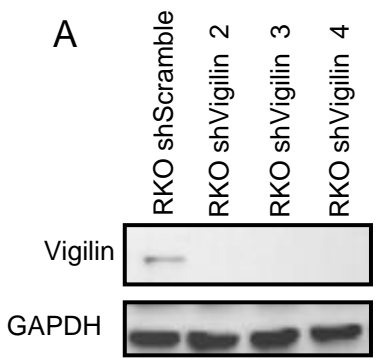
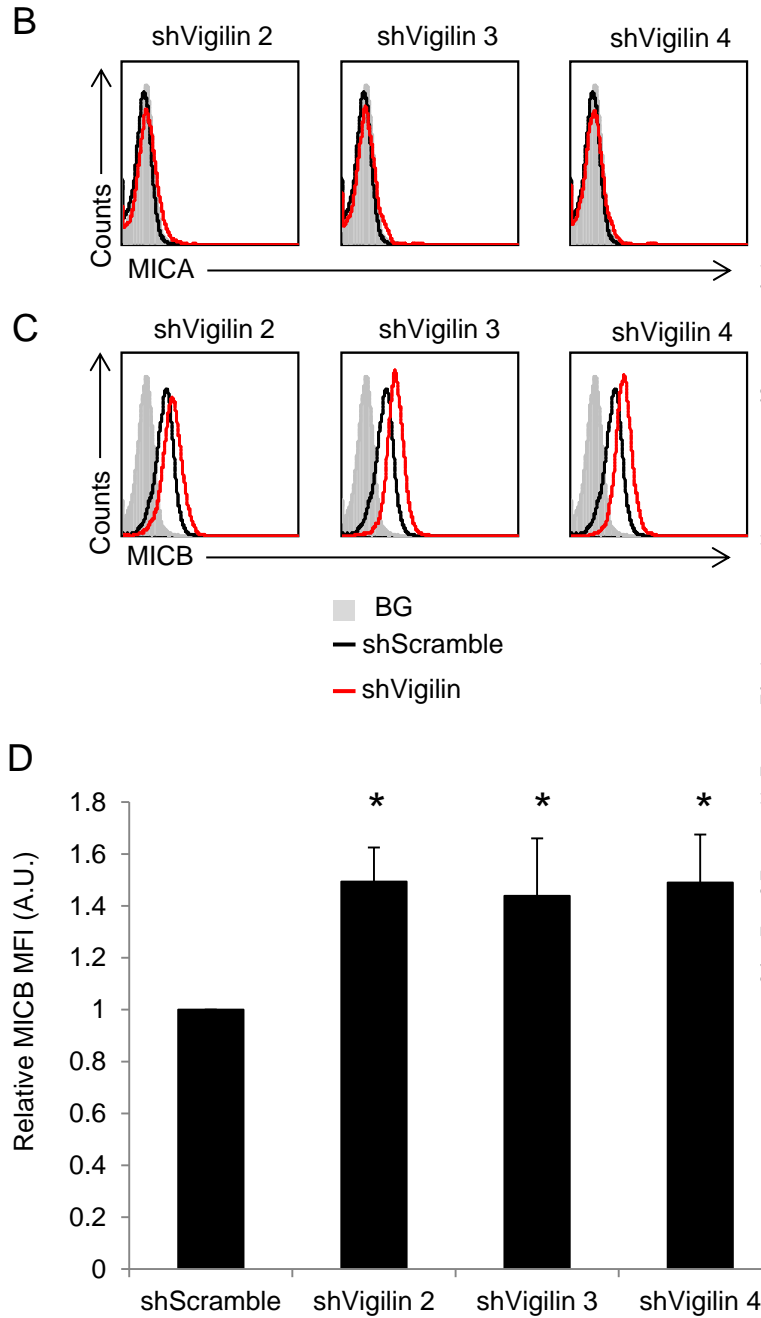


Supplementary Figure 1

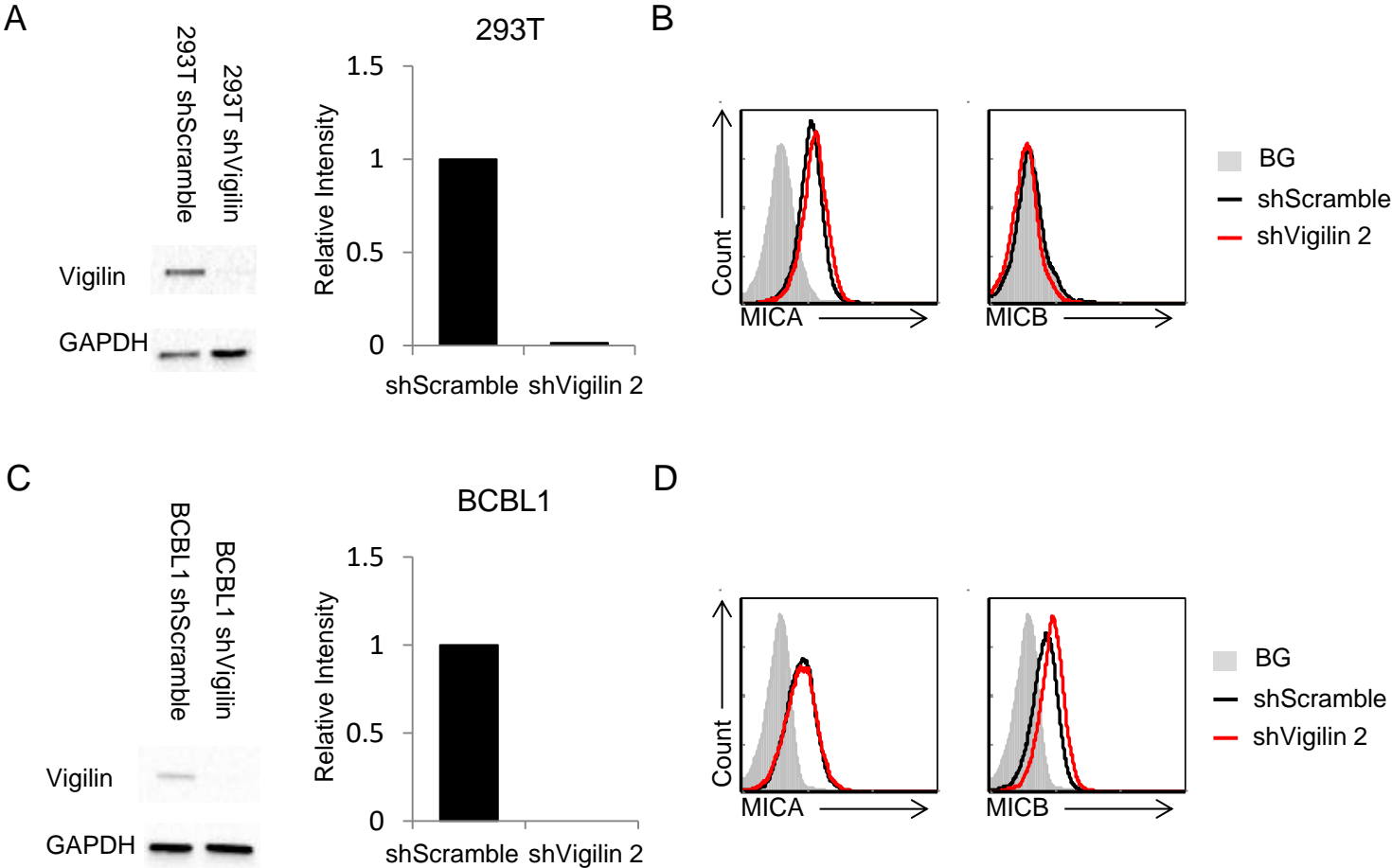


Supplementary Figure 1. Effect of vigilin knockdown on MICA and MICB surface expression

(A) Western blot analysis of vigilin (140kDa) in RKO cells transduced with either scrambled shRNA (RKO shScramble) or various shRNAs against vigilin (shVigilin 2, shVigilin 3, and shVigilin 4). GAPDH (36kDa) was used as a loading reference. Contrast in the WB was adjusted for clarity. (B-C) FACS analyses of MICA (B) and MICB (C) surface expression on RKO cells transduced with either scrambled shRNA (shScramble, black histogram) or different shRNA's against vigilin (shVigilin 2, 3, and 4, red histograms). The filled gray histogram represents staining with secondary antibody only of the RKO shScramble cells (BG). The backgrounds of the RKO shVigilin cells were similar and not shown in the figures. Figures show one representative FACS staining out of at least 4 performed. (D) Quantification of MICB expression on RKO transduced cells. Shown is the relative average MFIs of MICB on RKO shVigilin cells relative to RKO shScramble cells (set as 1) from 4 independent experiments. Applied single sample t-test where the two tailed $*p < 0.05$.



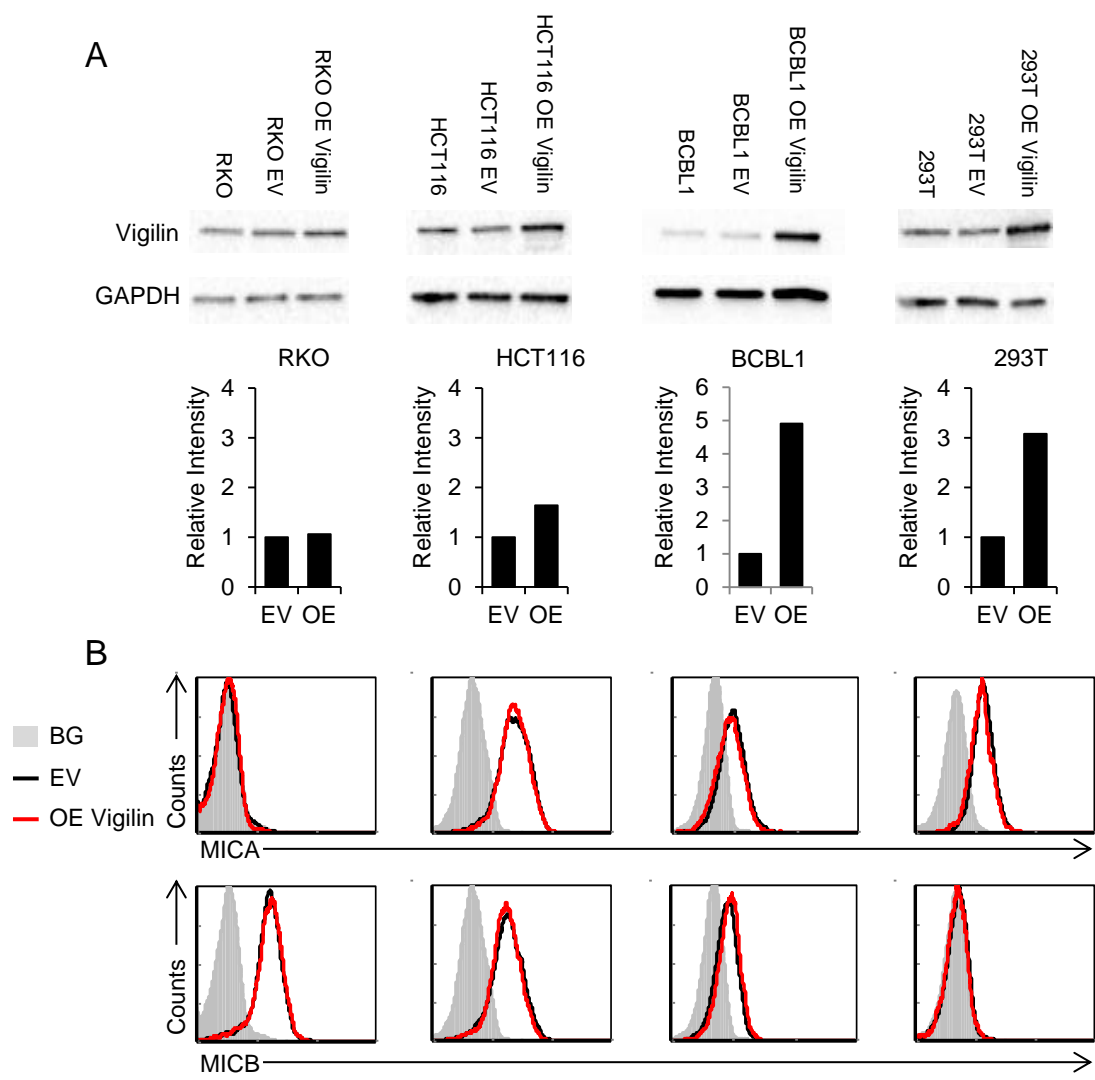
Supplementary Figure 2



Supplementary Figure 2. Effect of vigilin knockdown on MICA and MICB surface expression

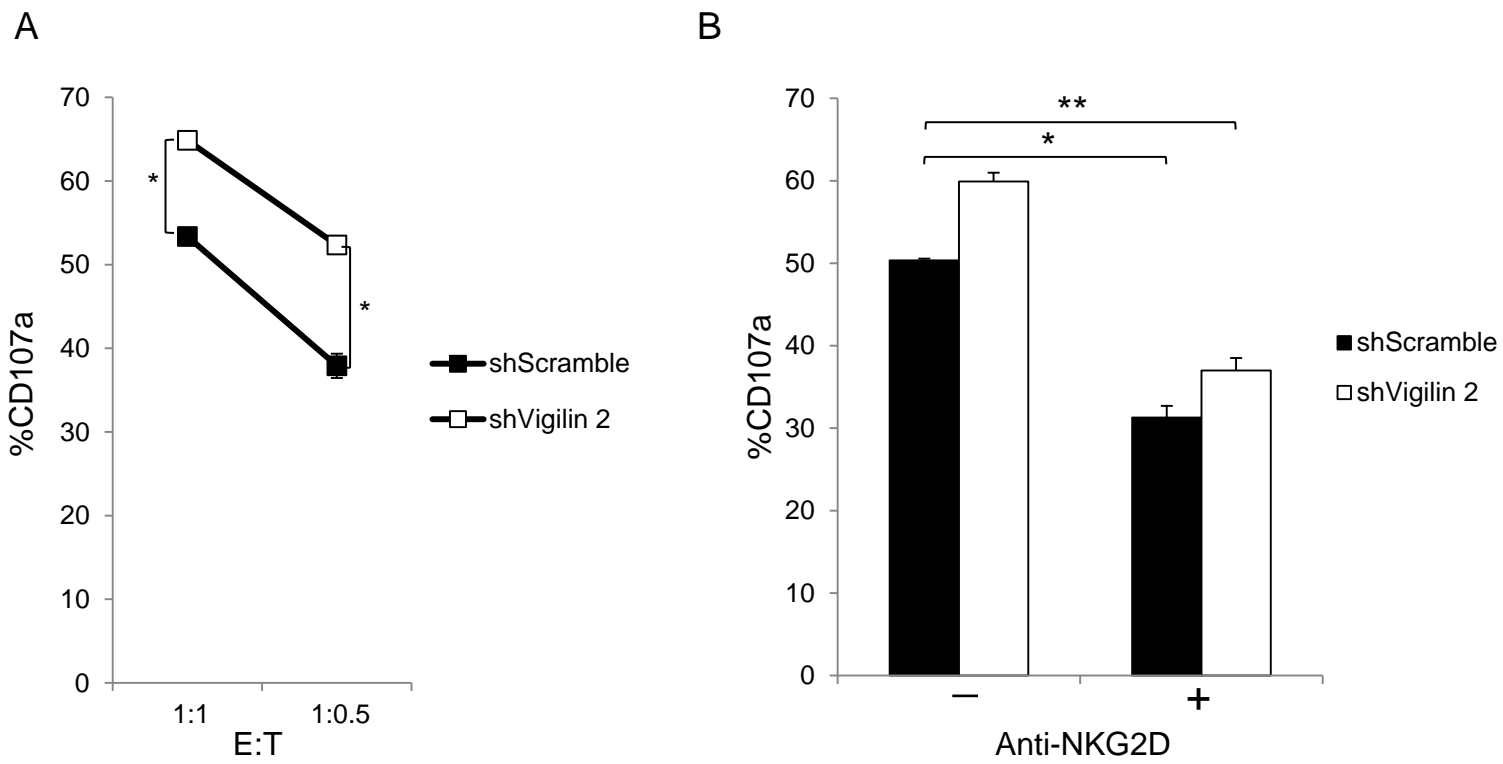
(A, C) Western blot analysis of vigilin (140kDa) in 293T (A) or BCBL1 (C) cells transduced with either scrambled shRNA (293T/BCBL1 shScramble) or shRNA against vigilin (293T/BCBL1 shVigilin 2). GAPDH (36kDa) was used as a loading reference. Contrast in the WB was adjusted for clarity. The graphs are quantification of the WB shown. (B, D) FACS analyses of MICA and MICB surface expression on 293T (B) or BCBL1 (D) shScramble (black histograms) and shVigilin 2 (red histograms) cells. The filled gray histogram represents staining with secondary antibody only (BG). The backgrounds of the shVigilin 2 cells were similar and not shown in the figures. Figures show one representative FACS staining out of at least 2 performed.

Supplementary Figure 3



Supplementary Figure 3. Effect of vigilin overexpression on MICA and MICB surface expression

(A) Western blot analysis of vigilin (140kDa) in RKO, HCT, BCBL1 and 293T cells transduced with either an Empty Vector (EV) plasmid or one overexpressing vigilin (OE Vigilin). GAPDH (36kDa) was used as a loading reference. Contrast in the WB was adjusted for clarity. The graphs are quantification of the WB shown. (B) FACS analyses of MICA and MICB surface expression on RKO, HCT, BCBL1 and 293T cells transduced with either EV (black histograms) and OE Vigilin (red histograms) plasmids. The filled gray histogram represents staining with secondary antibody only (BG). The backgrounds of the OE Vigilin cells were similar and not shown in the figures. Figures show one representative FACS staining out of at least 2 performed.



Supplementary Figure 4. Downregulation of vigilin in target cells increases NK cell activation against target cells

(A) Primary activated bulk human NK cells (activated NK cells) were incubated with either RKO cells transduced with scrambled shRNA (shScramble) or an shRNA against vigilin (shVigilin 2), and anti-CD107a APC and anti-CD56 PE antibodies for 2hrs at 37°C. Two ratios of NK cells to target cells were tested (1:1 and 1:0.5). The NK cells were then analyzed by FACS for %CD107a expression. Figure shows one representative experiment out of 3 performed. Shown are the mean values and SD derived from quadruplicates. *p< 0.005. (B) Activated NK cells were initially incubated either with or without an anti-NKG2D mAb for 1hour on ice. RKO shScramble or RKO shVigilin 2 cells were subsequently added, with anti-CD107a APC and anti-CD56 PE antibodies for 2hrs at 37°C. Figure shows one representative experiment out of 3 performed. Shown are the mean values and SD derived from quadruplicates. *p< 0.005, **p<0.0005.