supplementary material Fig. S1.

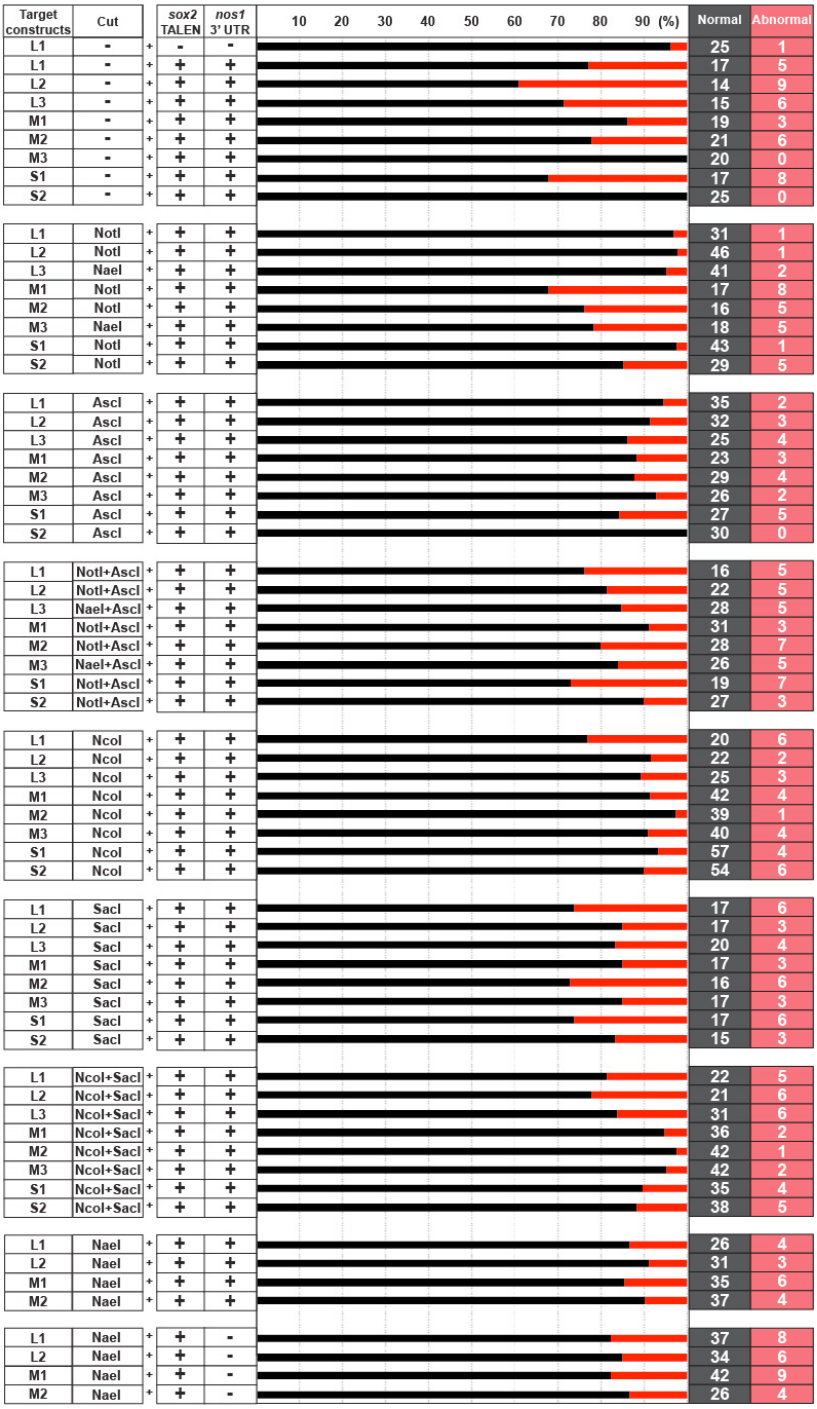

Fig. S1. Effect of TALEN and targeting construct co-injection. Graph depicting the percentage of normal (Black) and abnormal embryos (Red) in each co-injection condition. The constructs used and location of restriction sites are described in Fig. 2A-H.

supplementary material Fig. S2.

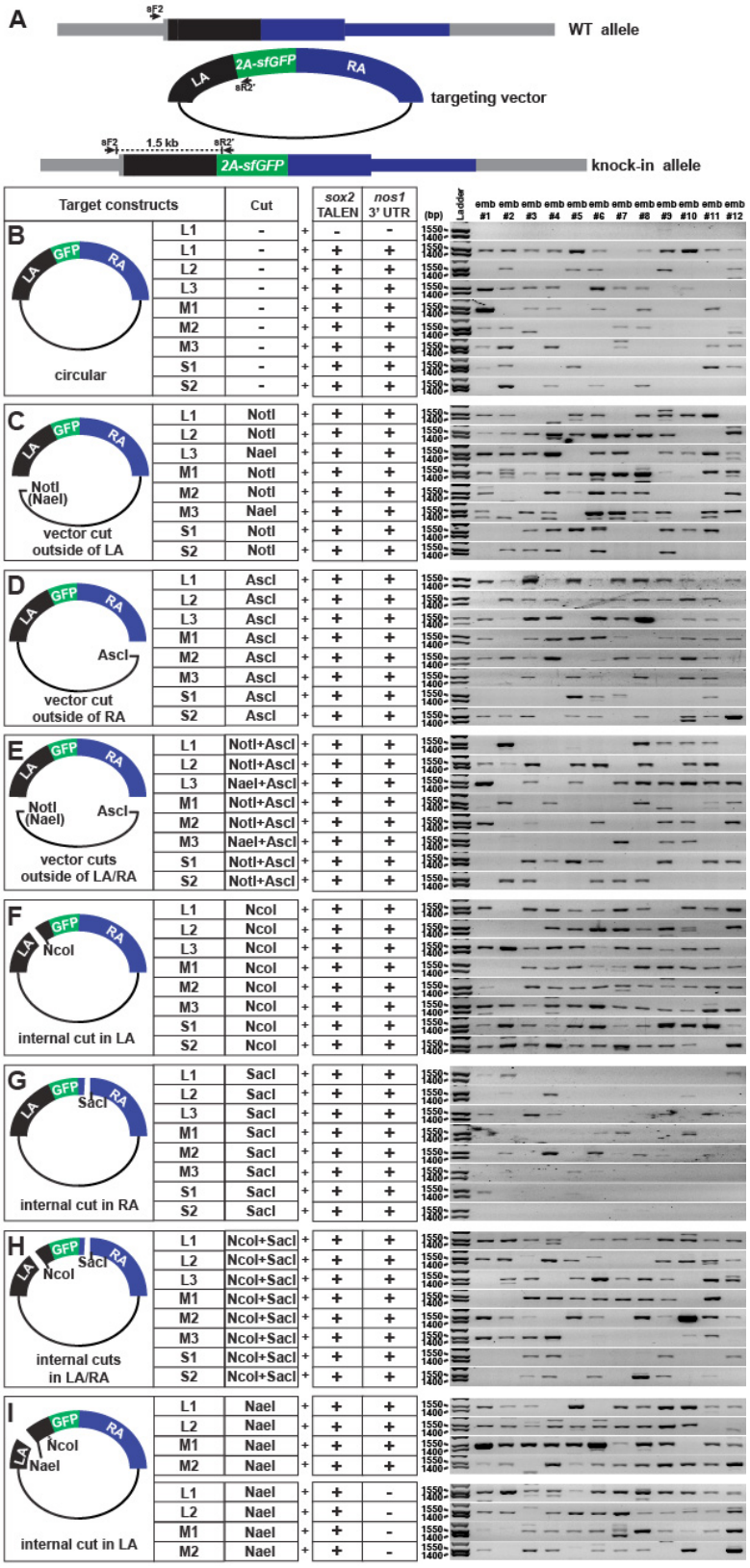

Fig. S2. PCR-based recombination detection assay. (A) Schematic representation of recombinant chromosome detection. A version of this data is shown in Fig. 2. Using a forward primer in the *sox2* gene just outside of the LA region (sF2) and a sfGFP-specific reverse primer (sR2'), the recombination event can be recognized by amplification of a 1.5 kb PCR product. (B-I) PCR-based recombination frequency analysis for each condition (12 embryos per each condition). Each lane represents a single embryo. Only 1.5 kb PCR products were counted as recombination-positive. (B) Circular form of targeting constructs. (C) NotI (NaeI)-digested targeting constructs. (D) AscI-digested targeting contructs. (E) NotI (NaeI)+AscI-digested targeting constructs. (F) NcoI-digested targeting constructs. (G) SacI-digested targeting constructs. (H) NcoI+SacI-digested targeting constructs. (I) Targeting constructs were digested with NaeI within the LA. *sox2* TALEN RNAs with or without *nos1* 3' UTR were used for each condition.

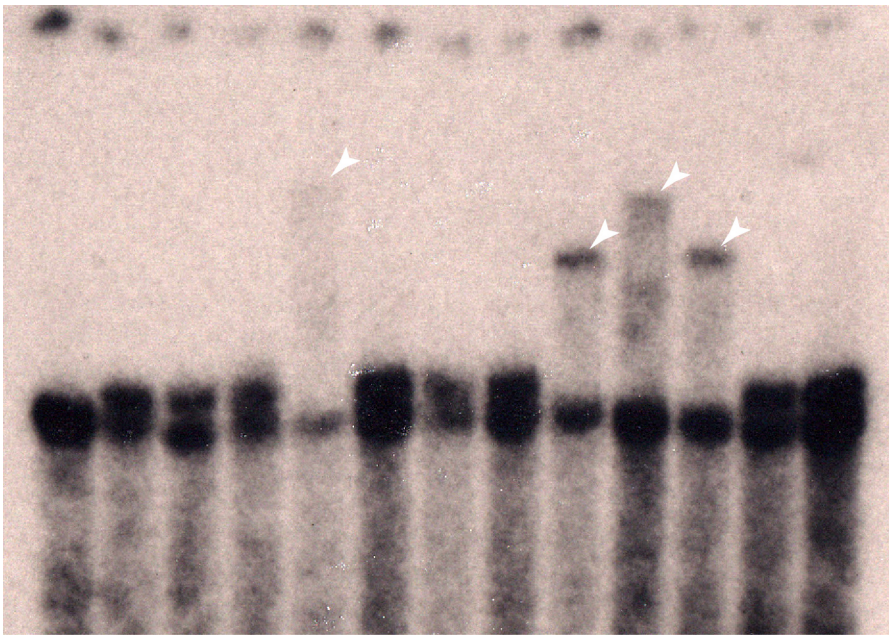

Fig. S3. Overexposure of the Southern blot analysis shown in Fig. 4B. A smear insertion band is detected with wild-type band in #4 line (see Results for detail).

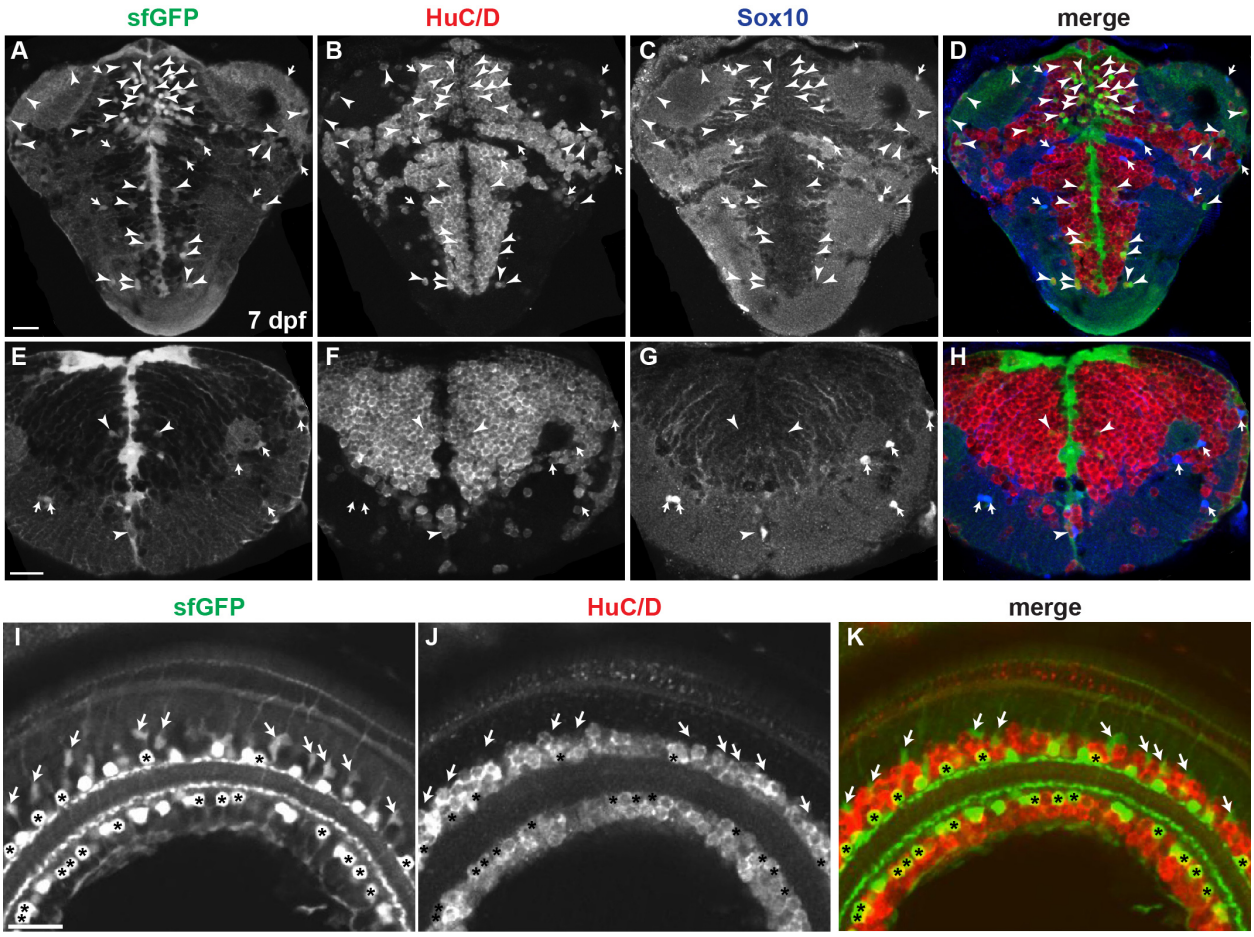

Fig. S4. Analysis of sfGFP-positive cells of *sox2-2a-sfGFP* line. Confocal microscope images. All images are transverse sections of 7 dpf *sox2-2a-sfGFP* larvae. (A-H) Arrowheads indicate HuC/D-positive, sfGFP-positive neurons. Arrows indicate Sox10-positive, sfGFP-positive OPCs. (A-D) Most sfGFP-positive cells are located in periventricular zones of the telencephalon and diencephalon. (E-H) Most sfGFP-positive cells are located in ventricular zones of the hindbrain. (I-K) Asterisks mark HuC/D-positive, sfGFP-positive cells that are putative amacrine cells. Arrows indicate HuC/D-negative, sfGFP-positive cells that are resembled as Müller glia morphologically.

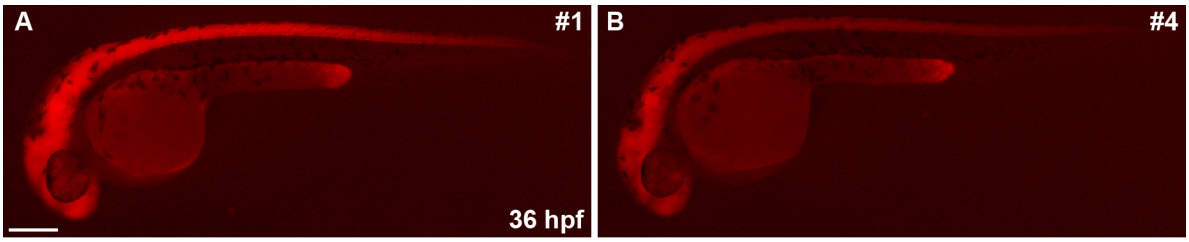

Fig. S5. Comparison of tdTomato expression between strongly and weakly expressing *gfap-2a-tdTomato* F1 lines. Lateral views of 36 hpf embryos. Epifluorescence stereomicroscope images of (A) a strongly expressing tdTomato-positive F1 embryo obtained from #1 line and (B) a weakly expressing tdTomato-positive F1 embryo obtained from #4 line. All images were taken with the same exposure time. Scale bar, 200 μm.

Table S1. Sequences of targeting constructs and *sox2* probe

L1 Targeting construct sequences

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L3 Targeting construct sequences

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M2 Targeting construct sequences

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M1 Targeting construct sequences

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agg

M3 Targeting construct sequences

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S1 Targeting construct sequences

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atcattattattattattattattattattattattattattaagttc
taaatgagagaacgaacagaggcactctccagctttttttaatttccacttgattattttt
cggtctcccactcttcatctgtt

S2 Targeting construct sequences

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g

gfap targeting construct sequences

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ataaacaatgaacaatatatgtattatattacttttaatcttttgtcaaatgttagttga
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sox2 probe sequences

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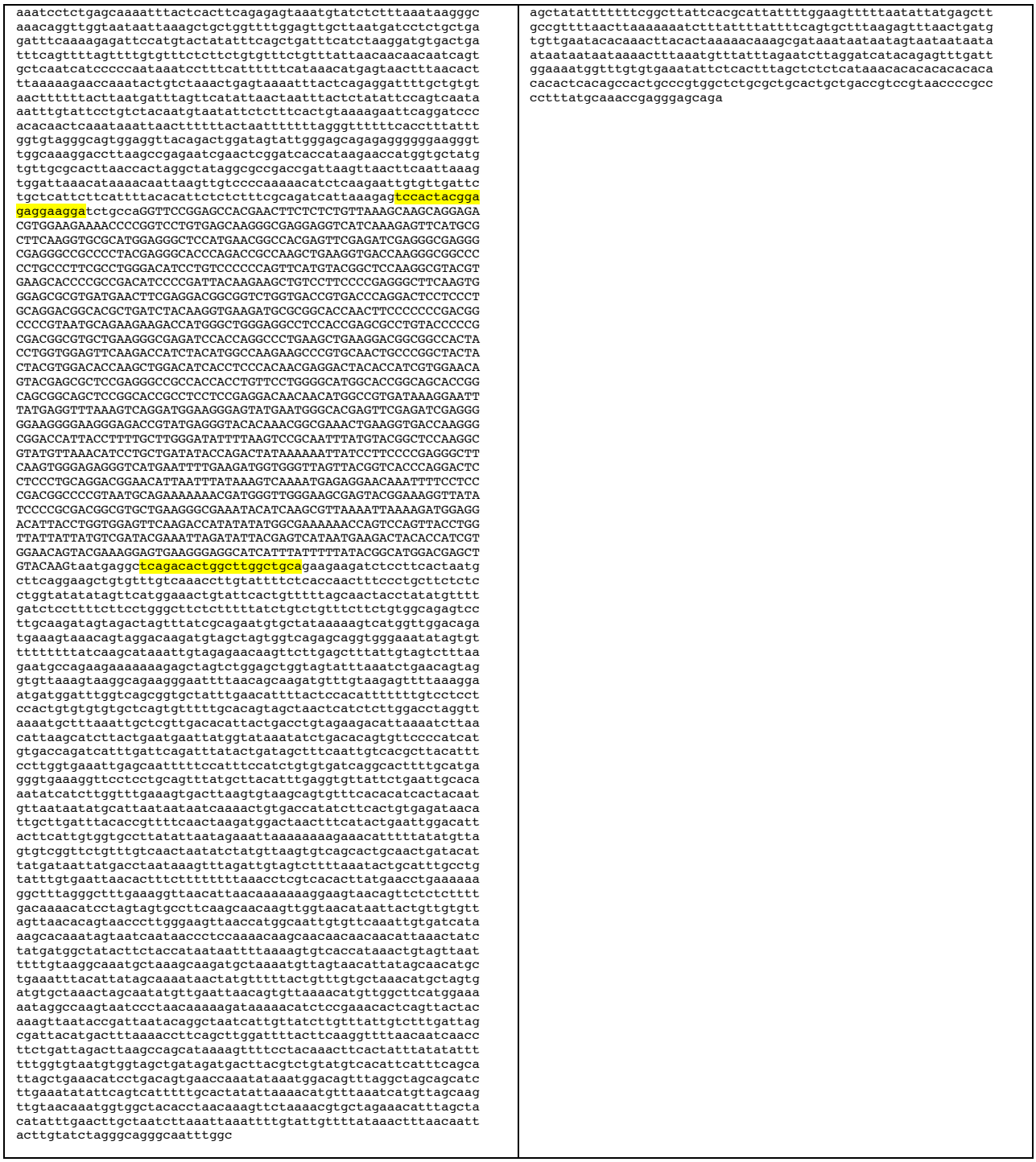

Lowercase indicates zebrafish genomic DNA sequences and uppercase indicates sfGFP and tdTomato sequences. The sequences highlighted in yellow mark indicate TALEN target sequences.

Table S2. Genotyping primers for knock-in analysis

Primer name	Primer Description	Primer sequence
sox2_geno_F	Forward primer for *sox2* TALEN target analysis	GACCTGCGGGACATGATCAGTATGTA
sox2_geno_R	Reverse primer for *sox2* TALEN target analysis	TCCCTCCCCAAAAGAAGTGTTCTGTA
sF1	*sfGFP* specific forward primer	CTTCAGCCGCTACCCCGACCACAT
sR1	*sfGFP* specific reverse primer	TCGCCTTGATGCCGTTCTTCTGCT
sF2	Forward primer outside of the LA region in *sox2* locus	GTAACCCCGCCCCTTTATGCAAACCG
sR2	*sfGFP* specific reverse primer	GTCTTGTAGGTGCCGTCGTCCTTG
sR2'	*sfGFP* specific reverse primer	TTGCCGGTGGTGCAGATGAACTTCAG
sF3	*sfGFP* specific forward primer	AGTCCGTCCTGAGCAAAGACCCCAAC
sR3	Reverse primer outside of the RA region in *sox2* locus	AGTGCTCCCTGACCCTTTGAGAGTCCG
F4	Vector specific forward primer	CTCTGGCCCGTGTCTCAAAATCTCTG
R4	Vector specific reverse primer	TTCAGAAACAACTCTGGCGCATCGG
qF	Forward primer for qPCR Reference	TGAGTTCGACTTTTGTTACACCACA
qR	Reverse primer for qPCR Reference	AGCGCTTCAGATTTTGTTTGTTTCA
gfap_geno_F	Forward primer for *gfap* TALEN target analysis	TTCGCAGATCATTAAAGAGTCCACTACGG
gfap_geno_R	Reverse primer for *gfap* TALEN target analysis	CAGGAGAGAAGCAGGGAAAGTTGGTG
gF1	*tdTomato* specific forward primer	GGGCGAGGAGGTCATCAAAGAGTTC
gR1	*tdTomato* specific reverse primer	AGCTTCTTGTAATCGGGGATGTCGG
gF2	Forward primer outside of the LA region in *gfap* locus	AACACTAGGAGGCGCTGTTCATCAACC
gR2	*tdTomato* specific reverse primer	TTTCTTCCACGTCTCCTGCTTGCTTT
gF3	*tdTomato* specific reverse primer	TTGAAGATGGTGGGTTAGTTACGGTCA
gR3	Reverse primer outside of the LA region in *gfap* locus	GGTGGAAGGTCGAAATTTAGGCAATGT
sox2_T7Egeno_F	Forward primer for *sox2* gRNA target analysis	CTTCCTTCCCCAGCAAAGTTACCTCC
sox2_T7Egeno_R	Reverse primer for *sox2* gRNA target analysis	TAATCCGGGTGTTCCTTCATGTGCAG
gfap_T7Egeno_F	Forward primer for *gfap* gRNA target analysis	CAGCGTTCCTTCTCATCCTACCGAAA
gfap_T7Egeno_R	Forward primer for *gfap* gRNA target analysis	CAGCATTGAGTCCATCCACCTGTCTG