Ellis et al., http://www.jcb.org/cgi/content/full/jcb.201212095/DC1

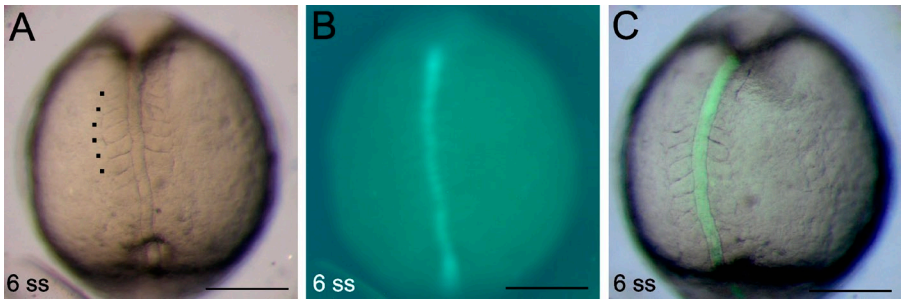

Figure S1. **The *rcn3* promoter turns on at 6 ss and is specific to the notochord.** (A) Whole-mount bright field image of a 6-ss transgenic GFP-CaaX embryo—*Tg(rcn3:GFP-CaaX)*. Dots indicate somites. (B) GFP-CaaX localizes specifically to the notochord. (C) Merge of brightfield and GFP-CaaX. Bars, 200 µm.

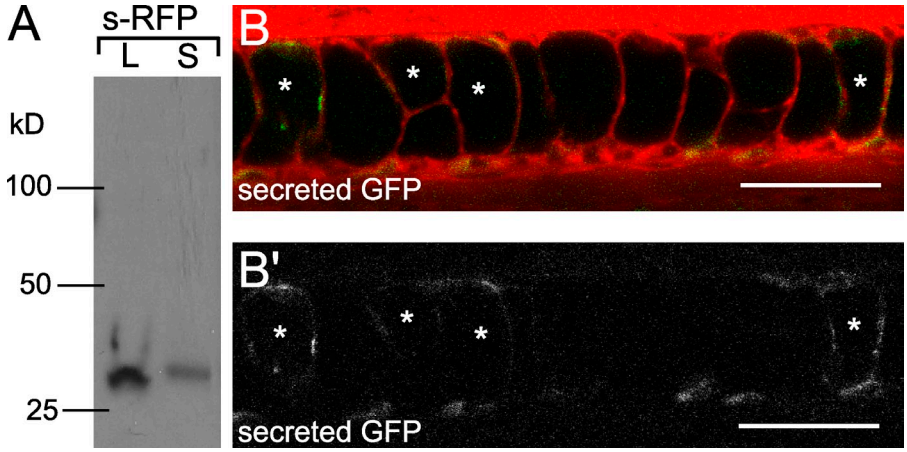

Figure S2. **Secreted GFP does not accumulate in the vacuole.** (A) Western blot of HEK293AD cells expressing secreted RFP. A GFP-tagged version of the same protein was used for the transgenic fish line. L, cell lysate; S, supernatant. (B and B′) Live confocal image of a 24-hpf embryo expressing secreted GFP (*Tg(rcn3:gal4); Tg(UAS:s-GFP)*), visualized with MED (B) and grayscale GFP alone (B′). Asterisks label expressing cells. Bars, 50 µm.

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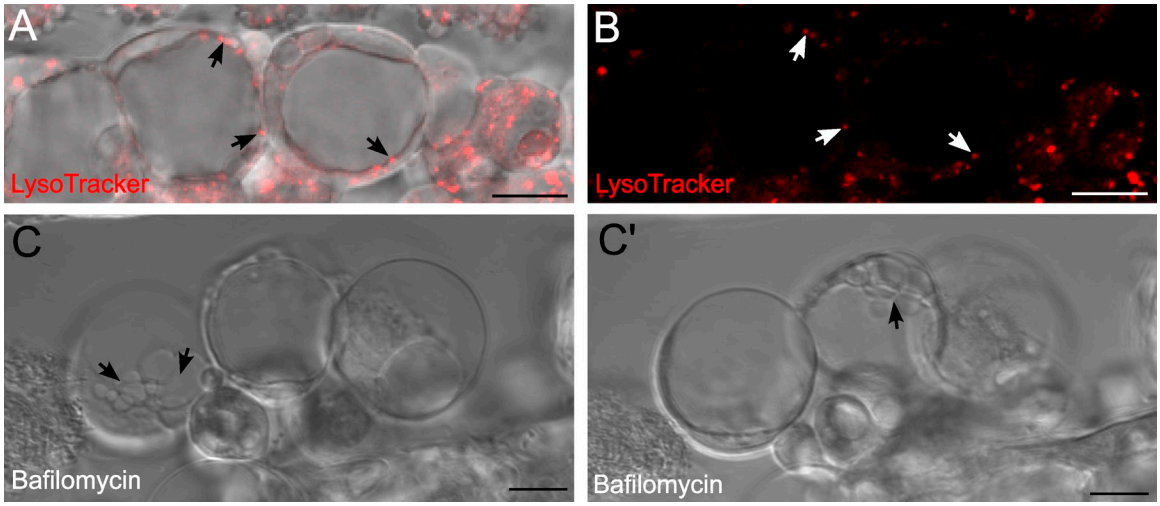

Figure S3. **The lumen of the vacuole is not acidic, yet acidification is necessary for vacuole maintenance.** (A) Confocal image (bright field and LysoTracker merged) of cells from a dissected notochord of a 24-hpf embryo incubated with LysoTracker for 20 min. (B) LysoTracker alone. (C and C′) Confocal images of cells from a dissected notochord of a 24-hpf embryo incubated with 1 µM bafilomycin for 2 h. Arrows indicate fragmenting vacuoles. The same cells are shown in C and C′ in different focal planes to show vacuole fragmentation. Bars, 10 µm.

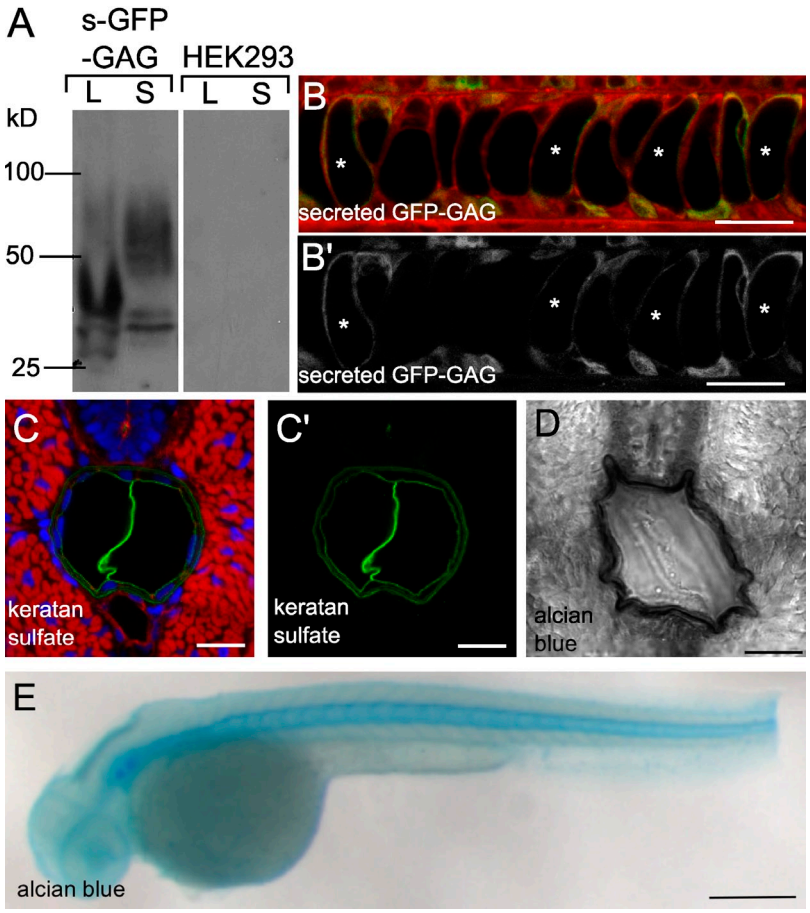

Figure S4. **Glycosaminoglycans are not present in the lumen of the vacuole.** (A) Western blot of HEK293AD cells expressing secreted GFP-GAG. The characteristic smear in the supernatant is indicative of GAG modification. L, cell lysate; S, supernatant. (B and B′) Live confocal image of a transgenic 24-hpf embryo expressing GAG-tagged secreted GFP (*Tg(rcn3:gal4); Tg(UAS:s-GFP-GAG)*), visualized with MED (B) and grayscale GFP-GAG alone (B′). Asterisks label expressing cells. (C) Confocal image of a cross section of a 2-dpf embryo immunostained with an antibody against keratan sulfate (green), phalloidin (red), and DAPI (blue). (C′) anti-keratan sulfate alone. (E) 2-dpf embryo labeled with Alcian blue reveals staining in developing otoliths and perinotochordal sheath. (D) Cross section of a 2-dpf Alcian blue–stained embryo. Alcian blue labels the perinotochordal sheath but not the lumen of the vacuoles. Bars: (B and B′) 50 µm; (C and D) 20 µm; (E) 250 µm.