Supplemental Data

Trafficking of α4* nicotinic receptors revealed by superecliptic phluorin: effects of a β4 ALS-associated mutation and chronic exposure to nicotine

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Running Head: nAChR Plasma Membrane Trafficking Dynamics

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Figure S1. Colocalization of nAChRs and Endoplasmic Reticulum Marker.

A representative cortical neuron (8 d in culture) expressing α4-SEP, β2wt, and dsRed with an ER localization sequence (KDEL). A-C, Extracellular pH of 7.4. A. Representative TIRF image with 488 nm excitation, which selectivity excites SEP; the emission was also filtered to isolate SEP. B. The same neuron excited with 561 nm excitation, which selectively activates dsRed; the emission was also filtered to isolate emission dsRed. C. Overlay of images A and B showing extensive colocalization of the SEP and dsRed signals. D. The same neuron in (A) 4 minutes after adjusting the extracellular solution to a pH of 5.4. D agrees with the interpretation that most of the α4 signal is in organelles, insensitive to extracellular pH.

Figure S2. ER localized Superecliptic phluorin.

A. Representative TIRF image of a neurons transfected with α4-SEP (in the absence of a beta subunit) with an extracellular pH of 7.4. B. The same neuron depicted in (A), after the extracellular solution was adjusted to a pH of 5.4. The images were acquired and shown at the same intensity scale. No loss of fluorescence was observed for control samples with an ER localized super ecliptic phluorin when the extracellular solution was adjusted from pH 7.4 to pH 5.4.

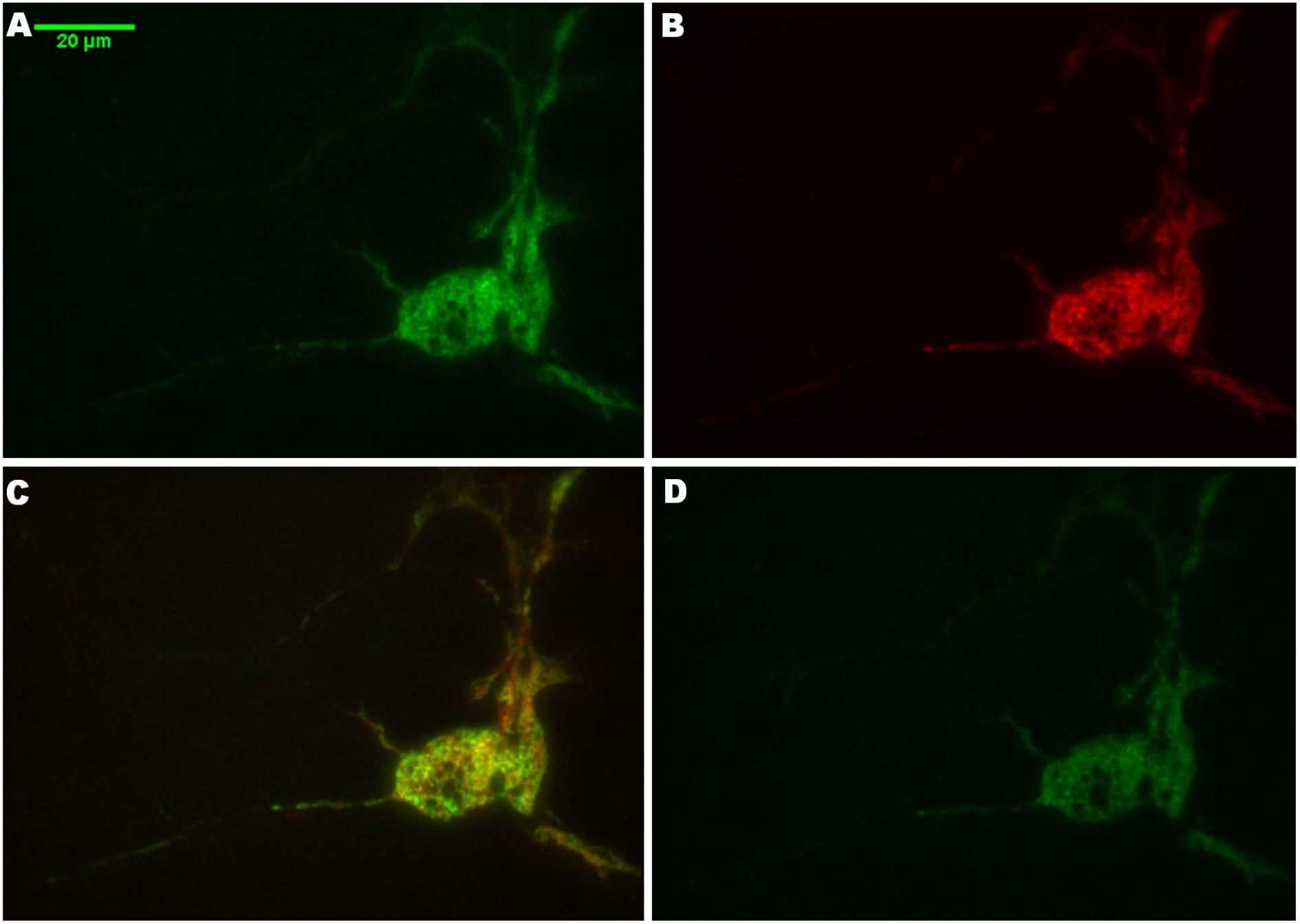
Figure S3. Superecliptic phluorin (SEP)-labeled α4β2 nAChRs.

A. Representative TIRF image of a dendritic section of a cortical neuron (8 d in culture) expressing α4-SEP and β2wt with an extracellular pH of 7.4. B. The same neuron depicted in (A), 4 minutes after the extracellular solution was adjusted to pH 5.4. The images were acquired and shown at the same intensity scale. The average ratio of fluorescence intensity (Fl. at pH 5.4 / Fl. at pH 7.4) was .61.

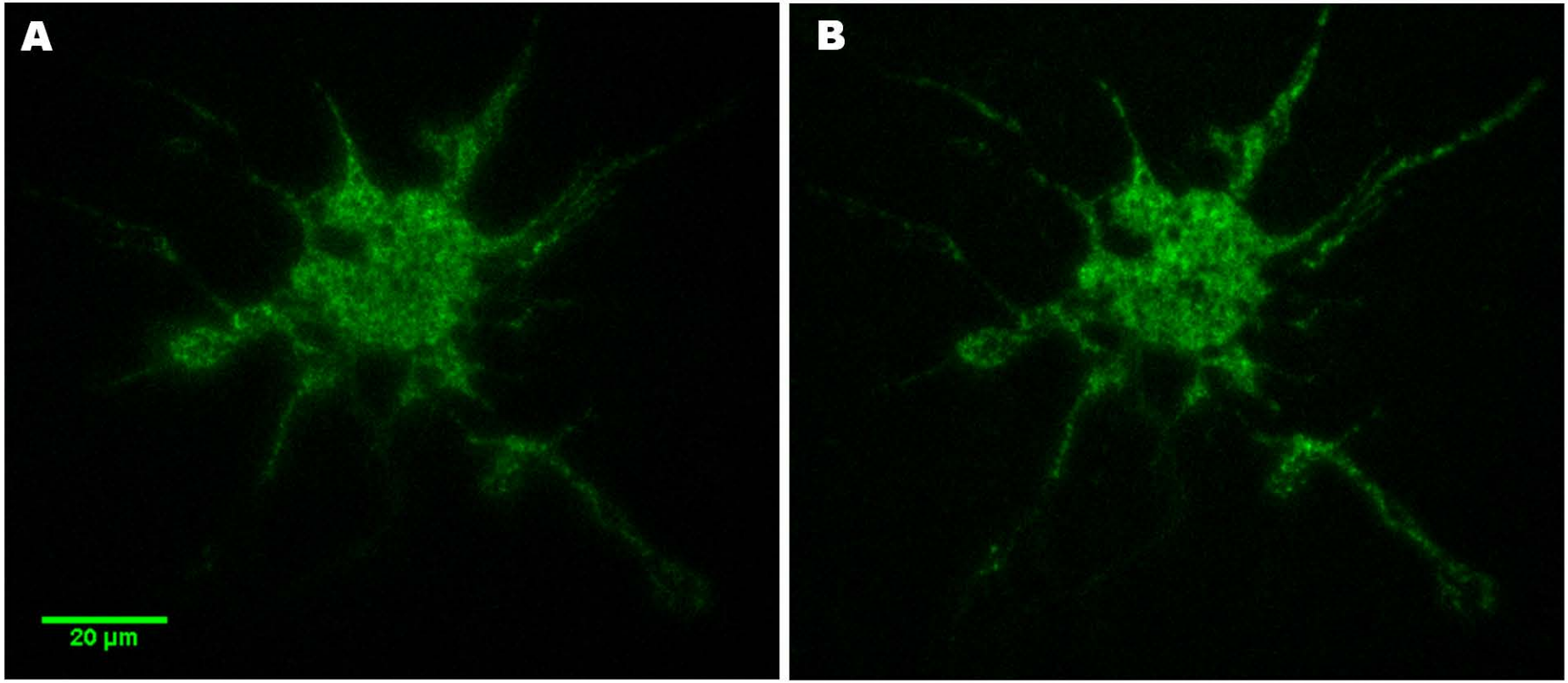
Figure S4. Acute nicotine leads to receptor endocytosis

A. A representative TIRF image of a cortical neuron transfected with α4-GFP β4-wt. The first panel shows the typical distribution of fluorescent receptors before exposure to nicotine. B.

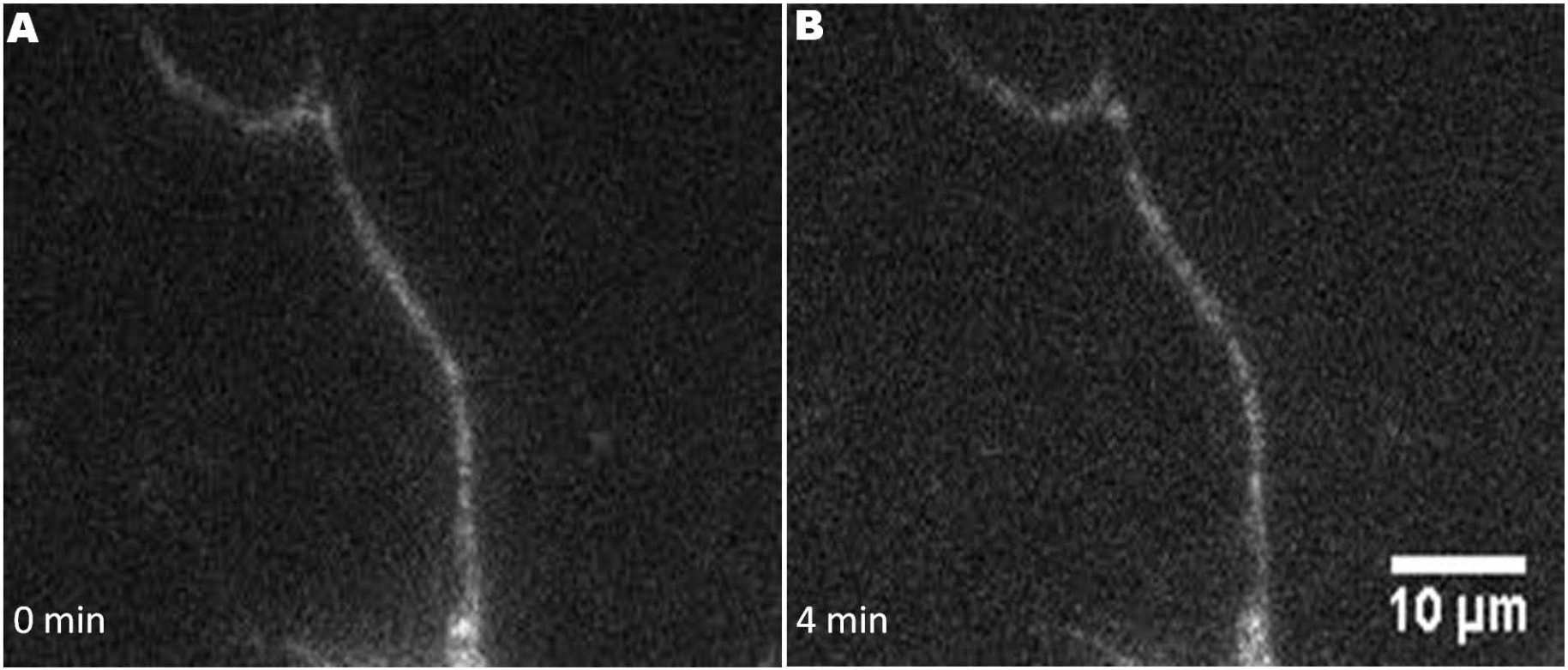
After incubation with 1 μM nicotine for 5 minutes, there is a reduction in average fluorescence, from 1690 (Figure S4A) to 1549 counts (Figure S4B). The eGFP marker is not pH sensitive and is therefore still visible within transport vesicles resulting in less of a reduction than seen with SEP. This is further illustrated in the post nicotine image where the receptors have aggregated into bright, presumably vesicle-like punctate structures. C. Shows the cell body region of A rescaled to demarcate regions with fluorescence above the average level. D. Shows the cell body region of B rescaled to demarcate regions with fluorescence above the average level. This clearly shows that the post nicotine image (Figure S4D) has a greater number of bright punctate features, indicating a pooling of receptors into vesicles. E. A representative TIRF image of a cortical neuron transfected with α4-SEPβ4wt before nicotine exposure. F. The same cell in E after 4min of 1 μM nicotine. While receptors labeled with SEP are recycled from the PM into vesicles, they lose their fluorescence after several seconds as the environment acidifies. Comparing the pre- and post-nicotine images provides a $F_{nic}/F_0 = .65 \pm .03$ (mean ± SEM, N=7).



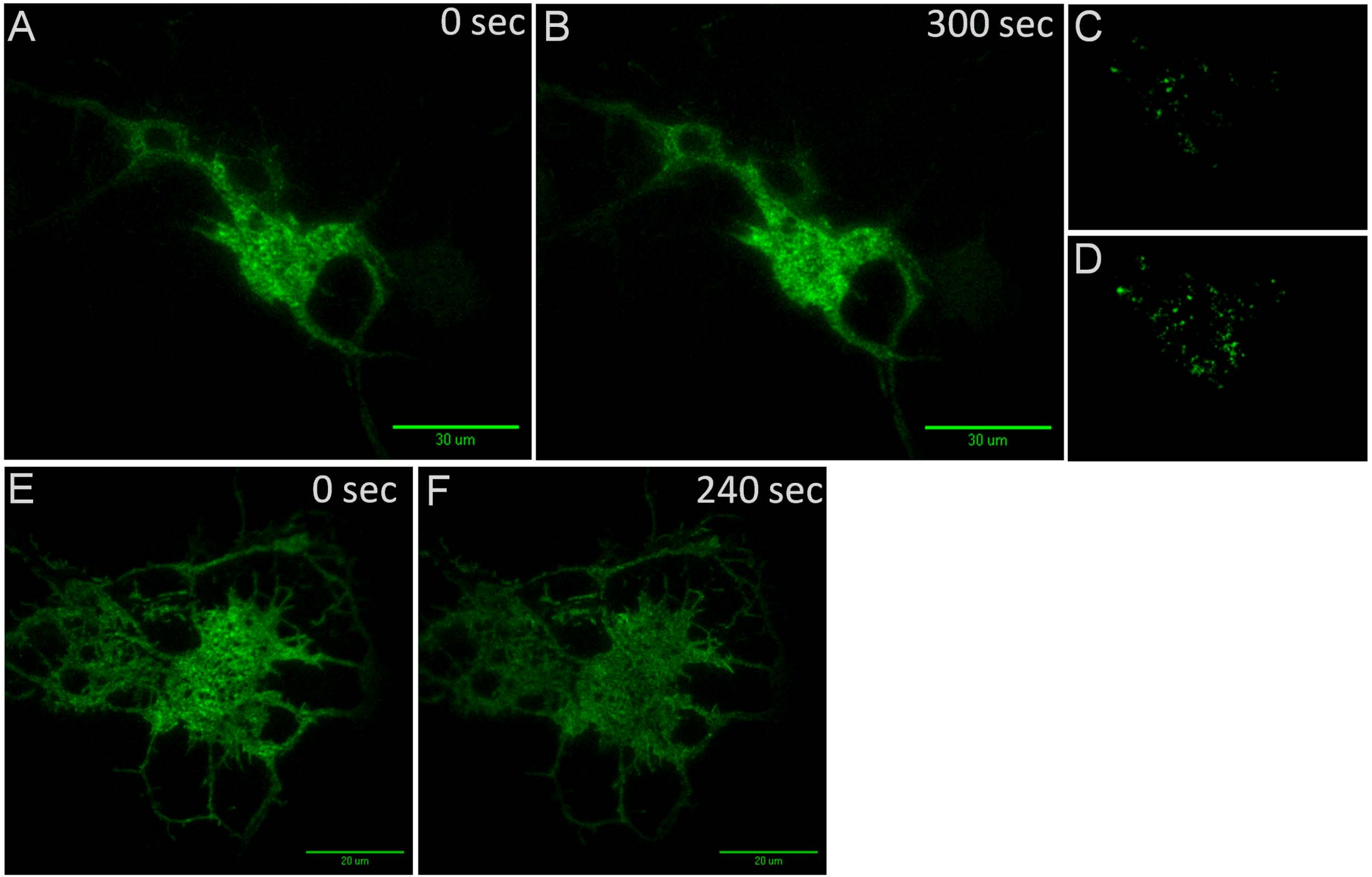
Richards et al. Supplemental Figure 1



Richards et al. Supplemental Figure 2



Richards et al. Supplemental Figure 3



Richards et al. Supplemental Figure 4