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Supplemental Material to:

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Autophagy modulates cell migration and β1-integrin membrane recycling

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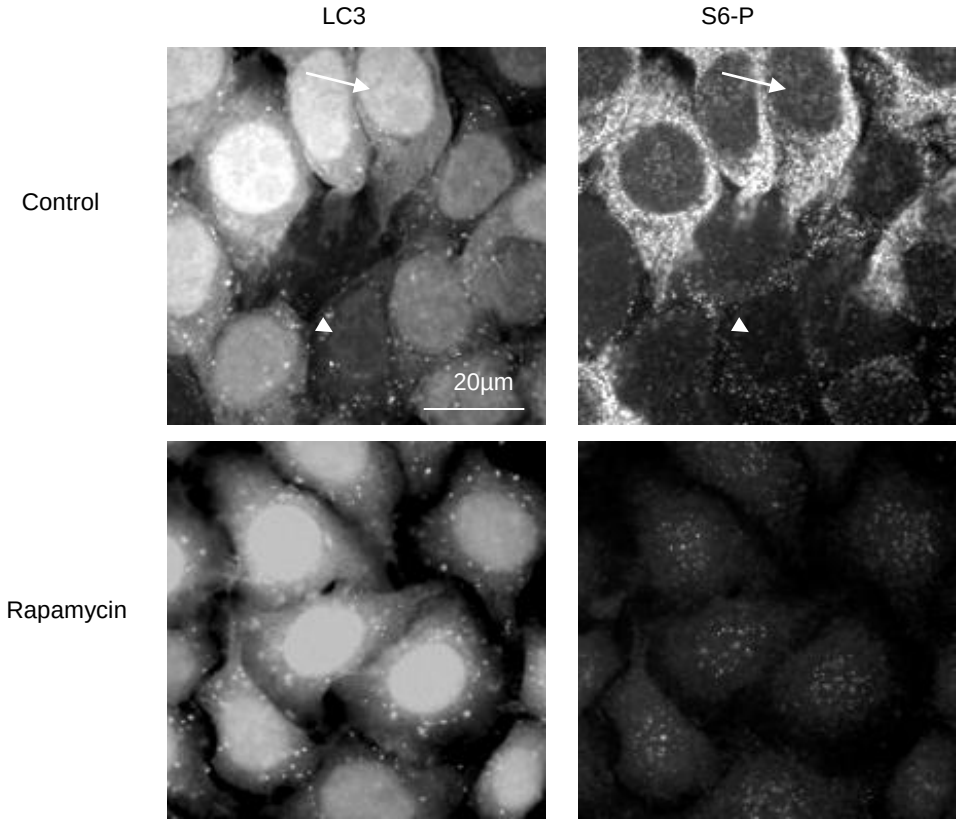

Figure S1

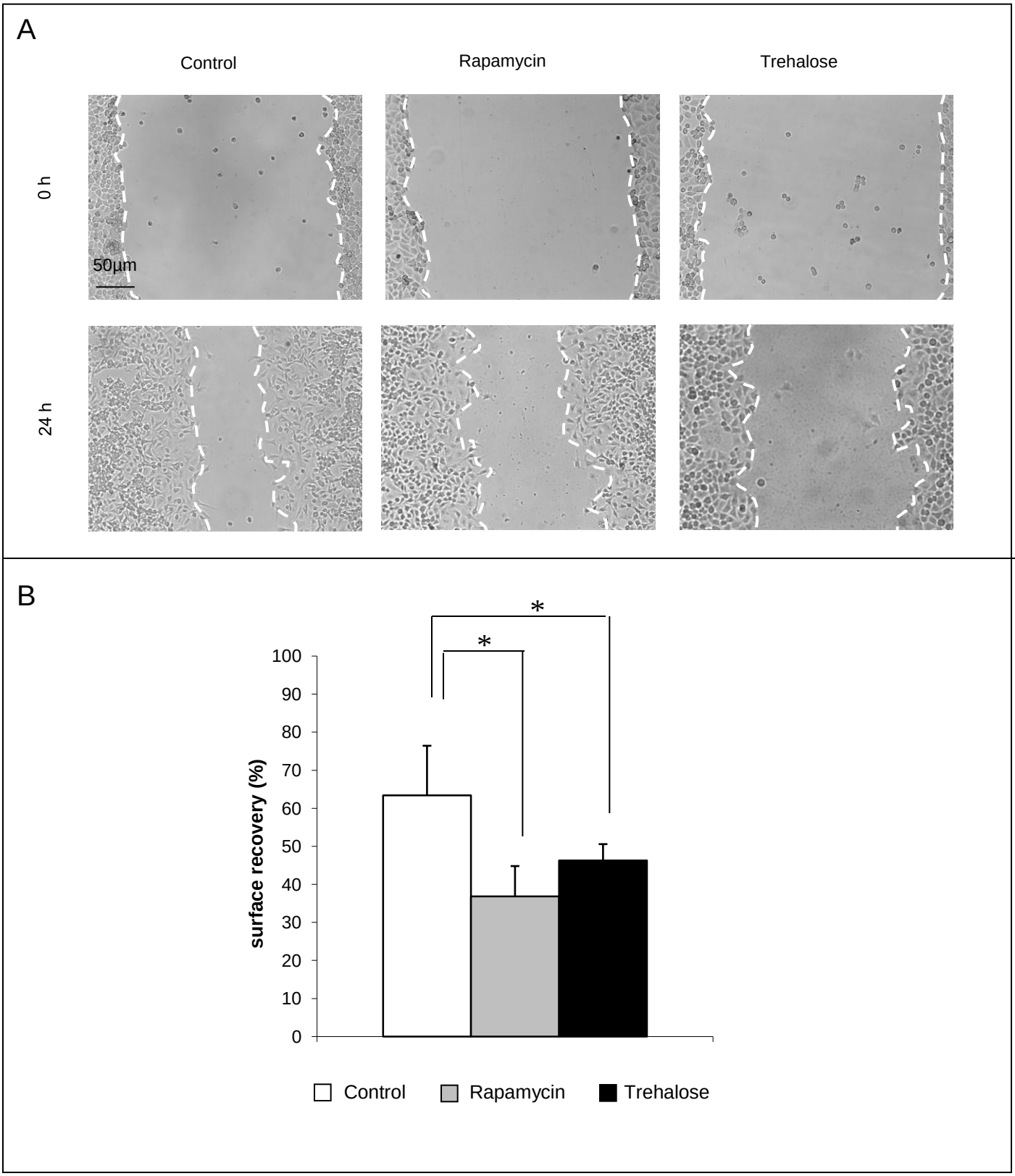
A
Control
Rapamycin
Trehalose
0 h
24 h
50µm
B
surface recovery (%)
100
90
80
70
60
50
40
30
20
10
0
*
*
Control
Rapamycin
Trehalose

Figure S2

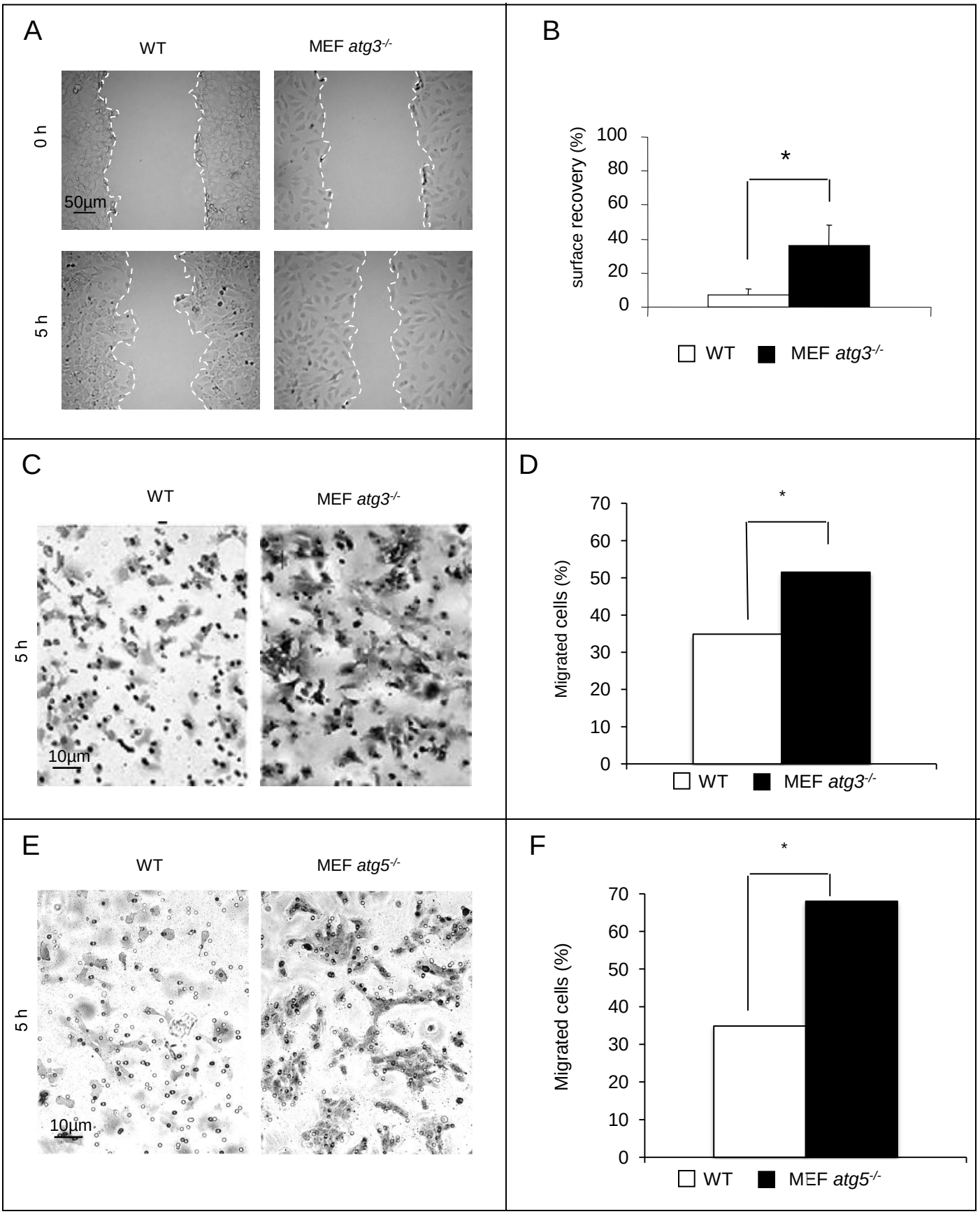
A
WT
MEF atg3-/-
0 h
5 h
50µm
B
surface recovery (%)
100
80
60
40
20
0
*
WT
MEF atg3-/-
C
WT
MEF atg3-/-
5 h
10µm
D
Migrated cells (%)
70
60
50
40
30
20
10
0
*
WT
MEF atg3-/-
E
WT
MEF atg5-/-
5 h
10µm
F
Migrated cells (%)
70
60
50
40
30
20
10
0
*
WT
MEF atg5-/-

Figure S3

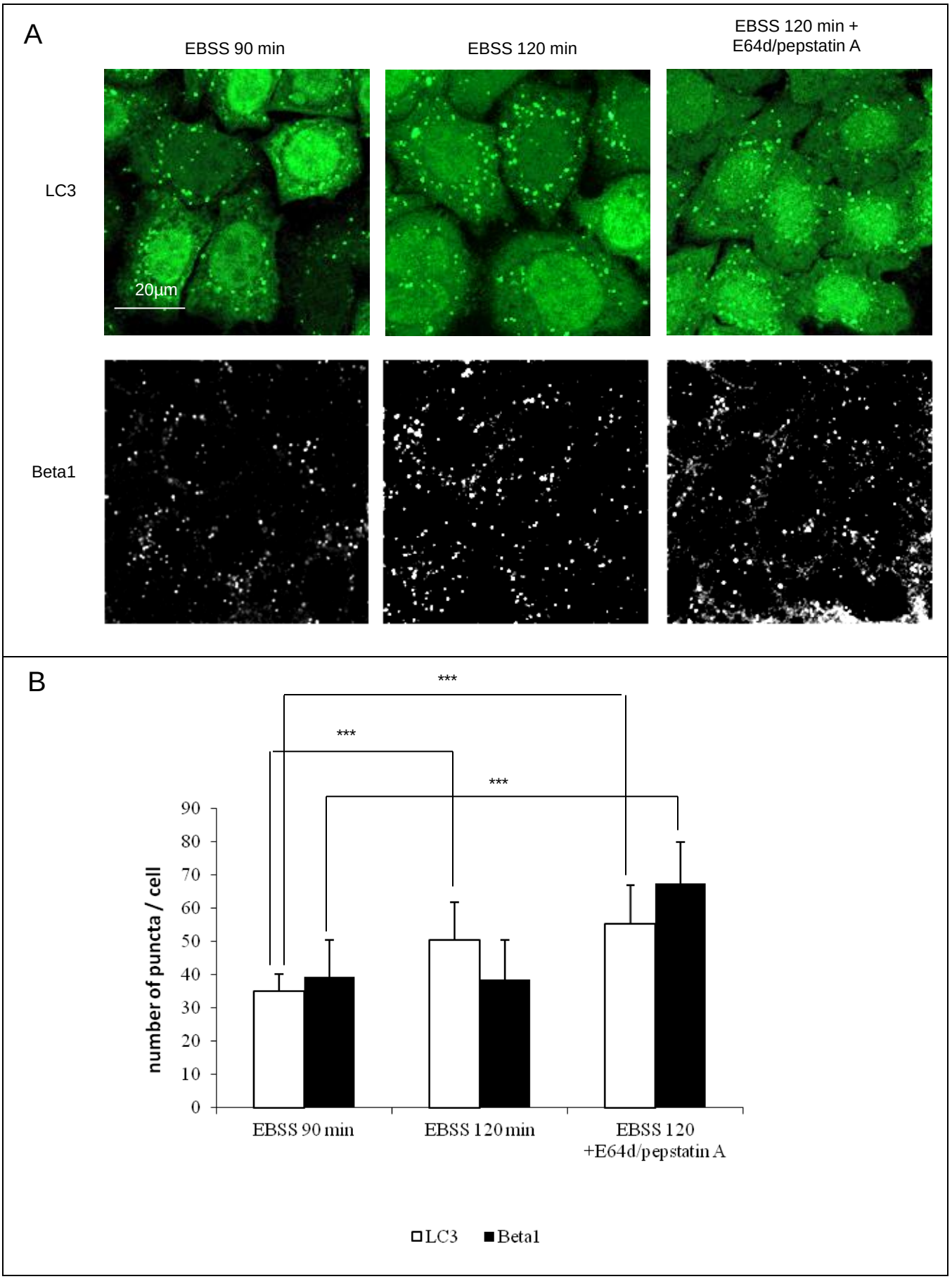
A
EBSS 90 min
EBSS 120 min
EBSS 120 min +
E64d/pepstatin A
LC3
Beta1
20µm
B
***
***
***
number of puncta / cell
90
80
70
60
50
40
30
20
10
0
EBSS 90 min
EBSS 120 min
EBSS 120
+E64d/pepstatin A
LC3
Beta1

Figure S4

Supplemental material

Figure S1: Activation of MTOR is correlated to autophagy inhibition in confluent Hela cells. HeLa-GFP-LC3 cells were grown to confluence in complete medium (Control) or medium containing 200 nM rapamycin (Rapamycin). The cells were fixed with 4% paraformaldehyde, and then stained with anti-S6-P antibody (1:100). The arrow indicates a low level of GFP-LC3 puncta correlated with high phosphorylation of S6. The arrowhead indicates a high level of GFP-LC3 puncta correlated with low phosphorylation of S6.

Figure S2: Effect of rapamycin and trehalose on the migration of HeLa cells. A. Cell monolayers were assayed for wound-healing migration in complete medium, medium containing 200 nM rapamycin and medium containing 10-µM trehalose. Phase-contrast microscopy was carried out to record the relative rate of wound closure immediately after the scratch (0 h) and after 24 h of migration (24 h). **B.** The surface of the wound was evaluated by Image J software, and the percentage of the surface to have recovered was determined. Results are representative of four independent experiments ± SD. *$p<0.05$.

Figure S3: Effect of Atg3 and Atg5 knock out on MEF migration. A. Control MEF (WT) and *atg3*$^{-/-}$ MEF (MEF *atg3*$^{-/-}$) monolayers were assayed for wound-healing migration in complete medium. Phase-contrast microscopy was carried out to record the relative rate of wound closure after the scratch (0 h) and after 5 h of migration (5 h). **B.** The surface of the wound was evaluated by Image J software, and the percentage of the

surface to have recovered was determined. Results are representative of four independent experiments ± SD. *p<0.05. **C** and **E.** Control MEFs (WT), *atg3*$^{-/-}$ MEFs (MEF *atg3*$^{-/-}$) and *atg5*$^{-/-}$ MEFs (MEF *atg5*$^{-/-}$) were plated at the top of a Transwell, and allowed to migrate for 5 h. They were then rinsed, fixed with 4% paraformaldehyde, stained with crystal violet, and counted. Images show cells on the undersurface of a filter. **D** and **F.** The percentage of migrated cells was evaluated using the migration after 24 h as equivalent to 100% migration. Results are representative of four independent experiments ± SD. *p<0.05

Figure S4: Quantification of autophagosomes and β1 integrins during starvation.

A. HeLa-GFP-LC3 cells were first incubated in starvation medium for 4 h. The cells were then stained with anti-β1 integrin antibody (P5D2 1:50) before either being treated with the lysosomal inhibitors E64d (10µM) and pepstatin A (10µg/ml) or not treated. After 90 or 120 min of endocytosis, the cells were fixed with 4% paraformaldehyde, and the autophagosomes and β1 integrins were observed by fluorescence microscopy. **B.** The graph indicates the number of autophagosomes per cell and the number of β1-stained puncta per cell. Results are representative of three independent experiments ± SD *p<0.05, **p<0.01, ***p<0.005.

Videos 1 and 2: Convergence of the β1 integrin endocytic pathway and the autophagic pathway in migrating cells. Hela-GFP-LC3 were wounded with a p200 pipet tip, and then incubated with an anti-ß1integrin monoclonal antibody (P5D2 1:100 and Alexa-Fluor 594 anti-IgG antibody) for 4 h at 37°C. **A.** Cells in complete medium:

control (video 1) or **B.** Cells treated with rapamycin 200 nM for 4 h: rapamycin (video 2) . After the incubation, the coverslips were placed in a temperature- and CO_2-controlled incubator chamber mounted on the microscope stage, and examined using time-lapse fluorescent microscopy. A total of approximatively 200 images were obtained; each had a timelapse of 1.2 sec, and was taken with the green and red filters sets.

Videos 3 and 4: Redistribution of β1 integrins on the adhesion sites at the plasma membrane. Hela-GFP-LC3 were incubated with anti-β1 integrin antibody (P5D2 1:50) and Alexa fluor 555 anti-IgG antibody (1:2000) for 30 min at 4°C, then washed and incubated in complete medium (video 3) or in EBSS (video 4) under the videomicroscope at 37°C **.** For TIRF microscopy-videos, frames were acquired every 3 minutes for two hours under physiological conditions, i.e. 5% CO_2 and 37°C. The focus was maintained by the Definite focus device.