

acetylcholine (Ach)⁵ were used as the cellular context to fairly compare basic sensor characteristics such as Ca²⁺ sensitivities, which turned out to be indistinguishable between GCaMP6 and GCaMP6-X_C (Supplementary Fig. 11a). To closely examine the kinetics, the approach we employed was to induce Ca²⁺ dynamics mimicking that of one single action potential (AP), by fast break-in with brief ZAP stimulus, aided with strong Ca²⁺ chelators of

10 mM BAPTA in patch-recording pipettes. This way, a Ca²⁺ transient was created with fast onset and offset. GCaMP6m and GCaMP6m-X_C resulted into indistinguishable characteristics of peak $\Delta F/F_0$, SNR, rise time t_r and decay time t_d (Fig. 6b). Their t_r values were about the same (~0.1 s), further confirmed by an alternative approach to induce faster ($t_r < 0.1$ s) Ca²⁺ influx via voltage-gated Ca_v2.2 channels (Supplementary Fig. 12), which

