Goo et al., https://doi.org/10.1083/jcb.201704068

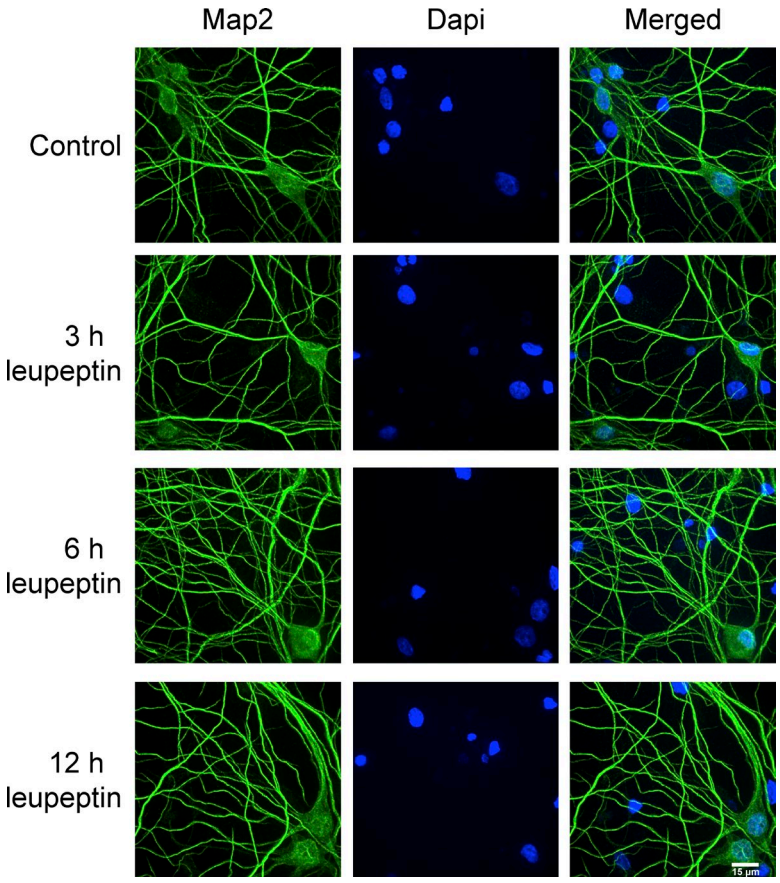


Figure S1. **Cell health is not compromised upon leupeptin treatment.** MAP2 immunostaining and DAPI staining of dissociated hippocampal neuron after 200 µM leupeptin treatment (3, 6, and 12 h).

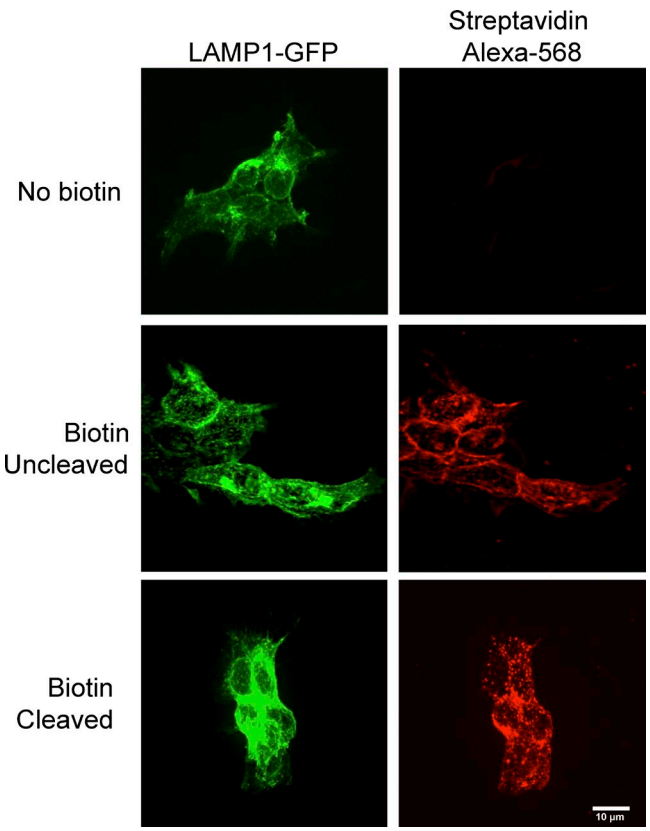


Figure S2. **Biotin-streptavidin staining of internalized membrane proteins in HEK293T cells.** HEK293T cells transfected with LAMP1-GFP, where cells were not treated with biotin (no biotin), not treated with stripping buffer (biotin, uncleaved), or treated with biotin and stripping buffer (biotin, cleaved). Subsequently, internalized biotinylated proteins were labeled with Streptavidin Alexa 568. Uncleaved cells show labeling on the cell membrane, whereas no biotin shows no labeling. Bottom image shows internalized membrane proteins.

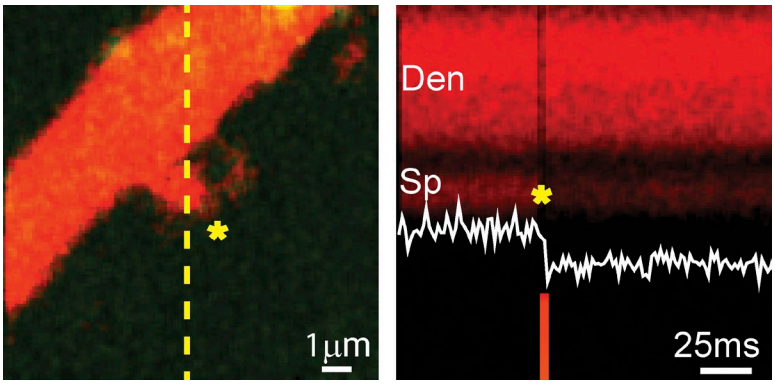
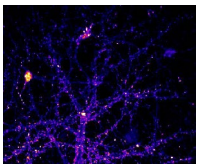
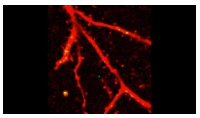


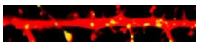
Figure S3. **Calibration of 2P uncaging power.** 2D image of a CA1 pyramidal neuron dendrite (Den; left). Yellow asterisk indicates spine (Sp) where uncaging was performed. Yellow dashed line indicates location of the line scan. Line scan through the spine and adjacent dendrite (right). Line represents red fluorescence profile of the spine, with the asterisk marking where uncaging occurred. Laser power was adjusted to achieve a 30% to 40% decrease in the red fluorescence signal.



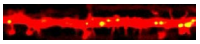
Video 1. **Time-lapse video of LysoTracker-labeled structures trafficking in a hippocampal neuron.** Movie corresponding to image in Fig. 1 B. An image was taken every second for 100 s. Movie plays at five frames per second.



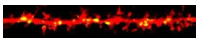
Video 2. **Time-lapse video of hippocampal neuron expressing mCherry and GCaMP3-TRPML1.** Movie corresponding to Fig. 2 (B–D). Movie plays at five frames per second.



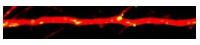
Video 3. **Time-lapse video of LAMP1-GFP–labeled lysosomes in a dendrite under basal conditions.** Movie corresponding to Fig. 3 (D and E). Dissociated hippocampal neuron expressing mCherry and LAMP1-GFP. An image was taken every second for 100 s. Movie plays at five frames per second.



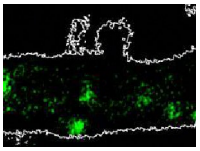
Video 4. **Time-lapse video of LAMP1-GFP–labeled lysosomes in a dendrite after 200 µM leupeptin treatment (3 h).** Movie corresponding to Fig. 3 (D and E). Dissociated hippocampal neuron expressing mCherry and LAMP1-GFP. An image was taken every second for 100 s. Movie plays at five frames per second.



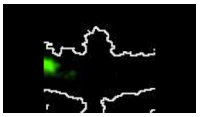
Video 5. **Time-lapse video of LAMP1-GFP–labeled lysosomes in a dendrite after nocodazole treatment.** Movie corresponding to Fig. 4 A. Dissociated hippocampal neuron expressing mCherry and LAMP1-GFP after treatment with 10 µg/ml nocodazole for 1 h. An image was taken every second for 100 s. Movie plays at five frames per second.



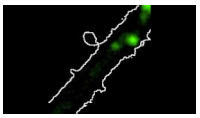
Video 6. **Time-lapse video of LAMP1-GFP–labeled lysosomes in a dendrite after Latrunculin A treatment.** Movie corresponding to Fig. 4 A. Dissociated hippocampal neuron expressing mCherry and LAMP1-GFP after treatment with 20 µM Latrunculin A for 10 min. An image was taken every second for 100 s. Movie plays at five frames per second.



Video 7. **Time-lapse video of LAMP1-GFP–labeled lysosomes in an organotypic hippocampal slice.** Rat organotypic hippocampal slice expressing dsRed and LAMP1-GFP. Movie indicates lysosomes move bidirectionally and a subset of lysosomes abruptly stop at the base of a dendritic spines. Movie plays at five frames per second.



Video 8. **Time-lapse video of LAMP1-GFP–labeled lysosomes in an organotypic hippocampal slice before and after glutamate uncaging.** Movie corresponding to Fig. 6 F. Rat organotypic hippocampal slice expressing dsRed and LAMP1-GFP. Images were taken 4 to 6 min and 0 to 2 min before stimulus. A single spine was stimulated with MNI-glutamate (2.5 mM MNI-glutamate) using 0.5-ms pulses at 1 Hz for 1 min. Then images were taken 0 to 2 min and 5 to 7 min after stimulation. Images were taken every second. Movie plays at five frames per second.



Video 9. **Time-lapse video of LAMP1-GFP–labeled lysosomes in an organotypic hippocampal slice before and after mock uncaging.** Movie corresponding to Fig. 6 G. Rat organotypic hippocampal slice expressing dsRed and LAMP1-GFP. Images were taken 4 to 6 min and 0 to 2 min before stimulus. A single spine was "mock stimulated" (no MNI-glutamate) using 0.5-ms pulses at 1 Hz for 1 min. Then images were taken 0 to 2 min and 5 to 7 min after stimulation. Images were taken every second. Movie plays at five frames per second.