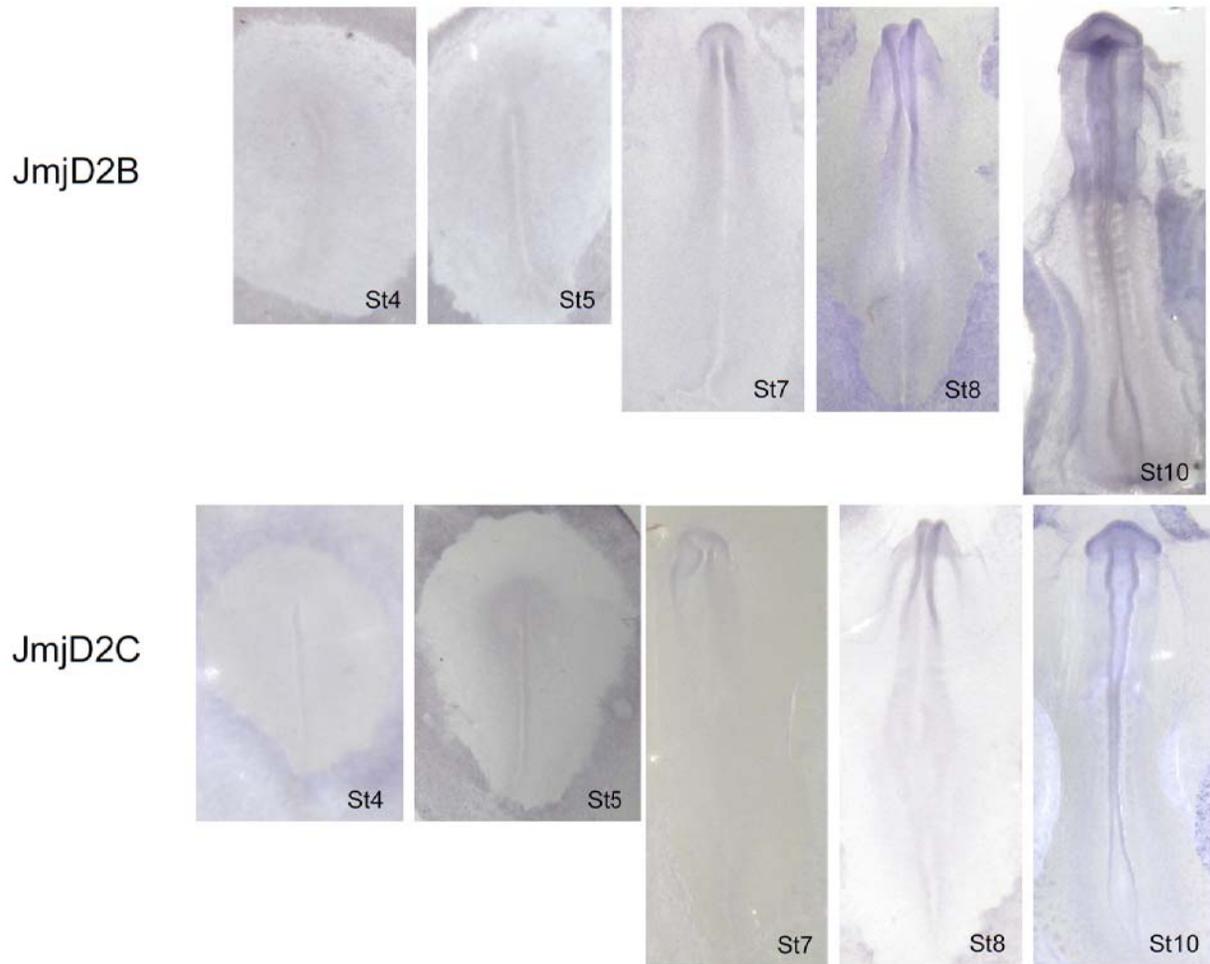


## Supplemental Information

### Histone Demethylase Jmjd2A Regulates

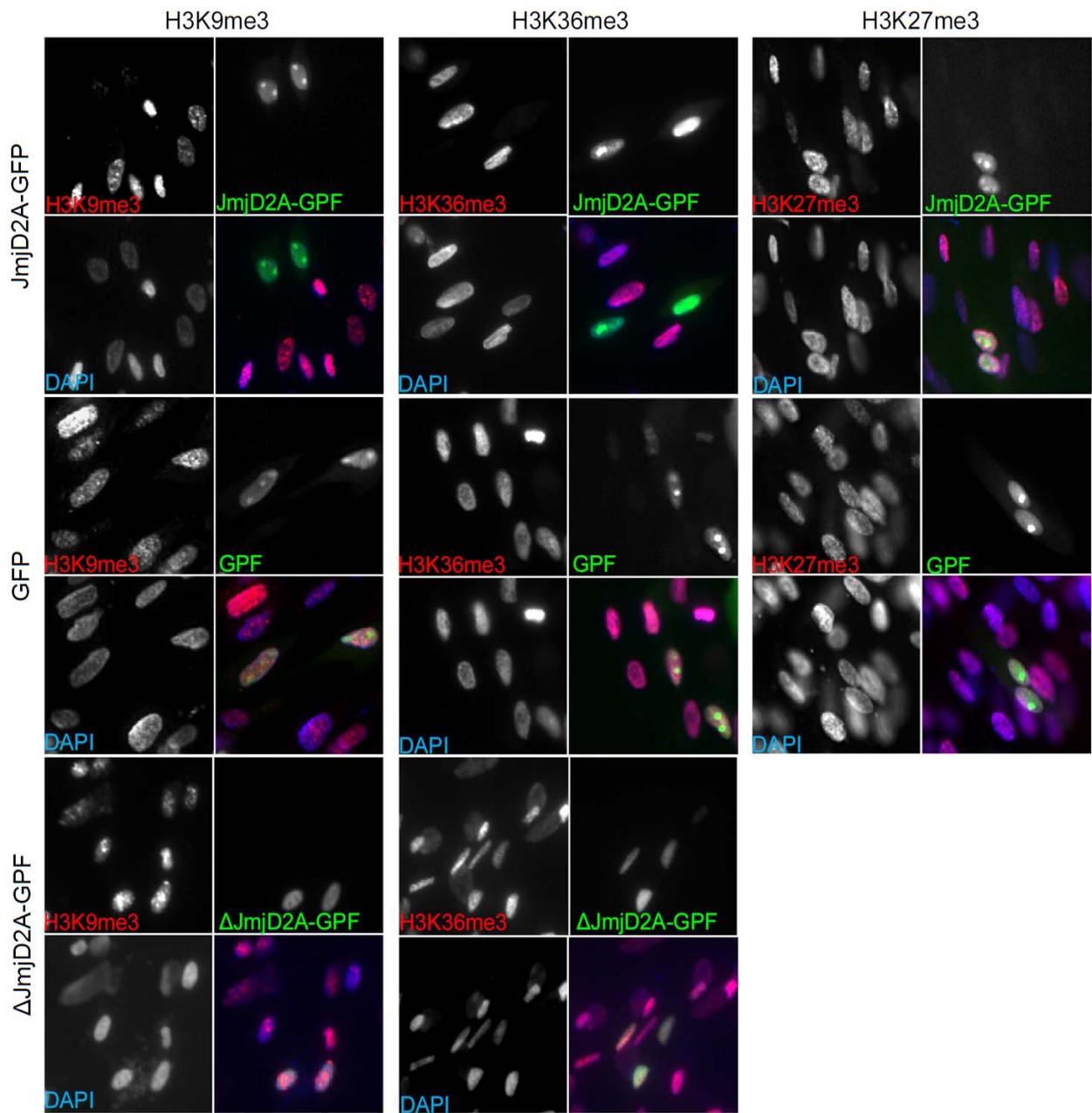
### Neural Crest Specification

Pablo Hernan Strobl-Mazzulla, Tatjana Sauka-Spengler, and Marianne Bronner-Fraser

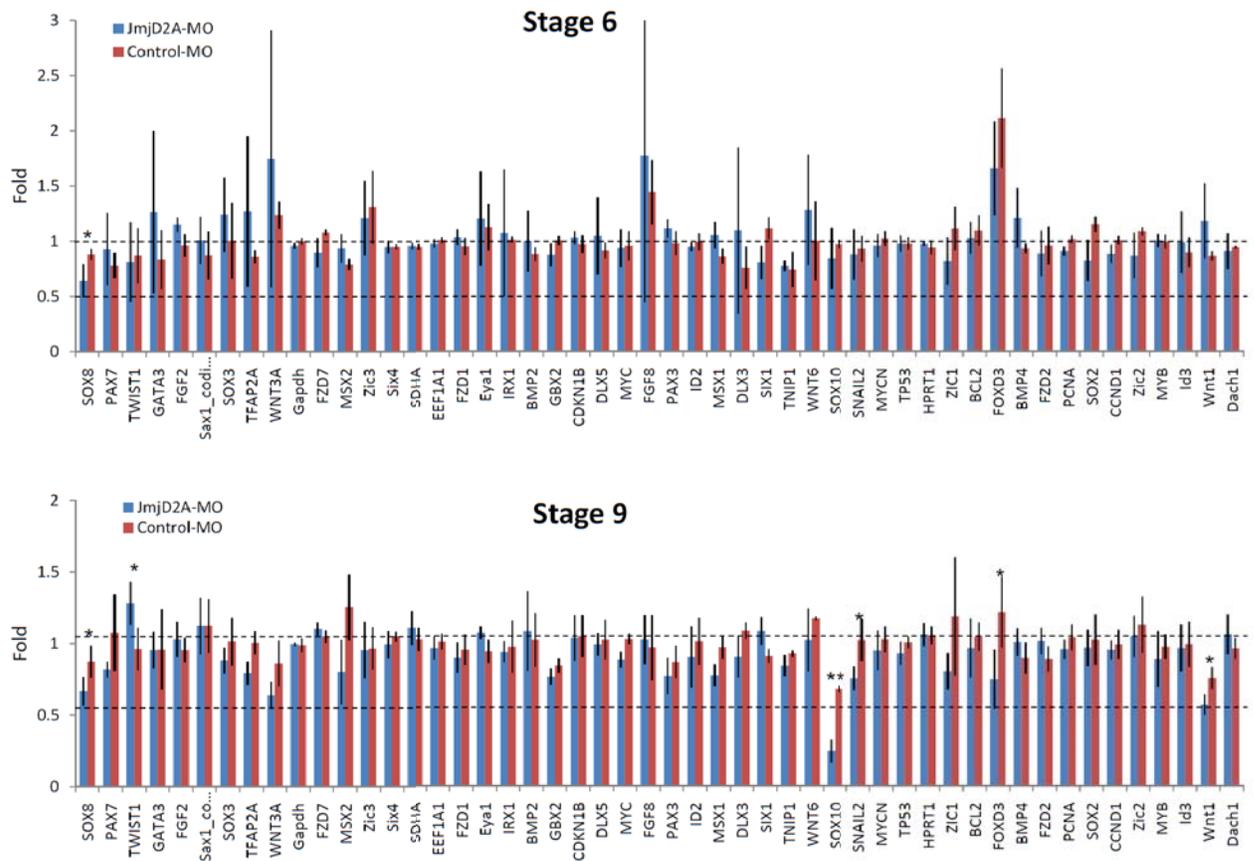


**Figure S1, related to Figure 1.**

Expression pattern of Jmjd2B and C in the early chick embryo by whole mount *in situ* hybridization at stages 4-10.



**Figure S2, related to Fig. 5:** Over-expression of JmjD2A (JmjD2A-GFP) reduces H3K9me3 and H3K36me3 but not H3K27me3 marks on transfected fibroblast chick cells, when compared with an empty vector (GFP). In contrast, a catalytically-dead mutant form of JmjD2A ( $\Delta$ JmjD2A-GFP) fails to alter any methylation marks.

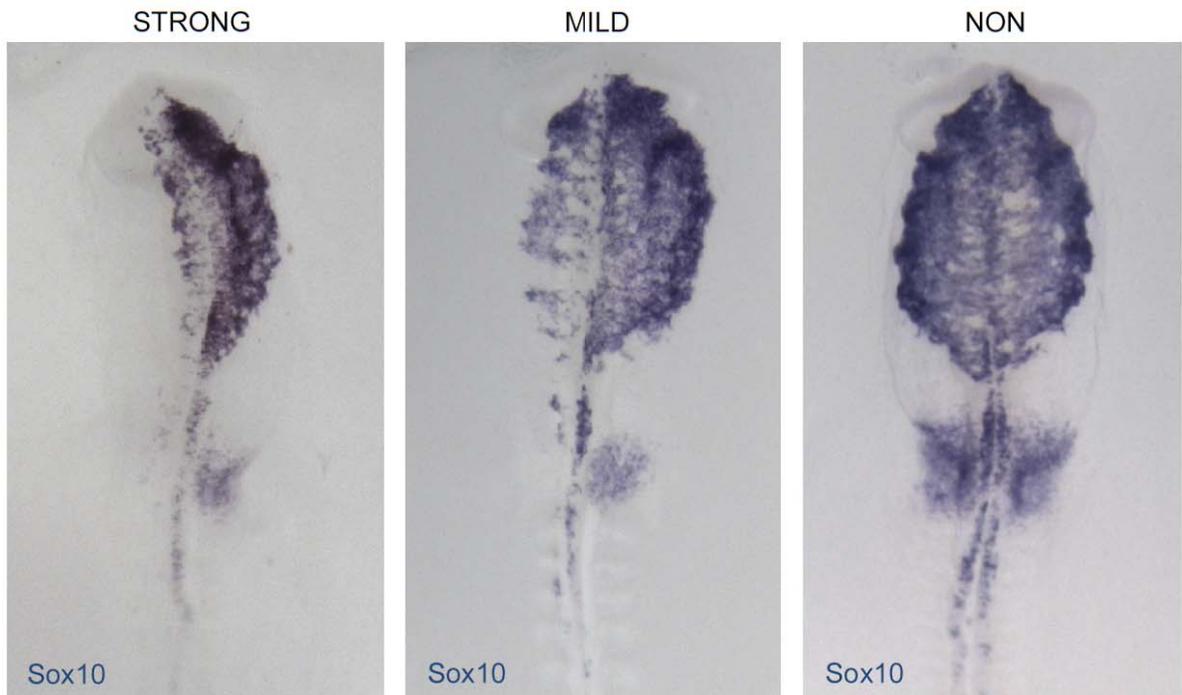
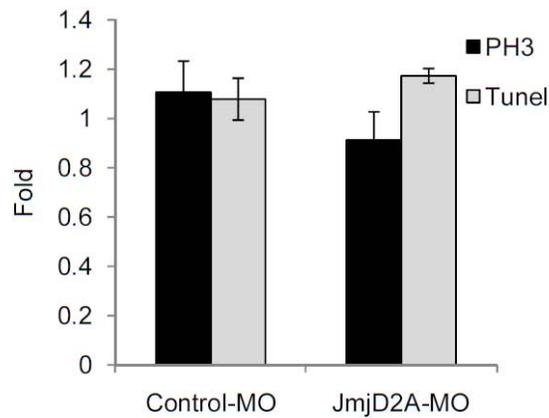


**Figure S3, related to Figure 2.**

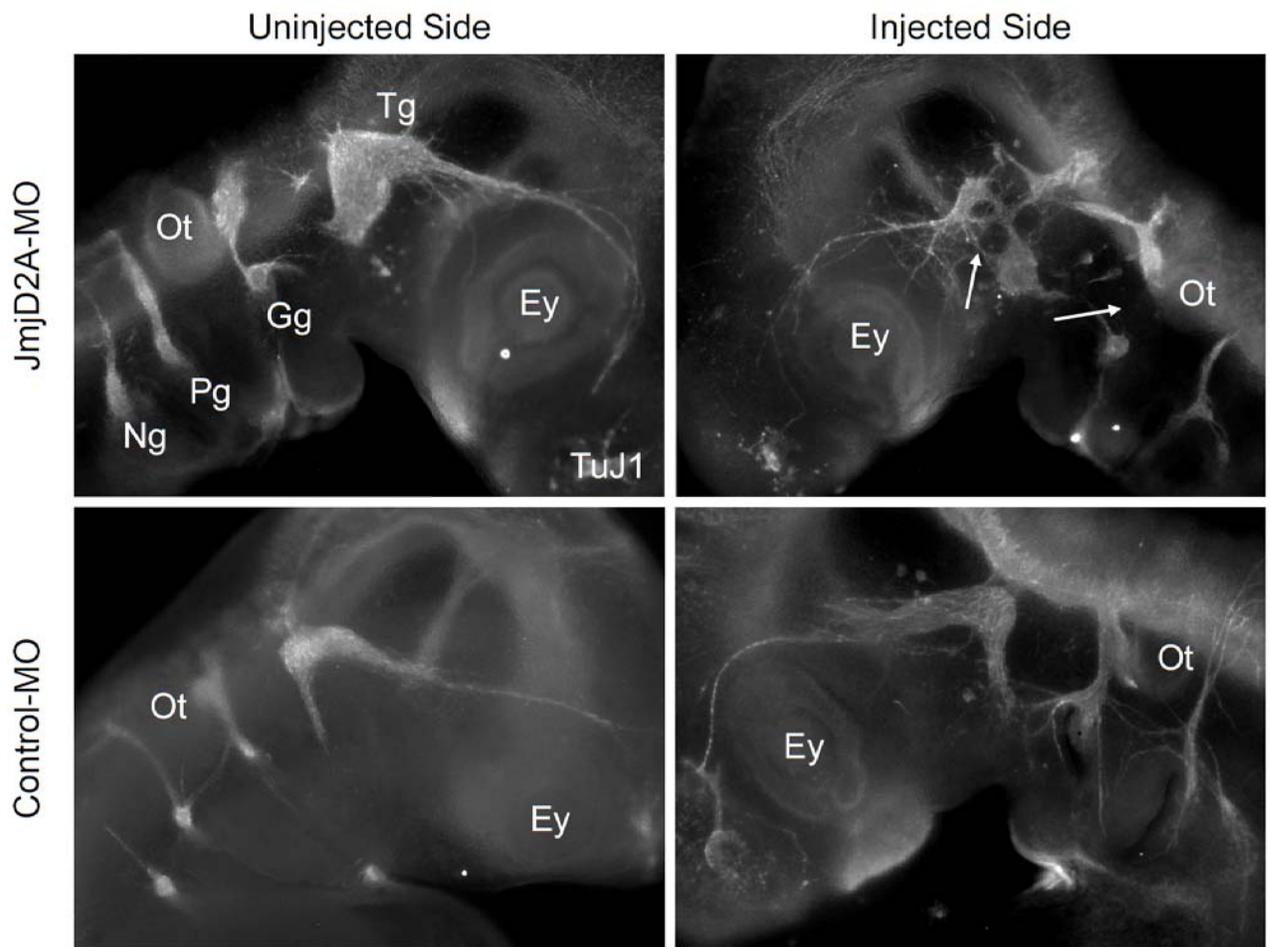
NanoString nCounter analysis of expression of 50 genes represented as a fold difference between the injection side (IS) and uninjected side (UIS), using an average of JmjD2A-MO (n=4) and Control-MO (n=3) treated embryos at stage 6 and 9. The graphs show a mean  $\pm$  SD. Asterisks (\*) and (\*\*)) indicates significant differences (P<0.05 and P<0.01, respectively) by *t*-Student test. Dotted line represents variation greater than 25% between IS and UIS.

**A**

## JmjD2A-MO Phenotypes

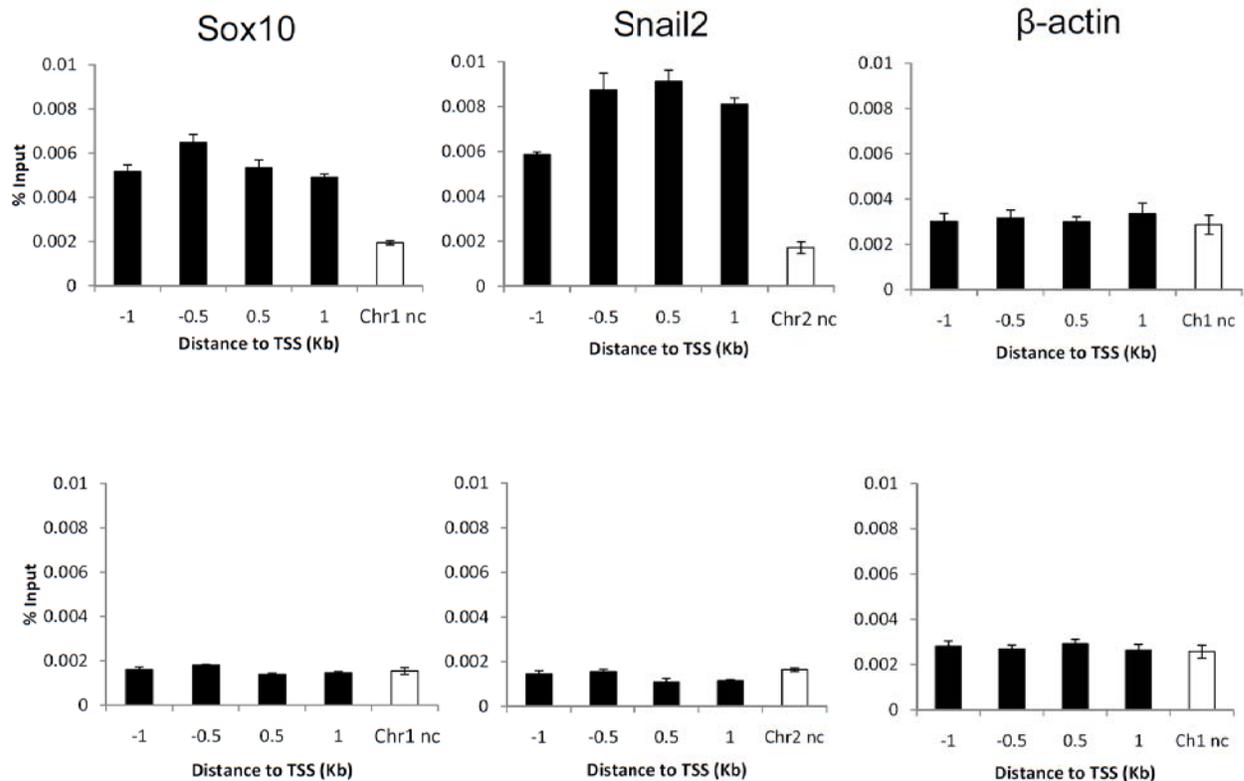
**B****Figure S4, related to Figure 3.**

(A) JmjD2A-MO treated embryos showing strong, mild and non phenotype on Sox10 expression. (B) JmjD2A knock-down fails to affect cell death (TUNEL.  $P = 0.32$ ) or proliferation (Phosphohistone 3, PH3.  $P = 0.29$ ). Data were obtained from five embryos and are presented as fold change compared with the contralateral side (mean  $\pm$  SEM).



**Figure S5, related to Figure 4.**

Long-term effect of the JmjD2A depletion cause a severe defect on cranial ganglia formation evidenced by the neuronal processes marker  $\beta$ -neurotubulin (TuJ1) on stage 16-17 embryos compared with control-MO treated embryos. Tg, Trigeminal ganglia; Gg, Geniculate ganglia; Pg, Petrosal ganglia; Ng, Nodose ganglia; Ey, Eye; Ot, Otic.



**Figure S6, related to Figure 6.**

Representative ChIP assay, out of three independent experiments, showing Jmjd2A binding around the TSS of Sox10, Snail2 and  $\beta$ -actin, and compared with two distant region in their respective chromosome 1 (Chr1) negative control (nc) and 2 (Chr2nc), which were used as a control. The graphs show a mean  $\pm$  SD.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Chick fibroblast transfection

Fibroblast cells were culture in 8-well slides coated with fibronectin until 50-80% confluence. Cells were trasfected adding 0.4ug of vector (pCIG- GFP, Jmjd2A-GFP and  $\Delta$ Jmjd2A-GFP) combined with lipofectamine/OPTIMEM and enriched DMEM media (1% L-GLu, 1x NEAA) during 4hs. The media was then change to a DMEM rich media (10% fetal bovine serum, 1%chicken serum, 0.1% L-Glu and 1% PenStrep) and incubated overnight. Slides were fixed in 4% PFA during 20min and used for immunohistochemistry with H3K9me3, H3K36me3, and H3K27me3 antibodies (Abcam).

### Whole-mount immunohistochemistry

Embryos were fixed after electroporation in 4% PFA ON at 4°C. Primary antibody anti-TuJ1 (1:200, Covance) was incubated ON at 4°C in PBS-T containing 5% sheep serum and then detected using the secondary Alexa Fluor goat anti-rabbit 594 (1:500).

## List of primers used for ChIP-QPCR

Target gene	Distance form TSS (bp)	Sequence 5'--3'
Sox10 -1kb	-831 to -657	tggcactgctctctgtcatc cattcactccttgctgctca
Sox10 -0.5 Kb	-423 to -200	cttgcaacccttctccata ctggtcacaggaagggatgt
Sox10 0.5kb	262 to 439	gcctttgctcctcattgact gcatcactgtcccctgattt
Sox10 1Kb	1305 to 1409	gaagaccaccactgcctctc acttcattgacccgaacag
Sox10 8Kb	8638 to 8793	acatgtcatggaggaggag gcagctgtgaaatcagcaa
Snail2 -1kb	-857 to -682	gcatttgtagcgcatcaaa ggagctgcctttgaaatctg
Snail2 -0.5kb	-315 to -154	aggagctgaaacctgaagca aaatacccgaagtcagggtg
Snail2 0.5kb	216 to 402	ttttgccgtgcttacagtg atgcagcagtgaaacctcct
Snail2 1kb	550 to 797	attctgagcgtttggcactt cggagagaggctcattgggta
$\beta$ -actin -1kb	-971 to 812	gagaaaccggaaagtgtca tcagctgctccacacatctc
$\beta$ -actin -0.5kb	-368 to 132	gtgtttgcttccaggaggag gttcccaagggaagagag
$\beta$ -actin 0.5kb	66 to 260	gtccaccttccagcagatgt agtcaagcgcaaaagaaaa
$\beta$ -actin 1kb	738 to 942	ggcttgactggtgttggtt acacactgatggcagctcag
Chr1nc		ctaatggcaactccgggta gccagcaccacactttaat
Chr2nc		actgcaggctggtgagtctt cttcagtgctgtcactcca