Supplemental Figure
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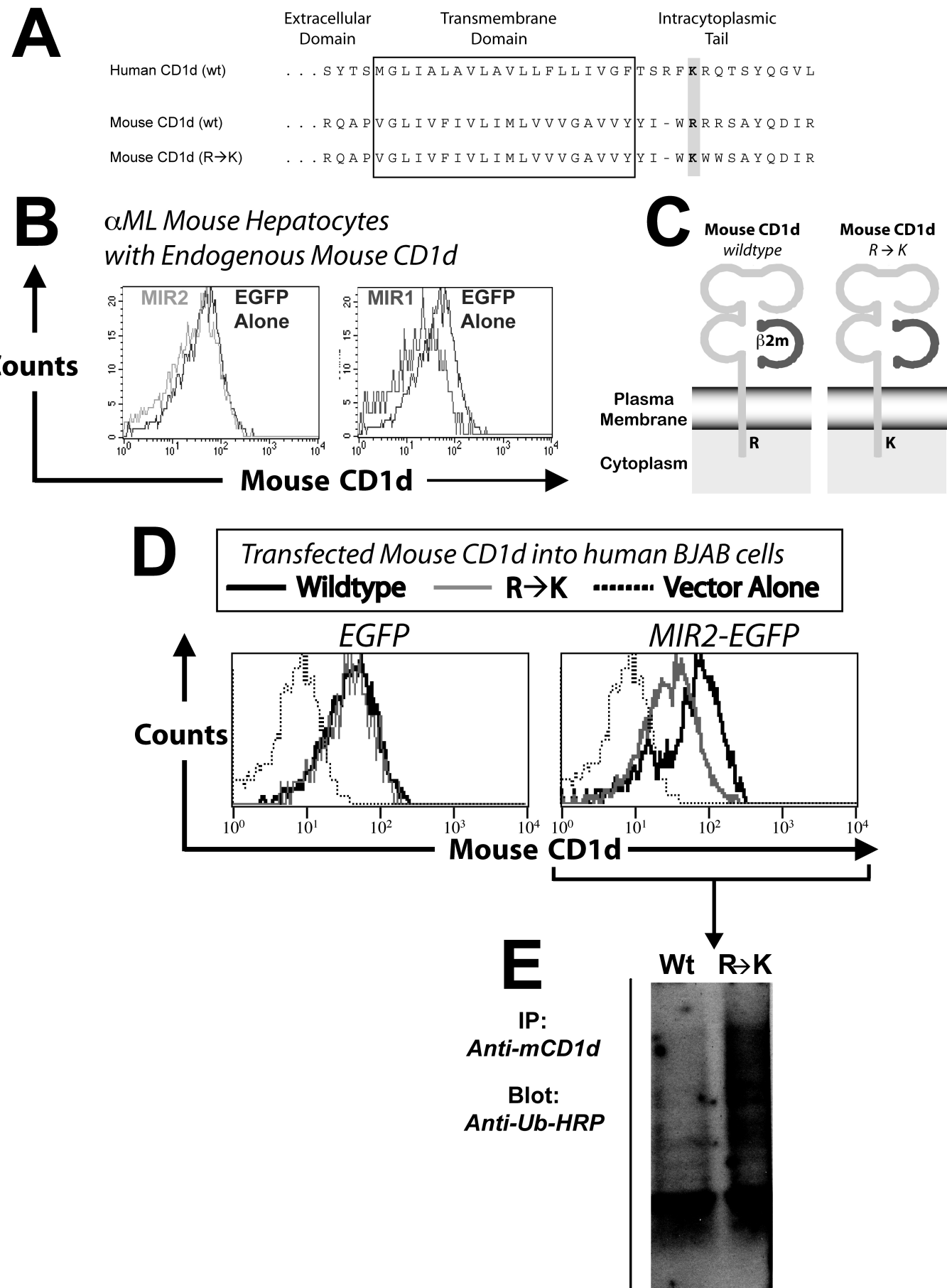

Supplemental Figure

Murine CD1's Lack of Cytoplasmic Lysines Make it Insensitive to MIR2 mediated downregulation and ubiquitination. A. Alignment of the transmembrane and cytoplasmic tails of CD1d of human and mouse origin show that a single lysine exists in human CD1d but not CD1d of mouse origin. B. αML cells were transfected with an expression vector for eGFP or MIR2 (left panel) or MIR1 (right panel) fused to eGFP (right panel). The levels of murine CD1d was determined by staining with anti-mouse CD1d antibodies conjugated to PE and the cells were analyzed by flow cytometry, gating upon eGFP positive cells. As shown, there was no dramatic change in CD1d level in the mouse cells when transfected with the KSHV proteins. A small, two to three fold decrease is seen in MIR1 transfected cells. C. A mutant of Murine CD1d was created to confirm the requirement of cytoplasmic lysines for the ability of the MIR proteins to downregulate CD1d. This schematic shows the theoretical domain organization of the human and mouse CD1d molecules that were constructed and the mutation that was introduced. D. BJAB cells were cotransfected with either of the murine CD1d (wildtype or arginine to lysine mutant) with an expression vector for eGFP (left panel) or MIR2 fused to eGFP (right panel). The levels of murine CD1d was determined by staining with anti-mouse CD1d antibodies and the cells were analyzed by flow cytometry, gating upon eGFP positive cells. E. BJAB cells from the right panel of part D that were transfected with MIR2 were lysed, and murine CD1d was immunoprecipitated and the immunoprecipitate was blotted for the presence of ubiquitin. As shown, only the mCD1d with the arginine to lysine mutation was able to coimmunoprecipitate ubiquitin.