Figure S1. Congruent expression of *foxq2, sfrp1/5* and *dkk3*. (A) Two-color in situ hybridization assay for *sfrp1/5* (green) and *foxq2* (red) mRNA transcripts in mesenchyme blastula staged embryos (24 hpf). **(B)** Two-color in situ hybridization assay for *sfrp1/5* (green) and *dkk3* (red) mRNA transcripts in mesenchyme blastula staged embryos.
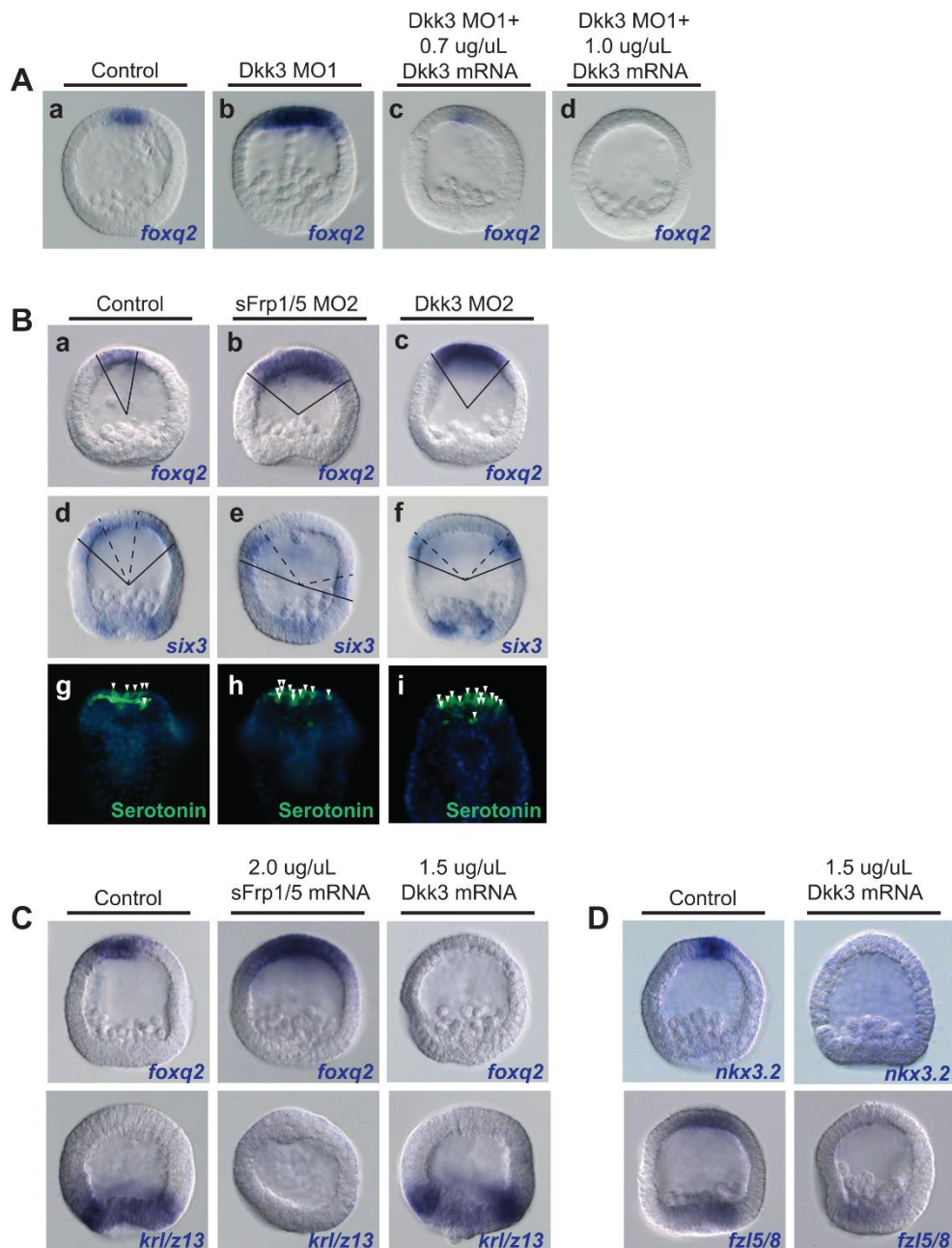
Figure S2. Additional morpholino and overexpression phenotypes. (A) Dkk3 overexpression rescues *foxq2* expression in embryos injected with Dkk3 MO1, which cannot bind to *dkk3* mRNA. **(B)** The ANE territory is expanded in embryos injected with sFrp1/5 Morpholino 2 and Dkk3 Morpholino 2. **(Ba-f)**The solid lines emanating from the center of the embryo mark the most posterior expression of *foxq2* and *six3*. The dotted lines originating from the center of the embryo indicate the anterior-most expression of the *six3* ring. **(Bg-i)** Serotonergic neurons (green) in control. sFrp1/5 and Dkk3 morpholino 2 knockdown 96 hpf pluteus larvae. Control = 5.95, n = 21; sFRP1/5 MO2 = 10.5 serotonergic neurons, n = 23; Dkk3 MO2 = 11.52 serotonergic neurons, n = 23. **(C)** *foxq2* is expanded (a, b) and the endomesoderm marker *krl/z13* is down regulated **(d,e)** in in embryos injected with sFRP1/5 mRNA. *foxq2* is down regulated **(a, c)** and the endomesoderm marker *krl/z13* is unaffected **(d, f)** in embryos injected with Dkk3 mRNA. **(D)** The ANE regulatory network genes, *nkx3.2* and *fzl5/8*, are down regulated in embryos injected with Dkk3 mRNA. MO; morpholino.
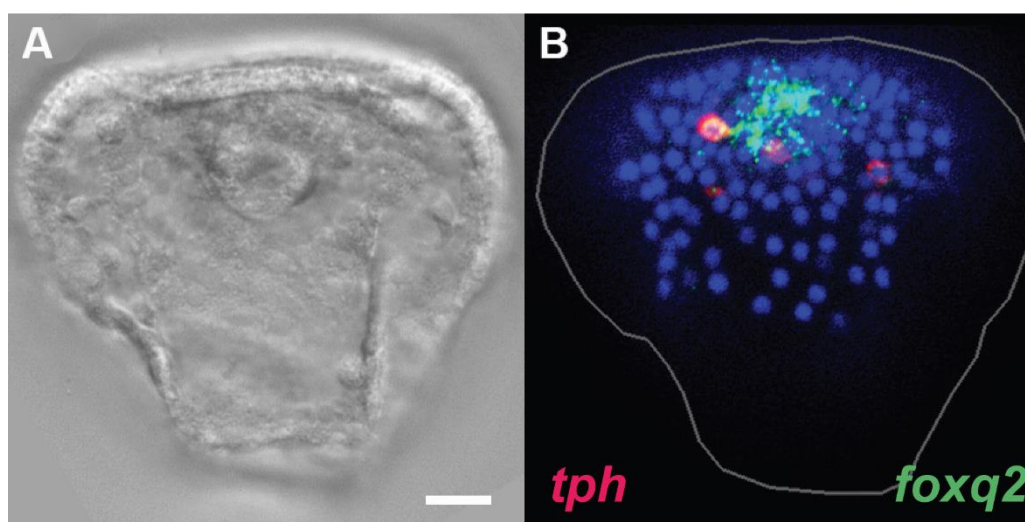
Figure S3. Serotonergic neural cells appear at the dorsal edge of FoxQ2-expressing cells in the ANE. A) DIC image of a gastrula stage (45hpf) embryo. Anterior view, dorsal is to the bottom. B) Whole mount fluorescence in situ hybridization for a serotonergic neural cell marker tryptophan hydroxylase (Tph) (red) and FoxQ2 (green) in the same embryo. Nuclei were labeled with DAPI (blue). Shown is a stack of optical sections through the animal pole. The white bar in A represents 20 μm .