A list of GFP-Protodin interaction proteins identified from coIP-LC/MS analysis after removal of those proteins coIPed by GFP alone.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP1A2</td>
<td>Soluble/proton-dependent anion transport protein ATP1A2 SPC212</td>
</tr>
<tr>
<td>CANX</td>
<td>Phosphorylated isoform of protein kinase C</td>
</tr>
<tr>
<td>VAPB</td>
<td>Vesicle-associated membrane protein-2</td>
</tr>
<tr>
<td>VAPA</td>
<td>Vesicle-associated membrane protein-2 - similar protein A</td>
</tr>
<tr>
<td>VAMP2</td>
<td>Vesicle-associated membrane protein-2 - synaptosomal protein A</td>
</tr>
<tr>
<td>SLC25A3</td>
<td>Phosphate carrier protein - mitochondrial</td>
</tr>
<tr>
<td>PDCD4</td>
<td>PDZ domain-containing protein 1</td>
</tr>
<tr>
<td>RAB14</td>
<td>Ras-related protein, Ras homolog 1</td>
</tr>
<tr>
<td>RAB18</td>
<td>Ras-related protein, Ras homolog 1</td>
</tr>
<tr>
<td>SARA</td>
<td>Ras-related protein, Ras homolog 1</td>
</tr>
</tbody>
</table>

**Fig. S1**

**Panel A**

**Panel B**

**Panel C**

**Panel D**

**Panel E**

**Panel F**
Fig. S1. PDZD8 interacted with the TM domain of Protrudin.

(A) A list of Protrudin-interacting protein candidates in COS7 cells identified by GFP-trap assays using GFP-Protrudin as the bait followed by mass-spectrometry.

(B) GFP-Trap assays were performed in COS7 cells expressing GFP-FYCO1 and PDZD8-myc-flag. Western blots with antibodies against Flag and GFP were performed. GFP empty vector were used as control.

(C) Representative confocal image of COS7 cells expressing GFP-Protrudin (green) and Lamp1-Halo (magenta) upon treatments with scrambled (top panel) or PDZD8 siRNAs (bottom panel) with yellow arrows indicating Protrudin decorated LE/lys.

(D) Western blots showing the knockdown efficiency of PDZD8-GFP by PDZD8 siRNAs used in this study.

(E) Quantification of number of Protrudin decorated LE/lys in scrambled (n=13) or PDZD8 siRNA treated cells (n=14). Two-tail unpaired student t test. Mean ± SD

(F) Flow cytometry analysis of PDZD8-GFP expression in WT COS7 cells (n=13348), COS7 cells expressing CMV-PDZD8-GFP (n=40000), sPDZD8-GFP (n=40000) or sPDZD8-GFP treated with PDZD8 siRNAs (n=40000).

Scale bar, 10 µm in whole cell image and 2 µm in insets in (C).
Fig. S2

Panel A: PDZD8-Halo, GFP-Mapper, Lamp1-Scar, Merge.

Panel B: Graph showing % PDZD8-Halo colocalization with Mapper and LE/lys in COS7 cells.****

Panel C: PDZD8-Halo, GFP-Mapper, Merge.

Panel D: Graph showing % PDZD8-Halo colocalization with Mapper and LE/lys in HeLa cells.****
Fig. S2: PDZD8 was not localized to ER-plasma MCSs.

Representative confocal image of live COS7 (A), or HeLa cells (C) co-transfected PDZD8-Halo (green), Lamp1-Snap (magenta), and GFP-mapper (red in (A); magenta in (C)). Top: merged image of the whole cell; Bottom: one inset with yellow arrows indicating that PDZD8 did not co-localize with GFP-mapper. (B, D) Percentage of PDZD8 co-localize with GFP-mapper or LE/lys in COS7 (B, n=10) or HeLa cells (D, n=15). ****, p<0.0001; Two-tail unpaired student t test. Mean ± SD

Scale bar, 10 µm in whole cell image and 2 µm in insets in (A) and (C).
Fig. S3: Supplemental data to Fig. 2.

(A) A Diagram demonstrating truncated PDZD8 mutations used in this study. (B-D) Representative confocal images of COS7 cells expressing GFP-SMP (B), GFP-PDZD8-ΔTM-ΔSMP (C) or GFP-PDZD8-ΔTM-ΔSMP-ΔC2-1-ΔPDZ-2 (D) (green) relative to LE/lys (magenta) or ER (red) with whole cell images on top and one inset on bottom. The quantification of frequency of truncated PDZD8 mutants on LE/lys was shown on the right. N represented cell number in this analysis.

Scale bar, 10 µm in whole cell image in (B-D); 2 µm in insets in (B-D).
Fig. S4: PDZD8-C1-CC localizes to LE/lys in HeLa, U2OS and N2A cells. Representative confocal images of HeLa (A), U2OS (B) or N2A (C) cells expressing GFP-C1-CC (green) and Lamp1-Halo (magenta).

Scale bar, 10 µm in whole cell images and 2 µm in insets.
A list of LEIysosomal membrane proteins colOpd with PDZD8-OPF after removal of those proteins colOpd by GFP alone.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDZD8</td>
<td>PDZ domain-containing protein 8</td>
</tr>
<tr>
<td>CHMP1A</td>
<td>Charged multivesicular body protein 1a</td>
</tr>
<tr>
<td>VPS28</td>
<td>Vacular protein sorting associated protein 28</td>
</tr>
<tr>
<td>VPS45</td>
<td>Vacular protein sorting associated protein 45</td>
</tr>
<tr>
<td>DCTD1</td>
<td>DCTD domain-containing protein 1</td>
</tr>
<tr>
<td>VPS27C</td>
<td>Vacular protein sorting associated protein 27C</td>
</tr>
<tr>
<td>VPS28</td>
<td>Vacular protein sorting associated protein 28</td>
</tr>
<tr>
<td>LAMP1</td>
<td>Lysosome-associated membrane glycoprotein 1</td>
</tr>
<tr>
<td>CD63</td>
<td>CD63 antigen LAMP3</td>
</tr>
</tbody>
</table>

**Fig. S5**

**A**

**B**

**C**

**D**

**E**

**F**

**G**

**H**

**I**
Fig. S5: PDZD8-C1-CC was recruited to LE/lys independent of Protrudin, VPS38B or PI3P.

(A) A list of PDZD8 interacting protein candidates on lysosomal membrane in COS7 cells identified by GFP-trap assays of PDZD8-GFP followed by mass-spectrometry.

(B-E) Representative confocal images of COS7 cells expressing GFP-C1-CC (green) and mCherry-Rab7 (magenta) upon treatments with scrambled (B), Rab7 siRNAs (C), Protrudin siRNAs (D) or VPS38B siRNAs (E). Quantification of frequency of C1-CC decorated LE/lys. N represented cell number in this analysis.

(F) Quantification of number of C1-CC decorated LE/lys. Scrambled (n=18); Rab7 siRNAs (n=16); Protrudin siRNAs (n=18); VPS38B siRNAs (n=18); Mean ± SD; ***, P<0.001; Two-tail unpaired student t test.

(G) Representative confocal images of COS7 cells expressing Flag-Rab7, and GFP-C1-CC (green) upon treatments with DMSO, wortmannin or VPS34IN-1.

(H) Western blots demonstrating the efficiency of Protrudin siRNAs mediated knockdown.

(I) Western blots demonstrating the efficiency of Rab7 siRNAs mediated knockdown.

Scale bar, 10 µm in whole cell images and 2 µm in insets in (B), (C), (D) and (E) and 10 µm in (G).
Fig. S6. Supplemental data to Fig. 6.

(A) Western blots of Rab7 expression in iRab7-sPDZD8 upon DOX addition.

(B) Ratio of the specific lipids bound by PDZD8-GFP (Left panel) or SMP-GFP (Right panel) to the total amounts of the specific lipids extracted from COS7 cells.

(C) Sequence alignment between the SMP domain of human PDZD8 and the SMP domain of human E-syt1. Two conserved hydrophobic residues (V166 and L285) were mutated to W in the SMP-mut, highlighted by magenta. The two conserved hydrophobic residues (V169 and L308) between E-syt1 and E-syt2 were highlighted by yellow.

(D) Dynamic light scattering assays confirmed the tethering efficiency of the tethered-SMP proteins. P<0.0001; Ordinary one-way ANOVA with Tukey’s multiple comparisons test. ****, significant; ns, not significant. Mean ± SD

(E) The dithionite assay demonstrated that tethered-PDZD8-SMP did not promote liposome fusion. In this assay, dithionite solution was added to the liposomes after the lipid transfer reactions. The NBD fluorescence was baseline subtracted. Mean ± SD

(F) GFP-Trap assay was performed in COS7 cells transiently expressing PDZD8-GFP or PDZD8 truncated mutants and PDZD8-myc-flag. Western blots with antibodies against GFP and Flag were performed with red asterisks showing a TM-SMP monomer and a putative TM-SMP dimer.
Fig. S7. Supplemental data to Fig.7.

(A) qPCR assays confirmed the efficiency of siRNAs-mediated PDZD8 suppression in COS7 and U2OS cells. All the data were obtained from three independent assays. Two-tail unpaired student t test. Mean ± SD

(B, C) Representative confocal images of U2OS cells (B) or HeLa cells (C) expressing Lamp1-OFP (magenta), ER-GFP (green) and mitoBFP (blue) upon treatments with scrambled (top) or PDZD8 siRNAs (bottom).

(D) Percentage of LE/lys at peri-nuclear regions in scrambled siRNAs treated U2OS (n=22), HeLa (n=22), and PDZD8 siRNAs treated U2OS (n=19), HeLa cells (n=19). All the data were obtained from three independent assays. ****, p<0.0001; Two-tail unpaired student t test. Mean ± SD

(E) Representative confocal images of COS7 cells expressing Lamp1-Halo (magenta), ER-RFP (green) and GFP-Protrudin (blue) upon treatments with scrambled (top) or PDZD8 specific siRNAs (bottom). Yellow arrows denote the LE/lys enrichments at cell periphery in cells expressing GFP-Protrudin.

(F) qPCR assays confirmed the efficiency of shRNAs-mediated suppression of PDZD8 (top panel) or Protrudin (bottom panel) in PC12 cells. All the data were obtained from three independent assays. ****, p<0.0001; Two-tail unpaired student t test. Mean ± SD

Scale bar, 10 µm in whole cell image in (B, C, E) and 2 µm in insets in (B, C, E).
**Movie 1:** Time-lapse images of PDZD8-GFP stable COS7 cells transiently transfected LE/lys marker (magenta) and ER marker (red) and showing PDZD8-GFP specifically accumulating at ER-LE/lys contact sites over time. Time intervals: 19 sec; Scar bar: 2 µm.