

SUPPLEMENTAL MATERIAL

PDE8 governs cAMP/PKA-dependent reduction of L-type calcium current in human atrial fibrillation

Nefeli Grammatika Pavlidou, Shokoufeh Dobrev, Kira Beneke, Franziska Reinhardt,
Simon Pecha, Eric Jacquet, Issam H. Abu-Taha, Constanze Schmidt, Niels Voigt,
Markus Kamler, Renate B. Schnabel, Istvan Baczkó, Anne Garnier, Hermann
Reichenspurner, Viacheslav O. Nikolaev, Dobromir Dobrev, Cristina E. Molina

SUPPLEMENTARY FIGURES

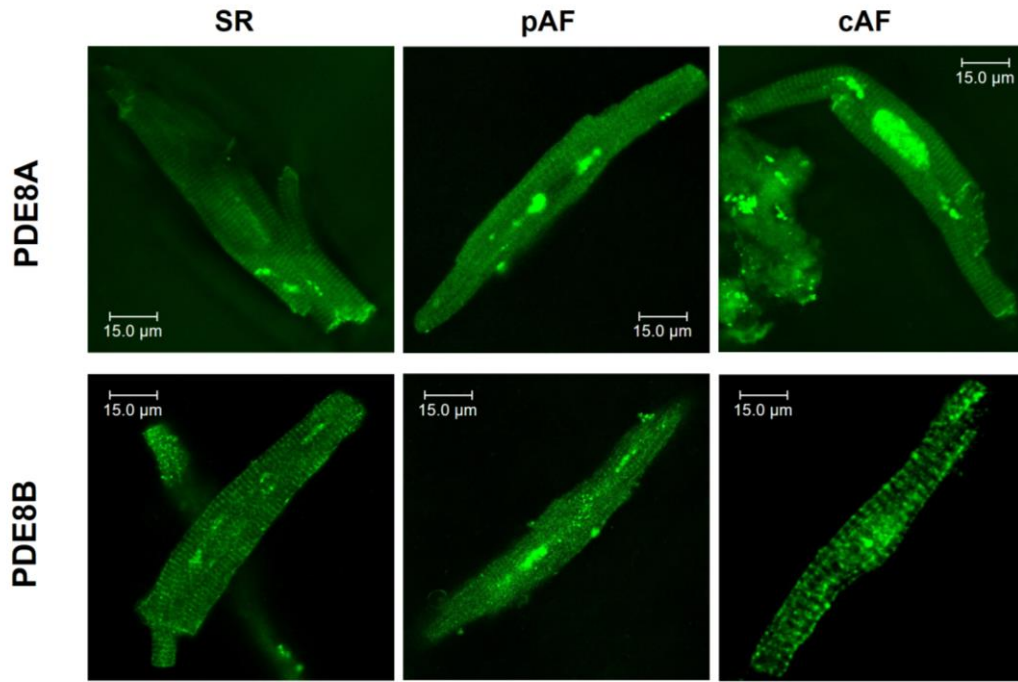


Figure S1. PDE8 localization in human atrial myocytes. Representative immunocytochemistry confocal images of PDE8A (top) showing its cytosolic distribution in isolated human atrial myocytes (HAMs) from sinus rhythm (SR), paroxysmal atrial fibrillation (pAF) and persistent (chronic) atrial fibrillation (cAF) patients (from left to right). Lower pictures show similar representative immunocytochemistry confocal images of PDE8B localization mainly at the plasma membrane, in HAMs from SR, pAF and cAF patients (from left to right). Gain was increased in order to obtain a better signal in images where the matched intensity was not sufficient to compare PDE8 isoform localizations.

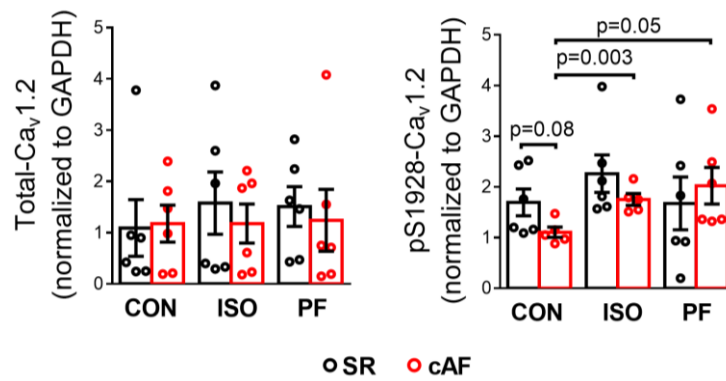


Figure S2. LTCC protein expression. Western-blot quantification of the protein expression of total-Ca_v1.2 (left, mean±SEM) and PKA phosphorylated pSer1928-Ca_v1.2 (right, mean±SEM) in atrial tissue homogenates from 6 SR and 6 cAF patients, at baseline and after 5 min stimulation with the selective PDE8 inhibitor PF-04957325 (30 nM) or the β-adrenoceptor agonist isoprenaline (ISO, 100 nM). GAPDH was used as loading control. *p<0.05 based on ANOVA with a Kruskal–Wallis test.

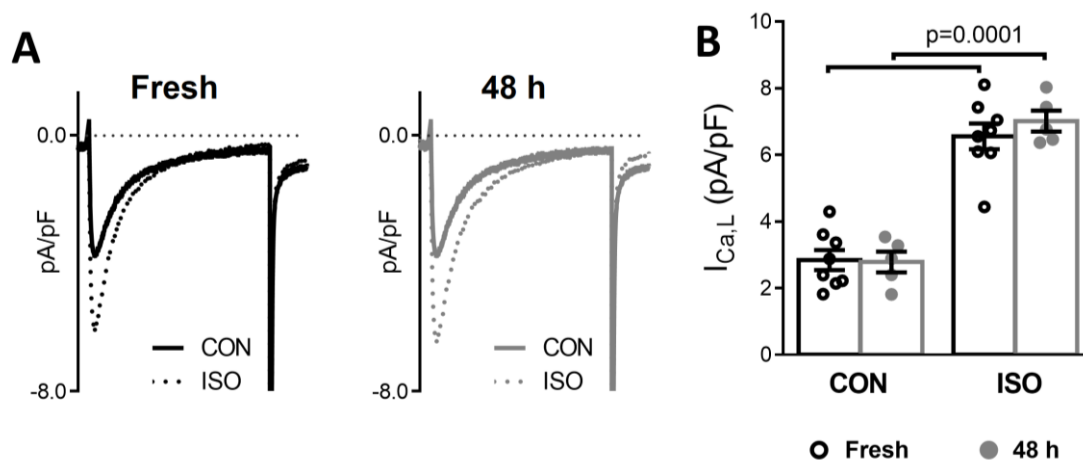


Figure S3. Functional characterization of human atrial myocytes in culture. From left to right: Representative L-type calcium current ($I_{Ca,L}$) patch-clamp recordings in freshly isolated (2 hours of culture) and after 48 hours of culture human atrial cardiomyocytes (HAMs). Quantification of $I_{Ca,L}$ density in cardiomyocytes from patients in sinus rhythm before (2 h) and after 48 hours of culture (48 h), at baseline (CON) and upon β -adrenoceptor stimulation with 100 nM isoprenaline (ISO). Individual and mean values of $I_{Ca,L}$ density in HAMs. # $p<0.05$ compared to CON based on ANOVA.

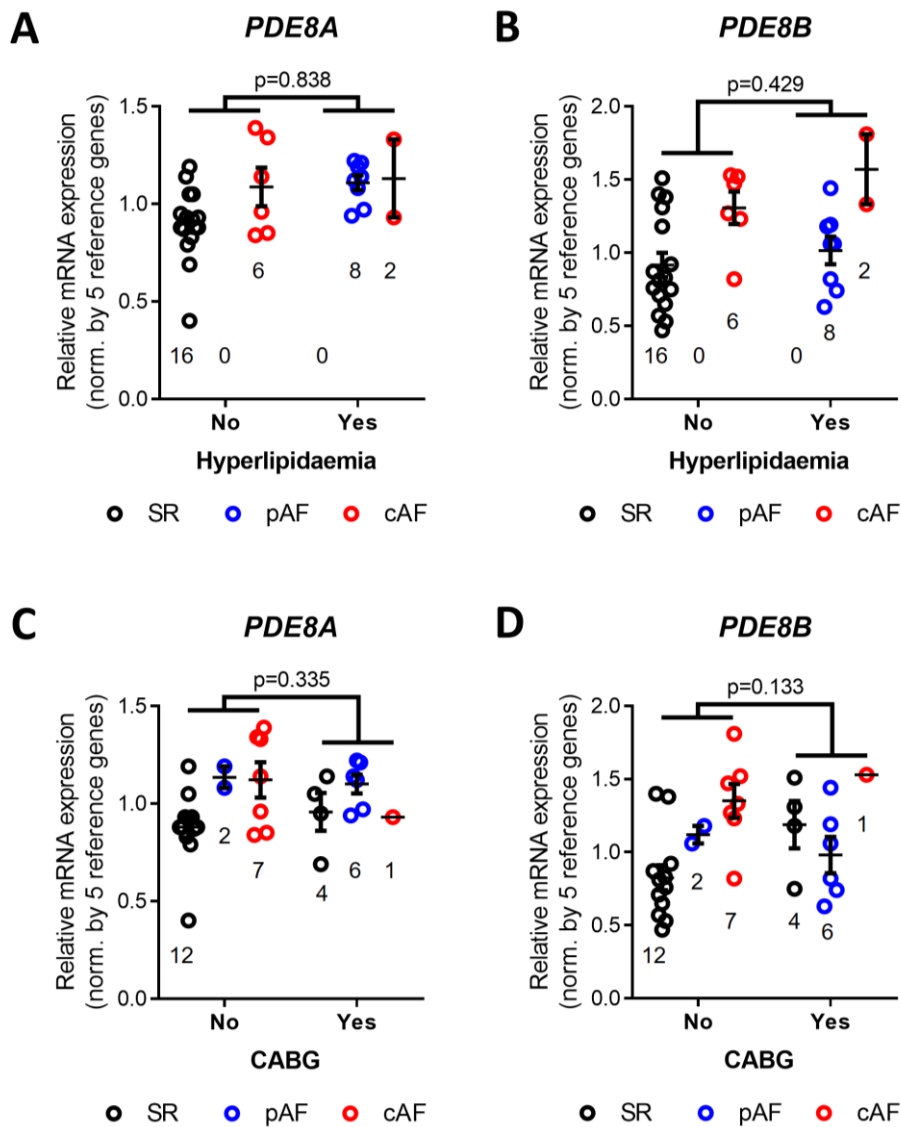


Figure S4. Relationship between hyperlipidaemia or cardiopulmonary bypass surgery and *PDE8A* or *PDE8B* mRNA expression. A-D, Two-way ANOVA analysis of atrial fibrillation rhythm (paroxysmal or persistent-chronic, pAF or cAF) status and hyperlipidaemia or bypass surgery (no vs. yes) for *PDE8A* (panels A and C) and *PDE8B* (panels B and D) mRNA expression levels. P values reflect significance level of the factor “hyperlipidaemia” or “bypass surgery” in two-way ANOVA. Numbers below symbols indicate number of patients. [Figure related to Supplementary Table 2.](#) Note that individual subgroups may be small, limiting the statistical power and robustness of these subanalyses.

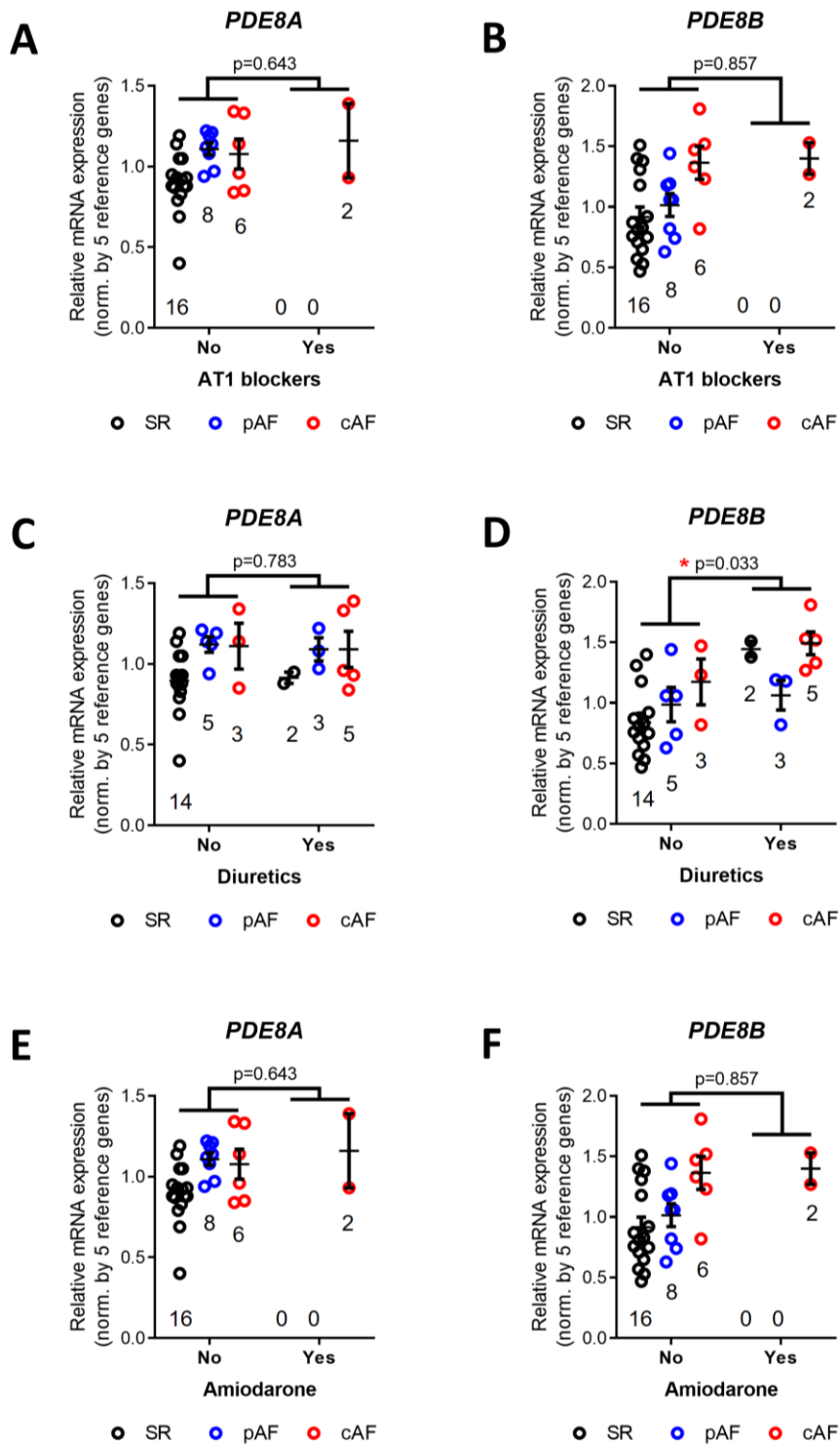


Figure S5. Relationship between angiotensin (AT) 1 blockers or diuretics or amiodarone use and *PDE8A* or *PDE8B* mRNA expression. A-F, Two-way ANOVA analysis of atrial fibrillation rhythm (paroxysmal or persistent-chronic, pAF or cAF) status and AT1 blockers or diuretics or amiodarone use (no vs. yes) for *PDE8A* (panels

A, **C** and **E**) and *PDE8B* (panels **B**, **D** and **F**) mRNA expression levels. P values reflect significance level of the factor “AT1 blockers” or “diuretics” or “amiodarone” in two-way ANOVA. Numbers below symbols indicate number of patients. **Figure related to Supplementary Table 2.** Note that individual subgroups may be small, limiting the statistical power and robustness of these subanalyses.

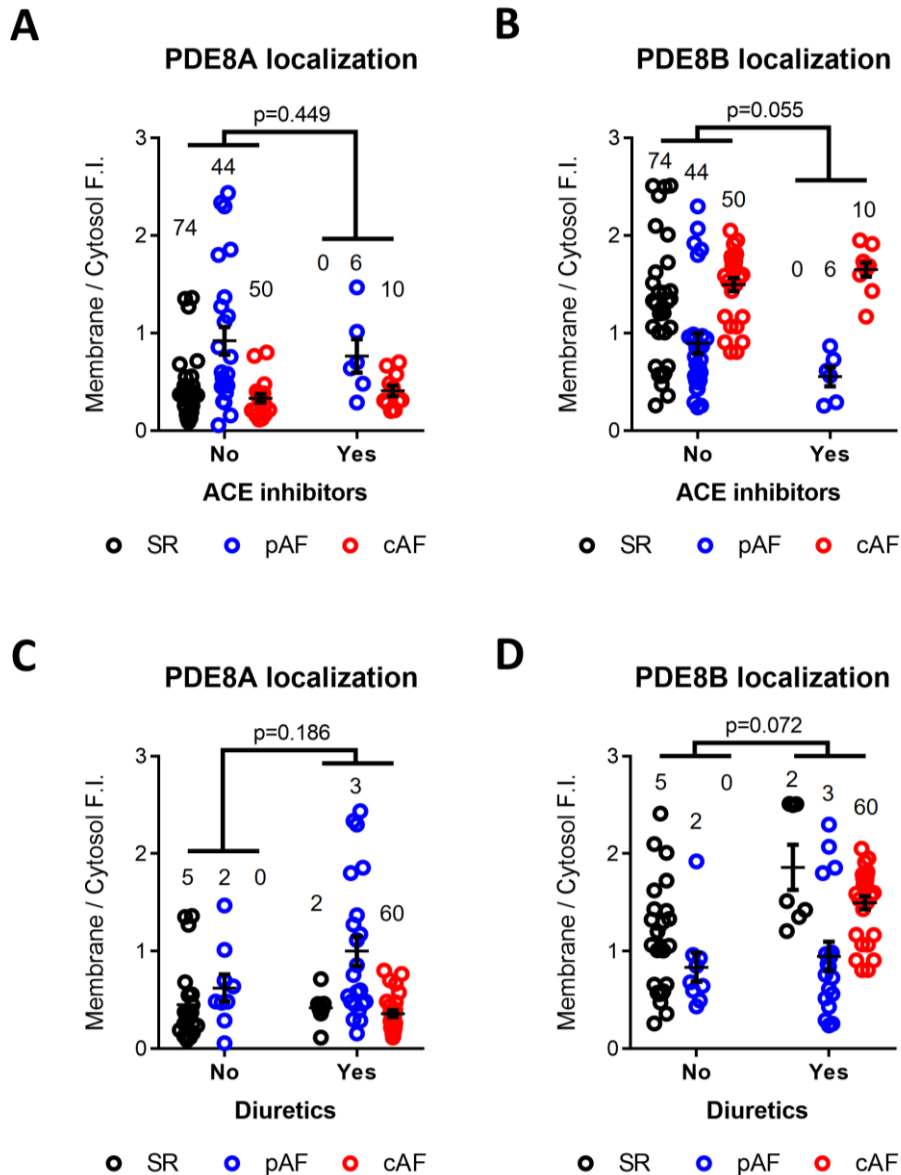


Figure S6. Relationship between angiotensin-converting enzyme (ACE) inhibitors or diuretics use and PDE8A or PDE8B localization. A-D, Two-way ANOVA analysis of atrial fibrillation rhythm (paroxysmal or persistent-chronic, pAF or cAF) status and ACE inhibitors or diuretics use (no vs. yes) for PDE8A (panels **A** and **C**) and PDE8B (panels **B** and **D**) localization. P values reflect significance level of the factor “ACE inhibitors” or “diuretics” in two-way ANOVA. Numbers above symbols indicate number of cells. **Figure related to Supplementary Table 5.** Note that individual subgroups may be small, limiting the statistical power and robustness of these subanalyses.

cAMP increase upon PDE8 inhibition

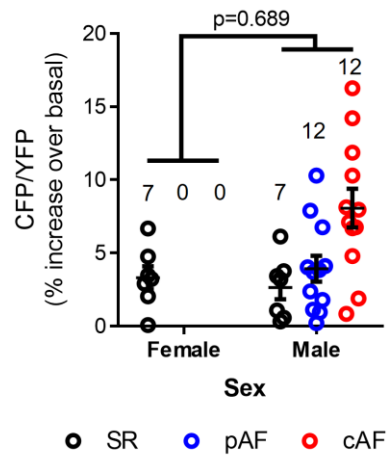


Figure S7. Relationship between sex and the increase in cAMP levels upon PDE8 inhibition. Two-way ANOVA analysis of atrial fibrillation rhythm (paroxysmal or persistent-chronic, pAF or cAF) status and sex (Female vs. Male) for the increase in cAMP levels upon PDE8 inhibition. P values reflect significance level of the factor “sex” in two-way ANOVA. Numbers above symbols indicate number of cells. **Figure related to Supplementary Table 6.** Note that individual subgroups may be small, limiting the statistical power and robustness of these subanalyses.

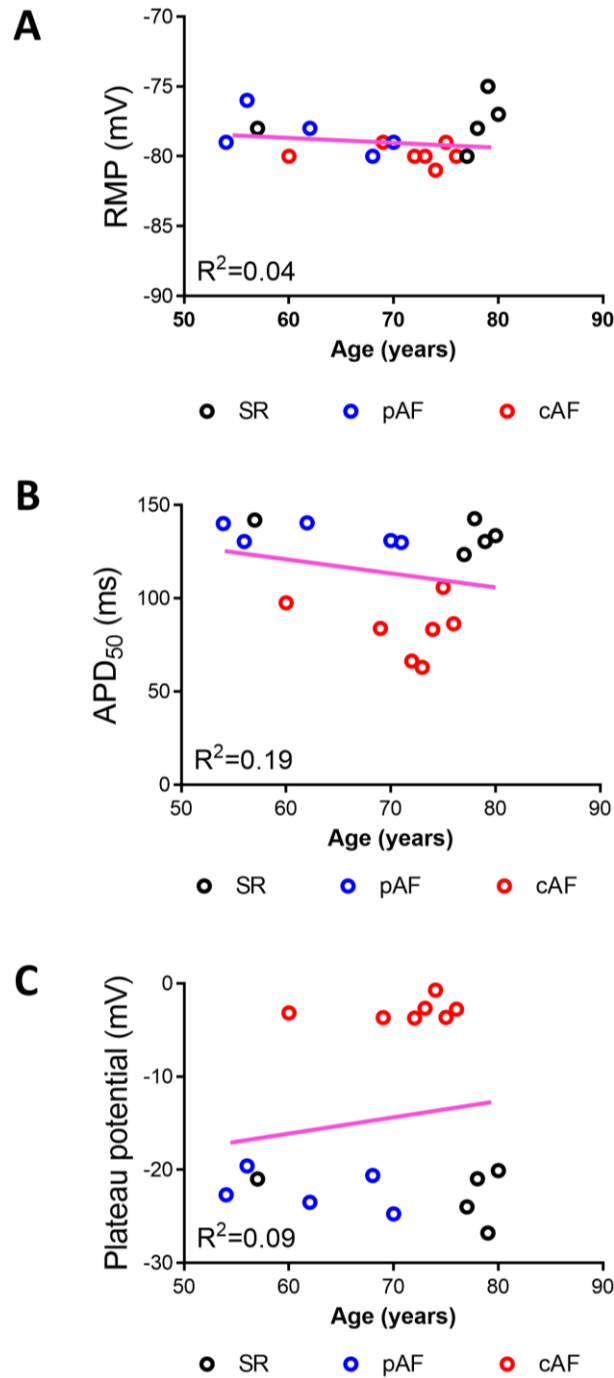


Figure S8. Age and rhythm dependency of the resting membrane potential (RMP) or the action potential duration at 50% of repolarization (APD₅₀) or the plateau potential parameters in human atrial trabeculae. Correlation between parameter value in sinus rhythm or paroxysmal or persistent-chronic (SR or pAF or cAF) and age, with corresponding correlation coefficients (R^2). **A**, RMP. **B**, APD₅₀. **C**, Plateau potential.

Figure related to Supplementary Table 8.