

Immunofluorescence

Tissues were harvested and embedded in OCT Compound and frozen in isopentane in liquid nitrogen. They were sectioned to 7 μ m and stained as previously described (21). LAMP1 (DSHB 1D4B) and LC3 (Novus NB100-2200) antibodies were applied overnight and identified with anti-rat AlexaFluorTM488 and anti-rabbit AlexaFluorTM 594 secondary antibodies (Invitrogen). Sections were imaged using an ECHO Revolve 4.

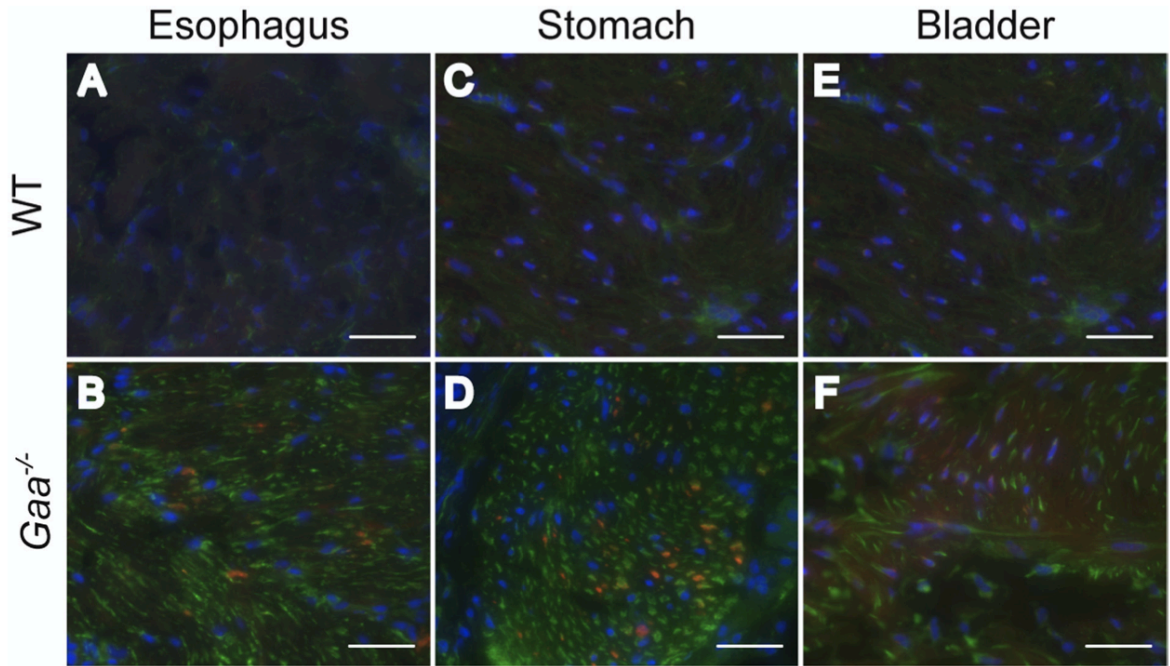


Fig. 4. Vacuolar accumulation in 6 month old *Gaa*^{-/-} mouse smooth muscle-containing tissues. Representative images of smooth muscle in the esophagus, stomach, and bladder from *Gaa*^{-/-} and WT mice, stained with LAMP1 (green) and LC3 (red) antibodies. In each tissue of the *Gaa*^{-/-} mouse there is an abundance of LAMP1+ puncta with fewer LC3+ puncta, indicating a significant accumulation of lysosomes and a moderate accumulation of autophagosomes compared to the WT tissues devoid of any positive staining. Scale bar=40 μ m.