Supplementary Figures

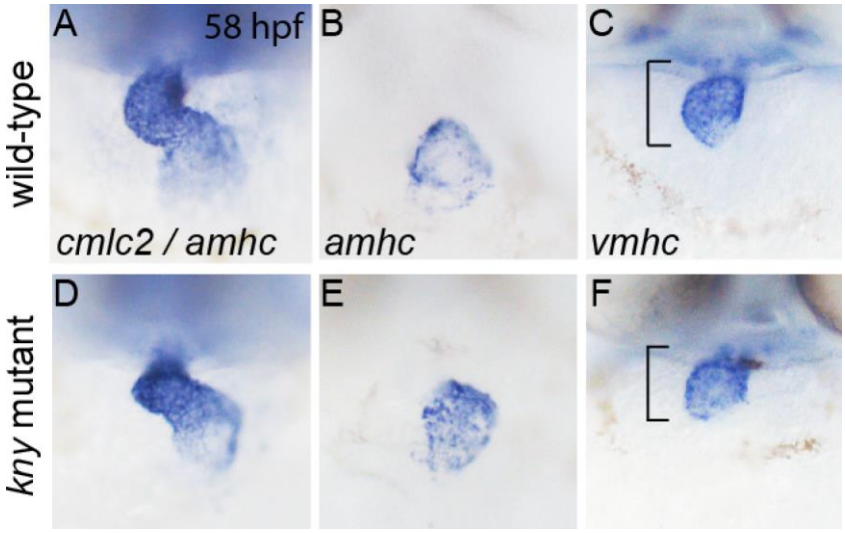

Figure S1, related to Figure 1: Reduced heart looping and reduced chamber sizes in *kny/gpc4* mutants. (A-F) Anterior view of *in situ* hybridized wild-type (A-C) and *kny/gpc4* mutant (D-F) at 58 hpf. *In situ* hybridization was carried out for *amhc/cmlc2* (A,D), *amhc* alone (B,E) and *vmhc* (C,F).

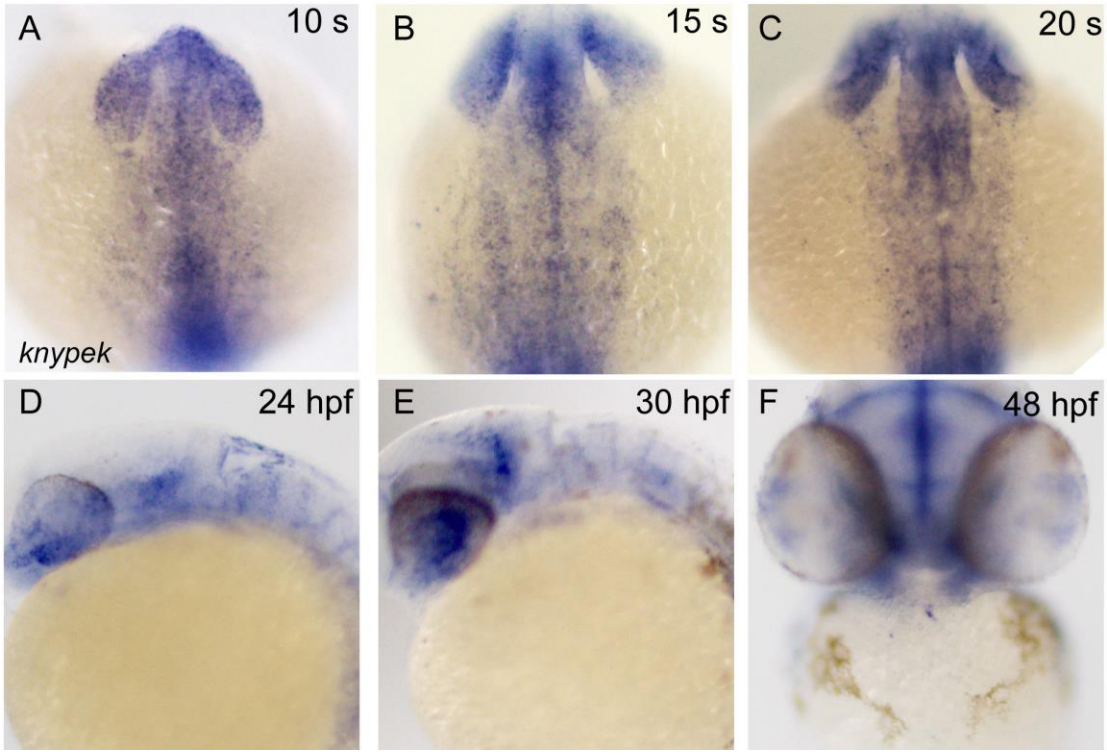

Figure S2, related to Figure 1: *Kny/gpc4* expression in wild-type embryos.

(A-C) Dorsal view of wild-type embryos at 10-somite, 15-somite and 20-somite stages, respectively. (D-F) Lateral view of wild-type embryos at 24, 30 and 48 hpf, respectively. *In situ* hybridization was performed for *kny/gpc4*.

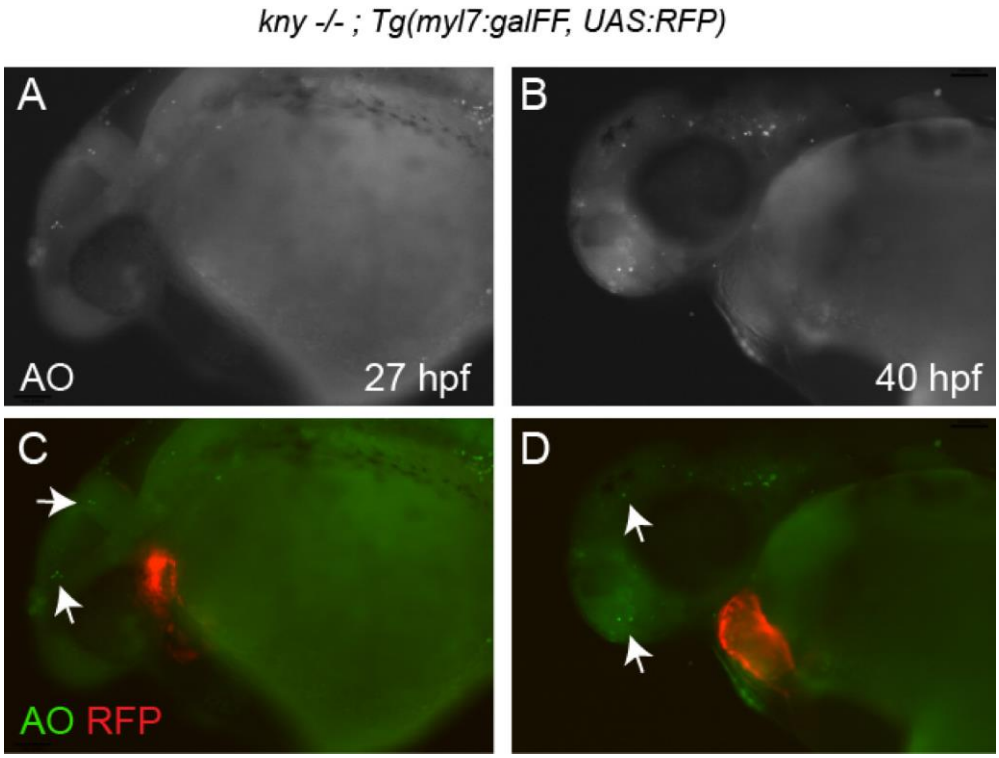

Figure S3, related to Figure 1: Acridine Orange staining of *kny/gpc4* mutants.

Live images of kny/gpc4 mutant embryos at 27 hpf (A,C) and 40 hpf (B,D) stained with Acridine Orange (AO) to visualize apoptotic cells (green). Arrows indicate apoptotic cells in brain region. No apoptotic cells were observed in the myocardium of *kny/gpc4* mutant hearts (red).

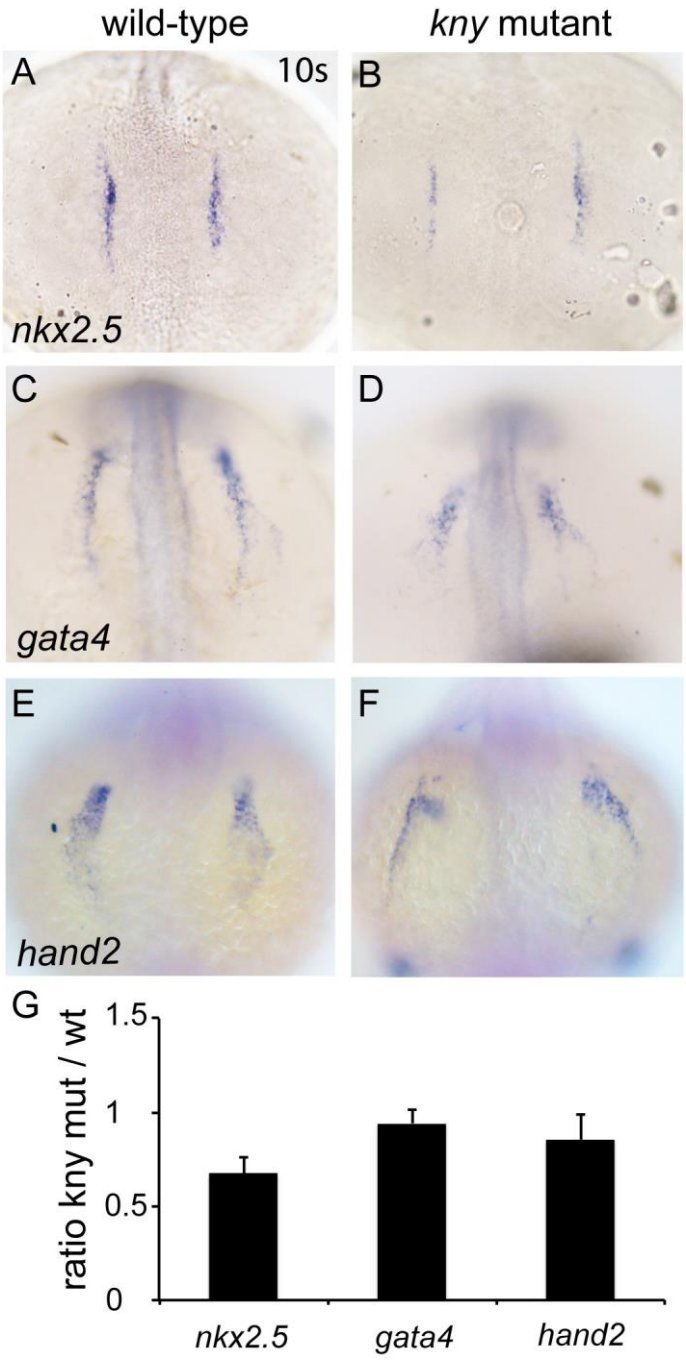

Figure S4, related to Figure 2: Cardiac specification in *kny/gpc4* mutants. (A-F) Dorsal view of wild-types (A,C,E) and *kny/gpc4* mutants (B,D,F) at 10-somite stage. *In situ* hybridization was carried out for *nkx2.5* (A and B), *gata4* (C and D) and *hand2* (E and F). (G) qPCR results of embryos from corresponding stages. Gene expression levels were normalized against *ef1a.* The *y*-axis represents the ratio of expression levels between *kny/gpc4* mutants and wild-type siblings (three biological repeats). Results are represented as mean±s.e.m.

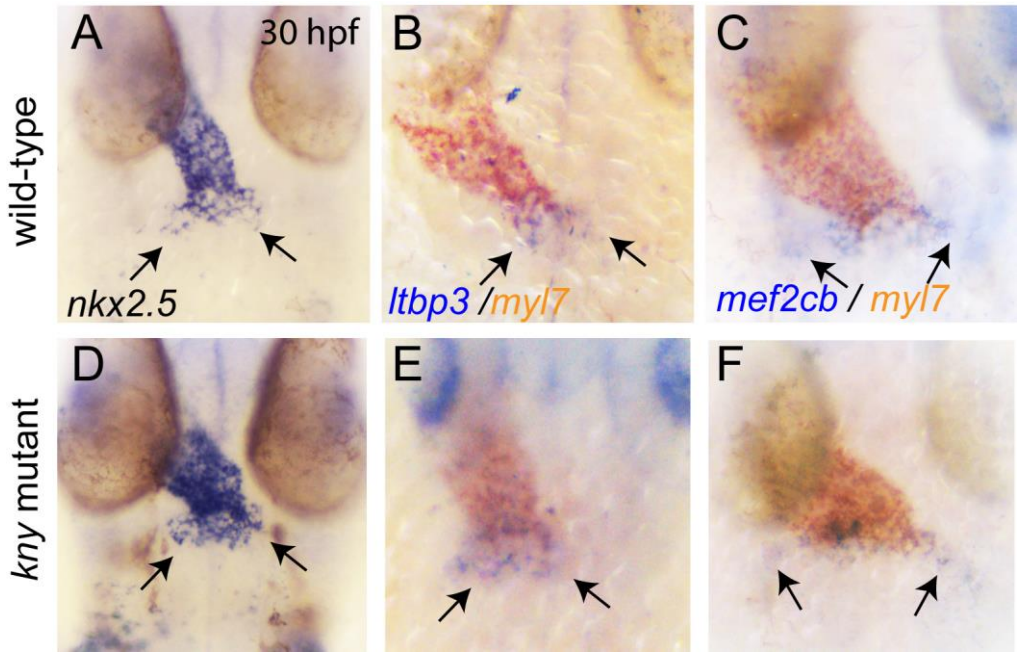

Figure S5, related to Figure 2: Specification of the SHF occurs in *kny/gpc4* mutants. (A-F) Dorsal view of wild-types (A-C) and *kny/gpc4* mutants (D-F) at 30 hpf. *In situ* hybridization was carried out for *nkx2.5* (A,D), *ltbp3* (blue) / *myl7* (orange) (B,E) and *mef2cb* (blue)/*myl7* (orange) (C,F). Arrows indicate staining in the SHF.

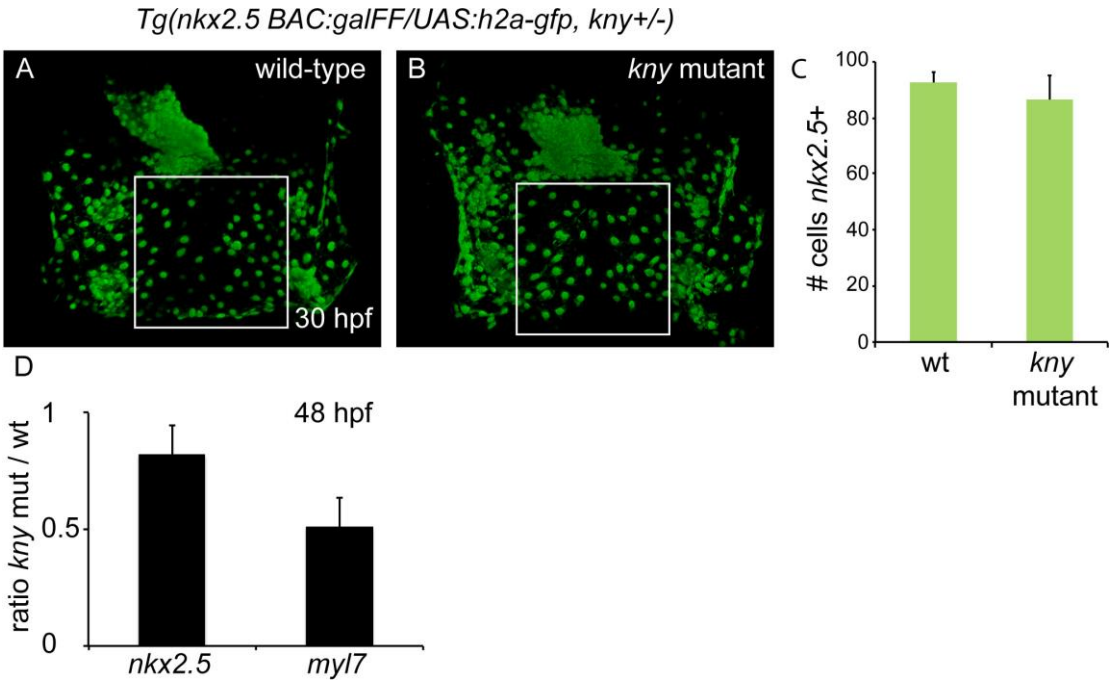

Figure S6, related to Figure 3: Nkx2.5+ cells in the anterior LPM of *kny/gpc4* mutants and wild-type siblings (A,B) Dorsal view of wild-type (A) and *kny/gpc4* mutant (B), expressing the *nkx2.5:galFF/UAS:h2a-gfp* transgene at 30 hpf. (C) Numbers of *nkx2.5*+ cells in boxed areas from (A,B). (D) qPCR results of *nkx2.5* and *myl7* expression levels at 48 hpf. Normalization against *ef1a*. The y-axis represents the ratio of expression level between *kny/gpc4* mutants and wild-type siblings (three biological repeats). Results are represented as mean±s.e.m.

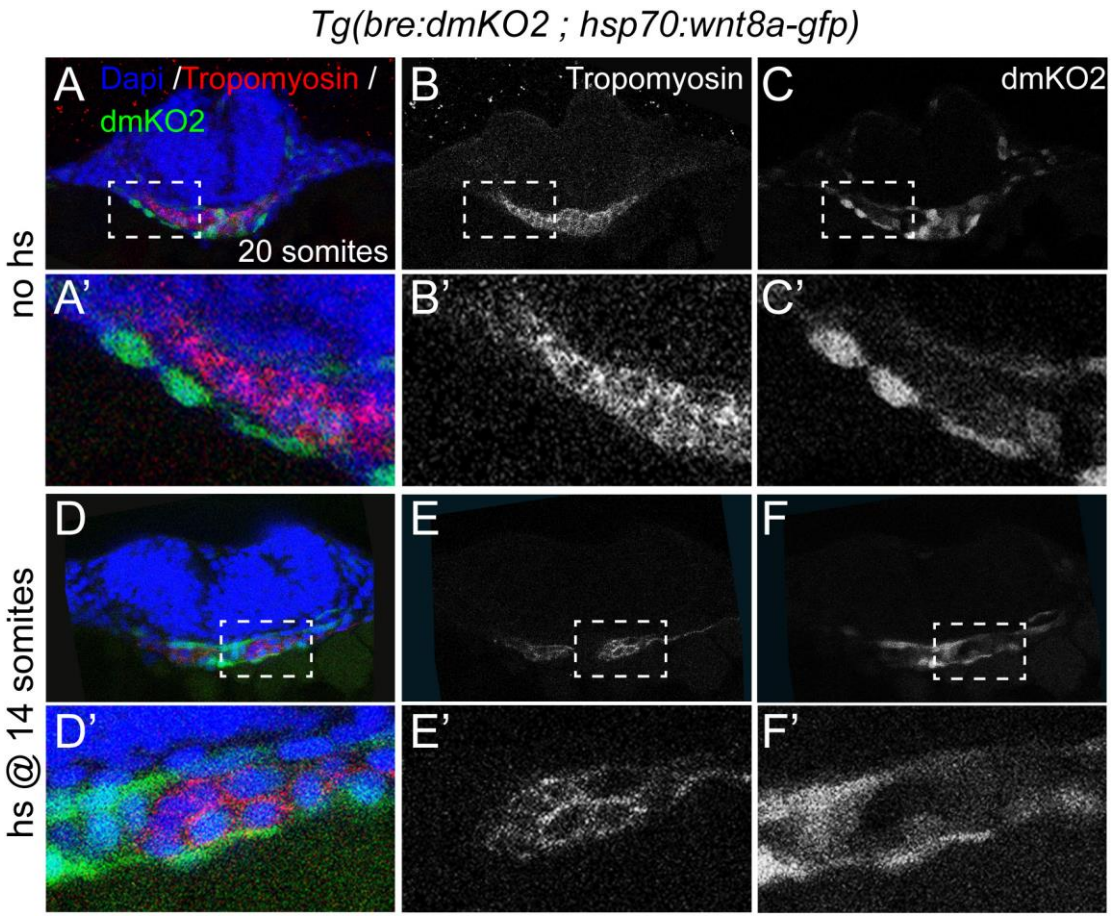

Figure S7, related to Figure 4: Ectopic Wnt8 expression does not induce *bre:dmKO2* activity. Cross-sections through anterior LPM of control (A-C') or heat-shocked (D-F') embryo of 20-somites with *Tg(bre:dmKO2 ; hsp70:wnt8a-gfp)*. Embryos in (D-F') were heat shocked at 16 hpf (14 somites). Cell nuclei are shown in blue (DAPI), cardiac tissue in red (tropomyosin) and Bmp activity in green (*bre:dmKO2* transgene). (A'-F') are magnifications of boxed areas in (A-F).

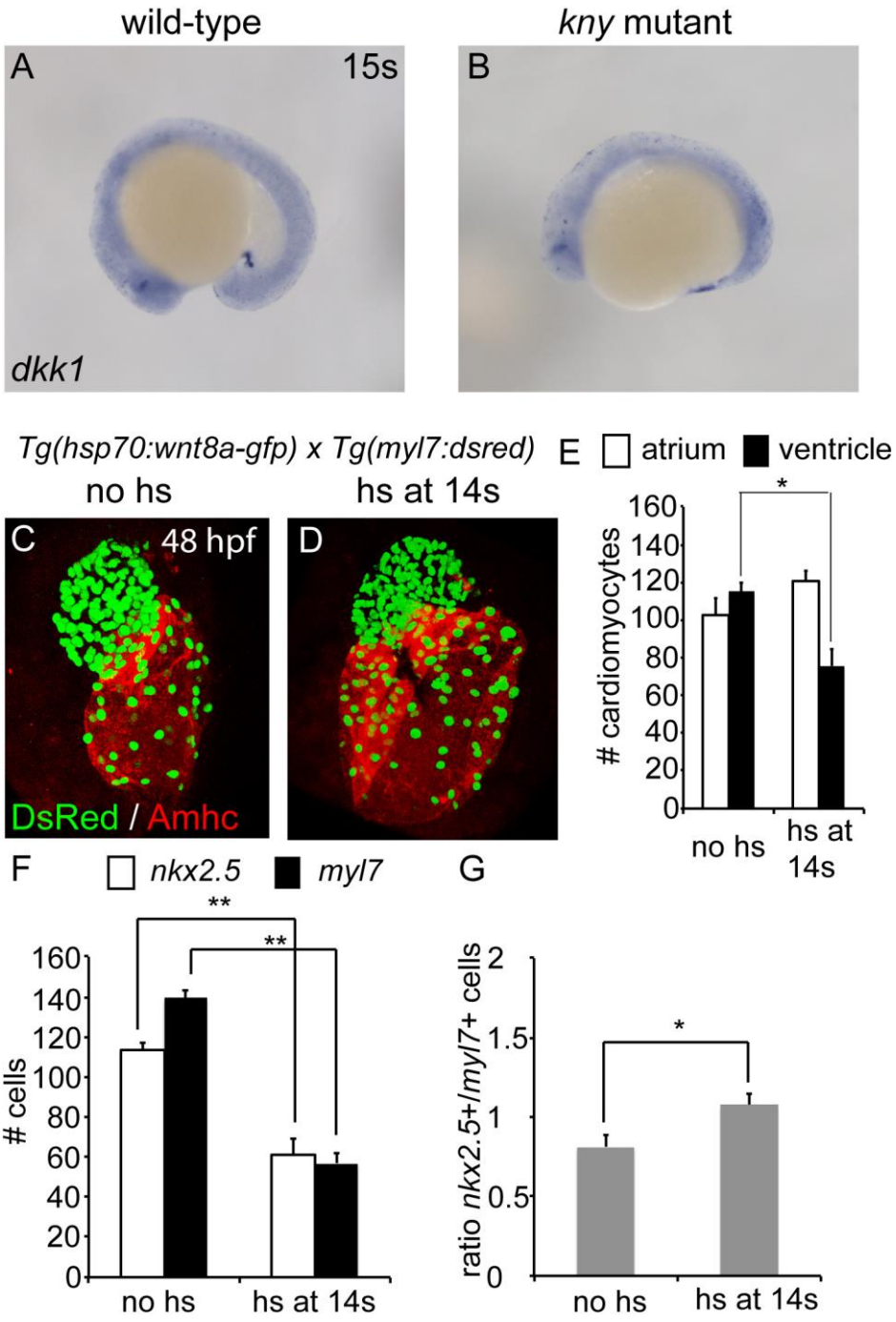

Figure S8, related to Figure 4: Elevation of canonical Wnt signaling after cardiac specification leads to a reduction of ventricular cardiomyocytes. (A,B) Lateral view of wild-type siblings and *kny/gpc4* mutants after in situ hybridization for *dkk1* at the 15 somite stage. (C,D) Hearts at 48 hpf of embryos derived from the *Tg(hsp70:wnt8a-gfp)* line crossed to *Tg(myl7:dsred),* stained for DsRed (false colored in green) and Amhc (red). (E)

Cardiomyocyte numbers of corresponding hearts (*n*=3). (F) Quantification of *nkx2.5+* and *myl7+* cells in the ventricles of embryos after *wnt8a* induction (n=3). (G) Ratio of *nkx2.5+/myl7+* cells in corresponding hearts after Wnt8 induction. Results are represented as mean ±s.e.m. Asterisks represents statistical significance according to a paired *t*-test: * $P<0.05$; ** $P<0.01$.