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Supplemental Material to:

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ATP is released from autophagic vesicles to the extracellular space in a VAMP7-dependent manner

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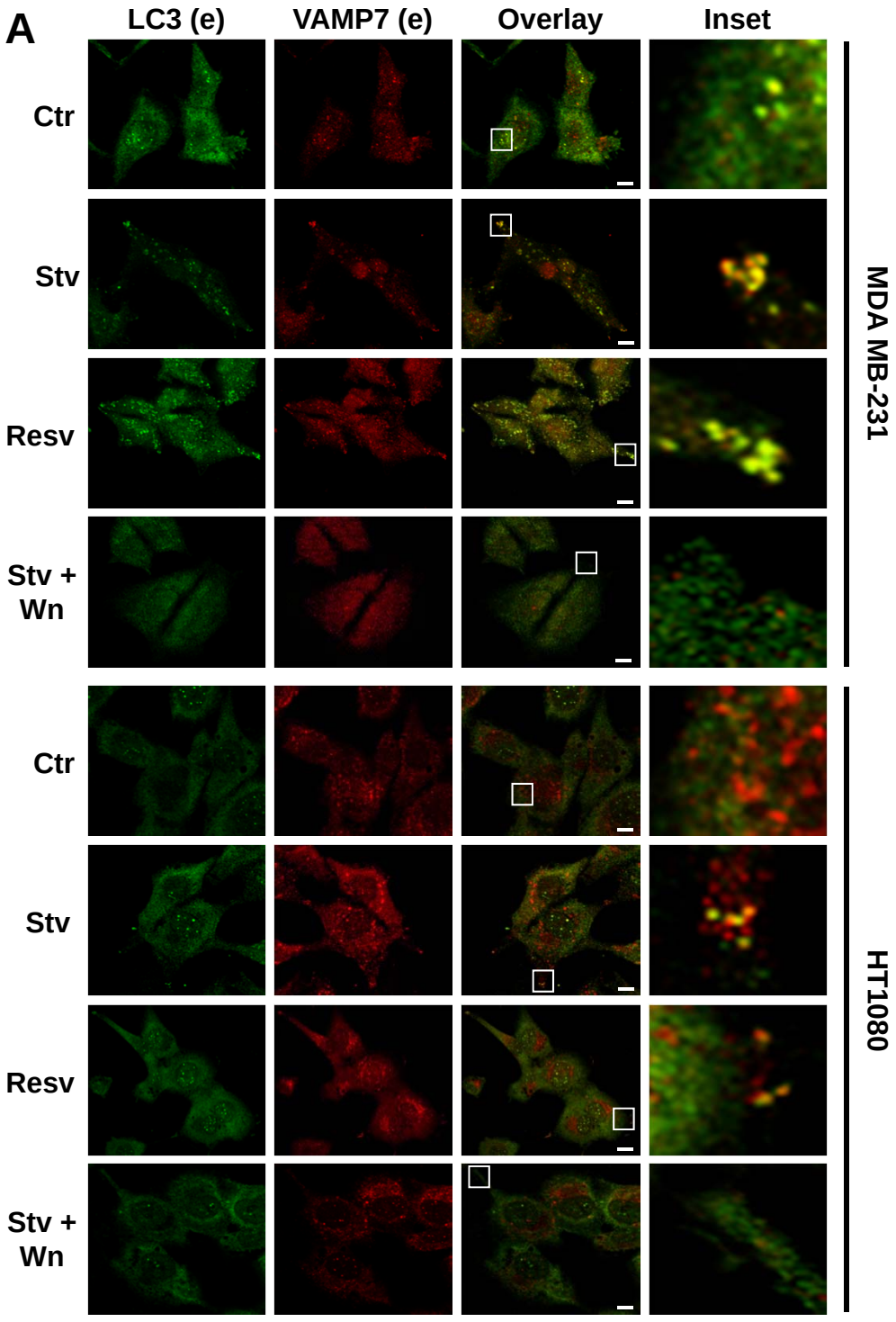
Figure S1


A
LC3 (e)
VAMP7 (e)
Overlay
Inset
Ctr
Stv
Resv
Stv + Wn
MDA MB-231
Ctr
Stv
Resv
Stv + Wn
HT1080

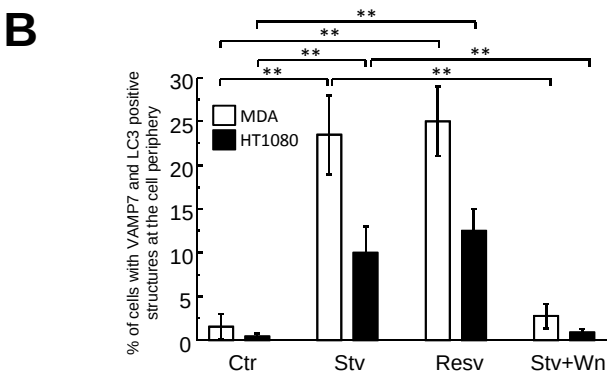

B
% of cells with VAMP7 and LC3 positive structures at the cell periphery
MDA
HT1080
Ctr
Stv
Resv
Stv+Wn
**

Figure S2

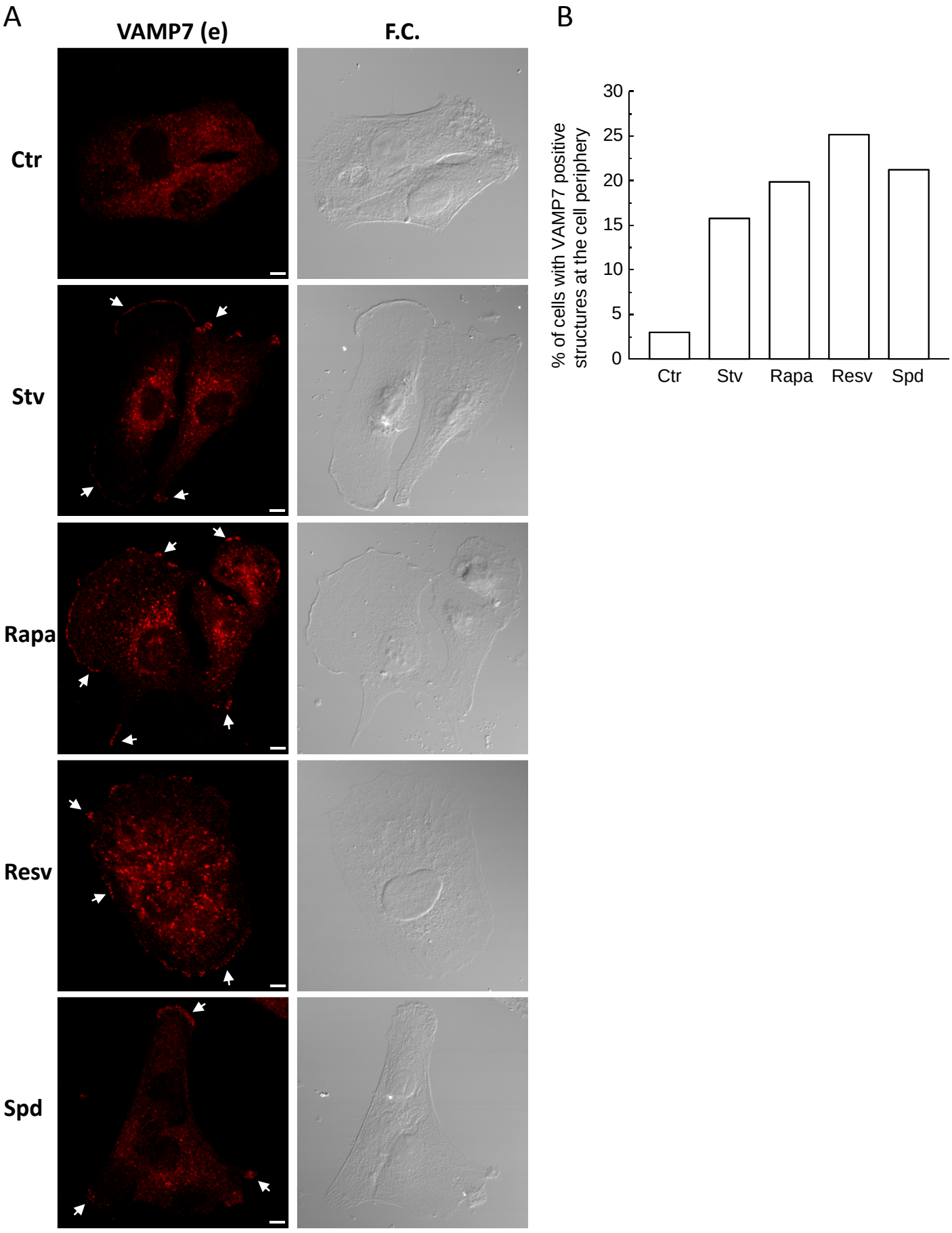

A
VAMP7 (e)
F.C.
Ctr
Stv
Rapa
Resv
Spd
B
% of cells with VAMP7 positive structures at the cell periphery
30
25
20
15
10
5
0
Ctr
Stv
Rapa
Resv
Spd

Figure S3

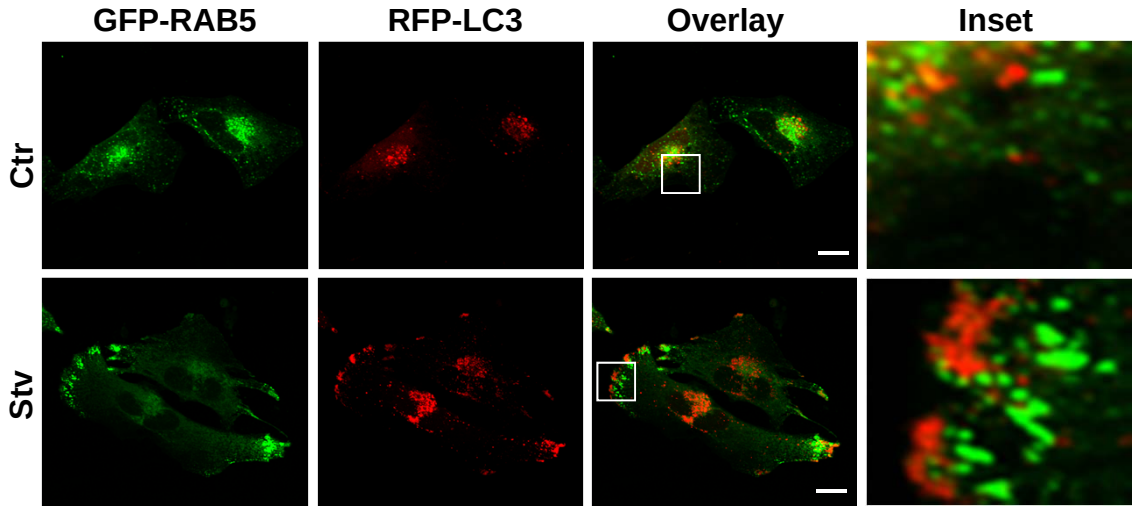


Figure S4

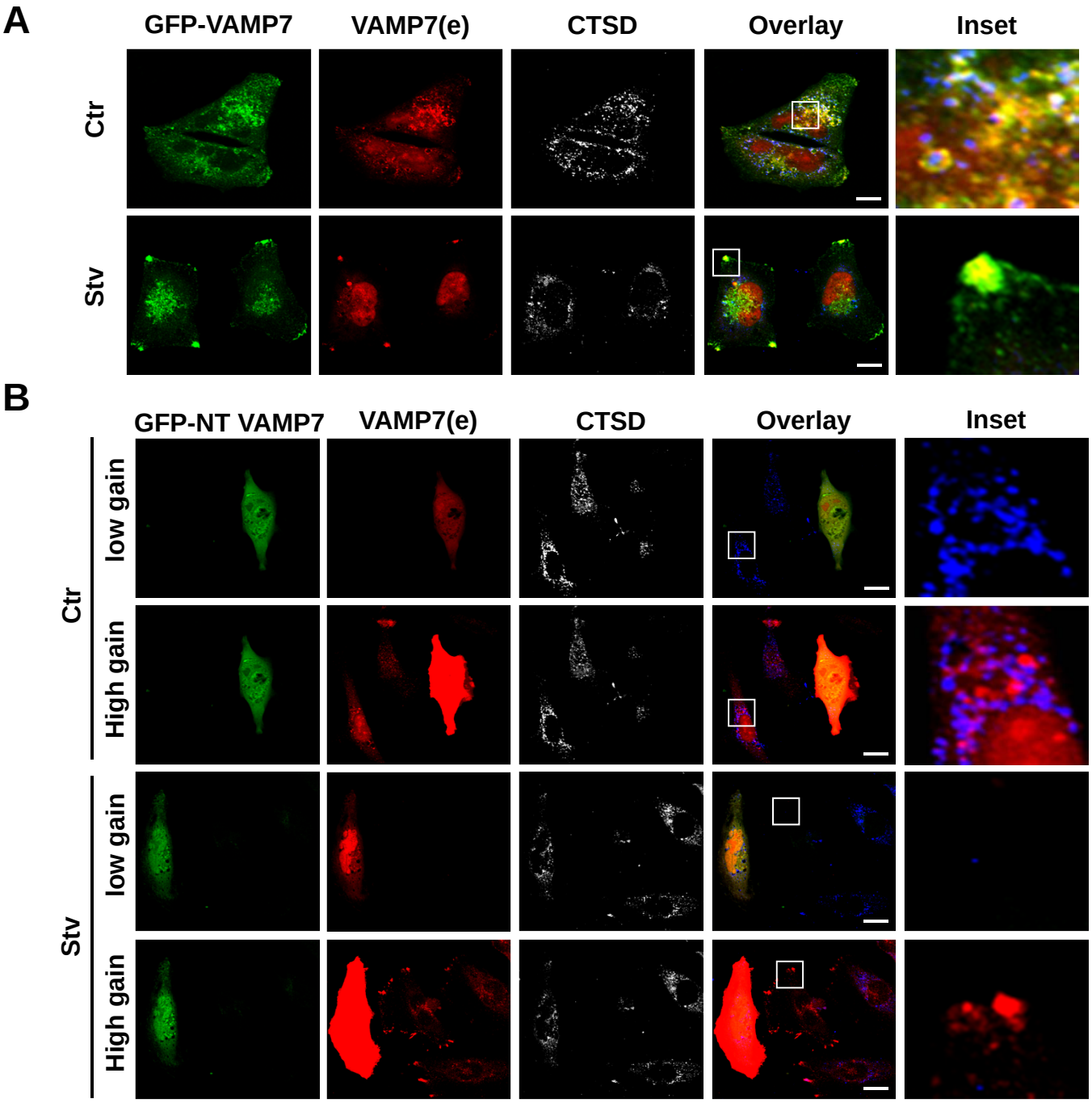
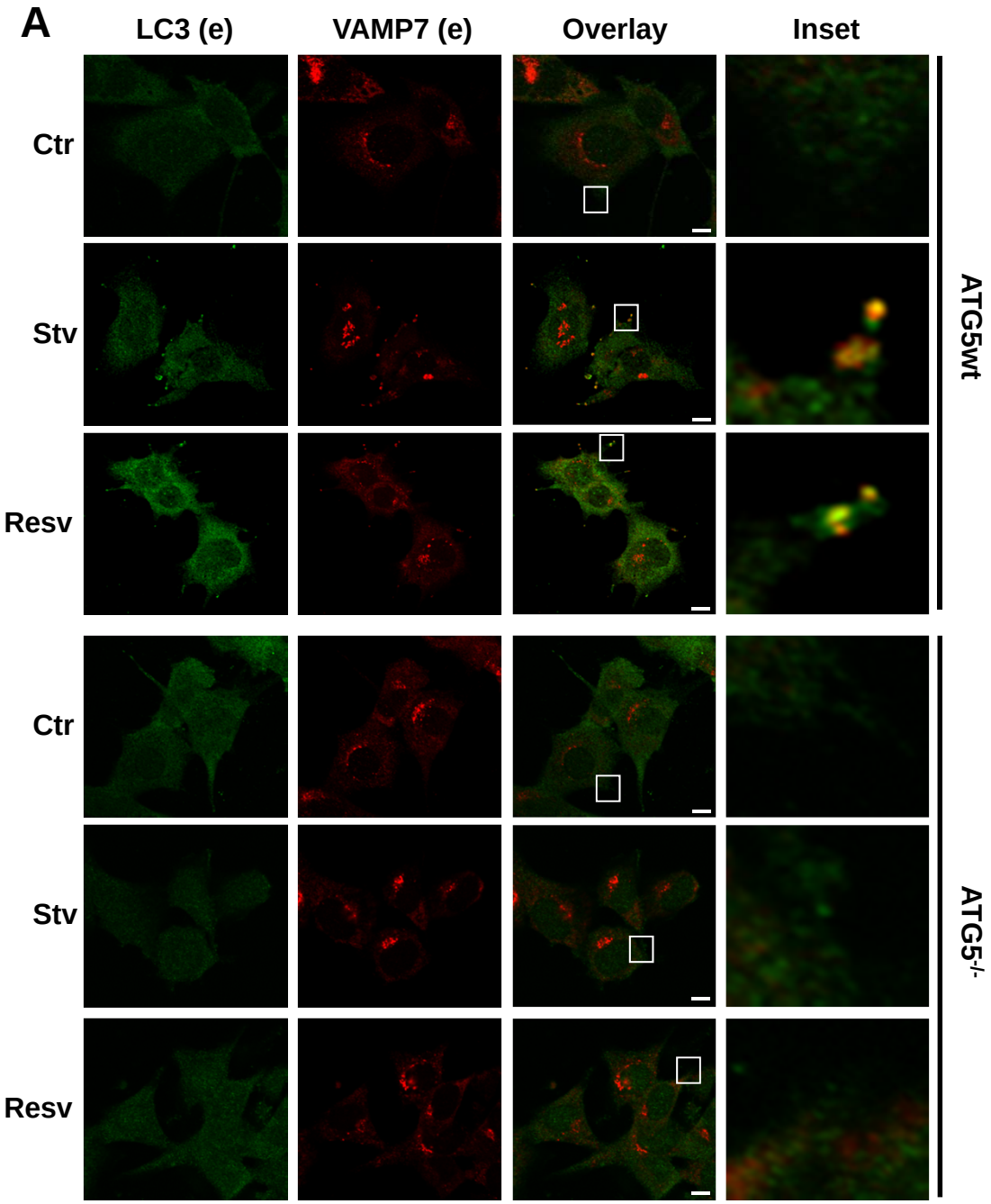

A
GFP-VAMP7
VAMP7(e)
CTSD
Overlay
Inset
Ctr
Stv
B
GFP-NT VAMP7
VAMP7(e)
CTSD
Overlay
Inset
Ctr
low gain
High gain
Stv
low gain
High gain
C
% of cells with VAMP7 peripheral structures
50
40
30
20
10
0
Ctr
Stv
Ct
Stv
VAMP7wt
NT-VAMP7

Figure S5


A
LC3 (e)
VAMP7 (e)
Overlay
Inset
Ctr
Stv
Resv
ATG5wt
Ctr
Stv
Resv
ATG5-/-

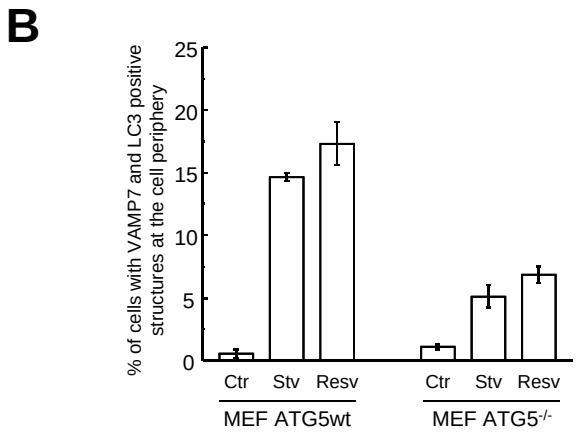

B
% of cells with VAMP7 and LC3 positive structures at the cell periphery
25
20
15
10
5
0
Ctr
Stv
Resv
Ctr
Stv
Resv
MEF ATG5wt
MEF ATG5-/-

Figure S1. *Autophagic inductors cause a redistribution of VAMP7-positive autophagosomes at the cell periphery.* A) MDA MB-231 (breast cancer cell) and HT-1080 (fibrosarcoma) cells were incubated 4h in starvation media in the absence (Stv) or in the presence of wortmannin (Wn) or 3h in complete media in the absence (Ctr) or in the presence of rapamycin (Rapa) or resveratrol (Resv). Cells were fixed and endogenous VAMP7 and LC3 were detected by indirect immunofluorescence. Mean of Pearson´s coefficient in MDA MB-231cells: Ctr:0.42, Stv:0.76, Resv:0.87, Wn:0,38 and in HT-1080 cells: Ctr:0.28, Stv:0.76, Resv:0.84, Wn:0,35. Images were obtained by confocal microscopy. Bars: 5 µm. The percentage of cells with VAMP7 and LC3-positive structures at the cell periphery was determined from images as the ones displayed in panels A. ** Significantly different from the control, P 0.005.

Figure S2. *Autophagic inductors cause a redistribution of VAMP7-positive structures at the cell periphery.* A) MIO-M1 cells (Muller stem cell line) were incubated in starvation media (Stv) or in complete media in the absence (Ctr) or presence of rapamycin (Rapa), resveratrol (Resv) or spermidine (Spd). Cells were fixed and VAMP7 was detected by indirect immunofluorescence. Images were obtained by confocal microscopy. Bars: 5 µm. The percentage of cells with VAMP7-positive structures at the cell periphery was determined from images as the ones displayed in panel A.

Figure S3. Autophagosomes redistributed at the cell periphery are not labeled by RAB5. Transiently cotransfected HeLa cells overexpressing GFP- RAB5 and RFP-LC3 where incubated in complete media or amino acid serum free media. Cells were mounted on coverslips and immediately analyzed by confocal microscopy. Bars: 5 µm.

Figure S4. Overexpression of NT-VAMP7 alters the endogenous VAMP7 localization at the the focal adhesions. A) HeLa cells overexpressing GFP-VAMP7 were incubated for 4 h in complete media (Ctr) or in starvation media (Stv) to activate autophagy. Cells were fixed and the overexpressed protein GFP-VAMP7 and the endogenous proteins (VAMP7 and CTSD) were detected by indirect immunofluorescence. B) HeLa cells overexpressing GFP NT-VAMP7 were incubated for 4 h in complete media or in starvation. Cells were subjected to indirect immunofluorescence to detect both endogenous proteins, VAMP7 and CTSD. Images were taken with high and low gain in each condition to visualize either endogenous or overexpressed VAMP7, respectively. Images were obtained by confocal microscopy. Bars: 5 µm. C) The number of VAMP7 vesicles close to the cell surface upon starvation-induced autophagy was quantified from images as those depicted in panels A and B. At least 50 cells were counted in each condition.

Figure S5. ATG5 is necessary for the redistribution of VAMP7 structures to focal adhesions upon autophagy induction. A) MEF *ATG*5wt and MEF *ATG*5 knockdown cells were incubated for 4 h in starvation media (Stv) or 3 h in complete media in the absence (Ctr) or presence of resveratrol (Resv). Cells were fixed and endogenous VAMP7 and LC3 were detected by indirect IF. Images were obtained by confocal microscopy. Mean of Pearson´s coefficient in MEF *ATG*5wt cells: Ctr:0.33, Stv:0.79, Resv:0.76 and in MEF *ATG*5$^{-/-}$ cells: Ctr:0.28, Stv=0.45, Resv=0.37. Bars: 5 µm. B) The percentage of cells with VAMP7 positive structures at the cell periphery were quantified from images as the ones displayed in panels A. At least 100 cells were counted in each condition. Images are representative of two independent experiments.

Video # 1. Fusion of the ATP-loaded autophagic with the plasma membrane. Cells were transiently transfected with RFP-LC3 (red) and autophagy was induced by starvation media for 6 h. Cells were incubated with 25µM of quinacrine for 20 minutes (green). A) Time in seconds corresponding to each frame is indicated. Cells were mounted on coverslips and immediately analyzed by TIRF microscopy. A total of 20 slides every 5 seconds were taken**.**