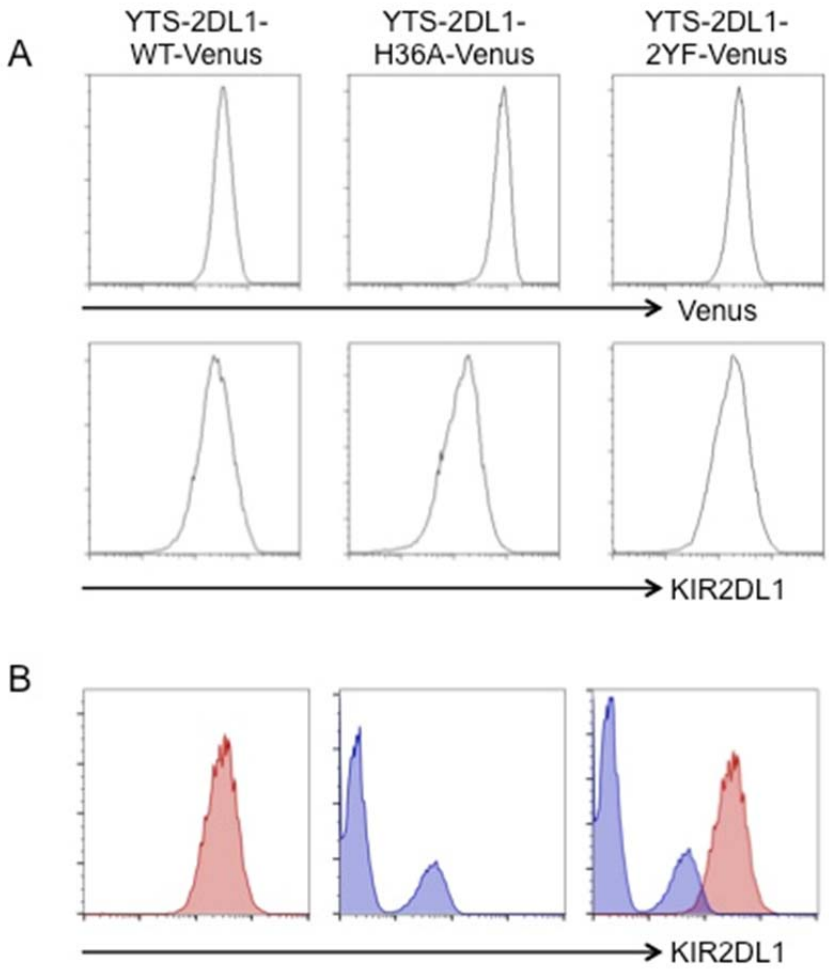
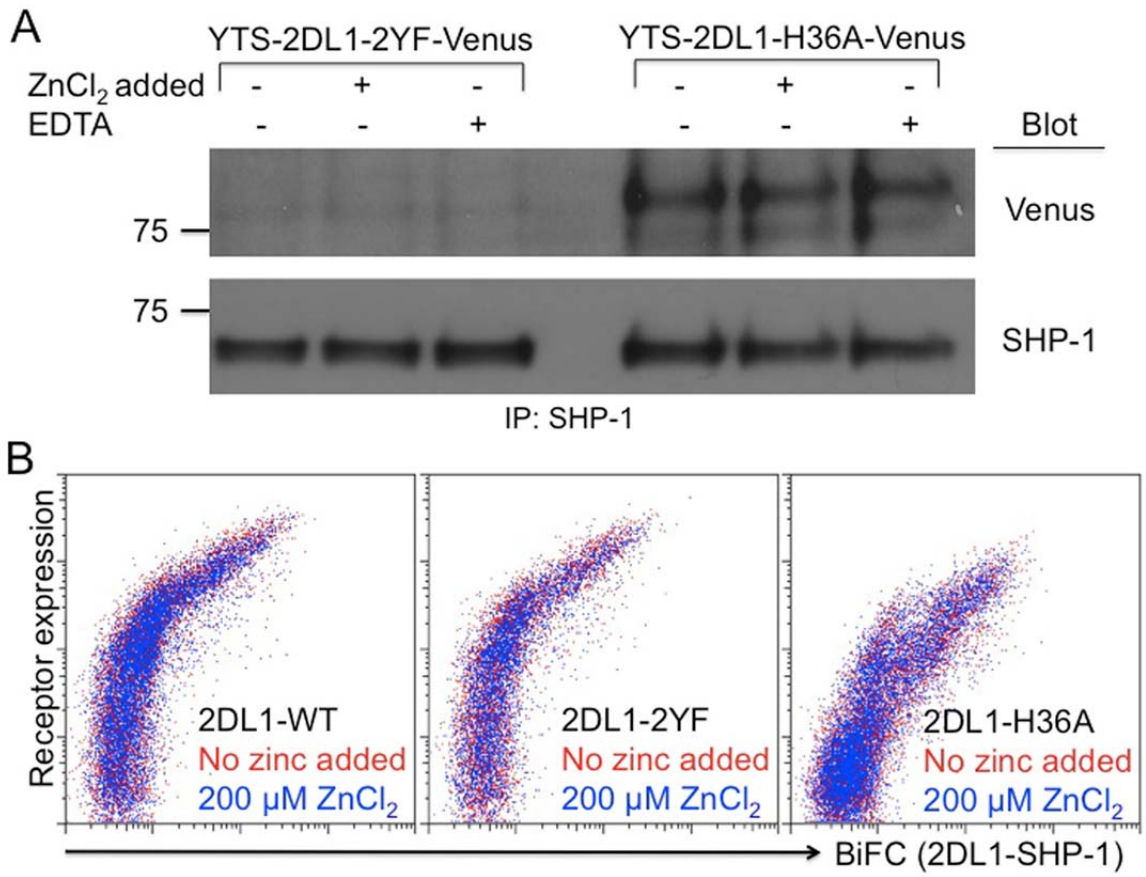
Supplemental Figure 1



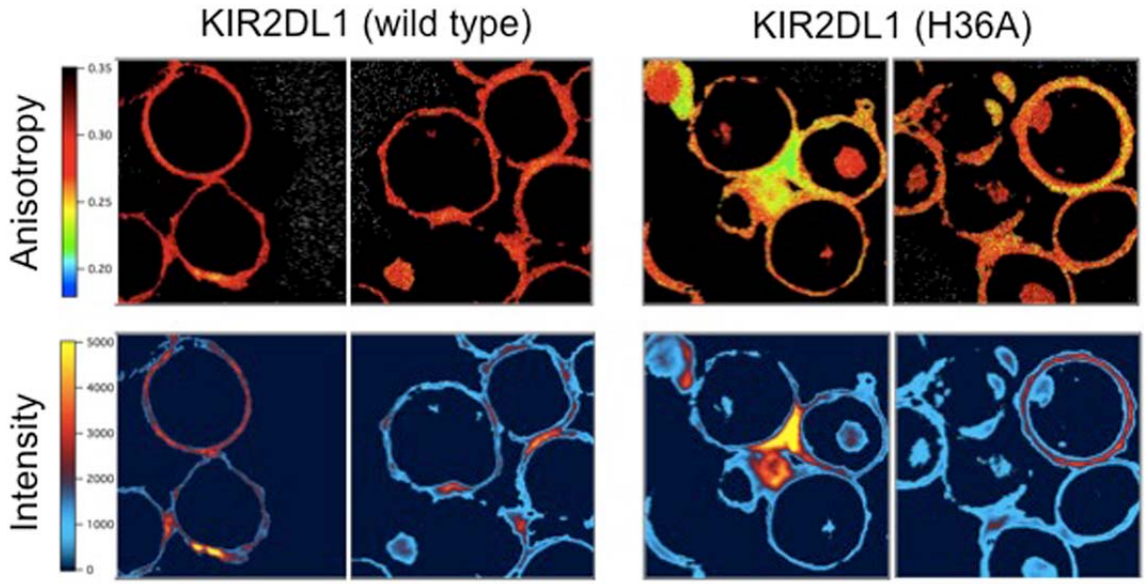
Supplemental Figure 1: (A) KIR2DL1-Venus expression in YTS cells stably transfected with 2DL1-WT-Venus, 2DL1-H36A-Venus and 2DL1-2YF-Venus. Top panels show Venus fluorescence and lower panels show surface KIR2DL1 stained with anti-2DL1-APC as monitored by flow cytometry (BD FACSCalibur). (**B**) Surface expression of KIR2DL1 in YTS-2DL1-WT-Venus cells (left-most panel) and primary NK cells (middle panel). The right-most panel shows an overlay (red: YTS-2DL1-WT-Venus cells; blue: primary NK cells.) KIR2DL1 surface expression was determined by staining with anti-2DL1-APC followed by flow cytometry (BD FACSCalibur). The mean fluorescence intensity (MFI) of the YTS transfectant and primary NK cells, as determined with anti-2DL1-APC, was 327 and 47, respectively. However, as the diameter of YTS cells (~ 15 μm) is about 3-fold greater than that of primary NK cells (~ 5 μm), which translates to a 9-fold greater surface area, the average density of KIR2DL1 on YTS transfectants and on primary NK cells is comparable. Primary NK cells were isolated from blood of an anonymized healthy donor (National Institutes of Health Blood Bank under a National Institutes of Health Institutional Review Board–approved protocol with informed consent).

Supplemental Figure 2



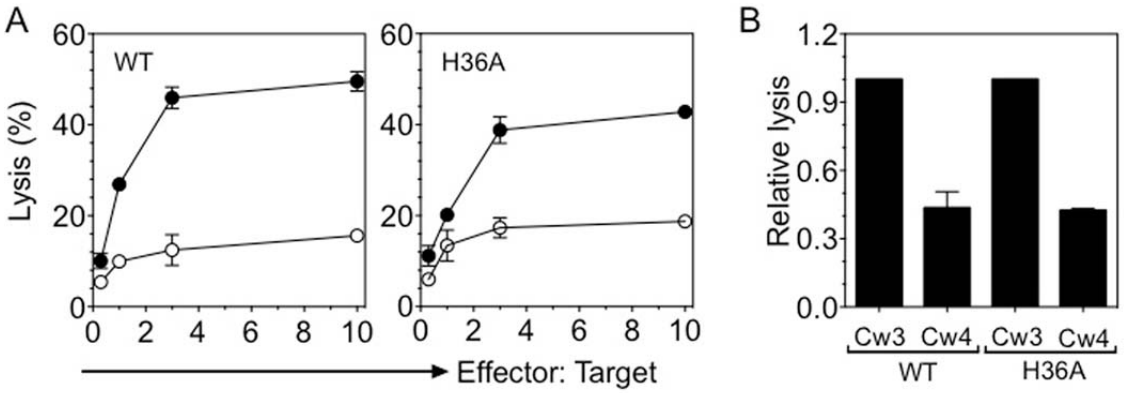
Supplemental Figure 2: Constitutive association of SHP-1 with 2DL1-H36A is independent of $ZnCl_2$. (**A**) Cells in RPMI + 10 % FBS (R10 medium) at 37^{o}C were either untreated, or treated with 200 μM supplemental $ZnCl_2$ for 1.5 h, or with 1 mM EDTA for 30 minutes. SHP-1 immuno-precipitates were probed with an anti-GFP antibody. (**B**) Constitutive SHP-1 association with KIR2DL1 in transfected YTS cells monitored by BiFC of reconstituted Venus using flow cytometry. BiFC signal of 2DL1-WT (left), 2DL1-2YF (middle) and 2DL1-H36A (right-most), incubated in R10 medium without (red) or with supplemental 200 μM $ZnCl_2$ (blue), is plotted against surface expression, as detected by anti-2DL1-APC binding.

Supplemental Figure 3



Supplemental Figure 3: Higher magnification images of YTS-2DL1-WT-Venus and YTS-2DL1-H36A-Venus, as in Figure 7. Anisotropy and fluorescence intensity were acquired simultaneously.

Supplemental Figure 4



Supplemental Figure 4: Lysis of 221 target cells by YTS-2DL1-WT and YTS-2DL1-H36A cells is inhibited by expression of HLA-Cw4. (**A**) Lysis of 221-HLA-Cw3 cells (filled circles) and 221-HLA-Cw4 cells (open circles) by YTS-2DL1-WT (left panel) and YTS-2DL1-H36A (right panel) cells. (**B**) To compare different experiments, lysis of 221-Cw4 is shown relative to lysis of 221-Cw3 (set to 1) at a NK to target ratio of 10.