Supplemental Materials

Molecular Biology of the Cell

Lin et al.

Supplementay metarial and method

Construction of truncated LIMCH1 and NM-IIA

GFP-LIMCH1 full length－Full length encompassing amino acid residues 1-1083 of LIMCH1 was PCR amplified from the *kiaa1102*/*limch1* cDNA with the primer pairs (Forward － 5'-CCAAGCTTGAAAGCCGTGTCACGAC-3' and Reverse － 5'-CCAAGCTTTCACAAGTTTGTACAAAAAAGCAGGC-3'), introducing HindIII sites on both terminals. This fragment was inserted in frame into the existing HindIII site in the pEGFP-C construct.

GFP-LIMCH1-delLIM－ To remove C-terminal amino acid residues 941-1083 of LIMCH1, GFP-LIMCH1 was digested with BamHI, followed by religating amino acid residues 1-441 and 442-940.

GFP-LIMCH1-delCH－ The removed N-terminal amino acid residues 1-250 of LIMCH1 was PCR amplified from the GFP-LIMCH1 full length construct, introducing N-terminal KpnI and C-terminal blunt sites. This fragment was inserted in frame into the existing KpnI/SmaI sites in the pEGFP-C construct.

GFP-LIMCH1-2xCoil－ The middle region encompassing amino acid residues 251-940 of LIMCH1 was PCR amplified from the GFP-LIMCH1 full length construct, introducing N-terminal EcoRI and C-terminal XhoI sites. This fragment was inserted in frame into the existing EcoRI/SalI sites in the pEGFP-C construct.

GFP-LIMCH1-CHCoil－To remove the C-terminal amino acid residues 442-1083 of LIMCH1, GFP-LIMCH1-delLIM was digested with BamHI, followed by religation.

GFP-LIMCH1-NCoil－ The N-terminal region encompassing amino acid residues 251-635 of LIMCH1 was generated by digesting GFP-LIMCH1-2xCoil with SacI. This fragment was inserted in frame into the existing SacI site in the pEGFP-C construct.

GFP-LIMCH1-CoilLIM－To remove the N-terminal amino acid residues 1-634 of LIMCH1, GFP-LIMCH1-delCH was digested with SacI, followed by religation.

GFP-LIMCH1-CCoil－The C-terminal region encompassing amino acid residues 441-940 of LIMCH1 was generated by digesting GFP-LIMCH1 full length with BamHI. This fragment was inserted in frame into the existing BamHI site in the

pEGFP-C construct.

FLAG-LIMCH1 full length— Full length of LIMCH1 was PCR amplified from the GFP-LIMCH1 full length construct, introducing N-terminal EcoRI and C-terminal KpnI sites. This fragment was inserted in frame into the existing EcoRI/KpnI sites in the p3×FLAG-*Myc*-CMV-26 construct.

FLAG-LIMCH1-delLIM— Similar to generating GFP-LIMCH1.

FLAG-LIMCH1-delCH — The delCH fragment was PCR amplified from the FLAG-LIMCH1 full length construct, introducing N-terminal HindIII and C-terminal BglII sites. This fragment was inserted in frame into the existing HindIII/BglII sites in the p3×FLAG-*Myc*-CMV-26 construct.

FLAG-LIMCH1-2xCoil — The 2xCoil fragment was generated by digesting N-terminal EcoRI and C-terminal KpnI sites of GFP-LIMCH1-2xCoil. This fragment was inserted in frame into the existing EcoRI/KpnI sites in the p3×FLAG-*Myc*-CMV-26 construct.

FLAG-LIMCH1-NCoil — The NCoil fragment was generated by digesting GFP-LIMCH1-NCoil with EcoRI. This fragment was inserted in frame into the existing EcoRI site in the p3×FLAG-*Myc*-CMV-26 construct.

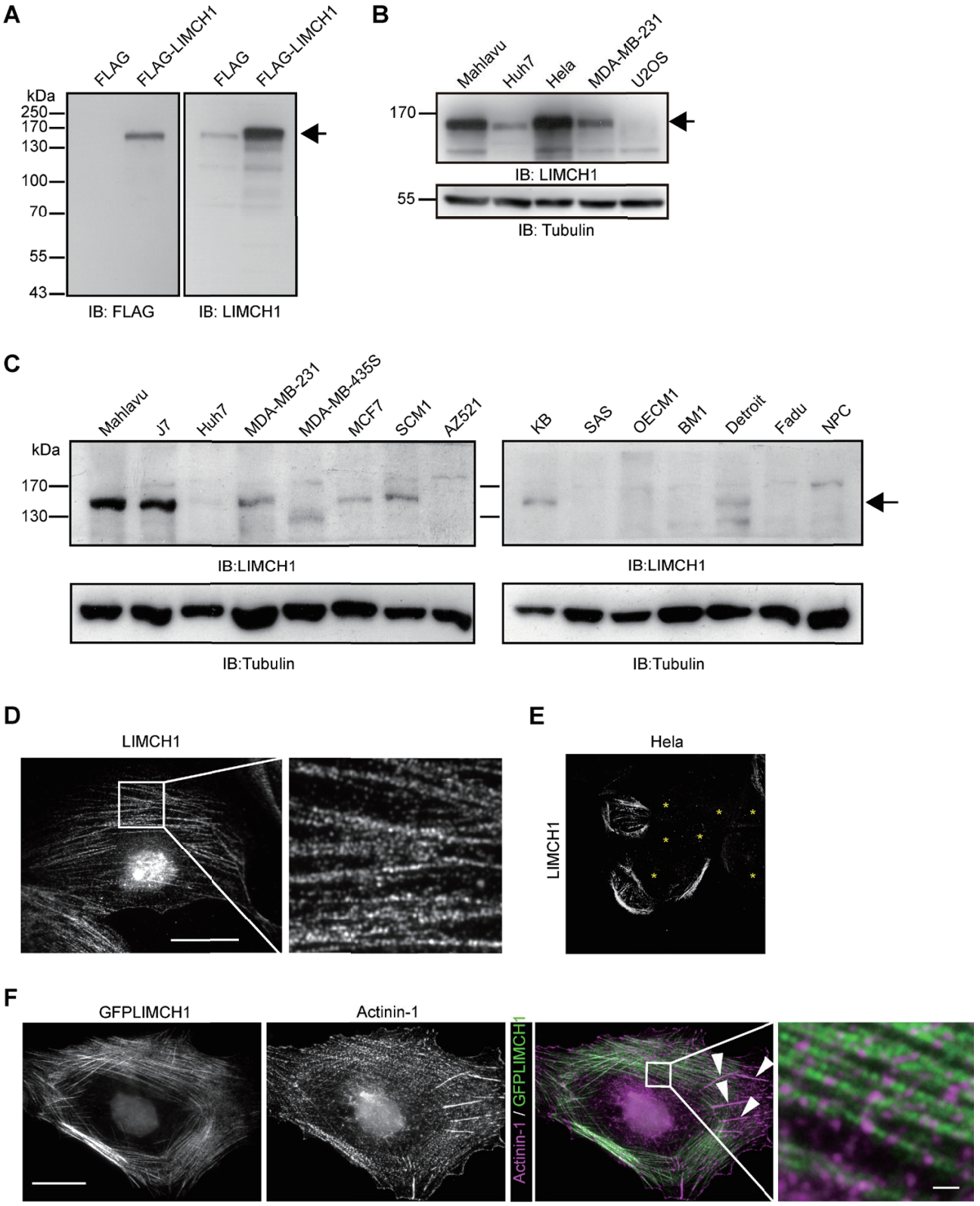
FLAG-LIMCH1-CCoil — The CCoil fragment was PCR amplified from the FLAG-LIMCH1 full length construct, introducing N-terminal HindIII and C-terminal BglII sites. This fragment was inserted in frame into the existing HindIII/BglII sites in the p3×FLAG-*Myc*-CMV-26 construct.

His-LIMCH1 full length— Full length of LIMCH1 was PCR amplified from the FLAG-LIMCH1 full length construct, introducing N-terminal XhoI and C-terminal EcoRI sites. This fragment was inserted in frame into the existing EcoRI/XhoI sites in the pET-32 construct.

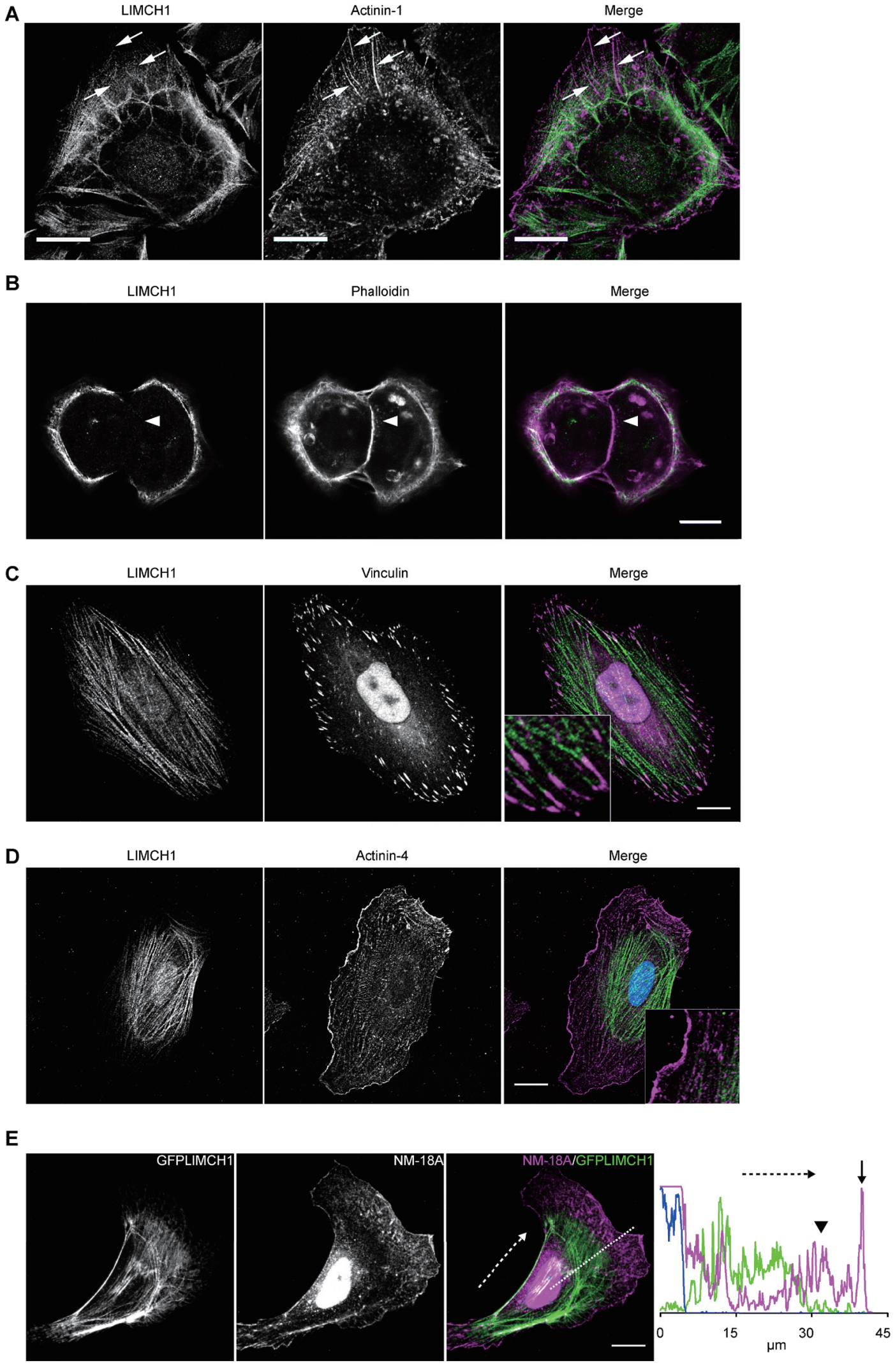
His-LIMCH1-CoilLIM— CoilLIM was PCR amplified from the FLAG-LIMCH1 full length construct, introducing N-terminal XhoI and C-terminal EcoRI sites. This fragment was inserted in frame into the existing EcoRI/XhoI sites in the pET-32 construct.

GST-IIA-head－ The N-terminal region encompassing amino acid residues 1-838 of NM-IIA was generated by PCR amplifying from the GFP-NM-IIA construct, introducing N-terminal EcoRI and C-terminal SalI sites. This fragment was inserted in frame into the existing EcoRI/SalI sites in the pGEX-4T construct.

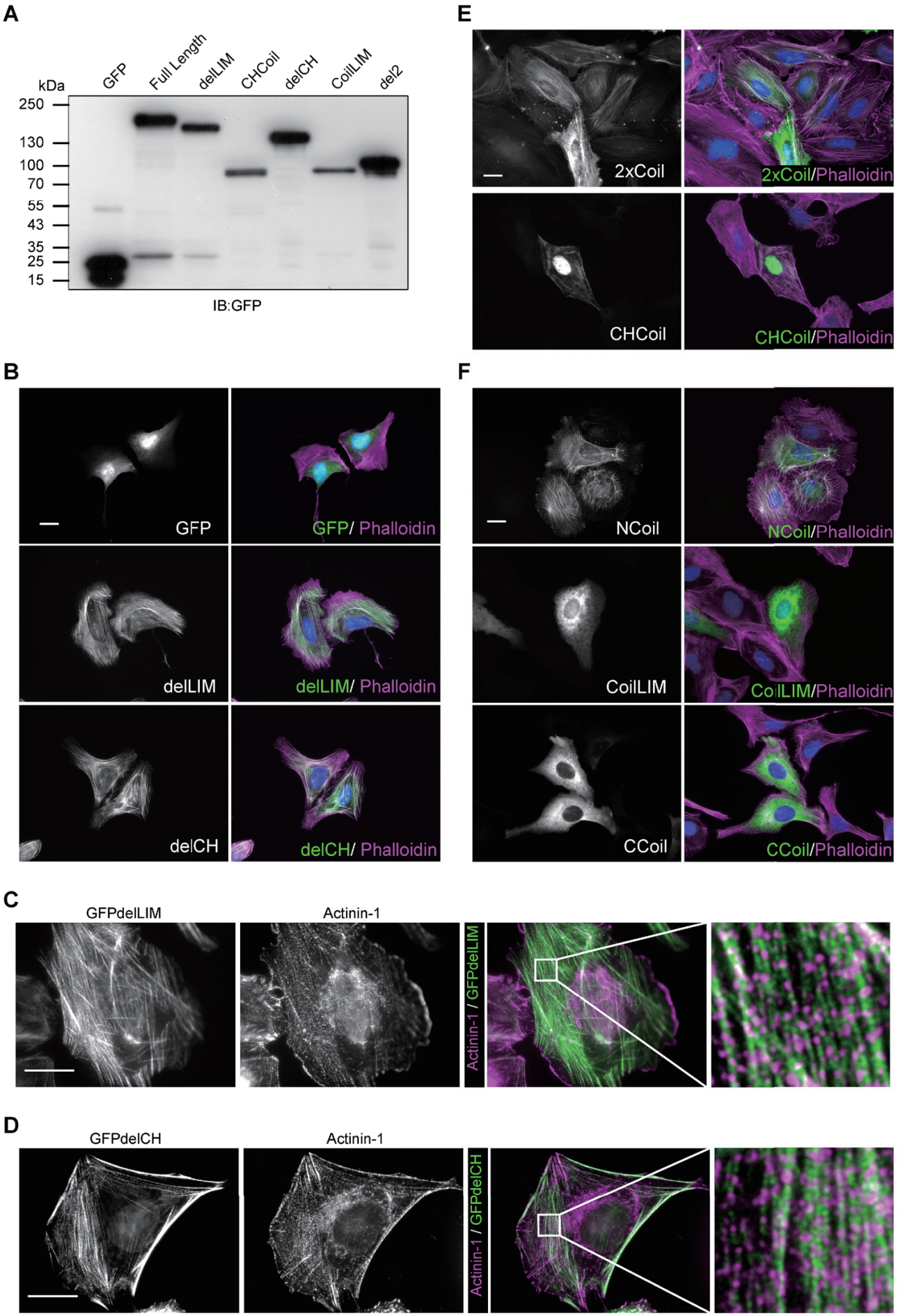
GST-IIA-coil－ The C-terminal region encompassing amino acid residues 830-1915 of NM-IIA was generated by PCR amplifying from the GFP-NM-IIA construct, introducing N-terminal SalI and C-terminal NotI sites. This fragment was inserted in frame into the existing SalI/NotI sites in the pGEX-4T construct.


A
FLAG
FLAG-LIMCH1
FLAG
FLAG-LIMCH1
kDa
250
170
130
100
70
55
43
IB: FLAG
IB: LIMCH1
B
Mahlavu
Huh7
Hela
MDA-MB-231
U2OS
170
IB: LIMCH1
55
IB: Tubulin
C
Mahlavu
J7
Huh7
MDA-MB-231
MDA-MB-435S
MCF7
SCM1
AZ521
KB
SAS
OECM1
BM1
Detroit
Fadu
NPC
kDa
170
130
IB:LIMCH1
IB:LIMCH1
IB:Tubulin
IB:Tubulin
D
LIMCH1
E
Hela
LIMCH1
F
GFPLIMCH1
Actinin-1
Actinin-1 / GFPLIMCH1

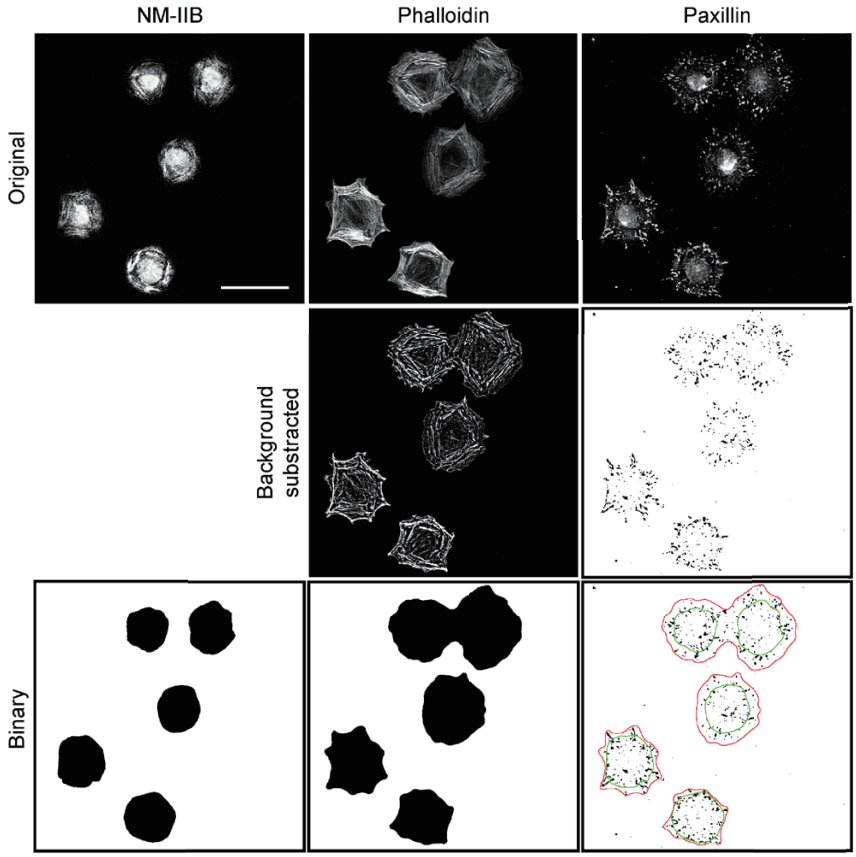
Supplementary figure 1. Expression pattern of LIMCH1 in various cancer cell lines. (A) Western blot analysis of HeLa cells transfected with FLAG-LIMCH1 and probed with anti-FLAG (*left*) and anti-LIMCH1 (*right*) antibodies. Endogenous and exogenous LIMCH1 proteins showed a similar molecular weight on SDS-PAGE, indicated by an arrow. (B) Western blot analysis of various cancer cell lines probed with anti-LIMCH1 antibody. Mahlavu and Huh7 are liver cancer cells; HeLa is cervical cancer cell; MDA-MB-231 is breast cancer cell; U2OS is osteoid cancer cell. Arrow indicates LIMCH1 protein. (C) Expression pattern of LIMCHI shown by western blot analysis in various cancer cell lines. Mahlavu, J7 and Huh7 are liver cancer cells; MDA-MB435S, MDA-MB-231 and MCF7 are breast cancer cells; SCM1 and AZ521 are gastric cancer cells; SAS and OECM1 are oral cancer cells; KB, BM1 and NPC are nasopharyngeal cancer cells; Detroit and Fadu are pharynx cancer cells. Arrow indicates LIMCH1 protein. (D) Fluorescence images of HeLa cell stained with anti-LIMCH1 antibody; bar, 20 μm. LIMCH1 displayed a dotted pattern (*right*, enlarged image). (E) Fluorescence images of HeLa cells treated with siRNA and stained with anti-LIMCH1 antibody. Asterisks indicate LIMCH1-depleted cells. (F) Representative image of U2OS cells transfected with GFP-LIMCH1 (green) and stained with anti-actinin-1 (magenta) antibody; bar, 20 μm. Magnified image display the twin signal of LIMCH1 between adjacent actinin-1 (*right*); bar, 1 μm. Arrows indicate dorsal stress fibers.


A
LIMCH1
Actinin-1
Merge
B
LIMCH1
Phalloidin
Merge
C
LIMCH1
Vinculin
Merge
D
LIMCH1
Actinin-4
Merge
E
GFPLIMCH1
NM-18A
NM-18A/GFPLIMCH1
0
15
30
45
μm

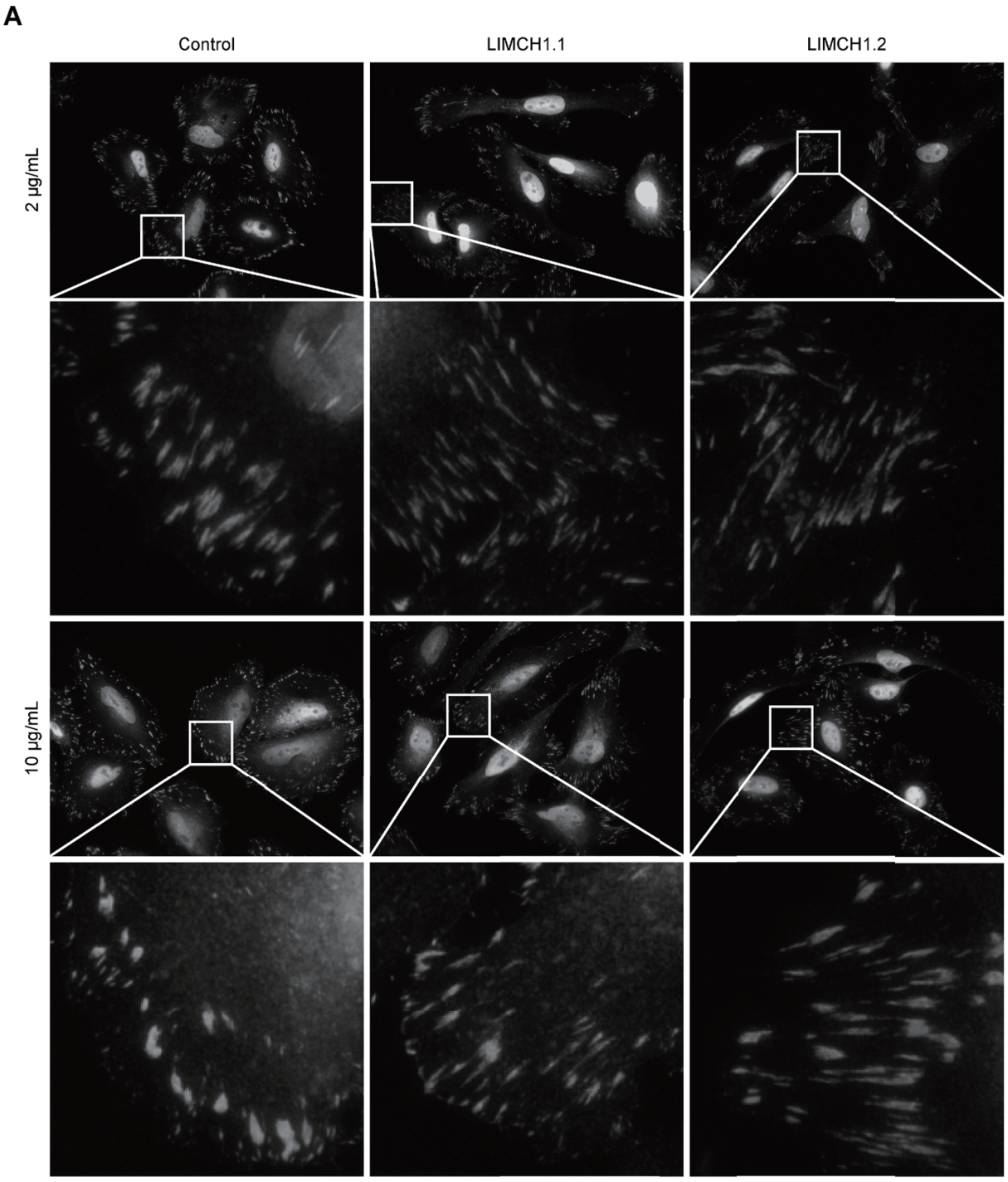
Supplementary figure 2. LIMCH1 is absence in dorsal stress fibers, cleavage furrow, focal adhesions, peripheral actin filaments, and mainly localized behind the lamella in a migrating cell. Confocal images in A-D were stained with anti-LIMCH1 (green) antibody. (A) Dorsal stress fibers (arrow) were recognized by staining with anti-actinin-1 (magenta) antibody in J7 cell. (B) Cleavage furrow (arrowhead) was recognized by staining with phalloidin (magenta) during cell division in J7 cell. (C) Focal adhesions were visualized by staining with anti-vinculin (magenta) antibody in HeLa cell. (D) Peripheral actin filaments were visualized by staining with anti-actinin-4 (magenta) antibody in HeLa cell. (E) HeLa cell was transfected with GFP-LIMCH1 and stained with anti-NM-18A (magenta) antibody. Arrow indicates the lamellipodia. Arrowhead indicates the lamellar; bar, 20 μm. Plot profiles are shown on the right.

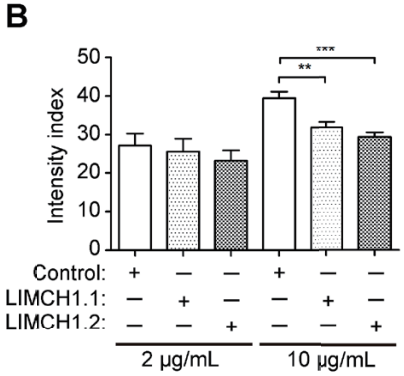

A
kDa
GFP
Full Length
delLIM
CHCoil
delCH
CoilLIM
del2
250
130
100
70
55
43
35
25
15
IB:GFP
E
2xCoil
2xCoil/Phalloidin
CHCoil
CHCoil/Phalloidin
B
GFP
GFP/ Phalloidin
delLIM
delLIM/ Phalloidin
delCH
delCH/ Phalloidin
F
NCoil
NCoil/Phalloidin
CoilLIM
CoilLIM/Phalloidin
CCoil
CCoil/Phalloidin
C
GFPdelLIM
Actinin-1
Actinin-1 / GFPdelLIM
D
GFPdelCH
Actinin-1
Actinin-1 / GFPdelCH

Supplementary figure 3. N-terminal coiled-coil domain of LIMCH1 is responsible for the association with actin stress fiber. (A) Western blot analysis of U2OS cells transfected with GFP tagged LIMCH1 truncations and probed with anti-GFP antibody. (B-F) Fluorescence images display the subcellular localization of GFP tagged LIMCH1 truncations (green) in U2OS cells; bar, 20 μm. The GFP-delLIM (C) and GFP-delCH (D) expressed U2OS cells displayed the twin signal between adjacent actinin-1(magenta). Actin stress fibers were visualized by staining with phalloidin (magenta) in B, E, and F.


NM-IIB
Phalloidin
Paxillin
Original
Background substracted
Binary

Supplementary figure 4. Demonstration of cell outline and cell center marks. Binary and background subtracted images were processed with Image J software. Original images were stained with NM-IIB, phalloidin and paxillin.


A
Control
LIMCH1.1
LIMCH1.2
2 µg/mL
10 µg/mL


B
Intensity index
50
40
30
20
10
0
***
**
Control: + − − + − −
LIMCH1.1: − + − − + −
LIMCH1.2: − − + − − +
2 µg/mL
10 µg/mL

Supplementary figure 5. LIMCH1 depletion in HeLa cells reduces the intensity of focal adhesions. (A) Immunofluorescence images of HeLa cells grown on the various concentration of fibronectin-coated coverslip for 24 h after siRNA treatment and stained with anti-vinculin antibody. Control cells exhibited the compact focal adhesions in the high adhesive condition and the loose or elongated focal adhesions in the low adhesive condition. In contrast, LIMCH1 depleted cells in both high and low adhesive condition exhibited thin and elongated focal adhesions. (B) Quantification of intensities of focal adhesion determined in A.