Supplemental Materials

Molecular Biology of the Cell

Merkel et al.

Supplemental Figure Legends

Supplemental Figure 1. Cre-mediated loss of N-cadherin in Ncad$^{fx/fx}$ cardiomyocytes (accompanies Figures 2 and 3). A. Representative western blot of cell lysates from Ncad$^{fx/fx}$ cardiomyocytes that were uninfected (WT), infected with Cre recombinase-expressing adenovirus (Cre) or GFP-expressing adenovirus (GFP). Cells were harvested 96 hours post-infection. Lysates were separated by SDS-PAGE and blotted for N-cadherin (top) and GAPDH (bottom). B. N-cadherin band intensities in A were measured, normalized to WT and plotted. Error bars represent standard deviation from at least three independent experiments. One-way ANOVA, $p<0.01$. (C-F) Neonatal cardiomyocytes from Ncad$^{fx/fx}$ mice were either uninfected (C, E) or infected with adenovirus expressing Cre recombinase (D, F), fixed and stained for desmosome components plakoglobin (C, E) and plakophilin (E, F). (G, H) Expression of N-cadherin-GFP in N-cadherin-null cardiomyocytes restored plakoglobin (G) and plakophilin (H) recruitment. Individual N-cadherin-GFP (green) and desmosome components (magenta) channels are shown along with the merge. Far right columns are higher magnifications of the boxed contact in the merge. Individual and merged channels are shown. (I-O) αE-catenin expression in control (I-L) and Cre/Ncad-GFP-M1-ABD infected Ncad$^{fx/fx}$ (M-O) cardiomyocytes. I-J. Uninfected Ncad$^{fx/fx}$ cardiomyocytes were fixed at four different time points post-infection and stained for αE-catenin and F-actin. (M-O) Ncad$^{fx/fx}$ cardiomyocytes were infected with Cre recombinase and Ncad-GFP-M1-ABD adenoviruses, fixed at three separate time points post-infection and stained for αE-catenin and F-actin. Individual and merged GFP (green), F-actin (red) and αE-catenin (blue) channels shown. αE-catenin expression was lost over 96 hours as Ncad-GFP-M1-ABD expression increased. Images are max projections of 2-3 μm deconvolved stacks. Scale bar is 10 μm in C-O, 5 μm in higher mag images in G, H.

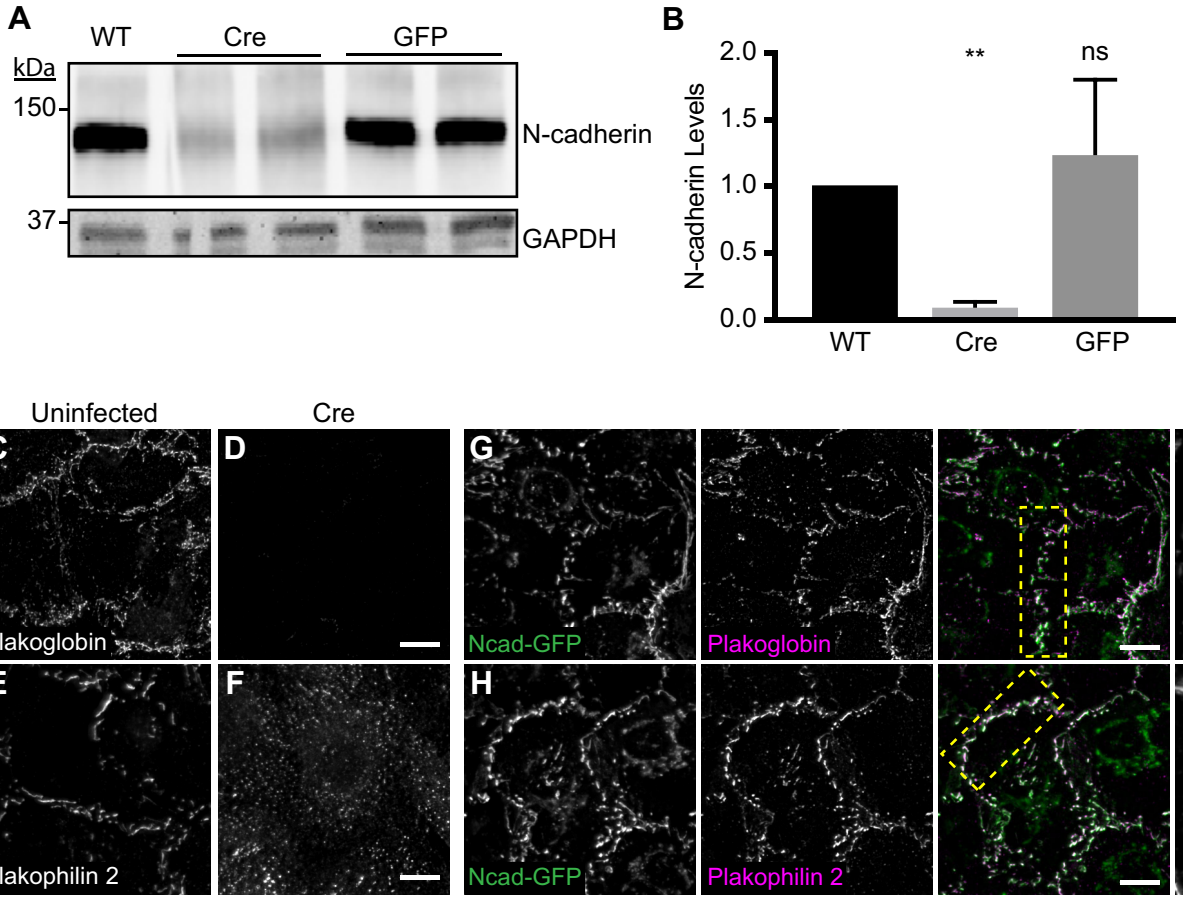
Supplemental Figure 2. Vinculin and Afadin recruitment to fusion constructs (accompanies Figure 4). (A-C) Neonatal cardiomyocytes isolated from Ncad$^{fx/fx}$ mice were infected with Cre and N-cadherin-GFP-αE-catenin fusion adenoviruses. Cells were fixed and stained for vinculin and afadin. Individual and merged GFP (green), vinculin (red) and afadin (blue) channels shown. Images are a max projection of 2-3 μm deconvolved stacks. Bottom image is a higher magnification of boxed contact. D-F. Quantification of vinculin and afadin intensities at cell-cell contacts. Signal intensity at contacts was divided by the average cytoplasmic intensity and a scatter plot of all data points is shown. The black horizontal line is the median and the error bars define the interquartile range. The shaded gray region in each plot defines the median (thick gray line) and interquartile range (thin gray lines) of vinculin or afadin recruitment observed with full-length N-cadherin-GFP (Fig. 4F) for comparison. One-way ANOVA, significance compared to recruitment with N-cadherin-GFP. $n \geq 40$ images from at least 2 independent experiments. (G-M) Vinculin or afadin contact/cytoplasmic ratio was plotted against the average GFP intensity of each fusion construct within the masked cell contact region. Linear regression analysis was performed to calculate the slope (red line), 95% confidence intervals (black lines surrounding slope) and R^2 value. The slope deviation from zero was analyzed for significance (p value). $n \geq 40$ images from at least 2 independent experiments. We observed a modest relationship between Ncad-GFP-M1-M2 expression levels and vinculin enrichment.

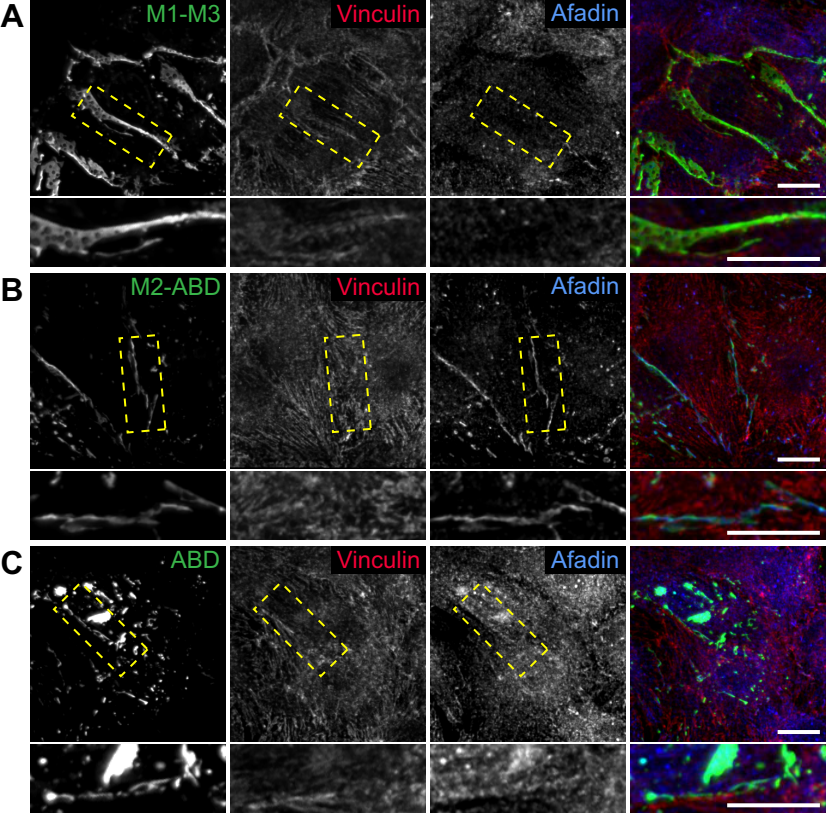
Supplemental Figure 3. TEM of N-cadherin-GFP-αE-catenin fusion constructs (accompanies Figure 4). A-C. $Ncad^{fx/fx}$ cardiomyocytes infected with Cre and N-cadherin-GFP-αE-catenin fusion adenoviruses were fixed and processed for thin section EM. TEM images are representative of >60 images from at least three independent experiments. Cell-cell contacts are highlighted in yellow. Scale bar is 1 μm.

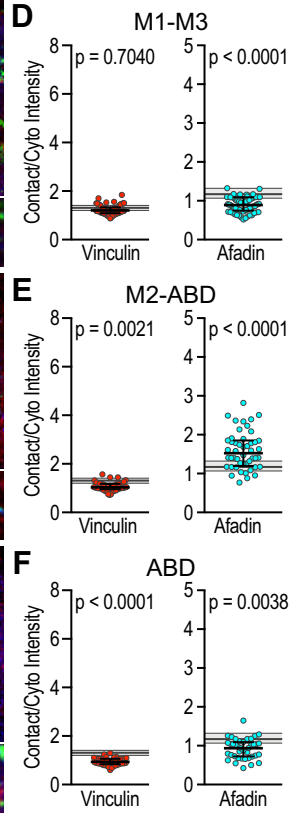
Supplemental Figure 4. Mena localization with N-cadherin fusion constructs. $Ncad^{fx/fx}$ cardiomyocytes were either uninfected (A) or infected with both Cre and N-cadherin-GFP-αE-catenin fusion constructs (B-D). Cells were fixed and stained for Mena and F-actin. Individual and merged GFP (green), Mena (red) and F- actin (blue) channels are shown. Images are a max projection of 2-3 μm deconvolved stacks. Bottom row in each panel set is a higher magnification of boxed contact. (E-H) Quantification of Mena intensity at cell-cell contacts. Signal intensity at contacts was divided by the average cytoplasmic intensity and a scatter plot of all data points is shown. The black horizontal line is the median and the error bars define the interquartile range. The shaded gray region in each plot defines the median (thick gray line) and interquartile range (thin gray lines) of Mena recruitment in the uninfected control (A) for comparison. One-way ANOVA, significance compared to recruitment with N-cadherin-GFP. $n \geq 15$ images from at least 1 independent experiment for each condition. Scale bar is 10 μm.

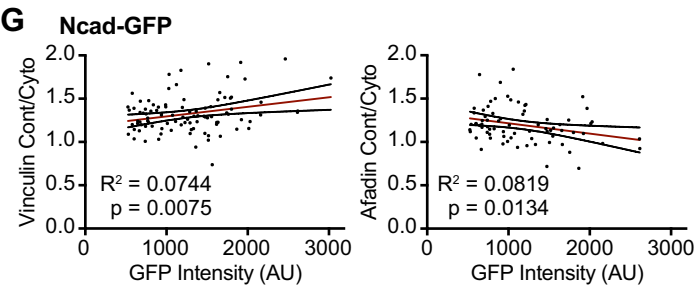
Supplemental Figure 5. α-Actinin localization with N-cadherin fusion constructs. $Ncad^{fx/fx}$ cardiomyocytes were either uninfected (A) or infected with Cre and N-cadherin-GFP-αE-catenin fusion constructs (B-D). Cells were fixed and stained for α-actinin and F-actin. Individual and merged N-cadherin fusion (green), α-actinin (red) and F-actin (blue) channels are shown. Images are a maximum projection of 2-3 μm deconvolved stacks. Bottom row in each panel set is a higher magnification of boxed contact. (E-H) Quantification of α-actinin intensities at cell-cell contacts. Signal intensity at contacts was divided by the average cytoplasmic intensity and a scatter plot of all data points is shown. The black horizontal line is the median and the error bars define the interquartile range. The shaded gray region in each plot defines the median (thick gray line) and interquartile range (thin gray lines) of α-actinin recruitment in the uninfected control (A) for comparison. One-way ANOVA, significance compared to recruitment with endogenous N-cadherin. $n \geq 12$ images from at least 1 independent experiment for each condition. Scale bar is 10 μm.

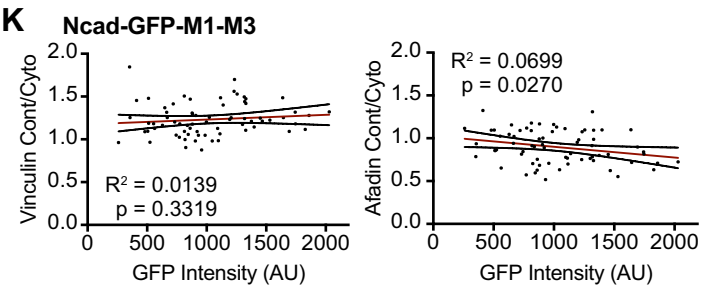
Supplemental Figure 1. Cre-mediated loss of N-cadherin in Ncad$^{fx/fx}$ cardiomyocytes

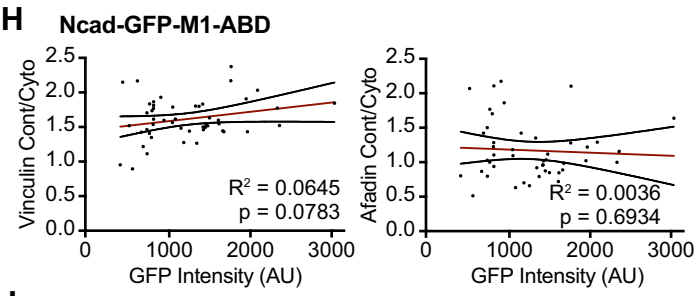


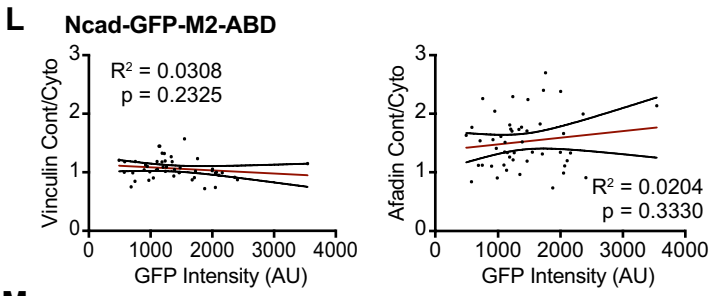


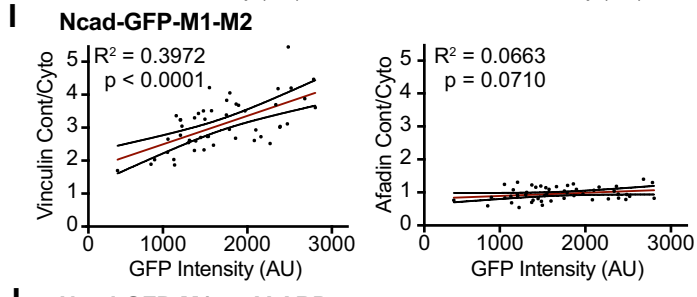


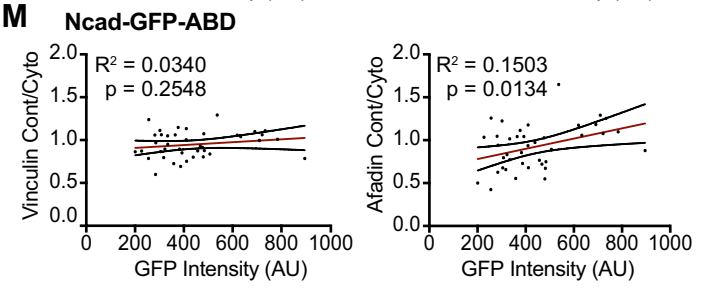


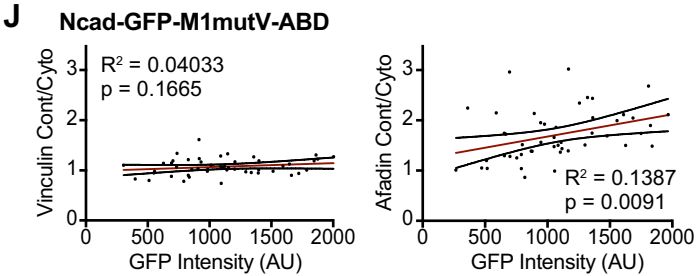












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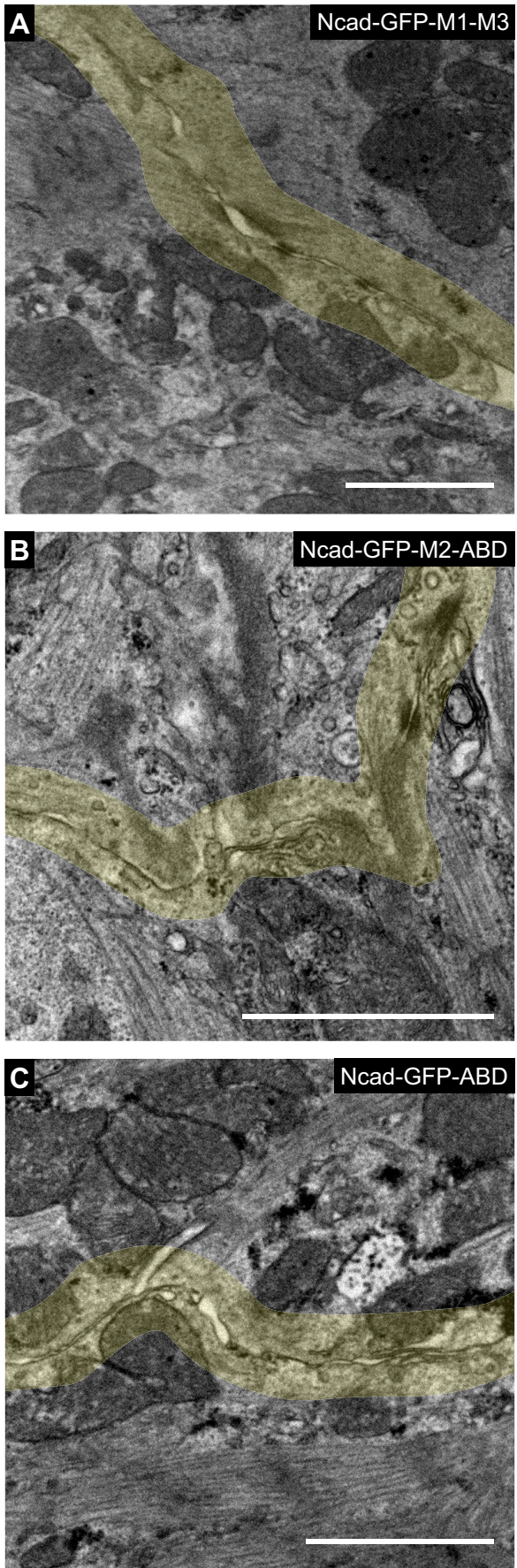
Supplemental Figure 3. TEM of N-cadherin-GFP-αE-catenin fusion constructs



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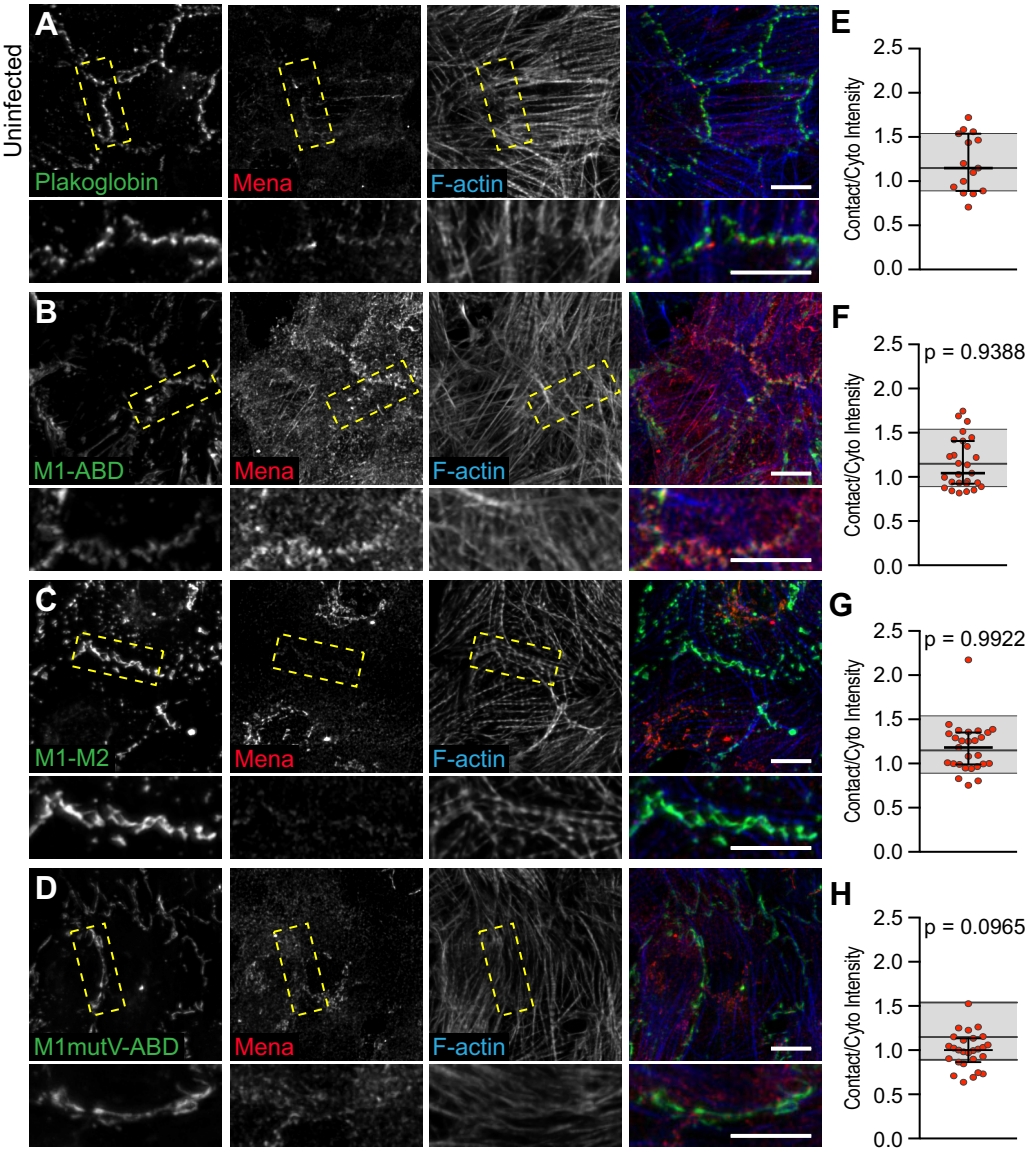

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