Multivalent role of human TFIID in recruiting elongation components at the promoter proximal region for transcriptional control

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Summary

Despite substantial progress in understanding of players involved and regulatory mechanisms controlling initiation and elongation steps of transcription, little is known about recruitment of elongation factors at promoter proximal region for the initiation to elongation transition. In this report, we show evidence that human TFIID, which initiates preinitiation complex (PIC) assembly, contributes to regulating recruitment of Super Elongation Complex (SEC) components at the promoter proximal region through interactions among selective TAF and SEC components. In vitro direct interactions, coupled with cell-based assays, identified important poly-Ser domain within SEC components that are involved in their interaction with TFIID. DNA template-based recruitment assay, using purified components, further show a direct role of poly-Ser domain-dependent TFIID interaction in recruiting SEC components on target DNA. Consistently, ChIP and RNA analyses have shown an importance of this mechanism in TFIID-dependent SEC recruitment and target gene expression within mammalian cells.

Introduction

The initiation step of RNA polymerase II (Pol II hereafter)-driven eukaryotic transcription begins with activator-dependent recruitment of TBP-containing TFIID complex (TFIID hereafter) at promoter region. Human TFIID contains ~14 other TBP-associated factors (TAFs) that help in recruiting TFIID and other general transcription factors (GTF) at the promoter region for assisting pre-initiation complex (PIC) assembly. Although regulation of

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Author Contributions

DY performed majority of the experiments in consultation with DB. KG performed initial ChIP experiments showing factor recruitments on target genes. SB generated baculoviruses expressing TAF subunits and performed their interactions with AF9 and EAF1. DY, RGR and DB wrote the manuscript.

Declaration of interest

The authors declare no competing interest.
transcription initiation is a key step for expression of majority of genes, studies from the last several years have shown that a substantial number of eukaryotic genes are also regulated at promoter proximal pausing step (Adelman and Lis, 2012; Henriques et al., 2013; Henriques et al., 2018; Williams et al., 2015).

Soon after promoter clearance, Pol II is subjected to pausing through action of two negative regulatory complexes i.e. DRB-sensitivity inducing factor (DSIF) and negative elongation factor (NELF), as well as Pol II-associated factor 1 (PAF1)(Chen et al., 2015; Chen et al., 2017; Yamaguchi et al., 2013; Zhou et al., 2012). Majority of the Pol II-mediated pausing events happen after synthesis of 20-50 bases nascent RNA (Yamaguchi et al., 2013; Zhou et al., 2012). It is widely believed that phosphorylation activity of positive transcription elongation factor b (P-TEFb) complex helps in relieving the paused Pol II to assist its entry into productive elongation. Human P-TEFb complex is a heterodimer consisting of CyclinT1/2 and Cyclin-dependent kinase 9 (CDK9)(Peng et al., 1998; Price, 2000). Upon recruitment, the P-TEFb complex phosphorylates the DSIF and NELF complexes along with C-terminal domain (CTD) of Pol II. Upon its phosphorylation, the NELF complex loses its ability to interact with the nascent RNA as well as the inhibitory DSIF complex and thereby relieves the paused Pol II to enter into productive elongation(Yamaguchi et al., 2013).

Majority of the human P-TEFb resides in an inactive complex, 7SK snRNP, in association with Hexim1, LARP1, MePCE2, and 7SK snRNA (He et al., 2008; Li et al., 2005; Yang et al., 2001). Transcriptionally-active P-TEFb complex has been shown to associate with Brd4 as well as components of the SEC. Human SEC was first reported as ~ 1.5 MDa complex containing multiple transcription elongation factors such as AF9, AF9 family protein ENL, AF4, AFF4, ELL and P-TEFb complex (Biswas et al., 2011; He et al., 2010; Lin et al., 2010; Yokoyama et al., 2010). Genome-wide association studies have shown strong presence of the SEC components at the promoter proximal region(Lin et al., 2011). A further study has shown a role for the SEC in regulating inducible gene expression (Takahashi et al., 2011). Although studies have shown a role for c-Myc as well as histone acetylation at H3K27 in SEC recruitment for transcriptional activation (Rahl et al., 2010; Zhang et al., 2017), role of transcription initiation factors in the SEC recruitment processes and thus helping in transition from initiation to elongation step is poorly known.

The current study demonstrates a novel role of human TFIID in recruiting the SEC components at the promoter proximal region for overcoming the pausing of Pol II. Our detailed analyses further show that specific domain-dependent interactions between the SEC components and TFIID are involved in this overall regulation. Thereby, our study has deciphered a novel role of TFIID in controlling transcription by regulating events beyond the promoter region.

Results

Interaction between AF9 and TFIID in mammalian cells

Our earlier demonstration of a physical association of several TFIID subunits with AF9 (Biswas et al., 2011), raised the possibility of existence of an actual functional interactions among these factors leading to transcriptional regulation. To address this question, we
initially asked whether other TAF subunits would also show similar association with AF9. Consistent with our earlier observations (Biswas et al., 2011), we have observed AF9 interaction with TBP and all tested TAFs (Fig. 1A). These interactions are specific since our analysis failed to show an interaction with ZMYND8 that positively regulates transcription in P-TEFb complex-dependent and SEC-independent manner (Ghosh et al., 2018). To address artifacts of protein interactions due to their over-expression, we immunoprecipitated AF9 using nuclear extract from 293T cells and AF9-specific antibody. Subsequent blotting analysis further confirmed AF9 association with tested TAF subunits along with other known AF9-interactors in endogenous context (Fig. 1B). To address reciprocal interactions, we immunoprecipitated TBP-interacting proteins using nuclear extract from a cell line that stably expresses FLAG-HA tagged TBP. Subsequent blotting analysis confirmed presence of AF9 along with known TAFs in TBP pull-down sample (Fig. 1C). Further immunoprecipitation analysis using nuclear extract from 293T cells and TBP-specific antibody also showed AF9 association with TFIID in endogenous context (Fig. 1D). Notably, both ectopic and endogenous TBP immunoprecipitation assays also revealed presence of other SEC subunits that included AF4, AFF4, EAF1, ELL and P-TEFb (Fig. 1C-D); and an in vitro kinase assay with a GST-CTD substrate demonstrated that the associated P-TEFb was functionally active (Fig. S1A). These results thus demonstrate an association of TFIID and SEC/P-TEFb complexes within mammalian cells.

**Extensive, but variable interactions between SEC components and TFIID**

Based on our observation of the presence of SEC components in ectopic and endogenous TBP-associated proteins (Fig. 1C-D), we further analyzed these interactions by reciprocal immunoprecipitations. Our further immunoprecipitation analysis using nuclear extract of ectopically-expressed FLAG-HA-CDK9 cells showed TFIID association along with known P-TEFb interactors (Fig.S1B). Subsequently, we also addressed TFIID association with other SEC components (ELL, AF4, EAF1, and EAF2) by immunoprecipitation analyses using nuclear extract of cells ectopically expressing these factors as FLAG-HA-tagged (Fig. 1E-F). These analyses further showed that, as observed for ectopic AF9 and CDK9, all tested TAF subunits were found to associate with ectopically expressed EAF1 (Fig. 1F), whereas, in contrast, ectopically expressed AF4, ELL, and EAF2 showed much weaker associations of TFIID components but not SEC components (Fig. 1E-F). Interestingly, although similar pull-down has been observed, the EAF1 protein showed much stronger association with TFIID components than the EAF2. Similar observation has also been noted for AF9 and AF4, wherein, the AF9 protein showed stronger association than the AF4. Therefore, we conclude that human TFIID and SEC associates extensively and different SEC components show variable interactions with TFIID components.

**Direct interactions of TFIID with specific SEC components**

To address whether TFIID directly interacts with specific SEC components, we purified TFIID from cells expressing FLAG-HA-tagged TBP and GST-tagged SEC components following expression from bacterial or baculoviral vectors(Fig. S1C). As shown in Fig. S1D, the purified TFIID did not show presence of SEC components that were tested in our assay. Consistent with our observation of a strong association of TFIID with ectopically-expressed AF9 (Fig. 1A and (Biswas et al., 2011), we observed a direct interaction of purified TFIID
with GST-AF9 but not with GST alone (Fig. 1G). Again, consistent with our immunoprecipitation analysis (Fig. 1F), we also observed similar direct interactions of TFIID with purified EAF1 (Fig. 1H). In contrast, further interaction assays with purified P-TEFb complex failed to reveal any TFIID interactions (Fig. S1E). Our failure to observe a direct P-TEFb interaction with TFIID indicates that the observed CDK9-TFIID association in our pull-down assay (Fig. S1B) is mediated by other CDK9-interacting proteins. Notably, although we failed to observe strong association of TFIID with ectopically-expressed ELL or EAF2 in our cell-based pull-down assays (Fig. 1E-F), we observed their direct interactions in our in vitro assays (Fig. 1H and S1F). This clearly reflects the existence of different modes of interaction between ELL and EAF2 components of SEC with TFIID in vitro and cell-based assays in vivo. Nonetheless, observation of direct interactions between some of the SEC components and TFIID sets stage for subsequent detailed understanding of the importance of these interactions in transcription functions. Since AF9 and EAF1 showed strong interaction with TFIID both in vitro and in vivo, subsequent studies of the role of TFIID-SEC interactions in transcriptional regulation have been focused on these components.

**Distinct domains of AF9 are involved in TFIID and SEC interactions**

Human AF9 contains multiple domains (Fig. 2A) through which it regulates several functions. The C-terminal domain of AF9 mediates its incorporation into the SEC through interactions with AF4 or AFF4 (Biswa et al., 2011; Bitoun et al., 2007; Yokoyama et al., 2010), whereas the N-terminal YEATS domain has been reported to interact both with the histone acetylation and crotonylation marks (Li et al., 2016; Li et al., 2014; Li et al., 2017) and with the PAF1 complex (He et al., 2011) to facilitate SEC recruitment and associated Pol II-mediated transcription events. Besides these, no other domains with specific functions have been described. AF9 also contains a serine rich region (hereafter poly-Ser), function of which is completely unknown (Fig. 2A). To investigate a potential function, various AF9 deletion mutants were purified (Fig. 2B) and tested for direct interactions with the purified TFIID. The results (Fig. 2C) show TFIID binding to N-terminal fragments (aa 1-208 and 1-196) containing the YEATS and poly-Ser domains (lanes 4 and 5) but not to a fragment (aa 1-148) lacking the poly-Ser domain (lane 3). Thus, these results clearly indicate a role for the poly-Ser domain of AF9 in its direct interaction with TFIID. Further emphasizing the importance of the poly-Ser region for the TFIID interaction, a central fragment (aa 201-380) showed no interaction (lane 6) while a C-terminal fragment (aa 381-568) showed a weak interaction (lane 7) owing to the presence of a short poly-Ser stretch in this region (Fig. S2A).

To establish a corresponding intracellular interaction, 293T cells were transfected with plasmids expressing different domains of AF9. Notably, C-terminal deletion fragments of AF9 that retained the poly-Ser domain also maintained TFIID interactions equivalent to those observed with intact AF9 (Fig. 2D, lanes 3-6), whereas N-terminal deletion fragments lacking the poly-Ser domain showed no interaction with TFIID (lanes 7 and 8). Interestingly, consistent with our earlier observation (Biswa et al., 2011), these N-terminal deletion constructs fully retained their ability to interact with the SEC as evidenced by their association with components of the P-TEFb complex, whereas the C-terminal deletion...
fragments failed to do so (Fig. 2D, lanes 4-6 and lanes 7-8). In further support of a role for the poly-Ser domain in AF9-TFIID interaction, an AF9 mutant with an internal 149-190 amino acid deletion of the poly-Ser domain showed no interaction with TFIID but retained its ability to associate with other components of the SEC (Fig. 2E, lane 6 vs. lane 5). Based on these collective interaction studies, we conclude that distinct regions of AF9 mediate interactions with TFIID (N-terminal poly-Ser domain) and the SEC (C-terminal domain) (Fig. S2B). These results thus establish a potential for SEC recruitment to promoter proximal regions, and subsequent transcription events, through interactions of AF9 with promoter-bound TFIID.

Distinct domains of EAF1 are involved in TFIID and SEC interactions

Our demonstration of a direct TFIID-EAF1 interaction led us to investigate responsible domain in EAF1 for TFIID interaction. Since EAF1, like AF9, also possesses a stretch of serine residues at the C-terminus (Fig. 2F and S2C), we tested a role for this poly-Ser domain in the EAF1-TFIID interaction following expression and purification of EAF1 deletion mutants (Fig. 2G). As shown in Fig. 2H, full-length EAF1 (lane 3) and a C-terminal EAF1 fragment (aa 181-268) containing the poly-Ser domain (lane 7) showed comparable TFIID interactions. In contrast, an N-terminal fragment containing sequences (aa 1-187) upstream of the poly-Ser domain (lane 5) and fragments containing the poly-Ser sequence but lacking either N-terminal (aa 188-268) or C-terminal (aa 1-216) flanking sequences failed to interact (lanes 6 and 4, respectively). These results indicate that the EAF1-TFIID interaction is mediated through a C-terminal EAF1 fragment that includes the poly-Ser domain and its flanking sequences.

To establish a corresponding intracellular interaction, 293T cells were transfected with plasmids expressing various EAF1 deletion constructs. As shown in Fig. 2I, an EAF1 fragment containing the poly-Ser domain but lacking downstream C-terminal sequences (aa 1-208), as well as fragments containing the poly-Ser sequences but lacking variable lengths of N-terminal sequences (aa 61-268, 121-268 and 181-268), showed normal or near normal TFIID interactions but no interactions with other SEC components (lanes 4, 5, 7-9). In contrast, an N-terminal fragment lacking the poly-Ser sequences (aa 1-148) showed no TFIID interaction (lane 5) but retained interactions with SEC components ELL and P-TEFb (lane 5) whereas a shorter N-terminal fragment (aa 1-88) showed no interactions (lane 6). Thus, like AF9, within cells, EAF1 also uses two distinct regions for its interactions with TFIID (C-terminal poly-Ser domain) and SEC (N-terminal domain) (Fig. S2D).

Poly-Ser domains are found in majority of the MLL fusion proteins (Luo et al., 2001; Simone et al., 2003). Like AF9 and EAF1, AF4 also possesses a poly-Ser domain (Fig. S2E) that has been reported to play a role in selectivity factor 1 (SL1)-mediated recruitment of pre-initiation factors for RNA polymerase I-mediated gene expression (Okuda et al., 2015). Based on these and our aforementioned results, we tested the possibility that the poly-Ser domain of AF4 might interact with TFIID, and thus contribute to the TFIID-SEC interaction. We purified different deletion mutants following their expression in bacterial system (Fig. S2F) and subjected them to in vitro interaction assay with the purified TFIID. As shown in Fig. S2G, an AF4 fragment containing the poly-Ser domain (aa 376-550) failed to interact
with TFIID whereas a more C-terminal fragment (551-725) showed a very weak TFIID interaction. Therefore, whereas the AF4 poly-Ser domain-containing fragment is not sufficient for TFIID interaction, the weak interaction of the C-terminal fragment might conceivably contribute to the observed weak intracellular interaction of ectopic AF4 with TFIID (Fig. 1E).

**Distinct TFIID subunits are involved in AF9 and EAF1 interactions**

For a deeper understanding of AF9 and EAF1 interactions with TFIID and its role in transcriptional regulation of target genes, we employed a baculovirus/Sf9 cell-based expression system to identify specific TFIID subunits (TAFs) that directly interact with AF9 and EAF1. We generated baculoviruses that express target TAF proteins and TBP as FLAG-tagged (Fig. 3A, input lanes). Co-infection of Sf9 cells with baculoviruses expressing TAF subunits and AF9 and subsequent immunoprecipitation analysis showed specific and strong interaction of AF9 with TAF5 and TAF6 (Fig. 3A, lanes 5 and 6). Although the other TAF subunits were expressed, they failed to show an interaction with AF9. This observation clearly shows the specificity of the interactions between the target AF9 and TAF subunits. A similar experiment further showed TAF7, TAF8, and TAF9-specific interaction with EAF1 (Fig. 3B, lanes 9-11). Therefore, we conclude that AF9 and EAF1 interact directly with distinct TAF subunits of TFIID.

**Domain analysis of TAF6 for its interaction with TFIID and SEC**

Next, using TAF6 as an example, we assessed AF9 interaction sites within TAF6. We created baculoviruses that express Flag-tagged TAF6 proteins with sequential ~150 amino acid N and C-terminal deletions. Co-immunoprecipitation analyses following co-expression of AF9 and individual TAF6 deletion mutants in Sf9 cells clearly showed that like full-length TAF6, the smallest C-terminal deletion fragment (1-227) containing the histone fold domain (Fig. 3C, upper diagram) retained its interaction with AF9 (Fig. 3C, lane 7). However, deletion of 150 amino acids from the N-terminus completely abolished TAF6 interaction with AF9 (Fig. 3C, lane 8). We conclude that the N-terminal 150 amino acids of TAF6 are important for its interaction with AF9.

To further investigate the function of TAF6 domains in TFIID-SEC interactions, we created similar TAF6 deletion constructs in a mammalian vector and expressed them in 293T cells. Consistent with the baculovirus expression-based interaction results, immunoprecipitation analyses showed that the minimal TAF6 fragment (1-227) required for the TFIID interaction also retained its interaction with AF9 and AF9-associated P-TEFb in 293T cells (Fig. 3D, lanes 3-6). Similarly, an N-terminal deletion of 150 amino acids completely abolished TAF6 interaction with TFIID as well as AF9 and associated SEC (P-TEFb) components (Fig. 3D, lane 7). Since similar patterns were observed for TAF6 interactions with TFIID and SEC/P-TEFb, we conclude that TAF6 uses overlapping N-terminal domain for its interactions with SEC and TFIID.

In order to further identify specific regions within the N-terminal TAF6 domain involved in TFIID versus SEC interactions, we created 30 amino acids deletion fragments of the minimal TAF6 domain (1-227), which is known to contain a histone fold domain that is
important for TFIID assembly and integrity (Grant et al., 1998; Nagy and Tora, 2007).

Immunoprecipitation analyses following expression of these deletion mutants in 293T cells
revealed that deletion of the N-terminal 30 amino acids abolished TAF6 association with
TFIID but not with the SEC components (Fig. 3E, lane 8), whereas, a further N-terminal
deletion (91-227) reduced the P-TEFb interaction but retained a weak AF9 interaction.

Therefore, we conclude that both TFIID and SEC interact with the N-terminus of TAF6,
wherein, the extreme N-terminus are selectively required for the TFIID interaction and more
C-terminal residues are required for interactions with SEC components.

**TFIID-dependent recruitment of EAF1 and AF9 proteins and associated higher order
complexes on DNA template**

To address a potential role of TFIID in directly recruiting SEC components to target gene
promoters, we monitored their binding to an immobilized DNA template containing five
GAL4 binding sites upstream of the adenovirus major late promoter (Fig. 4A) (Kim et al.,
2009; Kim et al., 2010). An initial test of the role of TFIID in AF9 recruitment showed
significant recruitment of AF9 only in the presence of TFIID (Fig. 4B, lane 3 versus lane 1).

Moreover, TFIID also facilitated the recruitment of a purified in vitro reconstituted AF9-
AF4 complex (Fig. 4C).

Using purified AF9 fragments, we next tested whether TFIID-mediated AF9 recruitment
depends upon the poly-Ser domain within AF9. As shown in Fig. 4D, the AF9 fragment
(1-208) containing the poly-Ser domain was recruited to the DNA template in the presence
of TFIID, whereas, other fragments lacking the poly-Ser domain were not (compare lane 4
vs lanes 5-6). To rule out the involvement of additional sequences present within the AF9
fragment (1-208) in this recruitment, we tested two additional N-terminal AF9 fragments
with or without the poly-Ser region. As shown in Fig. 4E, the N-terminal 1-208 and 1-196
fragments containing the poly-Ser domain showed TFIID-dependent recruitment (lanes 4
and 6) whereas, the AF9 fragment lacking the poly-Ser domain (1-148) showed no
recruitment (lane 5). From these analyses, we conclude that human TFIID is directly
involved in recruiting the SEC component AF9 on DNA template through specific domain-
dependent interactions between TFIID and AF9.

Given our demonstration of direct TFIID-EAF1 interactions, we investigated the possibility
of a TFIID-dependent EAF1 recruitment to the DNA template. As shown in Fig. 4F, EAF1
was recruited to the template in the presence (lane 3), but not in the absence (lane 2) of
TFIID, clearly showing a TFIID-dependent EAF1 recruitment. Related to this issue, and the
possibility of a similar recruitment mechanism for a higher-order EAF1 complex, we
reconstituted a recombinant ELL-EAF1 complex (Fig. 4G, left panel) that also showed a
TFIID-dependent recruitment to the DNA template (Fig. 4G, right panel, lane 1 vs lane 2).

Since our earlier analysis showed specific domain-dependent interaction of EAF1 and
TFIID, we also addressed the possibility of a similar domain-dependent recruitment of
EAF1 on DNA template. In support of this mechanism and as shown in Fig.4H, the EAF1
fragment containing the minimal 181-268 region that retained a TFIID interaction in both in
vitro and cell-based assays showed a TFIID-dependent recruitment (lane 10)comparable to
that of the full length EAF1 (lane 6). Moreover, and consistent with earlier observations,
further deletion of amino acids (181-187) completely abolished TFIIID-dependent EAF1 recruitment (Fig. 4H, lane 10 vs lane 9). Collectively, these results establish a role for TFIIID, through direct interactions involving specific domains, in recruiting SEC components on a DNA template.

**AF9-dependent SEC-TFIIID interactions in mammalian cells**

Towards deeper understanding of the role of TAF6 and AF9 in TFIIID and SEC-mediated regulation of target gene expression, we initially determined whether the TFIIID-SEC interaction within mammalian cells involves the TAF6-AF9 interaction. For that purpose, we generated shRNA constructs for knocking down TAF6 and AF9 in 293T cells (Fig. 5A-B). Subsequent experimental strategy (Fig. 5C) involved the stable shRNA-mediated knockdown of TAF6 and AF9 in a FLAG-HA TBP-expressing stable cell line (Fig. 5D, and 5E), followed by immunoprecipitation of TBP and immunoblot analysis of associated factors. As shown in Fig. 5D, and consistent with observations in Drosophila S2 cell line (Wright et al., 2006), TAF6 knockdown led to reduced expression of most of the other tested TAFs without affecting exogenously expressed TBP (Fig. 5D, input panel and S3A). This lower expression also resulted in a corresponding reduced association of the tested TAF components with TBP (Fig. 5D, IP panel). Notably, the reduced TAF6 association with TBP drastically reduced the normal SEC association with TBP (Fig. 5D, lanes 3 and 4). Interestingly, the AF9 knockdown had no significant effect on the expression(Fig. S3A) or the association (Fig. 5E, lanes 3 and 4) of endogenous TAFs and ectopic TBP. However, and reflecting the reduced expression and association of AF9 with TBP, the normal association of the P-TEFb components of SEC was completely abolished (Fig 5E, lanes 3 and 4). Thus, we conclude that AF9 interaction with TFIIID is important for TFIIID and SEC association within mammalian cells.

**MED26-independent TFIIID-SEC interaction within mammalian cells**

Earlier studies have shown a role of the MED26 subunit of the Mediator in EAF1-dependent recruitment of SEC components to certain target genes(Takahashi et al., 2011; Takahashi et al., 2015). Since human Mediator components also interact with TFIIID (Malik and Roeder, 2010, 2016), we determined whether our observed TFIIID and SEC association is dependent on MED26 within mammalian cells. We initially tested the efficiency of our generated shRNAs towards knocking down MED26 in 293T cells (Fig. 5F). Further knockdown MED26 in our Flag-TBP stable cell line (Fig. 5G, input panel) did not show any effect on expression of the TAF subunits that were tested (Fig. 5G, input lanes). Employing the strategy shown in Fig. 5G (left panel), we found that MED26 knockdown did not significantly impact SEC association with the TFIIID (Fig. 5G, IP lanes). However, this analysis consistently indicated a selective reduction of TFIIID-associated AF9 relative to other SEC components (P-TEFb, ELL) tested, suggesting the possibility of an alternative MED26-AF9 interaction outside of the context of the TFIIID-SEC complexes. Nevertheless, the results clearly indicate a significant MED26-independent SEC-TFIIID interaction in mammalian cells. A similar, albeit less efficient, knockdown of MED26 in the Flag-EAF1 stable cell line also did not have much impact on EAF1 interaction with either the TFIIID or ELL (Fig. 5H). Therefore, we conclude that the observed interactions of AF9 and EAF1 with
the TFIID are generally independent of MED26, although it remains possible that MED26 may play an additional role, beyond TFIID, in recruiting the SEC to selected promoters.

Co-regulation of expression of diverse set of genes by both TAF6 and AF9 within mammalian cells

Towards identification of target genes whose expression is directly co-regulated by AF9 and TAF6, we noted an earlier study reporting a set of genes whose expression is affected by knockdown of AF9 in HeLa cells (Li et al., 2014). We made stable shRNA-based TAF6 and AF9 knockdown cells (Fig. 5A, and 5B) and analyzed RNA expression of selected genes by qRT-PCR. As shown in Fig. 6A, upon knockdown of TAF6 and AF9, expression of most of these genes was significantly reduced. Once validated in 293T cells, we wanted to identify genes that would be co-regulated by both TAF6-AF9 pathway in a genome-wide manner in 293T cells. We performed RNA-Seq analysis for identifying genes that would show down-regulation of expression upon TAF6 and AF9 knockdown in 293T cells. We observe significantly high number of genes showing downregulation (≥1.3 fold with p value ≤0.05) upon AF9 knockdown than TAF6 (~1400 for TAF6 knockdown vs ~3500 for AF9 knockdown) (Fig. S3B). Gene Ontology analysis of AF9 and TAF6 co-regulated genes further indicated involvement of majority of these genes in controlling cell growth and motility (Fig. S3C). Accordingly, we checked the proliferation rates of the AF9 and TAF6 knockdown 293T cells relative to control scramble cells. As shown in Fig. S3D (upper panel), knockdown of both these factors significantly reduced proliferation rate relative to the control cells. Similar knockdown in HCT116 cells also had the same effects on cell proliferation, indicating that the effects are not cell type-specific (Fig. S3D, lower panel). Consistent with these observations, AF9 knockdown 293T cells also showed reduced colony forming ability as shown in Fig. S3E.

Since our in vitro assays showed a direct role of TFIID in recruitment of SEC components, we further tested for a similar function for TFIID on target genes within mammalian cells. As shown by ChIP analyses at promoter proximal region (primers flanking transcription start site) in Fig. 6B-D and S4A-B, knockdown of TAF6 greatly impaired TFIID recruitment (as measured by bound TBP). Since knockdown of TAF6 also affects expression of other TAF subunits (Fig. S3A), a reduction of TFIID on target genes could also be a reflection of reduced abundance of other TFIID components in TAF6 knockdown cells. Nevertheless, the reduced TFIID recruitment also leads to reduced recruitment of SEC components (e.g., CDK9 and ELL), as well as Pol II, on target genes. Thus, our data clearly show a role of TFIID-mediated SEC components recruitment to promoter proximal region for transcriptional activation by Pol II.

In a parallel analysis, and consistent with the complementary effects on expression of selected target genes, AF9 knockdown reduced recruitment of ELL and CDK9 to promoter proximal regions of these genes. (Fig. 6B-D and S4A-B). AF9 knockdown also led to increase in total Pol II accompanied by reductions in its Ser2 and Ser5 phosphorylated form at the promoter proximal regions of the tested genes (Fig. 6B-D, S4A-B). Since phosphorylated CTD Ser2 and Ser5 are hallmark signatures of transcriptionally-active Pol II, a reduction of these signatures indicate a role of AF9 in regulating release of paused Pol II
such that the AF9 knockdown results in increased abundance of paused Pol II at the promoter proximal regions. Indeed, pausing index analysis (a ratio of total Pol II present at promoter proximal region / coding region) further showed increased pausing of Pol II on all tested genes upon AF9 knockdown (Fig. S4C). Increase in paused Pol II is also correlated well with reduced P-TEFb recruitment at the promoter proximal region upon AF9 knockdown (Fig. 6B-D, S4A-B). Since recruitment of P-TEFb complex is essential for Pol II release from the promoter proximal region, we conclude that AF9-mediated SEC recruitment (containing P-TEFb complex) at the promoter proximal region is important for release of paused Pol II. Interestingly, although the AF9 knockdown did not change the level of TFIID components and TFIID integrity (Fig. S3A, and 5E), it dramatically reduced TFIID recruitment (monitored by TBP occupancy) at the promoter proximal regions of the tested target genes (Fig. 6B-D and S4A-B). Since paused Pol II has been shown to inhibit PIC assembly at the promoter region (Shao and Zeitlinger, 2017), the increase in paused Pol II following AF9 knockdown may reduce PIC assembly by reducing TFIID recruitment.

**AF9 is required for inducible gene expression**

As the above-described assays of transcriptional regulation by TFIID and SEC were in the context of basal level expression of target genes, we next investigated the role of TFIID-dependent SEC recruitment in regulation of inducible gene expression. Since recruitment of TFIID is required for p53 activator-dependent expression of \( CDKN1A \) gene (\( p21 \) gene) upon doxorubicin treatment (Lauberth et al., 2013), we used \( p21 \) as a model gene for an understanding of the role of TFIID-dependent SEC recruitment in regulation of inducible gene expression. Since TAF6 knockdown also affects overall TFIID integrity, we focused on the effect of AF9 knockdown on \( p21 \) gene expression. As shown in Fig. 6E, AF9 knockdown results in impaired doxorubicin-mediated induction of \( p21 \) gene based on qRT-PCR RNA analyses. In view of the time course of \( p21 \) RNA expression (Fig. 6E), subsequent studies of factor recruitment at promoter proximal region were carried out at 16 hrs time point.

As shown in Fig. 6F, AF9 knockdown results in impaired recruitment of both TFIID and SEC components at promoter proximal region of \( p21 \) gene. Further, and consistent with reduced recruitment of SEC components at the promoter proximal region, AF9 knockdown also led to lower TFIID and SEC levels in the coding region of \( p21 \) gene. Notably, AF9 knockdown also resulted in a substantial increase in Pol II occupancy at the promoter proximal region of the induced \( p21 \) gene along with decreased occupancies of the CTD Ser2- and Ser5-phosphorylated forms of Pol II (Fig. 6F) and thus further indicating accumulation of paused Pol II in AF9 knockdown cells upon doxorubicin treatment. We conclude that AF9-mediated SEC recruitment is also required for release of paused Pol II from the promoter proximal region of induced genes. Consistent with impaired \( p21 \) expression, AF9 knockdown cells showed reduced ability in colony formation after doxorubicin treatment for 8 hrs (Fig. S4D).
The AF9 poly-Ser domain is required for TFIID-dependent recruitment of AF9 and other SEC components on target genes

Since our in vitro and in vivo cell-based assays showed a direct role of the AF9 poly-Ser domain in TFIID-dependent SEC interactions and recruitment, we further addressed the role of this domain on AF9 recruitment to target genes. To this end, we used the AF9 knockdown cells and analyzed the effects of ectopically expressed full-length and poly-Ser domain-deleted AF9 (Fig. 7A) on target gene expression and factor recruitment. Notably, reintroduction of intact AF9 significantly restored expression of tested target genes whereas expression of poly-Ser domain-deleted AF9 failed to do so (Fig. 7B, compare EV (empty vector) vs WT and EV vs AF9ΔSer).

Next, we tested the recruitment of AF9, AF9-associated SEC components and TFIID (measured through TBP) on the target genes. As shown in Fig. 7C-E, although full-length AF9 gets recruited on target genes, the AF9 poly-Ser deletion fragment failed to do so. Owing to the restoration of AF9 recruitment, SEC recruitment is also restored. These observations clearly show a role of AF9 in recruitment of SEC components at the promoter proximal region of target genes for transcriptional activation. Interestingly, recruitment of SEC factors resulted in an increased transcriptional activity along with reduced abundance of paused Pol II at the promoter proximal region when compared to AF9 knockdown cells (compare Fig. 7C-E, RNA Pol II, EV vs full-length AF9). Increased transcriptional activity is further supported by increased abundance of phosphorylated Ser2 and Ser5 form of Pol II at the promoter proximal region (Fig. 7C-E). Despite retaining its ability to interact with SEC components (Fig. 2E), our observation that the poly-Ser domain deletion of AF9 results in its impaired recruitment, clearly shows a role for poly-Ser domain-mediated TFIID interaction in AF9 recruitment at the promoter proximal region of target genes. An impaired AF9 recruitment further resulted in failure to recruit SEC and restore target gene expression as evidenced in Fig. 7B and 7C-E. Owing to the restoration of target gene expression in AF9 knockdown cells by full-length AF9, we have observed restoration of impaired proliferative and colony forming ability of AF9 knockdown cells as shown in Fig. 7F and 7G. Since AF9 poly-Ser deletion fragment failed to restore impaired target gene expression in AF9 knockdown cells, it also failed to restore impaired proliferation and colony forming ability as shown in Fig. 7F and 7G.

Overall, our in vitro and in vivo cell-based assays clearly shows a role of human TFIID in specific domain-dependent recruitment of SEC at the promoter proximal region for release of paused Pol II for productive elongation. An impairment of this recruitment results in increased abundance of paused Pol II at the promoter proximal region. An increased paused polymerase, thus, in turn, further inhibits upstream pre-initiation complex assembly during transcriptional regulation as has been reported by a recent study (Shao and Zeitlinger, 2017). Therefore, beyond its proposed role in the initiation step of transcription, our study establishes a direct role for TFIID in regulating pause and release step through recruitment of SEC components at the promoter proximal region for transcriptional activation in mammalian cells.
Discussion

In this report, we show a role of human TFIID in recruiting SEC components at the promoter proximal region. Our results clearly indicate that multiple TAF subunits are involved -- through their specific interactions -- in overall SEC recruitment. Using in vitro interaction assays through purified proteins/complexes and in vivo cell-based assays, we clearly show that the SEC components and TAF subunits use specific domains for their interactions. Using AF9 as an example, we show that specific domain-mediated interaction between AF9 and TFIID is important for SEC recruitment at the promoter proximal region for transcriptional activation within mammalian cells. Our results further show that an impaired recruitment of SEC components results in increased abundance of paused Pol II at the promoter proximal region of both un-induced as well as induced genes in AF9 knockdown cells. Restoration of full-length AF9 expression in the AF9 knockdown cells results in restoration of target gene expression and factor recruitment, whereas, the poly-Ser-deleted AF9 fails to do so. Despite having its capability to interact with the SEC, the failure of poly-Ser-deleted AF9 to get recruited at the promoter proximal region further suggests existence of TFIID-mediated AF9/SEC recruitment and not vice versa for overall regulation. A model for this overall regulation is shown in Fig. 7H.

Promoter proximal pausing and role of different factors in overcoming pausing event

A few reports have described the role of factors including Brd4, c-Myc, and AF4/AFF4 in P-TEFb complex recruitment at the promoter proximal region (Biswa et al., 2011; He et al., 2010; Rahl et al., 2010; Winter et al., 2017; Yang et al., 2005; Yokoyama et al., 2010; Zhang et al., 2004). Besides P-TEFb-containing complexes, human PAF1 complex has also been shown to have roles in both pausing as well as release of paused Pol II from the promoter proximal region by two different studies (Chen et al., 2015; Yu et al., 2015). However, the role of initiation factors connecting the step of transcriptional initiation and events downstream of TSS is poorly understood.

In this regard, recent cryo-EM structures of human TFIID bound to promoter DNA hints at additional functions of TFIID in regulating transcription beyond the initiation step(Cianfrocco et al., 2013; Nogales et al., 2017; Plaschka et al., 2016). The fact that few TAF subunits reside well within the +30 to +40 nucleotide downstream of transcription start site (TSS), further indicates a possible role for TAF subunits -- residing within TFIID -- in regulating Pol II-mediated transcription events after promoter escape. Consistent with this model, our results have shown a direct role of TFIID in regulation of SEC recruitment at the promoter proximal region. An impaired recruitment of the SEC components results in pausing of escaped Pol II as evidenced by increased presence of total Pol II and decreased presence of its phospho-Ser2 and Ser5 form upon knockdown of AF9. However, knockdown of TAF6, which also affects overall TFIID integrity, fails to show any such pausing events (Fig. 6B-D and S3D-F). Knockdown of TAF6 also affects overall Pol II recruitment, thereby indicating that recruitment of active TFIID is a prerequisite for Pol II recruitment at the promoter region. Subsequent TFIID-mediated recruitment of AF9 and AF9-associated SEC components including P-TEFb complex, is absolutely essential for release of paused Pol II from the promoter proximal region.
The observation that AF9 knockdown results in reduced TBP recruitment at the promoter region indicates a possible role of AF9 and AF9-associated SEC in TFIID recruitment at the promoter proximal region. However, based on the recent report showing a role of paused Pol II towards inhibiting PIC formation (Shao and Zeitlinger, 2017), we believe the reduced TFIID recruitment is a result of increased paused Pol II at the promoter proximal region. This hypothesis is further substantiated by the observation that the AF9 poly-Ser deletion fragment fails to get recruited at the promoter proximal region despite retaining its capacity to interact with the SEC (Fig. 2E and Fig. 7C-E). Therefore, poly-Ser domain-mediated AF9 and TFIID interaction is essential for AF9 and associated SEC recruitment at the promoter proximal region for overall transcriptional activation.

**Important domain for connecting early events during transcriptional activation**

Earlier reports have shown a role of the N-terminal YEATS domain of AF9 in recognizing the acetylated and crotonylated histone H3 mark for its recruitment for transcriptional activation (Li et al., 2016; Li et al., 2014; Li et al., 2017). The YEATS domain has also been shown to interact with the PAF complex for PAF-dependent SEC recruitment for transcriptional activation (He et al., 2011). Our studies have deciphered another important domain (poly-Ser domain) that exclusively helps in TFIID-dependent AF9-containing SEC recruitment at the promoter proximal region. Similar poly-Ser rich domain present in other SEC components also plays a role in transcriptional activation (Luo et al., 2001; Simone et al., 2003). However, unlike AF9, the poly-Ser rich domain present in EAF1 is not sufficient for TFIID interactions as has been observed by our analysis both *in vitro* as well as *in vivo*. Therefore, our data deciphers an important function of poly-Ser domains within the SEC components that are involved in SEC recruitment for connecting transcriptional events at the promoter proximal region.

**Star Methods**
### Key Resources Table

| REAGENT or RESOURCEs | SOURCE | IDENTIFIER |
|----------------------|--------|------------|
| Antibodies           |        |            |
| CyclinT1             | Santa Cruz Biotechnology | Cat#sc-10750 |
| CDK9                 | Santa Cruz Biotechnology and Bethyl Lab | Cat# sc-8338 and Cat# A303-492A |
| FLAG epitope         | Sigma  | Cat# F7425 |
| GST                  |        |            |
| AF4                  | Abcam  | Cat# ab31812 |
| AF9                  | Bethyl Lab | Cat# A300-596 |
| Rpb1 (4H8 clone)     |        |            |
| Phospho Rpb1 CTD (Ser2) | Cell Signaling Technology | Cat# 13499 |
| Phospho Rpb1 CTD (Ser2) | Cell Signaling Technology | Cat# 13523 |
| Beta Actin           |        |            |
| EAF1                 | Santa Cruz Biotechnology | Cat# sc47778 |
| EAF2                 | Bethyl Lab | Cat# A302-503A |
| ELL                  | Bethyl Lab and Cell Signaling Technology | Cat# A301-645A, Cat# D7N6U |
| TAF1                 | Bethyl Lab and Cell Signaling Technology | Cat# A303-505A, Cat# D6J8B |
| TAF2                 | Bethyl Lab | Cat# A302-132A |
| TAF3                 | Abcam  | Cat# ab221633 |
| TAF5                 | Bethyl Lab | Cat# A303-687A |
| TAF6                 | Bethyl Lab | Cat# A301-276A |
| TAF7                 | This lab | N/A |
| TAF9                 | This lab | N/A |
| TBP                  | Bethyl Lab and Cell Signaling Technology | Cat# A301-229A |

### Bacterial and Virus Strains

|                | SOURCE | IDENTIFIER |
|----------------|--------|------------|
| DH5α E.Coli    | This lab | N/A |
| BL21 E.Coli    | This lab | N/A |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER | Description |
|---------------------|--------|------------|-------------|
| dNTP mix | BioBharati Life Science | Cat# BB-C0020B | Protein or Peptide |
| ATP | BioBharati Life Science | Cat# BB-C0030 | Protein or Peptide |
| T4 DNA ligase | NEB | Cat# M0503S | Protein or Peptide |
| XL Flag peptide | Sigma | Cat# F4799-25MG | Protein or Peptide |
| L-Glutathione reduced | Sigma | Cat# G425-25G | Protein or Peptide |
| Vero cDNA synthesis kit | Life Technologies | Cat# AB1453A | Protein or Peptide |
| TRIzol | Life Technologies | Cat# 15596026 | Protein or Peptide |
| 3X Flag peptide | Sigma | Cat# 15101G | Protein or Peptide |
| Gentamycin | Gold Biotechnology | Cat# G-400-100 | Protein or Peptide |
| Penicillin and Streptomycin | Life Technologies | Cat# 15760-026 | Protein or Peptide |
| Purified human TFIID complex | This study | N/A | Protein or Peptide |
| Purified recombinant GST-AF9 | This study | N/A | Protein or Peptide |
| Purified recombinant GST-EAF1 | This study | N/A | Protein or Peptide |
| Purified recombinant GST-EAF1.FLAG-ELL | This study | N/A | Protein or Peptide |
| Purified recombinant GST-AF9.FLAG-AF4 | This study | N/A | Protein or Peptide |
| Critical Commercial Assays | Qubit RNA HS assay kit | Cat# Q28852 | Protein or Peptide |
| Critical Commercial Assays | Agilent Bioanalyzer RNA Nano 6000 Kit | Cat# 5067-1511 | Protein or Peptide |
| Critical Commercial Assays | Agilent Bioanalyzer RNA Ultra II Library preparation kit for Illumina | Cat# 5067-1503 | Protein or Peptide |
| Critical Commercial Assays | Agilent Bioanalyzer DNA HS assay kit | Cat# 5067-1505 | Protein or Peptide |
| Critical Commercial Assays | Agilent Bioanalyzer RNA Ultra II Library preparation kit | Cat# 5067-1503 | Protein or Peptide |
| Critical Commercial Assays | Agilent Bioanalyzer DNA HS assay kit | Cat# 5067-1505 | Protein or Peptide |
| **REAGENT or RESOURCEs**                                                                 | **SOURCE**                  | **IDENTIFIER**               |
|-----------------------------------------------------------------------------------------|-----------------------------|------------------------------|
| Deposited Data                                                                           |                             |                              |
| List of genes showing downregulation of expression upon AF9 and TAF6 knockdown           | This study (Mendeley data)  | http://dx.doi.org/10.17632/3yz7xxkgw.1 |
| Unprocessed raw images of western blot                                                   | This study (Mendeley data)  | http://dx.doi.org/10.17632/bx4ky7pdy.c1 |
| Raw data for RNA-Seq analysis for differential RNA expression upon TAF6 and AF9 knockdown compared to control (Scramble) cells | This study (GenBank, NCBI)   | SRA Accession no: PRJNA512165 |
| **Experimental Models: Cell Lines**                                                      |                             |                              |
| Flp-In™ -293                                                                             | Invitrogen                  | Cat# R75007                   |
| Flp-In™ T-REX™ -293                                                                      | Invitrogen                  | Cat# R78007                   |
| SF9 insect cell line                                                                     | Invitrogen                  | Cat# B-82501                  |
| HEK293T                                                                                 | ATCC                        | Cat# CRL-3216                 |
| HCT116                                                                                  | ATCC                        | Cat# CCL-247                  |
| **Oligonucleotides**                                                                    |                             |                              |
| AF9 knockdown shRNA Upper oligo                                                          | This paper (IDT)            | N/A                          |
| (CCGGGCGGTGTTTACTCATTTTCGAGAAAGATGGATAACACACTGGCCTTTTG) lower oligo                      |                             |                              |
| (AATTCGAAAAAGCAGTGGTTACTCATTTTCGAGAAAGATGGATAACACACTGGC)                                |                             |                              |
| TAF6 knockdown shRNA # 4                                                                 | Open Biosystems             | N/A                          |
| (CCGGAAAATGCTGCTACCAGTTTACCTCGAGGTTAATCTGGATGCACTATTCTTTTCG) lower oligo                 | This paper (IDT)            | N/A                          |
| (AATTCGAAAAAGCAGTGGTTACTCATTTTCGAGAAAGATGGATAACACACTGGC)                                |                             |                              |
| MED26 knockdown shRNA # 4 Upper oligo                                                     | This paper (IDT)            | N/A                          |
| (CCGGGAAAATGCTGCTACCAGTTTACCTCGAGGTTAATCTGGATGCACTATTCTTTTCG) lower oligo                 |                             |                              |
| (AATTCGAAAAAGCAGTGGTTACTCATTTTCGAGAAAGATGGATAACACACTGGC)                                |                             |                              |
| MED26 shRNA # 5 Upper oligo                                                              | This paper (IDT)            | N/A                          |
| (CCGGATGACCTGAAGAGCCGCAATGCTGACGTGACGATATTCTTTTCG) lower oligo                           |                             |                              |
| (AATTCGAAAAAGCAGTGGTTACTCATTTTCGAGAAAGATGGATAACACACTGGC)                                |                             |                              |
| Primers for RNA analyses                                                                 | This paper (IDT)            | Supplemental Table 2          |
| Primers for ChIP analyses                                                                | This paper (IDT)            | Supplemental Table 3          |
| **Recombinant DNA**                                                                      |                             |                              |
| Plasmid constructs used in this study                                                     | This paper                  | Supplemental Table 1          |
| pLKO.1-TRC cloning vector                                                                | Addgene                     | Plasmid #10878                |
| pSIPAX2 packaging plasmid                                                                | Addgene                     | Plasmid # 12260               |
| pMD2.G envelope vector                                                                  | Addgene                     | Plasmid # 12259               |
| pcDNA5-FRT-TO mammalian expression vector                                                | Invitrogen                  | Cat # V6520-20                |
| pOG44 recombinase plasmid                                                                | Invitrogen                  | Cat # V6005-20                |
| pET-GST bacterial expression vector                                                     | This study                   | N/A                          |

### Software and Algorithms

| **Software and Algorithms** | **SOURCE** | **IDENTIFIER** |
|----------------------------|-------------|----------------|
| Yadav et al. Page 16       |             |                |
| REAGENT or RESOURCES                | SOURCE                     | IDENTIFIER |
|------------------------------------|----------------------------|------------|
| Excel                              | Excel software             | N/A        |
| DAVID                              | (Huang da et al., 2009)    | N/A        |
| DESeq2                             | Bioconductor               | N/A        |
| Graphpad prism                     | graphpad.com               | N/A        |
| Medcalc                            | medcalc.org                | N/A        |

**Others**

| Protein A agarose                  | Thermo fisher Scientific   | Cat# 20333 |
|------------------------------------|----------------------------|------------|
| Sure beads™ Protein G magnetic beads| Bio-Rad                    | Cat# 161-4013|
| Anti-flag M2 agarose beads         | Sigma                      | Cat# 8823-5ML |
| Anti-flag M2 magnetic beads        | Sigma                      | Cat# F2426  |
| GST agarose beads                  | GOLDBIO                    | Cat#G-250-10 |
| Ni-NTA agarose beads               | QIAGEN                     | Cat# 30210  |
| PCR purification kit               | QIAGEN                     | Cat# 28104  |
| Gel extraction kit                 | QIAGEN                     | Cat# 28706  |
| Plasmid miniprep kit               | QIAGEN                     | Cat# 27104  |
Contact for Reagent and Resource Sharing

Questions related to the key resources or request for reagents used in this study, should be addressed to corresponding author by email using ID dbiswas@iicb.res.in

Experimental Model and Subject Details

For all of our experiments, we have used either HEK293T or HCT-116 or Sf9 insect cell culture. HEK293T and HCT-116 cells were maintained in DMEM media supplemented with high glucose and 10% FBS and 1X penicillin-streptomycin solution as mentioned below. Sf9 cells were cultured in Grace’s insect media supplemented with 10% FBS and gentamicin (7μg/ml) at 26°C.

Method Details

Cell Culture and Transfection

All mammalian cell lines were cultured at 5% CO₂ and 37°C in DMEM media (GIBCO) containing high glucose along with 10% FBS (GIBCO) and 1% Penicillin and Streptomycin (GIBCO). For baculoviral expression system, Sf9 cells were cultured in Grace’s Insect media with 10% FBS and Gentamycin(7μg/ml) at 26°C. Mammalian cells were transfected using Fugene transfection reagent as per manufacturer's protocol. Insect cells were transfected with Cellfectin II reagent (Invitrogen) as per manufacturer’s protocol for generating bacloviruses for expression of recombinant proteins in Sf9 cells.

Plasmid construction

All the plasmids used in our study were cloned into appropriate expression vectors as mentioned in Supplemental Table 1. Since number of plasmids used in our study are relatively large, details of their cloning in appropriate vector is available upon request. All these plasmids were cloned either for their expression in mammalian or Sf9 or bacterial cells. Some of the TAF constructs cloned in pFAST-BAC vector for their expression in Sf9 cells, were PCR amplified from cDNA library prepared from 293T cells and were sequenced in its entirety before being used in our assays.

Stable Cell line generation

Generation of CDK9 stable cell line has been described earlier in our study (Ghosh et al., 2018). Stable cell line expressing FLAG-HA TBP was generated in Flp-In T-Rex 293T cells through co-transfection of TBP expressing plasmid with recombinase pOG44 plasmids. Subsequently, stable cells were selected in hygromycin-containing (200μg/ml) media. Individual colonies were screened for expressing FLAG-HA-TBP protein through western blot analysis using epitope-tag-specific antibodies.

Generation of stable knockdown cells

Individual sequences targeting AF9, MED26 (as indicated in Key Resources table) were cloned into pLKO.1 vector. shRNAs designed against TAF6 were obtained from Open Biosystems. The cloned plasmids of interest (500ng) were co- transfected with pMD2.G(125ng) and pPAX2 (375ng) plasmids in 3X 10⁵ cells in a single well of a 6-well
plate. Lentiviral supernatant was collected 72 hours after transfection and stored in -80°C for subsequent use. For generating stable knockdown, cells were transduced with 300μl of viral supernatant in presence of 8μg/ml polybrene. After 24hours of transduction, puromycin resistant stably-integrated knockdown cells were selected in presence of 3μg/ml puromycin containing media. Knockdown of target factors were tested by qRT-PCR analysis for checking RNA expression as well as by western blot analysis using factor-specific antibodies as indicated for protein expression.

**Nuclear Extract preparation**

Nuclear extract preparation starts with measuring packed cell volume (PCV) of harvested cells. Two volumes of PCV of NE1 buffer (10mM Tris-HCl pH7.3, 1.5mM MgCl2, 10mM NaCl and 0.7μl/ml β-Mercaptoethanol) was used for resuspending the harvested cells. Cells were allowed to swell in hypotonic buffer in ice for 15 mins. After resuspending, cells were passaged through 23 gauge syringe for 8 times. The cell lysate was spun at 6000 RPM for 5 mins at 4°C. The obtained pellet are referred as nuclear pellet. 2X of nuclear pellet volume (NPV) of pre-chilled NE2 (20mM Tris-HCl pH7.3, 1.5mM MgCl2, 20mM NaCl, 0.2mM EDTA, 25% glycerol 0.7μl/ml β-Mercaptoethanol and protease inhibitor cocktail from Roche) was added to resuspend the nuclear pellet. Subsequently, 1X NPV of pre-chilled NE3 (20mM Tris-HCl pH7.3, 1.5mM MgCl2, 1.2M NaCl, 0.2mM EDTA, 25% glycerol 0.7μl/ml βmercaptoethanol and protease inhibitor cocktail) was added gradually while vortexing simultaneously. For proper extraction, the lysate was kept in ice for 45 mins and vortexed intermittently at every 3 mins. To obtain nuclear extract, the lysate was spun at at 15000 RPM for 20 mins at 4°C. The supernatant was collected and stored in -80°C for further use or used directly, if required. The efficiency of nuclear extract preparation was confirmed by western blot analysis using target specific antibody against the factor or epitope tag or by Bradford assay before being used in downstream assays.

**Protein purification**

For recombinant expression of full-length AF9, EAF1, EAF2, ELL, deletion fragments of AF9, EAF1 and AF4 proteins, the constructs were cloned into pET-GST vector with GST tag at N-terminus. The mentioned factors were expressed in *E.coli* strain BL21 (DE3) upon induction by 1mM IPTG at 37°C for 4hrs. Cells were harvested and lysed in lysis buffer containing 20mM Tris pH-8, 20% glycerol, 2mM EDTA, 300mM KCl, 2mM β-Mercaptoethanol and 2mM PMSF. The lysed cells were further sonicated at 60% output level for 4min (with 30sec pulse on and off) on ice. The sheared lysate was centrifuged at 15000 RPM for 20 mins at 4°C. To obtain purified protein, the cleared lysate was incubated with glutathione agarose beads (Pierce) for 3-4 hours at 4°C and subsequently washed in the same buffer extensively. Glutathione bead-bound protein was competitively eluted using glutathione peptide in elution buffer containing 30mM reduced L-Glutathione (Sigma) in 100mM Tris pH 8.0.

Recombinant FLAG-tagged ELL, AF4 and CDK9 were expressed using Bac-to-Bac baculoviral expression system (Invitrogen). 48 hours after viral infection, Sf9 cells were harvested and lysed in lysis buffer containing 20mM Tris pH 8.0, 20% glycerol, 2mM EDTA, 300mM KCl, 2mM β-Mercaptoethanol and 2mM PMSF and rotated for 1.5 hours at
4°C. Subsequently lysate was centrifuged at 15000 RPM for 20 mins at 4°C and the supernatant was collected. The cleared lysate can be either stored in -80°C or directly used for downstream purification. For purification, the protein lysate was incubated with anti-FLAG M2 agarose beads (SIGMA) for overnight at 4°C. Protein-bound beads were washed with binding buffer extensively before eluting the bound proteins using elution buffer containing Flag peptide (250ng/μl) in 20mM Tris pH 8.0, 20% glycerol, 2mM EDTA, 100mM KCl.

Human TFIID complex purification

Nuclear extract from Flag-HA TBP stable cells were prepared using the protocol discussed above. For purifying human TFIID complex for *in vitro* studies, 5ml of nuclear extract was diluted two times with BC500 + 0.1% Nonidet P-40. The lysate was incubated with prewashed 100μl of anti-FLAG M2 agarose beads in a buffer containing 20mM Tris pH 8.0, 20% glycerol, 2mM EDTA, 500mM KCl, 2mM β-Mercaptoethanol, protease inhibitor and 0.1% Nonidet P-40 for overnight at 4°C. Protein-bound beads were washed with binding buffer extensively before competitively eluting the bound proteins using elution buffer containing Flag peptide (250ng/μl) in 20mM Tris pH 8.0, 20% glycerol, 2mM EDTA, 100mM KCl. Efficiency of TFIID purification was tested by western blot analysis using antibodies against TBP or various TAFs. Alternatively, silver staining was also used for quantifying the proteins after purifications.

Immunoprecipitation analysis of endogenous proteins

For immunoprecipitation of endogenous TBP and AF9 proteins, factor specific antibodies were used and a parallel analysis using IgG served as negative control for our experiments. Nuclear extracts obtained from 293T cells were used for this purpose. The nuclear extracts were initially pre-cleared with protein-A agarose beads (for 4hrs at 4°C) before being used for immunoprecipitation. In a parallel setup, protein-G magnetic beads were blocked with 1% BSA in buffer containing 20mM Tris pH-8, 20% glycerol, 2mM EDTA, 150mM KCl + 0.1% Nonidet P-40 for 4 hours at 4°C. The pre-blocked protein-G beads were incubated with factor-specific antibodies along with pre-cleared nuclear lysate for overnight 4°C. Protein-bound beads were washed with binding buffer extensively before eluting the bound proteins by boiling at 95°C for 5min in 1X SDS-loading dye for downstream western blot analysis.

Immunoprecipitation and western blot analysis

To identify interactions among different factors, the epitope-tagged full length and deletion constructs were over-expressed by transfection in 293T cells. The cells were harvested after 48 hours of transfection and subsequently subjected to nuclear extract preparation. The FLAG-tagged target factors were immunoprecipitated using anti-FLAG M2 agarose/magnetic beads for overnight at 4°C in a buffer containing 20mM Tris pH-8, 20% glycerol, 2mM EDTA, 300mM KCl (unless otherwise mentioned), 0.1% Nonidet P-40 for 4 hours at 4°C. Protein-bound beads were washed with binding buffer extensively before eluting the bound proteins by boiling at 95°C for 5min in 1X SDS-loading dye for downstream western blot analysis.
Interactions with the target factors were analysed by western blot analysis using factor-specific antibodies. The denatured boiled samples were run in SDS-PAGE gel at 100V until the required size of protein gets properly resolved. Subsequently, resolved proteins were transferred onto a nitro-cellulose membrane. The membrane with transferred proteins was blocked for 1 hour with 5% non-fat milk at room temperature. Primary antibody with appropriate dilution was added and incubated for overnight at 4°C. Next day, the antibody bound membranes were washed for 3X with 1X TBST. HRP conjugated secondary antibody (1:5000) dilution was added and incubated for 1 hour at RT followed by 3 times washing in 1X TBST. Finally, target proteins were detected by chemiluminescence method using ECL.

**Baculovirus expression-based interaction analyses**

Baculovirus expression-based interaction analyses between target factors were performed essentially following the same protocol as mentioned earlier (Ghosh et al., 2018). In brief, Sf9 cells were co-infected with the combination of viruses as indicated in results. 48 hours after infection, the cells were lysed in buffer containing 20mM Tris pH-8, 20% glycerol, 2mM EDTA, 300mM KCl (unless otherwise mentioned), 0.1% Nonidet P-40 for 4 hours at 4°C. The epitope tagged factor were immunoprecipitated using beads against the specific epitope tags by incubating for overnight at 4°C. Protein-bound beads were washed with binding buffer extensively before eluting the bound proteins by boiling at 95°C for 5min in 1X SDS-loading dye for downstream western blot analysis.

**In vitro binding assay**

For direct *in vitro* binding assay, the purified recombinant target factors such as GST-AF9, -EAF1, -EAF2, -ELL and deletion fragments were initially immobilized on Glutathione agarose beads. In all these analyses, purified GST protein served as control. Purified human TFIID complex (as mentioned above), was added to 1μg of immobilized target factors along with GST as control, in a binding buffer containing 20mM Tris pH-8, 20% glycerol, 2mM EDTA, 100mM KCl, 0.1% Nonidet P-40, 10ng/μl BSA, 0.05% Nonidet P-40. The target factors were allowed to bind with each other for overnight at 4°C. Protein-bound beads were washed with binding buffer extensively before eluting the bound proteins by boiling at 95°C for 5min in 1X SDS-loading dye for downstream western blot analysis using factor-specific antibodies as indicated.

**Factor recruitment assay on DNA template *in vitro***

Immobilized DNA template was generated by PCR amplification using pG5ML-Gless cassette containing five copies of GAL4 binding sites upstream of adenovirus major late promoter using a 5'-Biotin conjugated primer. 50μg of this PCR-amplified biotinylated DNA template was used for conjugating with streptavidin-coupled Dynabeads M280 (Invitrogen) in binding buffer (10mM Tris-Cl pH7.5, 1mM EDTA, 1M NaCl). 100ng of this immobilized DNA template was used for subsequent factor recruitment assay as mentioned. Binding assay was performed in a buffer containing 62mM KCl, 12.5% glycerol, 12.5mM HEPES (pH 7.9), 20mM Tris-HCl (pH 7.9), 0.06mM EDTA (pH 8.0), 7.5mM MgCl2, 0.5mg/ml BSA, 0.3mM DTT, and 0.05% Nonidet P-40. For TFIID-dependent factor recruitment assay, TFIID was incubated with immobilized DNA for 1 hour prior to adding the target factors. After incubation of reaction at RT for 2hrs, beads were washed with 1X binding buffer.
extensively. Streptavidin bead-bound proteins were eluted in 1X SDS loading dye by boiling at 95°C for 5’. The recruitment of target factors on DNA template were identified by western blotting using antibodies specific to target factors.

RNA Extraction, Reverse Transcription and quantitative Real-Time PCR Analysis

Total RNA was extracted from a 6-well plate containing 1.2X10⁶ cells using TRIzol Reagent (Invitrogen) following manufacturer’s protocol. 1ug of RNA was reverse transcribed using verso cDNA synthesis kit (Thermo Scientific) using manufacturer’s protocol. cDNA was diluted 25X for quantitative real-time PCR (qRT-PCR) analyses. qRT-PCR was performed using Universal SYBR green supermix (BIORAD). Gene expression level was calculated after normalizing with 18s rRNA, actin, and GAPDH as appropriate.

Chromatin Immunoprecipitation (ChIP) analysis

For ChIP analysis, essentially same protocol was followed as described earlier (Ghosh et al., 2018). Target cells were cross-linked with 1% formaldehyde for 10 min and stopped by using 125mM glycine for 5min. The cross-linked cells were resuspended in lysis buffer (0.5% NP40, 1% TritonX-100, 300mM NaCl, 20mMTris 7.5, 2mM EDTA) and incubated on ice for 30 mins. Lysed cells were subsequently passed for 6 times through 23 gauge syringe. The passaged cells were spun at 6000 RPM for 10 mins at 4°C to obtain nuclear pellets. The isolated nuclear pellet was resuspended in shearing buffer (1% SDS, 10mM EDTA and 500mM Tris pH8.0 with protease inhibitor cocktail) and chromatin was sheared using Bioruptor™ UCD200 (Diagenode) Sonicator for 20 mins (30sec on or off pulse in between). The sheared lysate were centrifuged at 15000 RPM for 20 mins at 4°C. The obtained lysate was diluted 10 times in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.1mM EDTA, 20mM Tris-Cl (pH 8.0) and 167mM NaCl). The diluted lysate was pre-cleared using IgG for 1hour and subsequently incubated with Protein G Magnetic beads (BIORAD) for additional 1 hour. The pre cleared lysate was incubated with required antibody (2μg) for overnight at 4°C. Subsequently, antibody-bound proteins were immunoprecipitated using Protein G Magnetic beads and incubating for 2hrs at 4°C. Bead-bound proteins were extensively washed with low salt buffer (0.1% SDS, 1% Troton X-100, 2mM EDTA, 20mM Tris-Cl (pH 8.0), 150mM NaCl, and freshly added Protease inhibitor cocktail); high salt buffer (0.1% SDS, 1% Troton X-100, 2mM EDTA, 20mM Tris-Cl (pH 8.0), 500mM NaCl, and freshly added Protease inhibitor cocktail); lithium chloride buffer (0.5M LiCl, 1% NP40, 1% deoxycholate, 20 mM Tris-Cl (pH8.0) and 1mM EDTA) and finally 2X with TE buffer. The complex bound DNA was eluted using elution buffer (1% SDS, 0.1M NaHCO₃). Reverse cross-linking was performed in 200mM NaCl by incubating at 65°C for overnight. DNA was purified using PCR purification kit (QIAGEN). The eluted DNA was analyzed using quantitative Real-Time PCR analysis (BIORAD CFX96™ Real-Time-System) for quantifying the enrichment of factor-bound DNA when compared to control IgG (mouse or rabbit as appropriate).

RNA-Seq Analysis

RNA-Seq analyses were performed following the steps as mentioned below.
A  RNA QC—The RNA quantity was checked using Qubit RNA HS assay kit (Thermo Fisher Scientific) and RNA quality was checked with Agilent Bioanalyzer RNA Nano 6000 Kit (Agilent Technologies). The samples with > 7 RNA integrity number (RIN) was taken further for library preparation.

B  Library Preparation—The NGS libraries were prepared using NEB NEXT RNA Ultra II Library preparation kit for Illumina (NEB). In brief, 800 ng of total RNA was used as input for poly (A) mRNA enrichment using NEB Next Poly(A) mRNA Magnetic Isolation Module (NEB) followed by fragmentation and reverse transcription to generate cDNA. Adapter index primers with unique barcodes for each sample was ligated to fragmented double strand cDNA. Ampure beads were used to purify adapter ligated fragments and the purified product was amplified using Illumina primers to generate sequencing library.

C  Library QC—The library quality was checked with Agilent Bioanalyzer DNA 1000 kit (Agilent Technologies) and quantity was checked using Qubit DNA HS assay kit (Thermo Fisher Scientific). Libraries were diluted to 2 nM and pooled to make equimolar sequencing library pool.

D  Sequencing—The pooled libraries were sequenced using Illumina HiseqX to generate 150 bp paired end reads. The library pool was diluted to 250 pM before loading it into the flowcell. Illumina bcl2fastq v 2.19 was used to generate fastq files.

E  Raw sequencing data analysis—After checking sequence data quality, raw sequence reads were processed to remove adapter sequences and low quality bases. The QC passed reads were mapped onto indexed Human reference genome(GRCh38.p7). After removing PCR and optical duplicates, gene level expression values were obtained as read counts. Expression similarity between biological replicates was checked by spearman correlation. For differential expression analysis, the biological replicates were grouped as Control and Test. Differential expression analysis was carried out using DESeq2 package after normalizing the data based on trimmed mean of M values (TMM).

Gene Ontology Analysis

Gene ontology analysis to identify TAF6 and AF9 co-regulated biological processes was performed as described previously (Ghosh et al., 2018). Briefly, RNA-seq data of TAF6 and AF9 was used as the basis to identify common TAF6 and AF9 regulated genes. Identified genes showing significant differential expression and p value <0.05 were classified as per their regulated biological processes. Gene functional annotation was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (Huang et al., 2009). GO terms with p values less than 0.05 were considered as significant.

Cell proliferation assay

For cell proliferation assay, 1X 10^4 stable knockdown HCT116 and 293T cells against control (Scr), TAF6 and AF9 were seeded in 24-well plate. Cells were counted on third and sixth day by using haemocytometer. The restoration of proliferation rate was studied by
transfecting AF9 (WT and poly-Ser del) in AF9 knockdown 293T cells. Counting was followed as described above.

**Colony formation assay**

To study colony formation potential of TAF6 and AF9 knock down cells, 2x10^4 cells were seeded in a 6-well plate and left them to grow until individual colonies were formed (~1-2 weeks). Colonies were fixed using fixing solution containing methanol: glacial acetic acid (3:1) and stained using 0.05% crystal violet stain in methanol for 15 mins. The stained cells were washed thoroughly with distilled water to obtain images of cell colonies with clear background.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**References**

Adelman K, Lis JT. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. Nat Rev Genet. 2012; 13:720–731. [PubMed: 22986266]

Biswas D, Milne TA, Basrur V, Kim J, Elenitoba-Johnson KS, Allis CD, Roeder RG. Function of leukemogenic mixed lineage leukemia 1 (MLL) fusion proteins through distinct partner protein complexes. Proc Natl Acad Sci U S A. 2011; 108:15751–15756. [PubMed: 21896721]

Bitoun E, Oliver PL, Davies KE. The mixed-lineage leukemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. Hum Mol Genet. 2007; 16:92–106. [PubMed: 17135274]

Chen FX, Woodfin AR, Gardini A, Rickels RA, Marshall SA, Smith ER, Shiekhattar R, Shilatifard A. PAF1, a Molecular Regulator of Promoter-Proximal Pausing by RNA Polymerase II. Cell. 2015; 162:1003–1015. [PubMed: 26279188]

Chen FX, Xie P, Collings CK, Cao K, Aoi Y, Marshall SA, Rendleman EJ, Ugarenko M, Ozark PA, Zhang A, et al. PAF1 regulation of promoter-proximal pause release via enhancer activation. Science. 2017; 357:1294–1298. [PubMed: 28860207]

Cianfrocco MA, Kassavetis GA, Grob P, Fang J, Juven-Gershon T, Kadonaga JT, Nogales E. Human TFIID binds to core promoter DNA in a reorganized structural state. Cell. 2013; 152:120–131. [PubMed: 23332750]

Ghosh K, Tang M, Kumari N, Nandy A, Basu S, Mall DP, Rai K, Biswas D. Positive Regulation of Transcription by Human ZMYND8 through Its Association with P-TEFb Complex. Cell Rep. 2018; 24:2141–2154.e2146. [PubMed: 30134174]

Grant PA, Schieltz D, Pray-Grant MG, Steger DJ, Reese JC, Yates JR 3rd, Workman JL. A subset of TAF(I)IIs are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. Cell. 1998; 94:45–53. [PubMed: 9674426]

He N, Chan CK, Sobhian B, Chou S, Xue Y, Liu M, Alber T, Benkirane M, Zhou Q. Human Polymerase-Associated Factor complex (PAFe) connects the Super Elongation Complex (SEC) to RNA polymerase II on chromatin. Proc Natl Acad Sci U S A. 2011; 108:E636–645. [PubMed: 21873227]
He N, Jahchan NS, Hong E, Li Q, Bayfield MA, Maraia RJ, Luo K, Zhou Q. A La-related protein modulates 7SK snRNP integrity to suppress P-TEFb-dependent transcriptional elongation and tumorigenesis. Mol Cell. 2008; 29:588–599. [PubMed: 18249148]

He N, Liu M, Hsu J, Xue Y, Chou S, Burlingame A, Krogan NJ, Alber T, Zhou Q. HIV-1 Tat and host AFF4 recruit two transcription elongation factors into a bifunctional complex for coordinated activation of HIV-1 transcription. Mol Cell. 2010; 38:428–438. [PubMed: 20471948]

Henriques T, Gilchrist DA, Nechaev S, Bern M, Muse GW, Burkholler A, Fargo DC, Adelman K. Stable pausing by RNA polymerase II provides an opportunity to target and integrate regulatory signals. Mol Cell. 2013; 52:517–528. [PubMed: 24184211]

Henriques T, Scruggs BS, Inouye MO, Muse GW, Williams LH, Burkholler AB, Lavender CA, Fargo DC, Adelman K. Widespread transcriptional pausing and elongation control at enhancers. Genes Dev. 2018; 32:26–41. [PubMed: 29378787]

Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009; 4:44–57. [PubMed: 19131956]

Kim J, Guermah M, McGinty RK, Lee JS, Tang Z, Milne TA, Shilatifard A, Muir TW, Roeder RG. RAD6-Mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. Cell. 2009; 137:459–471. [PubMed: 19410543]

Kim J, Guermah M, Roeder RG. The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS. Cell. 2010; 140:491–503. [PubMed: 20178742]

Lauberth SM, Nakayama T, Wu X, Ferris AL, Tang Z, Hughes SH, Roeder RG. H3K4me3 interactions with TAF3 regulate preinitiation complex assembly and selective gene activation. Cell. 2013; 152:1021–1036. [PubMed: 2342851]

Li Q, Price JP, Byers SA, Cheng D, Peng J, Price DH. Analysis of the large inactive P-TEFb complex indicates that it contains one 7SK molecule, a dimer of HEXIM1 or HEXIM2, and two P-TEFb molecules containing Cdk9 phosphorylated at threonine 186. J Biol Chem. 2005; 280:28819–28826. [PubMed: 15965233]

Li Y, Sabari BR, Panchenko T, Wen H, Zhao D, Guan H, Wan L, Huang H, Tang Z, Zhao Y, et al. Molecular Coupling of Histone Crotonylation and Active Transcription by AF9 YEATS Domain. Mol. Cell. 2016; 62:181–193. [PubMed: 27105114]

Li Y, Wen H, Xi Y, Tanaka K, Wang H, Peng D, Ren Y, Jin Q, Dent SY, Li W, et al. AF9 YEATS domain links histone acetylation to DOT1L-mediated H3K79 methylation. Cell. 2014; 159:558–571. [PubMed: 25417107]

Li Y, Zhao D, Chen Z, Li H. YEATS domain: Linking histone crotonylation to gene regulation. Transcription. 2017; 8:9–14. [PubMed: 27661789]

Lin C, Garrett AS, De Kumar B, Smith ER, Gogol M, Seidel C, Krumlauf R, Shilatifard A. Dynamic transcriptional events in embryonic stem cells mediated by the super elongation complex (SEC). Genes Dev. 2011; 25:1486–1498. [PubMed: 21764852]

Lin C, Smith ER, Takahashi H, Lai KC, Martin-Brown S, Florens L, Washburn MP, Conaway JW, Conaway RC, Shilatifard A. AFF4, a component of the ELL/P-TEFβ elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. Mol Cell. 2010; 37:429–437. [PubMed: 20159561]

Luo RT, Lavau C, Du C, Simone F, Polak PE, Kawamata S, Thirman MJ. The elongation domain of ELL is dispensable but its ELL-associated factor 1 interaction domain is essential for MLL-ELL-induced leukemogenesis. Mol Cell Biol. 2001; 21:5678–5687. [PubMed: 11463848]

Malik S, Roeder RG. The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. Nat Rev Genet. 2010; 11:761–772. [PubMed: 20940737]

Malik S, Roeder RG. Mediator: A Drawbridge across the Enhancer-Promoter Divide. Mol Cell. 2016; 64:433–434. [PubMed: 27814466]

Nagy Z, Tora L. Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. Oncogene. 2007; 26:5341–5357. [PubMed: 17694077]
Nogales E, Patel AB, Louder RK. Towards a mechanistic understanding of core promoter recognition from cryo-EM studies of human TFIID. Curr Opin Struct Biol. 2017; 47:60–66. [PubMed: 28624568]

Okuda H, Kanai A, Ito S, Matsu H, Yokoyama A. AF4 uses the SL1 components of RANP1 machinery to initiate MLL fusion- and AEP-dependent transcription. Nat Commun. 2015; 6

Peng J, Zhu Y, Milton JT, Price DH. Identification of multiple cyclin subunits of human P-TEFb. Genes Dev. 1998; 12:757–762. [PubMed: 9499409]

Plaschka C, Hantsche M, Dienenmann C, Burzinski C, Plitzko J, Cramer P. Transcription initiation complex structures elucidate DNA opening. Nature. 2016; 533:353–358. [PubMed: 27193681]

Price DH. P-TEFb, a cyclin-dependent kinase controlling elongation by RNA polymerase II. Mol Cell Biol. 2000; 20:2629–2634. [PubMed: 10733565]

Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA. c-Myc regulates transcriptional pause release. Cell. 2010; 141:432–445. [PubMed: 20439848]

Shao W, Zeitlinger J. Paused RNA polymerase II inhibits new transcriptional initiation. Nat Genet. 2017; 49:1045–1051. [PubMed: 28504701]

Simone F, Luo RT, Polak PE, Kaberlein JJ, Thirman MJ. ELL-associated factor 2 (EAF2), a functional homolog of EAF1 with alternative ELL binding properties. Blood. 2003; 101:2355–2362. [PubMed: 12446457]

Takahashi H, Parmely TJ, Sato S, Tomomori-Sato C, Banks CA, Kong SE, Szutorisz H, Swanson SK, Martin-Brown S, Washburn MP, et al. Human mediator subunit MED26 functions as a docking site for transcription elongation factors. Cell. 2011; 146:92–104. [PubMed: 21729782]

Takahashi H, Takigawa I, Watanabe M, Anwar D, Shibata M, Tomomori-Sato C, Sato S, Ranjan A, Seidel CW, Tsukiyama T, et al. MED26 regulates the transcription of snRNA genes through the recruitment of little elongation complex. Nat Commun. 2015; 6

Williams LH, Fromm G, Gokey NG, Henriques T, Muse GW, Burkholder A, Fargo DC, Hu G, Adelman K. Pausing of RNA polymerase II regulates mammalian developmental potential through control of signaling networks. Mol Cell. 2015; 58:311–322. [PubMed: 25773599]

Winter GE, Mayer A, Buckley DL, Erb MA, Roderick JE, Vittori S, Reyes JM, di Iulio J, Souza A, Ott CJ, et al. BET Bromodomain Proteins Function as Master Transcription Elongation Factors Independent of CDK9 Recruitment. Mol Cell. 2017; 67:5–18 e19. [PubMed: 28673542]

Wright KJ, Marr MT 2nd, Tjian R. TAF4 nucleates a core subcomplex of TFIID and mediates activated transcription from a TATA-less promoter. Proc Natl Acad Sci U S A. 2006; 103:12347–12352. [PubMed: 16895980]

Yamaguchi Y, Shibata H, Handa H. Transcription elongation factors DSIF and NELF: promoter-proximal pausing and beyond. Biochim Biophys Acta. 2013; 1829:98–104. [PubMed: 23202475]

Yang Z, Yok JH, Chen R, He N, Jang MK, Ozato K, Zhou Q. Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. Mol Cell. 2005; 19:535–545. [PubMed: 16109377]

Yang Z, Zhu Q, Luo K, Zhou Q. The 7SK small nuclear RNA inhibits the CDK9/cyclin T1 kinase to control transcription. Nature. 2001; 414:317–322. [PubMed: 11713532]

Yokoyama A, Lin M, Naresh A, Kitabayashi I, Cleary ML. A higher-order complex containing AF4 and ENL family proteins with P-TEFb facilitates oncogenic and physiologic MLL-dependent transcription. Cancer Cell. 2010; 17:198–212. [PubMed: 20153263]

Yu M, Yang W, Ni T, Tang Z, Nakadai T, Zhu J, Roeder RG. RNA polymerase II-associated factor 1 regulates the release and phosphorylation of paused RNA polymerase II. Science. 2015; 350:1383–1386. [PubMed: 26659056]

Zhang J, Kalkum M, Yamamura S, Chait BT, Roeder RG. E protein silencing by the leukemogenic AML1-ETO fusion protein. Science. 2004; 305:1286–1289. [PubMed: 15333839]

Zhang Z, Nikolai BC, Gates LA, Jung SY, Siwak EB, He B, Rice AP, O’Malley BW, Feng Q. Crosstalk between histone modifications indicates that inhibition of arginine methyltransferase CARM1 activity reverses HIV latency. Nucleic Acids Res. 2017; 45:9348–9360. [PubMed: 28637181]

Zhou Q, Li T, Price DH. RNA polymerase II elongation control. Annu Rev Biochem. 2012; 81:119–143. [PubMed: 22404626]
Figure 1. Extensive and direct interaction between TFIID and SEC both in vivo and in vitro.
A. Immunoblot analysis showing presence of TAF subunits of TFIID in AF9 pull-down sample. Nuclear extract from 293T cells transfected with plasmid expressing FLAG-HA-AF9 protein, was subjected to anti-FLAG(M2) affinity pull-down and subsequently to western blotting analysis with indicated antibodies for identifying interacting proteins.
B. Immunoblot analysis showing endogenous interaction of TFIID and SEC through AF9 pull-down by using AF9-specific antibody. Parallel immunoprecipitation experiment using IgG was used as control. Immunoprecipitated samples were subjected to western blot analysis using indicated factor-specific antibodies.
C. Immunoblot analysis showing presence of SEC components in TBP pull-down sample. Nuclear extract from FLAG-HA-TBP expressing stable cell line was subjected to anti-FLAG(M2) affinity pull-down and subsequently to western blotting analysis with indicated antibodies for identifying interacting proteins.
D. Immunoblot analysis showing endogenous interaction of TFIID and SEC through TBP pull-down by using TBP-specific antibody. Parallel immunoprecipitation experiment using IgG was used as control. Immunoprecipitated samples were subjected to western blot analysis using indicated factor-specific antibodies.
E. Immunoblot analysis showing presence of TAF subunits of TFIID and other known interactors in ELL and AF4 pull-down samples. Nuclear extracts from 293T cells transfected with plasmids expressing FLAG-HA-ELL and FLAG-HA-AF4 proteins, were subjected to anti-FLAG(M2) affinity pull-down and subsequently to western blotting analysis with indicated antibodies for identifying interacting proteins.

F. Immunoblot analysis showing presence of TAF subunits of TFIID and other known interactors in EAF1 and EAF2 pull-down samples. Nuclear extracts from 293T cells transfected with plasmids expressing FLAG-HA-EAF1 and FLAG-HA-EAF2 proteins, were subjected to anti-FLAG(M2) affinity pull-down and subsequently to western blotting analysis with indicated antibodies for identifying interacting proteins.

G. Immunoblot analysis showing direct interaction between AF9 and TFIID. Purified recombinant GST-AF9 protein was incubated with purified TFIID for their interactions and subsequently, interacting proteins were identified by western blotting using indicated antibodies.

H. Immunoblot analysis showing direct interaction between EAF1 and EAF2 with TFIID. Purified recombinant GST-EAF1 and GST-EAF2 proteins were incubated with purified TFIID for their interactions and subsequently, interacting proteins were identified by western blotting using indicated antibodies.
Figure 2. Specific and distinct domains of AF9 and EAF1 are involved in their interactions with TFIID and SEC.
A. Cartoon diagram depicting functionally important domains of AF9.
B. SDS-PAGE coomassie staining of purified recombinant AF9 fragments as indicated. All these proteins were expressed and purified using bacterial expression system.
C. Immunoblot analysis showing specific domain of AF9 that directly interacts with purified TFIID. As seen in blots, the AF9 fragments that harbor poly-Ser domain, interact with TFIID, whereas, other fragments fail to do so.
D. Immunoblot analyses showing specific domains of AF9 that interacts with TFIID and SEC in mammalian cells. 293T cells were transfected with plasmids expressing the indicated AF9 fragments and associated proteins were pulled-down using anti-FLAG(M2) affinity raisin and were identified by western blot analysis using indicated antibodies.
E. Immunoblot analysis showing TFIID interaction with full-length AF9 and poly-Ser domain-deleted AF9 proteins. Plasmids expressing these proteins were expressed in 293T
cells and interacting proteins were identified by anti-FLAG immunoprecipitation and subsequent blotting analysis with indicated antibodies.

F. Cartoon diagram depicting functionally important domains of EAF1.

G. SDS-PAGE coomassie staining of purified recombinant EAF1 fragments as indicated. All these proteins were expressed and purified using bacterial expression system.

H. Immunoblot analysis showing specific domain of EAF1 that directly interacts with TFIID. As seen in blots, the EAF1 fragments that harbor 181-268 region including the poly-Ser domain, interact with TFIID, whereas, other fragments fail to do so.

I. Immunoblot analyses showing specific domains of EAF1 that interacts with TFIID and SEC. 293T cells were transfected with plasmids expressing the indicated EAF1 fragments and associated proteins were pulled-down using anti-FLAG(M2) affinity raisin and were identified by western blot analysis using indicated antibodies.
Figure 3. AF9 and EAF1 proteins interact with specific TAF subunits of TFIID.

A-B. Sf9 expression-based interaction analysis showing specific interactions between individual TAF subunits including TBP with AF9 and EAF1 respectively. Baculoviruses expressing indicated TFIID subunits were co-infected in Sf9 cells with AF9 and EAF1-expressing baculoviruses. Cell lysate of the infected Sf9 cells were subjected to immunoprecipitation by anti-FLAG (M2) beads and association of TAF subunits with AF9 and EAF1 were identified by western blotting using indicated antibodies.

C. Sf9 expression-based interaction analysis showing specific domains of TAF6 that interact with AF9. Baculoviruses expressing indicated TAF6 domains were co-infected in Sf9 cells with AF9-expressing baculovirus. Cell lysate of the infected Sf9 cells were subjected to immunoprecipitation by anti-FLAG (M2) beads and AF9 association was identified by western blotting.

D. Immunoblot analyses showing specific domains of TAF6 that interact with TFIID and SEC in mammalian cells. 293T cells were transfected with plasmids expressing the indicated TAF6 fragments and associated proteins were pulled-down using anti-FLAG(M2) affinity raisin and were identified by western blot analysis using indicated antibodies.
E. Immunoblot analyses showing specific region of N-terminus of TAF6 that interacts with TFIID and SEC in mammalian cells. 293T cells were transfected with plasmids expressing the indicated TAF6 N-terminal fragments and associated proteins were pulled-down using anti-FLAG(M2) affinity raisin and were identified by western blot analysis using indicated antibodies.
Figure 4. TFIID-dependent recruitment of AF9 and EAF1 and associated higher order complexes on DNA template.

A. Cartoon diagram depicting important elements within the DNA fragment used in the recruitment assay.

B. DNA template-based recruitment assay showing TFIID-dependent recruitment of GST-AF9 protein on template DNA.

C. TFIID-dependent recruitment of AF9-AF4 complex on template DNA.

D. DNA template-based recruitment assay showing TFIID-dependent recruitment of GST-AF9 fragments on template DNA. Only the GST-AF9 fragment (1-208) that contains poly-Ser domain, gets recruited and others do not.

E. DNA template-based recruitment assay showing exclusive requirement of poly-Ser domain for TFIID-dependent recruitment of GST-AF9 fragments on template DNA. Only the GST-AF9 fragments (1-196 and 1-208) that contain poly-Ser domain, get recruited and other fragment (1-148) lacking poly-Ser domain does not.

F. DNA template-based recruitment assay showing TFIID-dependent recruitment of GST-EAF1 protein on template DNA.

G. DNA template-based recruitment assay showing TFIID-dependent recruitment of ELL-EAF1 complex on template DNA.

H. DNA template-based recruitment assay showing requirement of specific EAF1 domain (181-268 amino acids) for TFIID-dependent recruitment of EAF1 protein on template DNA.
Figure 5. Mediator-independent and TAF6 and AF9-dependent interaction of TFIIID and SEC in mammalian cells.

A. Stable knockdown of TAF6 in 293T cells using TAF6-specific shRNA. Knockdown efficiency was tested by western blotting (upper panel) as well as RNA analysis by qRT-PCR.

B. Stable knockdown of AF9 in 293T cells using AF9-specific shRNA. Knockdown efficiency was tested by western blotting (upper panel) as well as RNA analysis by qRT-PCR.

C. Schematics of experimental strategy that has been employed for experiments mentioned in the panel D and E.

D. Immunoblot analysis showing the effect of TAF6 knockdown on association of other TAF and SEC subunits with ectopically expressed FLAG-TBP. TBP-associated proteins were pulled-down using anti-FLAG M2 beads and proteins were identified by immunoblotting using specific antibodies.

E. Immunoblot analysis showing the effect of AF9 knockdown on association of other TAF and SEC subunits with ectopically expressed TBP. TBP-associated proteins were pulled-down using anti-FLAG M2 beads and proteins were identified by immunoblotting using specific antibodies.

F. Stable knockdown of Med26 in 293T cells using Med26-specific shRNA. Knockdown efficiency was tested by western blotting.

G. Immunoblot analysis showing the effect of Med26 knockdown on association of other TAF and SEC subunits with ectopically expressed FLAG-TBP. TBP-associated proteins...
were pulled-down using anti-FLAG M2 beads and proteins were identified by immunoblotting using specific antibodies. The left panel shows experimental strategy that was used for this assay.

H. Immunoblot analysis showing the effect of Med26 knockdown on association of other TAF and SEC subunits with ectopically expressed EAF1. EAF1-associated proteins were pulled-down using anti-FLAG M2 beads and proteins were identified by immunoblotting using specific antibodies. The left panel shows experimental strategy that was used for this assay.
Figure 6. TFIID-mediated recruitment of SEC at promoter proximal region is required for basal level as well as inducible expression of target genes.

A. qRT-PCR analysis showing effect of TAF6 and AF9 knockdown on basal level expression of target genes when compared to control scramble knockdown cells. Statistical significance for RNA analysis in TAF6 and AF9 knockdown samples are shown over control (Scramble) sample.

B-D: ChIP analysis showing recruitment of several factors at promoter proximal region of indicated target genes upon knockdown of TAF6 and AF9. Statistical significance for ChIP analysis in TAF6 and AF9 knockdown samples are shown over control (Scramble) sample.

E. qRT-PCR analysis showing effect of AF9 knockdown on induced expression of target p21 gene at different time points after doxorubicin treatment.

F. ChIP analysis showing effect of AF9 knockdown on recruitment of target factors at promoter proximal and ~4kb downstream (coding) region of p21 gene after 16 hrs of doxorubicin treatment. Statistical significance for ChIP analysis in AF9 knockdown sample are shown over control (Scramble) sample. PP denotes promoter proximal region.
All of our RNA expression and ChIP data represents mean ± SEM., a minimum of two biological and three PCR replicates. Statistical analysis was performed using one-tailed Student's t test wherein * denotes $p \leq 0.05$, ** denotes $p \leq 0.01$, *** denotes $p \leq 0.001$, and ns denotes not significant.
Figure 7. TFIID-mediated SEC recruitment at promoter proximal region is required for expression of target gene.

A. Western blot analysis showing expression of full-length and poly-Ser domain-deleted AF9 proteins in AF9 knockdown cells.

B. Effect of restoration of expression of full-length and poly-Ser domain-deleted AF9 proteins in AF9 knockdown cells on expression of selected target genes as analyzed by qRT-PCR analysis. EV represents empty vector control in our assay. Statistical significance for RNA analysis in AF9 (FL) was calculated with reference to empty vector and for AF9 (poly-Ser) with reference to AF9 (FL).

C-E. ChIP analysis showing effect of restoration of expression of full-length and poly-Ser domain-deleted AF9 proteins in AF9 knockdown cells on factor binding at indicated target genes. EV represents empty vector control in our assay. Ectopically-expressed AF9 recruitment was analyzed through FLAG pull-down using M2 beads in this experiment. Enrichment of full-length AF9 and poly-Ser-deleted AF9 fragment was calculated by normalizing over empty vector. Statistical significance for ChIP analysis in AF9 (FL) was calculated with reference to empty vector and for AF9 (poly-Ser) with reference to AF9 (FL).

F-G. Proliferation and colony formation assay showing effect of restoration of expression of full-length AF9 and poly-Ser domain-deleted AF9 on growth potential and colony formation of AF9-depleted 293T cells respectively.
H. Overall model showing regulation of pause and release of transcription event through TFIID-mediated AF9 and AF9-associated SEC components. Poly-Ser domains present within the AF9 and EAF1 play a major role in TFIID-mediated SEC recruitment for overcoming DSIF and NELF-mediated pausing of Pol II and its entry into productive elongation.

All of our RNA expression and ChIP data represents mean ± SEM., a minimum of two biological and three PCR replicates. Statistical analysis was performed using one-tailed Student's t test wherein * denotes p ≤ 0.05, ** denotes p ≤ 0.01, *** denotes p ≤ 0.001, and ns denotes not significant.
### Key Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| CyclinT1            | Santa Cruz Biotechnology | Cat#sc-10750 |
| CDK9                | Santa Cruz Biotechnology and Bethyl Lab | Cat#sc-8338 and Cat# A303-492A |
| FLAG epitope        | Sigma  | Cat#F7425 |
| GST                 | Santa Cruz Biotechnology | Cat#sc-53909 |
| AF4                 | Abcam  | Cat#ab31812 |
| AF9                 | Bethyl Lab | Cat# A300-596 |
| Rpb1 (4H clone)     | Cell Signaling Technology | Cat#2629 |
| Phospho Rpb1 CTD (Ser2) | Cell Signaling Technology | Cat#13499 |
| Phospho Rpb1 CTD (Ser2) | Cell Signaling Technology | Cat#13523 |
| Beta Actin          | Santa Cruz Biotechnology | Cat#sc-6778 |
| EAF1                | Santa Cruz Biotechnology | Cat#Sc-373832 |
| EAF2                | Bethyl Lab | Cat# A302-503A |
| ELL                 | Bethyl Lab and Cell Signaling Technology | Cat# A301-645A, Cat# D7N6U |
| TAF1                | Bethyl Lab and Cell Signaling Technology | Cat# A301-505A, Cat# DG53B |
| TAF2                | Bethyl Lab | Cat# A302-132A |
| TAF3                | Abcam  | Cat#ab21633 |
| TAF5                | Bethyl Lab | Cat# A301-607A |
| TAF6                | Bethyl Lab | Cat# A301-236A |
| TAF7                | This lab | N/A |
| TAF9                | This lab | N/A |
| TBP                 | Bethyl Lab and Cell Signaling Technology | Cat# A301-229A |
| Bacterial and Virus Strains |        |            |
| DH5α E.Coli         | This lab | N/A |
| BL21 E.Coli         | This lab | N/A |
| Chemicals, Peptides, and Recombinant Proteins |        |            |
| dNTP mix            | BioBharati Life Science | Cat#BI2-C0020B |
| REAGENT or RESOURCEs                      | SOURCE                  | IDENTIFIER |
|-----------------------------------------|-------------------------|------------|
| ATP                                     | BioBharati Life Science | Cat#BB-C0030 |
| Phusion high fidelity DNA polymerase    | NEB                     | Cat#M0530S |
| T4DNA ligase                            | NEB                     | Cat#M0202S |
| 3X Flag peptide                         | Sigma                   | Cat#F799-25MG |
| L-Glutathione reduced                   | Sigma                   | Cat#G425-25G |
| Vent cDNA synthesis kit                 | Life Technologies       | Cat#A1453A |
| iTaq Universal SYBR Green Mix           | BIo-Rad                 | Cat#172-5201AP |
| TRIreel                                 | Life Technologies       | Cat#1559026 |
| Gentamycin                              | Gold Biotechnology      | Cat#G-400-100 |
| Penicillin and Streptomycin             | Life Technologies       | Cat#151063 |
| Purified human TFIID complex            | This study              | N/A        |
| Purified recombinant GST-AF9            | This study              | N/A        |
| Purified recombinant GST-EAF1           | This study              | N/A        |
| Purified recombinant GST-EAF1           | This study              | N/A        |
| Purified recombinant GST-ELL            | This study              | N/A        |
| Purified recombinant GST-AF9.FLAG-AF4   | This study              | N/A        |
| Purified recombinant GST-EAF1.FLAG-ELL  | This study              | N/A        |
| Purified recombinant GST-AF9.deletion constructs | This study | N/A |
| Purified recombinant GST-EAF1.deletion constructs | This study | N/A |
| Critical Commercial Assays              |                         |            |
| Qubit RNA HS assay kit                  | Thermo Fisher Scientific | Cat#Q32852 |
| Agilent Bioanalyzer RNA Nano 6000 Kit   | Agilent Technologies    | Cat# 5067-1511 |
| NEB NEXTRNA Ultra II Library preparation kit for Illumina | NEB | Cat#E7760 |
| NEB Next Poly(A) mRNA Magnetic Isolation Module | NEB | Cat#E7490 |
| Agilent Bioanalyzer DNA 3000 kit        | Agilent Technologies    | Cat# 5067-1505 |
| Qubit DNA HS assay kit                  | Thermo Fisher Scientific | Cat#Q32851 |

**Deposited Data**

- List of genes showing downregulation of expression upon AF9 and TAF6 knockdown
  
  [http://dx.doi.org/10.17632/3yz7xxksgw.1](http://dx.doi.org/10.17632/3yz7xxksgw.1)
### REAGENT or RESOURCEs

| Source | Identifier |
|--------|------------|
| Unprocessed raw images of western blot | This study (Mendeley data) |
| Raw data for RNA-Seq analysis for differential RNA expression upon TAF6 and AF9 knockdown compared to control (Scramble) cells | This study (GenBank, NCBI) |

#### Experimental Models: Cell Lines

| Source | Identifier |
|--------|------------|
| Flp-In™ -293 | Invitrogen |
| Flp-In™ T-REX™ -293 | Invitrogen |
| SF9 insect cell line | Invitrogen |
| HEK293T | ATCC |
| HCT116 | ATCC |

#### Oligonucleotides

| Source | Identifier |
|--------|------------|
| AF9 knockdown shRNA Upper oligo (CCGGGCGTCTACAGATGTGAAGAAGATGAGTAACACACTGCTTTTTG) lower oligo (AATTCAAAAAGCCAGTGTGTTACTCATCTTTCTCTCGAGAAAGATGAGTAACACACTGCC) | This paper (IDT) |
| TAF6 knockdown shRNA # 4 | N/A |
| MED26 knockdown shRNA # 4 Upper oligo (CCGGGAAATGCTGCTACCAGTTTACCTCGAGGTAAACTGGTAGCAGCATTTCTTTTTG) lower oligo (AATTCAAAAAATGACCTGAAGAGCCGCAATGCTCGAGCATTGCGGCTCTTCAGGTCAT) | This paper (IDT) |
| MED26 shRNA # 5 Upper oligo (CCGGATGACCTGAAGAGCCGCAATGCTCGAGCATTGCGGCTCTTCAGGTCATTTTTTG) lower oligo (AATTCGAAATGCTGCTACCAGTTTACCTCGAGGTAAACTGGTAGCAGCATTTCTTTTTG) | This paper (IDT) |

#### Primers for RNA analyses

| Source | Identifier |
|--------|------------|
| Primers for RNA analyses | This paper (IDT) |

#### Primers for ChIP analyses

| Source | Identifier |
|--------|------------|
| Primers for ChIP analyses | This paper (IDT) |

#### Recombinant DNA

| Source | Identifier |
|--------|------------|
| Plasmid constructs used in this study | This paper |
| pLKO.1-TRC cloning vector | Addgene Plasmid #10878 |
| pS-PAX2 packaging plasmid | Addgene Plasmid #12260 |
| pMD2.G envelope vector | Addgene Plasmid #12259 |
| psvDANS-FRTTO mammalian expression vector | Invitrogen Cat # V5620-20 |
| pOG44 recombinase plasmid | Invitrogen Cat # V6035-20 |
| pET-GST bacterial expression vector | This study |

#### Software and Algorithms

| Source | Identifier |
|--------|------------|
| Excel | Excel software |
| DAVID | (Huang da et al., 2009) |
| DESeq2 | Bioconductor |
| Graphpad Prism | graphpad.com |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Protein A agarose   | Thermo Fisher Scientific | Cat# 20333 |
| Protein G monoclonal antibodies | Sigma | Cat# 20333 |
| Protein G magnetic beads | Sigma | Cat# 28020 |
| GST agarose beads   | Goldbio | Cat# G-250-10 |
| Ni-NTA agarose beads | Qiagen | Cat# 30210 |
| PCR purification kit | Qiagen | Cat# 28706 |
| Plasmid miniprep kit | Qiagen | Cat# 27104 |
| Gel extraction kit   | QiaGen | Cat# 28104 |
| Gel extraction kit   | QiaGen | Cat# 28020 |
| Gel extraction kit   | QiaGen | Cat# 27104 |