SUPPLEMENTAL FIGURES

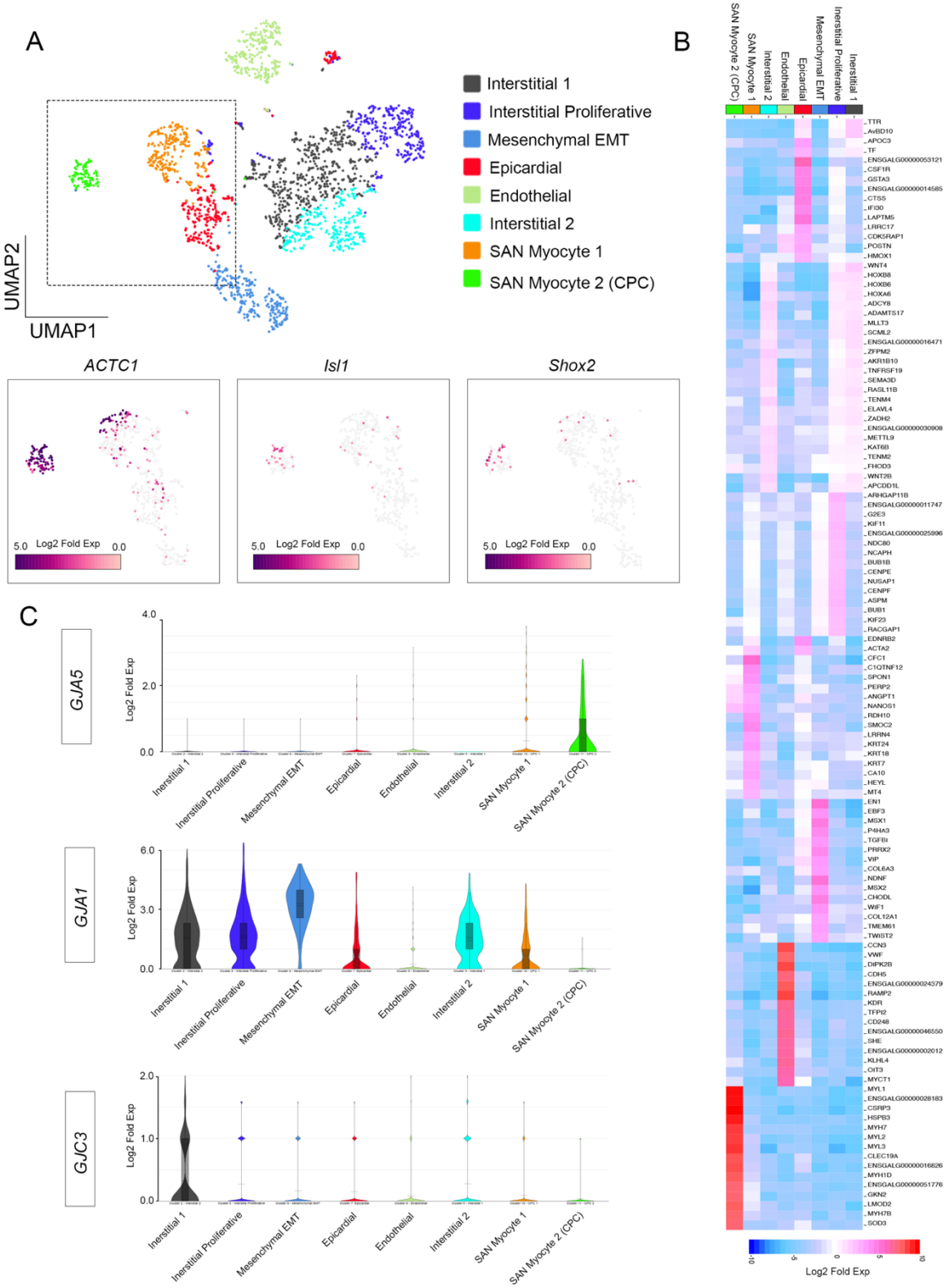

FIGURE s1: scRNA sequencing Analysis of the SAN. A) UMAP projection of the major cell types identified in the HH stage 30 SAN. **B)** Top 15 genes used for bioinformatic segregation of SAN cell clusters. **C)** Distribution of the major GJ transcripts (identified by bulk sequencing) among cell types in the SAN.

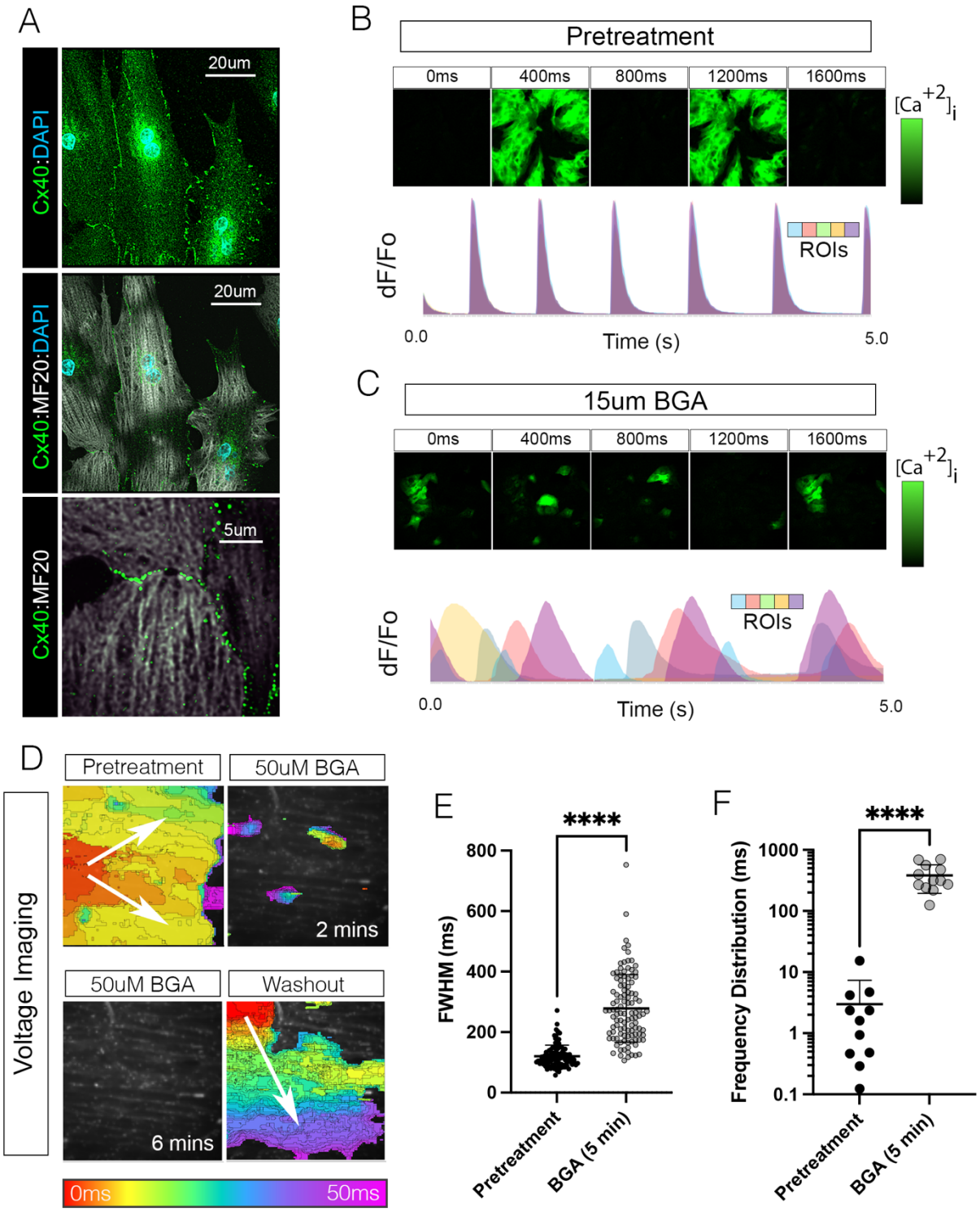
A
Cx40:DAPI
20um
Cx40:MF20:DAPI
20um
Cx40:MF20
5um
B
Pretreatment
0ms
400ms
800ms
1200ms
1600ms
[Ca+2]i
dF/Fo
ROIs
0.0
Time (s)
5.0
C
15um BGA
0ms
400ms
800ms
1200ms
1600ms
[Ca+2]i
dF/Fo
ROIs
0.0
Time (s)
5.0
D
Voltage Imaging
Pretreatment
50uM BGA
2 mins
50uM BGA
Washout
6 mins
0ms
50ms
E
****
FWHM (ms)
800
600
400
200
0
Pretreatment
BGA (5 min)
F
****
Frequency Distribution (ms)
1000
100
10
1
0.1
Pretreatment
BGA (5 min)

FIGURE s2: Gap Junction Uncoupler BGA Disrupts Electrical Coupling in Cultured Atrial Monolayers. A) CX40 (green) and MF20 (white) staining preformed in cultured monolayers of primary chick embryonic atrial myocytes. **B)** Time series of Ca^{+2} imaging performed on primary atrial myocyte monolayer pretreatment with BGA. **C)** Time series of Ca^{+2} imaging performed on primary atrial myocyte monolayer 5 minutes after treatment with BGA (15uM). **D)** Isochronal maps of primary embryonic chick atrial monolayers voltage imaged following exposure to 50uM BGA. Washout imaging was performed 10 minutes after removal of BGA. **E)** Quantification of Ca^{+2} transient duration (FWHM) in monolayers of primary chick embryonic atrial myocytes pretreatment and following BGA exposure (n=110 pretreatment and 109 posttreatment cells). **F)** Quantification of Ca^{+2} transient frequency distribution within a single field of view pretreatment and following BGA exposure (n=110 pretreatment and 109 posttreatment cells). Significance was calculated using a Welch's t test; p-values *<0.05, **<0.01,*** <0.001, **** < 0.0001.

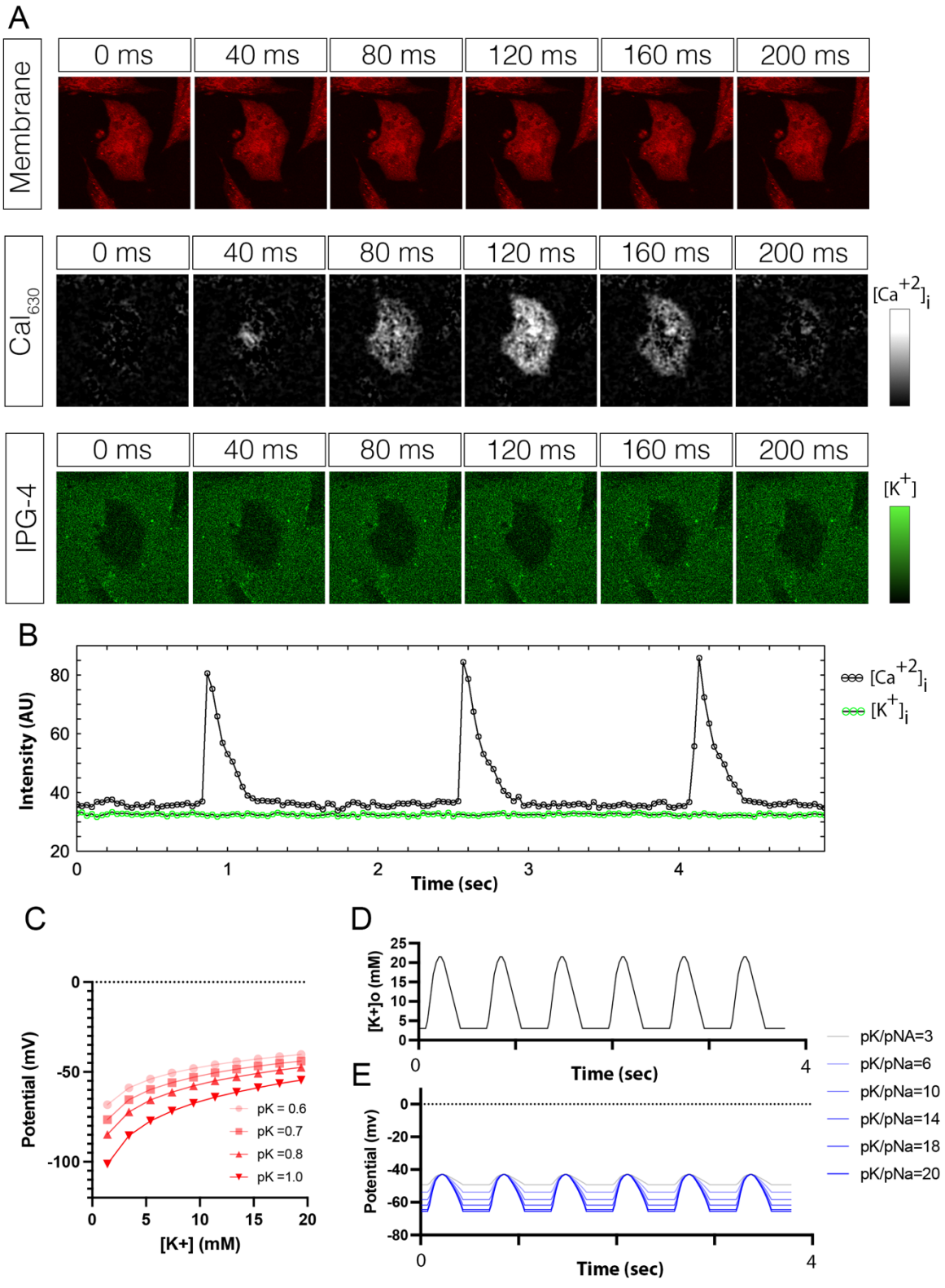
A
Membrane
0 ms
40 ms
80 ms
120 ms
160 ms
200 ms
Cal630
[Ca+2]i
IPG-4
[K+]
B
Intensity (AU)
Time (sec)
[Ca+2]i
[K+]i
C
Potential (mV)
[K+] (mM)
pK = 0.6
pK =0.7
pK =0.8
pK =1.0
D
[K+]o (mM)
Time (sec)
E
Potential (mv)
Time (sec)
pK/pNA=3
pK/pNa=6
pK/pNa=10
pK/pNa=14
pK/pNa=18
pK/pNa=20

FIGURE s3: IPG-4 Validation and Predicted Effects $[K^+]_o$ of on Membrane Potential. A) Time series of membrane staining, intracellular Ca^{+2} staining (using the calcium sensitive dye Cal_{630}), and IPG-4 staining in an isolated embryonic chick CPC. Staining was performed for 60 mins. Note that IPG-4 staining intensity is higher in the media (5.4mM) then the cytoplasm (~135mM) indicating that IPG-4 is membrane impermeant. **B)** Plot of Ca^{+2} dye and IPG-4 cytoplasmic intensity over time. **C)** Calculation of membrane potential vs $[K^+]_o$ concentration given o range of potassium membrane permeabilities. **D)** Calculation of membrane potential overtime given imposed fluctuations in $[K^+]_o$ for six theoretical cells with vary ratios of Na^+/K^+ permeabilities.

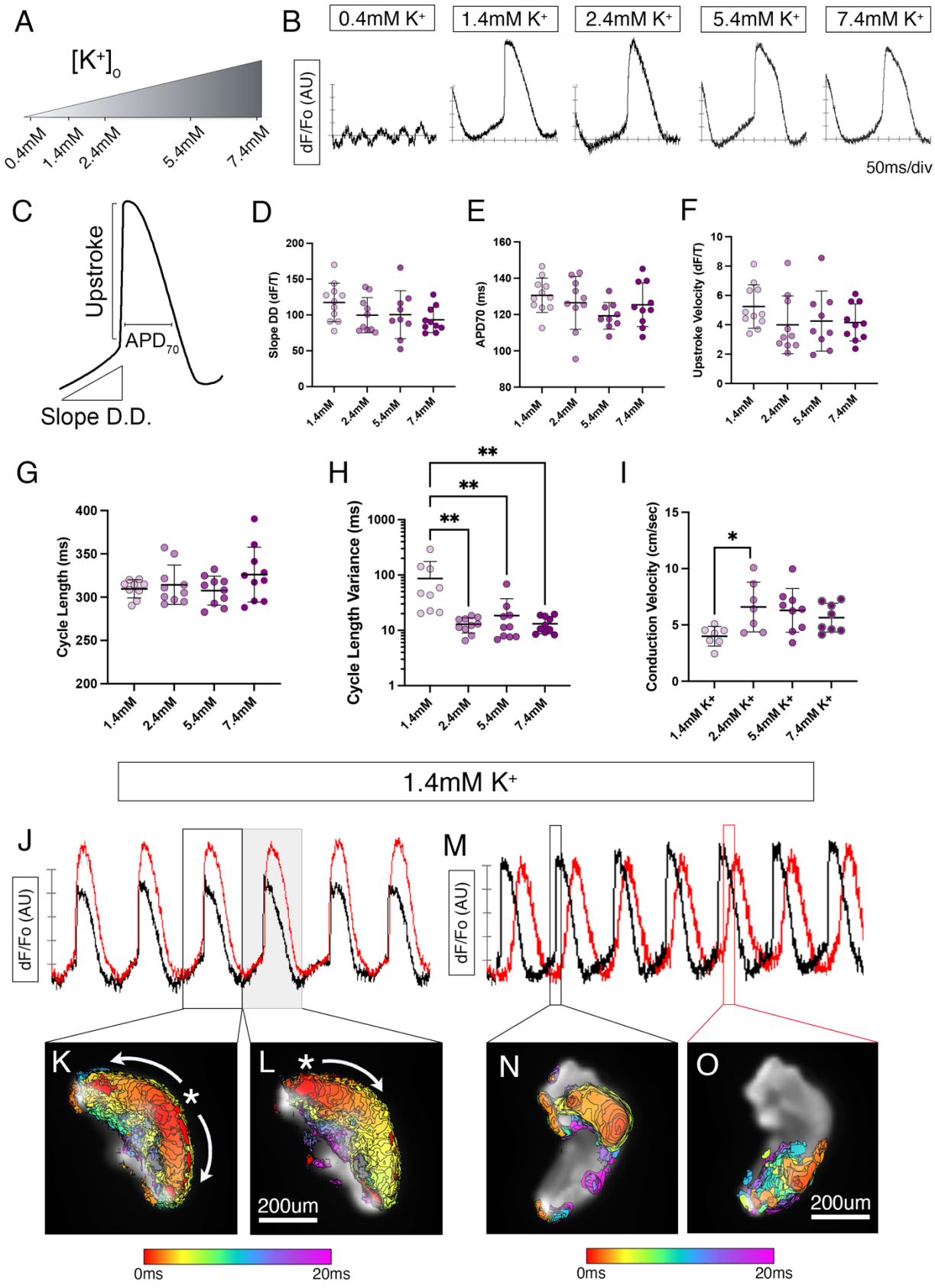
A
[K+]o
0.4mM
1.4mM
2.4mM
5.4mM
7.4mM
B
0.4mM K+
1.4mM K+
2.4mM K+
5.4mM K+
7.4mM K+
dF/Fo (AU)
50ms/div
C
Upstroke
APD70
Slope D.D.
D
Slope DD (dF/T)
E
APD70 (ms)
F
Upstroke Velocity (dF/T)
G
Cycle Length (ms)
H
Cycle Length Variance (ms)
I
Conduction Velocity (cm/sec)
1.4mM K+
J
M
dF/Fo (AU)
K
L
N
O
200um
0ms
20ms

FIGURE s4: Impact of $[K^+]_o$ on SAN Electrical Activation. A) Gradient of $[K^+]_o$ concentrations that primary embryonic SAN tissue was exposed to. **B)** Imaging based recordings of SAN action potential wave forms from 0.4mM - 7.4mM $[K^+]_o$. **C)** Action potential parameters quantified in D-F. **D)** Quantification of SAN diastolic depolarization (n=40). **E)** Quantification of SAN Action potential duration (n=40). **F)** Quantification of San upstroke velocity (n=40). **G)** Quantification of SAN cycle length (n=39). **H)** Quantification of SAN cycle length variance (n=39). **I)** Quantification of SAN conduction velocity (n=33). J) Action potential wave trace and isochronal maps (K,L) of SAN sample imaged at 1.4mM $[K^+]_o$. Two successive action potentials are highlighted. **K,L**) demonstrate that the action potential initiation site switched location. M) Action potential wave trace for a separate SAN sample imaged at 1.4mM $[K^+]_o$. **N,O**) Demonstrate that multiple foci of electrical activity have emerged in this sample. Significance was calculated using a one-way ANOVA, p-values *<0.05, **<0.01,*** <0.001, **** < 0.0001.

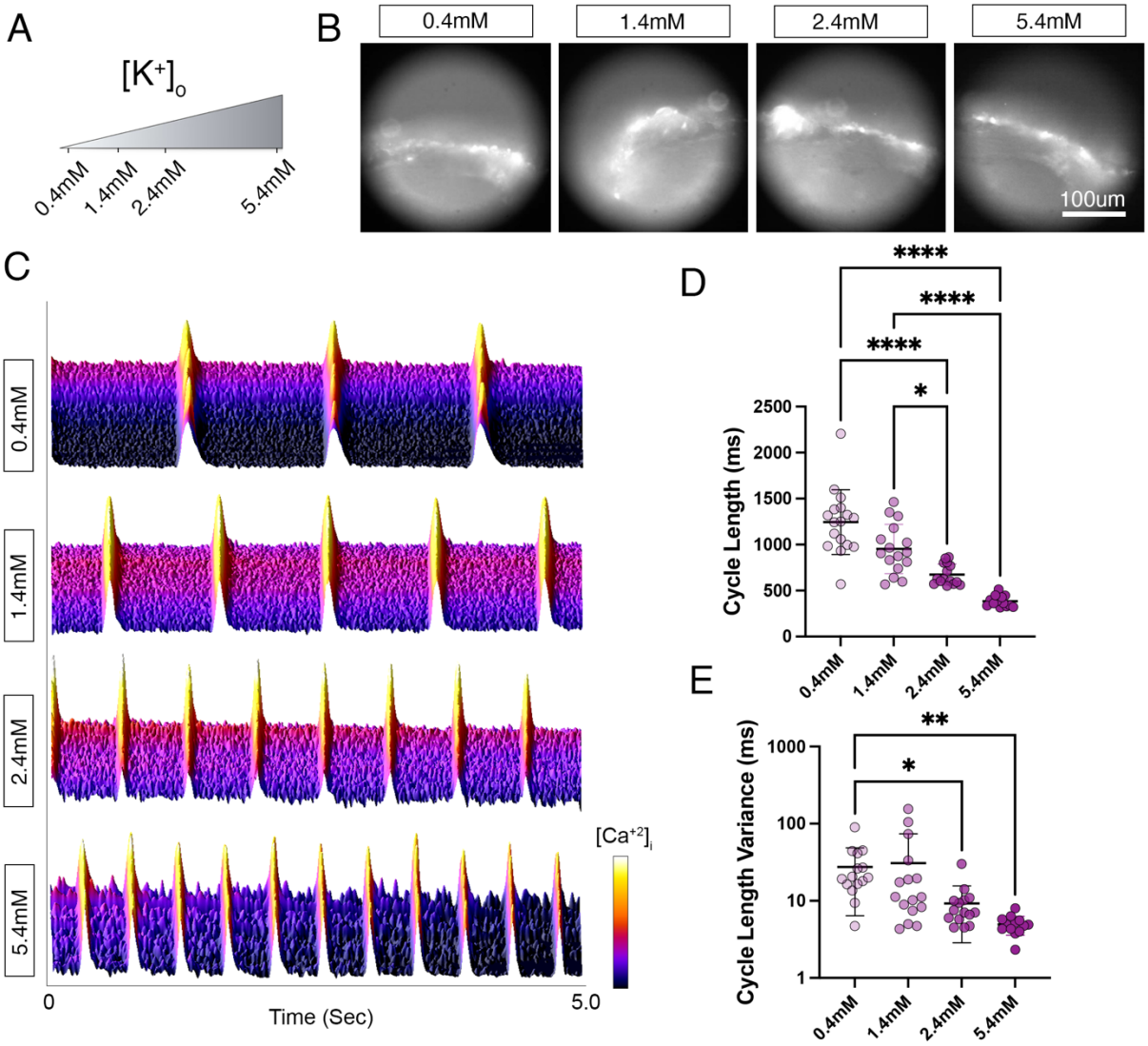
A
[K+]o
0.4mM
1.4mM
2.4mM
5.4mM
B
0.4mM
1.4mM
2.4mM
5.4mM
100um
C
0.4mM
1.4mM
2.4mM
5.4mM
[Ca+2]i
0
5.0
Time (Sec)
D
****
****
****
*
Cycle Length (ms)
2500
2000
1500
1000
500
0
0.4mM
1.4mM
2.4mM
5.4mM
E
**
*
Cycle Length Variance (ms)
1000
100
10
1
0.4mM
1.4mM
2.4mM
5.4mM

FIGURE s5: Impact of $[K^+]_o$ on Atrial Tissue Ca^{+2} Transient Activity. **A)** Gradient of $[K^+]_o$ concentrations that primary embryonic atrial tissue was exposed to. **B)** Low magnification images of an atria tissue preparation as it was moved up the $[K^+]_o$ gradient. **C)** Five second recordings of Ca^{+2} imaging from samples in "B." **D)** Quantification of atrial cycle length (n=61). **I)** Quantification of atrial cycle length variance (n=61). Significance was calculated using a one-way ANOVA, p-values *<0.05, **<0.01,*** <0.001, **** < 0.0001.

VideoS1: BGA Disrupts Electrical Communication Among Atrial Myocytes.

VideoS2: Validation of IPG-4. Live imaging of Ca^{+2} transient activation recorded using Cal_{630} and K+ dynamics in myocytes recorded ex vivo. Note, no change in IPG-4 intensity.

VideoS3: Lowering $[K^+]_o$ Leads to Desynchronization of SAN but Not Atrial Tissue. Schematic of experimental preperations and live Ca+2 imaging of Atrial and SAN tissue preparations before, during, and following drops in $[K_+]_o$ to 0.4mM.

VideoS4: Loss of SAN Entrainment as $[K^+]_o$ is Decreased from 5.4-0.4mM. Ca+2 imaging of a SAN tissue preparations as $[K_+]_o$ is serially depleted.

VideoS5: Lowering $[K^+]_o$ Leads to Desynchronization with hiPSC-CPC Spheroids. Ca+2 imaging of a hiPSC-CPC spheroids maintained at 2.4mM or 0.4mM $[K_+]_o$ for 10 mins.