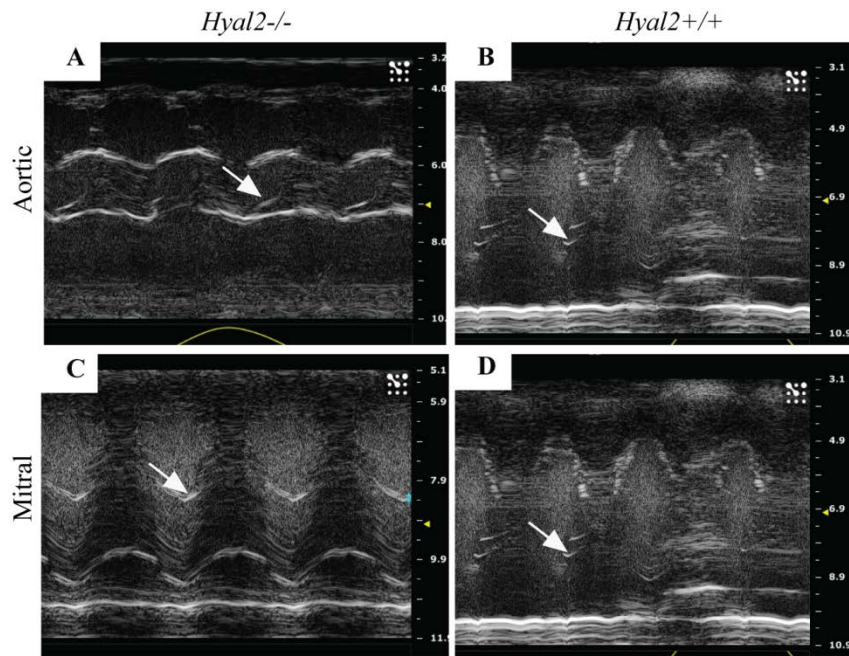
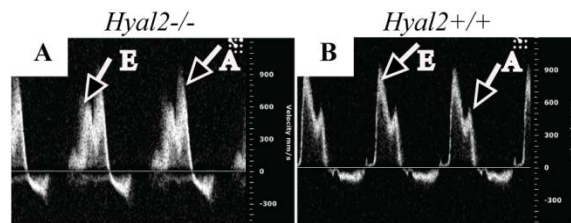


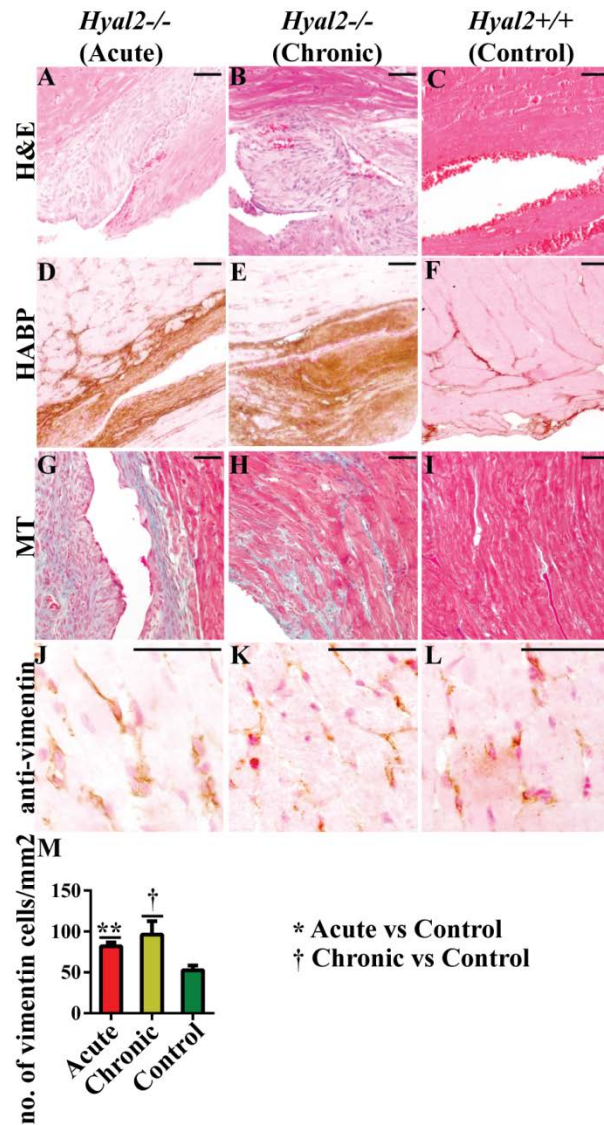
## SUPPLEMENTAL MATERIAL



**Figure S1.** Valve thickening in *Hyal2*<sup>-/-</sup> and control mice. B-mode image of high frequency ultrasound exhibiting thickened aortic and mitral valves in the *Hyal2*<sup>-/-</sup> mouse (arrow, A,C) compared to the control (arrow, B, D). This is a representative image from n=14 pairs of mice.

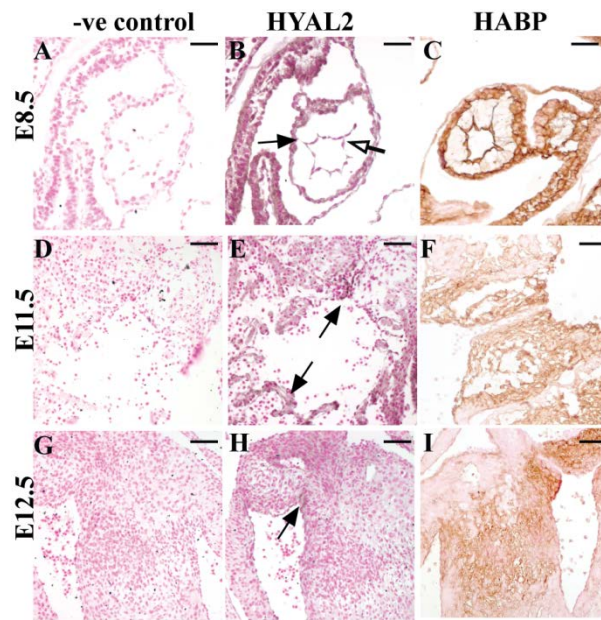


**Figure S2.** E/A ratio in *Hyal2*<sup>-/-</sup> and control mice. M-mode image of mitral early (E) and late atrial (A) flow was calculated to evaluate the E/A ratio of *Hyal2*<sup>-/-</sup> and controls. *Hyal2*<sup>-/-</sup> (A) showed reduced E and increased A in comparison to that of the control mouse (B).



**Figure S3.** Histological analysis of the ventricle of *Hyal2*<sup>-/-</sup> and control hearts. Transverse sections of hearts from *Hyal2*<sup>-/-</sup> (acute and chronic) and control mice were stained for different cellular components. (A-C) H & E staining of the heart revealed fibrous tissues in the ventricle region of the acute (A) and chronic (B) *Hyal2*<sup>-/-</sup> mice compared to control mice (C). (D-F) Detection of HA in *Hyal2*<sup>-/-</sup> and control ventricles using the HABP. HA (brown) was abundant in the ventricle of the *Hyal2*<sup>-/-</sup> acute and chronic groups (D-E) compared to the control (F). (G-I) Masson's trichrome staining of the ventricle of *Hyal2*<sup>-/-</sup> and control mice. The atrium of *Hyal2*<sup>-/-</sup> (G,H) show increased collagen (light blue) and accumulation of GAGs (white) compared to

controls (I). (J-L) Detection of mesenchymal cells in *Hyal2*<sup>-/-</sup> and control heart. Vimentin was detected using anti-vimentin (ab45939) and detected as a brown colour. Increased numbers of vimentin positive (brown color) cells are evident in the *Hyal2*<sup>-/-</sup> heart (J,K) compared to control (L). Scale bar = 50  $\mu$ m. †  $P < 0.05$ ; \*\*  $P < 0.001$ .



**Figure S4:** HYAL2 and HA distribution in wild type embryos. Sections of the embryonic heart at E8.5, 11.5 and 12.5 from a previous study<sup>1</sup> were used for the detection of HYAL2 and HA using histochemical approaches. (A) HYAL2 (brown) in the wall of the bulbus cordis (open arrow) and endocardial lining of the truncal region (arrow) of the E8.5 heart. (E) HYAL2 in the endocardial cushions (arrow) of the outflow tract and the trabeculated wall of the ventricular chamber of the E11.5 heart. (H) HYAL2 in the endocardial cushions and the aortic-pulmonary spiral septum at E12.5. (A,D,G) HYAL2 was not detected in sections where no primary antibody

was used. (C, F, I) HABP staining in the heart showed a progressive decrease in HA with increasing embryo age.