Supplementary Note on Sensor Design and Use:

The spatiotemporal dynamics of PKC isozymes were measured using newly developed isozyme-specific sensors, ITRACK and IDOCKS. Unlike previous PKC sensors which measure substrate phosphorylation or conformational change of PKC, we measured membrane translocation or the substrate-docking of eGFP-tagged PKC isozymes. Because overexpressed eGFP-PKC isozymes are functional[30-32] and rescue sLTP phenotypes in isozyme specific manner, measuring the activity of eGFP-tagged PKC isozymes can probe PKC activity in an isozyme-specific manner. The bimolecular sensor approaches used here enable greater sensitivity than previously used unimolecular approaches, as unbound donor and acceptor cause negligible FRET. Although a bimolecular approach can be challenging with traditional FRET measurements, it can be quantitatively assessed with fluorescence lifetime imaging (FLIM), which measures FRET efficiency without effects of local fluorophore concentrations and wavelength dependent scattering [24]. This provided highly sensitive and specific readouts of PKC isozyme activity with millisecond kinetics in single dendritic spines embedded in brain tissue. As any sensor can affect endogenous signaling, care must be taken in order to ensure that any effect of sensor are minimal. The use of multiple sensors with different designs and experimental controls to determine if biological activity is modulated in the presence of sensor demonstrated that the expression of ITRACK and IDOCKS have minimal perturbation to endogenous signaling related to sLTP (Fig. S2).