



FIGURE 5. In vivo β -lactamase PCA applications. (A) In vivo microtiter plate assay with CCF2/AM in transiently transfected 293 cells. Interaction between Ras and the Ras Binding Domain of Raf (Raf RBD), and absence of interaction between Ras or Raf RBD with FKBP as negative controls, are shown as an example. pcDNA3.1 is the empty vector. (B) Protein-interaction modulation measured by microscopy on live cells. Stably transfected 293 cells expressing the interacting partners PKB and PDK1 were stimulated with insulin. Blue/green (hydrolyzed CCF2/intact CCF2) fluorescence intensity ratios representing β -lactamase activity were obtained for each cell in populations of \sim 300 cells. Cell distribution as a function of β -lactamase activity (blue/green ratios) is concentrated around higher ratio values for the cell population that was stimulated (Insulin) compared to the nonstimulated population (NS: not stimulated), indicating an increase of the number of PKB–PDK1 complexes formed.