

SUPPLEMENTARY FIGURES AND MOVIE**Supplementary Figure 1. Downregulation of Myh6 in *Gata4*^{+/-} *Tbx5*^{+/-} embryos.**

Radioactive section in situ hybridization demonstrates decreased levels of Myh6 mRNA in ventricular myocardium of *Gata4*^{+/-} *Tbx5*^{+/-} embryonic heart (B) when compared to wildtype littermate (A). Coronal section through heart of E11.5 embryo is shown. ra, right atrium; la, left atrium; ivs, interventricular septum; rv, right ventricle; lv, left ventricle. (C) At E13.5, semi-quantitative RT-PCR demonstrates downregulation of Myh6 in only *Gata4*^{+/-} *Tbx5*^{+/-} embryonic hearts when compared to wildtype, *Gata4*^{+/-} single heterozygotes, and *Tbx5*^{+/-} single heterozygotes.

Supplementary Figure 2. Synergistic activation of ANF-luciferase reporter by *Gata6* and

***Tbx5*.** Luciferase activity directed by ANF promoter in HeLa cells transfected with *Tbx5* expression plasmid (100ng) and indicated amounts of *Gata6* expression plasmid.

Supplementary Movie. Histologic 3-D reconstruction of *Gata4*^{+/-} *Tbx5*^{+/-} E14.5 embryo. (A)

Still image demonstrating atrioventricular septal defect (asterisk) and thin myocardium

(arrowheads). (B) 3-D reconstruction demonstrating single atrioventricular valve, septation

defect and abnormal ventricular myocardium. ra, right atrium; la, left atrium; rv, right ventricle;

lv, left ventricle, ao, aorta; pa, pulmonary artery.