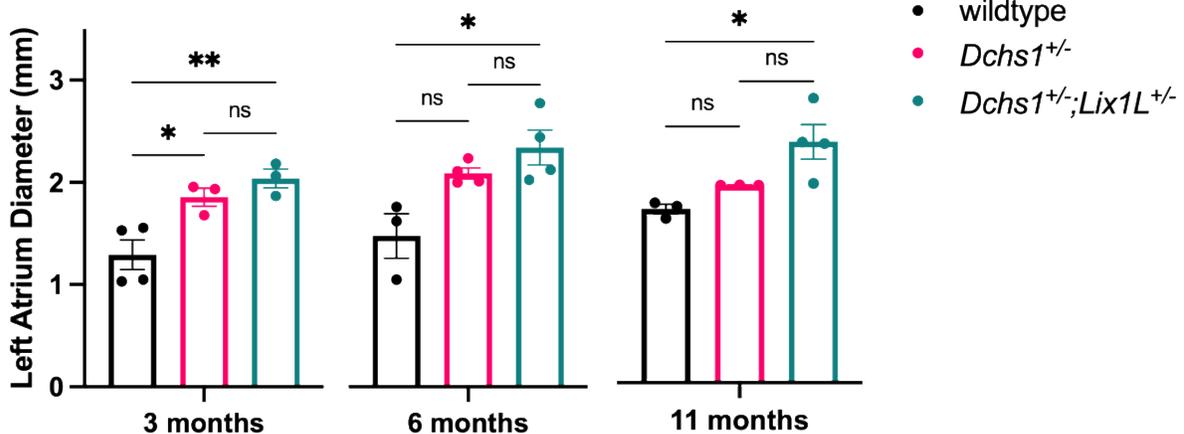
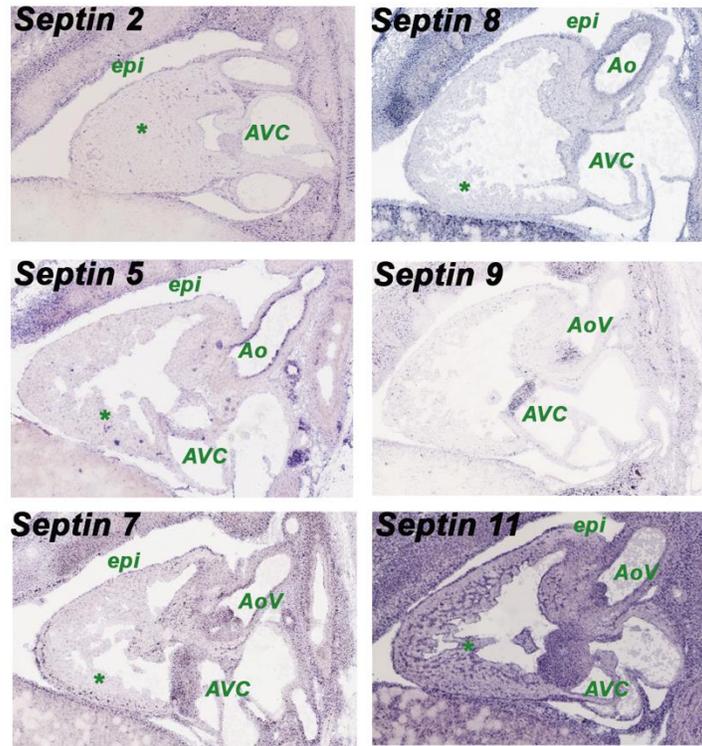


**Figure S1. Septin 9 expression is decreased in compound heterozygotes. (A)** IHC of SEPT9 (red) and nuclei (Hoechst, blue) depicts loss of Septin-9 expression in the interstitium of compound hets (*Dchs1*<sup>+/-</sup>;*Lix1L*<sup>+/-</sup>) compared to controls (*Dchs1*<sup>+/+</sup>;*Lix1L*<sup>+/+</sup>). **(B)** Quantification of staining intensity reveals significant decreases in SEPT9 expression. n=3 per genotype, \*\*=p<0.005 with Student's t-test.

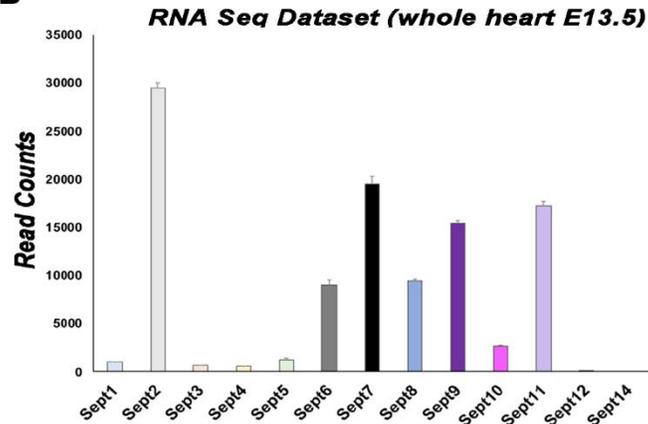


**Figure S2. Compound heterozygote mice display mitral valve prolapse and increased left atrium diameter throughout adulthood.** Quantification of left atrium (LA) diameter measured in 2D images in M-Mode exhibit significant increases in compound heterozygotes compared to wildtype controls at 3, 6, and 11 months of age. N=3,4 animals per genotype, \*=p<0.05, \*\*=p<0.005 with a One-Way Anova, graphs depict individual data points, mean and SEM.

**A E14.5 in situ hybridization (RNA)**



**B**



**Figure S3. Septin RNA expression in developing hearts. (A)** In situ hybridization (ISH) data obtained from GenePaint of wildtype E14.5 hearts depict expression of Septin 2, 5, 7, 8, 9, and 11 RNA in various regions (AVC=atrioventricular canal, Ao=aorta, Ao= Aortic Valve, epi=epicardium, \*=myocardium). **(B)** RNAseq data of whole heart E13.5 supports expression of Sept 2, 5, 7, 8, 9, 10, and 11.