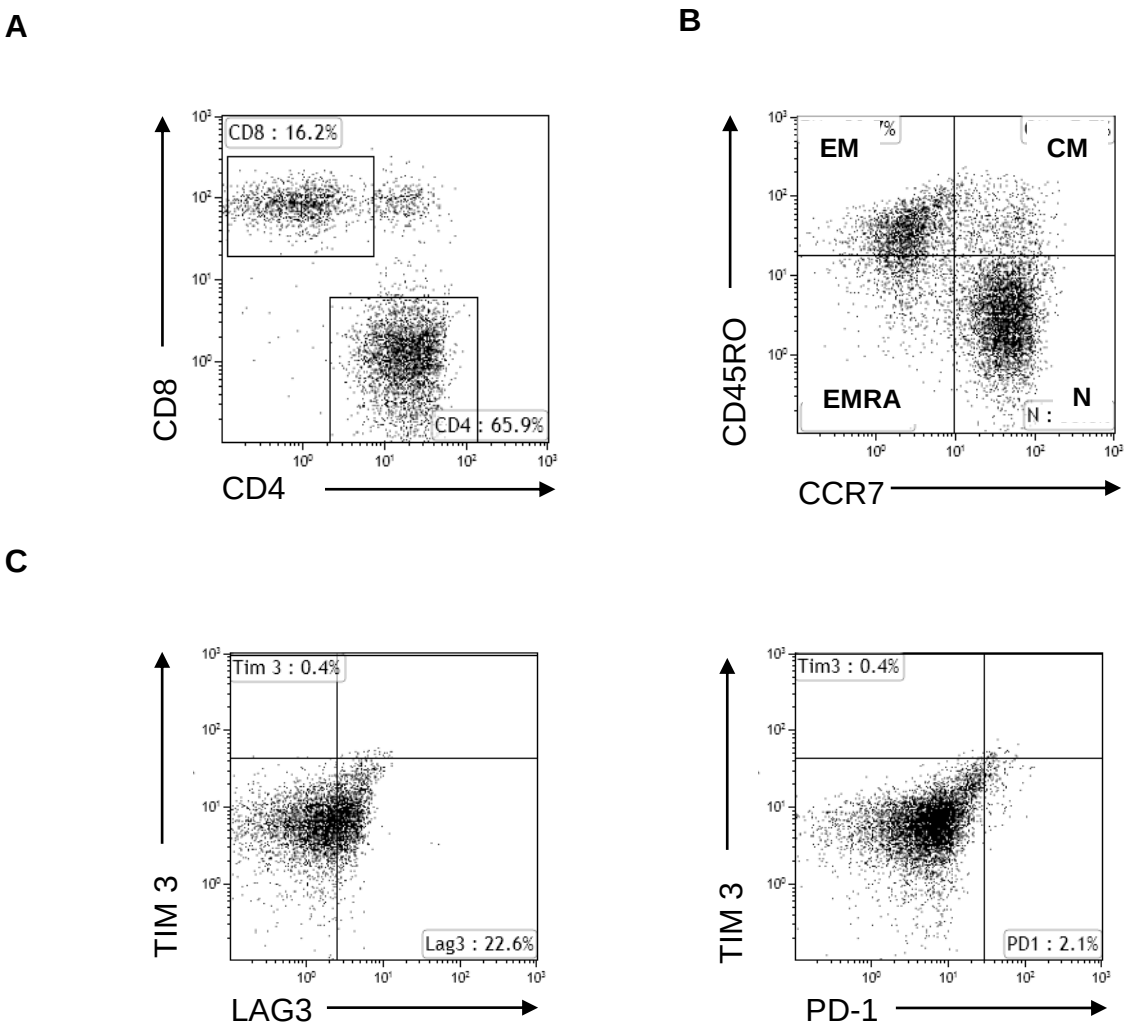
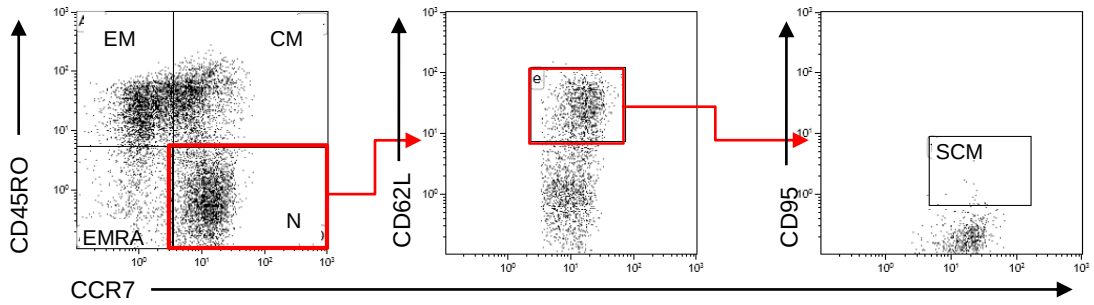


Supplementary Figure 1: T-cell immunophenotyping by flow cytometry. (**A**) Determination of CD8+ and CD4+ distribution using CD4 PE and CD8 APC antibodies. (**B**) CCR7 and CD45 RO gating strategy using CCR7 FITC and CD45 RO PercP Cy5.5. (**C**) Determination of expression of exhaustion markers (TIM3 PercP Cy5.5, Lag3 Pacific Blue and PD1 PE Cy7).

A



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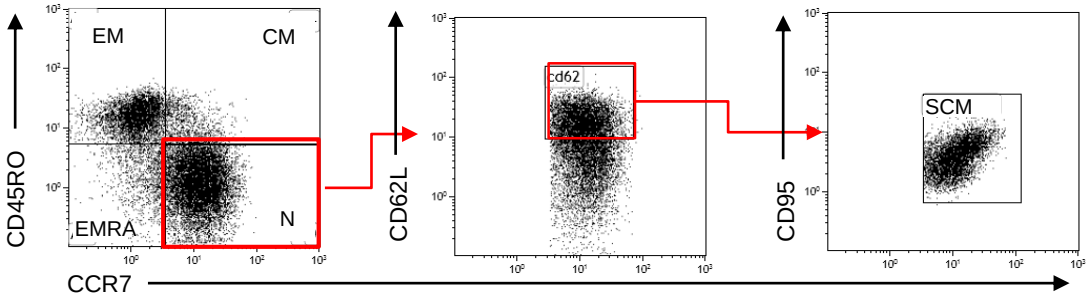

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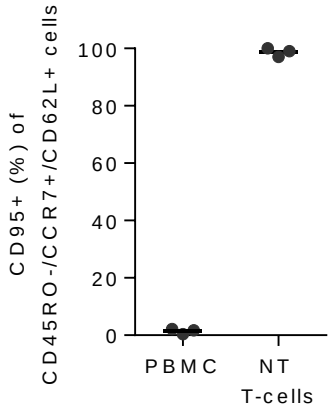
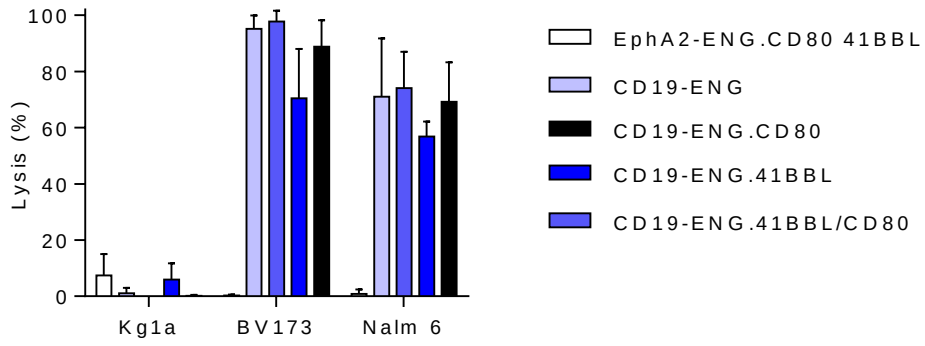
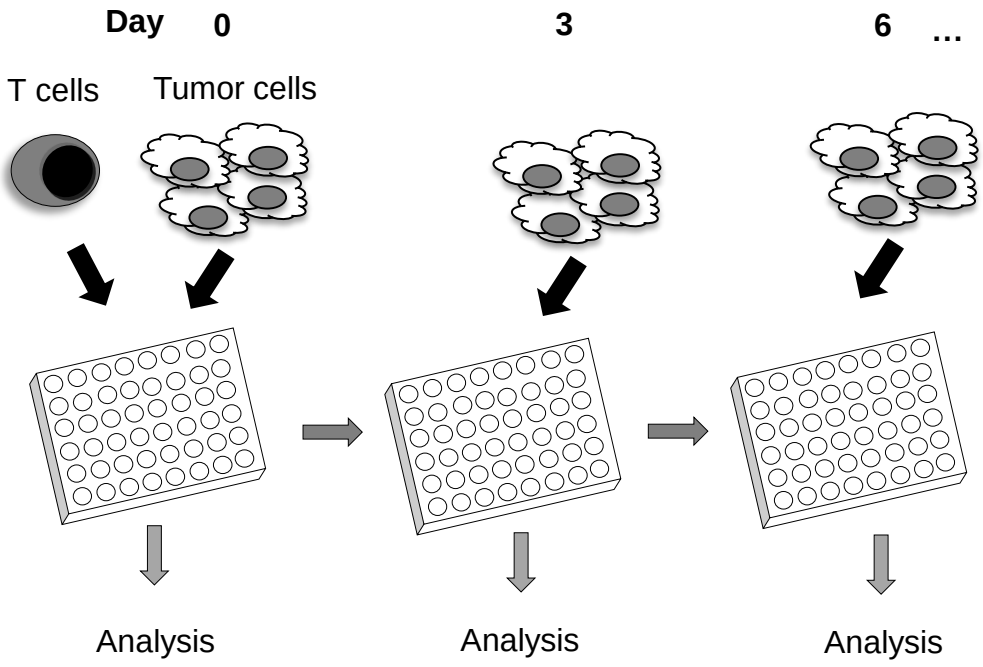


Supplementary Figure 2: Determination of TSCM presence in PBMC and NT T-cells by flow cytometry. Determination of TSCM cells (CD45RO-/CCR7+/CD95+) using CCR7 FITC, CD45 RO PercP Cy5.5, CD62L APC and CD95 Pac Blue. (**A**) CD3+ T-cells were selected from PBMCs using Miltenyi beads prior to staining. Representative example. (**B**) Nontransduced T-cells (NT) were activated with CD3/CD28 MAbs and expanded with IL7/IL15 prior to staining. Representative example. (**C**) Summary data.



Supplementary Figure 3: CD19-ENG T-cells and CD19-ENG T-cells expressing CD80 and/or 41BBL kill Nalm 6 cells efficiently. Cytotoxicity assays were performed using CD19-ENG, CD19-ENG.CD80, CD19-ENG.41BBL, CD19-ENG.41BBL/CD80 and EphA2-ENG,41BBL/CD80 T cells as effectors and CD19-positive (Nalm 6, BV173) and negative (KG1a) tumor cells as targets at a E:T ratio of 20:1 (n=3; done in triplicate; BV173 vs Nalm 6 for each effector T-cell population: ns).

A



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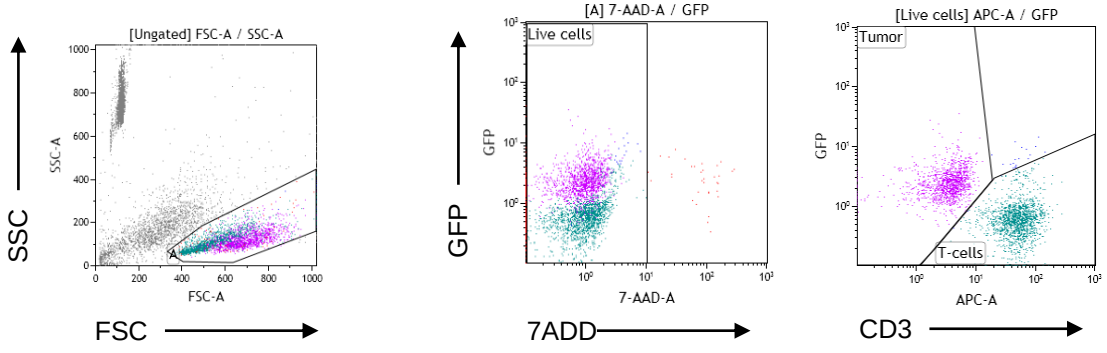
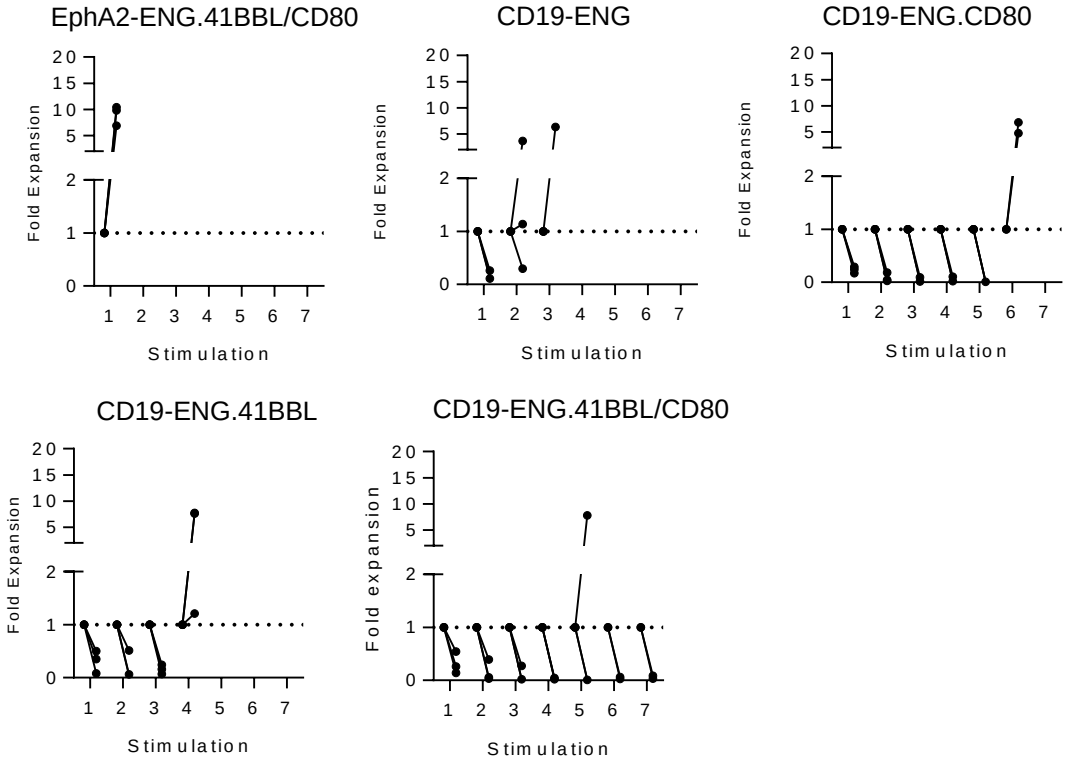


Supplementary Figure 4: Experimental set up of Serial Killing Assay. (**A**) T cells and GFP+ tumor cells (BV173 or Nalm6 cells) are plated at either 1:4 or 1:8 E:T ratio. An aliquot of cells is then analyzed using flow cytometry. Remaining cells are kept in culture and analyzed every 3-4 days. Fresh tumor cells are added at each time point. (**B**) Flow cytometry gating strategy.

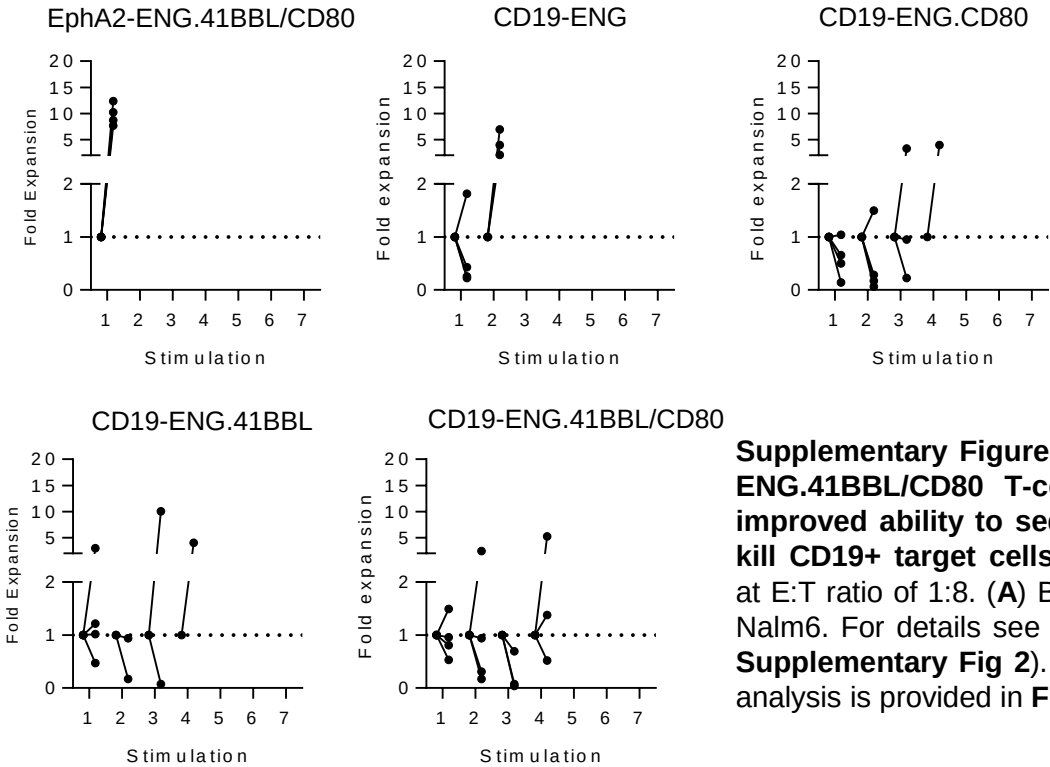
A

BV173; E:T ratio 1:8



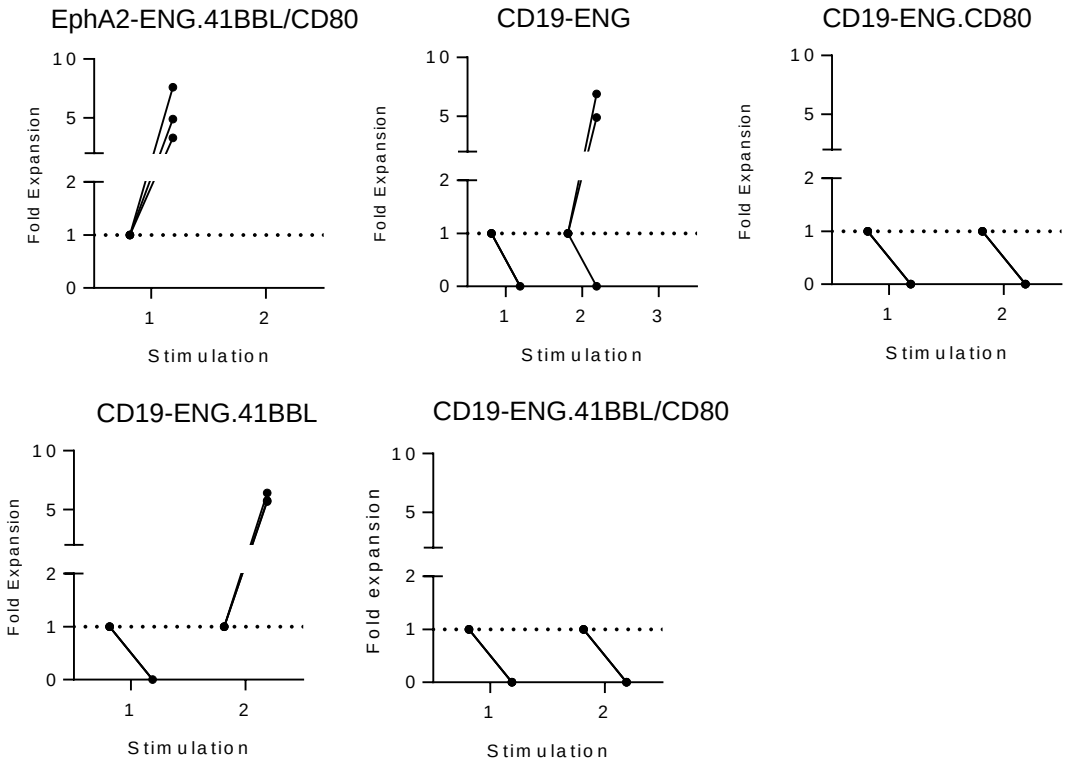
B

Nalm6; E:T ratio 1:8

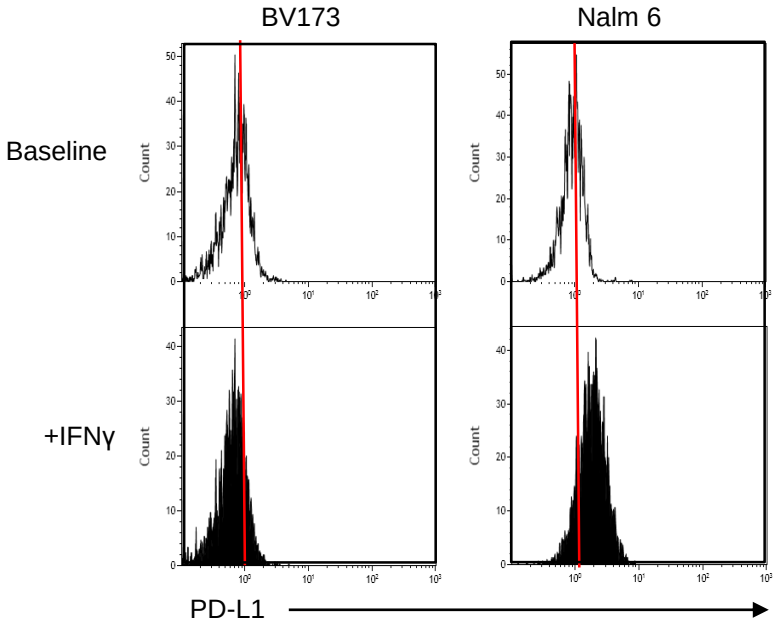


Supplementary Figure 5: CD19-ENG.41BBL/CD80 T-cells have improved ability to sequentially kill CD19+ target cells. Analysis at E:T ratio of 1:8. (**A**) BV173. (**B**) Nalm6. For details see **Fig 4** and **Supplementary Fig 2**). Statistical analysis is provided in **Fig 5B**.

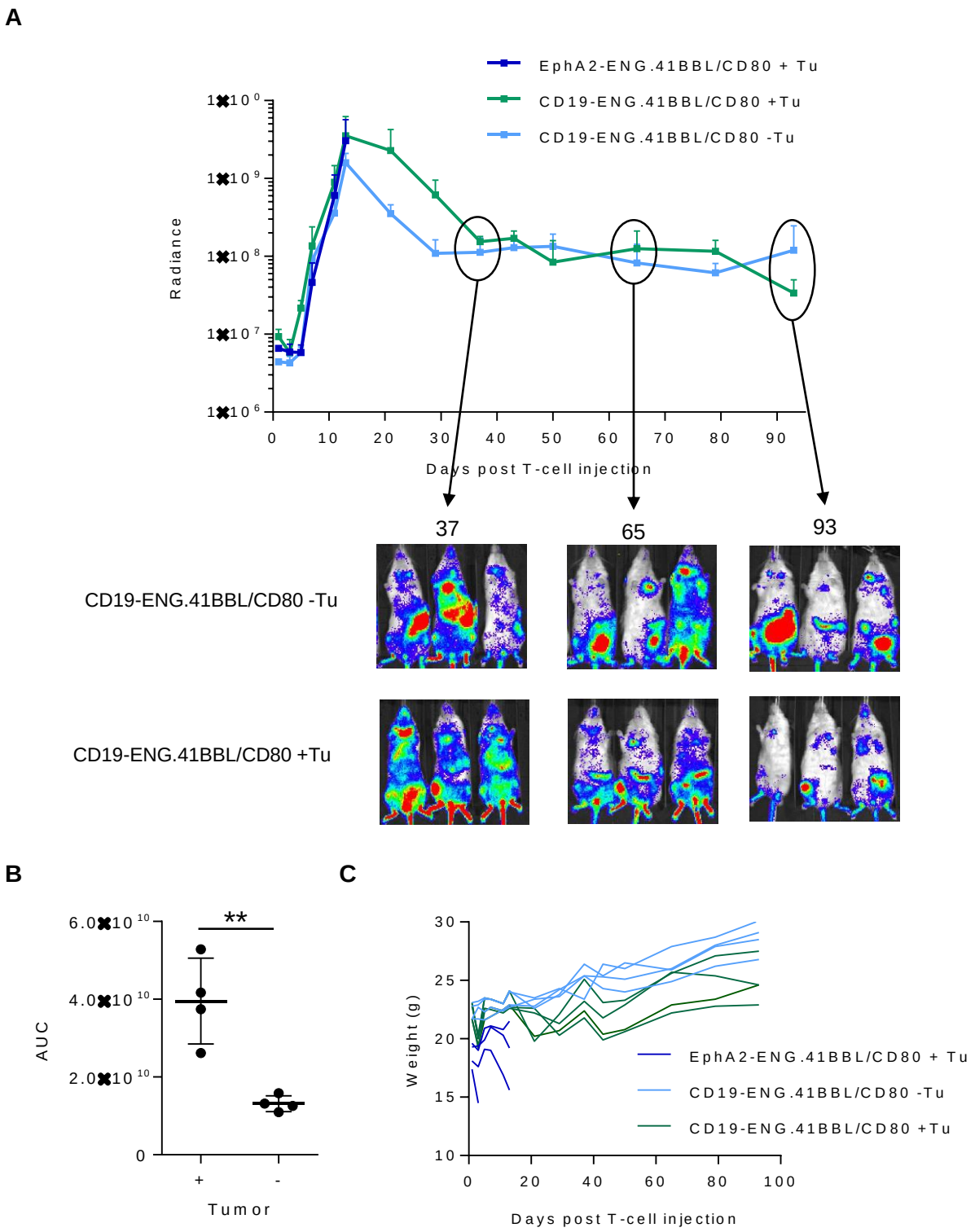
Supplementary Figure 5



Supplementary Figure 6: CD19-ENG T-cells expressing CD80 or 41BBL and CD80 have improved ability to sequentially kill CD19+ target cells. T cells were cocultured wiith tumor cells at E:T ratio of 1:1 with BV173 cells. After 5 days, cells were harvested and the presence of tumor cells enumerated by FACS analysis. If greater than 90% of tumor cells were killed, T cells were cocultured again with BV173 cells and after 5 days number of tumor cells were enumerated (n=3).



Supplementary Figure 7: Nalm 6 express PD-L1 after exposure to IFNγ. Nalm 6 and BV173 cell lines were stained for PD-L1 (BD Biosciences) at baseline (open curve) and 48 hours after exposure to IFNγ (7500 pg/mL; filled curve).



Supplementary Figure 8: CD19 ENG.41BBL/CD80 T-cells expand *in vivo* and persist long-term at low levels. This is the long-term follow up of the experiment shown in Figure 7. Please see figure legend for detail. (**A**) Bioluminescence imaging (mean and SD) of whole mouse. (**B**) Area under curve (AUC) analysis until day 37 post T-cell injection using a base line radiance of $1x10^7$ for CD19 ENG.41BBL/CD80 T-cells +/- tumor (+ vs - tumor: **p<0.01). (**C**) Weight measurements (each line represents an individual mouse).

Supplementary Figure 8