Supplementary Figure Legends

Figure S1. ERC1a, LL5α and LL5β can be efficiently silenced by specific siRNAs. (**A**) Blots on lysates from COS7 cells co-transfected with either mouse GFP-LL5α or mouse GFP-LL5β in combination with control (Luc) or specific siRNAs for either human LL5α or LL5β: the mouse LL5β protein was resistant to silencing by the siRNA targeting the human LL5β protein. (**B**) Immunofluorescence to show the decrease of the endogenous proteins in cells transfected with the corresponding specific siRNAs. Scale bar, 20 μm. (**C**) Upper blots: co-immunoprecipitations with antibodies for endogenous LL5β (left), liprin-α1 (center), or ERC1a (right) from lysates of HeLa cells transfected with the indicated GFP-tagged proteins. Lower blot: aliquots of lysates of cells transfected with the indicated GFP-tagged constructs. Lysates, 50 μg of protein/lane; 200 μg of protein/immunoprecipitation. (**D**) Left blot: co-immunoprecipitations with anti-Flag antibody from lysates of COS7 cells co-transfected with Flag-Liprin-α1, GFP-LL5β, GFP-LL5β^{M}, and mCherryERC1a or mCherry as control. Right blot: aliquots of lysates (30 μg) from transfected cells. 300 μg of protein lysate/immunoprecipitation were used. (**E**) Immunoprecipitations with anti-FLAG antibody from lysates of cells cotransfected with either FLAG-Liprin-α1, GFP-LL5β and mCherry-ERC1a, or with FLAG-Liprin-α1, GFP-LL5β and mCherry as control. (**F-H**) SiRNAs for either ERC1a or LL5 proteins negatively affects MDA-231 cell spreading (**F**) and migration on FN (**G**) (n=24-44 cells). (**H**) The inhibition of cell migration by silencing of endogenous liprin-α1 is rescued by the overexpression of the silencing-resistant liprin-α1^{SR} mutant (Lip^{SR}). (**I-J**) Analysis of the effects of silencing of the proteins of the complex with a second set of specific siRNAs for ERC1a and LL5 proteins: (**I**) migration on FN (n=18-32 cells); (**J**) lamellipodia stability (n=8-10 cells). Bars are means ± s.e.m.. *P<0.05; **P<0.005. Viability and growth of cell lines overexpressing GFP-Liprin-α1. (**K**) Levels of endogenous and overexpressed liprin-α1 in control (GFP) and GFP-Liprin-α1 expressing clones. (**L-N**) Increased migration on FN by liprin-α1 overexpression is prevented by ERC1a and LL5 silencing. Tracks (**L**) and quantification of the speed (**M**) of wildtype cells and of cells from clones expressing either GFP (C9) or GFP-Liprin-α1 (L4, L12, L17 clones). Bars are the normalized means ± s.e.m. (n=46-85 cells). (**N**) Tracks of cells migrating on FN show that the enhanced migration of cells overexpressing liprin-α1 (clone L12) is prevented by silencing either liprin-α1, ERC1a or LL5 proteins (see **Figure 1H** for quantification).

Figure S2. Distribution of ERC1a and LL5β (**A**), liprin-α1 and ERC1a (**B**), and LL5β and liprin-α1 (**C**) at active protrusions of MDA-231 migrating cells. Frames from time-lapses of cells cotransfected with the indicated mCherry- and GFP-tagged constructs. The arrows in the lower magnifications on the left (bars, 10 μm) point to protruding cell edges. A partial colocalization between the two proteins in each pair is observed near the sites of protrusion. Scale bar for higher magnification frames, 2.5 μm.

Figure S3. ERC1a does not evidently colocalize with any of several markers for endocytic and exocytic compartments. The ERC1a-positive clusters do not colocalize with the endocytic markers EEA1 (early endosomes), mannose 6-phosphate receptor (late endosomes), and LAMP1 (late endosomes/lysosomes) (**A**), nor with Golgi markers including the cis-Golgi protein GM130, the golgin tether Giantin, Golgi 58K, and the Golgi-associated GTPase Rab6 (**B**). No colocalization of the peripheral ERC1a-positive clusters was observed with any of 3 different functional endocytic markers: transferrin for the clathrin-mediated endocytic pathway, dextran for fluid phase uptake, and cholera toxin B for clathrin- and dynamin-independent carriers (CLICs) (**C**). Scale bars, 10 μm (**A**); 20 μm (**B,C**). **(D-I)** Cells transfected with siRNAs for ERC1a and GFP were incubated for 20 minutes with the indicated fluorescently-labelled endocytic markers. The internalization was evaluated after removal of residual surface-bound markers, fixation and immunofluorescence for GFP. Silencing of ERC1a did not affect the internalization of transferrin (**D,E**) and CTxB (**H,I**), while it partially affected the internalization of dextran (**F,G**), although this effect was not observed after normalization of the internalized dextran for the projected cell area. Bars are means ± s.e.m. (n=32-67 cells). *P<0.05; **P<0.005. Scale bars, 20 μm.

Figure S4. Immunoblots from lysates of cell preparations used for the experiments shown in **Figure 7** and **Fig. S1**. Effective silencing of the endogenous proteins by specific siRNAs for liprin-α1, ERC1a, or LL5 proteins. Lysates from cells transfected to silence either one (**A**) and two types of proteins (**B**) are shown. (**C**) Quantification of the endogenous protein levels after silencing with the indicated siRNAs. Bars are means ± s.e.m. (n=4).

Supplementary Movie Legends

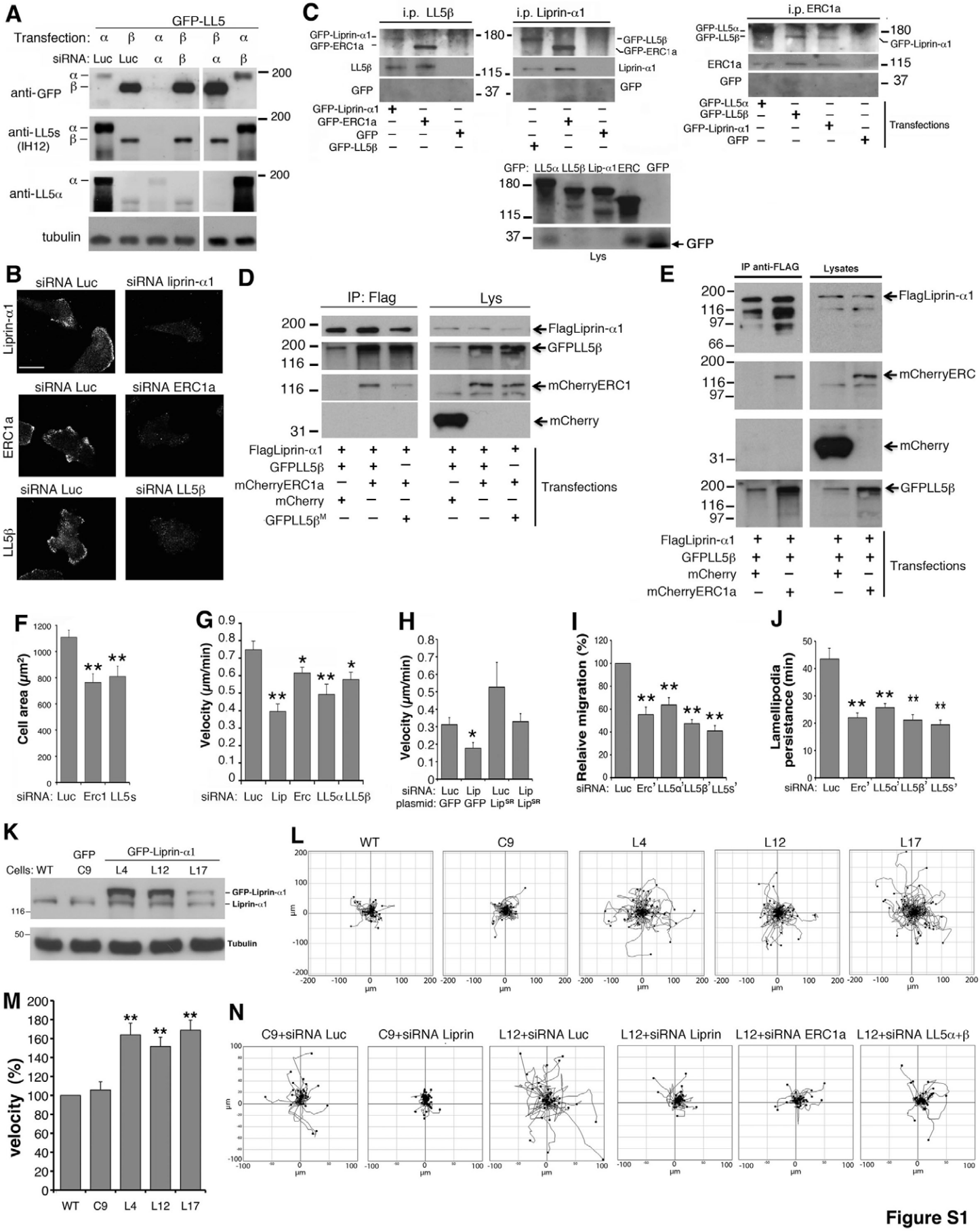
Movie 1. Migration in reconstituted 3D matrices of MDA-231 cells transfected with either control siRNA (left), or with siRNAs specific for ERC1a (center), or LL5α and LL5β proteins (right). One picture every 7 minutes. 20x lens; 8 hours.

Movie 2. MDA-231 cells transfected with mCherry together with either GFP-LL5β (left), GFP-LL5α (second from left), GFP-Liprin-α1 (third from left), or GFP-ERC1a (right) were plated on glass coverslip pre-coated with 2.5 μg/ml FN. Time lapses: one picture every minute. 63x lens; 2 hours.

Movie 3. Left: time lapse of a cell transfected with GFP-ERC1a. The GFP-ERC1a-positive compartment is highly dynamic in cells migrating on a substrate coated with 2.5 μg/ml FN. Right: detail of the periphery of the cell shown on the left, showing GFP-ERC1a-positive vesicle-like structures forming near the protruding edge of the migrating cell, and moving centripetally. Coalescence of the small vesicle-like structures into larger dynamic GFP-ERC1a-positive structures can be observed. One picture every 2 seconds. 63x lens; 9.5 minutes.

Movie 4. Time lapse of a cell transfected with GFP-ERC1a. Detail of the protruding cell periphery from **Movie 3**, showing coalescence of the small GFP-ERC1a-positive vesicle-like structures forming near the protruding edge of the migrating cell, into larger dynamic GFP-ERC1a-positive structures, as well as dispersal of smaller structures detaching from the larger ones. One picture every 2 seconds; 9.5 minutes.

Movie 5. Time lapse of a cell transfected with mCherry-Zyxin and GFP-LL5β. One picture every 30 seconds. 63x lens; 40 minutes.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anti-LL5s (IH12)
anti-LL5α
tubulin
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C
i.p. LL5β
i.p. Liprin-α1
i.p. ERC1a
GFP-Liprin-α1
GFP-ERC1a
GFP-LL5β
LL5β
Liprin-α1
GFP
ERC1a
GFP-LL5α
180
115
37
Transfections
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Lys
B
siRNA Luc
siRNA liprin-α1
siRNA ERC1a
siRNA LL5β
Liprin-α1
ERC1a
LL5β
D
IP: Flag
Lys
FlagLiprin-α1
GFPLL5β
mCherryERC1
mCherry
31
E
IP anti-FLAG
Lysates
FlagLiprin-α1
mCherryERC
mCherry
GFPLL5β
97
66
116
F
Cell area (μm²)
siRNA: Luc Erc1 LL5s
G
Velocity (μm/min)
siRNA: Luc Lip Erc LL5α LL5β
H
siRNA: Luc Lip Luc Lip
plasmid: GFP GFP Lip^SR Lip^SR
I
Relaive migration (%)
siRNA: Luc Erc' LL5α' LL5β' LL5s'
J
Lamellipodia persistance (min)
K
Cells: WT C9 L4 L12 L17
GFP
GFP-Liprin-α1
Tubulin
L
WT
C9
L4
L12
L17
μm
M
velocity (%)
N
C9+siRNA Luc
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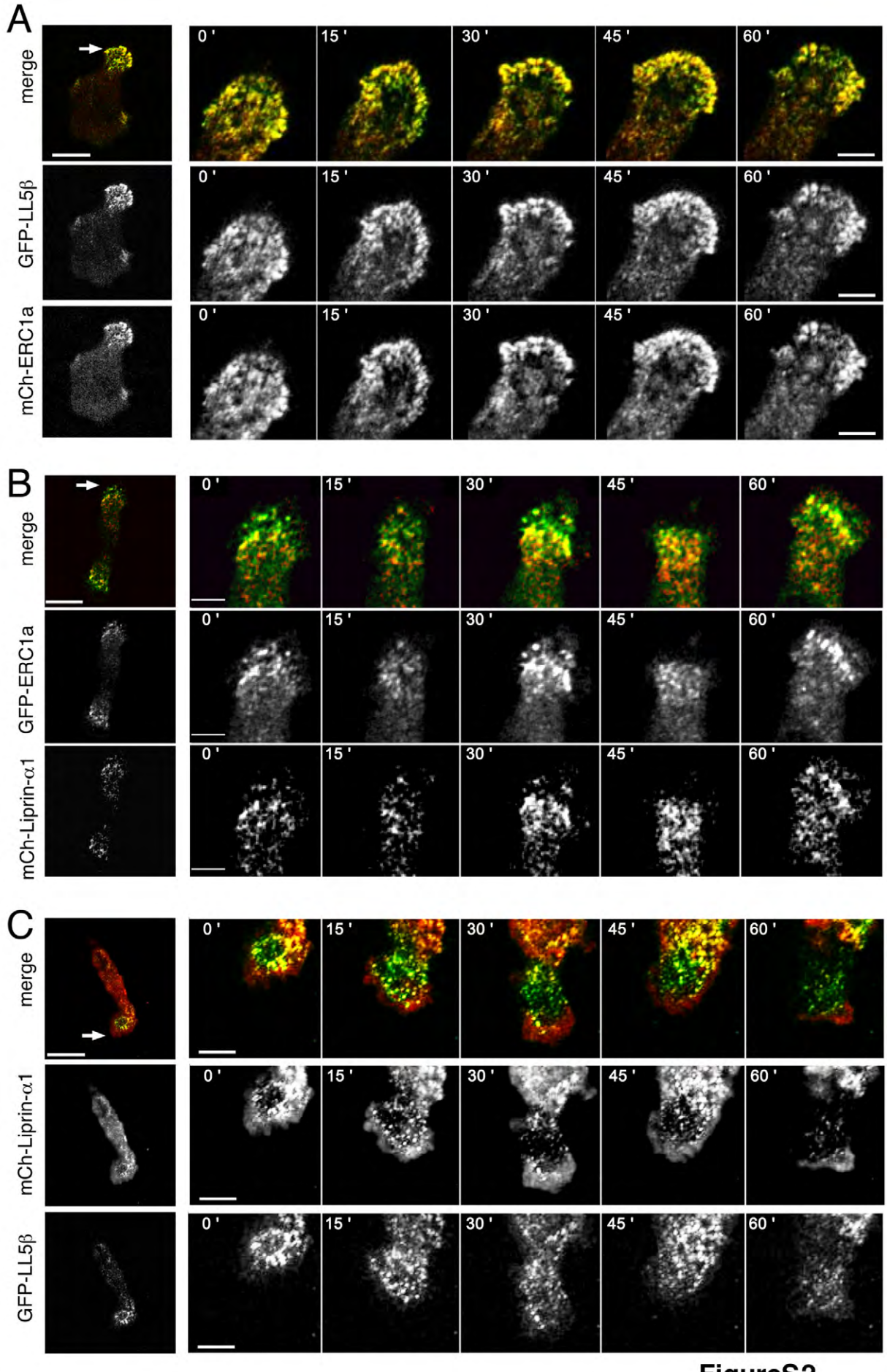
L12+siRNA Liprin
L12+siRNA ERC1a
L12+siRNA LL5α+β

Figure S1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mCh-ERC1a
B
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C
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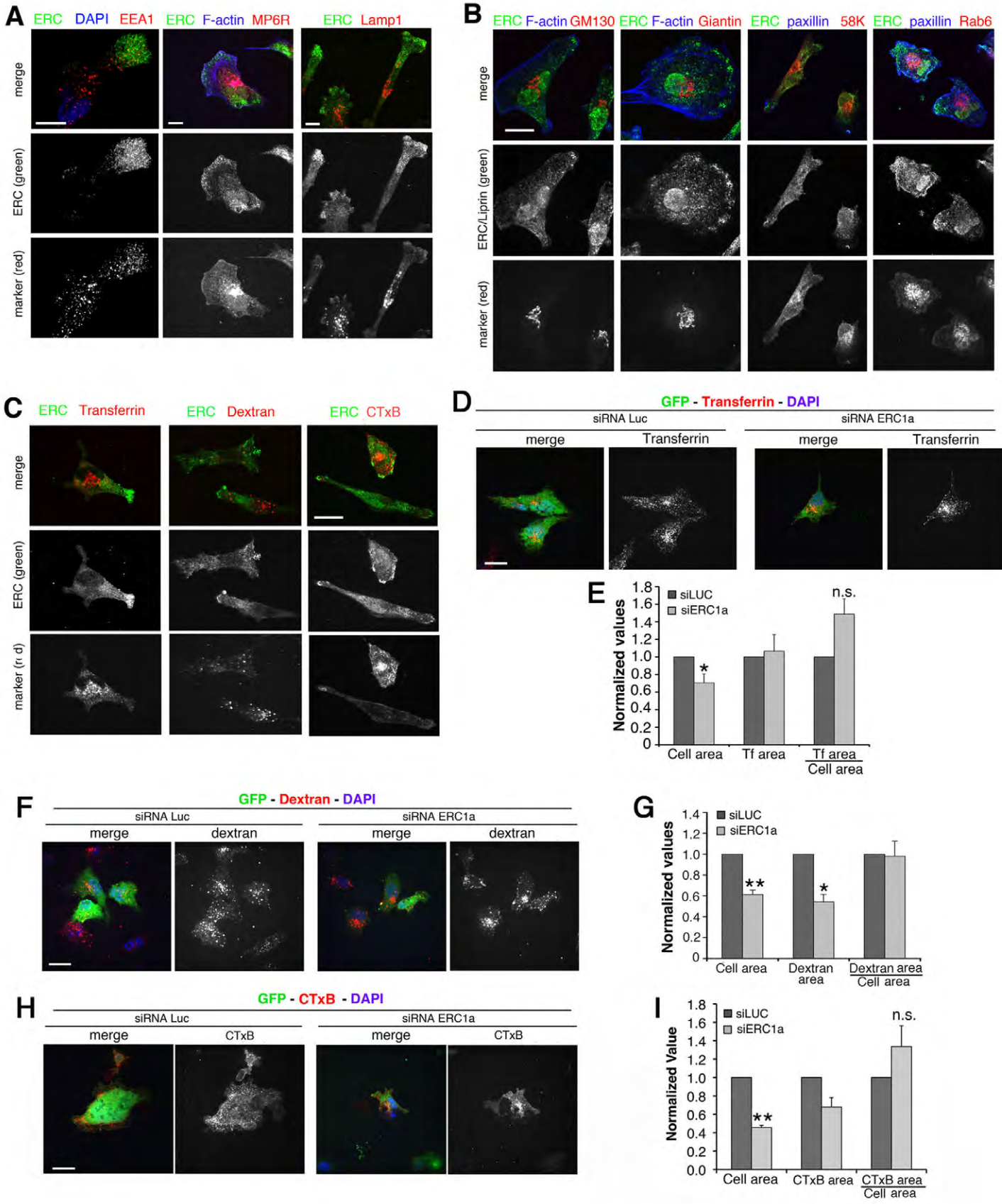
0 '
15 '
30 '
45 '
60 '

FigureS2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ERC DAPI EEA1
ERC F-actin MP6R
ERC Lamp1
merge
ERC (green)
marker (red)
B
ERC F-actin GM130
ERC F-actin Giantin
ERC paxillin 58K
ERC paxillin Rab6
merge
ERC/Liprin (green)
marker (red)
C
ERC Transferrin
ERC Dextran
ERC CTxB
merge
ERC (green)
marker (r d)
D
GFP - Transferrin - DAPI
siRNA Luc
siRNA ERC1a
merge
Transferrin
merge
Transferrin
E
siLUC
siERC1a
Normalized values
n.s.
*
Cell area
Tf area
Tf area / Cell area
F
GFP - Dextran - DAPI
siRNA Luc
siRNA ERC1a
merge
dextran
merge
dextran
G
siLUC
siERC1a
Normalized values
**
*
Cell area
Dextran area
Dextran area / Cell area
H
GFP - CTxB - DAPI
siRNA Luc
siRNA ERC1a
merge
CTxB
merge
CTxB
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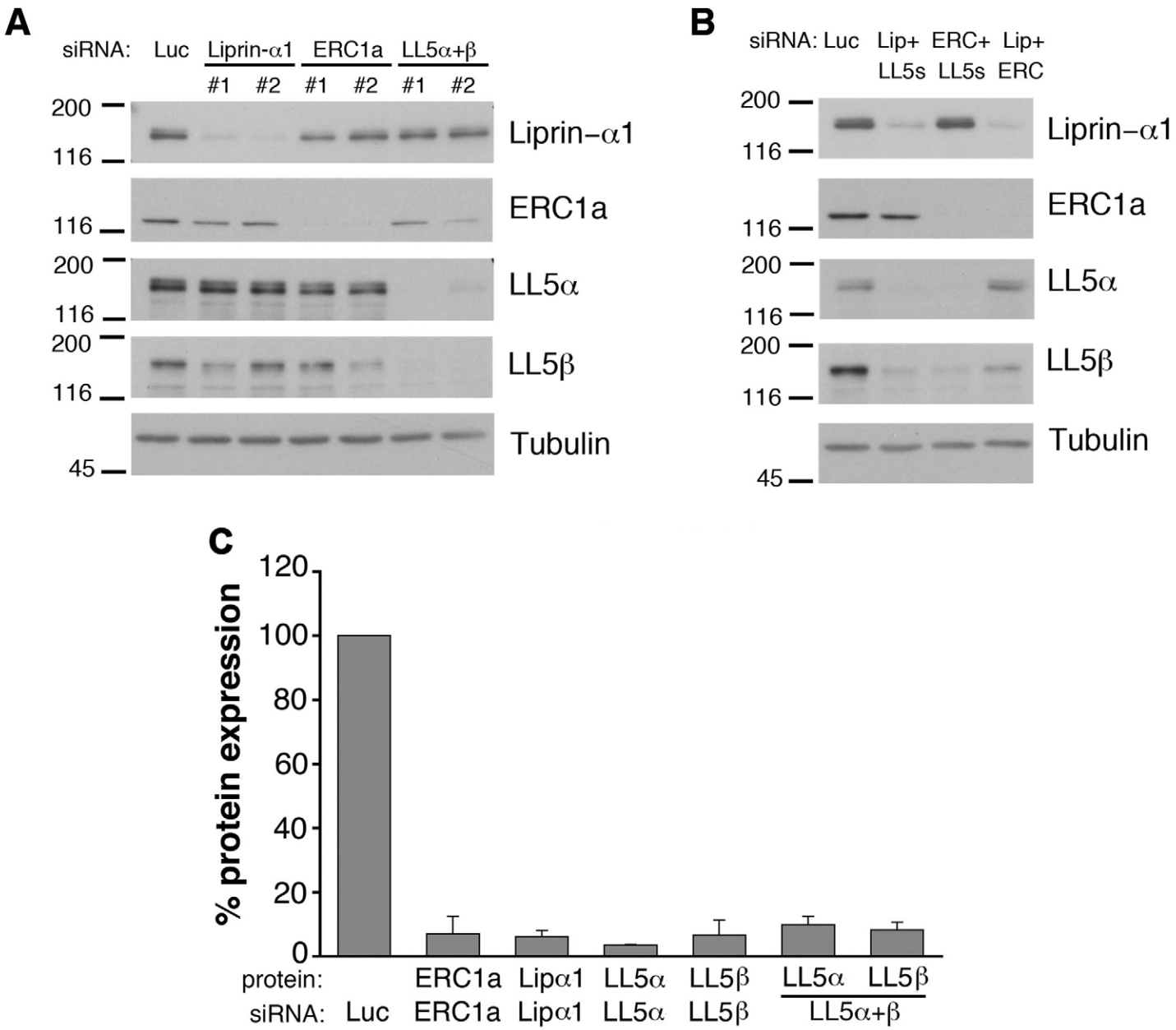
siLUC
siERC1a
Normalized Value
n.s.
**
Cell area
CTxB area
CTxB area / Cell area

FigureS3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Liprin–α1
116
ERC1a
200
116
LL5α
200
116
LL5β
45
Tubulin
B
siRNA: Luc Lip+ LL5s ERC+ LL5s Lip+ ERC
200
116
Liprin–α1
116
ERC1a
200
116
LL5α
200
116
LL5β
45
Tubulin
C
% protein expression
120
100
80
60
40
20
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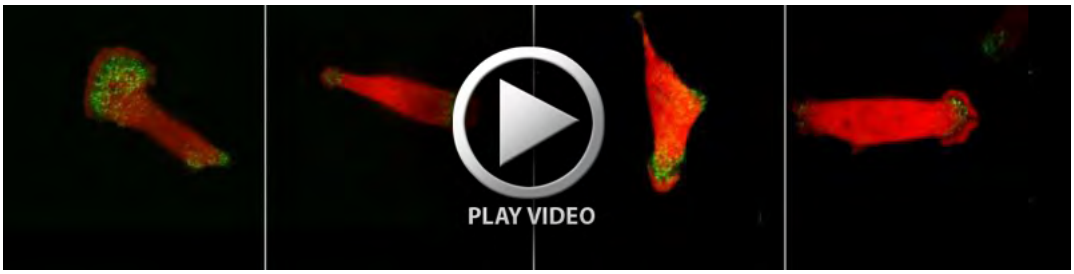
Figure S4

Movie 1.



Movie 2.

Movie 3.

Movie 4.

Movie 5.