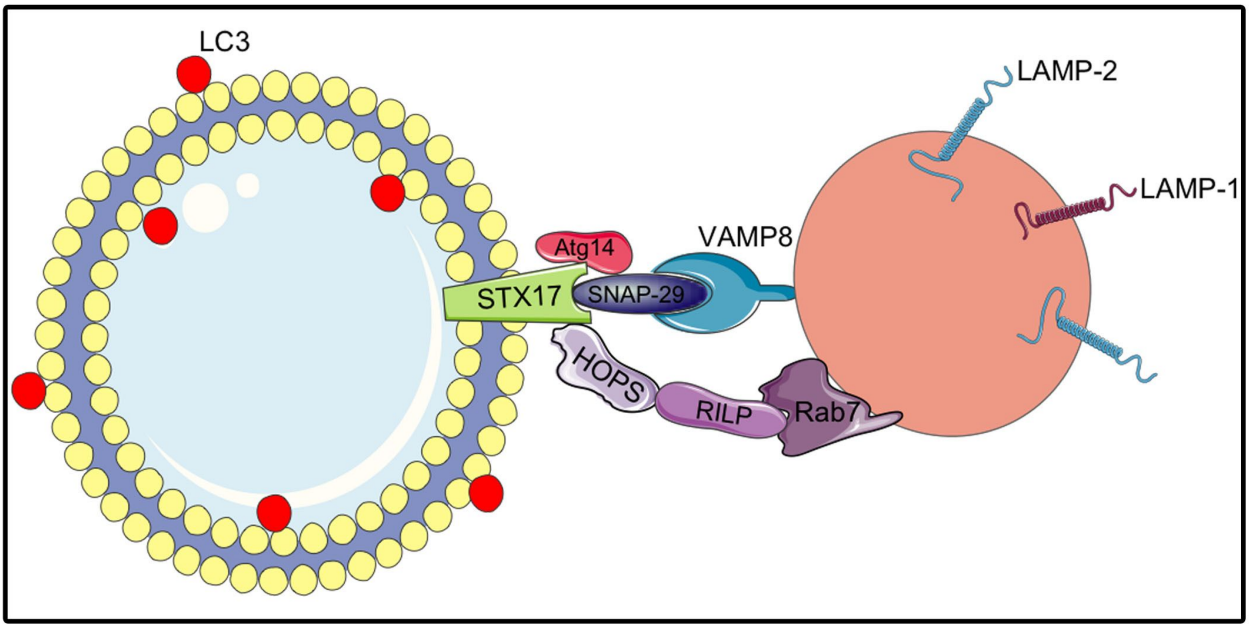
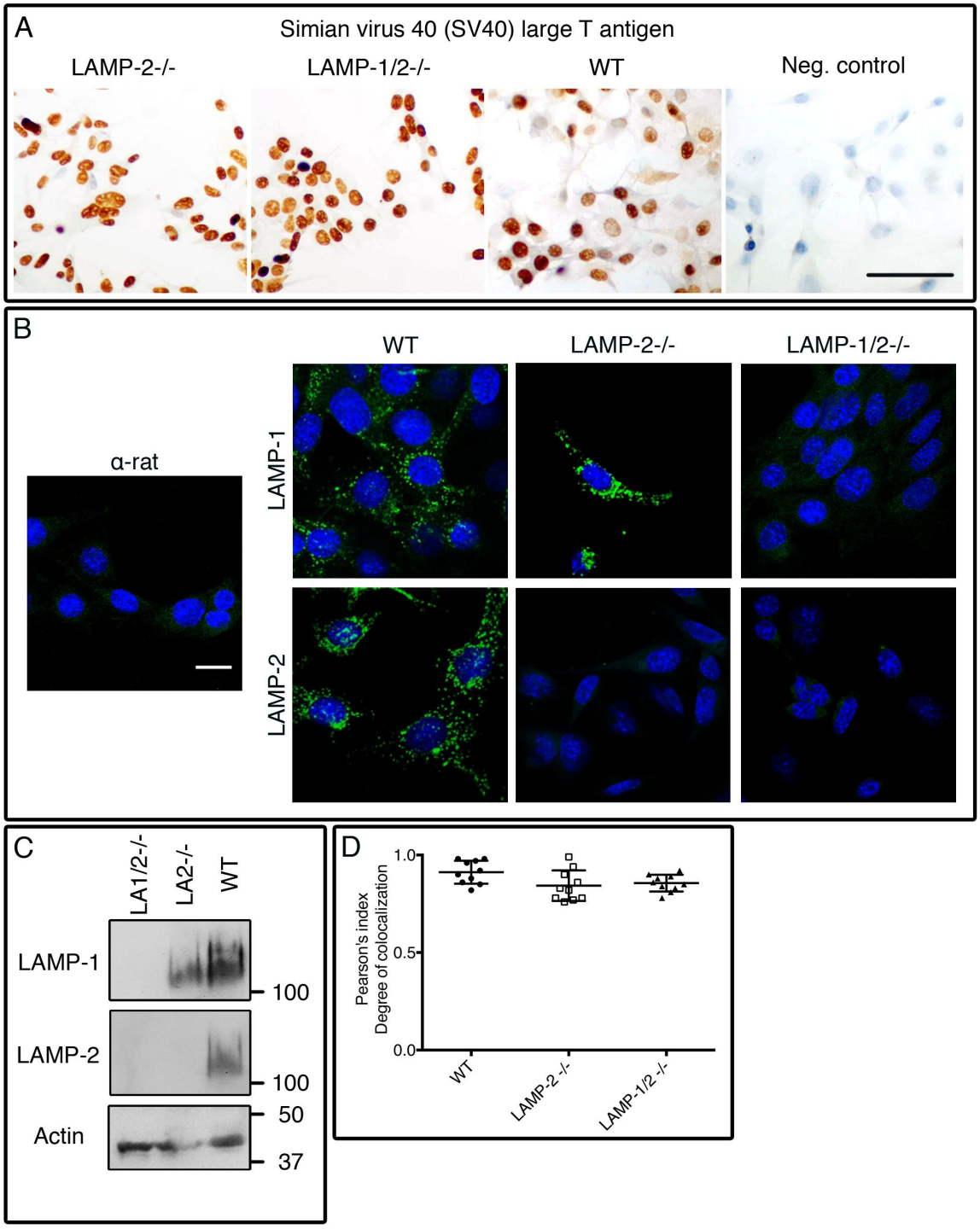
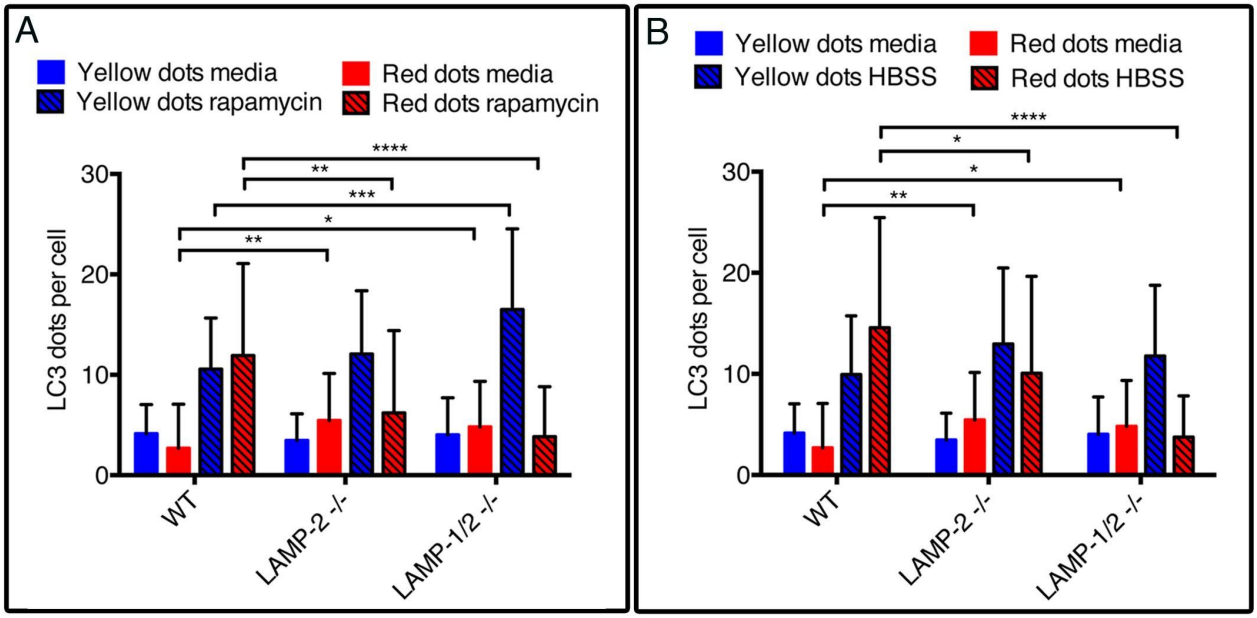


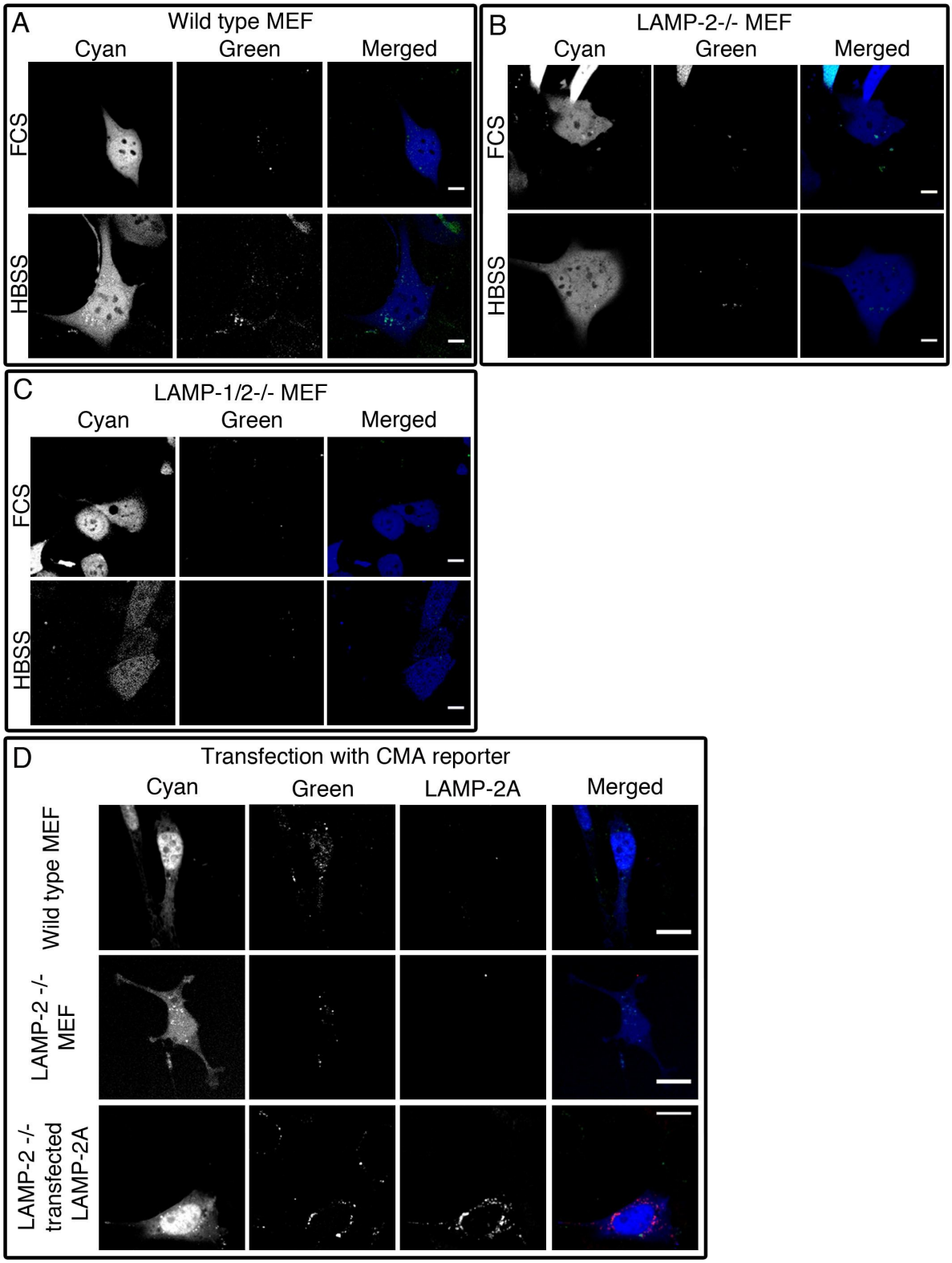
Sup. Figure 1: Model for autophagosome-lysosome fusion. Tethering of the autophagosome with the lysosomes is mediated by the interaction of the Rab7 effector RILP which respectively binds the HOPS complex. The autophagosomal SNARE syntaxin 17 binds HOPS and mediates fusion with the lysosome through its interaction with SNAP-29 and lysosomal VAMP8. Further stabilization of the syntaxin 17-SNAP-29 complex is achieved by Atg14.



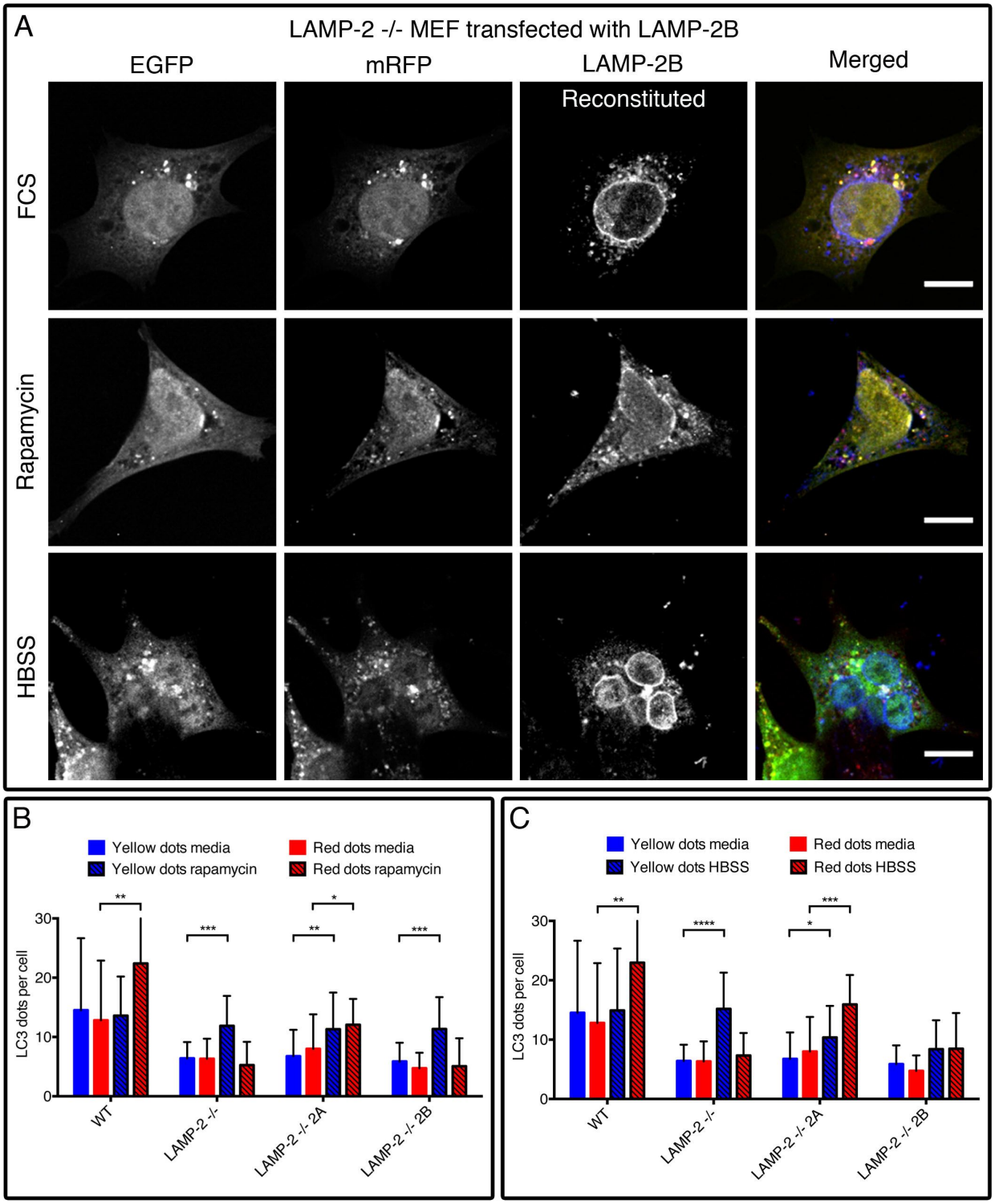
Sup. Figure 2: Characterization of cell lines. (A) Immortalization of the three cell lines by insertion of the SV40 large T antigen was confirmed by immunohistochemistry. Scale bar = 100μm. (B) LAMP-2 is expressed in wild type MEF while LAMP-1 is expressed in wild type and LAMP-2 deficient cells but not in doubly deficient fibroblast. Scale bar = 20μm. (C) Western blot analysis of total cell extracts confirm the absence of LAMP-2 expression in single and double knock out while LAMP-1 was not expressed in LAMP-1/2 -/- fibroblasts (D). A Pearson's correlation coefficient confirms the high degree o f colocalization of EGFP with mRFP and thus the efficiency of the construct. Datas are expressed as mean±SD of at least 10 cells.



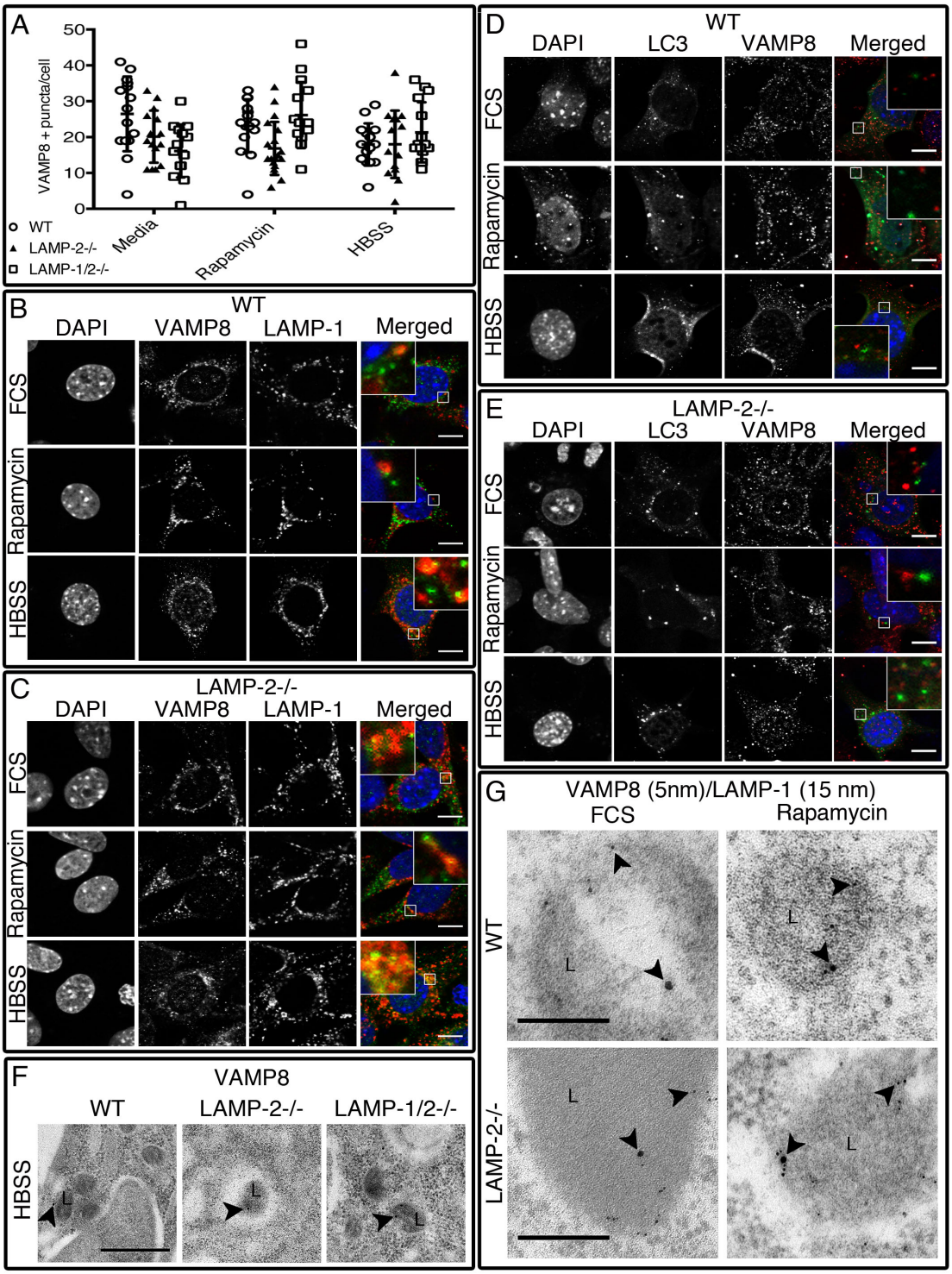
Sup. Figure 3: The autophagic flux is impaired in LAMP-2 single and LAMP-1/2 doubly deficient cells after autophagy induction. Autophagy induction in wild type MEF is reflected by an increased number of autophagosomes and autolysosomes while in LAMP-2 single deficient and LAMP-1/2 double deficient cells the number of autolysosomes remains unchanged after treatment with rapamycin (A) or HBSS (B). The number of LC3 dots was quantified from at least 25 cells in each condition and is representative of 3 independent experiments. Datas are expressed as mean±SD. *, P<0.05; **, P<0.01***; P<0.001; ****, P<0.0001 (Mann Whitney test).



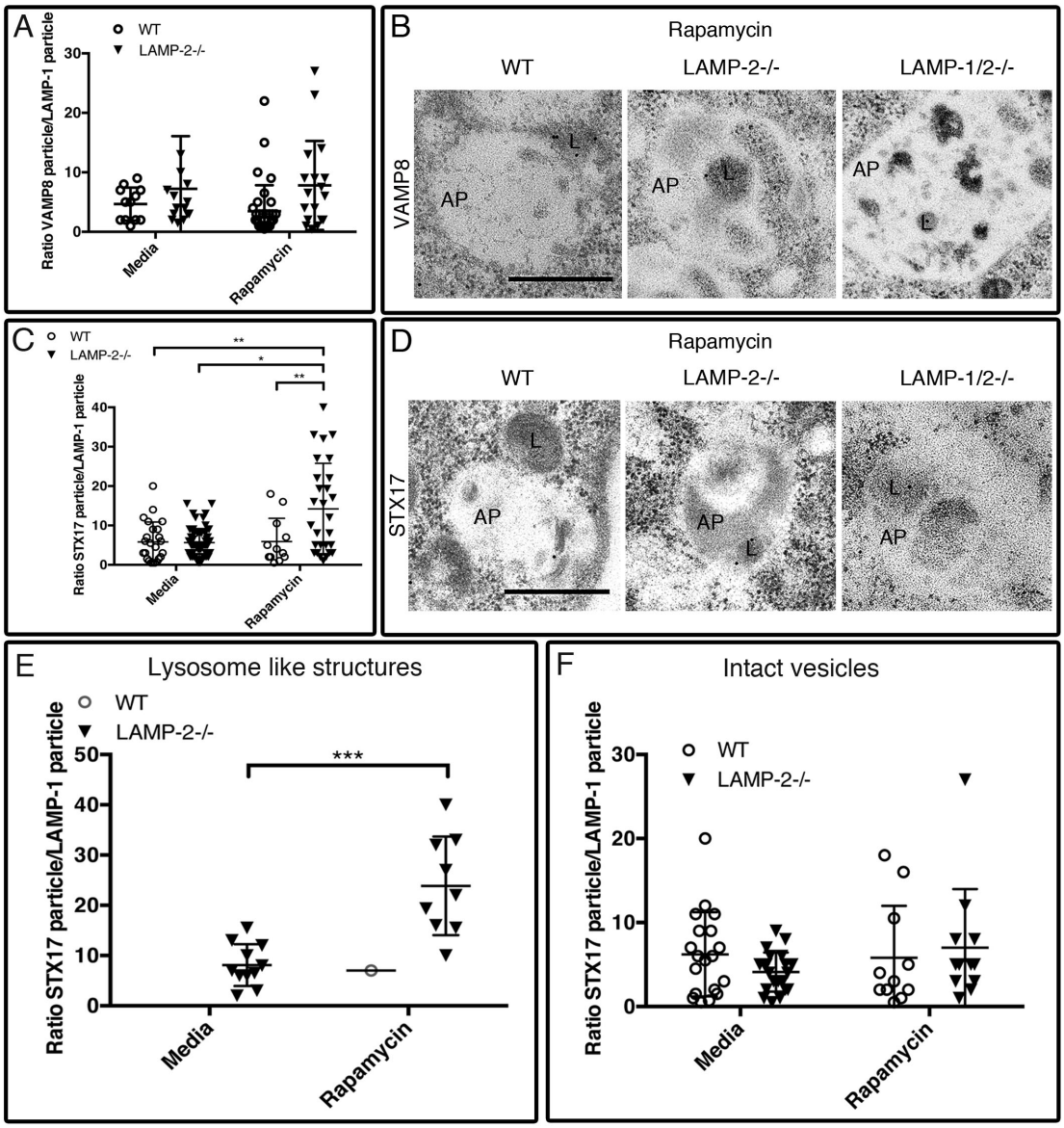
Sup. Figure 4: Complementation of LAMP-2 and LAMP-1/2 deficient cells with LAMP-2A restores CMA. Wild type (A), LAMP-2 deficient (B) and LAMP-1/2 deficient (C) cells were transfected with the photoswitchable reporter pKFERQ-PS-CFP2 able to monitor CMA (B). While CMA was efficiently induced in wild type MEF, LAMP-2 single and LAMP-1/2 doubly deficient cell were unable to conduct it. Scale bar = 5μm. Complementation of the deficient cell lines with LAMP-2A restores CMA (D).). Scale bar = 10μm.



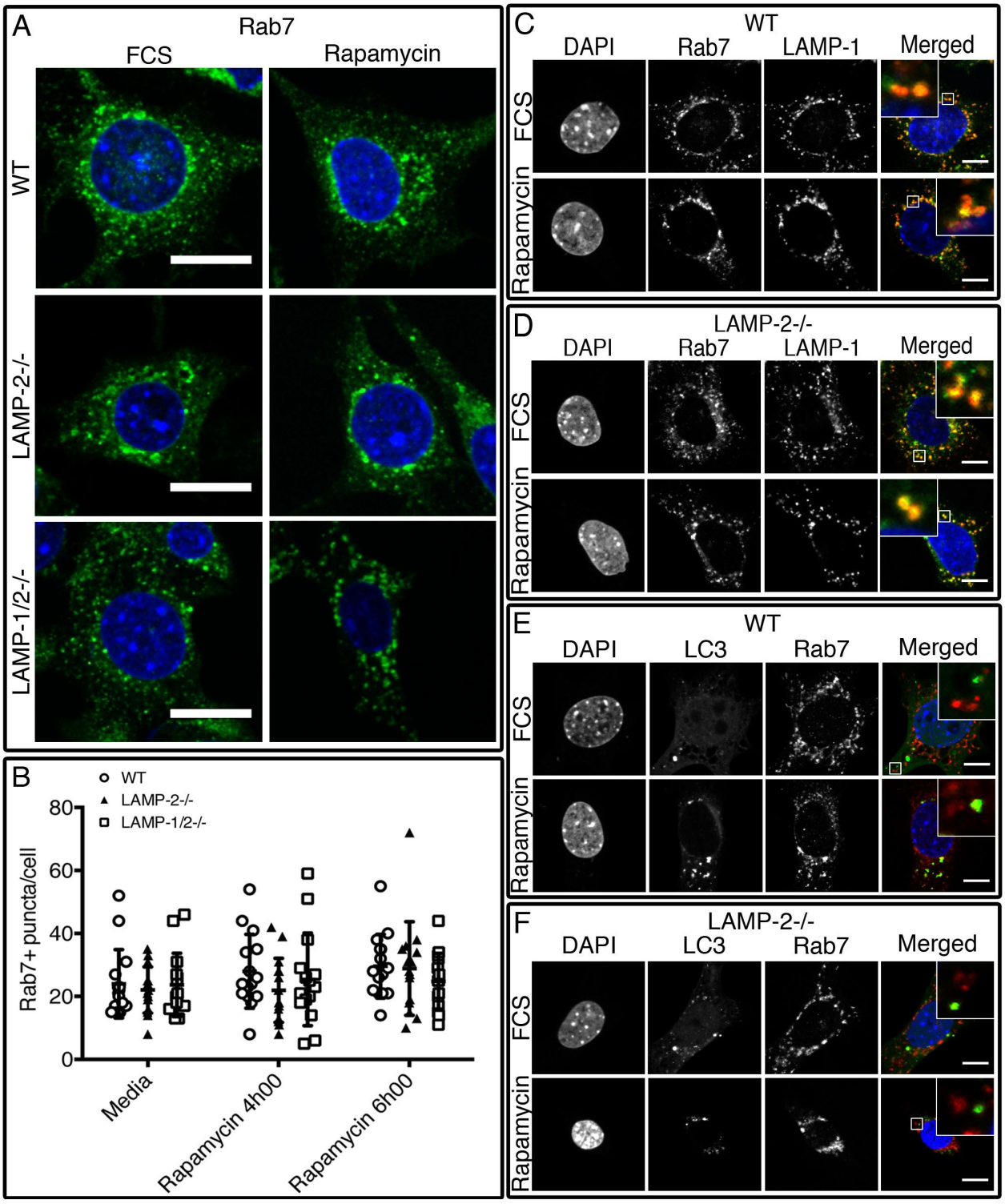
Sup. Figure 5: Autophagic flux is not restored by LAMP-2B. Autophagic flux remains altered in LAMP-2 deficient MEF reconstituted with LAMP-2B (A) both after treatment with rapamycin (B) or HBSS (C). Scale bar = 10μm. Datas are expressed as mean±SD of 3 independent experiments. *, P<0.05; **, P<0.01***; P<0.001; ****, P<0.0001 (Mann Whitney test).



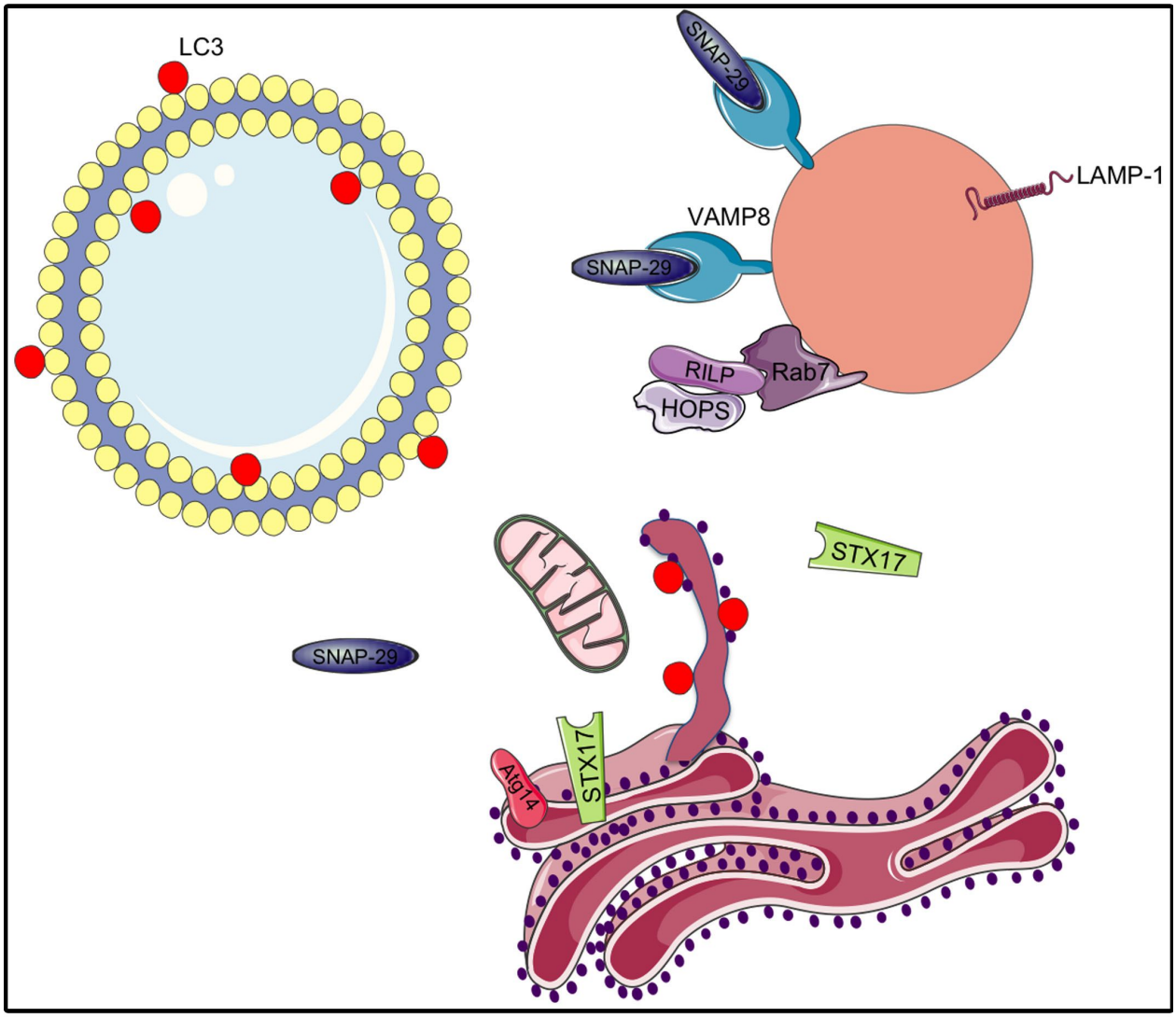
Sup. Figure 6: VAMP8 expression and localization is not altered in the absence of LAMP-2. The number of VAMP8 positive puncta is similar in the three cell lines before and after autophagy induction with rapamycin or HBSS for 4h00 (A). VAMP8 co-localized with LAMP-1 (B-C) but not with EGFP-LC3 (D-E) indicating its lysosomal localization both in LAMP-2 sufficient (B and D) or deficient cells (C and E). Scale bar = 10µm. Immunoelectron microscopy confirms the localization of VAMP8 in the lysosomes in the three cell lines after treatment with HBSS (F) (Scale bar = 500nm) as well as its co-localization with LAMP-1 particles (G) (Scale bar = 200nm). Datas are expressed as mean±SD from at least 15 cells in each conditions and is representative of 3 independent experiments.



Sup. Figure 7: Intact lysosome like structures are observed in the autophagosomes of LAMP-2 single and LAMP-1/2 doubly deficient MEF. A VAMP8/LAMP-1 ratio was established by counting positive particles per vesicles and was similar in both cell lines, independently of the cell treatment (A). Under rapamycin treatment, undigested VAMP8 (B) lysosome like structures were observed in the autophagosomes of LAMP-2 and LAMP-1/2 deficient fibroblast while fusion of the autophagosome with the lysosomes was observed in wild-type. The ratio STX17 particles/LAMP-1 particles was increased in LAMP-2 deficient cells under autophagy induction with rapamycin (C). The presence of STX17 positive lysosome like structures (D) in LAMP-2 single deficient and LAMP-1/2 double deficient cells was observed and associated with an increased ratio of STX17 particles/LAMP-1 particles in these vesicles (E). This ratio was similar in both cell lines when quantification was performed uniquely in normal vesicles (F). Scale bar = 500nm. $P<0.01$*** (Mann Whitney test).



Sup. Figure 8: The number of Rab7 positive vesicles is similar in the three cell lines despite an altered distribution in LAMP-1/2 deficient cell switching form perinuclear to diffuse staining (A-B). Scale bar = 20µm. The number of Rab7 positive vesicles was quantified from at least 15 cells in each condition and is representative of 3 independent experiments. In wild-type (C) and LAMP-2-/- cell (D) Rab7 co-localized with LAMP-1 but not with EGFP-LC3 (E-F). Scale bar = 10µm.



Sup. Figure 9: Blockage of autophagosome-lysosome fusion in LAMP-2 deficient cells. In these cells, STX17 does not translocated to the autophagosome and therefore cannot be bound by the HOPS complex. The absence of binding between HOPS and STX17 prevents the tethering and the fusion of the autophagosome with the lysosome.

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
CMA	Wild type	Control media	5.125±3.938
CMA	Wild type	HBSS	9.222±3.606
CMA	LAMP-2 -/-	Control media	5.532±2.504
CMA	LAMP-2 -/-	HBSS	4.294±3.016
CMA	LAMP-1/2 -/-	Control media	3.4±2.063
CMA	LAMP-1/2 -/-	HBSS	3.267±1.751

Sup. Table 1: Quantitative evaluation of CMA by transfection of cell with the CMA reporter pKFERQ-PS-CFP2.

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
CMA	Wild type	HBSS	8.429±3.625
CMA	LAMP-2 -/-	HBSS	3.050±1.504
CMA	LAMP-2 -/- transfected with LAMP-2A	HBSS	9.909±4.323

Sup. Table 2: Confirmation of the ability of LAMP-2A to restore CMA. Cells were co-transfected with the CMA reporter pKFERQ-PS-CFP2 and LAMP-2 followed by quantitation of CMA.

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
Macroautophagy Autophagosome	Wild type	Control media	14.524±12.135
Macroautophagy Autolysosome	Wild type	Control media	12.810±10.083
Macroautophagy Autophagosome	Wild type	Rapamycin	13.6±6.597
Macroautophagy Autolysosome	Wild type	Rapamycin	22.4±10.515
Macroautophagy Autophagosome	LAMP-2 -/-	Control media	6.4±2.746
Macroautophagy Autolysosome	LAMP-2 -/-	Control media	6.333±3.374
Macroautophagy Autophagosome	LAMP-2 -/-	Rapamycin	11.875±5.045
Macroautophagy Autolysosome	LAMP-2 -/-	Rapamycin	5.25±3.941
Macroautophagy Autophagosome	LAMP-2 -/- transfected with LAMP-2A	Control media	6.765±4.452
Macroautophagy Autolysosome	LAMP-2 -/- transfected with LAMP-2A	Control media	8±5.831
Macroautophagy Autophagosome	LAMP-2 -/- transfected with LAMP-2A	Rapamycin	11.316±6.183
Macroautophagy Autolysosome	LAMP-2 -/- transfected with LAMP-2A	Rapamycin	12.053±4.365
Macroautophagy Autophagosome	LAMP-2 -/- transfected with LAMP-2B	Control media	5.867±3.159
Macroautophagy Autolysosome	LAMP-2 -/- transfected with LAMP-2B	Control media	4.733±2.604
Macroautophagy Autophagosome	LAMP-2 -/- transfected with LAMP-2B	Rapamycin	11.353±5.373
Macroautophagy Autolysosome	LAMP-2 -/- transfected with LAMP-2B	Rapamycin	5.059±4.723

Sup. Table 3: Quantitative evaluation of macroautophagy using the tfLC3 construct in wild-type or LAMP-2 deficient cells reconstituted or not with LAMP-2A or LAMP-2B, following treatment with rapamycin.

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
Macroautophagy Autophagosome	Wild type	Control media	14.524±12.135
Macroautophagy Autolysosome	Wild type	Control media	12.810±10.083
Macroautophagy Autophagosome	Wild type	HBSS	14.923±10.423
Macroautophagy Autolysosome	Wild type	HBSS	22.962±12.571
Macroautophagy Autophagosome	LAMP-2 -/-	Control media	6.4±2.746
Macroautophagy Autolysosome	LAMP-2 -/-	Control media	6.333±3.374
Macroautophagy Autophagosome	LAMP-2 -/-	HBSS	15.188±6.102
Macroautophagy Autolysosome	LAMP-2 -/-	HBSS	7.313±3.807
Macroautophagy Autophagosome	LAMP-2 -/- transfected with LAMP-2A	Control media	6.765±4.452
Macroautophagy Autolysosome	LAMP-2 -/- transfected with LAMP-2A	Control media	8±5.831
Macroautophagy Autophagosome	LAMP-2 -/- transfected with LAMP-2A	HBSS	10.375±5.303
Macroautophagy Autolysosome	LAMP-2 -/- transfected with LAMP-2A	HBSS	15.938±4.959
Macroautophagy Autophagosome	LAMP-2 -/- transfected with LAMP-2B	Control media	5.867±3.159
Macroautophagy Autolysosome	LAMP-2 -/- transfected with LAMP-2B	Control media	4.733±2.604
Macroautophagy Autophagosome	LAMP-2 -/- transfected with LAMP-2B	HBSS	8.4±4.867
Macroautophagy Autolysosome	LAMP-2 -/- transfected with LAMP-2B	HBSS	8.467±6.010

Sup. Table 4: Quantitative evaluation of macroautophagy using the tfLC3 construct in wild-type or LAMP-2 deficient cells reconstituted or not with LAMP-2A or LAMP-2B, following treatment with HBSS.

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
Lysosomes number	Wild type	Control media	26.571±15.142
Lysosomes number	LAMP-2 -/-	Control media	24.917±15.294
Lysosomes number	LAMP-1/2 -/-	Control media	25.2±10.611
Lysosomes number	Wild type	Rapamycin	28.743±15.038
Lysosomes number	LAMP-2 -/-	Rapamycin	24.250±13.487
Lysosomes number	LAMP-1/2 -/-	Rapamycin	22.7±9.734
Lysosomes number	Wild type	HBSS	26.915±12.228
Lysosomes number	LAMP-2 -/-	HBSS	29.966±14.918
Lysosomes number	LAMP-1/2 -/-	HBSS	24.850±8.635

Sup. Table 5: Quantitative evaluation of the number of lysosomes per cell.

Parameter quantified	Cell	Treatment	Value expressed as me SD
VAMP8+ vesicles	Wild type	Control media	26.467±10.412
VAMP8+ vesicles	LAMP-2 -/-	Control media	20.133±7.279
VAMP8+ vesicles	LAMP-1/2 -/-	Control media	16.133±7.376
VAMP8+ vesicles	Wild type	Rapamycin	19.4±6.555
VAMP8+ vesicles	LAMP-2 -/-	Rapamycin	15.533±5.768
VAMP8+ vesicles	LAMP-1/2 -/-	Rapamycin	22.667±7.287
VAMP8+ vesicles	Wild type	HBSS	17.467±10.528
VAMP8+ vesicles	LAMP-2 -/-	HBSS	14.533±6.696
VAMP8+ vesicles	LAMP-1/2 -/-	HBSS	15.333±8.682

Sup. Table 6: Quantitative evaluation of the number of VAMP8 positive vesicles per cell.

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
STX17+ vesicles	Wild type	Control media	12.250±7.452
STX17+ vesicles	LAMP-2 -/-	Control media	3.091±2.212
STX17+ vesicles	LAMP-1/2 -/-	Control media	4.714±4.514
STX17+ vesicles	Wild type	Rapamycin	24.889±12.471
STX17+ vesicles	LAMP-2 -/-	Rapamycin	5.857±6.075
STX17+ vesicles	LAMP-1/2 -/-	Rapamycin	7.067±3.615
STX17+ vesicles	Wild type	Rapamycin	28.625±10.288
STX17+ vesicles	LAMP-2 -/-	Rapamycin	8.067±6.692
STX17+ vesicles	LAMP-1/2 -/-	Rapamycin	6.692±5.282

Sup. Table 7: Quantitative evaluation of the number of STX17 positive vesicles per cell.

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
STX17+ vesicles	Wild type	Control media	10.267±6.713
STX17+ vesicles	Wild type	Rapamycin	19.200±5.846
STX17+ vesicles	LAMP-2 -/-	Control media	7±6.245
STX17+ vesicles	LAMP-2 -/-	Rapamycin	6.692±4.111
STX17+ vesicles	LAMP-2 -/- transfected with LAMP-2A	Control media	9.765±3.784
STX17+ vesicles	LAMP-2 -/- transfected with LAMP-2A	Rapamycin	13.235±8.467

Sup. Table 8: Quantitative evaluation of the number of STX17 positive vesicles in wild-type, LAMP-2 deficient and LAMP-2 deficient cells reconstituted with LAMP-2A

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
Colocalization STX17 with LC3 Manders'coefficient	Wild type	Control media	0,27±0,066
Colocalization STX17 with LC3 Manders'coefficient	LAMP-2 -/-	Control media	0,081±0,068
Colocalization STX17 with LC3 Manders'coefficient	Wild type	Rapamycin	0,198±0.079
Colocalization STX17 with LC3 Manders'coefficient	LAMP-2 -/-	Rapamycin	0,101±0,116

Sup. Table 9: Quantitation of co-localization using the mander's coefficient and describing the fraction of STX17 overlapping with LC3.

Parameter quantified	Cell	Treatment	Value expressed as mean± SD
Colocalization STX17 with LAMP-1 Manders'coefficient	Wild type	Control media	0.066±0.051
Colocalization STX17 with LAMP-1 Manders'coefficient	LAMP-2 -/-	Control media	0.048±0.056
Colocalization STX17 with LAMP-1 Manders'coefficient	Wild type	Rapamycin	0.083±0.042
Colocalization STX17 with LAMP-1 Manders'coefficient	LAMP-2 -/-	Rapamycin	0.094±0.095

Sup. Table 10: Quantitation of co-localization using the mander's coefficient and describing the fraction of STX17 overlapping with LAMP-1.

Parameter quantified	Cell	Treatment	Value expressed as mean±SD
SNAP-29 + vesicles	Wild type	Control media	15.8±7.37
SNAP-29 + vesicles	LAMP-2 -/-	Control media	29.333±10.601
SNAP-29 + vesicles	LAMP-1/2 -/-	Control media	32.067±14.320
SNAP-29 + vesicles	Wild type	Rapamycin	28.200±12.043
SNAP-29 + vesicles	LAMP-2 -/-	Rapamycin	30.200±9.190
SNAP-29 + vesicles	LAMP-1/2 -/-	Rapamycin	33.600±15.519
SNAP-29 + vesicles	Wild type	Rapamycin	24.067±13.052
SNAP-29 + vesicles	LAMP-2 -/-	Rapamycin	31.667±10.554
SNAP-29 + vesicles	LAMP-1/2 -/-	Rapamycin	28.933±10.166

Sup. Table 11: Quantitative evaluation of the number of SNAP-29 positive vesicles per cell.

Staining	Permeabilization	Primary antibody	Secondary antibody	Primary antibody	Secondary antibody	Validation WB and immunostaining for all
LAMP-1	Saponin 0.05% 10 min	1DB4 1h00	Alexa Fluor 488 Goat anti-rat IgG	N/A	N/A	**Huynh, K. K.** et al. 2007
LAMP-2	Saponin 0.05% 10 min	ABL-93 1h00	Alexa Fluor 488 Goat anti-rat IgG	N/A	N/A	**Eskelinen, E. L.** et al. 2002 **Schneede, A.** et al. 2011
VAMP8	Methanol -20°C 6 min	Rabbit anti-VAMP8 ON	Alexa Fluor 488 Goat anti-rabbit IgG	N/A	N/A	Protein Atlas
Synthaxin17	Methanol -20°C 6 min	Rabbit anti-STX17 ON	Alexa Fluor 488 Goat anti-rabbit IgG	N/A	N/A	Protein Atlas **Diao, J.** et al. 2015 **Jiang, P.** et al. 2014 **Muppirala, M.** et al. 2012
SNAP-29	Methanol -20°C 6 min	Rabbit anti-SNAP29 ON	Alexa Fluor 488 Goat anti-rabbit IgG	N/A	N/A	Protein Atlas
Rab7	Methanol -20°C 6 min	Rabbit anti-Rab7 ON	Alexa Fluor 488 Goat anti-rabbit IgG	N/A	N/A	**Jean, S.** et al. 2015
VPS33A	Methanol -20°C 6 min	Rabbit anti-VPS33A ON	Alexa Fluor 488 Goat anti-rabbit IgG	N/A	N/A	**Whyte, J. R. C. and Munro, S.**2002
VAMP8 LAMP-1	Methanol -20°C 6 min	1DB4 1h00	Alexa Fluor 546 Goat anti-rat IgG	Rabbit anti-VAMP8 ON	Alexa Fluor 488 Goat anti-rabbit IgG	
Synthaxin17 LAMP-1	Methanol -20°C 6 min	1DB4 1h00	Alexa Fluor 546 Goat anti-rat IgG	Rabbit anti-STX17 ON	Alexa Fluor 488 Goat anti-rabbit IgG	
SNAP-29 LAMP-1	Methanol -20°C 6 min	1DB4 1h00	Alexa Fluor 546 Goat anti-rat IgG	Rabbit anti-SNAP29 ON	Alexa Fluor 488 Goat anti-rabbit IgG	
Rab7 LAMP-1	Methanol -20°C 6 min	1DB4 1h00	Alexa Fluor 546 Goat anti-rat IgG	Rabbit anti-Rab7 ON	Alexa Fluor 488 Goat anti-rabbit IgG	
VPS33A LAMP-1	Methanol -20°C 6 min	1DB4 1h00	Alexa Fluor 546 Goat anti-rat IgG	Rabbit anti-VPS33A ON	Alexa Fluor 488 Goat anti-rabbit IgG	
Synthaxin17 LC3	Methanol -20°C 6 min	Goat anti-LC3 ON	Alexa Fluor 488 Donkey anti-goat IgG	Rabbit anti-STX17 ON	Alexa Fluor 594 Donkey anti-rabbit IgG	**Gohla, A.** et al. 2007 **Tannous, P.** et al. 2008 **Tannous, P.** et al. 2008
SNAP-29 LC3	Methanol -20°C 6 min	Goat anti-LC3 ON	Alexa Fluor 488 Donkey anti-goat IgG	Rabbit anti-SNAP29 ON	Alexa Fluor 594 Donkey anti-rabbit IgG	**Gohla, A.** et al. 2007 **Tannous, P.** et al. 2008 **Tannous, P.** et al. 2008
VPS33A LC3	Methanol -20°C 6 min	Goat anti-LC3 ON	Alexa Fluor 488 Donkey anti-goat IgG	Rabbit anti-VPS33A ON	Alexa Fluor 594 Donkey anti-rabbit IgG	**Gohla, A.** et al. 2007 **Tannous, P.** et al. 2008 **Tannous, P.** et al. 2008

Sup. Table 12: Method of permeabilization and antibody used to stain untransfected cells in this study.

Transfection	Permeabilization	Primary antibody	Secondary antibody	Validation WB and immunostaining for all
tfLC3	No permeabilization	N/A	N/A	
tfLC3 LAMP-2A	Digitonin (50ug/ml) 5 min	Human LAMP-2 (clone H4B4) 1h00	Alexa Fluor 405 Goat anti-mouse	**Peschel, A.** et al. 2014
pKFERQ-PS-CFP2 LAMP-2A	Digitonin (50ug/ml) 5 min	Human LAMP-2 (clone H4B4) 1h00	Alexa Fluor 546 Goat anti-mouse	**Peschel, A.** et al. 2014
pEGFP-LC3 VAMP8 staining	Methanol -20°C 6 min	Rabbit anti-VAMP8 ON	Alexa Fluor 647 Goat anti-rabbit IgG	Protein Atlas
pEGFP-LC3 Rab7 staining	Methanol -20°C 6 min	Rabbit anti-Rab7 ON	Alexa Fluor 647 Goat anti-rabbit IgG	**Jean, S.** et al. 2015
LAMP-2A	Methanol -20°C 6 min	Rabbit anti-STX17 ON Human LAMP-2 (clone H4B4) 1h00	Alexa Fluor 488 Goat anti-rabbit IgG Alexa Fluor 405 Goat anti-mouse	Protein Atlas **Diao, J.** et al. 2015 **Jiang, P.** et al. 2014 **Muppirala, M.** et al. 2012
FLAG-Stx17	Combined fixation/permeabilization: Methanol -20°C 10 min Acetone -20°C 1 min	Rabbit anti-FLAG 1h00	Alexa Fluor 488 Goat anti-rabbit IgG	**Carmona-Mora, P.** et al. 2015 **Goodchild, R. E. et al. 2015**

Sup. Table 13: Method of permeabilization and antibody used to stain transfected cells in this study.