Supplemental Material to:

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M98K-OPTN induces transferrin receptor degradation and RAB12-mediated autophagic death in retinal ganglion cells

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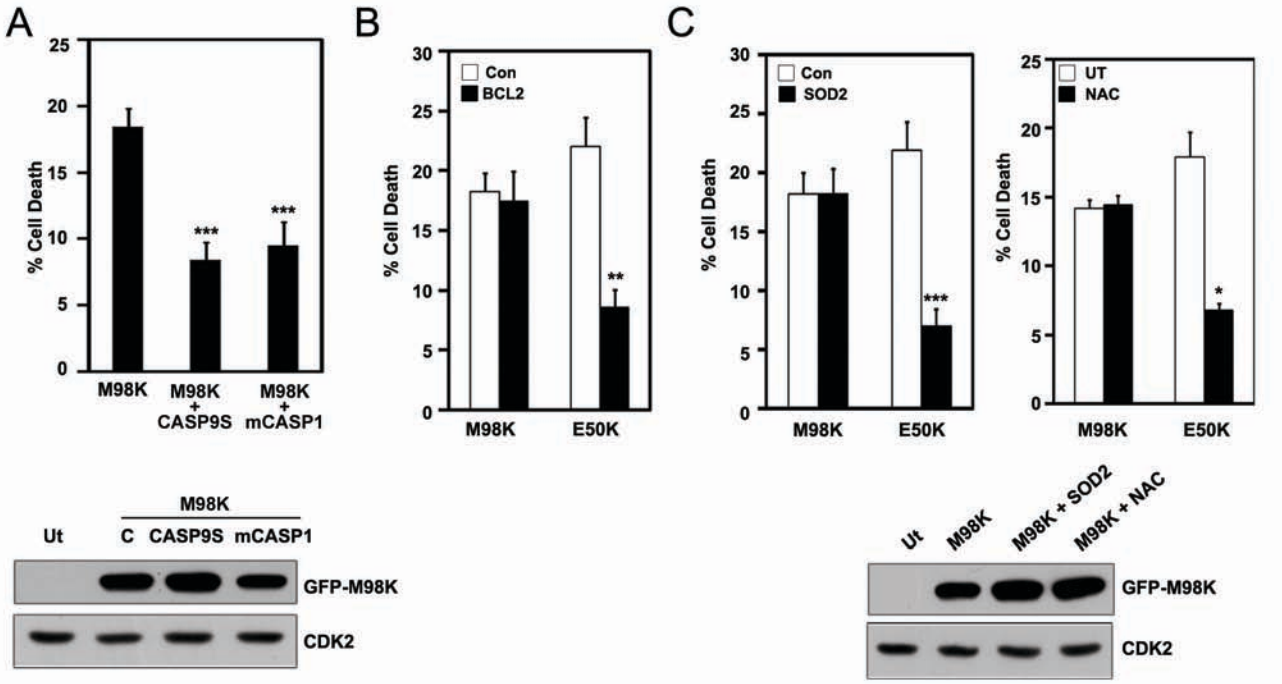

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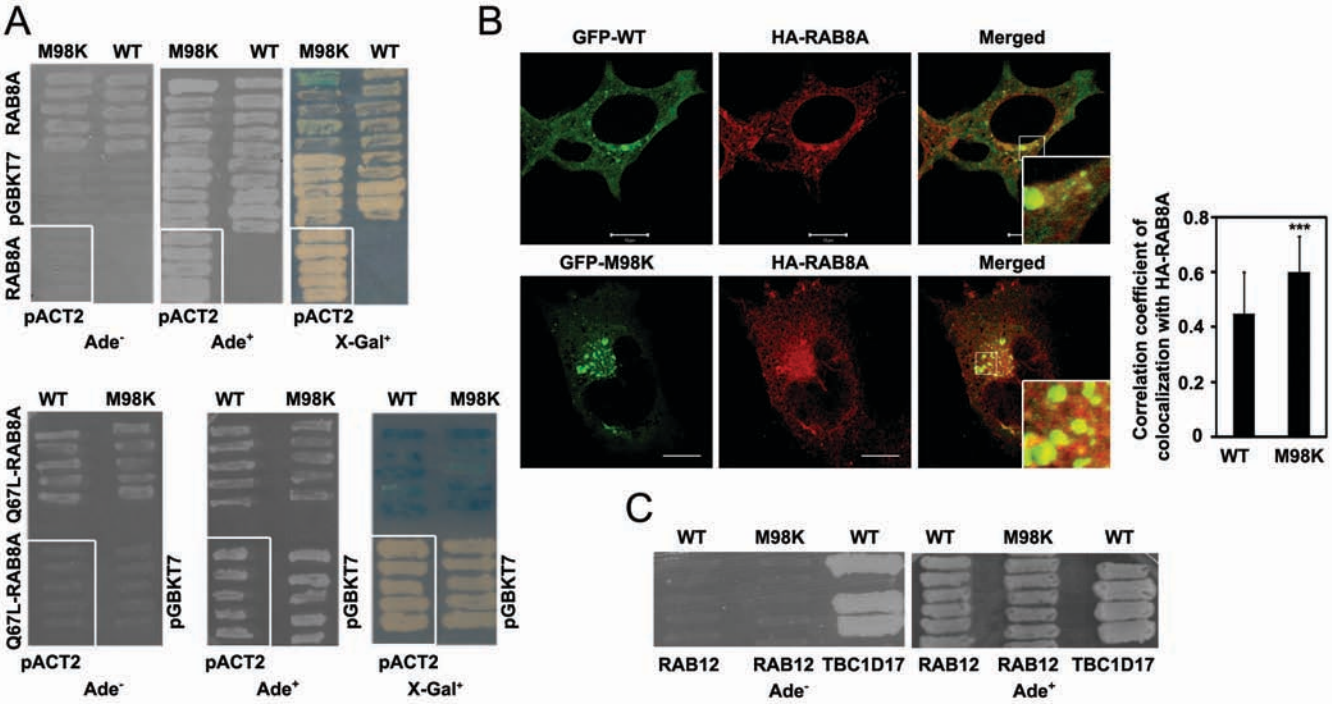


Figure S2. Interaction of WT-OPTN and M98K mutant with RAB8A and RAB12. (A) WT-OPTN and M98K do not differ in their interaction with Rab8A. Yeast strain cotransformed with RAB8A (upper panel) or activated RAB8A (Q67L-RAB8A) (lower panel) and WT-OPTN or M98K mutant showed growth on selection media (Ade^-) and blue color on $X\text{-}Gal^+$ media. (B) Left panel, representative confocal images show colocalization of WT-OPTN and M98K with HA-RAB8A. Right panel, The graph represents correlation coefficient of colocalization between GFP-WT, or GFP-M98K and HA-RAB8A. n=30, ***, $P<0.001$. (C) WT-OPTN and M98K do not show direct interaction with RAB12. Yeast strain cotransformed with RAB12 and WT-OPTN or M98K mutant do not show growth on selection media (Ade^-). Interaction of WT-OPTN with TBC1D17 was used as a positive control.

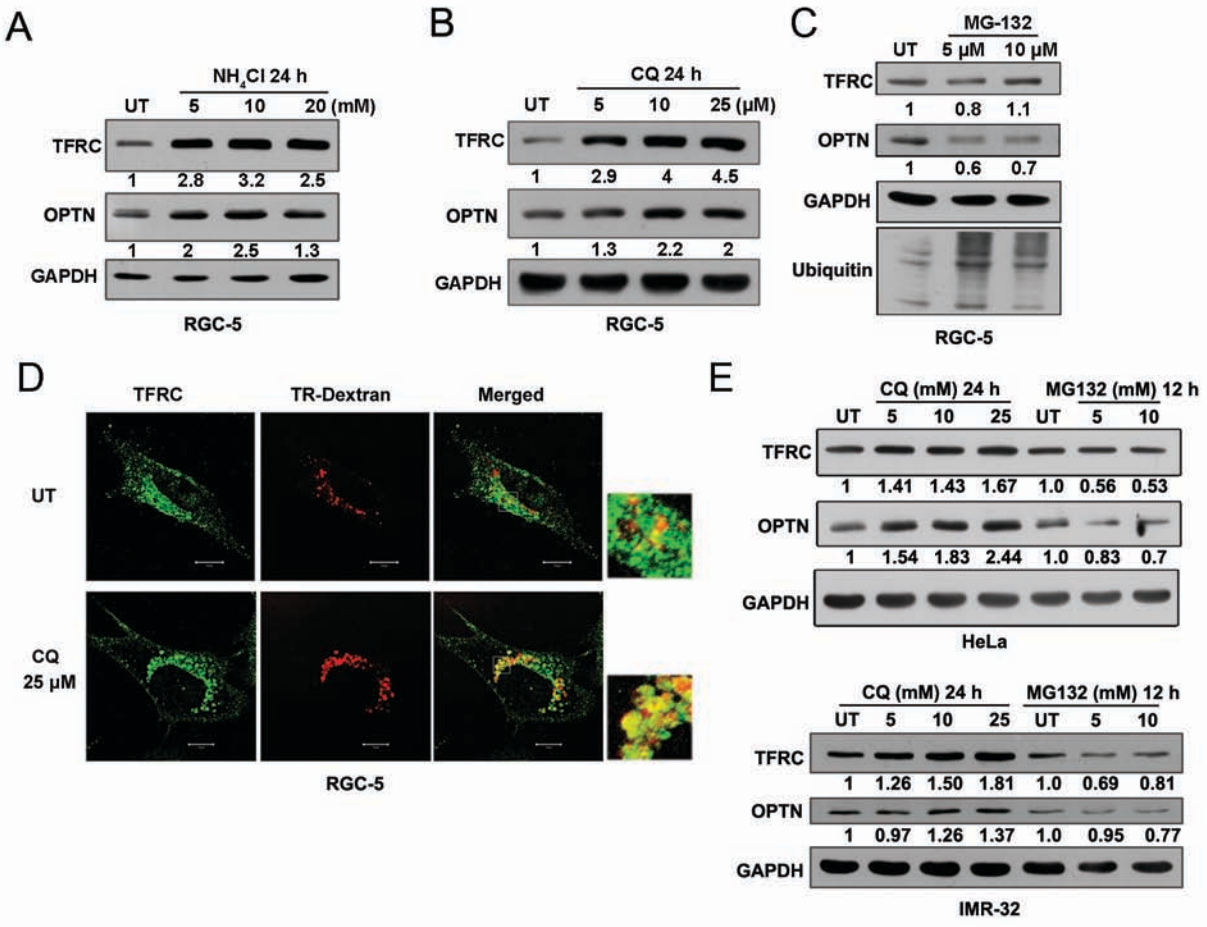


Figure S3. Cellular TFRC and OPTN turnover is regulated through the lysosomal pathway in RGC-5 cells. (A, B) Effect of lysosomal inhibitors on TFRC and OPTN levels in RGC-5 cells. RGC-5 cells were treated with indicated concentrations of ammonium chloride (A) or chloroquine (B) for 24 h and cell lysates were subjected to western blotting with TFRC, OPTN and GAPDH antibodies. The numbers below TFRC and OPTN blots indicate relative levels after normalization. UT, untreated; CQ, chloroquine. (C) RGC-5 cells were treated with 5 and 10 μM of MG-132 for 12 h and the cell lysates were subjected to western blotting with TFRC, OPTN, GAPDH and ubiquitin antibodies. UT, untreated. (D) Treatment with lysosomal inhibitor causes accumulation of TFRC in dextran positive lysosomal compartment. RGC-5 cells were treated with Texas Red conjugated Dextran (TR-Dextran) for 8 h and left untreated or treated with 25 μM of chloroquine for 18 h. Cells were analyzed by confocal microscopy. Areas shown in higher magnification are indicated. Scale bar: 10 μm. UT, untreated; CQ, chloroquine. (E) Western blots showing the effect of lysosomal and proteosomal inhibitors on TFRC and OPTN levels in HeLa (upper panels) and IMR-32 (lower panels). UT, untreated; CQ, chloroquine.

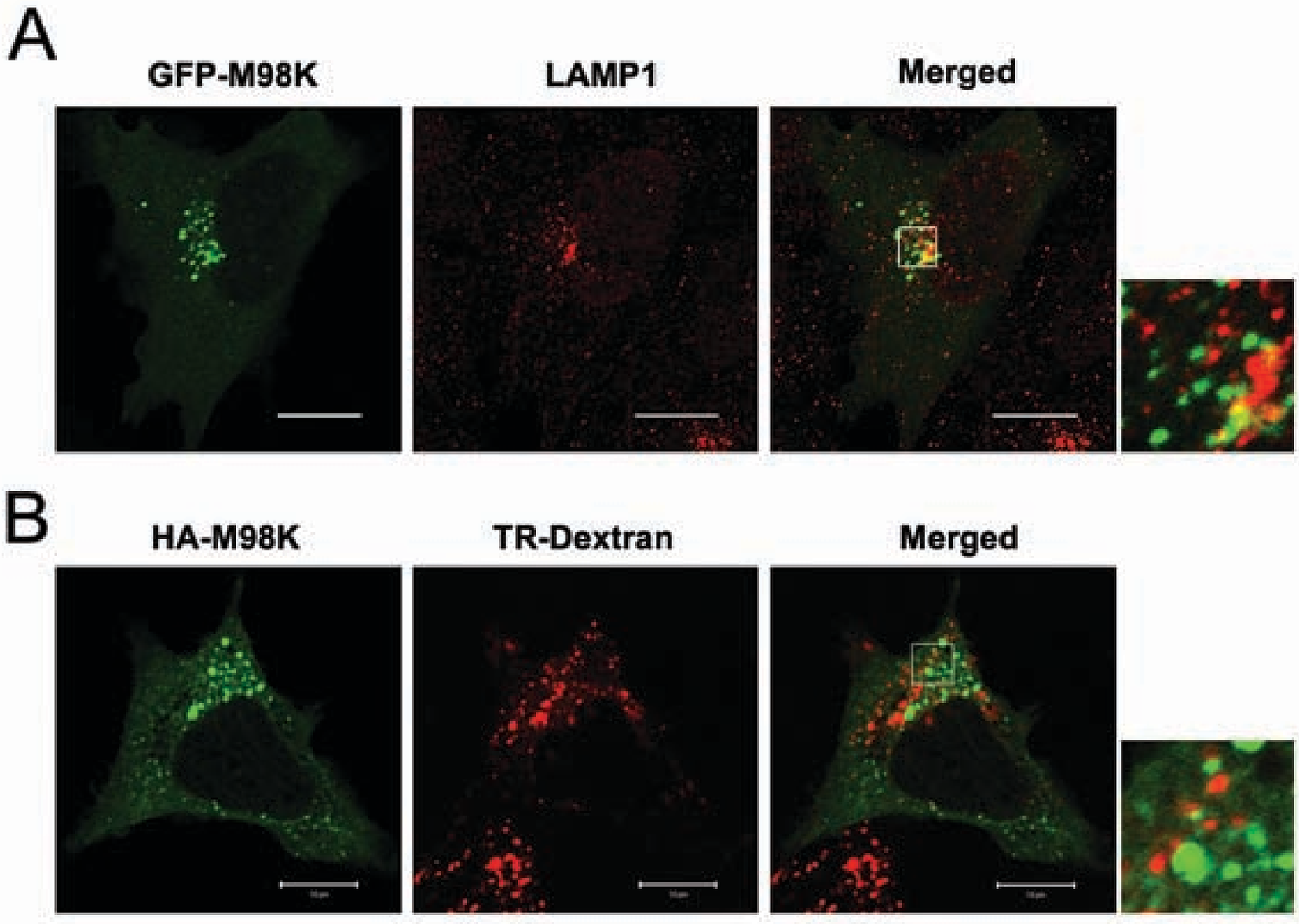


Figure S4. Colocalization of M98K with lysosomal markers. Representative confocal images showing colocalization of M98K with LAMP1 (A) and with Texas-conjugated Dextran Red (TR-Dextran) (B). Magnified areas are shown as insets. Scale bar: 10 μm.

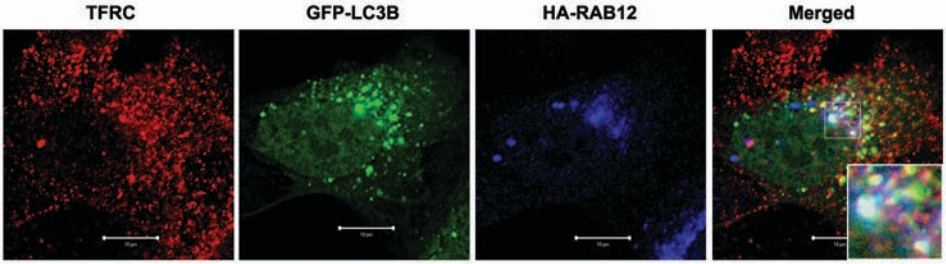
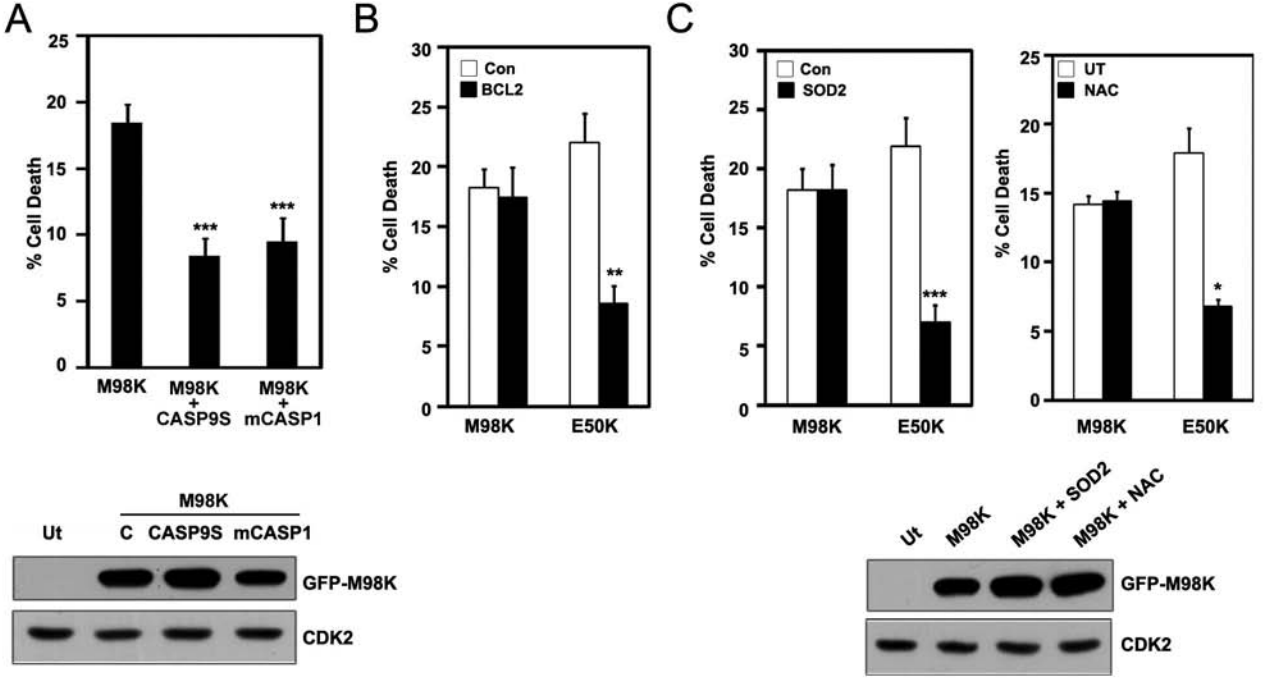


Figure S5. RAB12 colocalizes with TFRC in autophagosomes. Representative confocal images of colocalization of HA-RAB12 with endogenous TFRC in GFP-LC3B-positive structures (autophagosomes) represented by white spots in merged image. Inset shows the enlarged view.



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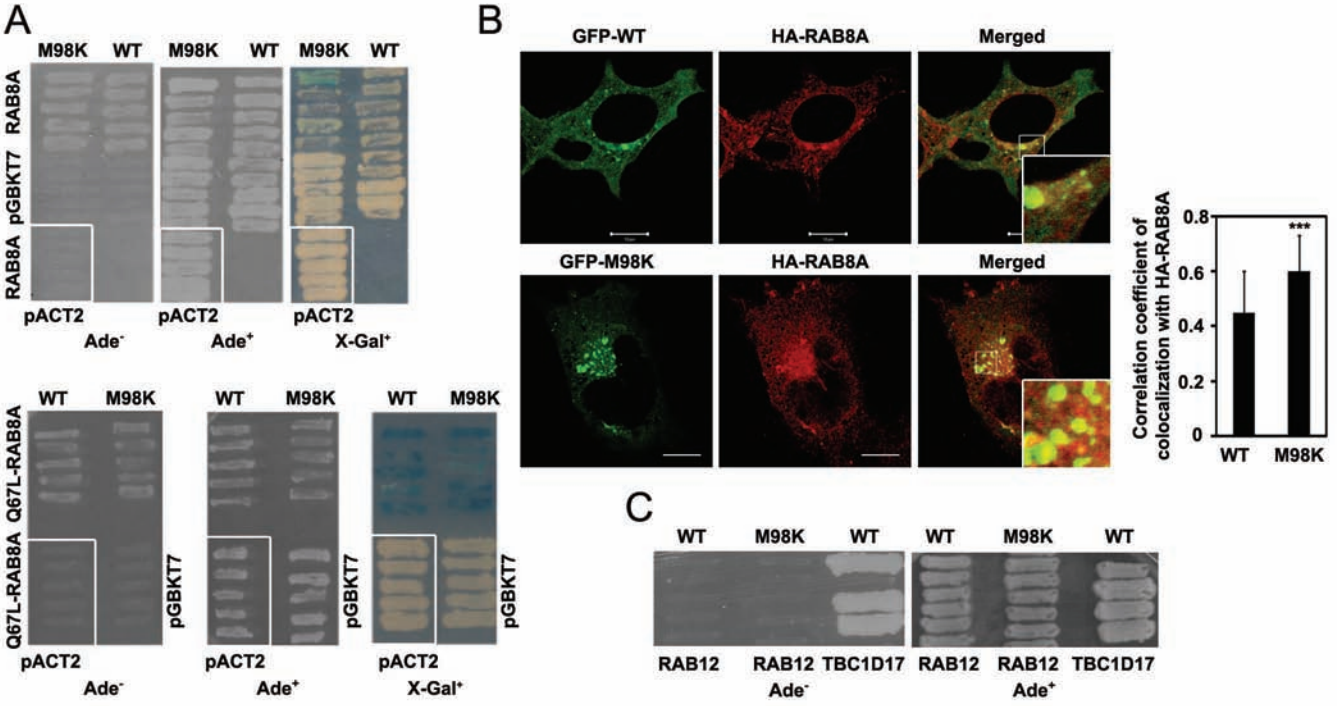

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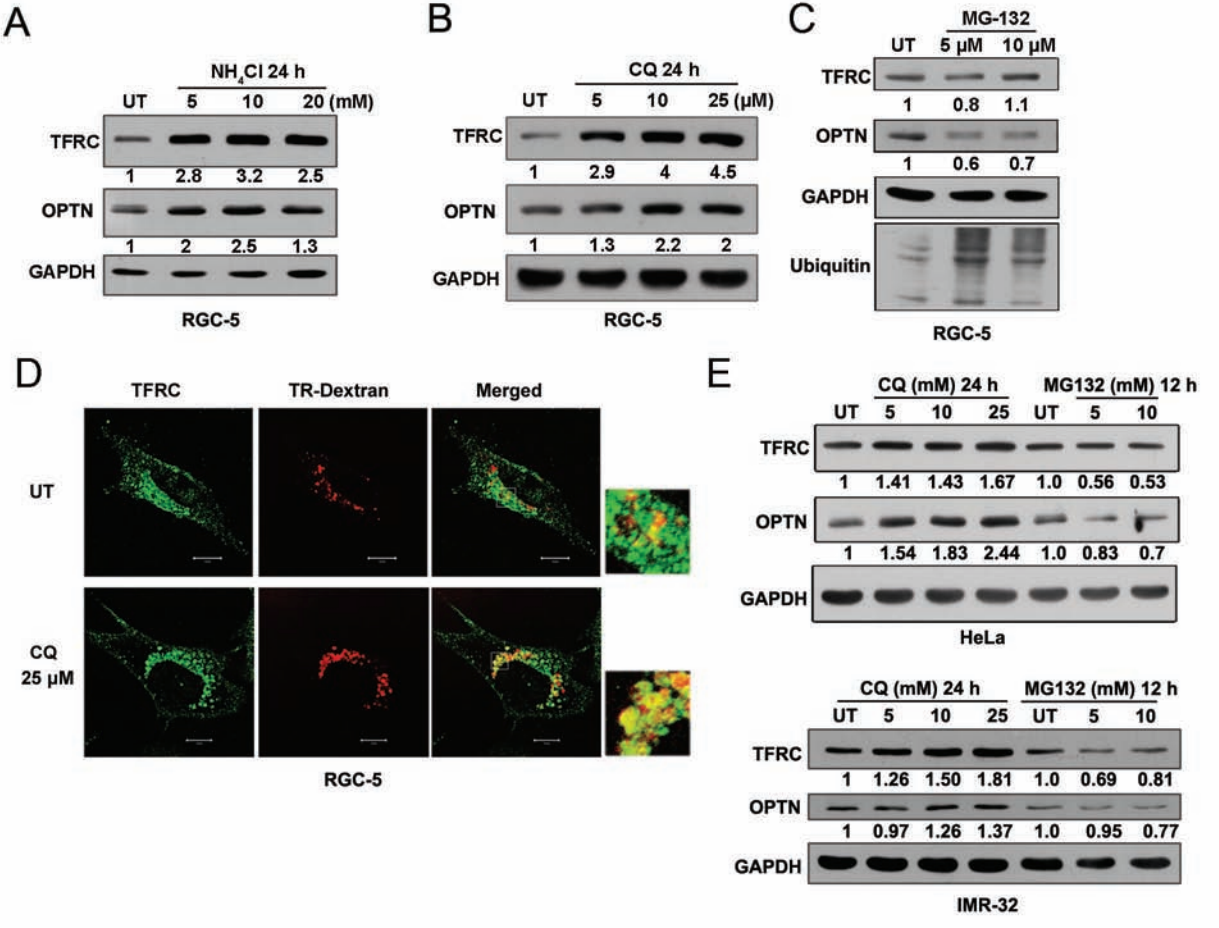

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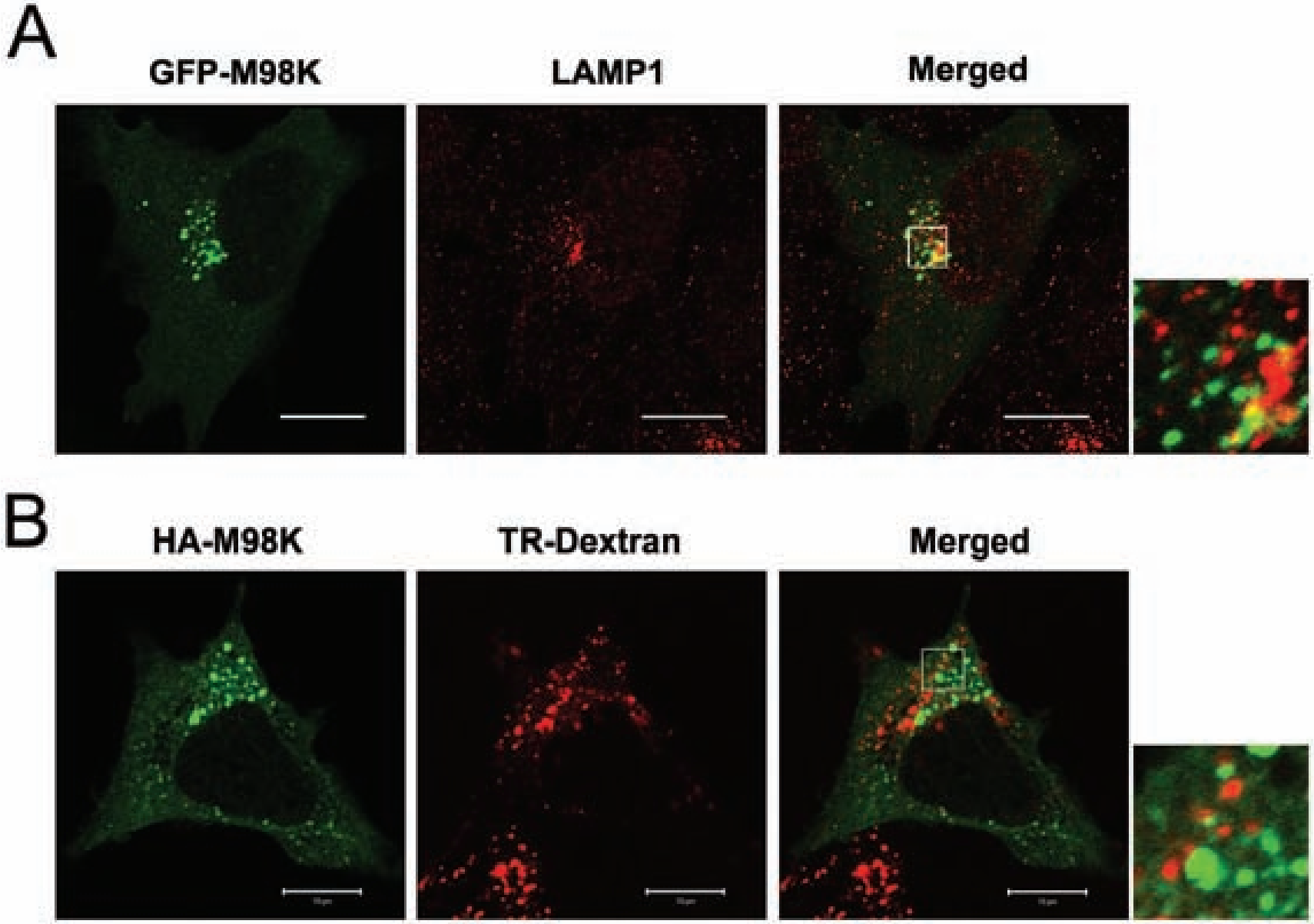

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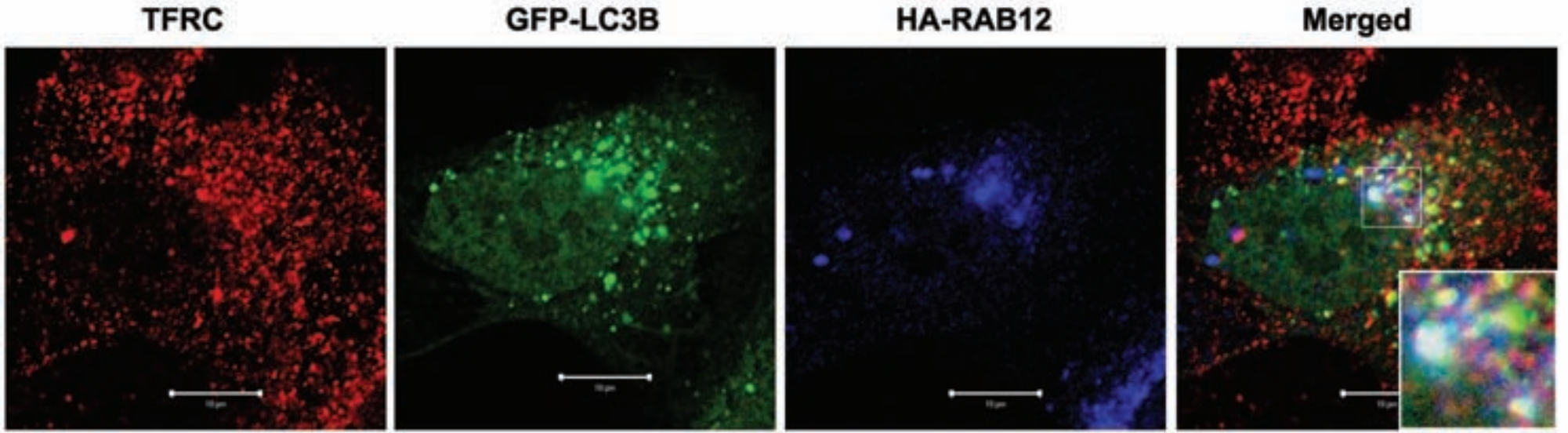

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