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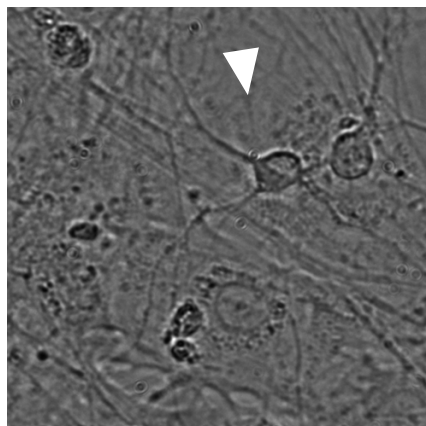
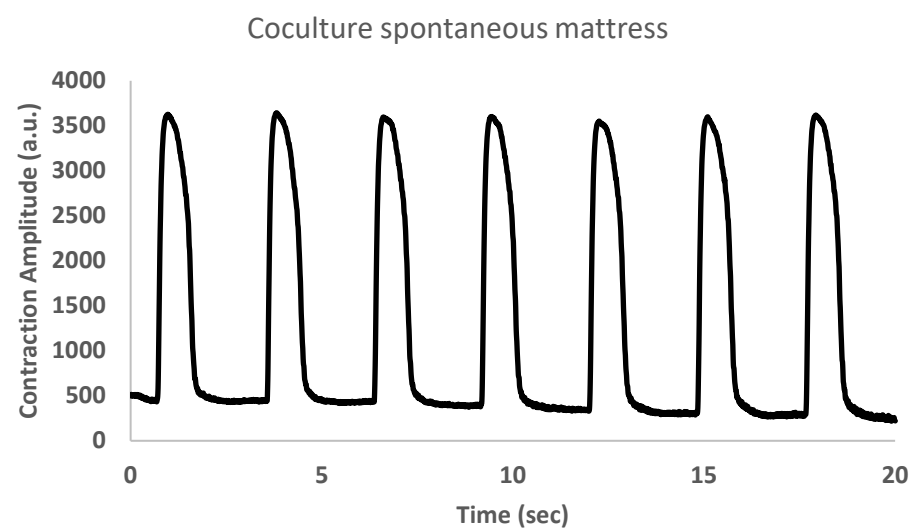
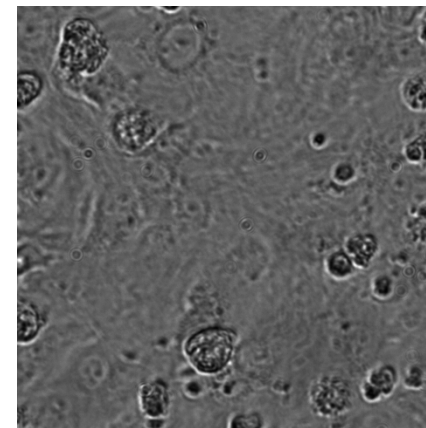
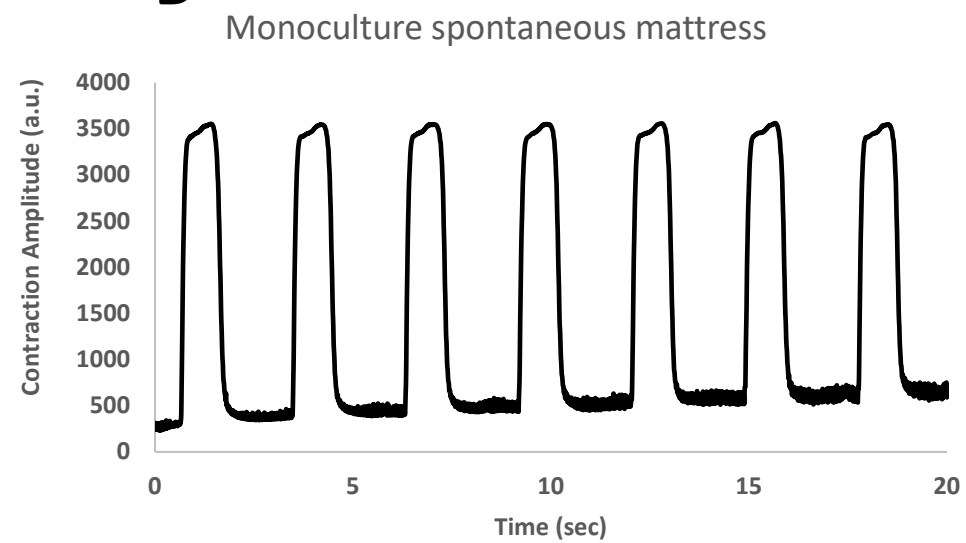
Supplemental Figure S1. Spontaneous contraction. **A.** Representative phase contrast image neurocardiac coculture with neurons in frame indicated by (white arrowhead). **B.** Representative spontaneous contractility trace for neurocardiac coculture on matrigel mattress $n \geq 6$. Similar image and trace for monoculture alone on mattress (**C, D**).

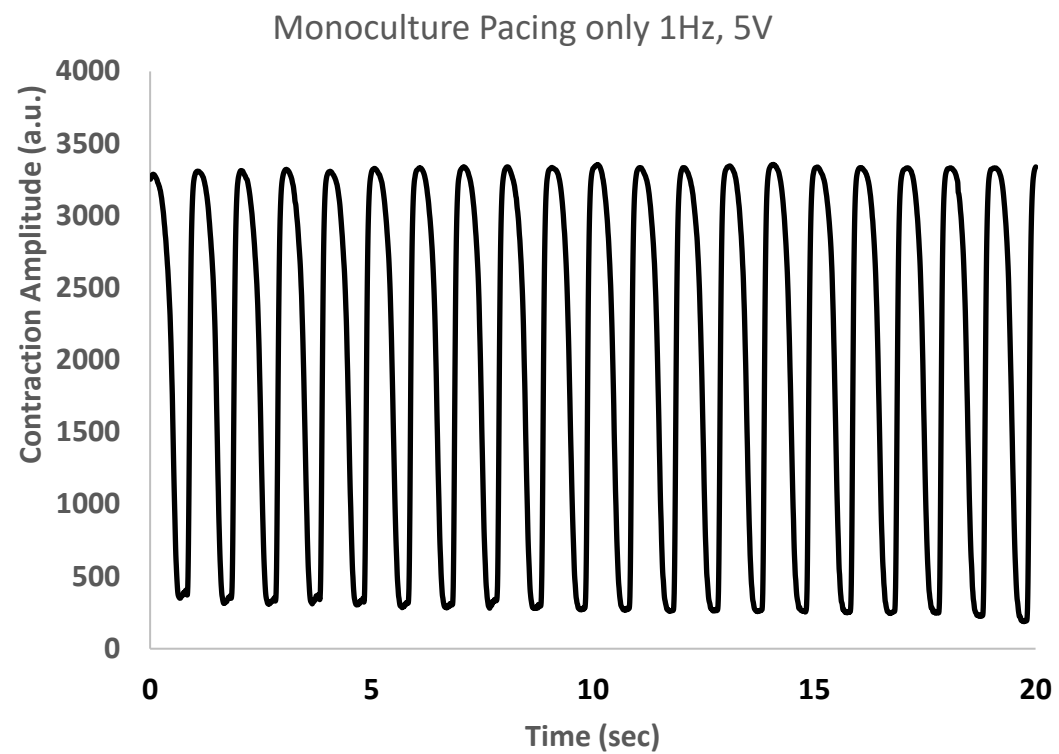
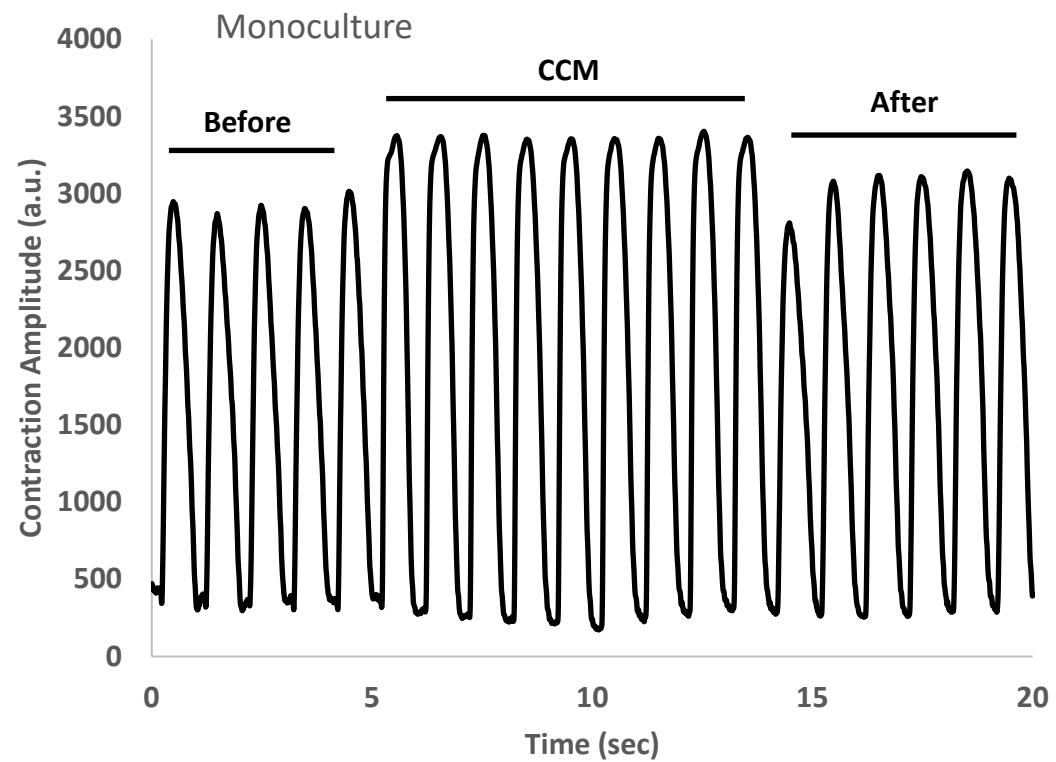
Supplemental Figure S2. Monoculture pacing alone and CCM. **A.** Representative contractility trace of hiPSC-CMs paced at 5V, 1Hz frequency (monophasic) pulses and 2 ms pulse duration, recording 20 sec with 40x objective. **B.** Representative contraction trace recording for monoculture before (5V), during CCM (7V), and after (5V), $n \geq 6$ collected with 20 sec recordings with 40x objective.

Supplemental Figure S3. Additional contractility changes post nicotine and metoprolol treatment. **A.** Quantification for contractility Interval changes post nicotine exposure and metoprolol in coculture and monoculture. **B.** Quantification for Contraction Duration (CD90) post nicotine exposure and metoprolol in coculture and monoculture. Similar summary bar graphs for contractility parameters including Contraction Amplitude and Relaxation Time Dn90 for monoculture and coculture (**C, D**). Data are mean \pm SEM. $n \geq 6$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Figures:

- **Figure S1.** Spontaneous contraction
- **Figure S2.** Monoculture Pacing alone and CCM
- **Figure S3.** Additional contractility changes post nicotine and metoprolol treatment

A**B****C****D****Figure S1**

A**B****Figure S2**

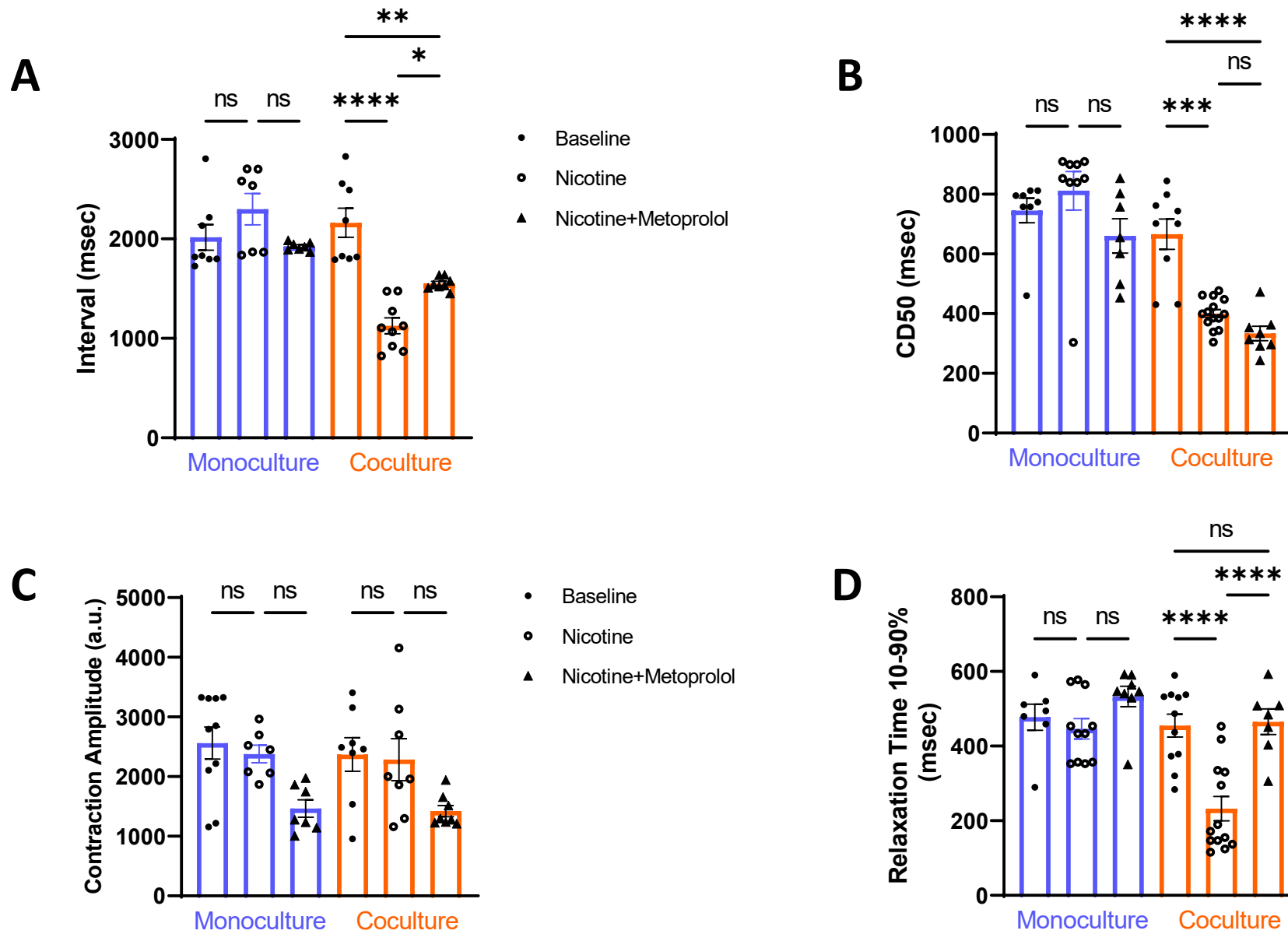


Figure S3

Supplementary Table Legends:

Table S1. Coculture CCM contractile properties. Percent change relative to before (5V), data are mean \pm SEM for all beats in each group CCM (7V), and After (5V). $n \geq 7$. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

Table S2. Monoculture CCM contractile properties. Percent change relative to before (5V), data are mean \pm SEM for all beats in each group CCM (7V), and After (5V). $n \geq 7$. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

Table S3. Baseline coculture contractility. Absolute values of baseline contraction, data are mean \pm SEM; $n \geq 7$, collected with 20 sec recordings with 40x objective.

Table S4. Baseline monoculture contractility. Absolute values of baseline contraction, data are mean \pm SEM; $n \geq 7$, collected with 20 sec recordings with 40x objective.

Supplementary Tables

Table S1: Coculture CCM Contractile Properties (Related to Figure 3)

Parameter	CCM	After
TUp50	$-26 \pm 12\%$ **	$-4 \pm 2\%$
TUp90	$-27 \pm 11\%$ ***	$-8 \pm 5\%$
TDown50	$-12 \pm 1\%$ *	$-3 \pm 1\%$
TDown90	$-15 \pm 5\%$ *	$-5 \pm 2\%$
Contraction Duration CD10	$-16 \pm 6\%$ *	$-5 \pm 3\%$
Contraction Duration CD50	$-8 \pm 1\%$	$-3 \pm 1\%$
Contraction Duration CD90	$-4 \pm 4\%$	$0 \pm 1\%$

Table S2: Monoculture CCM Contractile Properties (Related to Figure 3)

Parameter	CCM	After
TUp50	$-15 \pm 6\%$ *	$-0.5 \pm 3\%$
TUp90	$-16 \pm 5\%$ **	$-1 \pm 2\%$
TDown50	$-4 \pm 1\%$	$0 \pm 1\%$
TDown90	$-12 \pm 2\%$ *	$0 \pm 1\%$
Contraction Duration CD10	$-0.65 \pm 3\%$	$-0.11 \pm 1\%$
Contraction Duration CD50	$-2 \pm 1\%$	$-0.9 \pm 1\%$
Contraction Duration CD90	$-4.7 \pm 1\%$	$3.3 \pm 1\%$

Table S3: Coculture Baseline Contractile Properties (Related to Figure 3, 4 and S1)

Parameter	Values
TUp50 (ms)	93.08 ± 3.6
TUp90 (ms)	163.0 ± 8.6
TDown50 (ms)	802.9 ± 28.1
TDown90 (ms)	535.9 ± 28.6
Contraction Duration CD10 (ms)	477.4 ± 29.5

Table S4: Monoculture Baseline Contractile Properties (Related to Figure 3, 4 and S1)

Parameter	Values
TUp50 (ms)	114.4 ± 5.9
TUp90 (ms)	235.6 ± 20.0
TDown50 (ms)	896.5 ± 7.5
TDown90 (ms)	661.5 ± 20.07
Contraction Duration CD10 (ms)	602.3 ± 19.9