Johnson et al., http://www.jcb.org/cgi/content/full/jcb.201507112/DC1

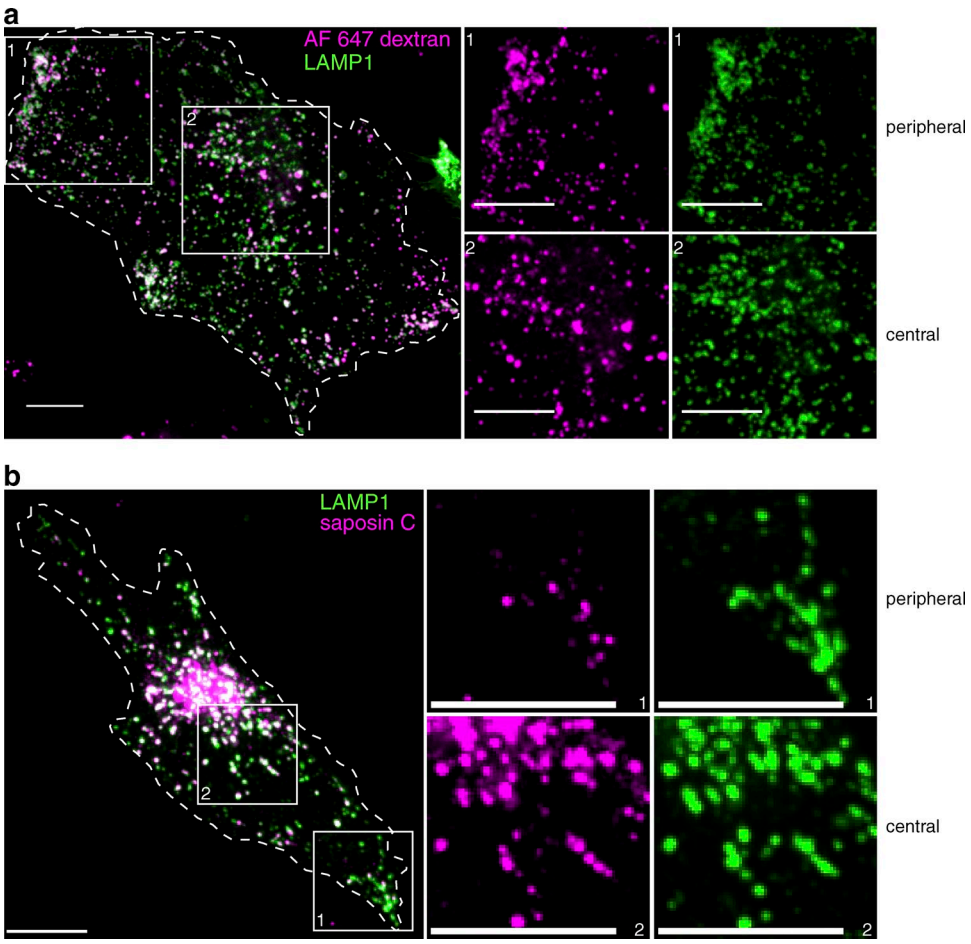

Figure S1. **LAMP1 and saposin C distribution in lysosomes.** (a) Lysosomes in HeLa cells were loaded with Alexa Fluor 647–dextran (magenta) and then transfected with LAMP1-RFP (green), fixed, and imaged by confocal microscopy. Overlap was 90 ± 1% near the nucleus and 82 ± 2% at the cell periphery. (b) Cells were fixed, permeabilized, and immunostained for LAMP1 and saposin C and then imaged by confocal microscopy. Overlap was 79 ± 2% near the nucleus and 64 ± 4% in the periphery. The cells of interest are outlined with dashed lines. The numbered white boxes indicate the regions magnified in the right-hand insets. Bars, 10 µm.

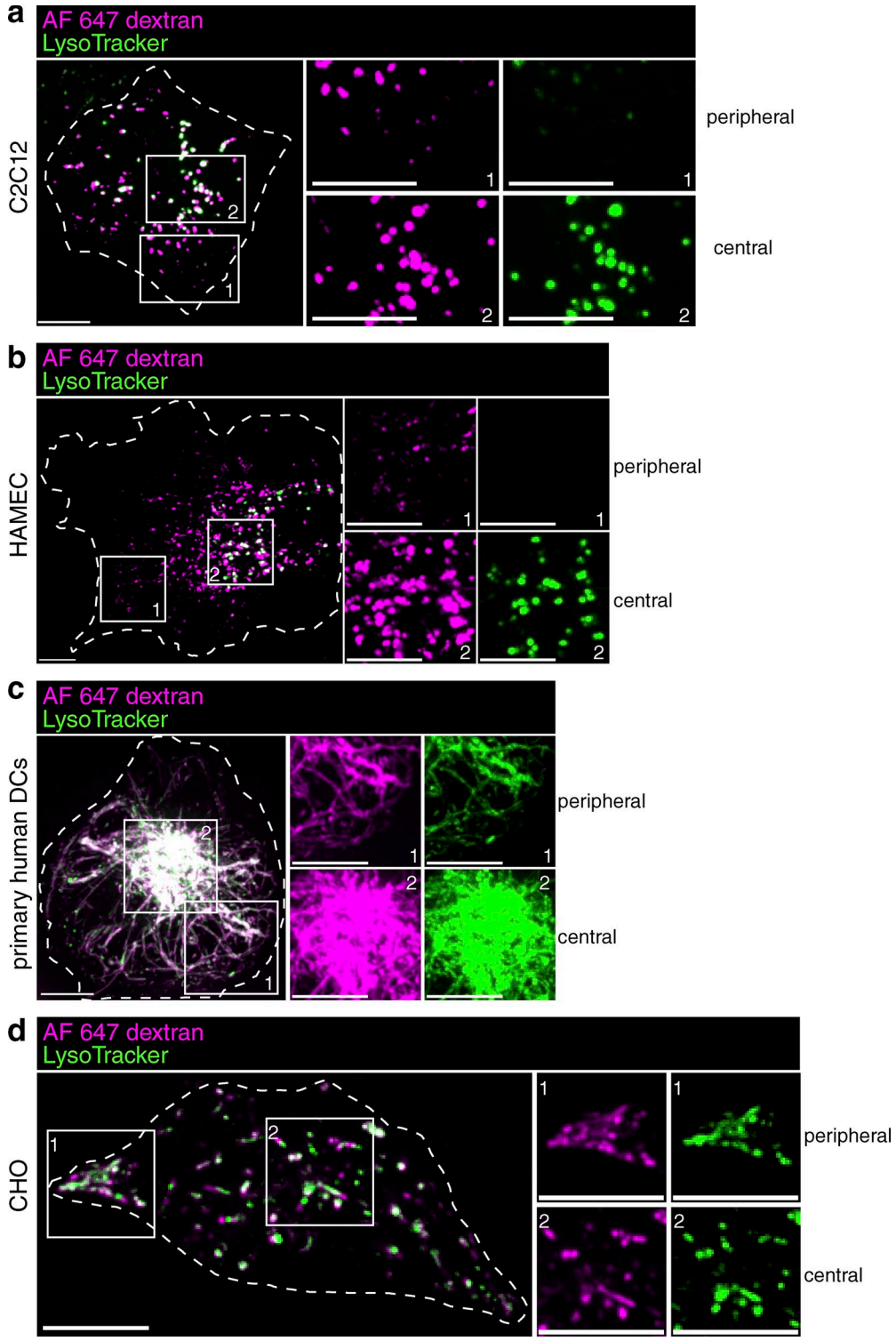

Figure S2. **Lysosomal pH heterogeneity is dependent on cell type.** The lysosomes of C2C12 murine myoblasts (a), human adipose microvascular endothelial cells (HAMEC; b), primary human dendritic cells (DCs; c), and CHO cells (d) were loaded with Alexa Fluor 647–dextran (magenta) and then loaded with the acidotropic dye LysoTracker (green) and imaged by confocal microscopy. The cells of interest are outlined with dashed lines. The numbered white boxes indicate the regions magnified in the right-hand insets. Bars, 10 µm.

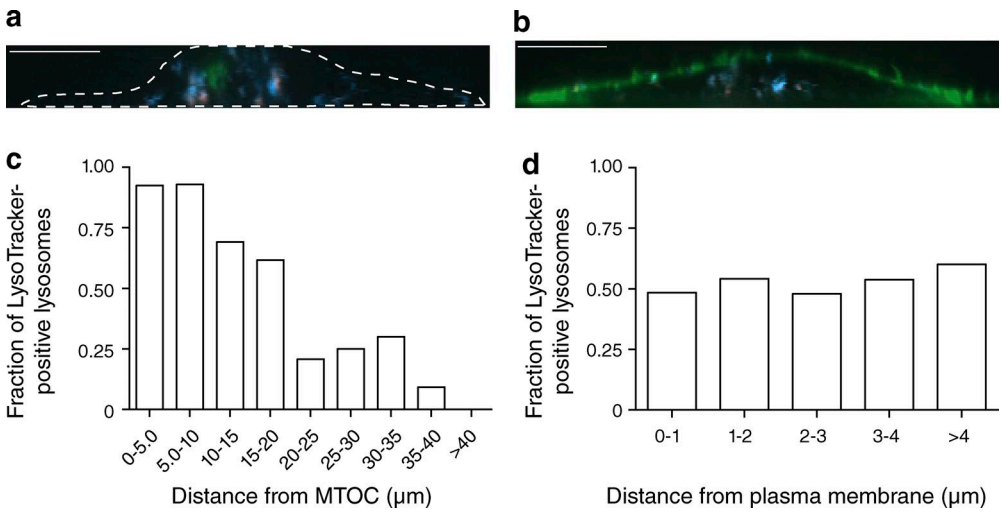

Figure S3. **Localization of lysosomes.** (a) Lysosomes of HeLa cells were loaded with Alexa Fluor 647–dextran (blue), transfected with pericentrin (green), and chased overnight. The next day, cells were loaded with 200 nM LysoTracker (red) for 5 min, washed, and imaged immediately. (b) Lysosomes were loaded with Alexa Fluor 647–dextran (blue) and chased overnight. The next day, cells were loaded with LysoTracker (red), washed, and then labeled with 10 µg/ml Oregon green 488–wheat germ agglutinin (green) at 4°C to highlight the plasmalemma and imaged immediately. (c and d) Fraction of LysoTracker-positive lysosomes as a function of their distance from either the MTOC (marked by pericentrin; c) or the plasma membrane (marked by wheat germ agglutinin; d). Bars, 10 µm.

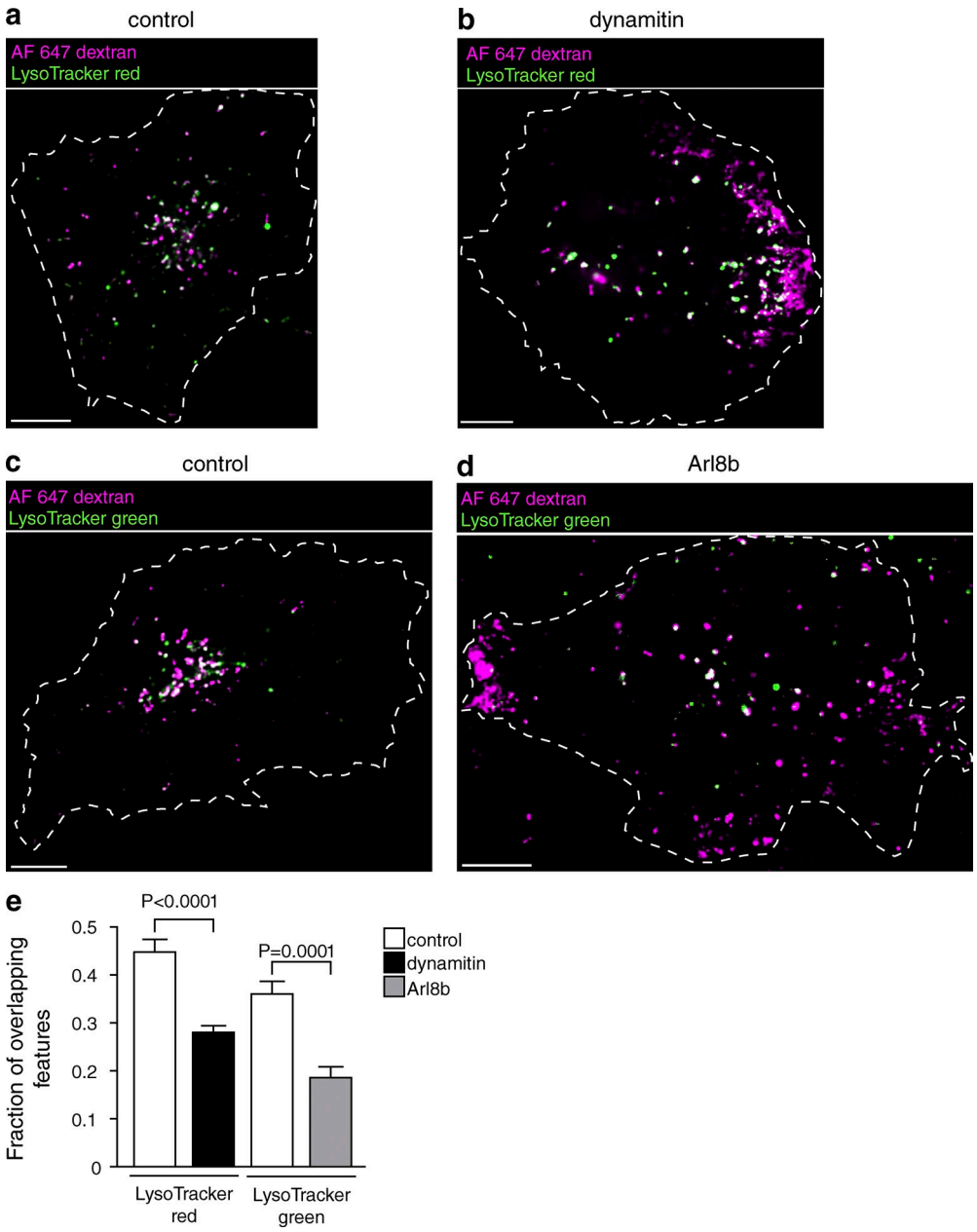

Figure S4. **Lysosomes displaced to the cell periphery by expression of Arl8b or dynamitin accumulate reduced levels of LysoTracker.** (a–d) The lysosomes of HeLa cells were loaded with Alexa Fluor 647–dextran (magenta) and then mock transfected (control) or transfected with dynamitin-GFP or Arl8b-mCherry as indicated. Lysosomes were then loaded with the acidotropic dye LysoTracker red or green (both shown as green in the figures) and imaged by confocal microscopy. The cells of interest are outlined with dashed lines. Bars, 10 µm. (e) Fraction of overlapping features (dextran-positive lysosomes containing LysoTracker) for control, dynamitin-, and Arl8b-expressing cells. Error bars represent SEM (n = 9 cells each from three independent experiments).

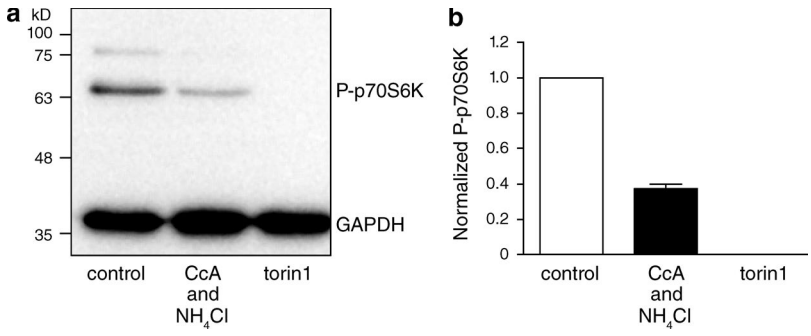

Figure S5. **Alkalinizing lysosomes decreases mTORC1 activity.** HeLa cells were left untreated, treated with 1 µM V-ATPase inhibitor CcA and 10 µM weak base NH_4Cl to alkalinize lysosomes, or treated with 1 µM mTORC1 inhibitor torin1 for 1 h. (a) Cell lysates were separated by SDS-PAGE, immunoblotted, and probed with antiphosphorylated p70 S6 kinase and anti-GAPDH. (b) Bands from four similar experiments were quantified by densitometry and normalized to control to enable comparison among blots. Error bar represents SEM (n = 4 independent blots).