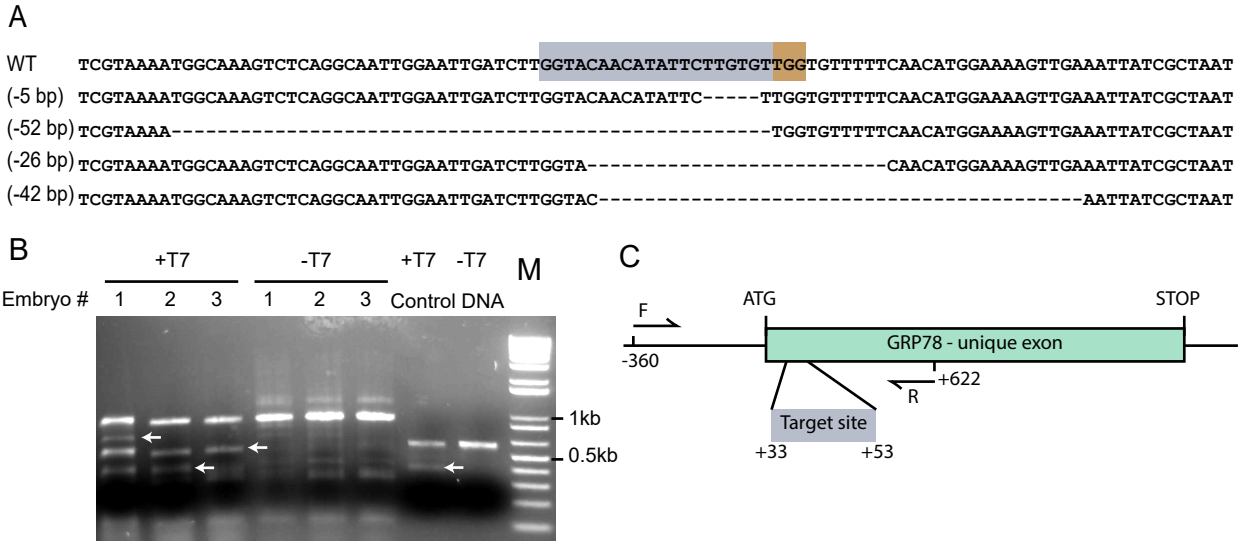
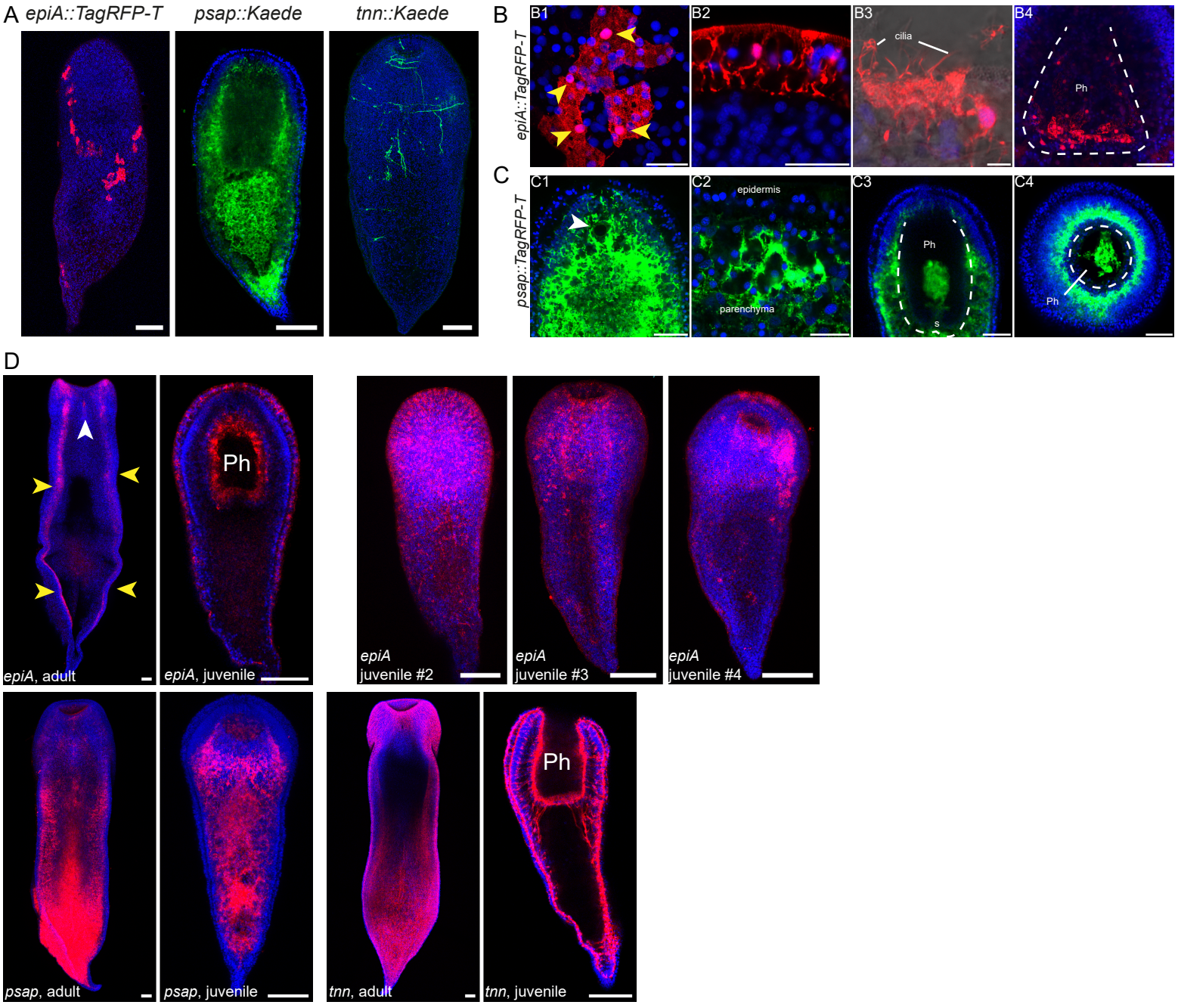
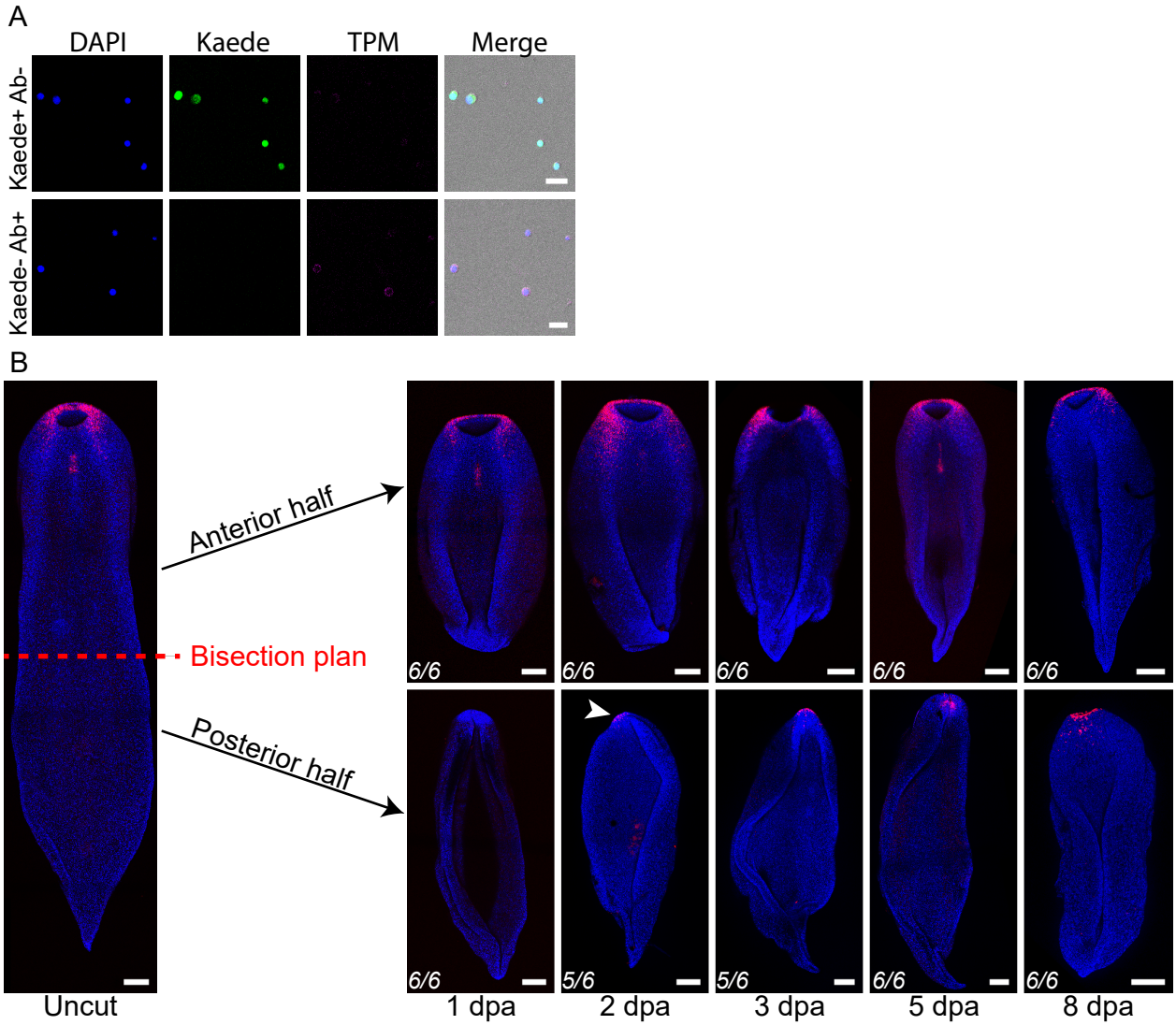
Supplemental Figure 1: Genome editing, related to Figures 1 and 3. (**A**) Sequences of the CAS9 targeted GRP78 locus, from 4 different injected embryos. Wild-type DNA sequence for the locus is shown on top; the number of deleted bases is indicated between parenthesis; grey and brown boxes show CAS9 target and PAM sites, respectively. (**B**) Gel migration of PCR amplified GRP78 locus with (+T7) or without (-T7) T7E1 treatment. White arrows show bands smaller than the expected amplicon, due to T7 activity on double strand DNA mismatch resulting from CRISPR/CAS9 indels (6 out of 9 injected embryos showed similar gel bands and indels); M, markers of molecular weight. (**C**) GRP78 locus, with the CAS9 target site and the primers used for pcr amplification.



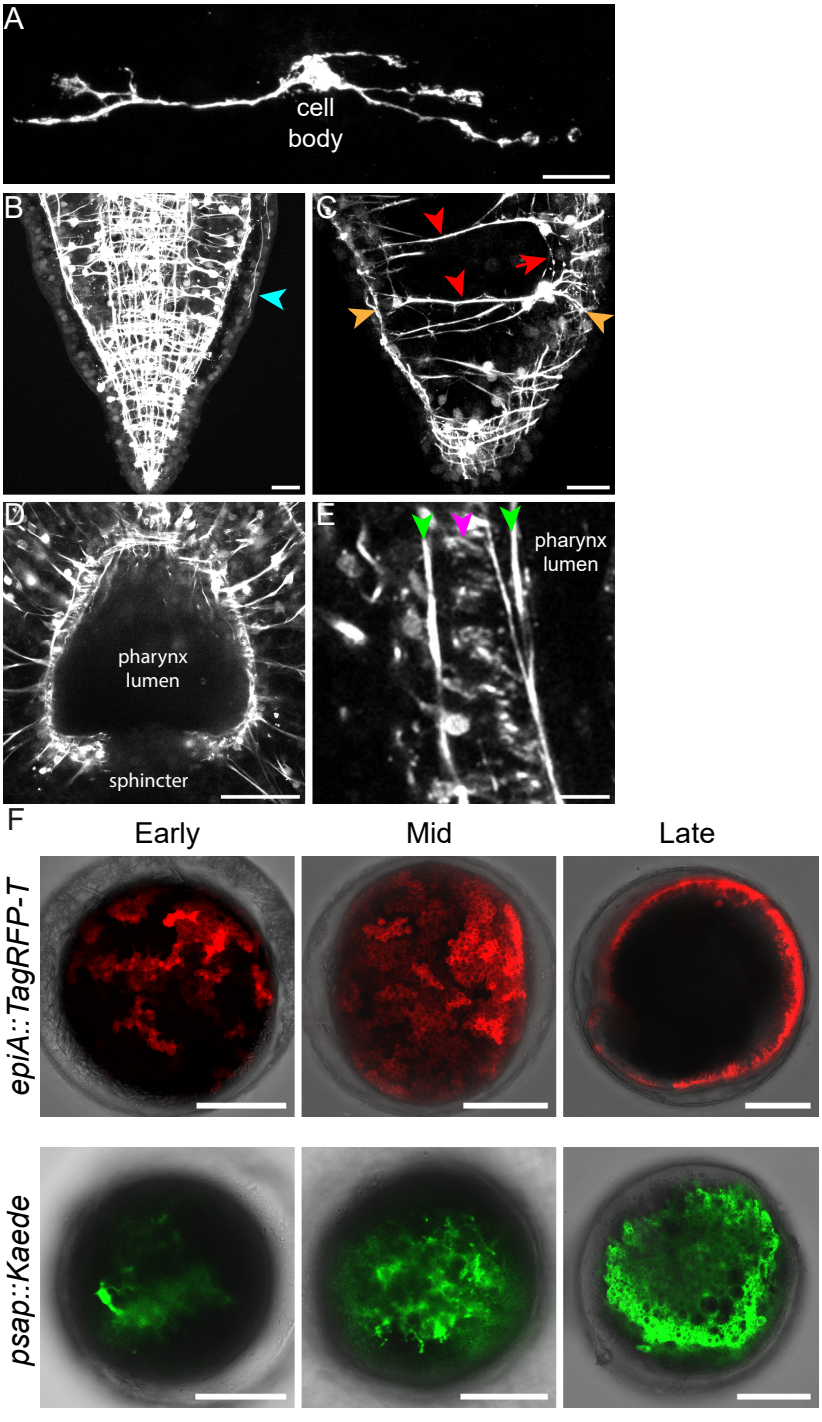
Supplemental Figure 2: Expression patterns for wild-type and transgenic *epi-a*, *psap* and *tnn* genes, related to Figure 2. (**A**) Mosaic transgene expression in juvenile worms. Transgene name is indicated in the bottom left corner. TagRFP-T (red); Kaede (green); DAPI (nuclei, blue). (**B**) Expression of *epiA::TagRFP-T* transgene in hatchlings. Top (**B1**) and side view (**B2**) of a patch of epidermal cells. Yellow arroheads point at epidermal cell nuclei positions. (**B3**) Ciliature of an epidermal cell. (**B4**) *EpiA*+ cells, lie on the pharynx wall. (**C**) Expression of *psap::Kaede* transgene in hatchlings. (**C1**) Kaede expressing cells in the hatchling head(white arrowhead indicates the statocyst position). (**C2**) Kaede expressing cells at the parenchyma-epidermis junction. Kaede+ digestive tissue protruding, through the pharynx sphincter, within the pharynx cavity, as seen in longitudinal (**C3**) and cross (**C4**) sections. White dashed lines delineate the pharynx cavity; P*h, pharynx lumen; s, pharynx sphincter*. TagRFP-T (red); Kaede (green); DAPI (nuclei, blue). Scale bars, B1, B2, C2, 20 µm; B3, 5 µm; B4, C1, C3, C4, 50 µm.(**D**) Fluorescent *in situ* hybridization of riboprobes (red) for *epiA*, *psap* and *tnn* are shown in adults and hatchlings of *Hofstenia* worms. White arrowhead points to *epiA*-expressing cells in the male penis stylet opening. Yellow arrowheads point at *epiA*-expressing cells in adult that were not consistently recapitulated by transgene expression. An example of the variety of expression patterns observed for *epiA* shown in four different hatchlings. All worms are oriented anterior side up and images of adult worms show their ventral side. Nuclei are counterstained with DAPI (blue). *Ph, pharynx lumen.* Scale bars, 100 µm. *Images shown are representative of at least 10 and 6 samples for FISH and transgenic, respectively.*



Supplemental Figure 3: Transgenic toolkit, related to Figure 3. (**A**) Controls for FACS and ICC. Anti-Tropomyosin (TPM) immunohistochemistry on FACS-isolated cells, from *tnn::Kaede* transgenics. Scale bars, 20 µm. (**B**) Regeneration in *epiA::TagRFP-T* transgenics. Regeneration time course of both anterior and posterior fragments after sub-pharyngeal bisection. Time elapsed since amputation is indicated at the bottom (dpa, days post amputation). White arrowhead points to *de novo* transgene expression in posterior fragment. For each time points, 6 worms were amputated, the frequency of the phenotype is indicated in the left bottom corner. All images are projection of confocal z-stacks, taken ventrally, and oriented anterior side up. Scale bars, 200 µm.

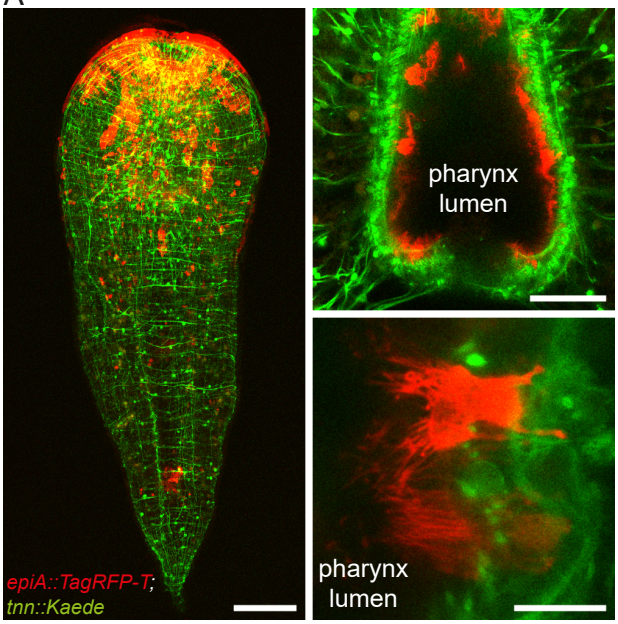


Supplemental Figure 4: Details of muscle anatomy and live studies, related to Figure 2 and 4. (**A**) Full length peripheral muscle fiber in G0 animal. (**B**) The tip of the tail appears deprived of peripheral muscles. Blue arrowhead points to the posterior extremity of a peripheral muscle. (**C**) Posterior parenchymal muscles (red arrowhead) connect body wall fibers together (orange arrowheads). Cellular projections can be observed connecting parenchymal fibers together (red arrow). (**D, E**) The pharynx is contained within a dense, basket-shaped (**D**), three-layered (**E**) muscular network. Green arrowheads indicate longitudinal pharyngeal muscles; magenta arrowhead point at circular pharyngeal muscles. Scale bars, A, E,10 µm; B, C, 20 µm; D, 50 µm. (**F**) Transgene expression as seen in G1 or G2 embryos at three different time points : right after detection (Early, 4.5 days post fertilization), then 1-2 days later (Mid) and finally, prior hatching (Late, 7-8 days post fertilization), when the embryos are constantly spinning within their capsule. Name of the transgene is indicated on the left side of the panel. Scale bars, 100 µm. *Images shown are representative of 6 (muscle, hatchlings) and at least 10 samples (gut and epidermis, embryos)*.

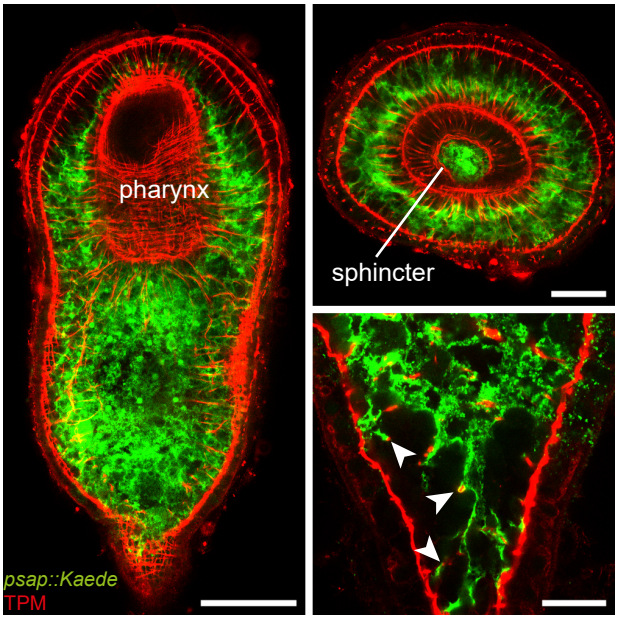


Supplemental Figure 5: Dual labeling of muscle with *epiA*+ and *psap*+ cells, related to Figures 2 and 5. (**A**) Crossing of G1 *epiA::-TagRFP-T* with G1 *tnn::Kaede* transgenic lines. (**Left**) Double transgenic *epiA::Tag-RFP-T* ; *tnn::Kaede*, hatchling, projection of confocal z-stack. (**Right, top**) Pharynx, longitudinal section. (**Right, bottom**) Ciliature of *epiA*+ pharyngeal cells. Kaede (green); TagRFP-T (red). Scale bars, left, 100 µm; top right, 50 µm; bottom right, 10 µm. (**B**) Dual abeling of muscle with *psap*+ cells. Anti-TPM immunohistochemistry on *psap::Kaede* G2 transgenic animals. (**Left**) Whole worm (hatchling), projection of confocal z-stack. (**Right, top**) Pharynx, posterior, cross section. (**Right, bottom**) Association of digestive tissue with muscle fibers (white arrowheads), in the tail of the animal. Kaede (green); TPM (red). Scale bars, left, 100 µm; top right, 50 µm; bottom right, 20 µm. *Images shown are representative of at least 5 samples.*

A



B



Supplemental Table 1: Injection troubleshooting guide, related to STAR methods

Step	Issue	Troubleshooting
DNA preparation	Low DNA concentration / incomplete digestion	• Decrease elution volume • Digest more plasmid in several reaction tubes, then pool them • Extend digestion time and/or reduce DNA concentration • Use fresh I-SceI enzyme • Minimize the amount of gel while cutting the band
	Low DNA purity	• Add extra wash buffer steps • Let the wash buffer incubate on the nucleospin membrane (5 min)
	Poor/no band separation	• Run the gel for a longer time • Check enzyme activity • Add another restriction enzyme to the digestion tube, specific to the plasmid backbone, to generate multiple, distinct fragments.
Injection dish	Embryo popping out of the agarose well	• Force the embryo deeper into the well • Make larger/longer pins to the injection mold • Reduce agarose concentration
	Embryo falling into the wells	• Make shorter pins to the injection mold • Increase agarose concentration
	Embryo rolling into the well while trying to inject	• Reorient the embryo and/or the injection dish • Increase agarose concentration • Put less agarose in the injection dish
	Soft embryo capsule and plasma membrane	• Wash the injection dish several times with SW prior adding the embryos
Injection	Clogged injection needle	• Make sure to spin, 30 s at max. speed, the following components : eluted DNA, FL-Dextran, I-SceI buffer, before assembling the injection solution. • Clear the needle • Re-break the needle tip • Load a new needle
	Reduced flow	• Clear the needle • Increase P balance • Re-break the needle tip • Make steeper needles
	Overflow	• Decrease P balance • Make thinner needles • Break the needle more gently

Supplemental Table 2: Primer informations, related to STAR Methods

Primers and restriction enzyme name (5' - 3') used for transgenesis		Amplicon size (bp)	Genome scaffold #	Position on the scaffold (**)
Promoter				
Epi-AF-AsiSI Epi-AR-AscI	gcgatcgcTACCTACTCTTGCACCCCTTTATA gcgatcgcTGCACTATCCTAACTACAACAA	2035	5	4293372 - 4295406
TnnF-AsiSI TnnR-AscI	gcgatcgcTCATATGCTGTGGAGGTTAATCTGT ggcgcgccCTAAAAATTCAATTATTTCATTT	4649	44	1808578 - 1813226
SapF-AsiSI SapR-AscI	ggcgcgccGTAACAACACCAAGCCATGTGTTAGCCC ggcgcgccCTTTCCTAATACTGATGCTATTTATCC	3109	96	1102963 - 1106071
3'UTR				
Epi-AF (*) Epi-AR-FseI	cttaaggcgtaaaggACGTAGACTGTATTAAACCATAA ggccggccGTAGACTAGGGAATAGATACACTTTGT	972	5	4297179 - 4298150
TnnF-SbfI TnnR-FseI	cctgcaggAGGAGTGACACTAACGCAGCTTAT ggccggccAGCCTTGGAAATGTTTGGAAGAA	1039	44	1801098 - 1802136
SapF-SbfI SapR-FseI	cctgcaggTAATCAGAAATTAAGAATCAATAGAAG ggccggccGTATATCACAATACTAGAATATAAATGC	1134	96	1101380 - 1102513
Primers used for FISH experiments		**Probe size**	**Transcript ID**	
Gene name				
Tnn B(***) Tnn C	AAGCTGGAGCTCCACCGCGGAAGGTCAACAGATCACTCAC GGGCGAATTGGGTACCGGGCTTATCACCAGTGAGATCCG	780 bp	98028242	
sap B sap C	AAGCTGGAGCTCCACCGCGGAGTTCTTCTTATCGCCTTTGCTG GGGCGAATTGGGTACCGGGATCGGAAGTTTATTCATCCCAATG	486 bp	98037255	
epiA B epiA C	AAGCTGGAGCTCCACCGCGGGTCAATTGGATTGATTGGAAGGCA GGGCGAATTGGGTACCGGGATTATTTGCAGCGAAAATGTAACTC	401 bp	98025858	
Primers used for CRISPR/CAS9 genome editing validation with T7 endonuclease				
F	GAACTTGTTCTTTTGGTAGTTG			
R	AAACATCAAATGTGCCACCAC			

(*) This primer contains 15 bases (lower case) overlapping to the 3' end of a Tag-RFP-T::SV40pA fragment to which it was ligated in a gibson assembly reaction. The resulting fragment was PCR-amplified, digested with AscI and FseI enzymes and ligated into a vector already containing the pEpi-A fragment.
(**) Hofstenia genome browser is accessible at http://srivastavalab.rc.fas.harvard.edu/
(***) For each riboprobe, a forward (B) and reverse (C) primer were designer, containing adapter sequences (in red) to facilitate cloning into a vector plasmid