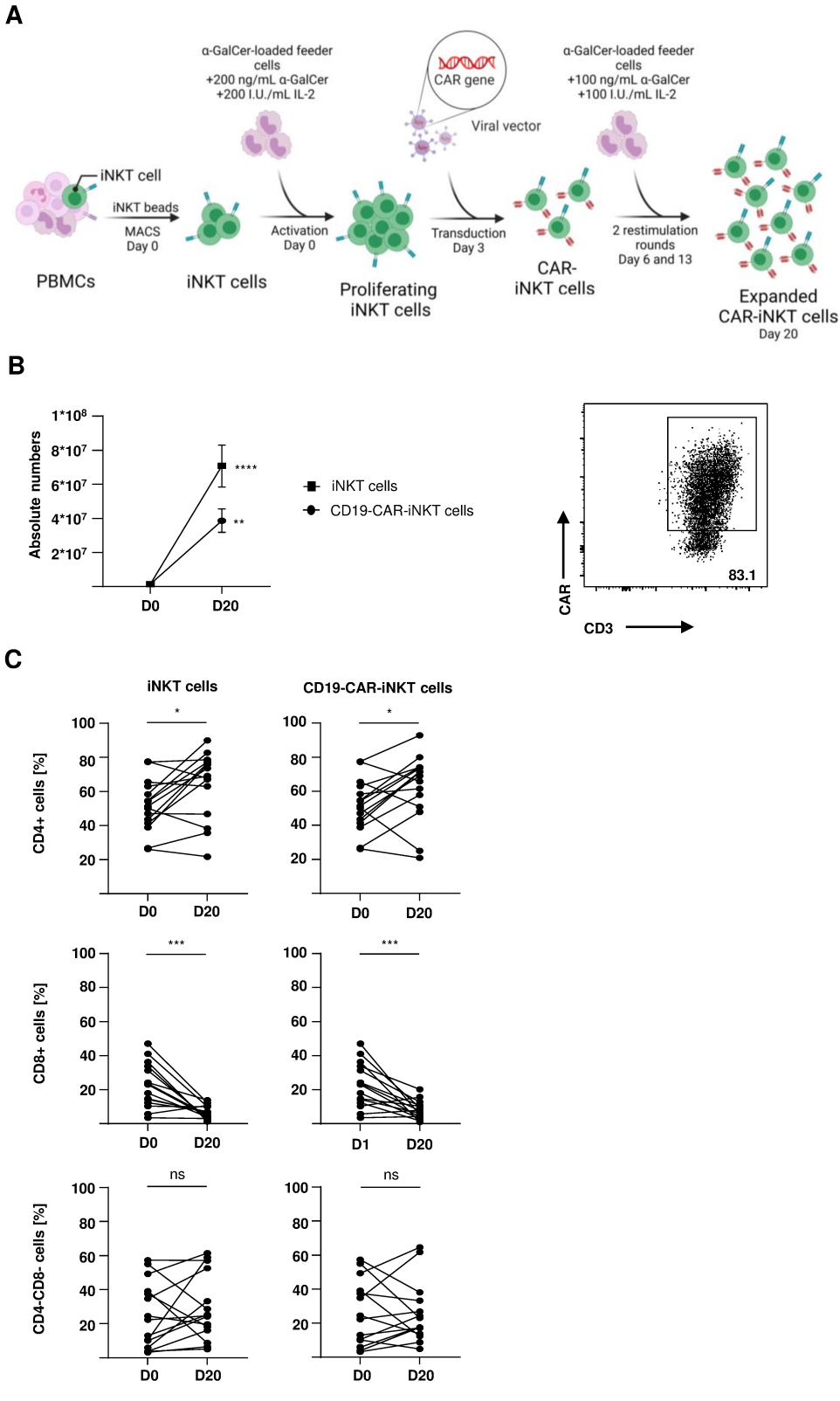
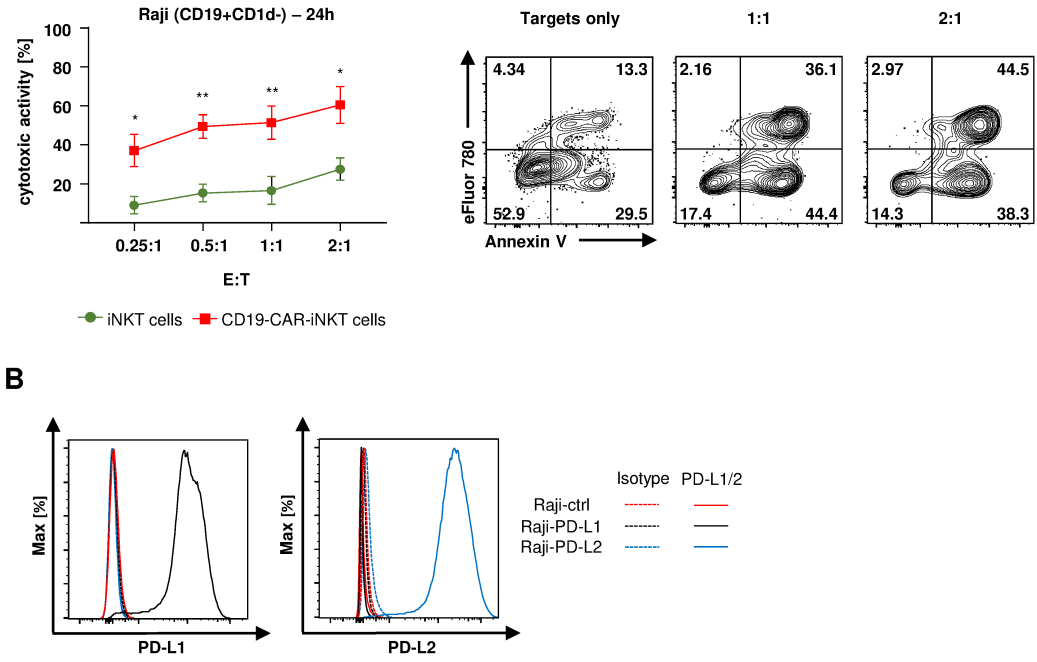
Supplementary data 1



Supplementary data 1. CD19-directed CAR-iNKT cells induce cell death of CD19+ cell lines and primary patient blasts. (A) Scheme of the generation of CD19-CAR-iNKT cells. The figure was generated with Biorender® **(B)** Absolute numbers of expanded untransduced iNKT cells and CD19-CAR-iNKT cells on day 20 (left, n=10) and representative data showing the transduction efficiency for one donor (right). Expansion was determined by flow cytometry and cell counting. **(C)** Percentage of CD4, CD8 and double negative (CD4-CD8-) untransduced iNKT cells and CD19-CAR-iNKT cells on day 0 and day 20. Only live (VD-) iNKT cells/CD19-CAR-iNKT cells were included in the analysis. Error bars show SEM. Two-way ANOVA for **(B)** and paired *t*-test for **(C)**. ns p>0.05, * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.

Supplementary data 2



Supplementary data 2. PD-1:PD-L1/PD-L2 interaction impairs the functionality of CD19-CAR-iNKT cells. (A) Cytotoxic activity of untransduced iNKT cells and CD19-CAR-iNKT cells against Raji cells for 24h (left, n=3) and representative plots for targets only and target cells challenged with CD19-CAR-iNKT cells at 1:1 and 2:1 ratios (E:T) (right). Annexin V/VD gates were set on singlets and CTV+ cells. The experiments were performed at least three times independently with at least three different iNKT-cell donors in two technical replicates. **(B)** PD-L1 (left) and PD-L2 (right) expression by modified Raji cells. Stainings with isotype controls are shown as dashed lines, while specific staining is shown with full lines. Gates were set on singlets and live CD19+ cells. Error bars show SEM. Two-way ANOVA. * p≤0.05, ** p≤0.01.

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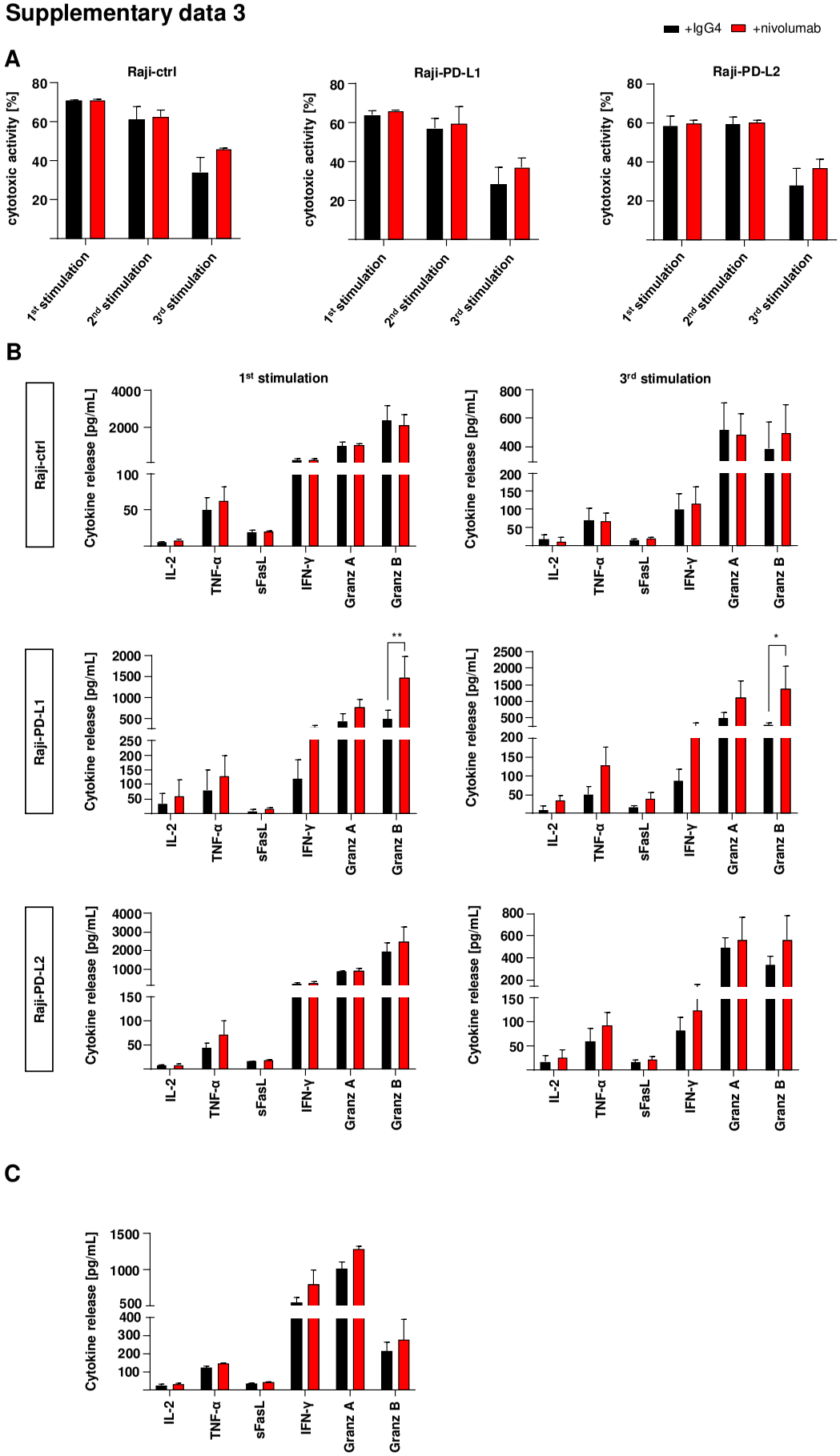
Supplementary data 3



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Supplementary data 3. Checkpoint inhibition increases cytokine release by CD19-CAR-iNKT cells. (A) Cytotoxicity of CD19-CAR-iNKT cells for one donor in the course of three stimulations with Raji-Ctrl (left), Raji-PD-L1 (middle) and Raji-PD-L2 (right). Target cells were discriminated from CD19-CAR-iNKT cells through the FSC-A vs. SSC-A gate and CTV+ staining. Only singlets were analyzed. Error bars show SD. **(B)** IL-2, TNF-α, sFasL, IFN-γ, granzyme A (Granz A) and granzyme B (Granz B) release of CD19-CAR-iNKT cells during 1st and 3rd stimulations with either Raji-ctrl, Raji-PD-L1 or Raji-PD-L2 in presence of IgG4 or nivolumab (n=3). Error bars show SEM. Two-way ANOVA. * p≤0.05, ** p≤0.01. Non-significant statistical analysis (p>0.05) is not depicted. **(C)** Representative cytokine release (IL-2, TNF-α, sFasL, IFN-γ, granz A and granz B from CD19-CAR-iNKT cells upon challenge with patient blasts in the presence of IgG4 or nivolumab after 24h of co-incubation (n=1). Error bars show SD. The experiments were performed at least three times independently with at least three different iNKT-cell donors in three technical replicates.