Supplementary Information for

Embryonic regeneration by relocalization of the Spemann Organizer during twinning in *Xenopus*

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This PDF file includes:

Figs. S1 to S3
Captions for Movies S1 to S4

Other supplementary materials for this manuscript include the following:

Movies S1 to S4

Supplementary Information Figures

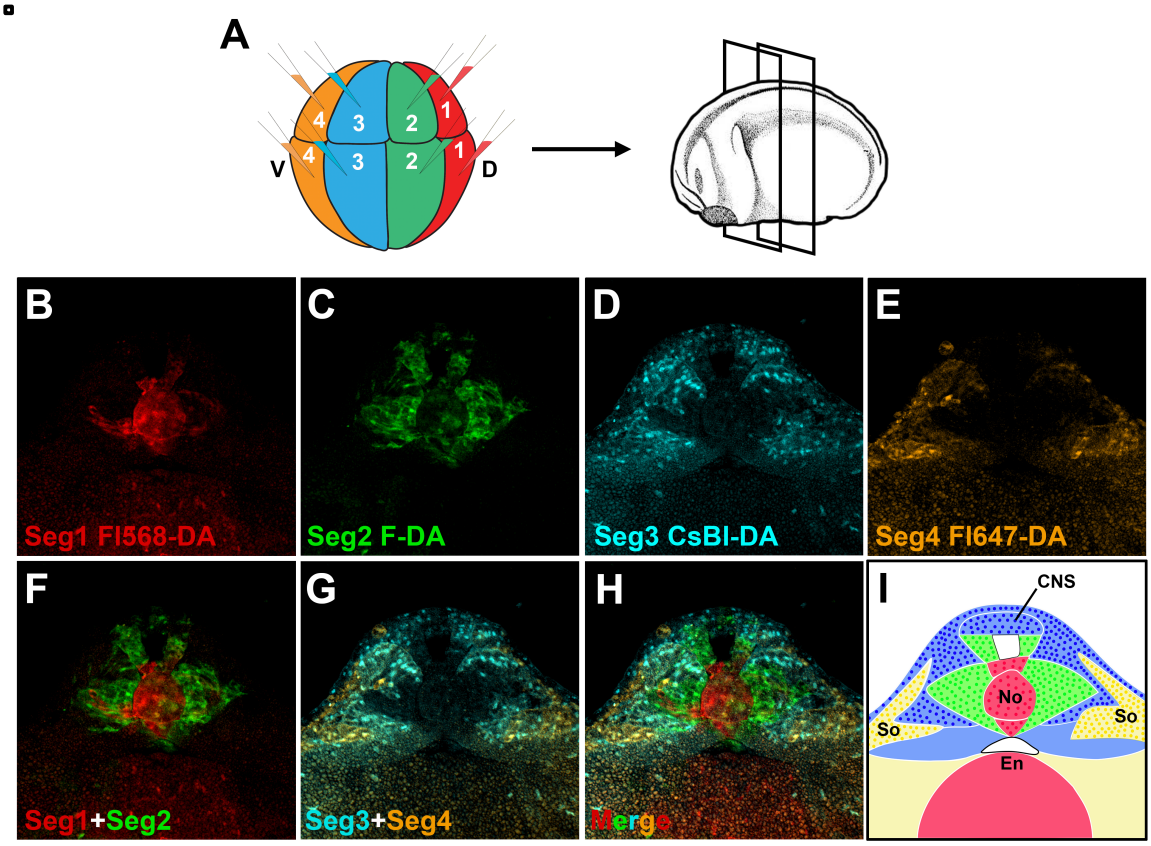

Fig. S1. The *Xenopus* somite has a highly stereotypical development that can be followed by labeling the four segments of the *Xenopus* gastrula at 16-cell stage with red, green, blue, and orange conjugated Dextran amine. (*A*) Experimental diagram; embryo received 16 injections and Vibratome sections were prepared at tailbud stage. (*B*) Segment 1 (red Fl568-DA) gave rise to notochord, hypochord, dorsal endoderm and ventral-most CNS, with only a small contribution to the medial somite. (*C*) Segment 2 (green, Fluorescein-Dextran amine, F-DA) gave rise to most of the medial somite and spinal cord. (*D*) Segment 3 (Cascade Blue-Dextran amine, CsBl-DA) gave rise to most of the lateral somite, dorsal CNS and epidermis. (*E*) Segment 4 gave rise to the outer-most parts of the somite, intermediate mesoderm, lateral plate and ventral epidermis (not shown). (*F*) Descendants of the dorsal segments 1 and 2. (*G*) Descendants of ventral segments 3 and 4. (*H*) Merge of the four-channel confocal image (n = 10, four independent experiments). (*I*) Diagram summarizing the origins of the somite in the *Xenopus* embryo. Images were acquired with an LSM880 Zeiss confocal microscope equipped with four lasers. Regrettably embryos injected in this way are fragile and photosensitive, and they do not survive bisection.

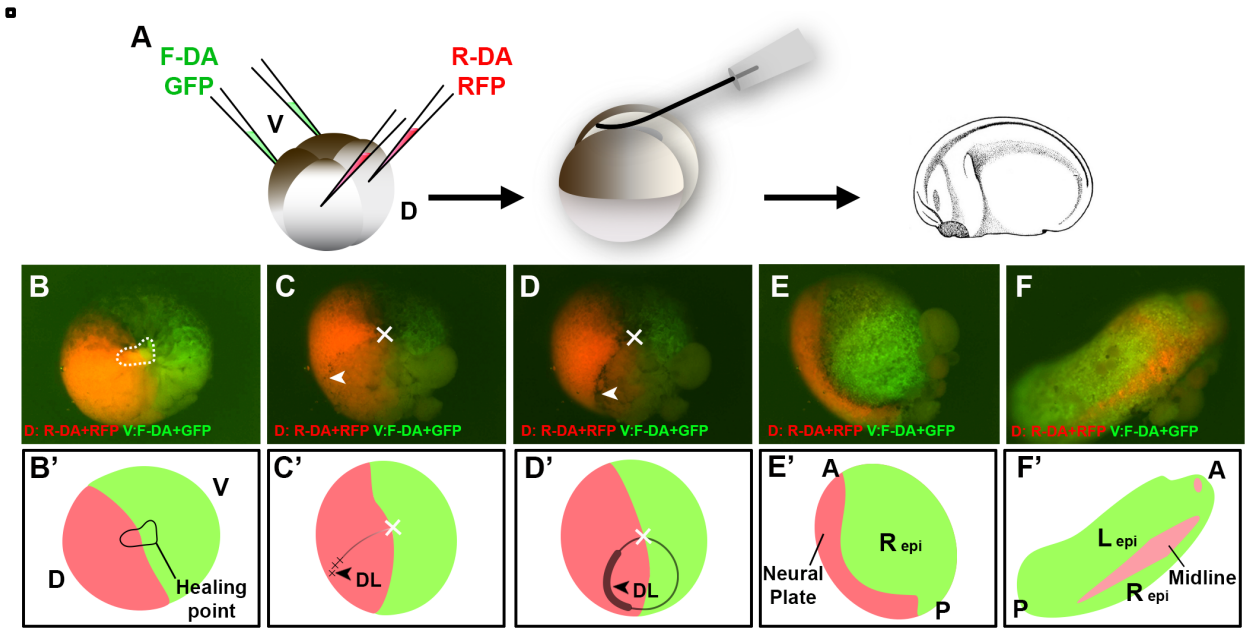

Fig. S2. Still images from Movie S3 showing that the dorsal lip was formed at a distance of about 90° from the healing point, and that all the epidermis was derived from ventral tissues in a regenerating *Xenopus* half-embryo. (*A*) Diagram of the experiment. At the 4-cell stage the two dorsal blastomeres were marked in red with a mixture of Rhodamine Dextran amine (R-DA) plus a cocktail of *RFP* mRNAs (consisting of soluble, membrane-targeted, and histone H2B RFP). The ventral side was marked in green with F-DA and a similar cocktail of *GFP* mRNAs to ensure high levels of fluorescence. Embryos were bisected at stage 8 and filmed until tailbud. (*B*-*B*') Embryo 60 min after bisection showing that the dorsal and ventral sides become juxtaposed at the healing point (indicated with a dotted line). (*C*-*C*') Half embryo about to start gastrulation; the position of the healing point is indicated with a cross. (*D*-*D*') At gastrula the dorsal blastopore lip (indicated with an arrowhead) is seen forming at a distance from the healing point (white cross). (*E*-*E*') At neurula, the half-embryo shows that the neural plate is of dorsal origin. (*F*-*F*') At tailbud, after neural plate closure, dorsal cells are seen in the midline and, importantly, both the left and right epidermis are derived from the ventral part of the embryo. DL, dorsal lip; D, dorsal; V, ventral; A, anterior; P, posterior; L epi; left epidermis; R epi, right epidermis.

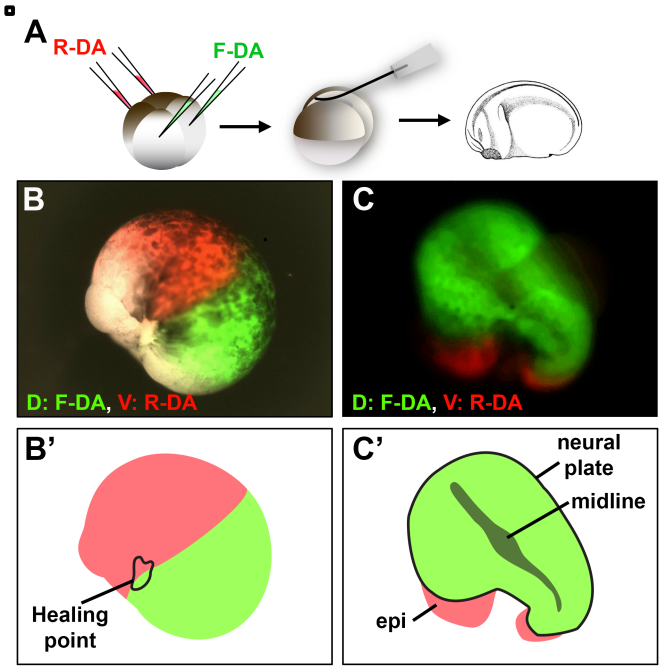

Fig. S3. Lineage tracing experiment of regenerating half embryo showing that the neural plate midline arises in the center of the original dorsal side. (*A*) Experimental design in which the two dorsal blastomeres were labeled with Fluorescein Dextran amine (F-DA) and the ventral ones with Rhodamine Dextran amine (R-DA) (note that the colors are reversed from those of the previous supplementary figure). (*B* and *B'*) Half embryo 60 min after bisection, note that the ventral and dorsal sides are juxtaposed to each other and only a small healing point is left at the junction of the two tissues. (*C* and *C'*) The neural plate of the half embryo derives from dorsal tissues that flank its midline. This implies that the point of invagination of the mesoderm that underlies the neural plate arises at a distance from the original dorsal-most side that was juxtaposed to the ventral-most cells at the start of the experiment. These still images were derived from Movie S4.

Supplementary Information Movies

Movie S1. Bisection of a midblastula *Xenopus laevis* embryo using an eyelash knife. Movie starts with removal of the chorion (the zona pellucida of the mouse) and ends with separation of vegetal cells with forceps.

Movie S2. Healing of half embryo after bisection by convergence of cells from multiple directions on a central healing point. The movie spans 60 min after bisection at midblastula. See also Fig. 1.

Movie S3. Regenerating embryo labeled in green in the ventral half and red in the dorsal half. The healing point can be seen at the beginning. Note that dorsal blastopore lip forms at a distance of the healing point, and that the skin ectoderm derives from ventral cells on both side of embryo. Still images of this movie are presented in Fig. S2.

Movie S4. The neural plate is derived from the dorsal side of the half embryo. Embryo was labeled at the 4-cell stage with green F-DA on the dorsal and red R-DA on the ventral. The neural plate midline forms within the dorsal region, not at the border of dorsal and ventral cells. Still images of this movie are presented in Fig. S3.