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Supplemental Data

A La-Related Protein Modulates 7SK snRNP Integrity to Suppress P-TEFb-Dependent Transcriptional Elongation and Tumorigenesis

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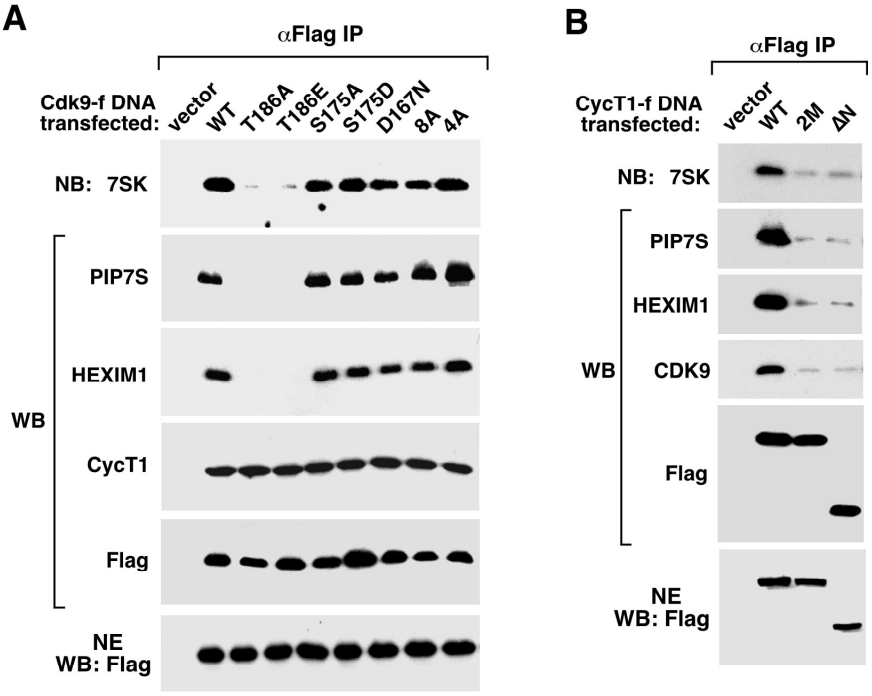

Figure S1. Both the Cdk9 T-loop and CycT1 cyclin-box are required for the interaction of PIP7S with P-TEFb. A. Mutation of the Cdk9 T-loop disrupts the 7SK-P-TEFb binding, which in turn dissociates PIP7S and HEXIM1 from P-TEFb. Anti-Flag IP derived from NEs (bottom panel) of HeLa cells transfected with the indicated wild-type or mutant Cdk9-f constructs were analyzed by western (WB) and northern blotting (NB). The Cdk9 mutants T186A and T186E,

which contain substitutions at the tip of the T-loop, are unable to interact with 7SK and HEXIM1 (Chen et al., 2004; Yik et al., 2003). The Cdk9 mutants S175A and S175D fail to bind Brd4 (Yang et al., 2005). D167N lacks kinase activity. The mutants 4A and 8A contain 4 and 8 Ser/Thr to Ala substitutions, respectively, in the Cdk9 C-terminal region and are unable to form the Tat-TAR-P-TEFb ternary complex (Fong and Zhou, 2000). **B.** The cyclin-box region in CycT1 is essential for the PIP7S-P-TEFb interaction. Factors associated with the indicated CycT1-f proteins were immunoprecipitated from transfected HeLa NEs (bottom panel) and analyzed as in A. The mutant ΔN has a deletion of the cyclin-box in CycT1 (Chen et al., 2004). The mutant 2M contains two point mutations (K93L and E96K) in the cyclin-box region (Bieniasz et al., 1999).

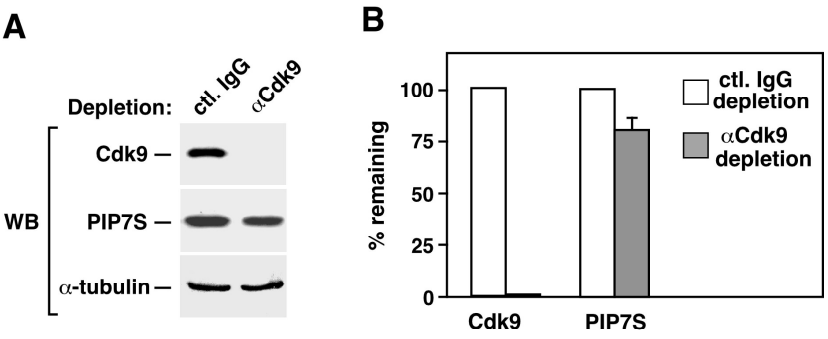

Figure S2. P-TEFb interacts with about 20% PIP7S protein in vivo. A. HeLa NEs were subjected to immunodepletion with the indicated antibodies and then analyzed by western blotting (WB). **B.** The levels of Cdk9 and PIP7S in the depleted NEs were normalized to those of α-tubulin and quantified based on serial dilutions, with those in mock-depleted NE artificially set to 100%. The error bars represent mean +/- SD.

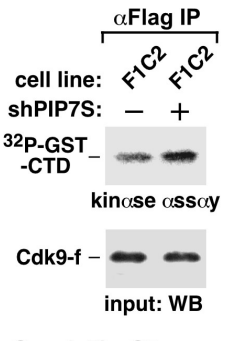

Figure S3. PIP7S knockdown increases P-TEFb's kinase activity toward GST-CTD. P-TEFb was affinity-purified by anti-Flag immunoprecipitation (IP) from NEs of F1C2 cells either expressing (+) or not expressing (-) shPIP7S, normalized for their Cdk9-f levels by western blotting (WB; lower panel), and analyzed in kinase reactions containing GST-CTD as a substrate (autoradiography; upper panel).

References for supplemental data:

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