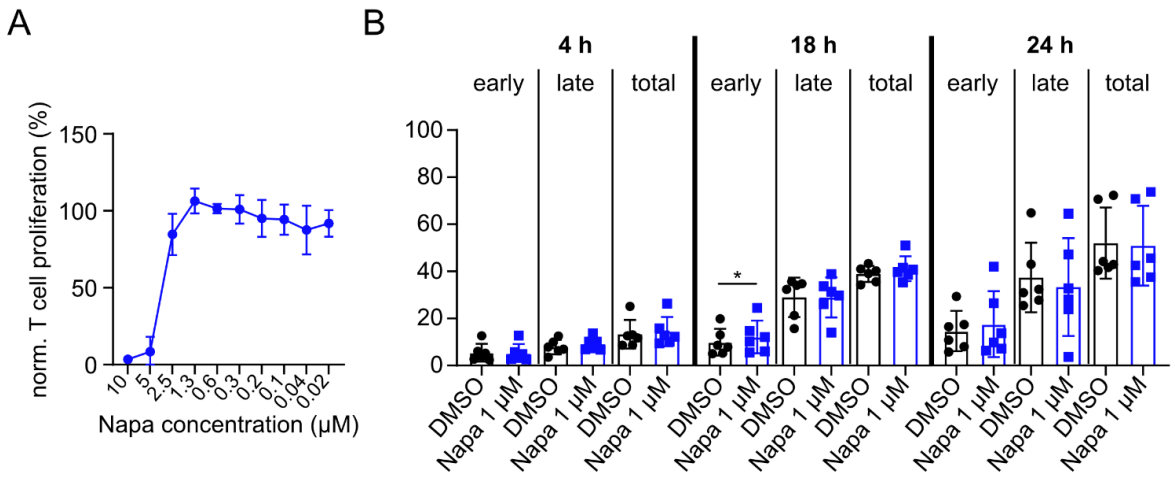

Fig. S1 Impact of Napabucasin on T cell proliferation or survival. (A) CFSE-labeled murine splenic $CD8^+$ T cells were activated by anti-CD3 and anti-CD28 antibodies and cultured for 72 h upon the addition of indicated concentrations of Napabucasin (Napa). Cumulative data for T cell proliferation are presented as the percentage of divided T cells normalized (norm.) to the respective control of stimulated T cells alone (mean ± SD; n=4). (B) Murine splenic $CD8^+$ T cells were treated with 1 µM Napabucasin (Napa) or DMSO at the respective concentration. Apoptosis was measured by flow cytometry. Results are presented as the percentage of early (Annexin V^+7AAD^-), late (Annexin V^+7AAD^+) and total apoptotic cells among all T cells upon 4 h, 18 h and 24 h incubation with Napa (mean ± SD; n=6). *$p < 0.05$.

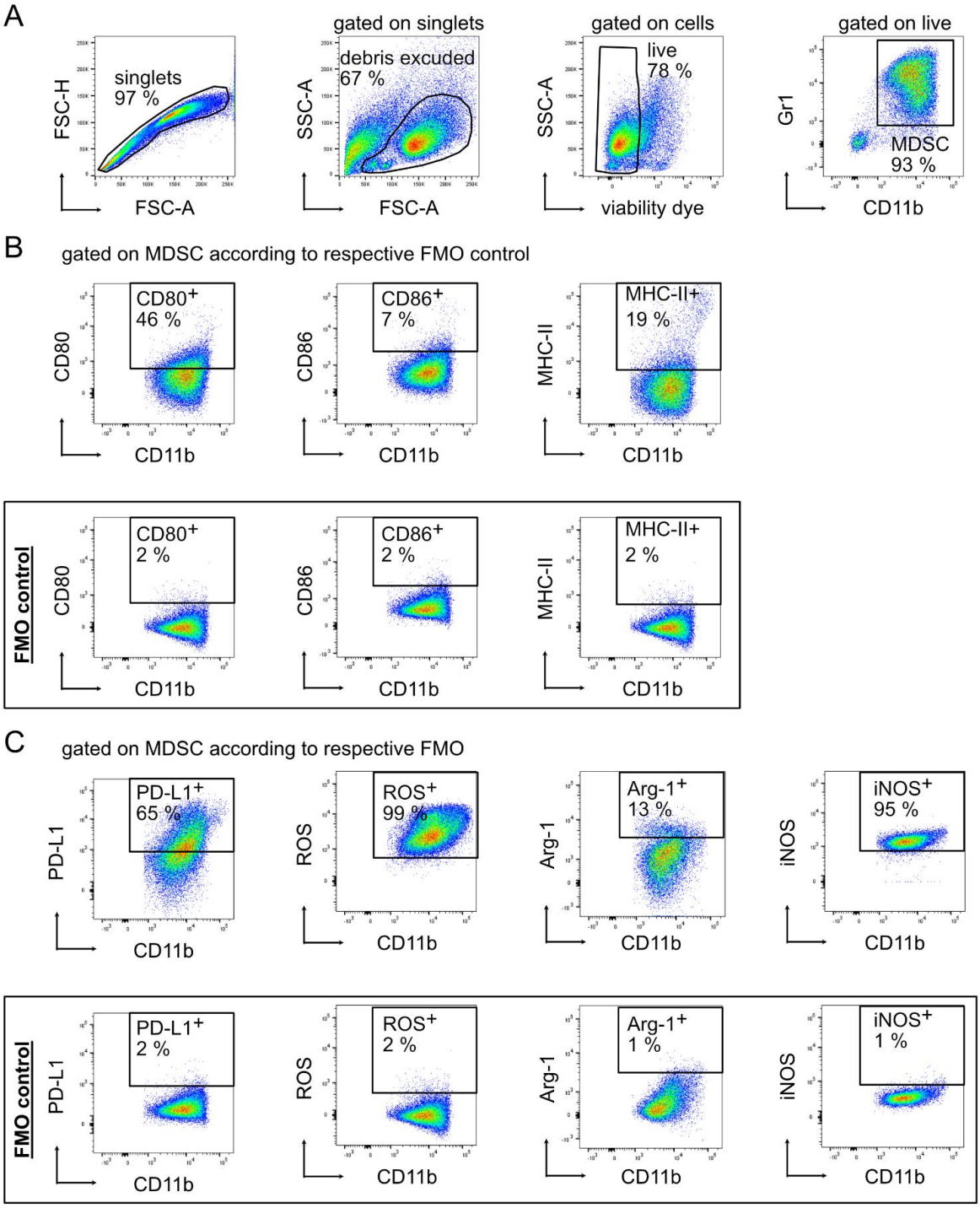

Fig. S2 Gating strategy for marker expression of *in vitro* generated MDSC. After generation, MDSC were incubated for 24 hours with Napabucasin or DMSO at the respective concentration followed by

flow cytometry. Representative dot plots are shown for DMSO treated MDSC. (A) $CD11b^+Gr1^+$ MDSC were gated after exclusion of doublets, debris and dead cells. (B) $CD80^+$, $CD86^+$ and MHC class II^+ (MHC-II) MDSC were gated according to the respective fluorescence minus one (FMO) control. (C) PD-$L1^+$, ROS^+, Arg-1^+ and $iNOS^+$ MDSC were gated according to the respective FMO control. As all cells were ROS^+, median fluorescence intensity (MFI) of total MDSC in the ROS channel was used to quantify ROS production.

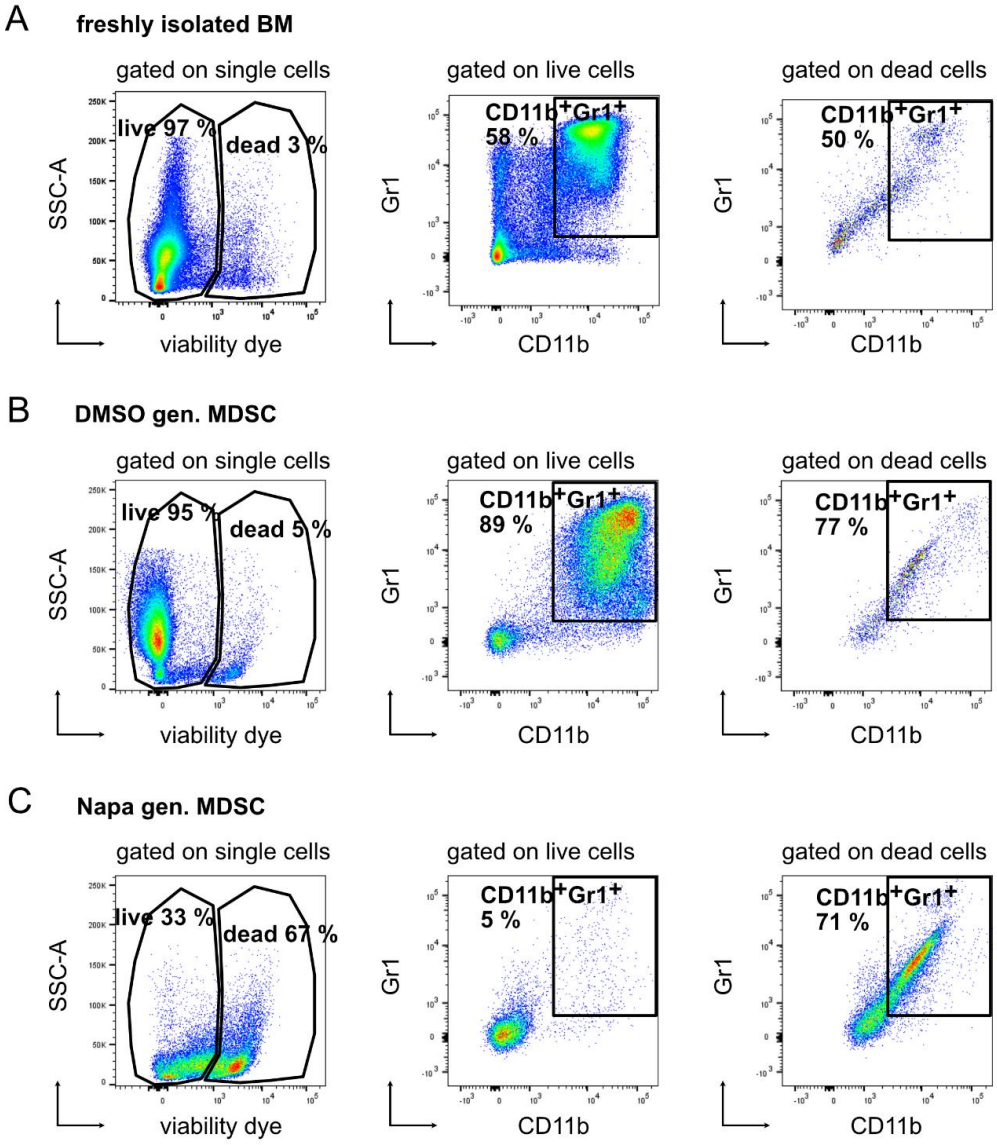

Fig. S3 Exemplary flow cytometry plots for cells treated with Napabucasin during MDSC generation. MDSC were generated *in vitro* from murine bone marrow (BM) cells by IL-6 and GM-CSF (40 ng/ml both). 1 µM Napabucasin (Napa) or DMSO at the respective concentration were added together with cytokines followed by FACS analysis. Representative dot plots for freshly isolated BM cells (A), MDSC after the generation with DMSO (B) or 1 µM Napabucasin (Napa) (C)

are shown. Data are presented as the percentage of live and dead cells within total cells as well as the percentage of $CD11b^{+}Gr1^{+}$ MDSC among live and dead cells.

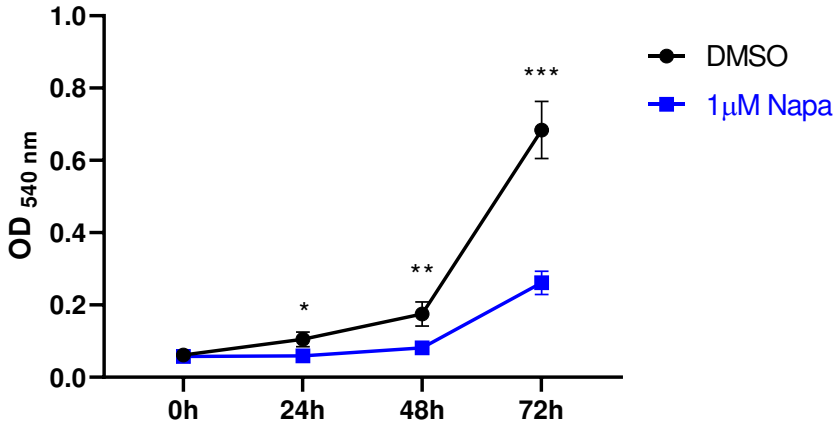

Fig. S4 Effect of Napabucasin on Ret melanoma cell growth *in vitro*. Cell proliferation was measured by MTT assay upon the treatment with 1 µM Napabucasin (Napa) or 0.01 % DMSO for 0 h, 24 h, 48 h and 72 h (mean ± SD; n=4). *p < 0.05, **p < 0.01, ***p < 0.001.

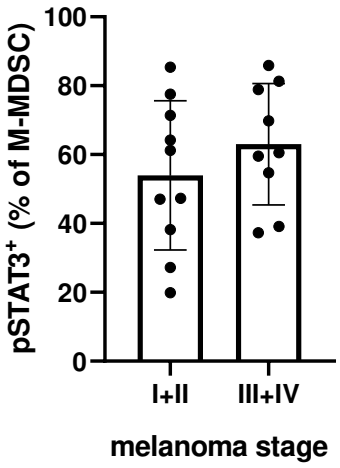

Fig. S5 STAT3 activation in M-MDSC from melanoma patients by disease stage. PBMC from untreated melanoma patients (stage I-IV) were analyzed by flow cytometry. M-MDSC were defined as $CD33^{high}HLA\text{-}DR^{low/neg}$. Cumulative data are shown as the percentage of $pSTAT3^{+}$ cells among M-MDSC (mean ± SD) in stage I and II (n=10) versus stage III and IV patients (n=9). $pSTAT3^{+}$ M-MDSC were gated according to isotype control.