Supplemental Figure Legends

Supplemental Figure 1. RAB26 does not localize to the immature secretory vesicle, endosomal, Golgi, or post-Golgi sorting compartments. EGFP-RAB26 (green) transfected HGC-27 cells immunostained for: **A.** immature secretory vesicle marker, Furin (red); **B.** endoplasmic reticulum marker, Calregulin (red); **C.** early endosomal marker, EEA1 (red); **D.** Golgi marker, Giantin (red); **E.** *cis*-Golgi marker, GM130 (red); **F.** *trans*-Golgi marker, TGN46 (red); and **G.** post-Golgi/late endosome marker, CI-M6PR (red). Confocal microscopy of EGFP-RAB26 (green) transfected HGC-27 cells immunostained for post-Golgi/endosomal markers: **B.** CI-M6PR (red) **C.** AP1 (red), **D.** GGA2 (red). Insets indicate RAB26 vesicles (white filled arrowheads) overlap with stained markers (open arrowheads) (Cell border outlined, "N" – nucleus). Scale bars = 20 µm.

Supplemental Figure 2. RAB26 not RAB3D localizes to lysosomes. A. Immunofluorescence microscopy of HGC-27 cells transfected with EGFP-RAB3D and co-stained for LAMP1 (red) and cathepsin D (purple**).** Epifluorescence images of EGFP-RAB26 transfected: **B.** AGS, gastric cells; **C.** 5637, bladder cells; and **D.** AR42J, pancreatic cells co-labeled for LAMP1 (red). Panels indicate unmerged green and red channels. **E.** Confocal microscopy of an AR42J cell transfected with EGFP-RAB26 and immunostained for LAMP1 (red). Arrowheads indicate areas of overlap (Cell border outlined, "N" – nucleus). Scale bars = 20 µm unless indicated.

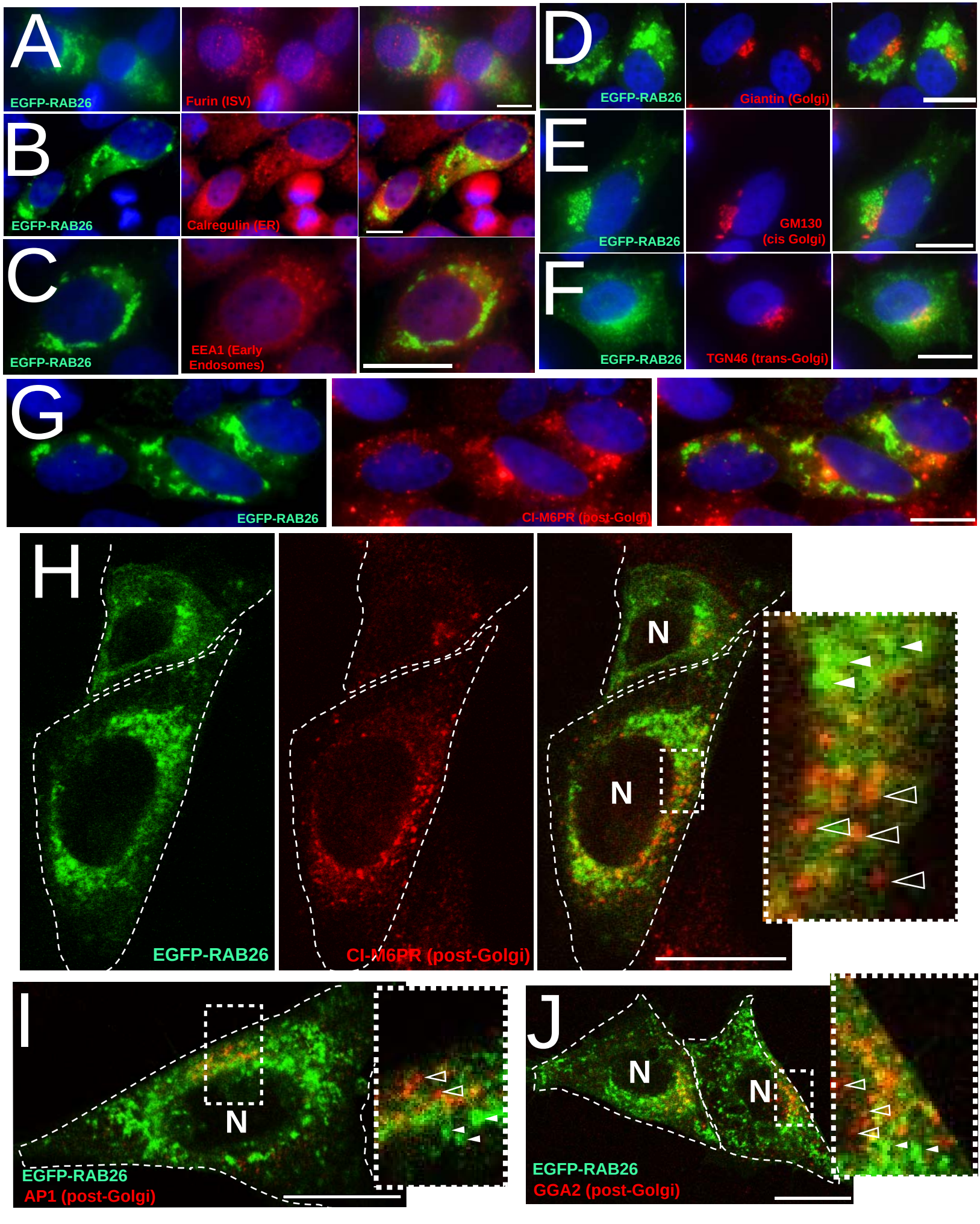
Supplemental Figure 3. RAB26 does not affect Golgi or late endosomal distribution. Fluorescence microscopy of EGFP-RAB26, EGFP-RAB26T77N, EGFP-RAB26Q123L, and EGFP transfected HGC-27 cells (green) co-stained for: **A.** giantin (red) and **B.** CI-M6PR (red). Low magnification panels of EGFP-RAB26 transfected cells highlight giantin and CI-M6PR distribution in transfected (green) and untransfected cells. Scale bars = 20 µm.

Supplemental Figure 4. RAB26 does not affect lysosomal or mitochondrial function. A. Western blots (triplicate experiments) of EGFP-RAB26, EGFP-RAB26T77N, EGFP-RAB26Q123L, and EGFP transfected HGC-27 cells comparing levels of LAMP1, cathepsin D, mTOR, phosphorylated S6 kinase, total S6 kinase, and tubulin. **B.** Fluorescence microscopy of

EGFP-RAB26, EGFP-RAB26T77N, EGFP-RAB26Q123L, and EGFP transfected HGC-27 cells (green) co-stained for: **B.** MitoTracker CMXRos (red) and **C.** MitoSOX Red (red) and MitoTracker Deep Red (purple). Scale bars = 20 μm.

Movie 1. RAB26 does not colocalize with secretory vesicles. Live confocal timelapse microscopy of HGC-27 cells transfected with EGFP-RAB26 (green) and PGC-RFP (red). Images were taken at 3 second intervals and movie is displayed at 5 frames/second. White arrows highlight RAB26 vesicle movement. Yellow arrows and circles show vesicle fusion or fission events. Time stamp is shown in upper left corner and scale bar = 20 μm.

Movie 2. RAB26 localizes with lysosomes in mitochondrial free subcellular areas. Live confocal timelapse microscopy of HGC-27 cells transfected with EGFP-RAB26 (green) stained for lysosomes (LysoTracker, red) and mitochondria (MitoTracker Deep Red, Blue). Images were taken at 40 second intervals and movie is displayed at 3 frames/second. White arrows highlight RAB26/LysoTracker vesicle movement. Time stamp is shown in upper left corner and scale bar = 20 μm.


A
EGFP-RAB26
Furin (ISV)
B
EGFP-RAB26
Calregulin (ER)
C
EGFP-RAB26
EEA1 (Early Endosomes)
D
EGFP-RAB26
Giantin (Golgi)
E
EGFP-RAB26
GM130 (cis Golgi)
F
EGFP-RAB26
TGN46 (trans-Golgi)
G
EGFP-RAB26
CI-M6PR (post-Golgi)
H
EGFP-RAB26
CI-M6PR (post-Golgi)
N
N
I
N
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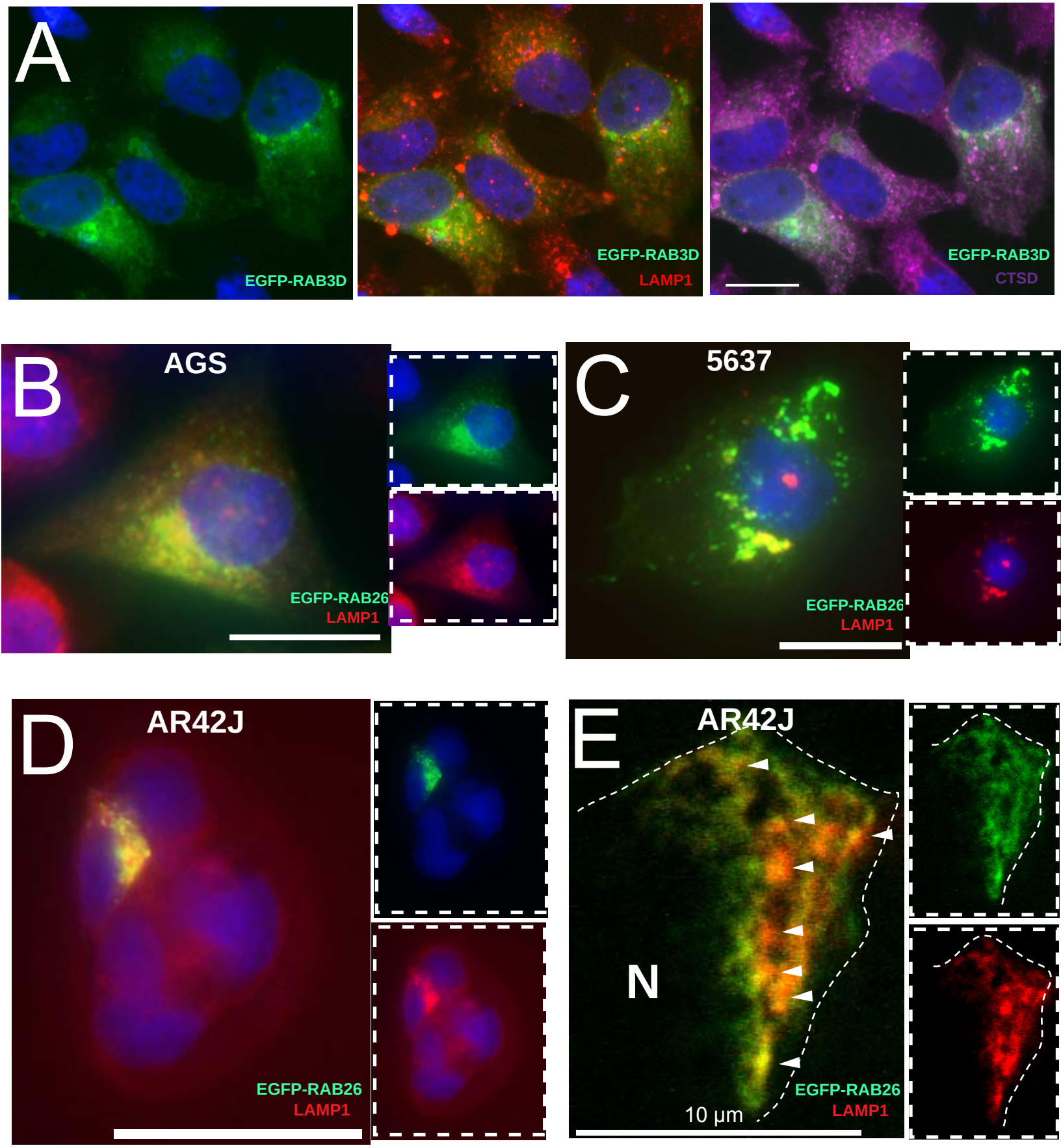
J
N
N
EGFP-RAB26
GGA2 (post-Golgi)


A
EGFP-RAB3D
EGFP-RAB3D
LAMP1
EGFP-RAB3D
CTSD
B
AGS
EGFP-RAB26
LAMP1
C
5637
EGFP-RAB26
LAMP1
D
AR42J
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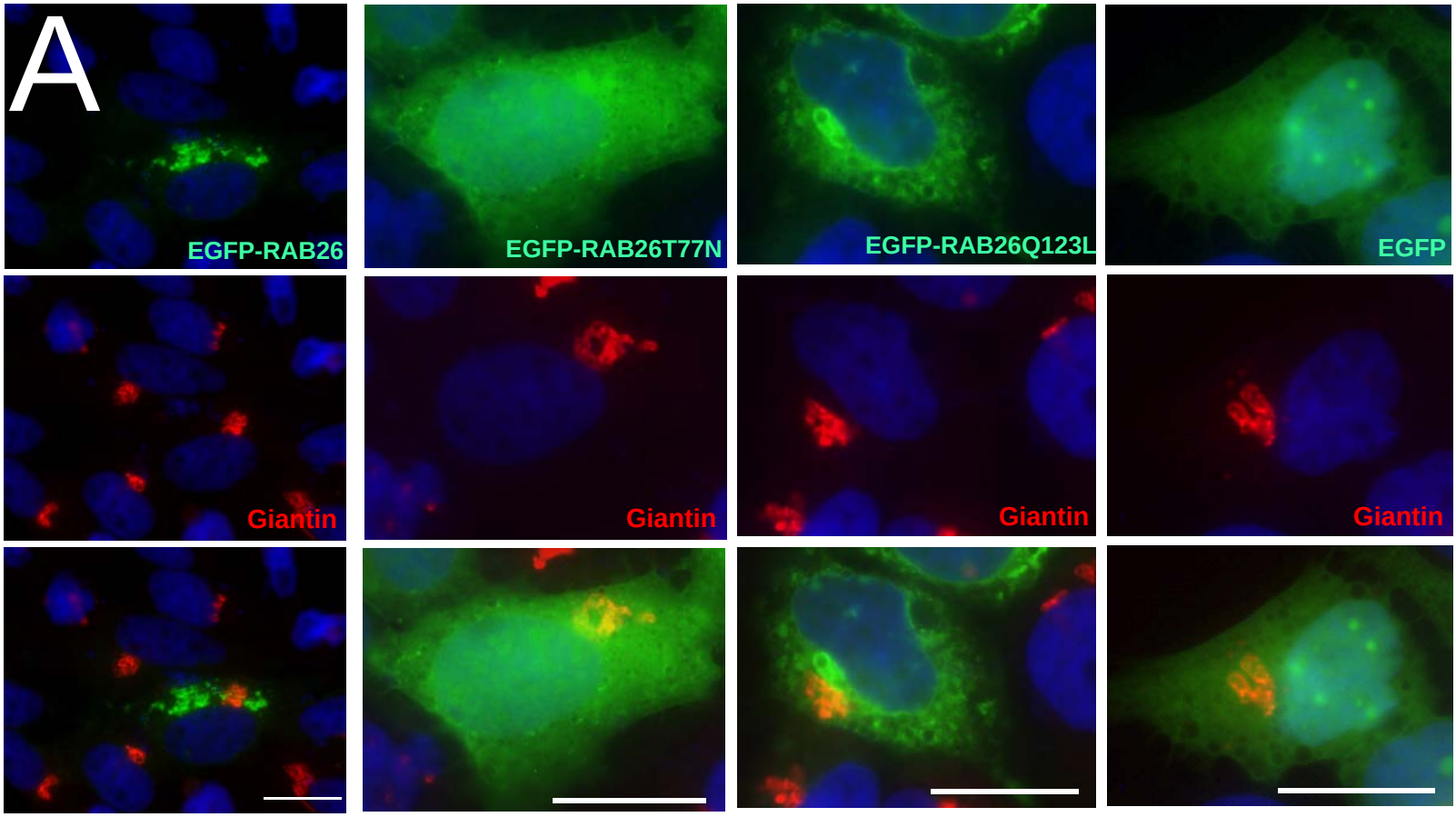
LAMP1
E
AR42J
N
10 µm
EGFP-RAB26
LAMP1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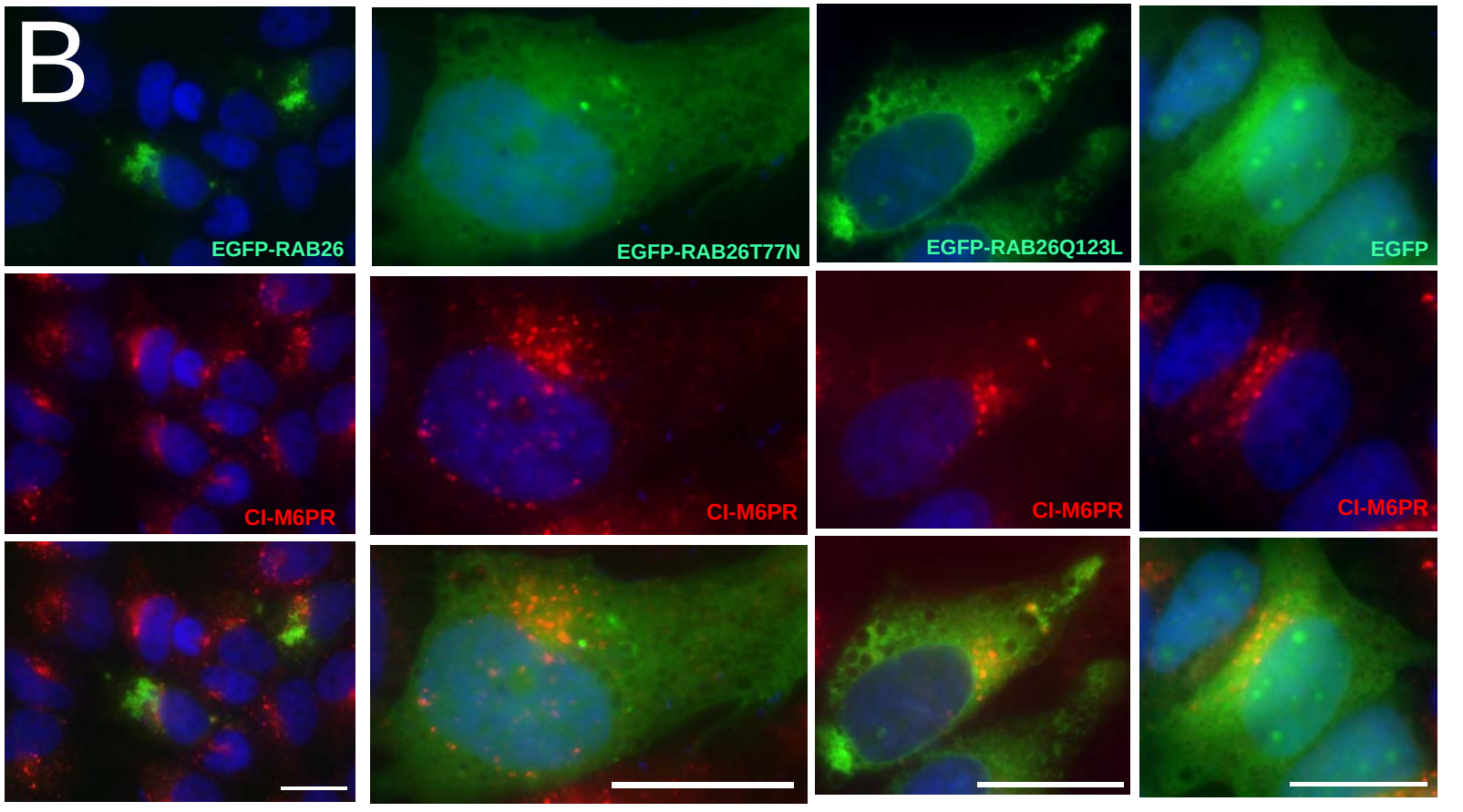
EGFP-RAB26T77N
EGFP-RAB26Q123L
EGFP
Giantin
Giantin
Giantin
Giantin


B
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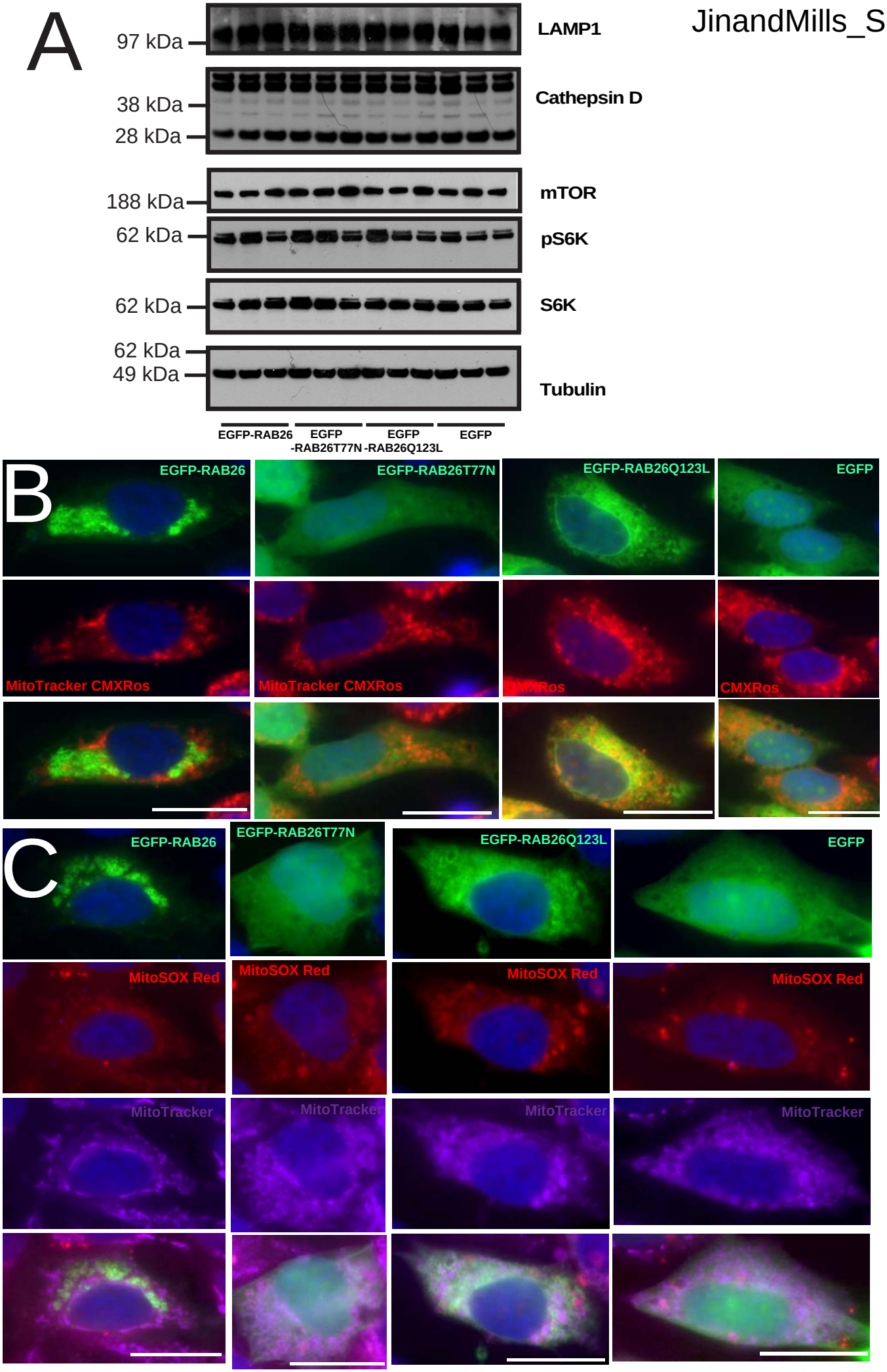
EGFP-RAB26Q123L
EGFP
CI-M6PR
CI-M6PR
CI-M6PR
CI-M6PR


A
97 kDa
LAMP1
38 kDa
28 kDa
Cathepsin D
188 kDa
mTOR
62 kDa
pS6K
62 kDa
S6K
62 kDa
49 kDa
Tubulin
EGFP-RAB26
EGFP -RAB26T77N
EGFP -RAB26Q123L
EGFP
B
EGFP-RAB26
EGFP-RAB26T77N
EGFP-RAB26Q123L
EGFP
MitoTracker CMXRos
MitoTracker CMXRos
CMXRos
CMXRos
C
EGFP-RAB26
EGFP-RAB26T77N
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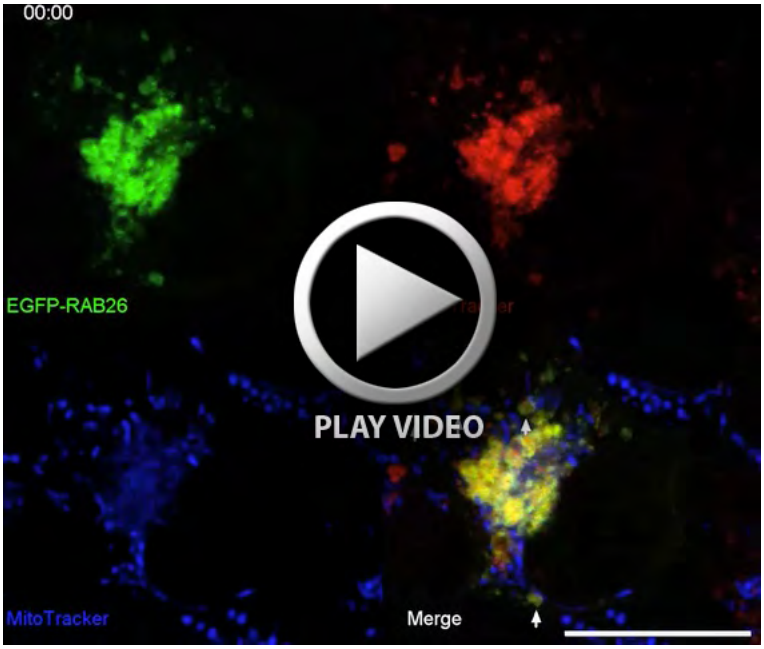
MitoTracker

Movie 1.



Movie 2.