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Supporting Information for

Growth Hormone–Releasing Hormone Agonists Ameliorate Chronic Kidney Disease-Induced Heart Failure with Preserved Ejection Fraction

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Author Contributions
Angela C. Rieger – Planned and carried out the experiments. Performed surgeries and analyzed data. Took the lead in writing the manuscript.
Luiza L Bagno – Carried out some experiments, performed surgeries, analyzed data, and helped to write the manuscript.
Alessandro Salerno – Assisted in the surgeries and performed hemodynamic analysis
Victoria Florea – Performed hemodynamic analysis.
Jose Rodriguez – Performed the MRI, handled and medicated animals.
Marcos Rosado – Handled and medicated the animals.
Darren Turner – Performed cine analysis.
Raul Dulce – Performed calcium analysis in cardiomyocytes.
Lauro M. Takeuchi – Performed and analyzed immunostaining and Western blots.
Rosemeire Kanashiro-Takeuchi – Analyzed immunostaining and Western blots.
Peter Buchwald – Analyzed kidney function data
Amarylis Wanchel – performed and analyzed gene expression data
Wayne Balkan - Encouraged the team to investigate the effect of the GHRH in a swine HFpEF model, supervised the findings of this work and helped to write the manuscript.
Ivonne H. Schulman – Conceived the present idea and helped to write the manuscript.
Andrew Schally – Provided the material for the study.
Joshua M. Hare – Conceived the present idea, analyzed data, and wrote the manuscript.
*All authors discussed the results and contributed to the final manuscript

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Supporting Information

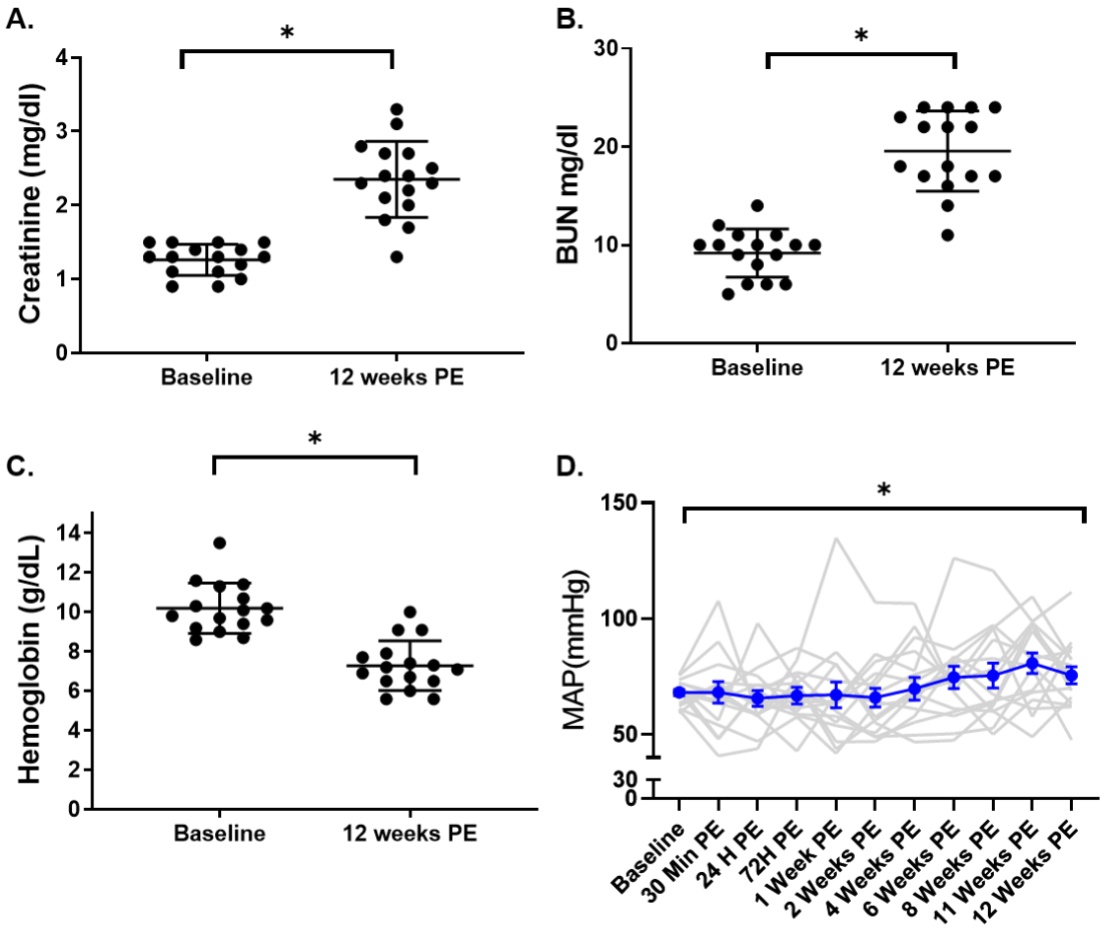


Figure S1. Development of Chronic Kidney Disease, 12-week follow up post embolization

Swine Yorkshires (n=16) developed signs of CKD after 5/6 embolization, evidenced by increased **A**. creatinine, ($p<0.0001$) **B**. Blood urea nitrogen (BUN, $p<0.0001$) increased **C**. decreased hemoglobin ($p<0.0001$) and **D.** mean arterial pressure (MAP, $p=0.04$). All data are expressed as mean ± SEM.

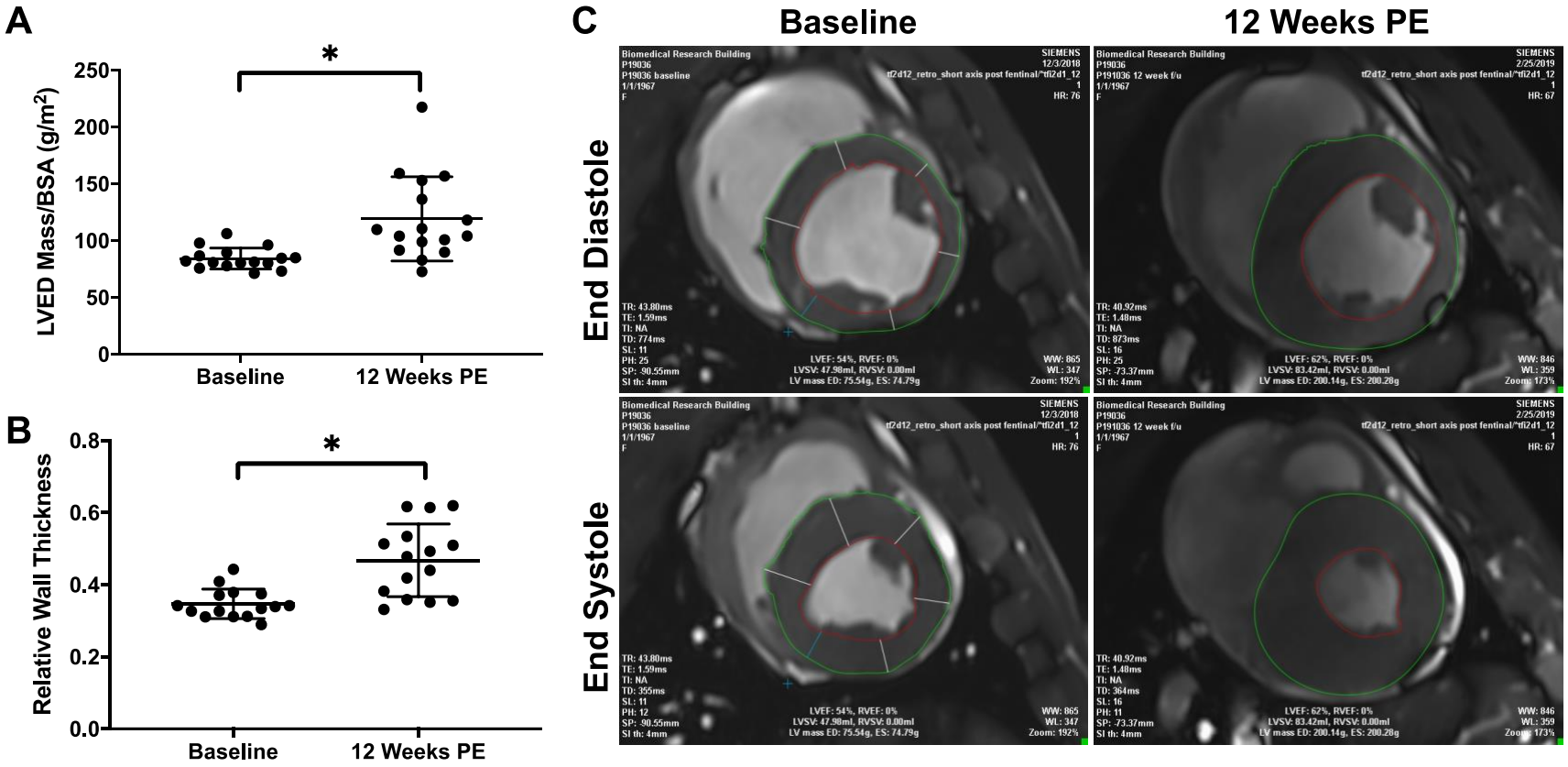


Figure S2. Left ventricular hypertrophy, 12-week follow up post embolization.

After renal embolization CKD induced left ventricular hypertrophy evidenced by **A**. left ventricular end-diastolic (LVED)-Mass corrected by body surface area (BSA) (p=0.0002), **B**. relative wall thickness (RWT; p<0.0001), **C**. representative MRI at baseline and 12 weeks post embolization in end diastole and end systole. All data are expressed as mean ± SEM.

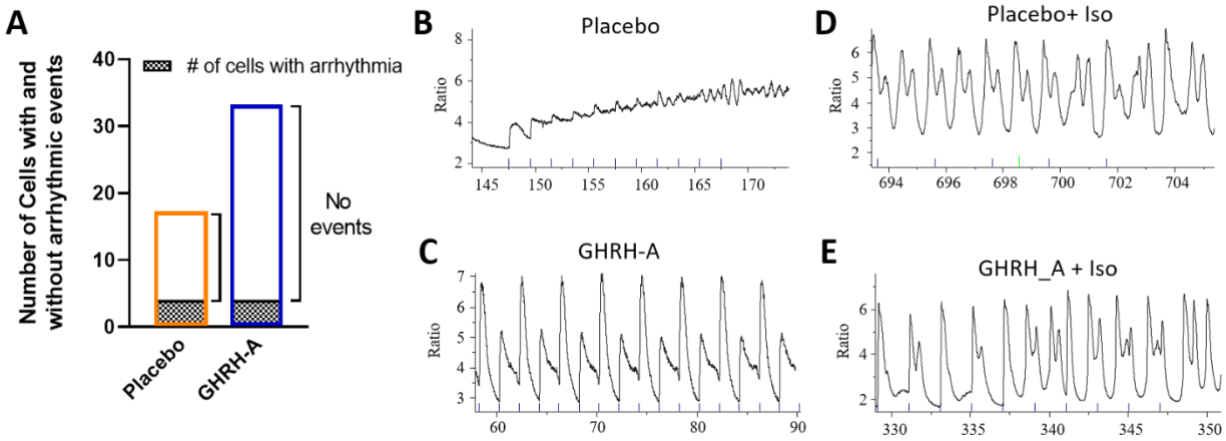
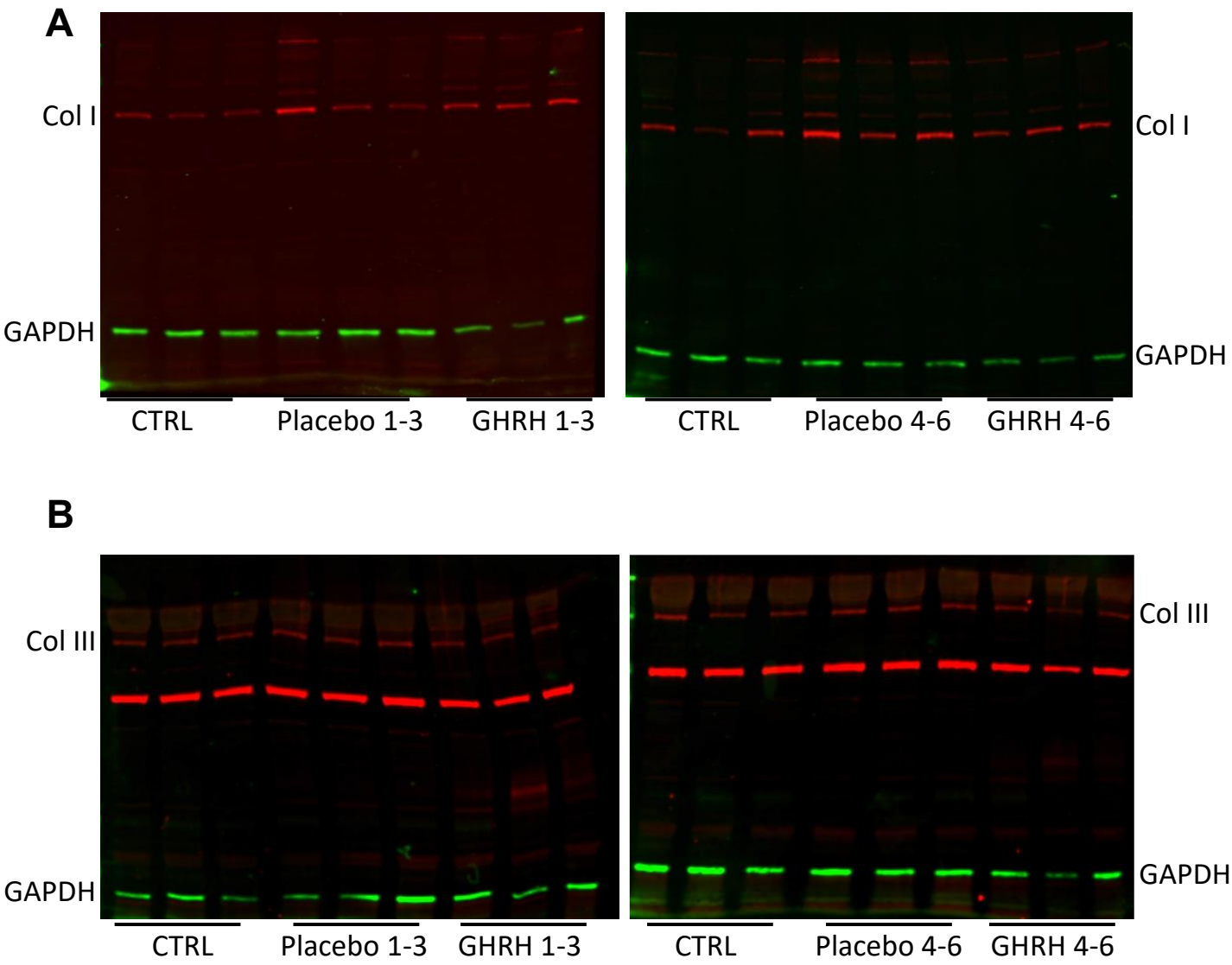


Figure S3. Arrhythmogenic behavior in intracellular calcium recordings.

The criteria for selecting isolated cardiomyocyte suitable for calcium recording are based on the structural and physiologic criteria. Structural criteria include cardiomyocyte rod shape, clearly organized striations and cytoplasm that does not contain vacuoles. Physiological health includes response to electrical field stimulation, a properly shaped calcium transient twitch and the absence of massive unstimulated activity (arrhythmia). Using these criteria, twice as many healthy cardiomyocytes were obtained (analyzed) from GHRH-A-treated animals than from the Placebo group. However, once the cardiomyocytes undergo the experiment, spontaneous depolarization and arrhythmic activity might be triggered. Thus, we were able to quantify the proportion of cells that exhibited arrhythmic activity, which included extrasystole-like twitches and dysregulated calcium gating inducing fibrillation-like activity (A). Overall, we did not see significant differences in the occurrence arrhythmia [Placebo: 4/17 cells; GHRH-A: 4/34 cells] (p=0.4156, Fisher's Exact test). (B) Calcium overload induced by the increase of pacing rate in one Placebo cell. (C) Electrical alternans in one GHRH-A cell. Three out of four cells, from either group, presented early after depolarization (EAD) induced by Isoproterenol. Representative traces EAD in cardiomyocytes from (D) placebo- and (E) GHRH-A-treated animals.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GAPDH
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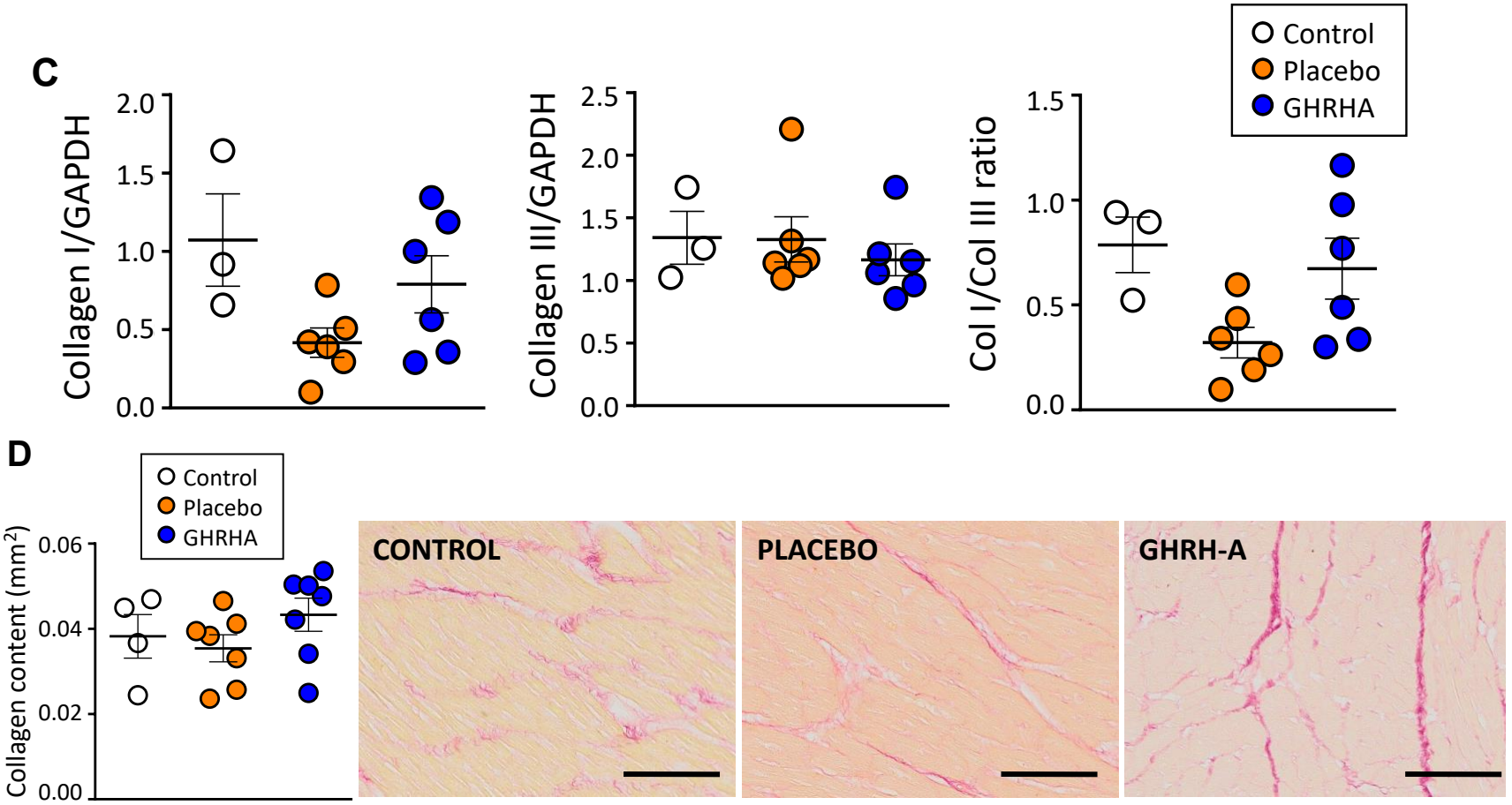

Figure S4. Collagen expression and staining

Western blot for (A) Collagen I and (B) Collagen III from swine hearts. (C) Quantification of Collagen I and Collagen III Western blots and the Collagen I/Collagen III ratio. (D). Collagen content area measured by Picro-Sirius Red staining and representative micrographs of a left ventricle from Control, Placebo-treated and GHRH-A-treated animals (Scale bars correspond to 100 µm).

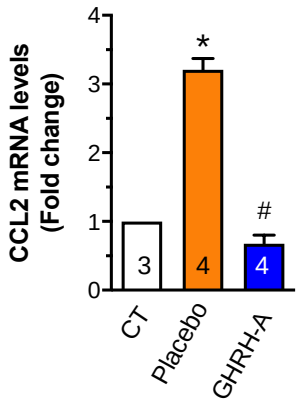

Figure S5. Expression of *CCL2* in the myocardium of control, and placebo- and GHRH-A-treated CKD-HFpEF swine.

Quantitative Rt-PCR analysis of cardiac biopsies from control, and placebo- and GHRH-A-treated animals. Numbers within bars represent number of samples analyzed; *p<0.05 compared to control; #p<0.01 compared to placebo; Student's t-Test.