

Supplementary Materials

The Histamine 3 Receptor is Expressed in the Heart and its Activation Opposes Adverse Cardiac Remodeling in the Angiotensin II Mouse Model

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Short Title: Histamine 3 Receptor and Cardiac Remodeling

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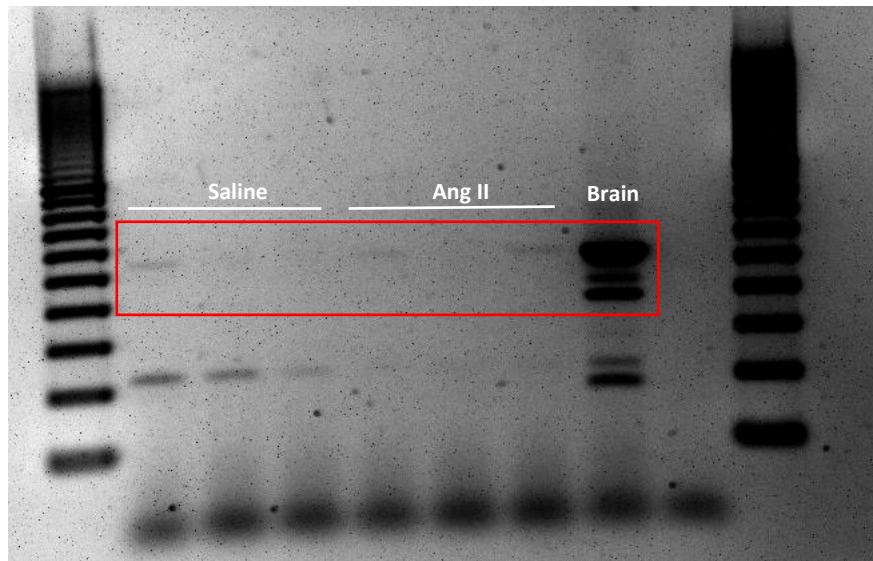


Figure S1. Full PCR gel that appears in cropped form in Figure 1A. Shows the ~530 bp product for the left ventricle from Saline and Ang II-infused mice, as well as the ~530, 430 and 400 bp products for the mouse brain (red box).

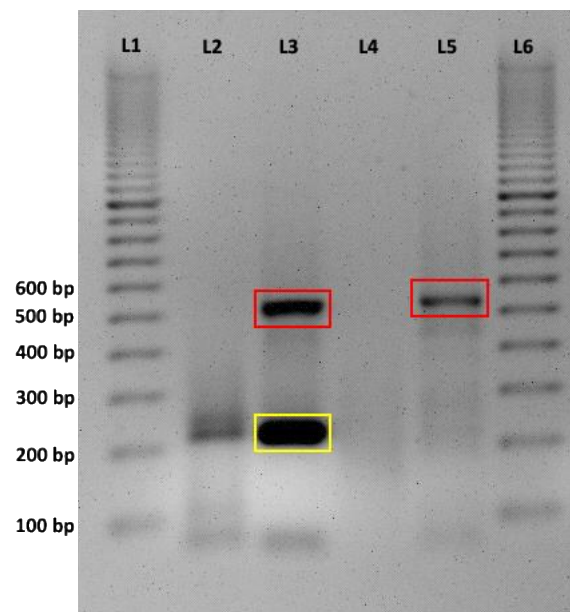


Figure S2. Full PCR gel showing the ~530 bp product (red box) from the Ang II LV (L3) and brain (L5) that were subsequently purified and identified by Sanger sequencing as a 99% match for the mouse H₃R. The ~200 bp product (yellow box) from the LV was also purified, sequenced and identified as a 98% match for mouse endoplasmic reticulum chaperone SIL1 homolog. L1 = ladder, L2 = saline LV, L4 = empty, L6 = ladder.

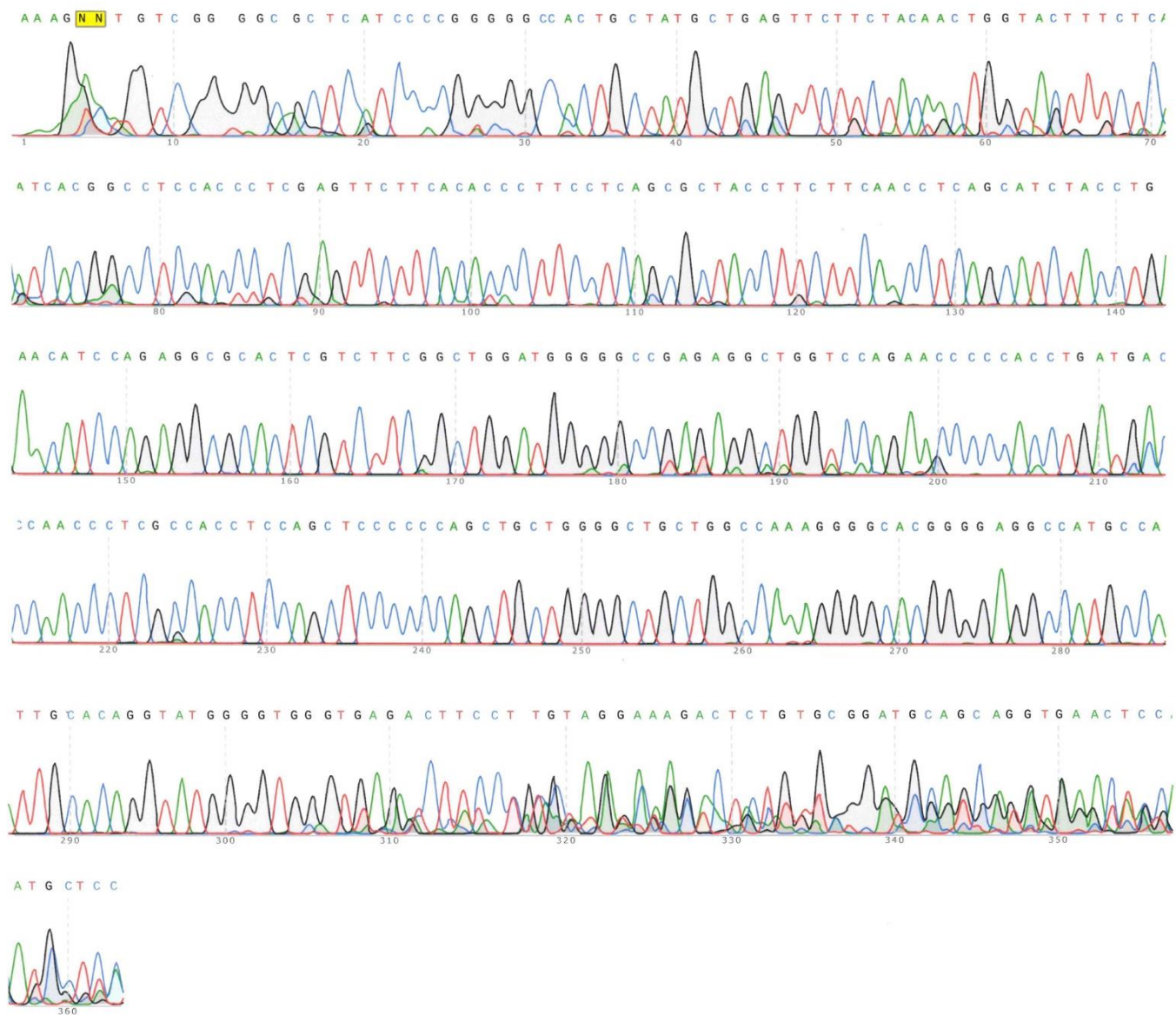


Figure S3. (A) Electrophoretogram identifying the amplicon of the 530 bp PCR product identified as a 99% match for *mus musculus histamine 3 receptor* – forward primer.

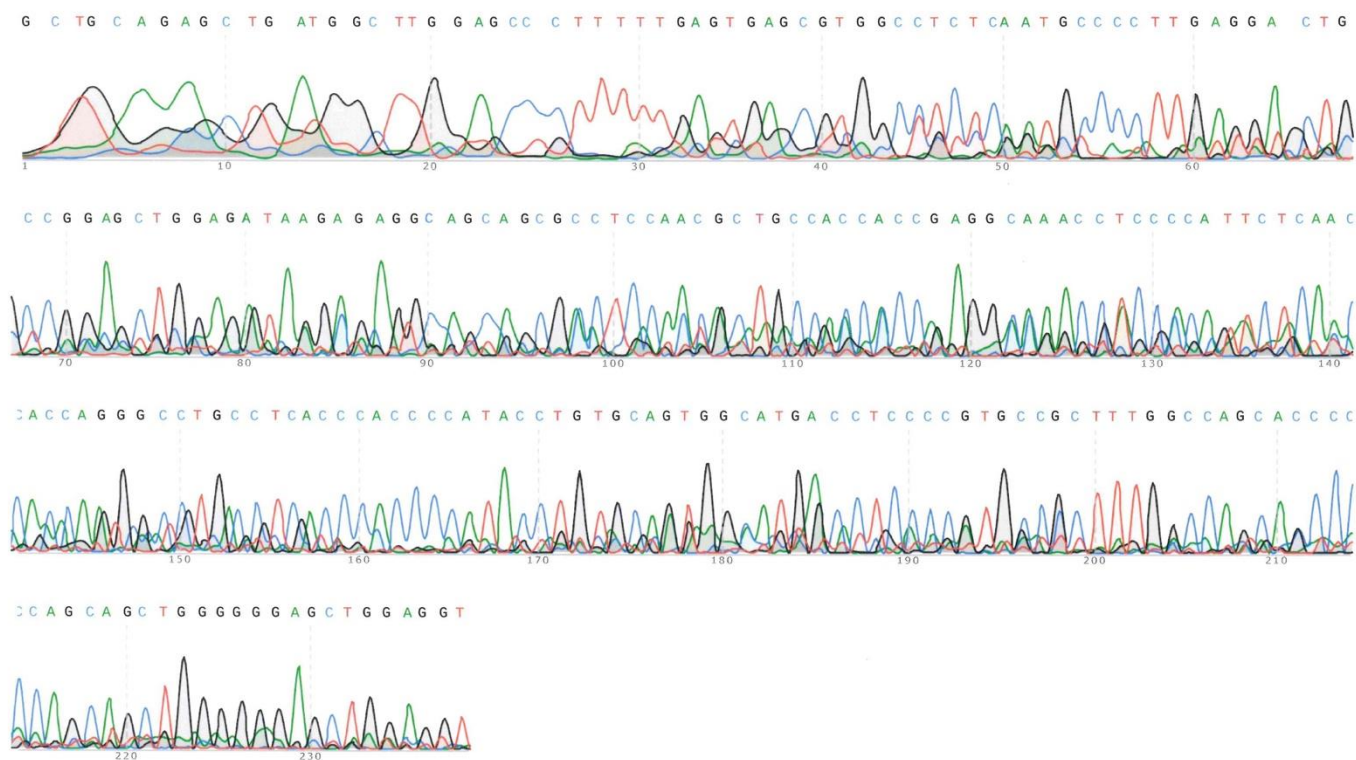


Figure S3. (B) Electrophoretogram identifying the amplicon of the 530 bp PCR product identified as a 99% match for *mus musculus histamine 3 receptor* – reverse primer.

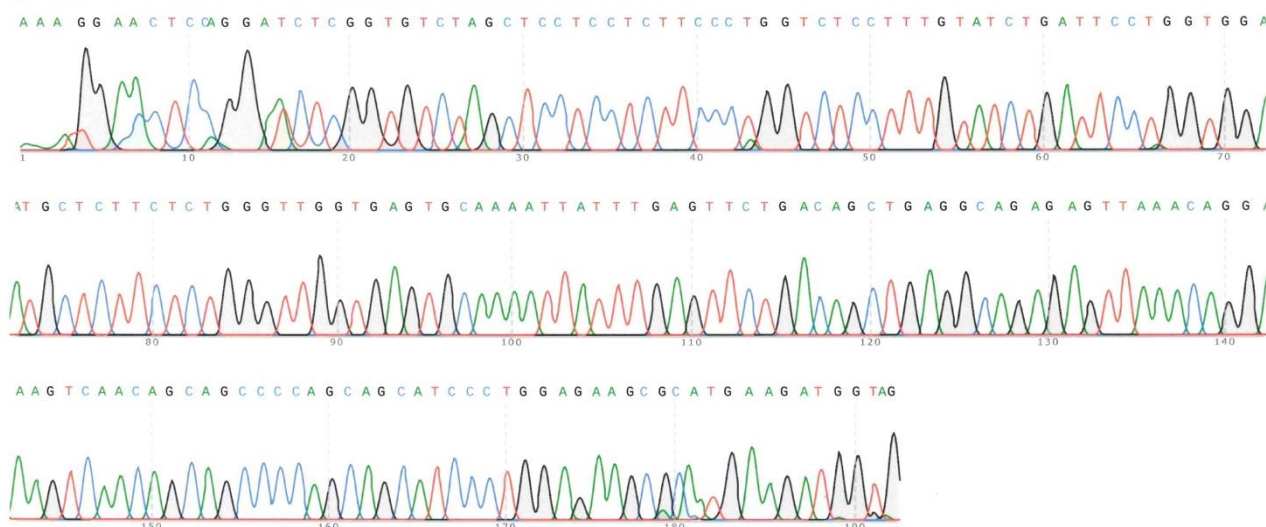


Figure S3. (C) Electrophoretogram identifying the amplicon of the 200 bp PCR product identified as a 98% match for *mus musculus* endoplasmic reticulum chaperone *SIL1* homolog – forward primer.

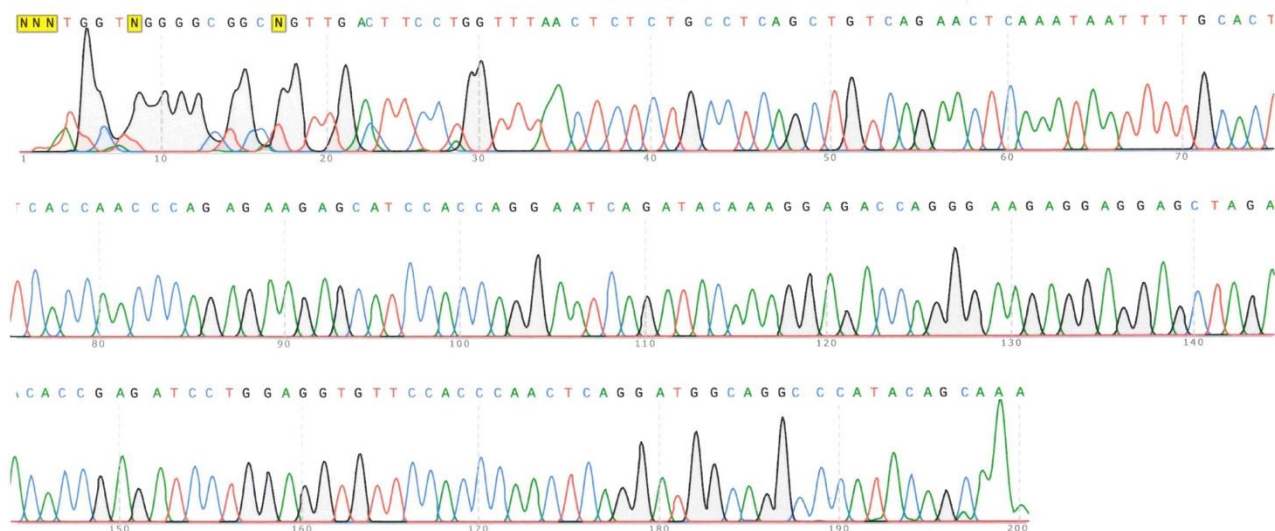


Figure S3. (D) Electrophoretogram identifying the amplicon of the 200 bp PCR product identified as a 98% match for *mus musculus* endoplasmic reticulum chaperone *SIL1* homolog – reverse primer.

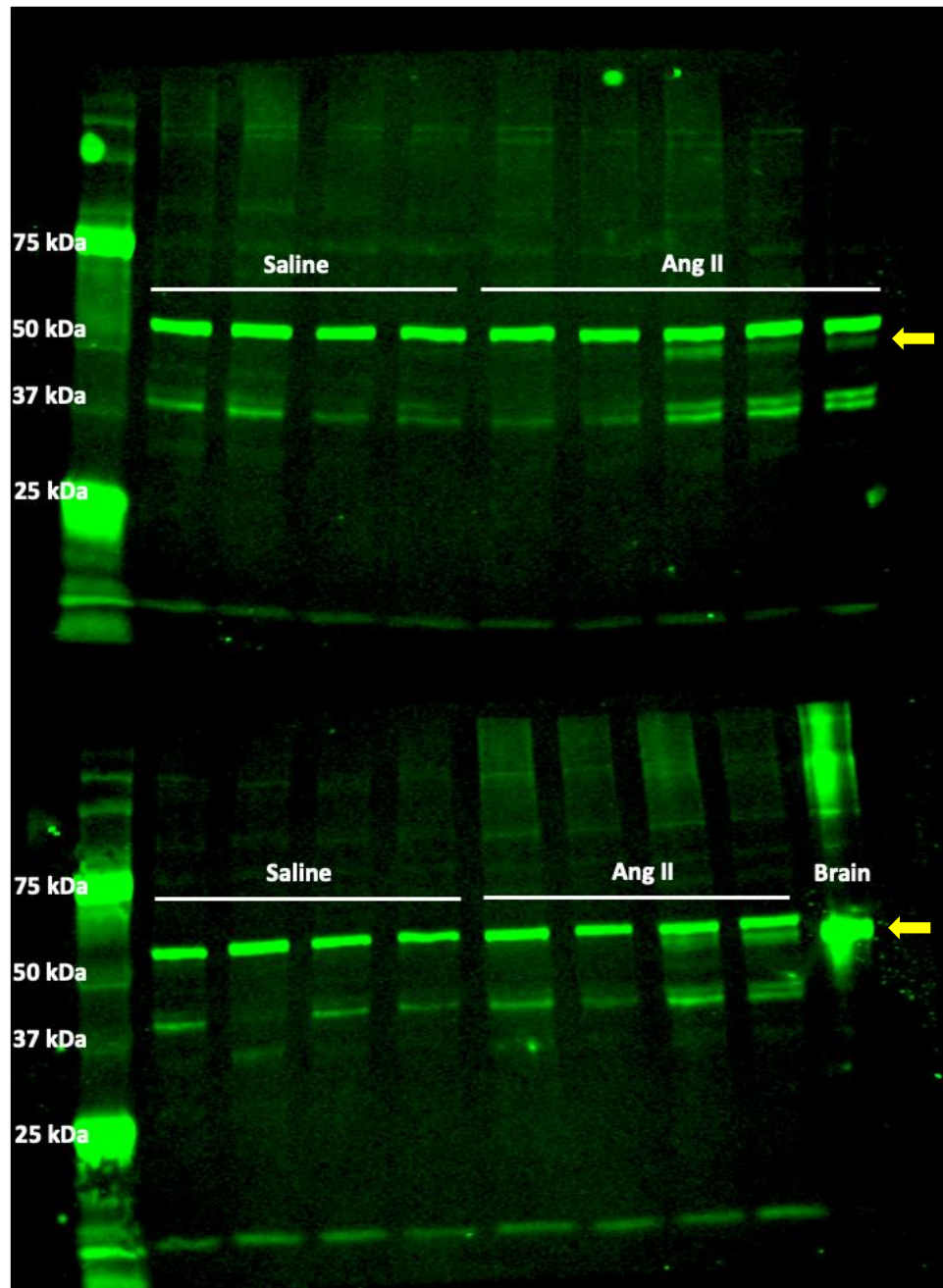


Figure S4. Image of full-length western blots for the H₃R (yellow arrow) in LV lysate from saline and Ang II-infused mice that appears in cropped form in Figure 1C.

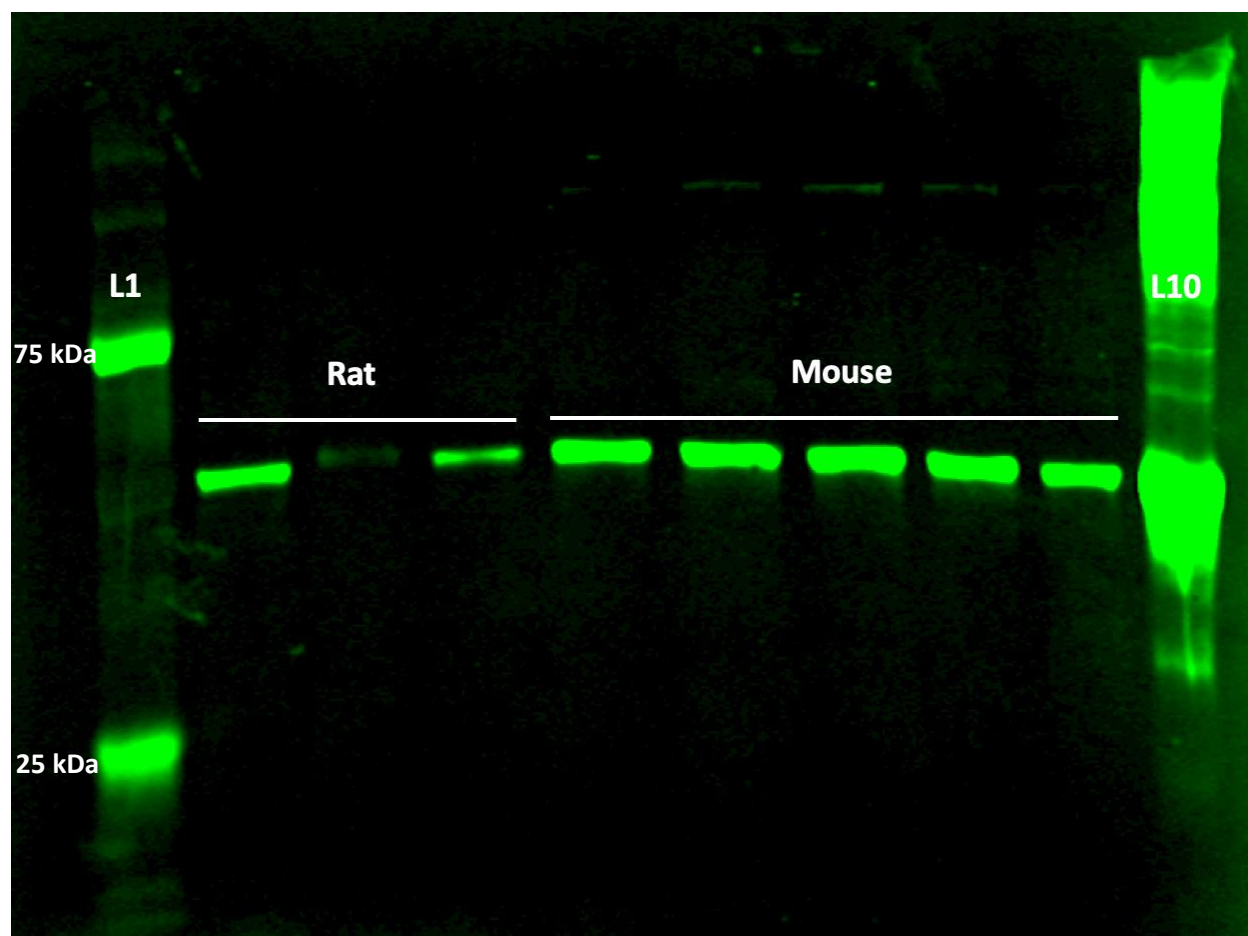


Figure S5. Image of full-length western blot for the H₃R in protein lysate from isolated rat and mouse cardiac (myo)fibroblasts that appears in cropped form in Figure 2A. Lane 1 (L1) is the molecular weight ladder. Lane 10 (L10) is brain extract as a positive control for the H₃R. Lane specifics are contained in the figure legend for Figure 2.

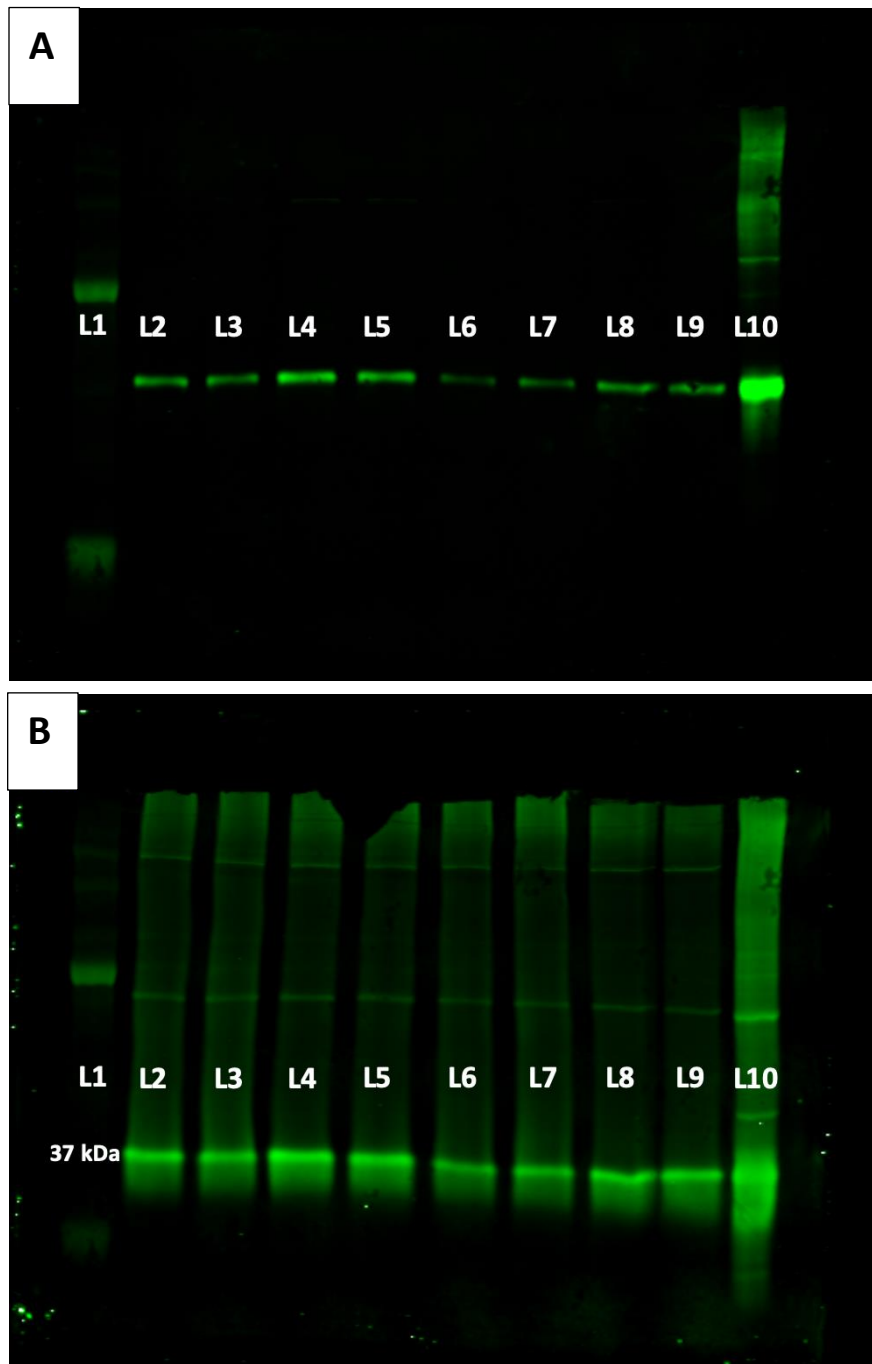
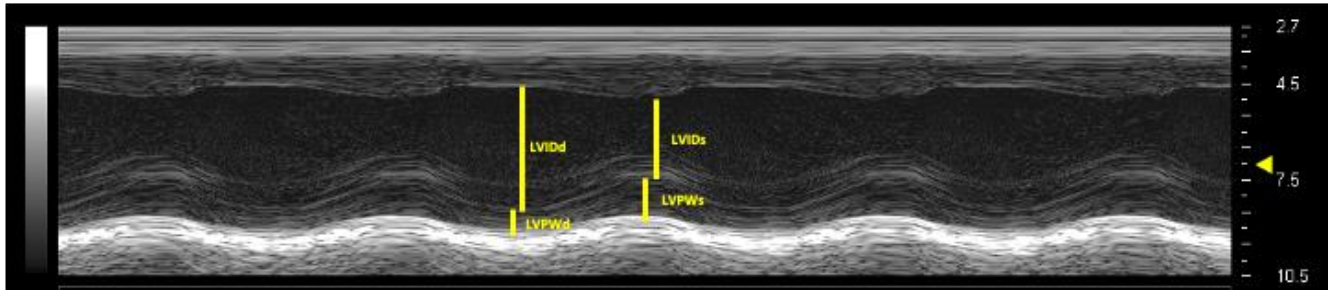
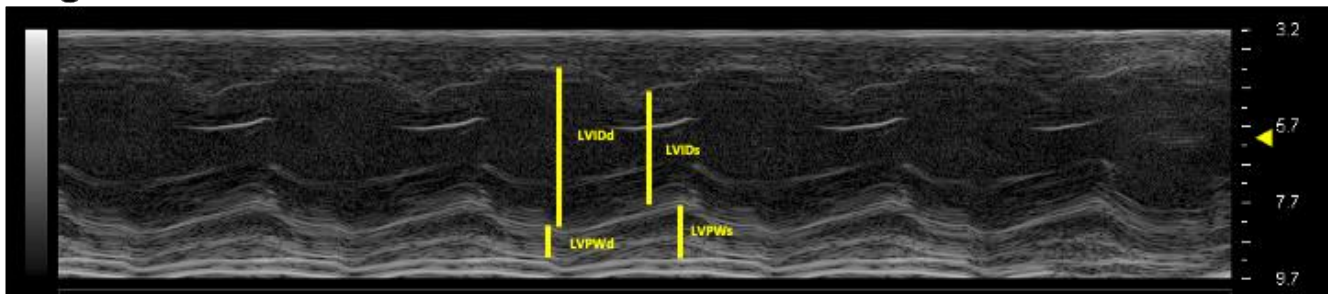


Figure S6. Images of full-length western blots for (A) the H₃R and (B) GAPDH in control cardiac fibroblast and cardiac fibroblasts treated with Ang II (300 nM) that appears in cropped form in Figure 2B. Lane 1 (L1) = molecular weight ladder, L2/3 = control, L4/5 = Ang II treated, L6/7 = control, L8/9 = Ang II treated, L10 = brain extract as a positive control for H₃R.

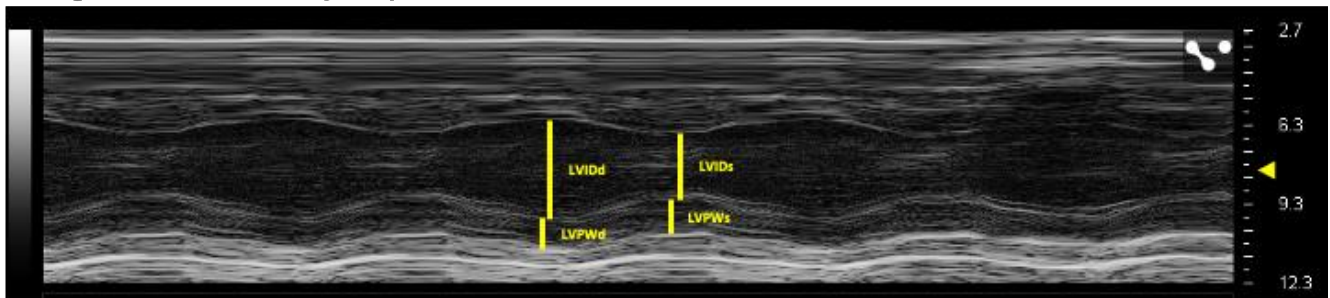
Saline



Ang II



Ang II+A331440 (HD)



Ang II+Imetit (HD)

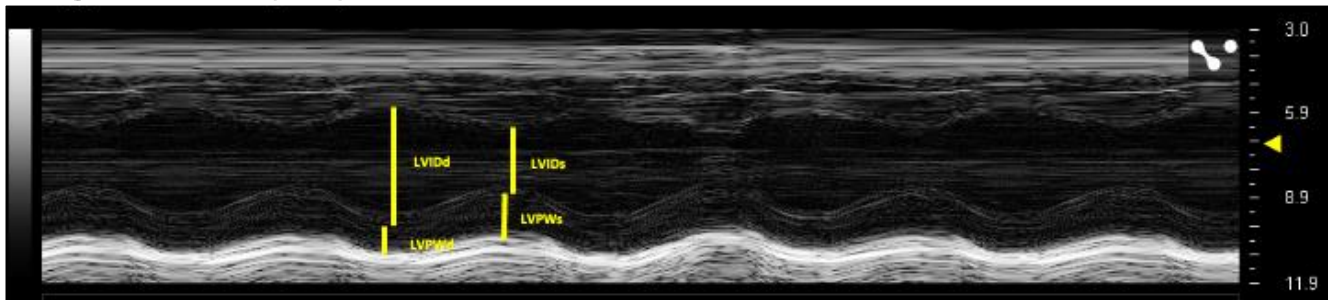


Figure S7. Example echocardiographic images for Saline, Ang II, Ang II+A331440 (HD) and Ang II+Imetit (HD) groups.

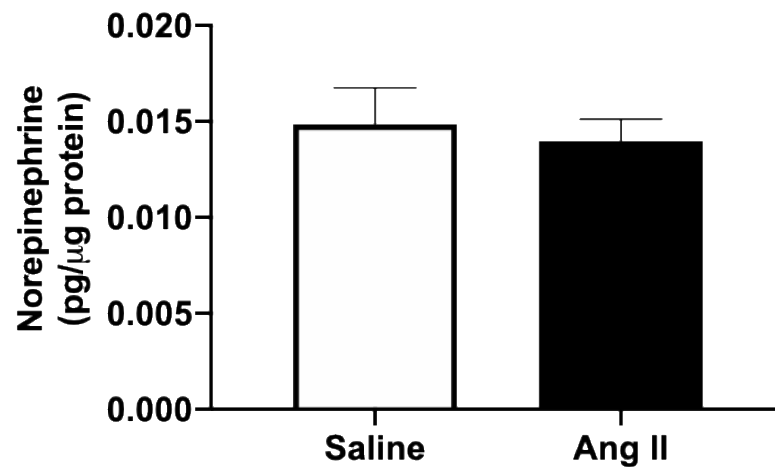


Figure S8. Norepinephrine levels from Saline and Ang II-infused mouse LV lysates. Data are mean \pm SEM.

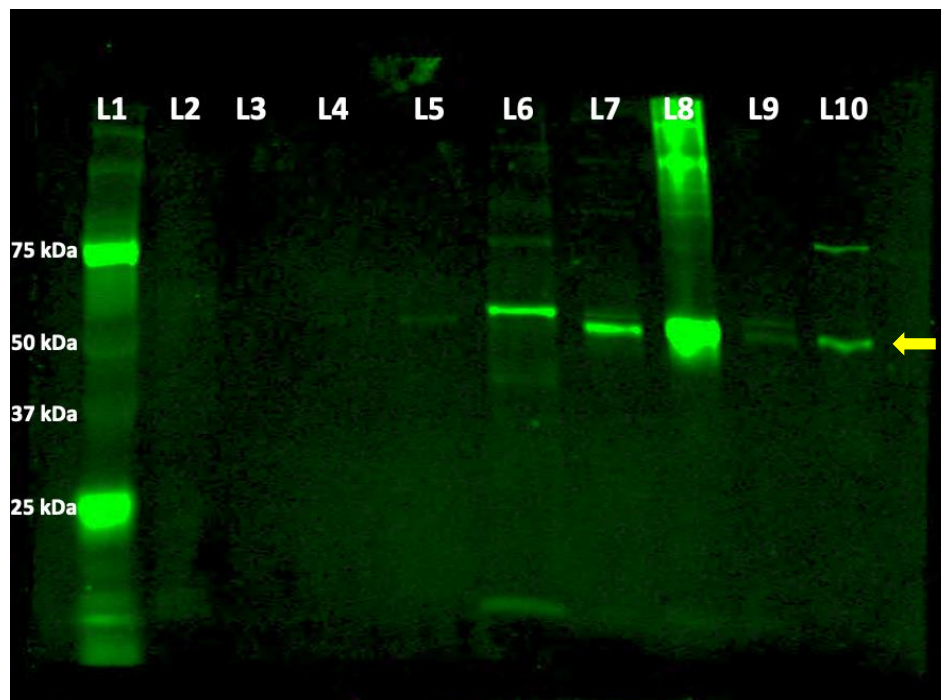


Figure S9. Western blot for the H₃R (50 kDa, yellow arrow). L1 - Ladder, L2 - Bone marrow-derived mast cells, L3 - Bone marrow-derived macrophages, L4 - H9c2 cells (immature cardiomyocytes), L5 - Cardiac fibroblasts, L6 - LV, L7 - Lung, L8 - Brain, L9 - Thyroid, L10 - Thyroid cell line (NThy)

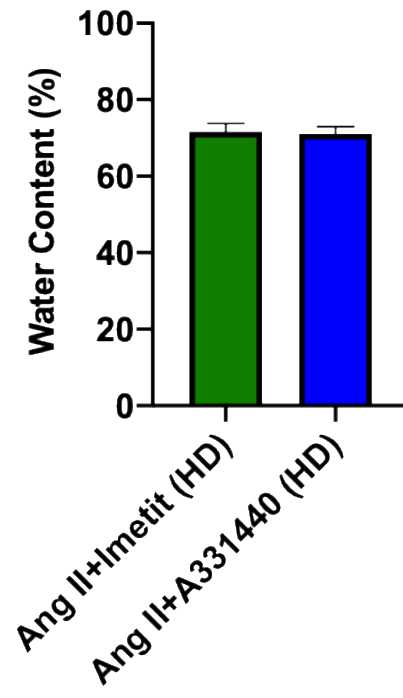


Figure S10. LV water content following Ang II-infusion for high dose (HD) Imetit and A331440 groups. Data are mean \pm SD.