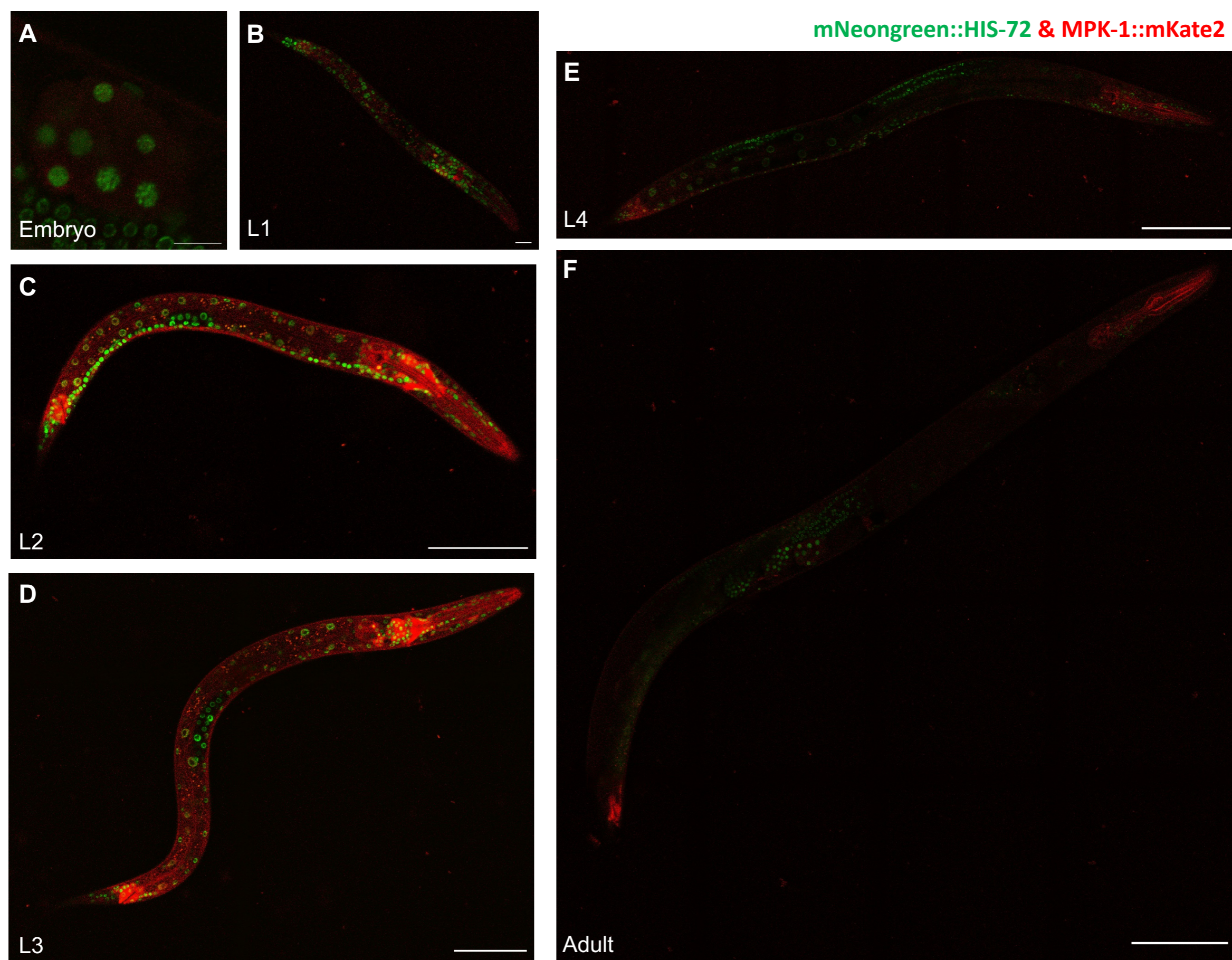
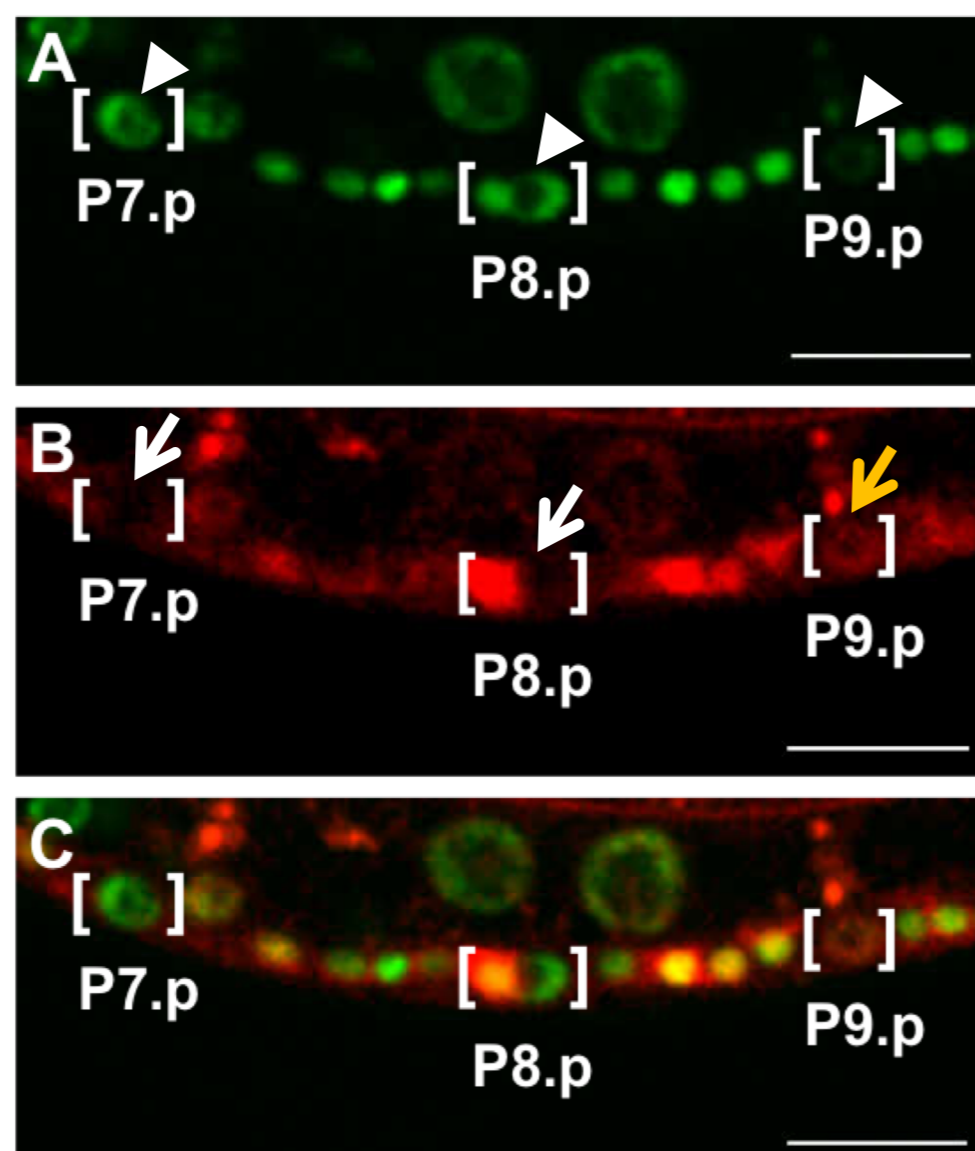


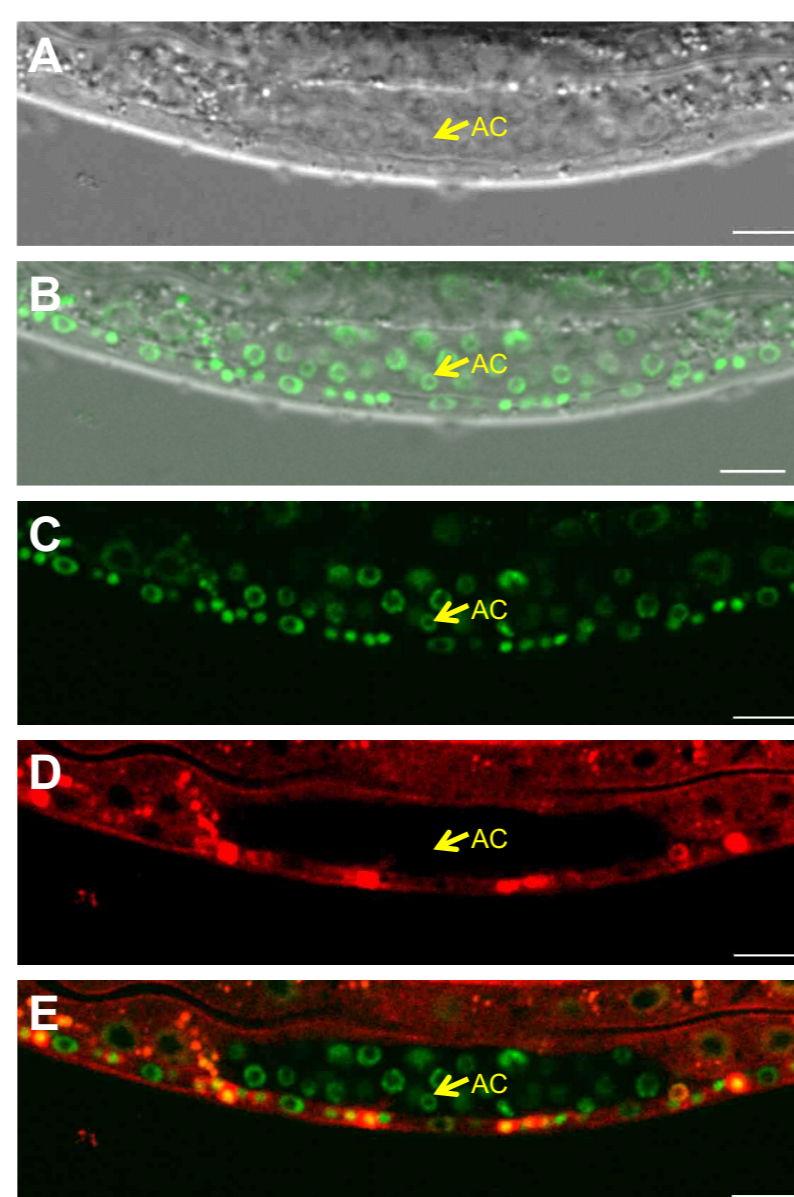
**Fig. S1. Endogenous MPK-1 is expressed throughout the animal and is elevated in certain tissues and at certain stages.** Confocal micrographs of MPK-1::mKate2 animals at different stages of the life cycle. \*\*\*\*All images were captured with the same exposure levels.\*\*\*\*\* **A)** An early stage embryo in the uterus of its mother with low background MPK-1. **B)** L1 larva. **C)** L2 larva evincing global elevation of MPK-1 expression and increased expression in the pharynx, anterior intestine and posterior intestine. **D)** L3 larva with similarly elevated expression. **E)** L4 larva with decreased global expression but relative expression still elevated in certain tissues. **F)** Adult animal with decreased expression except in rectal epithelia and the pharynx. Scale bars = 10 μm (**A,B**), 50 μm (**C,D,E**), and 100 μm (**F**).



**Fig. S2. Endogenous MPK-1 with a nuclear marker as a reference point.** The same images as in Supplementary Figure 1 but with mNeonGreen::HIS-72 nuclear marker in green. **A)** An early stage embryo in the uterus of its mother. **B)** L1. **C)** L2. **D)** L3. **E)** L4. **F)** Adult animal. Scale bars = 10 μm (A,B), 50 μm (C,D,E), and 100 μm (F).



**Fig. S3.** Tagged MPK-1::mKate2 translocates to the nucleus of posterior non-VPC P9.p prior to induction of VPCs P7.p and P8.p. **A)** Green channel nuclei, mNeonGreen::HIS-72. **B)** Red channel MPK-1::mKate2. A ventral cord neuron (bright red puncta) overlaps with the P8.p nuclei **C)** Merged images. Brackets are included around nuclei for references. Many but not all such nuclei have nuclear MPK-1 (smaller ventral nuclei in the figure). Orange arrows indicate nuclei with nuclear MPK-1, white arrows nuclei without nuclear MPK-1. The white arrowhead indicates VPC and non-VPC nuclei with differing levels of mNeonGreen::HIS-72 intensity. Scale bars = 10  $\mu$ m.



**Fig. S4.** The gradient of relocalization of MPK-1::mKate2 to the nucleus centers on P6.p, the VPC closest the the Anchor Cell in the developing ventral somatic gonad. All spinning disk confocal photomicrographs are of the same animal. **A)** Location of the AC by DIC and **B,C)** merge and mNeonGreen::HIS-72, respectively. **D)** MPK-1::mKate2 and **E)** merge. Yellow arrows point to the AC. Scale bars = 10  $\mu$ m.

**Table S1.**

Genotype:	WT	<i>mpk-1(re172)</i>
L1/L2 Healthy Animals	380	365
Unhatched	0	0
L1/L2 rods	0	0

**Table S2.**

Genotype:	WT	<i>mpk-1(re172)</i>
Normal Vulvae	59	52
Vulvaless	0	0
Ectopic Pseudovulvae	0	0

**Table S3. Strains**

Strain	Genotype
DV3261	<i>mpk-1(re171[mpk-1::mKate2<sup>SEC</sup>3xFlag])</i> III
DV3262	<i>mpk-1(re172[mpk-1::mKate2<sup>3xFlag</sup>])</i> III
DV3285	<i>his-72(cp76[mNeonGreen<sup>3xFlag</sup>::his-72]), mpk-1(re172[mpk-1::mKate2<sup>3xFlag</sup>])</i> III
BS3760	<i>rskn-1(ok159)</i> I
DV3317	<i>rskn-1(ok159)</i> I; <i>his-72(cp76[mNeonGreen<sup>3xFlag</sup>::his-72]), mpk-1(re172[mpk-1::mKate2<sup>3xFlag</sup>])</i> III

**Table S4. Primers**

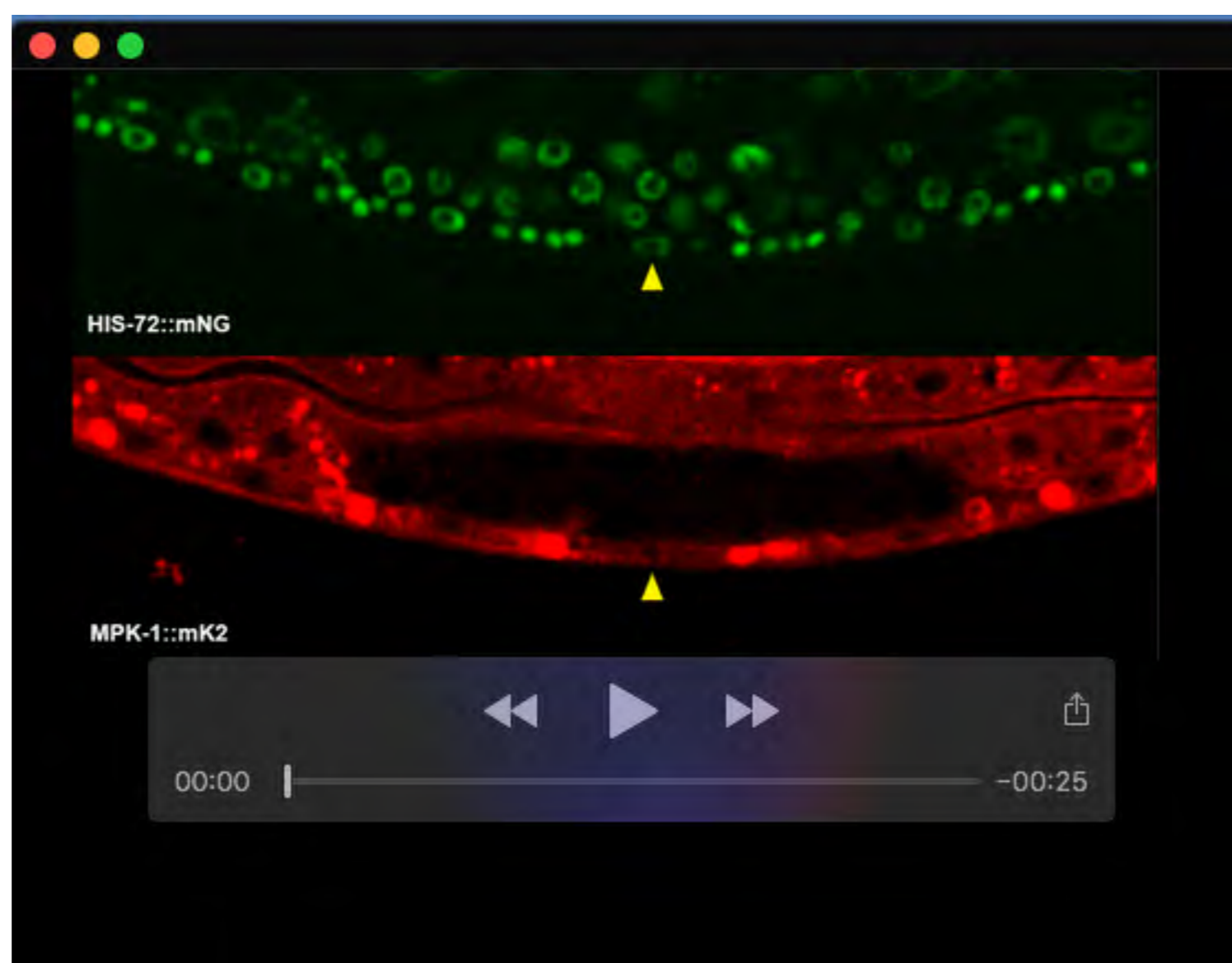
Name	Sequence	Used for
oNR065	GGCTCATCACAGACGAATGGGTTTAAGAGCT ATGCTGGAAACAG	<i>mpk-1</i> sgRNA-1 mutagenesis
oNR066	AGCTTTTCAGCGGGAACGGGGTTTAAGAGCT ATGCTGGAAACAG	<i>mpk-1</i> sgRNA-2 mutagenesis
oNR023	CAAGACATCTCGCAATAGG	<i>mpk-1</i> sgRNA mutagenesis
oNR059	CGTGATTACAAGGATGACGATGACAAGAGA ATTAGTAGTATTTACCCACTAAATTAG	<i>mpk-1</i> homology arm-2 FW
oNR060	GGAAACAGCTATGACCATGTTATCGATTTC GCTATGATTGTTGTGTAACCC	<i>mpk-1</i> homology arm-2 RV
oNR067	CGATTTTCAGTATCGACATCGAGCAAGCATT GGCTCACCCATACTTGGAGCAATACTACGAT CCAGGAGATGAGCCAGTTTGTGAGGAACCAT TCACTTTGAAAATGGAATTCGACGATTTACC GAAGGAGAAGCTGAAGGAGCTGATTTGGGA AGAAGCCGAGGCTCATCACAGACGAATGGA GGCAGAAGCGGCTGCAAGGAATAATGGAGG GCAGAATCCTGTTTCAGCCGGAGGTAGCGCC GGCGGAAGTGCTGGTGAATGGTCTCCGAGC TCATTAAGAAAACATG	<i>mpk-1</i> homology arm-1 gBlock
oNR094	ACCAAAACAACCATGGGCTCG	<i>mpk-1</i> genotyping FW
oNR095	GCTCCAAGTATGGGTGAGCC	<i>mpk-1</i> genotyping RV-1
oNR096	GGTCCCTCGTATGGCTTTCC	<i>mpk-1</i> genotyping RV-2

**Table S5. Plasmids**

Name	Description	Used for
pNR9	Cas9 and MPK-1 sgRNA-1	MPK-1 sgRNA-1
pNR10	Cas9 and MPK-1 sgRNA-2	MPK-1 sgRNA-2
pNR11	MPK-1::mKate2 <sup>3xFlag</sup>	SEC CRISPR plasmid repair template
pCFJ104	<i>P<sub>myo-3</sub>::mCherry</i>	Co-injection marker
pJW1236	Cas9 and sgRNA expression plasmid	mpk-1 SEC-CRISPR tagging
pDD285	mKate2::3xFlag SEC repair template	mpk-1 SEC CRISPR repair template

**Table S6. sgRNA sequences and PAMs**

Sequence	Used for
GGCTCATCACAGACGAATGG	mpk-1 SEC-CRISPR tagging
AGCTTTTCAGCGGGAACGGG	mpk-1 SEC-CRISPR tagging



**Movie 1.** Time lapse film of MPK-1::mKate2 entering P6.p first. Arrows indicate P6.p and other VPCs.