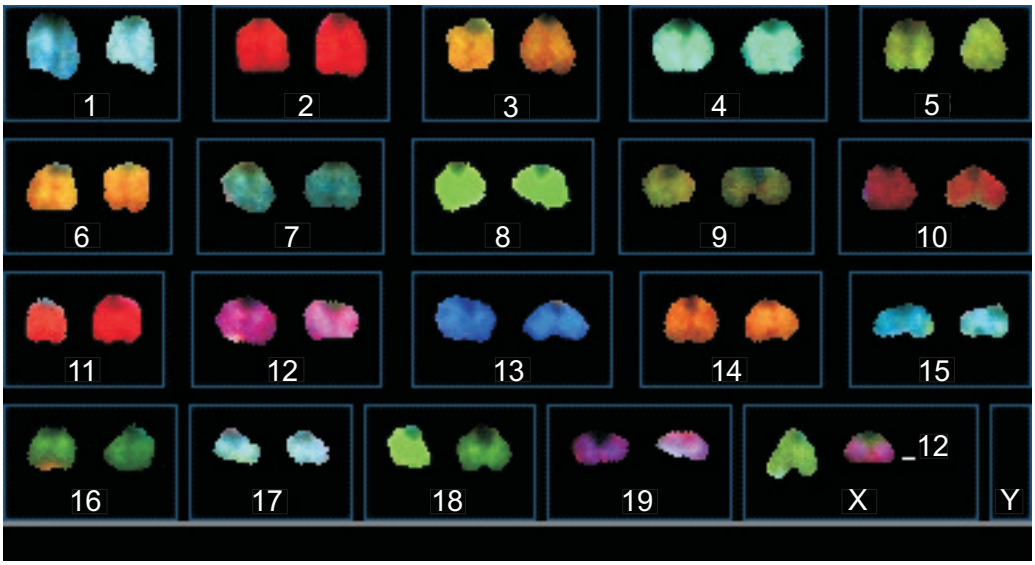
**Supplemental Figure 1.**

**A**

| Cell-Type | Cell line | Passage Number | Metaphases analysed | Metaphases with 40 chromosomes |
|---|---|---|---|---|
| ESC | R1 | 40 | 25 | 24 |
| PSC | B2 | 8 | 20 | 18 |
| | B3 | 8 | 20 | 19 |
| | A3 | 25 | 20 | 18 |
| | A3 | 40 | 20 | 20 |

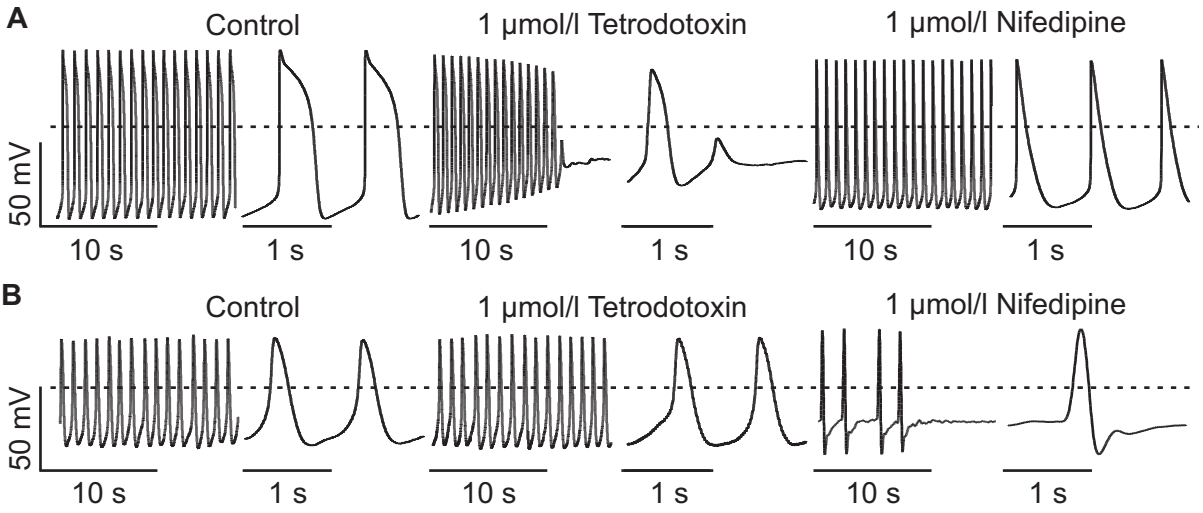
**B**




| PSC-Line | Clonal karyotype [metaphases] | passage |
|---|---|---|
| A3 | 40,X,Der(X)T(XA1.1;12D1) [14] | 24 |
| A6 | 39,X,-X [13] | 25 |
| B2 | 40,XX [5]<br>39,X,-X [7] | 25 |
| B3 | 40,XX [2]<br>39,X,-X [7]<br>40,X,-X,+1 [3] | 25 |
| C4 | 40,XX [9]<br>39,X,-X [3] | 25 |

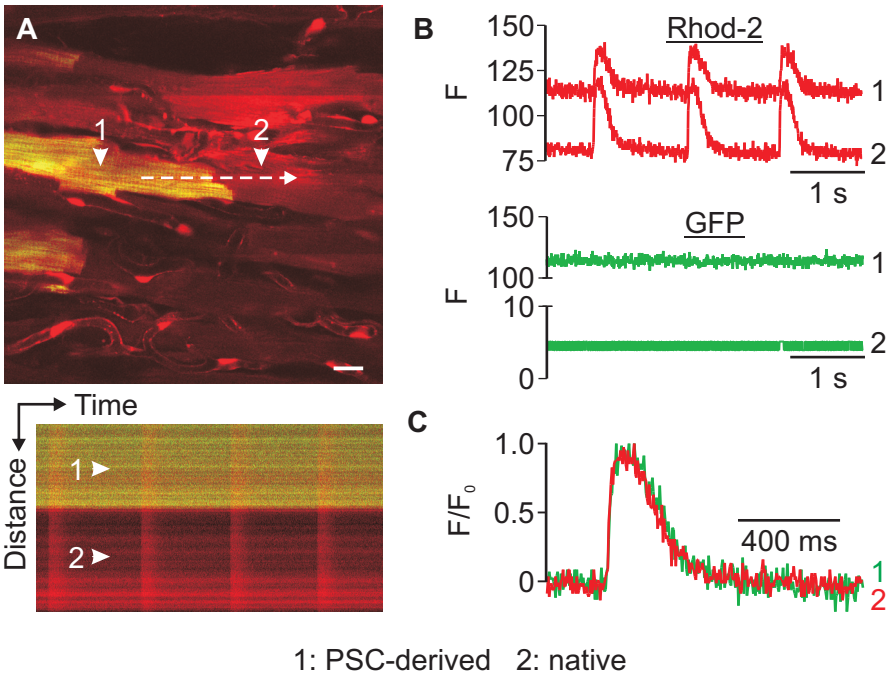
**Supplemental Figure 1. Karyotype analysis.** (A) Representative euploid metaphase spread from PSC line B3 and overview of the results of the metaphase analyses in ESCs and PSCs (PSC line A6 was initially not evaluated in detail). (B) Spectral karyotyping (SKY) of PSC lines. The upper panel shows the A3 karyotype; the lower table summarizes the karyotype data from 2 transgenic (A3 and A6) and 3 non-transgenic PSC lines (B2, B3, and C4; PSC line C4 was one of the 2 non-transgenic lines that did not show beating activity upon the initial screen). Array-CGH analyses confirmed the unbalanced translocation of parts of chromosome 12 to the partially deleted X-chromosome and identified the break and fusion points (12D1 to XA.1).

**Supplemental Figure 2.**




**Supplemental Figure 2. Electrophysiological identification of distinct myocyte populations.** (A) Purkinje-like cell (n=3): $Na^+$-channel blockade with tetrodotoxin (TTX, 1 µmol/l) resulted in a reduction of upstroke velocity and ultimately a complete loss of spontaneous APs (middle panel); subsequent washout reversed the TTX effects; addition of nifedipine (1 µmol/l) abolished the plateau and thus shortened the AP (right panel). (B) Pacemaker-like cell (n=3): TTX did not influence fast depolarization (middle panel) while nifedipine abolished spontaneous activity (right panel).

**Supplemental Figure 3.**




1: PSC-derived 2: native

**Supplemental Figure 3. Electrical integration of parthenogenetic cardiomyocytes from αMHC-EGFP-PSCs into native myocardium in chimeric mice.** (A) Two-photon laser scanning microscopy of intracellular $Ca^{2+}$-transients: 2D- (upper panel) and line- (lower panel) scan images obtained during sinus rhythm (arrows 1 and 2: EGFP-positive and -negative cell, respectively; the dotted line indicates the location of the line-scan). (B) Plots of EGFP and rhod-2 line scan data in the EGFP-expressing cardiomyocyte 1 and the EGFP-negative (native) cardiomyocyte 2 as function of time. (C) Superimposed tracings of AP-evoked changes in rhod-2 fluorescence as function of time from cardiomyocytes 1 (green) and 2 (red). For each cell, the relative changes in fluorescence were normalized such that 0 represents the prestimulus fluorescence intensity and 1 represents the peak fluorescence intensity. F: fluorescence intensity; F: prestimulus fluorescence intensity. Scale bar: 20 μm

| | Ventricle-like | Purkinje-like | Atrial-like | Pacemaker-like | Intermediate |
|---|---|---|---|---|---|
| n | 49 | 13 | 11 | 15 | 14 |
| $C_m$ (pF) | 64 ± 7 | 80 ± 14 | 60 ± 14 | 95 ± 24 | 58 ± 9 |
| MDP (mV) | -66 ± 1 | -64 ± 1 | -62 ± 1 | -48 ± 1 | -52 ± 2 |
| APD 20 (ms) | 756 ± 110 | 364 ± 40 | 178 ± 26 | 462 ± 90 | 685 ± 152 |
| APD 50 (ms) | 950 ± 128 | 487 ± 45 | 243 ± 30 | 563 ± 98 | 685 ± 152 |
| APD 90 (ms) | 1031 ± 132 | 540 ± 45 | 352 ± 37 | 731 ± 108 | 1080 ± 229 |
| Max dV/dt (V/s) | 7.7 ± 1.2 | 21.9 ± 5.7 | 9.6 ± 2.2 | 1.9 ± 0.3 | 6.3 ± 1.7 |
| AP amplitude (mV) | 113 ± 1 | 115 ± 2 | 99 ± 3 | 77 ± 3 | 100 ± 2 |

Data are presented as mean ± SEM; Cm: cell capacity; MDP: maximal diastolic potential; APD 20, APD 50, APD90: AP duration measured at 20%, 50% or 90% repolarisation, respectively; max dV/dt: maximum rate of rise of AP

**Supplemental Table 1. AP-characteristics of PSC-derived cardiomyocytes.**

| | Ventricle | EHT |
|---|---|---|
| n | 4 | 8 |
| max TT (mN) | 0.08 ± 0.03 | 0.17 ± 0.02 * |
| $T1_{90\%}$ (ms) | 38 ± 2 | 65 ± 3 * |
| $T2_{50\%}$ (ms) | 38 ± 2 | 69 ± 4 * |
| $Ca^{2+}$ $EC_{50}$ (mmol/L) | 1.52 ± 0.06 | 0.82 ± 0.03 * |

Data are presented as mean ± SEM; max TT: maximal twitch tension at 2.4 mmol/L calcium; $T1_{90\%}$: contraction time from 10% to max TT; T2: relaxation time from max TT to 50% relaxation; $Ca^{2+}$ $EC_{50}$: calcium concentration at half max TT. *$P$<0.05 vs. ventricle (unpaired, two-tailed student's t-test).

**Supplemental Table 2. Contractile properties of mouse ventricular myocardium and PSC-EHT.**

| | SHAM | Ctr. | F-EHT | EHT |
|---|---|---|---|---|
| n | 8 | 8 | 8 | 7 |
| AWTh diastole (mm) | 0.77 ± 0.05 | 0.76 ± 0.08 | 0.87 ± 0.06 | 1.09 ± 0.10 * |
| AWTh systole (mm) | 1.13 ± 0.05 * | 0.80 ± 0.09 | 0.92 ± 0.06 | 1.28 ± 0.14 * |
| AWTh fraction (%) | 32 ± 3 * | 4 ± 2 | 5 ± 1 | 13 ± 3 * |
| PWTh diastole (mm) | 0.74 ± 0.03 | 0.95 ± 0.08 | 0.81 ± 0.06 | 0.85 ± 0.08 |
| PWTh systole (mm) | 1.06 ± 0.07 | 1.14 ± 0.08 | 0.90 ± 0.09 | 0.96 ± 0.11 |
| PWTh fraction (%) | 28 ± 3 * | 16 ± 3 | 7 ± 4 | 10 ± 4 |
| LVID diastole (mm) | 3.68 ± 0.10 * | 4.49 ± 0.13 | 4.97 ± 0.11 * | 4.32 ± 0.14 |
| LVID systole (mm) | 2.69 ± 0.12 * | 4.09 ± 0.09 | 4.80 ± 0.16 * | 3.84 ± 0.25 |
| LV Area diastole (mm) | 10.9 ± 0.4 * | 17.2 ± 0.9 | 21.1 ± 1.1 * | 17.3 ± 1.2 |
| LV Area systole (mm) | 6.0 ± 0.5 * | 13.5 ± 1.0 | 17.6 ± 1.0 * | 13.4 ± 1.3 |
| FAS (%) | 45 ± 4 * | 22 ± 2 | 17 ± 2 | 23 ± 4 |
| LVEDV (µl) | 66 ± 3 * | 109 ± 6 | 136 ± 8 * | 109 ± 8 |
| LVESV (µl) | 32 ± 3 * | 83 ± 6 | 110 ± 7 * | 82 ± 9 |
| EF (%) | 51 ± 4 * | 25 ± 2 | 20 ± 3 | 25 ± 4 |

Data are presented as mean ± SEM; AWTh: anterior wall thickness; PWTh: posterior wall thickness; LVID: left ventricular inner diameter; FAS: fractional area shortening; LVEDV: left ventricular end diastolic volume; LVESV: left ventricular end systolic volume; EF: ejection fraction; SHAM: mock ligation was placed around the LAD and mock sutures were placed as if EHT was implanted; Ctr.: LAD was permanently ligated and mock sutures were placed as if EHT was implanted; F-EHT: LAD was permanently ligated followed by implantation of formaldehyde fixed (non-viable) EHT; EHT: LAD was permanently ligated followed by implantation of a viable EHT. ANOVA with Dunnett's multiple comparions test; * $P<0.05$ vs Ctr.

**Supplemental Table 3. Echocardiography 14 days after EHT implantation or control intervention.**

**Chromosome 5**

| Marker | Distance from centro-mere (cM) | Forward primer 5'-3' | Reverse primer 5'-3' |
|---|---|---|---|
| **D5Mit193** | 1 | TGTCTTTAAAGTGGCCCAGG | TGTTTTCTATGTGTTTTATATGCTTCA |
| **D5Mit294** | 8 | TGCAAACTAGCAGCCAACTG | GTCAACCTCTGATCTACACCCC |
| **D5Mit352** | 20 | CCCAGAGCCCACATCAAG | TAGGTGGGTGTGTCTCTCCC |
| **D5Mit81** | 28 | GGGAGTTCCAGGTTCATTGA | ATGTGCATTATGGCATGTAAATG |
| **D5Mit135** | 42 | TACACAGGGAAAGGACAGGG | AGGGAGATTTTGGATTAGAGGC |
| **D5Mit18** | 45 | CTGTAGTGGGTGGTTTTAAAATTG | ATGCCACTGGTGCTCTCTG |
| **D5Mit136** | 65 | CTTCCAGGATGATTTACAGTATAACTG | AAACTTGCCCACTCCCATC |
| **D5Mit168** | 78 | CAGGTGACAGTTGTTCTCTTCC | CATGCATGAACACACATCACA |

**Chromosome 17**

| Marker | Distance from centro-mere (cM) | Forward primer 5'-3' | Reverse primer 5'-3' |
|---|---|---|---|
| **D17Mit113** | 6.5 | GAAACCTTTATGTAACCATCTGGC | AATTTATTTTCATAGTGCATGTGTTG |
| **D17Mit198** | 16 | TGCTTCTACCTCCCAAGGG | CCAACCTTTCAAGTCAGATGTG |
| **H2-Q4** | 19.16 | CCTGCAGGAATATCAATAGTG | ATACAGAGAAACCCTATCTCAA |
| **D17Mit24** | 20.43 | ACCTCTCACCTCTCTCTGTG | TGGAGAGACGTCCTATGATG |
| **D17Mit178** | 24.5 | ACACAATTTCTTTTAGTGGGTTCC | TGTGGAAGACACTCAATATCAACC |
| **D17Mit139** | 30.2 | AGACATGTGAGTACTGCACAGACA | ATGATGACATACCTCCTAGTAGTCCC |
| **D17Mit93** | 44.5 | TGTCCTTCGAGTGTTTGTGTG | TCCCCGGTGAATGAGTTATC |
| **D17Mit130** | 55.7 | CTCAACTCCCCCTCTGCTTT | TGTCTGAACTCCTCAGGTACCA |

**Supplemental Table 4. Primers used for haplotype analyses of chromosomes 5 and 17.**

| | Forward primer 5'-3' | Reverse primer 5'-3' | Annealing temp. (°C) |
|---|---|---|---|
| **Peg1 1st** | TAGGGGTTTGTTTTGTTGTTTATTT | AACCTATAAATATCTTCCCATATTC | 45 |
| **Peg1 2nd** | GATATGATAGAAAATATTTTGAAATTAAAA | TAAAAATACCAACACCTAAAAAAAA | 55 |
| **Igf2r 1st** | GTAGAGTTTTTTGAATTTTTTTGTT | TAAACTATAATTCTAATTATACCAAATTAC | 45 |
| **Igf2 2nd** | TGGTATTTTTATGTATAGTTAGGATAG | AAAAATTCTATAATCAAAACCAAC | 55 |
| **H19 (CTCF1) 1st** | TAAGGAGATTATGTTTTATTTTTGGA | CCCCCTAATAACATTTATAACCCC | 45 |
| **H19 (CTCF1) 2nd** | AAGGAGATTATGTTTTATTTTTGGA | AAACTTAAATAACCCACAACATTACC | 55 |
| **Igf2 (DMR2) 1st** | TTTAATATGATATTTGGAGATAGTT | AAAAAACAACCTAATATAAAAAAAC | 45 |
| **Igf2 (DMR2) 2nd** | GAGTTTAAAGAGTTTAGAGAGGTTAAA | TAAAACTATCCCTACTCAAAAAAAA | 55 |
| **Dlk1-Gtl2 (IG-DMR) 1st** | GGTGGGATTGTTTTAGGTTTTTATT | AAATTTCTCCAACCCCAATATAACT | 45 |
| **Dlk1-Gtl2 (IG-DMR) 2nd** | TGGATTTGGTTTTATGAATGAAGATA | AAATAATCACCCTAACCCAACCTAC | 55 |

**Supplemental Table 5. Primers used for nested PCR to determine DNA methylation.**

| | Forward primer 5'-3' | Reverse primer 5'-3' | TaqMan probe 5'FAM-3'TAMRA |
|---|---|---|---|
| **α-MHC (Myh6)** | GCT GAC AGA TCG GGA GAA TCA G | CCC CTA TGG CTG CAA TGC | TCC TCA TCA CCG GAG AAT CCG GAG |
| **β-MHC (Myh7)** | TCC TCA CAT CTT CTC CAT CTC TGA | GCA AAA TAT TGG ATG ACC CTC TTA G | ATG CTG ACA GAT CGG GAG AAT CAG TCC AT |
| **Nkx2.5** | CTT TGT CCA GCT CCA CTG C | CAA GTG CTC TCC TGC TTT CC | TTC TGC AGC GCG CAC AGC TCT TT |
| **Oct3/4** | GCC CCA ATG CCG TGA AG | CAG CAG CTT GGC AAA CTG TTC | TGG AAC CAA CTC CCG AGG AGT CCC |
| **Nanog** | TGC TAC TGA GAT GCT CTG CAC A | TGC CTT GAA GAG GCA GGT CT | AGG CTG CCT CTC CTC GCC CTT C |
| **Brachyury** | AGC AAG AAA GAG TAC ATG GCA TTG | GCA GCG AGA AGG GAG ACC | AAC ATC CTC CTG CCG TTC TTG GTC A |
| **Flk1** | TGA TTG CCA TGT TCT TCT GG | TGT GTG TTG CTC CTT CTT TCA | CTA CGG ACC GTT AAG CGG GCC AAT |
| **Isl-1** | CAT TTG ATC CCG TAC AAC CTG ATA | AAA TTC ACG ACC AGT ATA TTC TGA GG | TTG GAG TGG CAT GCA GCA TGT TTG A |
| **GAPDH** | ATG TTC CAG TAT GAC TCC ACT CAC G | GAA GAC ACC AGT AGA CTC CAC GAC A | AAG CCC ATC ACC ATC TTC CAG GAG CGA GA |
| **Casq2** | CGG GAC AAC ACT GAC AAT CC | CCC AAT CTG TGG CTT GAA CA | TGA CTT TCC ACT GCT TGT TGC TTA CTG GG |
| **Rex1** | GGC CAG TCC AGA ATA CCA GA | GAA CTC GCT TCC AGA ACC TG | |
| **Sox2** | GGC AGC TAC AGC ATG ATG CAG GAG C | CTG GTC ATG GAG TTG TAC TGC AGG | |
| **cTnT** | CAG AGG AGG CCA ACG TAG AAG | CTC CAT CGG GGA TCT TGG GT | |

**Supplemental Table 6. TaqMan primer/probe sets and SYBR Green primer sequences (Rex1, Sox2, cTnT).**

| Antibody | Origin | Clone | Dilution | Source |
|---|---|---|---|---|
| **Primary antibodies** | | | | |
| Neurofilament protein M, 160 kDa | mouse | NF-09 | 1/1000 | abcam |
| Sarcomeric myosin | mouse | MF 20 | 1/50 | DSHB |
| Pan-cytokeratin | mouse | C 11 | 1/100 | Sigma |
| Nebulin | mouse | NB2 | 1/400 | Sigma |
| α-Sarcomeric actinin | mouse | EA-53 | 1/800 | Sigma |
| Pan-cadherin | rabbit | polyclonal | 1/250 | Sigma |
| alpha-Fetoprotein | rabbit | polyclonal | 1/500 | DAKO |
| Cytokeratin 18 | mouse | ks18.04 | 1/20 | Progen Biotechnik |
| Cardiac troponin-I | rabbit | polyclonal | 1/2000 | Chemicon |
| Connexin43 | mouse | 2/connexin-43 | 1/250 | Translab |
| Connexin43 | rabbit | polyclonnal | 1/100 | Chemicon |
| Nkx2.5 | rabbit | polyclonal | 1/200 | Santa Cruz |
| GATA4 | rabbit | polyclonal | 1/200 | Santa Cruz |
| Oct3/4 | rabbit | polyclonal | 1/200 | abcam |
| Nanog | rabbit | polyclonal | 1/200 | abcam |
| SSEA1 | mouse | MC-480 | 1/20 | DSHB |
| **Secondary antibodies** | | | | |
| Anti-mouse IgG Alexa 488 | goat | polyclonal | 1/800 | Molecular Probes |
| Anti-mouse IgG Alexa 546 | goat | polyclonal | 1/800 | Molecular Probes |
| Anti-mouse IgG Alexa 633 | goat | polyclonal | 1/800 | Molecular Probes |
| Anti-rabbit IgG Alexa 546 | goat | polyclonal | 1/800 | Molecular Probes |
| Anti-rabbit IgG Alexa 633 | goat | polyclonal | 1/800 | Molecular Probes |

DSHB: Developmental Study Hybridoma Bank at the University of Iowa

**Supplemental Table 7. Applied primary and secondary antibodies.**