Figure S1. Association between Rab10 and Dyn2 on LD associated membranes within primary hepatocytes.

(A-C) Confocal images of primary rat hepatocytes expressing mCherry-Rab10, Dyn2-GFP and co-stained with MDH to label cytosolic LDs (blue channel). (A'-C") Insets below are increased magnifications of boxed regions within panels (A-C). Quantitation of these cells revealed that of the LDs counted in 21 cells, 2.6% and 26.9% of the LDs had either Dyn2 or Rab10 respectively, while 11.7% possessed both proteins. Micron bars for (A-C)=5µm and (A'-C'')=1µm. (D) Immunoblots of GFP-trap based immunoprecipitations from primary rat hepatocytes expressing either GFP alone, WT-GFP Rab10 or the GFP-Rab10(Δ3) proteins. Endogenous Dyn2 protein has a higher affinity for WT Rab10 than for the Rab10(Δ3) mutant.

Figure S2. Dyn2 localization at the site of degradative membrane tubule scission extending from the surface of a LD.

(A) Still images taken from a time lapse series captured of a Hep3B cell expressing Dyn2-GFP and Lamp1-mCherry to mark the degradative autophagic/lysosomal membranes often seen on the periphery of MDH co-stained cytoplasmic LDs. Dyn2 (arrows) can be seen at the site of membrane tubule scission, indicating a direct influence on the Lamp1 positive membranes that have engulfed a LD (arrowhead). (B) Still images from a live Hep3B cell with an identical treatment as shown in (A) where the appearance of Dyn2 on LD associated Lamp1 membranes is coincident with a separation of this degradative compartment from the LD surface (arrows). Micron bars=2µm.

Figure S3. Colocalization of Rab10 and Dyn2 proteins on LD-associated degradative autophagic membranes within nutrient depleted Hep3B cells.

(A-B) Confocal images of Hep3B cells expressing mCherry-tagged Rab10 and GFP-tagged-LC3 that were treated for 4h in HBSS then fixed and co-stained for Dyn2 with a rabbit polyclonal antibody and LDs using the MDH compound. For display purposes, the MDH stained LDs were pseudo-colored white and the Dyn2 stain was pseudo-colored blue. (A'-B') Hep3B cells labeled with mCherry-tagged Rab10, GFP-tagged-LC3, and Dyn2 where the pseudo-colored MDH-LD

channel from panels (A-B) has been removed. Increased magnification of boxed regions display individual color channels. (C-D) Confocal images of 4h HBSS starved Hep3B cells expressing mCherry-tagged Rab10 and GFP-tagged-Atg16 co-stained for Dyn2 with a rabbit polyclonal antibody and LDs using the MDH compound. For display purposes, the MDH stained LDs were pseudo-colored white and the Dyn2 stain was pseudo-colored blue. (C'-D') Hep3B cells labeled with mCherry-tagged Rab10, GFP-tagged-Atg16, and Dyn2 where the pseudo-colored MDH-LD channel from panels (C-D) has been removed. Increased magnification of boxed regions display individual color channels. Micron bars denote 5μm in (A-D) and 2μm in enlargements of boxed regions.

Figure S4. Dyn2 and Rab10 proteins are found on LD associated autophagic membranes in primary hepatocytes.

(A-C) mCherry-tagged Rab10 is found on LD associated LC3-GFP positive membranes in isolated primary rat hepatocytes (arrows). (D-F) Dyn2-mCherry is also found on autophagosome membranes in close association with lipid droplets (arrows). These LC3 positive autophagosome membranes often display tubules that extend away from the droplet surface (arrowheads). Panels (A'-C') and (D'-F') represent increased magnifications of the boxed regions in panels (A-F). Quantitation of these cells revealed that of the LDs counted in 15 cells, 90.7% and 86.8% of associated LC3-GFP spots were co-labeled with Dyn2 and Rab10 proteins respectively. Micron bars for (A,B, D-F)=15μm, for (C)=10μm and for (A'-F')=3μm .

Figure S5. Tubulated lipid droplet-associated membranes are numerous in Dyn2 knockdown cells as depicted by low magnification image tracings.

Hep3B cells were treated with NT siRNA or Dyn2 siRNA as described Fig. 5. Low magnification of en face sections were analyzed for all HRP positive compartments and graphically depicted by tracings (depicted in red). Micron bars=10μm.

Figure S6. LD associated membranes are altered in Rab10 knock out cells.

Normal and Rab10-KO MEF cells were loaded with 400µM oleate overnight and subjected to a 24hr starvation period in low serum media (0.1% FBS). For visualizing the late degradative compartments, cells were loaded with HRP for 90' prior to processing for imaging by TEM that included a peroxidase reaction step for visualizing the terminal lysosomes (denoted by the electron dense reaction product in all images and annotated with arrows). Normal MEF cells contain small lysosomes distributed throughout the cytoplasm (A-B). Rab10-KO MEF cells have swollen lysosomes that contain dense membranous material and often display numerous lipid aggregates that appear to be remnants of cytoplasmic lipid droplets (C-G). Micron bars in (A,B)=2µm; (C-G)=1µm.

Supplemental Movies:

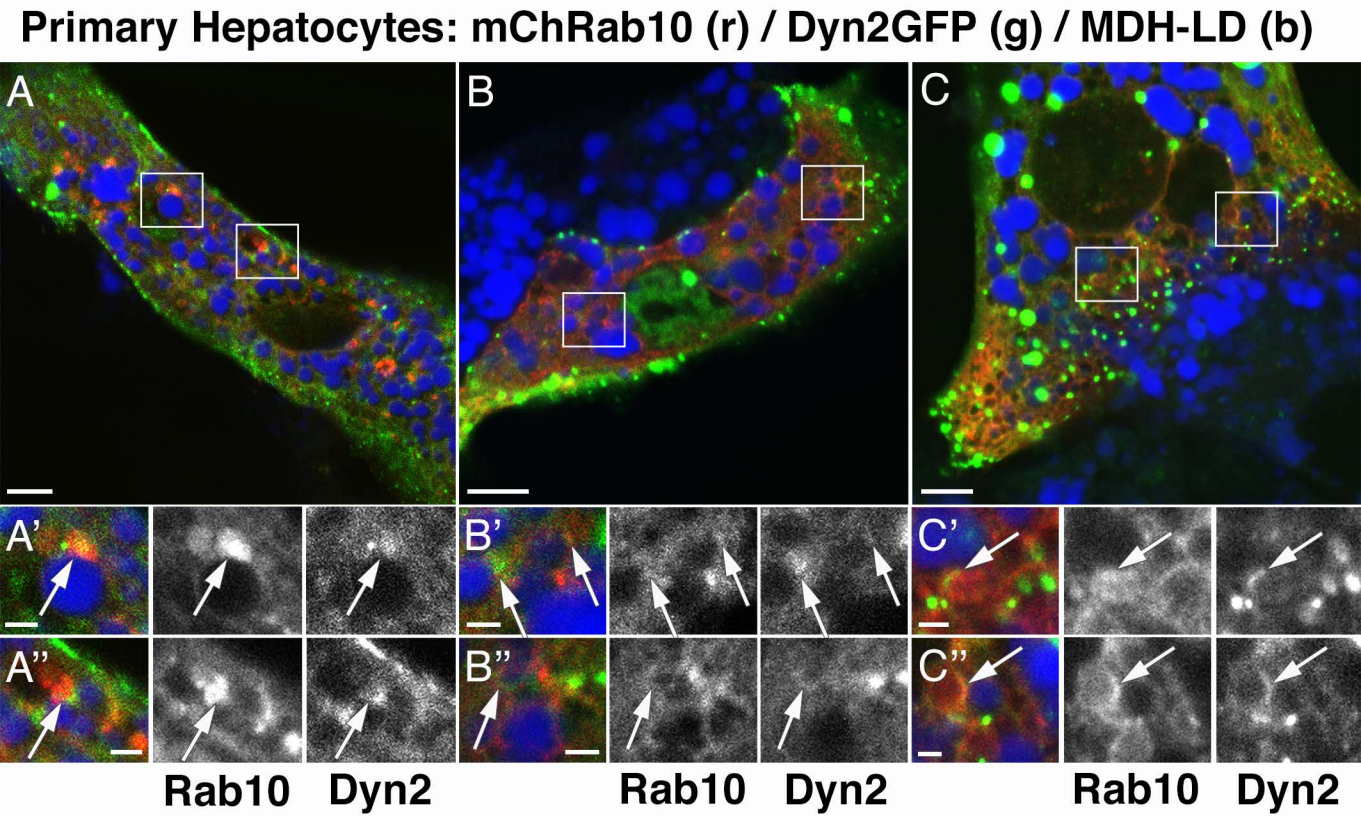
Movie 1) Live-cell confocal fluorescence microscopy of a 24h low serum starved Hep3B hepatoma cell co-transfected with Dyn2-GFP and mcherry-Rab10. Lysosomes are stained by lysotracker-blue. Note the transient association between Dyn2 and Rab10, and the dynamic remodeling of Dyn2 positive membranes.

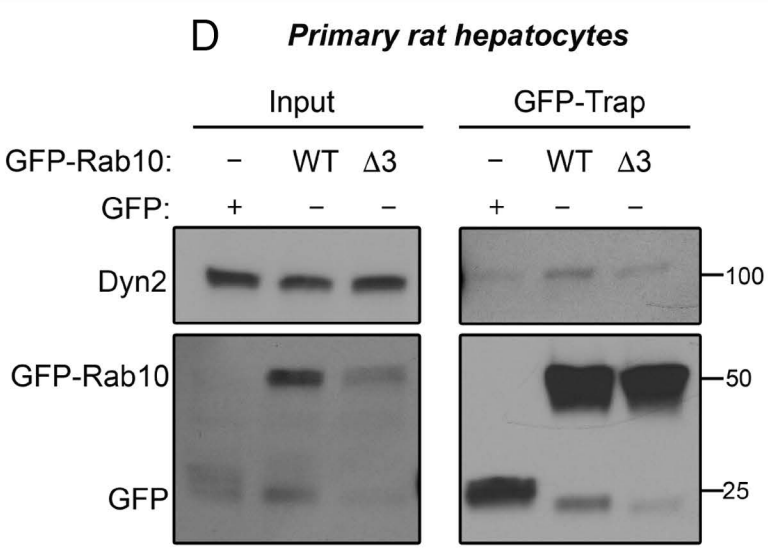
Movie 2) Time series of a Hep3B cell following a 24h starvation in low serum showing the transient recruitment of GFP-tagged Dyn2 to a scission point of a mCherry-Rab10 coated membrane tubule.

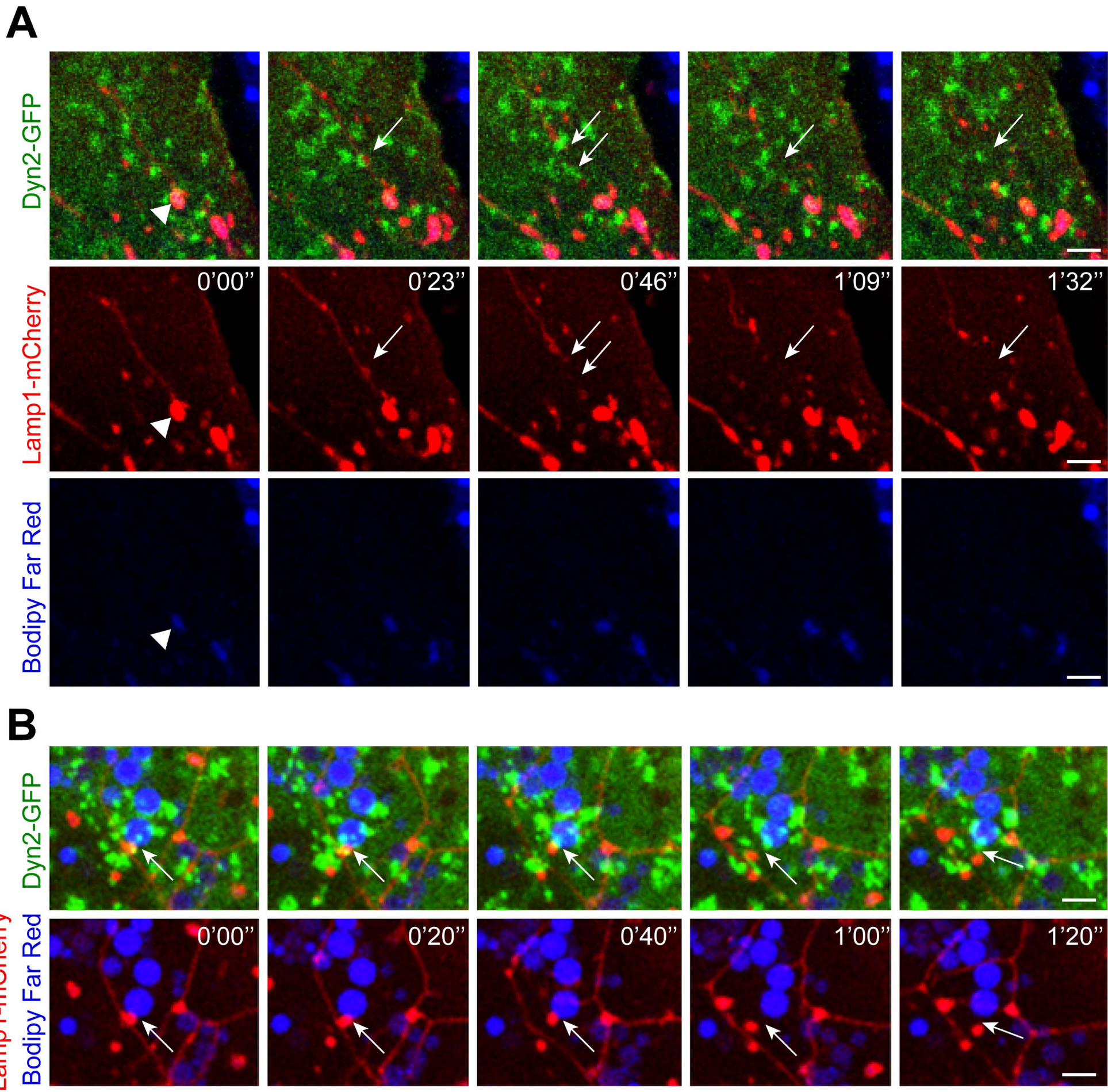
Movie 3-5) Hep3B cells showing dynamic extensions of GFP-Rab10 coated membrane tubules that are associated with red fluorescent BODIPY-C12 labeled LDs.

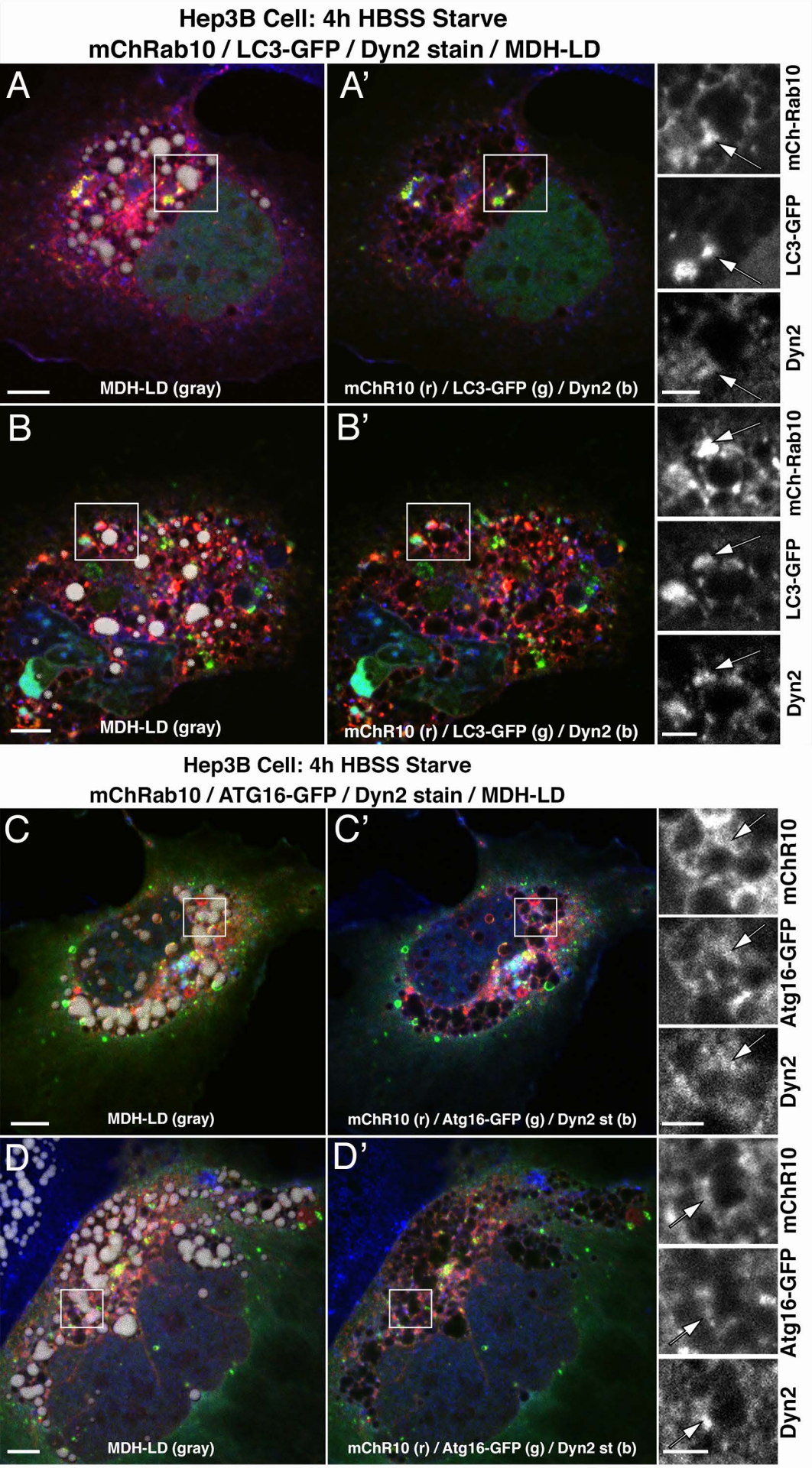
Movie 6-7) Live confocal fluorescence microscopy of Hep3B cells expressing GFP-Dyn2 and mCherry-Rab10. LDs are labelled by MDH (in blue).

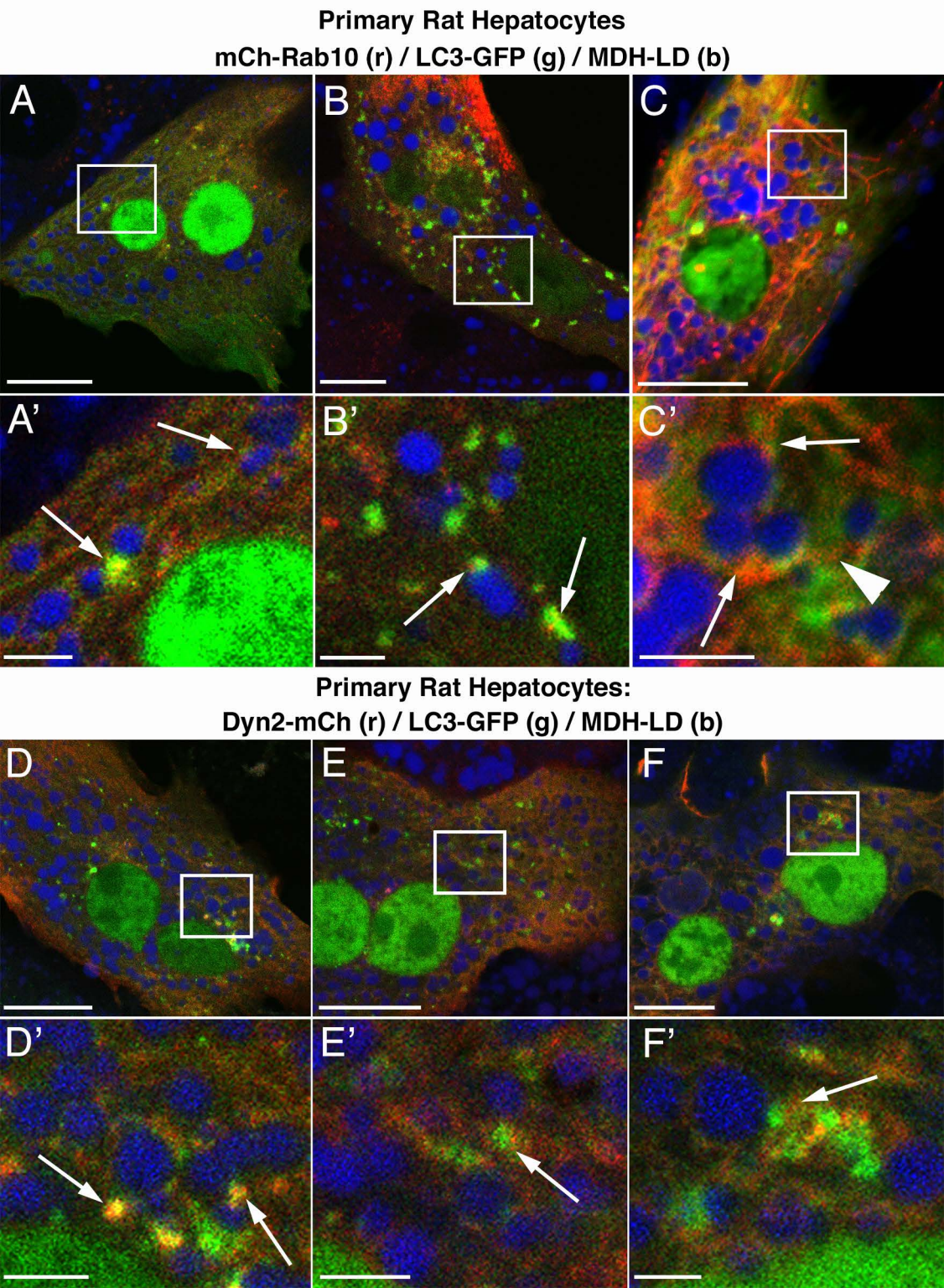
Movie 8) A Hep3B cell expressing GFP tagged Dyn2, Lamp1-mCherry proteins and labeled with MDH for visualization of lipid droplets. Note the Lamp1 decorated tubule extending away from the surface of a cytoplasmic LD that undergoes scission coincident with the recruitment of Dyn2. Still frames for this movie can be found in Fig. S2 panel A.

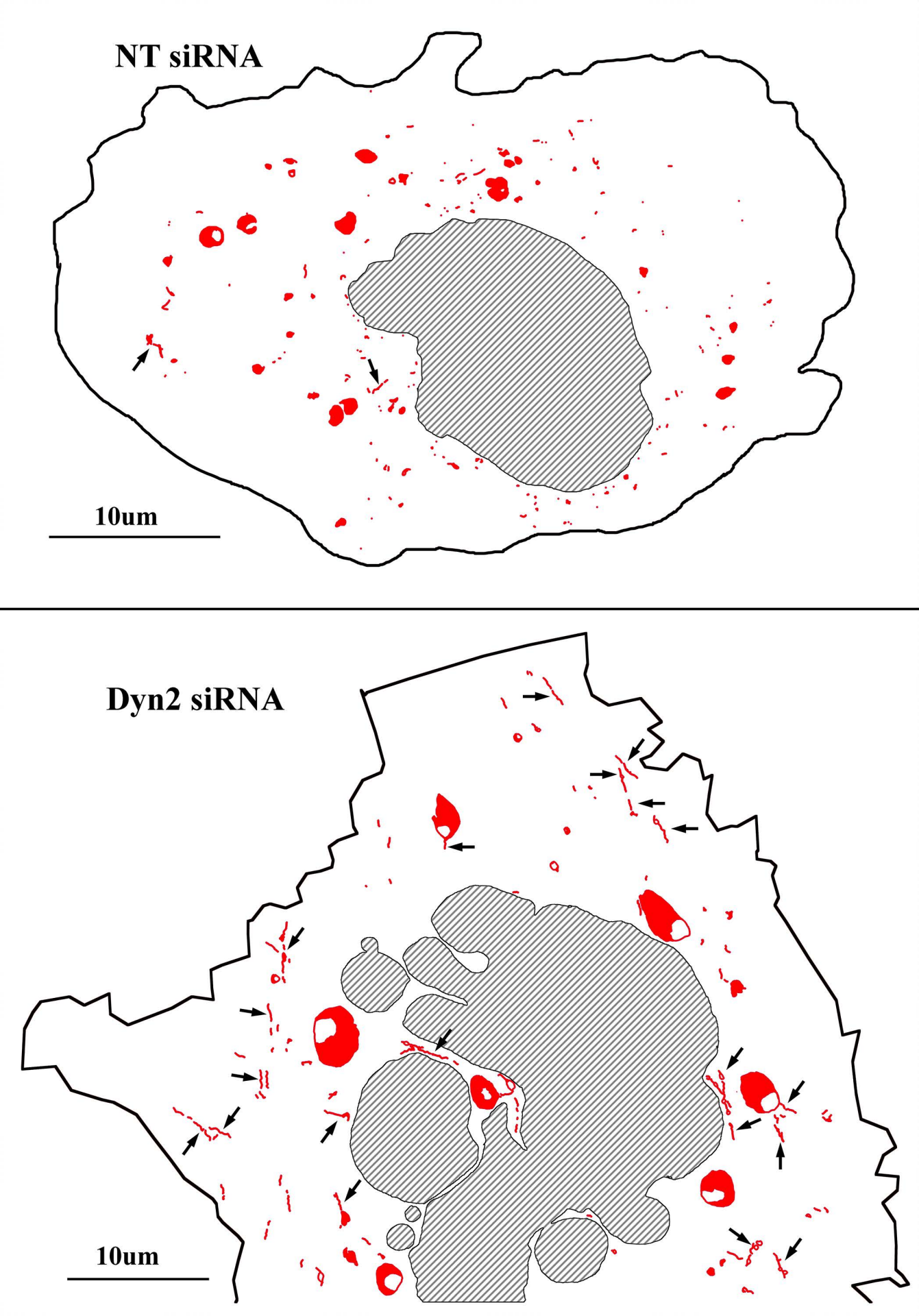

Primary Hepatocytes: mChRab10 (r) / Dyn2GFP (g) / MDH-LD (b)
A
B
C
A'
B'
C'
A''
B''
C''
Rab10
Dyn2
Rab10
Dyn2
Rab10
Dyn2

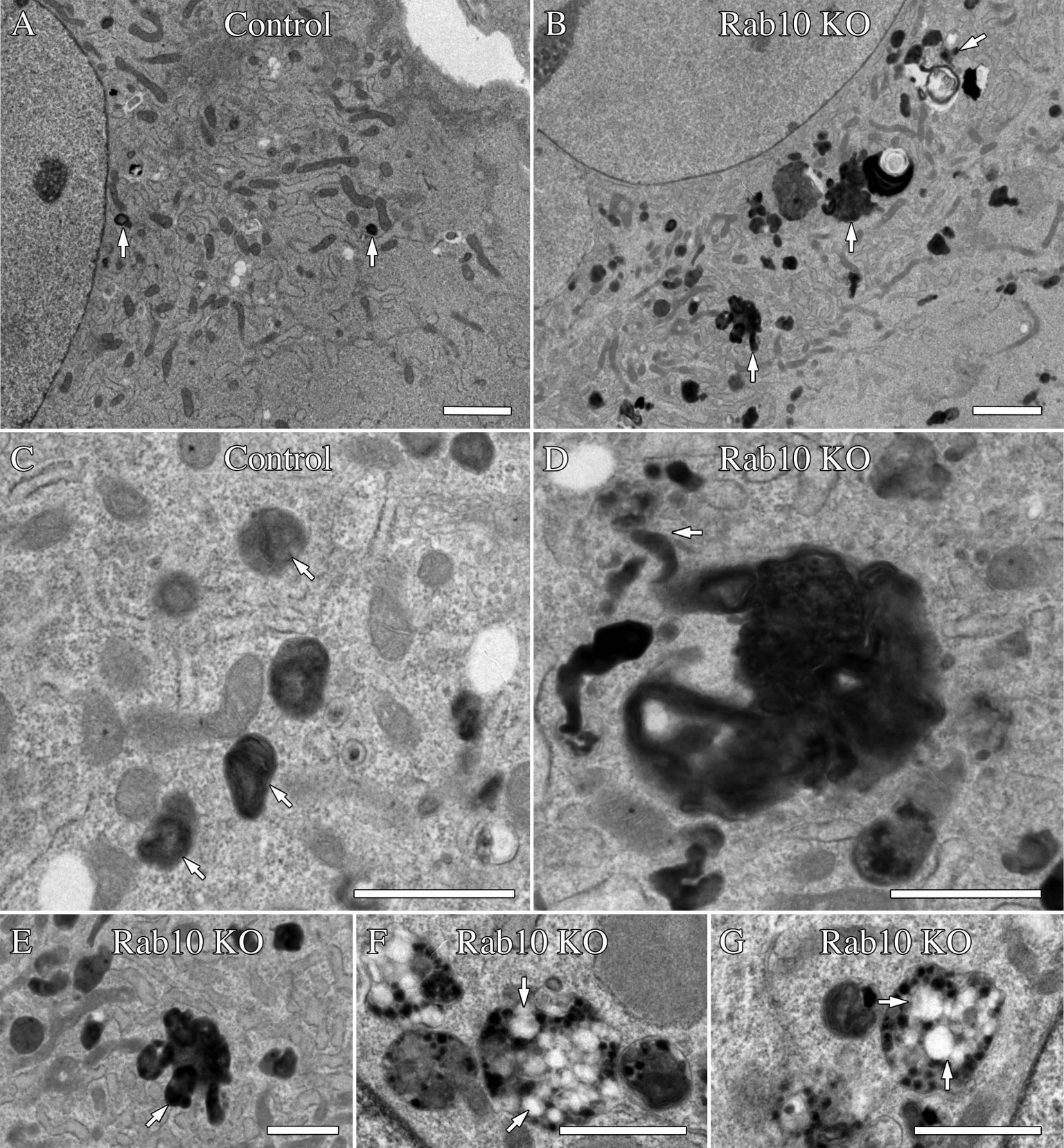

D
Primary rat hepatocytes
Input
GFP-Trap
GFP-Rab10:
−
WT
Δ3
−
WT
Δ3
GFP:
+
−
−
+
−
−
Dyn2
GFP-Rab10
GFP
100
50
25


A
Dyn2-GFP
Lamp1-mCherry
Bodipy Far Red
0'00"
0'23"
0'46"
1'09"
1'32"
B
Dyn2-GFP
Lamp1-mCherry
Bodipy Far Red
0'00"
0'20"
0'40"
1'00"
1'20"


Hep3B Cell: 4h HBSS Starve
mChRab10 / LC3-GFP / Dyn2 stain / MDH-LD
A
A'
B
B'
MDH-LD (gray)
mChR10 (r) / LC3-GFP (g) / Dyn2 (b)
mCh-Rab10
LC3-GFP
Dyn2
Hep3B Cell: 4h HBSS Starve
mChRab10 / ATG16-GFP / Dyn2 stain / MDH-LD
C
C'
D
D'
MDH-LD (gray)
mChR10 (r) / Atg16-GFP (g) / Dyn2 st (b)
mChR10
Atg16-GFP
Dyn2


Primary Rat Hepatocytes
mCh-Rab10 (r) / LC3-GFP (g) / MDH-LD (b)
A
B
C
A'
B'
C'
Primary Rat Hepatocytes:
Dyn2-mCh (r) / LC3-GFP (g) / MDH-LD (b)
D
E
F
D'
E'
F'


NT siRNA
10um
Dyn2 siRNA
10um


A
Control
B
Rab10 KO
C
Control
D
Rab10 KO
E
Rab10 KO
F
Rab10 KO
G
Rab10 KO