Photoactivation localization microscopy of cardiomyopathy associated plakophilin-2 mutants

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic heart disease characterized by ventricular arrhythmia, dilation of the right ventricle and fibro-fatty replacement of the myocardium [1]. ARVC is mainly caused by mutations in genes encoding desmosomal proteins. Desmosomes are cell-cell connections which are linked to the intermediate filament system contributing to the mechanical stabilization of cardiomyocytes [2]. The most prominent ARVC gene is *PKP2*, encoding plakophilin-2. Most ARVC associated *PKP2* mutations cause premature termination codons leading to a putative truncation of the protein [3]. Plakophilin-2 is a linker protein of the Armadillo family and connects desmosomal cadherins with desmoplakin.

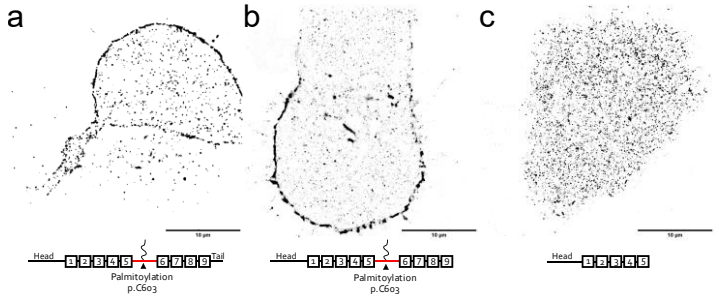

Fig 1: Photoactivation Localization Microscopy (PALM) reconstructions of mEosFP thermo (red fluorescence) fused to Plakophilin-2. The wild-type Plakophilin-2 mEosFP thermo signals is localized at the plasma membrane (a). Removing the tail domain has no visible effect on the localization position (b). Without Armadillo repeats Plakophilin-2 is not specifically localized at the membrane (c). Scale bar 10 µm.

However, the detailed cellular assembly of the desmosomal proteins is incompletely understood. Plakophilin-2 consists of a head, a central Armadillo and a short tail domain. Here, we generated a series of truncation mutations and investigated the localization in transfected cells by photoactivation localization microscopy (PALM), when switching mEosFP thermo from its green fluorescent into its red fluorescent channel by irradiation with violet light (405 nm). Wild-type plakophilin-2 is localized at the plasma membrane (Fig. 1a). Deletion of the tail domain has no obvious effect on membrane localization of plakophilin-2 (Fig. 1b). However, different mutants without the Armadillo repeats lose their membrane localization (Fig. 1c).

Our study indicates that the desmosomal assembly is disturbed by truncation mutations of plakophilin-2, which might have relevance for the molecular and cellular understanding of ARVC.

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