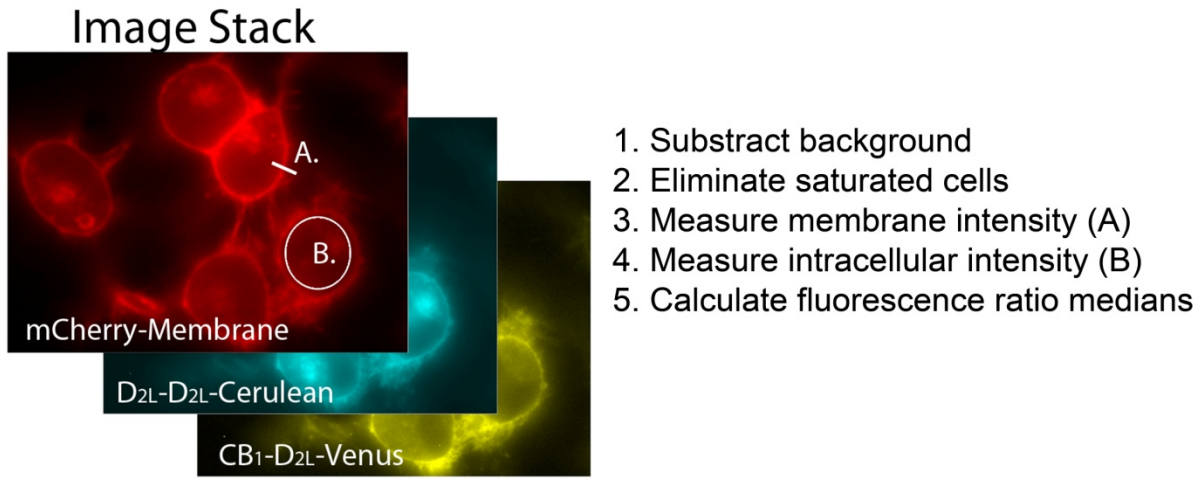
Authors: Julie A. Przybyla and Val J. Watts

Title: Ligand-Induced Regulation and Localization of Cannabinoid CB_1 and Dopamine D_{2L} Receptor Heterodimers

Journal of Pharmacology and Experimental Therapeutics

Supplemental Figure 1



Flowchart of the analysis of microscopic multicolor BiFC measurements. Fluorescent BiFC signals (CB_1-VN/D_{2L}-CC and D_{2L}-CN/- D_{2L}-CC), as well as mCherry-Mem fluorescent signals, are imaged by epifluorescence microscopy. The mCherry-Mem membrane marker signals were used to select cells for image analysis and to normalize BiFC signals. The fluorescent signal intensity maximum at the membrane was determined by drawing a perpendicular line through the membrane using the mCherry-Mem image (see A.). The average BiFC signal intensity in the intracellular space was determined by outlining the intracellular compartment (excluding the plasma membrane, see B.).

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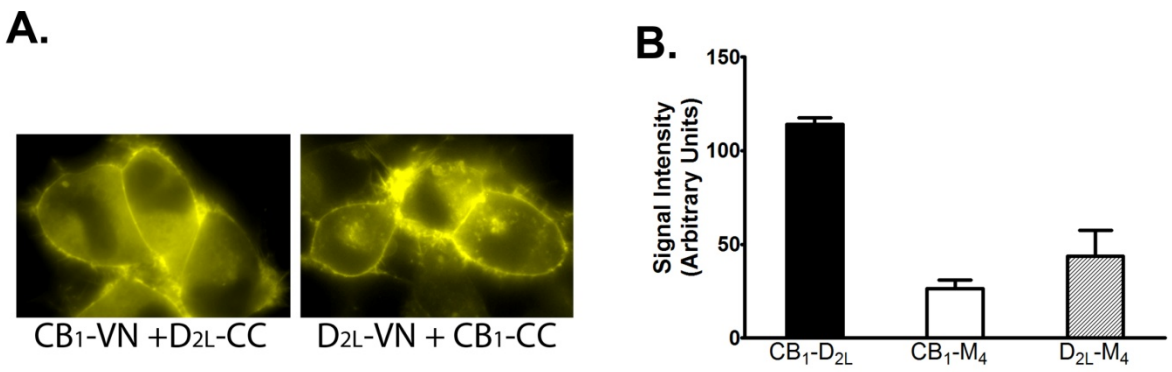
Supplemental figure 2



A. BiFC complementation in CAD cells. Co-expression of CB_1-VN + D_{2L}-CC or D_{2L}-VN + CB_1-CC in CAD cells produced robust Venus signals in CAD cells. **B.** Fluorescent signals from Venus complementation (VN-VC) in CAD cells transfected with CB_1-VN + D_{2L}-CC, CB_1-VN + M_4-CC, or D_{2L}-CC + M_4-VN. Whole cell signals were quantified by measuring the average signal intensity in the Venus channel.

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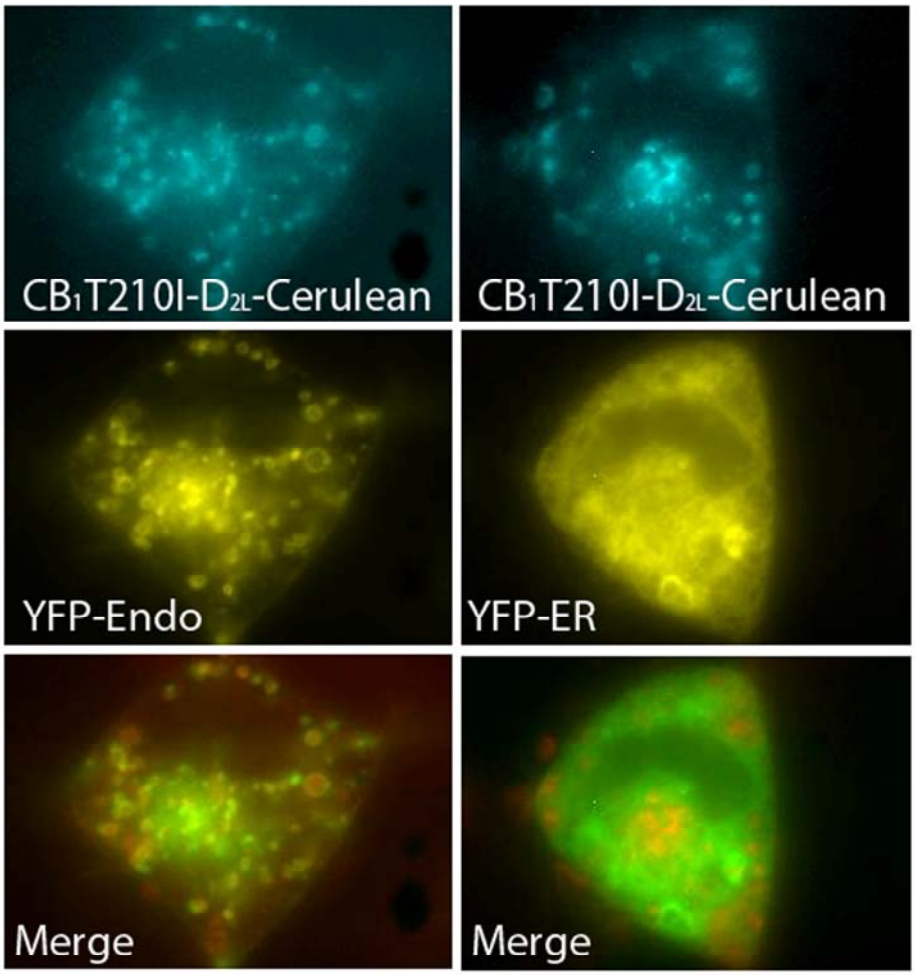
Supplemental Figure 3



Intracellular localization patterns of CB_1T210I-D_{2L} heteromers. CAD cells were transiently transfected with CB_1T210I-CC and D_{2L}-CN (cyan signal) along with YFP fluorescent marker proteins for the endosomes or the ER (yellow signal). The merged image (overlapping signal in yellow) represents an overlap of the BiFC signal (depicted in red) and the fluorescent marker signal (depicted in green). Images are representative of 2 independent transfections.