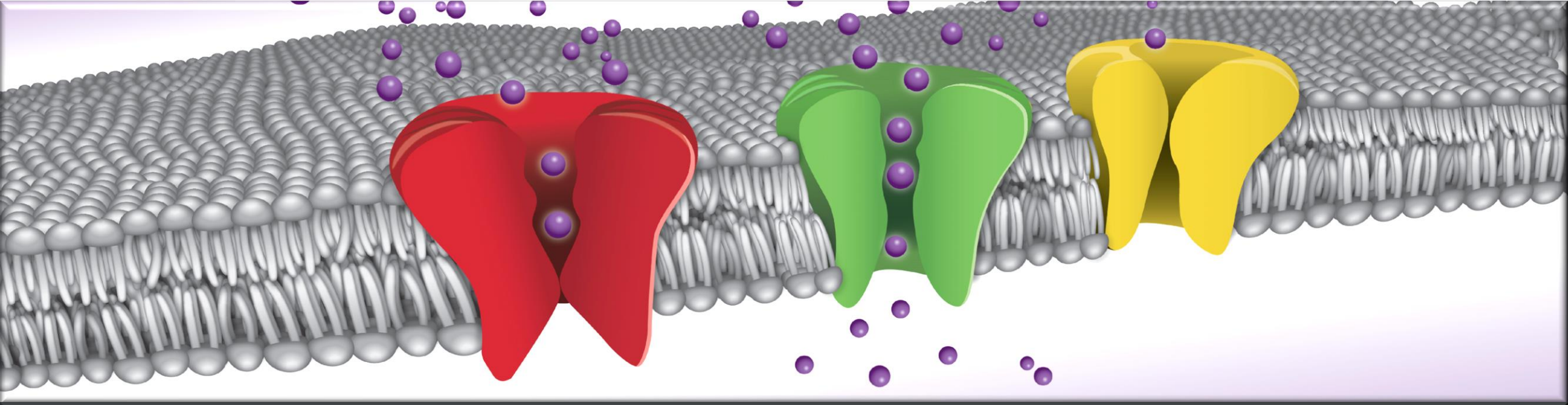

# Metrion Biosciences: the ion channel specialists

## Utility of human iPSC-derived cardiomyocytes for preclinical safety assays and disease modelling

Sarah Williams, PhD

23 May 2018

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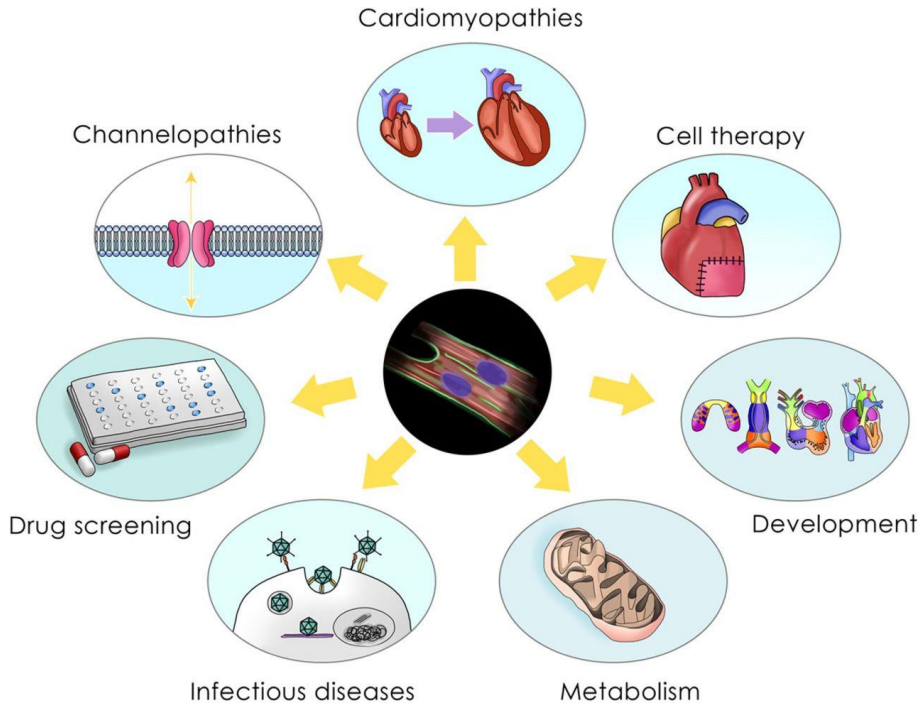
# Applications of human iPSC-derived cardiomyocytes



- Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) can be differentiated from healthy or diseased donors

- Many applications of iPSC-CM including:
  - Study of channelopathies e.g. long QT syndromes
  - Development of patient-specific pharmacology
  - Stem cell therapies
  - Drug screening and preclinical safety assessment

- To successfully utilise iPSC-CM as a model system they need thorough functional characterisation




Karakikes *et al.*, Circulation Research. 2015;117:80-88

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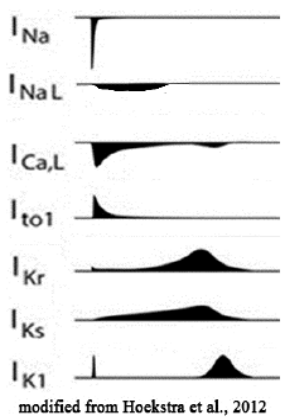
# Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative



- CiPA initiative is a regulatory proposal sponsored by USA, European, and Japanese safety bodies to augment current cardiac safety testing regimes
- The scientific proposal for CiPA involves 'four pillars'

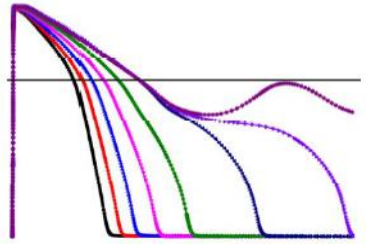
**1**




**Analysis of compound activity in a full panel of human cardiac ion channels**

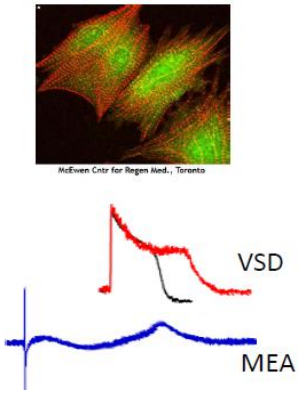
**2**

$$I_{stim} = C\frac{dV_m}{dt} + I_m$$



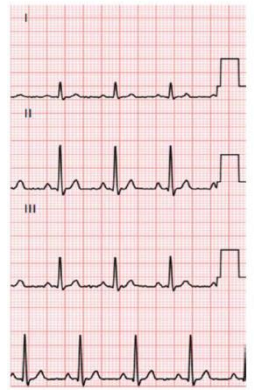
**Use *in vitro* data for *in silico* modelling to evaluate proarrhythmic risk**

**3**




**Confirm proarrythmia signals in human iPSC-derived cardiomyocytes**

**4**



**Clinical evaluation of unanticipated effects**

http://cipaproject.org

- Metrion have established assays for CiPA pillars 1 - 3

# Contents



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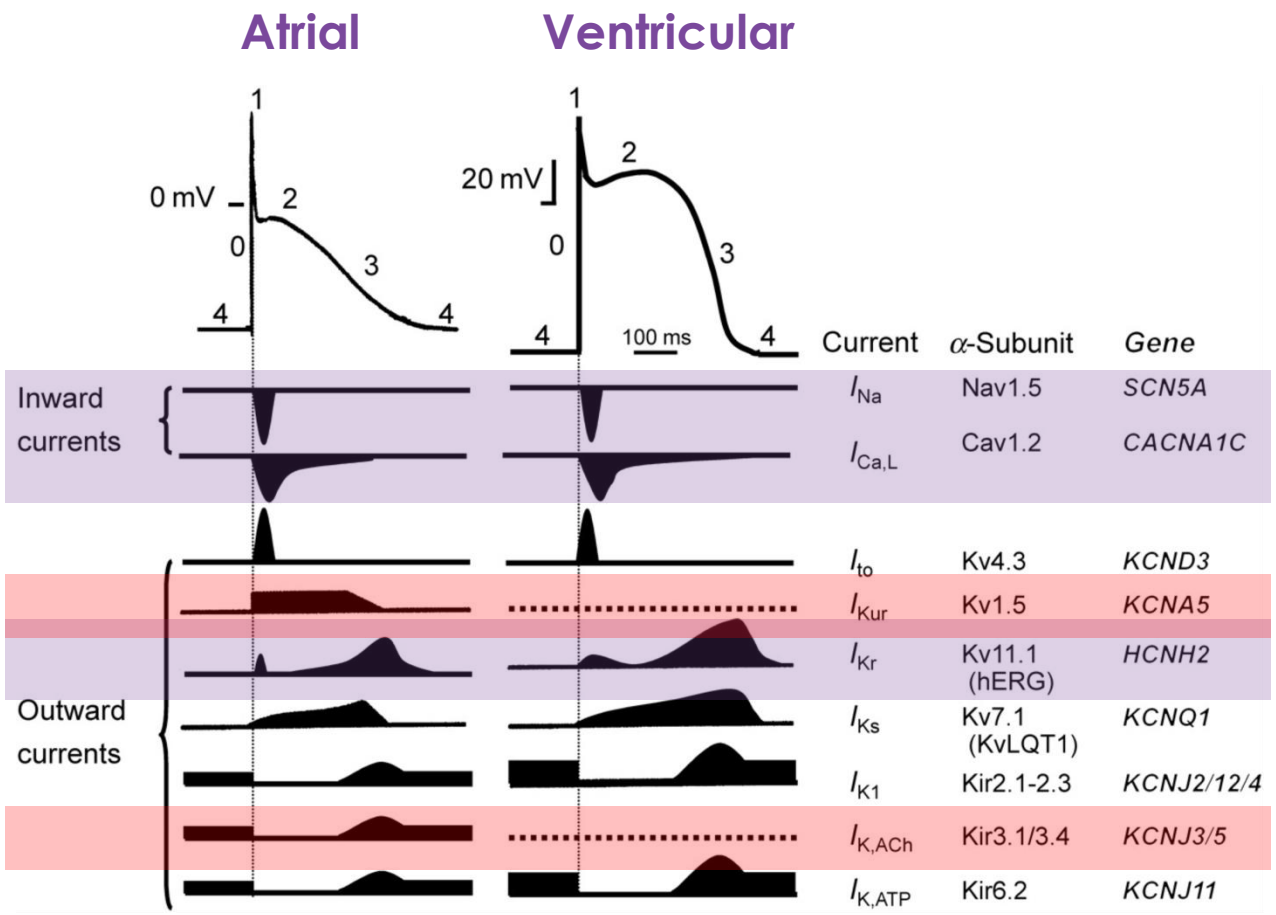
# Establishing Axol's iPSC-CM as model systems






Role of potassium currents in cardiac arrhythmias. Europace. 2008;10(10):1133-1137

- Electrophysiological characterisation of channel expression
  - Gold-standard manual patch clamp
- Three core cardiac currents
  - $I_{Na}$ ($Na_V1.5$)
  - $I_{Ca,L}$ ($Ca_V1.2$)
  - $I_{Kr}$ (hERG)
  - Present in both chambers and essential for cardiomyocyte action potentials
- Two chamber specific currents
  - $I_{KACh}$ ($K_{ir}3.1/K_{ir}3.4$)
  - $I_{Kur}$ ($K_V1.5$)
  - Functional expression only observed in human atrial cardiomyocytes

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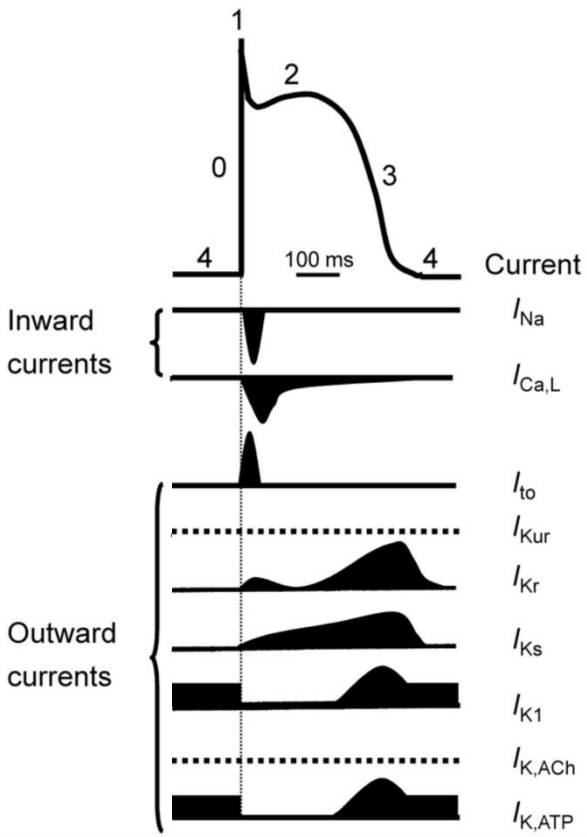
# Ventricular cardiomyocytes

## Ventricular




Europace. 2008;10(10):1133-1137

## Applications of ventricular iPSC-CM at Metrion

- Preclinical cardiac safety screening – CiPA
- Profiling compound effects in a translational human model system
- Disease modelling

## Aims of electrophysiological characterisation

1. Determine control properties of Axol ventricular iPSC-CM

2. Confirm the functional expression of three core cardiac currents
   - $I_{Na}$ ($Na_V1.5$), $I_{Ca,L}$ ($Ca_V1.2$), and $I_{Kr}$ (hERG)

3. Confirm the absence of two chamber specific currents
   - $I_{KACh}$ ($K_{ir}3.1/K_{ir}3.4$) and $I_{Kur}$ ($K_V1.5$)

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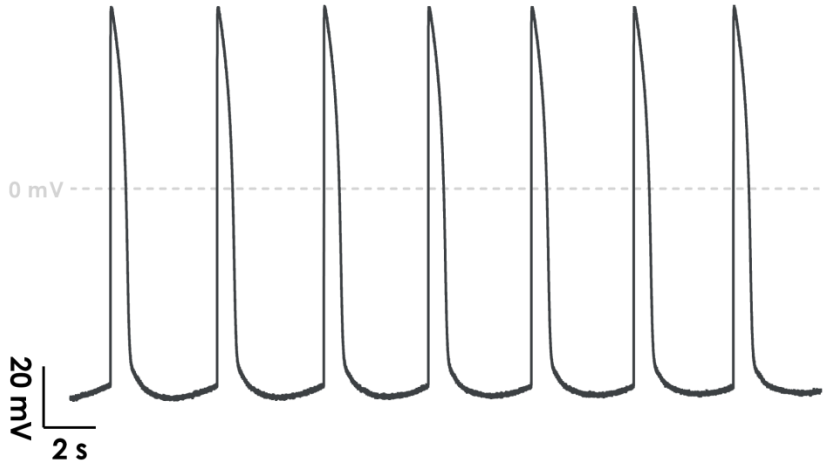
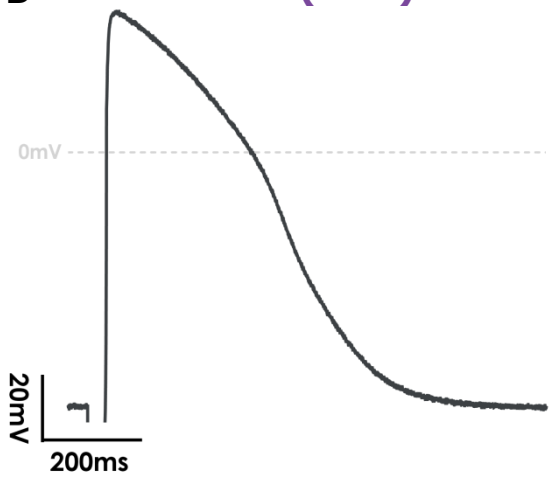
# Ventricular action potential characteristics



**A** Spontaneous



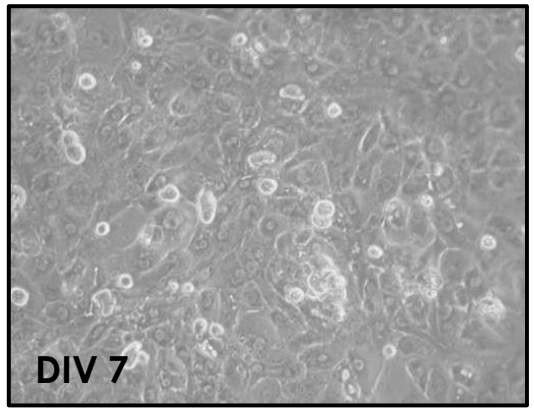

**B** Evoked (1 Hz)




**C**

| AP parameter | Spontaneous (N = 38) | Evoked 1 Hz (N = 31) |
|---|---|---|
| **MDP (mV)** | -66.2 ± 0.9 | -71.2 ± 1.9 |
| **$dV/dt_{max}$ (V/s)** | 24.0 ± 2.8 | 19.5 ± 3.6 |
| **APA (mV)** | 115.4 ± 1.4 | 118.9 ± 2.3 |
| **APD20 (ms)** | 274.3 ± 11.1 | 203.4 ± 7.0 |
| **APD50 (ms)** | 421.6 ± 14.9 | 366.0 ± 10.6 |
| **APD90 (ms)** | 532.7 ± 14.8 | 547.5 ± 12.1 |
| **Frequency (Hz)** | 0.26 ± 0.03 | 1 |

- Suitable for recording of spontaneous & evoked activity (0.2 – 1 Hz)
- Good maximum diastolic potential ~ -71 mV
- Upstroke velocity of ~ 20 V/s
- Consistent APD90 ~ 550 ms

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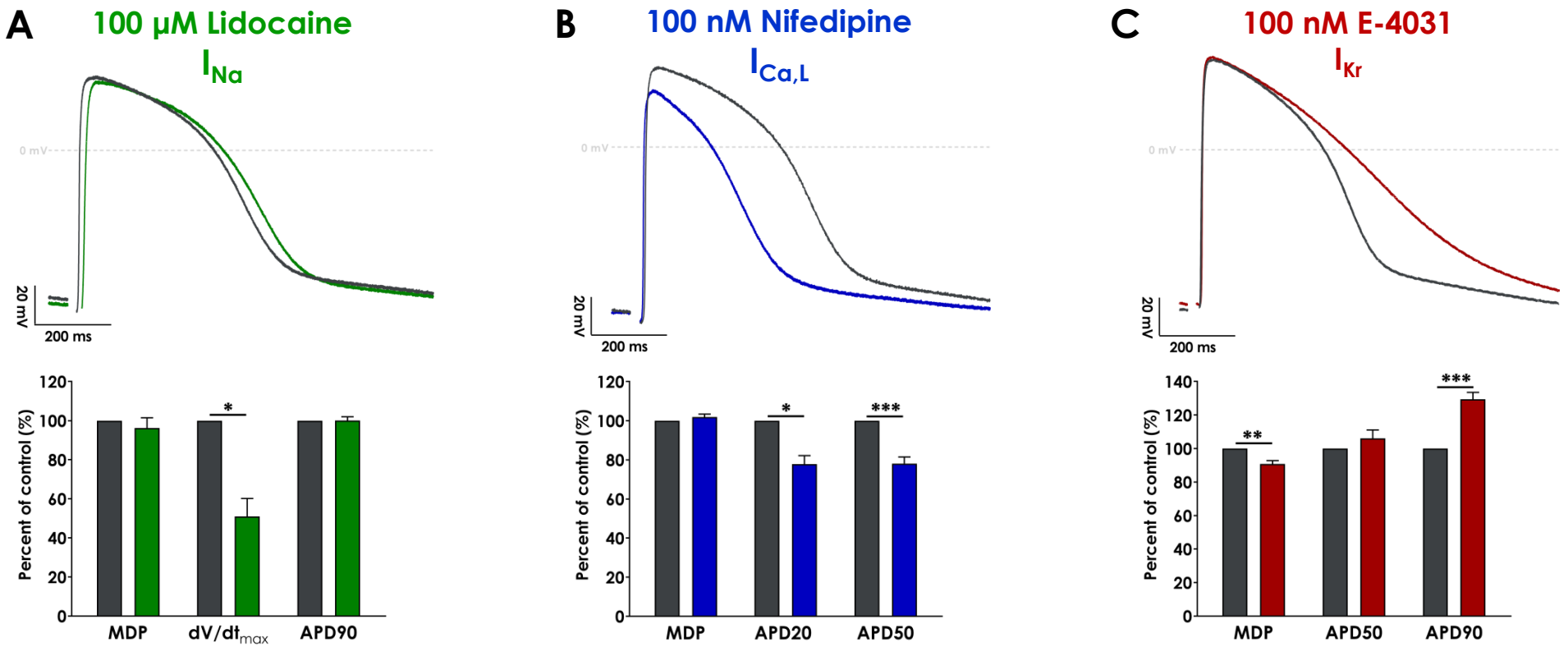
Spontaneous APD values are rate-corrected using Fridericia's formula

# Expression of core cardiac currents






- Confirmed functionality of core cardiac currents in Axol ventricular iPSC-CM
  - Expected effects of three reference compounds on evoked action potentials
    (A) Lidocaine slowed the upstroke velocity by ~50 %
    (B) Nifedipine decreased all APD values
    (C) E-4031 prolonged APD90 by ~30 % but did not trigger EADs at 1 Hz pacing

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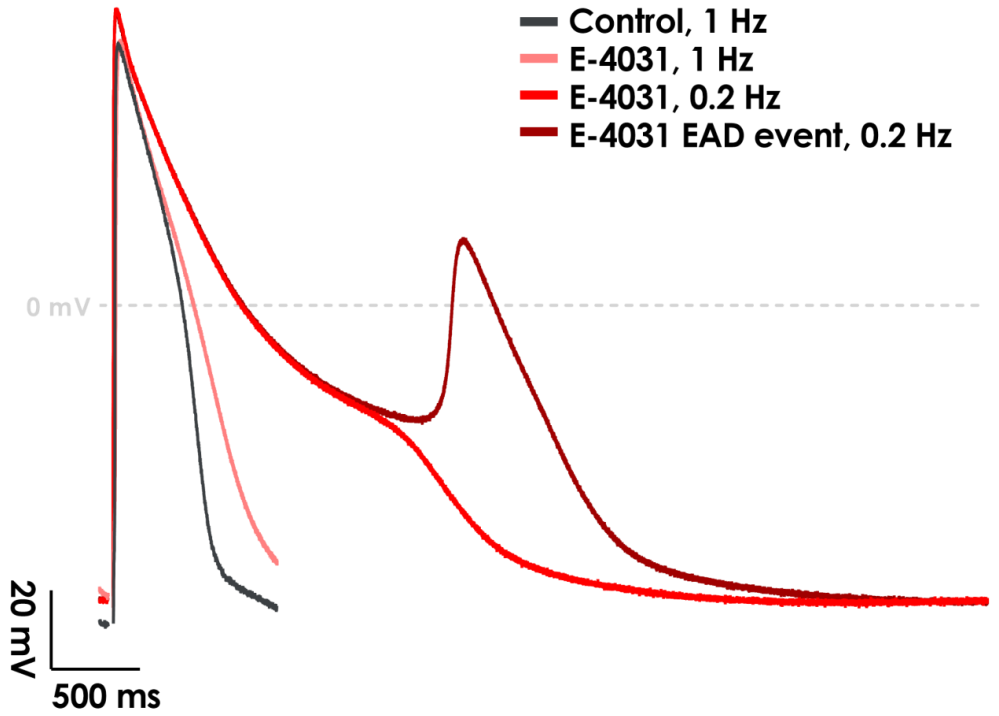
Significance tested using paired Student's t-test; * P<0.05, ** P<0.01, *** P<0.001

# Generation of arrhythmic events



- No early after-depolarisation (EAD) events were observed at 1 Hz pacing
- But, 100 nM E-4031 resulted in further prolongation and early after-depolarisation (EAD) events at lower pacing rates




- Confirms arrhythmic capability of Axol ventricular iPSC-CM

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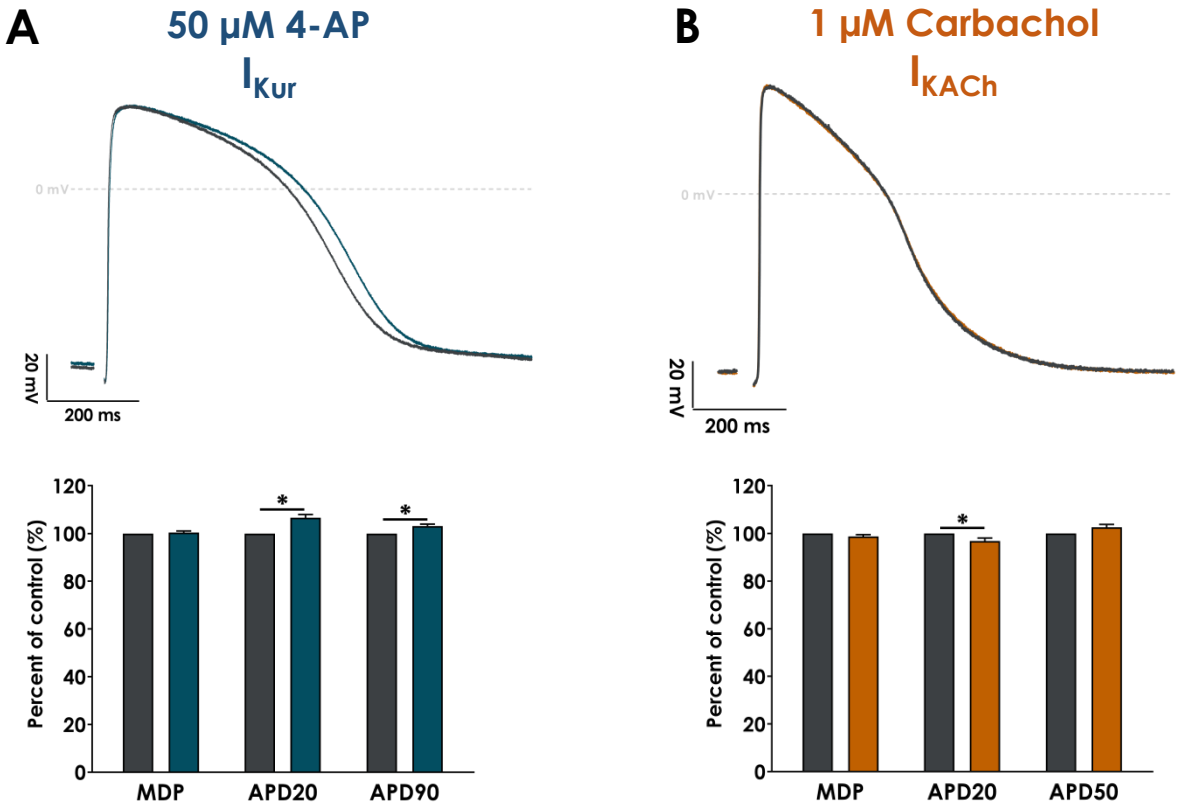
# Confirmation of predominantly ventricular phenotype






Pharmacological tools targeted against atrial specific currents show minimal effects

(A) $I_{Kur}$

- Small effect (6 % prolongation) of 4-AP suggesting low level functional expression of $K_V1.5$ channels
- $K_V1.5$ protein is expressed in the human ventricle, but no functional activity has been detected (Mays *et al.*, 1995 J Clin Invest)
- Common feature of the majority of human iPSC-CM tested at Metrion

(B) $I_{KACh}$

- Carbachol ($I_{KACh}$ activator) is expected to shorten APD and hyperpolarise the membrane potential
- Carbachol resulted in a 3 % decrease of APD20

**Suggests majority of cells have a ventricular phenotype**

Significance tested using paired Student's t-test; * P<0.05, ** P<0.01, *** P<0.001



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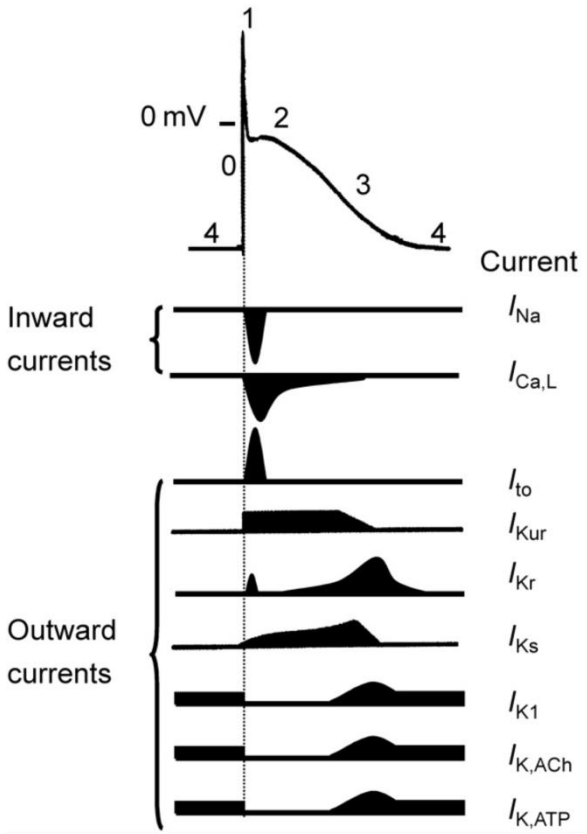
# Atrial cardiomyocytes



## Atrial




Europace. 2008;10(10):1133-1137

## Applications of atrial iPSC-CM at Metrion

- Disease modelling – atrial fibrillation
- Profiling compound effects in a translational human model

## Aims of electrophysiological characterisation

1. Determine control properties of Axol atrial iPSC-CM

2. Confirm the functional expression of three core cardiac currents
   - $I_{Na}$ ($Na_V1.5$), $I_{Ca,L}$ ($Ca_V1.2$), and $I_{Kr}$ (hERG)

3. Confirm the presence of two chamber specific currents
   - $I_{KACh}$ ($K_{ir}3.1/K_{ir}3.4$) and $I_{Kur}$ ($K_V1.5$)

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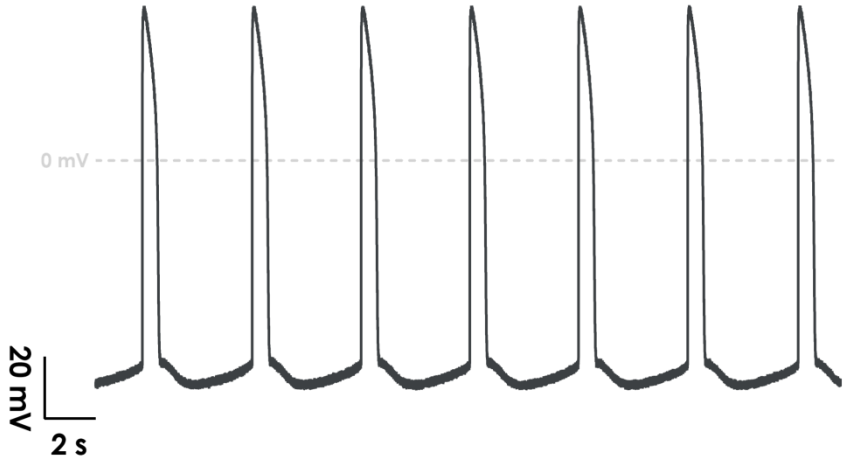
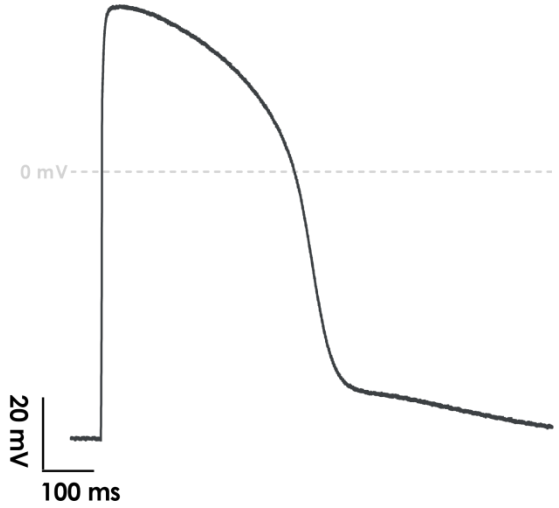
# Atrial action potential characteristics



**A** **Spontaneous**



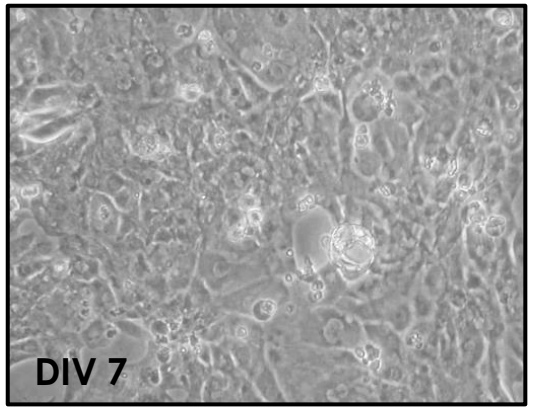

**B** **Evoked (1 Hz)**




**C**

| AP parameter | Spontaneous (N = 31) | Evoked 1 Hz (N = 28) |
|---|---|---|
| **MDP (mV)** | -73.6 ± 0.8 | -73.0 ± 0.9 |
| **$dV/dt_{max}$ (V/s)** | 44.6 ± 3.4 | 30.9 ± 3.8 |
| **APA (mV)** | 120.2 ± 1.0 | 126.3 ± 1.5 |
| **APD20 (ms)** | 252.4 ± 6.3 | 235.2 ± 5.6 |
| **APD50 (ms)** | 338.0 ± 6.7 | 352.1 ± 4.8 |
| **APD90 (ms)** | 419.0 ± 7.0 | 468.7 ± 11.4 |
| **Frequency (Hz)** | 0.28 ± 0.01 | 1 |

- Suitable for recording of spontaneous & evoked activity (0.5 – 2 Hz)
- Good maximum diastolic potential ~ -73 mV
- Upstroke velocity of ~ 31 V/s
- Consistent APD90 ~ 470 ms

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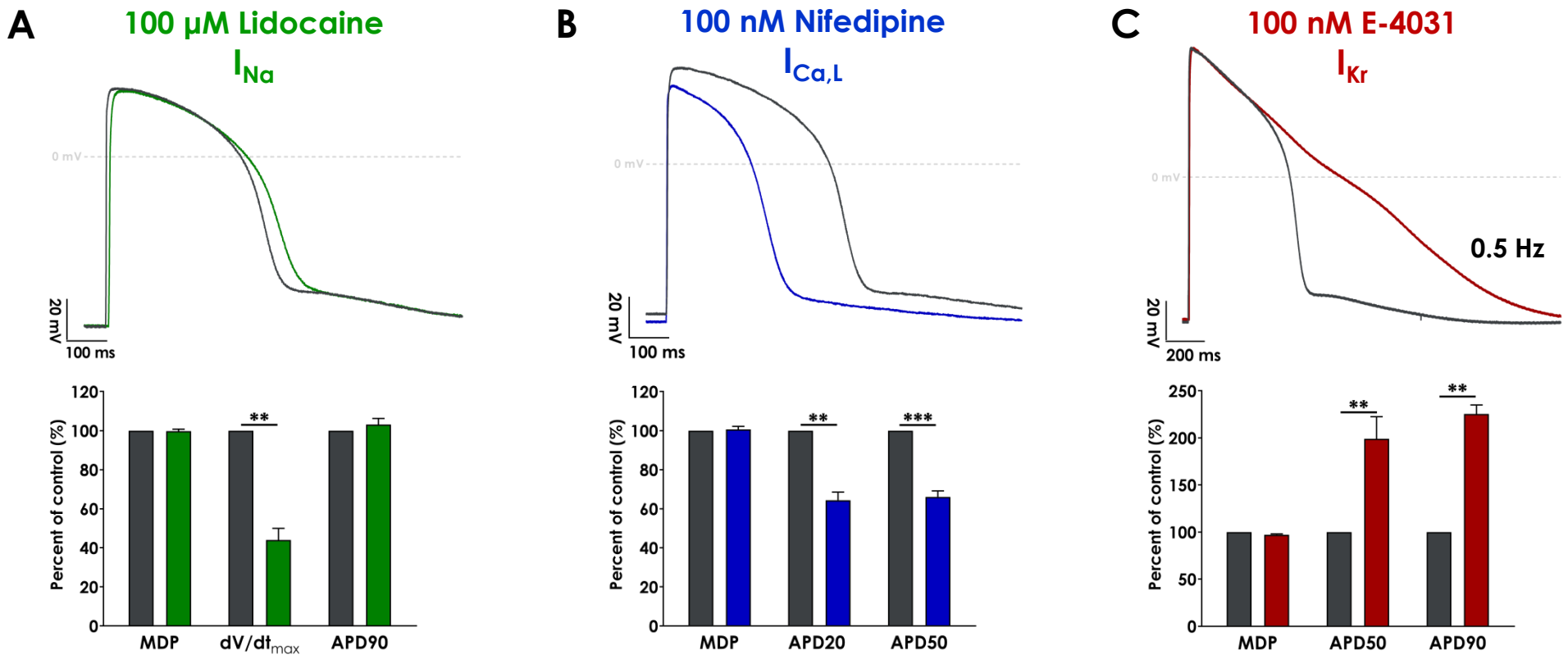
Spontaneous APD values are rate-corrected using Fridericia's formula

# Expression of core cardiac currents






- Confirmed functionality of core cardiac currents in Axol atrial iPSC-CM
  - Expected effects of three reference compounds on evoked action potentials
    (A) Lidocaine slowed the upstroke velocity by ~50 %
    (B) Nifedipine decreased all APD values
    (C) E-4031 prolonged APD90 by ~125 % at 0.5 Hz pacing

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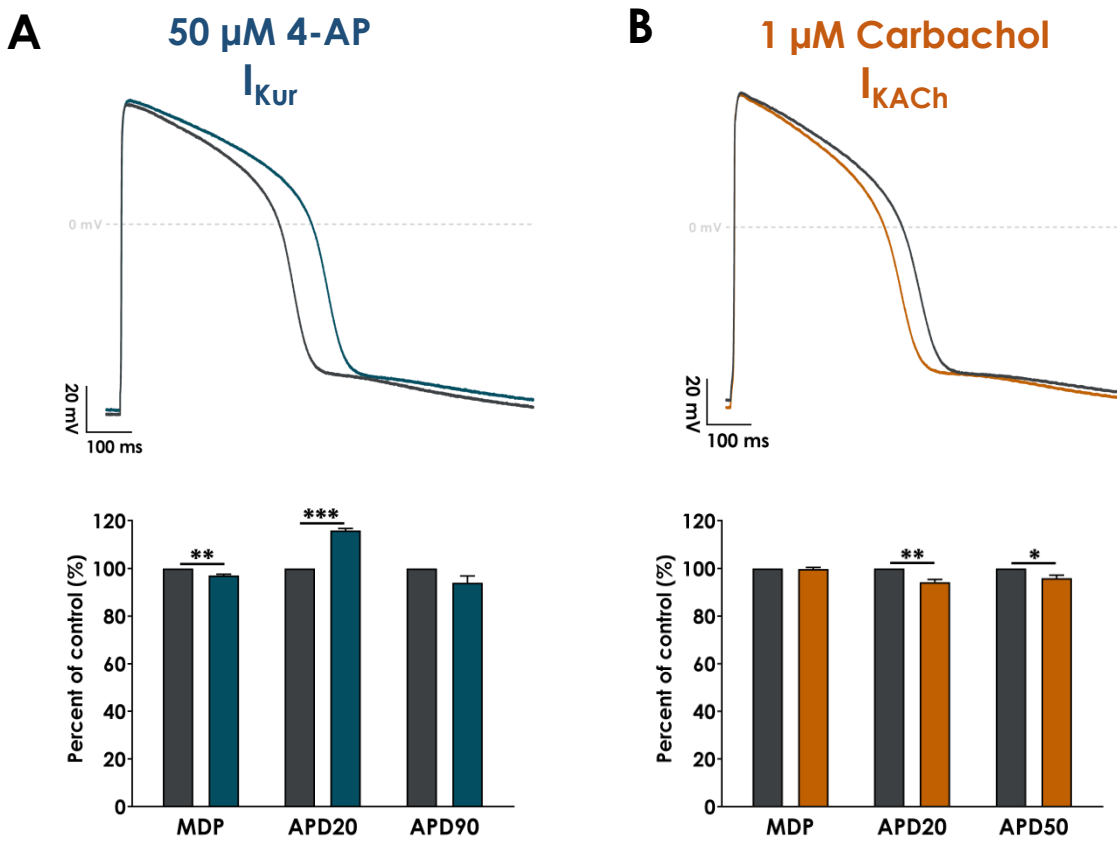
Significance tested using paired Student's t-test; * P<0.05, ** P<0.01, *** P<0.001

# Confirmation of atrial phenotype






Pharmacological tools targeted against atrial specific currents showed significant effects

(A) $I_{Kur}$

- 4-AP resulted in significant APD20 prolongation (14 %) suggesting functional expression of $K_V1.5$ channels

(B) $I_{KACh}$

- Carbachol ($I_{KACh}$ activator) is expected to shorten APD and hyperpolarise the membrane potential
- Carbachol resulted in a 6 % decrease of APD20

**Suggests cells have an atrial phenotype**

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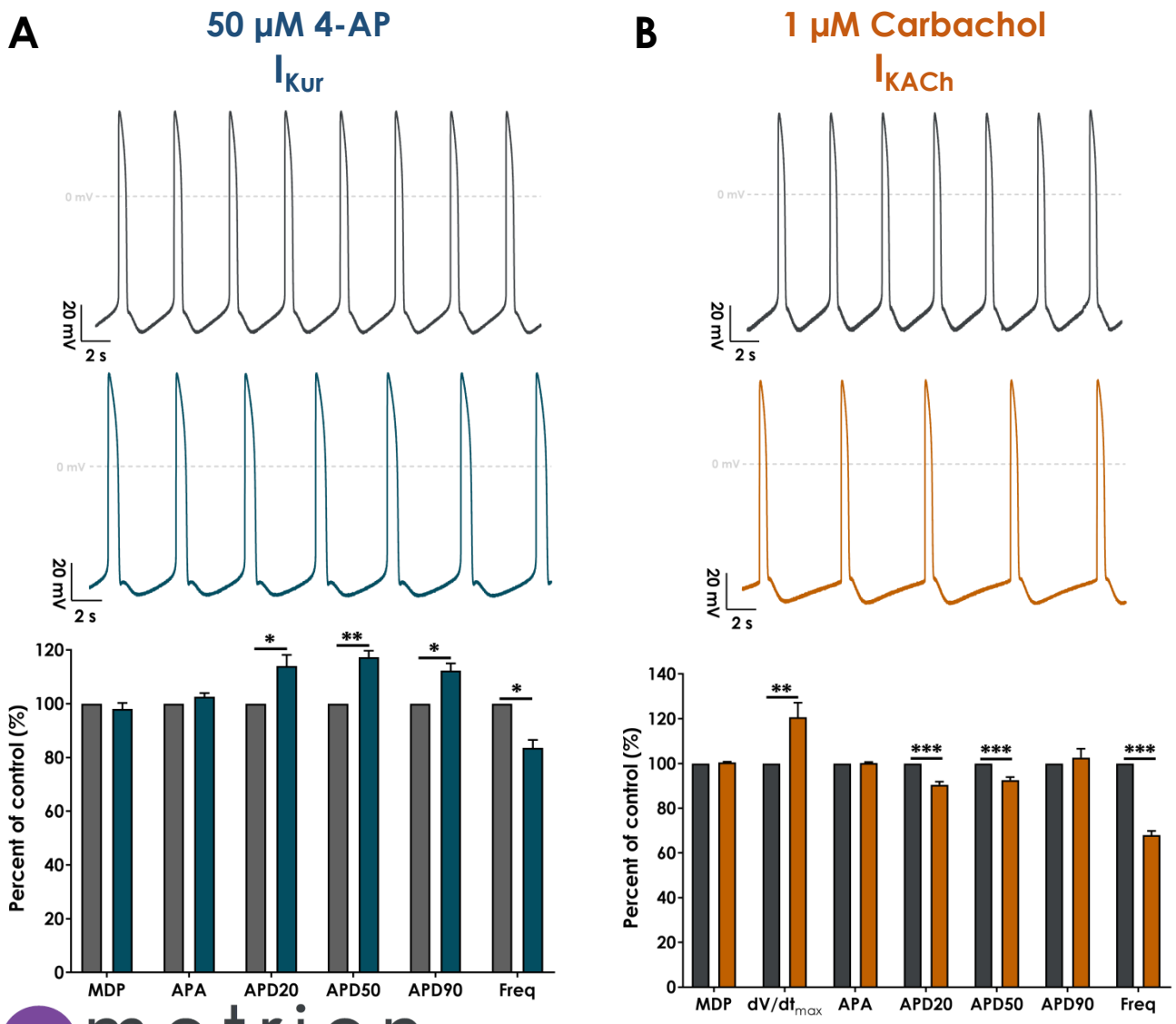
Significance tested using paired Student's t-test; * P<0.05, ** P<0.01, *** P<0.001

# Further confirmation of atrial phenotype






Further confirmation of atrial phenotype using spontaneous action potential recordings

(A) $I_{Kur}$

- 4-AP resulted in characteristic prolongation of APD values
- ~15 % increase in APD20, 50 and 90
- Corresponding decrease in firing rate

(B) $I_{KACh}$

- Carbachol resulted in characteristic negative chronotropic effects and shortening of APDs
- 32 % decreasing in firing rate
- ~10 % decrease in APD20 and 50

**Further suggests cells have a atrial phenotype**

APD values are rate-corrected using Fridericia's formula
Significance tested using paired Student's t-test; * P<0.05, ** P<0.01, *** P<0.001

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# Summary

- Axol iPSC-CM express functional ion channels which are key components of human cardiomyocyte action potentials
  - Both ventricular and atrial cells have functional $I_{Na}$, $I_{Ca,L}$, and $I_{Kr}$
- Chamber specific pharmacology was determined and this confirmed the ventricular and atrial phenotypes of each cell line
  - Atrial iPSC-CM show functional $I_{KACh}$ and $I_{kur}$
- Full characterisation of commercial human iPSC-CM allows Metrion to provide a range of validated assays
  - Proarrhythmic risk assessment as part of our integrated range of CiPA-ready assays
  - Profiling of compound effects in a translational human system
  - Characterisation of human iPSC-CM cell lines

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# Acknowledgments


- Metrion Biosciences
  - Saïd El-Haou
  - John Ridley
  - Louise Webdale
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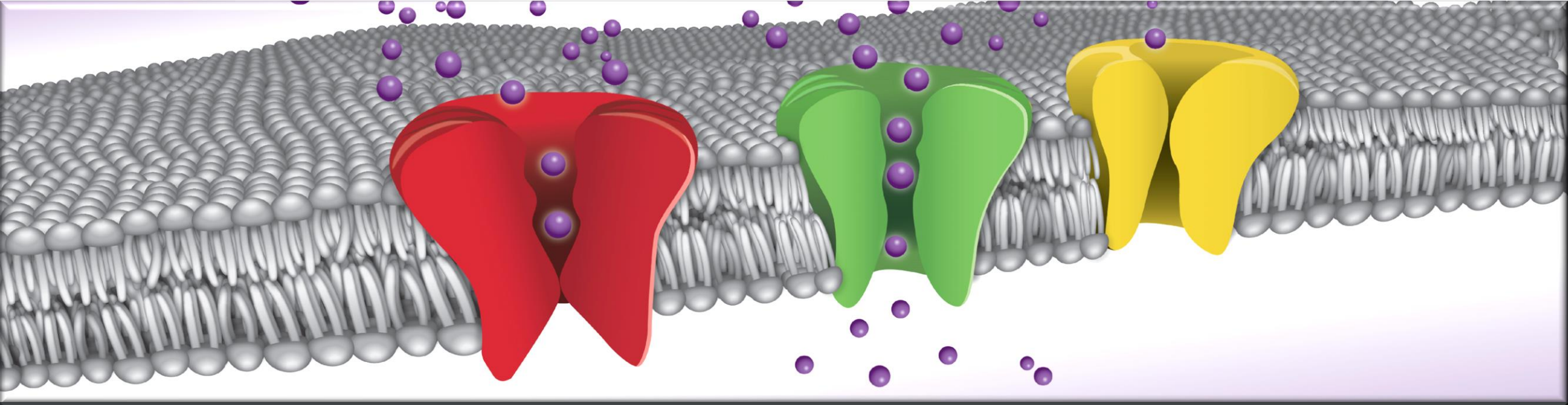
  - Robert Kirby

- Axol Biosciences
  - Zoe Nilsson
  - Yichen Shi

- This project received funding from the Eurostars-2 joint program with co-funding from the European Union Horizon 2010 research and innovation program



# Metrion Biosciences: the ion channel specialists

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