SUPPLEMENTAL

Methods:

Cloning:

Human IP6K2 and 3 were cloned from IMAGE clones using the primers listed below:

IP6K2 5'-GCATGAATTCATGAGCCCAGCCTTCAG-3'
5'- TGAGGCAGTGGGGAGTGAGCGGCCGCAGC-3'
IP6K3 5'-GCATGAATTCATGGTTGTGCAAAACAGCG -3'
5'-GCATGTCGACTCATTCTCCCTCTTGGAT-3'

The primers include the recognition sequences for EcoRI and SalI respectively. The gene was amplified using PCR and cloned into the mammalian vector pEGFP (Clontech). Plasmid DNA was transfected into HeLa cells and stably expressing clones were selected as explained in Materials and Methods.

In vitro kinase assays:

The purification of all enzymes used in the protein kinase panel and the conditions for the *in vitro* assays have been enlisted and reviewed in the references (27-29).

LEGENDS TO SUPPLEMENTAL FIGURES AND TABLES

Figure S1:
A Structure of the compound used in this study
N2-(*m*-(Trifluoromethyl)benzyl), N6-(*p*-nitrobenzyl)purine (TNP)
B and C Molecules structurally related to TNP.
B N2-(*m*-(Trifluoromethyl)benzyl), N6-(*p*-aminobenzyl)purine (TAP) and
C N2-(*m*-(Trifluoromethyl)benzyl), N6-benzyl purine (TBP), see discussion.

Figure S2: Inhibition of $InsP_7$ synthesis by TNP in cells over-expressing IP6K1, 2 and 3.
Bar chart depicting change in $InsP_7$ as a percentage of $InsP_6$ in HeLa cells expressing GFP-IP6K1, GFP-IP6K2 and GFP-IP6K3 in absence or presence of 10 μM TNP. In each case, cells were labeled with [2-^{3}H] inositol and inositol phosphates were extracted as explained under Materials and Methods. Data are average of triplicate experiments and error bars represent the standard deviation of the data.

Figure S3: HPLC analysis of inositol phosphates extracted from yeast cells.
Yeast cells were labeled with [^{3}H]inositol. Cells were treated with DMSO or TNP (10 μM). The soluble inositol phosphates were extracted and separated as explained in Materials and Methods. The HPLC of $InsP_6$ and $InsP_7$ from Wt, Wt+TNP and *kcs1*Δ;*vip1*Δ cells, *vip1*Δ+TNP and *kcs1vip1*ΔΔ cells and *ddp1*Δ, *ddp1*Δ+TNP and *ddp1kcs1*ΔΔ cells.

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Table S1: Effect of TNP on protein kinase activity

The panel of protein kinases was tested for loss of activity against 3 concentrations of the TNP *viz.,* 100 µM, 10 µM and 1µM. Results are presented as kinase activity as a percentage of that in control incubations (average of duplicate determinations). For each concentration, left hand column shows the percentage activity with respect to controls and right hand column shows % variation in the activity within duplicates.

	Concentration of TNP					
Kinase Name	**1 µM**		**10 µM**		**100µM**	
	%	**+/-**	**%**	**+/-**	**%**	**+/-**
MKK1	**92**	0	**110**	2	**131**	0
ERK1	**75**	5	**87**	5	**86**	5
ERK2	**88**	7	**97**	2	**99**	1
JNK1	**81**	3	**86**	2	**82**	4
JNK2	**87**	4	**94**	2	**78**	1
JNK3	**78**	8	**87**	2	**83**	3
P38αMAPK	**143**	6	**139**	2	**139**	1
P38βMAPK	**125**	4	**119**	8	**108**	7
p38γ MAPK	**81**	3	**90**	4	**88**	1
p38δMAPK	**73**	2	**84**	2	**85**	9
ERK8	**88**	3	**99**	6	**113**	0
RSK1	**99**	6	**120**	5	**90**	7
RSK2	**103**	7	**91**	1	**94**	3
PDK1	**83**	1	**85**	5	**91**	2
PKBα	**117**	0	**123**	1	**114**	1
PKBβ	**80**	2	**89**	4	**94**	4
SGK1	**105**	3	**117**	9	**122**	6
S6K1	**119**	9	**110**	1	**101**	2
PKA	**78**	1	**82**	3	**92**	4
ROCK 2	**86**	4	**95**	2	**86**	2
PRK2	**93**	3	**102**	7	**109**	1
PKCα	**80**	5	**87**	0	**83**	3
PKC zeta	**79**	3	**96**	6	**94**	6
PKD1	**86**	6	**93**	3	**92**	7
PKD1	**86**	6	**93**	3	**92**	7
MSK1	**93**	4	**101**	2	**112**	2

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MNK1	**106**	5	**125**	1	**148**	1
MNK2	**81**	4	**94**	7	**85**	9
MAPKAP-K2	**77**	0	**83**	5	**95**	4
MAPKAP-K3	**80**	8	**93**	1	**104**	14
PRAK	**117**	8	**126**	3	**126**	8
CAMKKa	**151**	6	**162**	1	**138**	2
CAMKKb	**89**	2	**95**	4	**101**	2
CAMK1	**77**	3	**93**	0	**90**	6
SmMLCK	**77**	3	**83**	8	**73**	6
PHK	**103**	4	**123**	4	**110**	1
CHK1	**69**	1	**67**	2	**86**	2
CHK2	**77**	2	**100**	0	**95**	7
GSK3b	**96**	2	**128**	3	**120**	7
CDK2-Cyclin A	**110**	6	**108**	2	**111**	1
PLK1	**117**	1	**112**	2	**111**	7
PLK1 (Okadaic Acid)	**94**	2	**107**	6	**99**	2
AURORA B	**42**	2	**67**	7	**69**	1
AURORA C	**91**	3	**101**	13	**106**	1
AMPK	**87**	2	**97**	9	**99**	1
MARK3	**100**	8	**117**	12	**111**	6
BRSK2	**80**	8	**92**	3	**93**	1
MELK	**94**	3	**109**	12	**105**	9
CK1	**96**	1	**89**	1	**90**	8
CK2	**92**	1	**94**	5	**98**	7
DYRK1A	**100**	6	**118**	7	**115**	1
DYRK2	**99**	8	**103**	2	**100**	2
DYRK3	**80**	2	**88**	4	**92**	2
NEK2a	**101**	1	**108**	5	**110**	9
NEK6	**88**	2	**85**	6	**81**	5
NEK7	**71**	8	**83**	6	**87**	9
IKKb	**97**	3	**102**	1	**93**	3
PIM1	**76**	2	**92**	8	**98**	4
PIM2	**103**	2	**107**	1	**107**	6
PIM3	**89**	2	**110**	1	**109**	2

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SRPK1	**133**	2	**137**	1	**131**	1
MST2	**99**	9	**101**	1	**111**	7
EFK2	**82**	1	**90**	2	**95**	2
HIPK2	**99**	1	**100**	1	**104**	2
HIPK3	**96**	1	**100**	8	**103**	3
PAK4	**101**	2	**108**	1	**111**	2
PAK5	**98**	1	**95**	1	**100**	2
PAK6	**94**	5	**94**	1	**90**	7
Src	**77**	2	**83**	9	**87**	6
Lck	**73**	2	**108**	1	**115**	1
CSK	**100**	2	**95**	6	**99**	2

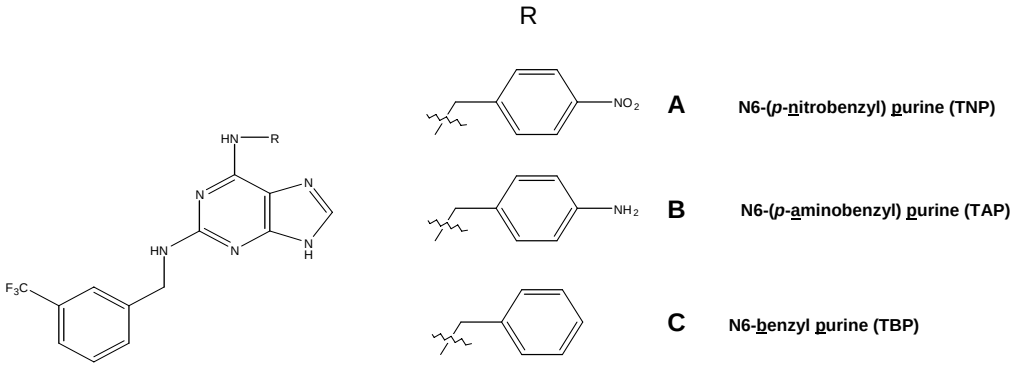

Figure S1: Padmanabhan, U et al.

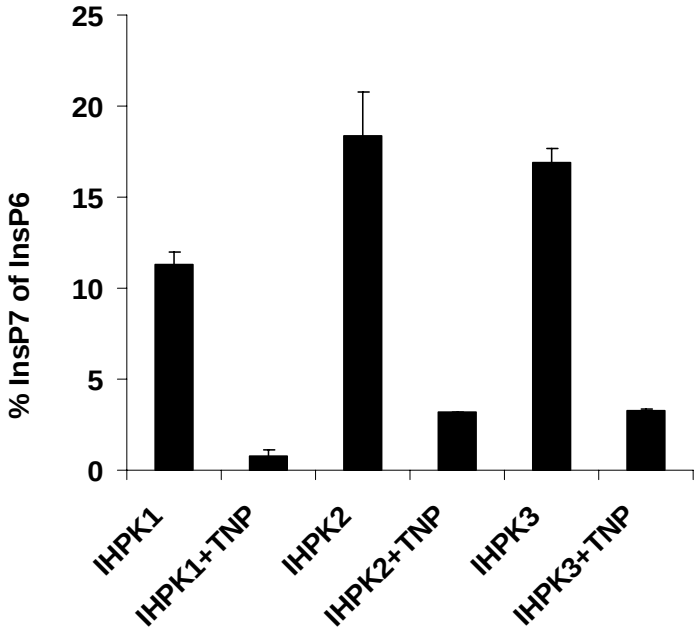

Figure S2: Padmanabhan, U et al.

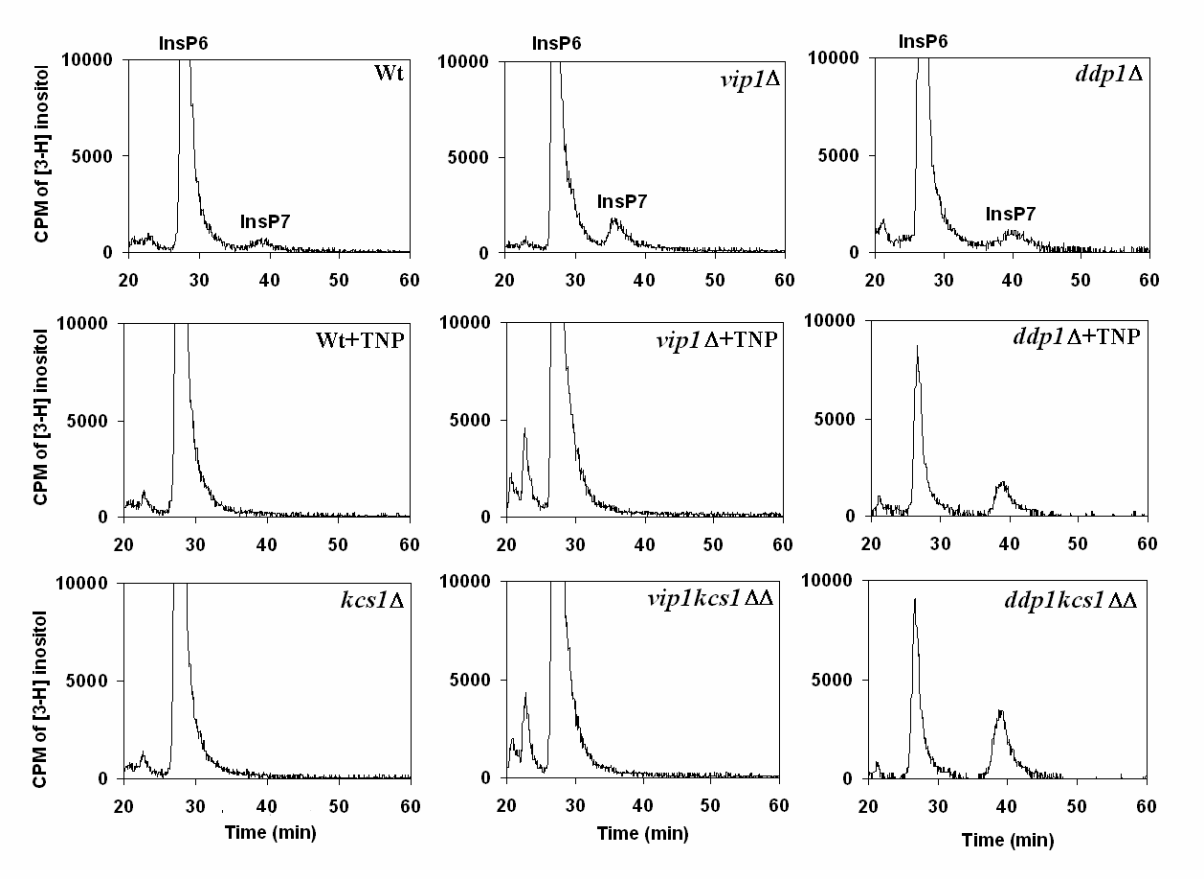

Figure S3: Padmanabhan, U et al.