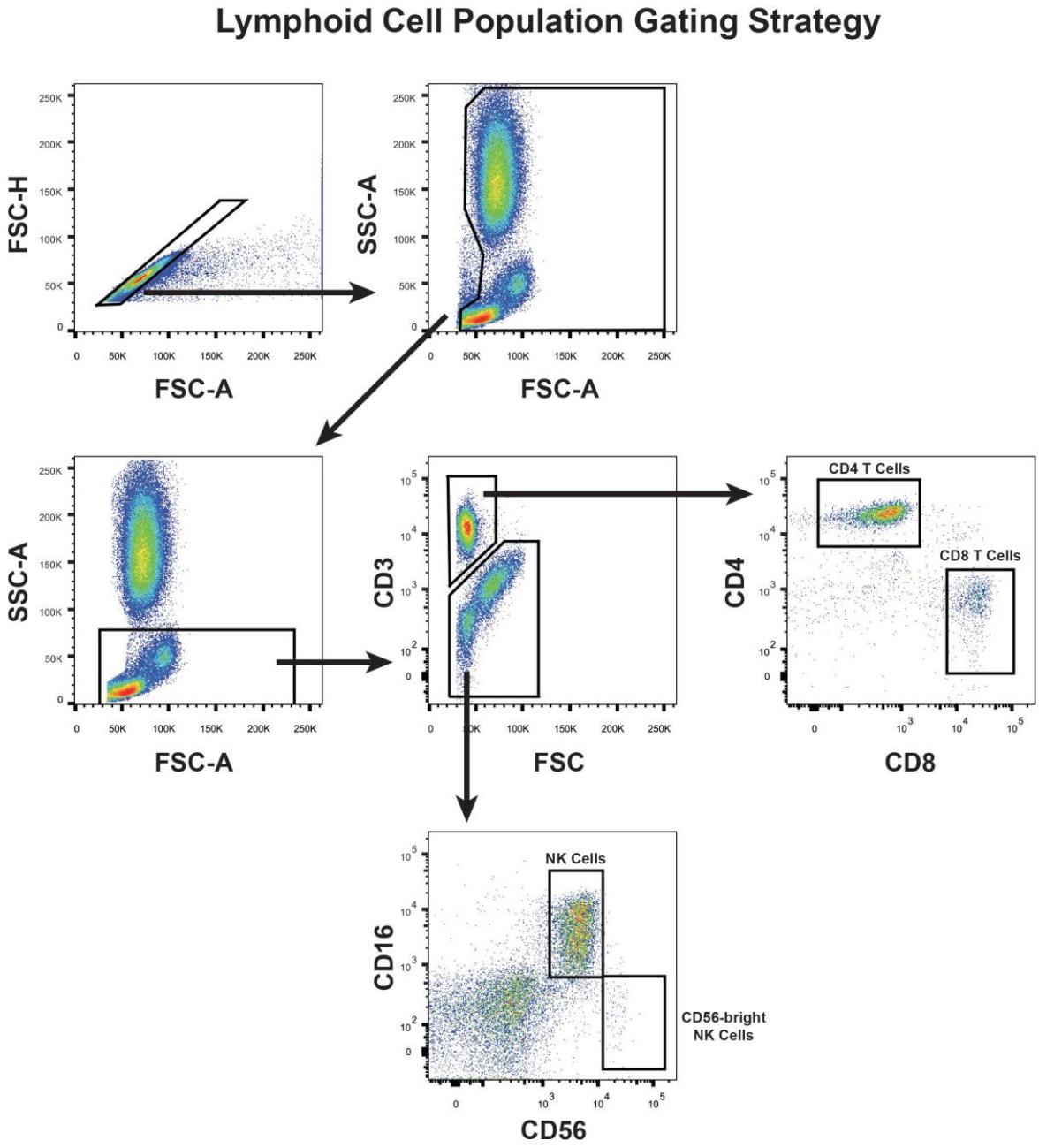
Abbreviations: MFI = Median Fluorescent Intensity; SD = Standard Deviation

Supplementary Figure 1



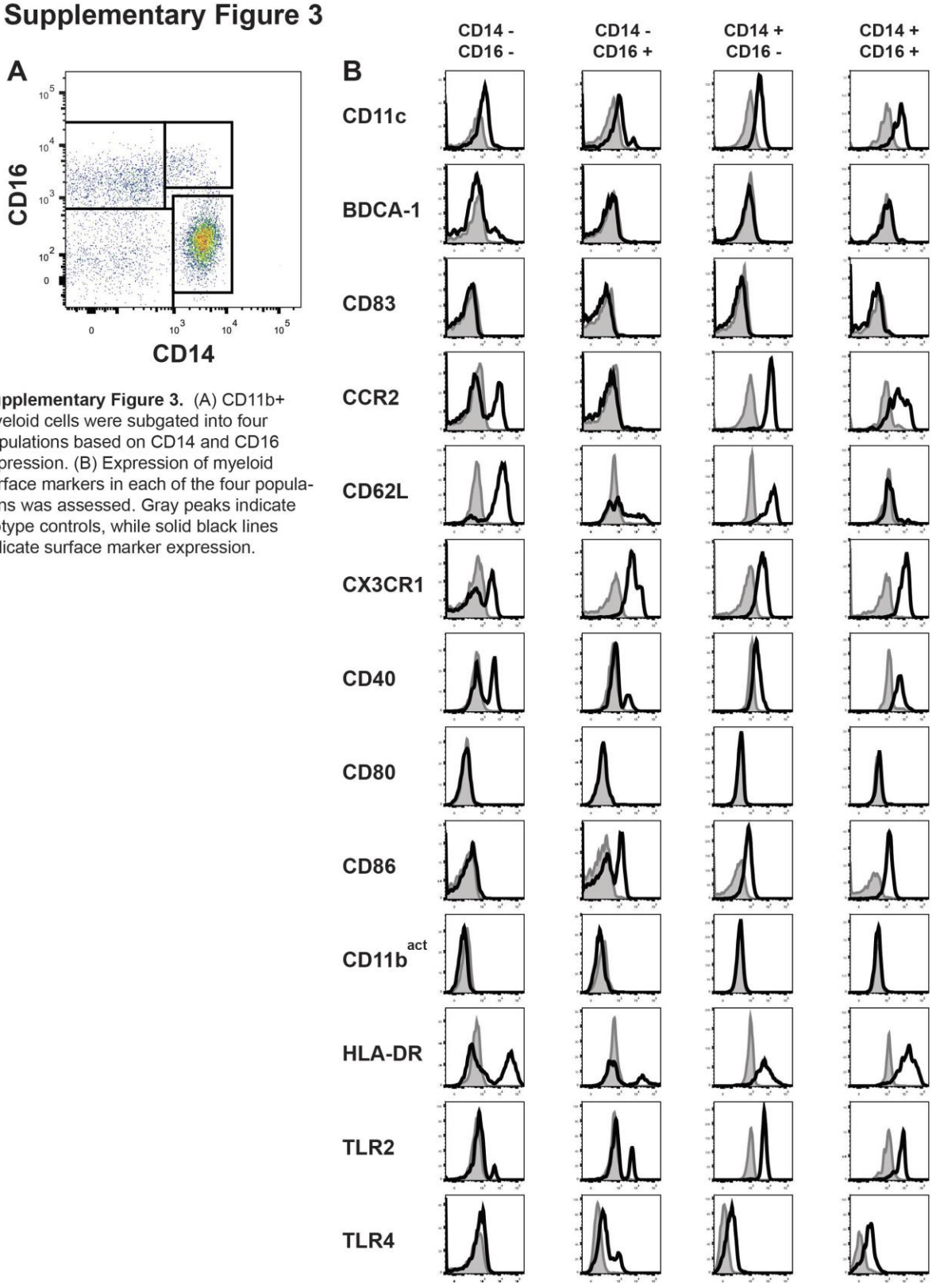
Supplementary Figure 1. Following isolation, peripheral blood immune cells were stained with a combination of CD11b, CD14, and CD16. Doublets and debris were first excluded, and then cells were gated into SSC-high and SSC-low populations. The SSC-hig populatin was further subdivided into CD16-high (neutrophil) and CD16-low (eosinophil) populations. CD11b+ cells were selected from the SSC-low population, and further subdivided into four groups based on CD14 and CD16 expression.

Supplementary Figure 2



Supplementary Figure 2. Following isolation, peripheral blood immune cells were stained with a combination of CD3, CD4, CD8, CD16, and CD56. Doublets and debris were first excluded, and then SSC-low cells were selected. Cells were divided into CD3+ and CD3- populations; CD3+ cells were analyzed for CD4 and CD8 expression while CD3- cells were analyzed for CD56 and CD16 expression to assess NK cell levels.

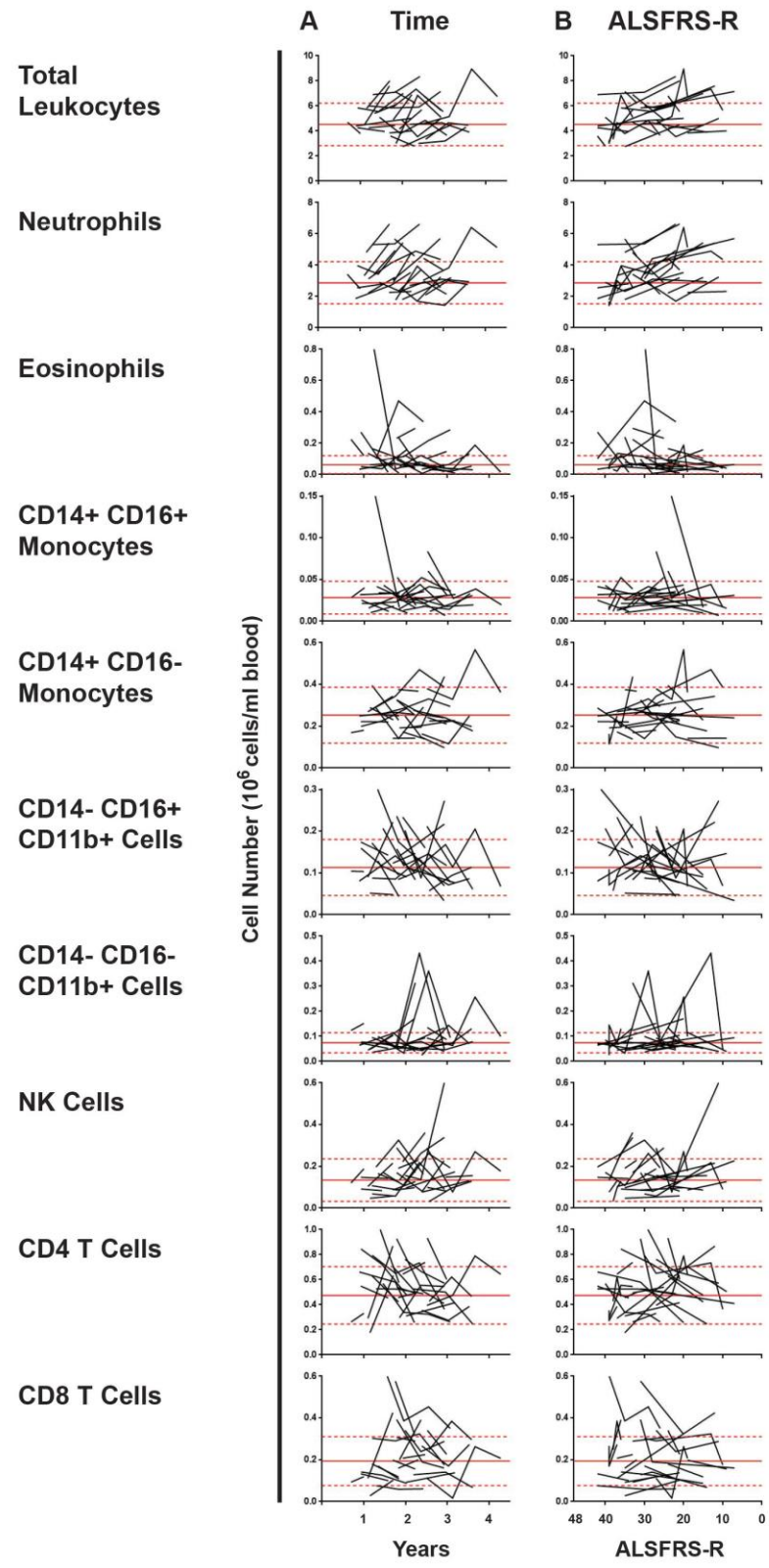
Supplementary Figure 3



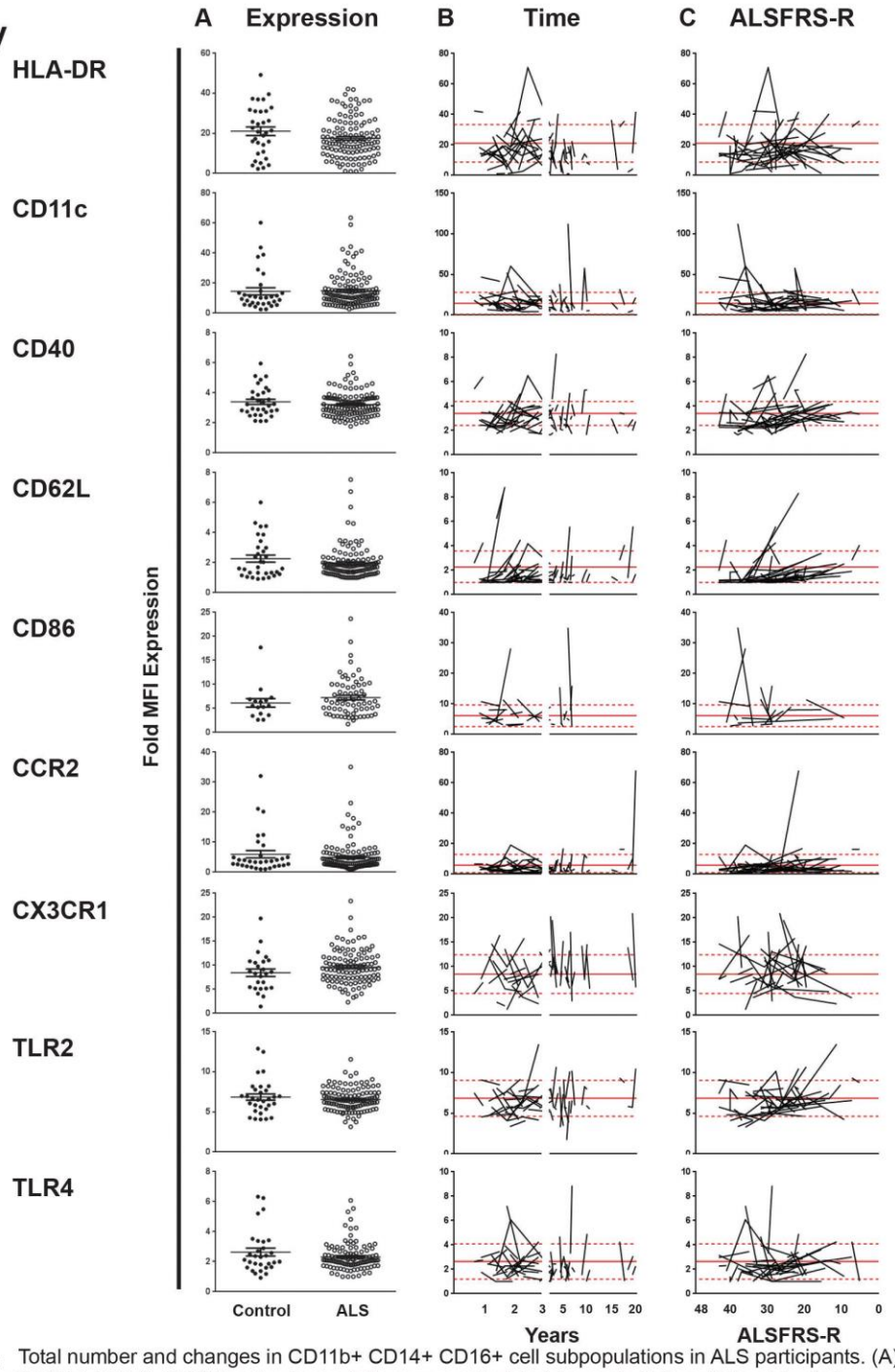
Supplementary Figure 3. (A) CD11b+ myeloid cells were subgated into four populations based on CD14 and CD16 expression. (B) Expression of myeloid surface markers in each of the four populations was assessed. Gray peaks indicate isotype controls, while solid black lines indicate surface marker expression.

Supplementary Figure 4

Supplementary Figure 4. Changes in immune cell subpopulations in ALS participants. (A) The time from ALS onset and (B) participant ALSFRS-R score was calculated for each participant visit and plotted vs. population number over multiple visits. Black lines represent changes between visits. Red lines represent the average number of cells in control participant (solid line) ±1 standard deviation (dashed lines). These lines are intended as reference points and do not indicate change over time, as control participants did not make multiple visits to the clinic. ALS participants with a single visit to the clinic are not included in the kinetic data.

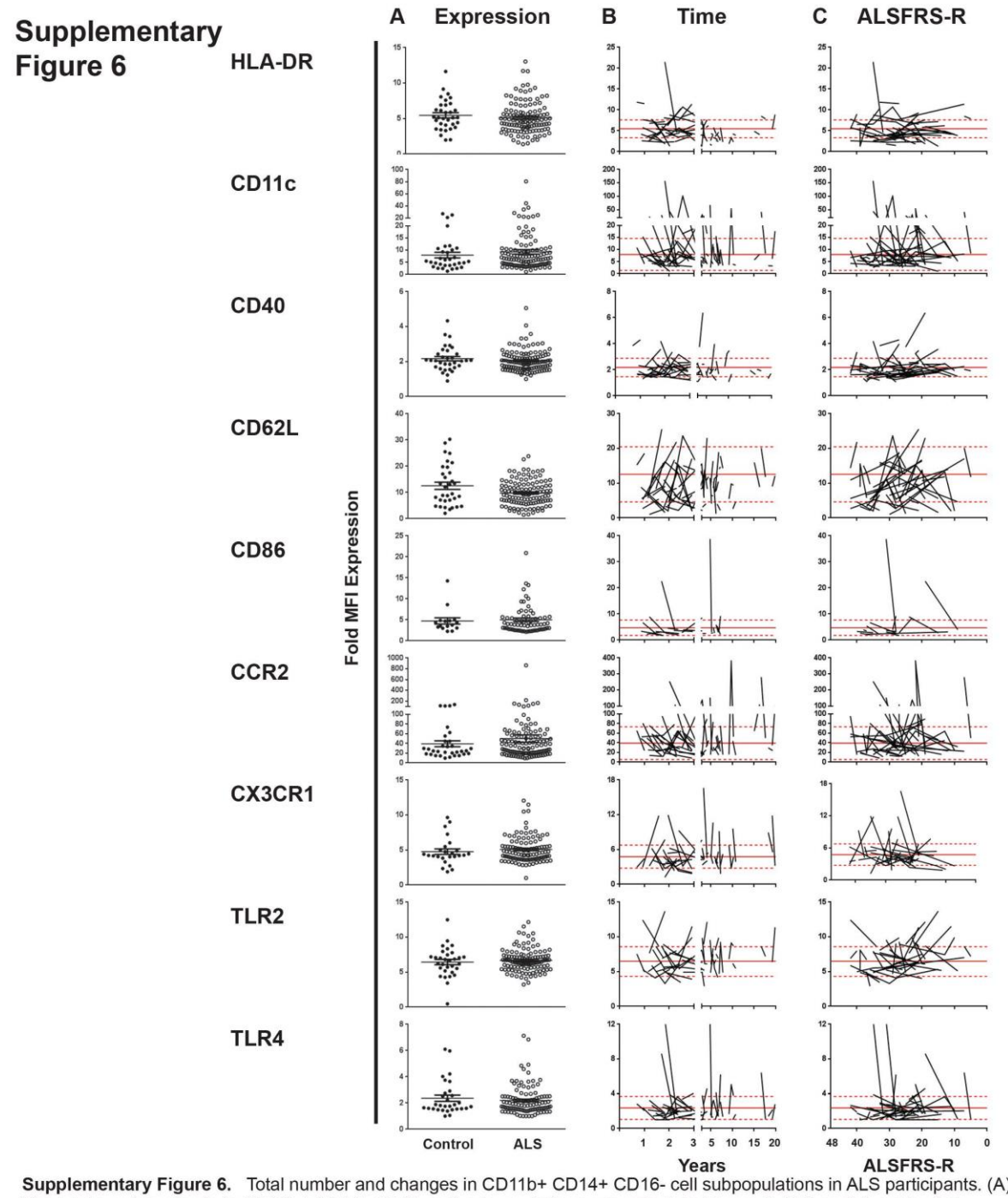


Supplementary Figure 5



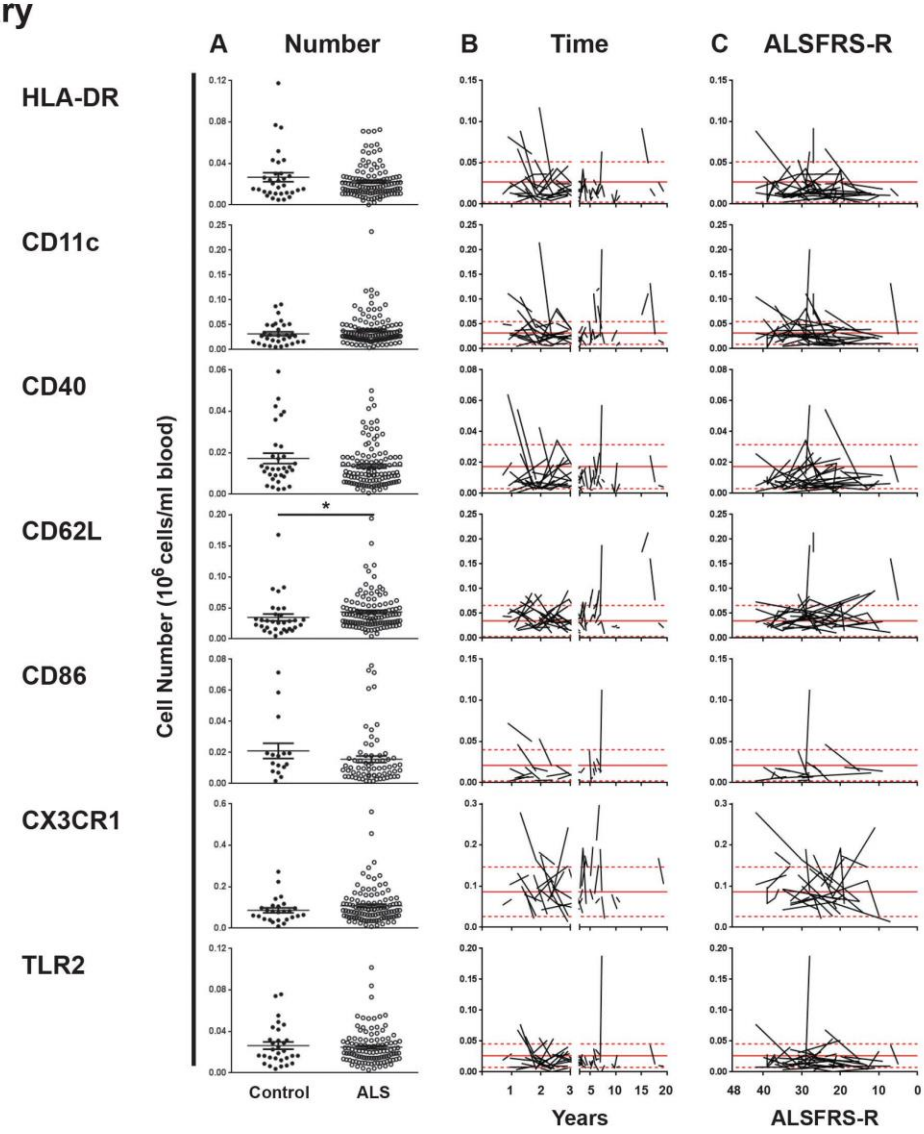
Supplementary Figure 5. Total number and changes in CD11b+ CD14+ CD16+ cell subpopulations in ALS participants. (A) The total number of cells in CD11b+ CD14+ CD16+ cell subpopulations was calculated for each ALS participant by first averaging the population numbers at each visit to create a single value per participant. Cell populations in ALS participants (open circles) were then compared to healthy control participants (filled circles). The mean and SEM is displayed for each population. (B) The time from ALS onset and (C) participant ALSFRS-R score was calculated for each participant visit and plotted vs. subpopulation number over multiple visits. Black lines represent changes between visits. Red lines represent the average number of cells in control participant (solid line) ±1 standard deviation (dashed lines). These lines are intended as reference points and do not indicate change over time, as control participants did not make multiple visits to the clinic. ALS participants with a single visit to the clinic are not included in the kinetic data.

Supplementary Figure 6



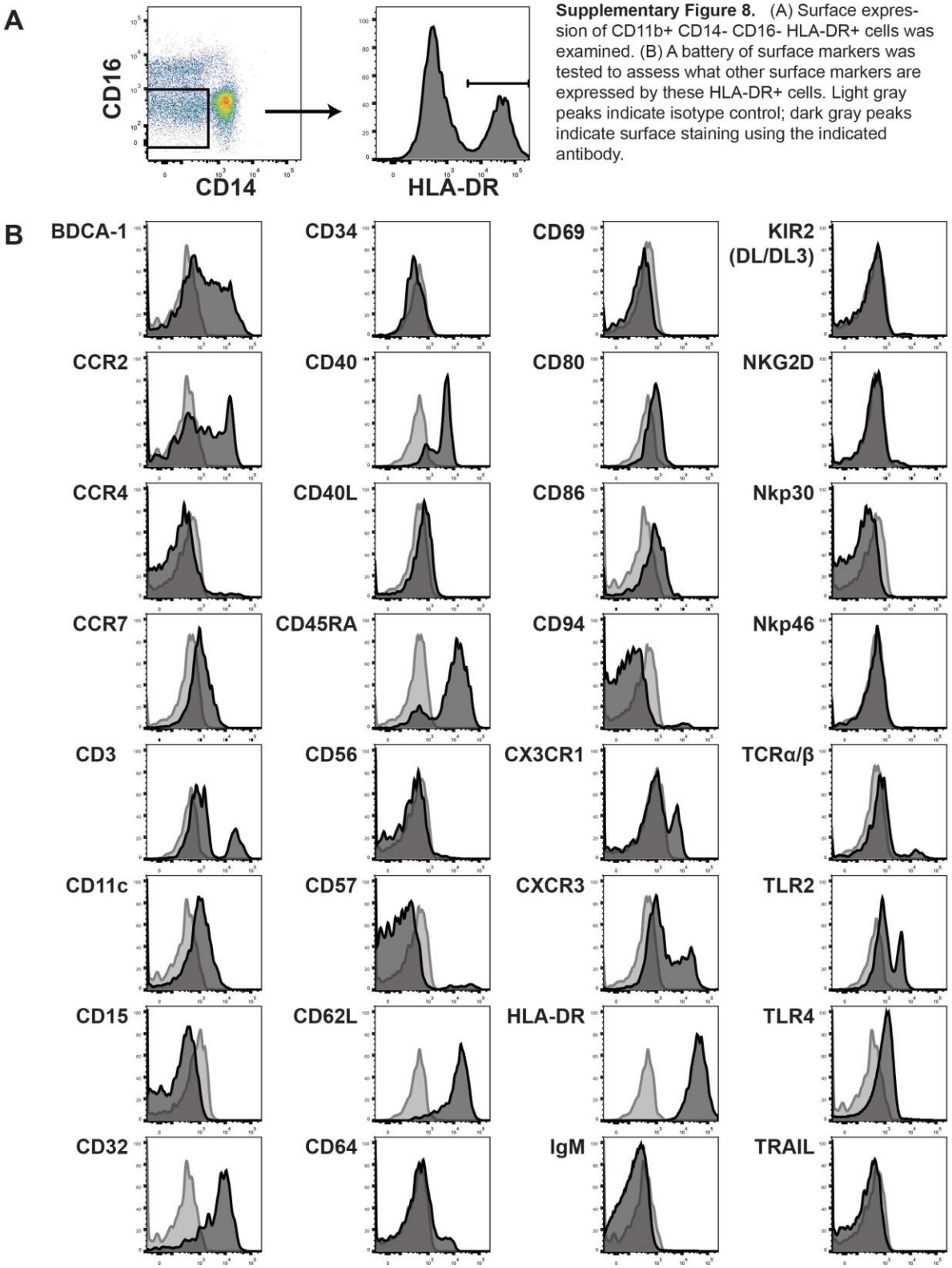
Supplementary Figure 6. Total number and changes in CD11b+ CD14+ CD16- cell subpopulations in ALS participants. (A) The total number of cells in CD11b+ CD14+ CD16- cell subpopulations was calculated for each ALS participant by first averaging the population numbers at each visit to create a single value per patient. Cell populations in ALS participants (open circles) were then compared to healthy control participants (filled circles). The mean and SEM is displayed for each population. (B) The time from ALS onset and (C) participant ALSFRS-R score was calculated for each participant visit and plotted vs. subpopulation number over multiple visits. Black lines represent changes between visits. Red lines represent the average number of cells in control participants (solid line) ±1 standard deviation (dashed lines). These lines are intended as reference points and do not indicate change over time, as control participants did not make multiple visits to the clinic. ALS participants with a single visit to the clinic are not included in the kinetic data.

Supplementary Figure 7



Supplementary Figure 7. Total number and changes in CD11b+ CD14- CD16+ cell subpopulations in ALS participants. (A) The total number of cells in CD11b+ CD14- CD16+ cell subpopulations was calculated for each ALS participant by first averaging the population numbers at each visit to create a single value per participant. Cell populations in ALS participants (open circles) were then compared to healthy control participants (filled circles). The mean and SEM is displayed for each population. * $P < .05$ (B) The time from ALS onset and (C) participant ALSFRS-R score was calculated for each participant visit and plotted vs. subpopulation number over multiple visits. Black lines represent changes between visits. Red lines represent the average number of cells in control participants (solid line) ±1 standard deviation (dashed lines). These lines are intended as reference points and do not indicate change over time, as control participants did not make multiple visits to the clinic. ALS participants with a single visit to the clinic are not included in the kinetic data.

Supplementary Figure 8



Supplementary Figure 8. (A) Surface expression of CD11b+ CD14- CD16- HLA-DR+ cells was examined. (B) A battery of surface markers was tested to assess what other surface markers are expressed by these HLA-DR+ cells. Light gray peaks indicate isotype control; dark gray peaks indicate surface staining using the indicated antibody.