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Supplemental information

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

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Fig. S1.

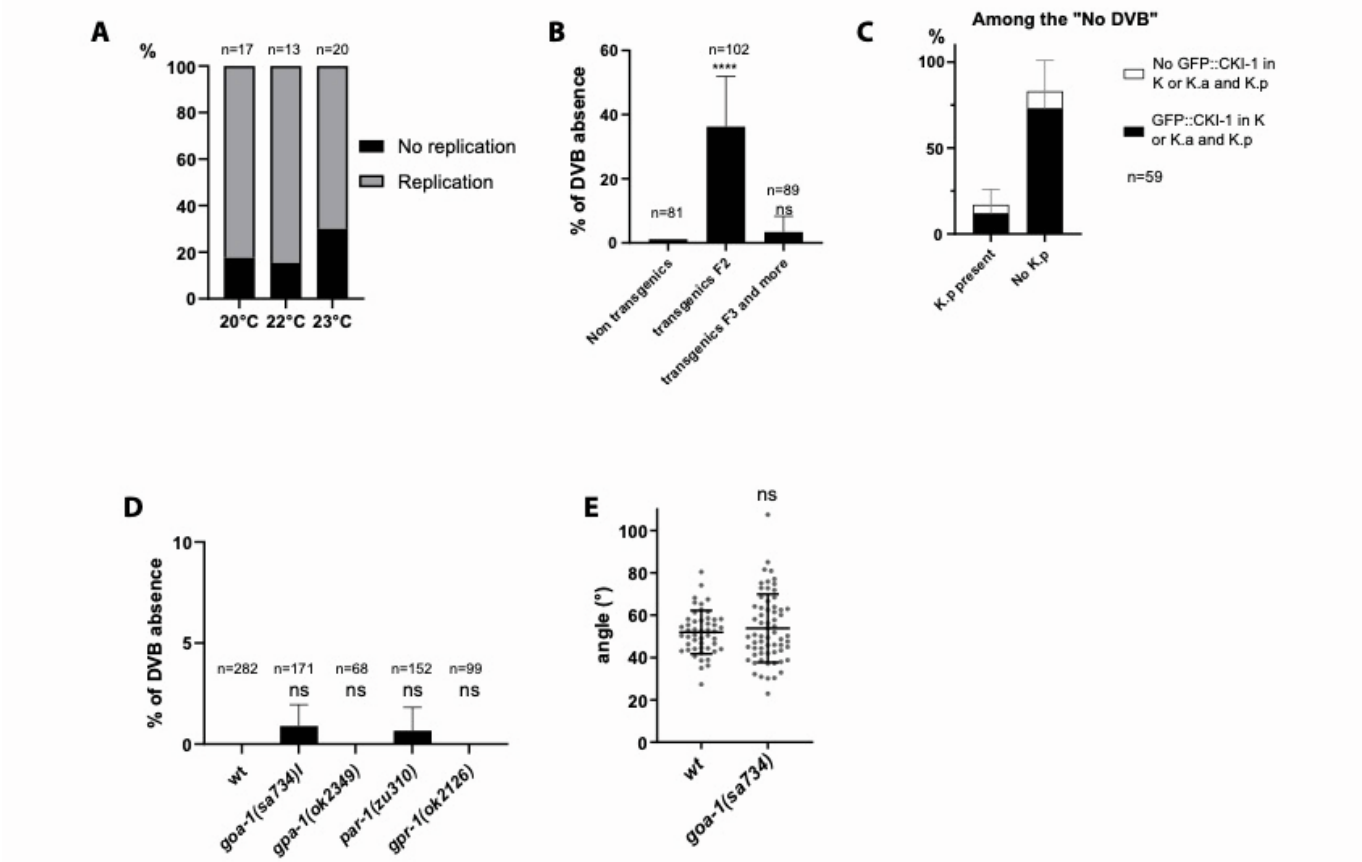


Figure S1. While DNA replication in K is not sufficient for DVB formation and division orientation mutants do not impact K-to-DVB, K division is required for K-to-DVB. Related to Figure 2.

(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.

Fig. S2.

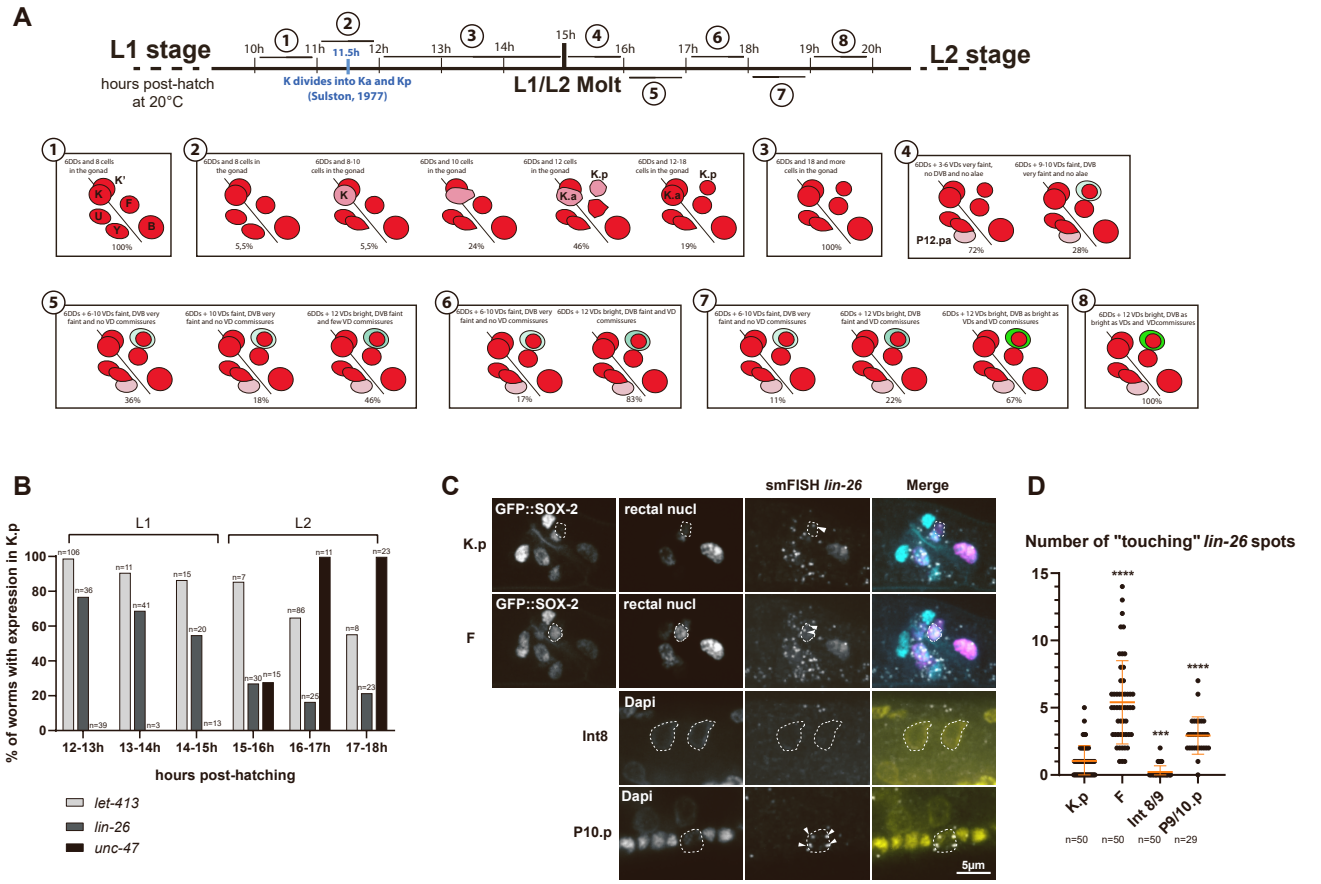


Figure S2. K divides into K.a and K.p in late L1 stage and K.p still shows epithelial features after division. Related to Figure 2.

(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 *oxIs12[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 *gaIs245*, 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).

Fig. S3.

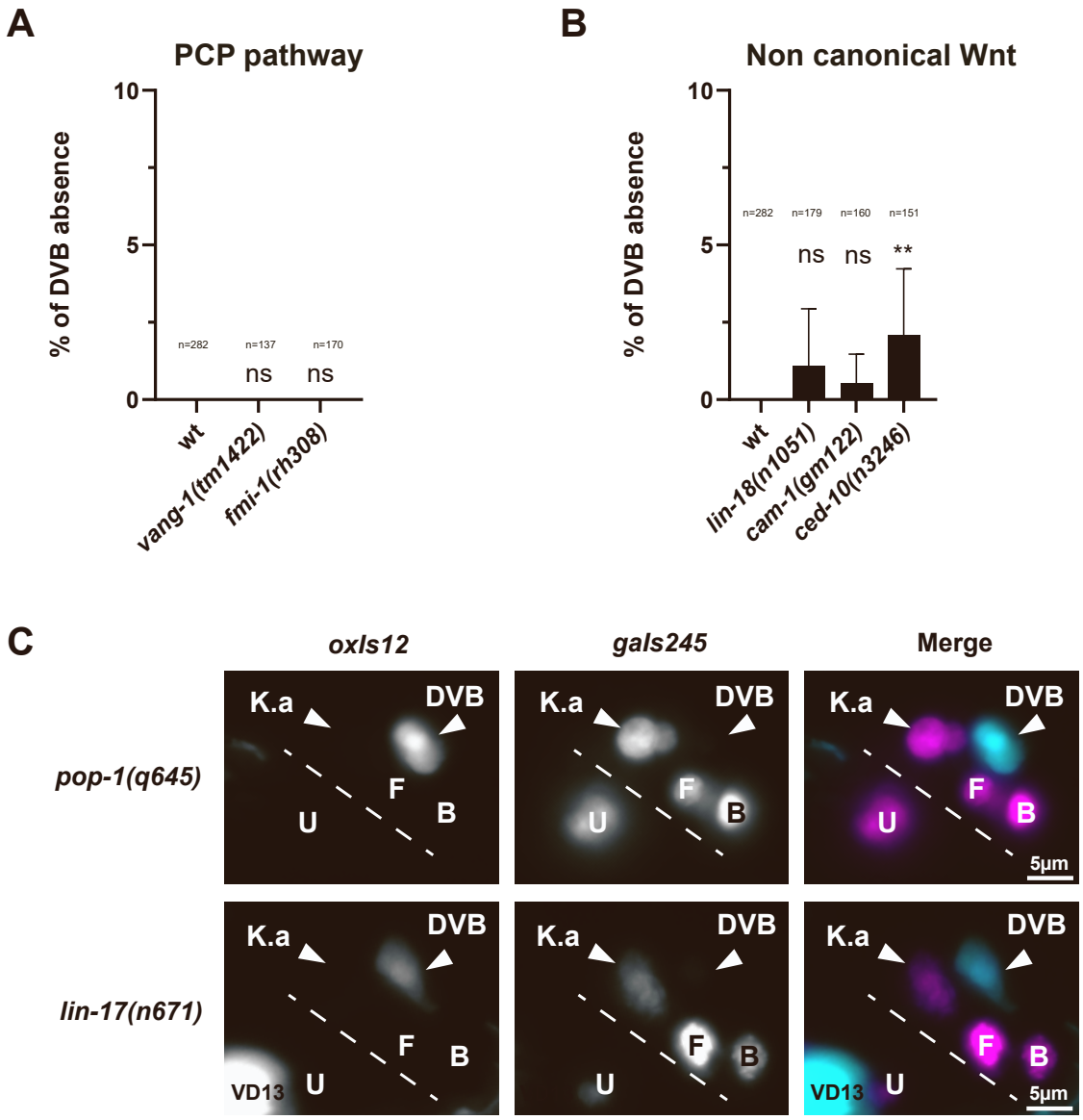


Figure S3. The non-canonical Wnt pathways are not required for K-to-DVB. Related to Figure 3.

(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 o*xIs12,* and of the K.a, U, F and B rectal cells, as observed using *gaIs245*, are indicated. VD13, GABAergic neuron. Dash line, rectal slit. Anterior is to the left and ventral to the bottom.

Fig. S4.

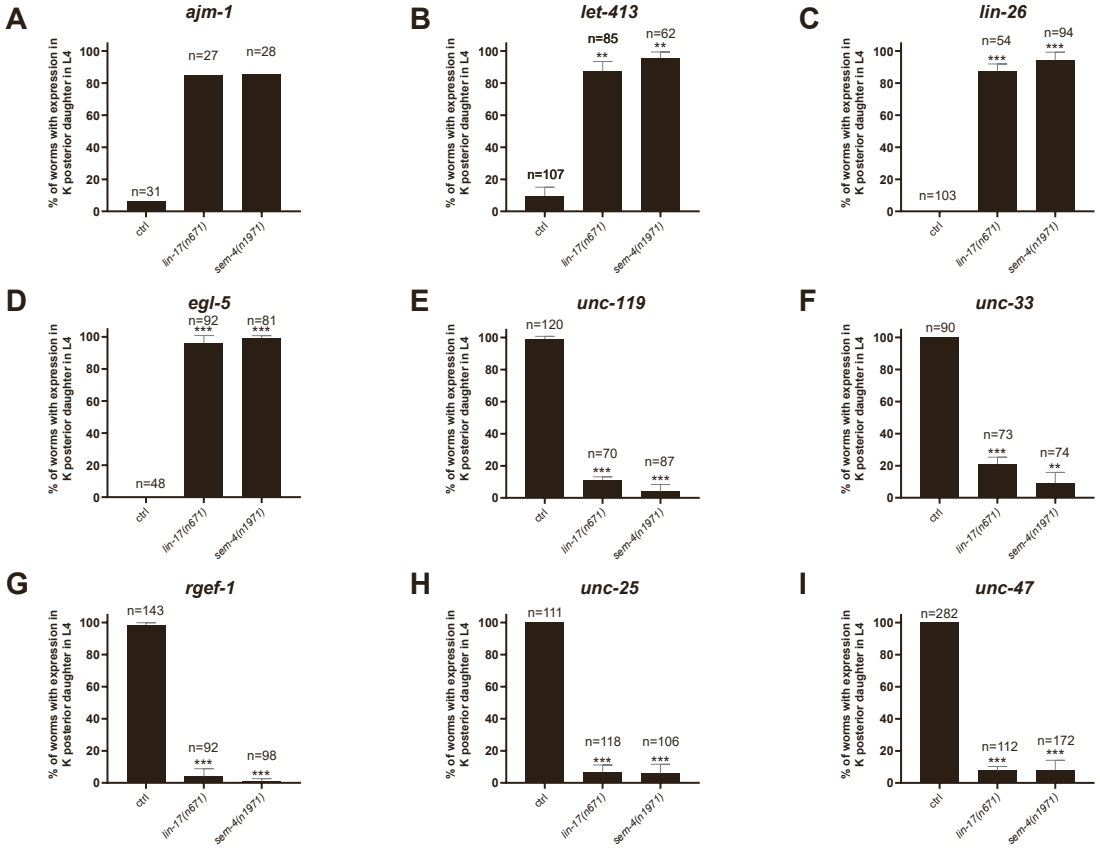


Figure S4. K.p cell remains rectal-epithelial in *lin-17/FZD* and *sem-4/SALL* mutants. Related to Figure 3 and Figure 4.

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/F*ZD and *sem-4/SALL* mutant backgrounds, or DVB in wild type, in L4 larvae. n, total animal scored.

Fig. S5.

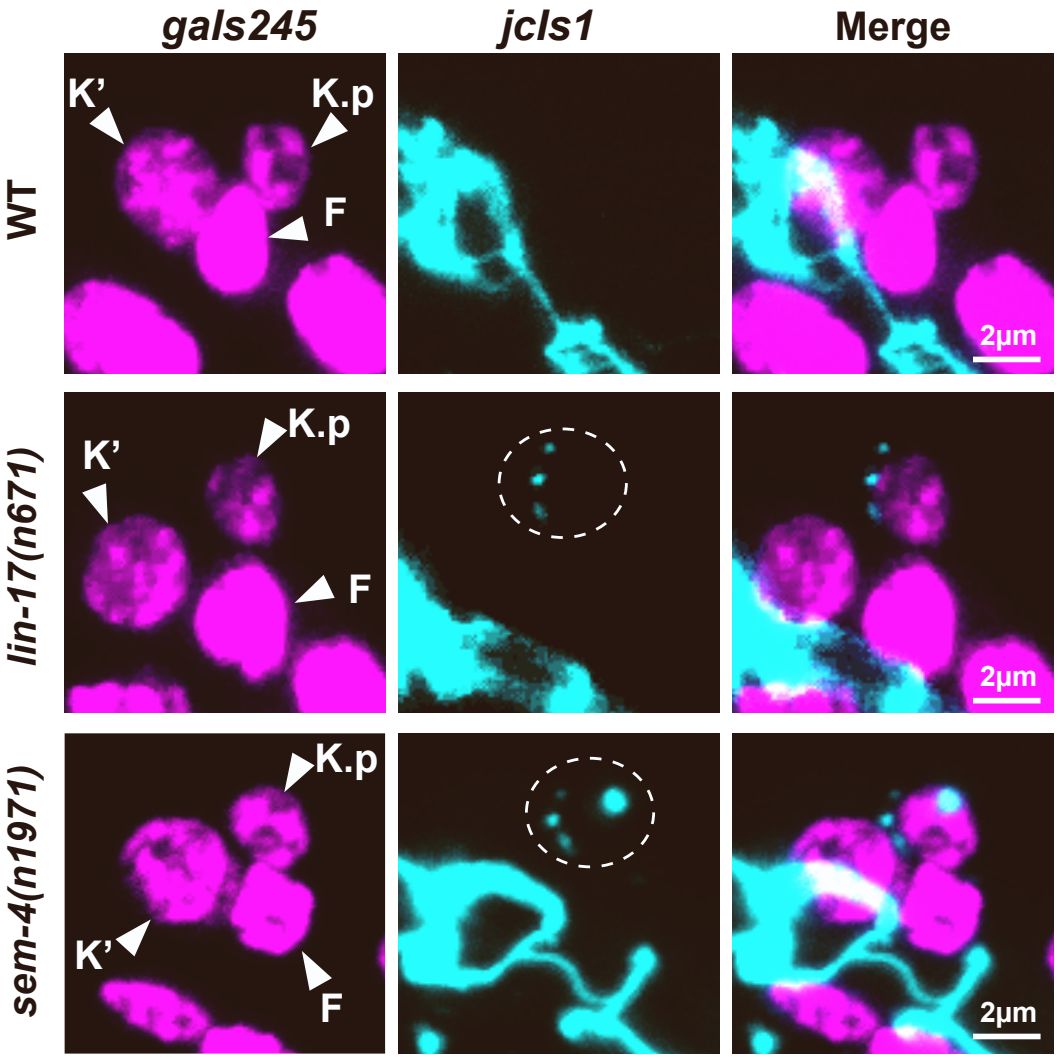


Figure S5. K.p cell expresses the apical junction protein AJM-1 in *lin-17/FZD* and *sem-4/SALL* mutants. Related to Figure 3 and Figure 4.

Confocal images of wild-type, *lin-17/FZD* and *sem-4/SALL* mutant backgrounds in L3 larvae carrying *gaIs245[col-34p::his-24::mcherry]* to visualize the rectal cell nuclei and *jcIs1[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.

Fig. S6.

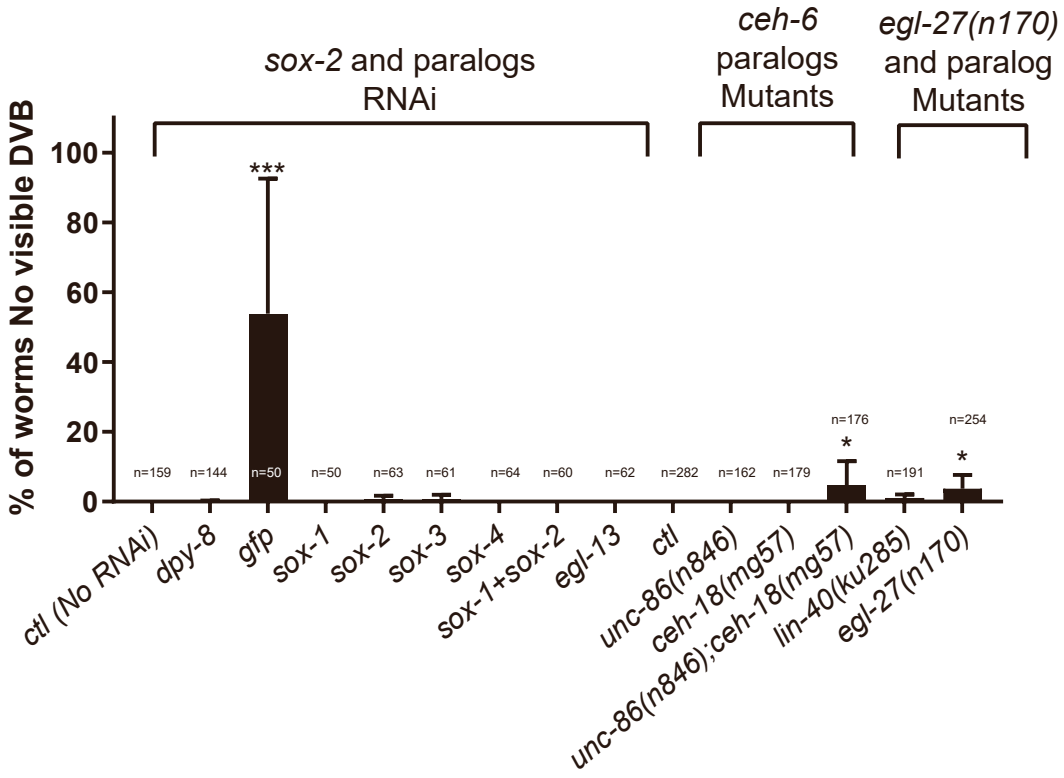


Figure S6. *sox-2*, *ceh-6* and *egl-27* paralogs do not seem to be required to form DVB. Related to Figure 4. Quantification of DVB defective L4 animals (as observed by *unc-47* expression) using RNAi in a sensitized *rrf*-3 mutant background to target *sox-2* paralogs (*dpy-8* and *gfp* RNAi represent 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.

Fig. S7.

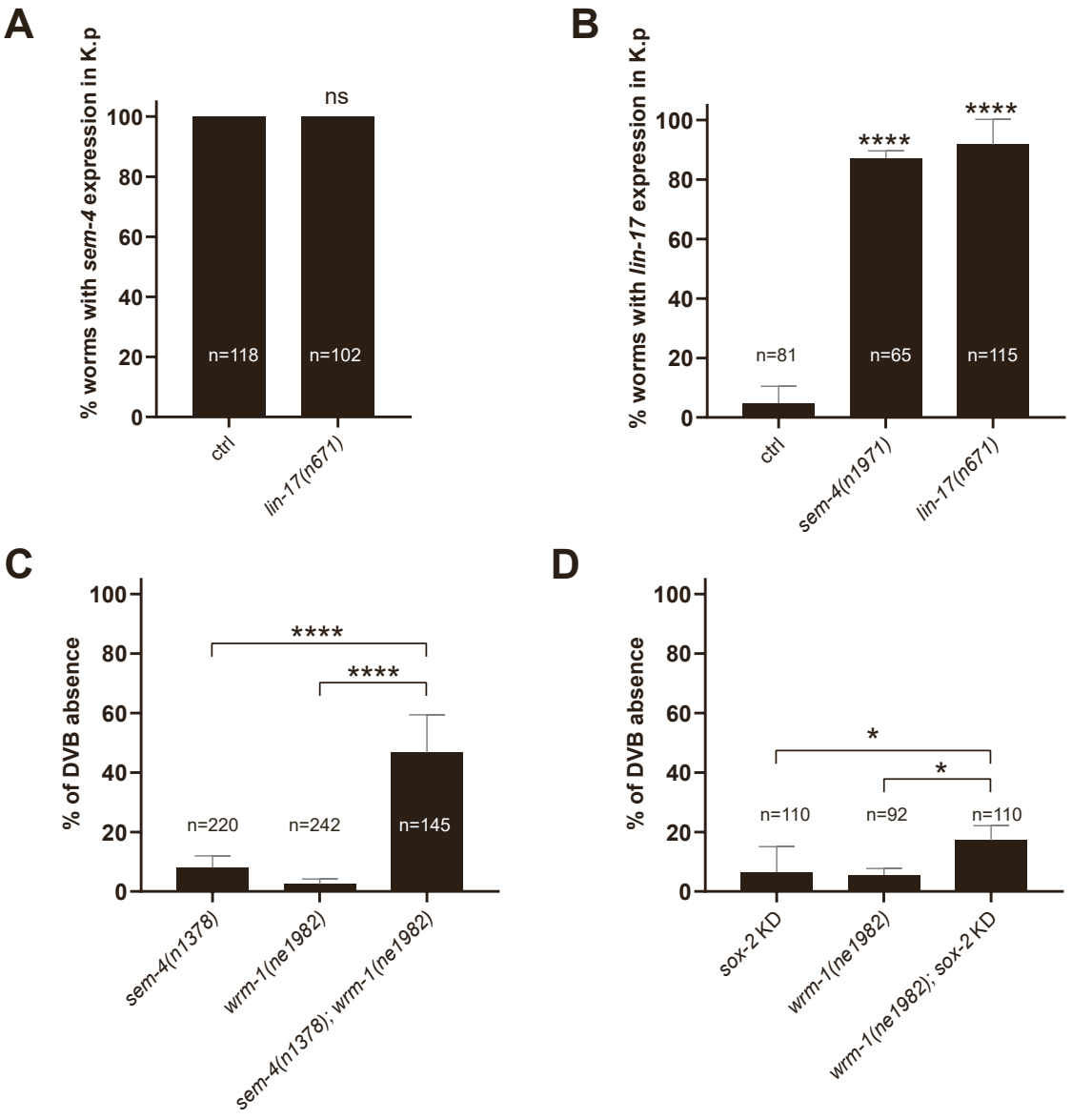


Figure S7. *sem-4/SALL* and the Wnt signaling pathway act in parallel to drive K-to-DVB Td. Related to Figure 5.

(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.

Fig. S8.

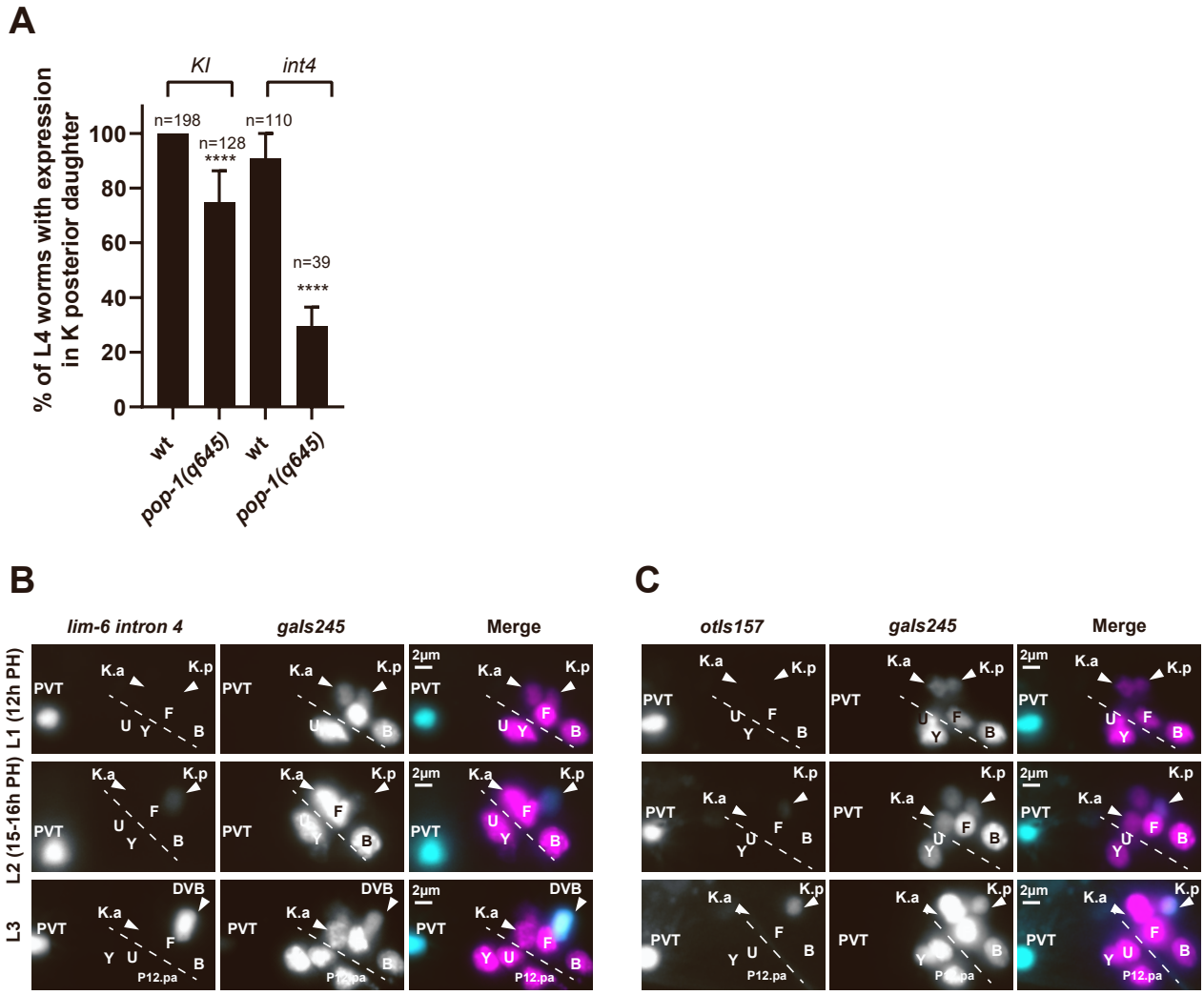


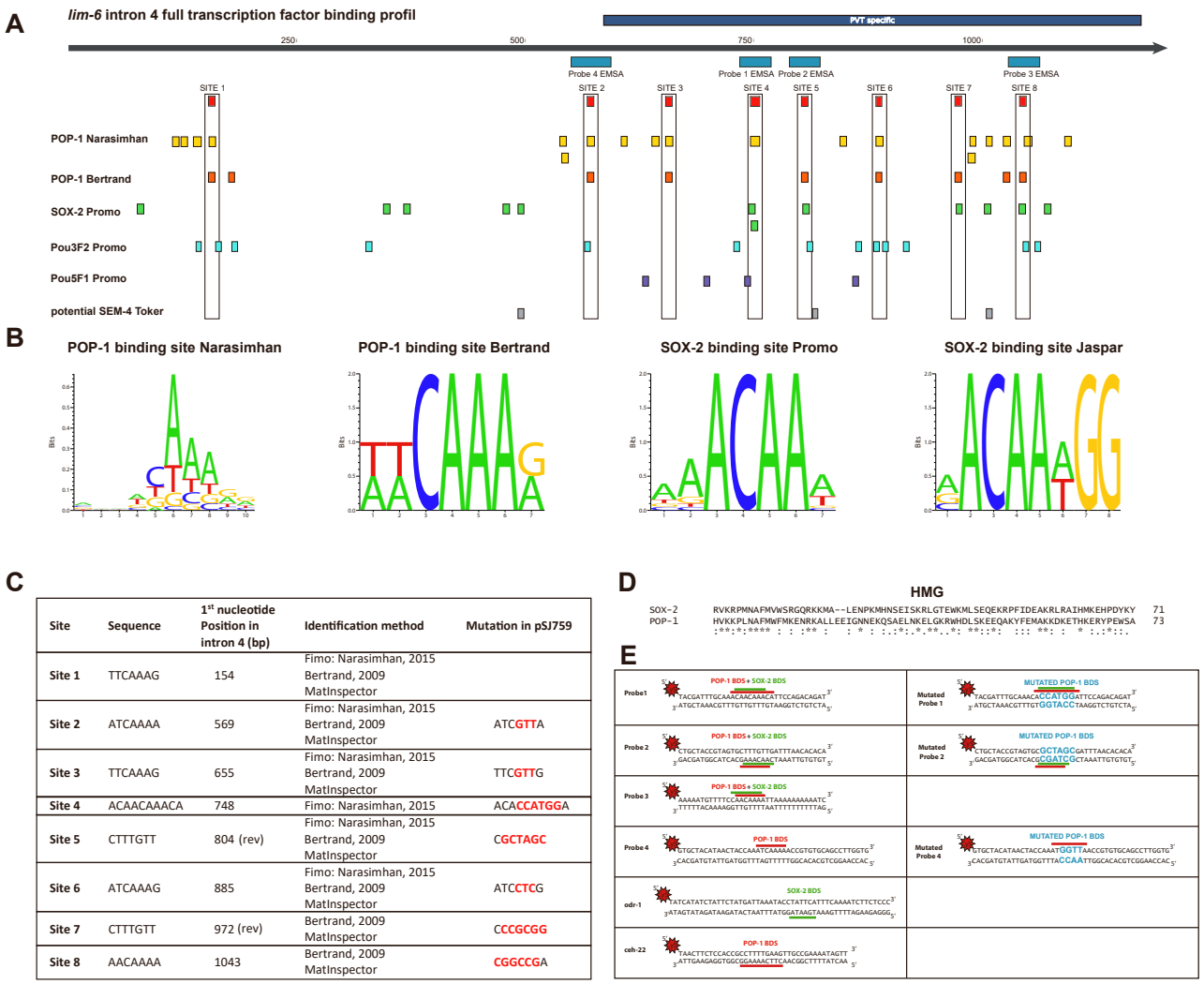
Figure S8. *lim-6* is expressed early in K.p and its expression is affected in *pop-1/TCF* mutant. Related to Figure 5.

(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.

Fig. S9.



Site	Sequence	1st nucleotide Position in intron 4 (bp)	Identification method	Mutation in pSJ759
Site 1	TTCAAAG	154	Fimo: Narasimhan, 2015 Bertrand, 2009 MatInspector	
Site 2	ATCAAAA	569	Fimo: Narasimhan, 2015 Bertrand, 2009 MatInspector	ATC**GTT**A
Site 3	TTCAAAG	655	Fimo: Narasimhan, 2015 Bertrand, 2009 MatInspector	TTC**GTT**G
Site 4	ACAACAAACA	748	Fimo: Narasimhan, 2015	ACA**CCATGG**A
Site 5	CTTTGTT	804 (rev)	Fimo: Narasimhan, 2015 Bertrand, 2009 MatInspector	C**GCTAGC**
Site 6	ATCAAAG	885	Fimo: Narasimhan, 2015 Bertrand, 2009 MatInspector	ATC**CTC**G
Site 7	CTTTGTT	972 (rev)	Bertrand, 2009 MatInspector	C**CCGCGG**
Site 8	AACAAAA	1043	Bertrand, 2009 MatInspector	**CGGCCG**A

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.

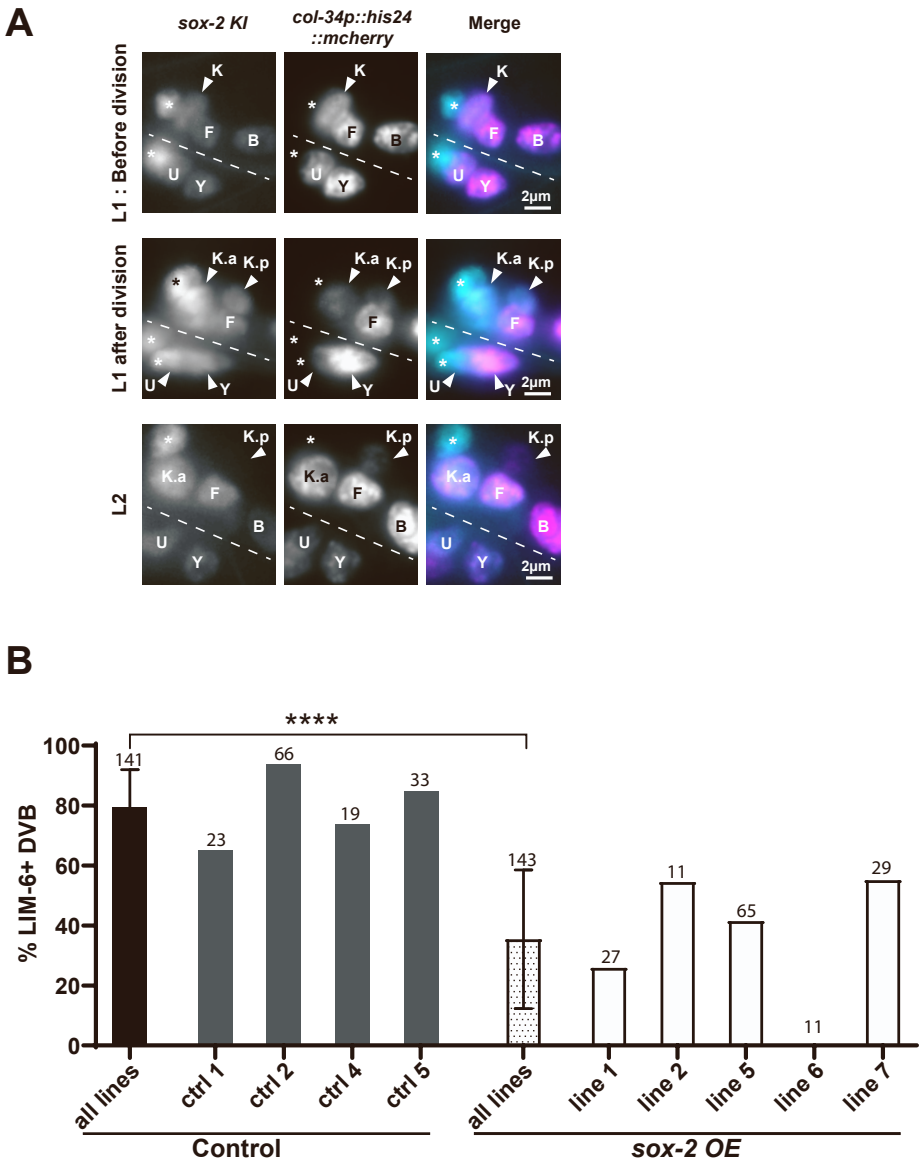


Figure S10. *sox-2* is expressed in K.p after K division and it is subsequently downregulated during DVB differentiation. Overexpression of *sox-2* prevents *lim-6* expression. Related to Figure 5A.

(A) Fluorescent images of *gfp::sox-2* KI and *col-34p::his-24::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. ****, $p<0.0001$.

Fig. S11.

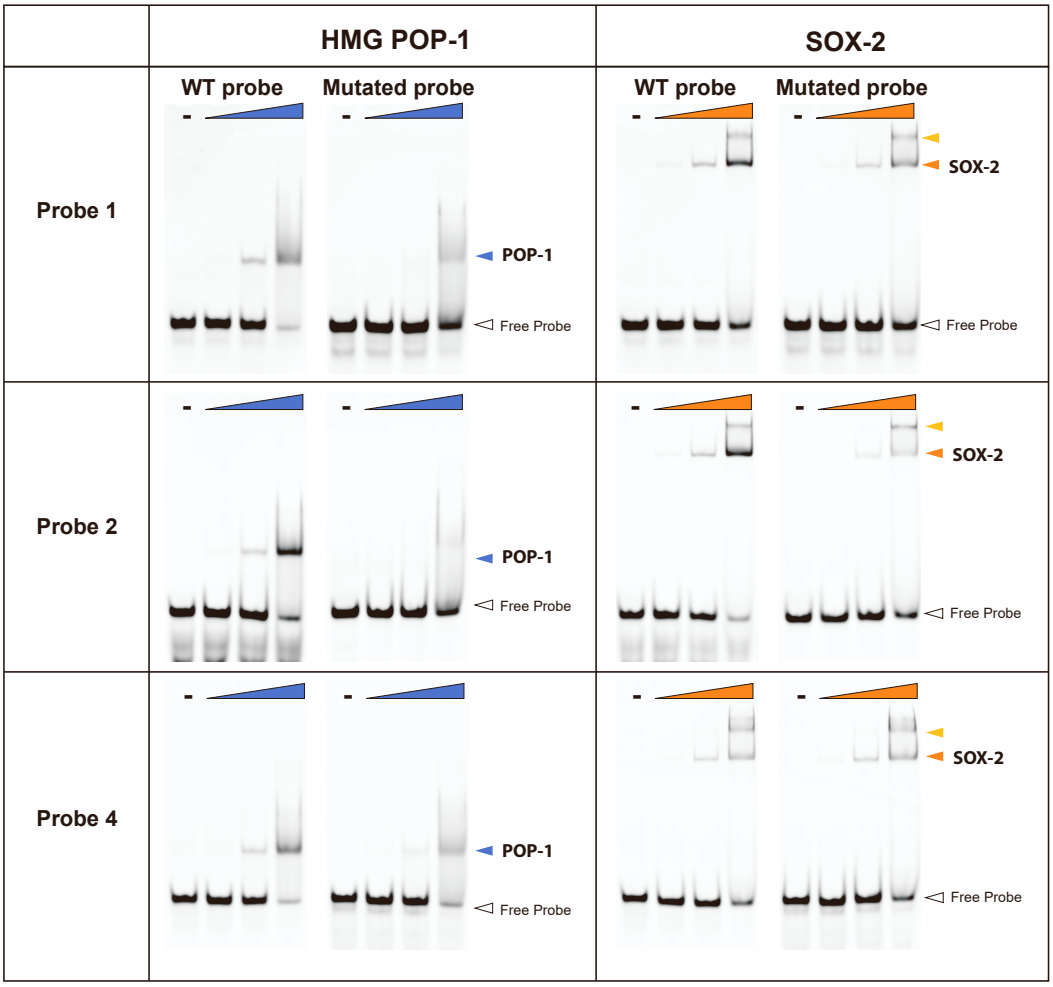


Figure S11. Gel shift experiments show independent binding capacities of POP-1 and SOX-2 to probes 1, 2 and 4. Related to Figure 5E, F.

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.

Fig. S12.

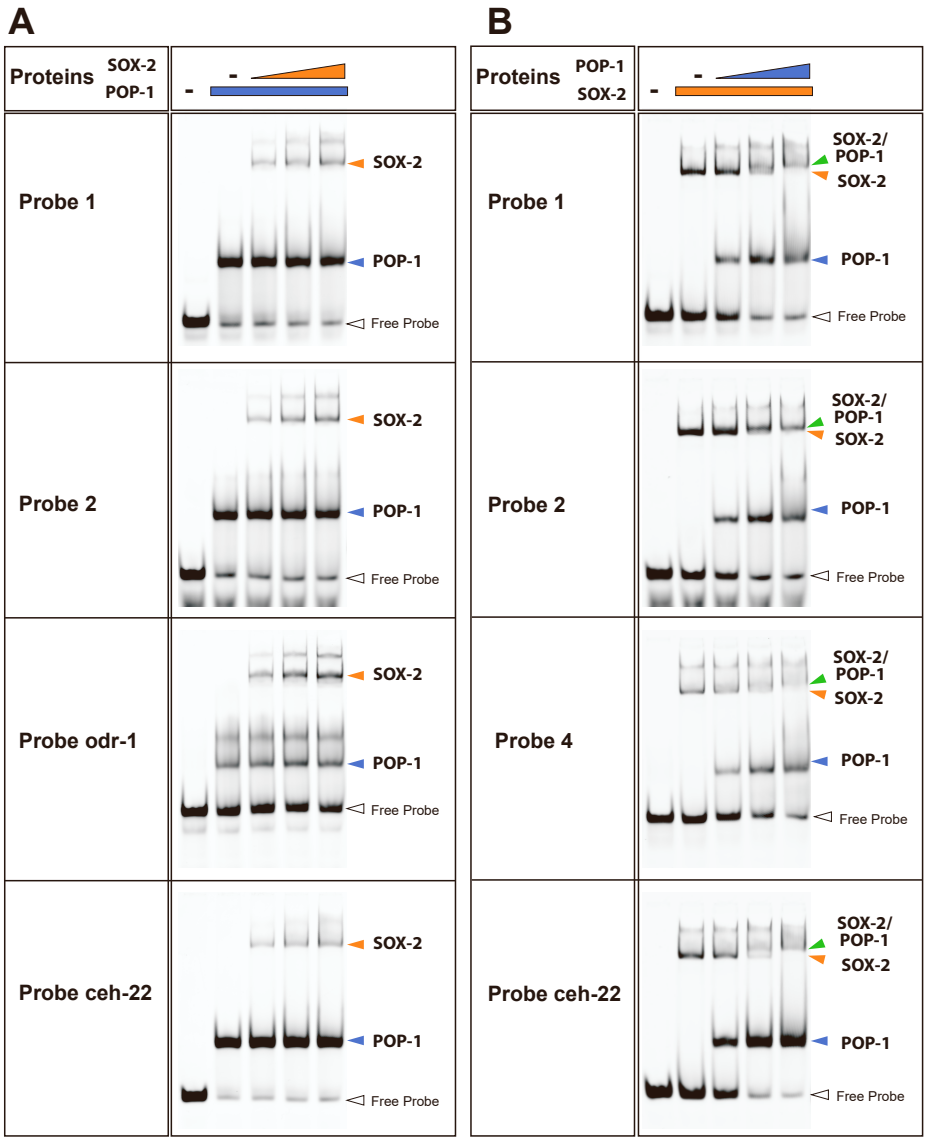


Figure S12. Gel shift experiments show binding capacities of POP-1 and SOX-2 when co-incubated. Related to Figure 5E, F.

(**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.

Fig. S13.

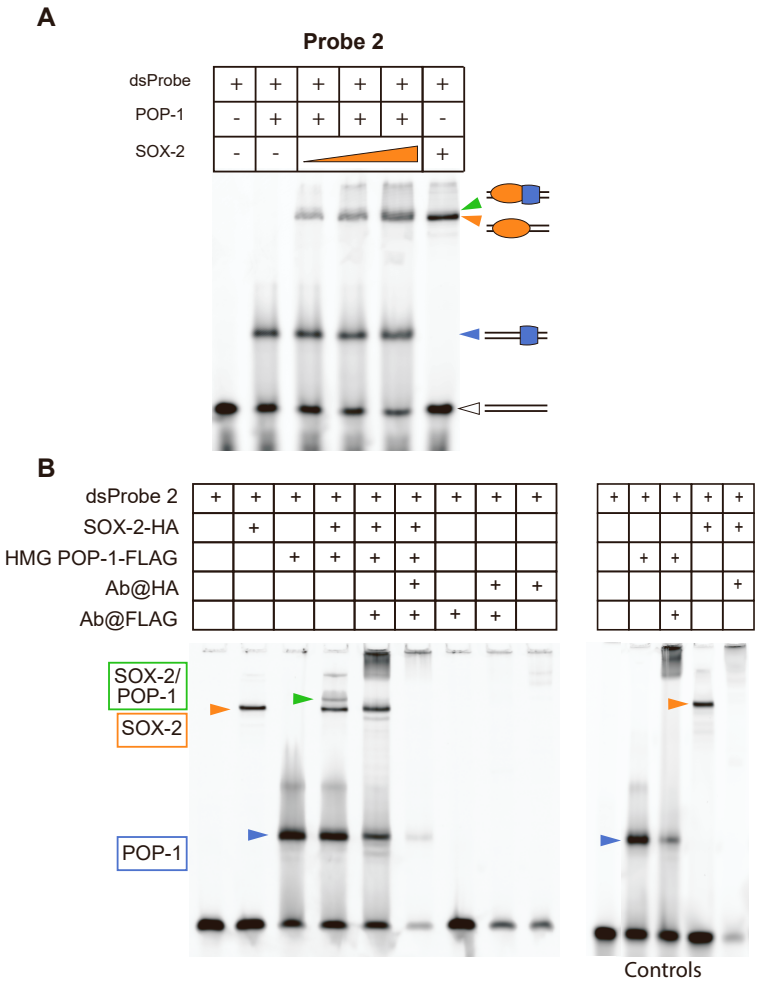


Figure S13. Antibody supershift EMSA analysis of SOX-2 and POP-1 co-binding. Related to Figure 5E, F.

(**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				*sem-4*	*lin-17*	References reporter	Ref observation
			L1	early L2		L4	L4	L4		
			K	K.a	K.p	DVB	K post. daughter	K post. daughter		
Epithelial markers										
dlg-1	*DLG*	*mcIs46[dlg-1::rfp]*	+	+	-	-	N.D	N.D	Diogon et al. (2007)	This study
ajm-1	*AJM1*	*jcIs1[ajm-1::gfp]*	+	+	-	-	+	+	Mohler et al. (1998)	This study; Mohler et al. (1998)
hmr-1	Cadherin	*fpIs17[hmr-1::gfp]*	+	+	-	-	N.D	N.D	This study	This study
let-413	*SCRIB*	*fpEx1062[let-413a::gfp::pest]*	+	+	+	-	+	+	This study	This study
lin-26	Zinc-finger transcription factor	*fpIs110[lin-26rectalp::gfp]*	+	+	+	-	+	+	Labouesse et al. (1996); this study	Labouesse et al. (1996); this study
Rectal markers (also in other cells, to visualise the rectal cells)										
sem-4	*SALL* transcription factor	*syb1287[sem-4::gfp]*	+	+	+	+	ND	+	This study	This study
sox-2	*SOX* transcription factor	*syb737[gfp::sox-2]*	+	+	+*	-	+	+	This study	This study
ceh-6	*POU* transcription factor	*syb972[gfp::ceh-6]*	+	+	+*	-	+	+	This study	This study
egl-5	*HOX* transcription factor	*bxIs7[egl-5::gfp]*	+	+	+	-	ND	ND	Teng et al. (2004)	This study
col-34	Cuticle collagen gene	*gaIs245[col-34p::his-24::mcherry]*	+	+	+	-	+	+	Zuryn et al. (2014)	This study
got-1.2	*GOT1*	*sIs11174[rCesT01C8.5::gfp+pCeh361]*	+	+	+	-	ND	ND	McKay et al. (2003)	This study
Pan-neuronal markers										
unc-33	*DPYS*	*otIs117[unc-4(+) ; unc-33p::GFP]* *otIs118[unc-33::GFP; unc-4(+)]*	-	-	-	+	-	-	McKay et al. (2003)	This study
unc-119	*UNC119*	*edIs6[unc-119::gfp; rol-6]*	-	-	-	+	-	-	Maduro and Pilgrim (1995); Praitis et al. (2001)	This study
rgef-1	RASGRP3	*otIs173 [F25B3.3::DsRed2; ttx-3promB::GFP]*	-	-	-	+	-	-	Benard et al. (2009)	This study
DVB Terminal selector										
lim-6	*LMX1B*	*syb971[lim-6::gfp]* *otIs157[lim-6r::GFP]*	-	-	+	+	-	-	This study	Hobert et al. (1999); this study
GABAergic markers										
unc-47	*SLC32A1*	*oxIs12[unc-47p::gfp]*	-	-	-	+	-	-	McIntire et al. (1997)	McIntire et al. (1997); this study
		krIs6[unc-47p::DsRed2]							Teuliere et al. (2011)	Teuliere et al. (2011)
unc-25	*GAD*	*juIs8[unc-25p::gfp]*	-	-	-	+	-	-	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 ; oxIs12[unc-47::gfp; lin-15(+)] X	**IS17**
lin-5(ev571) II; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V ; oxIs12[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; otIs117[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; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS1299**
egl-5(n945) III; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS1332**
lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[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; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS1432**
sem-4(n1971) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS2968**
unc-86(n846) III; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3097**
fpIs110[lin-26p::gfp; rol-6(su1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V	**IS3107**
egl-27(ok1670) II; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V ; oxIs12[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; oxIs12[unc-47p::gfp; lin-15(+)] X; fpEx955[Δ(-2846pb to -102)ceh-6p::gfp::ceh-6; odr-1::rfp]	**IS3120**
ceh-6(gk665) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[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; oxIs12[unc-47p::gfp; lin-15(+)] X; fpEx788[egl-5p(1,3kb)::sox-2(antisens); rol-6(su1006)]	**IS3142**
lin-40(ku285) V; oxIs12[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(su1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V	**IS3327**
lin-17(n671)I; edIs6[unc-119p::gfp; rol-6(su1006)] 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(+)]	**IS3329**
lin-17(n671)I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; otIs118[unc-33p::gfp; unc-4(+)]	**IS3330**
lin-17(n671)I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; juIs8 [unc-25p::gfp; lin-15(+)]	**IS3335**
jcIs1[ajm-1::gfp; rol-6(su1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V	**IS3339**
lin-17(n671) I; fpIs110[lin-26p::gfp; rol-6(su1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V	**IS3349**
lin-17(n671) I; jcIs1[ajm-1::gfp; rol-6(su1006)] IV; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V	**IS3357**
gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1111[lim-6int4::gfp; coel::dsred]	**IS3379**
lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1062[let-413a::gfp::pest; myo-2p::gfp]	**IS3383**
lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1111[lim-6int4::gfp; coel::dsred]	**IS3420**
gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; sox-2(syb737[gfp::linker::sox-2]) X	**IS3423**
wyIs75[unc-47p::dsred; exp-1p::gfp; odr-1p::rfp] III; vang-1(tm1422)X	**IS3433**
unc-73(e936) dpy-5(e61) I ; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V ; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3452**
lin-17(n671) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; sox-2(syb737[gfp::linker::sox-2]) X	**IS3457**
sem-4(n1971) I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; sox-2(syb737[gfp::linker::sox-2]) X	**IS3458**
dsh-1(ok1445) II; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3464**
egl-27(ok1670) II; wyIs75[unc-47p::dsred; exp-1p::gfp; odr-1p::rfp] III; him-5(e1490)V	**IS3469**
egl-5(n945) III; syIs50[cdh-3p::gfp; dpy-20(+)]	**IS3475**
egl-20(n585) IV ; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3485**
lin-44(n1792)I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3486**
lin-44(n1792) I ; egl-20(n585) IV ; gaIs245[col-34p::his-24::mCherry; unc-119(+)] V ; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3487**
gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; lim-6(nr2073) oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3490**
par-1(zu310) gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3491**
fmi-1(rh308) gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3511**
dsh-1(ok1445) mig-5(tm2639) II; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3512**
wyIs75[unc-47p::dsred; exp-1p::gfp; odr-1p::rfp] III; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; sox-2(syb737[gfp::linker::sox-2]) X; fpEx1156[egl-5p(6.5kb)::nanobodyGFP::zif-1; coel::gfp; pBSK]	**IS3521**

gpr-1(ok2126) III; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[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**
lin-17(n671) bxIs7[egl-5(6.5kb)::gfp; lin-15(+)] I; otIs173[rgef-1p::dsred2; ttx-3pB::gfp]III	**IS3583**
hT2[bli-4(e937) let-?(q782) qIs48] (I;III)/pop-1(q645)I ; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3596**
hT2[bli-4(e937) let-?(q782) qIs48] (I;III)/pop-1(q645)I; gaIs245[col-34p::his-24::mcherry; unc-119(+)] V; fpEx1111[lim-6int4::gfp; coel::dsred]	**IS3600**
otIs173[rgef-1p::dsred2; ttx-3pB::gfp]III; oxIs12[unc-47p::gfp; lin-15(+)] X	**IS3604**
lin-5(ev571)II; 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; oxIs12[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**

Table S3. Oligonucleotides list.

Oligo name	Sequence	Use
BDT950	CTGAATCCGGATCCATCATCATGTCGACTGCAGAATTCGAAGCTT GTCGACGGAGCTC	*sox-2* antisense construct
BDT952	CTTGGAGGGTACCTAGGAGCTCGATATCTAGAAGAGGTAACATG GGATTGGGA	*sox-2* antisense construct
EB110F	AGAAGACCGCCCCTCTTTTGA	Genotyping *ceh-6(syb972)*
EB110R	GGCTGCCTCCATCTCGTTCT	Genotyping *ceh-6(syb972)*
EB5F	TCCAGTCTCTTCAGGTCAGTGATCT	Genotyping *egl-27(ok1670)*
EB5R	CGAGATTTCCAAATTCTTACCCGACTG	Genotyping *egl-27(ok1670)*
EB6R	GTGTAATTGACAGCGATGATGATGAAGG	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	GAACTACAACTACTTTGGTCAACCATTGGGCCCTGCCACGTTTCC CCCAT	Genotyping *egl-5(n945)*, digest w/ NcoI
mcm124R	CGTAAGATAGCATATAGGGTCAGACG	Genotyping *egl-5(n945)*, digest w/ NcoI
mcm125F	CCGCGCCATTGACACCGATTTGGTAC	Genotyping *sem-4(n1971)*, digest w/ Acc65I
mcm125R	CCTAACAAGCTAGCCTTTTCAGTTACAAAAACATCTCTCTTAACTG GGTA	Genotyping *sem-4(n1971)*, digest w/ Acc65I
oCG347 rev début GFP	CCACTGACAGAAAATTTGTGCCC	Genotyping *sem-4 (syb1287)* and sequencing
oCG368 sens PEST	CTTAGCCATGGCTTCCCGCCGGCGGTGGCGGCGCAGGATGATG GCACGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGATGGACCGT CACCCTGCAGCCTGTGCTTCTGCTAGGATCAAT	PEST sequence fw
oCG369 rev PEST	ATTGATCCTAGCAGAAGCACAGGCTGCAGGGTGACGGTCCATCC CGCTCTCCTGGGCACAAGACATGGGCAGCGTGCCATCATCCTGC GCCGCCACCGCCGGCGGGAAGCCATGGCTAAG	PEST sequence rv
oCG370 sens MW PEST	GGATTACACATGGCATGGATGAACTATACAAACTTAGCCATGGCT TCCCGCCGGCGGTGGC	Cloning *pest* sequence into pML801
oCG371 rev MW PEST	GGTAGCGACCGGCGCTCAGTTGGAATTCTACGAATGCTACACATT GATCCTAGCAGAAGCACAGGCTG	Cloning *pest* sequence into pML801
oCG381 lin-26p f	CAACTTGGAAATGAAATAAGCTTGCATGGATCCGACGTCTTCCCA TTGTCTTCCATTCATCTT	Cloning of the lin-26 rectal specific promoter into pPD97.82
oCG382 lin-26p r	GCTGAAAAGTGTCTAGAGTCGACCAAGGCCTGCAGCTGAAAATAA TCAATTAAAAATTTTAAAAAAAGTAAGCGAGGG	Cloning of the lin-26 rectal specific promoter into pPD97.82
oCG390 for NLS1 kpnI	AGGGTACCGAGCTCAGAAAAAATGACAGC	Cloning *2nls* into pPD95.75
oCG391 rev GFP XhoI	GGGTATCTCGAGAAGCATTGAACACCATAACAGAAAG	Cloning *2nls* into pPD95.75
oCG411 sens egr-1 ku285	GCCCCAAAAGCCTGAAAAAGCCCAAAATTTCTCAATTTCCA	Genotyping *egr-1(ku285)*, digest w/ Hpy188III
oCG412 rev egr-1 ku285	GACGTCTCCGCAGAAGCTTCGGTGGC	Genotyping *egr-1(ku285)*, digest w/ Hpy188III
oCG444 lim-6 3int sens	GGATACGCTAACAACTTGGAAATGAAATAGGCGCCCTTCTTGAGA TTGCG	Cloning *lim-6 intron4* into pPD95.75
oCG445 lim-6 3int rev	CGACCTGCAGGCATGCAAGCTAAAGATTGACATATTGGAGACATC TGCC	Cloning *lim-6 intron4* into pPD95.75
oCG461 sens sox-2 CRISPR	GGTTGTCTTTTGCAGTGTCCGG	Genotyping *sox-2(syb737)*

oCG462 rev sox-2 CRISPR	CAGAGCCATTTTCTTCCGCTGTC	Genotyping *sox-2(syb737)*
oCG463 sens sem-4 CRISPR	GACGACGAATCTTCGATGTGGC	Genotyping *sem-4 (syb1287)*
oCG464 rev sem-4 CRISPR	GGGGGAAAGAGGGAAAATTAGCTG	Genotyping *sem-4 (syb1287)*
oCG556 sens HMG	GACAGCCCAGATCTGGGTACCCAAGGAGGTGGAAAAGCGAAGA	cloning of POP-1 HMG
oCG557 rev HMG	GACGGAGCTCGAATTCGGATCCTTAACTCTTATCCCTTCGTTTCTTCG	cloning of POP-1 HMG
oCR017 ceh-6 fw	GGCGGATGCAAGATTTTACG	Genotyping *ceh-6(gk665)*
oCR018 ceh-6 rv wt	GGATGACGACGAAGGTATGAG	Genotyping *ceh-6(gk665)*
oCR019 ceh-6 rev gk665	CTGTGACAATGTTCCCGGAG	Genotyping *ceh-6(gk665)*
oCR029 fw ceh-18(mg57)	CCCACACCAGTTTCCACAAATGGC	Genotyping *ceh-18(mg57)*
oCR030 rv ceh-18(mg57)	AGGCTAGAAAGTTCTACGGG	Genotyping *ceh-18(mg57)*
oCR036 rv ceh-18 wt	GCTCGCCGCCTCAATTCTTGAT	Genotyping *ceh-18(mg57)*
oCR061 fw AfeI EpiDeg	GAGGGTACCAGAGCTCAAGCGCTATTCACCTGGCACCGACTAC	Cloning AfeI and XhoI sites into pOD1988
oCR062 rv XhoI EpiDeg	CCAGACTCCACCAGTTGGACTTGATCCATCTCGAGTTATCTGGAACAAAATGTAAG	Cloning AfeI and XhoI sites into pOD1988
oCR073 AscI nanob	ATAAAAGGCGCGCCAAAAAATGGATCAAGTCCAACTGGT	Cloning *nanobodyGFP::zif-1* into pSJ671
oCR074 U54 ApaI	GTAATAGGGCCCTTAACCCTCACTAAAGGGAACAAAA	Cloning *nanobodyGFP::zif-1* into pSJ671
oCR075 tm1422 fw	GGGCCAGAAGATTGCACCAC	Genotyping *vang-1(tm1422)*
oCR076 tm1422 rv wt	GCATGCTGAAGCCGAAACGT	Genotyping *vang-1(tm1422)*
oCR077 tm1422 rv	CGCAATCGGTAGAATTGAAAATTTCGG	Genotyping *vang-1(tm1422)*
oCR087 nr2073 fw	GTAATGCGCGAAGCTTCCTG	Genotyping *lim-6(nr2073)*
oCR088 nr2073rv wt	GGGAGCCTATAGGTCAGCTCT	Genotyping *lim-6(nr2073)*
oCR089 nr2073 rv	CCTCCGCTTGGAAGGACAAAA	Genotyping *lim-6(nr2073)*
oCR092 rh308 fw	GTGATAATGCTCGTATTGTCTATTCCATTGATTCCTAT	Genotyping *fmi-1(rh308)*, digest w/ AseI
oCR093 rh308 rv	GTGGATGAGATCCGCCGTCAG	Genotyping *fmi-1(rh308)*, digest w/ AseI
oCR094bis	CCTCTTAAAAACTTACCTCTCAAATTTGAACTTATTCAAGC	Genotyping *egl-20(n585)*, digest w/ HindIII
oCR095 n585 rv	GAACATTGGCATTTGTGGGTTCAAAC	Genotyping *egl-20(n585)*, digest w/ HindIII
oCR096 n1792 fw	CTTCAAAACTGTGCGAATCGTTTGAGATTTCAGCCCT	Genotyping *lin-44(n1792)*, digest w/ AvrII
oCR097 n1792 rv	CCTTTTGACCCTACCCGCCGAAC	Genotyping *lin-44(n1792)*, digest w/ AvrII
oCR105 zu310 fw	CCCACATTCATCCATCGATCTTTCATAAT	Genotyping *par-1(zu310)*, digest w/ SspI
oCR106 zu310 rv	GTCTCTGCTGTTCAATATTTGCATTCG	Genotyping *par-1(zu310)*, digest w/ SspI
oCR107 ok2126fw wt	CTGAACTGCCTGCTGCCAGA	Genotyping *gpr-1(ok2126)*
oCR108 ok2126rv	CACGAAAGTCATCAACGTATGTAGTAAAG	Genotyping *gpr-1(ok2126)*
oCR109 ok2126fw mu	CCAAGGCTCGACGGTTTGC	Genotyping *gpr-1(ok2126)*
oCR113 ga80 fw	GCATAGTGAGTTCTGGAATTGCTCGAACTGTGTTATACTGCCC	Genotyping *bar-1(ga80)*, digest w/ BclI
oCR114 ga80 rv	CATCCATGGCCGACTATGAGCCGATCCCCACTCTTTCTGAT	Genotyping *bar-1(ga80)*, digest w/ BclI

oCR122 q645 fw	CGATGGATTTCGACCGGCACC	Genotyping *pop-1(q645),* digest w/ ClaI
oCR123 q645 rv	GATATAAAAATACACAAAAATGATGGCCGACGAAGAGCTCATCGA	Genotyping *pop-1(q645),* digest w/ ClaI
oCR128 n1378 fw	CAACACCGAATCCAAAAAACGAAAATCCACTGCTTGGCATG	Genotyping *sem-4(n1378),* digest w/ SphI
oCR129 n1378 rv	CCACGAGTTGTGAATGCGCGTCCAC	Genotyping *sem-4(n1378),* digest w/ SphI
oCR138 syb971fw	GACATTCGAAGCTCTGATGATG	Genotyping *lim-6(syb971)*
oCR139 syb971rv wt	GTGCAAAGATTAGAGCTCTGAC	Genotyping *lim-6(syb971)*
oCR140 syb971rv mu	GGGTATCTCGAGAAGCATTG	Genotyping *lim-6(syb971)*
oCR144 n1051 fw	CACTACAGAGTTATGGCAAACATCGACTACCTCTCGTTCCCAT	Genotyping *lin-18(n1051),* digest w/ NcoI
oCR145 n1051 rv	CCTGTCGCAATTTCACTTTCAACGGCTC	Genotyping *lin-18(n1051),* digest w/ NcoI
oCR149 gm122 fw	GACCACGATTTACTTCGGCAACG	Genotying *cam-1(gm122),* digest w/ BclI
oCR150 gm122 rv	CATCATATGTATAAAGTTTGCGAATCGGATTCTAATGAT	Genotying *cam-1(gm122),* digest w/ BclI
oCR151 q544 fw	CCTGTTGGCGGAGGAGGTTGATCATGTGG	Genotyping *sys-1(q544),*digest w/ AflIII
oCR152 q544 rv	GGCAAAAAGATCCTCACATGAAACACTGCGCAAATCACGT	Genotyping *sys-1(q544),*digest w/ AflIII
oCR153 n671	CCGCATTTTTCGTAGATCACACC	Sequencing *lin-17(n671)*
oCR154 n671	CGAGCACATTCCACAGAAGATG	Sequencing *lin-17(n671)*
oCR155 lin-17p fw	CTGAAGCTTACACTTTGTTCGCTC	Cloning *lin-17p* reporter
oCR156 lin-17p rv	CGGCTGCAGTTTGGAGAAGGAGCCAGTCTCTC	Cloning *lin-17p* reporter
oCR157 wrm-1 fw	GATGTTCTTCCGACTGAATGC	Sequencing and genotyping *wrm-1(ne1982ts)*
oCR158 wrm-1 rv	CTTGTGCTCCACCCATTTG	Sequencing and genotyping *wrm-1(ne1982ts)*
pLG7F	ACGCGTCGACGTGAAAACATAGTGTTTCCCAGTAC	*sox-2* antisens construct
pLG7R	GCTCTAGAGATATTATACATATTTCATAAAGCCAAC	*sox-2* antisens construct
oSKS-233	ATGAACTATACAAAGCTTCGAATTCTGCAGTCGACA TGTCTTCTGCTCGTCGTTGC	To amplify cki-1 for construction of pSJ1108
oSKS-234	ATTCATGCATAGGCCTGCGGCCGCGCTAGCCTAGTAT GGAGAGCATGAAGATCGAGTTC	To amplify cki-1 for construction of pSJ1108
oSKS-235	ATGGACTACAAGGACGACGATGACAAGTAAGGATCC GAATTCGAGCTCC	To insert Flag at the C-terminal of hmg-pop1 in pSJ769, for construction of pSJ1107
oSKS-236	ACTCTTATCCCTTCGTTTCTTCGTC	To insert Flag at the C-terminal of hmg-pop1 in pSJ769, for construction of pSJ1107
oSKS-237	AACCAAAGTTCTCACTGTCAGAACCA	To delete intron from cki-1 for construction of pSJ1112
oSKS-238	CTGTAGAACTCCGGAACACAATTCTCT	To delete intron from cki-1 for construction of pSJ1112
psj6094sox-2 F	TCGACATGATGATGGATCCGGATTCAGC	*6XHis::sox-2* construct
psj6094sox-2 R	GTGCGGCCGCAAGCTTGGTACC	*6XHis::sox-2* construct