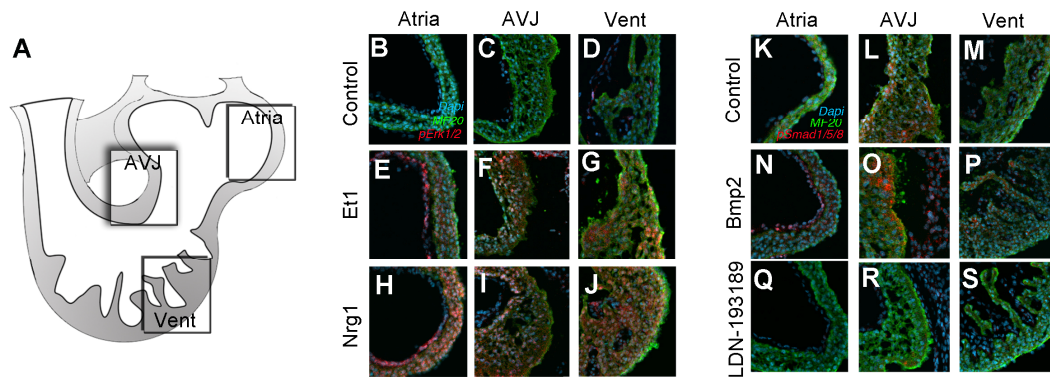
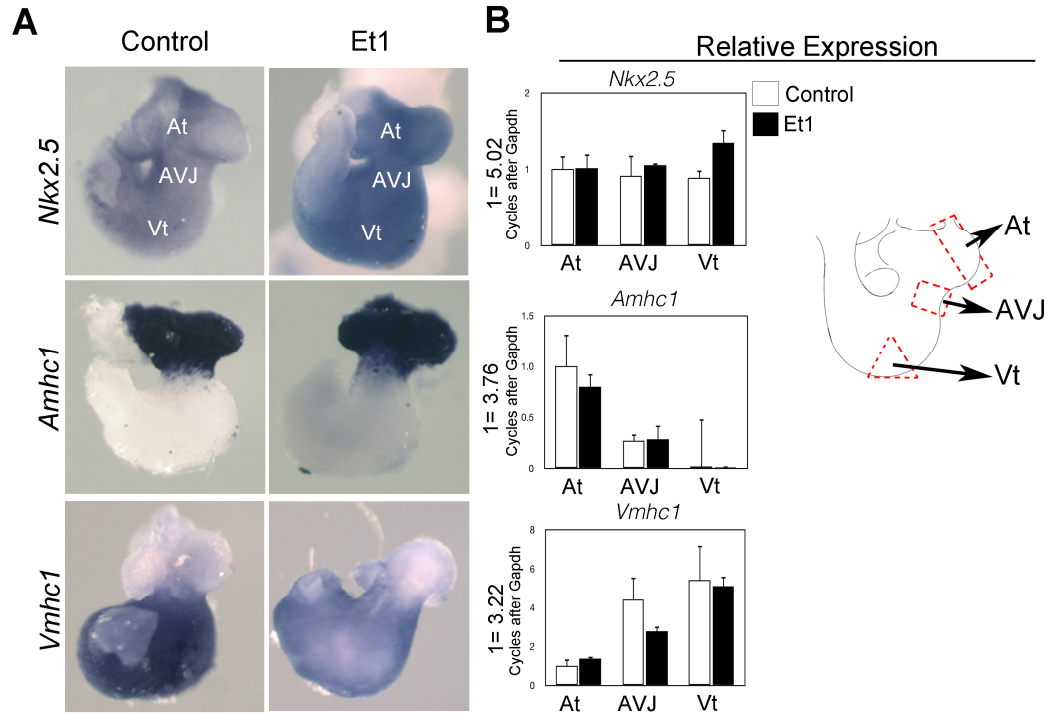


Supplemental Figures

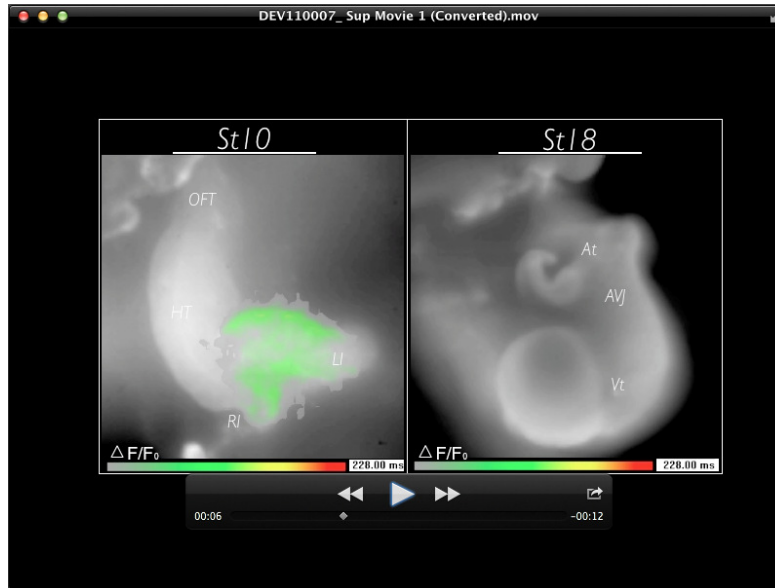


Supplemental Figure 1. Downstream signaling effectors are activated in all regions of the heart following growth factor treatment. (A) Schematic of a looping stage heart, boxed regions indicate areas of atria, AVJ, and ventricles presented in (BS). All treatments were for 30 minutes. (B-J) MF20 (green), Dapi (blue), and pErk1/2 (red) staining in control (B-D), Et1 (15uM) (E-G), and Nrg1 (50 nM) (H-J) treated hearts. (K-S) MF20 (green), Dapi (blue), and pSmad1/5/8 (red) staining in control (K-M), Bmp2 (25 nM) (N-P), and LDN-193189 (1 uM) (Q-S)-treated hearts.

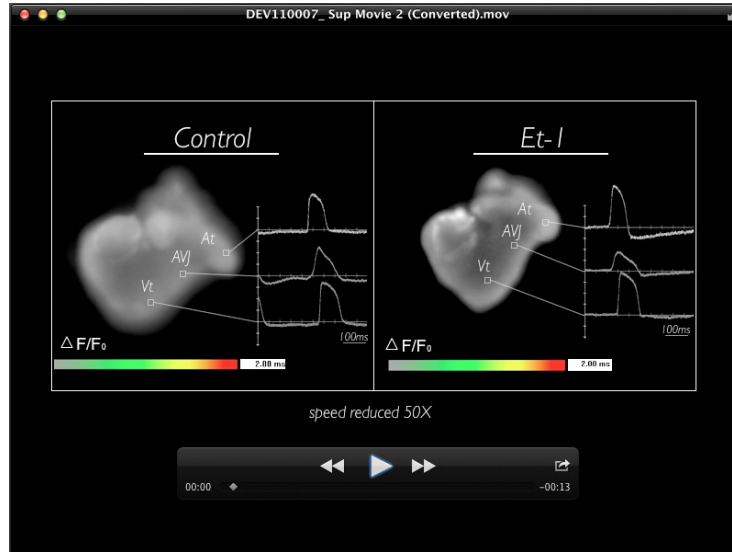


Supplemental Figure 2. Patterning of cardiac myocyte fate is maintained in Et1-treated hearts. (A) Whole-mount *in situ* hybridization for indicated genes in control and Et1 (15 uM)-treated hearts. (B) RT-PCR quantification of mRNA transcript levels relative to control atria for indicated genes in atria (At), atrioventricular junction (AVJ), and ventricle (Vt). Areas isolated for RNA extraction are indicated in schematic. Cycle number after Gapdh is indicated on the y-axis, n = 3 pools of 4 hearts. At - atria, AVJ - atrioventricular junction, Vt - ventricle. Bars = mean + s.d.

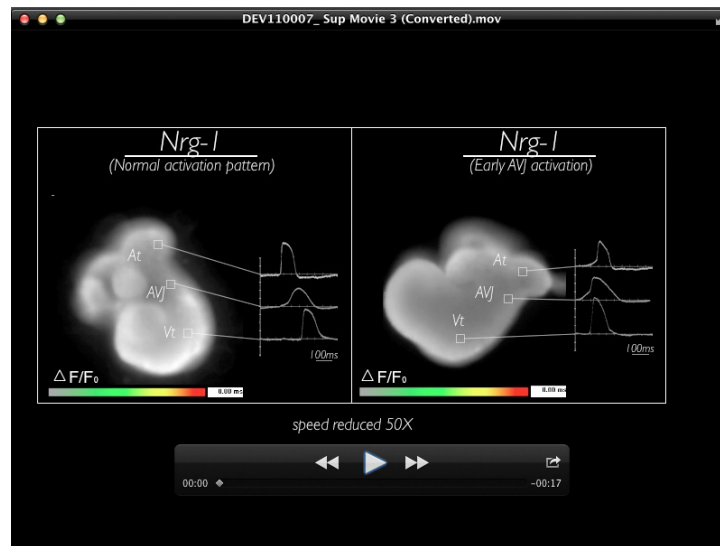
Supplemental Movies



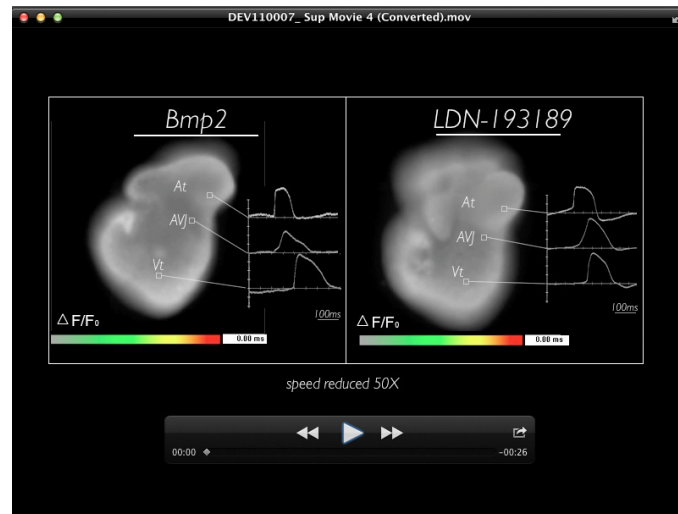
Supplemental Movie 1. Development of conduction delay during cardiac morphogenesis. (A) Optical mapping of linear stage chick heart (~33 h of development). Depolarization is depicted by change in color (change in fluorescence/original fluorescence). Note slow, even propagation initiating in the left inflow and moving anteriorly towards the outflow. Scale bar = 250 μm . **(B)** Optical mapping of a looping stage embryonic chick heart (~68 h of development). Scale bar = 500 μm . Note action potential velocity is much faster than in heart tube stages (A). Further action potentials are delayed as they cross the AVJ. OFT - outflow tract, RI - right inflow, LI - left inflow, At - atria, AVJ - atrioventricular junction, Vt - ventricle.



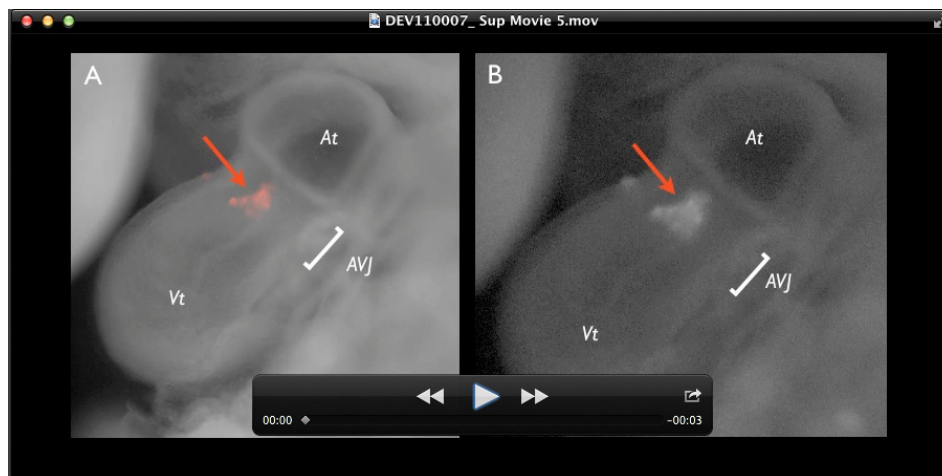
Supplemental Movie 2. Et1 treatment results in increased AVJ conduction velocity. (A) Optical mapping of isolated looping stage heart cultured in control media for 16 h. (B) Optical mapping of looping stage heart cultured with 15 μ M Et1 for 14 h. Note that action potentials reach the ventricle much faster in Et1-treated heart.



Supplemental Movie 3. Nrg1 treatment in looping stage hearts. (A) Optical mapping of isolated looping stage heart cultured in control media for 16 hrs. (B) Optical mapping of looping stage heart treated with (50 nM) Nrg1 displays no change in conduction (representative of 5/11 imaged hearts). (C) Optical mapping of Nrg1-treated heart that displays ectopic early depolarization (representative of 6/11 imaged hearts). Note initiation of action potential in the AVJ.



Supplemental Movie 4. Neither stimulation nor suppression of BMP signaling affects conduction pattern through the AVJ. (A) Optical mapping of isolated looping-stage heart cultured in control media for 16 h. (B) Optical mapping of looping-stage heart treated with 25 nM BMP2. (C) Optical mapping of looping-stage heart treated with 1 μ M BMP antagonist, LDN-193189. Conduction delay is present in both B and C.



Supplemental Movie 5. AVJ myocytes injected into host endocardial cushions beat independently of the host heart. (A) Donor AVJ myocytes (red) present within the host superior endocardial cushion. (B) Monochrome video of donor cells beating within the host endocardial cushion. The host heart was cooled to room temperature to lower sinus rhythm. Note that donor cells beat independently of the host heart, suggesting that they are not electrically coupled.