Online Supplemental Data

Online Supplemental Materials and Methods

Exome sequencing and variant filtering

DNA was extracted from blood or saliva samples and sequenced as previously described [4,72-74]. Sequence reads were mapped to the reference genome (hg19), and further processed using the GATK Best Practices workflows as previously described[4,72-74]. Single nucleotide variants (SNVs) and small indels were called with GATK HaplotypeCaller. The MetaSVM algorithm was used to predict deleteriousness of missense mutations (annotated as "D-Mis") using software defaults[75]. *De novo* mutations were independently called and filtered for quality using the Trio Denovo program and a custom pipeline, which have been shown to yield a specificity of 96.3%[2]. Candidate LoF heterozygous variants were filtered for rarity (Allele frequency ≤ 10^{-5}) and quality as previously described[4].

CRISPR gene editing and mutation confirmation

NAA15 LoF iPSCs were generated by non-homologous end joining (NHEJ). Two micrograms of plasmid expressing Cas9 (PX459v2 from Addgene) were co-transfected with 2 µg plasmid expressing guide RNA using a stem cell 4-D core nucleofector unit (Lonza). *NAA15*$^{+/R276W}$ hiPSCs were generated using homology directed repair (HDR), 2 µg plasmid expressing Cas9 (PX459v2 from Addgene) was co-transfected with 2 µg plasmid expressing guide RNA and 2 µg single-stranded oligonucleotide (HDR template) using a stem cell nucleofector kit (Lonza, cat. no. AAF-1002B). Edited clones were selected using puromycin (Gibco), expanded and genotyped as previously described[27].

Gene-edited cells were subcloned twice and validated by Sanger sequencing and next generation sequencing. Subcloning involved dissociation of iPSCs by pipetting and filtering

through a 60-μm strainer. Cells were then plated onto a 15-cm dish containing MTESR (Stemcell Technologies) + Rock inhibitor (10 nM) (R&D systems). Individual colonies were allowed to grow to 300 cells, clones were picked and separately placed into individual wells of a 96-well plate. After clones grew to 85% confluency, iPSCs were collected and processed for PCR where the sequence of the variant was amplified. To ensure purity of iPSCs clone, iPSCs were then subjected to an additional set of sub-cloning and processed for PCR. PCR amplified fragments were submitted for Sanger sequencing and next generation sequencing. Confirmation of sequencing was analyzed by bioinformatic analysis, DNA-star software, and Integrated Genomics Viewer (IGV). Two independent clones were created for each genotype (*NAA15$^{-/-}$: NAA15$^{K336*/G348*}$, NAA15$^{L314*/L314*}$; NAA15$^{+/-}$: NAA15$^{+/K336*}$, NAA15$^{+/G348*}$; NAA15$^{+/R276W}$ (Online Table II)*). All PGP1 iPS cells used in experiments were within passage ranges of 65-77.

Western Blotting

iPSCs were harvested and lysed in RIPA lysis buffer (Thermoscientific). Cells were sonicated for 2 min in 6 x 16 mm AFA microtubes (Covaris) using a Covaris E210 focused ultrasonicator, duty factor 5%, and 200 Cycles/burst at 4 °C. Protein concentration was determined using a BCA Protein Assay Kit (Pierce) and NanoDrop 2000/2000c Spectrophotometer. For immunoblotting protocol, approximately 30 μg of protein per sample were mixed with NuPAGE Reducing Buffer (Invitrogen) and LDS Sample Buffer (Pierce). After denaturation, samples were loaded onto a Novex 4-20% Tris-Glycine Mini Gel cassettes (Thermo Scientific). Gels were transferred overnight at 16°C at 100mA onto a PVDF membrane and blocked for 1 h. Primary antibodies used were: Rabbit Anti-NAA10 (Novus, cat. no. NBP2-19461) and Rabbit Anti-NAA15 (Sigma, cat. no. HPA023589). Beta

actin (Thermoscientific, cat no. MA5-15739) was used as a loading control. Goat anti-rabbit IgG HRP secondary antibodies (Thermo Scientific, cat no. A21207) and Goat Anti-Mouse IgG peroxidase labelled secondary antibody (cat. no. 115-035-003; Jackson ImmunoResearch Laboratories, Inc.) were used for western blot visualization. Bands were visualized using a ChemiDocTM XRS+ and ImageLab v3.0.

RNAseq library prep and analysis

RNA was purified from iPSCs using Trizol (Life Technologies) protocols. All RNA samples had an RNA integrity number of >8. Library preparation was conducted using the Nextera Library preparation method (Illumina). RNA-Seq library samples were pooled and run on four lanes (one flow cell). Libraries were sequenced using the Illumina NextSeq500 platform. All data was combined into a single fastq file. Reads were aligned to the hg19 reference genome using STAR. Mitochondrial and duplicate reads were discarded using Samtools and Picard's MarkDuplicates, respectively. The aligned reads were quantitated by Feature counts and counts were normalized. Gene expression was determined by calculating reads per gene per million aligned reads (rpkm). Differentially expressed genes were identified using DESeq2 and Student's T-test for comparison between datasets. RNAseq data sets are available in GEO public data repository; GEO accession number GSE160809.

Yeast Strains and Plasmids

The *Saccharomyces cerevisiae* strain W303-1A (MAT**a**; *ade2-1; ura3-1; his3-11,15; leu2-3,112; trp1-1; can1-100*) was used to construct a y*NatAΔ* strain (*ard1-Δ::LEU2*; *nat1-Δ::kanMX*)[8].Yeast was grown and manipulated according to standard procedures[8].

Expression vectors for h*NAT1* (= h*NAA15*), h*ARD1* (= h*NAA10*) and h*NAT1* h*ARD1* were made by subcloning full length h*NAT1* and/or h*ARD1* genes from pDNA3.1-ARD1-V5His and pDNA3.1-NATH-V5His to pBEVY-U bidirectional yeast expression vector [8], using *Xma*I and *Eco*RI sites for inserting h*ARD1* after the *ADH1* promoter and *Bam*HI and *Sal*I sites for inserting h*NAT1* after the GPD promoter. The genotype of the *yNatAΔ* strain (*ard1-Δ::LEU2; nat1-Δ::kanMX*) was confirmed by colony PCR using *ARD1* and *NAT1* primers. *ARD1*-specific PCR product 1024 bp (*ARD1* 717 bp + gene specific sequence 307 bp) and *NAT1*-specific PCR product 2976 bp (*NAT1* 2565 bp + gene specific sequence 411 bp). *yNatAΔ* mutant cells were transformed with a bidirectional expression vector encoding wild-type or mutated variants of the human NatA complex (HsNatA). Yeast transformants were selected and maintained on SD-Ura [0.67 % (w/v) yeast nitrogen base without amino acids, 0.2 % (w/v) yeast drop-out mix without uracil, 2 % (w/v) glucose, and 2 % (w/v) agar]. HsNatA expression was confirmed by immunoblot analysis using anti-HsNAA10 and anti-HA as previously described.[5, 58]

Yeast growth assay

Yeast strains were grown in SD-URA at 30 °C to early log phase (OD_{600} 0.8-1.0). The cultures were diluted to 1 OD_{600}/ml. Subsequently, tenfold serial dilutions were spotted onto YPD and SD-Ura agar plates and incubated for 2 days at 30 °C or 38 °C. The plates were imaged using the spImager from S&P Robotics.

iPSC-CM Differentiation

For cardiac differentiation, iPSCs were maintained in feeder-free conditions with MTESR (Stemcell Technologies) media and differentiated to the CM lineage by sequential targeting

of the WNT pathway as described[30-31]. CMs were purified using glucose metabolic selection and studied on day 30-40 after initiation of differentiation except when mentioned otherwise [30-31].

Immunofluorescence

iPS cardiomyocytes (iPSC-CMs) were seeded onto sterile, acid-treated, 18-mm #1.5 glass coverslips in 24-well plates. iPSC-CMs were fixed in 2% paraformaldehyde in PBS for 10 min. The cells were washed with PBS. Permeabilization was accomplished by placing cells for 5 min in PBS containing 0.1% Triton X-100 at 37 °C. Cells were incubated with primary antibodies, rabbit polyclonal cardiac troponin T (Abcam, cat. no. ab45932) and mouse monoclonal α–actinin (Sigma-Aldrich, cat. no. a7811), for 1 h at room temperature, followed by three washes to remove unbound antibody. Cells were then incubated with secondary antibodies, goat anti-rabbit FITC (Jackson laboratories, cat. no. 656111) and goat anti-mouse rhodamine (Jackson laboratories, cat no. 31663), for 1 h. DNA was stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma, cat no. D9564) at a 1:5000 dilution for a 2 min incubation. As a negative control, antibody buffer without primary antibody was used to assess nonspecific binding of secondary antibodies (FITC and Rhodamine). Cells were imaged in a Yokogawa CSU-W1 spinning disk scan head with a 50-μm pinhole disk mounted on a Nikon Ti inverted microscope (Nikon Ti), equipped with a Nikon motorized stage with a Physik Instrument piezo Z motor, a Plan Apo Lambda 100x/1.45 DIC objective, and a Andor Zyla 4.2 plus sCMOS camera. Images were acquired using NIS Elements AR 5.02. Signal from FITC and Rhodamine channels was collected using a Chroma ET 525/36 and ET 605/52 emission filters, respectively. For immunofluorescent analysis, we chose representative images that best depicted the sarcomere phenotype of each cell line.

For live cell imaging analysis, iPSC-CMs were differentiated in 6-well plates (Corning) using RPMI-1640 media containing B27 supplement. Live cell images were collected using a Keyence BZ-X710 microscope at room temperature using a 60X objective.

2D Contractile Functional Assays of Day 30 iPSC-CMs

For unloaded contractile measures, iPSC-CM were transfected with GFP-actinin lentivirus and five seconds videos were acquired around day 30 of differentiation[35]. Contractile measures of iPSC-CMs were performed on 3 separate wells of cardiac differentiations by using SarcTrack as previously described[35]. iPSC-CM were paced at 1Hz, 12 volts at 0.5 ms.

Cardiac Microtissue (CMT) platform

Polydimethylsiloxane (PDMS) micro-tissue devices with tissue wells each containing two cylindrical micro-pillars with spherical caps were casted from a 3D printed mold (Protolabs). Two days prior to tissue seeding, devices were plasma treated for 60 seconds, treated with 0.01% Poly-L-Lysine (ScienCell) for 2 hours, 0.1% Glutaraldehyde (EMS) for 15 minutes, washed three times with DI water, and let sit in DI water at 4^{o}C overnight. Immediately prior to seeding, devices were dried and sterilized under UV for 15 minutes. After sterilization, 3 µL of 5% BSA was added to each tissue well and devices were spun in a tabletop centrifuge at 3000rpm for 1.5 minutes to evenly distribute the BSA. After one hour incubation at room temperature, BSA was removed and 2 µL of 2% Pluronic F-127 (Sigma) was added to each well and incubated for 30 minutes at room temperature to prevent tissues sticking to the bottom and side surface of the tissue well.

A total of 60,000 cells per tissue, consisting of 90% iPSC-CMs and 10% human mesenchymal stem cells (hMSCs), were mixed in 7.5 µL of an ECM solution, consisting of 4 mg/ml of human fibrinogen (Sigma), 10% Matrigel (Corning) and 0.4 unit of thrombin (Sigma)

per mg of fibrinogen, 5 μM Y-27632 (Tocris), and 0.033mg/mL aprotinin (Sigma). The cell-ECM mixture was pipetted into each tissue well, and after gel polymerization for five minutes, tissue maintenance growth media containing high glucose Dulbecco's Modified Medium (DMEM, Fisher) supplemented with 10% Fetal Bovine Serum (Sigma), 1% Penicillin Streptomycin (Fisher), 1% Non-essential Amino Acids (Fisher), 1% Glutamax (Fisher), 5 μM Y-27632, and 0.033mg/mL Aprotinin was added. The growth media was replaced every other day. Y-27632 was removed two days following seeding.

Seven days post seeding, the tissues were electrically stimulated at 1Hz using the IonOptix C-Pace EP Culture Pacer (IonOptix) with a 10ms square pulse. Time-lapse videos of the tissue contraction were acquired at 30 frames per second using a 4x objective on a Nikon Eclipse Ti (Nikon Instruments, Inc.) with an Evolve EMCCD Camera (Photometrics) equipped with a temperature and CO2 equilibrated environmental chamber. Maximum contractile stress was calculated using a custom Matlab Script based on the deflection of the pillars and the measured pillar spring constant of 2.68 μN/μm, as described previously[37]. For statistical analysis, ANOVA was used with multiple comparisons tested by post hoc Tukey HSD using a R script with a statistical significance cutoff of $p < 0.05$.

Label-free quantitative shotgun proteomics and data analysis

Cell pellets (1 x 10^6 cells each) were lysed in a urea lysis buffer containing 8 M urea, 20 mM HEPES pH 8.0 and PhosSTOP phosphatase inhibitor cocktail (Roche, 1 tablet/10 ml buffer). The samples were sonicated with 3 pulses of 15 s at an amplitude of 20% using a 3 mm probe, with incubation on ice for 1 minute between pulses. After centrifugation for 15 minutes at 20,000 x g at room temperature to remove insoluble components, proteins were reduced by addition of 5 mM DTT and incubation for 30 minutes at 55˚C and then alkylated by addition

of 10 mM iodoacetamide for 15 minutes at room temperature in the dark. Samples were diluted with 20 mM HEPES pH 8.0 to a final urea concentration of 4 M and proteins were digested with 1 µg LysC (Wako) (1/200, w/w) for 4 hours at 37°C. Samples were again diluted to 2 M urea and digested with 2 µg trypsin (Promega) (1/100, w/w) overnight at 37˚C. The resulting peptide mixture was acidified by addition of 1% trifluoroacetic acid (TFA) and after 15 minutes incubation on ice, samples were centrifuged for 15 minutes at 1,780 x g at room temperature to remove insoluble components. Next, peptides were purified on SampliQ SPE C18 cartridges (Agilent). Columns were first washed with 1 ml 100% acetonitrile (ACN) and pre-equilibrated with 3 ml of solvent A (0.1% TFA in water/ACN (98:2, v/v)) before samples were loaded on the column. After peptide binding, the column was washed again with 2 ml of solvent A and peptides were eluted twice with 750 µl elution buffer (0.1% TFA in water/ACN (40:60, v/v)).

Purified peptides were dried, re-dissolved in 20 µl solvent A and from each sample 5 µl was injected for LC-MS/MS analysis on an Ultimate 3000 RSLCnano system (Thermo) in-line connected to a Q Exactive HF mass spectrometer (Thermo) equipped with a Nanospray Flex Ion source (Thermo). The peptides were first loaded on a trapping column (made in-house, 100 µm internal diameter (I.D.) × 20 mm, 5 µm beads C18 Reprosil-HD, Dr. Maisch, Germany) and after flushing from the trapping column, peptides were separated on an analytical column in the needle (made in-house, 75 µm I.D. × 400 mm, 1.9 µm beads C18 Reprosil-HD, Dr. Maisch) using a non-linear 145 min gradient of 2 to 56% solvent B (0.1% formic acid in water/acetonitrile, 20/80 (v/v)) at a constant flow rate of 250 nL/min and at a constant temperature of 50°C (CoControl 3.3.05, Sonation). Following a 10 min wash reaching 99% solvent B, the column was re-equilibrated with solvent A (0.1% formic acid in water).

The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the 16 most abundant ion peaks per MS spectrum. Full-scan MS spectra (375-1500 m/z) were acquired at a resolution of 60,000 in the orbitrap analyser after accumulation to a target value of 3,000,000. The 16 most intense ions above a threshold value of 22,000 were isolated (window of 1.5 Th) for fragmentation at a normalized collision energy of 32% after filling the trap at a target value of 100,000 for maximum 45 ms. MS/MS spectra (200-2000 m/z) were acquired at a resolution of 15,000 in the orbitrap analyser. The S-lens RF level was set at 55 and we excluded precursor ions with single and unassigned charge states from fragmentation selection.

Data analysis was performed with MaxQuant (version 1.5.4.1) using the Andromeda search engine with default search settings including a false discovery rate set at 1% on both the peptide and protein level. Spectra were searched against the human proteins in the Uniprot/Swiss-Prot database (database release version of August 2016 containing 20,210 human protein sequences, downloaded from www.uniprot.org). The mass tolerance for precursor and fragment ions was set to 4.5 and 20 ppm, respectively, during the main search. Enzyme specificity was set as C-terminal to arginine and lysine, also allowing cleavage at proline bonds with a maximum of two missed cleavages. Variable modifications were set to oxidation of methionine residues and acetylation of protein N-termini, while carbamidomethylation of cysteine residues was set as fixed modification. Matching between runs was enabled with a matching time window of 0.7 minutes and an alignment time window of 20 minutes. Only proteins with at least one unique or razor peptide were retained leading to the identification of 5,196 proteins. Proteins were quantified using the MaxLFQ algorithm[76]

integrated in the MaxQuant software. MaxLFQ protein intensity values were calculated from two biological replicates and at least three technical replicates for each genotype. A minimum ratio count of two unique or razor peptides was required for quantification. Further data analysis was performed with the Perseus software (version 1.5.5.3) after loading the protein groups file from MaxQuant. Proteins only identified by site, potential contaminants and reverse database hits were removed and replicate samples were grouped by mean MaxLFQ intensity. Proteins with less than three valid values in at least one group were removed and missing values were imputed from a normal distribution around the detection limit leading to a list of 3,910 quantified proteins that was used for further data analysis. To reveal proteins of which the expression level was significantly affected in either the mutant conditions relative to the wildtype condition, sample groups were defined based on genotype: $NAA15^{+/+}$, $NAA15^{+/-}$, $NAA15^{-/-}$. Then, pairwise SAM t-tests (or pairwise statistical testing using the SAM method[29]) were performed on the log2 transformed MaxLFQ protein intensity values and significant hits determined using as cutoff values a permutation based FDR of 0.01 (1000 permutations) and a background variance parameter s0 of 1. R studio program was used to generate volcano plots.

N-terminal peptide enrichment and LC-MS/MS analysis

Pellets from 1x10^7 cells of two biological replicates for NAA15 mutant iPSCs and wildtype iPSCs were lysed for 30 min on ice in 1.5 ml of 50 mM sodium phosphate pH 7.5, 100 mM NaCl, 0.8% (wt/vol) CHAPS lysis buffer containing protease inhibitor cocktail. The lysates were centrifuged at 16,000 g at 4°C and the supernatant was collected. Sample volumes were adjusted to 2 ml of 50 mM sodium phosphate buffer pH 7.9 containing 4 M of guanidinium hydrochloride. Cysteines were reduced and S-alkylated for 15 min at 37°C in the dark using

15 mM TCEP and 30 mM iodoacetamide respectively. This step was followed by desalting on a NAP-10 gel filtration column to remove reagents and lower the guanidinium hydrochloride concentration to 2 M, also in 50mM Sodium phosphate buffer. Then, all α- and ε-amines of the proteins were acetylated using 5 mM of an N-hydroxysuccinimide (NHS) ester of $^{13}CD_3$-acetate (Acetyl + 4 Da) for 1 h at 30°C. This step was once repeated and allows to distinguish *in vivo* from *in vitro* acetylation. The NHS-ester was quenched with 40mM of glycine and possible O-acetylation of the side-chains of serine, threonine and tyrosine was reversed with 100mM of hydroxylamine. This was followed by a desalting step over a NAP-10 column and overnight digestion with trypsin in a trypsin/protein ratio of 1/50 (w/w) – which now only cleaved after Arg residues as since Lys residues were acetylated – in 10 mM ammonium bicarbonate pH7.6. Following digestion, N-terminal pyroglutamates were removed prior to the Strong Cation Exchange (SCX) step which enriches for N-terminal peptides. Note that this step was performed to prevent pyroglutamate-starting peptides from running through the SCX-column and thereby being enriched. In this step 25µl purified pGAPase (625 mU) was incubated at 37°C for 10 minutes with 1 µl of 800 mM NaCl, 1 µl of 50 mM EDTA (pH 8.0) and 11 µl of freshly prepared 50 mM cysteamine for activation. The peptide sample was resuspended in 212 µl of freshly made pyro-glu buffer containing 16 mM NaCl, 0.5 mM EDTA, 3 mM cysteamine and 50 µM aprotinin. The activated pGAPase (625 mU) was added to each sample together with 25 µl of Q-cyclase (1250 mU) and incubated for 1 h at 37 °C. Next, N-terminally blocked peptides were enriched by SCX (mixed mode SCX cartridge, Bond Elut certify 200 mg, 3 ml, 40µm) in 10 mM sodium phosphate buffer pH 3 (SCX buffer A) as such peptides will not bind to the SCX resin at this pH. The SCX run-through fractions that were collected in 6 ml of 10 mM sodium phosphate buffer pH 3 with 70% acetonitrile (SCX buffer B) were vacuum-dried. Methionine residues were oxidized to their sulfoxide forms in 0.6%

hydrogen peroxide for 30 min at 30°C and the peptides were then purified on OMIX C18 pipette tips (Agilent). These peptides were vacuum-dried and re-suspended in loading solvent (0.1% TFA in water/acetonitrile, 98/2 (v/v)) and about 3 µg was injected for LC-MS/MS analysis on an Ultimate 3000 RSLC nano LC (Thermo) in-line connected to a Q Exactive HF mass spectrometer (Thermo). The peptides were first loaded on a trapping column (made in-house, 100 µm internal diameter (I.D.) × 20 mm, 5 µm beads C18 Reprosil-HD, Dr. Maisch, Germany) and, after flushing from the trapping column, the peptides were separated on an analytical column in the needle (made in-house, 75 µm I.D. × 400 mm, 1.9 µm beads C18 Reprosil-HD, Dr. Maisch) using a non-linear 150 min gradient from 2 to 56% solvent B (0.1% formic acid in water/acetonitrile, 20/80 (v/v)) at a constant flow rate of 250 nL/min and at a constant temperature of 50°C (CoControl 3.3.05, Sonation). Following a 10 min wash reaching 99% solvent B, the column was re-equilibrated with solvent A (0.1% formic acid in water).

The mass spectrometer was operated in data-dependent, positive ionization mode, automatically switching between MS and MS/MS acquisition for the 16 most abundant peaks in a given MS spectrum. The source voltage was set to 2.5 kV and the capillary temperature was 250°C. One MS1 scan (m/z 375-1500, AGC target 3E6 ions, maximum ion injection time of 60 ms), acquired at a resolution of 60,000 (at 200 m/z) was followed by up to 16 tandem MS scans, acquired at a resolution of 15,000 (at 200 m/z) of the most intense ions fulfilling predefined selection criteria: AGC target 1E5 ions, maximum ion injection time of 80 ms, isolation window of 1.5 m/z, fixed first mass of 145 m/z, spectrum data type: centroid, under fill ratio 2%, intensity threshold 1.3E4, exclusion of unassigned and singly charged precursors, peptide match preferred, exclude isotopes on, dynamic exclusion time of 12 s. The normalized

collision energy was set to 28% and the polydimethylcyclosiloxane background ion at 445.12003 Da was used for internal calibration (lock mass).

Materials required for N-terminal peptide enrichment assay

1. NAP-10 columns (GE Haelthcare, cat no. 17-0854-02)
2. Glycine (Bio-Rad Laboratories, cat. no. 161-0718)
3. Tagzyme kit (Qiagen, cat. no. 34342). Purify the pGAPase by means of Ni^{2+}-IMAC.
4. Ammonium bicarbonate (Sigma-Aldrich, cat. no. A-6141)
5. Sodium chloride (Sigma-Aldrich, cat. no. S-6191)
6. Guanidinium hydrochloride (Sigma-Aldrich, cat. no. G3272-500Gr or Roth , Cat no. 0037.1, 1 kg)
7. Tris(2-carboxyethyl)phosphine (TCEP; Pierce, cat. no. 20490)
8. Iodoacetamide (Sigma-Aldrich, cat. no. 57670)
9. Hydroxylamine, 50% (wt. %) in water (Sigma-Aldrich, cat. no. 438227)
10. Sequencing grade modified trypsin (Promega, cat. no. V5111)
11. Aprotinin (Roche, cat. no. 10981532001)
12. EDTA (Serva, cat. no. 11278)
13. Cysteamine hydrochloride (Sigma-Aldrich, cat. no. M6500)
14. Protease inhibitor cocktail Complete, EDTA-free (Sigma Aldrich, cat no. 11873580001)
15. Hydrogen peroxide, 30% (w/w) in water (Sigma-Aldrich, cat. no. H1009)

16. Cell lysis buffer: 50 mM sodium phosphate pH 7.5, 100 mM NaCl, 0.8% CHAPS (w/v) in water, protease inhibitor cocktail tablet
17. Pyro-glu removal buffer: 16 mM NaCl, 0.5 mM EDTA, 3 mM cysteamine and 50 µM aprotinin
18. 12-port vacuum manifold for SPE cartridges (Agilent, cat. no. 5982-9110)
19. Bond Elut Certify cartridges 200 mg, 3 ml, 40µm (Agilent, cat no. 12102145)
20. SCX solvent A1: 60 mg sodium phosphate monobasic (Sigma-Aldrich,#S8282) + 50 ml H_2O
21. SCX solvent B1: 60 mg sodium phosphate monobasic + 15 ml H_2O + 35 ml ACN
22. SCX solvent A2: 57.6 mg H_3PO_4 (Sigma-Aldrich, #345245) + 50 ml H_2O
23. SCX solvent B2: 57.6 mg H_3PO_4 + 15 ml H_2O + 35 ml ACN
24. SCX buffer A: Combine SCX solutions A1 and A2 until the pH reaches 3.0 (approximately A1/A2 3/1 v/v)
25. SCX buffer B: Combine SCX solutions B1 and B2 until the pH reaches 3.0 (approximately B1/B2 1/2.5 v/v)
26. Loading solvent A: 0.1% TFA in 98/2 water/acetonitrile.

N-terminal peptide data analysis

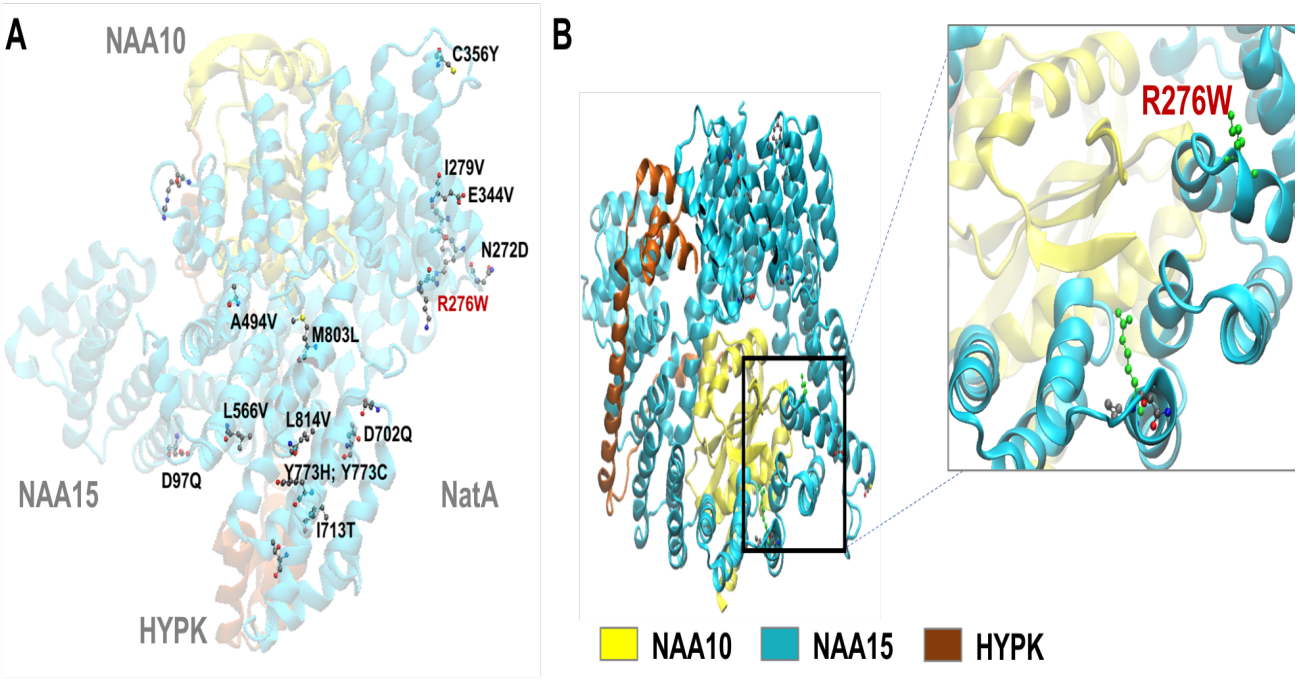
Recorded MS/MS spectra were searched against the human proteins in the Swiss-Prot database by the Mascot algorithm (version 2.5.1). Carbamidomethylation of cysteine residues, oxidation of methionine residues and $^{13}CD_3$-acetylation on lysine residues (Acetyl +4 Da) were set as fixed modifications. Acetylation (Ace) and $^{13}CD_3$-acetylation on the

peptide N-terminus (Acetyl +4 Da) were used for quantitation. The enzyme was set to semiArgC/P. A second, similar search was done in parallel to identify the unlabeled peptides, allowing no modification/label on the peptide N-terminus. In this search internal peptides can be identified. The same settings as above were used, supplemented with pyro-glutamine on N-terminal glutamine residues as a variable modification. Only peptides that were ranked first and scored above the threshold score set at 99% confidence were withheld. Identified peptides were quantified automatically by the Mascot Distiller software. This software tries to fit an ideal isotopic distribution on the experimental data based on the peptide average amino acid composition. This is followed by extraction of the XIC signal (extracted ion current) of both peptide components (light and heavy) from the raw data. Ratios are calculated by integrating the area below the light and heavy isotopic envelope of the corresponding peptide. If the software was able to perform a high-quality isotopic pattern match leading to reliable ratio calculation, this ratio is flagged valid = 'TRUE'. 'FALSE' ratios were not used for further data analysis. If required, manual ratio validation can be performed for selected peptides. The degree of acetylation was calculated using the following equation: Ac % = (ratio (L/H) / ratio (L/H) + 1)x100.

In some cases, peptides were identified and quantified multiple times. In these cases, the mean of the acetylation degree was taken and, additionally, the standard deviation on this mean value was calculated.

A list containing all identified and successfully quantified acetylated N-terminal sequences was created. Acetylation degrees were calculated separately for all samples. Only N-termini

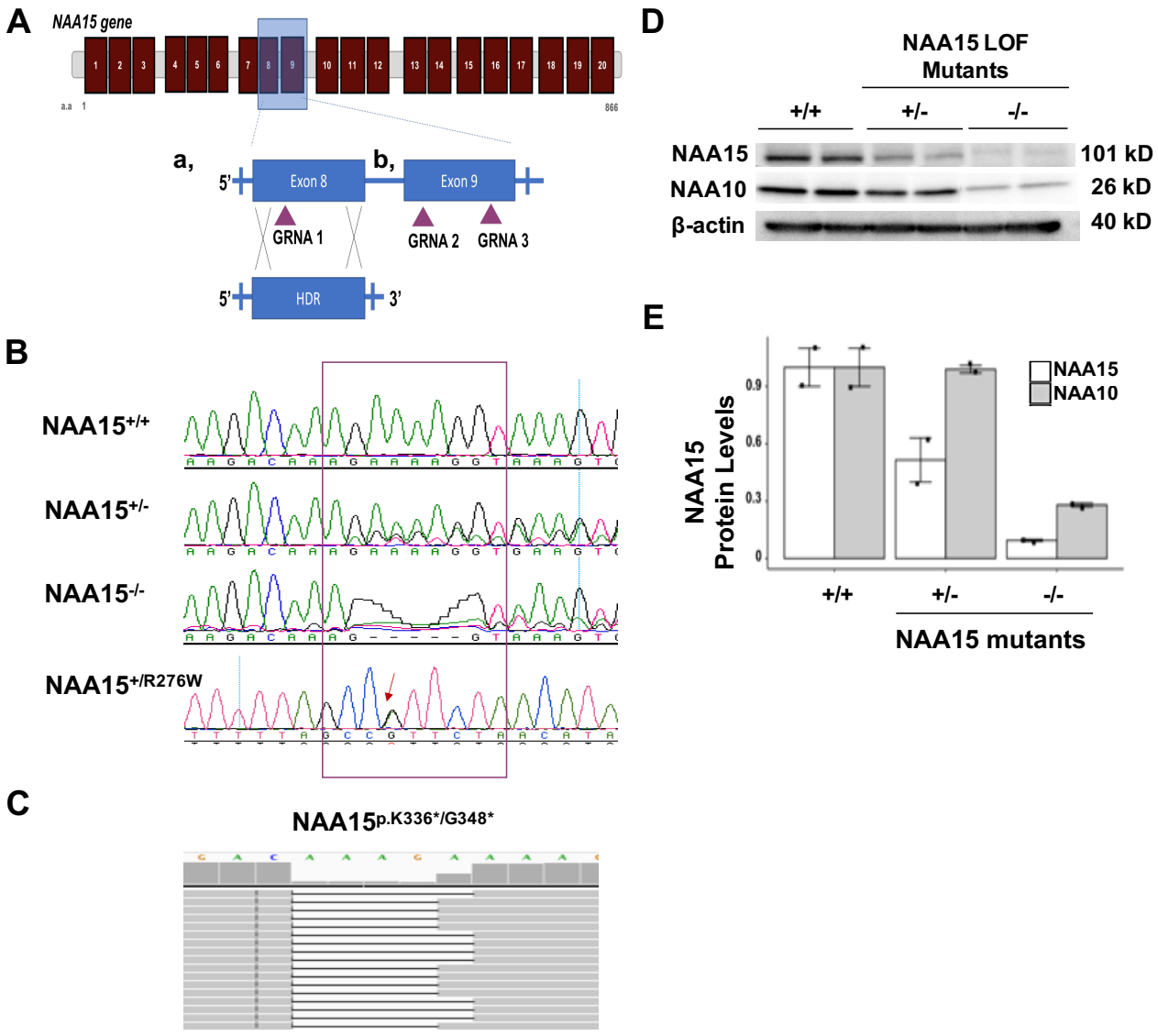
(n=989) which were semi-tryptic and start at amino acid position 1 or 2 (considering iMet-cleavage) were considered.



Online Figure I. NAA15 variants in CHD patients

A) Model of the human NatA complex (subunits: NAA15, NAA10, and HYPK) using visual molecular dynamics. Location of NAA15 variants discovered in CHD patients are indicated on the model. This image of PDB:6C95 includes NAA15 amino acids 1-841.

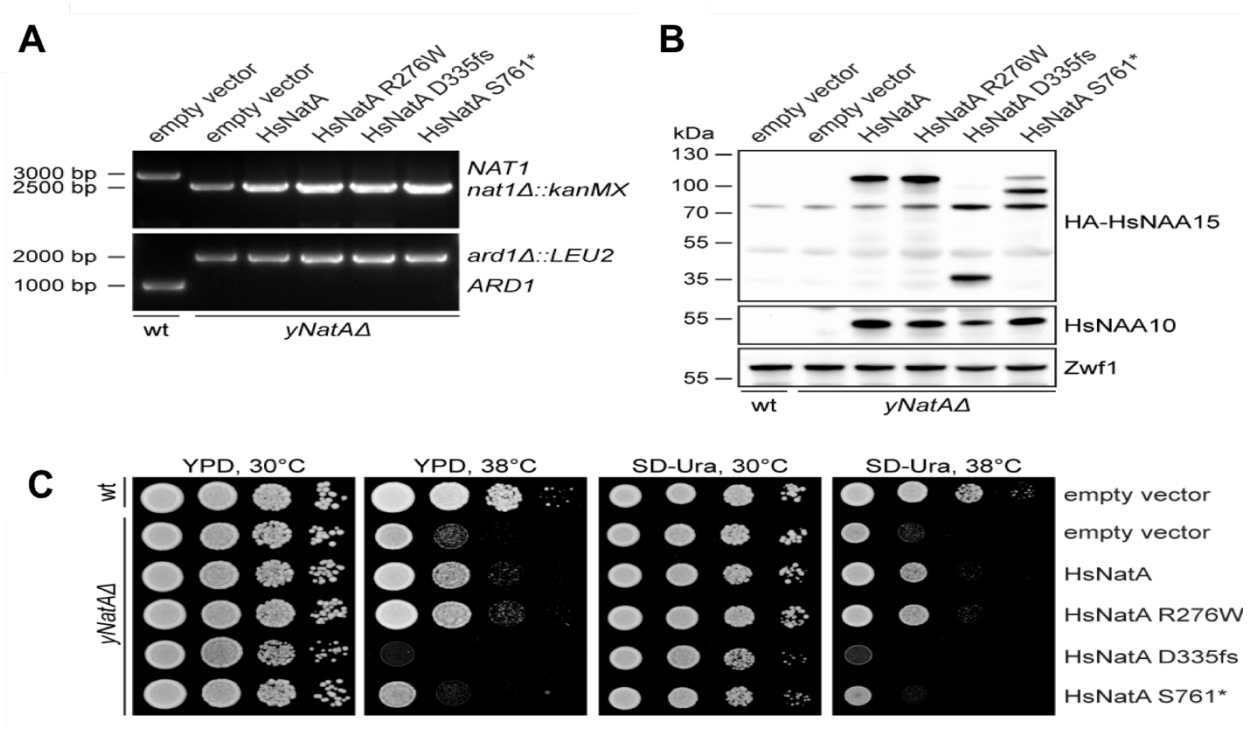
B) Location of missense NAA15$^{+/R276W}$ variant introduced into iPS cells using CRISPR/Cas9.



Online Figure II. *NAA15* variants affect protein expression of the NatA and NatE complex.

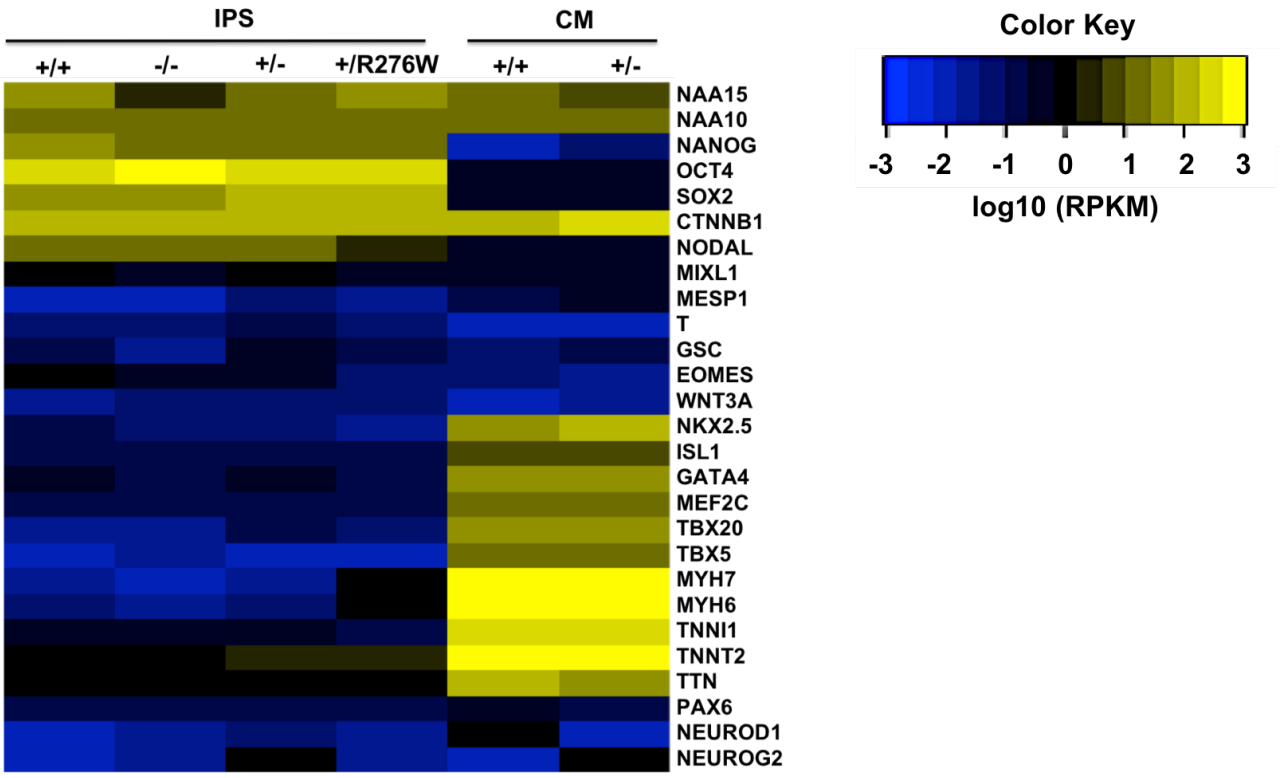
A)Schematic overview of *NAA15* gene and locations of guide RNAs used to introduce CRISPR mutations. Panel a, NAA15 **missense variant (*NAA15*$^{+/R276W}$) was created by homologous directed repair using GRNA 1 (Guide RNA: AGAACGGCTAAAAATTTATGAGG). Panel b, *NAA15* LoF variants (*NAA15*$^{K336*/G348*}$,**

$NAA15^{L314*/L314*}$, $NAA15^{+/K336*}$, and $NAA15^{+/G348*}$) were created using nonhomologous end joining using GRNA 2 (Guide RNA: ATTATACAAAGACAAAGAAAAGG) or GRNA 3 (Guide RNA: CCTTGCTGAAATTCATCCTTAGG). B) Representative images display confirmed sanger sequencing of $NAA15^{+/+}$, $NAA15^{+/-}$, $NAA15^{-/-}$, and $NAA15^{+/R276W}$ iPS cells C) Validation of sequencing was further confirmed by MiSeq. IGV image displaying alignments of MiSeq reads from the $NAA15^{-/-}$ iPS cell line, $NAA15^{p.K336*/G348*}$. D) Western analysis of *NAA15* LoF mutant iPSCs. Proteins were detected on immunoblots using NAA15, NAA10, and Beta-actin antibodies. Compared to wildtype iPS cells, NAA15 protein levels are reduced in the $NAA15^{+/-}$ and $NAA15^{-/-}$ iPS cells. NAA10 protein levels were reduced in $NAA15^{-/-}$ iPS cells only. E) Densitometry of western blots of two biological replicates of wildtype, $NAA15^{+/-}$, and $NAA15^{-/-}$ iPS cells normalized to Beta-actin loading control.



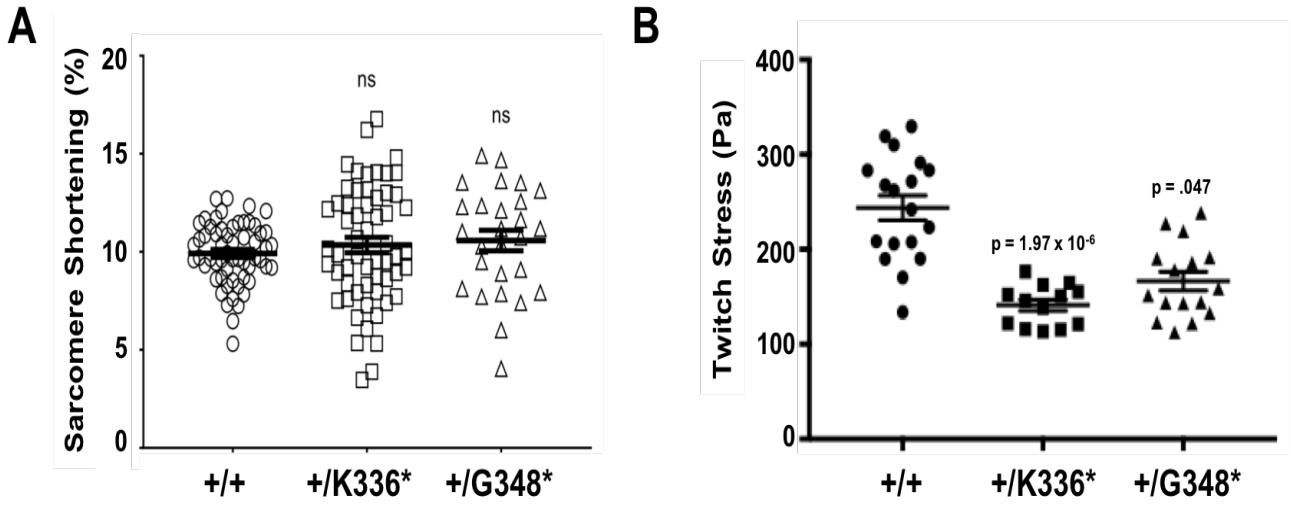
Online Figure III. Human NAA15 D335fs and S761* mutants display impaired NatA functionality *in vivo*.

A) The genotype of the *yNatAΔ* strain (*ard1-Δ::LEU2; nat1-Δ::kanMX*) was confirmed by colony PCR using *ARD1* and *NAT1* primers. *ARD1*-specific PCR product 1024 bp (*ARD1* 717 bp + gene specific sequence 307 bp) and *NAT1*-specific PCR product 2976 bp (*NAT1* 2565 bp + gene specific sequence 411 bp). B) *yNatAΔ* mutant cells were transformed with a bidirectional expression vector encoding wild-type or mutated variants of the human NatA complex (HsNatA). HsNatA expression was confirmed by immunoblot analysis using anti-HsNAA10 and anti-HA (to detect HA-HsNAA15) and anti-Zwf1 served as loading control. C) The indicated strains were grown to early log-phase in SD-Ura medium and tenfold serial dilutions were spotted onto YPD or SD-Ura plates and incubated for 2 days at permissive (30°C) or non-permissive (38°C) temperature.

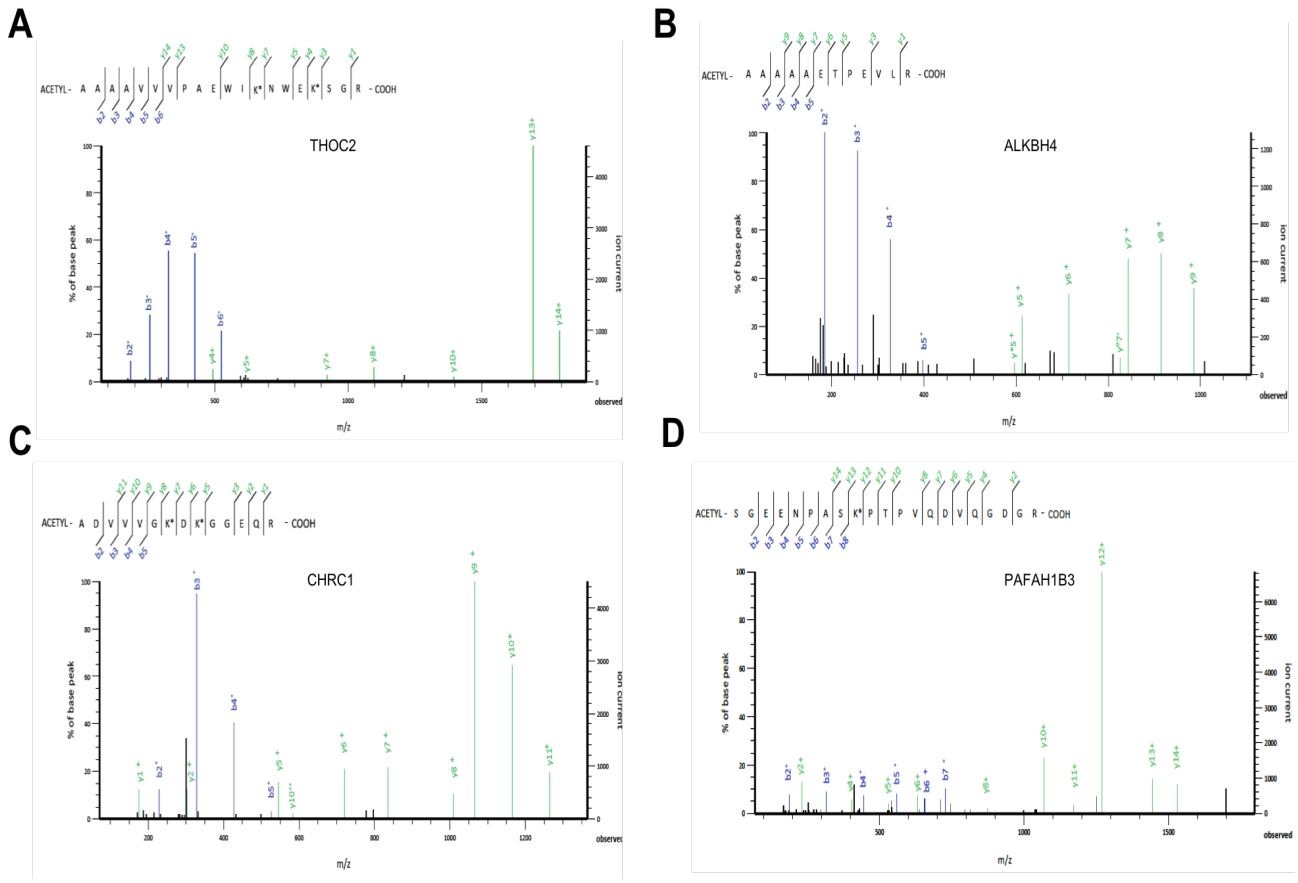


Online Figure IV. NAA15$^{+/-}$ and NAA15$^{+/R276W}$ iPS cells develop into cardiomyocytes, while NAA15$^{-/-}$ iPS cells do not.

Expression of significant cardiac differentiation markers in iPS and cardiomyocytes. Downregulation of TTN was observed in heterozygous cells.

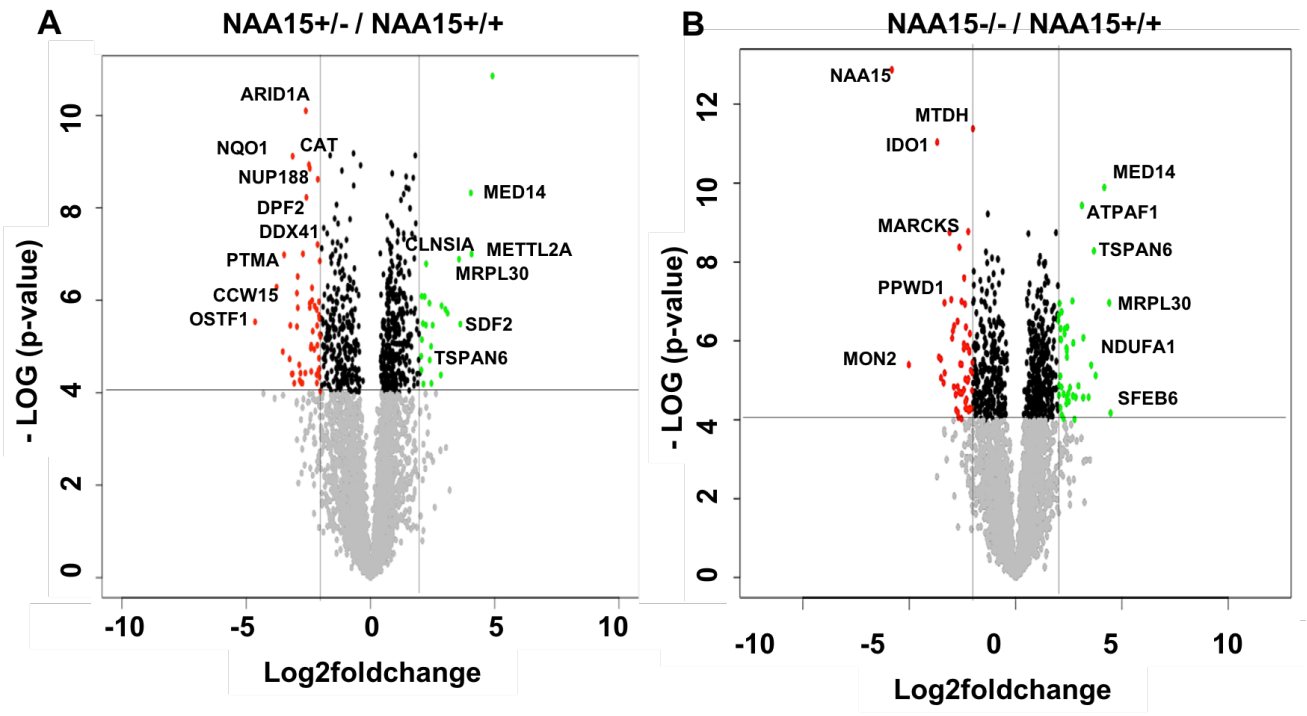


Online Figure V. Contractile function of NAA15$^{+/-}$ and wildtype iPS cardiomyocytes grown on 2D ('unloaded') and 3D microtissues ('loaded'). A) Sarcomere shortening is shown as a percentage of initial sarcomere length, of wildtype (NAA15$^{+/+}$) iPSC-CMs and two biological replicates of NAA15$^{+/-}$ iPSC-CMs grown in 2D culture. IPSCs were differentiated into cardiomyocytes to day 30. Unloaded contractile measures show no statistical differences (denoted as NS) in sarcomere shortening. Measurements were collected in NAA15$^{+/+}$ and NAA15$^{+/K336*}$ (n= 58 videos), and NAA15$^{+/G348*}$ (n=27 videos) from 3 tissue culture wells. All values are stated as mean±SEM. B. Loaded contractile force measurements from one biological replicate of NAA15$^{+/+}$ iPSC-CMs and two biological replicates of NAA15$^{+/-}$ iPSC-CMs paced at 1Hz. Each 'dot' indicates mean systolic stress of (>3) twitch cycles on a particular 3D microtissue. Systolic stress was calculated using a custom Matlab Script[37]. All values are stated as mean ± SEM (see Online METHODs).



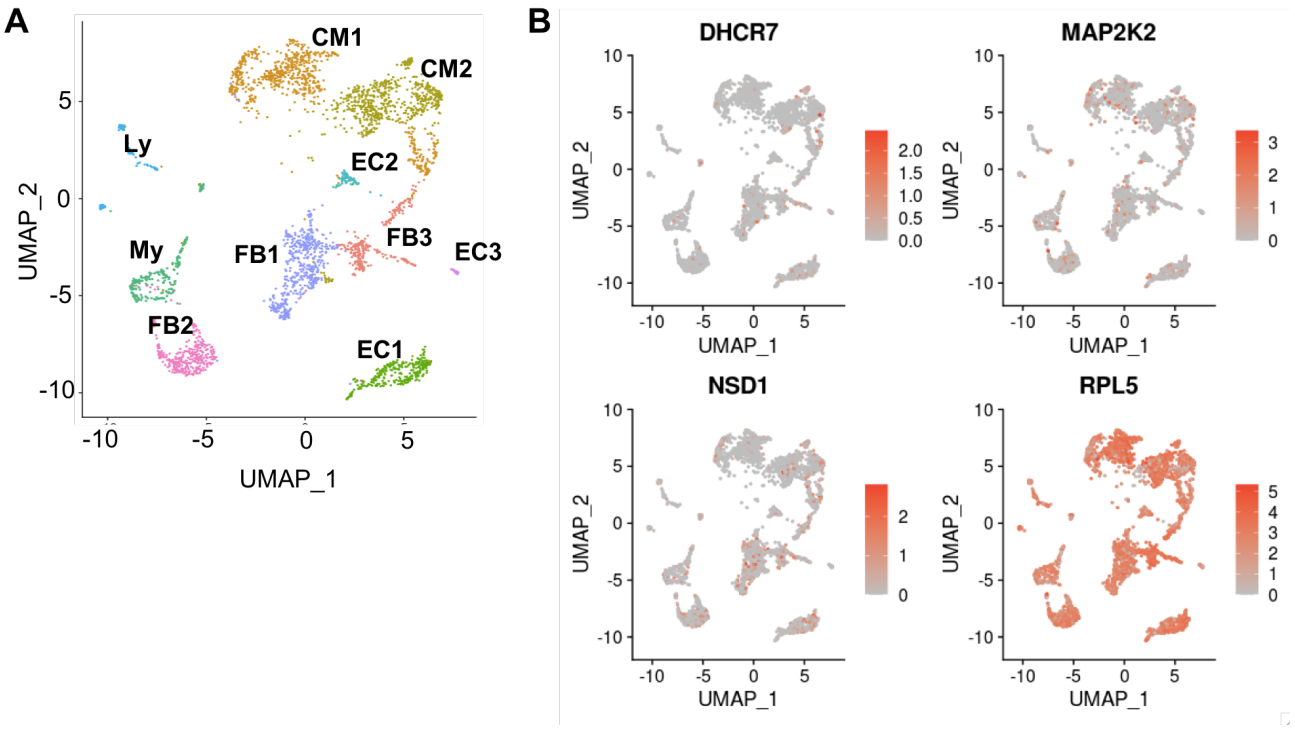
Online Figure VI. MS/MS validation of N-terminal acetylation sites in tryptic peptides affected in NAA15 mutant iPSCs.

Representative MS spectrums in NAA15$^{+/+}$ iPSCs of A) THO complex subunit 2 (Acetyl-AAAAVVVPAEWIKNWEKSGR-COOH (1158.6293 m/z, 2+) B) Alpha-ketoglutarate-dependent dioxygenase (Acetyl-AAAAAETPPEVLR-COOH (620.8278, 2+) C) Chromatin accessibility complex protein 1 (Acetyl-ADVVVGKDKGGEQR-COOH (796.4279, 2+) D) Platelet-activating factor acetylhydrolase IB subunit gamma (Acetyl-SGEENPASKPTPVQGDGR-COOH (1128.5358 m/z, 2+). The fragmentation spectrums are shown with annotated y-ions (green) and b-ions (blue). The b-ion series covers the acetylation site. * In-vitro 13CD3-acetylation on lysine residues.



Online Figure VII. Proteins are differentially expressed in NAA15 mutated iPS cells

A-B) Volcano plot of proteins differentially expressed in NAA15 mutated iPS cells. Proteins with increased expression are highlighted in green (-LOG(p value) > 4 and Log2foldchange > 2) Proteins with decreased expression are highlighted in red (LOG(p value) > 4 and Log2foldchange < -2).



Online Figure VIII. Fetal Heart Gene Expression of CHD associated proteins downregulated in NAA15$^{-/-}$ and NAA15$^{+/-}$ iPSCs.

A) UMAP plot showing distribution of cells in the fetal heart. CM1;CM2 = Cardiomyocytes, EC1;EC2;EC3 = Endothelial cells, FB1;FB2;FB3 = Fibroblasts; My=Myeloid cells, Ly=Lymphoid cells B) Fetal RNA expression of genes encoding for proteins downregulated in both NAA15$^{-/-}$ iPSCs and NAA15$^{-/-}$ iPSCs. All four genes are known to cause autosomal dominant CHD.