

**Figure S1. CD56+CD27+ cells from LTBI+ donors proliferate after stimulation with Mtb.** **A.** Gating strategy used to analyze memory like NK cells. **B.** proliferation, **C.** frequencies of IFN- $\gamma$ + cells and **D.** frequencies of CD107a+ cells in CD3-CD45+CD56+CD27+ cells from PBMC of LTBI+ and LTBI- donors stimulated with  $\gamma$ -irradiated Mtb (n=6). **E-H.** Proliferative profile of non-memory CD56+CD27- cells or memory like CD56+CD27+ cells from LTBI- (**E,F**) or LTBI+ (**G,H**) after stimulation with  $\gamma$ -irradiated Mtb for 5 days. **I-K.** PBMC from LTBI+ people were stimulated with  $\gamma$ -Mtb and treated with isotype or blocking CD226 antibody. On day 5, frequencies and MFI for CD69, CD16 and HLA-DR in CD56+CD27+ NK cells were determined. n=4. of Mean  $\pm$  s.d. two-sided Student's t-test. \*p< 0.05, \*\*p< 0.01, \*\*\*p< 0.001, \*\*\*\*p< 0.0001.

**Figure S2. CD226 gene deletion strategy and verification.** **A.** Purified NK cells (CD3-CD45+CD56+ cells) were deleted or not for CD226 gene using CRISPR/Cas9 technology (Synthego) and maintained for 5 days in recombinant human IL-15. **B,C** Validation of gene deletion was performed using flow cytometry (**B**) and western blotting (**C**).

**Figure S3. Blocking CD226 antibody reduces expression of several cytokines in supernatant of PBMC stimulated with Mtb.** PBMC from LTBI+ donors were stimulated with  $\gamma$ -irradiated Mtb for 5 days and treated with isotype antibody or CD226 blocking antibody every other day. The supernatant was analyzed for **A.** IFN- $\gamma$ , **B.** TNF $\alpha$ , **C.** MIP-1 $\beta$ , **D.** MCP-1 and, **E.** IL-10 by ELISA to determine differential cytokine profile (n=5). Mean  $\pm$  s.d. ANOVA. \*p< 0.05, \*\*p< 0.01, \*\*\*p< 0.001, \*\*\*\*p< 0.0001.

**Figure S4. Gene expression of Wnt/TCF1 pathway and ER stress genes.** Enriched NK cells after 24 hours of stimulation with  $\gamma$ -irradiated Mtb and treatments with isotype or CD226 blocking antibody were probed for quantification of genes using TaqMan probes. Expression of Wnt/TCF1 pathway genes (**A**) (*Tcf7*, *Dll1*, *Jag1*, *Axin2*) and ER stress genes (**B**) (*Chop*, *Atf4*, *BiP*, *IRE1 $\alpha$* , *Xbp1* and spliced *XBP1*) (n=5). Mean  $\pm$  s.d. ANOVA. \*p< 0.05, \*\*p< 0.01.

**Figure S5. cMyc inhibition with MYCi975 reduces expression of several cytokines in supernatant of PBMC stimulated with Mtb.** PBMC from LTBI+ donors were stimulated for 5 days and treated with 6 $\mu$ M and 12 $\mu$ M of cMyc inhibitor MYCi975 every other day or DMSO vehicle. The supernatant was analyzed for **A.** IFN- $\gamma$ , **B.** TNF $\alpha$ , **C.** MIP-1 $\beta$ , **D.** MCP-1 and, **E.** IL-10 by ELISA to determine differential cytokine profile (n=5). Mean  $\pm$  s.d. ANOVA. \*p< 0.05, \*\*\*p< 0.001, \*\*\*\*p< 0.0001.

**Figure S6. NK cells viability after cMyc inhibitor MYCi975 and 2DG treatments and effect of CD226 blockade on glycolysis.** **A.** PBMC from LTBI+ donors were stimulated for 5 days with  $\gamma$ -irradiated Mtb and treated with 6  $\mu$ M, 12  $\mu$ M and 18  $\mu$ M of cMyc inhibitor MYCi975 or mock every other day, and viability of CD3-CD45+CD56+CD27+ cells was determined by flow cytometry (n=5). **B.** PBMC from LTBI+ donors were stimulated for 5 days with  $\gamma$ -irradiated Mtb and treated with 0.5 mM, 1 mM, 10 mM and 20 mM of glycolysis inhibitor 2DG or mock every three days, and viability of CD3-CD45+CD56+CD27+ cells was determined by flow cytometry (n=5). **C-E** PBMC from LTBI+ donors were stimulated with  $\gamma$ -irradiated Mtb for 48 hours and treated with isotype or blocking CD226 antibody. After this, 5x10<sup>5</sup> enriched NK cells were plated to perform glycolysis assay using seahorse analyzer. **C.** Basal glycolysis, **D.** glycolytic capacity and **E.** glycolytic reserve were determined (n=4). Mean  $\pm$  s.d. ANOVA. \*p< 0.05, \*\*p<0.01.











