SUPPLEMENTARY INFORMATION

VPS13C/PARK23 initiates lipid transfer and membrane remodeling for efficient lysosomal repair

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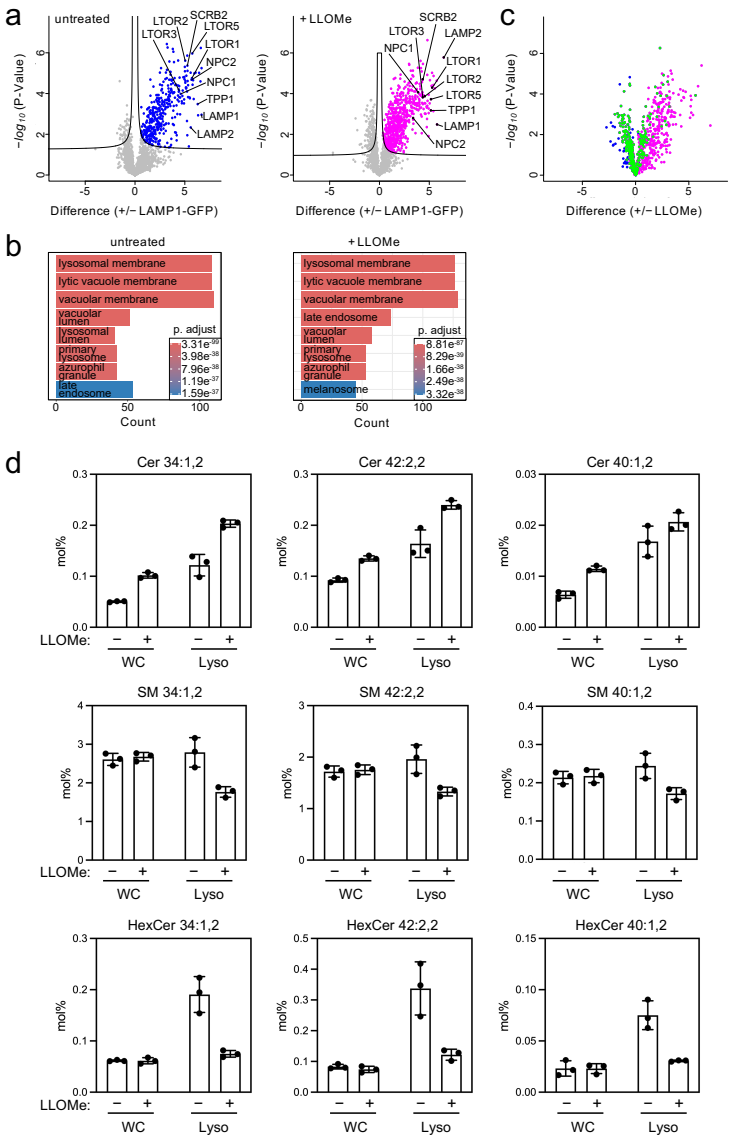


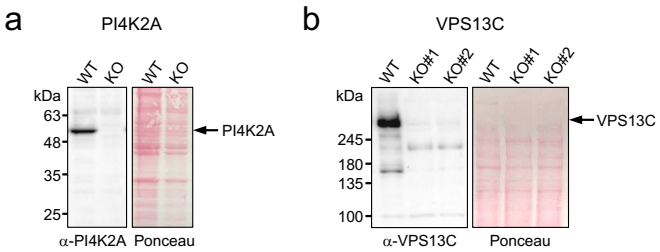
Supplementary Figure 1. Protein and lipid composition of lysosomes affinity-purified from control and LLOMe-treated HeLa cells.

(**a**) Volcano plots showing enrichment of various lysosomal membrane proteins in lysosome isolates from untreated or LLOMe-treated (500 µM, 30 min) LAMP1-GFP-expressing HeLa cells relative to background HeLa cells. Fold changes were calculated from three independent biological replicates and plotted on the x-axis against the negative logarithmic *P*-values on the y-axis.

(**b**) Gene Ontology (GO) enrichment analysis of lysosome isolates relative whole cells. Shown are data from LAMP1-GFP-expressing HeLa cells treated as in (a).

(**c**) Volcano plot of proteins enriched in lysosome isolates from untreated and LLOMe-treated LAMP1-GFP HeLa cells and grouped according to how lysosomal damage affects their relative levels. *Green*, proteins enriched in both intact (–LLOMe) and damaged (+LLOMe) lysosomes; *blue*, proteins selectively depleted in damaged lysosomes; *magenta*, proteins selectively enriched in damaged lysosomes.

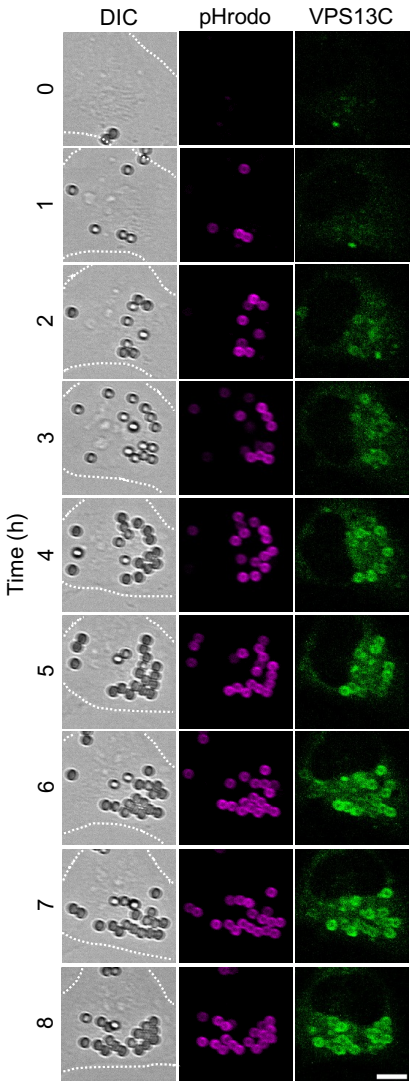
(**d**) Lipid composition of whole cell lysates (WC) and lysosomes purified from untreated and LLOMe-treated (500 µM, 30 min) HeLa cells expressing LAMP1-GFP was determined by mass spectrometry-based shotgun lipidomics. Levels of the different lipid species are expressed as mol% of total identified lipids. SM, sphingomyelin; Cer, ceramide; HexCer, hexosylceramide.



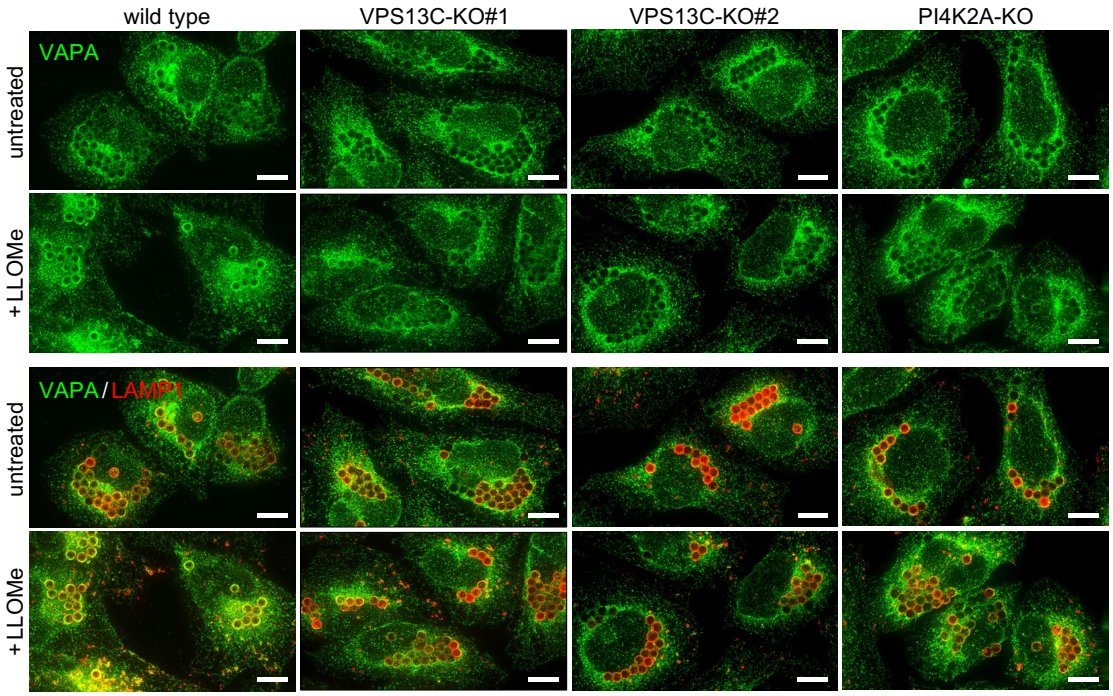
Supplementary Figure 2. Validation of PI4K2A-KO and VPS13C-KO cell lines by immunoblot analysis.

(**a**) U2OS cells lacking PI4K2A were created by CRISPR/Cas9. Loss of PI4K2A was confirmed by immunoblot analysis with an anti-PI4K2A antibody, using Ponceau S staining as loading control. Migration of the PI4K2A protein is marked by an arrow.

(**b**) U2OS cells lacking VPS13C were created by CRISPR/Cas9. Loss of VPS13C was confirmed by immunoblot analysis with an anti-VPS13C antibody, using Ponceau S staining as loading control. Migration of the VPS13C protein is marked by an arrow.

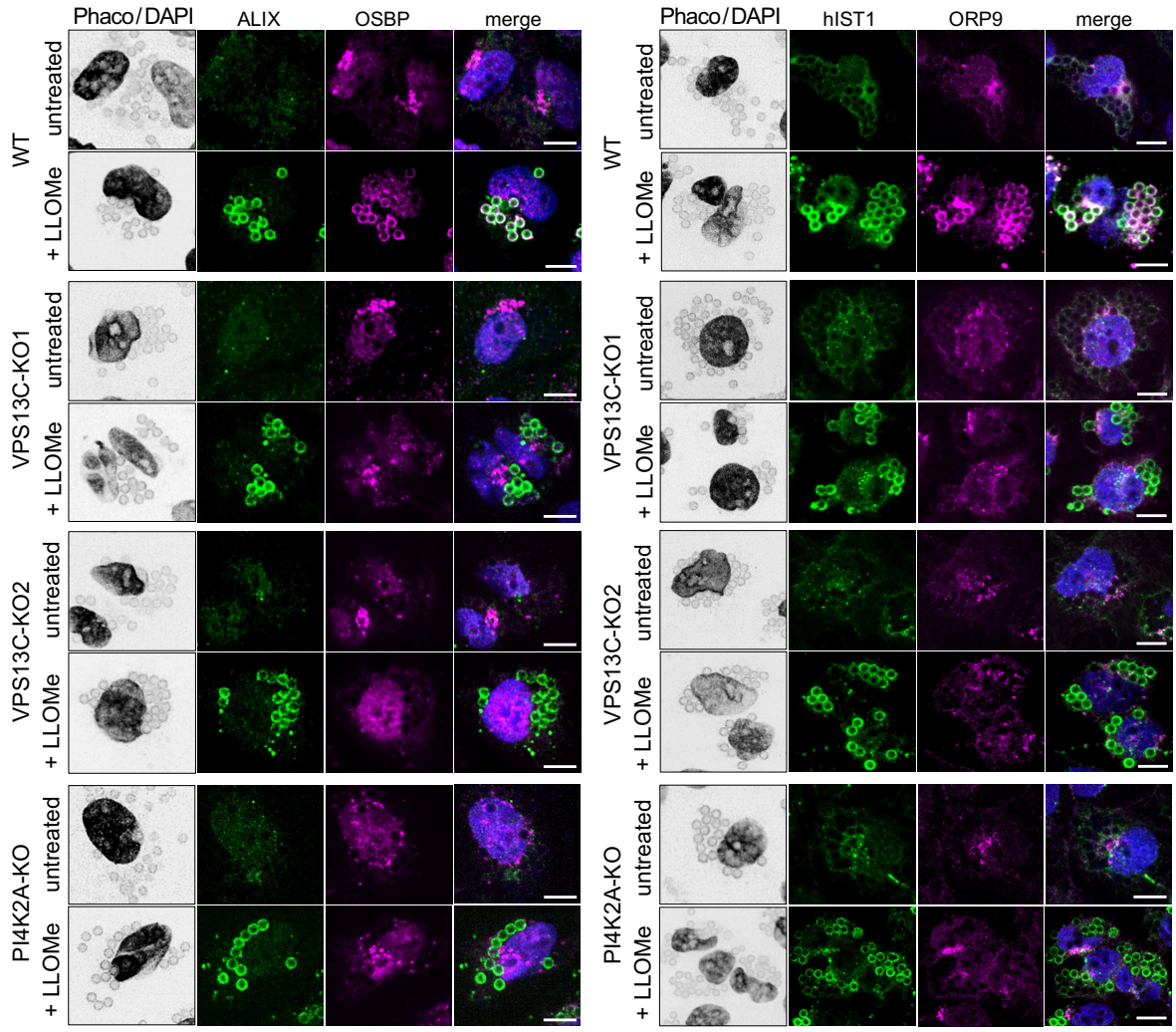


Supplementary Figure 3. pHrodo-conjugated beads internalized by U2OS cells readily gain pHrodo fluorescence and induce large-scale mobilization of VPS13C.
Time-lapse images of U2OS cells transfected with VPS13C-mClover (*green*) and incubated with 3 µm pHrodo-labeled beads (*magenta*). Cells were imaged by spinning disk microscopy. Scale bar, 10 µm.



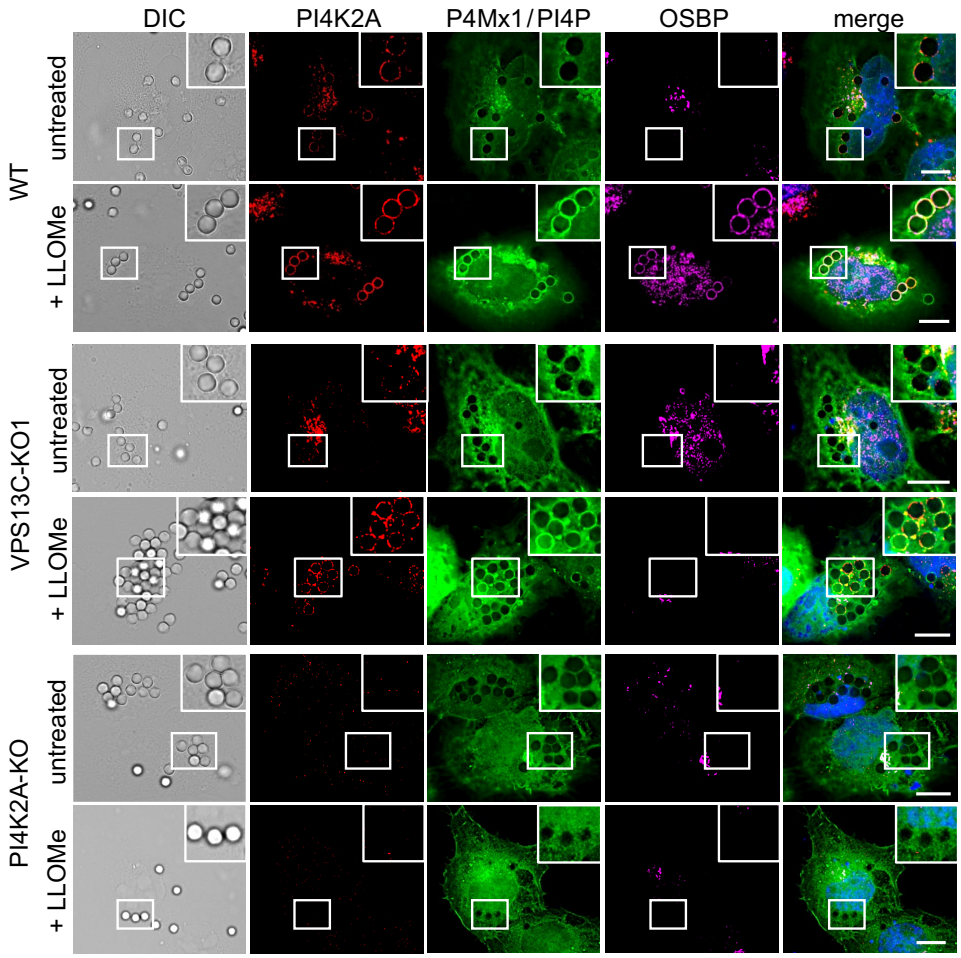
Supplementary Figure 4. VPS13C is required for tethering damaged microbead-containing lysosomes to the ER.

U2OS wildtype (WT), VPS13C-KO1, VPS13C-KO2 and PI4K2A-KO cells were fed 3 μm polystyrene beads, treated with LLOMe (1 mM, 20 min) as indicated, immunostained for LAMP1 (*red*) and VAPA (*green*), and imaged by DeltaVision microscopy. Scale bar, 10 μm.

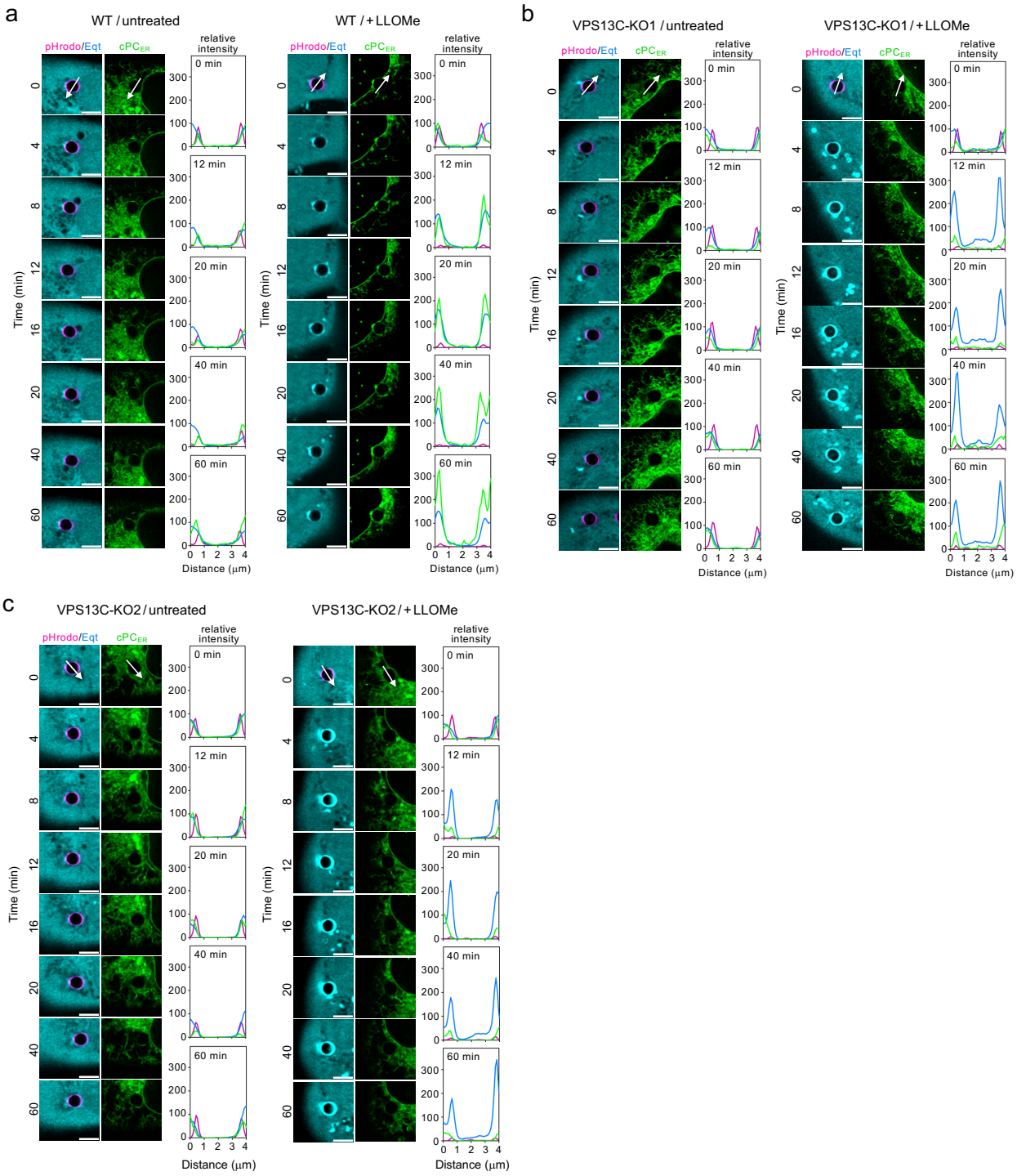


Supplementary Figure 5. VPS13C is essential for recruiting OSBP and ORP9 to damaged lysosomes.

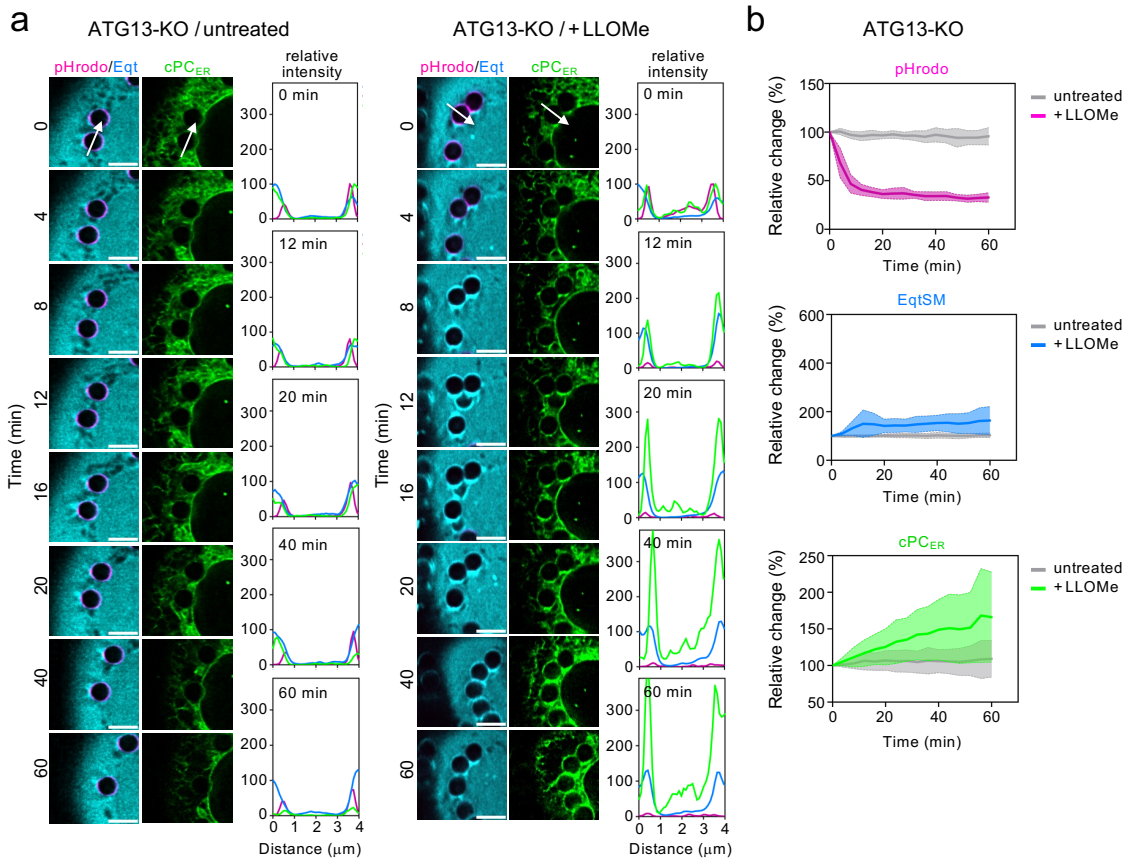
Phase contrast (Phaco) and fluorescence images of polystyrene bead-containing untreated or LLOMe-treated (1 mM, 10 min) U2OS wildtype (WT), VPS13C-KO1, VPS13C-KO2 or PI4K2A-KO cells immunostained for OSBP (*magenta*) and ALIX (*green*) or ORP9 (*magenta*) and hIST1 (*green*) and counterstained with DAPI (*blue*). Cells were imaged by spinning disk microscopy. Scale bar, 10 µm.



Supplementary Figure 6. Mobilization of PI4K2A to damaged lysosomes is independent VPS13C. Differential interference contrast (DIC) and fluorescence images of polystyrene bead-containing untreated or LLOMe-treated (1 mM, 10 min) U2OS wildtype (WT), VPS13C-KO1 or PI4K2A-KO cells expressing P4MX1-GFP (*green*) and immunostained for PI4K2A (*red*) and OSBP (*magenta*) and counterstained with DAPI (*blue*). Cells were imaged by DeltaVision microscopy. Scale bar, 10 µm.



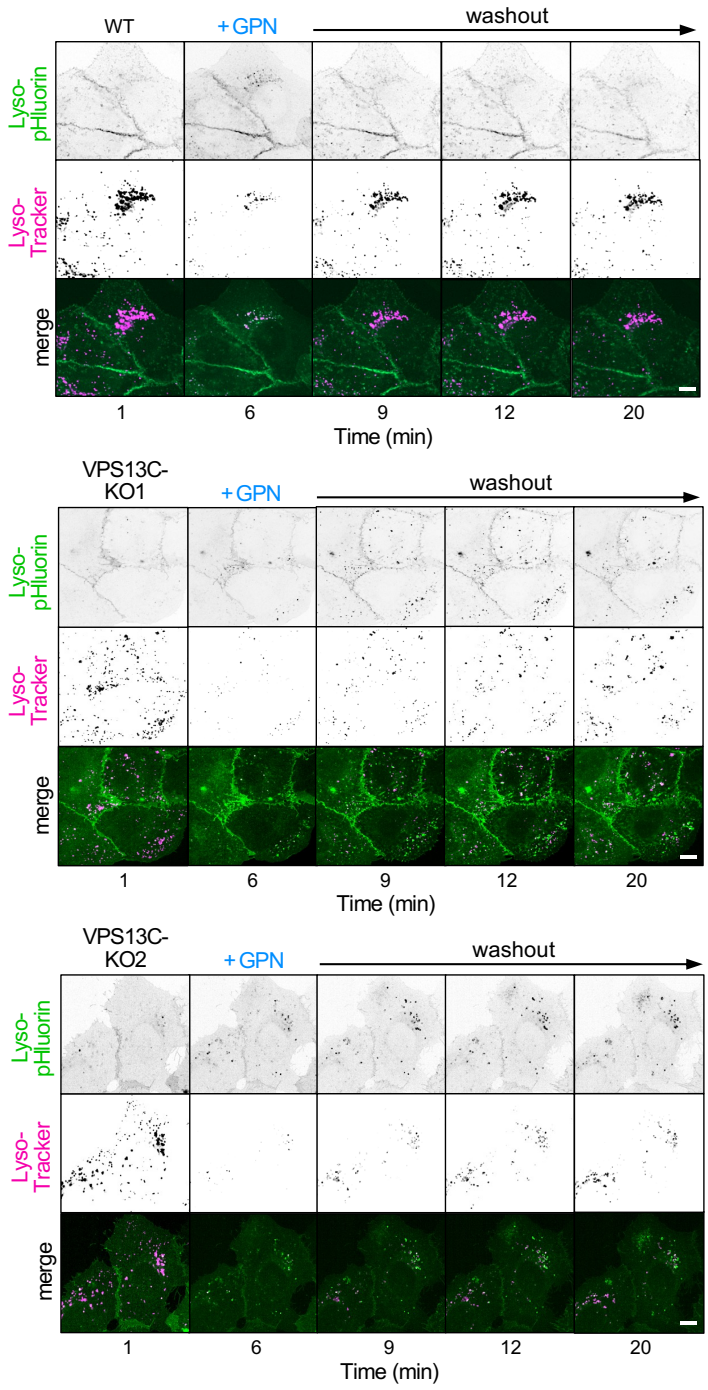
Supplementary Figure 7. VPS13C removal disrupts delivery of cPC$_{ER}$ to damaged lysosomes. (**a, b, c**) U2OS wildtype, VPS13C-KO1 and VPS13C-KO2 cells expressing EqtSM-Halo (*cyan*) were fed pHrodo-beads (*magenta*), incubated with N_3-Chol, stained with ER-DBCO (cPC$_{ER}$, *green*) and then subjected to time-lapse imaging in the absence (untreated) or presence of 1 mM LLOMe. Cells were imaged by LLSM and only zoom-ins of imaged cells are shown. Line scans show the intensity profiles of EqtSM-Halo (*cyan*), pHrodo (*magenta*) and cPC$_{ER}$ (*green*) signals along the path of the arrows. Profiles are plotted as relative intensities for each channel normalized to the 0 min time point.



Supplementary Figure 8. Accumulation of cPC$_{ER}$ on damaged lysosomes is not affected in cells defective in autophagy.

(**a**) U2OS ATG13-KO cells expressing EqtSM-Halo (*cyan*) were fed pHrodo-beads (*magenta*), incubated with N_3-Chol, stained with ER-DBCO (cPC$_{ER}$, *green*) and then subjected to time-lapse imaging in the absence (untreated) or presence of 1 mM LLOMe. Cells were imaged by LLSM and only zoom-ins of imaged cells are shown. Line scans show the intensity profiles of EqtSM-Halo (*cyan*), pHrodo (*magenta*) and cPC$_{ER}$ (*green*) signals along the path of the arrows. Profiles are plotted as relative intensities for each channel normalized to the 0 min time point. Scale bar, 5 μm.

(**b**) Time course plotting the relative changes in pHrodo, EqtSM-Halo, and cPC$_{ER}$ signals on the surface of pHrodo-bead containing lysosomes in U2OS ATG13-KO cells treated as in (a). A 3D-surface was generated around the pHrodo-positive beads using Imaris software, and the mean fluorescence intensities of the respective channels on this surface were quantified. For each cell, signals from all bead-associated surfaces were averaged. Values were normalized to the signal at timepoint 0 min and plotted as mean ± SD over time. ATG13-KO (n = 3): control, 10 cells; +LLOMe, 9 cells.



Supplementary Figure 9. VPS13C removal disrupts lysosomal repair.
Time-lapse images of LysoTracker-labelled (*magenta*) and Lyso-pHluorin-expressing (*green*) U2OS wildtype (WT), VPS13C-KO1 and VPS13C-KO2 cells pulse-treated with GPN (200 μM, 2 min). Cells were imaged by spinning disk microscopy. Scale bar, 10 μm.

Supplementary Table 1. Primers used for cloning and site-directed mutagenesis.

Primer name	Primer sequence (5'-3')
hCalnexin-ORF_fwd	gaatgatatcatggaagggaagtggttgctgtgtatg
hCalnexin-ORF_rev	gttagaattcctctcttcgtggctttctgtttcttgg
$EqtSM_{cyto}$-Halo_fwd	cacagaattcaccatgtccgcagacgtgg
$EqtSM_{cyto}$-Halo_rev	cacacagcggccgcctaaccggaaatctccagagtagac
VPS13C ORF_fwd	gctgtacaaggaccatctcaaagaacaagaag
VPS13C ORF_rev	tgctcacgccaggtacacttgaaacatcaac
Halo-ATG2C_SbfI_fwd	cacacctgcaggtgaattaatggagacttcaatgactg
Halo-ATG2C_XhoI_rev	cacactcgagcctatgccctctgaatgccttg
VPS13C-V3563Q_fwd	tgtgggtgctcaggcccgtc
VPS13C-V3563Q_rev	agccctttccaattcctttaaagaatc
FLAG-GFP-OSBP_fwd	cacagaaccaattcctaccggtgccatggactac
FLAG-GFP-OSBP_rev	cacagatatcgtcgagaattgatccgcgc
mEGFP w/o Met_fwd	aagtgtacctggcgtgagcaagggctgagcaagggcg
mEGFP w/o Met_rev	tgagatggtccttgtacagctcgtccatgc

Image J Macros

Quantification of VPS13C-mClover, GFP-OSBP, and LysopHluorin puncta

1. //set Threshold manually
2. setOption("BlackBackground", false);
3. run("Convert to Mask", "method=Default background=Dark");
4. run("Fill Holes","stack");
5. run("Watershed", "stack");
6. run("Analyze Particles...", "size=0.1-5 circularity=0.50-1.00 show=Outlines display exclude summarize stack");
7. run("Next Slice [>]")
8. //Repeat step 6 and 7 until all time points are analyzed

Quantification of LysoTracker puncta

1. run("Subtract...", "value=20 stack");
2. setAutoThreshold("Default dark no-reset");
3. setOption("BlackBackground", false);
4. run("Convert to Mask", "method=Default background=Dark");
5. run("Convert to Mask", "method=Default background=Light");
6. run("Watershed", "stack");
7. run("Analyze Particles...", "size=0.2-5 circularity=0.5-1.00 show=Outlines display exclude summarize stack");
8. run("Next Slice [>]")
9. // repeat step 7 and 8 until all time frames have been quantified

Quantification of pHrodo, cPC_{ER} andCNX-Halo and EqtSM-Halo signals on pHrodo-microbead containing lysosomes

Macro based on Maib, H. and Murray, D.H. (2022) 'A mechanism for exocyst-mediated tethering via Arf6 and PIP5K1C-driven phosphoinositide conversion', *Current Biology*, 32(13), pp. 2821-2833.e6 (https://doi.org/10.1016/j.cub.2022.04.089) with modifications by Steffen Wolke-Hanenkamp (Ultraphysics Division, Osnabrück University).

```
// Check for the Excel plugin
if (File.exists(getDirectory("plugins") + "/Read_and_Write_Excel-1.1.7.jar") != 1) {
    exit("ResultsToExcel Plugin not found. Make sure you activated the plugin in the
ImageJ updater. ('Help' > 'Update...' > 'Manage update sites')");
}
// Get input image and basic parameters
dir1 = getDirectory("image");
name = getTitle();
selectWindow(name);
getDimensions(width, height, channels, slices, frames);
run("Split Channels");
// Open results Excel sheet
run("Read and Write Excel", "file_mode=read_and_open file=[" + dir1 +
"/CS_BeadsResults.xlsx] sheet=[C1]");
// Iterate over all time frames
for (i = 1; i <= frames; i++) {
    // --- MASK GENERATION (Channel 2) ---
    selectWindow("C2-" + name);
    Stack.setFrame(i);
    run("Duplicate...", "title=C2-mask-frame_" + i + ".tif frames=" + i);
    maskID = getImageID();
    setAutoThreshold("Li dark");
    run("Convert to Mask", "method=Li background=Dark calculate");
    setOption("BlackBackground", false);
    run("Fill Holes");
    run("Watershed");
    run("Analyze Particles...", "size=5-250 circularity=0.1-1.00 show=Overlay clear add
stack");
    // --- ROI ENLARGEMENT ---
    upperROI = roiManager("count");
    for (index = 0; index < upperROI; index++) {
        roiManager("Select", index);
        run("Enlarge...", "enlarge=-1");
        run("Make Band...", "band=1.25");
        roiManager("Update");
    }
    // --- MEASURE CHANNEL 1 ---
    selectWindow("C1-" + name);
```

```
    Stack.setFrame(i);
    run("Duplicate...", "title=C1-double.tif frames=" + i);
    C1doubleID = getImageID();
    roiManager("Deselect");
    roiManager("Measure");
    run("Read and Write Excel", "file_mode=queue_write dataset_label=[Frame " + i + "]
sheet=[C1]");
    run("Clear Results");
    close(C1doubleID);
    // --- MEASURE CHANNEL 2 ---
    selectWindow("C2-" + name);
    Stack.setFrame(i);
    run("Duplicate...", "title=C2-double.tif frames=" + i);
    C2doubleID = getImageID();
    roiManager("Deselect");
    roiManager("Measure");
    run("Read and Write Excel", "file_mode=queue_write dataset_label=[Frame " + i + "]
sheet=[C2]");
    run("Clear Results");
    close(C2doubleID);
    // --- MEASURE CHANNEL 3 ---
    selectWindow("C3-" + name);
    Stack.setFrame(i);
    run("Duplicate...", "title=C3-double.tif frames=" + i);
    C3doubleID = getImageID();
    roiManager("Deselect");
    roiManager("Measure");
    run("Read and Write Excel", "file_mode=queue_write dataset_label=[Frame " + i + "]
sheet=[C3]");
    run("Clear Results");
    close(C3doubleID);
    // --- SAVE MASK ---
    selectImage(maskID);
    saveAs("PNG", dir1 + File.separator + i + "C2-Masks");
    close(maskID);
}
// Finalize Excel export and clean up
run("Read and Write Excel", "file_mode=write_and_close");
close("*");
close("Results");
close("ROI Manager");
// Notify user
waitForUser("End", "Macro has finished");
```

Uncropped blots

Figure 1c

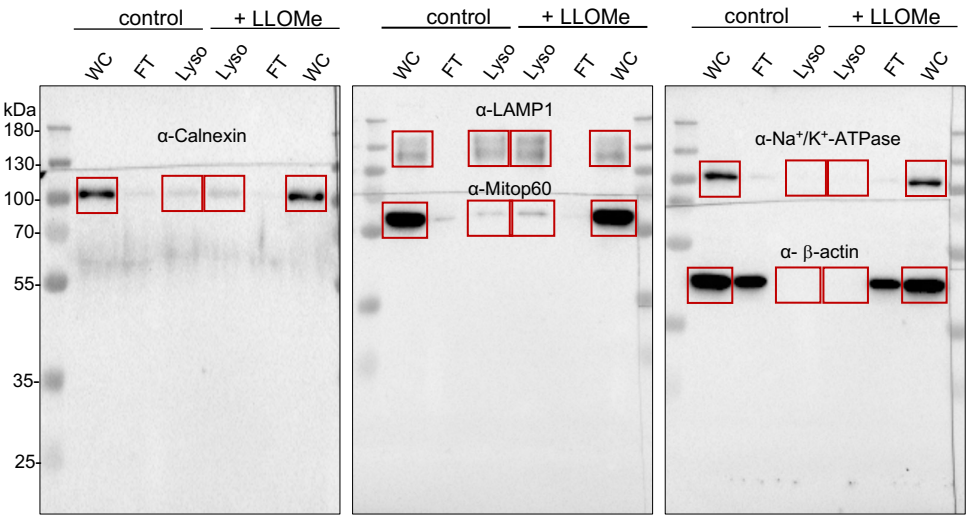


Figure 2b