Figure S1: Supplementary functional, signaling, and transcriptional figures.

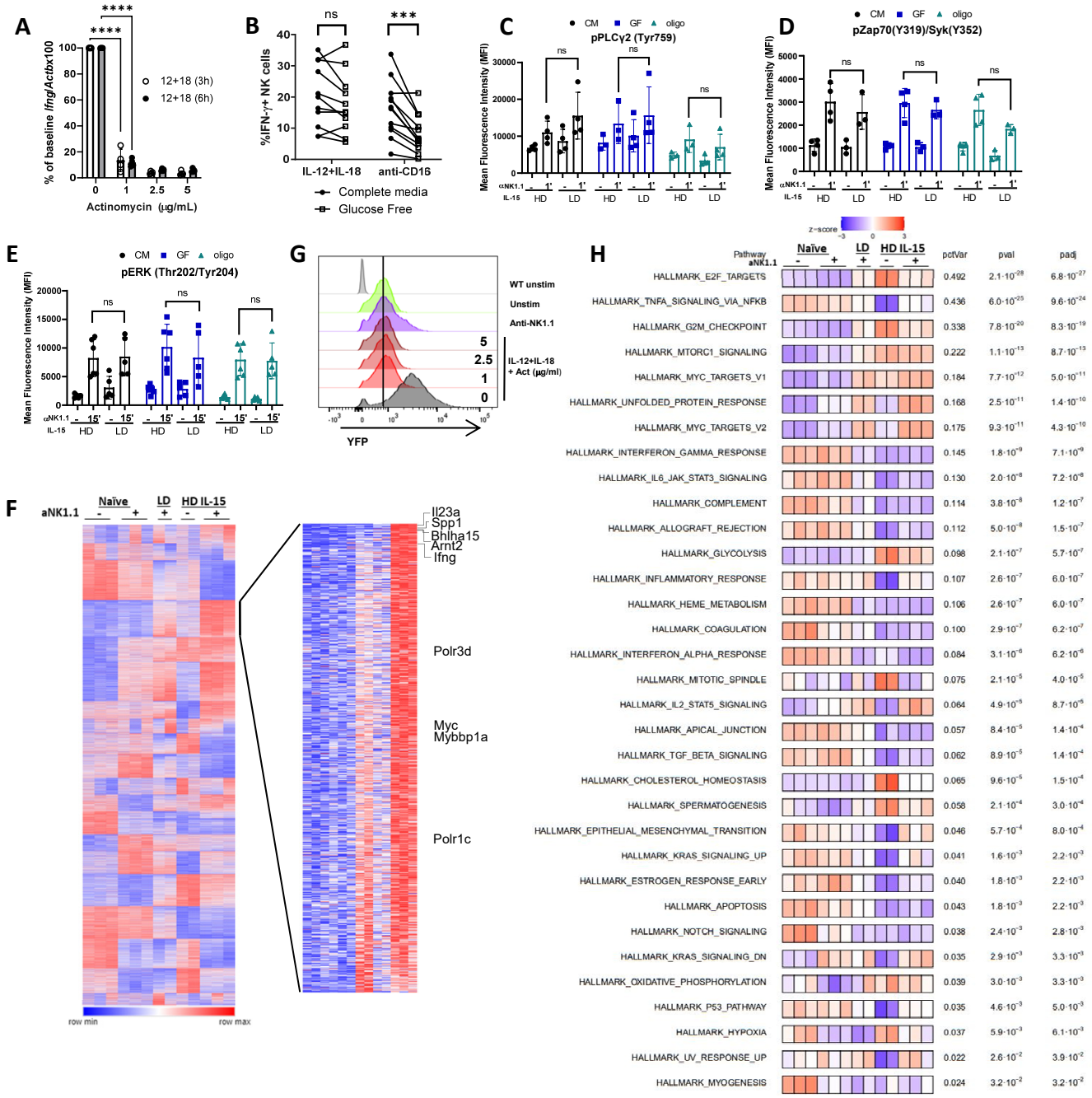

Figure S1. Supplementary functional, signaling, and transcriptional figures. A) Freshly isolated NK cells were stimulated low dose IL-12+IL-18 (1 ng/ml each) in the presence of indicated doses of actinomycin. % maximal Ifng relative to Actb x 100 shown compared to baseline (no actinomycin). n=3 independent experiments, 5 mice per experiment; significance only indicated for the first actinomycin dose, but $p<0.001$ for all doses compared to baseline. **B)** Human NK cells stimulated with either IL-12+IL-18 (10 ng/ml and 50 ng/ml, respectively) or with plate-bound anti-CD16 (1 mg/ml) in complete or glucose-free media for 6 hours. n=7 donors, 6 independent experiments. **C-E**) NK cells were cultured for 72 hours with 10 ng/ml (LD) or 100 ng/ml (HD) IL-15, followed by anti-NK1.1 stimulation for the indicated time, in complete media (CM), glucose-free media (GF), or complete media + oligomycin (oligo). Phosphorylation of Zap70/SYK (**C**), PLCγ2 (**D**), and ERK (**E**) was quantified by flow cytometry. Results shown are from a minimum of 3 independent experiments, 1-2 mice per experiment. **F**) and **H**) NK cells from the spleens of 15 male mice were pooled. One independent experiment, 2-3 technical replicates per condition. **F**) k-means clustering strategy highlighting a cluster of genes uniquely upregulated in primed NK1.1-stimulated NK cells. Select genes are annotated. **G**) Representative histogram of NK cells from *Ifng* reporter mice, +/- 6 h stimulation in the presence or absence of indicated doses of actinomycin (Act), 2 independent experiments. **H**) Gene set co-regulation analysis, results shown for the Hallmark collection. Statistical analysis: (**A**) 2way ANOVA, (**B**) Paired t-test, (**C-E**) Wilcoxon test (paired t-test, non-parametric).

Figure S2. Myc inhibition in murine NK cells.

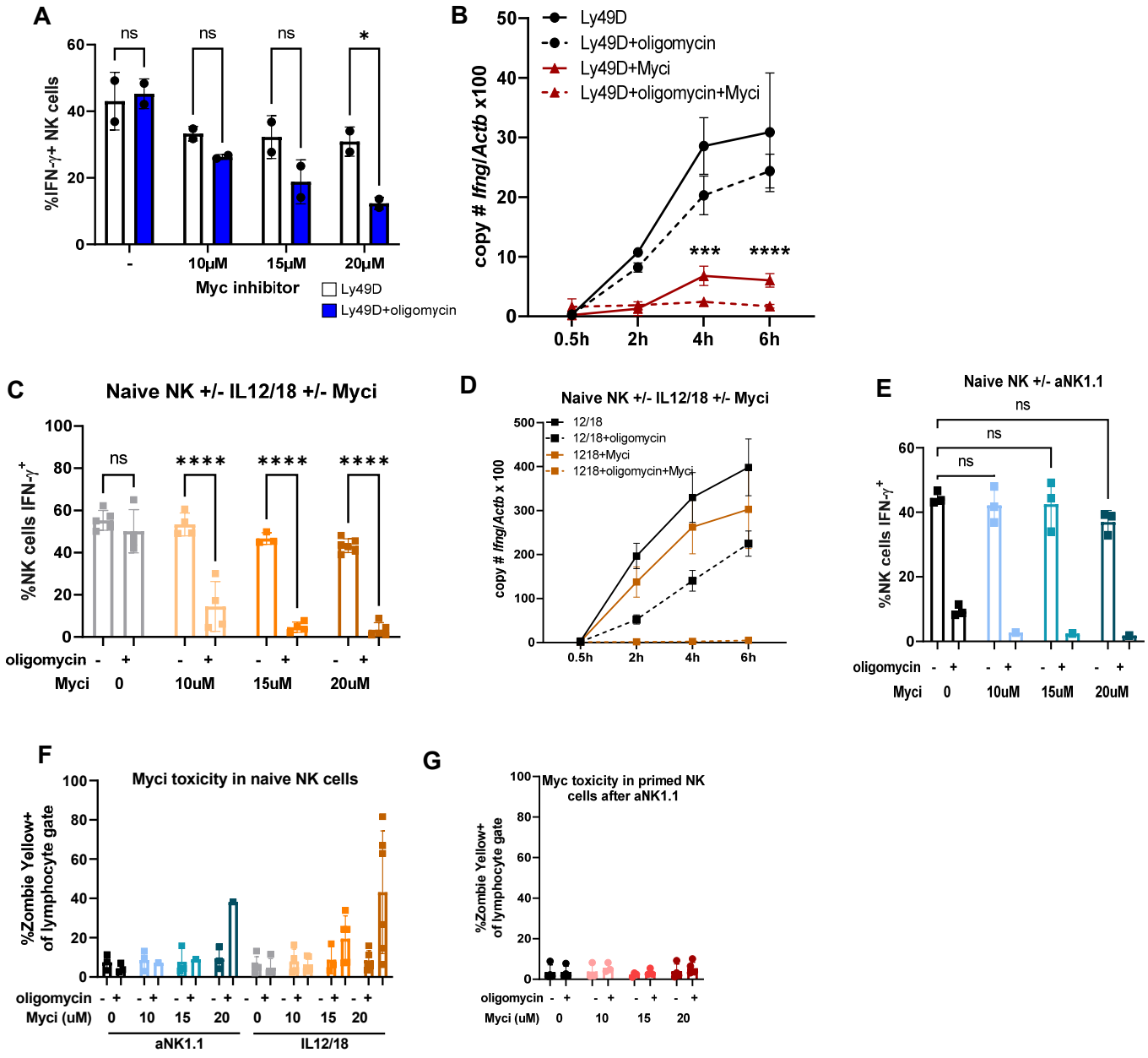

Fig S2. Myc inhibition in murine NK cells. Purified mouse NK cells were cultured for 72 hours in 100 ng/ml IL-15, "washed" to remove the cytokine, then stimulated with anti-Ly49D (**A-B**) in the absence or presence of Myc inhibitor KJ-Pyr-9 (Myci) +/- OXPHOS inhibitor oligomycin. n=10 mice, 2 independent experiments. **A**) % IFN-γ+ NK cells were measured by flow cytometry. **B**) *Ifng* transcript was measured by quantitative RT-PCR normalized to beta-actin (*Actb*). * denote significance from NK1.1 vs. NK1.1+Myci comparison. **C-D**) Purified mouse NK cells were stimulated with IL-12 (1ng/ml) and IL-18 (1ng/ml) or anti-NK1.1 for 6 hours +/- of Myc inhibitor KJPyr-9 (Myci) +/- OXPHOS inhibitor oligomycin. **C**) % IFN-γ+ NK cells were measured by flow cytometry after IL12/18 stimulation. 2-4 independent experiments, 5 mice per experiment. **D**) *Ifng* transcript was measured by quantitative RT-PCR normalized to beta-actin (*Actb*). n=4 independent experiments, 5 mice per experiment. * denotes significance from NK1.1 vs. NK1.1+Myci comparison. **E**) % IFN-γ+ NK cells were measured by flow cytometry after anti-NK1.1 stimulation; n=3 independent experiments, 5 mice per experiment. Myci toxicity in naïve (**F**) or primed (**G**) NK cells after 6 h stimulation, as measured by Zombie Yellow dye. 3-4 independent experiments, 5 mice per experiment. Statistical analysis: 2 way ANOVA.