FIGURE LEGENDS FOR SUPPLEMENTARY DATA

Suppl.Fig. 1. SLP-1 co-localizes with late endosomal markers. A, Single channel images and overlays of GFP-Rab constructs (green channels) and SLP-1-myc (red channels) are shown, supplementing the overlays in Fig. 3B. Images are projections of z-stacks onto the xy-plane. The overlays show large overlaps between GFP-Rab7 and GFP-Rab9, respectively, and SLP-1-myc but only little overlap between GFP-Rab5a and SLP-1-myc. B, Co-staining of SLP-1-GFP with the Golgi marker GM130 (upper panel) and the peroxisomal marker PMP70 (lower panel) reveals that SLP-1 is not targeted to the Golgi complex nor to peroxisomes. Scale bars: 10 µm.

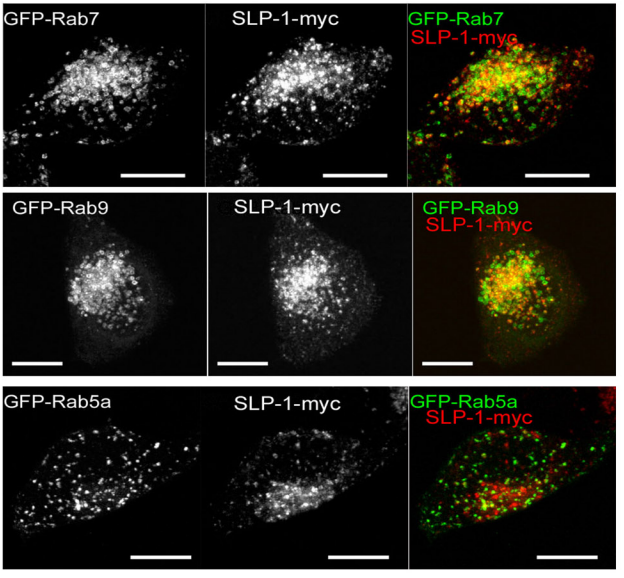
Suppl.Fig. 2. SLP-1(1-224)-GFP is localized to tubulovesicular structures. A, Two cells stably expressing high levels of SLP-1(1-224)-GFP are shown. A very strong signal was observed in cytoplasmic aggregates (red and white boxes). The inserts show these regions after reduction of the detector gain and reveal that these structures are aggregates of smaller, SLP-1(1-224)-GFP positive vesicles. B, A different cell from the same clone is shown, where the SLP-1(1-224)-GFP signal was detected on characteristic, filamentous structures (often several µm in length). Cells stably expressing SLP-1(1-224)-GFP either contained aggregates or filamentous structures or both. Cells expressing low amounts of SLP-1(1-224)-GFP predominantly showed filamentous structures. Co-localization with late endosomal markers was not detected in aggregates or filamentous structures and was low in residual perinuclear vesicles. Scale bars: 10 µm.

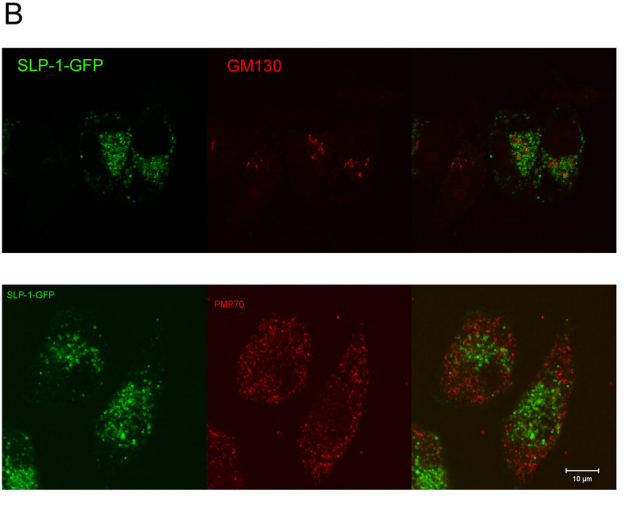
Suppl.Fig. 3. The truncation mutants SLP-1(1-288)-GFP and SLP-1(1-224)-GFP were stably expressed in HeLa cells and the respective DRMs were isolated as described in Experimental Procedures. Western blots with anti-GFP- and anti-stomatin antibody are shown, as indicated. Both C-terminal truncation mutants of SLP-1 are enriched in the DRM fractions, indicating that the C-terminus of SLP-1 is not required for DRM targeting.

Suppl.Fig. 4. Analysis of transient transfections of the chimeric SLP-1/stomatin fusions by Western blotting. Equal amounts of protein from transient transfections with the indicated GFP-tagged constructs were probed with an anti-GFP antibody. STOM(21-287)-GFP migrates slightly below 60 kDa, as expected. The fusion proteins containing residues 1-49 of SLP-1 (WT and point mutants Y6A and L9S, respectively) show increased molecular weight. Transfection efficiency was lowest with the STOM(21-287)-GFP construct. The positions of the 100, 72, and 50 kDa marker bands are indicated.

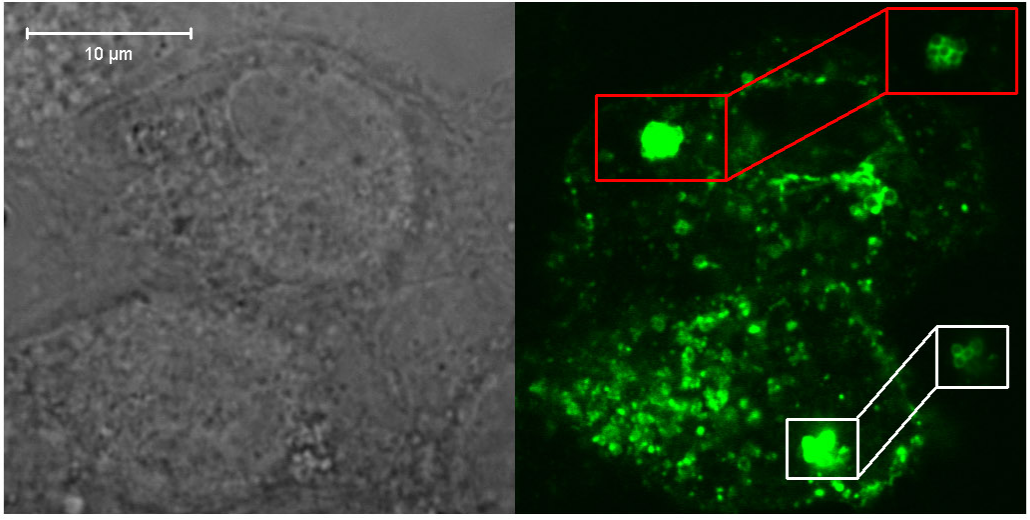
Suppl.Fig. 5. Treatment of SLP-1-myc expressing cells with U18666A results in the formation of large, cholesterol-rich vesicles. Cells stably expressing SLP-1-myc were treated with 3 µg/ml U18666A for 48 h, stained with anti-myc antibody and filipin. Two enlarged structures containing SLP-1-myc (green) and filipin (blue) were marked by arrows. These vesicles were clearly visible in the phase contrast image. Scale bar: 10 µm.

Suppl.Fig. 6. Reduced LAMP-2 localization to the large, cholesterol-rich SLP-1-GFP-positive vesicles. A, Normal HeLa cells were incubated with 3 µg/ml U18666A for 48 h and stained with anti-LAMP-2 antibody and filipin. LAMP-2 was detected in most filipin-positive vesicles. B, HeLa cells stably expressing SLP-1-GFP were treated equally and analyzed by SLP-1-GFP, LAMP-2, and filipin staining. Three enlarged, SLP-1-GFP-positive vesicles are marked with arrows. These structures contain minute, but clearly detectable amounts of LAMP-2, revealing that they are derived from the late endosomal compartment. C, Co-localization of SLP-1-GFP and LTR in large vesicles confirms the association with late endosomes. Scale bars: 10 µm.

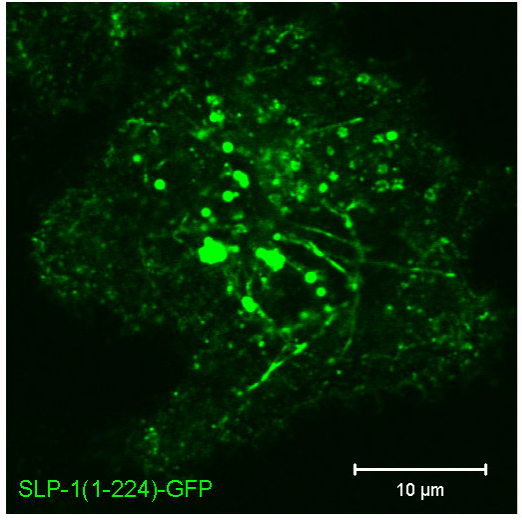

A
GFP-Rab7
SLP-1-myc
GFP-Rab7
SLP-1-myc
GFP-Rab9
SLP-1-myc
GFP-Rab9
SLP-1-myc
GFP-Rab5a
SLP-1-myc
GFP-Rab5a
SLP-1-myc

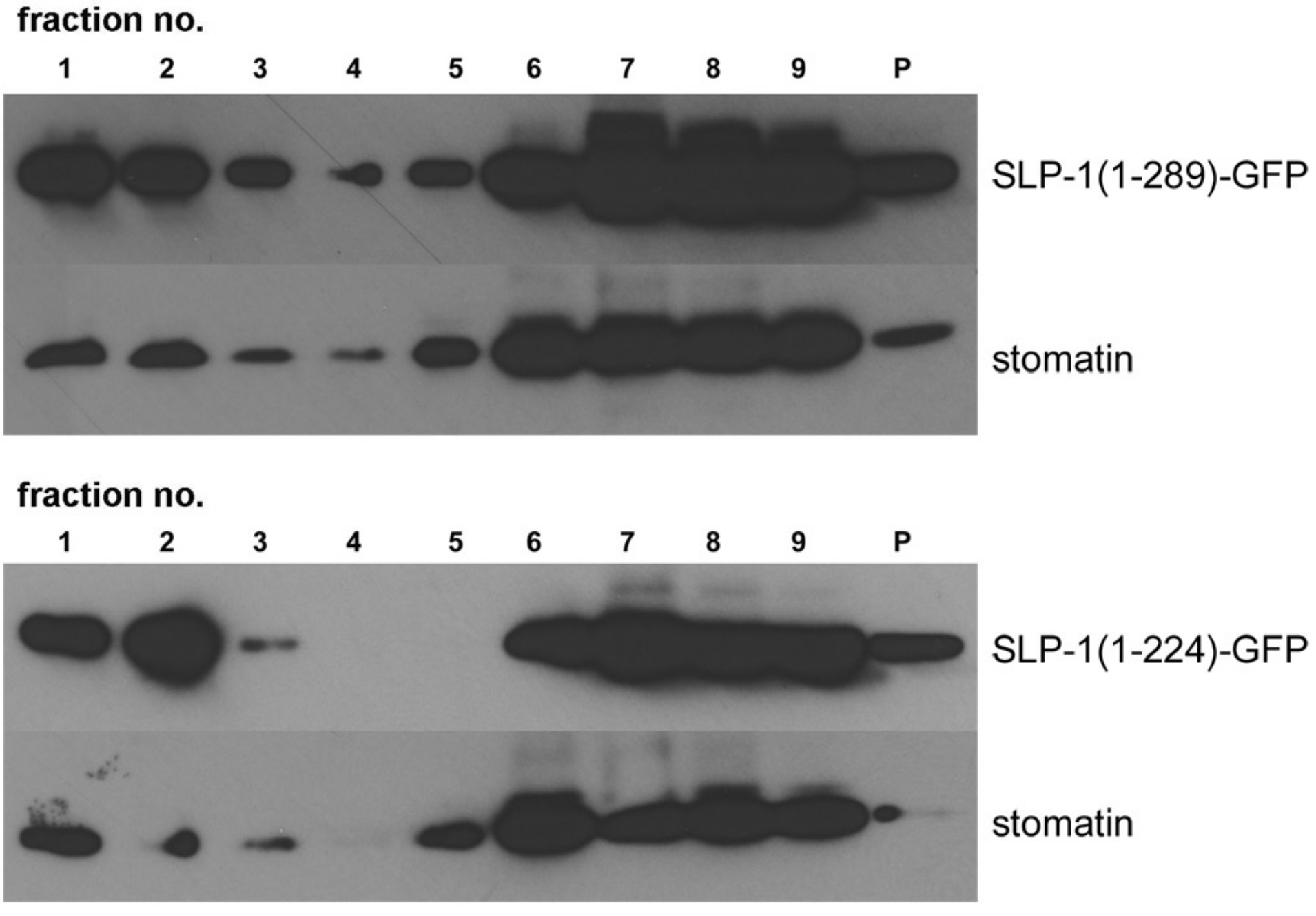

B
SLP-1-GFP
GM130
SLP-1-GFP
PMP70
10 µm

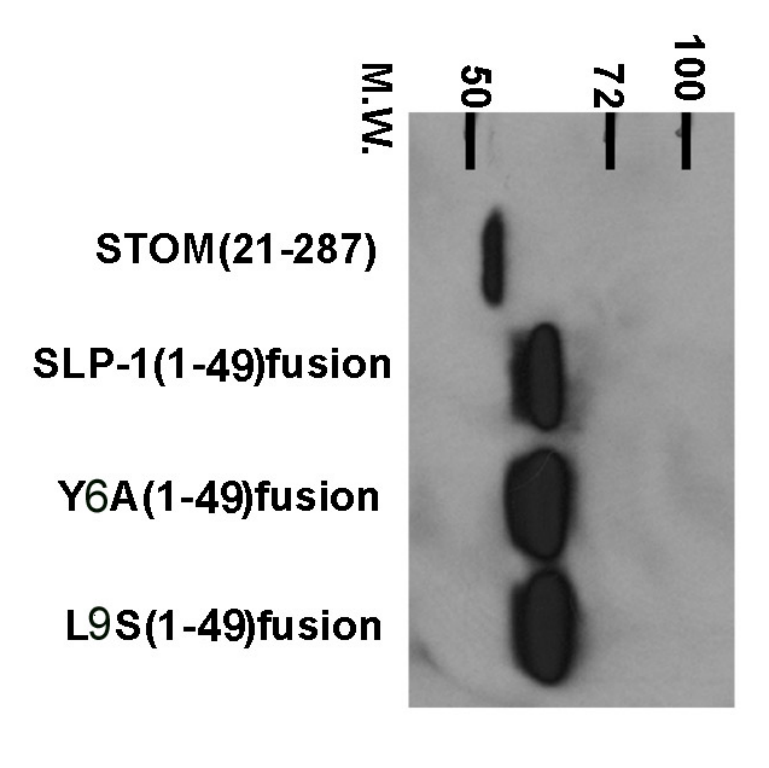
A

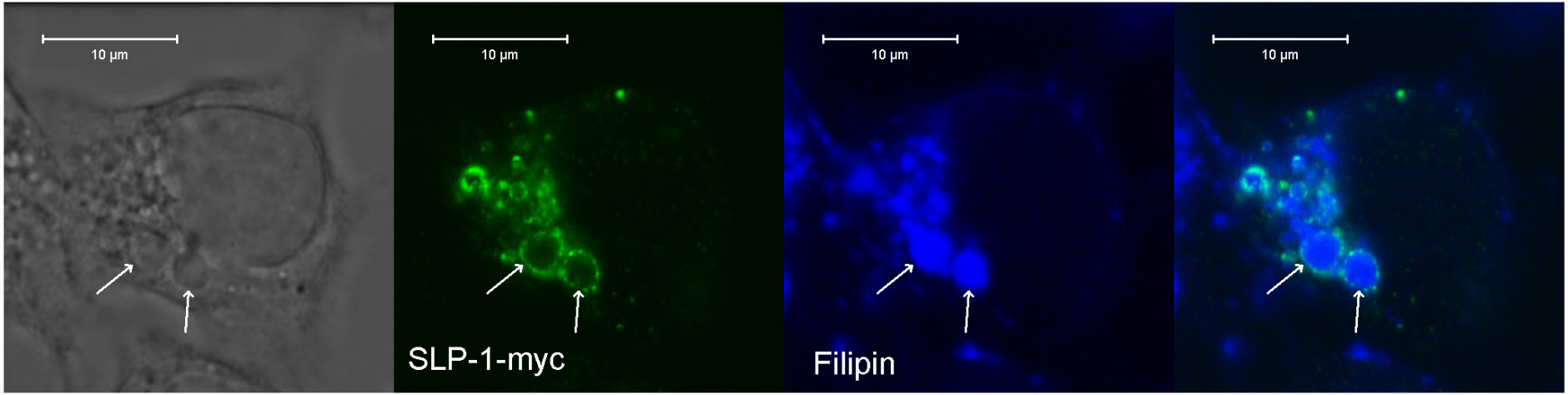


B

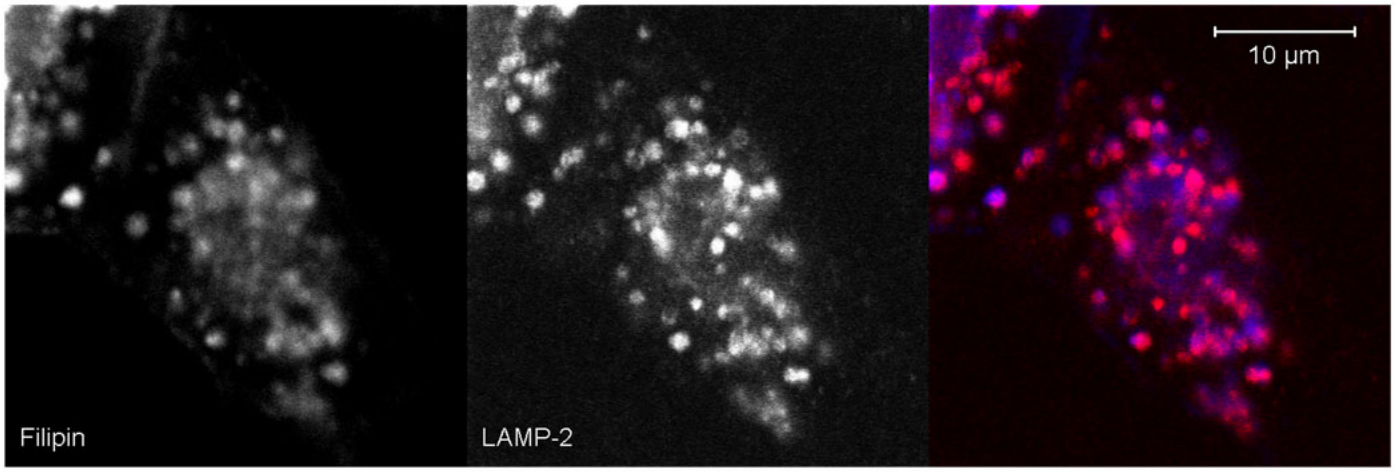



fraction no.
1
2
3
4
5
6
7
8
9
P
SLP-1(1-289)-GFP
stomatin
fraction no.
1
2
3
4
5
6
7
8
9
P
SLP-1(1-224)-GFP
stomatin

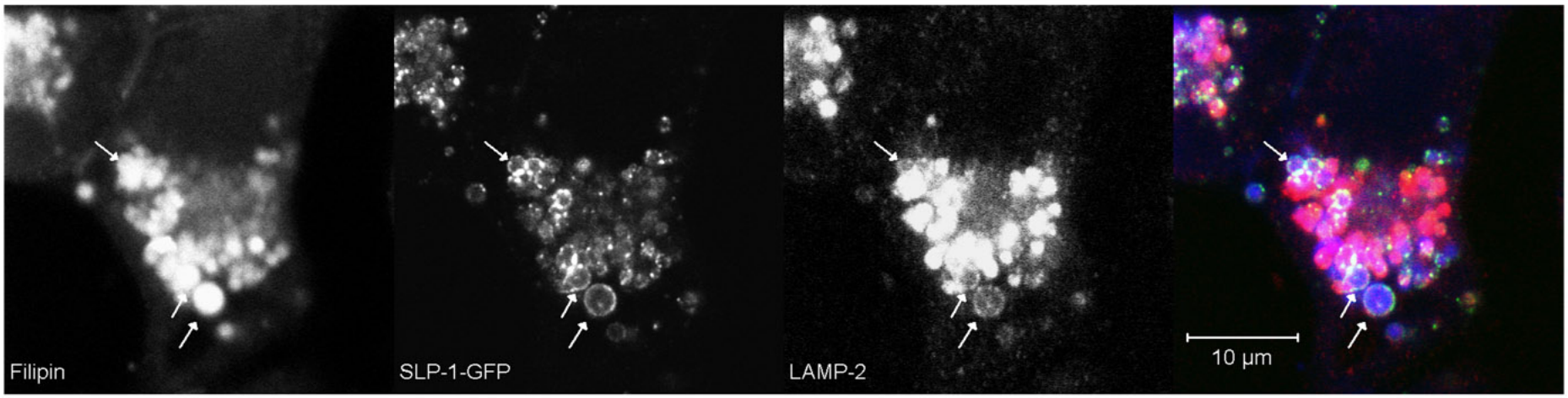

M.W.
50
72
100
STOM(21-287)
SLP-1(1-49)fusion
Y6A(1-49)fusion
L9S(1-49)fusion


10 µm
10 µm
SLP-1-myc
10 µm
Filipin
10 µm

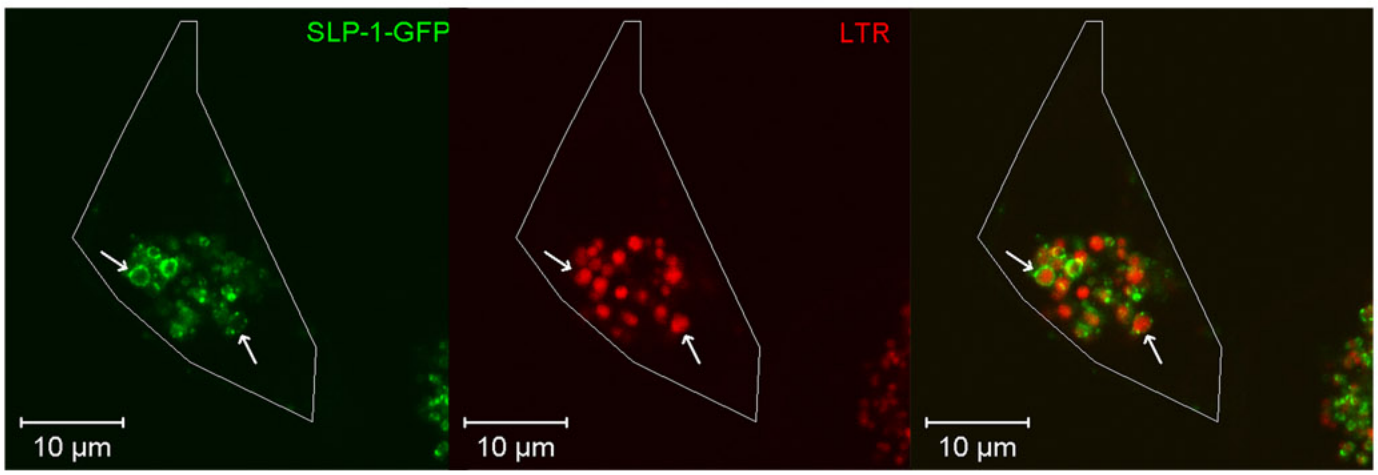
A


Filipin
LAMP-2
10 µm

B


Filipin
SLP-1-GFP
LAMP-2
10 µm

C


SLP-1-GFP
LTR
10 µm
10 µm
10 µm