**Supplementary Materials**

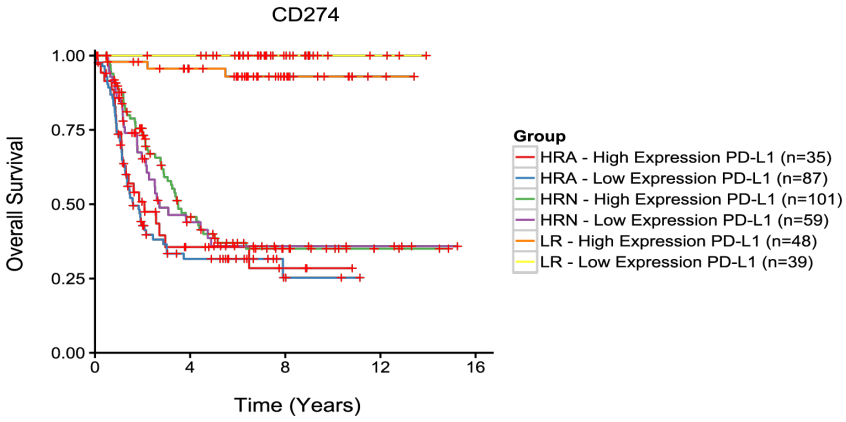
## Supplemental Figure 1.

**A**

| Risk Group* | Number of samples |
|---|---|
| **Low risk**<br>*Stage 1, 2 and 4S  All tumors are MYCN-non-amplified* | 30 |
| **High risk MYCN-non-amplified**<br>*Stage 3 >18mo with UH, Stage 4 12-18 with UH or diploid, all Stage 4 >18 mo* | 151 |
| **High risk MYCN-amplified**<br>*Stage 2+ MYCN-A* | 68 |
| **Cell Lines**<br>(*MYCN Amplified:* BE2, KAN, KCNR, LHN (MYCN overexpressing), SAN, CHLA-90, CHLA-119, CHLA-122, CHLA-136, *MYCN Non-Amplified:* LAN6, CHLA-15, CHLA20, CHLA-42, CHLA-51, CHLA-79, CHLA-140, CHLA-172) | 17 |

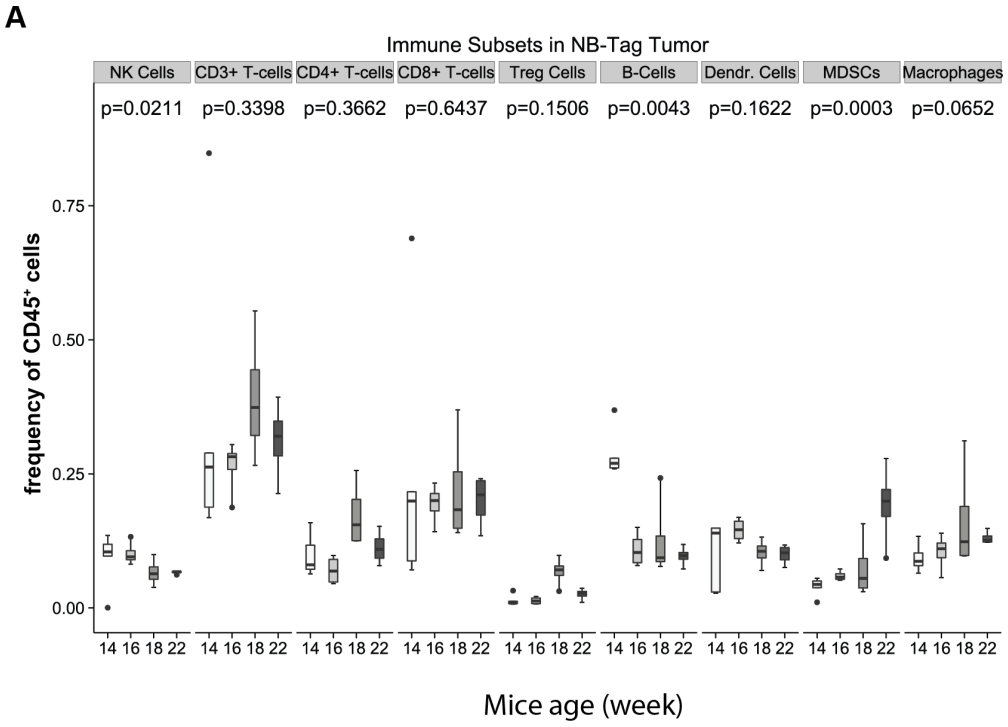
**All tumors except Stage 1 tumors with MYCN-amplification are superceded into the high-risk MYCN-amplified category. UH: unfavorable histology. MYCN-A: MYCN-amplified. Clinical information as well as selected open-access genomic data are available at the TARGET website: <https://ocg.cancer.gov/programs/target/projects/neuroblastoma>*

**B**



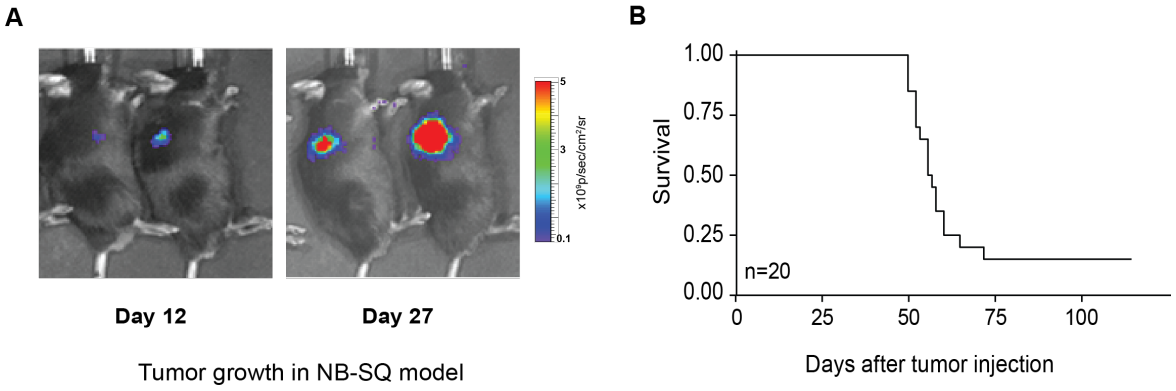

**Supplementary Figure 1. Characteristics of neuroblastoma patient cohort and cell lines. (A)** Characteristics of neuroblastoma patient tumor cohort (n=249) and cell lines (n=17) used in gene expression analysis. **(B)** No difference was seen in the overall survival of 249 patients grouped by *CD274*/*PD-L1* gene expression above the 50$^{th}$ percentile (High Expression) or below the 50$^{th}$ percentile (Low expression) (p-values were 0.71, 0.62 and 0.11 for HRA, HRN and LR, respectively). HRA, High risk *MYCN*-amplified; HRN, High risk *MYCN*-non-amplified; LR, Low risk.

## Supplemental Figure 2.

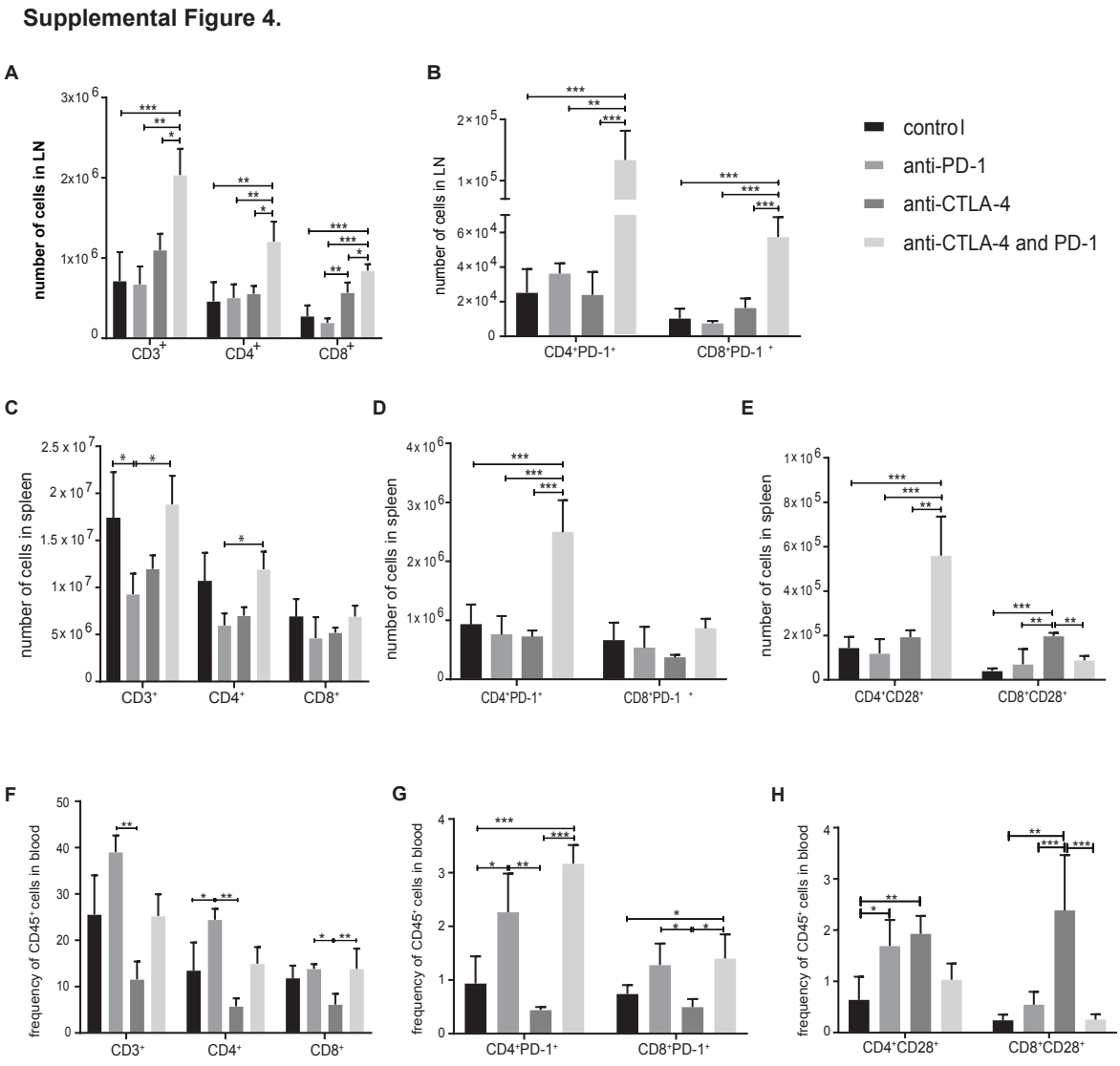



**Supplementary Figure 2. Cellular composition of the tumor microenvironment in NB-Tag mice over time. (A)** Flow cytometric analysis of single cell suspensions of tumors obtained from NB-Tag mice at 14, 16, 18, and 22 weeks of age. Significance: *: < 0.05, **: < 0.005, ***: < 0.0005.

## Supplemental Figure 3.



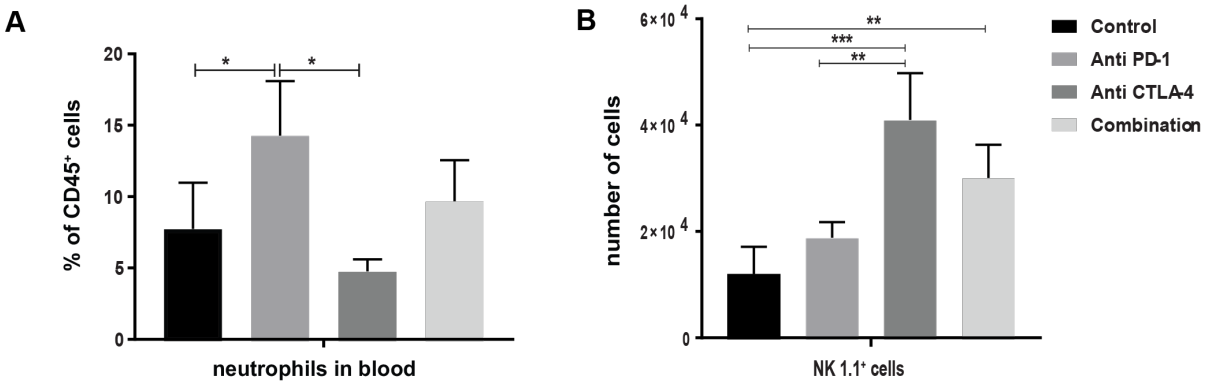
**Supplementary Figure 3. Tumor development in the syngeneic subcutaneous model. (A, B)** $4x10^6$ Luciferase-expressing NB-Tag-derived cells (NBT3L) were injected into syngeneic mice and forms subcutaneous tumors. Tumors are lethal, similar to transgenic NB-Tag mice (n=20, survival of 15.3%)




**Supplementary Figure 4. Changes in different T cell subpopulations following early immune checkpoint treatment.** Flow cytometric analysis of tumor draining LNs, spleens, and blood for different T cells population. **(A)** Average number (+SD) of $CD3^+$, $CD4^+$, $CD8^+$ T-cell subsets in draining LNs. **(B)** Average number (+SD) of $CD4^+PD\text{-}1^+$ $CD8^+PD\text{-}1^+$ T cells in draining lymph nodes; **(C)** Average number. (+SD) of $CD3^+$, $CD4^+$, $CD8^+$ T-cell subsets in spleen. **(D)** Average number (+SD) of $CD4^+PD\text{-}1^+$ and $CD8^+PD\text{-}1^+$ T cells in spleen. **(E)** Average number (+SD) of $CD4^+CD28^+$ and $CD8^+CD28^+$ T cells in spleen. **(F)** Average frequency (+SD) of $CD3^+$, $CD4^+$ and $CD8^+$ T cells among

CD45+ blood cells. **(G)** Average frequency (+SD) of circulating PD-1$^{+}$ CD4$^{+}$ and CD8$^{+}$ T-cells among CD45+ blood cells; **(H)** Average frequency (+SD) of CD28$^{+}$ CD4$^{+}$ and CD8$^{+}$ T-cells among CD45+ blood cells. Significance: *: < 0.05, **: < 0.005, ***: < 0.0005.

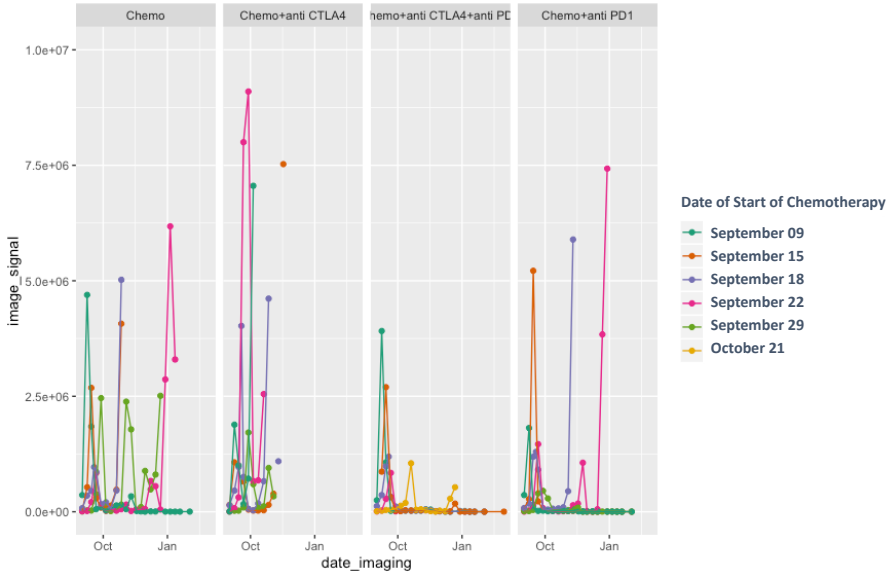
## Supplemental Figure 5.



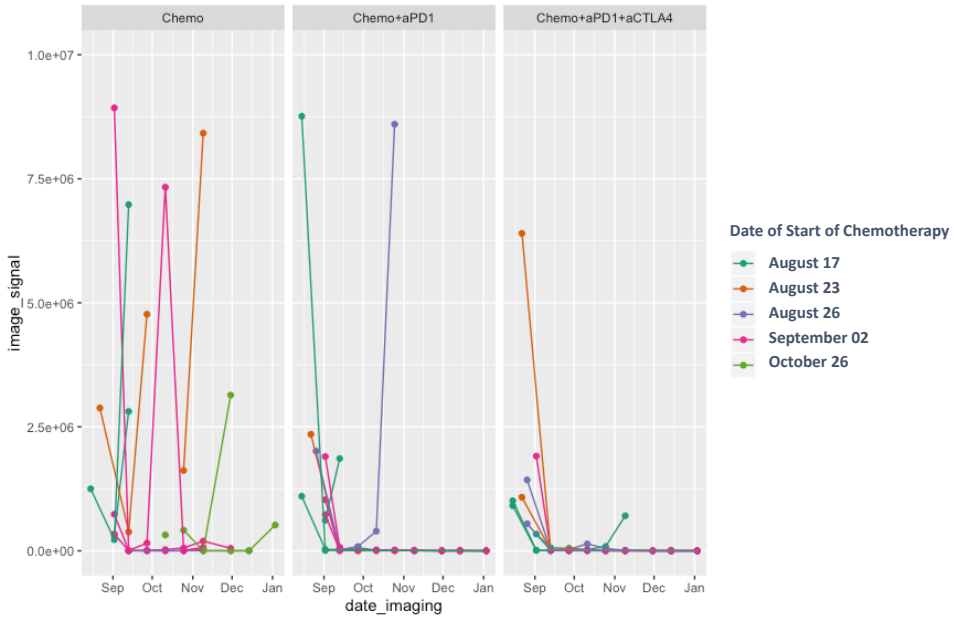

**Supplementary Figure 5. Modulation of the innate immune system by immune checkpoint treatment at an early stage of tumor growth.** Mice were subcutaneously injected with 4x10$^{6}$ NBT3L cells and treated with single or combination immune checkpoint antibodies or isotype controls the next day. On day 5, spleen, tumor draining LNs and blood were collected and single cells were analyzed by flow cytometry. **(A)** Frequency of circulating neutrophils; **(B).** Total number of NK cells in draining lymph nodes. Significance: *: < 0.05, **: < 0.005, ***: < 0.0005

**Supplementary Figure 6. Bioluminescent imaging (BLI) signal values for experiments combining chemotherapy and immune checkpoint therapy. A)** Experiment #1 in Year 1 and B) Experiment #2 in Year 2 with colors denote groups of mice starting at particular treatment timepoint (when BLI > 100 p/s (log10))

**A)**




**B)**



**Supplemental Table 1.**

| Specificity | Fluorochrome | Clone | Vendor | Catalogue # |
|---|---|---|---|---|
| Fixable Viability Dye | eFluor 450 | NA | e Biosciences | 65-0863-14 |
| anti-Mouse CD3e | FITC | 145-2C11 | e Biosciences | 11-0031-85 |
| anti-Mouse CD4 | PerCP-Cyanine5.5 | 45-0042-82 | e Biosciences | RM4-5 |
| anti-Mouse CD8 | APC | 53-6.7 | BD Biosciences | 563035 |
| anti-Mouse CD25 | PE | PC61-5 | e Biosciences | 12-0251-83 |
| Anti-Mouse NK1.1 | PE-Cyanine7 | PK136 | e Biosciences | 25-5941-82 |
| anti-Mouse/Rat Foxp3 | APC | FJK-16s | e Biosciences | 17-5773-82 |
| anti-Mouse Ly-6G (Gr-1) | Alexa Fluor 488 | RB6-8C5 | e Biosciences | 53-5931-82 |
| anti-Mouse CD19 | PerCP-Cyanine5.5 | 6D5 | BioLegend | 115534 |
| anti-Mouse CD11b | PE | M1/70 | BD Biosciences | 12-0112-83 |
| anti-Mouse Ly-6C | PE-Cyanine7 | HK1.4 | e Biosciences | 25-5932-82 |
| anti-Mouse CD11c | Alexa Fluor 700 | N418 | e Biosciences | 56-0114-82 |
| anti-Mouse CD45 | APC-Cyanine7 | 30-F11 | BD Biosciences | 103116 |
| anti-Mouse F4/80 | APC | BM8 | BioLegend | 123116 |
| anti-Mouse CTLA-4 | PE | UC10-4B9 | BioLegend | 106305 |
| anti-Mouse PD-1 | BV421 | 29F.1A12 | BioLegend | 135221 |
| anti-Mouse B7H1(PD-L1) | PE | MIH5 | e Biosciences | 12-5982-81 |