SUPPORTING INFORMATION

Coupling of Smoothened to inhibitory G proteins reduces voltage-gated K^+ currents in cardiomyocytes and prolongs the cardiac action potential duration

Lan Cheng[1], Moza Al-Owais[2], Manuel L. Covarrubias[3], Walter J. Koch[4], David. R. Manning[5], Chris Peers[2], and Natalia A. Riobo-Del Galdo[1,6,*]

Index:

Fig. 1S

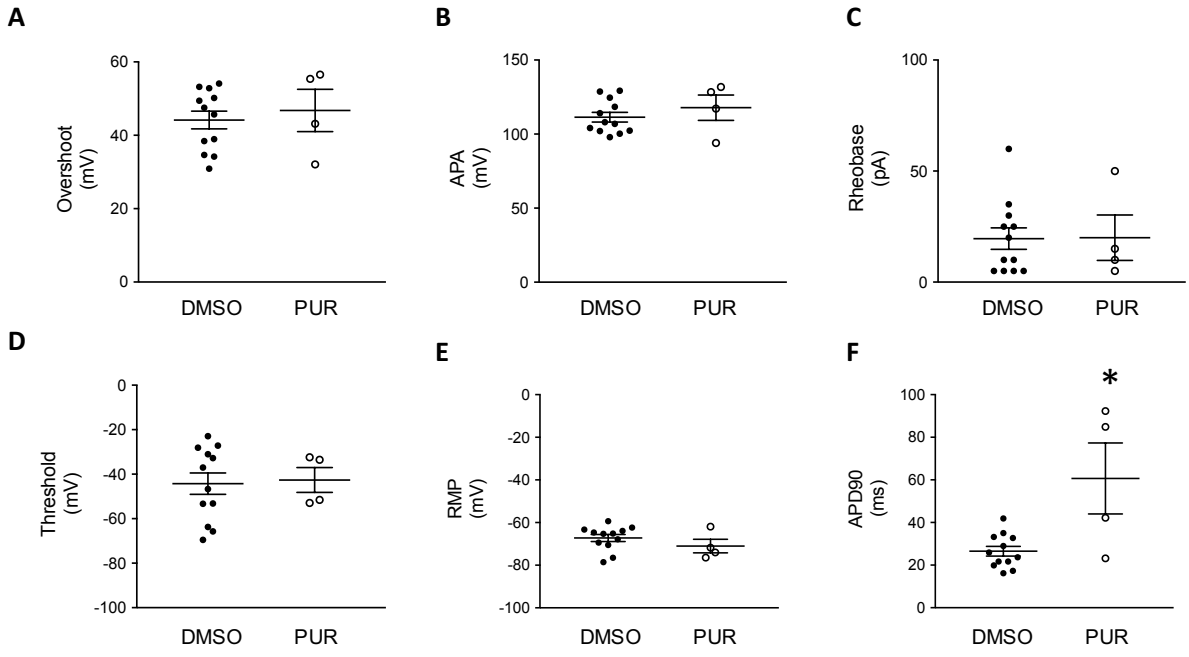

Fig. 1S. Action potential parameters in cardiomyocytes isolated from TTA mice after 10 min incubation with 0.1% DMSO or 5 μM purmorphamine (PUR). Averaged data at 1 Hz for overshoot (A), maximal AP amplitude (APA) (B), rheobase (C), threshold (D), resting membrane potential (RMP) (E), and AP duration (APD) at 90% repolarization (APD90) (F). Data represent the mean ± SEM (n=4-9). * $P<0.05$

Fig. 2S

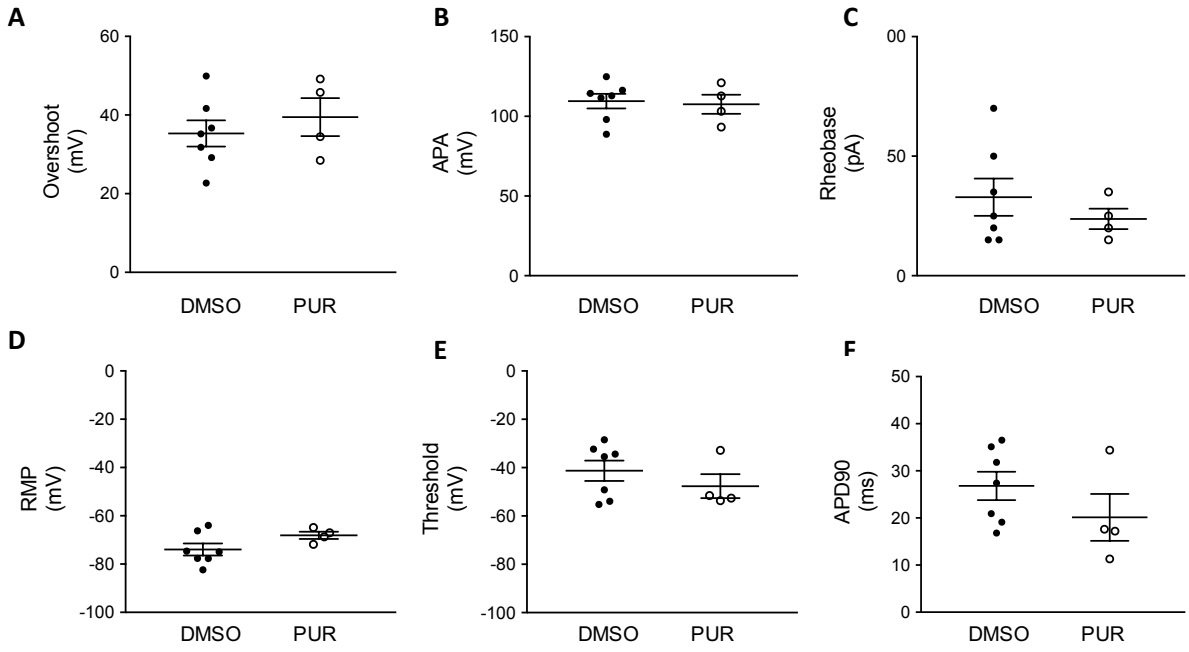

Fig. 2S. Action potential parameters in cardiomyocytes isolated from GiCT/TTA mice after 10 min incubation with 0.1% DMSO or 5 μM purmorphamine (PUR). Averaged data at 1 Hz for overshoot (A), maximal AP amplitude (APA) (B), rheobase (C), threshold (D), resting membrane potential (RMP) (E), and AP duration (APD) at 90% repolarization (APD90) (F). Data represent the mean ± SEM (*n*=4-9).

Fig. 3S

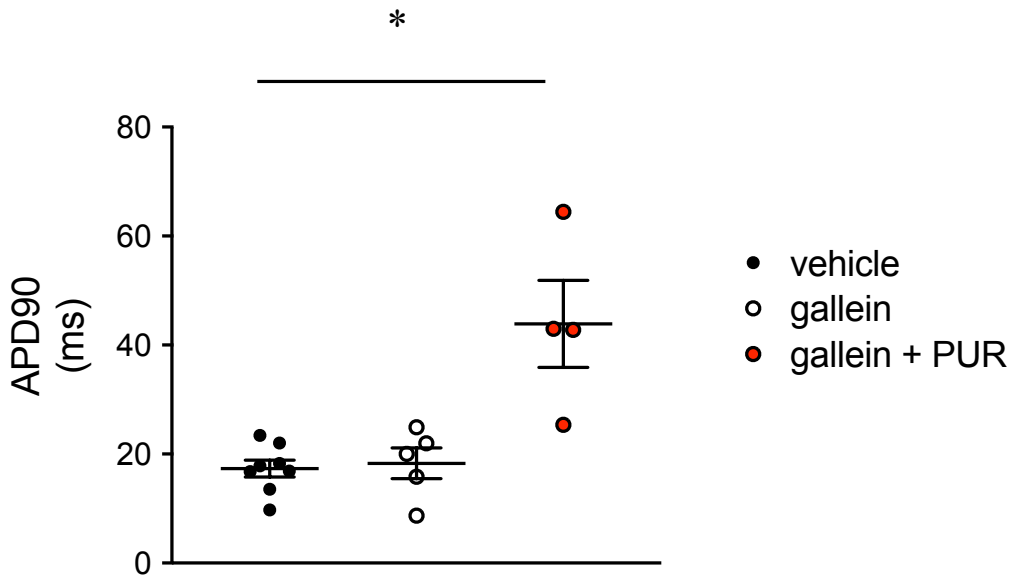

Fig. 3S. Action potential duration at 90% of action potential amplitude (APD90) of cardiomyocytes pre-treated with 50 μM gallein for 10 min before addition of vehicle or 5 μM purmorphamine (PUR) for 5 min. * P<0.001 (*n*=4-9).

Fig. 4S

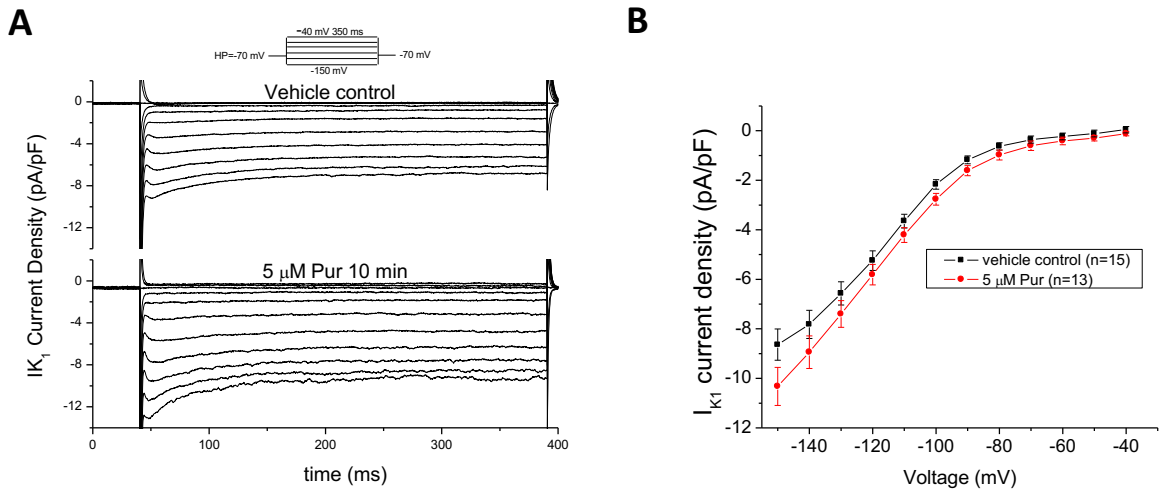

Fig. 4S. A. Representative whole-cell inwardly rectifying K^+ currents, recorded at room temperature from vehicle control (DMSO) and 5 μM purmorphamine (PUR)-treated ventricular myocytes in response to 350 ms depolarizing voltage steps to test potentials between −150 mV and -40 mV from a holding potential of −70 mV. Recorded currents were normalized for differences in cell size (whole-cell membrane capacitance) to obtain current densities. B. Peak I_{K1} current densities as a function of voltage in vehicle-treated (black trace) vs. PUR-treated cardiomyocytes (red trace). Data are expressed as mean ± SEM, *n* = 13-15.

Fig. 5S

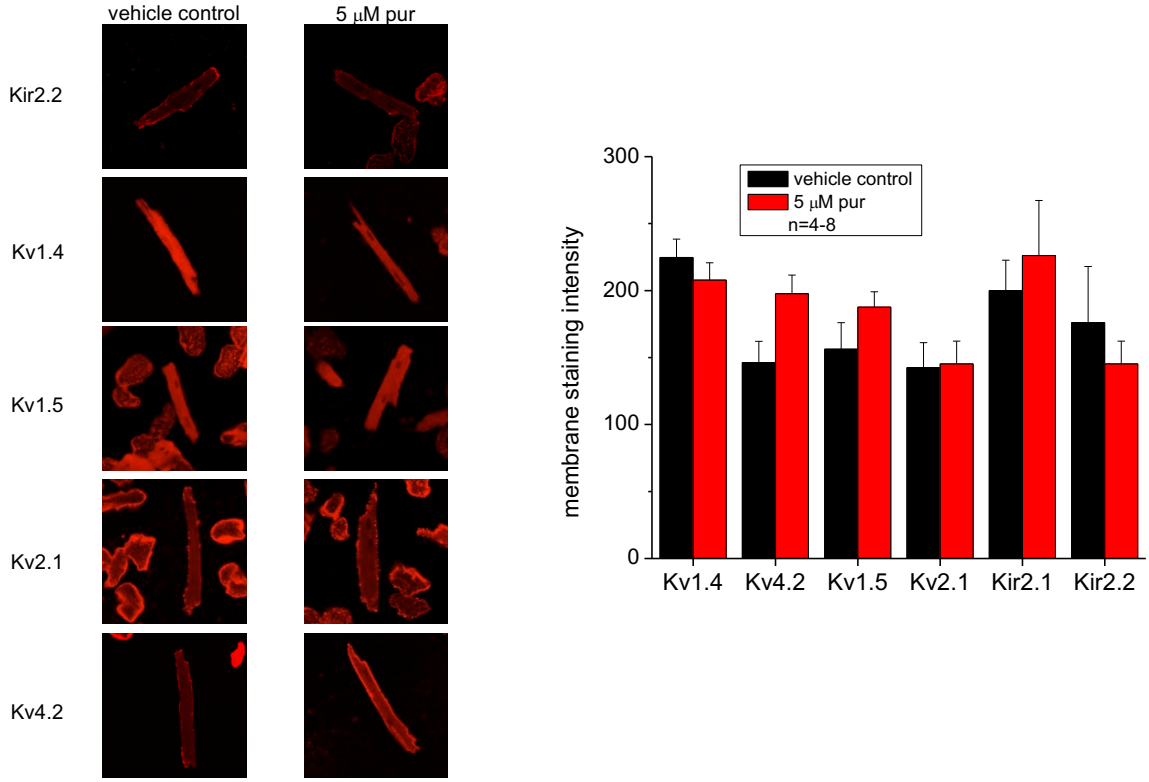

Fig. 5S. Immunofluorescent staining of indicated Kv channel subunits in fixed cardiomyocytes isolated from TTA or GiCT/TTA mice after 15 min incubation with 0.1% DMSO or 5 μM purmorphamine (PUR). Digital quantification of the Kv channel staining intensity at the plasma membrane in cells treated with 0.1% DMSO (vehicle, black bars) or 5 μM purmorphamine (PUR, red bars).

Fig. 6S

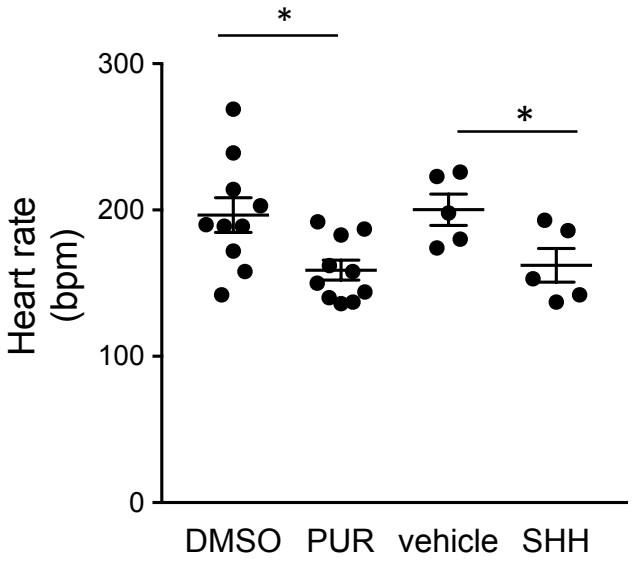

Fig. 6S. Spontaneous heart rate (HR) in isolated hearts from wild-type mice perfused with Tyrode's solution containing 0.1% DMSO (DMSO), 5 μM purmorphamine (PUR), 20% DMEM (vehicle) or Shh in 20% DMEM (Shh) for 20 min. Data represent mean ± SEM. One-tail t-test, * $P<0.05$ DMSO vs. PUR (n=10-12) and vehicle vs. Shh (n=5).

Fig. 7S

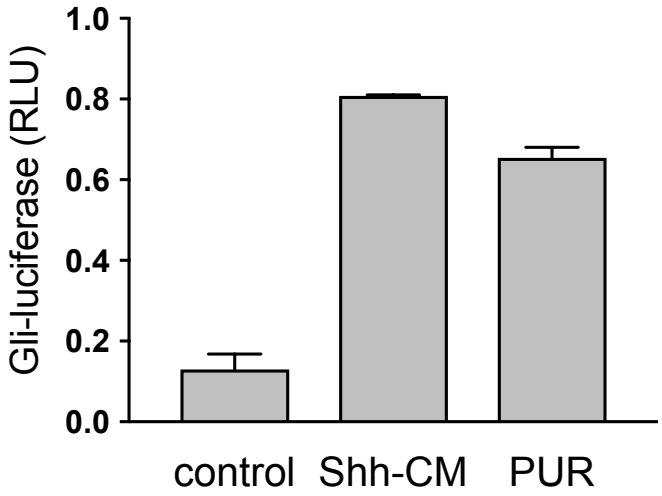

Fig. 7S. NIH 3T3 cells were transfected with 8XGli-*Firefly* luc and pTK-*Renilla* luc vectors as previously described (3). After 48 h, when cells were confluent, the growth medium was changed to DMEM with 0.5% FBS alone (control), Shh-conditioned medium (Shh-CM) at 1:5 dilution, or 5 μM purmorphamine (PUR). Cells were lysed 24 h later and the Gli-luciferase activity (ratio of Firefly/Renilla luminescence) was measured on a T20/20 luminometer. Data represent mean ± SEM.

Table S1

Table S1. Baseline ECG analysis of hearts from TTA and GiCT/TTA mice.

Parameter	TTA *(n=8)*	GiCT/TTA *(n=5)*
HR (rpm)	193 ± 14	152 ± 12
PR interval (ms)	53.1± 5.9	59.7± 4.9
QT interval (ms)	22.4 ± 3.0	24.9 ± 5.0

Hearts from TTA or GiCT/TTA mice were perfused for 20 min in Tyrode's solution. Volume-conducted ECG was recorded and analysed as described in Materials and Methods.