a. DYRK1a is localized to the nucleus.

GFP (upper panels) or GFP-DYRK1a (lower panels) was transfected into 293T cells, which were fixed and stained with DAPI. DYRK1a expression is restricted to the nucleus, suggesting that its activity on the NFAT pathway is to target nuclear NFATc for phosphorylation and export.

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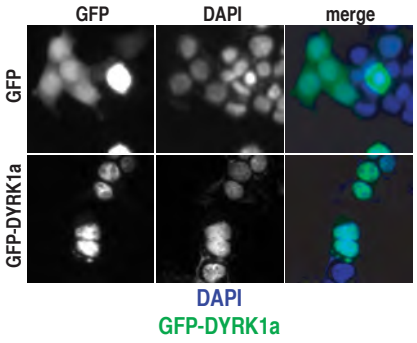
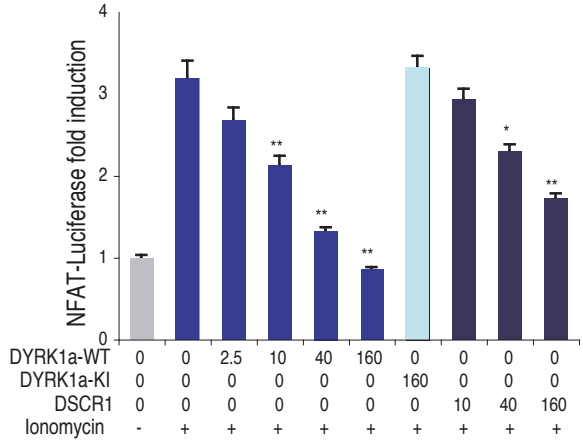
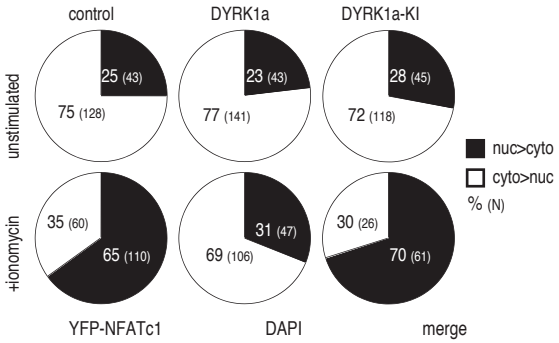


b. DYRK1a and DSCR1 inhibit NFAT transcriptional activity in a dose-dependent manner.

293T cells in 12-well plates were transfected in triplicate with an NFAT-luciferase reporter construct, a Renilla-luciferase internal standard, and DYRK1a (WT or Kinase inactive, KI) or DSCR1 as indicated. Cells were treated with ionomycin (2.5 μM) as indicated and analysed 24h after transfection. Numbers below graph represent ng of plasmid transfected. Error bars denote S.E.M.
*p < 0.07 **p < 0.02.





c. DYRK1a kinase activity prevents nuclear accumulation of NFATc1.

YFP-tagged NFATc1 (YFP-NFATc1) was transfected with empty vector (control), DYRK1a, or DYRK1a-KI into 293T cells. Cells were unstimulated or stimulated with ionomycin (2 μM) for 15 minutes, then fixed and stained with DAPI. Fluorescent cells were counted and scored as having a majority of YFP-NFATc1 in the nucleus or cytoplasm (top panels). Representative cells are shown in lower panels. DYRK1a promoted cytoplasmic accumulation of YFP-NFATc1 in stimulated cells while DYRK1a-KI did not.