SUPPLEMENTAL DATA

Desmosomal COP9 regulates proteome degradation in arrhythmogenic right ventricular dysplasia/cardiomyopathy

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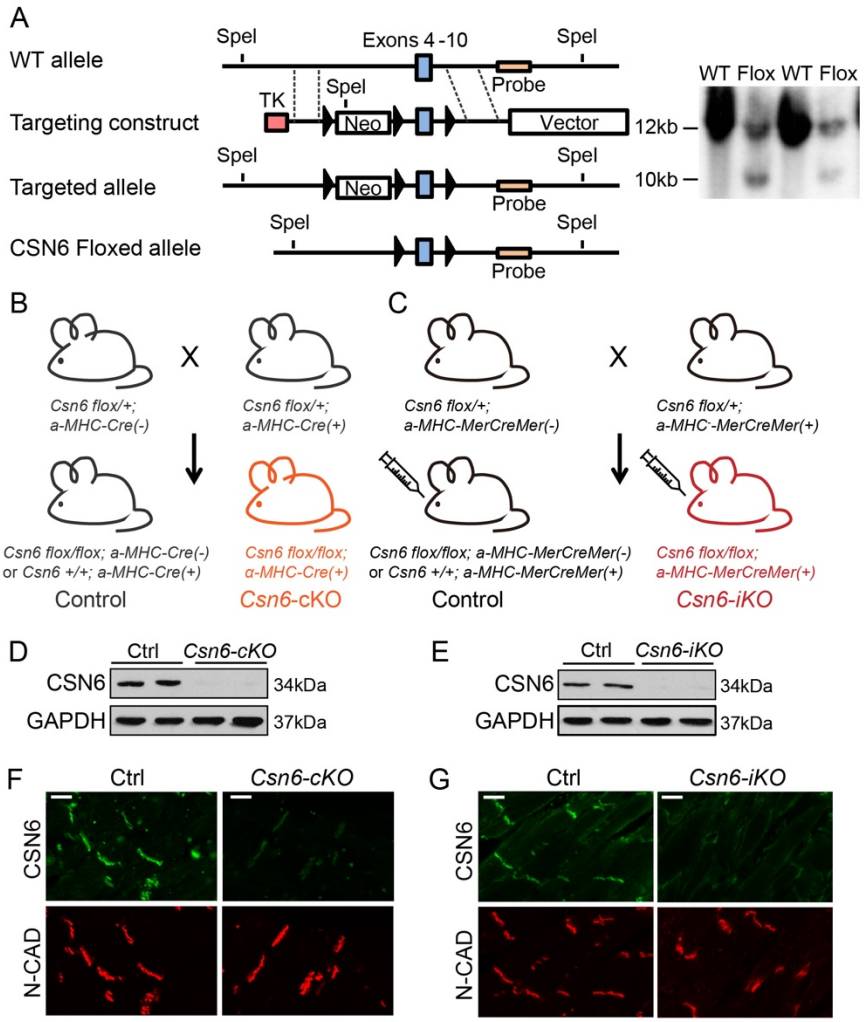
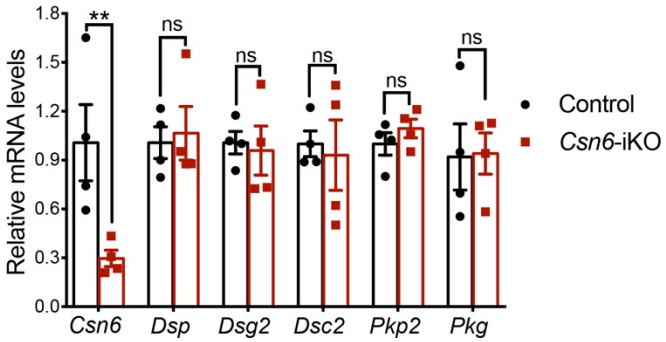
Supplemental Figure 1



Supplemental Figure 1: Generation and validation of CSN6 loss in cardiac specific and cardiac inducible *Csn6* knockout mice. (**A**) Restriction map of the relevant genomic region of *Csn6*, targeting construct, the mutated locus following recombination and *Csn6* floxed allele (left). TK: thymidine kinase gene, Neo: neomycin resistance gene. Detection WT and mutant (target) alleles by southern blot analysis (right). (**B**) Schematic representation of breeding strategy to generate *Csn6*-cKO (*Csn6 flox/flox*; *a-MHC-Cre(+)*) and littermate control (*Csn6 flox/flox; a-MHC-Cre(-)* or *Csn6*$^{+/+}$*; a-MHC-Cre(+)*) mice. (**C**) Schematic representation of breeding strategy to generate *Csn6*-iKO (*Csn6 flox/flox; a-MHC-MerCreMer(+)*) and littermate control (*Csn6 flox/flox; a-MHC-MerCreMer(-)* or *Csn6 +/+; a-MHC-MerCreMer(+)*) mice. To induce ablation of *Csn6*, 5- to 6-week-old mice were treated with daily intraperitoneal injection of tamoxifen (Sigma, 50μg/g per day) for 5 consecutive days. (**D** and **E**) Protein blot analysis of CSN6 in total

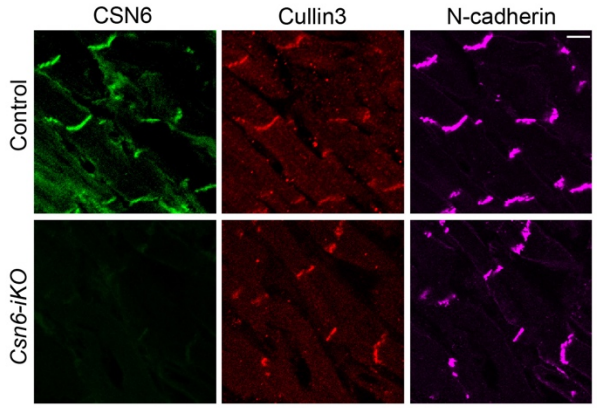
protein extracts from *Csn6*-cKO and control mice at 2 weeks of age (**D**) and *Csn6*-iKO mice at 2 weeks post tamoxifen injection (**E**) (n=4 mice). GAPDH is used as a loading control. (**F** and **G**) Immunofluorescence staining of CSN6 (green) and N-cadherin (red) in cardiac sections from *Csn6*-cKO and control mice at 2 weeks of age (**F**) and *Csn6*-iKO at 2 weeks post tamoxifen injection (**G**). Scale Bar: 10 μm.

Supplemental Figure 2



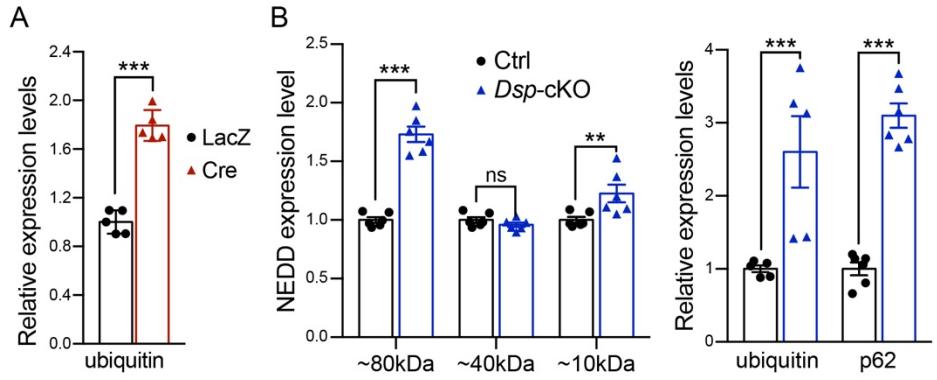
Supplemental Figure 2: RNA levels of desmosomal genes are not impacted in *Csn6* knockout hearts. Quantitative RT-PCR analysis of *Csn6* and desmosomal gene expression in *Csn*6-iKO and control mouse hearts at 6 weeks post-tamoxifen injection (n=4 mice). *18S* and *Gapdh* RNA were used as loading controls and showed similar results. Data are mean ± s.e.m; two-way ANOVA with Sidak multiple comparison test, $^{**}P<0.01$, ns, not significant.

Supplemental Figure 3



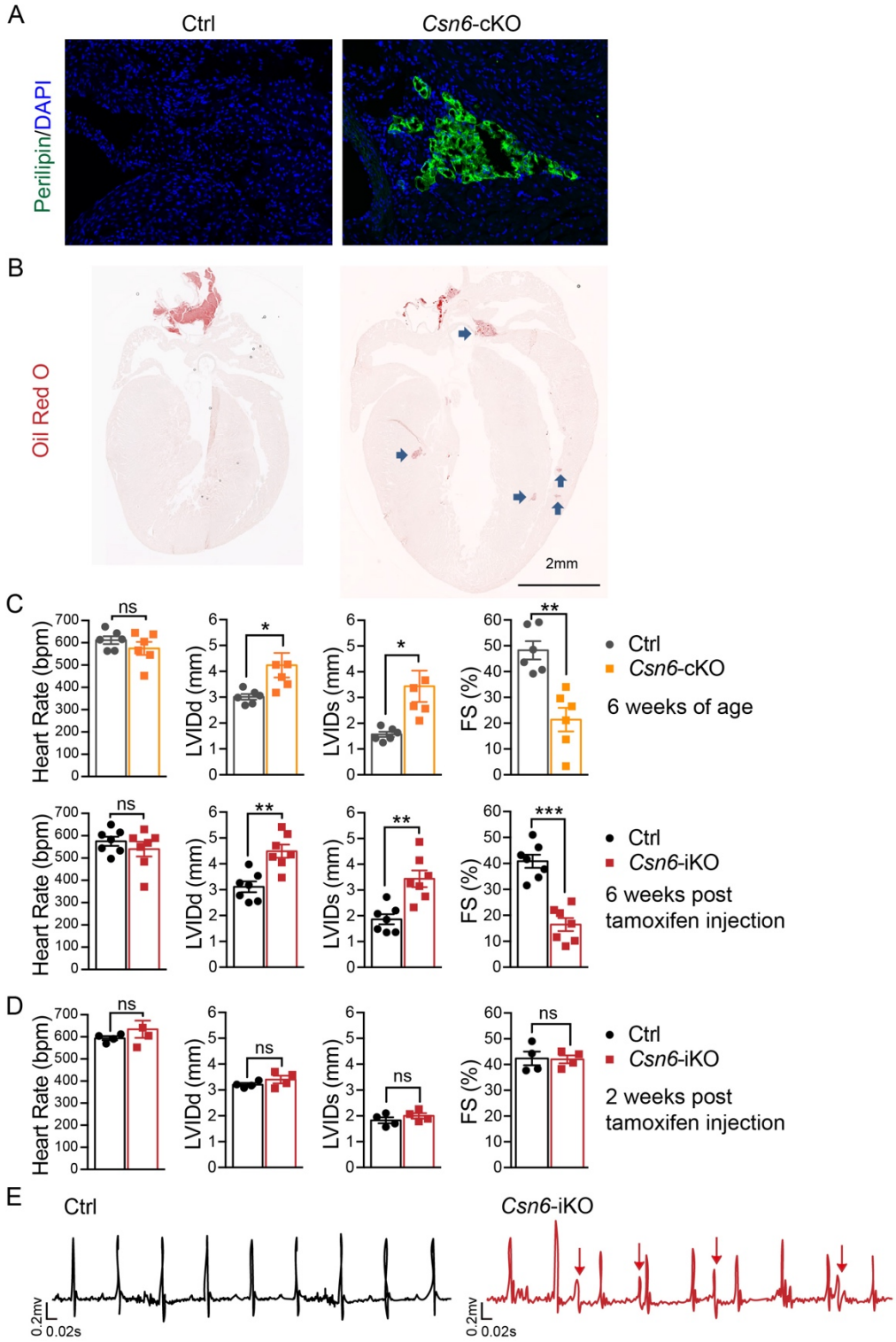
Supplemental Figure 3: Cullin 3 is localized to the cardiac cell-cell junction in adult WT and *Csn6*-iKO mouse hearts. Immunofluorescence staining of Cullin 3 (red), CSN6 (green) and N-cadherin (magenta) in control and *Csn6*-iKO mouse hearts at 6 weeks post tamoxifen injection (n=3 per group). Scale bar: 10μm.

Supplemental Figure 4



Supplemental Figure 4: Quantification of protein expression levels in Figure 4 and Figure 5. (**A**) Quantification of protein expression levels in Figure **4A**. The expression levels of the proteins were normalized to the loading control GAPDH. Data are mean ± s.e.m; two-tailed Student's t-test. ***P<0.001. (**B**) Quantification of protein expression levels in Figure **5A**. The expression levels of the proteins were normalized to the loading control GAPDH. Data are mean ± s.e.m;, two-way ANOVA with Sidak multiple comparison test, **P<0.01, ***P<0.001, ns, not significant.

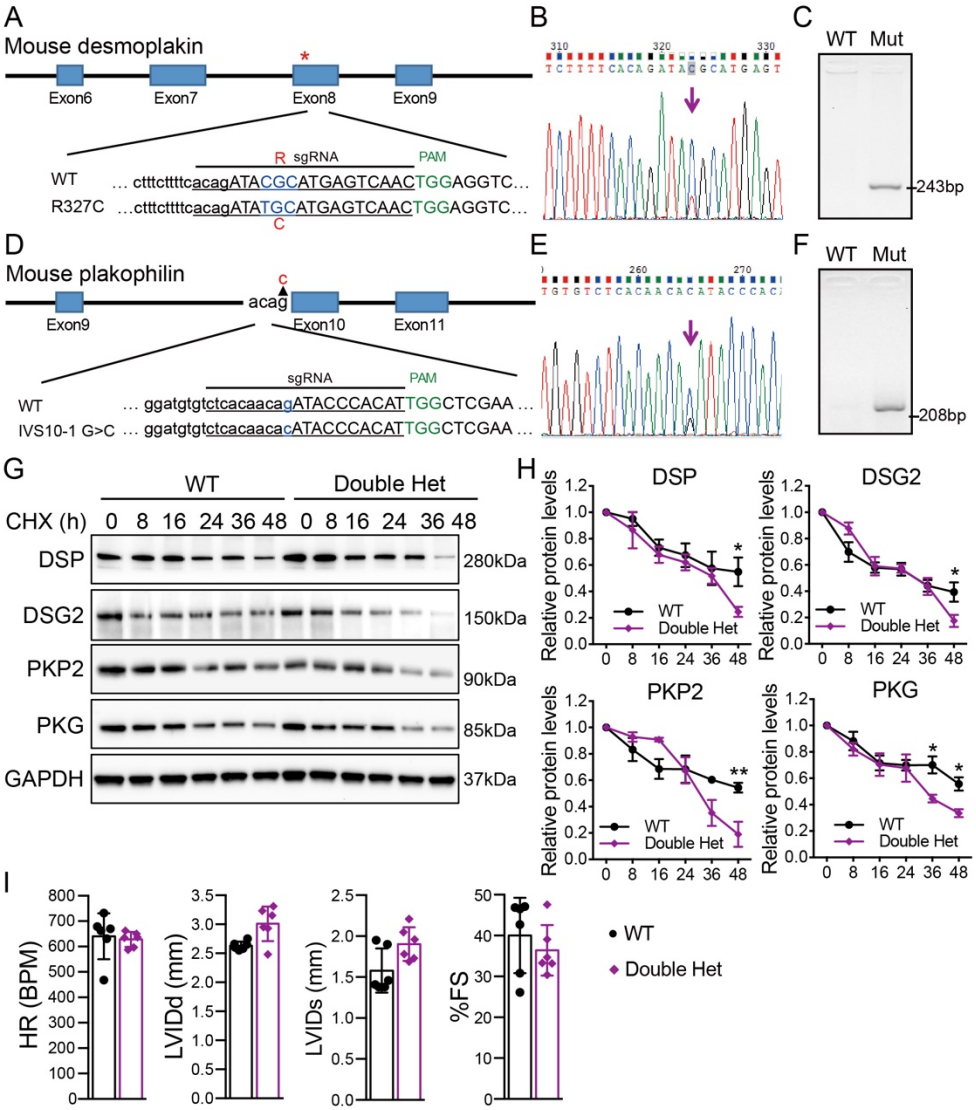
Supplemental Figure 5



Supplemental Figure 5: Characterization of cardiac specific *Csn6* knockout mice. (**A**) Immunofluorescence staining of perilipin in triangular area of cardiac sections in *Csn6*-cKO and control mice at 6 weeks of age. Cardiac sections were counterstained with DAPI nuclear stain

(blue). Scale Bar: 100 μm. (**B**) Representative Oil Red O stained whole cardiac sections from *Csn6*-cKO and control mice at 6 weeks of age (n=4 per group). Scale bar: 2mm.(**C**) Echocardiographic M-mode analysis of heart function in *Csn6* deficient and control mice at 6 weeks of age and post-tamoxifen injection (n=6 mice). FS %: percent fractional shortening, LVIDd: left ventricular internal dimension at end-diastole. LVIDs: left ventricular internal dimension at end-systole. Data are mean ± s.e.m.; two-tailed Student's t-test, $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; ns, not significant. (**D**) Echocardiographic M-mode analysis of heart function in *Csn6*-iKO and control mice at 2 weeks post tamoxifen injection (n=4 mice). Data are mean ± s.e.m.; two-tailed Student's t-test, ns, not significant. (**E**) Representative conscious ECG tracings from *Csn6*-iKO (red) and control mice (black) at 2 weeks post tamoxifen injection (n=4 mice). Arrows denotes ectopic beats.

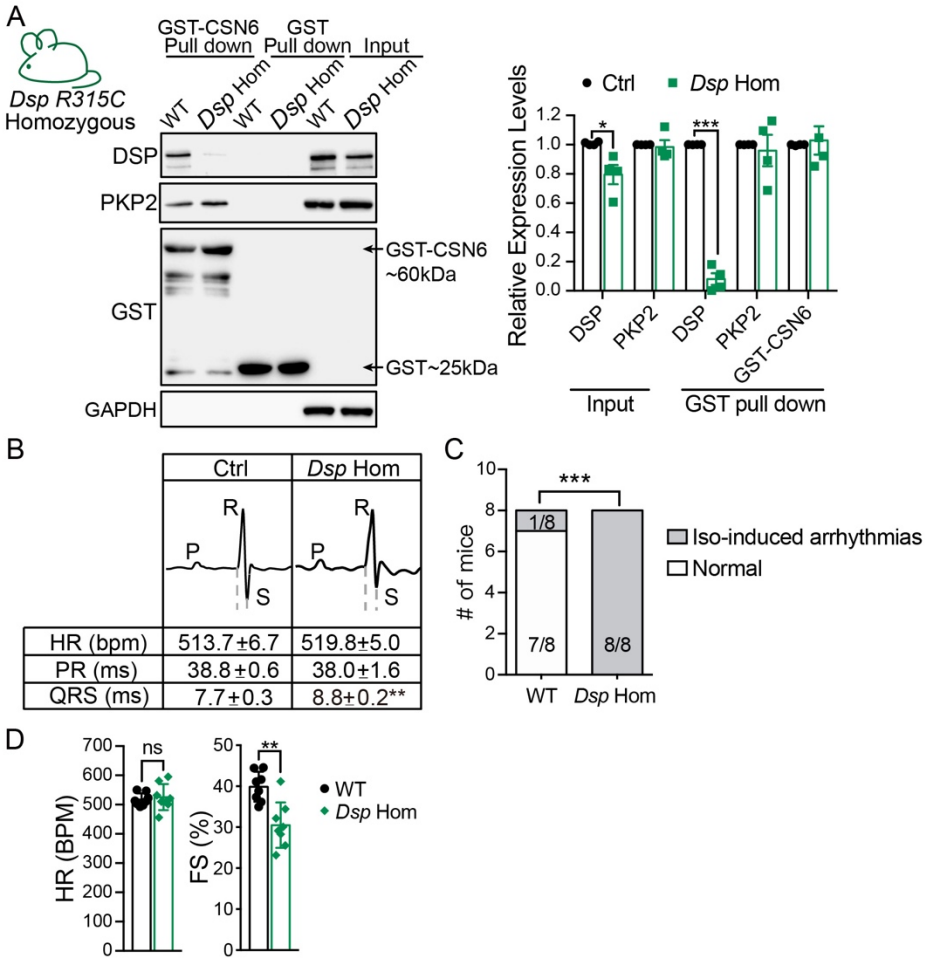
Supplemental Figure 6



Supplemental Figure 6: Generation and characterization of knock-in mice harboring human equivalent *DSP R315C* and *PKP2 IVS10-1 G>C* mutations. (**A** and **D**) Schematic of the Cas9/sgRNA targeting site for the *Dsp R327C* (**A**) and *Pkp2 IVS10-1 G>C* (**D**) which are equivalent to the human *R315C* mutation and *PKP2 IVS10-1 G>C* respectively. The sgRNA coding sequence is underlined. The protospacer-adjacent motif (PAM) sequence is labeled in green. Uppercase: exon sequences. Lowercase: intron sequences. (**B** and **E**) Validation of *Dsp R327C* (**B**) and *Pkp2 IVS10-1 G>C* (**E**) allele by sequencing. (**C** and **F)** PCR using mutant-specific primers confirmed newborn mice contained the correct gene variant allele. (**G** and **H**) Representative protein blots (**G**) and quantification analyses (**H**) of desmosomal proteins in ventricular cardiomyocytes which isolated from Double Het and WT pups. The cardiomyocytes

were treated with cycloheximide (CHX, 10 μg/ml) for 0, 8, 16, 24, 36 and 48 hours. The expression levels of proteins were normalized to the loading control GAPDH. Data are mean ± s.e.m; two-way ANOVA with Sidak multiple comparison test, $^{*}P<0.05$, $^{**}P<0.01$. (**I**) Echocardiographic M-mode analysis of left ventricular dimensions and function in Double Het and WT mice at 4 months of age (n=6 mice). FS %: percent fractional shortening, LVIDd: left ventricular internal dimension at end-diastole. LVIDs: left ventricular internal dimension at end-systole. Data are mean ± s.e.m.; two-tailed Student's t-test.

Supplemental Figure 7



Supplemental Figure 7: Characterization of *Dsp R315C* homozygous mice. (**A**) Protein blot analyses and quantification of protein expression levels following GST pulldown assay. GST (~25kDa) and GST-CSN6 (~60kDa) protein were incubated with the heart extracts from WT and *Dsp R315C* homozygous mice (*Dsp* Hom) at 1 year of age (n=4 per group). The expression levels of input proteins were normalized to the loading control GAPDH. Data are mean ± s.e.m; two-way ANOVA with Sidak multiple comparison test, **P*<0.05, ****P*<0.001. (**B**) Representative surface ECG tracings in *Dsp R315C* homozygous and WT mice at 1 year of age (n=8 per group). Quantification of heart rate (HR), PR and QRS intervals from ECG tracings was performed. Data from 100 ECG tracings per mouse were averaged for analysis. Data are mean ± s.e.m; Student's two-tailed t-test, ***P*<0.01. QRS duration is indicated by the dotted line. (**C**) Quantification of number of *Dsp R315C* homozygous and WT mice which displayed arrhythmia with 2.5mg/kg

isoproterenol treatment during ECG recording at 1 year of age (n=8 per group). Chi-square test, ***P<0.05. (**D**) Echocardiographic M-mode analysis of left ventricular dimensions and function in *Dsp R315C* homozygous and WT mice at 1 year of age (n=8 per group). HR: heart rate; FS %: percent fractional shortening. Data are mean ± s.e.m.; Student's two-tailed t-test. **P<0.01, no significant differences.

Supplemental Table 1: *Dsp R315C* and *Pkp2 IVS10-1 G>C* crRNA, trans-activating crRNA (tracrRNA), and single-strand oligodeoxynucleotides sequences used to generate knockin mice.

Oligo Name	Sequence (5' to 3')
Dsp R315C crRNA	acagauacgcaugagucaacGUUUUAGAGCUAUGCUGUUUUG
Pkp2 IVS10-1 G>C crRNA	cucacaacagauacccacauGUUUUAGAGCUAUGCUGUUUUG
tracrRNA	AAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGGUGCU
Dsp R315C DNA	AACAGAAATCTAACTGTGACTTGCTGTATGGACTGGTCCTTTCTTTTC ACAGATA**T**GCATGAGTCAACTGGAGGTCAAGGAAAAGGAACTCAAT AAGCTTAAACAAGAAAG
Pkp2 IVS10-1 DNA	AGAGAACTTCTCTGGTAGCAAATGTGATAGCATTTACAGGATGTGTC TCACAACA**C**ATACCCACATTGGTGGCTCGAATGGTTGTCCAAAAGGA AAATGGTCTTCAGCATA

Supplemental Table 2: Primer sequences for real-time PCR analyses.

Gene	Primer 5'-3'
Csn6-F	GAGCTGGAGTTTCTGGGTTG
Csn6-R	GATCCGTTCAGCTTCCTCAG
Dsp-F	GCTCCATTACCAAGACTTCATC
Dsp-R	TGTCGTCGTCTCCAAACATCT
Dsg2-F	GAGGAATTGAGTGCAGCACATAC
Dsg2-R	CTTGCTTCCACCGTCAAGG
Dsc2-F	ATGCAGATGGGAGAAGCTGT
Dsc2-R	TGCAACAATTTCAGCAGAGG
Pkp2-F	GGCTCTCCAGAACCTCACAG
Pkp2-R	GGGAAAGATTCCGTGACAAA
Pkg-F1	CCTGTGGACTCTGCGCAAT
Pkg-R1	GACCAGGATCTTCAGCACACTCT