Supplements

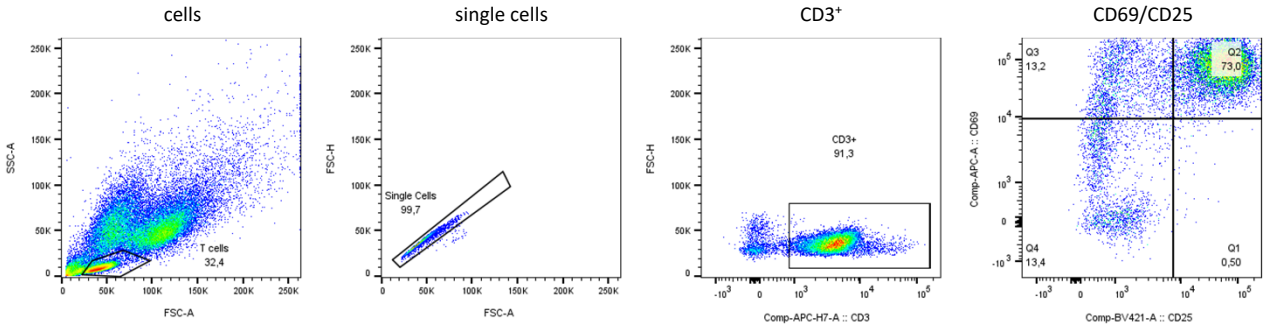

Figure S1: Gating strategy for co-culture of TCR-transfected CD8+ T cells and tumor cells. The gating strategy for all for all experiments where tumor cells were co-incubated with $CD8^+$ T cells. First a cell gate excluding cell debris and tumor cells was drawn, followed by single cells selection and finally $CD3^+$ cells were selected. The $CD3^+$ cells were then analyzed for their expression of CD25 and CD69. Shown here is the gating for one representative sample of A375M cells electroporated with CD80-RNA and loaded with the MAGE-A3 peptide and incubated with $CD8^+$ T cells that express the respective MAGE-A3 T cell receptor.

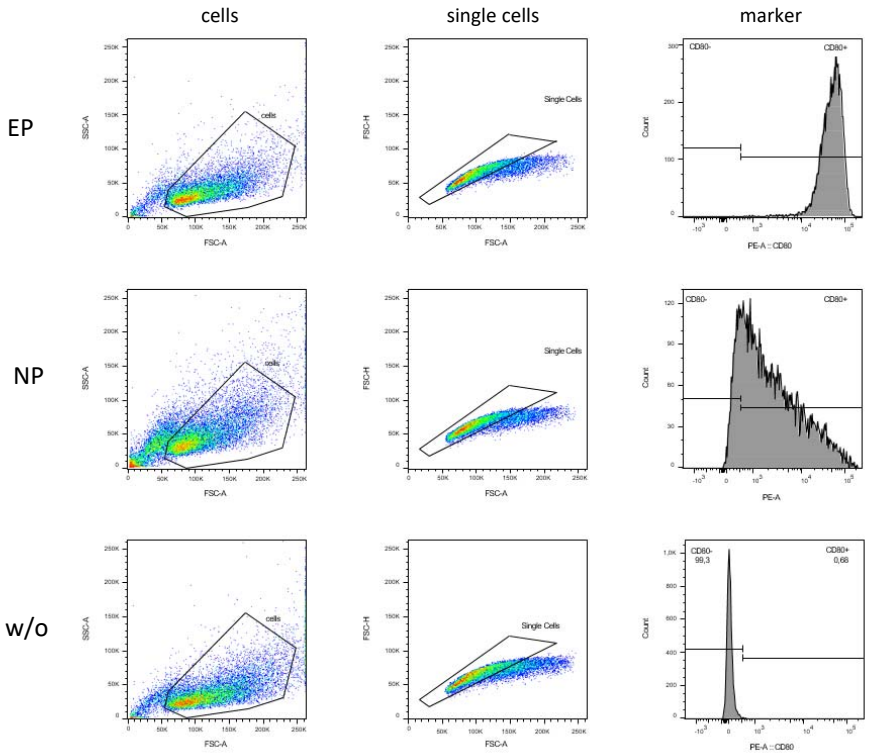

Figure S2: Gating strategy of tumor cells electroporated and nanoparticle transfected. The gating for all experiments where tumor cells were electroporated or transfected with nanoparticles was conducted equally. First a cell gate excluding cell debris was drawn, followed by single cells selection and finally the marker expression was evaluated, either the CD80 expression detected by antibody staining or GFP expression without additionally antibody staining. Shown is the gating for one representative sample of mel526 for CD80 expression after 24h.

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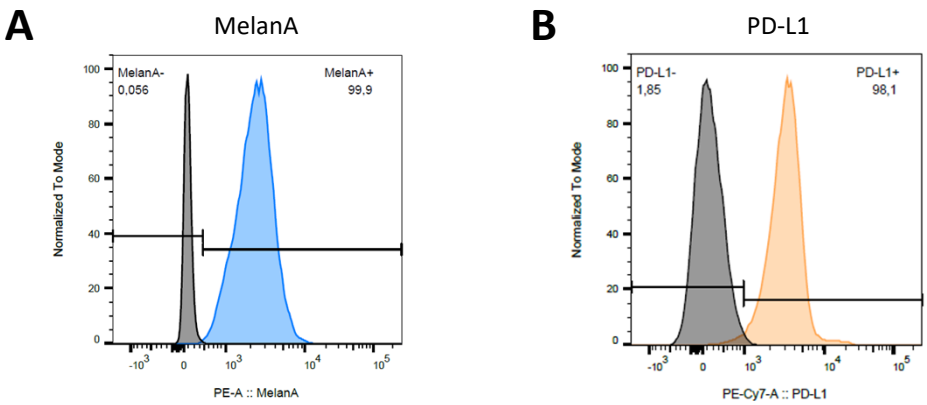

Figure S3: Characterization of Mel526 cells by flow cytometry. (A) Intracellular MelanA expression in Mel526. Cells were harvested, fixated, permeabilized, and stained with a directly labeled anti-MelanA antibody. MelanA expression was subsequently analyzed by flow cytometry. The unstained control is shown in grey, and stained cells are shown in blue. The experiment was conducted three times; one representative experiment is shown. **(B)** Surface PD-L1 expression of Mel526 cells. Cells were harvested and stained with an anti-PD-L1 antibody, followed by flow cytometry analysis. The unstained control is shown in grey, and stained cells are shown in orange. The experiment was conducted three times; one representative experiment is shown.

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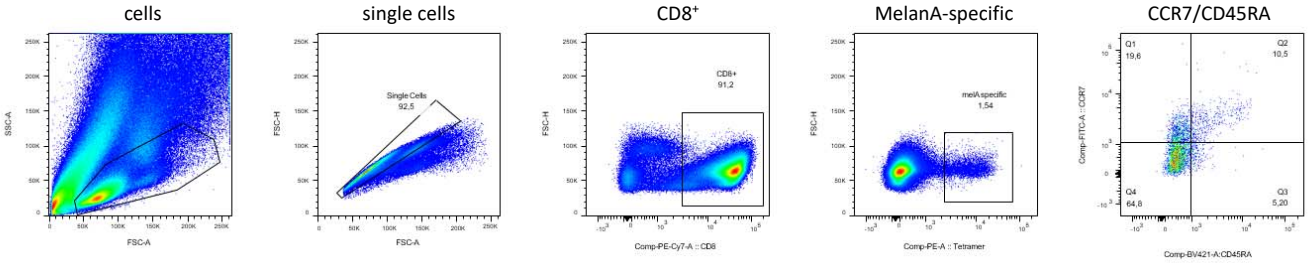

Figure S4: Gating strategy for T cells of priming experiments using CD80 electroporation or nanoparticle transfected mel526. First a cell gate broadly selecting cells in T cell size range was drawn. Next, single cells were selected, from these CD8 positive cells were selected to exclude mel526 cells completely. Further, MelanA specific T cells were selected which were tetramer/dextramer positive. MelanA-specific cells where further divided by their CCR7 and CD45RA expression.

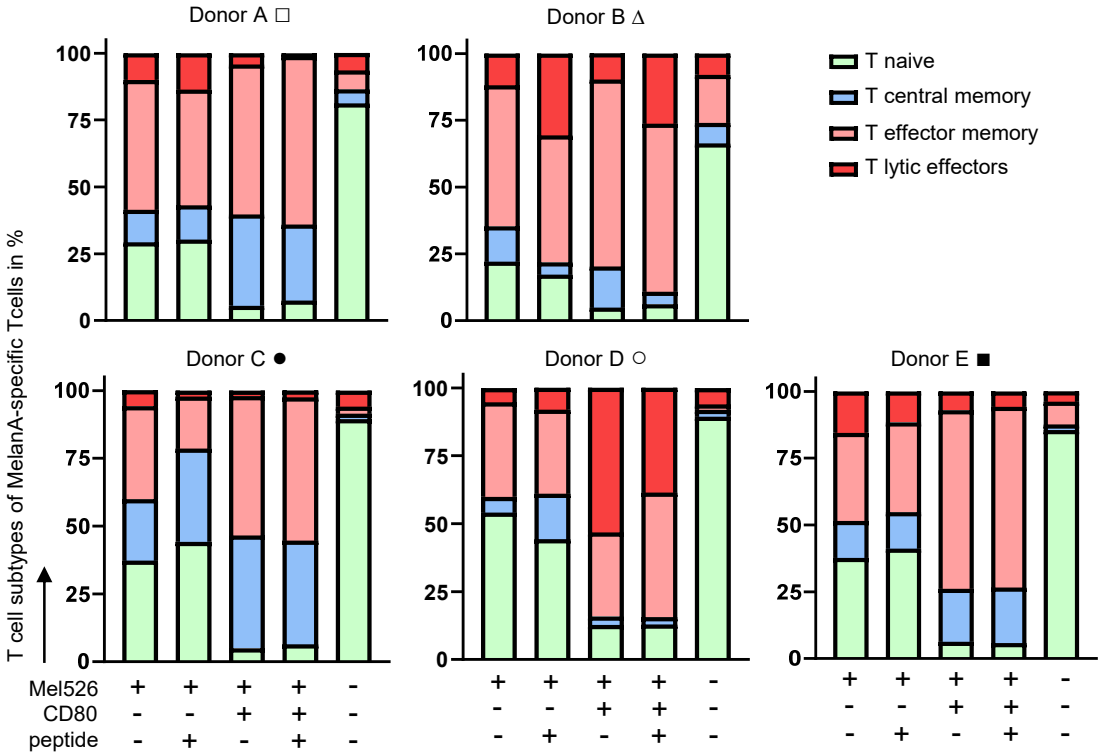

Figure S5: Normalized T cell subtypes of MelanA specific T cells EP experiment. Depicted are the subpopulations of MelanA specific T cells normalized to 100 percent for each condition. T cell subtypes are divided into naïve T cells (CD45RA+,CCR7+), central effector T cells (CD45RA-, CCR7+), effector memory T cells (CD45RA-, CCR7-) and lytic effector T cells (CD45+, CCR7-). Shown are 5 donors independently.

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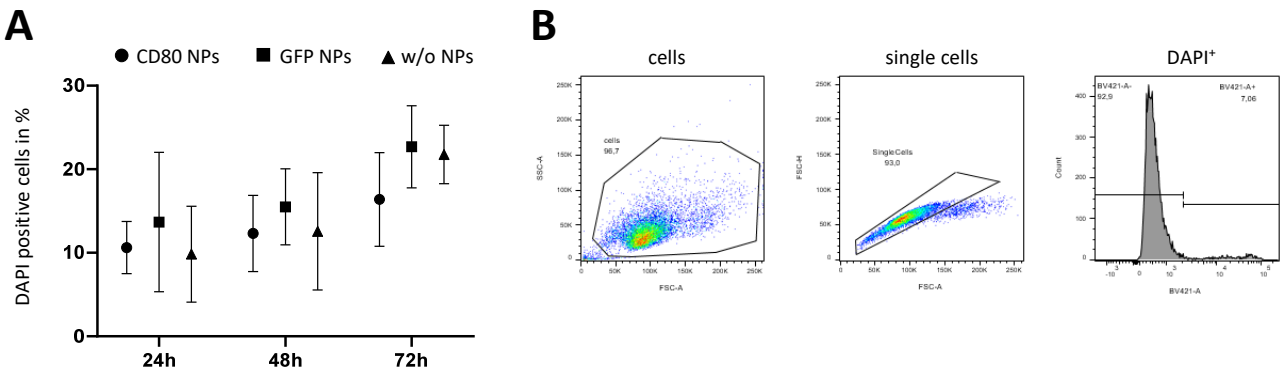

Figure S6: Live/Dead staining of mel526 cells transfected with nanoparticles. (A) 100.000 mel526 were seeded in a 24-well plate, when the cells were adherent, CD80 nanoparticles (CD80 NPs) or GFP nanoparticles (GFP NPs) were given into the well. As control served mel526 without nanoparticle addition (w/o NPs). After 24h, 48h or 72h cells were harvested and prepared for flow cytometry analysis. CD80 NPs samples were stained with CD80 antibodies to confirm the transfection, for GFP NP samples no additional staining was necessary to proof confirm the transfection. All samples were stained with DAPI shortly before the flow cytometry analysis. Depicted is the mean value ±SD for 3-4 independent experiments. **(B)** gating strategy for experiment (A), the cell gate size was selected to impede that dead cells were excluded in the beginning. Then single cells were gated and from that DAPI positive cells selected.

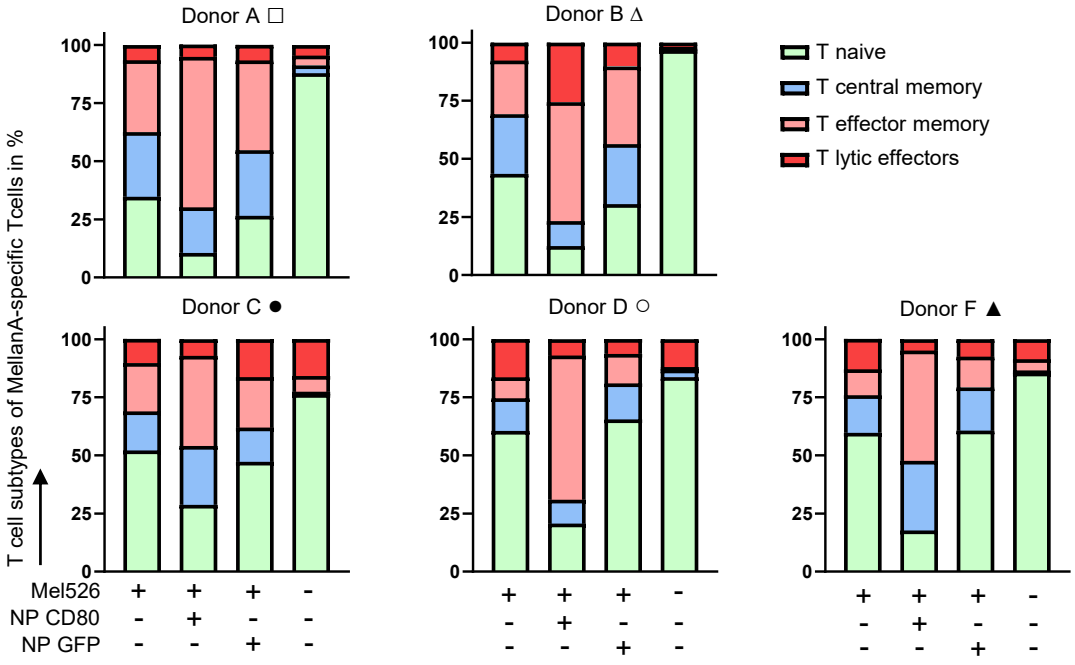

Figure S7: Normalized T cell subtypes of MelanA specific T cells EP experiment. Depicted are the subpopulations of MelanA specific T cells normalized to 100 percent for each condition. T cell subtypes are divided into naïve T cells (CD45RA+,CCR7+), central effector T cells (CD45RA-, CCR7+), effector memory T cells (CD45RA-, CCR7-) and lytic effector T cells (CD45+, CCR7-). Shown are 5 donors independently.

Supplements

Figure S8: Vascularization of CAM explanted tumors. A Mel526-spheroid was grafted onto the chorioallantoic membrane and allowed to grow for 5 days before nanoparticle treatment. After 32 h, tumors were excised and imaged by light microscopy to show vascularization.