Supplemental information

A natural transdifferentiation event involving mitosis is empowered by integrating signaling inputs with conserved plasticity factors

Claudia Riva, Martina Hajduskova, Christelle Gally, Shashi Kumar Suman, Arnaud Ahier, and Sophie Jarriault
Figure S1. While DNA replication in K is not sufficient for DVB formation and division orientation mutants do not impact K-to-DVB, K division is required for K-to-DVB. Related to Figure 2.

(A) Histogram summarizing the percentage of animals in which K DNA underwent replication in the lin-5(ev571ts) mutant at different restrictive temperatures.

(B) Histogram showing the percentage of animals with a “NO DVB” defect, and without (Non transgenics) or with (transgenics) overexpression of gfp::cki-1 in the rectal cells. Note that for the transgenics, the F2 generation of the transgenic lines was first scored and displayed more penetrant defects than the following generations, due to transgene silencing.

(C) Histogram showing the percentage animals displaying an absence (No K.p) or presence (K.p present) of the K.p cell among the gfp::cki-1 overexpressing animals without DVB. Note that for each category, the GFP expression was assessed in K, K.a and K.p, when present. Solid bar: GFP expression was observed; white bar, No GFP expression. Error bars, SD of the GFP-expressing animals.

(D) Histogram showing the percentage of worms without DVB in mutants for the Gα and gpr-1/LGN genes involved in spindle orientation in C. elegans zygote and for the Par gene par-1. The low penetrance of DVB absence is due to an impairment in K cytokinesis.

(E) Dot plot representing K division angle in the goa-1(sa734) mutant. n=64.

For all the histograms, ns, not significant; n, total animal scored.
Figure S2. K divides into K.a and K.p in late L1 stage and K.p still shows epithelial features after division. Related to Figure 2.

(A) Time course of the cellular events during K division and DVB formation along the developmental timeline. The col-34p::mCherry positive rectal cells and the rectal slit (line) are represented. Each box corresponds to a point of one hour where different characteristic landmarks have been observed in addition to K division: number of GABAergic neurons, VD commissures, presence of alae, number of cells in the gonad, col-34p::mCherry and unc-47p::GFP intensities, rectal cell shapes. The percentage of animals (n%) with the corresponding landmarks is indicated at each particular time point. Worms were synchronized by hatch pulse (see Methods). Left is towards front, anterior is left, and dorsal is up.

(B) Quantification of worms with expression of fpEx1062[let-413::gfp::pest], fpIs110[lin-26p::GFP] and oxisIs12[unc-47p::GFP] in K.p over time, in L1 and L2 grown at 20°C. n, total animal scored.

(C) lin-26 smFISH staining (white spots on the merge) on 26h post hatching L1 larvae expressing a gfp::sox-2 (CRISPR KI syb737, shown in cyan) and the rectal nuclear reporter gals245, shown in magenta. Intestinal cells and Pn.p cells in the same area were identified with the DAPI staining, in yellow. Scale bar for all in bottom right picture.

(D) Quantifications of the lin-26 smFISH spots were performed on K.p and F rectal cells as well as on intestinal cells (Int 8/9) and Pn.p cells (P9/10.p). We considered only the spots in contact with the nuclei to reflect the most recently transcribed mRNAs. Statistical test compares the number of spots in the K.p cell to the others. Very few lin-26 mRNA molecules are detected in close proximity to the nucleus in epithelial cells generally (from 3 spots to 5 spots on average; P9/10.p where lin-26 has been shown to be expressed (Labouesse et al., 1996), 3 spots; rectal F cell, 5 spots; K.a, which is on the left side, was very difficult to image because of photobleaching (n=9) and exhibits 4 spots on average). Intestinal cells, where lin-26 is not expressed, show no lin-26 mRNA spots (0, 22 spots on average).
Figure S3. The non-canonical Wnt pathways are not required for K-to-DVB. Related to Figure 3.

(A) Histograms showing the percentage of “No DVB” worms in mutant backgrounds for genes of the PCP pathway.

(B) Histograms showing the percentage of “No DVB” worms in mutant backgrounds for the non-canonical Wnt-dependent pathways (*lin-18* and *cam-1*) or their downstream effectors (*ced-10*). n, total animal scored; ns, non-statistically significant; **, p<0.005.

(C) When present, in a small percentage of *pop-1/TCF* (top) or *lin-17/FZD* (bottom) L4 mutants, the DVB neuron is formed from K posterior daughter. The positions of DVB, as observed with *oxIs12*, and of the K.a, U, F and B rectal cells, as observed using *gals245*, are indicated. VD13, GABAergic neuron. Dash line, rectal slit. Anterior is to the left and ventral to the bottom.
Figure S4. K.p cell remains rectal-epithelial in lin-17/FZD and sem-4/SALL mutants. Related to Figure 3 and Figure 4.
Quantification of the % of animals expressing (A-C) epithelial (ajm-1, let-413 and lin-26), (D) rectal (egl-5), (E-G) pan-neuronal (unc-119, unc-33 and rgef-1) and (H, I) GABAergic (unc-25, unc-47) reporters in K posterior daughter in lin-17/FZD and sem-4/SALL mutant backgrounds, or DVB in wild type, in L4 larvae. n, total animal scored.
**Figure S5.** K.p cell expresses the apical junction protein AJM-1 in *lin-17/FZD* and *sem-4/SALL* mutants. Related to Figure 3 and Figure 4.

Confocal images of wild-type, *lin-17/FZD* and *sem-4/SALL* mutant backgrounds in L3 larvae carrying *gaIs245[+34p::his::mcherry]* to visualize the rectal cell nuclei and *jclIs1[ajm-1::GFP]*. Patches of AJM-1 proteins are present in the K.p cell (dashed oval) in the mutant backgrounds, consistently with the mutant K.p retaining its epithelial identity.
Figure S6. *sox*-2 and *ceh*-6 paralogs do not seem to be required to form DVB. Related to Figure 4.
Quantification of DVB defective L4 animals (as observed by *unc*-47 expression) using RNAi in a sensitized *rrf*-3 mutant background to target *sox*-2 paralogs (*dpy*-8 and *gfp* RNAi represent controls). Mutants were used for paralogs of *ceh*-6 (*unc*-86(n846) and *ceh*-18(mg57)) and *egl*-27 (*lin*-40(ku285)). No obvious defects were observed, although RNAi was found to work poorly in the rectal cells. n, total animal scored.
Figure S7. *sem-4/SALL* and the Wnt signaling pathway act in parallel to drive K-to-DVB Td. Related to Figure 5.

(A) Quantification of *sem-4/SALL* expression in K.p in wild type L4s and in *lin-17/Frizzled* mutant L4s.

(B) Quantification of *lin-17/FZD* expression in K.p in wild type L4s *vs* *sem-4/SALL* and *lin-17/FZD* mutants.

(C-D) Quantification of DVB defective L4 animals (as observed by *unc-47* expression using *krIs6* in A and *oxIs12* in B) in simple *sem-4(n1378)* (C), *sox-2* knock-down (using a nanobody strategy, D) and *wrm-1(n1982)* (C, D) mutants, or in *sem-4(n1378);wrm-1(n1982)* (C) and *wrm-1(n1982);sox-2 KD* (D) double mutants, all raised at 25°C.

n, total animal scored.
Figure S8. *lim-6* is expressed early in *K.p* and its expression is affected in *pop-1/TCF* mutant. Related to Figure 5.

(A) *lim-6* expression is impaired in *pop-1* mutant. Quantification of the % of L4 larvae expressing *lim-6::gfp* CRISPR (KI) and *lim-6 intron 4* transcriptional reporter (*int4*) in wild-type (DVB) and *pop-1(q645)* mutant (persistent *K.p*) backgrounds. Note that for the *pop-1(q645)* mutant, only viable homozygote (not balanced) mutant worms were analyzed. n, total animal scored.

(B-C) Time course expression of *lim-6(int4)::gfp* (*fpEx1111*) (B) and *lim-6r::gfp* (*otIs157*) (C) reporters in *K.p*/DVB (Cyan) in L1, 1h after the division (top), in an early L2 animal (middle) and in an L3 larva (bottom) where rectal cells are visualized with *gaIs245* (*col-34p::his-24::mcherry*; magenta).

For all pictures, dashed line, rectal slit. Anterior is to the left and ventral to the bottom.
**Figure S9. Analysis of SOX-2 and POP-1/TCF binding sites in *lim-6* intron 4. Related to Figure 5.**

(A) The intron 4 of *lim-6* was analyzed using different tools (See Methods). Here the POP-1 binding site prediction using the Matrix of Narasimhan and the consensus of Bertrand, 2009, are represented. The SOX-2, POU3F2 and POU5F1 binding sites were predicted by Promo and the SEM-4 consensus site published in Toker, 2003 was used.

(B) Sequence logo for POP-1 and SOX-2 binding sites showing the sequence similarities.

(C) Table summarizing the binding sites on which we have focused our efforts in this study. Binding sites predicted by more than one approach, or because of the presence of two consecutive binding sites for SOX-2 (site 4), were selected. The mutations introduced into the *lim-6* transcriptional reporter to abolish POP-1 binding are presented on the right. Note that these mutations most probably abolish also SOX-2 binding due to the very close similarity of their predicted binding sites.

(D) This binding site similarity can be explained by the sequence similarity of the HMG domains present in SOX-2 and POP-1.

(E) Sequence of the probes used in this study for the gel shift experiments. Note that probe 3 displayed poor annealing due to its AT rich sequence and therefore was not further used.
Fig. S10.  

**A**  

sox-2 KI  

col-34p::his24 ::mcherry  

Merge  

L1 : Before division  

L1 after division  

L2  

Figure S10. sox-2 is expressed in K.p after K division and it is subsequently downregulated during DVB differentiation. Overexpression of sox-2 prevents lim-6 expression. Related to Figure 5A.  

(A) Fluorescent images of gfp::sox-2 KI and col-34p::his24::mcherry in the rectum of a wild-type L1 animal before K division (top), in an L1 animal after K division (14 cells in the gonad; middle) and in an L2 animal (bottom). Note that K.a continues to express sox-2 over time whereas expression fades away in K.p during its conversion. White stars indicate the rectal gland cells; the rectal cell position is indicated on the pictures; dashed line, rectal slit; anterior is left and ventral is bottom.  

(B) The rectal col-34 promoter was used to overexpress (OE) SOX-2 in K.p along with a co-injected lim-6(int4)::mCherry reporter. The % of L4 animals displaying lim-6 expression in DVB are represented. Black bar, all results obtained for the control lines (lim-6(int4)::mCherry alone) and dark grey bars, each individual line data respectively; Dotted bar, all results obtained for the SOX-2 overexpressing lines, followed by each individual SOX-2(OE) line data (white bars). Note that transgenic lines overexpressing SOX-2 are difficult to retrieve and maintain, and throw few transgenic animals; transgenics in the F2 generation were usually the only animals that could be scored. The total number of animals scored is indicated above each bar. ****, p<0.0001.
Figure S11. Gel shift experiments show independent binding capacities of POP-1 and SOX-2 to probes 1, 2 and 4. Related to Figure 5E, F.

Increasing concentration (5nm, 50nM and 500nM) of purified HMG-POP-1 and SOX-2 were incubated with wild type or mutated probes 1, 2 and 4 bound to the Cy5 fluorophore. Note that the probe 4 which does not bear canonical SOX-2 binding site is able to bind SOX-2. As, in addition, the mutation of the POP-1 binding site does not seem to affect this binding, it is likely that a non-predicted SOX-2 binding site is present. Probe 3 was also able to bind both SOX-2 and HMG-POP-1, although results for are not presented because this probe annealed poorly, most probably due to its AT-rich sequence. Blue arrowhead, POP-1 bound to the probe; orange arrowhead, SOX-2 bound to the probe; light orange arrowhead, a second SOX-2 shifted band appears at high SOX-2 concentrations; open arrowhead, unbound probe.
Figure S12. Gel shift experiments show binding capacities of POP-1 and SOX-2 when co-incubated. Related to Figure 5E, F.

(A) Increasing quantity of SOX-2 (125nM-250nM-500nM) was added to a mix of HMG-POP-1 and Cy-5-dsProbe #1, #2, odr-1 (known SOX-2 target, Alqadah, 2015) and ceh-22 (known POP-1 target, Lam, 2007, Bhambhani, 2014).

(B) Increasing quantity of HMG-POP-1 (125nM-250nM-500nM) was added to a mix of SOX-2 and Cy-5-dsProbe #1, #2, #4 and ceh-22. Increasing quantity of HMG-POP-1 shows an increasing binding to all the probes as well as an upper shift, most probably corresponding to a HMG-POP-1-SOX-2-Probe complex.

Blue arrowhead, POP-1 bound to the probe; orange arrowhead, SOX-2 bound; green arrowhead, POP-1 and SOX-2 bound; open arrowhead, unbound probe.
Figure S13. Antibody supershift EMSA analysis of SOX-2 and POP-1 co-binding. Related to Figure 5E, F.

(A) Representative EMSA assay on Probe 2 revealing single binding of SOX-2 (orange arrow head) and HMG-POP-1 (blue arrow head) as well as co-binding (upper band, green arrow head).

(B) This upper band was totally upshifted after pre-incubation of the complex with an anti-FLAG antibody (Ab@FLAG) against HMG-POP-1-FLAG.

Various combinations of antibodies/protein/probe complexes were used for controls as indicated.
Table S1. Summary of all cell markers expression.

| C. elegans genes | Human ortholog | Reporter | WT L1 | early L2 | L4 | sem-4 L4 | lin-17 L4 | References | Ref. observation |
|------------------|----------------|----------|--------|----------|----|----------|----------|------------|----------------|
|                  |                |          | K     | K.a      | K.p| DVB      | K post. daughter |            |               |

**Epithelial markers**

| dpy-1 | DSG | mec-4(e54[dpy-1::gfp]) | + | + | - | - | N.D | N.D | Diogen et al. (2007) | This study |
| qjm-1 | AUM1 | jcm-1[qjm-1::gpa] | + | + | - | - | + | + | Mohler et al. (1998) | This study; Mohler et al. (1998) |
| fem-2-2 | Cadherin | gpa-12[hem-1::gpa] | + | + | - | - | N.D | N.D | This study | This study |
| let-413 | SCRAB | psek-1662[lin-14:spg-1:prem1] | + | + | + | - | + | + | This study | This study |
| lin-26 | Zinc-finger transcription factor | P862[gpa-26:rest:dp] | + | + | + | - | + | + | Labousse et al. (1996); this study | This study; Labousse et al. (1999); this study |

**Rectal markers (also in other cells, to visualise the rectal cells)**

| sem-4 | SALL transcription factor | cph-189[sem-4::gpa] | + | + | + | + | ND | + | This study | This study |
| iwe-2 | SOK transcription factor | nph-777[aba::iwe-2] | + | + | - | + | + | + | This study | This study |
| ceh-6 | PDZ transcription factor | pge-97[ghi::ceh-6] | + | + | + | - | + | + | This study | This study |
| cgl-3 | HOOK transcription factor | bh-3[prio-3::gpa] | + | + | - | ND | ND | ND | Teng et al. (2000) | This study |
| cos-34 | Cuticle collagen gene | gpa-245[cal-34::ihe-26::mcherry] | + | + | + | - | + | + | Zurn et al. (2014) | This study |
| gap-1-2.1 | GDF2 | croy-174[ceh-10:gpa-5::gfp::p F1022] | + | + | + | - | ND | ND | McKay et al. (2007) | This study |

**Pan-neuronal markers**

| unc-33 | DRM5 | eto-227[unc-33::gpa]; unc-4::dpy-9 | - | - | - | + | - | - | McKay et al. (2003) | This study |
| unc-119 | LCC129 | edeto[unc-119::gpa; rol-6] | - | - | - | + | - | - | Muroya and Pilgrim (1995); Pratts et al. (2001) | This study |
| rgef-1 | RASGERP3 | ceh-73[ces233::dpy-12::dsf::gfp; ceh-30::p::gpa] | - | - | - | + | - | - | Benard et al. (2009) | This study |

**DVB Terminal selector**

| lin-6 | LIK22B | syg-172[lin-6::gfp] | - | - | + | + | - | - | Herbert et al. (1999); this study |
|       | och-157[lin-6::dpy-9] | - | - | + | + | - | - | |

**GABAergic markers**

| unc-47 | 3CR2A1 | mec-47[unc-4::gpa] | - | - | - | + | - | - | McIntire et al. (1997) | McIntire et al. (1999); this study |
|        | kna(unc-4::gpa::chp-4) | - | - | - | + | - | - | Teuliere et al. (2011) | Teuliere et al. (2011) |
| unc-25 | GAD | pr5[unc-25::gpa] | - | - | - | + | - | - | Jin et al. (1999) | Jin et al. (1999); this study |

* expression is seen in K.p after its birth, and disappears as lim-6 expression appears (see Fig. 5A).
### Table S2. Strain list.

| C. elegans strain | Identifier |
|-------------------|------------|
| rrf-3(pk1426) II ; axIs12[unc-47::gfp; lin-15(+)] X | IS17 |
| lin-5(ev571) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V ; axIs12[unc-47p::gfp; lin-15(+)] X | IS1118 |
| sem-4(n1971) bxIs7[egl-5p(6,5kb)::gfp; lin-15(+)] I; otIs173[rgef-1p::dsred2; ttx-3p::gfp] III | IS1208 |
| sem-4(n197) I; otIs17[unc-4(+); unc-33p::gfp] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V | IS1210 |
| gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X | IS1299 |
| egl-5(n945) III; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X | IS1332 |
| lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X | IS1370 |
| fpIs17[hmr-1::gfp]; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V | IS1374 |
| wrm-1(ne1982) III; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X | IS1432 |
| sem-4(n1971) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X | IS2968 |
| unc-86(n846) III; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X | IS3097 |
| fpIs110[lin-26p::gfp; rol-6(sa1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V | IS3107 |
| egl-27(ok1670) II; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X | IS3113 |
| gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1062[let-413a::gfp::pest; myo-2p::gfp] | IS3119 |
| gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X; fpEx955[Δ(−2846pb to -102)ceh-6p::gfp::ceh-6; odr-1::rfp] | IS3120 |
| ceh-6(ok665) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X; fpEx955[Δ(−2846pb to -102)ceh-6p::gfp::ceh-6; odr-1::rfp] | IS3122 |
| gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X; fpEx788[egl-5p(1,3kb)::sox-2(antisens); rol-6(sa1006)] | IS3142 |
| lin-40(ku285) V; axIs12[unc-47p::gfp; lin-15(+)] X | IS3146 |
| sem-4(n1971) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1062[let-413a::gfp::pest; myo-2p::gfp] | IS3176 |
| gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; juIs8[unc-25p::gfp; lin-15(+)] | IS3298 |
| edIs6[unc-119p::gfp; rol-6(sa1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V | IS3327 |
| lin-17(n671) I; edIs6[unc-119p::gfp; rol-6(sa1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V | IS3328 |
gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; otIs118[unc-33p::gfp; unc-4(+)]

lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; otIs118[unc-33p::gfp; unc-4(+)]

lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; juIs8 [unc-25p::gfp; lin-15(+)]

jcIs1[ajm-1::gfp; rol-6(su1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V

lin-17(n671) I; fpIs111[lin-26p::gfp; rol-6(su1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V

lin-17(n671) I; jcIs1[ajm-1::gfp; rol-6(su1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V

gas245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1111[lim-6int4::gfp; coel::dsred]

lin-17(n671) I; gas245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1062[let-413a::gfp::pest; myo-2::gfp]

lin-17(n671) I; gas245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1111[lim-6int4::gfp; coel::dsred]

gas245[col-34p::his-24::mcherry; unc-119(+)] V; sax-2(syb737[gfp::linker::sax-2]) X

wyIs75[unc-47p::dsred; exp-1p::gfp; odr-1p::rfp] III; yng-1(tm1422)]X

unc-73(e936) dpy-5(e61)] I ; gas245[col-34p::his-24::mcherry; unc-119(+)] V ; oxIs12[unc-47p::gfp; lin-15(+)] X

lin-17(n671) I; gas245[col-34p::his-24::mcherry; unc-119(+)] V; sax-2(syb737[gfp::linker::sax-2]) X

sem-4(n1971) I; gas245[col-34p::his-24::mcherry; unc-119(+)] V; sax-2(syb737[gfp::linker::sax-2]) X

dsh-1(ok1445) II; gas245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X

egl-27(ok1670) II; wyIs75[unc-47p::dsred; exp-1p::gfp; odr-1p::rfp] III; him-5(e1490)]V

egl-5(n945) III; syIs30[cdh-3p::gfp; dpy-20(+)]

egl-20(n585) IV ; gas245[col-34p::his-24::mcherry; unc-119(+)] V ; oxIs12[unc-47p::gfp; lin-15(+)] X

lin-44(n1792) III; gas245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X

lin-44(n1792) I ; egl-20(n585) IV ; gas245[col-34p::his-24::mCherry; unc-119(+)] V ; oxIs12[unc-47p::gfp; lin-15(+)] X

gas245[col-34p::his-24::mCherry; unc-119(+)] V; lim-6(nr2073) oxIs12[unc-47p::gfp; lin-15(+)] X

par-1(u3110) gas245[col-34p::his-24::mCherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X

fmi-1(rh308) gas245[col-34p::his-24::mCherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X

dsh-1(ok1445) mig-5(m2639) II; oxIs12[unc-47p::gfp; lin-15(+)] X

wyIs75[unc-47p::dsred; exp-1p::gfp; odr-1p::rfp] III; gas245[col-34p::his-24::mCherry; unc-119(+)] V; sox-2(syb737[gfp::linker::sax-2]) X; fpEx1156[eegl-5p(6.5kb)::nanobodyGFP::zif-1; coel::gfp; pBSK]

wyIs75[unc-47p::dsred; exp-1p::gfp; odr-1p::rfp] III; gas245[col-34p::his-24::mCherry; unc-119(+)] V; sox-2(syb737[gfp::linker::sax-2]) X; fpEx1156[eegl-5p(6.5kb)::nanobodyGFP::zif-1; coel::gfp; pBSK]
gpr-1(ok2126) III; gaIs245[col-34p::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X

IS3530

sem-4(n1971) I; edIs6[unc-119p::gfp; rol-6(su1006) IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V

IS3537

sem-4(n1971) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; otIs118[unc-33p::gfp; unc-4(+)]

IS3539

gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; ceh-6(syb972[gfp::linker::ceh-6]) X

IS3540

IS3537

lin-17(n671) hxs7[egl-5(6.5kb)::gfp; lin-15(+)] I; otIs173[rgef-1p::dsred2; ttx-3p8::gfp] III

IS3583

hT2[bl-4(e937) let-2(q782) qIs48] (I;III)/pop-1(q645) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; coel::dsred]

IS3596

IS3600

oIs173[rgef-1p::dsred2; ttx-3pB::gfp] III; axIs12[unc-47p::gfp; lin-15(+)] X

IS3604

lin-5(ev571) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; fpIs101[col-34p::ph::gfp; odr-1p::dsRed] X

IS3619

sem-4(n1971) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; lim-6(syb971[lim-6::linker::gfp]) X

IS3632

lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; lim-6(syb971[lim-6::linker::gfp]) X

IS3669

gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; lim-6(syb971[lim-6::linker::gfp]) X

IS3677

lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; ceh-6(syb972[gfp::linker::ceh-6]) X

IS3702

sys-1(q544) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; axIs12[unc-47p::gfp; lin-15(+)] X

IS3718

krIs6[unc-47::DsRed2; lin-15(+)] II; gaIs245[col-34p::HIS-24::mCherry; unc-119(+)] V; fpEx1295[pcr fragment col-34p::gfp::cki-1(gDNA), myo-2p::mCherry]

IS3950

krIs6[unc-47::DsRed2; lin-15(+)] II; gaIs245[col-34p::HIS-24::mCherry; unc-119(+)] V; fpEx1296[pcr fragment col-34p::gfp::cki-1(gDNA), myo-2p::mCherry]

IS3951

krIs6[unc-47::DsRed2; lin-15(+)] II; gaIs245[col-34p::HIS-24::mCherry; unc-119(+)] V; fpEx1297[pcr fragment col-34p::gfp::cki-1(cDNA), myo-2p::mCherry]

IS3952

krIs6[unc-47::DsRed2; lin-15(+)] II; gaIs245[col-34p::HIS-24::mCherry; unc-119(+)] V; fpEx1298[pcr fragment col-34p::gfp::cki-1(gDNA), myo-2p::mCherry]

IS3972

krIs6[unc-47::DsRed2; lin-15(+)] II; gaIs245[col-34p::HIS-24::mCherry; unc-119(+)] V; fpEx1299[pcr fragment col-34p::gfp::cki-1(gDNA), myo-2p::mCherry]

IS3973

krIs6[unc-47::DsRed2; lin-15(+)] II; gaIs245[col-34p::HIS-24::mCherry; unc-119(+)] V; fpEx1300[pcr fragment col-34p::gfp::cki-1(gDNA), myo-2p::mCherry]

IS3974
| Oligo name | Sequence | Use |
|------------|----------|-----|
| **BDT950** | CTGAATCCGGATCCATCATCATGTCGACTGCAGAATTCGAAGCTT GTCGACGGAGCTC | sox-2 antisense construct |
| **BDT952** | CTTGGAAGGGTACCTAGGAGCTCGATATCTAGAAGAGGTAACATG GATTGGGA | sox-2 antisense construct |
| **EB110F** | AGAAGACCGCCCCTCTTTTGA | Genotyping ceh-6(syb972) |
| **EB110R** | GGCTGCTCATTCTGGTCT | Genotyping ceh-6(syb972) |
| **EB5F** | TCCAGTCTCTCAGGTCAATGGT | Genotyping egl-27(ok1670) |
| **EB5R** | CGAGATTCCAATACTTATCCGACTG | Genotyping egl-27(ok1670) |
| **EB6R** | GGTAAATTCAGCGATGATGATGAAGG | Genotyping egl-27(ok1670) |
| **LIN5 FW 01** | GACAAGACCAAGTTATCGGC | Genotyping lin-5(ev571), digest w/ BglII |
| **LIN5 RV 01** | CCCATTGACTGAAATTCTTCG | Genotyping lin-5(ev571), digest w/ BglII |
| **mcm124F** | GAACATACAACCTTTGTGACCAACATTTGCCACGCTTTCC CCCAT | Genotyping egf-5(n945), digest w/ Ncol |
| **mcm124R** | CGTAAGATAGCATATAGGGTCAGACG | Genotyping egf-5(n945), digest w/ Ncol |
| **mcm125F** | CCGCGCATGACACGGATTGGTAC | Genotyping sem-4(n1971), digest w/ Acc65I |
| **mcm125R** | CCTAACAAGCTAGCCTTTACAGTTACAAAAACATCTCCTTTACT GGTA | Genotyping sem-4(n1971), digest w/ Acc65I |
| **oCG347 rev début GFP** | CCACGTGACAGAAATTTTGTGCC | Genotyping sem-4 (syb1287) and sequencing |
| **oCG368 sens PEST** | CTTGACATGCTCTCCGGCGCGCGCGCGCGATGATG | Cloning of the lin-26 rectal specific promoter into pPD97.82 |
| **oCG369 rev PEST** | AGGCTGCTCATTCTGGTCAATGGT | Cloning of the lin-26 rectal specific promoter into pPD97.82 |
| **oCG370 sens MW PEST** | GGTTGTCTTTTGCAGTGTCCGG | Cloning of the lin-26 rectal specific promoter into pPD97.82 |
| **oCG371 rev MW PEST** | ATGGATCATCACATGACAGTAAACCTTTGATGACTACATTGGATC | Cloning of the lin-26 rectal specific promoter into pPD97.82 |
| **oCG381 lin-26p f** | CAACCTTGGAAATGAAATAGGCTGATCGAGCTCAGGCACTTTCCA TTGCTTCTTACATCTT | Cloning of the lin-26 rectal specific promoter into pPD97.82 |
| **oCG382 lin-26p r** | GCTGAAAAGTGTCTAGAGTCGACCAAGGCAGAGCTGAGAGCACTGAGGGAGGGTGAGAGCTAGTGG | Cloning of the lin-26 rectal specific promoter into pPD97.82 |
| **oCG390 for NLS1 kpnI** | AGGGTACCATGCTCAAGAAGAAATGACCGAGGTCGAGT | Cloning 2nls into pPD95.75 |
| **oCG391 rev GFP Xhol** | GGGATCTACGGAAGCATTGAAACACCATAACAGAAAG | Cloning 2nls into pPD95.75 |
| **oCG411 sens egr-1 ku285** | GCCCCAAAAGCCTGAAAAAACGCGAAAAATTTTCTAAATTCTT | Genotyping egr-1(ku285), digest w/ Hpy188III |
| **oCG412 rev egr-1 ku285** | GAGGTCTCCGACAGAAGCTGATGG | Genotyping egr-1(ku285), digest w/ Hpy188III |
| **oCG444 lim-6 3int sens** | GATAGCCTAACAACTTGGAAATGAAATAGGCTGAGAGCACTGAGGGAGGGTGAGAGCTAGTGG | Cloning lmr-6 intron4 into pPD95.75 |
| **oCG445 lim-6 3int rev** | CGACCTGACAGGCATGCAAGCTAAATATTGACTATGGGAGACATC TGCC | Cloning lmr-6 intron4 into pPD95.75 |
| **oCG461 sens sox-2 CRISPR** | GGTGTCTCTTTCGACGAGTCCGG | Genotyping sox-2(syb737) |
| CRISPR | Genotyping | Notes |
|--------|------------|-------|
| CAGAGCCATTTTCCTCCGCTGTC | sox-2 (syb737) | |
| GACGACGAATCTCGATGTTGCG | sem-4 (syb1287) | |
| GGGGAAAGAGGGGAATAGCTG | sem-4 (syb1287) | |
| GACGACGAATCTTCGATGTGGC | cloning of POP-1 HMG | |
| GGGGGAAAGAGGGAAAATTAGCTG | cloning of POP-1 HMG | |
| GACAGCCCAGATCTGGGTACCCAAGGAGGTGGAAAAGCGAAGA | cloning of POP-1 HMG | |
| GACGGAGCTGCAATTCCTCCCGAGAGGTGGAAAAGCGAAGA | cloning of POP-1 HMG | |
| CCCACACAGTTCACAAATGGCC | cloning of POP-1 HMG | |
| AGGCTAGAAAGTTCTACGGG | cloning of POP-1 HMG | |
| GCTCGACACATGTTCCCGGAG | cloning of POP-1 HMG | |
| GTTGACAATGTTCCCGGAG | cloning of POP-1 HMG | |
| CCCACACAGTTCACAAATGGCC | cloning of POP-1 HMG | |
| ACCAGCTCAGGATTTGGAATCTCGAATCTCAGATTATCTGGAA | cloning of POP-1 HMG | |
| ACCAGCTCAGGATTTGGAATCTCGAATCTCAGATTATCTGGAA | cloning of POP-1 HMG | |
| GTTGACAATGTTCCCGGAG | cloning of POP-1 HMG | |
| GTTGACAATGTTCCCGGAG | cloning of POP-1 HMG | |
| CCCACACAGTTCACAAATGGCC | cloning of POP-1 HMG | |
| ACCAGCTCAGGATTTGGAATCTCGAATCTCAGATTATCTGGAA | cloning of POP-1 HMG | |
| ACCAGCTCAGGATTTGGAATCTCGAATCTCAGATTATCTGGAA | cloning of POP-1 HMG | |
| GTTGACAATGTTCCCGGAG | cloning of POP-1 HMG | |
| GTTGACAATGTTCCCGGAG | cloning of POP-1 HMG | |
| oCR122 q645 fw | CGATGGATTTCGACCGGCACC | Genotyping pop-1(q645), digest w/ ClaI |
| oCR123 q645 rv | GATATAAAATACACAAAAATGAGGCACGAGTCATCGA | Genotyping pop-1(q645), digest w/ ClaI |
| oCR128 n1378 fw | CAACCGGATCCAAAACCGAAATCCCTGCTGGCATG | Genotyping sem-4(n1378), digest w/ SphI |
| oCR129 n1378 rv | CCCAGGTGGATGGAATGCGCGTGCAAC | Genotyping sem-4(n1378), digest w/ SphI |
| oCR138 syb971fw | GATATAAAAATACACAAAAATGATGGCCGACGAAGAGCTCATCGA | Genotyping pop-1(q645), digest w/ ClaI |
| oCR139 syb971fw wt | GTGCAAAGATTAGAGTCATCTGAC | Genotyping lim-6(syb971) |
| oCR140 syb971rv mu | GGTTATCTGAGAAGCATTG | Genotyping lim-6(syb971) |
| oCR144 n1051 fw | CACTACAGGTTATGGCAAACATCGACTACCTCTCGTTCCCAT | Genotyping lim-18(n1051), digest w/ NcoI |
| oCR145 n1051 rv | CCTGTCGCAATTTCACTTTCAACGGCTC | Genotyping lim-18(n1051), digest w/ NcoI |
| oCR149 gm122 fw | GACCAGATTATTCTTCGCGCAACG | Genotyping cam-1(gm122), digest w/ BclI |
| oCR150 gm122 rv | CATCATATGTATAAAGTTTGCGAATCGGATTCTAATGAT | Genotyping cam-1(gm122), digest w/ BclI |
| oCR151 q544 fw | CCTGTGGCGGAGGAGGTTGATCATGTGG | Genotyping sys-1(q544), digest w/ AffIII |
| oCR152 q544 rv | GCCAAAAAGATCTCCTCACATGAAACACTGCGAAAATCAGT | Genotyping sys-1(q544), digest w/ AffIII |
| oCR153 n671 | CGCATTTTTTCAGATCAGACC | Sequencing lin-17(n671) |
| oCR154 n671 | CGACACATTCTCCAGAGAAGGT | Sequencing lin-17(n671) |
| oCR155 lin-17p fw | CTGAAAGCTTACCTTTGTCGTC | Cloning lin-17p reporter |
| oCR156 lin-17p rv | CGGCTGCAAGTTGGAAGAGACGCTCTCT | Cloning lin-17p reporter |
| oCR157 wrm-1 fw | GATGTCTTCCGGACTGAATGC | Sequencing and genotyping wrm-1(ne1982ts) |
| oCR158 wrm-1 rv | CTGTGCTCCACCCATTG | Sequencing and genotyping wrm-1(ne1982ts) |
| pLG7F | ACGCGTCGACGTAAACATGATGTTTCCCCAGTAC | sox-2 antisens construct |
| pLG7R | GCCTAGAGATATTTACATTTTATACATCAAAGGCAACC | sox-2 antisens construct |
| oSKS-233 | ATGAACTATAACAAGCTCTCGAATTCTGCAGTCGACA TGTCTTTCTGCTCGTCTGTGC | To amplify cki-1 for construction of pSJ1108 |
| oSKS-234 | ATTTCATGCAAGCCCTCGGCGCCGCTGACTCCTGATGATGAAAGCATGAAGATCAGGAGGTCGTC | To amplify cki-1 for construction of pSJ1108 |
| oSKS-235 | ATGGACTACAAAGGACGACGTTAAGTAGAAGATCAGAGCTCC GAATTCGAGCTCC | To insert Flag at the C-terminal of hmg-pop1 in pSJ769, for construction of pSJ1107 |
| oSKS-236 | ACTCTTATCCCTTGCCTCTTGGTGTG | To insert Flag at the C-terminal of hmg-pop1 in pSJ769, for construction of pSJ1107 |
| oSKS-237 | ACCAAAAATTTCTCAGTCAGAACCAC | To delete intron from cki-1 for construction of pSJ1112 |
| oSKS-238 | CTGAGAAACTCCGGAACACAATTCCTCT | To delete intron from cki-1 for construction of pSJ1112 |
| pSJ6094sox-2 F | TCGACATGATGATGAGTCGACGTACGATCCGATCCGAC | 6XHis::sox-2 construct |
| pSJ6094sox-2 R | GTGCCGGCAGGTCTTGGTACC | 6XHis::sox-2 construct |