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online data

SUPPLEMENTARY ONLINE DATA

A robust methodology to subclassify pseudokinases based on their nucleotide-binding properties

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Supplementary Figures S1–S3 and Supplementary Table S1 are on the following pages.

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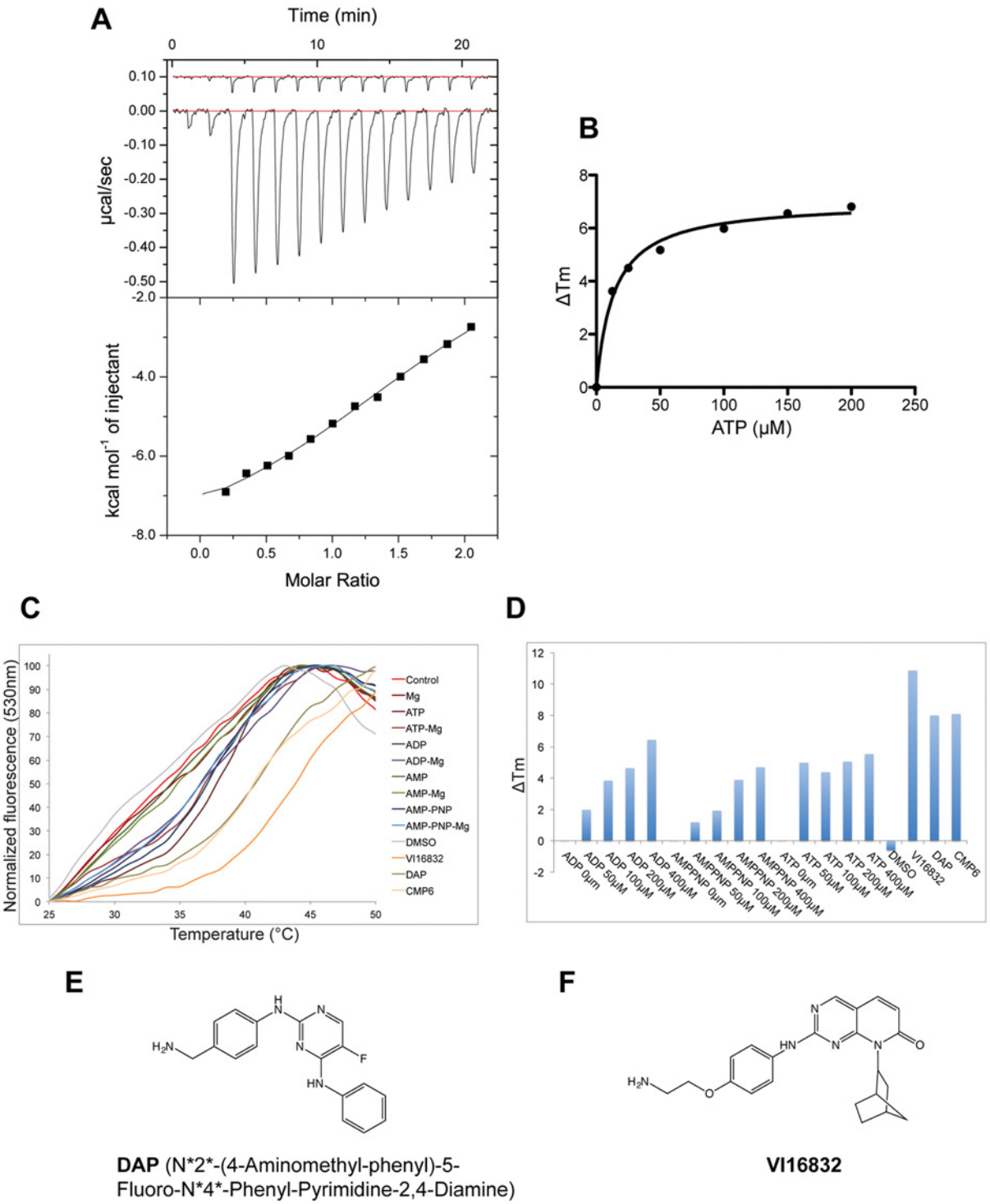

Figure S1 Validation of the thermal-shift assay for detecting ligand binding

(A) ITC data demonstrating ATP binding to MLKL. Upper panel: the upper isotherm represents a buffer control and the lower isotherm represents the injection and binding of ATP to 50 µM MLKL. The K_d value for the experiment shown was calculated as 20 µM. The K_d value calculated from a second experiment (results not shown) was 15 µM. (B) Titration of MLKL with increasing concentrations of ATP in the thermal-shift assay. The data from two independent experiments yielded a K_d value (mean±S.E.M.) of 13.6±1.9 µM. (C) Thermal denaturation curves for the tyrosine kinase (JH1) domain of JAK2 in the presence of 200 µM nucleotide in the presence or absence of 1 mM Mg^{2+}. The inhibitors VI16832 and DAP, as well as the JAK-speci c inhibitor CMP6, all at concentrations of 40 µM, were included as positive controls. (D) □T_m analyses of JAK2(JH1) nucleotide-binding titrations (0 400 µM nucleotide concentration) in the presence of 1 mM Mg^{2+}. At a 200 µM nucleotide concentration (the concentration used elsewhere in the present study), we could detect ADP, ATP and AMP-PNP binding to JAK2(JH1), consistent with a limit of detection corresponding to K_d values in excess of 200 µM. (E and F) Chemical structures of the promiscuous inhibitors DAP [5] (E) and VI16832 [6,7] (F).

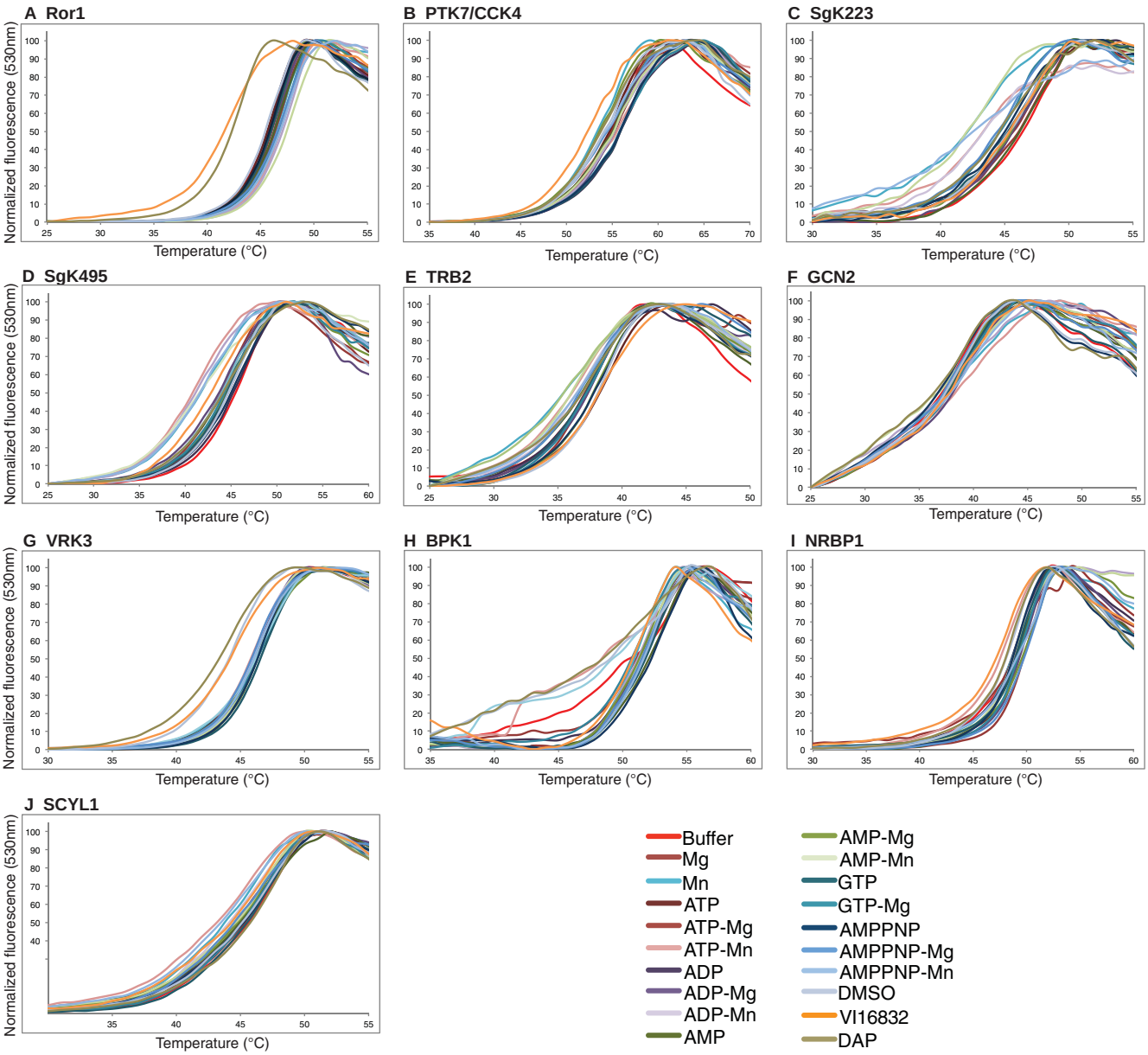

Figure S2 Thermal denaturation curves of Class 1 (no detectable nucleotide or cation binding) pseudokinases

Selected data from this pseudokinase subclass are presented in Figure 2 of the main text. Thermal denaturation curves for Ror1 (A), PTK7/CCK4 (B), SgK223 (C), SgK495 (D), TRB2 (E), GCN2 (F), VRK3 (G), BPK1 (H), NRBP1 (I) and SCYL1 [SCY1-like 1 (*S. cerevisiae*)] (J) did not detectably shift in the presence of nucleotides with or without cations. A colour key for the curve labelling is underneath the curves. Control experiments establishing sensitivity of the assay for nucleotide binding are shown in Figure S1.

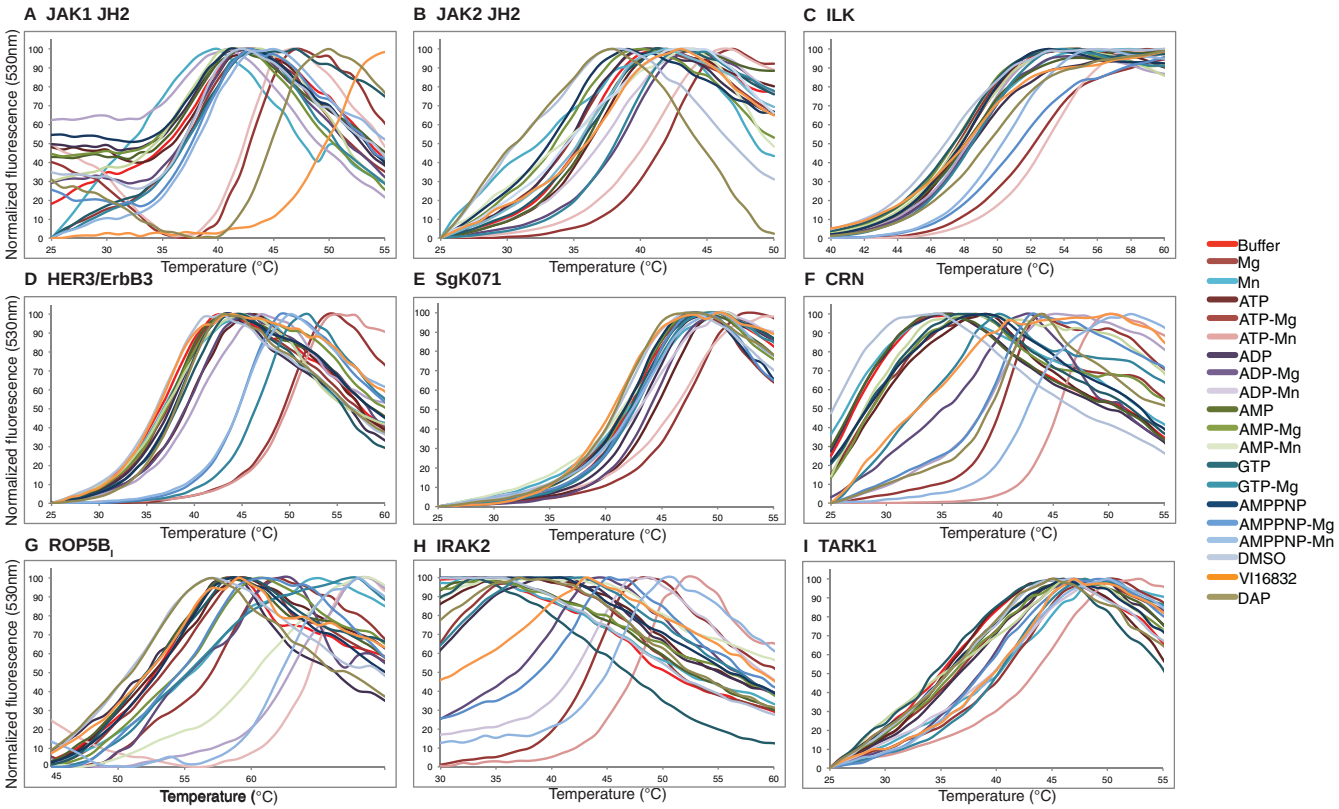

Figure S3 Thermal denaturation curves of Class 4 (nucleotide- and cation-binding) pseudokinases

The ΔT_m values calculated from these curves are presented as histograms in Figure 4 of the main text.

Table S1 Sources of cDNA templates for previously undescribed expression constructs

Gene	GenBank® accession number	Template source	Reference
PTK7/CCK4	U40271	Professor S.-T. Lee	[1]
Ror1	NM_005012	Dr A. Gentile	[2]
IRAK3	NM_007199	Dr William Hahn* via Addgene (plasmid 23627)	[3]
GCN2	BC072637	IMAGE clone 30354433	
ULK4	NM_017886	Dr William Hahn* via Addgene (plasmid 23849)	[3]
IRAK2	NM_001570	Dr William Hahn* via Addgene (plasmid 23412)	[3]
MLKL	NM_152649	Synthetic; DNA2.0 (CA)	
VRK3	BC095449	IMAGE clone 30721912	
TARK1	FJ176293	M.B. Mudgett	[4]
EphB6	NM_004445	Dr William Hahn* via Addgene (plasmid 23931)	[3]
NRBP1	NM_013392	Dr William Hahn* via Addgene (plasmid 23568)	[3]
SCYL1	BC069233	IMAGE clone 6581110	
RYK	BC032275	IMAGE clone 5370100	
CASK	NM_003688	IMAGE clone 40125862	
SgK495/STK40	NM_032017	IMAGE clone 4109652	
TRB2	BC002637	IMAGE clone 3607549	
SgK071/C9orf96	NM_153710	IMAGE clone 5265506	

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