	WT	Pak1$^{-/-}$	WT +CCh 3'	Pak1$^{-/-}$ +CCh 3'
HR	745 ± 9	743 ± 12	511 ± 102*	457 ± 51*
CV	6.7 ± 0.7	6.6 ± 0.9	17.4 ± 8.6	61 ± 17*,#
PQ	18.9 ± 0.3	17.5 ± 0.4	30.7 ± 5.6	23.8 ± 3.5
QRS	10.1 ± 0.2	9.8 ± 0.2	15.7 ± 3.7	13.0 ± 2.9
QT	41.1 ± 0.7	40.1 ± 0.9	66.3 ± 13	61.3 ± 10.7
QTC	45.8 ± 0.7	44.6 ± 0.8	56.6 ± 6.1	56.2 ± 4.1
ST	31.6 ± 0.7	30.8 ± 0.9	51.1 ± 10	53.5 ± 5.6
QTcD	15.7 ± 2.1	22.6 ± 2.4	36.8 ± 8.4*	49.2 ± 12

Table 1: heart rate (HR, (beats/min)); Coefficient of variability (CV; %), PQ, QRS, QT, ST-duration; ms), corrected QT duration (QTc; ms) and dispersion of corrected QT (QTcD; ms) from WT and Pak1$^{-/-}$ mice before and 3 minutes after intraperitoneal injection of carbachol (CCh: 150 ng/g) (*p < 0.05 compared to t = 0 min; #p < 0.05 compared to WT)

Figure 1, *1.5 column*

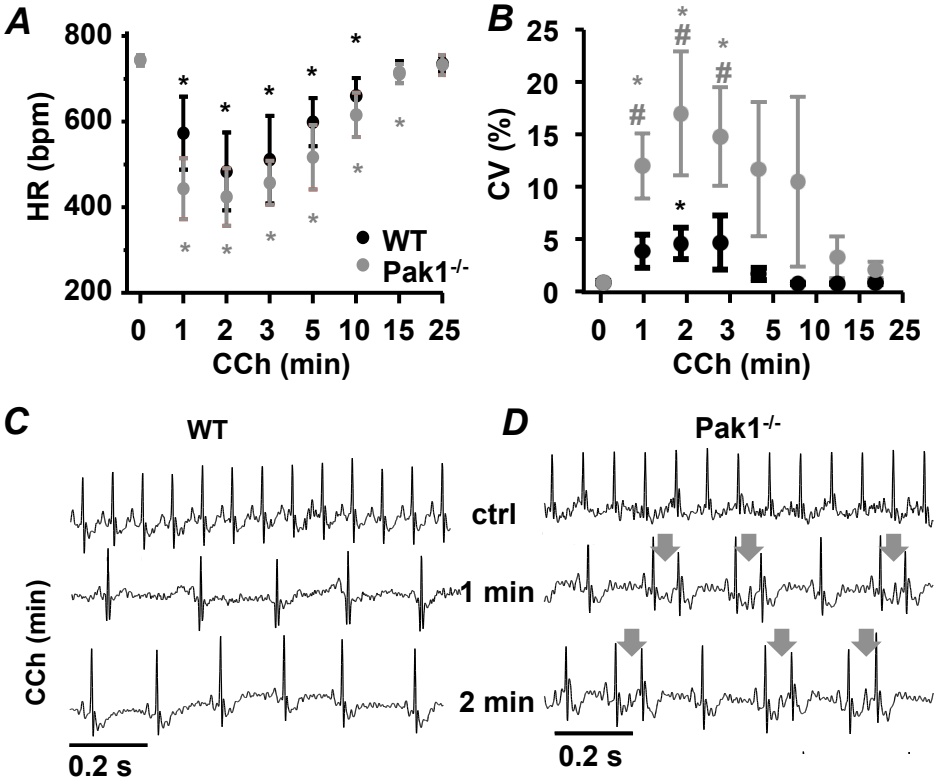

Figure 1: Reduced Pak1 activity enhances the propensity for atrial arrhythmic events. (A) Heart rate (HR, beats per minute: bpm) and **(B)** Coefficient of variation (CV) recorded in conscious WT and Pak1$^{-/-}$ mice after intraperitoneal injection of carbachol (CCh: 150 ng/g). Representative ECG recording in WT (C) and Pak1$^{-/-}$ (D) mice show spontaneous arrhythmic events originating in the atria of Pak1$^{-/-}$ mice (arrows indicate P-wave). (*p < 0.05 compared to t = 0 min; #p < 0.05 compared to WT)

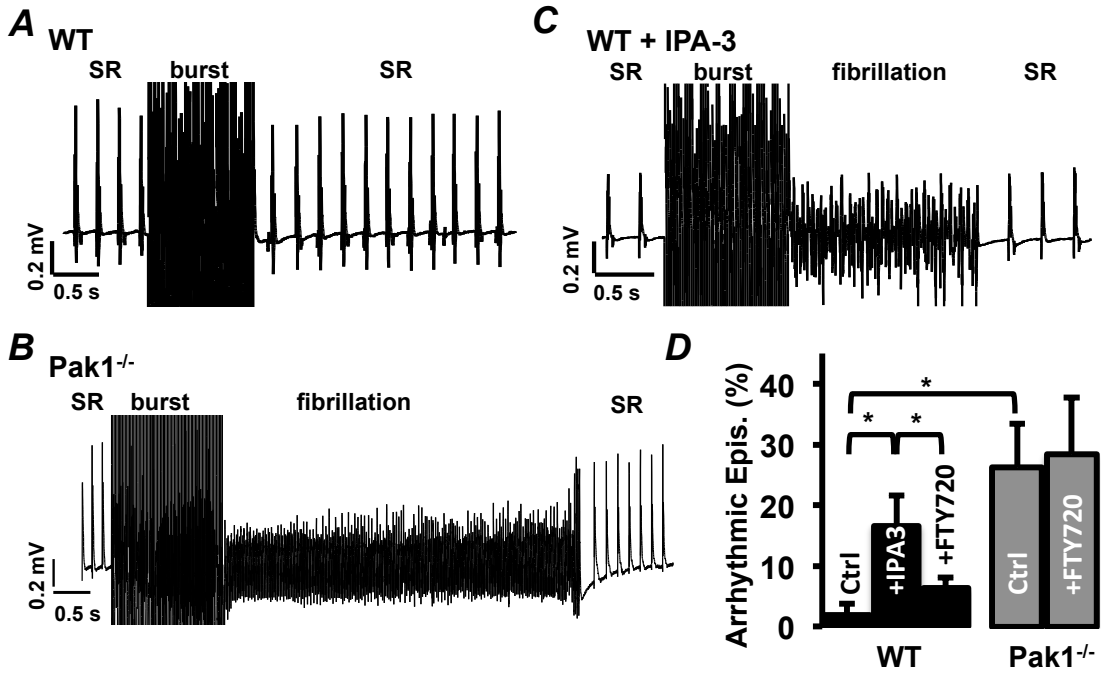

Figure 2: Representative examples of left atrial electrograms showing spontaneous activity (SR: sinus rhythm) and arrhythmic episodes induced by burst pacing (burst) in WT (**A**), Pak1$^{-/-}$ (**B**) and WT hearts perfused with IPA-3 (**C**). (**D**) Percent of burst pacing episodes that induced arrhythmic events in Pak1$^{-/-}$ and WT hearts during Ctrl conditions, Pak1 inhibition with IPA-3 (10 μM), or Pak1 stimulation with FTY720 (1 μM). (*$p < 0.05$).

Figure 3, *1.5 column*

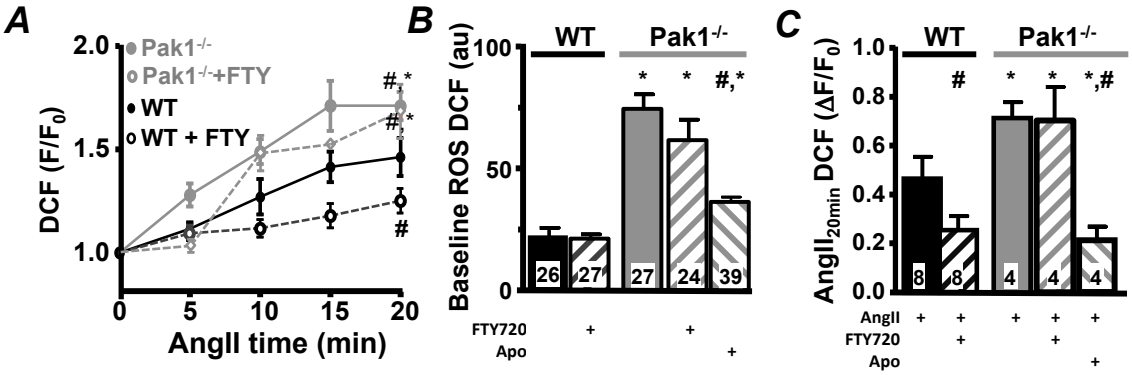

Fig. 3: Attenuated Pak1 activity increases NOX2-dependent ROS production. (**A**) Change in fluorescence recorded in WT (black) and Pak1$^{-/-}$ (grey) AMs loaded with DCF during stimulation with AngII in the presence and absence of FTY720 (open symbols). Bar graphs show DCF fluorescence at baseline (**B**) and (**C**) 20 min after AngII, AngII+FTY720, or AngII+Apo (Apo:1 µmol/L) superfusion in WT and Pak1$^{-/-}$ cells. (*: $p < 0.05$ compared to WT; #:$p < 0.05$ compared to own Ctrl)

Figure 4, *1.5 column*

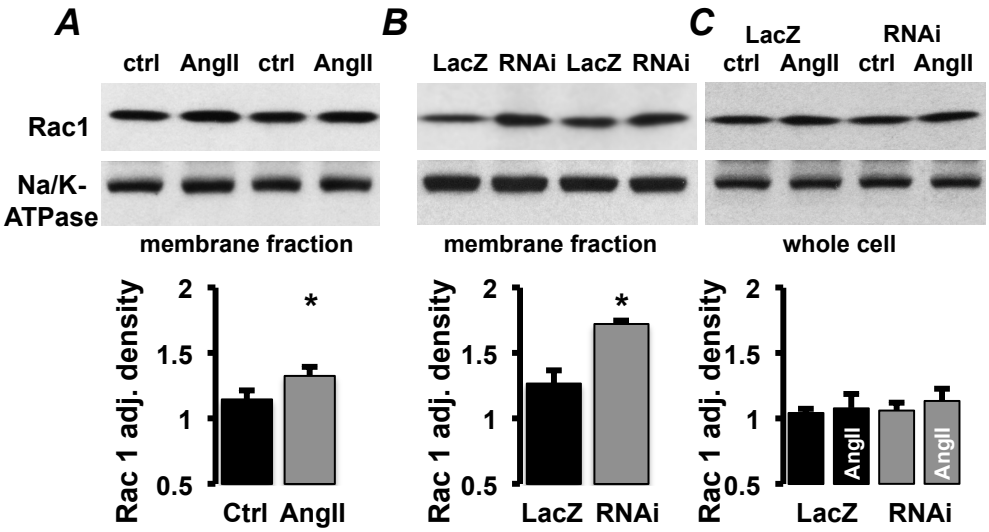

Fig. 4: Attenuated Pak1 promotes Rac1 translocation to the plasma membrane. Western blotting results of AngII (**A**) and Pak1-RNAi (**B**) treated HL-1 cells and (**C**) LacZ or Pak1-RANi treated HL-1 cells that were stimulated with AngII. Densiometric quantification (below) presented as relative density adjusted to the Na^+/K^+-ATPase (NKA) loading control. (*: $p < 0.05$ compared to Ctrl or LacZ, respectively).

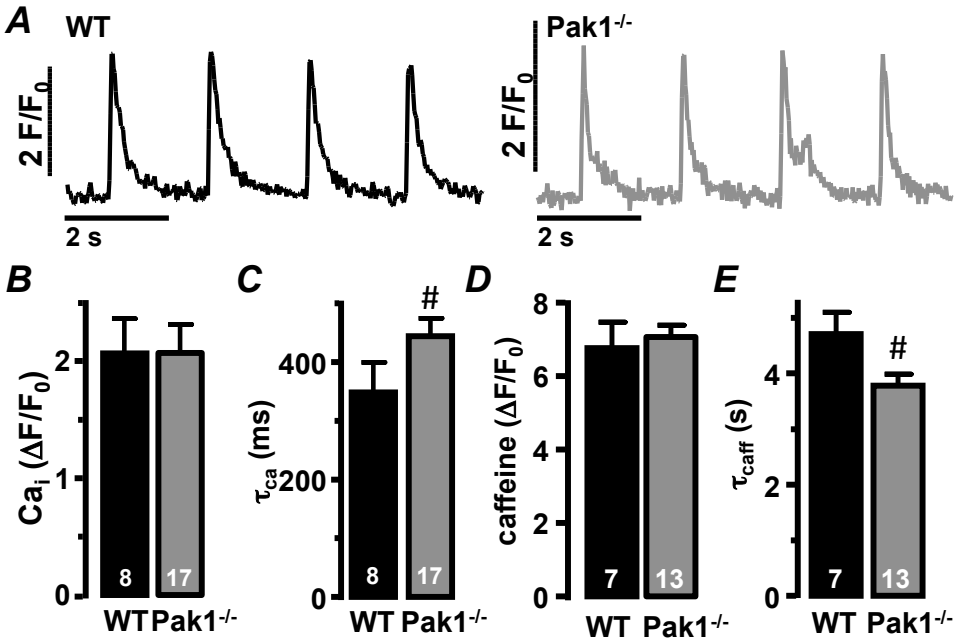

Figure 5: Ca^{2+} removal shifts from SERCA to NCX in Pak1$^{-/-}$ myocytes. (**A**) Representative field stimulation-induced Ca^{2+} transients recorded in WT (black) and Pak1$^{-/-}$ (grey) AMs under control conditions. Bar graphs show (**B**) the Ca^{2+} transient amplitude, (**C**) the Ca^{2+} transient decay constant (τ_{Ca}), (**D**) the caffeine transient amplitude and (**E**) the caffeine transient decay constant (τ_{Ca}). (#: $p < 0.05$ compared to WT).

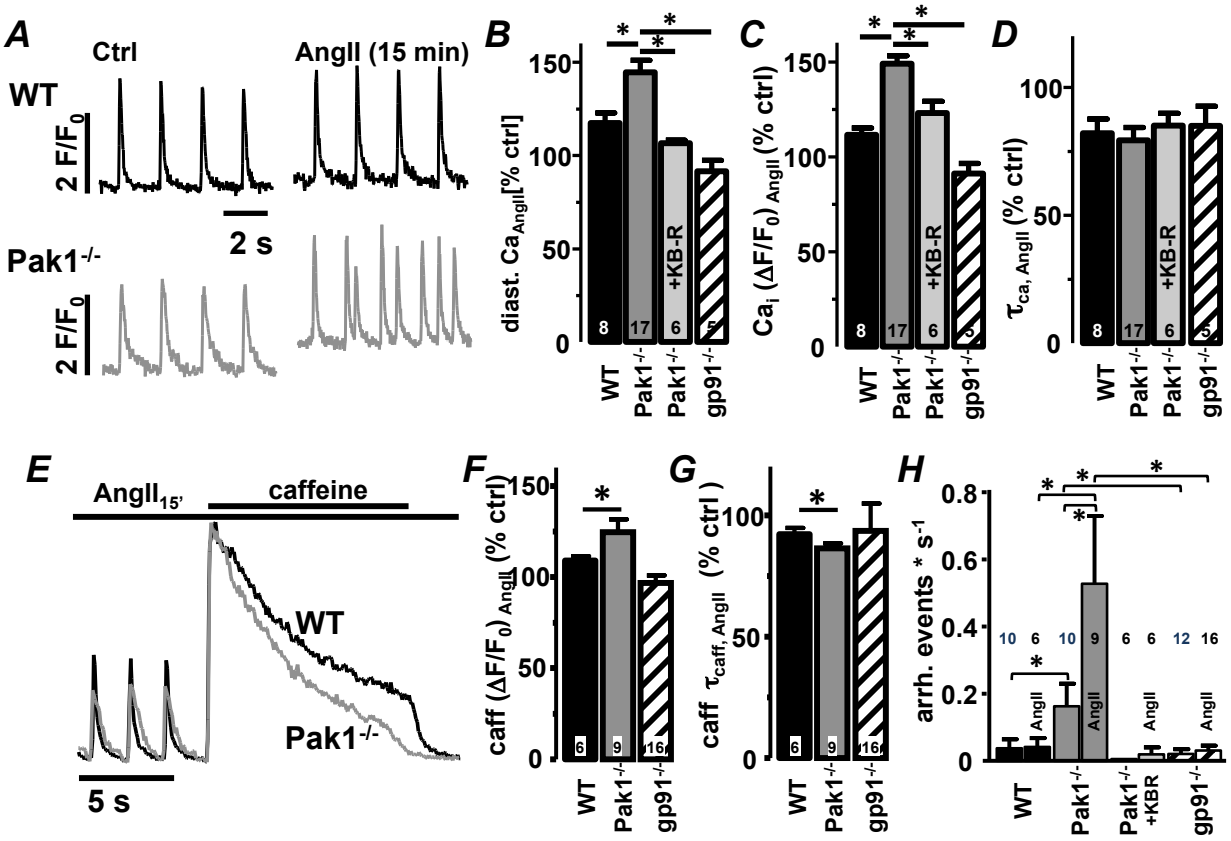

Fig. 6. Pak1$^{-/-}$ AMs exhibit exaggerated AngII induced arrhythmic events.
(**A**) Representative field stimulation induced Ca^{2+} transients recorded in WT and Pak1$^{-/-}$ (grey) AMs. Bar graphs represent the percent change after 15 min of AngII superfusion of the (**B**) diastolic Ca^{2+}, (**C**) Ca^{2+} transient amplitude, and (**D**) Ca^{2+} transient decay constant (τ_{caff}) in AMs from WT, Pak1$^{-/-}$, gp91$^{phox-/-}$, and Pak1$^{-/-}$ AMs treated with NCX inhibitor KB-R7943 (KBR; 1 μM). (**E**) Representative, normalized caffeine transients (10 mmol/L) after 15 min of AngII superfusion. Bar graphs show (**F**) caffeine transient amplitude and (**G**) decay constant. (**H**) Average number of spontaneous arrhythmic events per second in AMs before and after AngII superfusion for the cells and treatment conditions described in A-D. (*: $p < 0.05$)

Figure 8

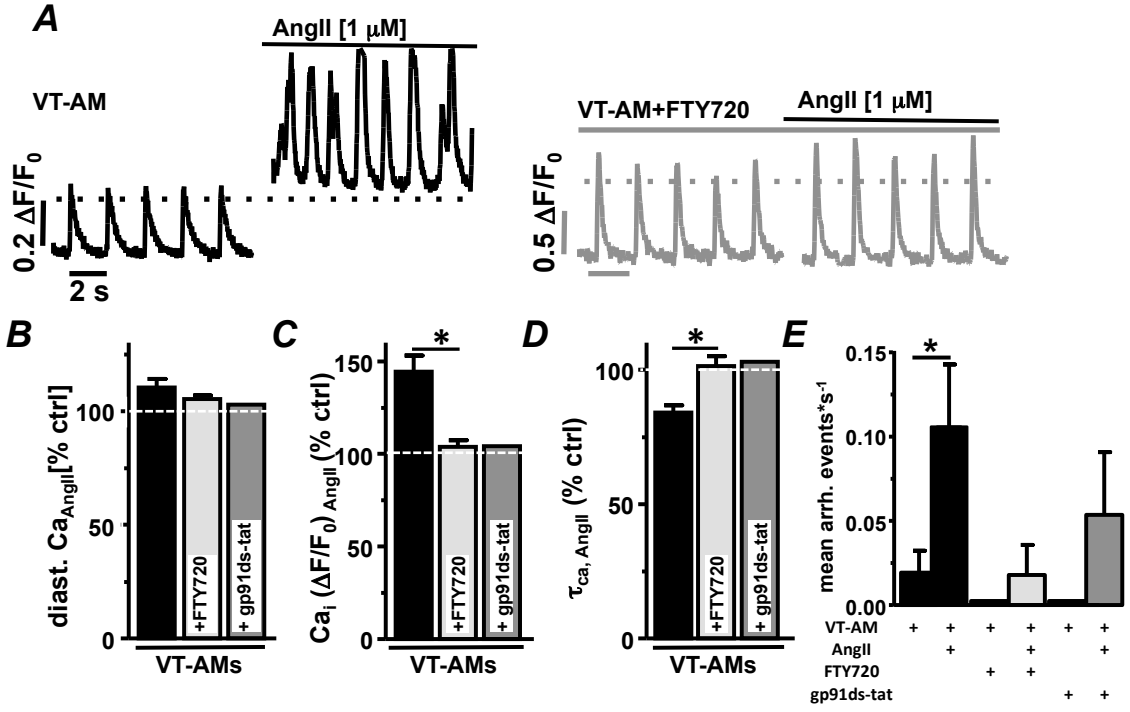

Fig. 7: Pak1 stimulation prevents arrhythmic events in AMs from a canine AF model. (**A**) Representative field stimulation induced Ca^{2+} transients before and 20 min after stimulation with AngII under Ctrl conditions (black) and in presence of Pak1 stimulation (FTY720: 200 nM; grey). Bar graph show the percent change in (**B**) diastolic $[Ca]_i$, (**C**) Ca transient amplitude and (**C**) Ca transient decay constant after AngII stimulation under Ctrl conditions and in presence of FTY720 or gp91ds-tat (1 μM). (**E**) Mean arrhythmic events per second in VT-AMs under the indicated treatment conditions (*: $p < 0.05$ compared to Ctrl).

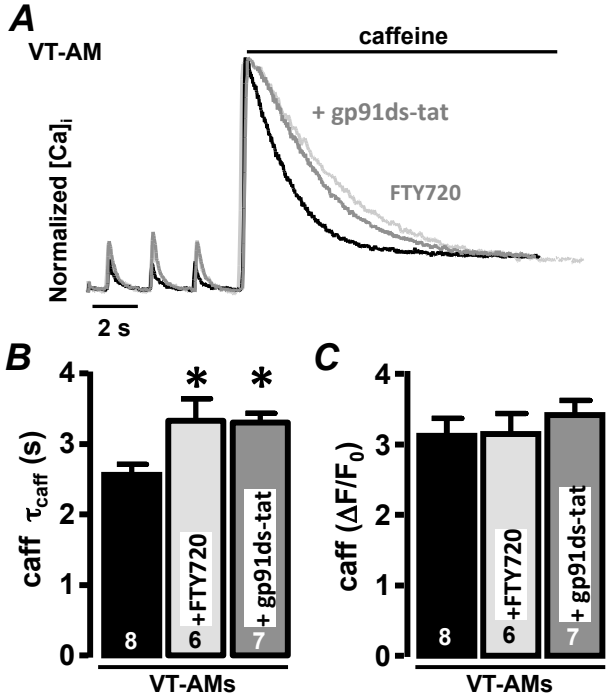

Fig. 8: Pak1 stimulation and NOX2 inhibition attenuate NCX activity in VT-AMs. (**A**) Representative normalized caffeine induced Ca^{2+} transients before and 20 min after stimulation with AngII under Ctrl conditions (black), in presence of gp91ds-tat (1 μM, grey) and FTY720 (200 nM, light-grey). Bar graphs show the (**B**) caffeine transient decay constant and (**C**) caffeine transient amplitude under Ctrl conditions and in presence of gp91ds-tat or FTY720. (*: $p < 0.05$ compared to Ctrl).