



Fig. 2 GCaMP interferes with $\text{Ca}_v1.3$ gating. **a** Schematic summary of GCaMP series. Upgrades of GCaMP (from GCaMP3 to GCaMP5 and GCaMP6) were achieved by mutations of the EGFP and CaM domains at the sites indicated by vertical letters (GCaMP5G/6f/6m vs. GCaMP3), or by horizontal letters (GCaMP5G/6f/6m vs. GCaMP3). Details see Supplementary Fig. 1. **b** Effects on I_{Ca} were examined for neurons infected with AAV-Syn-GCaMP6f. Representative traces of Ca^{2+} current (left), S_{Ca} and $J_{\text{Ca, peak}}$ analyses (right) for native $\text{Ca}_v1.3$ in cortical neurons expressing GCaMP6. Neurons were treated with a cocktail recipe to isolate Ca_v1 current (mostly $\text{Ca}_v1.3$) and recorded at the membrane potential (V) of -10 mV. S_{Ca} (quantified as $1 - I_{\text{Ca}, 50} / I_{\text{Ca, peak}}$, where $I_{\text{Ca}, 50}$ and $I_{\text{Ca, peak}}$ represent the currents measured at 50 ms and the instantaneous peak, respectively) and $J_{\text{Ca, peak}}$ (pA/pF , the current density of $I_{\text{Ca, peak}}$) serve as the indices for CDI and VGA respectively. **c** Effects of GCaMP3 on recombinant $\alpha_{1\text{DL}}$. Representative Ca^{2+} current traces were compared for I_{Ca} recorded from HEK293 cells expressing long variant $\alpha_{1\text{DL}}$ alone (left), or with GCaMP3 (right) at -10 mV. Ba^{2+} currents (rescaled) and Ca^{2+} currents (I_{Ca}) were shown as grey and red traces, respectively, with scale bars indicative of I_{Ca} amplitudes. CDI (S_{Ca}) and VGA ($J_{\text{Ca, peak}}$) profiles at different membrane potentials are compared between $\alpha_{1\text{DL}}$ control and $\alpha_{1\text{DL}}$ overexpressed with GCaMP3 (differences highlighted by orange areas). **d** Effects of GCaMP3 on recombinant $\alpha_{1\text{DS}}$ alone (left), or with GCaMP3 (right), in a similar fashion to **c**. 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, and n.s. denotes "not significant"