

Fitzwilliam Seibert^{1,2}, Markus Rapedius³, Funsho Fakuade^{1,2}, Philipp Tomsits^{4,5,6}, Lukas Cyganek^{2,7}, Nadine Becker³, Sebastian Clauß^{4,5,6}, Niels Fertig³, Niels Voigt^{1,2,8}

¹Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Georg-August University Göttingen, Germany. ²DZHK (German Center for Cardiovascular Research), partner site Göttingen, Germany. ³Nanon Technologies GmbH, Munich, Germany. ⁴Department of Medicine I, University Hospital, LMU Munich, Munich, Germany. ⁵Institute of Surgical Research at the Walter-Brendel-Centre of Experimental Medicine, University Hospital, LMU Munich, Munich, Germany. ⁶DZHK (German Centre for Cardiovascular Research), Partner Site Munich, Munich Heart Alliance (MHA), Munich, Germany. ⁷Stem Cell Unit, Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Georg-August University Göttingen, Germany. ⁸Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Georg-August University Göttingen, Germany

BACKGROUND

Automated patch-clamp (APC) technology is emerging as a promising method to increase the throughput of functional electrophysiological measurements. Typical APC devices use microfluidic cell handling channels, which has historically limited this method to the study of smaller cellular constructs such as expression systems.

The newly-designed APC device, Syncropatch 384 (Nanon Technologies), operates using a fixed well mechanism, with no microfluidics present. We aim to test this device using induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) and primary atrial cardiomyocytes (CM) to assess the extent to which APC can contribute in the future study of atrial fibrillation associated remodelling. This could be essential for potential therapeutic strategy development, which to date remains substantially scarce.



Figure 1. Automated Patch Clamp (APC) overview. A, Traditional patch clamp (upper) and fixed-well APC (lower). B, Syncropatch 384. C, Consumable 384-well chip (upper) with a software screenshot during an example experiment with a representative trace highlighted (lower).

RESULTS

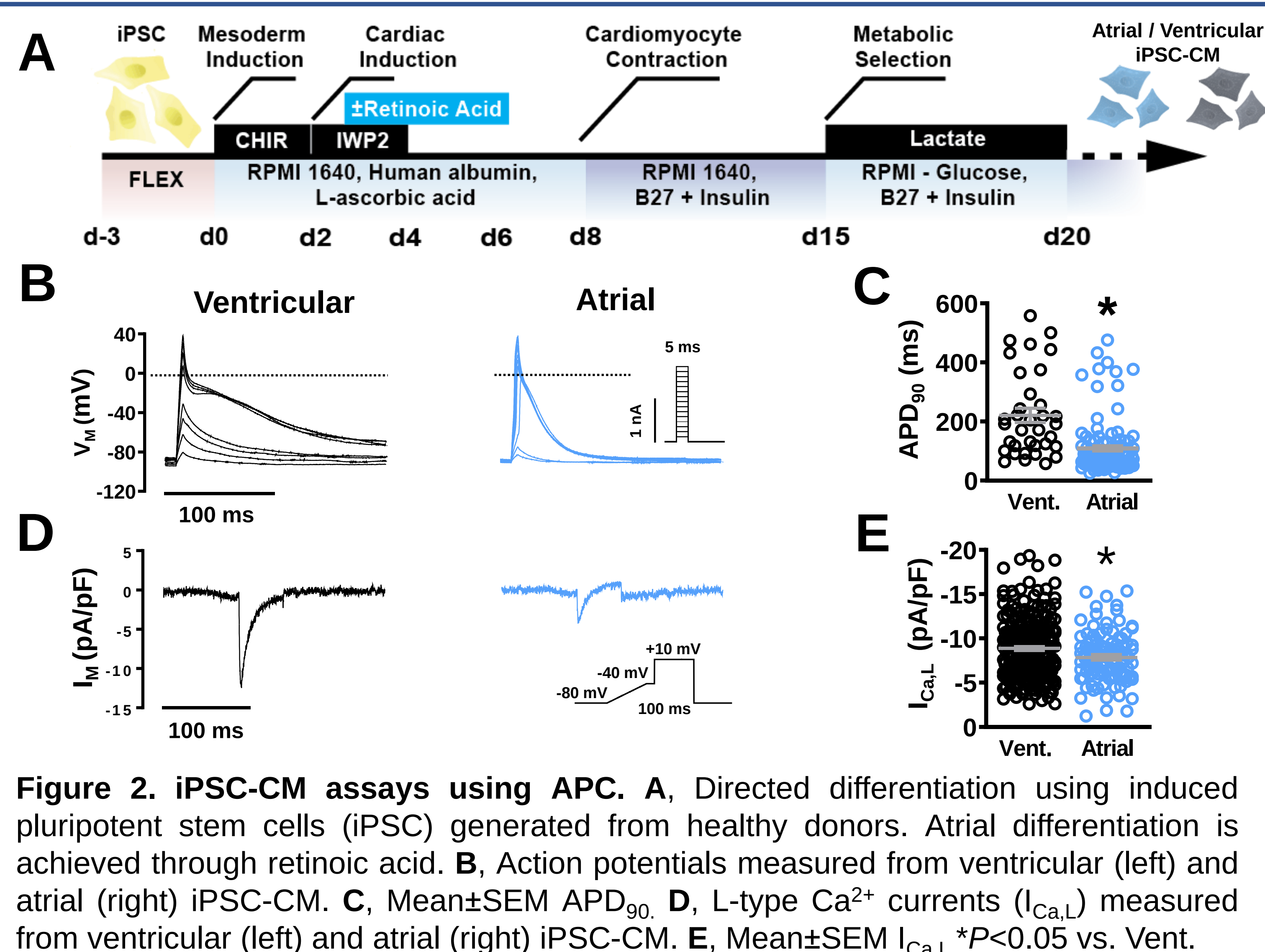


Figure 2. iPSC-CM assays using APC. A, Directed differentiation using induced pluripotent stem cells (iPSC) generated from healthy donors. Atrial differentiation is achieved through retinoic acid. B, Action potentials measured from ventricular (left) and atrial (right) iPSC-CM. C, Mean±SEM APD₉₀. D, L-type Ca²⁺ currents (I_{CaL}) measured from ventricular (left) and atrial (right) iPSC-CM. E, Mean±SEM I_{CaL}. *P<0.05 vs. Vent.

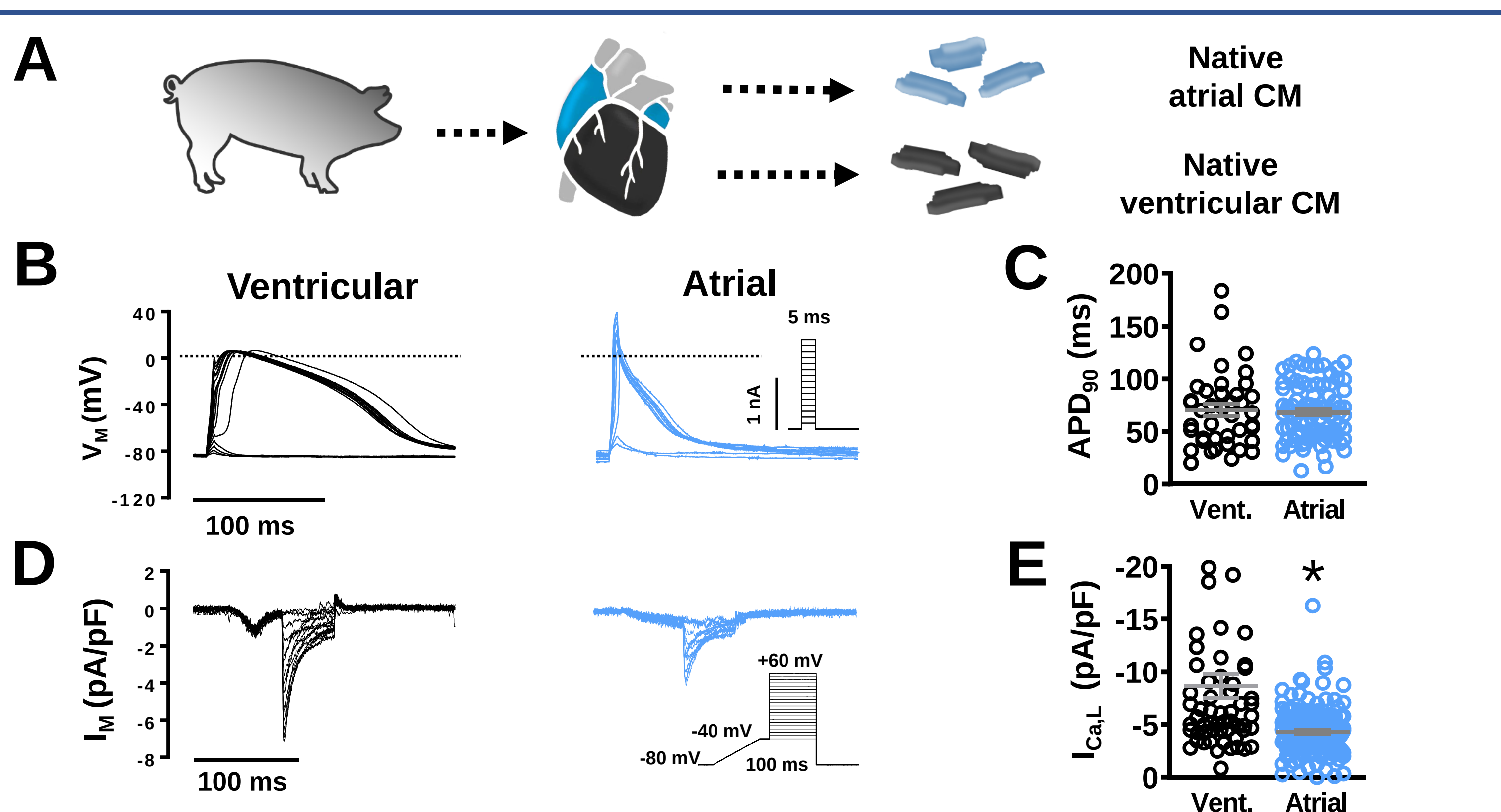


Figure 3. Native CM assays using APC. A, CM origin and isolation. B, Action potentials measured from ventricular (left) and atrial (right) CM. C, Mean±SEM APD₉₀. D, I_{CaL} measured from ventricular (left) and atrial (right) CM. E, Mean±SEM I_{CaL}. *P<0.05 vs. Vent.

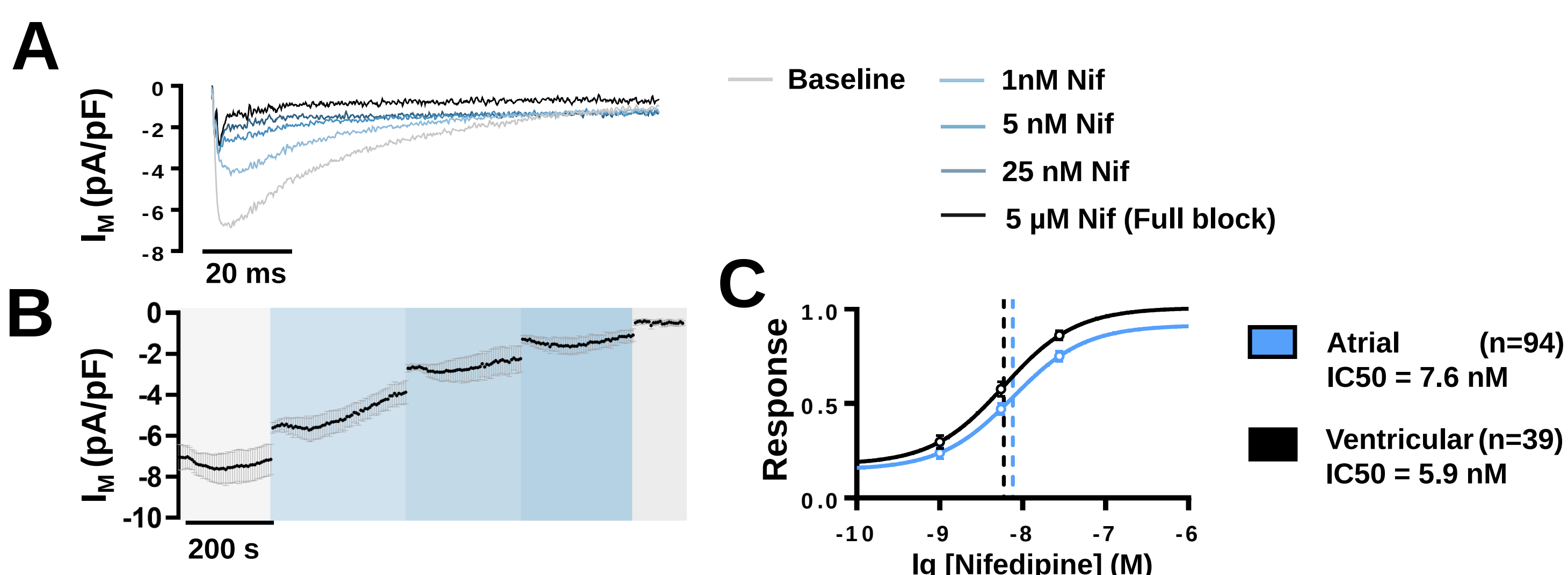


Figure 4. Pharmacological modulation of I_{CaL} in native CM. A, Representative atrial I_{CaL} with increasing Nifedipine (Nif) concentrations. B, Time course of a single atrial APC plate (n=10). C, Normalised concentration response curve. n=Number of CM.

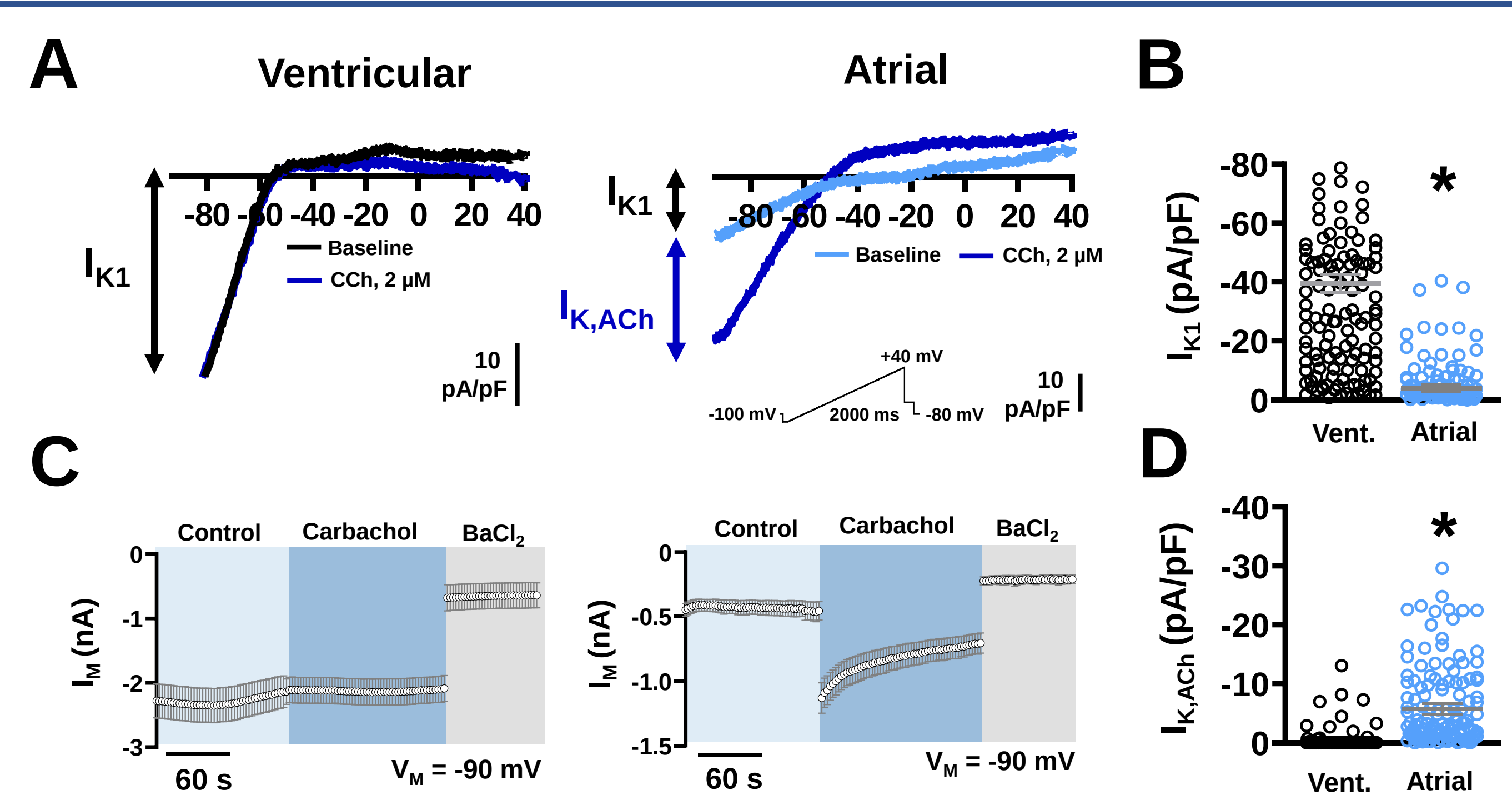


Figure 5. Native CM inward rectifier assays using APC. A, Inward rectifier currents in ventricular (left) and atrial (right) CM before and after Carbachol (CCh) application. A current increase after CCh indicates the presence of acetylcholine activated inward rectifiers (I_{K,ACh}). B, Mean±SEM inward component of I_{K1}. C, Time course of a single APC plate for both ventricular (left; n=11) and atrial (right; n=10) CM measured at -90mV. D, Mean±SEM inward component of I_{K,ACh}. *P<0.05 vs. Vent.

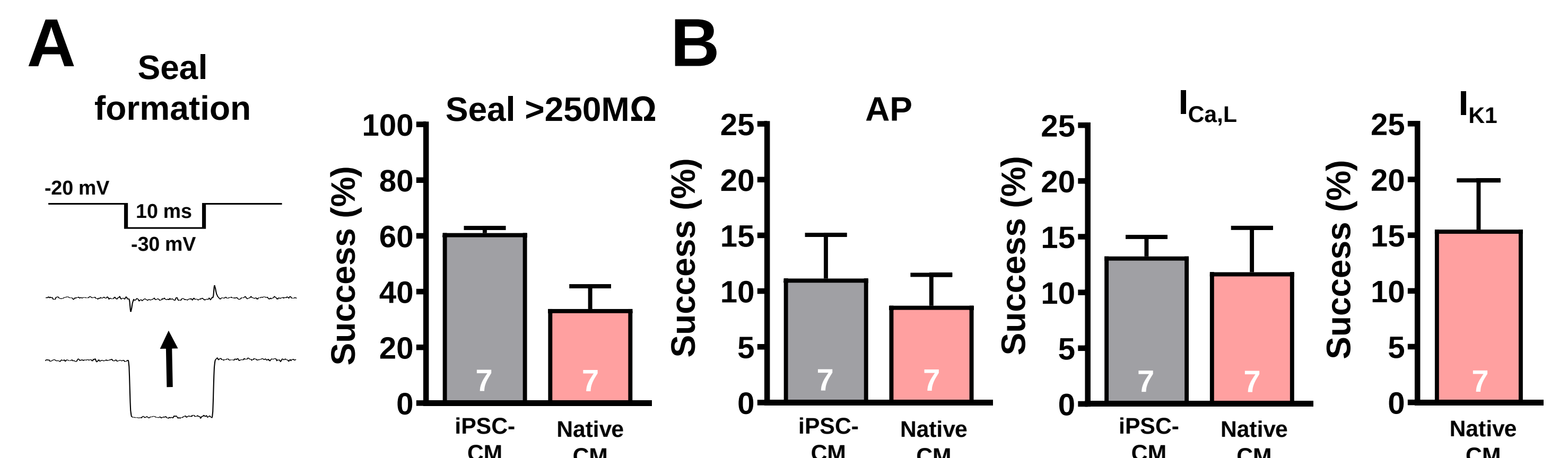


Figure 6. Preliminary success rates. A, Seal formation example (left) and percentage of cells with a successful seal per APC run in iPSC-CM and native CM (right). B, Absolute success rate of robust recording detection for action potential (AP; left), I_{CaL} (middle) and I_{K1} (right) per APC run. n=Number of APC runs.

BREAKING NEWS APRIL 2022

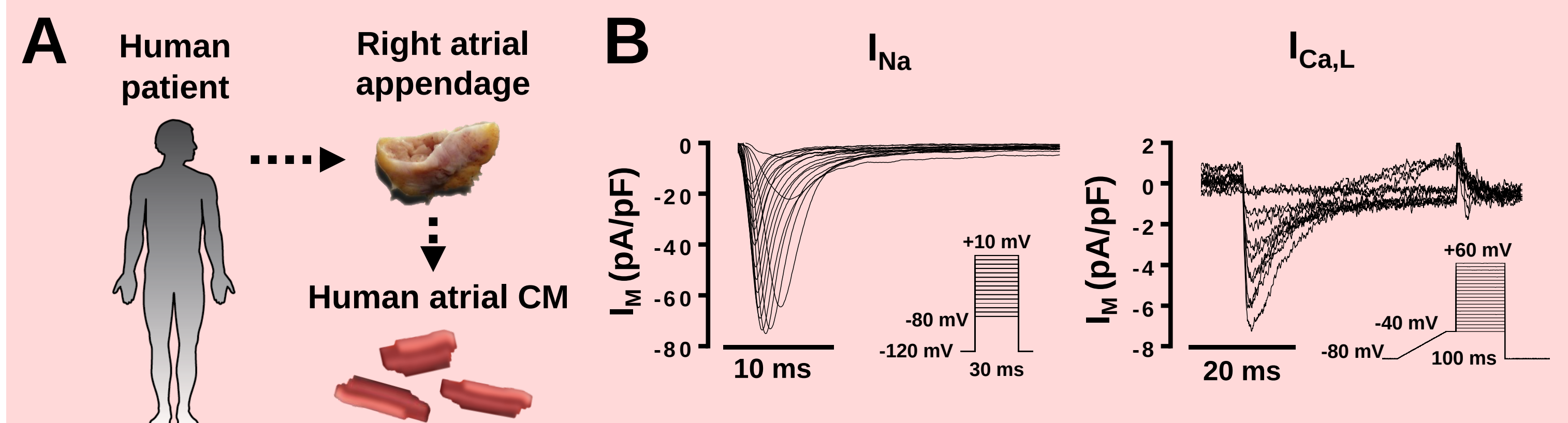


Figure 7. Successful recording of human atrial CM using APC. A, Isolated atrial CM from a right atrial appendage procured during open heart surgery. B, Representative peak sodium current (I_{Na}; left) and I_{CaL} (right) recording using APC.

CONCLUSION AND OUTLOOK

- For the first time, APC has successfully recorded currents and action potentials from native cardiomyocyte material.
- Sensitive discrimination of typical atrial phenotypes shows promise for future studies of atrial fibrillation.
- Optimisation of overall success rates will further increase throughput.