Supporting Information

Semiflexible Immunobrushes Induce Enhanced T Cell Activation And Expansion

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Content

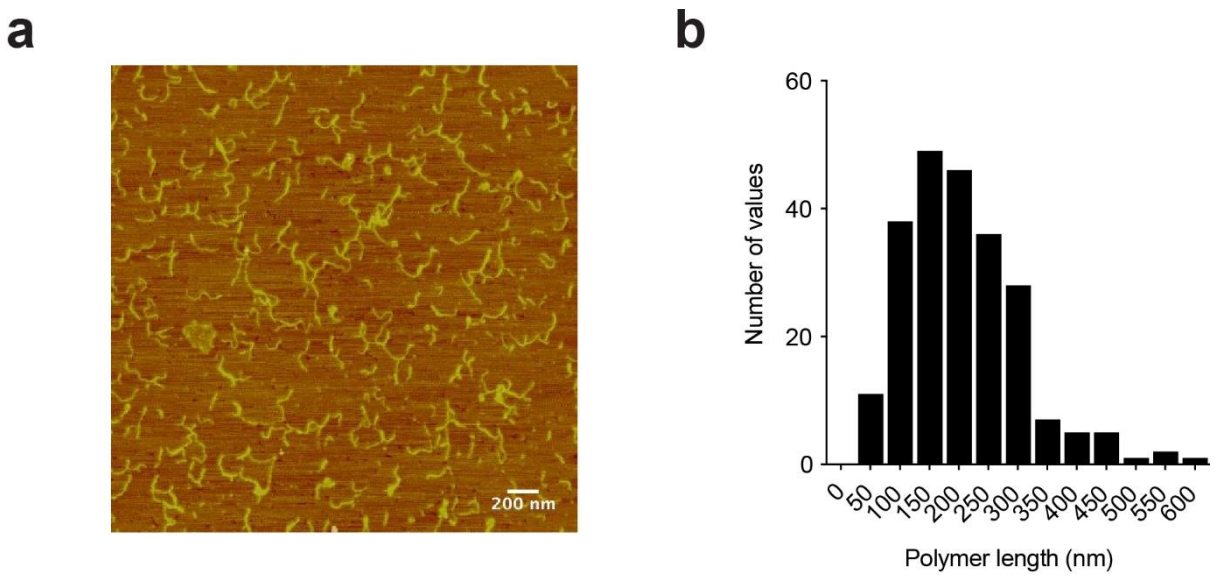

Figure S1. AFM analysis of PIC length. (a) Representative AFM image showing PIC polymers on a mica surface, scale bar is 200 nm. (b) Histogram of PIC length distribution after analysis of 229 polymers. Mean polymer length is 205 nm.

Table S1: Overview of all formulations used in this study

Figure	Bead type	PIC (ng/10^6 beads)	Total antibody (ng/10^6 beads)	αCD28/αCD3 ratio	Average # antibody per PIC	Average spacing PIC (nm)	Average spacing antibodies (nm)
1b,c,d	Ab beads		14.5	3.60			33.1
1b,d,c	PIC beads	3.65(1)	12.2	4.20	16.8(2)	148	36.1
1e,f	Flat PIC	56			12.4	37.8	10.7
1e,f	Crosslinked PIC	591			16.8(3)	11.6(3)	2.84
1e,f	PIC beads	15			16.8(1)	73.0	17.8
S2	PIC beads	15.5	51.6	4.76	16.8(1)	71.8	17.5
2a,b	Ab beads		35.6	0.54			21.1
2a,b	Ab beads		38.8	0.98			20.2
2a,b	Ab beads		44.6	2.0			18.8
2a,b	Ab beads		37.4	5.0			20.6
2a,b	PIC beads	3.74	9.78	0.39	13.2	146	40.2
2a,b	PIC beads	4.41	11.7	0.46	13.3	135	36.9
2a,b	PIC beads	4.91	12.9	0.75	13.2	128	35.0
2a,b	PIC beads	4.77	12.7	1.4	13.4	129	35.3
2a,b	PIC beads	4.29	11.5	4.0	13.5	136	37.1
2c-h	Ab beads		67.5	4.39			15.3
2c-h	Ab beads		60.4	3.48			16.2
2c-h	Ab beads		43.4	3.17			19.1
2c-h	Ab beads		22.5	3.43			26.5
2c-h	Ab beads		14.5	3.60			33.1
2c-h	Ab beads		5.56	3.44			53.4
2c-h	Ab beads		2.38	2.82			81.6
2c-h	Ab beads		1.06	4.23			122.5
2c-h	PIC beads	15.9(1)	53.0	4.81	16.8(2)	70.9	17.3
2c-h	PIC beads	15.5(1)	51.6	4.76	16.8(2)	71.8	17.5
2c-h	PIC beads	11.4(1)	38.0	4.66	16.8(2)	83.7	20.4
2c-h	PIC beads	3.65(1)	12.2	4.20	16.8(2)	148	36.1
2c-h	PIC beads	2.26(1)	7.53	3.69	16.8(2)	188	45.9
2c-h	PIC beads	1.06(1)	3.52	3.64	16.8(2)	275	67.1
2c-h	PIC beads	0.36(1)	1.20	4.31	16.8(2)	471	114.9
2c-h	PIC beads	0.21(1)	0.70	3.82	16.8(2)	617	150.3
3/4	PIC beads	2.5(1)	13.1	10	32.1	179.7	34.8

(1): These values are based on the antibody per polymer values found after purification of the polymer.
(2): This value is not determined by stripping assay but based on analysis after polymer purification and assumed similar for all samples that use this polymer.
(3): This value is assumed similar as the value of the corresponding PIC beads used in the same figure because the same polymer was used.

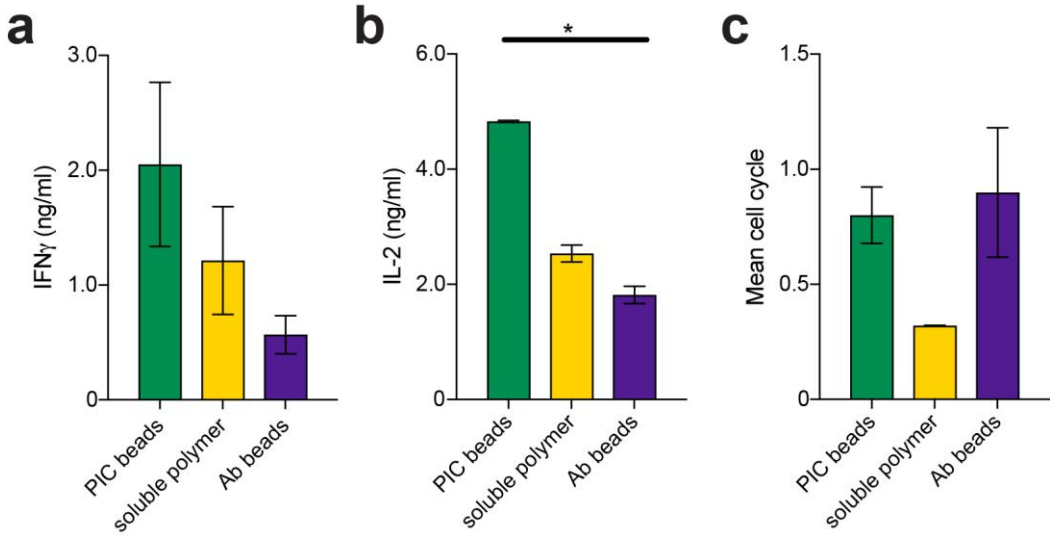

Figure S2. Comparison with soluble PIC. (a) IFNγ and (b) IL-2 concentration in the supernatant of T cells stimulated with PIC beads, soluble PIC or Ab beads with conjugated αCD28/ αCD3 after 24h of culture. (c) Mean cell cycle of proliferated human T cells after 3 days of stimulation with αCD28/ αCD3 modified PIC beads, soluble PIC or Ab beads. n=2 in 1 independent experiment for all conditions. (a-c) Data was analyzed using a RM one-way ANOVA with Geisser-Greenhouse correction, followed by Dunnett's multiple comparison test on log2 transformed data.

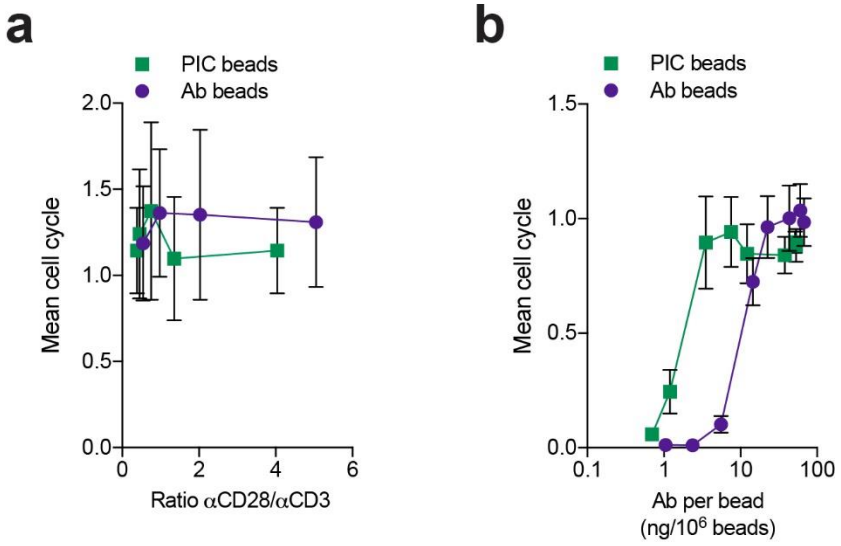

Figure S3. Influence of αCD28/ αCD3 ratio and density on proliferation. (a) Mean cell cycle of proliferated T cells stimulated with Ab or PIC beads with different αCD28/ αCD3 ratios after 3 days of culture n=3 in 2 independent experiments. (b) Mean cell cycle of proliferated T cells stimulated with Ab or PIC beads with different antibody densities after 3 days of culture n=4 in 2 independent experiments.

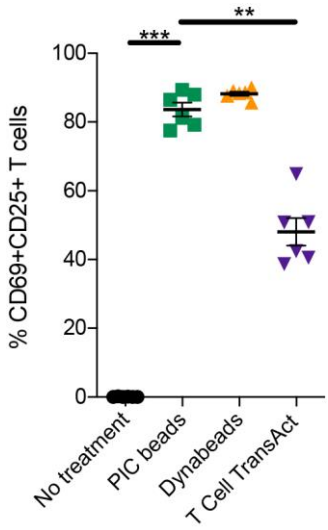

Figure S4. CD69 and CD25 expression of stimulated T cells. Percentage of $CD69^{+}CD25^{+}$ T cells after 1 day of stimulation with PIC beads, Dynabeads, T Cell TransAct or no treatment n=6 in 3 independent experiments. Significance was analyzed using a RM one-way ANOVA with Geisser-Greenhouse correction followed by a Dunnett's multiple comparison test on log2 transformed data.

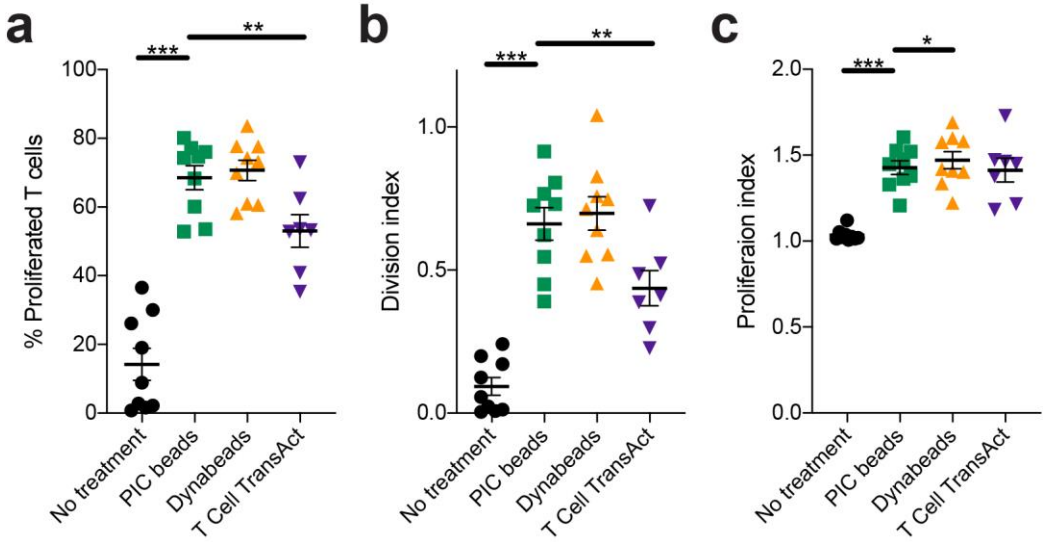

Figure S5. Proliferation of expanded T cells. (a) Percentage of viable T cells after expansion with PIC beads, Dynabeads, T Cell TransAct. (b) Calculated division index, defined as the average number of division cycles of all (both proliferating and non-proliferating) T cells. (c) Calculated proliferation index, defined as the average number of division cycles of only the proliferating T cells. (a-c) n=7 for T cell Transact in 3 independent experiments, n=9 for all other conditions in 4 independent experiments. Data was analyzed using a mixed-effects analysis with the Geisser-Greenhouse correction, followed by Dunnett's multiple comparisons test on log2 transformed data.

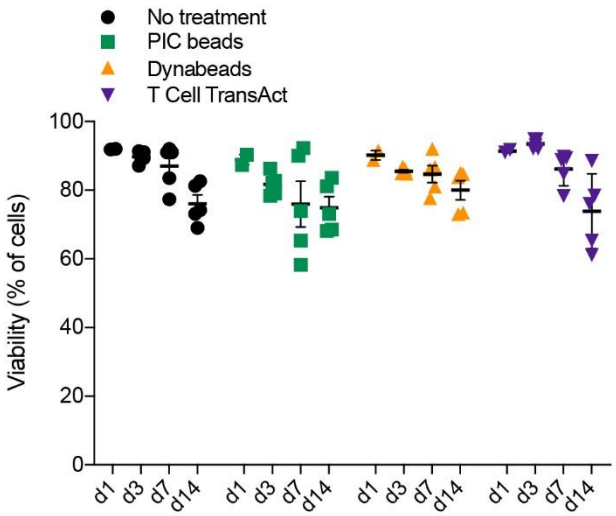

Figure S6. Viability of expanded T cells. Percentage of viable T cells after expansion with PIC beads, Dynabeads, T Cell TransAct. d1 n=2 in 1 independent experiment, all other days n=5 in 2 independent experiments.

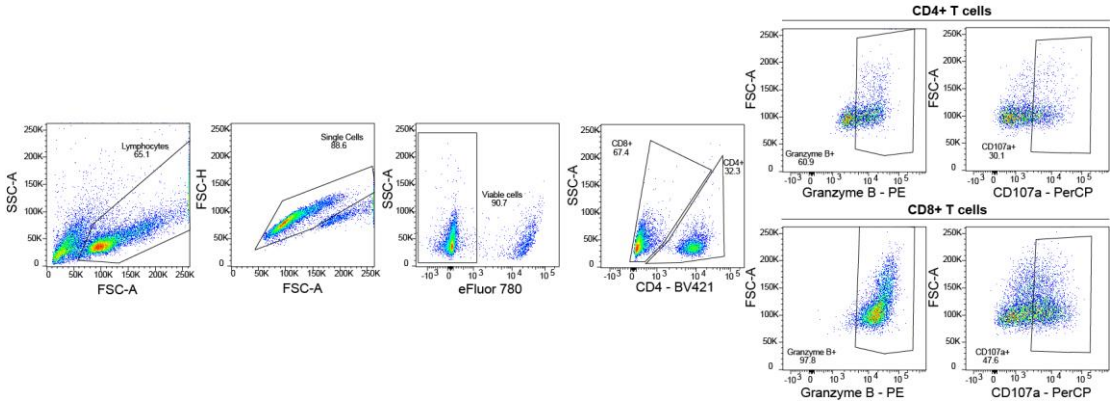

Figure S7. Gating strategy to determine T cell differentiation on day 14, based on FMO controls.

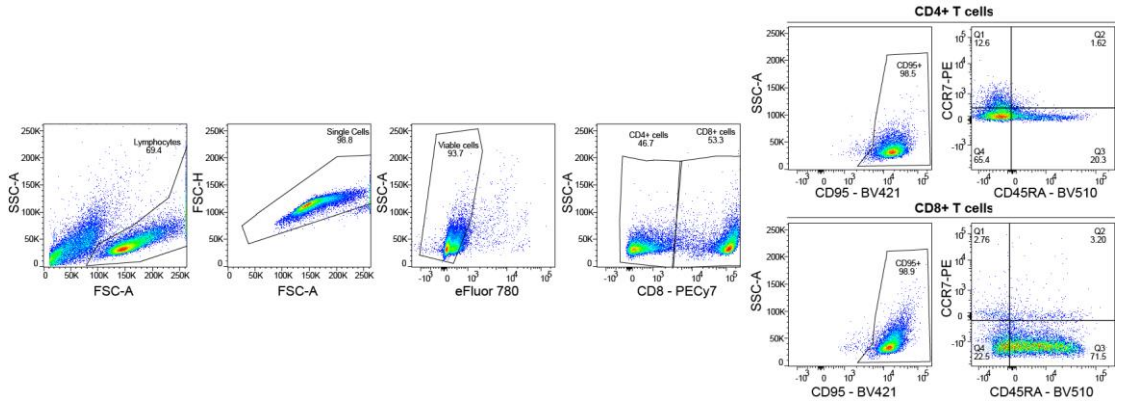

Figure S8. Gating strategy to determine the T cell cytotoxicity on day 14, based on FMO controls.

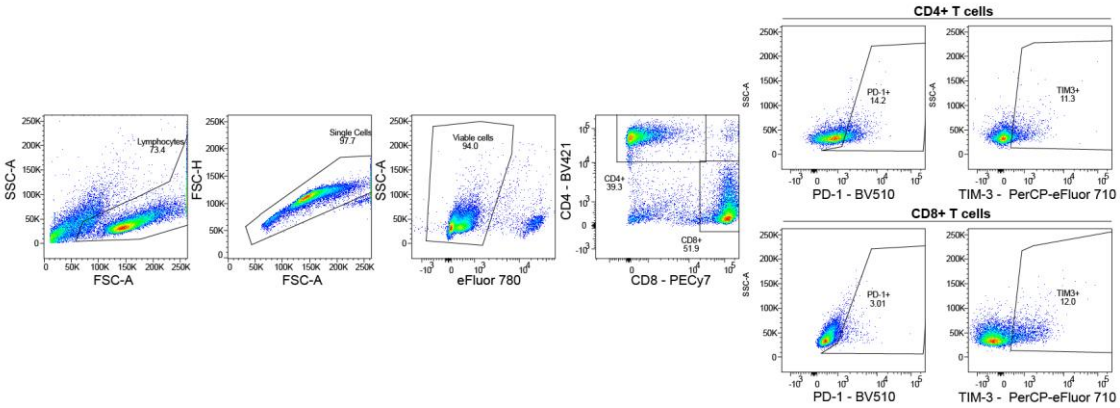

Figure S9. Gating strategy for to determine T cell exhaustion on day 14, based on FMO controls.

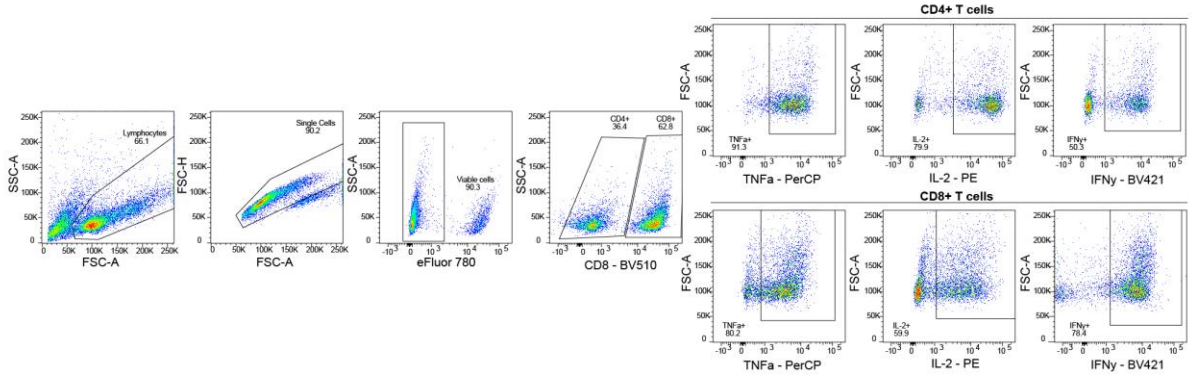

Figure S10. Gating strategy to determine the intracellular cytokine production of T cells on day 14, based on FMO controls.

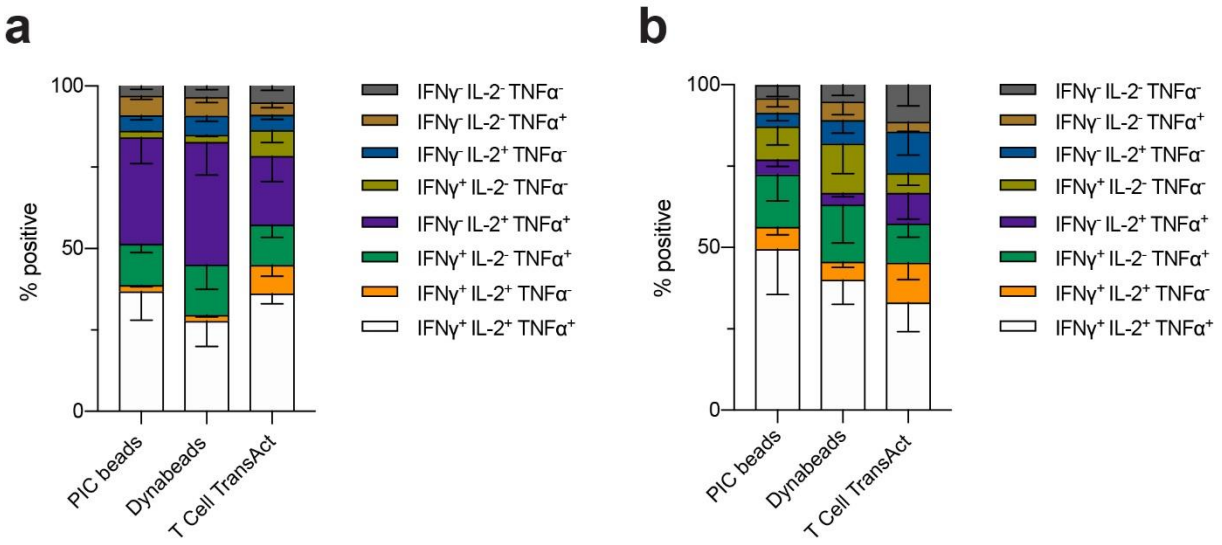

Figure S11. Detailed overview of intracellular cytokine production. (a) Intracellular cytokine staining for $CD4^+$ T cells. (b) Intracellular cytokine staining for $CD8^+$ T cells. n=5 in 2 independent experiments.