

SUPPLEMENTAL MATERIAL

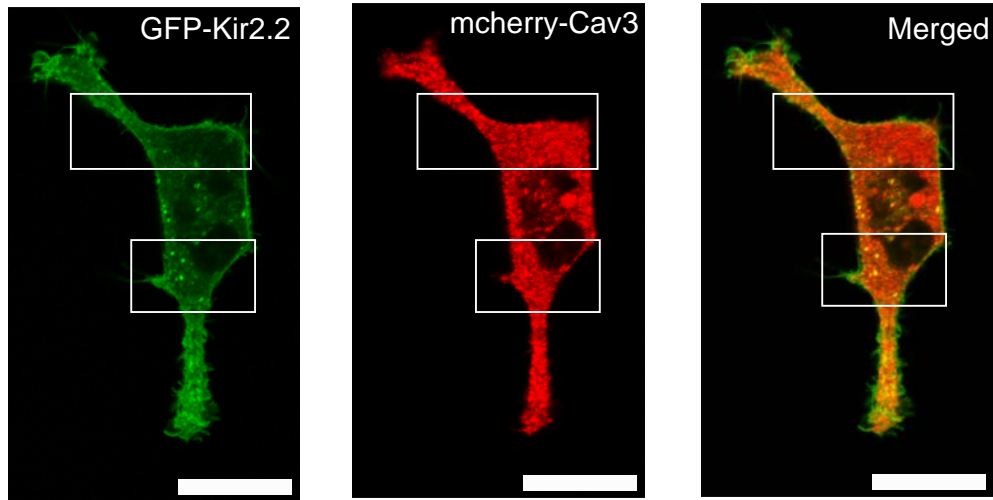
Methods for supplemental figure 2:

HEK cells were transfected with either HA-Kir2.2 and WT-Cav3 or HA-Kir2.2 and F97C-Cav3. Cells were fixed with 4% paraformaldehyde in 1X PBS and then permeabilized with Triton X-100 0.1%. Prepared slides were blocked in 5% normal goat serum for 1 hour and primary antibodies chicken anti HA, (Abcam), mouse anti Cav3 (BD Transduction Laboratories) and rabbit anti Golgi-97 (Abcam) were incubated overnight at 4 °C. Slides were washed with PBS (0.1% Tween-20) and incubated in secondary antibodies (Invitrogen) for 1 hour at room temperature. Samples were then washed in PBS (0.1% Tween-20) and mounted using ProLong Gold anti-fade mounting kit containing DAPI (Invitrogen). A Leica SP5 confocal microscope was used for image acquisition and image analysis and co-localization by ImageJ software (NIH free-ware).

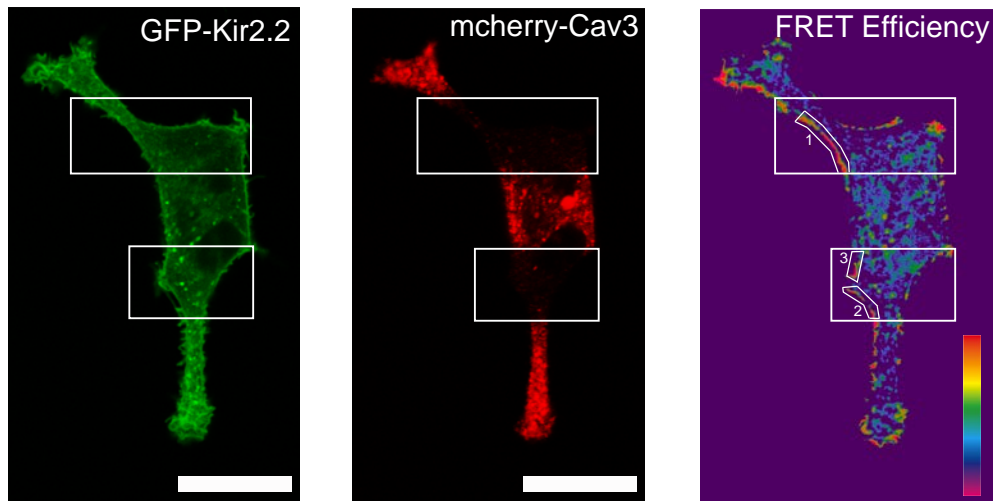
Supplemental Figure 1: Example FRET Images of Kir2.2 + WT-Cav3: Pre-bleached, live cells expressing both fluorophores are shown in the top panels and bleached areas are denoted by white rectangles. Regions of interest (ROI) were selected at the cell membrane. Post-bleach images are shown on the lower panels and ROIs outlined in white.

Supplemental Figure 2: Golgin-97 identifies Kir2.2 with F97C-Cav3 Golgi accumulation but not Kir2.2 with WT-Cav3. Confocal images of HEK-293 cells co-transfected with WT-Cav3 and HA-Kir2.2 (top panel) and F97C-Cav3 and HA-Kir2.2 (bottom panel) stained for the Nuclei (DAPI) in panels a and f, Golgin-97 in panels b and g, Cav3 in panels c and h, Kir2.2 in panels d and i. The merged for the WT-Cav3 and HA-Kir2.2 is shown in e and that for F97C-Cav3 and HA-Kir2.2 is shown in j. Scale bar=15µM.

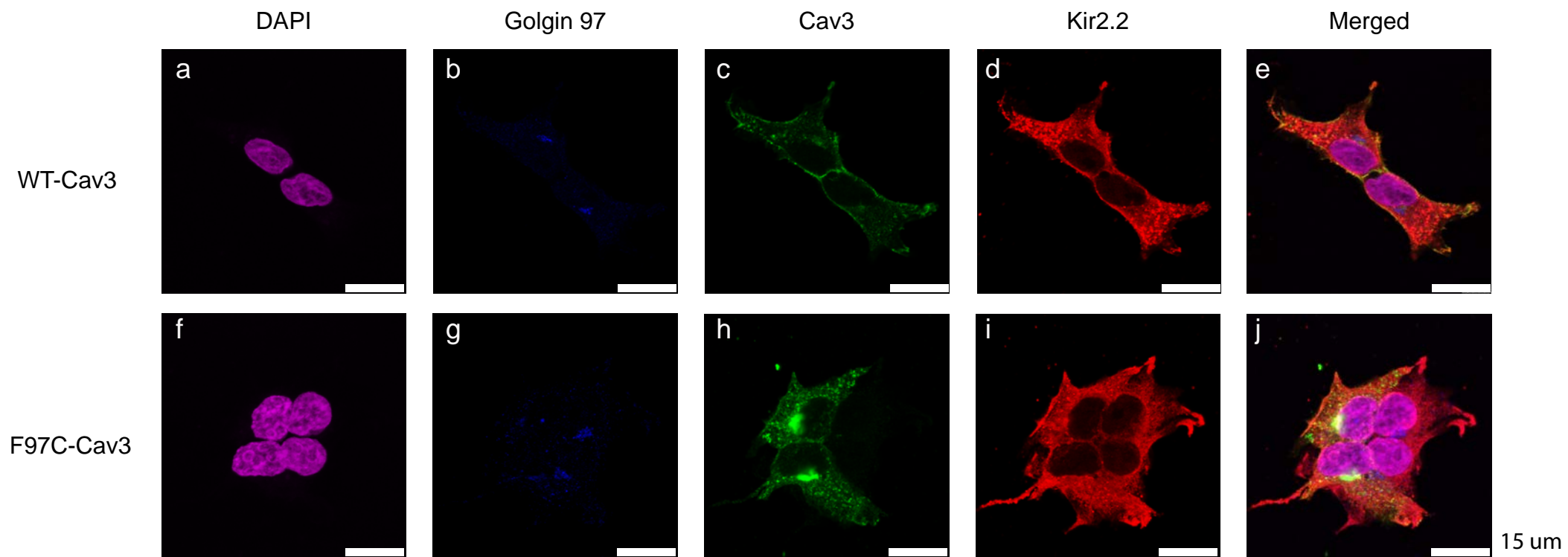
Pre-bleach



Post-bleach



FRET Efficiency : ROI-1 = 0.26 ROI-2 = 0.12 ROI-3 = 0.42



Supplemental figure 2