Caspase-Activated Cell-Penetrating Peptides Reveal Temporal Coupling Between Endosomal Release and Apoptosis in an RGC-5 Cell Model of Retinal Degeneration

Supporting Information

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Abbreviations: RGC; retinal ganglion cell, CPP; cell-penetrating peptide, SS; staurosporine

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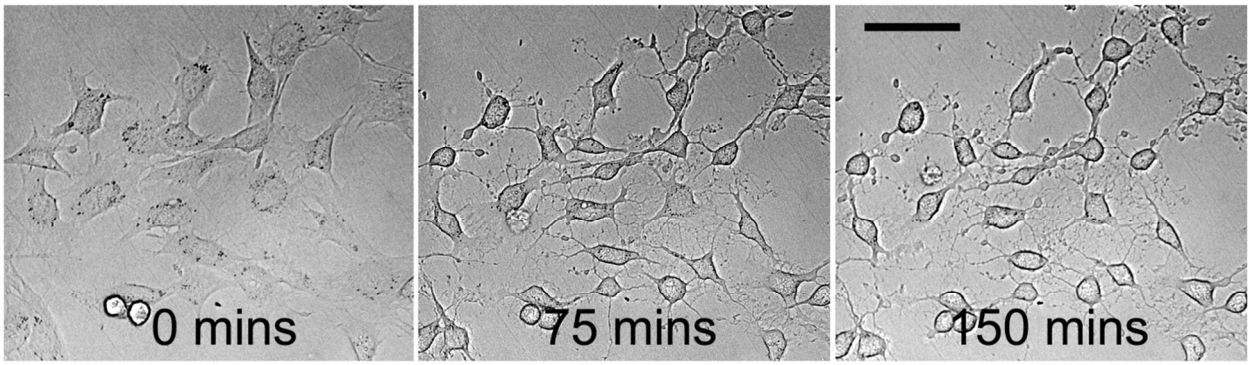

Figure S1. Transmitted light microscopy montage produced from a time lapse movie of RGC-5 cells undergoing SS-induced (0.5 μM) differentiation. Times correspond to movie acquisition time (min). Image acquisition began 1 minute following SS exposure (50 μm scale bar).

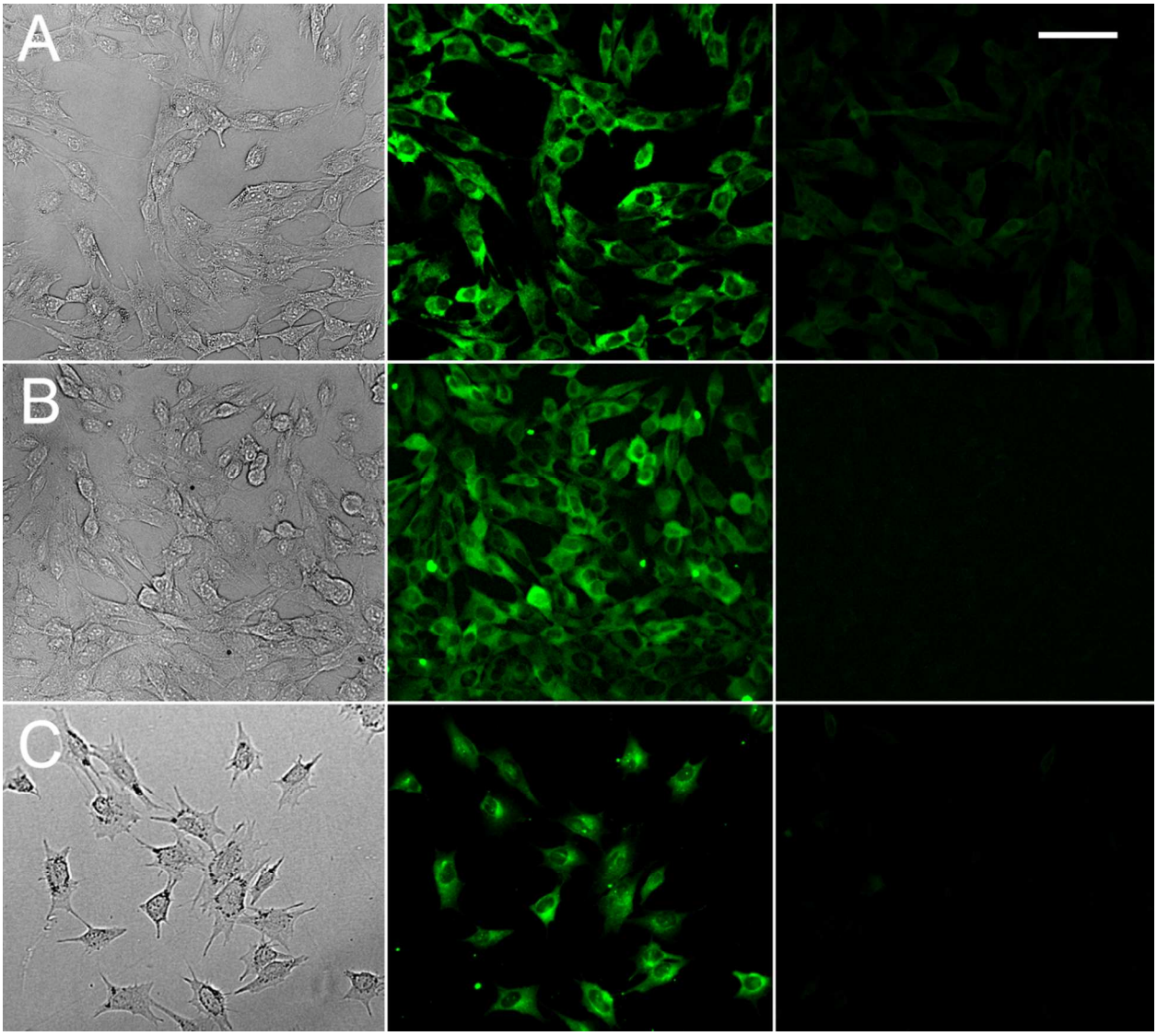

Figure S2. Immunohistochemical staining of neuronal markers in native RGC-5 cells. Fluorescence microscopy images of NF70 (row A), Thy-1 (row B) and NMDAR1 (row C) expression (bright field left, primary antibody labeling middle, and secondary antibody only right; 50 µm scale bar).

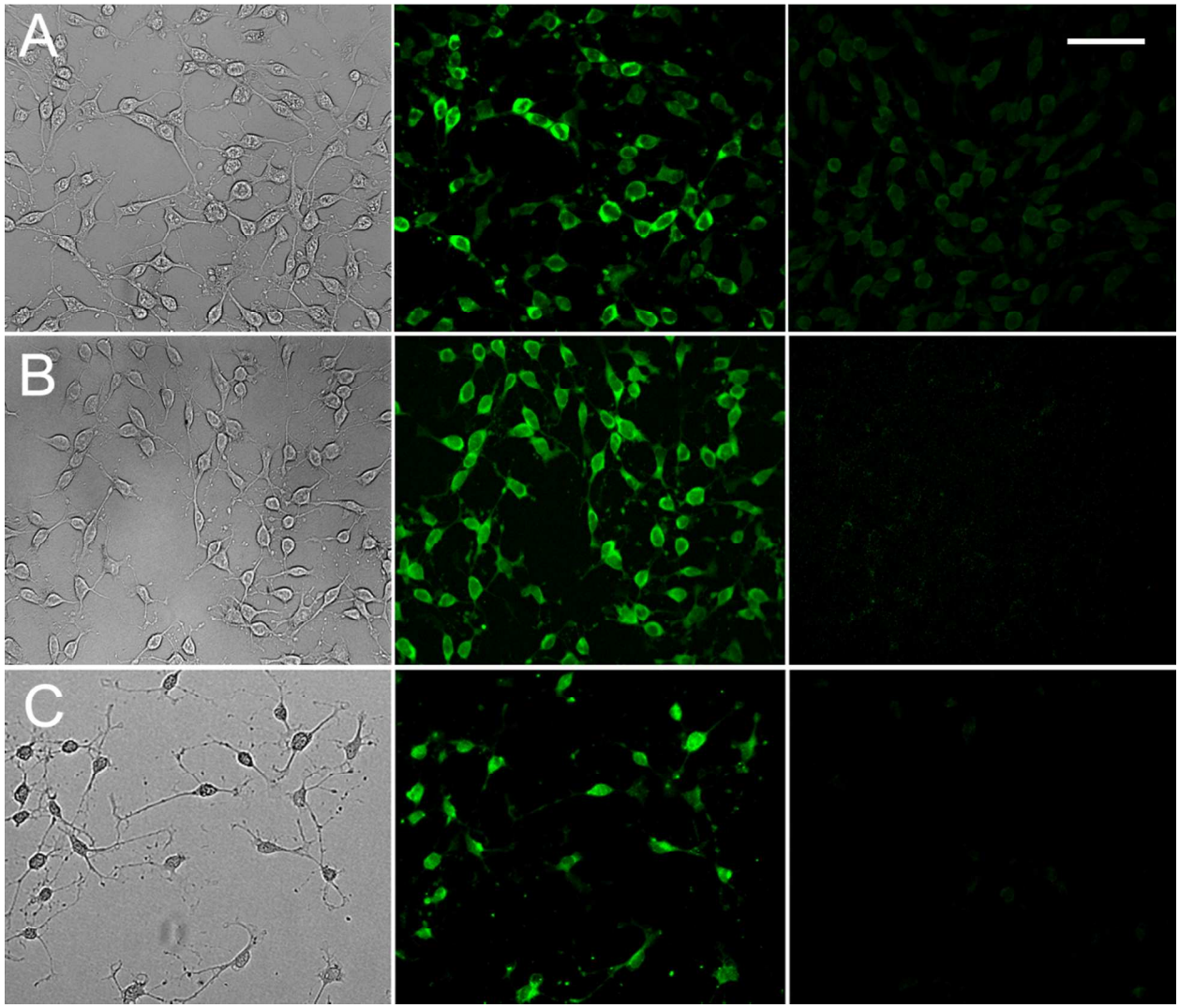

Figure S3. Immunohistochemical staining of neuronal markers in SS-differentiated RGC-5 cells. Fluorescence microscopy images of NF70 (row A), Thy-1 (row B) and NMDAR1 (row C) expression (bright field left, primary antibody labeling middle, and secondary antibody only right; 50 μm scale bar).

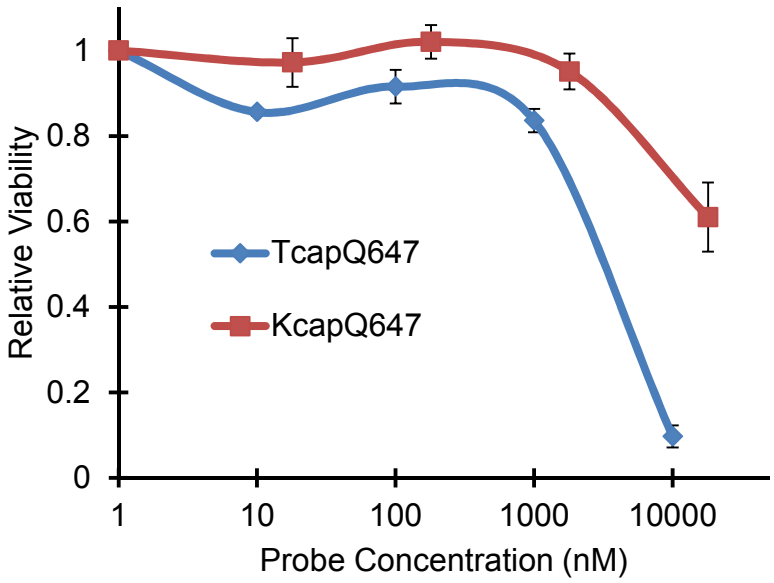

Figure S4. Alamar Blue cytotoxicity assays of CPP probes in native RGC-5 cells. RGC-5 cells were incubated with increasing concentrations of KcapQ647 (**1**) or TcapQ647 for 72 h before the addition of resazurin to assay cell viability. Data are presented as mean values ± SEM (*n* = 3 each).

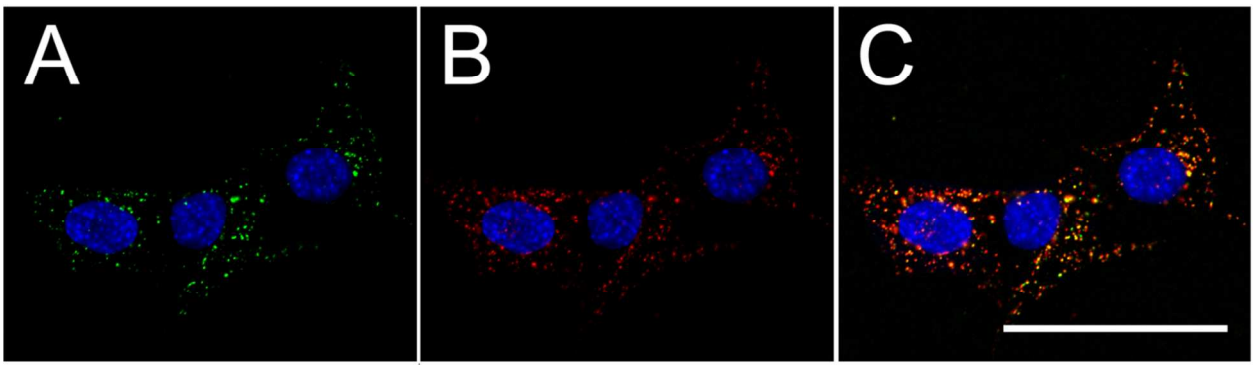

Figure S5. Fluorescence microscopy images of native RGC-5 cells following a 3 hour co-incubation with Kcap488 (**4**) (A) and Tcap647 (B). Overlay of panels A and B (C) showing co-localization (yellow) of each probe (50 μm scale bar).

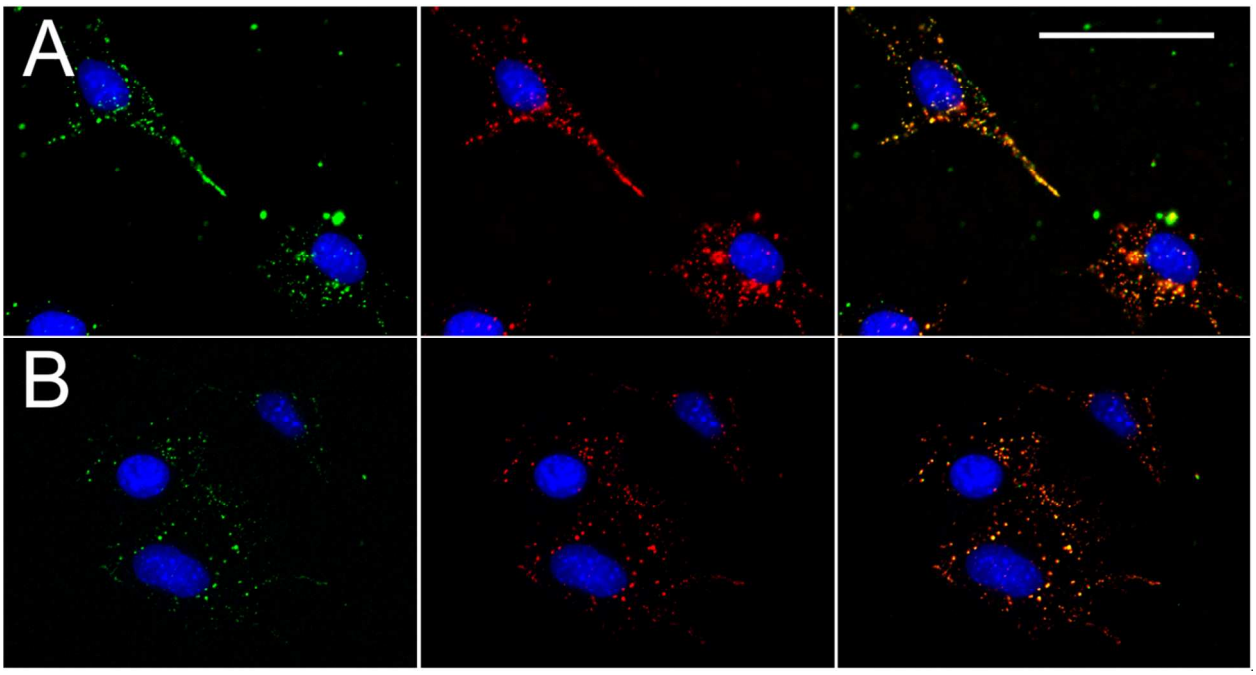

Figure S6. Fluorescence microscopy images of native RGC-5 cells following a 3 hour incubation with CF-albumin (A, left panel) or CF-dextran (B, left panel) and 10 μM Kcap647 (**2**) (A and B, middle panels). Composite image showing co-localization (yellow) of internalized Kcap647 (red) and the respective endosomal marker (green) (A and B, right panels; 50 μm scale bar).

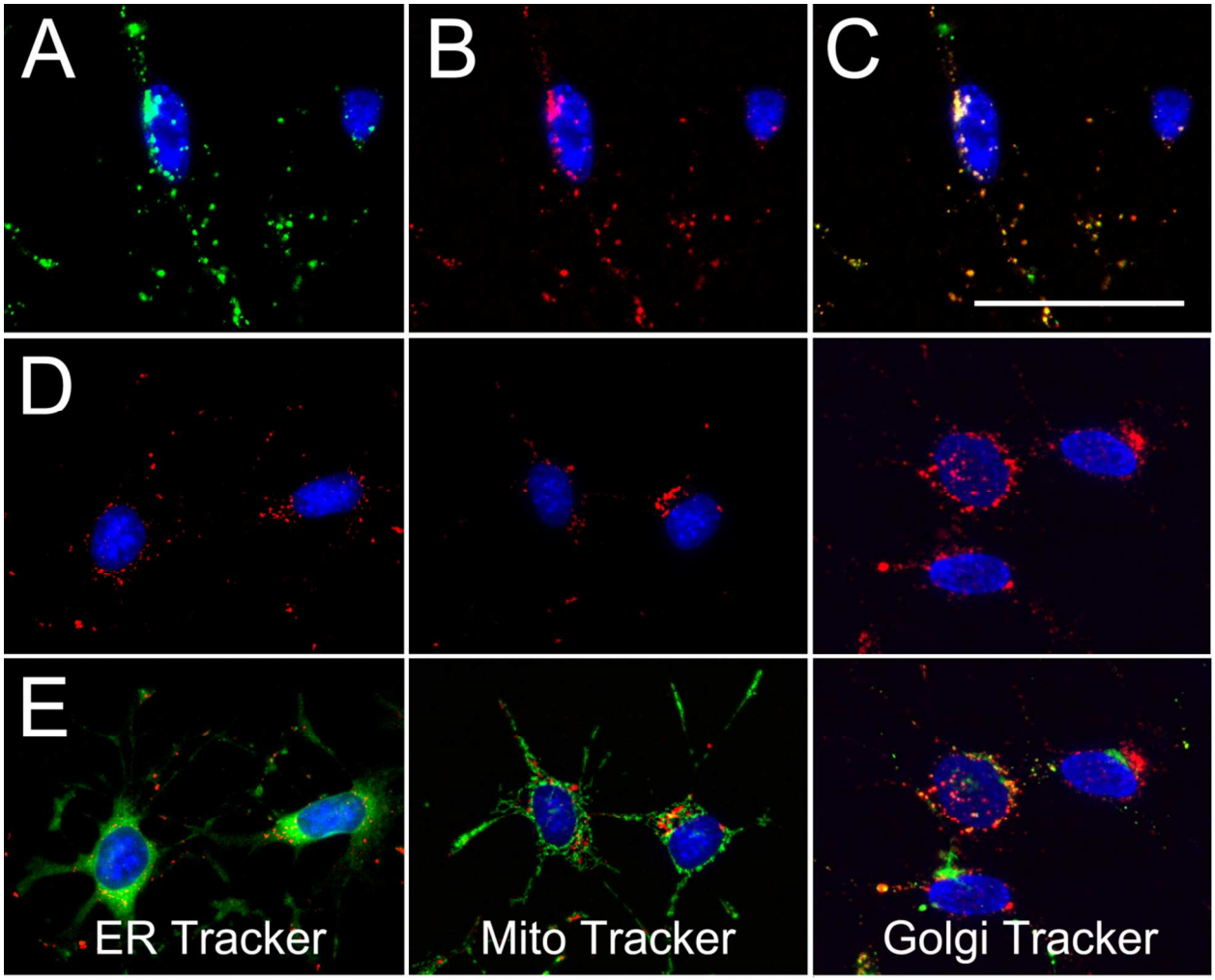

Figure S7. Fluorescence microscopy images of SS-differentiated RGC-5 cells following a 3 hour co-incubation with 10 μM **4** (A) and 10 μM **2** (B). Overlay of panels A and B (C) showing co-localization of each probe. Images of native RGC-5 cells following co-exposure to **2** (10 μM, 3hrs) (row D) and ER Tracker Green (1 μM, 30 min) (E, left), Mito Tracker Green (50 nM, 30 min) (E, middle), or C_6-ceramide-NBD (Golgi Tracker, 2.5 μM, 30 min) (E, right); overlay image of fluorescence fields (row E). Cells were counterstained with H33342 (pseudocolor blue) to show subcellular distribution relative to nuclei (50 μm scale bar).

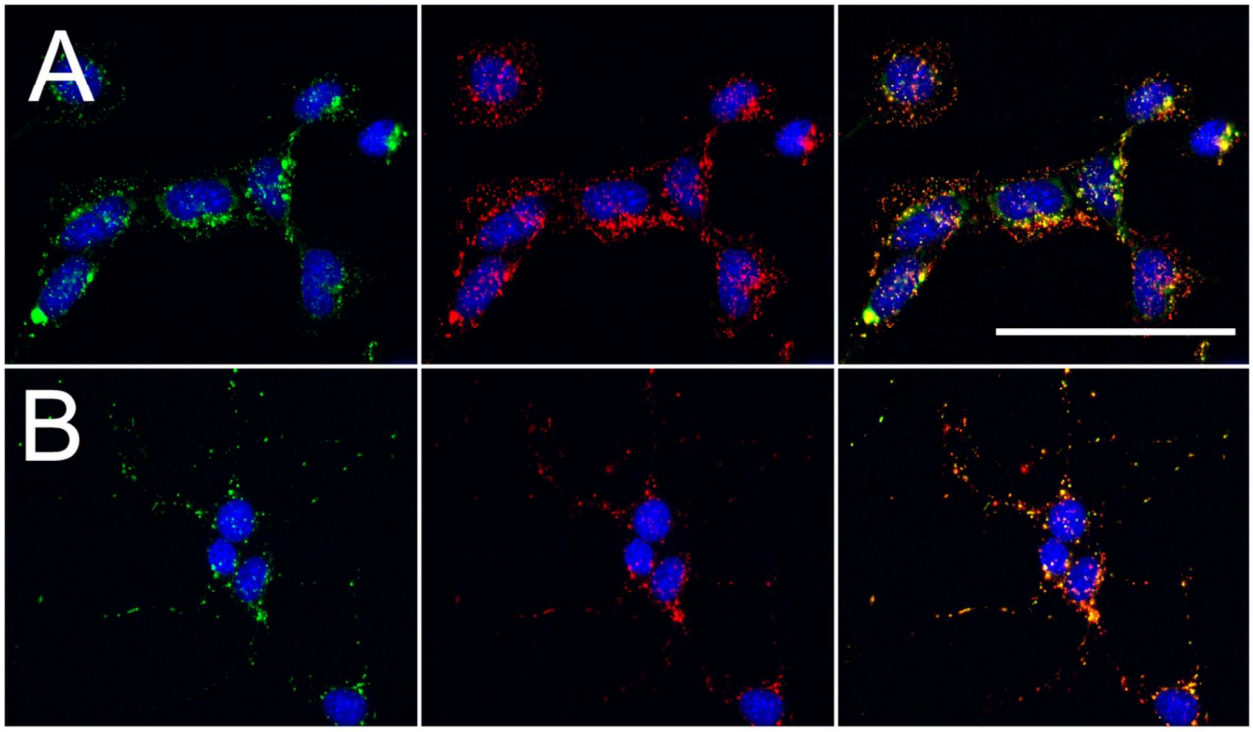

Figure S8. Fluorescence microscopy images of native (row A) and SS-differentiated RGC-5 (row B) cells following a 3 hr labeling with LysoTracker Red (left panels, pseudocolored green) and 10 μM KcapQ647 (**1**) (middle panels, pseudocolored red). Right, composite images showing co-localization (yellow) of activated KcapQ647 (red) and LysoTracker (green) (50 μm scale bar).

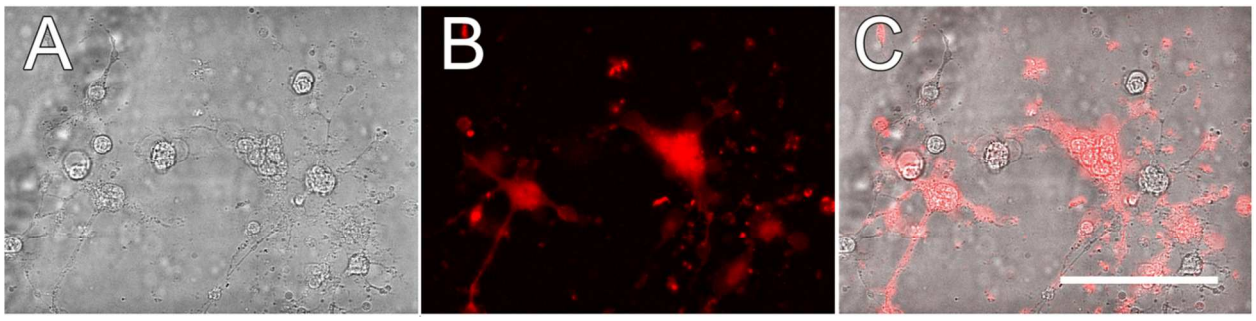

Figure S9. Transmitted light image of SS-differentiated RGC-5 cells undergoing ionomycin-induced apoptosis (10 μM, 1 hr) (A) with concurrent activation of **1** (10 μM, pseudocolor red, B). Overlay of panels A and B (C) (50 μm scale bar).

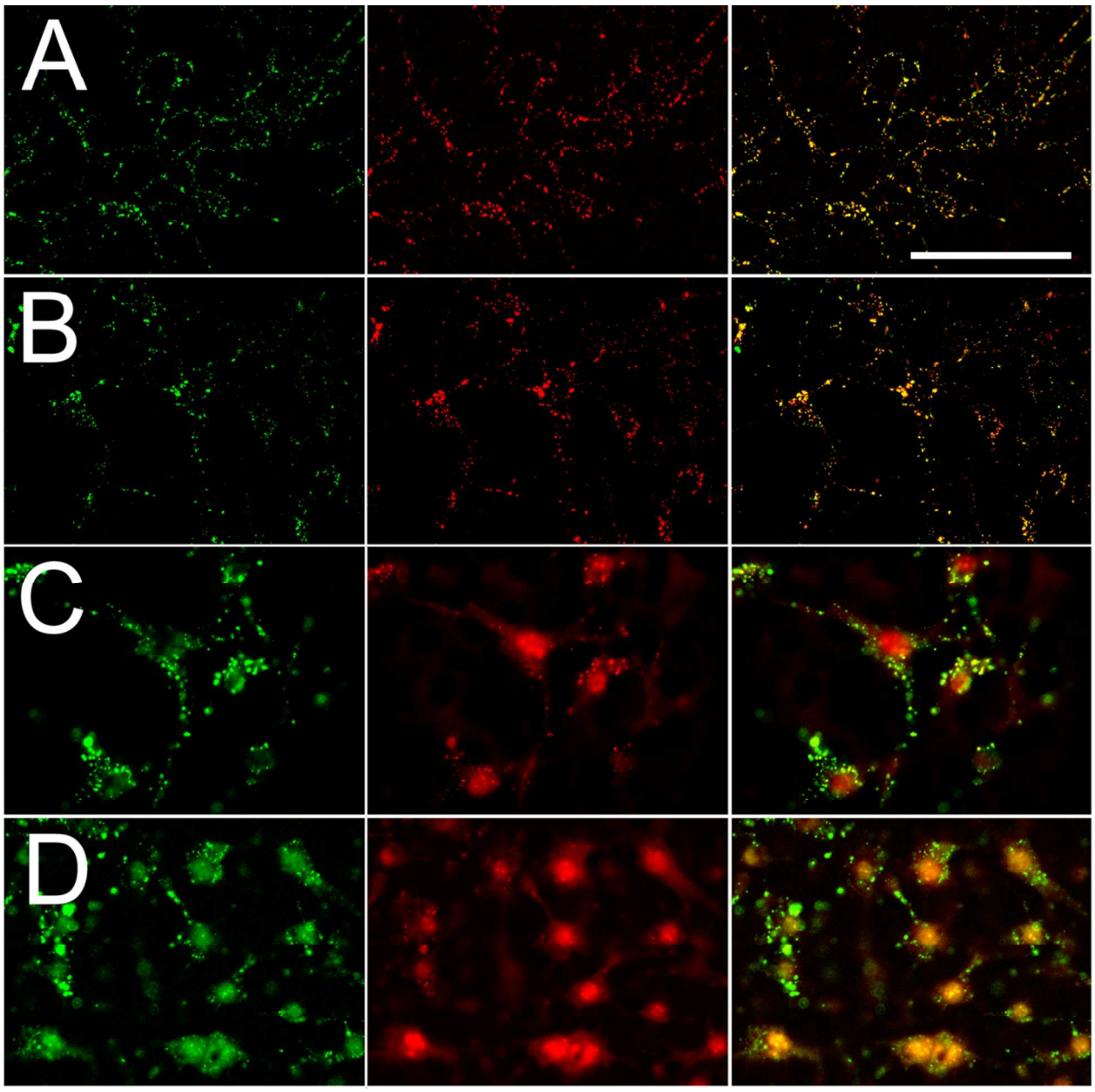

Figure S10. Fluorescence microscopy images of **5** (12 μM) distribution in SS-differentiated RGC-5 cells (row A); *D*-**5** (12 μM) distribution in SS-differentiated RGC-5 cells (row B); **5** (12 μM) distribution in ionomycin-treated (10 μM) SS-differentiated RGC-5 cells (row C); *D*-**5** (12 μM) distribution in ionomycin-treated (10 μM) SS-differentiated RGC-5 cells (row D). Left, fluorescence field for the Alexa Fluor 488-labeled C-terminus (pseudocolored green); middle, fluorescence for the Texas Red-labeled N-terminus (pseudocolored red); right, overlays of Alexa Fluor 488 and Texas Red images (50 μm scale bar).