

signals of each neuron were normalized by the mean intensity of pCREB fluorescence obtained from the control group. Normalized pCREB of GCaMP3-expressing neurons exhibited strong (negative) correlations with the N/C ratio (Fig. 3b) or expression level (Supplementary Fig. 5a) of GCaMP, resembling the high correlations previously obtained from neurite outgrowth (Fig. 1g and Supplementary Fig. 3). Grouped data from nuclear-excluded

(N/C ratio < 0.6) and nuclear-filled (N/C ratio > 1 or NLS-tagged) neurons revealed that pCREB signals under normal physiological conditions (control group) were significantly decreased by nuclear GCaMP3 but aberrantly enhanced by cytosolic GCaMP3 (Fig. 3c), while the total CREB levels remained the same for all neurons (Supplementary Fig. 5b). Such pCREB changes in dual directions were mostly attributed to GCaMP effects on native I_{Ca}

