Supplemental Materials

Supplemental Figures:

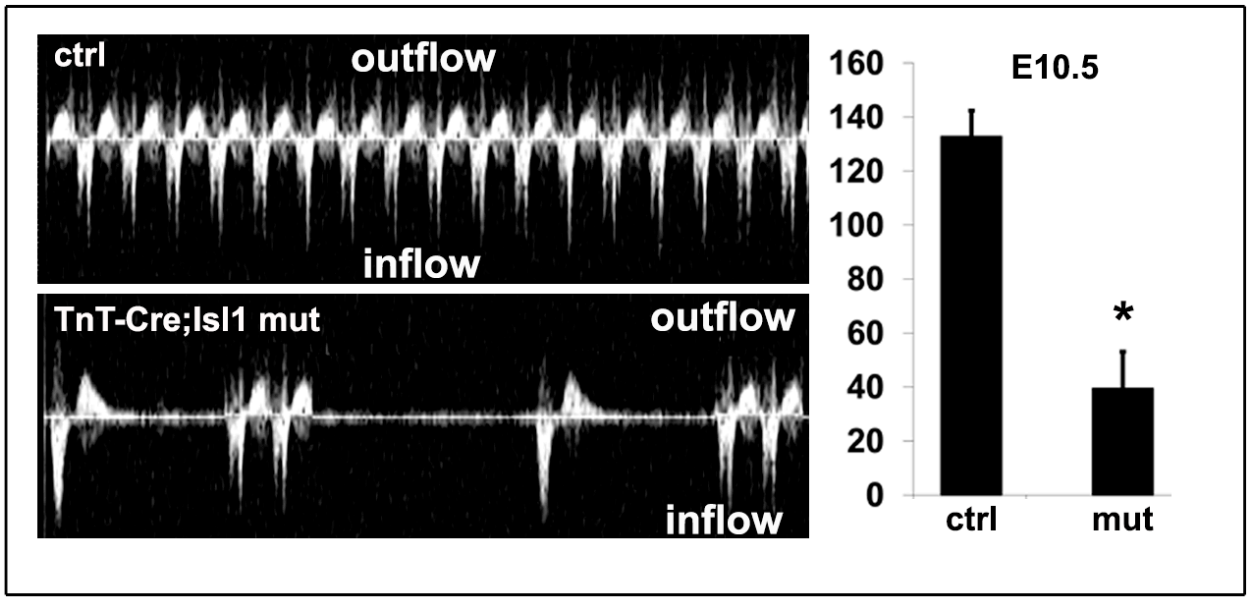

Figure S1

Figure S1. Requirement for Isl1 in differentiated myocytes for pacemaker function of the SAN. Ablation of *Isl1* specifically in myocardial cells using *Troponin T-Cre* resulted in severe bradycardia and irregular heart rate as revealed by echocardiography. Pulse wave Doppler revealed well coupled outflow and inflow wave, suggesting no AV block.

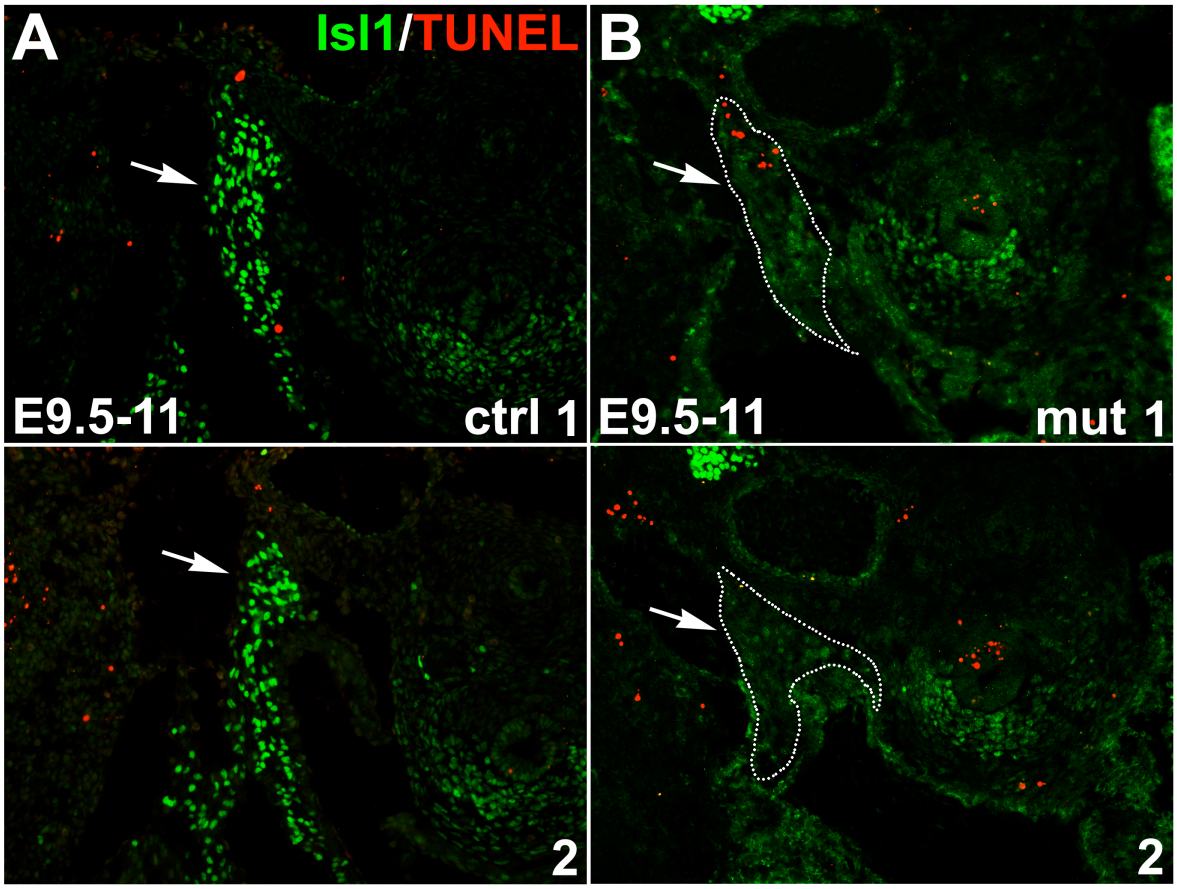

Figure S2

Figure S2. Ablation of *Isl1* using *Hcn4-CreERT2* at E9.5 leads to increased cell death in the SAN. Immunostaining demonstrated effective ablation of ISL1 expression in *Isl1* mutants at E11 (B1 and 2), but ISL1 expression in pharyngeal region and dorsal mesocardium appeared to be normal. TUNEL revealed increased cell death in *Isl1* mutant SAN (B1 and 2) compared to control littermate (A1 and 2).

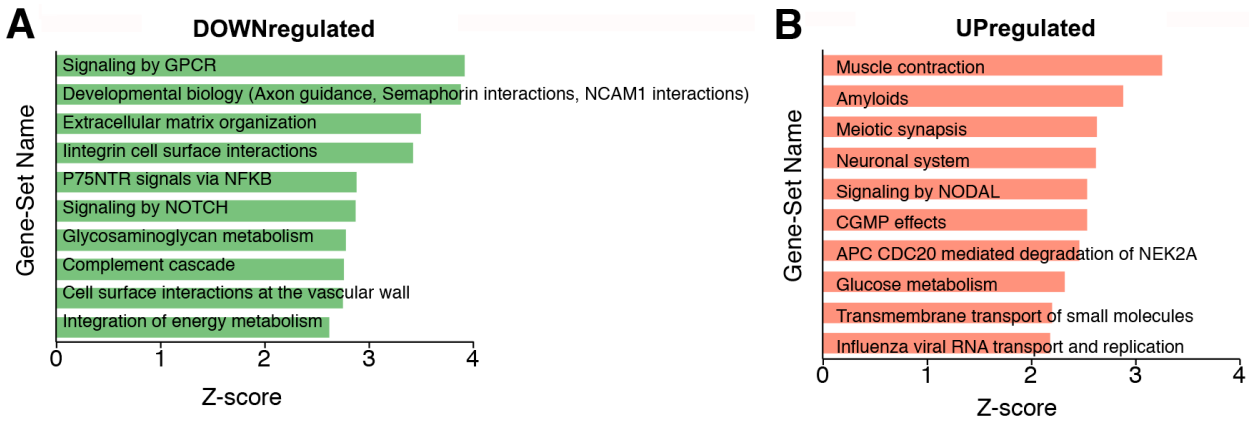

Figure S3

Figure S3. REACTOME pathway enrichment analysis of DE genes in *Hcn4-CreERT2*;*Isl1* mutant SAN. A) Top 10 REACTOME categories enriched of genes downregulated in *Isl1* mutant SAN. B) Top 10 REACTOME categories enriched of genes upregulated in *Isl1* mutant SAN.

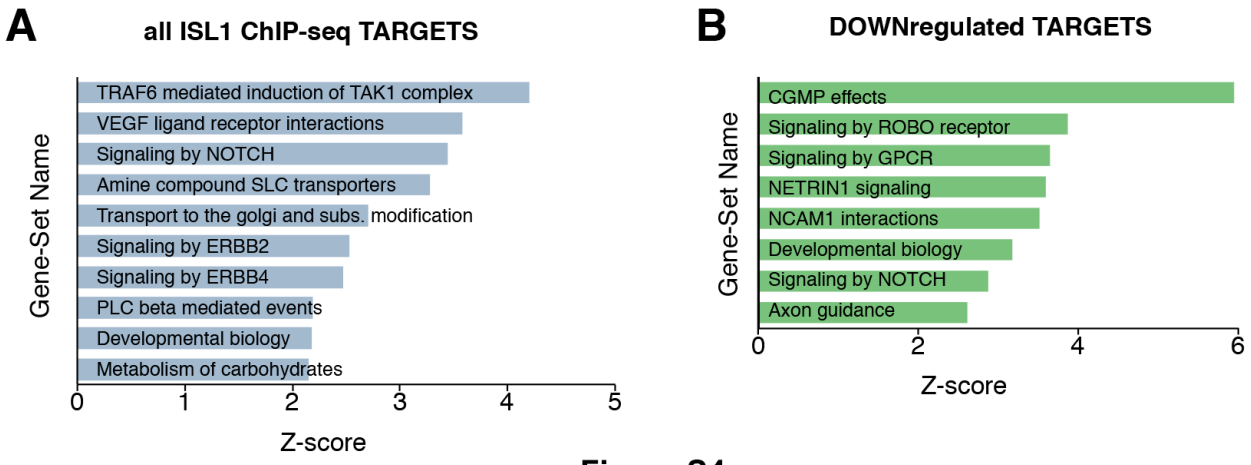

Figure S4

Figure S4. REACTOME pathway enrichment analysis of ISL1 targets. A) Top 10 REACTOME categories significantly enriched of all genes annotated with ISL1 ChIP-seq peaks. **B)** REACTOME categories significantly enriched of genes downregulated in *Hcn4-CreERT2*;*Isl1* mutant SAN cells, associated to ISL1 ChIP-seq peak. No REACTOME categories found significant enriched of genes upregulated in *Hcn4-CreERT2;Isl1* mutant SAN cells, associated to ISL1 ChIP-seq peak.