Supplemental Information

Transcription Factors Mediate the Enzymatic Disassembly of Promoter-bound 7SK snRNP to Locally Recruit P-TEFb for Transcription Elongation

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SUPPLEMENTAL DATA

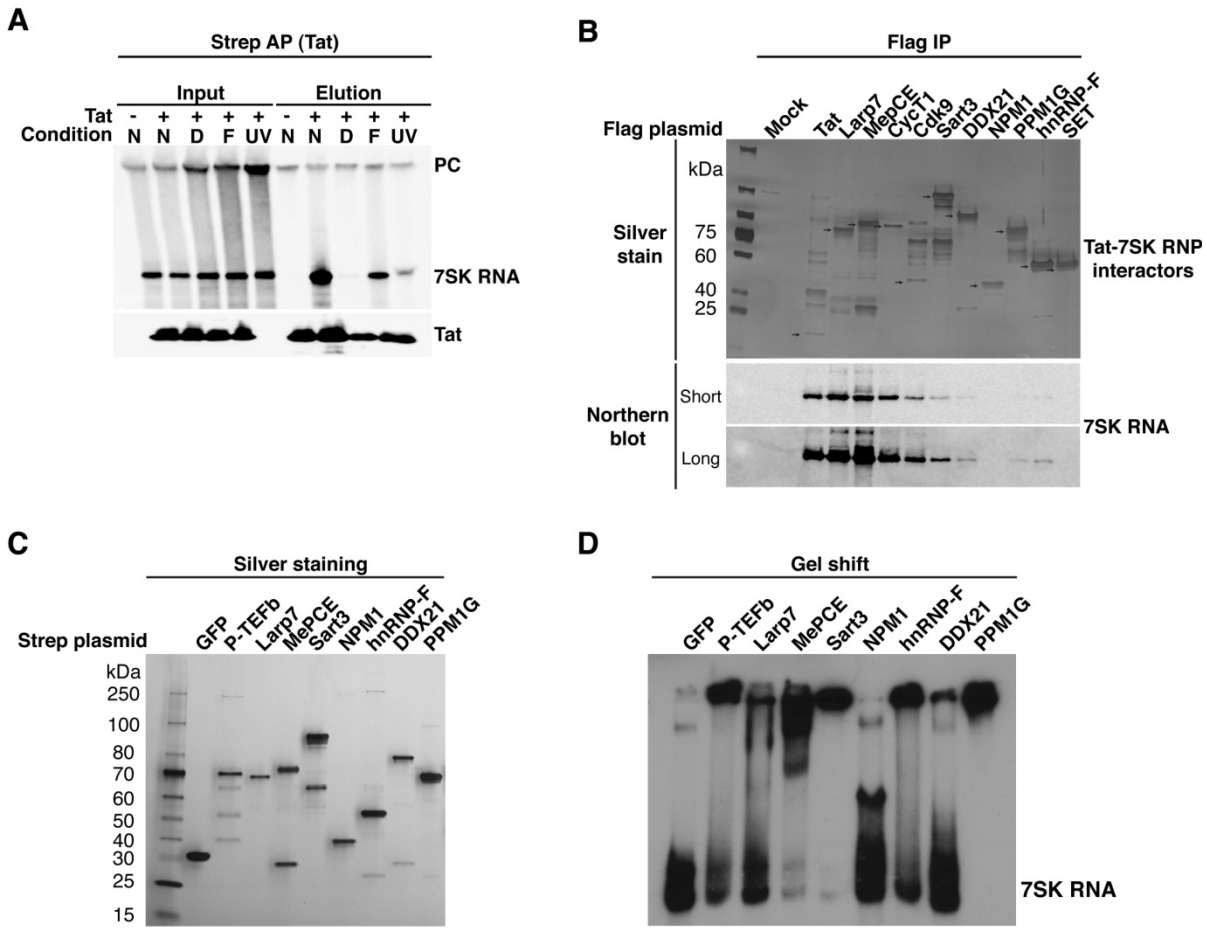

Figure S1. Interactions between Tat and the 7SK RNP interactors with 7SK RNA *in vitro* and in cells. Related to Figure 1

(A) Tat was affinity purified from 293T cells under different conditions: using a Native (N) buffer or from cells previously chemically crosslinked with formaldehyde (F), irradiated with UV (UV) or left untreated using a Denaturing (D) buffer. (D) is a negative control for F and UV samples. PC denotes an RNA used as an internal precipitation control used in the affinity purification (AP) step to control for RNA degradation. Tat was detected with a Strep antibody.

(B) 293T cells were transfected with the indicated Flag plasmids. Cells were lysed in native conditions and total lysates used to purify the Flag-tagged factors (Flag IP). The eluted samples were directly electrophoresed on SDS-PAGE and silver stained or used to extract co-purifying

RNAs to monitor levels of 7SK RNA by Northern blot. Short and long denote two exposure time points.

(C) 293T cells were transfected with the indicated Strep plasmids and proteins were purified in the presence 1 μg/mL of RNase A (to eliminate any co-purifying RNA). A fraction of the elutions was visualized by silver staining.

(D) Gel shift assays between *in vitro* synthesized 7SK RNA and the interactors shown in panel (C). P-TEFb binds 7SK RNA in the presence of co-purifying Hexim1 protein (Li et al., 2005).

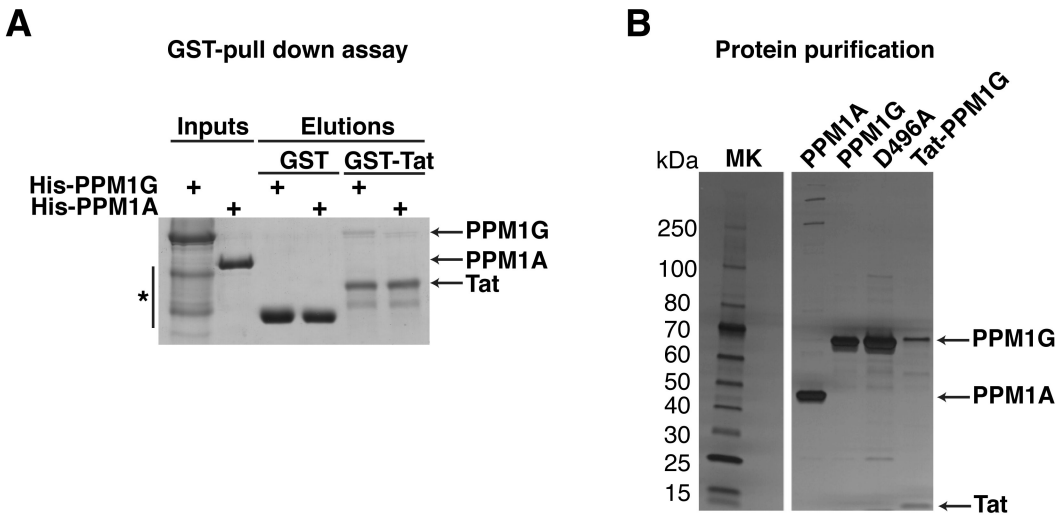

Figure S2. Tat directly binds PPM1G but not PPM1A. **Related to Figure 2**

(A) GST-pull down assay of GST or GST-Tat and His-tagged PPM1G or PPM1A. The indicated proteins were incubated *in vitro* and elutions were stained with coomassie blue. The asterisk denotes the presence of PPM1G cleavage products.

(B) 293T cells were transfected with the indicated plasmids (Strep-PPM1A, Strep-PPM1G, Strep-PPM1G D496A mutant or co-transfected with Strep-Tat and Flag-PPM1G). Cell lysates were used to purify PPM1A, PPM1G and D496A using a single Strep purification step or the Tat-PPM1G complex using a Strep-Flag tandem affinity purification protocol. A fraction of the elutions was visualized by silver staining. Proteins are indicated with arrows.

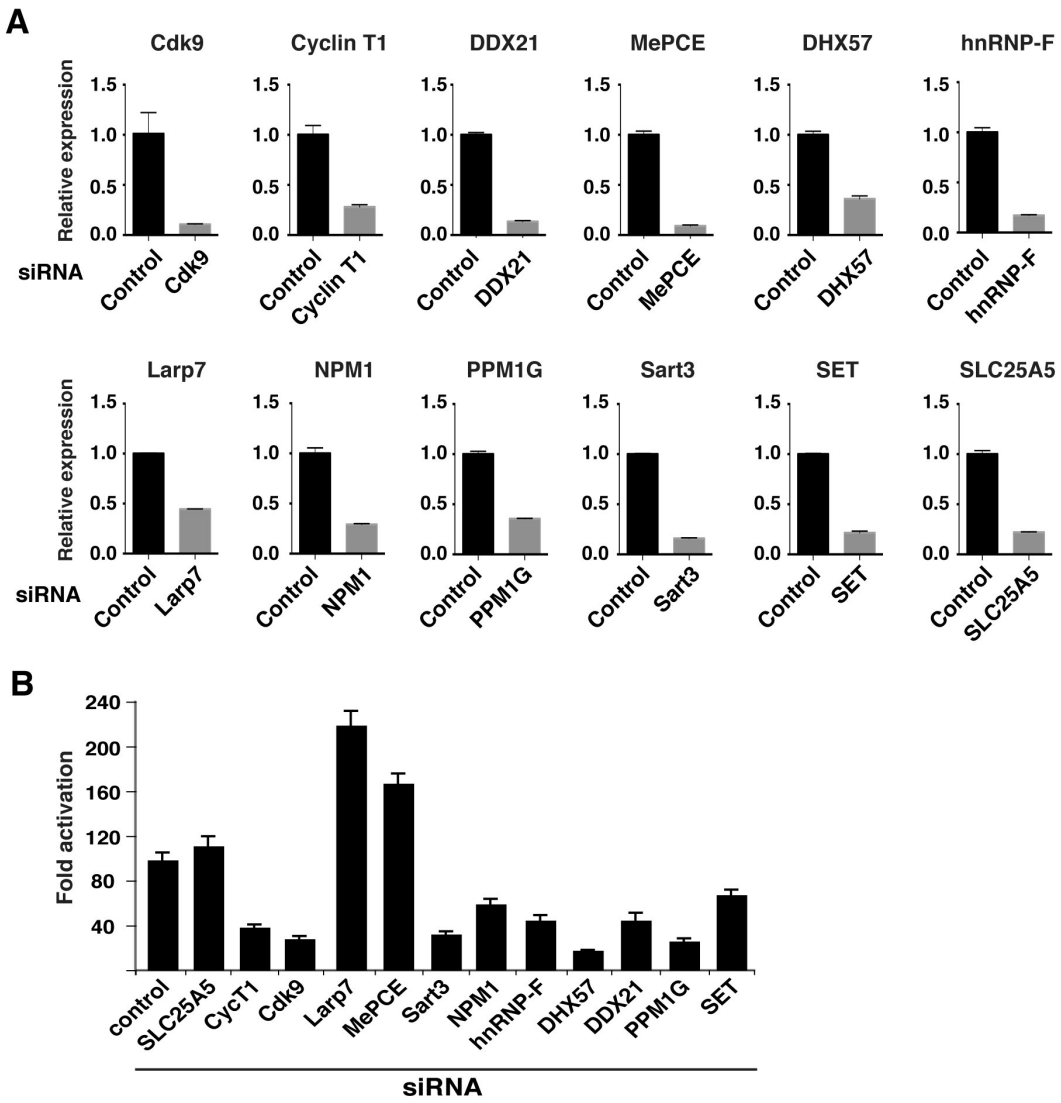

Figure S3. RNAi knockdown of the Tat-7SK RNP interactors decreases Tat activity from an HIV LTR reporter. **Related to Figure 3**

(A) HeLa cells were transfected with the indicated siRNAs. Levels of the indicated mRNAs were quantified by RT-qPCR using gene-specific primers. Relative expression denotes relative gene expression levels normalized to the Rpl19 gene (Mean ± SEM, n=3).

(B) HeLa cells transfected with siRNAs as in panel (A) were subsequently transfected with a Flag-tagged Tat and an HIV LTR-luciferase plasmids (24 hrs post-siRNA transfection) to measure transcription reporter activity. The fold activation (+/– Tat) normalized to a CMV-RL activity is shown (Mean ± SEM, n=3).

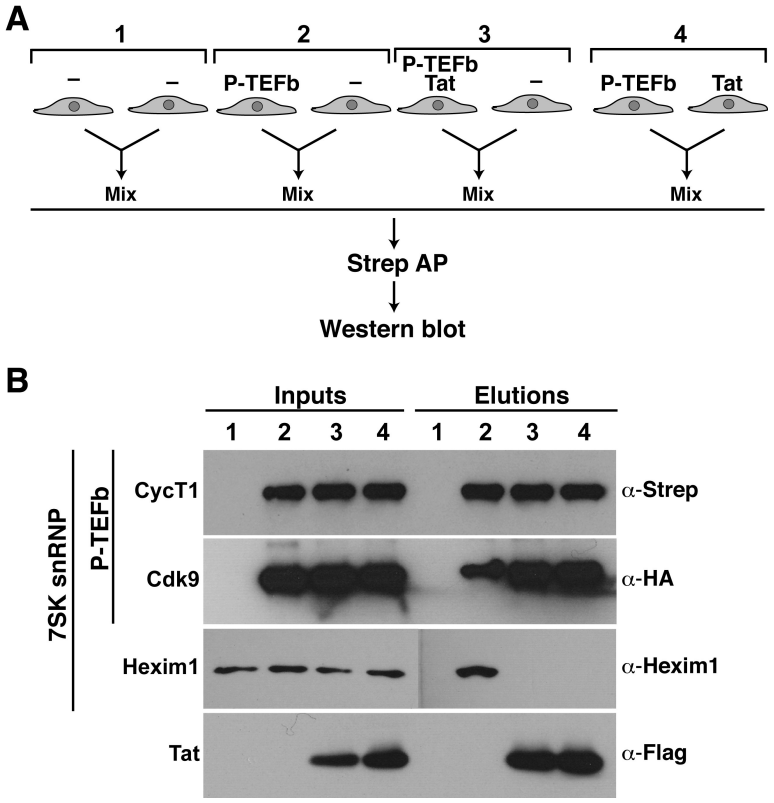

Figure S4. *In vitro* association of P-TEFb and Tat leads to Hexim1 physical competition from the 7SK snRNP. Related to Figure 4

In the competition model, the release of P-TEFb from the 7SK snRNP by Tat occurs primarily *in vitro* but not in cells, since Tat and P-TEFb ectopically expressed in separate plates can efficiently reassort *in vitro* during affinity purification of P-TEFb leading to Hexim1 displacement. (A) Schematic diagram of the population of 293T cells examined in the mixing experiments. Cells were transfected with the indicated plasmids 48 hrs before harvesting. P-TEFb is CycT1:S and Cdk9:HA; Tat is Flag-tagged; (–) denotes cells transfected with an empty pcDNA4 vector. (B) The cells described in panel (A) were mixed as indicated before the cell lysis. Protein lysates were subjected to a Strep AP for 2 hrs at 4°C and the eluted material was probed in Western blots with the indicated antibodies.

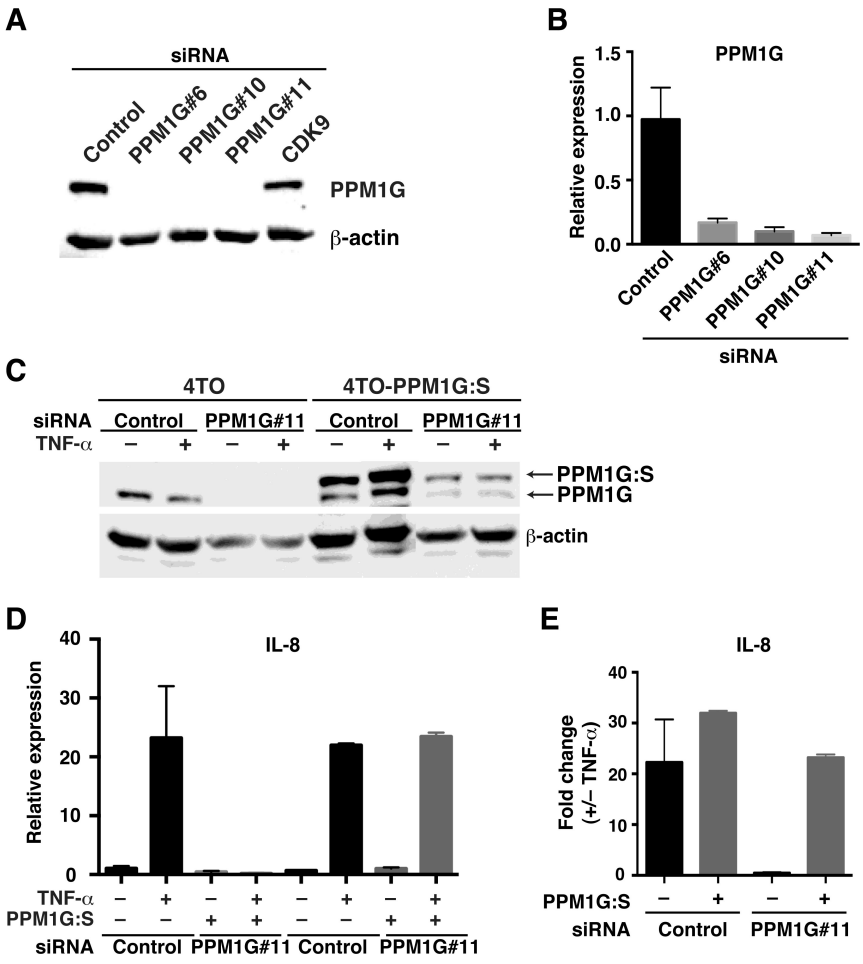

Figure S5. PPM1G RNAi with different siRNA duplexes and rescue experiment. Related to Figure 5

(A) HeLa cells were transfected with the indicated siRNAs and the expression of PPM1G and β-actin (loading control) was analyzed by Western blot.

(B) The relative expression of IL-8 (normalized to β-actin) in the samples from panel (A) was determined by RT-qPCR (Mean ± SEM, n=3).

(C) siRNA rescue experiment. HeLa cells were transfected with the indicated plasmids (4TO or 4TO-PPM1G:S) and one day later re-transfected with the indicated siRNAs. The expression of endogenous and transfected PPM1G and β-actin was analyzed by Western blot.

(D) A fraction of the samples transfected as in panel (C) was used to extract RNA and prepare cDNA. The relative expression of IL-8 (normalized to β-actin) was determined by RT-qPCR (Mean ± SEM, n=3).

(E) IL-8 expression levels from panel (D) expressed as fold change (+/– TNF-α) (Mean ± SEM, n=3).

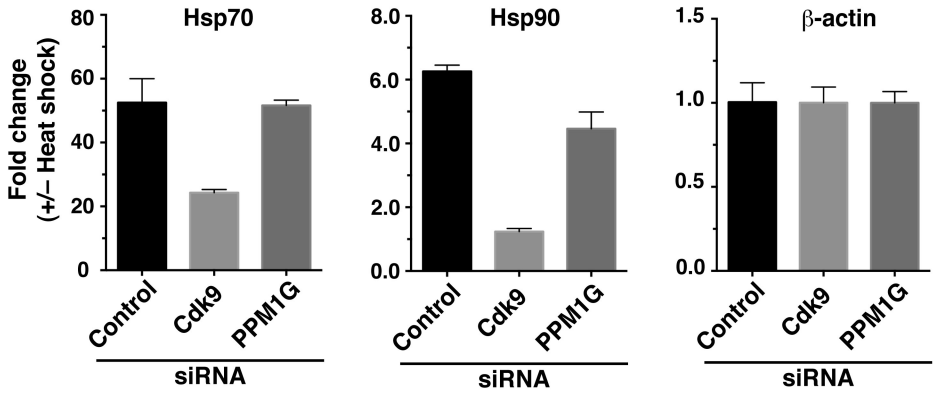

Figure S6. PPM1G is not involved in activation of heat shock-responsive genes. Related to Figure 6

RT-qPCR analysis of gene expression in response to heat shock. HeLa cells were exposed (or not) to heat shock stress (42°C) and harvested 2 hrs post-treatment. Total RNA was isolated, and RT-qPCR was performed with gene-specific primers (**Table S4**). Values were normalized to Rpl19 and are expressed as fold change over untreated cells (Mean ± SEM, n=3).