

Title

CKI isoforms α and ϵ regulate Star-PAP target messages by controlling Star-PAP poly (A)
polymerase activity and phosphoinositide stimulation

Rakesh S. Laishram¹, Christy A. Barlow², and Richard A. Anderson^{1*}

¹ *University of Wisconsin-Madison, Department of Pharmacology, 1300 University Ave.
University of Wisconsin Medical School, Madison, Wisconsin, 53706*

² *Present address: ChemRisk, LLC, 4840 Pearl East Circle, Suite 300 West, Boulder, CO 80301*

* Corresponding Author

Richard A. Anderson

Department of Pharmacology

1300 University of Wisconsin-Madison

Madison, WI 53706

Phone: 608-262-3573

Fax: 608-262-1257

E-mail: raanders@wisc.edu

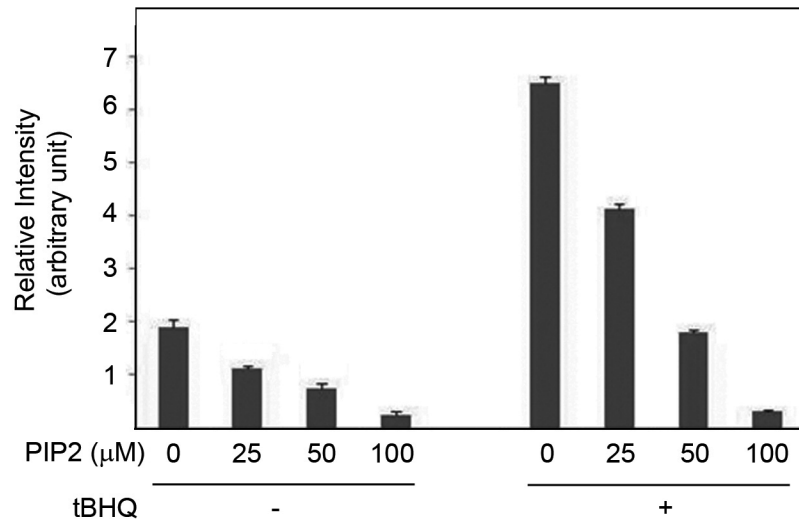
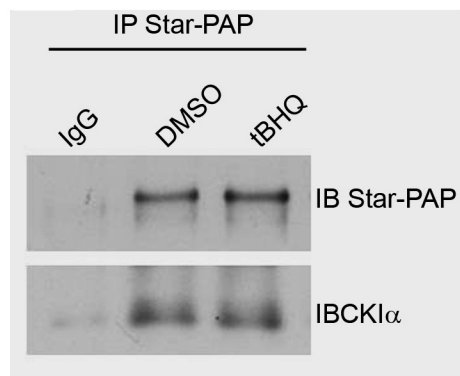
Supplementary Figure 1. (A) Quantification of the kinase activity toward Flag-Star-PAP in the presence of increasing amounts of PI4,5P₂ from the autoradiogram in Fig. 1C. The intensity in arbitrary unit is expressed relative to the amount of total protein in each lane. (B) Immunoprecipitation of Star-PAP from the HEK-293 cells in presence of tBHQ stimulation and immunoblotted for Star-PAP and CKI α . Control cells were treated with DMSO.

Supplementary Figure 2: (A) *In vitro* kinase assay of Flag Star-PAP complex purified from HEK 293 cells stably expressing Flag-tagged Star-PAP after treatment with tBHQ (100 μ M for 4 h) or the solvent control DMSO with no exogenous substrate as in Fig. 1D, but with 5, 10 or 15 μ M CKI7 and IC261 treatment prior to initiation of the kinase reaction. (B) Quantification of the kinase activity toward Star-PAP from the autoradiogram in A. The intensity in arbitrary unit normalized for the total protein in each lane is expressed relative to the control untreated sample.

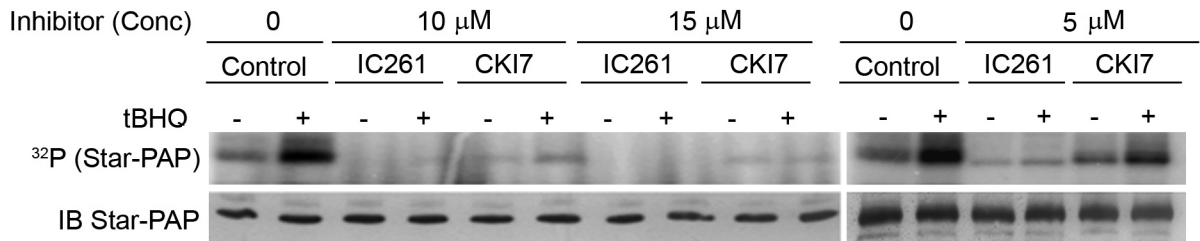
Supplementary Figure 3: (A) Western analysis of siRNA knockdown of CKI α and control actin. (B) The Flag Star-PAP complex was affinity purified from cell lysates by Flag affinity chromatography of Flag Star-PAP wild-type or a mutant with a deletion of the proline rich region (residues 223 to 275). Prior to purification, cells were treated with CKI α inhibitors, tBHQ, or DMSO as indicated and then analyzed by Western blot for Star-PAP. Sup=supernatant, F/T=Flow through, E1-E3=elution fractions of the Star-PAP complex, St=Strip of the column.

Supplementary Figure 4: (A) Western blot analysis of Star-PAP and other associated members of the Star-PAP polyadenylation complex (RNAPII, CPSF 73, PIPKI α , CKI α) as indicated (left panel) after HEK-293 cells were immunoprecipitated (IP) with a rabbit polyclonal antibody specific for Star-PAP or with normal rabbit IgG after treatment with IC261 (100 μ M for 2.5 h), tBHQ (100 μ M for 4 h), and DMSO as indicated in the top panel. β -tubulin was used as a

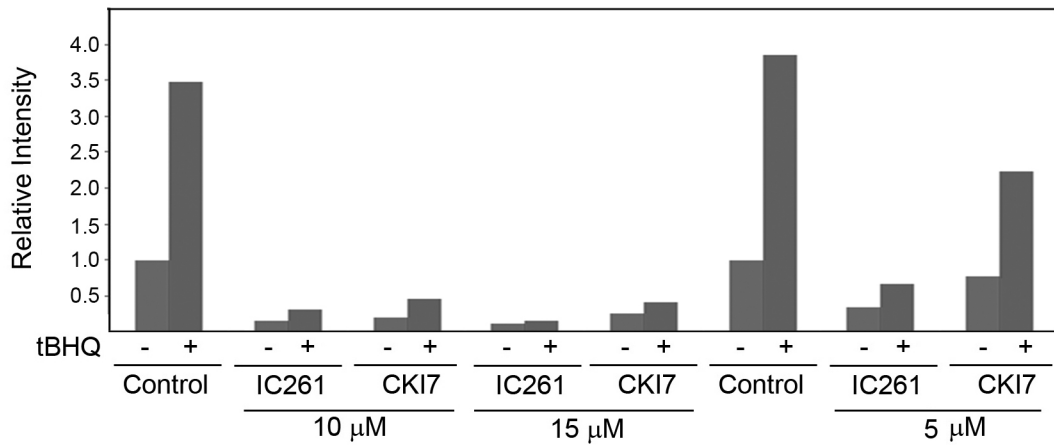
loading control. (B) *In vitro* kinase assay of CKIε with its generic kinase substrate casein. The ³²P incorporated and the total protein (coomassie-stained) are indicated.

A**B**

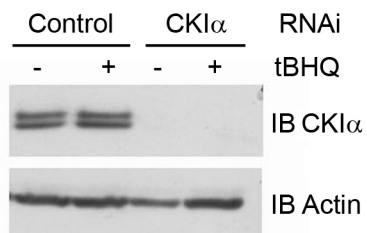
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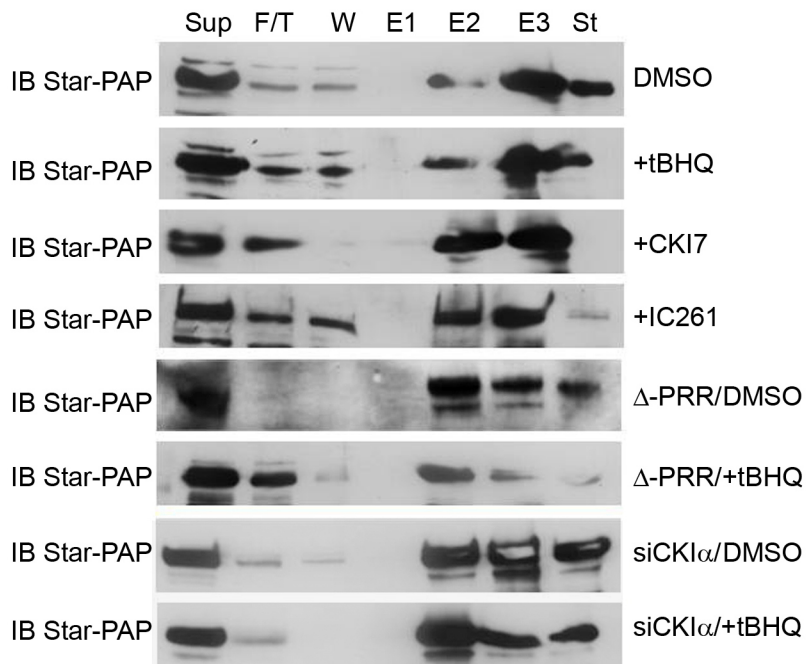
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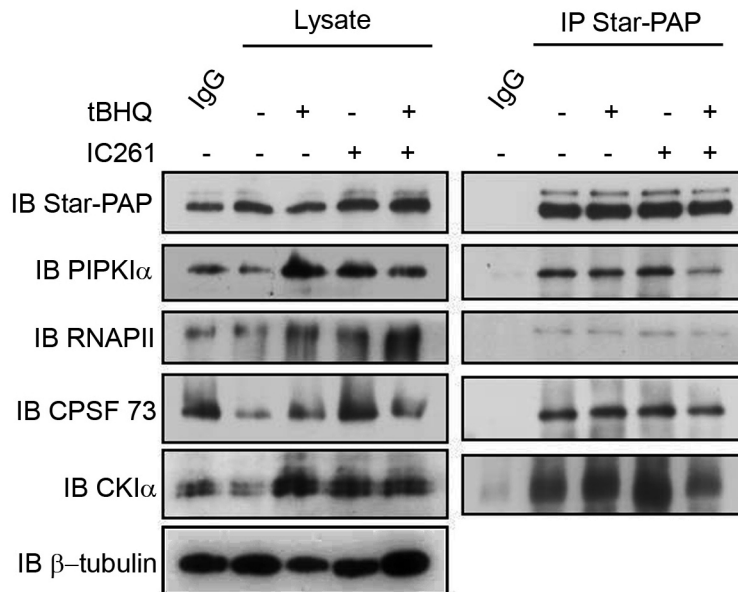
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