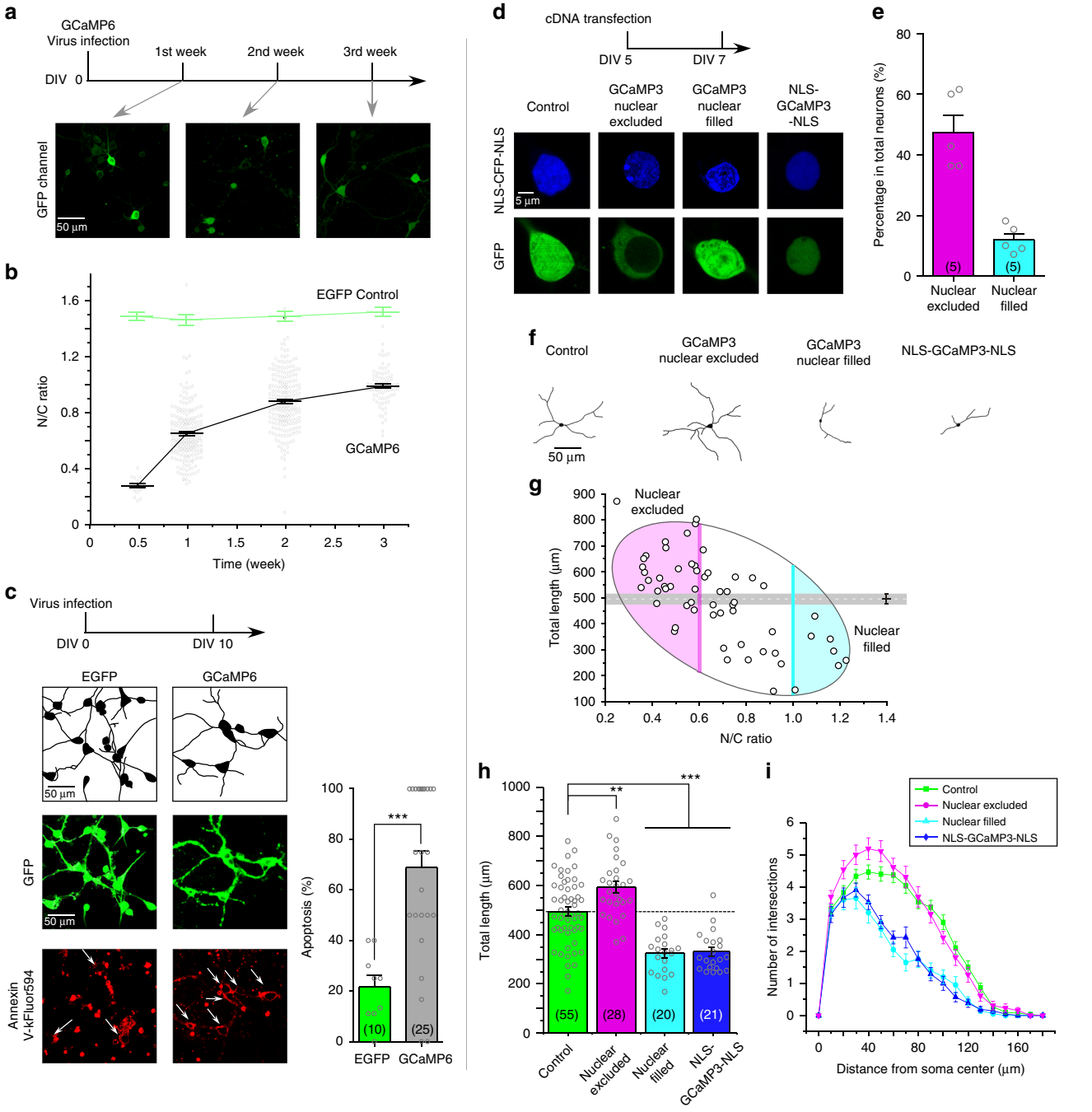

Fig. 1 Side-effects of GCaMP on cortical neurons. **a** Abnormal nuclear accumulation of GCaMP. Cultured cortical neurons infected with GCaMP6 (AAV-*Syn*-GCaMP6f) virus were examined with confocal live-cell imaging. **b** Analyses indexed with nuclear/cytosolic (N/C) fluorescence ratio indicate time-dependent nuclear accumulation of GCaMP6, in contrast to stable GFP distribution. **c** GCaMP6 caused apoptosis. For cortical neurons infected with AAV-*Syn*-EGFP and AAV-*Syn*-GCaMP6f (green), tracing images of dendritic morphology (black and white) and confocal fluorescence images of Annexin V-kFluor594 (red) are shown. Arrows are to identify apoptotic neurons where both green and red fluorescence are present and the percentage of such neurons (per view) are counted (right, number of views in parentheses). **d** Subcellular distributions of YFP, GCaMP3 or NLS-GCaMP3-NLS overexpressed in cortical neurons. CFP fluorescence (NLS-CFP-NLS, upper) indicates the nucleus (blue) in cortical neurons. GFP images (lower row) of neurons expressing GCaMP3 (2 days after cDNA transfection) could be categorized into two major subgroups of interest: nuclear-excluded (N/C ratio < 0.6) and nuclear-filled (N/C ratio > 1.0), with the latter mimicked by neurons expressing NLS-GCaMP3-NLS. Such criteria (N/C ratio < 0.6 and >1) were applied throughout this study (unless indicated otherwise). **e** Based on the above criteria neurons of nuclear-filled group and nuclear-excluded group accounted for ~10% and ~50% of the total number of GCaMP3-expressing neurons, respectively (5 experiments). **f** Representative images tracing neurite morphology for cortical neurons from different subgroups. **g** Correlations between N/C ratio of GCaMP3 and neurite outgrowth. The grey line/area represents the total neurite length per neuron (control group). The eclipse enclosing most neurons contains two major areas representing the subgroups of nuclear-filled (cyan) and nuclear-excluded (pink). Nuclear GCaMP accumulation (N/C ratio) and neurite outgrowth (neurite length per neuron) are highly correlated (the correlation coefficient is −0.7). **h**, **i** Additional experiments and analyses for neurite morphology. Neurite outgrowth was quantified for the four subgroups by neurite length (**h**) and Sholl analysis (**i**), with the total number of cells in parentheses. Standard error of the mean (S.E.M.) and Student's *t*-test (two-tailed unpaired with criteria of significance: $*p < 0.05$; $**p < 0.01$, and $p < 0.001$) were calculated when applicable