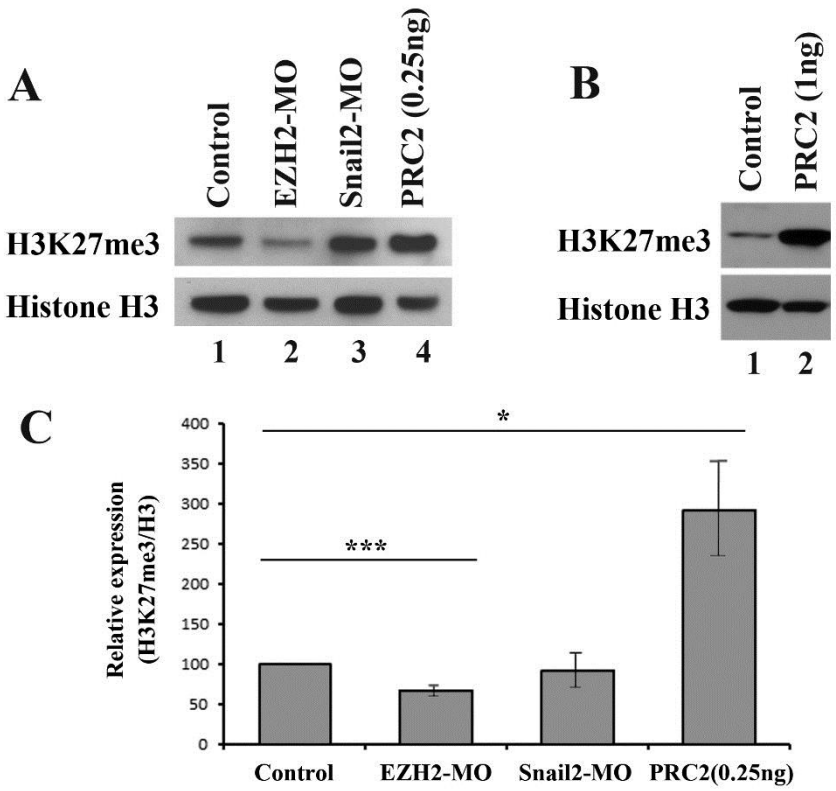
SUPPLEMENTARY MATERIALS

Materials and methods

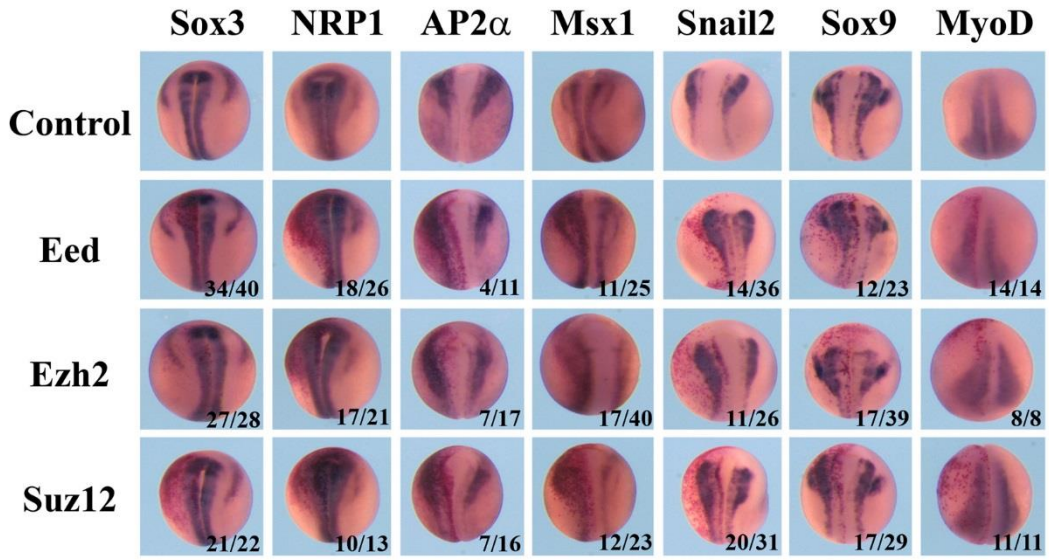
In vitro methylation assay

GST-Snail2 (prepared as described above, 5µg) or isolated mammalian nucleosomes (2.3µg) were cultured with the PRC2 complex (BPS Bioscience 51003, 2µg) and 2µl ^{3}H-SAM (14 Ci/mM; NEN Life Science Products) for in vitro methyltransferase assay. The reaction was carried out in the HMT buffer (4mM Tris-HCl, pH 8.0, 0.8mM EDTA, 0.2mM PMSF, and 0.1mM DTT) at 30°C for 1.5 hours. The reaction was stopped with the SDS loading dye and the proteins were separated by 15% SDS-PAGE. The gel was stained with Coomassie blue and treated with Entensify (NEN Life Science Products) following manufacturer's instruction. The methylation signal was detected by autoradiography.

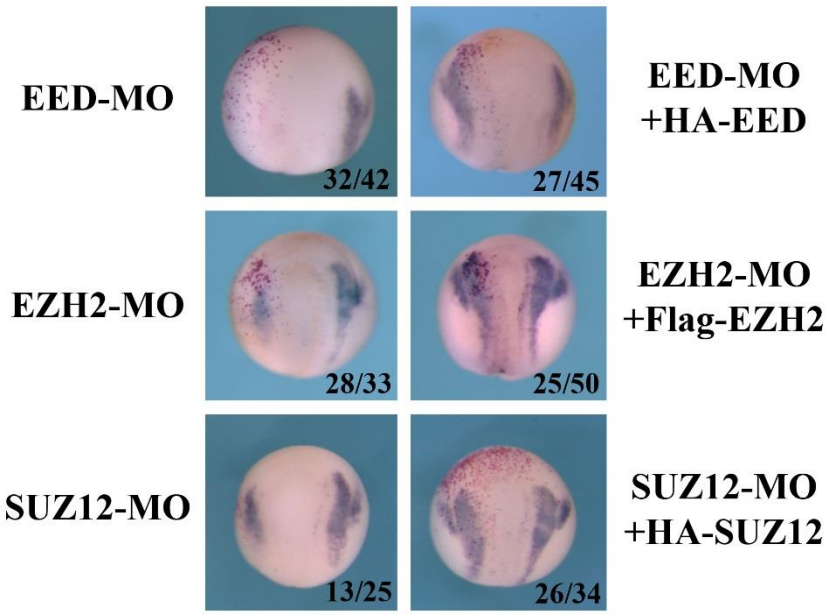
Figures



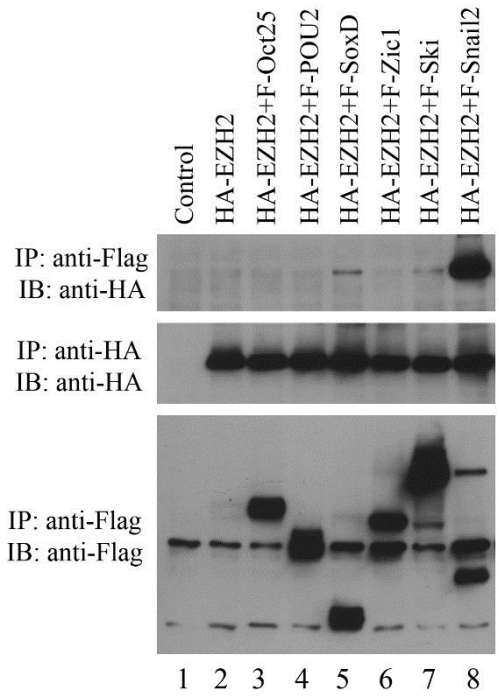
Suppl. Fig. 1. PRC2 regulates histone H3K27 tri-methylation in Xenopus embryos. RNAs encoding 0.25ng or 1ng mixed PRC2 components or 20ng MOs against EZH2 or Snail2 were injected into 1-or 2-cell stage embryos. Histones from the head region of late neurula stage embryos (stages 20-21) were obtained using acid extraction buffer, separated on 15% SDS-PAGE, and analyzed by Western blot for H3K27me3 levels. A) PRC2 enhanced, and EZH2-MO reduced, H3K27me3 levels. B) At higher doses of PRC2 RNAs, more dramatic increase in H3K27me3 levels was seen. C) Quantification of H3K27me3 over total histone H3 levels revealed that alteration of PRC2 amount led to significant changes in H3K27me3 levels, but knockdown of Snail2 did not result in statistically significant changes in H3K27me3 level.



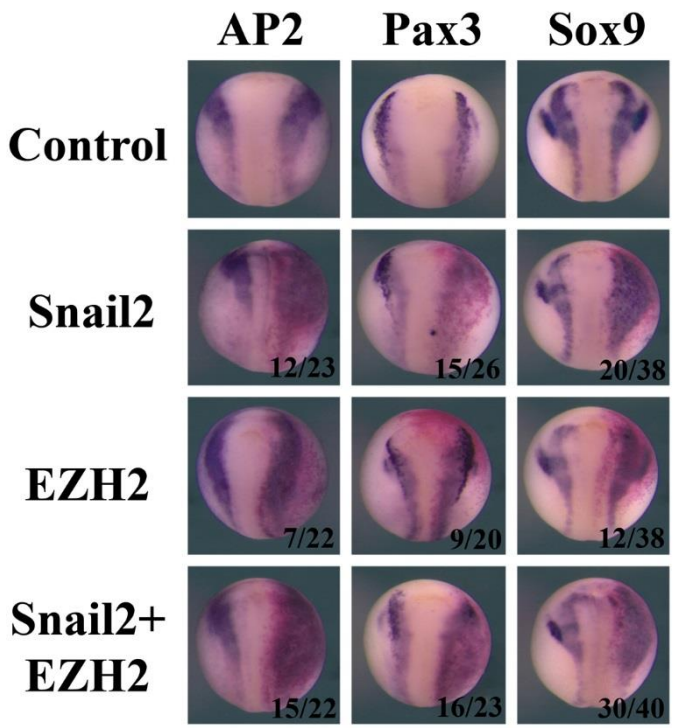
Suppl. Fig. 2. Ectopic expression of PRC2 components moderately expands neural crest markers, but does not alter neural gene expression. RNAs encoding 1ng Eed, Ezh2 or Suz12 were injected into the animal region of one of the two blastomeres of 2-cell stage embryos. In situ hybridization was performed at neurula stages (stages 17 to 19) to examine expression of neural (Sox3 and NRP1) and neural crest markers. While none of the PRC2 genes affected the expression of the neural genes (the number of the embryos indicated in the panel), all could moderately expand the neural crest markers. The mesodermal marker MyoD was not affected by ectodermal expression of the PRC2 genes.



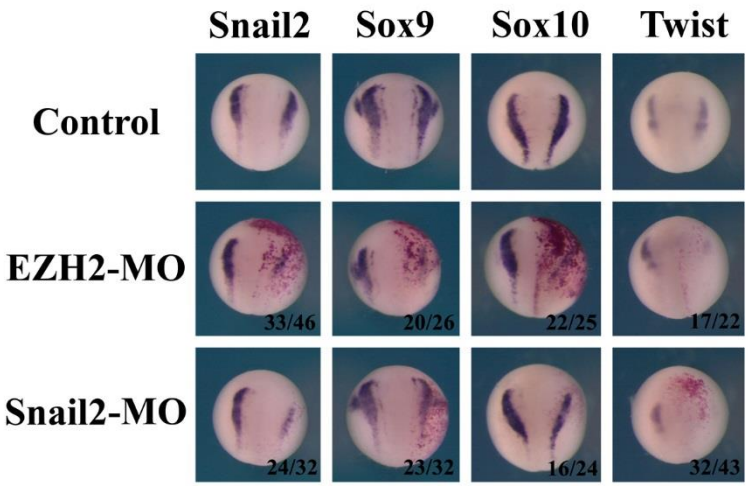
Suppl. Fig. 3. Defects in neural crest induction in PRC2 morphant embryos are rescued by PRC2 RNAs that are not sensitive to the MOs. 20ng PRC2-MO was co-injected with 1ng epitope tagged PRC2 RNAs that were insensitive to the MOs into the animal region of one blastomere of 2-cell stage embryos. The embryos were collected at mid-neurula stages (stages 15 to 16) and assayed for the expression of Sox9. The number of the embryos displaying the shown in situ patterns was indicated in the panel.



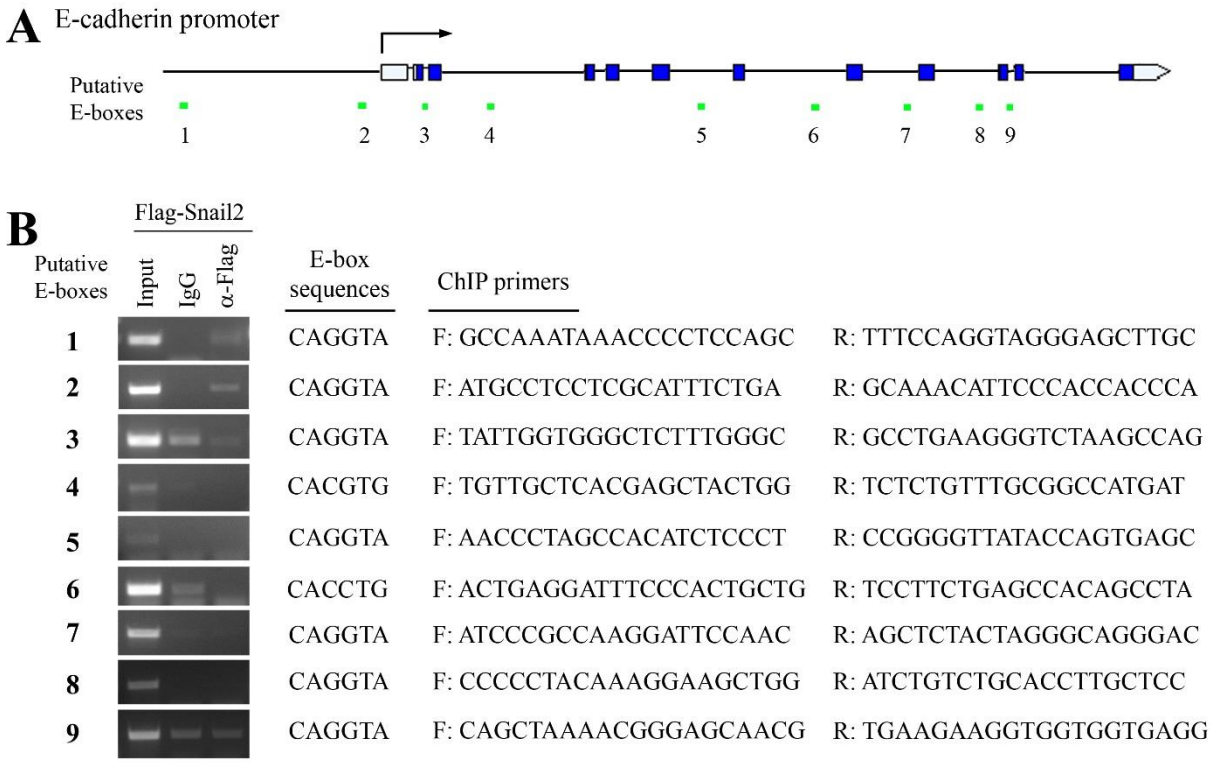
Suppl. Fig. 4. EZH2 interacts with Snail2. 1-2ng RNAs encoding Flag-tagged transcription factors known to regulate neural or neural crest development were co-injected with 1ng RNA encoding HA-tagged EZH2 into 1- to 2-cell stage embryos. The embryos were cultured to the gastrula stages (stages 10 to 11) before protein extract was obtained for immunoprecipitation assay with anti-Flag antibody (1:1000). Among the transcription factors, Flag-tagged Snail2 efficiently pulled down HA-tagged EZH2, demonstrating that the two proteins could interact with each other in gastrula Xenopus embryos.



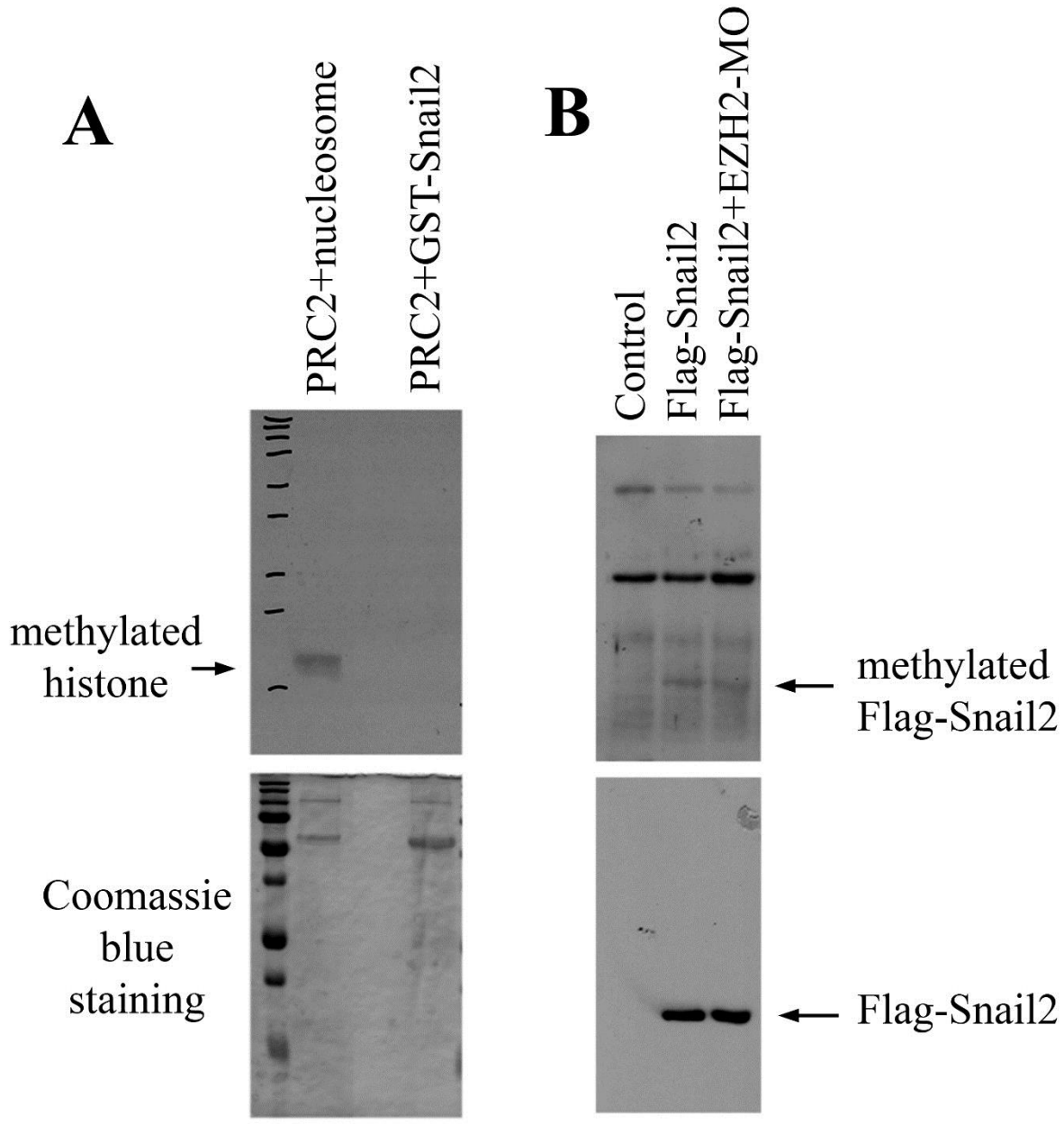
Suppl. Fig. 5. Regulation of neural crest marker expression by Snail2 and EZH2. RNAs encoding 0.2ng Snail2 or 1ng EZH2 were injected alone or together into one of the two blastomeres of 2-cell stage embryos. The embryos were cultured to neurula stages 16-17 and analyzed by in situ hybridization for neural crest marker expression. The number of the embryos displaying shown in situ patterns was indicated in the panels. Co-injection of the two RNAs led to expansion of the neural crest domains similarly to that when Snail2 RNA was injected alone.



Suppl. Fig. 6. Both Snail2 and EZH2 are required for neural crest specification. 20ng MOs against Snail2 or EZH2 were injected into one of the two blastomeres of 2-cell stage embryos. The embryos were cultured to neurula stages 15-16 and analyzed by in situ hybridization for the expression of the neural crest markers. Knockdown of either Snail2 or EZH2 led to similar reduction in neural crest marker expression in the embryos.



Suppl. Fig. 7. Snail2 binds to the E-boxes at the E-cadherin promoter. A) Genomic structure of the E-cadherin promoter and the gene body. The positions of the putative E-boxes were labeled underneath. B) 1ng RNA encoding Flag-Snail2 was injected into both blastomeres of 2-cell stage embryos. The embryos were cultured to the neurula stage 19 when neural crest cells underwent EMT. ChIP assay was performed using mouse anti-Flag antibody (Sigma). IgG was used as a negative control. Snail2 associated with the two E-boxes upstream of the transcription start site, but did not bind appreciably to the putative E-boxes within the intronic regions in the E-cadherin gene.



Suppl. Fig. 8. PRC2 does not methylate Snail2. A) In vitro methylation assay was performed using commercial PRC2 complex and ^{3}H-SAM as methyl group donor. Purified nucleosomes from the HeLa cells and purified GST-Snail2 were used as the substrates. PRC2 methylated histones in the nucleosomes, but did not methylate GST-Snail2. B) RNA encoding 1ng Flag-Snail2 was injected alone or with EZH2-MO into early Xenopus embryos. Proteins from injected embryos were extracted at neurula stages (stages 19-20),

immunoprecipitated with anti-flag antibody, and subjected to Western blot analysis for pan-lysine methylation. Although Snail2 was methylated in Xenopus embryos, its methylation was not affected by EZH2 knockdown.

Suppl. Movies 1 and 2. Time lapse movies of neural crest cell migration in DMSO-treated samples.
Suppl. Movies 3 and 4. Time lapse movies of neural crest cell migration in GSK126-treated samples.

The supplemental movies are stored on the website with the following links:

http://cdib.uab.edu/faculty/cchang/movie_1.avi

http://cdib.uab.edu/faculty/cchang/movie_2.avi

http://cdib.uab.edu/faculty/cchang/movie_3.avi

http://cdib.uab.edu/faculty/cchang/movie_4.avi