Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figure Legends

Figure S1 A) HeLa cells overexpressing GFP-NESRLIM, GFP-NESRnf6 or GFP-NESHIV/Rev were treated with Leptomycin B (LMB) and stained with anti-GFP antibodies. Note increased nuclear localization of GFP fusion proteins in cells treated with LMB. **B)** Plasmids expressing GFP-RLIMNLS 205-230 with wt sequence (SSSS) and the corresponding RLIM NLS (205-230) in which serine residues at positions 212, 214, 227 and 229 have been replaced by alanine (AAAA). Transfected proteins were visualized using anti-GFP antibodies (green). Note that GFP-RLIMNLS (205-230) is expressed strictly nuclear, whereas the GFP-RLIMNLS (205-230-AAAA) protein is also localized in the cytoplasm, similar to GFP alone (compare with Fig. 2B).

Figure S2

Specificity of the RLIM-pS214 antiserum in cross competition experiments using immunohistochemical stainings. Scale bars = 25μm. **A, B)** Stainings were performed on sections of a human microinvasive squamous cell carcinoma using A) antibodies made against the unphosphorylated RLIM (205-230) peptide (RLIM non-phospho-specific) or B) the 205-230 peptide phosphorylated at S214 (RLIM-pS214; phospho-specific). Staining reactions were competed (comp) by adding excess amounts of RLIM peptides containing the unphosphorylated NLS (RLIM-205-230) or phosphorylated at S214 (RLIM-205-230-pS214). Note that both peptides compete staining of the non-phospho-specific RLIM antibody whereas only the phospho-peptide is able to compete the staining reaction of the RLIM-pS214 antibody. **C)** Stainings on sections of normal human mammary gland using phospho-specific RLIM-pS214 antibodies. Staining reactions were competed by adding excess amounts of RLIM-205-230 or RLIM-205-230-pS214 peptides. Note that similar to B) only the phospho-peptide is able to

compete the staining reaction of the RLIM-pS214 antibody. **D)** RLIM is phosphorylated at S214 in nuclei of cells. Untreated HeLa cells were stained with antibodies directed against RLIM or with RLIM-pS214-specific antibodies. While RLIM is detected mostly nuclear using the non-phospho-specific RLIM antiserum, low levels of cytoplasmic RLIM can also be detected (compare with Fig. 1A). However, in stainings with the RLIM-pS214-specific antibodies RLIM is strictly nuclear.

Figure S3

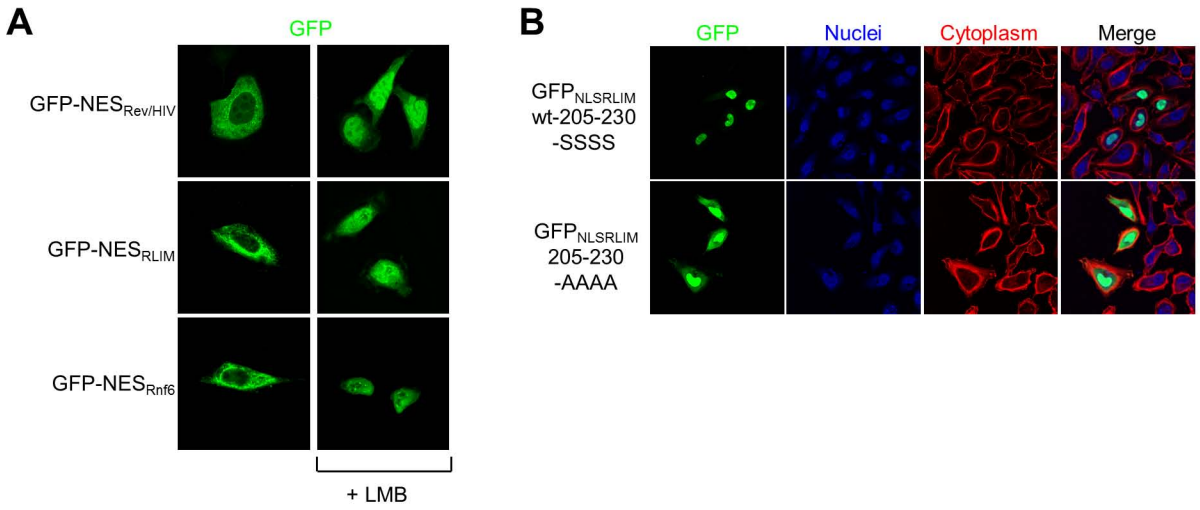
RLIM promotes cell motility in MDA-MB-231 breast cancer cells. MDA-MB-231 cells were transfected with siRNA against RLIM or control siRNA. Left panel: Motility of transfected MDA-MB-231 cells as measured in trans-well migration assays. Shown are averages +/- SEM for three independent measurements. Right panel: Representative Western blot of cell extracts. The same blot was hybridized with antibodies against RLIM and GAPDH.

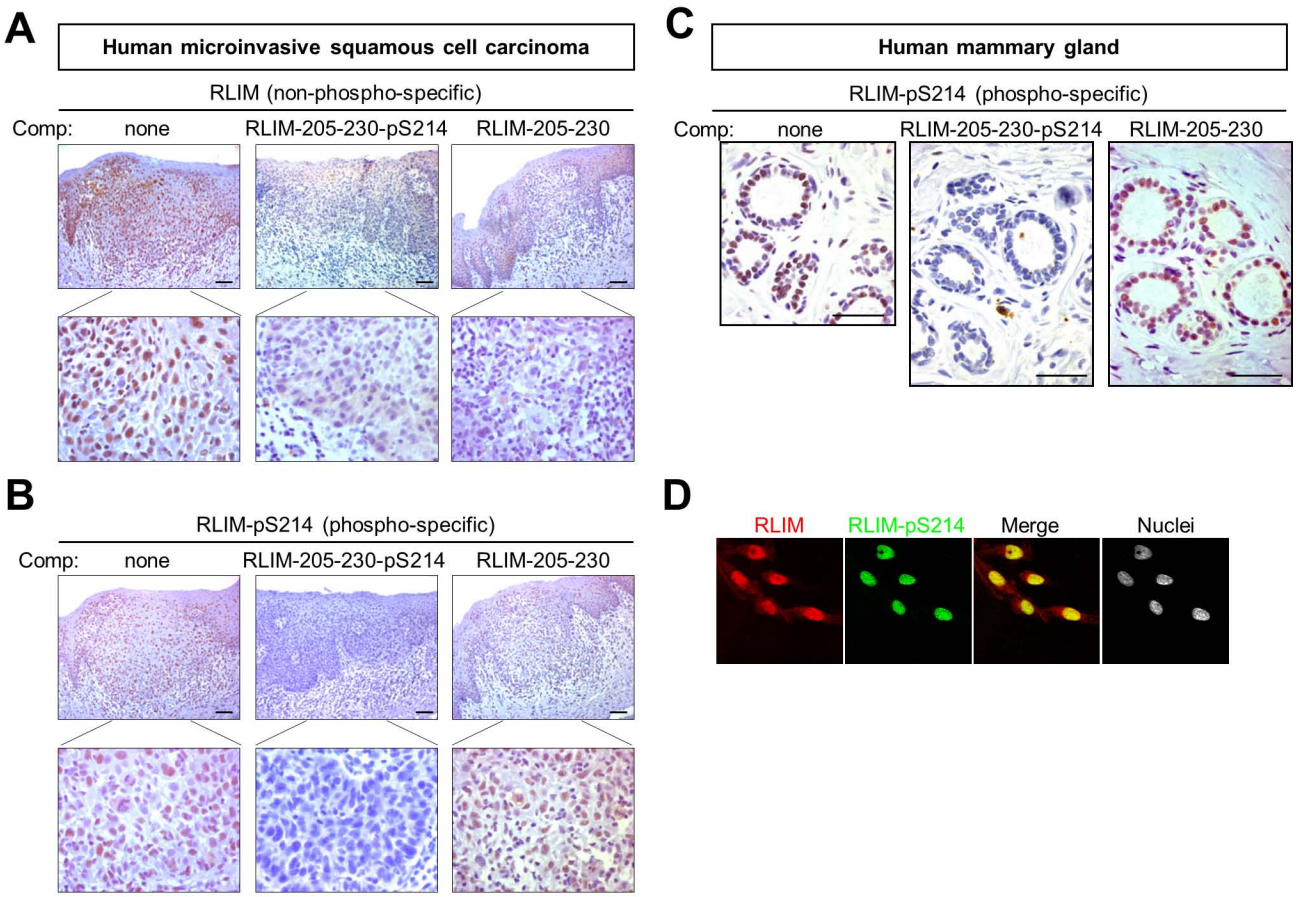
Figure S4

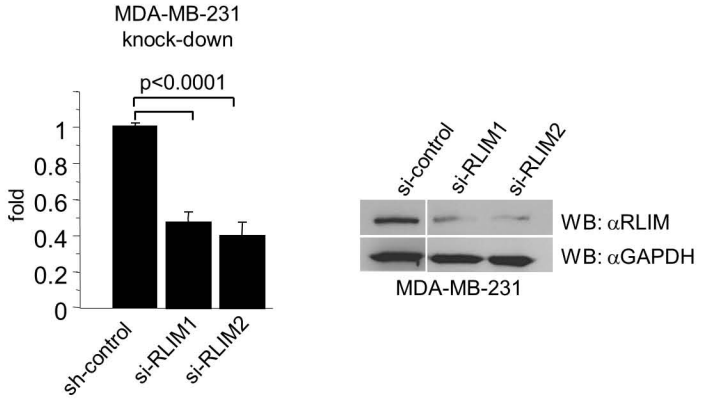
Infection of primary mammary epithelial cells with lentivirus expressing 6xMyc-tag, Myc-tagged RLIM wt (SSSS) and shuttling-deficient RLIM mutants Myc-ΔNES and Myc-SASS. Infection rates were > 90%.

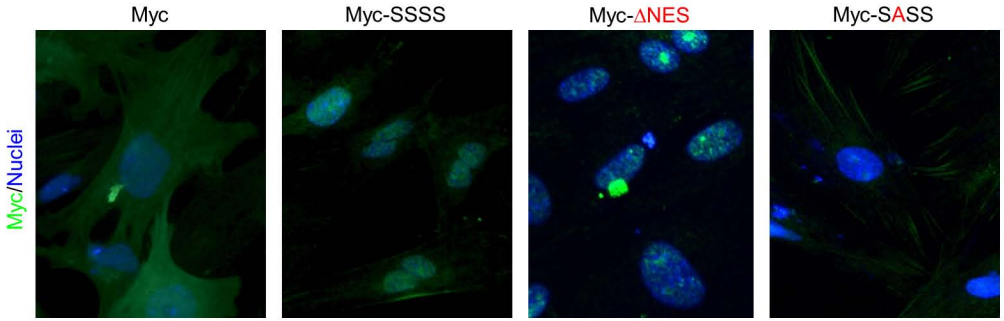
Figure S5

BLAST analysis of the tandem RSRSP motifs against the Sequence data base reveals many nuclear proteins which contain tandem RSRSP motifs.


A
GFP
GFP-NES$_{Rev/HIV}$
GFP-NES$_{RLIM}$
GFP-NES$_{Rnf6}$
+ LMB
B
GFP
Nuclei
Cytoplasm
Merge
GFP$_{NLSRLIM}$ wt-205-230 -SSSS
GFP$_{NLSRLIM}$ 205-230 -AAAA


A
Human microinvasive squamous cell carcinoma
RLIM (non-phospho-specific)
Comp:
none
RLIM-205-230-pS214
RLIM-205-230
B
RLIM-pS214 (phospho-specific)
Comp:
none
RLIM-205-230-pS214
RLIM-205-230
C
Human mammary gland
RLIM-pS214 (phospho-specific)
Comp:
none
RLIM-205-230-pS214
RLIM-205-230
D
RLIM
RLIM-pS214
Merge
Nuclei


MDA-MB-231
knock-down
p<0.0001
fold
1
0.8
0.6
0.4
0.2
0
sh-control
si-RLIM1
si-RLIM2
si-control
si-RLIM1
si-RLIM2
WB: αRLIM
WB: αGAPDH
MDA-MB-231


Myc
Myc-SSSS
Myc-ΔNES
Myc-SASS
Myc/Nuclei

Proteins containing tandem RSRSP motifs

Amino Acid Sequence	Protein (species)	Locus
VPPTRGQRRA**RSRSP**DHRRTRARAE**RSRSP**LHPMSEIPRR	Ubiquitin ligase RLIM/RNF12 (human)	(NP_899196)
PINRWSPTRR**RSRSP**IRR**RSRSP**LR**RSRSP**RR**RSRSP**RRRDRSRRSK	PRP4 Pre-mRNA processing factor (mouse)	(NP_038858)
PSPYSSSSWR**RSRSP**YSPVLRRSGKS**RSRSP**YSSRHSRSRS	CDC2-related protein kinase 5 (human)	(Q14004)
SLSASDRSYS**RSRSP**YESRSRGRSYS**RSRSP**ESRFRGRSDS**RSRSP**GYELERAAKK	Pre-mRNA-splicing factor PRP45 (Y.lipolytica)	(Q6CC77)
WRRSPPRMRR**RSRSP**RRRSPVRR**RSRSP**GRRRHRSRSS	Splicing related factor RNPS1 (human)	(AAL56665)
LYSRRRRARS**RSRSP**GRRRGSRS**RSRSP**GRRGGGRGDG	Splicing factor U2af 38kDa subunit (Drosophila)	(Q94535)
RSRSHGYHRS**RSRSP**PYRRYHS**RSRSP**QAFRGQSPTK	RBBP 6 Retinoblastoma-binding protein 6 (human)	(Q7Z6E9)
RRSSSGHRIR**RSRSP**VRYIYRP**RSRSP**RICHRFISKY	Zinc finger protein 638 (mouse)	(Q61464)
RSRSRERRRS**RSRSP**HRR**RSRSP**RRHRSSSISP	U4/U6.U5 tri-snRNP-associated prot 3 (X.laevis)	(Q6GLZ8)
SRRSRSRSSS**RSRSP**VRESRRRSE**SRSP**SPKRDLKREAS**RSRSP**LPAKDRSRTR	RNA-binding protein srp-3 (C.elegans)	(Q10021)
QTHPPPQTLP**RS**K**SP**SGQKRS**RSRSP**HEAGFCVYLK	RNA binding protein 12 (human)	(Q9NTZ6)
KYRSRSRSRS**RSRSP**YRSRNLLR**RSP**KSY**RS**A**SP**ERTSRKSVRS	Zinc finger protein 638 (mouse)	(Q61464)
FSQRPQRMRS**RSRS**FSRHRSC**SRSP**YSRS**RSRSP**GSRSSSRSCY	PPAR gamma coactivator 1-alpha (human)	(Q9UBK2)
SISRPRSSRSK**SRSP**SPK**RSRSP**SGSPR**RS**A**SP**ERMD	Splicing factor SFRS7 (human)	(Q16629)
RSQSSRSKKEK**SRSP**SKDNKSRS**RSRSP**DKSRSKSKDH	SFRS4 Splicing factor 4 (mouse)	(Q8VE97)
SRTPKRSRRS**RSRSP**KKSGKKSRSQ**SRSP**HRSHKKSKKN	U2-associated SR140 protein (human)	(NP_001073884)
HSRSPRRGRS**RSRSP**KRRSVSSQRS**RSRS**RRSYRS**SRSP**RSSSS**RS**S**SP**YSKSPVSKRR	BCL2-associated transcription factor 1 (human)	(AAI32781)
QAYSPRRGRS**RSRSP**KRRSPSP**RSRS**HSRNSDKSSSD	Thyroid horm recept assoc prot 3 (Trap150;human)	(Q9Y2W1)
KKGSDRNRGI**RSRS**RSRAESSRRS**RSRSP**YRQKHREVRS	CBF1-interacting corepressor (human)	(Q86X95)
SVRSVSRCRS**RS**L**SP**ICPRSQIGLNTMS**RSRSP**SPIRCGLPRF	Spermatogenesis-associated protein 18 (human)	(Q8TC71)
ASRGRSGSRS**RSRSP**SDKRSKRGDDR**RSRS**RDRDRRRERS	Prob ATP-dependent RNA helicase DDX46 (human)	(Q7L014)
SLRRRSYRGD**RSRSP**SRSRSRSYSP**SRSP**RRKRRRHRTS	Pre-mRNA-splicing factor CWC22 (Y.lipolytica)	(Q6C8C5)
SPCRRADSRS**RSRSP**ERGSRRKSYRE**RSRS**RSRERSERRGR	Pre-mRNA-splicing factor CWC21 (Y.lipolytica)	(Q6C0M9)
RSLDEIHPTR**RSRSP**TRHHDA**SRSP**VDHRTRDVDS	Rab3-interacting molecule 1 (RIM1; human)	(Q86UR5)
ARRDSPTYDPYK**RSP**SESSSESRS**RSRSP**TPGREEKITF	Splicing factor 16 (human)	(Q8N2M8)
TSFRQRKGGST**SS****SP**SRRRGSRSRS**RSRSP**GRPPKSARRS	Lamin B receptor (human)	(NP_919424)
RRPERSYQHS**RSRSP**HSSQS**R**NQ**SP**QRLASQASRP	Msx2-interact prot (SMART/HDAC1 assoc prot;mouse)	(Q62504)
RIPKNIGNDKE**SN****SP**ALYGRNARSRS**RSRSP**DRGKSAEPQH	Histone methyl transferase SET2 (D.hansenii)	(Q6BM04)
EDDAEKNEER**RS**A**S**VDSRQSGGSYLDAECSRHRRD**RSRSP**HKRKRNKDKD	U11/U12 snRNP 48kDa protein (human)	(Q6IEG0)