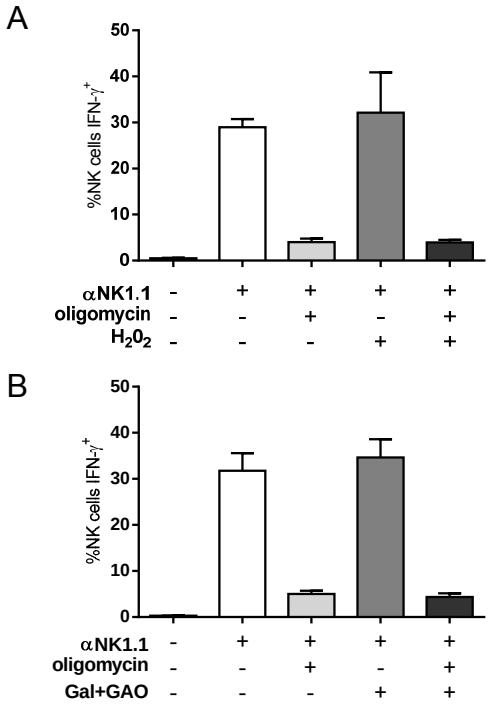
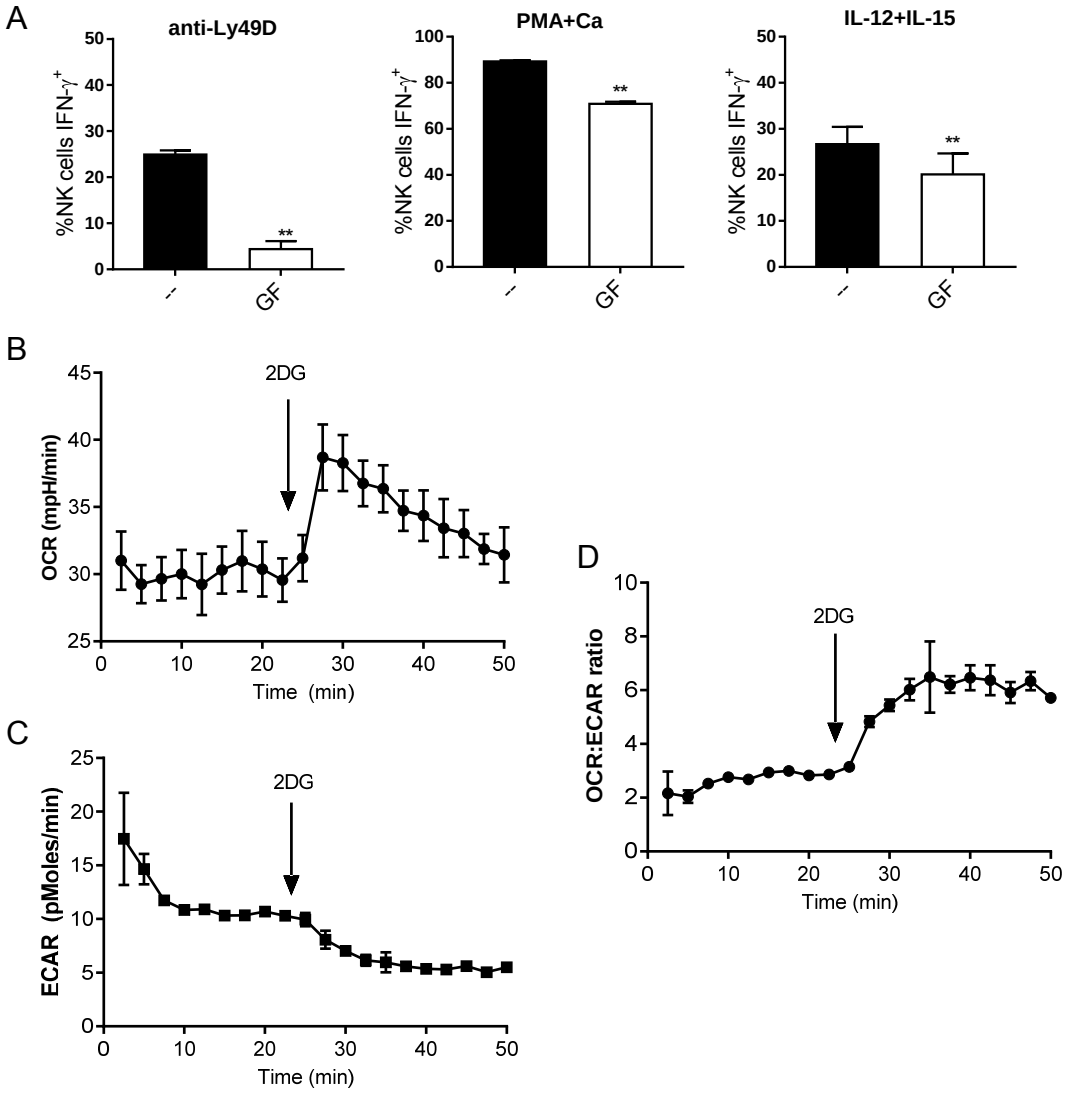
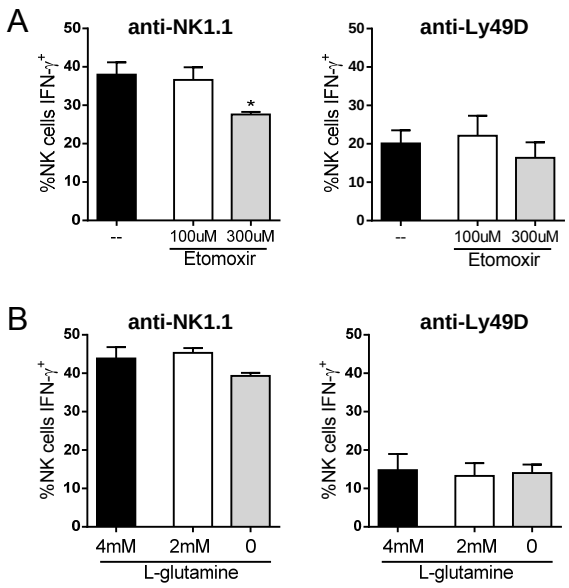


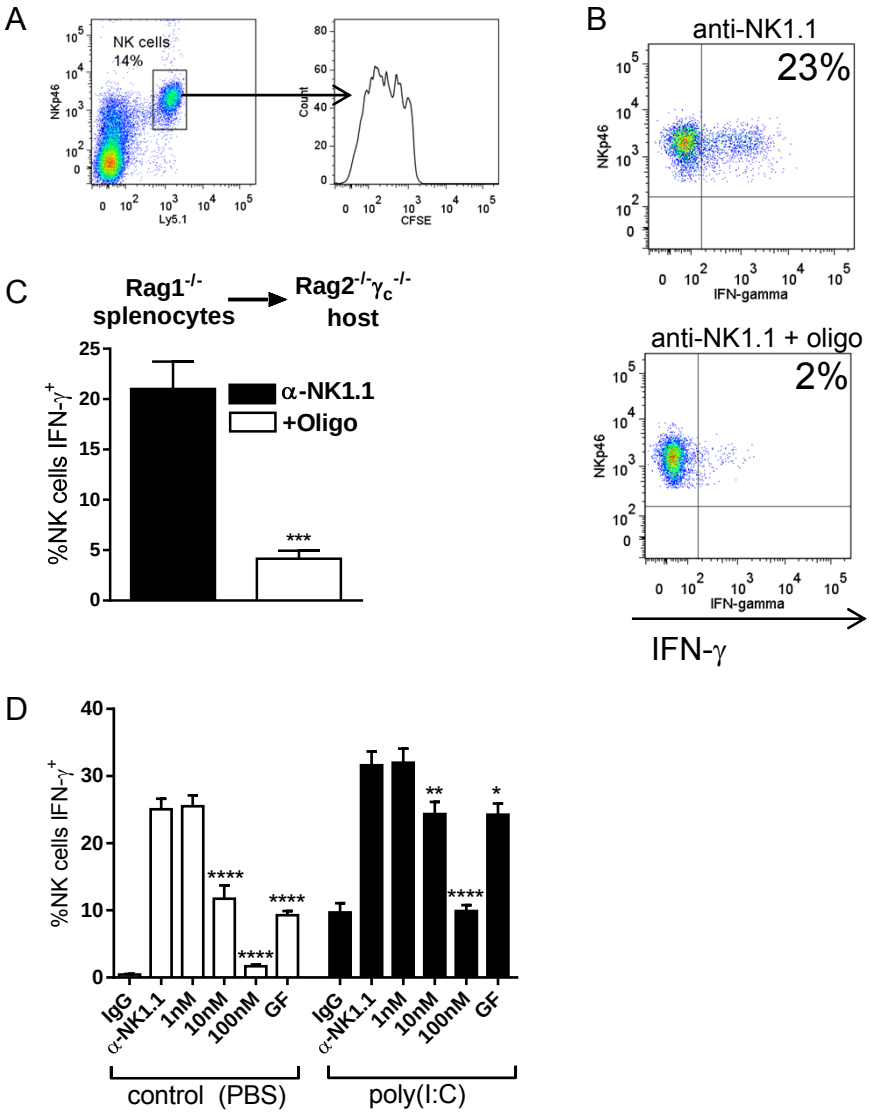
Supplemental Figure 1. Addition of reactive oxygen species does not alter receptor-mediated NK cell activation. Enriched splenic NK cells were stimulated with plate-bound anti-NK1.1 for 6h and NK cell production of IFN-γ measured by intracellular flow cytometry. A) NK cells were activated in the presence of oligomycin (100nM) and/or H_20_2 (1μM). NK cell survival was intact with this optimized dose of H_20_2, but cell death was observed with increasing concentrations of H_20_2 (not shown). B) Stimulation of NK cells in the presence of oligomycin and/or galactose (Gal, 500μM) plus galactose oxidase (GAO, 0.045U/mL) which generates H_20_2 (reference 18).



Supplemental Figure 2. Glucose is required for receptor-stimulated IFN-γ and inhibition of glucose metabolism leads to upregulation of OXHPOS. (A) NK cells were stimulated for 4-6h with anti-Ly49D, PMA + calcimycin (PMA+CA), or IL-12+IL-15 in complete (--) or glucose-free (GF) media and IFN-γ measured by flow cytometry. **p≤0.007. Results represent the mean +/-SEM of 3 independent experiments (B-D) Freshly isolated enriched murine splenic NK cells were assayed for oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) by extracellular flux assay before and after addition of the glycolytic inhibitor 2DG (50mM, shown by the arrow). Results represent the mean +/- SEM of 3 replicate wells at each timepoint and are representative of 4 independent experiments.



Supplemental Figure 3. **Fatty acid oxidation or L-glutamine are not required for receptor-stimulated IFN-γ production.** Enriched NK cells were stimulated for 6hr with receptors (anti-NK1.1 or anti-Ly49D) (A) in the presence or absence (--) of etomoxir, a fatty acid oxidation inhibitor, or (B) with limiting concentrations of L-glutamine. *p<0.05; **p≤0.007. Results represent the mean +/-SEM of 3 independent experiments.



Supplemental Figure 4. Metabolic in inhibition of NK cell recpetor-stimulated IFN-γ after in vivo proliferation or activation with poly(I:C). (A-C) CFSE-labeled splenocytes from Rag-1$^{-/-}$ mice were adoptively transferred into Rag-2$^{-/-}$g$_c^{-/-}$ hosts and harvested 3 days later. (A) Representative flow plot of Ly5.1$^+$ adoptively transferred NK cells (NKp46$^+$) and CFSE dilution. (B & C) NK cells were stimulated with anti-NK1.1 in the absence or presence of oligomycin (oligo, 100nM). Results represent the mean +/-SEM of 8 mice from 3 independent experiments. (D) Wt mice were treated with poly(I:C) (black bars) or control PBS (white bars). Splenocytes were stimulated 14-16h later with plate-bound anti-NK1.1 without or with oligomycin (1nM, 10nM, or 100nM) or in glucose free media (GF). Results represent the mean +/- SEM of 7 independent experiments with 2-3 mice per group. Statistics represent comparison between anti-NK1.1 versus each inhibitor for control-treated or poly(I:C) treated splenocytes. *p<0.05, **p≤0.01, ****p≤0.0001.