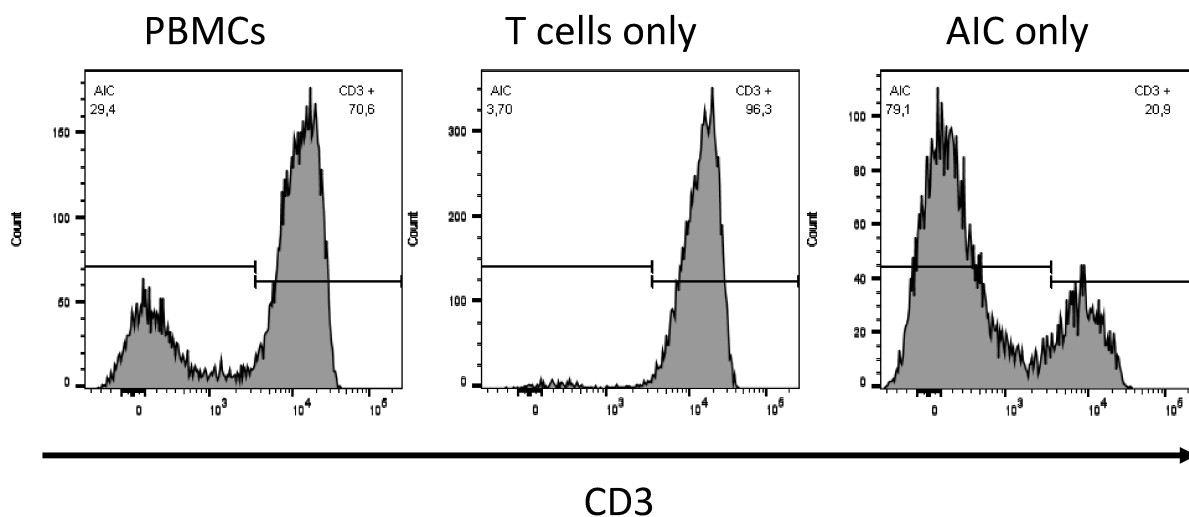


# Online supplementary material

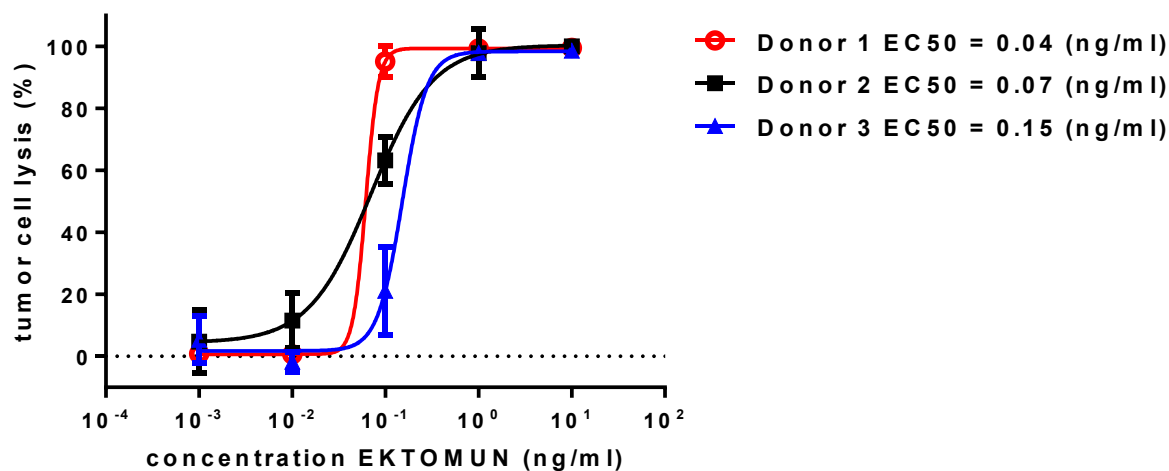
Zirngibl F *et al*, “GD2-directed bispecific trifunctional antibody outperforms dinutuximab beta in a murine model for aggressive metastasized neuroblastoma”

# Supplementary Figure S1



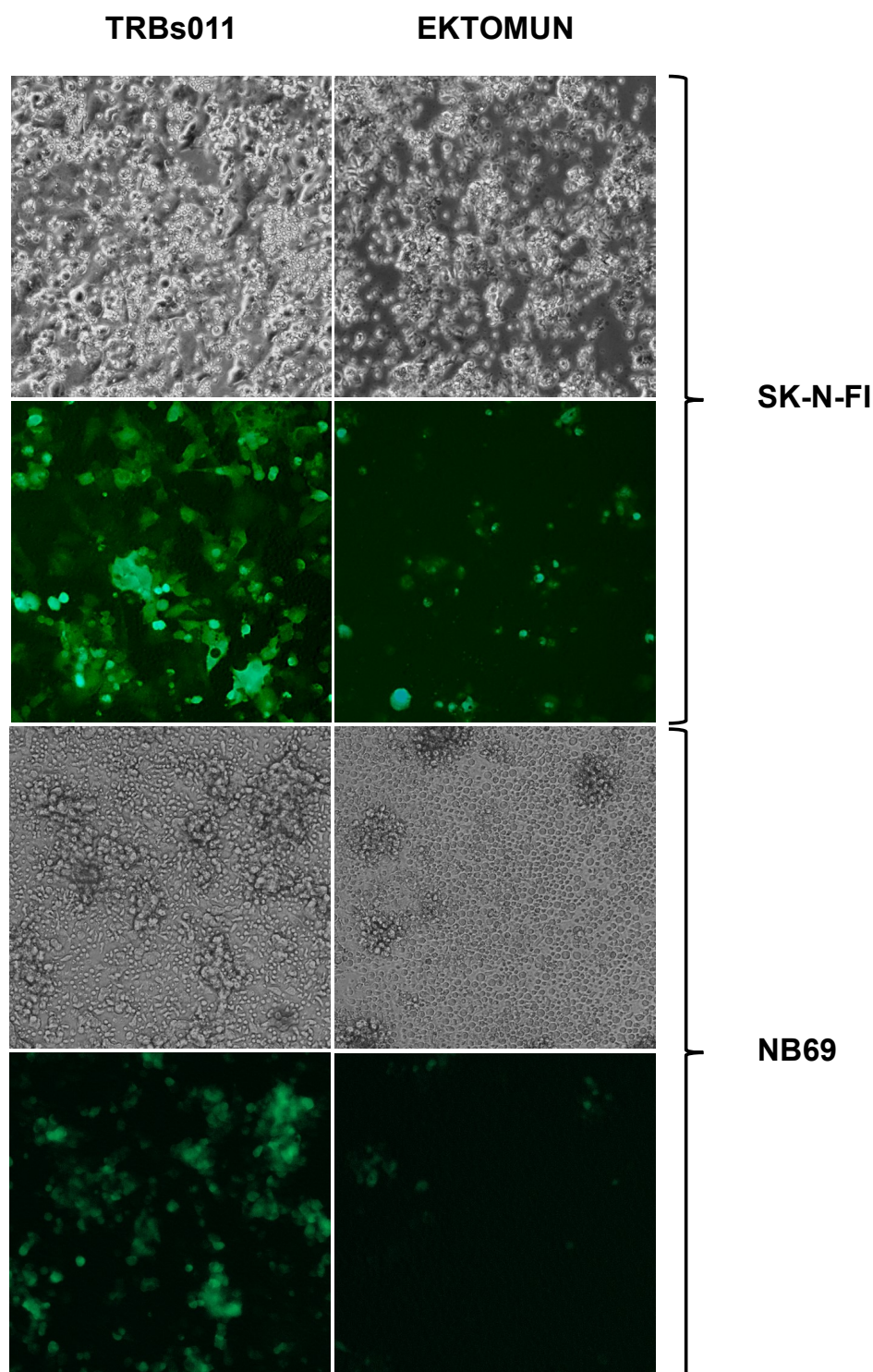
**Supplementary Figure S1. Flow cytometric composition of peripheral blood mononuclear cells (PBMCs).** T cells from a healthy donor were separated from PBMCs using MACS technology. Cells left over after separation were considered to be accessory immune cells (AIC). Cells were stained with an anti-CD3 antibody and analysed by flow cytometry. CD: cluster of differentiation.

## Supplementary Figure S2



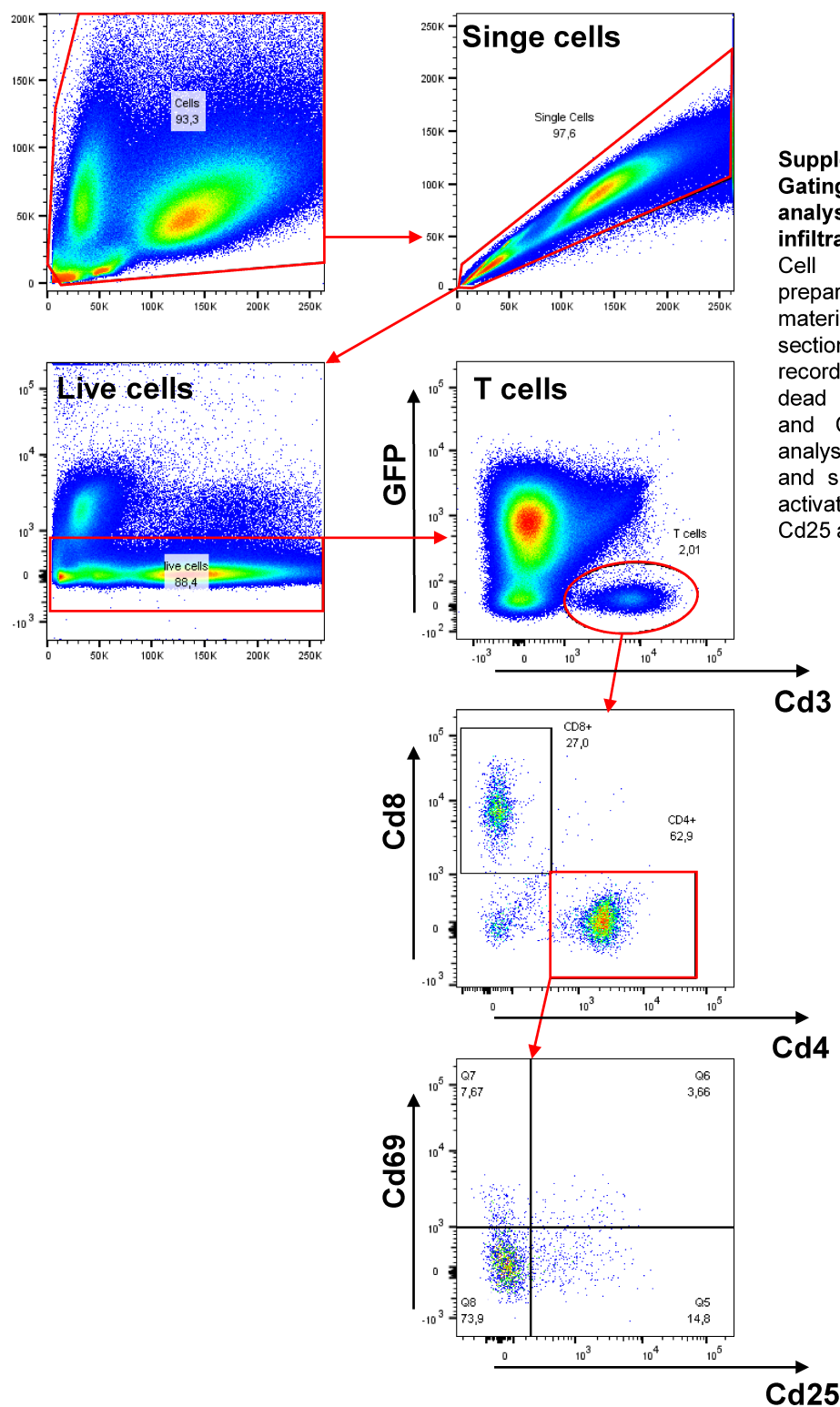
**Supplementary Figure S2. Different PBMC donors show comparable cytotoxic efficacy in co-culture with a neuroblastoma cell line.** The neuroblastoma cell line NB69 was stably transfected with a GFP\_ffluc construct, co-cultivated with PBMCs from 3 healthy donors (effector:target = 10:1) and treated with the trAb EKTOMUN (GD2 x human CD3) in different concentrations. Tumor cell lysis was determined by bioluminescent flux relative to an untreated co-culture after 72 hours (h).

# Supplementary Figure S3

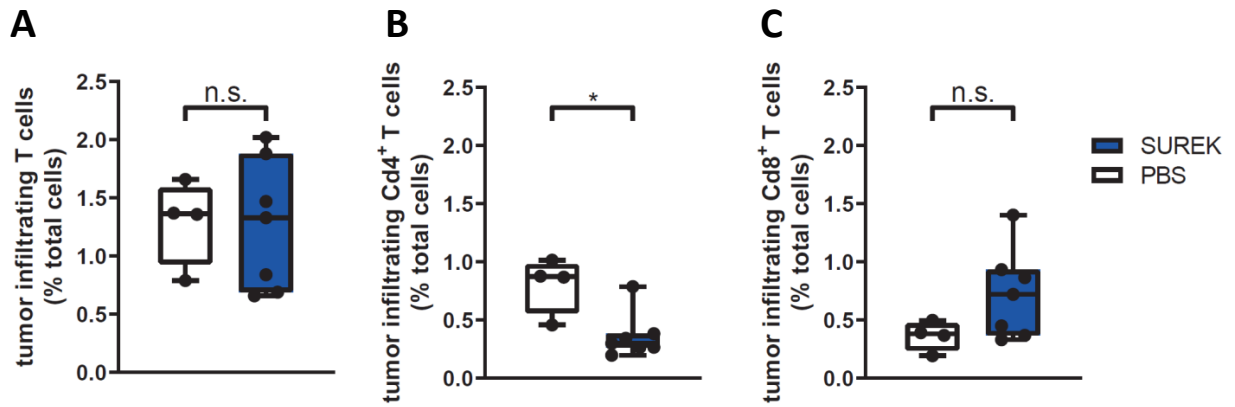


**Supplementary Figure S3. EKTOMUN treatment leads to diminished green fluorescing SK-N-FI and NB69 cells in the presence of PBMCs.** The SK-N-FI and NB69 neuroblastoma cell lines were stably transfected with a GFP\_ffluc construct, co-cultivated with PBMCs (effector:target = 10:1), and treated with 0.1 ng/ml of TRBs011 or EKTOMUN. Micrographs were taken 72 hours after initiation of the co-culture.

## Supplementary Figure S4



# Supplementary Figure S5



**Supplementary Figure S5. The proportion of tumor infiltrating lymphocytes 4 days after treatment with SUREK or PBS in experimental neuroblastoma metastases.** A/J mice were treated as described in figure 5A. Quantification of (A) tumor infiltrating Cd3<sup>+</sup> T cells (B) Cd4<sup>+</sup> T cells and (C) Cd8<sup>+</sup> T cells as percent of total live cells by flow cytometry. \*: p<0.05; n.s.: not significant using Mann-Whitney test.