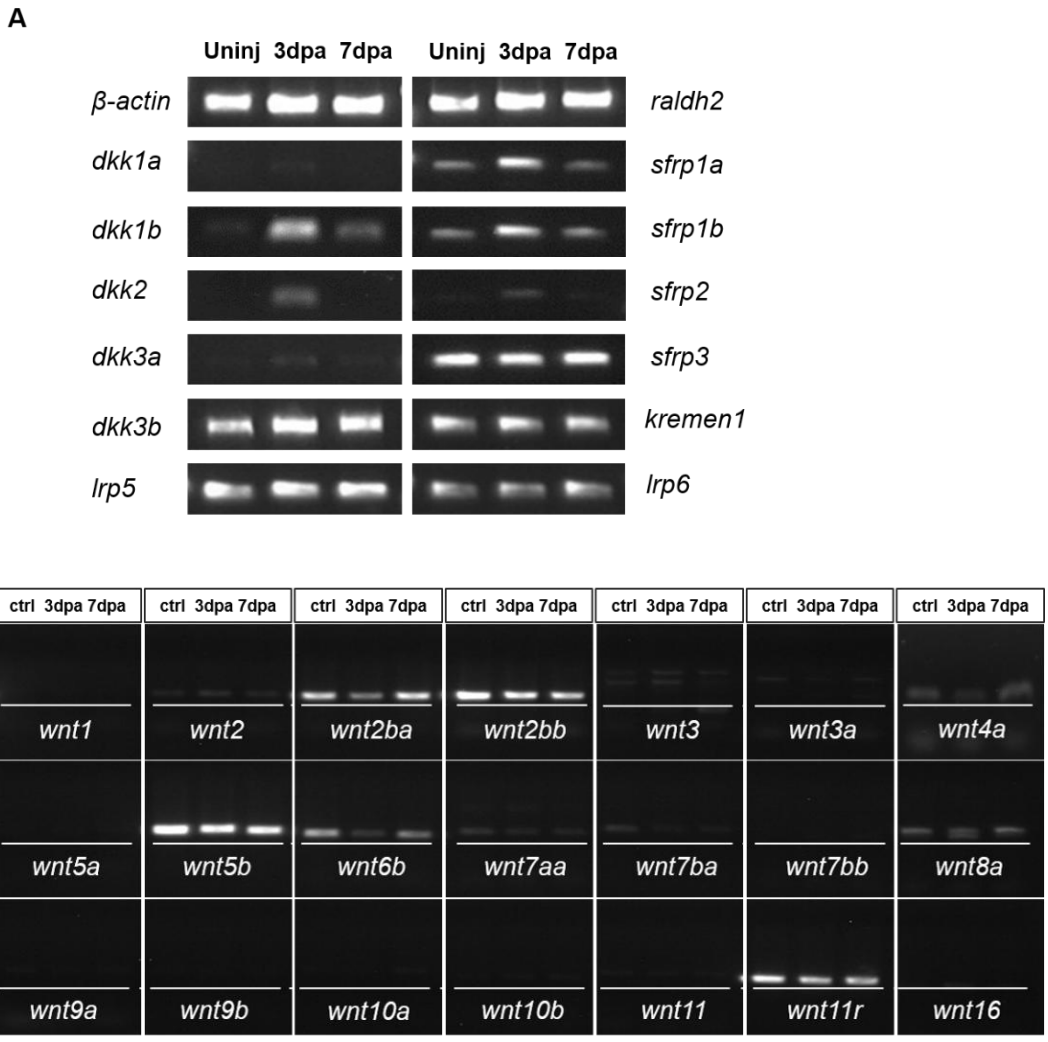
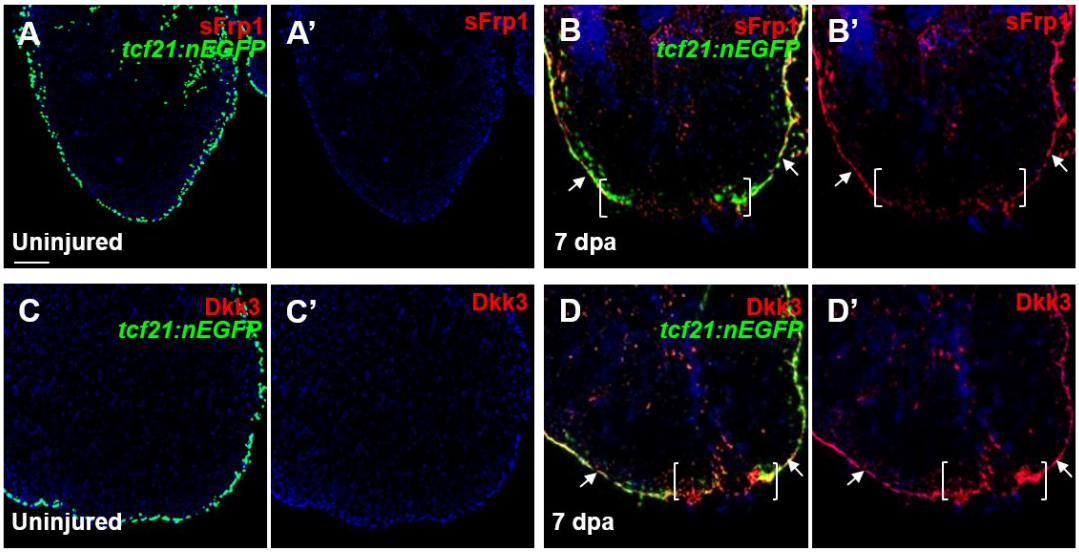
Supplementary material

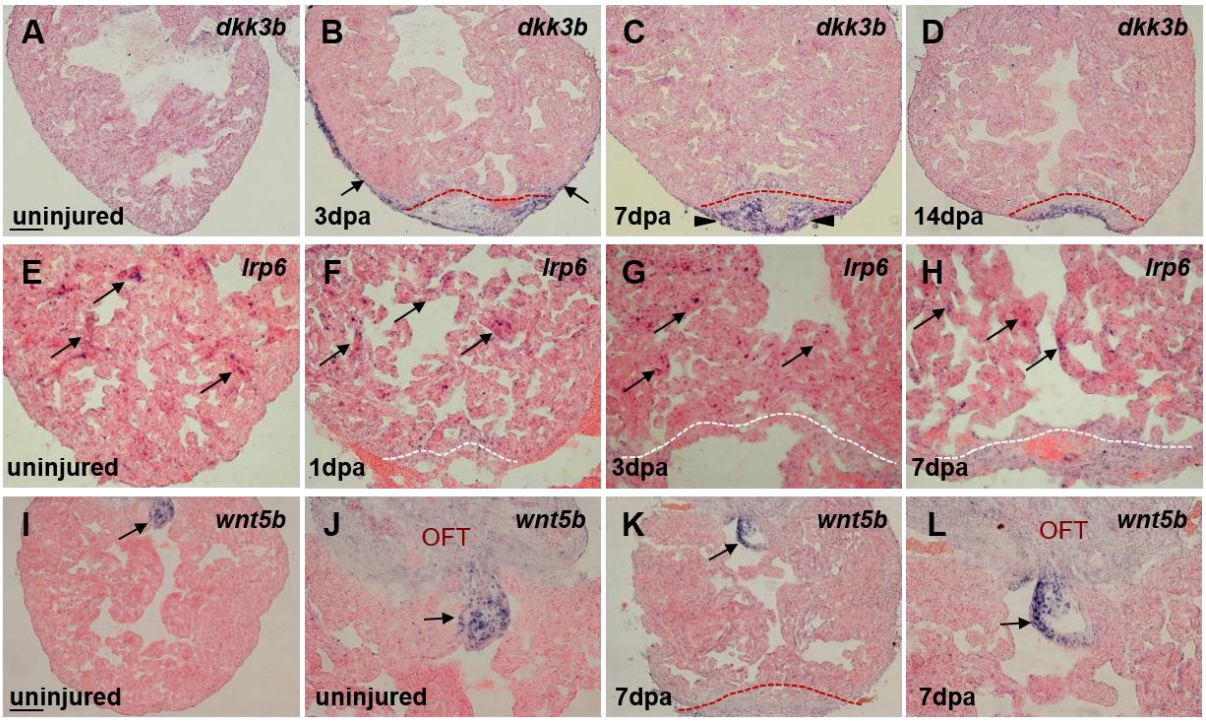
Induction of Wnt signaling antagonists and P21-activated kinase enhances cardiomyocyte proliferation during zebrafish heart regeneration



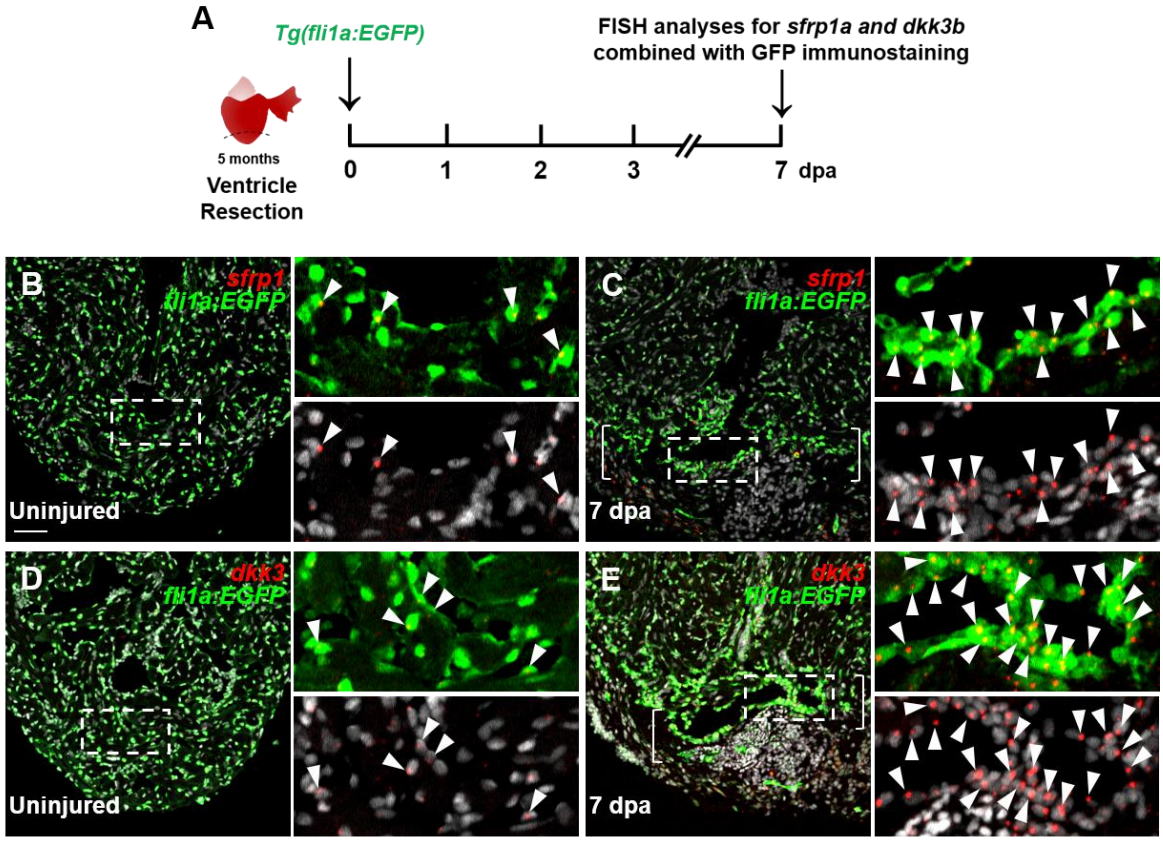
Supplementary Figure S1 Expression of Wnt inhibitors and Wnt ligands during cardiac regeneration by RT-PCR analyses. (**A**) Expression of Wnt inhibitor genes, *dkk1a, dkk1b, dkk2, dkk3a, dkk3b, sfrp1a, sfrp1b, sfrp2, sfrp3;* Wnt receptor genes, *kremen1, lrp5, lrp6* were analyzed using semi-quantitative RT-PCR analyses. (**B**) Wnt ligands, including *wnt1, wnt2, wnt2ba, wnt2bb, wnt3, wnt3a, wnt4a, wnt4b, wnt5a, wnt5b, wnt6b, wnt7aa, wnt7ba, wnt7bb, wnt8a, wnt8b, wnt9a, wnt9b, wnt10a, wnt10b, wnt11, wnt11r, wnt16* were analyzed using semi-quantitative RT-PCR analyses. mRNAs were isolated from uninjured and amputated hearts at 3 days and 7 days.



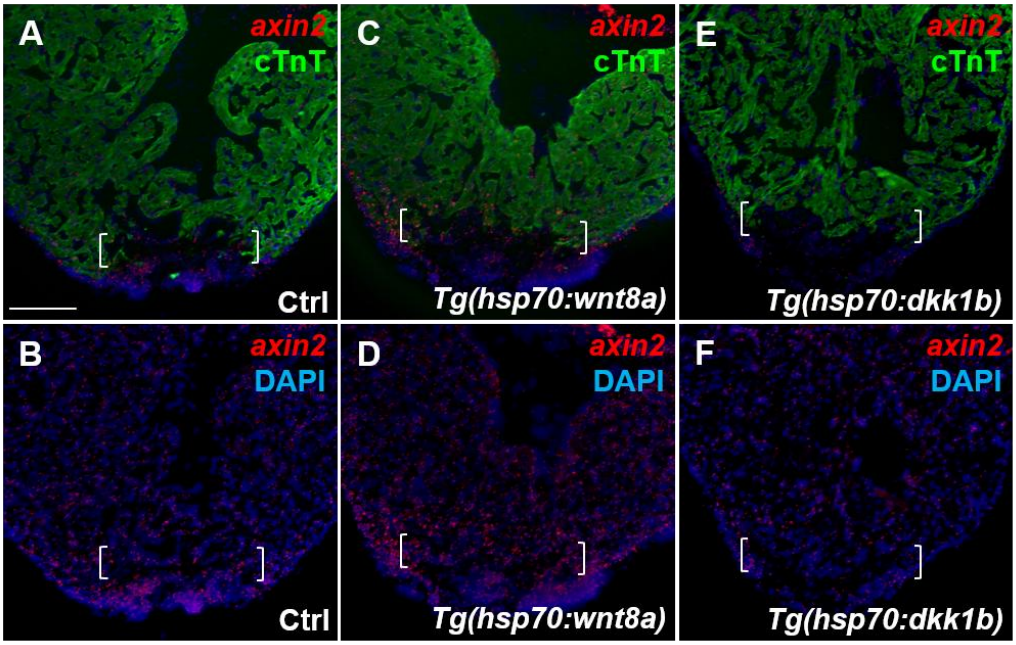
Supplementary Figure S2 Dkk3 and sFrp1 are induced in epicardium during heart regeneration. (**A**-**D**) Representative confocal images of heart sections from uninjured and 7 dpa *Tg(tcf21:nEGFP)* animal co-stained with antibodies for GFP (green) and sFrp1 (red) or Dkk3 (red). Arrows point to sFrp1^{+} epicardium (**B** and **B'**) or Dkk3^{+} epicardium (**D** and **D'**). **A'**, **B'**, **C'**, and **D'** are separate channels of **A**, **B**, **C** and **D**, respectively. Brackets indicate amputation plane. Scale bars: 100 μm.



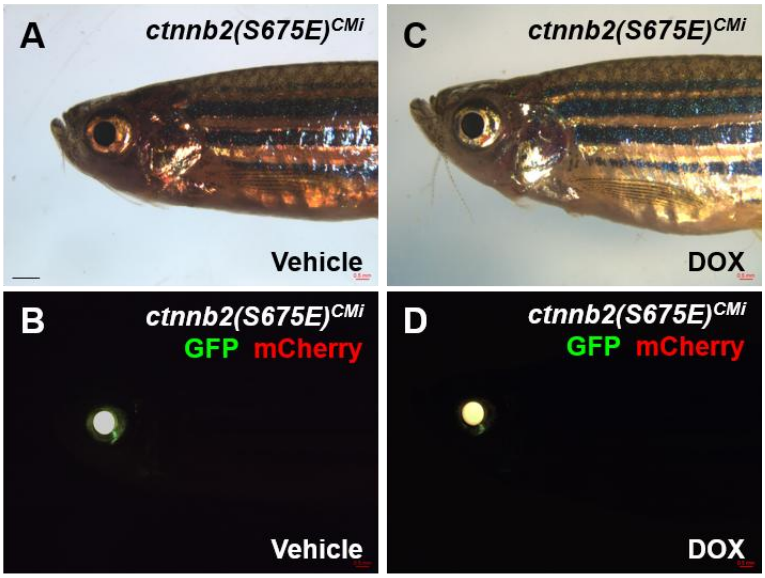
Supplementary Figure S3 Expression of *dkk3b*, *lrp6* and *wnt5b* during heart regeneration by *in situ* hybridization analyses. (**A**-**D**) While the uninjured ventricle shows no *dkk3b* expression, epicardial cells induce *dkk3b* expression (arrows) at 3 dpa. *dkk3b* expression is detectable in the regenerate (arrowheads) at 7 dpa, and *dkk3b* expression remains in the epicardial sheet enclosing the wound by 14 dpa. (**E**-**H**) ISH analyses reveal expression of *lrp6* (arrows) in myocardium in uninjured hearts, 1 dpa, 3 dpa and 7 dpa hearts. (**I**-**L**) *wnt5b* is expressed at the junctional region between the ventricle and the outflow tract (OFT) in uninjured hearts (arrow in **J** and **L**); Following resection at 7 dpa, *wnt5b* expression remains unaltered (arrow in **K** and **L**). Dotted line indicates amputation plane. Scale bars: 100 μm (**A**-**L**).



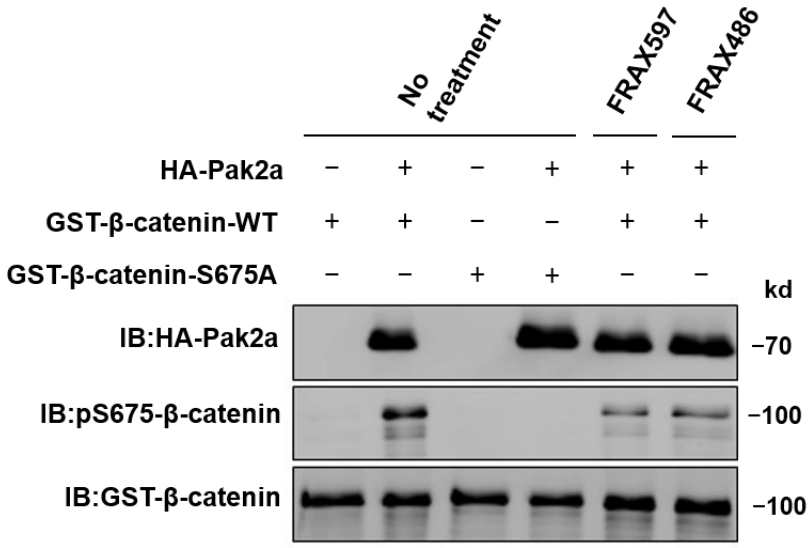
Supplementary Figure S4 *dkk3b* and *sfrp1* transcripts are induced in endocardium during heart regeneration. (**A**-**D**) Schematic of experimental procedures for FISH analyses combined with GFP immunostaining. (**B**-**E**) Representative images of FISH of *sfrp1* (**B** and **C**, red) or *dkk3* (**D** and **E**, red) combined with immunofluorescence for GFP on heart sections from uninjured and 7 dpa *Tg(fli1a:EGFP)* animal. Boxed regions are magnified in adjacent right panels. Arrowheads point to *sfrp1*$^{+}$Fli1a^{+} endocardial cells or *dkk3*$^{+}$Fli1a^{+} endocardial cells. Brackets indicate amputation plane. Scale bars: 100 μm.



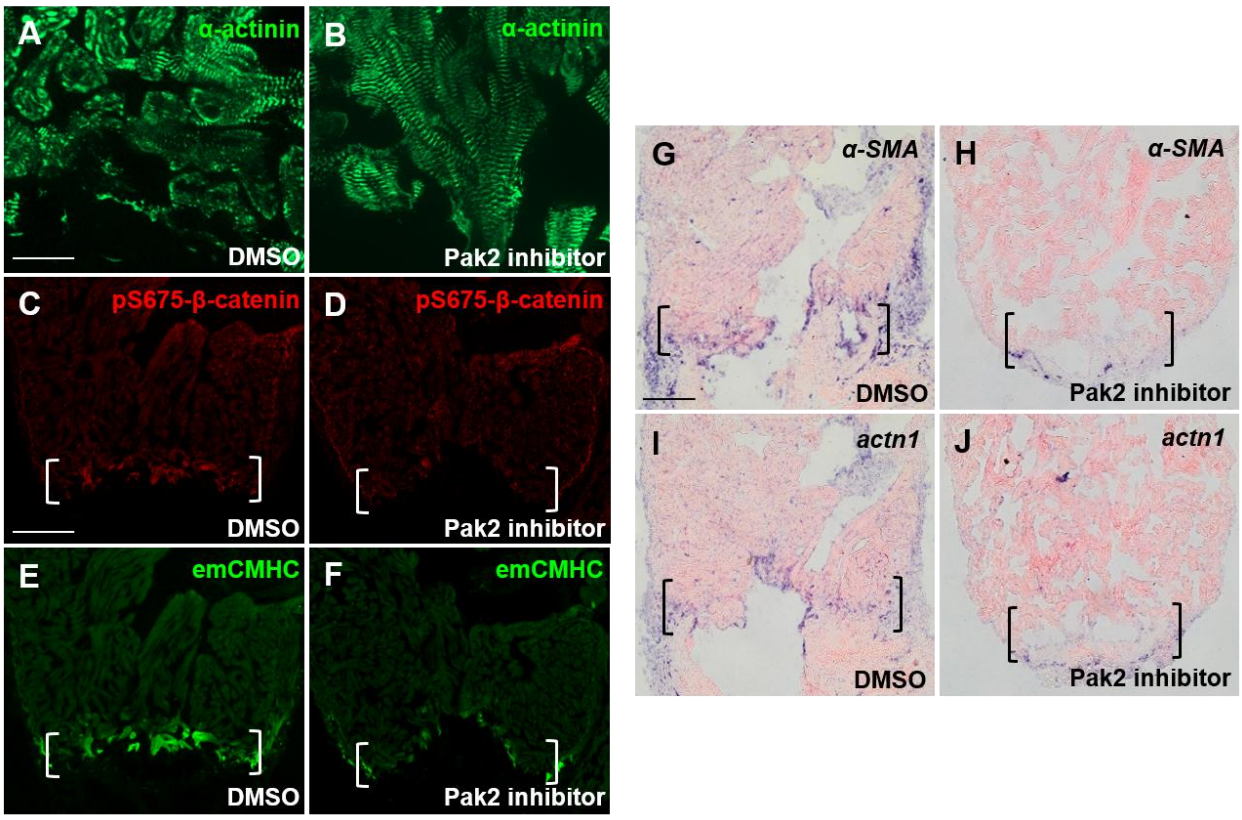
Supplementary Figure S5 Increased or reduced Wnt pathway activity in *Tg(hsp70:wnt8a)* or *Tg(hsp70:dkk1b)* hearts following ventricular resection by *axin2* RNAscope analyses, respectively. (**A**-**F**) RNAscope analyses showing *axin2* expression in control, *Tg(hsp70:dkk1b)* and *Tg(hsp70:wnt8a)* hearts following ventricular resection at 7 dpa. *axin2* transcripts are detectable in the injured heart and slightly enriched at the injury region (**A** and **B**). *axin2* expression is upregulated in *wnt8a*-overpexpressing hearts in heat shocked *Tg(hsp70:wnt8a)* animals (**C** and **D**), but reduced in *dkk1b*-overexpressing hearts in *Tg(hsp70:dkk1b)* zebrafish (**E** and **F**), when compared with control animals (**A** and **B**). Brackets indicate amputation plane. Scale bars: 100 μm.



Supplementary Figure S6 Identification of *ctnnb2(S675E)^{CMi}* transgenic zebrafish using lens-expressed fluorescent proteins. (**A**-**D**) Adult transgenic *ctnnb2(S675E)^{CMi}* zebrafish showed the selectable markers of yellow lens (overlap of GFP with mCherry). No mScarlet-I fluorescence were observed in zebrafish muscle, skin or fin upon DOX or vehicle treatment. Scale bars: 250 μm.



Supplementary Figure S7 Pak2a kinase phosphorylates zebrafish β-catenin at the Ser675 residue in cells. Kinase phosphorylation assay shows that Ser675 residue of β-catenin is phosphorylated by zebrafish Pak2a, but completely abrogated by β-catenin S675A mutant. Administration of FRAX597 or FRAX486 reduces the Ser675 phosphorylation of β-catenin. The immunoprecipitated HA-Pak2 was subjected to kinase assay in the presence of 500 μM ATP and 1.5 μg of zebrafish GST-tagged WT or S675A-β-catenin purified from *Escherichia coli* as substrates. 2 μM Pak2 inhibitor FRAX597 or FRAX486 was added to the reaction mixtures by incubating for 60 min. The reactions were subjected to analysis by SDS-PAGE with related antibodies.



Supplementary Figure S8 Inhibition of Pak2 activity attenuates injury-induced CM dedifferentiation. (**A** and **B**) Confocal high magnification image analyses exhibiting disassembled sarcomeres in DMSO-treated hearts (**A**), and relatively normal striated sarcomeres in FRAX597-treated hearts (**B**). (**C**-**F**) Separate fluorescence channel analyses revealing the reduction of pS675-β-catenin levels (**D**) and emCMHC expression (**F**) at injured myocardial cell edges in FRAX597-treated hearts, compared to DMSO-treated hearts (**C** and **E**). (**G**-**J**) ISH analyses display a reduction of *α-SMA* (**H**) and *actn1* (**J**) expression at wound edges in FRAX597-treated hearts, compared to that in DMSO-treated hearts (**G** and **I**) at 7 dpa. 1 μM FRAX597 treatment from 4 to 6 dpa. Scale bars: 10 μm (**A**-**B**), 100 μm (**C**-**J**).