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kinase screen data for Nature Communications publication

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***In vitro* kinase screening:** 245 individual Ser/Thr kinases were evaluated for their ability to phosphorylate S502 of TNK1 using a radiometric KinaseFinder assay (ProQinase GmbH). A biotinylated TNK1 peptide that included S502 or carried an S502A substituteion (Biotin-RMKGISRSLESVL-OH, or Biotin-RMKGISRALESVL-OH; New England Peptide) was reconstituted in 50 nM HEPES pH 7.5 at 200 µM stock solution. Reaction buffer (60 mM HEPES-NaOH pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 1 µM ATP/[γ-^{33}P]-ATP), protein kinase (1-400 ng/50 µL) and TNK1 peptide (1 µM) were pipetted into 96-well, V-shaped polypropylene microtiter plates (assay plate). All PKC assays (except the PKC-mu and the PKC-nu assay) additionally contained 1 mM $CaCl_2$, 4 mM EDTA, 5 µg/ml phosphatidylserine and 1 µg/ml 1.2-dioleyl-glycerol. The MYLK2, CAMK1D, CAMK2A, CAMK2B, CAMK2D, CAMK4, CAMKK2, and DAPK2 assays additionally contained 1 µg/ml calmodulin and 0.5 mM $CaCl_2$. The PRKG1 and PRKG2 assays additionally contained 1 µM cGMP. One well of each assay plate was used for a buffer/substrate control containing no enzyme. The assay plates were incubated at 30°C for 60 minutes. Subsequently, the reaction cocktails were stopped with 20 µl of 4.7 M NaCl/35 mM EDTA. The reaction cocktails were transferred into 96-well streptavidin-coated FlashPlate® HTS PLUS plates (PerkinElmer, Boston MA), followed by 30 min incubation at room temperature on a shaker to allow for binding of the biotinylated peptides to the streptavidin-coated plate surface. Subsequently, the plates were aspirated and washed three times with 250 µl of 0.9% NaCl. Incorporation of radioactive $^{33}P_i$ was determined with a microplate scintillation counter (Microbeta, Perkin Elmer). For evaluation of the results of the FlashPlate® PLUS-based assays, the background signal of each kinase (w/o biotinylated peptide) was determined in parallel. 7 protein kinases were selected from the screen described above to repeat at three peptide concentrations (1 µM, 0.5 uM and 0.25 uM) in triplicate. The assays were performed as described above.