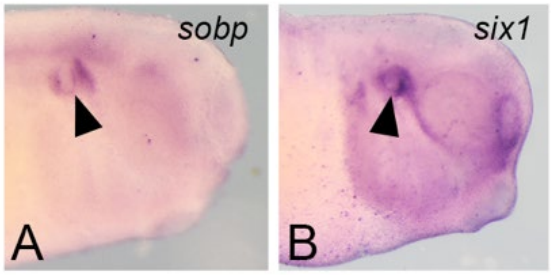

Fig. S1. In situ hybridization for *sobp* and *six1* at larval stages. Whole-mount view of embryos sectioned in Fig. 1C (*sobp*) and D (*six1*) showing expression in the otic vesicle (arrowheads)

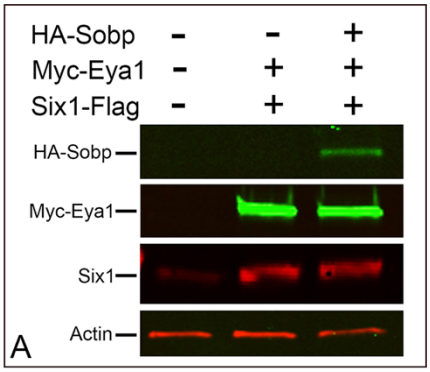

Fig. S2. Control multiplex fluorescence Western blot for luciferase assays. Constructs for HA-Sobp, Myc-Eya1 and Six1-Flag are properly expressed in HEK293T cells in different combinations tested in luciferase assays. Actin is used as loading control.

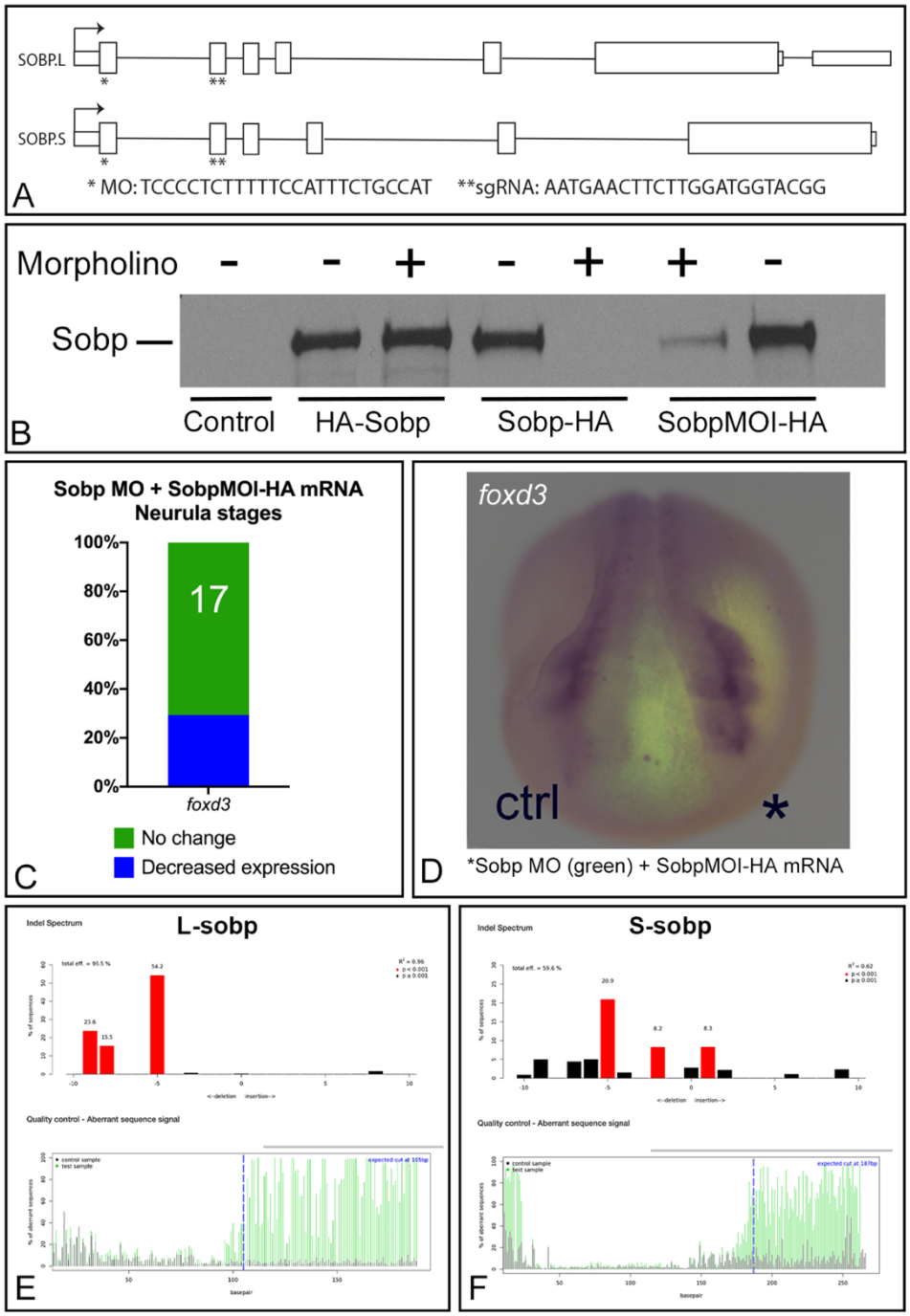

Fig. S3. Control experiments for *in vivo* studies using a translation-blocking antisense morpholino oligonucleotide (MO) against Sobp or F0 analysis after CRISPR/Cas9-mediated genome editing. A. Schematic representation of exons and introns in *Xenopus laevis* Sobp.L and Sobp.S genes. The MO binds at the ATG start site for both L- and S-homeologs. The sgRNA targets the L- and S-homeologs in the

second exon. **B.** Western blot detection of the HA tag showing the ability of the MO to block endogenous *sobp* translation (represented by Sobp-HA). The HA-*sobp* transcript is expected to avoid translation blockage because the 5'HA tag prevents MO binding at the translational start site. Translation of *sobp*-HA is expected to be blocked in the presence of the MO because the 3'HA tag does not interfere with MO binding. *sobpMOI*-HA is translated in the presence of MO because it has a deletion of the third codon in the *sobp* ORF (and a 3'HA tag) making it insensitive to a translation-blocking MO. **C.** Graph showing decrease in the frequency of *foxd3* reduction (29.4%) after partial rescue with unilateral injection of MO plus *sobpMOI*-HA mRNA (compare to 90.2% reduction in MO-only embryos). **D.** An embryo in which *foxd3* expression on the MO+*sobpMOI*-HA side (*) is similar to control side. **E-F.** Calculation of insertion/deletion frequencies with the TIDE software package in an injected embryo after CRISPR/Cas9 editing showing that sgRNA targets both homeologs at the predicted site.

Table S1. Summary of gene expression changes analyzed at neural plate stages

Gene	Sobp MO	Sobp CRISPR	Sobp mRNA	p.R651X mRNA
sox2	qPCR: no significant change ISH: broader, fainter	qPCR: no significant change ISH: broader, fainter	qPCR: no significant change ISH: no change in 67.5%	qPCR: no significant change ISH: no change in 71.4%*
foxd3	qPCR: decrease ISH: decrease	qPCR: decrease ISH: decrease	qPCR: decrease ISH: decrease in 39%; increase in 39%	qPCR: no significant change* ISH: decrease in 47.4%; increase in 7.9%*
six1	qPCR: decrease ISH: decrease	qPCR: decrease ISH: decrease	qPCR: decrease ISH: decrease in 86.9%	qPCR: decrease ISH: decrease in 95.0%
krt12.4	qPCR: no significant change ISH: decrease	qPCR: no significant change ISH: decrease	qPCR: increase ISH: no change in 54.2%; ectopic in 33.3%	qPCR: increase ISH: no change in 77.3%; ectopic in 19.7%*

* Significantly different compared to full-length sobp mRNA

Table S2. Summary of gene expression changes at larval stages

Gene	Sobp CRISPR	Sobp mRNA	p.R651x mRNA
six1	qPCR: no significant change ISH: no change in 68.6%; decrease in 25.7%	qPCR: no significant change ISH: no change in 43.3%; decrease in 50.0%	qPCR: no significant change ISH: no change in 51.3%; decrease in 35.9%
dlx5	qPCR: decrease ISH: decrease in 42.9%	qPCR: no significant change ISH: increase in 46.4%; decrease in 39.3%	qPCR: no significant change ISH: no change in 42.1%; decrease in 36.8% *
pax2	qPCR: decrease ISH: decrease in 54.0%	qPCR: increase ISH: increase in 59.5%; decrease in 16.2%	qPCR: no significant change ISH: no change in 56.7%; increase in 30.3% *

* ignificantly different compared to full-length sobp mRNA

Table S3. CRISPR primer and genotyping sequences

	Forward	Reverse
PCR: L-sobp	GGATTACGTTCAACCGGGC	CCCATCTGCATGATAGTTCC
PCR: S-sobp	GTGCCTTACTTTTGCCAATCC	CTTCCACTTCAGAACAAACC
Sequencing L	GCATGATGAACCCATACTCC	
Sequencing S	CACTTTCTAAAAGTCCTACT	

Table S4. qPCR primer sequences

	Forward	Reverse
dlx5	GGAGCGTATAACAGGGTGCA	CGTCTTTGTAACGCTGCGAG
foxd3	GAGGACATGTTCGACAATGG	CAAAGCTTTGCATCATGAGAG
krt12.4	CACCAGAACACAGAGTAC	CACCAGAACACAGAGTAC
odc	CATTGCAGAGCCTGGGAGATA	TCCACTTTGCTCATTCACCATAAC
pax2	ATCTGCGACAATGACACGGT	GGGTTGGATGGAATGGCTGT
six1	CAGGTCAGCAATTGGTTCAAG	CAGGTCAGCAATTGGTTCAAG
sobp	GCCTTCAAGAATAACTGCGAAC	TTGATTTAGACACTTTGCACTGC
sox2	TCACCTCTTCTTCCCATTCG	CGACATGTGCAGTCTGCTTT