A TLR7 agonist strengthens T and NK cell function during BRAF-targeted therapy in a preclinical melanoma model

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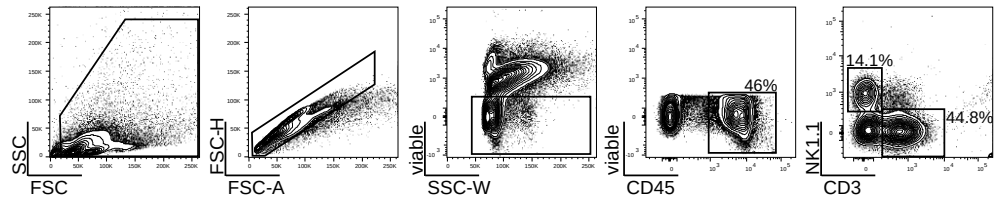
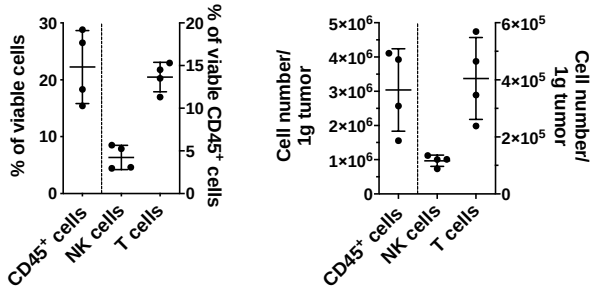
Supplementary Material

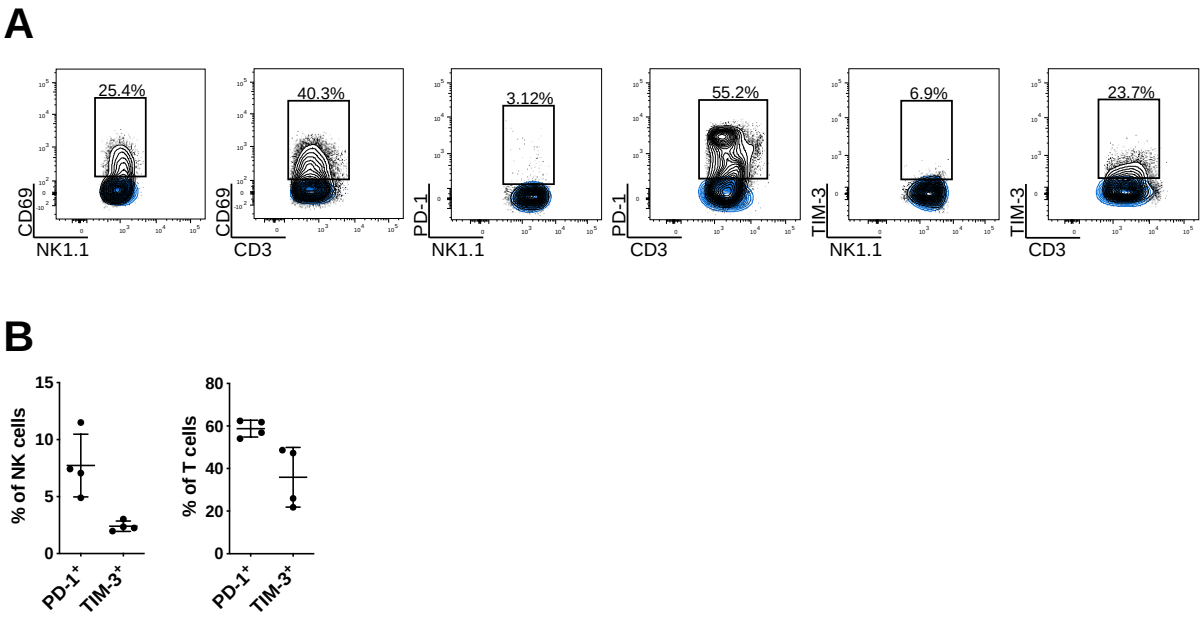
A



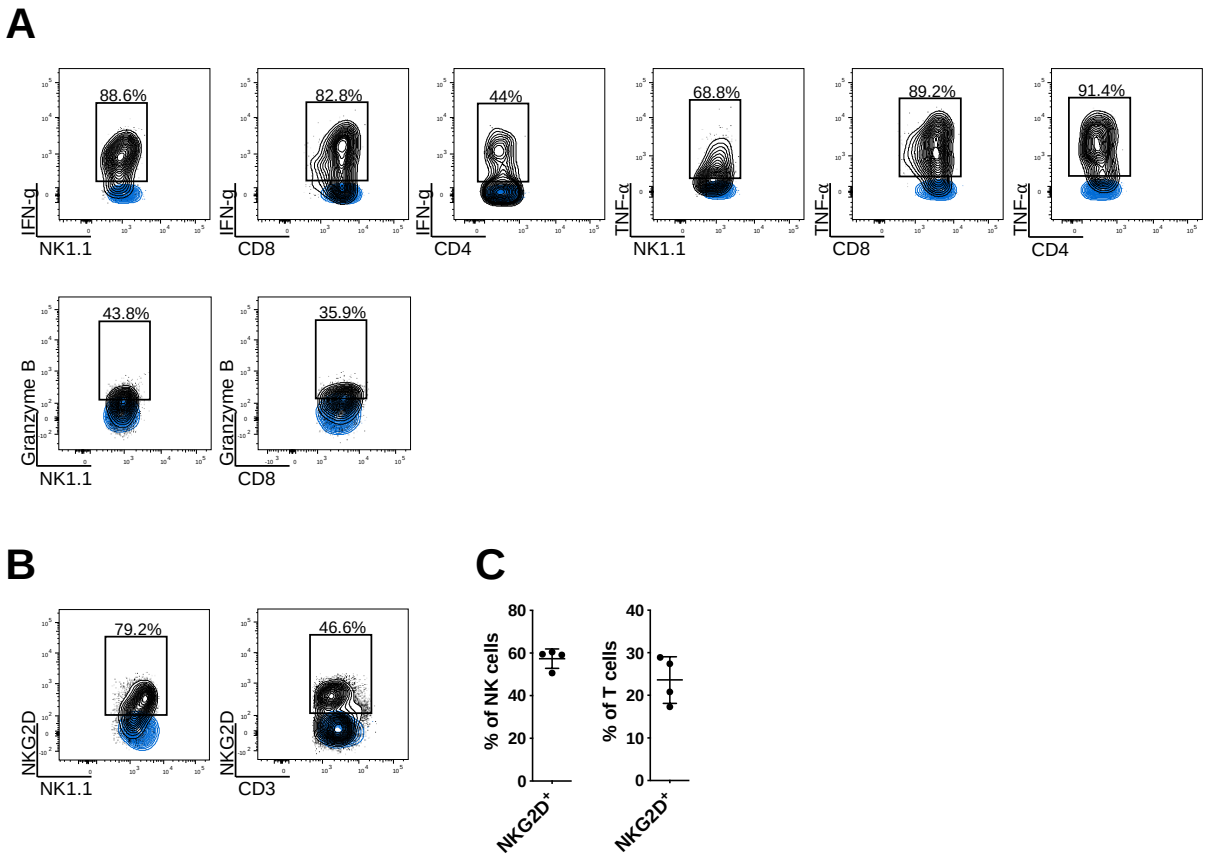
B



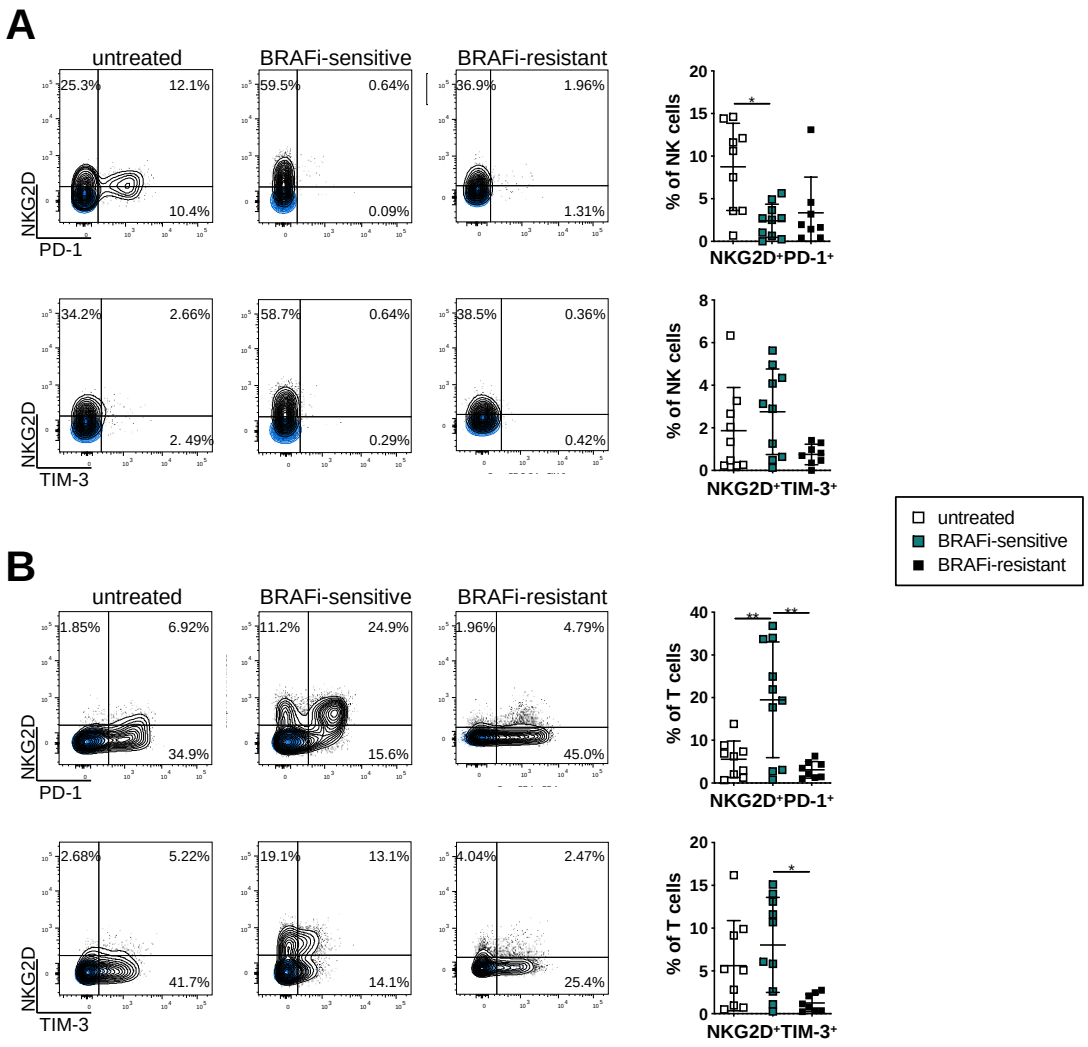
Supplementary Figure 1: (A) Gating strategy for the immune cell infiltrate. Immune cells are pre-gated on single, viable cells, then CD45 for all immune cells, followed by NK ($NK1.1^+$) as well as ($CD3^+$) T cells. Representative example for a BRAFi-sensitive tumor is shown. **(B)** Tumor-infiltrating $CD45^+$ cells, NK and T cells from tumors analyzed on day 8 after tumor transplantation, the time point when BRAFi treatment was initiated. Results are displayed as percentage as well as cell numbers/gram tumor. Summary graphs for one experiment are shown (n = 4).



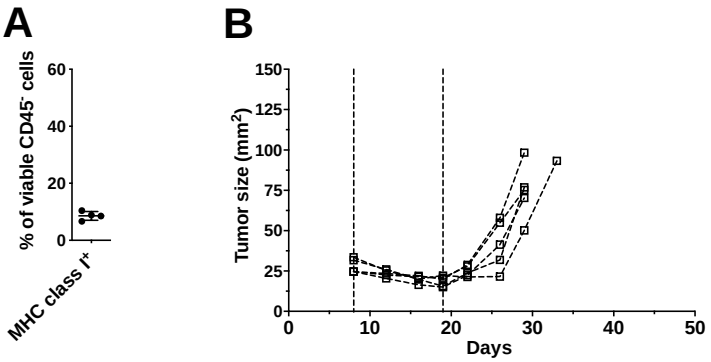
Supplementary Figure 2: (A) Gating strategy for CD69, PD-1 and TIM-3 is shown. Cells were pre-gated on single, viable $CD45^+$ cells. Representative example for a BRAFi-sensitive tumor is shown. FMO or isotype-matched antibodies served as controls (blue). **(B)** Percentages of $PD\text{-}1^+$ and $TIM\text{-}3^+$ cells of NK and T cells infiltrating tumors dissected on day 8 after tumor transplantation were determined by flow cytometry analysis. Summary graphs for one experiment are shown (n = 4).



Supplementary Figure 3: (A and B) Gating strategy for (A) IFN-γ, TNF-α and granzyme B and (B) NKG2D is shown. Cells were pre-gated on single, viable CD45$^+$ cells. Representative example for a BRAFi-sensitive tumor is shown. FMO or isotype-matched antibodies served as controls (blue). **(C)** Percentages of NKG2D$^+$ cells of NK and T cells infiltrating tumors analyzed on day 8 after tumor transplantation were determined by flow cytometry analysis. Summary graphs for one experiment are shown (n = 4).



Supplementary Figure 4: (A and B) Gating strategy to determine co-expression of NKG2D with PD-1 or NKG2D with TIM-3. Percentages of double-positive cells of (A) NK cells and (B) T cells are shown. Dot plots show representative examples for an untreated, BRAFi-sensitive and BRAFi-resistant tumor. Cells were pre-gated on single, viable $CD45^+$ cells. FMO or isotype-matched antibodies served as controls (blue). Summary graphs for at least 2 independent experiments are shown ($n \geq 8$/group).



Supplementary Figure 5: (A) Percentages of MHC-class I^+ cells of viable $CD45^-$ tumor cells from tumors analyzed on day 8 after tumor transplantation were determined by flow cytometry analysis. Summary graph for one experiment is shown (n = 4). **(B)** Tumor growth curve upon s.c. injection with $3x10^5$ murine melanoma D4M cells. On day 8 (dotted vertical line), mice were switched to BRAFi-containing chow and on day 19 (second dotted vertical line) mice were switched back to control chow (no BRAFi) and given s.c. peritumorally 2 mg/kg imiquimod (TLR7A). Summary graph for one experiment is shown (n = 5).