

Figure S1

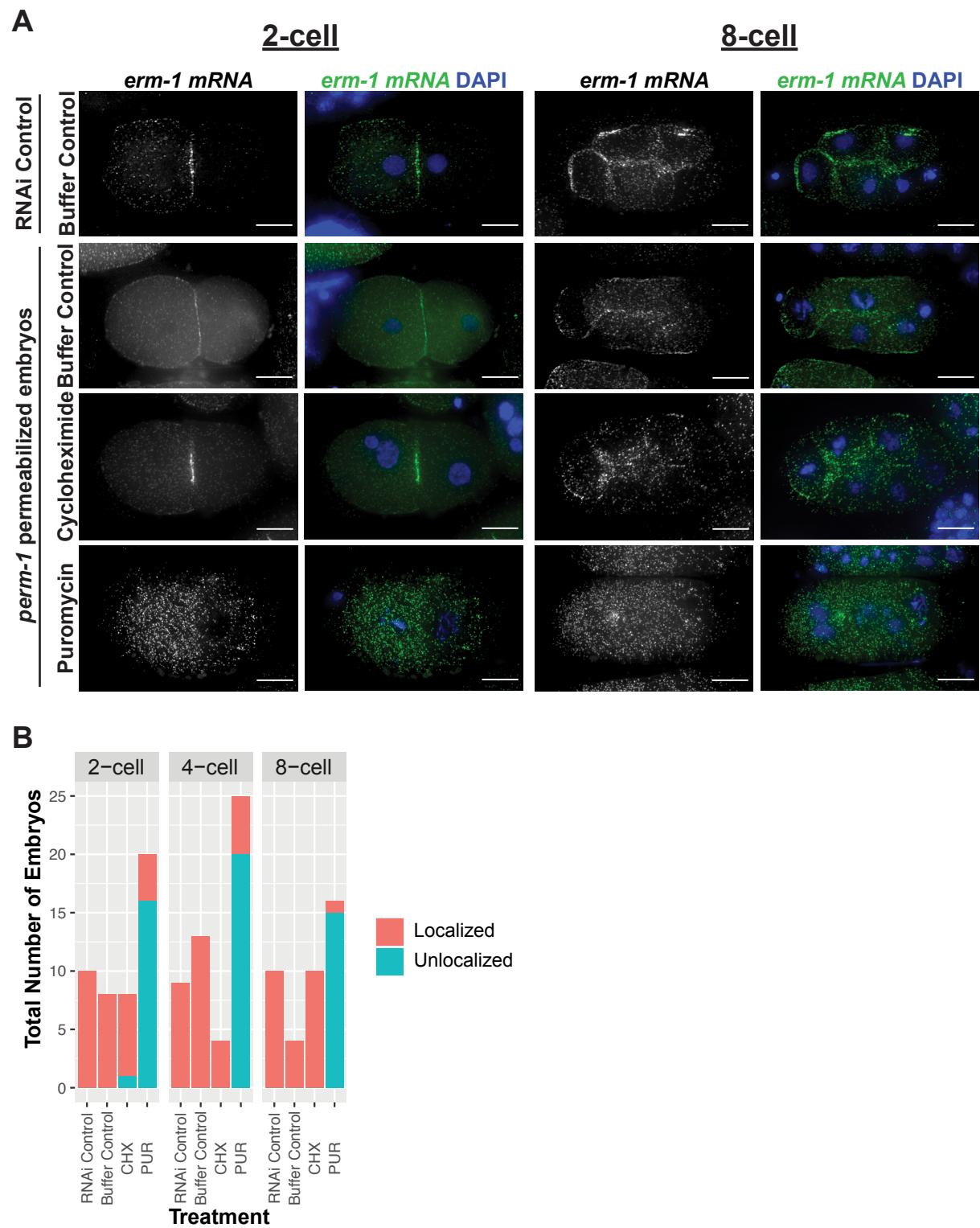


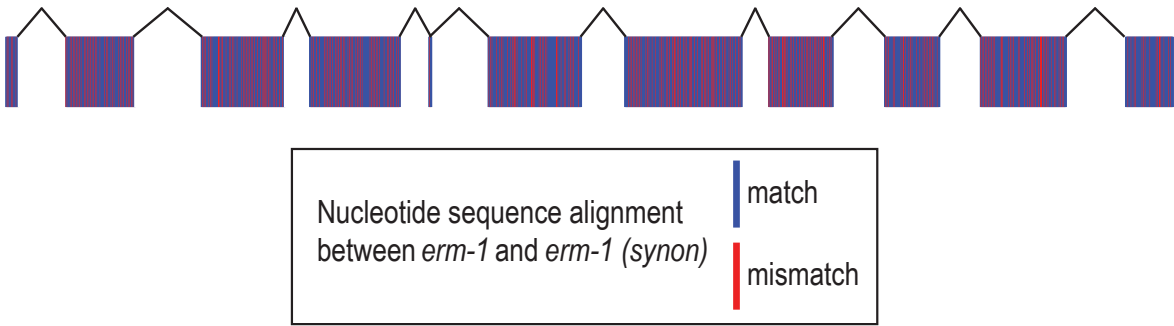
Fig. S1. The ERM-1 nascent peptide is required for *erm-1* mRNA cell membrane localization. **(A)** Fluorescent micrographs of 2-cell and 8-cell *C. elegans* embryos are shown in which embryos were permeabilized by *perm-1* RNAi and subsequently treated with small molecule translation inhibitors, in comparison to RNAi and drug treatment controls. *erm-1* mRNA (green) was imaged by smFISH, under control, cycloheximide (500 µg/mL, 20min), or puromycin (500 µg/mL, 20min) treatment conditions. Scale bars 10 µm. **(B)** Bar plot indicating the number of embryos displaying *erm-1* mRNA enriched at cell membranes (localized) or homogenously distributed through the cell (unlocalized) for 2-cell, 4-cell, and 8-cell embryos subjected to the indicated treatments.

Figure S2

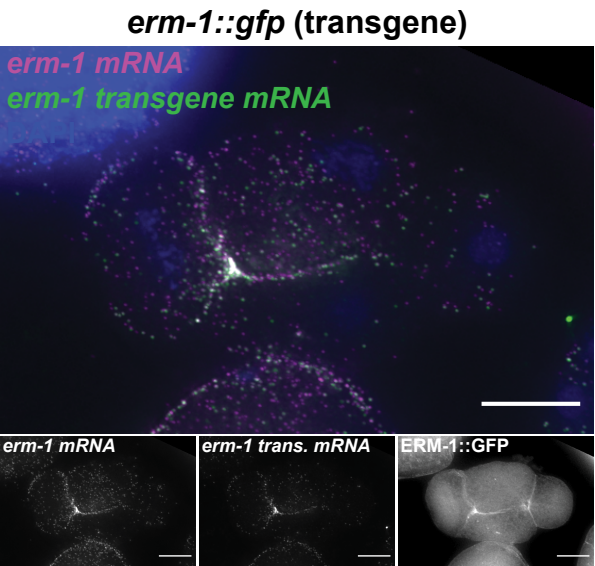
A

Figure 3 worm strains		
<i>erm-1::gfp</i> (endogenous)	<i>erm-1p::erm-1::gfp::erm-1 3'UTR I</i>	<i>gfp</i> knock-in at endogenous <i>erm-1</i> locus on Chr I (Ramalho et al., 2021)
<i>erm-1::gfp</i> (transgene)	<i>erm-1p::erm-1::gfp::erm-1 3'UTR IV</i>	single copy insertion at Chr IV MosSCI locus (this study)
<i>erm-1 synon::gfp</i> (transgene)	<i>erm-1p::erm-1 synon::gfp::erm-1 3'UTR IV</i>	single copy insertion at Chr IV MosSCI locus (this study)

B



C



D

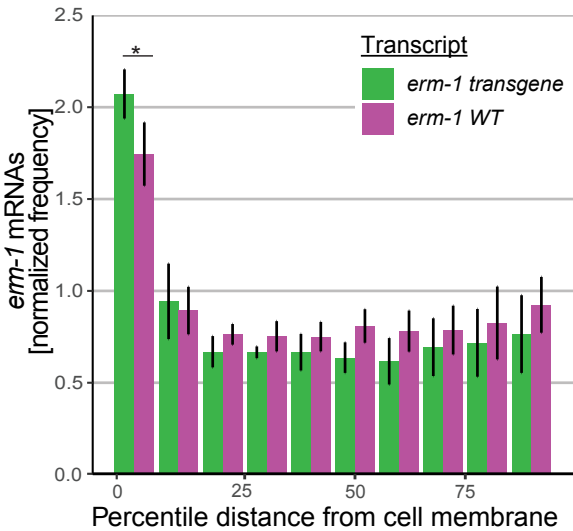


Fig. S2. *erm-1* mRNA localizes at the cell membrane when expressed from a transgenic MosSCI locus. (A) Description of the three worm strains used in the synonymous *erm-1* assays. **(B)** nucleotide sequence alignment between *erm-1* and the synonymous *erm-1(erm-1 (synon))* showing nucleotide matches in blue and mismatches in red. **(C)** smFISH micrographs of 4-cell staged *C. elegans* embryos imaging *erm-1::gfp* transgene probed by *erm-1* (*erm-1* mRNA, magenta) and the transgenic *erm-1::gfp* mRNA probed by *nemamatrix gfp* (*erm-1* transgene mRNA, green). DNA (DAPI, blue) and membranes marked by ERM-1::GFP expressed at the MosSCI locus are also shown. **(D)** Quantification of endogenous and MosSCI-expressed transgenic *erm-1* mRNA ($n = 6$) indicating the volume normalized frequency of mRNAs within binned, normalized distances from the cell membrane. Welch Two Sample t-test p-value = 0.006 at the cell membrane. Scale bars 10 μ m. *P* value legend: NS>0.05; 0.05>*>0.005; 0.005>**>0.0005; 0.0005>***>0.00005; 0.00005>****

Figure S3

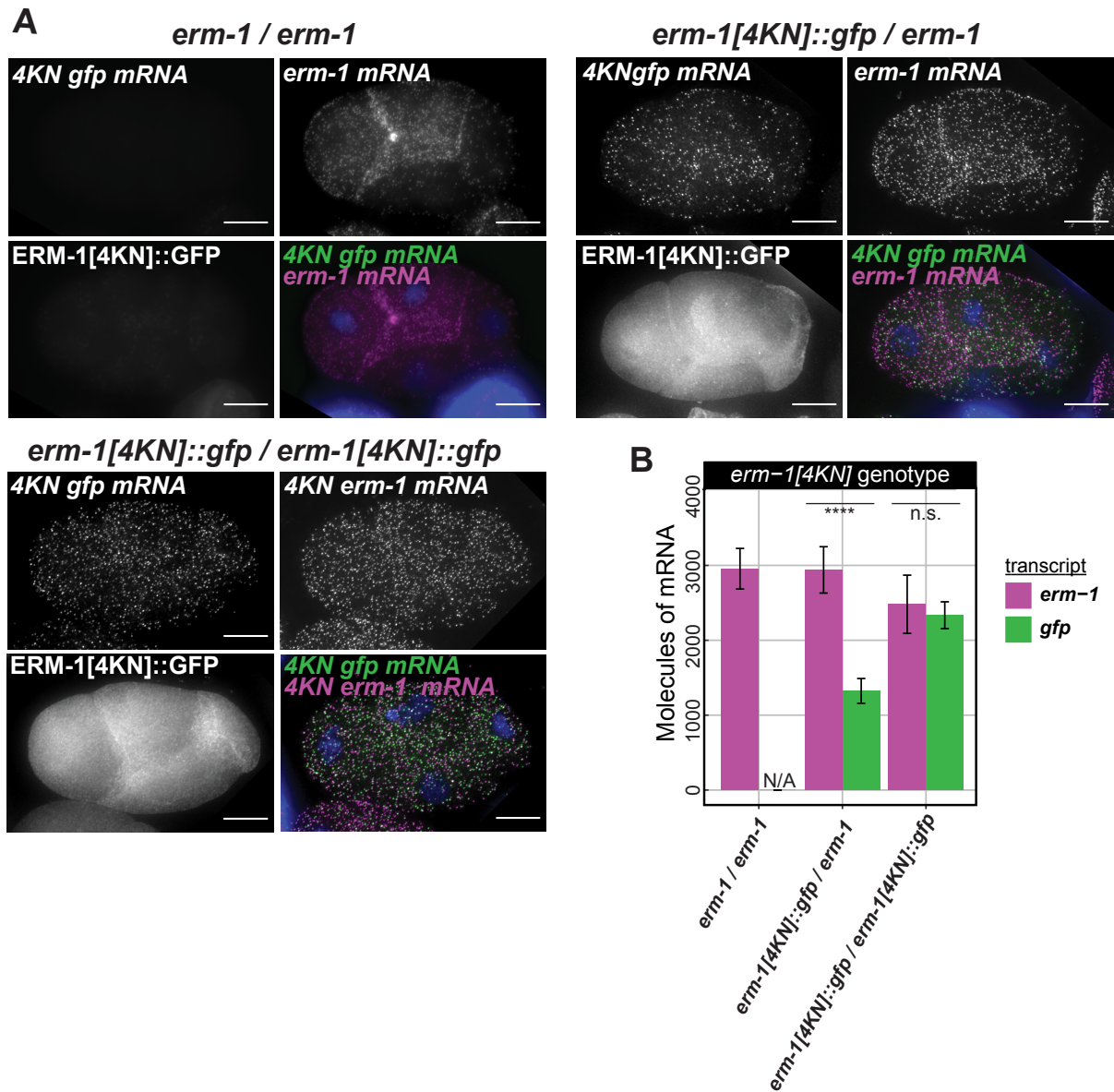


Fig. S3. ERM-1[4KN]::GFP RNA abundance corresponds to genomic copy-number. **(A)** 4-cell embryos of the ERM-1[4KN]::GFP mutant probing for *erm-1* mRNA (magenta) and *4KN gfp* (green) are shown in *erm-1/erm-1* homozygotes ($n = 9$), *erm-1[4KN]::gfp/erm-1* heterozygotes ($n = 9$), or *erm-1[4KN]::gfp/erm-1[4KN]::gfp* homozygous mutants ($n = 9$). Scale bars 10 μm . **(B)** Total number of *erm-1* and *gfp* mRNA molecules P -values derived from Welch Two Sample t -tests comparing the number of *gfp* and *erm-1* mRNA molecules detected in the same strains. P value legend: NS>0.05; 0.05>*>0.005; 0.005>**>0.0005; 0.0005>***>0.00005; 0.00005>****

Figure S4

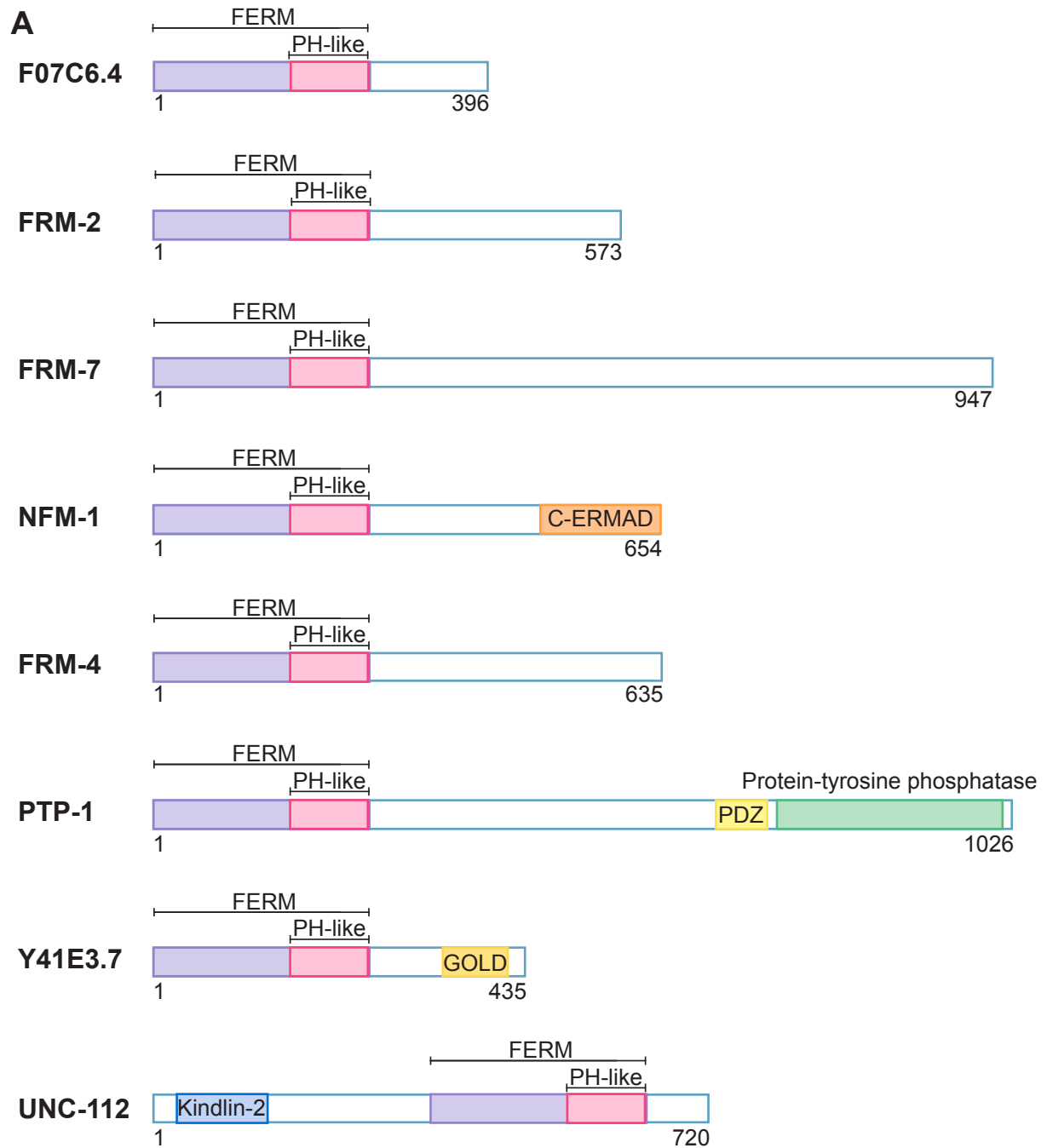


Fig. S4. Depictions of the encoded protein domains for the surveyed FERM domain-containing transcripts.

Figure S5

A

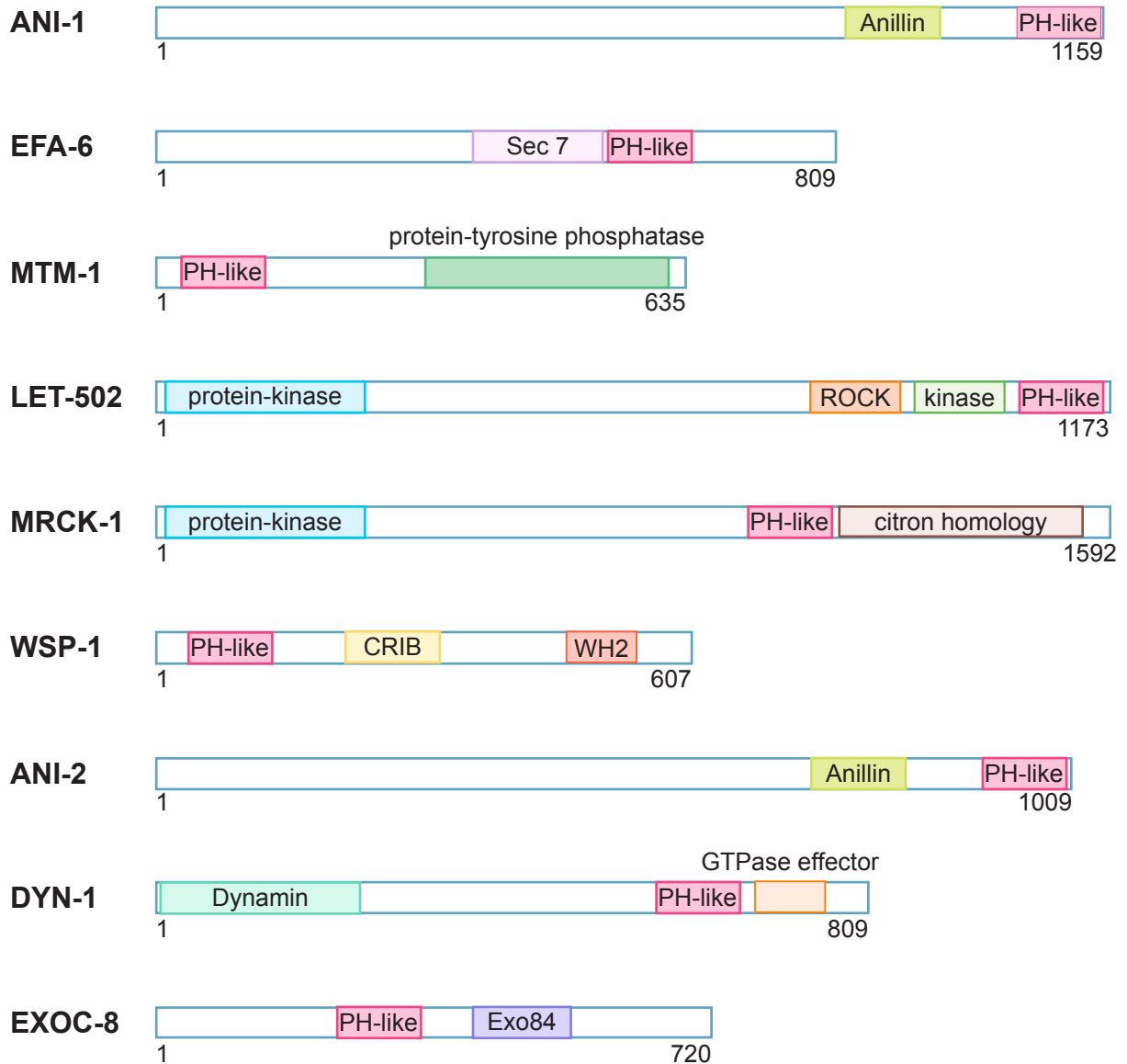


Fig. S5. Depictions of the encoded protein domains for the surveyed PH-like domain-containing transcripts.

Figure S6

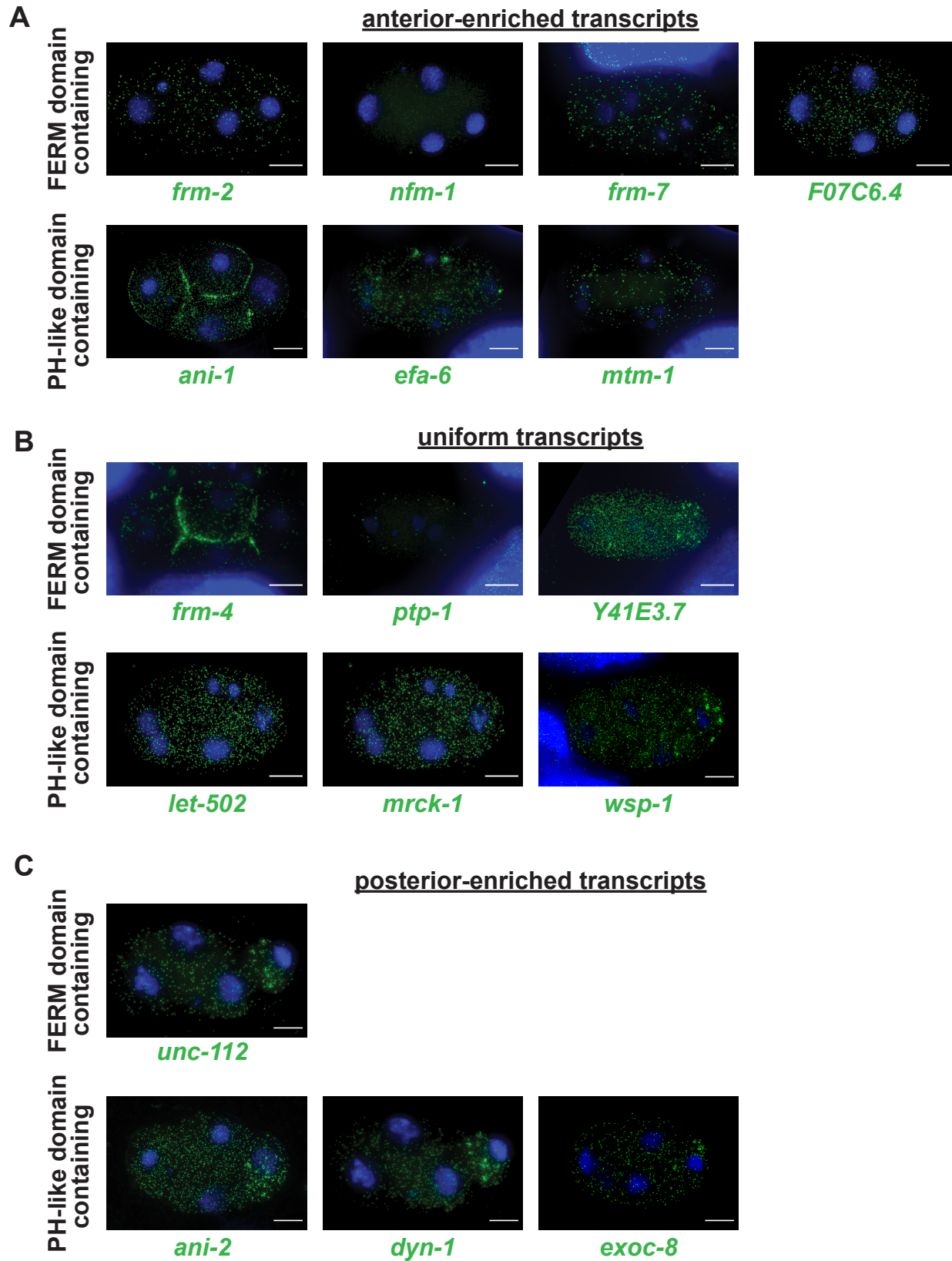


Fig. S6. Subcellular localization patterns of surveyed FERM and PH domain-containing transcripts. (A) Seven anterior-enriched transcripts *F07C6.4*, *frm-2*, *frm-7*, *nfm-1*, *ani-1*, *efa-6*, and *mtm-1*, **(B)** six uniformly distributed transcripts *frm-1*, *ptp-2*, *Y41E3.7*, *let-502*, *mrck-1*, and *wsp-1*, and **(C)** four poster-enriched transcripts *unc-112*, *ani-2*, *dyn-1*, and *exoc-8* are shown.

Figure S7

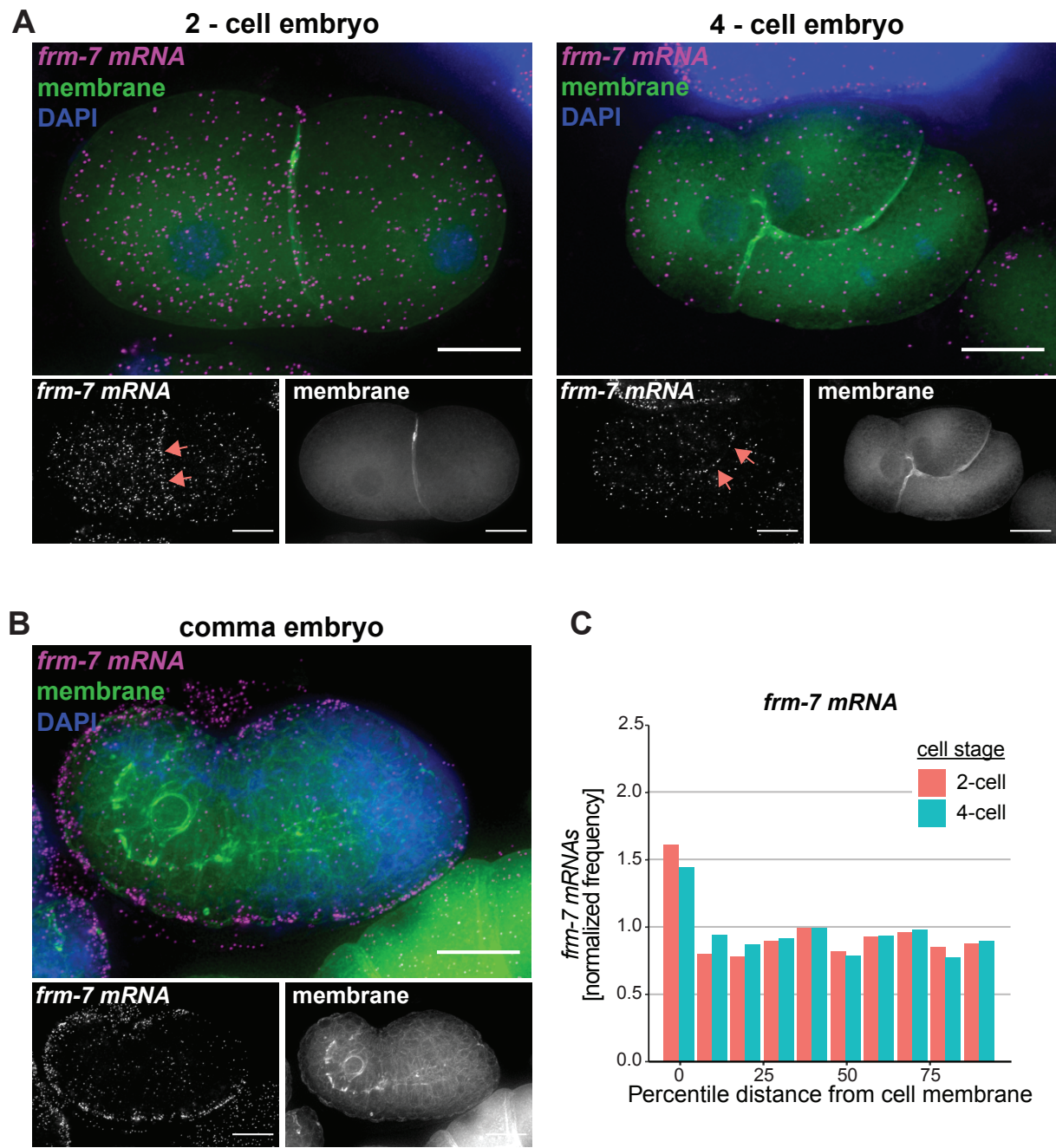
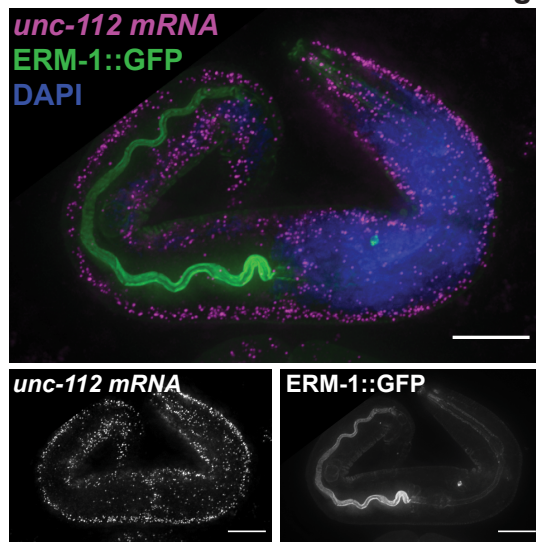


Fig. S7. Subcellular localization patterns of *frm-7* in early and mid-stage embryos.

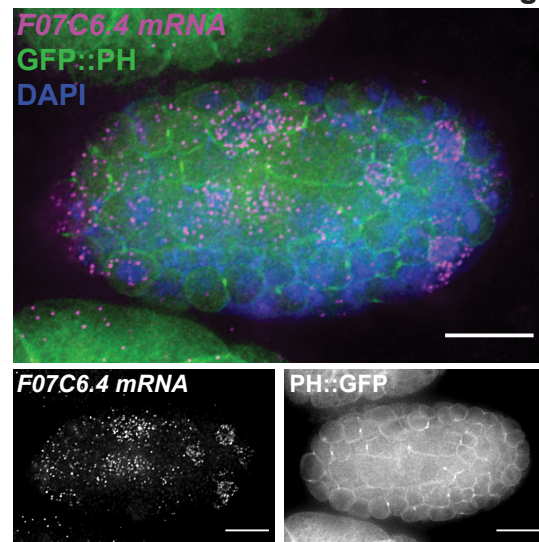
(A) smFISH micrographs of a 2-cell and 4-cell embryo imaging the anterior-enriched, FERM domain encoding transcript *frm-7* mRNA (magenta) in a GFP::PH membrane marker transgenic background (GFP::PH, green). **(B)** smFISH micrograph of a comma stage *C. elegans* embryo imaging the FERM domain encoding transcript *frm-7* (magenta) in ERM-1:GFP background (green) with DNA (DAPI, blue). **(C)** Quantification of *frm-7* mRNA in a 2-cell embryo ($n=1$) and 4-cell embryo ($n=1$) indicating the normalized frequency *frm-7* mRNA at increasing, normalized distances from the cell periphery.

Figure S8

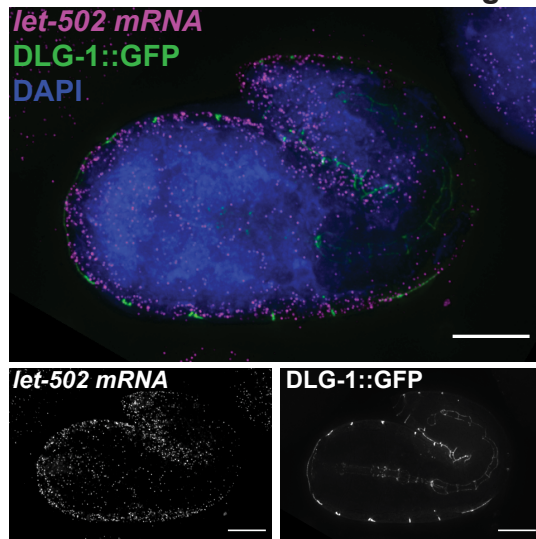
A *unc-112* FERM domain-containing



B *F07C6.4* FERM domain-containing



C *let-502* PH domain-containing



D *ani-2* PH domain-containing

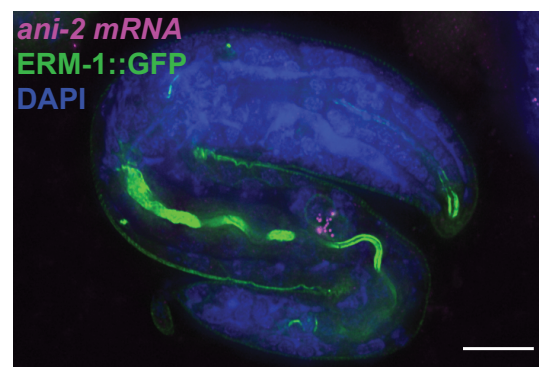
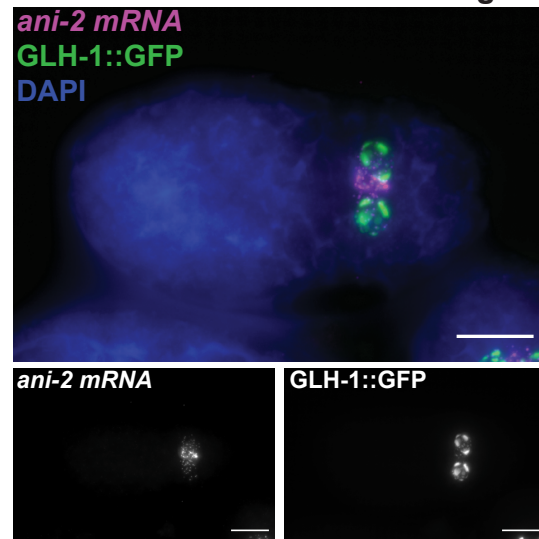


Fig. S8. Subcellular localization patterns of surveyed FERM and PH domain-containing transcripts at mixed later stages. (A) smFISH micrograph of 3-fold *C. elegans* embryo imaging the posterior-enriched, FERM domain encoding transcript *unc-112* (magenta) in ERM-1:GFP background (green) with DNA (DAPI, blue). **(B)** smFISH micrograph of 100-cell *C. elegans* embryo imaging the anterior-enriched, FERM domain encoding transcript *F07C6.4* (magenta) in GFP::PH membrane marker background (green) with DNA (DAPI, blue). **(C)** smFISH micrograph of 1.5-fold *C. elegans* embryo imaging the uniformly-distributed, PH-like domain encoding transcript *let-502* (magenta) in DLG-1:GFP background (green) with DNA (DAPI, blue). **(D)** smFISH micrograph of a bean (top) and 3-fold (bottom) *C. elegans* embryo imaging the posterior-enriched, PH-like domain encoding transcript *ani-2* (magenta) in GLH-1::GFP P granule marker background (green, top) and ERM-1:GFP background (green, bottom) with DNA (DAPI, blue).

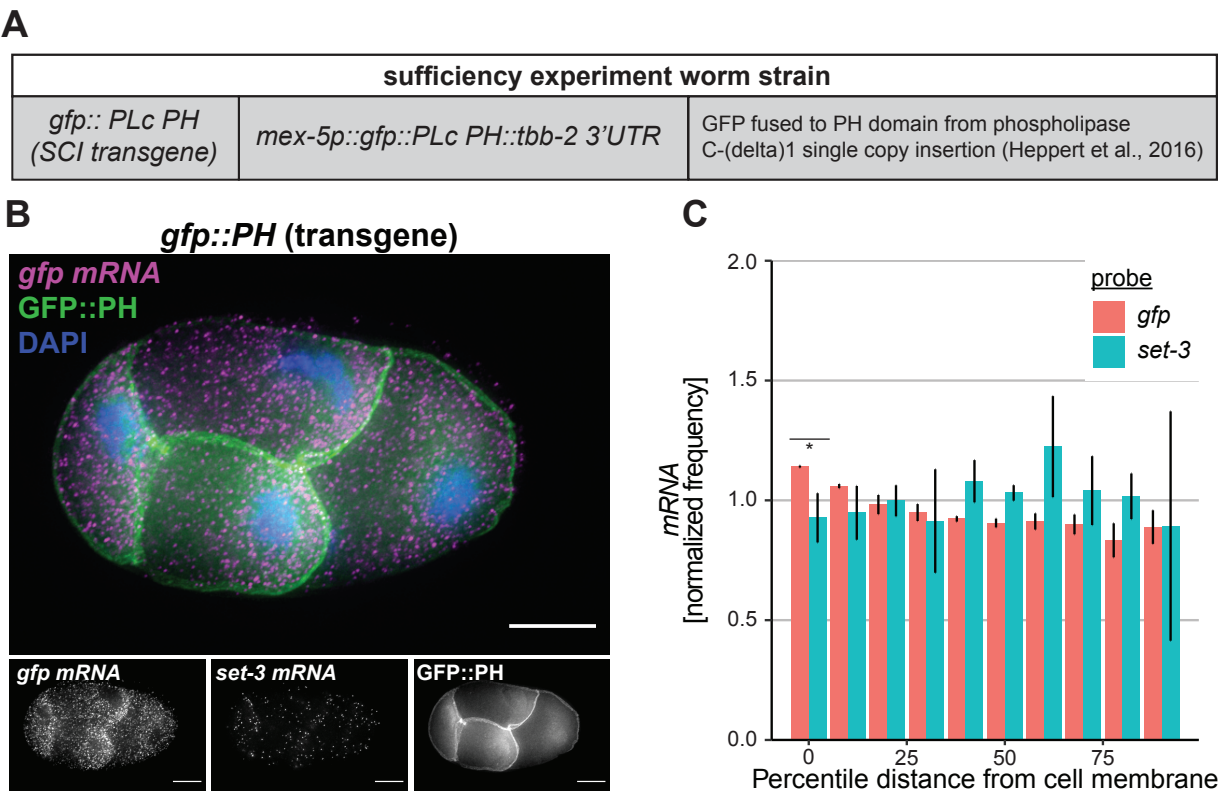


Fig. S9. Sufficiency of the phospholipase C-delta-1 PH domain to direct *gfp* localization to plasma membranes at the 4-cell stage. (A) description of the GFP::PH reporter construct. (B) smFISH micrographs of 4-cell embryos imaging *gfp::PH* by probing *gfp*, (magenta) in a GFP::PH membrane marker transgenic background (GFP::PH, green). (C) Quantification of transcripts *frm-4* and *ani-1* compared to uniform control transcript *set-3* (for each transcript $n=3$) in 4-cell embryos displaying the normalized frequency of *frm-4*, *ani-1*, and *set-3* mRNA at increasing, normalized distances from the cell periphery. Significance indicates P -values derived from Welch Two Sample t -tests comparing the cell membrane localization of *gfp::PH* and uniform *set-3*. P value legend: $* < 0.05$.

Table S1. Worm strains used in study

[Click here to download Table S1](#)

Table S2. *E. coli* strains used in study.

[Click here to download Table S2](#)

Table S3. smFISH and smiFISH probe sets used in study.

[Click here to download Table S3](#)

Table S4. Total list of FERM and PH-like domain containing genes at the 2-cell stage based on scRNA-Seq.

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