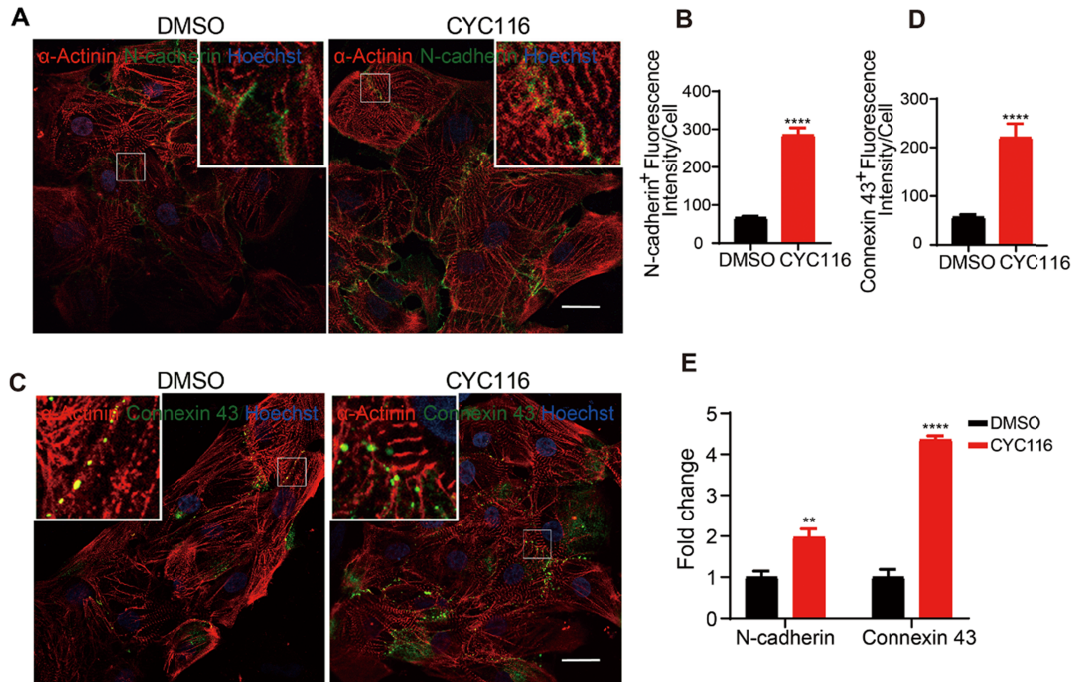


Supplementary Table S1. Primers for cardiomyocyte specific expression genes used in qRT-PCR

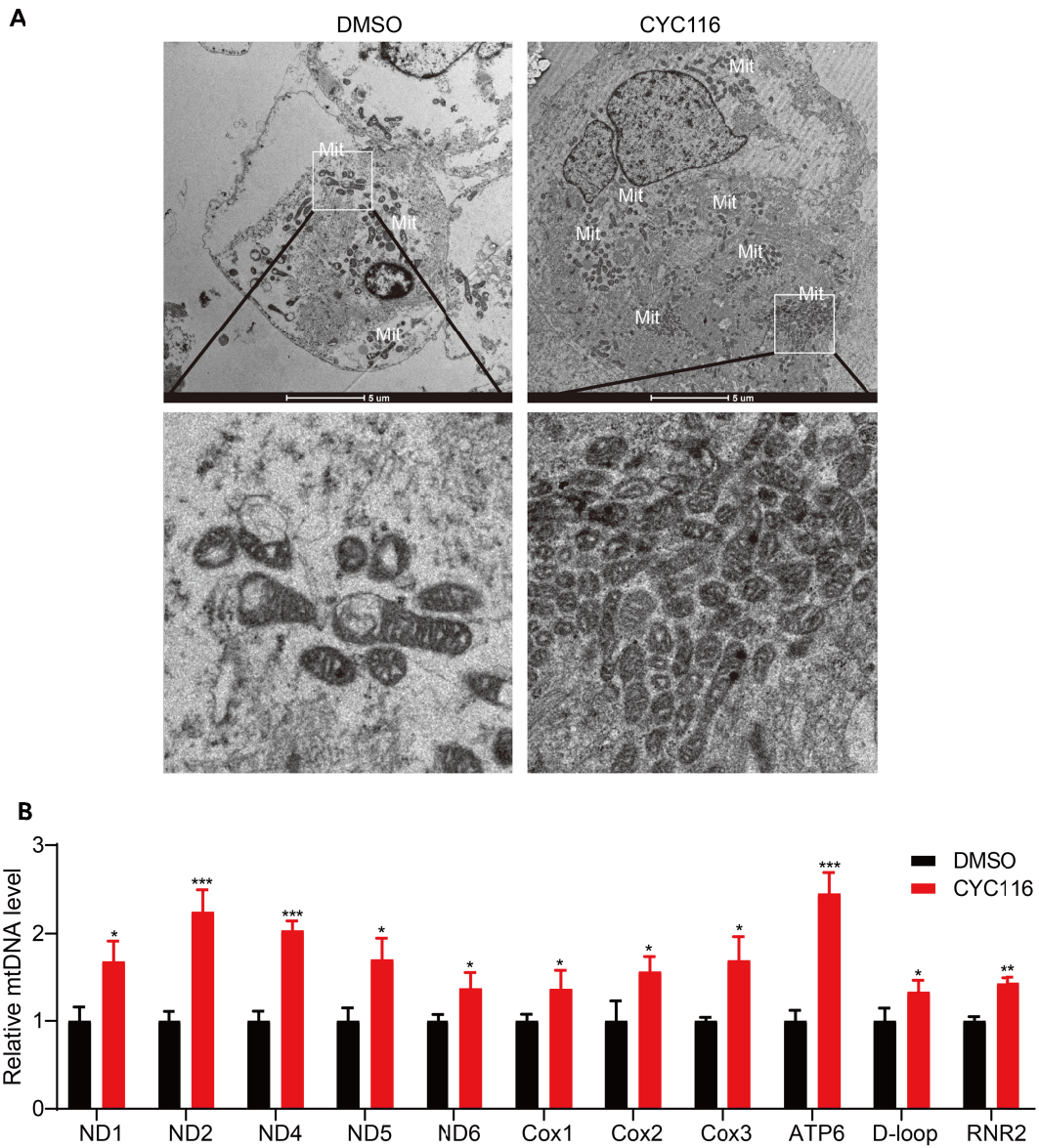
Primers	Sequence (5'-3')
TNNT2 F	GCGGGTCTTGGAGACTTTCT
TNNT2 R	TTCGACCTGCAGGAGAAGTT
MYH6 F	CTTCTCCACCTTAGCCCTGG
MYH6 R	GCTGGCCCTCAACTACAGA
MYH7 F	CGCACCTTCTTCTTGCTC
MYH7 R	GAGGACAAGGTCAACACCCT
MYL7 F	GCCCAACGTGGTTCTTCAA
MYL7 R	CTCCTCTCTGGGACACTC
MYL2 F	CGTTCTGTCAATGAAGCCA
MYL2 R	CAACGTGTCTCCATGTTCG
TNNI1 F	CCGGAAGTCGAGAGAAAACCC
TNNI1 R	TCAATGTCGTATCGTCCTCA
TNNI3 F	TTTGACCTTCGAGGCAAGTTT
TNNI3 R	CCCGGTTTTCTTCTCGGTG
GJA5 F	AGAGTGTGAAGAAGCCCACG
GJA5 R	AACAGATGCCAAAACCTTCTGCT
KCNH2 F	CAACCTGGGCGACCAGATAG
KCNH2 R	GGTGTGGGAGAGACGTTGC
KCND3 F	GCATCCTCCCGGAGATCATC
KCND3 R	CCGAGTCGTTGTGTCGTCAT
KCNQ1 F	TGTCCACCATCGAGCAGTATG
KCNQ1 R	CCGTCCCGAAGAACCAC
KCNJ2 F	CTGGCTTTCGTCTGTATGG
KCNJ2 R	GCCCACGATTGACTGGAACA
CACNA1C F	TGATTCCAACGCCACCAATTC
CACNA1C R	GAGGAGTCCATAGGCGATTACT
RYR2 F	CATCGAACACTCCTCTACGGA
RYR2 R	GGACACGCTAACTAAGATGAGGT
SERCA2a F	TTTCTACAGTGTAAGAGGACAACC
SERCA2a R	TTCCAGGTAGTTGCGGGCCACAAA
SCN5A F	AGCTGGCTGATGTGATGGTC
SCN5A R	CACTTGTGCCTTAGGTTGCC
PPARGC1A F	GCTTCTGGGTGGACTCAAGT
PPARGC1A R	GAGGGCAATCCGTCTTCATCC
TFAM F	ATGGCGTTTCTCCGAAGCAT
TFAM R	TCCGCCCTATAAGCATTTGA
N-cadherin F	TGAGCCTGAAGCCAACCTTA
N-cadherin R	AGGTCCCCTGGAGTTTTCTG
Connexin 43 F	CAATCACTTGGCGTGACTTC
Connexin 43 R	AAAGGCAGACTGTCATCTC
GAPDH F	CTGGGCTACACTGAGCACC
GAPDH R	AAGTGGTCGTTGAGGGCAATG
Nd1 F	AACCCGCCACATCTACCATC
Nd1 R	AGGAGGCCTAGGTTGAGGTT
Nd2 F	TCAGCTAAATAAGCTATCGGGC
Nd2 R	GAGTGGGGTTTTGCAGTCCT
Nd4 F	CAGCCACATAGCCCTCGTAG
Nd4 R	GCGAGGCTTGCTAGAAGTCA
Nd5 F	CTGCTAACTCATGCCCCAT
Nd5 R	GGAGGATCCTATTGGTGCGG
Nd6 F	CCTCTTTCTTCTTCCCCTCA
Nd6 R	CGATGGTTTTTCATATCATTGGTCCG

Supplementary Table S1. Continued

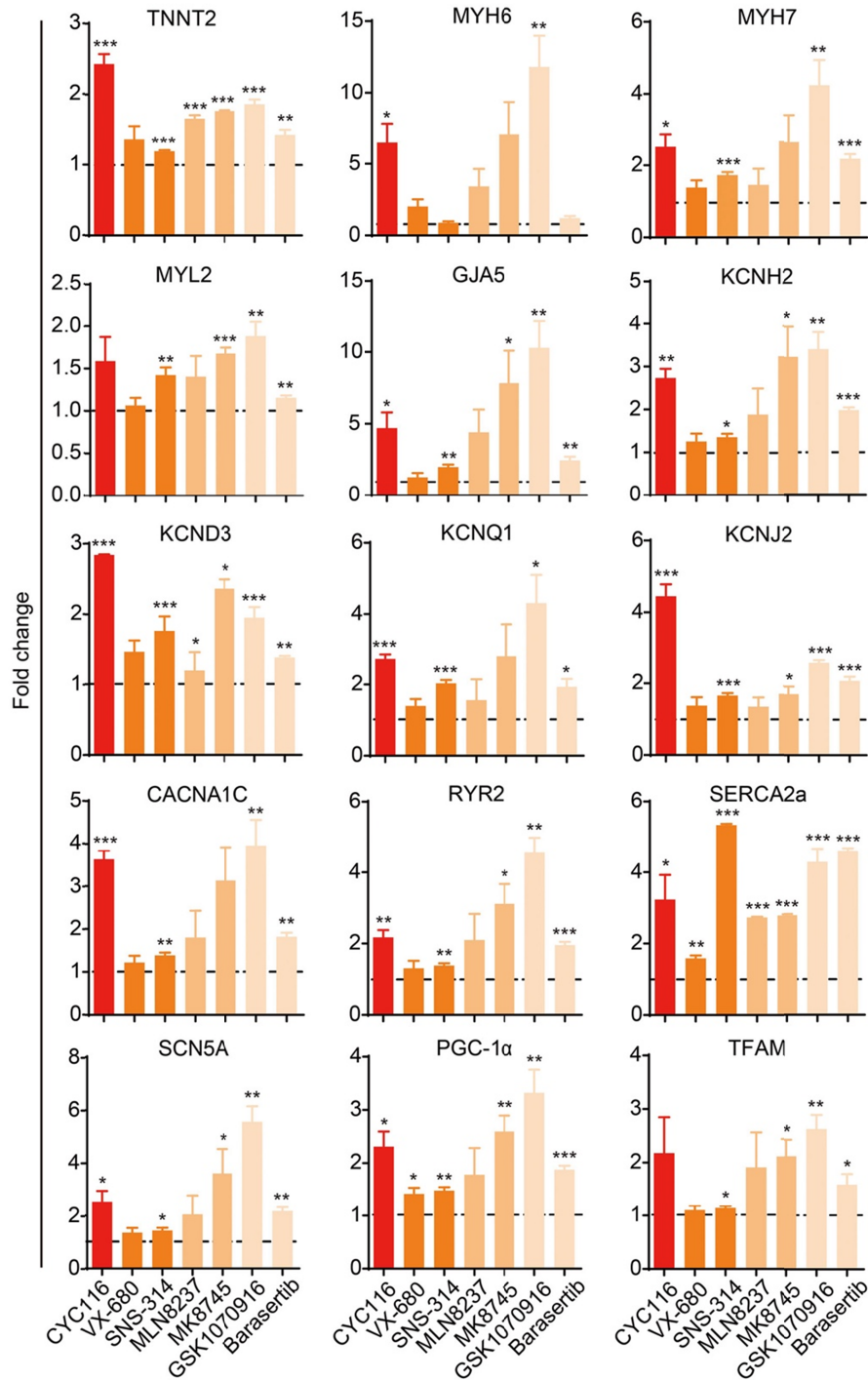
Primers	Sequence (5'-3')
Cox1 F	CCGACCGTTGACTATTCTCT
Cox1 R	GCTCAGACCATACCTATGTA
Cox2 F	CAAACCACTTTCACCGCTACAC
Cox2 R	GGACGATGGGCATGAAACTGT
Cox3 F	AGGCATCACCCCGCTAAATC
Cox3 R	GGTGAGCTCAGGTGATTGATACTC
Atp6 F	AGCCCACTTCTTACCACAAG
Atp6 R	TACTATATGATAGGCATGTGA
D-loop F	CTCCACCATTAGCACCCAAA
D-loop R	GGTGAACACTGGAACGGG
RNR2 F	CCAAACCCACTCCACCTTAC
RNR2 R	TCATCTTTCCTTGCGGTA
β-globin F	CTGGGCATGTGGAGACAGAGAAGACT
β-globin R	AGGCCATCACTAAAGGCCACCGAGC



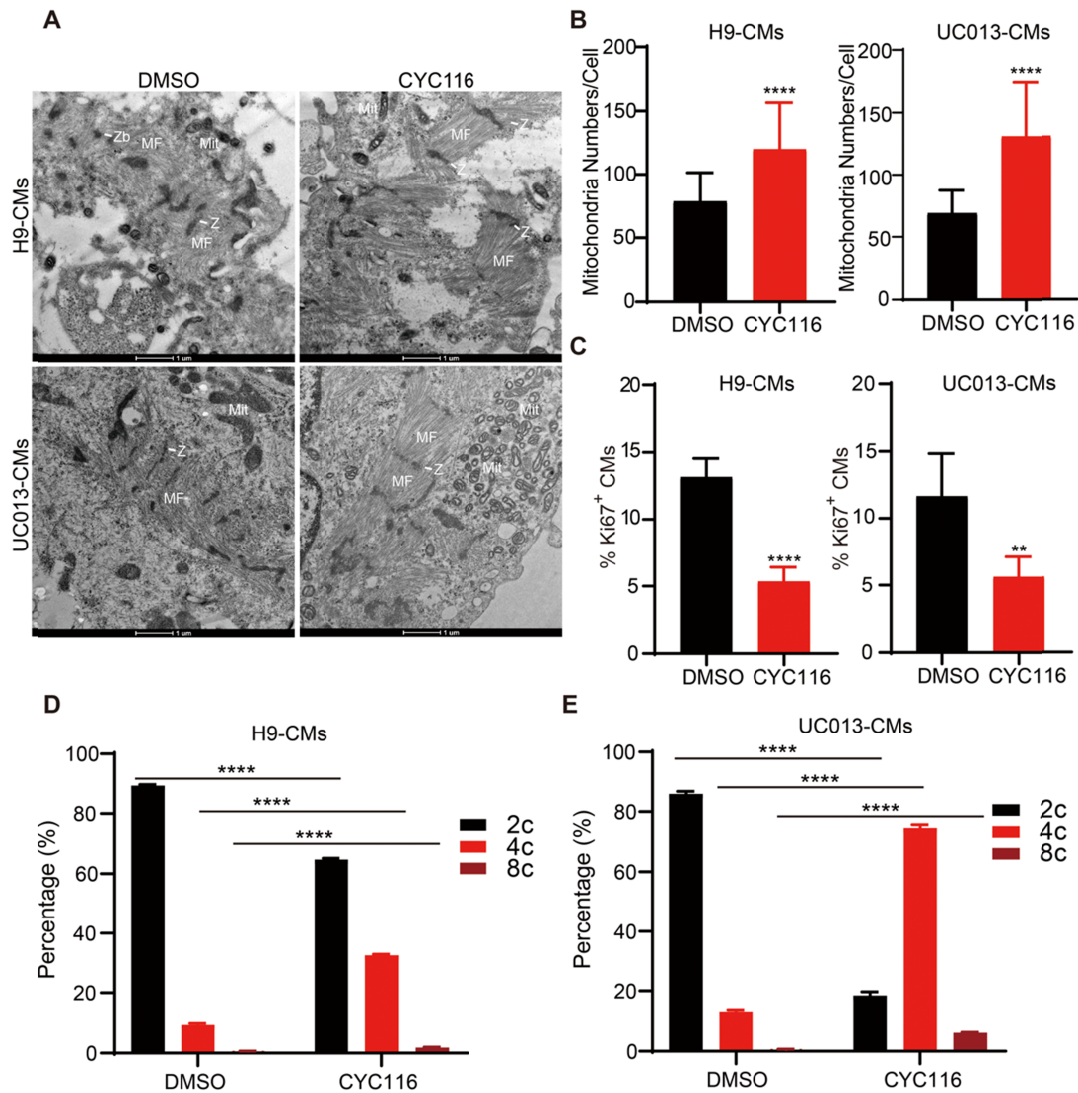
Supplementary Fig. S2. CYC116 improves structural maturation in H1-CMs. (A and B) Representative images (A) and statistical quantification (B) of the immunofluorescence staining of N-cadherin at Day 30 in H1-CMs treated with 5 μ M CYC116 or DMSO from Days 22-30 ($n = 10$ fields). Scale bar = 25 μ m. (C and D) Representative images (C) and statistical quantification (D) of the immunofluorescence staining of Connexin 43 at Day 30 in H1-CMs treated with 5 μ M CYC116 or DMSO from Days 22-30 ($n = 10$ fields). Scale bar = 25 μ m. (E) qRT-PCR analysis of the expression of N-cadherin and Connexin 43 at Day 30 in H1-CMs treated with 5 μ M CYC116 or DMSO from Days 22-30 ($n = 3$). Data are presented as the mean \pm SEM. ** $P < 0.01$; **** $P < 0.0001$.



Supplementary Fig. S3. CYC116 improves mitochondria maturation in H1-CMs. (A) TEM images of H1-CMs treated with DMSO or CYC116. Mit, mitochondria; Scale bars = 5 μ m. (B) The relative mtDNA copy number was evaluated by the ratio of *ND1*, *ND2*, *ND4*, *ND5*, *ND6*, *Cox1*, *Cox2*, *Cox3*, *ATP6*, *D-loop*, and *RNR2* to β -globin of H1-CMs treated with DMSO or CYC116 at Day 30 (n = 3). Data are presented as the mean \pm SEM. * P < 0.05; ** P < 0.01; *** P < 0.0001.



Supplementary Fig. S4. Other Aurora kinase inhibitors also increases the expression of cardiac genes in H1-CMs. qRT-PCR analysis of the mRNA levels of genes related to cardiac structure, ion channels and mitochondrial functions in H1-CMs treated with DMSO or Aurora kinase inhibitors (n = 3). Data are presented as the mean ± SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.



Supplementary Fig. S5. CYC116 promotes the maturation of cardiomyocytes derived from other hPSCs. (A) Transmission electron microscopy images of H9-CMs and UC013-CMs treated with DMSO or CYC116. MF, myofibrils; Z, Z-bands; Zb, Z-bodies; Mit, mitochondria. Scale bars = 1 μ m. (B) Quantitative analyses of mitochondria number per cell from TEM images of H9-CMs and UC013-CMs treated with DMSO or CYC116 (n = 25 cells). (C) Flow cytometry analysis of Ki67⁺ cells in CYC116-treated H9-CMs and UC013-CMs (n = 3). (D and E) Flow cytometry analysis of ploidy ratio with PI staining in DMSO- or CYC116-treated H9-CMs (D) and UC013-CMs (E) (n = 3). Data are presented as the mean \pm SEM. ***P* < 0.01; *****P* < 0.0001.