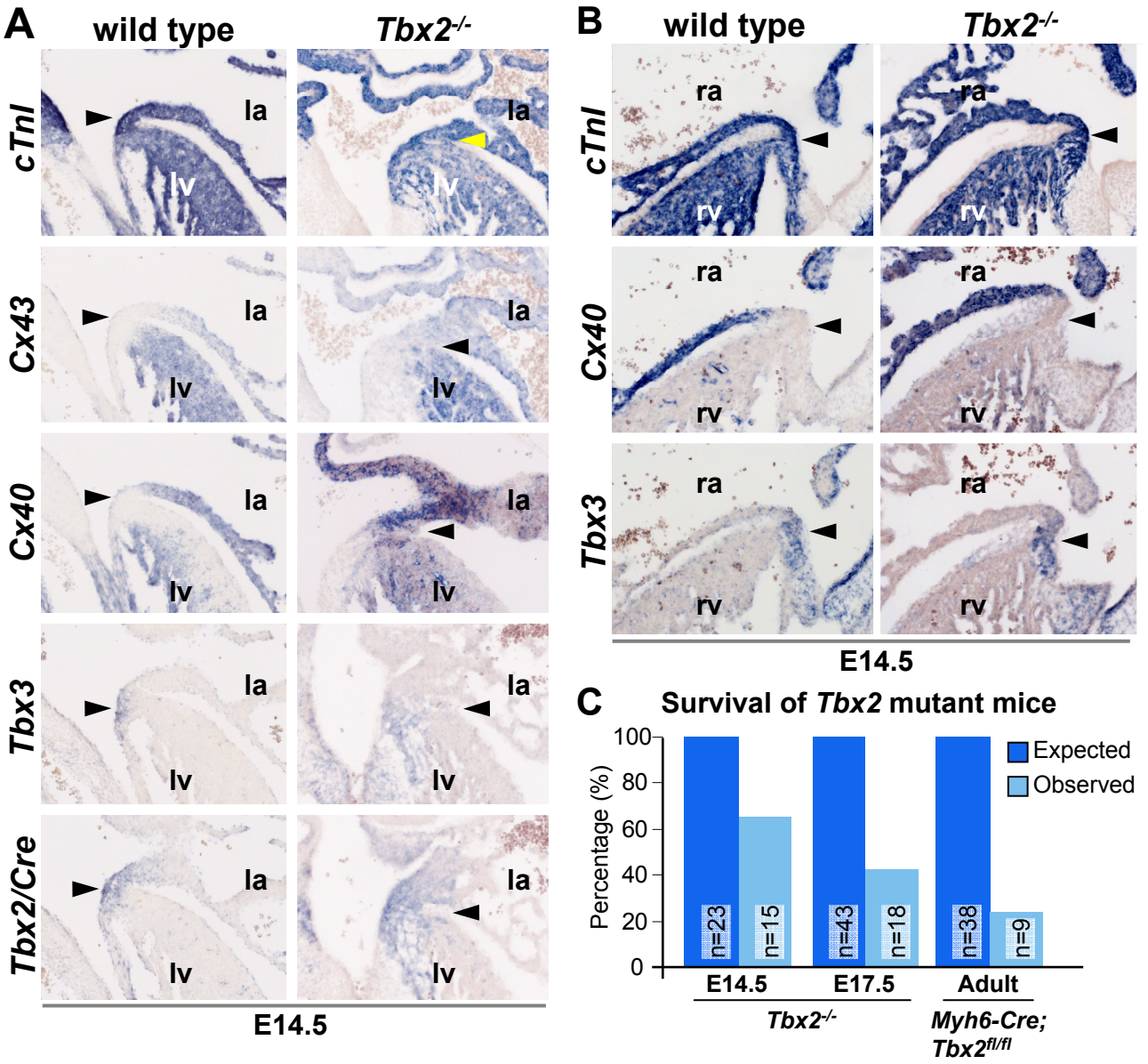
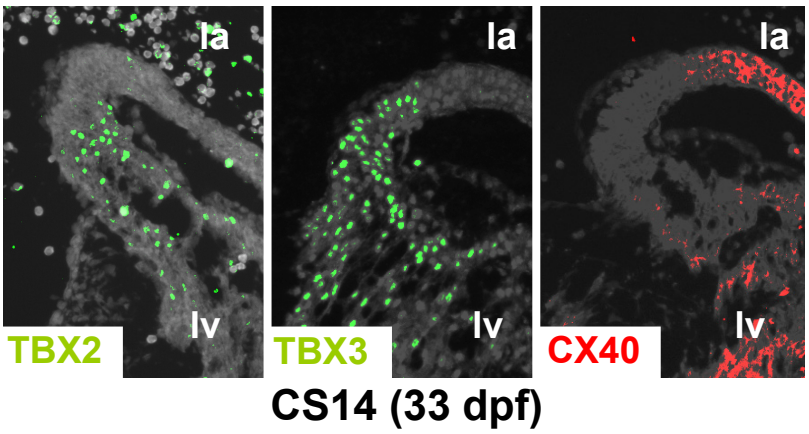
Supplemental Figure 1



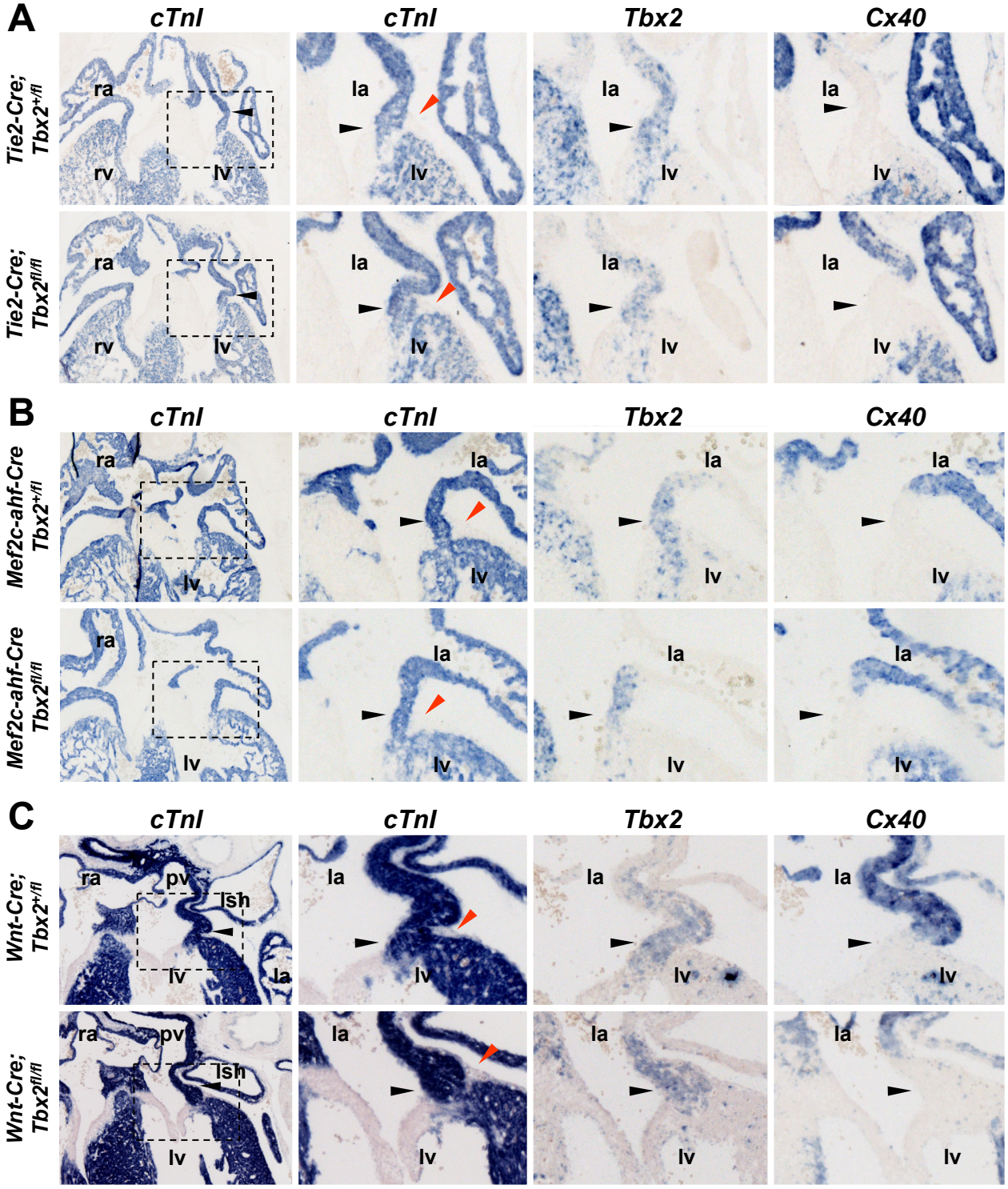
Supplemental Figure 1. Images of i*n situ* hybridization in serial sections of wild type and *Tbx2*$^{-/-}$ embryos of the (A) left and (B) right atrioventricular canal (both E14.5). In wild type the myocardium of the atrioventricular canal expressed *Tbx2* and *Tbx3*, whereas *Cx40* and *Cx43* are complementarily expressed in the working myocardium of the atria and ventricles. In *Tbx2*$^{-/-}$ embryos the left atrioventricular canal myocardium has broadened and shortened. In addition, *Cx40* and *Cx43* are ectopically expressed in the atrioventricular canal myocardium complementary to the remaining *Tbx3* expression. The right atrioventricular canal is unaffected in the mutants. Arrowheads point to the atrioventricular canal myocardium. (C) Expected (based on Mendelian inheritance) and observed numbers of *Tbx2*$^{-/-}$ fetuses (E14.5 and E17.5) and adult *Myh6-Cre;Tbx2*$^{fl/fl}$. la, left atrium; lv, left ventricle; ra, right atrium; rv, right ventricle.

Supplemental Figure 2



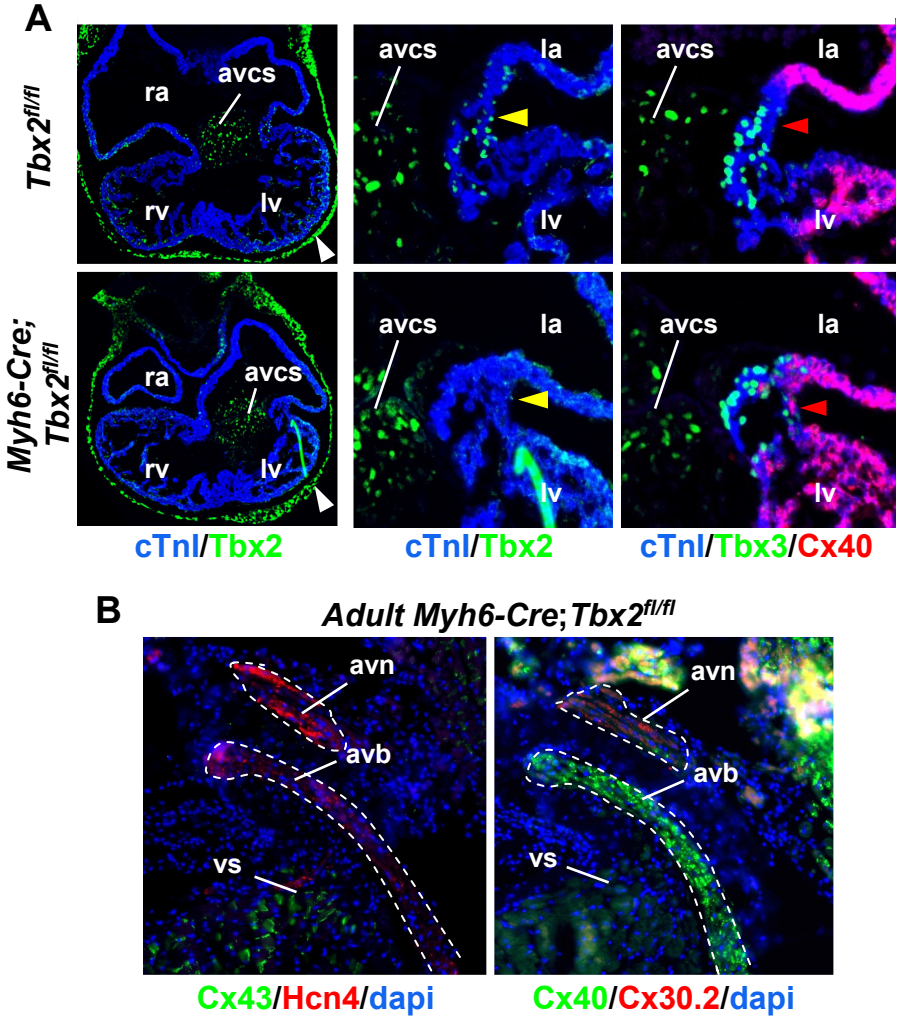
Supplemental Figure 2. Images of immunohistochemical analyses in sections of human embryonic hearts at Carnegie stage 14 (33 dpf)(comparable to mouse E11.5)k. TBX2 and TBX3 are both expressed in the atrioventricular canal myocardium. CX40 is expressed in the working myocardium of the atria and ventricles and not in the atrioventricular canal myocardium. This suggests a similar role for TBX2 and TBX3 in human and mice to maintain the atrioventricular canal myocardial phenotype by repression of working myocardial genes. la, left atrium; lv, left ventricle.

Supplemental Figure 3



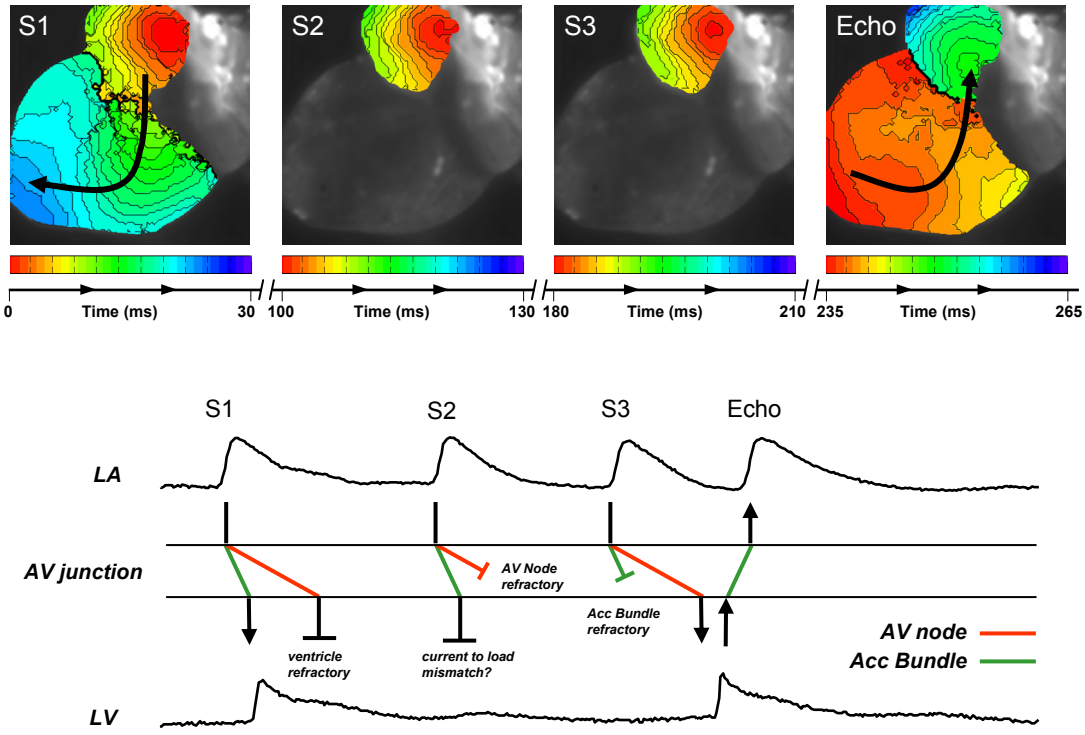
Supplemental Figure 3. *In situ* hybridization images of serial sections of wild type and after specific inactivation of *Tbx2* in (A) endothelial derived tissue (*Tie2-Cre*), (B) anterior secondary heart field and dorsal mesenchymal protrusion derived tissue (*Mef2c-AHF-Cre*), and (C) neural crest derived tissue (*Wnt-Cre*). The atrioventricular canal patterning and morphology was unaffected in all three lineage-specific knock-outs. The boxes indicate the area that is enlarged in the related sections. Black arrowheads indicate the atrioventricular canal myocardium. Red arrowheads indicate the epicardium and epicardium derived mesenchyme. ra, right atrium; rv, right ventricle; la, left atrium; ra, right atrium; pv, pulmonary vein; lsh, left sinus horn.

Supplemental Figure 4



Supplemental Figure 4. Myocardial specific deletion of *Tbx2* results in ectopic expression of working myocardial proteins in the left AV canal myocardium. (A) Immunohistochemical analyses in serial section of *Tbx2*$^{fl/fl}$ and *Myh6-Cre;Tbx2*$^{fl/fl}$ embryos at E10.5. In the *Tbx2*$^{fl/fl}$ embryo Tbx2 protein is expressed in the atrioventricular (AV) canal myocardium, the AV cushions and the body wall (white arrowhead). Tbx3 is expressed in the AV canal myocardium and Cx40 is expressed in the atria and ventricles but not in the AV canal myocardium. In the *Myh6-Cre;Tbx2*$^{fl/fl}$ littermate Tbx2 protein is expressed in the AV cushions and body wall but is lost from the AV canal myocardium (yellow arrowhead). Cx40 is ectopically expressed in the left AV canal complementary to the remaining Tbx3 (red arrowhead). (B) Images of immunohistochemical analyses in serial section of a 3 months old *Myh6-Cre;Tbx2*$^{fl/fl}$ mouse. The central conduction system is demarcated by Tbx3 and Hcn4 expression. The atrioventricular bundle is also Cx40-positive while the atrioventricular node does not express Cx40. The left panel reveals that Cx30.2 is expressed in the atrioventricular node but not in the atrioventricular bundle. This suggests that the atrioventricular conduction axis is intact in *Myh6-Cre;Tbx2*$^{fl/fl}$ mice. ra, right atrium; la, left atrium; rv, right ventricle; avcs, atrioventricular cushion; avn, atrioventricular node; avb, atrioventricular bundle; vs, ventricular septum.

Supplemental Figure 5



Supplemental Figure 5. An induced atrial echo beat in an adult mouse heart with a myocardial specific deletion of *Tbx2*. This heart showed ventricular preexcitation during sinus rhythm (not shown) and atrial stimulation (S1) and premature stimulation. After premature stimulation (S2) in the presences Ajmaline ventricular activation failed. This means that both the AV node and the accessory pathway were not able to activate the ventricles. After a third stimulation (S3) the ventricle was activated after a normal AV delay followed by atrial activation. We hypothesize that the accessory pathway was activated during the first premature stimulus (S2), but was not able to activate the ventricles because of current-to-load mismatch, while the AV node was still refractory. After a second premature stimulus (S3) the accessory bundle is refractory while the AV node is able to propagate the impulse to the ventricle (note the apex to base activation pattern) followed by a retrograde activation of the atria via the accessory bundle. la, left atrium, lv, left ventricle, acc bundle, accessory bundle.