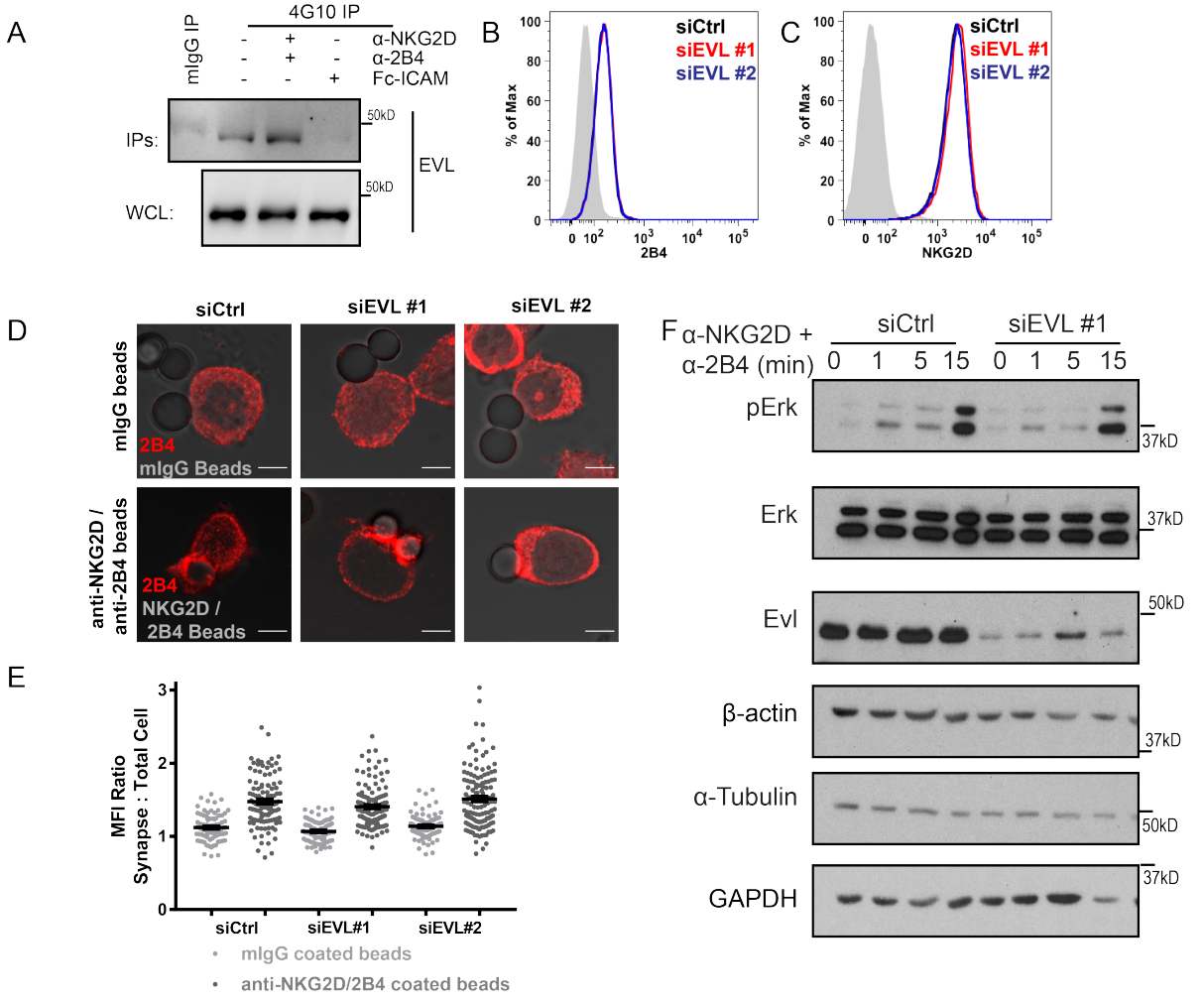

Fig. S1: EVL depletion does not impact NKG2D or 2B4 receptor expression

(A) NKL cells were stimulated with mouse Immunoglobulin (mIgG) antibody, anti-NKG2D/anti-2B4 antibody, recombinant human Fc-ICAM or a combination. The cells were lysed and anti-phospho-tyrosine antibody (clone 4G10) was used to immunoprecipitate activated protein complexes, which were separated by SDS-PAGE and immunoblotted as indicated. **(B-C)** NKL cells nucleofected with EVL siRNAs or a control siRNA were evaluated by flow cytometry for surface 2B4 (**B**) and NKG2D (**C**). (**D-E**) NKL cells nucleofected with control siRNA or one of two siRNAs targeting EVL were allowed to form conjugates with mIgG coated negative control beads or anti-NKG2D/anti-2B4 coated stimulatory beads for 30 minutes at 37°C. Conjugates were fixed and stained for the presence of 2B4, as indicated. Results are quantified in (**E**). (**F**) NKL nucleofected with an EVL-targeting siRNAs or a control siRNA were stimulated by crosslinking with a combination of FcICAM, anti-NKG2D and anti-2B4 over the indicated time course. Cell lysates were prepared and equivalent amounts of protein were separated by SDS-PAGE and immunoblotted with the indicated antibodies. Confocal scale bars indicate 5 µm unless otherwise indicated. All images are representative of 3 independent experiments. Dot graph is combined analysis of 3 independent experiments. Error bars indicate S.E.M. around the indicated mean.

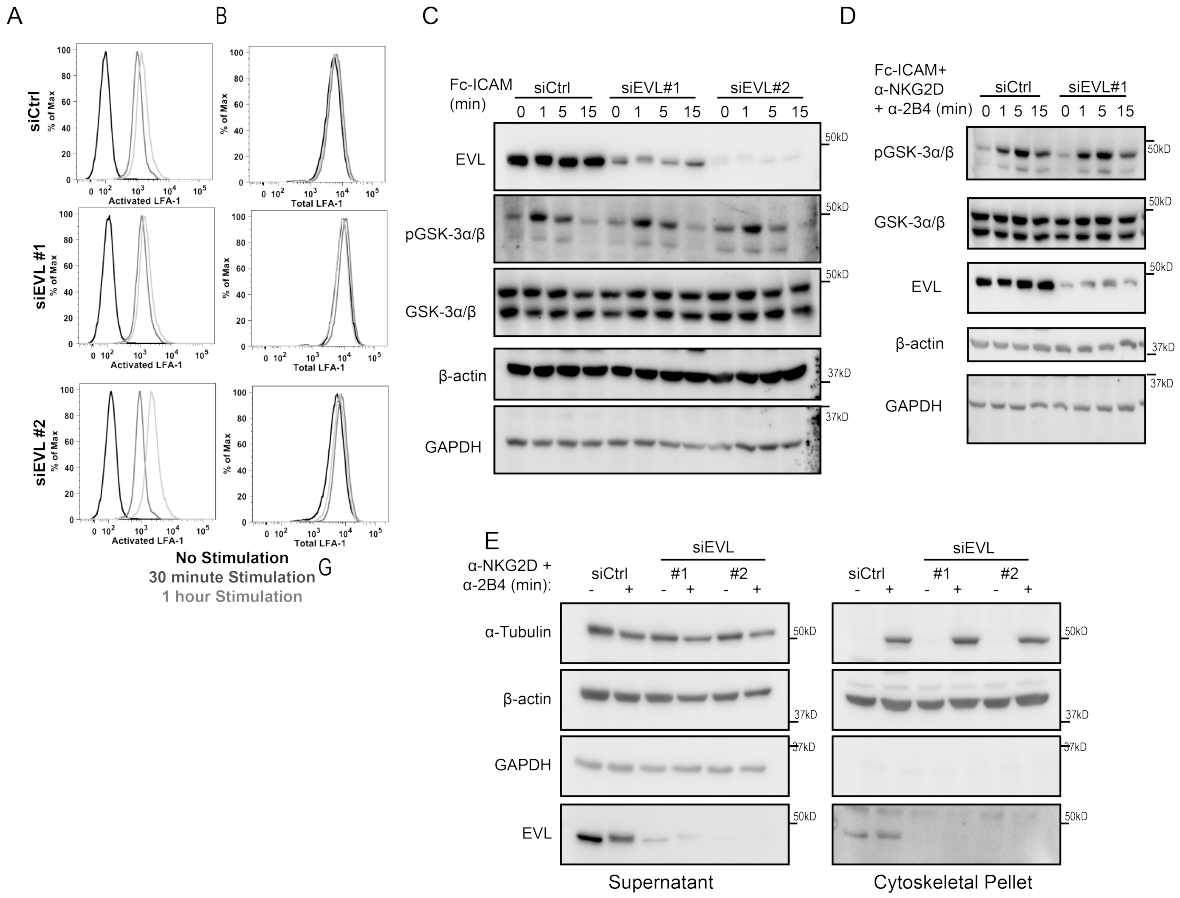

Fig. S2: Integrin LFA-1 expression and signaling are intact in EVL knockdown NKL cells

(A-B) EVL or control siRNA nucleofected NKL were stimulated for the indicated times with anti-NKG2D and anti-2B4, fixed and evaluated by flow cytometry for levels of activated **(A)** and total surface LFA-1 **(B). (C-D).**NKL nucleofected with an EVL-targeting siRNAs or a control siRNA were stimulated by crosslinking with FcICAM alone or a combination of FcICAM, anti-NKG2D and anti-2B4 over the indicated time course. Cell lysates were prepared and equivalent amounts of protein were separated by SDS-PAGE and immunoblotted with the indicated antibodies. **(E)** NKL nucleofected with either control siRNA or siRNA targeting EVL were stimulated for 15 minutes and then their cytoskeletal components and cytoplasmic supernatant were isolated, as indicated in the methods. The resulting fractions were then evaluated by immunoblot for the indicated proteins. Shown are representative experiments chosen from a minimum of 3 independent experiments.

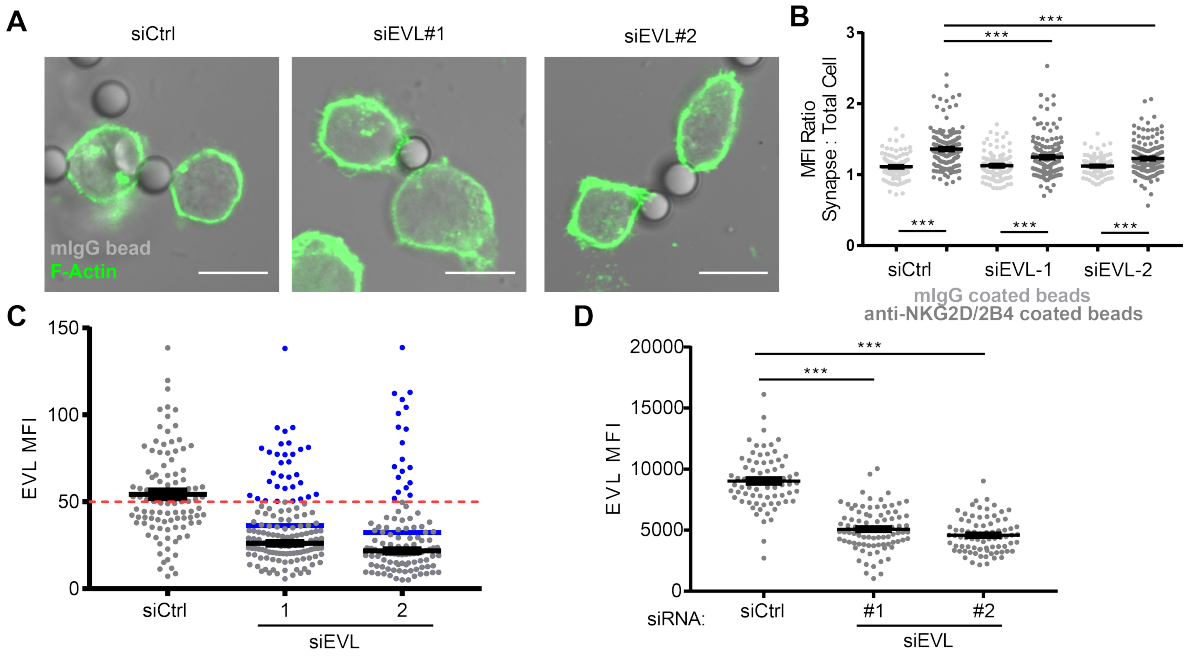

Fig. S3: Control experiments in support of data shown in Figure 4

(A-B) EVL-targeting or control siRNA-treated NKL cells were allowed to form conjugates with mIgG coated beads for 30 minutes, then fixed and stained for F-actin (via phalloidin staining) and EVL. Representative images are shown (A). Quantification of the mean fluorescence intensity (MFI) of F-actin present at the bead interface over the total cell F-actin MFI of the NKL is shown in (B). This data shows a complete comparison of NKL conjugated to mIgG coated beads and NKL conjugated to anti-NKG2D/anti-2B4 coated beads (representative images shown in **Fig. 4**). (C) EVL staining in the NKL-bead conjugates was quantified for each experiment and a threshold set for exclusion. In the representative experiment shown, the red dotted line represents the threshold chosen. Blue data points were excluded based on this threshold. The average and S.E.M. of the EVL MFI is indicated by the black (removing excluded points) and blue horizontal bars (excluded points removed). Of note, significance is not shown in this graph due to complexity. Overall EVL knockdown was significant in all comparisons ($p<0.05$). (D) The MFI of the primary NK cells allowed to spread on coated coverslips in **Fig. 4** were evaluated by immunofluorescence for efficiency of EVL knockdown. Confocal scale bars indicate 10 µm unless otherwise indicated. ***$p<0.0005$ by Student's t-test. Error bars indicate S.E.M. around indicated means. All images are representative of three independent experiments.

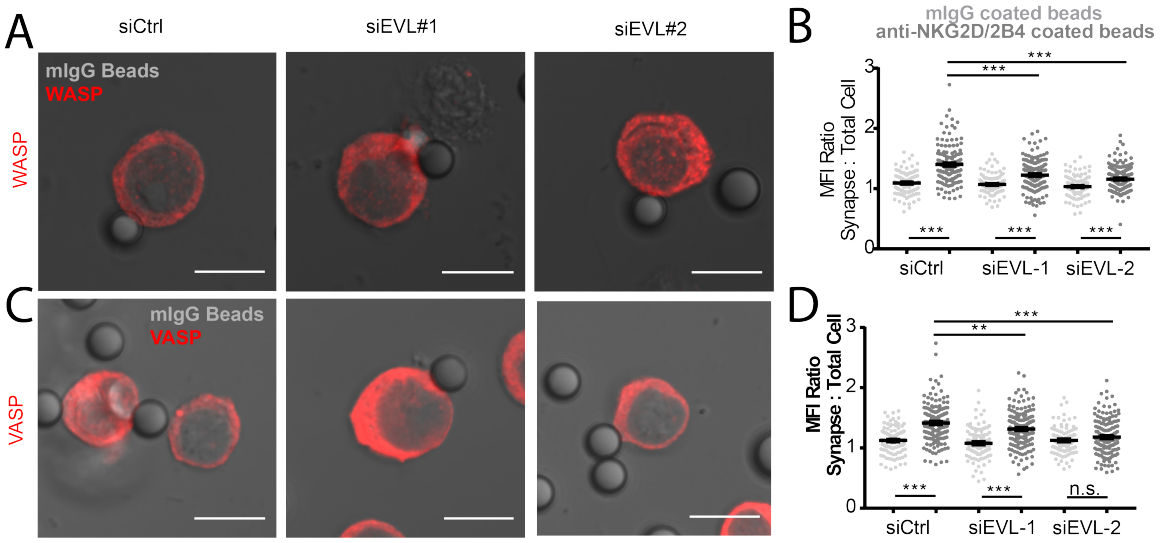

Fig. S4: Control Data in Support of Figure 5

NKL cells nucleofected with EVL-targeting siRNA or control siRNA were allowed to interact with mIgG coated latex beads for 30 minutes. The resulting cell- mIgG bead complexes were fixed and imaged for the presence of (A-B) WASP and (C-D) VASP in order to serve as a control for **Fig. 5**. Representative images are shown (A, C). Quantification of NKL conjugated to mIgG-coated latex beads (light grey) in comparison to NKL conjugated to anti-NKG2D/anti-2B4 coated latex beads (dark grey) is shown in (B, D). Additional data about the NKL adhered to anti-NKG2D/anti-2B4 coated latex beads can be found in **Fig. 5**. Confocal scale bars indicate 10 µm unless otherwise indicated. **p<0.005; ***p<0.0005 by Student's t-test. n.s. indicates p>0.05, and is only indicated for the mIgG-NKG2D/2B4 paired groups. Error bars indicate S.E.M. Images are representative of three independent experiments. Dot graphs show all data collected in three independent experiments.

Table S1 : Detailed Information about Antibodies Used and their Applications

Antibody	Company	Location	Catalog Number	Dilution	Verification / Previous Use
Immunoblot Antibodies					
alpha-Tubulin	Sigma-Aldrich	St. Louis, MO	T6557	1 to 5000	Standard loading control
b-Actin	Sigma-Aldrich	St. Louis, MO	A2228	1 to 5000	Standard loading control
DOCK8	Cocalico	Reamstown, PA	--	1 to 1000	(Ham et al., 2013)
ERK	Cell Signaling Technologies	Danver, MA	9102	1 to 3000	(Li et al., 2008)
EVL	Cocalico	Reamstown, PA	--	1 to 3000	**Figure 2**
GAPDH	Genetex	Irvine, CA	GTX627 408-01	1 to 5000	Standard loading control
GSK-3	Cell Signaling Technologies	Danver, MA	5676	1 to 1000	(Zhang et al., 2014)
pErk	Cell Signaling Technologies	Danver, MA	43705	1 to 1000	(Li et al., 2008)
pGSK-3 Ser 21/9	Cell Signaling Technologies	Danver, MA	9931	1 to 1000	(Schrecengost et al., 2018)
VASP	Cocalico	Reamstown, PA	--	1 to 3000	**Figure 6** and (Wilton and Billadeau, 2018)
VAV1	Cocalico	Reamstown, PA	--	1 to 5000	(Billadeau et al., 1998)
WASP	Cocalico	Reamstown, PA	--	1 to 3000	(Ham et al., 2013)
Immunofluoresence Antibodies					
2B4	R&D Systems	Minneapolis, MN	AF1039	1 to 200	(Wilton and Billadeau, 2018)
EVL	Cocalico	Reamstown, PA	--	1 to 500	**Supplemental Figure 3**
Perforin	BD Pharmingen	San Diego, CA	556434	1 to 200	(Ham et al., 2013); (Wilton and Billadeau, 2018)
VASP	Cocalico	Reamstown, PA	--	1 to 500	(Wilton and Billadeau, 2018)
WASP	Cocalico	Reamstown, PA	--	1 to 500	(Ham et al., 2013); (Wilton and Billadeau, 2018)
Immunoprecipitation					
DOCK8	Cocalico	Reamstown, PA	--	1 to 200	(Ham et al., 2013)
EVL	Cocalico	Reamstown, PA	--	1 to 200	**Figure 5**
pTyr 4G10	Millipore	Burlngton, MA	05-321	1 to 200	(Foy et al., 2007)
VASP	Cocalico	Reamstown, PA	--	1 to 200	(Wilton and Billadeau, 2018)
VAV1	Cocalico	Reamstown, PA	--	1 to 200	**(**Billadeau et al., 1998)
WASP	Cocalico	Reamstown, PA	--	1 to 200	(Ham et al., 2013)
Stimulation					
2B4	Hybridoma	--	Clone C1.7	As indicated per assay	**Supplemental Figure 1**
Anti-Flag	Sigma	St. Louis, MO	Clone M2	As indicated per assay	**Figure 7**
CD16	Hybridoma	--	Clone 3G8	As indicated per assay	(Mandelboim et al., 1999)
Fc-ICAM	R&D Systems	Minneapolis, MN	720-IC	As indicated per assay	**Supplemental Figure 2**
NKG2D	R&D Systems	Minneapolis, MN	MAB139	As indicated per assay	**Supplemental Figure 1**

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Table S2 : Short interfering RNAs used for knockdown experiments

siRNA	Sequence	Company
siCtrl	5’ – UUCUCCGAACGUGUCACGU-3’	ThermoFisher
	Negative Control Medium GC Content	Invitrogen
siEVL #1	5’-UGUUGAUCCGGCUGAAUCCCUGCUG-3’	Invitrogen
siEVL #2	5’-CAGCAGGGAUUCAGCCGGAUCAACA-3’	Invitrogen
siVASP #1	5’ – UGGCGUUCAUCUCUUCCAUGAGUCC-3’	Invitrogen
siVASP #2	5’ – GCCAAGGAUGAAGUCGUCUUCUUCG – 3’	Invitrogen