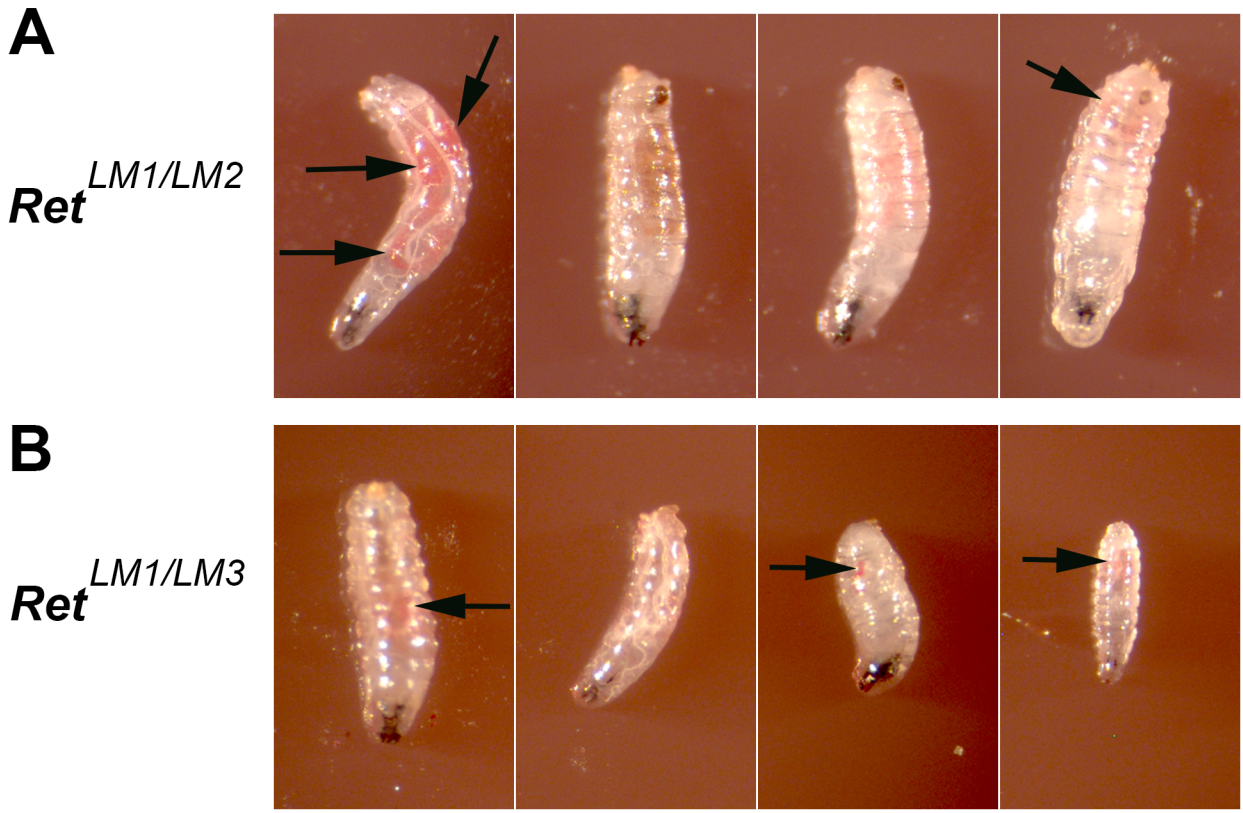
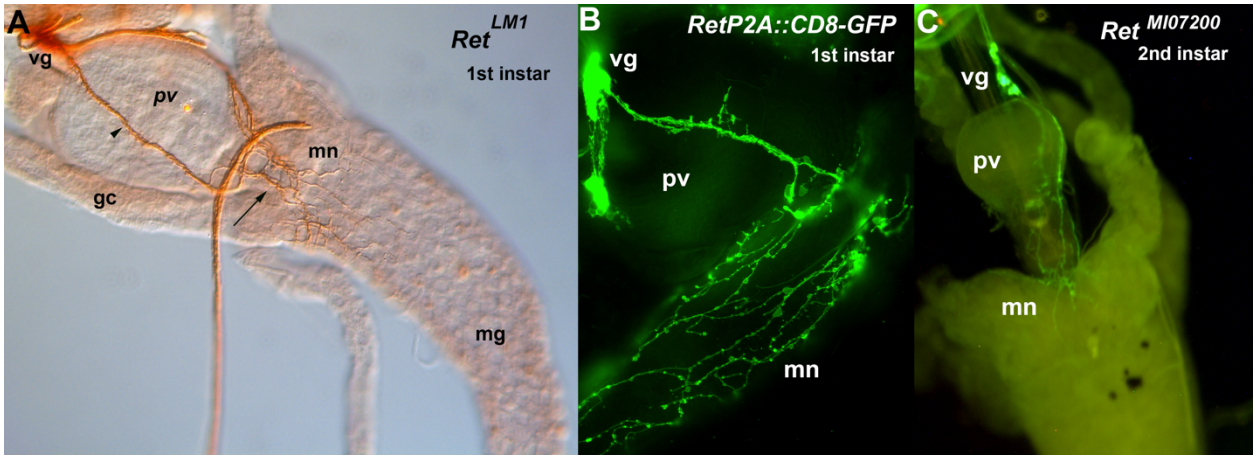
Supplemental Information



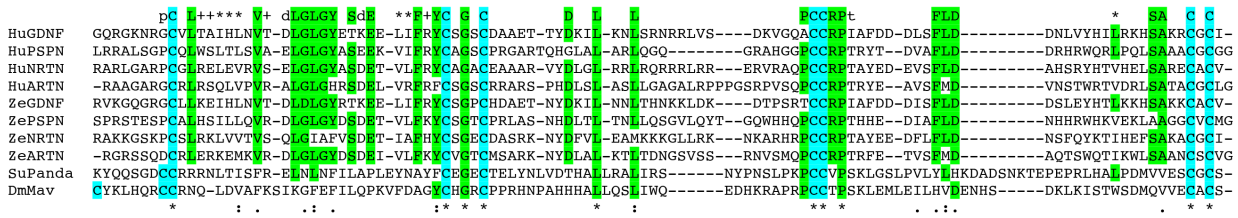
Supplemental Figure 1. Feeding defects in CRISPR allele combinations.

CRISPR alleles of Ret were crossed and the homozygous larvae examined for feeding defects. Homozygous larvae were selected on the basis of foraging behavior (moving away from the central food source). Larvae are oriented with the anterior down (the head is distinguished by black mouthhooks). *(A)* Ret^{LM1}/Ret^{LM2} larvae showing a range of food ingestion as revealed by pink coloring inside the larvae. The food is most visible in the leftmost larvae (arrows) and the tubular nature of the midgut is evident towards the anterior. Other larvae show markedly reduced levels of food intake; n=55. *(B)* Ret^{LM1}/Ret^{LM2} larvae showing a range of food ingestion (arrows) and retarded growth; n=40.



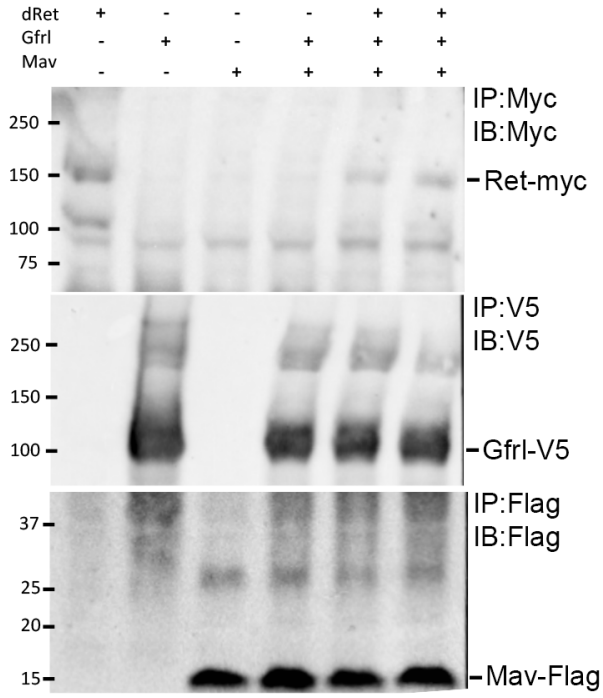
Supplemental Figure 2. Larval midgut axons

(A) Ret^{LM1} homozygous first instar larva stained with 22c10 to reveal the midgut axons (mn) and the ventricular ganglion (vg). The midgut axons are tightly fasciculated crossing the proventriculus (pv, arrowhead), but rapidly start branching on encountering the anterior midgut and do not project very far into the midgut (mg). The prep is partially obscured a 22c10 positive bundle from another part of the nervous system (entering from the bottom of the panel and projecting vertically). *(B)* Expression of *RetP2A-GAL4* in a first instar larva visualized with *UAS-CD8-GFP*. Although wild type, a minor amount of branching can be seen as the midgut axons cross the proventriculus, although this might be a difference between CD8-GPF and 22c10 antigen distribution. Varicosities in the mn are clearly visible. There is no detectable expression in the underlying gut tissues. *(C)* A transposon insertion into the *Ret* gene (*MI07200*) appears to act as a transcriptional fusion, expressing GPF in the pattern of the *Ret* mRNA in the embryo and larvae. Fluorescence in a second instar larva (bright green) is visible in the vg and mn, but there is no fluorescence in the underlying tissues. Lipid auto-fluorescence from the gut tissue is visible as a dull yellow-green that is distinct from GFP fluorescence.



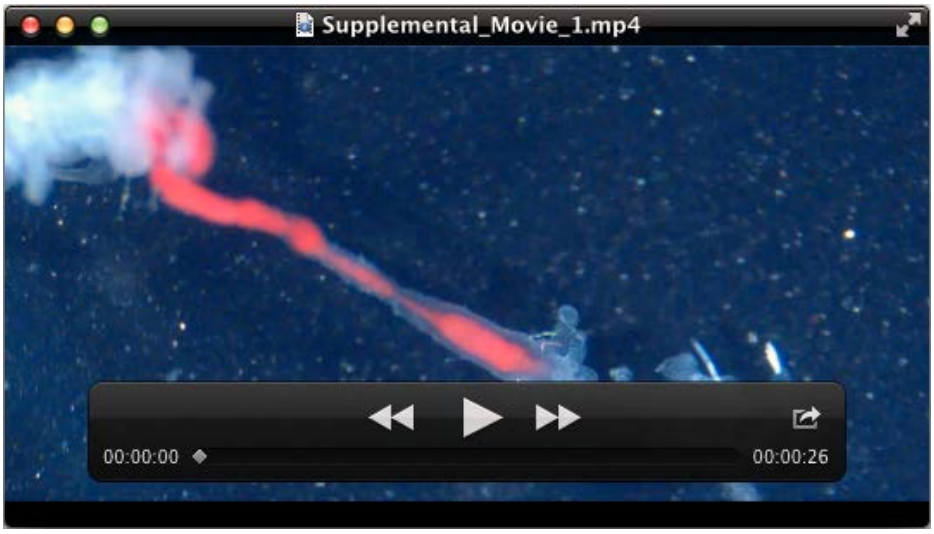
Supplemental Figure 3. Alignment of GDNF family proteins with Maverick.

CLUSTAL alignment of human (Hu) and zebrafish (Ze) GDNF, Neurturin (NRTN), Artemin (ARTN) and Persephin (PSPN) with *Drosophila* Maverick (DmMav) and *Paracentrotus lividus* Panda/Maverick (SuPanda). Conserved cysteines are highlighted in blue. Highly conserved amino acids in the GDNF family are in green and indicated above the alignment. Conservation between GDNF and Mav proteins are shown below. Asterisks indicate fully conserved residues, colons residues of strongly similar properties, periods are weakly similar residues.



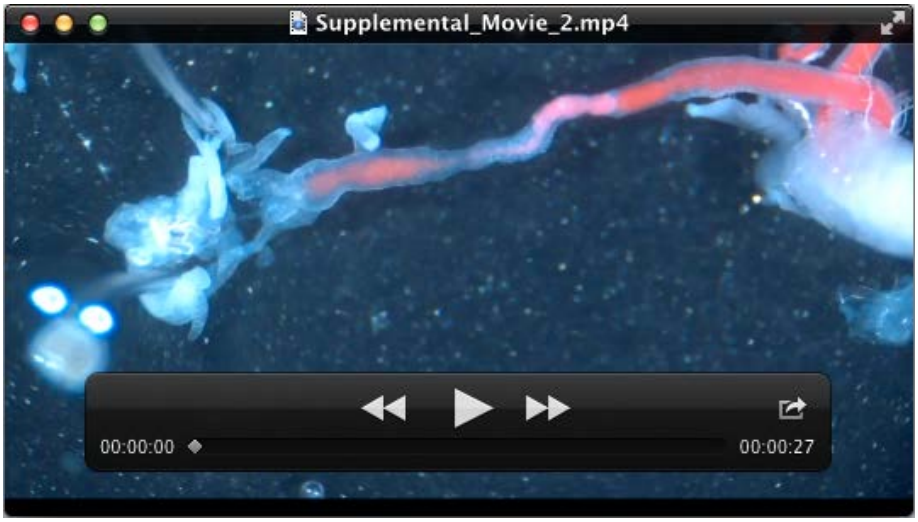
Supplemental Figure 4. Cell culture expression of Ret, Gfrl and Mav.

Full-length Ret, Gfrl, and Mav proteins were expressed in HEK293 and immunoprecipitated using the following epitope tags: Ret – C-terminal myc tag, Gfrl – N-terminal V5 tag, Mav – C-terminal DYK/Flag tag. Observed proteins sizes were 150kDa, 100kDa, and 14.2kDa respectively. Despite sample preparation by boiling in the presence of SDS, apparent dimers for Gfrl and Mav can be observed.



Supplemental Movie 1. Peristalsis of a dissected w^{1118} third instar midgut.

A w^{1118} third instar midgut was dissected in Schneider's medium and pinned in place. The head is to the right. Yeast colored with Carmine Red is visible in the midgut (center) and also the esophagus (thinner section to the right disappearing into the head tissues). The proventriculus is a bulge connected to the thin esophagus in center right and identifiable by the tentacle like gastric caeca projecting from it. Pink food is not retained in the proventriculus. The most visible peristaltic contractions initiate just posterior to the proventriculus and propagate as a wave down the midgut. This region is innervated by the larval axons. Contractions are also visible in the head and esophagus to the right.



Supplemental Movie 2. Peristalsis of a dissected *Ret* third instar midgut.

A Ret^{LM1} mutant third instar larval midgut dissected and observed. The head is to the left with two metal pins inserted into it. The thin esophagus is visible connecting the head to the proventriculus and pink food is visible immediately posterior in the start of the midgut. Peristaltic contractions are visible in the esophagus, proventriculus and the start of the midgut but fail to propagate as a wave into the rest of the midgut.

Host and target	Working dilution	Company
22c20 mouse anti-futsch	1:10	DSHB, University of Iowa, IA
AlexaFluro Bovine anti-goat 488 (805-545-180)	1:1000	Jackson Immuno Research, West Grove, PA
AlexaFluor Goat anti-mouse 568 (ab175473)	1:1000	Abcam, Cambridge, MA
AlexaFluro Goat anti-rabbit 594 (111-585-144)	1:1000	Jackson Immuno Research, West Grove, PA
Donkey anti-goat: Biotin (705-065-147)	1:250	Jackson Immuno Research, West Grove, PA
Donkey anti-goat: HRP (705-035-147)	1:1000	Jackson Immuno Research, West Grove, PA
Donkey anti-mouse: Biotin (715-065-150)	1:250	Jackson Immuno Research, West Grove, PA
Donkey anti-rabbit: Biotin (711-065-152)	1:500	Jackson Immuno Research, West Grove, PA
Goat anti-flag/DDDDK (ab1257)	1:1000	Abcam, Cambridge, MA
Goat anti-mouse: HRP (115-035-146)	1:1000	Jackson Immuno Research, West Grove, PA
Goat anti-rabbit: HRP (111-035-144)	1:1000	Jackson Immuno Research, West Grove, PA
Mouse anti-c-myc (ab32)	1:500	Abcam, Cambridge, MA
Mouse anti-V5 (R96025)	1:1000	Invitrogen, Carlsbad, CA
Rabbit anti-βgalactosidase (559762)	1:5000	MP Biomedicals, Solon, OH
Rabbit anti-dRet (NP-477044)	1:500	Genscript Biotech Corp., Piscataway, NJ
Rabbit anti-GFP (A11122)	1:500	Life technologies, Carlsbad, CA

Supplemental Table 1. Antibodies used in the study.

Name	Sequence (5`->3`)	Application/Construct
Ret_ex3-1_F	GTCGTGTTTACTTTCCCACCACGT	Ret gRNA 3 oligo^{sense}
Ret_ex3-1_R	AAACACGTGGTGGGAAAGTAAACA	Ret gRNA 3 oligo^{antisense}
Ret_ex5-1_F	GTCGGAACTGCGATTCTCCCGAAA	Ret gRNA 5-1 oligo^{sense}
Ret_ex5-1_R	AAACTTTCGGGAGAATCGCAGTTC	Ret gRNA 5-1 oligo^{antisense}
Ret_ex5-2_F	GTCGGCCTCCAAGGACACACAATA	Ret gRNA 5-2 oligo^{sense}
Ret_ex5-2_R	AAACTATTGTGTGTCCTTGGAGGC	Ret gRNA 5-2 oligo^{antisense}
pCFD3_pcr_F	GCCACCTACTCAGCCAAGAG	Colony PCR screening
pCFD3_pcr_31_R	CTAAAACACGTGGTGGGAAAG	Colony PCR screening
pCFD3_pcr_51_R	GAATCGCAGTTCCGACGTTA	Colony PCR screening
pCFD3_pcr_52_R	GGAGGCCGACGTTAAATTG	Colony PCR screening
dU6-3:gRNA F	ACCTACTCAGCCAAGAGGC	5` sequencing
dU6-3:gRNA R	TGCATACGCATTAAGCGAAC	3` sequencing
Ret_ex3_CRISPR_F	TTCATTGATTGCGGCACAGTG	Fly gDNA screening
Ret_ex3_CRISPR_R	CGTGATCCAAGGTTTGTCGG	Fly gDNA screening
Ret_ex5_CRISPR_F	GGAGGCAAATGCTCACCTTG	Fly gDNA screening
Ret_ex5_CRISPR_R	CAAGCCGCAATATCGAACGC	Fly gDNA screening

Supplemental Table 2. DNA oligonucleotides used in this study.

Table S3. Quantification of embryonic phenotypes and larval mortality.

Raw data with statistical analysis for larval feeding and mortality. Embryonic phenotypes: (FN: frontal nerve, FG: frontal ganglion, RN: recurrent nerve, EG: esophageal ganglia). Measurements of axon length and branching in larval midgut nerves were derived from the Simple Neurite Tracer plugin in Image J and recorded as pixels, converted to millimeters and the branches per 0.1mm calculated. Statistics are shown in the boxes below. Larval gut motility was calculated by observing the number of contractions for a minute and a half. Statistical analysis is below.

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