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Supporting Information

Title Cell-templated Supported Lipid Bilayers for T Cell Activation

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Supporting Tables

Table S1. Molar composition of lipid bilayers

Molar %	Liquid Crystalline (Fluid)	Ordered Gel (Stationary)
77.5	DOPC (T_m = **-17**°C)	DSPC (T_m = **55**°C)
20	Cholesterol	Cholesterol
0.02-2	DSPE-PEG-Biotin	DSPE-PEG-Biotin
0.5-2.48	18:1 PEG-2000 PE	18:0 PEG-2000 PE

Table S2. Size and polydispersity of liposomes

Formulation	Z-average diameter (nm)	PDI
DOPC 2%	86.91	0.251
DOPC 0.2%	104.7	0.361
DOPC 0.02%	87.61	0.337
DSPC 2%	81.13	0.172
DSPC 0.2%	81.22	0.161
DSPC 0.02%	93.74	0.213

Table S3. Antibody clones and vendors for intracellular cytokine staining (ICCS) panel

Panel: ICCS	Clone	Dye	Vendor
Live/Dead	N/A	BD Horizon Fixable Viability Stain 520	BD Biosciences
CD4	SK3	APC	Biolegend
CD8	SK1	PacBlue	Biolegend
4-1BB	4B4-1	PE	Biolegend
TNFa	MAb11	BUV395	BD Biosciences
IFNg	4S.B3	BV786	BD Biosciences
IL-2	MQ1-17H12	PE-Cy7	Biolegend

Table S4. Antibody clones and vendors for outgrowth panel

Panel:	Clone	Dye	Vendor

Outgrowth			
Live/Dead	N/A	Zombie NIR Fixable Viability Stain	Biolegend
CD3	HIT3a	PE	Biolegend
CD4	RPA-T4	APC	Biolegend
CD8	RPA-T8	FITC	Biolegend

Table S5. Antibody clones and vendors for differentiation panel

Panel: Differentiation	Clone	Dye	Vendor
Live/Dead	N/A	BD Horizon Fixable Viability Stain 520	BD Biosciences
CD4	OKT4	PE-Dazzle594	Biolegend
CD8	SK1	PacBlue	Biolegend
CD45RA	HI100	BV785	Biolegend
CD62L	DREG-56	PE	Biolegend
PD-1	EH12.2H7	BV711	Biolegend
CCR7	150503	BUV395	BD Biosciences

Table S6. Summary of statistical analysis on aAPC design parameters and T cell response

|	Antibody Density	Lipid	Shape
Outgrowth	$p<0.0001$, positive linear trend	$p<0.0001$, DOPC>DSPC	$p=0.34$
CD4/CD8 Ratio	$p=0.21$	$p=0.0035$, DOPC>DSPC	$p<0.0001$, HeLa>RBC>uSphere
CD4$^+$ Differentiation (CD45RA$^+$/CD62L$^+$)	$p=0.0016$, positive linear trend	$p=0.15$	$p=0.0013$, RBC>uSphere
CD8$^+$ Differentiation (CD45RA$^+$/CD62L$^+$)	$p=0.011$, positive linear trend	$p=0.037$, DSPC>DOPC	$p<0.0001$, RBC≈HeLa>uSphere
PD-1$^+$, CD4$^+$	$p=0.13$	$p=0.49$	$p<0.001$, uSphere>HeLa≈RBC
PD-1$^+$, CD8$^+$	$p=0.14$	$p=0.004$, DSPC>DOPC	$p=0.32$

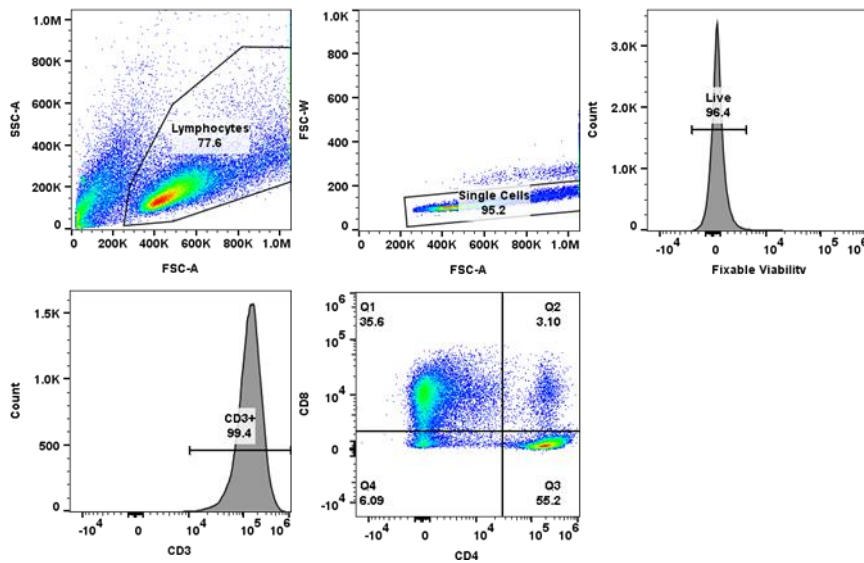**Supporting Figures**
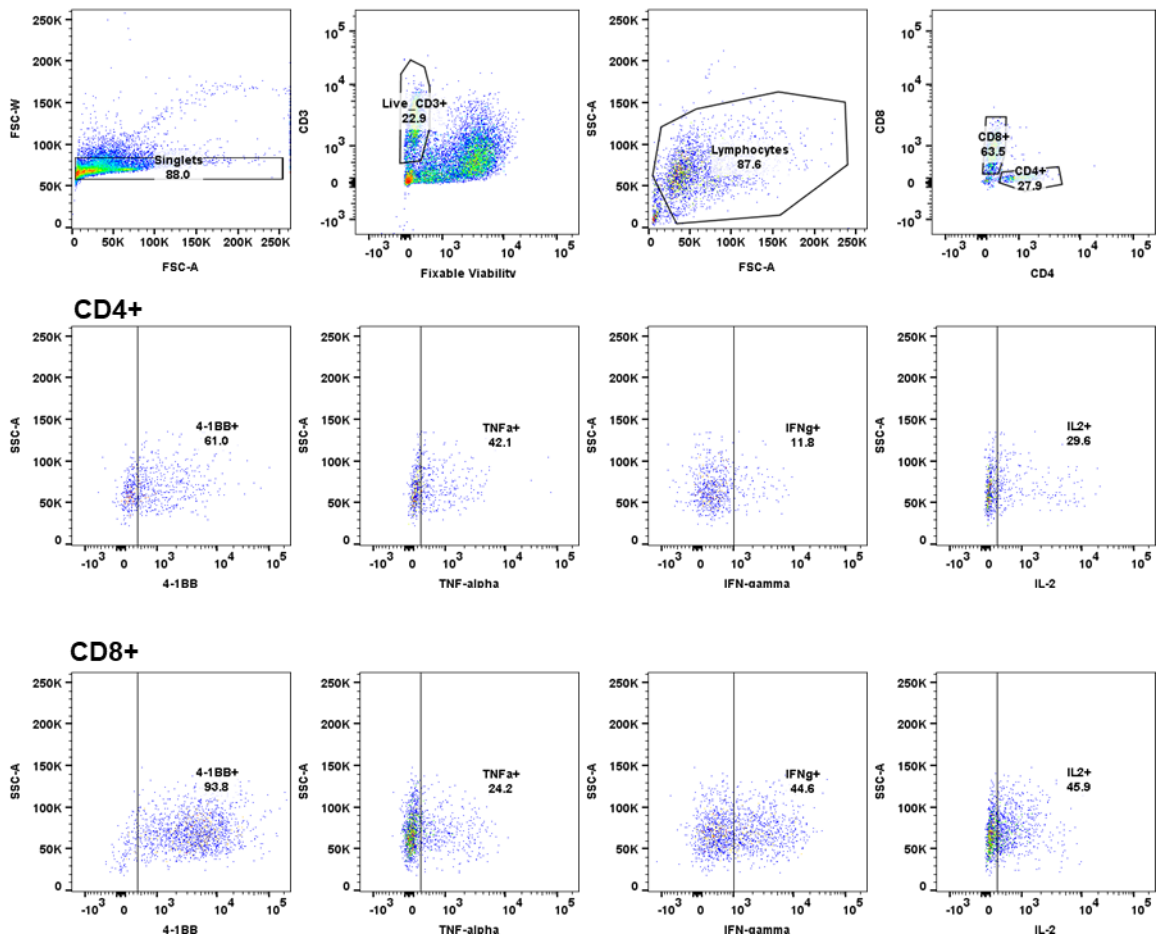
Figure S1. Gating strategy for ICCS flow analysis
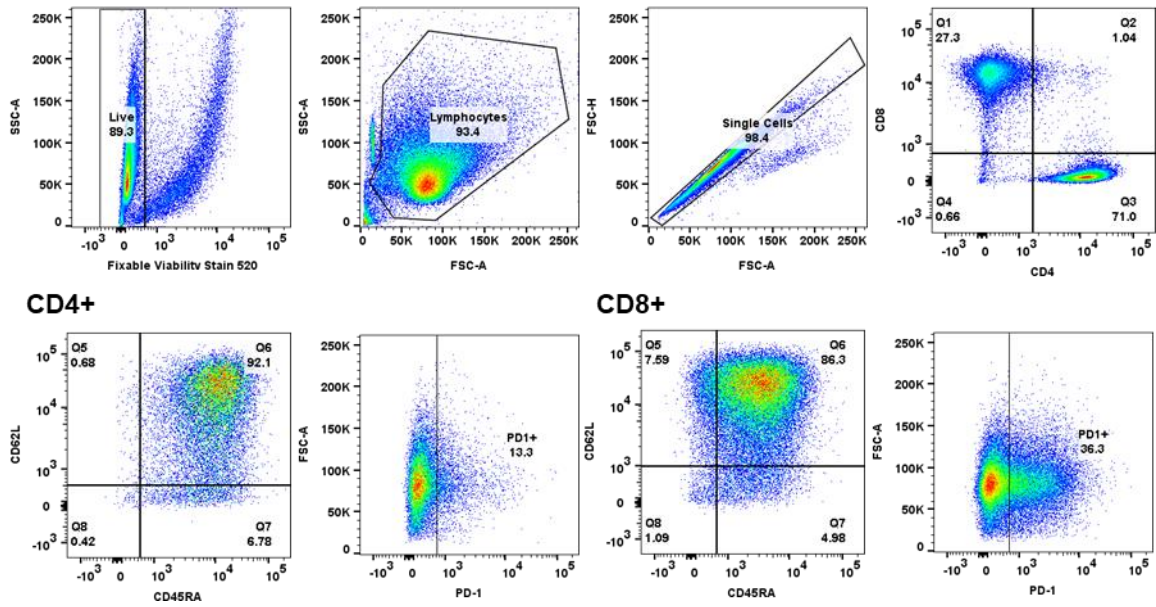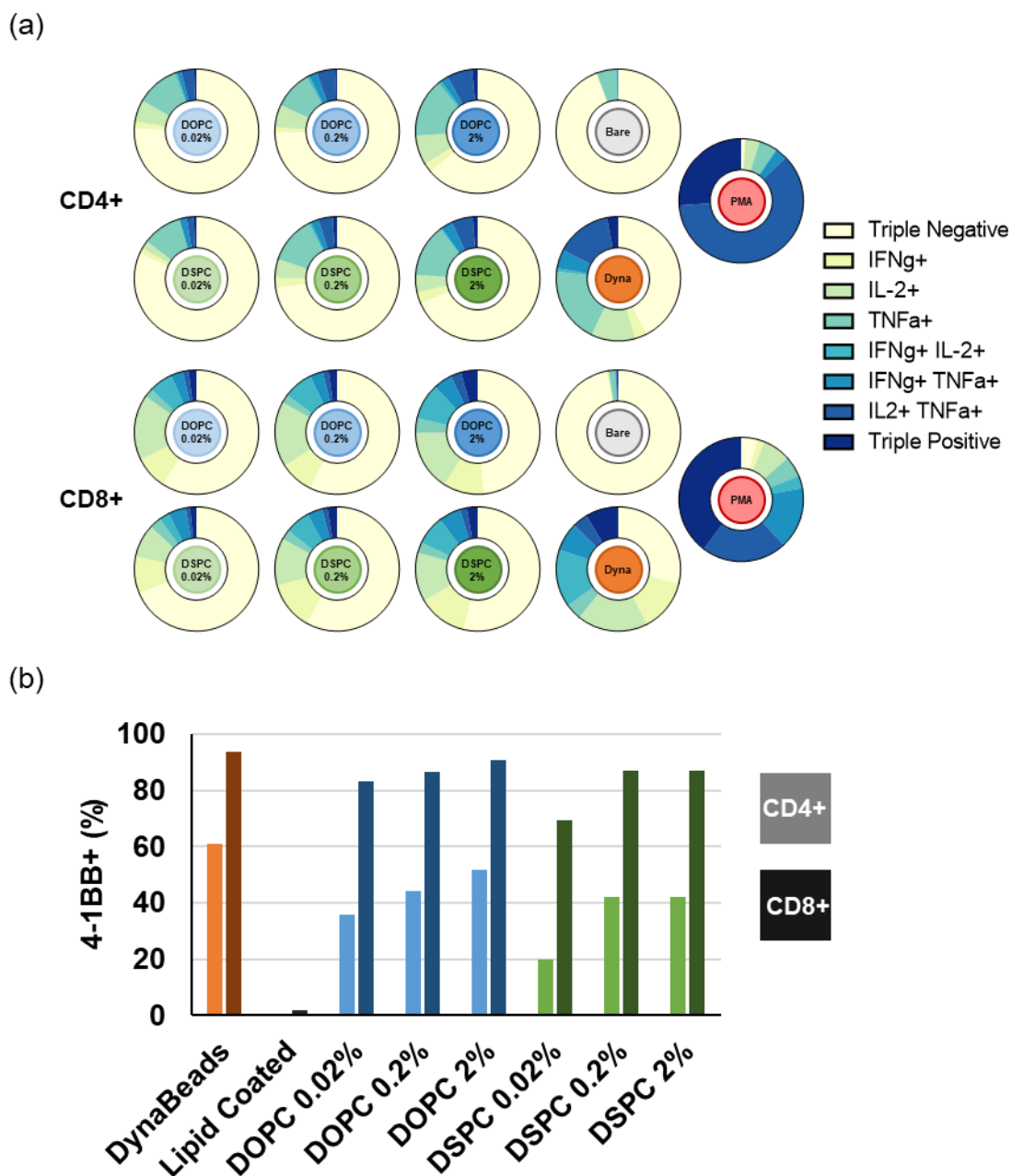

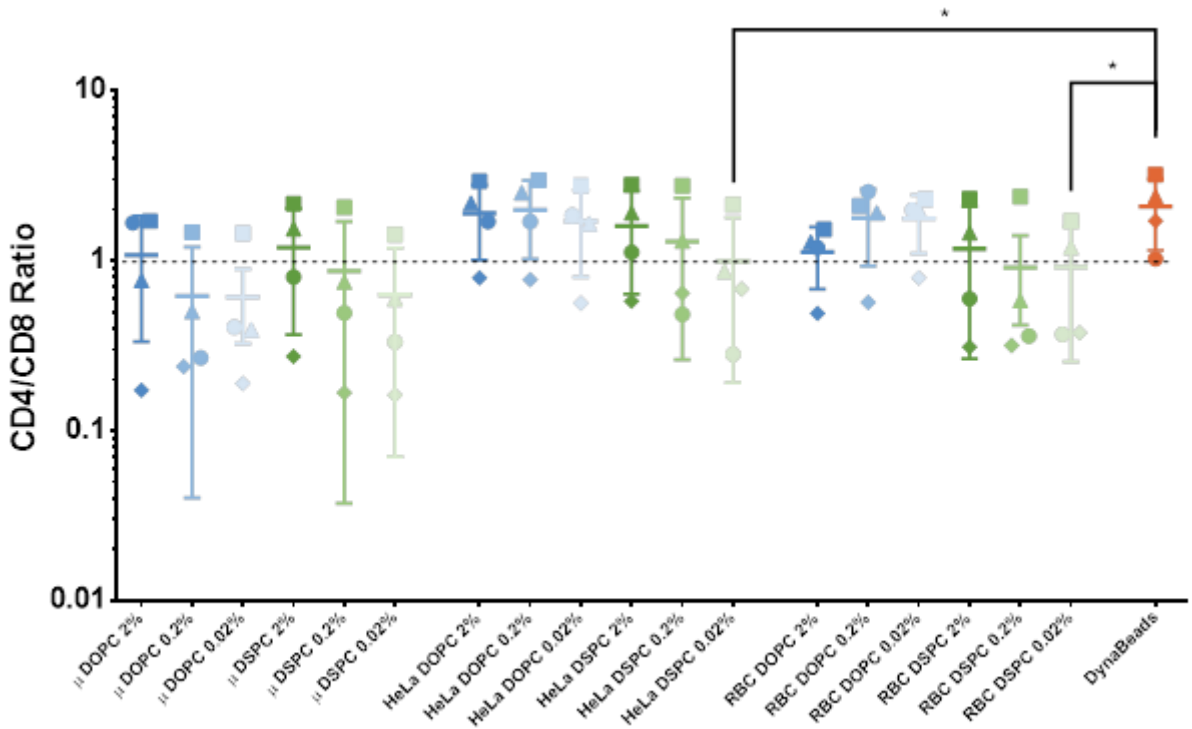**Figure S2.** Gating strategy for outgrowth flow analysis

**Figure S3.** Gating strategy for differentiation flow analysis

Figure S4. 72-hour microsphere particle activation. (a) Basal cytokine production and (b) 4-1BB expression in CD4$^+$ and CD8$^+$ T cells co-cultured with microsphere activation particles (n = 1).

Figure S5. Ratio of CD4+ to CD8+ T cells after 9-day outgrowth. Data represented as mean ± SD (n = 4 biological replicates; paired one-way ANOVA with Dunnett's multiple comparisons test, *P < 0.05).
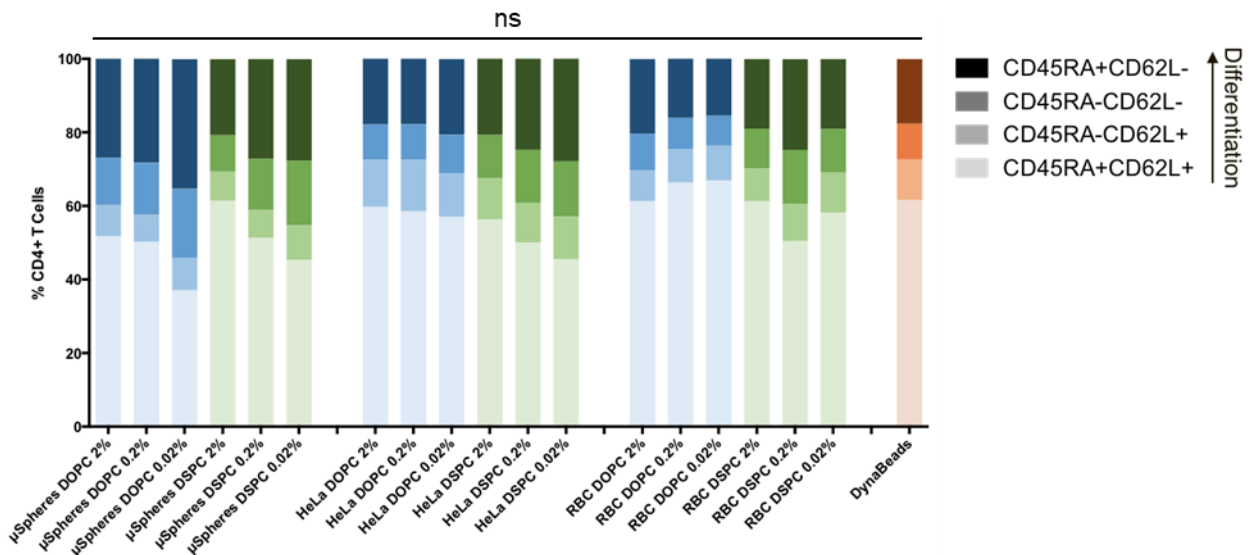

Figure S6. CD4+ T cell differentiation at day 12 post-stimulation. Data are shown as mean of 3 independent experiments with cells from 3 donors (error bars omitted for legibility, paired two-way ANOVA with Bonferroni's multiple comparisons test, ns = not significant).
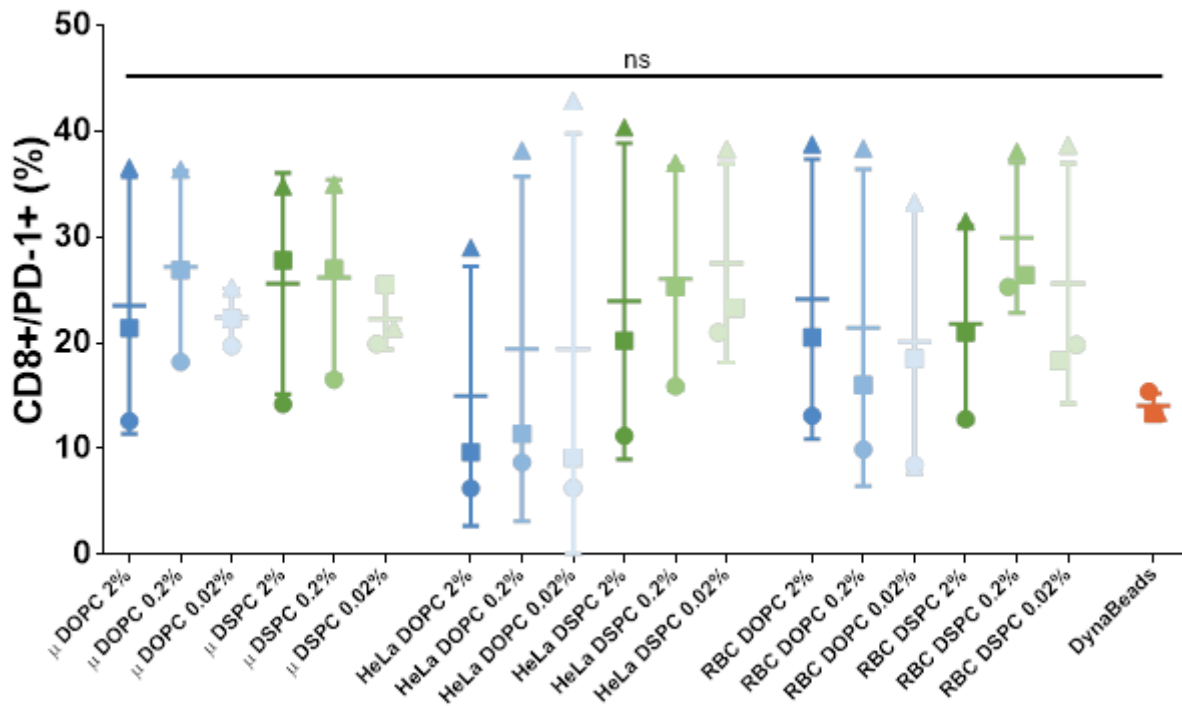
Figure S7. CD8^{+} T cell PD-1 expression at day 12 post-stimulation. Data are shown as mean of 3 independent experiments with cells from 3 donors (Paired one-way ANOVA with Tukey's multiple comparisons test, ns = not significant, $P = 0.33$).
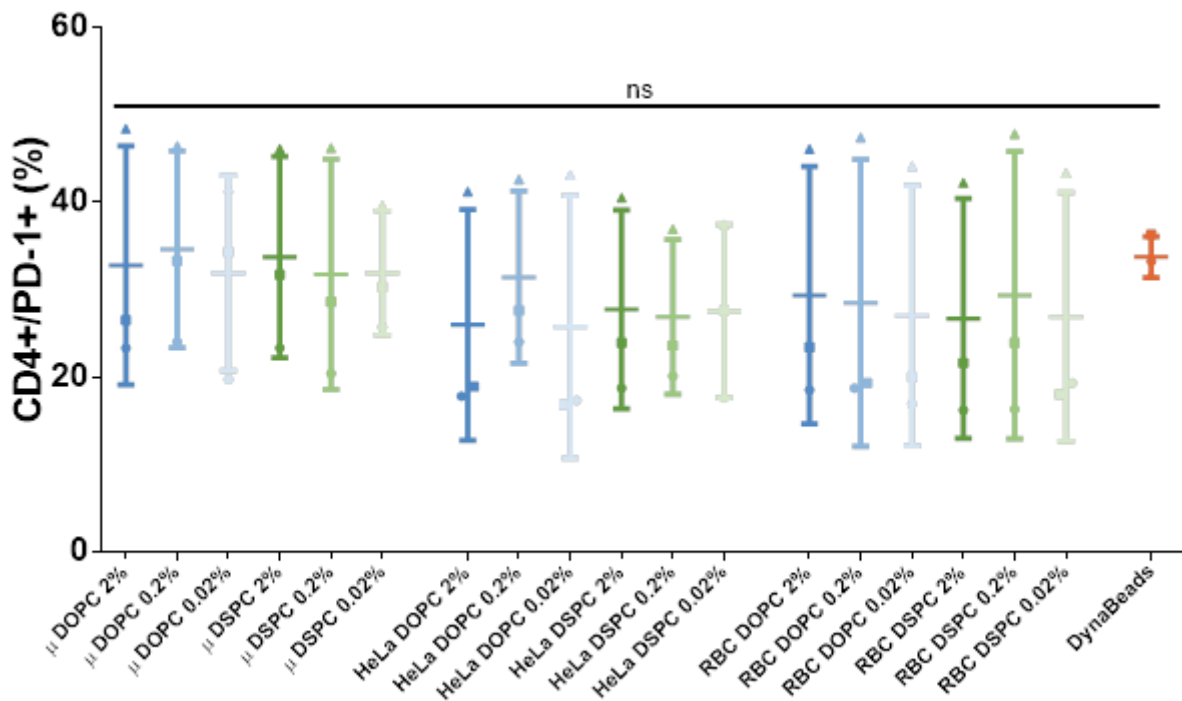

Figure S8. CD4^{+} T cell PD-1 expression at day 12 post-stimulation. Data are shown as mean of 3 independent experiments with cells from 3 donors (Paired one-way ANOVA with Tukey's multiple comparisons test, ns = not significant, $P = 0.40$).
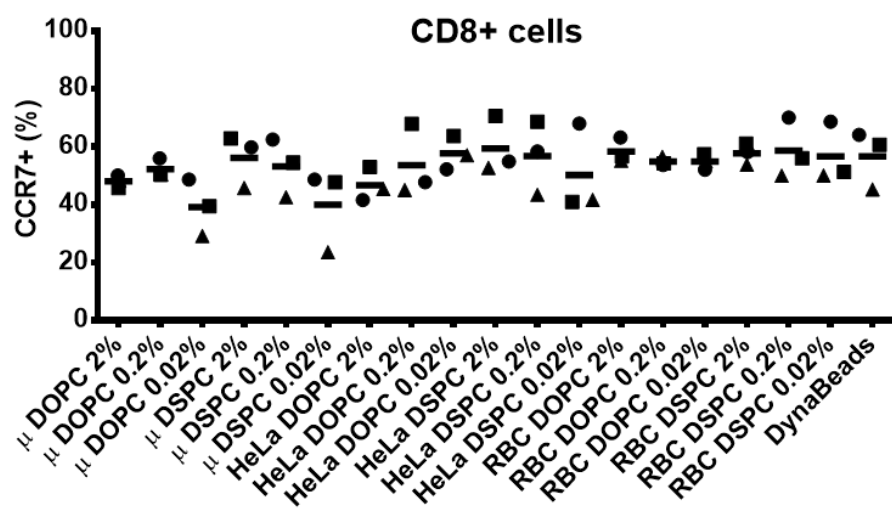
(a)

(b)
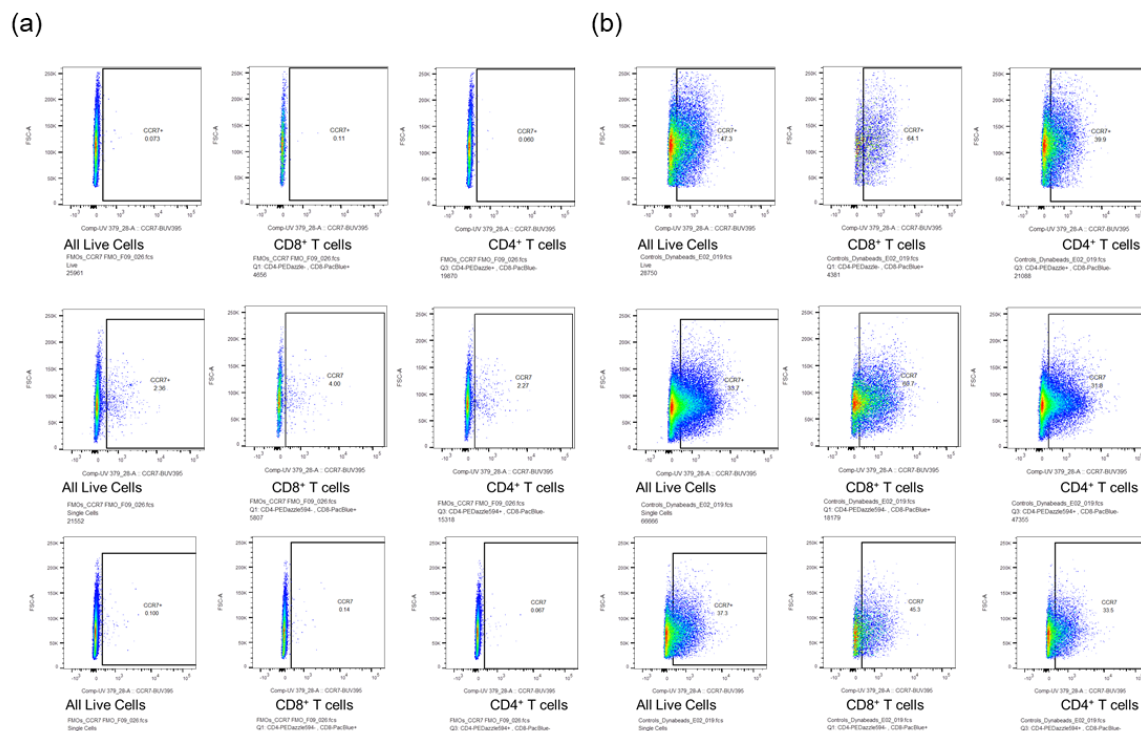
Figure S9. (a) Fluorescence-minus-one (FMO) gating and (b) DynaBeads control gating of CCR7$^+$ cells in live cell, CD4$^+$, and CD8$^+$ T cell populations of three independent donors.
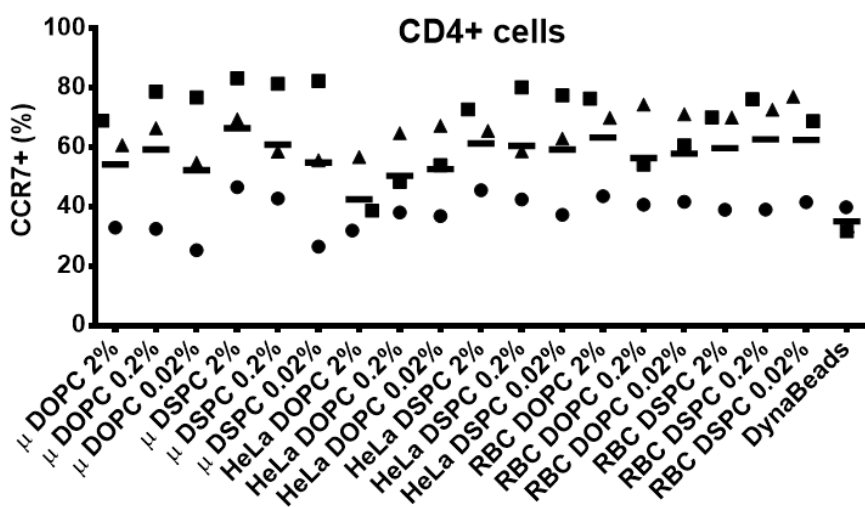
(a)
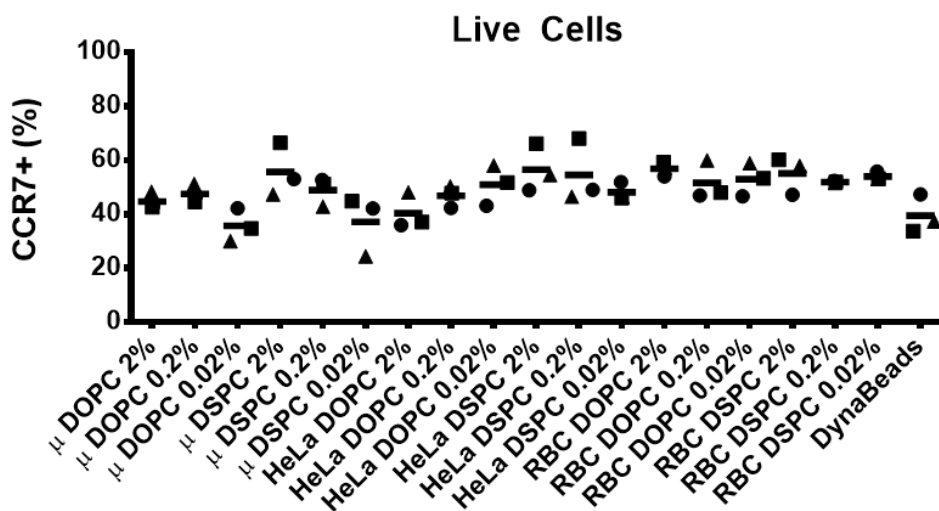
(b)

(c)

Figure S10. Percent of population that is CCR7^{+} of (a) live cells, (b) CD8^{+} T cells, and (c) CD4^{+} T cells. Data represented as mean and individual data points of three independent donors with each symbol representing a different donor.