Receptor Expressed	Bmax (fmol/μg)	SEM		Kd (nM)	SEM
F-MOR	1.071	0.16		0.32	0.22
F-MOR-0cK	1.065	0.07		0.28	0.09
F-MORΔ17	2.076	0.16		0.31	0.11
F-MORΔ17-0cK	2.081	0.14		0.35	0.11

Supplemental Table 1 – Receptor expression levels of HEK293 stable cell lines

Receptor levels were measured by saturation binding to [3H]-diprenorpnine, and curves fitted using nonlinear regression (GraphPad Prism).

Supplemental Figures

Supplemental Figure 1. Colocalization of receptors with early and then late endosomes.
HEK293 cells expressing F-MORΔ17 (A and C) or F-MORΔ17-0cK (B and D) were treated with 10μM DADLE for 30 (A and B) or 90 minutes (C and D) before being fixed and stained. A and B) Cells were stained with anti-FLAG (magenta, left panel) to label receptor and anti-EEA1 (green, middle panel). C and D) Cells were stained with anti-opioid receptor (magenta, left panels) and anti-LAMP1 (green, middle panels).

Supplemental Figure 2. Image stack of enlarged Rab5-Q79L endosomes.
HEK293 cells were transiently transfected with CFP-Rab5Q79L and either F-MORΔ17-GFP (A) or F-MORΔ17-0cK-GFP (B), and replated onto to coverslips before treatment for 90 minutes with 10μM DADLE. Cells were then imaged by spinning disc confocal microscopy as described in the methods section. Shown are representative image stacks of either GFP (top) or FLAG (middle) fluorescence to localize the N or C termini respectively. Cross sections of the endosome were taken every 0.8μm, scale bars represent 10μm.

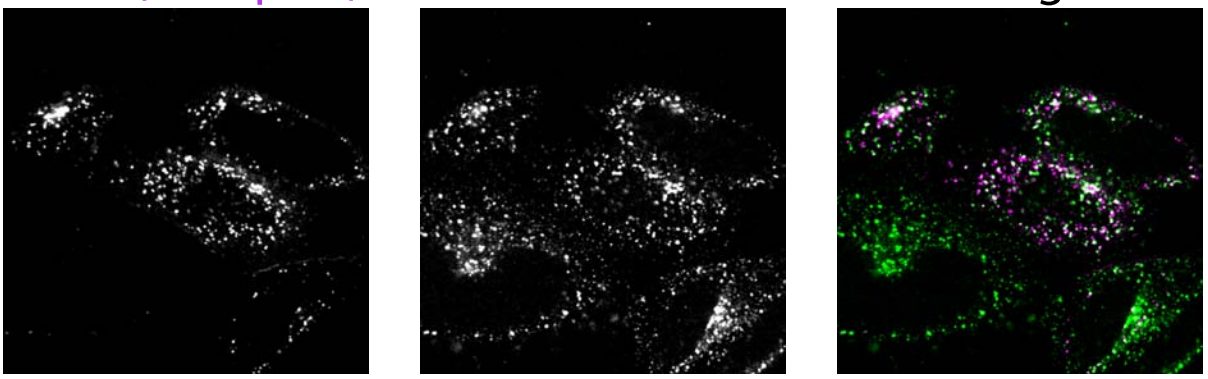
Supplemental Figure 3. Specific lysine residues within the first intracellular loop mediate transfer to ILVs. A and B) Cartoon depicting the F-MORΔ17-1st loop 2K/R-GFP (A) or F-MORΔ17-0cK-1st loop K-GFP (B) to show position of the lysine (**K**) and arginine (**R**) residues and the GFP. C and D) HEK293 cells were transiently transfected with CFP-Rab5Q79L and either F-MORΔ17-1st loop 2K/R-GFP (C) or F-MORΔ17-0cK-1st loop K-GFP (D), and replated onto to coverslips before treatment for 90 minutes with 10μM DADLE. Cells were then imaged by spinning disc confocal microscopy as described in the methods section. Shown are representative still images of the representative acquired image series, scale bars are 10μm. C and D) Line scan analysis to quantify involution was performed as described (see Methods and Fig.5 legend), and data of line scan analysis compiled. E) shows mean and SEM, F) shows the distribution of internal fluorescence values *** $p<0.001$, Student's t-test, n=100 endosomes for each receptor, ≥ 12 cells).

Supplemental 1

A

FLAG (Receptor) EEA1 Merge

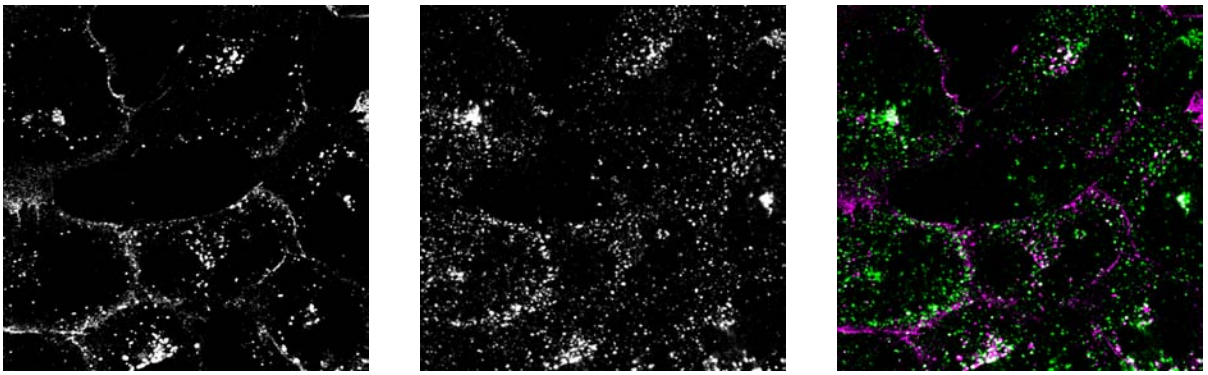
F-MORΔ17



B

FLAG (Receptor) EEA1 Merge

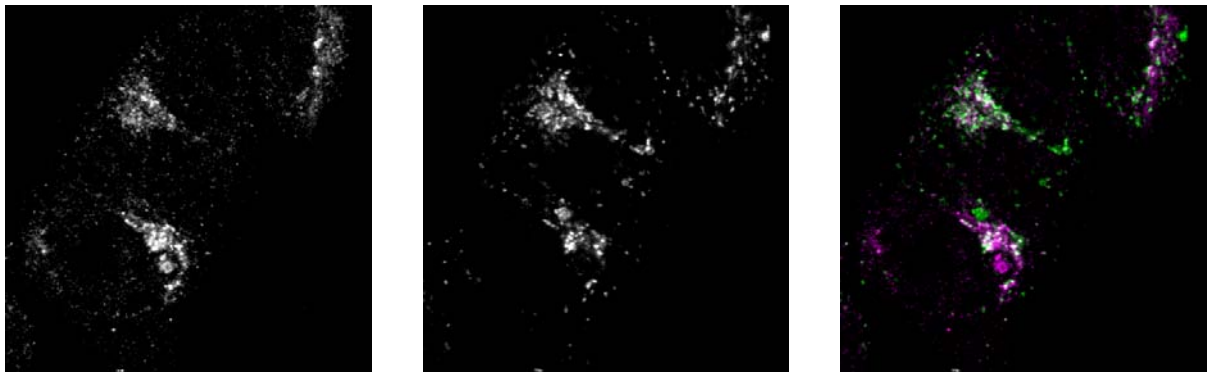
F-MORΔ17
-0cK



C

Opioid (Receptor) LAMP Merge

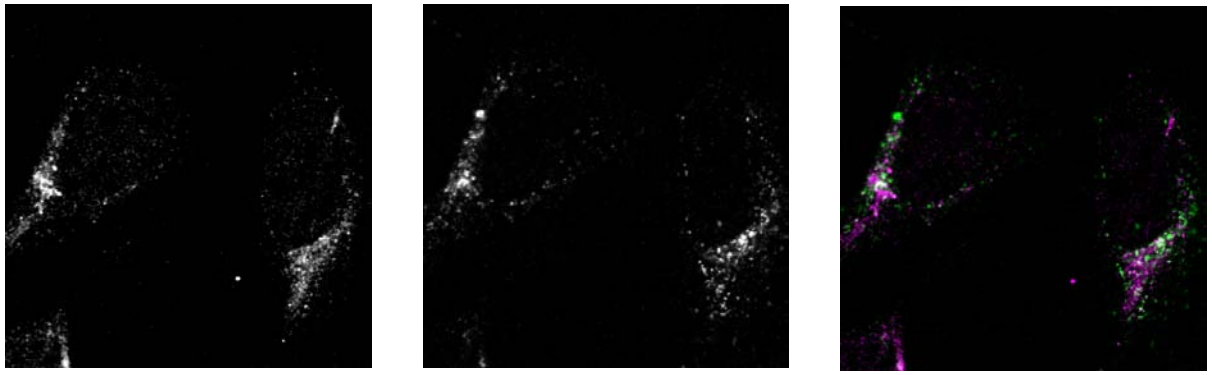
F-MORΔ17



D

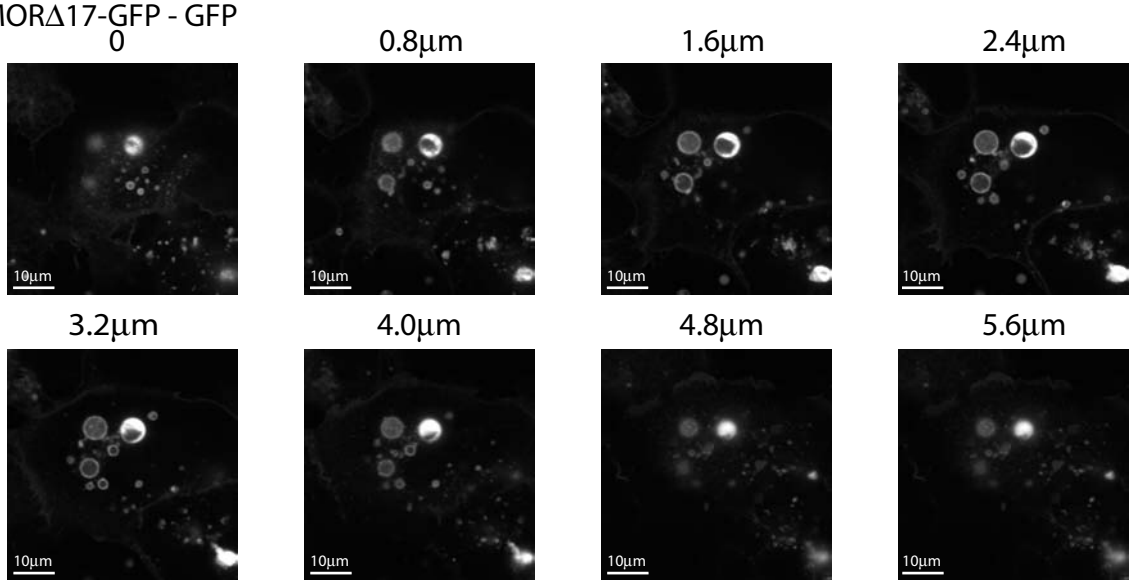
Opioid (Receptor) LAMP Merge

F-MORΔ17
-0cK

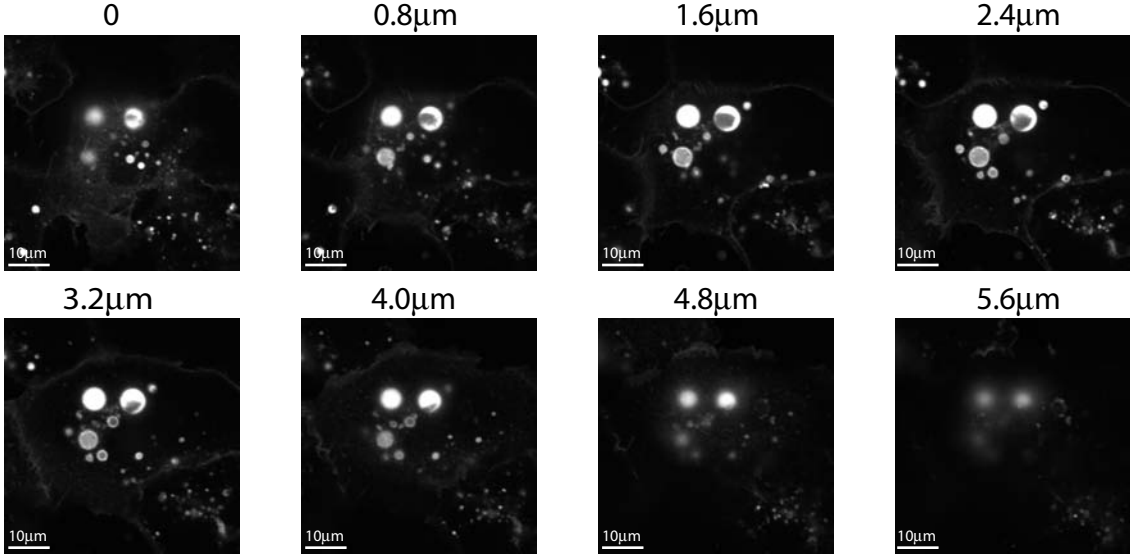


Supplemental 2A

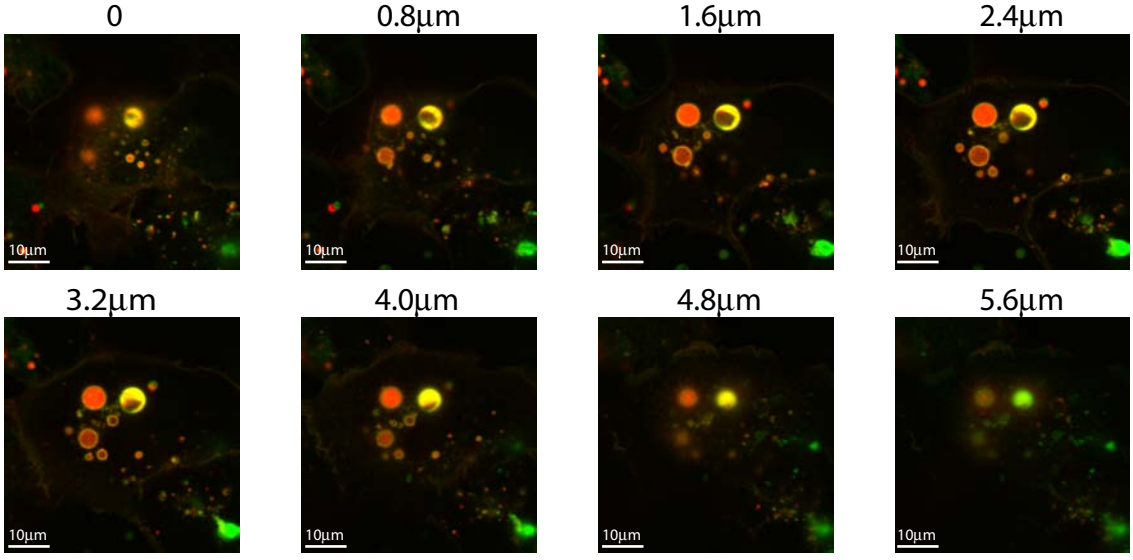
F-MORΔ17-GFP - GFP



F-MORΔ17-GFP - M1-594

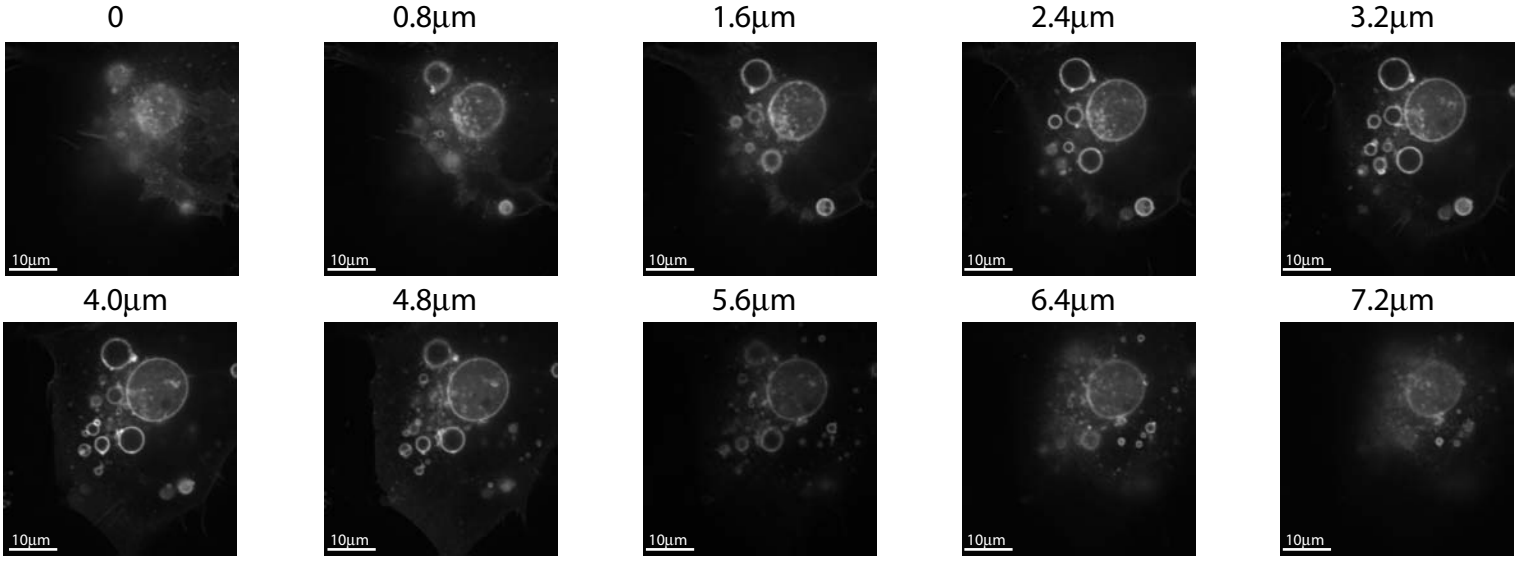


F-MORΔ17-GFP - Merge

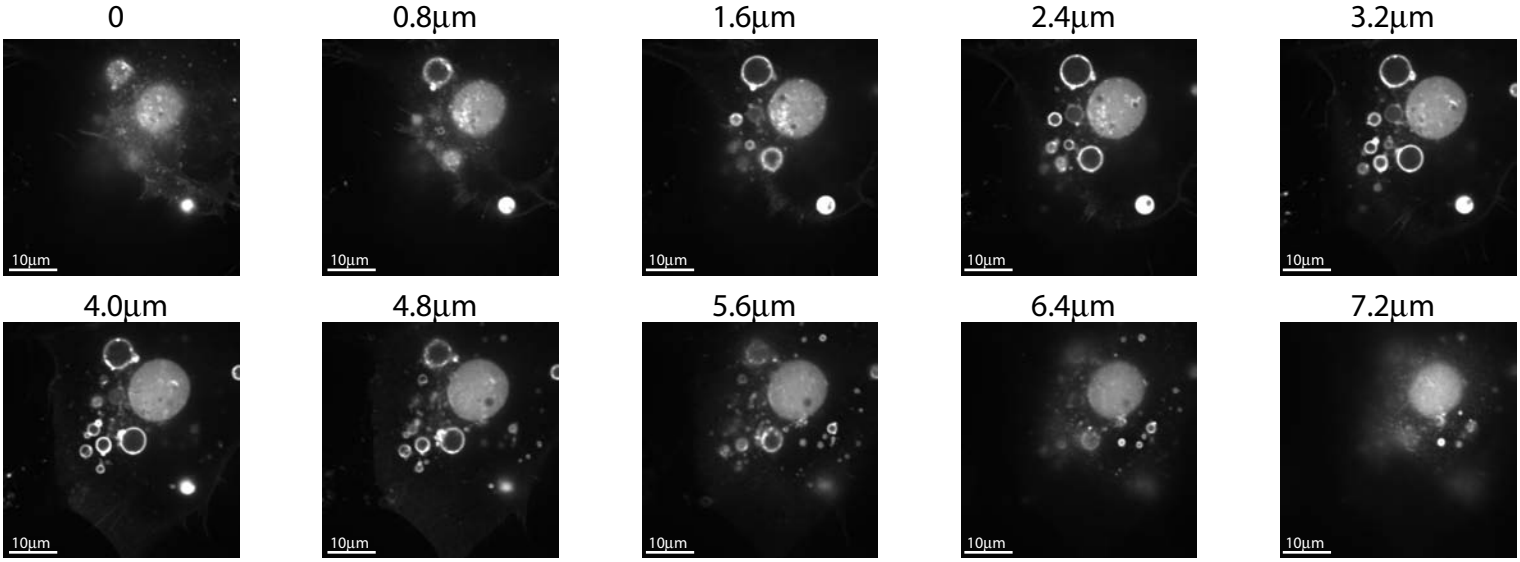


Supplemental 2B

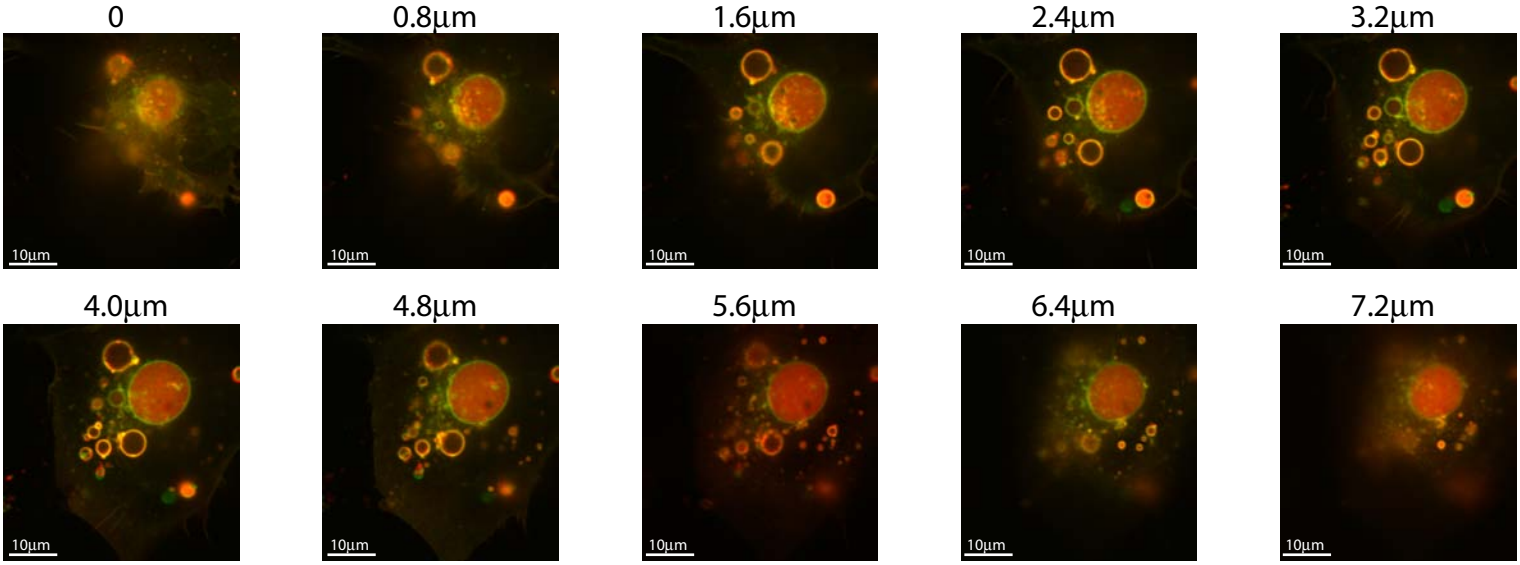
F-MORΔ17-0cK-GFP - GFP



F-MORΔ17-0cK-GFP - M1-594

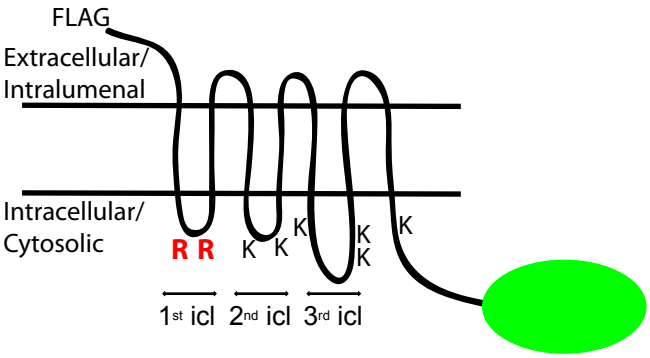


F-MORΔ17-0cK-GFP - Merge



Supplemental 3

A



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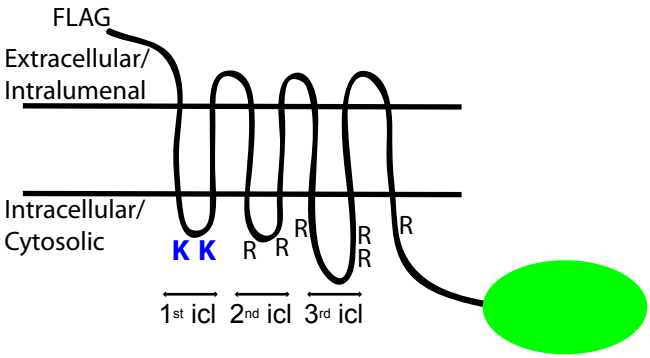
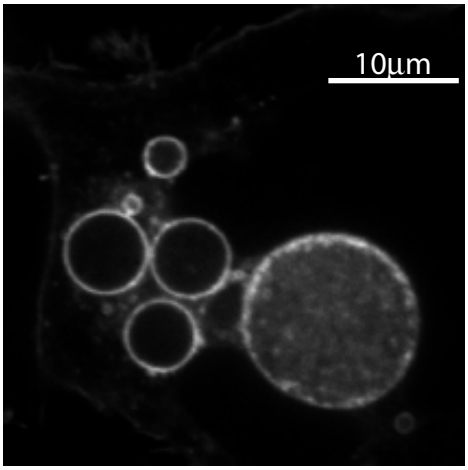


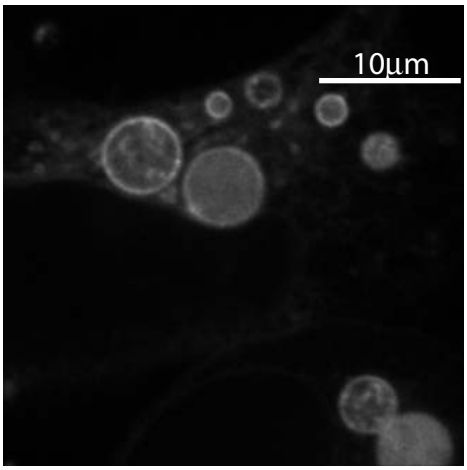
C

F-MORΔ17-2K/R-GFP

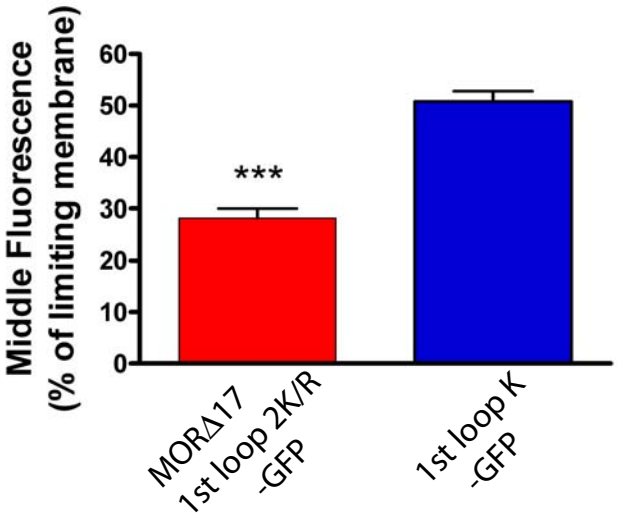


D

F-MORΔ17-0cK-1st loop K-GFP



E



F