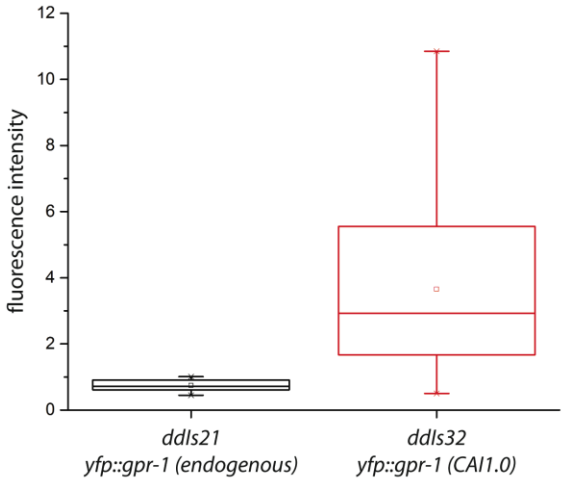
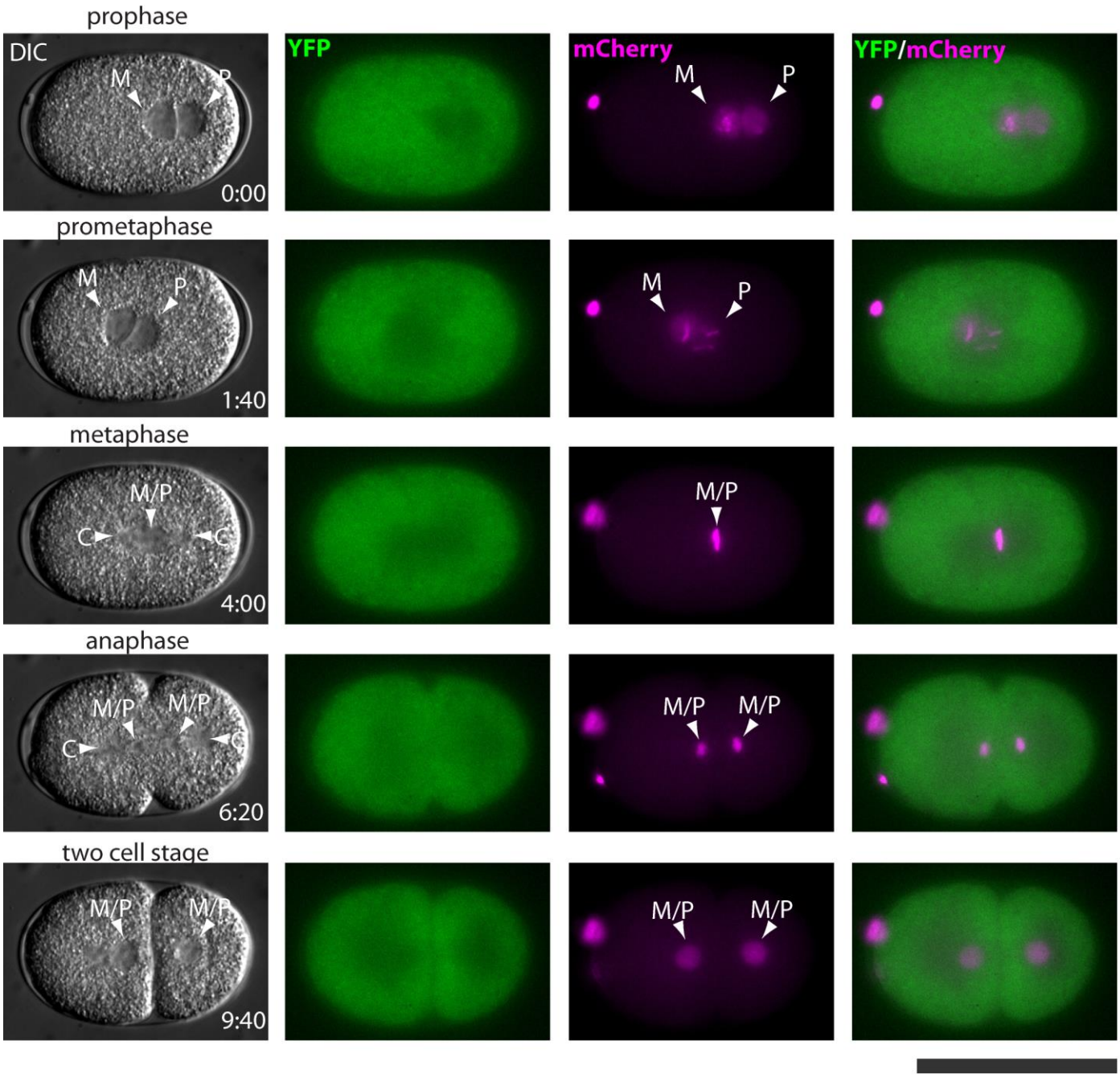


Supplementary Figure 1

Overexpression of YFP::GPR-1 in the germline.

The *pie-1* promoter and 3'utr were used to express *yfp::gpr-1* in the germline. Expression levels from the *yfp::gpr-1(CAI 1.0)*-expressing transgene *ddIs32* were measured and compared with the *yfp::gpr-1(endogenous)-expressing* transgene *ddIs21*. We quantified YFP::GPR-1 expression by measuring the mean intensity of YFP fluorescence of the embryo. For this, the image region containing the embryo was cut out and the mean intensity over background was determined using iQ software. To subtract background fluorescence that was not derived from YFP, we quantified 20 control embryos that did not express YFP and subtracted the averaged intensity of each embryo that contained a *yfp::gpr-1* transgene. Shown are box plots with whiskers displaying the maximum range. The two expression levels were significantly different according to a Mann-Whitney test (N = 13 for *ddIs21* and N = 70 for *ddIs32*, $p < 0.001$). To maintain overexpression of the YFP::GPR-1 from the *ddIs32* transgene, worms were regularly checked for expression and batches of worms that did not show YFP::GPR-1 overexpression were discarded as described in the methods section.



Supplementary Figure 2

Fluorescence imaging of a control embryo that did not overexpress GPR-1.

For visualizing chromatin in Fig 1A and S2-4, a germline-expressed histone::mCherry transgene was used. For quantitative data embryos without mCherry expression were used. Contrast was adjusted to visualize crucial cell biological structures. These images correspond to the images shown in Figure 1A. Scale bar is 50 µm.

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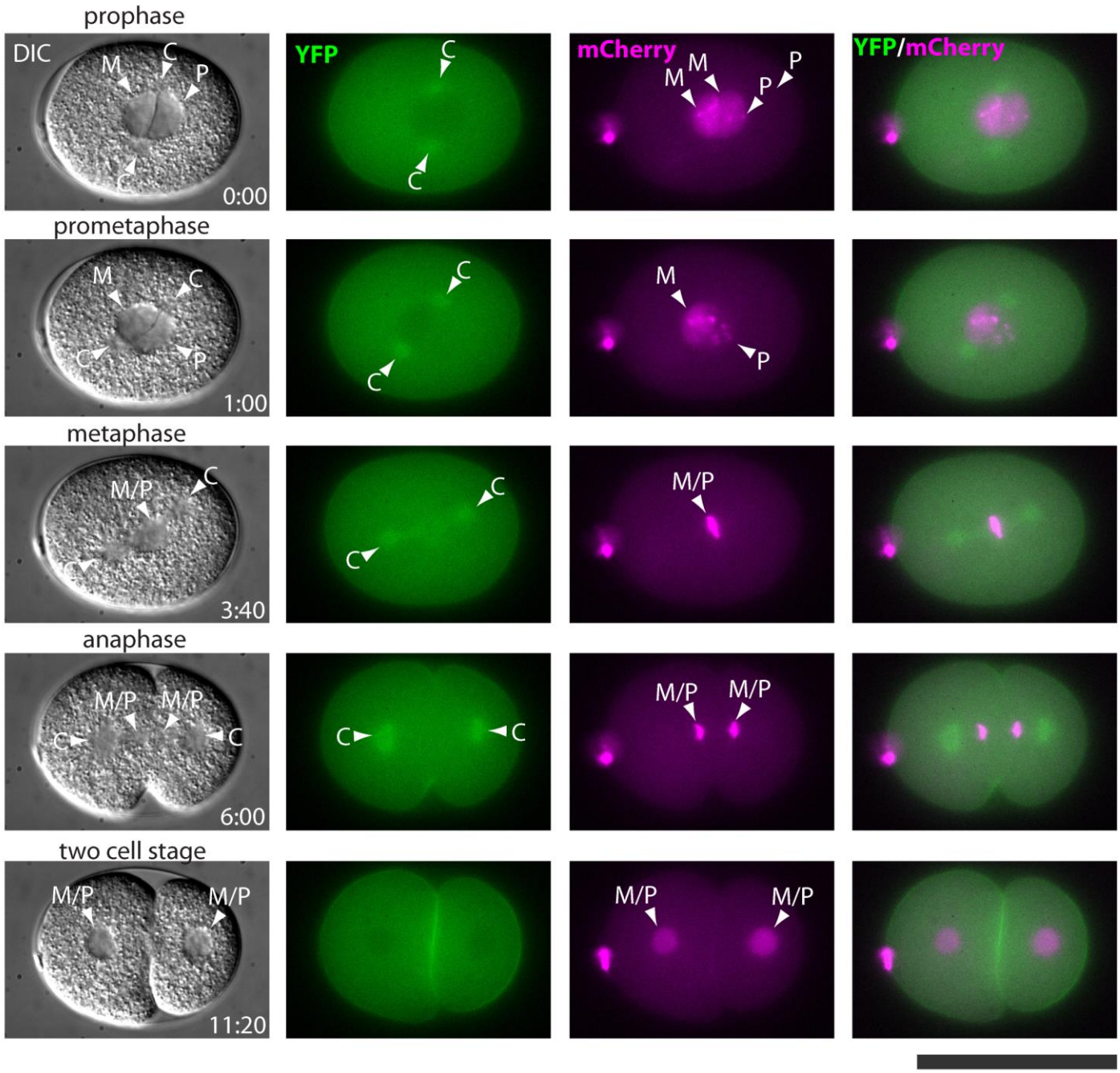
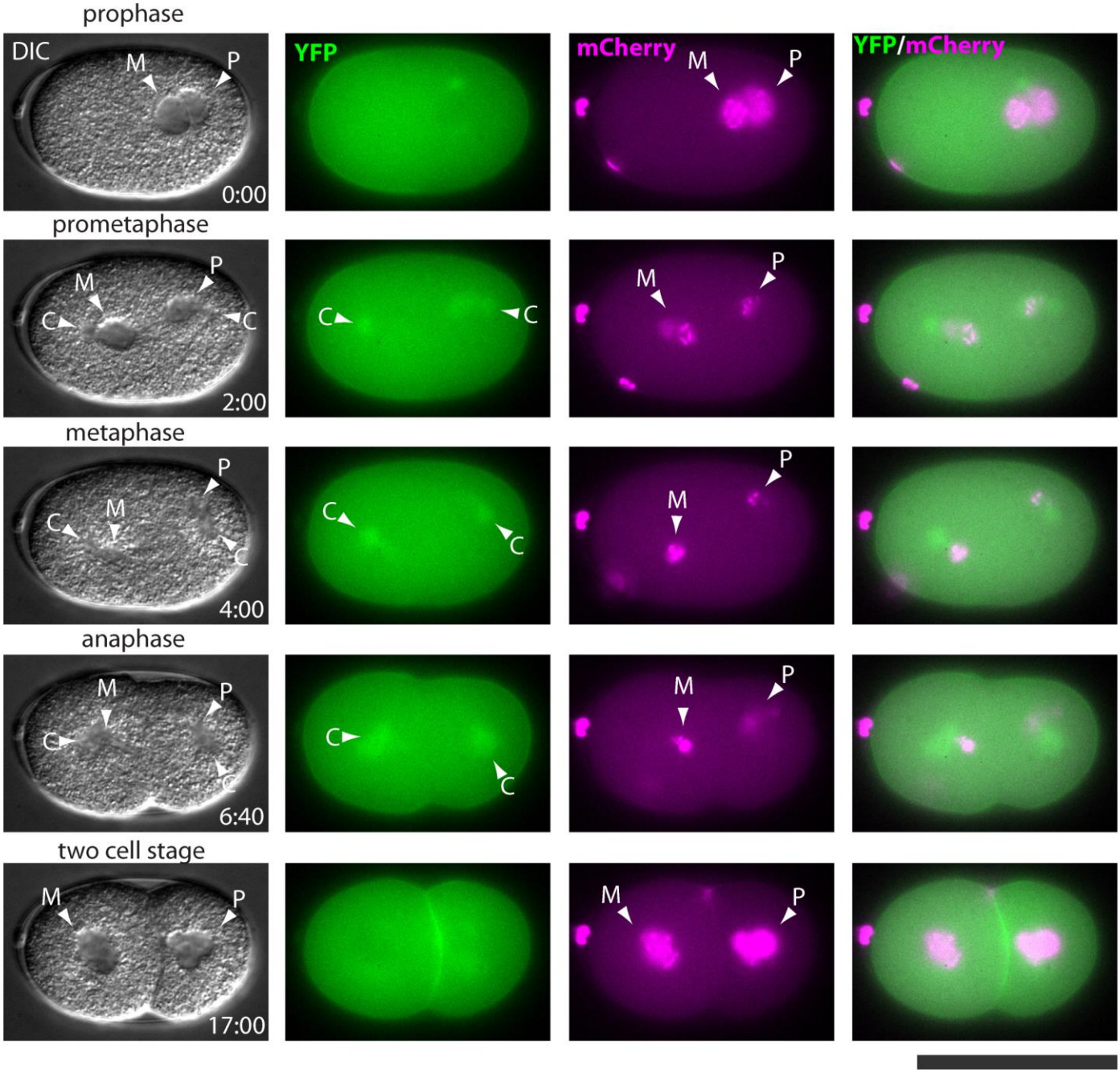


Supplementary Figure 3

Fluorescence imaging of a GPR-1 overexpressing embryo that formed a bipolar spindle.

YFP fluorescence is derived from transgenically expressed YFP::GPR-1 (*ddIs32* insertion). For quantitative data embryos without mCherry expression were used. Contrast was adjusted to visualize crucial cell biological structures. These images correspond to the images shown in Figure 1A. Scale bar is 50 µm.

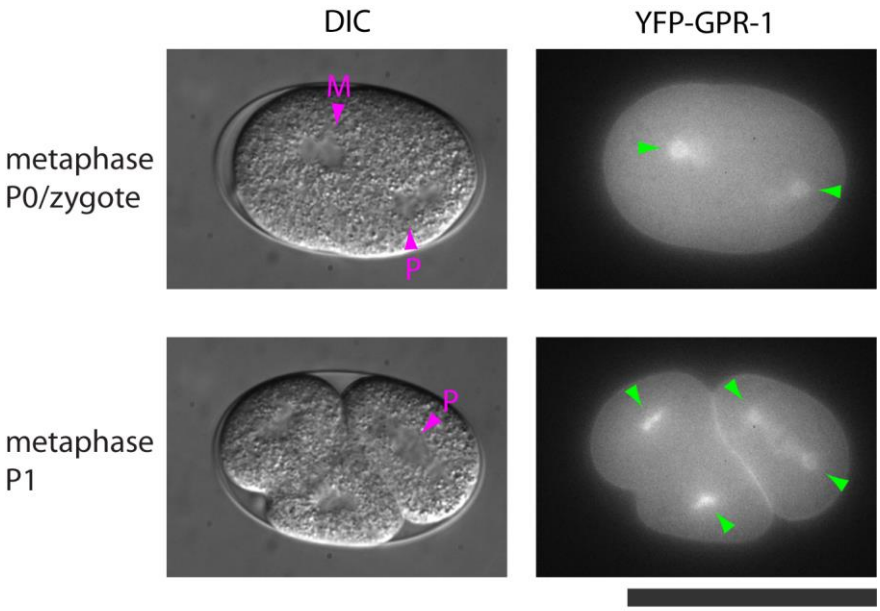
Nature Biotechnology: doi:10.1038/nbt.3643



Supplementary Figure 4

Fluorescence imaging of a GPR-1 overexpressing embryo that formed two monopolar spindles in the zygote.

YFP fluorescence is derived from transgenically expressed YFP::GPR-1 (*ddIs32* insertion). For quantitative data embryos without mCherry expression were used. Contrast was adjusted to visualize crucial cell biological structures. These images correspond to the images shown in Figure 1A. Scale bar is 50 µm.



Supplementary Figure 5

Despite forming two monopolar spindles in the P0 zygote, embryos formed normal bipolar spindles in subsequent divisions.

Left top panel shows a DIC image of an embryo during P0 metaphase. Two monopolar spindles have formed, each from either the paternal (P) or maternal (M) pronucleus (magenta arrowheads). Right top panel shows a fluorescent image of YFP::GPR-1. The spindle poles are indicated with green arrowheads. Bottom left panel shows the same embryo 20 minutes later, during the second round of cell division. Shown is the time of P1 metaphase. The P1 metaphase plate that is derived only from paternal DNA is indicated with a magenta arrowhead. Right bottom panel shows YFP fluorescence with spindle poles indicated by green arrowheads. Scale bar is 50 μm.

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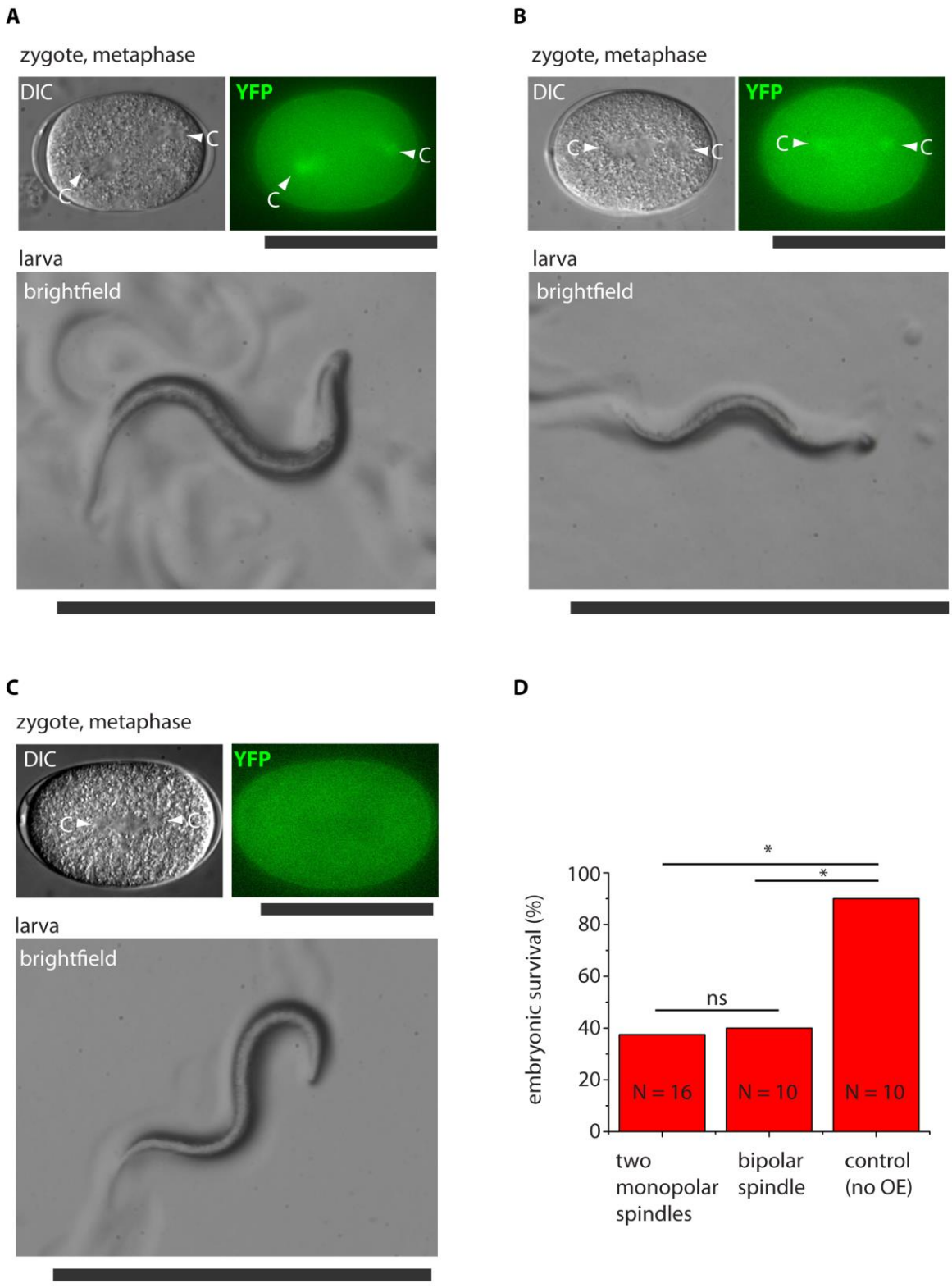


Supplementary Figure 6

Some zygotes that formed two monopolar spindles survived after imaging and recovery.

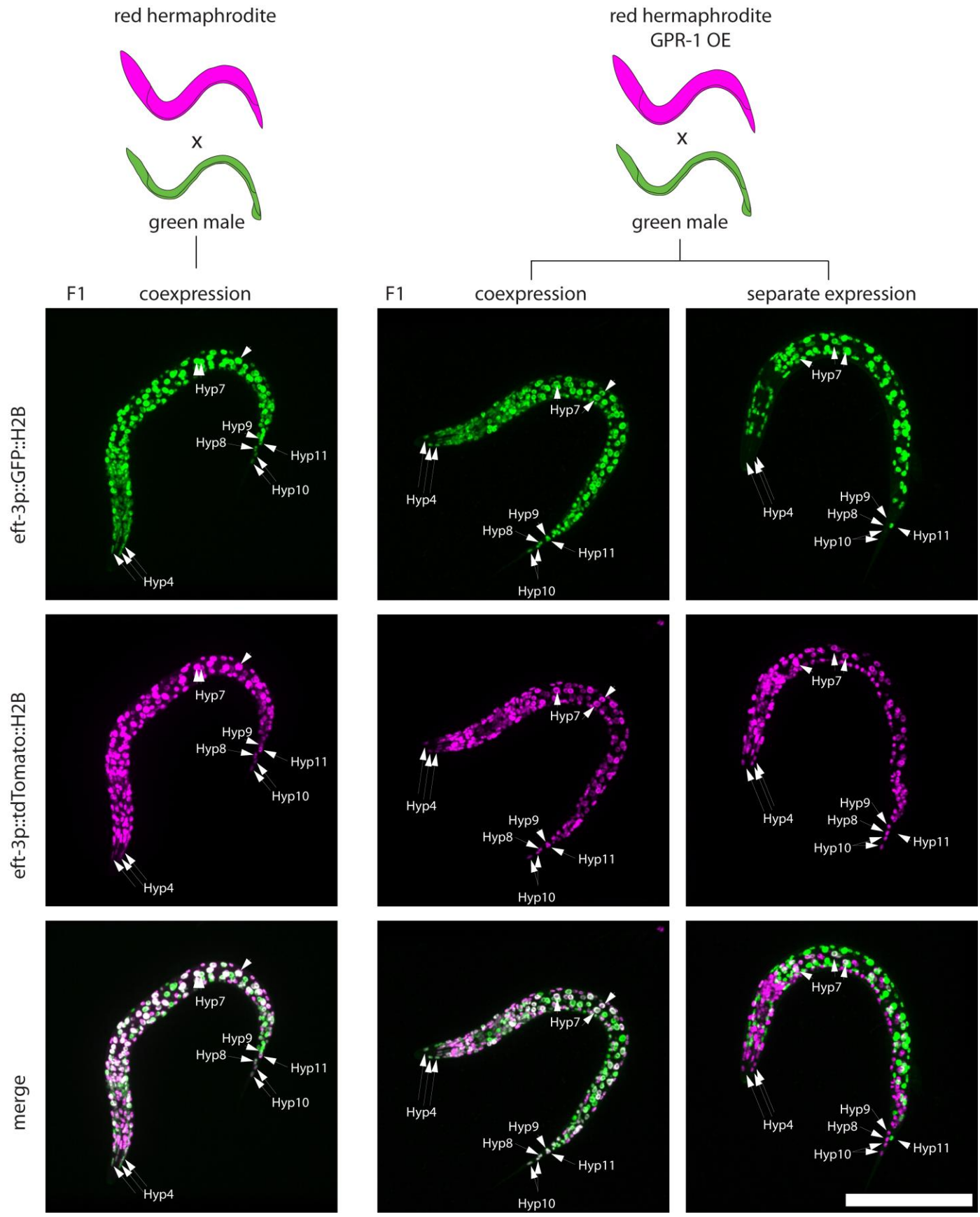
Hermaphrodite mother animals were placed into S-Basal and were cut open using steel needles to obtain the embryos, which were then transferred to an agar pad on a microscope slide and covered with a glass coverslip. One embryo was then imaged during zygotic mitosis to identify the status of the spindle. Then, lifting the coverslip and transferring the embryo with a fine glass pipette using S-Basal recovered the embryo. The embryo was then placed onto an NGM plate and was later checked for survival.

(A) A DIC and a fluorescence image of an embryo that formed two monopolar spindles. For the embryos the scale bar is 50 μm. The brightfield image shows the larva that emerged from the embryo that formed two monopolar spindles and that is shown above. For the larva the scale bar is 500 μm.

(B) Recovery of an embryo that overexpressed YFP::GPR-1 and formed a bipolar spindle during zygotic mitosis.

(C) Control embryos that did not overexpress YFP::GPR-1 all formed bipolar spindles.

(D) Survival rate of the experiment. Of the GPR-1 OE embryos that formed two monopolar spindles, 6/16 completed embryogenesis and hatched. Of the GPR-1 OE embryos that formed bipolar spindles, 4/10 completed embryogenesis and hatched. Of control embryos that did not carry the overexpression construct and that all formed bipolar spindles, 9/10 completed embryogenesis and hatched. The statistical test used was Fisher's exact test. * denotes statistical significance with $p < 0.05$, ns: not significant.



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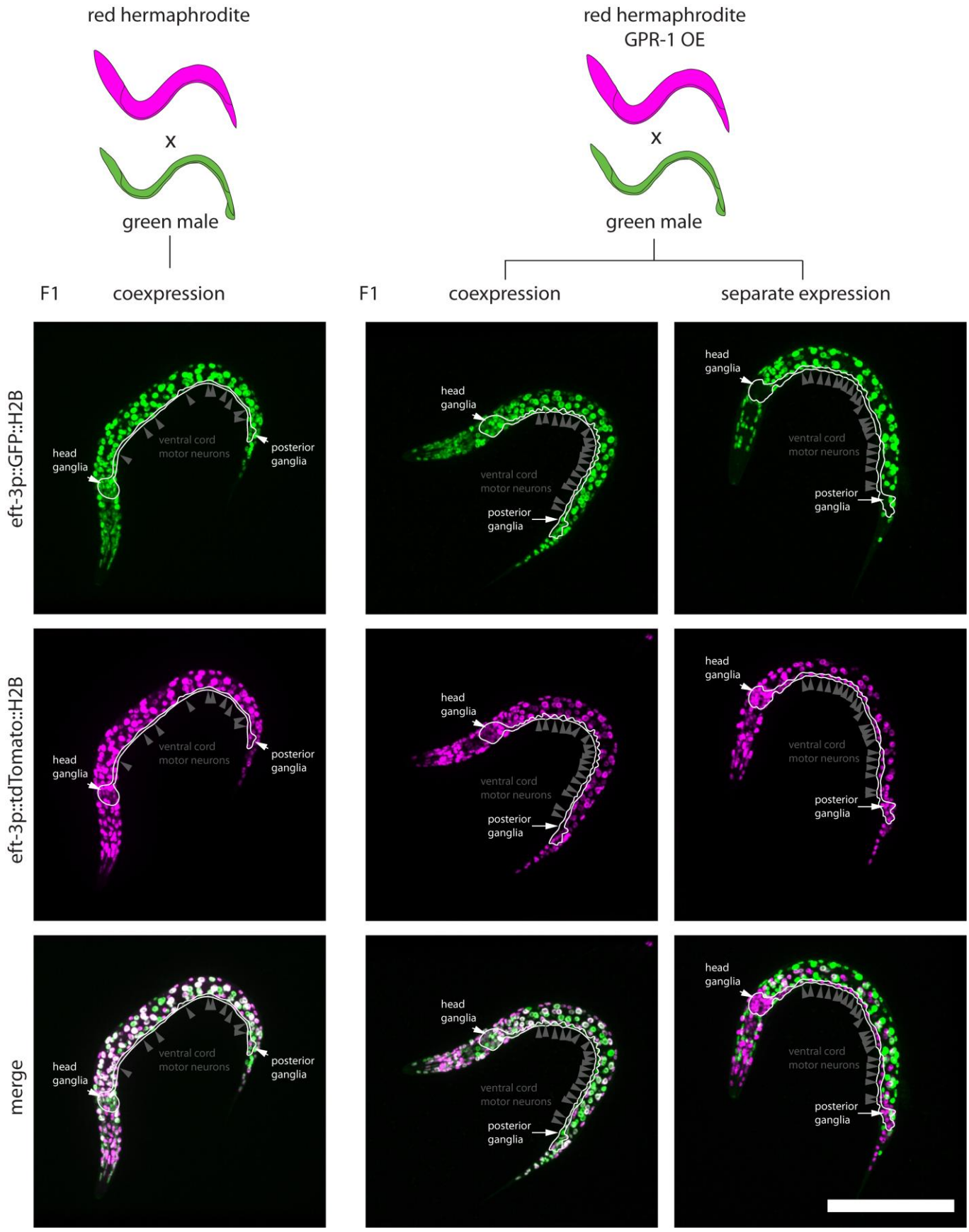
Supplementary Figure 7

Following unequal chromosome segregation into hypodermal cells.

Crossing hermaphrodites that overexpress GPR-1 and that ubiquitously express tdTomato-labeled histones with males that ubiquitously express GFP-labeled histones led to two types of progeny: individuals that coexpressed both markers in most cells and individuals that expressed the two markers separately in different tissues. Shown are example animals from Figure 1C. *C. elegans* has a fixed and invariant number of 959 somatic cells that can be identified based on their location, size, and shape (classic work by John Sulston, see citation main text). Hypodermal cells are annotated. The hyp4, hyp8, hyp9, and hyp10 cells are derived from the AB lineage and only express tdTomato after unequal segregation. Hyp-11 is derived from the P lineage and expresses GFP after unequal segregation. Several hyp-7 cells exist and some are derived from the AB lineage and some are derived from the P lineage. These cells form a syncytium and express both fluorescent markers even after unequal segregation. Hyp-7 cells appear to be the only cells to express both markers after unequal segregation. Transgene silencing can occur in *C. elegans* and tissue-specific reciprocal suppression, even if unlikely, could explain the expression pattern. The observation of a lineal expression in similar tissue types (hypodermis) strongly supports the view that the expression is lineal rather than tissue-specific. The fact that the only white nuclei in these animals resulted from cell fusion events in the syncytium of the hyp-7 hypodermis supports this view. The anterior (nose tip) is on the left, the posterior tail on the right side. Dorsal is top and ventral bottom. Scale bar is 100 µm.



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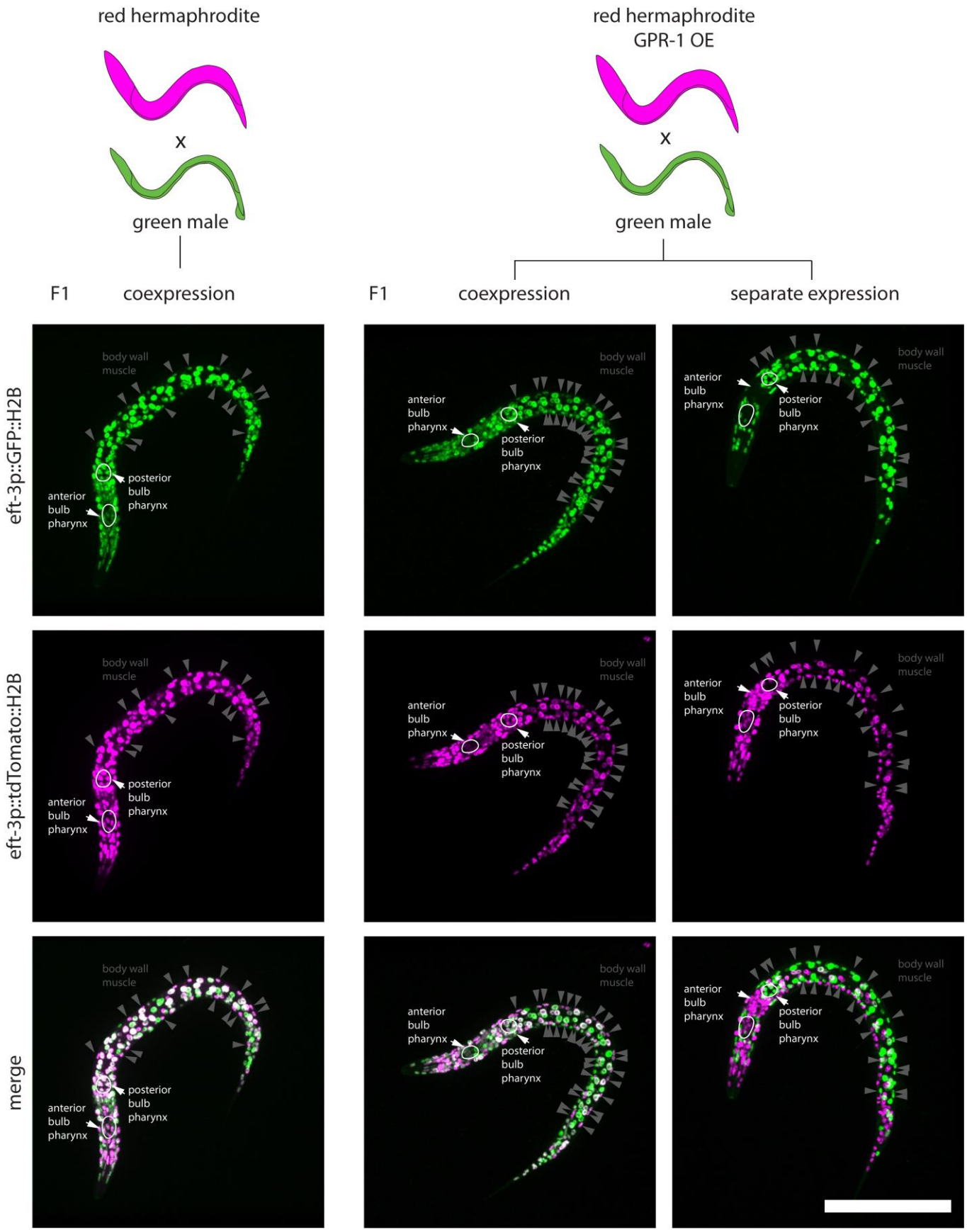
Supplementary Figure 8

Following unequal chromosome segregation into neurons.

Shown are again example animals from Figure 1C. The nervous system was annotated. Of the 959 cells, 302 are neurons. Almost all neurons are derived from the AB lineage and expressed tdTomato after unequal segregation. Most neurons are located in the head ganglia. Motor neurons can be found in the ventral cord. In addition, ganglia can be found in the tail. Scale bar is 100 μm.

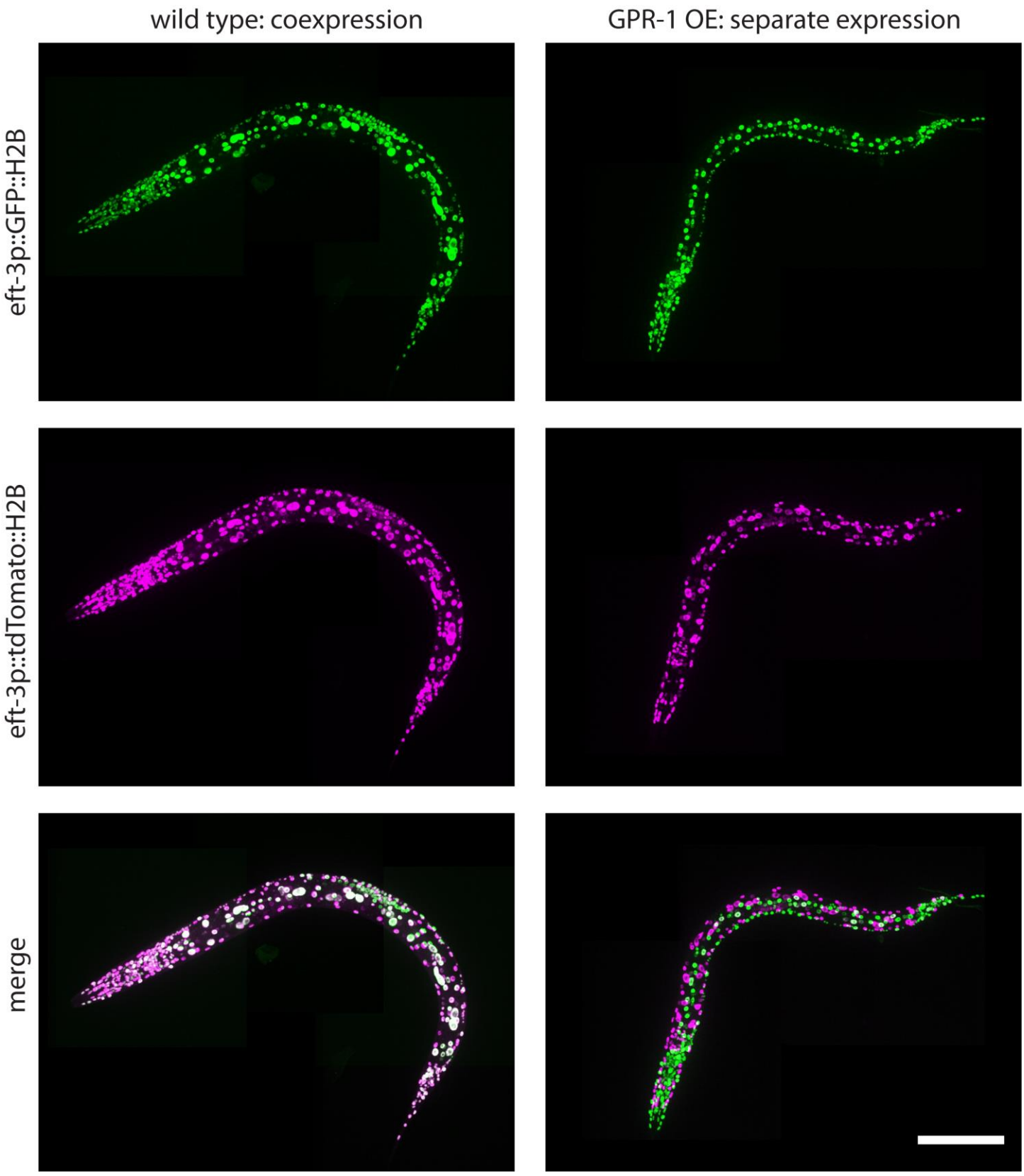


Nature Biotechnology: doi:10.1038/nbt.3643

Supplementary Figure 9

Following unequal chromosome segregation into cells of the pharynx and body wall muscles.

Shown are again example animals from Figure 1C. The pharynx contains two bulbs, an anterior and a posterior that contain muscle cells. The anterior bulb is located anterior to the head ganglia and the posterior bulb is located posterior to the head ganglia. In addition, body wall muscles run along the length of the worm. Most cells from the anterior bulb are derived from the AB lineage and expressed tdTomato after unequal segregation. Most cells from the posterior bulb are derived from the P lineage and expressed GFP after unequal segregation. Body wall muscle cells are derived from the P lineage and expressed GFP after unequal segregation. Scale bar is 100 μm.



Supplementary Figure 10

Following the segregation of maternal DNA into the P lineage.

Crossing hermaphrodites that overexpress GPR-1 and that ubiquitously express tdTomato-labeled histones with males that ubiquitously express GFP-labeled histones led to two types of progeny: individuals that co-expressed both markers in most cells and individuals that expressed the two markers separately in different tissues. Shown is a wild-type control animal resulting from a mating with a hermaphrodite that did not overexpress GPR-1 (left) and a rare example animal that expressed the maternal fluorescent marker in the cells of the P lineage (right). Note that the pattern is inverted compared with the individuals that express the paternal marker in the P lineage (Figure 1C, Supplementary Figures 7-9). Because the animals did not fit to one frame, the different body parts were imaged separately with 3-4 frames, and individual frames were then assembled to obtain the final image showing the entire animal at once on a black background. The animals were imaged around the L2 or L3 stage. Scale bar is 100 μm.

A

genotype	viable F1 embryos N (%)	dead F1 embryos N (%)	total number F1 N (%)	number of parents (P0) N
wild type N2	1890 (94.1%)	119 (5.9%)	2009 (100%)	9
oxTi75 II [histone::GFP]	1683 (93.7%)	114 (6.3%)	1797 (100%)	10
oxTi411 III [histone::tdTomato]	1093 (92.6%)	88 (7.5%)	1181 (100%)	6
oxTi411 III; ddIs32 [yfp::gpr-1OE]	1166 (72.2%)	450 (27.8%)	1616 (100%)	18
oxTi75 II [histone::GFP] x oxTi411 III [histone::tdTomato]	2041 (97%)	63 (3%)	2104 (100%)	10
oxTi75 II [histone::GFP] x oxTi411 III; ddIs32 [yfp::gpr-1OE]	438 (36.9%)	748 (63.1%)	1186 (100%)	11

B

individual cross no. #	*oxTi411; oxTi75* separate expression (paternal P lineage) N (%)	*oxTi411; oxTi75* separate expression (maternal P lineage) N (%)	*oxTi411; oxTi75* co-expression N (%)	number of F1 N
1	0	0	158 (100%)	158
2	0	0	105 (100%)	105
3	0	0	198 (100%)	198
4	0	0	188 (100%)	188
5	0	0	95 (100%)	95
6	0	0	228 (100%)	228
7	0	0	205 (100%)	205
8	0	0	206 (100%)	206
9	0	0	237 (100%)	237
10	0	0	161 (100%)	161
all worms	**0**	**0**	**1781 (100%)**	**1781**

C

individual cross no. #	*oxTi411; oxTi75* separate expression (paternal P lineage) N (%)	*oxTi411; oxTi75* separate expression (maternal P lineage) N (%)	*oxTi411; oxTi75* co-expression N (%)	number of F1 N
1	14(35%)	0(0%)	26 (65%)	40
2	12(60%)	0(0%)	8 (40%)	20
3	23(74.2%)	1(3.2%)	7 (22.6%)	31
4	4(18.2%)	0(0%)	18 (81.8%)	22
5	6(54.5%)	0(0%)	5 (45.5%)	11
6	15(53.6%)	1(3.6%)	12 (42.9%)	28
7	11(52.4%)	0(0%)	10 (47.6%)	21
8	15(38.5%)	0(0%)	24 (61.5%)	39
9	9(19.1%)	1(2.1%)	37 (78.7%)	47
10	13 (46.4%)	0(0%)	15 (53.6%)	28
11	10 (22.7%)	0 (0%)	34 (77.3%)	44
all worms	**132 (39.9%)**	**3 (0.9%)**	**196 (59.2%)**	**331**

Supplementary Figure 11

Crossing red GPR-1 OE hermaphrodites with green males resulted in two types of cross-progeny: F1 that co-expressed both markers, and F1 that expressed the markers in separate tissues.

(A) Background lethality was measured for wild type and the different strains that were used for crossing. The two marker strains showed lethality in the control genetic background in which the crosses were made. GPR-1 overexpression led to an increased lethality of 27.8%(450/1616) ($p < 0.001$ when comparing *oxTi411* with *oxTi411; ddIs32*). In the GPR-1 OE strain, mating with males further increased lethality to 63.1% (748/1186) ($p < 0.001$, compared with lethality in offspring resulting from self-fertilization). Increased lethality after mating may be due to a lack of the X chromosome, but other causes such as imprinting or strain differences may contribute to lethality as well. 40 dead embryos were checked for marker expression. From this sample, 80% co-expressed the markers and 20% expressed them separately, indicating that 80% of the dead embryos were derived from a zygote that unequally segregated the genomes.

(B) Crossing of the two marker strains in the absence of GPR-1 OE resulted exclusively in F1 cross progeny that were co-expressing both markers in all cells. For each cross, one red hermaphrodite was crossed with three green males.

(C) Crossing both markers combined with GPR-1 OE resulted not only in F1 cross progeny that were co-expressing both markers in all cells, but also in F1 cross progeny that were expressing the markers in separate tissues. For each cross, one GPR-1 OE hermaphrodite was crossed with three green males. The efficiency with which worms with separate marker expression were formed among the surviving cross progeny varied between 18 and 77 %, and separate marker expression was never observed in the control crossings [$N = 331$ (total number of surviving offspring resulting from all crosses in the presence of GRP-1 OE), $N = 1781$ (total number of surviving offspring resulting from all control crosses in the absence of GPR-1 OE), $p < 0.001$].

scoring of F2 broods derived from co-expressing F1

Type of cross: paternal genotype	F2 phenotype: no fluorescence N (%)		F2 phenotype: *oxTi411* N (%)		F2 phenotype: *oxTi75* N (%)		F2 phenotype: *oxTi411, oxTi75* N (%)		N (F1) N (F2)
	wt	mutant	wt	mutant	wt	mutant	wt	mutant	
dpy-5(-) I/+; oxTi75 II.	82 (5.4%)	34 (2.2%)	242 (15.9%)	77 (5.1%)	233 (15.3%)	65 (4.3%)	595 (39.2%)	191 (12.6%)	10 1519
oxTi75 II.	114 (7.3%)	0 (0%)	327 (20.9%)	0 (0%)	297 (19.0%)	0 (0%)	823 (52.7%)	0 (0%)	9 1561
oxTi75 II; dpy-17(-) III/+.	12 (0.6%)	116 (5.8%)	349 (17.4%)	16 (0.8%)	33 (1.6%)	301 (15%)	1124 (56%)	55 (2.7%)	10 2006
oxTi75 II; bli-6 (-) IV/+.	100 (5.9%)	18 (1.1%)	347 (20.6%)	36 (2.1%)	260 (15.4%)	52 (3.1%)	780 (47.4%)	73 (4.3%)	10 1686
oxTi75 II; dyp-11(-) V/+.	100 (4.8%)	30 (1.5%)	266 (12.9%)	80 (3.9%)	257 (12.4%)	141 (6.8%)	939 (45.4%)	254 (12.3%)	10 2067
oxTi75 II; lon-2(-) X/0.	78 (5.1%)	12 (0.8%)	256 (16.9%)	78 (5.1%)	234 (15.4%)	46 (3%)	709 (46.9%)	102 (6.7%)	9 1515

Supplementary Figure 12

Non-Mendelian genetics: Following the transmission of recessive alleles from F1 to F2 (scoring of F2 broods derived from co-expressing F1)

Crosses were set up as described in Figure 2. Some F1 were singled out onto individual plates and all F2 were scored for fluorescence and recessive phenotype. We scored some control F2 brood derived from animals that co-expressed the two markers and some control F2 broods for those F2 populations that emerged from worms that expressed the two markers separately. For the recessive allele experiments we counted the precise number of F2 for those broods that contained the recessive phenotype. For each individual F2 broods derived from F1 that expressed the two markers separately, either 100% of F2 worms showed the recessive phenotype or zero F2 animals showed the recessive phenotype. F1 that co-expressed the two markers produced all phenotypic combinations of F2 and only a fraction showed the recessive phenotype.

scoring of F2 broods derived from separately expressing F1

Type of cross: paternal genotype	F2 phenotype: no fluorescence N (%)		F2 phenotype: *oxTi411* N (%)		F2 phenotype: *oxTi75* N (%)		F2 phenotype: *oxTi411, oxTi75* N (%)		N (F1) N (F2) p value
	wt	mutant	wt	mutant	wt	mutant	wt	mutant	
dpy-5(-) I/+; oxTi75 II.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1119 (100%)	0 (0%)	0 (0%)	10 1119 p < 0.001
oxTi75 II.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1114 (100%)	0 (0%)	0 (0%)	0 (0%)	9 1114 p < 0.001
oxTi75 II; dpy-17(-) III/+.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1117 (100%)	0 (0%)	0 (0%)	10 1117 p < 0.001
oxTi75 II; bli-6 (-) IV/+.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	842 (100%)	0 (0%)	0 (0%)	8 842 p < 0.001
oxTi75 II; dyp-11(-) V/+.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1168 (100%)	0 (0%)	0 (0%)	10 1196 p < 0.001
oxTi75 II; lon-2(-) X/0.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1072 (100%)	0 (0%)	0 (0%)	10 1098 p < 0.001

Supplementary Figure 13

Non-Mendelian genetics: Following the transmission of recessive alleles from F1 to F2 (scoring of F2 broods derived from separately expressing F1)

The same analysis as described in Supplementary Figure 12, but scored for F2 broods that were derived from separately expressing F1. F1 that separately expressed the two markers produced exclusively F2 broods that contained only worms with the same phenotype. All F2 worms showed the GFP phenotype. An overview on the numbers of F1 obtained can be found in Supplementary Figure 12. For this experiment we counted only those F2 broods that contained the recessive phenotype. To follow the inheritance of only the GFP marker transgenic insertion we counted all broods resulting form a cross with GFP males in the absence of a recessive allele. We statistically tested the fractions of the F2 worms that had the recessive/GFP phenotype in B versus the fraction of F2 worms in A using Fisher's exact test. All conditions were significantly different at $p < 0.001$.