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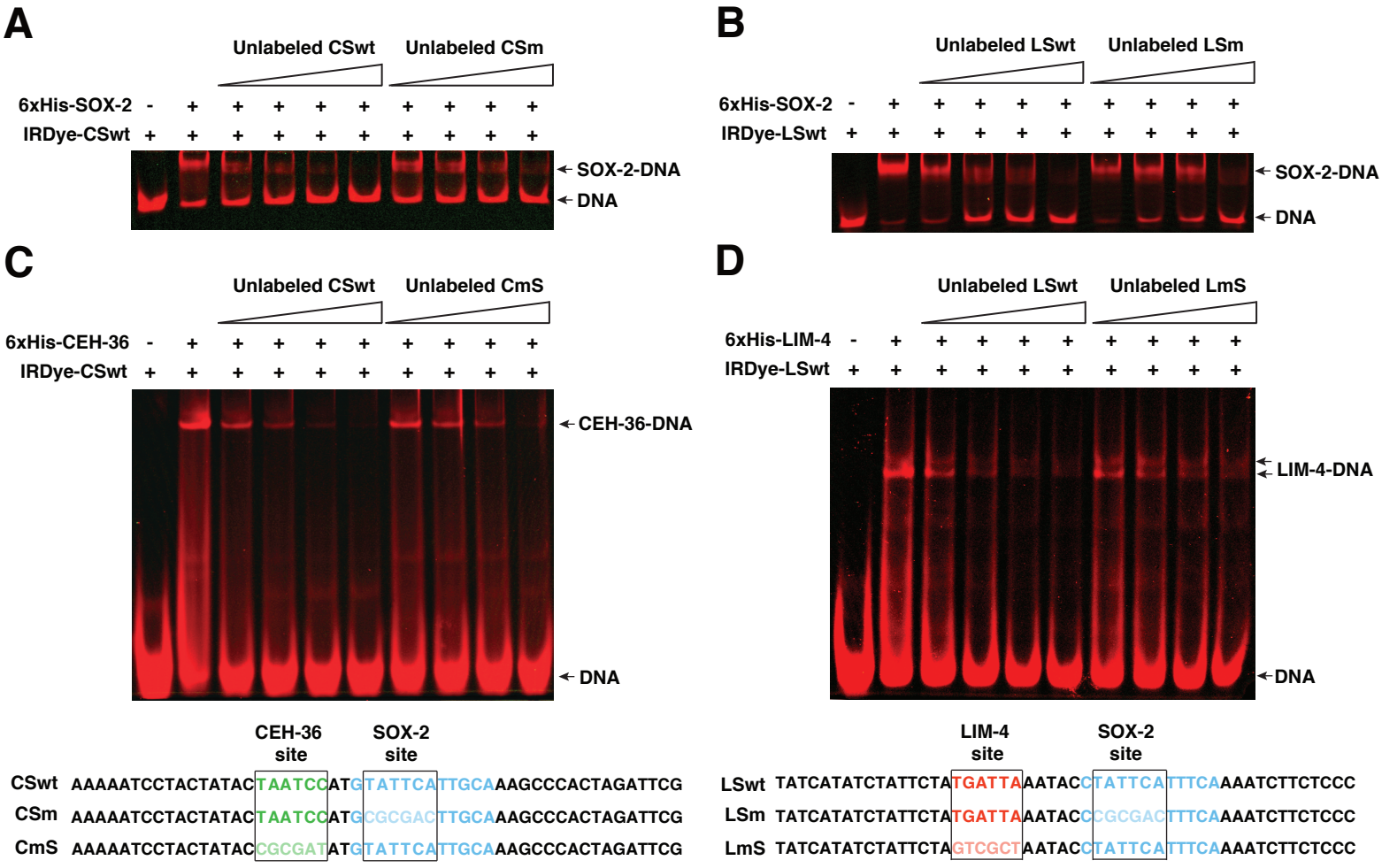
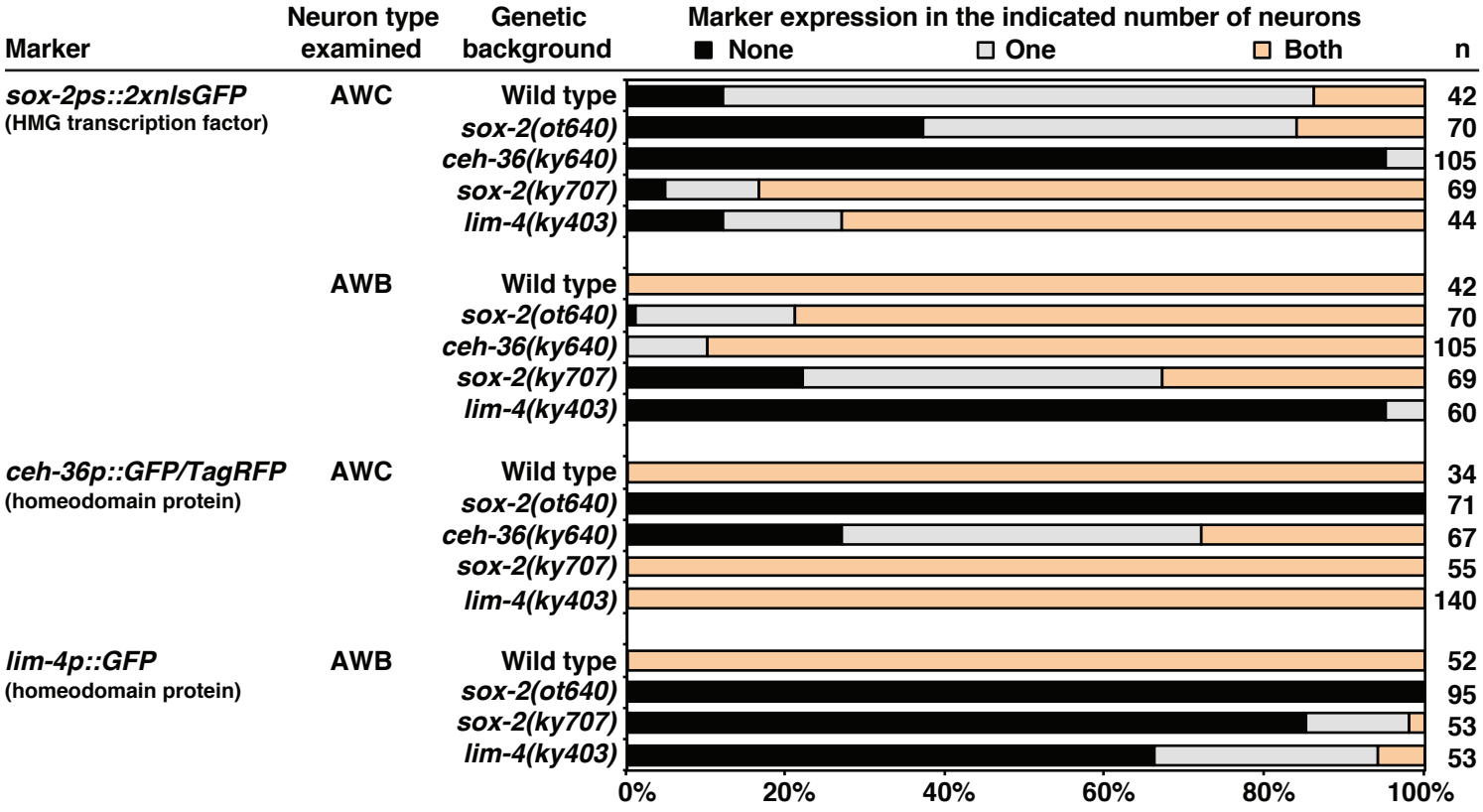
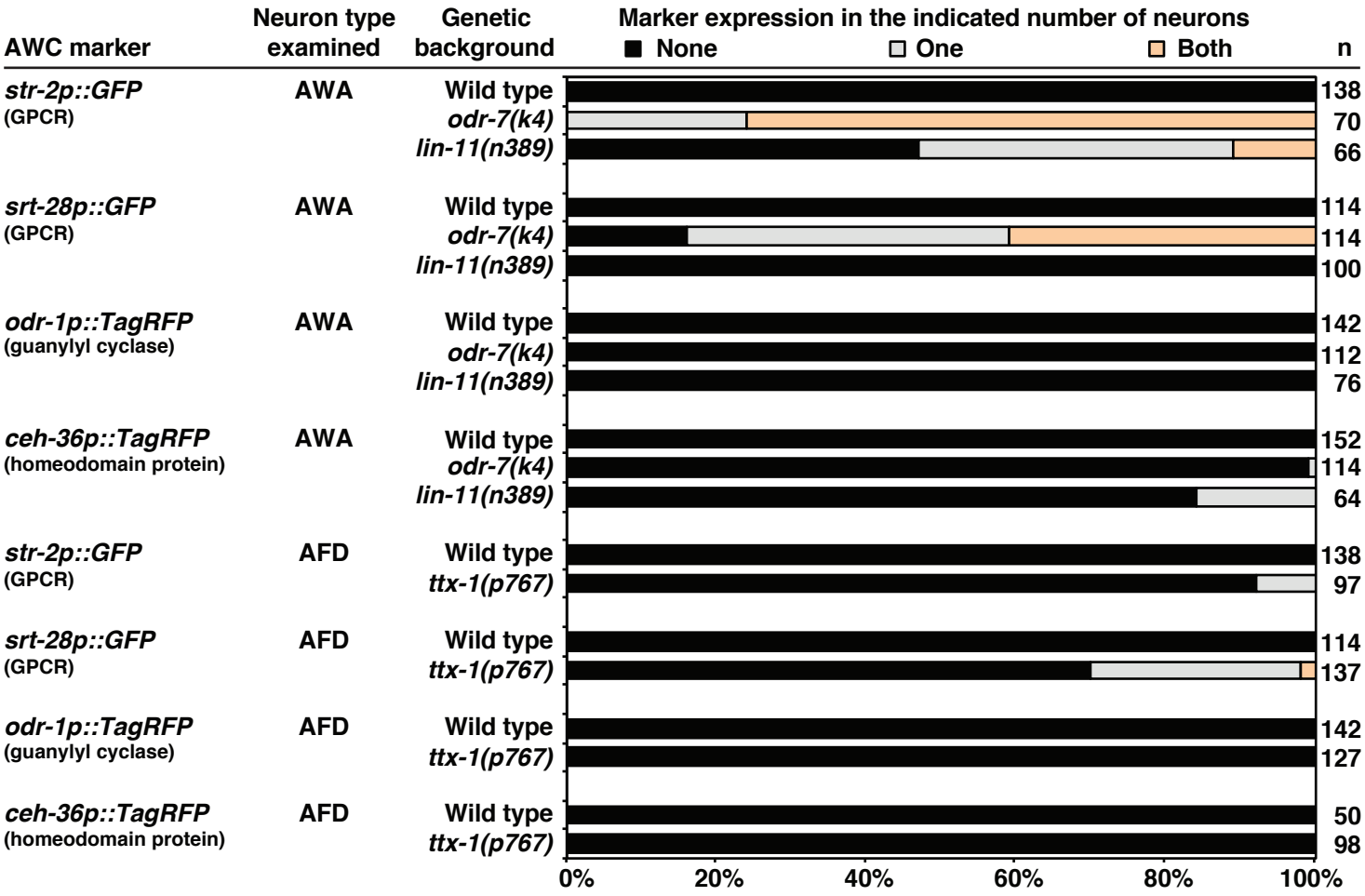
Appendix Figure S1



Appendix Figure S2



Appendix Figure S3



Appendix Figure Legends

Appendix Figure S1. SOX-2, CEH-36, and LIM-4 specifically bind to CEH-36/SOX-2 and LIM-4/SOX-2 sites.

(A) EMSA assays of 6xHis-tagged SOX-2 with an IRDye-labeled CSwt probe containing CEH-36/SOX-2 adjacent sites. 2x, 5x, 10x, or 25x cold competitor was added in lanes 3-6 (CSwt) and lanes 7-10 (CSm with a mutated SOX-2 site).

(B) EMSA assays of 6xHis-tagged SOX-2 with an IRDye-labeled LSwt probe containing LIM-4/SOX-2 adjacent sites. 2x, 5x, 10x, or 25x cold competitor was added in lanes 3-6 (LSwt) and lanes 7-10 (LSm with a mutated SOX-2 site).

(C) EMSA assays of 6xHis-tagged CEH-36 with an IRDye-labeled CSwt probe. 5x, 7.5x, 10x, or 15x cold competitor was added in lanes 3-6 (CSwt) and lanes 7-10 (CmS with a mutated CEH-36 site).

(D) EMSA assays of 6xHis-tagged LIM-4 with an IRDye-labeled LSwt probe. 1x, 2x, 3x, or 4x cold competitor was added in lanes 3-6 (LSwt) and lanes 7-10 (LmS with a mutated LIM-4 site).

Appendix Figure S2. Expression of *sox-2*, *ceh-36*, and *lim-4* markers in *sox-2*, *ceh-36*, and *lim-4* mutants

Animals were scored in the first larval stage. n = total number of animals.

Appendix Figure S3. Expression of AWC markers in *odr-7*, *lin-11*, and *ttx-1* mutants. Animals were scored in the adult stage. n = total number of animals.

Appendix Supplemental Materials and Methods

Strains and transgenes

Balanced mutant

sox-2(ot640null); otEx4454 [*sox-2 fosmid::mCherry; elt-2p::DsRed*] (Vidal et al, 2015)

Mutants

nsy-5(ky634) I (Chuang et al, 2007)

lin-11(n389) I (Ferguson & Horvitz, 1985)

nsy-1(ky542) II (Sagasti et al, 2001)

tir-1(tm3036) III (Chang et al, 2011)

tir-1(ky648gf) III (Chang et al, 2011)

unc-36(e251) III (Brenner, 1974)

unc-43(n498gf) IV (Park & Horvitz, 1986)

nsy-4(ky627) IV (Vanhoven et al, 2006)

nsy-7(ky630) IV (Lesch et al, 2009)

unc-76(e911) V (Bloom & Horvitz, 1997)

ttx-1(p767) V (Satterlee et al, 2001)

mls-2(cc615) X (Jiang et al, 2005)

odr-1(n1936) X (Bargmann et al, 1993)

odr-7(ky4) X (Sengupta et al, 1994)

sox-2(ky707) X (this study)

lim-4(ky403) X (Sagasti et al, 1999)

ceh-36(ky640) X (Lanjuin et al, 2003)

Integrated transgenes

Integrated transgenes	Figures
kyIs140 [*str-2p::GFP; lin-15(+)*] I (Troemel et al, 1999)	1,3,EV3,Appendix S3
kyIs408 [*str-2p::DsRed; srsx-3p::GFP; elt-2::GFP*] II (Lesch et al, 2009)	1,5
vyIs68 [*str-2p::TagRFP*; *srsx-3p::GFP*] III (Cochella et al, 2014)	5
vyIs56 [*odr-1p::TagRFP*] III	EV1,6,Appendix S3
mgIs18 [*ttx-3p::GFP*] IV (Altun-Gultekin et al, 2001)	EV1
ynIs66 [*flp-7p::GFP*] IV (Kim & Li, 2004)	EV1, EV4
vyIs59 [*str-2p::TagRFP; str-1p::GFP*] V	5
otIs3 [*gcy-7p::GFP; lin-15(+)*] V (Chang et al, 2003)	EV1

ntIs1 [*gcy-5p::GFP*] V (Chang et al, 2003)	EV1
oyIs14 [*sra-6p::GFP; lin-15(+)*] V (Sarafi-Reinach et al, 2001)	EV1
oyIs17 [*gcy-8p::GFP*] V (Satterlee et al, 2001)	EV1
kyIs84 [*srg-2p::GFP*] V	EV1
otIs92 [*flp-10p::GFP*] V (Mehta et al, 2004)	EV1
vyIs55 [*str-2p::TagRFP; str-1p::GFP*] X	1
mgIs19 [*lim-4p::GFP; pRF4 rol-6(su1006)*] X(Sagasti et al, 1999)	1,5, Appendix S2
vyIs66 [*sox-2ps::2xnlsGFP; ofm-1p::DsRed*] X	6,Appendix S2
vyIs64 [*odr-3p::2xnlsGFP; ofm-1p::DsRed*] X	EV4
otIs264 [*ceh-36p::TagRFP*] (Patel et al, 2012)	1,5,S7,Appendix S3
otIs185 [*ceh-36p::GFP::cog-1 3'; rol-6(su1006)*] (Sarin et al, 2007)	Appendix S1
otIs392 [*eat-4p6::GFP; ttx-3p::DsRed*] (Serrano-Saiz et al, 2013)	1,5,EV4
xrIs32 [*mec-4p::lin-14; odr-1p::GFP*]	5,EV4
kyIs170 [*srh-220::GFP; lin-15(+)*](Chang et al, 2006)	EV1
pkIs586 [*gpa-9p::GFP*] (Kim et al, 2010)	EV1
otIs393 [*ift-20p::2xnlsTagRFP*]	EV4
otIs356 [*rab-3p::2xnlsTagRFP*]	EV4

Extrachromosomal arrays

Extrachromosomal arrays	Figures
kyEx388 [*odr-3p::lim-4; pRF4 rol-6(su1006)*] (Sagasti et al, 1999)	1
vyEx1513 [*odr-3p::lim-4 (20ng/ul); ofm-1p::DsRed (30ng/ul)*]	1
vyEx2502, 2503, 2505 [*sox-2p::sox-2* (2 ng/µl); *odr-1p::DsRed* (15 ng/µl);	2,5,6
vyEx2562-2564 [*sox-2ps::sox-2 (3 ng/µl); odr-1p::DsRed (15 ng/µl); ofm-1p::DsRed (30 ng/µl)*]	2
vyEx2583-2585 [*sox-2ps::mSox2 (3 ng/µl); ofm-1p::DsRed (30ng/µl)*]	2
vyEx1736-1739 [*sox-2ps::mSox1 (3 ng/ul); ofm-1p::DsRed (30 ng/ul)*]	2
vyEx1740-1742 [*sox-2ps::mSox3 (3 ng/ul); ofm-1p::DsRed (30 ng/ul)*]	2
vyEx2501, 2506, 2507 [*sox-2p::sox-2^{G73E} (5 ng/µl); odr-1p::DsRed (15 ng/µl); ofm-1p::DsRed (30 ng/µl)*]	2

kyEx599 [*odr-3p::nsy-1(gf); myo-3p::DsRed*] (Chuang & Bargmann, 2005; Sagasti et al, 2001)	3
vyEx499 [*odr-3p::calbindin D28K (100ng/ul); odr-1p::DsRed (12ng/ul); ofm-1p::DsRed (30ng/ul)*] (Schumacher et al, 2012)	3
vyEx2521 [*odr-3p::sox-2 (5 ng/µl); odr-1p::DsRed (15 ng/µl); ofm-1p::DsRed (30 ng/µl)*]	5
vyEx2512, 2515, 2516 [*odr-3p::sox-2^{G73E}* (5 ng/µl); *odr-1p::DsRed* (15 ng/µl); *ofm-1p::DsRed* (30 ng/µl)]	5
vyEx2607, 2608, vy2612, vy2614 [*odr-3p::ceh-36 (30 ng/µl); ofm-1p::DsRed (30 ng/µl)*]	5
vyEx1396, 1397 [*odr-1p(-1027-1)::GFP (50ng/ul); ofm-1p::DsRed (30ng/ul)*]	7
vyEx1616, 1617 [*odr-1p(-393-1)::GFP (50ng/ul); ofm-1p::DsRed (30ng/ul)*]	7
vyEx1678, 1679 [*odr-1p(-393-1LmS)::GFP (50ng/ul); ofm-1p::DsRed*	7
vyEx1723, 1724 [*odr-1p(-393-1LSm)::GFP (50ng/ul); ofm-1p::DsRed*	7
vyEx1609, 1610 [*odr-1p(-393-170)::GFP (50ng/ul); ofm-1p::DsRed*	7
vyEx1632, 1643 [*odr-1p(-393-170CmS)::GFP (50ng/ul); ofm-1p::DsRed*	7
vyEx1650, 1657 [*odr-1p(-393-170CSm)::GFP (50ng/ul); ofm-1p::DsRed*	7
kyEx3187 [*srt-28p::GFP; elt-2p:;mCherry*] (Lesch & Bargmann, 2010)	EV1,Appendix S3
PY4881 [*sru-38p::GFP; ofm-1p::RFP*] (Nokes et al, 2009)	EV1,EV4
kyEx3208 [*odr-7p::GFP (15ng/ul); ofm-1p::DsRed (15ng/ul)*]	EV1
adEx1290 [*gcy-33p::GFP*] (Yu et al, 1997)	EV1
PY2978 [*srv-33p::RFP*] (Nokes et al, 2009)	EV1
kyEx329 [*srh-142p::GFP; pRF4 rol-6(su1006)*]	EV1

Plasmid construction

sox-2p::sox-2

A 7046 PCR fragment amplified from wild-type worm genomic DNA, containing 4756 bp of the *sox-2* promoter region, 1494 bp of the *sox-2* coding region, and 796 bp of the *sox-2* 3' UTR.

sox-2p::sox-2^{G73E}

A 7046 bp fragment amplified from *sox-2*(*ky707)* mutant genomic DNA.

sox-2ps::sox-2

A 5155 bp PCR fragment amplified from wild-type worm genomic DNA, containing 2865 bp of the *sox-2* promoter region, 1494 bp of the *sox-2* coding region, and 796 bp of the *sox-2* 3' UTR.

sox-2ps::mSox2

mSox2 was amplified from mouse brain cDNA and subcloned into a *sox-2ps* vector.

sox-2ps::mSox1

The *mSox1* fragment was amplified from pMXs-ms-Sox1 (Nakagawa et al, 2008), a gift from Shinya Yamanaka (Addgene plasmid # 50778), and cloned into a *sox-2ps* vector.

sox-2ps::mSox3

The *mSox3* fragment (NP_033263) was generated from postnatal day 1 mouse brain cDNA and pMXs-ms-Sox3 (Nakagawa et al, 2008), a gift from Shinya Yamanaka (Addgene plasmid # 50779), and cloned into a *sox-2ps* vector.

odr-3p::2xnlsGFP

A *str-2p::2xnls* DNA fragment was digested from *str-2p::2xnlsTagRFP* (Chang et al, 2011) and subcloned into a GFP vector to make *str-2p::2xnlsGFP*. A *2xnlsGFP* fragment was then digested from *str-2p::2xnlsGFP* and subcloned into an *odr-3p* vector (Roayaie et al, 1998).

sox-2ps::2xnlsGFP

A *2xnlsGFP* fragment was digested from *str-2p::2xnlsGFP* (see above) and subcloned into a *sox-2ps* vector.

odr-3p::sox-2

A 1491 bp of *sox-2* coding region was amplified from wild-type genomic DNA and subcloned into an *odr-3p* vector (Roayaie et al, 1998).

odr-3p::sox-2^{G73E}

sox-2^{G73E} coding regions were amplified from *sox-2*(*ky707)* mutant genomic DNA and subcloned into an *odr-3p* vector (Roayaie et al, 1998).

odr-3p::ceh-36

A 771 bp of *ceh-36* coding region was amplified from worm cDNA and subcloned into an *odr-3p* vector (Roayaie et al, 1998).

odr-3p::lim-4

A 1065 bp of *lim-4* coding region was amplified from worm cDNA and subcloned into an *odr-3p* vector (Roayaie et al, 1998).

CMVp::sox-2

An 849 bp of *sox-2* coding region was amplified from worm cDNA and cloned into pcDNA6 V5 6xHis A (Invitrogen).

CMVp::ceh-36

A 771 bp of *ceh-36* coding region was amplified from worm cDNA and cloned into pcDNA6 V5 6xHis A (Invitrogen).

CMVp::lim-4

A 1065 bp of *lim-4* coding region was amplified from worm cDNA and cloned into pcDNA6 V5 6xHis A (Invitrogen).

CMVp::sox-2^{G73E}

sox-2^{G73E} was generated by site directed mutagenesis of *sox-2* cDNA and subcloned into pcDNA6 V5 6xHis A.

odr-1p::luciferase

A 1027 bp PCR fragment of *odr-1* promoter was subcloned into the pGL4.23[luc2/minP] vector (Promega).

odr-1p::GFP

To make *odr-1p(-1027-1)::GFP*, the *mir-71* promoter of *mir-71p::GFP* (Hsieh et al, 2012) was replaced with a 1027bp *odr-1* promoter fragment amplified from N2 genomic DNA. Other GFP reporter constructs driven by shorter *odr-1* promoters were generated in a similar manner. Mutations of binding sites in the *odr-1* promoter GFP reporter constructs were generated using a QuikChange II XL site directed mutagenesis kit (Stratagene). All constructs were verified by DNA sequencing.

Germ line transformation

To generate transgenic strains, DNA constructs were injected into the syncytial gonad of adult worms as previously described (Mello & Fire, 1995).

Appendix Supplemental References

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