TABLE S1. Comparative FACS analysis of different expansion[a] protocols for γδ-TCR, CD3, and CD8 expression

|	PBMC + zoledronic acid n = 10		PBMC + OKT3 n = 10		MACS[b] CD8+ + OKT3 n = 7		MACS[c] γδ+ + OKT3 n = 7	
	start	end	start	end	start	end	start	end
γδ- / CD3+	59.2 ± 8.14	11.73 ± 9.29	59.2 ± 8.14	74.66 ± 5.85	90.08 ± 5.21	86.43 ± 4	5.32 ± 5.62	2.96 ± 1.89
γδ+ / CD3+	11.42 ± 6.99	84.61 ± 10.68	11.42 ± 6.99	18.92 ± 6.05	3.68 ± 3.41	7.91 ± 2.99	84.8 ± 10.08	91.65 ± 5.08
γδ- / CD3-	28.47 ± 8.8	2.67 ± 1.6	28.47 ± 8.8	5.93 ± 2.99	5.93 ± 4.41	5.38 ± 3.86	2.99 ± 2.44	2.31 ± 1.53
γδ+ / CD3-	0.91 ± 0.51	0.99 ± 0.78	0.91 ± 0.51	0.5 ± 0.27	0.31 ± 0.28	0.28 ± 0.19	6.89 ± 7.71	3.08 ± 1.93
γδ- / CD8+	26.05 ± 6.86	6.94 ± 6.39	26.05 ± 6.86	70.42 ± 7.98	96.15 ± 3.19	90.62 ± 3.43	1.5 ± 1.53	1.77 ± 1.72
γδ+ / CD8+	1.51 ± 0.78	15.2 ± 7.92	1.51 ± 0.78	13.19 ± 5.03	2.16 ± 1.17	7.6 ± 2.92	15.92 ± 6.96	15.87 ± 11.82
γδ- / CD8-	60.67 ± 6.36	7.81 ± 4.29	60.67 ± 6.36	10.14 ± 3.24	0.45 ± 0.22	1.32 ± 0.71	7.05 ± 6.93	3.84 ± 2.08
γδ+ / CD8-	11.77 ± 7.23	70.5 ± 17.4	11.77 ± 7.23	6.25 ± 4.87	1.24 ± 2.42	0.46 ± 0.97	75.52 ± 9.94	78.52 ± 13.52

[a]Expansion duration varied from ten to eleven days
[b]CD8+ T cells were extracted from donor-derived PBMC using magnetic-activated cell sorting
[c]γδ+ T cells were extracted from donor-derived PBMC using magnetic-activated cell sorting
[d]Mean ± standard deviation are displayed
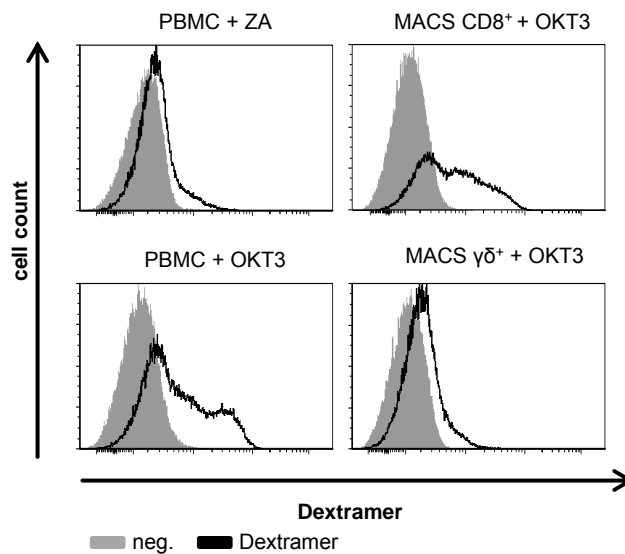
Figure S1: Expression of a gp100/HLA-A2-specific TCR by different T-cell populations after electroporation.

Zoledronic acid (ZA)-activated PBMC, OKT3-activated PBMC, OKT3-stimulated MACS-isolated CD8$^+$ T cells, and OKT3-stimulated MACS-isolated γ/δ T cells were expanded as described in figure 1. After 10-11 days, these cells were electroporated with RNA coding for the gp100/HLA-A2-specific TCR or with RNA encoding the MCSP-specific CAR. After receptor transfer, T cells were rested for one day and subsequently cryopreserved. After thawing TCR expression was detected using a PE-conjugated MHC-Dextramer HLA-A*0201/YLEPGPVTV (black lines). CAR-transfected T cells served as negative controls (neg.; filled grey histograms). Presented histograms are representatives out of three independent experiments.

TABLE S2: P-values corresponding to figure 2 g-j calculated by unpaired Student's t-test   **time points**

		conditions[#]	4h	24h	48h	72h	96h	120h
g	**TCR vs CAR**[a]	**PBMC + ZA** (TCR vs. CAR)	ns	***	*	ns	ns	ns
		MACS γ/δ + OKT3 (TCR vs. CAR)	***	***	***	***	**	*
h	**CAR vs TCR**[b]	**PBMC + ZA** (CAR vs. TCR)	**	***	**	*	*	0.0950
		MACS γ/δ + OKT3 (CAR vs. TCR)	***	***	***	***	***	***
i	**TCR vs CAR**	**PBMC + OKT3** (TCR vs. CAR)	0.0774	***	ns	ns	ns	ns
		MACS CD8 + OKT3 (TCR vs. CAR)	**	***	**	ns	ns	ns
j	**CAR vs TCR**	**PBMC + OKT3** (CAR vs. TCR)	*	***	**	***	***	***
		MACS CD8 + OKT3 (CAR vs. TCR)	***	**	***	***	***	***

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

[#]Conditions: PBMC + ZA = zoledronic-acid expanded PBMC; PBMC + OKT3 = OKT3-expanded PBMC; MACS CD8 + OKT3 = magnetic-activated cell sorting isolated CD8 T cells expanded with OKT3; γδ + OKT3 = magnetic-activated cell sorting isolated γδ T cells expanded with OKT3; all conditions were either transfected with the gp100 TCR or the MCSP CAR

[a]TCR vs CAR: TCR expression on TCR-transfected T cells against TCR expression on CAR-transfected T cells

[b]CAR vs TCR: CAR expression on CAR-transfected T cells against CAR expression on TCR-transfected T cells

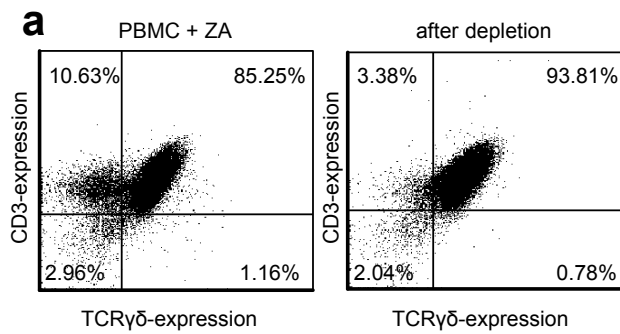**TABLE S3: P-values corresponding to figure 3 calculated by unpaired Student's t-test** **cytokines**

TARGET		CONDITION	IL-2	TNF	IFNγ
a					
Mel526	mock vs TCR	Mel526 **PBMC + ZA** (mock vs. TCR)	ns	ns	ns
		Mel526 **PBMC + OKT3** (mock vs. TCR)	ns	ns	ns
		Mel526 **MACS CD8 + OKT3** (mock vs. TCR)	ns	ns	ns
		Mel526 **MACS γ/δ + OKT3** (mock vs. TCR)	ns	ns	ns
	TCR vs TCR	Mel526 **PBMC + ZA vs. PBMC + OKT3** (TCR vs. TCR)	ns	ns	ns
		Mel526 **PBMC + ZA vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns
		Mel526 **PBMC + ZA vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns
		Mel526 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns
Mel526 + gp100	mock vs TCR	Mel526+gp100 **PBMC+ ZA** (mock vs. TCR)	0.0713	***	***
		Mel526+gp100 **PBMC+ OKT3** (mock vs. TCR)	***	***	***
		Mel526+gp100 **MACS CD8 + OKT3** (mock vs. TCR)	***	***	***
		Mel526+gp100 **MACS γ/δ + OKT3** (mock vs. TCR)	*	*	***
	TCR vs TCR	Mel526+gp100 **PBMC + ZA vs. PBMC + OKT3** (TCR vs. TCR)	***	**	***
		Mel526+gp100 **PBMC + ZA vs. MACS CD8 + OKT3** (TCR vs. TCR)	***	ns	**
		Mel526+gp100 **PBMC + ZA vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	***
		Mel526 +gp100 **PBMC + OKT3 vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	0.0949
		Mel526+gp100 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	***	**	***
		Mel526+gp100 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	***	*	***
A375M	mock vs TCR	A375M **PBMC+ ZA** (mock vs. TCR)	ns	ns	ns
		A375M **PBMC+ OKT3** (mock vs. TCR)	ns	ns	ns
		A375M **MACS CD8 + OKT3** (mock vs. TCR)	ns	ns	ns
		A375M **MACS γ/δ + OKT3** (mock vs. TCR)	ns	ns	ns
	TCR vs TCR	A375M **PBMC + ZA vs. PBMC + OKT3** (TCR vs. TCR)	ns	ns	ns
		A375M **PBMC + ZA vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns
		A375M **PBMC + ZA vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns
		A375M **PBMC + OKT3 vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns
		A375M **PBMC + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns
		A375M **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns
A375M + gp100	mock vs TCR	A375M+gp100 **PBMC+ ZA** (mock vs. TCR)	***	***	***
		A375M+gp100 **PBMC+ OKT3** (mock vs. TCR)	***	***	***
		A375M+gp100 **MACS CD8 + OKT3** (mock vs. TCR)	***	***	***
		A375M+gp100 **MACS γ/δ + OKT3** (mock vs. TCR)	**	***	***
	TCR vs TCR	A375M+gp100 **PBMC + ZA vs. PBMC + OKT3** (TCR vs. TCR)	***	***	***
		A375M+gp100 **PBMC + ZA vs. MACS CD8 + OKT3** (TCR vs. TCR)	***	*	**
		A375M+gp100 **PBMC + ZA vs. MACS γ/δ + OKT3** (TCR vs. TCR)	***	***	***
		A375M+gp100 **PBMC + OKT3 vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	**	ns
		A375M+gp100 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	***	***	***
		A375M+gp100 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	***	***	***
b					
Mel526	mock vs CAR	Mel526 **PBMC+ ZA** (mock vs. CAR)	*	**	***
		Mel526 **PBMC+ OKT3** (mock vs. CAR)	***	***	***
		Mel526 **MACS CD8 + OKT3** (mock vs. CAR)	***	***	***
		Mel526 **MACS γ/δ +O KT3** (mock vs. CAR)	ns	0.0678	**
	CAR vs CAR	Mel526 **PBMC + ZA vs. PBMC + OKT3** (CAR vs. CAR)	***	***	***
		Mel526 **PBMC + ZA vs. MACS CD8 + OKT3** (CAR vs. CAR	***	***	**
		Mel526 **PBMC + ZA vs. MACS γ/δ + OKT3** (CAR vs. CAR)	*	*	**
		Mel526 **PBMC + OKT3 vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	***	***	***
		Mel526 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	***	***	***
T2.A1	mock vs CAR	T2.A1 **PBMC + ZA** (mock vs. CAR)	ns	ns	ns
		T2.A1 **PBMC + OKT3** (mock vs. CAR)	ns	ns	ns
		T2.A1 **MACS CD8 + OKT3** (mock vs. CAR)	ns	ns	ns
		T2.A1 **MACS γ/δ + OKT3** (mock vs. CAR)	ns	ns	ns
	CAR vs CAR	T2.A1 **PBMC + ZA vs. PBMC + OKT3** (CAR vs. CAR)	ns	ns	ns
		T2.A1 **PBMC + ZA vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns
		T2.A1 **PBMC + ZA vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns
		T2.A1 **PBMC + OKT3 vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns
		T2.A1 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns
		T2.A1 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns
A375M	mock vs CAR	A375M **PBMC+ ZA** (mock vs. CAR)	*	***	***
		A375M **PBMC+ OKT3** (mock vs. CAR)	***	***	***
		A375M **MACS CD8 + OKT3** (mock vs. CAR)	***	***	***
		A375M **MACS γ/δ + OKT3** (mock vs. CAR)	*	***	***
	CAR vs CAR	A375M **PBMC + ZA vs. PBMC + OKT3** (CAR vs. CAR)	***	***	***
		A375M **PBMC + ZA vs. MACS CD8 + OKT3** (CAR vs. CAR)	***	***	**
		A375M **PBMC + ZA vs.MACS γ/δ + OKT3** (CAR vs. CAR)	**	***	**
		A375M **PBMC + OKT3 vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns
		A375M **PBMC + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	***	***	***
		A375M **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	***	***	***

Significance was calculated by unpaired student's t test from 7 to 10 independent experiments, * p≤0.05, ** p≤0.01, *** p≤0.001, ns p>0.1, p-values between 0.05 and 0.1 are specified.
Conditions: PBMC + ZA = zoledronic-acid expanded PBMC; PBMC + OKT3 = OKT3-expanded PBMC; MACS CD8 + OKT3 = magnetic-activated cell sorting isolated CD8 T cells expanded with OKT3; γδ + OKT3 = magnetic-activated cell sorting isolated γδ T cells expanded with OKT3; all conditions were either electroporated with no RNA (mock) or transfected with the gp100 TCR or the MCSP CAR

a

PBMC + ZA

CD3-expression

10.63%   85.25%

2.96%   1.16%

TCRγδ-expression

after depletion

CD3-expression

3.38%   93.81%

2.04%   0.78%

TCRγδ-expression

Figure S2: Zoledronic acid-expanded γ/δ T cells retain their cytokine secretory capacity after depletion of γδ⁻ cells.

a Donor-derived PBMC were expanded with ZA (PBMC + ZA) as explained above (Fig. 1). Following 10 days of expansion, untouched γ/δ T cells were isolated from an aliquot of stimulated cells via negative selection using the TCR γ/δ T Cell Isolation Kit (after depletion). Subsequently, a γδ and CD3 double staining was employed to flow-cytometrically verify the successful depletion procedure. **b+c** On day 11, negatively isolated γ/δ T cells (after depletion, grey bars) and the remaining ZA-expanded T cells (black bars) were electroporated with RNA coding for the gp100/A2-specific TCR **(b)** or with RNA encoding the MCSP-specific CAR **(c)**. T cells electroporated without RNA (mock) served as controls **(b+c)**. Antigen-specific cytokine secretion was determined as described above (Fig. 3). Data represent means ± SEM from 4 independent experiments. P values calculated by unpaired Student's t test are presented in additional file 6: Table ST4.

b

concentration [ng/ml]

IL-2 TCR

TNF TCR

IFNγ TCR

mock TCR — Mel526
mock TCR — Mel526 gp100
mock TCR — A375M
mock TCR — A375M gp100

c

IL-2 CAR

TNF CAR

IFNγ CAR

mock CAR — Mel526
mock CAR — T2.A1
mock CAR — A375M
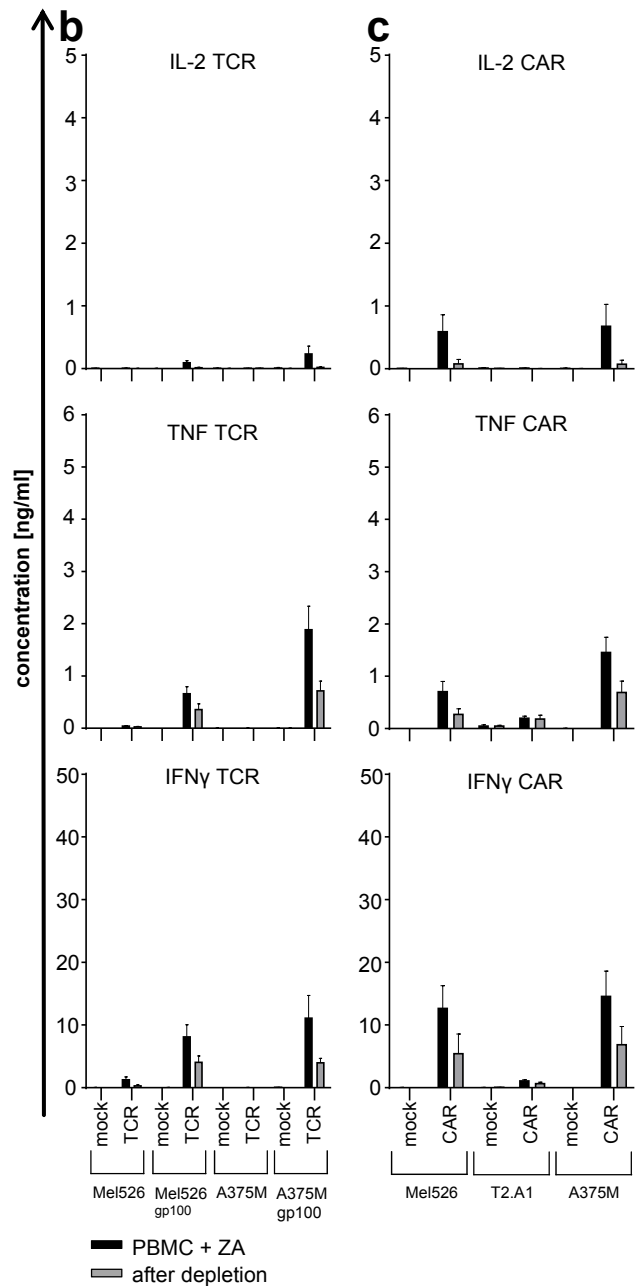
■ PBMC + ZA
▨ after depletion

TABLE S4: P-values corresponding to figure S2b and c calculated by unpaired Student's t-test **cytokines**

TARGET		CONDITION	IL-2	TNF	IFNγ
b					
Mel526	**mock vs TCR**	Mel526 **PBMC + ZA** (mock vs. TCR)	ns	ns	ns
		Mel526 **after depletion** (mock vs. TCR)	ns	ns	ns
	TCR vs TCR	Mel526 **PBMC + ZA vs. after depletion** (TCR vs. TCR)	ns	ns	ns
Mel526 + gp100	**mock vs TCR**	Mel526+gp100 **PBMC+ ZA** (mock vs. TCR)	0.0713	***	***
		Mel526+gp100 **after depletion** (mock vs. TCR)	ns	**	**
	TCR vs TCR	Mel526+gp100 **PBMC + ZA vs. PBMC + after depletion** (TCR vs. TCR)	ns	ns	*
A375M	**mock vs TCR**	A375M **PBMC+ ZA** (mock vs. TCR)	ns	ns	ns
		A375M **after depletion** (mock vs. TCR)	ns	ns	ns
	TCR vs TCR	A375M **PBMC + ZA vs. after depletion** (TCR vs. TCR)	ns	ns	ns
A375M + gp100	**mock vs TCR**	A375M+gp100 **PBMC+ ZA** (mock vs. TCR)	***	***	***
		A375M+gp100 **after depletion** (mock vs. TCR)	0.0682	***	***
	TCR vs TCR	A375M+gp100 **PBMC + ZA vs. after depletion** (TCR vs. TCR)	***	***	***
c					
Mel526	**mock vs CAR**	Mel526 **PBMC+ ZA** (mock vs. CAR)	*	**	***
		Mel526 **after depletion** (mock vs. CAR)	ns	0.0794	*
	CAR vs CAR	Mel526 **PBMC + ZA vs. after depletion** (CAR vs. CAR)	0.0853	0.0786	0.0603
T2.A1	**mock vs CAR**	T2.A1 **PBMC + ZA** (mock vs. CAR)	ns	ns	ns
		T2.A1 **after depletion** (mock vs. CAR)	ns	ns	ns
	CAR vs CAR	T2.A1 **PBMC + ZA vs. after depletion** (CAR vs. CAR)	ns	ns	ns
A375M	**mock vs CAR**	A375M **PBMC+ ZA** (mock vs. CAR)	*	***	***
		A375M **after depletion** (mock vs. CAR)	ns	***	*
	CAR vs CAR	A375M **PBMC + ZA vs. after depletion** (CAR vs. CAR)	*	**	*

Significance was calculated by unpaired student's t test from 4 independent experiments, * p ≤0.05, ** p≤0.01, *** p≤0.001, ns p>0.1, p-values between 0.05 and 0.1 are specified.
Conditions: PBMC + ZA = zoledronic-acid expanded PBMC; after depletion = negatively selected γδ T cells from ZA-expanded PBMC; all conditions were either electroporated with no RNA (mock) or transfected with the gp100 TCR or the MCSP CAR

TABLE S5: P-values corresponding to figure 5 a and b calculated by unpaired Student's t-test — effector to target ratio

a

TARGET	Comparison	CONDITION	60 : 1	20 : 1	6 : 1	2 : 1
Mel526	mock vs TCR	Mel526 **PBMC + ZA** (mock vs. TCR)	**	ns	ns	ns
		Mel526 **PBMC + OKT3** (mock vs. TCR)	***	*	ns	ns
		Mel526 **MACS CD8 + OKT3** (mock vs. TCR)	***	**	ns	ns
		Mel526 **MACS γ/δ + OKT3** (mock vs. TCR)	0.0790	0.0621	ns	ns
	mock vs mock	Mel526 **PBMC + ZA vs. PBMC + OKT3** (mock vs. mock)	ns	ns	ns	ns
		Mel526 **PBMC + ZA vs. MACS CD8 + OKT3** (mock vs. mock)	***	*	*	ns
		Mel526 **PBMC + ZA vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS CD8 + OKT3** (mock vs. mock)	**	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns	ns
		Mel526 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	**	ns	ns	ns
	TCR vs TCR	Mel526 **PBMC + ZA vs. PBMC + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		Mel526 **PBMC + ZA vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		Mel526 **PBMC + ZA vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		Mel526 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
A375M	mock vs TCR	A375M **PBMC+ ZA** (mock vs. TCR)	ns	ns	ns	ns
		A375M **PBMC+ OKT3** (mock vs. TCR)	ns	ns	ns	ns
		A375M **MACS CD8 + OKT3** (mock vs. TCR)	ns	ns	ns	ns
		A375M **MACS γ/δ + OKT3** (mock vs. TCR)	ns	ns	ns	ns
	mock vs mock	A375M **PBMC + ZA vs. PBMC + OKT3** (mock vs. mock)	*	ns	*	ns
		A375M **PBMC + ZA vs. MACS CD8 + OKT3** (mock vs. mock)	*	0.0693	0.0560	ns
		A375M **PBMC + ZA vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns	ns
		A375M **PBMC + OKT3 vs. MACS CD8 + OKT3** (mock vs. mock)	ns	ns	ns	ns
		A375M **PBMC + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns	ns
		A375M **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns	ns
	TCR vs TCR	A375M **PBMC + ZA vs. PBMC + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M **PBMC + ZA vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M **PBMC + ZA vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M **PBMC + OKT3 vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M **PBMC + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	*	ns	ns	ns
A375M + gp100	mock vs TCR	A375M+gp100 **PBMC+ ZA** (mock vs. TCR)	***	***	ns	ns
		A375M+gp100 **PBMC+ OKT3** (mock vs. TCR)	***	***	**	ns
		A375M+gp100 **MACS CD8 + OKT3** (mock vs. TCR)	***	***	*	ns
		A375M+gp100 **MACS γ/δ + OKT3** (mock vs. TCR)	***	***	0.0896	ns
	mock vs mock	A375M+gp100 **PBMC + ZA vs. PBMC + OKT3** (mock vs. mock)	***	*	*	*
		A375M+gp100 **PBMC + ZA vs. MACS CD8 + OKT3** (mock vs. mock)	***	**	*	0.0527
		A375M+gp100 **PBMC + ZA vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns	ns
		A375M+gp100 **PBMC + OKT3 vs. MACS CD8 + OKT3** (mock vs. mock)	**	ns	ns	ns
		A375M+gp100 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	***	*	0.0709	ns
		A375M+gp100 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	***	**	0.0748	ns
	TCR vs TCR	A375M+gp100 **PBMC + ZA vs. PBMC + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M+gp100 **PBMC + ZA vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M+gp100 **PBMC + ZA vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M+gp100 **PBMC + OKT3 vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns	ns
		A375M+gp100 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	0.0658	ns	ns	ns
		A375M+gp100 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns	ns

b

TARGET	Comparison	CONDITION	60 : 1	20 : 1	6 : 1	2 : 1
Mel526	mock vs CAR	Mel526 **PBMC+ ZA** (mock vs. CAR)	***	**	ns	ns
		Mel526 **PBMC+ OKT3** (mock vs. CAR)	***	***	***	0.0645
		Mel526 **MACS CD8 + OKT3** (mock vs. CAR)	***	***	*	ns
		Mel526 **MACS γ/δ +O KT3** (mock vs. CAR)	*	**	ns	ns
	CAR vs CAR	Mel526 **PBMC + ZA vs. PBMC + OKT3** (CAR vs. CAR)	ns	0.0937	ns	ns
		Mel526 **PBMC + ZA vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		Mel526 **PBMC + ZA vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	0.0652	ns	ns
		Mel526 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
T2.A1	mock vs CAR	T2.A1 **PBMC + ZA** (mock vs. CAR)	ns	ns	ns	ns
		T2.A1 **PBMC + OKT3** (mock vs. CAR)	ns	ns	0.0880	*
		T2.A1 **MACS CD8 + OKT3** (mock vs. CAR)	**	*	ns	ns
		T2.A1 **MACS γ/δ + OKT3** (mock vs. CAR)	ns	ns	ns	ns
	mock vs mock	T2.A1 **PBMC + ZA vs. PBMC + OKT3** (mock vs. mock)	*	*	ns	ns
		T2.A1 **PBMC + ZA vs. MACS CD8 + OKT3** (mock vs. mock)	***	**	*	ns
		T2.A1 **PBMC + ZA vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns	ns
		T2.A1 **PBMC + OKT3 vs. MACS CD8 + OKT3** (mock vs. mock)	0.0792	ns	ns	ns
		T2.A1 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	*	ns	ns	ns
		T2.A1 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	**	*	0.0644	0.0734
	CAR vs CAR	T2.A1 **PBMC + ZA vs. PBMC + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		T2.A1 **PBMC + ZA vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		T2.A1 **PBMC + ZA vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		T2.A1 **PBMC + OKT3 vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns	0.0919
		T2.A1 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		T2.A1 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
A375M	mock vs CAR	A375M **PBMC+ ZA** (mock vs. CAR)	***	***	ns	ns
		A375M **PBMC+ OKT3** (mock vs. CAR)	***	***	***	**
		A375M **MACS CD8 + OKT3** (mock vs. CAR)	***	***	*	ns
		A375M **MACS γ/δ + OKT3** (mock vs. CAR)	*	*	ns	ns
	CAR vs CAR	A375M **PBMC + ZA vs. PBMC + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		A375M **PBMC + ZA vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		A375M **PBMC + ZA vs.MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		A375M **PBMC + OKT3 vs. MACS CD8 + OKT3** (CAR vs. CAR)	ns	ns	0.0641	ns
		A375M **PBMC + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns	ns
		A375M **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns	ns

Significance was calculated by unpaired student's t test from 4 to 7 independent experiments, * p≤0.05, ** p≤0.01, *** p≤0.001, ns p>0.1, p-values between 0.05 and 0.1 are specified.
Conditions: PBMC + ZA = zoledronic-acid expanded PBMC; PBMC + OKT3 = OKT3-expanded PBMC; MACS CD8 + OKT3 = magnetic-activated cell sorting isolated CD8 T cells expanded with OKT3; γδ + OKT3 = magnetic-activated cell sorting isolated γδ T cells expanded with OKT3; all conditions were either electroporated with no RNA (mock) or transfected with the gp100 TCR or the MCSP CAR

TABLE S6: P-values corresponding to figure 5 c and d calculated by unpaired Student's t-test   **effector to target ratio**

	TARGET		CONDITION	60 : 1	20 : 1	6 : 1	2 : 1
c	Mel526	mock vs TCR	Mel526 **PBMC + ZA** (mock vs. TCR)	**	ns	ns	ns
			Mel526 **after depletion** (mock vs. TCR)	ns	ns	ns	ns
		mock vs mock	Mel526 **PBMC + ZA vs. after depletion** (mock vs. mock)	ns	ns	ns	ns
		TCR vs TCR	Mel526 **PBMC + ZA vs. after depletion** (TCR vs. TCR)	ns	ns	ns	ns
	A375M	mock vs TCR	A375M **PBMC+ ZA** (mock vs. TCR)	ns	ns	ns	ns
			A375M **PBMC+ after depletion** (mock vs. TCR)	ns	ns	ns	ns
		mock vs mock	A375M **PBMC + ZA vs. after depletion** (mock vs. mock)	ns	ns	ns	ns
		TCR vs TCR	A375M **PBMC + ZA vs. after depletion** (TCR vs. TCR)	ns	ns	ns	ns
	A375M + gp100	mock vs TCR	A375M+gp100 **PBMC+ ZA** (mock vs. TCR)	***	***	ns	ns
			A375M+gp100 **after depletion** (mock vs. TCR)	***	0.0587	ns	ns
		mock vs mock	A375M+gp100 **PBMC + ZA vs. after depletion** (mock vs. mock)	ns	ns	ns	ns
		TCR vs TCR	A375M+gp100 **PBMC + ZA vs. after depletion** (TCR vs. TCR)	ns	ns	ns	ns
d	Mel526	mock vs CAR	Mel526 **PBMC+ ZA** (mock vs. CAR)	***	**	ns	ns
			Mel526 **PBMC+ after depletion** (mock vs. CAR)	ns	0.0707	ns	0.0645
		CAR vs CAR	Mel526 **PBMC + ZA vs. after depletion** (CAR vs. CAR)	ns	ns	ns	ns
	T2.A1	mock vs CAR	T2.A1 **PBMC + ZA** (mock vs. CAR)	ns	ns	ns	ns
			T2.A1 **PBMC + after depletion** (mock vs. CAR)	ns	ns	ns	ns
		mock vs mock	T2.A1 **PBMC + ZA vs. after depletion** (mock vs. mock)	ns	ns	ns	ns
		CAR vs CAR	T2.A1 **PBMC + ZA vs. after depletion** (CAR vs. CAR)	ns	ns	ns	ns
	A375M	mock vs CAR	A375M **PBMC+ ZA** (mock vs. CAR)	***	***	ns	ns
			A375M **PBMC+ after depletion** (mock vs. CAR)	ns	ns	ns	ns
		CAR vs CAR	A375M **PBMC + ZA vs. after depletion** (CAR vs. CAR)	*	ns	ns	ns

Significance was calculated by unpaired student's t test from 3 independent experiments, * p≤0.05, ** p≤0.01, *** p≤0.001, ns p>0.1, p-values between 0.05 and 0.1 are specified.
Conditions: PBMC + ZA = zoledronic-acid expanded PBMC; after depletion = negatively selected γδ T cells from ZA-expanded PBMC; all conditions were either electroporated with no RNA (mock) or transfected with the gp100 TCR or the MCSP CAR

TABLE S7: P-values corresponding to figure 6 calculated by unpaired Student's t-test **effector to target ratio**

TARGET		CONDITION	20 : 1	6 : 1	2 : 1
Daudi	mock vs TCR	Mel526 **PBMC + ZA** (mock vs. TCR)	ns	ns	ns
		Mel526 **PBMC + OKT3** (mock vs. TCR)	ns	ns	ns
		Mel526 **MACS CD8 + OKT3** (mock vs. TCR)	ns	ns	ns
		Mel526 **MACS γ/δ + OKT3** (mock vs. TCR)	ns	ns	ns
	mock vs mock	Mel526 **PBMC + ZA vs. PBMC + OKT3** (mock vs. mock)	*	*	*
		Mel526 **PBMC + ZA vs. MACS CD8 + OKT3** (mock vs. mock)	**	**	0.0667
		Mel526 **PBMC + ZA vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS CD8 + OKT3** (mock vs. mock)	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	ns	ns	ns
		Mel526 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (mock vs. mock)	0.0860	0.0778	ns
	TCR vs TCR	Mel526 **PBMC + ZA vs. PBMC + OKT3** (TCR vs. TCR)	0.0602	0.0857	ns
		Mel526 **PBMC + ZA vs. MACS CD8 + OKT3** (TCR vs. TCR)	**	*	0.0697
		Mel526 **PBMC + ZA vs. MACS γ/δ + OKT3** (TCR vs. TCR)	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS CD8 + OKT3** (TCR vs. TCR)	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	0.0646	ns	ns
		Mel526 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (TCR vs. TCR)	*	ns	ns
	mock vs CAR	Mel526 **PBMC+ ZA** (mock vs. CAR)	ns	ns	ns
		Mel526 **PBMC+ OKT3** (mock vs. CAR)	ns	ns	ns
		Mel526 **MACS CD8 + OKT3** (mock vs. CAR)	ns	ns	ns
		Mel526 **MACS γ/δ +O KT3** (mock vs. CAR)	ns	ns	ns
	CAR vs CAR	Mel526 **PBMC + ZA vs. PBMC + OKT3** (CAR vs. CAR)	ns	ns	ns
		Mel526 **PBMC + ZA vs. MACS CD8 + OKT3** (CAR vs. CAR	**	**	ns
		Mel526 **PBMC + ZA vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS CD8 + OKT3** (CAR vs. CAR)	0.0659	ns	ns
		Mel526 **PBMC + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	ns	ns	ns
		Mel526 **MACS CD8 + OKT3 vs. MACS γ/δ + OKT3** (CAR vs. CAR)	**	*	0.0670

Significance was calculated by unpaired student's t test from 3 to 5 independent experiments, * p≤0.05, ** p≤0.01, *** p≤0.001, ns p>0.1, p-values between 0.05 and 0.1 are specified.
Conditions: PBMC + ZA = zoledronic-acid expanded PBMC; PBMC + OKT3 = OKT3-expanded PBMC; MACS CD8 + OKT3 = magnetic-activated cell sorting isolated CD8 T cells expanded with OKT3; γδ + OKT3 = magnetic-activated cell sorting isolated γδ T cells expanded with OKT3; all conditions were either electroporated with no RNA (mock) or transfected with the gp100 TCR or the MCSP CAR