CaM has been encoded into various CaM-based GECIs, with GCaMP as the representative. Vast efforts were put into updating GCaMP generation by generation through screening thousands of structure-based or random mutagenesis to improve baseline fluorescence, photostability, dynamic range, and Ca^{2+} affinity[5–7,43]. However, until this work the major drawback of GCaMP has not yet been specifically addressed and resolved, such as the well-known nuclear accumulation and related cell damages. We went beyond simply attributing these side-effects to over-buffering which supposedly could happen to almost any probes of excessive amount[15,16], unveiling that the true mechanisms underlying GCaMP effects are due to its CaM motif, which interferes with Ca_V1-dependent E–T coupling thus damaging neurons (Figs. 1–3 and Fig. 6a).