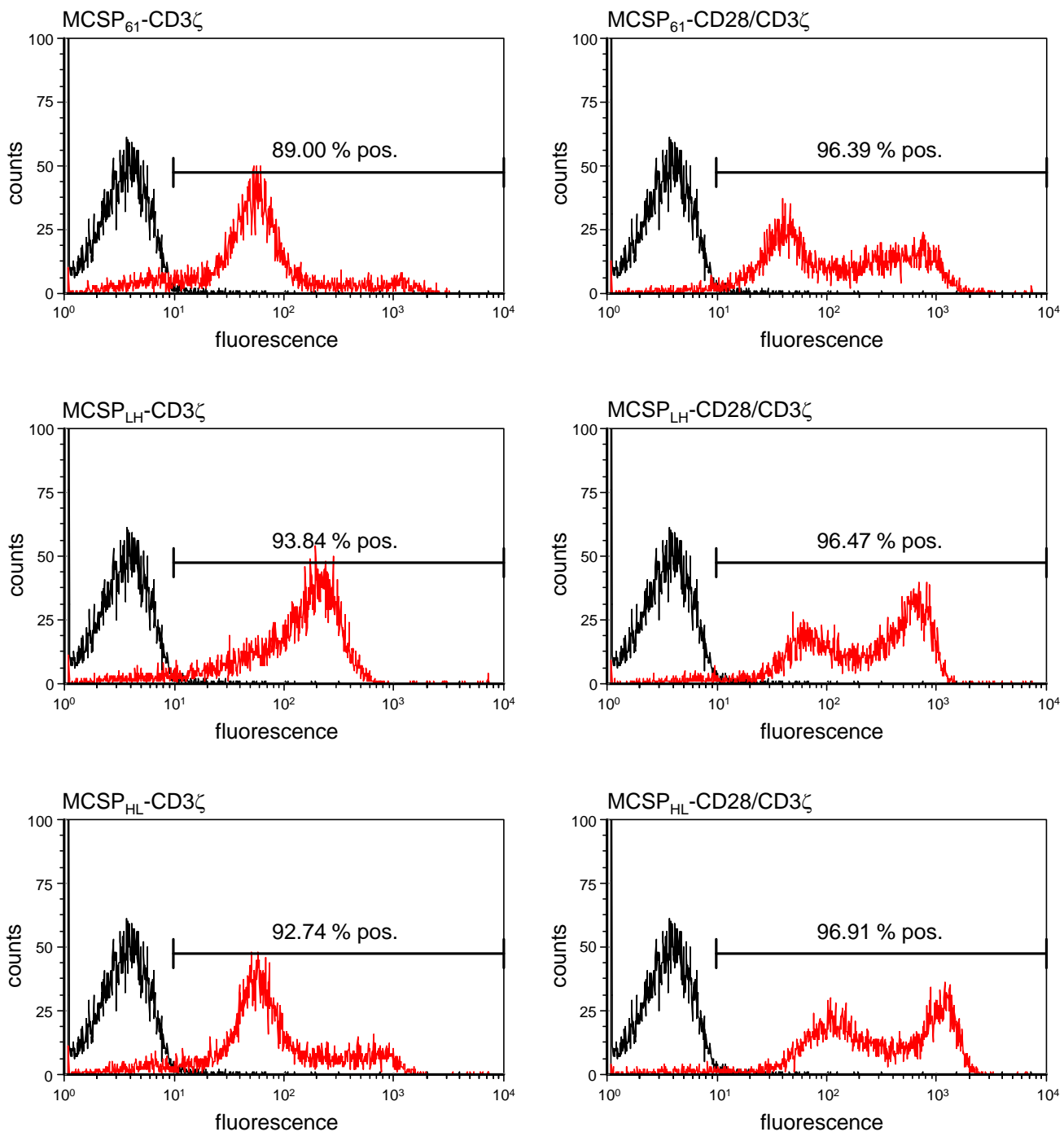


Supplementary Fig. 1 Expression level of MCSP-specific CARs on CD4⁺ T cells after RNA electroporation

CD4⁺ T cells were isolated from blood of healthy donors and activated for ten days using the agonistic anti-CD3 antibody OKT3 and IL-2. T cells were electroporated subsequently with mRNA encoding the different MCSP-specific 1st (left panels) or 2nd (right panels) generation CARs or without RNA. CAR-surface expression was determined 4 h after electroporation, by flow cytometry using a fluorescence-labeled antibody targeting the extracellular Fc-fragment. Histogram overlays showing the mock-electroporated T cells (black) and T cells transfected with RNA encoding the indicated CAR (red) are depicted. Markers were set to mock-electroporated T cells; percentages of positive cells (% pos.) are related to total CD4⁺ T cells. One representative out of three individual experiments is shown.

Supplementary Figure 2



Supplementary Fig. 2 Expression level of MCSP-specific CARs on CD8⁺ T cells after RNA electroporation

CD8⁺ T cells were isolated from blood of healthy donors and activated for ten days using the agonistic anti-CD3 antibody OKT3 and IL-2. T cells were electroporated subsequently with mRNA encoding the different MCSP-specific 1st (left panels) or 2nd (right panels) generation CARs or without RNA. CAR-surface expression was determined 4 h after electroporation, by flow cytometry using a fluorescence-labeled antibody targeting the extracellular Fc-fragment. Histogram overlays showing the mock-electroporated T cells (black) and T cells transfected with RNA encoding the indicated CAR (red) are depicted. Markers were set to mock-electroporated T cells; percentages of positive cells (% pos.) are related to total CD8⁺ T cells. One representative out of three individual experiments is shown.

Supplementary table S1:

p-values¹ corresponding to figure 1b, CD4⁺ T cells

conditions ²	time after electroporation									
	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	144 h	216 h
61-CD3ζ vs mock	**	**	**	**	*	0.075	ns	ns	0.085	ns
LH-CD3ζ vs mock	0.062	*	*	*	0.084	ns	ns	ns	0.075	ns
HL-CD3ζ vs mock	*	**	*	**	*	ns	ns	ns	*	ns
61-CD28-CD3ζ vs mock	**	**	**	**	*	0.054	ns	ns	ns	ns
LH-CD28-CD3ζ vs mock	*	*	*	**	**	**	**	*	*	*
HL-CD28-CD3ζ vs mock	**	***	**	***	**	**	***	**	*	0.089
61-CD3ζ vs 61-CD28-CD3ζ	*	ns	ns	0.05	0.093	*	ns	ns	ns	ns
LH-CD3ζ vs LH-CD28-CD3ζ	*	**	*	*	*	**	**	0.092	ns	ns
HL-CD3ζ vs HL-CD28-CD3ζ	**	**	**	**	**	**	***	**	*	ns
61-CD3ζ vs LH-CD3ζ	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
61-CD3ζ vs HL-CD3ζ	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
LH-CD3ζ vs HL-CD3ζ	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
61-CD28-CD3ζ vs LH-CD28-CD3ζ	ns	ns	ns	*	*	**	**	*	ns	ns
61-CD28-CD3ζ vs HL-CD28-CD3ζ	ns	0.057	*	*	*	**	**	**	ns	ns
LH-CD28-CD3ζ vs HL-CD28-CD3ζ	*	0.072	0.099	0.082	0.057	0.093	**	*	*	ns

¹ calculated by paired student's t test from 3 independent experiments, *** p<0.001, ** p<0.01, * p<0.05, ns p>0.1, p-values between 0.05 and 0.1 are specified. Calculations were performed based on the original, not on the normalized data.

² 61: MCSP₆₁, LH: MCSP_{LH}, HL: MCSP_{HL}

Supplementary table S2:

p-values¹ corresponding to figure 1b, CD8⁺ T cells

conditions ²	time after electroporation									
	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	144 h	216 h
61-CD3ζ vs mock	*	***	***	**	**	**	ns	ns	*	ns
LH-CD3ζ vs mock	ns	0.089	*	*	*	ns	0.064	ns	ns	ns
HL-CD3ζ vs mock	*	*	**	*	*	0.06	ns	ns	ns	ns
61-CD28-CD3ζ vs mock	0.058	**	**	**	**	*	ns	ns	*	ns
LH-CD28-CD3ζ vs mock	0.062	*	*	*	**	**	*	**	*	*
HL-CD28-CD3ζ vs mock	*	**	**	**	**	**	**	***	*	*
61-CD3ζ vs 61-CD28-CD3ζ	ns	ns	ns	ns	*	ns	ns	ns	0.082	ns
LH-CD3ζ vs LH-CD28-CD3ζ	**	*	*	*	**	**	*	*	ns	*
HL-CD3ζ vs HL-CD28-CD3ζ	*	**	*	**	**	**	***	***	*	0.062
61-CD3ζ vs LH-CD3ζ	ns	ns	ns	ns	ns	0.054	ns	ns	ns	ns
61-CD3ζ vs HL-CD3ζ	ns	ns	ns	ns	ns	0.075	ns	ns	ns	ns
LH-CD3ζ vs HL-CD3ζ	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
61-CD28-CD3ζ vs LH-CD28-CD3ζ	ns	ns	ns	ns	0.076	0.055	***	0.061	0.069	ns
61-CD28-CD3ζ vs HL-CD28-CD3ζ	ns	ns	0.091	*	*	0.051	**	*	*	ns
LH-CD28-CD3ζ vs HL-CD28-CD3ζ	*	ns	ns	ns	ns	*	*	*	0.055	0.065

¹ calculated by paired student's t test from 3 independent experiments, *** p<0.001, ** p<0.01, * p<0.05, ns p>0.1, p-values between 0.05 and 0.1 are specified. Calculations were performed based on the original, not on the normalized data.

² 61: MCSP₆₁, LH: MCSPLH, HL: MCSPLH

Supplementary table S3:

p-values¹ corresponding to figure 2

conditions ²	E:T 60:1			E:T 20:1		
	time after electroporation					
	2 h	12 h	24 h	2 h	12 h	24 h
61-CD3ζ vs mock	0.06	ns	*	ns	*	ns
LH-CD3ζ vs mock	*	*	*	*	**	ns
HL-CD3ζ vs mock	**	***	**	*	***	***
61-CD28-CD3ζ vs mock	ns	**	*	ns	0.07	*
LH-CD28-CD3ζ vs mock	**	**	**	**	***	**
HL-CD28-CD3ζ vs mock	**	**	**	**	***	***
61-CD3ζ vs 61-CD28-CD3ζ	ns	ns	ns	ns	ns	0.08
LH-CD3ζ vs LH-CD28-CD3ζ	ns	ns	**	ns	ns	**
HL-CD3ζ vs HL-CD28-CD3ζ	ns	ns	**	ns	ns	**
61-CD3ζ vs LH-CD3ζ	**	*	ns	*	**	ns
61-CD3ζ vs HL-CD3ζ	**	****	*	*	***	*
LH-CD3ζ vs HL-CD3ζ	ns	ns	*	ns	*	**
61-CD28-CD3ζ vs LH-CD28-CD3ζ	***	*	*	**	**	*
61-CD28-CD3ζ vs HL-CD28-CD3ζ	***	*	*	**	**	**
LH-CD28-CD3ζ vs HL-CD28-CD3ζ	ns	ns	ns	ns	ns	ns

¹ calculated by paired student's t test from 4 independent experiments, **** p<0.0001, *** p<0.001, ** p<0.01, * p≤0.05, ns p>0.1, p-values between 0.05 and 0.1 are specified.

² 61: MCSP₆₁, LH: MCSP_{LH}, HL: MCSP_{HL}

Supplementary table S4:

p-values¹ corresponding to figure 3,

conditions ²	IL-2			TNF			IFN _γ		
	time after electroporation								
	2 h	12 h	24 h	2 h	12 h	24 h	2 h	12 h	24 h
61-CD3ζ vs mock	ns	ns	ns	ns	ns	ns	0.06	ns	ns
LH-CD3ζ vs mock	ns	ns	ns	0.06	ns	ns	ns	ns	ns
HL-CD3ζ vs mock	0.06	ns	ns	0.08	ns	ns	*	ns	0.09
61-CD28-CD3ζ vs mock	**	ns	ns	ns	*	ns	**	**	ns
LH-CD28-CD3ζ vs mock	**	**	ns	*	**	0.06	**	*	*
HL-CD28-CD3ζ vs mock	*	0.07	**	ns	0.06	0.08	*	0.07	*
61-CD3ζ vs 61-CD28-CD3ζ	**	ns	ns	ns	*	ns	*	**	ns
LH-CD3ζ vs LH-CD28-CD3ζ	0.08	**	ns	*	**	0.06	**	*	*
HL-CD3ζ vs HL-CD28-CD3ζ	*	0.06	**	ns	0.07	0.08	ns	0.07	*
61-CD3ζ vs LH-CD3ζ	ns	ns	ns	ns	ns	ns	0.07	ns	ns
61-CD3ζ vs HL-CD3ζ	ns	ns	ns	0.09	ns	ns	*	ns	ns
LH-CD3ζ vs HL-CD3ζ	ns	ns	ns	0.08	ns	ns	*	ns	0.08
61-CD28-CD3ζ vs LH-CD28-CD3ζ	ns	**	ns	ns	**	0.09	0.06	*	*
61-CD28-CD3ζ vs HL-CD28-CD3ζ	0.06	0.07	**	ns	0.07	0.09	0.05	0.09	*
LH-CD28-CD3ζ vs HL-CD28-CD3ζ	*	ns	**	ns	ns	ns	0.1	ns	*

¹ calculated by paired student's t test, **** p<0.0001, *** p<0.001, ** p<0.01, * p≤0.05, ns p>0.1, p-values between 0.05 and 0.1 are specified.

² 61: MCSP₆₁, LH: MCSP_{LH}, HL: MCSP_{HL}

Supplementary table S5:

p-values¹ corresponding to figure 5

conditions ²	target							
	T2.A1				A375M			
	effector:target ratio							
	60:1	20:1	6:1	2:1	60:1	20:1	6:1	2:1
CEA vs mock	ns	ns	ns	*	**	ns	ns	ns
MCSP vs mock	ns	ns	ns	*	**	*	0.06	ns
MCSP vs CEA	ns	ns	0.061	ns	*	*	*	*

¹ calculated by paired student's t test from 4 independent experiments, ** p<0.01, * p≤0.05, ns p>0.1, p-values between 0.05 and 0.1 are specified.

² CEA: CEA CD28-CD3ζ CAR, MCSP: MCSP_{HL} CD28-CD3ζ CAR, mock: no RNA

Supplementary table S6:

p-values¹ corresponding to figure 6a

conditions ²	cytokine		
	IL-2	TNF	IFNγ
mock on T2.A1 vs mock on A375M	ns	ns	ns
mock on T2.A1 vs mock on KATO III	0.096	0.063	0.072
mock on A375M vs mock on KATO III	ns	**	ns
CEA on T2.A1 vs CEA on A375M	ns	ns	0.072
CEA on T2.A1 vs CEA on KATO III	0.085	*	*
CEA on A375M vs CEA on KATO III	0.084	*	*
MCSP on T2.A1 vs MCSP on A375M	0.05	ns	0.077
MCSP on T2.A1 vs MCSP on KATO III	ns	*	ns
MCSP on A375M vs MCSP on KATO III	*	*	*
mock on T2.A1 vs CEA on T2.A1	ns	ns	*
mock on T2.A1 vs MCSP on T2.A1	ns	*	*
CEA on T2.A1 vs MCSP on T2.A1	ns	ns	ns
mock on A375M vs CEA on A375M	*	*	*
mock on A375M vs MCSP on A375M	0.055	*	0.06
CEA on A375M vs MCSP on A375M	0.058	*	0.06
mock on KATO III vs CEA on KATO III	0.081	*	*
mock on KATO III vs MCSP on KATO III	ns	ns	ns
CEA on KATO III vs MCSP on KATO III	0.079	*	*

¹ calculated by paired student's t test from 3 independent experiments, * p≤0.05, ns p>0.1, p-values between 0.05 and 0.1 are specified.

² CEA: CEA CD28-CD3ζ CAR, MCSP: MCSP_{HL} CD28-CD3ζ CAR, mock: no RNA

Supplementary table S7:

p-values¹ corresponding to figure 6b

conditions ²	cytokine											
	IL-2				TNF				IFNγ			
	time after thawing [h]											
	6	24	48	72	6	24	48	72	6	24	48	72
CEA on T2.A1 vs CEA on A375M	ns	ns	ns	0.064	ns	*	ns	ns	0.051	0.067	ns	ns
MCSP on T2.A1 vs MCSP on A375M	**	ns	ns	ns	0.072	0.082	ns	ns	**	*	0.074	0.092
CEA on T2.A1 vs MCSP on T2.A1	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CEA on A375M vs MCSP on A375M	**	ns	ns	ns	ns	0.095	ns	ns	0.057	*	0.074	ns

¹ calculated by paired student's t test from 3 independent experiments, ** p<0.01, * p≤0.05, ns p>0.1, p-values between 0.05 and 0.1 are specified.

² CEA: CEA CD28-CD3ζ CAR, MCSP: MCSP_{HL} CD28-CD3ζ CAR, mock: no RNA