Regulation of late endosomal/lysosomal maturation and trafficking by cortactin affects Golgi homeostasis.

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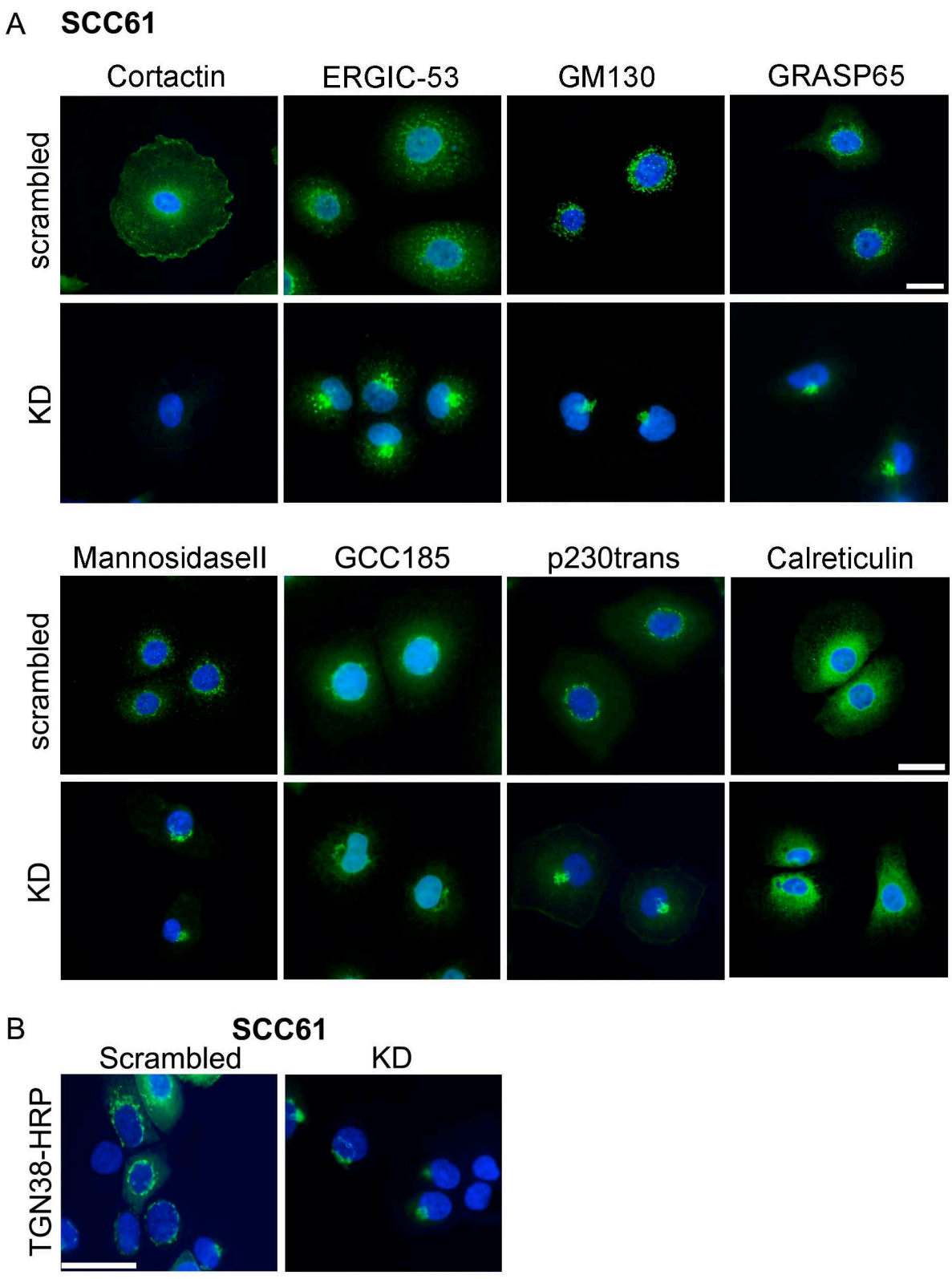

Figure S1. Cortactin affects the size of the Golgi, ERGIC, and TGN compartments. A. SCC61 control (scrambled) and cortactin-knockdown (KD) cells were immunostained for cortactin or the indicated markers of the secretory pathway (green). Nuclei are stained with Hoechst (blue). **B.** Control and KD cells were transiently transfected with TGN38-HRP for 24 hours followed by immunostaining against-HRP (green) and Hoechst (blue). n = 2 independent experiments.

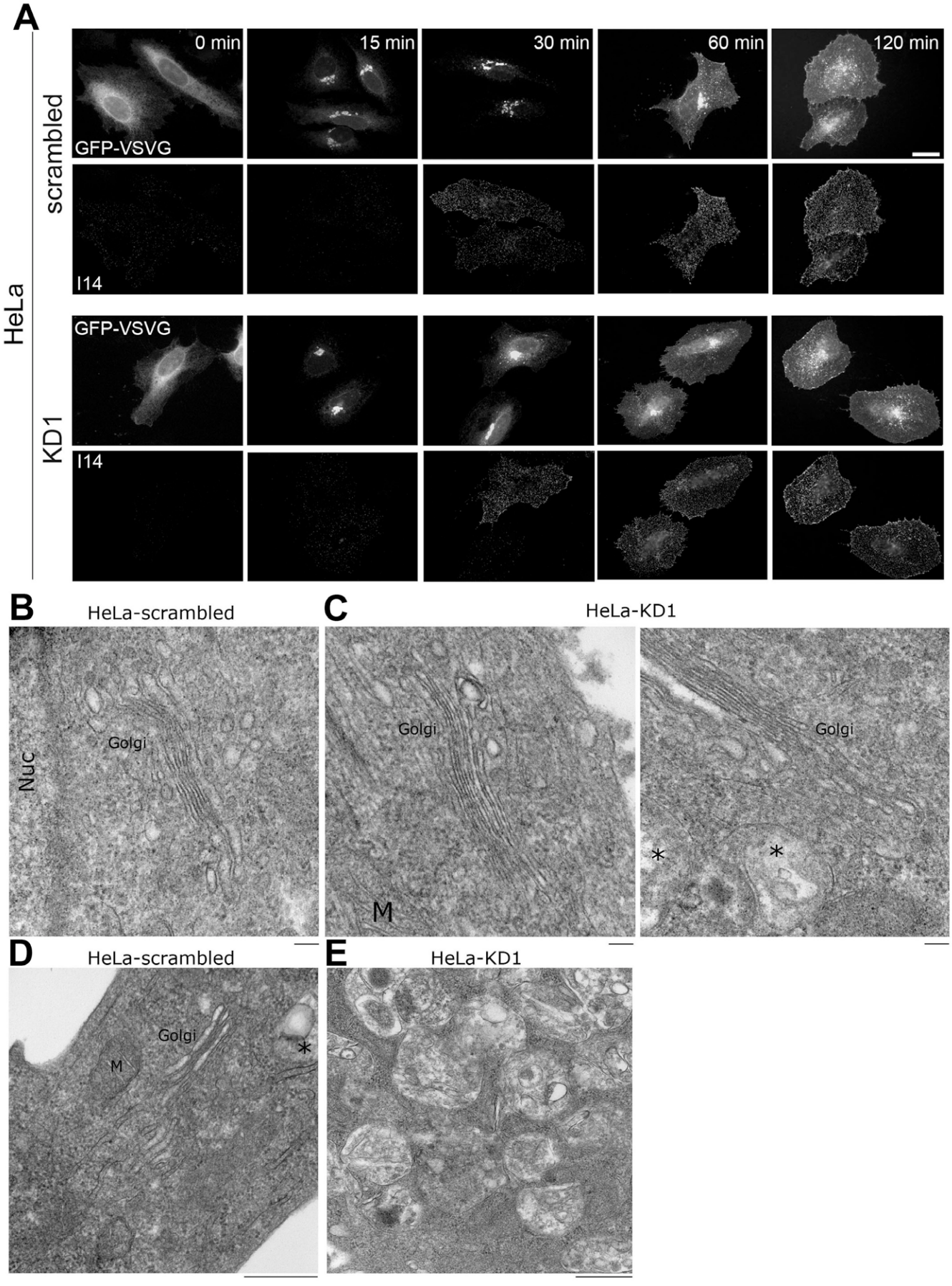
A
HeLa
scrambled
KD1
0 min
15 min
30 min
60 min
120 min
GFP-VSVG
I14
GFP-VSVG
I14
B
HeLa-scrambled
Nuc
Golgi
C
HeLa-KD1
Golgi
M
Golgi
*
*
D
HeLa-scrambled
Golgi
M
*
E
HeLa-KD1

Figure S2. Representative images from HeLa cells stably transfected with scrambled or cortactin-specific shRNA. A. VSV-G transport in HeLa cells. Representative images from stable control (scrambled, top panels) or cortactin-KD (KD1, bottom panels) HeLa cells showing total expressed VSV-G (green, GFP-VSVG) and cell surface VSV-G (red, I14) at the indicated times following a shift of the cells to the permissive temperature of 32°C. Scale bar = 25 μm. **B-E. Representative EM images of Golgi and LE/Lys ultrastructure in HeLa cortactin-KD cells. B and C:** Representative high power images of the Golgi for Hela-scrambled and HeLa-KD1 cells, as indicated. Scale bars = 100 nm. **D and E:** Lower power images show cytoplasmic contents of HeLa-scrambled and HeLa-KD1 cells. Note the large number of LE/Lys hybrid organelles filling the cytoplasm in HeLa KD cells (**E**). In other images * indicates LE/Lys, M indicates mitochondria, and Nuc indicates nucleus. Scale bars = 500 nm.

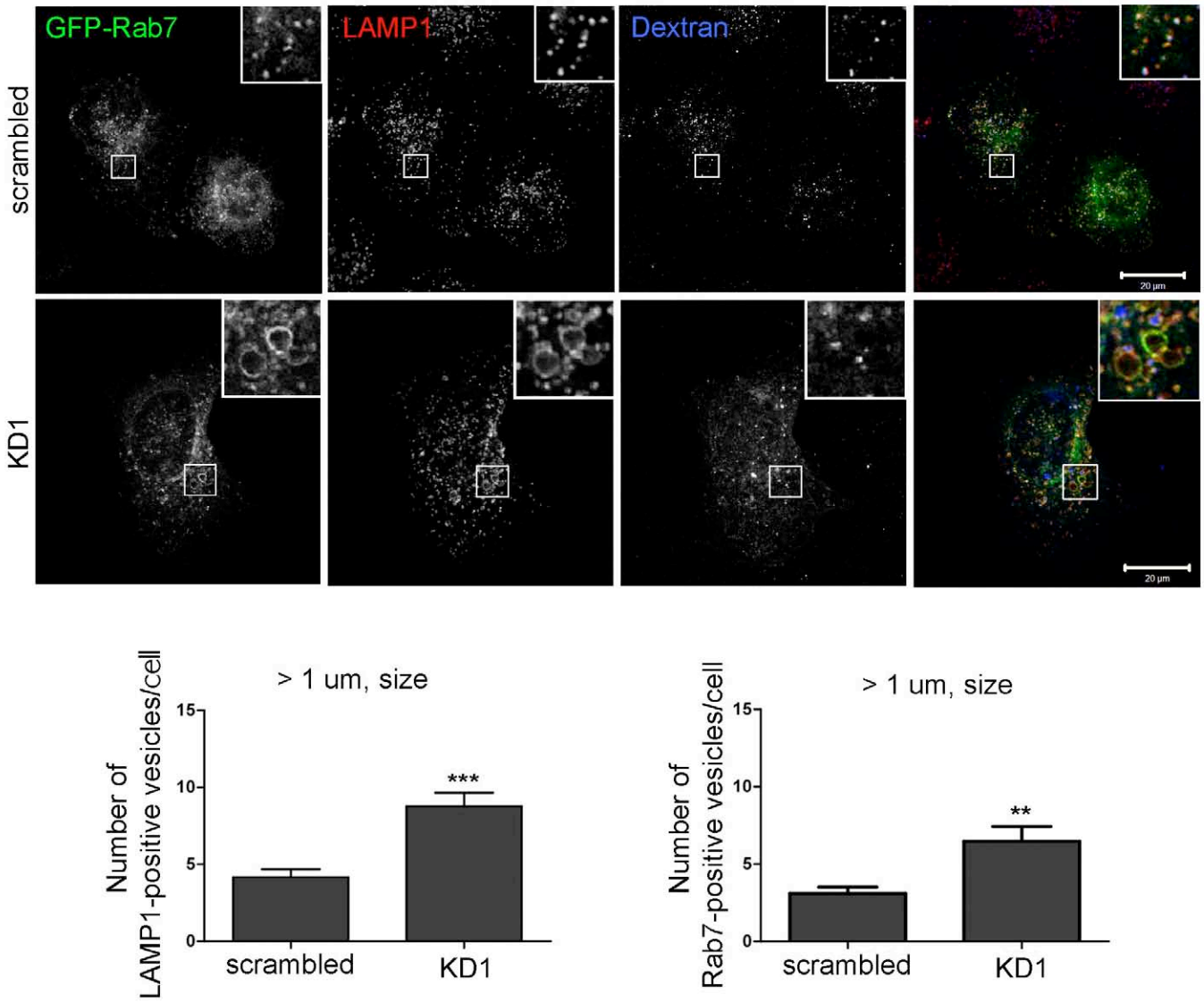

Figure S3. Enlarged hybrid compartments containing late endosomal and lysosomal markers are increased in cortactin-KD cells and these compartments are inaccessible to fluid-phase marker. Control (scrambled) and cortactin-KD (KD1) SCC61 cells were transiently transfected with a GFP-Rab7 construct, then incubated for 15 minutes at 37°C in serum free medium containing 1 mg/ml Alexa Fluor 546-conjugated Dextran 10,000 followed by a 4 hour chase period in medium lacking dextran. Then, cells were fixed and immunostained for LAMP1 to label lysosome. **A.** Representative images showing enlarged Rab7 (green) and LAMP1 (red) double positive compartments in KD1 cells. Boxed areas are zoomed to show dextran (blue) at the LE/Lys compartments in control cells (top right) and to show Rab7 and LAMP1 double positive enlarged vesicles that are inaccessible for dextran in KD cells (bottom right). **B.** Quantitation of the number of enlarged LAMP1-positive and Rab7-positive vesicles per cell with size > 1 μm. n=20 from 2 independent experiments. Mean +/-SE shown. ** $p<0.01$, *** $p< 0.001$. Scale bar = 20 μm

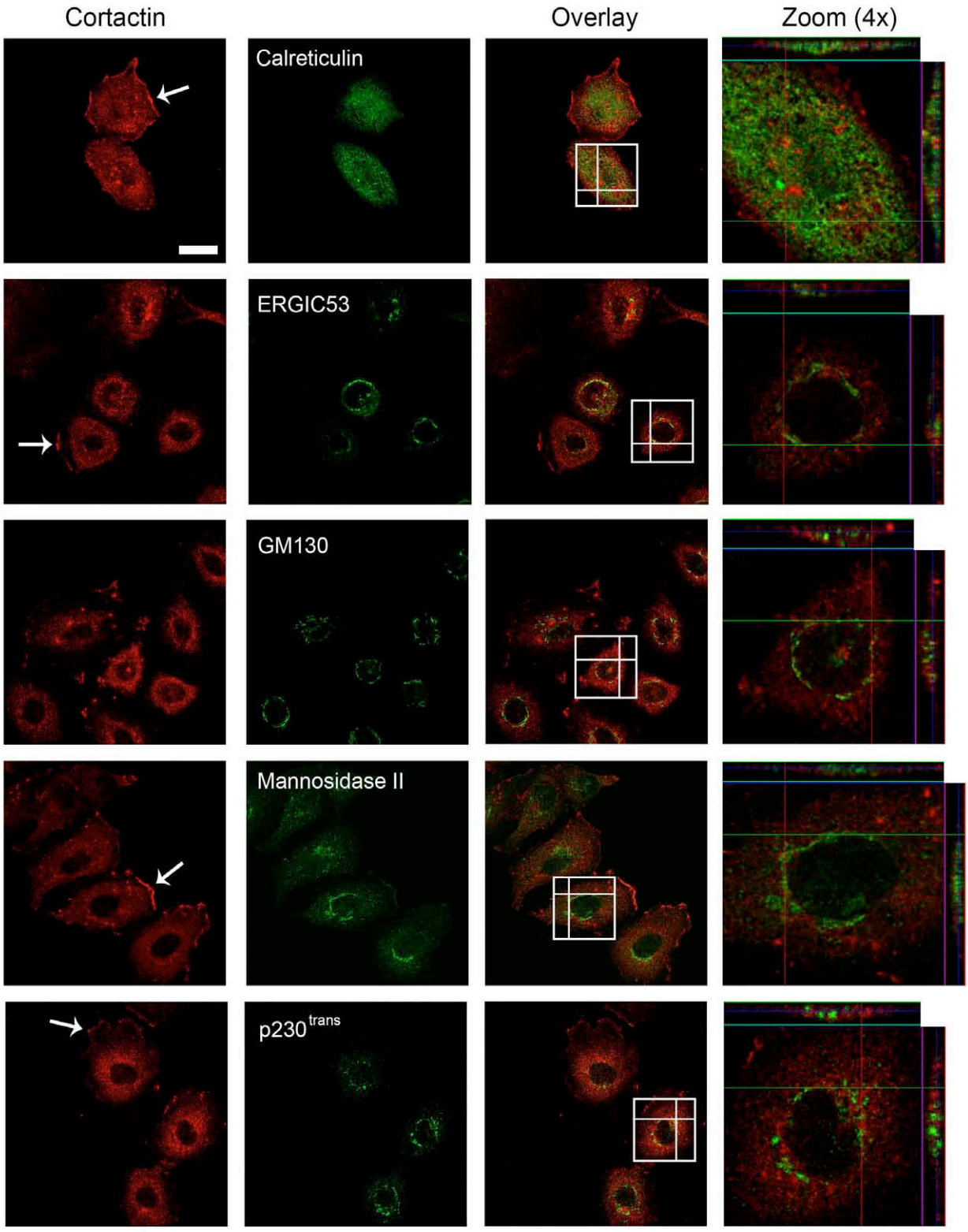

Figure S4. Cortactin does not localize with most ER or Golgi markers. SCC61 parental cells were fixed and stained for both cortactin (left panel, red) and the indicated markers of ER (Calreticulin), ERGIC (ERGIC53) or Golgi (GM130, Mannosidase II, p230^{trans}) (green). Images are overlaid and zoomed at 4x (white boxes indicate zoomed area, far right panel) to show subcellular localization of cortactin and the indicated markers. Z-slices of specific lines through the cell (indicated by the white lines) are shown along the top and right side of the zoomed images. Arrows indicate cortactin staining in lamellipodia. Scale bar = 25 μm.

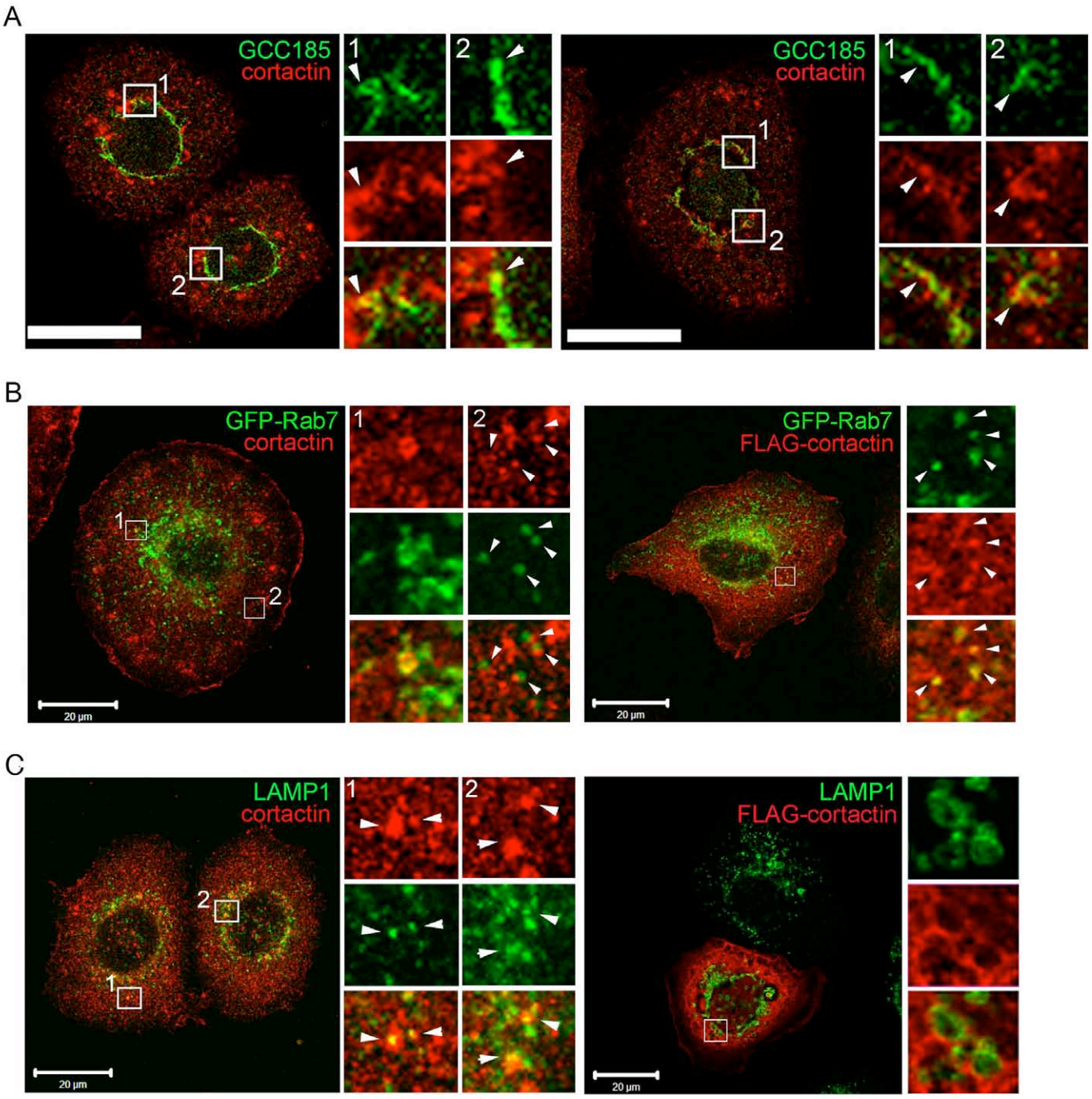

Figure S5. Cortactin colocalizes with a subset of GCC185, Rab7 and LAMP1-positive vesicles. **A.** SCC61 parental cells were fixed and stained for cortactin (red) and the TGN marker GCC185 (green). Scale bar = 25 µm. **B, C.** Representative images of endogenous (left) or exogenously expressed (Flag-tagged, right) cortactin (red) localization at GFP-Rab7- (**B**, green) or LAMP1-positive (**C**, green) late endosomal and lysosomal compartments in parental SCC61 cells. Scale bar = 20 µm. Boxed areas are zoomed to show colocalization (arrowheads) of cortactin with GCC185, Rab7 or LAMP1.