**Title:** PKCα diffusion and translocation are independent of an intact cytoskeleton

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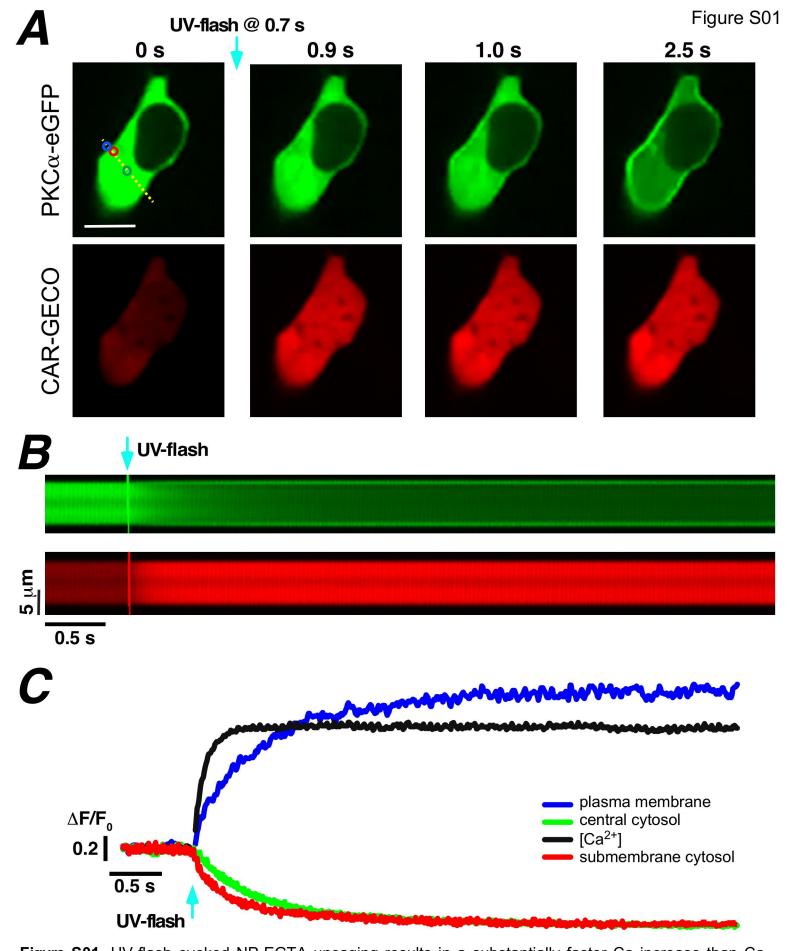
One Sentence Summary: Employing pharmacological and optical tools, we quantitatively demonstrate that the subcellular dynamicity of PKC $\alpha$  is driven by sole diffusion and independent of the integrity of the cytoskeleton.

## Affiliations:

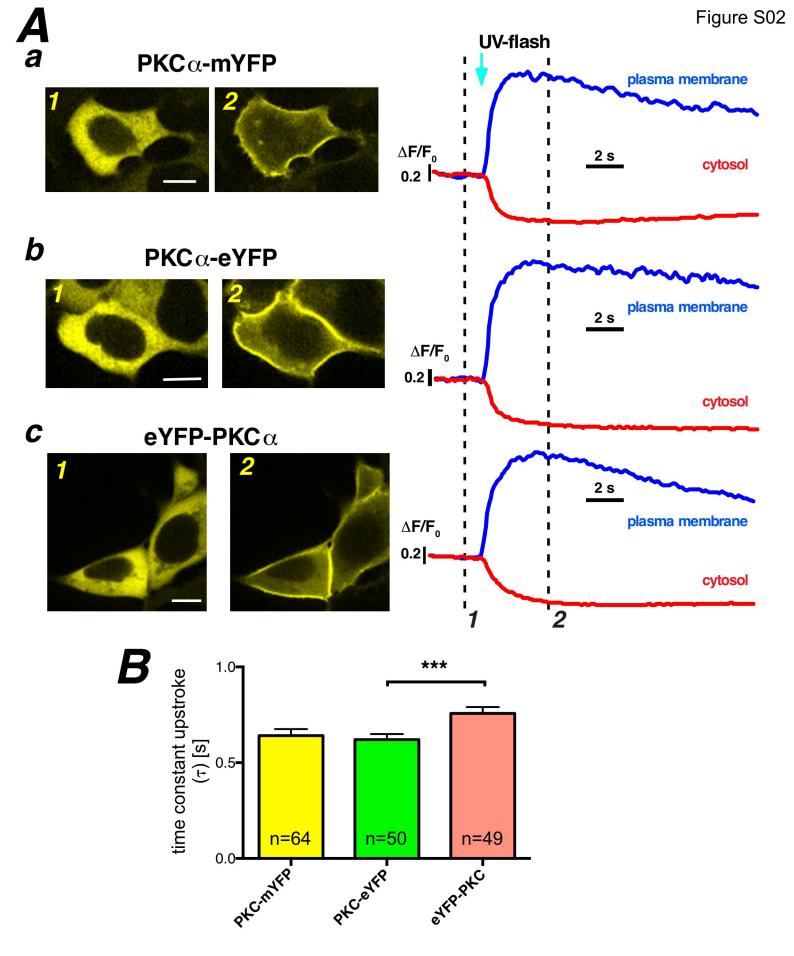
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**Figure S01.** UV-flash evoked NP-EGTA uncaging results in a substantially faster Ca increase than Cadependent translocation of PKC $\alpha$ -eGFP. NP-EGTA-AM loaded HEK cells expressing PKC $\alpha$ -eGFP and the red-shifted genetically encoded Ca sensor CAR-GECO were subjected to a birght UV-flash at the time indicated by the turkis arrow. (A) upper row redistribution of PKC $\alpha$ -eGFP and lower row fluorescence changes of the CAR-GECO for the time points given. Scale bar depicts 10 μm. (B) Psudeo-linescan along the yellow dahsed line in (A) shows the redistribution of PKC $\alpha$ -eGFP and the fluorescence change of CAR-GECO. (C) fluorescence over time traces for the subcellular locations given. The flash artefact was removed for display reasons. Similar results were obtained from additional 15 cells from 3 independent experiments.



**Figure S02.** UV-flash evoked redestribution of PKC $\alpha$ -mYFP (Aa, B-yellow bar), PKC $\alpha$ -eYFP (Ab, B-green bar) and eYFP-PKC $\alpha$  (Ac, B-red bar). In (A) left panels depict exemplified confocal sections at the time points highlighted in the fluorescence over time traces in the right panels.