## Supplemental material

**JCB** 

Petropoulos et al., http://www.jcb.org/cgi/content/full/jcb.201510036/DC1

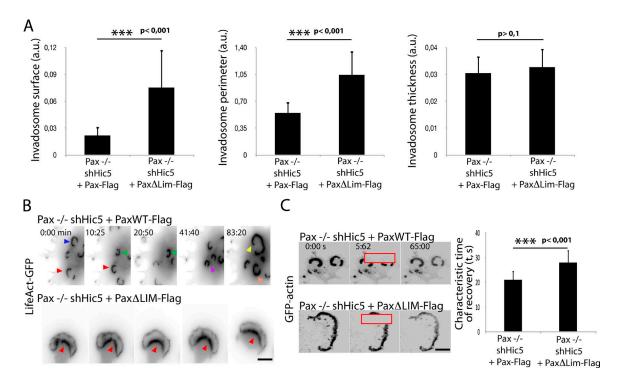


Figure S1. Structure-function studies revealed the functions of LIM domains of paxillin in invadosome morphometry and dynamics. (A) Morphometric analysis (surface, perimeter, and thickness) of the invadosome induced by either pax-Flag-WT or pax-ΔLIM-Flag mutant expressed in MEF-SrcY527F pax-/- shHic-5 cells (50 invadosome rings were quantified per condition). a.u., arbitrary units. (B) Representative images extracted from the time series of MEF-SrcY527F pax-/- shHic-5 cells expressing LifeAct-GFP revealed a highly dynamic invadosome turnover (multicolored arrowheads) in the presence of pax-Flag-WT, whereas the pax-ΔLIM-Flag mutant induced long-lasting poorly dynamic large rings (red arrowhead). (C) Representative images of a FRAP experiment (same imaging conditions and setup) on MEF-SrcY527F cells expressing GFP actin in the presence of pax-Flag-WT and pax-ΔLIM-Flag. Red rectangles show the area where the photobleaching is induced. Quantification of GFP-actin characteristic time of recovery showed that pax-ΔLIM-Flag stabilized actin dynamics in invadosomes in comparison with pax-WT-Flag. \*\*\*\*, P < 0.001. Bars, 4 μm.

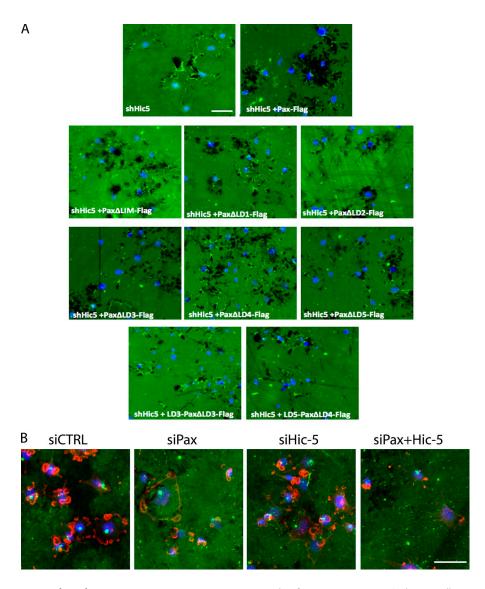


Figure S2. Representative ECM degradation patterns. (A) Representative micrographs of MEF-SrcY527F pax $^{-/-}$  shHic-5 cells expressing different paxillin mutants and spread on Oregon-green gelatin layer for 10 h. Cells were fixed and stained for DAPI before imaging with a 10x objective to measure ECM degradation, normalized to the number of nuclei in large cell numbers. (B) Representative micrographs of MEF-SrcY527F cells treated with the indicated siRNA before being spread on Oregon-green gelatin layer for 10 h, fixed, and stained for DAPI and F-actin-TRITC. Bars: (A) 50  $\mu$ m; (B) 25  $\mu$ m.

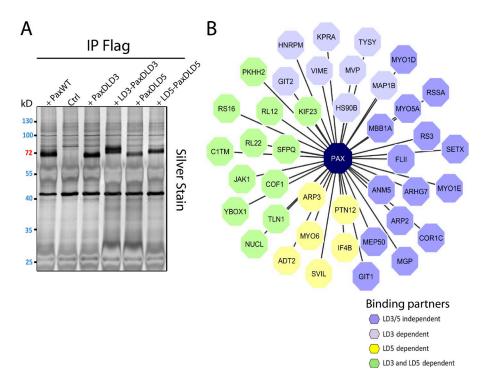


Figure S3. **Specific LD3 and LD5 interactors of paxillin.** (A) SDS-PAGE (10% gel) and silver staining analysis of paxillin binding partners copurified with pax-WT-Flag, pax-Flag mutants deleted for LD3 or LD5, and pax-Flag mutants pax-LD3\_\(D3\)-Flag or pax-LD5\_\(D4\)-Flag using an anti-flag M2 anti-body. The control condition corresponds to cell lysates of paxillin-deficient cells (\(pax^{-/-}\) and \(Hic.5^{-/-}\) cells) with nonspecific IgG but the same isotype as anti-Flag M2. (B) Protein interaction diagram of all interactors of paxillin. Based on a comparative study of the interactors of pax-WT-Flag and pax-Flag mutants deleted for LD3 or LD5, it was possible to identify the interactors independent of LD3 and LD5, dependent on LD3, dependent on LD5, and dependent on LD3 and LD5.

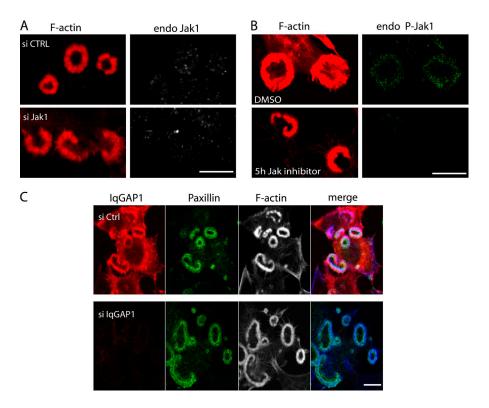


Figure S4. **Specific localization of endogenous JAK1 or IQGAP1 in invadosome.** (A) Confocal imaging of F-actin and endogenous JAK1 (total JAK1) in MEF-SrcY527F cells treated with CTRL or specific JAK1 siRNA. Cells were fixed and stained with the same procedure before performing quantitative confocal imaging performed with the same optical path and setup of the photomultiplier tube to compare fluorescence intensity. (B) The same protocol was applied on MEF-SrcY527F cells treated with DMSO or Jak inhibitor I for 5 h before fixation and staining with a specific antibody against phospho-JAK1. (C) Confocal imaging of endogenous IQGAP1 in MEF-SrcY527F cells treated with control or specific IQGAP1 siRNA. Silencing of IQGAP1 in MEF-SrcY527F cells validates the specificity of endogenous IQGAP1 immunodetection (red staining), which was highly enriched in invadosome rings (visualized by paxillin staining in green). Bars: (A and B) 5 µm; (C) 8 µm.

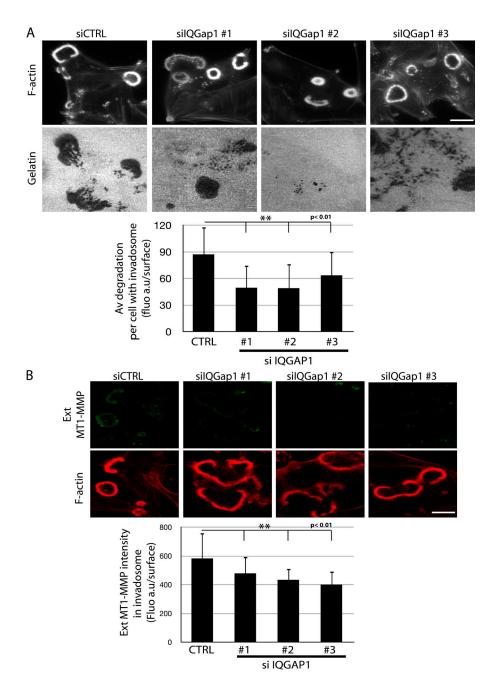


Figure S5. IQGAP1 silencing decreases ECM degradation of MEF-SrcY527F cells and is associated with a decrease of MT1-MMP at the surface of invado-somes. (A) F-actin staining of cells treated by different IQGAP siRNAs and spread on fluorescent gelatin layer for 12 h. IQGAP1 silencing induces large invadosomes that poorly digest ECM in comparison to control cells. To quantify this effect, the mean value (fluorescence in arbitrary units [fluo a.u.]/surface) of the digested ECM was quantified under cells forming invadosomes (representative of three different experiments, 50–75 cells per condition). (B) Based on the ability of MT1-MMP antibody to recognize the extracellular domain of this protease, its surface expression was detected in MEF-SrcY527F cells treated by different IQGAP siRNAs. Cells were fixed and stained without permeabilization and with the same procedure before performing quantitative confocal imaging. The same optical path and setup of the photomultiplier tube have been used to compare fluorescence intensity. Note the decrease of MT1-MMP accumulation after IQGAP1 depletion. \*\*, P < 0.01. Bars: (A) 4 μm; (B) 3 μm.

Table S1. Specific interactors of Hic-5-WT-Flag

Protein abbreviation	Negative control	Hic-5-WT-Flag	Protein name		
ANM5_MOUSE	0.0	9.5	Protein arginine N-methyltransferase 5		
ARP2_MOUSE	0.5	4.0	Actin-related protein 2		
DDB1_MOUSE	0.5	2.5	DNA damage-binding protein 1		
DJB11_MOUSE	0.0	3.5	DnaJ homolog subfamily B member 11		
EF1D_MOUSE	0.5	2.5	Elongation factor 1-δ		
FLII_MOUSE	0.0	6.5	Protein flightless-1 homolog		
GIT1_MOUSE	0.0	2.5	ARF GTPase-activating protein GIT1		
GLYR1_MOUSE	1.0	5.0	Putative oxidoreductase GLYR1		
QGAP1_MOUSE	0.5	2.5	Ras GTPase-activating-like protein IQGAP1		
(PRA_MOUSE	0.0	5.5	Phosphoribosyl pyrophosphate synthase-associated protein 1		
.RRF1_MOUSE	0.0	2.5	Leucine-rich repeat flightless-interacting protein 1		
MBB1A_MOUSE	1.0	7.0	Myb-binding protein 1A		
MEP50_MOUSE	0.0	4.0	Methylosome protein 50		
MGP_MOUSE	0.0	2.5	Matrix Gla protein		
NPM_MOUSE	0.5	3.5	Nucleophosmin		
PRPS1_MOUSE	0.0	2.5	Ribose-phosphate pyrophosphokinase 1		
PTN12_MOUSE	0.0	9.0	Tyrosine-protein phosphatase nonreceptor type 12		
20VEI6_MOUSE	0.0	6.0	Advillin		
RBM39_MOUSE	0.0	2.0	RNA-binding protein 39		
RSSA_MOUSE	0.0	3.0	40S ribosomal protein SA		
SERPH_MOUSE	0.0	3.0	Serpin H		
TOP2A_MOUSE	0.0	6.0	DNA topoisomerase 2-α		
TR150_MOUSE	0.5	3.0	Thyroid hormone receptor-associated protein 3		
TGFI1_MOUSE	0.0	36.0	Hic-5		

Table S2. Specific interactors of pax-WT-Flag

Protein abbreviation	Negative control	Pax-WT-Flag	Protein name		
ADT2_MOUSE	0.3	1.7	ADP/ATP translocase 2		
ANM5_MOUSE	0.0	15.0	Protein arginine N-methyltransferase 5		
ARHG7_MOUSE	0.0	5.7	Rho guanine nucleotide exchange factor 7 (Beta-Pix)		
ARP2_MOUSE	0.3	2.0	Actin-related protein 2		
ARP3_MOUSE	0.3	2.3	Actin-related protein 3		
B1ASP2_MOUSE	0.3	3.0	Tyrosine-protein kinase-JAK1		
C1TM_MOUSE	0.3	2.3	Monofunctional C1-tetrahydrofolate synthase.		
COF1_MOUSE	0.3	2.7	Cofilin-1 (Cofilin, nonmuscle isoform)		
COR1C_MOUSE	0.3	3.3	Coronin-1C (Coronin-3)		
9Q175_MOUSE	0.0	3.0	Unconventional myosin-VI		
9Q5G3_MOUSE	0.0	2.7	Protein Kif23		
LII_MOUSE	0.0	7.3	Protein flightless-1 homolog		
GIT1_MOUSE	0.0	7.0	ARF GTPase-activating protein GIT1		
GIT2_MOUSE	0.0	2.3	ARF GTPase-activating protein GIT2		
hnrpm_mouse	1.7	9.0	Heterogeneous nuclear ribonucleoprotein		
HS90B_MOUSE	0.0	3.0	Heat shock protein HSP 90-β		
F4B_MOUSE	0.7	4.3	Eukaryotic translation initiation factor 4B		
(PRA_MOUSE	0.0	3.3	Phosphoribosyl pyrophosphate synthase-associated protein 1		
MAP1B_MOUSE	0.0	3.3	Microtubule-associated protein 1B		
MBB1A_MOUSE	1.3	8.7	Myb-binding protein		
MEP50_MOUSE	0.0	8.0	Methylosome protein 50		
AGP_MOUSE	0.0	3.0	Matrix Gla protein		
AVP_MOUSE	0.7	4.3	Major vault protein		
MYO1D_MOUSE	1.3	10.3	Unconventional myosin-ld		
MYO1E_MOUSE	0.3	7.0	Unconventional myosin-le		
MYO5A_MOUSE	0.0	6.0	Unconventional myosin-Va		
NUCL_MOUSE	1.0	5.0	Nucleolin		
KHH2_MOUSE	0.0	3.0	Pleckstrin homology domain-containing family		
TN12_MOUSE	0.0	2.0	Tyrosine-protein phosphatase non-receptor type 12		
RL12_MOUSE	0.3	1.7	60S ribosomal protein L12		
RL22_MOUSE	0.3	2.3	60S ribosomal protein L22		
S16_MOUSE	0.3	1.7	40S ribosomal protein S16		
RS3_MOUSE	0.3	2.0	40S ribosomal protein S3		
RSSA_MOUSE	0.0	2.0	40S ribosomal protein SA		
SETX_MOUSE	0.0	5.3	Probable helicase senataxin		
SFPQ_MOUSE	0.3	2.0	Splicing factor, proline- and glutamine-rich		
VIL_MOUSE	0.0	2.3	Supervillin (Archvillin; p205/p250)		
"LN1_MOUSE	0.0	2.0	Talin-1		
YSY_MOUSE	0.0	2.0	Thymidylate synthase		
/IME_MOUSE	1.0	10.3	Vimentin		
/BOX1_MOUSE	0.0	2.0	Nuclease-sensitive element-binding protein		
PXN_MOUSE	0.7	44.0	Paxillin		

Table S3. Specific interactors of pax-WT-Flag, pax-ΔLD3-Flag, pax-ΔLD5-Flag, pax-LD3\_ΔLD3-Flag, and pax-LD5\_ΔLD5-Flag

Protein abbreviation	Negative control	Pax-WT-Flag	Pax-ΔLD3- Flag	Pax-LD3_ ΔLD3-Flag	Pax-∆LD5- Flag	Pax-LD5_ALD5-Flag	Protein name
ADT2_MOUSE	0.3	1.7	3.0	0.0	0.0	0.0	ADP/ATP translocase 2
ANM5_MOUSE	0.0	15	15	0.0	14.5	0.0	Protein arginine <i>N</i> -methyltransferase 5
ARHG7_MOUSE	0.0	5.7	8.0	7.0	6.0	9.0	Rho guanine nucleotide exchange factor 7 (Beta-Pix)
ARP2_MOUSE	0.3	2.0	2.5	0.0	2.0	0.0	Actin-related protein 2
ARP3_MOUSE	0.3	2.3	2.5	0.0	0.0	0.0	Actin-related protein 3
AVIL_MOUSE	0.0	0.0	2.5	0.0	6.0	0.0	Advillin
B1ASP2_MOUSE	0.3	3.0	0.0	0.0	0.0	0.0	Tyrosine-protein kinase-JAK1
C1TM_MOUSE	0.3	2.3	0.0	0.0	0.0	0.0	Monofunctional C1-tetrahydrofolate synthase
COF1_MOUSE	0.3	2.7	0.0	0.0	0.0	0.0	Cofilin-1 (Cofilin, non-muscle isoform)
COR1C_MOUSE	0.3	3.3	5.0	0.0	5.5	0.0	Coronin-1C (Coronin-3)
DHX9_MOUSE	0.0	0.0	0.0	0.0	2.5	0.0	ATP-dependent RNA helicase A
DJB11_MOUSE	0.0	0.0	0.0	3.0	0.0	0.0	DnaJ homolog subfamily B member 11
DNJA2_MOUSE	0.0	0.0	0.0	0.0	2.0	0.0	DnaJ homolog subfamily A member 2
DPYL3_MOUSE	0.0	0.0	0.0	0.0	0.0	3.0	Dihydropyrimidinase-related protein 3 (DRP-3)
E9Q175_MOUSE	0.0	3.0	0.0	0.0	0.0	0.0	Unconventional myosin-VI
E9Q5G3_MOUSE	0.0	2.7	0.0	0.0	0.0	0.0	Protein Kif23
ENOA_MOUSE	1.0	0.0	0.0	0.0	0.0	5.0	Alpha-enolase (EC 4.2.1.11)
FLII_MOUSE	0.0	7.3	4.5	5.0	5.0	0.0	Protein flightless-1 homolog
GIT1_MOUSE	0.0	7.0	7.0	4.0	3.0	5.0	ARF GTPase-activating protein GIT1
GIT2_MOUSE	0.0	2.3	0.0	3.0	2.5	0.0	ARF GTPase-activating protein GIT2
H2AV_MOUSE	0.0	0.0	2.5	0.0	0.0	0.0	Histone H2A.V
H2AX_MOUSE	0.0	0.0	2.5	0.0	0.0	0.0	Histone H2A.x
H32_MOUSE	0.0	0.0	2.0	0.0	0.0	2.0	Histone H3.2
H4_MOUSE	0.0	0.0	8.5	0.0	9.5	0.0	Histone H4
hnrpm_mouse	1.7	9.0	0.0	0.0	16	0.0	Heterogeneous nuclear ribonucleo- protein
HS90B_MOUSE	0.0	3.0	0.0	0.0	3.5	0.0	Heat shock protein HSP 90-β
IF4B_MOUSE	0.7	4.3	5.5	0.0	0.0	0.0	Eukaryotic translation initiation factor 4B
KPRA_MOUSE	0.0	3.3	0.0	0.0	2.5	6.0	Phosphoribosyl pyrophosphate synthase-associated protein 1
LDHA_MOUSE	0.0	0.0	0.0	0.0	0.0	2.0	L-lactate dehydrogenase A chain
LMNA_MOUSE	0.0	0.0	0.0	0.0	3.5	0.0	Prelamin-A/C
MAP1B_MOUSE	0.0	3.3	0.0	0.0	2.5	0.0	Microtubule-associated protein 1B
MBB1A_MOUSE	1.3	8.7	6.0	0.0	6.5	0.0	Myb-binding protein 1A
MED1_MOUSE	0.0	0.0	0.0	4.0	0.0	0.0	Mediator of RNA polymerase II transcription subunit 1
MED12_MOUSE	0.0	0.0	0.0	3.0	0.0	0.0	Mediator of RNA polymerase II transcription subunit 12
MED14_MOUSE	0.0	0.0	0.0	2.0	0.0	0.0	Mediator of RNA polymerase II transcription subunit 14
MED15_MOUSE	0.0	0.0	0.0	3.0	0.0	0.0	Mediator of RNA polymerase II transcription subunit 15
MED17_MOUSE	0.0	0.0	0.0	3.0	0.0	0.0	Mediator of RNA polymerase II transcription subunit 17
MED23_MOUSE	0.0	0.0	0.0	5.0	0.0	0.0	Mediator of RNA polymerase II transcription subunit 23
MED24_MOUSE	0.0	0.0	0.0	6.0	0.0	0.0	Mediator of RNA polymerase II transcription subunit 24
MED31_MOUSE	0.0	0.0	0.0	2.0	0.0	0.0	Mediator of RNA polymerase II transcription subunit 31
MEP50_MOUSE	0.0	8.0	8.5	0.0	4.0	0.0	Methylosome protein 50
MGP_MOUSE	0.0	3.0	3.0	0.0	3.0	0.0	Matrix Gla protein
MVP_MOUSE	0.7	4.3	0.0	0.0	5.0	0.0	Major vault protein
MYL9_MOUSE	0.0	0.0	0.0	0.0	0.0	5.0	Myosin regulatory light polypeptide 9
MYO1D_MOUSE	1.3	10.3	12	0.0	15	0.0	Unconventional myosin-ld
							•
MYO1E_MOUSE	0.3	7.0	9.0	0.0	7.0	0.0	Unconventional myosin-le

Table S3. Specific interactors of pax-WT-Flag, pax-ALD3-Flag, pax-ALD5-Flag, pax-LD3\_ALD3-Flag, and pax-LD5\_ALD5-Flag (Continued)

Protein abbreviation	Negative control	Pax-WT-Flag	Pax-∆LD3- Flag	Pax-LD3_ ΔLD3-Flag	Pax-∆LD5- Flag	Pax-LD5_ALD5-Flag	Protein name
MYO6_MOUSE	0.0	0.0	2.5	0.0	0.0	0.0	Unconventional myosin-VI
NPM_MOUSE	0.0	0.0	0.0	0.0	2.5	0.0	Nucleophosmin
NUCL_MOUSE	1.0	5.0	0.0	0.0	0.0	0.0	Nucleolin
OAT_MOUSE	0.0	0.0	0.0	0.0	0.0	2.0	Ornithine aminotransferase, mitochondrial
PGK1_MOUSE	0.0	0.0	0.0	0.0	0.0	2.0	Phosphoglycerate kinase 1 (EC 2.7.2.3)
PKHH2_MOUSE	0.0	3.0	0.0	0.0	0.0	2.0	Pleckstrin homology domain-con- taining family H member 2
PPIA_MOUSE	0.0	0.0	0.0	0.0	0.0	2.0	Peptidyl-prolyl cis-trans isomerase A
PTN12_MOUSE	0.0	2.0	2.0	0.0	0.0	5.0	Tyrosine-protein phosphatase non-receptor type 12 (EC 3.1.3.48; MPTP-PEST)
RBM39_MOUSE	0.0	0.0	0.0	0.0	0.0	2.0	RNA-binding protein 39
RL5_MOUSE	0.0	0.0	0.0	0.0	2.0	0.0	60S ribosomal protein L5
RL7_MOUSE	0.0	0.0	0.0	0.0	3.5	0.0	60S ribosomal protein L7
RL7A_MOUSE	0.0	0.0	0.0	0.0	2.5	0.0	60S ribosomal protein L7a
RL12_MOUSE	0.3	1 <i>.7</i>	0.0	0.0	0.0	0.0	60S ribosomal protein L12
RL15_MOUSE	0.0	0.0	0.0	0.0	0.0	2.0	60S ribosomal protein L15
RL22_MOUSE	0.3	2.3	0.0	0.0	0.0	0.0	60S ribosomal protein L22
RLA1_MOUSE	0.0	0.0	0.0	2.0	0.0	0.0	60S acidic ribosomal protein P1
ROA1_MOUSE	0.0	0.0	0.0	0.0	2.5	0.0	Heterogeneous nuclear ribonucleo- protein A1
RS13_MOUSE	0.0	0.0	2.5	0.0	2.5	0.0	40S ribosomal protein S13
RS16_MOUSE	0.3	1 <i>.7</i>	0.0	0.0	0.0	0.0	40S ribosomal protein S16
RS2_MOUSE	0.0	0.0	2.0	0.0	3.0	0.0	40S ribosomal protein S2
RS3_MOUSE	0.3	2.0	2.5	0.0	3.0	0.0	40S ribosomal protein S3
RSSA_MOUSE	0.0	2.0	0.0	0.0	3.0	0.0	40S ribosomal protein SA
SERPH_MOUSE	0.0	0.0	0.0	0.0	0.0	6.0	Serpin H1
SETX_MOUSE	0.0	5.3	3.0	0.0	3.0	0.0	Probable helicase senataxin
SFPQ_MOUSE	0.3	2.0	0.0	0.0	0.0	0.0	Splicing factor, proline- and glutamine-rich
SVIL_MOUSE	0.0	2.3	2.0	0.0	0.0	0.0	Supervillin (Archvillin; p205/p250)
TERA_MOUSE	0.0	0.0	0.0	0.0	0.0	3.0	Transitional endoplasmic reticulum ATPase
THIM_MOUSE	0.0	0.0	0.0	0.0	2.5	0.0	3-ketoacyl-CoA thiolase
TLN1_MOUSE	0.0	2.0	0.0	1.0	0.0	7.0	, Talin-1
TYSY_MOUSE	0.0	2.0	0.0	0.0	2.5	0.0	Thymidylate synthase
VIME_MOUSE	1.0	10.3	0.0	0.0	11.5	0.0	Vimentin
YBOX1_MOUSE	0.0	2.0	0.0	0.0	0.0	0.0	Nuclease-sensitive element-binding protein
PXN_MOUSE	0.7	44.0	58.5	88	35.5	49	Paxillin

Data are presented as mean peptide spectral count.