fluorochrome	target species	specificity	clone	company	catalog no.	concentration
BV421	human	CD19	HIB19	BioLegend	302234	1:100
	mouse	CD4	RM4-5	BioLegend	100544	1:400
SB436	human	CD31	WM-59	Invitrogen	62-0319-42	1:100
	mouse	CD31	390	Invitrogen	62-0311-82	1:400
eFluor450	human	CD3	Okt 03	Invitrogen	48-0037-42	1:100
	human/mouse	CD11b	M1/70	Invitrogen	48-0112-82	1:400
BV480	human	CD4	RPA-T4	BD Biosciences	746541	1:100
	mouse	CD4	RM4-5	BD Biosciences	565634	1:400
	mouse	Tim4	RMT4-54	BD Bioscience	746499	1:400
	mouse	MerTK	108928	BD Bioscience	747896	1:400
V500	human	CD16	3G8	BD Biosciences	561394	1:100
	mouse	CD45	30-F11	BD Biosciences	557659	1:400
SV538	human	CD3	UCHT1	BioLegend	300484	1:100
	mouse	B220	RA3-6B2	BioLegend	103283	1:400
BV570	human/mouse	CD11b	M1/70	BioLegend	101233	1:100
BV605	human	CD38	HIT2	BioLegend	303532	1:100
	mouse	CX3CR1	SA011F11	BioLegend	149027	1:400
BV650	human	CD15	W6D3	BioLegend	323033	1:100
	mouse	CX3CR1	SA011F11	BioLegend	149033	1:400
BV711	human	CD3	OKT3	BioLegend	317328	1:100
	mouse	CD8	53-6.7	Biolegend	100748	1:400
BV750	human	CD56	5.1H11	BioLegend	362556	1:100
	mouse	TCRb	H57-597	BD Biosciences	747006	1:400
BV785	human	TLR2	11G7	BD Biosciences	742771	1:100
	mouse	CD8	53-6.7	BioLegend	100749	1:400
	human	CD141	M80	BioLegend	344115	1:100
	mouse	CD206	C068C2	BioLegend	141729	1:400
	mouse	CD25	PC61	BioLegend	102051	1:400
BB515	human	CD8	RPA-T8	BD Biosciences	564526	1:400
	mouse	CD8	53-6.7	BD Biosciences	564459	1:400
FITC	human/mouse	CD11b	M1/70	BioLegend	101206	1:100
	mouse	CD4	RM4-5	BD Biosciences	553047	1:400
Alexa532	human	CD45	HI30	Invitrogen	58-0459-42	1:100
	mouse	CD45	30-F11	Invitrogen	58-0451-82	1:400
PE	human	CX3CR1	2A9-1	BD Biosciences	565796	1:100
	mouse	CD4	RM4-5	BioLegend	100512	1:400
PE/Dazzle594	human	CCR2	K036C2	BioLegend	357222	1:100
	human/mouse	CD11b	M1/70	BioLegend	101255	1:400
PE/Fire640	human	CD45RO	UCHL1	BioLegend	304263	1:100
	mouse	CD11c	QA18A72	BioLegend	161104	1:400
PE-Cy5	human	CD19	HIB19	BioLegend	302210	1:100
	mouse	CD19	eBio 1D3	Invitrogen	15-0193-83	1:400
BB700	human	CD127	HLA-7R-M21	BD Biosciences	566398	1:100
	mouse	CD11c	N418	BD Biosciences	745852	1:400
PerCP-Cy5.5	human	CD14	M5E2	BioLegend	301824	1:100
	human/mouse	CD11b	M1/70	BD Biosciences	550993	1:100
	mouse	CD4	RM4-5	Invitrogen	45-0042-82	1:400
	mouse	CD103	2EF	BioLegend	121416	1:400
	mouse	CD11c	N418	Invitrogen	45-0114-82	1:400
PerCP-eFl710	human	CD45RA	GRT22	Invitrogen	46-0468-42	1:100
	human	CD11c	3,9	Invitrogen	46-0116-42	1:100
	human	LAG3	3DS223H	Invitrogen	46-2239-42	1:100
	mouse	CD137	17B5	Invitrogen	46-1371-82	1:400
	mouse	CD369	bg1fpj	Invitrogen	46-5859-82	1:400
PE-Cy7	human	CD161	HP-3G10	Invitrogen	25-1619-42	1:100
	mouse	CD8	53-6.7	Invitrogen	25-0081-82	1:400
PE/Fire810	human	CXCR3	G025H7	BioLegend	353759	1:100
	mouse	CD3	17A2	BioLegend	100277	1:400
APC	human	CD14	M5E2	BioLegend	301807	1:100
	mouse	CD4	RM4-5	Invitrogen	17-0042-82	1:400
Al647	human	CD66b	G10F5	BioLegend	305109	1:100
	mouse	CD8	53-6.7	BioLegend	100724	1:400
SparkNIR 685	human	CD45	2D1	BioLegend	368551	1:100
	mouse	CD8	53-6.7	BioLegend	100781	1:400
Al700	human	CD19	HIB19	BioLegend	302225	1:100
	human	CD127	A7R34	Invitrogen	56-1271-82	1:100
	mouse	CD8	53-6.7	BD Biosciences	557959	1:400
	mouse	Ly6G	1A8	BioLegend	127622	1:400
APC-Cy7	human	CD33	WM53	BioLegend	303442	1:100
	mouse	CD45	30-F11	BD Biosciences	557659	1:400
APC/Fire810	human	CD25	M-A251	BioLegend	356150	1:100
	mouse	Gr1	RB6-8C5	BioLegend	108469	1:400

Suppl. Table 1. Antibodies used in this study

Laser	Detector	center Wavelength (nm)	bandwidth (nm)
Violet (405nm)	V1	428	15
	V2	443	15
	V3	458	15
	V4	473	15
	V5	508	20
	V6	525	17
	V7	542	17
	V8	581	19
	V9	598	20
	V10	615	20
	V11	664	27
	V12	692	28
	V13	720	29
	V14	750	30
	V15	780	30
	V16	812	34
Blue (488nm)	B1	508	20
	B2	525	17
	B3	542	17
	B4	581	19
	B5	598	20
	B6	615	20
	B7	661	17
	B8	679	18
	B9	697	19
	B10	717	20
	B11	738	21
	B12	760	23
	B13	783	23
	B14	812	34
Red (635nm)	R1	661	17
	R2	679	18
	R3	697	19
	R4	717	20
	R5	738	21
	R6	760	23
	R7	783	23
	R8	812	34

Suppl. Table 2. Cytek Aurora ® configuration.

Laser	fluorochrome	peak channel	antibody			
			human blood leukocytes	Biolegend Compensation Beads	UltraComp eBeads Plus	mouse splenocytes
			anti-human			anti-mouse
Violet (405nm)	BV421	V1	CD19	CD19	CD19	CD4
	SB436	V2	CD31	CD31	CD31	CD31
	eFl450	V3	CD3	CD3	CD3	CD11b
	BV480	V5	CD4	CD4	CD4	CD4
	V500	V7	CD16	CD16	CD16	CD45
	SV538	V7	CD3	CD3	CD3	B220
	BV570	V8	CD11b	CD11b	CD11b	CD11b
	BV605	V10	CD38	CD38	CD38	CX3CR1
	BV650	V11	CD15	CD15	CD15	CX3CR1
	BV711	V13	CD3	CD3	CD3	CD8
	BV750	V14	CD56	CD56	CD56	TCRb
	BV785	V15	TLR2	TLR2	TLR2	CD8
Blue (488nm)	BB515	B1	CD8	CD8	CD8	CD8
	FITC	B2	CD11b	CD11b	CD11b	CD4
	Alexa532	B3	CD45	CD45	CD45	CD45
	PE	B4	CX3CR1	CX3CR1	CX3CR1	CD4
	PE/Dazzle594	B6	CCR2	CCR2	CCR2	CD11b
	PE/Fire640	B7	CD45RO	CD45RO	CD45RO	CD11c
	PE-Cy5	B8	CD19	CD19	CD19	CD19
	BB700	B9	CD127	CD127	CD127	CD11c
	PerCP-Cy5.5	B9	CD14	CD14	CD14	CD4
	PerCP-eFl710	B10	CD45RA	CD45RA	CD45RA	CD369
	PE-Cy7	B13	CD161	CD161	CD161	CD8
	PE/Fire810	B14	CXCR3	CXCR3	CXCR3	CD3
Red (635nm)	APC	R1	CD14	CD14	CD14	CD4
	Al647	R2	CD66b	CD66b	CD66b	CD8
	SparkNIR 685	R3	CD45	CD45	CD45	CD8
	Al700	R4	CD19	CD19	CD19	CD8
	APC-Cy7	R7	CD33	CD33	CD33	CD45
	APC/Fire810	R8	CD25	CD25	CD25	Gr1

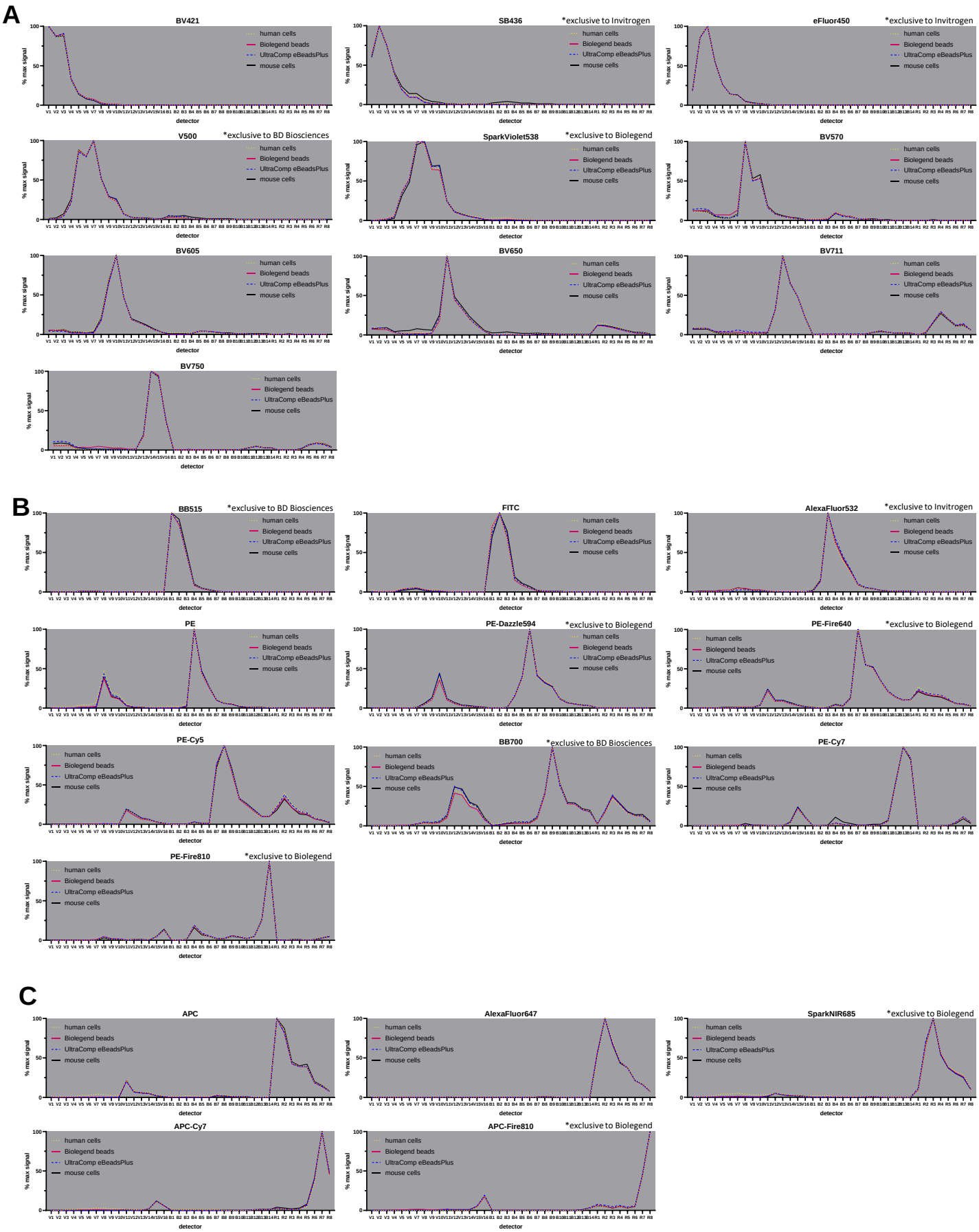
Suppl. Table 3. Antibodies used in Figure 1 and Supplementary Figure 1

Manufacturer	Product name	Catalog #	species reactivity					
			mouse	rat	rabbit	hamster	human	donkey
Biolegend	Compensation Beads	424601	x	x	x	x	x	x
ThermoFisher Scientific	Ultracomp eBeads Plus	01-3333-41	x	x	x	x	x	
	Ultracomp eBeads	01-2222-42	x	x		x		
	AbC Total Antibody Compensation Bead kit	A10513	x	x	x	x		
Slingshot Biosciences	SPECTRACOMP®	SSB-05-A	x	x		x		
	SPECTRACOMP® XT	SSB-21-A	x	x	x	x	x	
	HYPARCOMP™	SSB-20-A	x	x		x		
	HYPARCOMP™ XT	SSB-22-A	x	x	x	x	x	
Beckman Coulter	VersaComp Antibody Capture Kit	B22804	x	x	x	x		
Cytek Biosciences	Cytek® FSP™ CompBeads	B7-10011	x	x		x		
Novus Biologicals	Anti-Mouse Ig (H+L) Comp-Bead 2 Population (3.0-3.4 um) Kit	NBP3-11302	x	x		x		
	Anti-Mouse Ig (H+L) Comp-Bead 3 Population (5.5 µm) Kit	NBP3-00497	x	x		x		
	Anti-Mouse Ig (H+L) Comp-Bead 3 Population (7.5 µm) Kit	NBP3-00499	x	x		x		
	Blank Comp-Bead Particles (negative control)	NBP3-00500						
Miltenyi Biotec	MACS®Comp Bead Kit, anti-mouse Igκ	130-097-900	x					
	MACS®Comp Bead Kit, anti-human Igκ	130-104-187					x	
	MACS®Comp Bead Kit, anti-rat Igκ	130-107-755	x					
	MACS®Comp Bead Kit, anti-REA	130-104-693					x	
BD Biosciences	BD™ CompBeads Anti-Rat Ig, κ/Negative Control Compensation Particles Set	552844		x				
	BD™ CompBeads Anti-Rat and Anti-Hamster Ig κ /Negative Control Compensation Particles Set	552845		x		x		
	BD™ CompBeads Anti-Mouse Ig, κ/Negative Control Compensation Particles Set	552843	x					
	BD™ CompBead Plus Anti-Rat Ig, κ/Negative Control (BSA) Compensation Plus (7.5 µm) Particles Set	560499		x				
	BD™ CompBead Plus Anti-Mouse Ig, κ/Negative Control (BSA) Compensation Plus (7.5 µm) Particles Set	560497	x					
Bangs Laboratories	Simply Cellular® Compensation Standard (anti-Mouse IgG)	550	x					
	Simply Cellular® Compensation Standard - High (anti-Mouse IgG)	556	x					
	Simply Cellular® anti-Mouse for Violet Laser	835	x					
	Simply Cellular® Compensation Standard (anti-Rat IgG)	551		x				
	Simply Cellular® Compensation Standard (anti-Human IgG)	552						
Spherotech	COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 2 populations (Negative & High)	MIgP-08-2K	x					
	COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 2 populations (Negative & High)	CMIgP-30-2K	x					
	COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 2 populations (Negative & High)	CMIgP-30-5	x					
	COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 3 populations (Negative, Low, & High)	CMIgP-50-3K	x					
	COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 3 populations (Negative, Low, & High)	CMIgP-70-3K	x					

Suppl. Table 4. commercially available compensation beads

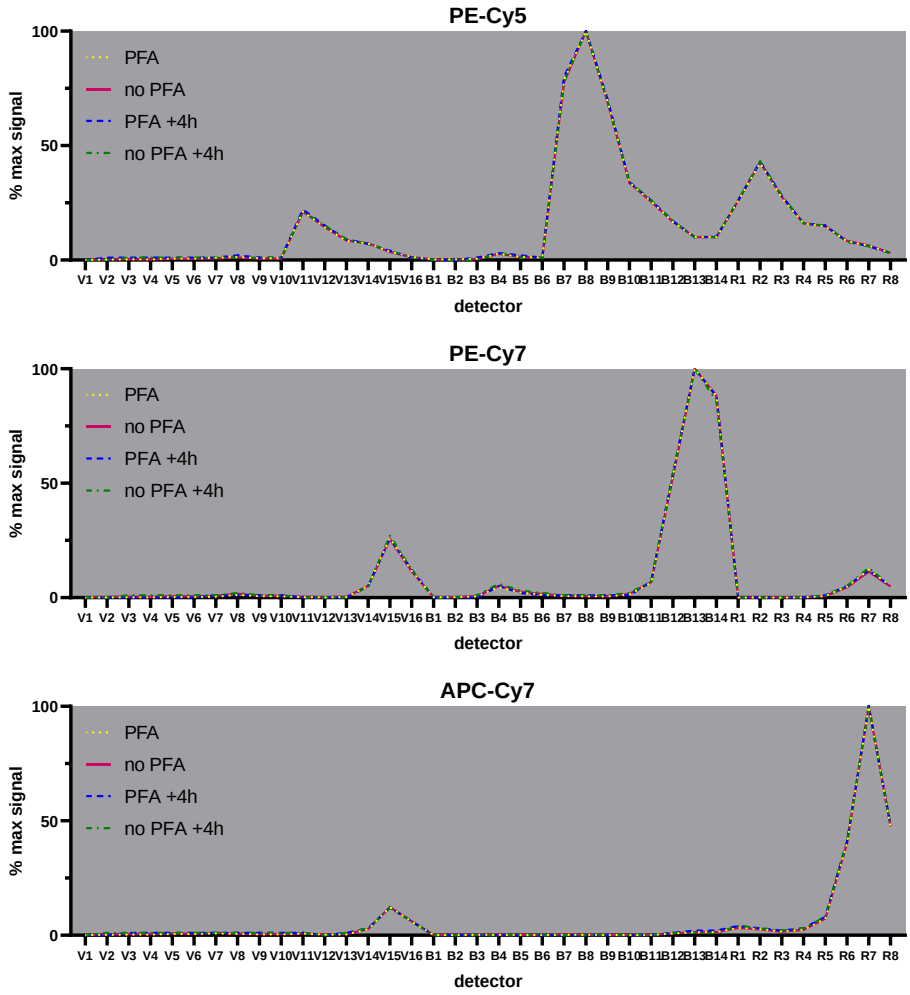
fluorochrome	Biolegend® Compensation Beads	UltraComp eBeads™ Plus
BV421	✓	✓
Super Bright™ 436	✓	✓
Pacific Blue™	✓	✓
BV480	✓	X
V500	✓	✓
Spark Violet™ 538	✓	✓
BV570	✓	✓
BV605	✓	✓
BV650	✓	✓
BV711	✓	✓
BV750	✓	✓
BV785	✓	X
BB515	✓	✓
FITC	✓	✓
Alexa Fluor™ 532	✓	✓
PE	✓	✓
PE/Dazzle™ 594	✓	✓
PE/Fire™ 640	✓	✓
PE-Cy5	✓	✓
BB700	✓	✓
PerCP-Cy5.5	X	X
PerCP-eFluor™ 710	✓	X
PE-Cy7	✓	✓
PE/Fire™ 810	✓	✓
APC	✓	✓
Alexa Fluor™ 647	✓	✓
Spark NIR™ 685	✓	✓
Alexa Fluor™ 700	X	X
APC-Cy7	✓	✓
APC/Fire™ 810	✓	✓

Suppl. Table 5. optimal reference control types for full spectrum cytometry



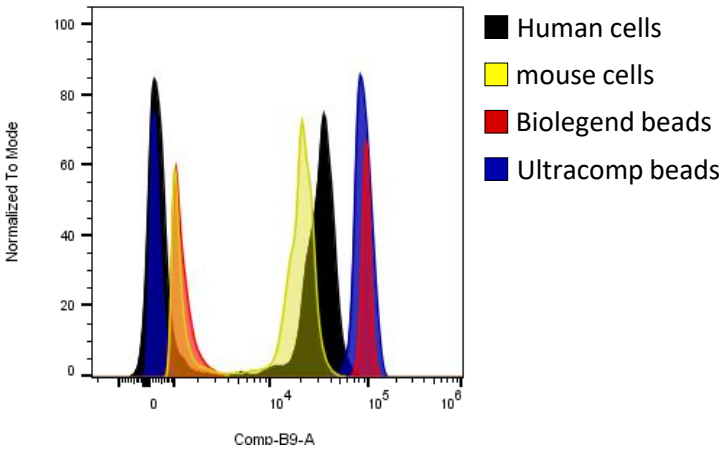
Supplementary Figure 1. Normalized emission spectra of fluorochromes showing identical emission spectra on cells and beads. Indicated fluorochromes were stained on cells (human peripheral blood leukocytes or murine splenocytes), Biolegend® Compensation Beads or UltraComp eBeads™ Plus Compensation Beads (ThermoFisher Scientific). Emission spectra were extracted from Spectroflow software, normalized to the maximum signal and overlaid in GraphPad Prism. **A** Fluorochromes excited by the violet laser (405nm). **B** Fluorochromes excited by the blue laser (488nm). **C** Fluorochromes excited by the red laser (635nm).

Supplementary Figure 2. similarity matrices of fluorochromes showing identical emission spectra on cells and beads. Indicated fluorochromes were stained on cells (human peripheral blood leukocytes or murine splenocytes), Biolegend® Compensation Beads or UltraComp eBeads™ Plus Compensation Beads (ThermoFisher Scientific). Similarity was determined in Spectroflow software. **A** Fluorochormes excited by the violet laser (405nm). **B** Fluorochormes excited by the blue laser (488nm). **C** Fluorochormes excited by the red laser (635nm). BL= Biolegend compensation beads, human= human peripheral blood leukocytes, mouse= murine splenocytes, UC= UltraComp eBeads Plus



Supplementary Figure 3. Normalized emission spectra of protein-based tandem dyes with and without PFA fixation. Indicated fluorochromes were stained on Biolegend® Compensation Beads and either fixed with 2% PFA or left unfixed. Samples were run immediately and again 4h later. Emission spectra were extracted from Spectroflo software, normalized to the maximum signal and overlaid in GraphPad Prism.

A



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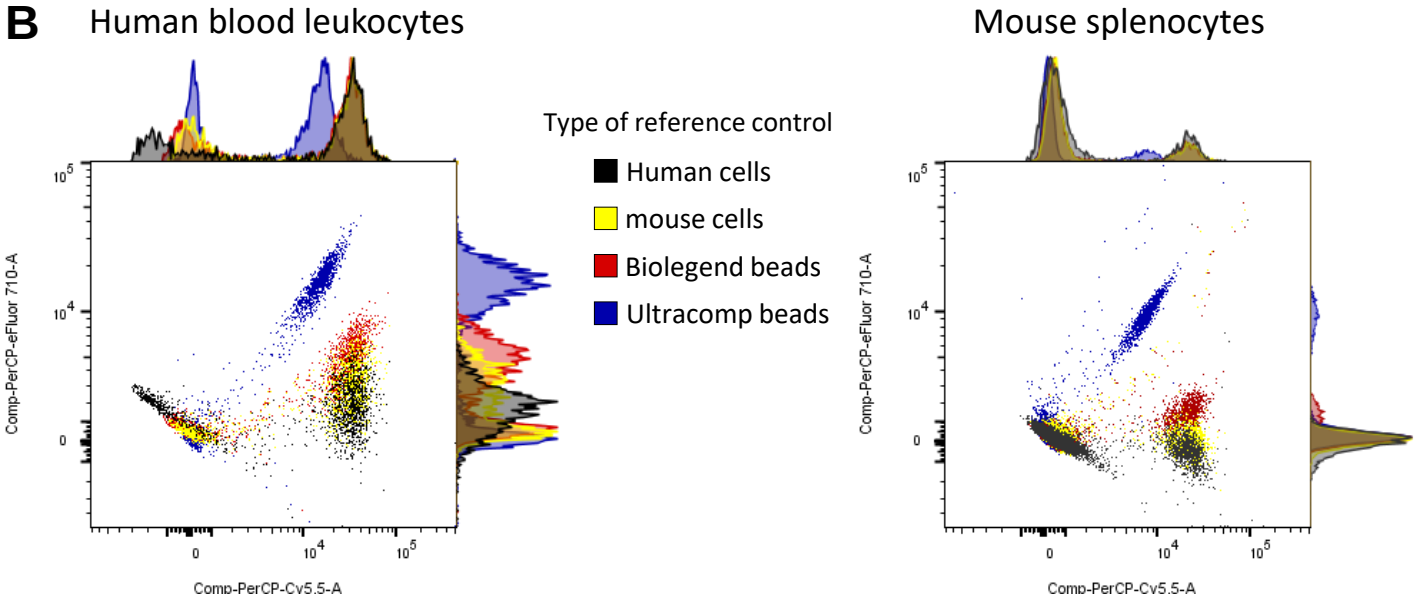
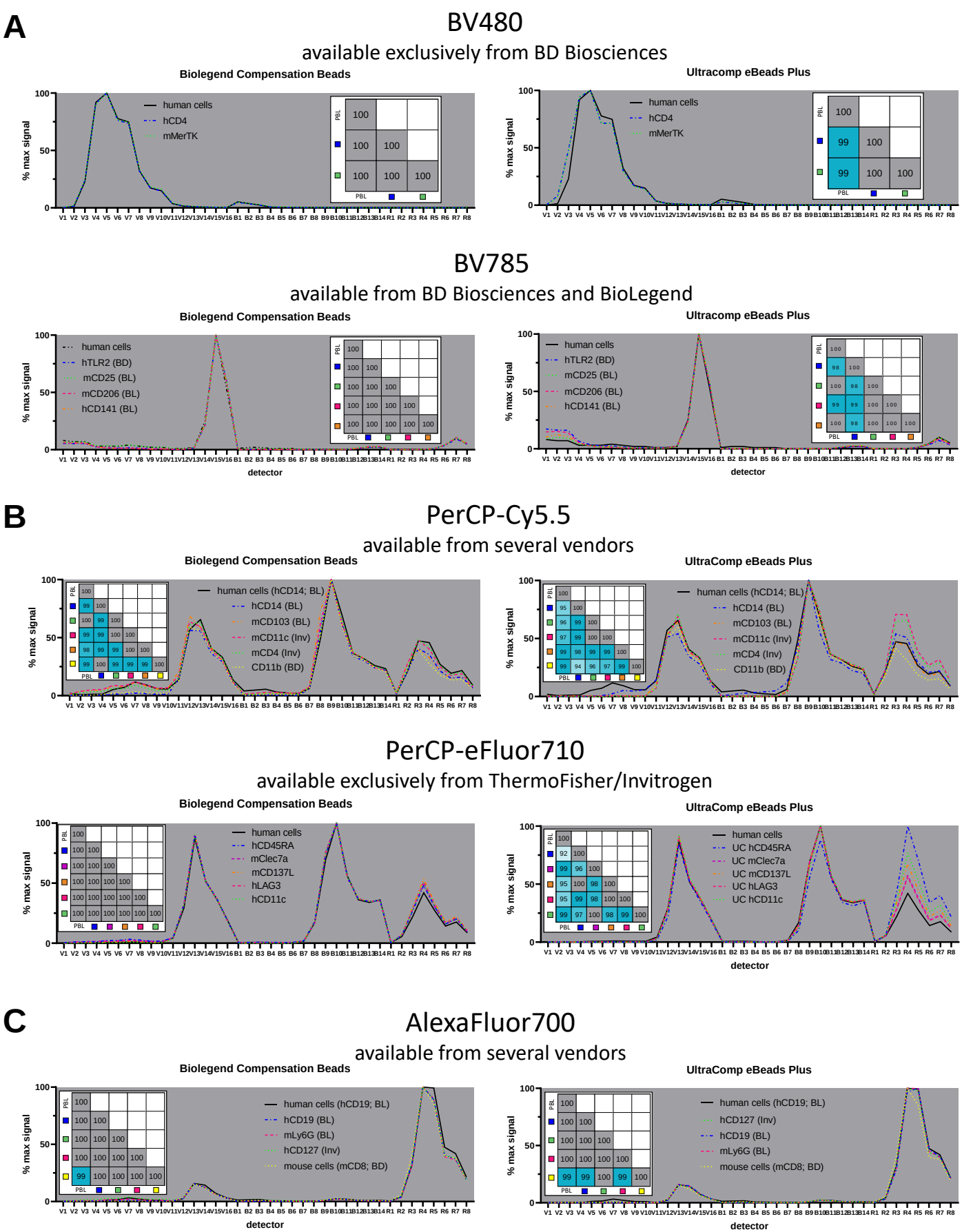


Supplementary Figure 4. A Histogram overlays of the indicated single stain control types stained with PerCP-Cy5.5 demonstrating signal intensity in the detector channel (B9). **B** Dot plot overlays with adjunct histograms of PerCP-Cy5.5 single stained human peripheral blood leukocytes (left) or murine splenocytes (right) unmixed with either Biolegend® Compensation Beads (red), UltraComp eBeads™ Plus (blue), human cells (black) or mouse cells (yellow) as reference controls.



Supplementary Figure 5. Indicated fluorochromes were stained on cells (human peripheral blood leukocytes), Biolegend® Compensation Beads or UltraComp eBeads™ Plus Compensation Beads (ThermoFisher Scientific). Emission spectra were extracted from Spectroflow software, normalized to the maximum signal and overlaid in GraphPad Prism. **A** Fluorochormes excited by the violet laser (405nm). **B** Fluorochromes excited by the blue laser (488nm). **C** Fluorochormes excited by the red laser (635nm). Antibody vendors are indicated in parentheses behind each antibody. h=anti-human, m=anti-mouse, PBL= peripheral blood leukocytes, BD= BD Biosciences, BL= Biolegend®, Inv= Invitrogen