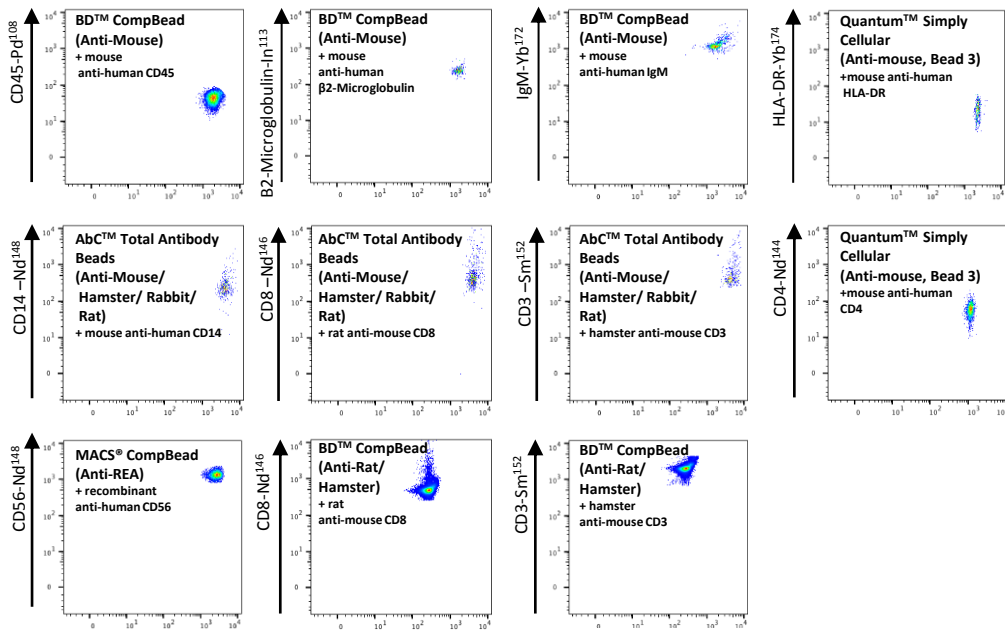


Supplementary Material

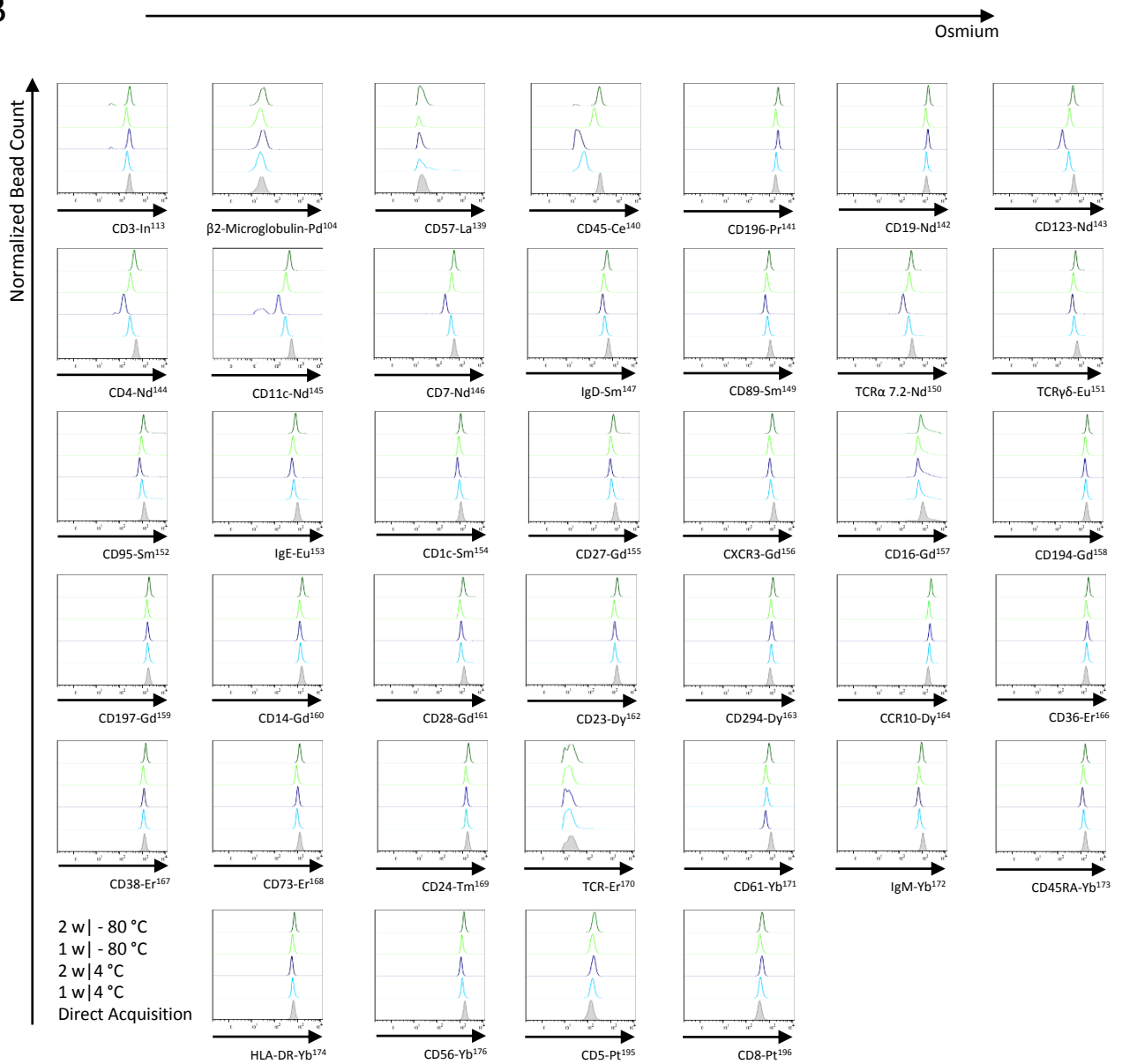
Osmium-labeled Microspheres for Bead-based Assays in Mass Cytometry

Lisa Budzinski, Axel R Schulz, Sabine Baumgart, Tyler Burns, Thomas Rose,
Heike Hirseland, and Henrik E Mei

A



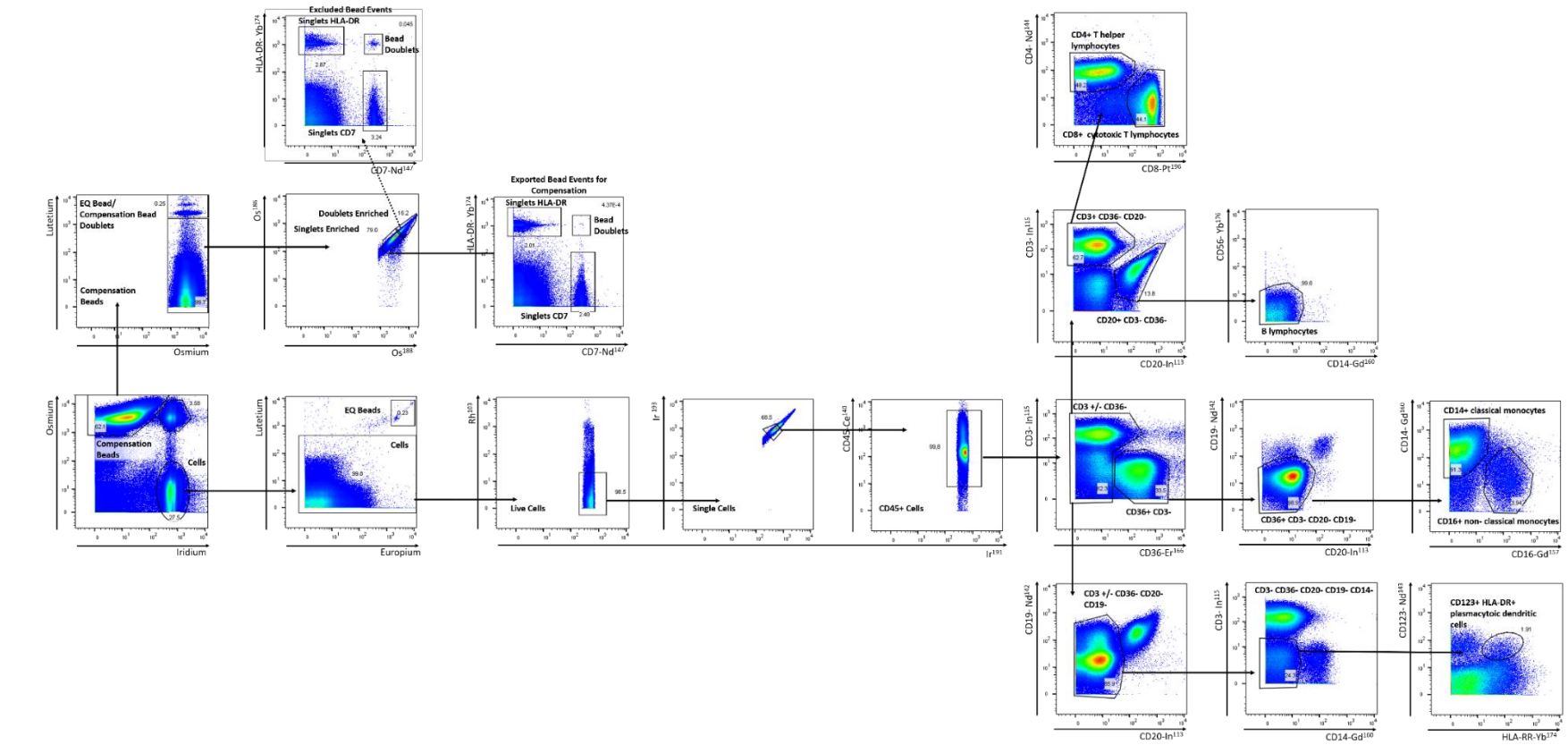
B



Supplementary Figure 1 | Osmium labeling of antibody-capture beads and storability of osmium-labeled beads with captured antibody-conjugate.

Supplementary Figure 1 | Osmium labeling of antibody-capture beads and storability of osmium-labeled beads with captured antibody-conjugate. A

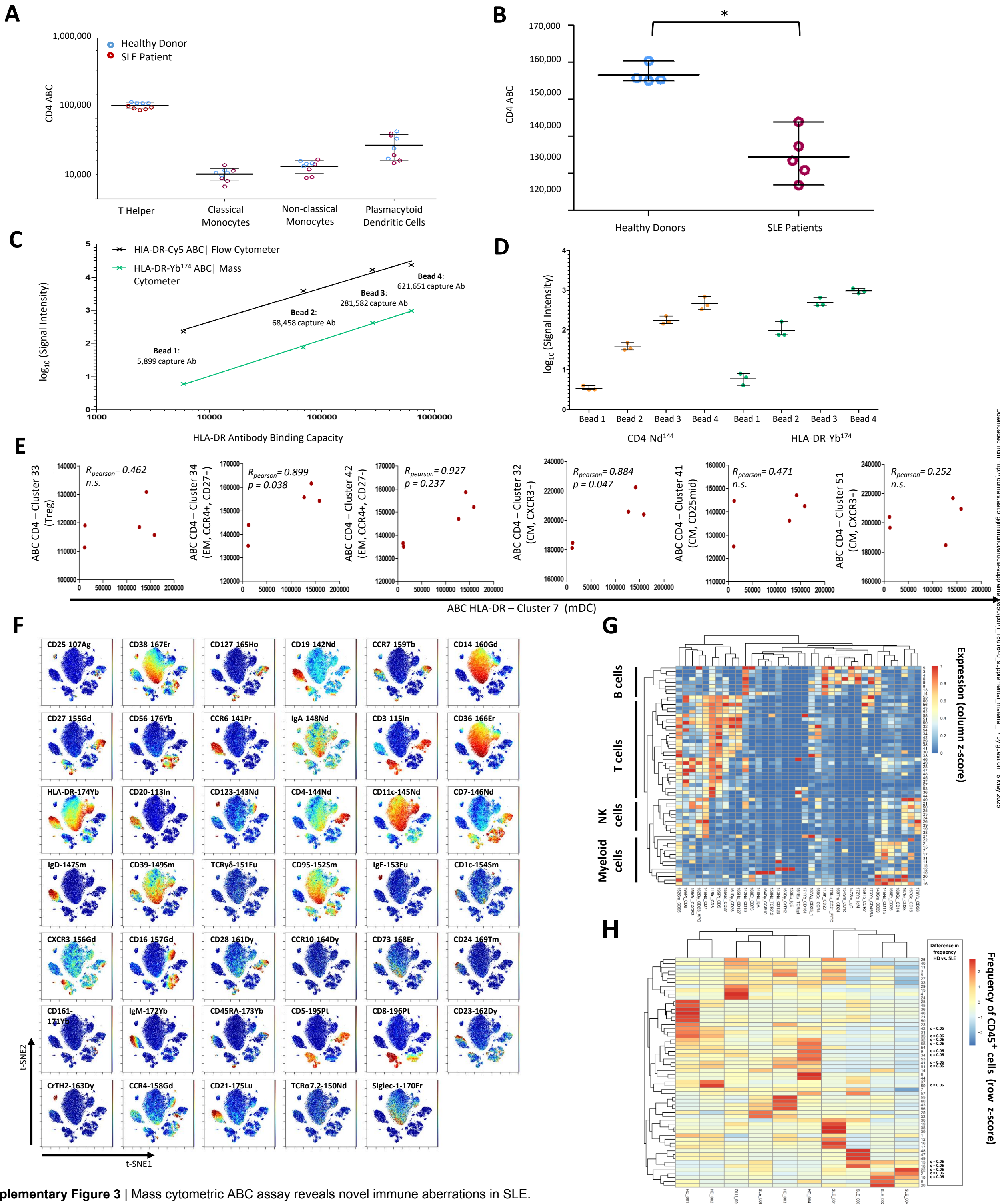
Versatility of bead labeling with osmium tetroxide. Various commercially available antibody capture beads from different vendors and representing different functionalizations were successfully labeled with OsO_4 and maintained their ability to capture antibody conjugates. Seven different functionalized polystyrene bead products were evaluated, five of which are shown. BDTM CompBeads (Fig. 1, 2, 3) and QSC beads (Fig. 4) were used throughout the study. **B** Storage of a mixture of osmium-labeled beads carrying individual antibody conjugates for 2 weeks without significant loss of the captured antibody signal. Aliquots of one batch of OsO_4 -labeled antibody-capture beads were individually stained with 39 antibody conjugates (panel B0, Suppl. Table), pooled and stored at 4 °C and at -80 °C. Bead pools were acquired after one and two weeks of storage.



Supplementary Figure 2 | Gating strategy for PBMC combined with osmium-labeled antibody-capture beads.

Supplementary Figure 2 | Gating strategy for PBMC combined with osmium-labeled antibody-capture beads. A representative gating is shown for the distinction of beads and cells, and for the identification of PBMC subsets in Figures 3, 4, and S3. PBMC and beads were prepared and stained as described in Material and Methods and summarized in Figure 3A. After data normalization, osmium-labeled compensation beads were first discriminated from nucleated cells stained by Ir-DNA intercalator. Doublets/aggregates formed between osmium-labeled beads and EQ calibration beads were removed, followed by discrimination of beads formed between individual osmium-labeled beads according to signals in the Os¹⁸⁶ and Os¹⁸⁸ channels. Note, that the Os^{high} bead fraction was enriched for doublets and excluded from analysis, while the remaining beads were devoid of doublets and further used for spillover calculation using CATALYST.

As for PBMC, doublets/aggregates with EQ calibration beads were removed. For the ABC assay in Fig. 4, data of the 10 PBMC samples were retrieved by manual debarcoding. For the spillover correction experiment in Fig. 3, the CD45⁺ PBMC fraction of a single random PBMC sample was used. Rh¹⁰³-mDOTA-labeled dead cells and cell aggregates were excluded as shown. After gating on CD45⁺ cells, CD4⁺ T cells, B cells, classical monocytes, non-classical monocytes, and plasmacytoid dendritic cells were identified as illustrated. Cell subsets obtained in the final gates were used for calculating median signal intensity for the ABC assay.



Supplementary Figure 3 | Mass cytometric ABC assay reveals novel immune aberrations in SLE.

Supplementary Figure 3 | Mass cytometric ABC assay reveals novel immune aberrations in SLE. This figure expands data provided in Figure 4. **A** Absolute quantification of CD4 ABC of the indicated, manually gated, immune cell subsets in 5 SLE patients and 4 control donors. **B** SLE patients (red open circles) show significantly lower CD4 ABC compared to controls (blue open circles) on CD4⁺ T cells. Medians and interquartile ranges are shown. Mann-Whitney-U-test was applied; p-value = 0.0095 **C** Comparison of reference bead-based calibration data for the HLA-DR ABC assay (clone L243) in flow (black; Cy5) and mass cytometry (blue; Yb¹⁷⁴). Lines exhibit similar slopes (flow cytometry: 1.094, mass cytometry: 1.026). **D** Inter-assay variation of osmium-labelled QSC beads for CD4-Nd¹⁴⁴ and HLA-DR-Yb¹⁷⁴ conjugates. Three independent experiments were performed and acquired on different days. All experiments were performed with a single lot of QSC beads. **E** Correlation between CD4 ABC of selected CD4⁺ T cell clusters (32, 33, 34, 41, 42, 51) and HLA-DR ABC of cluster 7 for all 5 SLE patients. **F** Individual marker expression profile of PBMC analyzed in Fig. 4. The t-SNE map was calculated based on live, CD45⁺, B2M⁺ cells using panel C1 markers (Suppl. Table 1), except Siglec-1, CD45, B2M and CD57. Blue color indicates no/low expression, dark red maximum expression for each marker. Data of Pat_001 is shown. **G** Heatmap showing median marker expression (z-scored) of all FlowSOM clusters analyzed in Figure 4. **H** Heatmap showing z-scored cluster frequencies (percentage of CD45⁺ cells) of SLE patients, one IBM patient (marked “OLU”), and controls. Clusters that showed differential abundance in 5 SLE patients vs. 4 healthy controls are indicated (FDR threshold, 10%). For both datasets, hierarchical clustering using Euclidian distances and complete linkage was performed.

Table S1: Mass and flow cytometry panels. The application of each conjugate in bead- and/or cell-based assays is indicated. Panel identifiers are indicated.

Antibody	Specificity	Clone	Vendor	Label	Vendor	Panel	On Cells	On Beads
-	-	-	-	Streptavidin-Ag ^{107/109}	Cytodiagnostics	C1	PBMC	
β 2-Microglobulin	mouse monoclonal IgG	2M2	Biologend	Pd ¹⁰⁴	Trace Sciences	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
β 2-Microglobulin	mouse monoclonal IgG	2M2	Biologend	Pd ¹⁰⁶	Trace Sciences	C1, B1	PBMC	BD™ CompBead (a-mouse)
β 2-Microglobulin	mouse monoclonal IgG	2M2	Biologend	Pd ¹⁰⁸	Trace Sciences	C1, B1	PBMC	BD™ CompBead (a-mouse)
β 2-Microglobulin	mouse monoclonal IgG	2M2	Biologend	Pd ¹¹⁰	Trace Sciences	C1, B1	PBMC	BD™ CompBead (a-mouse)
β 2-Microglobulin	mouse monoclonal IgG	2M2	Biologend	Pt ¹⁹⁸	Fluidigm	C1, B1	PBMC	BD™ CompBead (a-mouse)
β 2-Microglobulin	mouse monoclonal IgG	2M2	Biologend	In ¹¹³	Trace Sciences	C1, B1	PBMC	BD™ CompBead (a-mouse)
CCR10 (GPR-2)	rat monoclonal IgG	314305	Fluidigm	Dy ¹⁶⁴		C1, B0, B1	PBMC	BD™ CompBead (a-rat)
CD11c	mouse monoclonal IgG	Bu-15	Biologend	Nd ¹⁴⁵	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD123 (IL-3R)	mouse monoclonal IgG	6H6	Fluidigm	Nd ¹⁴³		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD127 (IL-7R)	mouse monoclonal IgG	A019D5	Fluidigm	Ho ¹⁶⁵		C1, B1	PBMC	BD™ CompBead (a-mouse)
CD14	mouse monoclonal IgG	RMO52	Beckman Coulter	Nd ¹⁴⁸	Fluidigm	B1		BD™ CompBead (a-mouse), AbCTM Total Antibody Beads (a-Mouse/ Hamster/ Rabbit/Rat)
CD14	Mouse monoclonal IgG	RMO52	Beckman Coulter	Gd ¹⁶⁰	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD14	Mouse IgG	M5E2	Biologend	APC-Fire750		C2	PBMC	
CD16	mouse monoclonal IgG	3G8	Biologend	Gd ¹⁵⁷	Trace Sciences	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD161	mouse monoclonal IgG	HP-3G10	Biologend	Yb ¹⁷¹	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD169 (Siglec-1)	Mouse monoclonal IgG	7-239	Miltenyi	Er ¹⁷⁰	Fluidigm	C1, B1	PBMC	BD™ CompBead (a-mouse), Quantum™ Simply Cellular
CD169 (Siglec-1)	Mouse monoclonal IgG	7-239	Biologend	PE		C2	PBMC	Quantum™ Simply Cellular
CD19	mouse monoclonal IgG	HIB-19	Fluidigm	Nd ¹⁴²		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD194 (CCR4)	mouse monoclonal IgG	205410	Fluidigm	Gd ¹⁵⁸		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD196 (CCR6)	mouse monoclonal IgG	G034E3	Fluidigm	Pr ¹⁴¹		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD196 (CCR6)	mouse monoclonal IgG	G034E3	Biologend	PE-Cy7		C2	PBMC	Quantum™ Simply Cellular
CD197 (CCR7)	mouse monoclonal IgG	G043H7	Fluidigm	Tb ¹⁵⁹		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD1c	mouse monoclonal IgG	AT5-8E7	Miltenyi	Sm ¹⁵⁴	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD20	human	RTX	Roche	In ¹¹³	Trace Sciences	C1, B0, B1	PBMC	MACS® CompBead (a-REA)
CD20	Mouse monoclonal IgG	2H7	Fluidigm	Nd ¹⁴⁷				BD™ CompBead (a-mouse)
CD21	mouse IgG1	HB5	Miltenyi	FITC		C1		
CD23	mouse IgG	M-L23.4	Miltenyi	APC		C1		
CD24	mouse monoclonal IgG	ML5	Fluidigm	Tm ¹⁶⁹		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD25	mouse monoclonal IgG	BC96	Biologend	Biotin		C1	PBMC	BD™ CompBead (a-mouse)
CD27	mouse monoclonal IgG	2,00E+04	Sanquin	Gd ¹⁵⁵	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD28	mouse monoclonal IgG	L293	BD	Dy ¹⁶¹	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD294 (CRTH2)	rat monoclonal IgG	BM16	Fluidigm	Dy ¹⁶³		C1, B0, B1	PBMC	BD™ CompBead (a-rat)
CD3	mouse monoclonal IgG	UCHT1	DRFZ	In ¹¹⁵	Trace Sciences	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD3	mouse monoclonal IgG	UCHT1	DRFZ	Er ¹⁷⁰	Fluidigm			BD™ CompBead (a-mouse)
CD3	mouse monoclonal IgG	UCHT1	Biologend	Brilliant Violet 510		C2	PBMC	
CD36	mouse monoclonal IgG	AC106	Miltenyi	Er ¹⁶⁶	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD38	mouse monoclonal IgG	HIT2	Biologend	Er ¹⁶⁷	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD39	mouse monoclonal IgG	A1	Biologend	Sm ¹⁴⁹	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD3e	hamster monoclonal IgG	145-2C11	Fluidigm	Sm ¹⁵²				AbCTM Total Antibody Beads, AbCTM Total Antibody Beads (a-Mouse/ Hamster/ Rabbit/ Rat)
CD4	mouse monoclonal IgG	RPA-T4	Biologend	Nd ¹⁴⁴	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse), Quantum™ Simply Cellular
CD4	mouse monoclonal IgG	RPA-T4	Biologend	Alexa 488		C2	PBMC	Quantum™ Simply Cellular
CD45	mouse monoclonal IgG	Hi30	Biologend	Ce ¹⁴⁰	Sigma	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD45RA	mouse monoclonal IgG	4G11	DRFZ	Yb ¹⁷³	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD5	mouse monoclonal IgG	UCHT2	Biologend	Pt ¹⁹⁵	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD56	Human recombinant IgG	REA196	Miltenyi	Nd ¹⁴⁸	Fluidigm			MACS® CompBead (a-REA)
CD56 (NCAM)	mouse monoclonal IgG	NCAM16.2	Fluidigm	Yb ¹⁷⁶		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD57	mouse IgM	HCD57	Biologend	La ¹³⁹	Sigma	C1	PBMC	BD™ CompBead (a-mouse)
CD7	mouse monoclonal IgG	CD7-6B7	Biologend	Nd ¹⁴⁶	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD73	mouse monoclonal IgG	AD2	Fluidigm	Er ¹⁶⁸		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD8	mouse monoclonal IgG	GN11/134D7	DRFZ	Pt ¹⁹⁶	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CD8	mouse monoclonal IgG	GN11/134D7	DRFZ	Ce ¹⁴⁰	Sigma			BD™ CompBead (a-mouse)
CD8	rat monoclonal IgG	53-6.7	Fluidigm	Nd ¹⁴⁶				AbCTM Total Antibody Beads (a-Mouse/ Hamster/ Rabbit/ Rat)
CD8	Mouse monoclonal IgG	BW135/80	Miltenyi	PE-Cy7		C2	PBMC	
CD95	mouse monoclonal IgG	DX2	Biologend	Sm ¹⁵²	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
CXCR3	mouse monoclonal IgG	G025H7	Fluidigm	Gd ¹⁵⁶		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
HLA-DR	mouse monoclonal IgG	L243	DRFZ	Yb ¹⁷⁴	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse), Quantum™ Simply Cellular
HLA-DR	mouse monoclonal IgG	L243	DRFZ	Cy5	GE Healthcare	C2	PBMC	Quantum™ Simply Cellular
IgA	goat polyclonal	Polyclonal	Fluidigm	Nd ¹⁴⁸		C1	PBMC	
IgD	mouse monoclonal IgG	IgD26	Miltenyi	Sm ¹⁴⁷	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
IgE	mouse monoclonal IgG	MB10-5C4	Biologend	Eu ¹⁵³	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
IgM	mouse monoclonal IgG	MHM-88	Fluidigm	Yb ¹⁷²		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
TCR α 7.2	mouse monoclonal IgG	3C10	Biologend	Nd ¹⁵⁰	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
TCR γ 6	mouse monoclonal IgG	11F2	Miltenyi	Eu ¹⁵¹	Fluidigm	C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
α -APC	mouse monoclonal IgG	APC003	Fluidigm	Dy ¹⁶²		C1, B0, B1	PBMC	BD™ CompBead (a-mouse)
α -Cy5/A647	mouse monoclonal IgG	AM1-7H6.16.44	Miltenyi	Er ¹⁷⁰	Fluidigm	B0		BD™ CompBead (a-mouse)
α -FITC	mouse monoclonal IgG	FIT22	Biologend	Lu ¹⁷⁵	Fluidigm	C1	PBMC	BD™ CompBead (a-mouse)