Supplemental Figure Legends and Figure S1-6

Figure S1, Related to Figure 1. (A) Sequences of identified peptides from Mass spec analysis of the 120 kDa band corresponding to Tti1. (B) Co-IP between GST-IP6K2 and myc-Tti2. (C) Co-IP between myc-IP6K2 and Flag-Tti1. (D) Co-IP between GST-IP6K2 and myc-Tel2.

Figure S2, Related to Figure 2. (A) Flag-Tti1 immunoprecipitation followed by immunobloting using anti-K63 linkage-specific ubiquitin antibody. **(**B) PAGE of IP6 and IP7 showing the purity of synthesized IP7. Gel was stained with toluidine blue. (C-F) Effect of IP6 or IP7 (25 μM each) on CK2 phosphorylation of purified Flag-Tti1, GST-Tti1MC, Flag-Tel2, and the CK2 substrate peptide (RRRADDSDDDDD). Phospho-Tti1 (S828) and phosphor-Tel2 (S487/491) antibodies were used for western analysis to determine CK2 activity. Phosphorylation of the CK2 substrate peptide was assayed using [^{32}P]ATP followed by scintillation counting. (G) Dose-dependent inhibition of CK2 phosphorylation of Gst-Tti1MC by purified recombinant His_6-hNopp140. Reaction conditions were: 20 mM $MgCl_2$ (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 200 μM ATP, 30 °C, 15 min. (H) [^{3}H]IP6 binding to myc-tagged CK2α, CKβ, and Tel2 immunoprecipitated from HEK293 cells. (I) The IP7 binding sites in CK2. Location of K77, R80 and R155 in the 3D structure of CK2α-IP6 complex (PDB id: 3W8L)

Figure S3, **Related to Figure 3.** (A) ShRNA knockdown of IP6K2 decreases the levels of DNA-PKcs and ATM. (B) Reverse-transcription PCR detection of mRNA level of DNA-PKcs and ATM, with β-actin and HPRT1 used as controls. (C) Relative levels of DNA-PKcs and ATM in Tti1 wildtype and S828A rescued HCT116 cells following CHX treatment. (D) Leupeptin (100 μM), but not Mg132 (20 μM) or z-VAD-fmk (100 μM), inhibits DNA-PKcs degradation in IP6K2$^{-/-}$ cells.

Figure S4. Related to Figure 4. (A) Western-blot analysis of wild-type and IP6K2$^{-/-}$ cells with/without 5-FU treatment. **(**B) p21 protein levels following treatment with Nu7026 (5 μM) for 2 h. (C) Western-blot analysis of p53 and various forms of phospho-p53 in wild-type, IP6K2$^{-/-}$, and p53$^{-/-}$ HCT116 cells after treatment with 5-FU (400 μM) for 16 h. (D) ShRNA knockdown of IP6K2 in U2OS cells attenuates p53 induction and p53 S15 phosphorylation. (E) p53 induction and S15 phosphorylation in Tti1 depleted HCT116 cells that were rescued with wildtype TTi1 or Tti1S828A. (F) Viability of wild-type and IP6K2$^{-/-}$ HCT116 cells after drug treatment, measured by MTT assay. (G) Knockdown of IP6K2 increases cell viability as measured by MMT assay. (H) IP6K2 knockdown by ShRNA does not improve viability of p53$^{-/-}$ HCT116 cells upon 5-FU treatment. (I) ATM inhibitor Ku55933 does not differentially influence 5-FU induced cell death of wild-type and IP6K2$^{-/-}$ cells.

Figure S5, Related to Figure 1. (A) Comparison of cellular IP7 levels in wild-type (Black circle) and Ip6k2$^{-/-}$ (While circle) MEFs. Experiment was performed three times with similar results. (B) Western-blot analysis of DNA-PKcs expression level in mouse neuroblastoma N2A cells, MEFs, and human neuroblastoma SH-SY5Y cells.

Figure S6, Related to Figure 6. (A) Distribution of IP6K2 and DNA-PKcs in model organisms. (B) Phylogenetic analysis of IP6K sequence alignments. *Hs: homo sapiens* ; *Mm: Mus musculus*;

Gg: Gallus gallus; *Xl: Xenopus laevis*; *Dr: Danio reiro*; *Dm: Drosophila melanogaster*; *Ce. Caenorhabditis elegans*; *Sc. Saccharomyces cerevisiae*

Supplemental Figures S1-6

Figure S1

A

Peptide	Mascot ion score	modifications	start	stop
(K)QGHSIVVSSLK(I)	32.3		227	237
(R)VAELMVYR(E)	38.7		268	275
(K)LTILIK(K)	22.9		287	292
(K)INFVLNSVAHLQR(L)	66.6		415	427
(K)QAAMILNELVTGAAGLEVEDLHEK(H)	110.3	Deamidation (+1)	521	544
(R)HLALHPHTPK(V)	31.8		708	717
(K)NQLLPLAHQAWPSLVHR(L)	34.1		901	917
(K)LAGSLVTQAPISAR(A)	68.6		956	969

gi|24307961 (100%), 122,071.9 Da
TELO2-interacting protein 1 homolog [Homo sapiens]
8 unique peptides, 11 unique spectra, 19 total spectra, 103/1089 amino acids (9% coverage)

MAVFDTPEEA FGVLRPVCVQ LTKTQTVENV EHLQTRLQAV SDSALQELQQ YILFPLRFTL
KTPGPKRERL IQSVVECLTF VLSSTCVKEQ ELLQELFSEL SACLYSPSSQ KPAAVSEELK
LAVIQGLSTL MHSAYGDIIL TFYEPSILPR LGFAVSLLLG LAEQEKSKQI KIAALKCLQV
LLLQCDCQDH PRSLDELEQK QLGDLFASFL PGISTALTRL ITGDFKQGHS IVVSSLKIFY
KTVSFIMADE QLKRISKVQA KPAVEHRVAE LMVYREADWV KKTGDKLTIL IKKIIECVSV
HPHWKVRLEL VELVEDLLLK CSQSLVECAG PLLKALVGLV NDESPEIQAQ CNKVLRHFAD
QKVVVGNKAL ADILSESLHS LATSLPRLMN SQDDQGKFST LSLLLGYLKL LGPKINFVLN
SVAHLQRLSK ALIQVLELDV ADIKIVEERR WNSDDLNASP KTSATQPWNR IQRRYFRFFT
DERIFMLLRQ VCQLLGYYGN LYLLVDHFME LYHQSVVYRK QAAMILNELV TGAAGLEVED
LHEKHIKTNP EELREIVTSI LEEYTSQENW YLVTCLETEE MGEELMMEHP GLQAITSGEH
TCQVTSFLAF SKPSPTICSM NSNIWQICIQ LEGIGQFAYA LGKDFCLLLM SALYPVLEKA
GDQTLLISQV ATSTMMDVCR ACGYDSLQHL INQNSDYLVN GISLNLRHLA LHPHTPKVLE
VMLRNSDANL LPLVADVVQD VLATLDQFYD KRAASFVSVL HALMAALAQW FPDTGNLGHL
QEQSLGEEGS HLNQRPAALE KSTTTAEDIE QFLLNYLKEK DVADGNVSDF DNEEEEQSVP
PKVDENDTRP DVEPPLPLQI QIAMDVMERC IHLLSDKNLQ IRLKVLDVLD LCVVVLQSHK
NQLLPLAHQA WPSLVHRLTR DAPLAVLRAF KVLRTLGSKC GDFLRSRFCK DVLPKLAGSL
VTQAPISARA GPVYSHTLAF KLQLAVLQGL GPLCERLDLG EGDLNKVADA CLIYLSVKQP
VKLQEAARSV FLHLMKVDPD STWFLLNELY CPVQFTPPHP SLHPVQLHGA SGQQNPYTTN
VLQLLKELQ

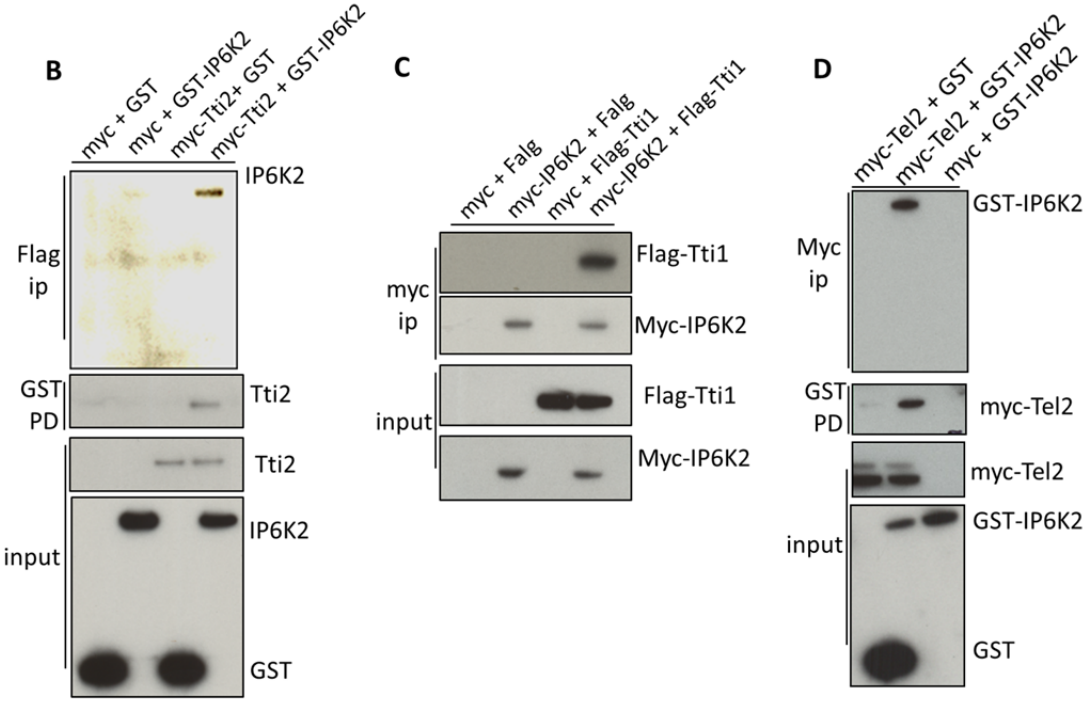


Figure S2

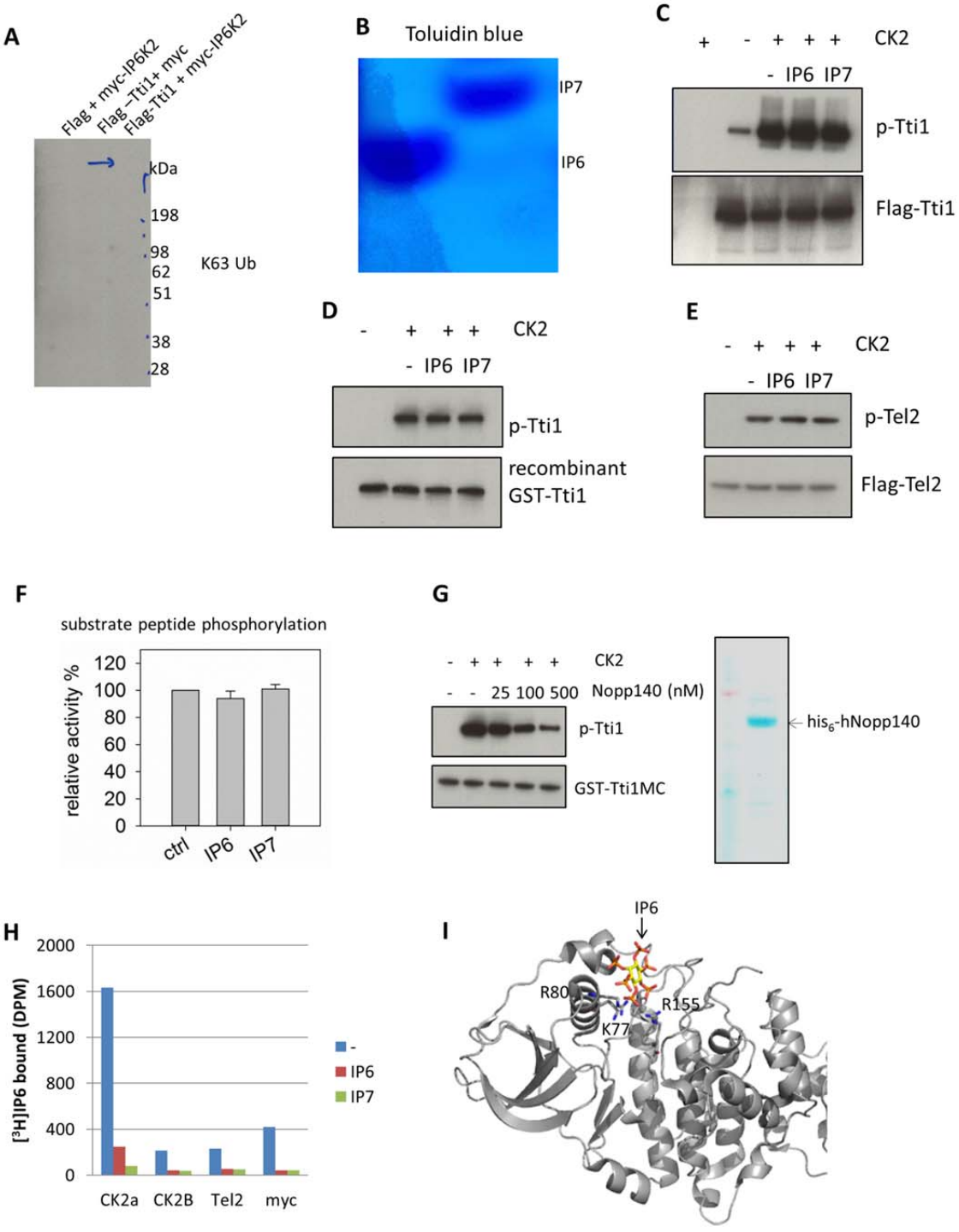

A
Flag + myc-IP6K2
Flag–Tti1+ myc
Flag-Tti1 + myc-IP6K2
kDa
198
98
62
51
38
28
K63 Ub
B
Toluidin blue
IP7
IP6
C
+ - + + + CK2
- IP6 IP7
p-Tti1
Flag-Tti1
D
- + + + CK2
- IP6 IP7
p-Tti1
recombinant
GST-Tti1
E
- + + + CK2
- IP6 IP7
p-Tel2
Flag-Tel2
F
substrate peptide phosphorylation
relative activity %
120
100
80
60
40
20
0
ctrl
IP6
IP7
G
- + + + + CK2
- - 25 100 500 Nopp140 (nM)
p-Tti1
GST-Tti1MC
his6-hNopp140
H
[3H]IP6 bound (DPM)
2000
1600
1200
800
400
0
CK2a
CK2B
Tel2
myc
-
IP6
IP7
I
IP6
R80
R155
K77

Figure S3

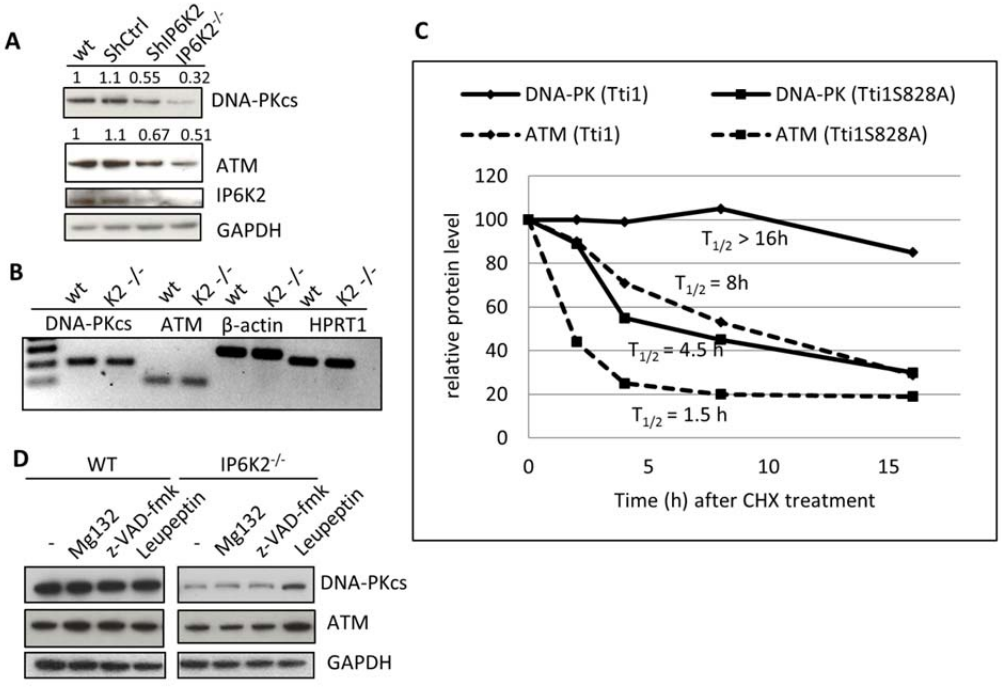

A
wt
ShCtrl
ShIP6K2
IP6K2-/-
1 1.1 0.55 0.32
DNA-PKcs
1 1.1 0.67 0.51
ATM
IP6K2
GAPDH
B
wt K2-/- wt K2-/- wt K2-/- wt K2-/-
DNA-PKcs ATM β-actin HPRT1
C
DNA-PK (Tti1)
DNA-PK (Tti1S828A)
ATM (Tti1)
ATM (Tti1S828A)
relative protein level
120
100
80
60
40
20
0
T1/2 > 16h
T1/2 = 8h
T1/2 = 4.5 h
T1/2 = 1.5 h
0 5 10 15
Time (h) after CHX treatment
D
WT
IP6K2-/-
- Mg132 z-VAD-fmk Leupeptin
- Mg132 z-VAD-fmk Leupeptin
DNA-PKcs
ATM
GAPDH

Figure S4

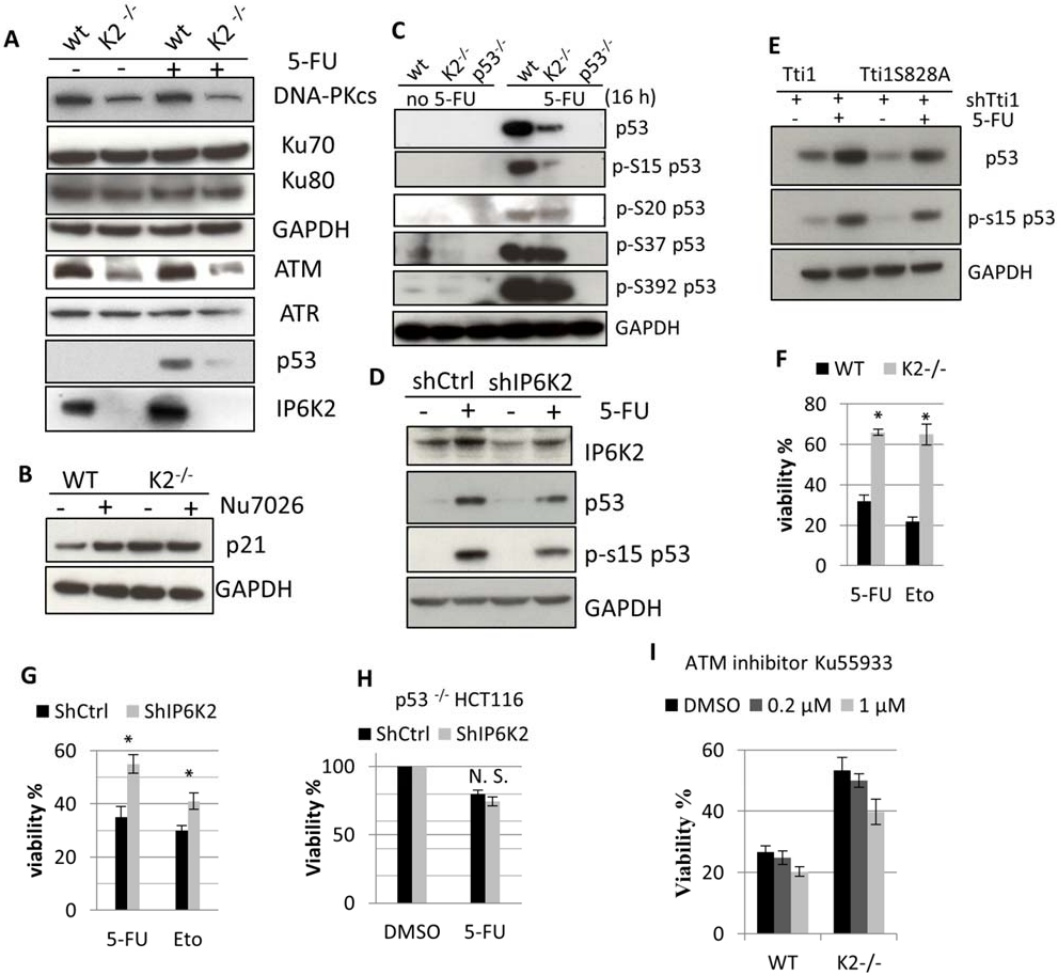

A
wt
K2-/-
wt
K2-/-
- - + +
5-FU
DNA-PKcs
Ku70
Ku80
GAPDH
ATM
ATR
p53
IP6K2
B
WT
K2-/-
- + - +
Nu7026
p21
GAPDH
C
wt
K2-/-
p53-/-
wt
K2-/-
p53-/-
no 5-FU
5-FU
(16 h)
p53
p-S15 p53
p-S20 p53
p-S37 p53
p-S392 p53
GAPDH
D
shCtrl
shIP6K2
- + - +
5-FU
IP6K2
p53
p-s15 p53
GAPDH
E
Tti1
Tti1S828A
+ + + +
- + - +
shTti1
5-FU
p53
p-s15 p53
GAPDH
F
WT
K2-/-
viability %
80
60
40
20
0
*
*
5-FU
Eto
G
ShCtrl
ShIP6K2
viability %
60
40
20
0
*
*
5-FU
Eto
H
p53 -/- HCT116
ShCtrl
ShIP6K2
Viability %
100
50
0
N. S.
DMSO
5-FU
I
ATM inhibitor Ku55933
DMSO
0.2 μM
1 μM
Viability %
60
40
20
0
WT
K2-/-

Figure S5

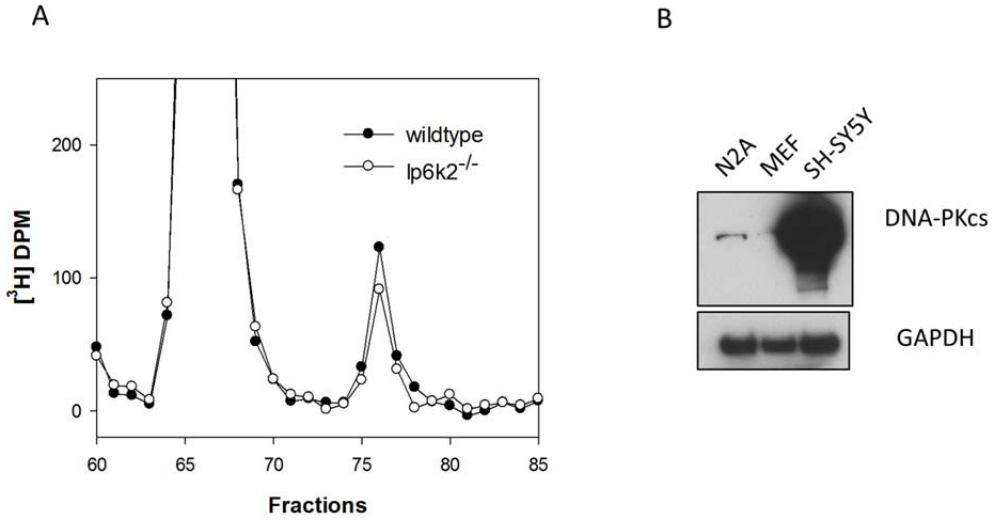
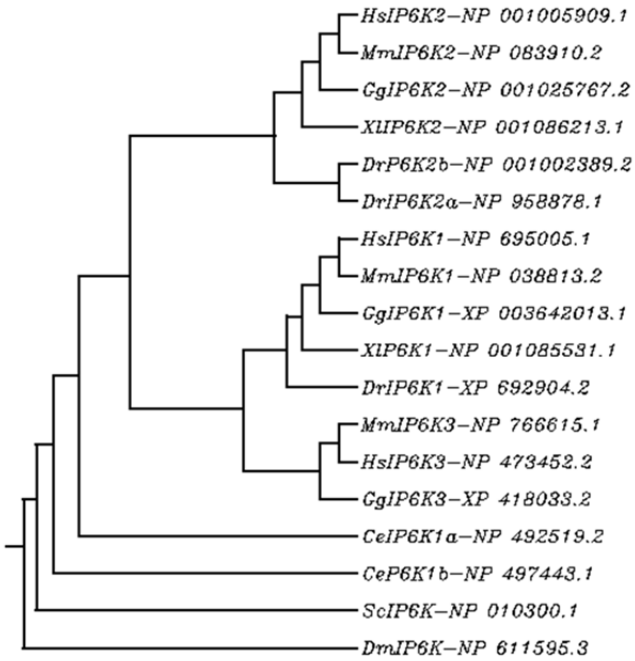

A
B
wildtype
Ip6k2-/-
[3H] DPM
Fractions
0
100
200
60
65
70
75
80
85
N2A
MEF
SH-SY5Y
DNA-PKcs
GAPDH

Figure S6

A

Organism	IP6K2	DNA-PKcs
Yeast	-	-
C elegans	-	-
Drosophila	-	-
Zebrafish	+	+
Xenopus	+	+
Mouse	+	+
Human	+	+

B



Supplemental Experimental Procedures

The supplemental experimental procedures contain detailed description of each section of the Experimental Procedures.

Reagents

5-FU, etoposide, cycloheximide, TNP, Mg132, leupeptin, Nu7026, novobiocin, IP6, IS6, Neocarzinostatin were from Sigma; Ku55933, TBB were from Tocris Biosciences. z-VAD-fmk was from Promega. IP7 and PCP-IP5 were synthesized as previously described (Wu et al., 2013; Zhang et al., 2009), the purity of IP7 was analyzed by PAGE as previously described (Losito et al., 2009) . Silver-Stain kit was from Pierce.

Antibodies and plasmids

The primary antibodies used were: DNA-PKcs (Abcam, Cell Signaling), ATM (ECM Biosciences), , IP6K2, p21, ATR, p-DNA-Pkcs (T2609) (Santa Cruz), p-ATM (S1981) (Epitomics), PUMA, MDM2 and GAPDH (Calbiochem), p53, phospho-p53 (S15, S20, S37, S392), Ku70, Ku80, mTOR, SMG1, TRRAP, p-Chk2, p-Chk1, p-H2AX, p-KAP1 (Cell Signaling), Noxa (Imgenex), Tel2 (Proteintech Group), Tti1 (Bethyl labs), β-tubulin (Hybridoma bank), Flag and GST (sigma), Myc (Roche). The secondary antibodies used were: Anti-rabbit IgG-HRP, anti-mouse IgG-HRP (GE healthcare), light chain specific anti-rabbit IgG-HRP, light chain specific anti-mouse IgG-HRP, anti-goat IgG-HRP (cell signaling).

Plasmids p3xFLAG-CMV10-hTel2, p3xFLAG-CMV10-hTti1, pLPC MYC hTel2, pGEX-CK2α′, Pet28-CK2β, pMD2.G, pAX2 and PG13-luc were from Addgenes. pDNR-dual vector encoding His-tagged hNopp140 was from DF/HCC DNA resource core. IP6K2, full-length or the C-terminal domain (aa 212-426), were cloned into pGEX6p2 vector at SalI/NotI restriction sites. Tti2 gene, purchased from Source Biosciences, was cloned into pCMV-myc at SalI/NotI restriction sites. The N-terminus (aa 1-460), Middle (aa 460-825), and C-terminus (aa

826-1089) portions of Tti1 were cloned into pCMV-myc at SalI/NotI restriction sites. Plasmids encoding GST-CK2α, GST-CK2β were generated by cloning into pGEX6p2 at SalI/NotI restriction sites. The shRNA construct targeting Tti1 was as described (Hurov et al., 2010), and the siRNA-resistant Tti1-Flag expression vector was constructed after 7 silent mutations in the targeting region. All point mutants were made using a Site-directed mutagenesis kit (Stratagene).

Cloning and tandem affinity purification

The STAP-IP6K plasmids used for tandem-affinity purification were generated by cloning IP6Ks into the pcDNA3.1-STAP vector using XhoI/BamHI restriction sites. Each of the constructs, which contain tandem streptavidin binding peptide and Protein A at the C-terminus, were then transfected in to HEK293 cells in five 10 cm dishes. Two days after transfection, each plate was lysed in 1ml of lysis buffer: 50 mM Tris (pH7.4), 125 mM NaCl, 2 mM EDTA, 0.2% NP-40, with protease and phosphatase inhibitors. After rotating at 4 °C for 15 min, cell lysates were cleared of debris by centrifuging for 30 min. The supernatant was incubated with IgG beads (GE Healthcare) for 2 h at 4°C with gentle rotation. The beads were spun down and washed twice in lysis buffer followed by two washes in TEV protease cleavage buffer: 100 mM Tris (pH 7.4), 100 mM NaCl, 0.2% NP-40%. The beads were incubated with TEV protease (Invitrogen) overnight at 4°C. After TEV reaction, the beads were spun down and the supernatant was incubated with streptavidin beads (Pierce) at 4°C for 2 h with gentle rotation. The beads were spun down, washed four times with TEV protease cleavage buffer and the purified complexes were eluted twice using 5 mM D-biotin at 4°C, for 30 min. The eluted sample was then denatured and fractionated by SDS-PAGE, bands were cut and sent for LC-MS/MS analysis at the Proteomics Core Facility (Johns Hopkins).

Immunoblotting and Immunoprecipitation

Cells were disrupted in lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% Triton X-100, 5% glycerol) supplemented with a Complete Protease Inhibitor tablet (Roche) and a phosphatase inhibitor tablet (Roche) on ice followed by passing through a 26 gauge syringe for 10 times. After centrifugation at 13, 000 rpm for 15 min, lysates were boiled in 1× NuPAGE lithium dodecyl sulfate sample buffer and subjected to SDS-PAGE using the Novex system (Invitrogen) following manufacturer's instructions, transferred to PVDF membranes (Millipore), blocked in 5% nonfat milk in Tris-buffered saline/0.1% Tween-20 for 1 h at room temperature, incubated with primary antibodies overnight at 4 °C, and with HRP-conjugated secondary for 1 h. Blots were developed using ECL reagents (Millipore) and quantified using ImageJ.

Tagged IP6K2 and Tel2/Tti1 co-IP employed lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 5% glycerol. The endogenous co-IP between IP6K2 and Tel2/Tti1, used the same buffer but with 0.5% Triton X-100. The antibody used for IP6K2 immunoprecipitation was generated in our lab. IP was done overnight after pre-clearing with IgG plus Protein A/G agarose (Calbiochem) for 2 h. IP of Myc- or Flag-tagged proteins employed EZview anti-Myc/Flag Affinity Gel (Sigma). Following five wash cycles, IP samples were boiled in 2 x LDS buffer and used for immunoblotting.

For direct binding between purified Tti1 and recombinant IP6K2, Flag-Tti1 and Flag-Tel2 were overexpressed in HEK293 cells, purified using EZview anti-Flag Affinity Gel, washed extensively with RIPA buffer supplemented with 600 mM NaCl, reconstituted in normal lysis buffer (1% Triton X-100), and incubated with purified recombinant GST-IP6K2$_{212\text{-}426}$. In the reverse experiment, recombinant IP6K2 purified after GST cleavage was incubated with GST-Tti1MC followed by pulldown using gluthathione beads.

Cell culture and transfection conditions.

HEK293 cells (ATCC) were cultured in DMEM supplemented with 10% FBS. Transient transfections were carried out using Polyfect (Qiagen). U2OS and HCT116 cells were cultured following standard procedures (Koldobskiy et al., 2010). Transient transfections were carried out using Lipofectamine 2000 (Invitrogen). The $p53^{-/-}$, $IP6K2^{-/-}$ and wild-type HCT116 cell lines used were described before (Koldobskiy et al., 2010). The U2OS Tet-inducible IP6K2 cell line was employed as described (Koldobskiy et al., 2010), and the IP6K2-K222A cell line was generated using the same method. For IP6K inhibition studies, unless otherwise stated, TNP (Sigma) was used at 10 μM. Cell viability was assessed in six-well plates by MTT assay, as described previously (Chakraborty et al., 2011b; Koldobskiy et al., 2010). Each experiment was repeated at least three times. For TUNEL assay, cells were plated on to glass-bottom 6-well plates, treated with Etoposide (40 μM) for 24 h. Cells were then processed and stained using the *In Situ* Cell Death Detection Kit, Fluorescein (Roche). Images were acquired using a Zeiss LSM 510 Meta Confocal Microscope at 10x magnification.

For IP6K2 knockdown studies, five shRNA constructs from Sigma (NM_016291.2) were tested. Results obtained from the best construct (clone ID: 1682s21c1) are presented, with scramble shRNA as control. Transfection was done for 48 h, before the addition of indicated drugs overnight.

Luciferase reporter assay

Plasmid PG13-luc (el-Deiry et al., 1993), from Addgene, was transfected into WT and $K2^{-/-}$ HCT116 cells for 28 h. Transfected cells were treated with 5-FU (400 μM) for 16 h and lysed for luciferase assay using the Luciferase Assay System (Promega). Luminescence was measured using SpectraMax M3 (Molecular Devices). For luciferase assay in the shRNA knock-down preparations, scrambled or IP6K2 shRNA were transfected in to HCT116 cells, selected

with puromycin for 10 d to obtain stably transfected cells. PG13-luc was then transfected and assayed as above.

In vitro protein kinase assay

To determine dsDNA dependent kinase activity for p53 S15-phosphorylation, wild-type and K2-/- HCT116 whole cell lysates were prepared as described (Achari and Lees-Miller, 2000). The assay employed the SignaTECT DNA-dependent Protein Kinase Assay System (Promega). Reactions were quenched at 10 mins. Radioactivity was measured by a scintillation counter.

To determine effects of hNopp140 and IP6/IP7 on CK2 kinase activity, CK2 holoenzyme (NEB) was incubated and hNopp140 and/or IP6/IP7 for 30 min in reaction buffer, followed by the addition of purified Tti1 and Tel2 substrates bound on Flag or GST beads. Reactions were allowed to proceed for various time periods with gentle agitation (300 g) at 30 °C in a thermomixer (Eppendorf), before being stopped using LDS lysis buffer and heating. The extent of phosphorylation was determined by western-blotting using phospho-specific antibodies.

Expression and purification of recombinant protein from *E. coli*

For GST-tagged proteins constructs, the plasmids were transformed into BL21 (DE3). Initial purification used Glutathione Sepharose 4B (GE healthcare). The beads were then washed and either incubated with 15 mM reduced glutathione (Sigma) for elution, or with Precission Protease to leave off the GST tag. The supernatants were collected and analyzed by SDS-PAGE, followed by staining using SimplyBlue SafeStain (Invitrogen). For His_6-tagged proteins, purification was done as previously described (Rao et al., 2010). Holo CK2 was prepared according to Turowec et al (Turowec et al., 2010).

[^{3}H] IP6 binding

Recombinant GST-tagged CK2 proteins on glutathione agarose beads were incubated with [^{3}H] IP6 (Perkin Elmer, specific activity: 10-20 Ci/mmol) overnight in binding buffer containing 40 mM Tris (pH 8.0), 150 mM NaCl, 1mM $MgCl_2$, 4% glycerol and 0.1% Triton. Beads were then washed 5 times in the same buffer. Radioactivity was measured by a scintillation counter. For competition assays, various concentrations of cold IP6, IP7 were added during incubation. The IC_{50} was determined by fitting the data with a one site competition Equation (GraphPad):

Reverse Transcription PCR

RNA was extracted using the RNeasy Plus Mini Kit (Qiagen). Two µg of RNA were used for reverse transcription with the iScript Reverse Transcription Supermix (Bio-Rad). PCR employed 2XPCR master polymerase (Fermentas) for 30-33 cycles. The primer sequences were: ATM-F 5'-AGCTGTCTCCATTACTGATGATACT-3', ATM-R 5'-TCCGTAAGGCATCGTAACACATA-3'; DNA-PKcs-F 5'-CATGGAAGAAGATCCCCAGA-3', DNA-PKcs-R 5'-TGGGCACACCACTTTAACAA-3'; HPRT1-F 5'-TTGCGACCTTGACCATCTTTG-3', HPRT1-R 5'-CTTTGCTGACCTGCTGGATTAC-3'; β-actin-F 5'-TCACCCACACTGTGCCCATCTACGA-3', β-actin-R: 5'-CAGCGGAACCGCTCATTGCCAATGG-3'

Generation of IP6K2 Knockout Mice, MEFs and B cell prepration.

IP6K2$^{flox/flox}$ mice were generated using a targeting construct based on the sequence of the C57BL/6 strain *Ip6k2* gene (Ensembl gene ID: ENSMUSG00000032599). Mouse Ip6k2 contains 6 exons. Exon 6 is composed of 794 bp, of which the first 501 bp encodes the C-terminal catalytic domain of IP6K2 (amino acids 282–448), and the remaining 295 bp gives rise to the 3′ untranslated region (UTR). A LoxP site was inserted between exons 5 and 6. A phosphoglycerine kinase (PGK) Neo cassette flanked by FLP recombinase target (FRT)

sequences and another LoxP site was inserted downstream of exon 6. The targeting vector was electroporated into 129SV/J ES cells, and neomycin-resistant ES cells were microinjected into C57BL/6 blastocysts and implanted into pesudo-pregnant female mice. The resulting chimeric mice ($Ip6k2^{wt/flox}$) were interbred to generate $Ip6k2^{flox/flox}$ mice, which was then crossed with knockin C57BL/6 mice carrying Cre recombinase driven by a PGK promoter to generate mice carrying the IP6K2 knockout alleles and Cre recombinase ($IP6K2^{-/-}$/*Cre*). Cre-mediated loxP recombination generates transcripts that lack exon 6 (including the 3′UTR) and are unstable, resulting in loss of expression. These mice were crossed with C57BL/6 mice to remove *Cre* ($IP6K2^{+/-}$), $IP6K2^{+/-}$ mice were subsequently backcrossed to C57BL/6 for eight generations. Mice were housed in a 12-h light/12-h dark cycle, at an ambient temperature of 22°C, and fed standard rodent chow. Experimental protocols were approved by the Johns Hopkins University Animal Care and Use Committee. Primary MEFs were prepared from Het/Het paired pups as previously described (Xu et al., 2013). Radiolabeling with [^{3}H]inostiol and inositol phosphate detection were done as previously described (Luo et al., 2003). B cells were prepared from 8-week old mice. Spleens were mashed in a cell strainer (70 μm) in the presence of ACK lysis buffer to removed red blood cells. Lymphocytes were then incubated with CD43 Microbeads (Milteny Biotech) to counter-select resting B cells. Equal amounts of B cells (2-2.5 x 10^7 per spleen) were isolated from wildtype and $Ip6k2^{-/-}$ mice. To examine ionizing radiation-mediated descreases in cell survival, cells were irradiated (2 and 5 Gray) using ^{137}Cs as radiation source. Cell viability was measured by the trypan blue exclusion method as previously described (Vandiver et al., 2013).

Statistical analysis

All results are presented as the mean and standard error of at least three independent experiments. Statistical significance was calculated by Student's t-test (**$p < 0.05$, *$p < 0.01$).

Achari, Y., and Lees-Miller, S.P. (2000). Detection of DNA-dependent protein kinase in extracts from human and rodent cells. Methods Mol Biol *99*, 85-97.
Losito, O., Szijgyarto, Z., Resnick, A.C., and Saiardi, A. (2009). Inositol pyrophosphates and their unique metabolic complexity: analysis by gel electrophoresis. PLoS One *4*, e5580.
Rao, F., Qi, Y., Murugan, E., Pasunooti, S., and Ji, Q. (2010). 2',3'-cAMP hydrolysis by metal-dependent phosphodiesterases containing DHH, EAL, and HD domains is non-specific: Implications for PDE screening. Biochem Biophys Res Commun *398*, 500-505.
Turowec, J.P., Duncan, J.S., French, A.C., Gyenis, L., St Denis, N.A., Vilk, G., and Litchfield, D.W. (2010). Protein kinase CK2 is a constitutively active enzyme that promotes cell survival: strategies to identify CK2 substrates and manipulate its activity in mammalian cells. Methods Enzymol *484*, 471-493.
Vandiver, M.S., Paul, B.D., Xu, R., Karuppagounder, S., Rao, F., Snowman, A.M., Ko, H.S., Lee, Y.I., Dawson, V.L., Dawson, T.M., *et al.* (2013). Sulfhydration mediates neuroprotective actions of parkin. Nat Commun *4*, 1626.
Xu, R., Sen, N., Paul, B.D., Snowman, A.M., Rao, F., Vandiver, M.S., Xu, J., and Snyder, S.H. (2013). Inositol polyphosphate multikinase is a coactivator of p53-mediated transcription and cell death. Sci Signal *6*, ra22.
Zhang, H., Thompson, J., and Prestwich, G.D. (2009). A scalable synthesis of the IP7 isomer, 5-PP-Ins(1,2,3,4,6)P5. Org Lett *11*, 1551-1554.