Supplementary Information

Spatial and Temporal Variations in Hemodynamic Forces Regulate Cardiac Trabecular Initiation and Consequent Contractile Function

Juhyun Lee[1,2*], Vijay Vedula[3*], Kyung In Baek[2*], Junjie Chen[2], Jeffrey J. Hsu[1], Yichen Ding[1], Chih-Chiang Chang[2], Hanul Kang[4], Peng Fei[5], Cheng-ming Chuong[6], Rongsong Li[1], René R. Sevag Packard[1, 4], Alison L. Marsden[3], Tzung K. Hsiai[1,2,4,7]

[1]Division of Cardiology, Department of Medicine, UCLA, Los Angeles, CA
[2]Department of Bioengineering, UCLA, Los Angeles, CA
[3]Department of Pediatrics and Bioengineering, Stanford University, Stanford, CA
[4]Division of Cardiology, VA Greater Los Angeles Healthcare System, Los Angeles, CA
[5]School of Optical and Electronic Information, Huazhong University of Science and Technology, Wuhan, Hubei, China
[6]Department of Pathology, University of Southern California, Los Angeles, CA
[7]Medical Engineering, California Institute of Technology, Pasadena, CA

*These authors contributed equally to this work.

Corresponding Author:
Tzung K. Hsiai, M.D., Ph.D.

Department of Medicine and Bioengineering

University of California, Los Angeles

Los Angeles, CA 90073

Email: thsiai@mednet.ucla.edu

Telephone: 310-268-3839

Fax: 310-268-4288

Supplementary materials:

Fig. S1	Sequential Notch in WT zebrafish from confocal mircoscopy
Fig. S2	NICD mRNA injection to control zebrafish
Fig. S3	Pulse-wave (PW) Doppler image of adult wild type zebrafish
Video S1	Registration of 4-D beating heart with segmented fluid domain
Video S2	4-D WSS profile of WT zebrafish
Video S3	4-D streamline traces of WT zebrafish heart
Video S4	Cardiomyocytes proliferation of WT zebrafish at 5 dpf
Video S5	Cardiomyocytes proliferation of gata1a MO injected zebrfish at 5 dpf
Video S6	Cardiomyocytes proliferation of rescue zebrafish with NICD mRNA injection at 5 dpf

Fig. S1

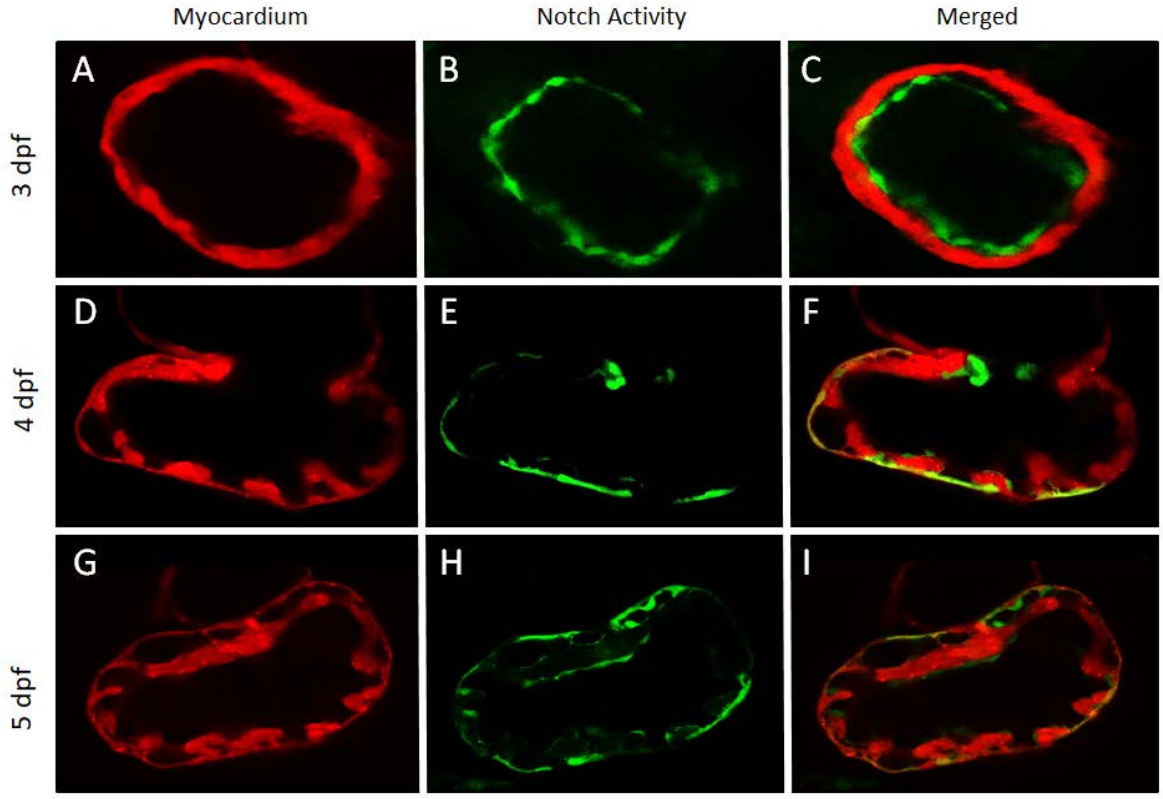

Fig. S1. Sequential Notch in WT zebrafish from confocal microscopy. (A-C) At 3 dpf, Tp1 signal, Notch promoter, activity initially resided in the endocardial layer and AV canal. (D-F) At 4 dpf, Notch activity appeared to be more prominent in the epicardium than in the endocardium. Epicardial Tp1 signal and trabecular ridges were organizing into an interspersed pattern. (G-H) As trabeculae developed to form a network structure, Notch activity was prominent in both the epi- and endocardium. Notch activity was absent in the myocardium.

Fig. S2

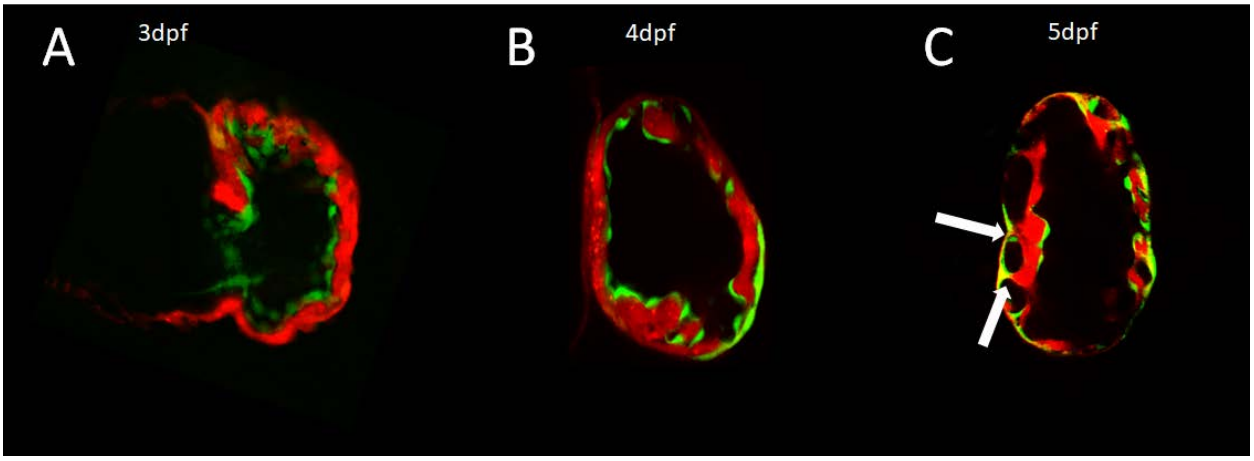

Fig. S2. NICD mRNA injection to WT zebrafish *Tg(cmlc:mcheery;tp1:gfp).* (A) At 3dpf, tp1 signal was initially expressed in endocardium as other groups. However, ventricular thickness was thicker than WT. (B) At 4 dpf, ventricle was over trabeculated and Notch activity was shown in both endocardium and myocardium. (C) Large amount of myocardium was separated from ventricular wall and form a trabecular network by interconnecting with thin bridges (arrow).

Fig. S3

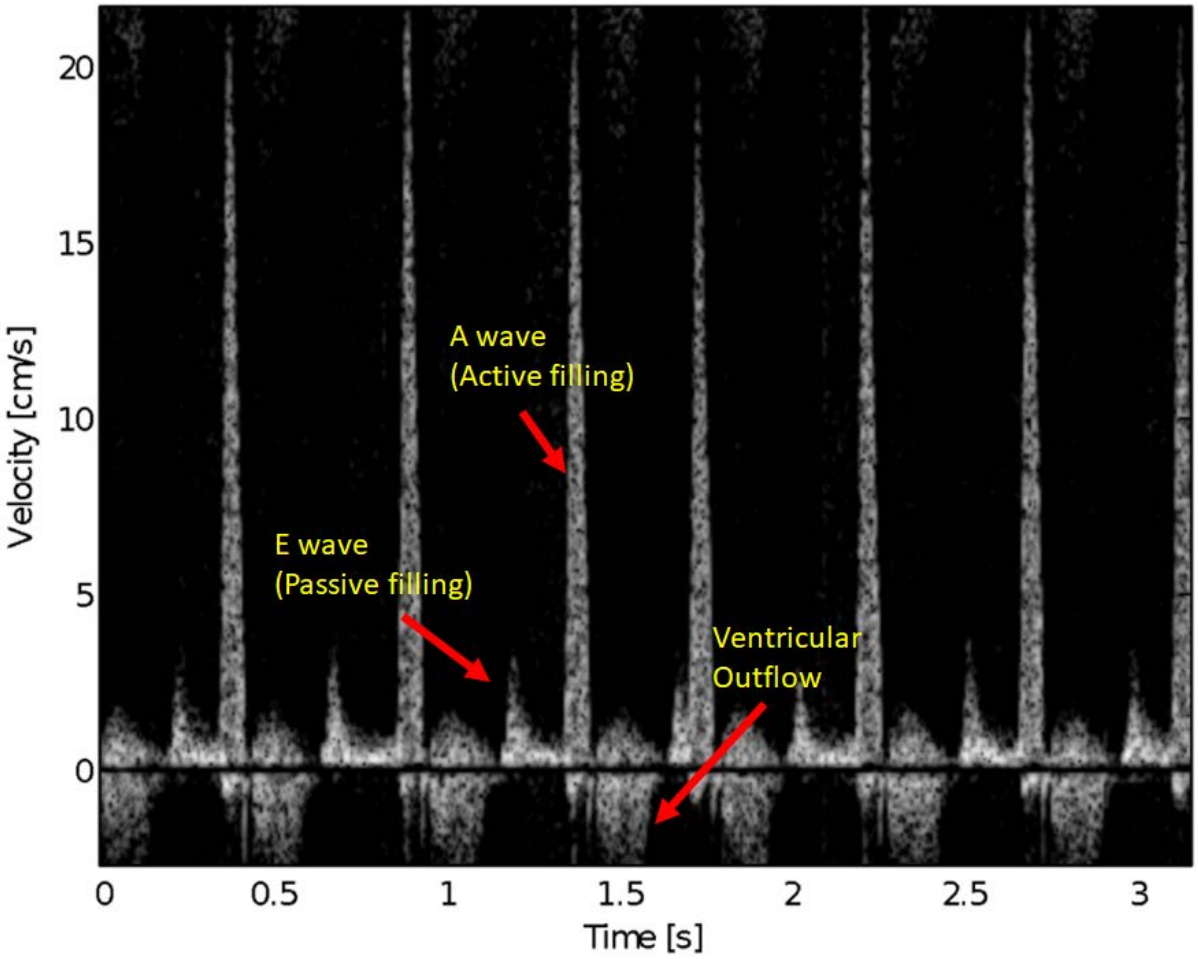

Fig. S3. Pulse-wave (PW) Doppler image of adult wild type zebrafish. PW Doppler provides assessment of passive filling of the ventricle (early [E]-wave velocity) and active filling during atrial systole (atrial [A]-wave velocity). Unlike in human hearts, atrial contraction (A-wave) is stronger than passive filling by ventricular relaxation, therefore, E/A ratio is < 1 at baseline. For *wea* mutant, A-wave would be negligible due to lack to atrial contraction.

Supplementary Video Legends

Video S1. Registration of 4-D beating heart with segmented fluid domain.

Video S2. 4-D WSS profile of WT zebrafish

Video S3. 4-D streamline traces of WT zebrafish heart

Video S4. Cardiomyocytes proliferation of WT zebrafish at 5 dpf

Video S5. Cardiomyocytes proliferation of gata1a MO injected zebrfish at 5 dpf

Video S6. Cardiomyocytes proliferation of rescue zebrafish with NICD mRNA injection at 5 dpf