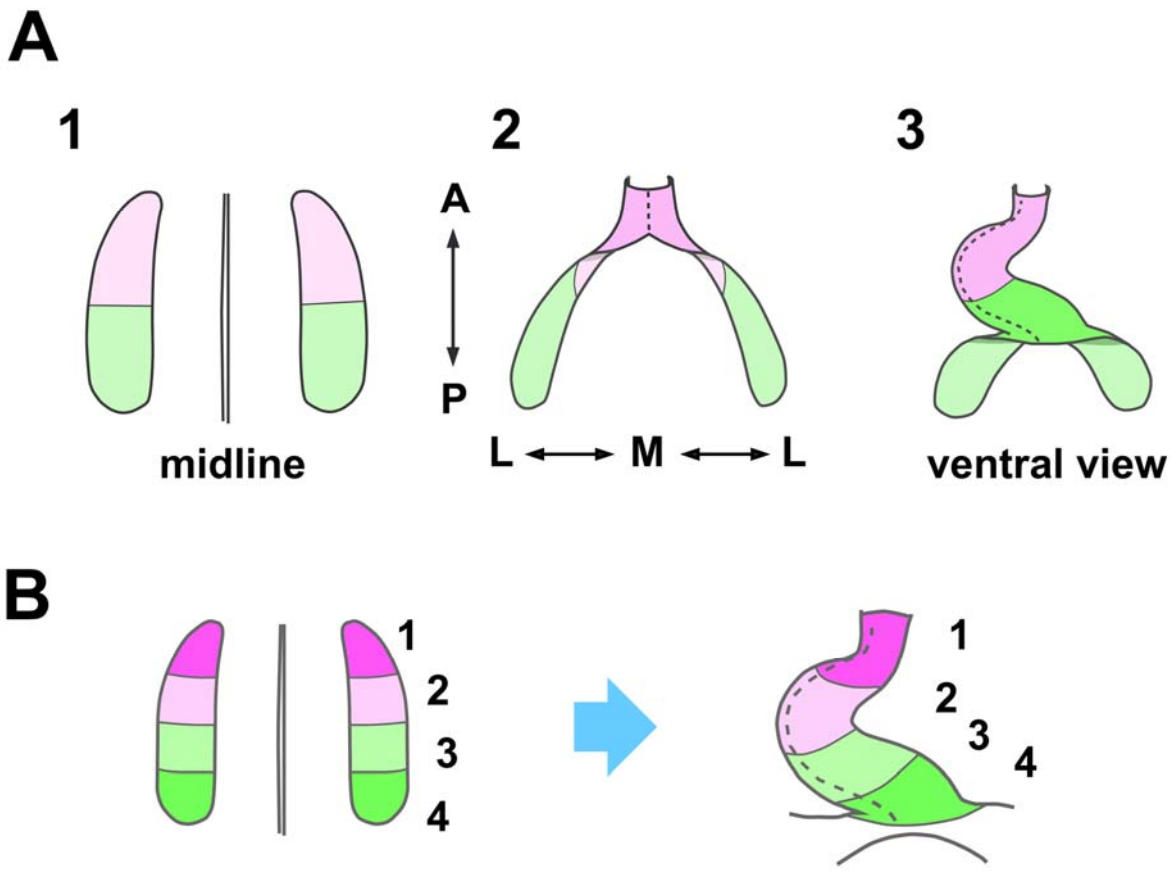
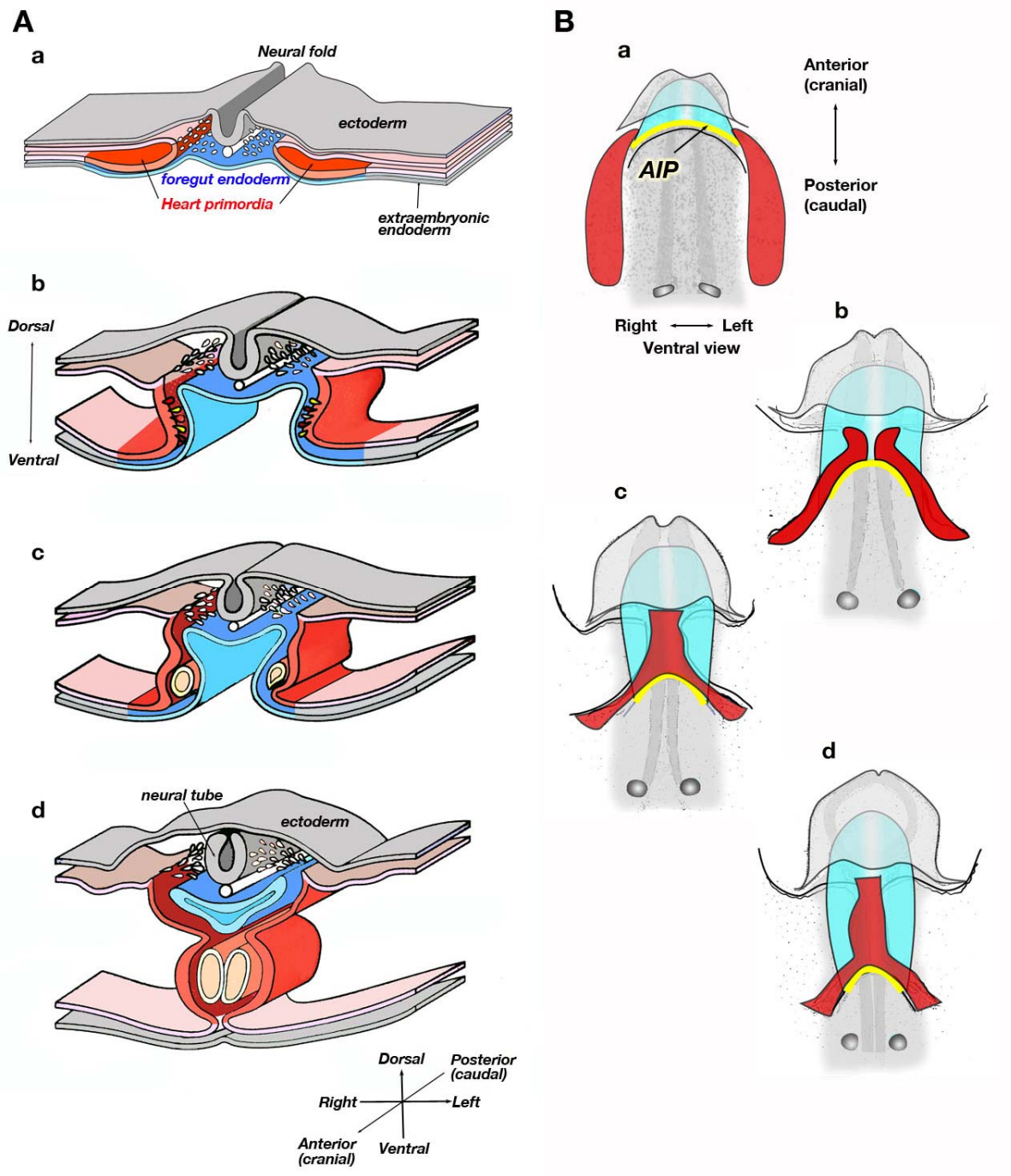
Supplementary Figures

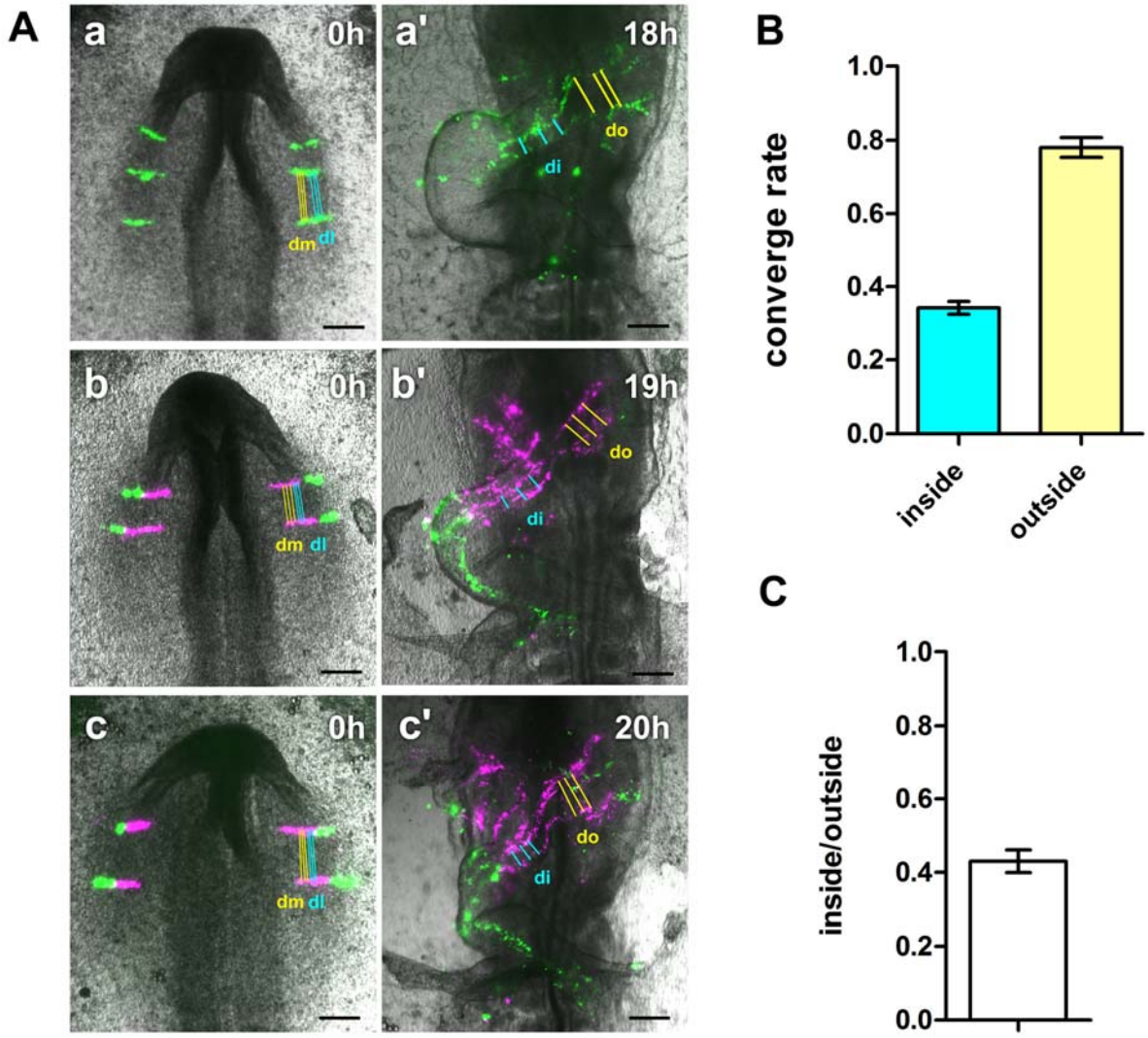


Supplementary Figure 1: Classical model of heart tube formation (ventral views). (**A**) The paired oblong heart primordia were believed in this model simply to fuse across the midline in anterior-to-posterior sequence, thereby extending the heart tube posteriorly. (**B**) Schematic heart fate map based on this model (DeHaan, 1965). A, anterior (cranial); P, posterior (caudal); M, medial; L, lateral sides of the heart primordia.



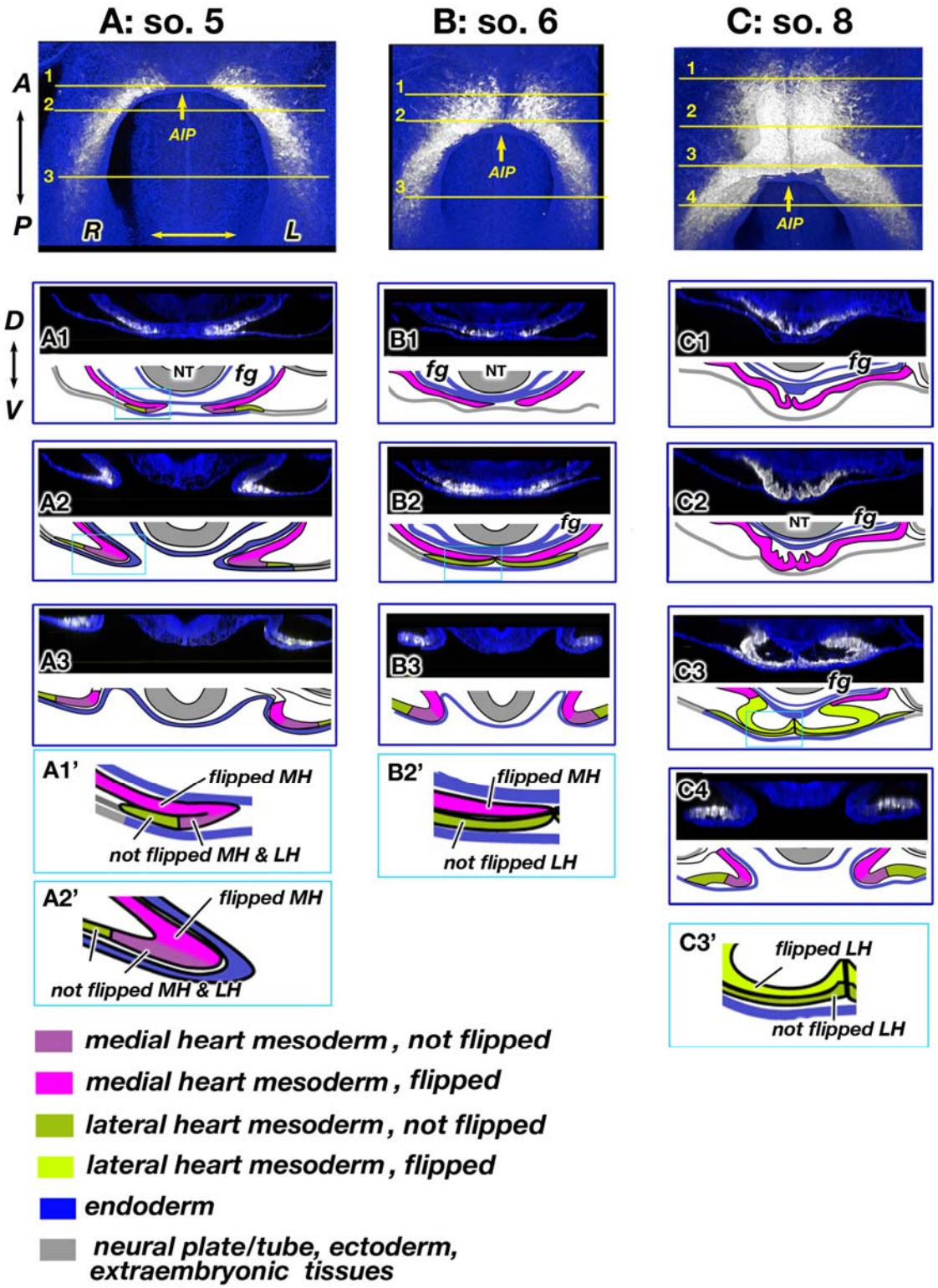
Supplementary Figure 2: Formation of the heart tube and foregut in the chick embryo. (**A**) Drawings of transverse slices through the developing heart region at around the 3- to 4-somite (Aa), 5-somite (Ab), 6- to 7-somite (Ac), and 9-somite (Ad) stages. The heart mesoderm (red), non-heart mesoderm (pink), endoderm (blue), notochord (white),

extraembryonic endoderm (gray), and ectoderm (gray) are shown. (Aa) The flat, bilateral heart primordia are separate structures lying on the left and right sides of the embryo. The foregut endoderm is located immediately ventral to the heart mesoderm. (Ab-d) As the endoderm folds to form the foregut in the midline, the bilateral heart mesoderm also fold toward the ventral midline and fuse, forming the heart tube ventral to the foregut. (**B**) Drawings of the heart primordia and foregut shown ventrally and illustrating the concomitant formation and elongation of the mesodermal heart tube and the endodermal foregut. (Ba) At the 3- to 4-somite stage, a small foregut (light blue) is already formed within the ectodermal pocket (gray) at the anterior (cranial) end of the embryo. The heart primordia (red) are flat, separate, left and right structures. During subsequent development, the anterior intestinal portal (AIP, yellow arc) descends posteriorly, lengthening the foregut. Concomitant with this, the left and right heart primordia merge medially, forming and lengthening the heart tube. (Bb) At the 5-somite stage, the length of the foregut is slightly increased compared to that at the earlier stage (Ba). The left and right heart primordia are now approximated at the ventral midline of the embryo. (Bc) At the 7-somite stage, the paired heart primordia have just merged, forming the nascent, short heart tube at the midline. (Bd) The 9-somite stage. With further descent of the AIP, the heart tube and foregut lengthen posteriorly together. The drawings are modified and colored from pictures in 1920 “The Early Embryology of the Chick. by Patten BM.” Philadelphia: P. Blakiston's Son and Co., and Figure 15.3 in “Developmental Biology” 7th ed. By Scott Gilbert, Sinauer Association Inc.

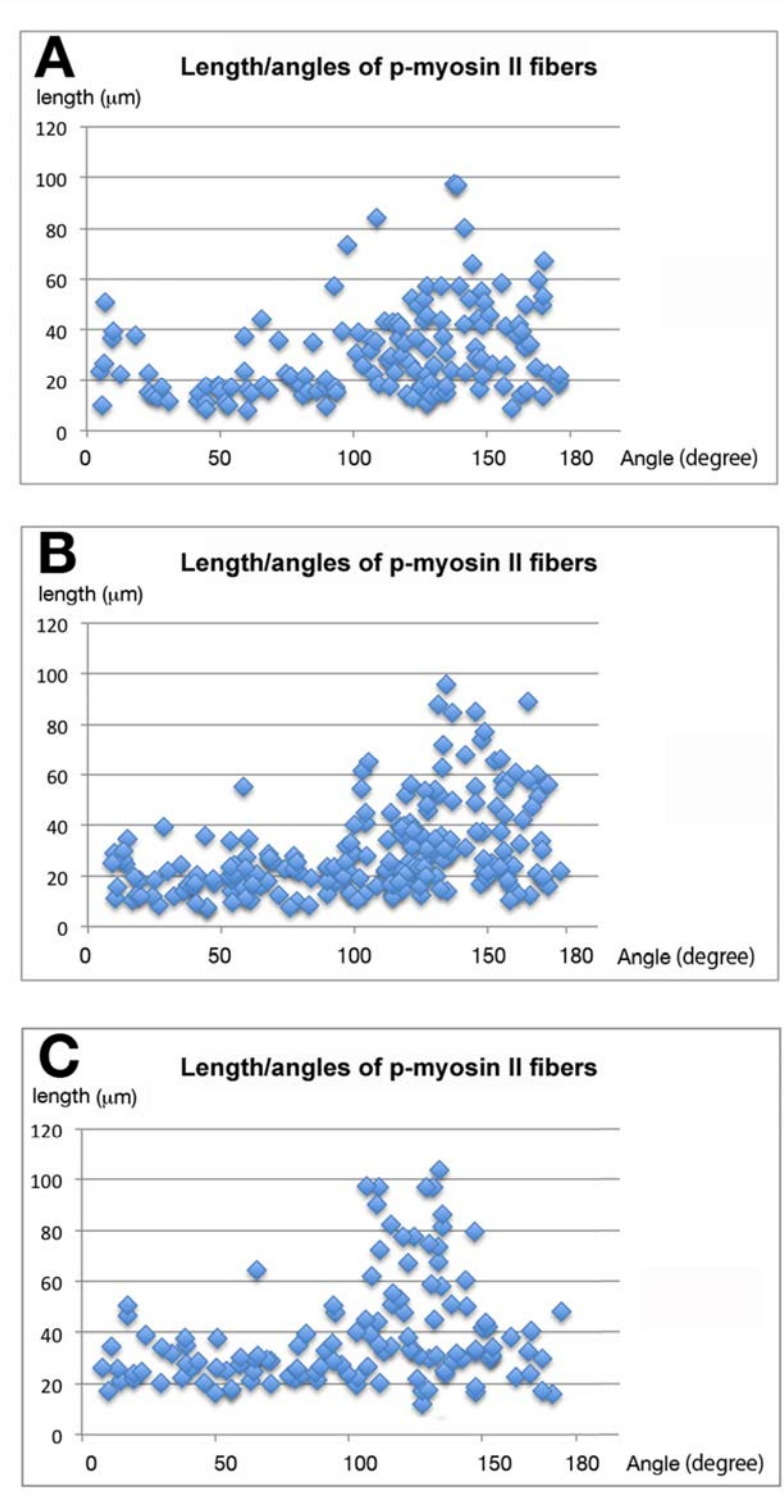


Supplementary Figure 3: The heart primordia converge along their original AP axis when forming the tubular heart. (**A**) The distances between the labeled cell stripes in the MHP were measured just after labeling (a-c: t0) and after the heart tube was formed (a'-c'). The embryos shown in a,a' and b,b' are the same ones shown in Figure 2 and Figure 3A in the main paper, respectively. The measurements were performed at three positions for each inside of the heart tube (di: distance at inside, cyan lines in a',b',c') and outside of the heart tube (do: distance at outside, i.e., the dorsal heart mesoderm, yellow lines in a',b',c'). Similarly, for the initial time point (t0, a,b,c), distances between cell stripes were measured at three positions in each medial (dm: distance at the medial side, yellow lines in a,b,c) and lateral (dl: distance at the lateral side: cyan lines in a,b,c) parts of the MHP. The averages of the distances (di, do, dm,

dl) at these three positions were calculated for each embryo (n=3). These average values were used for the following calculations. (**B**) The converge rate was computed by dividing di and do (distance at the end time-point) by dl and dm (distance at t0), respectively. Note that the distance between cell stripes at the inside of the heart tube (di) is markedly reduced compared to the distance at t0 (dl): (di/dl = 0.3414 ± 0.0174 (SD)), whereas the reduction of the distance at the outside of the heart tube (do) is relatively moderate (do/dm = 0.7802 ± 0.02704 (SD)). (**C**) di was divided by do (di/do = 0.4305 ± 0.03080(SD)) to compare the distance between stripes at inside of the heart tube (di) with the one at outside of the heart tube (do) at the end time-point. Scale bars represent 200 μm.

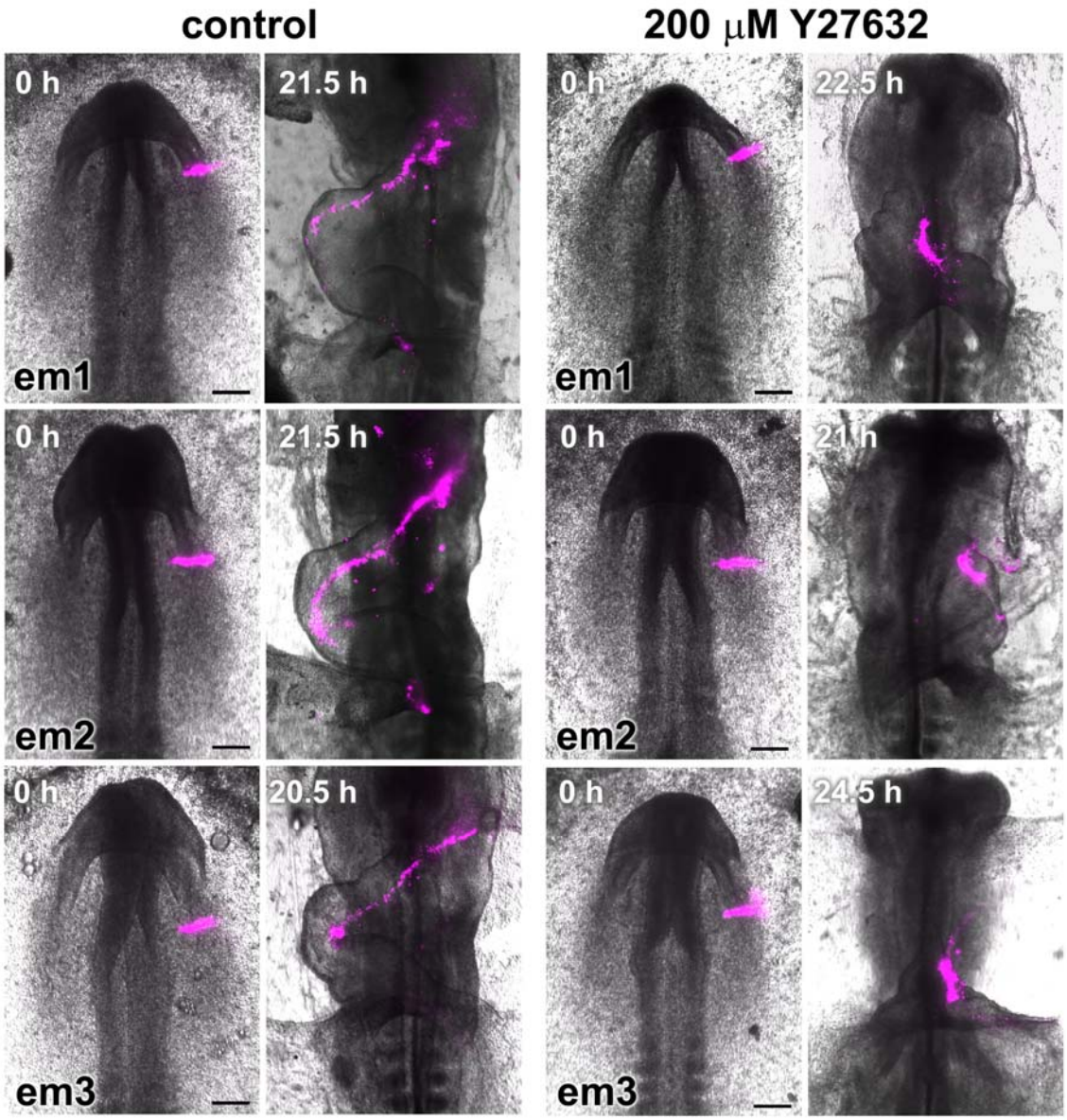


Supplementary Figure 4: The course of folding and merging of the heart primordia during heart tube formation in the chick. The heart primordia were labeled by whole-mount immunohistochemistry (IHC) with a cocktail of antibodies to tropomyosin (CH1) and myosin heavy chain (MF20) and were imaged using a confocal microscope. The top panels show volume renderings in ventral view. The lower panels show cross sections of the renderings at the levels indicated by yellow lines in the top panels. The actual heart fields are larger than the IHC labeling because the antibodies only recognize differentiating cardiac myocytes. The medial and lateral heart mesoderm are color-coded based on previous reports (Abu-Issa and Kirby 2007, 2008), as well as on our cell labeling experiments. Folding/flipping of the entire heart mesoderm progressively occurs from its medial (magenta) to lateral (green) sides, diagonally in the medioposterior direction. By this diagonal folding, the lateral heart primordia (LHP: green) are displaced to the posterior to the medial heart primordia (MHP: magenta).
(**A**) The embryo at the 5-somite stage corresponding to the ones shown in Figures 2D, 3Ad. (1) At the anterior region, the foregut is already formed and most of the extent of the MHP (magenta) is flipped, except for its lateral-most part. (2, 3) At more posterior levels, either a lesser extent of the MHP has completed flipping (2) or almost no flipping has occurred yet (3). At this stage, the entire LHP remains unflipped at all anteroposterior levels (1-3).
(**B**) The 6-somite stage embryo corresponding to the embryos in Figures 2E, 3Ae. The diagonal folding brings the mediolateral midpoint of each heart primordium (the boundary between the MHP (magenta) and the LHP (green)) to the embryonic midline for initial fusion of the paired primordia. (1) The MHP have completed folding, lying ventral to the foregut, but the left and right MHP have not yet met at the midline. (2) Near the boundary of the MHP and LHP, the paired heart primordia are merged at the midline. The LHP are not yet flipped at this level. (3) At the more posterior level, folding/flipping of the heart primordia has just started.
(**C**) The 8-somite stage embryo corresponding to the ones shown in Figures 2G, 3Af. Fusion of the heart primordia proceeds bidirectionally. At the anterior portion of the nascent heart tube, the paired MHP, which have completed folding, are now undergoing progressive fusion in the anterior direction. (1, 2) At both anterior-posterior levels shown here, the left and right MHP are merged, forming the anterior heart tube. (3) In contrast to the MHP, folding and fusion of the LHP occur simultaneously. The paired LHP are merged, forming the posterior heart tube. (4) The LHP are still separated on the left and right sides, remaining unflipped.
The areas indicated by rectangles in A1, A2, B2, and C3 are enlarged and shown in A1', A2', B2', and C3', respectively, to show flipped and unflipped heart primordia. AIP: anterior intestinal portal, fg: foregut, NT: neural tube, L: left, R: right, MH: medial heart mesoderm, LH: lateral heart mesoderm.



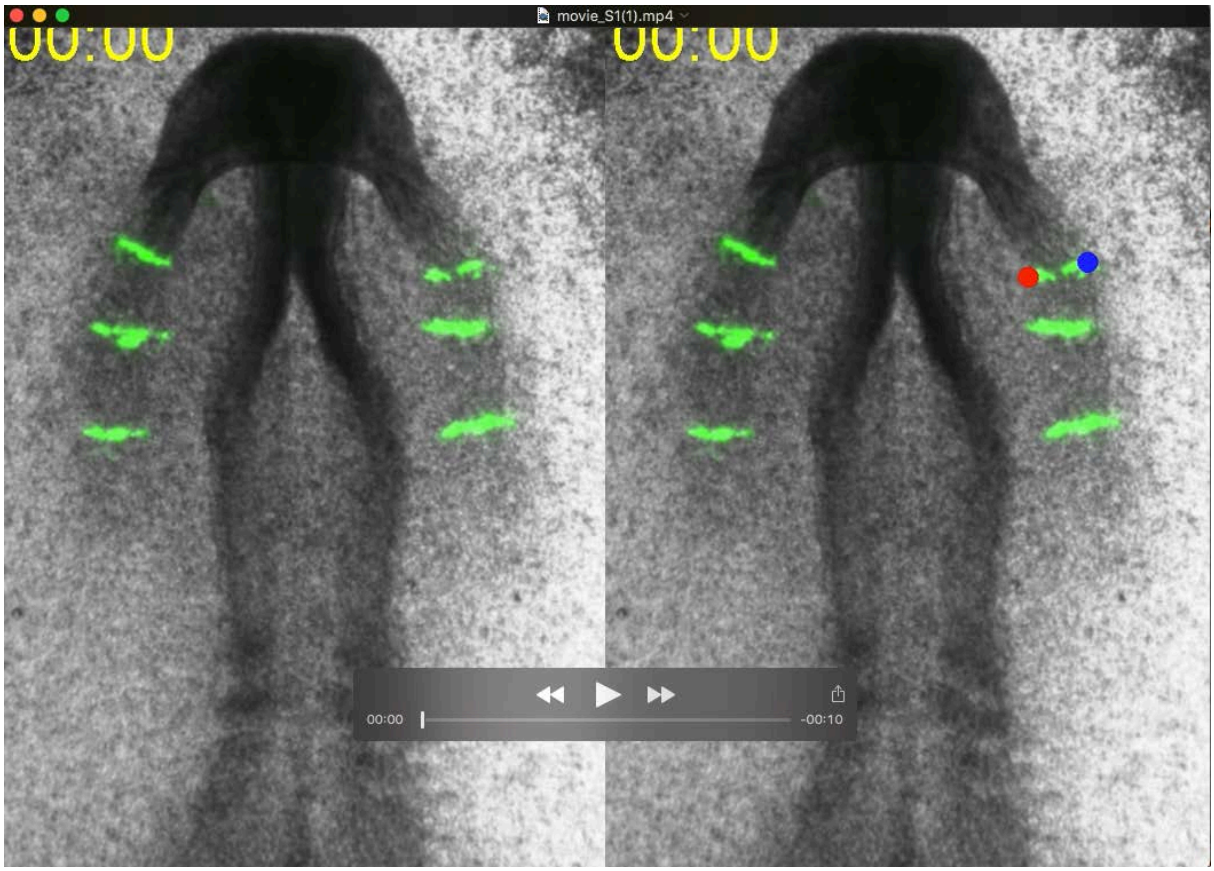
Supplementary Figure 5: Phospho-myosin supracellular cables are aligned perpendicularly to the direction of the extension of the heart mesoderm. The angle and

length of phosphorylated myosin (p-myoII) cables visualized by whole-mount immunofluorescence are plotted in the X and Y axis, respectively. Each diamond indicates an individual p-myoII fiber. Short p-myoII cables were localized in cell boundaries without bias in their orientation; however, long p-myoII fibers (> 50 μm) were aligned between 100º to 180º, with a peak of 120º to 130º. The peak angles are concordant with an orientation perpendicular to the direction of the observed extension of the heart mesoderm, which is approximately 36º (Fig. 4Ba).

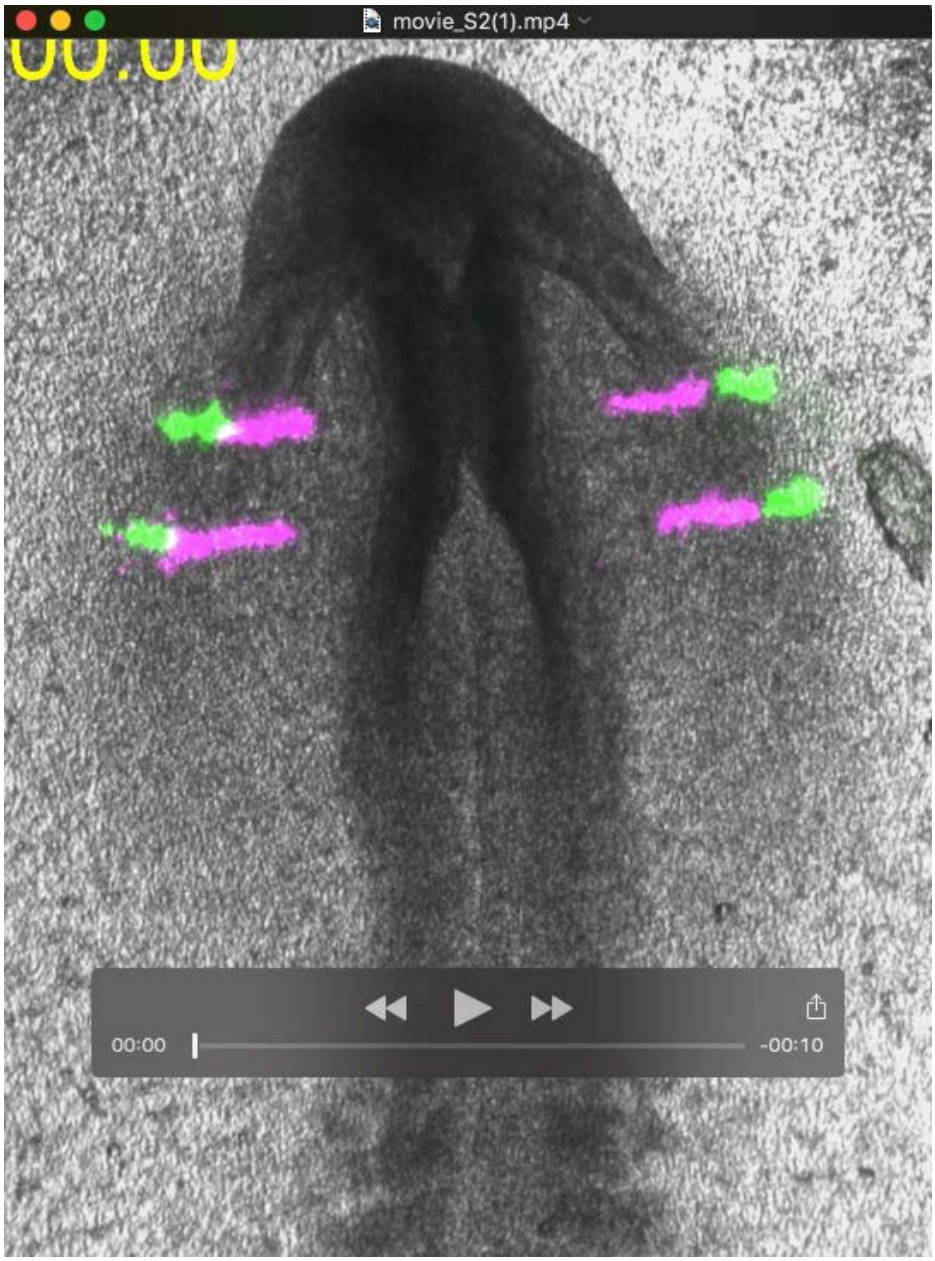


Supplementary Figure 6: ROCK-inhibitor treatment consistently blocked heart extension. Three examples of embryos treated with the ROCK inhibitor Y27632 are shown. Control embryos with similar dye labeling are aligned for comparison. Em1 of Y27632-treated embryo is the same embryo as the one shown in Figure 4C in the main paper. The inhibitor-treated embryos consistently exhibited failure of extension of the labeled cell cluster and striking shortening of the heart tube (n=7). Based on confocal imaging of fixed samples (the embryo from movie S6, as well as from other Y27632-treated samples) and paraffin sectioning, normal spatial relationships seemed to be maintained between the heart and endoderm or other adjacent structures after Y27632 treatment. Thus, it seems unlikely that the inhibition of CE in these embryos was due to a disconnection of the heart mesoderm surrounding tissues, preventing its stretching by the endoderm. Scale bars represent 200 μm.

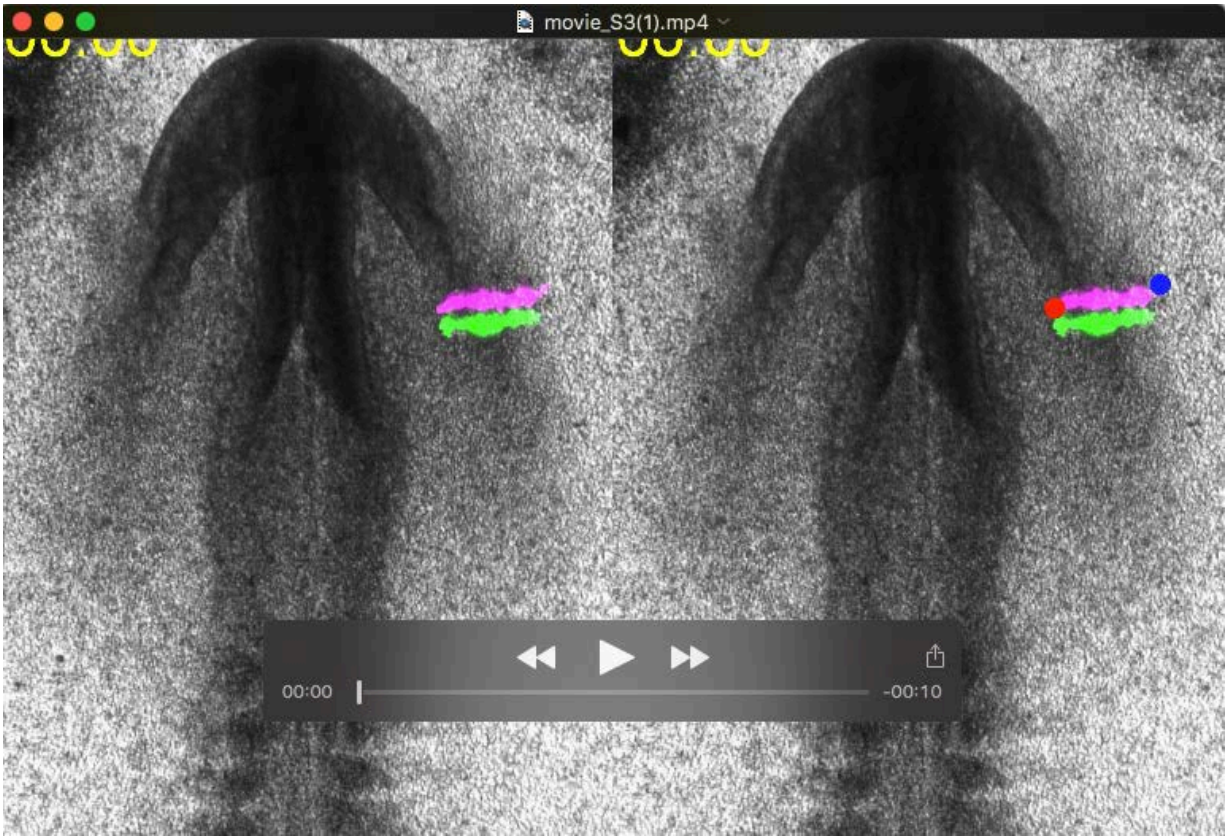
Supplementary Movies



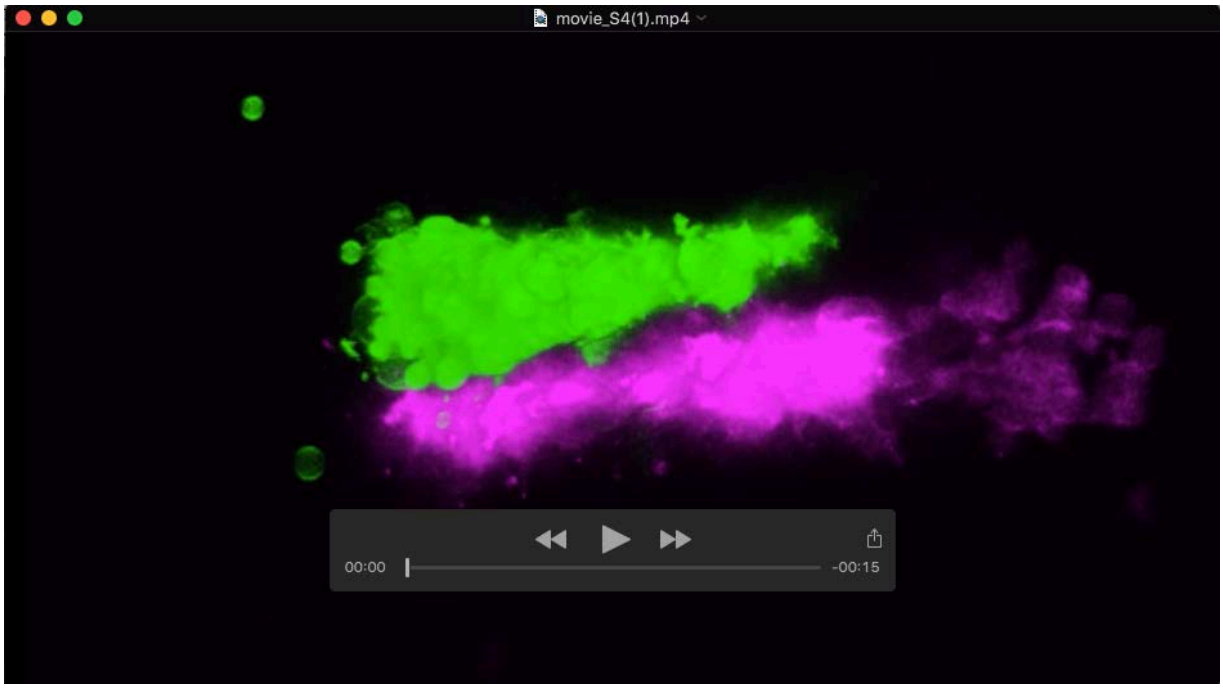
Movie S1. Time-lapse imaging of the dynamic movements of labeled cell clusters in the medial heart primordia (MHP), showing tissue dynamics during heart tube formation. Stripes of cells in the paired MHP were labeled with DiO (green) at three anteroposterior levels. Shown here and in subsequent supplementary movies, are ventral views (embryo's right side is on the left side of each frame; anterior is at the top of each frame), with relative times after the initiation of recording indicated in the upper left corner of each frame. Selected images are shown in Figure 2. Annotated, duplicated time-lapse images are shown in the right panel. Red and blue dots depict the original medial and lateral edges of the labeled cell stripe, respectively.



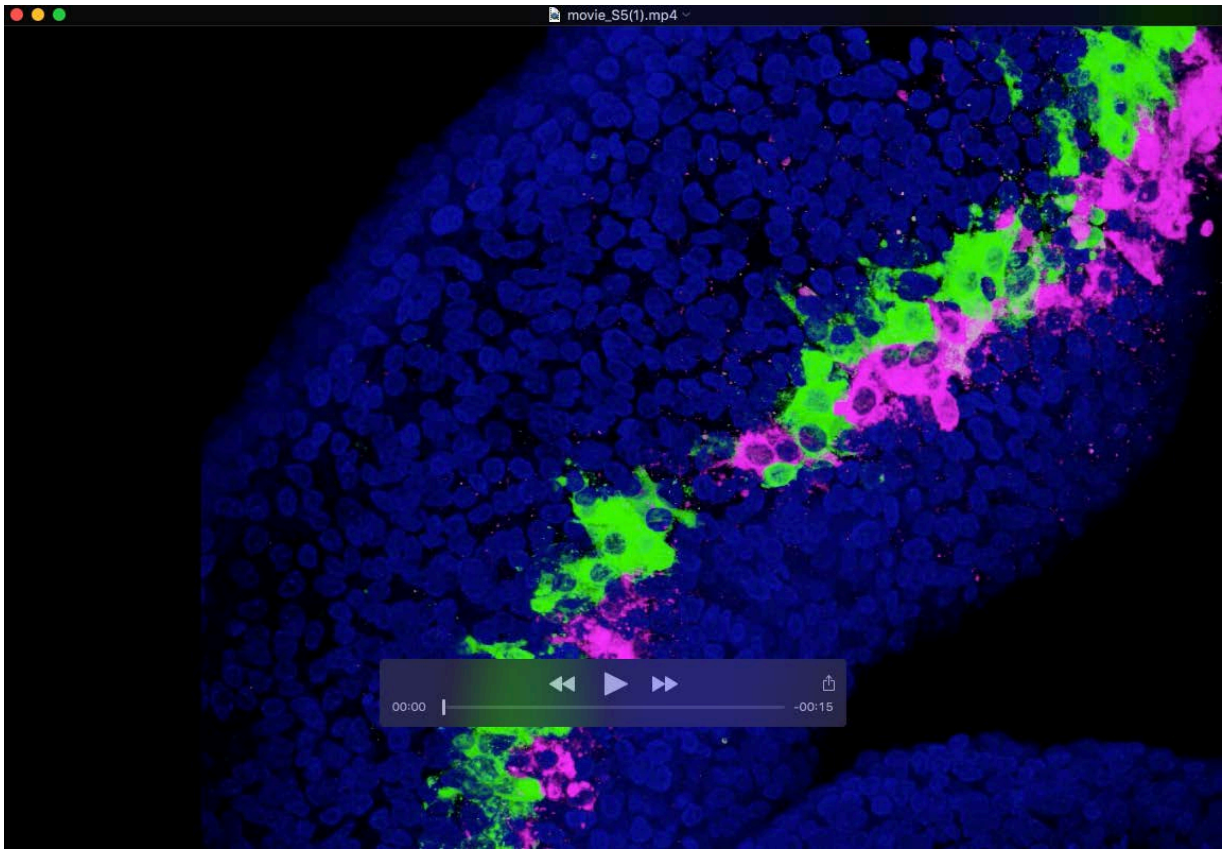
Movie S2. Time-lapse imaging of the dynamic movements of medial and lateral cell populations in the bilateral heart primordia during heart tube formation. Stripes of cells were labeled with DiI (magenta) or DiO (green) in the primordia at two mediolateral positions and two anteroposterior levels prior to their fusion. Selected images are shown in Figure 3A.



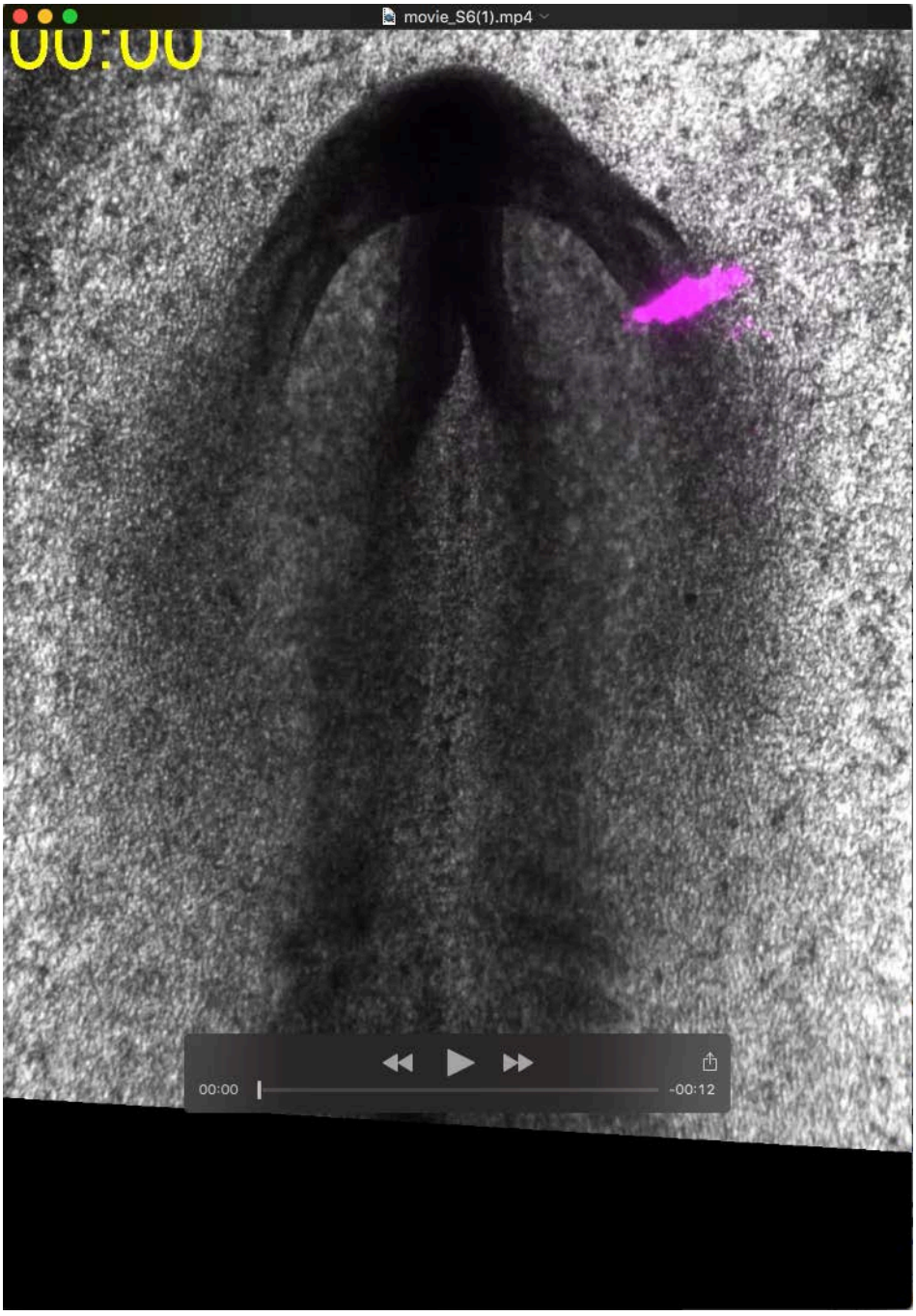
Movie S3. Time-lapse imaging of cells labeled at two neighboring anteroposterior levels with DiI (magenta) or DiO (green) in one of the paired heart primordia. This pattern of labeling clearly shows that cells initially arrayed anteroposteriorly reorient mediolaterally during heart tube formation. Selected images are shown in Figure 3B. Annotated, duplicated time-lapse images are shown in the right panel. Red and blue dots depict the original medial and lateral edges of the DiI-labeled cell stripe (magenta), respectively.



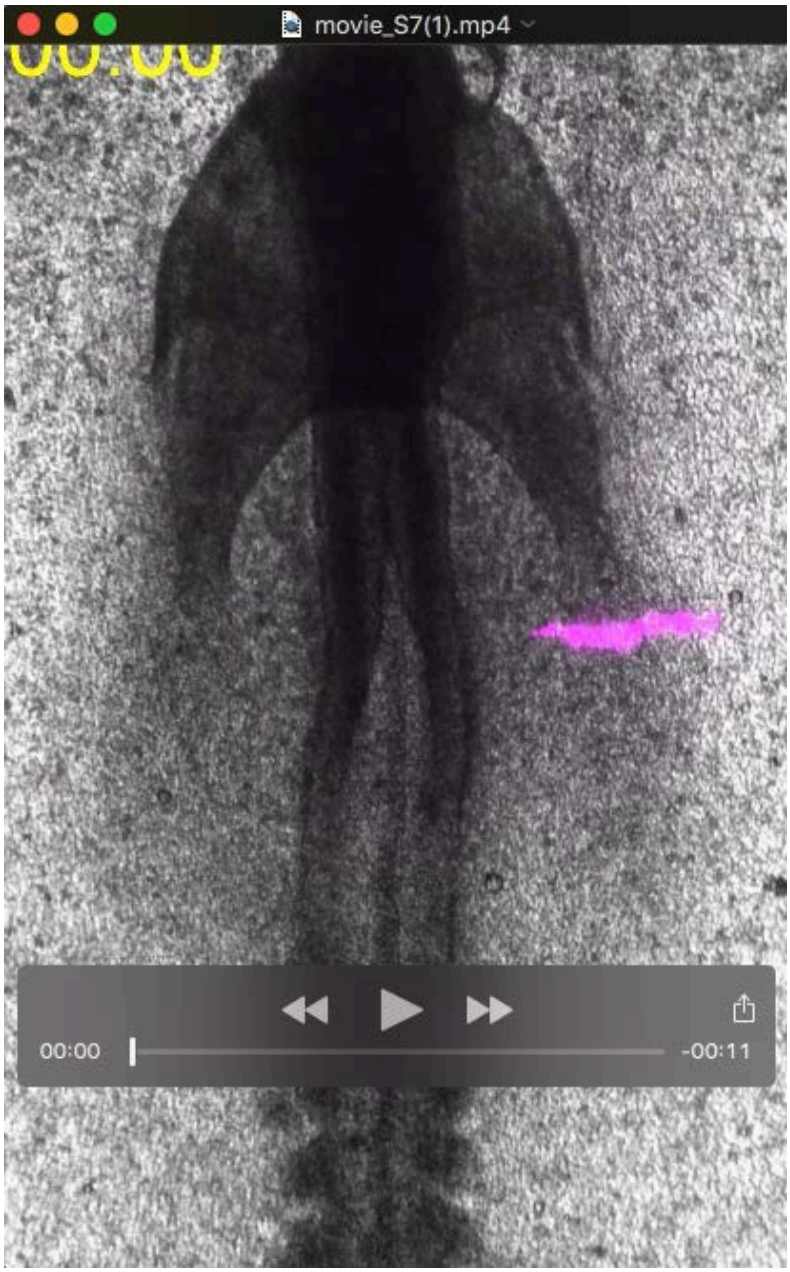
Movie S4. 3D rendering of confocal images showing two neighboring anteroposterior stripes of cells in one heart primordium immediately after labeling with DiI (magenta) or DiO (green). The rendering is rotated over 360^0 to show the spatial relationships between cells in the two stripes at the beginning of the experiment. A still image is shown in Figure 4Aa.



Movie S5. 3D rendering of confocal images showing the two neighboring anteroposterior stripes of cells from Movie S4 about 24 hours after labeling with DiI (magenta) or DiO (green). Images are rotated over 360^0 to show the intermingling of cells from the two stripes. Selected images are shown in Figures 4Ab-b".



Movie S6. Time-lapse imaging of the impaired extension of the heart mesoderm in the Y27632-treated embryo. One of the heart primordia was labeled with DiI (magenta), and the embryo was treated with Y27632 when reaching the 4-6 somite stage. Selected images are shown in Figure 4C.



Movie S7. Time-lapse imaging showing coupled folding of the heart mesoderm and the overlying endoderm during formation of the heart tube and foregut. A stripe of cells was labeled with DiI (magenta) in one of the heart primordia; unintentionally, a few cells of the adjacent endoderm were labeled and subsequently tracked. Selected images are shown in Figure 5A.

Supplementary references

Abu-Issa, R. and Kirby, M. L. (2007). Heart Field: From mesoderm to heart tube. *Annu. Rev. Cell Dev. Biol.* **23**, 45–68.

Abu-Issa, R. and Kirby, M. L. (2008). Patterning of the heart field in the chick. *Dev. Biol.* **319**, 223–233.

DeHaan, R. L. (1965). Development of pacemaker tissue in the embryonic heart. *Ann. N. Y. Acad. Sci.* **127**, 7–18.