Pu et al., https://doi.org/10.1083/jcb.201703094

	CE1		CE2
	216	221	349 352
H. sapiens	RHGDLTH	FVANN	LQLKIR QVRDIRRT LEV LEALC
D. rerio	HDGDLTH	FITKN	LEQKIK QIRDIKRT LDA LETIC
C. elegans	LSNKQTS	FVVDD	LEERIR QIKEMRRL VEL FETLF
D. melanogaster	DSDGMRH	FVAHD	LEAKLR RIRQIKRL VDM LDSTM

Figure S1. **Conserved structural elements of the lyspersin DUF2365.** Amino acid sequence alignment of CE1 and CE2 in the lyspersin DUF2365 from different species (InterPro IPR019314). Conserved residues targeted for mutagenesis are highlighted in red. Amino acid numbers are indicated. *H. sapiens*, *Homo sapiens*; *X. tropicalis*, *Xenopus tropicalis*; *D. rerio*, *Danio rerio*; *C. elegans*, *Caenorhabditis elegans*; *D. melanogaster*, *Drosophila melanogaster*.

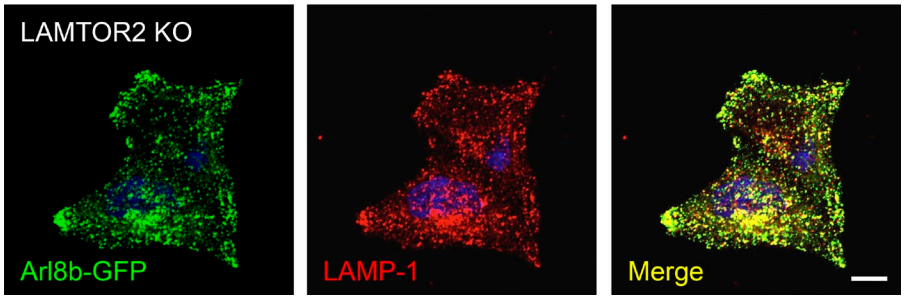


Figure S2. **LAMTOR2 KO redistributes lysosomes toward the cell periphery.** The gene encoding LAMTOR2 was knocked out in HeLa cells using CRISPR/Cas9. Cells were transfected with a plasmid encoding Arl8b-GFP and immunostained with antibodies to GFP and LAMP1. Bar, 10 µm.

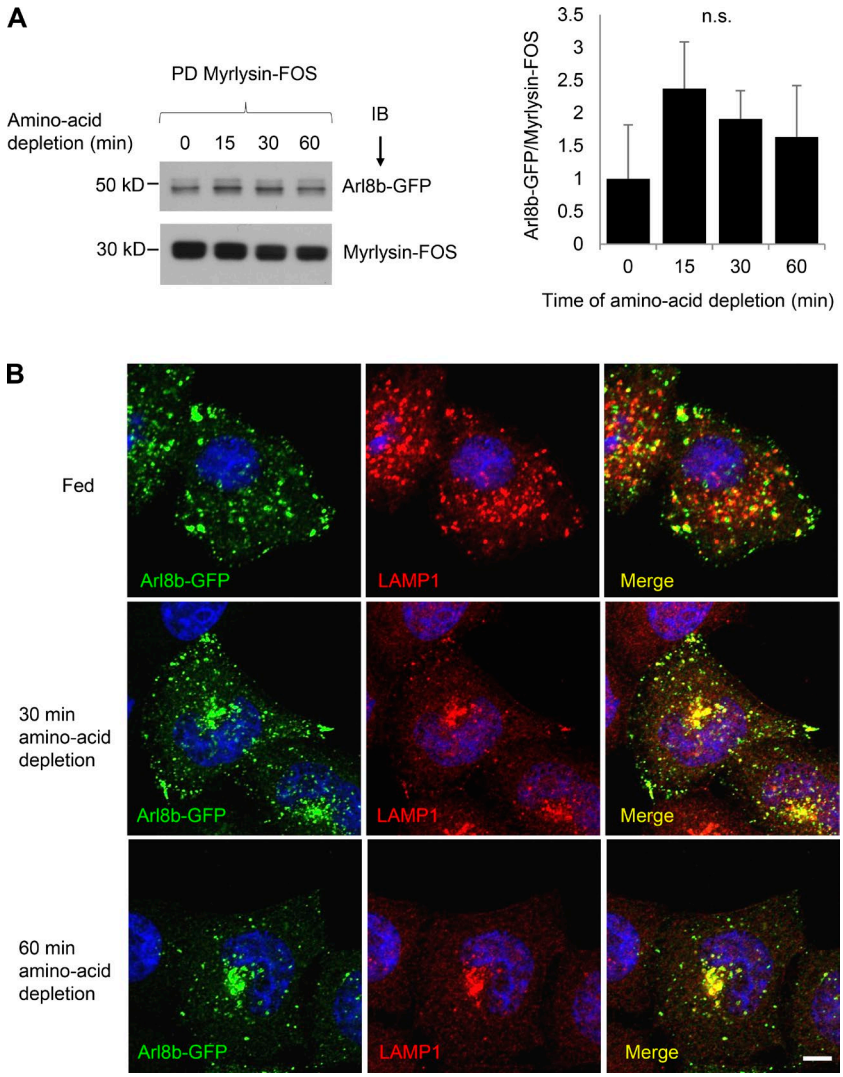
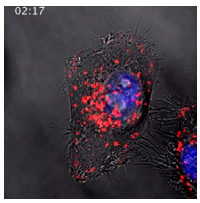
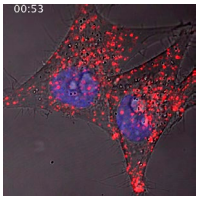


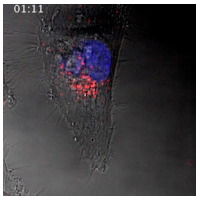
Figure S3. **Arl8b remains associated with lysosomes during amino acid depletion.** (A) Arl8b-GFP was expressed by transfection in myrlysin-KO HeLa cells stably rescued with myrlysin-FOS. Cells were incubated with medium without amino acids for the indicated times and subjected to pull down (PD) with Strep-Tactin beads, followed by immunoblotting (IB) with antibodies to GFP and FLAG. The positions of molecular mass markers (in kilodaltons) are indicated on the left. Bar graphs show the ratios of Arl8b-GFP to myrlysin-FOS quantified by densitometry. Values are the mean ± SD from six independent experiments. n.s., not significant (ANOVA). Note that starvation did not significantly change the association of Arl8b-GFP with myrlysin-FOS. (B) Arl8b-GFP was transiently expressed by transfection in WT HeLa cells and incubated with medium without amino acids for the indicated times. Immunostaining was performed with antibodies to GFP and endogenous LAMP1. Bar, 10 µm. Note that amino acid depletion causes juxtanuclear clustering of lysosomes, but Arl8b remains associated with the clustered lysosomes.



Video 1. **Lysosome movement in control cells.** WT HeLa cells transfected with a plasmid encoding nontargeting shRNA (control) were allowed to internalize dextran–Alexa Fluor 555 for 6 h at 37°C. Cells were chased overnight and analyzed by live-cell imaging with a Zeiss LSM780 confocal microscope. Images were captured with a 63× 1.4 oil objective over a period of 1 min at 1-s intervals. Videos were generated using ImageJ at 12 frames/s.



Video 2. **Lysosome movement in LAMTOR1-KD cells.** WT HeLa cells transfected with a plasmid encoding LAMTOR1 shRNA were analyzed as described in the legend to Video 1.



Video 3. **Lysosome movement in lyspersin-KO cells.** Lyspersin-KO cells were analyzed as described in the legend to Video 1.

Provided online are Tables S1–S3 in Excel showing MS data from affinity purifications using OSF-lyspersin, lyspersin-FOS, and OSF-pallidin as baits. Affinity purifications were performed from stably transfected H4 cells (OSF-lyspersin and lyspersin-FOS) or HeLa cells (OSF-Pallidin).