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The linkage phase of the polymorphism KCNH2-K897T influences the electrophysiological phenotype in hiPSC models of LQT2

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Supplementary Material

SUPPLEMENTARY FIGURES

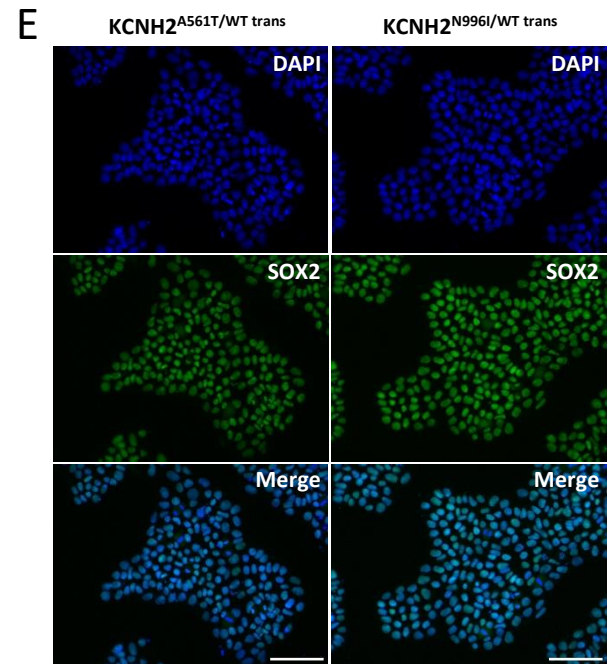
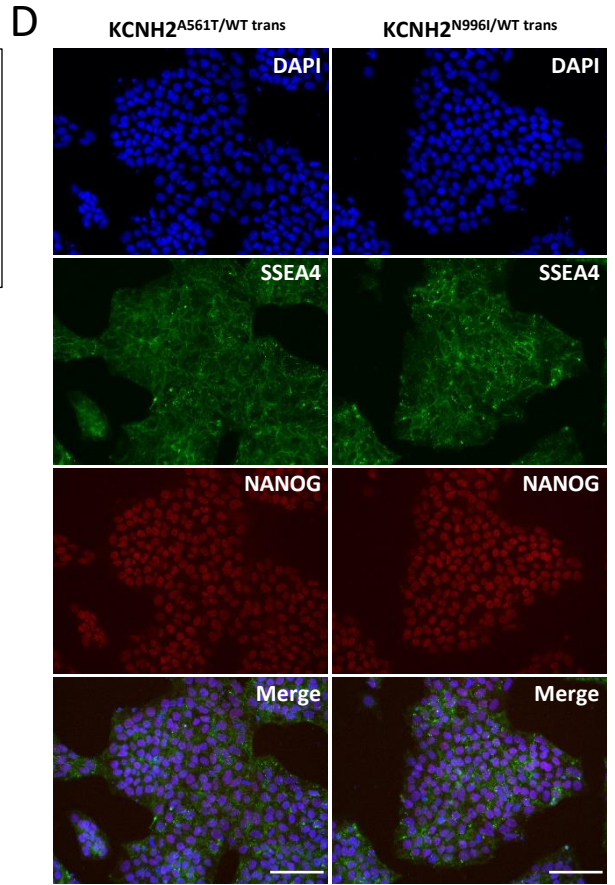
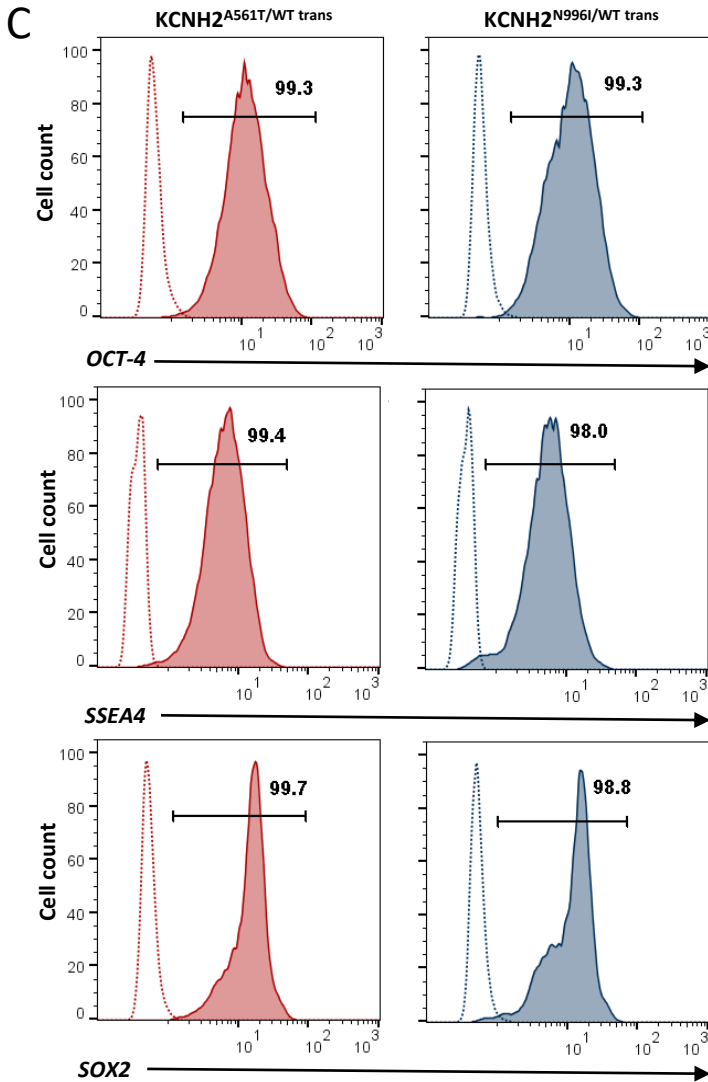
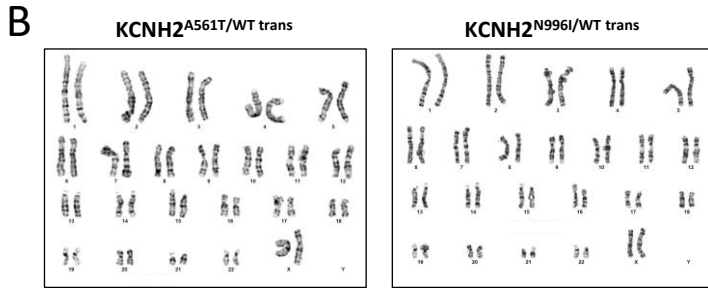
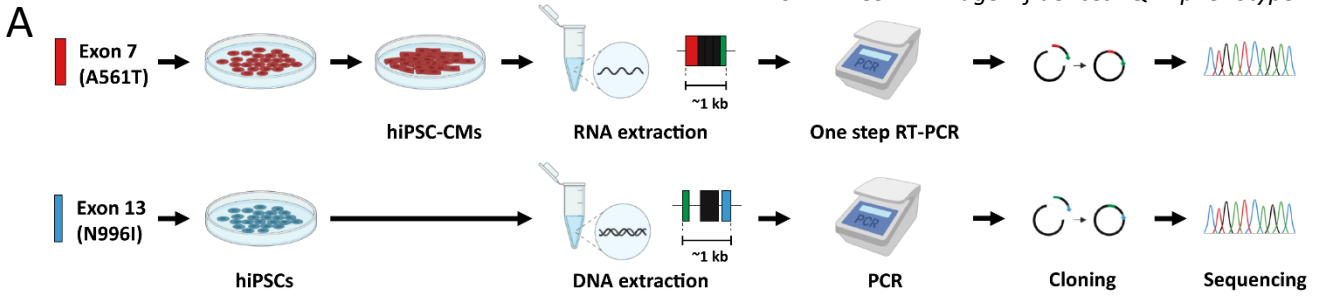


FIGURE S1 | Characterization of the hiPSC lines KCNH2^{A561T/WT} and KCNH2^{N996I/WT} with KCNH2-K897T in *trans* to the primary mutation. (A) Schematic outlining the strategy to identify the KCNH2-K897T linkage phase from either cDNA or genomic DNA for the KCNH2^{A561T/WT} and KCNH2^{N996I/WT} lines respectively. **(B)** G-banding karyograms for the indicated hiPSC lines exhibited a normal 46, XX karyotype. **(C-E)** Flow cytometry **(C)** and immunofluorescence **(D-E)** analysis of the pluripotency markers OCT-4, SSEA4, SOX2, and NANOG in the indicated hiPSC lines. Dashed lines in histogram plots represent the control hiPSC population. Scale bar in images: 100 μ m.

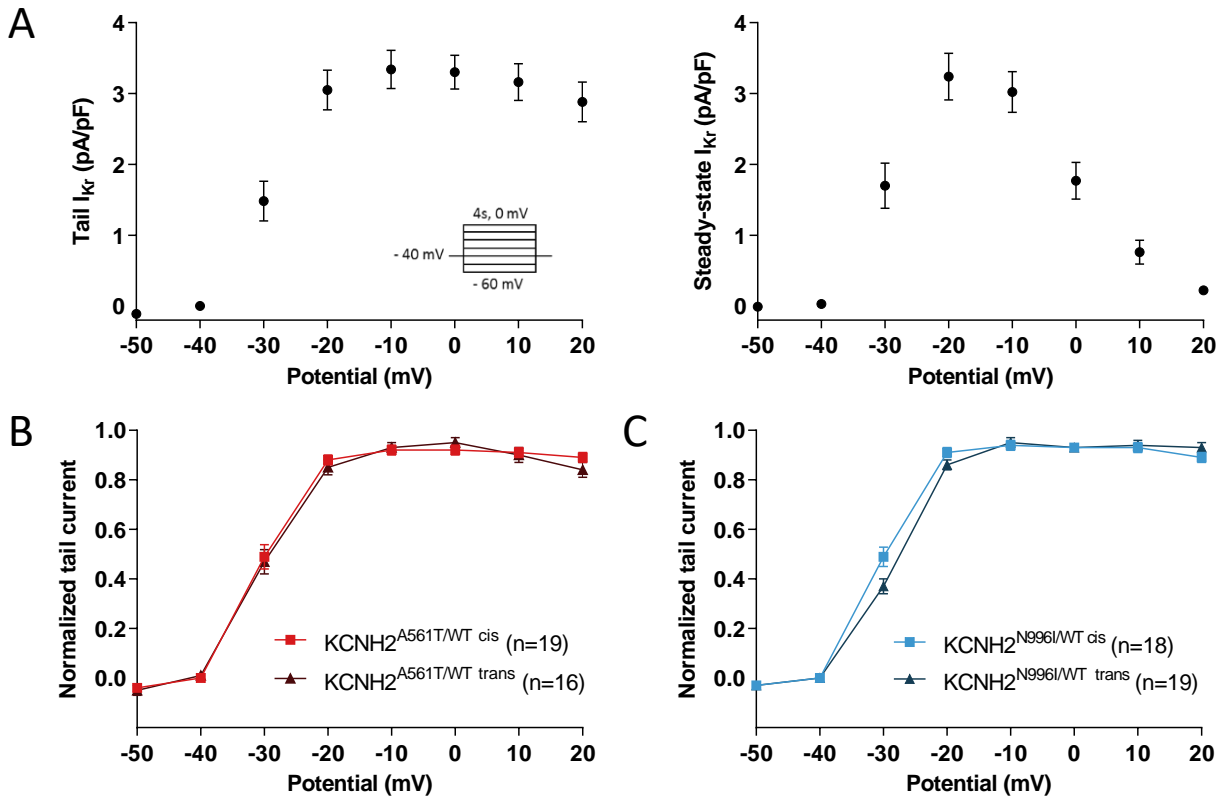


FIGURE S2 | I_{Kr} current characteristics in $KCNH2^{WT/WT}$ and $KCNH2^{Mut/WT}$ hiPSC-CMs. (A) Average current-voltage relationships for tail I_{Kr} current (left panel) and steady-state (right panel) densities in the $KCNH2^{WT/WT}$ hiPSC-CMs. Inset: voltage protocol. n=16 from four differentiations. (B & C) Average tail I_{Kr} current normalized to the maximal current following repolarization to holding potential -40 mV in $KCNH2^{A561T/WT}$ (B) and $KCNH2^{N996I/WT}$ (C) pairs of hiPSC-CMs. n, number of individual hiPSC-CMs analyzed from three or four differentiations.

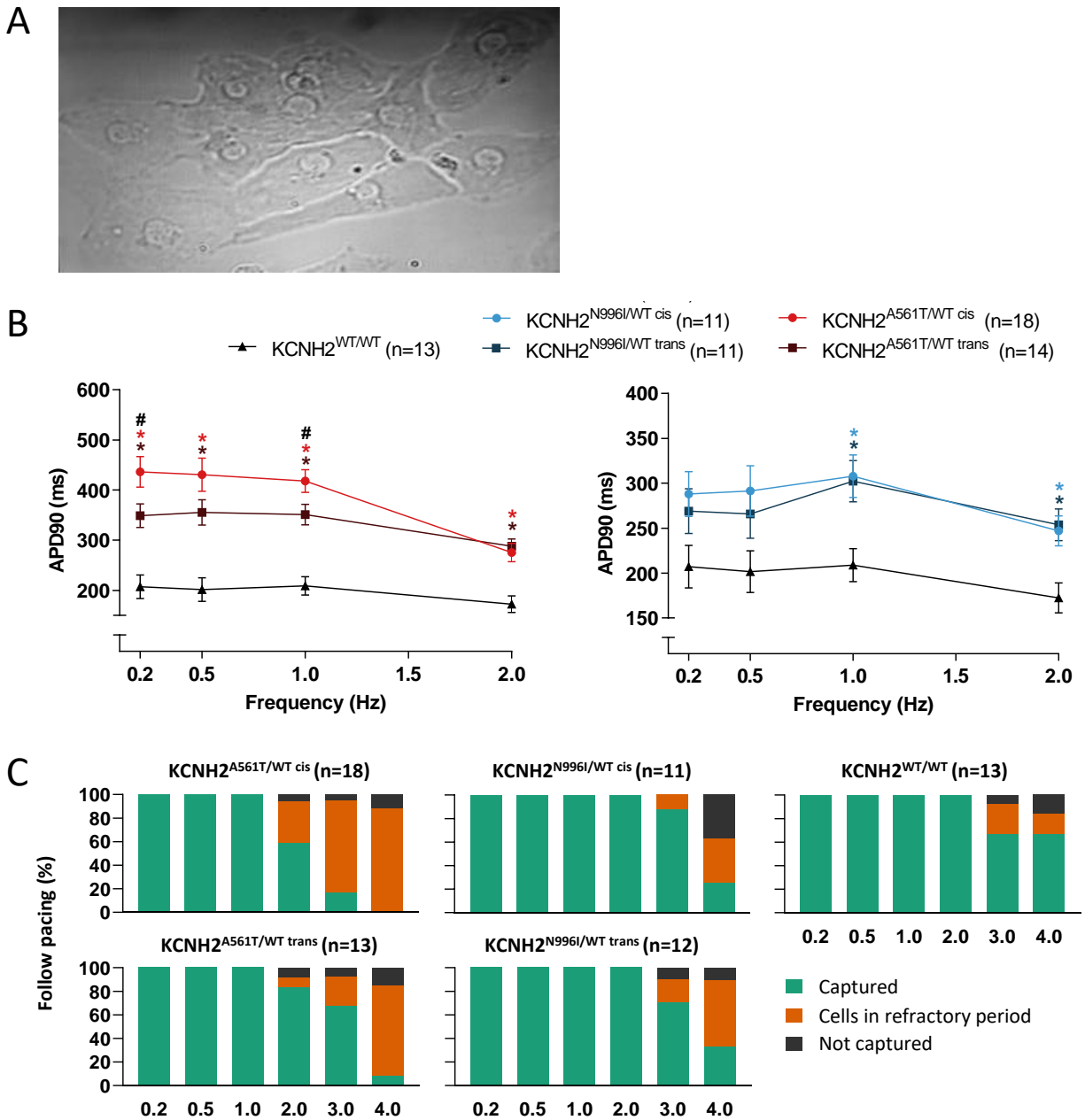


FIGURE S3 | Action potential duration (APD) and pacing responsiveness of the hiPSC-CMs from the indicated lines. (A) Example of a typical hiPSC-CM cluster measured by patch clamp. **(B)** Average APD₉₀ for the indicated *KCNH2*^{A561T/WT} (left panel), *KCNH2*^{N996I/WT} (right panel), and *KCNH2*^{WT/WT} hiPSC-CMs paced at various frequencies between 0.2-2 Hz. The data from the *KCNH2*^{WT/WT} hiPSC-CMs are shown in both panels for comparison. * indicates statistical significance to *KCNH2*^{WT/WT} hiPSC-CMs ($p \leq 0.01$); # indicates statistical significance between *KCNH2*^{A561T/WT cis} and *KCNH2*^{A561T/WT trans} hiPSC-CMs ($p \leq 0.05$); one-way ANOVA with Tukey's multiple comparisons test per indicated frequency. **(C)** Differences in the pacing responsiveness of the indicated hiPSC-CMs between 0.2-4 Hz (x-axis). hiPSC-CMs classified as 'in refractory period' could not follow the pacing frequency as they were generally still in phase 3 of repolarization when applying the stimulus. n, number of individual hiPSC-CM clusters analyzed from two or three differentiations.

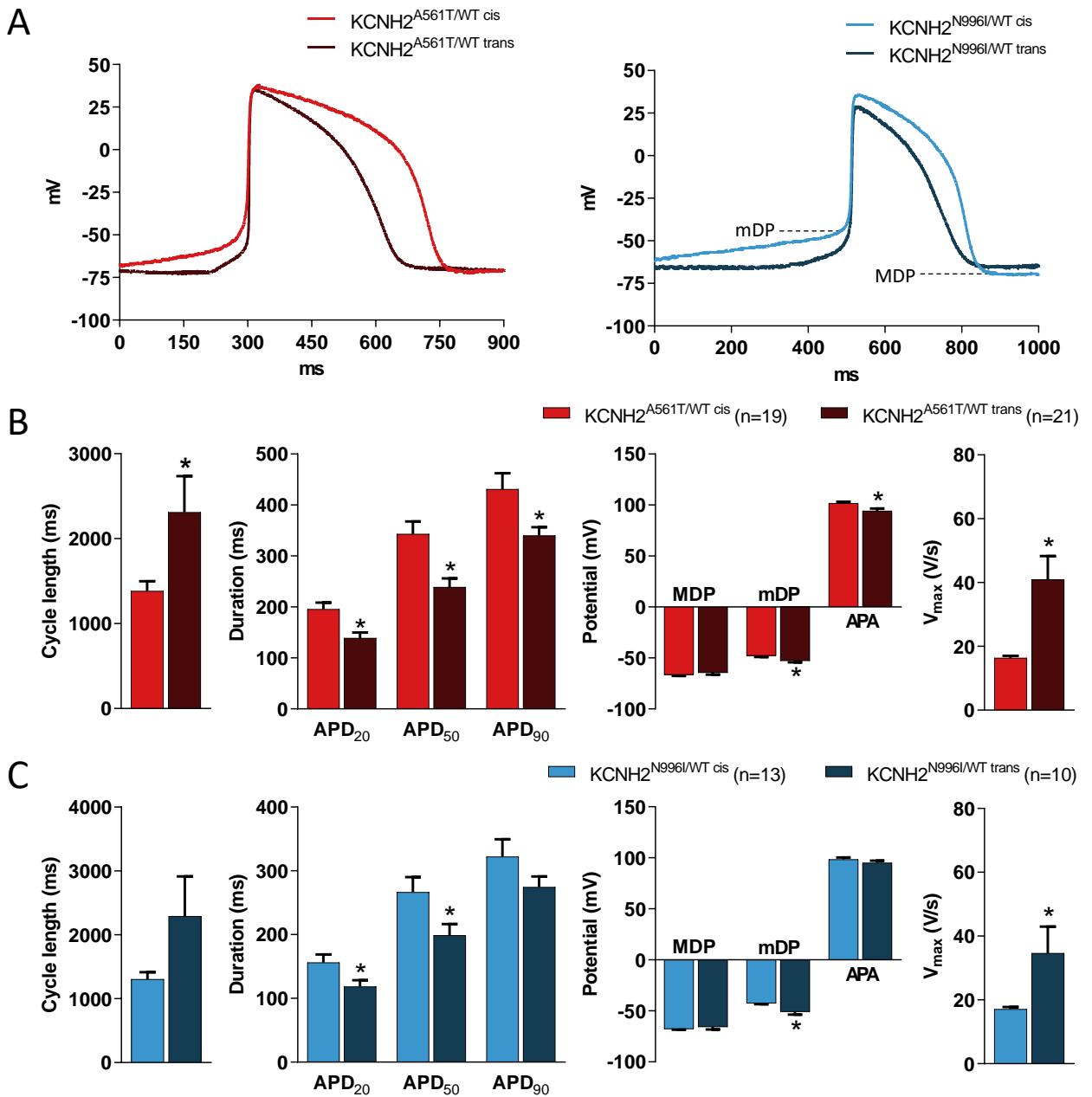


FIGURE S4 | Action potential (AP) characteristics of spontaneously beating hiPSC-CMs from the indicated lines. (A) Representative AP traces of the pairs of KCNH2^{A561T/WT} (left panel) and KCNH2^{N996I/WT} (right panel) hiPSC-CMs measured. Dotted lines in right panel indicate examples of MDP and mDP potentials. **(B & C)** Averaged data for the pairs of KCNH2^{A561T/WT} **(B)** and KCNH2^{N996I/WT} **(C)** hiPSC-CMs for cycle length, APD₂₀, APD₅₀, APD₉₀, MDP, mDP, APA, and V_{max}. n, number of individual hiPSC-CMs analyzed from two or three differentiations. * indicates statistical significance (p ≤ 0.04; unpaired Student's t-test).

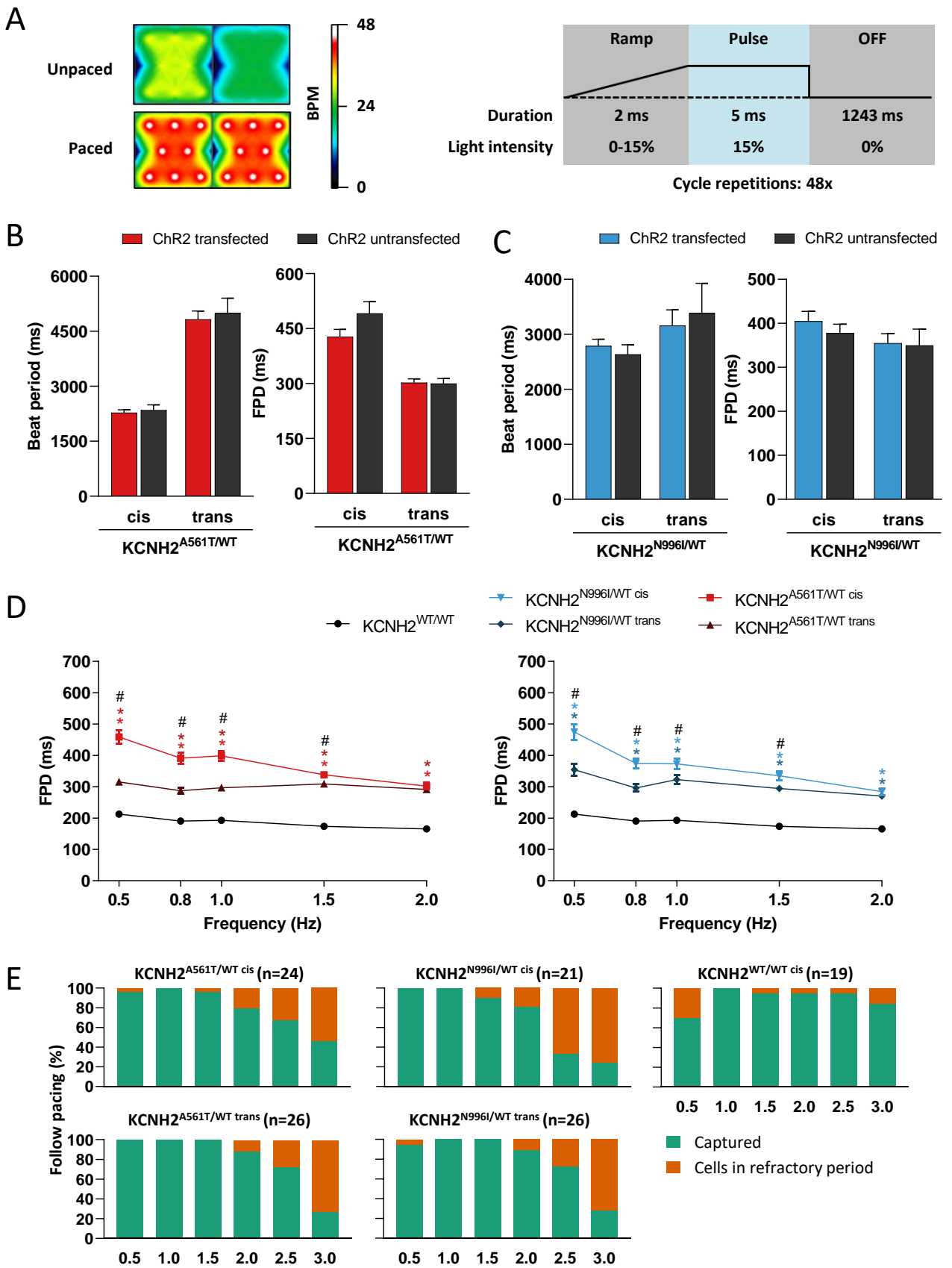


FIGURE S5 | Field potential duration (FPD) and pacing responsiveness of the hiPSC-CMs from the indicated cell lines. (A) Representative beat per minute (BPM) heatmaps for two adjacent wells of confluent KCNH2^{N996I/WT trans} hiPSC-CMs either unpaced or paced at 0.8 Hz. The optical pacing protocol (right panel) that was applied to pace the cells at 0.8 Hz included three phases (ramp, pulse, and light off), with the cycle continuously repeated for 60 seconds. Details for all frequencies are provided in **Table S4**. (B & C) Average spontaneous beat period (left panel) and FPD (right panel) values for the KCNH2^{A561T/WT} (B) and KCNH2^{N996I/WT} (C) pairs of hiPSC-CMs untransfected or transfected with of channelrhodopsin (ChR2) [for (B), transfected and untransfected respectively; KCNH2^{A561T/WT cis} n = 31 and n = 13; KCNH2^{A561T/WT trans} n = 31 and n = 12; for (C), transfected and untransfected respectively; KCNH2^{N996I/WT cis} n = 23 and n = 9; KCNH2^{N996I/WT trans} n = 38 and n = 14; numbers of individual recordings from three differentiations]. (D) Average FPD for the indicated KCNH2^{A561T/WT} (left panel) and KCNH2^{N996I/WT} (right panel) pairs of hiPSC-CMs paced between 0.2-2 Hz. The data from the KCNH2^{WT/WT} hiPSC-CMs are shown in both panels for comparison [KCNH2^{WT/WT} n = 16-19; KCNH2^{A561T/WT cis} n = 21-30; KCNH2^{A561T/WT trans} n = 21-27; KCNH2^{N996I/WT cis} n = 17-26; KCNH2^{N996I/WT trans} n = 23-32; number of individual recordings analyzed from three differentiations]. * indicates statistical significance to KCNH2^{WT/WT} (p < 0.0001); # indicates statistical significance between *cis* and *trans* hiPSC-CMs (p ≤ 0.03); one-way ANOVA with Tukey's multiple comparisons test per indicated frequency. (E) Differences in the pacing responsiveness of the indicated hiPSC-CM monolayers to 0.5 - 3 Hz (x-axis). n, number of individual recordings analyzed from three differentiations.

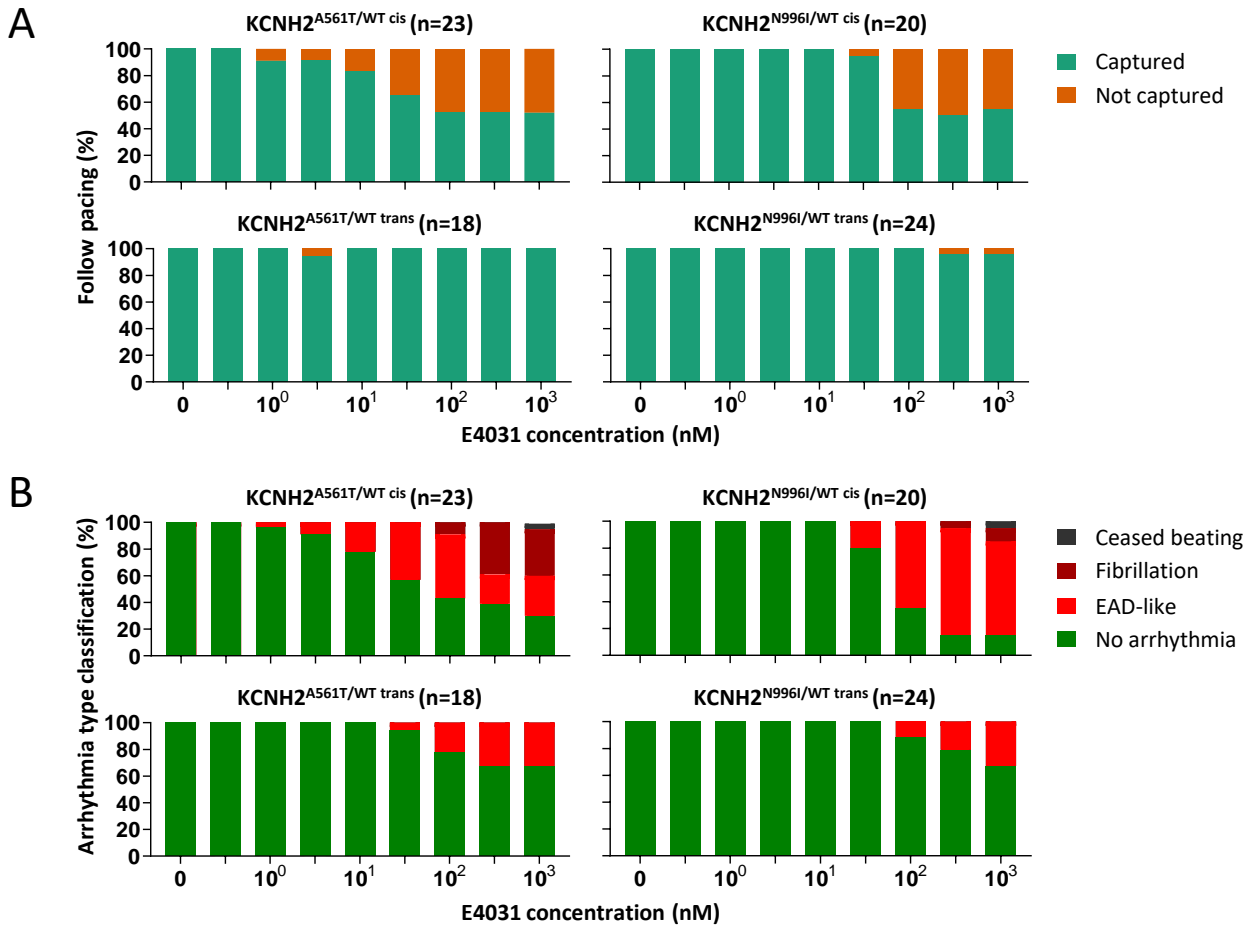


FIGURE S6 | Analysis of the effect of E4031 on $KCNH2^{A561T/WT}$ and $KCNH2^{N996I/WT}$ hiPSC-CMs with $KCNH2-K897T$ either in *cis* or *trans* orientation. (A) Quantification of the pacing responsiveness upon increasing concentrations of E4031 (300 pM - 1 μ M) for the indicated cell lines. n, number of individual recordings analyzed from three differentiations. (B) Assessment of the arrhythmia subtypes detected with increasing doses of E4031 (300 pM - 1 μ M) for the indicated cell lines. n, number of individual recordings analyzed from three differentiations.

SUPPLEMENTARY TABLES

TABLE S1 | AP characteristics of paced (1 Hz) and spontaneous beating hiPSC-CM clusters.

| Parameter | Unit | KCNH2 ^{A561T} /WT cis | KCNH2 ^{A561T} /WT trans | KCNH2 ^{N996I} /WT cis | KCNH2 ^{N996I} /WT trans |
|--------------------|------|-----------------------------------|-------------------------------------|-----------------------------------|-------------------------------------|
| 1 Hz | | | | | |
| APD ₂₀ | ms | 181.5 ± 10.0 | 136.0 ± 10.3* | 145.0 ± 12.8 | 130.0 ± 12.7 |
| APD ₅₀ | ms | 318.6 ± 17.3 | 244.0 ± 19.4* | 249.3 ± 23.1 | 220.1 ± 21.4 |
| APD ₉₀ | ms | 418.2 ± 23.5 | 351.0 ± 21.3* | 307.9 ± 24.8 | 302.2 ± 24.3 |
| MDP | mV | -70.0 ± 0.9 | -70.5 ± 1.7 | -70.7 ± 0.9 | -71.0 ± 2.3 |
| mDP | mV | -67.3 ± 1.8 | -69.5 ± 2.1 | -65.9 ± 1.8 | -69.4 ± 2.8 |
| APA | mV | 106.2 ± 1.4 | 102.0 ± 2.7 | 100.7 ± 1.7 | 100.4 ± 2.7 |
| V _{max} | V/s | 30.4 ± 5.5 | 44.4 ± 9.2 | 22.0 ± 3.4 | 45.7 ± 9.6* |
| n | | 18 | 14 | 11 | 11 |
| Spontaneous | | | | | |
| Cycle length | ms | 1383 ± 119 | 2311 ± 437* | 1305 ± 116 | 2290 ± 658 |
| APD ₂₀ | ms | 139.1 ± 11.0 | 195.6 ± 13.3* | 156.3 ± 13.0 | 118.4 ± 10.8* |
| APD ₅₀ | ms | 238.9 ± 17.6 | 342.9 ± 25.5* | 266.4 ± 24.9 | 199.0 ± 18.5 |
| APD ₉₀ | ms | 340.1 ± 16.7 | 431.0 ± 32.2 | 322.1 ± 28.4 | 274.7 ± 17.6 |
| MDP | mV | -64.6 ± 1.7 | -66.5 ± 1.1 | -67.7 ± 0.7 | -65.8 ± 2.6 |
| mDP | mV | -47.9 ± 1.3 | -52.9 ± 1.4* | -42.5 ± 0.9 | -50.9 ± 2.6* |
| APA | mV | 94.3 ± 2.4 | 101.7 ± 1.5* | 98.4 ± 2.0 | 95.2 ± 2.2 |
| V _{max} | V/s | 16.3 ± 0.7 | 41.0 ± 7.4* | 17.0 ± 0.7 | 34.6 ± 8.4* |
| n | | 17 | 21 | 13 | 10 |

Values expressed as mean ± SEM. n, number of individual hiPSC-CMs analyzed from 2 or 3 independent differentiations. * indicates statistical significance between *cis-trans* hiPSC-CM pairs ($p \leq 0.04$); unpaired Student's t-test. **Abbreviations:** AP duration at 20, 50, and 90% of repolarization (APD₂₀, APD₅₀, and APD₉₀, respectively), maximum diastolic potential (MDP), minimum diastolic potential (mDP), AP amplitude (APA), maximum upstroke velocity (V_{max}).

TABLE S2 | Sequences of primers and probes used in this study.

| Purpose | Sequence forward primer (5' – 3') | Sequence reverse primer (3' – 5') |
|------------------------------------|--|-----------------------------------|
| Primers | | |
| Amplification of KCNH2 exon 11-13 | GTGATTGGCTAAGAGGGTGTT | GGTGGTCACAGCACTGTAGG |
| Sequencing of exon 11 and 13 | GTGATTGGCTAAGAGGGTGTT | GGCCCTCCTTTGTTCTATGT |
| Amplification of KCNH2 exon 7-11 | CTGATCGGGCTGCTGAAGACT | GGTGGTCACAGCACTGTAGG |
| Sequencing of exon 7 and 11 | CTGATCGGGCTGCTGAAGACT | GGTGGTCACAGCACTGTAGG |
| ddPCR K897T primer | GATCACCTTCAACCTGCG | CACCTCCCCTGGCTG |
| ChR2 <i>in vitro</i> transcription | GAATTCTAATACGACTCACTAT AGGGAGAGGAGCCACCATGGA CTATGGC | CAGCGTATCCACATAGCGTA AAAGGAG |
| Probes | | |
| ddPCR K897T probe (HEX conjugated) | ACG GAC +A+C+G GAC A* | |
| ddPCR K897K probe (FAM conjugated) | ACG GAC +A+A+G +GAC A* | |

* + indicates the inclusion of locked nucleic acids to increase the specificity of the probes.
Abbreviations: carboxyfluorescein (FAM), channelrhodopsin-2 (ChR2), droplet digital polymerase chain reaction (ddPCR), hexachlorofluorescein (HEX).

TABLE S3 | Overview of the number of replicates performed per cell line for each of the experiments.

| Technique | Parameter measured | Cell format | Age* (days post thawing) | Number of replicates (# differentiations) | | | |
|----------------|-----------------------|-------------|--------------------------|---|--------------|---------------------------|--------------|
| | | | | KCNH2 ^{A561T/WT} | | KCNH2 ^{N996I/WT} | |
| | | | | <i>cis</i> | <i>trans</i> | <i>cis</i> | <i>trans</i> |
| Flow cytometry | % (ventricular) CMs | Monolayer | 7-8 | 4 (4) | 3 (3) | 5 (5) | 4 (4) |
| ddPCR | Allelic balance | N/A | 7-8 | 6 (2) | 6 (2) | 3 (1) | 3 (1) |
| Voltage clamp | I _{Kr} | Single | 7-10 | 19 (3) | 16 (3) | 18 (4) | 19 (3) |
| Current clamp | AP | Cluster | 7-12 | 18 (3) | 14 (2) | 11 (2) | 11 (2) |
| MEA | FP, arrhythmic events | Monolayer | 11-12 | 23 (4) | 18 (3) | 20 (3) | 24 (4) |

* All analyses were performed on hiPSC-CMs that were first cryopreserved at day 20 or 21 of differentiation. **Abbreviations:** Action potential (AP), cardiomyocytes (CMs), droplet digital polymerase chain reaction (ddPCR), field potential (FP), multielectrode array (MEA).

TABLE S4 | Settings for optical stimulation of Channelrhodopsin-2 (ChR2) messenger RNA (mRNA) transfected hiPSC-CMs using the Lumos™ system.

| Frequency (Hz) | Ramp duration (ms) | Pulse duration (ms) | OFF time (ms) | Light intensity |
|-----------------------|---------------------------|----------------------------|----------------------|------------------------|
| 0.5 | 2 | 5 | 1993 | 15-60% |
| 0.8 | 2 | 5 | 1243 | 15-60% |
| 1.0 | 2 | 5 | 993 | 15-60% |
| 1.5 | 2 | 5 | 659.7 | 15-60% |
| 2.0 | 2 | 5 | 493 | 15-60% |
| 2.5 | 2 | 5 | 393 | 15-60% |
| 3.0 | 2 | 5 | 326.3 | 15-60% |