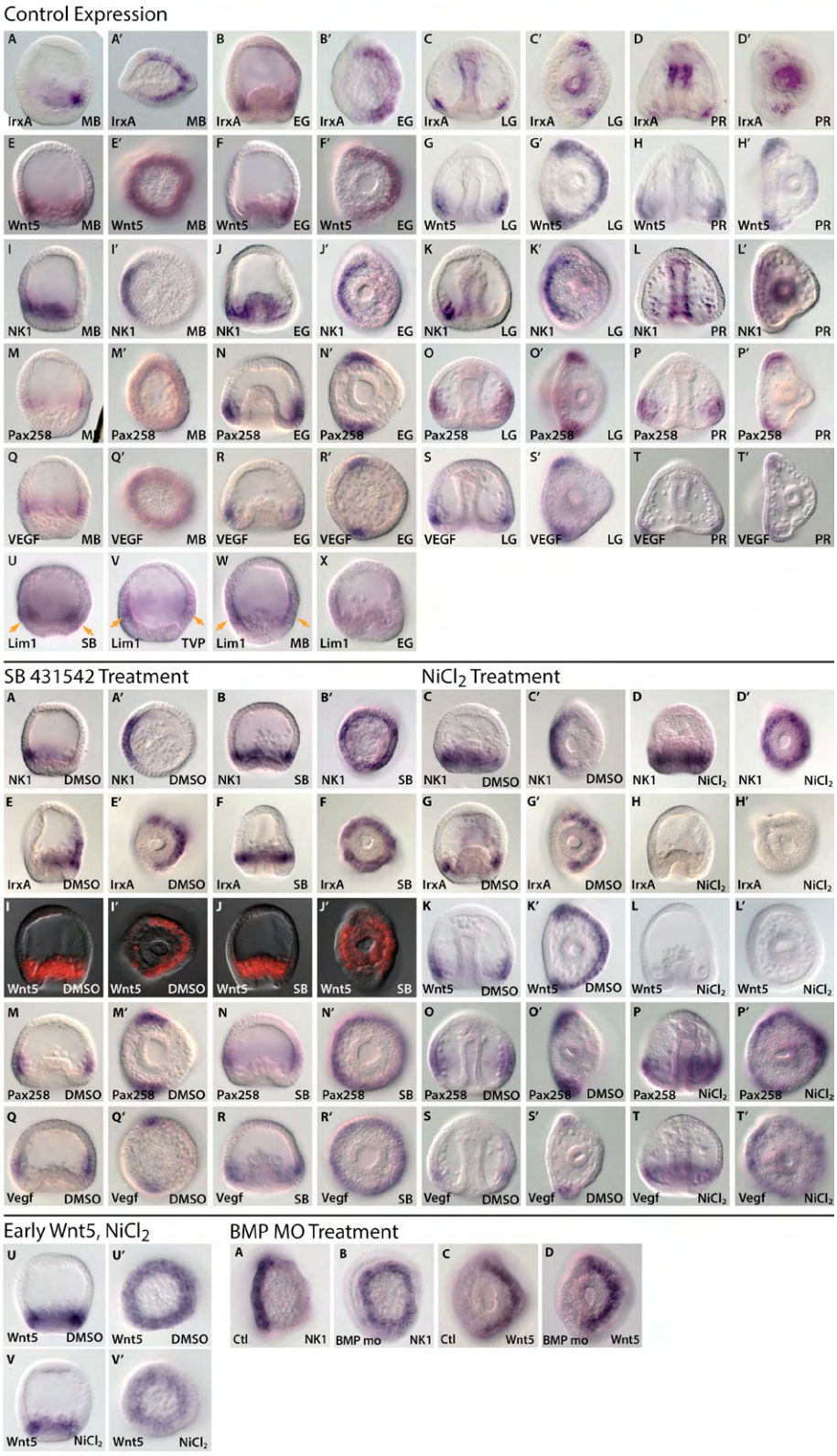

Fig. S1. Expression patterns of BE genes from 9 to 18 hpf. *In situ* hybridization was used to detect expression patterns of IrxA (A-D′), Wnt5 (E-H′), NK1 (I-L′), Pax2/5/8 (M-P′), VEGF (Q-T′) and Lim1 (U-X). Expression patterns are shown in front/lateral views as well as vegetal views for each gene. Each gene is shown at the following stages: 9 hpf (MB), 12 hpf (EG), 15 hpf (LG) and 18 hpf (Prism); except for Lim1 which is shown at earlier stages, as indicated. These data are summarized in the graphic shown as Fig. 1H. Expression of BE genes following perturbations to TGFbeta signaling. SB 431542 was used to disrupt Nodal signaling. $NiCl_2$ was used to activate Nodal signaling throughout the ectoderm. Expression of each BE gene was assayed by *in situ* hybridization under each condition during gastrulation, when BE expression was normally restricted to sub-regions of the BE. These results are shown for IrxA (A-D′), Wnt5 (E-H′), NK1 (I-L′), Pax2/5/8 (M-P′) and VEGF (Q-T′). For each gene/perturbation combination, frontal or lateral, and vegetal, views are shown. These results are summarized in Fig. 2A,B and E. We also assayed the effect of NiCl2 on endodermal Wnt5 at mesenchyme blastula, and observed no changes in expression (U-V′). Effect of BMP2/4 MO perturbations on BE gene expression. The oral BE marker NK1 expanded when BMP activity was blocked. The aboral marker WNT5 was unaffected.

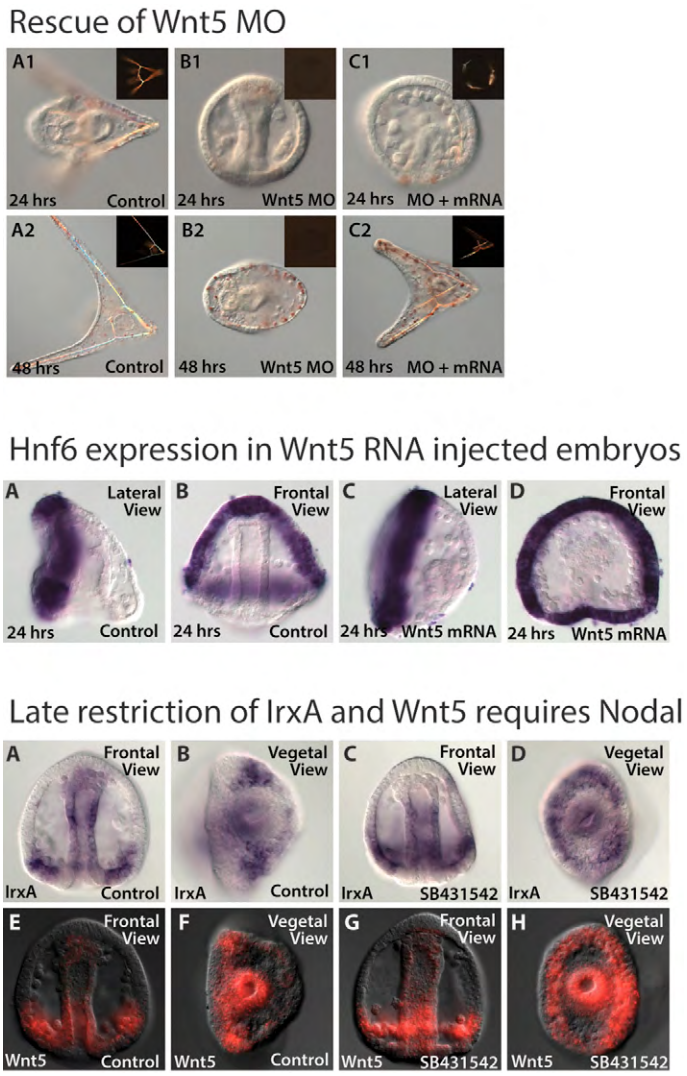

Fig. S2. Rescue of the Wnt5 MO with mRNA not sensitive to the MO. Control injected embryos developed normally at 24 and 48 hpf (A1 and A2). Wnt5 MO injected embryos arrested at late gastrula and had not produce skeleton at 48 hpf (panels B1 and B2). Embryos receiving both Wnt5 MO and mRNA were delayed, but had produced a robust skeleton by 48 hpf (panels C1 and C2). Inserts show birefringent skeletons in control and rescued embryos. Effect of Wnt5 rna injection on Ventral-Dorsal Specification Hnf6 staining on Wnt5 rna injected embryos at 24 hpf. Frontal and lateral views are shown. No changes in Hnf6 expression were observed indicating specification of the DV axis by TGFB signaling was not disrupted. Late restriction of IrxA and Wnt5 required Nodal. *In situ* hybridization for IrxA (A-D) and Wnt5 (E-H) at 18 hpf in embryos treated with SB431542. Frontal and vegetal views are shown. Late restriction of IrxA and Wnt5 to lateral patches of the BE was not observed in SB treatment. Instead, expression was radial.