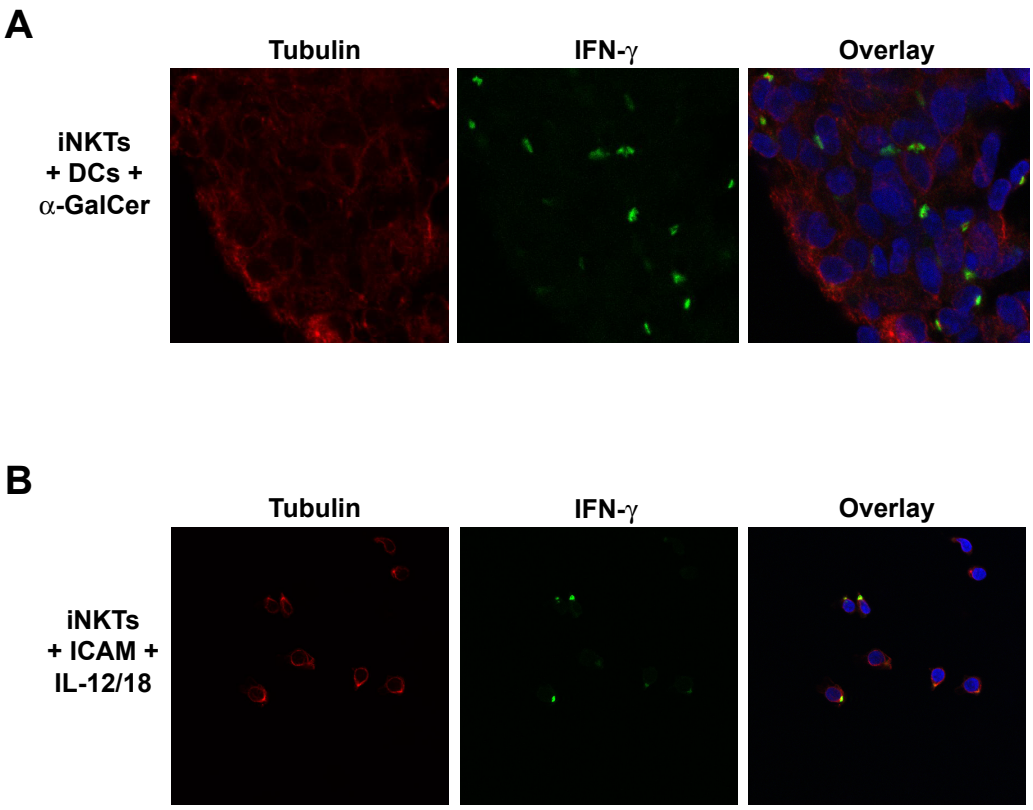
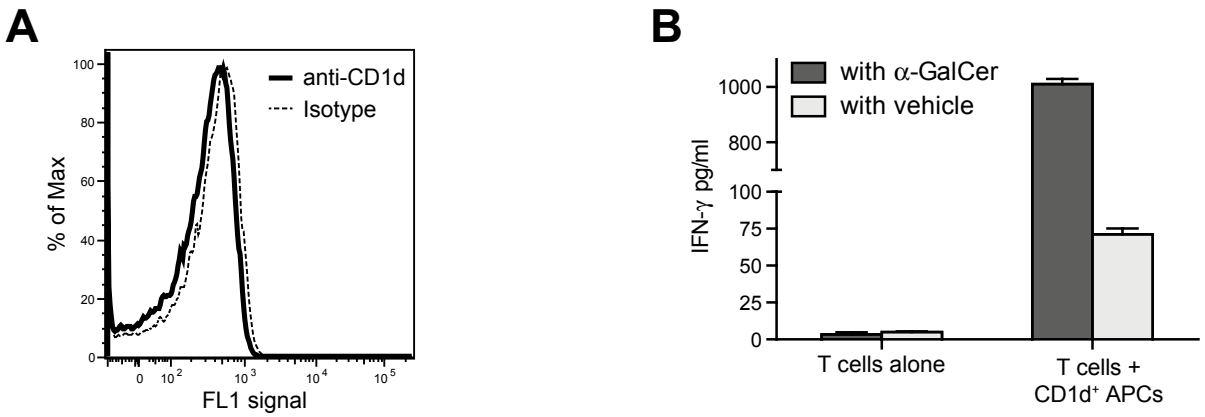


Supplementary Figure 1. Fluorescence microscopy showing IFN-γ production by human iNKT cells. A) Human iNKT cells were co-incubated with a 1:1 ratio of α-GalCer pulsed monocyte-derived dendritic cells, then stained with DAPI (blue) to identify cell nuclei, anti-tubulin (red), and anti-IFN-γ (green). Similar to what has been previously established for conventional T cells (52), the IFN-γ staining appears tightly localized at the interface between iNKT cells and DCs. **B)** Analysis of iNKT cells incubated on slides coated with ICAM-1-Fc in medium containing IL-12p70. IFN-γ staining appears to co-localize with a nexus of tubulin staining that corresponds to the microtubule organizing center (MTOC).



Supplementary Figure 2. CD1d expression and presentation of α-GalCer by cultured human iNKT cells. **A)** Human iNKT cell clonal lines (PP1.3 and GG1.2) and short-term polyclonal expansions (FoB and 318D) were stained for cell surface expression of CD1d and analyzed by flow cytometry. The two polyclonal iNKT cell expansions and clone PP1.3 showed no detectable CD1d staining above background (histogram plot shows results for the 318D line), while some positive CD1d staining was observed for clone GG1.2 (data not shown). **B)** Cultured human iNKT cells (clone J3N.5) were incubated for 24 hr alone or with a 1:1 ratio of CD1d-transfected 721.221 cells in medium containing 100 ng/ml α-GalCer or vehicle, and culture supernatants were harvested and assayed for IFN-γ by ELISA. The plot shows means and standard deviations of 3 replicate culture wells. Similar results were obtained from two additional CD1d-restricted T clones (J24N.70 and J24N.22).