**Supplemental data**

**Supplemental figures and legends**

**Fig. S1: PD-1-TALEN activity screen in murine thymoma EL4 cells.** (**A**) Optimized transfection with BTX electroporation using mRNA GFP *in vitro* transcribed from control plasmid EGFP-pCIpA102. (**B**) Three pairs of PD-1-TALEN expression in EL4 cells by western blot analysis, 24h after transfection. (**C**) T7EI assay showing NHEJ mutagenesis assessment in EL4 cells. Gene modification quantification was based on relative band intensities (Image J) (ND: non-digested; D: digested). (**D**) Percentage of PD-1 expression in EL4 cells transfected with mRNA GFP (CTL=control), or mRNA TALEN pair 1 to 3. Statistical analysis was performed using one-way ANOVA \*\**P*<0.01. (**E**) Flow plots depicting PD-1 expression 3 days after transfection in EL4 cells. Data represent 3 independent experiments.

**Fig. S2: PD-1 gene inactivation in pmel-1 CD8+T cells and functional analysis.** (**A**)Plots depictingCD8+ T cells phenotype (congenic marker: CD45.1+ and TCR gp100 specific: vβ13+) from SJL pmel-1 mice splenocytes. (**B**) Pmel-1 CD8+ T cells transfection efficacy(>88%) using BTX Agile Pulse electroporation with GFP IVT mRNA. (**C**) Flow plots depicting PD-1 expression 3 days after transfection (pre-infusion) and PD-1 negative subset enrichment with magnetic in Pmel-1 CD8+ T cells. (**D**) Absolute number of CD8wt or CD8PD-1Ex2 T cells showing the transferred cells engraftment in periphery (DLN). (**E**) T7EI assay showing NHEJ-induced mutagenesis in Pmel-1 CD8+ T cells 7 days after ACT. (**F, G, H**) Adjusted percentage of KI67+, GZB+ and IFN-γ+ CD8wt or CD8PD-1Ex2 T cells from the PD-1+ and the PD-1- sub-populations. Each dot represents an individual mouse, with mean ± SD of two independent experiments (n=10), statistical analysis was performed by one-way ANOVA (\*\*P <0.01).

**Fig. S3: PD-1-TALEN activity in TRL from MCA205.** (**A**) TRL (TILs and TDLN) transfection efficiency (>65%) using mRNA GFP and BTX electroporation and flow plots showing PD-1 expression 3 days after transfection. (**B**) PD-1-TALEN mutagenesis by Miseq analysis in TRLPD-1Ex2, 72h after transfection. (**C**) Absolute number of TRLwt or TRLPD-1Ex1 showing a similar peripheral engraftment (DLN). (**D, E, F**) Adjusted percentage of KI67+, GZB+ and IFN-γ+ TRLwt or TRLPD-1Ex2 Tcells from the PD-1+ and the PD-1- sub-populations. Each dot represents an individual mouse, with mean ± SD of 3 independent experiments (n=15), statistical analysis was performed by one-way ANOVA.

**Fig. S4: Effector/memory phenotype of TRL from MCA205. (A)** T-bet/Eomes and (**B**) CD62L/CD44 expressions and adjusted percentage on TRLwt and TRLPD-1Ex2, 6 days after transfer *in vivo*. **(C)** PMA/ionomycin re-stimulated TRL from peripheral blood of cured mice at day 40 after tumor challenge. (TRLwt n=2; TRLPD-1Ex2 n=7). Data represent 2 independent experiments (n=10), statistical analysis was performed by one-way ANOVA, \**P*<0.05.

**Supplemental table**

**Table S1. List of primers used for the T7 Endonuclease I assay and the Miseq analysis**

|  |  |  |
| --- | --- | --- |
| **T7 Endonuclease I assay** | | |
| FW | | 5’- AGGACAGAATAGTAGCCTCC-3’ |
| RV | | 5’-CCGTGTGTCAAGGATGTTCA-3’ |
| **Miseq** | | |
| FW | 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGgcacattcctctccaggggg-3’ | |
| RV | 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGatcctccgaccagttggaca-3’ | |