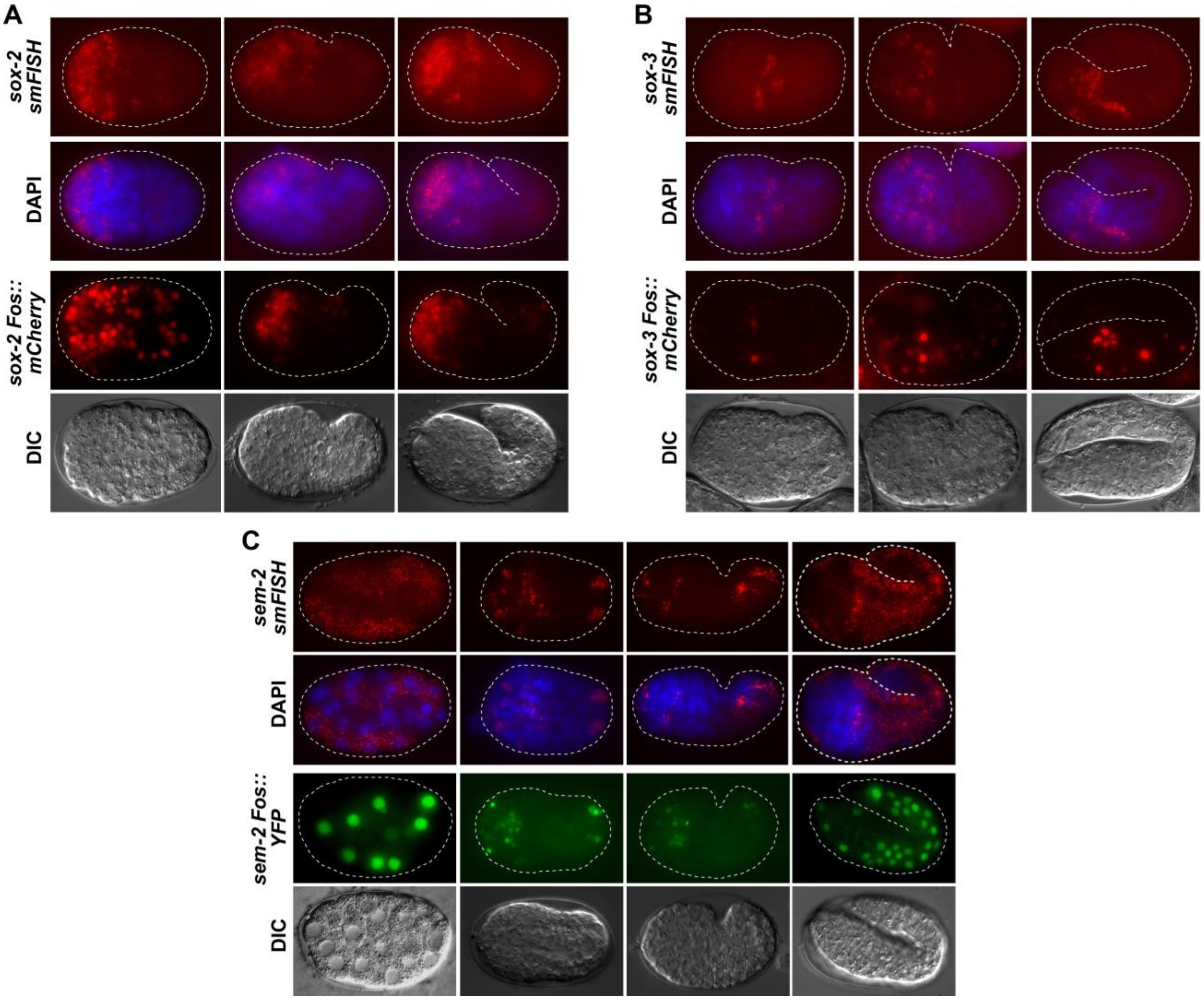
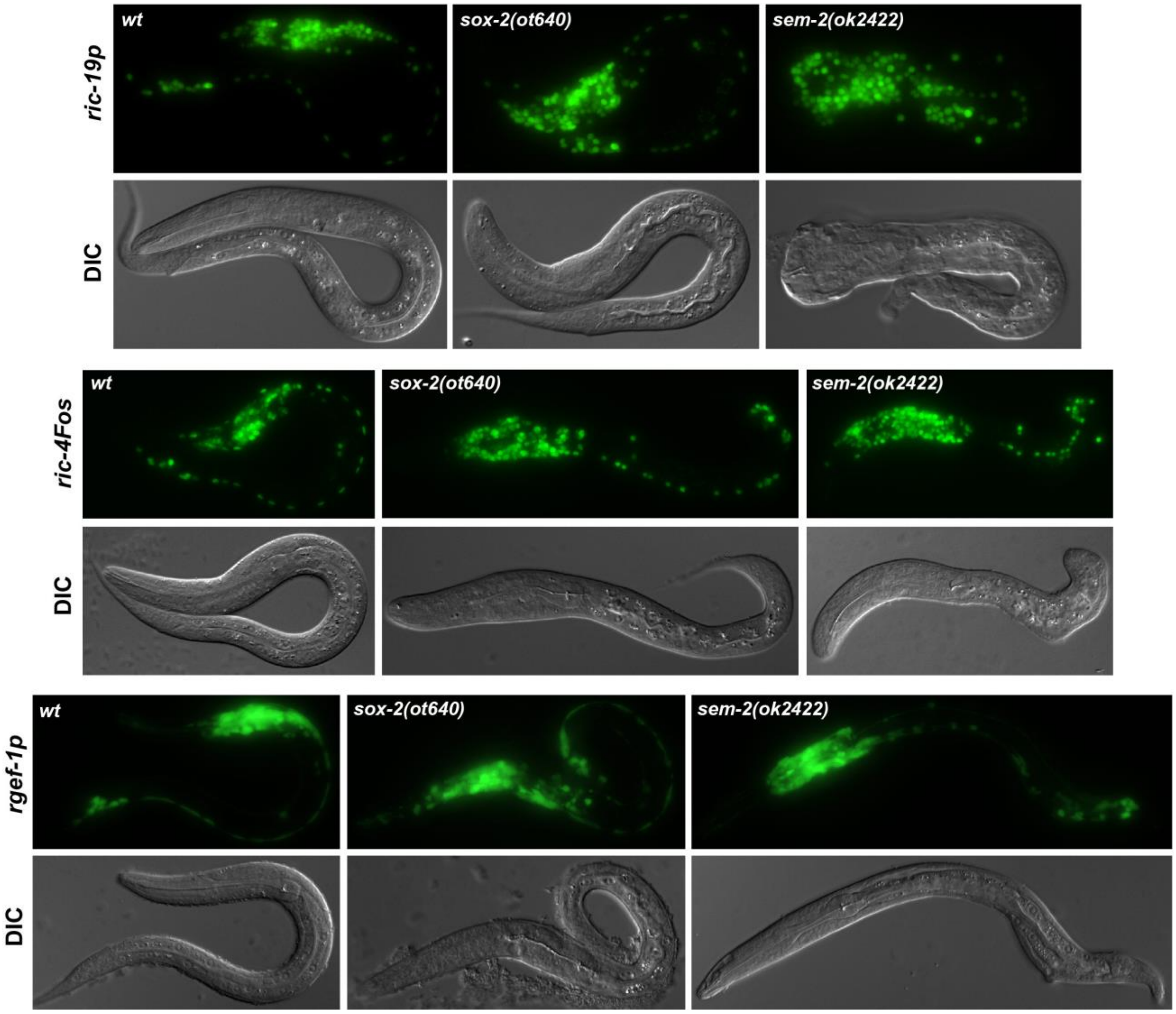
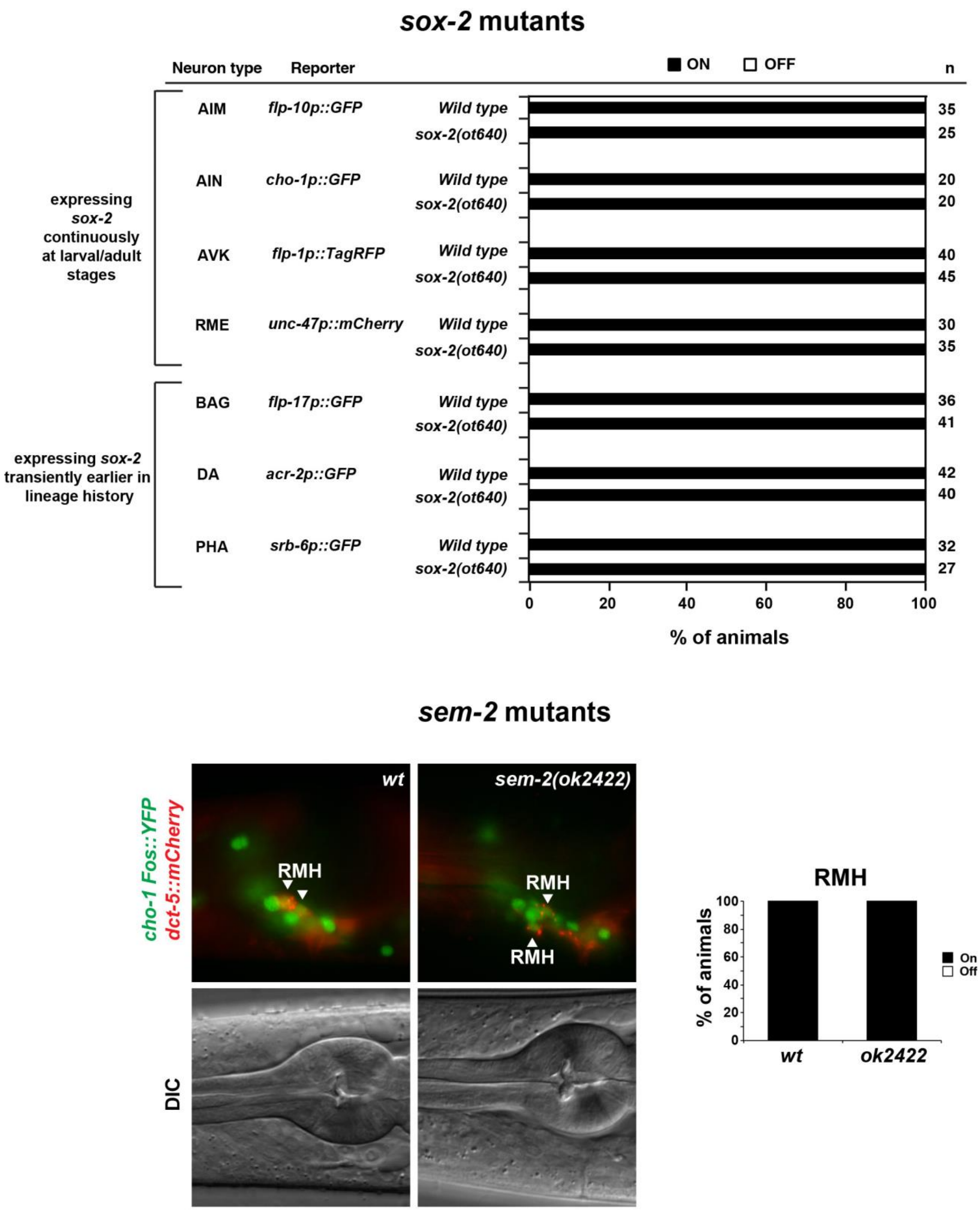


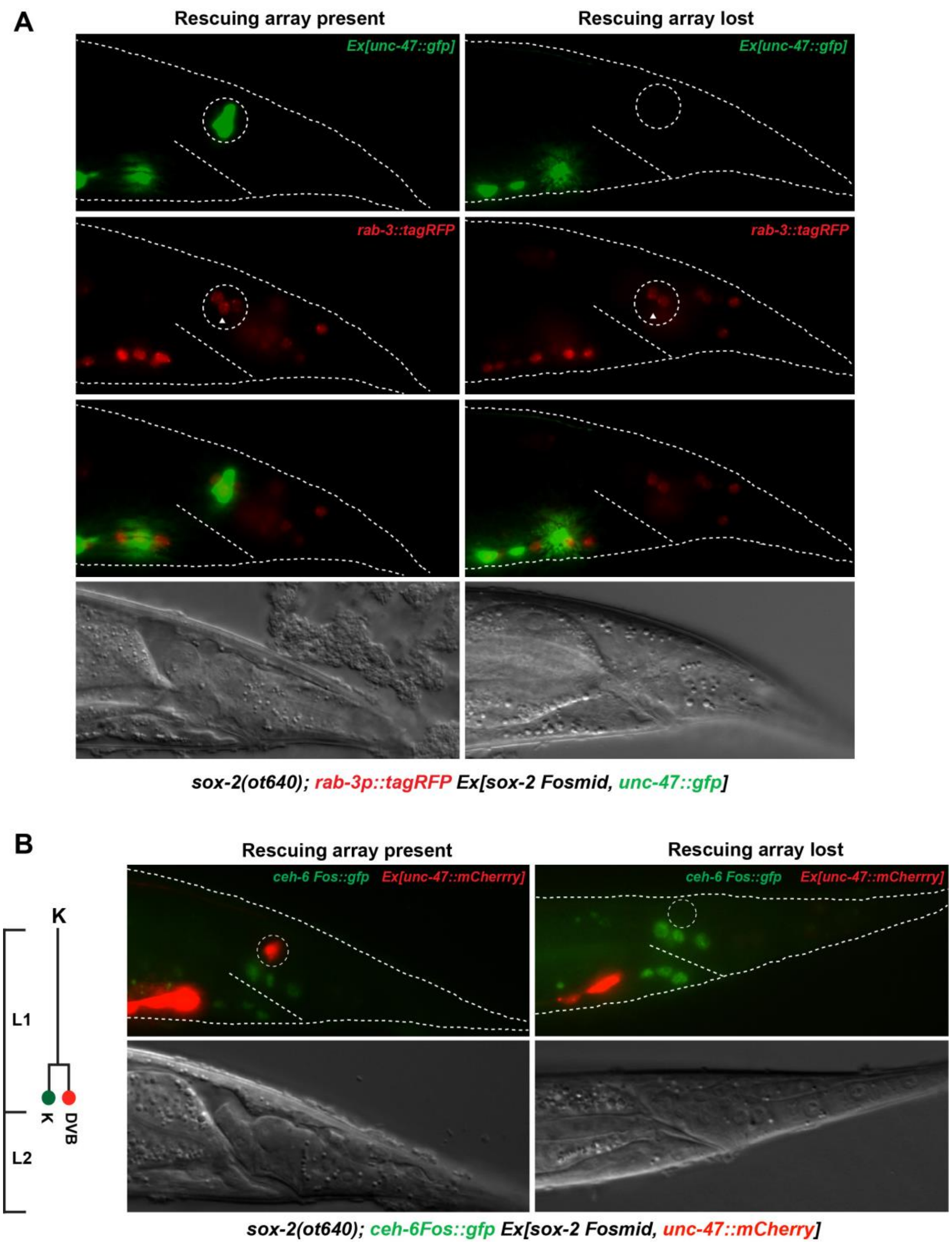
Supplementary Figure 1: soxB/C fosmid-based reporter genes largely recapitulate endogenous gene expression. smFISH against *sox-2* (A), *sox-3* (B) and *sem-2* (C) was performed at different embryonic stages in order to detect the endogenous transcript. Expression patterns obtained by smFISH were very similar to the ones observed with fosmid reporters. smFISH was done as previously described (Ji and van Oudenaarden, 2012). All sets of probes were designed by using the Stellaris RNA FISH probe designer and were obtained, already conjugated and purified, from Biosearch Technologies. All probes were conjugated to Quasar 670.



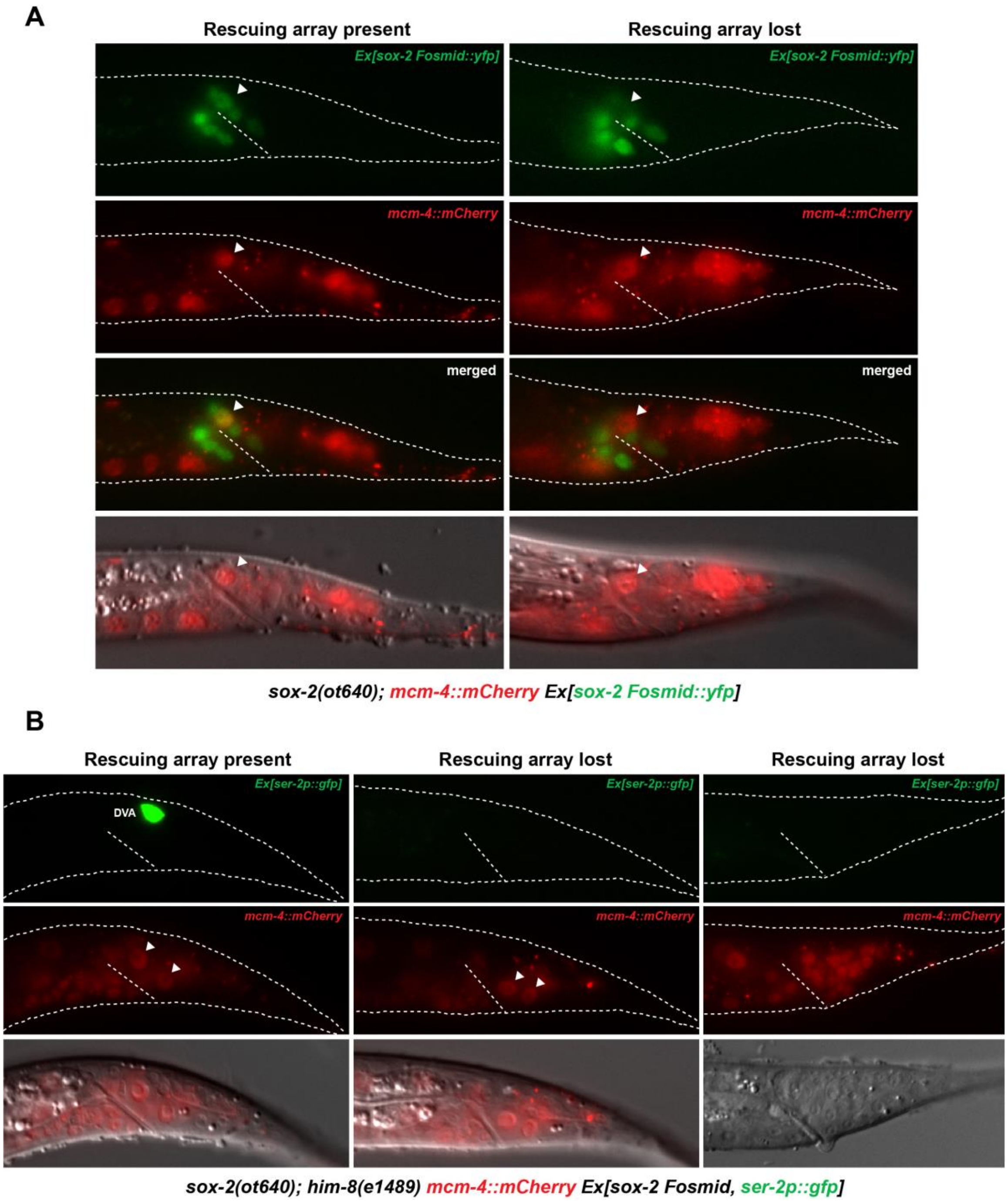
Supplementary Figure 2: Embryonic neurogenesis is unaffected in SoxB and SoxC mutants. Expression of the panneuronal genes *ric-19, ric-4 and rgef-1* is not affected in SoxB and SoxC mutants at the L1 stage. *sox-2* mutant animals analyzed are maternal/zygotic null.



Supplementary Figure 3: Expression of other markers in *sox-2* and *sem-2* mutants.

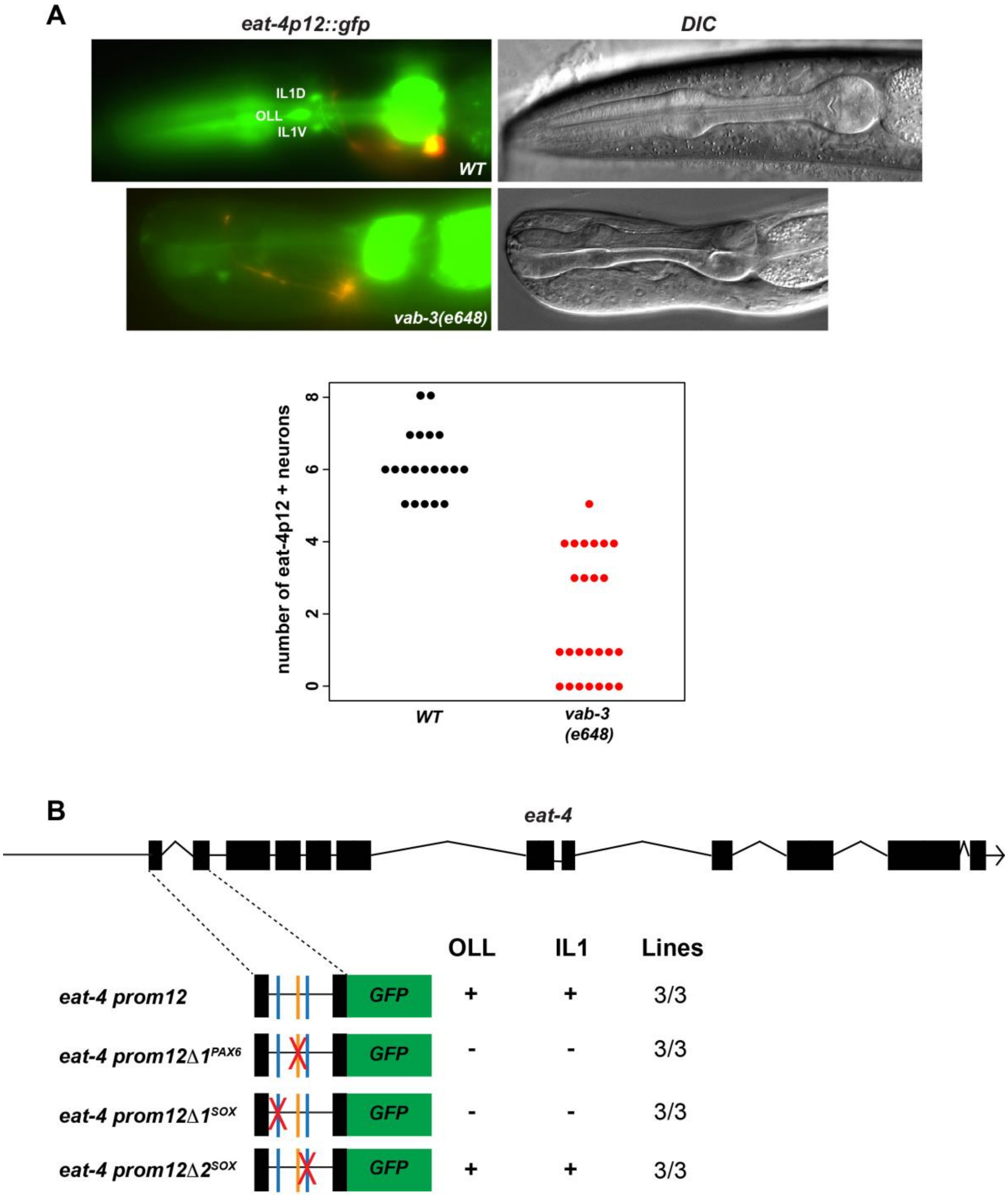


Supplementary Figure 4: Mosaic analysis shows K lineage defects in *sox-2* mutants. (A) The DVB motorneuron is not generated in *sox-2* mutants. L1-lethal *sox-2(ot640)* animals were rescued with an extrachromosomal array (Ex) containing a fosmid with the *sox-2* locus and the DVB reporter *unc-47p::GFP* to be able to follow the array in the K lineage. For phenotypic output, the expression of the integrated panneuronal marker *rab-3::tagRFP* was analyzed. The dashed circle shows the dorsorectal ganglion, which is formed by the DVA, DVB and DVC neurons in *wt* animals. In *sox-2* mosaic animals the dorsorectal ganglion shows only two neurons (DVA and DVC) since DVB is not generated. (B) The DVB neuron does no adopt the fate of its sister cell in *sox-2* mutants. A scheme of the postembryonic K lineage is shown on the left. L1-lethal *sox-2(ot640)* animals were rescued with an extrachromosomal array (Ex) containing a fosmid with the *sox-2* locus and the DVB reporter *unc-47p::mCherry* to be able to follow the array in the K lineage. For phenotypic output, the expression of the integrated K marker *ceh-6Fos::GFP* was analyzed.



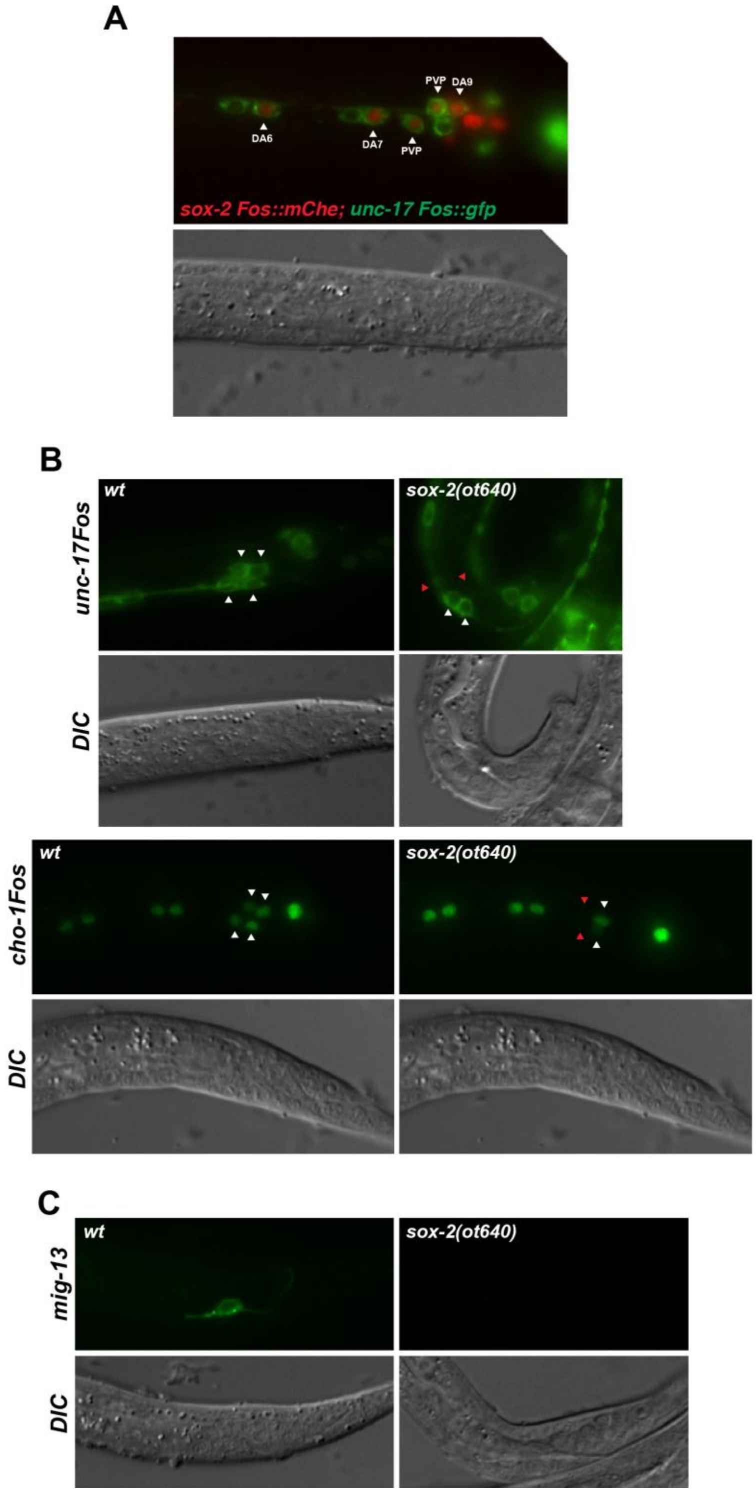
Supplementary Figure 5: Mosaic analysis shows that rectal epithelial blast cells can enter the cell cycle in the absence of *sox-2*. (A) The K rectal epithelial cell enters the cell cycle in *sox-2* mutants. L1-lethal *sox-2(ot640)* animals were rescued with an extrachromosomal array (Ex) containing a *yfp* tagged *sox-2* fosmid to be able to follow the array in the K lineage. For phenotypic output, the expression of the integrated *mcm-*

4::mCherry reporter which marks dividing cells was analyzed. (B) The B rectal epithelial cell enters the cell cycle in *sox-2* mutants. Males were generated by crossing the *sox-2(ot640) mutant* into a *him-8(e1489)* mutant background. L1-lethal *sox-2(ot640)* animals were rescued with an extrachromosomal array (Ex) containing a fosmid with the *sox-2* locus and the DVA (sister of B) reporter *ser-2p::GFP* to infer the presence or absence of the rescuing array in the B lineage. For phenotypic output, the expression of the integrated *mcm-4::mCherry* reporter which marks dividing cells was analyzed. The left and middle panels show B.a and B.p while the left panel shows a later stage.



Supplementary Figure 6: *sox-2* and *vab-3* specify OLL and IL1 fate. (A) *vab-3* affects the expression of eat-4/VGLUT in OLL and IL1. *eat-4* reporter gene was crossed into *vab-3(e648)* null mutant. The total number of cells expressing *eat-4p::gfp* was counted in *wt* and *vab-3(e648)* animals and represented in the lower graph. Animals were scored at the L4 or adult stage, N >20. (B) *eat-4prom12* expression in the OLL and IL1 neurons is abolished upon mutagenesis of the MatInspector predicted pax-6 motif

(+177 to +195) and the predicted sox motif 1 (+128 to +134). Mutagenesis of the predicted sox motif 2 (+196 to +201) does not effect expression of this reporter. Sox predicted motifs followed the consensus sox binding site (Narasimhan et al., 2015). Motifs were deleted with the QuickChangeII XL Site-Directed Mutagenesis Kit (Stratagene). Constructs were injected at 50 ng/μl with *unc-122::GFP* as coinjection marker. The resulting transgenic arrays are listed in the Supplementary table. All strains were scored as young adults.



Supplementary Figure 7: *sox-2(ot640)* mutants show defects in PVP and DA9 terminal differentiation. (A) *sox-2* is expressed in DA6, DA7, DA9 and PVP neurons at the L1 stage but its expression is not maintained at adult stages. (B) The expression of terminal differentiation markers of the cholinergic interneuron PVP is affected in *sox-2* mutants. Cholinergic fate reporter genes were crossed into *sox-2(ot640)* null mutants. Representative pictures are shown. Animals were scored at the first larval stage, N >20. (C) Expression of the DA9-specific marker *mig-13* is affected in *sox-2* mutants. DA9 cholinergic fate does not seem to be affected in the absence of *sox-2* as shown in panel B. Representative pictures are shown. Animals were scored at the first larval stage, N >20.

Supplementary references

Ji, N. and van Oudenaarden, A. (2012). Single molecule fluorescent in situ hybridization (smFISH) of C. elegans worms and embryos. WormBook: the online review of C. elegans biology, 1-16.

Narasimhan, K., Pillay, S., Huang, Y.H., Jayabal, S., Udayasuryan, B., Veerapandian, V., Kolatkar, P., Cojocaru, V., Pervushin, K. and Jauch, R. (2015). DNA-mediated cooperativity facilitates the co-selection of cryptic enhancer sequences by SOX2 and PAX6 transcription factors. Nucleic Acids Research 43, 1513-1528.

Supplementary Table S1

Strains and transgenes	Reference
Mutants:	
unc-119(ed3)III; unc-119(+) sox-2(ot640)/sox-2(+) X	This study
sox-2(ot640) X otEx4454	This study
sox-3(ok510) X	Kindly provided by the C. elegans knockout consortium.
sem-2(ok2422) I/hT2[bli-4(e937) let-?(q782) qIs48] (I;III)	Kindly provided by the C. elegans knockout consortium.
sem-2(ok2422) I otEx5881	This study
him-8(e1489) IV	Phillips, C. M., Wong, C., Bhalla, N., Carlton, P. M., Weiser, P., Meneely, P. M., & Dernburg, A. F. (2005). HIM-8 binds to the X chromosome pairing center and mediates chromosome-specific meiotic synapsis. Cell, 123, 1051-63.
vab-3(e648) X	Chisholm, A. D., & Horvitz, H. R. (1995). Patterning of the Caenorhabditis elegans head region by the Pax-6 family member vab-3. Nature, 377, 52-5.
Integrated transgenes:	
ynIs64 [flp-17p::GFP] I	Kim, K., and Li, C. (2004). Expression and regulation of an FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. The Journal of comparative neurology *475*, 540-550.
eIs34 [mab-9::GFP] III	Woollard, A. C., & Hodgkin, J. A. (2000). The caenorhabditis elegans fate-determining gene mab-9 encodes a T-box protein required to pattern the posterior hindgut. Genes Dev, 14, 596-603.
otIs333 [sox-2 fosmid::mCherry, pRF4 rol-6(su1006)] II	This study
gmIs12 [srb-6p::GFP, pRF4 rol-6(su1006)] III	Hawkins, N.C., Ellis, G.C., Bowerman, B., and Garriga, G. (2005). MOM-5 frizzled regulates the distribution of DSH-2 to control C. elegans asymmetric neuroblast divisions. Developmental biology *284*, 246-259.
otIs181 [dat-1p::mCherry, ttx-3p::mCherry] III	Flames, N., and Hobert, O. (2009). Gene regulatory logic of dopamine neuron differentiation. Nature *458*, 885-889.
otIs221 [cat-1p::GFP] III	Flames, N., and Hobert, O. (2009). Gene regulatory logic of dopamine neuron differentiation. Nature *458*, 885-889.
otIs388 [eat-4 fosmid::SL2::YFP::H2B, pha-1(+)] III	Serrano-Saiz, E., Poole, R.J., Felton, T., Zhang, F., De La Cruz, E.D., and Hobert, O. (2013). Modular control of glutamatergic neuronal identity in C. elegans by distinct homeodomain proteins. Cell *155*, 659-673.
ynIs66 [flp-7p::GFP] IV	Kim, K., and Li, C. (2004). Expression and regulation of an FMRFamide-related neuropeptide gene family inCaenorhabditis elegans. *J. Comp. Neurol. 475, 540-550*
otIs348 [unc-47p::mCherry, pha-1(+)] IV	Kindly provided by Marie Gendrel.
juIs14 [acr-2p::GFP, lin-15(+)] IV	Hallam, S., Singer, E., Waring, D., and Jin, Y. (2000). The C. elegans NeuroD homolog cnd-1 functions in multiple aspects of motor neuron fate specification. Development (Cambridge, England) *127*, 4239-4252.
ynIs34 [flp-19p::GFP] IV	Kim, K., and Li, C. (2004). Expression and regulation of an FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. The Journal of comparative neurology *475, 540-550*.
kuIs34 [sem-4::GFP, unc-119(+)] IV	Jarriault, S., Schwab, Y., and Greenwald, I. (2008). A Caenorhabditis elegans model for epithelial-neuronal transdifferentiation. Proceedings of the National Academy of Sciences of the United States of America *105*, 3790-3795.

heIs45 [mcm-4::mCherry, unc-119(+)] IV	Korzelius, J., The, I., Ruijtenberg, S., Portegijs, V., Xu, H., Horvitz, H.R., van den Heuvel, S. (2011). C. elegans MCM-4 is a general DNA replication and checkpoint component with an epidermis-specific requirement for growth and viability. Developmental Biology *350, 358-369.*
otIs92 [flp-10p::GFP] V	Mehta, N., Loria, P.M., and Hobert, O. (2004). A genetic screen for neurite outgrowth mutants in Caenorhabditis elegans reveals a new function for the F-box ubiquitin ligase component LIN-23. Genetics *166*, 1253-1267.
otIs291 [rab-3p::2xnlsYFP, pRF4 rol-6(su1006)] V	Kindly provided by Inés Carrera.
otIs381 [ric-19p::2xnlsGFP, elt-2::dsRed] V	Kindly provided by Inés Carrera.
otIs396 [ace-1p2::NLS::tagRFP, pha-1(+)] V	Serrano-Saiz, E., Poole, R.J., Felton, T., Zhang, F., De La Cruz, E.D., and Hobert, O. (2013). Modular control of glutamatergic neuronal identity in C. elegans by distinct homeodomain proteins. Cell *155*, 659-673.
ynIs49 [flp-5p::GFP] V	Kim, K., and Li, C. (2004). Expression and regulation of an FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. The Journal of comparative neurology *475*, 540-550.
muIs42 [mig-13::GFP, dpy-20(+)] X	Sym, M., Robinson, N., Kenyon, C. (1999). MIG-13 positions migrating cells along the anteroposterior body axis of C. elegans. Cell 98, 25-36.
otIs138 [ser-2p3::GFP, pRF4 rol-6(su1006)] X	Tsalik, E.L., Niacaris, T., Wenick, A.S., Pau, K., Avery, L., and Hobert, O. (2003). LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the C. elegans nervous system. Developmental biology *263*, 81-102.
oxIs12 [unc-47p::GFP] X	McIntire, S.L., Reimer, R.J., Schuske, K., Edwards, R.H., and Jorgensen, E.M. (1997). Identification and characterization of the vesicular GABA transporter. Nature *389*, 870-876.
otIs356 [rab-3p::2xnlsTagRFP]	Kindly provided by Inés Carrera.
ynIs21 [flp-3p::GFP, pRF4 rol-6(su1006)]	Kim, K., and Li, C. (2004). Expression and regulation of an FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. The Journal of comparative neurology *475*, 540-550.
otIs334 [sox-3 fosmid::mCherry, pRF4 rol-6(su1006)]	This study
otIs313 [sem-2 fosmid::YFP, pRF4 rol-6(su1006)]	This study
otIs452 [flp-1p::miniSOG, slp-1p::TagRFP]	Kindly provided by Abhishek Bhattacharya.
otIs502 [hlh-2 fosmid::yfp, myo-3p::mCherry]	Kindly provided by Neda Masoudi.
jcIs1 [ajm-1p::GFP, pRF4 rol-6(su1006)]	Koppen, M., Simske, J.S., Sims, P.A., Firestein, B.L., Hall, D.H., Radice, A.D., Rongo, C., and Hardin, J.D. (2001). Cooperative regulation of AJM-1 controls junctional integrity in Caenorhabditis elegans epithelia. Nature cell biology *3*, 983-991.
vtIs1 [dat-1p::GFP, pRF4 rol-6(su1006)]	Nass, R., Hahn, M.K., Jessen, T., McDonald, P.W., Carvelli, L., and Blakely, R.D. (2005). A genetic screen in Caenorhabditis elegans for dopamine neuron insensitivity to 6-hydroxydopamine identifies dopamine transporter mutants impacting transporter biosynthesis and trafficking. Journal of neurochemistry *94*, 774-785.
ynIs82 [flp-12p::GFP]	Kim, K., and Li, C. (2004). Expression and regulation of an FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. The Journal of comparative neurology *475*, 540-550.
otIs353 [ric-4 fosmid::SL2::YFP::H2B, pha-1(+)]	Kindly provided by Nikos Stefanakis.
otIs379 [cho-1p::GFP, pRF4 rol-6(su1006]	Zhang, F., Bhattacharya, A., Nelson, J.C., Abe, N., Gordon, P., Lloret-Fernandez, C., Maicas, M., Flames, N., Mann, R.S., Colon-Ramos, D.A., Hobert, O. (2014). The LIM and POU homeobox genes ttx-3 and unc-86 act as terminal selectors in distinct cholinergic and serotonergic neuron types. Development 141, 422-435.
otIs440 [lad-2p::GFP, pha-1(+)]	This study

otIs534 [cho-1 fosmid::SL2::NLS::H2B::YFP]	Kindly provided by Nikos Stefanakis.
otIs544 [cho-1 fosmid::SL2::NLS::H2B::mCherry, pha-1(+)]	Kindly provided by Laura Pereira.
otIs548 [unc-46p::GFP, pha-1(+)]	Kindly provided by Marie Gendrel.
otIs513 [unc-25::GFP, pha-1(+)]	Kindly provided by Marie Gendrel.
otIs576 [unc-17 fosmid::GFP, lin-44p::YFP]	Kindly provided by Laura Pereira.
otIs604 [eat-4prom12::GFP, ttx-3p::mCherry]	This study
evIs111 [(rgef-1p::GFP, dpy-20(+)]	Altun-Gultekin, Z. F., Andachi, Y., Tsalik, E. L., Pilgrim, D., Kohara, Y., & Hobert, O. (2001). A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in C. elegans. Development, 128, 1951-69.
wgIs87 [ceh-6 fosmid:: TY1::EGFP::3xFLAG, unc-119(+)]	Sarov, M., Schneider, S., Pozniakovski, A., Roguev, A., Ernst, S., Zhang, Y., Hyman, A.A., and Stewart, A.F. (2006). A recombineering pipeline for functional genomics applied to Caenorhabditis elegans. Nat. Methods *3*, 839–844.
wgIs54 [egl-5 fosmid::TY1::EGFP::3xFLAG, unc-119(+)]	Sarov, M., Schneider, S., Pozniakovski, A., Roguev, A., Ernst, S., Zhang, Y., Hyman, A.A., and Stewart, A.F. (2006). A recombineering pipeline for functional genomics applied to Caenorhabditis elegans. Nat. Methods *3*, 839–844.
wgIs171 [egl-38::TY1::EGFP::3xFLAG, unc-119(+)]	Sarov, M., Schneider, S., Pozniakovski, A., Roguev, A., Ernst, S., Zhang, Y., Hyman, A.A., and Stewart, A.F. (2006). A recombineering pipeline for functional genomics applied to Caenorhabditis elegans. Nat. Methods *3*, 839–844.
wgIs82 [ceh-16 fosmid::TY1::EGFP::3xFLAG, unc-119(+)]	Sarov, M., Schneider, S., Pozniakovski, A., Roguev, A., Ernst, S., Zhang, Y., Hyman, A.A., and Stewart, A.F. (2006). A recombineering pipeline for functional genomics applied to Caenorhabditis elegans. Nat. Methods *3*, 839–844.
Extrachromosomal arrays:	
otEx4454 [sox-2 fosmid::mCherry, elt-2p::DsRed]	This study
otEx4609 [sox-2 fosmid::mCherry, myo-3p::mCherry]	This study
otEx4877 [sox-2 fosmid, unc-47p::GFP]	This study
otEx4876 [sox-2 fosmid, ser-2p2::GFP]	This study
otEx4878 [sox-2 fosmid, unc-47p::mCherry]	This study
otEx4302 [sra-9p::GFP, pRF4 rol-6(su1006)]	Goldsmith, A.D., Sarin, S., Lockery, S., and Hobert, O. (2010). Developmental control of lateralized neuron size in the nematode Caenorhabditis elegans. Neural development *5*, 33.
otEx5321 [sox-2 fosmid, rab-3p::2xnlsTagRFP]	This study
otEx5881 [sem-2 fosmid::YFP, myo-3p::mCherry]	This study
otEx4306-4309 [egl-13 fosmid::mCherry, pRF4 rol-6(su1006)]	This study
otEx4424-4426 [sox-4 fosmid::mCherry, pRF4 rol-6(su1006)]	This study
otEx5883 [rig-5::SL2::GFP, pha-1(+)]	This study
otEx6281-6283 [eat-4prom12::GFP, unc-122::GFP]	This study
otEx6284-6286 [eat-4prom12Δ^{PAX6}*::GFP, unc-122::GFP]*	This study
otEx6287-6289 [eat-4prom12$\Delta 1^{SOX}$ *::GFP, unc-122::GFP]*	This study
otEx6290-6291 [eat-4prom12$\Delta 2^{SOX}$*::GFP, unc-122::GFP]*	This study