Science Signaling
AAAS
www.sciencesignaling.org/cgi/content/full/3/111/ra16/DC1

Supplementary Materials for

Characterization of a Domain that Transiently Converts Class 2 DYRKs into Intramolecular Tyrosine Kinases

Ross Kinstrie, Nathan Luebbering, Diego Miranda-Saavedra, Gary Sibbet, Jingfen Han, Pamela A. Lochhead, Vaughn Cleghon*

*To whom correspondence should be addressed. E-mail: vaughn.cleghon@cchmc.org

Published 2 March 2010, *Sci. Signal.* **3**, ra16 (2010)
DOI: 10.1126/scisignal.2000579

The PDF file includes:

Fig. S1. Full-length dDYRK2 does not complement ΔN2-dDYRK2 in trans.
Fig. S2. Autophosphorylation of the active loop tyrosine of dDYRK2 is not affected by inhibitors of Hsp90.
Table S1. Sequences of the forward primers used in site-directed mutagenesis of dDYRK2 and MNB.

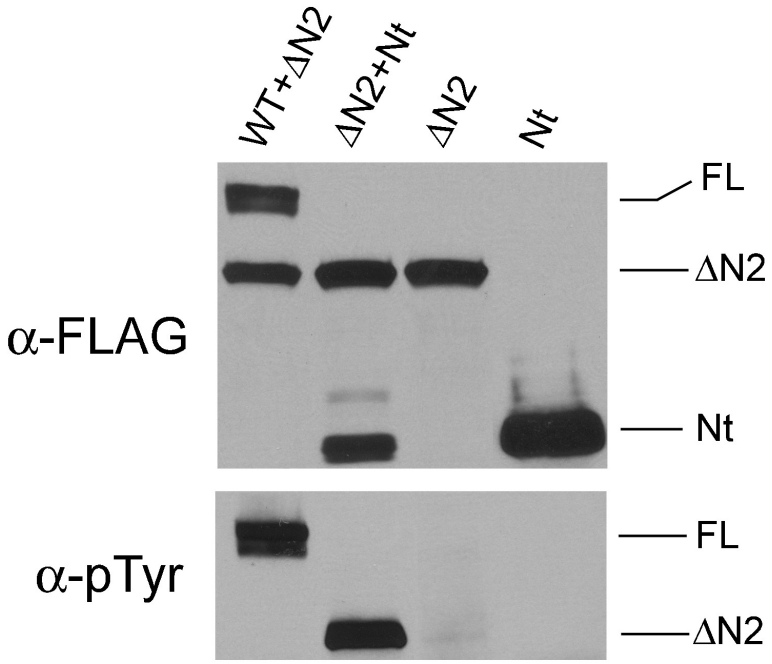

Fig S1. Full-length dDYRK2 does not complement ΔN2-dDYRK2 in trans. ΔN2-dDYRK2 (consisting of residues 168 to 722) was expressed alone or was coexpressed with either full-length (FL) WT dDYRK2 or with the isolated N-terminus (Nt, residues 1 to 197) of WT dDYRK2. Expression of the various dDYRK2 proteins was assessed by incubating Western blots with an antibody against the FLAG epitope (α-FLAG) (top panel). Phosphorylation of the activation loop was determined by immunoprecipitation and Western blotting analysis of FL dDYRK2 and ΔN2-dDYRK2 proteins with antibodies against phosphotyrosine residues (α-pTyr) (bottom). The positions of the various molecules are indicated to the right of the panels. The data shown are representative of three experiments.

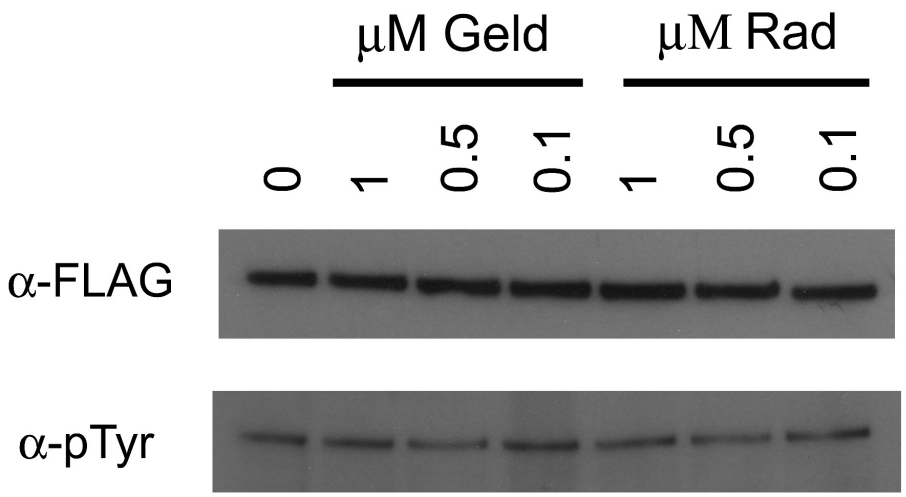

Fig S2. Autophosphorylation of the active loop tyrosine of dDYRK2 is not affected by inhibitors of Hsp90. WT dDYRK2 was expressed in an in vitro rabbit reticulolysate translation system in the presence of the Hsp90 inhibitors radicicol (Rad) or geldanamycin (Geld) at the indicated concentrations. dDYRK2 was immunoprecipitated from each sample and the abundance of dDYRK2 was assessed by Western blotting with an antibody against the FLAG epitope (α-FLAG), whereas autophosphorylation of the activation loop tyrosine was analyzed with an antibody against pTyr residues (α-pTyr). The data shown are representative of two experiments.

Table S1. Sequences of the forward primers used in site-directed mutagenesis of dDYRK2 and MNB.

Primer Name	Sequence
dDYRK2ΔN1	5'-GGCAACTACAAGATCATCGAATTCAACCATGGACTACA AGGACGACGATGACAAGGAACACGATCACATAGCTTTTC-3'
dDYRK2ΔN2	5'-AAGAACTACAACAAAGAATTCAACCATGGACTACAAG GACGACGATGACAAGCCCGCCCCCACAGCC-3'
dDYRK2ΔN3	5'-GGCCAGCAGCACGGGAAGTGGCGAGGAATTCAACCAT GGACTACAAGGACGACGATGACAAGCTCAAATGCAAT ACGCCCATGACGC-3'
dDYRK2ΔC	5'-GTTTCTGCAGCCATCTGCCTCCAGTTGAATTCCGCCAT CGCAGCTGCCGCATGTCG-3'
dDYRK2ΔNAPA	5'-GCTGGTGAAGAAGTTCCGCAACTACAACTACAACAAA CCCGCCCCCACAGC C-3'
dDYRK2Nt	5'-CGATCACATAGCTTTTCGTTACTGATGACTGGAGGTAA TTGGCAAGGGCAG-3'
dDYRK2L140E	5'-GAAGAAGTTCCGCAACTATGAAACCGATCTTGAGTTC GAAGAGC-3'
dDYRK2E144L	5'-CTACCTAACCGATCTTTTGTTCGAAGAGCTCAAGGTGT AC-3'
dDYRK2E147L	5'-CCGATCTGGAGTTCGAATTGCTCAAGGTGTACAAGGA AGTG-3'
dDYRK2K149I	5'-CTTGAGTTCGAAGAGCTCATTGTGTACAAGGAAGTGT GG-3'
dDYRK2G158E	5'-GGAAGTGTGGTATTTTGAACAGCACGCCAGCAAGAA C-3'
dDYRK2Y165A	5'-CACGCCAGCAAGAACGCCAACAAACCCGCCC-3'
dDYRK2D192V	5'-CAAGATCATCGAACACGTTCACATAGCTTTTCGTTAC G-3'

dDYRK2H193R	5’-CAAGATCATCGAACACGATCGCATAGCTTTTCGGTAC GAG-3’
MNBΔN1	5’-CGATAATCACGACTATATAATCAAGGCGGCCGCAACC ATGGACTACAAGGACGACGATGACAAGAATGGCGAAA AGTTTTTGGATCGC-3’
MNBΔN2	5’-CGACGACTCATCTAACAAAAAGGAGGCGGCCGCAACC ATGGACTACAAGGACGACGATGACAAGCGAAAACTTT ATAACGACGGCTACG-3’
MNBΔC	5’-CAAGCGCACGGCAGACGAGGCAACCTGAATTCAATAC GAGCGGCGCGGGCGCCAC-3’