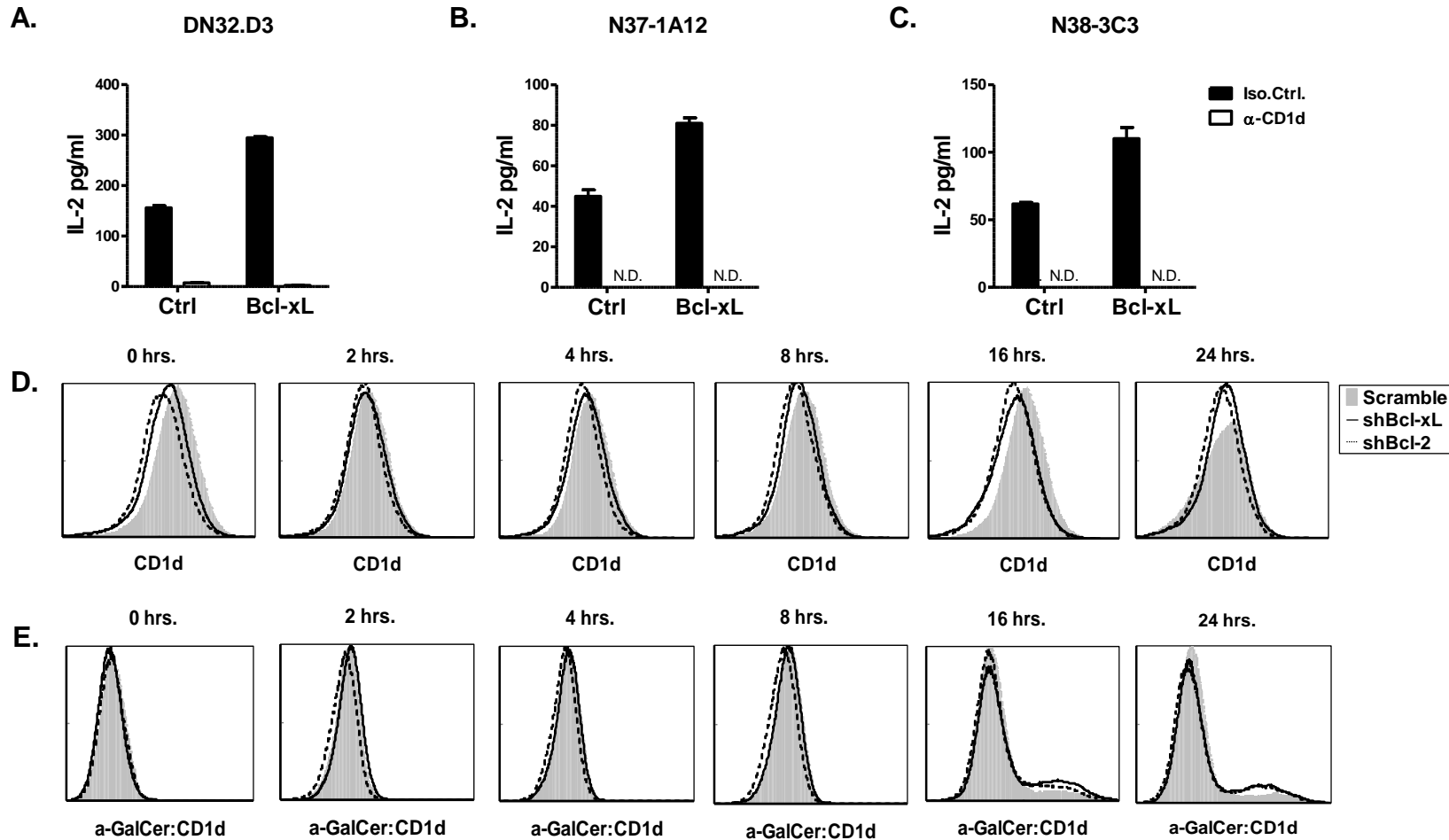
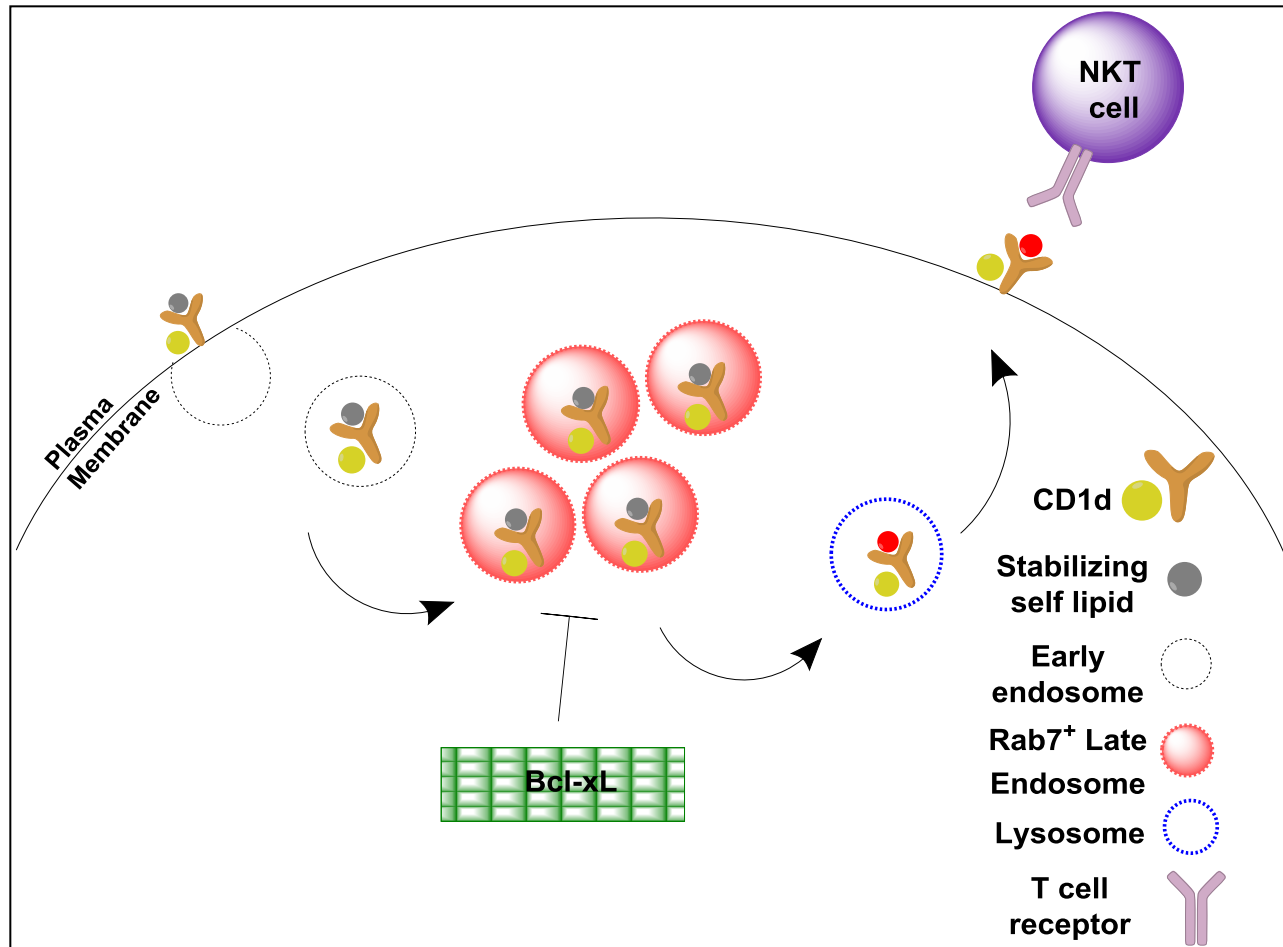


Supplementary Figure 1. The over-expression or knockdown of Bcl-xL does not alter cellular morphology, viability or proliferation. **(A)** LMTK-CD1d cells stably transfected with control vector (Ctrl) or Bcl-xL containing vector or **(B)** L-CD1d cells transduced with scramble shRNA or shRNA targeting Bcl-xL or Bcl-2 were cultured in complete medium with appropriate selection agents. These cells were grown in tissue-culture grade flasks and imaged under an Olympus CK2 light microscope from Olympus Corporation (Tokyo, Japan) using a 10x objective. Images were captured using an Olympus Pen E-PL1 digital camera from Olympus Corporation (Tokyo, Japan). **(C)** LMTK-CD1d cells stably transfected with empty vector (Ctrl) or Bcl-xL containing vector or **(D)** L-CD1d cells stably transduced with scramble shRNA or shRNA targeting Bcl-xL or Bcl-2 were incubated for the indicated time periods in WST-1 cell proliferation assay reagent as per the manufacturer's directions. The difference between absorbance at 450 nm and 680 nm was measured as per the manufacturer's directions.



Supplementary Figure 2. Bcl-xL-mediated regulation of antigen presentation to NKT cells is CD1d dependent. (A-C) LMTK-CD1d control or Bcl-xL transfected cells were treated with 10 μ g/ml purified CD1d blocking antibody (clone 1B1) before the addition of the indicated NKT cell hybridomas. IL-2 in the supernatant was measured by ELISA. N.D.- not detected. (D-E) Time course of cell surface expression of CD1d and CD1d: α -GalCer complexes. L-CD1d cells stably transduced with scramble shRNA or shRNA targeting Bcl-xL or Bcl-2 were incubated with medium alone or 100 ng/ml α -GalCer at 37°C for the indicated time periods. At the end of the incubation, cells were stained with fluorophore-conjugated antibodies to (D) CD1d (clone 1B1) or (E) CD1d: α -GalCer complexes (clone L363). Immediately after staining, cells were fixed using paraformaldehyde and analyzed by flow cytometry.



Supplementary Figure 3. Proposed mechanism by which Bcl-xL regulates CD1d-mediated antigen processing and presentation. Under normal conditions, CD1d molecules traffic through the early endosomes, late endosomes and finally the lysosomal compartment, are loaded with antigens and expressed on the cell surface. In Bcl-xL knock down cells, there is upregulation of Rab7 and expansion of the late endosomal compartment. This leads to accumulation of CD1d molecules in the late endosomes and ultimately results in reduced antigen presentation to NKT cells. Thus, the data from this study show that Bcl-xL plays a role in the regulation of CD1d-mediated antigen processing and presentation by altering CD1d trafficking through the endocytic pathway.