Full unedited gels for

Figure 1B

CAV3

kDa
250 —
130 —
100 —
70 —
55 —
35 —
25 —
15 —

◀ V5-APEX2-CAV3

◀ CAV3

Figure 1C

CAV3

kDa
250 —
130 —
100 —
70 —
55 —
35 —
25 —
15 —

◀ V5-APEX2-CAV3
◀ V5-APEX2
◀ CAV3

Figure 1D

V5   CAV3

kDa

720 —
545 —
146 —
67 —
45 —

Figure 2C

Steptavidin

kDa
250 —
130 —
100 —
70 —
55 —
35 —
25 —
15 —

CAV3

kDa
250 —
130 —
100 —
70 —
55 —
35 —
25 —
15 —

◀ V5-APEX2-CAV3
◀ V5-APEX

Figure 3A

CAV1

kDa
250 —
130 —
100 —
70 —
55 —
35 —
25 —
15 —

◀ CAV1

GAPDH

kDa
250 —
130 —
100 —
70 —
55 —
35 —

◀ GAPDH

Figure 3E

CAV1 / CAV3

kDa
55 —
35 —
25 —
15 —

◀ CAV1
◀ CAV3

Figure 4C

Ncx1

kDa
250 —
130 —
100 —

◀ Ncx1

TfR1

kDa
250 —
130 —
100 —

◀ TfR1

Na,K α1

kDa
250 —
130 —
100 —

◀ Na,K α1

McT1

kDa
70 —
55 —
35 —

◀ McT1

Cx43

kDa
70 —
55 —
35 —

◀ Cx43

CAV1 / CAV3

kDa
35 —
25 —
15 —

◀ CAV1
◀ CAV3

Figure 5 A

McT1

kDa
55 —
35 —

◀ McT1

β-Actin

kDa
55 —
35 —

◀ β-Actin

CAV3

kDa
25 —
15 —

◀ CAV3

Figure 5 B

McT1

kDa
250 —
130 —
100 —
55 —

◀ McT1

β-Actin

kDa
55 —
35 —
25 —

◀ β-Actin

Figure 7A

FLAG

kDa
250 —

◀ FLAG

Na$_V$1.5

250 —

◀ Na$_V$1.5

CAV3

kDa
55 —
35 —
25 —
15 —

◀ CAV3

Figure 7 B

Na$_V$1.5

kDa
250 —

◀ Na$_V$1.5

CAV3

kDa
25 —
15 —

◀ CAV3

SAP97

130 —
100 —
70 —
55 —
35 —

◀ SAP97

Online Figure VI

Na$_V$1.5

kDa
250 —

◀ Na$_V$1.5

CAV1 / CAV3

25 —
15 —

◀ CAV1
◀ CAV3

Online Figure XIII Documentation of full scans of Western blots.

SUPPLEMENTAL MATERIAL

Caveolin3 Stabilizes McT1-mediated Lactate/Proton Transport in Cardiomyocytes

Jonas Peper, Daniel Kownatzki-Danger, Gunnar Weninger, Fitzwilliam Seibertz, Julius Ryan D. Pronto, Henry Sutanto, David Pacheu Grau, Robin Hindmarsh, Sören Brandenburg, Tobias Kohl, Gerd Hasenfuss, Michael Gotthardt, Eva A. Rog-Zielinska, Bernd Wollnik, Peter Rehling, Henning Urlaub, Jörg Wegener, Jordi Heijman, Niels Voigt, Lukas Cyganek, Christof Lenz, Stephan E. Lehnart

Detailed Methods

Supplemental Figures and Figure Legends

Supplemental Tables and Supporting Information

DETAILED METHODS

Human Stem Cell Study Ethical Approval

The study was approved by the Ethics Committee of University Medical Center Göttingen (approval number 10/9/15) and carried out in accordance with approved guidelines. Written informed consent was obtained from all donors prior to participation in the study.

Mouse Study Approval

The generation of the CAV1 knock-out mouse model has been described previously.[56] Transgenic cardiac-specific FLAG-epitope tagged human -Na_V1.5-F1759A mice were used for FLAG-Na_V1.5 co-immunoprecipitation in Figure 7 and kindly supplied by Dr. Steven O. Marx described previously by Wan et al.[38] Breeding and humane euthanasia for organ harvesting were carried out according to guidelines for the care and use of laboratory animals, following directive 2010/63/EU of the European Parliament and in keeping with NIH guidelines. All procedures were reviewed by the institutional animal committee of the University Medical Center Göttingen and approved by the veterinarian state authority (LAVES, Oldenburg, Germany; 33.9-42502-04-18/2975 and 33.9-42502-04-18/2905).

APEX2 Plasmids and Recombinant Adenoviral Vectors for Cardiomyocyte Transfection

The V5 epitope and APEX2 were N-terminally tagged to wild-type mouse Caveolin3 (V5-APEX2-CAV3). The CAV3 cDNA (MR226246, Origene) was amplified according to the manufacturer's instructions (In-Fusion HD Cloning Kit, Clontech) using the following primers:
  - Fwd-5′-GGGGCAGCGGCTCGAGCATGATGACCGAAGAGCACA-3′
  - Rev-5′-TAGATGCATGCTCGAGTTAGCCTTCCCTTC-3′

The CAV3 insert was cloned into the V5-APEX2 tagged pcDNA3 vector using a XhoI restriction digest (NEB). The construct was transformed in Stellar Competent Cells (Clontech). For adenoviral transfection, a custom-designed bicistronic subtype-5 vector (pO6A5-CMV, Sirion Biotech) was used to express eGFP and V5-APEX2 or V5-APEX2-CAV3 in cultured neonatal rat cardiomyocytes. Adenoviral transduction of cardiomyocytes was monitored by eGFP fluorescence (Axiovert A1, Zeiss).

Neonatal Rat Cardiomyocyte (NRCM) Isolation and Purification

Hearts from 40 Wistar rat pups (P0-P3) were collected on ice in CBFHH buffer (Table I). The atria were excised with scissors (914012-12, FST) under magnification, the ventricles harvested for enzymatic digestion (Enzyme D, Neonatal Heart Dissociation Kit, Miltenyi Biotech), and NRCMs dissociated (gentleMACS Dissociator, Miltenyi Biotech) according to the manufacturer's instructions (Neonatal Heart Dissociation Kit, Miltenyi Biotech). To enrich isolated ventricular NRCMs, the raw cell suspension was filtered by gravity through a stainless steel mesh (grid size 250 µm, Thermo Fisher Scientific). The NRCMs were pelleted by centrifugation (60 x g for 20 min at 4 °C), resuspended in 5 mmol/L ice-cold PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco), and 2.5 mmol/L of the suspension layered on top of a Percoll density gradient (63 %, 40.5 % Centrifugation Media, pH 8.5 to 9.5, GE-Healthcare) using previously published protocols,[57] and centrifuged at 3,000 x g for 30 min at RT (acceleration speed 9; deceleration

speed 0; Heraeus Multifuge X1R, Thermo Fisher Scientific). NRCM enriched at the Percoll layer interface were collected with a 10 mL glass pipette (10 mL wide tip, Ratiolab) and suspended in 10 mL of 37 ºC NRCM cultivation medium (Table I).

We used flow cytometry to quantify the purity of isolated NRCMs. For this, freshly isolated NRCMs were fixed in 4% paraformaldehyde (PFA, Sigma-Aldrich) for 10 min at RT and permeabilized with chilled 90% methanol (Methanol, Sigma-Aldrich) for 15 min followed by a blocking step with 0.5 % BSA (BSA; Sigma-Aldrich) diluted in PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco) for 20 min at RT. Cells were stained with a mouse cardiac Troponin T (cTnT) antibody at a dilution of 1:50 and with mouse IgG1 at a dilution of 1:50 as isotype control for 1 h at RT (Major Resources Table). Donkey anti-mouse IgG-cy3 (Major Resources Table) was used at a dilution of 1:100 for 1 h at RT as secondary antibody. Cy3 stained cells were counted by flow cytometry (BD Accuri C6 plus, BD Biosciences) and the amount of cTnT positive cells analyzed with FlowJo Software (Tree Star Inc) resulting in 95.3 % cTnT positive cells.

3-State SILAC Cardiomyocyte Culture Conditions

SILAC containing DMEM (Flex Media, Gibco) without L-arginine and L-lysine was supplemented with penicillin-streptomycin (100 U/mL), D-glucose (1 g/L), Na-pyruvate (100 mmol/L), and BRDU (10 mmol/L) containing either heavy, medium, or light isotope lysine and arginine as follows: for *heavy* SILAC labeling, L-lysine [$^{13}C_6$,$^{15}N_2$]HCl (Lys-8) and L-arginine [$^{13}C_6$,$^{15}N_4$]HCl (Arg-10) were added; for *medium* SILAC labeling, L-lysine-4,4,5,5-d_4 (Lys-4) and L-arginine [$^{13}C_6$]HCl (Arg-6); and for *light* SILAC labeling, DMEM liquid medium with 1 g/L D-glucose was used. All solutions were vacuum-filtered (Steritop, Merck). For NRCM culture, 10% (vol/vol) heat inactivated FBS (Gibco) was added to the medium. NRCMs were seeded at a density of 500,000 cells on 35 mm dishes (CELLSTAR 6-well plate, Greiner) coated with collagen (13.96 mg/mL Collagen I rat tail, Corning) diluted 1:100 in PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco) and cultivated for 13 days in 2 mL light (non-labeled), medium, or heavy SILAC medium in 5% CO_2 / 21% O_2 at 37 ℃ (Heracell VIOS, Thermo Fisher Scientific). SILAC media were completely exchanged every second day. Mass spectrometry determined SILAC incorporation (%) for up to 20 days in culture. SILAC incorporation reached a plateau (>95%) after 13 days culture (Figure IIA). For proximity proteomic analysis (described in the next chapter) we used an experimental design for systematic label switching with three biological replicates as outlined in Figure 2A.

Ratiometric APEX2 Mediated Biotinylation in NRCMs

For ratiometric APEX2 mediated biotinylation of endogenous NRCM proteins, SILAC labeled NRCM were transfected with recombinant adenoviral vectors expressing V5-APEX2-CAV3 for 48 h using MOI 1 between day 11 and 13 in SILAC culture (for higher MOI doses please see Figure IC). In parallel, adenoviral vectors expressing soluble V5-APEX2 or eGFP were used as controls. Based on protocols published previously for ratiometric APEX biotinylation in heterologous cell systems,[15,16] 1 mL of each SILAC medium (chapter above) was exchanged by the same SILAC medium containing 500 µmol/L biotin-phenol. After 30 min equilibration, a final concentration of 1 mmol/L H_2O_2 was added and the medium gently mixed for 1 minute. After 1 min, the biotinylation reaction was quenched by replacing the medium with 1 mL quenching buffer (Table II). NRCMs were washed thrice with quenching buffer, scraped (Cell Scraper 25 cm, Sarstedt), and collected in 250 µL RIPA quenching buffer (Table II). The NRCM

suspension was passed 15 times through a 27 gauge syringe on ice and centrifuged at 13,000 x g for 10 min at 4 °C to collect the solubilized proteins in the supernatant. The protein concentration was determined by absorption measurement (Pierce 660 nm protein assay, Thermo Fisher Scientific). Heavy, medium and light labeled NRCM lysates were mixed at a 1:1:1 ratio at a total protein concentration of 250 µg.

Avidin Capture and Elution of Biotinylated Proteins

Avidin beads (Pierce Monomeric Avidin Agarose, Thermo Fisher Scientific) were equilibrated with RIPA quenching buffer (Table II) and 40 µL avidin beads added to 250 µg of NRCM lysate. The suspension was gently rotated for 1 h at 4 °C in a spin column (Pierce Spin Columns Screw Cap, Thermo Fisher Scientific). Next, beads were washed twice with 500 µL RIPA quenching buffer, once with 500 µL Tris/HCl buffer containing 2 mml/L urea (pH 8.0), and again twice with 500 µL RIPA quenching buffer (Table II). Two centrifugation steps at 100 x g for each 30 s and 2 min at 2000 x g (Heraeus, Fresco 21 centrifuge, Thermo Fisher Scientific) were used to harvest the beads, while the supernatant was discarded. Biotinylated proteins were eluted in 75 µL biotin buffer (Table II) for 15 min at RT, followed by 15 min at 70 °C. Beads were pelleted by centrifugation for 1 min at 1000 x g and the supernatant containing the eluted proteins was collected. The eluted proteins were analyzed by mass spectrometry as described below.

Sample Preparation for NanoLC-MS/MS Analysis of SILAC Labeled Samples

Eluted protein samples were fractionated on 4-12 % Bis-Tris minigels (NuPAGE Novex, Invitrogen). Gels were stained with Coomassie Blue overnight (Coomassie Brilliant Blue R-250 Staining Solution, BioRad) for protein visualization, and each lane sliced into 11 equal-sized gel pieces. After washing the gel pieces with 50 mmol/L ammonium bicarbonate (TEAB, Sigma Aldrich), gel slices were reduced with 10 mmol/L dithiothreitol (1,4-dithiothreitol, Sigma-Aldrich), alkylated with 55 mmol/L 2-iodoacetamide Sigma-Aldrich, and digested with endopeptidase trypsin (sequencing grade, Promega) diluted 1:50 in 55 mmol/L iodoacetamide overnight. Post-trypsinization the peptides were solubilized in MS loading buffer (Table III), dried (SpeedVac, Thermo Fisher Scientific), reconstituted in MS loading buffer and prepared for NanoLC-MS/MS analysis as described previously.[58]

NanoLC-MS/MS Analysis of SILAC Labeled Samples

For mass spectrometric analysis of solubilized peptides, samples were enriched on a self-packed reversed phase-C18 precolumn (0.15 mm ID x 20 mm, Reprosil-Pur120 C18-AQ 5 µm, Dr. Maisch, Ammerbuch-Entringen, Germany) and separated on an analytical reversed phase-C18 column (0.075 mm ID x 200 mm, Reprosil-Pur 120 C18-AQ, 3 µm, Dr. Maisch, Ammerbuch-Entringen, Germany) using a 30 min linear gradient of 5-35% acetonitrile/0.1% formic acid (v/v) at 300 nl min⁻¹. The eluent was analyzed on a hybrid quadrupole/orbitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific) equipped with a FlexIon nanoSpray source and operated under Excalibur 2.5 software using a data-dependent acquisition method. Each experimental cycle was of the following form: one full MS scan across the 350-1600 *m/z* range was acquired at a resolution setting of 70,000 full width at half maximum (FWHM), and AGC target of $1*10e6$ and a maximum fill time of 60 ms. Up to 12 most abundant peptide precursors of charge states 2 to 5 above a $2*10e4$ intensity threshold were then sequentially isolated at 2.0 FWHM isolation

width, fragmented with nitrogen at a normalized collision energy setting of 25%, and the resulting product ion spectra recorded at a resolution setting of 17,500 FWHM, and AGC target of 2*10e5 and a maximum fill time of 60 ms. Selected precursor *m/z* values were then excluded for the following 15 s. Two technical replicates per sample were acquired.

APEX2 Assay Data Processing

Raw data were processed using quantitative proteomic software (MaxQuant Software version 1.5.7.4, Max Planck Institute for Biochemistry). Proteins were identified against a UniProtKB-derived *rattus norvegicus* protein sequence database (v2018.02, 37830 protein entries) along with a set of common lab contaminants. The search was performed with trypsin as enzyme and iodoacetamide as cysteine blocking agent. Up to two missed tryptic cleavages and methionine oxidation as a variable modification were allowed for. Instrument type 'Orbitrap' was selected to adjust for MS acquisition specifics. The Arginine Arg-10, Arg-6 and Lysine Lys-8, Lys-6 labels including the 'Re-quantify' option were specified for relative protein quantitation. For identification of APEX2 biotinylated proteins, the ratios of V5-APEX2-CAV3 versus V5-APEX2 or eGFP were calculated and log2 transformed. The V5-APEX2-CAV3/V5-APEX2 ratio was plotted on the X-axis and the V5-APEX2-CAV3/eGFP ratio on the Y-axis (Figure 2D). Scatter plots were generated with Prism version 7.03 (GraphPad). Enriched biotinylated proteins were tested for statistical significance ($p<0.05$) by one sample z-test (Excel2007, Microsoft Office) and visualized as 'positive' or 'negative' hits, including previously established proteins-of-interest (POIs). Mass spectrometry results are provided as Excel file (Excel Table I). Mass spectrometry data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository[59] (http://www.ebi.ac.uk/pride) with the dataset identifier PXD019398.

Immunoblotting and Streptavidin Blotting for Protein Analysis

Mouse heart ventricular tissue, NRCMs, or human iPSC-cardiomyocytes were homogenized in ice-cold RIPA buffer (Table IV) by 20 strokes on ice using a Potter homogenizer (RW20 digital, IKA). The homogenate was centrifuged at 10,000 x g for 10 min at 4 °C to pellet insoluble materials, and the protein concentration determined (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific). For immunoblotting, 30 µg of cleared homogenate was loaded per lane onto a 4-20% Tris-Glycine gradient gel (Novex 4-20% Tris-Glycine, Thermo Fisher Scientific) and resolved by SDS gel electrophoresis at constant 200 V for 45 min. Proteins were transferred onto PVDF membranes (0.45 mm, Immobilon-FL, Merck Millipore) using an electrophoretic transfer cell (Mini Trans-Blot Electrophoretic Transfer Cell, Bio-Rad) at constant 100 V for 1 h in transfer buffer (Table IV) at 4 °C. PVDF membranes were blocked for 1 h in 5% w/v non-fat milk (Milkpowder, Roth) in Tris-buffered saline with 0.05% v/v Tween (Tween 20, Sigma Aldrich). PVDF membranes were incubated with the primary antibodies (Major Resources Table) at 4°C overnight, washed thrice with PBS (pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco) and incubated with fluorescent anti-mouse or anti-rabbit secondary antibodies at a dilution of 1:15,000 for at least 1 h at RT (P/N 926-32212, P/N 926-68072, P/N 926-32213, P/N 926-68073, IRDye LI-COR). Fluorescence signals were captured with an Odyssey CLx imaging system (LI-COR) and band intensities analyzed with Image Studio Lite Version 5.2 (LI-COR).

To analyze biotin-phenol labeled proteins (Figure 2C, Figure IA and IB), PVDF membranes were incubated with streptavidin (RD680, LI-COR) for at least 1 h at RT and the

fluorescence detected with the Odyssey CLx imaging system (LI-COR) and analyzed as described above.

Blue Native (BN)-PAGE Analysis

BN-PAGE was used to investigate oligomeric complexes of endogenous CAV3 and V5-APEX2-CAV3 transfected in NRCMs (Figure 1D). Transfected NRCMs were centrifuged at 13,000 x g for 10 min at 4 °C and 100 mg of the cell pellet resuspended in 1 mL homogenization buffer (Table VI). NRCMs were homogenized at 4 °C by 50 strokes on ice using a Potter homogenizer (RW20 digital, IKA). Homogenates were centrifuged at 1,000 x g for 10 min at 4 °C to remove cell debris. The cleared supernatant was centrifuged at 100,000 x g for 1 h (Optima Max-XP, MLA-150 rotor, Beckman) to enrich the membrane fraction. The plasma membrane fraction was resuspended in 30 µL solubilization buffer (Table VI), snap-frozen and stored at -80 °C. Solubilized membranes were thawed on ice and the protein concentration determined by absorption measurement (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific). Digitonin (Digitonin, Sigma Aldrich) was added as detergent (6 g digitonin/g protein), and insoluble membranes were removed by centrifugation at 13,000 x g for 10 min at 4 °C. The cleared supernatant was mixed 1:10 with a Coomassie blue solution (Coomassie Brilliant Blue R-250, 5% w/v, Sigma Aldrich) and a glycerol solution (Glycerol, 50% w/v, Sigma Aldrich). Anode/cathode buffers were prepared according to manufacturer's instructions (NativePAGE Bis-Tris Mini Gel Electrophoresis Protocol, Thermo Fisher Scientific). For BN-Page, 50 µg of solubilized membrane proteins were separated on a 3-12% Bis-Tris gradient gel (NativePAGE 3-12% Bis-Tris Gel, Thermo Fisher Scientific) at constant 150 V for 1 h, followed by replacing the cathode buffer (Dark Blue Cathode Buffer, Novex) by cathode buffer light (Light Blue Cathode Buffer, Novex), and electrophoresis at constant 250 V for 1 h. Native markers (Serva Native Marker, Serva) were used to estimate molecular weight. Solubilized membrane proteins were transferred onto PVDF membranes (0.45 mm, Immobilon-FL, Merck Millipore) using an electrophoretic transfer cell (Mini Trans-Blot Electrophoretic Transfer Cell, Bio-Rad) at constant 50 V for 2 h in transfer buffer (Table IV) at 4 °C. PVDF membranes were blocked for 1 h in 5% w/v non-fat milk (Milkpowder, Roth) in Tris-buffered saline with 0.05 % v/v Tween (Tween 20, Sigma Aldrich). Immunoblotting (Figure 1D) was performed using V5 and CAV3 antibodies (Major Resources Table).

Co-Immunoprecipitation of CAV1 and CAV3 Interacting Proteins

Mouse ventricular heart lysates were solubilized with CHAPS co-IP buffer (Table V), and 500 µg was incubated with 3 µg anti-CAV3 antibody (ab2912, Abcam), 3 µg anti-CAV1 antibody (ab2910, Abcam), or normal rabbit IgG antibody (12-370, Merck) at 4 °C overnight. The samples were incubated with magnetic beads (Dynabeads Protein G, 15 µL, Thermo Fisher Scientific) in 100 µL CHAPS co-IP buffer for 2 h at 4 °C. The magnetic beads were extracted with a magnet (DynaMag-2 Magnet, Thermo Fisher Scientific), the solution discarded and the beads washed thrice with 500 µL ice-cold CHAPS co-IP buffer to minimize unspecific binding. Precipitated proteins were eluted in 60 µL of 2 × SDS buffer containing β-mercaptoethanol (Table IV). For the McT1 co-IP, 60 µL of 2x LDS buffer without β-mercaptoethanol (1× NuPAGE, Invitrogen) was used to optimize IgG signals at 55 kDa. Eluted samples were heated to 70 °C for 5 min and resolved on 4-20% Tris-Glycine gradient gels (Novex 4-20% Tris-Glycine, Thermo Fisher

Scientific). For SDS gel electrophoresis and protein transfer see **Immunoblotting and Streptavidin Blotting for Protein Analysis**. After transfer and blocking, primary antibodies (Major Resources Table) against the proteins shown in Figure 3E, Figure 4C, and Figure XI were applied at 4 °C overnight. To reduce unspecific background signals, for Aquaporin1, McT1, Ncx1, and TfR1 antibody incubation the IRDye-680 detection reagent was added according to the manufacturer's instructions (Quick Western Kit, LI-COR).

Co-Immunoprecipitation of Transgenic Human FLAG-Na$_v$1.5

FLAG-based immuno-precipitation was used to investigate transgenic mouse heart FLAG-tagged human F1759A-Na$_v$1.5 in a mouses model described by Wan et al.[38] (labeled 'FLAG-hNa$_v$1.5' in Figure 7), and WT mice for endogenous mouse Na$_v$1.5 (negative control). Mouse ventricular heart lysates were solubilized with co-IP buffer according to the protocol of Vatta et al.[9] (Table V). Cardiac lysates were centrifuged at 10,000 x g for 15 min at 4 °C to pellet insoluble materials, and the protein concentration determined (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific). For human FLAG-hNa$_v$1.5 immunoprecipitation, 500 µg of cleared lysates were incubated with 3 µg anti-FLAG antibody (f1804, Sigma-Aldrich) at 4 °C overnight. The samples were incubated with magnetic beads (Dynabeads Protein G, 50 µL, Thermo Fisher Scientific) for 2 h at 4 °C. The beads were extracted with a magnet (DynaMag-2 Magnet, Thermo Fisher Scientific), the solution discarded, and the beads washed thrice with 500 µL ice-cold co-IP buffer (Table V) to minimize unspecific binding and as described by Vatta et al previously.[9] Precipitated proteins were eluted in 60 µL of 2 × SDS buffer containing β-mercaptoethanol (Table IV). For SDS gel electrophoresis and protein transfer protocols see **Immunoblotting and Streptavidin Blotting for Protein Analysis**. After transfer and blocking, the primary antibodies were applied (Figure 7A, Major Resources Table) at 4 °C overnight.

Co-Immunoprecipitation of WT Mouse Na$_v$1.5

For endogenous mouse Na$_v$1.5 immunoprecipitation, we used a protocol reported by Milstein et al.[39] Mouse ventricular heart lysates were solubilized with IGEPAL co-IP buffer (Table V) and 500 µg was incubated with 3 µg anti-Nav1.5 antibody (ASC-005, Alomone Lab) or normal rabbit IgG (12-370, Merck) antibody at 4 °C overnight. The samples were incubated with 50 µL recombinant protein A/G-Sepharose beads (sc-2003, Santa Cruz) for 4 h at 4 °C. Protein–antibody complexes were centrifuged at 1,000 × g and washed thrice with IGEPAL co-IP buffer (Table V). Protein–antibody complexes were finally centrifuged at 10,000 × g and precipitated proteins were eluted in 60 µL of 2 × SDS buffer containing β-mercaptoethanol (Table IV). For SDS gel electrophoresis and protein transfer see **Immunoblotting and Streptavidin Blotting for Protein Analysis**. After transfer and blocking, primary antibodies against the proteins indicated in Figure 7B were applied (Major Resources Table) at 4 °C overnight.

Co-Immunoprecipitation of V5-APEX2-CAV3

Adenovirally transfected NRCMs expressing V5-APEX2-CAV3 or V5-APEX2 were solubilized with sodium deoxycholate co-IP buffer (Table V) and incubated with 3 µg anti-V5 antibody (R960-25, Thermo Fisher Scientific) at 4 °C overnight. Magnetic beads (Dynabeads Protein G, Thermo Fisher Scientific) were added to the sample, incubated for 2 h at 4 °C, washed and resuspended in 2× SDS buffer containing β-mercaptoethanol as described above. Samples were

heated to 70 °C for 5 min and resolved on 4-20% Tris-Glycine gradient gels (Novex 4-20% Tris-Glycine, Thermo Fisher Scientific). For SDS gel electrophoresis and protein transfer see **Immunoblotting and Streptavidin Blotting for Protein Analysis**. After transfer on PVDF membranes and blocking, these were incubated with the antibodies anti-V5 (R960-25, Thermo Fisher Scientific) and anti-CAV3 (ab2912, Abcam) (Figure 1C).

Sample Preparation for Label-Free Data Independent Acquisition-Mass Spectrometry

Label-free DIA-MS quantification was performed according to published protocols.[60, 61] Samples were run on 4-12% NuPAGE Novex Bis-Tris Minigels (4-12% NuPAGE, Invitrogen) over a relatively short distance (~1 cm), cut out as a single fraction, and trypsinized as described under **In-Gel Tryptic Digestion**. Post-trypsin peptides were solubilized in MS loading buffer (Table III), dried (SpeedVac, Thermo Fisher Scientific), reconstituted in MS loading buffer, and prepared for nanoLC-MS/MS as described previously.[58] All samples were spiked with a synthetic peptide standard for retention time alignment (iRT Standard, Biognosys).

Affinity purification (AP) followed by label-free quantification (AP-MS) was performed as previously described with few modifications.[60] CAV1 and CAV3 were immunoprecipitated from 500 µg mouse ventricular tissue. Normal rabbit IgG (12-370, Merck) was used as negative control. Immunoprecipitated samples were run on a 4-12% NuPAGE Novex Bis-Tris Minigels (4-12% NuPAGE, Invitrogen) as a single fraction and prepared for nanoLC-MS/MS as described above.

NanoLC-MS/MS Analysis by Label-Free DIA-MS

Digested proteins were analyzed on a nanoflow chromatography system (Eksigent nanoLC425, SCIEX) hyphenated to a hybrid triple quadrupole-TOF mass spectrometer (TripleTOF 5600+, SCIEX) equipped with a Nanospray III ion source (Ionspray Voltage 2400 V, Interface Heater Temperature 150 °C, Sheath Gas Setting 12) controlled by Analyst (Analyst TF 1.7.1 software build 1163, SCIEX). Peptides were dissolved in MS loading buffer (Table III) to a concentration of 0.3 µg/µL. For each analysis 1.5 µg of digested protein were enriched on a precolumn (0.18 mm ID x 20 mm, Symmetry C18, 5 µm, Waters) and separated on an analytical RP-C18 column (0.075 mm ID x 250 mm, HSS T3, 1.8 µm, Waters) using a 90 min linear gradient of 5-35% acetonitrile/0.1% formic acid (v/v) at 300 nl min^{-1}.

Qualitative LC/MS/MS analysis was performed using a Top25 data-dependent acquisition method with an MS survey scan of *m/z* 350–1250 accumulated for 350 ms at a resolution of 30,000 full width at half maximum (FWHM). MS/MS scans of *m/z* 180–1600 were accumulated for 100 ms at a resolution of 17,500 FWHM and a precursor isolation width of 0.7 FWHM, resulting in a total cycle time of 2.9 s. Precursors above a threshold MS intensity of 125 cps with charge states 2+, 3+, and 4+ were selected for MS/MS, the dynamic exclusion time was set to 30 s. MS/MS activation was achieved by collision-induced dissociation using nitrogen as a collision gas and the manufacturer's default rolling collision energy settings. Two technical replicates per sample were analyzed to construct a spectral library.

For quantitative DIA analysis, MS/MS data were acquired using 65 variable size windows[61] across the 400-1,050 *m/z* range. Fragments were produced using rolling collision energy settings for charge state 2+, and fragments acquired over an *m/z* range of 350–1400 for

40 ms per segment. Including a 100 ms survey scan this resulted in an overall cycle time of 2.75 s. 3x3 replicates (biological x technical) were acquired for each biological state.

Data Processing for Label-Free DIA-MS

Protein identification was achieved (ProteinPilot Software version 5.0 build 4769, SCIEX) at "thorough" settings. The combined qualitative analyses were searched against the UniProtKB mouse reference proteome (revision 04-2018, 61,290 entries) augmented with a set of 52 known common laboratory contaminants to identify proteins at a False Discovery Rate (FDR) of 1%.

Spectral library generation and peak extraction were achieved in PeakView Software version 2.1 build 11041 (SCIEX) using the SWATH quantitation microApp version 2.0 build 2003. Following retention time correction using the iRT standard, peak areas were extracted using information from the MS/MS library at an FDR of 1%.[60] The resulting peak areas were then summed to peptide and finally protein area values per injection, which were used for further statistical analysis.

CAV1 versus CAV3 interacting proteins (Figure 4A) detected by DIA-MS were statistically evaluated in Perseus version 1.5.6.0 (Max Planck Institute for Biochemistry). Observed fold changes were log2 transformed and plotted on the X-axis, while the permutation-based false-discovery rate (t-test, $p > 0.05$, FDR=5%, S0=0.1) corrected p values were $-\log_{10}$ transformed and plotted on the Y-axis to generate a volcano plot. Log2 fold changes in protein areas between samples were evaluated for statistical significance ($p < 0.05$) by permutation-based false-discovery rate analysis (t-test, $p > 0.05$, FDR=5%, S0=0.1).[28] Furthermore, a $\log_2$ fold change ratio ≥1 was used as cutoff.[62] An Excel table of putative binding partners (dataset Excel Table II) was generated, with the most specific binding partners highlighted in grey. Mass spectrometry proteomic data have been deposited to the ProteomeXchange Consortium[59] (http://www.ebi.ac.uk/pride) with the dataset identifier PXD019399 (Major Resources Table).

STRING Analysis

Proteins of interest identified by MS were analyzed for subcellular compartments using Gene Ontology (GO) terms (Figure IIB) and known protein/protein interactions (Figure 2E) based on the STRING database (string-db.org) as described previously.[20] We applied the STRING tool (https://string-db.org/, version 11.0) [20] to generate context-specific protein/protein interaction networks, as well as for functional annotations. We used a scoring cut-off of ≥0.7 to define positive interactions for MS identified POIs following published workflows.[63]

Cell Culture of Human Induced Pluripotent Stem Cells (iPSCs)

Cell culture and ventricular cardiomyocyte differentiation of human iPSC-CMs was performed by the Stem Cell Unit Göttingen. The human iPSC lines isWT1.14 (UMGi014-C.14; abbreviated as WT iPSC) and isWT1-CAV3-KO.34 (UMGi014-C-3.34; abbreviated as CAV3 KO iPSC) were maintained on Matrigel-coated (Matrigel, growth factor reduced, BD Biosciences) 35 mm plates (CELLSTAR 6-well plate, Greiner), passaged every 4-6 days with a non-enzymatic cell dissociation reagent (Versene solution, Thermo Fisher Scientific) and cultured in iPSC medium (Table IX) for 24 h after passaging, and with daily complete medium changes. For cell culture a humidified incubator with 5% CO_2 and 21% O_2 at 37 °C was used (Heracell VIOS, Thermo Fisher Scientific).

Stem Cell Differentiation

Directed differentiation of human iPSCs into ventricular iPSC cardiomyocytes (iPSC-CMs) was performed via WNT signaling modulation as described previously.[64] The ventricular differentiation was initiated at 80%–90% confluence on Matrigel-coated 35 mm plates using the cardiac differentiation medium (Table IX) and sequential treatment with 4 µM of a GSK-3α/β inhibitor (CHIR-99021, Merck Millipore) for 48 h, followed by 5 µmol/L PORCN Inhibitor (IWP2, Merck Millipore) for 48 h. The complete medium was replaced by cardio culture medium (Table IX) at day 8. Differentiated cultures around day 15 were digested with 0.25% trypsin (Trypsin/EDTA, Thermo Fisher Scientific) and replated in 35 mm plates (CELLSTAR 6 Well plate, Greiner). Metabolic ventricular iPSC-CM selection was done with cardio selection medium (Table IX) for 5 days. Afterwards, iPSC-CMs were cultured in cardio culture medium (Table IX) at least to day 60 for further maturation. iPSC-CMs were washed thrice with PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco) and scraped (Cell Scraper 25 cm, Sarstedt) in 500 µL ice cold PBS. iPSC-CMs were pelleted at 13,000 x g for 10 min at 4 °C, snap-frozen in liquid N_2, and stored at -80 °C until further use.

CRISPR/Cas9 Mediated Genome Editing

For genome editing of human iPSC lines we used ribonucleoprotein (RNP)-based CRISPR/Cas9 to target exon 1 of the human CAV3 gene to generate CAV3 knock-out iPSCs (Figure VIA). The guideRNA target sequences, with PAM in bold, were:
- CRISPR#1 5'-TCCCCCCAGCTCTGCGATGA**TGG**-3'
- CRISPR#2 5'-CACCGCCCAGATGTGGCAGA**AGG**-3'

The human iPSC line is WT1.14 (UMGi014-C.14; abbreviated as WT iPSC) was cultured in StemFlex medium (StemFlex medium, Thermo Fisher Scientific) on Matrigel-coated (growth factor reduced, BD Biosciences) plates and transfected by nucleofection according to the manufacturer's instructions (P3 Primary Cell 4D-Nucleofector X Kit, Lonza) between passage 12 to 15. The CRISPR/Cas9 RNP complex was assembled by mixing of the individual Alt-R CRISPR-Cas9 crRNA and the Alt-R CRISPR-Cas9 tracrRNA, preassembled in a 1:1 ratio, with the Alt-R S.p. HiFi Cas9 Nuclease 3NLS (all: IDT DNA Technologies) at a 1:3 molar ratio, incubated for 10 min at RT and diluted in nucleofector solution (P3 4D-Nucleofector X Solution, Lonza). 1 h before nucleofection, iPSCs were pretreated with 2 µmol/L of Rho inhibitor (Thiazovivin, Merck Millipore) and dissociated using a non-enzymatic cell dissociation reagent (Versene solution, Thermo Fisher Scientific) at a confluence of 70-80%. For each approach, 2×10^6 iPSCs, quantified by cell counter (CASY, OMNI Life Science), were used according to the manufacturer's instructions. Following nucleofection, iPSCs were replated in two Matrigel-coated (Matrigel, growth factor reduced, BD Biosciences) wells on 35 mm plates (CELLSTAR 6 Well plate, Greiner) and cultured in StemFlex medium (Thermo Fisher Scientific) supplemented with 2 µmol/L of Rho inhibitor (Thiazovivin, Merck Millipore). After 48 h, transfected iPSCs were replated as single cells by limited dilution as described previously,[63] and cultured in StemFlex medium (Thermo Fisher Scientific) for one week. Individual iPSC colonies were manually picked and expanded for approximately one to three weeks in StemMACS iPS-Brew XF medium (Miltenyi Biotech) with daily medium change. Expanded colonies were analyzed for genetic modification by Sanger sequencing (Figure VIB) and positive clones were selected for further

analysis. The normal karyotype was determined in post-edited cells (Figure VIF) and clones were re-sequenced for purity every 5-10 passages. Genomic stability of human iPSC cultures was assessed between passage 25 and 30 using the G-banding method according to previous protocols.[65] At least 15 metaphase cells per sample were analyzed, all of which were concluded to have no structural abnormality (Figure VIE).

Immunocytochemical Staining and Flow Cytometry of Stem Cells

For immunocytochemical studies, cells were cultured on glass coverslips (Ø 18mm, width 1.5, Menzel), fixed (Roti-Histofix 4%, Carl Roth) at RT for 20 min, and blocked with stem cell blocking solution (Table X) at 4 °C overnight. Cells were incubated with primary antibodies against OCT4, NANOG, and TRA-1-60 (Major Resources Table) diluted in stem cell blocking solution (Table X) at 4 °C overnight, washed thrice with stem cell blocking solution, and finally incubated with secondary antibodies in stem cell blocking solution at RT for 1 h. For nuclear or cytosolic proteins, cells were permeabilized with 0.1 % Triton-X100 (Triton-X100, Carl Roth) in 1 % BSA (BSA; Sigma-Aldrich) in PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco). Nuclei were stained with 4.8 µmol/L DAPI (DAPI solution, Thermo Fisher Scientific) at RT for 10 min. Samples were mounted (Fluoromount-G, Thermo Fisher Scientific) and images collected by light microscopy (Axio Imager M2 microscopy system, Zen 2.3 software, Carl Zeiss) (Figure VID).

For flow cytometry, cells were manually agitated with a 10 mL glass pipette (Serological Pipette 10 mL, Sarstedt), fixed (Roti-Histofix 4%, Carl Roth) at RT for 20 min, and blocked with stem cell blocking solution (Table X) at 4 °C for at least 2 h. iPSCs were permeabilized with 0.1% Triton-X100 (Triton-X100, Carl Roth) in stem cell blocking solution, and co-incubated with fluorescence-conjugated antibodies against OCT4 and TRA-1-60 at RT for 1 h (Figure VIC; Major Resources Table). Nuclei were co-stained with 8.1 µmol/L Hoechst (Hoechst 33342, Thermo Fisher Scientific). Subsequently, cells were analyzed using the flow cytometry (LSRII, BD Biosciences) using BD FACSDiva software (BD Biosciences). Gating of cells was applied based on forward scatter area (FSC-A) and sideward scatter area (SSC-A) as well as on gating of single cells based on DNA signal width. At least 10,000 events were analyzed per sample.

iPSC-Cardiomyocyte 3-Bromopyruvate (3-BP) Uptake and Cell Viability Assay

Differentiated iPSC-CMs cultured for 30 days from the WT and CAV3 KO iPSC lines were digested with 0.25% trypsin (Trypsin/EDTA, Thermo Fisher Scientific) and 1 million iPSC-CMs quantified by cell counter (CASY, OMNI Life Science). iPSC-CMs were seeded on Matrigel-coated (growth factor reduced, BD Biosciences) 35 mm dishes (CELLSTAR 6-well plate, Greiner). WT and CAV3 KO iPSC-CMs were cultured in cardio culture medium (Table IX) until day 60 and the cardio culture medium exchanged by the same medium containing 50 µmol/L of the glycolysis disrupting McT1-specific substrate 3-bromopyruvate (3-BP). After incubation for 3 h in 5% CO_2 and 21% O_2 at 37 °C (Heracell VIOS, Thermo Fisher Scientific) the culture medium was collected, and cell death of iPSC-CMs c assessed by release of lactate dehydrogenase (LDH) into the media quantified by a coupled enzymatic reaction (LDH Cytotoxicity Assay Kit, Thermo Fisher Scientific). For this purpose, 50 µL culture media were transferred into a 96-well plate (Cellstar 96 well plates, Greiner) for triplicate LDL measurements, and the enzymatic reaction initiated by adding 50 µL reaction mixture (LDH Cytotoxicity Assay Kit, Thermo Fisher Scientific). After 30 min incubation, the absorbance was measured at 490 nm

and 680 nm (Spark 10M, Tecan), and LDH release calculated according to the manufacturer's instructions (LDH Cytotoxicity Assay Kit, Thermo Fisher Scientific) (Figure 5C).

iPSC-Cardiomyocyte Cell Surface Biotinylation and Elution of Biotinylated Surface Membrane Proteins

60 day cultured human iPSC-CMs were washed thrice with 500 µL PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco). For cell surface biotinylation, iPSC-CMs were incubated for 1 h at 4 °C with 2 mmol/L tagging solution (EZ-Link Sulfo-NHS-Biotin, Thermo Fisher Scientific) or PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco), the latter as negative control. Cell surface biotinylation was quenched after 1 h by adding 100 µmol/L of 1 mol/L Tris (pH 7.5) to the tagging solution and following incubating for 5 min at RT. The iPSC-CMs were washed twice with ice cold PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco), scraped (Cell Scraper 25 cm, Sarstedt) in 250 µmol/L ice cold PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco), and centrifuged at 13,000 x g for 1 min. The pellet was resuspended in 500 µL RIPA buffer (Table IV) and homogenized by 20 strokes on ice using a Potter homogenizer (RW20 digital, IKA). The homogenate was centrifuged at 10,000 x g for 10 min at 4 °C to remove insoluble contents and the protein concentrations determined by absorption measurement (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific). Biotinylated surface proteins were precipitated by adding 40 µg avidin beads to 500 µg lysate for 1 h at 4 °C in a spin column (Pierce Spin Columns Screw Cap, Thermo Fisher Scientific). Next, beads were washed thrice with 500 µL RIPA buffer. Two centrifugation steps at 100 x g for 30 s and 2 min at 2000 x g (Heraeus, Fresco 21 centrifuge, Thermo Fisher Scientific) were used to harvest the beads and the supernatant was discarded. Biotinylated proteins were eluted in 100 µL 2 x SDS buffer containing β-mercaptoethanol (Table IV). For Immunoblotting, 15 µg of input and 15 µL eluate sample were loaded onto a 4-20% Tris-Glycine gradient protein gel (Novex 4-20% Tris-Glycine, Thermo Fisher Scientific). For SDS gel electrophoresis and protein transfer see **Immunoblotting and Streptavidin Blotting for Protein Analysis**. Immunoblotting (Figure 5A and 5B) was performed with McT1 and β-actin antibodies (Major Resources Table).

iPSC-Cardiomyocyte Seahorse Studies

Initially, iPSC-CMs were cultured for 7 days in cardio culture medium (Table IX) to form a confluent synchronously beating monolayer. After 60 days of culture, iPSC-CMs were prepared for metabolic studies by exchanging the medium for the Seahorse XF assay buffer (Table XI). To determine the respiratory capacity, 20,000 iPSC-CMs were seeded per Matrigel-coated well (growth factor reduced, BD Biosciences) using a Seahorse 96-well plate (XF96 cell culture microplate, Agilent). The oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) were measured with a Seahorse Extracellular Flux Analyzer (XF96, Seahorse Bioscience). Periodic measurements of OCR and ECAR were repeated under basal conditions and after inhibition of the ATP synthase (Oligomycin, 3 µmol/L, Sigma Aldrich), after mitochondrial oxidative phosphorylation uncoupling with FCCP (carbonyl cyanide-*4*-(trifluoromethoxy)phenylhydrazone), 1 µmol/L, Sigma Aldrich), and finally after complex-I/complex-III inhibition (rotenone, 2 µmol/L, and antimycin A, 1 µmol/L, Sigma Aldrich) according to a previously published protocol (Figure 5D and 5E). Mitochondrial and glycolytic ATP levels were calculated based on experimentally determined OCR and ECAR values according to the

manufacturer's protocol (Quantifying Cellular ATP Production Rate Using Agilent Seahorse XF Technology, Agilent).

iPSC-Cardiomyocyte Optical Recording of Spontaneous Action Potentials and Early Afterdepolarizations

Monolayer cultured iPSC-CMs were trysinized and re-plated on 1 mm glass coverslips at a density of 15,000 cells per coverslip. Between days 60 and 70 post-differentiation, cells were loaded for 20 minutes at 37 °C with the voltage sensitive probe FluoVolt (VoltageFluor2.1Cl Voltage Sensitive Dye Kit, Thermofisher Scientific). For dye loading we added 3 µL from an aliquot of 1:10 diluted dye to the pluronic 'PowerLoad' solution (supplied as kit), and 3 mL Tyrode solution containing in mmol/L: 140 NaCl, 10 HEPES, 10 Glucose, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 2 Probenecid, pH 7.35. Optical action potentials (APs) were recorded from individually masked cells on the heated stage of an epifluorescence microscope (λEx=470 nm, λEm=535 nm) optimized for high speed signal capture with a photomultiplier. Signals were digitized at 10 kHz. Time of excitation light exposure was limited to 20 s long periods. Field stimulation was used for AP standardization (5 ms, 20-30 V, 0.5 Hz). 3 APs from each cell were ensemble averaged for temporal analysis. Analysis of time to peak and AP duration (APD) were conducted offline using Clampfit 10.7 (Molecular Devices).

CAV1 Knock-out Mouse Ventricular Cardiomyocytes

All animal procedures were reviewed by the by the institutional animal care and use committee of the University Medical Center Göttingen and the veterinarian state authority (LAVES, Oldenburg, Germany; 33.9-42502-04-18/2975). CAV1 KO mice were purchased from The Jackson Laboratory (B6.Cg-*Cav1[tm1MLs]*/J, 007083) and back-crossed into the C57BL/6N background. We harvested the hearts from adult mice of 12-14 weeks age and both genders. Mice were anesthetized with 3% isoflurane, euthanized, and the heart quickly extracted following IACUC approved procedures (T2/11). For ventricular tissue preparation for biochemical analysis, intact mouse hearts were perfused as detailed in the next chapter for 2 min to clear blood cells. The ventricular tissue was dissected under a binocular microscope (Stemi 305, Zeiss), snap-frozen in liquid N2, and stored at -80 °C until further use for analysis.

Adult Mouse Ventricular Cardiomyocyte Isolation

We used a customized, published protocol for isolation of adult ventricular cardiomyocytes.[66] The proximal aorta was tied to a 21 gauge cannula and connected to a modified Langendorff perfusion setup.[67] Mouse hearts were perfused at a constant flow rate of 4 mL/min with a nominally Ca^{2+} free perfusion buffer (Table VII) for 4 min at 37 °C, followed by a collagenase containing buffer (Table VII) for 9 min at 37 °C. The ventricles were dissected *using* a binocular microscope (Stemi 305, Zeiss) in 2 mL digestion buffer, followed by adding 3 mL stopping buffer to terminate collagenase activity (Table VII). Isolated ventricular cardiomyocytes were washed twice with the stopping buffer, cells sedimented for 8 min by gravity at RT, the supernatant discarded, and the cells resuspended. Cell quality was documented by transmitted light imaging (Zeiss LSM 710 or 880, Jena, Germany) using Fiji (https://imagej.net/Fiji) and based on criteria published previously.[67]

Immunofluorescence Confocal Microscopy and Superresolution STED Nanoscopy

Isolated cardiomyocytes were plated on glass coverslips (diameter 18 mm, width 1.5 mm, Menzel) coated with laminin (2 mg/mL) at a dilution of 1:10 in perfusion buffer (Table VII). Cardiomyocytes were fixed with 4% paraformaldehyde (PFA, Sigma-Aldrich) for 5 min at RT followed by three PBS washing steps (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco). Fixed samples were incubated overnight at 4 °C in blocking/permeabilization buffer followed by incubation with the primary antibodies (Major Resources Table) diluted in blocking buffer overnight at 4 °C. After washing thrice with blocking buffer, samples were incubated with secondary antibodies diluted 1:1000 overnight at 4 °C. For confocal immunofluorescence microscopy, Alexa Fluor 633 and Alexa Fluor 514 conjugated antibodies were used (Major Resources Table). For confocal and STED microscopy, STAR 635P and STAR 580 conjugated antibodies were used (Major Resources Table). After washing thrice with PBS, samples were embedded in mounting medium with DAPI (ProLong Gold Antifade Mountant with DAPI, Thermo Fisher Scientific) for confocal microscopy or DAPI-free mounting medium (ProLong Gold Antifade Mountant, Thermo Fisher Scientific) for STED nanoscopy. Embedded samples were stored overnight at RT and imaged the next day.

Confocal images were acquired with a Zeiss LSM 710 microscope using a Plan-Apochromat 63x/1.40 oil objective and a pixel size of 50 x 50 nm. Alexa Fluor 514 was excited at 514 nm and detected at 520–620 nm. AlexaFluor 633 was excited at 633 nm and detected at 640–740 nm. The confocal laser power was adjusted to maximize resolution following established workflows.[67]

For combined confocal microscopy and STED nanoscopy a Leica TCS SP8 system with a HC PL APO C2S 100x/1.40 oil objective and a pixel size 16.23 x 16.23 nm has been used. STAR 635P was excited at 635 nm and detected at 650-700 nm. STAR 580 was excited at 580 nm and detected at 600-630 nm. For STED depletion of STAR 580 and STAR 635P a 775 nm laser beam was used. The STED laser power was adjusted to maximize resolution following previously established workflows.[67] Raw images were processed in Fiji (https://imagej.net/Fiji) following established protocols.[67]

Patch-Clamp Recording of Peak and Late Na^+ Currents in 2 Months Matured iPSC-CMs

From day 60 post-differentiation, sodium currents including peak I_{Na} and late $I_{Na,L}$ in iPSC-CMs were measured by voltage-clamp in the whole-cell ruptured-patch configuration at RT.[68, 69] Bath solution for I_{Na} measurements contained in mmol/L: NaCl 5, HEPES 10, $MgCl_2$ 1, CsCl 10, glucose 10, $CaCl_2$ 0.5, and TEA-Cl 120 (pH 7.4 adjusted with CsOH). Bath solution for $I_{Na,L}$ measurements contained in mmol/L: NaCl 120, HEPES 10, $MgCl_2$ 1, CsCl 10, glucose 10, and $CaCl_2$ 0.5 (pH 7.4 adjusted with CsOH). Microelectrodes (3-5 MΩ) were filled with a solution containing in mmol/L: NaCl 5, cesium methanesulfonate 90, CsCl 20, HEPES 10, Mg-ATP 4, Tris-GTP 0.4, EGTA 10, and $CaCl_2$ 3 (pH 7.2, adjusted with CsOH). Current measurements occurred in the presence of 1 µmol/L nifedipine to block L-type Ca^{2+} currents. For current-voltage (IV) curve measurements, cells were held at -80 mV, and a 1000 ms pre-pulse at -110 mV preceded 30 ms long voltage steps from -80 to +20 mV. For $I_{Na,L}$ measurements cells were held at -120 mV, followed by a step to -80 mV, and a 5 ms activating step to +50 mV prior to a 250 ms step to -30 mV.[70] 10 µmol/L tetrodotoxin (TTX) was applied to inhibit $I_{Na,L}$, and subtracted from the current without TTX as readout of the TTX-sensitive sodium current following previously

established protocols. The calculated current was integrated from 50 ms to 250 ms after the depolarising pulse at -30 mV.

Patch-Clamp recording of Na,K-ATPase Pump Currents in 2 Months Matured iPSC-CMs

Monolayer cultured iPSC-CMs were trypsinized and re-plated on 1 mm glass coverslips at a density of 15.000 cells/per coverslip. Cells were washed 4 times with the standard bath solution before patch-clamp recording. Ruptured whole-cell patch-clamp recording was performed in iPSC-CMs between days 60 and 70 post-differentiation at RT following protocols published previously.[71,72] The resistance of the patch pipettes ranged between 2 to 4 GΩ. The solutions applied for Na,K-ATPase pump current measurements are designed to minimize other confounding membrane currents (Table XII). Measurements were started at least 60 s after rupture of the cell membrane to allow for equilibration and stabilization between the intracellular and pipette solutions. Membrane currents were continuously recorded at a holding potential of 0 mV using 240 ms long sweep episodes at 1 Hz and a sampling frequency of 1 kHz. Membrane capacitance was measured by imposing a 20 ms voltage square step from 0 to 10 mV and by integrating the area under the capacitance transient.

Computational Modeling of hiPSC-CMs

To investigate the contribution of $I_{Na,L}$ on the generation of EADs, a multiscale computational study was performed using the Kernik human hiPSC-CM model (Major Resources Table).[17] Both the cellular and tissue-level simulations were done in Myokit.[73] The maximal conductance of I_{Kr} was decreased to reduce repolarization reserve and $I_{Ca,L}$ inactivation was reduced (Table XIII) to enable EAD generation in the iPSC-CM model, consistent with impaired local $I_{Ca,L}$ regulation in the absence of CAV3 reported previously.[35,36] Moreover, to reproduce the CAV3 KO phenotype measured in iPSC-CMs, the experimentally observed upregulation of $I_{Na,L}$ was incorporated (Figure VIII).

For the cellular simulations, steady-state action potentials were recorded for 10 s following a stabilization period of 200 s in the absence of pacing stimuli to capture spontaneous activities in the control WT model or the EAD-prone model with or without upregulation of $I_{Na,L}$. A hyperpolarizing current of 0.1 µA/µF was injected into the model cell to approximate the experimentally observed spontaneous beating rate.

For the two-dimensional tissue simulations, an 8x8 cm (400x400 units) virtual tissue was created. A 0.3 µA/µF hyperpolarizing current was applied to the cells to fully suppress the automaticity of the hiPSC-CMs in the model, and the biological-experimentally observed $I_{Na,L}$ upregulation was incorporated in the CAV3 KO model. An S1 stimulus of 80 µA/µF was applied to the left-hand side of the tissue, producing a propagating wave towards the right side of the tissue. As shown in Figure 6F, an EAD-prone region (100x250 units) was introduced in the center of the tissue, surrounded by an EAD-resistant region. To further investigate the impact of the size, location and interaction of multiple EAD-prone regions in the proarrhythmic response, a heterogenous virtual tissue with multiple EAD-prone regions was simulated (Figure IX and X). All models are freely accessible at: www.github.com/jordiheijman.

Cross-Linking of Native Protein Complexes in Living Mouse Ventricular Cardiomyocytes

Ventricular cardiomyocytes were isolated from adult WT mouse hearts as described in **Adult Mouse Ventricular Cardiomyocyte Isolation**. For cross-linking, a 50 mmol/L stock solution of disuccinimidyl suberate (DSS, Thermo Fisher Scientific) was prepared by dissolving 2 mg DSS in 108 µL dimethyl sulfoxide (DMSO, Thermo Fisher Scientific) and isolated ventricular cardiomyocytes isolated from individual hearts were incubated at the final concentration of 0.1 mmol/L DSS or the carrier solution as control for 1 h at 4 °C. Cross-linking was quenched by adding 50 µL of 1 mol/L Tris (pH 7.5) for 5 min at RT. The isolated ventricular cardiomyocytes were centrifuged at 500 x g for 5 min and washed thrice with ice-cold PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco). The pellet was resuspended in 1 mL homogenization buffer (Table VI). Cells were homogenized at 4 °C by 50 strokes on ice using a Potter homogenizer (RW20 digital, IKA). Homogenates were centrifuged at 1,000 x g for 10 min at 4 °C to remove cell debris. The cleared supernatant was centrifuged at 100,000 x g for 1 h (Optima Max-XP, MLA-150 rotor, Beckman) to enrich the membrane fraction, resuspended in 30 µL solubilization buffer (Table VI), snap-frozen and stored at -80 °C. Solubilized membranes were thawed on ice and the protein concentration determined by absorption measurement (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific). Digitonin (Digitonin, Sigma Aldrich) was added as detergent (6 g digitonin/g protein) and insoluble membranes were removed by centrifugation at 13,000 x g for 10 min at 4 °C. The cleared supernatant was mixed 1:10 with a Coomassie blue solution (Coomassie Brilliant Blue R-250, 5% w/v, Sigma Aldrich) and a glycerol solution (Glycerol, 50% w/v, Sigma Aldrich). Anode/cathode buffers were prepared according to the manufacturer's instructions (NativePAGE Bis-Tris Mini Gel Electrophoresis Protocol, Thermo Fisher Scientific). For BN-Page, 50 µg of solubilized membrane proteins were separated on a 3-12% Bis-Tris gradient gel (NativePAGE 3-12% Bis-Tris Gel, Thermo Fisher Scientific) at constant 150 V for 1 h, followed by replacing the cathode buffer (Dark Blue Cathode Buffer, Novex) to cathode buffer light (Light Blue Cathode Buffer, Novex) and electrophoresis at constant 250 V for 1 h. For mass spectrometric analysis, the gel was cut into 23 equal-sized gel pieces and each individually processed using the workflow described below.

Sample Preparation for Complexome Profiling by NanoLC-MS/MS

Gel pieces obtained by BN-PAGE were washed with 50 mmol/L ammonium bicarbonate (TEAB, Sigma-Aldrich), reduced with 10 mmol/L dithiothreitol (1,4-dithiothreitol, Sigma-Aldrich), alkylated with 55 mmol/L iodoacetamide (2-iodoacetamide, Sigma-Aldrich), and digested with endopeptidase trypsin (sequencing grade, Promega) diluted 1:50 in 55 mmol/L iodoacetamide overnight. Post-trypsin peptides were solubilized in MS loading buffer (Table VI), dried (SpeedVac, Thermo Fisher Scientific), reconstituted in MS loading buffer and prepared for NanoLC-MS/MS analysis as described previously.[58]

NanoLC-MS/MS Analysis for Complexome Profiling

For mass spectrometric analysis of solubilized trypsin peptides, samples were enriched on a self-packed reversed phase-C18 precolumn (0.15 mm ID x 20 mm, Reprosil-Pur120 C18-AQ 5 µm, Dr. Maisch, Ammerbuch-Entringen, Germany) and separated on an analytical reversed phase-C18 column (0.075 mm ID x 200 mm, Reprosil-Pur 120 C18-AQ, 3 µm, Dr. Maisch, Ammerbuch-Entringen, Germany) using a 30 min linear gradient of 5-35% acetonitrile/0.1%

formic acid (v/v) at 300 nL min⁻¹. The eluent was analyzed on a hybrid quadrupole/orbitrap mass spectrometer (Q Exactive Plus, Thermo Fisher Scientific) equipped with a FlexIon nanoSpray source and operated under Excalibur 2.5 software using a data-dependent acquisition method. Each experimental cycle was of the following form: one full MS scan across the 350-1600 *m/z* range was acquired at a resolution setting of 70,000 FWHM, and AGC target of 1*10e6 and a maximum fill time of 60 ms. Up to the 15 most abundant peptide precursors of charge states 2 to 5 above a 2*10e4 intensity threshold were then sequentially isolated at 2.0 FWHM isolation width, fragmented with nitrogen at a normalized collision energy setting of 25%, and the resulting product ion spectra recorded at a resolution setting of 17,500 FWHM, and AGC target of 2*10e5 and a maximum fill time of 60 ms. Selected precursor *m/z* values were then excluded for the following 15 s. Two technical replicates per sample were acquired.

Data Processing for Complexome Profiling

Raw data were processed using MaxQuant Software version 1.6.5.0 (Max Planck Institute for Biochemistry). Proteins were identified against a UniProtKB-derived mouse protein sequence database (v2019.02, 61291 protein entries) along with a set of common lab contaminants. The search was performed with trypsin as enzyme and iodoacetamide as cysteine blocking agent. Up to two missed tryptic cleavages and methionine oxidation as a variable modification were allowed for. Instrument type 'Orbitrap' was selected to adjust for MS acquisition specifics, and the LFQ algorithm using default parameters employed for peptide and protein quantitation. See dataset for mass spectrometry results (Excel Table IV). The ass spectrometry proteomic data have been deposited to the ProteomeXchange Consortium[59] (http://www.ebi.ac.uk/pride) with the dataset identifier PXD019380 (Major Resources Table).

High-Pressure Freezing and Electron Tomography

High-pressure freezing was performed at the EMBL Heidelberg electron microscopy core facility according to published protocols.[74] Isolated mouse ventricular cardiomyocytes were placed in 200 µm aluminium type A specimen carriers coupled with type B lids (HPF carrier, Leica) and the specimens were rapidly frozen (HPM100, Leica). Specimens were freeze substituted for 24 h in 1% OsO4 in acetone (AFS2, Leica), dehydrated in graded acetone and embedded (Epon-Araldite resin, EMS). Semi-thick (280 nm) sections were placed on formvar-coated slot-grids (TEM Grids, Science Services), post stained with 2% aqueous uranyl acetate (2% Uranyl Acetate Solution, Science Services) and Reynold's lead citrate (Table VIII). Colloidal gold particles (Gold nanoparticles 15 nm, Sigma Aldrich) were added to both surfaces of the sections to serve as fiducial markers for tilt series alignment.

For imaging an intermediate voltage electron microscope (Tecnai TF30, FEI) was operated at 300 kV. Electron tomography was performed according to published protocols.[74] Images were captured on a 4K x 4K charge-coupled device camera (UltraScan, SerialEM software package; Gatan). For imaging, the specimen holder was tilted from +60 ° to -60 ° at 1 ° intervals. For dual-axis tilt series the specimen was then rotated by 90° in the X-Y plane, and another +60 ° to -60 ° tilt series was taken. The images from each tilt-series were aligned by fiducial marker tracking and back-projected to generate two single full-thickness reconstructed volumes (tomograms), which were then combined to generate a single high-resolution 3D reconstruction of the original partial cell volume.[75] Isotropic voxel size ranged from 0.765-1.206

nm. In some instances, tomograms were computed from montaged stacks, to increase the total reconstructed area to up to 10 µm x 10 µm in XY. Biologically meaningful resolution was approximately 4 nm in X-Y. All tomograms were processed and analyzed using IMOD software,[76] which was also used to generate 3D models of relevant structures of interest.[77] Models were smoothed and meshed to obtain the final 3D representation, in which spatial relations between caveolar and mitochondrial structures were quantified.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM) unless indicated otherwise. Unpaired 2-tailed Student's t-test or 1-way-ANOVA was applied as specified in the figure legends. Mass spectrometry data were found to be normally distributed in logarithmic space. A p value <0.05 was considered as statistically significant.

SUPPLEMENTAL FIGURES AND FIGURE LEGENDS
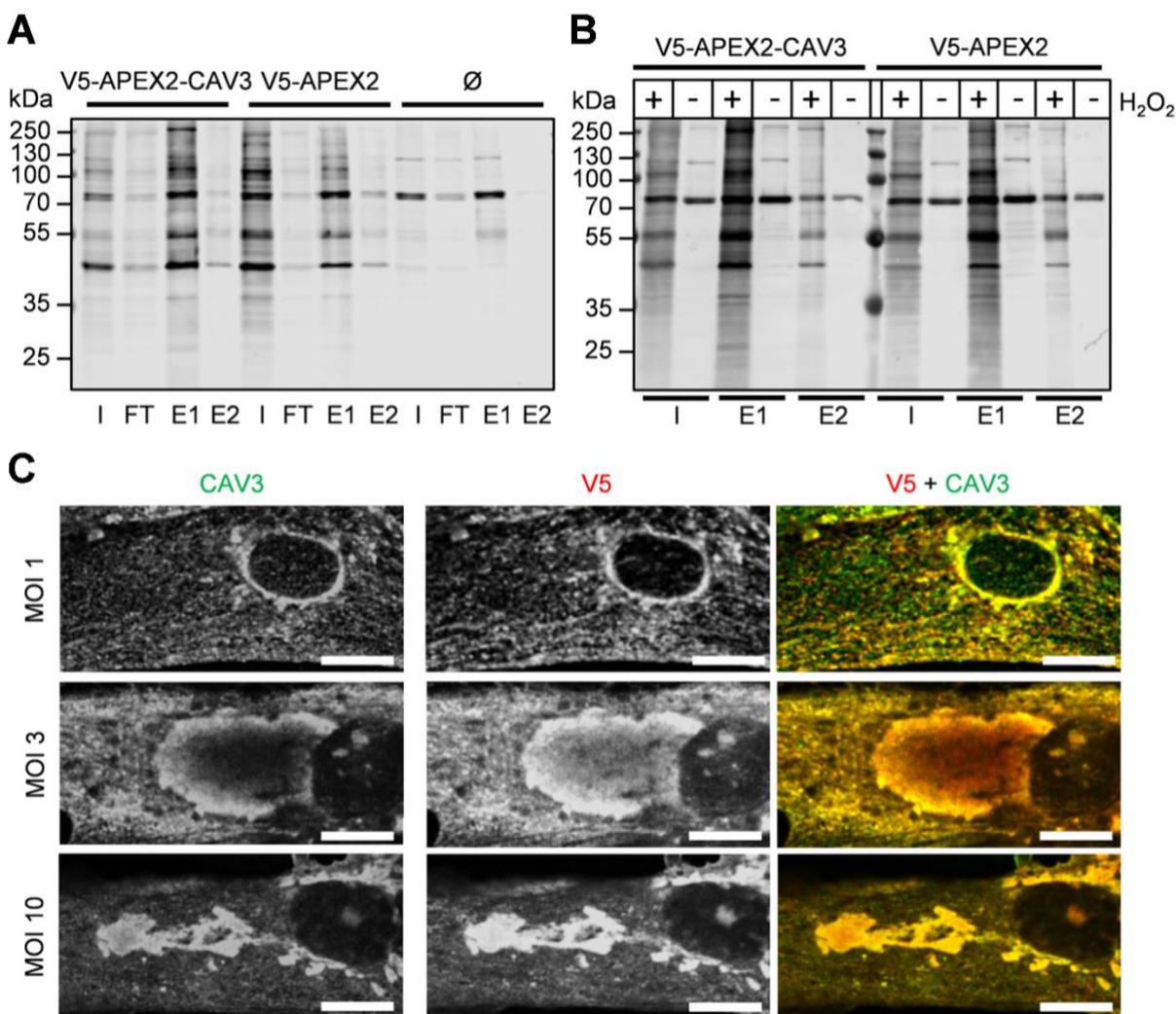
Online Figure I. CAV3 proximity labeling in living neonatal rat cardiomyocytes.
A, APEX2 proximity labeling in V5-APEX2-CAV3 versus V5-APEX2 transfected or untransfected (Ø) neonatal rat cardiomyocytes (NRCMs) as indicated. Biotinylated proteins were enriched by affinity purification and detected with streptavidin IRDye 680 RD. Darker signals in E1 lanes indicate proteins eluted by the biotin buffer (see Table II); subsequent E2 step with 2x SDS buffer excluding incomplete elution in E1 (note weaker signals in E2 compared to E1). Untransfected NRCMs (Ø) were used as negative control and document few signals from endogenously biotinylated proteins. I, input; FT, flow through; E, eluate; n=3. **B**, Biotinylation analyzed in V5-APEX2-CAV3 or V5-APEX2 transfected NRCMs after 1 min treatment versus omission of H_2O_2 confirming APEX2 biotinylation reactions. n=2. **C**, Confocal microscopy imaging showing immuno-labeled CAV3 and V5 signals following adenoviral transfection of NRCMs with V5-APEX2-CAV3 at MOI 1 through 10 as indicated. Of note, only the lowest MOI 1 resulted in subcellular V5-APEX2-CAV3 signals overlapping with physiological endogenous CAV3 signals. In contrast, the higher MOIs 3 and 10 resulted in large ectopic, sharply demarked signal regions indicative of Golgi aggregation potentially disrupting trafficking. Scale bars all images, 10 μm.
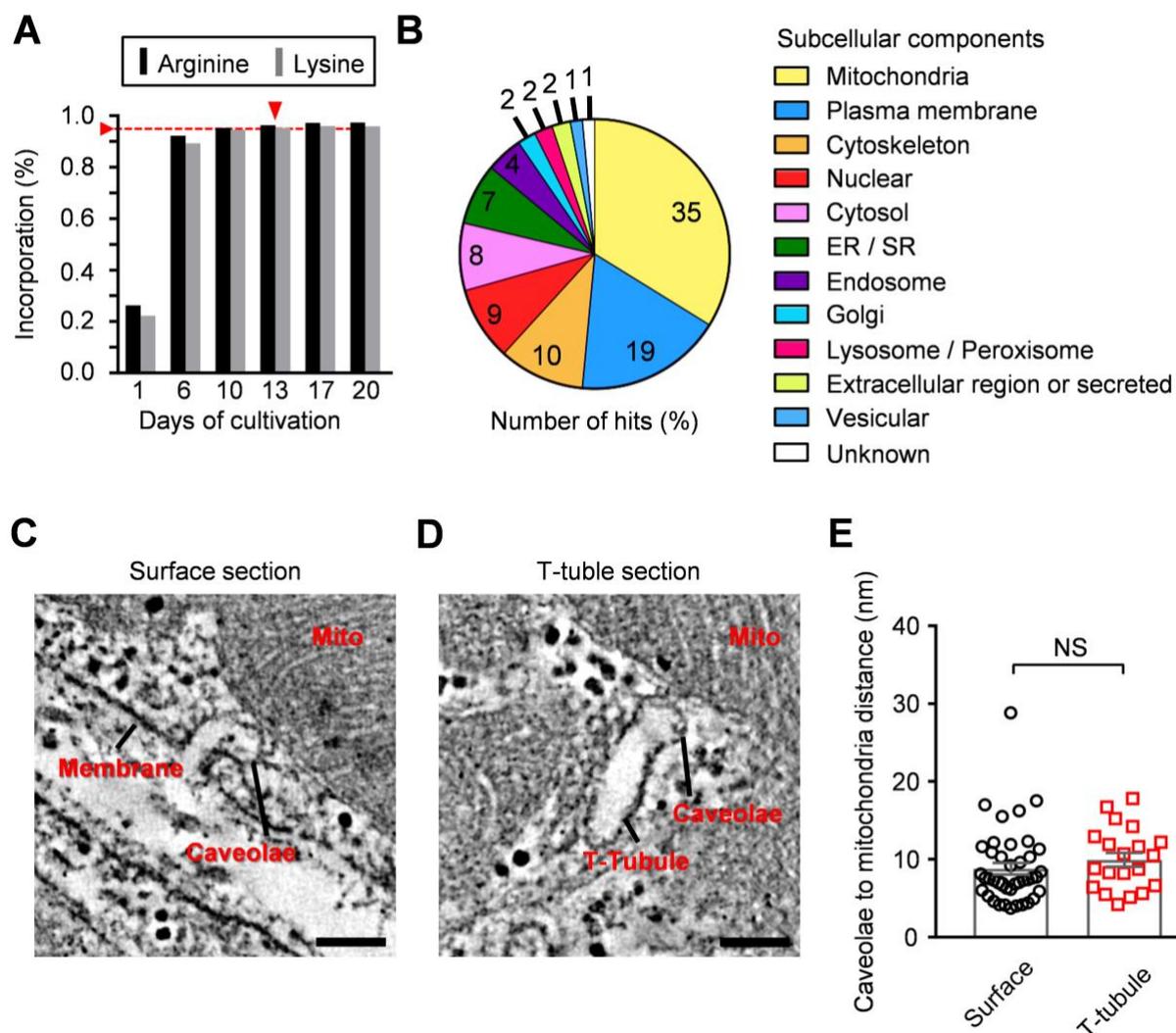
Online Figure II. SILAC incorporation, GO term analysis, and Electron Tomography.

A, Mass spectrometry (LC-MS/MS) analysis of heavy isotope-labeled ($^{13}C_6$,$^{15}N_4$-Arg and $^{13}C_6$,$^{15}N_2$-Lys), trypsin-digested NRCM cell lysates. SILAC incorporation reached ≥96% after 13 days of culture (red arrowhead). n=1. **B**, Pie chart summarizing the subcellular component classification of V5-APEX2-CAV3 enriched proteins based on Gene Ontology (GO) annotation. The number of identified hits and their relative percent area are indicated by color legend. **C-D**, Electron tomography images showing caveolae bulbs at the surface membrane (**C**), and at the transverse (T-)tubule membrane (**D**), and each in nanometric proximity to mitochondria. Scales, each 100 nm. **E**, Bar graph summarizing the caveolae to mitochondria distance at the outer surface membrane (8.8 nm) and at T-tubules (9.9 nm). t-test, Prism version 7.03; NS, not significant. Surface sections n=41, T-tubules n=20.

A

B
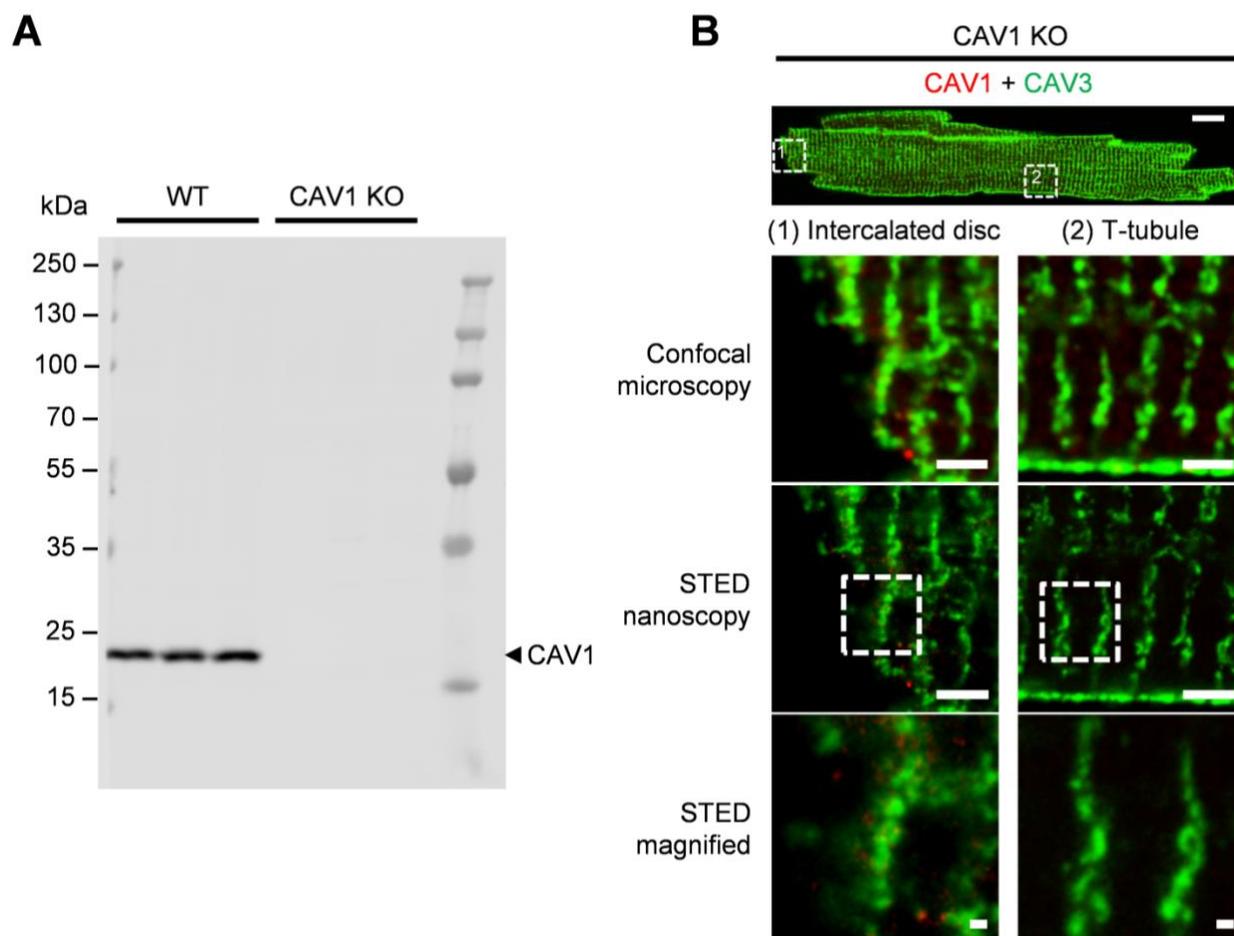
Online Figure III. CAV1 expression in isolated mouse ventricular cardiomyocytes.
A, Immunoblot (full gel) showing a single CAV1 band below 25 kDa in ventricular cardiomyocyte samples isolated from wild-type mouse hearts. In contrast, in ventricular cardiomyocytes from CAV1 knockout mouse hearts the CAV1 signal was completely absent confirming antibody specificity. n=3. **B**, Confocal and STED co-immunofluorescence images of a ventricular CAV1 knockout cardiomyocyte. Whereas the CAV1 staining resulted in few unspecific background signals at intercalated disc, punctate CAV1 signals were excluded at transverse (T-)tubule membrane structures stained by CAV3 excluding unspecific antibody binding at T-tubules. Dashed boxes indicate magnified regions each at the intercalated disk and T-tubules. Scale bars, top panels each 10 µm; middle panels each 2 µm; bottom panels each 200 nm.
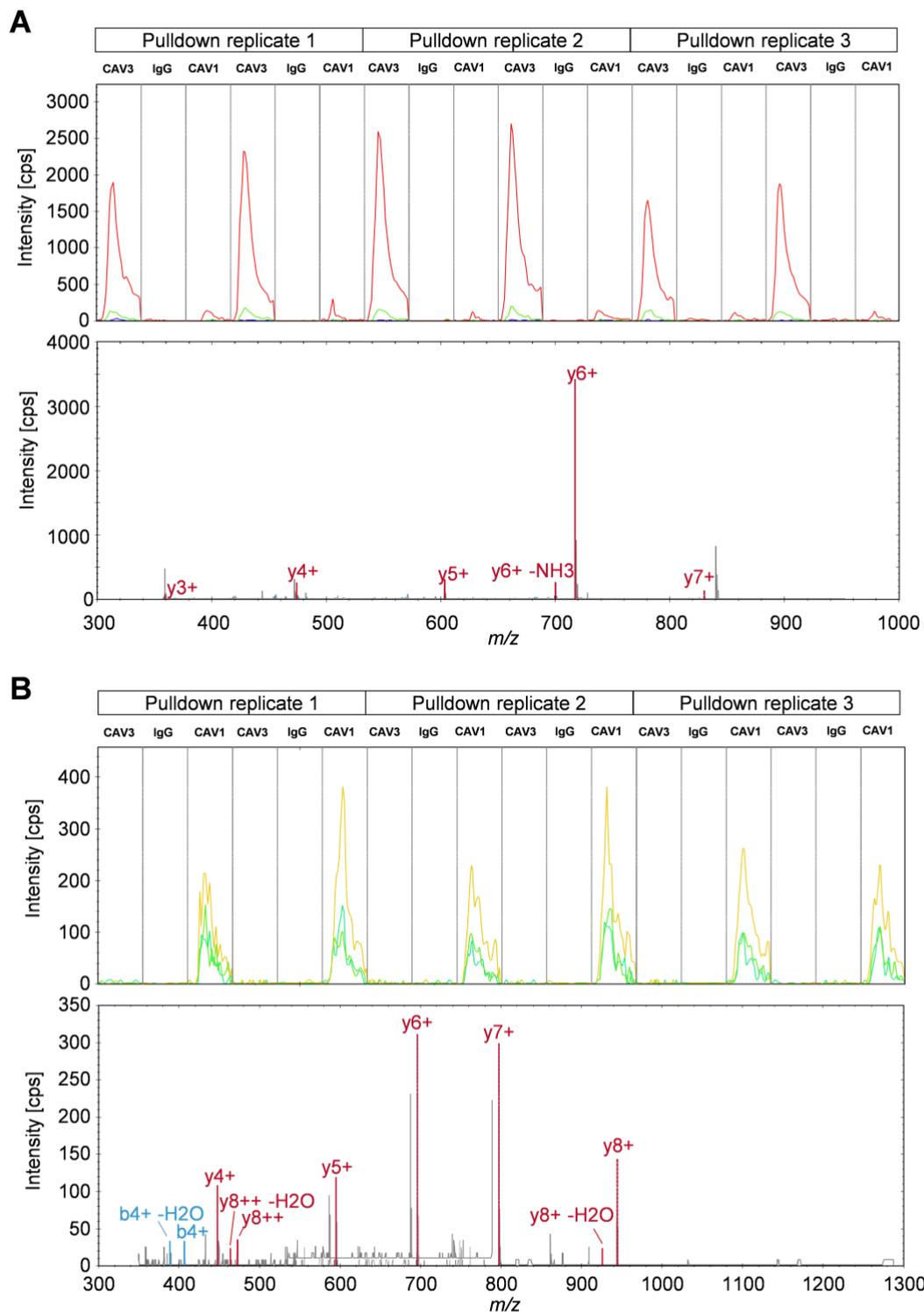
Online Figure IV. CAV1/CAV3 isoform-specific interactome analysis by DIA-MS.
A, NINEDIVK (*m*/z 472.7556^{2+}) from mouse CAV3 (P51637), precursor window *m*/z 469.2-477.8. *Top*: XICs for MS2 top3 interference-free fragments y6, y5, y3. *Bottom*: annotated DIA-MS/MS @ 33.0 min. **B**, ASFTTFTVTK (*m*/z 551.7927^{2+}) from CAV1 (P49817), precursor window *m*/z 550.2-557.5. *Top*: XICs for MS2 top3 interference-free fragments y7, y8, y5. *Bottom*: annotated DIA-MS/MS @ 41.0 min.
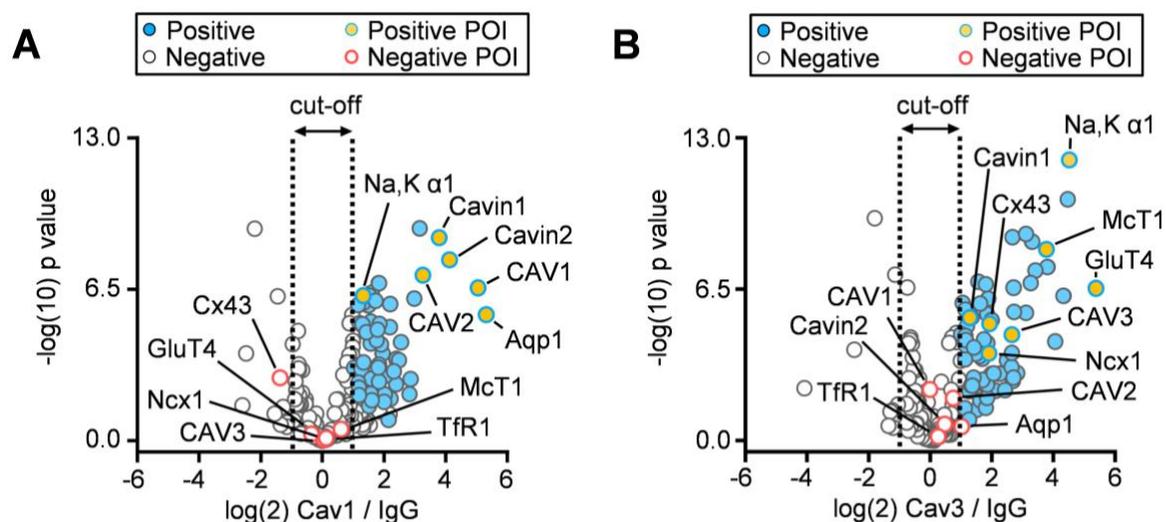
Online Figure V. CAV1 versus CAV3 protein interactions identified by AP-DIA-MS.
A-B, Volcano plots summarizing affinity-enriched CAV1 (**A**) and CAV3 (**B**) interactors identified by AP-DIA-MS. Significantly enriched proteins were identified by permutation-based false-discovery rate analysis (t-test, FDR=5%, S0=0.1, Perseus version 1.5.6.0) and logarithmic cut-off ≥1. n=3. Positive hits (blue circles), including a subgroup of positive hits with previously established proteins of interest (POI, yellow circles) functions, negative hits (white circles), and not enriched negative POI (red circles). Plotted are indicated in the legend (top).
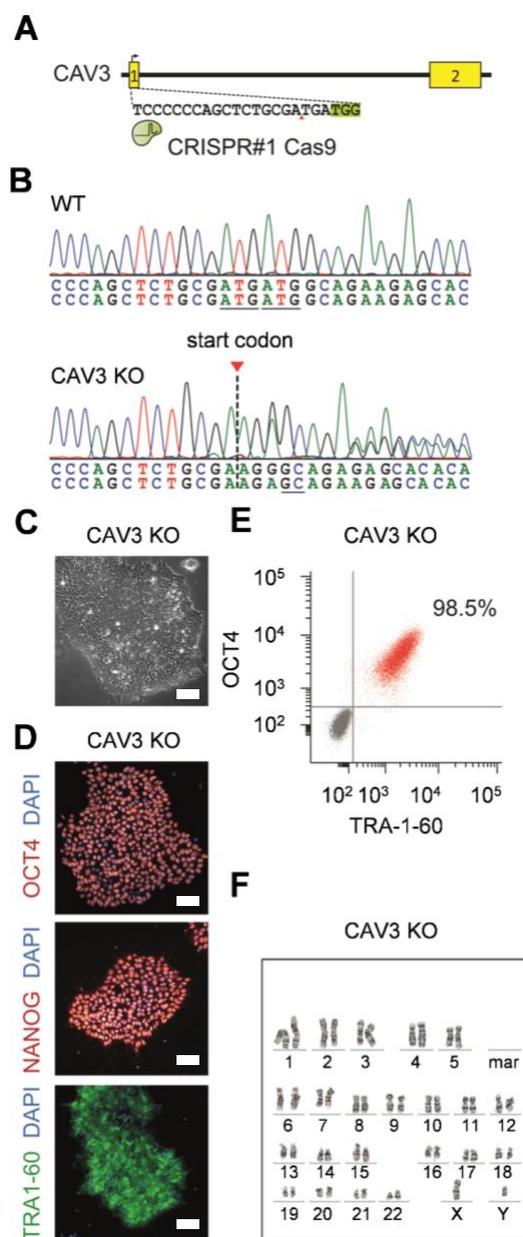
Online Figure VI. CRISPR/Cas9 mediated CAV3 knockout in human iPSC.
A, Human CAV3 knockout iPSCs were generated with a CRISPR guide RNA targeting the start codon of the CAV3 gene and a knockout (KO) clone with a disabled start codon on both alleles was selected for further analysis. **B**, Sanger sequencing of genomic DNA confirmed the deleted start codon in CAV3 KO iPSCs. **C**, Bright field imaging of CAV3 KO iPSCs documented typical human stem cell-like morphology and proliferation characteristics. Scale bar, 100 µm. **D**, Immunofluorescence staining of the key pluripotency markers OCT4, NANOG and TRA1-60 in CAV3 KO iPSCs. Nuclei were counter-stained with DAPI. Scale bar, 100 µm. **E**, Analysis of edited iPSC line by flow cytometry for the pluripotency markers OCT4 and TRA1-60 documenting 98.5% purity. Gray dots: negative controls. **H**, Karyotyping of the CAV3 KO iPSC line between passages 25-30 demonstrated chromosomal stability after CRISPR/Cas9-based genomic editing and passaging.
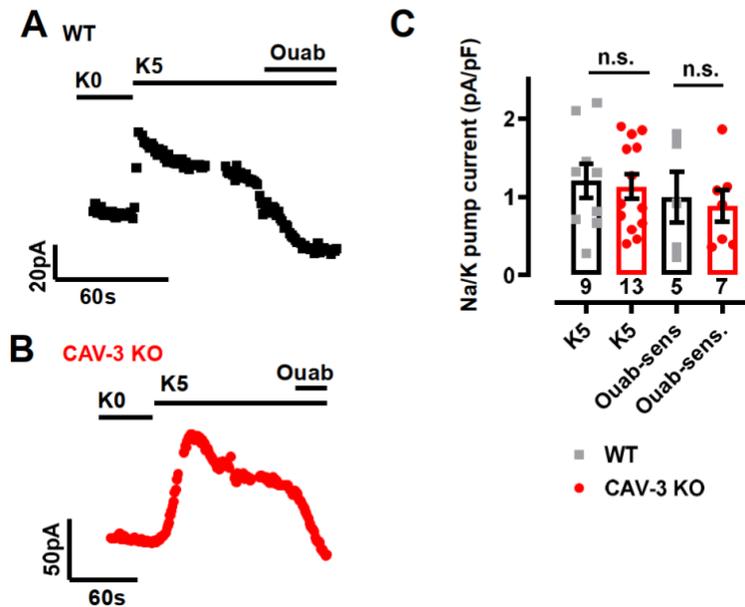
Online Figure VII. Na,K-ATPase pump current recording in human iPSC-CMs.
Human WT and CAV3 KO iPSC-CMs matured 2 months in culture followed by patch-clamp recording. For details please refer to the Detailed Methods and Table XII. **A-B**, Representative pump current time-course traces from WT (**A**) and CAV3 KO iPSC-CMs (**B**) at 0 mV holding potential. Data points represent membrane current values averaged sequentially over 100 ms at 1 kHz. Horizontal bars indicate extracellular solution changes starting with 0 mmol/L K$^+$ (K0), followed by a step increase to 5 mmol/L K$^+$ (K5) activating the Na,K-ATPase pump current, and finally 5 mmol/L K$^+$ plus 10 μmol/L ouabain (Ouab) to selectively inhibit and, thus, confirm Na,K-ATPase pump currents. **C**, Dot/bar blot summarizing off-line the calculated Na,K-ATPase pump current data for WT versus CAV3 KO iPSC-CMs (mean±SEM). Na,K-ATPase current changes were calculated as the difference in pump currents between K0 and K5 (*left*: K5); or as the difference in pump currents before/after K5±10 μmol/L ouabain (*right*: Ouab-sensitive). Numbers indicate individual cells from successfully completed experiments. These data have passed normality and log normality testing (D'Agostino & Pearson). Group comparison by Student's t-test; n.s., not significant.
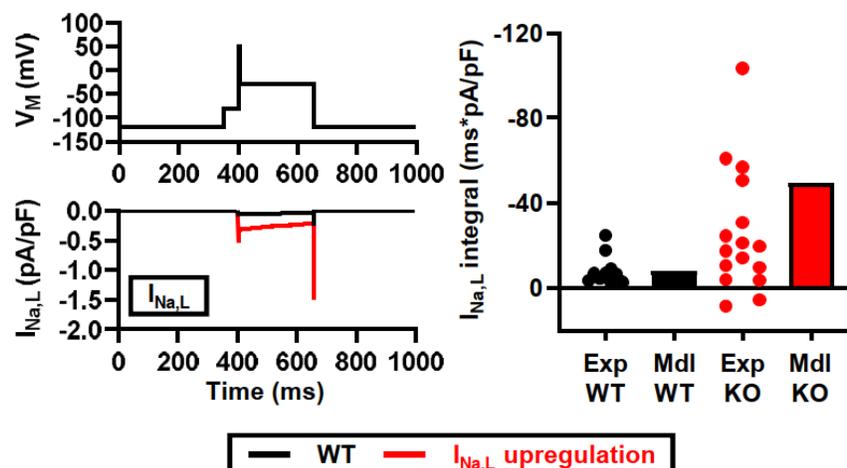
Online Figure VIII. Computational modeling of I_{Na,L} in human iPSC-CMs.

Left panels show the experimental voltage-clamp protocol applied to the iPSC-derived cardiomyocyte model (top) and the resulting I_{Na,L} currents (bottom) for the WT (black) versus the CAV3 KO model, the latter reproducing the I_{Na,L} increase (red). *Right* panel showing the simulated model (Mdl) integrated I_{Na,L} each in the WT and CAV3 KO models (black/red bars), and as validation compared to the experimental data measured by patch-clamp in human WT or CAV3 KO iPSC-CMs (filled circles; we note that the experimental data are reproduced from Figure 6G for comparison).
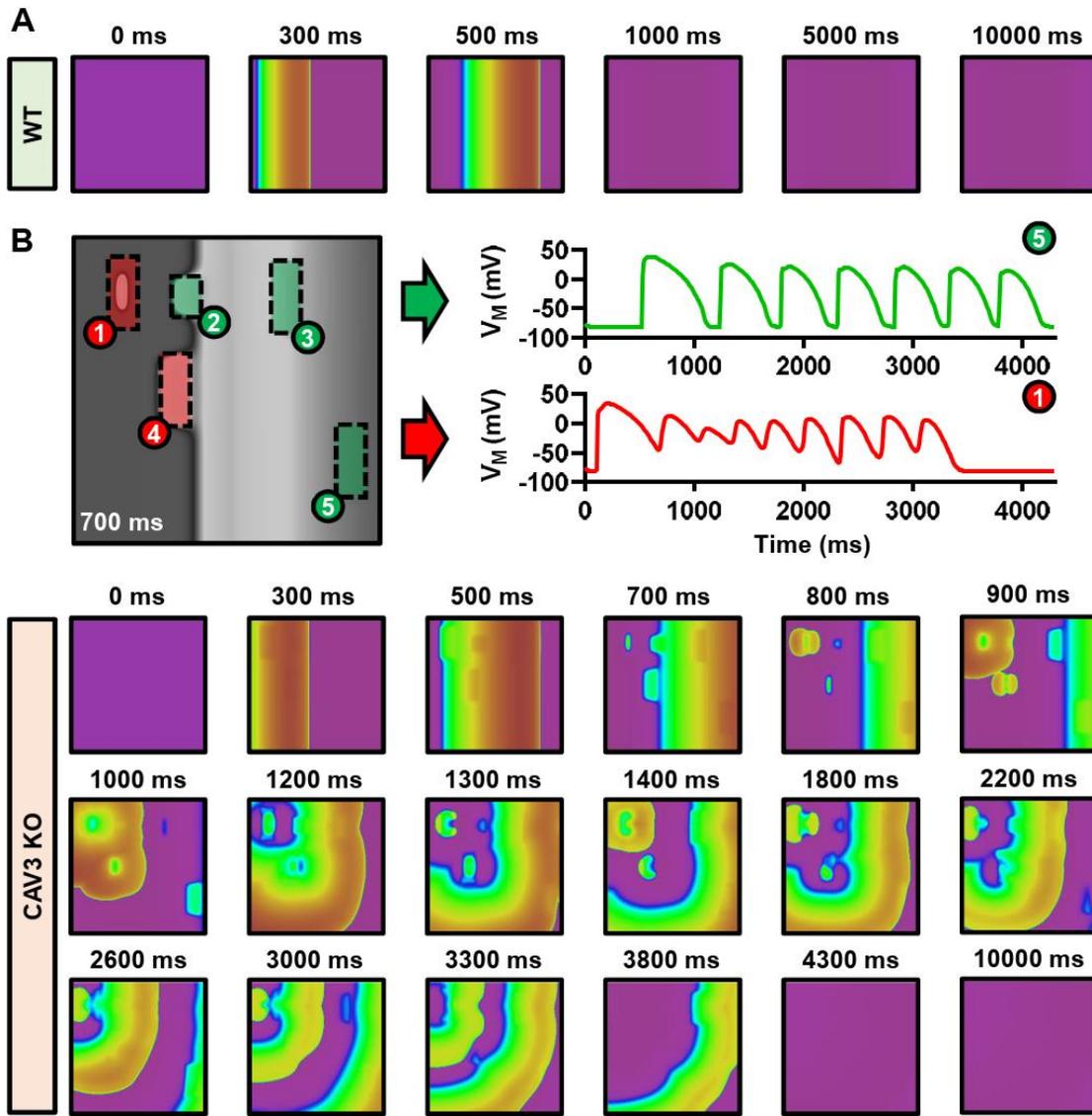
Online Figure IX. Computational modeling of $I_{Na,L}$ in two-dimensional tissue.
A, Snapshots of the membrane potential in the two-dimensional virtual tissue at the indicated time points following S1 pacing stimulation of the left-hand side of the tissue in the WT model. **B**, Two-dimensional virtual tissue simulations of the CAV3 KO model with 5 cluster regions of EAD-prone cells (marked 1 to 5 in the top-left panel) following S1 pacing stimulation of the left-hand side of the tissue. The traces from regions 1 and 5 (top-right panels) and snapshots of the membrane potential show repetitive focal activity originating from region 1. A movie of the simulation is available online (Video I).
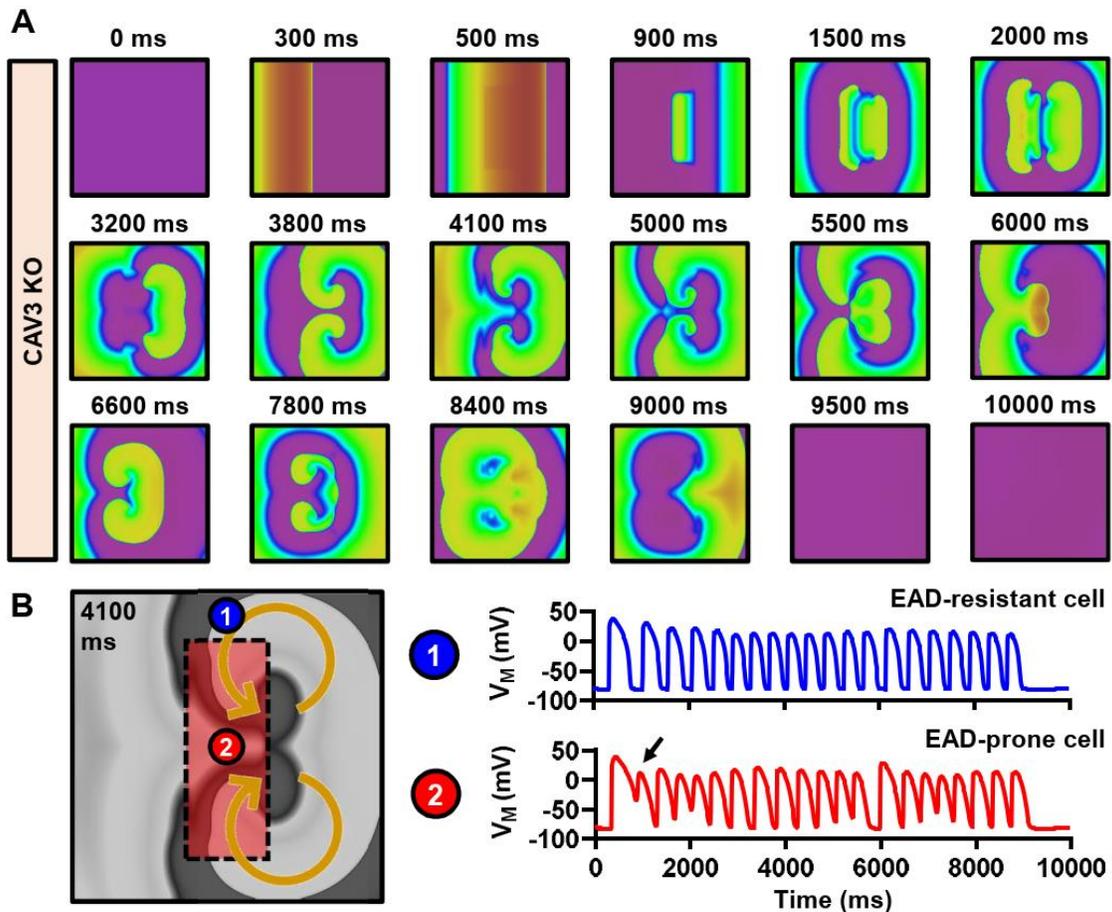
Online Figure X. Computational modeling figure-of-eight two-dimensional tissue reentry pattern.
Two-dimensional tissue simulations using the CAV3 KO iPSC-CM model with a central cluster of early afterdepolarization (EAD)-prone cells. **A**, Snapshots of the membrane potential in the two-dimensional virtual tissue at the indicated time points following S1 pacing stimulation of the left-hand side of the tissue, revealing ectopic activity emerging from the EAD-prone region that triggers a figure-of-eight reentry. The corresponding movie is available online as Video II. **B**, Schematic summary of the resultant reentry and action potential traces of the EAD-resistant (blue) and EAD-prone (red) regions, showing the initial EAD that triggered the reentry (arrow).
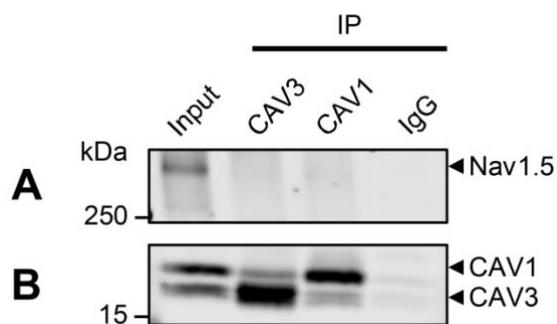
Online Figure XI. Na$_V$1.5 does not co-immunoprecipitate with CAV1 or CAV3.
A, Ventricular tissue lysates from adult WT mouse hearts were used for immunoprecipitation, and Na$_V$1.5 immunoblotting was performed after CAV1 or CAV3 immunoprecipitation using a CHAPS solubilization protocol. Input: immunoblot confirming Na$_V$1.5 in the input fraction. IP: immunoblot excluding Na$_V$1.5 detection at the physiological endogenous level in the mouse heart. Rabbit IgG was used as a negative control. n=3 independent experiments and mouse heart ventricular samples. **B**, Immunoblot confirming CAV3 as positive control each in the input fraction and following immunoprecipitation (we note panel **B** is reproduced from Figure 4C for comparison).
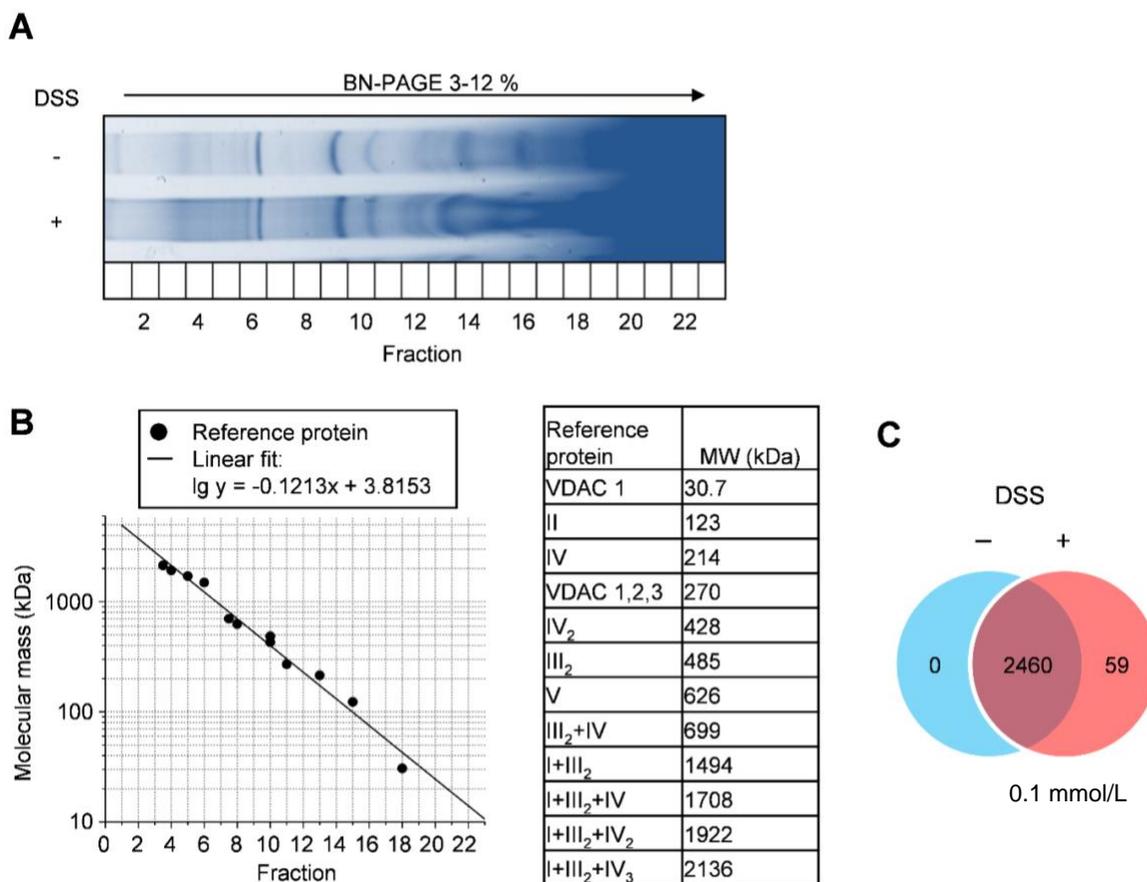
Online Figure XII. BN-PAGE separation of cross-linked complexes and mass calibration.
A, Blue Native polyacrylamide gel electrophoresis (BN-PAGE) was used to separate the solubilized membrane protein fractions from mouse ventricular cardiomyocytes isolated individually from three adult mouse hearts. Furthermore, each isolated cardiomyocyte suspension was split into two separate treatment groups: either treated for 1 h with (-) the carrier solution or (+) 0.1 mmol/L DSS for live in-cell cross-linking. Each lane was cut into 23 equally sized pieces for in-gel digestion and BN-MS analysis (complexome profiling). **B**, *Left*: logarithmic mass calibration graph showing the relation between the molecular weight (MW) versus gel piece #based on predefined membrane reference proteins and protein complexes. R^2= 0.977, Prism version 7.03. *Right*: Table listing the reference proteins and protein complexes according to their molecular weight in kDa. VDAC 1: monomeric voltage-dependent anion channel 1; VDAC 1,2,3: hetero-nonameric VDAC complex; II / IV / IV_2 / III_2 / V: mitochondrial respiratory complexes; III_2+IV / I+III_2 / I+III_2+IV / I+III_2+IV_2 / I+III_2+IV_3: mitochondrial super-complexes. **C**, Venn diagram summarizing the number of proteins identified by BN-MS in carrier-treated (-) cardiomyocytes or DSS cross-linker treated (+) cardiomyocytes.

SUPPLEMENTAL TABLES AND SUPPORTING INFORMATION

Online Table I. NRCM isolation buffer and cultivation medium

CBFHH buffer

	MW (g/mol)	Final concentration
NaCl	58.44	37 mmol/L
KCl	74.56	5.4 mmol/L
KH_2PO_4	136.09	0.44 mmol/L
$Na_2HPO_4 \cdot 2\,H_2O$	177.99	33.5 mmol/L
Glucose	180.16	5.6 mmol/L
HEPES	238.31	20 mmol/L
$MgSO_4$	120.37	0.8 mmol/L
in 500 mL ddH$_2$O, pH 7.4		

NRCM cultivation medium

	MW (g/mol)	Final concentration
FBS	-	10% (v/v)
5-Bromo-2'-deoxyuridine	307.1	10 mmol/L
Penicillin/streptomycin	647	1% (v/v)
in 500 mL cell culture medium		
(DMEM-1 g/L D-glucose, Thermo Fisher Scientific)		

Online Table II. APEX2 biotinylation buffer compositions

Quenching buffer

	MW (g/mol)	Final concentration (mmol/L)
6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic-acid (Trolox)	250.29	5
Sodium azide	65	10
Sodium ascorbate	136.09	10
in 50 mL PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco)		

RIPA quenching buffer

	MW (g/mol)	Final concentration
Tris HCl, pH 7.4	157.60	50 mmol/L
NaCl	58.44	150 mmol/L
Triton-X-100	647	1% (v/v)
Sodium deoxycholate	414.55	0.5% (w/v)
Sodium dodecyl sulfate	288.37	0.2% (v/v)
6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic-acid (Trolox)	250.29	5 mmol/L
Sodium azide	65	10 mmol/L
Sodium ascorbate	136.09	10 mmol/L
in 10 mL ddH$_2$O, pH 7.4		
1 tablet of protease inhibitors (Complete Mini EDTA free, Sigma Aldrich)		

Tris/HCl buffer containing urea

	MW (g/mol)	Final concentration
Tris HCl, pH 8	157.60	50 mmol/L
Urea	60.06	2 mmol/L
in 1 L ddH$_2$O, pH 8		

Biotin buffer

	MW (g/mol)	Final concentration
Biotin	244.31	2 mmol/L
Sodium dodecyl sulfate	288.37	2% (v/v)
in 1 mL ddH$_2$O, pH 8		

Online Table III. Mass spectrometry loading buffer

MS loading buffer

	MW (g/mol)	Final concentration
Acetonitrile	41.05	2% (w/v)
Formic acid	46.03	0.1% (w/v)
in 50 mL ddH$_2$O		

Online Table IV. Cell lysis and protein transfer buffers

RIPA buffer

	MW (g/mol)	Final concentration
Tris HCl, pH 7.4	157.60	50 mmol/L
NaCl	58.44	150 mmol/L
Triton-X-100	647	1% (v/v)
Sodium deoxycholate	414.55	0.5% (w/v)
Sodium dodecyl sulfate	288.37	0.2% (v/v)
in 10 mL ddH₂O, pH 7.4		
1 tablet of protease inhibitors (Complete Mini EDTA free, Sigma Aldrich)		

Transfer buffer (immunoblot)

	MW (g/mol)	Final concentration
Tris HCl, pH 7.4	157.60	191.6 mmol/L
Glycine	75.07	192 mmol/L
in 1 L ddH₂O, pH 7.4		

5 x SDS buffer (immunoblot)

	MW (g/mol)	Final concentration
Tris HCl, pH 7.4	157.60	191.6 mmol/L
Glycine	75.07	1.92 mol/L
SDS	288.37	34.69 mmol/L
in 1 L ddH₂O, pH 8.3		
(dilution 2 mL 5x SDS buffer in 3 mL to yield 2x SDS buffer)		

Online Table V. Immunoprecipitation buffers

CHAPS co-IP buffer

	MW (g/mol)	Final concentration
Tris HCl, pH 7.4	157.60	50 mmol/L
NaCl	58.44	150 mmol/L
CHAPS	614.88	0.15% (w/v)
EGTA	380.35	1 mmol/L
in 10 mL ddH$_2$O, pH 7.4		
1 tablet of protease inhibitors (Complete Mini EDTA free, Sigma Aldrich)		

Sodium deoxycholate co-IP buffer

	MW (g/mol)	Final concentration
Tris HCl, pH 7.4	157.60	50 mmol/L
NaCl	58.44	150 mmol/L
Triton-X-100	647	1% (v/v)
Sodium deoxycholate	414.55	0.5% (w/v)
in 10 mL ddH$_2$O, pH 7.4		
1 tablet of protease inhibitors (Complete Mini EDTA free, Sigma Aldrich)		

co-IP buffer, Vatta et al.[9]

	MW(g/mol)	Final concentration
Tris HCl, pH 7.4	157.60	25 mmol/L
NaCl	58.44	150 mmol/L
EDTA	292.24	5 mmol/L
Triton-X-100	647	1% (v/v)
Nonidet P-40	600	1% (v/v)
Octyl β-D-glucopyranoside	292.37	60 mmol/L
in 10 mL ddH$_2$O, pH 7.4		
1 tablet of protease inhibitors (Complete Mini EDTA free, Sigma Aldrich)		

IGEPAL co-IP buffer

	MW(g/mol)	Final concentration
HEPES, pH 7.5	238.30	20 mmol/L
NaCl	58.44	150 mmol/L
IGEPAL CA-630	603.00	1% (v/v)
SDS	288.37	0.1% (v/v)
in 10 mL ddH$_2$O, pH 7.4		
1 tablet of protease inhibitors (Complete Mini EDTA free, Sigma Aldrich)		

Online Table VI. Blue Native (BN)-PAGE buffers

Homogenization buffer

	MW (g/mol)	Final concentration
Sucrose	342.30	250 mmol/L
Tris HCl, pH 7.4	157.60	10 mmol/L
EDTA	292.24	1 mmol/L
PMSF	174.19	1 mmol/L
in 50 mL ddH$_2$O, pH 7.4		

Solubilization buffer

	MW (g/mol)	Final concentration
NaCl	58.44	50 mmol/L
Imidazole	68.08	50 mmol/L
EDTA	292.24	1 mmol/L
Aminocaproic acid	414.55	2 mmol/L
in 50 mL ddH$_2$O, pH 7.4		

Online Table VII. Mouse cardiomyocyte isolation, blocking, and permeabilization buffers

Perfusion buffer (mouse heart)

	MW (g/mol)	Final concentration
NaCl	58.44	120.4 mmol/L
KCl	74.56	14.7 mmol/L
KH_2PO_4	36.09	0.6 mmol/L
$Na_2HPO_4 \cdot 2\,H_2O$	177.99	0.6 mmol/L
$MgSO_4 \cdot 7\,H_2O$	246.48	1.2 mmol/L
HEPES	238.31	10 mmol/L
$NaHCO_3$	84.01	4.6 mmol/L
Taurin	125.20	30 mmol/L
2,3-Butanedione monoxime	101.1	10 mmol/L
Glucose	180.16	5.5 mmol/L
in 1 L ddH$_2$O, pH 7.4		

Digestion buffer (mouse heart)

	MW (g/mol)	Final concentration
Collagenase type II	-	2 mg/mL
$CaCl_2$	110.98	40 µmol/L
in 50 mL perfusion buffer, pH 7.4		

Stop buffer (mouse heart)

	MW (g/mol)	Final concentration
Bovine calf serum	-	10% (v/v)
$CaCl_2$	110.98	12.5 µmol/L
in 50 mL perfusion buffer, pH 7.4		

Blocking/permeabilization buffer for mouse cardiomyocyte immunofluorescence

	MW (g/mol)	Final concentration
Bovine calf serum	-	10% (v/v)
Triton X-100	74.56	0.2% (v/v)
in 50 mL PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco)		

Online Table VIII. Electron tomography buffer

Reynold's lead citrate

	MW (g/mol)	Final concentration
$Pb(NO_3)_2$	331.23	80 mmol/L
$Na_3C_6H_5O_7$	258.06	136 mmol/L
NaOH, 1N	40.00	8 mL (v/v)
in 50 mL ddH$_2$O, pH 12		

Online Table IX. Stem cell differentiation and human iPSC-CM culture media

iPSC culture medium

	MW (g/mol)	Final concentration
Thiazovivin	311.4	2 µmol/L
in 500 mL cell culture medium		
(StemMACS iPS-Brew stem cell culture media, Miltenyi Biotec)		

Human iPSC-CM differentiation medium

	MW (g/mol)	Final concentration
Human recombinant albumin	-	0.5 mg/mL
L-ascorbic acid 2-phosphate	289.54	0.2 mg/mL
in 500 mL cell culture medium		
(RPMI 1640 cell culture medium with Glutamax and HEPES, Thermo Fisher Scientific)		

Human iPSC-CM selection medium

	MW (g/mol)	Final concentration
Human recombinant albumin	-	0.5 mg/mL
L-ascorbic acid 2-phosphate	289.54	0.2 mg/mL
Lactate	89.07	4 mmol/L
in 500 mL cell culture medium		
(RPMI 1640 cell culture medium, no glucose, Thermo Fisher Scientific)		

Human iPSC-CM culture medium

	MW (g/mol)	Final concentration
B27	-	2% (v/v)
in 500 mL cell culture medium		
(RPMI 1640 cell culture medium with Glutamax and HEPES, Thermo Fisher Scientific)		

Online Table X. Stem cell blocking buffer

Stem cell blocking buffer (immunofluorescence)

	MW (g/mol)	Final concentration
Bovine Serum Albumin	-	1% (v/v)
in 50 mL PBS (PBS, pH 7.4, without Ca^{2+} and Mg^{2+}, Gibco)		

Online Table XI. Seahorse XF assay buffer

Seahorse XF assay buffer

	MW (g/mol)	Final concentration
Pyruvate	88.06	1 mmol/L
Glucose	180.16	4.5 mg/mL
in 500 mL Seahorse assay medium (Seahorse XF assay medium, Agilent)		

Online Table XII. Solutions Na,K-ATPase patch-clamp recording in human iPSC-CMs

Pipette solution	MW (g/mol)	Final concentration
$CsCH_3O_3S$	228.01	85 mmol/L
$NaCH_3O_3S$	118.09	25 mmol/L
CsCl	168.36	20 mmol/L
EGTA	468.3	5 mmol/L
HEPES	238	10 mmol/L
$ATP-Mg_2$	529.47	5 mmol/L
$CaCl_2 H_2O$	147	1 mmol/L
$CTP-Na_2$ dibasic	563.15	5 mmol/L
$MgCl_2$	203	1 mmol/L
in 100 mL, pH 7.35 with CsOH		

Bath solution	MW (g/mol)	Final concentration
NaCl	58.44	140 mmol/L
KCl	74.56	0 or 5 mmol/L
HEPES	238	10 mmol/L
Glucose	180.2	10 mmol/L
$BaCl_2$	208.23	1 mmol/L
$NiCl_2$	129.6	2 mmol/L
$MgCl_2$	203	1 mmol/L
in 100 mL, pH 7.35 with NaOH		

Online Table XIII. Modifications to the human Kernik iPSC-CM model

Parameter	Model				
	Original	**WT EAD-resistant**	**WT EAD-prone**	**KO EAD-resistant**	**KO EAD-prone**
I_{hyper}	0 µA/µF	0.1 µA/µF **(0.3 µA/µF in 2D simulations)**			
I_{stim}	80 µA/µF, 0.5 ms	0 pA/pF for spontaneous activity, 80 µA/µF for paced simulations			
k_fca	if(fCa_inf>fCa,if(V>**k_fca_thresh**,0,if(V>-60,**0.1**,1)),1)				
k_fca_thres	-60	-60 mV	-30 mV	-60 mV	-30 mV
f_inf	1 / (1 + exp((V-(-44)+**shift_inact**)/5))				
shift_inact	0 mV	0 mV	-10 mV	0 mV	-10 mV
G_{Kr}	100%	100%	55%	100%	55%
G_{NaL}	100%	100%	100%	600%	600%

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (ex vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse adult	University Medical Center Göttingen	C57BL/6N	Female, Male	Does not apply
Rat neonatal	University Medical Center Göttingen	Wistar	Female, Male	Does not apply

Genetically Modified Animals (ex vivo studies)

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Male	Mouse	Jackson labs	C57BL/6N	CAV1 KO	https://www.jax.org/strain/007083
Female	Mouse	Jackson labs	C57BL/6N	CAV1 KO	https://www.jax.org/strain/007083
Male	Mouse	Columbia University	C57BL/6N	Tg FLAG-F1759A-Na$_V$1.5	https://www.jci.org/articles/view/84669
Female	Mouse	Columbia University	C57BL/6N	Tg FLAG-F1759A-Na$_V$1.5	https://www.jci.org/articles/view/84669
Male	Mouse	Columbia University	C57BL/6N	Tg Myh6-rtTA	https://www.jci.org/articles/view/84669
Female	Mouse	Columbia University	C57BL/6N	Tg Myh6-rtTA	https://www.jci.org/articles/view/84669

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
α-Actinin	Sigma Aldrich	A7811	1:1000 for WB	https://www.sigmaaldrich.com/catalog/product/sigma/a7811?lang=de&region=DE
β-Actin	Santa cruz	sc-47778	1:1000 for WB	https://www.scbt.com/de/p/beta-actin-antibody-c4
Aqp1	Santa cruz	sc-25287	1:500 for WB	https://www.scbt.com/de/p/aqp1-antibody-b-11
Caveolin-1	Abcam	ab2910	1:1000 for WB	https://www.abcam.com/caveolin-1-antibody-caveolae-marker-ab2910.html
Caveolin-1	Abcam	Ab17052	1:50 for IF	https://www.abcam.com/caveolin-1-antibody-7c8-ab17052.html
Caveolin-3	Abcam	ab2912	1:1000 for WB 1:500 for IF	https://www.abcam.com/caveolin-3-antibody-caveolae-marker-ab2912.html
Caveolin-3	Abcam	ab182739	1:500 for WB	https://www.abcam.com/caveolin-3-antibody-epr18975-ab182739.html
cTnT	Thermo Fisher Scientific	MS-295-P0	1:50 for FC	https://www.fishersci.de/shop/products/troponin-t-cardiac-isoform-ab-1-mouse-monoclonal-antibody/p-4563797
Cx43	BD transduction	610062	1:500 for WB	https://www.bdbiosciences.com/us/applications/research/stem-cell-research/mesoderm-markers/mouse/purified-mouse-anti-connexin-43-2connexin-43/p/610062
FITC	Jackson	115-097-020	-1:1000 for IF	https://www.jacksonimmuno.com/catalog/p

DOI [to be added]

	Immuno Research Labs			roducts/115-097-020
FLAG	Sigma Aldrich	F1804	1:1000 for WB	https://www.sigmaaldrich.com/catalog/product/sigma/f1804?lang=de&region=DE
GAPDH	Biotrend	5G4 Mab 6C5	1:160000 for WB	https://www.biotrend.com/-186/gapdh-mouse-monoclonal-antibody-181891778.html
IgG-cy3	Jackson Immuno Research Labs	715-165-150	1:100 for FC	https://www.jacksonimmuno.com/catalog/products/715-165-150
McT1	Abcam	ab90582	1:500 for WB	https://www.abcam.com/monocarboxylic-acid-transporter-1-antibody-ab90582.html
McT1	Bioss Antibodies	BS-10249R	1:1000 for WB 1:1000 for IF	https://www.biossantibodies.com/datasheets/bs-10249R
Mouse IgG	DAKO	X0931	1:50 for FC	https://www.agilent.com/store/productDetail.jsp?catalogId=X093101-2
NANOG	Thermo Fisher Scientific	PA1-097	1:1000 for IF	https://www.thermofisher.com/antibody/product/Nanog-Antibody-Polyclonal/PA1-097
Na/K-ATPase α1	Santa Cruz	sc-21712	1:1000 for WB	https://www.scbt.com/p/na-k-atpase-alpha1-antibody-c464-6?gclid=CjwKCAiAudD_BRBXEiwAudakX9yrcVyZQHKpmkOHO6oe7-5fhHZfo-TTtgWFytAljxPo-g0FfHRcLhoCUnQQAvD_BwE
Na$_V$1.5	Alomone	ASC-005	1:500 for WB 1:250 for IF	https://www.alomone.com/p/anti-nav1-5/ASC-005?gclid=CjwKCAiAudD_BRBXEiwAudakXxmCuOtm3SCOo5VHGGmbo-_E3KM5x0eyDy2Pt7LJHvA8j1Q3Kg-fLBoCVGwQAvD_BwE
Ncx1	Swant	π 11-13	1:1000 for WB	https://www.swant.com/pdfs/Rabbit_anti_Na-Ca-exchanger.pdf
OCT4	R and D Systems	AF1759	1:1000 for IF	https://www.rndsystems.com/products/human-mouse-oct-3-4-antibody_af1759
OCT4 conjugated Alexa Fluor 647	BD Biosciences	560329	1:50 for FC	https://www.bdbiosciences.com/eu/applications/research/stem-cell-research/cancer-research/human/alexa-fluor-647-mouse-anti-oct34-40oct-3/p/560329
p115	BD Biosciences	612261	1:1000 for IF	https://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/cell-biology-reagents/cell-biology-antibodies/purified-mouse-anti-p115-15p115/p/612261
TfR1	Invitrogen	13-6800	1:500 for WB	https://www.thermofisher.com/antibody/product/Transferrin-Receptor-Antibody-clone-H68-4-Monoclonal/13-6800
TRA-1-60	Abcam	ab16288	1:1000 for IF	https://www.abcam.com/tra-1-60-r-antibody-tra-1-60-ab16288.html
TRA-1-60 conjugated Alexa Fluor 488	BD Biosciences	560173	1:50 for FC	https://www.bdbiosciences.com/eu/applications/research/stem-cell-research/pluripotent-stem-cell-markers-esc-and-ipsc/human/alexa-fluor-488-mouse-anti-human-tra-1-60-antigen-tra-1-60/p/560173

DOI [to be added]

SAP97	Abcam	ab3437	1:1000 for WB	https://www.abcam.com/sap97-antibody-ab3437.html
V5	Invitrogen	R960-25	1:1000 for WB 1:1000 for IF	https://www.thermofisher.com/antibody/product/R960CUS.html?gclid=CjwKCAiAudD_BRBXEiwAudakX3ZY6ql6BiNQnTOvy8bwYYV5BRP_NMIsQ98QXa1HyEkqccZnAH_FCxoCQZcQAvD_BwE&ef_id=CjwKCAiAudD_BRBXEiwAudakX3ZY6ql6BiNQnTOvy8bwYYV5BRP_NMIsQ98QXa1HyEkqccZnAH_FCxoCQZcQAvD_BwE:G:s&s_kwcid=AL!3652!3!459736943987!b!!g!!
goat anti-mouse Alexa Fluor 633	Thermo Fisher Scientific	A-21052	1:1000 for IF	https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21052
goat anti-rabbit Alexa Fluor 514	Thermo Fisher Scientific	A-31558	1:1000 for IF	https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31558
donkey anti-goat Alexa Fluor 555	Thermo Fisher Scientific	A-21432	1:1000 for IF	https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21432
donkey anti-rabbit Alexa Fluor 555	Thermo Fisher Scientific	A-31572	1:1000 for IF	https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572
goat anti-rabbit STAR 635P	Abberior Instruments	2-0012-007-2	1:1000 for IF	https://abberior.shop/abberior-STAR-635P
goat anti-mouse STAR 635P	Abberior Instruments	2-0002-007-5	1:1000 for IF	https://abberior.shop/abberior-STAR-635P
goat anti-rabbit STAR 580	Abberior Instruments	2-0012-005-8	1:1000 for IF	https://abberior.shop/abberior-STAR-580
goat anti-mouse STAR 580	Abberior Instruments	2-0002-005-1	1:1000 for IF	https://abberior.shop/abberior-STAR-580

DNA/cDNA Clones/Adenoviral plasmids/ guid RNA in 5'–3' orientation

Clone Name	Sequence	Source / Repository	Persistent ID / URL
CAV3 cDNA	atgatgaccgaagagcacacggatctggaagctcggatcatcaaggacattcactgcaaggagatagacttggtgaaccgcgaccccaagaacatcaatgaggacattgtgaaggtagattttgaagacgtgattgcggagcccgagggcacctacagcttcgacggtgtatggaaggtgagcttcaccacgttcaccgtctccaagtactggtgctaccgcctgttgtctacactgctgggtgttccactggccctgctctggggattcctgttcgcctgtatctccttctgccacatctgggccgtggtgccctgcattaagagctacctgatcgagatccagtgcatcagccacatctactcactgtgtatccgcaccttctgcaacccgctcttcgctgcgttgggccaggtctgcagcaacattaaggtggtgctgcgaagggaaggc	MR226246, Origene	https://cdn.origene.com/datasheet/mr226246.pdf
V5-APEX2-CAV3 cDNA	atgggcaagcccatccccaaccccctgctgggcctggacagcaccggaaagtcttaccaactgtgagtgctgattaccaggacgccgttgagaaggcgaagaagaagctcagaggcttcatcgctgagaagagatgcgctcctctaatgctccgtttggcattccactctgctgaacctttgacaagggcacgaagaccggtggacccttcggaaccatcaagcaccctg	University Medical Center Göttingen	Does not apply

DOI [to be added]

	ccgaactggctcacagcgctaacaacggtcttgacatcgctgttaggctttggagccactcaaggcggagttccctattttgagctacgccgatttctaccagttggctggcgttgttgccgttgaggtcacgggtggacctaaggttccattccaccctggaagagaggacaagcctgagccaccaccagagggtcgcttgcccgatcccactaagggttctgaccatttgagagatgtgtttggcaaagctatggggcttactgaccaagatatcgttgctctatctgggggtcacactattggagctgcacacaaggagcgttctggatttgagggtccctggacctctaatcctcttattttcgacaactcatacttcacggagttgttgagtggtgagaaggaaggtctccttcagctaccttctgacaaggctcttttgtctgaccctgtattccgccctctcgttgataaaatatgcagcggacgaagatgccttcttgctgattacgctgaggctcaccaaaagctttccgagctttgggtttgctgatgccggggggcagcggctcgagcatgatgaccgaagagcacacggatctggaagctcggatcatcaaggacattcactgcaaggagatagacttggtgaaccgcgaccccaagaacatcaatgaggacattgtgaaggtagattttgaagacgtgattgcggagcccgagggcacctacagcttcgacggtgtatggaaggtgagcttcaccacgttcaccgtctccaagtactggtgctaccgcctgttgtctacactgctgggtgttccactggccctgctctggggattcctgttcgcctgtatctccttctgccacatctgggccgtggtgccctgcattaagagctacctgatcgagatccagtgcatcagccacatctactcactgtgtatccgcaccttctgcaacccgctcttcgctgcgttgggccaggtctgcagcaacattaaggtggtgctgcgaagggaaggctaa		
CAV3 Fwd Primer	gggggcagcggctcgagcatgatgaccgaagagcaca	Invitrogen	Does not apply
CAV3 Rev Primer	tagatgcatgctcgagttagccttcccttc	Invitrogen	Does not apply
pO6A5-CMV-GFP	N/A	Sirion Biotech	https://www.sirion-biotech.com/viral-vectors/adenovirus/
pO6A5-CMV-V5-APEX2-CAV3-IRES-GFP	Please see above for V5-APEX2-CAV3 sequence	Sirion Biotech	https://www.sirion-biotech.com/viral-vectors/adenovirus/
pO6A5-CMV-V5-APEX2-IRES-GFP	Please see above for V5-APEX2 sequence	Sirion Biotech	https://www.sirion-biotech.com/viral-vectors/adenovirus/
CRISPR#1	tccccccagctctgcgatgatgg	Invitrogen	Does not apply
CRISPR#2	caccgcccagatgtggcagaagg	Invitrogen	Does not apply

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Ventricular differentiated human induced pluripotent stem cells	Stem Cell Unit in Göttingen	unknown	https://herzzentrum.umg.eu/forschung/arbeitsgruppen/stem-cell-unit/
Ventricular neonatal rat cardiomyocytes	Isolated from neonatal wistar rats	F, M	Does not apply
Ventricular mouse cardiomyocytes	Isolated from WT mice	F, M	Does not apply

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
Mass spectrometry proteomic data have been deposited to the ProteomeXchange Consortium	ProteomeXchange Consortium	PXD019399 http://www.ebi.ac.uk/pride
MS dataset APEX2 Proximity Assay by 3-state SILAC-MS	EMBL-EBI PRIDE Archive	https://www.ebi.ac.uk/pride/archive/projects/PXD019398
MS dataset interactome Cav1/Cav3 by AP-DIA-MS	EMBL-EBI PRIDE Archive	https://www.ebi.ac.uk/pride/archive/projects/PXD019399
MS dataet complexome profiling by BN-PAGE-MS	EMBL-EBI PRIDE Archive	https://www.ebi.ac.uk/pride/archive/projects/PXD019380

Other

Description	Source / Repository	Persistent ID / URL
Computational model code	GitHub	https://github.com/JordiHeijman/hIPSC-CM-CAV3/

DOI [to be added]