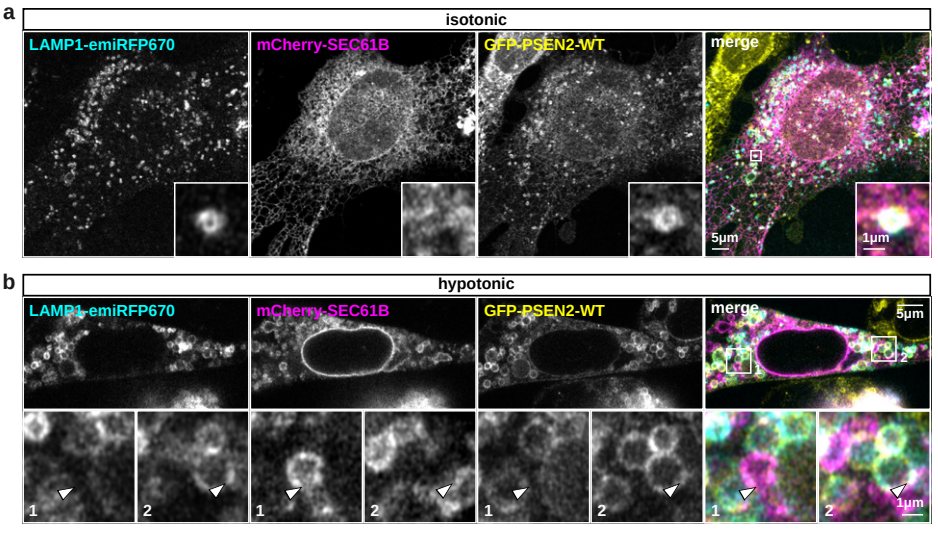
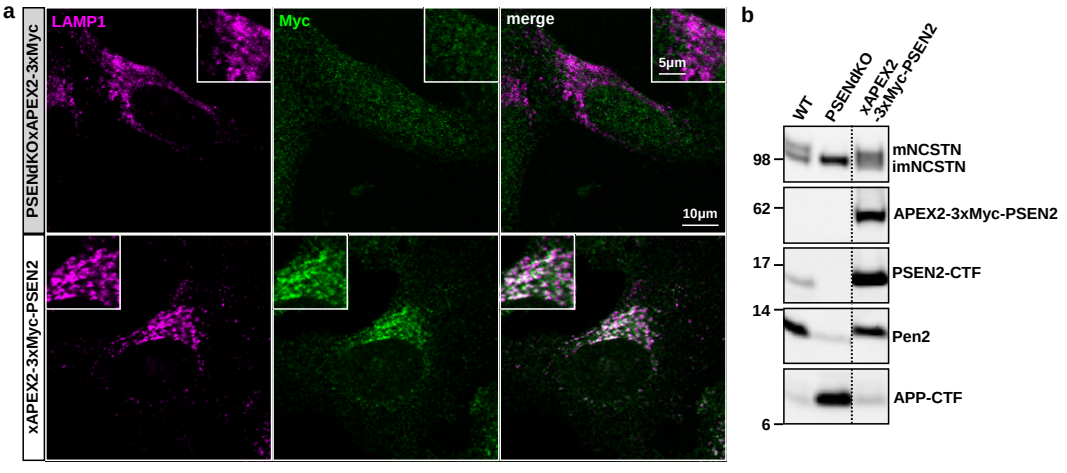
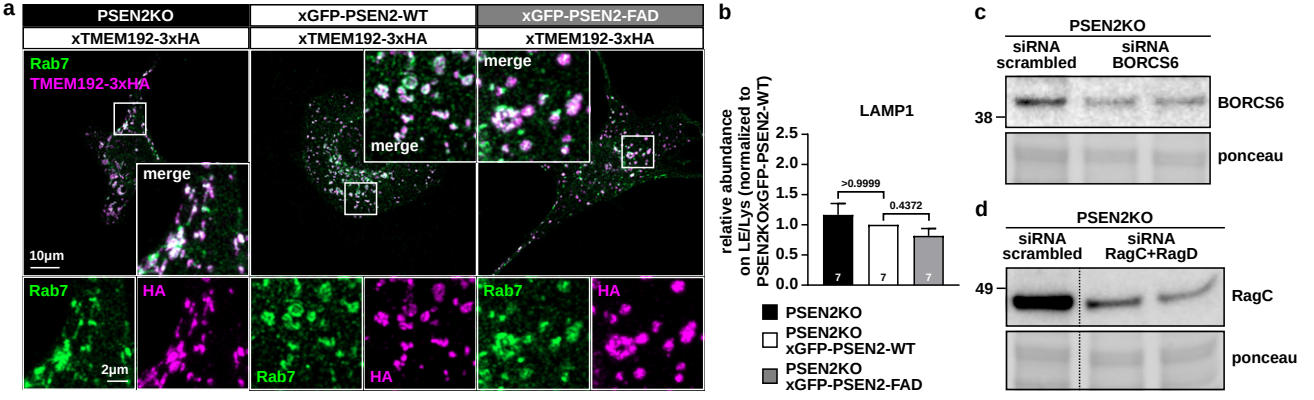

a
isotonic
LAMP1-emiRFP670
mCherry-SEC61B
GFP-PSEN2-WT
merge
5μm
1μm
b
hypotonic
LAMP1-emiRFP670
mCherry-SEC61B
GFP-PSEN2-WT
merge
5μm
1
2
1μm

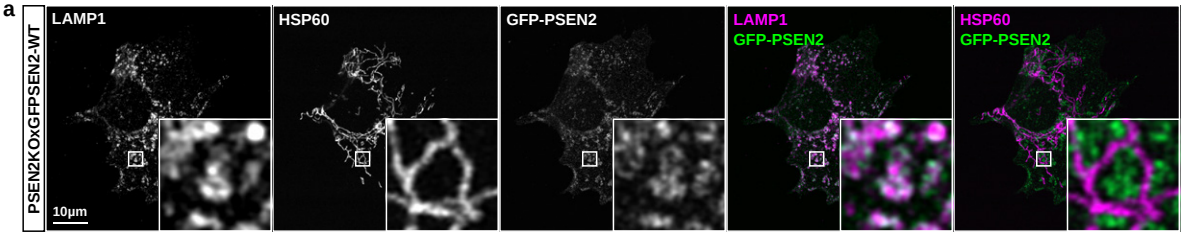
**Supplementary Figure 1. PSEN2 localizes on LAMP1 organelles, at LE/Lys-ER contacts. a–b**. Live-cell Airyscan imaging of PSEN-deficient MEFs expressing LAMP1-emiRFP670, mCherry-SEC61B and GFP-PSEN2-WT reveals that GFP-PSEN2 resides on LAMP1+ organelles closely apposed to the ER in isotonic conditions (**a**); hypotonic swelling highlights narrow MCSs (**b**).


a
LAMP1
Myc
merge
5μm
10μm
PSENdKOxAPEX2-3xMyc
xAPEX2-3xMyc-PSEN2
b
WT
PSENdKO
xAPEX2-3xMyc-PSEN2
98
62
17
14
6
mNCSTN
imNCSTN
APEX2-3xMyc-PSEN2
PSEN2-CTF
Pen2
APP-CTF

**Supplementary Figure 2. APEX2-3xMyc tagging preserves PSEN2 localization, complex formation, and catalytic activity. a**. Immunolabeling of fixed PSEN1/2 double-knockout (PSENdKO) HeLa cells expressing soluble APEX2-3xMyc or APEX2-3xMyc-PSEN2 confirmed that the APEX2-3xMyc tag does not interfere with proper subcellular localization of PSEN2 to LAMP1+ LE/Lys. **b**. HeLa wild-type (WT) cells were compared to PSENdKO and PSENdKOxAPEX2-3xMyc-PSEN2 cells; maturation of the γ-secretase subunit nicastrin (NCSTN), stabilization of the γ-secretase subunit Pen2, and cleavage of the γ-secretase substrate APP-CTF were restored upon APEX2-3xMyc-PSEN2 expression, indicating that the APEX2-3xMyc tag does not interfere with PSEN2's catalytic function. Dashed lines separate non-adjacent lanes from the same blot.


a
PSEN2KO
xGFP-PSEN2-WT
xGFP-PSEN2-FAD
xTMEM192-3xHA
xTMEM192-3xHA
xTMEM192-3xHA
Rab7
TMEM192-3xHA
merge
merge
merge
10µm
Rab7
HA
2µm
Rab7
HA
Rab7
HA
b
relative abundance on LE/Lys (normalized to PSEN2KOxGFP-PSEN2-WT)
LAMP1
2.5
2.0
1.5
1.0
0.5
0.0
>0.9999
0.4372
7
7
7
PSEN2KO
PSEN2KO xGFP-PSEN2-WT
PSEN2KO xGFP-PSEN2-FAD
c
PSEN2KO
siRNA scrambled
siRNA BORCS6
38
BORCS6
ponceau
d
PSEN2KO
siRNA scrambled
siRNA RagC+RagD
49
RagC
ponceau

**Supplementary Figure 3. TMEM192-3xHA fusion proteins localize to LE/Lys and enable comparable LysoIP efficiency across PSEN2 genotypes. a**. Cell lines used for LysoIP (MEF PSEN2KO, PSEN2KOxGFP-PSEN2-WT and PSEN2KOxGFP-PSEN2-FAD transduced with TMEM192-3xHA) were assessed for subcellular localization. Super-resolution Airyscan imaging confirmed TMEM192-fusion proteins localize to Rab7+ LE/Lys. **b**. LysoIP samples were quantified for LAMP1 enrichment vs PNS to verify comparable LE/Lys isolation efficiency between genotypes relative to PSEN2KOxGFP-PSEN2-WT. Data are mean with S.E.M., n as indicated in the figure, corresponding to N=7 independent experiments. According to data distribution normality, statistical significance was assessed with the Kruskal-Willis test and Dunn's multiple comparisons test, where the mean of each column was compared to the mean of the control column (PSEN2KOxGFP-PSEN2-WT). **c–d**. Western blot validation of siRNA-mediated knockdown in MEF PSEN2KO for (**c**) BORCS6 or (**d**) RagC/RagD, compared to scrambled siRNA control. RagC immunoblot reflects the combined depletion of RagC and RagD. Dashed lines separate non-adjacent lanes from the same blot.


a
PSEN2KOxGFPSEN2-WT
LAMP1
HSP60
GFP-PSEN2
LAMP1
GFP-PSEN2
HSP60
GFP-PSEN2
10μm

**Supplementary Figure 4. PSEN2 localizes to LE/Lys, not mitochondria. a**. MEF PSEN2KO cells stably expressing GFP-PSEN2 were fixed and co-stained for late endosomal/lysosomal marker LAMP1 and mitochondrial marker HSP60. Super-resolution Airyscan imaging shows perfect overlap of GFP-PSEN2 with LAMP1, but no overlap with HSP60.

## Supplementary Movies Legends

**Supplementary Movie 1. LE/Lys and ER dynamics in PSEN2-WT cells.** Live-cell imaging of LysoTracker+ organelles (magenta) and ER (green) in PSEN2-WT expressing cells. LysoTracker+ organelles are highly motile, while ER tubules undergo constant reshaping. Related to Fig. 1g.

**Supplementary Movie 2. 'Sliding' LE/Lys-ER contacts in PSEN2-WT cells.** LysoTracker+ organelles (magenta) slide along ER tubules (green) to change position. Related to Fig. 1h(i).

**Supplementary Movie 3. 'Dragging' LE/Lys-ER contacts in PSEN2-WT cells.** LysoTracker+ organelles (magenta) appear to drag ER tubules (green) to new locations. Related to Fig. 1h(ii).

**Supplementary Movie 4. Reduced LE/Lys motility and enlarged contacts in PSEN2KO cells.** In PSEN2KO cells, LysoTracker+ organelles (magenta) are less motile and appear trapped within the ER network (green), with increased contact site size. Related to Fig. 1i.

**Supplementary Movie 5. Immobile LE/Lys surrounded by ER sheets in PSEN2KO cells (example 1).** LE/Lys (magenta) remain stationary and are surrounded by ER sheets (green). Related to Fig. 1j(i).

**Supplementary Movie 6. Immobile LE/Lys surrounded by ER sheets in PSEN2KO cells (example 2).** A second example of stationary LE/Lys (magenta) enveloped by ER sheets (green). Related to Fig. 1j(ii).

**Supplementary Movie 7. Nutrient-driven redistribution of LE/Lys in PSEN2-WT cells.** Live-cell imaging of LysoTracker+ organelles in PSEN2-WT-expressing MEFs. Upon refeeding after 1h amino acid and serum starvation, perinuclear LE/Lys progressively redistribute towards the cell periphery. Related to Fig. 3e.

**Supplementary Movie 8. Altered nutrient-driven LE/Lys positioning in PSEN2KO cells.** Live-cell imaging of LysoTracker+ organelles in PSEN2KO MEFs. Following refeeding after 1h amino acid and serum starvation, LE/Lys remain clustered in peripheral regions irrespective of nutrient availability. Related to Fig. 4b.

**Supplementary Movie 9. Growth cone dynamics in WT neurons.** Live-cell brightfield imaging of a representative WT mouse primary neuron from 5h to 14h30 post-plating, acquired at 30min intervals and displayed at 3 frames per second. Related to Supplementary Fig. 4a.

**Supplementary Movie 10. Growth cone dynamics in PSEN2KO neurons.** Live-cell brightfield imaging of a representative PSEN2KO mouse primary neuron from 5h to 14h30 post-plating, acquired at 30min intervals and displayed at 3 frames per second. Related to Supplementary Fig. 4b.

**Supplementary Movie 11. Growth cone dynamics in PSEN2-FAD neurons.** Live-cell brightfield imaging of a representative PSEN2-FAD mouse primary neuron from 5h to 14h30 post-plating, acquired at 30min intervals and displayed at 3 frames per second. Related to Supplementary Fig. 4c.