

Supplementary information

**Potent Apoptosis Induction by a Novel Trispecific B7-H3xCD16xTIGIT 2+1 Common Light Chain
Natural Killer Cell Engager**

Michael Ulitzka¹, Julia Harwardt¹, Britta Lipinski¹, Hue Tran¹, Björn Hock¹, Harald Kolmar^{1,2} *

1 Institute for Organic Chemistry and Biochemistry, Technical University of
Darmstadt, Peter-Grünberg-Str. 4, 64287 Darmstadt, Germany

2 Centre of Synthetic Biology, Technical University of Darmstadt

* Correspondence: harald.kolmar@tu-darmstadt.de

1. Screening of anti-B7-H3 Fab

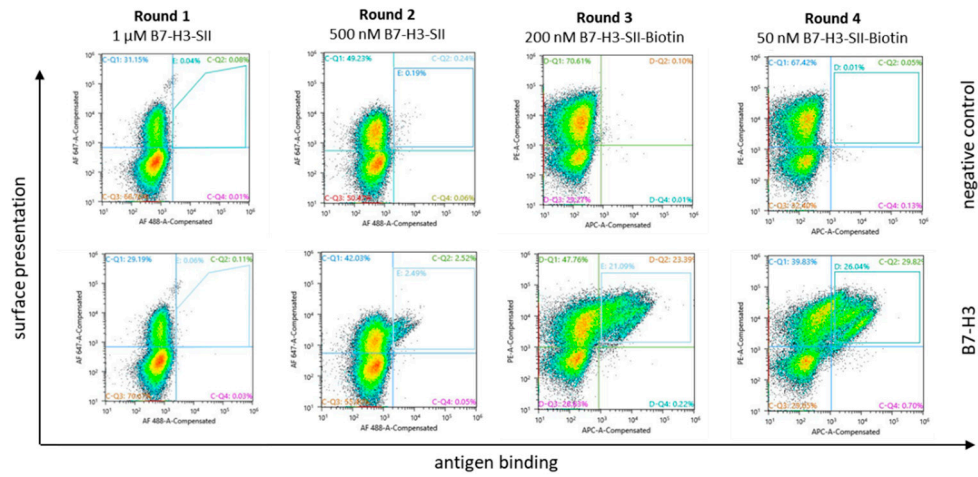


Figure S1. FACS plots of the anti-B7-H3 screening campaign.

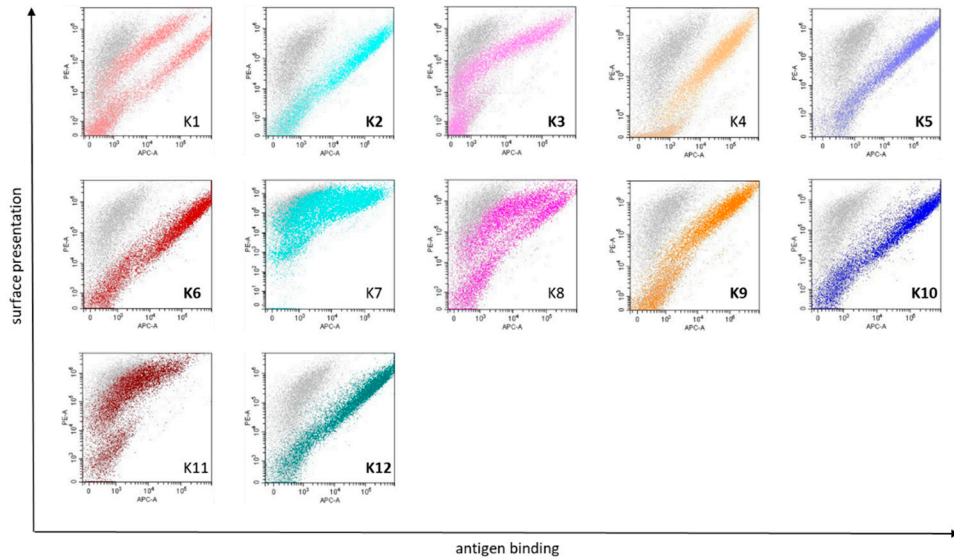


Figure S2. Single clone analysis of anti-B7-H3 Fabs.

2. Screening of anti-TIGIT Fab

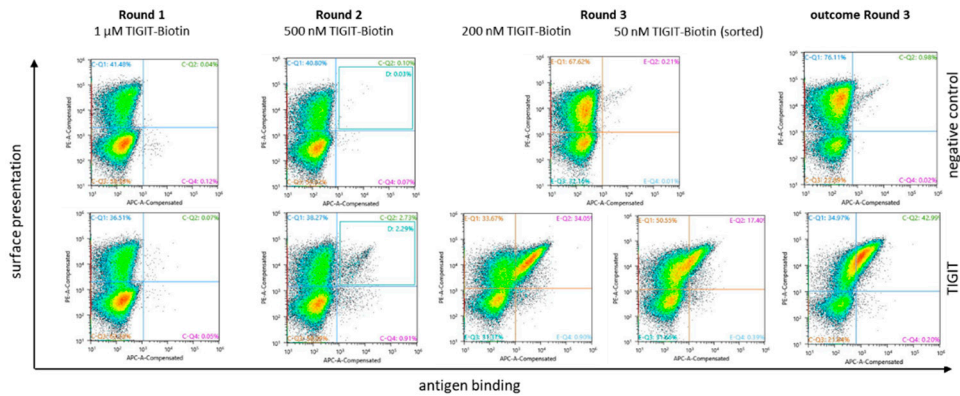


Figure S3. FACS plots of the anti-TIGIT screening campaign.

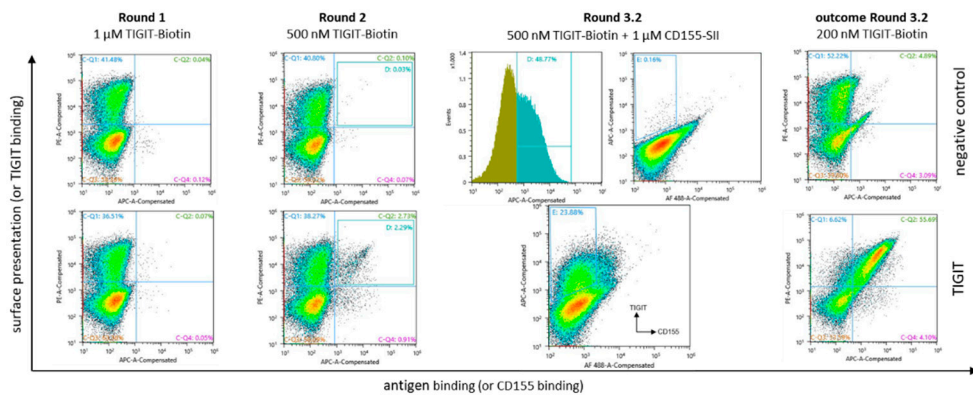


Figure S4. FACS plots of the anti-TIGIT screening campaign focusing on blocking antibodies.

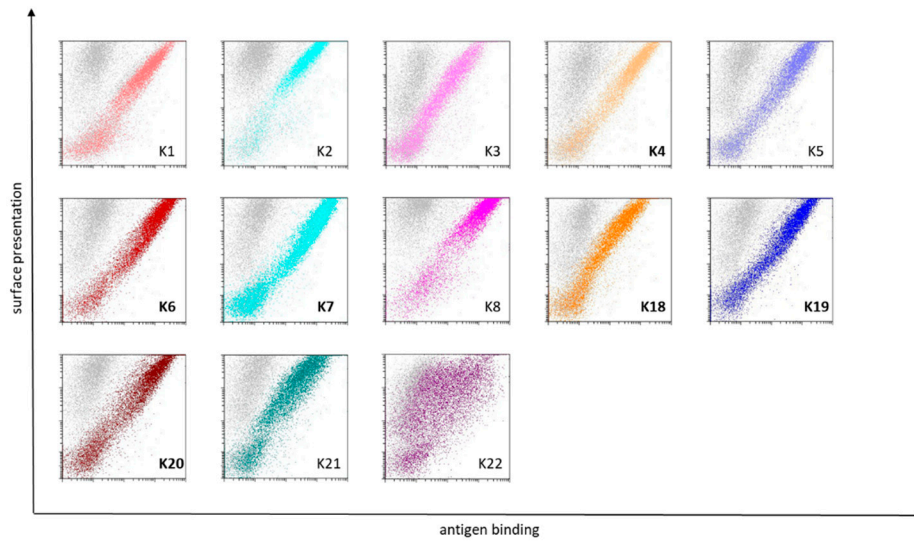


Figure S5. Single clone analysis of anti-TIGIT Fabs derived from both screening campaigns.

3. Killing assay

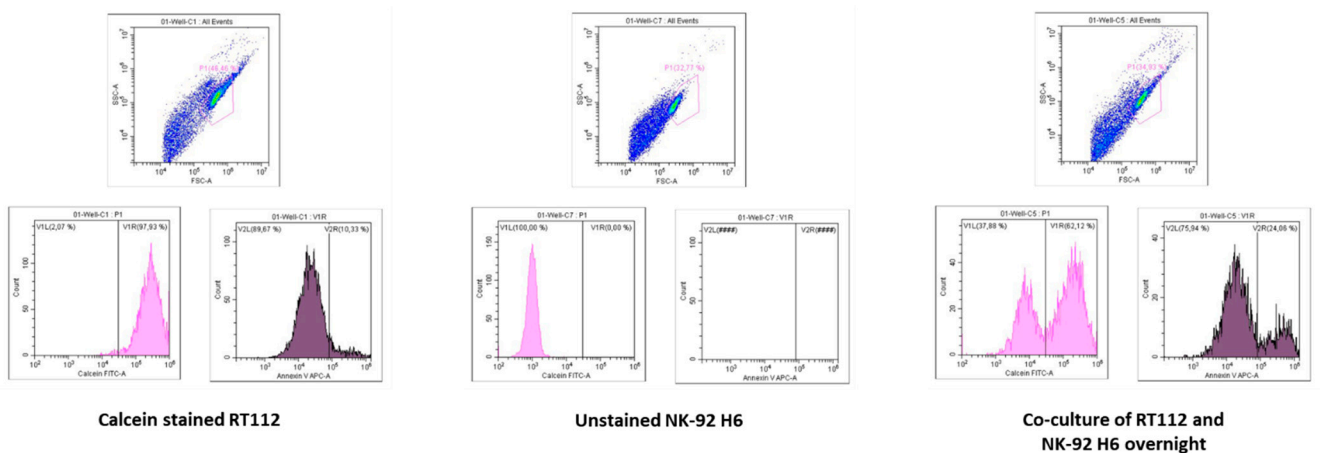


Figure S6. Gating strategy used for the analysis of apoptosis induction in the NK-92 H6-based assay.

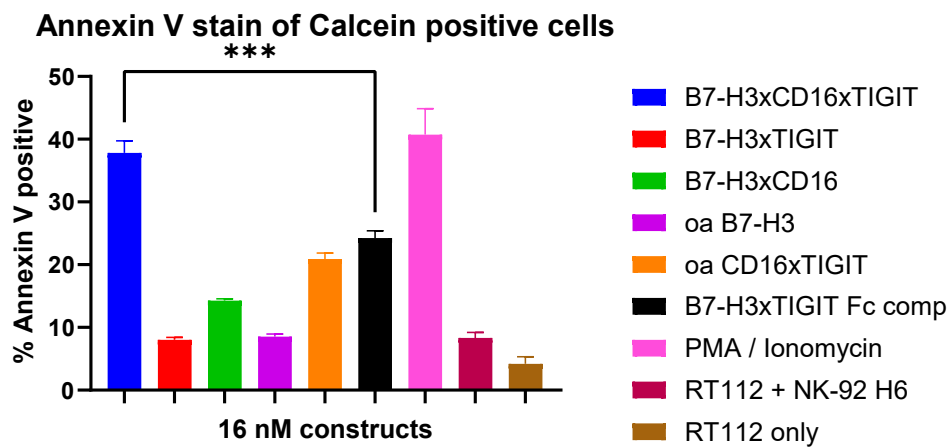
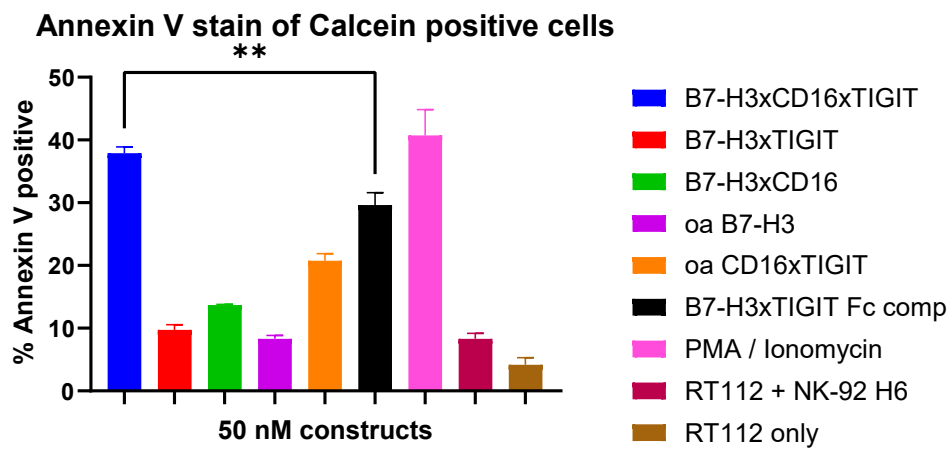
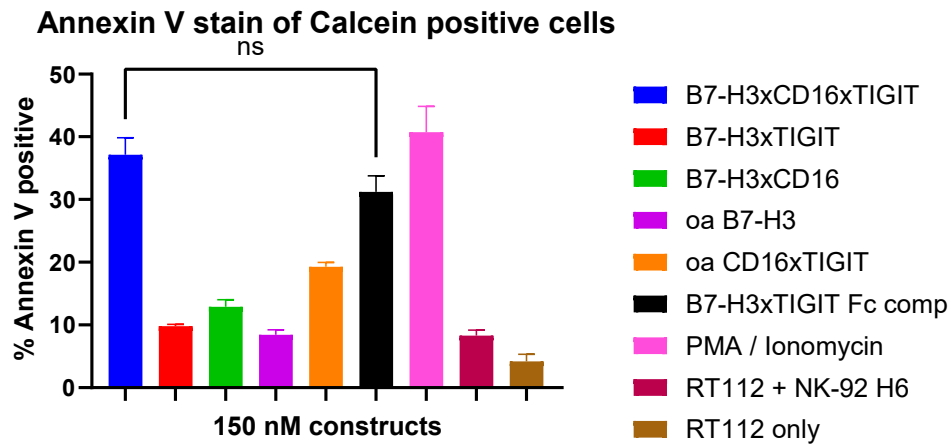


Figure S7. Apoptosis induction in RT112 cells by different antibody constructs in the NK-92 H6-based assay.