Figure S1

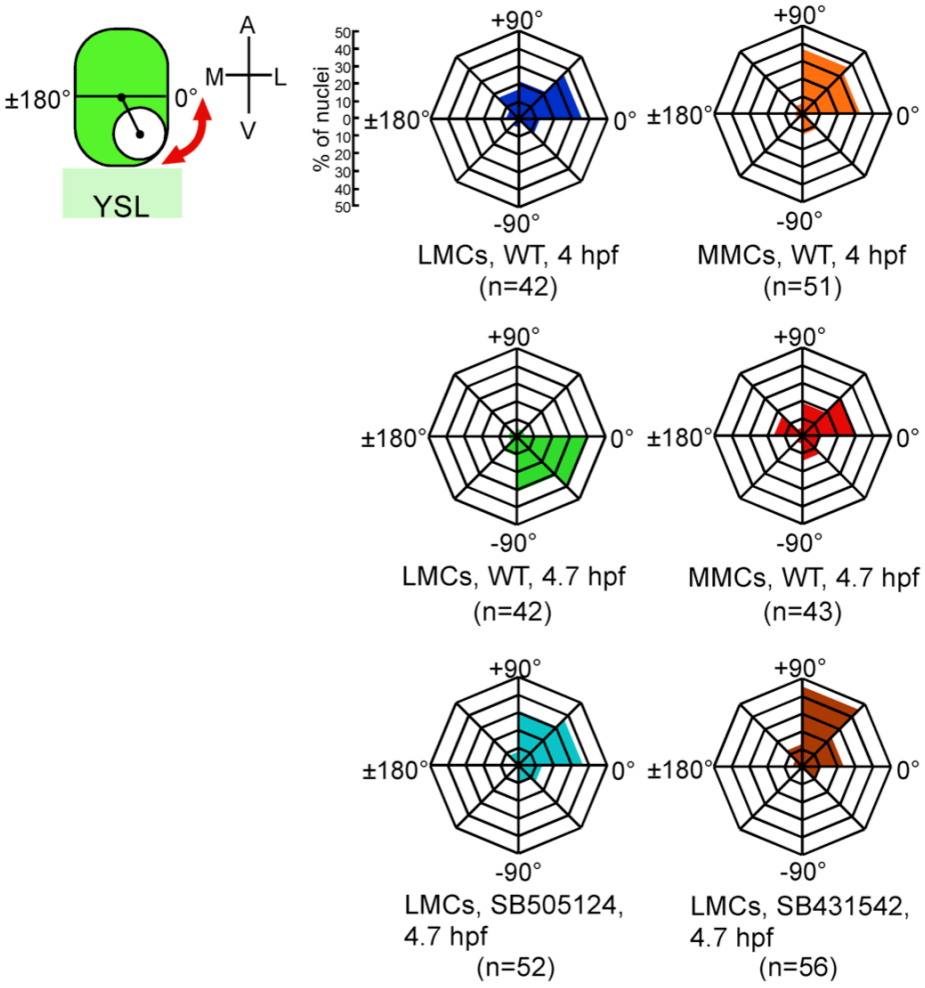

Figure S1. Nuclear positional changes in LMCs and MMCs between 4.0 and 4.7 hpf

Angles between the center of the cell and the nucleus relative to the medial-lateral axis were measured (red line). n = number of nuclei examined.

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Figure S2

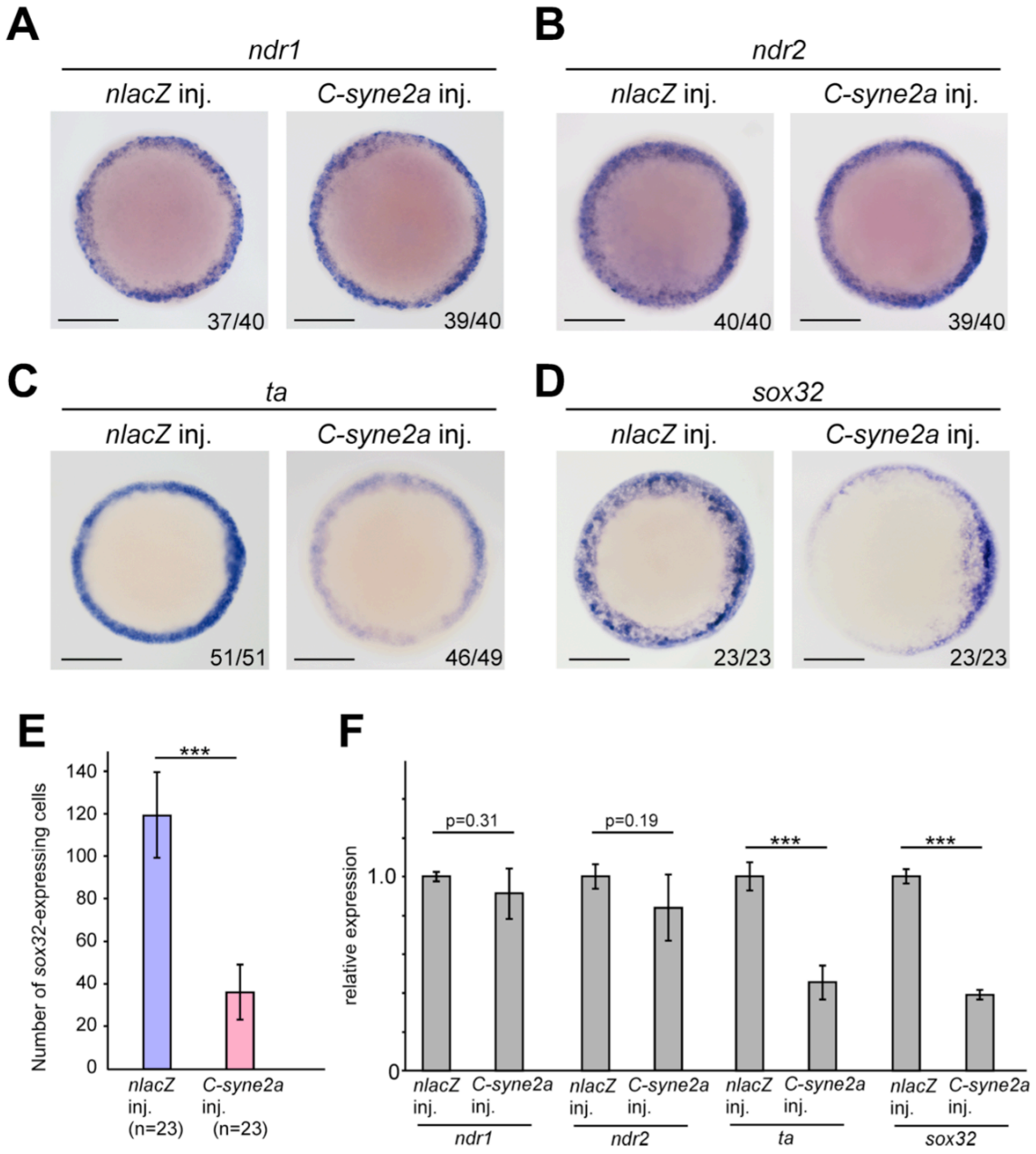

Figure S2. Expression of *ndr*, mesoderm, and endoderm genes in *C-syne2a*-overexpressing embryos

(A-D) Expression of *ndr1*, *ndr2*, *ta*, and *sox32* was examined by WISH in *nlacZ* or *C-syne2a* mRNA overexpressing embryos at 5.3 hpf. The number of embryos examined is shown at the lower right corner of each panel. Animal pole views. Scale

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bars: 200 μm. (E) Number of *sox32*-expressing endodermal cells in *nlacZ*- or *C-syne2a*-overexpressing embryos at 5.3 hpf. n = number of embryos examined. ***$p < 0.001$. (F) Expression of *ndr1*, *ndr2*, *ta*, and *sox32* was examined by qPCR in *nlacZ* or *C-syne2a* mRNA-overexpressing embryos at 5.3 hpf. Error bars represent standard deviations of three or four independent experiments. ***$p < 0.001$.

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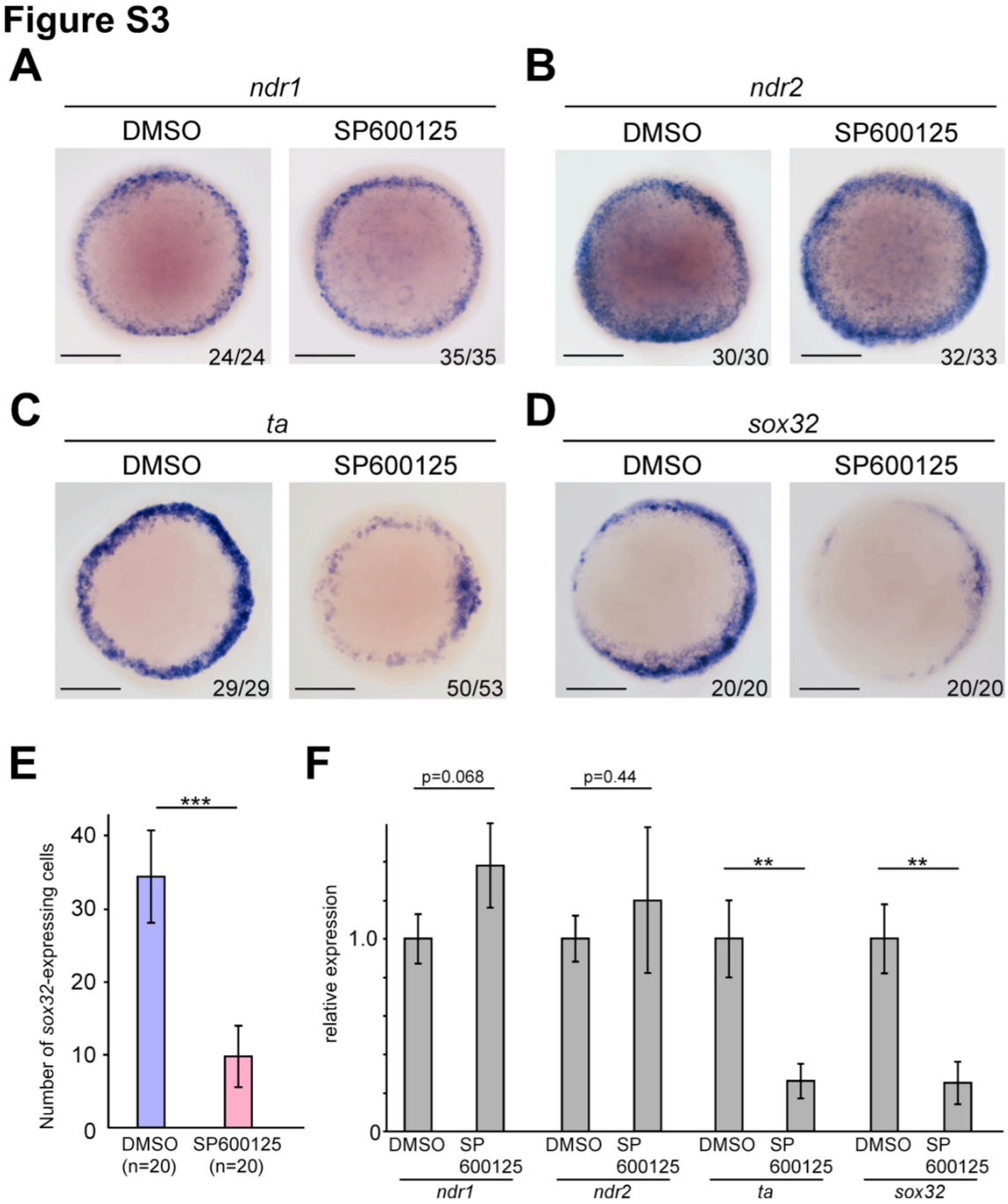

Figure S3. Expression of *ndr*, mesoderm, and endoderm genes in SP600125-treated embryos

(A-D) Expression of *ndr1*, *ndr2*, *ta*, and *sox32* was examined by whole-mount *in situ* hybridization in DMSO- or SP600125-treated embryos at 4.7 hpf. Number of embryos

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examined is shown at the lower right corner of each panel. Animal pole views. Scale bars: 200 μm. (E) Number of *sox32*-expressing endodermal cell was counted in DMSO-, or SP600125-treated embryos. n = number of embryos examined. ***$p <$ 0.001. (F) Expression of *ndr1*, *ndr2*, *ta*, and *sox32* was examined by qPCR in DMSO- or SP600125-treated embryos at 4.7 hpf. Error bars represent standard deviations of three or four independent experiments. ***$p < 0.001$. ** $p < 0.05$.

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Figure S4

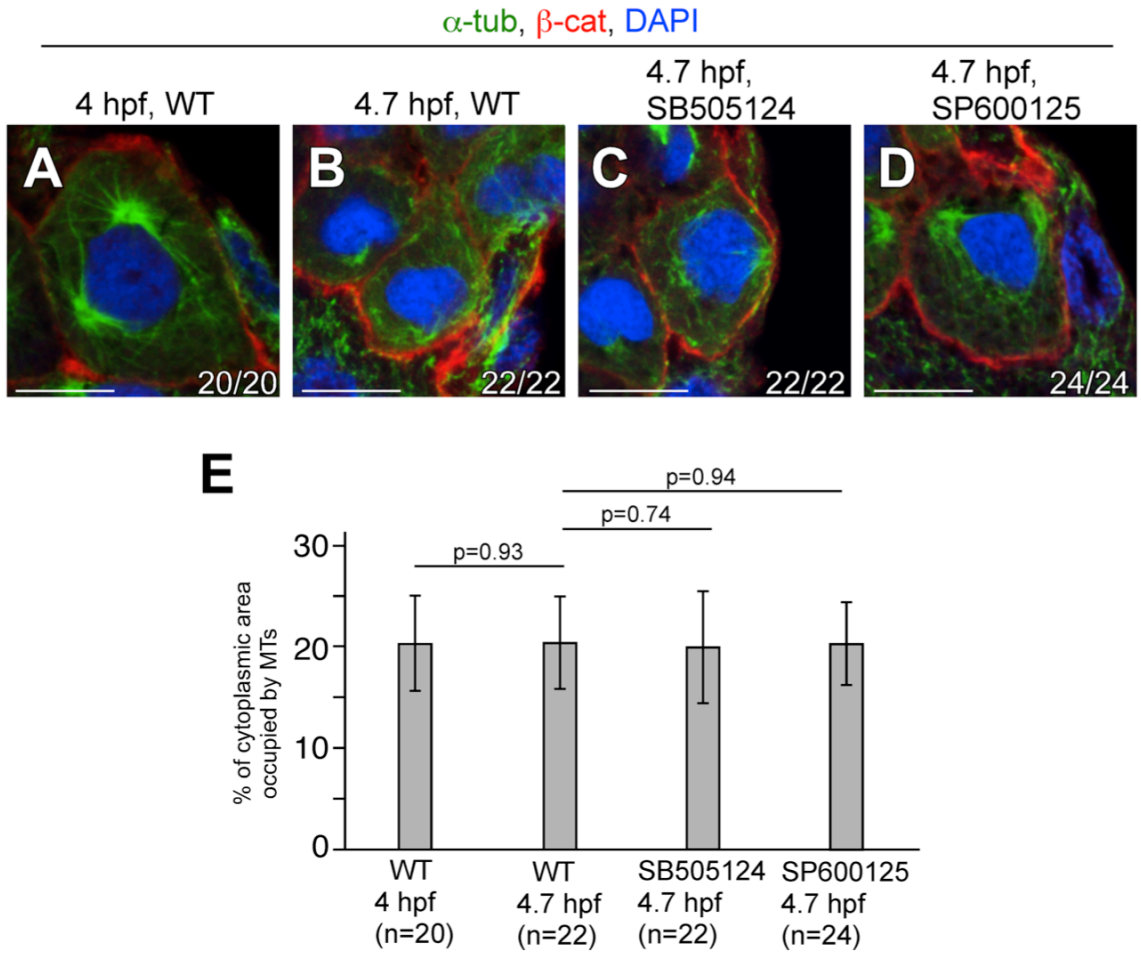

Figure S4. MT formation was not affected by inhibition of Nodal or JNK signaling

(A-D) Cross sections of WT, SB505124-, and SP600125-treated embryos; MTs, cell membrane, and nuclei were visualized by α-tubulin (α-tub), β-cat, and DAPI staining, respectively at 4.0 or 4.7 hpf. Number of nuclei examined is shown at the lower right corner of each panel. Scale bars: 10 μm. (E) Percentage of cytoplasmic area occupied by MTs in LMCs of WT, SB505124-, and SP600125-treated embryos.

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Figure S5

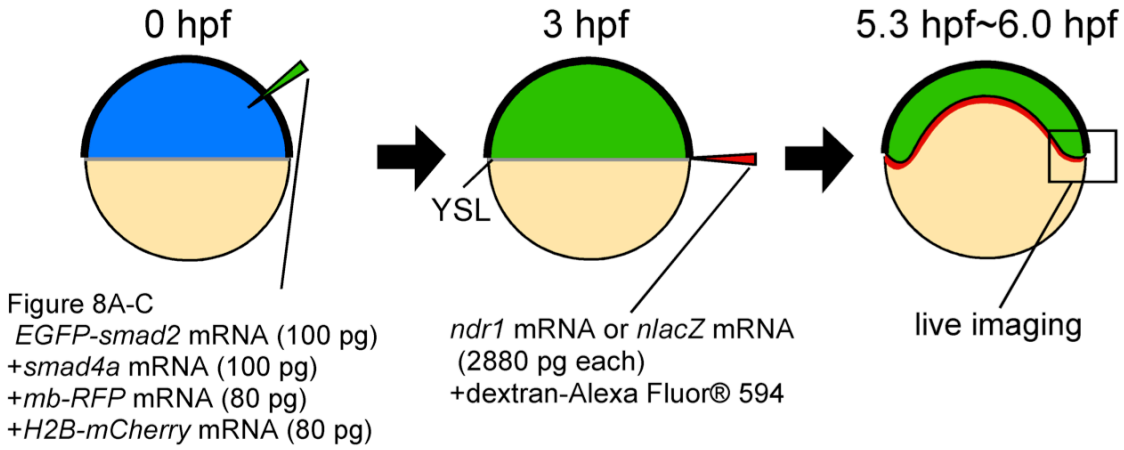

Figure S5. Experimental design of work presented in Figure 8

A mixture of *EGFP-smad2*, *smad4a*, *mb-RFP*, and *H2B-mCherry* mRNAs was injected into WT embryos at the one-cell stage. At 3 hpf, *ndr1* or *nlacZ* mRNA was injected with dextran-Alexa Fluor® 594 into the YSL, and confocal live images were captured at 5.3-6.0 hpf.

Table S1. Primer sequences used for quantitative real-time PCR

Click here to Download Table S1

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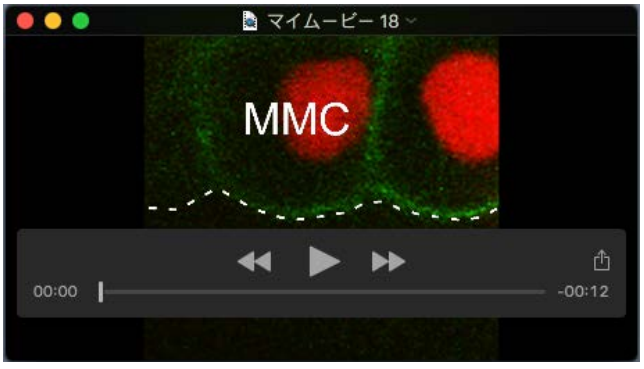
Movie 1

Time-lapse image of the nucleus in LMC between 4.0 and 4.7 hpf. A white dotted line indicate the boundary between the blastoderm and the YSL. Thick white lines indicate DNBs.



Movie 2

Time-lapse image of the nucleus in MMC between 4.0 and 4.7 hpf. A white dotted line indicate the boundary between the blastoderm and the YSL. Thick white lines indicate DNBs.

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