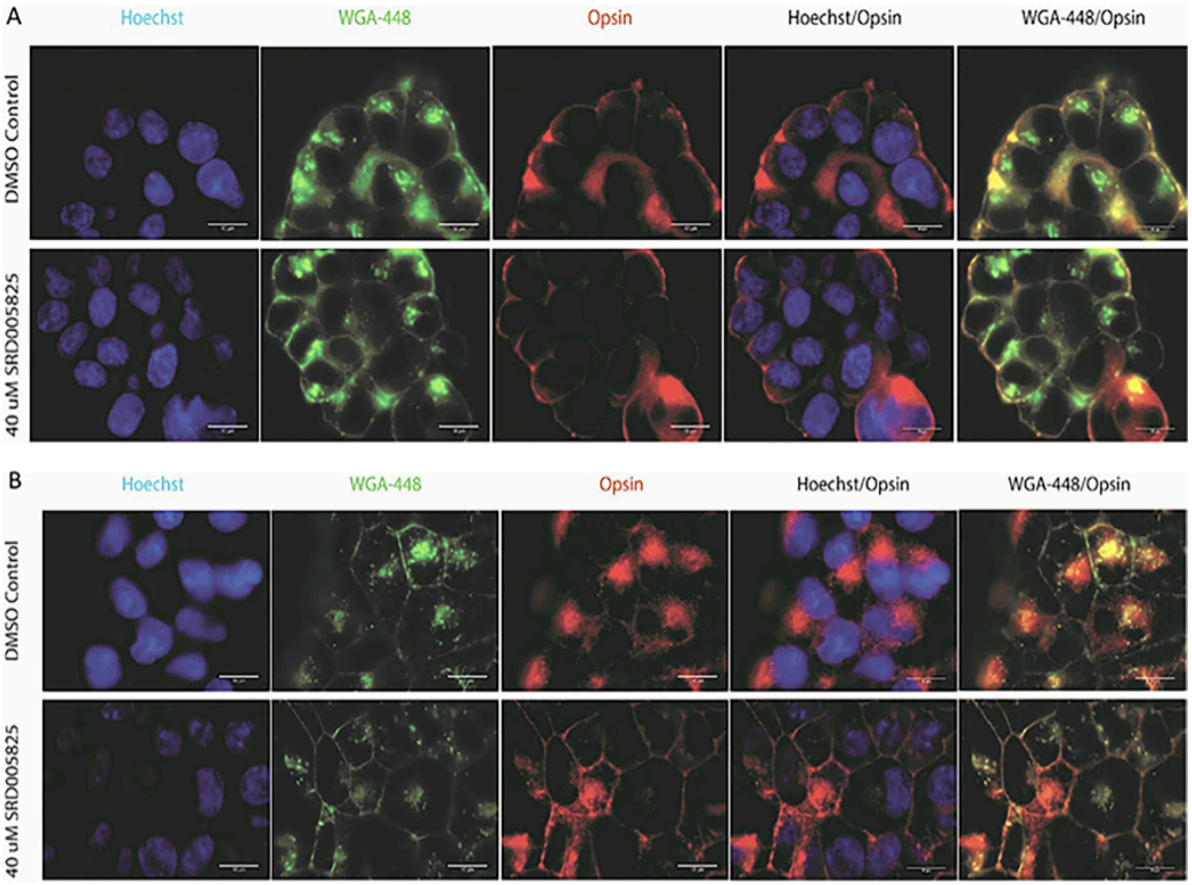

Confocal Microscopy of Membrane-Associated Opsin

Wildtype and T17M mutant opsin-expressing cells cultured as described above were induced with tetracycline in the presence of increasing concentrations of SRD005825, and 24 hours later SRD005825 was reapplied. One day later, cells were fixed in 4% paraformaldehyde in PBS and analyzed by confocal microscopy. Staining with 1D4 monoclonal antibody was used to detect rhodopsin. The plasma membrane was marked by fluorescently tagged wheat germ agglutinin (WGA). Images from 10 replicate wells per treatment were acquired using the Thermo CX7 HCS instrumentation (ThermoFisher) with a 40× objective. Automated quantitative analysis of relative opsin intensity was determined via colocalization analysis of opsin with the membrane fraction of WGA-Alexa Fluor 488 on the analysis suite of the CX7 HCS platform. High-resolution images were taken with a 100× objective on an Echo Revolve microscope (Echo, San Diego, CA). Fluorescence intensity of membrane-bound opsin in each chaperone treated sample was measured and the value compared to the value of treatment with dimethyl sulfoxide (DMSO).