**Supplementary Methods**

**Gene targeting and generation of OriNax and FuseNax Mice**

OriNax mice:

Plakoglobin genomic DNA was amplified from mouse R1 ES cells using PCR. 5' homology arm consisting of a 4.3kbp SalI-SalI fragment was generated using forward primer: GTCGACCCTGGGAAAAGAGAGATATTTGAA and reverse primer: GTCGACCATCTTAGGAAAGAGAAAGCCAAG. 3' homology arm consisting of 4.2kbp ApaI-NotI/ PspOMI fragment was generated using forward primer: GGGCCCTCCCTGCTGTGGGGGTGTAGGGAG and reverse primer: GCGGCCGCAGGAATAAAATCTGTGGGAATCAA. Both arms were cloned in to pBluescript KS+ containing FRT-Neomycin cassette and Diptheria Toxin A (DTA) gene (See Supplementary Figure 1). A 2kb EcoRV- NotI fragment for mutagenesis was amplified using forward primer: GATATCCTTGGCTTTCTCTTTCCTAAGATG and reverse primer: GCGGCCGCTCTAGACTGGAACACACTGGCTAC and cloned in to pBluescript KS+. Mutagenesis was performed in 2 steps, the first of which was using primers to delete 2bp: ***G***GAGGCTGTGAGTATCAGACCACA and GGCAGCTGGGTCATGCTTGAAGAG. Following which the specific bp mutations were modified using primers: GCATGATTCCCATCAATGAACCCTATGCAGACGGTGAG and TCTGGGCCTGAGGAAGGACAAAAA. This fragment was then cloned in to pBluescript KS+.

The targeting construct was verified by sequencing and linearized with NotI before electroporation into R1 ES cells at the Transgenic Core Facility at the University of California, San Diego. G418-resistant ES clones were screened for homologous recombination by Southern blot analysis as described previously (1). The 257bp probe for Southern blotting was amplified using forward primer GCAAGCACTTGACAAGCAACAG and reverse primer

CAAACCCTAAGCAGGACGAAAGC. The Neomycin cassette was deleted using flipase deleter mice (2)

FuseNax mice:

(A) A 751bp Nar-1-EcoRV fragment of mouse Plakoglobin cDNA was amplified from a mouse heart cDNA library using the forward primer: GCGGCCGCGGGCGCCTCTGCCCCTCTCATGGAGC, and reverse primer: CTTGGATATCCCAGGAGGTCAAGTGAAAAACTGG. The fragment was cloned in to pBluescript II KS+ using NotI and EcoRV. Once cloned, mutagenesis was performed using 2 primers: CATGACCCA**GCTGCCGGAGGCT**GCCCAGAGCATGATTCCCATCAATGACCCTATG CAG and CTGCATAGGGTCATTGATGGGAATCATGCTCTGGGCAGCCTCCGGCAGCTGGGTC ATG. After mutagenesis, the cDNA fragment was excised using NarI and EcoRV. Alternatively, for the unmutated cDNA control, no mutagenesis was performed and the cDNA was excised as above.

(B) A 4.0kbp BglII-BglII fragment of genomic Plakoglobin DNA fragment including the last 5 exons was amplified from R1 ES cells into a separate pBluescript II KS+ using primers ggagatCTTGGCTTTCTCTTTCCTAAGATGG and GCAGATCTCATTTGCCTCTGCAACCCTGAG. This construct was cut with NarI and EcoRV to leave 209bp and 591bp of genomic DNA at 5' and 3' ends respectively to generate pBS-Gen.

(C) The mutated and wild-type cDNAs from (A) were then inserted in to the pBS-Gen plasmid using NarI and EcoRV sites to make pBS-Gen-CM and pBS-CW, respectively. Following which the insert in pBS-Gen-CM was amplified using forward primer: gtgtcgaCTTGGCTTTCTCTTTCCTAAGATGG containing SalI site and reverse primer:

GCCATATGATTTGCCTCTGCAACCCTGAG containing NdeI. The PCR product was subsequently cleaved with SalI and NdeI. Whereas pBS-CW was cleaved with BglII and the wild-type insert purified.

(D) PCR and digestion products from (C) were ligated into pDNL vector containing the DTA gene and FRT-Neomycin cassette using NdeI and SalI sites for mutated cDNA and BamHI sites for wild-type cDNA.

(E) The 5' arm of homology consisting of a 4.27 kb Not1-Not1 fragment was generated using forward primer: gagcggccgcctgggaaaagagagatatttgaattag and reverse primer: GTGCGGCCGCCATCTTAGGAAAGAGAAAGCCAAGG. The 3' arm of homology was a 3.19kb NdeI-KpnI fragment generated using forward primer: TTCATATGAAGAGGAGGGAGGGTTTGAAGAGCAGG and reverse primer: GCGGTACCGAGGGCATCGGATCTCATTACAGATG GTTG. Both arms were cloned into pDNL.

(F) The targeting construct was verified by sequencing and linearized with NotI before electroporation into R1 ES cells at the Transgenic Core Facility at the University of California, San Diego. G418-resistant ES clones were screened for homologous recombination by Southern blot analysis as described previously (1).

(G) The 257bp probe for Southern blotting was amplified using forward primer GCAAGCACTTGACAAGCAACAG and reverse primer CAAACCCTAAGCAGGACGAAAGC.

(H) Chimeric mice were crossed with Sox2 Cre deleter mice to delete the wild-type construct.

**Animals procedures, Echocardiography, Hemodynamics, Telemetry ECG**

The UCSD Animal Care Personnel maintained all animals and the UCSD Institutional Animal Care and Use Committee approved all experimental procedures.

Mice were anesthetized with 1% isoflurane and underwent echocardiography using VisualSonics, SonoSite FUJIFILM, Vevo 2100 ultrasound system with a linear transducer 32-55MHz. Measurements of heart rate (HR), left ventricular end-diastolic dimensions (LVED) and left ventricular end-systolic dimensions (LVESD), end-diastolic interventricular septal thickness (IVSd) and LV posterior wall thickness (LVPWd) were determined from the LV M-mode tracing. Percentage fractional shortening (%FS) was used as an indicator of systolic cardiac function. N=16 mice were used for each genotype.

For hemodynamics, 10 week old adult mice were anesthetized using 100mg/kg ketamine and 10mg/kg xylazine and subjected to hemodynamic measurements as described previously (3). N=8 mice were used for each genotype.

Conscious mouse ECG was measured using the telemetry system (Data Sciences International/ Ponemah) on 14-month-old mice as previously described (4). N=4 mice were used for each genotype.

**Immunofluorescence**

Hearts were isolated from 10 month old mice and were rinsed in PBS, immersed in cold isopentane and embedded in optical cutting temperature (OCT) compound on dry ice. Cryostat sections (10μm) were cut and fixed in acetone at -20°C for 5 mins. After permeabilisation with washing buffer (PBS with 0.2% TX-100) sections were incubated with the indicated antibodies (Supplementary Table 1) overnight in blocking buffer (PBS with 5% normal donkey serum, 1% BSA) in a humidified chamber at 4°C. Sections were rinsed in wash buffer and incubated at room temperature with the indicated fluorescently-conjugated secondary antibodies and DAPI (1:500) diluted in blocking buffer for 2 h. Slides were rinsed in wash buffer and mounted in mounting buffer (Dako). Sections were imaged as described previously (5). Briefly, using an oil-immersed 100X

objective, with 1.35 numerical aperture, on an inverted microscope controlled by a DeltaVision system (Applied Precision, Washington, USA).

**Subcellular Fractionation**

Whole ventricles were dissected and snap frozen in liquid nitrogen. Subcellular fractions were extracted using ProteoExtract® Subcellular Proteome Extraction Kit according to manufacturer's recommendations. N=6 mice were used for each genotype.

**Western Blotting**

Protein lysates were run on 4-12% SDS-PAGE gels (Life Technologies, Carlsbad, CA) and transferred overnight at 4°C on to PVDF membrane (Biorad). Following which, they were blocked for an hour in wash buffer (Tris Buffered Saline with 0.1% Tween 20 (TBS-T)) containing 5% Milk and incubated overnight at 4°C with the indicated primary antibody (listed in Supplementary Table 1) in wash buffer supplemented with 2% milk. Blots were washed and incubated with HRP-conjugated secondary antibody generated in Rabbit (1:5000) or Mouse (1:2000) (Dako) for 1hr at room temperature. Blots were visualized using ECL Chemiluminescence. N=6 mice were used for each genotype.

**Antibodies**

Antibodies used for immunofluorescence and western blotting are listed in Supplementary Table 1.

**Histology**

Hearts were isolated from age and sex matched littermates and washed in PBS before being fixed overnight in Formalin. Hearts were subsequently dehydrated in 70% Ethanol and embedded in paraffin and cut into 10mm sections. Sections were stained using

either Masson's Trichrome or Hematoxylin and Eosin, mounted and imaged using a Hamamatsu NanoZoomer 2.0HT Slide Scanning System (Hamamatsu).

**Real-time PCR**

Total RNA was extracted from 10 month old mouse hearts using Trizol reagent according to manufacturer's recommendations (Life Technologies). cDNA was synthesized using MMLV Reverse Transcriptase (Bio-Rad). Primers for RT-PCR are listed in Supplementary Table 2. RT-PCR reactions were performed using Sso-Fast EvaGreen Real Time PCR (Bio-Rad) master mix in 96-well low profile PCR plates in the CFX96 Biorad Thermocycler. N=8 mice were used for each genotype.

## Statistics

Data are presented as mean ± SEM unless indicated otherwise. We used 2-tailed Student's *t* test or ANOVA for comparisons among groups as indicated. Analysis was performed using Microsoft Excel software. *P* values of less than 0.05 were considered significant.

## Study approval

All animal procedures were approved by the UCSD Animal Care and Use Committee. UCSD has an Animal Welfare Assurance (A3033-01) on file with the Office of Laboratory Animal Welfare and is fully accredited by AAALAC International.

| Antibody | Source | Catalogue Number |
|---|---|---|
| Plakoglobin N-term | Santa Cruz | H80 |
| Plakoglobin N-term | Sigma | P8087 |
| Plakoglobin C-term | BD | 610253 |
| Plakophilin 2 | Fitzgerald | 10R-P130B |
| Desmoplakin | Serotec | 2722-5204 |
| Desmocollin 2 | Fitzgerald | 20R-DR004 |
| Desmoglein 2 | Fitzgerald | 10R-D105A |
| Alpha E Catenin | Sigma | C2081 |
| Beta Catenin | Sigma | C2206 |
| N-cadherin | Sigma | C3678 |
| Connexin 43 | Sigma | C6219 |
| ZO1 | Life Technologies | 40-2200 |
| ILK | Cell Signalling | 3862 |
| Pinch 1 | Wang-Rodriguez et al. (6) | NA |
| B1d Integrin | Pham et al. (7) | NA |
| Desmin | Sigma | D33 |
| Vinculin | Sigma | V9131 |
| GAPDH | Santa Cruz | SC32233 |
| Titin (m8) | Kind gift from M. Gautel, (KCL) (8) | NA |
| Alpha actinin | Sigma | A7811 |

**Supplementary Table 1. Antibodies used in this study.** NA, not applicable.

| Gene | Orientation | Sequence |
|---|---|---|
| ANP | 5'-3' | GAT AGA TGA AGG CAG GAA GCC GC |
| ANP | 3'-5' | AGG ATT GGA GCC CAG AGT GGA CTA GG |
| BNP | 5'-3' | TGTTTCTGCTTTTCCTTTATCTGTC |
| BNP | 3'-5' | CTCCGACTTTTCTCTTATCAGCTC |
| Myh 6 | 5'-3' | CTG CTG GAG AGG TTA TTC C TCG |
| Myh 6 | 3'-5' | GGA AGA GTG AGC GGC GCA TCA AGG |
| Myh 7 | 5'-3' | TGC AAA GGC TCC AGG TCT GAG GGC |
| Myh 7 | 3'-5' | GCC AAC ACC AAC CTG TCC AAG TTC |
| Collagen 1a1 | 5'-3' | TCACCAAACTCAGAAGATGTAGGA |
| Collagen 1a1 | 3'-5' | GACCAGGAGGACCAGGAAG |
| Collagen 3a1 | 5'-3' | ACAGCAGTCCAACGTAGATGAAT |
| Collagen 3a1 | 3'-5' | TCACAGATTATGTCATCGCAAAG |
| TGF beta 1 | 5'-3' | CTCCCGTGGCTTCTAGTGC |
| TGF beta 1 | 3'-5' | GCCTTAGTTTGGACAGGATCTG |
| C-Myc | 5'-3' | ATGCCCCTCAACGTGAACTTC |
| C-Myc | 3'-5' | GTCGCAGATGAAATAGGGCTG |
| Cyclin D1 | 5'-3' | ATGGAAGGACCCTTGAGGC |
| Cyclin D1 | 3'-5' | CTTCACGGCTTGCTCGTTCT |
| Adiponectin | 5'-3' | TTGCAAGCTCTCCTGTTCCT |
| Adiponectin | 3'-5' | CAGCTCCTGTCATTCCAACA |
| 18S | 5'-3' | GGA AGG GCA CCA CCA GGA GT |
| 18S | 3'-5' | TGC AGC CCC GGA CAT CTA AG |
| GAPDH | 5'-3' | CTCAAGATTGTCAGCAATGCATCC |
| GAPDH | 3'-5' | CCAGTGGATGCAGGGATGATGTTC |
| Lipoprotein lipase | | TaqMan primer/ probeset Mm00495368_m1 |
| NF-KB | | TaqMan primer/ probeset Mm00476361_m1 |
| GAPDH | | TaqMan primer/ probeset Mm99999915_g1 |

**Supplementary Table 2. Primers used for q-RT-PCR in this study.**

**References**

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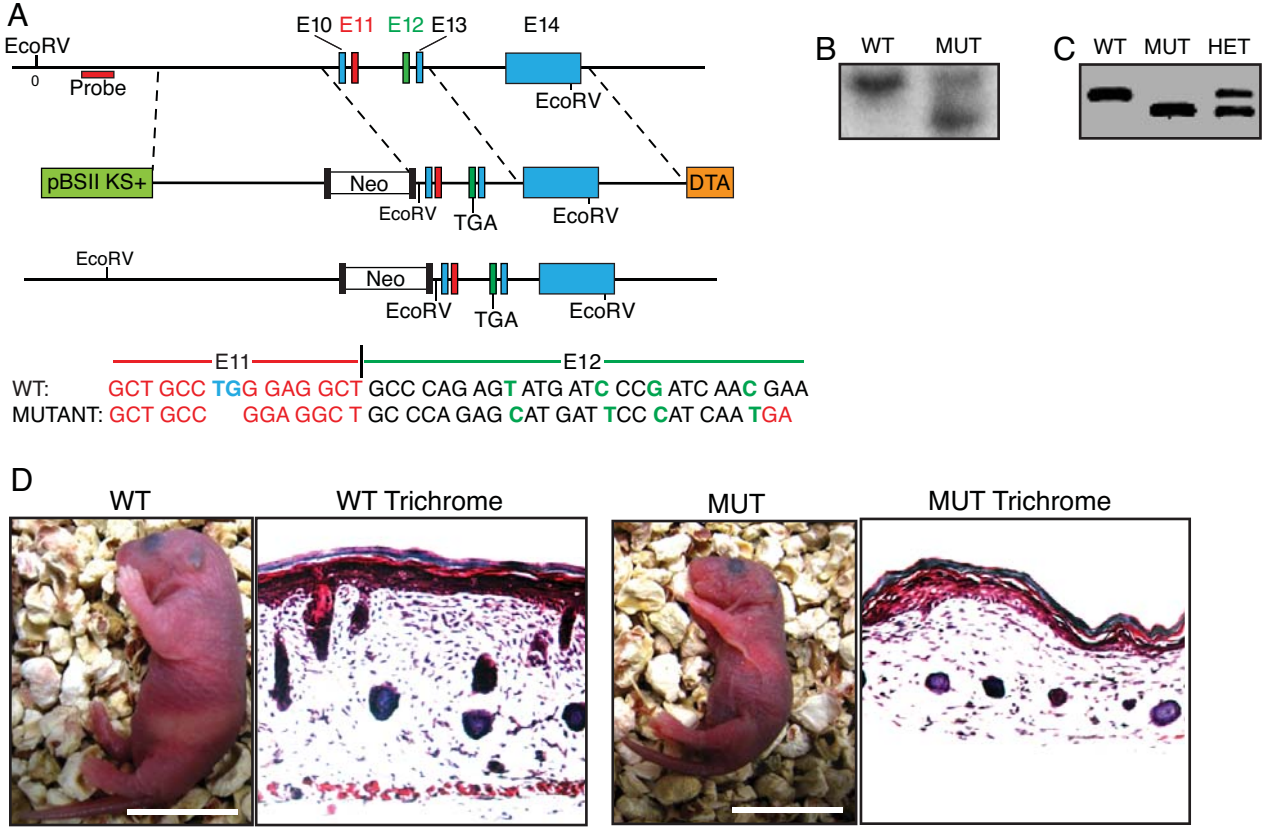
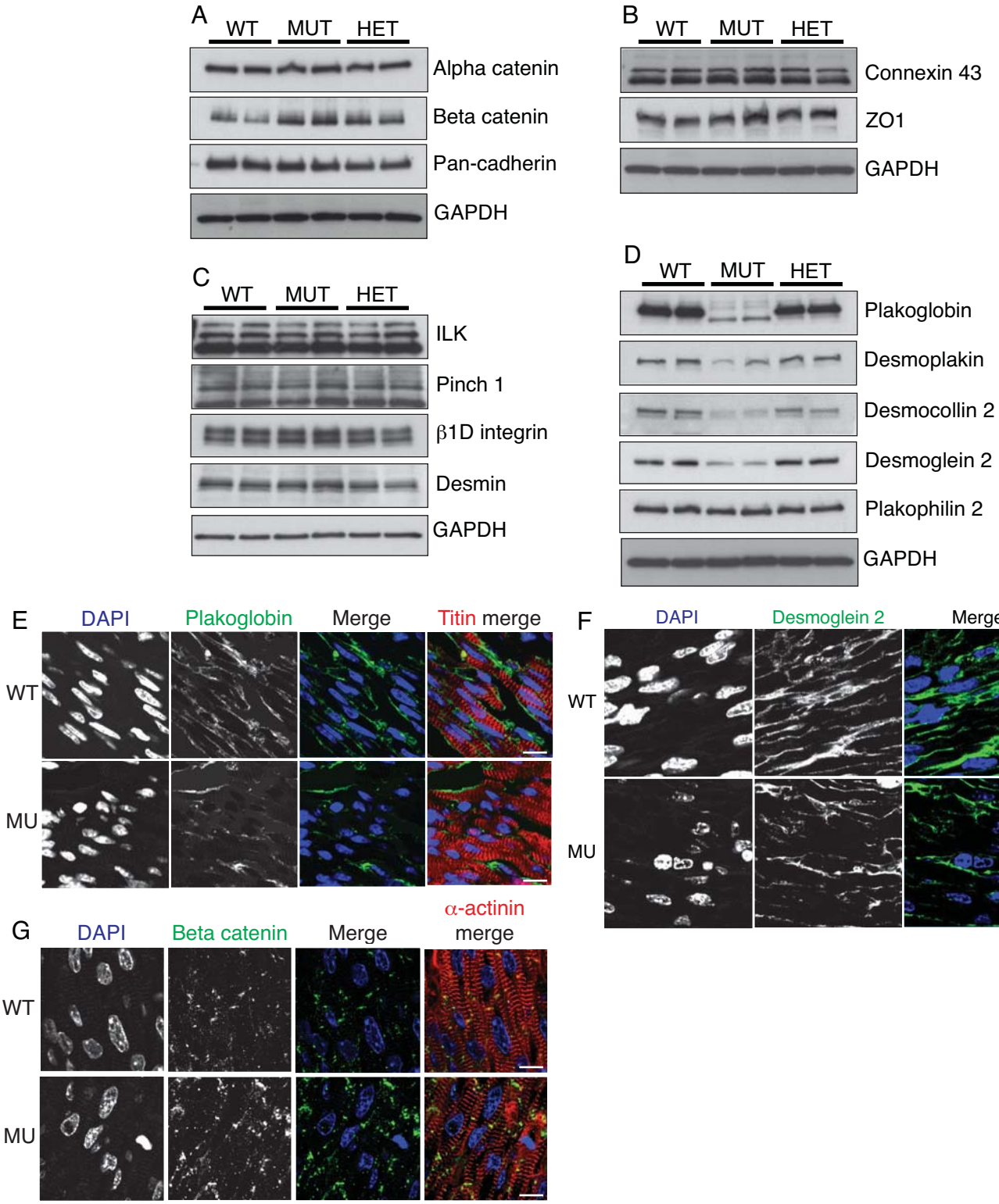
# Supplementary Figure 1




**Validation of OriNax mice**

**A** Schematic of the original Naxos construct. Exons are depicted in blue, flipase sites are denoted as black rectangles. Red and green rectangles denote the exons with the TG deletion and premature stop codons, respectively. Note the removal of 2 base pairs (bps) (TG) denoted in blue from the wildtype (WT) allele and mutation of the indicated bps (green) to modify the mouse genome to exactly mimic the human mutation found in Naxos disease. **B, C** Southern blot and genotyping confirmation, respectively. **D** Macroscopic view of WT (left) and OriNax (right) and Masson's Trichrome stained epidermis isolated from postnatal day 1 mice. Note the more wrinkled, looser skin in the OriNax mouse and the thinning of the epidermis as well as the lack of milk in the stomach compared to its WT littermate. Images are representative of 4 mice per genotype. Scale bars, 10mm.

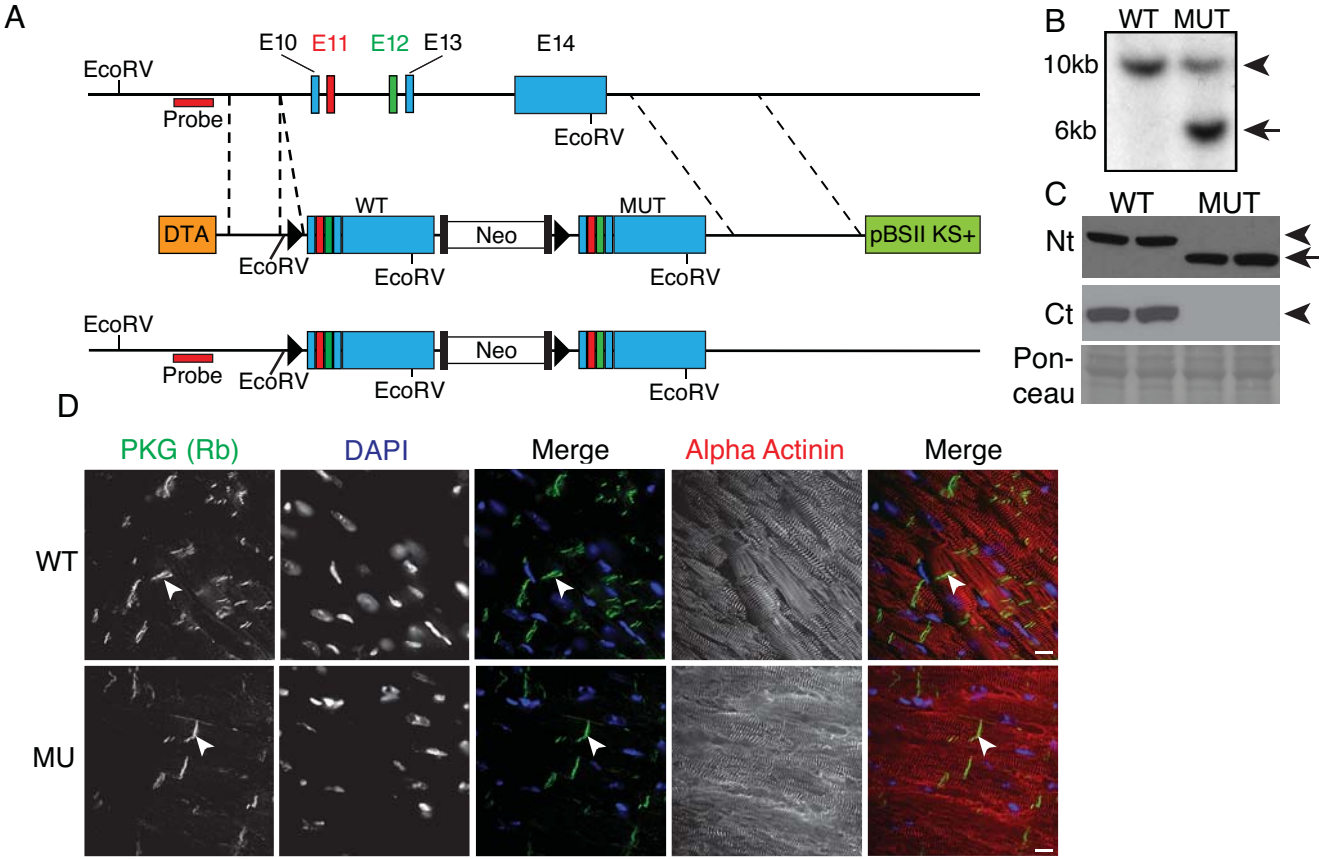
Supplementary Figure 2




**Molecular characterization of OriNax mice**

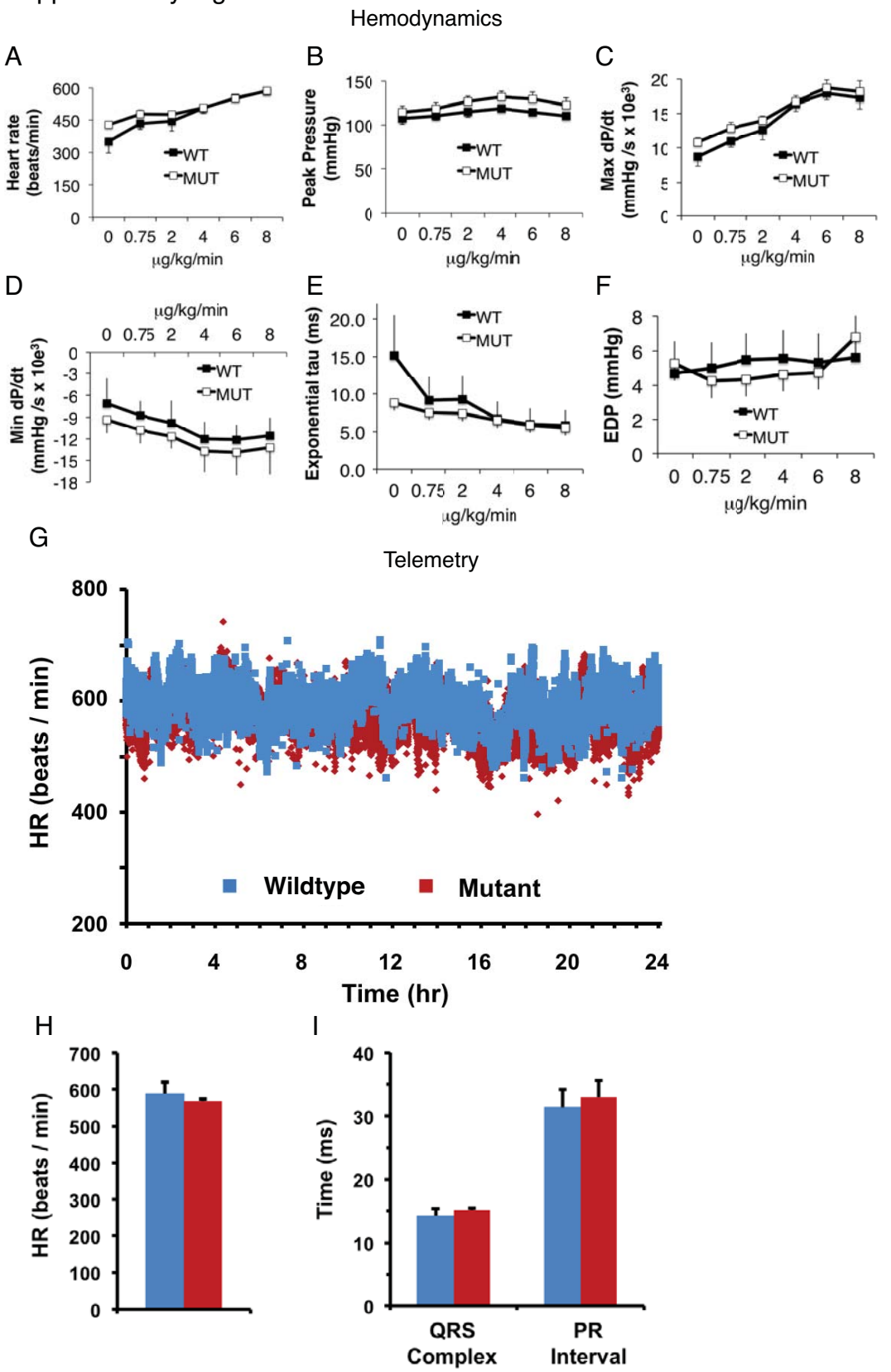
**A-D** Western blots of the different protein components of the intercalated disc in wildtype (WT), OriNax (MUT), and heterozygote (HET) mice. Note that β-catenin levels increased in the OriNax mice and levels of Desmoplakin, Desmocollin 2 and Desmoglein 2 decreased. Each WB is representative of at least 6 mice per genotype.**E-G** Immunofluorescence of Plakoglobin (E), Desmoglein 2 (F) and β-catenin (G) in mouse myocardium. Each image is representative of at least 3 mice per genotype. Scale bars, 10μm.

Supplementary Figure 3




**Validation and characterization of the Fused Exon Naxos (FuseNax) knock-in mouse**
**A** Schematic of the FuseNax construct. Exons are depicted in blue, LoxP and flipase sites are denoted as black triangles and rectangles, respectively. Red and green rectangles denote the location of the exons with the TG deletion and premature stop codons, respectively. Wildtype (WT) and mutant (MUT) denote the last 5 exons that are fused from either WT or mutated cDNA. **B** Southern blot confirmation of the FuseNax allele (MUT). Arrowhead indicates the predicted size of the WT band, arrow indicates the predicted size of the mutant band. **C** Western blot of the WT and homozygote FuseNax (MUT) mice using antibodies that recognize the N-terminus (Nt) and C-terminus (Ct) of Plakoglobin. Note the reduced MW of Plakoglobin in the FuseNax mouse (black arrow) and the absence of a band using PKG Ct indicating the protein has been truncated at the correct site. Arrowheads denote the predicted MW of full-length Plakoglobin. Each WB is representative of at least 6 mice per genotype. **D** Immunofluorescence of mouse myocardium using antibodies generated against Plakoglobin in rabbit (PKG Rb). Note the localization of Plakoglobin (green) to the intercalated disc (white arrowheads). Each image is representative of at least 3 mice per genotype. Scale bars, 10µm.

Supplementary Figure 4




**Hemodynamics and Telemetry analysis of FuseNax mice**

**A-F** Hemodynamics measurements of contractile function in wildtype (WT) and FuseNax (MUT) mice in response to increasing levels of beta 1 adrenergic agonist dobutamine. Note that no defects in contractile function between WT and FuseNax mice were observed. N=8 mice per genotype. **G** Heart rates (HR) of wildtype and FuseNax (Mutant) mice were monitored over 24 hrs. **H** Average heart rates of WT and FuseNax over 24 hrs. **I** Duration of the QRS complex and PR interval were monitored and averaged over 24 hrs. Note there were no significant differences between WT and FuseNax mice. N=4 mice per genotype. Data represent mean ± SEM. Two-tailed Student's t-test was performed on the data.