Supplementary Materials

***In Vitro* Assays** - Scintillation proximity assay (SPA) based G-protein coupled receptor assay: Cell membrane was prepared from CHO cells stably expressing the human APJ (hAPJ) receptor (Amgen generated) following 3-5 passages. 5 ug of membrane was incubated with 0.1 mg of wheat-germ agglutinin (WGA) coated SPA beads (RPNQ0260, Perkin Elmer). AM-8123, AMG 986 or pyr-apelin-13 was added, and the reaction was initiated by the addition of 0.1 nM non-hydrolysable ^{35}S GTPγS in reaction buffer (20 mM HEPES, pH 7.5, 5 mM $MgCl_2$, and 0.1% (w/v) BSA] with 100 mM NaCl, 3 μM GDP - 60 uL total volume). The level of receptor activation was assessed by measuring the amount of ^{35}S GTPγS that accumulates on the membrane surface over the course of the reaction (3-6 hours).

HitHunter cAMP assay: Following 1-12 passages, CHO cells that overexpress the hAPJ receptor (Amgen generated) were seeded at 4,000 cells/well in 25 uL of plating medium (DMEM-F12 containing 10% FBS, 1% Penicillin/Streptomycin, 1% L-glutamine and 400 ug/ml G418). Small molecule APJ agonists were resuspended in DMSO and diluted to cell media containing Forskolin. Cell media was replaced with media containing a 2-fold serial dilution of the small molecule agonists (AM-8123 or AMG 986), Forskolin and DMSO to achieve a final concentration of 15 uM Forskolin, 1% DMSO, and up to 1 uM small molecule agonist. Pyr-apelin-13 was used as a positive control. Cells were subsequently incubated at 37 ^{0}C for 45 minutes, then media was replaced with lysis buffer and the plate read according to the product manual.

APJ internalization assay: U2OS cells expressing hAPJ (DiscoveRx) were seeded at 5,000 cells/ well in cell plating medium following 3-4 passages. The following day, cell media was replaced with media containing a 2-fold serial dilution of the small molecule agonists to achieve up to 1 uM small molecule agonist and 1% DMSO – final concentrations. Reactions were incubated for 3

hours at 37 ^{0}C before the addition of the Path Hunter detection reagent. The plate was read according to the assay kit product manual.

β-arrestin recruitment assay: 293 cells stably expressing the hAPJ receptor – ProLink fusion and complementary N-terminal truncated β-gal (Enzyme Acceptor (EA)) – β-arrestin fusion were used for these studies (DiscoveRx). APJ receptor activation led to the recruitment of β-arrestin and formation of the ProLink β-gal complex to induce β-galactosidase activity. Cells were seeded at 10,000 cells/well in starvation media (OptiMEM + 0.1% BSA) overnight. The following day, cell media was replaced with OptiMEM containing a 3-fold serial dilution of the small molecule agonists to achieve up to 1 uM small molecule, <0.1% DMSO – final concentrations. Reactions were incubated for 1.5 hours at 37 ^{0}C. PathHunter detection reagent was subsequently added and chemiluminescent signal was measured according to the assay kit product manual.

Data Analysis: For the cAMP, GTPγS binding, β-arrestin, and internalization assays, raw data was normalized to DMSO with pyr-apelin-13 serving as a positive control. Data was analyzed with Genedata Screener using a four-parameter logistical (4PL) Hill model described by the following equation.

$$y = S_{\text{inf}} + \frac{S_0 - S_{\text{inf}}}{1+10^{(\log AC50 - \log x)nHill}}$$

For the calculation of EC50 values, the N per group is: for pyr-apelin-13, N = 2,160 (GTPγS), 134 (cAMP), 1,519 (internalization), and 487 (β-arrestin); for AM-8123, N = 47 (GTPγS), 8 (cAMP), 34 (internalization), and 4 (β-arrestin); for AMG 986, N = 33 (GTPγS), 24 (cAMP), 13 (internalization), and 4 (β-arrestin), respectively.

***In Vivo* Animal Pharmacology Studies** – Rats: All rats were housed and maintained under standard conditions with free access to food and water and a 12-hour light-dark cycle. Male Lewis (Harlan) and ZSF1 obese rats (Charles River) fed a chow diet (Research Diets) were used for all studies utilizing rats.

Permanent ligation of the left anterior descending coronary artery (LAD) was performed on 2-3-month-old Lewis rats to induce a myocardial infarction (MI) with a 7% mortality rate for the surgical procedure. Sham operations consisting of opening, then suturing the chest cavity were performed on a control cohort. Echocardiography was performed one-week post-MI for infarct size verification. Animals with an ejection fraction (EF) > 45% and animals with no visible infarct were excluded from the study. Non-invasive echocardiograms were obtained on anesthetized rats using isoflurane, up to 3% for induction and 1.5% for maintenance. Animals were shaved, placed on a stage, and a thermo-couple rectal probe was used to assess body temperature and to adjust the temperature of the platform to maintain normothermia. Sonography gel was applied and parasternal long-axis B-mode imaging data were acquired using a Vevo 2100 imaging system (Visual Sonics).

For invasive hemodynamic assessments animals underwent general anesthesia using 80-100 mg/kg ketamine and 5-10 mg/kg diazepam (IP) followed by a ketamine boost (10 to 20 mg/kg) as necessary to maintain the plane of anesthesia. Body temperature was monitored and maintained using a rectal temperature probe that controlled the temperature of the surgical platform. Arterial pressure was measured by femoral artery catheterization using either a pressure-volume conductance catheter or a pressure catheter (SPR-839 or SPR-671, Millar). Intravenous infusions were performed via the jugular vein using either PE10 or PE50 tubing with a constant infusion rate of 50 uL/kg.minute. LV pressure – volume measurements (PV loops) were acquired using a

pressure-volume conductance catheter (SPR-838, Millar) following carotid artery cannulation and advancement of the catheter into the LV. Data were acquired using the MPVS ultra / PowerLab 8/35 system (AD Instruments) with the supplied LabChart software. At study termination, whole blood was collected by cardiac puncture. Animals were euthanized by bilateral thoracotomy together with major organ removal under anesthesia. Hearts were excised, flash frozen and stored at -80 C until further use.

Histological evaluation post chronic treatment in MI rats: The hearts were soaked in 4% neutral-buffered formalin fixation for 24-48 hour prior to being placed in 70% ethanol for storage prior to further processing. Formalin fixed hearts serially sampled from apex to base at 2mm steps. Tissue blocks were embedded in paraffin. Heart tissue blocks were sectioned and followed by picrosirius red (PSR) staining. Stained slides were scanned into Aperio (bright-field) and imported into Visiopharm. Each 2mm step profile area was analyzed in its entirety for reassembly into volume. The final 3D volume reconstruction based on established Cavalieri stereology methods. PSR positive volume fraction was expressed as percentage of total heart volume.

Canines: Healthy beagles (Marshall Farms, North Rose, NY) were surgically instrumented with a radiotelemetry transmitter (TL11M3-D70 PCTP, Data Sciences, Int. St. Paul, MN) for recording of single-lead ECGs (e.g., Lead II) and systemic (arterial, AoP)/left-ventricular pressures (LVP). Additionally, these animals had chronic instrumentation (flow probe and sono-micrometry crystals) providing aortic flow and left-ventricular (LV) dimension/volume signals, allowing the generation of pressure-volume (PV) relationships during heterometric autoregulation along with pneumatic vessel occluders around the inferior (caudal) vena cava to induce controlled reductions in preload. In addition, chronic pacing leads were placed in the right ventricular epicardium and/or endocardium. A programmable (either external or subcutaneous) high-rate pacing-device was

used to induce left-ventricular dysfunction/remodeling and heart failure. Instrumented animals were acclimated to a restraining device (sling) prior to the start of the study. Subsequently, heart failure (see below) was induced to establish/confirm the *in vivo* cardiovascular profile of AM-8123 in conscious (sling-restrained) and anesthetized animals.

Heart Failure Induction Protocol: Following recovery, animals were subjected to a well-established over-drive pacing protocol (180-240 bpm for 3-5 weeks) aimed to trigger left-ventricular dysfunction/remodeling consistent with heart failure via the implanted pacemaker/right-ventricular lead. Over the course of the induction protocol, left-ventricular remodeling and heart failure induction were confirmed by echocardiographic (e.g., EF decrease, LV dilatation). Overdrive pacing (at ~210 bpm) was maintained throughout the HF experiment phase, unless it was deemed necessary to decrease the rate in order to rescue/improve overt heart failure.

In the control group beagles were infused with the vehicle (25% HPbCD, 10% PEG 400, pH 9.5) for 2 hrs at 1 mL/kg/hr (time-matched vehicle control) intravenously via a peripheral vein. In the AM-8123 group all beagles were infused with escalating doses of AM-8123 (0.035, 0.09, 0.9, 9 mg/kg) with each dose being infused over 30 mins. Whole blood specimens were collected once during baseline conditions, at the end of vehicle infusion and at 30 minutes during each AM-8123 infusion period. Plasma samples were prepared and analyzed for AM-8123 concentrations.

Toxicology Studies - For all *in vivo* toxicology studies, AMG 986 was formulated in 2% hydroxpropyl methylcellulose, 1% Pluronic F68, pH 10.

Sprague Dawley (SD) rats (Charles River Laboratories, Hollister, CA) were group-housed in solid bottom cages with bedding and equipped with an automatic watering valve. Rats had ad libitum

access to water and food (PMI Nutrition International Certified Rodent Chow 5CR4), except for an overnight food fast prior to blood collection at scheduled necropsy. Beagles (Marshall BioResources, North Rose, NY) were group-housed in stainless steel cages equipped with an automatic watering valve. Beagles had ad libitum access to water and rationed food (PMI Nutrition International Certified Canine Chow 5007), except for an overnight food fast prior to blood collection at scheduled necropsy. All animals were maintained on a 12:12 hour light:dark cycle in rooms with appropriate temperature and humidity controls.

AMG 986 was formulated in vehicle and administered once daily for 28 days by oral gavage to 9 to 10-week old rats (n = 10/sex/group) at doses of 0, 100, 300 and 1000 mg/kg, or to 15 to 16-month old beagles (n = 3/sex/group) at doses of 0, 10, 100 and 300 mg/kg. The following study parameters were evaluated in both rat and canine studies: mortality, clinical observations, body weight, food consumption, toxicokinetics, clinical chemistry, hematology, coagulation, ophthalmology, urinalysis, organ weights, macroscopic observations, and light microscopic evaluation of tissues. Surface ECG recordings were evaluated in canines pre-test, week 2 and 4 approximately 0.5 to 1 hr postdose.

Genotoxicity: AMG 986 was evaluated for genotoxicity in accordance with ICH S2(R1), which included a bacterial reverse mutation assay in Salmonella typhimurium (strains TA98, TA100, TA1535, and TA1537) and Escherichia coli (strain WP2 uvrA) in the presence and absence of a metabolic activation system derived from Aroclor-induced rat liver S9 fraction. AMG 986 was tested for genotoxicity *in vivo* using the erythrocyte micronucleus test and liver comet assay in male SD rats (Charles River Laboratories, St. Constant, QC; 7-weeks of age) and dosed by oral gavage with vehicle, 100, 300 or 1000 mg/kg (n = 5/group), the maximum tolerated dose, at 48, 24 and 3 hrs prior to termination.

Bioanalysis and Toxicokinetics: In the pivotal toxicology studies, analytical studies confirmed dose formulation concentrations, homogeneity, and stability using a validated high-performance liquid chromatography method with ultraviolet detection. Plasma drug levels were quantified using a sensitive high-performance liquid chromatography method with tandem MS/MS detection. Toxicokinetic parameters were derived from noncompartmental analysis of the individual plasma concentration-time data using Phoenix WinNonlin (Pharsight Corp., Sunnyvale, CA).

Safety Pharmacology: Safety pharmacology evaluations were in accordance with ICH S7A/B. The effect of AMG 986 on the human ether-a-go-go (hERG) potassium channel current was evaluated in a HEK-293 cell line stably transfected with hERG cDNA using the whole-cell patch clamp assay at concentrations up to 300 uM (n = 4).

Cardiovascular effects of AMG 986 were initially evaluated in conscious female beagles (Marshall Bioresources, North Rose, NY) in a 28 day repeat dose exploratory (non-GLP) study conducted at Charles River Laboratories (Reno, NV). Beagles were co-housed, except during cardiovascular monitoring periods, and had ad libitum access to water and rationed food (PMI Nutrition International Certified Canine Chow 5007). AMG 986 was formulated in vehicle and administered once daily for 28 days by oral gavage to 6-month old beagles (n = 4/group) at doses of 0, 10 and 300 mg/kg. Cardiovascular evaluations were conducted twice during the pretest period and on days 2 and 12 and included ECGs (collected with a Jacketed External Telemetry, Data Sciences International) and hemodynamics in 30 sec averages (systolic, diastolic, and mean arterial blood pressure, pulse pressure, and heart rate using an implanted blood pressure transmitter, Data Sciences International). Electrocardiographic tracings from 0 to 2 hrs postdose were evaluated qualitatively by a veterinary cardiologist for waveform abnormalities. Cardiovascular function and chamber volumes were also evaluated by echocardiography twice pretest and on days 3 and

13 using a GE Vivid i echocardiographic recorder. Two-dimensional, M-mode and Doppler measurements were performed with a 3.5/6.9 MHz phased array sector transducer. Toxicokinetics were evaluated on days 1 and 14.

In the definitive GLP cardiovascular safety pharmacology study, AMG 986 was evaluated in conscious male beagles (Covance Research Products, Cumberland, VI) surgically implanted with telemetry devices to evaluate arterial blood pressures, body temperature and ECGs. The study was conducted at Covance, Madison, WI. Beagles were co-housed, except during cardiovascular monitoring periods, and had ad libitum access to water and rationed food (PMI Nutrition International Certified Canine Chow 5007). AMG 986 was formulated in vehicle and administered once orally to 11-12-month-old beagles (n = 8/group) on Study Days 1, 8, 15, and 22 in a double Latin square design. On each dosing occasion, the blood pressure and ECG measurements were recorded continuously 90 min before dosing and 25 hours after dosing. The data were captured using the Data Sciences International Dataquest OpenART telemetry equipment and analyze on the Ponemah Physiology Platform software system in 15 min or 1 hr segment averages for QRS, PR, QT(c) and RR intervals, and RR-derived heart rate. Electrocardiographic tracings of approximately 60 to 90 seconds duration were evaluated qualitatively by a veterinary cardiologist for waveform abnormalities and arrhythmias from all beagles prior to dosing and at 0.5, 2, 4, 8, 12, 16 and 24 hr post dose.

Central nervous and respiratory system effects were evaluated in pair-housed female SD rats (Charles River Laboratories, Raleigh, NC; 14-16 weeks of age). The study was conducted at Covance, Madison, WI. Rats were given a single oral dose of vehicle or 100, 300, or 1000 mg/kg of AMG 986 (n=8/group) and evaluated for central nervous system effects using a modified Irwin test, which included group home cage, hand-held, open-field, elicited response observations, and

body temperature. After a 2-week washout, the same rats were acclimated to plethysmography chambers and dosed similarly for evaluation of respiratory effects, which included tidal volume, respiration rate, or minute volume.

Selectivity of AMG 986 was evaluated *in vitro* against a panel of human recombinant phosphodiesterase enzymes (PDE1-5, 7-11; BPS Biosciences, San Diego, CA) based on functional inhibition of enzyme activity using the IMAP TR-FRET assay system (time resolved fluorescence resonance energy transfer) (Molecular Devices, Sunnyvale, CA). Selectivity of AMG 986 was also evaluated *in vitro* against a panel of human GPCRs using an aequorin luminescence assay.

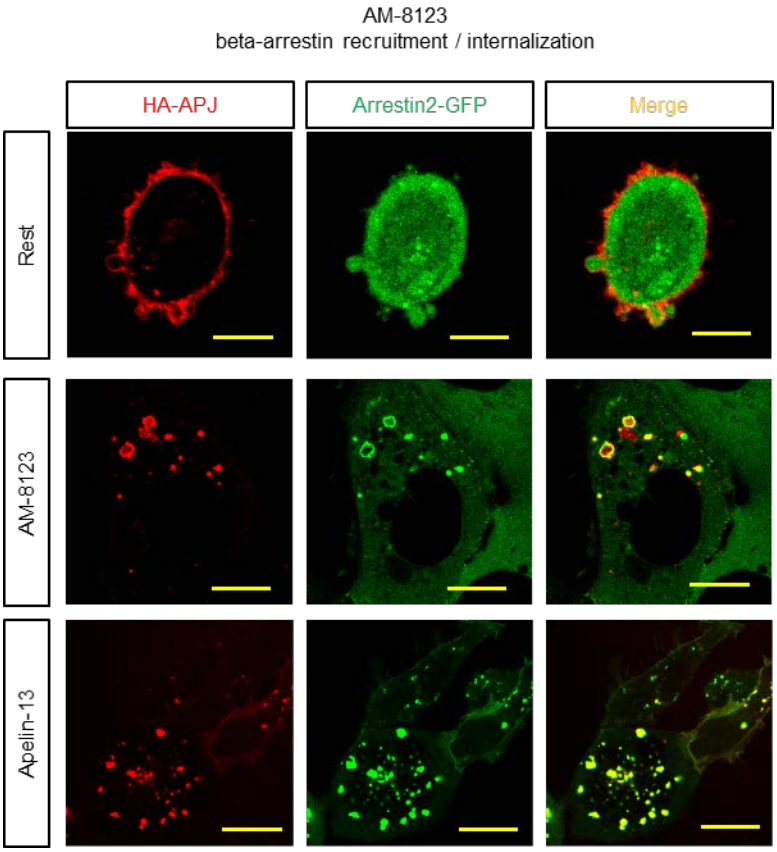


Fig. S1. AM-8123 and apelin-13 stimulated β-arrestin recruitment and receptor internalization of APJ. β-arrestin and APJ trafficking were monitored with confocal fluorescence microscopy using GFP (green) and Alexa-594 conjugated anti-HA antibody (red). The yellow bar represents 10 um.

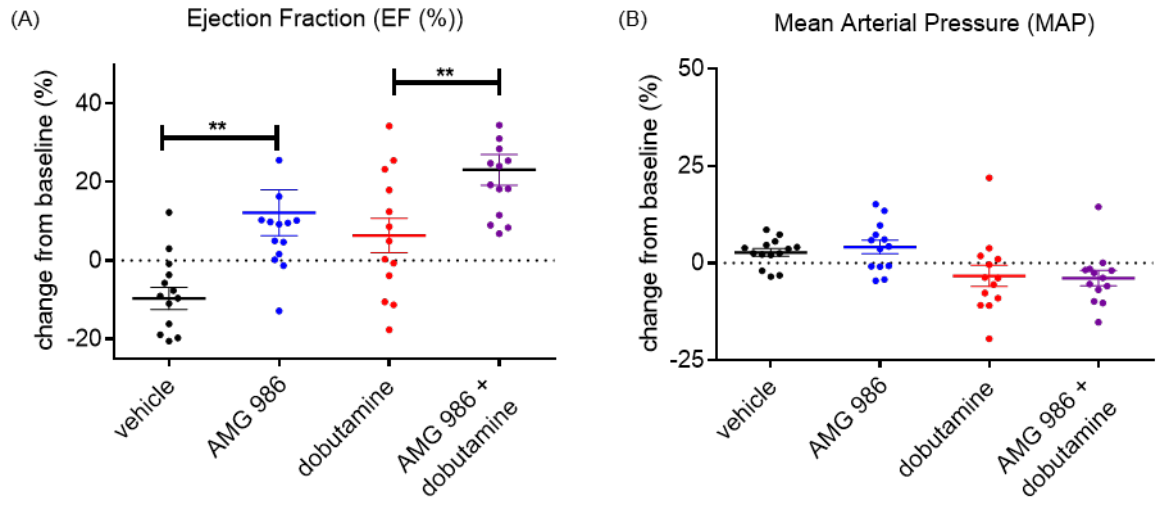


Fig. S2. Acute infusion of the APJ small molecule agonist AMG 986 increases (A) ejection fraction but not (B) mean arterial pressure in a rat model of impaired metabolic function. These data were collected from the same study as shown in Fig. 3. Data represented as individual animals (circles) together with the group mean (bars) ± SEM. N= 13-14 animals per group. Data analyses performed blinded. Significance measured by a two-tailed unpaired t-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$).

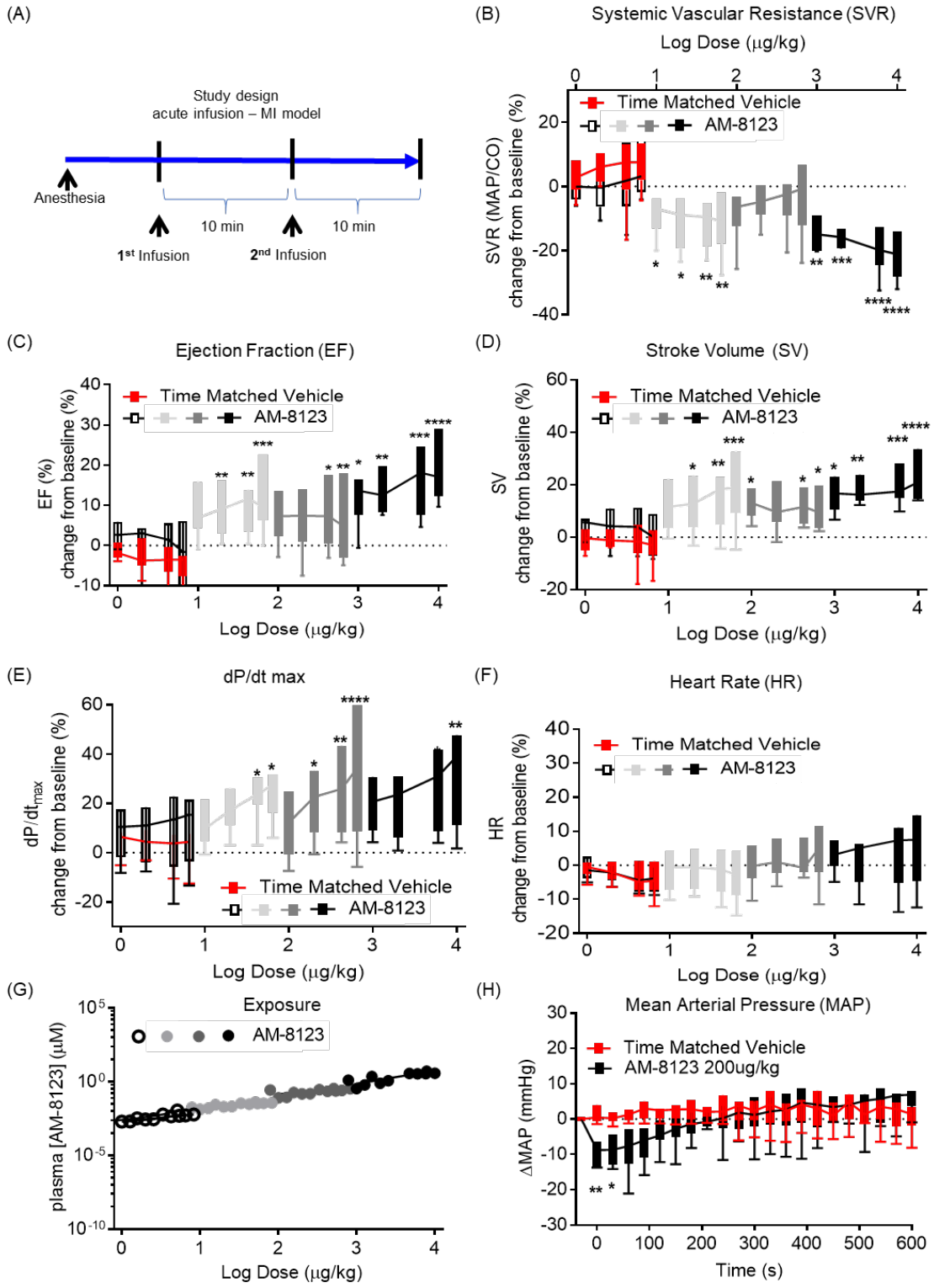


Fig. S3. Acute infusion of the APJ small molecule agonist AM-8123 increases cardiac function in a MI rat model. (A) Left anterior descending coronary artery (LAD) ligation was used to induce a MI in 2-3-month-old Lewis rats. Animals with an EF ≤ 45% and a medium infarct as measured by echocardiography 6-8

weeks post MI were randomly assigned to one of the five groups (Grp A (red): vehicle, Grp B (white): AM-8123 (0.002 mg/kg, 0.0045 mg/kg), Grp C (light grey): AM-8123 (0.02 mg/kg, 0.045 mg/kg), Grp D (dark grey): AM-8123 (0.2 mg/kg, 0.45 mg/kg), or Grp E (black): AM-8123 (2 mg/kg, 8 mg/kg). Following catherization and baseline stabilization, rats were infused with AM-8123 or vehicle (25% HPbCD, 10% PEG400, pH9.5 w/NaOH) for 20 minutes, 10 minutes at the low dose followed by 10 minutes at the higher dose using a constant 50 uL/kg.min infusion rate. Cardiovascular parameters reported as the percentage change over baseline for each data point, corresponding to each dose level at 5, 10, 15, and 20 minute infusion intervals. Vehicle control (red) is a time-matched control for each dose of AM-8123. Acute infusion of AM-8123 decreased in (B) SVR and increased parameters of systolic function: (C) EF, (D) SV, and (E) dP/dt max, without a discernable increase in (F) HR from sub-micromolar unbound plasma (G) exposure. An initial high dose of AM-8123 (0.2 mg/kg) resulted in a transient drop in (H) MAP that returns to and in some instances overshoots baseline values. Data represented as a box and whiskers plot with a line indicating the median, the box representing the 25th-75th interquartile range and the Tukey method to calculate the whiskers. For panel G, individual animals are shown. N = 6-10 animals per group. Data analyses were performed blinded. Significance measured by a two-way ANOVA, Tukey post-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$).

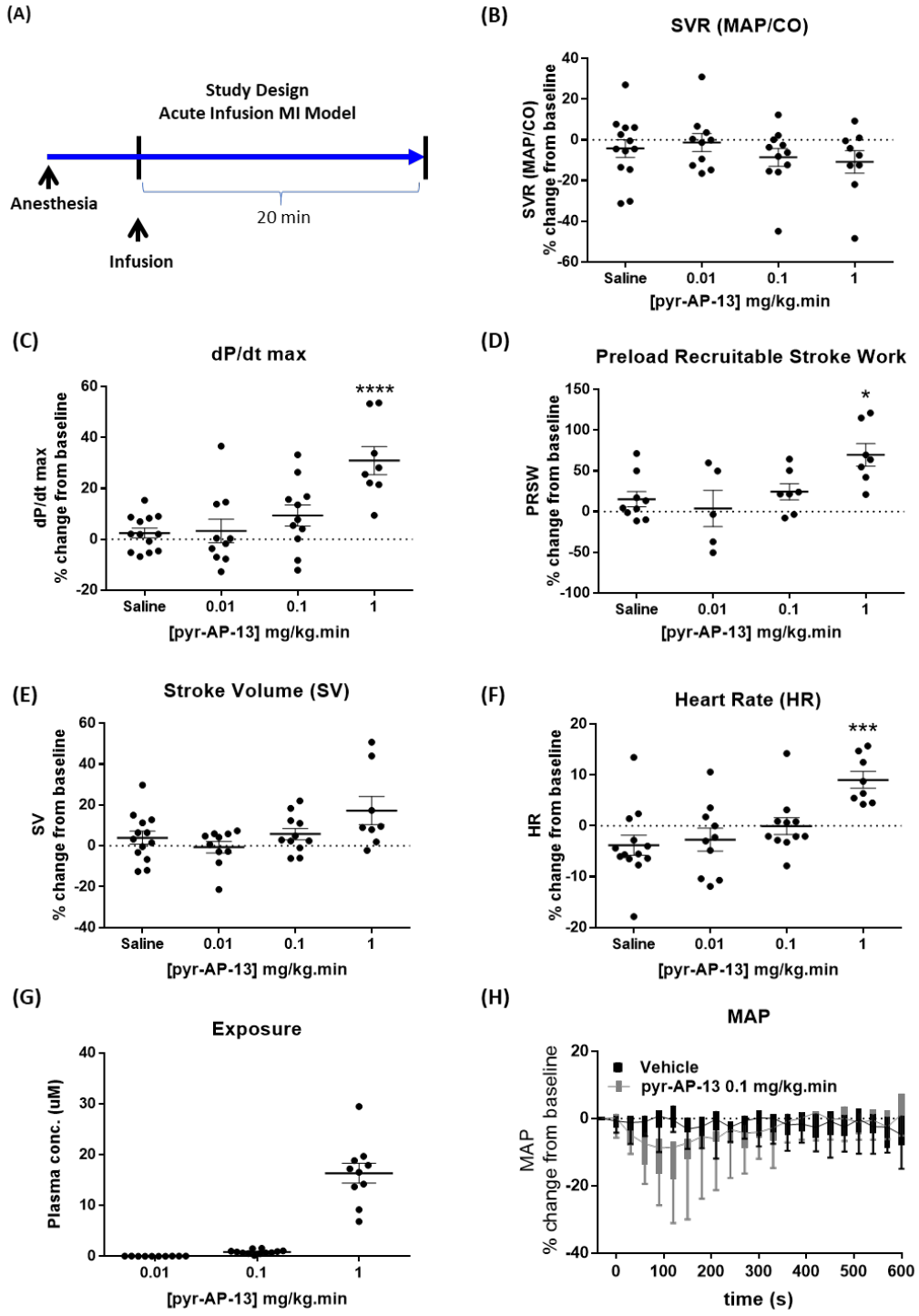


Fig. S4. Acute administration of pyr-AP-13 improves several markers of cardiac function in an MI rat model of HF. (A) Left anterior descending coronary artery (LAD) ligation was used to induce a MI in 2-3-month-old Lewis rats. Animals with an EF ≤ 45% and a medium infarct as measured by echocardiography 6-8 weeks post MI were randomly assigned to one of the four groups (Grp A: vehicle, Grp B: 0.01 mg/kg pyr-AP-13, Grp C: 0.1 mg/kg pyr-AP-13, Grp D: 1 mg/kg pyr-AP-13). Following catherization and baseline stabilization, rats were infused with pyr-AP-13 or vehicle (saline) for 20 minutes using a constant 50

uL/kg.min infusion rate. Cardiovascular parameters reported as the percentage change over baseline for each data point, corresponding to each dose level at the end of the 20 minute infusion interval. (B) SVR, (C) dP/dt max, (D) PRSW, (E) SV, (F) HR, (G) plasma exposure and (H) MAP are shown. Data shown as the percent change from baseline for individual animals (circles) and the group mean (bars) ± SEM. For panel H, data represented as a box and whiskers plot with a line indicating the median, the box representing the 25th-75th interquartile range and the Tukey method to calculate the whiskers. N = 8-13 animals per group. Significance measured by a one-way ANOVA, Tukey post-test. For panel H, significance was measured a two-way ANOVA – Tukey post-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). Data analyses were performed blinded.

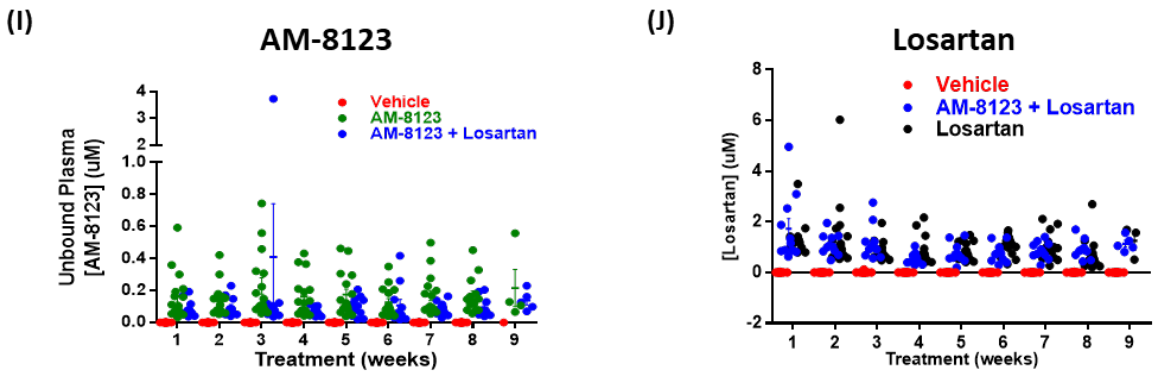


Fig. S5. Sustained levels of exposure were maintained for both (A) AM-8123 and (B) losartan for the relevant treatment groups. These data were collected from the same study as shown in Fig. 4. Data represented as individual animals (circles) with the group mean (bars) ± SEM. N = 6-10 animals per group.

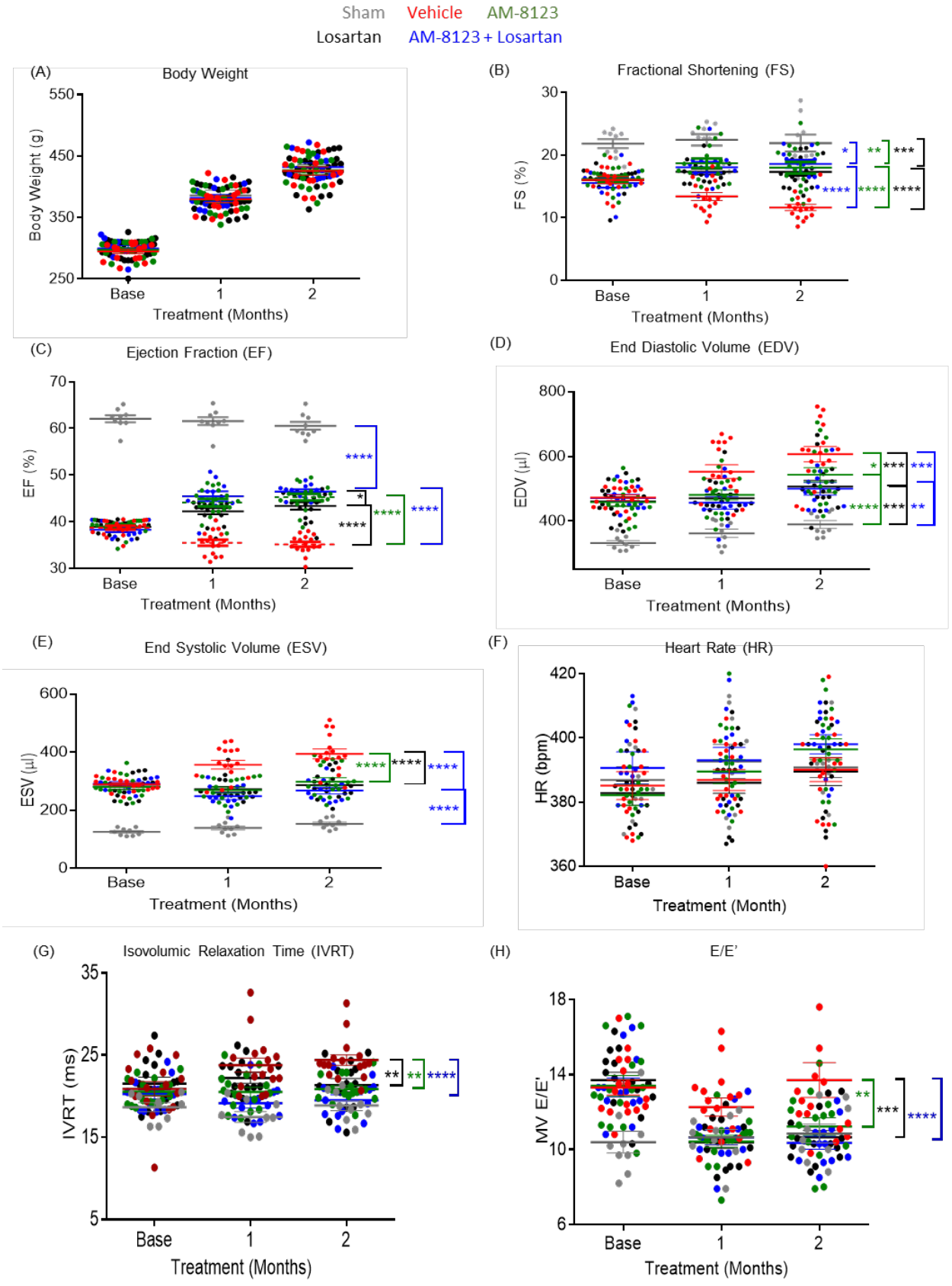

Sham
Vehicle
AM-8123
Losartan
AM-8123 + Losartan
(A)
Body Weight
Body Weight (g)
Treatment (Months)
(B)
Fractional Shortening (FS)
FS (%)
Treatment (Months)
(C)
Ejection Fraction (EF)
EF (%)
Treatment (Months)
(D)
End Diastolic Volume (EDV)
EDV (µl)
Treatment (Months)
(E)
End Systolic Volume (ESV)
ESV (µl)
Treatment (Months)
(F)
Heart Rate (HR)
HR (bpm)
Treatment (Month)
(G)
Isovolumic Relaxation Time (IVRT)
IVRT (ms)
Treatment (Months)
(H)
E/E'
MV E/E'
Treatment (Months)

Fig. S6. Echocardiography reveals that chronic administration of the APJ small molecule agonist AM-8123 improves several markers of cardiac function. (A) Comparable increases in body weight were observed across groups. (B) FS and (C) EF were significantly improved for AM-8123, losartan and combination treated animals relative to vehicle. This was associated with a reduction in (D) EDV and (E) ESV with no change in (F) HR. Additionally, two markers of diastolic function, (G) IVRT (H) and E/E' were lower for the treated groups relative to control. These data were collected from the same study as shown in Fig. 4. Data represented as individual animals (circles) together with the group mean (bars) ± SEM. N = 13-14 animals per group. N = 16 for vehicle and AM-8123 treatment groups, N = 15 for the losartan treated group, N = 11 for the AM-8123 + losartan combination group, and N = 9 for sham cohorts. Significance measured by a two-tailed unpaired t-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). Data analyses performed blinded.

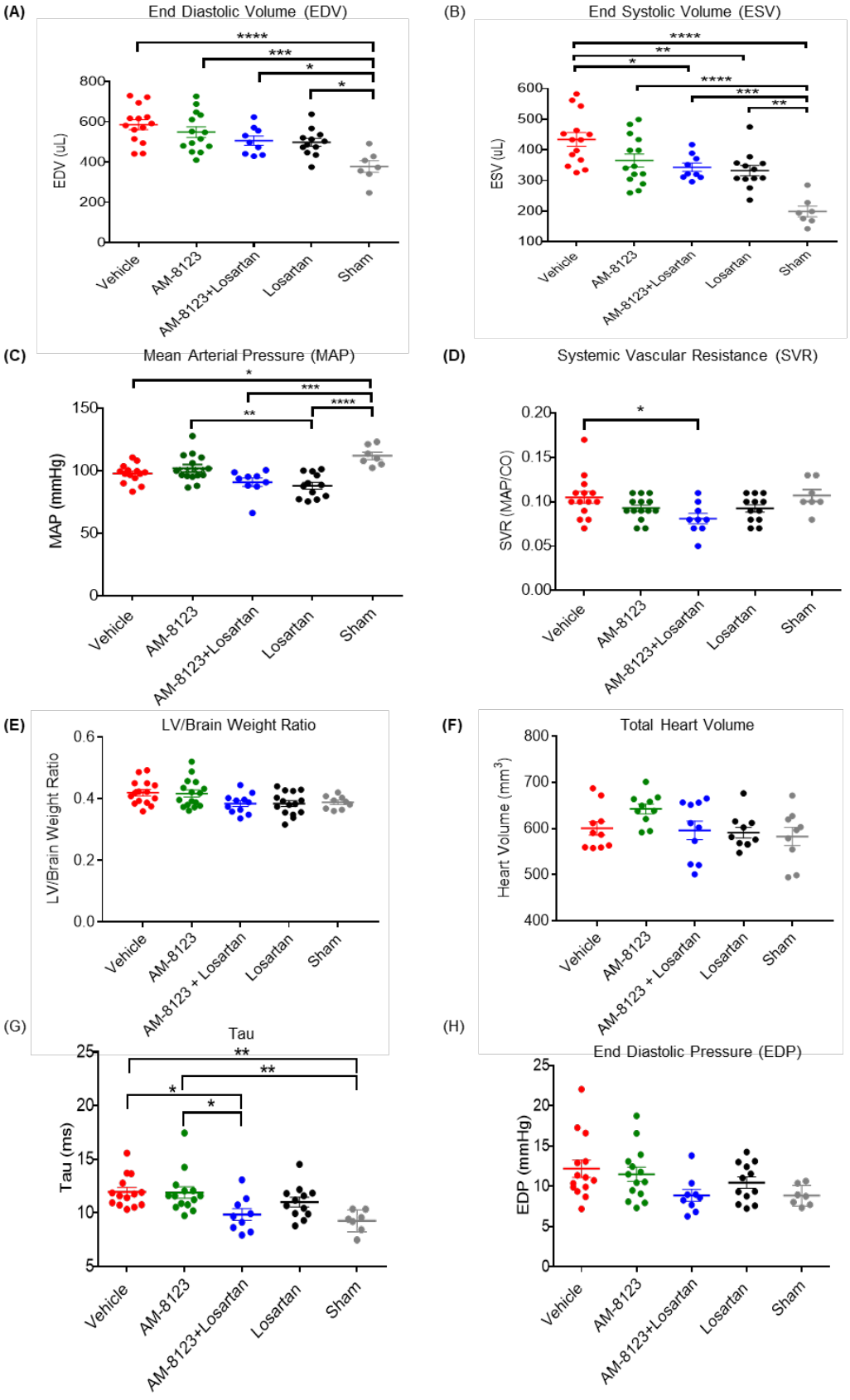

(A)
End Diastolic Volume (EDV)
EDV (uL)
Vehicle
AM-8123
AM-8123+Losartan
Losartan
Sham
(B)
End Systolic Volume (ESV)
ESV (uL)
(C)
Mean Arterial Pressure (MAP)
MAP (mmHg)
(D)
Systemic Vascular Resistance (SVR)
SVR (MAP/CO)
(E)
LV/Brain Weight Ratio
LV/Brain Weight Ratio
AM-8123 + Losartan
(F)
Total Heart Volume
Heart Volume (mm3)
(G)
Tau
Tau (ms)
(H)
End Diastolic Pressure (EDP)
EDP (mmHg)

Fig. S7. Invasive hemodynamic assessment reveals that chronic administration of the APJ small molecule agonist AM-8123 improves several markers of cardiac function. These data were collected from the same study as shown in Fig. 4. Data represented as individual animals (circles) together with the group mean (bars) ± SEM. N = 13-14 animals per group. N = 14 for vehicle and AM-8123 treatment groups, N = 12 for the losartan treated group, N = 9 for the AM-8123 + losartan combination group, and N = 7 for sham cohorts. Significance measured by a two-tailed unpaired t-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). Data analyses performed blinded.

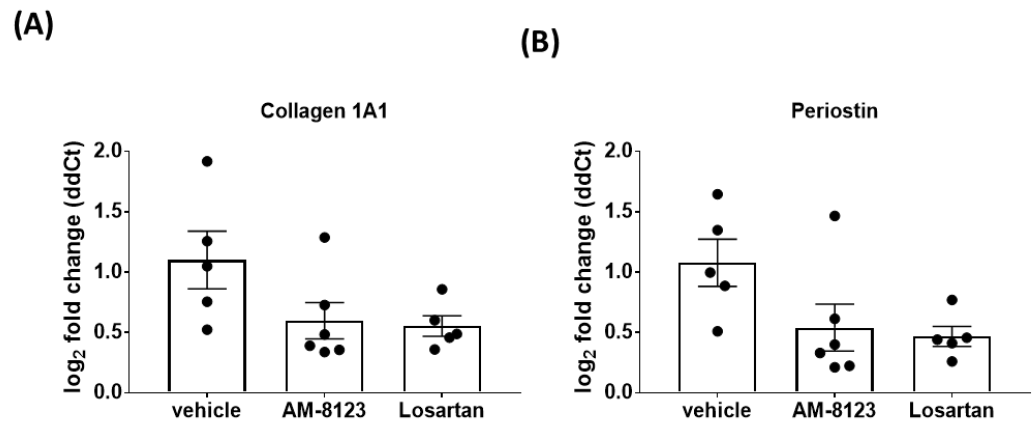


Fig. S8. Chronic administration of the APJ small molecule agonist AM-8123 results in a trend toward lower collagen 1A1 and periostin expression. Rats (N = 5 / group) were administered vehicle, losartan or AM-8123 as part of the study presented within Fig. 4. Gene expression was assessed using qRT-PCR (TaqMan) from RNA isolated from flash frozen LV tissue collected from a subset of animals at study termination. Data represented as the log2 fold change (ddCt) relative to the vehicle average. Individual animals (circles) and the group means (bars) are shown. Significance measured by a one-way ANOVA, Tukey post-test.

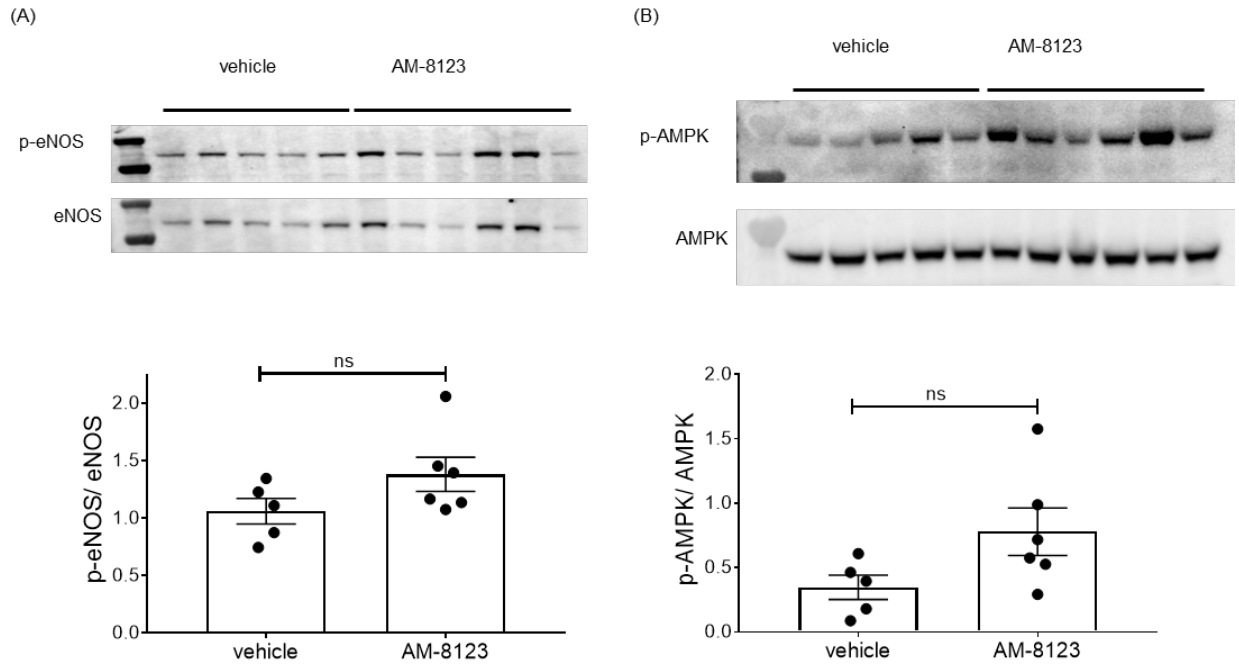


Fig. S9. Chronic administration of the APJ small molecule agonist AM-8123 results in a trend toward increased (A) eNOS and (B) AMPK phosphorylation. Rats (N = 5 -6/ group) were administered vehicle or AM-8123 as part of the study presented within Fig. 4. Westerns were performed using protein isolated from flash frozen LV tissue collected from a subset of animals at study termination. Individual animals (circles) and the group means (bars) are shown. Significance measured by a two-tailed unpaired student t-test. See full blots in Supplemental Figures 21 and 22.

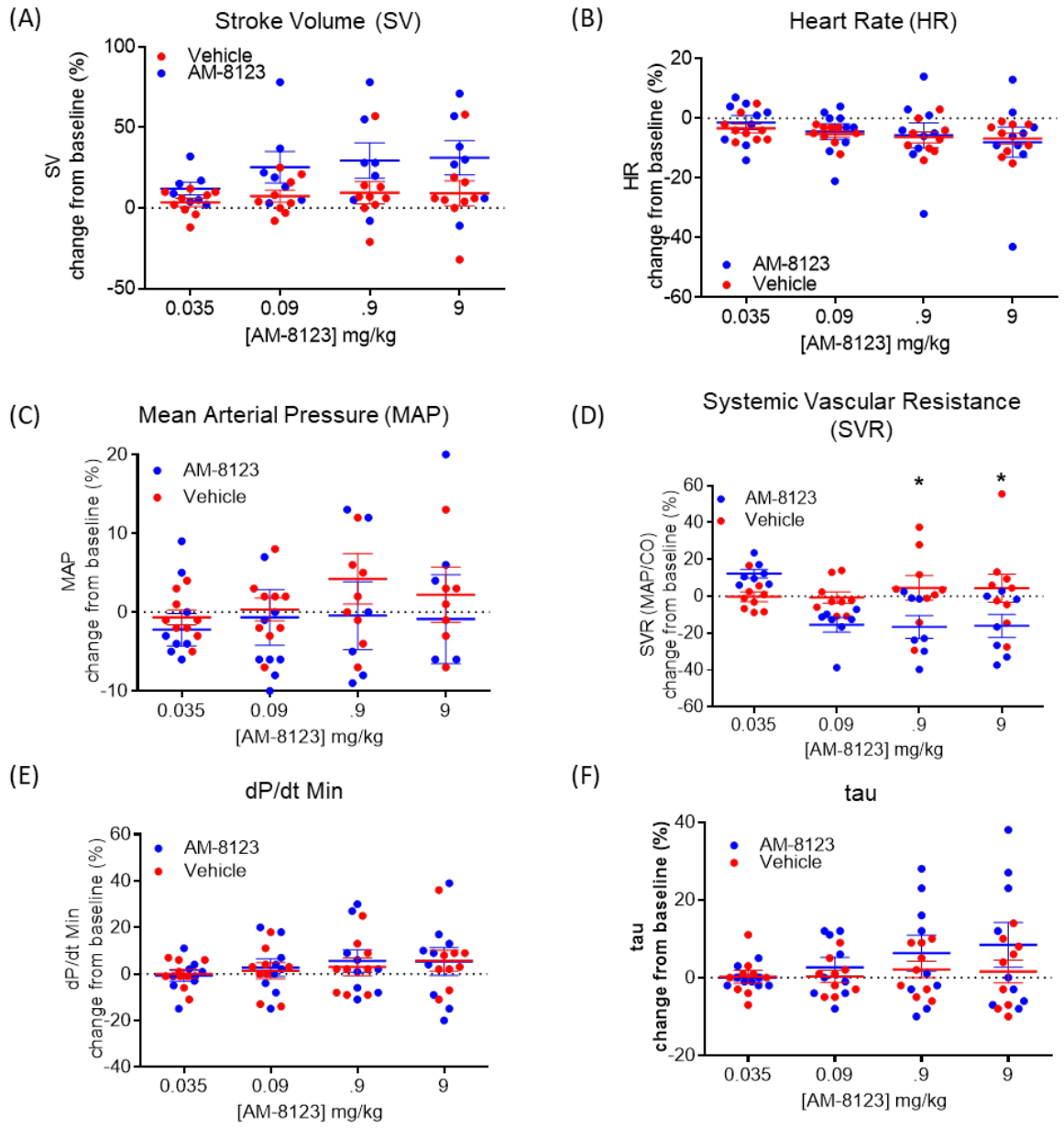
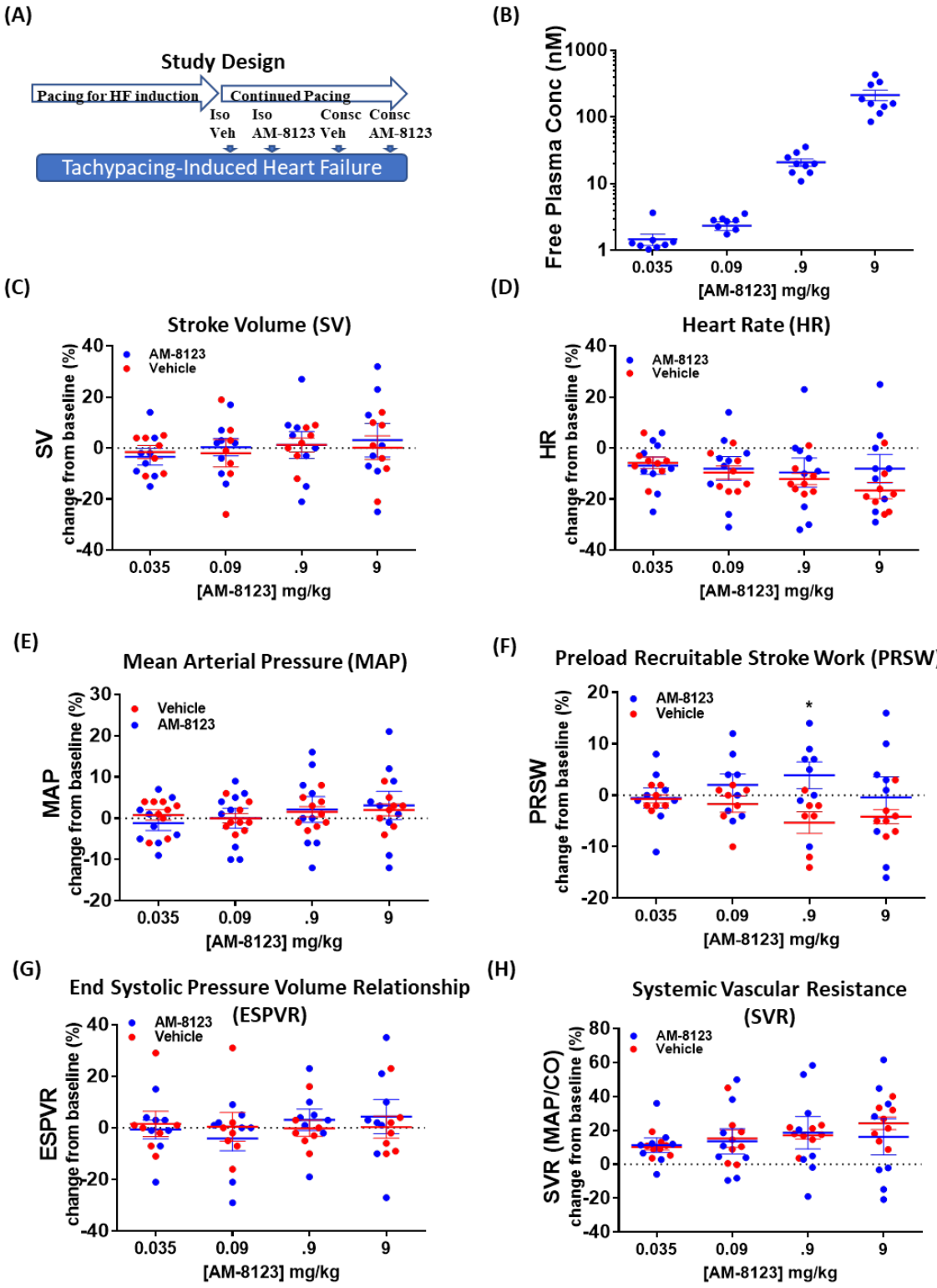


Fig. S10. Additional endpoints from the study presented in Fig. 6 are shown. Data represented as individual animals (circles) with the group mean (bars) ± SEM. N = 5-9 animals per group. Significance was measured by a two-way ANOVA, Tukey post-test (* $P < 0.05$). Data analyses were performed blinded.


(A)
Study Design
Pacing for HF induction
Continued Pacing
Iso Veh
Iso AM-8123
Consc Veh
Consc AM-8123
Tachypacing-Induced Heart Failure
(B)
Free Plasma Conc (nM)
1000
100
10
1
0.035
0.09
.9
9
[AM-8123] mg/kg
(C)
Stroke Volume (SV)
AM-8123
Vehicle
SV
change from baseline (%)
40
20
0
-20
-40
0.035
0.09
.9
9
[AM-8123] mg/kg
(D)
Heart Rate (HR)
AM-8123
Vehicle
HR
change from baseline (%)
40
20
0
-20
-40
0.035
0.09
.9
9
[AM-8123] mg/kg
(E)
Mean Arterial Pressure (MAP)
Vehicle
AM-8123
MAP
change from baseline (%)
30
20
10
0
-10
-20
0.035
0.09
.9
9
[AM-8123] mg/kg
(F)
Preload Recruitable Stroke Work (PRSW)
AM-8123
Vehicle
*
PRSW
change from baseline (%)
20
10
0
-10
-20
0.035
0.09
.9
9
[AM-8123] mg/kg
(G)
End Systolic Pressure Volume Relationship (ESPVR)
AM-8123
Vehicle
ESPVR
change from baseline (%)
40
20
0
-20
-40
0.035
0.09
.9
9
[AM-8123] mg/kg
(H)
Systemic Vascular Resistance (SVR)
AM-8123
Vehicle
SVR (MAP/CO)
change from baseline (%)
80
60
40
20
0
-20
-40
0.035
0.09
.9
9
[AM-8123] mg/kg

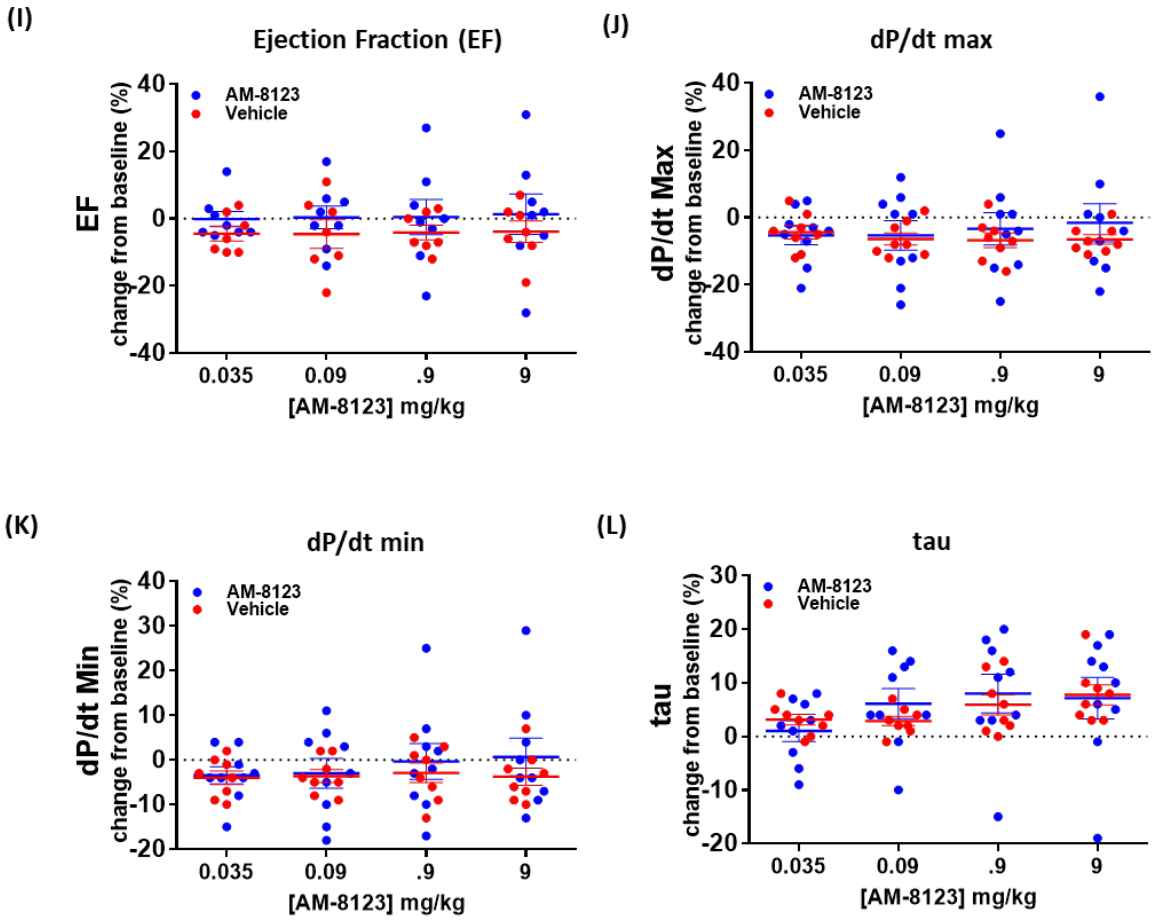


Fig. S11. Acute administration of the APJ small molecule agonist AM-8123 did not improve cardiac function in conscious beagles with tachypaced-induced HF. HF was induced by an overdrive pacing protocol, where the pace maker was turned-off at least 15 minutes prior to the initiation of treatment and data acquisition. (A) Each animal received both vehicle and AM-8123 sequentially as indicated. While escalating doses of AM-8123 resulted in a clear dose dependent increase in (B) exposure, no appreciable dose-dependent changes in any hemodynamic parameters were observed (C-L). Data represented as individual animals (circles) with the group mean (bars) ± SEM. N = 7-9 animals per group. Data analyses were performed blinded. Significance was measured by a two-way ANOVA, Tukey post-test.

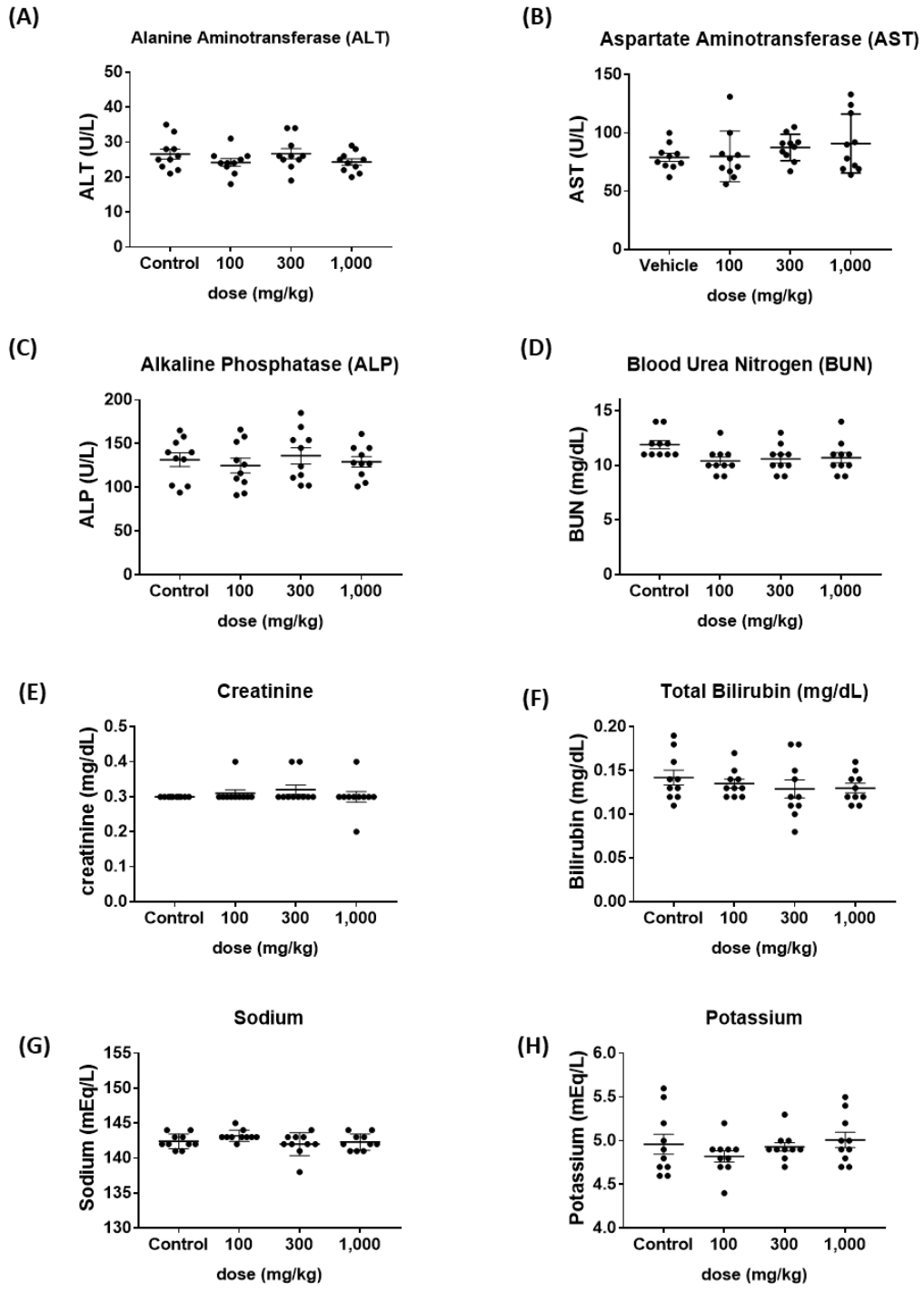


Fig. S12. No change in clinical chemistry toxicology markers. AMG 986 was administered once daily for 28 days by oral gavage to 9-10 week old male rats (N = 10/group) at doses of 0, 100, 300 and 1000 mg/kg. Data represented as individual animals (circles) with the group mean (bars) ± SEM. Treated groups were determined to be not statistically significant and comparable to the control group at each time point (two-way ANOVA, Tukey's multiple comparison test).

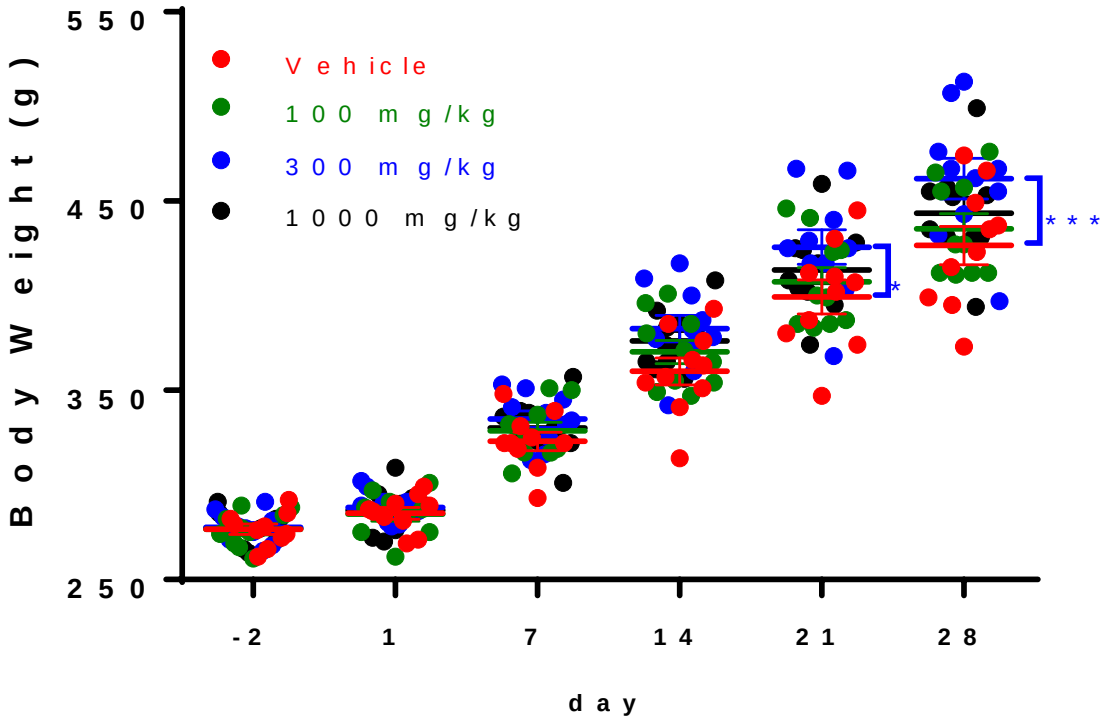


Fig. S13. Body weights. AMG 986 was administered once daily for 28 days by oral gavage to 9–10 week old male rats (N = 10/group) at doses of 0, 100, 300 and 1000 mg/kg. Data represented as individual animals (circles) with the group mean (bars) ± SEM. The 300 mg/kg dose group exhibited a slight but significant increase in BW relative to control at the 21 and 28 day timepoints. (* $p < 0.05$, *** $p < 0.001$, two-way ANOVA, Tukey's multiple comparison test).

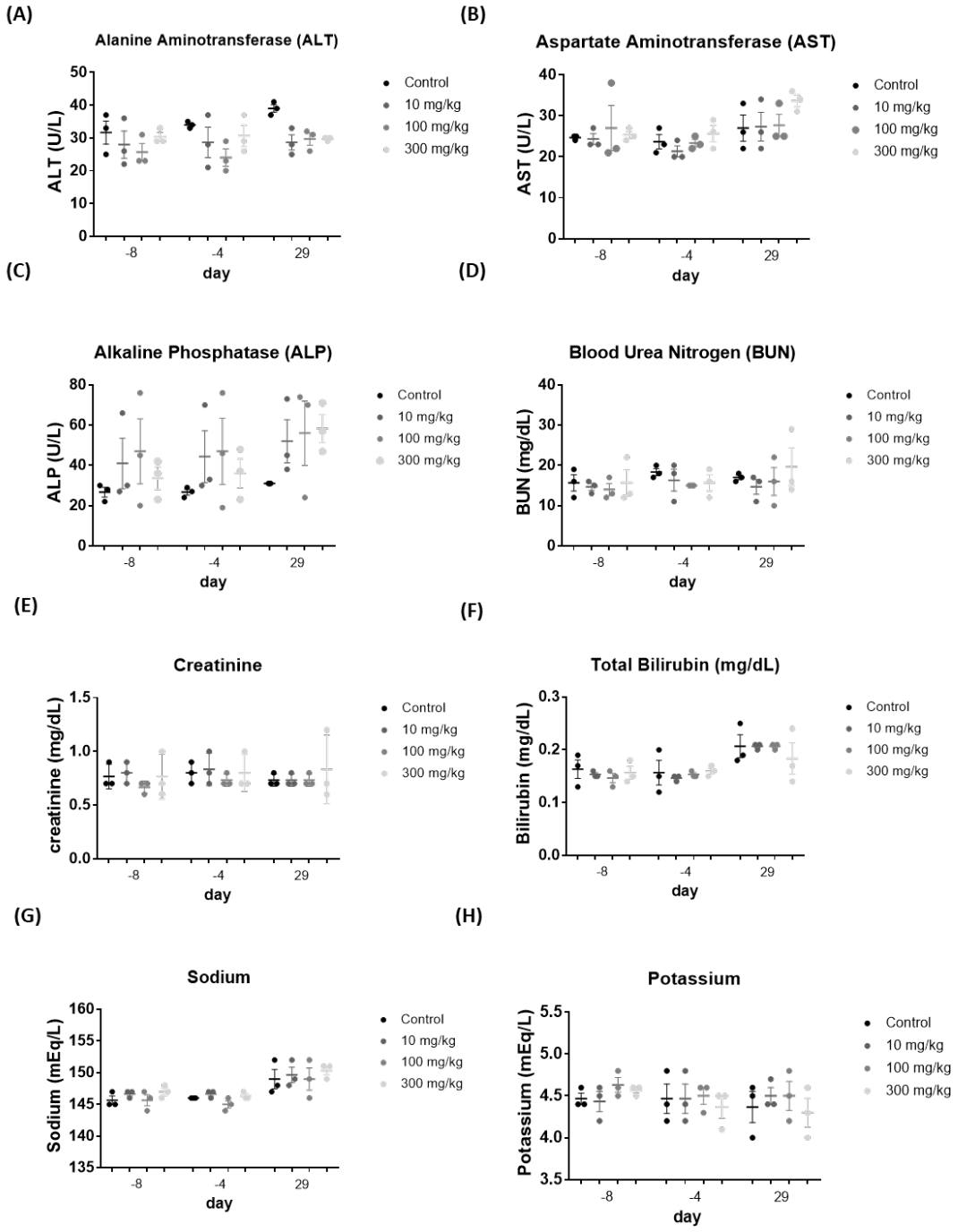


Fig. S14. No change in clinical chemistry toxicology markers. AMG 986 was administered once daily for 28 days by oral gavage to 15-16 month old male beagles (N = 3/group) with either a 0, 10, 100 or 300 mg/kg dose. Data represented as individual animals (circles) with the group mean (bars) ± SEM. Treated groups were determined to be not statistically significant and comparable to the control group at each time point (two-way ANOVA, Tukey's multiple comparison test.

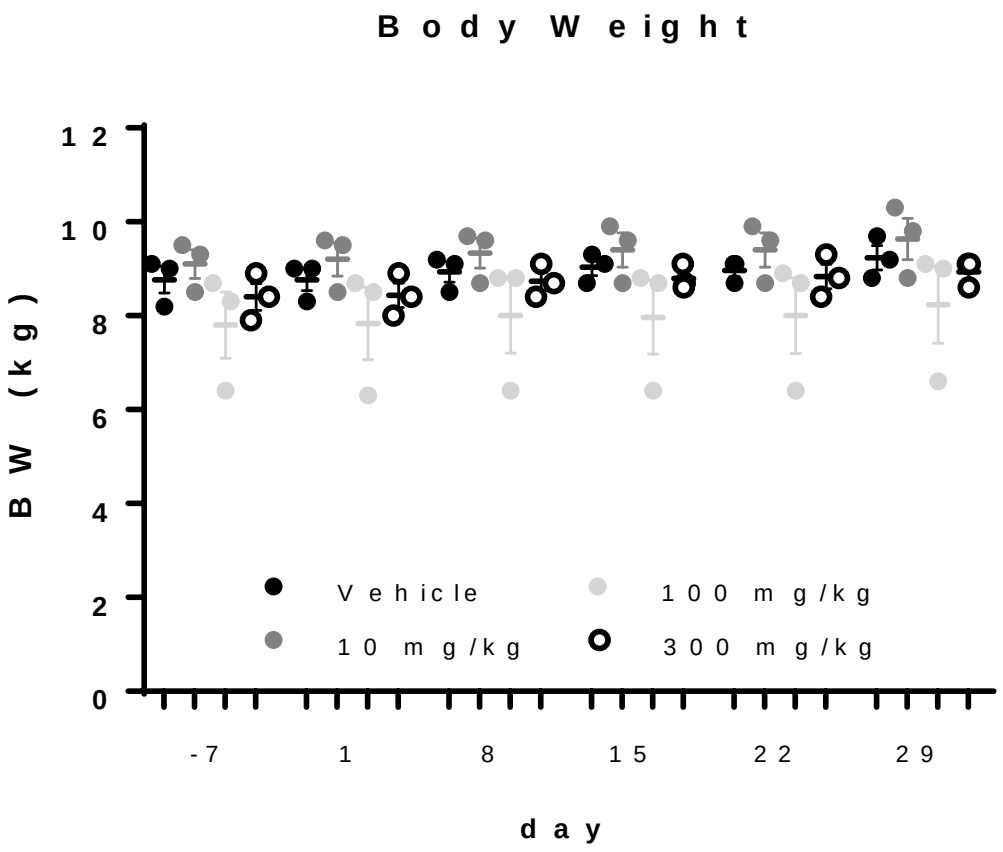


Fig. S15. No change in body weight. AMG 986 was administered once daily for 28 days by oral gavage to 15-16 month old male beagles (N = 3/group) with either a 0, 10, 100 or 300 mg/kg dose. Data represented as individual animals (circles) with the group mean (bars) ± SEM. Treated groups were determined to be not statistically significant and comparable to the control group at each time point (two-way ANOVA, Tukey's multiple comparison test.

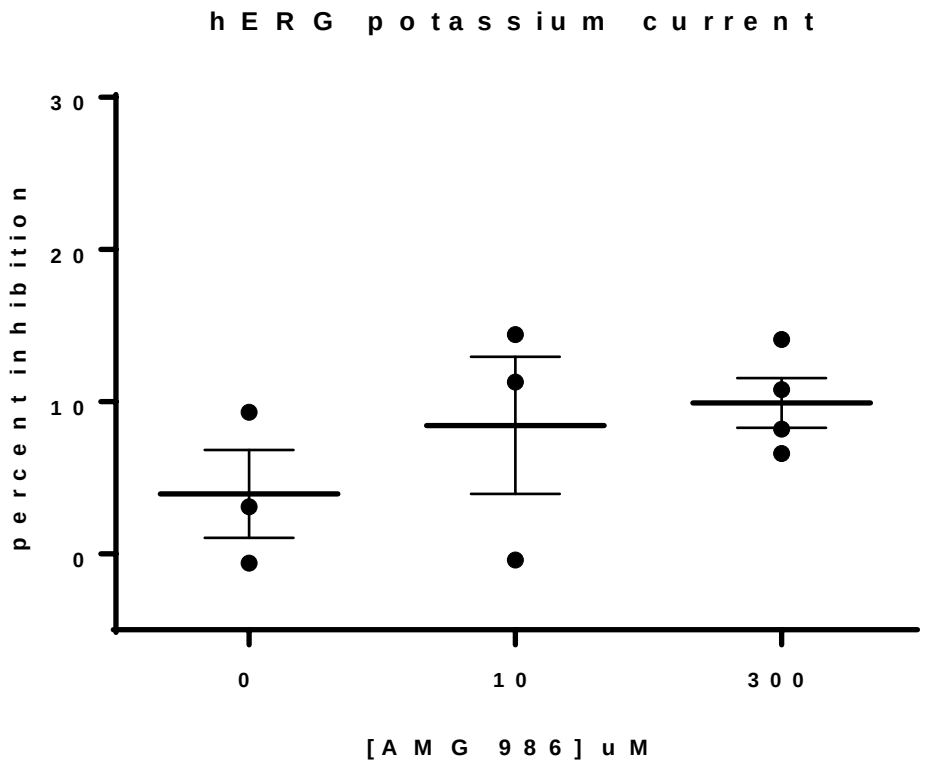


Fig. S16. Percent inhibition of hERG potassium current. AMG 986 was tested at 10 and 300 uM. The maximum percent inhibition achieved was ~10% and determined to be not statistically significant and comparable to the control group. N = 3 (0 uM), 3 (10 uM), and 4 (300 uM). Data represented as individual animals (circles) with the group mean (bars) ± SEM. Treated groups were determined to be not statistically significant and comparable to the control group at each time point (one-way ANOVA, Tukey's post-test).

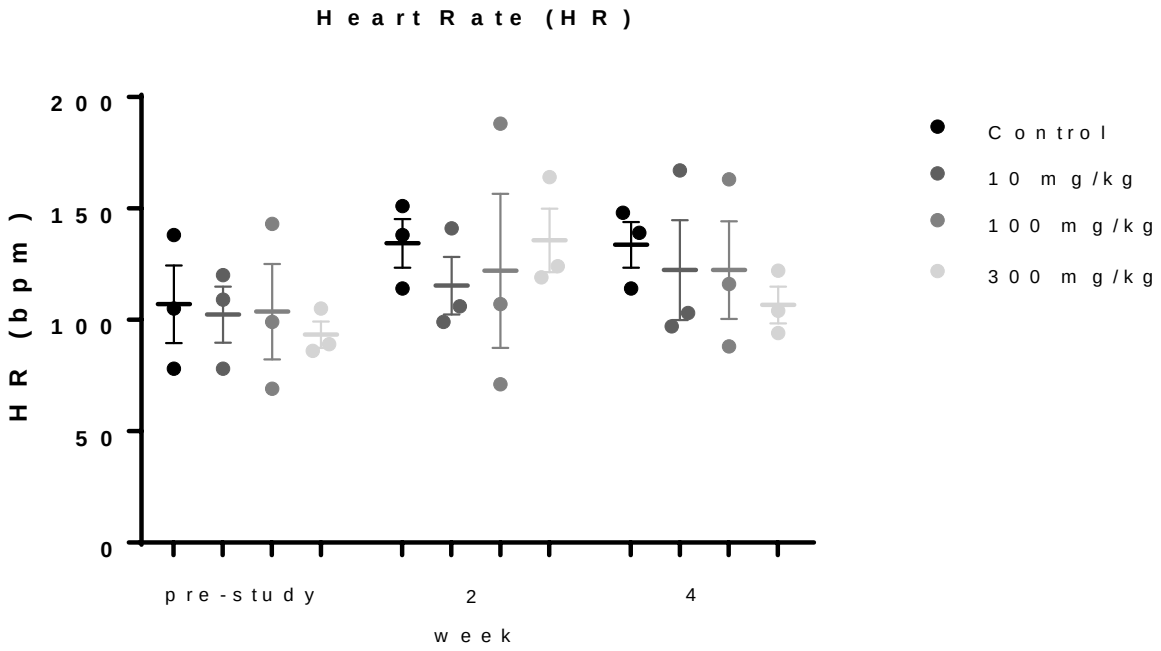


Fig. S17. No change in heart rate. AMG 986 was administered once daily for 28 days by oral gavage to 15-16 month old male beagles (N = 3/group) with either a 0, 10, 100 or 300 mg/kg dose. Data represented as individual animals (circles) with the group mean (bars) ± SEM. Treated groups were determined to be not statistically significant and comparable to the control group at each time point (two-way ANOVA, Tukey's multiple comparison test).

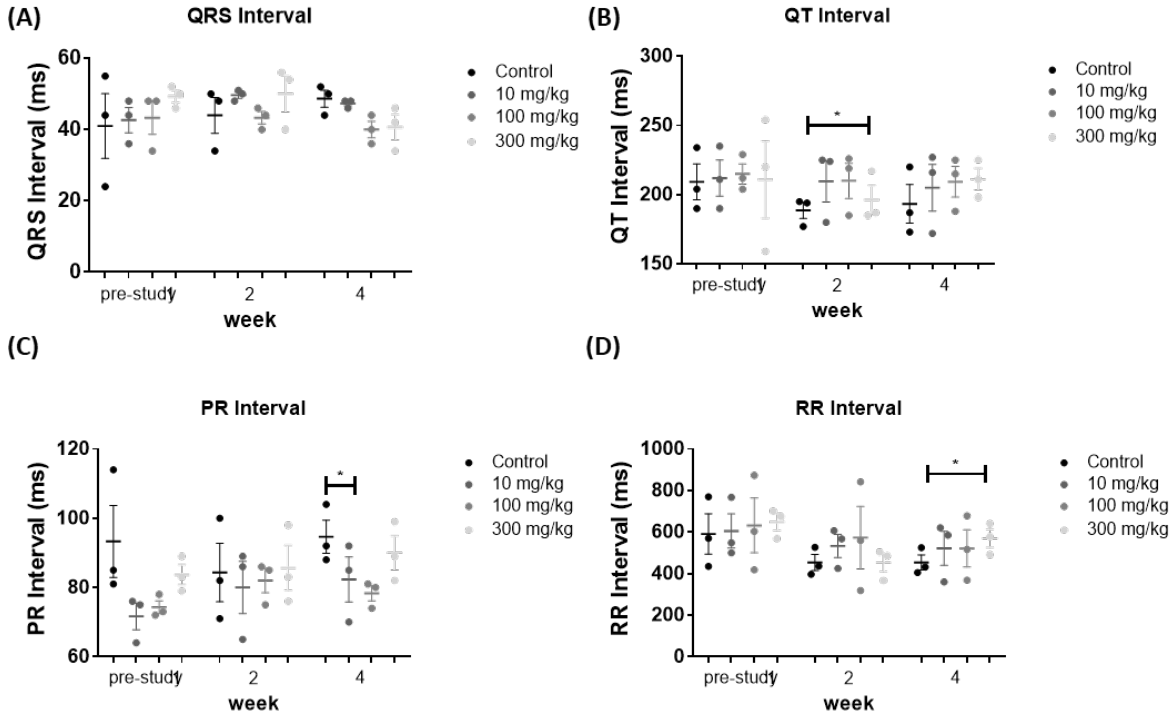


Fig. S18. AMG 986 had no meaningful dose dependent impact on (A) QRS, (B) QT, (C) PR and (D) RR intervals when evaluated in an exploratory 28 day repeat dose toxicity study in telemetered canines at doses up to 300 mg/kg. AMG 986 was administered once daily for 28 days by oral gavage to 15-16 month old male beagles (N = 3/group). Data represented as individual animals (circles) with the group mean (bars) ± SEM. Significance measured by a two-way ANOVA, Tukey's multiple comparison test (* $P < 0.05$).

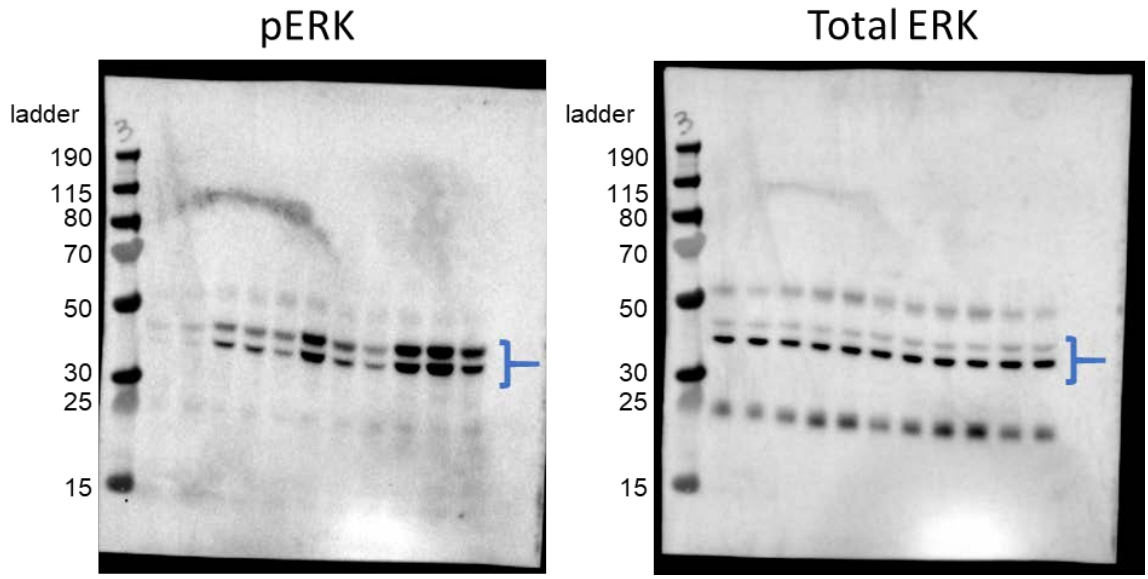


Fig. S19. Images for the entire western blot presented in figure 5A.

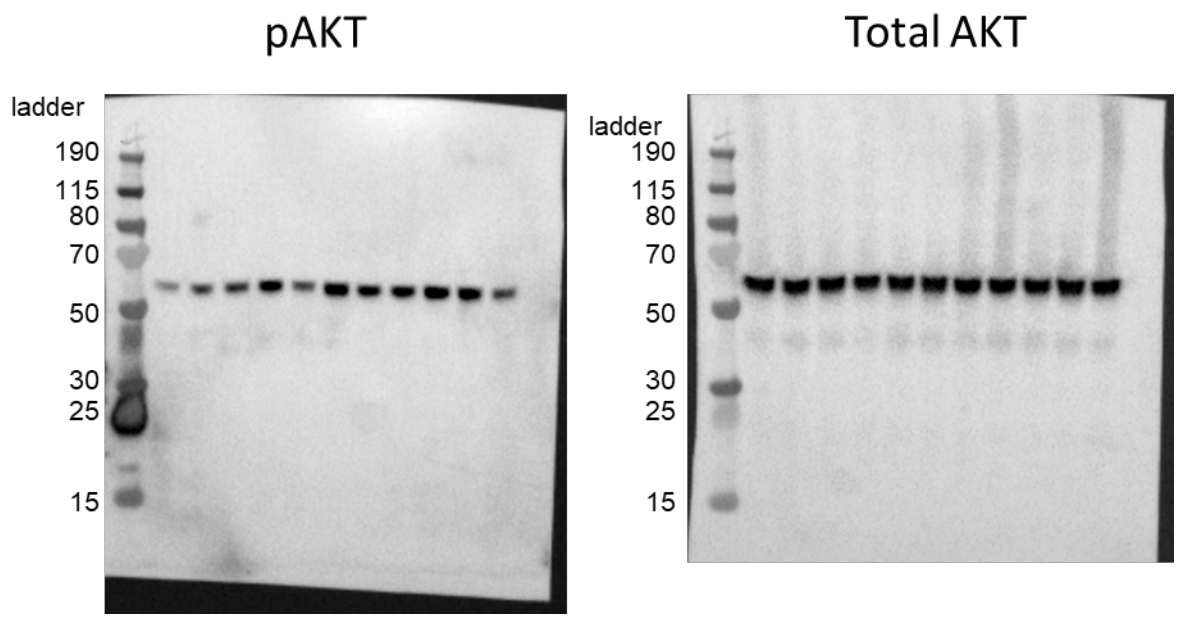


Fig. S20. Images for the entire western blot presented in figure 5B.

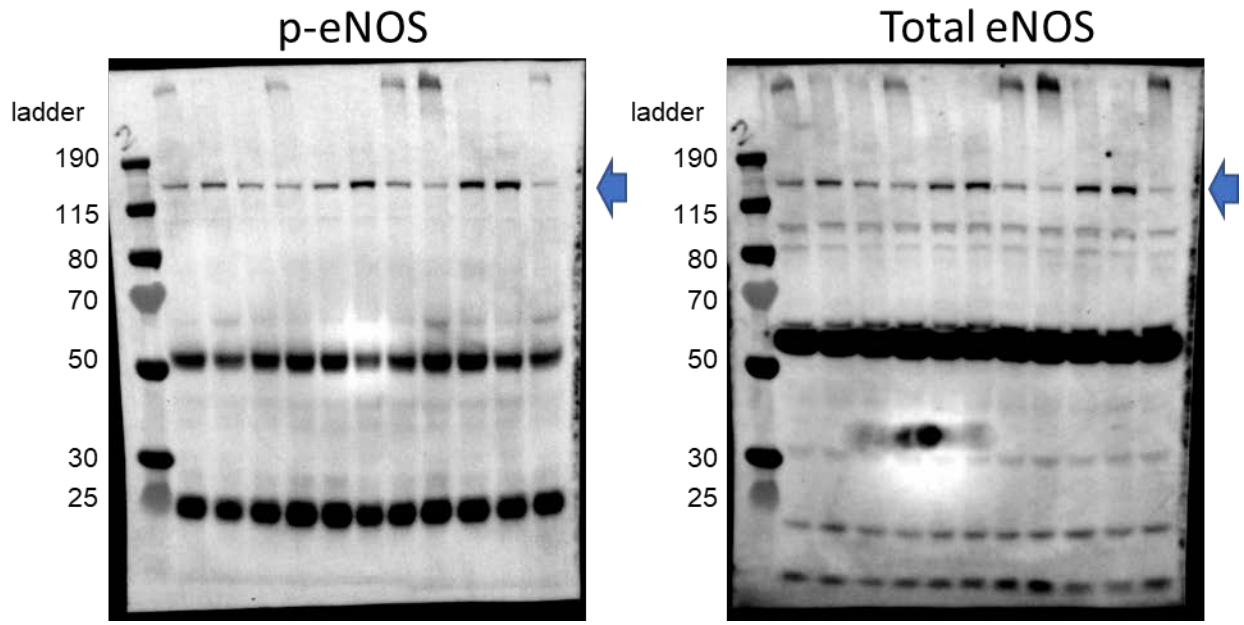


Fig. S21. Images for the entire western blot presented in figure S9A.

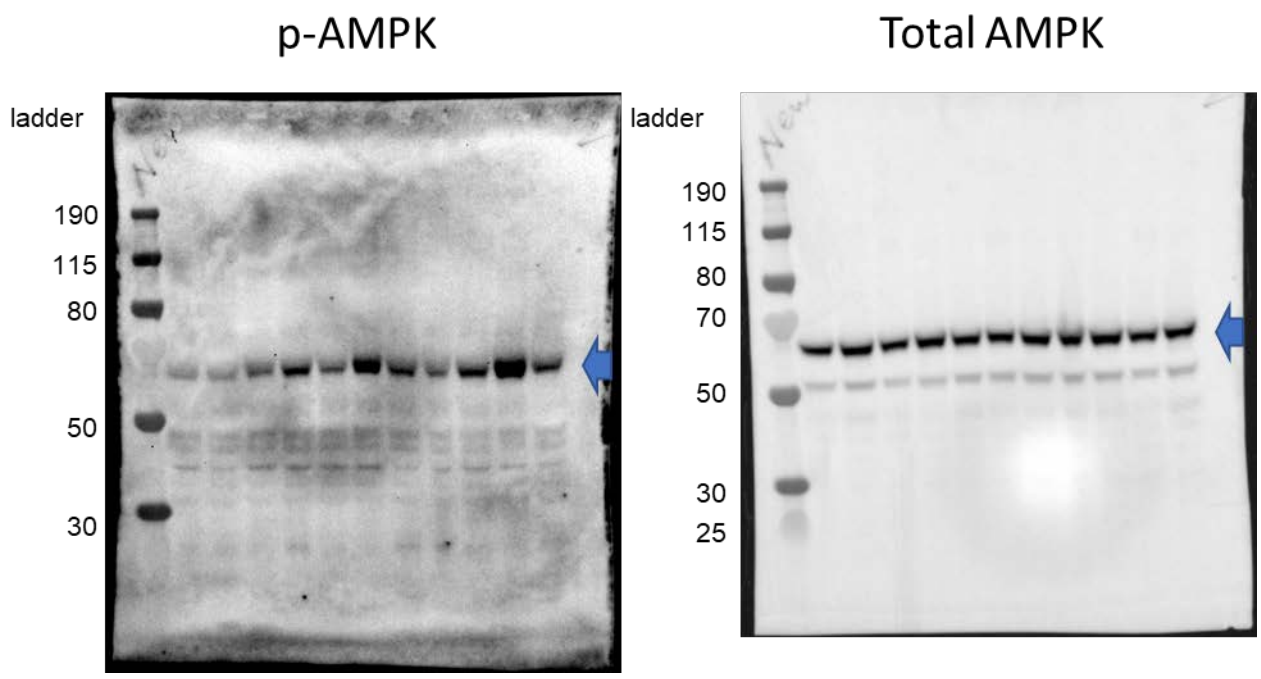


Fig. S22. Images for the entire western blot presented in figure S9B.

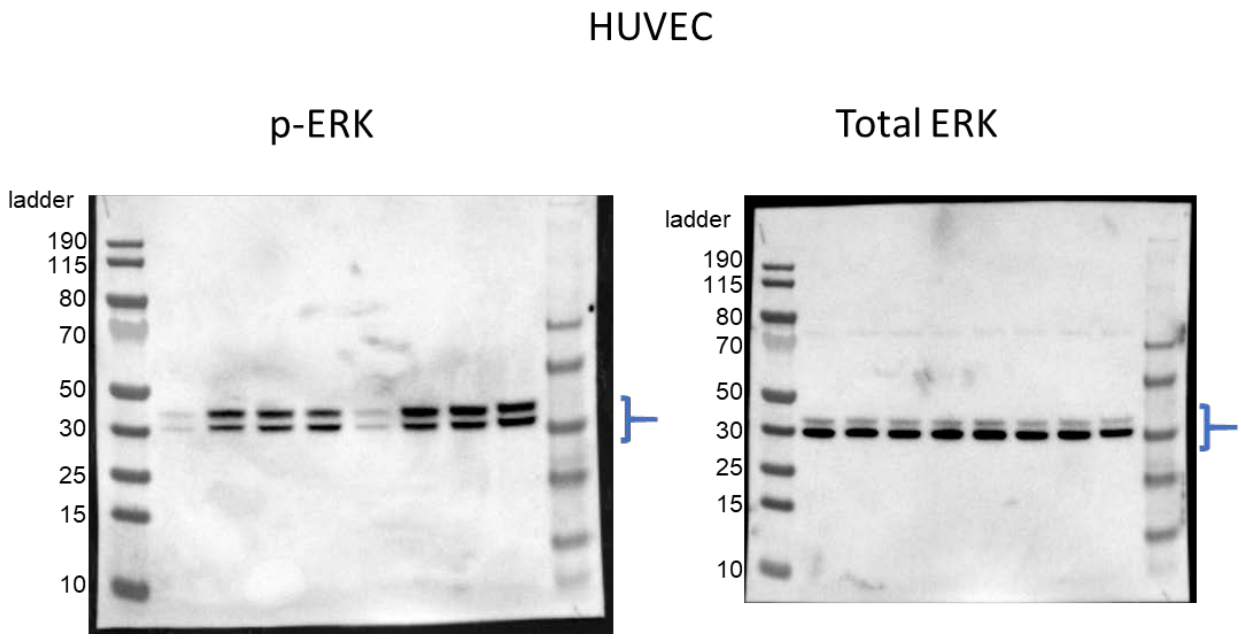


Fig. S23. Images for the entire western blot presented in figure 6A.

Table S1. Effect of AMG 986 on *In Vitro* Phosphodiesterase Enzyme Inhibition

Enzyme (Human Isoform)	Final Conc.	Substrate	IC50 (μM)
PDE1 (1B)	0.375 nM	cAMP	6.5
PDE2 (2A1)	1.38 nM	cAMP	>30
PDE3 (3A)	0.15 nM	cAMP	>30
PDE4 (4D2)	0.031 nM	cAMP	>30
PDE5 (5A)	0.105 nM	cGMP	>30
PDE7 (7A1)	0.19 nM	cAMP	>30
PDE8 (8A1)	0.05 nM	cAMP	>30
PDE9 (9A2)	0.038 nM	cGMP	>30
PDE10 (10A2)	0.008 nM	cAMP	>30
PDE11 (11A4)	0.038 nM	cAMP	>30

Table S2. AMG 986 activity against a panel of 13 GPCRs

Receptor	**EC_{50} (uM)**	**Functional Response**
Muscarinic M1	>48.5	No
Histamine H1	>48.5	No
Chemokine CCR2	>48.5	No
Somatostatin sst4	>48.5	No
Beta A1 adrenergic	>48.5	No
Mu Opioid	>48.5	No
Chemokine CXCR2	>48.5	No
Serotonin 5HT1A	>48.5	No
Dopamine D2	>48.5	No
Tachykinin NK1	>48.5	No
Cannabinoid CB1	>48.5	No
Alpha A1 adrenoceptor	>48.5	No
A2A Adenosine	>48.5	No