Supplementary Figures

Figure S1. ALMS1 conservation across species, computational pathogenicity prediction of the *ALMS1*:c.10003G>C variant and CRISPR-Cas9 base editing components.
(a) Alignment showing conservation of feline *ALMS1* exon 17 harbouring the *ALMS1*:c.7384G>C variant and human *ALMS1* exon 13. *ALMS1* exonic sequences were excerpted from Ensembl (www.ensembl.org): domestic cat, ENSFCAG00000008756; human, ENSG00000116127. Full conservation is shown in red.
(b) Partial alignment of ALMS1 showing evolutionary conservation of ALMS1 proteins across species. Protein sequences were excerpted from Ensembl: cat, ENSFCAT00000085744.1; cow, ENSBTAT00000047267.5; pig, ENSSSCT00000064085.3; dog, ENSCAFT00000014029.6; chimpanzee, ENSPTRT00000093268.1; human, ENST00000613296.6; mouse, ENSMUST00000213058.2. Fully conserved residues are shown with dots. The conserved region around the G residue mutated in cats is surrounded in green and the position of the feline ALMS1:p.(G2462R) (*ALMS1*:c.7384G>C) variant is shown.
(c) *In-silico* pathogenicity predictions for the human *ALMS1*:c.10003G>C variant (homologous to the feline *ALMS1*:c.7384G>C variant) generated using multiple computational tools (e.g., PolyPhen-2: http://genetics.bwh.harvard.edu/pph2, MutationTaster: www.mutationtaster.org, CADD: https://cadd.gs.washington.edu, and AlphaMissense: https://alphamissense.hegelab.org).
(d) CRISPR-Cas9 base editing components used to introduce the *ALMS1*:c.10003G>C variant in hiPSCs. gRNA sequence: GTTAACATTAAACATAAAGA; ssODN sequence: AGCTCAGGTGCTAGGCACAAGAGATGATGACCTCTCAGCCACTGTTAACATTAAACATAAAGACGAATCTACAGTAA GAGGGTAGTGACTAAGGCATCCTTGCCAGTGGGAGAAAAACC
(e) Representative Sanger electropherograms from the *ALMS1*:c.10003G>C homozygous mutant clone and the isogenic control. The site of the G>C change is surrounded.

Figure S2. Validation of pluripotency of the hiPSC clones.
Flow cytometry gating strategy and marker expression are shown. Briefly, doublets and clumps were excluded based on forward scatter (FSC) and side scatter (SSC) profiles. Single cells were then gated, and live cells were selected for analysis. Within the live population, expression of the pluripotency surface markers (antigens TRA-1-60 and SSEA-4) was assessed. The final plot illustrates the proportion of cells co-expressing TRA-1-60 and SSEA-4 (approximately 75% of cells in both lines), confirming maintenance of pluripotency in the hiPSC clones.

Figure S3. Assessment of mitotic nuclei in isogenic control and *ALMS1*-mutant hiPSC lines.
(a) Representative image depicting proliferative hiPSCs immunolabelled with DAPI (blue), phalloidin (grey) and ALMS1 (red) with a zoomed mitotic spindle marked in yellow box.
(b) Quantification showing percentage of mitotic nuclei in proliferating isogenic control and *ALMS1*-mutant hiPSCs. N=6. The graph represents box plot, with the minimum and maximum values indicated by lines, and the median marked by a line at the centre. Mann-Whitney test for non-normal distributions between *ALMS1*-mutant and isogenic control. ns = not significant.

Figure S4: Relative mRNA expression of *TNNT2 (Troponin T2, Cardiac Type)*, *TTN (Titin)*, *MYL7 (Myosin Light Chain 7)* and *MYL3* ventricular marker (*Myosin Light Chain 3*) at day 30 of differentiation in hiPSC-CMs.
Five independent biological replicates were analysed for the isogenic control. Seven independent biological replicates were analysed for the *ALMS1*-mutant line. The graphs represent box plots, with the minimum and maximum values indicated by lines, and the median marked by a line at the centre. For normalization, ΔCt values were calculated by subtracting the Ct values of each target gene from those of the three housekeeping genes. The average ΔCt from the three housekeeping genes was then used to calculate the ΔΔCt relative to the isogenic condition. Statistical significance was assessed using the Mann-Whitney test for non-normal distributions. ns = not significant.

Figure S5. Relative mRNA expression of *TNNT2 (Troponin T2, Cardiac Type)* at different timepoints of differentiation.

Days 0, 20 and 30 were analysed. Data are presented as mean±SEM. For normalization, ΔCt values were calculated by subtracting the Ct values of each target gene from those of the three housekeeping genes. The average ΔCt from the three housekeeping genes was then used to calculate the ΔΔCt relative to the isogenic condition. The relative expression is shown in comparison to day 0 to illustrate the expression profile along the differentiation. Independent biological replicates were four for the isogenic control at each timepoint and four at day 0, three at day 20 and four at day 30 for the *ALMS1*-mutant line. Statistical significance was assessed using the Mann-Whitney test for non-normal distributions. ns = not significant.