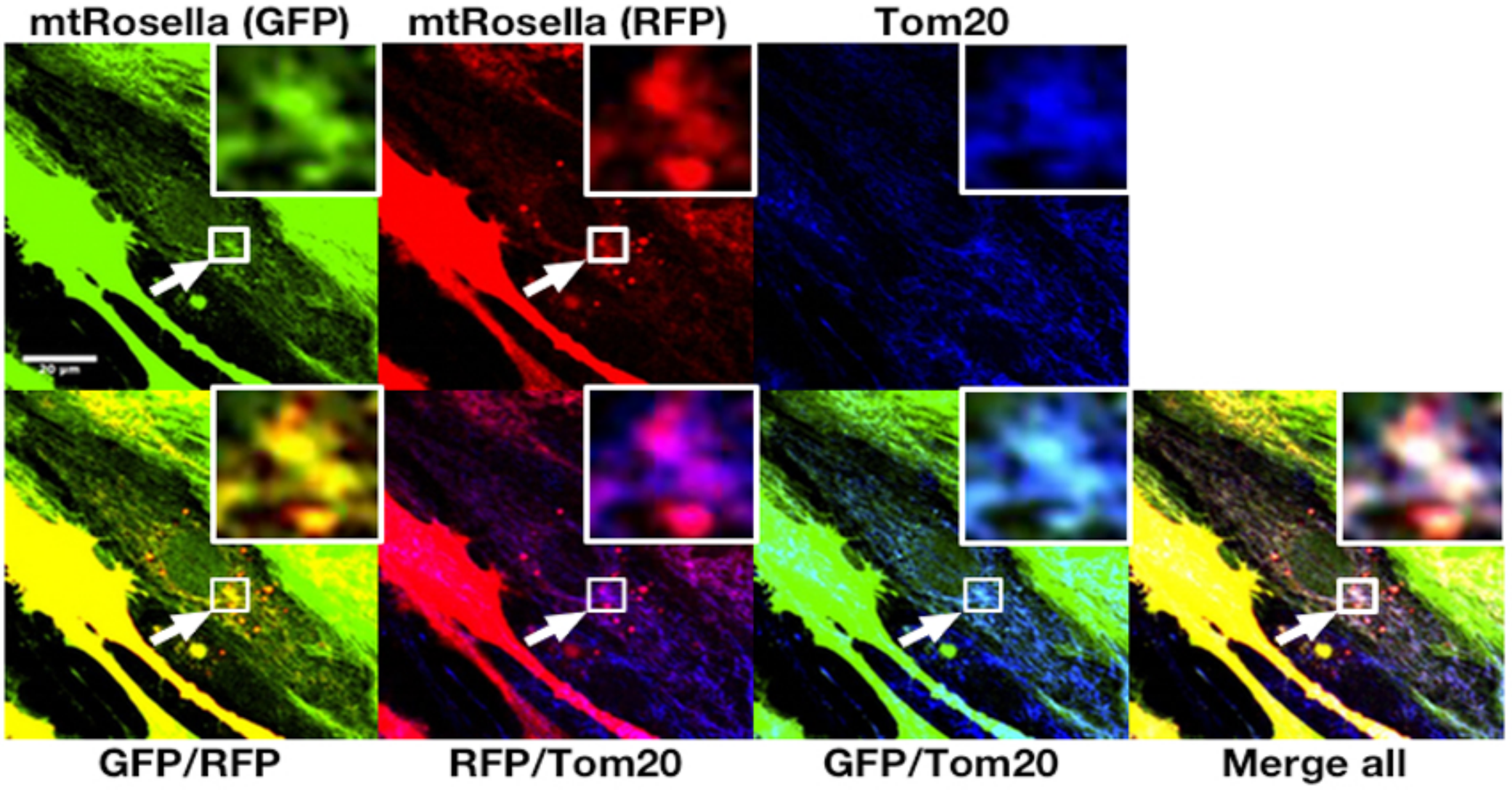
Supplemental Figure Legend

Supp Fig. 1. Characterization of the mitophagy reporter mt-Rosella. H9c2 cardiac myoblasts were infected with Ad-mt-Rosella and examined under a confocal microscope. The mitochondria glowing green or yellow in the merged confocal images are located in the cytosolic compartments, while the fragmented mitochondria fluorescing only red are being degraded within the lysosomes where the pH is low and the GFP is quenched. The red signal or dots colocalized with mitochondrial protein TOM20 (A), autophagosomal marker LC3 (B), and lysosomal protein LAMP1 (C), confirming the specificity of the reporter.

Supp Fig. 2. Parkin overexpression or knockdown affected DOX-induced mitophagy. **A**. H9c2 cells were cultured in DMEM with 10% bovine serum and co-infected with the adenoviruses encoding mt-Rosella or Parkin for 48 hrs and then exposed to either DOX (750nM) or saline for 24 hrs. **B**. H9c2 cells were cultured in DMEM with 10% bovine serum and were pretreated with Parkin-targeted siRNA (siParkin) or control siRNA (siCON) for 24 hrs, and then exposed to either DOX (750nM) or saline for an additional 24 hrs. Mitophagy was observed with confocal microscopy and analyzed using ImageJ. After image optimization, particle analysis allowed for the calculation of the number of mitophagy foci per cell. Between 3 and 8 images (totaling between 5 and 15 cells) were captured per treatment over 3 separate experiments. To determine mitophagy flux, experiments were repeated with addition of lysosomal inhibitors (Pepstatin A and E64D) or DMSO 4 hours after DOX treatment. Data are expressed as mean ± SE and were analyzed by one-way ANOVA (p values were indicated in the bar graphs, n=3).

Supplemental Figure 1

A



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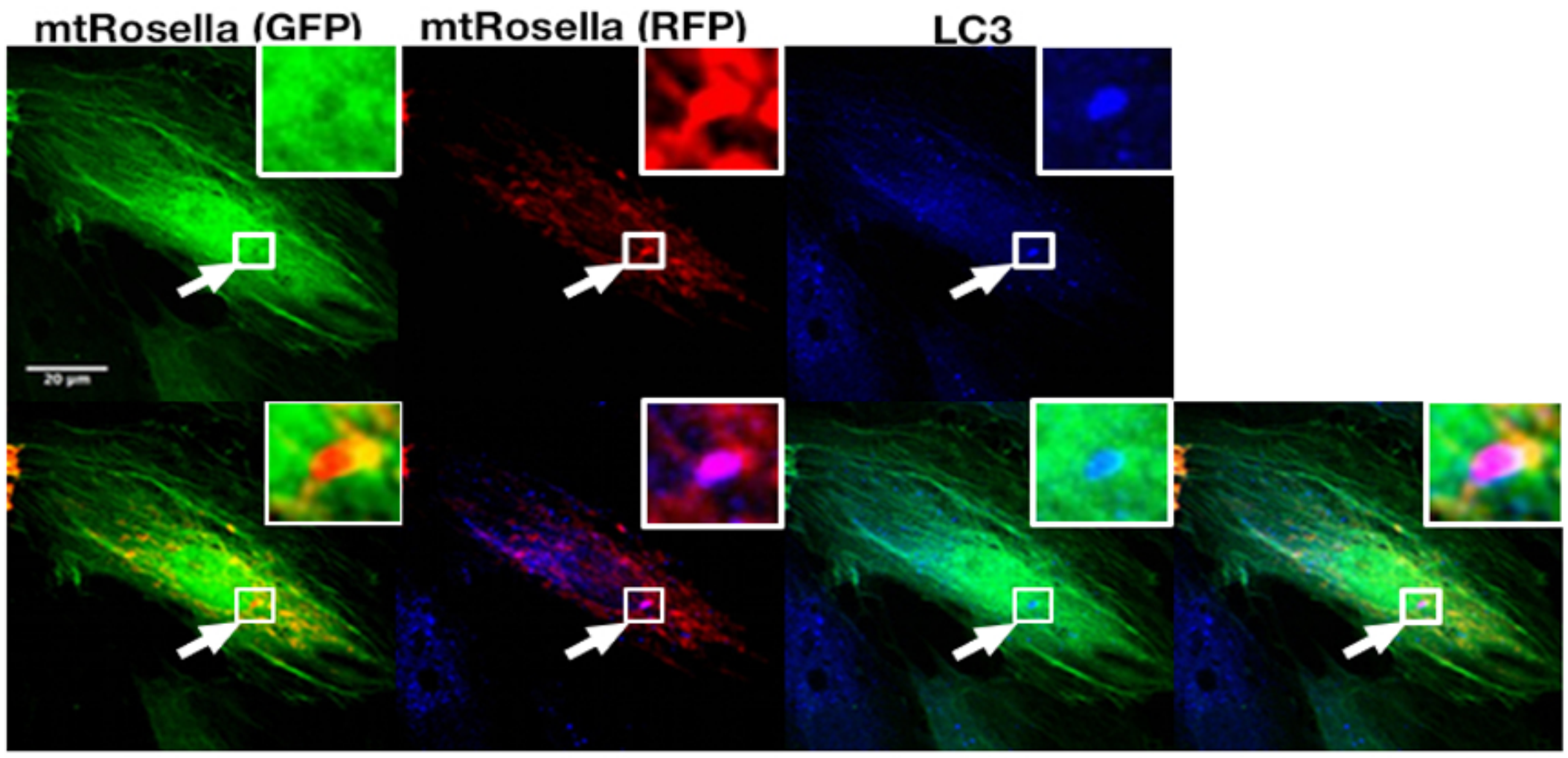
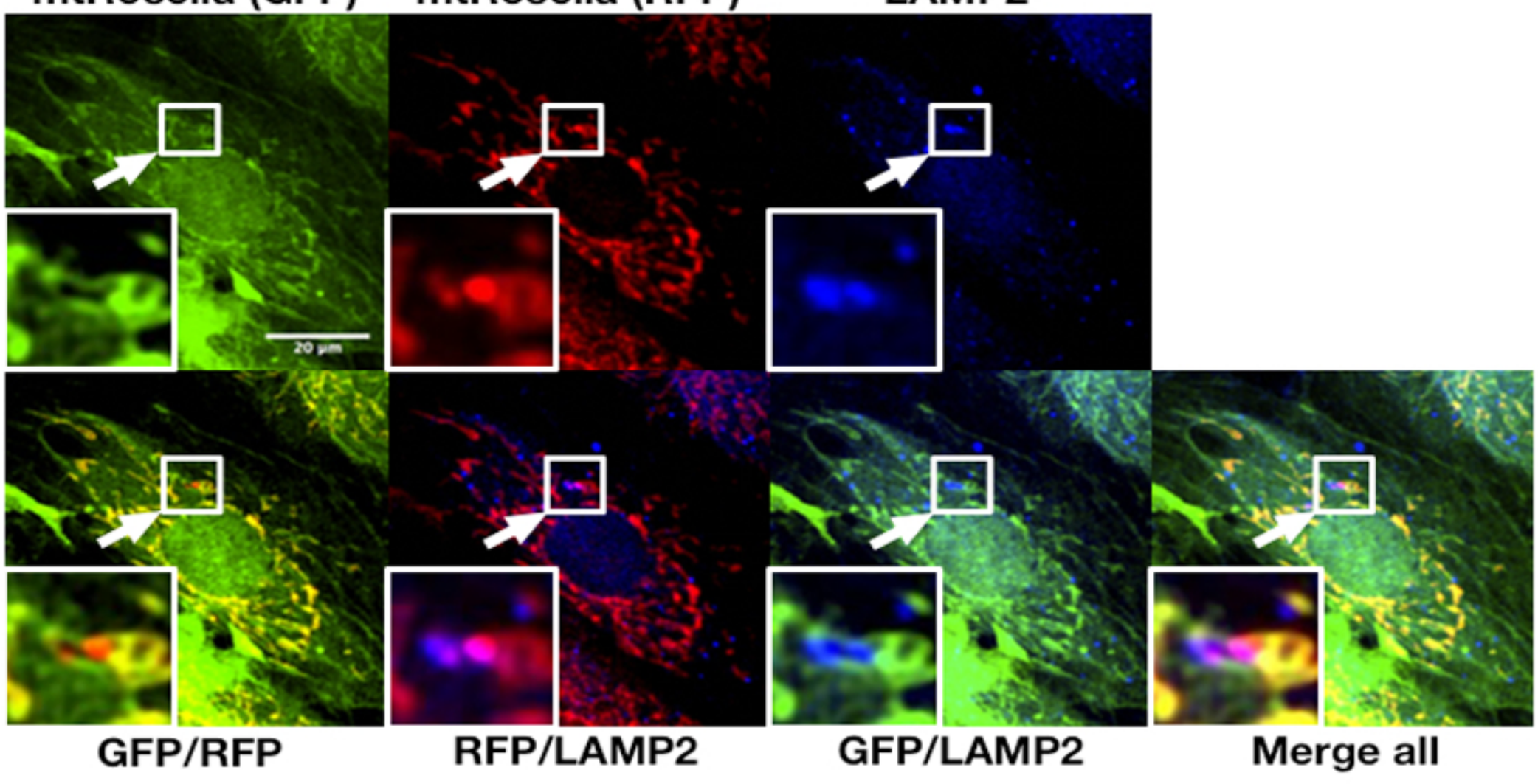


C



Supplemental Figure 2