

Supplemental Material

Figure S1. ENH expression at E8.5 and E10.5

Whole-mount RNA *in situ* hybridization analysis of ENH expression in a wild type embryo (right and left lateral views) at (A and B) E8.5 and (C and D) E10.5 using an ENH-specific probe.

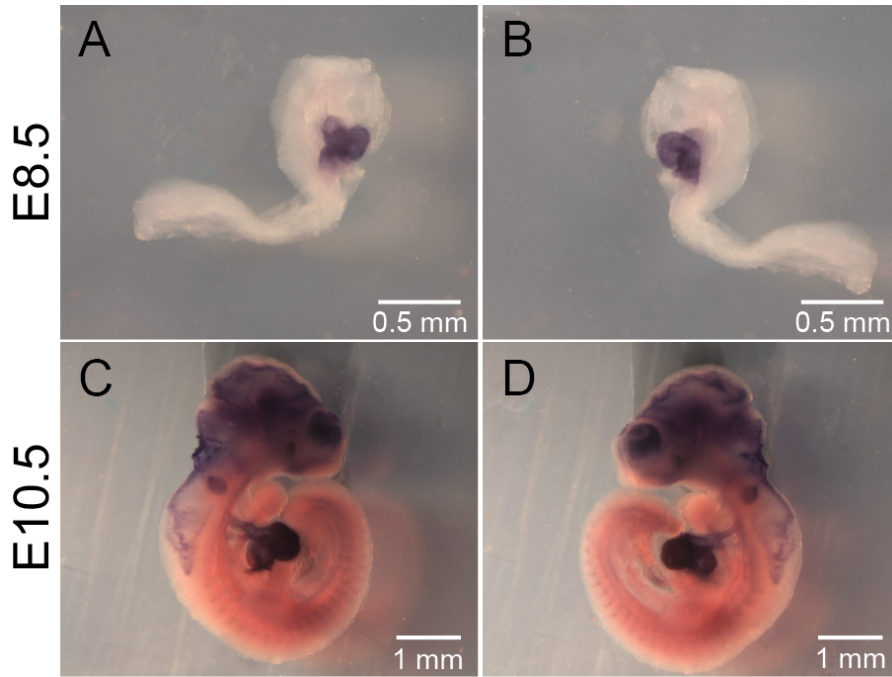


Figure S2. Cypher ablation does not affect early heart development

Whole-mount microscopic assessment of wild type and Cypher-null ($Cypher^{-/-}$) embryos (left lateral view) at different developmental stages. (A) Wild type embryo and (B) somite-matched $Cypher^{-/-}$ embryo at E9.5. (C) Wild type embryo and (D) somite-matched $Cypher^{-/-}$ embryo at E10.5.

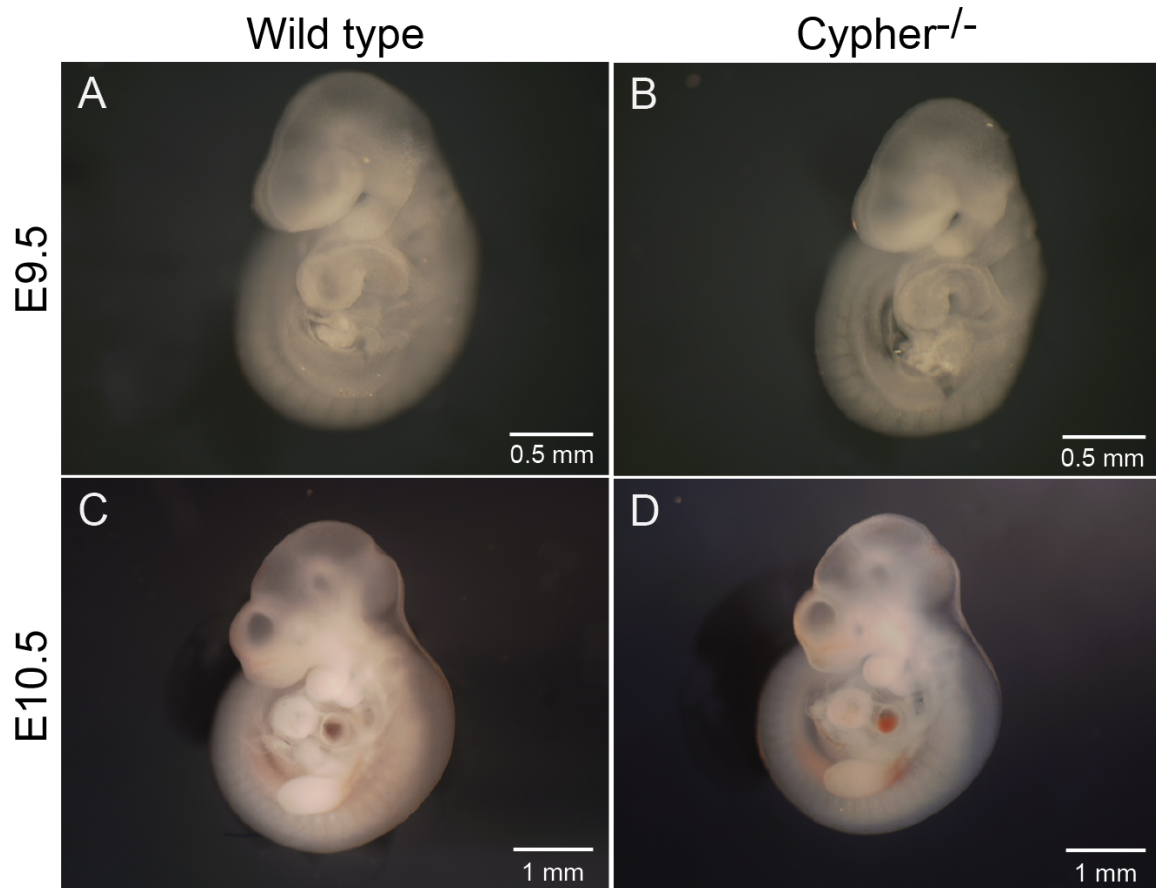


Figure S3. ENH^{-/-}/Cypher^{-/-} hearts display no abnormalities in cardiomyocyte apoptosis or proliferation

Immunofluorescence analysis of a transverse cryosection of the heart of (A and C) control and (B and D) somite-matched ENH^{-/-}/Cypher^{-/-} double knockout (dKO) embryos at E9.0-E9.5. Apoptosis and proliferation were stained using antibodies against (A and B) cleaved caspase 3, cCASP3 (green), and (C and D) phospho-histone H3, pHH3 (green), respectively. Sarcomeres were stained with anti- α -Actinin (red); DNA is stained with DAPI (blue). Note: due to thinning of the myocardium and severe dilation of the heart chamber, dKO hearts shrunk during sucrose processing.

