# GPER mediated estrogenic amelioration of sodium channel dysfunction in stressed human induced pluripotent stem cell-derived cardiomyocytes

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## **Running Title**

Estrogen alters cardiac sodium currents through GPER

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Supplemental material Supplementary figures / tables



Supplemental Figure 1 Abundant expressions of cardiac troponin T and decreasing expressions of GPER gene after siRNA interference in hiPSC-CMs. (A) immunofluorescence of cTnT (green) and DAPI (blue). cTnT, cardiac troponin T; Bars=100  $\mu$ m. (B) Relative expression of GPER gene after siRNA knockdown. Unpaired t test was used. \*\*P < 0.01. Data are shown as Mean  $\pm$  S.E.M.



Supplemental Figure 2 ISO elevates sodium currents and alteres  $I_{\rm Na}$  kinetics as well as action potential parameters in hiPSC-CMs. (A) Average  $I_{\rm NaL}$  density. (B) Current-voltage curve of the sodium channel. (C) Peak  $I_{\rm Na}$  density. (D) Time constant of recovery from inactivation curve of the sodium channel (E)  $\tau$  value of recovery from inactivation. (F) Activation and inactivation curve of the sodium channel of hiPSC-CMs. (G-J) Values for k and  $V_{1/2}$  of (in) activation. n=17-37 cells, from 9-11 petri dishes. (K) Representative single AP and slope curve of hiPSC-CMs. (L-Q) Values for APA, RMP, dV /dt max, APD<sub>10</sub>, APD<sub>50</sub> and APD<sub>90</sub>. n=20-34 cells, from 4 petri dishes, separately. An unpaired t test was used. Welch's correction was selectively used in cases of uneven variance. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001. ISO, isoproterenol;  $I_{\rm NaL}$ , late sodium current;  $I_{\rm Na}$ , sodium current; k of (in) activation, slope factor of (in) activation;  $V_{1/2}$  of (in) activation;  $\tau$ , time constant of recovery from inactivation; APA, action potential amplitude; RMP, resting membrane potential; dV /dt max, maximal action potential upstroke velocity; APD<sub>10, 50, 90</sub>, action potential duration at 10%, 50% and 90% repolarization. pA, pico Ampere; pF, pico Farad. Insets: voltage clamp protocols.



Supplemental Figure 3 Effects of estrogen on the time constant of recovery from inactivation of sodium current in hiPSC-CMs. (A and B) Representative traces were showed in each group. Insets: voltage clamp protocols.



Supplemental Figure 4 E2 modifies the action potential parameters of hiPSC-CMs. (A-C) Typical APs and slope curve of hiPSC-CMs. (D-I) Values for APA, RMP, dV /dt max, APD<sub>10</sub>, APD<sub>50</sub> and APD<sub>90</sub>. n=19-40 cells, from 4-6 petri dishes. \*p < 0.05, \*\*p < 0.01, \*\*\*\*p < 0.001.



Supplemental Figure 5 E2 improves ISO induced abnormal action potential parameters of hiPSC-CMs. (A-C) Typical APs and slope curve of hiPSC-CMs. (D-I) Values for APA, RMP, dV /dt max, APD<sub>10</sub>, APD<sub>50</sub> and APD<sub>90</sub>. n=22-34 cells, from 4-5 petri dishes. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.



Supplemental Figure 6 Effects of GPER on the time constant of recovery from inactivation of sodium current in hiPSC-CMs. (A and B) Representative traces were showed in each group. Insets: voltage clamp protocols.



Supplemental Figure 7 Silence of GPER cancels the effect of estrogen on regulating peak  $I_{\rm Na}$  and  $I_{\rm NaL}$ . (A) Average  $I_{\rm NaL}$  density. n=10-25 cells, from 4-9 Petri dishes. (B) Peak  $I_{\rm Na}$  density. n=13-32 cells, from 4-11 Petri dishes. (C-F) Values for k and  $V_{1/2}$  of (in) activation. n=9-26 cells, from 4-11 Petri dishes. (G)  $\tau$  value of recovery from inactivation. n=5-17 cells, from 4-10 Petri dishes. (H) Average  $I_{\rm NaL}$  density. n=9-26 cells, from 4-10 Petri dishes. (I) Peak  $I_{\rm Na}$  density. n=12-37 cells, from 4-11 Petri dishes. (J-M) Values for k and  $V_{1/2}$  of (in) activation. n=8-34 cells, from 4-11 Petri dishes. (N)  $\tau$  value of recovery from inactivation. n=5-32 cells, from 5-11 Petri dishes. One-way ANOVA was used. Kruskal-Wallis test was used selectively in the case of uneven variance. \*P < 0.05, \*\*P < 0.01, \*\*\*\*P < 0.0001. GPER, G protein coupled estrogen receptor. Insets: voltage clamp protocols.



Supplemental Figure 8 Effects of silenced GPER on the time constant of recovery from inactivation of sodium current in hiPSC-CMs. (A and B) Representative traces were showed in each group. Insets: voltage clamp protocols.

Supplemental Table 1 : Details in values of statistical analysis and the number of cells for average  $I_{\rm NaL}$  density.

Group	Mean $\pm$ S.E.M	n (cells)	Petri dishes
Control	$-1.409 \pm 0.1558$	25	9
0.01  nmol/L E2	$-2.299 \pm 0.4903$	8	3
1  nmol/L E2	$-2.386 \pm 0.4755$	10	4
100  nmol/L E2	$-1.938 \pm 0.2423$	14	4
ISO	$-2.600 \pm 0.2790$	26	10
0.01  nmol/L E2+ISO	$-2.209 \pm 0.4861$	12	4
1  nmol/L E2+ISO	$-1.108 \pm 0.2739$	9	6

Group	Mean $\pm$ S.E.M	n (cells)	Petri dishes
100 nmol/L E2+ISO	$-1.290 \pm 0.2608$	9	4
G1	$-1.547 \pm 0.1888$	13	4
G15	$-1.287 \pm 0.2632$	12	4
G15+E2	$-2.426 \pm 0.3696$	20	4
G1+ISO	$-1.201 \pm 0.1890$	16	4
G15+ISO	$-2.284 \pm 0.3592$	10	4
G15+E2+ISO	$-2.644 \pm 0.3551$	12	4
Negative GPER-siRNA	$-1.218 \pm 0.1891$	19	5
GPER-siRNA	$-1.600 \pm 0.2371$	18	4
GPER-siRNA+E2	$-1.572 \pm 0.2237$	18	5
GPER-siRNA+ISO	$-2.417 \pm 0.3920$	15	4
GPER-siRNA+E2+ISO	$-2.559 \pm 0.3509$	18	4

 $I_{\text{NaL}}$ , late sodium current; E2,  $\beta$ -Estradiol; ISO, isoproterenol; G1, G protein-coupled estrogen receptor agonists; G15, G protein-coupled estrogen receptor antagonists; GPER, G protein-coupled estrogen receptor.

Suppler	mental	Tabl	e 2	:	Detail	s in	val	ues	of	statistical	anal	lysis	and	$^{\mathrm{the}}$	e num	$\mathbf{ber}$	of	cells	s f	or	peak	ΞI	Na
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Group	Mean $\pm$ S.E.M	n (cells)	Petri dishes
Control	$-577.7 \pm 40.21$	32	11
0.01  nmol/L E2	$-1086 \pm 119.4$	10	4
1  nmol/L E2	$-1191 \pm 133.8$	13	4
100  nmol/L E2	$-865.3 \pm 82.75$	14	4
ISO	$-811.8 \pm 43.41$	37	11
0.01  nmol/L E2+ISO	$-793.5 \pm 74.50$	12	4
1  nmol/L E2+ISO	$-384.2 \pm 41.11$	12	6
100  nmol/L E2+ISO	$-588.0 \pm 69.50$	13	4
G1	$-885.8 \pm 75.24$	9	4
G15	$-651.3 \pm 57.12$	17	4
G15+E2	$-676.6 \pm 74.12$	21	4
G1+ISO	$-536.5 \pm 44.83$	16	4
G15+ISO	$-793.8 \pm 103.4$	13	4
G15+E2+ISO	$-812.7 \pm 56.97$	19	4
Negative GPER-siRNA	$-466.3 \pm 82.09$	17	5
GPER-siRNA	$-603.8 \pm 54.23$	15	4
GPER-siRNA+E2	$-462.6 \pm 53.29$	17	5
GPER-siRNA+ISO	$-714.5 \pm 61.06$	13	4
GPER-siRNA+E2+ISO	$-694.2 \pm 66.75$	14	4

 $I_{\text{Na}}$ , sodium current; E2,  $\beta$ -Estradiol; ISO, isoproterenol; G1, G protein-coupled estrogen receptor agonists; G15, G protein-coupled estrogen receptor antagonists; GPER, G protein-coupled estrogen receptor.

Supplemental Table 3: Details in values of statistical analysis and the number of cells for k of activation.

Group	Mean $\pm$ S.E.M	n (cells)	Petri dishes
Control	$0.6123 \pm 0.07342$	24	10
0.01  nmol/L E2	$0.4784 \pm 0.03594$	8	4
1  nmol/L E2	$0.5839 \pm 0.1201$	12	4
100  nmol/L E2	$0.5838\pm0.1079$	12	4
ISO	$0.6146\pm0.09461$	34	11
0.01  nmol/L E2+ISO	$1.018\pm0.3049$	9	4
1  nmol/L E2+ISO	$0.9925 \pm 0.4239$	8	5
100  nmol/L E2+ISO	$1.217 \pm 0.3018$	10	4
G1	$0.5483 \pm 0.1588$	8	4
G15	$0.6775\pm0.09204$	15	4
G15+E2	$0.5658\pm0.08662$	15	4
G1+ISO	$0.4632 \pm 0.1084$	13	4
G15+ISO	$0.5109\pm0.08299$	12	4
G15+E2+ISO	$0.6402\pm0.1313$	18	4
Negative GPER-siRNA	$0.8718 \pm 0.1446$	15	5
GPER-siRNA	$0.7959\pm0.09635$	10	4
GPER-siRNA+E2	$1.353 \pm 0.3517$	13	5
GPER-siRNA+ISO	$0.3773 \pm 0.02849$	10	4
GPER-siRNA+E2+ISO	$0.6217\pm0.2054$	9	4

k of activation, slope factor of activation; E2,  $\beta$ -Estradiol; ISO, isoproterenol; G1, G protein-coupled estrogen receptor agonists; G15, G protein-coupled estrogen receptor antagonists; GPER, G protein-coupled estrogen receptor.

Supplemental Table 4 : Details in values of statistical analysis and the number of cells for  $V_{1/2}$  of activation.

Group	Mean $\pm$ S.E.M	n (cells)	Petri dishes
Control	$-51.95 \pm 0.9018$	24	10
0.01  nmol/L E2	$-52.63 \pm 2.250$	8	4
1  nmol/L E2	$-51.37 \pm 1.660$	12	4
100  nmol/L E2	$-54.44 \pm 1.769$	12	4
ISO	$-56.16 \pm 1.297$	34	11
0.01  nmol/L E2+ISO	$-51.65 \pm 1.385$	9	4
1 nmol/L E2+ISO	$-52.42 \pm 2.842$	8	5
100 nmol/L E2+ISO	$-53.00 \pm 2.709$	10	4
G1	$-54.60 \pm 2.306$	8	4
G15	$-51.14 \pm 1.310$	15	4
G15+E2	$-49.89 \pm 1.385$	15	4
G1+ISO	$-56.98 \pm 1.607$	13	4
G15+ISO	$-57.33 \pm 1.956$	12	4
G15+E2+ISO	$-55.11 \pm 1.302$	18	4
Negative GPER-siRNA	$-50.91 \pm 0.9862$	15	5
GPER-siRNA	$-47.32 \pm 1.015$	10	4
GPER-siRNA+E2	$-53.44 \pm 2.278$	13	5
GPER-siRNA+ISO	$-56.68 \pm 1.960$	10	4
GPER-siRNA+E2+ISO	$-55.68 \pm 1.644$	9	4

 $V_{1/2}$  of activation, half-voltage of activation; E2,  $\beta$ -Estradiol; ISO, isoproterenol; G1, G protein-coupled estrogen receptor agonists; G15, G protein-coupled estrogen receptor antagonists; GPER, G protein-coupled estrogen receptor.

Mean $\pm$ S.E.M	n (cells)	Petri dishes
$4.255 \pm 0.08697$	26	11
$4.123 \pm 0.04982$	9	4
$4.074 \pm 0.08570$	9	4
$4.174 \pm 0.08291$	13	4
$4.278 \pm 0.09169$	33	11
$4.150\pm0.1370$	9	4
$4.243 \pm 0.1257$	8	5
$4.106 \pm 0.08571$	12	4
$4.070\pm0.09689$	9	4
$4.225 \pm 0.09732$	14	4
$4.301 \pm 0.1188$	17	4
$4.195 \pm 0.1151$	15	4
$4.177 \pm 0.1031$	13	4
$4.302 \pm 0.1036$	17	4
$4.583 \pm 0.1292$	12	5
$4.638 \pm 0.08516$	10	4
$4.490 \pm 0.1358$	10	4
$4.275 \pm 0.2412$	9	4
$4.499\pm0.1053$	13	4
	$\begin{array}{r} \text{Mean} \pm \text{S.E.M} \\ \hline 4.255 \pm 0.08697 \\ 4.123 \pm 0.04982 \\ 4.074 \pm 0.08570 \\ 4.174 \pm 0.08291 \\ 4.278 \pm 0.09169 \\ 4.150 \pm 0.1370 \\ 4.243 \pm 0.1257 \\ 4.106 \pm 0.08571 \\ 4.070 \pm 0.09689 \\ 4.225 \pm 0.09732 \\ 4.301 \pm 0.1188 \\ 4.195 \pm 0.1151 \\ 4.177 \pm 0.1031 \\ 4.302 \pm 0.1036 \\ 4.583 \pm 0.1292 \\ 4.638 \pm 0.08516 \\ 4.490 \pm 0.1358 \\ 4.275 \pm 0.2412 \\ 4.499 \pm 0.1053 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

Supplemental Table 5: Details in values of statistical analysis and the number of cells for k of inactivation.

k of inactivation, slope factor of inactivation; E2,  $\beta$ -Estradiol; ISO, isoproterenol; G1, G protein-coupled estrogen receptor agonists; G15, G protein-coupled estrogen receptor antagonists; GPER, G protein-coupled estrogen receptor.

Supplemental Table 6 : Details in values of statistical analysis and the number of cells for  $V_{1/2}$  of inactivation.

Group	Mean $\pm$ S.E.M	n (cells)	Petri dishes		
Control	$-68.06 \pm 0.6328$	26	11		
0.01  nmol/L E2	$-69.75 \pm 0.9422$	9	4		
1  nmol/L E2	$-69.36 \pm 0.6028$	9	4		
100  nmol/L E2	$-66.86 \pm 0.9896$	13	4		
ISO	$-70.54 \pm 0.8001$	33	11		
0.01  nmol/L E2+ISO	$-67.75 \pm 1.809$	9	4		
1  nmol/L E2+ISO	$-69.77 \pm 1.604$	8	5		
100 nmol/L E2+ISO	$-69.41 \pm 1.444$	12	4		
G1	$-70.03 \pm 0.9939$	9	4		
G15	$-69.32 \pm 0.7582$	14	4		
G15+E2	$-70.11 \pm 0.9584$	17	4		

Group	Mean $\pm$ S.E.M	n (cells)	Petri dishes
G1+ISO	$-68.95 \pm 0.7643$	15	4
G15+ISO	$-69.87 \pm 0.7944$	13	4
G15+E2+ISO	$-71.15 \pm 0.7586$	17	4
Negative GPER-siRNA	$-71.58 \pm 1.278$	12	5
GPER-siRNA	$-69.61 \pm 0.8926$	10	4
GPER-siRNA+E2	$-68.98 \pm 1.557$	10	4
GPER-siRNA+ISO	$-66.90 \pm 1.183$	9	4
GPER-siRNA+E2+ISO	$-69.36 \pm 1.231$	13	4

 $V_{1/2}$  of inactivation, half-voltage of inactivation; E2,  $\beta$ -Estradiol; ISO, isoproterenol; G1, G protein-coupled estrogen receptor agonists; G15, G protein-coupled estrogen receptor antagonists; GPER, G protein-coupled estrogen receptor.

Supplemental Table 7 : Details in values of statistical analysis and the number of cells for  $\tau$  of recovery from inactivation.

Group	Mean $\pm$ S.E.M	n (cells)	Petri dishes
Control	$3.544 \pm 0.2019$	17	10
0.01  nmol/L E2	$3.594 \pm 0.1980$	8	4
1  nmol/L E2	$3.641 \pm 0.2212$	10	4
100  nmol/L E2	$3.350 \pm 0.4407$	11	4
ISO	$4.522 \pm 0.2312$	32	11
0.01  nmol/L E2+ISO	$4.518\pm0.6411$	8	3
1  nmol/L E2+ISO	$3.291 \pm 0.3461$	5	4
100  nmol/L E2+ISO	$4.553 \pm 0.6519$	8	4
G1	$5.207 \pm 0.7400$	7	3
G15	$4.973 \pm 0.2979$	16	4
G15+E2	$4.717 \pm 0.4154$	15	4
G1+ISO	$4.147 \pm 0.2508$	12	4
G15+ISO	$4.116\pm0.3768$	11	4
G15+E2+ISO	$4.991\pm0.2917$	14	4
Negative GPER-siRNA	$4.676\pm0.2341$	5	4
GPER-siRNA	$4.633 \pm 0.4730$	8	4
GPER-siRNA+E2	$4.779\pm0.5478$	8	4
GPER-siRNA+ISO	$3.386\pm0.1518$	8	4
GPER-siRNA+E2+ISO	$3.924 \pm 0.2886$	8	4

 $\tau$ , time constant of recovery from inactivation; E2,  $\beta$ -Estradiol; ISO, isoproterenol; G1, G protein-coupled estrogen receptor agonists; G15, G protein-coupled estrogen receptor antagonists; GPER, G protein-coupled estrogen receptor.

Supplemental Table 8: Details in values of statistical analysis and the number of cells for parameters of action potential.

Group	APA (mV)	RMP (mV)	$\mathrm{d}V/\mathrm{d}t_{\mathrm{max}} \; (\mathrm{mV/ms})$	$APD_{10} (ms)$	$APD_{50}$ (
Control (n=20-25 cells)	$113.8\pm0.9386$	$-69.83 \pm 0.4187$	$27.70 \pm 0.6309$	$117.3 \pm 4.879$	302.6 $\pm$
0.01  nmol/L E2 (n=31-34  cells)	$110.1 \pm 1.133$	$-68.17 \pm 0.7299$	$24.02 \pm 0.8293$	$127.2 \pm 8.815$	326.6 $\pm$
1  nmol/L E2 (n=19-21  cells)	$107.9 \pm 1.021$	$-67.57 \pm 1.012$	$20.35 \pm 0.9915$	$143.2 \pm 9.363$	376.6 $\pm$
100  nmol/L E2 (n=35-40  cells)	$108.4 \pm 0.9901$	$-66.57 \pm 0.7071$	$23.41 \pm 0.7410$	$130.8 \pm 5.379$	341.6 $\pm$
ISO $(n=29-34 \text{ cells})$	$109.4 \pm 1.482$	$-69.64 \pm 0.6279$	$21.74 \pm 0.7582$	$97.59 \pm 5.575$	264.5 $\pm$
0.01  nmol/L E2+ISO (n=22-28  cells)	$106.9 \pm 1.189$	$-64.09 \pm 0.7868$	$19.00 \pm 1.284$	$115.1 \pm 6.775$	326.4 $\pm$
1  nmol/L E2+ISO (n=24-27 cells)	$109.7 \pm 1.230$	$-66.91 \pm 0.5038$	$23.50 \pm 0.9637$	$109.8 \pm 5.416$	325.6 $\pm$
$100 \text{ nmol/L E2+ISO} (n{=}24{\text{-}}26 \text{ cells})$	$113.8 \pm 1.434$	$-69.04 \pm 0.7847$	$21.82 \pm 0.9924$	$118.5 \pm 5.812$	363.7 $\pm$

APA, action potential amplitude; RMP, resting membrane potential;  $dV/dt_{max}$ , maximal action potential upstroke velocity; APD<sub>10</sub>, action potential duration at 10% repolarization; APD<sub>50</sub>, action potential duration at 50% repolarization; APD<sub>90</sub>, action potential duration at 90% repolarization. E2,  $\beta$ -Estradiol; ISO, isoproterenol. Control, 0.01 nmol/L E2, 1 nmol/L E2, 100 nmol/L E2, ISO, 0.01 nmol/L E2+ISO, 1 nmol/L E2+ISO and 100 nmol/L E2+ISO group originated in 4, 5, 4, 6, 4, 5, 4, 4 Petri dishes respectively.