

### Online Figure I

***Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>* mice have modestly decreased cardiac function without evidence of gross or histologic changes.** A. Representative M-mode echocardiographs 10 d post tamoxifen injection. B. Time course of ejection fraction and heart rate in *Tjp1<sup>fl/fl</sup>* and *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>* mice following tamoxifen injection ( $p=5.3e-5$  for 20 d and  $p=0.0080$  for 40 d.  $n=8$  *Tjp1<sup>fl/fl</sup>*,  $n=10$  *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>* mice for ejection fraction and  $n=8$  for each genotype for heart rate). C. Hearts from of a cohort of 3 *Tjp1<sup>fl/fl</sup>* (top) and 3 *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>* mice (bottom) 42 d after tamoxifen injection. D. Heart weights of 3 *Tjp1<sup>fl/fl</sup>* and 3 *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>* mice 42 d after tamoxifen injection. E. Low and high power images of trichrome stained *Tjp1<sup>fl/fl</sup>* and *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>* hearts. Left panels: low magnification; Right panels: high magnification of boxed regions in left panels. (Red: *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>*, Blue: *Tjp1<sup>fl/fl</sup>*, \*\*\* $p<0.001$ , \*\*  $p<0.01$ ) Two-tailed Student's t-test was used for calculation of all p-values.

### Online Figure II

**Cardiac dimension changes in *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>* mice following tamoxifen induced knockout.** Time course of echocardiogram parameters in *Tjp1<sup>fl/fl</sup>* and *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>* mice following tamoxifen injection ( $n=8$  mice per genotype) A. Septal wall thickness during diastole ( $p=0.0094$  for 5 d and  $p=0.0054$  for 10 d). B. Septal wall thickness during systole. C. Left ventricular internal diameter during diastole ( $p=0.00093$  for 5 d and  $p=0.0032$  for 10 d). D. Left ventricular internal diameter during systole ( $p=0.0015$  for 5 d,  $p=0.0017$  for 20 d, and  $p=0.018$  for 40 d) E. Posterior wall thickness during diastole. F. Posterior wall thickness during systole. \*\*\* $p<0.001$ , \*\*  $p<0.01$ , \* $p<0.05$ . Two-tailed Student's t-test was used for calculation of all p-values.

### Online Figure III

**Connexin 40 and CAR colocalize with  $\beta$ -catenin in the AV node.** Representative immunofluorescence staining of *Tjp1<sup>fl/fl</sup>* AV nodes. A. Cx40 B. CAR. Arrows indicate regions of co-localization ( $n=3$  mice per genotype). All studies were performed 10 d post-tamoxifen injection.

### Online Figure IV

***Tjp1* deletion does not alter nodal expression of Nav1.5, Cx45,  $\beta$ -catenin or N-cadherin.** Representative trichrome and immunofluorescence staining of AV nodes. A. Nav1.5, B. Cx45, C.  $\beta$ -catenin, D. N-cadherin.  $n=3$  mice per genotype. All studies were performed 10 d post-tamoxifen injection. For each stain, trichrome was used to visualize the node (top) and low power (middle) and high power (bottom) images are shown. High power images reflect boxed areas within the circled AV nodal regions.

### Online Figure V

***Tjp1* deletion alters protein localization at the intercalated disc of ventricular cardiomyocytes.** Representative images immunofluorescent staining and quantification of immunofluorescent staining along lines perpendicular to the intercalated disc are shown. Peak intercalated disc intensity values were averaged per animal and compared. A. Cx43 (red,  $p=0.042$ , WRS:  $p=0.050$ ), JUP (green), B. CAR (red,  $p=0.0032$ , WRS:  $p=0.050$ , JUP (green), C.  $\beta$ -catenin ( $\beta$ -cat; red), JUP (green), D. JUP (green,  $p=0.033$ , WRS:  $p=0.050$ ), N-cad (red). Phalloidin stains (F-actin) are shown in grey. (Red: *Tjp1<sup>fl/fl</sup>*; *Myh6<sup>Cre/Esr1\*</sup>*, Blue: *Tjp1<sup>fl/fl</sup>*, \*\*  $p<0.01$ , \*  $p<0.05$ ,  $n=3$  mice per genotype). All studies were performed 10 d post-tamoxifen injection (Scale bars=10  $\mu$ m). Two-tailed Student's t-test was used to calculate p-values.

### Online Figure VI

**AV node ultrastructure was not significantly altered by *Tjp1* deletion.** A-B. Representative images of Toluidine blue stained sections of AV node containing tissues; bars: 10  $\mu$ m. Arrows indicate large AV nodal cell nuclei with prominent nucleoli. C-D. Representative TEM images of AV nodes. Arrows indicate large AV nodal cell nuclei with prominent nucleoli. Bars=5  $\mu$ m. E-F. Representative TEM images of AV nodes. Magnified views of boxed intercalated disc containing region are shown in insets. Bars=1  $\mu$ m.  $n=2$  per genotype. Samples were collected 10 d post-tamoxifen injection.

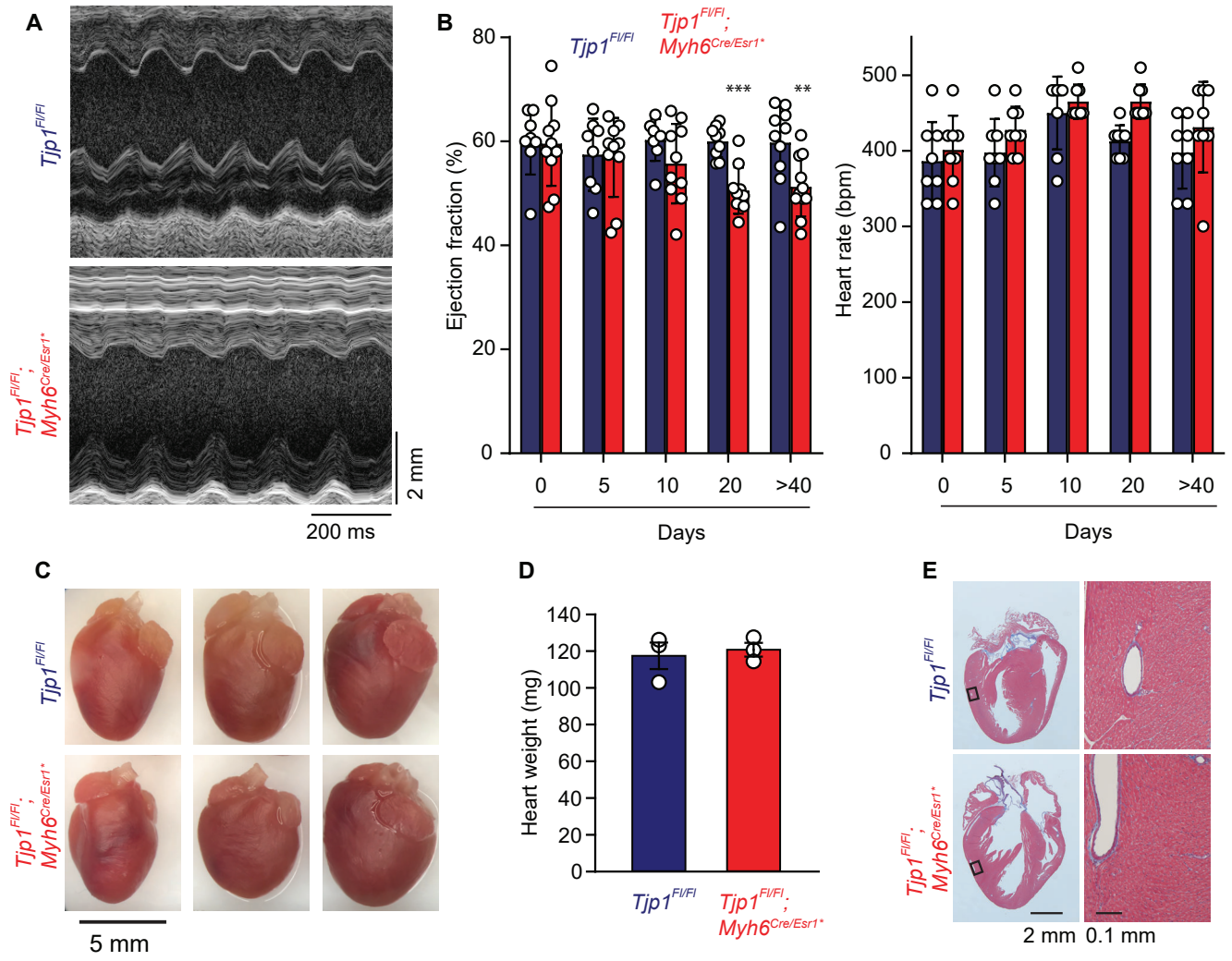
### Online Figure VII

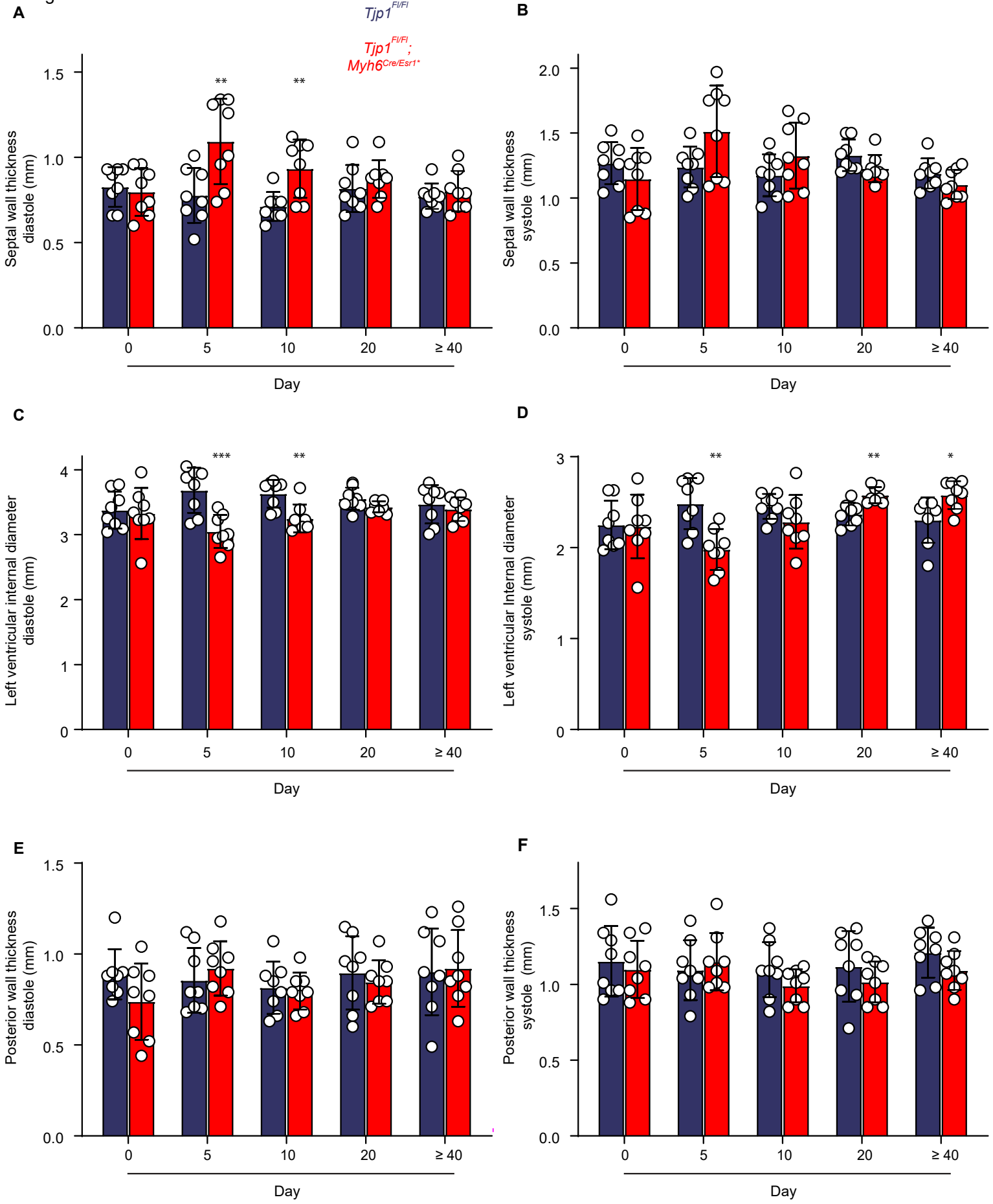
**ZO-1 is abundantly expressed in AV node, but not the His bundle in *Tjp1<sup>fl/fl</sup>* mice.** A. Immunohistochemical staining of HCN4 in AV node and His bundle in *Tjp1<sup>fl/fl</sup>* mice. B. Immunohistochemical staining of ZO-1 in AV node and His bundle. (left panels: scale bars=100  $\mu\text{m}$ , right panels: scale bars=10  $\mu\text{m}$ ). His bundles are circled by dashed lines.

### Online Figure VIII

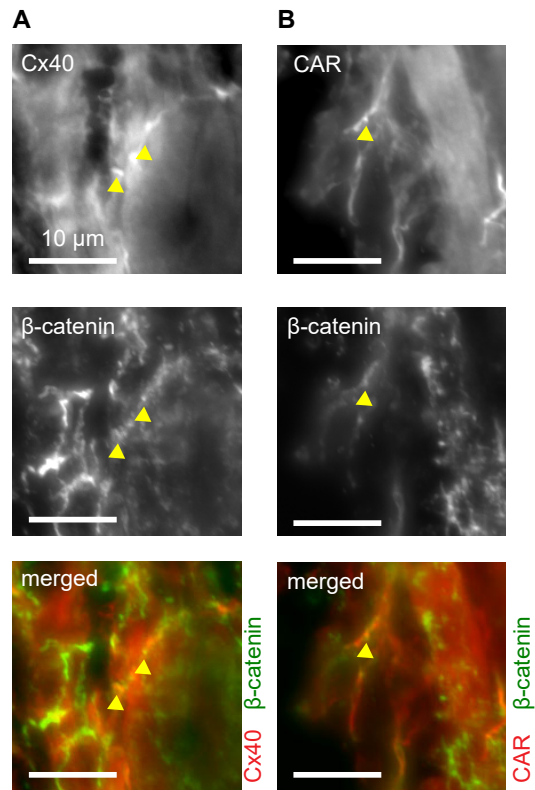
**Low ZO-2 expression in ventricular cardiomyocytes.** ZO-2 and N-cadherin staining in *Tjp1<sup>fl/fl</sup>* and *Tjp1<sup>fl/fl</sup>; Myh6<sup>Cre/Esr1\*</sup>* ventricular tissue sections. N-cadherin staining highlights intercalated discs (white arrows), with no colocalization with ZO-2. ZO-2 staining is present in cardiac endothelial cells (yellow arrowheads). Studies were performed 10 d post-tamoxifen injection (Scale bar=10  $\mu\text{m}$ , n=3 mice per genotype).

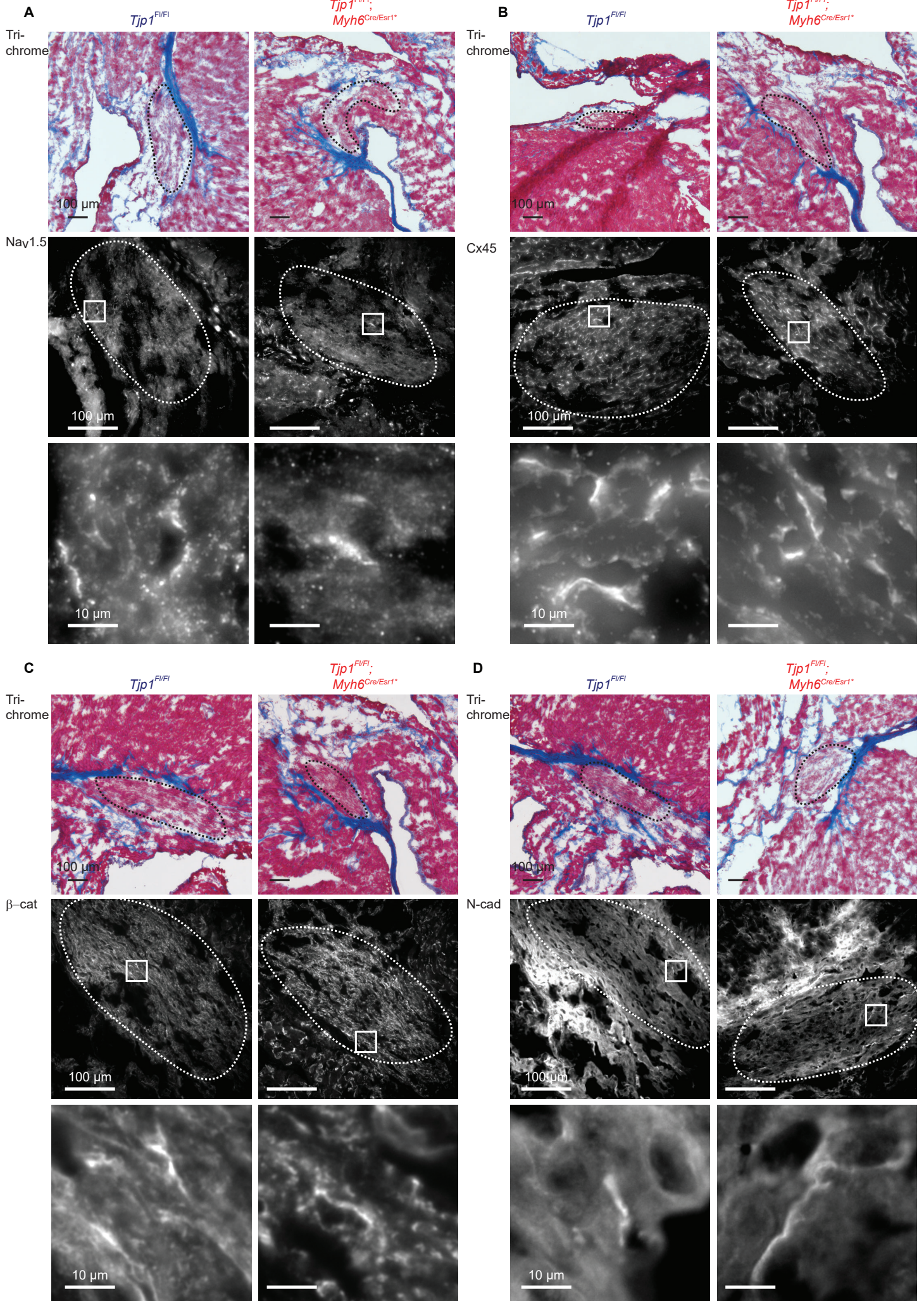
Online Figure 1



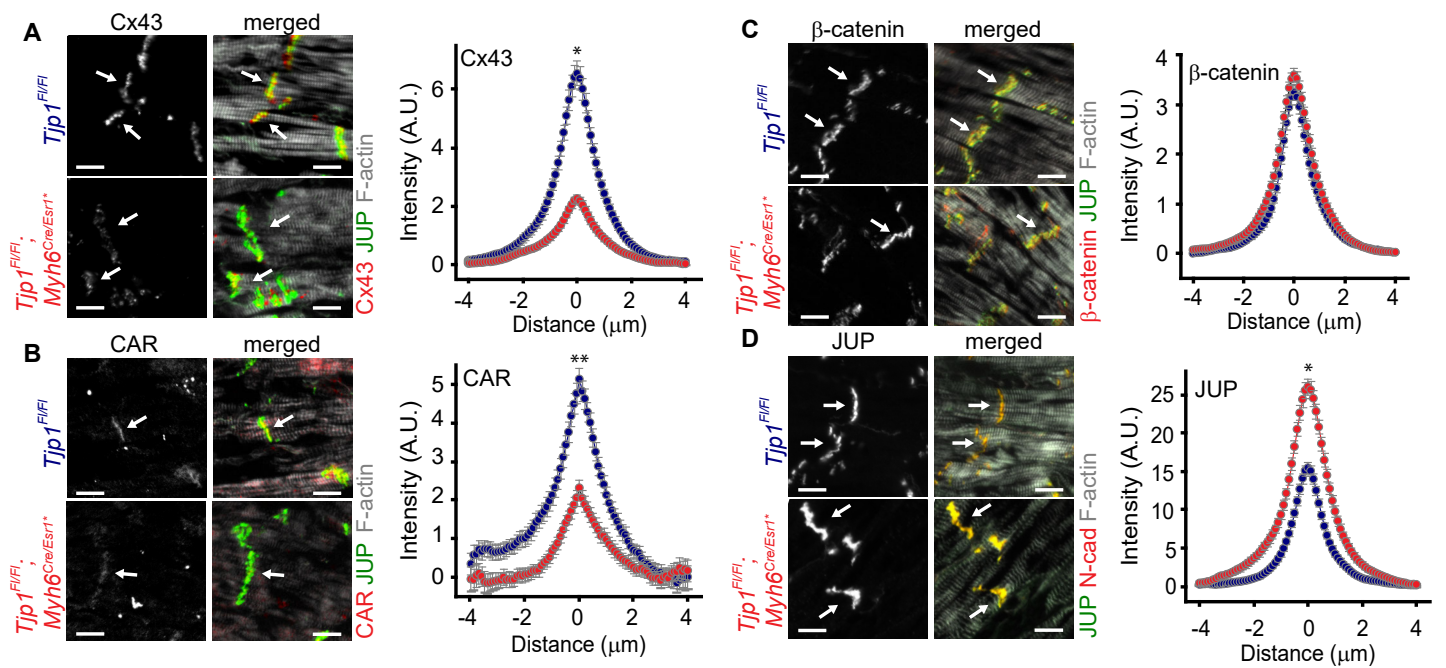


Online Figure III

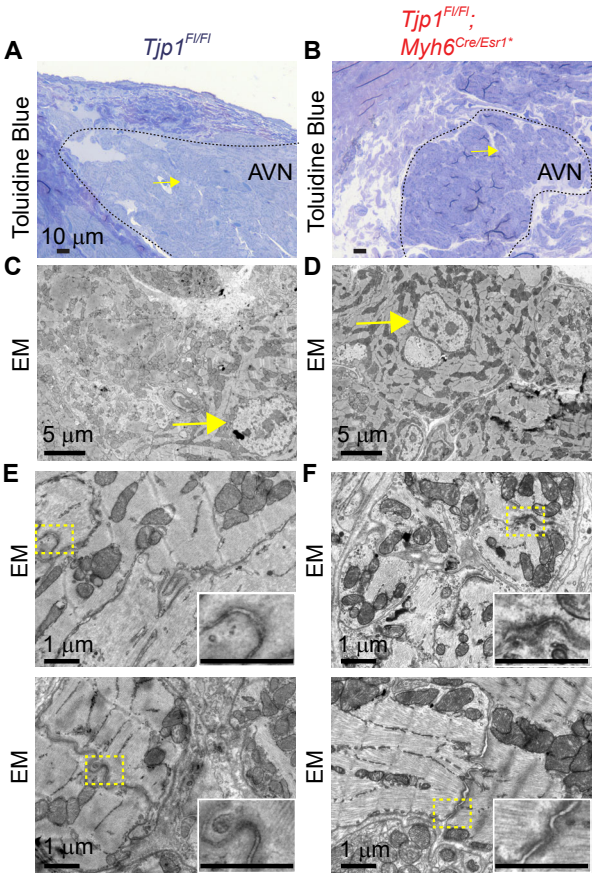




Online Figure V

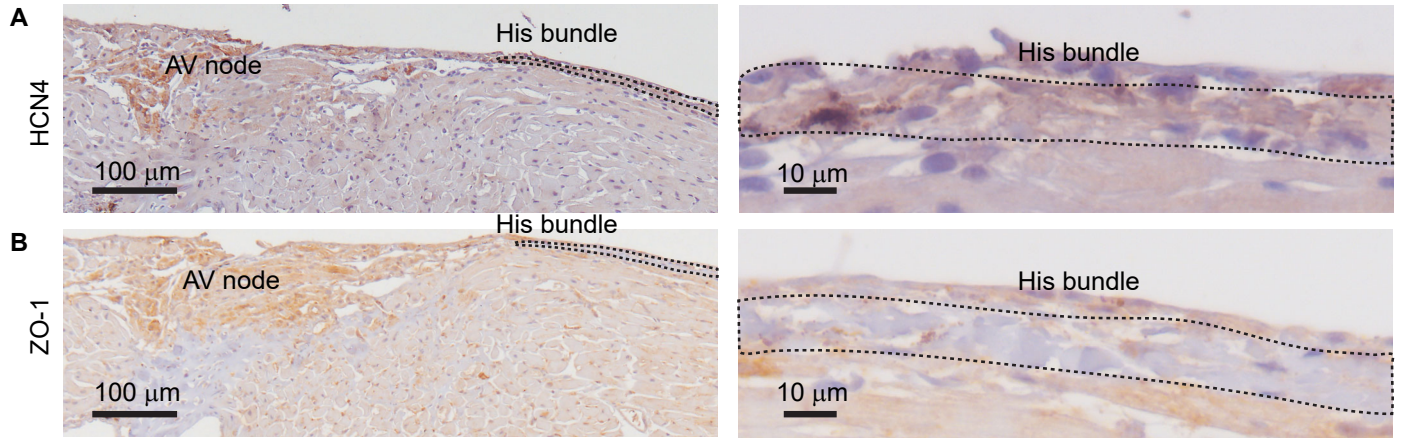


Online Figure VI





Online Figure VII



Online Figure VIII

