Supplementary Online Material

PER2 Differentially Regulates Clock Phosphorylation versus Transcription by Reciprocal Switching of CK1ε Activity

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Supplemental Figures

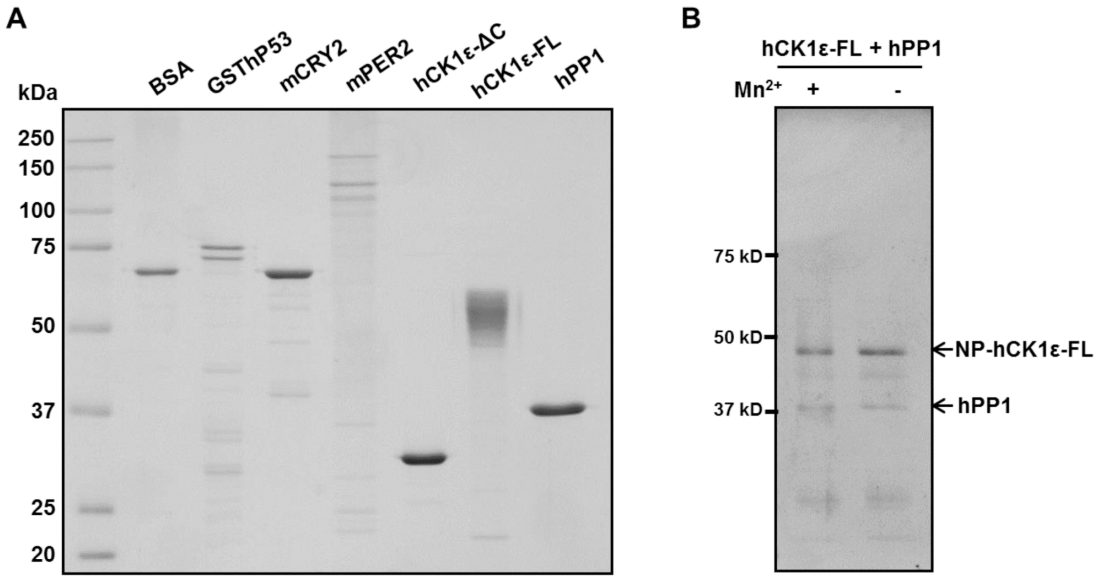

Figure S1. Proteins used in this study. (A) Proteins used in this study are resolved by 10% SDS-PAGE and Coomassie Brilliant Blue (CBB) staining. The hCK1ε-FL band is smeared due to autophosphorylation. **(B)** hCK1ε-FL can be converted to a single band by dephosphorylation with purified hPP1 human phosphatase and resolved by 10% SDS-PAGE. Note that the mobility shift of hCK1ε-FL was centered around the 50 kDa protein marker before hPP1 treatment (panel A), but converts to a single band below 50 kDa after hPP1 treatment (panel B). The dephosphorylation of hCK1ε-FL by hPP1 is Mn^{2+} independent, but requires Mg^{2+}.

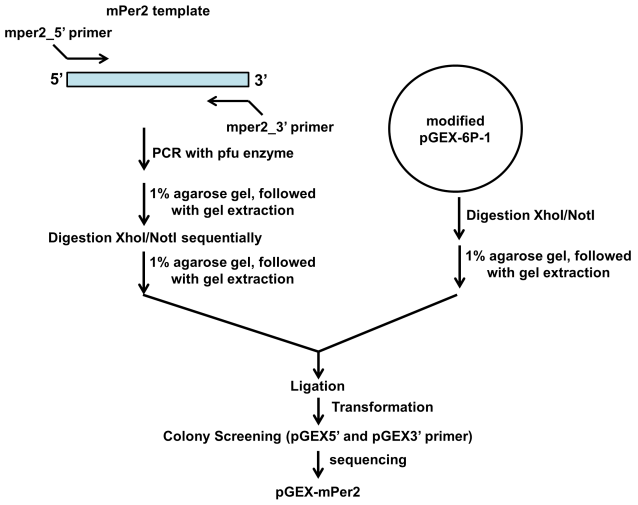

Figure S2. Procedure for cloning pGEX-mPer2.

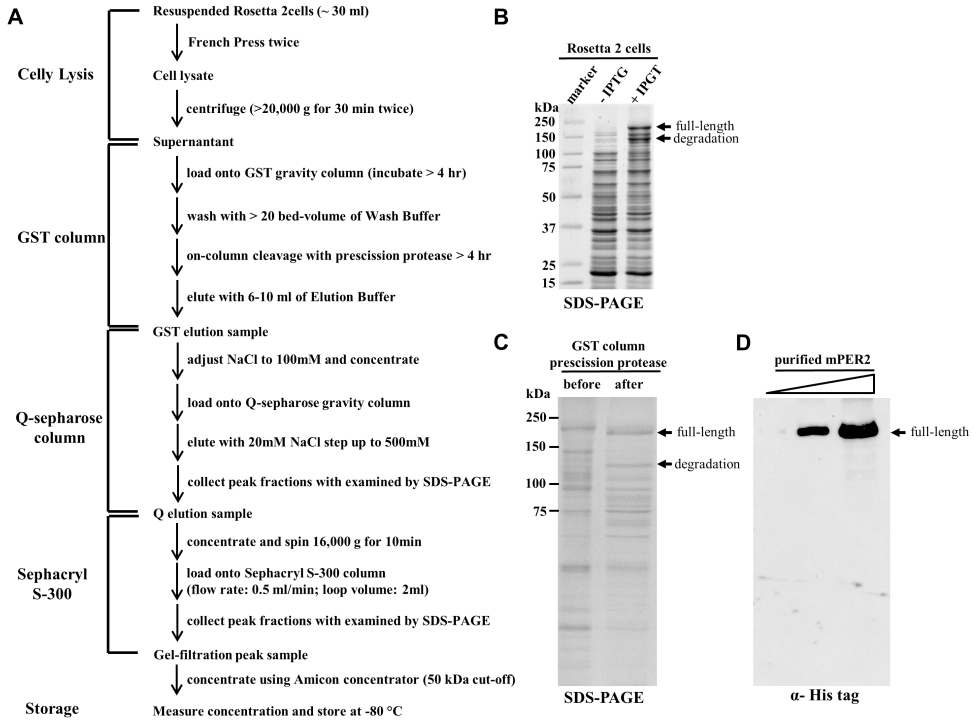

Figure S3. mPER2 purification process and confirmation of full-length mPER2. (A) Diagram for mPER2 purification with three steps, GST affinity, Q anion ion exchange, and S-300 gel-filtration. **(B)** The expression of GST-mPER2 in Rosetta2 cells in the presence of 0.5 mM IPTG. A degraded GST-mPER2 product is present during the expression process. **(C)** The full-length-mPER2 and degraded-mPER2 products after prescission protease cleavage on GST column. **(D)** Immunoblot to identify the full-length mPER2 by using anti-His serum to detect mPER2-His6. A CBB-stained SDS-PAGE gel of the final purified product is shown in Figure S1.

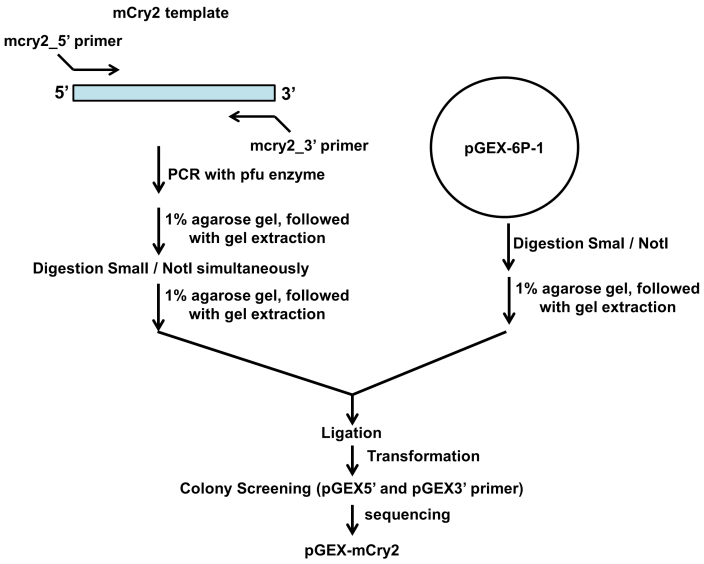

Figure S4. Procedure for cloning pGEX-mCry2.

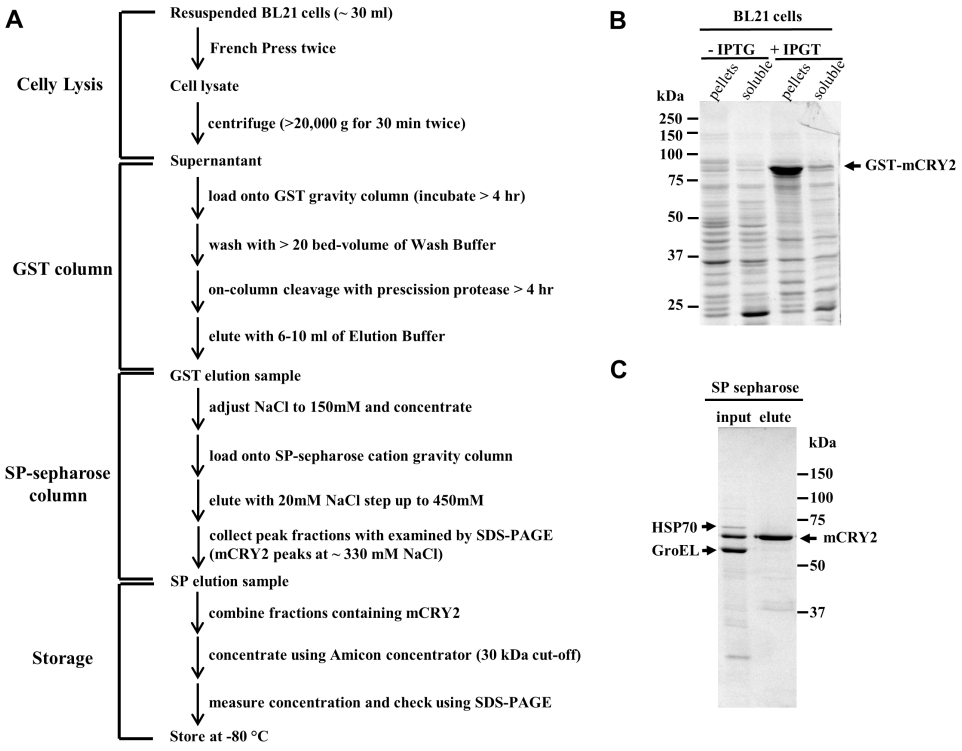

Figure S5. mCRY2 purification process. (A) Diagram for mCRY2 purification by two steps, GST affinity and SP cation ion exchange. **(B)** The expression of GST-mCRY2 in BL21 cells in the presence of 0.2 mM IPTG. A significant portion of the expressed GST-mCRY2 is insoluble ("pellets"). **(C)** SP sepharose cation ion exchange removes the HSP70 and GroEL contaminants from the mCRY2 after the GST affinity preparation (CBB-stained SDS-PAGE gel band).

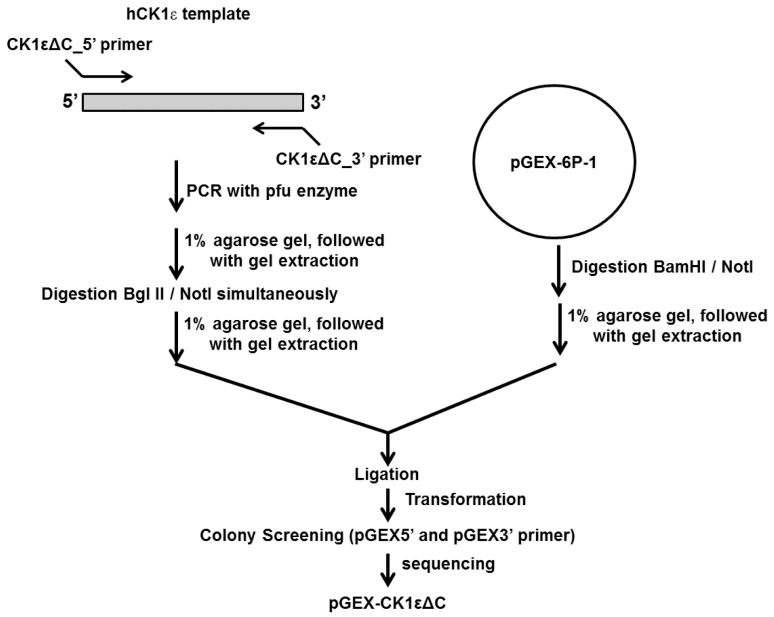

Figure S6. Procedure for cloning pGEX-CK1εΔC.

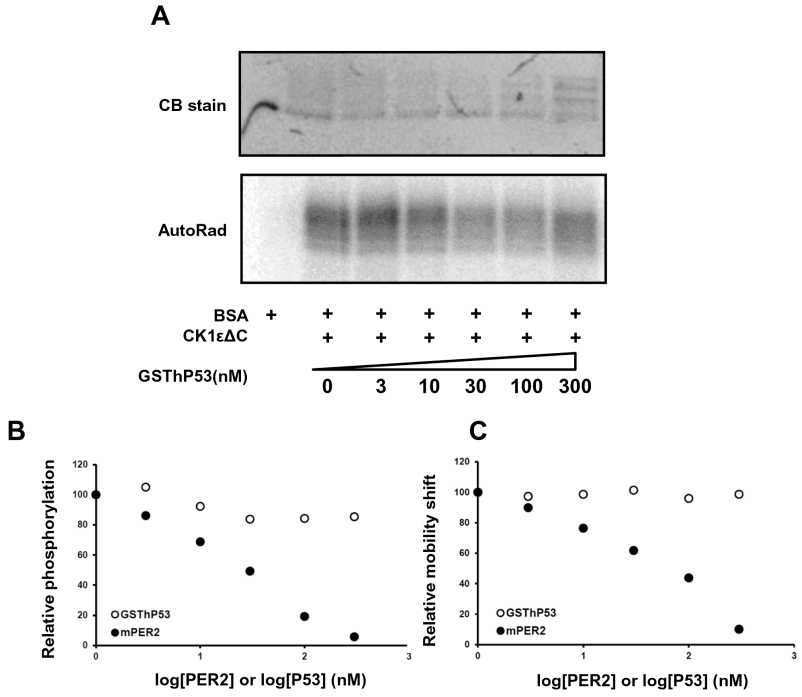

Figure S7. CK1ε is not inhibited by substrates other than mPER2. (A) Varying P53 concentration does not affect the kinase activity of CK1εΔC on the BSA substrate. **(B)** Quantification of the relative phosphorylation intensity in panel A (for P53) as compared with Figure 1 (for mPER2). **(C)** Quantification of the relative mobility shift in panel A (for P53) as compared with Figure 1 (for mPER2).

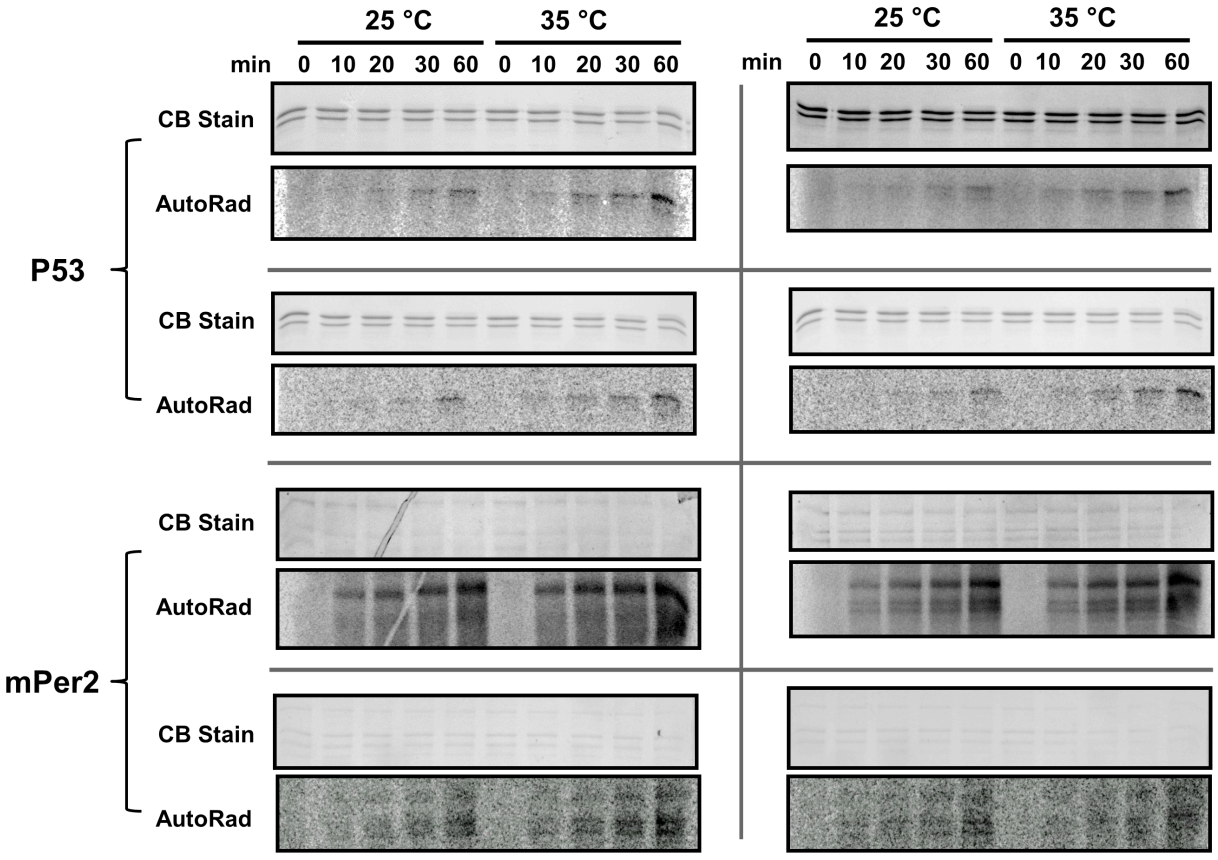

Figure S8. Substrate dependent temperature sensitivity/compensation of CK1εΔC.
Replicate experiment to that shown in Figure 5 to assess Q_{10} of CK1εΔC activity.

Supplemental Tables

Supplementary table S1. Primers and sites using to clone the expression constructs used in this study.

Construct	5' Primer	3' Primer	Clonning sites	Protein tag	Extra AA at N-terminus
pGEX-mPer2	GGACTCGAGATGAATGGATA CGTGGACTTCTCCCCAAG	GATGCGGCCGCTTAATGATGAT GATGATGATGGGATCCACCCGT CTGGGCCTCTATCCTGGGGCC	XhoI / NotI	His6 at C-terminus	Gly-Pro-Leu-Leu-Glu-
pGEX-mCry2	CCGCCCGGGTGCGGCGGCT GCTGTGGTGGCAGC	GATGCGGCCGCTTAGGAGTCC TTGCTTGCTGGCTC	SmaI / NotI	N/A	Gly-Pro-Leu-Gly-Ser-Pro-Glu-Phe-Pro-Gly-
pGEX-CK1εΔC	GGAAGATCTGAGCTACGTGT GGGGAACAAGTACCGC	AATAGCGGCCGCGAATTCTCA CTACTGCCCCATCCTCTCCTCG CGTTC	BamHI / NotI	N/A	Gly-Pro-Leu-Gly-Ser-

Other constructs used in this study are:
pGEX-human p53-(1-393) (Addgene ID 24860)
PP1 7-300 (Addgene ID 26566)
pRSET-CK1ε (Gift from Dr. Virshup)

Table S1. Primers and sites using to clone the expression constructs used in this study.

Supplementary table S2. Intracellular transcription factor concentrations.

Intracellular transcription factor (TF) concentrations. *

Protein Name	Value	Concentration over tatal cell volume **
P53 (TF)	21,000 – 195,700 molecules / cell	~21 – 200 nM
NF-κB P65 (TF)	120,000 molecules / cell	~ 120 nM
ER (TF)	260,000 molecules / cell	~ 11 nM
SMAD4 (TF)	93,000 – 120,000 molecules / cell	~ 93 – 120 nM
AHR (TF)	120,000 molecules / cell	~ 12 nM
MYC (TF)	60,000 molecules / cell	~ 60 nM
H2A (Histones)	1.5e+8 molecules / cell	~ 150 mM

* **Resource: Milo et al. Nucl. Acids Res. (2010) 38: D750-D753. "BioNumbers" (http://bionumbers.hms.harvard.edu/default.aspx).**

** **The concentration is estimated on the basis of an average mammalian cell volume of 2,000 μm^3; the nucleus is about 10% of the volume, so the concentration of the TFs if they are totally in the nucleus would be approximately 10X the value tabulated here.**

Table S2. Estimation of intracellular concentrations of typical transcription factors (TF) concentrations. The concentration is estimated on the basis of an average mammalian cell volume of 2,000 μm^3; the nucleus is about 10% of the volume, so the concentration of the TFs if they are totally in the nucleus would be approximately 10X the values tabulated here**.** Data from: Milo et al. Nucl. Acids Res. (2010) 38: D750-D753. "BioNumbers" (http://bionumbers.hms.harvard.edu/default.aspx).