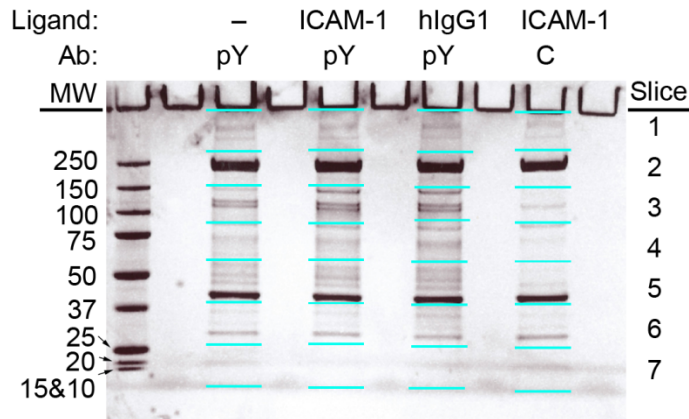
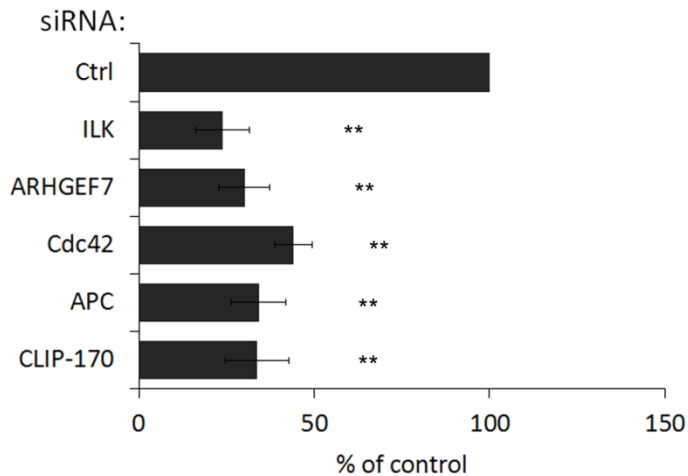


## SUPPLEMENTARY FIGURES AND LEGENDS



**Fig. S1. Preparative Gel for Phosphotyrosine Protein Complexes Analyzed by Mass Spectrometry**

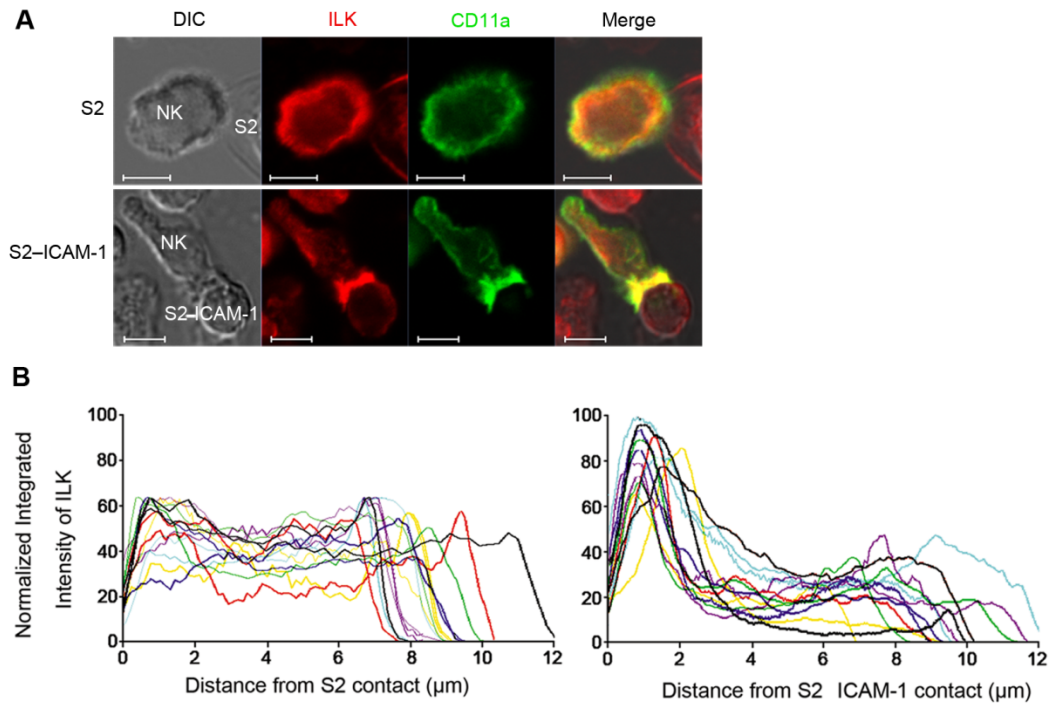
Lysates of cells described in Fig. 1A were incubated with mAb 4G10 (pY) coupled to agarose beads or with isotype control IgG2b mAb MOPC141 (C) coupled to agarose beads. After elution with sodium phenyl phosphate, samples were concentrated by Amicon Ultra-0.5 centrifugal filter with a 3K membrane, run over a 4%-12% Bis-Tris gel, and stained with colloidal blue. Each lane was cut into seven slices, as indicated with blue bars, and sent for mass spectrometry analysis. Spectral counts for each of the seven slices are shown in Table S1.



**Fig. S2. Granule Polarization toward 721.221 Cells**

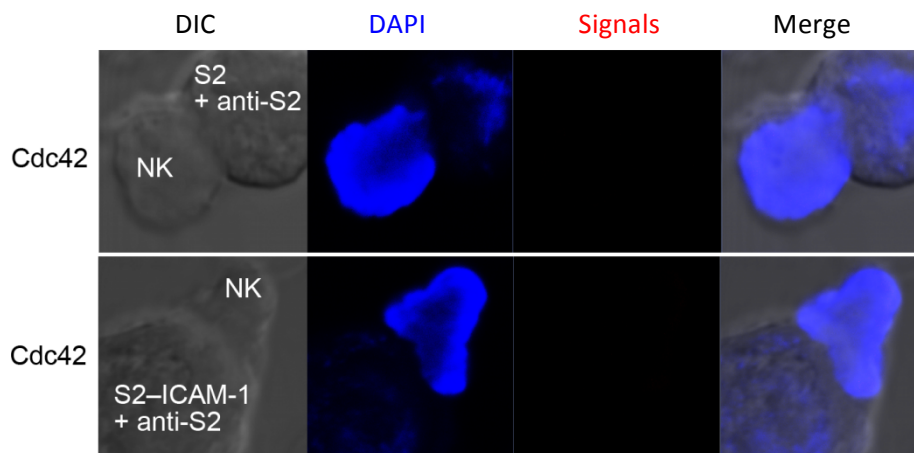
NK cells were transfected with control (Ctrl) or specific siRNA, as indicated, and were mixed with 721.221 cells for 20 min at 37°C. Cells were fixed, permeabilized and stained with mAb to perforin (IgG2b) and  $\beta$ -tubulin (IgG1) followed by isotype-specific Alexa Fluor 488-conjugated and Alexa Fluor 647-conjugated secondary antibodies. Quantitative analysis of MTOC and

granule polarization toward 721.221 cells after knock-downs is shown. Graphs show mean  $\pm$  SEM from 3 experiments. **\*\*p<0.01.**



**Fig. S3. ILK Recruitment to the Site of Contact with S2-ICAM-1 Cells**

(A) Representative images of ILK and LFA-1 in NK cells mixed with S2 or S2-ICAM-1 cells. (B) Tracings of the fluorescence intensity of ILK in primary NK cells mixed with S2 (n=16) or S2-ICAM-1 (n=13) cells for 20 min. Cells were fixed, permeabilized, and incubated with mouse mAb to ILK and rabbit mAb to CD11a, followed by specific Alexa Fluor 488-conjugated and Alexa Fluor 647-conjugated secondary antibodies.



**Fig. S4. Rabbit Anti-S2 Cell Antibodies Do not Generate Proximity Ligation Signals with Mouse mAb to Cdc42**

Primary NK cells were mixed for 20 min with S2 or S2–ICAM-1 cells that were pre-loaded with rabbit anti-S2 serum. Cells were fixed, permeabilized, incubated with mouse mAb to Cdc42, and tested by Proximity Ligation Assay. Analysis of 24 S2 cells and 22 S2–ICAM-1 cells is shown in Fig. 7.

**SUPPLEMENTARY TABLES**

Table S1. Spectral Count HeatMap of Mass Spectrometry Data. (See the supplemental excel file)

Table S2. Top Spectral Counts in Isotype Control Pull-down.

Ligand:	–	ICAM-1		hIgG1
mAb:	4G10	MOPC141	4G10	4G10
<i>MYH9</i>	1718	1654	1481	1441
<i>ACTB</i>	499	540	467	442
<i>TPM3</i>	152	180	145	127
<i>MYL6</i>	95	135	114	85
<i>MYO1G</i>	135	133	91	94
<i>GSN</i>	85	93	73	45
<i>TPM4</i>	86	89	53	55
<i>HSPA5</i>	25	75	18	20
<i>MYO18A</i>	70	69	50	47
<i>MYL12B</i>	41	57	61	32
<i>ACTR3</i>	44	54	55	50
<i>ARPC1B</i>	37	52	32	34
<i>HSPA9</i>	23	49	30	27
<i>HSPA8</i>	39	44	39	48
<i>VIM</i>	20	43	20	18
<i>HCLS1</i>	54	38	65	61
<i>ACTR2</i>	27	37	24	22
<i>MYO1D</i>	39	35	22	26
<i>TMOD3</i>	28	35	30	23
<i>CAPZB</i>	42	34	27	31

Table S3. Constitutive Phosphotyrosine Protein Complexes in NK Cells.

Ligand:	–	ICAM-1		hIgG1	Ligand:	–	ICAM-1		hIgG1
mAb:	4G10	MOPC141	4G10	4G10	mAb:	4G10	MOPC141	4G10	4G10
<i>FYB</i>	200	1	166	139	<i>PRKDC</i>	20	1	43	46
<i>INPP5D</i>	141	0	215	191	<i>TRAPPC10</i>	19	0	12	9
<i>LCK</i>	81	13	64	64	<i>RBM14</i>	19	1	19	22
<i>MICAL3</i>	61	0	51	46	<i>HNRNPD</i>	19	2	21	18
<i>LYN</i>	60	4	52	50	<i>PTPN6</i>	19	3	23	23
<i>PTK2B</i>	58	0	153	91	<i>PXN</i>	18	0	61	39
<i>PAG1</i>	55	5	64	53	<i>DOK2</i>	18	0	36	27
<i>ARHGAP27</i>	53	0	54	31	<i>DOK1</i>	18	0	26	17
<i>SKAP1</i>	51	0	72	54	<i>LAT2</i>	18	2	13	23
<i>CLNK</i>	47	0	55	54	<i>HNRNPUL2</i>	17	0	13	9
<i>KHDRBS1</i>	47	1	32	40	<i>RNF213</i>	17	1	33	52
<i>HNRNPF</i>	44	1	59	57	<i>ARHGAP9</i>	16	0	22	14
<i>FYN</i>	43	1	38	36	<i>ARHGAP12</i>	16	0	20	10
<i>TXK</i>	41	0	57	33	<i>ANXA11</i>	16	0	11	10
<i>AHSG Precursor</i>	41	0	1	0	<i>LASP1</i>	16	1	19	19
<i>SGK223</i>	39	0	23	18	<i>HGS</i>	15	0	19	22
<i>SNX18</i>	38	0	38	34	<i>SYNCRIP</i>	15	0	23	19
<i>HNRNPH3</i>	38	0	27	28	<i>FUS</i>	15	0	15	15
<i>TRAPPC9</i>	38	0	10	9	<i>DOCK8</i>	15	0	16	14
<i>CSK</i>	37	2	42	46	<i>G6PD</i>	15	2	10	13
<i>EPS15L1</i>	36	0	40	40	<i>ZAP70</i>	14	0	13	80
<i>SKAP2</i>	34	0	36	29	<i>CASS4</i>	14	0	54	25
<i>ARAP2</i>	30	0	32	31	<i>GRB2</i>	14	0	15	18
<i>HNRNPA2B1</i>	30	1	28	28	<i>JAK3</i>	14	0	17	9
<i>VAV1</i>	28	0	43	36	<i>DHX9</i>	14	0	3	5
<i>DBNL</i>	28	2	36	22	<i>RPS3</i>	13	4	8	23
<i>TRIP10</i>	26	0	24	35	<i>RAC2</i>	13	4	11	9
<i>RPS27A</i>	26	4	22	58	<i>PIK3R1</i>	12	0	10	12
<i>PSTPIP1</i>	25	0	38	33	<i>HNRNPH1</i>	12	2	12	9
<i>SEC16A</i>	25	0	32	31	<i>MAPK14</i>	11	0	16	18
<i>ITSN2</i>	25	0	15	19	<i>PIK3AP1</i>	11	0	18	13
<i>TNK2</i>	25	1	30	19	<i>CAD</i>	11	0	12	10
<i>AHNAK</i>	24	7	43	29	<i>AFAP1L2</i>	11	0	16	8
<i>NEDD9</i>	24	0	143	65	<i>JAK1</i>	11	0	7	4
<i>CBL</i>	24	0	18	32	<i>EZR</i>	11	1	13	8
<i>PTPRA</i>	24	0	19	14	<i>SLC25A5</i>	11	2	7	12
<i>SH2D3C</i>	22	0	119	66	<i>RASAL3</i>	11	2	7	12
<i>STAT5B</i>	20	0	17	16	<i>YWHAZ</i>	11	3	10	14

Proteins are ranked by spectral counts (cut-off at  $\geq 11$ ) in the 4G10 pull-down from NK cells incubated on plates without ligand (BSA only). Only proteins with  $\text{Log}(-/\text{MOPC141}) \geq 1.7$  are included.

Table S4. Top Spectral Counts in 4G10 Pull-down from ICAM-1 Stimulated NK Cells.

Protein	ICAM-1/- score	Spectral counts			Protein	ICAM-1/- score	Spectral counts		
		-	ICAM-1	hIgG1			-	ICAM-1	hIgG1
<i>LPXN</i>	5	0	13	6	<i>PTPRJ</i>	2	2	8	5
<i>CBLB</i>	4.6	0	10	26	<i>IL1RL2</i>	2	1	4	1
<i>PLCG2</i>	4.3	1	20	13	<i>USP9X</i>	2	1	4	12
<i>FLNA</i>	4.2	2	38	35	<i>ERAP1</i>	2	1	4	9
<i>CHN2</i>	4.1	0	7	8	<i>CASS4</i>	1.9	14	54	25
<i>RASA1</i>	3.6	0	5	8	<i>ILK</i>	1.9	8	30	18
<i>GRAP2</i>	3.3	1	10	12	<i>PXN</i>	1.8	18	61	39
<i>ATM</i>	3.3	0	4	14	<i>KLRB1</i>	1.8	2	7	3
<i>YWHAE</i>	3.3	0	4	6	<i>CLTC</i>	1.7	4	13	17
<i>SOS1</i>	3.3	0	4	7	<i>NCK1</i>	1.7	3	10	11
<i>MAPK1</i>	3.3	0	4	3	<i>LCP2</i>	1.6	10	30	37
<i>SMARCA2</i>	3	1	8	4	<i>RSU1</i>	1.6	3	9	9
<i>VAV3</i>	2.9	0	3	8	<i>ALO17</i>	1.6	3	9	10
<i>ARAP1</i>	2.9	0	3	7	<i>RPN1</i>	1.6	1	3	4
<i>CYTH4</i>	2.8	1	7	3	<i>H1FX</i>	1.6	1	3	2
<i>NEDD9</i>	2.6	24	143	65	<i>UQCRC2</i>	1.6	1	3	3
<i>SH2D3C</i>	2.4	22	119	66	<i>TRA2B</i>	1.6	1	3	5
<i>SEC23B</i>	2.3	2	10	4	<i>MYO1E</i>	1.6	1	3	2
<i>FCGR3A</i>	2.3	1	5	7	<i>CASP7</i>	1.6	1	3	1
<i>LIMS1</i>	2.3	1	5	1	<i>ARHGEF7</i>	1.6	1	3	0
<i>PLCG1</i>	2.2	4	18	16	<i>FGR</i>	1.5	5	14	13
<i>UTRN</i>	2	9	35	9	<i>PTK2B</i>	1.4	58	153	91

Proteins are ranked by log (ICAM-1/-) score. Proteins with a log (ICAM-1/-)  $\geq 1.4$ , spectral count  $\geq 3$  in ICAM-1 stimulation and log (ICAM-1/MOPC141)  $\geq 1$  are included.

Table S5. Top Spectral Counts in 4G10 Pull-down from Human IgG1 Stimulated NK Cells.

Protein	hlgG1/- score	Spectral counts			Protein	hlgG1/- score	Spectral counts		
		-	ICAM-1	hlgG1			-	ICAM-1	hlgG1
<i>ALB</i>	6.6	0	0	39	<i>TSG101</i>	2.3	1	2	5
<i>S100A9</i>	6.3	0	0	31	<i>TRA2B</i>	2.3	1	3	5
<i>CBLB</i>	6	0	10	26	<i>NCK2</i>	2.2	2	4	9
<i>ATM</i>	5.1	0	4	14	<i>UBASH3B</i>	2.2	2	5	9
<i>S100A8</i>	4.8	0	0	11	<i>CLTC</i>	2.1	4	13	17
<i>IGHG1</i>	4.5	0	0	9	<i>PLCG1</i>	2	4	18	16
<i>IGLC2</i>	4.5	0	0	9	<i>PPIB</i>	2	2	2	8
<i>VAV3</i>	4.3	0	3	8	<i>LAX1</i>	2	2	3	8
<i>RASA1</i>	4.3	0	5	8	<i>PRKCD</i>	2	2	3	8
<i>CHN2</i>	4.3	0	7	8	<i>UNC13D</i>	2	1	0	4
<i>FLNA</i>	4.1	2	38	35	<i>ATP1A1</i>	2	1	1	4
<i>SERPINB3</i>	4.1	0	0	7	<i>GZMK</i>	2	1	2	4
<i>ARAP1</i>	4.1	0	3	7	<i>CPT1A</i>	2	1	2	4
<i>SOS1</i>	4.1	0	4	7	<i>ELMO1</i>	2	1	2	4
<i>IGKC</i>	3.9	0	0	6	<i>FCRL3</i>	2	1	2	4
<i>LAT</i>	3.9	0	0	6	<i>TMEM109</i>	2	1	2	4
<i>YWHAE</i>	3.9	0	4	6	<i>RPN1</i>	2	1	3	4
<i>LPXN</i>	3.9	0	13	6	<i>SMARCA2</i>	2	1	8	4
<i>PLCG2</i>	3.7	1	20	13	<i>LCP2</i>	1.9	10	30	37
<i>USP9X</i>	3.6	1	4	12	<i>NCK1</i>	1.9	3	10	11
<i>GRAP2</i>	3.6	1	10	12	<i>GRAP</i>	1.8	2	3	7
<i>CASP14</i>	3.6	0	0	5	<i>TYROBP</i>	1.8	2	4	7
<i>SPRR2D</i>	3.6	0	0	5	<i>TBX21</i>	1.8	2	5	7
<i>GZMM</i>	3.6	0	1	5	<i>ALO17</i>	1.7	3	9	10
<i>FAM62B</i>	3.6	0	2	5	<i>SH2D3C</i>	1.6	22	119	66
<i>SYK</i>	3.5	4	8	45	<i>RNF213</i>	1.6	17	33	52
<i>CD247 transcript variant2</i>	3.3	0	0	4	<i>FCER1G</i>	1.6	7	17	21
<i>RPS19</i>	3.3	0	0	4	<i>RPS16</i>	1.6	4	7	12
<i>SPRR1B</i>	3.3	0	0	4	<i>RSU1</i>	1.6	3	9	9
<i>TXN</i>	3.3	0	0	4	<i>HMGB2</i>	1.6	2	2	6
<i>CD48</i>	3.3	0	1	4	<i>RNMT</i>	1.6	2	4	6
<i>XPO1</i>	3.3	0	1	4	<i>RCN2</i>	1.6	1	1	3
<i>ERAP1</i>	3.2	1	4	9	<i>AGTRAP</i>	1.6	1	1	3
<i>ANKRD13A</i>	3	1	0	8	<i>SFXN1</i>	1.6	1	1	3
<i>UNC84B</i>	2.9	0	0	3	<i>CD44</i>	1.6	1	2	3
<i>CALML5</i>	2.9	0	0	3	<i>PTPRC</i>	1.6	1	2	3
<i>TF</i>	2.9	0	0	3	<i>UQCRC2</i>	1.6	1	3	3
<i>DNAJC10</i>	2.9	0	2	3	<i>CYTH4</i>	1.6	1	7	3
<i>SFN</i>	2.9	0	2	3	<i>CD247</i>	1.5	10	9	29
<i>MAPK1</i>	2.9	0	4	3	<i>EPS15</i>	1.5	4	5	11
<i>FCGR3A</i>	2.8	1	5	7	<i>NEDD9</i>	1.4	24	143	65
<i>GZMB</i>	2.7	2	1	13	<i>UBASH3A</i>	1.4	6	10	16
<i>S100A7</i>	2.6	3	0	18	<i>FGR</i>	1.4	5	14	13
<i>TGM3</i>	2.6	1	0	6	<i>PRPF39</i>	1.4	3	6	8
<i>ZAP70</i>	2.5	14	13	80					

Proteins are ranked by log (hlgG1/-) score. Proteins with a log (hlgG1/-)  $\geq$  1.4, spectral count  $\geq$  3 in hlgG1 stimulation and log (hlgG1/MOPC141)  $\geq$  1 are included.

Table S6. Phosphotyrosine Protein Complexes Selectively Enriched in ICAM-1 Stimulated over Human IgG1 Stimulated NK Cells.

Protein	ICAM-1/hlgG1 score	Spectral counts		
		–	ICAM-1	hlgG1
<i>PEAK1</i> <sup>a</sup>	3.6	2	5	0
<i>ARHGEF7</i>	2.9	1	3	0
<i>C19orf35</i> <sup>a</sup>	2.8	3	7	1
<i>LIMS1</i>	2.3	1	5	1
<i>PARVG</i> <sup>a</sup>	2.3	2	5	1
<i>IL1RL2</i>	2	1	4	1
<i>UTRN</i>	2	9	35	9
<i>CASP7</i>	1.6	1	3	1
<i>TEC</i> <sup>a</sup>	1.6	5	9	3
<i>SEC23B</i>	1.3	2	10	4
<i>CYTH4</i>	1.2	1	7	3
<i>KLRB1</i>	1.2	2	7	3
<i>CASS4</i>	1.1	14	54	25
<i>LPXN</i>	1.1	0	13	6
<i>NEDD9</i>	1.1	24	143	65
<i>SMARCA2</i>	1	1	8	4
<i>SH2D3C</i>	0.9	22	119	66
<i>ILK</i>	0.7	8	30	18
<i>PTK2B</i>	0.7	58	153	91
<i>PTPRJ</i>	0.7	2	8	5
<i>H1FX</i>	0.6	1	3	2
<i>MYO1E</i>	0.6	1	3	2
<i>PXN</i>	0.6	18	61	39

Proteins are ranked by log (ICAM-1/hlgG1) score. Proteins from Table S5 were subtracted from Table S4, unless log (ICAM-1/hlgG1)  $\geq$  0.9, and proteins with a log (ICAM-1/hlgG1)  $\geq$  1.4 with  $\geq$  3 spectra, were added. <sup>a</sup>These 4 proteins are not in Table S3 due to low log (ICAM-1/–) scores..

Table S7. Primers Used in Experiments.

<i>GAPDH</i>	Forward	5'-CCATGAGAAGTATGACAACAGCC-3'
	Reverse	5'-GGGTGCTAAGCAGTTGGTG-3'
<i>ILK</i>	Forward	5'-TCAAACAGCTTAACTTCCTGACG-3'
	Reverse	5'-AGCACATTTGGATGCGAGAAAA-3'
<i>PARVG</i>	Forward	5'-GCCGGAGTTCTTGTACGACC-3'
	Reverse	5'-ATAGGTGGTGTAGGATGAGCC-3'
<i>LPXN</i>	Forward	5'-CATGGCTCACCTGACTGAGAT-3'
	Reverse	5'-GTTTCTGGCAGGATGCACAA-3'
<i>ARHGEF7</i>	Forward	5'-ACACTCAATTCAAGTTCACGCA-3'
	Reverse	5'-CTCATCCCAGGCAGGATCATT-3'
<i>SH2D3C</i>	Forward	5'-ATGACAGAGGGGACCAAGAAG-3'
	Reverse	5'-AGGATCGTCTCAGAGTGAAGG-3'
<i>NEDD9</i>	Forward	5'-ATGGCAAGGGCCTTATATGACA-3'
	Reverse	5'-TTCTGCTCTATGACGGTCAGG-3'
<i>PTPRJ</i>	Forward	5'-ACAGAGCCGATCCCAGTTTCT-3'
	Reverse	5'-CTTGAGTCTTGAGTCAACTCCTC-3'
<i>CDC42</i>	Forward	5'-CCATCGGAATATGTACCGACTG-3'
	Reverse	5'-CTCAGCGGTCGTAATCTGTCA-3'
<i>PAR6A</i> (Par6)	Forward	5'-AGCATCGTCGAGGTGAAGAG-3'
	Reverse	5'-GTATAGCCAAGTAGCACGTCC-3'
<i>APC</i>	Forward	5'-AGGCTGCATGAGAGCACTTGTG-3'
	Reverse	5'-CACACTTCCAATTCTCGCAACG-3'
<i>CLIP1</i> (CLIP-170)	Forward	5'-AGAAGACGCTGCTGGACACAGA-3'
	Reverse	5'-TGGCATCTCCGCTGTTTGAGC-3'
<i>SYK</i>	Forward	5'-TGCACTATCGCATCGACAAAG-3'
	Reverse	5'-CATTTCCCTGTGTGCCGATTT-3'



Table S8. Antibodies Used in Experiments.

Molecule (clone)	Isotype	Source	Catalog#
	H <sup>a</sup> IgG1	Sigma	5029
p-Tyr (4G10-biotin)	M <sup>a</sup> IgG2b	Millipore	16-103
(MOPC141)	M IgG2b	Sigma	M8894
Perforin (deltaG9)	M IgG2b	Thermo	MA1-91079
$\beta$ -tubulin (TUB2.1)	M IgG1	Sigma	T4026
ILK (65.1)	M IgG2b	Santa Cruz	sc-20019
$\gamma$ -parvin (8C5.2)	M IgG1	Millipore	MAB2621
Leupaxin (N-14)	G <sup>a</sup> polyclonal IgG	Santa Cruz	sc-241521
Pyk2 (C-19)	G polyclonal IgG	Santa Cruz	sc-1515
RhoGEF7	R <sup>a</sup> polyclonal IgG	Abcam	ab92657
GSK3 $\beta$ (H-76)	R polyclonal IgG	Santa Cruz	sc-9166
pSer9-GSK3 $\beta$	R polyclonal IgG	Cell Signaling	9336
Actin	R polyclonal IgG	Cytoskeleton	AAN01
$\beta$ 2 integrin, C-terminus	R polyclonal IgG	LSBio	LS-C117844
CD11a (EP1285Y)	R monoclonal IgG	Abcam	ab52895
Talin (8D4)	M IgG1	Sigma	T3287
Cdc42 (B-8)	M IgG3	Santa Cruz	sc-8401
WASp	R polyclonal IgG	Upstate	07-066

<sup>a</sup>H, human; M, mouse; G, goat; R, rabbit.

Table S9. siRNA Used in Experiments.

Molecule	siRNA target	Catalog#	Ref.
<i>ILK</i>	5'-GGGCAAUGACAUUGUCGUG-3'		(Durbin et al., 2009)
<i>PARVG</i> ( $\gamma$ -parvin)	5'-GGACGUCUUUGAUGAAUUA-3'	J-017821-17-0020	
<i>LPXN</i> (Leupaxin)	5'-UCCAGGAGCUCAAUGUCUA-3'	J-009746-08-0020	
<i>PTK2B</i> (Pyk2)	5'-GGAUCAUCAUGGAAUUGUA-3'		Designed by Dharmacon s iDESIGN Center
<i>ARHGEF7</i>	5'-GGACGAGCUUCCUUCUCA-3'	J-009616-07-0020	
<i>SH2D3C</i> (Nsp3)	5'-GGACUCAUCGCCAGAGAAA-3'	J-019971-08-0020	
<i>NEDD9</i> (CasL)	5'-CCAGGACAUUCGCAACAAA-3'		(Natarajan et al., 2006)
<i>PTPRJ</i> (CD148)	5'-GCAGUACAGCAGAAUCCUU-3'		(Takahashi et al., 2006)
<i>CDC42</i>	5'-GAUGACCCCUCUACUUAUUG-3'	J-005057-07-0020	
<i>PARD6A</i> (Par6)	5'-GCAAAUUUGACGCCGAGUU-3'	J-013859-05-0020	
<i>APC</i>	5'-AAGACGUUGCGAGAAGUUGGA-3'		(Hadjihannas et al., 2006)
<i>CLIP1</i> (CLIP-170)	5'-UGAAGAUGUCAGGAGAUAA-3'		(Watson and Stephens, 2006)
<i>SYK</i>	5'-CCUCAUCAGGGAAUAUCUGUU-3'		(Cheng et al., 2011)
Non-target	ON-TARGETplus Non-targeting siRNA #1	D-001810-01-50	

All siRNAs are from Dharmacon.