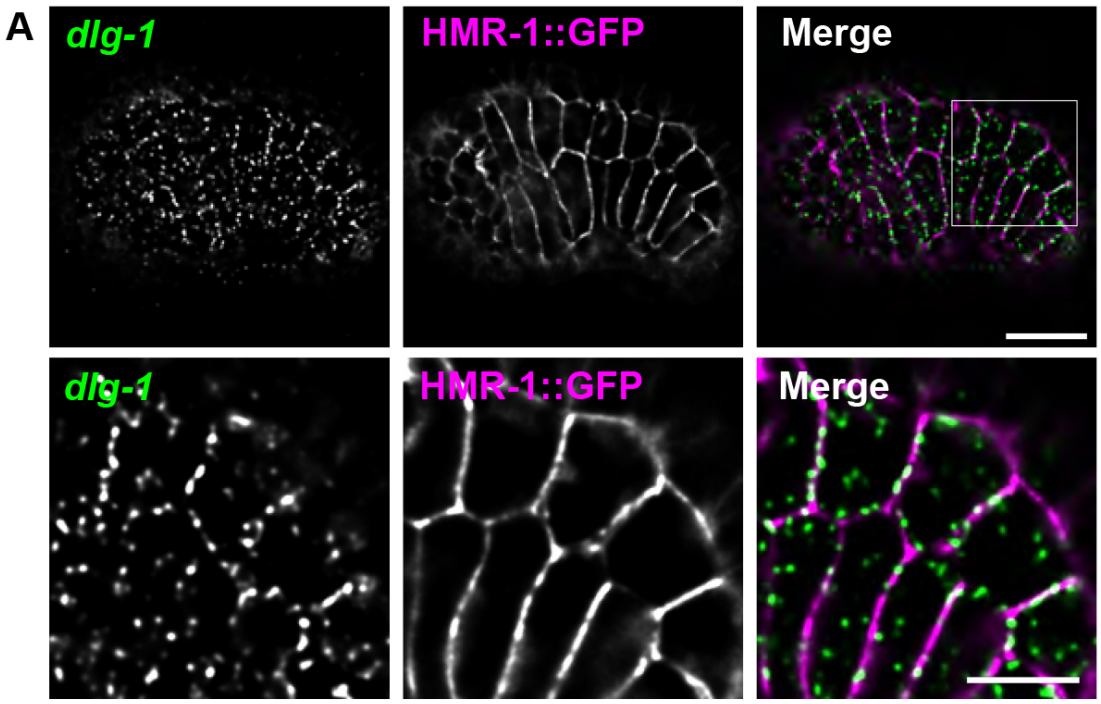


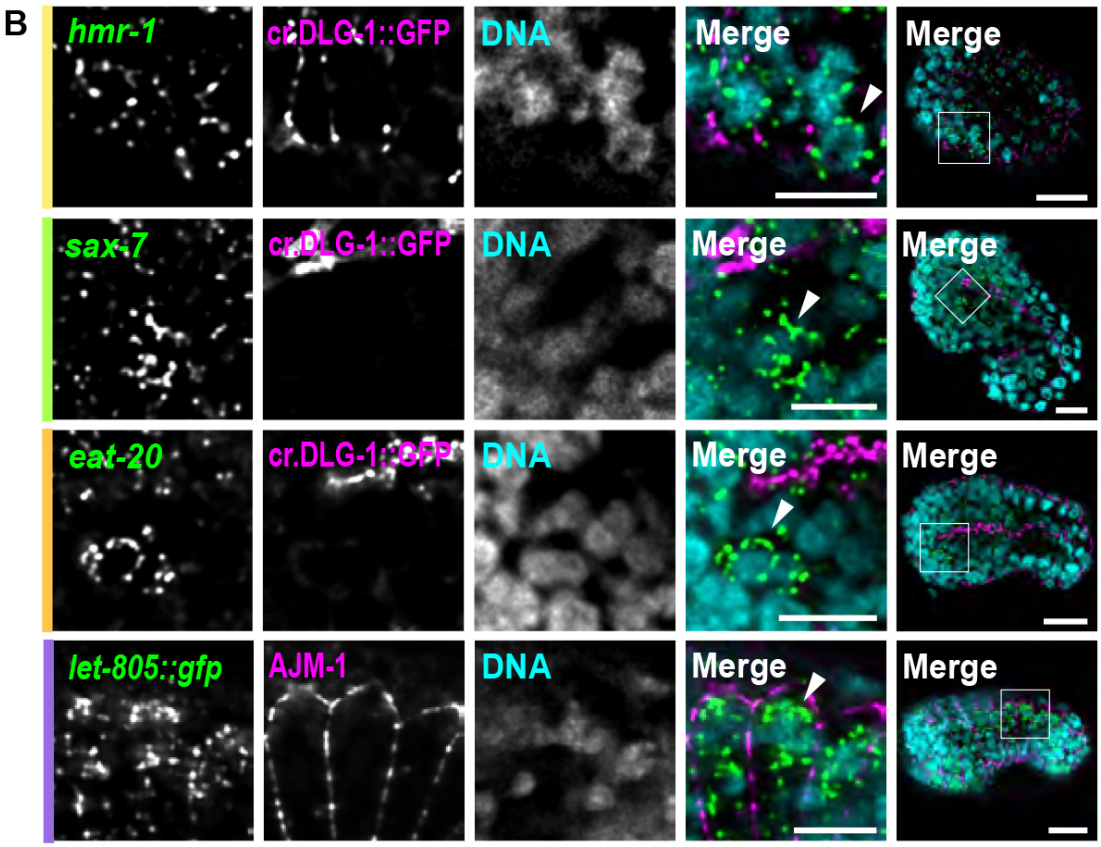

**Fig. S1. Endogenous *dlg-1* is enriched at the *Ce*AJ; transmembrane protein-coding mRNAs show instances of perinuclear localization. A.** Fluorescent micrographs of the entire C. elegans embryos (upper panels) and zoom-ins (lower panels) showing smFISH signal of endogenous dlg-1 mRNAs (green) in epidermal and seam cells of a bean stage, fluorescent signal of the transgenic GFP-tagged HMR-1 protein (HMR-1::GFP, magenta), and merges. Endogenous/non-tagged dlg-1 mRNA shows CeAJ/membrane localization like its endogenous/GFP CRISPR-tagged version. Scale bar (upper panels): 10 µm. Scale bars (lower panels): 5 µm. **B.** Fluorescent micrographs of portions and entire C. elegans embryos showing instances of perinuclearly localized mRNAs coding for transmembrane proteins (HMR-1, SAX-7, EAT-20, and LET-805::GFP). smFISH signal of localized mRNAs hmr-1, sax-7, eat-20, and let-805::gfp (green), fluorescent signal of the CRISPR-engineered DLG-1::GFP (cr.DLG-1::GFP) or AJM-1 (magenta), and merges. To the left of each image, bars color-coded as in Fig. 1A to indicate to which sub-class of factors the mRNAs code for. Arrowheads: examples of cells with perinuclearly localized mRNAs. Scale bars: 5 µm.

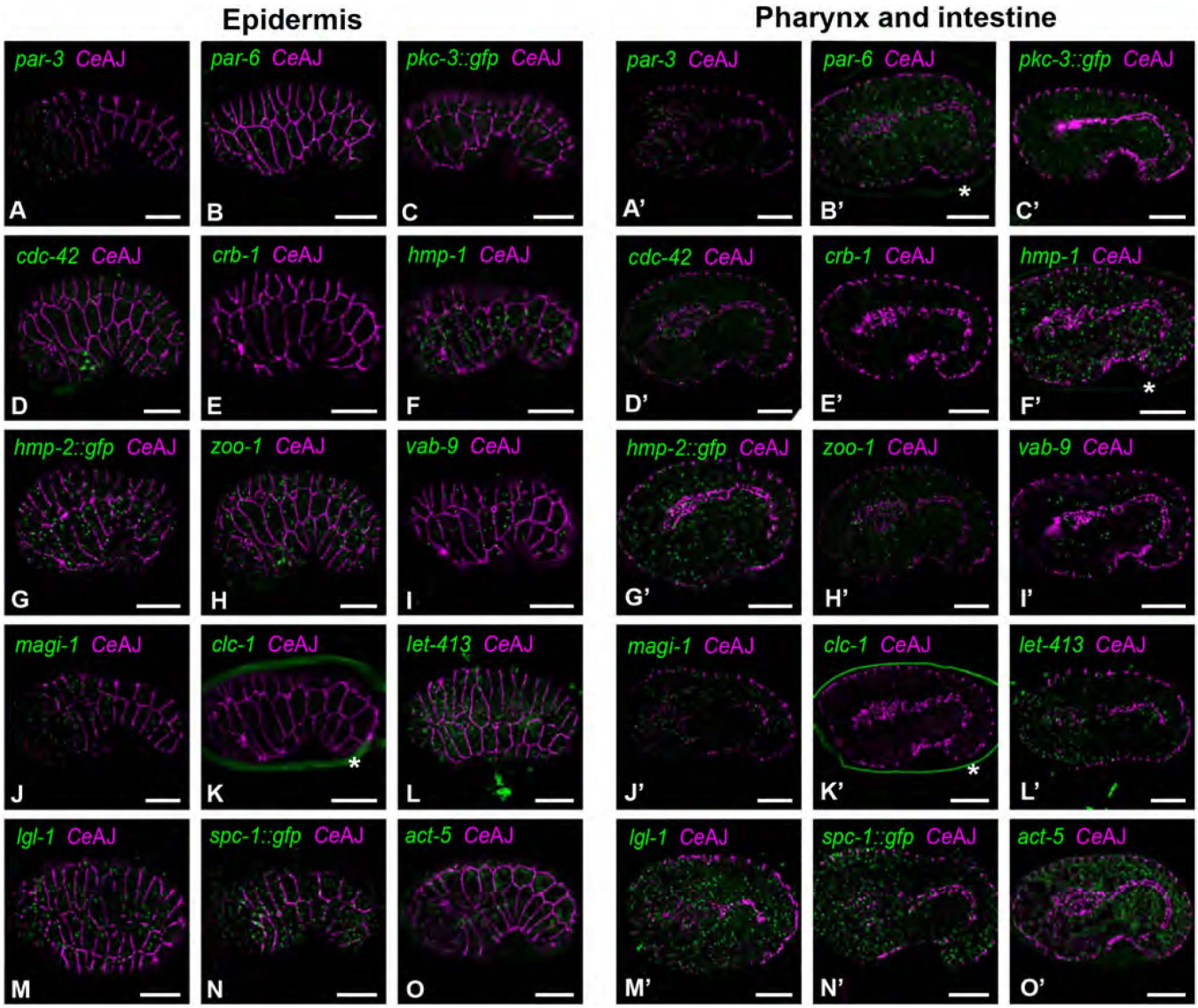


**Fig. S2. Fifteen mRNAs coding for *Ce*AJ, cell polarity, and related components do not show any evident subcellular enrichment. A-O.** Fluorescent micrographs of entire *C. elegans* embryos at the bean/comma stages focusing on the epidermis for the stated mRNAs in each image (green). Magenta: *Ce*AJ marker (cr.DLG-1::GFP or AJM-1 for GFP-tagged lines). White asterisks mark unspecific signal from eggshells. Scale bars: 10 µm. **A'-O'.** Same as in A-O but for the forming digestive track (pharynx and intestine).

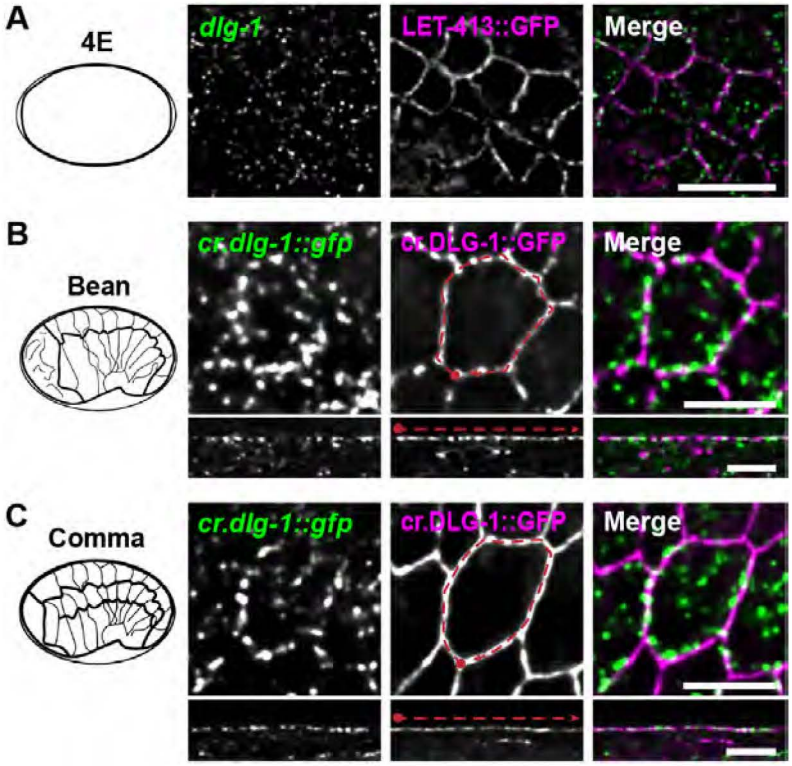


**Fig. S3. *dlg-1* mRNA is enriched at the cell membrane prior to *Ce*AJ maturation. A.** Fluorescent micrographs of developing epithelial cells of a portion of a *C. elegans* embryo at the 4E stage (schematics on the left) showing smFISH signal of the endogenous *dlg- 1* mRNA (green), fluorescent signal of the transgenic GFP-tagged LET-413 protein (LET- 413::GFP, magenta), and merges. Scale bars: 10 µm. **B-C.** Top views of fluorescent micrographs of a seam cell at the embryonic bean and comma stages (schematics on the left). Images show smFISH signal of the *dlg-1::gfp* mRNA (*cr.dlg-1::gfp*, green), fluorescent signal of the CRISPR-engineered GFP-tagged DLG-1 protein (cr.DLG-1::GFP, magenta), and merges. Red asterisks mark the site from which transverse projections shown below start from. Scale bar: 5 µm. Below: corresponding transverse sections on a flat plane of the sole rolled out membrane of the seam cell shown above. Red asterisks mark the horizontal location of *Ce*AJ and the starting site shown above. Scale bar: 5 µm.

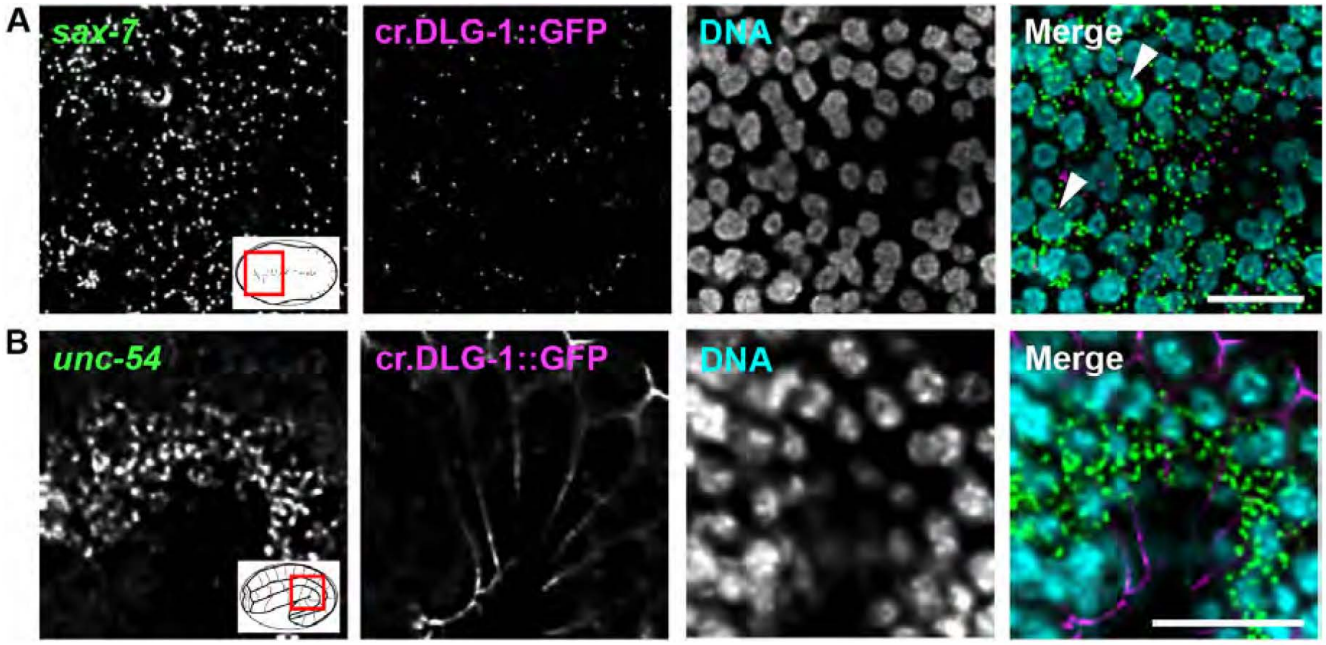


**Fig. S4. The UTRs enrolled in transgenic lines derive from unlocalized mRNAs. A-B.** Maximum intensity projections of 5 (A) and 3 (B) Z-stacks of fluorescent micrographs of portions (red box in the cartoon) of *C. elegans* embryos at the 16E (A) and 1.5-fold (B) stages showing smFISH signal of *sax-7* (A) and *unc-54* (B) mRNAs (green), fluorescent signal of the endogenous GFP-tagged DLG-1 protein (cr.DLG-1::GFP, magenta), DNA (cyan), and merges. Arrowheads: examples of cells with perinuclearly localized *sax-7* mRNA. Such a localization depends on the signal peptide located in the CDS of *sax-7* and not on its UTRs. Scale bars: 10 µm.

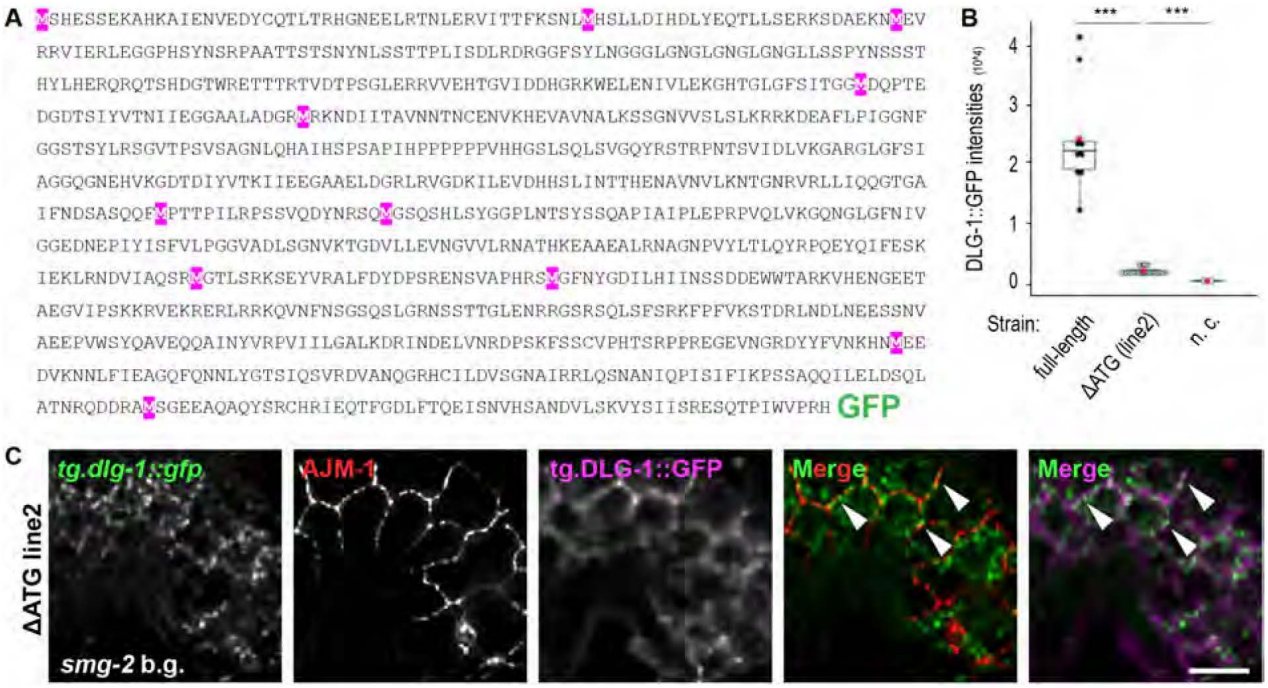


**Fig. S5. *dlg-1* mRNA coding sequence possesses putative alternative start codons that may allow its partial translation. A.** Transgenic GFP-tagged (green "GFP" at the carboxy-terminus) DLG-1 protein sequence. Highlighted in magenta are methionine amino acids corresponding to the green asterisks show in Fig. 4A. **B.** Dot plot with box plot: each dot represents the GFP intensity of pharynxes of adult worms carrying a "full-length" (n = 9; mean = 24.6 x 10^3; StDev = 9.4 x 10^3) or a "ΔATG line2" transgene (n = 15; mean = 2.0 x 10^3; StDev = 0.1 x 10^3). GFP intensities from animals of the "ΔATG line2" not carrying a transgene (recognizable through the "rolling" phenotype from the co-marker *rol-6*) were used as a negative control ("n. c.") (n = 3; mean = 0.4 x 10^3; StDev = 0.1 x 10^3). Red dots represent the mean. Significance of statistical analyses (*t*-test, one tail): *** = < 0.001. **C.** Fluorescent micrographs of a lateral portion of seam and epithelial cells at the late bean stage of a *C. elegans* embryo showing an example of a "ΔATG" transgene expressed in a null mutant background for an NMD component ("*smg-2* b.g.", bottom)., that is able to express its coded protein partially ("ΔATG line2"). As in Fig. 4C-E: smFISH signal of altered ATG ("ΔATG") *tg.dlg-1::gfp* mRNAs (green), immuno-fluorescent signal of the endogenous AJM-1 protein (red), fluorescent signal of the corresponding ΔATG tg.DLG-1::GFP protein (magenta), and merges. Arrowheads: examples of laterally localized mRNAs. Scale bar: 5 μm.

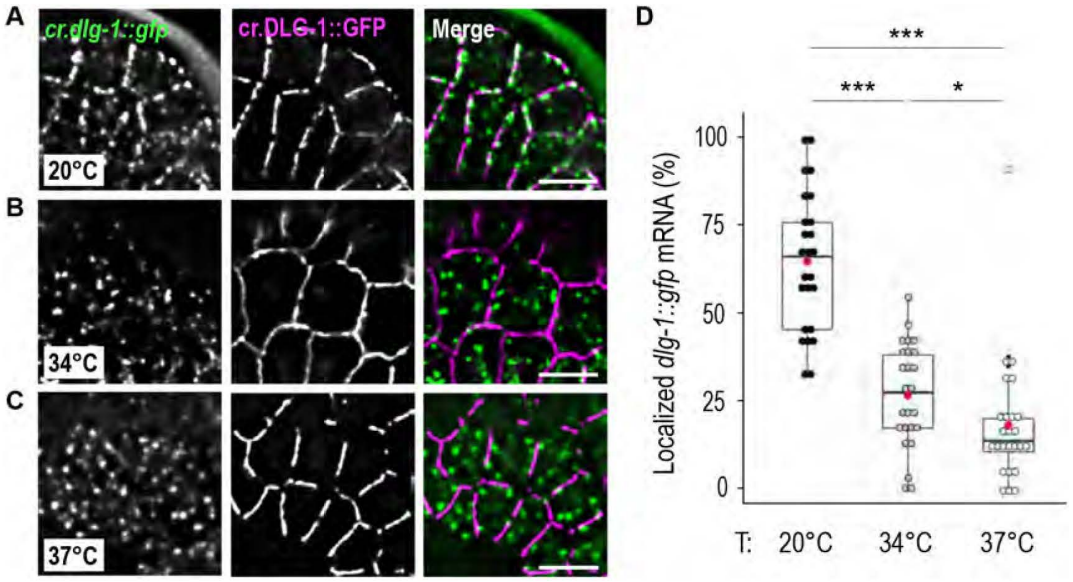


**Fig. S6. *dlg-1* mRNA localization is lost upon heat stress. A-C.** Fluorescent micrographs of seam cells of *C. elegans* embryos at the late bean stage that have been grown at 20°C (A) or subsequently subjected to a 1-hour heat-shock at 34°C (B) or 37°C (C) on plates. The images show smFISH signal of *cr.dlg-1::gfp* mRNA (green), fluorescent signal of the corresponding cr.DLG-1::GFP (magenta), and merges. Scale bars: 5 µm. **D.** Dot plot with box plot: each dot represents the percentage of laterally localized versus total cellular *cr.dlg-1::gfp* mRNA in each seam cell analyzed for embryos grown at 20°C (black; mean = 65.36; StDev = 19.78) or subsequently subjected to 34°C (grey; mean = 27.35; StDev = 14.85) or 37°C (white; mean = 18.70; StDev = 18.55) heat-shock (n = 25 for all conditions). Data derived from 5 different embryos. Significance of statistical analyses (*t*-test, one tail): * = < 0.05; *** = < 0.001.

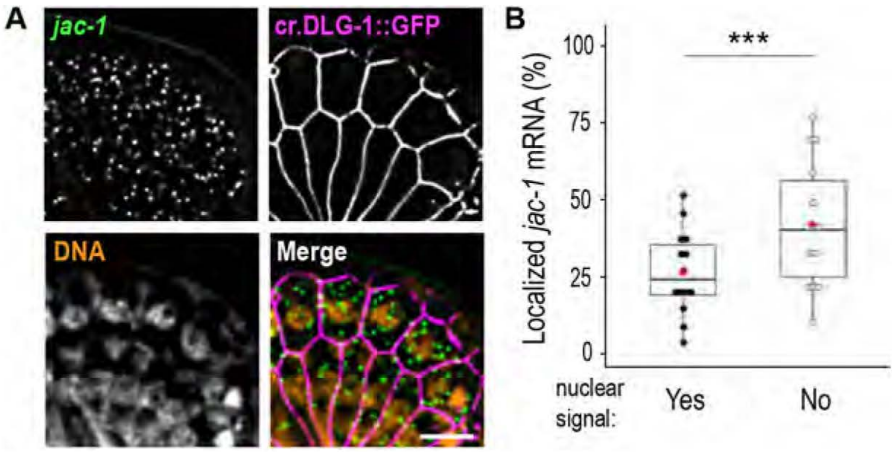


**Fig. S7. The baseline percentage of lateral localization to call an mRNA unlocalized varies if the nuclear signal is taken under consideration or not. A.** Fluorescent micrographs of the five posterior seam cells of a *C. elegans* embryo at the bean stage. The images show smFISH signal of *jac-1* mRNA (green), fluorescent signal of cr.DLG-1::GFP (magenta), DNA staining (orange), and merge. Scale bar: 5 µm. **B.** Dot plot with box plot: each dot represents the percentage of laterally localized versus "total" ("nuclear signal: yes"; black; mean = 25.87; StDev = 13.36) or versus "total" minus "nuclear" ("nuclear signal: no"; white; mean = 40.98; StDev = 20.05) *jac-1* mRNA in each seam cell at the bean stage analyzed (n = 14) (Table S2; Materials and methods). Data derived from 3 different embryos. Significance of statistical analyses (*t*-test, one tail): *** = < 0.001.

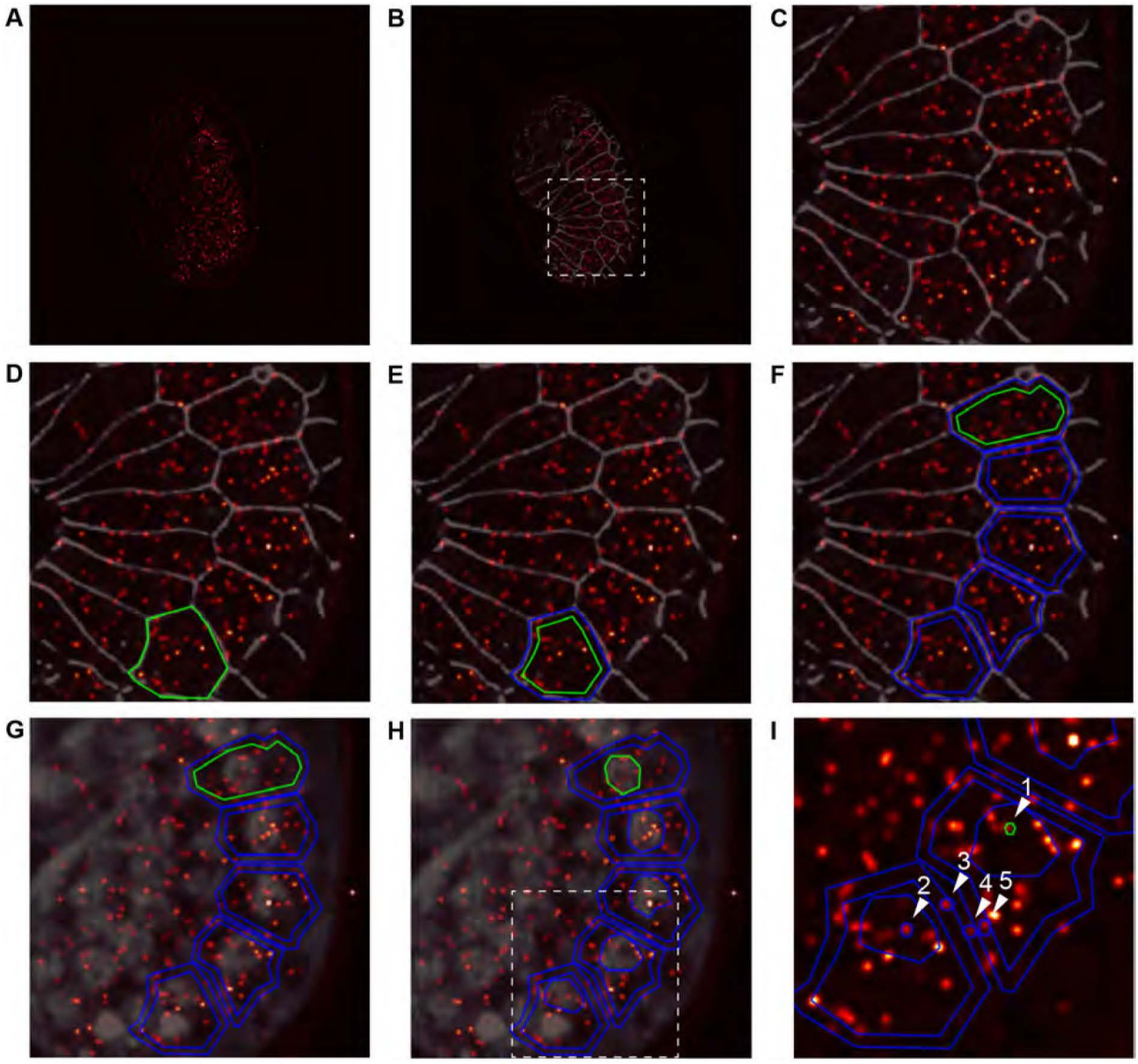


**Fig. S8. Quantitation through FISH-quant provides total, cytoplasmic, and nuclear mRNA counts. A.** Fluorescent micrograph (maximum intensity projection) of the same embryo shown in Fig. S7A showing smFISH signal of *jac-1* mRNA (red) from the FISH-quant platform. **B.** Same embryo as in (A) with cr.DLG-1::GFP signal (grey) to identify seam cells. Dashed square: portion of the embryo zoomed-in in (C). **C.** Zoom-in showing the five seam cells that will be undergoing the analysis through FISH-quant. **D.** Example of an outline (green) drawn along the border of a seam cell marked by cr.DLG-1::GFP signal. This outline provides the counts for the "total" cellular mRNA. **E.** Example of an outline (green) drawn parallel (± 500 nm) to the outer border (blue) of the same seam cell. This outline provides the counts for the "cytoplasmic + nuclear" mRNA. **F.** Outlines of the five seam cells that will be undergoing the analysis through FISH-quant. **G.** Same as (F), but showing the DNA signal (grey) instead of the cr.DLG-1::GFP signal. **H.** Same as in (G) showing the outlined DNA signals (small polygons in blue and green) to mark the "nuclear" mRNA. Dashed square: portion of the embryo zoomed-in in (I). **I.** Zoom-in on two of the five seam cells that will be undergoing the analysis through FISH-quant and showing the outlined mRNA dots chosen as units (small ellipses in blue and green).

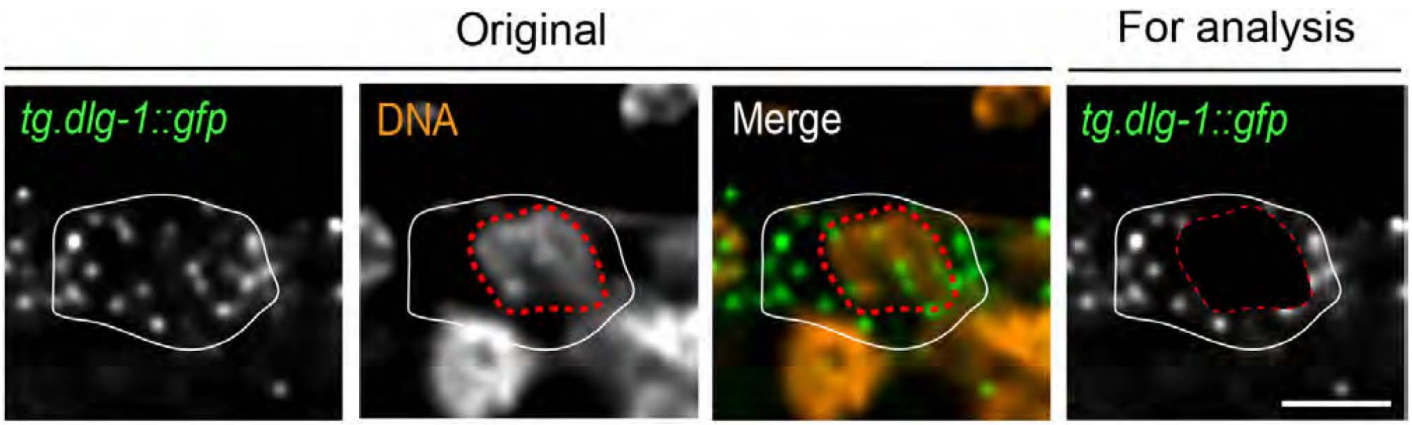


**Fig. S9. Removal of smFISH signal overlapping with DNA in apicobasal and apical profile analyses.** Fluorescent micrographs of a seam cell at the bean stage seen in frontal view (ΔL27 line). In the first three panels, from left to right: smFISH signal of *tg.dlg-1*$^{\Delta L27}$ mRNA (green), DNA staining (orange), and merge. White continuous lines: periphery of the cell. Red dashed lines: periphery of the DNA staining. On the very right: smFISH signal of *tg.dlg-1*$^{\Delta L27}$ mRNA, (same as on the very left) with the mRNA signal overlapping with the DNA staining being removed for profile analyses shown in Fig. 6 (Methods). Scale bar: 2.5 µm.

**Table S1. List of factors enrolled in the smFISH survey.** Names of the factors and their orthologues whose mRNAs were tested in our smFISH-based survey for localized mRNAs highlighted in the same color-code as in Fig. 1A to address to which group of localized protein they belong to (Factor); to what (if any) AS they belong to (Adhesion system); subcellular localization of the stated factor (Protein localization); WormBase identification number (WormBaseID); gene sequence name (Sequence name); mRNA localization observed in this study (Observed mRNA localization); target of smFISH probes (smFISH probes; for full list of probe sequences, see Table S4); strain that has been tested in our survey for the corresponding mRNA/factor (Strain tested); reference for the corresponding factor (Reference).

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**Table S2. Raw data for image quantitation.** Raw data (mRNA counts), percentages of localization, means, and standard deviations, for all the quantification analyses in this study.

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**Table S3. List of methionine amino acid within the DLG-1 sequence.** Amino acid positions and domain location of the 11 methionine amino acids found in the DLG-1 sequence, corresponding to the green asterisks in Fig. 4A.

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**Table S4. List of *C. elegans* strains.** Detailed list of names, genotypes, and references of C. elegans strains used in this work.

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**Table S5. List of smFISH probes.** Sequences of the smFISH primary probes used in this study.

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