Supplemental Materials

Identification of Drug Transporter Genomic Variants and Inhibitors that Protect Against Doxorubicin–Induced Cardiotoxicity

Running Title: *Magdy et al.; Desipramine attenuates doxorubicin cardiotoxicity*

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Expanded Methods

Human induced pluripotent cell derivation. All pluripotent and reprogramming cell cultures were maintained at 37 ºC in Heracell VIOS 160i humidified incubators (Thermo Scientific) with 5% CO_2 and 5% O_2. Differentiation cultures were maintained at 5% CO_2 and atmospheric O_2. Protocols were approved by the Northwestern University and University of British Columbia Institutional Review Boards. Patients had previously been genotyped with Illumina Infinium HumanOmniExpress array (738,432 SNPs). With informed written consent, ~9 ml of peripheral blood was taken from each volunteer and shipped at 4 ºC, samples were transferred to LeucoSep tubes (Greiner) filled with Histopaque–1077 (Sigma). 1×10^6 isolated peripheral blood mononuclear cells (PMBC) were grown in 24–well tissue culture–treated plates (Greiner) in 2 ml of SFEM II (Stem Cell Technologies) supplemented with 10 ng ml^{-1} IL3, 50 ng ml^{-1} SCF (KITLG), 40 ng ml^{-1} IGF1 (all Peprotech), 2 U ml^{-1} EPO, 1 µM dexamethasone (both Sigma)[1]. 50% medium was changed every other day. After 12 days of growth, 6×10^4 cells were transferred to a well of a 24–well plate in 500 µl of SFEM II with growth factors supplemented with CytoTune–iPS 2.0 Sendai Reprogramming Kit viral particle factors (Invitrogen)[2] diluted to 10% of the manufacturer's recommendations. Cells were treated with 3.5 µl, 3.5 µl, and 2.2 µl of hKOS (0.85×10^8 CIU ml^{-1}), hMYC (0.85×10^8 CIU ml^{-1}), and hKLF4 (0.82×10^8 CIU ml^{-1}), respectively at MOI of 5:5:3 (KOS: MYC: KLF4). 100% media was changed after 24 h by centrifugation (300 × *g* for 4 min) to 2 ml fresh SFEM II with growth factors, and cells were transferred to one well of a 6–well plate (Greiner) coated with 2 ml of 1:800 reduced growth factor Matrigel (Corning) diluted in DMEM (Corning). 50% medium was changed gently every other day. On d8 after transduction, 100% of medium was changed to B8 medium. B8 medium was

made in–house as previously described[3] and consisted of DMEM/F12 (10–092–CM, Corning), 5 μg ml^{-1} *E. coli*–derived recombinant human insulin (Gibco), 200 μg ml^{-1} L–ascorbic acid 2–phosphate trisodium salt (Wako), 5 μg ml^{-1} *Oryza sativa*–derived recombinant human transferrin (Sigma), 20 ng ml^{-1} sodium selenite (Sigma), 40 ng ml^{-1} recombinant human FGF2 (154 amino acids, *E. coli*–derived, made in–house), 0.1 ng ml^{-1} recombinant human TGFβ3 (113 amino acid, *E. coli*–derived, Cell Guidance Systems), and 0.1 ng ml^{-1} recombinant human NRG1 (65 amino acid, *E. coli*–derived, Peprotech). Medium was changed every day. At d17 individual colonies were picked in to a Matrigel–treated 12–well plate (one colony per well). Subsequently, cells were expanded in Matrigel–coated 6–well plates by passaging using 0.5 mM EDTA (Gibco) in DPBS without Ca^{2+} or Mg^{2+} (Corning) for 6 min at RT. Specific hiPSC clones used for this study were summarized in **Supplemental Table 2**. The genotypes for SNP rs7853758 were confirmed through *SLC28A3* gene sequencing in all hiPSC lines using Nanopore MinION sequencer.

Human induced pluripotent stem cell culture. Cells were routinely maintained in B8 medium (made as above) on 1:800 diluted growth factor reduced Matrigel. B8 was supplemented with 2 μM Rho kinase inhibitor (thiazovivin) (LC Labs), hereby referred to as B8T, for the first 24 h after passage. Cells were passaged at a ratio of 1:15 every 3 days using 0.5 mM EDTA, achieving 75% confluence. Cell lines were used between passages 20 and 80. All cultures (pluripotent and differentiation) were maintained with 2 ml medium per 9.6 cm^2 of surface area or equivalent. All cultures were routinely tested for mycoplasma using a MycoAlert PLUS Kit (Lonza) and a Varioskan LUX (Thermo Scientific) plate reader.

Karyotyping. Genomic DNA was extracted from the cell pellets using a Quick–DNA Miniprep Plus kit (Zymo). SNP karyotyping was performed using a whole–genome Infinium

HumanCytoSNP–12 BeadChip Array (Illumina) covering 300,000 SNP using a NextSeq 500 (Illumina). Data was analyzed using BlueFuse Multi software (Illumina).

CRISPR/Cas9–mediated gene knockout. To generate *SLC28A3* knockout gRNA expression vectors, gRNA targeting the start codon designed an online CRISPR design tool (http://tools.genome–engineering.org) with minimal predicted off–target effect[4]. Each gRNA with BbsI ligation overhangs was annealed and inserted into the BbsI restriction site of a pSpCas9(BB)-2A-Puro (PX459) V2.0 (48138, Addgene) plasmid that expresses puromycin resistance gene for downstream antibiotic selection, in addition to Cas9. The constructed gRNA expression plasmids were confirmed by Sanger sequencing (Eurofins) with LKO1_5_primer (5'–GACTATCATATGCTTACCG–3'). 10^6 cells were electroporated with 5 μg PX459 plasmid using Neon™ Transfection System (Invitrogen) using electroporation parameters,1400 V, 20 ms, and 2 pulses. Positive clones were selected 24 h post transfection using puromycin (0.5 μg ml^{-1}) treatment for 48 h. Indels introduced by Cas9 were confirmed by sanger sequencing after PCR amplification of target region using forward primer (5'-AAACTGAAGCAAGCTGTGCC-3') and reverse primer (5'-TTTGTCAACCCAGAAGAGCCC-3')

CRISPR/Cas9–mediated gene overexpression. To generate *SLC28A3* overexpressing cells, *SLC28A* cDNA (Mammalian Genome Collection (MGC) Human *SLC28A3* Sequence-Verified cDNA (insert sequence, BC09382; CloneId,7939666, Catalog number, MHS6278-202857241, Dharmacon) was first amplified and cloned into pENTR/D–TOPO® (Invetrogen) by TOPO cloning reaction performed according to the manufacturer protocol. SLC28A3 overexpression donor plasmid was generated by inserting *SLC28A3* cDNA under the CAG promoter of a

pAAVS1–Nst–CAG–DEST gateway cloning vector (80489, Addgene), which has a neomycin selection cassette in addition to homology arms for *AAVS1*, using Gateway LR Clonase II Enzyme Mix (Invitrogen). The constructed *SLC28A3* donor plasmid was confirmed by Sanger sequencing with the following primer set: P3–F (5'–GGCGCCGGCAGGAAGGAAAT–3') and P3–R (5'–AGCCAGGGCATTGGCCACAC–3'). *AAVS1* gRNA expression vector[5] (pXAT2, Addgene 80494), which expresses gRNA and Cas9, was used to target *AAVS1* locus in the first intron of the *PPP1R12C* gene[5]. Cells were then electroporated (as mentioned above) with 1 μg AAVS1 targeting plasmid and 3 μg SLC28A3 overexpression donor plasmid. Positive clones were selected using neomycin (100 μg ml^{-1}) treatment for 14 days.

Cardiac differentiation. Differentiation into cardiomyocytes was performed according to previously described protocol with some modifications (**Supplemental Fig. 3a**)[6,7]. All cell lines for each individual experiment were differentiated in parallel to further reduce experimental variability. Briefly, hiPSCs were split at 1:15 ratios using 0.5 mM EDTA as above and grown in B8 medium for 3 days reaching 75% confluence. At the start of differentiation (day 0), B8 medium was changed to R6C[6], consisting of RPMI 1640 (Corning) and 6 μM of the glycogen synthase kinase 3–β inhibitor CHIR99021 (LC Labs). On day 1, medium was changed to RPMI 1640 only and on day 2 medium was changed to RBA consisting of RPMI, 2 mg ml^{-1} fatty acid-free albumin (GenDEPOT) and 200 μg ml^{-1} L–ascorbic acid 2–phosphate (Wako) supplemented with 0.5 μM of the Wnt inhibitor Wnt–C59 (Biorbyt). Medium was then changed on day 4 to RBAI consisting of 0.5 mg ml^{-1} fatty acid-free albumin, 200 μg ml^{-1} L–ascorbic acid 2–phosphate, and 5 μg ml^{-1} insulin (Gibco). Medium was then changed every other day with RBAI. Contracting cells were noted from day 7. For each hiPSC line, we added a *TNNT2* promotor-driven neomycin resistant cassette

targeted to the AAVS1 locus[5] to guarantee cardiomyocyte purity to >80% $TNNT2^{+}$. On day 8-12, cells were selected with 100 μg/ml G418/geneticin (Gibco). On day 16, cardiomyocytes were dissociated using DPBS for 20 min at 37 °C followed by 1:200 Liberase TH (Roche) in DPBS for 20 min at 37 °C, manually triturated, centrifuged at 300 *g* for 5 min, filtered through a 100 μm cell strainer (Falcon). Live cells were counted using a LUNA–FL Dual Fluorescence cell counter (Logos Biosystems) then plated onto Matrigel–treated Nunc Lab–Tek II 8–chamber slides (50,000 cells per well), No 1.5 coverslips (100,000 cells per coverslip) in 12–well plates, 24–well plates (1×10^6 cells per well), or 384–well white–sided μClear plates (50,000 cells per well) (all Greiner), in RBAI medium supplemented with 10% Cosmic Calf Serum (U.S. Origin, Hyclone) for 48 h and changed back to RBAI medium thereafter. Cardiomyocytes were used for analysis 30 days after differentiation.

Immunofluorescent staining. Cardiomyocytes were dissociated with Liberase TH and plated onto Matrigel–coated No 1.5 coverslips as described previously and allowed to adhere and spread for 4 days. Cells were fixed with 4% paraformaldehyde (Electron Microscopy Services) in DPBS for 15 min at RT, permeabilized with 10 mg ml^{-1} (1%) saponin (Sigma) in DPBS for 15 min at RT, blocked with 30 mg ml^{-1} (3%) bovine serum albumin (BSA, Sigma) and saponin in DPBS for 30 min at RT, and stained for 3 h in 3% BSA/1% saponin/DPBS at RT with 1:200 polyclonal rabbit IgG TNNT2 (Abcam, ab45932), 1:500 monoclonal mouse IgG_1 ACTN2 (Sigma, A7811), 1:200 polyclonal rabbit IgG SLC28A3 (Origene, TA337177). Cells were washed three times in 1% saponin/DPBS and then stained with secondary antibodies 1:250 Alexa Fluor 488 goat anti–rabbit IgG, Alexa Fluor 594 goat anti–mouse IgG_1, or Alexa Fluor 488 goat anti–mouse IgG_1, Alexa Fluor 594 goat anti–rabbit IgG (all Invitrogen) in 3% BSA/1% saponin/DPBS for 1 h at RT

in the dark. Cells were washed three times with 1% saponin/DPBS, with NucBlue (Invitrogen) in the last wash for 20 min and mounted with ProLong Diamond Antifade Mountant (Invitrogen). Slides were imaged with a Ti–E inverted fluorescent microscope (Nikon Instruments) and a Zyla sCMOS camera (Andor) using NIS–Elements 4.4 Advanced software.

Flow cytometry. hiPSCs were dissociated with TrypLE Express (Gibco) for 3 min at RT and 1×10^6 cells were transferred to flow cytometry tubes (Falcon). For staining of surface marker, cells were stained 5 mg ml^{-1} (0.5%) BSA (Sigma) in DPBS using 1:20 mouse IgG_3 SSEA4–488 (BD Biosciences, 560308) for 30 min at RT then washed twice in DPBS by centrifugation. For intracellular staining, cells were fixed with 4% PFA for 20 min at RT, washed twice with DPBS, and permeabilized with 1% saponin for 15 min at RT, and stained using 1:20 mouse IgG_1 POU5F1–647 (BD Biosciences, 560307), and mouse IgG_1 NANOG–647 (BD Biosciences, 561300) for 30 min at RT then washed. Isotype controls mouse IgG_3–488 (BD Biosciences, 563636) and mouse IgG_1–647 (BD Biosciences, 565571) were used to establish gating. Cardiomyocytes were dissociated with Liberase TH as described above, fixed and permeabilized as above, and stained using 1:100 mouse monoclonal IgG_1 TNNT2–647 (BD Biosciences, 565744) for 30 min at RT and washed again. Isotype controls mouse IgG_1–647 (BD Biosciences, 565571) were used to establish gating. Primary human dermal fibroblasts showed no staining under these conditions. All cells were analyzed using a CytoFLEX (Beckman Coulter) with CytExpert 2.0 software. To account for autofluorescence, each and every sample had a negative untreated control for which the fluorescence is measured before DOX treatment. Exemplary flow cytometry plots for DOX uptake in hiPSC-CMs is shown in Supplemental Figure 11.

Flow cytometry–based doxorubicin uptake quantification. On day 14, cardiomyocytes were dissociated and then plated on 12-well plate (2×10^6 per well). On day 30, cells were treated for 24 h with either tested drugs in relevant concentration or RPMI 1640 medium (no phenol red, Corning) supplemented with 500 μg ml^{-1} recombinant human serum albumin (Oryzogen) as negative control (**Supplemental Figure 4**). Cells were then treated with either doxorubicin (1 and 3 μM) alone or in combination with tested drugs in relevant concentrations. Cells auto-fluorescence was assayed before doxorubicin treatment and serves as baseline fluorescence. Doxorubicin intrinsic fluorescence-PE was measured 1 and 3 h post doxorubicin treatment and normalized to baseline fluorescence. All cells were stained with NucRed Live ReadyProbes Reagent (Invitrogen) to monitor cell viability.

Doxorubicin treatment. Doxorubicin hydrochloride (HY–15142, MedChem Express) was resuspended to 10 mM in cell culture–grade water (Corning). Day 30 hiPSC–CMs were treated for 24 h or 72 h with doxorubicin (0.01–100 μM) diluted in RPMI 1640 medium (no phenol red, Corning) supplemented with 500 μg ml^{-1} recombinant human serum albumin (Oryzogen). For SLC transporter modulator drug screening, day 30 hiPSC–CMs were treated with respective drug 24 h prior to doxorubicin administration and then a second dose was co–administered with doxorubicin as above.

384–well plate–based cell viability, caspase 3/7 activity assays. To measure cell viability after 72 h of doxorubicin (0.01–100 μM) treatment, CellTiter–Glo 2.0 (Promega) was used per manufacturer's instructions. Luminescence was measured using a VarioSkan Lux Multi–Mode Reader (Thermo Scientific) with an integration time of 0.25 sec. Apoptosis was measured using

Caspase 3/7–Glo (Promega) respectively according to manufacturer's instructions with an integration time of 1 sec. 10 μM staurosporine (MedChemExpress) was used as a positive control. Data were analyzed using Prism 7.0 software (GraphPad) using standard dose–response guidelines.

RNA–seq gene expression. RNA was extracted using a TRI reagent and Direct–zol RNA microprep kit (Zymo) including on-column DNase digestion to remove genomic DNA. Samples were quantified using an Agilent 2100 Bioanalyzer and passed QC. Forward stranded library preparation was done after ribosomal RNA depletion and sequencing with DNBseq platform sequencing (BGI), generating ~90 million paired-end 100 bp reads for each sample. Reads were mapped to the GRCh38 reference human genome using HISAT2[8]. Gene expression levels and exon usage were estimated using featureCounts function in the Subread software[9]. Differential gene expression analysis was done using DEseq2 package[10] and R (v3.3.3). Bioinformatics script and codes for the analysis are available upon request.

Quantitative Real–time PCR. RNA was isolated using a TRI reagent and Direct–zol RNA microprep kit (Zymo) including on-column DNase digestion to remove genomic DNA. cDNA was produced from 1 μg of total RNA using the High Capacity RNA–to–cDNA kit (Applied Biosystems). All PCR reactions were performed in triplicate in a 384–well plate format using TaqMan Gene Expression Master Mix in a QuantStudio 5 Real–Time PCR System (both Applied Biosystems) with following TaqMan Gene Expression Assays (Applied Biosystems): 18S (Hs99999901_s1), *NANOG* (Hs02387400_g1), *POU5F1* (Hs00999632_g1), *SOX2* (Hs01053049_s1), *KLF4* (Hs00358836_m1), *LIN28* (Hs00702808_s1), *MYC* (Hs00153408_m1),

UTF1 (Hs00747497_g1), *DNMT3B* (Hs01003405_m1), *TERT* (Hs99999022_m1), *TP53* (Hs99999147_m1), *SLC28A3* (hs00910439_m1). Relative quantification of gene expression was calculated using $2^{-\Delta\Delta Ct}$ method[11], normalized to the reference 18S and untreated control samples as specified in the figure legends.

Western blot. Cells were washed twice with DPBS, then the supernatant was aspirated, and the cell pellets were flash frozen. Cells were lysed with lysis buffer (150 mM NaCl, 1% Triton X–100, protease inhibitor and 50 mM Tris–HCl, pH 8.0). Cell protein was isolated by centrifugation at 4 °C for 15 minutes at 15,000 rpm. Protein was quantified using Bradford assay (IBI scientific). 20–50 µg of protein was reduced and denatured in LDS sample buffer and reducing agent (Invitrogen) at 37 °C for 20 min, loaded onto the precast NuPage 10% Bis–Tris gel (Invitrogen) and run for 35 min at 200 V. Transfer to the nitrocellulose membrane (GE Healthcare) was performed at 10 V for 90 min. The membrane was blocked for 1 h at RT in the blocking buffer (5% BSA diluted with TBST) and incubated with 1:200 polyclonal rabbit SLC28A3 (Santa Cruz, sc134529), and 1:2000 monoclonal mouse IgG_{2a} β–Tubulin (Invitrogen, MA5–16308) at 4 °C overnight. The membrane was then washed three times with TBST and incubated with 1:2000 HRP–goat anti–mouse IgG or HRP–goat anti–rabbit IgG (both Invitrogen) for 1 h at RT. The membrane was washed three times with TBST and incubated with Chemiluminescent substrate for quantitative chemiluminescent Westerns (Azure Biosystems) according to the manufacturer's recommendation. The chemiluminescent signals were captured using a CCD camera–based imager (Azure Biosystems).

Breast cancer cell lines. Four human breast cancer cell lines were used, MCF7 (adenocarcinoma, ATCC HTB–22) and Hs 578T (carcinsarcoma, ATCC HTB–126) both cultured in RPMI 1640

(Hyclone) with 10% FBS (Seradigm), MDA–MB–231 (adenocarcinoma, ATCC HTB–26) and MDA–MB–468 (adenocarcinoma, ATCC HTB–131) both cultured in DMEM (Corning) with 10% FBS. All cells were cultured on uncoated tissue culture plates and passaged with TrypLE Express (Gibco).

***SLC28A3* candidate gene resequencing using MinION Nanopore sequencer.**

DNA extraction and purification. DNA was isolated from six patient derived human induced pluripotent stem cells, using QuickExtract DNA Extraction Solution (Epicenter) according to manufacturer protocol. Isolated DNA was then purified using Genomic DNA Clean & Concentrator–10 (Zymo) according to manufacturer protocol.

***SLC28A3* locus amplification and amplicons validation.** ~77 kb located on Chr9: 84,291,953-84,368,534 (NC_000009.12, GRCh38.p7) encompassing the coding region of *SLC28A3* gene in addition to 9 kb and 5 kb at the 5'UTR and 3`UTR, respectively was amplified using long range PCR. A set of primer pairs were designed to amplify nine overlapping amplicons covering the target region whereas, length of amplicons ranged between 5732 and 9908 bp (**Supplemental Table 3**). Generation of overlapping amplicons help compensate for the low depth of coverage associated with the start and the end of each sequence read. Using ~200 ng of DNA per reaction, amplicons were amplified using PrimeSTAR GXL DNA Polymerase (Takara) via three steps-PCR. PCR reaction mixture components and cycling conditions are mentioned in **Supplemental material online, Table S4.** Generated amplicons were then purified using Genomic DNA Clean & Concentrator-5 (Zymo research) according to the manufacturer protocol to get rid of

contaminants that might damage the pores of the Nanopore flow cell, which leads to a significant decrease in the number of sequence reads.

Amplicon validation prior sequencing. PCR product (amplicons) were run on 1% agarose gel and visualized by staining with GelGreen Nucleic Acid Stain (Biotium) (**Figure 1**). Gel bands equivalent to target amplicons were confirmed for all amplified amplicons. For further confirmation that we got the correct amplicons, about 1 kb of the start and the end of each purified amplicon were then Sanger sequenced, and *in silico* aligned to its corresponding reference sequence. The quality and concentration of generated amplicons was assessed using NanoDrop 8000 and Qubit 3.0 fluorometer, respectively **(Supplemental Table 3)**. It is important to generate amplicons with reasonable purity to avoid ruining the pores of the flow cell which decreases the number of generated sequencing reads. Thus, amplicons with 260/280 and 260/230 of less than 1.8 and 1.5 were excluded and regenerated **(Supplemental Table 5)**.

MinION library preparation and flow cell loading. Library preparation was done using ligation sequencing (Oxford, Nanopore, SQK-LSK108) and 1D Native barcoding (Oxford, Nanopore, EXP-NBD103) kits. Nine amplicons from relevant patients were pooled together in am equimolar amount. Amplicons were then repaired using NEBNext FFPE Repair Mix (New England Biolabs, M6630) to maximize the read length by adding 1mg DNA to 8.5 ml nuclease free water, 6.5 ml FFPE repair buffer, and 2 ml FFPE Repair. The reaction mix was then cleaned adding 62 ml AMPure XP beads (Beckman Coulter, A63880), DNA was then incubated on a hula mixer at room temperature for 5 min, spun down, and pelleted on a magnet, washed twice with 200 ml freshly prepared 70% ethanol. Samples was pun down again, placed back on a magnet, left to dry for ~ 30

sec. DNA was then removed from the magnet, re-suspended in 46 ml nucleases free water, incubated for 2 min at room temperature, and re-placed on a magnet until the elute is clear. Finally, 46 ml of clear elute was transferred to 1.5 ml Eppendorf DNA LoBind tube. End-repair and dA-tailing was then performed using NEBNext End repair / dA-tailing Module (New England Biolabs, E7546). Reaction mix was prepared by adding 45 ml eluted DNA to 7 ml Ultra II End-prep reaction buffer, 10 ml Ultra II End-prep enzyme mix, and 5 ml nuclease-free water. Reaction mix was then incubated for 5 min at 20 °C followed by 5 min at 65 °C. DNA was then purified using AMPure XP beads (see above). Finally, 25 ml clear elute was transferred into DNA LoBind tube. Each sample was barcoded using 1D Native barcoding (Oxford, Nanopore, EXP-NBD103), 2.5 ml native Barcode was added to 22.5 ml end-prepped DNA, and 25 ml Blunt/TA Ligase Master Mix (New England Biolabs, M0367). Reaction mix was then incubated for 10 min at room temperature, DNA was than purified using AMPure XP beads (see above), and 26 ml of clear elute was transferred into Eppendorf DNA LoBind tube.

Barcoded samples were pooled in an equimolar amount to a final concentration of 700 ng, then diluted by adding 24 ml nuclease free water. Adapter ligation was then performed using NEBNext Quick Ligation Module (New England Biolabs, E6056). 700 ng pooled DNA was mixed with 20 ml Barcode Adapter Mix, 20 ml NEBNext Quick Ligation Reaction Buffer, and 10 ml Quick T4 DNA Ligase. Reaction mix was then incubated for 10 min at room temperature, and DNA was then purified by adding 62 ml AMPure XP beads Beckman Coulter, A63880), incubated on a hula mixer at room temperature for 5 min, spun down, and pelleted on a magnet, and Supernatant was discarded. Beads were then resuspended in 140 ml Adapter Bead Buffer (ABB) by flicking the tube, pelted on magnet, and supernatant was discarded (resuspension step was repeated). Pellet was resuspended in 15 ml Elution Buffer, incubated for 10 min at room

temperature, pellet on magnet until the elute is clear, and finally 15 ml clear elute was transferred into Eppendorf DNA LoBind tube.

Priming mix was prepared by adding 576 µl RBF to 624 µl nuclease-free water, then 800 µl priming mix was loaded on the flow cell using priming port dropwise to avoid the introduction of air bubbles. Five minutes later, SpotON sample cover on MinION was opened and 200 µl priming mix was loaded. DNA library was prepared for loading by adding 12 µl DNA library to 35 µl RBF, 25.5 µl LLB, and 2.5 µl nuclease-free water. DNA library was gently mixed, loaded on the flow cell (FLO-MIN 106 R9 version, FAF19356) through SpotON port. Library was then sequenced for 48 hours with live base-calling.

Raw sequencing data and SNPs functional analysis. Raw barcoded sequence reads were demultiplexed into six fastaq files using Porechop[12]. Quality of demultiplexed sequence reads were assessed using Nanopack[13]. Sequence reads were then aligned to reference human genome (GRCh38.p92) using minimap2[14] "-ax map-ont", sam files were then sorted and converted into bam files using SAMtools[15]. Bam files were down-sampled using SAMtools "-s 0.1 to -s 0.9", and the quality of aligned reads were assessed using Nanopack. Depth of coverage analysis was done using deepTools2[16]. Sequence reads were indexed and variants were called using Nanopolish[17]. Variant call format files containing called SNPs were processed and analyzed using several tools including VCFtools[18], SnpSift[19], and BCFtools[20]. SNPs functional annotation analysis was done using DeepSEA[21], R (RCoreTeam) and BiomaRt[22] Bioconductor package that includes multiple ensemble gene regulation database. Conservation analysis was done using SnpSift[19] and PhastCons dataset that includes genome-wide multiple alignments with other 99 vertebrate species. (http://hgdownload.cse.ucsc.edu/goldenpath/hg38/phastCons100way)

Editing of the causal variant, rs11140490 in hiPSC derived form study patients. Locus-specific base-editor protein complex and the gRNA were designed using Beditor [23], and the designed gRNA was cloned in the gRNA expressing plasmid (73797, Addgene). Then 1 x 10^6 cells were electroporated with 4 µg of the base editor expressing plasmid (pSI-Target-AID-NG, 119861, Addgene) and 4 µg of the gRNA expressing plasmid (lenti sgRNA (MS2) _puro, 73797, Addgene). Cells were then selected with 0.3 µg/ml puromycin 24 h post transfection for 48 h, clones were picked, the target locus was PCR-amplified and sanger-sequenced to confirm the SNP editing in all clones.

SLC28A3-AS1 overexpression in isogenic hiPSCs. The *SLC28A3-AS1* cDNA was cloned into pLenti-C-Myc-DDK-IRES-Puro lentiviral vector (Origene) which was then co–transfected with packaging plasmids psPAX2 (Addgene 12260) and pMD2.G (Addgene 12259) into Lenti-X 293T cells (Takara) to generate lentivirus. Virus–containing supernatant was collected at 48– and 72–-hours post–transfection. Lentivirus was concentrated 1:100 from cleared supernatant using PEG-iT (SBI). Isogeneic hiPSCs were then transduced and positive clones were selected with puromycin for seven days to generate $ISO^{SLC28A3\text{-}AS1}$. *SLC28A3-AS1* overexpression was confirmed using stranded RNA–Seq after ribosomal RNA depletion.

Mouse model of doxorubicin–induced cardiomyopathy and drug administration. C57BL/6J 10 weeks old male mice were co-treated with doxorubicin (NovaPlus) and water as a control vehicle (n = 100), or with desipramine (Sigma) as experimental groups (n = 8). At day 0, mice were treated with doxorubicin (3 mg kg^{-1}) intraperitoneally twice a week alone or with desipramine

by Alzet pump infusion (20 mg kg^{-1} day^{-1}) for 3 weeks (day 0–day 21). For the control group, we treated mice with corn oil in the same schedule as desipramine administration. We recorded an echocardiogram once a week (day 0, day 7, day 14, and day 21) and terminated the experiment at day 21.

Echocardiographic evaluation. Mice were studied at baseline and weekly during the protocol under light anesthesia with isoflurane (induction 3%, maintenance 1.5%). 2D images in the parasternal short axis were obtained with a GE Vivid 7 ultrasound system (GE Healthcare) equipped with a 13 MHz transducer. Left ventricular end–systolic (LVESD) and end–diastolic (LVEDD) dimensions were measured and left ventricular fractional shortening (FS) was calculated.

Statistical methods. Data were analyzed in R version 4.0.3 and graphed in GraphPad Prism 6. Detailed statistical information is included in the corresponding figure legends. Data were presented as mean ± SEM. Comparisons were conducted via one way–ANOVA test, or an unpaired two–tailed Student's t–test with significant differences defined as $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****). Our sample size (3 patients in each category) was based on the feasibility of handling this number of hiPSC lines. For dose response curves, log-logistic non-linear regression model was used to estimate the value of the four parameters, and t–statistic was used to test for significant difference in LD_{50} between different groups using "drc" package[24] in R. Patient exclusion criteria are outlined in **Supplemental Table 1**. No statistical methods were used to predetermine sample size. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

Supplemental Table 1: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Diagnosis of cancer	Patients who have not been treated with doxorubicin
Treatment with doxorubicin (Adriamycin)	Unwilling to consent/assent to ≤15 ml blood draw (≤5 ml for children under 5 years)
Age <21 years at time treatment	
Must have previously participated in the research of CPNDS	
Must be European ancestry	
Documentation of pre–chemotherapy shortening fraction of ≥30%	
For DIC patients only: SF of ≤26%. Only echocardiograms ≥21 days after a doxorubicin dose are to be considered.	
For control patients: SF of ≥30% and no symptoms of cardiac compromise for at least 5 years after treatment	

Date provided by the Canadian Pharmacogenomics Network of Drug Safety (CPNDS); SF, shortening fraction

Supplemental Table 2: Doxorubicin–treated patients recruited in this study

ID	Anthracycline	Cardiotoxicity	Gender	Age at Treatment	Cancer diagnosis	Heart radiation	rs7853758 genotype
SLC^{ref1}	Yes	Yes	Male	5.1	Wilm's Tumor	Yes	GG
SLC^{ref2}	Yes	Yes	Male	1.6	ALL	No	GG
SLC^{ref3}	Yes	Yes	Female	4.3	ALL	No	GG
SLC^{var1}	Yes	No	Female	2.7	ALL	No	AG
SLC^{var2}	Yes	No	Female	2.2	ALL	No	AG
SLC^{var3}	Yes	No	Male	1.6	ALL	No	AG
ISO	No	NA	Male	NA	NA	No	GG

Heart radiation therapy includes significant radiation exposure to the heart or surrounding tissue. This includes mantle and mediastinal radiation, whole–lung radiation, whole–abdomen or upper abdominal radiation, left–side flank radiation and total–body irradiation. SF, shortening fraction; NA, not applicable. ISO is the control hiPSC line used to generate knockout and overexpression lines.

Supplemental Table 3: primers for *SLC28A3* amplicons amplification

Primer ID	Sequence 5'>3'	Direction	Amplicon length (bp)
Amp_1_fw	AGTTGCATGTTGCCATTCTG	Forward	9218
Amp_1_rw	GTTGCTGTAGCCCTCAGCTC	Reverse	
Amp_2_fw	CTCCCCAGGAGTGCAAATAG	Forward	9908
Amp_2_rv	TCAAGGGGAATCACTTCAGG	Reverse	
Amp_3_fw	TCAAGTTTGCATGATCACACC	Forward	8979
Amp_3_rv	CAGGAAATATGGCTTCAGCTC	Reverse	
Amp_4_fw	AAGGAAGATCCCACGTTGTG	Forward	9286
Amp_4_rv	AAGTGATGCTTCCCATCAGG	Reverse	
Amp_5_fw	GCTGTTTGTTGAATCGGATG	Forward	9306
Amp_5_rv	TCCAACTGTCTGAGCACCAG	Reverse	
Amp_6_fw	TGTTGCAGGTGTTTGGAAAG	Forward	5732
Amp_6_rv	ACATTATGAGCCCACCGAAG	Reverse	
Amp_7_fw	CGGCCGCTGGTGAGGTCCCCCAA	Forward	8668
Amp_7_rv	TGGGCAGTGGTGCTGGCAAGCGT	Reverse	
Amp_8_fw	TTGGCAATGTCCGGATTC	Forward	9420
Amp_8_rv	TTCCCCTTTCCAGGGATAAC	Reverse	
Amp_9_fw	GGACCTCTTCTCCCTGGAAC	Forward	9509
Amp_9_rv	AGACCCTAAGGCCTCTCCAG	Reverse	

Supplemental Table 4: PCR reaction mixture and conditions

Amplicon	Composition of reaction mixture	PCR condition
Amp1, Amp2, Amp4, Amp5, and Amp9	10 µl 5X PrimeSTAR GXL Buffer, 4 µl dNTP Mixture (2.5 mM each), 1 µl of 100 µM primer, 300 ng DNA template, and 1 µl PrimeSTAR GXL DNA Polymerase 1.25 U/50 µl, and Sterile distilled water to 50 µl	30 cycles 98 °C 10 sec 60 °C 15 sec 68 °C 10 min Hold at 4 °C
Amp3, and Amp 8	10 µl 5X PrimeSTAR GXL Buffer, 4 µl dNTP Mixture (2.5 mM each), 1 µl of 100 µM primer, 300 ng DNA template, and 1 µl PrimeSTAR GXL DNA Polymerase 1.25 U/50, and Sterile distilled water to 50 µl	30 cycles 98 °C 10 sec 58 °C 15 sec 68 °C 10 min Hold at 4 °C
Amp6	10 µl 5X PrimeSTAR GXL Buffer, 4 µl dNTP Mixture (2.5 mM each), 1 µl of 100 µM primer, 300 ng DNA template, and 1 µl PrimeSTAR GXL DNA Polymerase 1.25 U/50, and Sterile distilled water to 50 µl	30 cycles 98 °C 10 sec 60 °C 15 sec 68 °C 6 min Hold at 4 °C
Amp7	10 µl 5X PrimeSTAR GXL Buffer, 4 µl dNTP Mixture (2.5 mM each), 1 µl of 100 µM primer, 300 ng DNA template, and 1 µl PrimeSTAR GXL DNA Polymerase 1.25 U/50, and Sterile distilled water to 50 µl	30 cycles 98 °C 10 sec 66 °C 15 sec 68 °C 6 min Hold at 4 °C

Supplemental Table 5: Quality assessment of *SLC28A3* amplicons

Sample ID	Amplicon	Conc (ng/µl)	A260	A280	260/280	260/230
SLCref1	Amp01	117.2	2.344	1.232	1.9	1.72
	Amp02	7.525	0.15	0.095	1.58	1.69
	Amp03	74.43	1.489	0.771	1.93	1.73
	Amp04	48.37	0.967	0.514	1.88	2.08
	Amp05	128.3	2.566	1.342	1.91	1.94
	Amp06	59.79	1.196	0.649	1.84	1.62
	Amp07	90.3	1.806	0.971	1.86	1.69
	Amp08	7.217	0.144	0.074	1.95	1.52
	Amp09	146.8	2.936	1.546	1.9	2.13
***SLC*^{ref2}**	Amp01	148.3	2.967	1.57	1.89	2.14
	Amp02	80.62	1.612	0.88	1.83	1.72
	Amp03	51.97	1.039	0.549	1.89	1.75
	Amp04	107.7	2.154	1.142	1.89	1.96
	Amp05	98.96	1.979	1.06	1.87	1.95
	Amp06	122.6	2.453	1.288	1.9	1.94
	Amp07	103.4	2.069	1.123	1.84	1.88
	Amp08	19.44	0.389	0.217	1.79	2.04
	Amp09	81.35	1.627	0.855	1.9	2.02
***SLC*^{ref3}**	Amp01	96.78	1.936	1.016	1.91	2.09
	Amp02	60.44	1.209	0.672	1.8	1.57
	Amp03	73.76	1.475	0.796	1.85	1.72
	Amp04	134.1	2.681	1.424	1.88	2.07
	Amp05	84.22	1.684	0.887	1.9	1.92
	Amp06	41.24	0.825	0.439	1.88	1.86
	Amp07	104.5	2.089	1.095	1.91	2.11
	Amp08	45.57	0.911	0.496	1.84	1.79
	Amp09	73.19	1.464	0.81	1.81	1.67
***SLC*^{var1}**	Amp01	120.1	2.403	1.284	1.87	2.07
	Amp02	40.18	0.804	0.449	1.79	1.61
	Amp03	58.98	1.18	0.631	1.87	1.8
	Amp04	91.2	1.824	0.973	1.87	1.92
	Amp05	114.6	2.293	1.241	1.85	1.72
	Amp06	93.09	1.862	0.979	1.9	2.01
	Amp07	106.4	2.128	1.11	1.92	1.77
	Amp08	64.44	1.289	0.711	1.81	1.91
	Amp09	61.63	1.233	0.651	1.89	2.08
***SLC*^{var2}**	Amp01	147	2.94	1.551	1.9	1.79
	Amp02	38.41	0.768	0.405	1.9	1.78
	Amp03	35.4	0.708	0.387	1.83	1.56
	Amp04	155.2	3.105	1.664	1.87	1.91
	Amp05	101.4	2.028	1.045	1.94	1.98
	Amp06	46.66	0.933	0.494	1.89	1.88
	Amp07	82.67	1.653	0.911	1.81	1.9
	Amp08	14.07	0.281	0.15	1.88	1.81
	Amp09	61.4	1.228	0.663	1.85	2.11
***SLC*^{var3}**	Amp01	72.96	1.459	0.771	1.89	1.72
	Amp02	116.3	2.327	1.246	1.87	2.13
	Amp03	125.4	2.509	1.312	1.91	2.2

	Amp04	59.99	1.2	0.644	1.86	1.55
	Amp05	33.31	0.666	0.353	1.89	1.79
	Amp06	141.3	2.826	1.515	1.87	1.73
	Amp07	27.08	0.542	0.295	1.84	1.59
	Amp08	41.91	0.838	0.44	1.91	1.72
	Amp09	90.51	1.81	0.978	1.85	1.83

Supplemental Table 6: Identified *SLC28A3* SNP genotypes across study samples

Position	SNP Id	REF	ALT	*SLC*^{ref1}	*SLC*^{ref2}	*SLC*^{ref3}	*SLC*^{var1}	*SLC*^{var2}	*SLC*^{var3}	Location	AA alteration
84273903	rs1332538	C	T	0	0	0	1	1	0	3'-UTR	
84274601	**rs12003403**	G	A	0	0	0	1	1	1	3'-UTR	
84274729	**rs12003423**	G	A	0	0	0	1	1	1	3'-UTR	
84275091	**rs11140488**	A	T	0	0	0	1	1	1	3'-UTR	
84275843	rs17426961	C	T	0	1	0	0	0	0	3'-UTR	
84276016	**rs11140489**	T	A	0	0	0	1	1	1	3'-UTR	
84276158	NA	C	T	1	1	1	1	1	1	3'-UTR	
84276679	**rs10868133**	T	C	0	0	0	1	1	1	3'-UTR	
84276696	rs1036176955	C	A	0	0	0	0	0	1	3'-UTR	
84277372	**rs4877272**	G	A	0	0	0	1	1	1	3'-UTR	
84277979	**rs3750406**	A	C	0	0	0	1	1	1	3'-UTR	
84278156	**rs7858075**	T	C	0	0	0	1	1	1	3'-UTR	
84278398	**rs11140490**	A	G	0	0	0	1	1	1	I17	
84278763	NA	G	A	1	1	1	1	1	1	I17	
84279527	rs7862562	T	C	0	1	0	1	1	1	I16	
84279858	rs1290966405	C	T	1	0	0	0	0	1	I16	
84280938	**rs10868135**	T	C	0	0	0	1	1	1	I14	
84282506	NA	C	T	1	0	1	1	1	1	I14	
84283431	rs973302715	A	G	0	0	0	0	1	0	I14	
84284969	**rs4877831**	C	G	0	0	0	1	1	1	I14	
84285032	rs4877832	A	C	0	1	0	1	1	1	I14	
84285101	**rs4877833**	T	C	0	0	0	1	1	1	I14	
84285427	NA	G	A	1	1	1	1	1	1	E14	A522V
84285698	**rs7853066**	A	G	0	0	0	1	1	1	I13	
84286011	**rs7853758**	G	A	0	0	0	1	1	1	E13	L489L
84286220	rs937635656	G	A	0	0	0	0	0	1	I12	
84287089	**rs7030019**	A	G	0	0	0	1	1	1	I12	
84288640	NA	G	A	1	0	0	0	0	0	I11	
84289166	NA	G	A	1	1	1	1	1	1	I11	
84290636	**rs4877834**	T	C	0	0	0	1	1	1	I10	
84291093	**rs7047315**	A	G	0	0	0	1	1	1	I10	
84291502	**rs7047898**	A	C	0	0	0	1	1	1	I10	
84291663	rs1050069561	C	T	1	1	1	1	1	1	I10	

84291698	NA	G	A	1	1	1	1	1	1	I10	
84291702	NA	G	A	1	1	1	1	1	1	I10	
84294167	**rs10868137**	A	G	0	0	0	1	1	1	I9	
84294635	**rs885004**	G	A	0	0	0	1	1	1	I8	
84295359	rs530032784	C	T	0	2	0	0	0	0	I8	
84296355	NA	C	T	1	1	1	1	1	1	I8	
84297553	NA	C	T	0	0	0	0	1	0	I7	
84298559	NA	G	A	0	0	0	1	0	0	I6	
84299856	rs530032784	G	A	0	0	1	0	0	0	I5	
84300626	rs144419201	C	T	0	1	0	0	0	0	I5	
84301200	rs12379959	A	T	1	0	1	0	0	0	I5	
84301258	rs12377274	G	A	1	0	0	0	0	0	I5	
84301936	**rs4877835**	T	G	0	0	0	1	1	1	I5	
84302092	rs17087056	C	A	0	0	0	1	1	0	I5	
84302173	**rs4877836**	T	C	0	0	0	1	1	1	I5	
84303804	rs1021699143	C	T	1	1	1	1	1	1	I4	
84305321	**rs7867504**	T	C	0	0	0	1	1	1	E4	T89T
84305796	rs4242626	T	C	0	0	0	0	1	0	I3	
84306347	rs989230152	C	T	1	1	1	1	1	1	I3	
84307078	**rs12237803**	C	T	0	0	0	1	1	1	I3	
84307083	rs1262441955	G	A	0	1	1	0	0	0	I3	
84307315	rs142007597	C	T	1	0	0	0	0	0	I3	
84307845	rs150776148	T	C	1	0	0	0	0	0	I3	
84308361	rs141695271	C	T	0	0	0	0	1	0	I3	
84308737	NA	C	T	1	1	1	1	1	1	I3	
84313793	rs13291905	A	G	0	1	0	1	0	1	I1	
84313852	rs7866821	C	G	2	1	1	0	2	0	I1	
84314849	rs4877843	T	C	0	0	0	0	1	0	I1	
84319068	rs1051842387	T	C	0	0	1	0	0	0	I1	
84319815	rs10735568	T	C	2	2	1	0	2	0	I1	
84321516	rs12347278	G	A	0	0	1	0	0	0	I1	
84322400	rs11140525	G	A	1	0	0	0	1	0	I1	
84323144	rs12004882	C	G	0	0	0	0	1	0	I1	
84324414	rs7046305	T	C	0	2	0	0	2	0	I1	
84324908	rs4877845	A	C	0	2	0	0	2	0	I1	
84326000	rs1331168053	G	A	1	1	1	1	1	1	I1	
84326705	rs4588940	A	G	0	0	0	0	2	0	I1	
84327052	rs7019546	A	G	0	2	0	0	2	0	I1	

84327889	rs10868148	T	G	0	2	0	0	2	0	I1	
84328654	rs4877846	G	A	0	0	0	0	2	0	I1	
84328682	rs4877273	T	C	0	2	0	0	2	0	I1	
84328768	rs4877274	G	A	0	2	0	0	2	0	I1	
84328814	rs11789143	G	A	1	1	1	1	1	1	I1	
84329641	NA	C	T	1	1	1	1	1	1	I1	
84330006	rs4242627	C	T	0	0	0	0	2	0	I1	
84330082	rs4242628	G	A	1	0	0	1	2	0	I1	
84330800	rs58075154	C	T	0	0	0	0	2	0	I1	
84330820	rs57409783	A	G	0	0	0	0	2	0	I1	
84331158	rs17343066	G	A	1	1	1	0	2	1	I1	
84331502	rs4877847	A	C	1	1	1	1	2	1	I1	
84331509	rs75663843	T	G	0	1	0	0	0	0	I1	
84331692	rs980292	T	C	1	2	1	1	2	1	I1	
84332442	rs1972245	T	C	1	1	1	1	2	1	I1	
84332615	NA	G	A	0	0	0	0	1	0	I1	
84333013	rs79257653	C	T	0	0	0	0	0	1	I1	
84333038	rs1248714397	C	T	1	1	1	1	1	1	I1	
84333357	rs118104816	A	G	0	1	1	0	0	0	I1	
84333380	rs4448361	T	C	1	1	1	1	2	1	I1	
84333660	rs76940186	A	C	0	1	1	0	0	0	I1	
84333701	rs4266723	C	T	1	2	2	1	2	1	I1	
84335058	rs10868149	G	A	0	1	1	0	0	0	I1	
84335955	rs4877848	C	T	0	1	1	0	0	0	I1	
84336700	rs4877850	C	T	1	2	2	1	2	1	I1	
84337348	rs6559781	T	C	1	2	2	1	2	1	I1	
84337448	rs149980849	G	A	0	1	0	0	0	0	I1	
84338592	NA	G	A	0	0	1	0	0	0	I1	
84338706	rs17428030	A	G	0	1	0	0	0	0	I1	
84338759	rs7043257	T	C	1	2	1	1	2	1	I1	
84339395	rs4877852	A	G	1	2	1	1	2	1	I1	
84339551	rs7027983	C	T	1	2	1	1	2	1	I1	
84339776	rs7031310	C	G	1	2	1	1	2	1	I1	
84339802	rs7031197	A	G	2	2	2	1	2	2	I1	
84340111	NA	T	C	0	0	0	1	0	0	I1	
84340242	rs3812509	C	T	1	2	1	1	2	1	I1	
84340301	rs1175981076	C	T	1	0	0	0	0	0	I1	
84340767	rs7035753	C	T	1	0	1	1	0	1	5-UTR	

84340824	rs562029530	C	T	0	0	1	0	0	0	5'-UTR	
84341021	rs4604528	T	C	1	1	1	1	1	1	5'-UTR	
84341181	NA	G	A	0	0	0	0	1	0	5'-UTR	
84341186	NA	T	G	0	0	0	0	1	0	5'-UTR	
84341202	NA	C	T	0	0	0	0	1	0	5'-UTR	
84341213	NA	C	T	0	0	0	0	1	0	5'-UTR	
84341214	NA	A	G	0	0	0	0	1	0	5'-UTR	
84341215	NA	C	T	0	0	0	0	1	0	5'-UTR	
84341217	NA	T	C	0	0	0	0	1	0	5'-UTR	
84341405	rs57404564	C	A	0	1	0	0	0	0	5'-UTR	
84341428	rs28629238	A	G	0	1	0	0	0	0	5'-UTR	
84341697	rs17343456	A	G	0	0	0	0	1	0	5'-UTR	
84342889	NA	C	T	1	0	1	0	1	1	5'-UTR	
84343833	rs12335574	A	G	0	1	0	0	0	0	5'-UTR	
84344334	rs144927764	G	A	0	0	0	1	0	0	5'-UTR	
84345145	rs10780664	C	A	0	1	0	0	0	0	5'-UTR	
84345715	rs11140535	A	G	0	1	0	0	0	0	5'-UTR	
84347396	rs77681349	C	T	0	1	0	0	0	0	5'-UTR	
84347715	NA	C	T	0	0	0	0	1	1	5'-UTR	
84349384	NA	C	A	0	0	0	0	1	0	5'-UTR	
84349394	rs1298053988	G	A	0	0	0	0	1	0	5'-UTR	
84349402	NA	A	G	0	0	0	0	1	0	5'-UTR	
84349404	NA	T	C	0	0	0	0	1	0	5'-UTR	
84349741	rs13298157	G	A	0	1	0	0	0	0	5'-UTR	

REF, reference allele; ALT, alternative allele; 0, homozygous reference; 1, heterozygous variant; and 2, homozygous variant; AA, amino acid. SNPs in bold are SNPs coinherited in cardio protected patients but not in cardiotoxicity patients. Variants are annotated in relevance to *SLC28A3* transcript NM_001199633.1.

Supplemental Table 7: Regulatory properties of *SLC28A3* SNPs coinherited only in cardioprotected patients

rs Id	No. of altered chromatin feature binding sites
rs11140490	206
rs4877835	204
rs4877836	141
rs7867504	134
rs4877272	107
rs885004	105
rs12237803	52
rs3750406	41
rs12003403	40
rs10868135	33
rs4877831	32
rs4877833	31
rs10868137	30
rs7853758	11
rs7858075	6
rs7047315	4
rs7853066	4
rs7030019	3
rs12003423	2
rs7047898	2
rs11140488	1
rs4877834	1
rs11140489	0
rs10868133	0

Supplemental Table 8: *SLC28A3* SNPs coinherited only in cardio protected patient affecting chromatin feature binding sites (showing only SNPs with Log2 fold change value >=1)

SNP Id	Cell type| chromatin| treatment	E-value	Log2 fold change
rs4877272	ECC-1|ERalpha|BPA_100nM	0.01	-1.01
	H1-hESC|TEAD4|None	0.01	-1.60
	NT2-D1|DNase|None	0.03	-1.22
	NHEK|DNase|None	0.03	-1.01
	H7-hESC|DNase|None	0.03	-1.22
	H1-hESC|DNase|None	0.04	-1.22
	RWPE1|DNase|None	0.05	-1.09
rs7867504	GM12878|JunD|None	0.00	-1.11
	PrEC|DNase|None	0.01	-1.55
	GM12878|BATF|None	0.01	-1.34
	GM12865|DNase|None	0.01	-1.04
	GM12864|DNase|None	0.01	-1.00
	SAEC|DNase|None	0.01	-1.62
	HMEC|DNase|None	0.01	-1.12
	HEEpiC|DNase|None	0.01	-1.52
	pHTE|DNase|None	0.01	-1.06
	NHEK|DNase|None	0.01	-1.17
	HRCEpiC|DNase|None	0.02	-1.18
	HRE|DNase|None	0.02	-1.21
	HPDE6-E6E7|DNase|None	0.02	-1.22
	MCF10A-Er-Src|STAT3|4OHTAM_1uM_12hr	0.02	-1.30
	MCF10A-Er-Src|STAT3|EtOH_0.01pct_12hr	0.02	-1.25
	MCF10A-Er-Src|c-Fos|4OHTAM_1uM_12hr	0.02	-1.81
	MCF10A-Er-Src|c-Myc|4OHTAM_1uM_4hr	0.02	-1.06
	MCF10A-Er-Src|STAT3|EtOH_0.01pct_4hr	0.02	-1.18
	MCF10A-Er-Src|STAT3|4OHTAM_1uM_36hr	0.02	-1.21
	MCF10A-Er-Src|STAT3|EtOH_0.01pct	0.02	-1.00
	MCF10A-Er-Src|c-Fos|4OHTAM_1uM_4hr	0.02	-1.67
	RWPE1|DNase|None	0.02	-1.17
	HUVEC|c-Fos|None	0.03	-1.09
	MCF10A-Er-Src|c-Fos|EtOH_0.01pct	0.03	-1.66
	MCF10A-Er-Src|c-Fos|4OHTAM_1uM_36hr	0.03	-1.82
	HMVEC-dBl-Ad|DNase|None	0.03	-1.13

	RPTEC|DNase|None	0.03	-1.01
	HMVEC-dLy-Neo|DNase|None	0.03	-1.02
	WI-38|DNase|4OHTAM_20nM_72hr	0.04	-1.28
	HMVEC-LBl|DNase|None	0.04	-1.18
	HUVEC|c-Jun|None	0.04	-1.01
	HFF-Myc|DNase|None	0.05	-1.03
	NHLF|DNase|None	0.05	-1.16
rs11140490	Melano|DNase|None	0.00	1.05
	HSMM_emb|DNase|None	0.00	1.22
	HSMMtube|DNase|None	0.00	1.42
	NHDF-neo|DNase|None	0.00	1.76
	NHDF-Ad|DNase|None	0.00	1.73
	AG10803|DNase|None	0.00	1.56
	ProgFib|DNase|None	0.00	1.34
	FibroP|DNase|None	0.00	1.25
	HGF|DNase|None	0.00	1.56
	HPdLF|DNase|None	0.00	1.58
	Stellate|DNase|None	0.00	1.36
	HCF|DNase|None	0.00	1.42
	AG09319|DNase|None	0.00	1.46
	HSMM|DNase|None	0.00	1.36
	SK-N-SH|TAF1|None	0.00	1.07
	HFF|DNase|None	0.00	1.35
	BJ|DNase|None	0.00	1.42
	HCM|DNase|None	0.00	1.42
	AG09309|DNase|None	0.00	1.45
	Myometr|DNase|None	0.00	1.16
	AG04449|DNase|None	0.00	1.37
	HPF|DNase|None	0.00	1.51
	AoAF|DNase|None	0.00	1.36
	AoSMC|DNase|None	0.00	1.40
	SKMC|DNase|None	0.00	1.29
	PanIsletD|DNase|None	0.00	1.18
	HMF|DNase|None	0.00	1.42
	HPAF|DNase|None	0.00	1.31
	HConF|DNase|None	0.00	1.37
	HAc|DNase|None	0.00	1.07
	HFF-Myc|DNase|None	0.00	1.08

	HBMEC|DNase|None	0.00	1.29
	WI-38|DNase|4OHTAM_20nM_72hr	0.00	1.17
	NH-A|DNase|None	0.01	1.16
	WI-38|DNase|None	0.01	1.25
	NHLF|DNase|None	0.01	1.15
	AG04450|DNase|None	0.01	1.21
	HCFaa|DNase|None	0.01	1.13
	HNPCEpiC|DNase|None	0.01	1.16
	HVMF|DNase|None	0.01	1.26
	HCPEpiC|DNase|None	0.01	1.05
	HIPEpiC|DNase|None	0.01	1.06
	HAEpiC|DNase|None	0.01	1.10
rs4877835	NHDF-Ad|DNase|None	0.01	1.07
	NHDF-neo|DNase|None	0.01	1.06
	BE2_C|DNase|None	0.01	1.08
	SK-N-SH_RA|DNase|None	0.01	1.08
rs10868137	H1-hESC|TCF12|None	0.00	1.07
	GM12878|ZEB1|None	0.00	1.06

E-value, expect value stands for the significance of each individual chromatin feature predicted score; Log2 fold change, measure the fold change in the probability of observing a binding site for relevant chromatin feature between reference and alternative allele for a particular SNP[21].

Supplemental Table 9: *SLC28A3* SNPs coinherited only in cardioprotected patients located at regulatory regions and histone marks in cardiac tissues, and at transcription factor binding sites using ensemble regulatory build

SNP Id	Position	Histone marks in cardiac tissue	Regulatory region in cardiac tissue	Motifs present at SNP locus
rs3750406	84277979	NA	Open chromatin	TEAD4::RFX5, FOXJ3::TBX21, SOX6::TBX21, ELK1::FOXI1, ETV2::FOXI1, MGA, TBX2, TBX4, TBX5, ONECUT1, ONECUT2, ONECUT3, HOXB2::EOMES,HOXB2::TBX21, HOXB2::TBX3, MGA::DLX2, MGA::DLX3, MGA::EVX1, PITX1::HES7, E2F3::ONECUT2,TFAP2C::ONECUT2, ETV2::SREBF2, CUX1::SOX15, HOXB13::EOMES, HOXB13::TBX21,HOXD12::TBX21, TBX20, KLF13, KLF14,SREBF2, GLIS1, EOMES, SNAI2, TCF3, TCF4, THRB (n = 36)
rs7858075	84278156	NA	Open chromatin	TEAD4::FOXI1, IRF3, ETV2::SOX15, POU2F1::FOXO6, POU2F1::DLX2, TEAD4::FOXI1 (n = 6)
rs11140490	84278398	NA	Open chromatin	CLOCK::FIGLA, TEAD4::EOMES, TEAD4::TBX21, ETV2::DRGX, ZIC1, ZIC3, ZIC4, HOXB2::NHLH1,TEAD4::TCF3, GCM2::SOX15, and TEAD4::FIGLA (n = 11)
rs4877831	84284969	H3K4me1	NA	NA
rs7047898	84291502	H3K36me3	NA	NA
rs10868137	84294167	NA		TFAP2C::DLX3, FOXO1::HOXB13, MGA::DLX3, HOXB2::TCF3 (n = 4)
rs885004	84294635	NA	CTCF binding site	THRB, TEAD4::CEBPD, ERF::PITX1, ETV2::GSC2, ERF::ONECUT2, ETV2::ONECUT2, FLI1::ONECUT2, POU2F1::DLX2, R, X3::SRF, TEAD4::PAX5, PITX1::HES7, HESX1, LHX9, HOXD12::HOXA3, ZBED1, BARHL2, E2F1, E2F2, E2F3, BARX1, MSX1, MSX2, TBX1, TBX20, HOXB13::EOMES, HOXB13::TBX21, TEAD4::HOXB13, PBX4::HOXA1, PBX4::HOXA10, ONECUT1, ONECUT2, HMX1, HMX2, HMX3, CUX1::SOX15, TFAP2C::ONECUT2 (n = 36)
rs4877835	84301936	NA	NA	POU2F1::FOXO6, POU2F1::EOMES, CLOCK::BHLHA15, MAX, TFAP4::MAX, HOXD12::EOMES, FOXO1, FOXO3, FOXO4, FOXO6, CTCF, ZNF238, ASCL2, BHLHA15, BHLHE22, BHLHE23,MESP2, MSC, MYF6, NEUROD2, NEUROG2, NHLH1, OLIG1, OLIG2, OLIG3, TCF15, TFAP4, ESRRA, ESRRG, FOXJ2::HOXB13 (n = 30)
rs4877836	84302173	NA	NA	MYBL1, MYBL2, IRF4, IRF5, IRF8, IRF9, ELK1::FOXI1, ERF::FOXI1, ETV2::FOXI1, ETV5::FOXI1, FLI1::FOXI1, FOXO1::ELF1, FOXO1::ELK1, ELK1::HOXA3 (n=14)

Supplemental Table 10: eQTL functional annotation of *SLC28A3* SNPs coinherited only in cardioprotected patients

SNP Id	*P*-value	NES	Tissue
rs10868133	2.10E-07	-0.22	Cells - Cultured fibroblasts
	4.50E-07	0.21	Thyroid
rs10868135	4.10E-07	-0.22	Cells - Cultured fibroblasts
	0.0000034	0.2	Thyroid
rs10868137	3.80E-07	0.23	Thyroid
	6.70E-07	-0.22	Cells - Cultured fibroblasts
rs11140488	1.60E-07	-0.22	Cells - Cultured fibroblasts
	2.60E-07	0.22	Thyroid
rs11140489	1.50E-07	-0.22	Cells - Cultured fibroblasts
	4.20E-07	0.21	Thyroid
rs11140490	1.40E-07	-0.22	Cells - Cultured fibroblasts
	6.30E-07	0.21	Thyroid
rs12003403	1.60E-07	-0.22	Cells - Cultured fibroblasts
	2.60E-07	0.22	Thyroid
rs12003423	1.60E-07	-0.22	Cells - Cultured fibroblasts
	7.20E-07	0.21	Thyroid
rs12237803	4.70E-08	-0.24	Cells - Cultured fibroblasts
	7.50E-08	0.24	Thyroid
rs3750406	1.40E-07	-0.22	Cells - Cultured fibroblasts
	6.30E-07	0.21	Thyroid
rs4877272	6.40E-08	-0.23	Cells - Cultured fibroblasts
	5.60E-07	0.21	Thyroid
rs4877831	6.00E-09	-0.21	Cells - Cultured fibroblasts
	0.000021	0.16	Thyroid
rs4877833	5.30E-07	-0.21	Cells - Cultured fibroblasts
	8.90E-07	0.21	Thyroid
rs4877834	4.70E-08	0.24	Thyroid
	7.40E-07	-0.21	Cells - Cultured fibroblasts
rs4877835	4.20E-07	0.23	Thyroid
	5.20E-07	-0.22	Cells - Cultured fibroblasts
rs4877836	3.10E-07	-0.23	Cells - Cultured fibroblasts
	3.30E-07	0.23	Thyroid
rs7030019	1.70E-08	0.25	Thyroid
	8.70E-08	-0.23	Cells - Cultured fibroblasts

	0.000014	0.64	Brain - Amygdala
rs7047315	3.80E-07	0.23	Thyroid
	6.70E-07	-0.22	Cells - Cultured fibroblasts
rs7047898	3.80E-07	0.23	Thyroid
	6.70E-07	-0.22	Cells - Cultured fibroblasts
rs7853066	1.50E-07	0.23	Thyroid
	7.00E-07	-0.21	Cells - Cultured fibroblasts
rs7853758	3.10E-08	0.23	Thyroid
	0.0000019	-0.2	Cells - Cultured fibroblasts
	0.000014	0.61	Brain - Amygdala
rs7867504	0.000003	-0.16	Cells - Cultured fibroblasts
rs885004	1.30E-07	-0.23	Cells - Cultured fibroblasts
	1.90E-07	0.23	Thyroid

NES, normalized effect size; This analysis was done using GTEX eQTL database

Supplemental Table 11: Linkage disequilibrium pattern of Nanopore-identified cardioprotective haplotype SNPs (*n* = 24) in 99 control individuals

SNP 1	SNP 2	D'	R^2	SNP 1	SNP 2	D'	R^2
rs12003403	rs12003423	1.00	1.00	rs11140488	rs11140489	1.00	1.00
rs12003403	rs11140488	1.00	1.00	rs11140488	rs10868133	1.00	1.00
rs12003403	rs11140489	1.00	1.00	rs11140488	rs4877272	1.00	1.00
rs12003403	rs10868133	1.00	1.00	rs11140488	rs3750406	1.00	1.00
rs12003403	rs4877272	1.00	1.00	rs11140488	rs7858075	1.00	1.00
rs12003403	rs3750406	1.00	1.00	rs11140488	rs11140490	1.00	1.00
rs12003403	rs7858075	1.00	1.00	rs11140488	rs10868135	1.00	1.00
rs12003403	rs11140490	1.00	1.00	rs11140488	rs4877831	0.96	0.65
rs12003403	rs10868135	1.00	1.00	rs11140488	rs4877833	1.00	0.97
rs12003403	rs4877831	0.96	0.65	rs11140488	rs7853066	1.00	0.85
rs12003403	rs4877833	1.00	0.97	rs11140488	rs7853758	0.97	0.82
rs12003403	rs7853066	1.00	0.85	rs11140488	rs7030019	1.00	0.85
rs12003403	rs7853758	0.97	0.82	rs11140488	rs4877834	1.00	0.88
rs12003403	rs7030019	1.00	0.85	rs11140488	rs7047315	1.00	0.88
rs12003403	rs4877834	1.00	0.88	rs11140488	rs7047898	1.00	0.88
rs12003403	rs7047315	1.00	0.88	rs11140488	rs10868137	1.00	0.88
rs12003403	rs7047898	1.00	0.88	rs11140488	rs885004	1.00	0.85
rs12003403	rs10868137	1.00	0.88	rs11140488	rs4877835	1.00	0.88
rs12003403	rs885004	1.00	0.85	rs11140488	rs4877836	1.00	0.88
rs12003403	rs4877835	1.00	0.88	rs11140488	rs7867504	0.95	0.37
rs12003403	rs4877836	1.00	0.88	rs11140488	rs12237803	1.00	0.82
rs12003403	rs7867504	0.95	0.37	rs11140489	rs10868133	1.00	1.00
rs12003403	rs12237803	1.00	0.82	rs11140489	rs4877272	1.00	1.00
rs12003423	rs11140488	1.00	1.00	rs11140489	rs3750406	1.00	1.00
rs12003423	rs11140489	1.00	1.00	rs11140489	rs7858075	1.00	1.00
rs12003423	rs10868133	1.00	1.00	rs11140489	rs11140490	1.00	1.00
rs12003423	rs4877272	1.00	1.00	rs11140489	rs10868135	1.00	1.00
rs12003423	rs3750406	1.00	1.00	rs11140489	rs4877831	0.96	0.65
rs12003423	rs7858075	1.00	1.00	rs11140489	rs4877833	1.00	0.97
rs12003423	rs11140490	1.00	1.00	rs11140489	rs7853066	1.00	0.85
rs12003423	rs10868135	1.00	1.00	rs11140489	rs7853758	0.97	0.82
rs12003423	rs4877831	0.96	0.65	rs11140489	rs7030019	1.00	0.85
rs12003423	rs4877833	1.00	0.97	rs11140489	rs4877834	1.00	0.88
rs12003423	rs7853066	1.00	0.85	rs11140489	rs7047315	1.00	0.88

rs12003423	rs7853758	0.97	0.82	rs11140489	rs7047898	1.00	0.88
rs12003423	rs7030019	1.00	0.85	rs11140489	rs10868137	1.00	0.88
rs12003423	rs4877834	1.00	0.88	rs11140489	rs885004	1.00	0.85
rs12003423	rs7047315	1.00	0.88	rs11140489	rs4877835	1.00	0.88
rs12003423	rs7047898	1.00	0.88	rs11140489	rs4877836	1.00	0.88
rs12003423	rs10868137	1.00	0.88	rs11140489	rs7867504	0.95	0.37
rs12003423	rs885004	1.00	0.85	rs11140489	rs12237803	1.00	0.82
rs12003423	rs4877835	1.00	0.88	rs10868133	rs4877272	1.00	1.00
rs12003423	rs4877836	1.00	0.88	rs10868133	rs3750406	1.00	1.00
rs12003423	rs7867504	0.95	0.37	rs10868133	rs7858075	1.00	1.00
rs12003423	rs12237803	1.00	0.82	rs10868133	rs11140490	1.00	1.00
rs4877272	rs3750406	1.00	1.00	rs10868133	rs10868135	1.00	1.00
rs4877272	rs7858075	1.00	1.00	rs10868133	rs4877831	0.96	0.65
rs4877272	rs11140490	1.00	1.00	rs10868133	rs4877833	1.00	0.97
rs4877272	rs10868135	1.00	1.00	rs10868133	rs7853066	1.00	0.85
rs4877272	rs4877831	0.96	0.65	rs10868133	rs7853758	0.97	0.82
rs4877272	rs4877833	1.00	0.97	rs10868133	rs7030019	1.00	0.85
rs4877272	rs7853066	1.00	0.85	rs10868133	rs4877834	1.00	0.88
rs4877272	rs7853758	0.97	0.82	rs10868133	rs7047315	1.00	0.88
rs4877272	rs7030019	1.00	0.85	rs10868133	rs7047898	1.00	0.88
rs4877272	rs4877834	1.00	0.88	rs10868133	rs10868137	1.00	0.88
rs4877272	rs7047315	1.00	0.88	rs10868133	rs885004	1.00	0.85
rs4877272	rs7047898	1.00	0.88	rs10868133	rs4877835	1.00	0.88
rs4877272	rs10868137	1.00	0.88	rs10868133	rs4877836	1.00	0.88
rs4877272	rs885004	1.00	0.85	rs10868133	rs7867504	0.95	0.37
rs4877272	rs4877835	1.00	0.88	rs10868133	rs12237803	1.00	0.82
rs4877272	rs4877836	1.00	0.88	rs3750406	rs7858075	1.00	1.00
rs4877272	rs7867504	0.95	0.37	rs3750406	rs11140490	1.00	1.00
rs4877272	rs12237803	1.00	0.82	rs3750406	rs10868135	1.00	1.00
rs7858075	rs11140490	1.00	1.00	rs3750406	rs4877831	0.96	0.65
rs7858075	rs10868135	1.00	1.00	rs3750406	rs4877833	1.00	0.97
rs7858075	rs4877831	0.96	0.65	rs3750406	rs7853066	1.00	0.85
rs7858075	rs4877833	1.00	0.97	rs3750406	rs7853758	0.97	0.82
rs7858075	rs7853066	1.00	0.85	rs3750406	rs7030019	1.00	0.85
rs7858075	rs7853758	0.97	0.82	rs3750406	rs4877834	1.00	0.88
rs7858075	rs7030019	1.00	0.85	rs3750406	rs7047315	1.00	0.88
rs7858075	rs4877834	1.00	0.88	rs3750406	rs7047898	1.00	0.88

rs7858075	rs7047315	1.00	0.88	rs3750406	rs10868137	1.00	0.88
rs7858075	rs7047898	1.00	0.88	rs3750406	rs885004	1.00	0.85
rs7858075	rs10868137	1.00	0.88	rs3750406	rs4877835	1.00	0.88
rs7858075	rs885004	1.00	0.85	rs3750406	rs4877836	1.00	0.88
rs7858075	rs4877835	1.00	0.88	rs3750406	rs7867504	0.95	0.37
rs7858075	rs4877836	1.00	0.88	rs3750406	rs12237803	1.00	0.82
rs7858075	rs7867504	0.95	0.37	rs11140490	rs10868135	1.00	1.00
rs7858075	rs12237803	1.00	0.82	rs11140490	rs4877831	0.96	0.65
rs10868135	rs4877831	0.96	0.65	rs11140490	rs4877833	1.00	0.97
rs10868135	rs4877833	1.00	0.97	rs11140490	rs7853066	1.00	0.85
rs10868135	rs7853066	1.00	0.85	rs11140490	rs7853758	0.97	0.82
rs10868135	rs7853758	0.97	0.82	rs11140490	rs7030019	1.00	0.85
rs10868135	rs7030019	1.00	0.85	rs11140490	rs4877834	1.00	0.88
rs10868135	rs4877834	1.00	0.88	rs11140490	rs7047315	1.00	0.88
rs10868135	rs7047315	1.00	0.88	rs11140490	rs7047898	1.00	0.88
rs10868135	rs7047898	1.00	0.88	rs11140490	rs10868137	1.00	0.88
rs10868135	rs10868137	1.00	0.88	rs11140490	rs885004	1.00	0.85
rs10868135	rs885004	1.00	0.85	rs11140490	rs4877835	1.00	0.88
rs10868135	rs4877835	1.00	0.88	rs11140490	rs4877836	1.00	0.88
rs10868135	rs4877836	1.00	0.88	rs11140490	rs7867504	0.95	0.37
rs10868135	rs7867504	0.95	0.37	rs11140490	rs12237803	1.00	0.82
rs10868135	rs12237803	1.00	0.82	rs7853066	rs7853758	0.97	0.90
rs4877831	rs4877833	1.00	0.68	rs7853066	rs7030019	0.97	0.93
rs4877831	rs7853066	1.00	0.59	rs7853066	rs4877834	0.97	0.90
rs4877831	rs7853758	0.92	0.52	rs7853066	rs7047315	0.97	0.90
rs4877831	rs7030019	0.96	0.54	rs7853066	rs7047898	0.97	0.90
rs4877831	rs4877834	0.96	0.56	rs7853066	rs10868137	0.97	0.90
rs4877831	rs7047315	0.96	0.56	rs7853066	rs885004	0.97	0.93
rs4877831	rs7047898	0.96	0.56	rs7853066	rs4877835	0.97	0.90
rs4877831	rs10868137	0.96	0.56	rs7853066	rs4877836	0.97	0.90
rs4877831	rs885004	0.96	0.54	rs7853066	rs7867504	0.94	0.31
rs4877831	rs4877835	0.96	0.56	rs7853066	rs12237803	0.97	0.90
rs4877831	rs4877836	0.96	0.56	rs7853758	rs7030019	1.00	0.97
rs4877831	rs7867504	0.97	0.55	rs7853758	rs4877834	0.97	0.93
rs4877831	rs12237803	0.96	0.52	rs7853758	rs7047315	0.97	0.93
rs4877833	rs7853066	1.00	0.88	rs7853758	rs7047898	0.97	0.93
rs4877833	rs7853758	0.93	0.79	rs7853758	rs10868137	0.97	0.93

rs4877833	rs7030019	0.96	0.82	rs7853758	rs885004	1.00	0.97
rs4877833	rs4877834	0.97	0.85	rs7853758	rs4877835	0.97	0.93
rs4877833	rs7047315	0.97	0.85	rs7853758	rs4877836	0.97	0.93
rs4877833	rs7047898	0.97	0.85	rs7853758	rs7867504	0.95	0.32
rs4877833	rs10868137	0.97	0.85	rs7853758	rs12237803	1.00	0.93
rs4877833	rs885004	0.96	0.82	rs7030019	rs4877834	1.00	0.97
rs4877833	rs4877835	0.97	0.85	rs7030019	rs7047315	1.00	0.97
rs4877833	rs4877836	0.97	0.85	rs7030019	rs7047898	1.00	0.97
rs4877833	rs7867504	0.95	0.36	rs7030019	rs10868137	1.00	0.97
rs4877833	rs12237803	0.96	0.79	rs7030019	rs885004	1.00	1.00
rs4877834	rs7047315	1.00	1.00	rs7030019	rs4877835	1.00	0.97
rs4877834	rs7047898	1.00	1.00	rs7030019	rs4877836	1.00	0.97
rs4877834	rs10868137	1.00	1.00	rs7030019	rs7867504	1.00	0.35
rs4877834	rs885004	1.00	0.97	rs7030019	rs12237803	1.00	0.97
rs4877834	rs4877835	1.00	1.00	rs7047898	rs10868137	1.00	1.00
rs4877834	rs4877836	1.00	1.00	rs7047898	rs885004	1.00	0.97
rs4877834	rs7867504	1.00	0.36	rs7047898	rs4877835	1.00	1.00
rs4877834	rs12237803	1.00	0.93	rs7047898	rs4877836	1.00	1.00
rs7047315	rs7047898	1.00	1.00	rs7047898	rs7867504	1.00	0.36
rs7047315	rs10868137	1.00	1.00	rs7047898	rs12237803	1.00	0.93
rs7047315	rs885004	1.00	0.97	rs10868137	rs885004	1.00	0.97
rs7047315	rs4877835	1.00	1.00	rs10868137	rs4877835	1.00	1.00
rs7047315	rs4877836	1.00	1.00	rs10868137	rs4877836	1.00	1.00
rs7047315	rs7867504	1.00	0.36	rs10868137	rs7867504	1.00	0.36
rs7047315	rs12237803	1.00	0.93	rs10868137	rs12237803	1.00	0.93
rs885004	rs4877835	1.00	0.97	rs4877835	rs4877836	1.00	1.00
rs885004	rs4877836	1.00	0.97	rs4877835	rs7867504	1.00	0.36
rs885004	rs7867504	1.00	0.35	rs4877835	rs12237803	1.00	0.93
rs885004	rs12237803	1.00	0.97	rs4877836	rs7867504	1.00	0.36
rs7867504	rs12237803	1.00	0.34	rs4877836	rs12237803	1.00	0.93

D', d prime; R^2, r-squared, linkage disequilibrium coefficients.

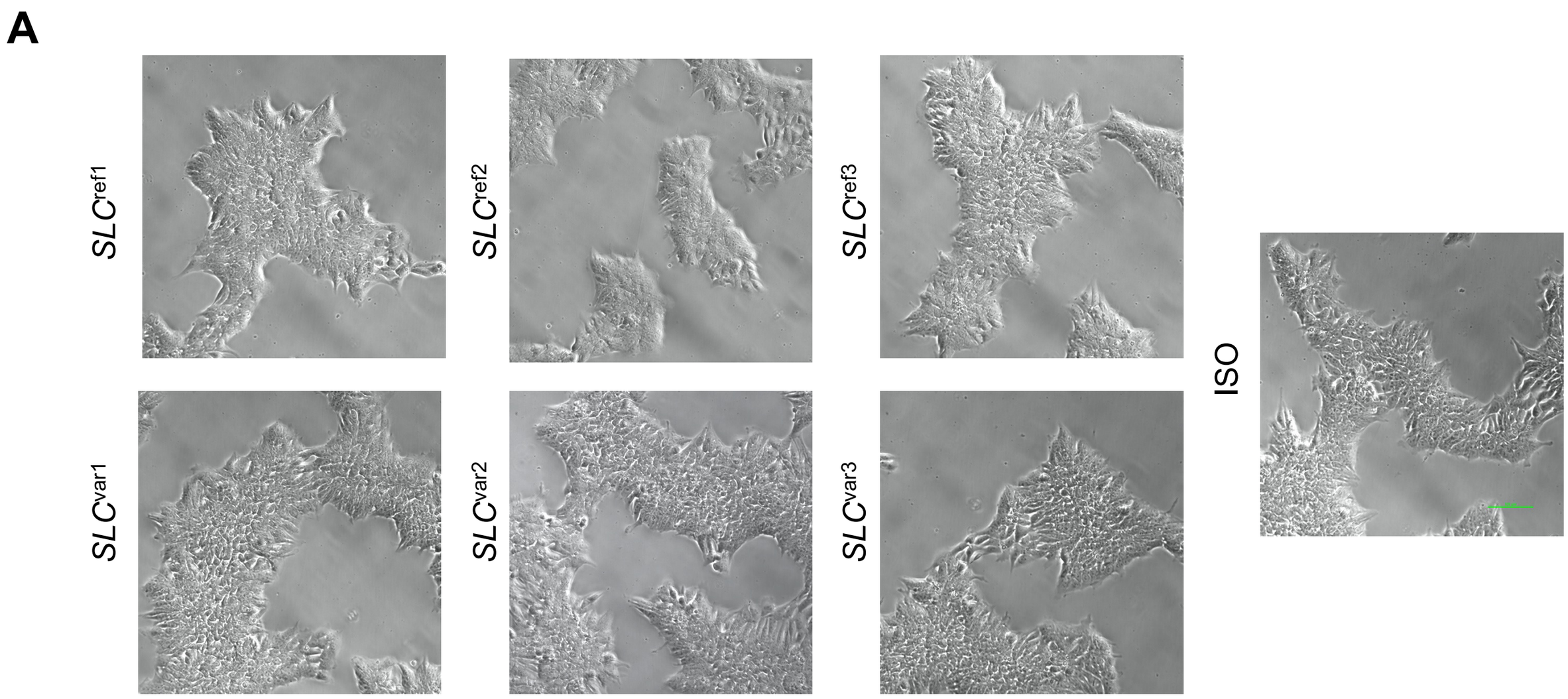
Supplemental Table 12: List of drugs previously associated with attenuating uptake via SLC transporters.

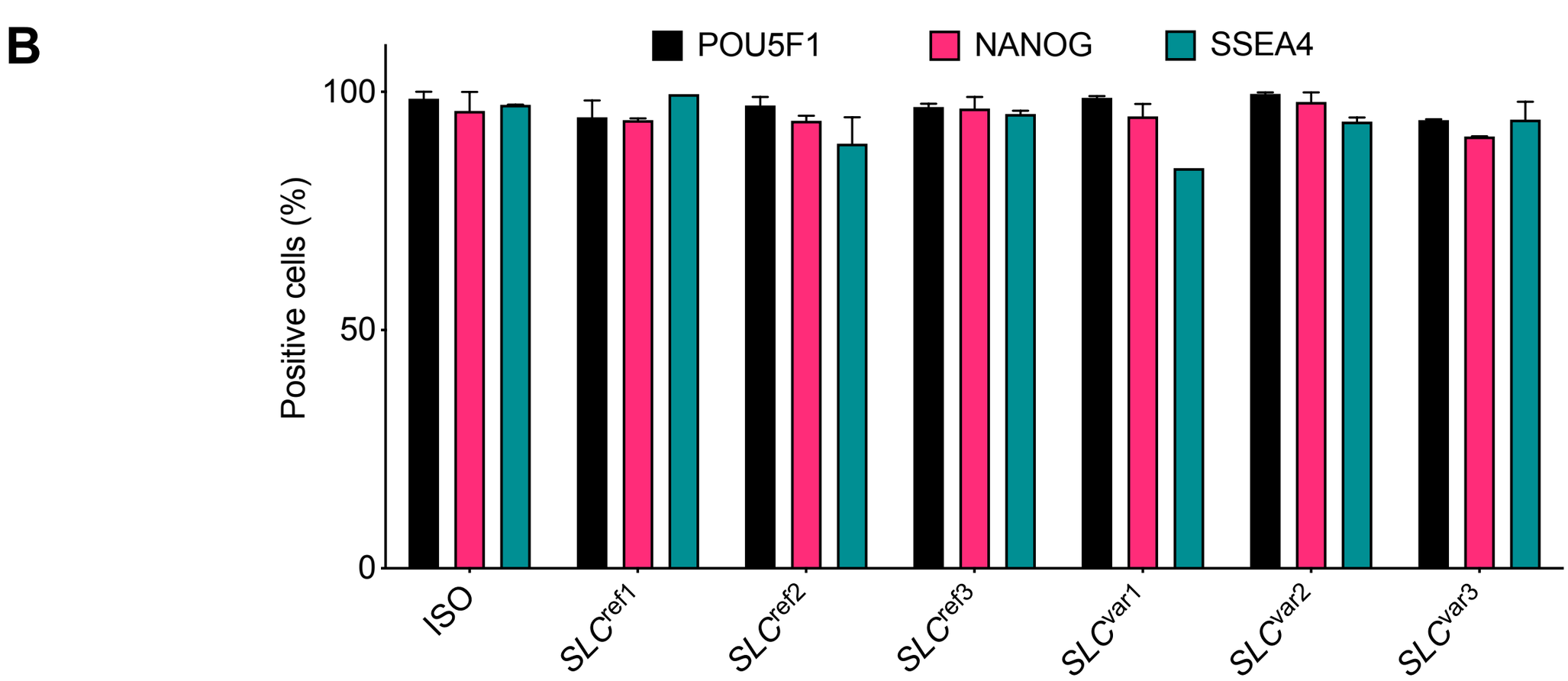
Drug	Transporter	References
Bosutinib	SLC16A2 and SLC29A1	25-27
Cimetidine	SLC22A2 and SLC47A2	28,29
Cyclosporin A	SLCO1B1, SLCO1B3, SLC10A2, SLC10A1, and SLC22A6,	30-34
Dasatinib	SLCO1B1, SLCO1B3, SLC29A1, and SLC16A2	25,26,35
Entecavir	SLC22A6 and SLC22A8	36
Indomethacin	SLC22A6, SLCO1A2, SLC10A1, and SLC22A6	37,38
Nilotinib	SLCO1B1 and SLC29A1	26,39
Pazopanib hydrochloride	SLCO1B1	39,40
Phlorizin dihydrate	SLC5A2 and SLC5A2	41,42
Quinidine	SLC22A1, SLC22A2, SLC2A4, SL C22A5, SLCO1A2, and SLC22A8	43-49
Rifampicin	SLC21A6, SLC21A8, SLC21A9, SLC21A3, SLCO1B3, SLC22A7, SLCO1B1, SLCO2B1, SLCO1A2, and SLCO1B3	50-53
Rifamycin SV sodium salt	SLC21A6, SLC21A8, SLC21A9, SLC21A3, SLCO1A2, SLCO1B1, SLCO1B3, SLCO2A1, SLCO2B1, and SLC47A1	50,51,54
Sulfobromophthalein sodium	SLC1A1 and SLCO1B2	50,51
Sunitinib	SLC22A1, SLC22A2 and SLC22A3	55
Vadentanib	SLC22A2	56
verapamil	SLC22A1, SLC22A4, SLC22A5, SLCO1B1, SLCO1A2, SLC47A1 and SLC47A2	48,57-60
Desipramine	SLC22A1, SLC22A2, SLC22A3, SLC22A4 and SLC22A5	61-65

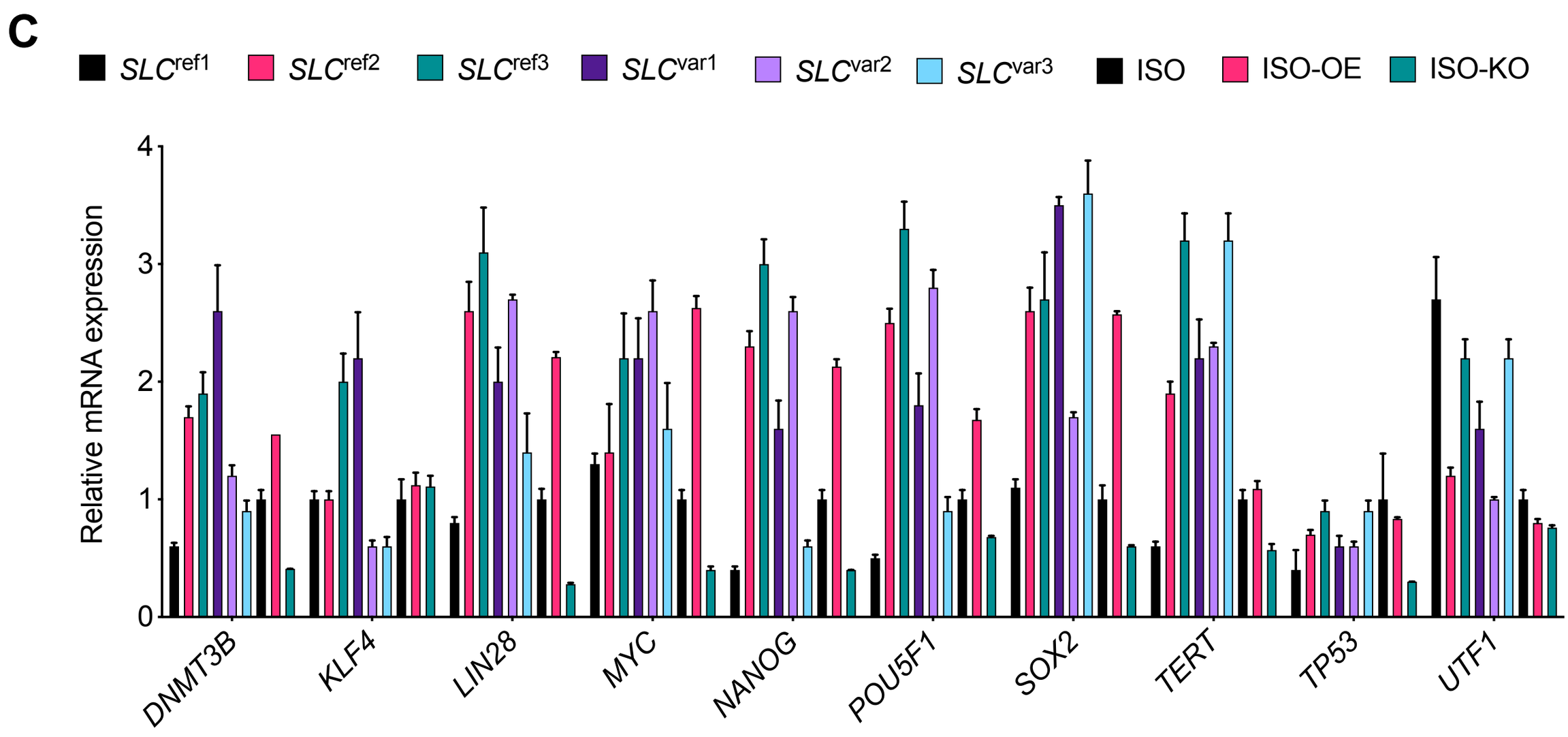
Supplemental Table 13: Major echocardiography parameters for mice treated with DOX and DOX+DESP at base line and three weeks post treatment.

Base line	DESP+DOX								Mean	SD	DOX										Mean	SD
Mouse ID	3453	3455	3456	3457	3458	3459	3460	3461			1	2	3	4	5	6	7	8	9	10		
IVSd (mm)	0.99	0.92	0.92	0.98	0.87	0.82	0.89	0.89	0.91	0.06	0.89	0.89	0.78	0.74	0.85	0.71	0.74	0.71	0.74	0.78	0.78	0.07
IVSs (mm)	1.38	1.27	1.30	1.33	1.31	1.06	1.36	1.21	1.28	0.10	1.17	1.24	1.10	1.03	1.06	1.13	1.03	0.82	0.96	0.85	1.04	0.13
LVIDd (mm)	3.48	4.03	3.15	3.58	3.75	3.96	3.84	3.80	3.70	0.29	3.26	3.55	3.62	3.62	3.62	3.87	3.79	3.62	3.72	3.76	3.64	0.17
LVIDs (mm)	1.82	2.66	1.74	2.14	2.12	2.39	2.14	2.28	2.16	0.30	2.23	2.23	2.34	2.09	2.09	2.41	2.27	2.09	2.30	2.38	2.24	0.12
LVPWd (mm)	0.89	0.82	0.92	0.89	0.95	0.96	0.96	0.95	0.92	0.05	0.92	0.96	1.03	0.92	1.03	0.78	0.82	0.78	0.92	0.82	0.90	0.09
LVPWs (mm)	1.11	1.10	1.05	1.11	1.03	1.15	1.04	1.06	1.08	0.04	1.10	0.99	1.21	1.21	1.28	1.17	0.89	1.31	1.03	0.96	1.12	0.14
FS (%)	47.33	34.00	45.00	40.33	43.67	39.67	44.00	40.00	41.75	4.14	32.33	37.67	35.67	42.00	42.00	38.00	40.67	42.33	38.50	36.00	38.52	3.29
Week 3	DESP+DOX								Mean	SD	DOX										Mean	SD
IVSd (mm)	0.83	0.74	0.83	0.78	0.92	0.92	0.84	0.96	0.85	0.08	0.78	0.74	0.92	0.82	0.78	0.85	0.74	0.78	0.78	0.82	0.80	0.05
IVSs (mm)	1.11	1.12	1.27	1.05	1.23	1.28	1.28	1.36	1.21***	0.11	0.82	1.03	1.21	0.82	1.10	0.89	0.85	0.96	1.06	0.92	0.97	0.13
LVIDd (mm)	3.97	4.22	3.31	3.88	3.90	3.53	3.76	4.09	3.83	0.30	3.83	3.23	4.18	4.15	3.90	2.80	3.83	3.55	3.58	3.33	3.64	0.43
LVIDs (mm)	2.70	2.73	1.75	3.24	2.36	2.45	2.34	2.41	2.50	0.42	2.59	2.30	3.12	3.19	2.45	1.84	2.70	2.48	2.55	2.48	2.57	0.39
LVPWd (mm)	0.75	0.83	0.89	0.89	0.83	0.81	0.77	0.89	0.83	0.05	0.82	0.89	0.89	0.71	0.82	1.06	0.82	0.78	0.82	1.03	0.86	0.11
LVPWs (mm)	1.00	1.02	1.12	1.06	0.91	0.96	1.05	1.10	1.03**	0.07	0.82	0.82	0.78	0.74	0.99	1.03	0.89	0.92	0.96	0.96	0.89	0.10
FS (%)	32.00	35.00	47.33	25.00	39.33	30.67	38.00	41.00	36.04***	6.92	32.00	28.00	25.00	23.50	36.50	34.50	30.25	29.50	28.33	26.67	29.43	4.06

IVSd, LV interventricular septum thicknesses at diastole; IVSs, LV interventricular septum thicknesses at systole; LVIDd, LV internal dimensions at diastole; LVIDs, LV internal dimensions at systole; LVPWd, LV posterior wall thicknesses at diastole; LVPWs, LV posterior wall thicknesses at systole; and FS, fractional shortening; and *, significant difference between groups (DESP+DOX *versus* DOX) by t-test, *$P < 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

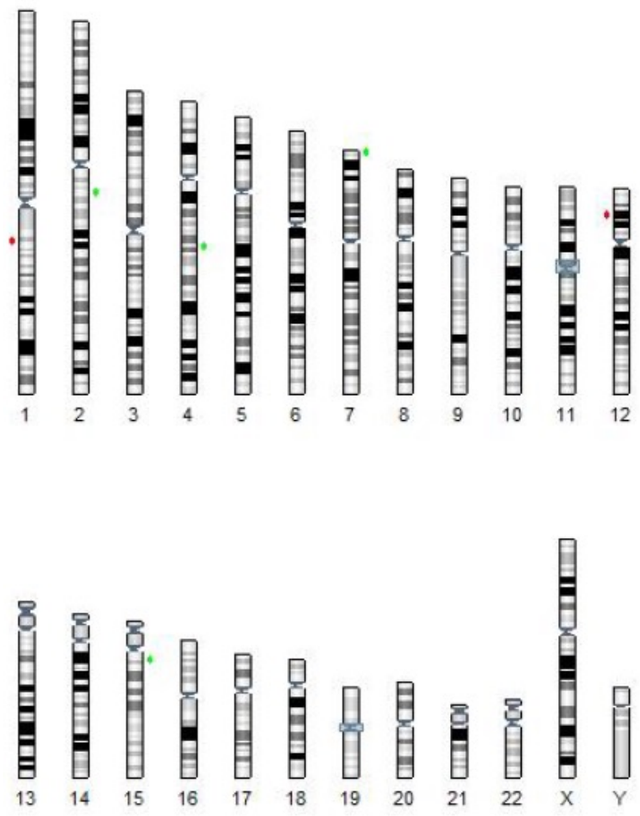






Supplementary Figure 1. Generation and characterization of patient–specific hiPSCs. A, Phase contrast images of patient-specific hiPSC lines derived under chemically defined conditions. Scale bar, 100 μm. **B,** Flow cytometry analysis of markers of undifferentiated cells, POU5F1, NANOG, and SSEA4, in all hiPSC lines. **C,** Real–time PCR assessment of the expression levels of genes associated with the undifferentiated state in all hiPSC lines, relative to control isogenic hiPSC line. *n* = 3 replicates for each hiPSC line. Error bars represent s.e.m. of experimental replicates.

*SLC*ref1



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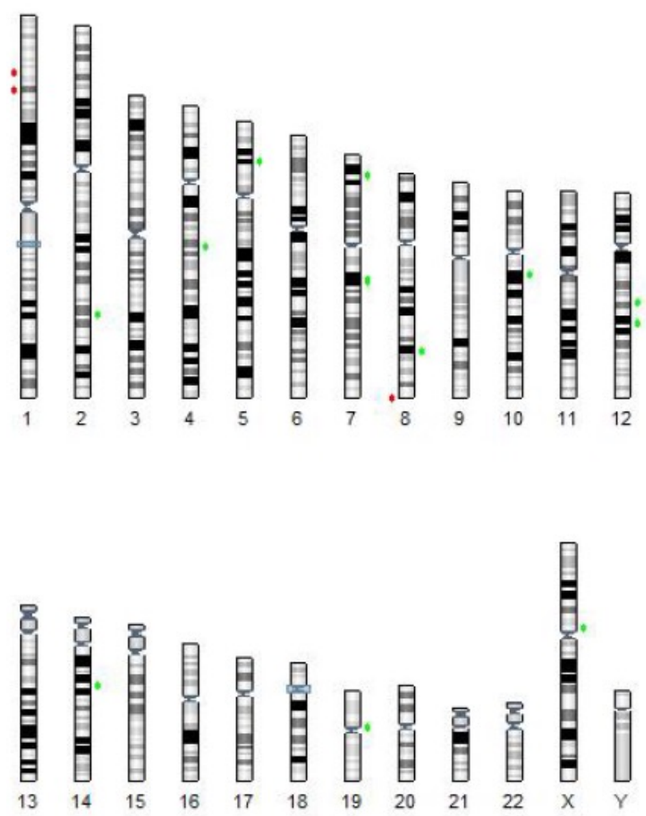
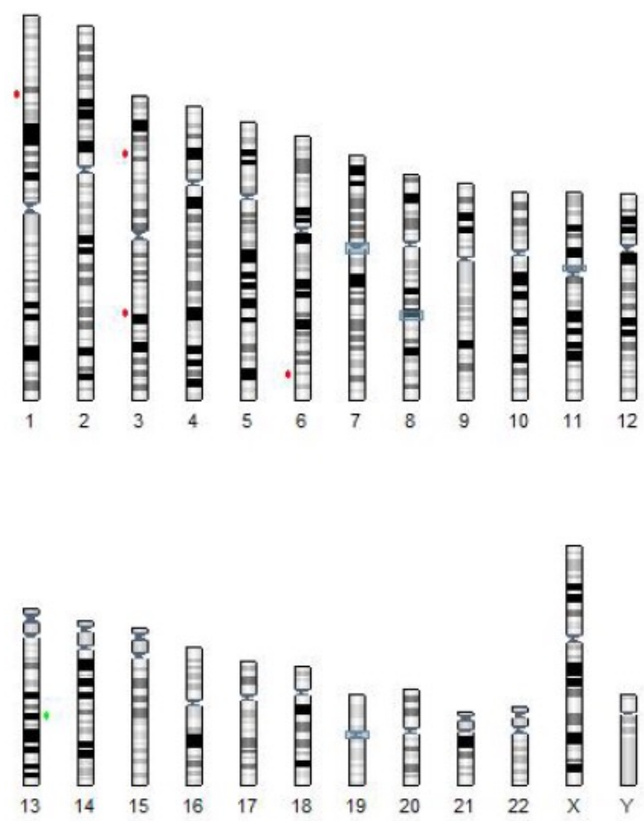
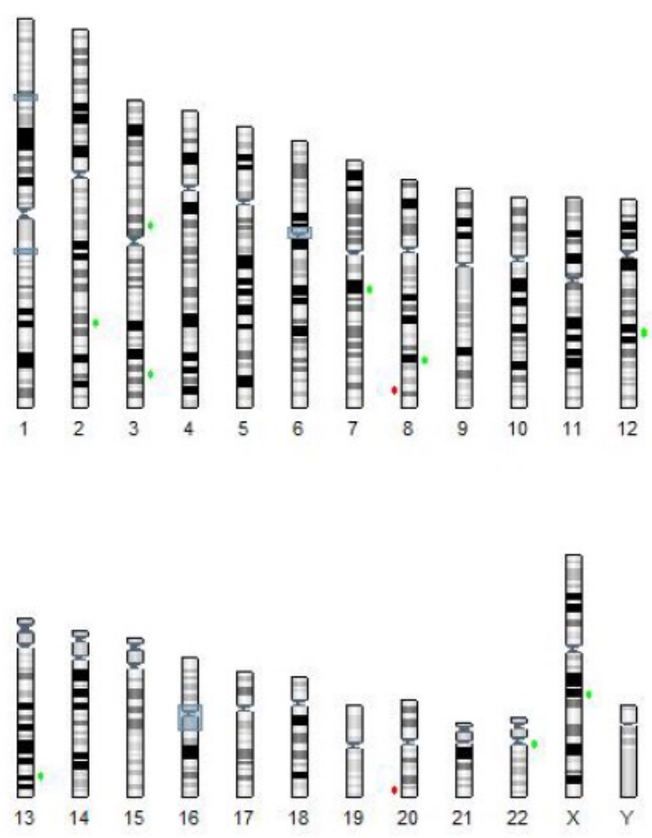


*SLC*ref3



*SLC*var1



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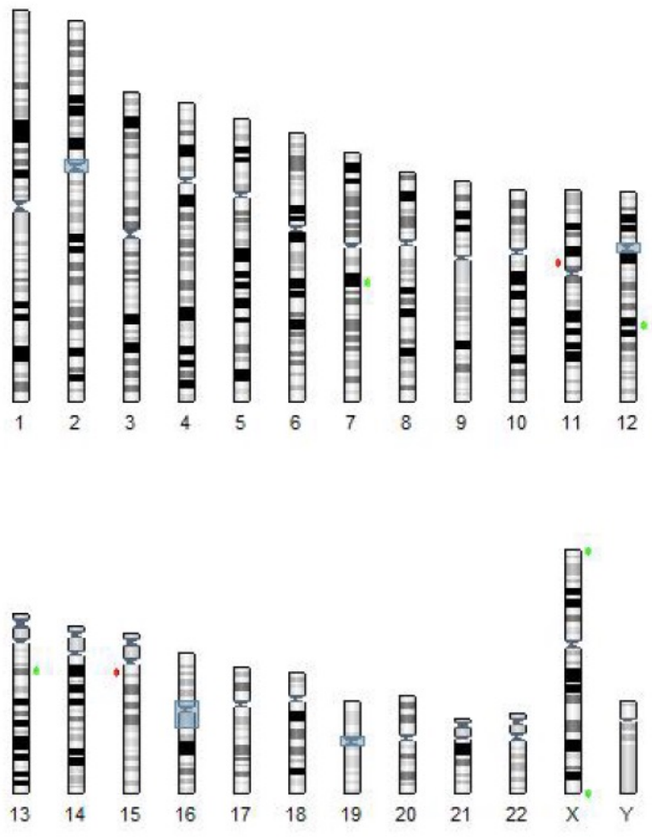

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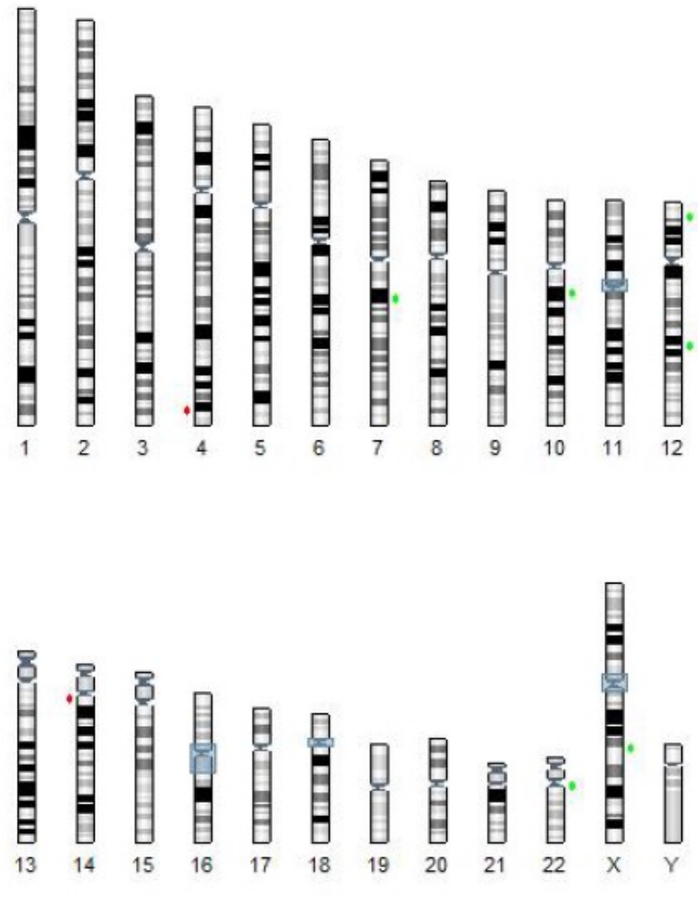

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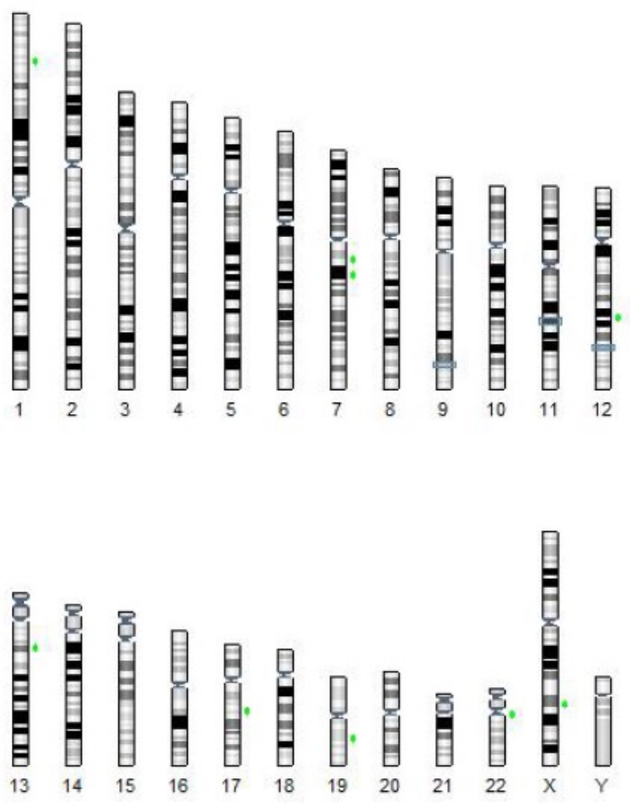
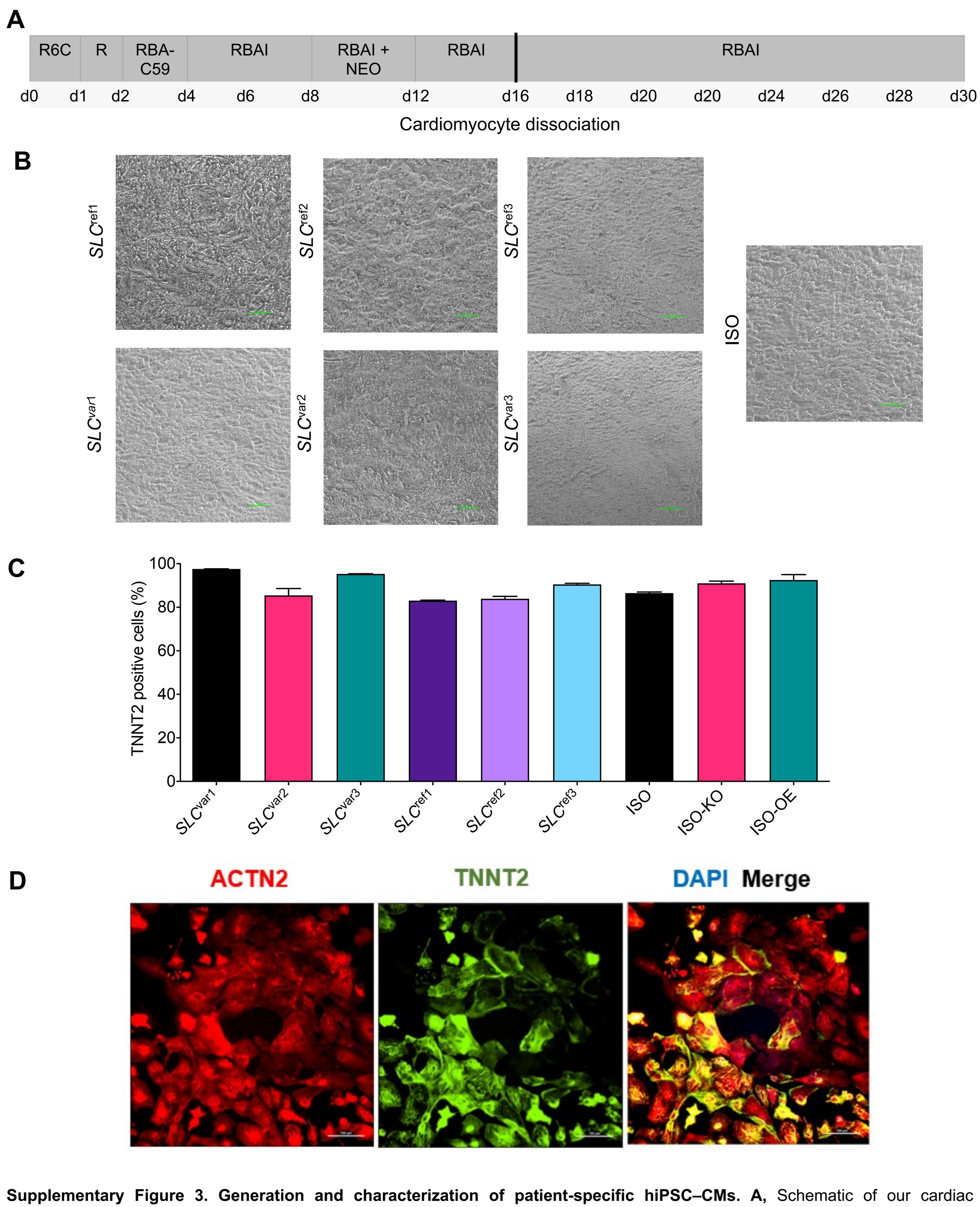
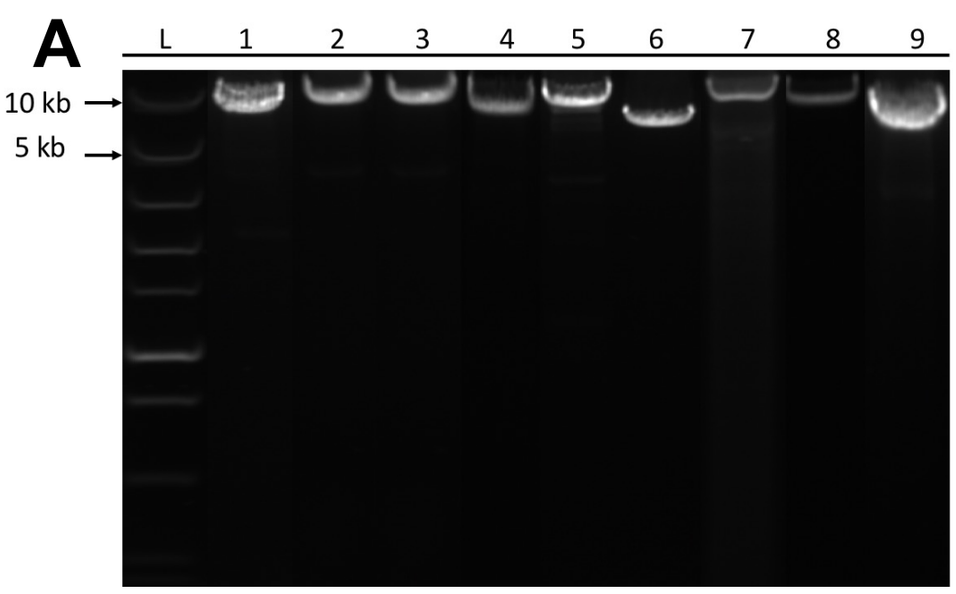
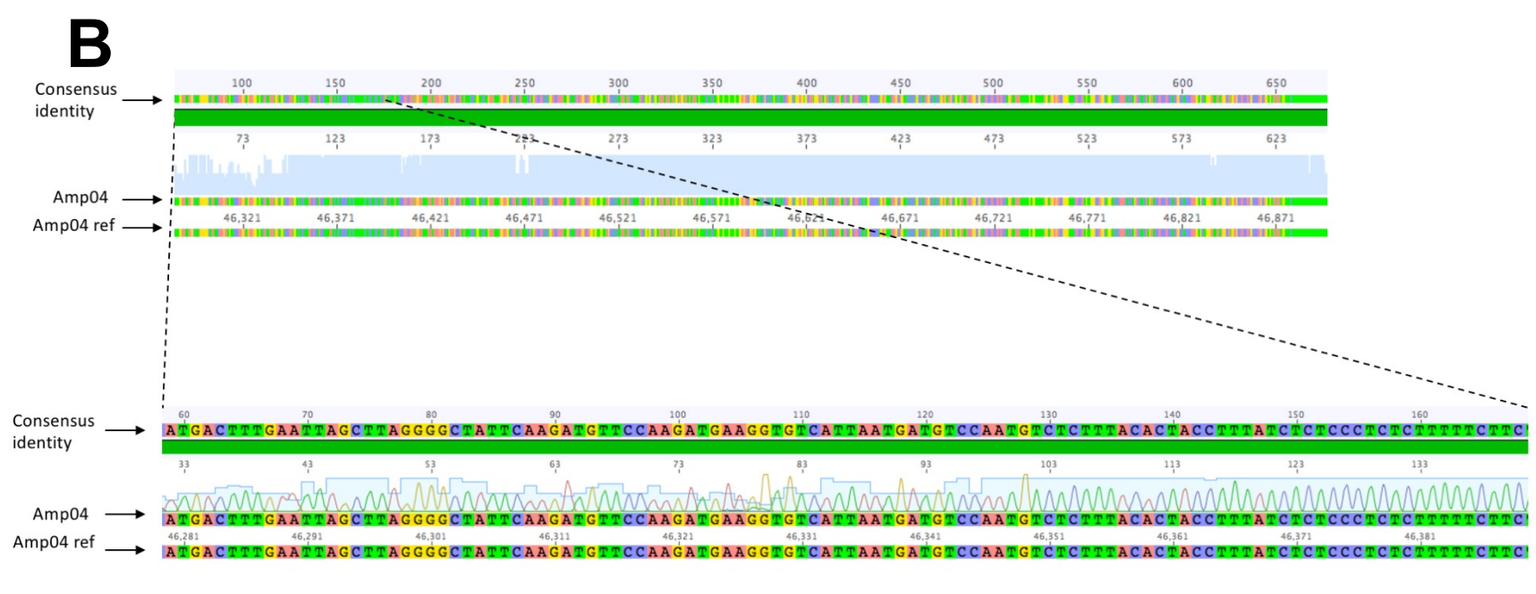


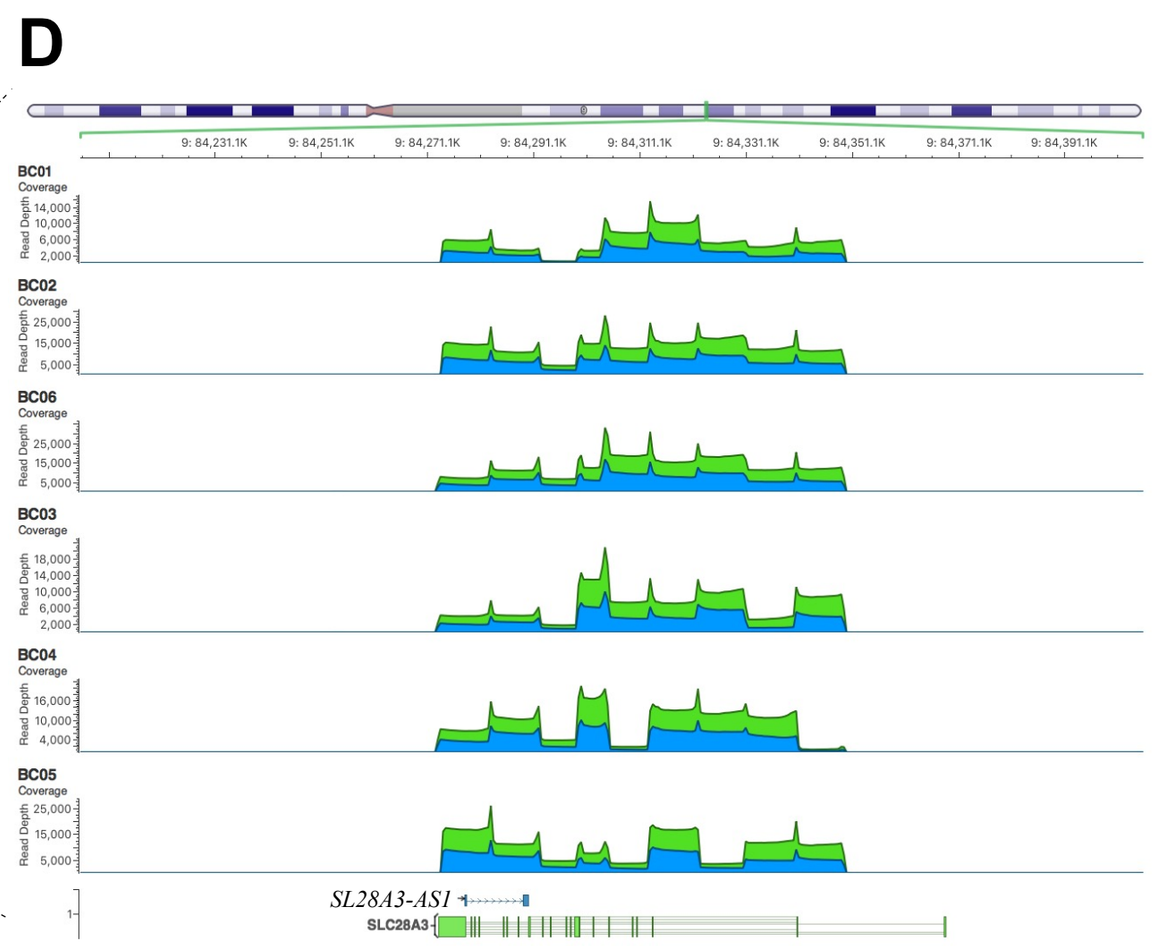
Supplementary Figure 2. Karyotype analysis of patient–specific hiPSC lines. SNP-based karyotype analysis of all patient-derived hiPSC lines (passage > 20) demonstrating normal karyotype after reprogramming. Karyotyping was assessed using a whole–genome Infinium HumanCytoSNP–12 BeadChip Array (Illumina) covering 300,000 SNPs.

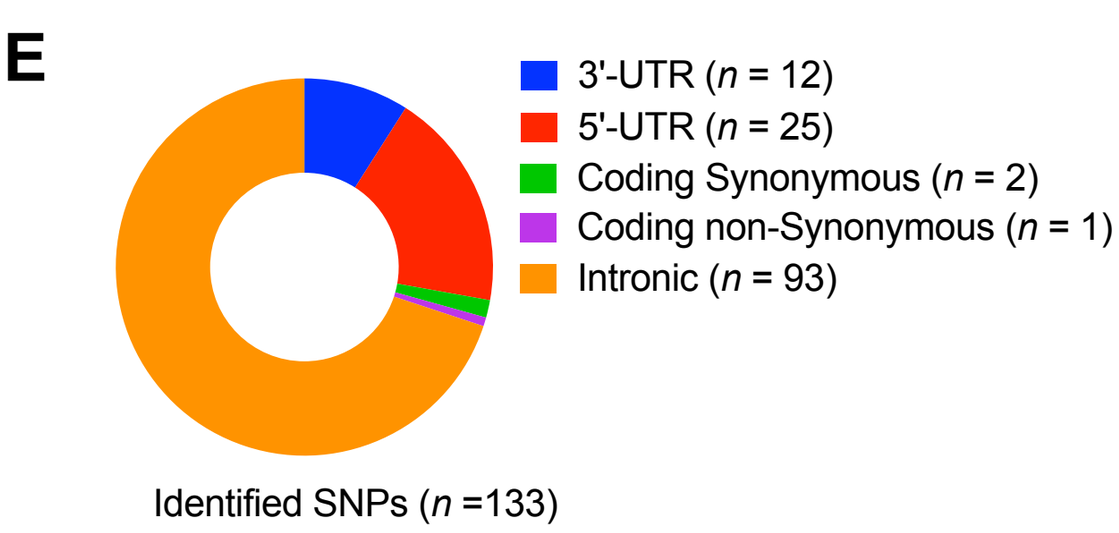


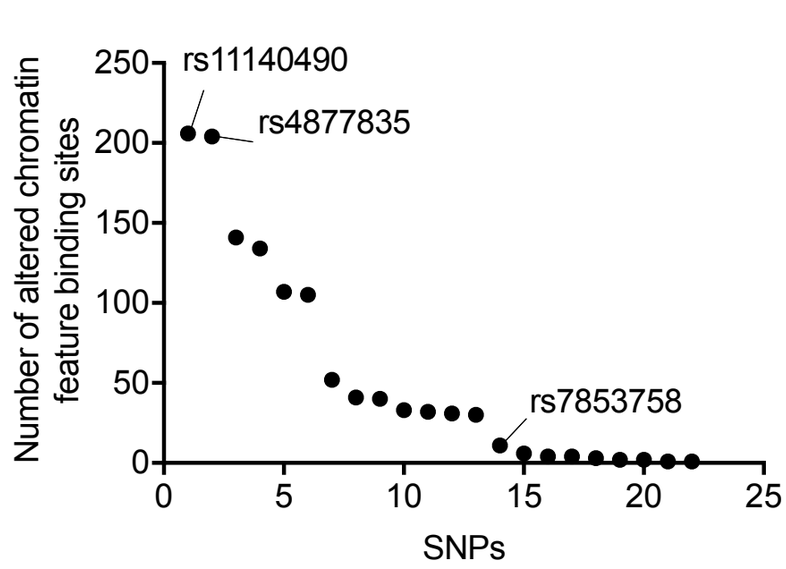
Supplementary Figure 3. Generation and characterization of patient-specific hiPSC–CMs. A, Schematic of our cardiac differentiation protocol (details in Methods). **B,** Representative phase contrast images of day 30 cardiomyocyte monolayers differentiated from all hiPSC lines. Scale bar, 100 μm. **C,** Flow cytometry analysis for the percentage of the cardiac troponin T (TNNT2) positive cells derived from all hiPSC lines, *n* = 3 replicates for each line. Error bars represent s.e.m. of experimental replicates. **D,** Representative immunofluorescent staining images for cardiac markers troponin T (TNNT2) and α-actinin (ACTN2). Scale bar, 25 μm.

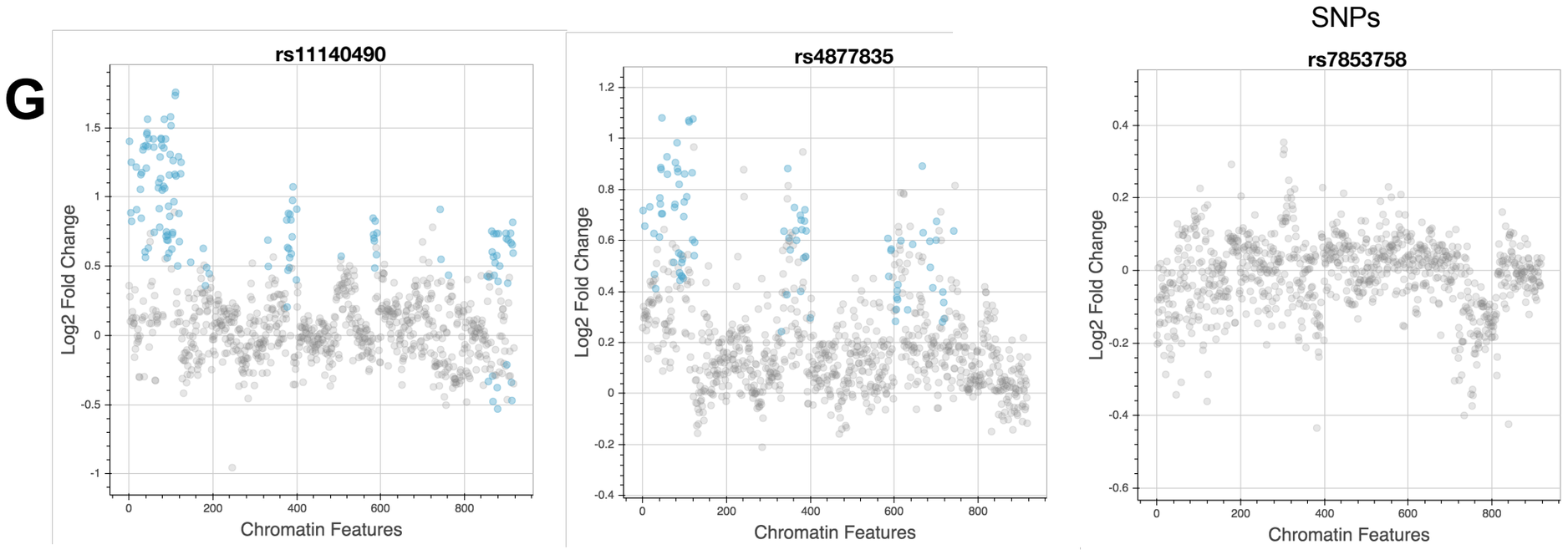






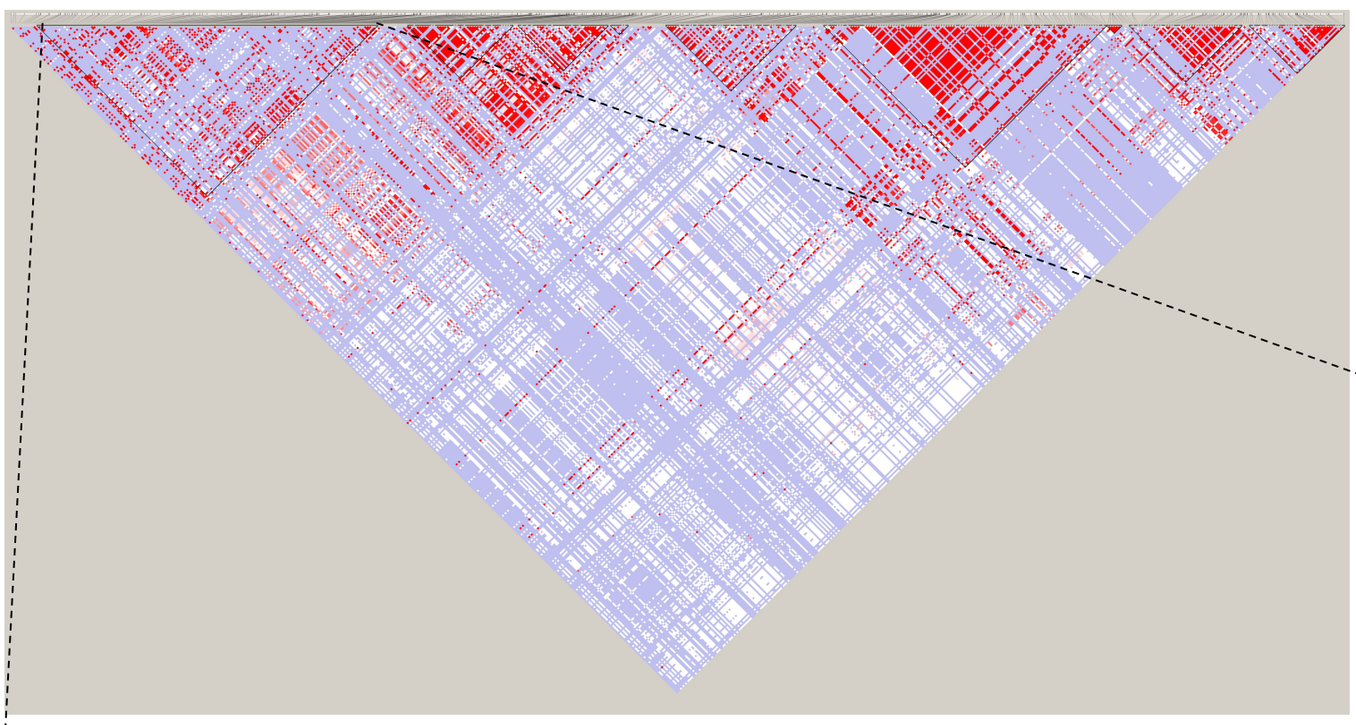






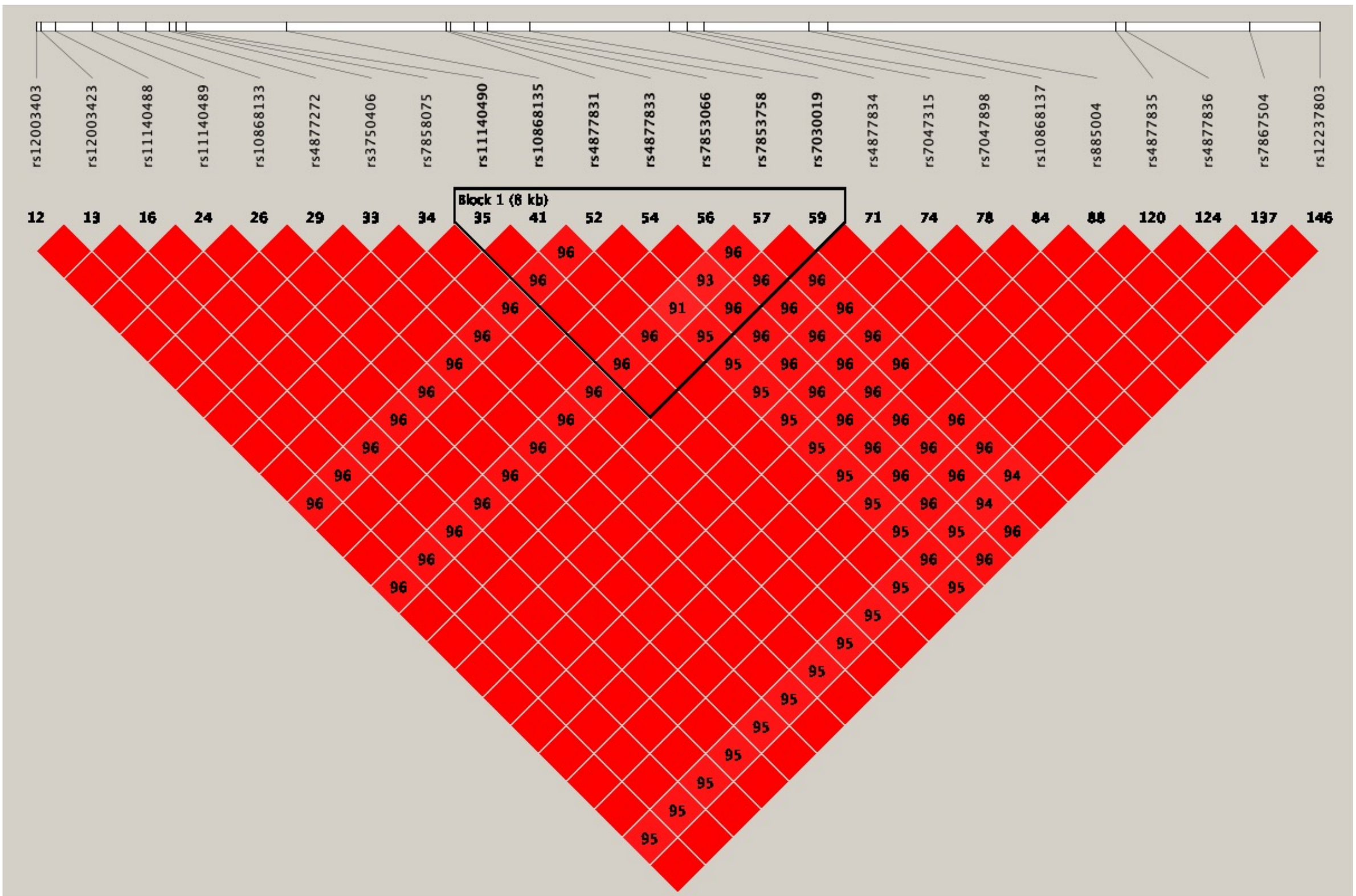
Supplementary Figure 4. *SLC28A3* gene resequencing. A, Exemplary agarose gel picture for all nine overlapping SLC28A3 amplicons generated from one sample. L, ladder; 1–9, amplicons one to nine. **B,** Exemplary pre-nanopore sequencing amplicon validation by sanger sequencing for amplicon number four (Amp04). Top panel shows the first ~600 bp of generated amplicon four (AMP04) aligned to its reference sequence (AMP04 ref). Bottom panel shows a zoom–in view for the first ~100 bp of generated amplicon four perfectly matching its reference sequence. **C,** Long range PCR–based target enrichment for *SLC28A3* amplicons aligned to reference human genome (GRCh38) showing depth of coverage peaks at chr9: 84,274,029- 545 84,349,802. **D,** Zoom–in view at locus chr9: 84,274,029-84,349,802 encompassing *SLC28A3*. **E**, Consequence and location of identified SNPs (*n* = 133). **F,** Functional chromatin regulatory analysis for the candidate SNPs showing the number of chromatin binding sites significantly altered by candidate SNPs. **G,** Effect of candidate SNPs, rs11140490, rs4877835, and rs7853758 on chromatin feature binding sites. Log2 fold change measure the fold change in the probability of observing a binding site for relevant chromatin feature between reference and alternative allele for a particular SNP (adapted from Magdy et al.[23]).

A



	D'<1	D'=1
LOD<2	White	Blue
LOD≥2	Shades of pink/red	Bright red

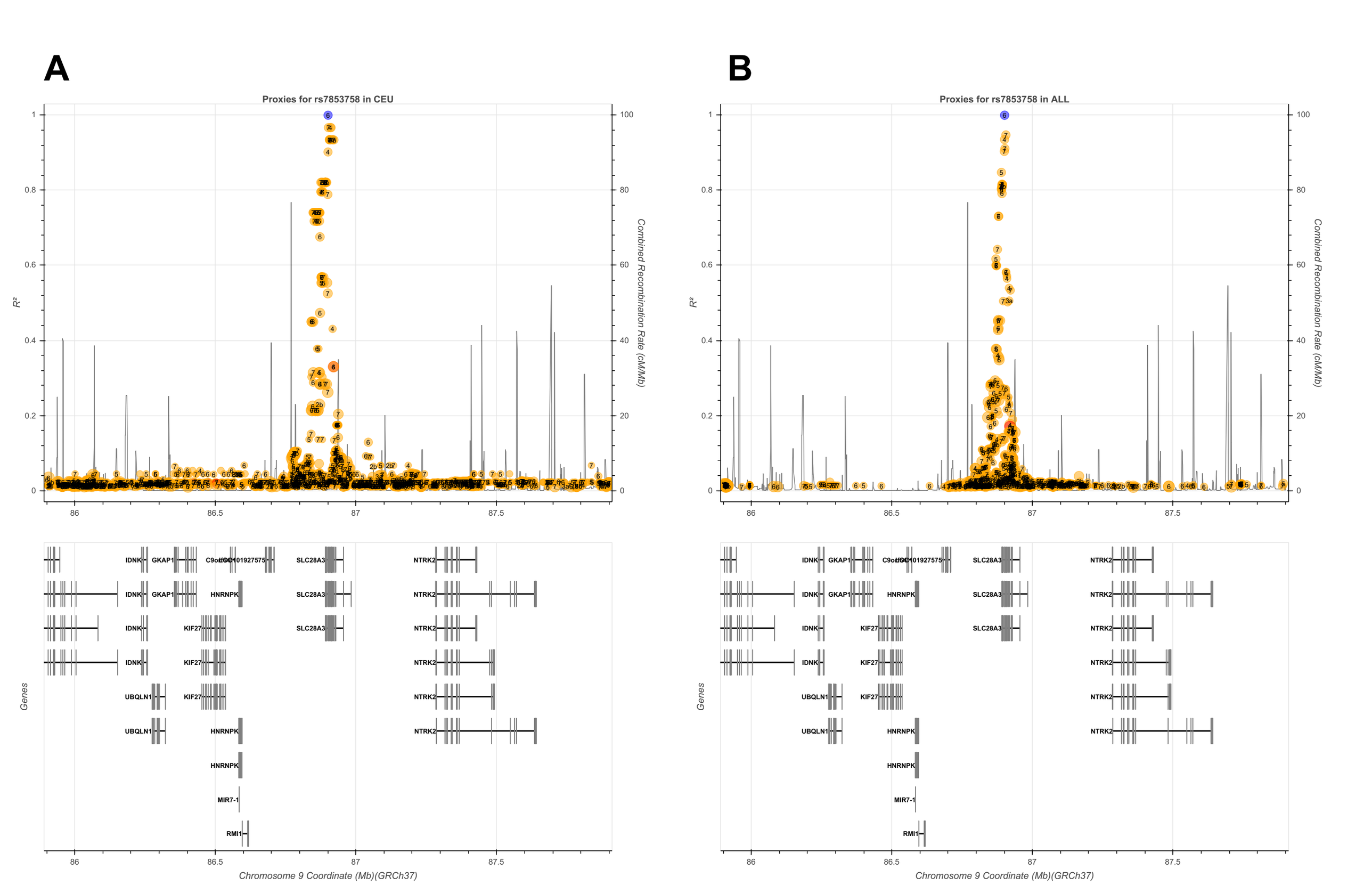
B



C

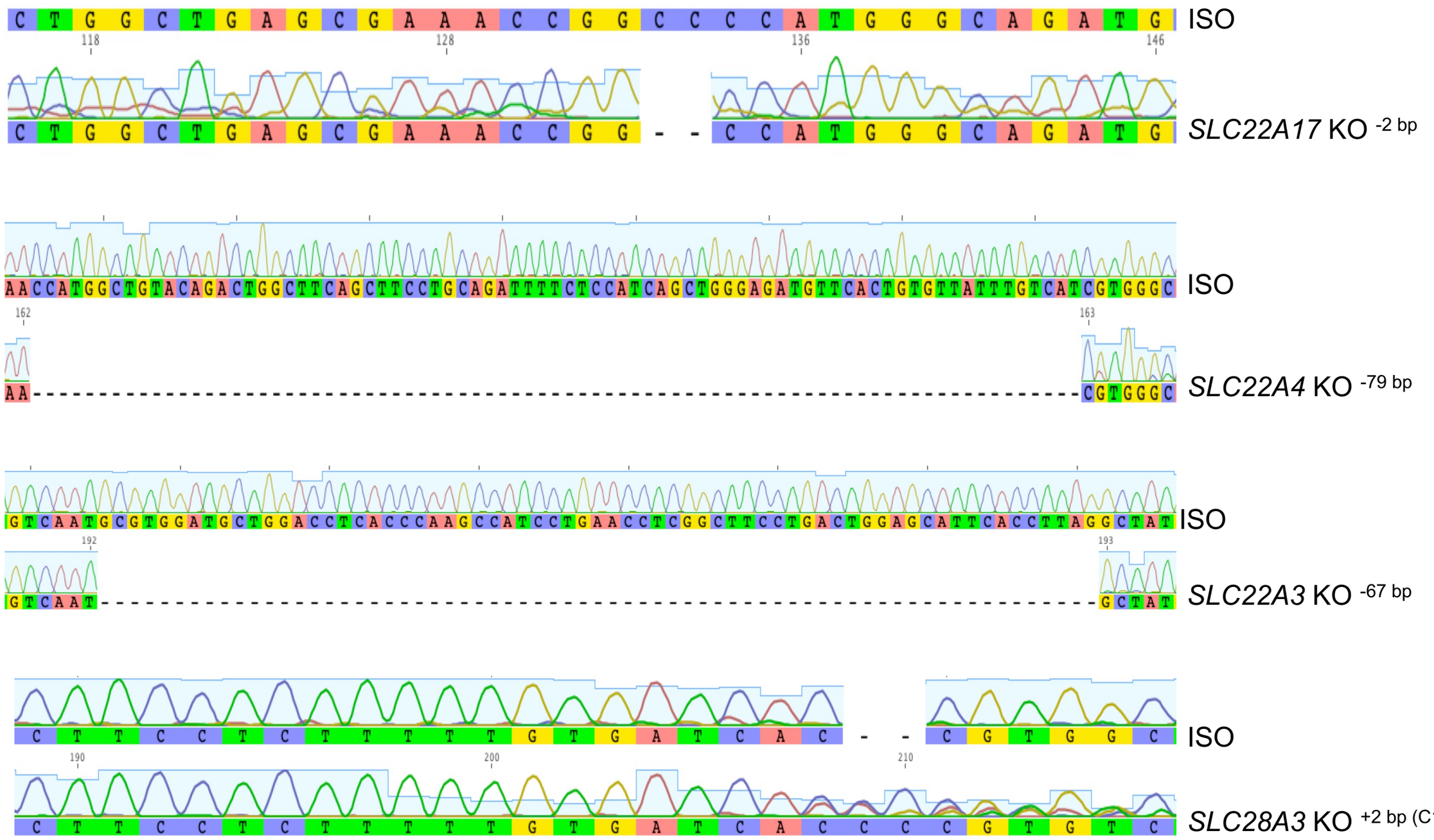
Haplotype Id	rs11140490, (35, A>G)	rs10868135 (41, T>C)	rs4877831 (52, C>G)	rs4877833 (54, T>C)	rs7853066 (56, A>G)	**rs7853758 (57, G>A)**	rs7030019 (59, A>G)	Haplotype Frequency (%)
I	A	T	C	T	A	G	A	71.7
II	G	C	G	C	G	A	G	17.7
III	A	T	G	T	A	G	A	7.1
IV	G	C	G	C	A	G	A	2
V	G	C	G	C	G	G	A	0.5
VI	A	T	C	T	A	A	A	0.5
VII	G	C	C	T	A	A	G	0.5

Supplementary Figure 5. Haplotype structure at *SLC28A3 / SLC28A3-AS1* locus. **A,** Pairwise linkage disequilibrium (D') for all SNPs spread over ~100 kb encompassing *SLC28A3 / SL28A3-AS1* locus. The linkage disequilibrium (D') is indicated in the small boxes colored red or blue (a color legend is provided). LOD, log of the likelihood odds ratio. **B,** LD haplotype structure for Hap^{SLC28A3} that is spread over 32 kb and comprising 24 SNPs that are co-inherited only in cardio protected patients. The reference SNP numbers (rs) are indicated on top. Hap$^{SL28A3\text{-}AS1}$ (outlined by black triangle) spread over 8 kb and is composed of seven SNPs that are located within a long non-coding RNA, *SL28A3-AS* that overlaps with *SLC28A3*. **C,** Haplotype structure and allelic frequency of Hap$^{SL28A3\text{-}AS1}$ showing seven haplotype structures, [Hap–I$^{SL28A3\text{-}AS1}$ to Hap–VII$^{SL28A3\text{-}AS1}$]. Each SNP is labeled as follow; rs id (SNP number on the LD block in Fig. b, reference allele > variant allele). SNP rs7853758 (in bold) is the primary GWAS hit. For each SNP, variant alleles are in red (adapted from Magdy et al.[23]).

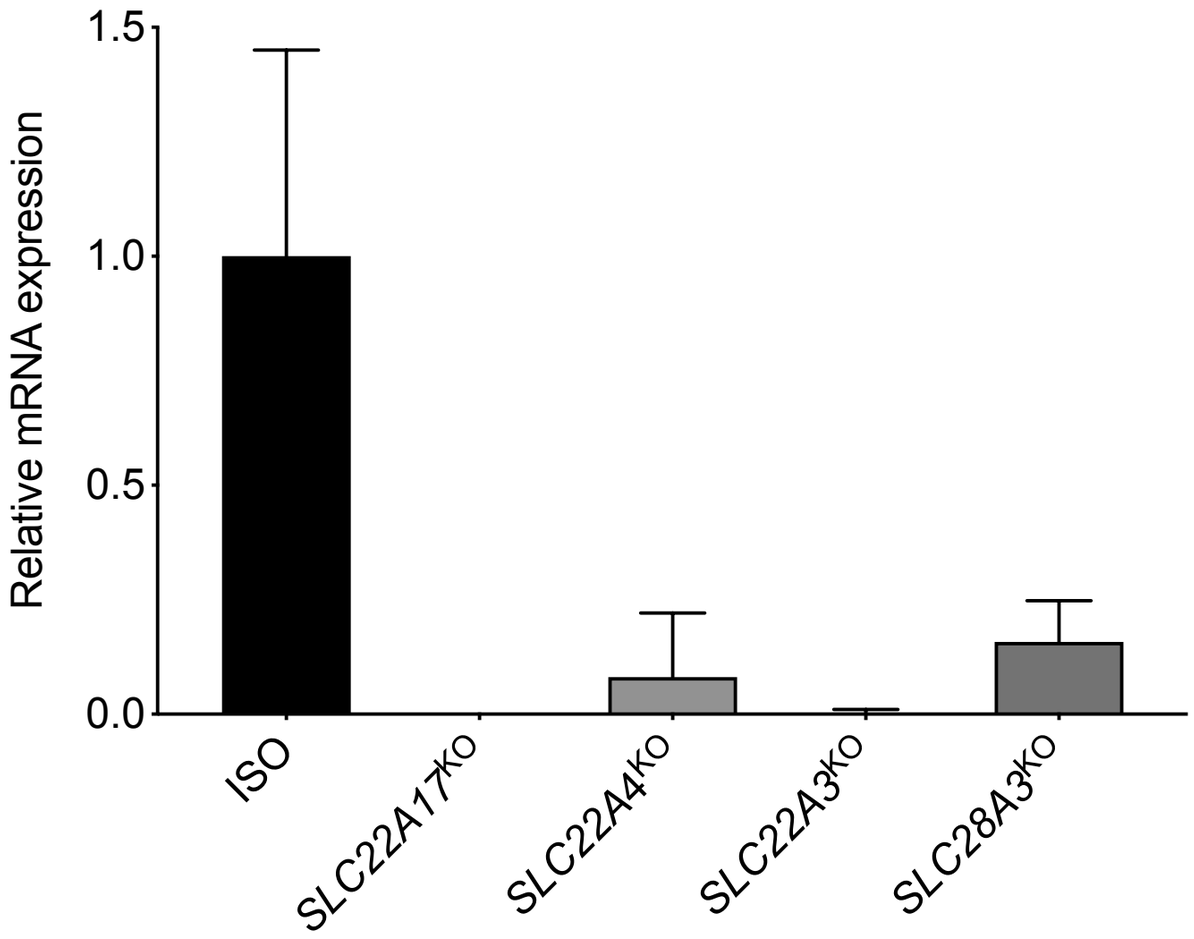


Supplementary Figure 6. Extended Linkage disequilibrium (LD) analysis over 2 Mb on chromosome 9. Linkage disequilibrium for variants located within 1 Mb up and down–stream the *SLC28A3 / SLC28A3-AS1* locus in CEU/European population **(A)** and in All ethnicity population **(B)**. For each plot the LD Co-efficient (R^2) is represented on the left Y-axis and the genomic coordinates are represented on the x-axis. Each yellow circle denotes for a single SNP within the target locus. The original CGAS hit, rs7853758 is represented by a purple circle.

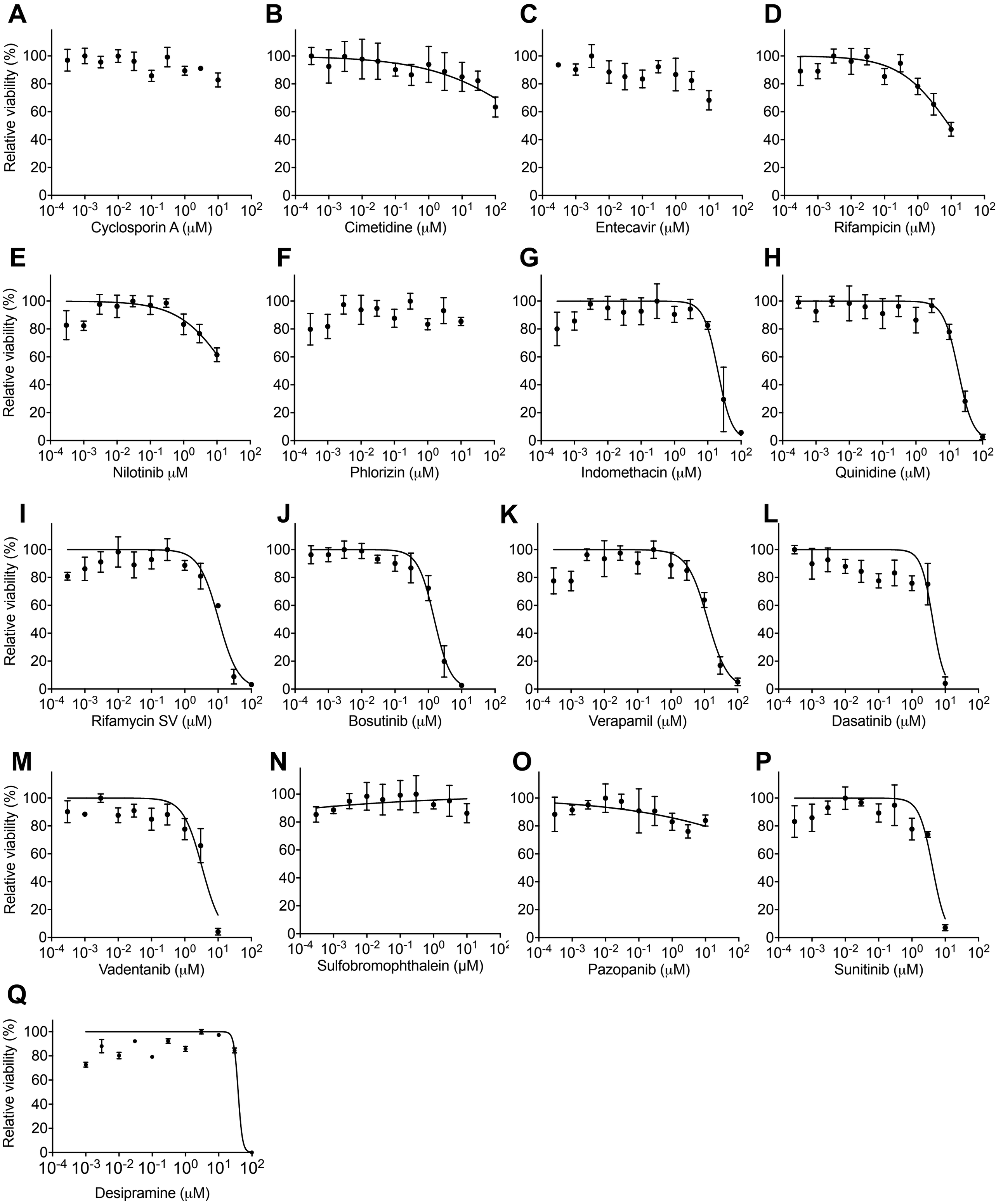
A



B

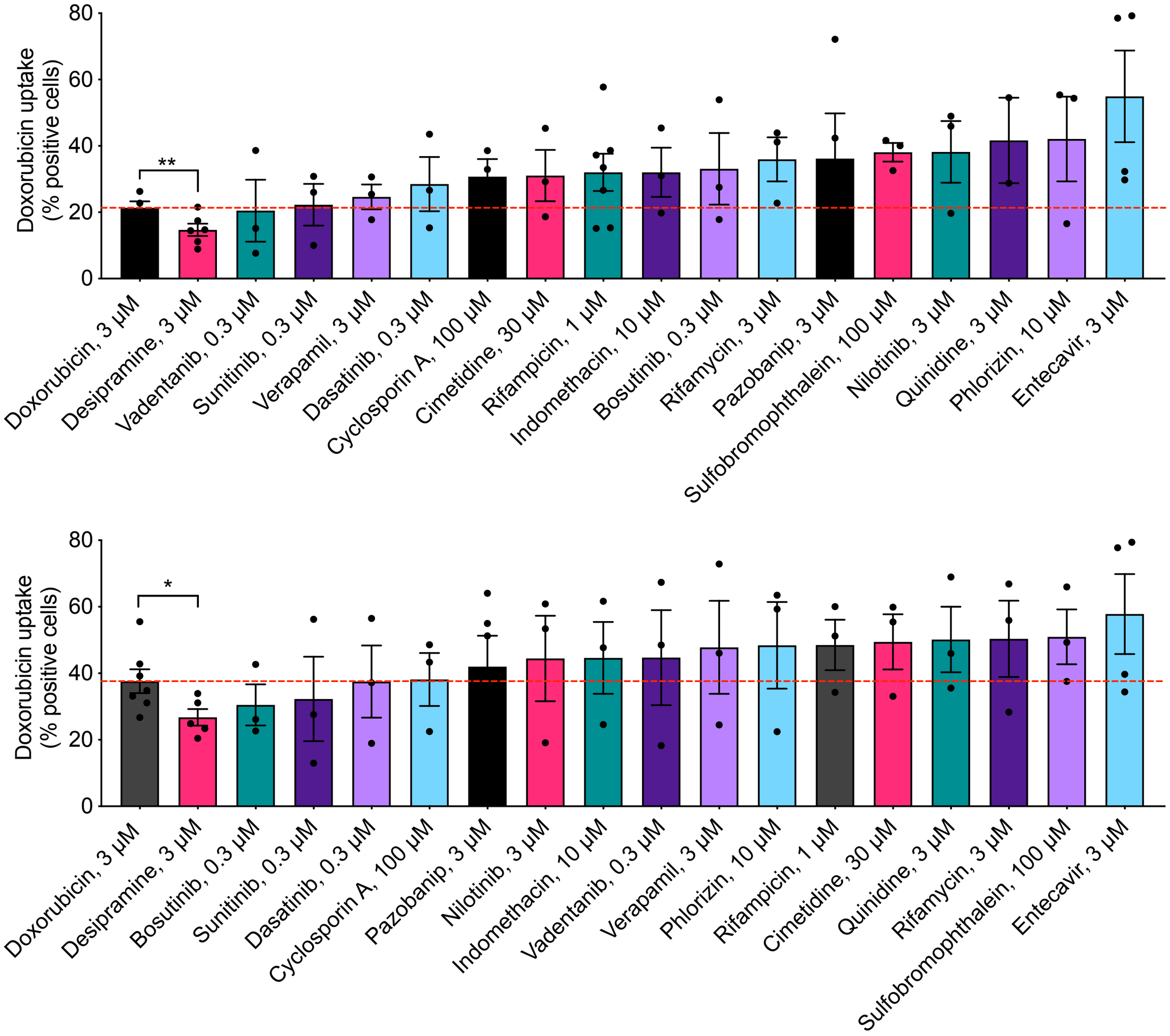


Supplementary Figure 7. Generation of DOX-relevant SLC transporter knockouts in an isogenic cell line. A, Validation of SLC transporters knockouts using Sanger sequencing showing disturbance on DNA level at target loci. **B,** and qPCR to quantify the mRNA expression of relevant transporters (*n* = 3). *n* = full independent experimental replicates, Error bars, s.e.m.



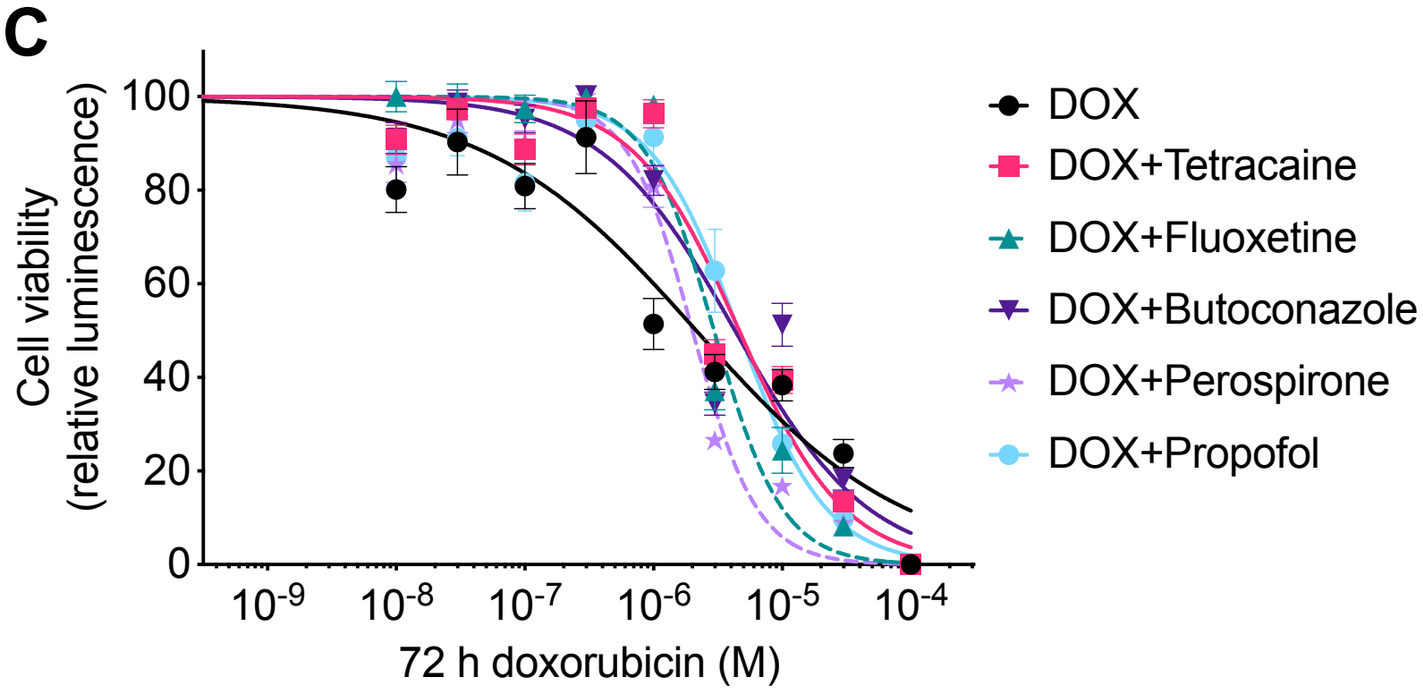
Supplementary Figure 8. Assessment of the toxicity of the cherry-picked SLC transporter modulators in hiPSC–CMs. a-q, The effect of 17 different transporter inhibitors on patient derived hiPSC-CMs viability were assessed 72 h post treatment. The drugs assessed were; **A,** cyclosporin A, **B,** cimetidine, **C,** entecavir, **D,** rifampicin, **E,** nilotinib, **F,** phlorizin, **G,** indomethacin, **H,** quinidine, **I,** rifamycin, **J,** verapamil, **K,** bosutinib, **L,** dasatinib, **M,** vadentanib, **N,** pazopanib, **O,** sunitinib, **P,** sulfobromophthalein, and **Q,** desipramine.

A



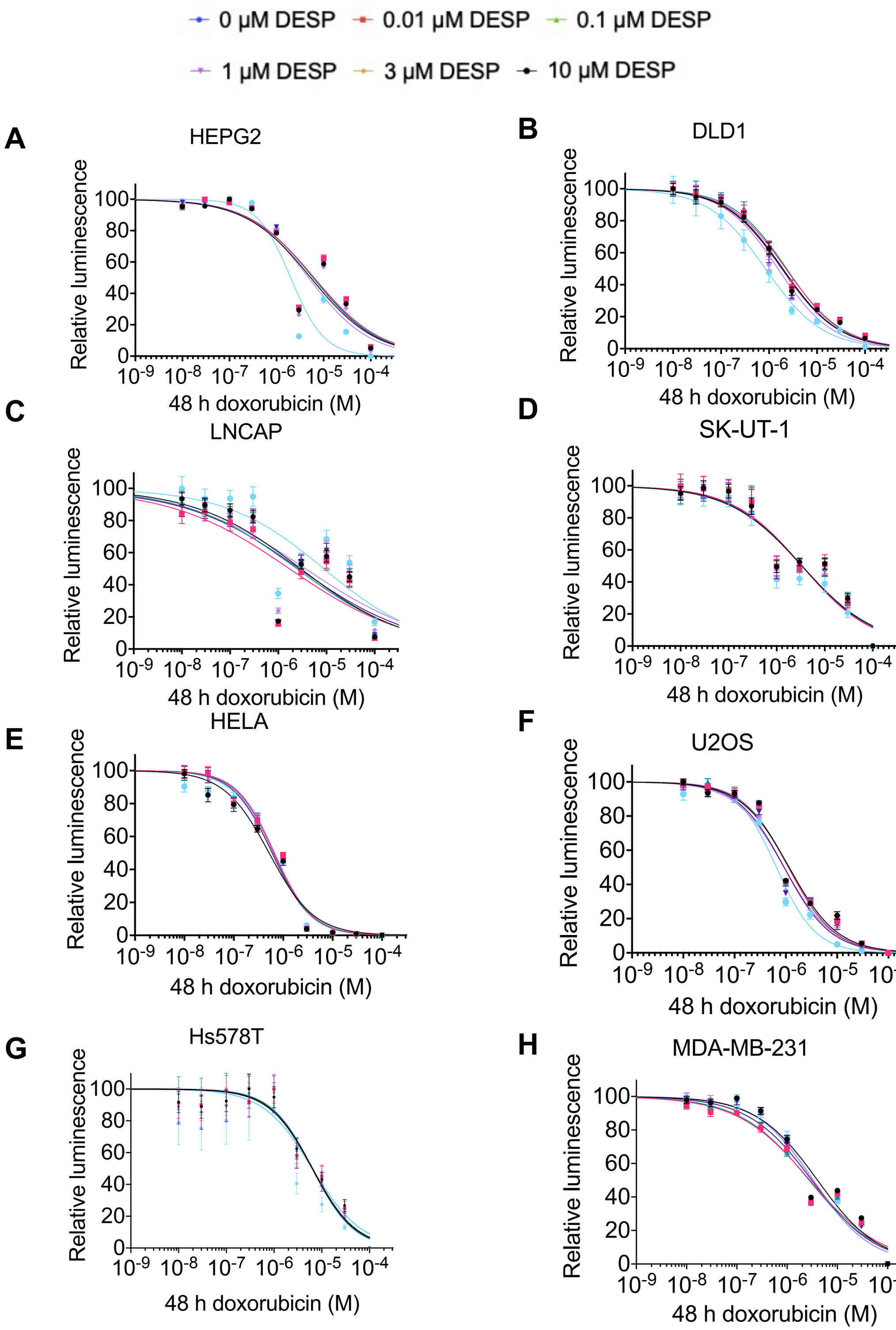
B

C

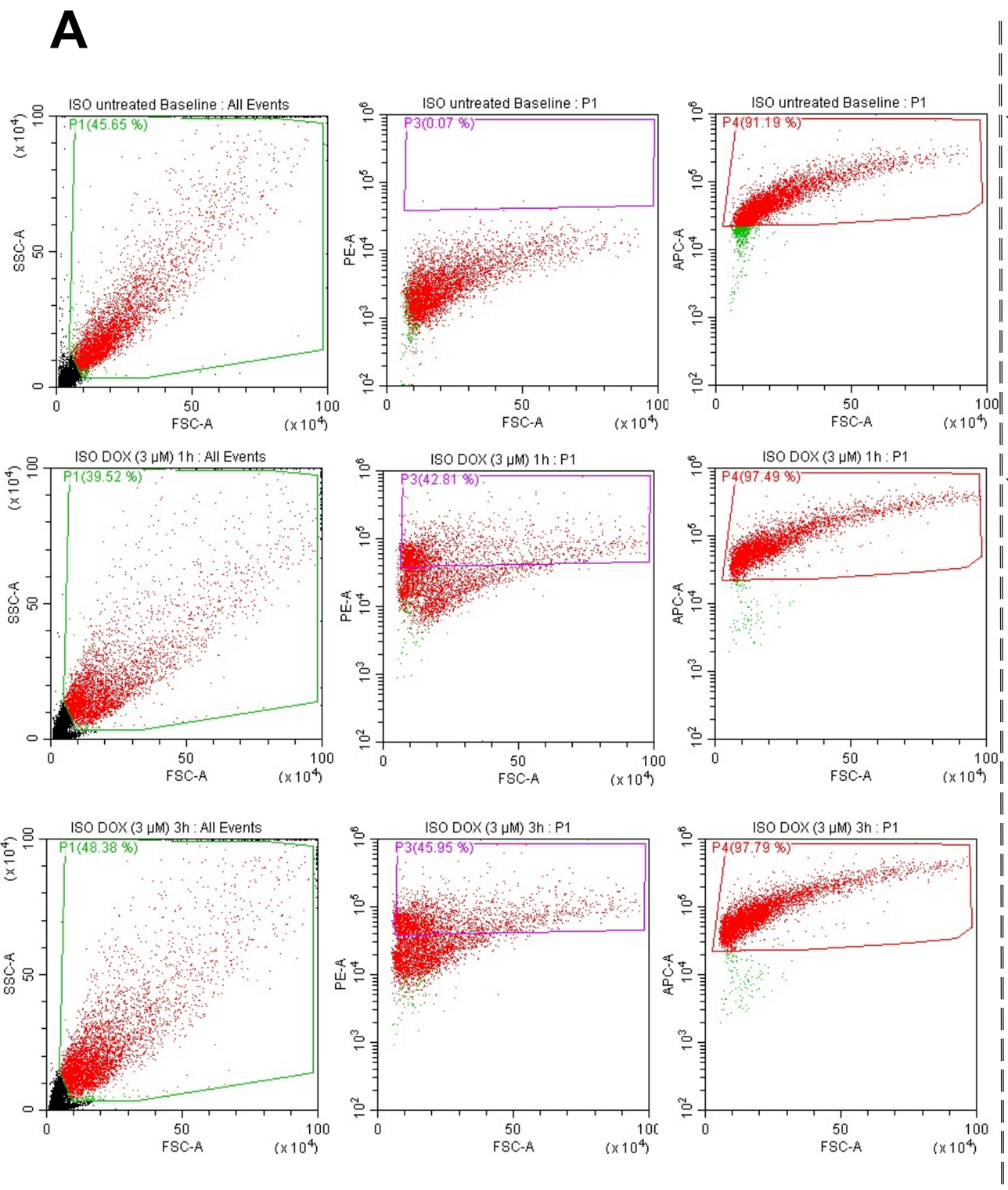


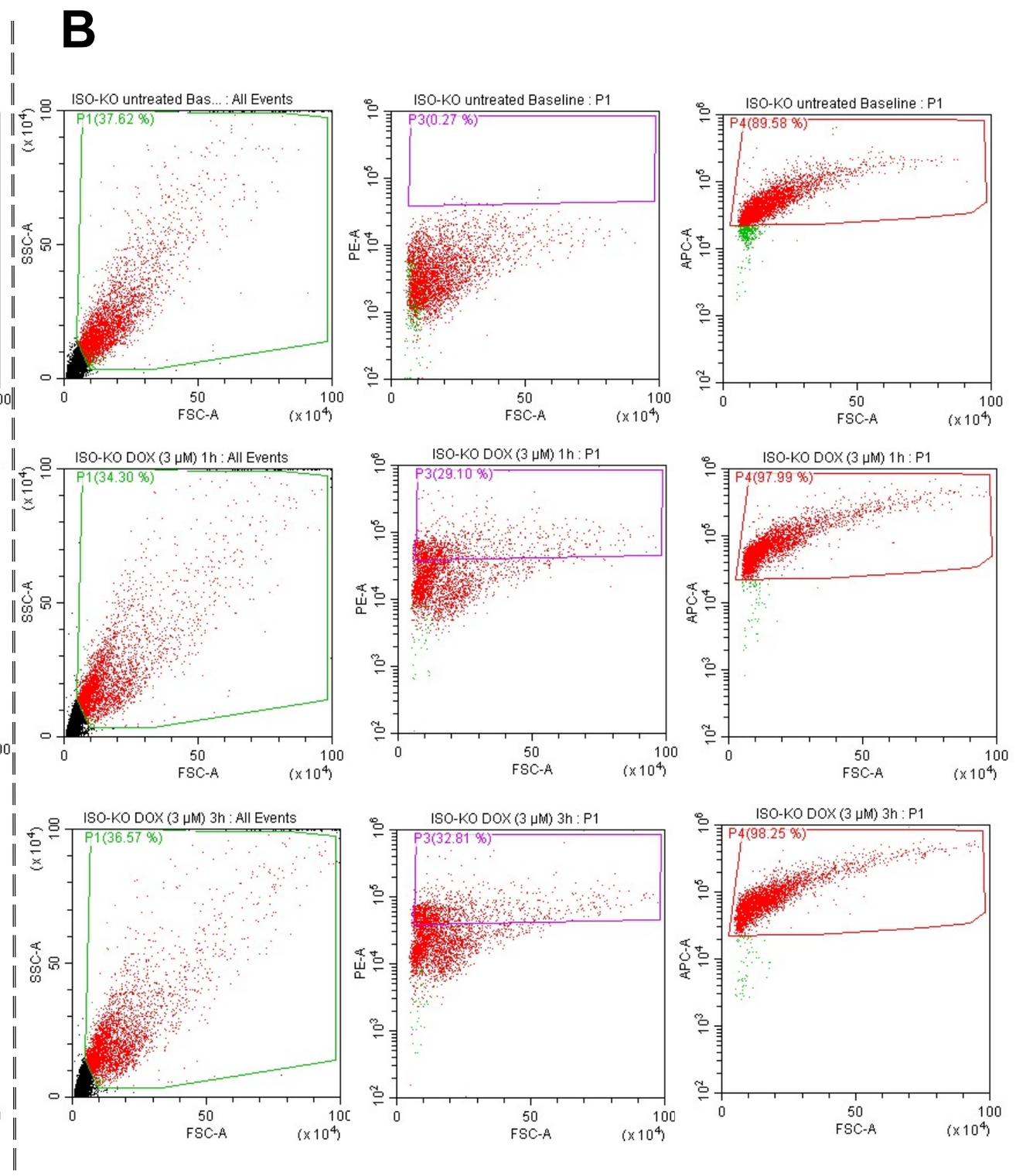
Treatment	LD_{50} (μM)	*P*-value
DOX	2.35	NA
DOX + Perospirone	1.98	0.35
DOX + Fluoxetine	2.69	0.76
DOX + Butoconazole	4.40	0.01
DOX + Propofol	4.77	0.003
DOX + Tetracaine	4.65	0.0007
DOX + Desipramine	10.66	<0.0001

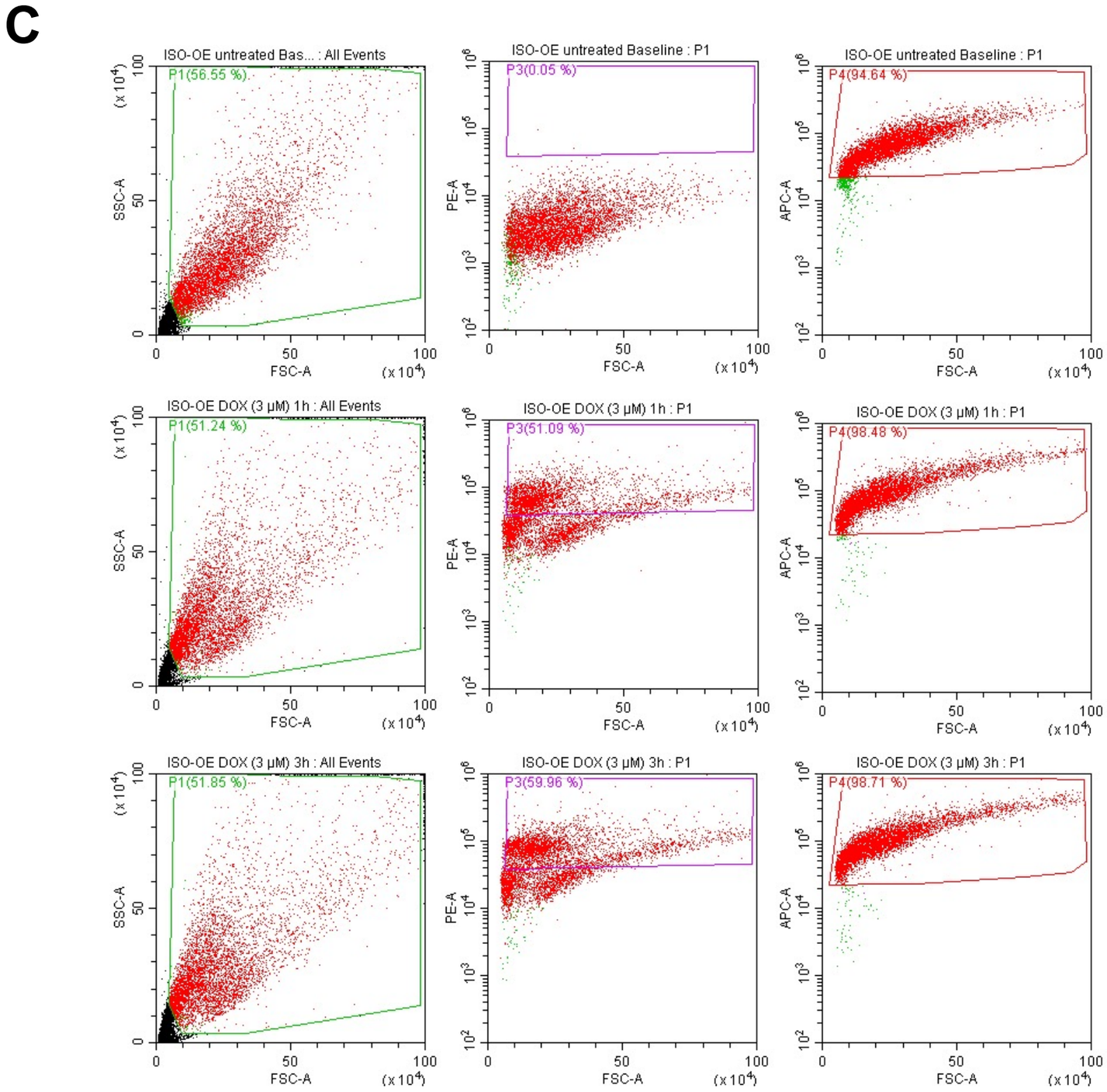
Supplementary Figure 9. Screening of cherry-pick *SLCs* modulators in relation to DIC in hiPSC–CMs. A–B, The effect of 17 *SLC* transporter modulators on DOX intracellular accumulation in hiPSC-CMs by quantification of DOX intrinsic fluorescence using a flow cytometry-based assay (*n* = 3–6). DOX uptake was quantified 1 h **(A)** and 3 h **(B)** post DOX treatment. **C,** Validation of Prestwick drug library screening-identified top FDA-approved cardioprotectants against 10 log-doses of doxorubicin. *n* = full independent experimental replicates, Error bars, s.e.m, **P* < 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, *****P* < 0.0001 by unpaired two-tailed Student's t-test **(a-b)**. For **(c)** log-logistic non-linear regression model was used to estimate the value of the four parameters, and t-statistic was used to test for significant difference in LD_{50} between different groups.



Supplementary Figure 10. Desipramine does not attenuate doxorubicin cytotoxicity in cancer cell lines. A-H, Assessment of cell viability after after 48 h of doxorubicin and desipramine co–treatment in **A** HEPG2. **B**, DLD1. **C**, LNCAP. **D**, SK-UT-1. **E,** HeLa. **F**, U2OS. **G,** Hs 578T. **H**, and MDA-MB-231 (*n* = 12–20). DESP, desipramine. *n* = full independent experimental replicates, Error bars, s.e.m, Log-logistic non-linear regression model was used to estimate the value of the four parameters, and t-statistic was used to test for significant difference in LD_{50} between different groups.







Supplementary Figure 11. Exemplary flow cytometry plots for DOX uptake in hiPSC-CMs. Flow cytometry-based DOX uptake quantification in ISO **(A)**, ISO-KO **(B)**, and ISO-OE **(C)**. For each subfigure, the top panel depicts DOX uptake in untreated cells at the baseline; the middle panel depicts DOX uptake 1 h post DOX treatment (3 µM); and the bottom panel depicts DOX uptake 3 h post DOX treatment (3 µM). P3 denotes DOX uptake (% positive cells) at baseline, 1 h, and 3 h post DOX treatment. P4 denotes live cells (% positive cells) at baseline, 1 h, and 3 h post DOX treatment.

Supplementary Figure 12. Effect of doxorubicin treatment in hiPSC-CMs and mice. **A,** Comparison of hiPSC–CMs derived from three patients harboring the heterozygous rs7853758 variant and were protected from DIC after DOX treatment (*SLC*^{var1} (*n* = 37), *SLC*^{var2} (*n* = 26), *SLC*^{var3} (*n* = 63); collectively *SLC*^{var}), to hiPSC-CMs from three control patients who did not carry this protective SNP and developed DIC upon same DOX treatment (*SLC*^{ref1} (*n* = 30), *SLC*^{ref2} (*n* = 29), *SLC*^{ref3} (*n* = 22); collectively *SLC*^{ref}). **B,** Western blot showing SLC28A3 expression in *SLC*^{ref} (*n* = 3), and *SLC*^{var} (*n* = 3) hiPSC-CMs. **C-D,** representative echocardiography images for m hearts after 3 weeks of doxorubicin treatment (3 mg/kg, ip, *n* = 10) compared co-treatment (*n* = 8) of desipramine (20 mg/kg/day, Alzet pump) and doxorubicin (3 mg/kg, ip) showing an increased end-systolic dimension in the Dox group when compared to DOX+DESP group. *n* = full independent experimental replicates, Error bars, s.e.m, *$P < 0.05$,