

Figure legends for Supplemental Data

Figure S1. *six3b* RNA, but not *six3b^{vu87}* RNA, Rescues the Eye Deficiency in *six3b/six7*-Deficient Embryos

Embryos from a cross between *six3b^{vu87/vu87}* parents. (A) Uninjected controls. (B) Embryos injected with MO^{*six7*} (*six3b/six7*-deficient embryos). (C) *six3b/six7*-deficient embryos injected with 500 pg synthetic *six3b^{vu87}* RNA. (D) *six3b/six7*-deficient embryos injected with 5 pg *six3b* RNA. This amount of *six3b* RNA often causes dorsalization. Embryos in top of each panel are representative individuals from the groups below. Scale bar for embryos in top part of each panel is 200 μm .

Figure S2. Brain-Specific Randomized Laterality in *six3b/six7*-Deficient Embryos

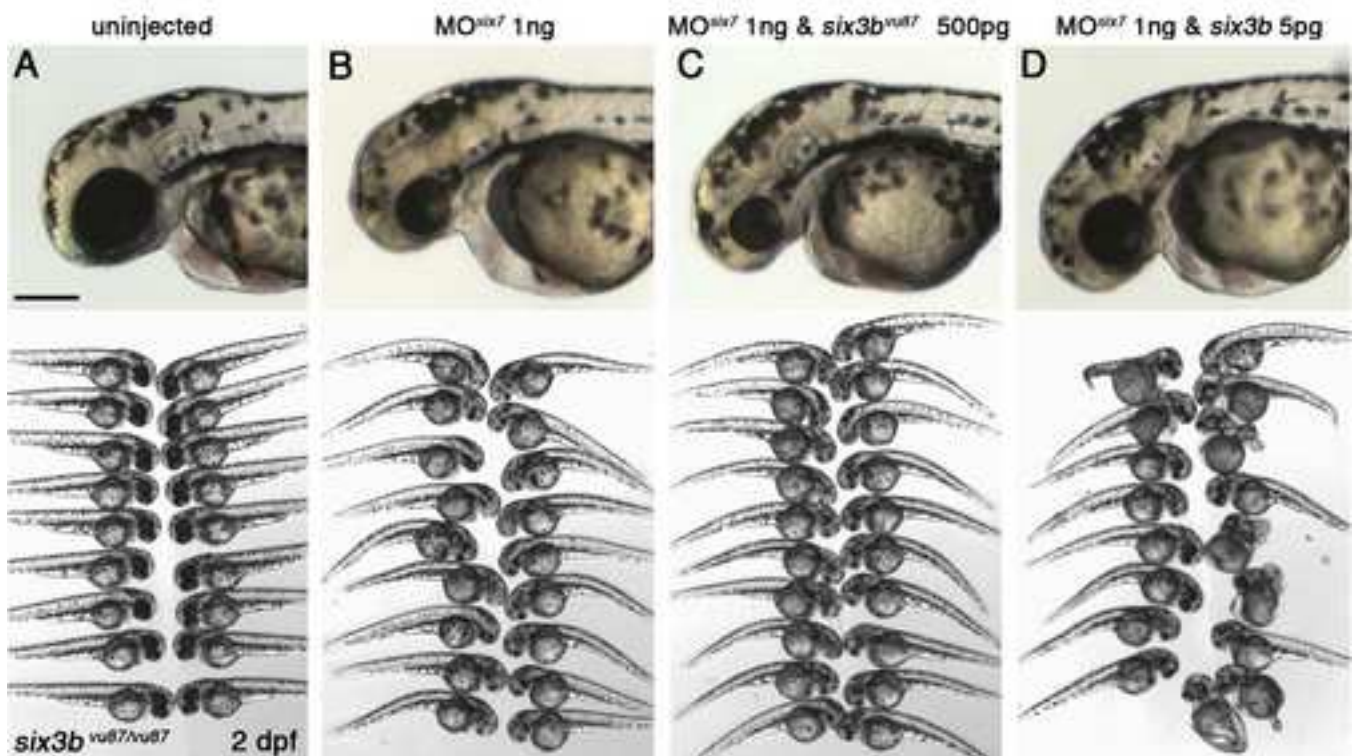
(A-D) Graphs representing left-right organ localization (A, B, D) or left-right differences (C) in control and *six3b/six7*-deficient embryos. Control embryos are *six3b^{vu87/+}* or *six3b^{vu87/vu87}*, and present wild-type phenotypes. For exact embryo numbers see text. Differences between control and *six3b/six7*-deficient embryos in (A-C) are highly significant (Chi Square test; $p < 0.001$). In (A) and (B), only embryos in which parpineal localization could be seen clearly were scored.

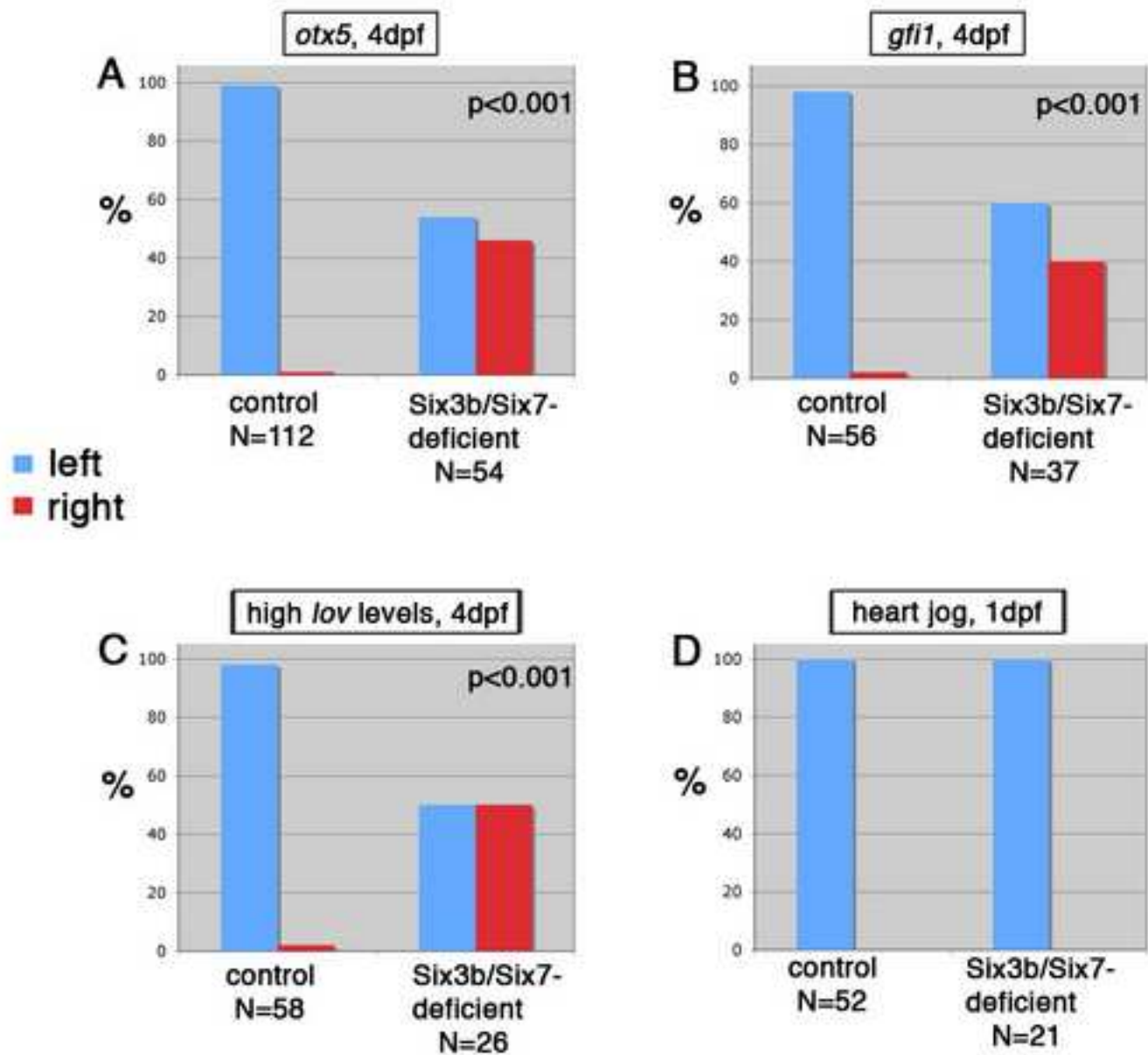
Figure S3. Precocious Nodal Pathway Activity in *six3b/six7*-Deficient Embryos

At 18-20s stage, only 5% of embryos in a clutch comprising approximately 1:1 ratio of *six3b*^{vu87/+} and *six3b*^{vu87/vu87} embryos express *lft1* in the diencephalon. When injected with MO^{six7}, the fraction of *six3b*^{vu87/vu87}, but not *six3b*^{vu87/+} embryos, that express *lft1* increases significantly (Chi Square test; p<0.001). Exact embryo numbers and percentages are depicted.

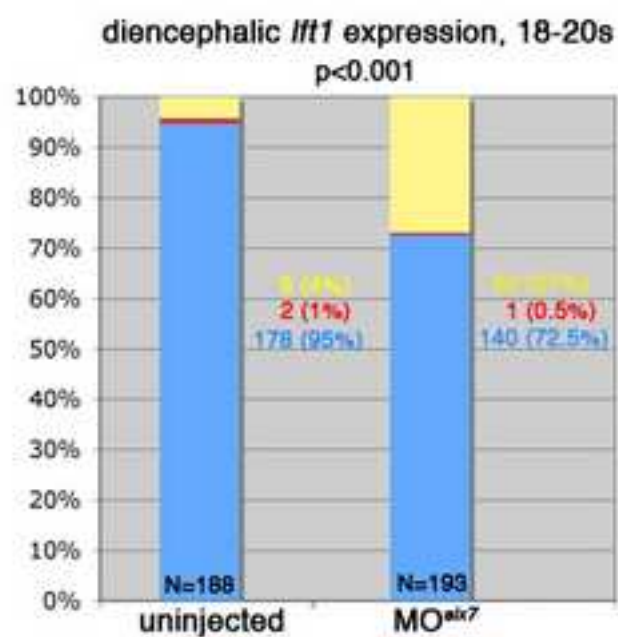
Figure S4. Prechordal Plate Appears Normal in *six3b/six7*-Deficient Embryos

(A, C, E, G) Control, uninjected embryos (*six3b*^{vu87/+} or *six3b*^{vu87/vu87}), stained with prechordal plate markers at the end of gastrulation (9.5-10 hpf). (B, D, F, H) Representative sibling embryos injected with MO^{six7}.





- no *lft1* expression, *six3b*^{vu87/+} or *six3b*^{vu87/vu87}
- *lft1* expression, *six3b*^{vu87/+}
- *lft1* expression, *six3b*^{vu87/vu87}



six3b^{vu87/+} , *six3b*^{vu87/vu87}

