Supplemental Figure Legends

Supplemental Figure 1. Activated CD47 does not regulate other HDAC family members. Western immunoblot analysis of HDAC1,-2, and -4 protein expression levels in LV tissue samples from wild type ($CD47^{+/+}$) (WT) and CD47 ($^{-/-}$) null animals 4 weeks post-TAC or control. Densitometry is presented as the mean (± S.E.M.) of n = 4 animals/group.

Supplemental Figure 2. CD47 promotes PE-stimulated upregulation of cardiac HDAC3. Rat neonatal cardiac myocytes RNCM were treated with phenylephrine (PE, 10 mM) ± a CD47 antagonist antibody (Ab) (clone OX101, 1 μg/ml) and Western immunoblot analysis of HDAC3 protein expression performed on cell lysates. A representative blot is shown. Densitometry is presented as the mean (± S.E.M.) of n = 4 experiments. * = statistically significant difference ($p < 0.05$) compared to untreated; # = statistically significant difference ($p < 0.05$) compared to PE treated.

Supplemental Figure 3. Oligonucleotide morpholino treatment effectively suppresses HDAC3 protein expression in cardiac myocytes. (A) RNCM were treated with a morpholino oligonucleotide to HDAC3 at the indicated concentrations or vehicle (CTRL) for 48 h, lysates prepared and protein expression determined via Western immunoblot. Densitometry is presented as the mean (± S.E.M.) of 4 separate experiments. * = statistically significant difference ($p < 0.05$) compared to CTRL. (B) Rat neonatal cardiac myocytes were treated with BAY58-2667 (1 μM) for 3 h, lysates prepared and protein expression of total and phosphorylated HDAC3 (p-HDAC3) determined via Western immunoblots. Densitometry is presented as the mean (± S.E.M.) of 3 separate experiments.

Supplemental Figure 4. Activated CD47 regulates CaMKII downstream targets. RT-PCR analysis of mRNA transcript expression levels of (A) beta myosin heavy chain (β-MHC), (B) atrial

natriuretic peptide (ANP), (C) alpha skeletal actin (α-SKA) and (D) brain natriuretic peptide (BNP) from LV tissue samples from age matched male wild type (WT) and CD47 ($^{-/-}$) null mice 4 weeks post-TAC or control. Data are presented as the mean (± S.E.M.). (n = 4 animals/group). Rat neonatal cardiac myocytes were treated with 7N3 (10 μM) for 3 h, lysates prepared and RT-PCR analysis of mRNA transcript expression levels of (E) β-MHC and (F) BNP performed. Data are presented as the mean (± S.E.M.) of 3 separate experiments.

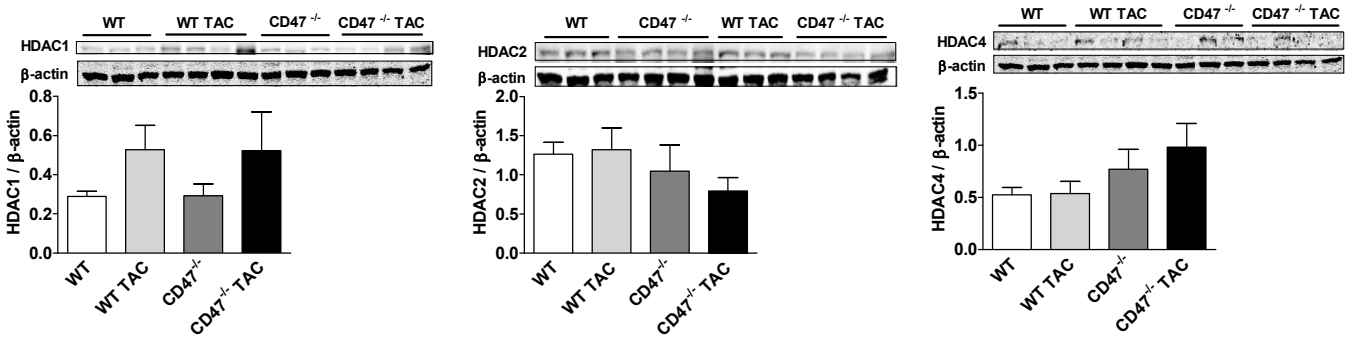
Supplemental Figure 5. Activated CD47 regulates cardiac myocyte Ca^{2+} to control CaMKII expression. (A) Western immunoblot analysis of phosphorylated CaMKII (p-CaMKII) and total CaMKII protein expression levels in LV samples from wild type (WT) mice ± an HDAC3 morpholino 4 weeks post-TAC or control. Densitometry is presented as the mean (± S.E.M.) of n = 7 animals/group. * = statistically significant difference ($p < 0.05$) compared to control; # = statistically significant difference ($p < 0.05$) compared to WT TAC. (B) Rat neonatal cardiac myocytes were treated with a amiloride (5 mM, 30 min) (B, C) or nifedipine (10 mM, 30 min) (D) ± peptide 7N3 or a control (CTRL) peptide (10 μM), lysates prepared and protein expression of CaMKII, phosphorylated CaMKII (p-CaMKII) (A, C), HDAC3 and phosphorylated HDAC3 (p-HDAC3, B) determined via Western immunoblot. Densitometry is presented as the mean (± S.E.M.) of 4 separate experiments. * = statistically significant difference ($p < 0.05$) compared to 7N3 + amiloride and 7N3 + nifedipine.

Supplemental Figure 6. Activated CD47, in a Ca^{2+} dependent manner, promotes autophagy marker accumulation. (A) Rat neonatal cardiac myocytes were treated with amiloride (5 mM, 30 min, A) or nifedipine (10 mM, 30 min, B) ± peptide 7N3, a control peptide (CTRL) (10 μM) or vehicle (control), lysates prepared and protein expression determined via Western

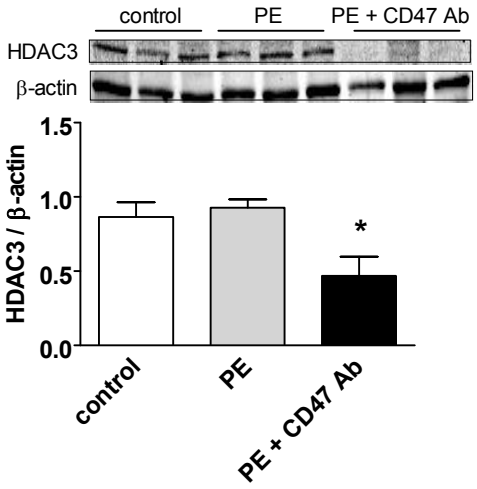
immunoblot. Densitometry is presented as the mean (± S.E.M.) of 4 separate experiments. * = statistically significant difference ($p < 0.05$) compared to 7N3 + amiloride.

Supplemental Figure 7. CD47 is expressed in LV biopsies from healthy subjects and dysregulated in end-stage LV HF. (A) Western immunoblot analysis of CD47 protein expression in left ventricular tissue samples from patients with non-ischemic LV HF (n = 4) and normal controls (n = 5) and mRNA levels of CD47 (B) and TSP1 (C) from the same. Data are presented as the mean of all samples (± S.E.M.). * = statistically significant difference ($p < 0.05$) compared to control.

Supplemental Figure 1

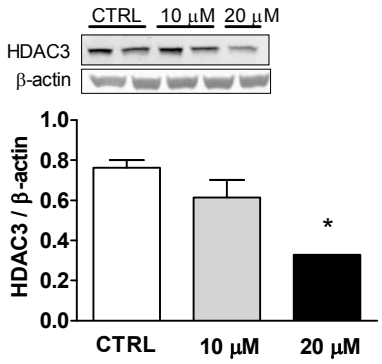


Supplemental Figure 2

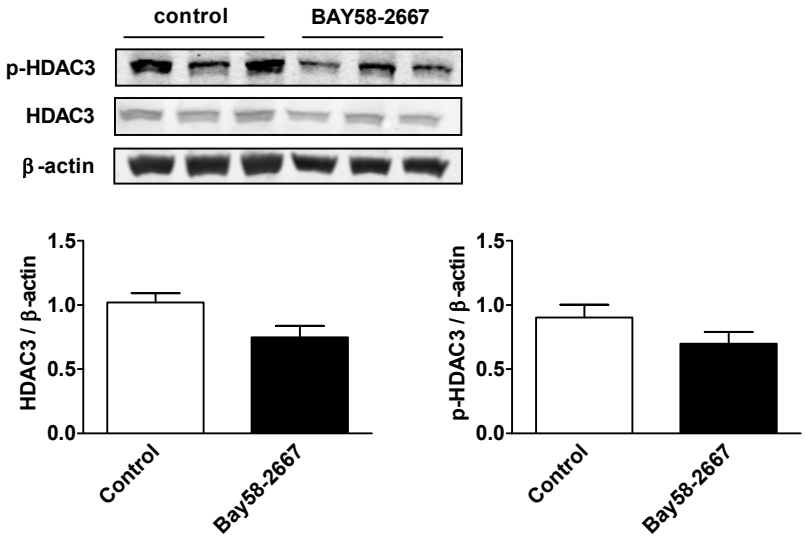


Supplemental Figure 3

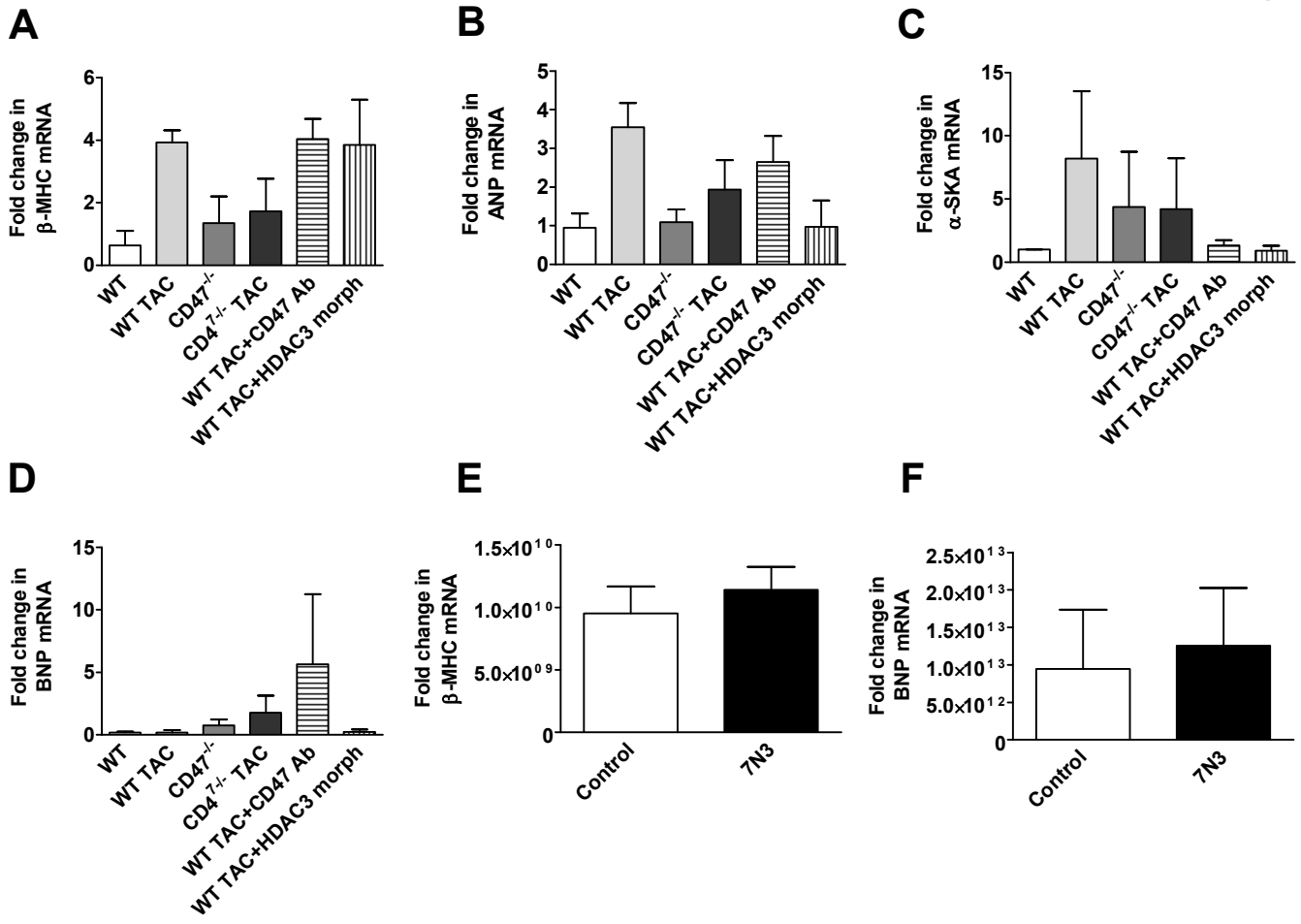
A



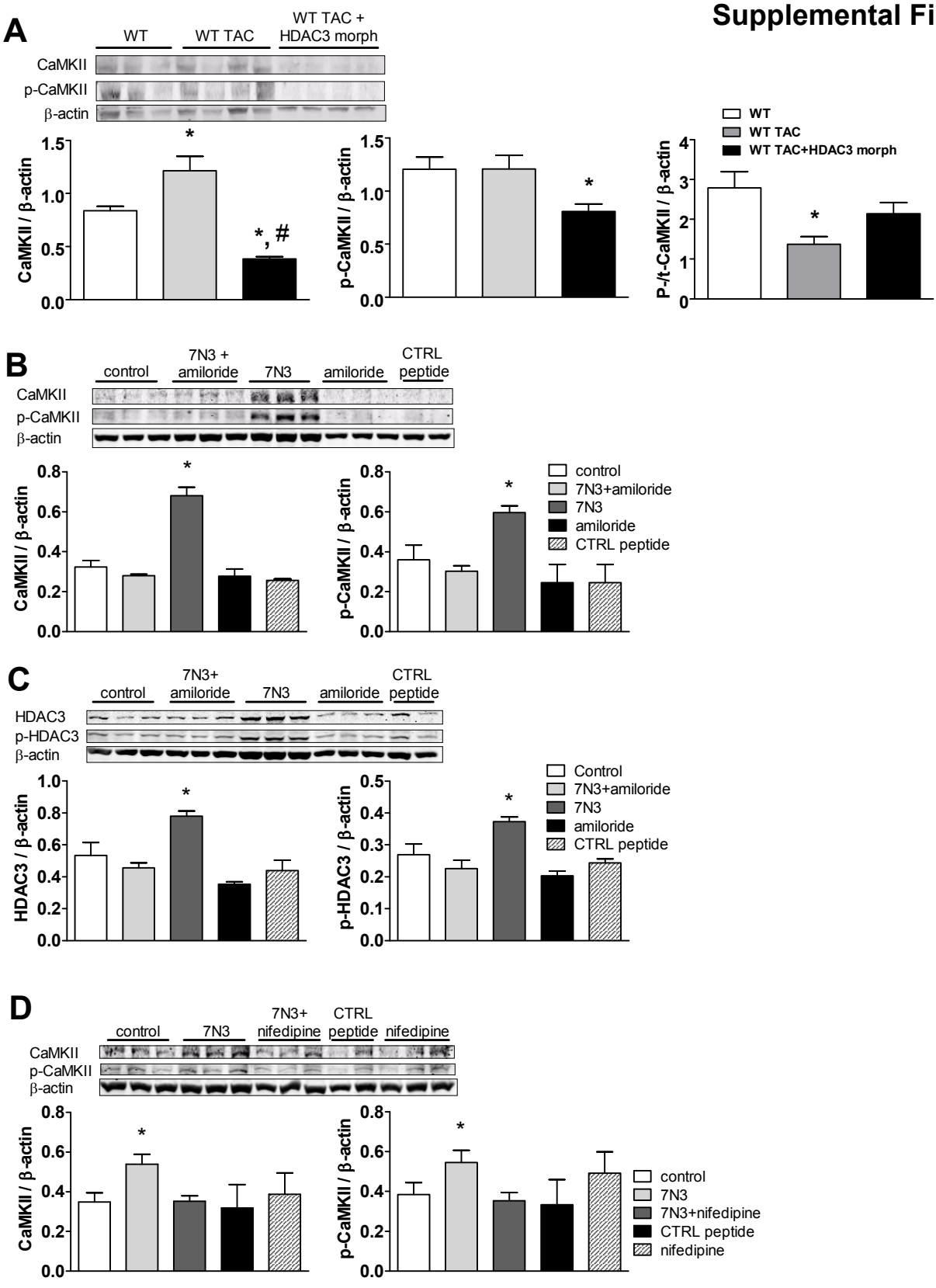
B



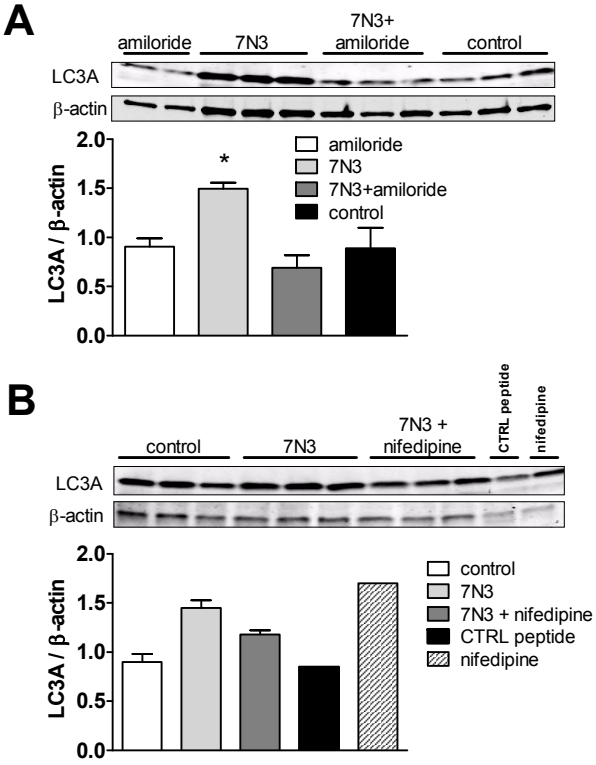
Supplemental Figure 4



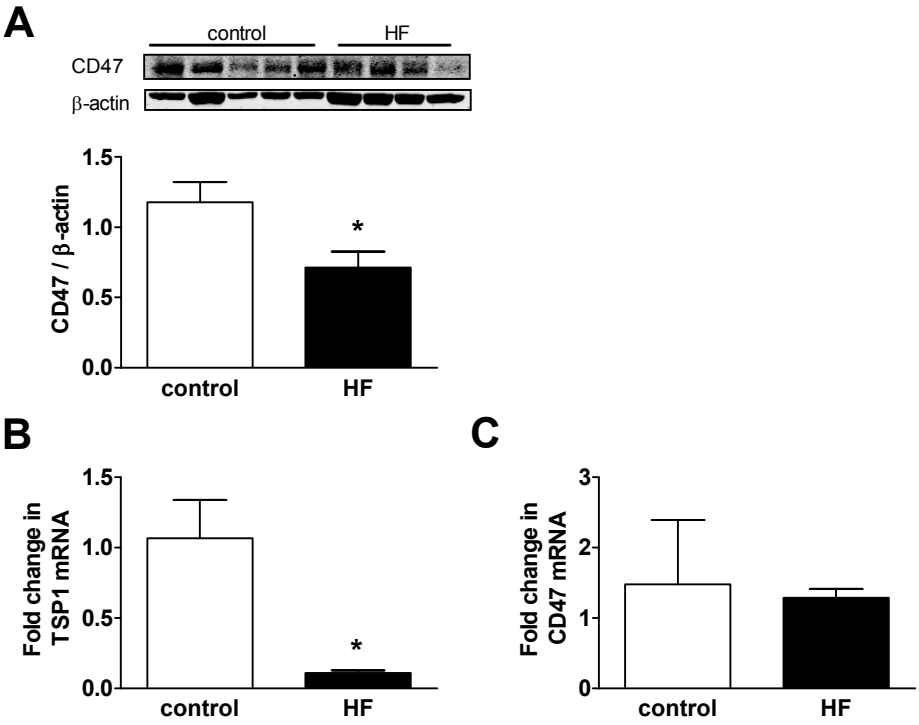
Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7



Supplemental Movie Legend.

Activation of CD47 results in a rapid increase in cytosolic calcium in cardiac myocytes. Real-time live cell imaging of RNCM shows a rapid increase in calcium-driven fluorescence following treatment with the CD47 specific activating peptide 7N3. RNCM were cultured on glass bottom dishes, loaded with the calcium sensitive dye Fluo-4 AM (5 μM), for 20 minutes, followed by treatment with peptide 7N3 (10 μM). Following the addition of peptide 7N3 to the culture medium data were collected every 3 minutes over a 30 minute period employing an inverted Nikon TiE fluorescent microscope.