Schulze et al., http://www.jcb.org/cgi/content/full/jcb.201306140/DC1

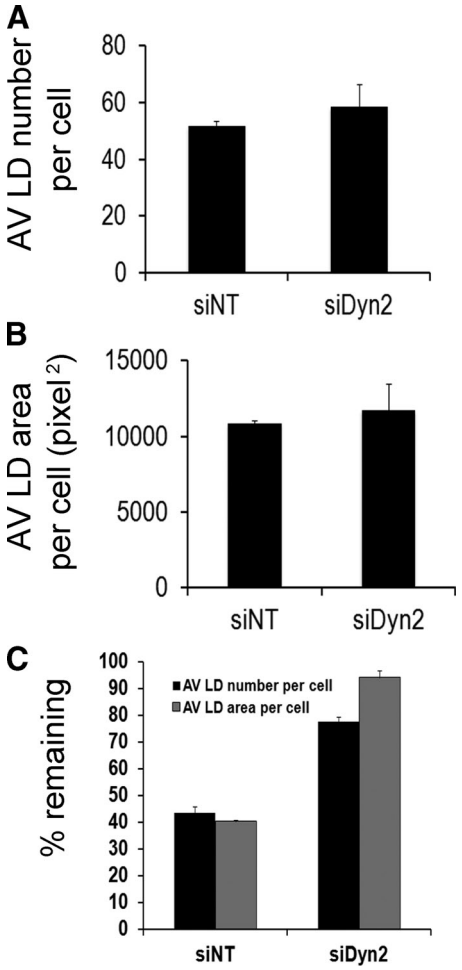


Figure S1. **Dyn2 depletion does not affect oleate loading of Hep3B hepatocytes.** (A and B) Hep3B cells were treated with nontargeting (siNT) siRNA or siRNA to deplete Dyn2 (siDyn) and then loaded with 150 µM oleate overnight. LDs were visualized by immunofluorescence using Oil Red O stain. Lipid loading was assessed by quantitation of the average LD number and size from four independent experiments and the data are represented as mean ± SE. No significant difference was observed in the LD loading capacity of control and Dyn2-depleted cells. (C) The percentage of LD breakdown was analyzed by comparing the average LD number and area obtained from three different experiments in control and Dyn2-depleted cells. The percentage of the remaining lipid was calculated as the amount of LDs after starvation divided by the amount of LDs after loading. Data are plotted as the number and area of LDs that remain after the above-mentioned calculation.

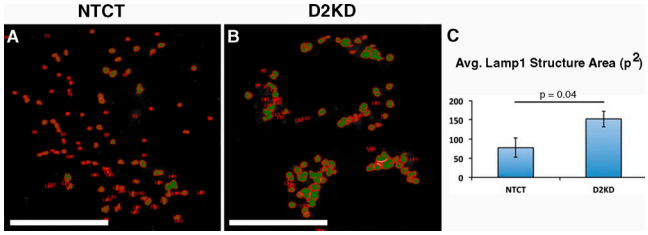


Figure S2. **Dynamin knockdown is associated with enlarged Lamp1-positive phagolysosomal structures.** Hep3B cells treated with either a nontargeting control siRNA (A, NTCT) or an siRNA targeting human Dyn2 (B, D2KD) were fixed and stained with an antibody specific for LAMP1. After Dyn2 knockdown, a juxtanuclear aggregation and enlargement of the LAMP1-positive compartment is observed (B). The sizes of LAMP1-positive structures (A and B, green segmentation label) were measured using an auto-segmentation tool within iVision software (red demarcation). (C) Quantitation of the average LAMP1 structure area (in pixels^2) taken from measurements of >65 cells in each treatment group over four independent experiments. The data are represented as mean ± SE. *, P = 0.04. Bars, 20 µM.

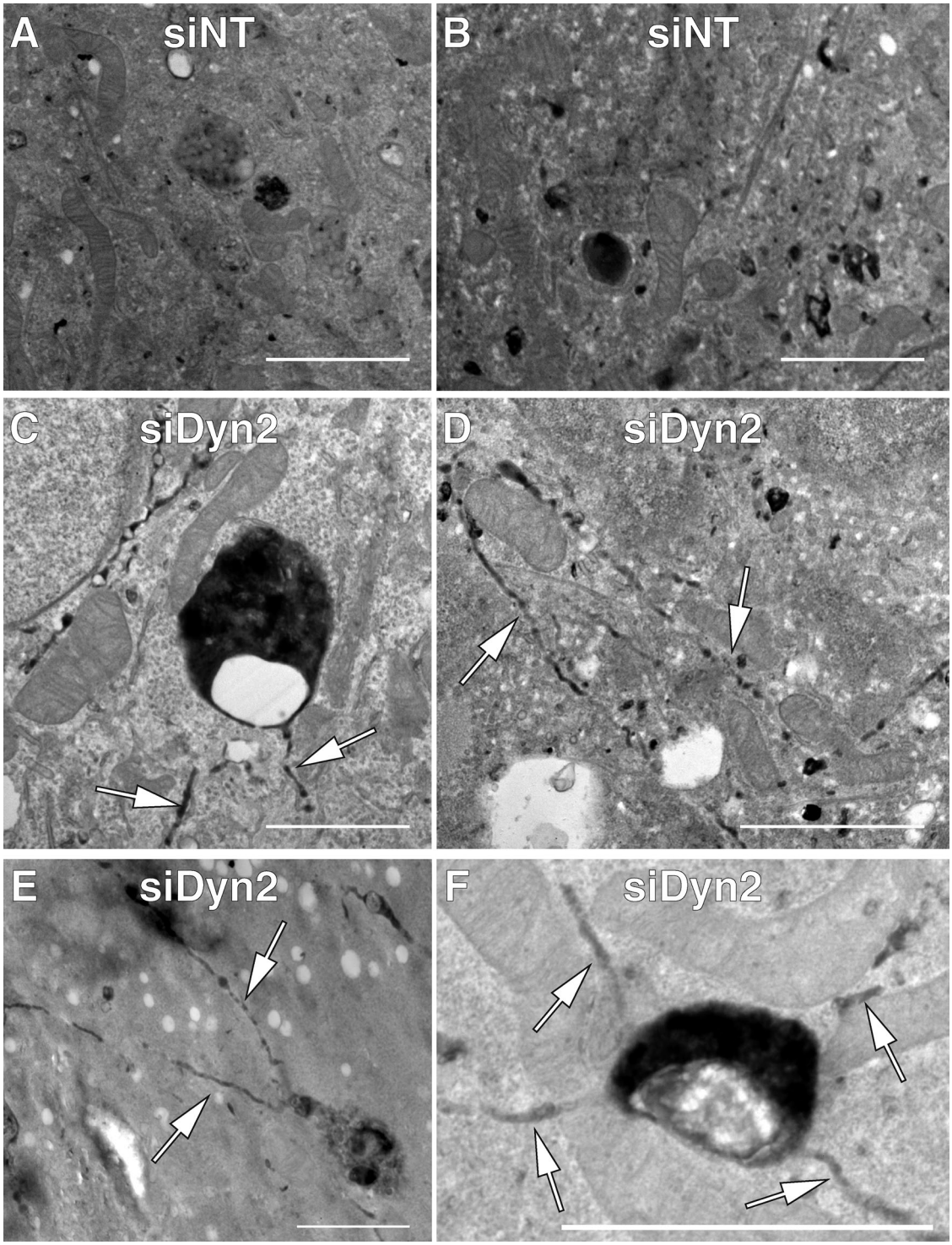


Figure S3. **Endocytic uptake of HRP confirms that the Dyn2-induced tubules extend from components of the late endosome pathway.** Hep3B hepatocytes were treated with nontargeting (siNT) siRNA (A and B) or siRNA to deplete Dyn2 (siDyn) (C–F) and then loaded with 150 µM oleate overnight. Horseradish peroxidase (HRP) was added as a fluid phase marker for 1 h and chased in media lacking HRP for 150 min to allow transport from the early to late compartments of the endocytic pathway. Thick EM sections were treated with diaminobenzidine (DAB), which is oxidized into an insoluble precipitate by HRP, for visualization of HRP-loaded organelles. DAB-treated thick sections reveal electron dense lysosomes/autolysosomes in the Dyn2 knockdown cells that possess numerous long constricted tubules (indicated by arrows) similar to those displayed in Fig. 5.

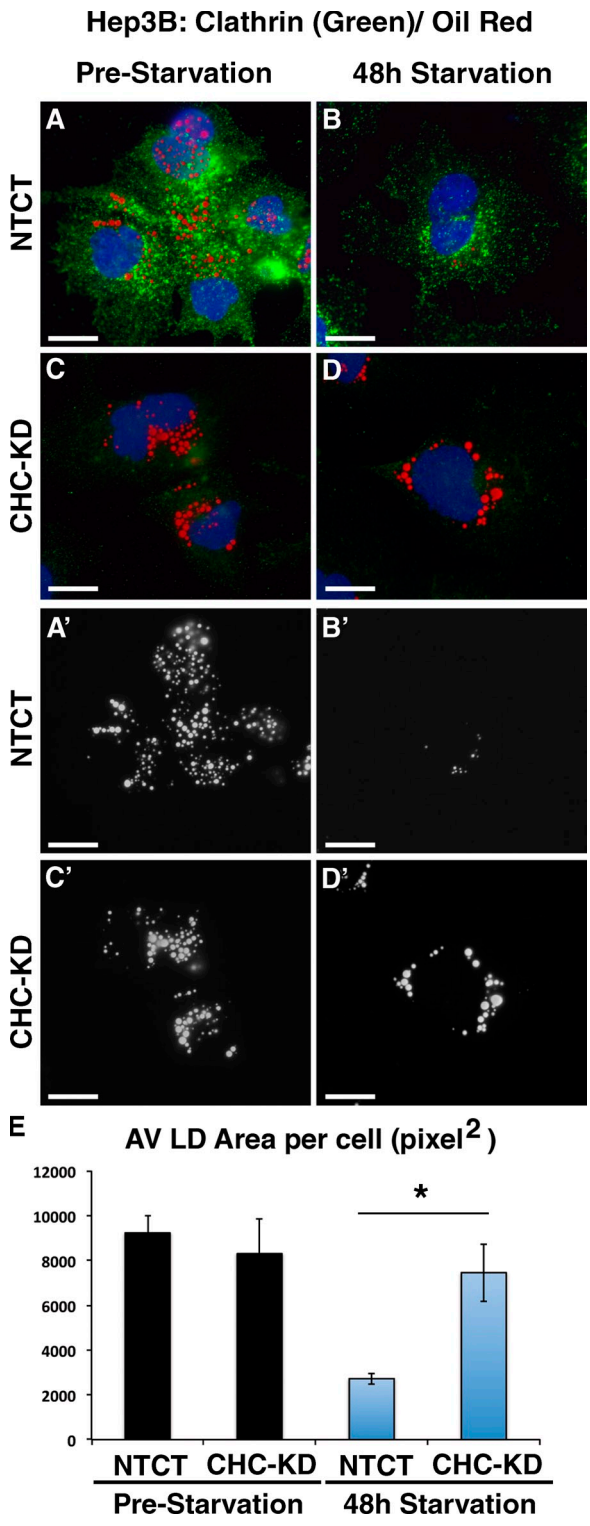
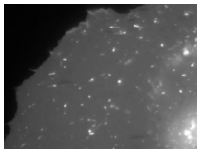
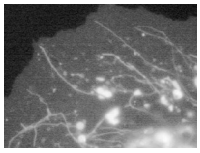


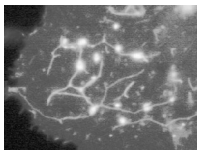
Figure S4. **Knockdown of clathrin heavy chain (CHC) interferes with starvation-induced LD breakdown in Hep3B hepatocytes.** Hep3B cells, treated with a nontargeting control siRNA (NTCT) or siRNA targeting the CHC (CHC-KD) were loaded with 150 µM oleate overnight and starved for 48 h in medium containing 0.1% FBS. (A–D′) LDs were visualized by Oil Red O staining (A–D, red channel and panels A′–D′). CHC knockdown was verified through immunofluorescence staining (A–D, green channel). (E) Quantitation of the average LD area (in pixels^2) per cell from four independent experiments. The data are represented as mean ± SE. *, P = 0.03. Bars, 20 µM.



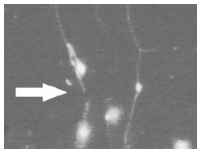
Video 1. **Live-cell microscopy of a Hep3B hepatocyte expressing LAMP1-mCherry and starved for 1 h 30 min in HBSS + Ca^{2+}/Mg^{2+} + 10 mM Hepes.** Images were acquired every 20 s using epifluorescence microscopy (Axio Observer; Carl Zeiss). Movie is displayed at 5 fps and represents 10 min total elapsed time.



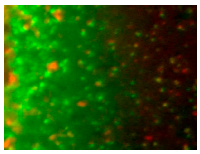
Video 2. **Live-cell microscopy of a Hep3B hepatocyte expressing LAMP1-mCherry and starved for 2 h in HBSS + Ca^{2+}/Mg^{2+} + 10 mM Hepes, followed by 30 min treatment with 40 µM Dynasore.** Images were acquired every 20s using epifluorescence microscopy (Axio Observer; Carl Zeiss). Movie is displayed at 5 fps and represents 10 min total elapsed time. Contrast was enhanced by histogram normalization in ImageJ (National Institutes of Health) for better visualization of the LAMP1-positive tubules.



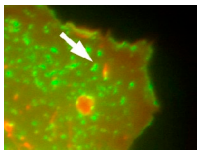
Video 3. **Live-cell microscopy of a Hep3B hepatocyte expressing LAMP1-mCherry and starved for 2 h in HBSS + Ca^{2+}/Mg^{2+} + 10 mM Hepes, followed by 30 min treatment with 40 µM Dynasore and subsequent drug washout and replacement with media containing 10% FBS.** Notice the marked reduction in overall number and size of LAMP1-positive tubules over time after Dynasore washout. Images were acquired every 20 s using epifluorescence microscopy (Axio Observer; Carl Zeiss). Movie is displayed at 10 fps and represents time elapsed between 10 and 45 min after drug washout. Contrast was enhanced by histogram normalization in ImageJ (National Institutes of Health) for better visualization of the LAMP1-positive tubules.



Video 4. **Live-cell microscopy of Hep3B hepatocyte expressing LAMP1-mCherry and starved for 2 h in HBSS + Ca^{2+}/Mg^{2+} + 10 mM Hepes, followed by 30 min treatment with 40 µM Dynasore and subsequent drug washout and replacement with media containing 10% FBS.** Movie shows a high-magnification crop of a LAMP1-positive tubule after Dynasore washout. Note the formation of a prominent varicosity along the tubule length from which the scission and release of a nascent protolysosome occurs (arrow). Images were acquired every 20 s using epifluorescence microscopy (Axio Observer; Carl Zeiss). Movie is displayed at 1 fps and represents time elapsed between 81′ 40″ and 84′ 40″ after drug washout. Contrast was enhanced by histogram normalization in ImageJ (National Institutes of Health) for better visualization of the LAMP1-positive tubules.



Video 5. **Live-cell microscopy of Hep3B hepatocytes expressing Dyn2-GFP and LAMP1-mCherry under resting conditions.** Note the extensive associations between Dyn2 and LAMP1 throughout the cell. Images were acquired every 5 s using epifluorescence microscopy (Axio Observer; Carl Zeiss). Movie playback is at 10× speed.



Video 6. **Hep3B hepatocyte expressing Dyn2-GFP and LAMP1-mCherry after 7 h 20 min starvation in media containing 0.1% FBS.** Note the colocalization of Dyn2 and LAMP1 at the base of the tubule, which undergoes a clear scissile event. Images were acquired every 5 s using epifluorescence microscopy (Axio Observer; Carl Zeiss). Movie playback is at 10× speed.