# Supplemental Data

## *TFIIH kinase places bivalent marks on the carboxyl-terminal domain of RNA polymerase II*

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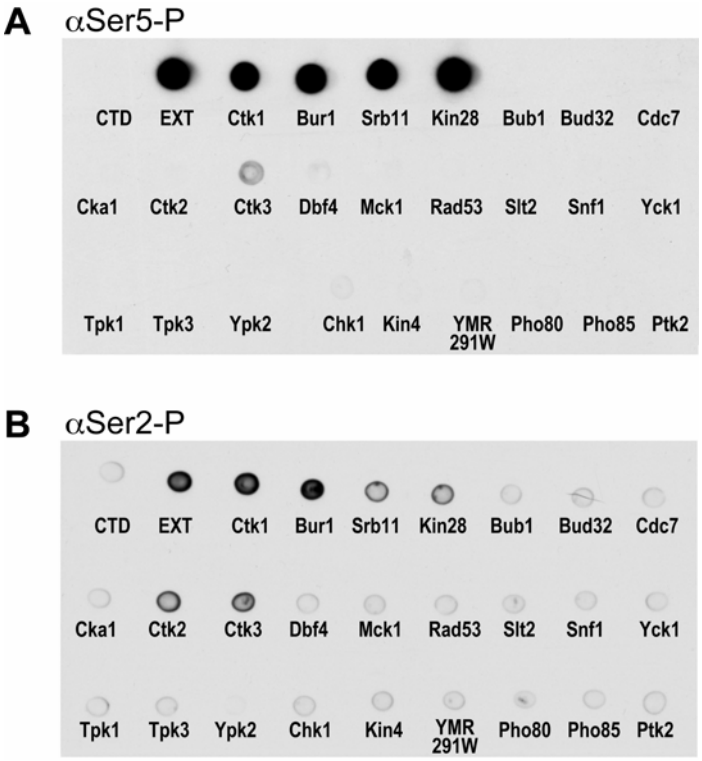
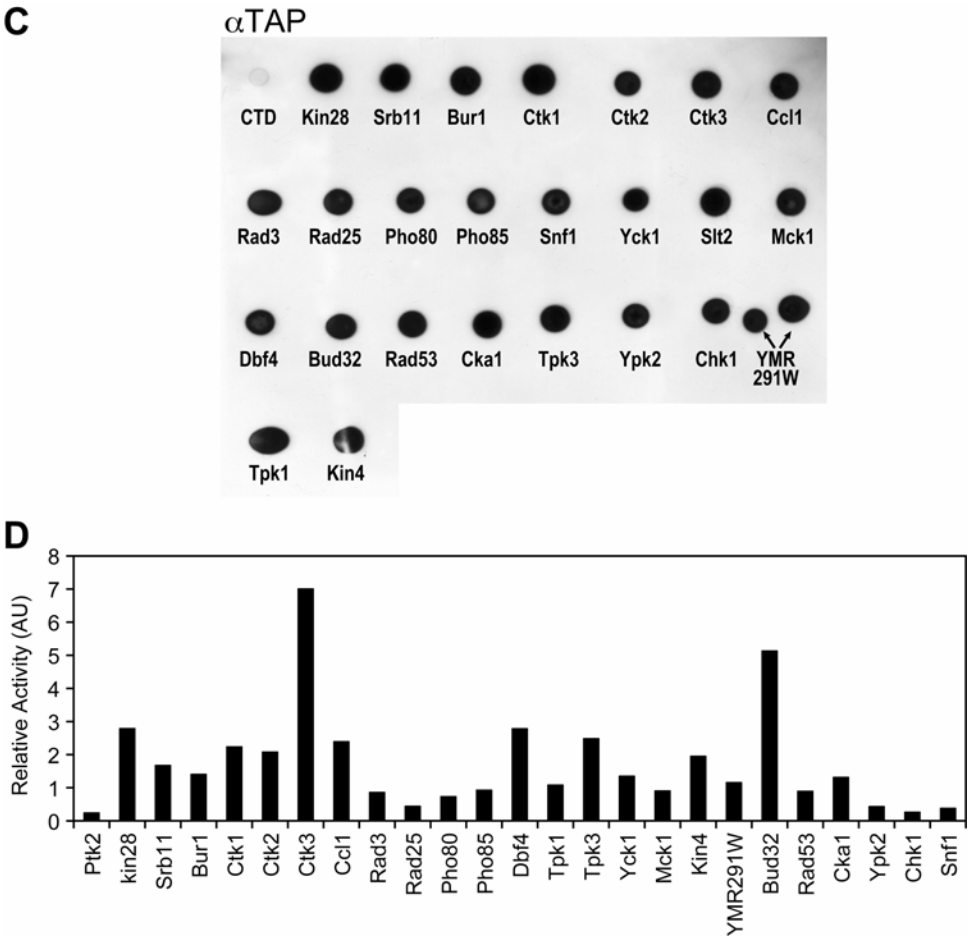



**Figure S1- Tandem affinity purified kinases phosphorylate the known substrates**

**(A)** Dot blot assay of GST-CTD phosphorylated by purified yeast kinases and probed with αSer5-P antibody. Names of the kinases are mentioned below their respective spot on the blot. CTD unphosphorylated (-) and phosphorylated by yeast cell extract (EXT) are used as negative and positive controls respectively.

**(B)** Dot blot assay of GST-CTD phosphorylated by purified yeast kinases and probed with αSer2-P antibody.




**(C)** Dot blot of purified nuclear kinases and cyclins with αTAP antibody (Open biosystems). Kinases and cyclins are mentioned below their respective spot on the blot. **(D)** Activity of purified yeast kinases against a common substrate, Ptk2. The reaction mix was resolved by SDS PAGE and the relative phosphorylation was quantitated using phosphoimager. Name of the kinases are mentioned below their respective bar.

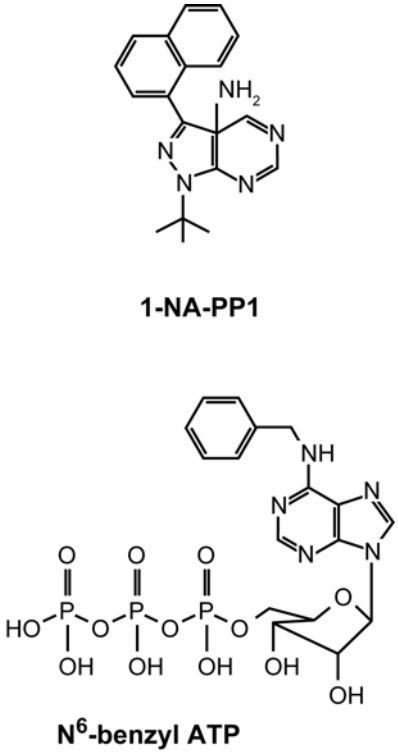



**Figure S2- Chemical structure of 1-NA-PP1 and $N^6$- benzyl ATP**

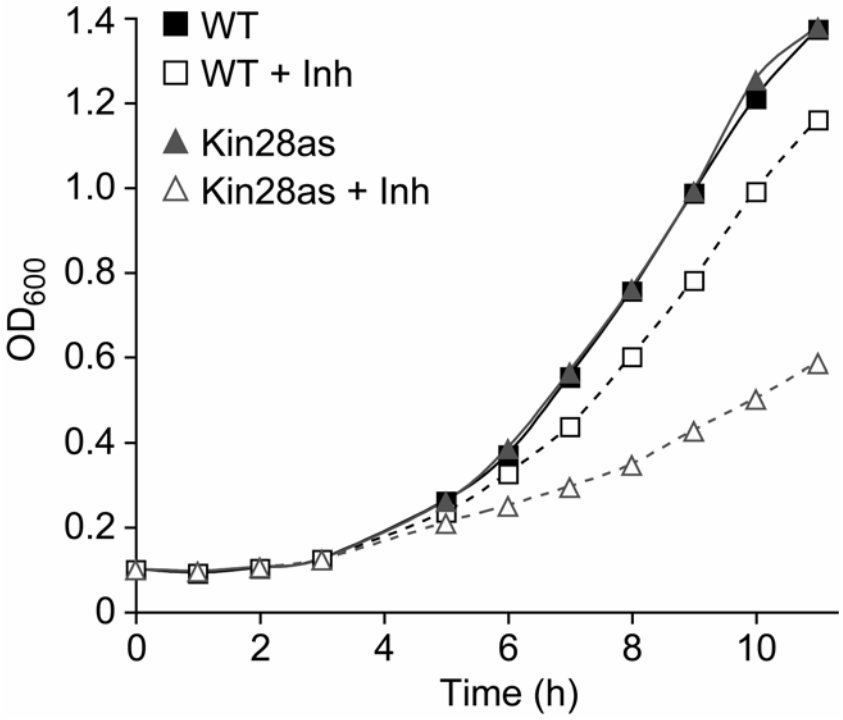



**Figure S3- Growth curve for wild type and mutant strains**

Growth curve for the wild type (Kin28) and its analog sensitive single mutant Kin28as (Kin28-L83G) in presence and absence of inhibitors. Inhibitors (2μM 1-NA-PP1 and 4μM 1-NM-PP1) were added at $OD_{600}$ of 0.5 for 30 minutes.

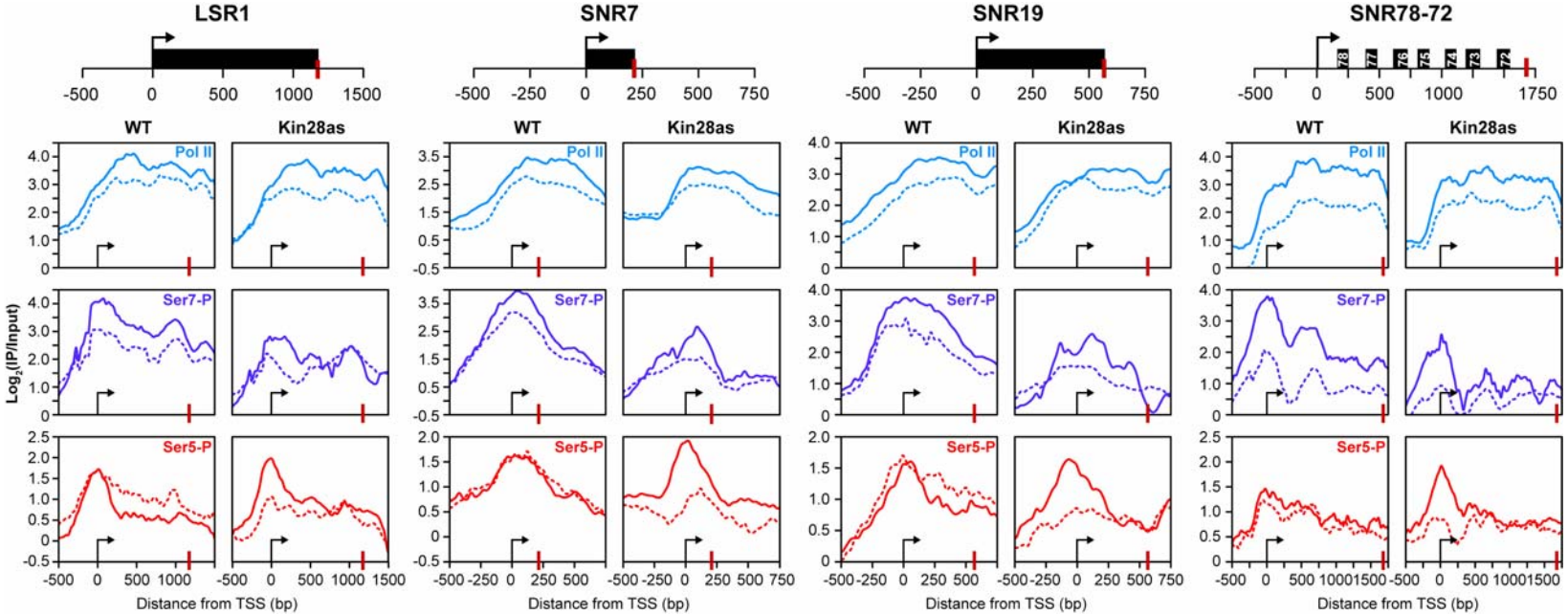



**Figure S4- ChIP chip profile for different representative snRNAs**

Inhibition effect on Pol II (blue), αSer7-P (green) and αSer5-P (red) occupancy profile shown for LSR1, SNR7, SNR19, and SNR72-78. Uninhibited profiles are shown as solid lines and inhibited profiles as dashed lines. TSS and 3' processing sites are marked by an arrow and a red bar. All x-axes are shown as the distance in base pairs relative to the TSS and y-axes are shown on a log2 scale for ChIP chip enrichment (IP/input).

## EXPERIMENTAL PROCEDURES

### *In vitro* kinase assay

The substrate (200ng) used for this assay were either GST-CTD3 (three repeats of YSPTSPS attached to GST) or GST-CTD16 (sixteen repeats of YSPTSPS attached to GST) or GST-CTD4 (four repeats of YSPTSPS attached to GST) or Ser2A (serine 2 is replaced with alanine in all four repeat) or Ser5A (serine 5 is replaced with alanine in all four repeat) or Ser7A (serine 7 is replaced with alanine in all four repeat) and were purified as described previously (Ansari et al., 2005; Patturajan et al., 1998). The assays were conducted in a 25µl reaction volume at 25°C for two hours in buffer containing 20mM HEPES [pH 7.5], 2.5mM EGTA, 15mM magnesium acetate, 0.8mM ATP, 10% glycerol, protease inhibitors and phosphatase inhibitors [1mM $NaN_3$, 1mM NaF, 0.4mM $Na_3VO_3$]. Four micro-liters from the reaction mix were directly spotted onto nitrocellulose membrane (Amersham Biosciences) and further processed as a standard dot blotting protocol.

For ELISA, The CTD-peptide (YSPTSPSYSPTSPSYSPTSPSYSPTSPSC; Peptide Specialty Laboratories GmbH, Heidelberg) was coupled on 96-well maleimide plates for 60 minutes at 37°C in carbonate buffer at pH 9.5. After washing, the kinase assay was performed using recombinant kinase (100ng) in 25µl kinase buffer containing 20mM TrisCl [pH 7.4], 20mM NaCl, 10mM $MgCl_2$, 1µM DTT and 2µM ATP, at 28°C for 60 minutes followed by washing and blocking with PBS/milk (1%) for 30 minutes. Primary antibodies were added and incubated for 30 minutes. After an additional

washing and blocking step, biotin-coupled secondary antibodies were added for 30 minutes. Following another washing and blocking step, peroxidase attached to avidin was added to the wells. After washing five times with PBS, 50 µl of substrate buffer (containing o-phenylenediamine and $H_2O_2$; pH 5) were added and after the color change, samples OD were measured at 405nm in the ELISA reader.

**ChIP-chip analysis**

For microarray analysis, the whole genome “input” samples were labeled with Cy3-dUTP and the “IP” samples were labeled with Cy5-dUTP. The reaction products were purified using the QIAquick PCR Purification Kit and the concentrations verified via nano-drop (Thermo Scientific).