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Supplemental Information

A critical role of retinoic acid concentration for the induction of a fully human-like atrial action potential phenotype in hiPSC-CM

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ERC	[RA] (µM)	BR (bpm)	MDP (mV)	TOP (mV)	APD_{20} (ms)	APD_{90} (ms)	APD_{90-20} /APD_{90}
001	**0**	54±11.5	-78.1±0.9	-77.7±2.6	130.2±19.2	267±33.6	0.54±0.1
	0.01	131.1±14.7 *	-76.6±3.8	-76.4±4.3	86.4±7.9 *	206±16 *	0.58±0.1
	0.1	187.5±68.7 #	-71.8±4.9	-71.3±4.2 #	76.3±11.4	161±26 #	0.53±0
	1	147±36 §	-68.6±0.7 §	-67.7±1.4	8.8±3.6 §	140±25.5	0.92±0 §
021	**0**	74.5±12.3	-74.1±2.1	-74±4.2	108.3±6.9	239±28.1	0.54±0
	0.01	77.2±32.5 *	-67.4±1.1 *	-65.3±4.1 *	114.7±46 *	212±76.1 *	0.39±0.2
	0.1	111±18.4 #	-64.3±2.1 #	-63.9±4.1	70.5±39.3 #	166±39.3 #	0.6±0.1#
	1	125.9±16.7 §	-65.3±4.9	-65±2	17.4±1.6 §	125±32.3 §	0.85±0 §

Supplement Table S1: Effects of different concentrations of retinoic acid (RA) on AP parameters. Mean values±SD for beating rate (BR), maximum diastolic potential (MDP), take-off potential (TOP), action potential duration at 20% (APD_{20}) and 90% repolarization (APD_{90}) and repolarization fraction (APD_{90-20} /APD_{90}) measured in intact EHT based on hiPSC-CM differentiated with different retinoic acid (RA) concentrations. * indicates p<0.05 vs. "0" RA, # indicates p<0.05 vs. 0.01 µM RA and § indicates p<0.05 vs. 0.1 µM RA (ANOVA, same n numbers as in Figure 2).

ERC	[RA] (µM)	ΔAPD_{20}	ΔAPD_{90}	$\Delta V_{Plateau}$
001	**0**	1.5±2	4.2±5.9	3.2±2.1
	0.01	18±14.1 *	27.3±12.4 *	2.3±5.7
	0.1	8.9±12.6	18.1±20.6	1.1±8.9 #
	1	20.26±5.6	-25.8±25.9 §	12±1.7 §
021	**0**	1.8±19.9	9±15	-0.3±.6
	0.01	16.3±8.2 *	24±12.4 *	3.1±14.3
	0.1	6.3±6.2	12.4±11.8	7.5±2.5
	1	18±7.8	-17.3±10 §	13.6±5.1 §

Supplement Table S2: Effects of different concentrations of retinoic acid (RA) on 4-amniopyridine effects. Mean values±SD for the effects of 50 µM 4-aminopyridine (4-AP, expressed as Δ-values) on action potential duration at 20% repolarization (APD_{20}), plateau voltage ($V_{Plateau}$) and action potential duration at 90% repolarization (APD_{90}) measured in intact EHT based on hiPSC-CM differentiated with different retinoic acid (RA) concentrations. * indicates p<0.05 vs. "0" RA, # indicates p<0.05 vs. 0.01 µM RA and § indicates p<0.05 vs. 0.1 µM RA (ANOVA, same n numbers as in Figure S3).

ERC	[RA] (µM)	ΔAPD$_{90}$	ΔTOP	ΔBR
001	**0**	3.1±4.2	0.1±2	3.3±4.3
	0.01	4.3±8.4 *	-0.03±1.4	-8.8±16 *
	0.1	0.1±9.3	-3.6±5.4	-15.9±25.3
	1	-57.1±33 §	-7.2±3.4	-45.6±28.1
021	**0**	4.1±30.7	0.1±1.1	-3.6±12.4
	0.01	-38.2±16.9 *	-1±2.1	-24.4±10.7 *
	0.1	-32±12.2	-1.2±3.3	-40.6±24
	1	-40±7.7 §	-7.8±2.3 §	-61.6±9.8 §

Supplement Table S3: Effects of different concentrations of retinoic acid (RA) on carbachol effects. Mean values±SD for the effects of 10 µM carbachol (CCh, expressed as Δ-values) on action potential duration at 20% repolarization (APD_{20}), take-off potential (TOP) and beating rate (BR) measured in intact EHT based on hiPSC-CM differentiated with different retinoic acid (RA) concentrations. * indicates $p<0.05$ vs. "0" RA, # indicates $p<0.05$ vs. 0.01 µM RA and § indicates $p<0.05$ vs. 0.1 µM RA (ANOVA, same n numbers as in Figure S4).

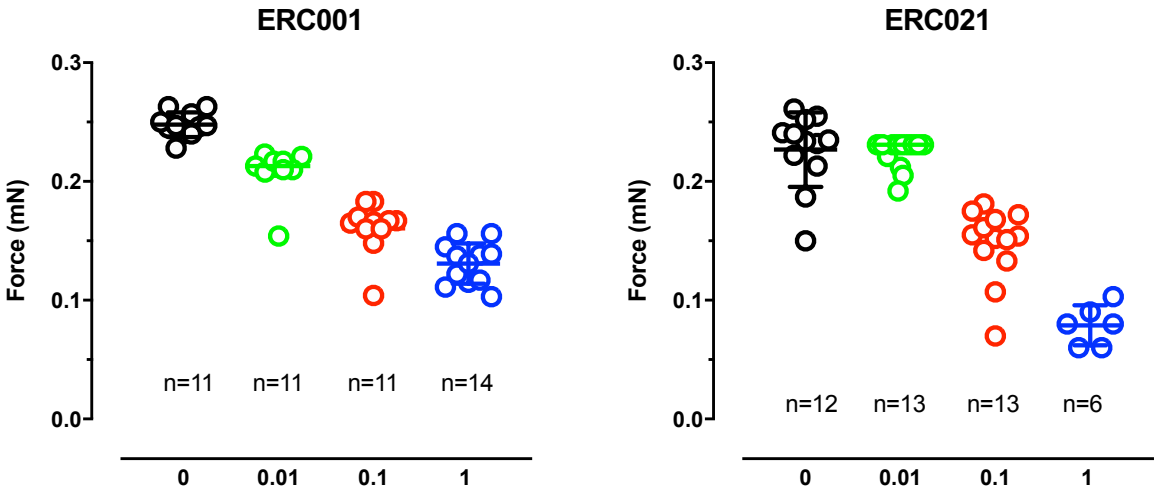
	Filtropur S® (n=9)	Millex-GP® (n=9)	Whatman® REZIST (n=9)
Recovery in %	29.8±2.4	28.6±7.6	0.1±0.01

Supplement Table S4: Loss of RA by sterile filtration
Mean values±SD for recovery rate of RA concentration in % of unfiltered controls (n=6, data not shown) for three different filters.

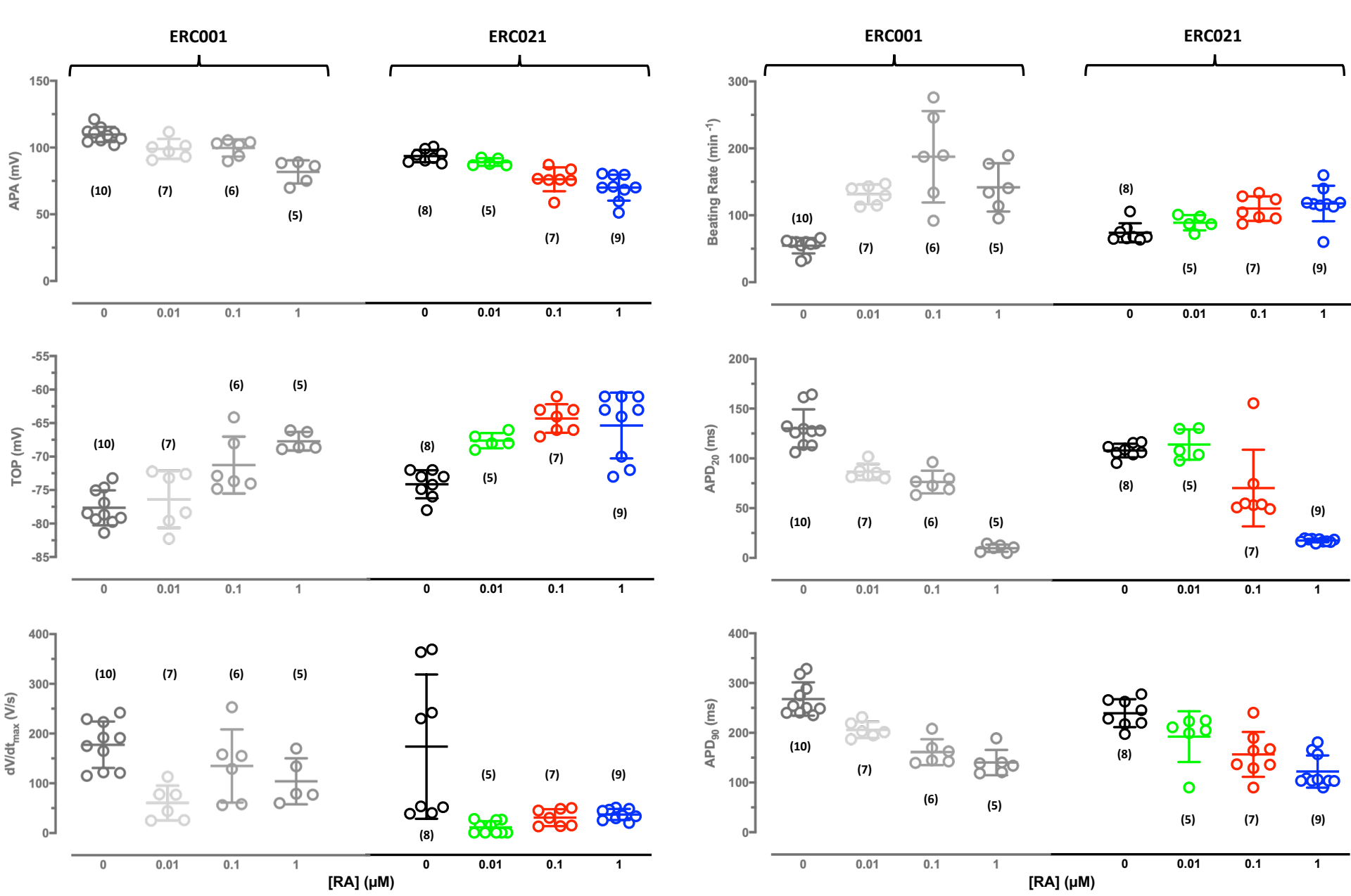
Cell type	Adult atrial tissue	**hiPSC-CM**	hiPSC-CM	hESC-CM	hiPSC-CM	hiPSC-CM	hESC-CM	hESC-CM	hiPSC-CM
Culture format	-	**3D**	3D	2D	2D	3D	2D	2D	3D
RA concentration (µM)	-	**1**	1 (?)	1	2	0.25-0.5	1	1	1
Recording technique	Sharp ME	**Sharp ME**	Sharp ME	Patch clamp	Patch clamp	Patch clamp	Patch clamp	Patch clamp	Voltage dye
Temperature	37 °C	**37 °C**	37 °C	36 °C	RT	31 °C	RT	RT	RT
MDP/RMP (mV)	-76	**-68**	-69	-72	-55	-63	-56	-56	n. d.
APA (mV)	105	**82**	80	80	85	n. d.	80	82	n. d.
APD_{20} (ms)	8	**10**	31	21	13*	115*	37*	n. d.	n. d.
APD_{90} (ms)	314	**140**	220	145	189	205	247	169	n. d.
APD_{20}/APD_{90}	0.02	**0.07**	0.14	0.14	0.07	0.56*	0.14	n. d.	0.12*
dV/dt_{max} (V/s)	219#	**104**	95	26	68	7	63	13	n. d.
$V_{Plateau}$	-16#	**-16**	n. d.	-10	n.d.	n. d.	n. d.	n.d.	n. d.
4-AP effect									
APD_{20} (% of baseline)	194	**300**	106	205	n. d.	n. d.	n. d.	n. d.	n. d.
APD_{90} (% of baseline)	88	**81**	100	120	n. d.	n. d.	n. d.	n. d.	n. d.
CCh effect									
APD_{90} (% of baseline)	55	60	82	97	n. d.	69	n. d.	n. d.	n. d.
MDP/RMP (% of baseline)	102	**110**	107	107	n. d.	n. d.	n. d.	n. d.	n. d.
Author/Year	Lemme 2018 Wettwer 2004	**This study**	Lemme 2018	Devalla 2015	Lee 2017	Goldfracht 2020	Laksman 2017	Zhang 2011	Cyganek 2018

Supplement Table S5: Action potential parameters in different types of atrial cardiomyocytes.

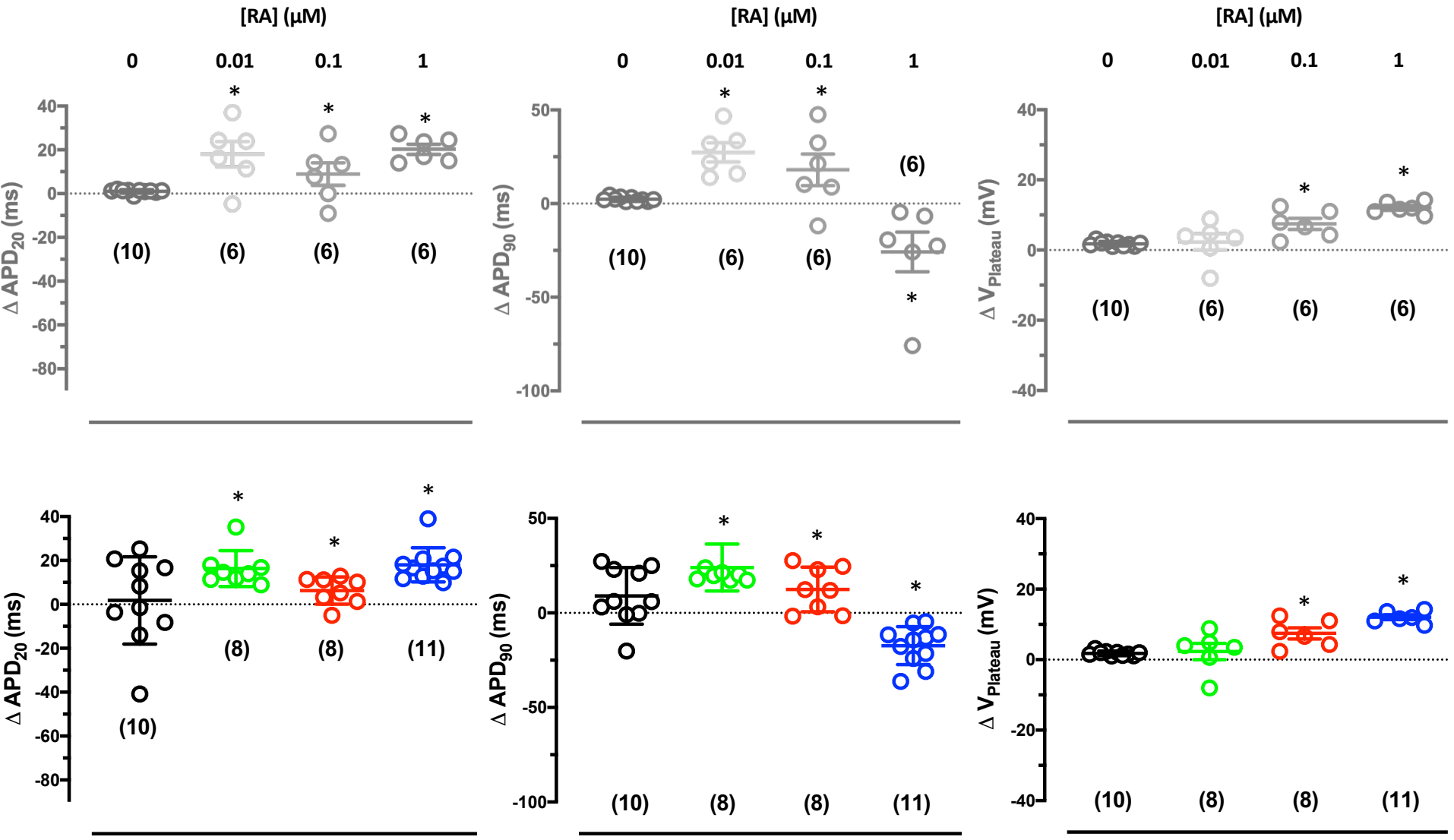
Overview about action potential (AP) parameters of different cell types and different studies. The different cell types included adult atrial tissue, cardiomyocytes differentiated from human induced pluripotent stem cells (hiPSC-CM) and cardiomyocytes differentiated from human embryonic stem cells (hESC-CM). The effects of carbachol (CCh) and 4-aminopyridine (4-AP) are given as percentage of baseline values. * indicates an estimated parameter from AP shape.



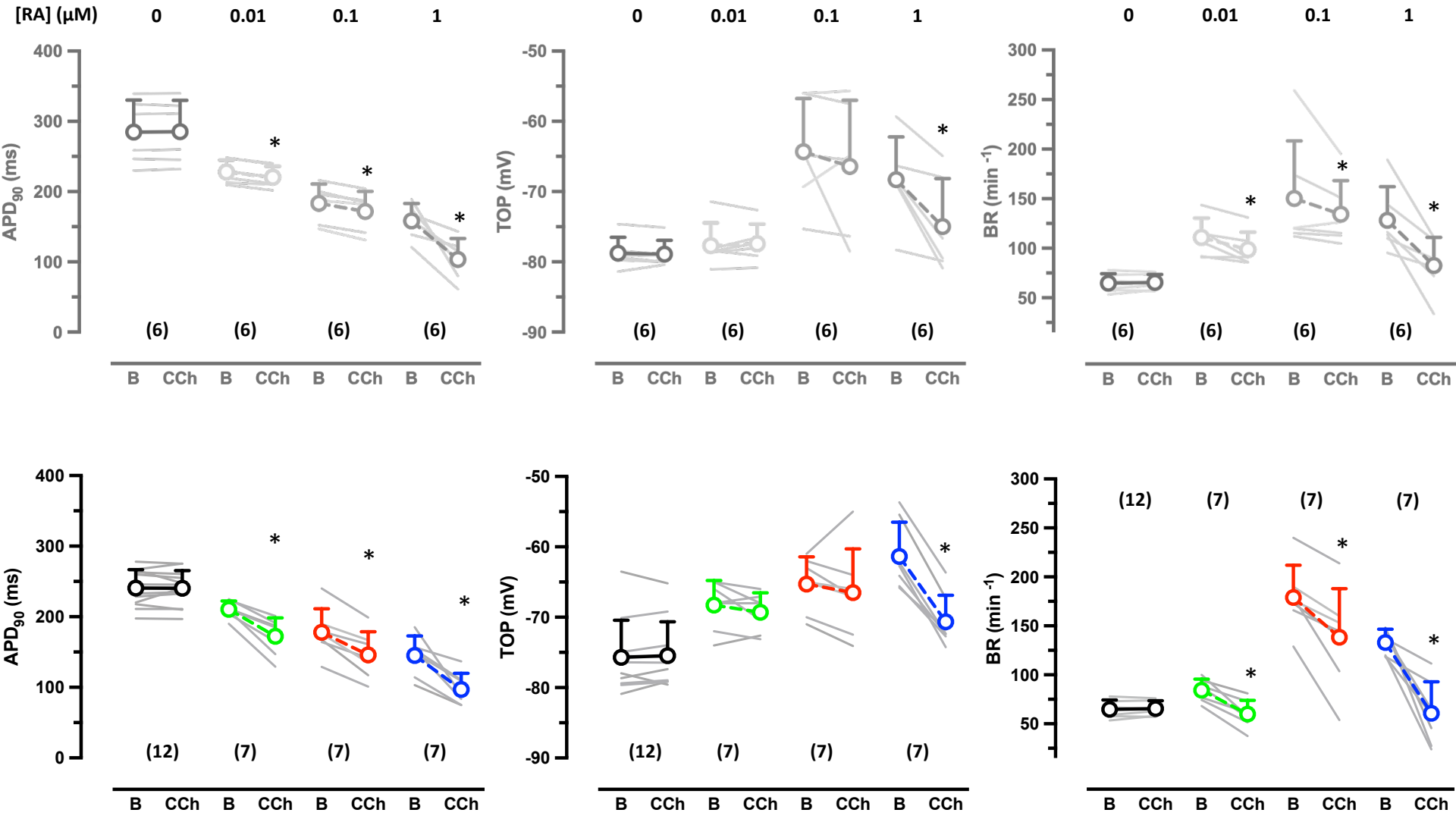
Supplement Figure S1



Supplement Figure S2



Supplement Figure S3



Supplement Figure S4

Legends to supplement figures

Figure S1: Concentration-dependency of RA on force in EHT

Mean values±SD force in EHT from ERC001 and ERC021.

Figure S2: Concentration-dependency of RA on AP in EHT in a second cell line (ERC021)

Summary of results: Mean values±SD for beating rate (**BR**), take-off potential (**TOP**), action potential duration (at 20 and 90% repolarization, **APD_{20}** and **APD_{90}**) and repolarization fraction (**APD_{90}-APD_{50}/APD_{90}**) in EHT from ERC021. * indicates $p<0.05$ vs. 0 RA, one way ANOVA of log transformed data. Number of EHTs resulting from one differentiation run is given in brackets. For better comparison, data for ERC001 (given already in the main manuscript as **Figure 2)** are plotted again in grey.

Figure S3: Concentration-dependency of RA on the AP-response to I_{Kur} block

Top: Data for ERC001 (given already in the main manuscript as **Figure 3)** are plotted in grey. **Bottom:** Data for ERC021. Summary of results for the effects of 4-AP on APD_{20} (**left**), on plateau voltage ($V_{Plateau}$, **middle**) and on APD_{90} in ERC021 (**right**). Open circles indicate mean values±SD. Numbers in brackets indicate number of EHTs resulting from one differentiation run. * indicates $p<0.05$ vs. 0 RA, one-way ANOVA of log transformed data.

Figure S4: Concentration-dependency of RA on carbachol effects on APD_{90} in EHT

Top: Data for ERC001 (given already in the main manuscript as **Figure 5)** are plotted in grey. **Bottom:** Data for ERC021. Summary of results for the effects of CCh on beating rate (BR**, left**), take-off potential (TOP, **middle**) and APD_{90} (**right**). Gray lines indicate individual EHT. Numbers in brackets indicate number of EHTs resulting from one differentiation run. Open circles indicate mean values±SD. * indicates $p<0.05$ vs. basal (paired t-test). Number of EHTs is given in brackets.