Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figure and Video Legends

Figure S1. Phagocytosis of PS-coated beads does not support cell survival or reactivate mTORC1 in macrophages cultured in amino acid-free media. (A) Graph shows cell fates of J774.1 macrophages cultured under the indicated conditions including full media, and amino acid (aa)-free media in the presence or absence of PS-coated latex beads or apoptotic cell corpses. 'Single' refers to individual macrophages without phagocytic engulfment. For Single in full media n=60; Single aa-free media n=60; Engulfed PS-Beads n=120; Engulfed Corpses n=104. *p<0.001; **p<0.001 (chi-square). Data are from 3 independent experiments. (B) PS-coated beads label with Annexin V. Control beads, PS-coated beads, and apoptotic cell corpses were incubated with fluorescent Annexin V-FITC (green) to label PS and examined by microscopy for green fluorescence (top panels), or by flow cytometry for green fluorescence (for Control and PS-labeled beads). (C) *FIP200* knockdown blocks autophagy induction in MCF10A cells. Western blot shows inhibition of LC3-II accumulation (indicated by LC3-II/LC3-I ratio) in starved cells by *FIP200* siRNA. Cells were starved in HBSS for 6 hours. (D) Western blot shows pS6K reactivation in J774.1 macrophages cultured in aa-free media by apoptotic corpse engulfment but not by engulfment of PS-coated beads. (E) mTOR does not recruit to phagosomes containing engulfed PS-coated beads. Image panels show immunofluorescence staining for mTOR (green) and Lamp1 (red). mTOR does not recruit to phagosome (arrow) (0% of PS-bead phagosomes recruited mTor, n=77).

Figure S2. Apoptotic cell phagocytosis in full media versus starvation media. Western blot shows levels of phosphorylation of S6K1 threonine 389 (pS6K1) in J774.1 macrophages cultured under the indicated conditions, including full or amino acid (aa)-free media, with or without apoptotic corpses, latex beads, or the mTOR inhibitor Torin1 (500nM). Torin1 was added to cultures 1 hour prior to cell lysis. Note that pS6K1 is not increased by the engulfment and digestion of apoptotic corpses when macrophages are cultured in full media. Note also that apoptotic cell corpses expressed H2B-mCherry, which was degraded into free mCherry by macrophages after engulfment.

Figure S3. Amino acid incorporation from apoptotic corpses into macrophage-expressed GFP. Blots show GFP immunoprecipitated from J774.1 macrophages cultured under the indicated conditions, including amino acid (aa)-free or full media, in the presence or absence of corpses with radio-labeled (35S) cysteine and methionine, and the actin inhibitor Latrunculin B that blocks phagocytosis. '35S' indicates GFP with incorporated radio-labeled cysteine and methionine as indicated by phosphor-imaging. 'Filtered media' is a control for leakage of amino acids from apoptotic cells into media during the assay, and refers to culture media that was incubated with radiolabeled corpses for the duration of the assay, and then filtered through 0.45μm pore filter and used for the assay that is shown. The amount of 35S incorporation into GFP from the 'filtered media' experiment indicates amino acid recovery from media rather than phagocytosed corpses. Note that 35S incorporation in the Latrunculin B-treated culture in the presence of

corpses is similar to ‘filtered media’, indicating the amount of amino acid uptake that occurs in an actin-independent manner and that does not result from phagocytosis. Note that macrophages recover amino acids from apoptotic corpses in both aa-free and full media, and use recovered amino acids in protein synthesis.

Figure S4. RagC localizes to corpse and latex bead-containing vacuoles in amino acid-free conditions. Image panels show immunofluorescence staining of MCF10A cells for RagC (green, top image) and Lamp1 (red, second image), merged fluorescent channels (DAPI-stained nuclei are blue) and DIC. Arrows indicate vacuoles containing a latex bead and a cell corpse.

Figure S5. Entotic corpses fuse with lysosomes during vacuole shrinkage. (A) Entotic vacuole, labeled with Lamp1-GFP (green), accumulates 10kD red fluorescent dextran (arrowhead) from media over the course of 10hr, even as the vacuole shrinks in size. Note this example is from the same experiment as in Figure 3E, but shows a smaller vacuole, representing a different stage of shrinkage. 10 out of 10 total vacuoles imaged accumulated red dextran similarly over time. (B) Two control entotic vacuoles containing cell corpses (arrows) at different stages of degradation (corpse 1, left; corpse 2, right) fuse with Lamp1-GFP-labeled lysosomes (green) after cell fusion initiated by PEG treatment. Arrowheads indicate Lamp1-GFP accumulated at entotic vacuole after cell fusion. Time indicates minutes after cell fusion. 10 out of 10 entotic vacuoles in

control cells recruited Lamp1-GFP within 3 hours after cell fusion. See Supplemental Video 4. (C) An entotic vacuole in a Brefeldin A-treated culture fuses with Lamp1-GFP-labeled lysosomes (green) after PEG-initiated cell fusion. Arrowhead indicates Lamp1-GFP accumulation at entotic vacuole. Time indicates minutes after cell fusion. Note this example is from the same experiment as the vacuole shown in Figure 3F, but shows a larger vacuole, representing a different stage of shrinkage. 10 out of 10 entotic vacuoles in cells treated with Brefeldin A recruited Lamp1-GFP within 3 hours after cell fusion. See Supplemental Video 5. (D) An entotic vacuole in a Torin1-treated culture fuses with Lamp1-GFP-labeled lysosomes (green) after PEG-initiated cell fusion. Arrowhead indicates Lamp1-GFP accumulation at entotic vacuole. Time indicates minutes after cell fusion. Note this example is from the same experiment as the vacuole shown in Figure 4E, but shows a smaller vacuole, representing a different stage of shrinkage. 10 out of 10 entotic vacuoles in cells treated with Torin1 recruited Lamp1-GFP within 3 hours after cell fusion. See Supplemental Video 9.

Figure S6. mTOR inhibition slows entotic vacuole shrinkage. (A) Entotic vacuole shrinkage in cells cultured in aa-free media quantified by time-lapse microscopy. Vacuole areas were quantified every hour for 10 hours. For control n=57; Torin1 n=56. Bars show SEM. $p<0.02$ for all timepoints from 2 to 10 hours (Students t-test). Data are from three independent experiments (B) Entotic vacuole shrinkage in cells cultured in full media. For control n=54; Torin1 n=55. Bars show SEM.

$p<0.02$ for all timepoints from 2 to 10 hours (Students t-test). Data are from three independent experiments.

Figure S7. Rab-7 localizes to entotic vacuoles during shrinkage. Entotic vacuoles of different sizes, representing different stages of shrinkage (large vacuole in top row, small vacuole in bottom row) show localization of endogenous Rab-7 (red) to the vacuole membrane marked by Lamp1 (green). Images show confocal microscopic images of immunofluorescence (IF) staining. DAPI-stained nuclei are shown in blue. 11 out of 11 entotic vacuoles that were examined by IF showed endogenous Rab-7 colocalizing with Lamp1 on the vacuole.

Figure S8. Tubulation from entotic vacuoles is not required for shrinkage and is mTOR-independent. (A) Entotic vacuole (asterisk) labeled with Lamp1-GFP (green) containing an H2B-mCherry-expressing cell corpse (red) exhibits tubulation (arrow). Panels show overlays of two z-plane confocal microscopic images. (B) Tubulation from entotic vacuoles quantified over time is uninhibited by Torin1 treatment but blocked by treatment with Nocodazole. Bars indicate means and SD; each individual data point is also shown. (C) Nocodazole treatment has no effect on entotic vacuole shrinkage. Bars show fold change in vacuole area over 10 hours; error bars depict SEM from three independent experiments. Total cell number analyzed for Control n=60; Nocodazole n=68.

Supplemental Video 1. Entotic vacuole undergoes fission. Entotic vacuole harboring H2B-mCherry-expressing cell corpse undergoes fission as corpse is degraded. Video shows confocal time-lapse analysis of MCF10A cells. Left image panel shows DIC from a single x-y plane, right image panel shows mCherry fluorescence (red) as a maximum projection. Acquisition times are shown as minutes (min). Note engulfing cell also expresses H2B-mCherry and is binucleate.

Supplemental Video 2. Apoptotic cell phagosome undergoes fission. Apoptotic cell phagosome undergoes fission as corpse is degraded. Video shows confocal time-lapse analysis of J774.1 macrophage phagocytosing an apoptotic cell expressing H2B-mCherry. Left image panel shows DIC and mCherry fluorescence (red) from a single x-y plane, right image panel shows mCherry fluorescence (red) as a maximum projection. White outline shows area of macrophage; video frames were aligned by tracking the movement of the engulfing cell. Times are shown as minutes (min).

Supplemental Video 3. Entotic vacuole undergoes fission into lysosome network. Video shows confocal time-lapse analysis of Lamp1-GFP-expressing MCF10A cell (green) harboring an entotic vacuole with an H2B-mCherry-expressing corpse (red). Times are shown as minutes (min). As entotic vacuole undergoes fission, the Lamp1-GFP-labeled lysosomes in the engulfing cell become labeled with mCherry.

Supplemental Video 4. Lysosomes fuse to entotic vacuoles. Two control entotic vacuoles containing cell corpses (arrows) at different stages of degradation (corpse 1, top; corpse 2, bottom) fuse with Lamp1-GFP-labeled lysosomes (green) after cell fusion initiated by PEG treatment. Arrows follow entotic vacuole in merged image. Time indicates minutes after cell fusion. 10 out of 10 entotic vacuoles in control cells recruited Lamp1-GFP within 3 hours after cell fusion.

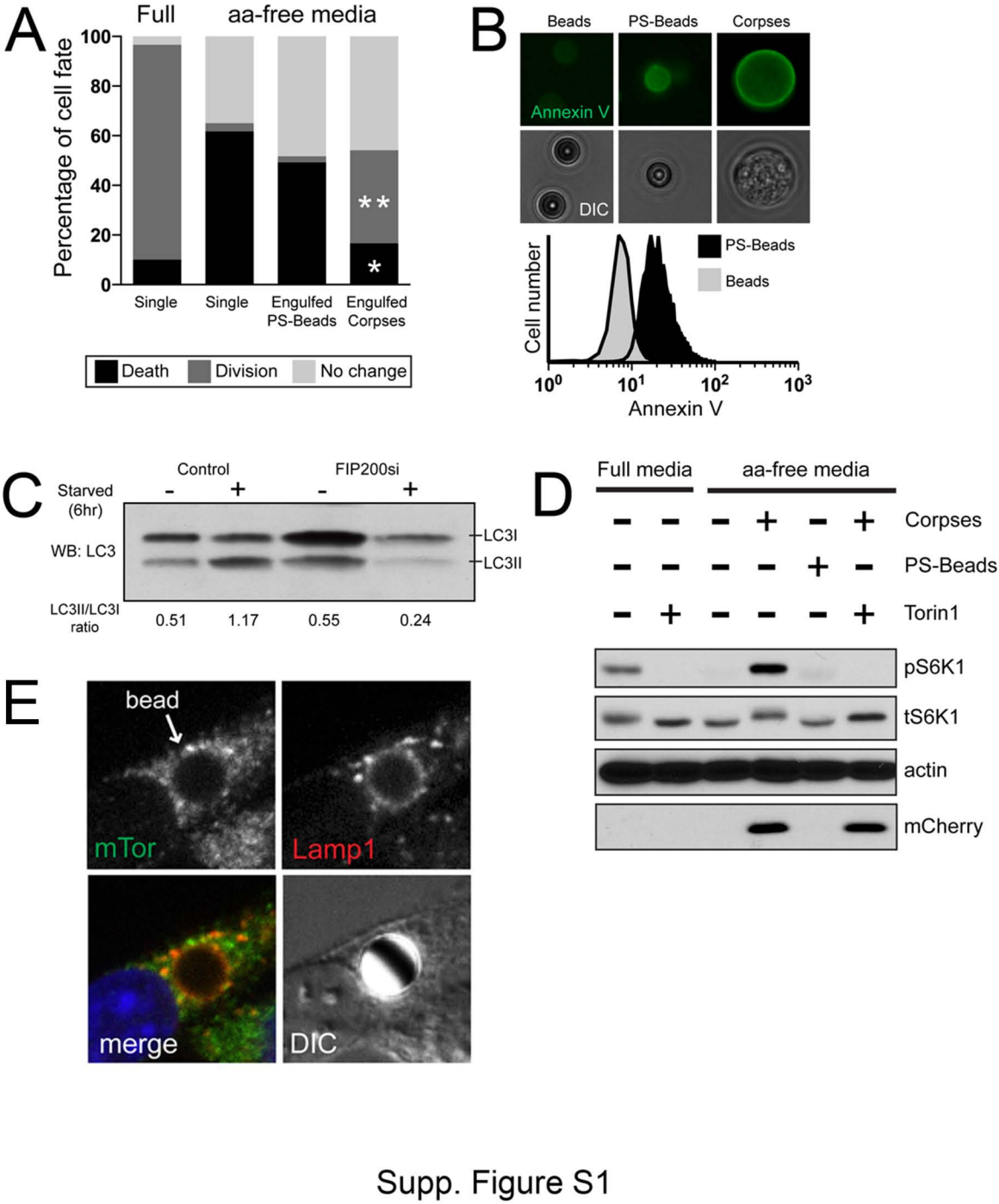
Supplemental Video 5. Lysosomes fuse to entotic vacuoles in Brefeldin A-treated cells. Two entotic vacuoles in containing cell corpses (arrows) in Brefeldin A-treated cells (corpse 1, top; corpse 2, bottom) fuse with Lamp1-GFP-labeled lysosomes (green) after cell fusion initiated by PEG treatment. Arrows follow entotic vacuole in merged image. Time indicates minutes after cell fusion. 10 out of 10 entotic vacuoles in Brefeldin A-treated cells recruited Lamp1-GFP within 3 hours after cell fusion.

Supplemental Video 6. Entotic vacuole exhibits tubulation. Video shows confocal time-lapse analysis of entotic vacuole in Lamp1-GFP-expressing cell (green) harboring an H2B-mCherry-expressing corpse (red). Images show maximum projections of merged green and red fluorescence acquired every 5.5 seconds through the entire z-stack covering the vacuole, at 0.5 micron steps. Time is shown as seconds (sec).

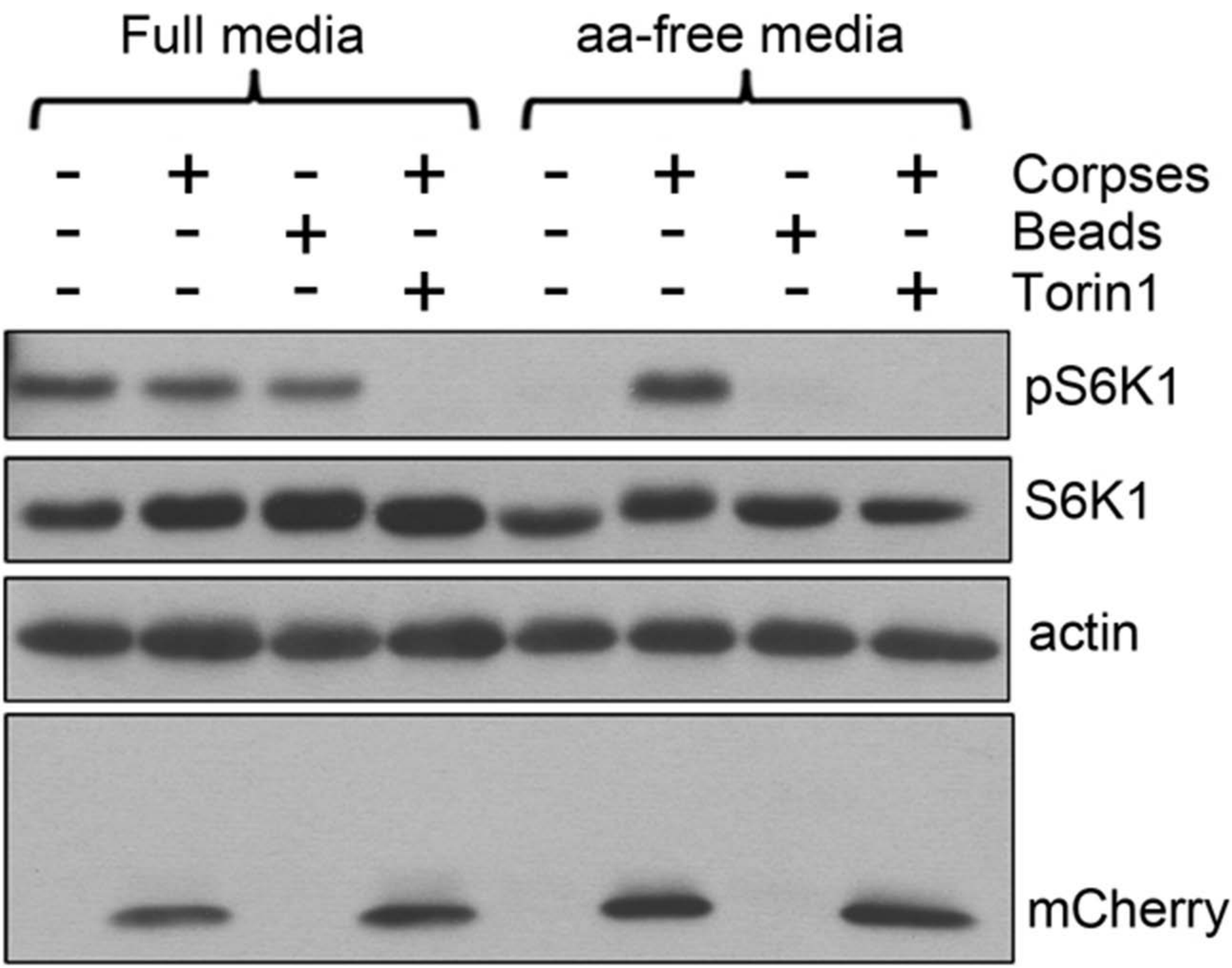
Supplemental Video 7. Entotic corpse degradation and vacuole shrinkage. Video shows entotic corpse expressing H2B-mCherry and entotic vacuole shrinking in a control MCF10A culture. Left image panel shows merged DIC and mCherry fluorescence (red), right image panel shows mCherry fluorescence (red). Times are shown as hours (hr).

Supplemental Video 8. mTOR inhibition slows entotic vacuole shrinkage. Video shows entotic corpse expressing H2B-mCherry and entotic vacuole shrinking in a Torin1-treated MCF10A culture. Left image panel shows merged DIC and mCherry fluorescence (red), right image panel shows mCherry fluorescence (red). Times are shown as hours (hr).

Supplemental Video 9. Lysosomes fuse to entotic vacuoles in Torin1-treated cells. Two entotic vacuoles in containing cell corpses (arrows) in Torin1-treated cells (corpse 1, top; corpse 2, bottom) fuse with Lamp1-GFP-labeled lysosomes (green) after cell fusion initiated by PEG treatment. Arrows follow entotic vacuole in merged image. Time indicates minutes after cell fusion. 10 out of 10 entotic vacuoles in Torin1-treated cells recruited Lamp1-GFP within 3 hours after cell fusion.



Supp. Figure S1



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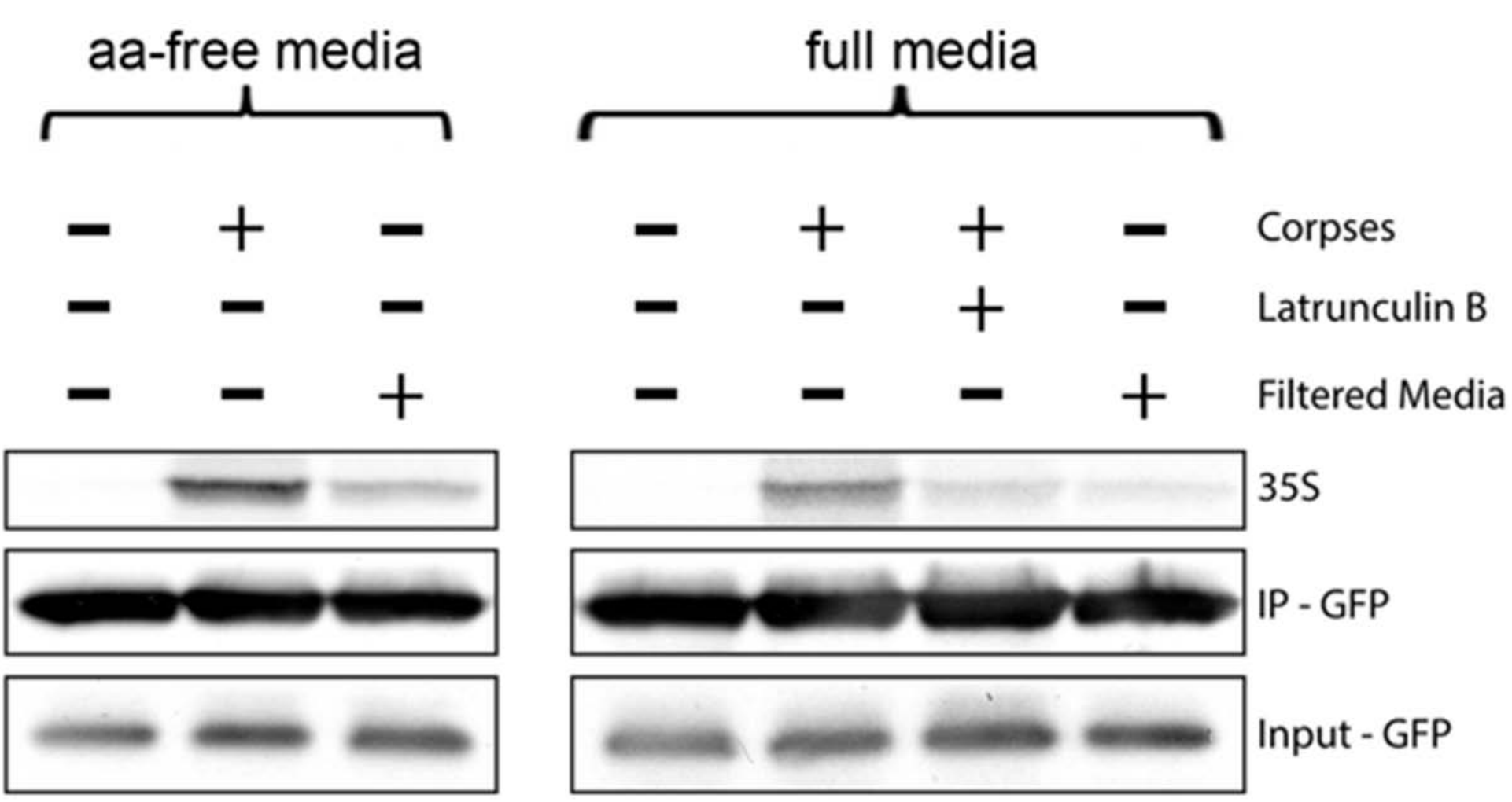

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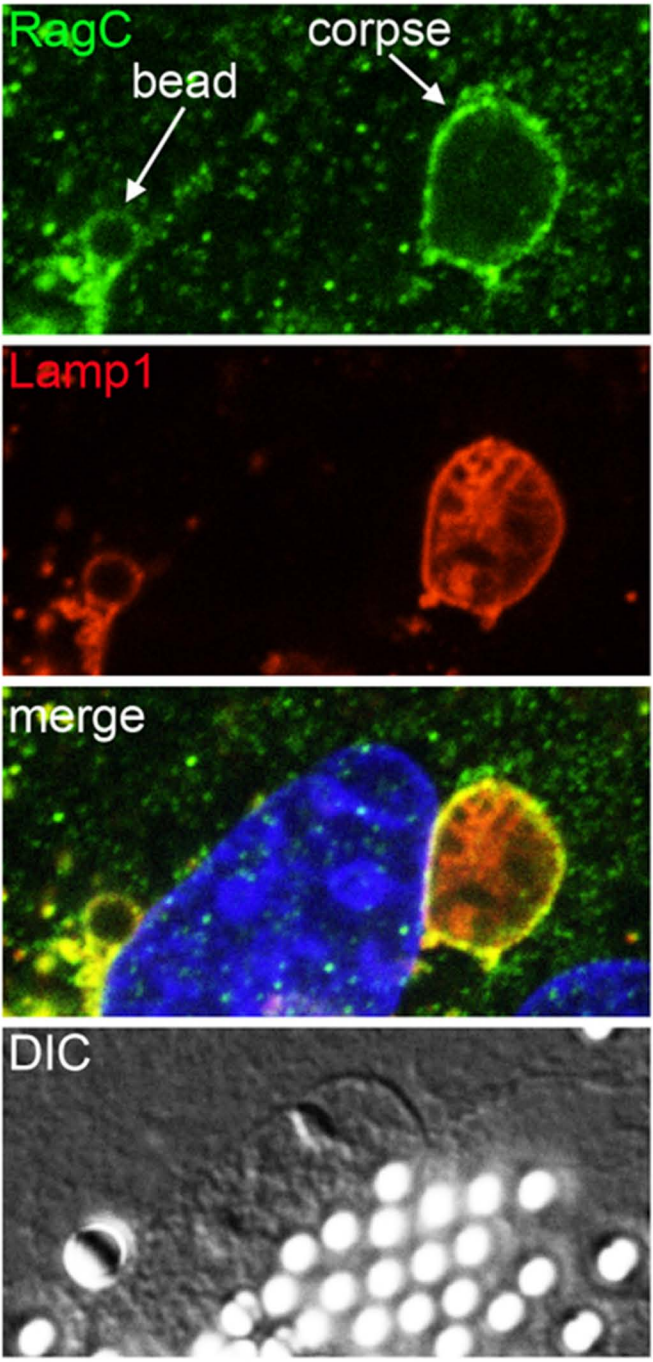

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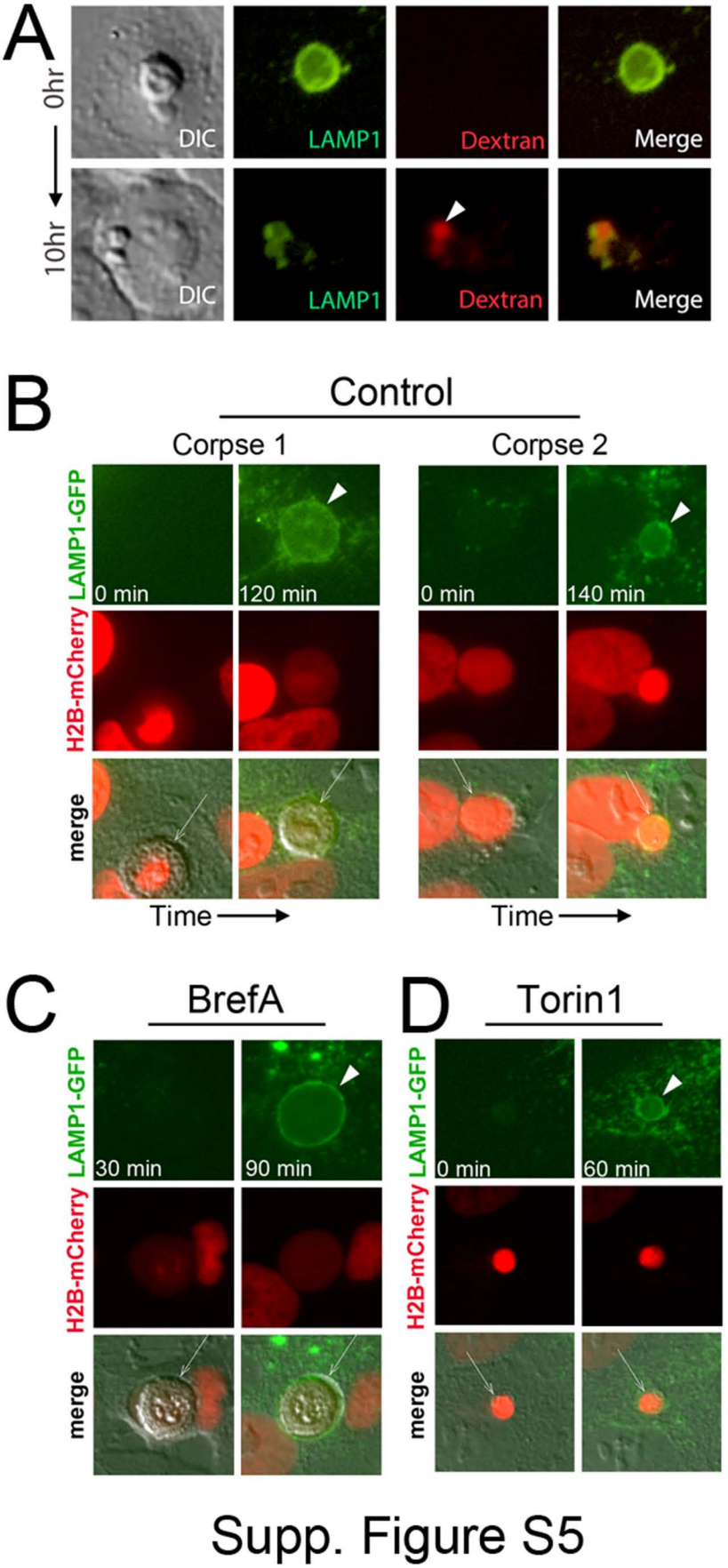

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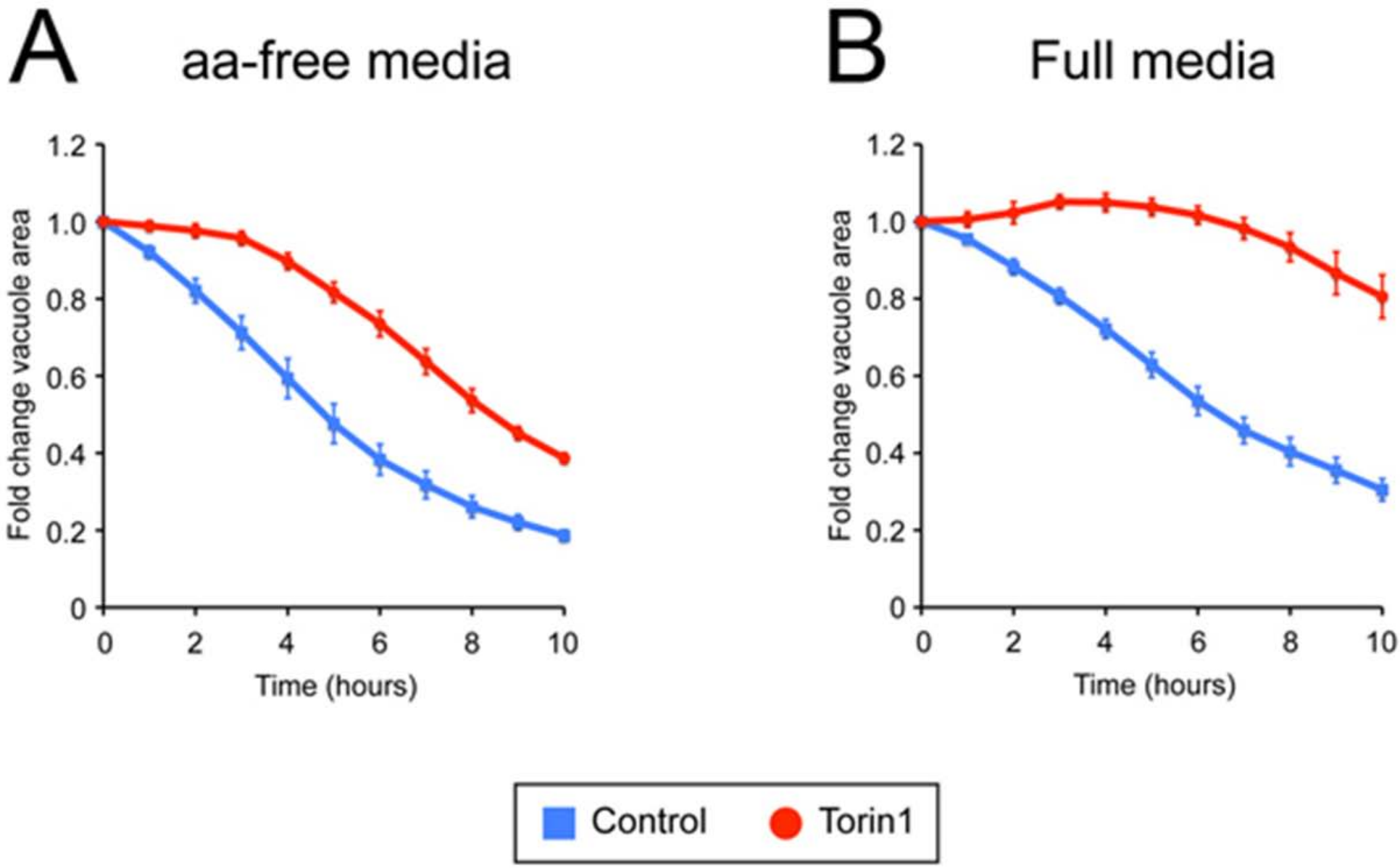

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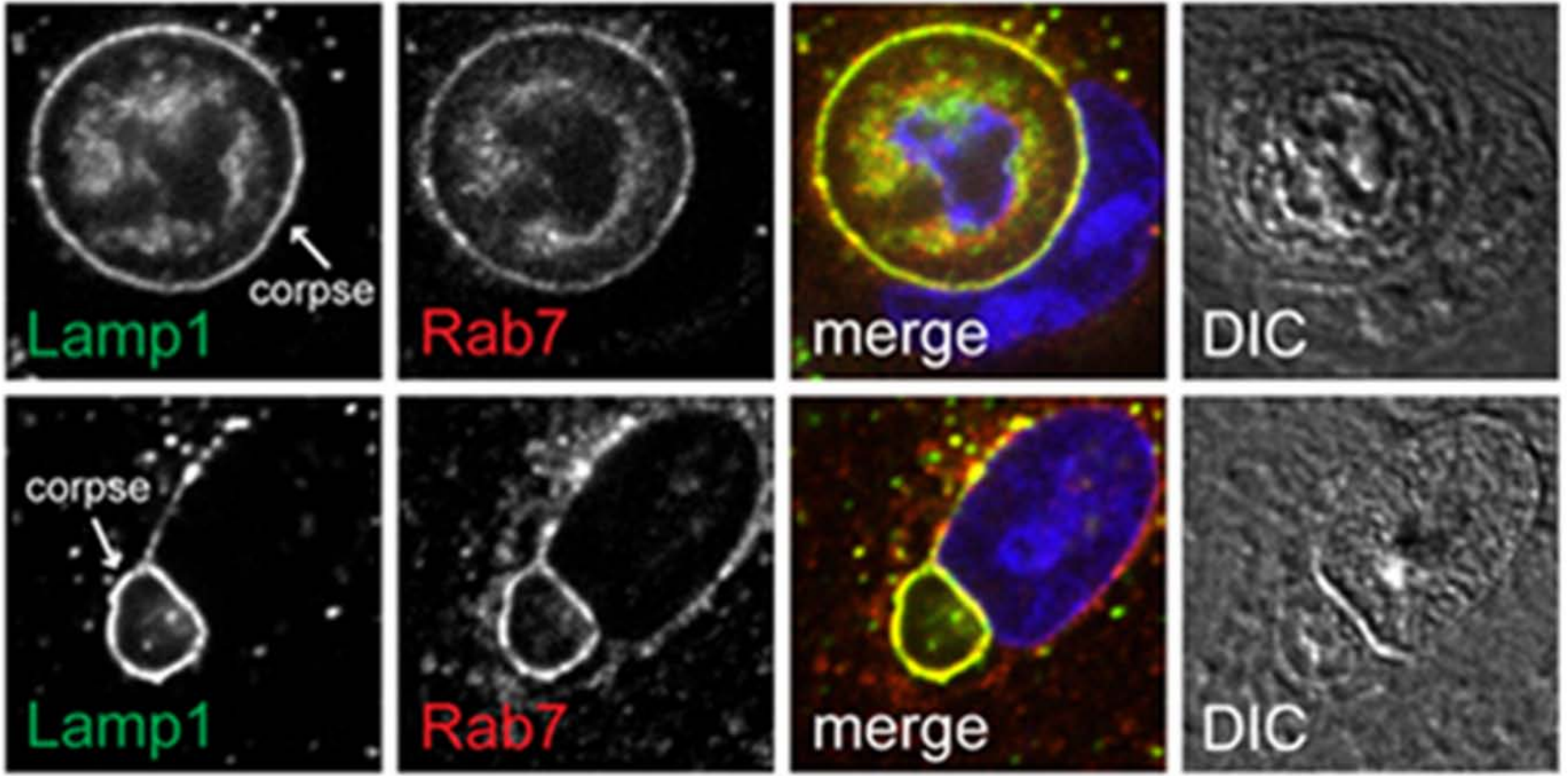

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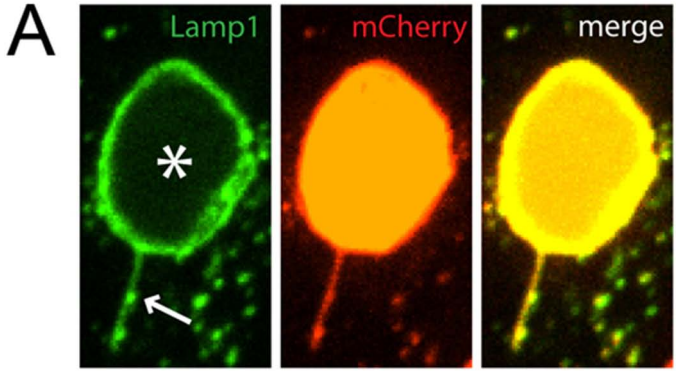

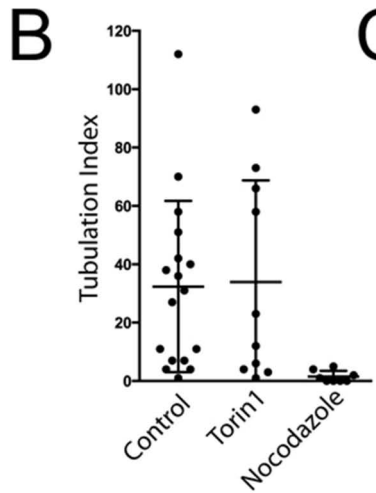

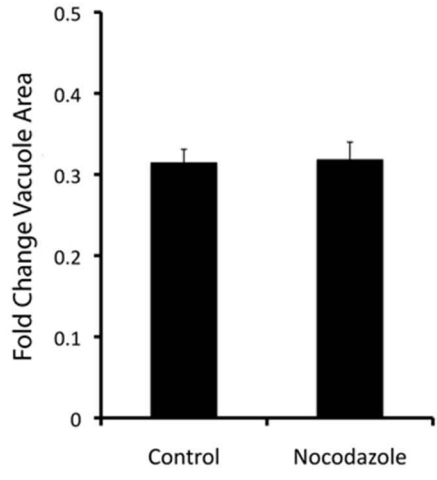

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