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## Supplemental information

# Septin 9 and phosphoinositides regulate lysosome localization and their association with lipid droplets

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Figure S1 Oleate treatment induces acidic vesicles redistribution, related to Figure 1

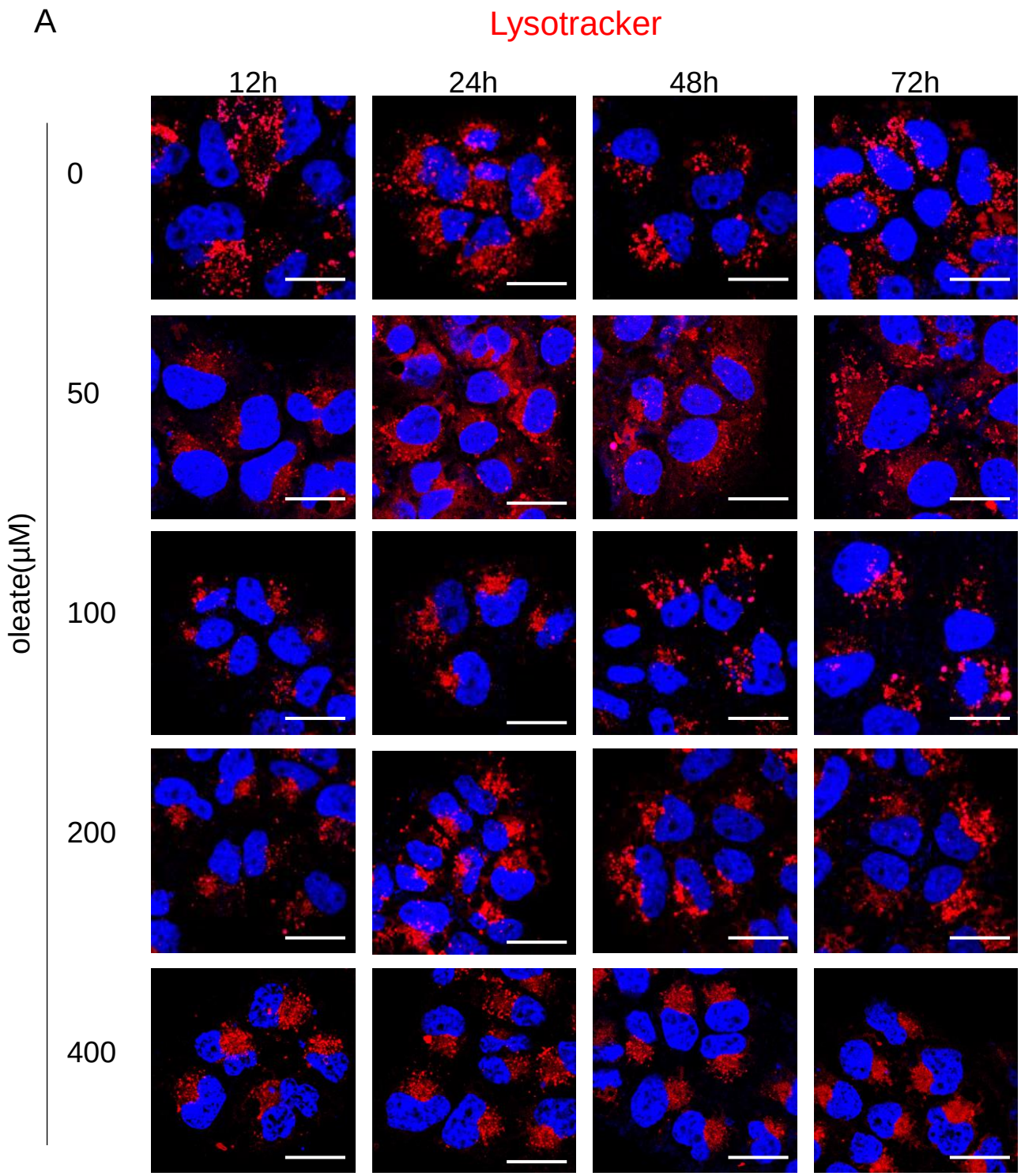



**Figure S1 Oleate treatment induces acidic vesicles redistribution**

**A**. Huh7 cells were grown for 24h. the culture medium was supplemented with or without 50, 100, 200, 400 μM sodium oleate complex for different time points (12h, 24h, 48h, 72h), then stained with Lysotracker(red). Scale bar, 20μm.

Figure S2 Analysis of lysosomal genes in oleate treated cells and LAMP1-Bodipy interaction, related to Figure 1

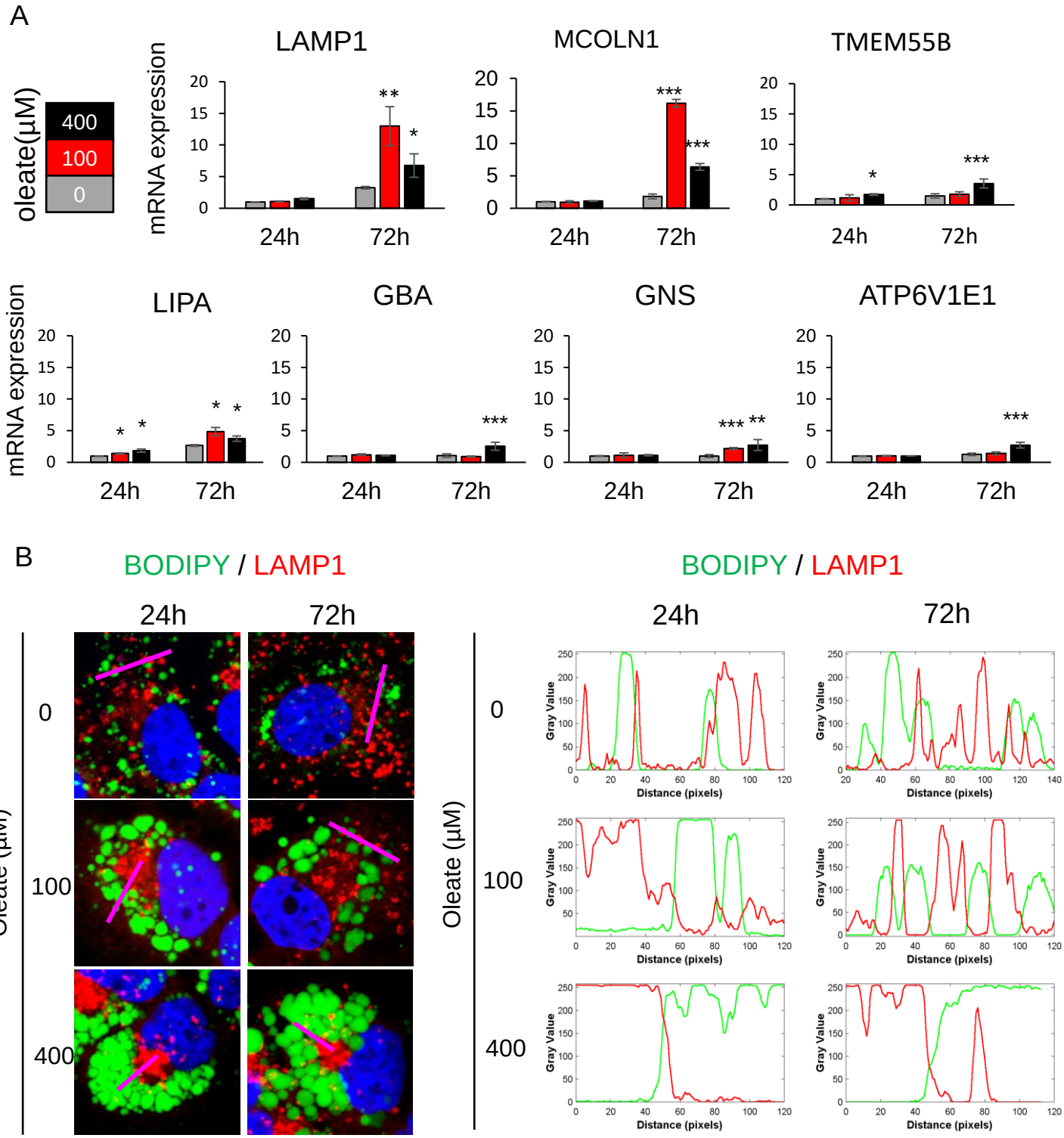



**Figure S2 Analysis of lysosomal genes in oleate treated cells and LAMP1-Bodipy interaction**

**A**. Huh7 cells were grown overnight. Then culture medium was supplemented without or with 100 and 400 μM sodium oleate complex for different time (24h, 72h), mRNA was extracted, and RT-PCR were performed for LAMP1, TMEM55B, MCOLN1, GBA, GNS, LIPA and ATP6V1E1.

**B.** The line plots for LAMP1 and BODIPY were performed for cells treated with 0,100 and 400 μM sodium oleate complex for different time (24h, 72h).

Data information: Bar graphs present Mean ± SEM. Student's t-test was used. *P < 0.05, **P< 0.001, ***P<0.0001.

Figure S3 Knockdown of septin 9 disrupts oleate-induced LDs accumulation and lysosome perinuclear clustering, related to Figure 2

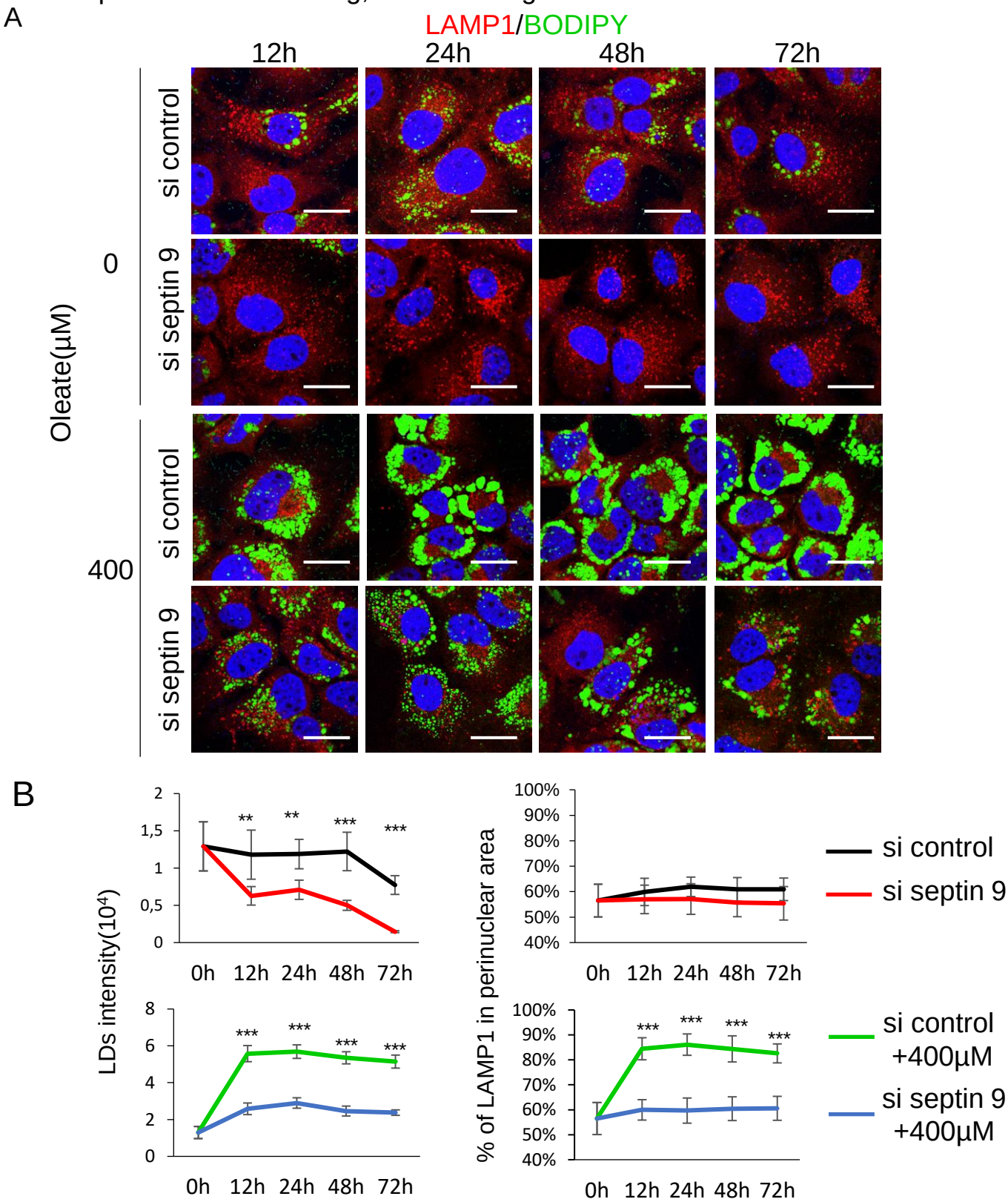



**Figure S3 Knockdown of septin 9 disrupts oleate-induced LDs accumulation and lysosome perinuclear clustering**

**A**. Huh7 cells were transfected with or not septin 9-siRNA for 24h then culture with medium supplemented with or not 400 μM oleate for different time. Then stained for BODIPY (green) and LAMP1 (red). Scale bar, 20μm.

**B**. Fluorescence intensity of LD and the percentage of LAMP1 in perinuclear area were analyzed in 25 cells from two experiments performed as described in A.

Data information: Line graphs present Mean ± SEM. Student's t-test was used. *P < 0.05, **P< 0.001, ***P<0.0001.

Figure S4 Septin 9_i1 overexpression maintains the colocalization of LAMP1 and TGN46, related to Figure 3

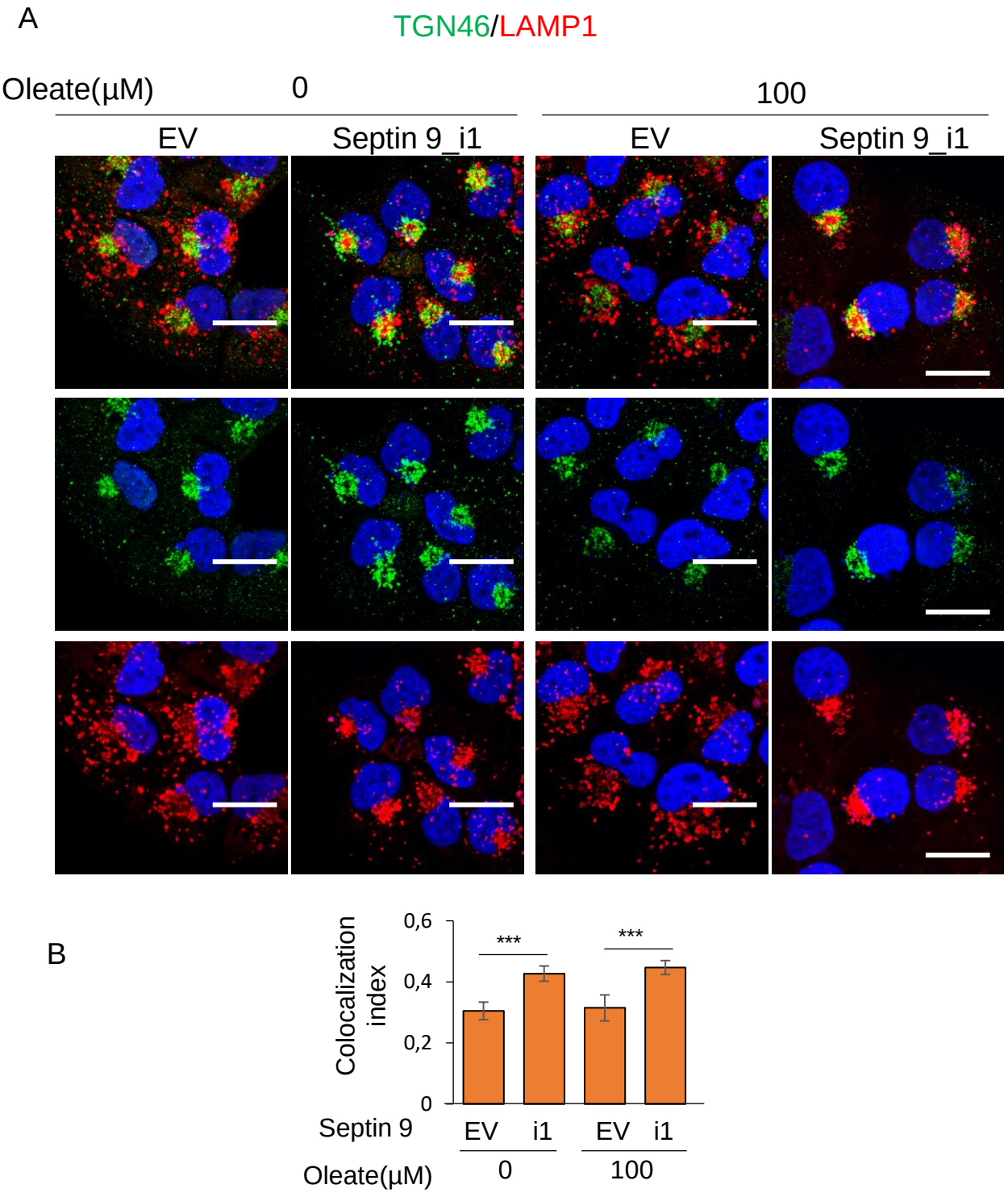



**Figure S4 Septin 9_i1 overexpression maintains the colocalization of LAMP1 and TGN46**

**A**. Huh7 cells were transfected with EV or septin 9_i1 were grown for 24h. Then culture medium was supplemented with 100 µM sodium oleate complex for 72h, after treatment, cells were stained for TGN46 (green) and LAMP1 (red). Scale bar, 20µm.

**B**. Bar graphs show Pearson's correlation coefficient (Rr) for co-localization between LAMP1 and TGN46.

Data information: Bar graph present Mean ± SEM. Student's t-test was used. *P < 0.05, **P< 0.001, ***P<0.0001.

Figure S5 MTs increase in LD accumulated cells and associate with LAMP1 perinuclear clusters, related to Figure 4

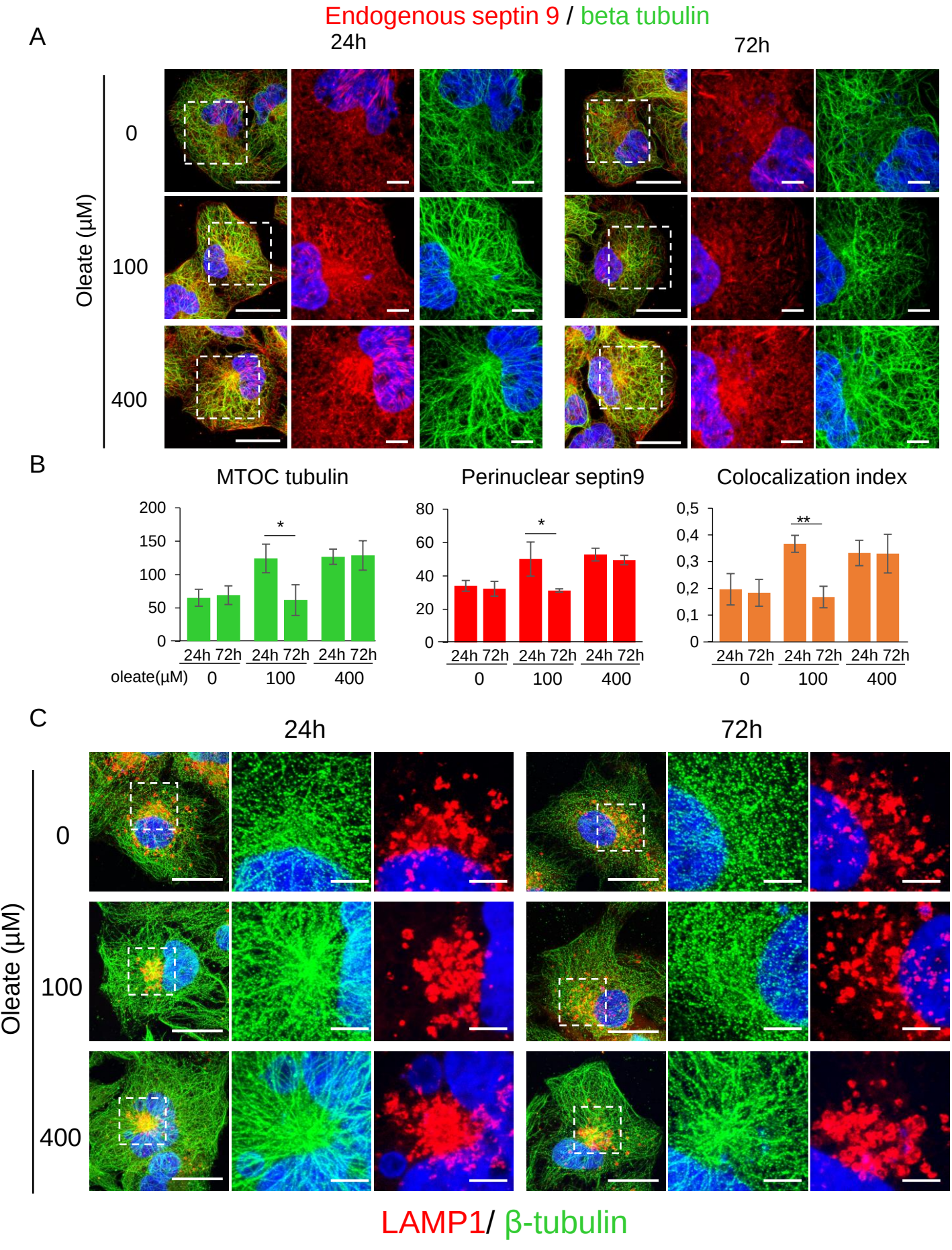


**Figure S5 MTs increase in LD accumulated cells and associate with LAMP1 perinuclear clusters**

**A**. Huh7 cells were culture with medium supplemented without or with 100 and 400 μM oleate complex for 24h or 72h then stained for β tubulin(green) and septin 9 (red). Scale bar, 20μm. The dot squares indicate the zoom area. Scale bar, 5μm.

**B**. Bar graphs show mean intensity of β-tubulin in MTOC area, mean intensity of septin 9 in perinuclear area and Pearson's correlation coefficient (Rr) for co-localization between β-tubulin and septin 9 from 3 independent experiments performed as described in A.

**C**. Huh7 cells were grown overnight. Then culture medium was supplemented without or with 100 and 400 μM sodium oleate complex for different time (24h, 72h), then stained for LAMP1 (red) and β tubulin (green). Scale bar, 20μm. The dot squares indicate the zoom area. Scale bar, 5μm.

Data information: Bar graphs present Mean ± SEM. Student's t-test was used. *P < 0.05, **P< 0.001, ***P<0.0001.

Figure S6 Oleate treatment promotes colocalization of Rab7 with LAMP1 in the perinuclear area, related to Figure 6

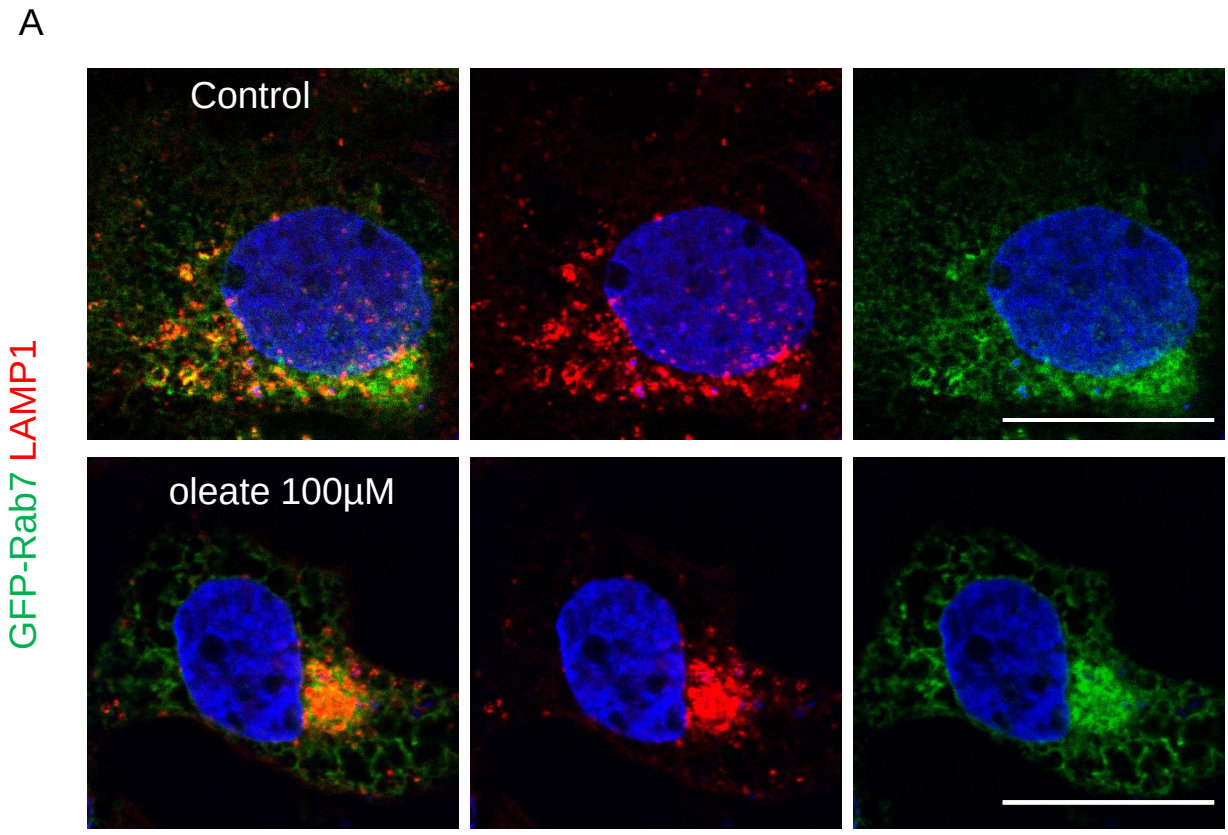



**Figure S6 Oleate treatment promotes colocalization of Rab7 with LAMP1 in the perinuclear area**

**A**. Huh7 cells expressed GFP-Rab7 were culture medium supplemented with or without 100 μM oleate for 24h, then stained for LAMP1 (red).

Figure S7 Septin 9 regulates the intracellular distribution of endogenous Rab7, related to Figure 6

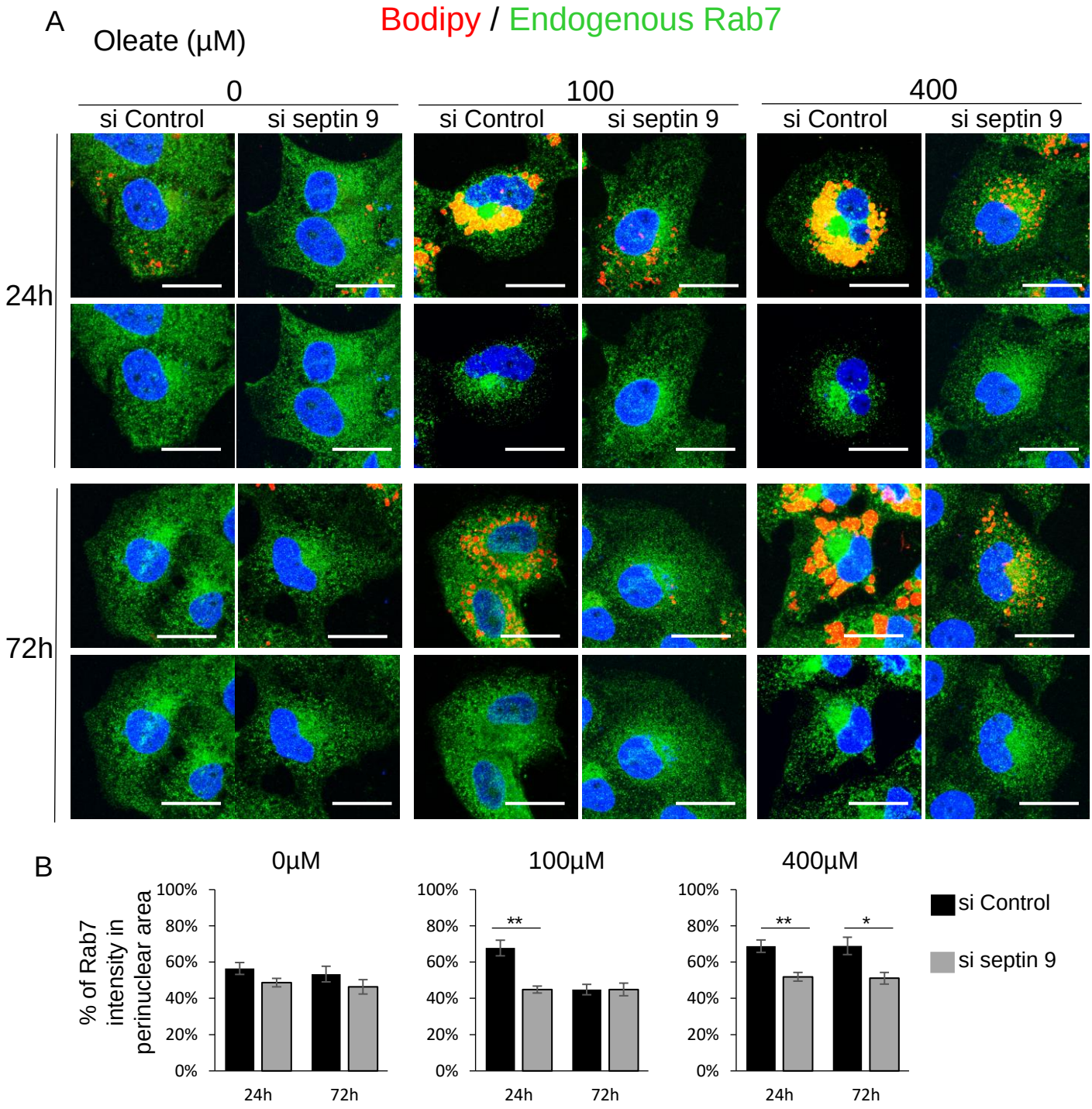



**Figure S7 Septin 9 regulates the intracellular distribution of endogenous Rab7**

**A**. Huh7 cells were transfected with or not septin 9-siRNA for 24h then culture with medium supplemented with or not 100, 400 µM oleate for different time (24, 72h). Then stained for Rab7 (green) and BODIPY (red). Scale bar, 20µm.

**B**. Bar graphs show the percentage of Rab7 intensity in perinuclear area from two different experiments.

Data information: Bar graphs present Mean ± SEM. Student's t-test was used. *P < 0.05, **P< 0.001, ***P<0.0001.

Figure S8 Septin 9 promotes Rab7 perinuclear clustering and its colocalization with Golgi, related to Figure 6

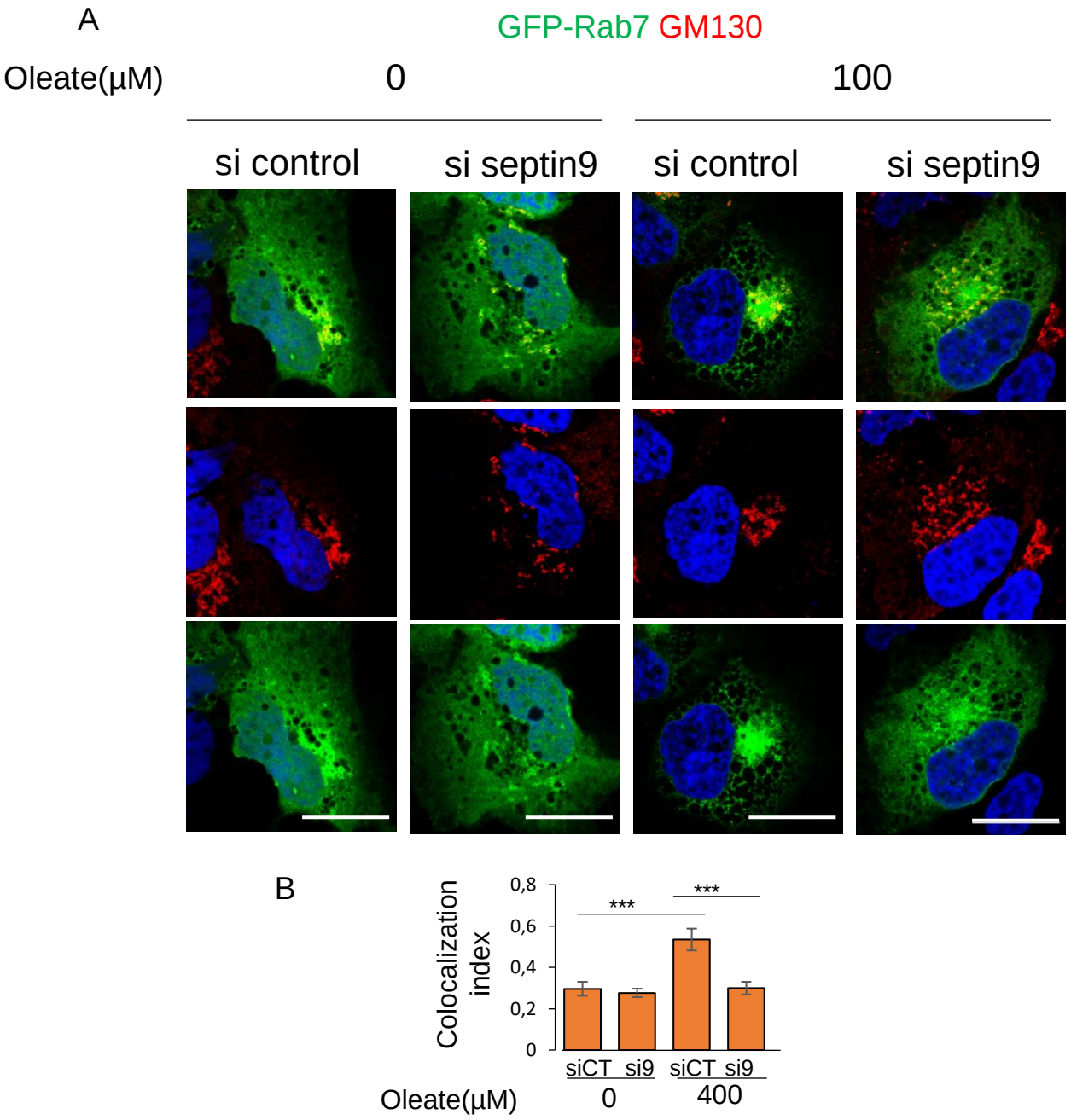



**Figure S8 Septin 9 promotes Rab7 perinuclear clustering and its colocalization with Golgi**

**A**. GFP-Rab7 expressed Huh7 cells were transfected with or not septin 9-siRNA for 24h then culture with medium supplemented with or not 400 μM oleate for 72h then stained for TGN46(red). Scale bar, 20μm.

**B**. Bar graphs show Pearson's correlation coefficient (Rr) for co-localization between Rab7 and TGN46.

Data information: Bar graphs present Mean ± SEM. Student's t-test was used. *P < 0.05, **P< 0.001, ***P<0.0001.

Figure S9 Septin 9 promotes the LC3B accumulation, related to Figure 6

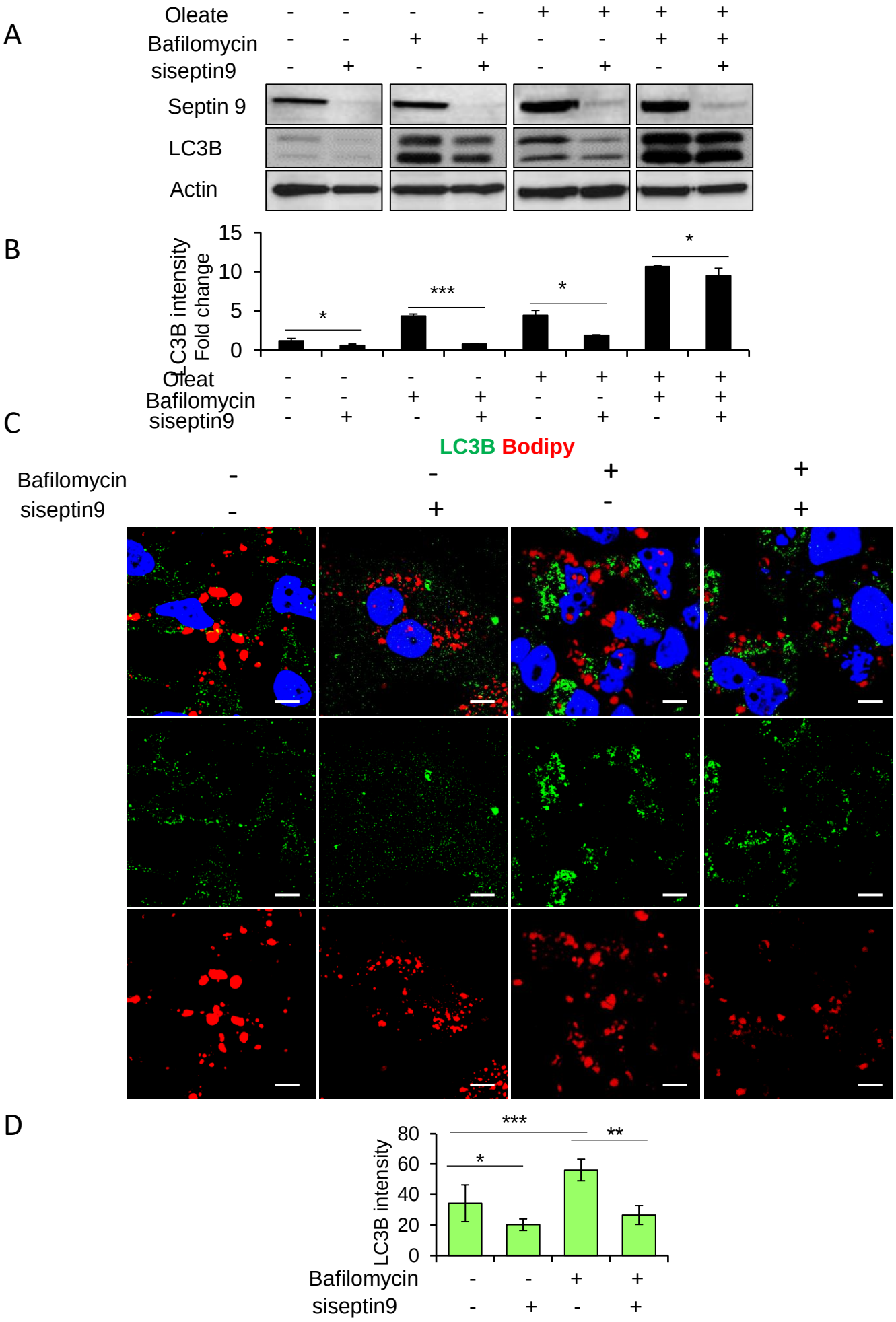


**Figure S9 Septin 9 promotes the LC3B accumulation**

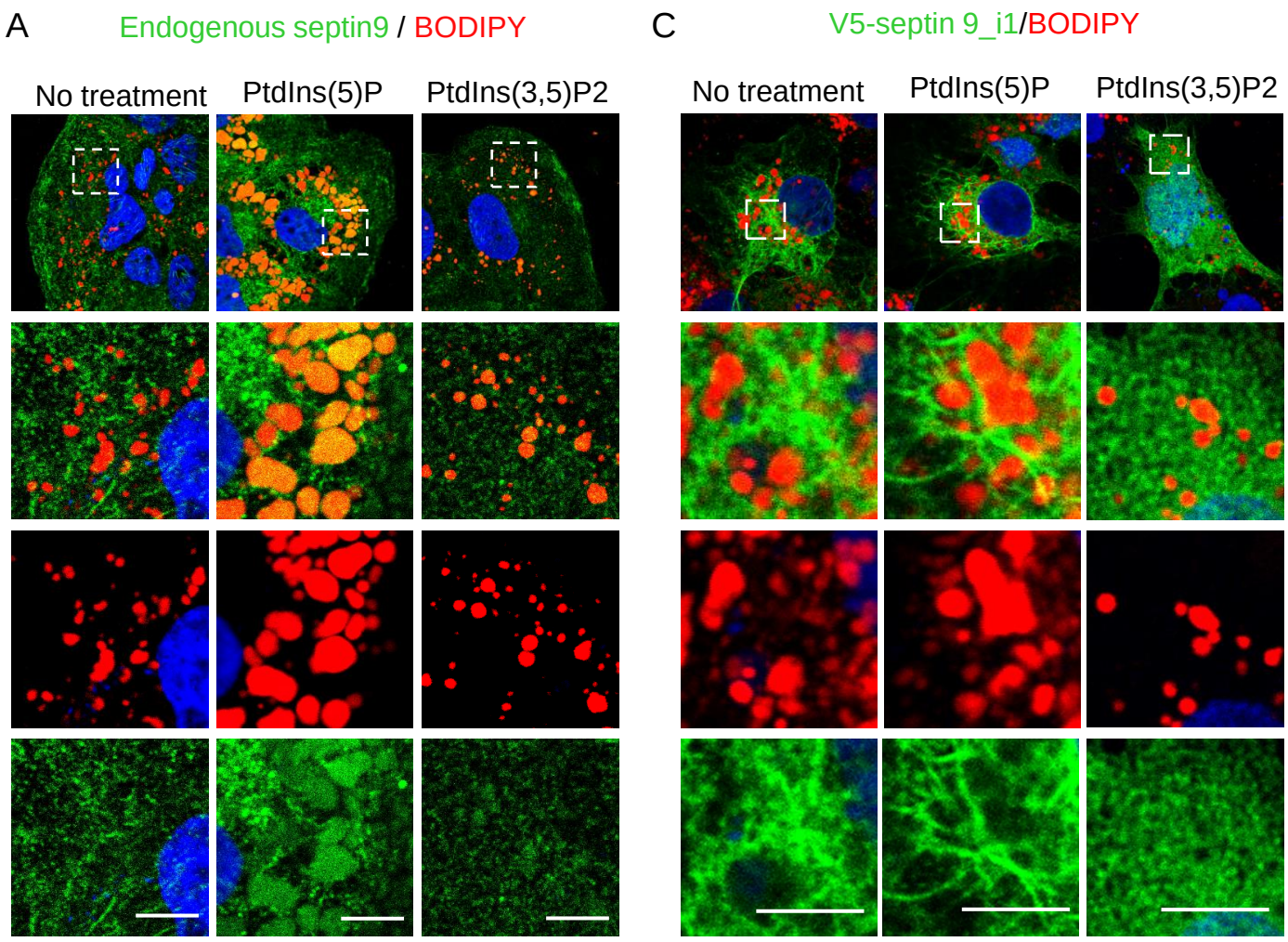
**A**. Huh7 cells were grown overnight. The cells were then transfected with siseptin9 for 24 h, then added 100nM Bafilomycin, 100μM Oleate, or not for further treatment. After another 24h, the cells were collected and analyzed by immunoblotting for septin 9 and LC3B.

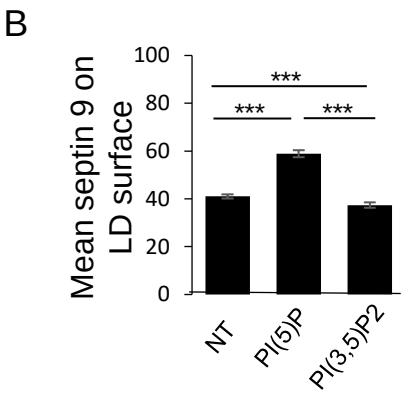
**B**. Three independent experiments were performed, Bar graphs present the mean ± SEM. A Student's t-test was used: ** $p < 0.001$, and *** $p < 0.0001$.

**C**. The cells are treated in the manner described in A, and then stained for LC3B(green) and Bodipy (red). Scale bar, 10 μm.

**D**. The LC3B intensity was analyzed at least 30 cells from the experiments. Bar graphs present the mean ± SEM. A Student's t-test was used: ** $p < 0.001$, and *** $p < 0.0001$.

Figure S10 PIs regulates septin 9 association with LDs, related to Figure 7



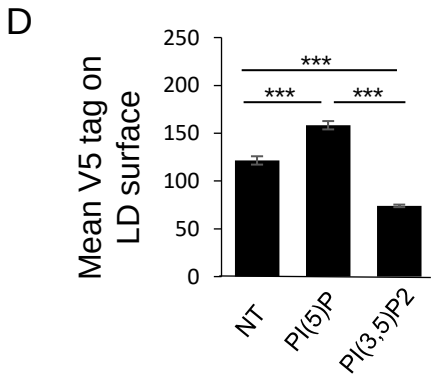




**Figure S10 PIs regulates septin 9 association with LDs**

**A**. Huh7 cells treated with cell-permeant PtdIns5P or PtdIns (3,5) P2 at 30 mM for 15 min before fixing and staining for endogenous septin 9 (green) and LDs (red). The dot squares indicate the zoom area. Scale bar, 5µm.

**B**. Bar graphs show the mean intensity of endogenous septin 9 on LDs from two independent experiments performed as described in A.

**C**. Huh7 cells transfected with or without septin 9_i1 were cultured for 24h. Then, cells were treated with cell-permeant PtdIns5P or PtdIns (3,5) P2 at 30 mM for 15 min before fixing and staining for V5-tag (green) and LDs (red). The dot squares indicate the zoom area. Scale bar, 5µm.

**D**. Bar graphs show the mean intensity of V5-tag on LDs from three independent experiments performed as described in C.

Data information: Bar graphs present Mean ± SEM. Student's t-test was used. *P < 0.05, **P< 0.001, ***P<0.0001.

Figure S11 PIs and MTMR3 regulate septin 9 and MT accumulation at MTOC,related to Figure 8

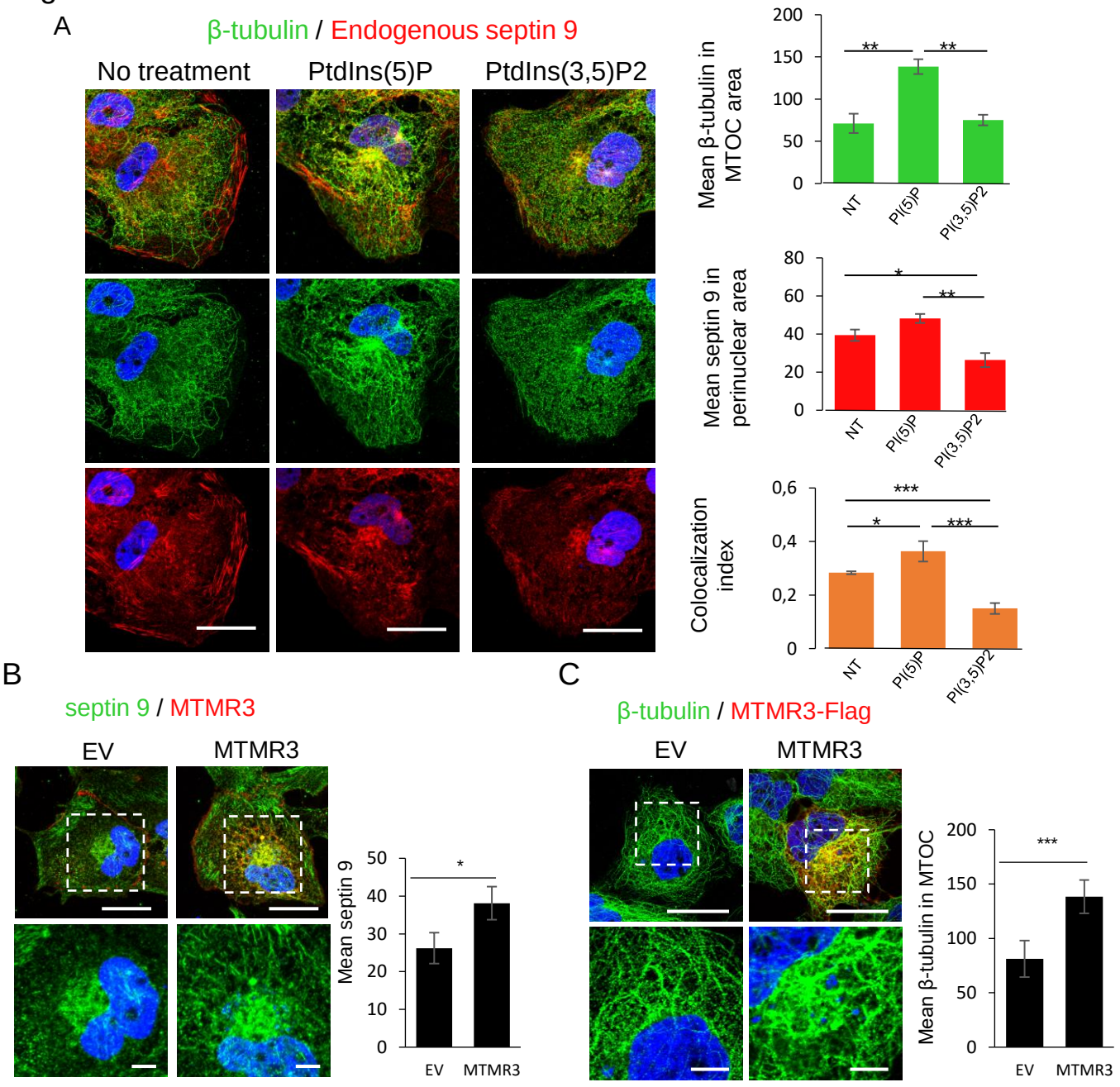



**Figure S11 PIs and MTMR3 regulate septin 9 and MT accumulation at MTOC**

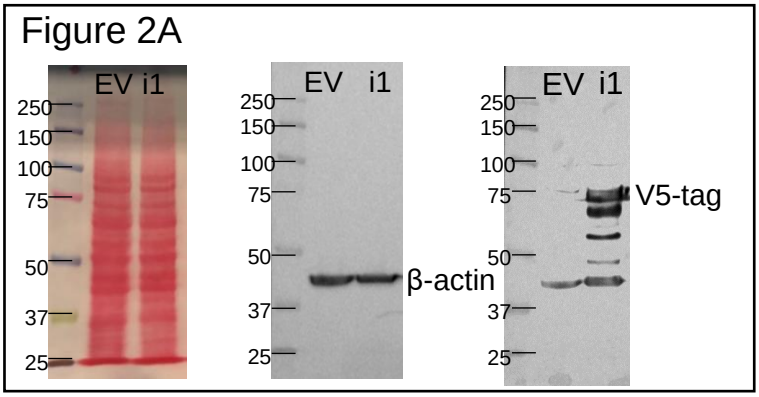
A. Huh7 cells treated with cell-permeant PtdIns5P or PtdIns (3,5) P2 at 30 mM for 15 min before fixing and staining for endogenous septin 9 (red) and β-tubulin (red). Scale bar, 20μm. Bar graphs show mean intensity of β-tubulin in MTOC area, mean intensity of septin 9 in perinuclear area and Pearson's correlation coefficient (Rr) for co-localization between β-tubulin and septin 9 from two independent experiments performed as described in A.

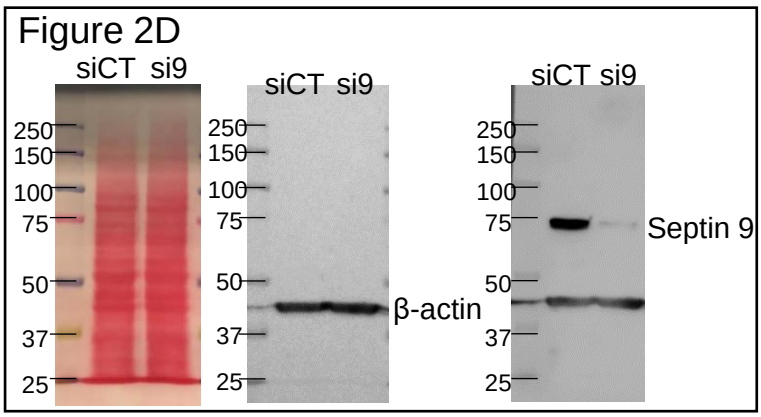
**B**. Huh7 cells were transfected with EV or MTMR3 were grown for 48h. Then, cells were stained for septin 9 (green)/ MTMR3 (red). Bar graphs show the mean intensity of septin 9 in perinuclear area from 2 independent experiments performed as described. Scale bar, 20μm. The dot squares indicate the zoom area. Scale bar, 5μm.

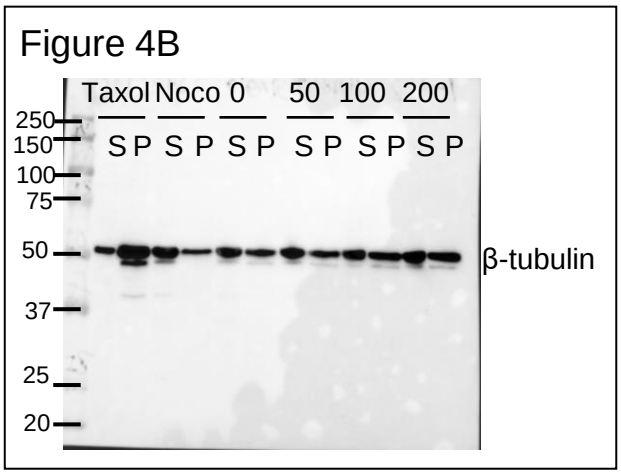
**C**. Huh7 cells were transfected with EV or MTMR3 were grown for 48h. Then, cells were stained for β-tubulin (green)/ Flag (red). Bar graphs show the mean intensity of β-tubulin in MTOC area from 2 independent experiments performed as described. Scale bar, 20μm. The dot squares indicate the zoom area. Scale bar, 5μm.

Data information: Bar and line graphs present Mean ± SEM. Student's t-test was used. *P < 0.05, **P< 0.001, ***P<0.0001.

Figure S12 uncropped scans, related to Figure 2, Figure 4 and Figure S9



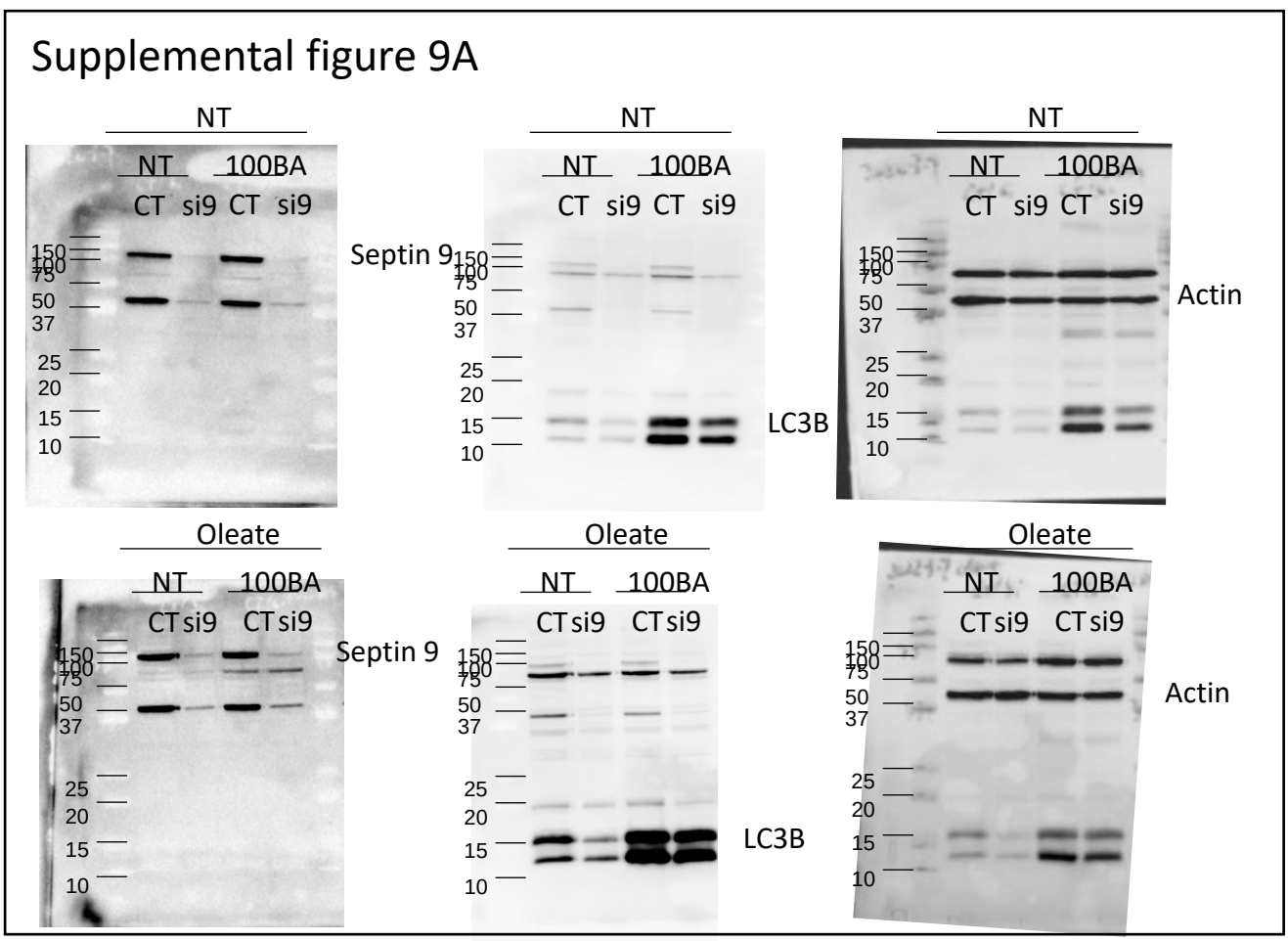
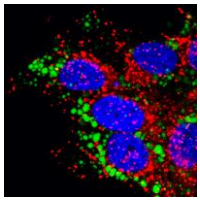



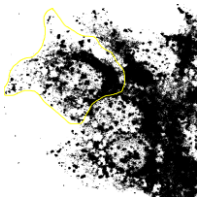
Figure S13 Schematic diagram of statistical methods for image signal calculation, related to STAR + Methods

A. Methods for protein signal in perinuclear and perinuclear area.

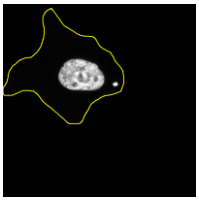
Steps



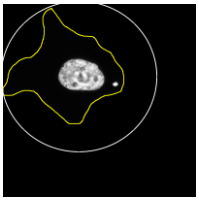
1.Images



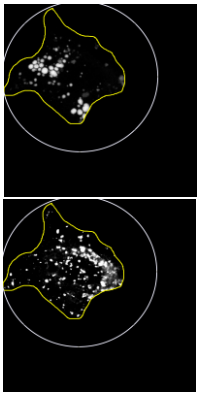
2.Threshold



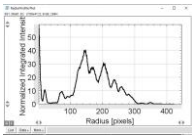
3.Total area



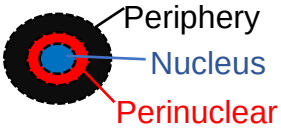
4.Define Radial profile circle



5.Apply Plugin on target channel



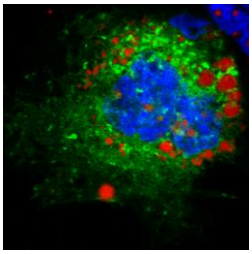
6.Data for calculation and analysis




$$\%\ \text{of intensity in perinuclear area} = \frac{\text{Intensity within perinuclear area}}{\text{Intensity within total cell area}} \times 100\%$$

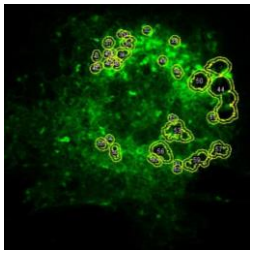
B. Method for protein signal around LDs.

Steps

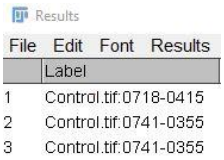


1.Images

2.Find LDs area (Area1)



3.Enlarge Area1 by 0.5μm (Area2)




4.Measure Intensity and area value

$$\text{Mean intensity} = \frac{(\text{Intensity within Area2})-(\text{Intensity within Area1})}{(\text{Area2 value})-(\text{Area1 value})}$$