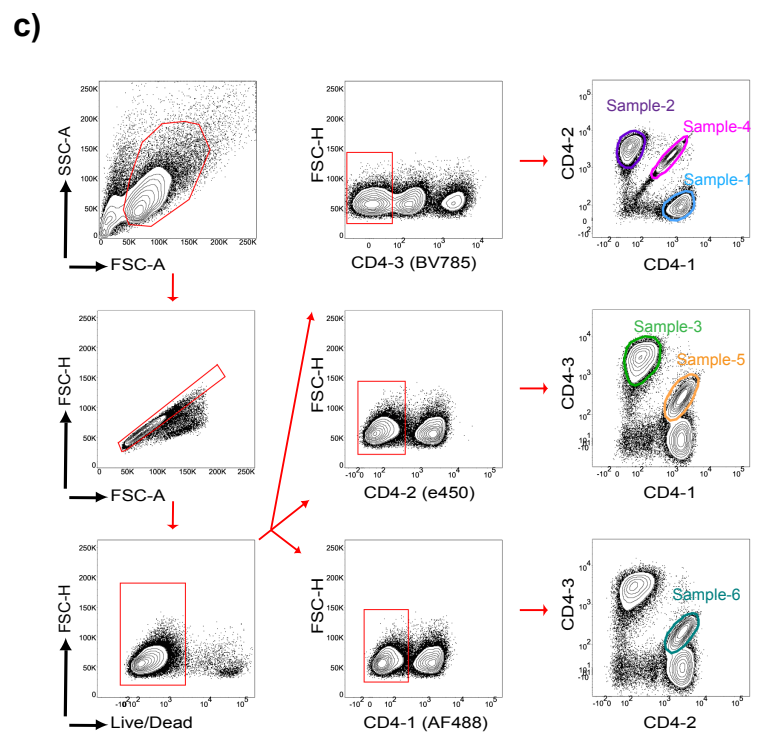
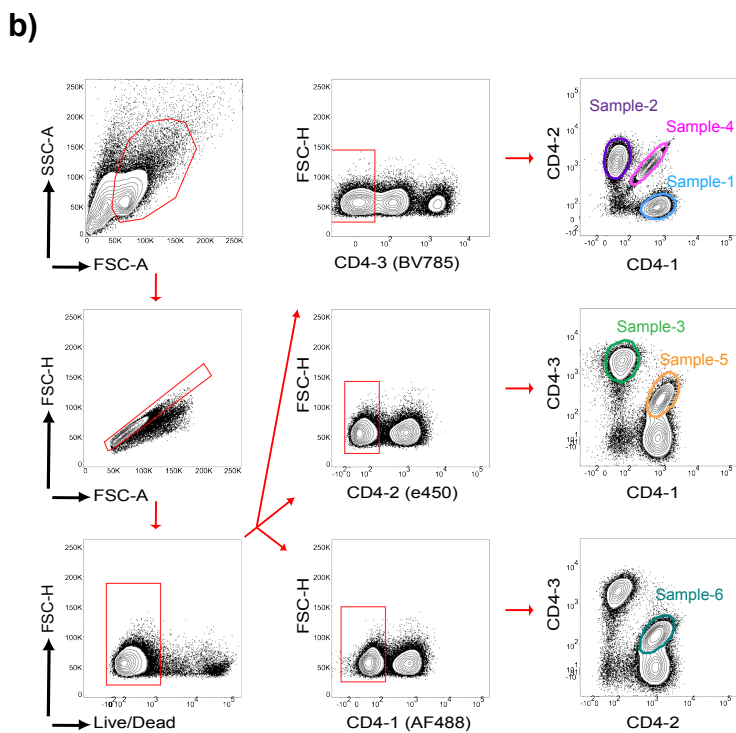
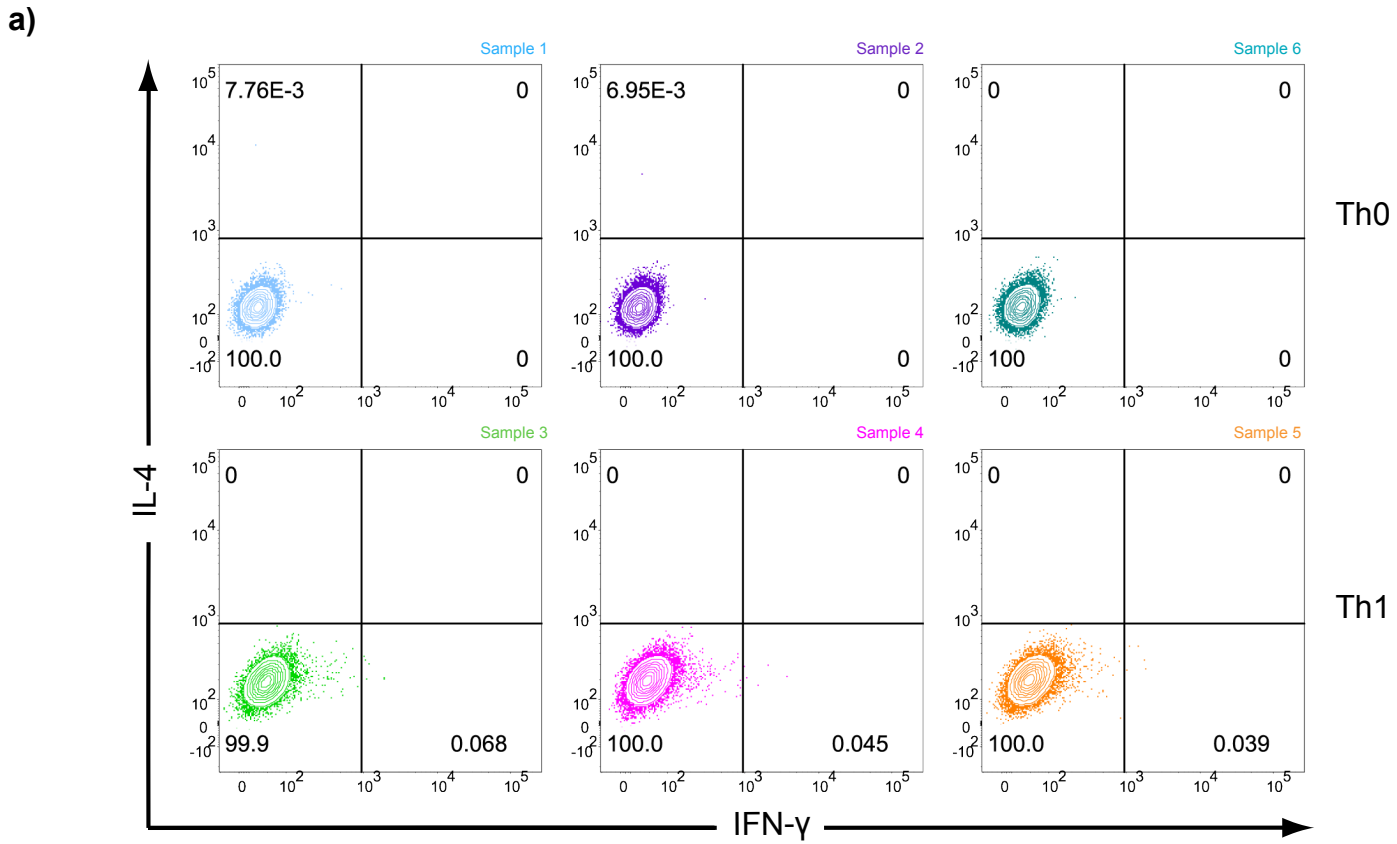
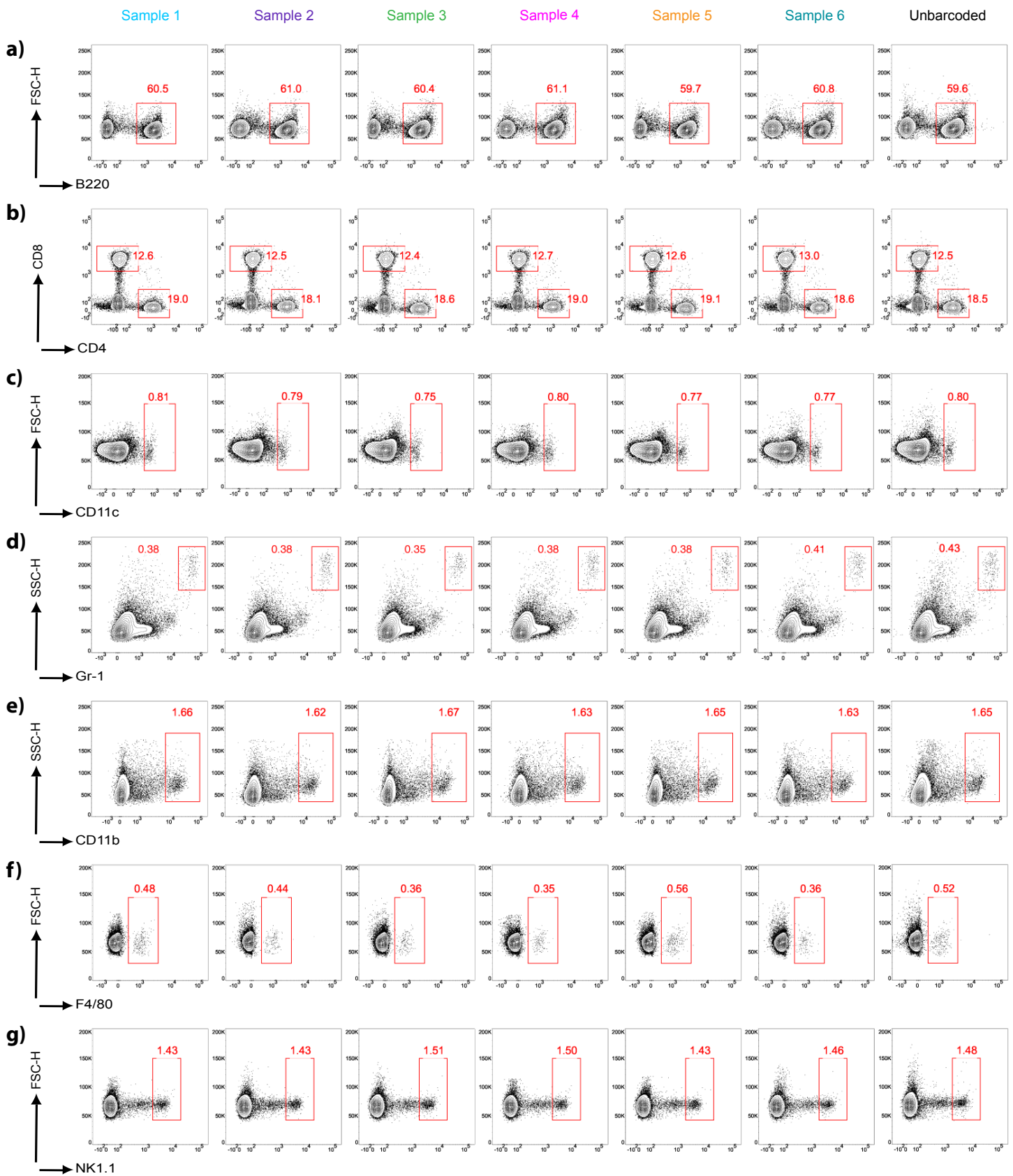


Supplementary Figure 1: a-b) Negative magnetic separation of B cells and naïve CD4<sup>+</sup> T cells generates highly viable pure lymphocyte populations. Representative flow cytometry plots showing the step wise gating of purified naïve CD4<sup>+</sup> T (a) and B (b) lymphocytes that were used in barcoding experiments. c-d) Actual flow cytometry plots showing the identification of individual barcoded B cell populations in phospho-flow and calcium flux assays. c) Fixed and methanol-permeabilized B cell samples were barcoded as shown and used in phospho-flow assays. d) Gates showing the individually-barcoded and pooled, live, Fluor4 stained and successively stimulated B cell populations used in real time calcium flux experiments.



Supplementary Figure 2: Barcoded T cell populations can be distinguished after 6 hours of culturing in both restimulating or non-restimulating conditions. a) Representative flow cytometry plots showing the percentages of IFN- $\gamma$ - and/or IL-4- expressing barcoded cells for both Th0 (samples 1,2,6) and Th1 (samples 3,4,5) differentiated in the non-restimulated group. b-c) Representative flow cytometry plots showing the actual gating of barcoded populations in the non-restimulated (b) and restimulated groups (c).



Supplementary Figure 3: Flow cytometry plots of a representative set of the six uniquely barcoded samples from the data in (Figure 6c). Red rectangular gates show B cells (a), CD4+ and CD8+ T cells (b), dendritic cells (c), granulocytes (d), monocytes (e), macrophages (f) and NK cell (g). Numbers refer to the percentage of the cells of each type within the live cell gate.

# Supplementary Table 1

	Clone	Conjugate	Vendor	Catalog no
Rat anti human/mouse B220	RA3-6B2	AF488	Biologend	103225
Rat anti human/mouse B220	RA3-6B2	BV785	Biologend	103246
Rat anti human/mouse B220	RA3-6B2	BV421	Biologend	103240
Rat anti human/mouse B220	RA3-6B2	PE-CF594	BD	562290
Rat anti human/mouse B220	RA3-6B2	AF700	Biologend	109822
Rat anti human/mouse B220	RA3-6B2	AF647	Biologend	103226
Rat anti human/mouse B220	RA3-6B2	PE	Biologend	103208
Rat anti mouse CD4	RM4-5	AF488	Biologend	100529
Rat anti mouse CD4	RM4-5	e450	Ebioscience	48-0042-82
Rat anti mouse CD4	RM4-5	BV785	Biologend	100552
Rat anti mouse CD4	RM4-5	BV421	Biologend	100544
Rat anti mouse CD4	RM4-5	AF647	Biologend	100530
Rat anti mouse CD4	RM4-5	APC	Biologend	100516
Rat anti mouse CD4	RM4-5	BV650	Biologend	100545
Rat anti mouse CD3	17A2	AF700	Biologend	100216
Rat anti mouse CD3	17A2	BV785	Biologend	100232
Rat anti human/mouse CD44	IM7	APC	Biologend	103012
Rat anti mouse CD62L	MEL-14	PE	Biologend	104408
Mouse anti mouse CD45.2	104	AF700	Biologend	109822
Mouse anti mouse CD45.2	104	FITC	Biologend	109816
Mouse anti mouse CD45.2	104	PE	Biologend	109808
Mouse anti mouse CD45.2	104	BV421	Biologend	109832
Mouse anti mouse CD45.2	104	APC	Biologend	109814
Mouse anti mouse CD45.2	104	AF647	Biologend	109818
Rat anti mouse CD8	53-6.7	BV785	Biologend	100750
Rat anti mouse CD8	53-6.7	BV711	Biologend	100748
Rat anti mouse CD19	6D5	BV421	Biologend	115538
Mouse anti mouse NK1.1	PK136	Percp	Biologend	108726
Mouse anti mouse NK1.1	PK136	AF647	Biologend	108418
Rat anti mouse Gr1	RB6-8C6	AF488	Biologend	108417
Rat anti mouse Gr1	RB6-8C6	BV605	Biologend	108440
Rat anti mouse F4/80	BM8	PE.Cy7	Biologend	123114
Rat anti mouse F4/80	BM8	Percp.Cy5.5	Biologend	123128
Rat anti mouse CD11b	M1/70	PE.Cy7	Biologend	101216
Hamster anti mouse CD11c	N418	PE-Dazzle 594	Biologend	117348
Hamster anti mouse CD11c	N418	BV421	Biologend	117330
Donkey anti mouse IgM (Fab'2) $\mu$ chain specific	Polyclonal	Unconjugated	Jackson Immuno research	715-006-020
Hamster anti mouse CD3	145-2C11	Unconjugated	Biologend	100331
Hamster anti mouse CD28	37.51	Unconjugated	Biologend	102112
Rat anti mouse IL-4	1B11	Unconjugated	Biologend	504108
Rat anti mouse IL-4	1B11	AF647	Biologend	504110
Rat anti mouse IFN- $\gamma$	XMG1.2	PE	Biologend	505808
Rat anti mouse CD16/32	93	Unconjugated	Biologend	101302
Rat IgG2a isotype control	RTK2758	AF700	Biologend	400528
Rat IgG2a isotype control	RTK2758	PE	Biologend	400507
Rat IgG2a isotype control	RTK2758	BV421	Biologend	400535
Rat IgG2a isotype control	RTK2758	AF488	Biologend	400525
Rat IgG2a isotype control	RTK2758	AF647	Biologend	400526

Biologend, San Diego, CA, USA

Ebioscience, San Diego, CA, USA

BD Biosciences, San Jose, CA, USA

Jackson Immunoresearch, West Grove, PA, USA