Connexin Defects Underlie Arrhythmogenic Right Ventricular Cardiomyopathy In a Novel Mouse Model

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SUPPLEMENTARY METHODS

TUNEL Analysis

Terminal deoxynucleotidyl transferasemediated biotinylated UTP nick end labeling (Roche Applied Science) staining assays were performed according to manufacturer’s instructions on cardiac sections counterstained with Alexa Fluor 488 phalloidin (1:200, Invitrogen) and 4’,6-Diamidino-2-Phenyldione, Dihydrochloride (DAPI) to visualize cardiac muscle and nuclei, respectively.

RNA Analysis

Total RNA was extracted from control and DSP-cKO hearts at 4 weeks of age with Trizol (Invitrogen Corp). cDNA was generated utilizing random hexamers and Superscript® III Reverse Transcriptase (Invitrogen). Real Time-PCR (RT-PCR) was performed in the CFX96 BioRad thermocycler (Bio-Rad) using the SsoFast EvaGreen RT-PCR Supermix (Bio-Rad). The oligonucleotide primers utilized for connexin 40 and GAPDH were the following: Cx40 Forward, 5’-CGATACCATTCAGCCTGGTT-3’, Cx40 Reverse, 5’-GAAGGCGTG GACACAAAGAT-3’, GAPDH Forward, 5’-GACGCGCCGCATCTTTGTT-3’ and GAPDH Reverse, 5’-CACACCACCTTCACCATTTTT-3’.
Figure S1. **TUNEL analysis of DSP-cKO hearts.** TUNEL staining of cardiac sections from control and DSP-cKO mice at 4 weeks of age. Cardiac midwall (A and B) and subepicardium (C and D) sections were TUNEL-labeled (red) and counterstained with DAPI (blue) and phalloidin (green) to visualize apoptotic nuclei and cardiac muscle, respectively. White arrows highlight TUNEL positive cells. Bar represents 40 μm.
Figure S2. Cx40 mRNA expression in DSP-cKO hearts. RT-PCR analysis of Cx40 mRNA expression relative to GAPDH in control (n=3) and DSP-cKO (n=3) hearts at 4 weeks. ns=not significant.
**Figure S3. Connexin 43 expression in DSP-cKO hearts.** Cardiac sections from control and DSP-cKO mice at 6 weeks were double labeled with antibodies against connexin 43 (red) and sarcomeric α-actinin (green), as well as being counterstained with DAPI nuclear stain (blue). Bar represents 40 µm.