FIGURE S1 Small molecule library screening set up a) IFN-γ concentration following IL15 titration on primary NK and A549 β2m$^{-/-}$ co-culture (n=4). b) IFN-γ secretion of primary human NK cell (n=3) to suboptimal and optimal IL15 concentrations. IFN-γ was measured with a screening optimized ELISA approach after overnight co-culture of NK cells and A549 β2m$^{-/-}$ cell

line. c) Flow cytometry histogram and statistics of HLA-ABC expression in CRISPR generated A549 $\beta2m^{-/-}$ d) Z prime value across screening plates. e) Granzyme B, IFN-γ and TNF-α concentrations following dose response treatment of I8 and K5 Cbl-b inhibitors compounds on primary NK cells in co-culture with A549 $\beta2m^{-/-}$ (n=3). f) IFN-γ concentration after Cbl-b inhibitor titration on A549β2m-/- or co-culture with primary human NK cells (n=3). g) Toxicity of Cbl-b inhibitor following titration (n=3).
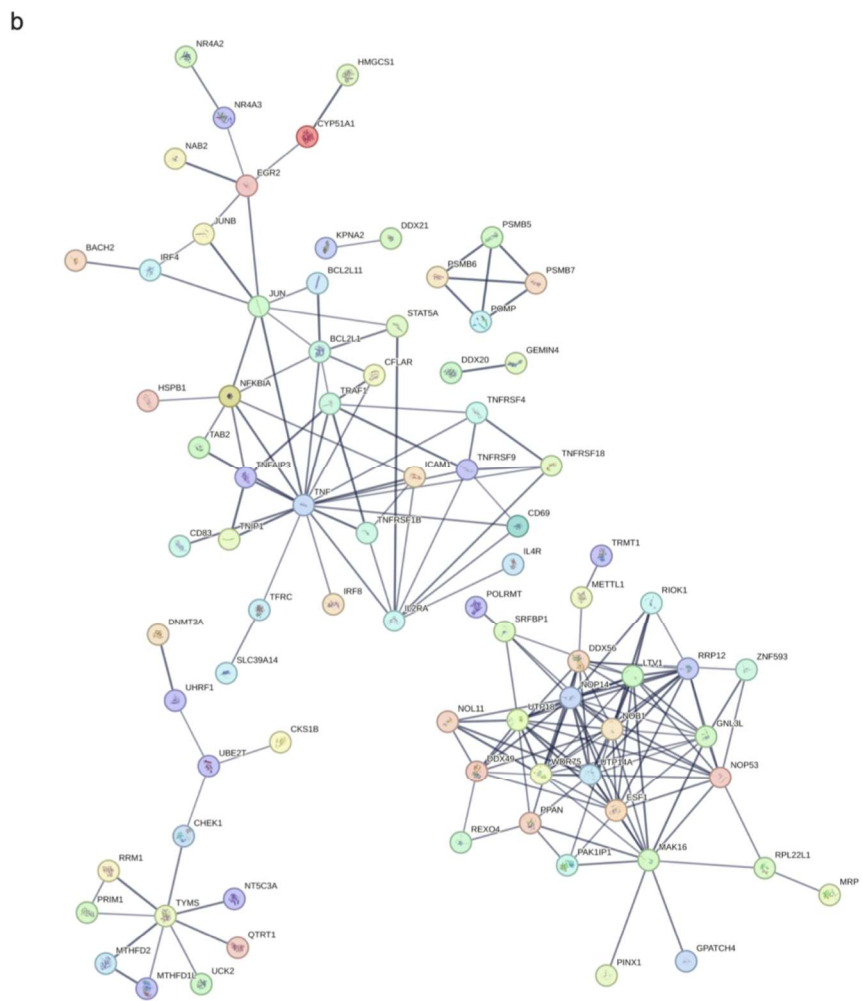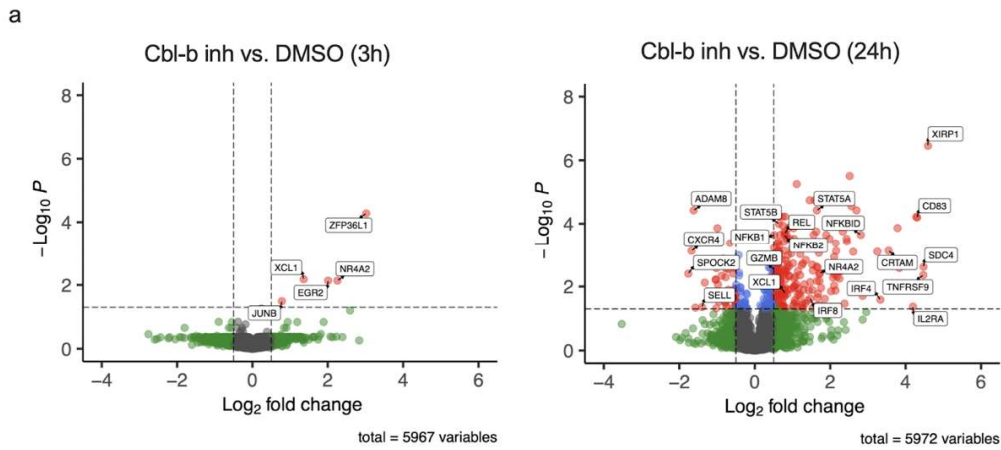
FIGURE S2 Proteomics short (3h) vs. long (24h) Cbl-b inhibitor treatment. a) Volcano plots displaying differentially expressed proteins following Cbl-b inhibitor treatment, comparing 3 h and 24 h treatments (n=6; grey=NS, green=Log2 FC, blue= adjusted P value, red= Log2 FC and adjusted P value. For statistical analyses see "Material and methods"). b) Multiple protein interaction analysis of top upregulated proteins (log2FC >1, p value <0.05).
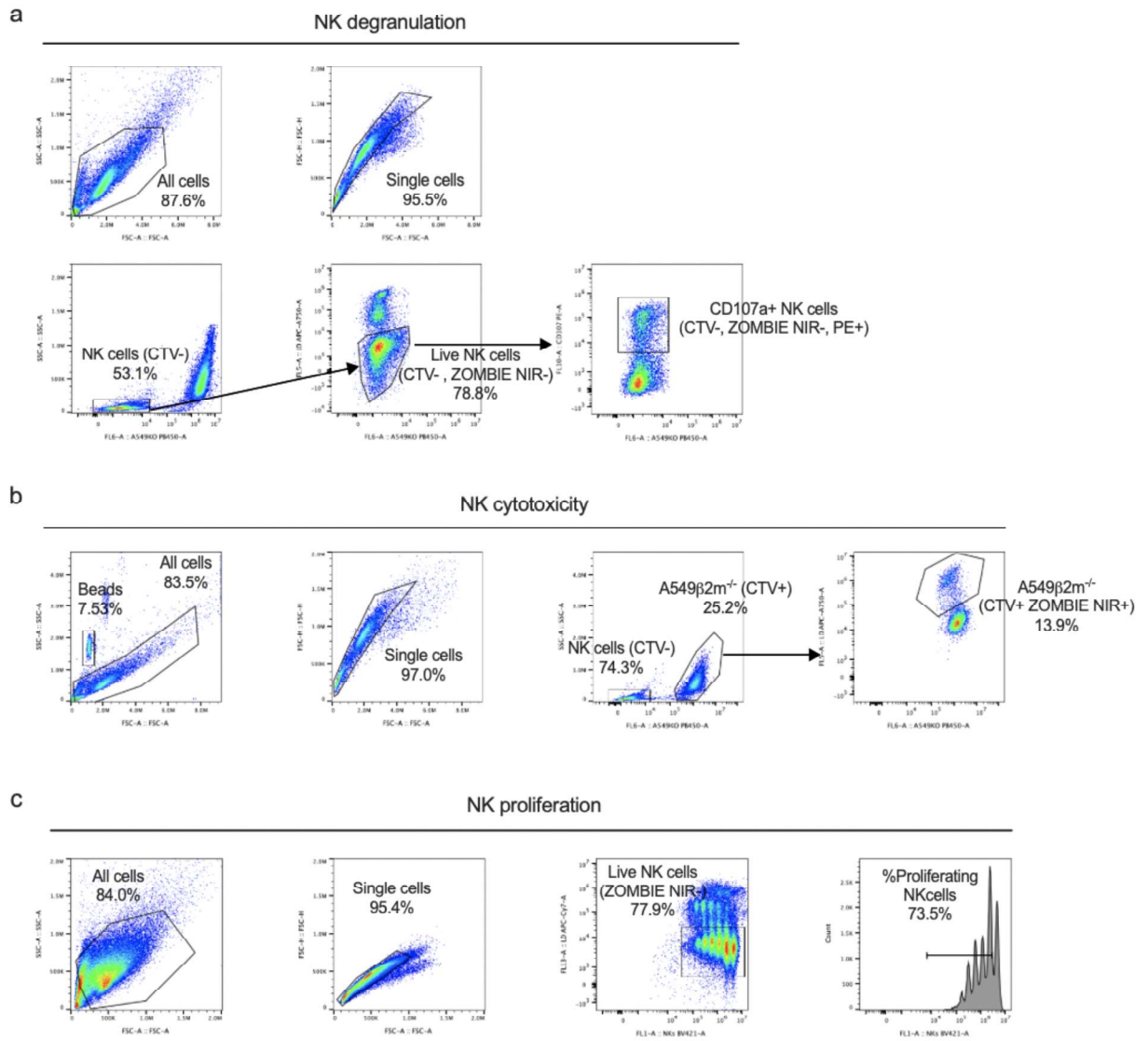
FIGURE S3 Gating strategy of NK functional assays. a) Example of gating strategy to calculate NK cell degranulation (CD107a). b) Example of gating strategy to calculate NK cell mediated killing. c) Example of gating strategy to assess NK proliferation.
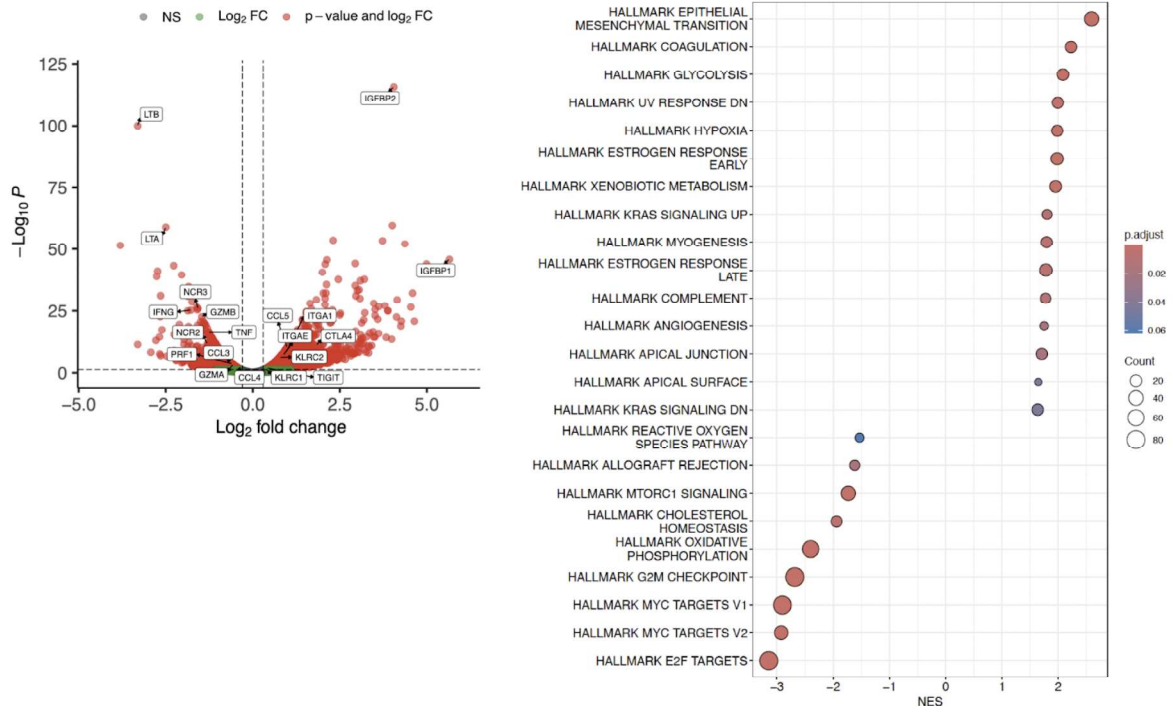
a

FIGURE S4 Bulk RNAseq of *in vitro* generated dysfunctional NK cells. a)Volcano plot of differentially expressed genes in NKD9 compared to NKD0 and hallmarks enrichment analysis of *in vitro* generated dysfunctional NK cells (n=6).
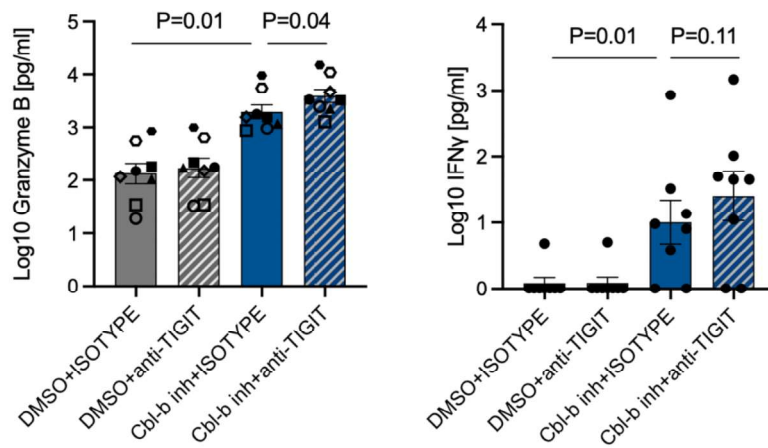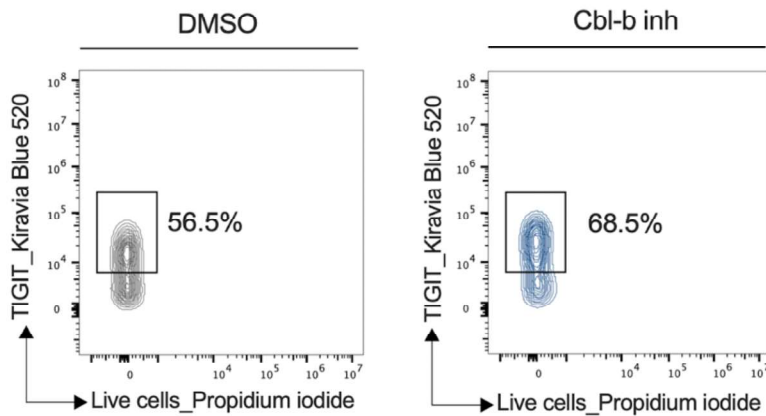
FIGURE S5 Cytokines production of *in vitro* generated dysfunctional NK cells after treatment. a) IFN-γ and granzyme B supernatant concentrations of Cbl-b inhibitor and anti-TIGIT treated *in vitro* generated dysfunctional NK cells (n=8, for P values see "Material and methods").
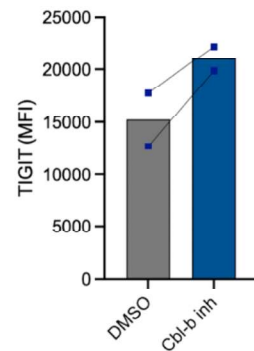

FIGURE S6 TIGIT upregulation on tumor infiltrating NK cells after Cbl b inhibitor treatment. a) Representative flow cytometry plot of TIGIT expression (%) on tumor infiltrating NK cells after Cbl-b inhibitor treatment. b) TIGIT expression on tumor infiltrating NK cells after Cbl-b inhibitor treatment of lung cancer patients (n=2, lung adenocarcinoma pleural effusions).