Supporting Information for

"Neuronal ER-PM junctions organized by Kv2 and VAP pairing recruit Nir proteins and impact phosphoinositide homeostasis"

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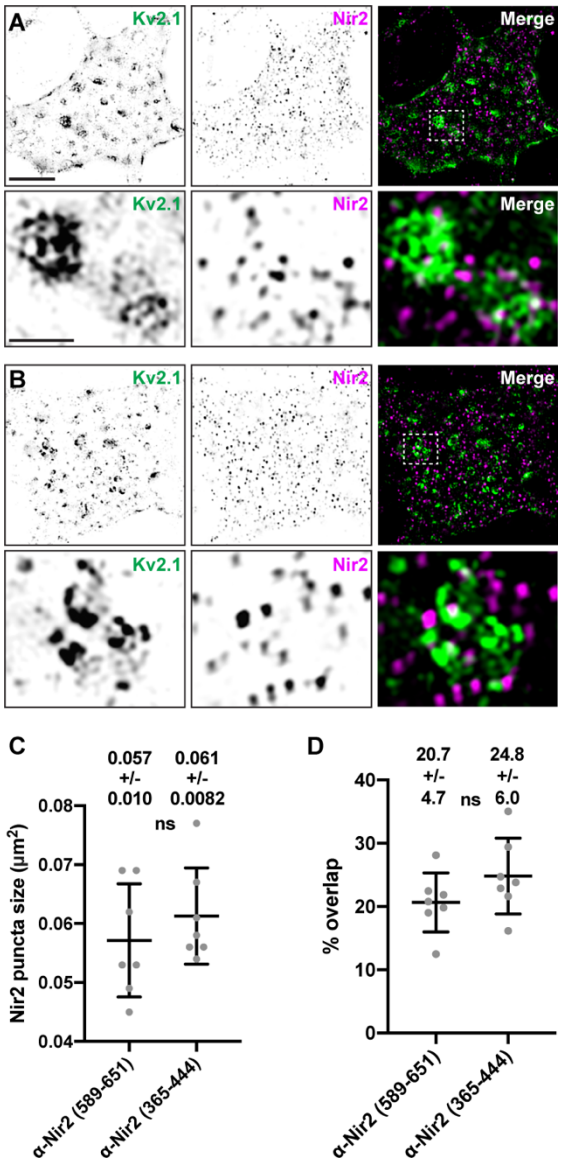
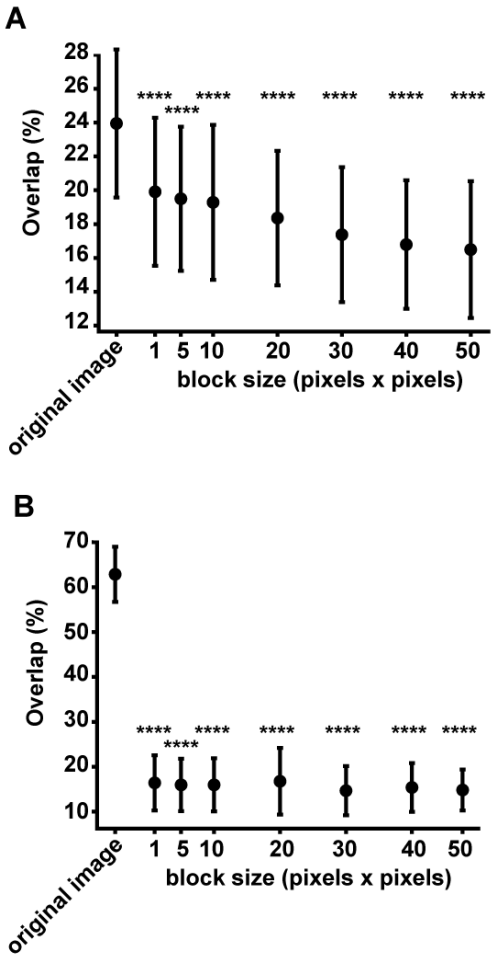
Supporting Figure 1. Comparison of Nir2 immunolabeling in CHNs using two distinct anti-Nir2 antibodies.

Supporting Figure 2. Comparison of block sizes used to segment and randomize Kv2.1 position on overlap with Nir2 or RyRs in immunolabeled CHNs imaged with N-SIM.



Supporting Figure 1. Comparison of Nir2 immunolabeling in CHNs using two distinct anti-Nir2 antibodies. (A and B) Super-resolution (N-SIM) optical sections taken at the basal membrane of cultured rat hippocampal neurons fixed and immunolabeled for endogenous Kv2.1 (shown in green) and Nir2 (shown in magenta; anti-Nir2 antibody used in A targets amino acids 365-444 of human Nir2; a different anti-Nir2 antibody was used in B and targets amino acids 589-651 of human Nir2). The scale bar in the upper left Kv2.1 panel is 5 µm and holds for all top panels of A and B. Merged images shown to the right. The bottom panels of A and B show the indicated magnified area from the merged images in top panel. Overlap between the Nir2 and Kv2.1 signals is shown at the far right. The scale bar in the magnified Kv2.1 panel is 1.25 µm and holds for all bottom panels of A and B. (C) Quantification of mean Nir2 puncta size from cultured rat hippocampal neurons immunolabeled for endogenous Kv2.1 and Nir2 with either anti-Nir2 (365-444) or anti-Nir2 (589-651) and imaged with N-SIM. Mean values are noted above bars. Bars are mean +/- SD (ns, p-value=0.4013, n=7 cells; two-tailed, unpaired t-test). (D) Quantification of the percent of Nir2 signal overlapping with Kv2.1 in cultured hippocampal neurons immunolabeled for endogenous Kv2.1 and Nir2 with either anti-Nir2 (365-444) or anti-Nir2 (589-651) and imaged with N-SIM. Mean values are noted above bars. Bars are mean +/- SD (ns, p-value=0.6399, n=7 cells; two-tailed, unpaired t-test).



Supporting Figure 2. Comparison of block sizes used to segment and randomize Kv2.1 position on overlap with Nir2 or RyRs in immunolabeled CHNs imaged with N-SIM. (A) Summary graph of Nir2 overlap with Kv2.1 segmented and randomized using blocks sized at 1 x 1, 5 x 5, 10 x 10, 20 x 20, 30 x 30, 40 x 40, and 50 x 50 pixel sized blocks. A single pixel is sized at 31.34 nm in our images. Values are mean +/- SD (all p-values are significant and are summarized in Table 3, n=11 cells; two-tailed paired t-test versus overlap in original image). (B) Summary graph of RyR overlap with Kv2.1 segmented and randomized using blocks sized at 1 x 1, 5 x 5, 10 x 10, 20 x 20, 30 x 30, 40 x 40, and 50 x 50 pixel sized blocks. Values are mean +/- SD (all p-values are significant and are summarized in Table 4, n=8 cells; two-tailed paired t-test versus overlap in original image).