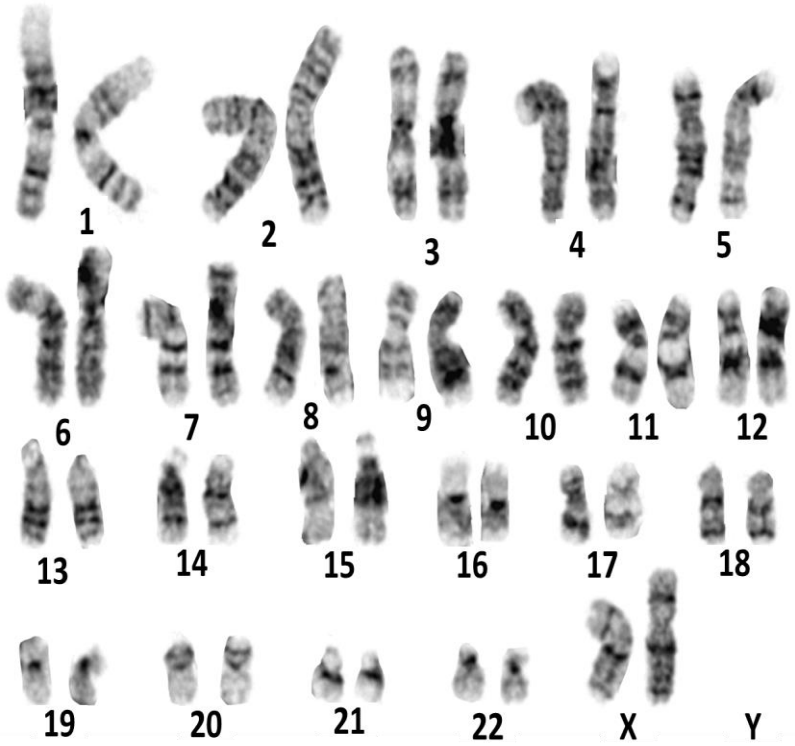
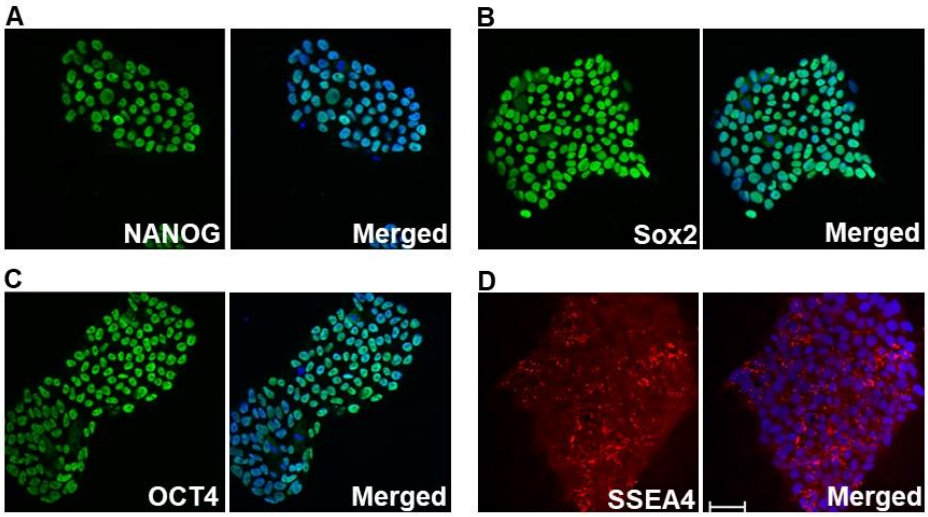
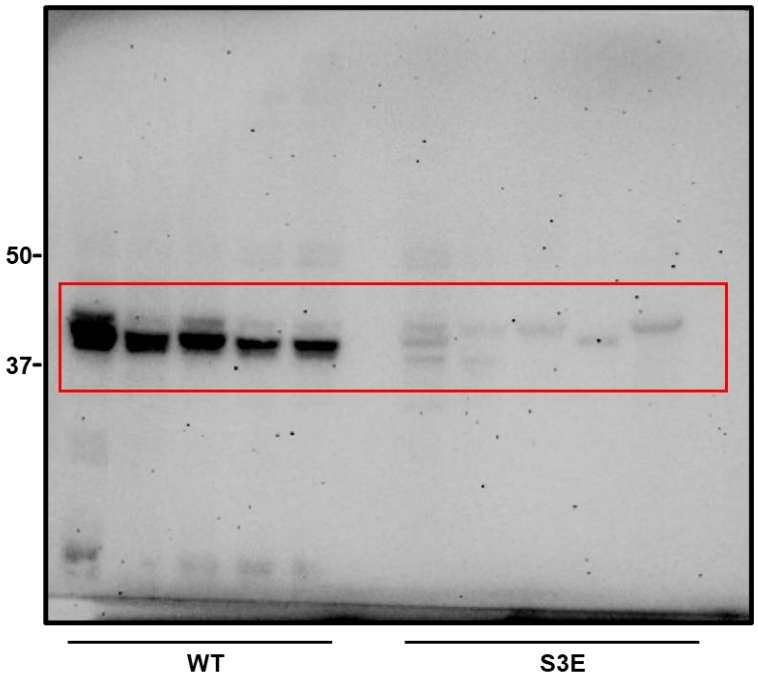
Supplemental information



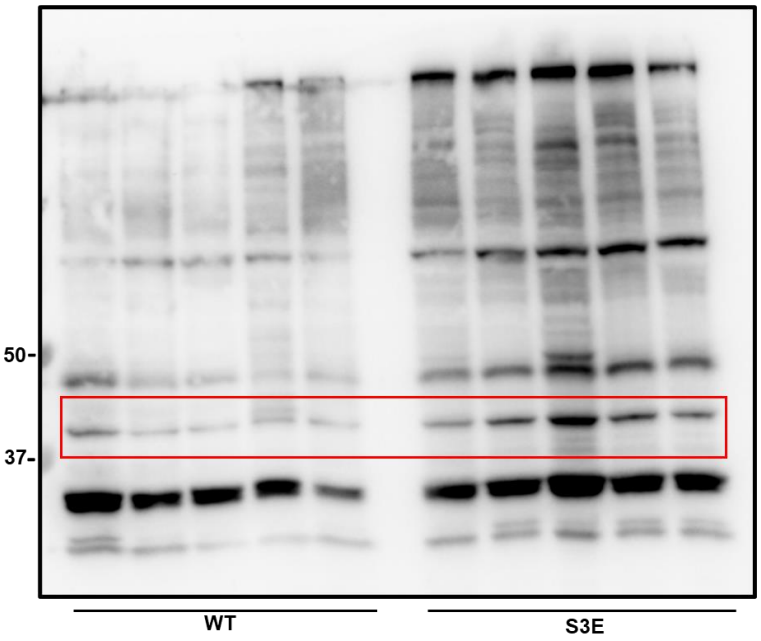
Supplemental Figure S1. Cx43-S3E hPSCs karyotype. G-banded karyotype of Cx43-S3E hPSCs demonstrating a normal (46, XX) karyotype.



Supplemental Figure S2 Cx43-S3E hPSCs express expected markers of pluripotency. Representative confocal photomicrographs of undifferentiated Cx43-S3E hPSCs confirming strong expression of pluripotency markers NANOG (**A**), SOX2 (**B**), OCT4 (**C**), and SSEA4 (**D**).



Supplemental Figure S3. Full length blot detecting total Cx43 protein. Molecular ladders are depicted to the left.



Supplemental Figure S4. Full length blot detecting pCx43 (pS279/282) protein. Molecular ladders are depicted to the left.

Supplemental Video S1. Spontaneous beating of compact hPSC-CM monolayers. Representative videos of WT **(A)** and Cx43-S3E **(B)** hPSC-CMs at 20 days post differentiation.

Supplemental Table 1: Primary antibodies employed.

Antigen	Antibody type	Titre	Vendor
CD56	Mouse Monoclonal (Cat#555518)	1:100	BD Biosciences

CD140a	Mouse Monoclonal (Cat# 556002)	1:100	BD Biosciences
Myosin light chain-2v (MLC2v)	Monoclonal human recombinant (clone REA401)	1:100	Miltenyi Biotec
Cardiac troponin T (cTnT)	Monoclonal human recombinant (clone REA400)	1:100	Miltenyi Biotec
Connexin-43 (Cx43)	Rabbit polyclonal (Cat # ab11370)	1:800	Abcam
Sarcomeric α-actinin	Mouse monoclonal (clone EA-53)	1:500	Sigma
SSEA4	Mouse monoclonal (Cat#4755)	1:500	Cell Signaling Technology
Sox2	Rabbit monoclonal (Cat#3579)	1:400	Cell Signaling Technology
OCT4	Rabbit monoclonal (Cat#2840)	1:200	Cell Signaling Technology
NANOG	Rabbit monoclonal (Cat#4903)	1:200	Cell Signaling Technology

Supplemental Table 2: List of primers used.

Target	Forward	Reverse
Cx30	CGTACACCAGCAGCATCTT	CGTCTGTGCTCTCTTTGATCTC
Cx40	AGAGTGTGAAGAAGCCCACG	AACAGATGCCAAAACTTCTGCT
Cx40.1	AGCGAAAAGCTGGGCAGAC	TCACACCCACTCAGACTTCCT
Cx43	GGTGACTGGAGCGCCTTAG	GCGCACATGAGAGATTGGGA
Cx45	GTCCACCCGTTTTATGTGTGC	AGTGAGTCTCGAATGGTCCCA
Cx58	GAAATGCCTAGGGATCGGA	AACCTAGGTGGAAAATTTCAAGA
Cx62	AAGGATGTCTGCTGCGTACTTA	GCCTGTTTCATCCTCAATGC
TNNI3	CCTCAAGCAGGTGAAGAAGG	CAGTAGGCAGGAAGGCTCAG
TNNT2	AGCATCTATAACTTGGAGGCAGAG	TGGAGACTTTCTGCTTATCGTTG
MYH6	GATAGAGAGACTCCTGCGGC	CCGTCTTCCCATTCTCGGTT
MYH7	TCGTGCCTGATGACAAACAGGAGT	ATACTCGGTCTCGGCAGTGACTTT
KCNQ1	CGCCTGAACCGAGTAGAAGA	TGAAGCATGTCGGTGATGAG
HCN1	CATGCCACCGCTTTAATCCAG	ATTGTAGCCACCAGTTTCCGA
HNC2	AGAAGGGCATTGACTCCGAG	TAGCGGATCAGGCGTGAGA
HCN4	TCTTCCTCATTGTGGAGACACGCA	TGAGGATCTTCGTGAAGCGGACAA
KCNH2	CAACCTGGGCGACCAGATAG	GGTGTTGGGAGAGACGTTGC
KCNJ8	GTGATTGCCGTCCGAAATGG	AGTTGGTGAATAGGAACCACCT
KCNJ2	CTTTGTAGTGCCAGAGACTTAG	CACTGTCGTCTTCCTCTTTG
KCND2	CTACCTGTTCCGGTGATTGTATCC	TCTTTTGTGCCCTTCGTTTGT
SCN5A	TCTCTATGGCAATCCACCCCA	GAGGACATACAAGGCGTTGGT
ATP2A2	CATCAAGCACACTGATCCCGT	CCACTCCCATAGCTTTCCCAG
CACNA1C	TGATTCCAACGCCACCAATTC	GAGGAGTCCATAGGCGATTACT
CAMKIIB	GCACACCAGGCTACCTGTC	CATACGCCTCTTTGCGAAGG
PLN	ACCTCACTCGCTCAGCTATAA	CATCACGATGATACAGATCAGCA
TRDN	TCACAGAAGACATAGTGACGACG	TGGCAATAGAGCTTGCTGAAA
RYR2	CATCGAACACTCCTCTACGGA	GGACACGCTAACTAAGATGAGGT
JPH2	ACTCTGGCTCCTGGAACTTTG	GCGCCCCTTGGTCTCTATG
DDB1	TCAACGGCATGATAGGGCTG	CGCTCGGTGTGAAAGGATCT