SUPPLEMENTAL DATA

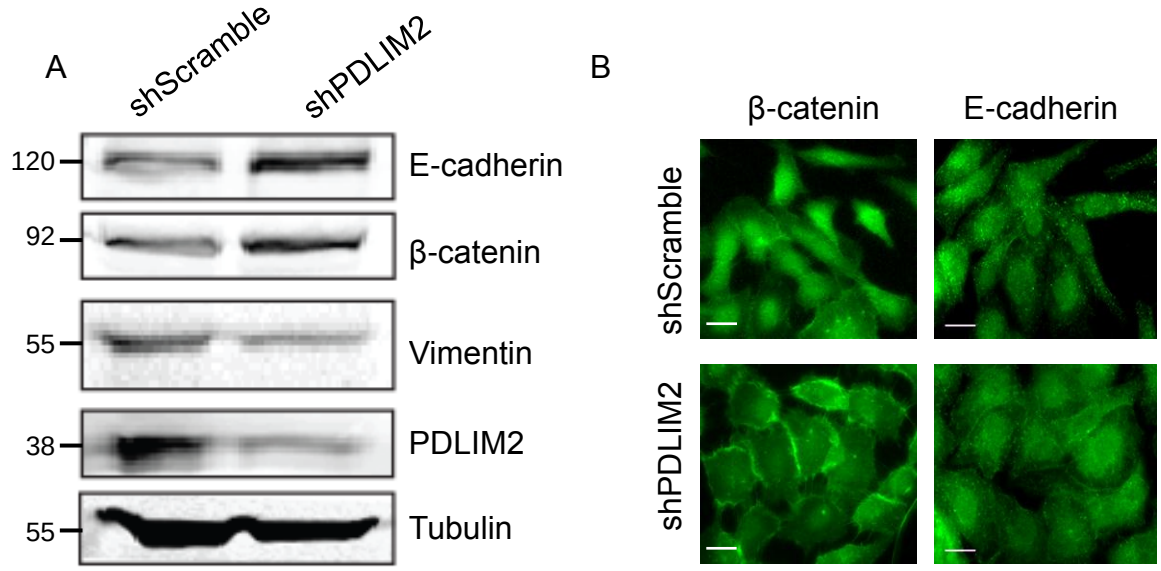
Supplemental Movies 1 and 2: (related to Figure 2)

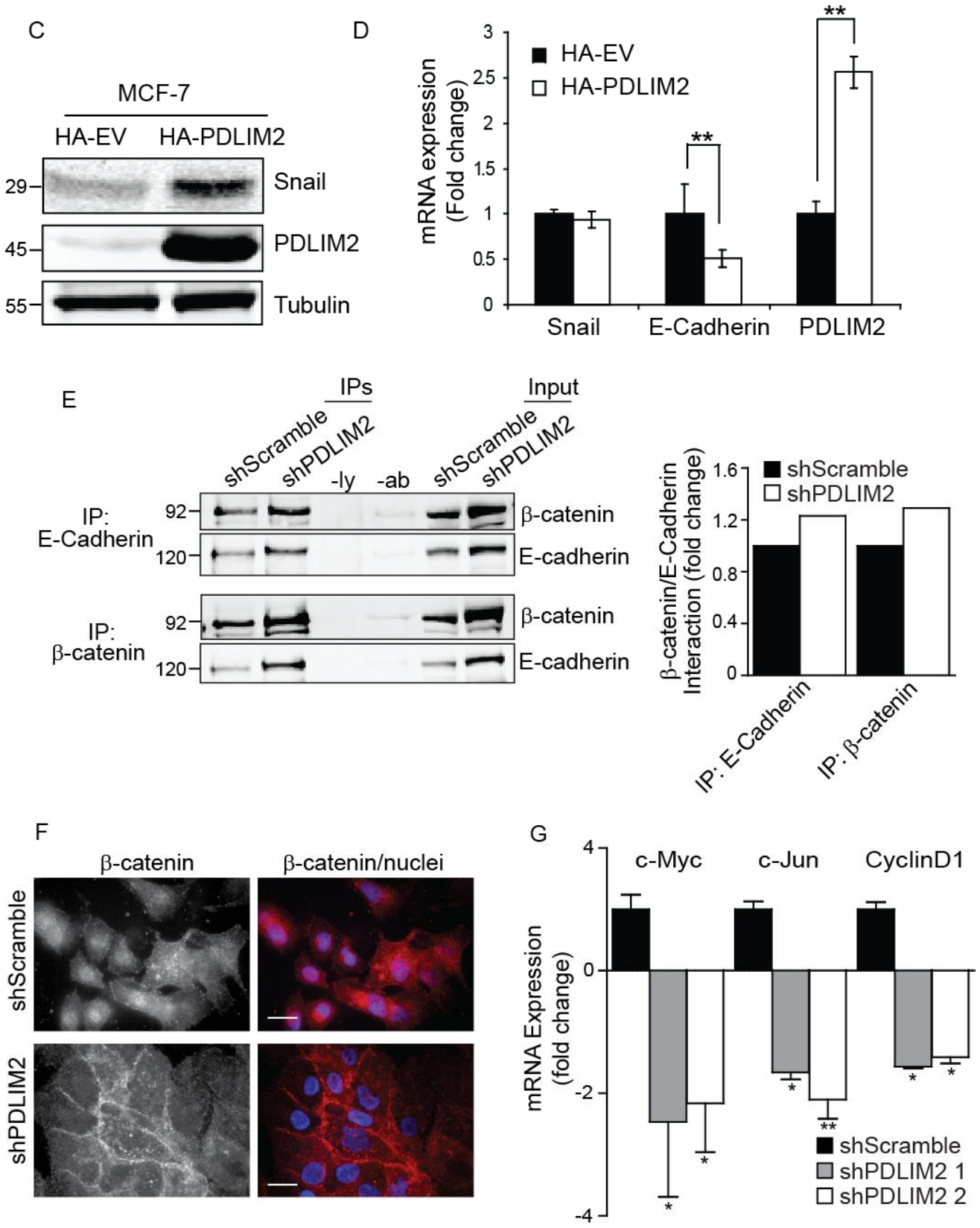
Lack of Directional migration in cells with suppressed PDLIM2

Confluent monolayers of shScramble and shPDLIM2 DU145 cells were wounded with a pipette tip and cells migrating into the wound tracks were visualised and photographed by Timelapse phase microscopy over 12h. Original magnification is 10x.

Supplemental Figure S1 (related to Figure 3)

PDLIM2 regulates expression of epithelial and mesenchymal markers and β-catenin transcriptional activity is decreased in cells with suppressed PDLIM2.



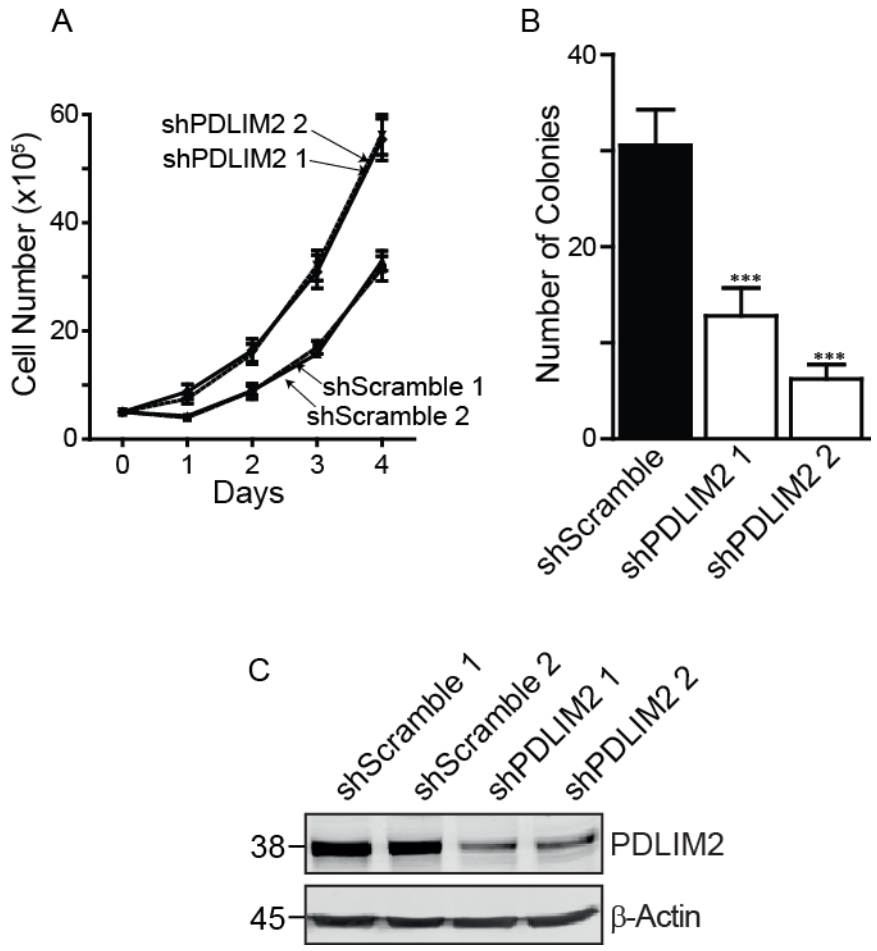


(A) shScramble and shPDLIM2 DU145 were grown on collagen-coated plates for 48h. Cells were lysed and expression of proteins indicated was analysed by western blotting, tubulin was used as loading control. **(B)** Cells cultured as in (A) were stained for β-catenin and E-

cadherin expression as described in methods. Scale bar is 20µm, representative fields of at least 3 independent experiments are shown. **(C)** MCF7 cells stably transfected with HA-Empty Vector (EV) or HA-PDLIM2 as described in supplemental procedures, were lysed and processed for protein expression of Snail, PDLIM2 and tubulin by western blotting. **(D)** qPCR on total RNA extracted from MCF7 cells stably expressing HA-EV or HA-PDLIM2 was performed to examine expression of Snail, E-cadherin and PDLIM2. **(E)** Immunoprecipitations (IPs) of cell lysates for E-cadherin (Top panels) or β-catenin (Bottom panels) were performed as described in Supplemental methods. Immune complexes were probed for both proteins, detected by Western blotting. Protein interaction relative to total levels was quantified by densitometry using Odyssey software, normalised to shScramble. Controls for IPs included no lysate (-ly) or beads plus lysate only, no antibody (-ab). Experiments were repeated at least twice, representative data are shown. **(F)** shScramble and shPDLIM2 DU145 were cultured overnight and stained for β-catenin as described in methods. Nuclei were stained with Hoechst (blue), representative fields of at least 3 independent experiments are shown, scale bar is 20µm. **(G)** mRNA expression of β-catenin target genes was assessed by qPCR as described in methods. mRNA expression in 2 clones of shPDLIM2 DU145 were normalised to 2 shScramble clones and expressed as mean fold change +/- SEM (*$P<0.05$, **$P<0.005$).

Supplemental Figure S2 (related to Figure 3)

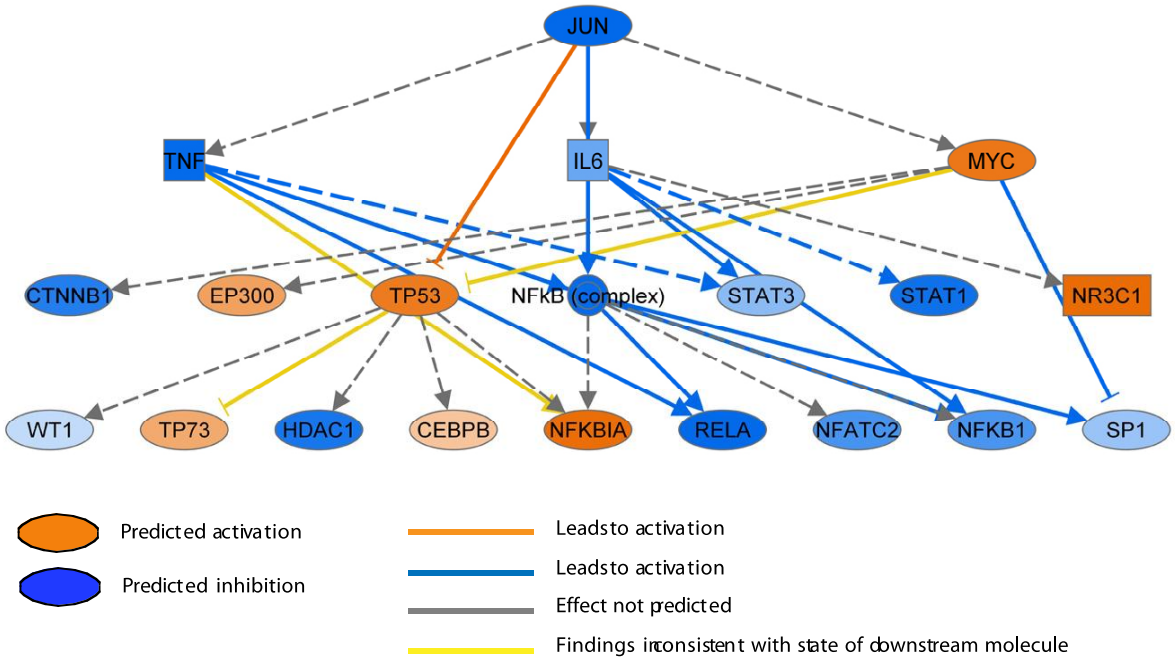
MDA-MB-231 breast cancer cells with suppressed PDLIM2 exhibit increased proliferation and decreased clonogenic growth.



(A) Proliferation of MDA-MB-231 cells stably expressing control scrambled shRNA (shScramble) or PDLIM2 shRNA (shPDLIM2) initially cultured at equal densities was measured by crystal violet staining over 5 days. Data represents mean cell numbers of 6 wells per cell type from a representative experiment of 4. **(B)** shScramble and shPDLIM2 MDA-MB-231 cells were plated at equal densities and grown in soft agarose for 7 weeks. Colonies were stained with crystal violet and counted as described in methods. Data are presented as mean number of colonies from triplicate wells per cell type (***P<0.0005). **(C)** Cell lysates from 2 clones of both shScramble and shPDLIM2 MDA-MB-231 cells were analysed for PDLIM2 expression by western blotting, with β-actin as loading control.

Supplemental Figure S3 (related to Figures 5 and 6)

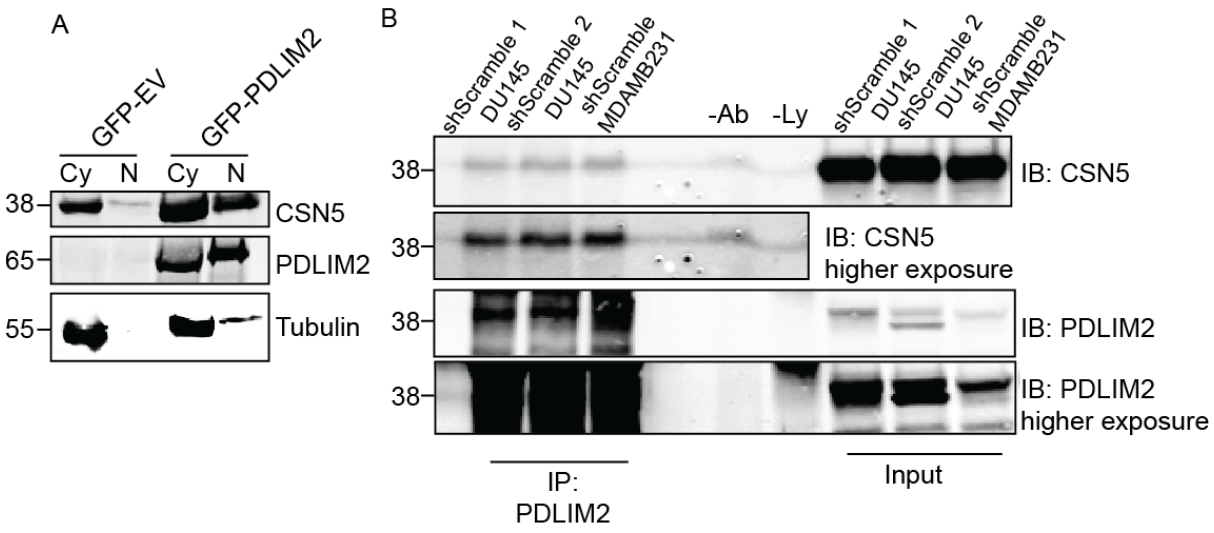
Mechanistic Network predicted using Ingenuity Pathway Analysis.



Mechanistic Network predicted using Ingenuity Pathway Analysis. Molecules predicted to be active are shown in orange, while molecules predicted to be inhibited are shown in blue. In both cases, the darker the colour, the stronger the activation or inhibition is. The relationships between molecules are colour-coded and indicated in the Figure key.

Supplemental Figure S4 (related to Figure 7)

Overexpression of PDLIM2 causes increased nuclear expression of CSN5 and endogenous PDLIM2 associates with CSN5 in other cell types.



(A) Cytoplasmic (Cy) and Nuclear (N) fractions were prepared from MCF7 cells expressing GFP-EV or GFP-PDLIM2 and probed for PDLIM2 and CSN5 and tubulin as loading control. **(B)** PDLIM2 IPs were performed from lysates of shScramble DU145 and MDA-MB-231 cells. IPs were probed with CSN5 antibody to detect interaction. Data are representative of at least 2 independent experiments with similar results.

Supplemental Table 1: (related to Figures 5 and 6)

List of genes differentially expressed in shPDLIM2 DU145 cells compoared with shScramble control cells.

Bowe et al_Supplemental Table 1 PDLIM2 Microarray Differential Gene expression.xls

The gene expression profile of DU145 cells with stable suppression of PDLIM2 was compared with control DU145 cells by RNA profiling and data analysis as described in Materials and Methods. Included are Affymetrix ID, fold change, p-value, gene accession number and sequence description for each differentially expressed gene.

Supplemental Table 2: (related to Figure 6)

Ingenuity Pathway Analysis of Biofunctions related to differentially expressed genes between shPDLIM2 and shScramble control DU145 cells.

Bowe et al_Supplemental Table 2 Biofunctions.xls

The list of genes differentially expressed in shPDLIM2 DU145 was subjected to Ingenuity Pathway analysis for associated signalling pathways and networks and a Biofunction category list generated. p-values validate the relationship between the Biofunctions and molecules involved. The top 20 Biofunctions from this list are shown in Fig. 6A.

Supplemental Table 3: (related to Figure 6)

Ingenuity Pathway analysis of the predicted activation status of cellular functions associated with Biofunction categories of differentially expressed genes in shPDLIM2 DU145 cells compared with shScramble control cells.

Bowe et al_Supplemental Table 3_ Cellular Functions Activation status.xls

Ingenuity Pathway Analysis (IPA) was performed to predict the activation state of specific cellular functions within affected Biofunctions determined by IPA analysis of genes differentially expressed in shPDLIM2 DU145 cells as described for Table 2. Data shows predicted activation state, regulation z-score, p-value and associated molecules.

Supplemental Table 4: (related to Figure 6)

Predicted activation state of Transcription Factor families determined by Ingenuity Pathway Analysis

Bowe et al_Supplemental Table 4 TFs_ActivationZ-score.xls

The list of genes differentially expressed in shPDLIM2 DU145 cells was analysed by Ingenuity Pathway analysis to predict the activation state of transcription factor families as described for Figure 6B. Data in the table includes predicted activation state, regulation z-score, p-value and associated molecules.

Supplemental Table 5: (related to Figure 7)

Peptide array Results summary

CSN subunit	Number of peptides with PDLIM2 interaction	Position on protein	Sequences	Fold increase compared to shPDLIM2 control
CSN1	0	none	none	-
CSN2	0	none	none	-
CSN3	7/59	15-32	LSAQGQMTQLCELINKSG	2.4
		197-214	NFERALYFYEQAITTPAM	3.4
		274-291	ELRNLVNKHSETFTRDNN	2.3
		281-298	KHSETFTRDNNMGLVKQC	2.8
		288-305	RDNNMGLVKQCLSSLYYK	6.3
		323-340	ASRVQLSGPQEAEKYVLH	3.0
		406-423	KSMGSQEDDSGNKPSSYS	2.6
CSN4	0	none	none	-
CSN5	13/46	8-24	MAQKTWELANNMQEAQSI	2.5
		78-95	MGLMLGKVDGETMIIMDS	6.1
		155-172	QMLNQQFQEPFVAVVIDP	2.2
		162-179	QEPFVAVVIDPTRTISAG	3.8
		169-186	VIDPTRTISAGKVNLGAF	2.4
		176-193	ISAGKVNLGAFRTYPKGY	4.7
		183-200	LGAFRTYPKGYKPPDEGP	3.4
		190-207	PKGYKPPDEGPSEYQTIP	2.1
		246-263	WVNTLSSSSLLTNADYTT	3.0
		253-270	SSLLTNADYTTTGQVFDLS	2.6
		260-277	DYTTTGQVFDLSEKLEQSE	2.3
		309-326	KTTIEAIHGLMSQVIKDK	4.0
		316-333	HGLMSQVIKDKLFNQINI	4.8
CSN6	0	none	none	-
CSN7	0	none	none	-
CSN8	23/29	1-18	MPVAVMAESAFSFKKLLD	8.9
		8-25	ESAFSFKKLLDQCENQEL	4.8
		15-32	KLLDQCENQELEAPGGIA	4.8
		29-46	GGIATPPVYGQLLALYLL	3.0
		36-53	VYGQLLALYLLHNDMNNA	7.7
		43-60	LYLLHNDMNNARYLWKRI	5.1
		50-67	MNNARYLWKRIPPAIKSA	2.7
		57-73	WKRIPPAIKSANSELGGI	2.8
		64-80	IKSANSELGGIWSVGQRI	3.1
		71-87	LGGIWSVGQRIWQRDFPG	7.0
		78-94	GQRIWQRDFPGIYTTINA	6.8
		85-101	DFPGIYTTINAHQWSETV	2.1
		99-115	SETVQPIMEALRDATRRR	3.7
		106-122	MEALRDATRRRAFALVSQ	3.2
		113-129	TRRRAFALVSQAYTSIIA	9.8
		120-136	LVSQAYTSIIADDFAAFV	17.9
		127-143	SIIADDFAAFVGLPVEEA	4.9
		148-165	ILEQGWQADSTTRMVLPR	3.9
		155-172	ADSTTRMVLPRKPVAGAL	2.4
		169-186	AGALDVSFNKFIPLSEPA	7.3
		190-207	PIPNEQQLARLTDYVAFL	3.2
		192-209	PNEQQLARLTDYVAFLEN	6.0

Supplementary Table 5: PDLIM2 interacts with CSN subunits 3, 5 and 8.

Summary of peptide array results for interactions of PDLIM2 with all CSN subunits. Peptide arrays of immobilized, overlapping 18-mer peptides were generated, each shifted to the right by 8 amino acids encompassing the entire sequence of the different subunits. Arrays were probed with DU145 shScramble lysates and PDLIM2 interaction was detected by immunoblotting with anti-PDLIM2 antibody. DU145 shPDLIM2 lysates were used to control for the specificity of interactions. Intensity of each peptide interaction was established by densitometry using ImageJ software. The threshold of positive peptides (spots), was set as a 2-fold increase in interaction compared to shPDLIM2 controls.

Supplemental Experimental Procedures:

Reagents and Antibodies:

MG132 inhibitor was from Calbiochem (La Jolla, CA, USA). Tumour Necrosis Factor-alpha (TNF-α was purchased from Sigma (Dublin, Ireland). Lipofectamine™ and Lipofectamine™ 2000 were from Invitrogen (Paisley, UK). The anti-PDLIM2 antibody was generated by Fusion antibodies (Belfast, Northern Ireland) using GST-tagged PDLIM2 (amino acids 79-352) as an immunogen. The anti-HA antibody (16B12 clone) was obtained from Covance (Berkeley, CA, USA). Anti-alpha-tubulin and anti-gamma-tubulin antibodies were from Sigma-Aldrich (Dublin, Ireland). Anti-PARP, anti-Snail and anti-phospho-cofilin (Serine3) antibodies were from Cell Signalling Technologies (Beverly, MA, USA). Anti-lamin B, anti-HSP90, anti-CSN5 (rabbit), anti-cullin 1 and 3 and anti-P27, were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-CSN5 (Mouse) was from Thermofisher Scientific, (Dublin, Ireland). Anti-CSN2 was from Abcam (Cambridge, UK) and anti-CSN8 was from Enzo life sciences LTD (Exeter, UK). Anti E-cadherin and β-catenin were from BD Biosciences (Oxford, UK). Cy2- and Cy3-conjugated secondary antibodies were purchased from Jackson ImmunoResearch Laboratories (Cambridgeshire, UK). IRDye® 680 and IRDye® 800CW-conjugated antibodies were from LI-COR Biosciences (Cambridge, UK). All other reagents were from Sigma (Dublin, Ireland), unless otherwise noted.

Primer sequences:

Primer sequences were as follows: (*for*: Forward, *rev*: Reverse primer, 5' to 3' sequence listed for each). PDLIM2: *for* 5'-CAGCCCGCAGGGTACTTT, *rev* GATGGGCGTGTGGAAATC, E-cadherin: *for* TGAAGGTGACAGAGCCTCTG, *rev*

TGGGTGAATTCGGGCTTGTT, b-catenin: *for* GAATTGGGCCCTTAATGGTT, *rev* CTTGTACCTGCTCTGGAGGC, B2M: *for* CCAGCAGAGAATGGAAAGTC, *rev* CCTCCATGATGCTGCTTACA, PLAU: *for* GCCCTGGTTTGCGGCCATCT, *rev* GGATGTGCTTGGGGTGGGGC, CXCL10: *for* GGAGGTAGGGGAGGGCGTGC, *rev* AGGTAGGTTTGCTGTTGCCTG, IL8: *for* TTCCCCCAACAGGTGCAGTTT, *rev* TGTGTTGGCGCAGTGTGGTC, ID1: *for* CCCTGACGGCCGAGGTGAGA, *rev* AGGCTCCTTAGGCACCCCCG, VEGFC: *for* GTCCCGTTGGGGAACTGCGG, *rev* GCAGCCCCCACTGGAGAGGA, WWOX1: *for* GAGTTCCTGAGCGAGTGGAC, *rev* TCTCCTGCCACTCGTTTTCT, FPN1: *for* CGAGATGGATGGGTCTCCTA, *rev* ACCACATTTTCGACGTAGCC, GNG2: *for* GTGGGCACCTTTCTAGTCCA, *rev* CAGGCAGTTCGTCAGAATCA, MYL9: *for* ACCCCACAGACGAATACCTG, *rev* CCGGTACATCTCGTCCACTT, CITED2: *for* GGCGGCTCTGGCAGCAGCTC, *rev* CGGGCAGCTCCTTGATGCGG, IGFBP3: *for* CCTGCCGTAGAGAAATGGAA, *rev* CAGAAGCCCCGCTTCCTGCC, c-MYC: *for* TCAAGAGGCCACAGCAAAC, *rev* AAAAGCTACGCTTCAGCTCG, c-JUN: *for* CCGGCTAGAGGAAAAAGTGA, *rev* TGAGTTGGCACCCACTGTTA, CyclinD1: *for* TCGCCACCTGGATGCTGGAGGTCTG, *rev* CACCAGGAGCAGCTCCATTTGCAGCAG-3'.

Immunoprecipitation of E-cadherin and β-catenin

For immunoprecipitation of proteins, all procedures were performed at 4°C as previously described (Loughran et al., 2005, Healy and O'Connor, 2009). Briefly, cell lysates prepared with NP40 lysis buffer (described in Materials and Methods), were precleared using bovine serum albumin-coated protein G-agarose beads (Pierce, Rockford, IL, USA) for 1h. The lysates were recovered from the beads by centrifugation, prior to incubation with primary antibody overnight. Immune complexes were obtained by adding 20µl of protein G-agarose beads for 3h. The beads were washed 3 times with ice-cold lysis buffer, and the immune complexes were removed from the beads by boiling for 5min in 2x concentrated SDS-PAGE sample buffer for Western blot analysis as described in Materials and Methods.

Phase contrast Timelapse movies

For wound healing assays, confluent monolayers of cells cultured on collagen-coated 6-well plates were wounded by scoring with a sterile pipette tip. Wounded cultures were washed, re-incubated with growth medium and allowed to migrate. Cell movement across the wound tracks was monitored by timelapse microscopy, with images taken at 5 minute intervals for up to 12h post wounding using Metamorph® software described in Materials and Methods for

phase contrast microscopy. Images were stacked and saved as .mov (movie) files using Metamorph® software.

Generation of MCF7 –HA-Empty vector and HA-PDLIM2 cell lines

MCF7 cells were transfected with pcDNA3-Empty Vector or pcDNA3-PDLIM2 constructs using lipofectamine and stable clones were generated following selection in 1mg/ml G418 as described in Materials and Methods.