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SUPPLEMENTAL METHODS

Clinical evaluation. The diagnosis of DCM was made based on current practice guidelines.^{1,2}

Biochemical analysis in both affected siblings included quantitative analysis of lactate, amino acids, organic acids, carnitine and acylcarnitines, oligosaccharides, and isoelectric focusing of transferrin and apolipoprotein C-III. Family members who participated in this study underwent cardiac screening with electrocardiogram and echocardiography.

Exome sequencing. Genomic DNA was extracted from peripheral blood samples using standard protocols, and fragmented by sonication. Exons were captured using the SureSelect Human All Exon V4 (Agilent Technologies). Sequencing was performed on a HiSeq 2000 system (Illumina) for 101 base pair paired-end runs. Reads were mapped to the human reference genome GRCh37/hg19 using the Burrows-Wheeler Aligner (BWA).³ Variants were called using the Genome Analysis Toolkit (GATK),⁴ and filtered using Cartagenia Bench Lab software. We selected for rare variants, defined as variants with a minor allele frequency <0.1% in public variant databases, including the Genome Aggregation Database (gnomAD), the NHLBI GO Exome Sequencing Project (ESP), and the Genome of the Netherlands (GoNL). We only included non-synonymous coding and splice site (± 10 bp from exon-intron boundaries) variants with a minimum coverage of 10 reads. Apparent *de novo*, homozygous and compound heterozygous variants were considered for further analysis.

Sanger sequencing. Bidirectional Sanger sequencing of the entire coding region and exon-intron boundaries of the candidate genes was performed using PCR primers designed by Primer3 software ([Supplemental Table 1](#)). PCR products were purified and subsequently sequenced using the BigDye Terminator v3.1 kit on an ABI 3730xl DNA Analyzer (Life Technologies). Sequence data was analyzed using SeqScape v2.5 software. For annotation of DNA and protein changes, the mutation nomenclature recommendations from the Human Genome Variation Society were followed. To describe variants at the cDNA level, the A of the translation initiation codon of the reference sequence was designated as position +1.

Histology and immunostaining. Paraffin-embedded, hematoxylin and eosin (H&E) stained myocardial tissue from both affected siblings was examined using standard techniques. For immunohistochemistry, sections were deparaffinized and rehydrated before antibody retrieval. Primary antibodies included: full-length rabbit polyclonal anti-TRC40 (non-commercial, dilution 1:400),⁵ mouse monoclonal anti-N-cadherin (Agilent Technologies #M3616, dilution 1:200), and mouse monoclonal anti-desmin (Ventana Medical Systems #760-2513, prediluted). The slides were counterstained with hematoxylin II for 8 minutes and bluing reagent for 8 minutes according to the manufacturer's instructions (Ventana Medical Systems). Immunostained preparations were analyzed by light microscopy. Glutaraldehyde-fixed myocardial tissue was prepared for electron microscopy. Immunolabeling was performed on cryosections as described previously.⁶ Primary antibodies included: mouse monoclonal anti-N-cadherin (Sigma-Aldrich #C3865, 1:800) and mouse monoclonal anti-emerin (Novocastra Laboratories #NCL-EMERIN). Secondary labeling was performed with appropriate Texas Red (N-cadherin) or fluorescein isothiocyanate-conjugated antibodies (emerin). After immunolabeling, sections were analysed with a Nikon Eclipse 80i epifluorescence microscope, and images were taken using a DS-2BWC digital sight camera and NIS-Elements BR 3.0 software (Nikon Instruments).

Western blotting. Cultured skin fibroblasts from patient II:2 and three control individuals were lysed in 100 μ L TNE buffer [50 mM Tris-HCl (pH 7.6), 100 mM NaCl, 50 mM NaF, 1% (v/v) Triton X-100] and cOmplete Protease Inhibitor Cocktail tablets (Roche Applied Science). Lysates were centrifuged for 10 minutes at 10,000 rpm to remove small cell debris. Equal amounts (20 μ g or 40 μ g) of protein were separated on a 4-15% precast polyacrylamide gel (Bio-Rad Laboratories). Rabbit polyclonal antibodies against the full-length (non-commercial; dilution 1:2000) and N-terminus (non-commercial; dilution 1:2000) of human ASNA1 were used for detection.⁵ Results were normalized to the GAPDH loading control. Note: these experiments were only performed in patient II:2, as we did not have access to cultured skin fibroblasts from patient II:3.

***In vitro* synthesis of mRNA.** Total RNA was extracted from human skin fibroblasts using the RNeasy Mini Kit (QIAGEN), and converted into cDNA using the iScript Reverse Transcription

Supermix (Bio-Rad Laboratories, #1708840). Products were ligated into the pCMV6-entry vector with C-terminal Myc-DDK tag, and subsequently transformed into XL10-Gold ultracompetent cells (Stratagene). All constructs were verified by DNA sequencing. Expression of recombinant proteins was checked after transfection into human embryonic kidney (HEK) 293 cells using previously described rabbit polyclonal antibodies raised against the full-length and N-terminal peptide of human ASNA1.⁵ Linearized constructs were used as a template for *in vitro* synthesis of capped mRNA using the mMMESSAGE mMACHINE T7 Transcription Kit (Thermo Fisher Scientific).

Purification of recombinant ASNA1. The construct for wild-type zebrafish ASNA1 (TRC40) expression in *E. coli* has been described previously.⁷ It contains an N-terminal 6xHis tag and tobacco etch virus (TEV) protease cleavage site, followed by the full-length ASNA1 open reading frame. The Val163Ala variant was introduced into this construct by site-directed mutagenesis and verified by sequencing. Expression and purification from *E. coli* used minor modifications of previously published methods.^{7, 8} Briefly, the Rosetta BL21(DE3) pLysS strain of *E. coli* (Novagen) was transformed with the plasmid for wild-type or mutant ASNA1, and a single colony was used to grow an overnight starter culture. This was expanded to either 1 L or 6 L (for the wild-type and mutant cultures, respectively), and when the absorbance at 600 nm was between 0.4 to 0.6, isopropyl β -D-1-thiogalactopyranoside (IPTG) was added to 1 mM. After 3 hours at 37°C, the cells were collected by centrifugation at 4°C, washed once in ice cold PBS supplemented with 250 mM NaCl, and re-collected by centrifugation. The washed cells were resuspended in 35 mL of ice cold lysis buffer [PBS with 250 mM NaCl, 5 mM 2-mercaptoethanol, and 1X cOmplete EDTA-free Protease Inhibitor Cocktail mix (Roche)]. After lysis by sonication, the insoluble material was sedimented by centrifugation at 18,000 rpm for 30 minutes at 4°C. The soluble extract was adjusted to 20 mM imidazole, then passed over a 3 mL column of Ni-NTA resin, washed three times in 10 mL of lysis buffer supplemented with 20 mM imidazole, and eluted with lysis buffer supplemented with 250 mM imidazole. The peak fractions (identified by absorbance at 280 nm) were pooled, mixed with TEV protease (at a protein ratio of 1:100), and dialyzed overnight against dialysis buffer (150 mM KAc, 50 mM HEPES, pH 7.4, 2 mM MgCl₂, 10% glycerol, 7 mM 2-mercaptoethanol). Insoluble material was

removed by centrifugation, and the dialyzed sample was passed over a 3 mL column of Ni-NTA to remove the cleaved tag and TEV protease. The flow-through was collected and concentrated to ~4 mg/mL by centrifugal concentrators (Amicon). The protein was snap-frozen in liquid nitrogen and stored in aliquots at -80°C.

Analysis of ASNA1 protein function *in vitro*. Thermal stability of the purified wild-type and Val163Ala mutant ASNA1 protein was analyzed using the Prometheus NT.48 system (NanoTemper Technologies). Purified protein at 0.8 mg/mL was monitored for intrinsic tryptophan fluorescence during a temperature ramp from 20°C to 95°C. A change in the ratio of emission at 330 nm and 350 nm was used to measure unfolding. The ability of ASNA1 protein to capture TA protein was assayed exactly as described previously.⁷ In short, ³⁵S-labeled TA protein containing the transmembrane domain of VAMP2 (referred to simply as VAMP2 hereinafter) was assembled with the upstream chaperone SGTA. The TA protein contained a photo-crosslinking residue within the transmembrane domain to monitor its interactions. The SGTA-VAMP2 complex was then mixed with the bridging cBAG6 complex and either wild-type or mutant ASNA1. After incubating at 32°C for 90 seconds, the reaction was transferred to ice and irradiated with UV to induce crosslinking for 10 minutes. The samples were analyzed by SDS-PAGE and autoradiography to determine whether VAMP2 was successfully transferred from SGTA to ASNA1. To test the functionality of ASNA1 for TA protein insertion, a complex between ASNA1 and VAMP2 was assembled as before,⁷ and incubated with ER microsomes for between 0 to 15 minutes at 32°C. The samples were then analyzed by SDS-PAGE and autoradiography. Insertion of the TA protein was monitored by its glycosylation at a site located near the C-terminus. The ER microsomes used for this assay were derived from HEK293 cells and were prepared as described before.⁹

CRISPR/Cas9 targeting of zebrafish *asna1*. Zebrafish *asna1* (ENSDARG00000018190) was targeted by Cas9/gRNA complex injection as described previously.¹⁰ The online program CRISPRscan (www.crisprscan.org) was used to design a single-stranded guide RNA (gRNA) targeting exon 5 in *asna1* (5'-CCAAACTGGAGGAGACGCTGC-3'), approximately in between the variants identified in the parents. The gRNAs were obtained by *in vitro* transcription of synthetic

oligonucleotides containing a minimal T7 RNA polymerase promoter using the mMACHINE mMACHINE T7 Ultra Kit (Thermo Fisher Scientific). SP-Cas9 plasmid was a gift from Niels Geijssen (Addgene plasmid #62731).¹¹ A mix of 100 pg of either gRNA and 650 pg Cas9 protein was injected into single-cell stage zebrafish embryos. Injected embryos were raised to adulthood (F0) and analyzed for genomic modifications at the target site by Sanger sequencing and the online tool Tracking Indel by DEcompensation (TIDE).¹² In two individual F0 founder fish, ~30% of the mapped reads contained indels at the target site in exon 5 (**Supplemental Figure 1**). We screened their offspring (F1) for germline transmission using PCR followed by restriction enzyme digestion (**Supplemental Table 2**), and identified three fish (25%) that carried a heterozygous 7 base pair deletion ($\Delta 7$). These fish were used for further breeding to create a stable mutant line. For rescue experiments, 300 pg wild-type or mutant human *ASNA1* mRNA (see above) was injected in the yolk at the single-cell stage. Expression of MYC-tagged human ASNA1 was confirmed by Western blot analysis with an anti-MYC primary antibody.

Phenotypic analysis of mutant zebrafish. Zebrafish were anesthetized with tricaine methanesulfonate (MS-222) and imaged using a Leica M165 FC stereo microscope connected to a Leica DFC550 digital camera. Zebrafish were positioned horizontally in 5% methylcellulose to obtain a lateral view of the ventricle (**Supplemental Figure 2**). Heart rate (beats/minute) was calculated by three independent counts of the number of beats in 15 second intervals. Fractional shortening (%) was derived from linear measurements of the ventricle at end-diastole and end-systole.¹³ Blood flow rate, that can be used an indirect measure of cardiac function, was determined by visual inspection of circulating red blood cells passing through the dorsal aorta and classified as “normal”, “decreased” or “absent”. For microscopic analysis, zebrafish larvae ($n=4$ for each group) were anesthetized, fixed in Karnovsky fixative (PBS containing 2% paraformaldehyde and 3% glutaraldehyde), and embedded in Epon. Semithin sections (1 μm) were stained with toluidine blue and studied under a light microscope. Ultrathin sections (70 nm) were stained with 5% uranyl acetate and 2.5% lead citrate, and photographically recorded using a JEOL 1200-EX II transmission electron microscope.

Bioinformatics. In order to find proteins that might be affected by defective ASNA1-mediated membrane insertion, we obtained a list of all human single-pass membrane proteins from UniProt.¹⁴ We first removed all proteins that contain an N-terminal signal sequence, and from the remainder, selected for proteins that contain a transmembrane domain within the last 50 residues from the C-terminus. The final list contained 286 predicted human TA proteins (**Supplemental Table 3**). We investigated the potential association between the corresponding genes and cardiomyopathy using the Online Mendelian Inheritance in Man database (<https://www.omim.org>).

Statistical analysis. Statistical analyses were performed using Microsoft Excel or GraphPad Prism software. Continuous variables were expressed as means \pm standard deviation, and compared using the Student's *t*-test. Categorical variables were expressed as counts and percentages, and compared using the Fisher's exact test. An asterisk (*) indicates *p*-values lower than 0.05.

SUPPLEMENTAL TABLES

Supplemental Table 1. List of primer sequences used for Sanger sequencing.

| Target | Direction | Primer sequence (5' - 3') | Product size (bp) |
|---------------------|-----------|---------------------------|-------------------|
| <i>ASNA1</i> exon 1 | F | tcctaaaaggcaagtaatgagga | 367 |
| | R | gtggaaaagccggccttg | |
| <i>ASNA1</i> exon 2 | F | ctgctccaggaacctacc | 389 |
| | R | tggtcccttgtagtatgttg | |
| <i>ASNA1</i> exon 3 | F | cccctgttttgaccctt | 470 |
| | R | AAGTTCATGCCCTTCACCAG | |
| <i>ASNA1</i> exon 4 | F | ATCGATGAGGCCATGAGCTA | 375 |
| | R | tgggaaggaaaggaattgt | |
| <i>ASNA1</i> exon 5 | F | ccactgggaggtatcaggag | 599 |
| | R | caggaggctagaggcagag | |
| <i>ASNA1</i> exon 6 | F | TCAAGGACCCTgtgagtgg | 400 |
| | R | caggaggctagaggcagag | |
| <i>ASNA1</i> exon 7 | F | cactctgtctctgccttctg | 299 |
| | R | GGCTCCCCCTGTATTATGG | |

F: forward; R: reverse.

Supplemental Table 2. List of oligonucleotide sequences used in zebrafish studies.

| Target | Direction | Primer sequence (5' - 3') | Product size (bp) |
|---------------------|------------------|----------------------------------|--------------------------|
| <i>asnal</i> exon 5 | F | TAAAGCCCATTCCTGAGTGC | 404 |
| | R | TTGAAGTGGATGGATGATGG | |

F: forward; R: reverse.

The PCR product was subjected to restriction enzyme digestion by BsrI. As a result of the 7 bp deletion induced by CRISPR/Cas9, one BsrI enzyme restriction site will be lost and the mutant allele will only be cut once. Subsequent gel electrophoresis will reveal three bands in wild-type (199, 131 and 74 bp), four bands in *asnal*^{Δ7/+} (266, 199, 131 and 74 bp), and two bands in *asnal*^{Δ7/Δ7} (266 and 131 bp) zebrafish.

Supplemental Table 3. List of predicted human tail-anchored proteins.

| Entry | Protein names | TMD sequence |
|--------------|---|-------------------------|
| E0CX11 | Short transmembrane mitochondrial protein 1 | GFTLGNVVGMVLAQNYD |
| Q8NDB6 | Protein FAM156A/FAM156B (Transmembrane protein 29/29B) | WETLVQGLSGLTSLGT |
| Q9H7X2 | Uncharacterized protein C1orf115 | VVIGLQGFAAAYSAPFAVATSVV |
| Q8TCY0 | Small integral membrane protein 11B | MEFPLCGCLSLILHHFA |
| Q96PS6 | Putative uncharacterized protein GAFA-1 (Gene associated with FGF-2 activity protein 1) | IHLYVMASAMSSSPIFFFFQ |
| O75438 | NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 1 (Complex I-MNLL) (CI-MNLL) (NADH-ubiquinone oxidoreductase MNLL subunit) | HWVHVLVPMGFVIGCYL |
| Q9H1C7 | Cysteine-rich and transmembrane domain-containing protein 1 | LGPSTCLTACWTALCCCC |
| Q9HDD0 | Phospholipid-metabolizing enzyme A-C1 (EC 2.3.1.-) (EC 3.1.1.-) (HRAS-like suppressor 1) (HRSL1) | ISTVEFVTAAGVFSFLGLFPGQ |
| L0R6Q1 | SLC35A4 upstream open reading frame protein | ASAVLGFAVGTCTGIYAAQAYAV |
| Q96I36 | Cytochrome c oxidase assembly protein COX14 | FSTSMMLLTVYGGYLCSVRVYHY |
| P21397 | Amine oxidase [flavin-containing] A (EC 1.4.3.4) (Monoamine oxidase type A) (MAO-A) | VSGLLKIIGFSTSVTALGFVL |
| O75452 | Retinol dehydrogenase 16 (EC 1.1.-.-) (Microsomal NAD(+)-dependent retinol dehydrogenase 4) (RoDH-4) (Short chain dehydrogenase/reductase family 9C member 8) (Sterol/retinol dehydrogenase) | LLYLPMSYMPFTFLVDAIMYWV |
| Q9BVW6 | Small integral membrane protein 2 | GHAISILFGFWTSFICDTYIVLA |

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| Q75NE6 | Putative microRNA 17 host gene protein (Putative microRNA host gene 1 protein) | LNVPKLVLIYLSHFVLFSSMC |
| Q9UMX3 | Bcl-2-related ovarian killer protein (hBOK) (Bcl-2-like protein 9) (Bcl2-L-9) | WLVAALCSFGRFLKAAFFVLL |
| Q8TCP9 | Protein FAM200A | ILLLLPFTTTYLCELGFSIL |
| O95167 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 3 (Complex I-B9) (CI-B9) (NADH-ubiquinone oxidoreductase B9 subunit) | LVVSFVVGGLAVILPPLSPYF |
| Q07812 | Apoptosis regulator BAX (Bcl-2-like protein 4) (Bcl2-L-4) | TVTIFVAGVLTASLTIWKKMG |
| Q8WXE9 | Stonin-2 (Stoned B) | IWLMLPTPFVHPTTLPLLFLLAM |
| Q9NX95 | Syntabulin (Golgi-localized syntaphilin-related protein) (Syntaxin-1-binding protein) | SFLVDLLAVAAPVVPTVLWAF |
| Q6ZSY5 | Protein phosphatase 1 regulatory subunit 3F (R3F) | VLAGLVVVPVALNSGVSLVL |
| Q3KP22 | Membrane-anchored junction protein | AATGFFGFLSSLFPFRYFF |
| A8MTT3 | Protein CEBPZOS (CEBPZ antisense RNA 1) (CEBPZ opposite strand) | GVLVAELVGVFAYFLFS |
| Q07817 | Bcl-2-like protein 1 (Bcl2-L-1) (Apoptosis regulator Bcl-X) | FNRWFLTGMTVAGVVLL |
| P56378 | 6.8 kDa mitochondrial proteolipid | VYQEIWIGMGLMGFIVYKI |
| O15079 | Syntaphilin | YIVDLLAVVVPVPTVAWLC |
| Q9NRY6 | Phospholipid scramblase 3 (PL scramblase 3) (Ca(2+)-dependent phospholipid scramblase 3) | VKAVLLGATFLIDYMF |
| Q9NUB4 | Uncharacterized protein C20orf141 | LLLLMGLGPLLRACGMPLTLL |
| O95139 | NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6 (Complex I-B17) (CI-B17) (NADH- | SIFVFTHVLVPVWIIHYYM |

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| | ubiquinone oxidoreductase B17 subunit) | |
| O43676 | NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 3 (Complex I-B12) (CI-B12) (NADH-ubiquinone oxidoreductase B12 subunit) | VFFKGFKWGFAAFVVAVGAEYYL |
| Q8NCU8 | Uncharacterized protein encoded by LINC00116 | LQLSVLVAFASGVLLGW |
| Q8N4H5 | Mitochondrial import receptor subunit TOM5 homolog | SIRNFLIYVALLRVTPFIL |
| Q9NRY7 | Phospholipid scramblase 2 (PL scramblase 2) (Ca(2+)-dependent phospholipid scramblase 2) | MKAVMIGACFLIDYMF |
| Q8N7S6 | Uncharacterized protein ARIH2OS (Ariadne-2 homolog opposite strand protein) | CILTALLAVSFHSIGVVIMTS |
| Q9UL19 | Retinoic acid receptor responder protein 3 (EC 3.1.1.-) (HRAS-like suppressor 4) (HRSL4) (RAR-responsive protein TIG3) (Retinoid-inducible gene 1 protein) (Tazarotene-induced gene 3 protein) | KVEVGVATALGILVVAGCSFAI |
| A2RU48 | Single-pass membrane and coiled-coil domain-containing protein 3 | IGASLLGSIGVAVLGLGIDMI |
| P03928 | ATP synthase protein 8 (A6L) (F-ATPase subunit 8) | VWPTMITPMLLTLFLIT |
| P0DJ07 | Protein PET100 homolog, mitochondrial | IFRMIIYLTFPVAMFWVS |
| Q5TGZ0 | MICOS complex subunit MIC10 (Mitochondrial inner membrane organizing system protein 1) | AVVKIGTGFGGLGIVFSLTFF |
| O15162 | Phospholipid scramblase 1 (PL scramblase 1) (Ca(2+)-dependent phospholipid scramblase 1) (Erythrocyte phospholipid scramblase) (MmTRA1b) | MKAVMIGACFLIDFMFF |
| O15239 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 1 (Complex I-MWFE) (CI-MWFE) (NADH-ubiquinone oxidoreductase MWFE subunit) | MWFEILPGLSVMGVCLLIPGL |

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| A6NCI5 | Putative transmembrane protein encoded by LINC00862 (Small integral membrane protein 16) | IMALILMPSLHCFGNILILLF |
| Q9NRQ2 | Phospholipid scramblase 4 (PL scramblase 4) (Ca(2+)-dependent phospholipid scramblase 4) (Cell growth-inhibiting gene 43 protein) (TRA1) | MKAMIFGACFLIDFMYF |
| Q9BSJ5 | Uncharacterized protein C17orf80 (Cell migration-inducing gene 3 protein) (Human lung cancer oncogene 8 protein) (HLC-8) | GFGGITMLFTGYFVLCCSWSF |
| P08574 | Cytochrome c1, heme protein, mitochondrial (Complex III subunit 4) (Complex III subunit IV) (Cytochrome b-c1 complex subunit 4) (Ubiquinol-cytochrome-c reductase complex cytochrome c1 subunit) (Cytochrome c-1) | MLMMMALLVPLVYTI |
| Q9HD87 | Putative uncharacterized protein C6orf50 (Nasopharyngeal carcinoma-associated gene 19 protein) | IISLLAIFIKMCLWLWKQFL |
| P60602 | Reactive oxygen species modulator 1 (ROS modulator 1) (Epididymis tissue protein Li 175) (Glyrichin) (Mitochondrial targeting GxxxG motif protein) (MTGM) (Protein MGR2 homolog) | GFVMGCAVGMAAGALFGTFSCLR |
| Q9P0U1 | Mitochondrial import receptor subunit TOM7 homolog (Translocase of outer membrane 7 kDa subunit homolog) | FAIRWGFIPLVIYLG |
| P0DMW3 | Small integral membrane protein 10-like protein 1 | FFYFYILASVILNVHLQVY |
| Q96HG1 | Small integral membrane protein 10 | FFYFYILASVILNVHLQVY |
| Q96IX5 | Up-regulated during skeletal muscle growth protein 5 (Diabetes-associated protein in insulin-sensitive tissues) (HCV F-transactivated protein 2) | TLTGRMNCVLATYGSIALIVLYF |

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| O95237 | Lecithin retinol acyltransferase (EC 2.3.1.135) (Phosphatidylcholine--retinol O-acyltransferase) | VLASAVLGLASIVCTGLVSYT |
| E9PQ53 | NADH dehydrogenase [ubiquinone] 1 subunit C2, isoform 2 (NDUFC2-KCTD14 readthrough transcript protein) | GLHRQLLYITAFFFAGYYLV |
| O95298 | NADH dehydrogenase [ubiquinone] 1 subunit C2 (Complex I-B14.5b) (CI-B14.5b) (Human lung cancer oncogene 1 protein) (HLC-1) (NADH-ubiquinone oxidoreductase subunit B14.5b) | GLHRQLLYITAFFFAGYYLV |
| P56134 | ATP synthase subunit f, mitochondrial | ISGITMVLACYVLFSYSFSY |
| A0A5B9 | T-cell receptor beta-2 chain C region | TILYEILLGKATLYAVLVSALVL |
| P53816 | HRAS-like suppressor 3 (HRSL3) (EC 3.1.1.32) (EC 3.1.1.4) (Adipose-specific phospholipase A2) (AdPLA) (Group XVI phospholipase A1/A2) (H-rev 107 protein homolog) (H-REV107) (HREV107-1) (HRAS-like suppressor 1) (HREV107-3) (Renal carcinoma antigen NY-REN-65) | VIIAASVAGMGLAAMSLIGVMFS |
| Q7Z412 | Peroxisome assembly protein 26 (Peroxin-26) | FFSLPFKKSLLAALILCLLVV |
| Q9NS69 | Mitochondrial import receptor subunit TOM22 homolog (hTom22) (1C9-2) (Translocase of outer membrane 22 kDa subunit homolog) | ALWIGTTSFMILVLPVVFET |
| Q9GZY8 | Mitochondrial fission factor | VMYSITVAFWLLNSWLWF |
| Q13505 | Metaxin-1 (Mitochondrial outer membrane import complex protein 1) | ILSVLAGLAAMVGYALLSGIV |
| P01848 | T-cell receptor alpha chain C region | VIGFRILLKLVAGFNLLMTL |
| P27338 | Amine oxidase [flavin-containing] B (EC 1.4.3.4) (Monoamine oxidase type B) (MAO-B) | PGLLRLLIGLTTIFSATALGFLAHRGL |

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| O00198 | Activator of apoptosis harakiri (BH3-interacting domain-containing protein 3) (Neuronal death protein DP5) | WPWLCAAAQVAALAAWLLG |
| B7Z8K6 | T-cell receptor delta chain C region | LGLRMLFAKTVAVNFLLTAKLFF |
| O95168 | NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4 (Complex I-B15) (CI-B15) (NADH-ubiquinone oxidoreductase B15 subunit) | LMGALCGFGPLIFIYYII |
| Q16611 | Bcl-2 homologous antagonist/killer (Apoptosis regulator BAK) (Bcl-2-like protein 7) (Bcl2-L-7) | ILNVLVVLGVVLLGQFVV |
| O60238 | BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like (Adenovirus E1B19K-binding protein B5) (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3A) (NIP3-like protein X) (NIP3L) | VFIPSLFLSHVLALGLGIYIG |
| Q96N68 | Putative uncharacterized protein C18orf15 | MCVCVHVCACVYVCMCVLVCM |
| Q07820 | Induced myeloid leukemia cell differentiation protein Mcl-1 (Bcl-2-like protein 3) (Bcl2-L-3) (Bcl-2-related protein EAT/mcl1) (mcl1/EAT) | IRNVLLAFAGVAGVGAGLAYL |
| Q09013 | Myotonin-protein kinase (MT-PK) (EC 2.7.11.1) (DM-kinase) (DMK) (DM1 protein kinase) (DMPK) (Myotonic dystrophy protein kinase) | LLLFAVVLSRAAALGCIGLVA |
| Q9Y3D6 | Mitochondrial fission 1 protein (FIS1 homolog) (hFis1) (Tetratricopeptide repeat protein 11) (TPR repeat protein 11) | LVGMAIVGGMALGVAGLAGLI |
| Q96JJ6 | Junctophilin-4 (JP-4) (Junctophilin-like 1 protein) | LVVGAVALLDLSLAFLFSQLLT |
| Q96K12 | Fatty acyl-CoA reductase 2 (EC 1.2.1.84) (Male sterility domain-containing protein 1) | NIHYLFNTALFLIAWRLLIA |

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| Q9H0X9 | Oxysterol-binding protein-related protein 5 (ORP-5) (OSBP-related protein 5) (Oxysterol-binding protein homolog 1) | SWFLLCVFLACQLFINHIL |
| F7VJQ1 | Alternative prion protein (AltPrP) | WWWLGAASWWLGAAPWWWLG |
| Q96KF7 | Small integral membrane protein 8 | PVMAFGLVTLSLCVAYIGYLHAI |
| Q8WVI0 | Small integral membrane protein 4 | FGIYRFLPFFFVLGGTMEWIMI |
| P37268 | Squalene synthase (SQS) (SS) (EC 2.5.1.21) (FPP:FPP farnesyltransferase) (Farnesyl-diphosphate farnesyltransferase) | PIYLSFVMLLAALSWQYLTTL |
| Q3B7S5 | Small integral membrane protein 21 | HIRFFTLLVLFHVMVLL |
| P10415 | Apoptosis regulator Bcl-2 | FSWLSLKTLLSLALVGACITLG |
| H7C350 | Coiled-coil domain-containing protein 188 | LLLGALLVWTAAYVYVV |
| Q14318 | Peptidyl-prolyl cis-trans isomerase FKBP8 (PPIase FKBP8) (EC 5.2.1.8) (38 kDa FK506-binding protein) (38 kDa FKBP) (FKBP-38) (hFKBP38) (FK506-binding protein 8) (FKBP-8) (FKBPR38) (Rotamase) | WLFGATAVALGGVALSVVIAA |
| Q8IVJ8 | AP20 region protein 1 | IALALAGPGAILILELSWFLG |
| P0DMT0 | Myoregulin | VGRLLKILFVIFVDLISIIYV |
| Q8N326 | Uncharacterized protein C10orf111 | MSLLLLPAFSGLTWAPFLFLF |
| P60059 | Protein transport protein Sec61 subunit gamma | FQKIAMATAIGFAIMGFIGFFVKLIHIPI |

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| Q12983 | BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 | VFLPSLLLSHLLAIGLGIYIG |
| O43677 | NADH dehydrogenase [ubiquinone] 1 subunit C1, mitochondrial (Complex I-KFYI) (CI-KFYI) (NADH-ubiquinone oxidoreductase KFYI subunit) | WLKVGFTLGTTVFLWIYLI |
| Q8N2K1 | Ubiquitin-conjugating enzyme E2 J2 (EC 2.3.2.23) (E2 ubiquitin-conjugating enzyme J2) (Non-canonical ubiquitin-conjugating enzyme 2) (NCUBE-2) | GLLGGALANL FVIVGFAAFAY |
| O96011 | Peroxisomal membrane protein 11B (Peroxin-11B) (Peroxisomal biogenesis factor 11B) (Protein PEX11 homolog beta) (PEX11-beta) | GIVGLCGLVSSILSILTLYPWL |
| Q86T96 | E3 ubiquitin-protein ligase RNF180 (EC 2.3.2.27) (RING finger protein 180) (RING-type E3 ubiquitin transferase RNF180) | MVIIYIYSVNWVIGFIVFCFL |
| Q5T8D3 | Acyl-CoA-binding domain-containing protein 5 | GVLTFAIWPFIAQWL VYLYY |
| Q9H4I9 | Essential MCU regulator, mitochondrial (Single-pass membrane protein with aspartate-rich tail 1, mitochondrial) | FGLLRVFSIVIPFLYVGTLI |
| Q8NA58 | Poly(A)-specific ribonuclease PNLDC1 (EC 3.1.13.4) (PARN-like domain-containing protein 1) (Poly(A)-specific ribonuclease domain-containing protein 1) (HsPNLDC1) | VNCLLQVCGIVTAWALLAFIL |
| O95169 | NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8, mitochondrial (Complex I-ASHI) (CI-ASHI) (NADH-ubiquinone oxidoreductase ASHI subunit) | LFGFLAFMIFMCWVGDVYPVY |
| O94966 | Ubiquitin carboxyl-terminal hydrolase 19 (EC 3.4.19.12) (Deubiquitinating enzyme 19) (Ubiquitin | FVLGTVAALVALVLNVFYPLV |

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| | thioesterase 19) (Ubiquitin-specific-processing protease 19) (Zinc finger MYND domain-containing protein 9) | |
| P01850 | T-cell receptor beta-1 chain C region | ILLGKATLYAVLVSALVLMAM |
| Q96A26 | Protein FAM162A (E2-induced gene 5 protein) (Growth and transformation-dependent protein) (HGTD-P) | ISYLMIALTVVGCIFMVI |
| Q969F0 | Fetal and adult testis-expressed transcript protein (Cancer/testis antigen 43) (CT43) (Tumor antigen BJ-HCC-2) | TLIIAVLVSASIANLWLWM |
| Q8N5G0 | Small integral membrane protein 20 (Mitochondrial translation regulation assembly intermediate of cytochrome c oxidase protein of 7 kDa) (MITRAC7) | TALIFGGFISLIGAAFYPIYF |
| Q86UQ5 | Gilles de la Tourette syndrome chromosomal region candidate gene 1 protein | AICMEVFLFLWFIAPYACVC |
| P00167 | Cytochrome b5 (Microsomal cytochrome b5 type A) (MCB5) | WWTNWWVIPAISAVAVALMYRLYM |
| Q9NPU4 | Uncharacterized protein C14orf132 | AVLLWIAIIATLGNIVVVGVV |
| P60468 | Protein transport protein Sec61 subunit beta | VPVLVMSLLFIASVFMLHIWG |
| Q9NWW9 | HRAS-like suppressor 2 (EC 2.3.1.-) (EC 3.1.1.-) | AVTTVGVAAGLLAAASLVGILLA |
| Q7Z3B0 | Small integral membrane protein 15 | YGFLTIVILALPLFLASAVL |
| Q9Y5L2 | Hypoxia-inducible lipid droplet-associated protein (Hypoxia-inducible gene 2 protein) | LYLLGVVLTLLSIFVRV |
| Q96FB5 | Protein RRNAD1 (Ribosomal RNA adenine dimethylase domain-containing protein 1) | VVAFFSLALLLAPLVETLILL |

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| B2RUZ4 | Small integral membrane protein 1 (Vel blood group antigen) | LGIAMKVLGGVALFWIIFILG |
| Q9Y2R0 | Cytochrome c oxidase assembly factor 3 homolog, mitochondrial (Coiled-coil domain-containing protein 56) (Mitochondrial translation regulation assembly intermediate of cytochrome c oxidase protein of 12 kDa) | IVTGLGIGALVLAIYGYTFYS |
| Q8IXI1 | Mitochondrial Rho GTPase 2 (MIRO-2) (hMiro-2) (EC 3.6.5.-) (Ras homolog gene family member T2) | GLLGVVGA AVAAVLSFSLYRVLV |
| POC6T2 | Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 4 | VQLAIFANMLGVSLFLLVVLVY |
| Q14BN4 | Sarcolemmal membrane-associated protein (Sarcolemmal-associated protein) | WMPMLAALVAVT AIVLYVPGL |
| Q8WVX9 | Fatty acyl-CoA reductase 1 (EC 1.2.1.84) (Male sterility domain-containing protein 2) | IRYGFNTILVILIWRIFI |
| Q14D33 | Receptor-transporting protein 5 (3CxxC-type zinc finger protein 5) (CXXC-type zinc finger protein 11) | FWIWVSMTVCVFWLMCM |
| Q8NI28 | Putative uncharacterized protein encoded by LINC01006 (Long intergenic non-protein coding RNA 1006) | WIPLLLVAGCVSCFVGLAVCV |
| I3L1I5 | Putative uncharacterized protein LOC100996504 | VLSIILLGSLLMCASSFCFAL |
| A4D256 | Dual specificity protein phosphatase CDC14C (EC 3.1.3.16) (EC 3.1.3.48) (CDC14 cell division cycle 14 homolog C) | ILLPSPLAVLTFTLCSVVIWWIV |
| Q9Y385 | Ubiquitin-conjugating enzyme E2 J1 (EC 2.3.2.23) (E2 ubiquitin-conjugating enzyme J1) (Non-canonical ubiquitin-conjugating enzyme 1) (NCUBE-1) (Yeast ubiquitin-conjugating enzyme UBC6 homolog E) (HsUBC6e) | DHGGSAVLIVILTLALAALIF |

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| Q6ZS62 | Colorectal cancer-associated protein 1 | LYGCFCVGLVSGMAISVLLLA |
| A6NFE2 | Single-pass membrane and coiled-coil domain-containing protein 2 | IFIMFDVLTVTGLLCYILFFG |
| Q9NS64 | Protein reprimo | VVQIAVMCVLSLTVVFGIFFL |
| Q16821 | Protein phosphatase 1 regulatory subunit 3A (Protein phosphatase 1 glycogen-associated regulatory subunit) (Protein phosphatase type-1 glycogen targeting subunit) (RG1) | YFLLFLIFLITVYHYDLMIGL |
| Q9NQG1 | Protein MANBAL | YGLFLGAIQFLICVLAIVPI |
| P58511 | Small integral membrane protein 11A | PLLLYILAAKTLILCLTFAGVKM |
| Q9Y228 | TRAF3-interacting JNK-activating modulator (TRAF3-interacting protein 3) | WLPVLMVVIAAALAVFLA |
| Q9BXU9 | Calcium-binding protein 8 (CaBP8) (Calneuron I) (Calneuron-1) | LICAFAMAFIISVMLIAANQI |
| A6NL05 | Protein FAM74A7 | LSLLLHLAVFLWIIIAINFSN |
| Q4VXF1 | Putative protein FAM74A3 | LSLLLHLAVFLWIIIAINFSN |
| Q5RGS3 | Protein FAM74A1 | LSLLLHLAVFLWIIIAINFSN |
| Q5T6X4 | Protein FAM162B | VKACYIMIGLTIACFAVIVS |
| Q9NXE4 | Sphingomyelin phosphodiesterase 4 (EC 3.1.4.12) (Neutral sphingomyelinase 3) (nSMase-3) (nSMase3) (Neutral sphingomyelinase III) | LLLAFFVASLFCVGPLPCTLL |
| P51648 | Fatty aldehyde dehydrogenase (EC 1.2.1.3) (Aldehyde dehydrogenase 10) (Aldehyde dehydrogenase family 3 member A2) (Microsomal aldehyde dehydrogenase) | LGLLLLTFLGIVA AVL V |

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| Q8IXI2 | Mitochondrial Rho GTPase 1 (MIRO-1) (hMiro-1) (EC 3.6.5.-) (Rac-GTP-binding protein-like protein) (Ras homolog gene family member T1) | WLRASFGATVFAVLGFAMYKALL |
| Q8N4K4 | Reprimo-like protein | VAQIAVLCVLSLTVVFGVFFL |
| Q8N6R1 | Stress-associated endoplasmic reticulum protein 2 (Ribosome-associated membrane protein RAMP4-2) | GPWLLALFVVFVCGSAIFQII |
| P58549 | FXYD domain-containing ion transport regulator 7 | TVQTVGMTLATILFLLGILIVIS |
| Q86V35 | Calcium-binding protein 7 (CaBP7) (Calneuron II) (Calneuron-2) | LICAFAlAFIISVMLIAANQV |
| Q6ZNB6 | NF-X1-type zinc finger protein NFXL1 (Ovarian zinc finger protein) (hOZFP) | YYLISVCGVVVVVFAWYI |
| O75056 | Syndecan-3 (SYND3) | AVIVGGVVGALFAAFLVTLII |
| P03986 | T-cell receptor gamma-2 chain C region (T-cell receptor gamma chain C region PT-gamma-1/2) | MYLLLLLKSVVYFAITCCLL |
| A1L1A6 | Immunoglobulin superfamily member 23 | LLAAGILGAGALIAGMCFIII |
| Q8N5Y8 | Mono [ADP-ribose] polymerase PARP16 (EC 2.4.2.30) (ADP-ribosyltransferase diphtheria toxin-like 15) (Poly [ADP-ribose] polymerase 16) (PARP-16) | SHWFTVMISLYLLLLLIVSVI |
| P61266 | Syntaxin-1B (Syntaxin-1B1) (Syntaxin-1B2) | IMIIICCVVLGVVLASSIGGTGL |
| Q9BZF1 | Oxysterol-binding protein-related protein 8 (ORP-8) (OSBP-related protein 8) | YFIIFLLILLQVIINFMF |
| A6NGB0 | Transmembrane protein 191C | VLGALQVLLTLPLLFLGLSLL |
| P0C7N4 | Transmembrane protein 191B | VLGALQVLLTLPLLFLGLSLL |
| Q7Z419 | E3 ubiquitin-protein ligase RNF144B (EC 2.3.2.-) (IBR domain-containing protein 2) (RING finger | VVGILVGLGIIALVTSPLLLL |

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| | protein 144B) (p53-inducible RING finger protein) | |
| Q9UPX6 | UPF0258 protein KIAA1024 | IAALIAAACTVILVIVVPIC |
| P54710 | Sodium/potassium-transporting ATPase subunit gamma (Na(+)/K(+) ATPase subunit gamma) (FXYD domain-containing ion transport regulator 2) (Sodium pump gamma chain) | GGLIFAGLAFIVGLLILL |
| Q96LL3 | Uncharacterized protein C16orf92 | PGLFHHLVGLLVVAFFLLF |
| Q9Y6X1 | Stress-associated endoplasmic reticulum protein 1 (Ribosome-attached membrane protein 4) | GPWLLALFIFVVCGSAIFQII |
| Q16623 | Syntaxin-1A (Neuron-specific antigen HPC-1) | IMIHCCVILGIVIASTVGGI |
| P60509 | Endogenous retrovirus group PABLB member 1 Env polyprotein (Endogenous retrovirus group PABLB member 1) (Envelope polyprotein) (HERV-R(b) Env protein) (HERV-R(b)_3p24.3 provirus ancestral Env polyprotein) [Includes: Surface protein domain (SU); Transmembrane protein domain (TM)] | ILIVLATLWSVGIALCCGLYF |
| P50876 | E3 ubiquitin-protein ligase RNF144A (EC 2.3.2.-) (RING finger protein 144A) (UbcM4-interacting protein 4) (Ubiquitin-conjugating enzyme 7-interacting protein 4) | VVGIFAGFGLLLVASPLLL |
| Q96DX8 | Receptor-transporting protein 4 (28 kDa interferon-responsive protein) (3CxxC-type zinc finger protein 4) | PLNICVFILLLVFIVVKCFTS |
| Q12846 | Syntaxin-4 (Renal carcinoma antigen NY-REN-31) | IAICVSITVVLLAVIIGVTVV |
| Q9UEU0 | Vesicle transport through interaction with t-SNAREs homolog 1B (Vesicle transport v-SNARE protein | LSIIILLELAILGGLVYYKFF |

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| | Vti1-like 1 (Vti1-rp1) | |
| A8MYB1 | Transmembrane and coiled-coil domain-containing protein 5B | YFQYLTFMVLVFIRLLAYVIFHL |
| Q9BXX5 | Bcl-2-like protein 13 (Bcl2-L-13) (Bcl-rambo) (Protein Mil1) | ILLFGGAAAVAILAVAIGVAL |
| Q6PJW8 | Consortin | CILLVLLCIATVFLSVGGTAL |
| H3BV60 | Transforming growth factor-beta receptor type 3-like protein (TGF-beta receptor type-3-like protein) (TGFR-3L) (Transforming growth factor-beta receptor type III-like protein) (TGF-beta receptor type III-like protein) | VVALVLAAFVLGAALAAGLGL |
| P17706 | Tyrosine-protein phosphatase non-receptor type 2 (EC 3.1.3.48) (T-cell protein-tyrosine phosphatase) (TCPTP) | ILTKMGFMSVILVGAFVGVWTLFF |
| Q8N111 | Cell cycle exit and neuronal differentiation protein 1 (BM88 antigen) | LVAGGVAVAAIALILGVAFLV |
| E7ERA6 | RING finger protein 223 | LVSALLMLFCVALWPVQCAL |
| O14653 | Golgi SNAP receptor complex member 2 (27 kDa Golgi SNARE protein) (Membrin) | YFMIGGMLLTCVVMFLVVQYL |
| Q9BZ97 | Putative transcript Y 13 protein | LLGWDLNLSLFLGLCLMLLLA |
| Q9P0L0 | Vesicle-associated membrane protein-associated protein A (VAMP-A) (VAMP-associated protein A) (VAP-A) (33 kDa VAMP-associated protein) (VAP-33) | LPSLLVVIAAIFIGFFLGKFI |
| Q8N8J7 | Uncharacterized protein C4orf32 | VIVIFFWVMLWFLGLQALGLV |
| P37287 | Phosphatidylinositol N-acetylglucosaminyltransferase subunit A (EC 2.4.1.198) (GlcNAc-PI synthesis) | PVTGYIFALLAVFNFLFLIFL |

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| | protein) (Phosphatidylinositol-glycan biosynthesis class A protein) (PIG-A) | |
| Q5VV42 | Threonylcarbamoyladenosine tRNA methylthiotransferase (EC 2.8.4.5) (CDK5 regulatory subunit-associated protein 1-like 1) (tRNA-t(6)A37 methylthiotransferase) | CALRMSVGLALLGLLFAFFVKVY |
| Q8WWG1 | Pro-neuregulin-4, membrane-bound isoform (Pro-NRG4) [Cleaved into: Neuregulin-4 (NRG-4)] | EAFVALAVLVTLIIGAFYFLC |
| Q96NA8 | t-SNARE domain-containing protein 1 | CFLSAGVTALLVIIIATSV |
| Q68G75 | LEM domain-containing protein 1 (Cancer/testis antigen 50) (CT50) (LEM domain protein 1) (LEMP-1) | FPVGLKLAVLGIFIIIVFVYL |
| P0CF51 | T-cell receptor gamma chain C region 1 | YYMYLLLLLKSVVYFAITCCLL |
| Q9NX14 | NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial (Complex I-ESSS) (CI-ESSS) (NADH-ubiquinone oxidoreductase ESSS subunit) (Neuronal protein 17.3) (Np17.3) (p17.3) | LVFFFGVSIILVLGSTFVAYL |
| Q8IU3 | GRAM domain-containing protein 2A | LLKVFFVLICFLVMSSSYLAF |
| Q9UNK0 | Syntaxin-8 | MIMVILLLLVAIVVVAV |
| Q9GZT6 | Coiled-coil domain-containing protein 90B, mitochondrial | TIRYLAASVFTCLAIALGFYRFW |
| O95249 | Golgi SNAP receptor complex member 1 (28 kDa Golgi SNARE protein) (28 kDa cis-Golgi SNARE p28) (GOS-28) | SLILGGVIGICTILLLLYAFH |
| Q9P0B6 | Coiled-coil domain-containing protein 167 | MLLSVAIFILLTLVYAYW |

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| Q9HDC5 | Junctophilin-1 (JP-1) (Junctophilin type 1) | IMIVLVMLLNIGLAILFVHFL |
| Q8NF91 | Nesprin-1 (Enaptin) (KASH domain-containing protein 1) (KASH1) (Myocyte nuclear envelope protein 1) (Myne-1) (Nuclear envelope spectrin repeat protein 1) (Synaptic nuclear envelope protein 1) (Syne-1) | AALPLQLLLLLLIGLACLVPM |
| Q8TBA6 | Golgin subfamily A member 5 (Cell proliferation-inducing gene 31 protein) (Golgin-84) (Protein Ret-II) (RET-fused gene 5 protein) | VFVIIMALLHLWVMIVLLTY |
| Q53EP0 | Fibronectin type III domain-containing protein 3B (Factor for adipocyte differentiation 104) (HCV NS5A-binding protein 37) | IIVLGFATLSILFAFILQYFL |
| Q13323 | Bcl-2-interacting killer (Apoptosis inducer NBK) (BIP1) (BP4) | VLLALLLLLALLPLLSGGLH |
| Q9Y6F6 | Protein MRVII (Inositol 1,4,5-trisphosphate receptor-associated cGMP kinase substrate) (JAW1-related protein MRVII) | WQVIWMMAAVMLVLTVVVGLY |
| Q8N912 | Nutritionally-regulated adipose and cardiac enriched protein homolog | GGSLLLQLCVCVLLVLALGLY |
| Q13948 | Protein CASP | IGFFYTLFLHCLVFLVLYKLA |
| Q8WXI7 | Mucin-16 (MUC-16) (Ovarian cancer-related tumor marker CA125) (CA-125) (Ovarian carcinoma antigen CA125) | FWAVILIGLAGLLGVITCLIC |
| Q14789 | Golgin subfamily B member 1 (372 kDa Golgi complex-associated protein) (GCP372) (Giantin) (Macrogolgin) | VPLLAAYFLMIHVLLILCFT |

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| Q8WXH2 | Junctophilin-3 (JP-3) (Junctophilin type 3) (Trinucleotide repeat-containing gene 22 protein) | LVVMVILLNIGVAILFINFFI |
| Q8TC41 | Probable E3 ubiquitin-protein ligase RNF217 (EC 2.3.2.-) (IBR domain-containing protein 1) (RING finger protein 217) | LIMVLGLALGAIAVVIGLFVF |
| Q9Y6H6 | Potassium voltage-gated channel subfamily E member 3 (MinK-related peptide 2) (Minimum potassium ion channel-related peptide 2) (Potassium channel subunit beta MiRP2) | YMYILFVMFLFAVTVGSLILG |
| Q8NCQ3 | Putative uncharacterized protein encoded by LINC00301 | SFGLAIIGILLIACEIILFLT |
| P0DN84 | Sarcoplasmic/endoplasmic reticulum calcium ATPase regulator DWORF (SERCA regulator DWORF) (Dwarf open reading frame) (DWORF) | VPILLLIGWIVGCIIMIYVVF |
| P0DL12 | Small integral membrane protein 17 | IVLVVCVFLFLVLTGMPMMF |
| Q86Z14 | Beta-klotho (BKL) (BetaKlotho) (Klotho beta-like protein) | LIFLGCCFFSTLVLLLSIAIF |
| P59025 | Receptor-transporting protein 1 (3CxxC-type zinc finger protein 1) | IPWCCLFWATVLLLIYQLQSF |
| Q5QGT7 | Receptor-transporting protein 2 (3CxxC-type zinc finger protein 2) | LSLRWCLFWASLCLLVVYLQFSF |
| Q6ZS82 | Regulator of G-protein signaling 9-binding protein (RGS9-anchoring protein) | ALAAILFGAVLLAAVALAVCV |
| Q9NYM9 | BET1-like protein (Golgi SNARE with a size of 15 kDa) (GOS-15) (GS15) (Vesicle transport protein GOS15) | LLCGMAVGLIVAFFILSYFLS |
| Q9NRQ5 | Single-pass membrane and coiled-coil domain-containing protein 4 (Protein FN5) | TVVLPTLAVVVLLIVVFVYVA |
| Q0VAQ4 | Small cell adhesion glycoprotein (Small transmembrane and glycosylated protein) | IAVVITVVFLTLLSVVILIFF |

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| Q96AG4 | Leucine-rich repeat-containing protein 59 (Ribosome-binding protein p34) (p34) [Cleaved into: Leucine-rich repeat-containing protein 59, N-terminally processed] | WAVLKLLLLLLLLFGVAGGLVA |
| Q9BR39 | Junctophilin-2 (JP-2) (Junctophilin type 2) | ILICMVILLNIGLAILFVHLL |
| P23763 | Vesicle-associated membrane protein 1 (VAMP-1) (Synaptobrevin-1) | MMIMLGAICAIHVVVIVVIYF |
| Q96JN2 | Coiled-coil domain-containing protein 136 (Nasopharyngeal carcinoma-associated gene 6 protein) | IFSLPLVGLVVISALLWCWWA |
| P51809 | Vesicle-associated membrane protein 7 (VAMP-7) (Synaptobrevin-like protein 1) (Tetanus-insensitive VAMP) (Ti-VAMP) | LTIIIIVSIVFIYIIVSPLC |
| Q9BQQ7 | Receptor-transporting protein 3 (3CxxC-type zinc finger protein 3) (Transmembrane protein 7) | SIFCCCVILIVIVVIVVKTAI |
| O00631 | Sarcolipin | LFLNFTIVLITVILMWLLV |
| A6NCQ9 | RING finger protein 222 | LITLIAVVAVVAAILPWVLLV |
| Q8WWP7 | GTPase IMAP family member 1 (Immunity-associated protein 1) (hIMAP1) | SWRLGLALLLGGALLFWVLL |
| Q96D05 | Uncharacterized protein C10orf35 | ILLFLLMMLGVRGLLLVGLV |
| Q9Y2H6 | Fibronectin type-III domain-containing protein 3A (Human gene expressed in odontoblasts) | ILVLFAPFSILIAFIIQYFVI |
| P04921 | Glycophorin-C (Glycoconnectin) (Glycophorin-D) (GPD) (Glycoprotein beta) (PAS-2) (Sialoglycoprotein D) (CD antigen CD236) | DIVVIAGVIAAVAIVLVSLLFVML |
| Q8N8N0 | E3 ubiquitin-protein ligase RNF152 (EC 2.3.2.27) (RING finger protein 152) (RING-type E3 ubiquitin transferase RNF152) | SGVCTVILVACVLVFLLGIVL |

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| A6NNC1 | Putative POM121-like protein 1-like | LGLFLLVFSFFLLTWASFSF |
| Q12912 | Lymphoid-restricted membrane protein (Protein Jaw1) [Cleaved into: Processed lymphoid-restricted membrane protein] | ALWLSIAFIVLFAALMSFLTG |
| P61566 | Endogenous retrovirus group K member 24 Env polyprotein (Envelope polyprotein) (HERV-K101 envelope protein) (HERV-K_22q11.21 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)] | IGSTTIINLILILVCLFCLLL |
| P61567 | Endogenous retrovirus group K member 7 Env polyprotein (Envelope polyprotein) (HERV-K(III) envelope protein) (HERV-K102 envelope protein) (HERV-K_1q22 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)] | IGSTTIINLILILVCLFCLLL |
| Q8N6L0 | Protein KASH5 (Coiled-coil domain-containing protein 155) (KASH domain-containing protein 5) | LIPAPVLGLLLLLLLLSVLLLG |
| Q8N6Q1 | Transmembrane and coiled-coil domain-containing protein 5A | IFCCLFFITLFFIRLLSYMFF |
| Q12981 | Vesicle transport protein SEC20 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 1) (Transformation-related gene 8 protein) (TRG-8) | TDKLLIFLALALFLATVLYIV |
| P59773 | UPF0258 protein KIAA1024-like | GLILLVVISILVTIVTIITFF |
| Q8WXH0 | Nesprin-2 (KASH domain-containing protein 2) (KASH2) (Nuclear envelope spectrin repeat protein 2) (Nucleus and actin connecting element protein) (Protein NUANCE) (Synaptic nuclear envelope protein 2) (Syne-2) | AALPLQLLLLLLLLLLACLPS |

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| Q8WVX3 | Uncharacterized protein C4orf3 (Hepatitis C virus F protein-transactivated protein 1) (HCV F-transactivated protein 1) | WLDLWLFILFDVVVFLFVYFL |
| P32856 | Syntaxin-2 (Epimorphin) | WIIIAVSVVLVAIIALIIGLSVGK |
| Q6IEE8 | Schlafen family member 12-like | IFLFVCLFRFCLFVCWFVCF |
| Q8NHP6 | Motile sperm domain-containing protein 2 | LLLSLTMLLLAFVTSFFYLLY |
| O95292 | Vesicle-associated membrane protein-associated protein B/C (VAMP-B/VAMP-C) (VAMP-associated protein B/C) (VAP-B/VAP-C) | RLLALVVLFFIVGVIIIGKIAL |
| Q96QK8 | Small integral membrane protein 14 | GISVTMILVAWMVIALILFLL |
| Q96JQ2 | Calmin (Calponin-like transmembrane domain protein) | MMYFILFLWLLVYCLLLFPQL |
| Q71RC9 | Small integral membrane protein 5 | IVAFSVIILFTATVLLLLLIA |
| A2A2Y4 | FERM domain-containing protein 3 (Band 4.1-like protein 4O) (Ovary type protein 4.1) (4.1O) | LLVVGLGLLLFFVPLLLLLLE |
| O42043 | Endogenous retrovirus group K member 18 Env polyprotein (Envelope polyprotein) (HERV-K(C1a) envelope protein) (HERV-K110 envelope protein) (HERV-K18 envelope protein) (HERV-K18 superantigen) (HERV-K_1q23.3 provirus ancestral Env polyprotein) (IDDMK1,2 22 envelope protein) (IDDMK1,2 22 superantigen) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)] | IRSTMIINLILIVVCLFCLLL |
| P50402 | Emerin | VPLWGQLLLFLVFVIVLFFIY |
| Q96D59 | Probable E3 ubiquitin-protein ligase RNF183 (EC 2.3.2.27) | IFAYLMAVILSVTLLLLIFSIF |

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| Q9P2W9 | Syntaxin-18 (Cell growth-inhibiting gene 9 protein) | AGFRVWILFFLLVMCSFSLFL |
| Q01629 | Interferon-induced transmembrane protein 2 (Dispanin subfamily A member 2c) (DSPA2c) (Interferon-inducible protein 1-8D) | IWALILGIFMTILLIIPVLV |
| Q86Y82 | Syntaxin-12 | KKMCILVLVLSVILLGLII |
| Q01628 | Interferon-induced transmembrane protein 3 (Dispanin subfamily A member 2b) (DSPA2b) (Interferon-inducible protein 1-8U) | IWALILGILMTILLIVIPVLI |
| POC2S0 | Cortixin-2 | TGFAFVGILCIFLGLLIIRCF |
| O75396 | Vesicle-trafficking protein SEC22b (ER-Golgi SNARE of 24 kDa) (ERS-24) (ERS24) (SEC22 vesicle-trafficking protein homolog B) (SEC22 vesicle-trafficking protein-like 1) | KLAAVAVFFIMLIVYVRFWWL |
| P42167 | Lamina-associated polypeptide 2, isoforms beta/gamma (Thymopoietin, isoforms beta/gamma) (TP beta/gamma) (Thymopoietin-related peptide isoforms beta/gamma) (TPRP isoforms beta/gamma) [Cleaved into: Thymopoietin (TP) (Splenin); Thymopentin (TP5)] | IPVWIKILLFVVVAVFLFLVYQAM |
| P13164 | Interferon-induced transmembrane protein 1 (Dispanin subfamily A member 2a) (DSPA2a) (Interferon-induced protein 17) (Interferon-inducible protein 9-27) (Leu-13 antigen) (CD antigen CD225) | IWALILGILMTIGFILLLVFG |
| Q0VDE8 | Adipogenin | FSFLVFWFCLPVGLLLLLLIW |
| Q9BZL3 | Small integral membrane protein 3 (NGF-induced differentiation clone 67 protein) (Small membrane protein NID67) | IWVIVLIIILATIVIMTSLLLC |

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|--------|---|------------------------|
| Q96AJ9 | Vesicle transport through interaction with t-SNAREs homolog 1A (Vesicle transport v-SNARE protein Vti1-like 2) (Vti1-rp2) | ILLVILGHIIVVITILMAITFS |
| O75379 | Vesicle-associated membrane protein 4 (VAMP-4) | IKAIMALVAAILLLVIIIIV |
| O95159 | Zinc finger protein-like 1 (Zinc finger protein MCG4) | LLLLLGLLGFLALLALMSRLG |
| Q86Y07 | Serine/threonine-protein kinase VRK2 (EC 2.7.11.1) (Vaccinia-related kinase 2) | VYYYRIIIPVLLMLVFLALFF |
| Q86W74 | Ankyrin repeat domain-containing protein 46 (Ankyrin repeat small protein) (ANK-S) | LGFWRVLLIFVIALLSLZIA |
| Q629K1 | Triple QxxK/R motif-containing protein (Triple repetitive-sequence of QXXK/R protein homolog) | VGLVLAAILALLAFYAFFYL |
| P0DKX4 | Small integral membrane protein 18 | CFVILLLFIFTVVSLVLAFL |
| Q8N8F7 | Leucine-rich single-pass membrane protein 1 | VGLLIVLIVSLALVFFVIFLI |
| O15155 | BET1 homolog (hBET1) (Golgi vesicular membrane-trafficking protein p18) | KLLCYMMLFSLFVFFIYWII |
| P63027 | Vesicle-associated membrane protein 2 (VAMP-2) (Synaptobrevin-2) | MMIILGVICAILIIIVYF |
| O15400 | Syntaxin-7 | CIILILVIGVAISLIWGL |
| A2A2V5 | Serine-rich and transmembrane domain-containing protein 1 | IYVSIFLSLLAFLLLLLIIAL |
| Q6ZMZ3 | Nesprin-3 (KASH domain-containing protein 3) (KASH3) (Nuclear envelope spectrin repeat protein 3) | VALPLQLLLLLFLLLLFLLPI |
| O60499 | Syntaxin-10 (Syn10) | WCAIAVLVGVLLLVLILFSL |
| P60606 | Cortexin-1 | TVFAFVLCLLVVLVLLMVRCV |
| Q13277 | Syntaxin-3 | LIIIVLVVVLLGILALIIGL |

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| O95183 | Vesicle-associated membrane protein 5 (VAMP-5) (Myobrevin) | VGLVVVGVLLIILIVLLVFL |
| Q7Z6J6 | FERM domain-containing protein 5 | LLLVTMGLLFVLLLLLIILTE |
| Q15836 | Vesicle-associated membrane protein 3 (VAMP-3) (Cellubrevin) (CEB) (Synaptobrevin-3) | MWAIGITVLVIFIIIIIVWVV |
| Q4LDR2 | Cortexin-3 (Kidney and brain-expressed protein) | MTFVVFVILLFIFLGILIVRCF |
| A9Z1Z3 | Fer-1-like protein 4 | LVLLLLVLLTVFLLLVFYTIP |
| Q9HCU5 | Prolactin regulatory element-binding protein (Mammalian guanine nucleotide exchange factor mSec12) | VPVWLLLLLCVGLIIVTILL |
| Q8N112 | Leucine-rich single-pass membrane protein 2 | GFLLLLALLVLTCLVLALLAV |
| Q13190 | Syntaxin-5 | WLMVKIFLILIVFFIIFVVFL |
| P0DI80 | Small integral membrane protein 6 | LAVIILFITAVLLLILFAIVF |
| Q96F15 | GTPase IMAP family member 5 (Immunity-associated nucleotide 4-like 1 protein) (Immunity-associated nucleotide 5 protein) (IAN-5) (hIAN5) (Immunity-associated protein 3) | IFVFLLLCSILFFIIFLFIFH |
| Q9NX77 | Endogenous retrovirus group K member 13-1 Env polyprotein (Envelope polyprotein) (HERV-K_16p13.3 provirus ancestral Env polyprotein) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)] | GSLLLLALLILVCLCCLLLVC |
| Q8N205 | Nesprin-4 (KASH domain-containing protein 4) (KASH4) (Nuclear envelope spectrin repeat protein 4) | FLILFLLFLLLVGAMFLLPA |
| P26678 | Cardiac phospholamban (PLB) | FINFCLILICLLLCIIVMLL |
| O14662 | Syntaxin-16 (Syn16) | MLVILILFVHIIIVLIVVLVGV |

| | | |
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| Q9BV40 | Vesicle-associated membrane protein 8 (VAMP-8) (Endobrevin) (EDB) | MIVLICVIVFIILFIVLFAT |
| Q9NZ43 | Vesicle transport protein USE1 (Putative MAPK-activating protein PM26) (USE1-like protein) (p31) | WLLWAMLIIVCFIFISMILFI |
| O43752 | Syntaxin-6 | WCAIAILFAVLLVVLILFLVL |
| Q9NZM1 | Myoferlin (Fer-1-like protein 3) | WVIIGLLFLLILLFVAVLLY |
| O75923 | Dysferlin (Dystrophy-associated fer-1-like protein) (Fer-1-like protein 1) | IILFIILFILLFLAIFIYAF |
| Q2WGJ9 | Fer-1-like protein 6 | IIAFILIIIFLVLFYTL |
| Q9HC10 | Otoferlin (Fer-1-like protein 2) | WLLKLLLLLLLLLALFLY |

TMD: transmembrane domain.

Supplemental Table 4. Animal models for ASNA1-mediated TA protein insertion related genes.

| Gene | Synonyms | Species | Genotype | Mechanism | Phenotype | Refs |
|------------|-------------------|--------------------------|--|-----------------|---|------|
| <i>WRB</i> | <i>CHD5, GET1</i> | <i>Mus musculus</i> | $Wr^{tm1.1(KOMP)Vlcg}/Wr^{tm1.1(KOMP)Vlcg}$ | homozygous ko | embryonic lethality (<E9.5) | 15 |
| | | <i>Mus musculus</i> | $Wr^{tm1.1(KOMP)Vlcg}/Wr^{+}$ | heterozygous ko | abnormal brain development | 15 |
| | | <i>Mus musculus</i> | $Wr^{fl/fl}:Vglut3-Cre$ $Wr^{fl/fl}:Vglut3-ires-Cre$ | conditional ko | progressive hearing impairment, tonic-clonic seizures | 16 |
| | | <i>Danio rerio</i> | $wr^{hi1482Tg}$ | homozygous ko | abnormal myocardial repolarization, bradycardia | 17 |
| | | <i>Danio rerio</i> | $wr^{hi1482Tg/hi1482Tg}$ $wr^{hi1482Tg/hi1482Tg}; n11Tg$ | homozygous ko | reduced auditory startle response, reduced visual evoked potentials | 18 |
| | | <i>Danio rerio</i> | $lri48Tg; wr^{hi1482Tg}$ $q16aTg; q16bTg; wr^{hi1482Tg/hi1482Tg}$ $wr^{hi1482Tg/hi1482Tg}$ | homozygous ko | photoreceptor synapse defects | 19 |
| | | <i>Danio rerio</i> | $wr^{hi1482Tg/hi1482Tg}$ | homozygous ko | impaired hair cell exocytosis and hearing | 16 |
| | | <i>Oryzias latipalis</i> | WT + MO <i>chd5</i> (ATG) | knockdown | cardiac looping defects, abnormal chamber differentiation, ocular abnormalities | 20 |

| | | | | | | |
|--------------|--------------------|---------------------------|---|----------------|--|------------|
| | | <i>Xenopus tropicalis</i> | Tg (actc1:GFP) ^{Mohun} + MO chd5 (ATG) Tg (actc1:GFP) ^{Mohun} + MO chd5 (SB) | knockdown | cardiac looping defects, abnormal chamber differentiation | 21 |
| <i>CAMLG</i> | <i>CAML, GET2</i> | <i>Mus musculus</i> | CamI ^{tm1Rjb} /CamI ^{tm1Rjb} | homozygous ko | embryonic lethality (E4.5-E7.5) | 22 |
| | | <i>Mus musculus</i> | CamI ^{tm1Rjb} /CamI ^{tm2Rjb} | conditional ko | abnormal T-cell development | 23 |
| <i>ASNA1</i> | <i>TRC40, GET3</i> | <i>Mus musculus</i> | Asna1 ^{tm1Hbha} /Asna1 ^{tm1Hbha} | homozygous ko | embryonic lethality (E3.5-E8.5) | 24 |
| | | <i>Danio rerio</i> | q16aTg ; q16bTg + MO1 asna1 WT + MO1 asna1 (ATG) WT + MO2 asna1 (SB) | knockdown | decreased visual perception, photoreceptor synapse defects, lack of swim bladder | 19 |
| | | <i>Danio rerio</i> | asna1 ^{Δ7/Δ7} | homozygous ko | impaired swim bladder inflation, decreased blood flow in dorsal aorta, impaired cardiac contractility, early lethality (6-8 dpf) | This study |

ATG, translation-blocking; dpf, days post fertilization; E, embryonic day; ko, knock-out; MO, morpholino; SB, splice-blocking; WT, wild-type.

SUPPLEMENTAL FIGURES

Supplemental Figure 1. CRISPR/Cas9-induced *asna1* deletion in zebrafish.

(A) Schematic representation of guide RNA target site (*asna1* exon 5). Protospacer is highlighted in cyan; PAM in red. BsrI recognition site used for genotyping is underlined. (B) Sequence and position of induced 7 bp deletion ($\Delta 7$) predicted to result in a frameshift and premature stop codon. (C) Chromatogram of PCR-amplified DNA from F1 fish showing wild-type and mutant sequence (reverse complement). The arrow indicates the position of the deletion.

A

5' -GACATGAATGCAGATCAGCTGGCGT **CCAAACTGGAGGAGACGCTGCC** TGTCATCCGCTCCGTCAGCGAGCAG-3'

B

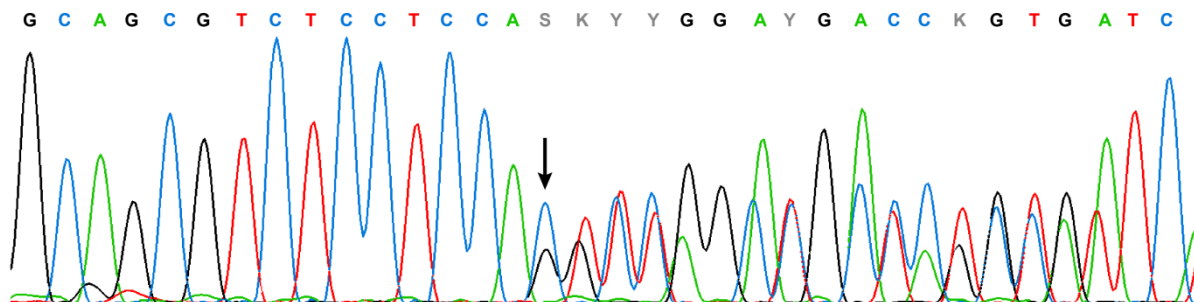
5' -GACATGAATGCAGATCAGCTGGCGTCCAAACTGGAGGAGACGCTGCCTGTTCATCCGCTCCGTCAGCGAGCAG-3' WT

D M N A D Q L A S K L E E T L P V I R S V S E Q

5' -GACATGAATGCAGATCAGCTGGCG-----TGGAGGAGACGCTGCCTGTTCATCCGCTCCGTCAGCGAGCAG-3' $\Delta 7$

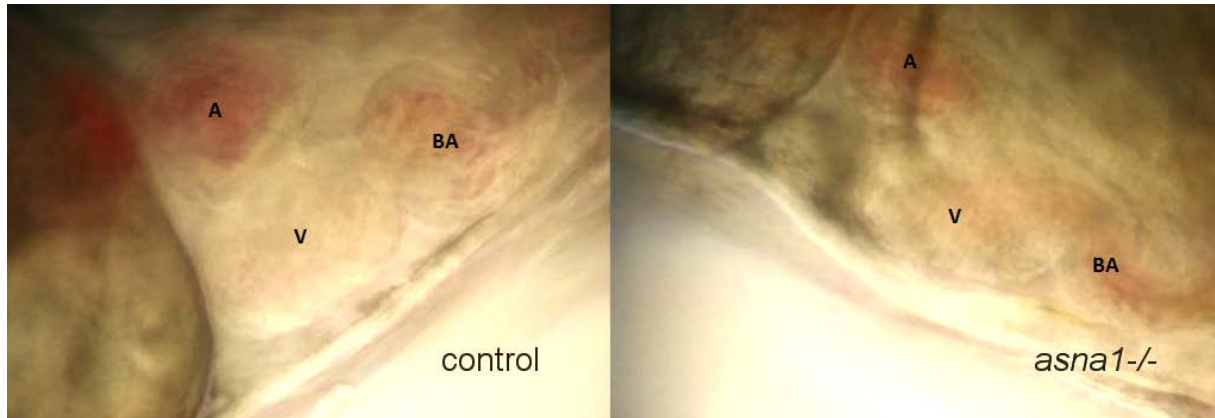
D M N A D Q L A W R R R C L S S A P S (missing 48 AA)

C



Supplemental Figure 2. Lateral views of the heart in wild-type and mutant zebrafish.

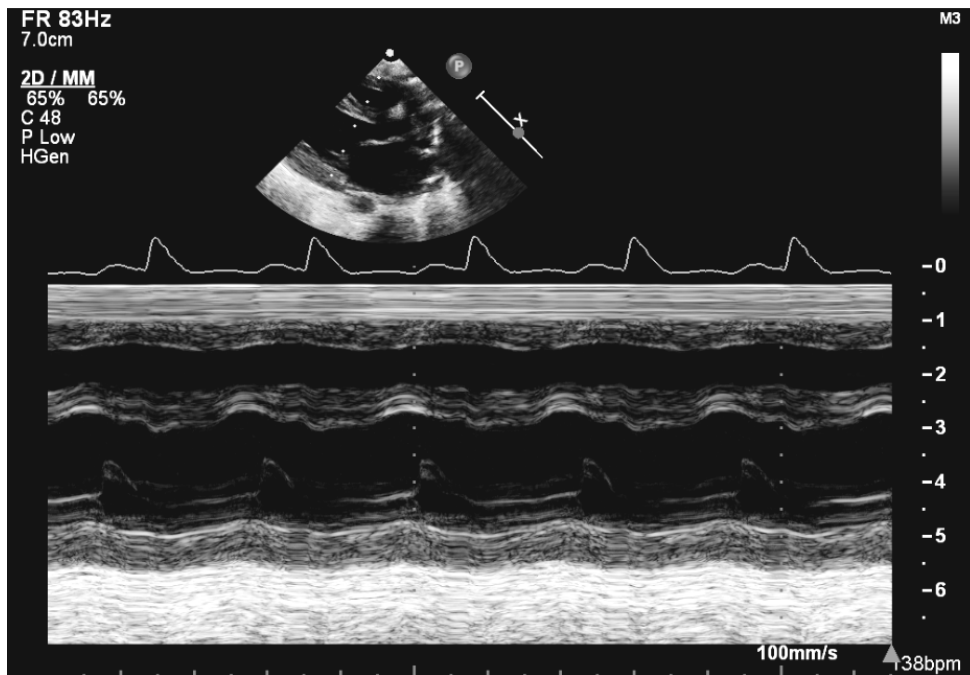
Microscopic images of the heart in wild-type and *asna1*^{Δ7/Δ7} zebrafish larvae. The atria and ventricles are marked as A and V, respectively. The bulbus arteriosus (outflow tract) is marked as BA.



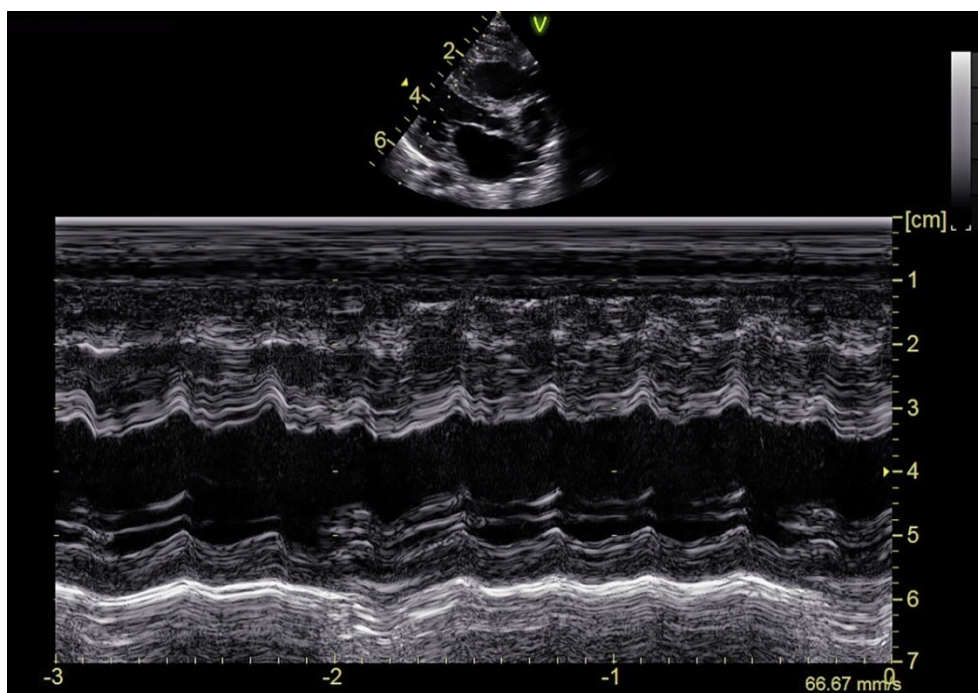
Supplemental Figure 3. M-mode imaging in both patients.

M-mode image of the heart in parasternal long axis view in (A) patient II:2 and (B) patient II:3 showing severely reduced left ventricular contractility.

A



B



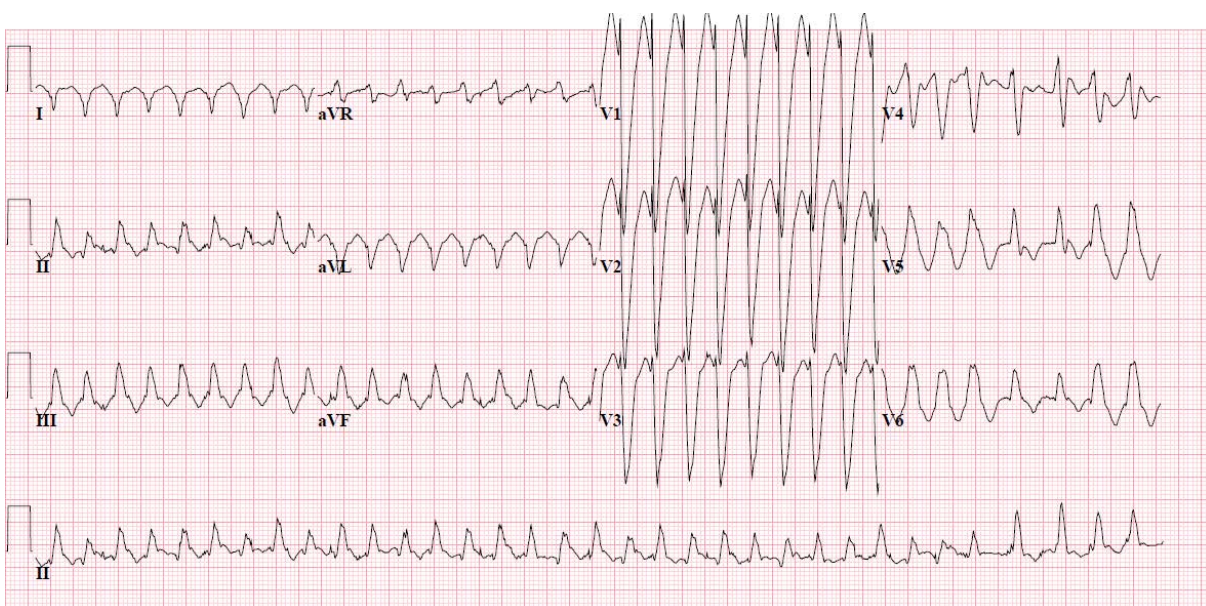
Supplemental Figure 4. Electrocardiography recordings of both patients.

(A) ECG of patient II:2 during hospital admission showing sinus rhythm at a rate of 130/min with extremely broad QRS complexes of 220 ms and normal QRS axis of 60 degrees. (B) ECG of patient II:3 during cardiopulmonary resuscitation (no prior ECG available).

A

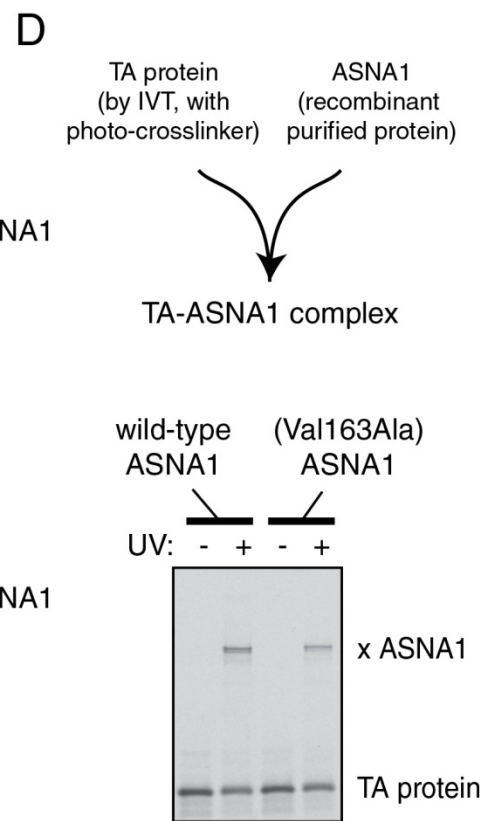
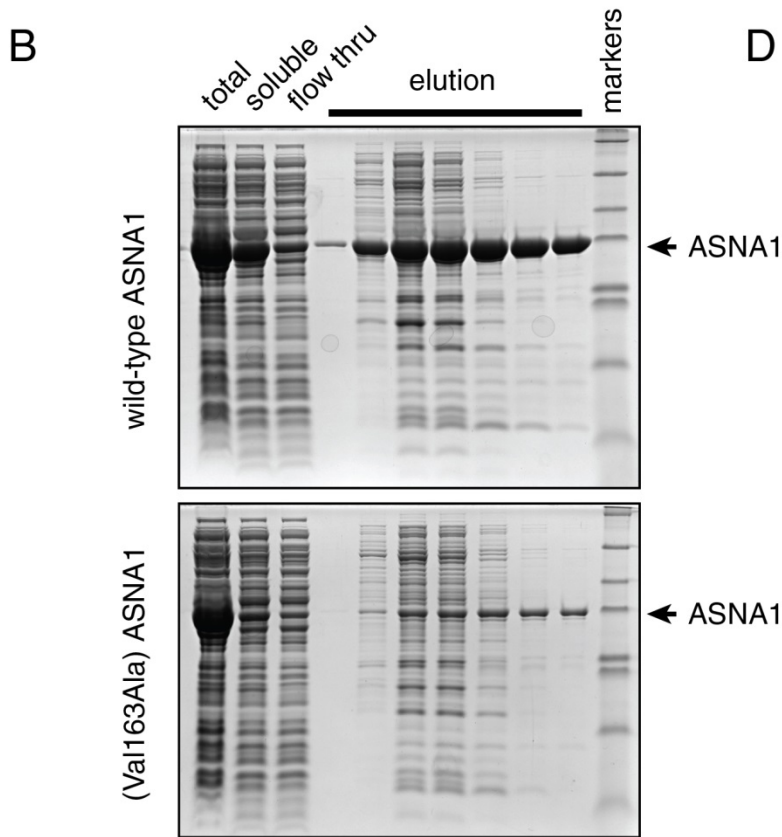
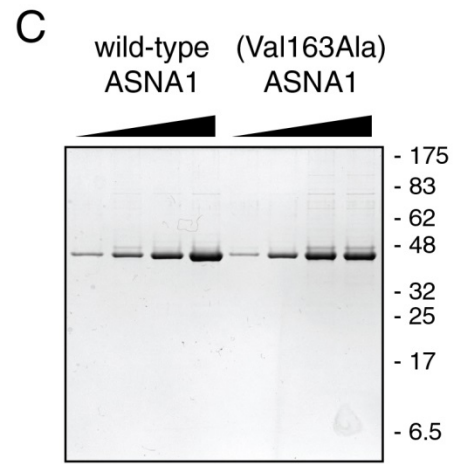
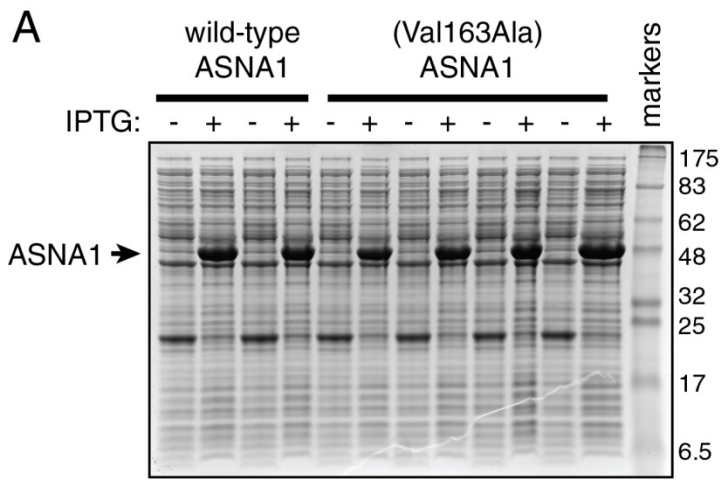


B



Supplemental Figure 5. Recombinant expression and purification of ASNA1 from *E. coli*.

(A) Expression tests of *E. coli* transformed with plasmids encoding either wild-type ASNA1 or the Val163Ala mutant. In each case, equal numbers of cells harvested before or after induction with 1 mM IPTG (for 3 hours at 37°C) were analyzed by SDS-PAGE and staining with Coomassie Blue. Two individual isolates of wild-type and four of mutant ASNA1 all show comparable expression levels of recombinant ASNA1 (indicated by the arrow). (B) The cells from a larger scale induction of wild-type and mutant ASNA1 (as in panel A) were collected, lysed by sonication, and subjected to chromatography using Ni-NTA columns. The total cells, soluble lysate, flow through, and elution fractions are shown. Note that a substantially higher proportion of wild-type ASNA1 is produced as a soluble protein, and recovered by chromatography. This is a consistent effect observed in more than six independent trials. (C) Increasing amounts of purified wild-type or mutant ASNA1 (ranging from 100 ng to 1 µg protein) were analyzed by SDS-PAGE and Coomassie staining to document concentration and purity. (D) A model TA protein containing the transmembrane domain from VAMP2 was translated in a purified *E. coli*-based translation system.²⁵ This system contains only recombinant translation factors and ribosomes, with no additional proteins. In addition, it contains ³⁵S-methionine to label the newly synthesized TA protein, and the photo-crosslinking amino acid benzyl-phenylalanine (BPA) and components for its incorporation at amber codons. A single amber codon in the transmembrane domain of the TA protein is used to incorporate this photo-crosslinking amino acid. The translation was supplemented with either wild-type or mutant ASNA1, which forms a complex with the newly made TA protein. The successful formation of the TA-ASNA1 complex was verified by UV irradiation to induce a covalent crosslink between these two proteins (indicated by “x ASNA1”). These recombinant TA-ASNA1 complexes were used for the insertion assay shown in Figure 4D.



SUPPLEMENTAL VIDEOS

1. Cardiac ultrasound examination in patient II:2 showing poor contractility and thrombus formation in the left ventricle prior to death at age 7 weeks.
2. Cardiac ultrasound examination in patient II:3 showing minor abnormalities at age 9 days.
3. Repeat examination in patient II:3 on day 12 showing ventricular dysfunction and dilatation.
4. Microscopic imaging of blood flow velocity in wild-type and *asna1*^{Δ7/Δ7} zebrafish larvae.
5. Microscopic imaging of heart contractions in wild-type and *asna1*^{Δ7/Δ7} zebrafish larvae.

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