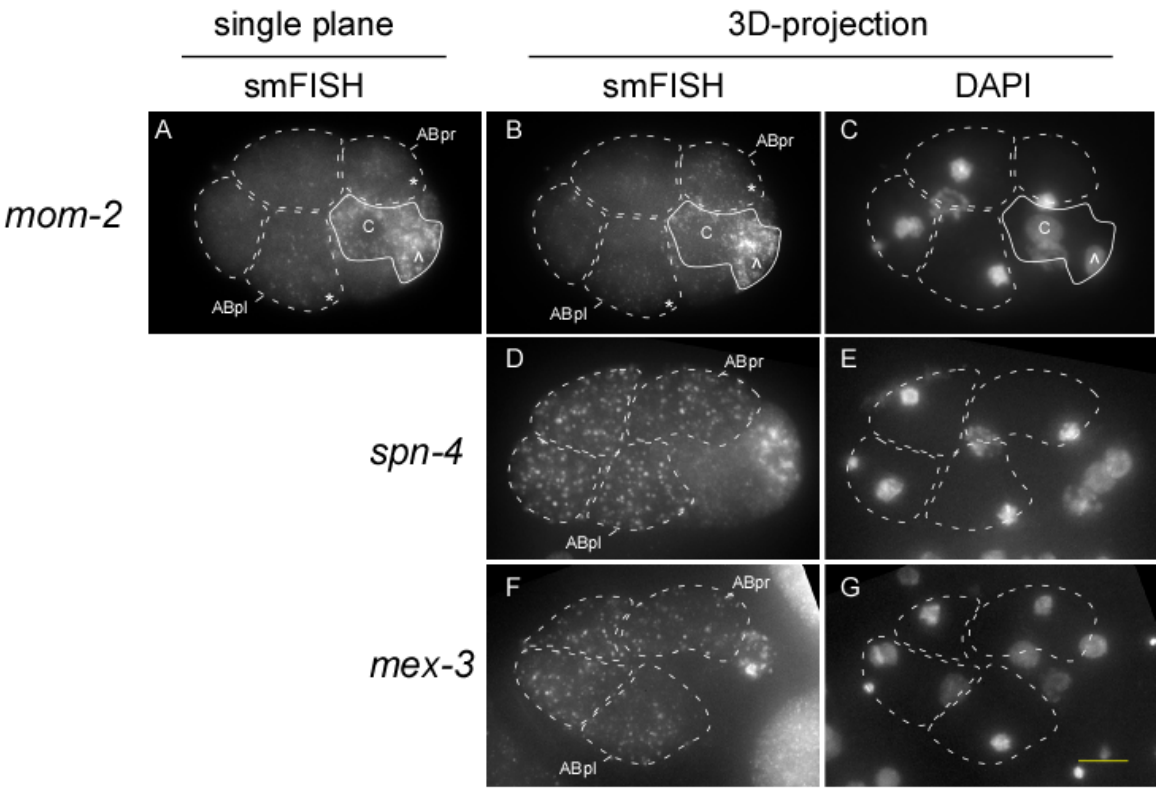

Figure S1. Related to Figure 2.

smFISH analyses of *mom-2, spn-4, and mex-3* mRNAs in 8-cell embryos
(A) Fluorescence image of *mom-2* smFISH from a single focal plane of a wildtype 8-cell embryo. (B-G) Fluorescence images of smFISH and DAPI staining are shown as 3D projected images, which show all nuclei in the embryo. P3 (^) and C, which contain maternally expressed *mom-2* mRNA, are in solid outline. The four AB-derived blastomeres in each panel are outlined by dashed lines. (A,B) The two AB-derived cells (ABpl and ABpr) with consistently elevated *mom-2* smFISH signal are indicated with asterisks. Bar: 10 um.

	ABal	ABar	ABpl	ABpr
8-cell embryo #1	49	59	205	193
8-cell embryo #2	113	82	220	281

	ABala	ABalp	ABara	ABarp	ABpla	ABplp	ABpra	ABprp
14-cell embryo	11	25	46	21	38	136	34	163
15-cell embryo #1	2	227	119	17	52	190	nd	nd
15-cell embryo #2	2	86	57	5	20	144	nd	nd

Table S1. Related to Figure 2.

***mom-2* smFISH quantitation.**
smFISH spot quantitation in individual cells of wildtype embryos. (Upper) Quantitation of *mom-2* smFISH spots in the 4 AB granddaughters of two 8-cell embryos. In both embryos, higher counts were obtained in the two ABp daughters, ABpl and ABpr, than in ABa daughters, ABal and ABar. (Lower) Quantitation of *mom-2* smFISH spots in the 8 AB great-granddaughters in one 14-cell embryo and two 15-cell embryos. Higher counts were obtained in ABalp and ABara than in their respective sister cells, ABala and ABarp. Higher counts were also obtained in ABplp than in its sister blastomeres, ABpla. nd: not able to be determined.

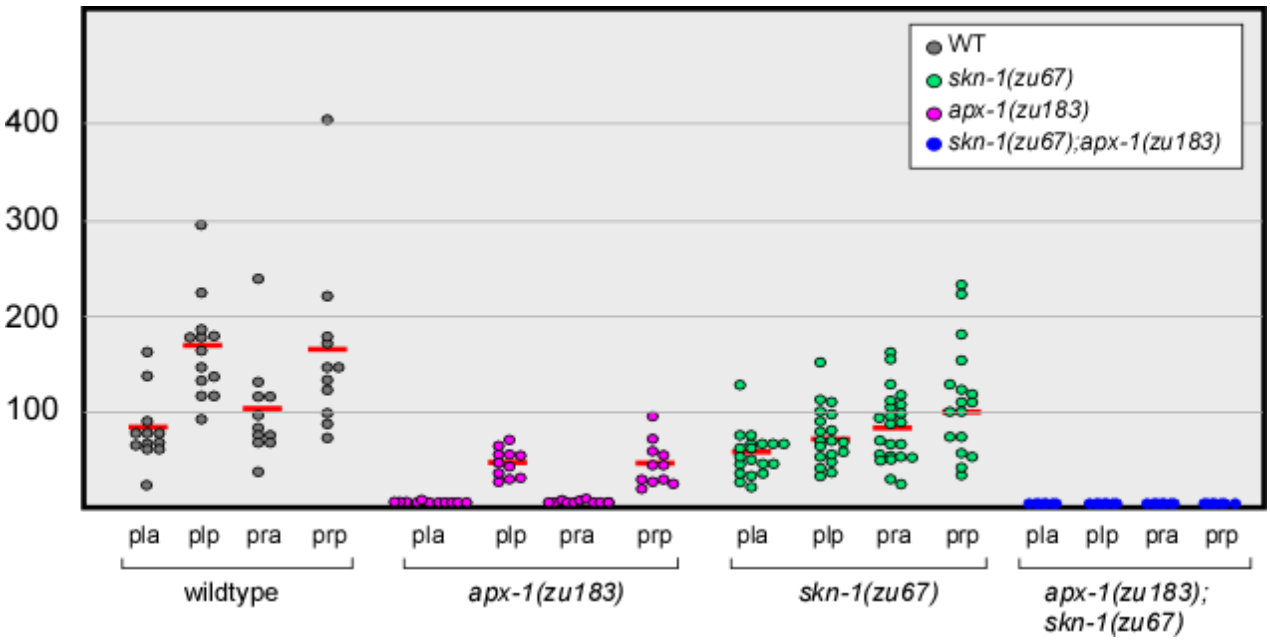

Figure S2. Related to Figure 3 (bottom two rows of panels).

Quantitation of GFP::H2B fluorescence from *mom-2* reporter transgene in ABp granddaughters (ABpla, ABplp, ABpra, ABprp) in the indicated genetic backgrounds as shown in Figure 3. All quantifications were performed on embryos of similar stages (26-28-cell). At this stage, each of the ABp granddaughters will have divided once. Each circle represents the average mean GFP intensity of the two daughter cells derived from that particular ABp granddaughter. Only embryos in which daughter cells at the same or adjacent focal planes were scored. Because GFP is fused to histone H2B, only cells that are not dividing were scored. These data show the spread of GFP intensity in ABp granddaughters among embryos. It does not show the differences of pla/p and pra/p pairs of any given embryo. In general, we observed a higher level of GFP in plp and prp compared to their respective anterior sisters in wildtype embryos. In *apx-1(zu183)* embryos, no GFP was detected in pla and pra and low but detectable GFP was observed in plp and prp. In *skn-1(zu67)* embryos, the differences between plpa/p and prpa/p pairs are less pronounced or not detected. In *skn-1(zu67);apx-1(zu183)* embryos, no GFP was detected in any of the four ABp granddaughters. Horizontal red bars indicate average value for each blastomere data set.

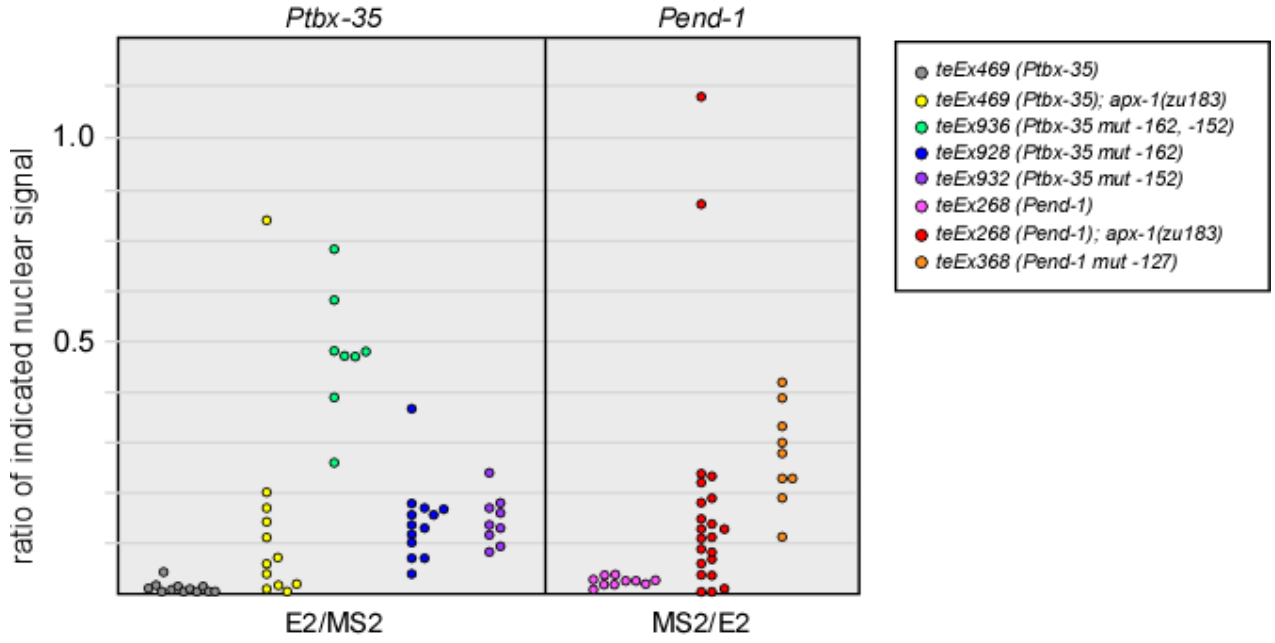

Figure S3. Related to Figure 5.

Quantitation of loss of lineage fidelity in *Ptbx-35* (left) and *Pend-1* (right) reporter transgenes following mutation of putative REF-1 binding sites. Reporter GFP intensity was quantified for the indicated reporter and genetic background shown in the box to the right. For *Ptbx-35* (left panel), each circle represents the ratio of average mean GFP intensity in the two E daughters to that in the two MS daughters of individual embryos. For *Pend-1* (right panel), each circle represents the ratio of average mean GFP intensity in the two MS daughters to that in the two E daughters. For both *Ptbx-35* and *Pend-1*, the ratio is close to zero in wildtype embryos, but is variable in individual *apx-1(zu183)* mutant embryos and for mutant transgenes. **Note**: Reversed ratios for *Ptbx-35* (MS-lineage-restricted) and *Pend-1* (E-lineage-restricted) are presented.

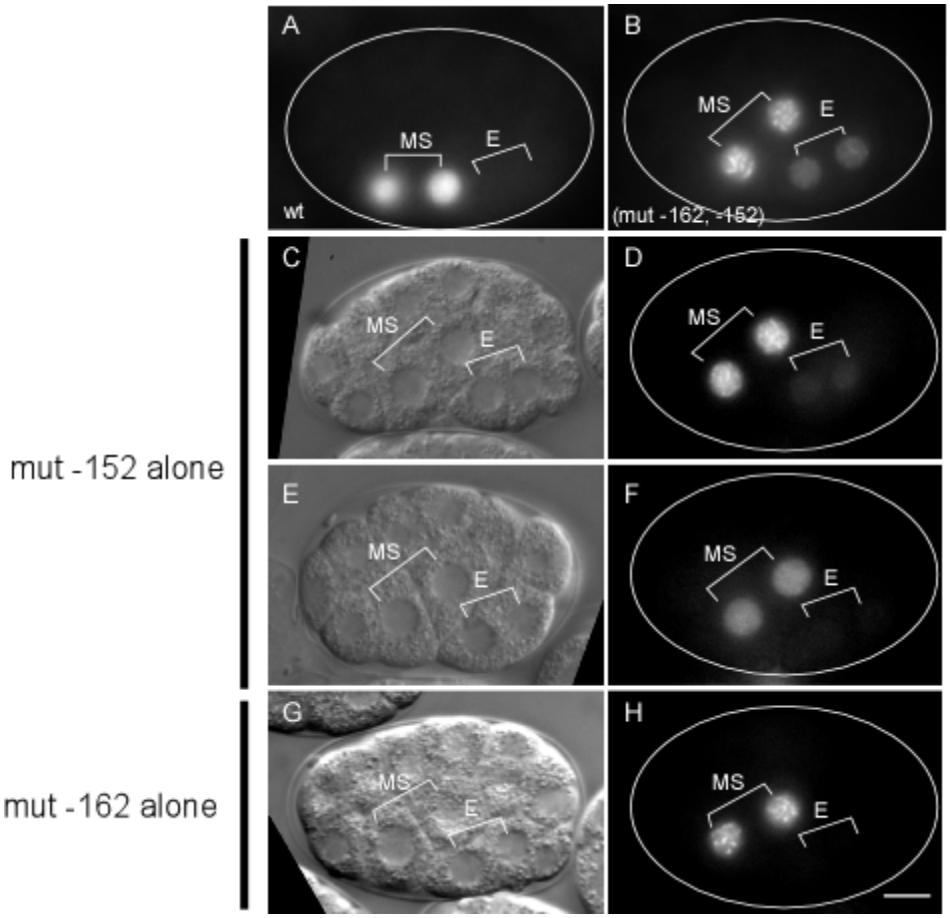

Figure S4. Related to Figure 5.

Effect of mutations in putative REF-1-binding sites on the expression of the *tbx-35* reporter transgene in wildtype embryos

All embryos are 2MS,2E (~15-cell) stage with anterior to the left. (A,B) Panels A and C from Figure 5 showing (A) wildtype *tbx-35* reporter expression and (B) reporter expression following mutation of the two putative REF-1 binding sites in the *tbx-35* promoter (see Fig. 5 for schematic and relevant promoter sequence). (C-F) Mutation of the putative REF-1 binding site at -152 alone. (C,E) DIC images of the corresponding GFP fluorescence images (D,F), respectively, from two different embryos, one with very weak and the other with no observable GFP in the 2E nuclei. (G,H) DIC and corresponding GFP fluorescence image following mutation of the putative REF-1 binding site at -162 alone. No derepression in the E blastomeres was observed. Bar: 7 um.

Transgenes used for single REF-1 binding site mutations: TX1963 [*unc-119(ed3)III; teEx928 (pRL3465 Ptbx-35*$^{(mut-162)}$*::gfp::H2B, pDPmm016)*], and TX1967 [*unc-119(ed3)III; teEx932 (pRL3466 Ptbx-35*$^{(mut-152)}$*::gfp::H2B, pDPmm016)*]. Mutation sequences are shown in Figure 5H.

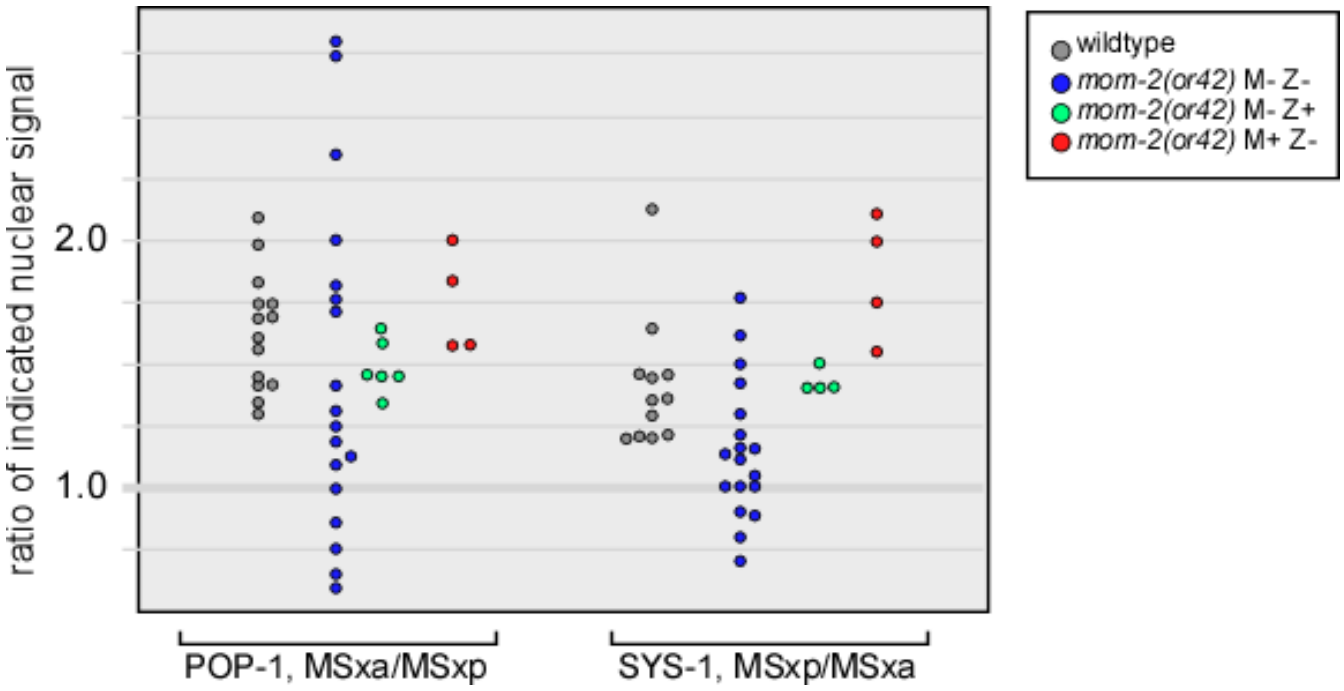

Figure S5. Related to Figure 6.

Zygotic versus maternal *mom-2* regulating nuclear POP-1 and SYS-1 asymmetry. We quantify anti-POP-1 and anti-GFP (SYS-1::GFP) staining in wild type embryos or embryos missing maternal, zygotic, or both *mom-2* as described in Figure 6. POP-1 staining is strictly nuclear. For POP-1, each circle represents the average ratio of anti-POP-1 mean intensity in MSaa to MSap and in MSpa to MSpp. SYS-1 signal is detected in nuclei, cytoplasm, and cytoplasmic membrane. For the purpose of this quantification, only nuclear signal was quantified. Each circle represents the average ratio of anti-GFP mean intensity in MSap to MSaa and in MSpp to MSpa. **Note**: Reversed ratios for POP-1 and SYS-1 are presented as POP-1 nuclear levels are higher in the anterior sister, whereas the SYS-1 nuclear levels are higher in the posterior sister.