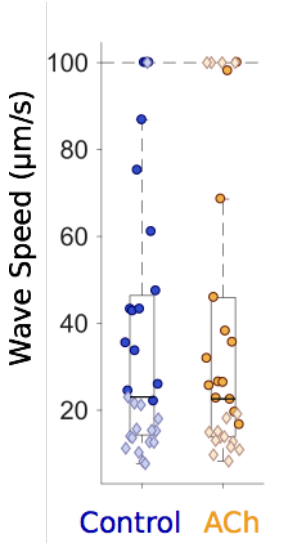
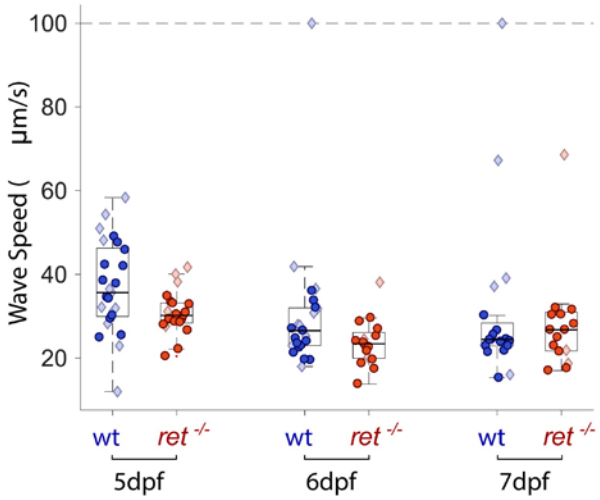
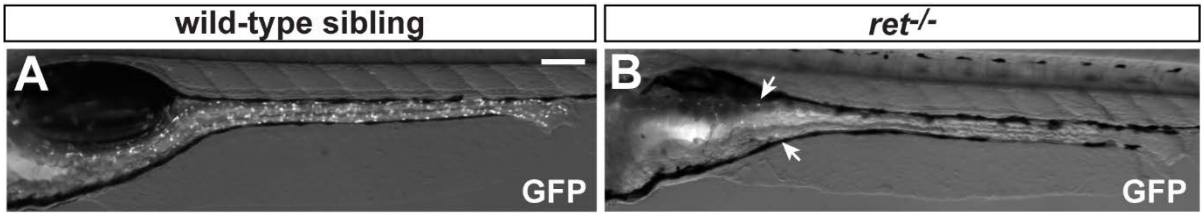


Supplementary Figure 1: Acetylcholine does not alter the wave speed of zebrafish gut motility. Wave propagation speeds for 6 dpf control larvae (blue, n=31) and larvae immersed in acetylcholine [ACh; 2.5mg/ml (orange, n=30)]. As larger velocities tend to be unreliable due to the low temporal resolution of our data, measured velocities are capped at a user-defined threshold, given by the dashed line. Each point is derived from a five minute video of a single larva. Darker circles and lighter diamonds represent two independent experiments.



Supplementary Figure 2: *ret* mutant zebrafish larvae show no noticeable difference in gut motility wave speed compared to wild-type (wt) siblings. Wave propagation speeds for wt (blue, n=25, 23, 20) and *ret*$^{-/-}$ (red, n=21, 16, 16) larvae over three days of development. As larger velocities tend to be unreliable due to the low temporal resolution of our data, measured velocities are capped at a user-defined threshold, given by the dashed line. Each point is derived from a five minute video of a single fish. Darker circles and lighter diamonds represent two independent experiments.



Supplementary Figure 3: *ret* mutant larvae lack ENS innervation. Lateral views of 6 dpf sibling larvae, from combined brightfield and fluorescence images. (A) Wild-type larva with ENS neurons expressing GFP driven by the *phox2b* promoter (*phox2b*:GFP) along the entire length of the gut. (B) *ret* mutant larva, which lacks ENS innervation except for a few GFP-positive ENS neurons in the anterior-most part of the gut (arrows). Scale bar = 100µm in A, B.

See file SupplMovie1.avi

Supplementary Movie 1: Larval gut motility and Particle Image Velocimetry. (Top) DIC movie of larval zebrafish gut motility with PIV vectors overlaid in red. The magnitude of the vector represents the instantaneous velocity of a small section of the gut and the angle represents the direction it is traveling. Total time: 22 seconds. (Bottom) Averaging the anterior-posterior component of the velocity along the dorsal-ventral direction generates a single curve at each time point. QSTMaps are the surfaces generated by these curves over time.