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3D Visualization of Structural and Protein Compositional Changes in Plakophilin-2 Deficient Hearts

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Many biological and pathological questions can be addressed by observing the morphological and protein compositional changes in high detail using various microscopy technologies. Super resolution microscopy has increased resolution dramatically for protein localization over the last decade, but immune-electron microscopy remains a powerful tool to localize a protein within its native structure landscape.

Plakophilin-2 (PKP2) is a component of desmosome in cardiac intercalated disc (ID) which plays an important role in intercellular adhesion (Grossmann *et al.*, 2004). Mutations in PKP2 might lead to arrhythmogenic right ventricular cardiomyopathy (ARVC) (Basso et al., 2009; Philips and Cheng, 2016) which is responsible for sudden death in the young. Previous studies demonstrated an abundance of functional Connexin 43 (Cx43) hemichannels consequent to loss of plakophilin-2 (PKP2) expression in adult murine ventricle (Kim et al, Circulation, 2019). The increased Cx43-mediated membrane permeability is likely responsible for the excess entry of calcium into the cells, leading to an arrhythmogenic/cardiomyopathic phenotype.

To study the role of PKP2 and Cx43 in cardiac tissue, we utilized a murine model of cardiomyocyte-specific, Tamoxifen (TAM) activated knockout of PKP2 previously developed in the laboratory (Cerrone et al, 2017; Kim et al, 2019), and applied super resolution microscopy, electron tomography, focus ion beam-scanning electron microscopy (FIB-SEM), and serial block face-scanning electron microscopy (SBF-SEM) coupled with immune-electron microscopy to observe structural and protein compositional changes in PKP2-heterozygous-null murine (PKP2cKO) hearts. Electron tomography and FIB-SEM clearly show the increased intercellular distance in intercalated disc of right ventricle in PKP2cKO mice. The implementation of pre-embedding immune-electron microscopy coupled with SBF-SEM enable us to demonstrate the separation of the ID membrane and presence of Cx43 hemi-channels. 3D reconstructed images allowed us to visualize the entire length of gap junction plaques seen as two parallel, closely packed strings of Cx43 -immunoreactive beads at the intercalated disc. In contrast, we discovered bulging of the intercellular space, and entire areas where only one of the two strings could be observed in PKP2-deficient hearts, indicating the presence of orphan Cx43. We conclude that pre-embedding coupled with SBF-SEM allowed visualization of cardiac Cx43 plaques in their native environment, providing for the first time a visual complement of functional data indicating the presence of orphan Cx43 hemichannels resulting from loss of desmosomal integrity in the heart.

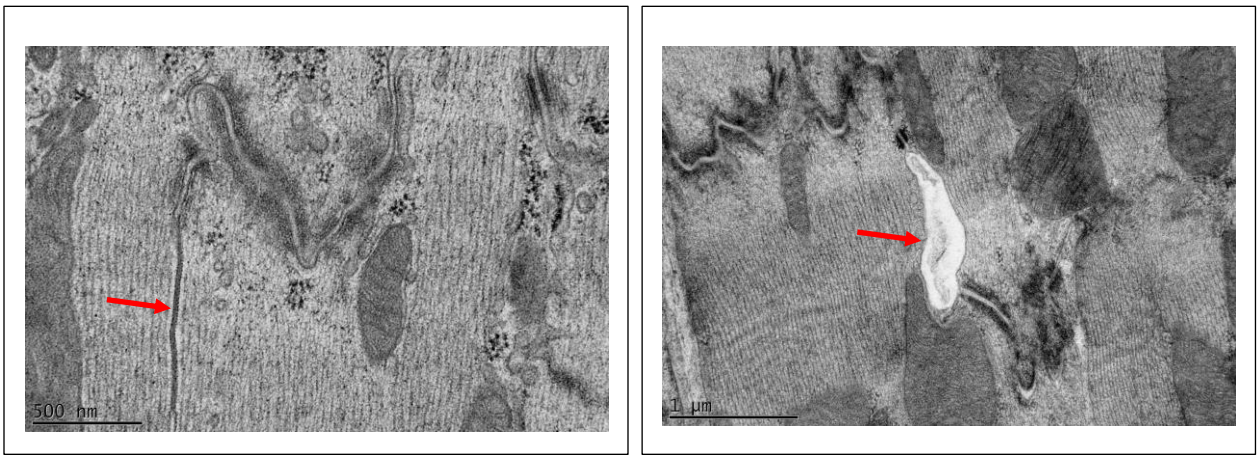

Figure 1. Morphological change of gap junction in intercalated disc of PKP2cKO murine heart. Gap junction (red arrow) in control mouse (left) demonstrates tight parallel membrane structure, while in PKP2cKO mouse shows enlarged intercellular space (right).

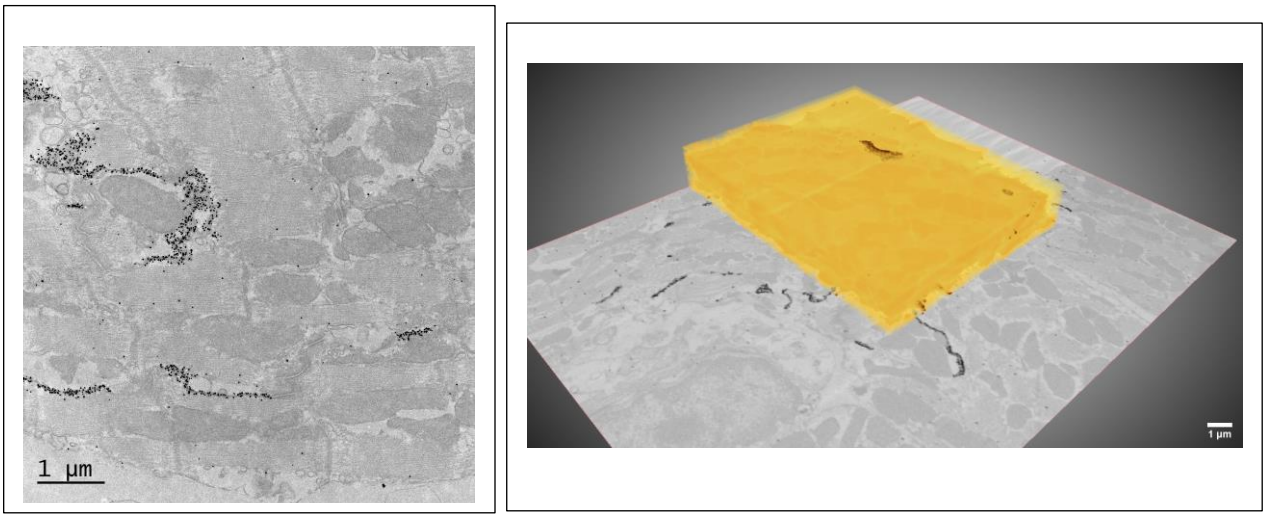

Figure 2. Cx43 localization revealed by pre-embedding immunolabeling and SBF-SEM imaging. Nanogold labeled Cx 43 shows specific labeling on intercalated disc in the heart (left), and 3D reconstruction documented the Cx43 localization in volumetric scale (right).

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