The effect of artificial antigen-presenting cells with preclustered anti-CD28/-CD3/-LFA-1 monoclonal antibodies on the induction of *ex vivo* expansion of functional human antitumor T cells

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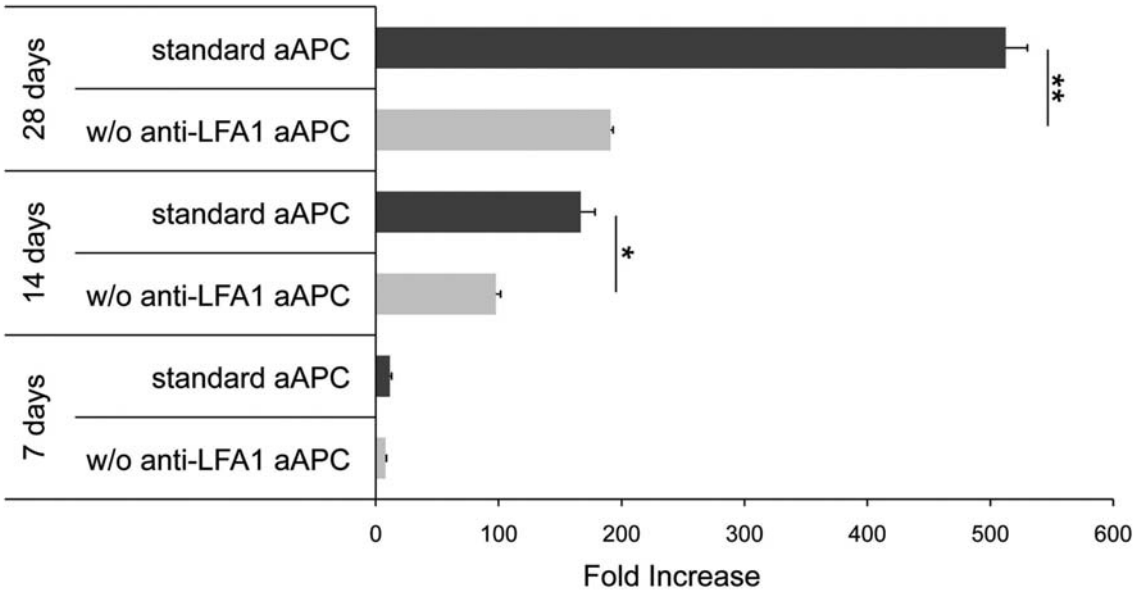
Supplementary Table S1. Maturation profile of artificial APC-expanded polyclonal CD3⁺ T cells as assessed by flow cytometric analyses of CD27, CCR7, CD62L and CD45RA expression. The average values of three independent experiments are reported.

POLYCLONAL CD3+ T-CELL EXPANSION WITH artificial APC and IL-2+IL-15

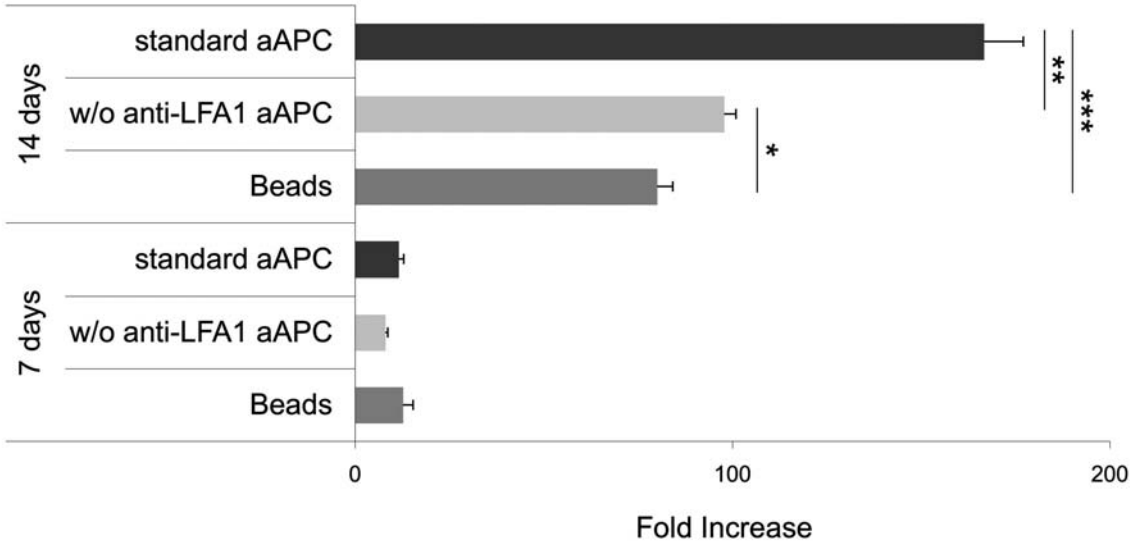
CELL SUBSET	IMMUNOPHENOTYPE	% + cells	MFI
CD4	CD27+	65.82	36.19
	CCR7+	42.69	40.46
	CCR7+CD45RA-CD62L+ (CM)	3.44	104.36 (CD62L)
	CCR7+CD45RA+CD62L+ (naive)	21.85	113.68 (CD62L)
	CCR7-CD45RA-CD62L- (EM)	7.48	5.70 (CD62L)
	CCR7-CD45RA+CD62L- (effector)	12.45	6.74 (CD62L)
CD8	CD27+	87.47	46.58
	CCR7+	28.63	41.54
	CCR7+CD45RA-CD62L+ (CM)	0.15	93.96 (CD62L)
	CCR7+CD45RA+CD62L+ (naive)	9.08	86.02 (CD62L)
	CCR7-CD45RA-CD62L- (EM)	2.58	3.13 (CD62L)
	CCR7-CD45RA+CD62L- (effector)	19.36	5.45 (CD62L)

Supplementary Table S2. Immunophenotype of polyclonal CD3⁺ T cells before expansion and 14 and 27 days after the initial stimulation with complete artificial APC (aAPC) or anti-CD3/-CD28 aAPC. Cultures were supplemented with high-dose IL-2 and IL-15. Reported values are the average of two independent experiments.

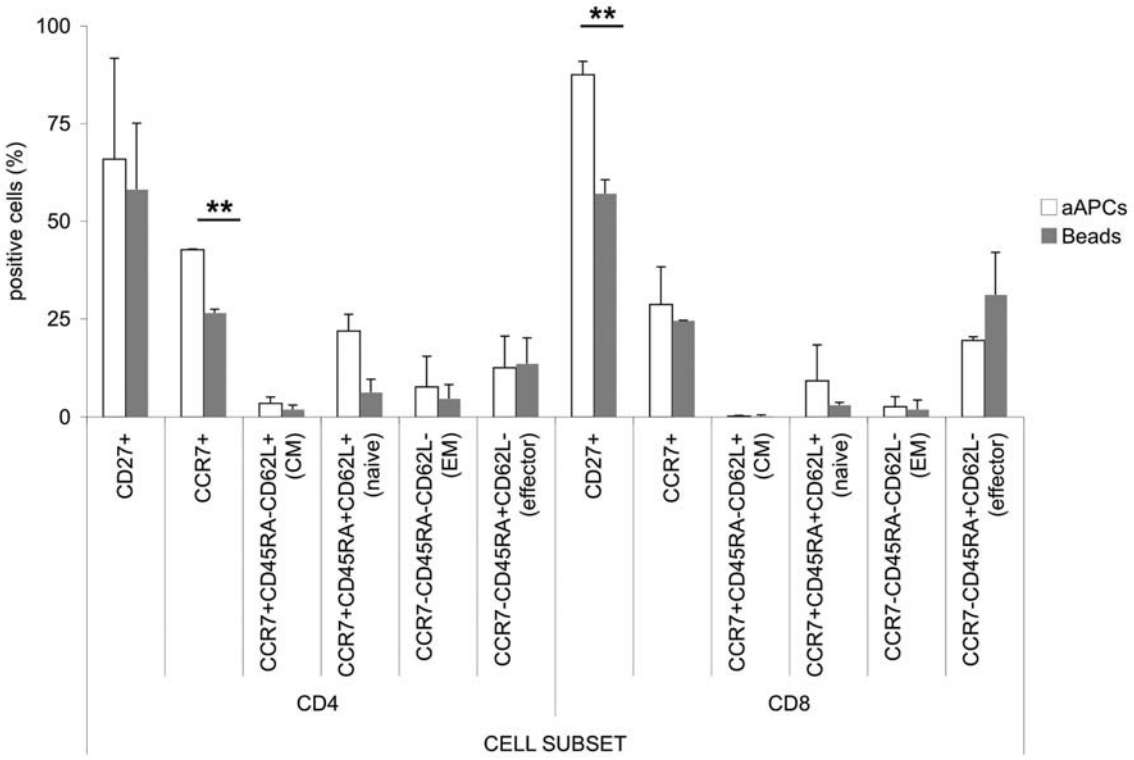
	FACS analyses	Freshly isolated CD3+T cells	aAPC stimulated CD3⁺ T cells			
			+14 days		+27 days	
			+ anti-LFA-1 mAb	- anti-LFA-1 mAb	+ anti-LFA-1 mAb	- anti-LFA-1 mAb
CD4	CD4	43,57	26,42	25,89	9,18	6,99
	CD62L+	70,27	88,53	88,43	78,54	77,09
	CCR7+	60,09	31,37	15,89	28,71	18,01
	CCR7+CD45RA+CD62L+	72,38	24,90	13,30	23,17	16,03
	CCR7-CD45RA+CD62L-	10,51	5,08	6,30	13,40	15,27
	CD69+	0,45	10,47	7,82	6,72	4,67
	CD27+	89,49	84,14	80,69	86,75	81,15
	CD25+	7,96	46,65	41,75	16,00	15,69
	CD25-FOXP3+	0,62	0,41	0,35	1,58	2,17
	CD25+FOXP3+	2,40	1,10	0,65	0,78	0,69
CD8	CD8	20,81	58,85	58,06	75,98	80,78
	CD62L+	51,99	77,82	74,22	53,87	47,44
	CCR7+	41,27	17,69	8,56	28,24	11,22
	CCR7+CD45RA+CD62L+	75,60	15,60	6,90	20,45	8,98
	CCR7-CD45RA+CD62L-	50,13	18,65	22,57	37,35	48,34
	CD69+	1,14	7,99	24,68	29,66	31,23
	CD27+	77,14	85,04	71,11	79,31	70,41
	CD25+	0,66	23,15	13,67	2,52	1,96
	CD25-FOXP3+	0,13	1,97	1,42	1,49	3,40
	CD25+FOXP3+	0,21	1,04	0,52	0,26	0,48



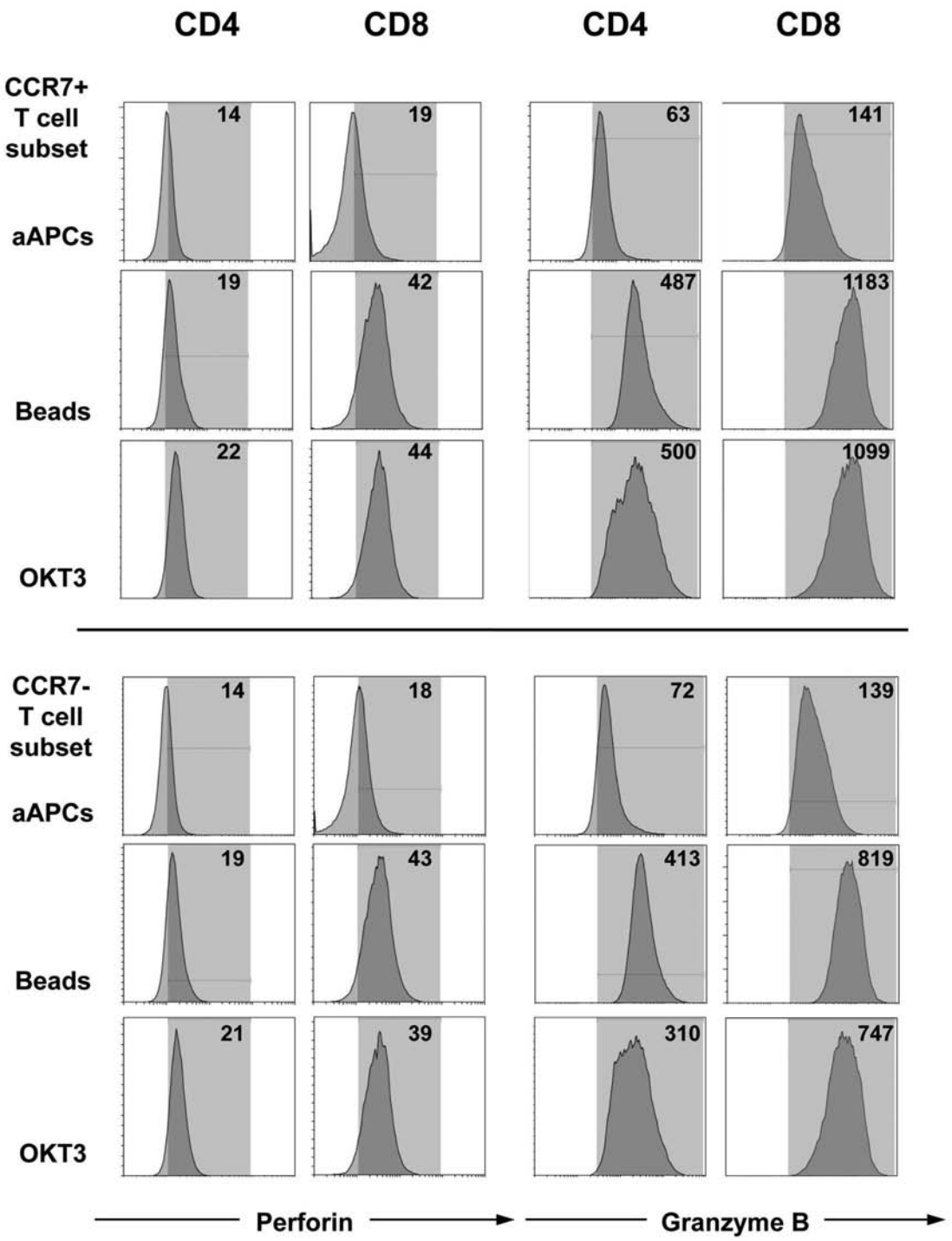
Supplementary Figure S1. Long-term expansion experiments. Comparison of $CD3^+$ T-cell expansion efficiencies obtained by a single stimulation with complete aAPC or anti-CD28/-CD3 aAPC (without [w/o] anti-LFA1 aAPC) in the presence of high-dose IL-2 and IL-15 for 28 days of culture. Fold increase values were calculated on the basis of the percentage of annexin V^-/propidium$^-$ viable cells detected by flow cytometry (*: $p \leq 0.05$; **: ≤ 0.02).



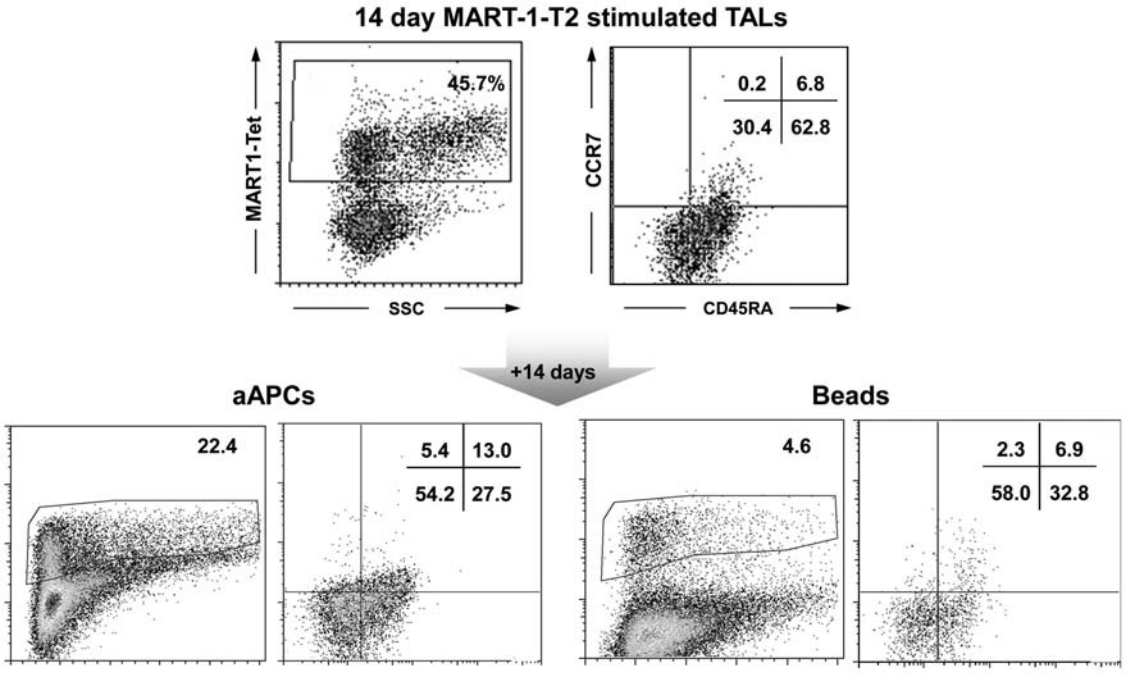
Supplementary Figure S2. Comparison of $CD3^+$ T-cell expansion efficiencies performed by a single stimulation with complete aAPC or anti-CD28/-CD3 aAPC (without [w/o] anti-LFA1 aAPC) or anti-CD28/-CD3 microbeads in the presence of high-dose IL-2 and IL-15 for 14 days of culture. Fold increase values were calculated on the basis of the percentage of annexin V^-/propidium iodide$^-$ viable cells detected by flow cytometry (*: $p \leq 0.05$; **:$p \leq 0.02$).



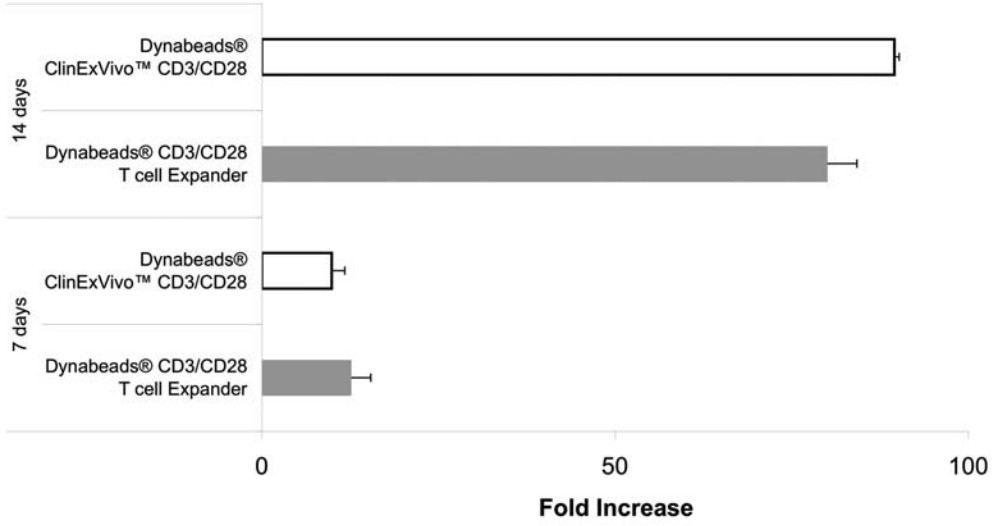
Supplementary Figure S3. Comparison of the maturation profiles of T cells expanded in the presence of high-dose IL-2 and IL-15 14 days after the initial stimulation with aAPC or microbeads. Central memory (CM) and naive T cells were defined, respectively, as the $CD62L^+CD45RA^-$ and $CD62L^+CD45RA^+$ cell subsets in the $CCR7^+$ gated population. Effector memory (EM) and effector T cells were defined, respectively, as the $CD62L^-CD45RA^-$ and $CD62L^-CD45RA^+$ cell subsets in the $CCR7^-$ gated population. The average values of three independent experiments and their standards deviations are reported. (**: $p \leq 0.02$).



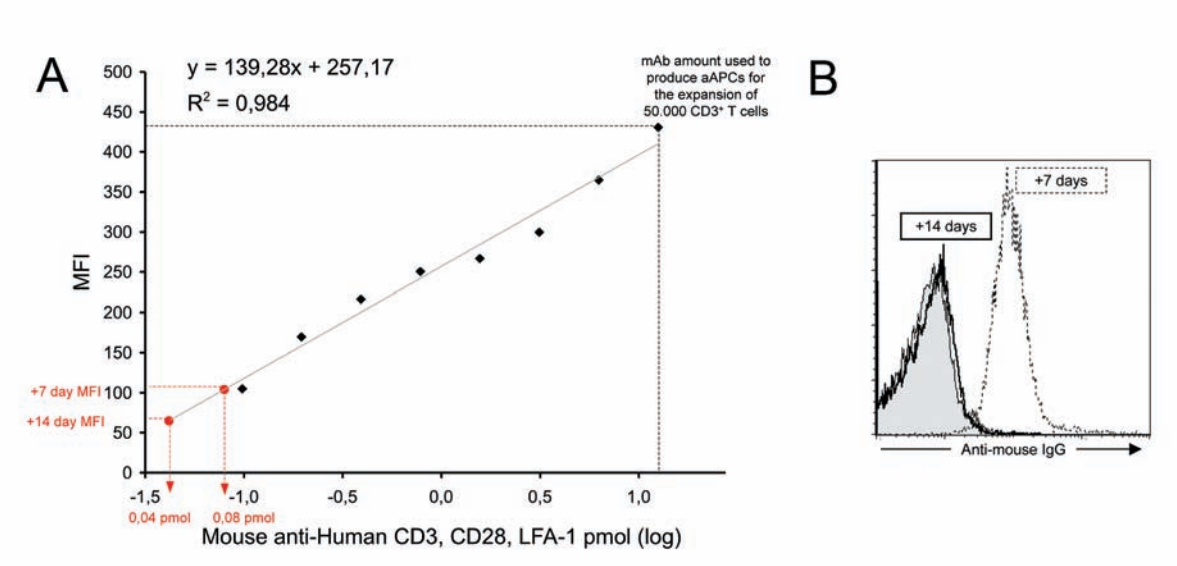
Supplementary Figure S4. Perforin and granzyme B expression in CCR7$^+$ and CCR7$^-$ T-cell subsets 14 days after aAPC or microbead or OKT3 stimulation and high-dose IL-2 plus IL-15. Histogram representation of perforin (left panel) and granzyme B (right panel) in CCR7$^+$ T cells (upper panel) or CCR7$^-$ T cells (lower panel) gated as CD3$^+$CD8$^+$ and CD3$^+$CD4$^+$. The mean fluorescence intensity values are reported.



Supplementary Figure S5. Immunophenotype of aAPC-expanded MART-1-specific T cells. T cells isolated from melanoma infiltrating lymph nodes were cultured for 2 weeks in the presence of MART-1-loaded T2 cells. The frequency of MART-1-specific T cells was then detected using MART-1 tetramers (MART-1-tet) and revealed by flow cytometry in the $CD3^+CD4^-$ gated T-cell subset (upper left panel). The maturation status of these cells was evaluated by flow cytometry detection of CCR7 and CD45RA in the $CD3^+CD4^-$MART-1-tet^+ gated T-cell subset (upper right panel). After another 2 weeks from aAPC (lower left panel) or microbead (lower right panel) stimulation in the presence of high-dose IL-2 and IL-15, the same parameters were evaluated again.



Supplementary Figure S6. Comparison of T-cell expansion efficiencies following a single stimulation with the research grade anti-CD28/-CD3 coated immunomagnetic microbeads used in the experiments previously described (*Dynabeads® CD3/CD28 T Cell Expander*) or with the clinical grade ones (*Dynabeads® ClinExVivo CD3/CD28*), in the presence of high-dose IL-2 and IL-15 for 14 days of culture. Fold increase values were calculated on the basis of the percentage of annexin V^-/propidium $iodide^-$ viable cells detected by flow cytometry.



Supplementary Figure S7. Persistence of artificial APC in T-cell cultures. (A) Regression line obtained by plotting mean fluorescence intensity (MFI) values of T cells pre-incubated with decreasing concentrations of anti-CD3, anti-CD28, anti-LFA-1 mAb and stained with a FITC-labeled antibody directed to the total mouse IgG. A fixed number (5×10^4) of freshly isolated T cells were incubated with serial dilutions of anti-CD3, anti-CD28, and anti-LFA-1 mAb, starting with the concentration used to prepare as many aAPC as required for the stimulation of such an amount of cells. The MFI (y axis) of the cells stained with FITC-labeled anti-mouse IgG were plotted against the known concentrations of mAb (x axis), creating a titration curve. Regression analysis of the data yielded a linear relationship between 0.1 and 10 pmol (described by the indicated equation). Experimentally derived MFI in T-cell cultures with aAPC at 7 and 14 days (red dots) allowed the extrapolation of the values of 0.08 and 0.04 pmol, respectively. Regression analysis was performed using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA). (B) The persistence of aAPC in expanded T-cell cultures was evaluated by flow cytometry, analyzing the reactivity of a FITC-labeled anti-mouse IgG antibody in T-cell cultures after 7 (dotted line) or 14 (black line) days from the initial aAPC stimulation. Unstimulated T cells stained with FITC-labeled anti-mouse IgG (gray filled histogram) were used as a control.