# Supplemental Materials

***Molecular Biology of the Cell***

Von Stetina et al.

Figure S1

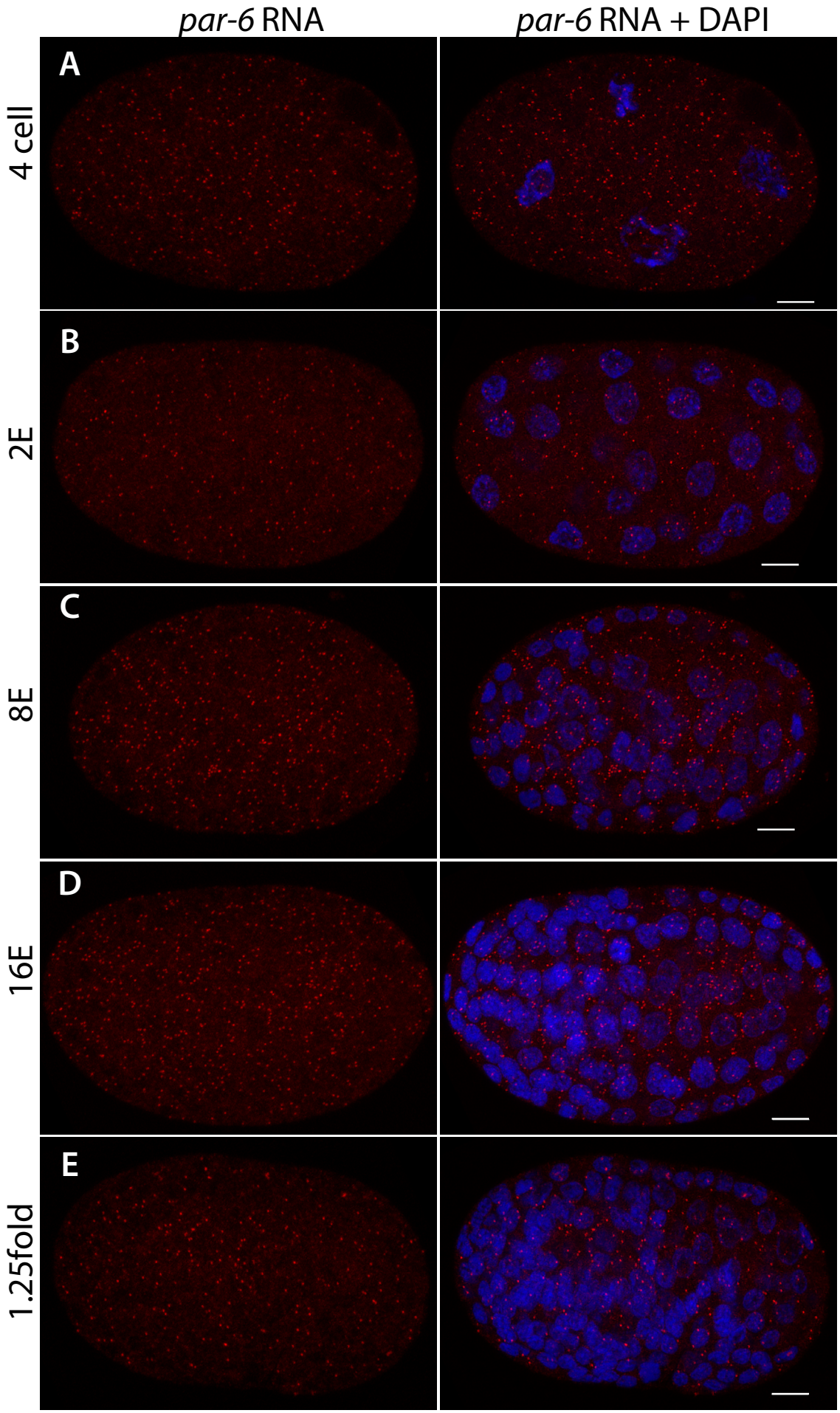


**Figure S1. *par-6* mRNA expression.** Maximum image projections of representative embryos. Developmental stage is on the left. *par-6* mRNA smFISH spots are in red, DAPI is in blue. *par-6* mRNA is maternally deposited, as evidenced by detection of the abundant mRNAs at the 4-cell stage (A). *par-6* is detected throughout embryogenesis (B-E), with no obvious onset of zygotic transcription. Anterior is to the left, scale bars 5 μm.

Figure S2

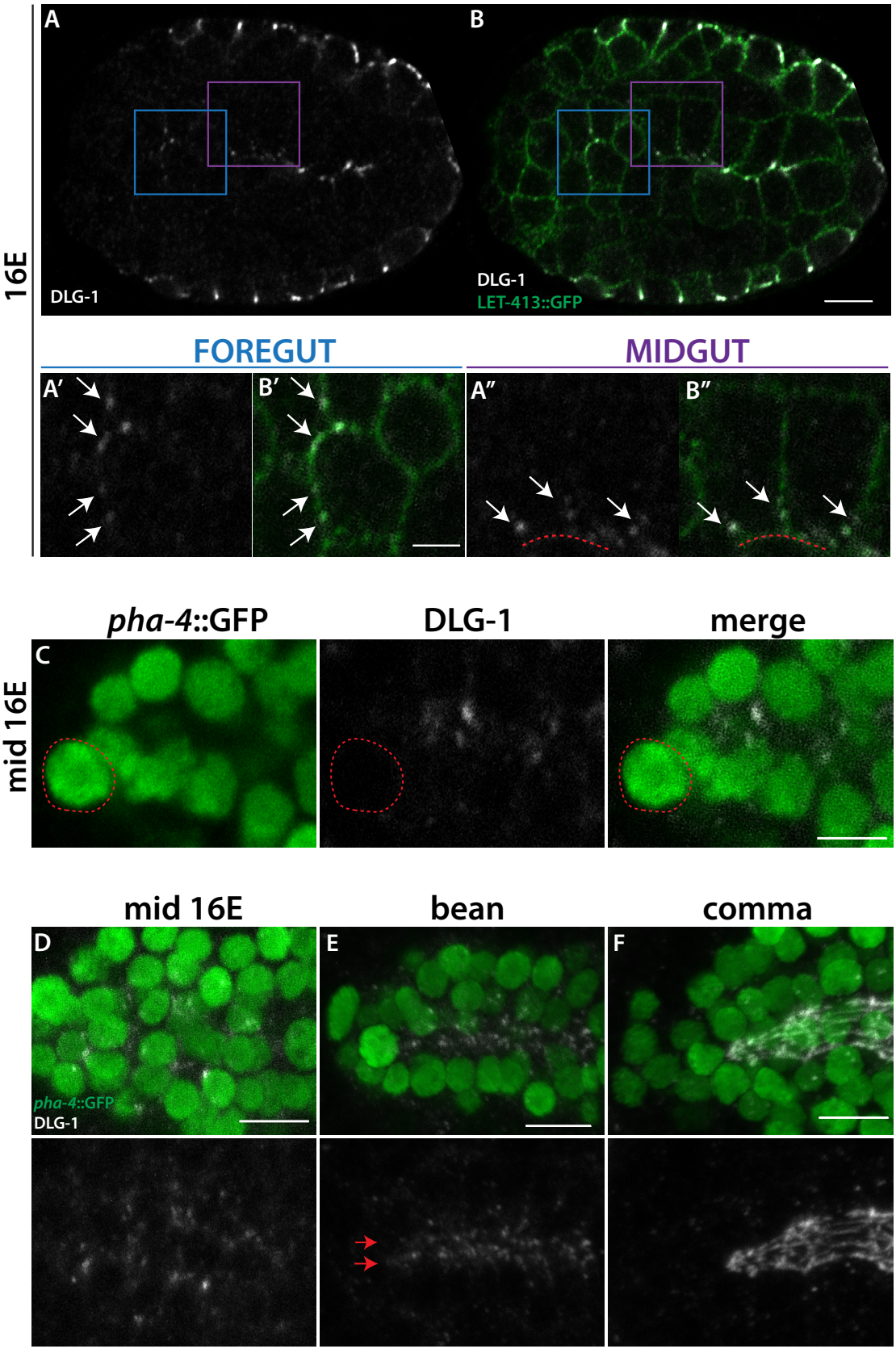


**Figure S2. DLG-1 protein localization during embryogenesis.** A, B. DLG-1 (white, detected by antibody staining) is initially found on the lateral surfaces (green, marked by LET-413::GFP) of polarizing foregut (blue box and A', B' insets) and midgut (purple box and A" and B" insets) cells. Arrows point to DLG-1 puncta at the membrane. Dashed red line in A" and B" refers to the nascent apical surface of these midgut cells. C. Single focal plane of a 16E foregut primoridium. Arcade cells are born at this stage but do not have detectable DLG-1 expression. A representative arcade cell is circled with a red dotted line. Nearby foregut cells have detectable DLG-1 protein (white). D-F. Time-course of DLG-1 localization (white) in the foregut, denoted by *pha-4*::GFP (green). Panels D, F are the same as Figure 2P, Q. At the mid-16E stage, DLG-1 puncta are found throughout the tissue. At the bean stage the cells have reoriented, forming a nascent apical lumen in the center of the epithelial cyst. At this stage the DLG-1 puncta are enriched at the nascent apical surfaces that surround the lumen (red arrows). By the comma stage the DLG-1 puncta have fused to form continuous apical junctions. Anterior is to the left, scale bars 5 μm.

Figure S3

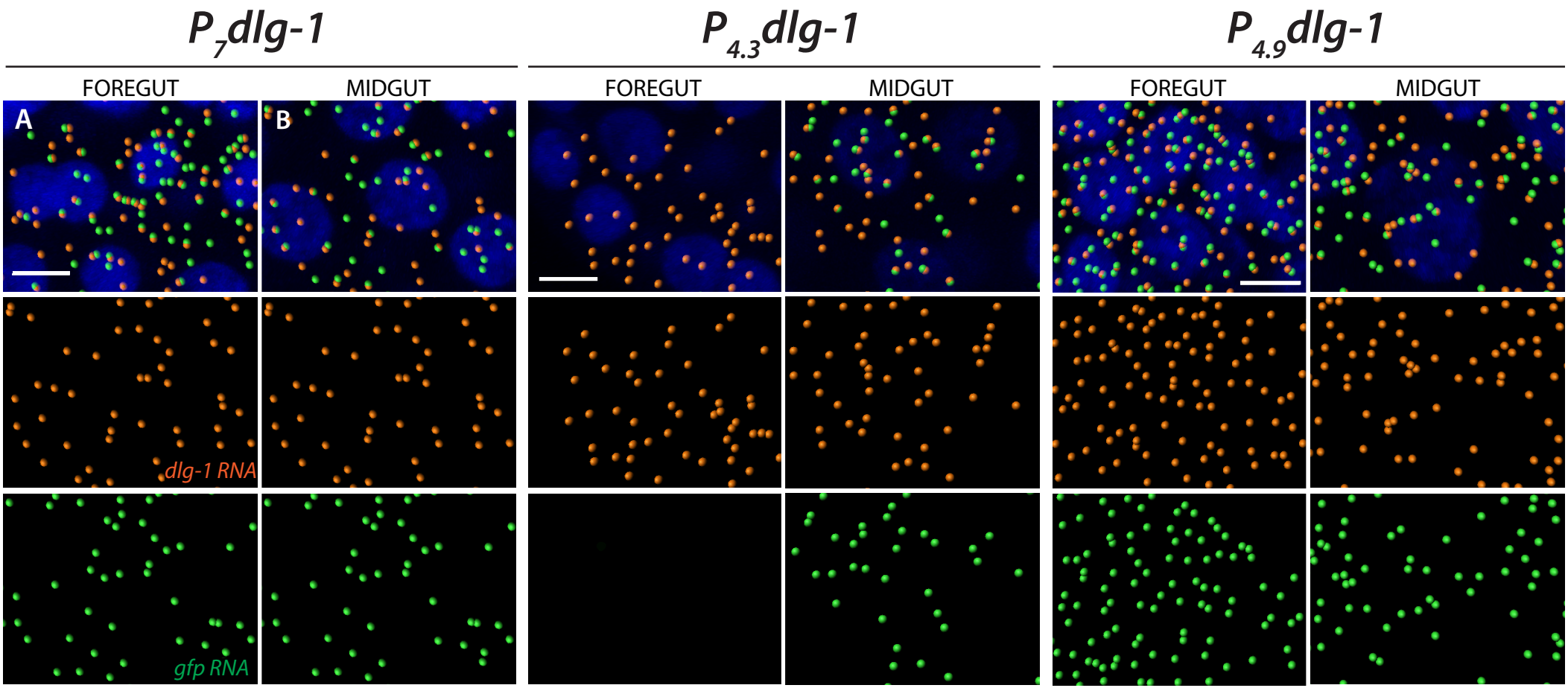


**Figure S3. Onset of *gfp* expression in 8E gut for $P_7$*dlg-1* and $P_{4.9}$*dlg-1*, but not $P_{4.3}$*dlg-1*, matches that of endogenous *dlg-1*.** A-F. Maximum intensity projection of the digestive tract, showing embryos expressing various *dlg-1* promoter transgenes at the 8E stage (see Figure 4 for description of the promoter constructs). Endogenous *dlg-1* (imported into Imaris and pseudocolored orange, see Methods) and transgenic gfp (pseudocolored green) mRNAs were detected using smFISH. A, B. $P_7$*dlg-1* transgenic embryos express *gfp* transcript at the same time as endogenous *dlg-1* in both the foregut (A) and midgut (B). C, D. $P_{4.3}$*dlg-1* transgenic embryos express *gfp* transcript at the same time as endogenous *dlg-1* in the midgut (D), but lack any expression in the foregut (C). E, F. $P_{4.9}$*dlg-1* transgenic embryos express *gfp* transcript at the same time as endogenous *dlg-1* in both the foregut (E) and midgut (F). Anterior is to the left, scale bar 3 μm.

Figure S4

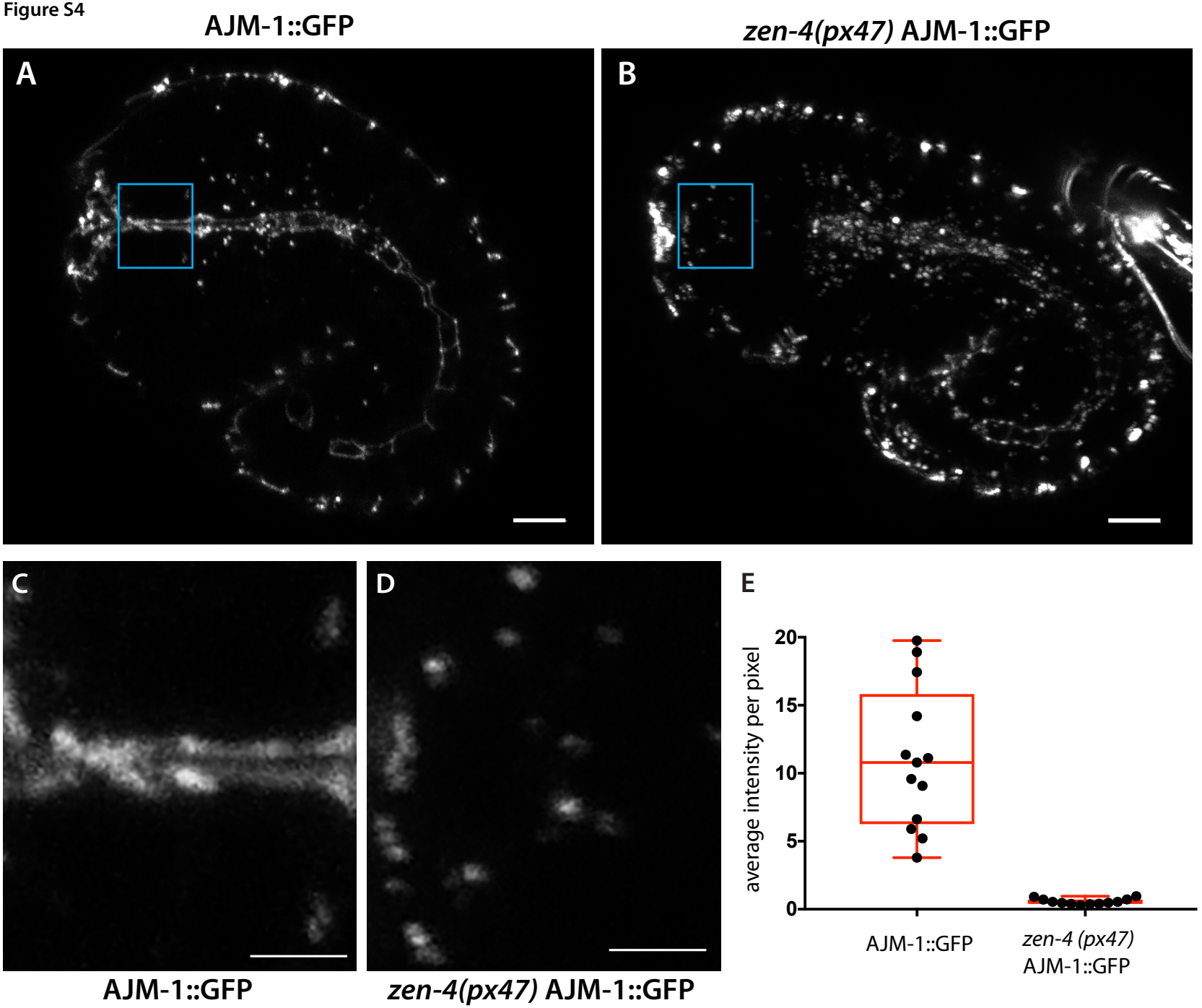


**Figure S4. 20-fold less AJM-1::GFP in *zen-4* mutant Arcade Cells compared to wildtype.** A, B. Maximum Intensity Projections through the digest tract of AJM-1::GFP in wildtype and *zen-4* mutant backgrounds, acquired via live-imaging. The boxed regions show the Arcade Cells, which are blown up in panels C, D. 14 wildtype and 12 *zen-4* mutants were quantified (see Methods for details), the results of which are shown in panel E. The average intensity per pixel values are displayed as a box and whisker plot. The line within the box plot displays the median value, while the highest and lowest values are shown at the end of the whiskers. Each dot within the box plot represents the value from a single quantified embryo. A comparison of the mean intensities reveals an average of 20-fold less signal in *zen-4* mutant Arcades versus wildtype. Anterior is to the left. Scale bars are 5 μm (A, B) and 2 μm (C, D).

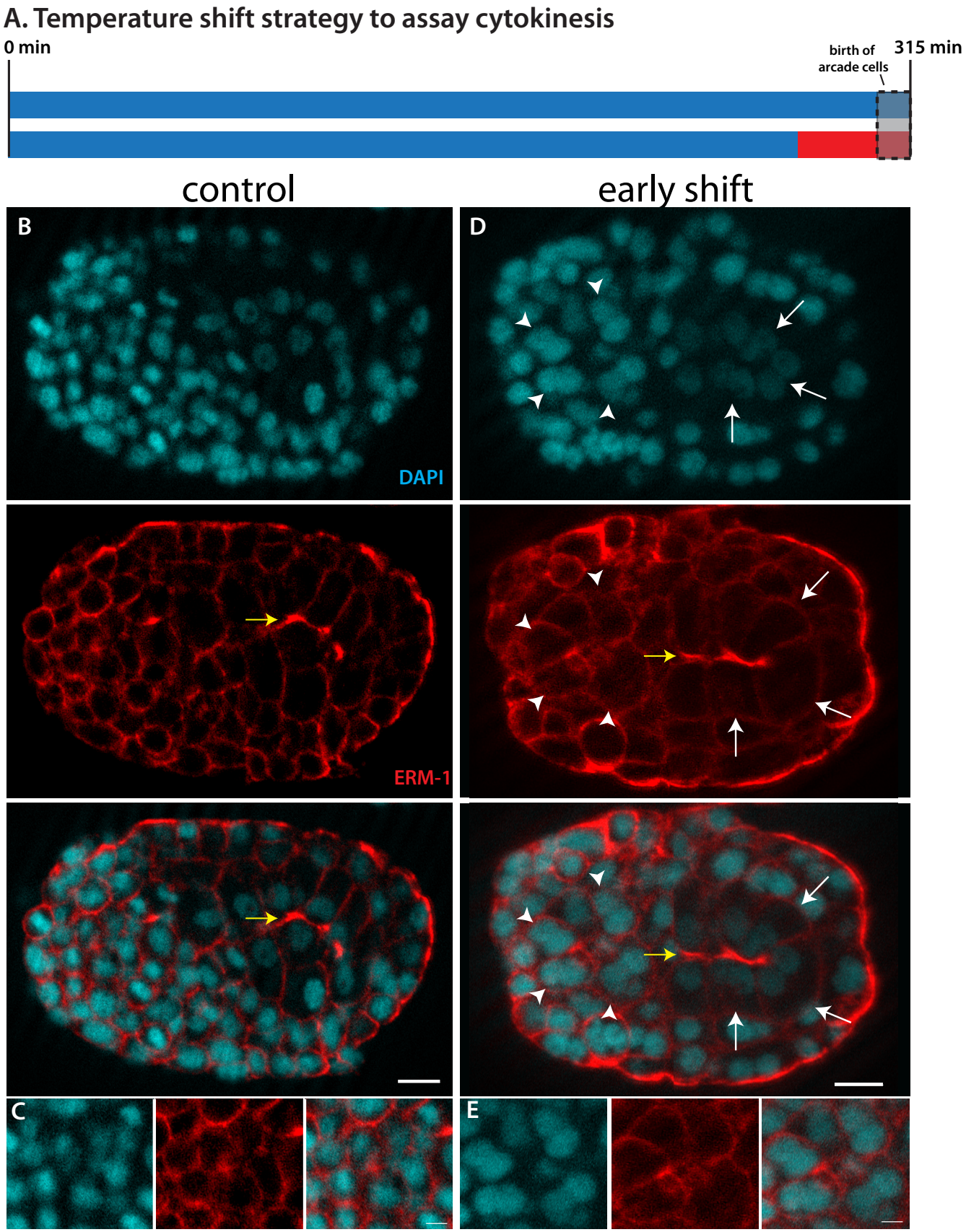


**Figure S5. *zen-4(or153ts)* displays cytokinesis defects when shifted at mid-embryogenesis.** A. Experimental design. Control embryos were held at the permissive temperature (15°C) for the entire experiment, which was timed to be at the same developmental stage as the experimental embryo. Shifted embryos were subjected to the restrictive temperature (26°C) 50 minutes prior to arcade cell birth, and removed for processing 4/5 of the way through the temperature shift shown in Figure 8. B-E. Embryos were immunostained for ERM-1 to show cell borders. At this stage ERM-1 is also enriched apically in the polarizing midgut (yellow arrows). B, C. Control embryos display mononucleate cells, including in a close-up in the developing foregut (C). D, E. Shifted embryos display cytokinesis defects, as evidenced by the bi- and tri-nucleate cells (arrowheads), including a close-up of the anterior foregut (E). Note that the midgut cells display cytokinesis defects but still polarize ERM-1 apically (arrows). Anterior is to the left. Scale bars are 5 μm (B,D) and 2 μm (C, E).

Figure S6

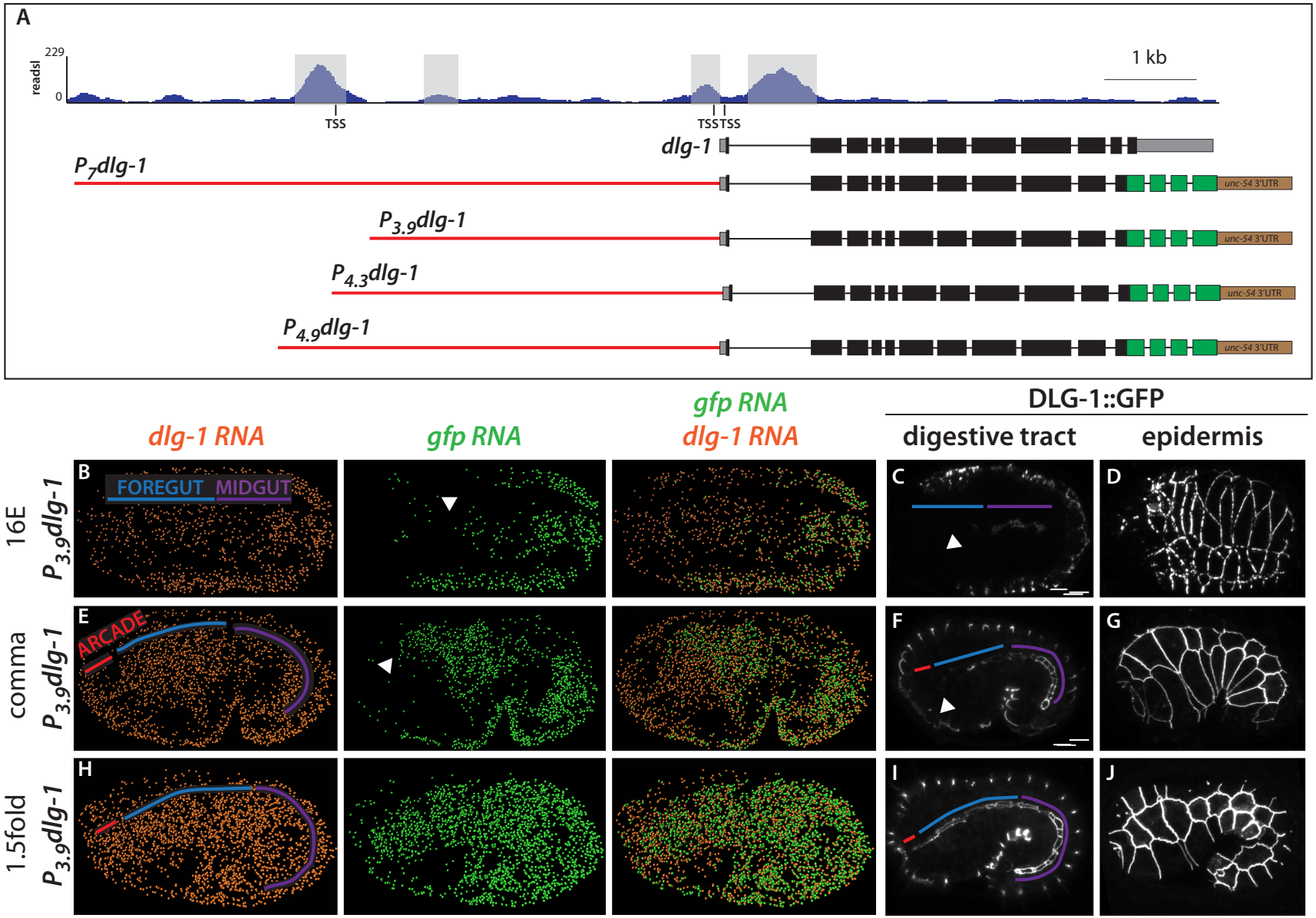


**Figure S6. *P*$_{3.9}$*dlg-1* reporter shows delayed expression in foregut/Arcade Cells.** As in Figure 4, PHA-4::GFP ChIP-Seq profile at the endogenous *dlg-1* locus (top). Exons are black boxes, 5' and 3' UTR sequence are gray boxes, and introns are black lines. There are four called PHA-4 ChIP peaks (highlighted in gray). The 3 constructs shown in Figure 4 are displayed for comparison, in addition to *P*$_{3.9}$*dlg-1* (contains 3.9kb of sequence), which lacks the upstream peak and the upstream TSS. B-J. Maximum intensity projections of worm embryos expressing the *P*$_{3.9}$*dlg-1* promoter construct at either the 16E, comma or 1.5fold stage. Transgenic embryos were co-hybridized with probes to detect *dlg-1* (pseudocolored orange) and *gfp* (pseudocolored green) transcripts via smFISH (columns 1-3). DLG-1::GFP protein (white) was detected by live imaging (columns 4-5). For every row, DLG-1::GFP panels are two focal planes from the same embryo. Note that the RNA and protein data are from different embryos. The areas corresponding to the arcade cells are labeled with a red line, the foregut with a blue line, and midgut with a purple line. Embryos expressing DLG-1::GFP under the *P*$_{3.9}$*dlg-1* promoter have transgenic gfp mRNA expression that matches endogenous *dlg-1* mRNA only in the epidermis and midgut. Expression in the foregut is delayed. In the 16E stage (E) *gfp* mRNA is nearly absent in the foregut (white arrowhead). By the comma stage (N) the posterior foregut has *gfp* mRNA but the anterior foregut and arcade cells have no expression (white arrowhead). Transgenic mRNA expression in the arcades and anterior foregut is seen by the 1.5fold stage (W). DLG-1::GFP (F, O, X) also follows this pattern (white arrowheads point to absent expression in foregut/arcades). All images are maximum intensity projections. Anterior is to the left and scale bars are 5 μm. smFISH data imported into Imaris 3D software.

| Table S1. Analysis of non-foregut epithelial genes for PHA-4 binding | | |
|---|---|---|
| **Gene** | **PHA-4 bound?** | **Location of peak** |
| ***git-1*** | yes | upstream near TSS |
| ***ifa-3*** | no | n/a |
| ***ifb-2*** | no | n/a |
| ***ifc-1*** | no | n/a |
| ***ifd-1*** | no | n/a |
| ***ifd-2*** | no | n/a |
| ***ife-3*** | no | n/a |
| ***mua-3*** | no | n/a |
| ***mua-6*** | no | n/a |
| ***mup-4*** | no | n/a |
| ***pak-1*** | no | n/a |
| ***rga-2*** | no | n/a |

This table shows the specificity of the PHA-4 ChIP-seq dataset, as all but one of the epithelial genes that are not expressed in the foregut lack PHA-4 binding.

| Table S3. ZEN-4 structure-function analysis | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Transgene negative | | | Transgene positive | | |
| **Construct** | **Array** | **% polarized (n)** | **% unpolarized (n)** | **% enclosure defective (n)** | **% polarized (n)** | **% unpolarized (n)** | **% enclosure defective (n)** |
| **No transgene control (SM1052)** | n/a | 75% (335/448) | 20% (91/448) | 5% (22/448) | n/a | n/a | n/a |
| **Wildtype** | *pxEx390* | 76% (88/115) | 15% (17/115) | 9% (10/115) | 96% (144/150) | 0.7% (1/150) | 3.3% (5/150) |
| | *pxEx392* | 72% (39/54) | 15% (8/54) | 13% (7/54) | 97% (89/92) | 0% (0/92) | 3% (3/92) |
| | *pxEx393* | 81% (54/67) | 12% (8/67) | 7% (5/67) | 95% (129/136) | 0% (0/136) | 5% (7/136) |
| | *pxEx396* | 78% (90/115) | 16% (18/115) | 6% (7/115) | 97% (124/128) | 0% (0/128) | 3% (4/128) |
| **P-loopΔ** | *pxEx395* | 71.5% (43/60) | 20% (12/60) | 8% (5/60) | 96% (50/52) | 0% (0/52) | 4% (2/52) |
| | *pxEx399* | 80% (144/181) | 12% (22/181) | 8% (15/181) | 96% (237/246) | 2% (5/246) | 1.5% (4/246) |
| | *pxEx402* | 80% (86/107) | 15% (16/107) | 5% (5/107) | 100% (89/89) | 0% (0/89) | 0% (0/89) |
| | *pxEx486* | 76% (63/83) | 17% (14/83) | 7% (6/83) | 95% (79/82) | 1% (1/82) | 2.5% (2/82) |
| | *pxEx488* | 76% (22/29) | 13% (4/29) | 10% (3/29) | 97% (70/72) | 1% (1/72) | 1% (1/72) |
| **Loop12Δ** | *pxEx422* | 79% (107/136) | 18% (25/136) | 3% (4/136) | 98% (126/129) | 1.5% (2/129) | 0.7% (1/129) |
| | *pxEx424* | 80% (145/181) | 17% (30/181) | 3% (6/181) | 98% (354/363) | 0% (0/363) | 2% (9/363) |
| | *pxEx489* | 80% (41/51) | 14% (7/51) | 6% (3/51) | 100% (157/157) | 0% (0/157) | 0% (0/157) |
| **NLS1Δ** | *pxEx397* | 73% (32/44) | 18% (8/44) | 9% (4/44) | 96% (79/82) | 2% (2/82) | 1% (1/82) |
| | *pxEx423* | 78% (79/101) | 16% (16/101) | 6% (6/101) | 99% (66/67) | 0% (0/67) | 1.5% (1/67) |
| | *pxEx425* | 86% (35/41) | 16% (6/41) | 0% (0/41) | 98% (41/43) | 5% (2/43) | 0% (0/43) |
| **NLS2Δ** | *pxEx398* | 75% (243/324) | 18% (58/324) | 7% (23/324) | 96% (315/327) | 0.6% (2/327) | 3% (10/327) |
| | *pxEx420* | 78% (52/67) | 19% (13/67) | 3% (2/67) | 99% (93/94) | 0% (0/94) | 1% (1/94) |
| **NLS1/2Δ** | *pxEx455* | 80% (73/91) | 16% (15/91) | 3% (3/91) | 96% (129/134) | 1.5% (2/134) | 2% (3/134) |
| | *pxEx456* | 82% (90/110) | 11% (12/110) | 7% (8/110) | 99% (131/132) | 0% (01/132) | 0.7% (1/132) |
| **NeckΔ** | *pxEx391* | 75% (137/182) | 17% (32/189) | 10% (20/189) | 77% (170/222) | 20% (44/222) | 4% (9/222) |
| | *pxEx444* | 70% (83/119) | 16% (39/119) | 15% (17/189) | 74% (184/249) | 19% (47/249) | 7% (18/249) |

This table shows complete data for the ZEN-4 structure-function analysis. SM1052 is the reference strain with genotype *zen-4 dpy-20/bli-6 unc-24*, maintained as a heterozygote. The progeny, by Mendelian genetics, should be 75% wildtype and 25% mutant, of which the majority displays the unpolarized Arcade Cell phenotype (assessed by Nomarski optics, looking for foregut attachment), while a subset display a failure in epidermal enclosure and subsequently elongation. To assess for rescue compare the % unpolarized between the transgene negative and transgene-positive columns for a given transgenic line. For example, *pxEx390* displays 15% unpolarized Arcades for the transgene-negative, while only 0.7% of the transgene-positive embryos have unpolarized Arcades and thus is considered to rescue the phenotype. The n for a given phenotype in a given transgenic line is displayed in parantheses.

| Table S4. ZEN-4 tether analysis | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Transgene negative | | | Transgene positive | | |
| **Construct** | **Array** | **% polarized (n)** | **% unpolarized (n)** | **% enclosure defective (n)** | **% polarized (n)** | **% unpolarized (n)** | **% enclosure defective (n)** |
| **No transgene control (SM1052)** | n/a | 75% (335/448) | 20% (91/448) | 5% (22/448) | n/a | n/a | n/a |
| **ZEN-4::CYK-4** | *pxEx518* | 81% (78/96) | 10% (9/96) | 9% (9/96) | 98% (166/170) | 0.6% (1/170) | 2% (3/170) |
| | *pxEx519* | 81% (65/80) | 16% (13/80) | 2.5% (2/80) | 94% (129/137) | 3.6% (5/137) | 2% (3/137) |
| | *pxEx541* | 85% (145/171) | 13% (22/171) | 2% (4/171) | 99% (103/206) | 0.5% (1/206) | 1% (2/206) |
| **ZEN-4(NeckΔ) ::CYK -4** | *pxEx516* | 80% (87/109) | 11% (12/109) | 9% (10/109) | 96% (71/74) | 0.1% (1/74) | 3% (2/74) |
| | *pxEx517* | 84% (336/398) | 12% (46/398) | 4% (16/398) | 98% (280/285) | 0.1% (3/285) | 0.7% (2/285) |
| **ZEN-4::AIR-2** | *pxEx542* | 79% (179/228) | 15% (34/228) | 6.5% (15/228) | 98% (140/145) | 0.7% (1/145) | 3% (4/145) |
| | *pxEx543* | 82% (27/33) | 18% (6/33) | 0% (0/33) | 100% (135/135) | 0% (0/135) | 0% (0/135) |
| | *pxEx544* | 75% (47/63) | 22% (14/63) | 3% (2/63) | 96% (79/82) | 4% (3/82) | 0% (0/82) |
| **ZEN-4(NeckΔ) ::AIR-2** | *pxEx523* | 75% (96/128) | 20% (26/128) | 5% (6/128) | 83% (161/194) | 13% (25/194) | 4% (8/194) |
| | *pxEx524* | 83% (118/142) | 12% (17/142) | 5% (7/142) | 81% (125/155) | 13% (20/155) | 6% (10/155) |
| | *pxEx556* | 73% (74/101) | 22% (22/101) | 5% (5/101) | 81% (140/172) | 15% (25/172) | 4% (7/172) |
| **CYK-4::AIR-2** | *pxEx635* | 80% (171/186) | 18% (13/186) | 2% (2/186) | 75% (237/314) | 20% (63/314) | 5% (14/314) |

This table shows complete data for the tethered dimer analysis. SM1052 is the reference strain with genotype *zen-4 dpy-20/bli-6 unc-24*, maintained as a heterozygote. The progeny, by Mendelian genetics, should be 75% wildtype and 25% mutant, of which the majority displays the unpolarized Arcade Cell phenotype (assessed by Nomarski optics, looking for foregut attachment), while a subset display a failure in epidermal enclosure and subsequently elongation. To assess for rescue compare the % unpolarized between the transgene negative and transgene-positive columns for a given transgenic line. For example, *pxEx518* displays 10% unpolarized Arcades for the transgene-negative, while only 0.6% of the transgene-positive embryos have unpolarized Arcades and thus is considered to rescue the phenotype. The n for a given phenotype in a given transgenic line is displayed in parantheses.

**Table S5.** Scoring of *ect-2; zen-4* double mutants

| Genotype | % polarized (n) | % unpolarized (n) | % enclosure defective (n) |
|---|---|---|---|
| ***zen-4(px47) dpy-20/mIs11*** | 77% (348/450) | 16% (72/450) | 7% (30/450) |
| ***ect-2(xs110); zen-4(px47) dpy-20/mIs11*** | 78% (267/341) | 17% (58/341) | 5% (16/341) |

Polarity determined by Nomarski optics, analyzing attachment of foregut to epidermis. Three independent experiments are grouped here.