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Supplemental Information

Dynamic and Irregular Distribution of RyR2 Clusters in the Periphery of Live Ventricular Myocytes

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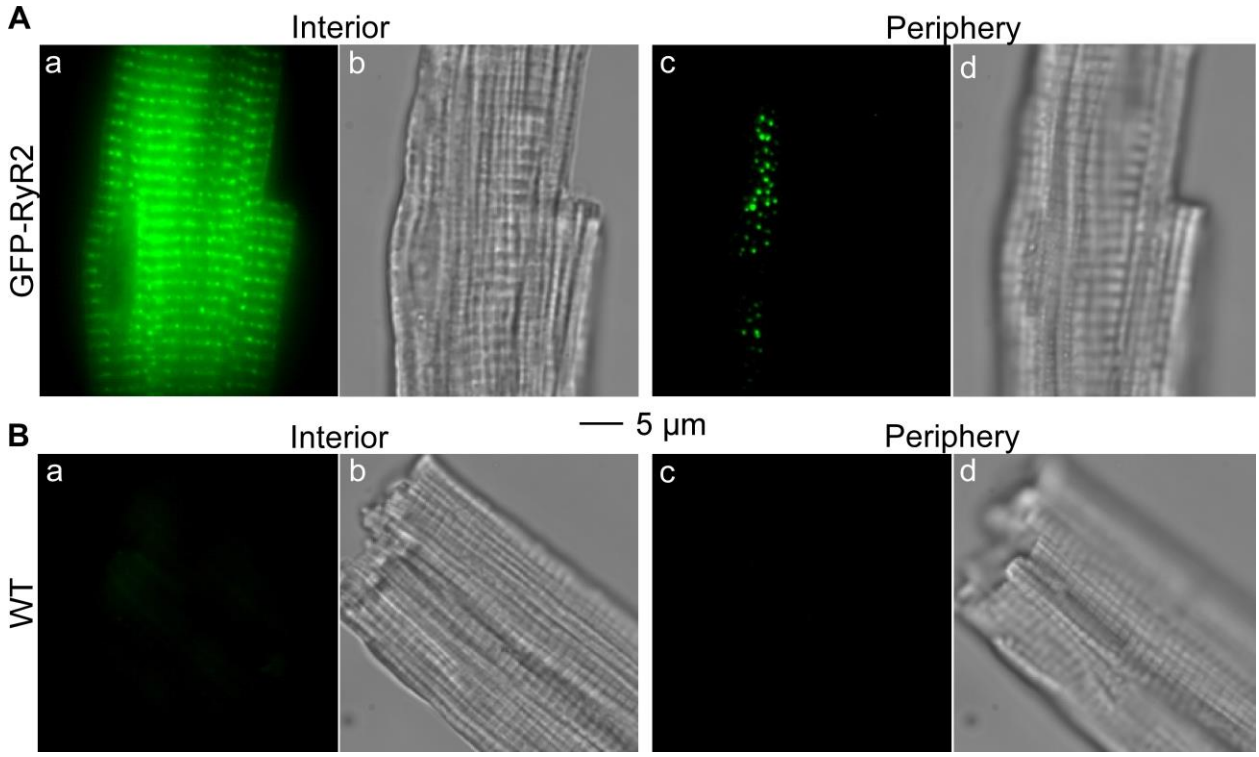
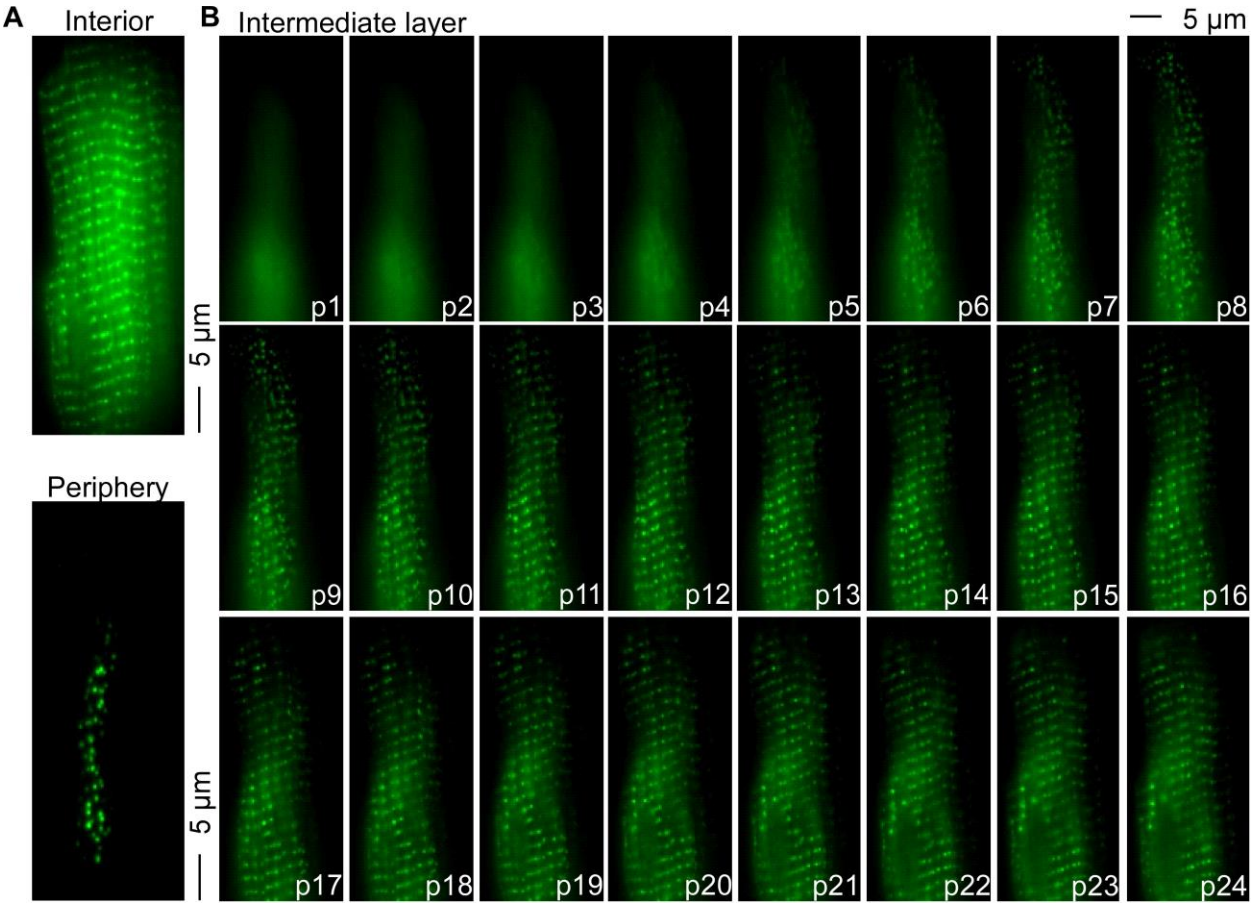
Supplemental Fig. 1



Supplemental Fig. 1. Fluorescence Signal Detection with a TIRF Microscope in the Interior and Periphery of Ventricular Myocytes Isolated from GFP-RyR2 Mice and WT Littermates

Representative fluorescence and transmission light images were taken under the same conditions in the same live ventricular myocytes isolated from GFP-RyR2 knock-in mice (A), or WT control littermate (B). GFP-fluorescence was only detected in myocytes isolated from the GFP-RyR2 knock-in mice, but not in WT mice (a & c). Membrane integrity and typical rod-shaped appearance in transmission light images indicate cell viability and allowed comparison of optical focus planes (b & d). (n = 6 GFP-RyR2 expressing cells and 15 WT cells) Scale bar applies to all images.

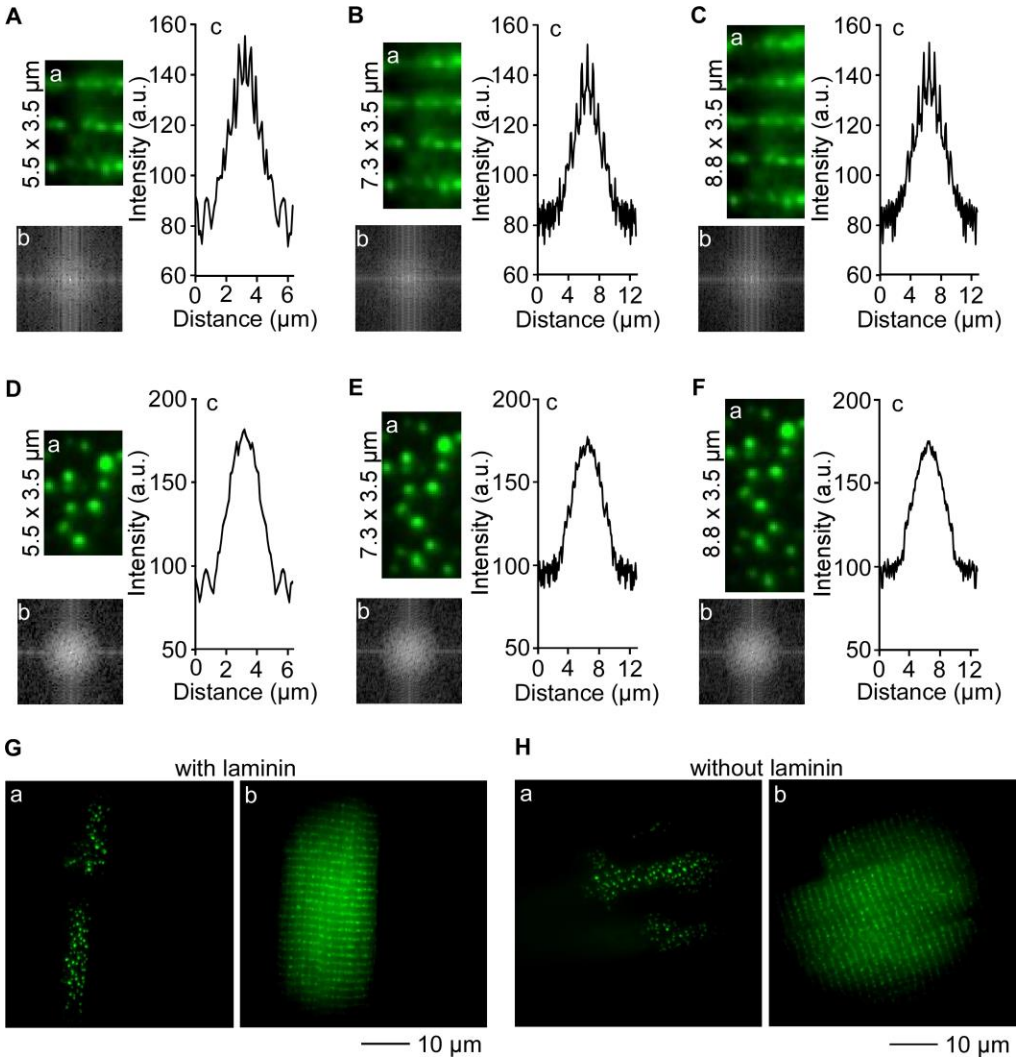
Supplemental Fig. 2



Supplemental Fig. 2. Imaging of Layers between the Interior and Periphery within the Same Ventricular Myocytes Detected using a TIRF Microscope

A. Representative images of periphery and interior in the same ventricular myocytes isolated from GFP-RyR2 mice were taken with a TIRF microscope. B, Optical z-stack planes (z stack distance 0.25um; plane *p1-24*) of layers with HILO illumination of the same cell. (n = 14 cells)

Supplemental Fig. 3



Supplemental Fig. 3. Fourier Transformation of Interior and Peripheral GFP-RyR2 Cluster Images with Different Area Sizes and Effects of Laminin on the Distribution of GFP-RyR2 Clusters in the Periphery and Interior of live Ventricular Myocytes

Fluorescence image areas with different sizes (A-F, panel a), corresponding 2D Fourier transforms (A-F, panel b), and intensity profiles derived from the Fourier transforms (A-F, panel c) of the interior (A-C) and the periphery (D-F) of live ventricular myocytes isolated from GFP-RyR2 mice are represented. Imaging of epi-fluorescent illuminated (angle = 0) interior showed highly-ordered, single-row arrays of GFP-RyR2 clusters (A-C, a). Irregular distribution of GFP-RyR2 clusters was found in the periphery through TIRF imaging (D-F, a). Distinct maxima were observed in all representative Fourier space of the interior (A-C, c), even in pattern analysis of areas as small as 5.5x3.5µm (A, c). In contrast, Fourier transform intensity profiles of the TIRF image of peripheral GFP-RyR2 clusters did not reveal intensity peaks in images with identical areas (D-F, c). Panels G and H shows TIRF images (a) and epi-fluorescence images (b) obtained from isolated GFP-RyR2 ventricular myocytes attached to glass coverslips with (G) and without (H) laminin coating.