Supplementary Figure 3: E1 inhibition of cells expressing CD4-WTLAT or CD4-2KRLAT. A. JCam2.5 cells stably expressing CD4-WTLAT or CD4-2KRLAT were treated with DMSO or indicated concentrations of TAK-243 E1 inhibitor for 60 or 120 min as indicated. Whole cell lysates were immunoblotted for ubiquitin (Ub) and β-actin. B and C. JCam2.5 cells stably expressing CD4-WTLAT or CD4-2KRLAT were treated with 1uM TAK-243 for 60 min and labeled with anti-CD4 (clone OKT4) at 4°C. Cells were left unstimulated or stimulated with indicated doses of anti-CD3 and transferred to 37°C for 120 min. at which total CD4 levels were measured by flow cytometry. Data is representative of three independent experiments. % surface labeled CD4 remaining in the cell was measured as described in Materials and Methods. Bars denote means ± SEM of three independent experiments. Statistical significance was determine using Student’s t test. ** p < 0.0005.