

Supporting Information

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SI Materials and Methods

Bisulfite Sequencing. Bisulfite treatment and sample recovery were carried out with the EpiTect Plus Bisulfite Conversion Kit (Qiagen) following the manufacturer's instructions. In brief, six dorsal neural tubes from control or 3B MO-treated sides were lysed and treated with proteinase K. Then 20 μ L of the lysis reactions was mixed with 85 μ L of bisulfite mix and 35 μ L of DNA protect buffer. Bisulfite conversion was performed on a thermocycler as follows: 99 $^{\circ}$ C for 5 min, 60 $^{\circ}$ C for 25 min, 95 $^{\circ}$ C for 5 min, 60 $^{\circ}$ C for 85 min, 95 $^{\circ}$ C for 5 min, 60 $^{\circ}$ C for 175 min, and 20 $^{\circ}$ C indefinitely. The bisulfite-treated DNA was recovered by an EpiTect spin column and quantified on a NanoDrop spectrophotometer. Two sets of nested primers located on the promoter regions of Sox10 and Snail2 genes were designed using Methyl Primer Express version 1.0 (Applied Biosystems). For this, 1 μ L of bisulfite-converted DNA was combined with 0.15 μ L of 10 mM primers (forward: Sox10bis F1, TTGGTGAGAGTTTATAGAGATGG; Snail2bis F1, GTTG-TGTTTGAAGGGGTAGTT; reverse: Sox10bis R1, CAAAC-

CCTATAAAAACTACAACCTCC; Snail2bis R1, TCGCATTAT-CTACTCCATACACTCC), 1.5 μ L of 10 \times Taq Buffer with 25 mM Cl_2Mg (Roche), 0.3 μ L of Taq polymerase (Roche), 0.2 μ L of dNTPs (10 mM each), and 0.75 μ L of DMSO in a total reaction volume of 15 μ L. The thermal cycler protocol included the following steps: 5 min at 95 $^{\circ}$ C; 45 cycles of 30 s at 95 $^{\circ}$ C, 1 min at 60 $^{\circ}$ C, and 1 min at 72 $^{\circ}$ C, followed by a final extension cycle of 3 min at 72 $^{\circ}$ C. Then 1 μ L of the products thus obtained were used for a second round of PCR with a set of nested primers (forward: Sox10bis F2, AAGAGTTTTAAGTGGGTTTAAGTTG; Snail2bis F2, AAGGTAGTTTTTAGGTAGAGTAGT; reverse: Sox10bis R2, AACTACAACCTCCTATCCCAATACC; Snail2bis R2, CTCCTCCAATCACTTCTAAATATTC). The PCR and thermal cycler protocols were set using the conditions described above. Two independent PCR products were combined and loaded on a gel, and the expected bands were gel-purified and cloned into the pGEM-T easy vector (Promega). Individual clones were sequenced, and the 3B MO-injected and uninjected sides were compared.

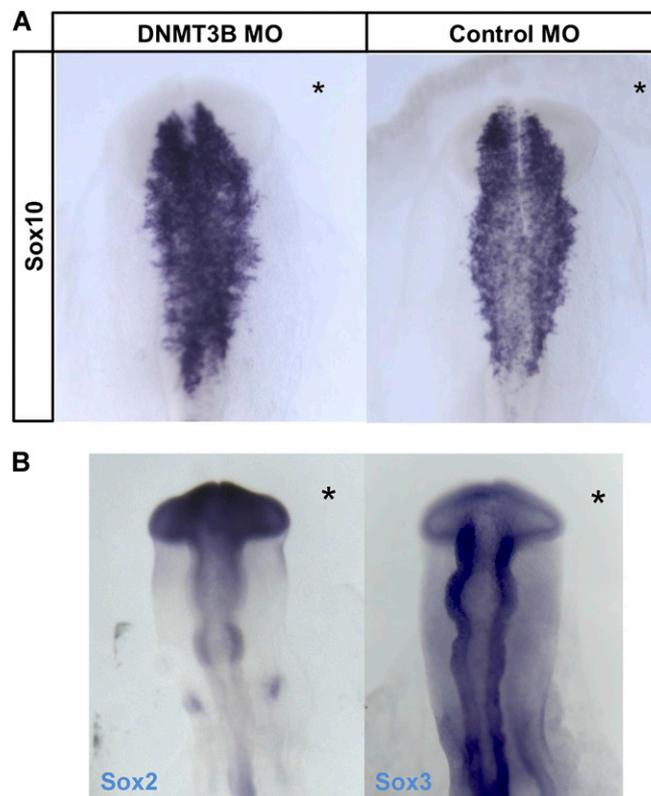


Fig. S1. Lack of effects of knockdown of DNMT3B on neural crest specification and neural gene expression. (A) The right side (asterisk) of each neural tube was electroporated with DNMT3B MO or control morpholino at stage 8, and embryos were scored at early stage 9, corresponding to the onset of neural crest migration. Cranial neural crest cells marked by Sox10 appear normal on both the control and injected sides of the embryo. (B) No obvious differences in expression of neural markers Sox2 or Sox3 were noted after 3B MO knockdown (indicated by an asterisk) compared with the control side.

