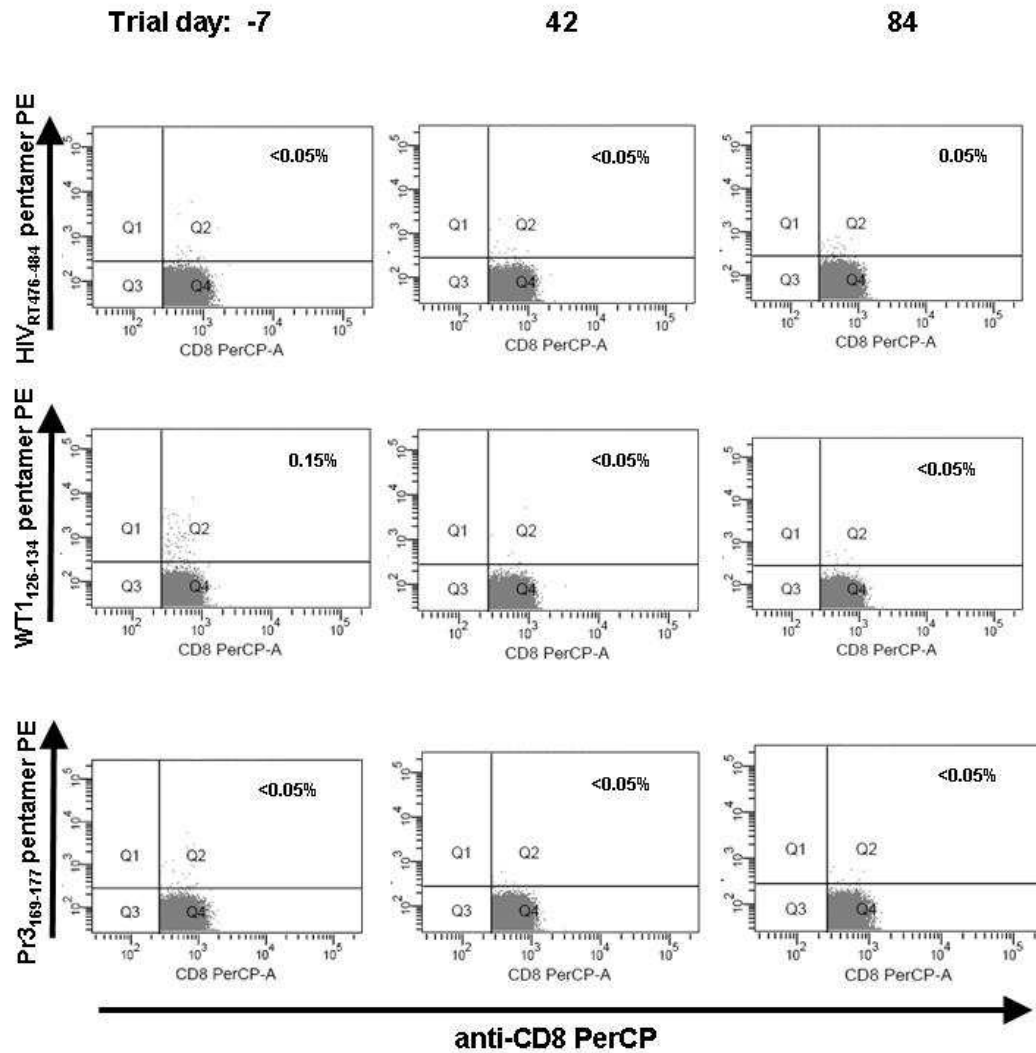


Supplementary Figure 1. Loss of WT1₁₂₆₋₁₃₄ pentamer positive CD8⁺ T-cells after vaccination

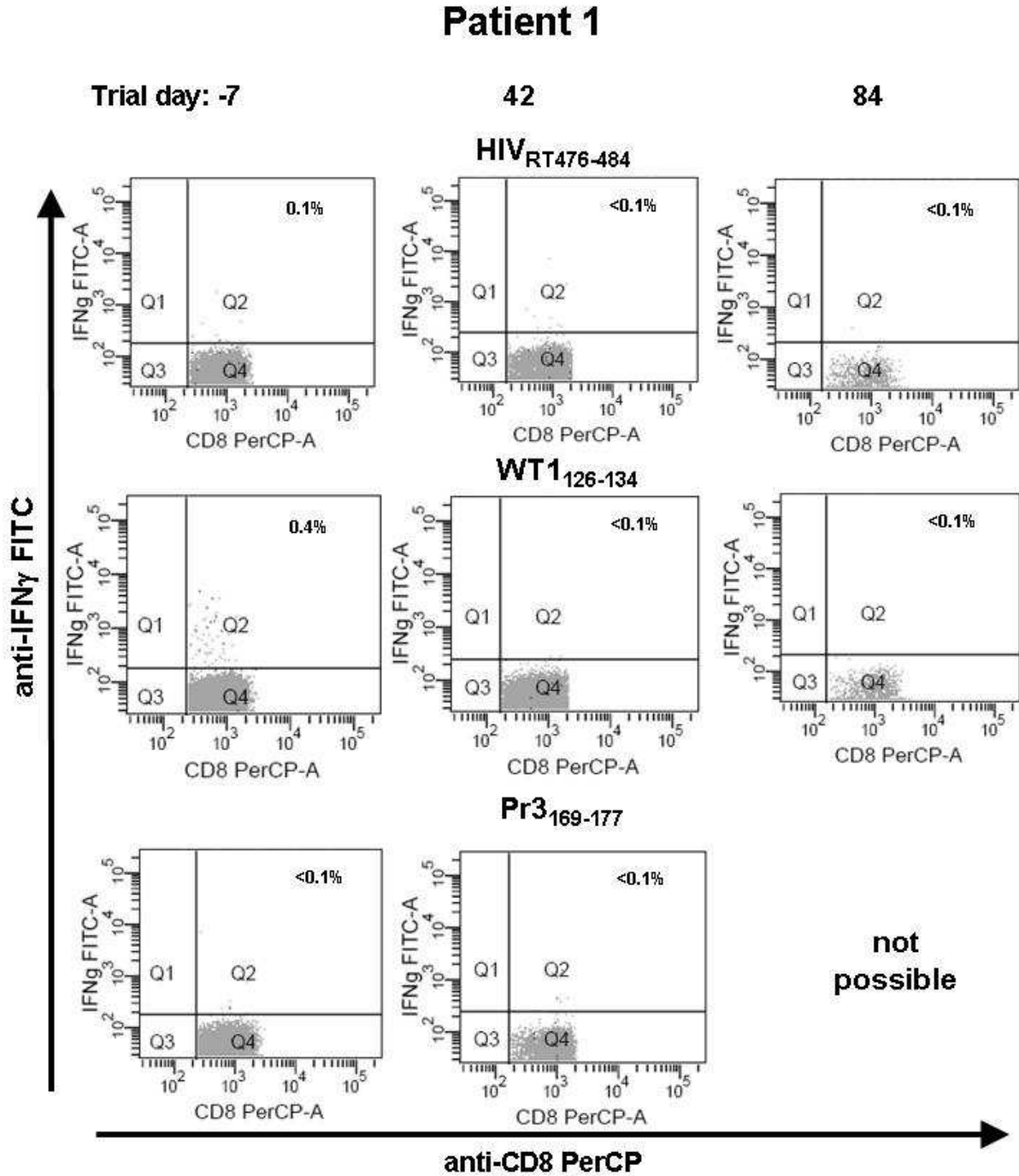
Flow cytometry analysis of patient 4 (AML)-derived PBMC samples collected prior to vaccination and at day 42 and 84. Cells were gated on CD3⁺CD8⁺ events within the lymphocyte gate and counterstained with WT1₁₂₆₋₁₃₄, Pr3₁₆₉₋₁₇₇, and HIV_{RT476-484} pentamer.

Patient 4



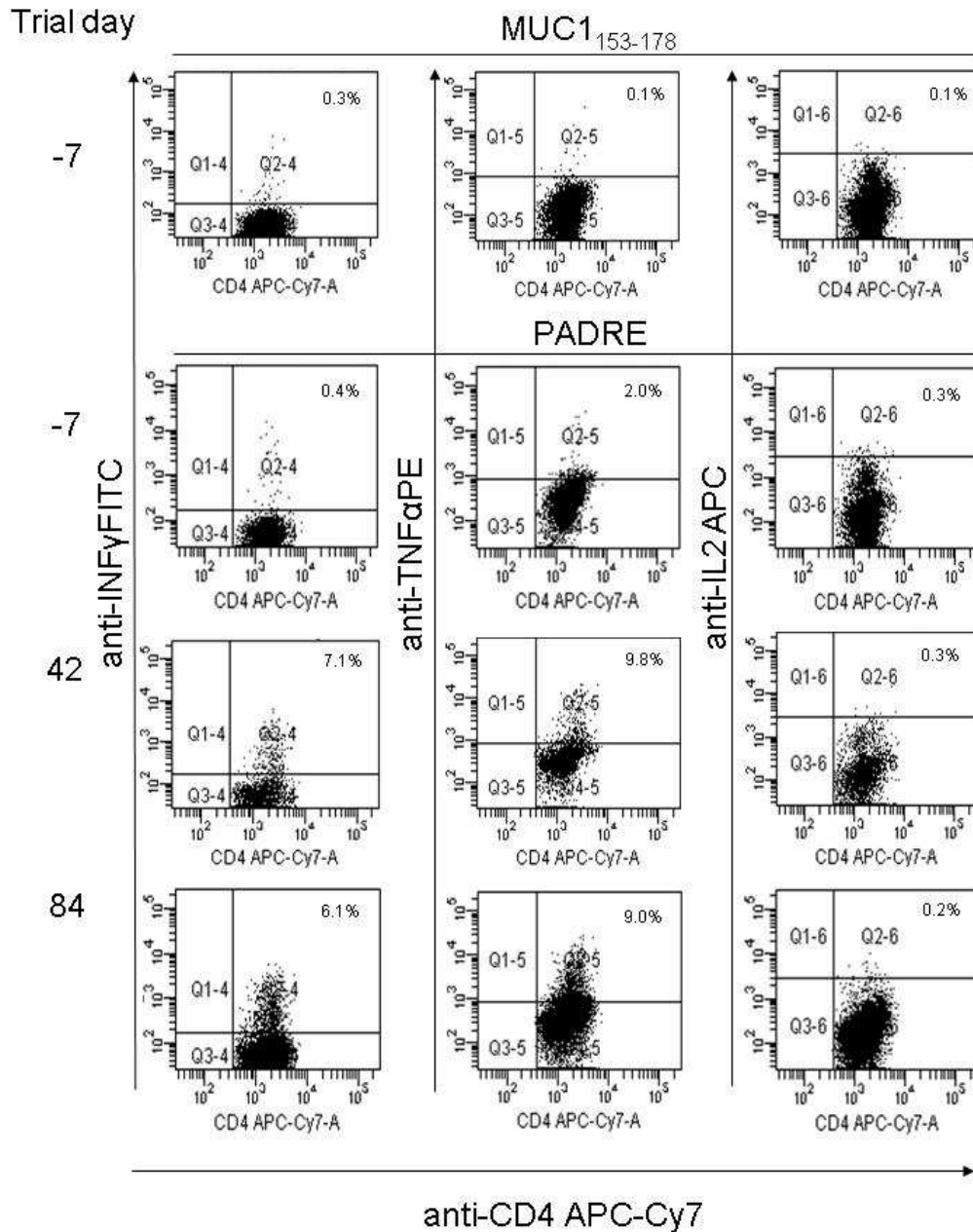
Supplementary Figure 2. Loss of IFN γ secreting WT1₁₂₆₋₁₃₄-specific CD8⁺ T-cells after vaccination.

Flow cytometry analysis of patient 1 (AML)-derived PBMC samples collected prior to vaccination and at day 42 and 84. IFN γ production of CD8⁺ T-cells after 7 days *in vitro* re-stimulation followed by ICC assay is presented. Cells were gated on CD3⁺CD8⁺ events within the lymphocyte gate, stimulating peptides are indicated.

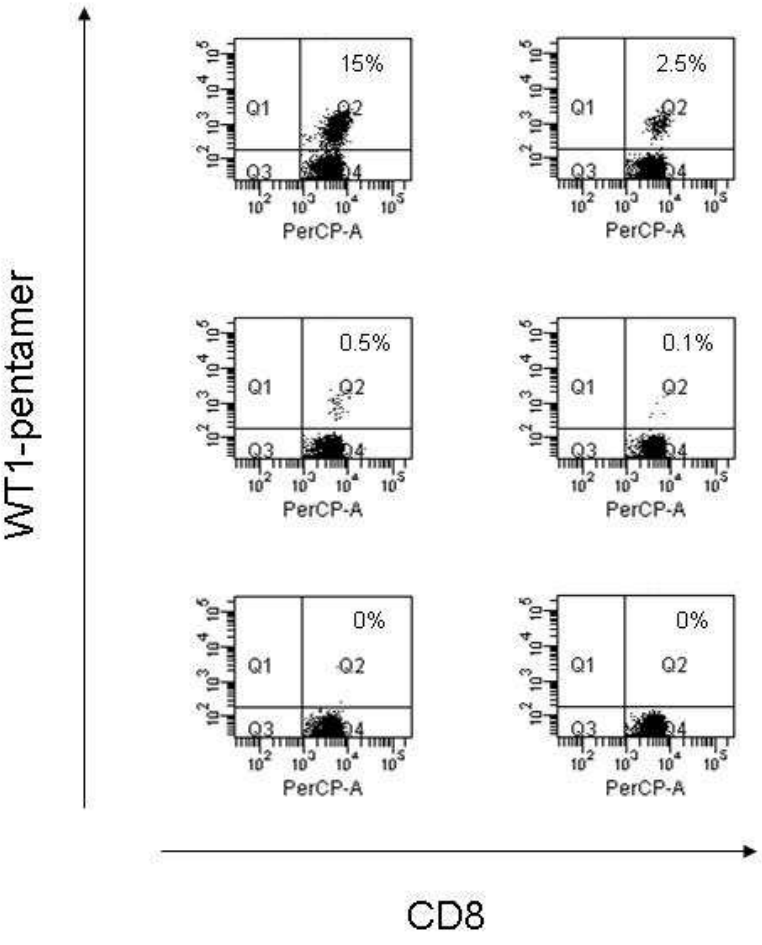


Supplementary Figure 3. Lack of expansion of IL2 secreting PADRE-specific CD4⁺ T-cells after vaccination.

Flow cytometry analysis of patient 2 (AML)-derived PBMC samples collected prior to vaccination and at day 42 and 84. IFN γ , TNF α and IL2 production of CD8⁺ T-cells after 7 days *in vitro* re-stimulation with PADRE followed by ICC assay is presented. As negative control MUC1₁₅₃₋₁₇₈ peptide pool was used. Cells were gated on CD3⁺CD4⁺ events within the lymphocyte gate, stimulating peptides are indicated.



Supplementary Figure 4. Sensitivity of WT1₁₂₆₋₁₃₄-specific pentamers. Titration of a WT1₁₂₆₋₁₃₄ specific T-cell clone into 10⁶ CD8⁺ T-cells. WT1₁₂₆₋₁₃₄ specific T-cell clones were detected until 1000 into 10⁶ CD8⁺ T-cells (0.1%) but not at 100 (lowest panel left). Lowest panel right are only CD8⁺T-cells without WT1₁₂₆₋₁₃₄ specific T-cell clones.



Supplementary Table 1. Cytokine production of MUC1-specific CD4⁺ T-cells before and after vaccination. MUC1-specific cytokine production of CD4⁺ T-cells expression was measured by intracellular cytokine production assay (ICC) without further extensive *in vitro* stimulation (left columns) or 7 days after PADRE-specific stimulation (right columns). Bold numbers indicate the specific detection of cytokine secreting CD4⁺ T-cells; underlined numbers indicate a 2 fold change as compared to day -7. * Only 1 of the indicated time points was analyzed. n.d.: not determined due to lack of material.

		Direct ex vivo analysis MUC1 ₁₅₃₋₁₇₈		After 7 days of MUC1 ₁₅₃₋₁₇₈ ⁻ specific ex vivo expansion	
	Day	-7	42 & 84	-7	42 & 84
6 MM	IFN γ	<0.1	<0.1	n.d.	n.d.
	TNF α	<0.1	<0.1	n.d.	n.d.
	IL2	<0.1	<0.1	n.d.	n.d.
7 MM	IFN γ	<0.1	<0.1	n.d.	n.d.
	TNF α	<0.1	<u>0.4 & 0.2</u>	n.d.	n.d.
	IL2	n.d.	n.d.	n.d.	n.d.
8 MM	IFN γ	<0.1	<0.1*	n.d.	n.d.
	TNF α	<0.1	<0.1*	n.d.	n.d.
	IL2	<0.1	n.d.	n.d.	n.d.
9 MM	IFN γ	<0.1	<0.1	0.1	<0.1
	TNF α	0.1	<0.1	<0.1	<0.1
	IL2	0.1	<0.1	<0.1	<0.1
		Direct ex vivo analysis MUC1 ₁₃₈₋₁₆₄		After 7 days of MUC1 ₁₃₈₋₁₆₄ specific ex vivo expansion	
6 MM	IFN γ	<0.1	0.1	n.d.	n.d.
	TNF α	<0.1	<0.1	n.d.	n.d.
	IL2	<0.1	<0.1	n.d.	n.d.
7 MM	IFN γ	<0.1	<0.1	n.d.	n.d.
	TNF α	<0.1	<0.1	n.d.	n.d.
	IL2	n.d.	n.d.	n.d.	n.d.
8 MM	IFN γ	<0.1	0.1*	n.d.	n.d.
	TNF α	<0.1	<0.1*	n.d.	n.d.
	IL2	<0.1	<0.1	n.d.	n.d.
9 MM	IFN γ	<0.1	<0.1	0.3	<u><0.1</u>
	TNF α	<0.1	<0.1	<0.1	<0.1
	IL2	<0.1	<0.1	<0.1	<0.1
Pre-existing		0/8		1/2	
Induction			1/8		0/2
Loss			0/8		1/2

Supplementary Table 2. Frequency of immature and mature plasmacytoid dendritic in the peripheral blood and prior to and after vaccination.

PBMCs were collected prior to and at day 42 and 84 after vaccination and percentages of anti-BDCA-2 (CD303)-FITC labelled (plasmacytoid dendritic) cells of total PBMCs are shown.

Anti-CD86-PE staining was used to assess the percentage of matured plasmacytoid dendritic cells of all plasmacytoid dendritic cells. n.d.: not determined.

Day	-7		42		84	
	%pDC total	%maturated	%pDC total	%maturated	%pDC total	%maturated
1 AML	0.1	22	0.1	21	0.2	20
2 AML	0.4	15	<u>0.1</u>	22	<u>0.1</u>	21
3 AML	0.1	16	0.1	24	0.1	23
4 AML	0.1	17	0.1	19	0.1	29
5 MM	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6 MM	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7 MM	0.1	17	0.1	12	0.1	13
8 MM	0.1	23	0.1	7	0.1	8
9 MM	0.3	37	0.2	39	0.3	22