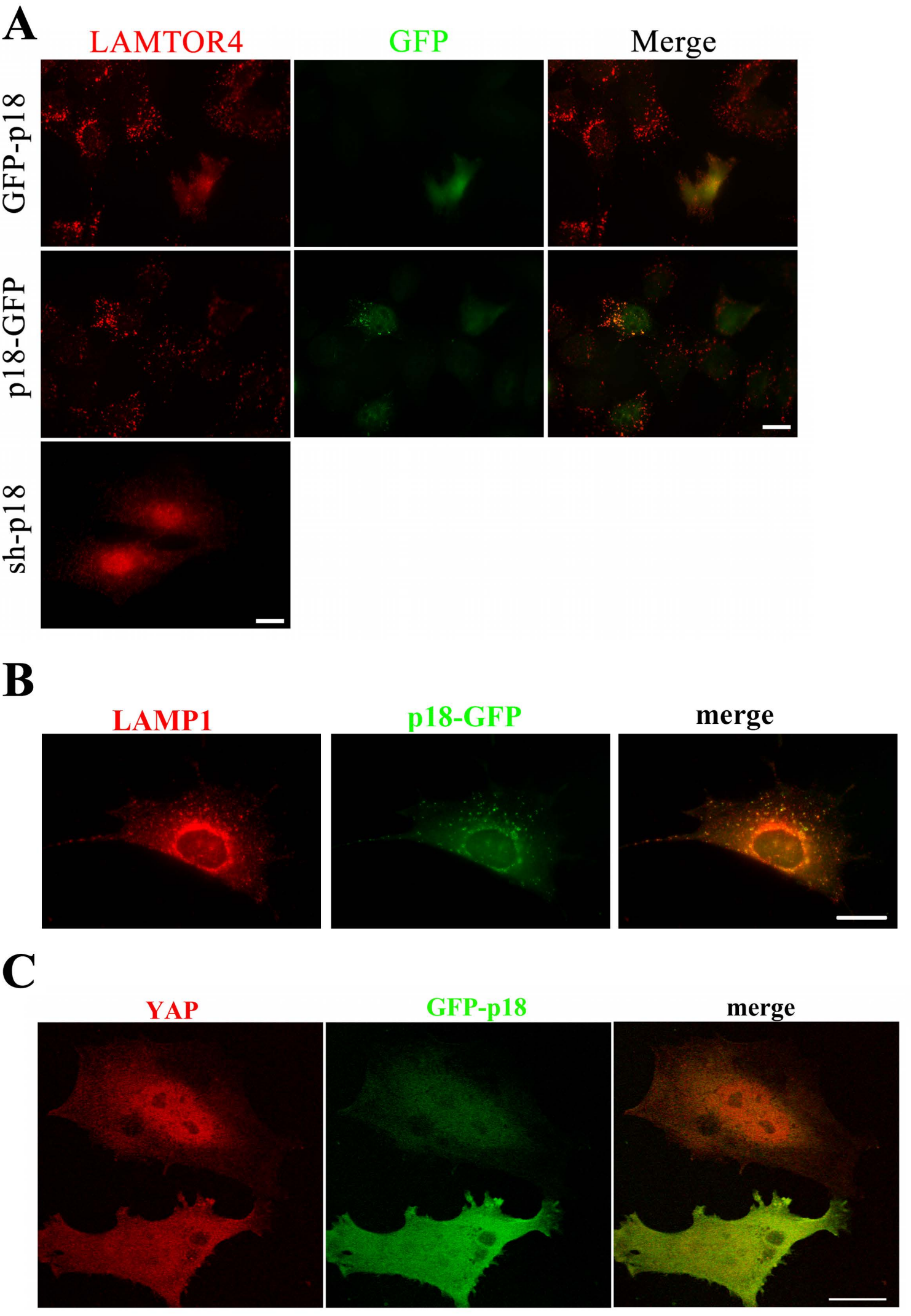
A
LAMTOR4
GFP
Merge
GFP-p18
p18-GFP
sh-p18
B
LAMP1
p18-GFP
merge
C
YAP
GFP-p18
merge

Figure S1

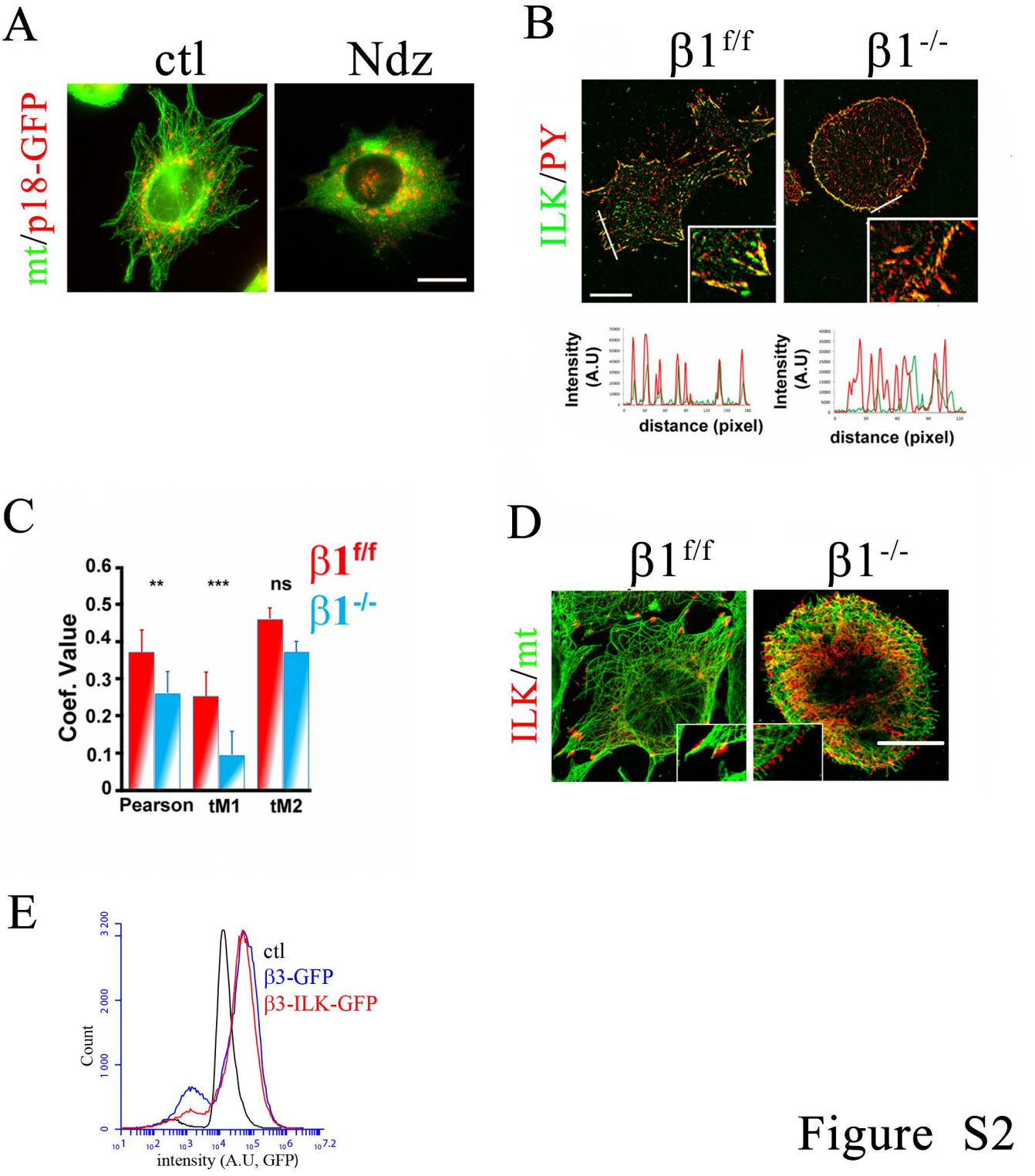
A
ctl
Ndz
mt/p18-GFP
B
β1f/f
β1-/-
ILK/PY
Intensitty (A.U)
distance (pixel)
Intensitty (A.U)
distance (pixel)
C
β1f/f
β1-/-
Coef. Value
0.6
0.5
0.4
0.3
0.2
0.1
0
**
***
ns
Pearson
tM1
tM2
D
β1f/f
β1-/-
ILK/mt
E
ctl
β3-GFP
β3-ILK-GFP
Count
3 200
2 000
1 000
0
intensity (A.U, GFP)

Figure S2

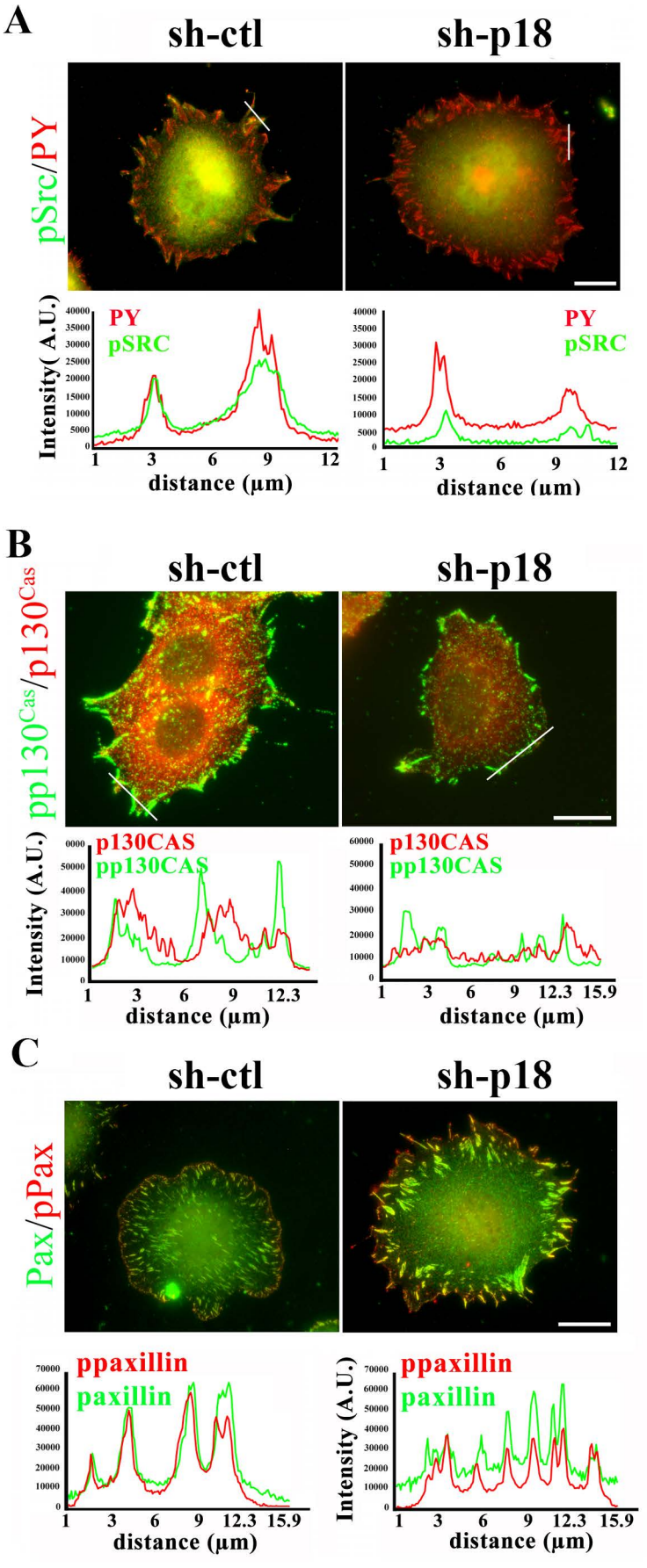
A
sh-ctl
sh-p18
pSrc/PY
PY
pSRC
Intensity(A.U.)
distance (μm)
B
sh-ctl
sh-p18
pp130Cas/p130Cas
p130CAS
pp130CAS
Intensity (A.U.)
distance (μm)
C
sh-ctl
sh-p18
Pax/pPax
ppaxillin
paxillin
Intensity (A.U.)
distance (μm)

Figure S3

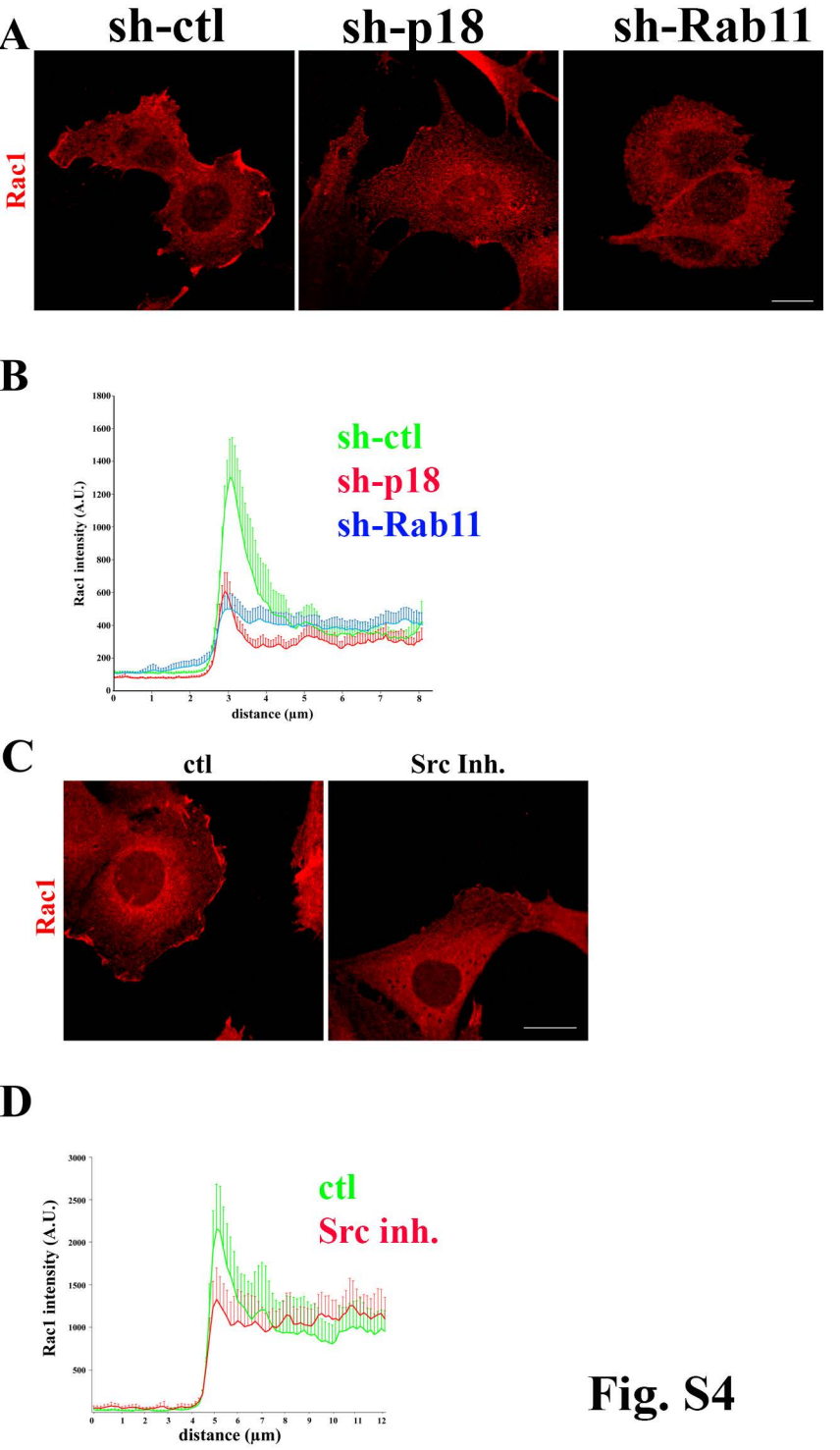
A
sh-ctl
sh-p18
sh-Rab11
Rac1
B
sh-ctl
sh-p18
sh-Rab11
Rac1 intensity (A.U.)
distance (μm)
C
ctl
Src Inh.
Rac1
D
ctl
Src inh.
Rac1 intensity (A.U.)
distance (μm)

Fig. S4

Legends to Supplementary Figures.

Figure S1 Loss of p18/LAMTOR1 or expression of dominant negative form of p18/LAMTOR1 affects LAMTOR complex docking to LE and YAP nuclear localization.

A. Pre-osteoblast cells expressing GFP-p18 (dominant negative form), p18-GFP (functional p18) and sh p18/LAMTOR1 were cultured overnight and stained for LAMTOR4. Note that GFP-p18 is diffusely distributed in the cytoplasm and LAMTOR4 no longer detected at typical vesicles. Bar: 10µm.

B. LAMP1 (late endosome marker) expression analysis in pre-osteoblast cells that stably express p18/LAMTOR1-GFP after overnight growth on glass coverslips. Bar: 10µm.

C. YAP (red) expression analysis in pre-osteoblast cells that express a dominant negative form of p18/LAMTOR1 (GFP-p18). Cells were grown overnight on glass coverslips. Scale bar: 10µm.

Figure S2. ILK is enriched in FAs in a β1 integrin-dependent manner.

A. Pre-osteoblasts that express p18-GFP were grown overnight on glass coverslips. p18/LAMTOR1 (red) and microtubules (green) were analyzed in control cells (ctl) and in cells incubated with nocodazole (Ndz, 10µg/ml, 1h). Bar: 10µm.

B. Control ($\beta1^{f/f}$) and β1 integrin-deficient ($\beta1^{-/-}$) pre-osteoblasts that stably express ILK-GFP were grown overnight on glass coverslips, and FAs stained using an anti-phosphotyrosine monoclonal antibody (PY, upper panel). The line intensity profiles (bottom) were obtained using the Icy software plugin. Bar: 10µm.

C. Histogram representing the Pearson and thresholded Manders coefficients (tM1, tM2) obtained from confocal microscopy images of $\beta1^{f/f}$ and $\beta1^{-/-}$ pre-osteoblasts that stably express ILK-GFP and stained with anti-phosphotyrosine antibody to label FAs. Data are the mean ±SD of n >20 cells/condition (two-tailed unpaired Student's *t*-test).

D. $\beta1^{f/f}$ and $\beta1^{-/-}$ pre-osteoblasts that stably express ILK-GFP (red) were grown overnight on glass coverslips and stained for microtubules (green). Bar: 10µm.

E. $\beta1^{-/-}$ pre-osteoblasts stably expressing β3-ILK-GFP, β3-GFP or not were analyzed by flow cytometry (BD Accuri™ C6).

Figure S3. Phosphorylation of Src and Src substrates in p18/LAMTOR1-silenced cells.

Control cells (sh-ctl) and p18/LAMTOR1-silenced cells (sh-p18) were grown overnight on glass coverslips and stained with antibodies against:

A. phosphorylated Src (pSrc, green) and phosphorylated tyrosines (PY, red),

B. phosphorylated $p130^{CAS}$ ($pp130^{CAS}$, green) and $p130^{CAS}$ (red),

C. paxillin, (green) and paxillin phosphorylated at Y31 ($pPaxillin^{Y31}$, red).

The line intensity profiles were obtained using the Icy software plugin. Bar: 10µm.

Figure S4. Rab-11, p18/LAMTOR1 and Src activity regulate Rac1 subcellular localization.

A. Pre-osteoblasts that express scramble (sh-ctl), and shRNAs against Rab-11 (sh-Rab-11) and p18/LAMTOR1 (sh-p18) were grown overnight on glass coverslips and stained for Rac1. Bar: 10µm.

B. Comparison of Rac1 distribution (fluorescence intensity) in sh-ctl (green), sh-Rab-11 (blue) and sh-p18 (red) cells. The line profiles were obtained using the Fiji analysis software (30 regions/condition were selected for quantification).

C. Pre-osteoblasts were grown overnight on glass coverslips and incubated or not with the specific Src inhibitor 5 (1µM for 2h) and stained for Rac1. Bar: 10µm.

D. Comparison of Rac1 distribution (fluorescence intensity) in pre-osteoblasts incubated (red) or not (green) with the Src inhibitor 5 (1µM for 2h) using fluorescence intensity. The line profiles were obtained using the Fiji analysis software (30 regions/conditions).