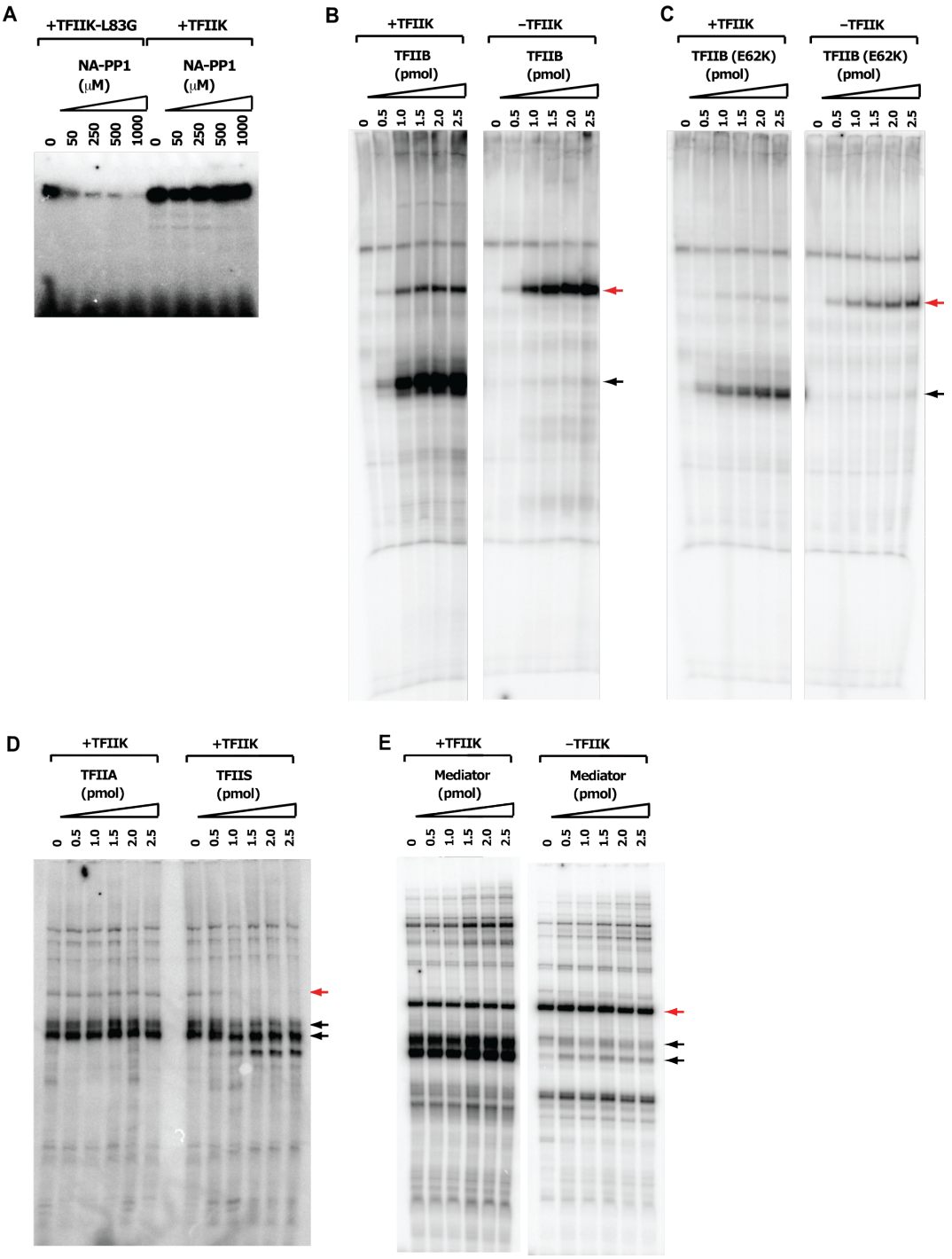

Figure S1 *In vitro* kinase assay and run-off transcription assay of PIC (related to Fig.1).
(A) *In vitro* kinase assay of PIC, titrated with NA-PP1. A PIC was assembled with *HIS4*(−96/+112) in the presence of TFIIK or inhibitor-sensitive TFIIK mutant (TFIIK-L83G) (2.0 pmol), and phosphorylation was initiated by adding 500 μM ATP, and 0.5 μCi/μl of [γ-^{32}P] and analyzed by SDS-PAGE. **(B, C)** Effect of TFIIB on TSS utilization. A PIC formed on *SNR20* 91W (−122/+97) was transcribed in the presence or absence of TFIIK (2.0 pmol), with increasing amounts of TFIIB (B) or a TFIIB mutant E62K (C). Transcripts initiated from upstream and downstream TSSs are indicated by red and black arrows.

(D) Run-off transcription with increasing amounts of TFIIA or TFIIS. A *HIS4*(−96/+112) fragment was combined with TFIIB, TBP, TFIIE, holo-TFIIH, pol II-TFIIF complex, and was titrated with the amounts of TFIIA or TFIIS indicated. The transcription reaction was performed as described in Experimental Procedures. Transcripts initiated from upstream and downstream TSSs are indicated by red and black arrows. **(E)** Run-off transcription with increasing amounts of Mediator. A *HIS4*(−96/+112) fragment was combined with TFIIA, TFIIB, TBP, TFIIE, TFIIH-ΔTFIIK, pol II-TFIIF complex in the presence or absence of TFIIK, and was titrated with the amounts of Mediator indicated.

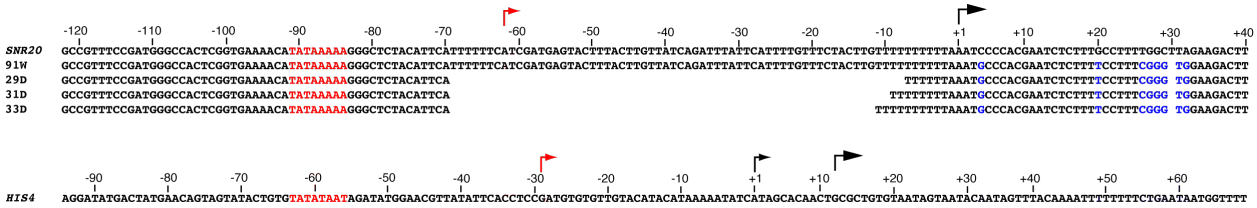

Figure S2 Promoter sequences for *SNR20* and *HIS4* (related to Fig. 2)

Promoter sequences for *SNR20*, *SNR20* deleted promoters (91W, 29D, 31D, 33D), and *HIS4*. Upstream and downstream *in vitro* TSSs are indicated by red and black arrows. TATA box is colored in red. Mutations are colored in blue.

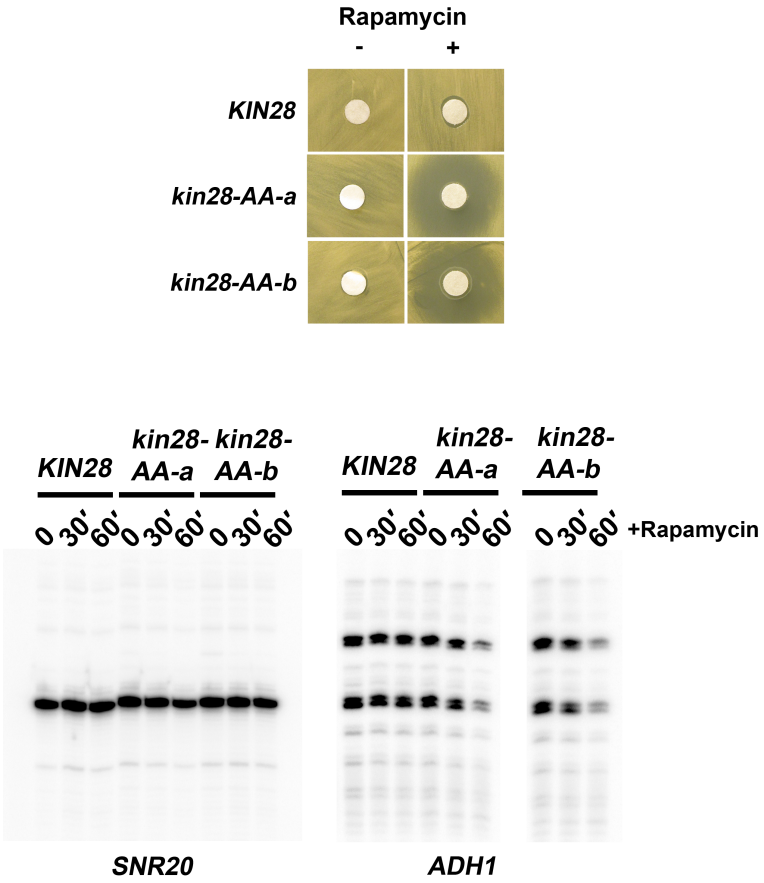

Fig S3. Anchors-away of Kin28 does not alter TSS usage at *SNR20* or *ADH1* (related the Experimental Procedures)

(Top) Halo assays for relevant yeast strains (KIN28 or kin28-FRB (replicate strains "a" and "b") plated as lawns with sterile filter discs indicating position of spotting with Rapamycin (10 µl of 10 mg/ml concentration) or vehicle (10 µl of DMSO). Rapamycin inhibition of growth due to nuclear depletion of Kin28 is dependent on presence of kin28-FRB. (Bottom) Primer extension analysis for start site usage on total RNA isolated from KIN28 or kin28-FRB strains upon time course of rapamycin (1 µg/ml) treatment. Results shown are representative of three independent experiments. TSSs for *SNR20* and *ADH1* are as follows (underlined/bold):

SNR20:TATAAAAAgggctctacattcatttttcatcgatgagtactttacttgttatcagatttattcattttgtttctacttgttttttttttaaatccccAcgaatctctttgccttttgg

ADH1:TATAAATAgacctgcaattattaatcttttgtttcctcgtcattgttctcgttccctttcttccttgtttctttttctgcacaatatttcAagctatacAagcatacaatcaactatctcatataca

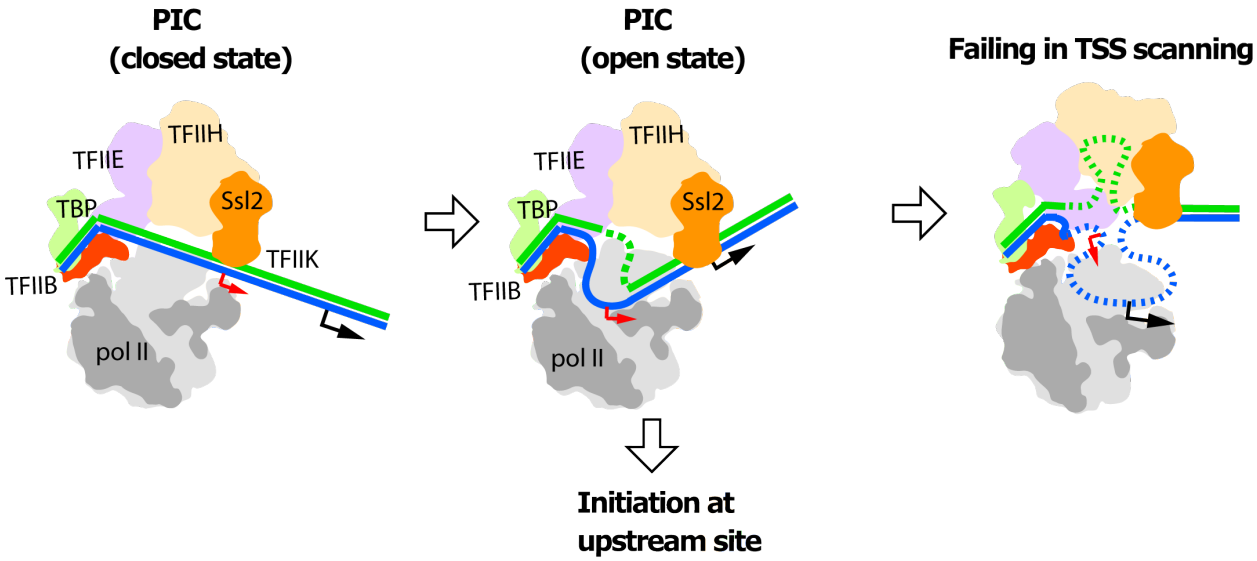

Figure S4 Schematic representation of transcription initiation in the absence of TFIIK (related to Fig. 4).
DNA is blue and green. Upstream and downstream TSSs are indicated by red and black arrows.