The extra-terminal domain drives the role of BET proteins in transcription

Iwona Pasionek[1,†], Agnieszka Machowska[1,†], John B. Ridenour[1], Magdalena Donczew[1], Rafal Donczew[1,2,*]

[1] Cell Cycle and Cancer Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, 73104, OK, USA.

[2] Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, 73104, OK, USA.

[†] These authors contributed equally to this work.

[*] To whom correspondence should be addressed. Tel: 405-271-7437; Email: rafal-donczew@omrf.org.

Abstract

BET proteins facilitate transcription of most eukaryotic genes but the specific mechanisms by which BET proteins perform their functions remain poorly understood. As chromatin readers, BET proteins use their tandem bromodomains to interact with acetylated lysine residues on histones and other protein partners. However, recent findings illustrate that bromodomain activity does not fully explain BET protein roles in transcription, highlighting the significance of other BET protein domains. We evaluated the importance of all conserved domains of BET proteins and determined that the extra-terminal (ET) domain is essential for cell viability, genome-wide transcription and BET protein chromatin occupancy. Furthermore, we provide evidence that the ET domain performs these functions serving as a hub for interactions with other factors involved in transcription regulation. Our findings expand current understanding of the complex biology of BET proteins and suggest the mechanisms by which cells can bypass bromodomain inhibition in pathologic states.

Introduction

Bromodomains (BDs) are reader modules that recognize acetylated lysine residues on histones and other protein targets (*1*–*3*). Proteins from the bromodomain and extra-terminal domain (BET) family are conserved chromatin readers with important roles in regulation of transcription by RNA polymerase II (Pol II) (*4*–*7*). Consistently, BET proteins were implicated in sustaining pathologic transcription in various human conditions (*8*–*10*). Chemical inhibition of BET BDs is a promising approach in treatment of cancer and other diseases, but resistance to BD inhibitors develops quickly and alternative strategies to target BET proteins are needed (*11*–*17*). However, a comprehensive model of BET protein functions in transcription remains to be determined.

All three mammalian BET proteins expressed in somatic tissues (BRD2, BRD3, and BRD4) contribute to gene transcription, but BRD4 is a dominant BET factor in transcription regulation (*7*, *18*–*20*). A current literature consensus is that BRD4 controls transcription primarily through its roles in transcription elongation (*5*–*7*, *21*). There are two BET proteins in budding yeast (Bdf1 and Bdf2, referred to here as Bdf1/2), and at least one is required for viability. Bdf1/2 have redundant contributions to transcription but, similarly to BRD4 in mammalian cells, yeast cells depend primarily on Bdf1 (*4*, *22*, *23*). On the mechanistic level, Bdf1/2 contribute both to the initiation and elongation stages of transcription (*4*). Importantly, mammalian BET proteins were

also implicated in transcription initiation, indicating that the roles of BET proteins in transcription are largely conserved across eukaryotic phyla (*6*, *24*).

According to the canonical model, tandem BDs are the primary module that supports BET protein function (*1*, *2*, *11*, *23*, *25*). Multiply acetylated histone H4 N-terminal tail is a preferred target of most BET BDs, including both BDs in Bdf1 (*23*, *26*, *27*). We previously showed that the rapid loss of H4 acetylation results in only ~50% decrease of Bdf1 chromatin occupancy (*4*). Consistent with this result, yeast cells are viable without acetylated residues on H4 tail (*28*, *29*), Bdf1 is recruited to immobilized promoter DNA in the absence of nucleosomes (*22*, *30*), Bdf1 mutant with deactivated BDs suppresses temperature sensitivity resulting from *BDF1* gene deletion (*26*), and chemical inhibition of BET BDs in mammalian cells does not cause a complete dissociation of BET proteins from chromatin or abolish their function (*6*, *21*, *24*, *31*). Altogether, these findings indicate that BET protein roles in transcription involve important but poorly understood mechanisms that do not rely on the BDs.

BET proteins have a modular architecture that includes conserved structured (BDs, motif B, and an extra-terminal (ET) domain) and unstructured regions (phosphorylated regions N and C terminal phosphorylation sites (NPS and CPS), and basic interaction domain (BID)), separated by unstructured regions with no sequence conservancy (**Fig. 1A**). The significance of the non-BD BET domains has not been comprehensively investigated although existing evidence supports their important roles. For example, the ET domain of mammalian BET proteins is an interaction site for several protein partners (*2*, *32*–*35*) and it was proposed that these interactions are important for BRD4 roles in transcription and BRD4 recruitment to chromatin (*31*, *32*, *36*, *37*). However, the significance of the ET domain for BET protein function *in vivo* remains to be determined. Further, BET protein phosphorylation in the NPS and CPS regions was proposed to lead to increased association with chromatin and protein partners (*15*, *16*, *25*, *38*). Other conserved domains, motif B and BID, were biochemically shown to facilitate BET protein dimerization (*39*, *40*).

Here, we defined the contributions of conserved domains of BET proteins to cell viability, transcription, BET protein chromatin association, and recruitment of partner proteins. Our key findings established the importance of the ET domain for BET protein function in living cells and allowed us to propose a new model of transcription regulation by BET proteins.

100 **Results**

101

102 **BET protein ET domain is essential for cell survival.**

103 Prior yeast genetic studies determined that BDs and NPS/CPS regions of BET proteins are
104 important for viability, but the significance of other domains is unknown (*26*, *38*). We previously
105 showed that while Bdf1 and Bdf2 have redundant functions in transcription, Bdf1 is the dominant
106 BET protein in yeast (*4*). Therefore, we chose Bdf1 as our model BET protein. We constructed a
107 collection of *BDF1* gene variants with deletions or targeted mutations in all conserved BET
108 protein domains. If prior studies suggested a functional overlap, we included combinations of
109 perturbations in more than one domain. Since intrinsically disordered regions (IDRs) were
110 proposed to be important for BRD4 roles in mammalian cells, we also constructed a *BDF1*
111 variant with truncations in IDRs that do not overlap with conserved unstructured domains NPS,
112 CPS, and BID (*41*, *42*). In case of BDs and NPS/CPS regions, we included targeted mutation of
113 residues essential for BD function and of serine residues with evidence of phosphorylation,
114 respectively (*26*, *38*, *43*, *44*). As a comparison, we prepared a *BDF1* phosphomimetic variant
115 with all phosphorylated serine residues substituted for glutamate residues. In total, we
116 constructed eighteen *BDF1* variants (**Fig. 1B**). To facilitate stable expression in yeast cells, we
117 cloned *BDF1* variants or unmodified *BDF1* into a minichromosomal plasmid pRS315 under the
118 control of endogenous *BDF1* promoter and 3' untranslated region (**Table S1**) (*45*).

119

120 We performed a complementation assay in a strain that relies solely on *BDF1* expressed from a
121 minichromosomal plasmid as endogenous *BDF1/2* are deleted. We attempted replacing the
122 plasmid carrying an unmodified copy of *BDF1* and the *URA3* gene with plasmids carrying *BDF1*
123 variants by selecting for viable transformants on a medium supplemented with 5-Fluoroorotic
124 acid (5-FOA), which inhibits growth of cells that contain the *URA3* gene (*46*). We found that five
125 *BDF1* variants did not support viability – a double BD deletion or mutation variants, variants with
126 deletion or mutation of both NPS/CPS regions or serine to alanine substitutions in both
127 NPS/CPS regions, and a variant with the ET domain deletion (**Fig. 1B**). Importantly, except for
128 the variant with double BD deletion (ΔBD1/2), the variants not supporting viability were
129 expressed at near endogenous levels (**Fig. S1A**). In summary, while prior studies addressed the
130 *in vivo* requirement for the BDs and NPS/CPS regions of BET proteins in yeast (*26*, *38*), we are
131 the first to show that the ET domain is essential for cell viability.

132

133

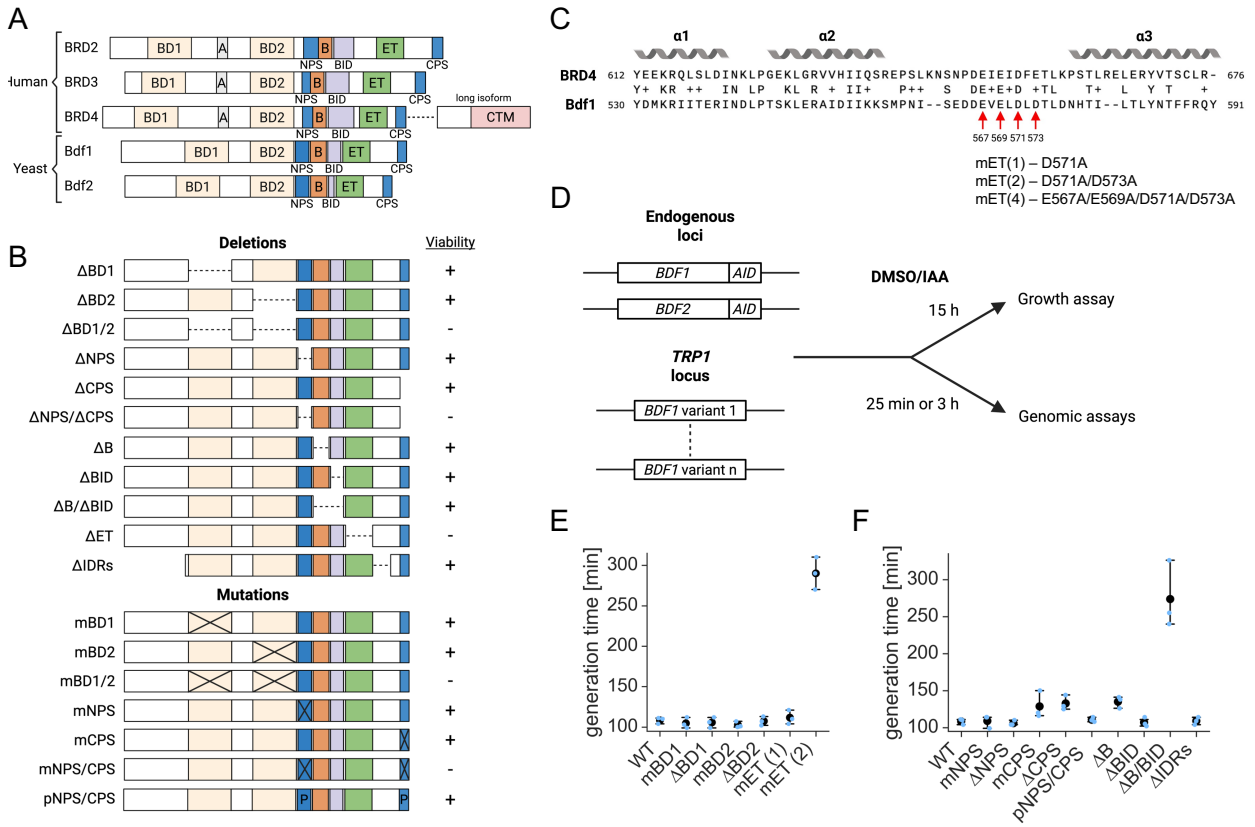


Fig. 1. **BET protein ET domain is essential for viability.** (**A**) Conserved domain organization of BET proteins. BD – bromodomain, A/B – motifs A/B, BID – basic interaction domain, ET – extra-terminal domain, NPS/CPS – N/C-terminal phosphorylation sites, CTM – C-terminal motif (BRD4 long isoform only). (**B**) *BDF1* variants constructed in this work. Ability to support growth in the absence of endogenous *BDF1/2* genes is indicated. (**C**) Sequence alignment of the ET domain of BRD4 (top) and Bdf1 (bottom). Four conserved acidic residues important for BRD4 interactions with protein partners are marked. Coordinates for Bdf1 are shown below the arrows. Three ET domain mutants selected for subsequent experiments are shown. (**D**) Schematic representation of the experimental strategy for the viability assay and genomic assays (SLAM-seq and ChEC-seq). Experiments were based on strains where endogenous Bdf1/2 carried auxin-inducible degron (AID) tags and *BDF1* variants (or unmodified *BDF1*) were expressed from the *TRP1* locus. During experiments, endogenous Bdf1/2 were depleted by the addition of 3-indolacetic acid (IAA). (**E**) Growth assay comparing the fitness of a control strain expressing unmodified *BDF1* (WT) to strains expressing *BDF1* variants after 15 h exposure to IAA. Black markers indicate mean generation times and error bard correspond to 95% confidence intervals.

A conserved stretch of acidic residues in the ET domain is required for its function. Conserved acidic residues between second and third helices of the ET domain were shown to mediate BRD4 interactions with several protein partners (*32–34*). To evaluate the *in vivo*

154 significance of this region of the ET domain, we designed six additional *BDF1* variants wherein
155 different number of four conserved acidic residues (amino acids 567, 569, 571, 573 in Bdf1)
156 were substituted alanine (**Figs. 1C and S1B**). We tested the new *BDF1* variants in the
157 complementation assay and found that mutation of three or more acidic residues in the ET
158 domain is lethal for yeast cells, indicating that this region of the ET domain is essential for its
159 function (**Fig. S1B**). For the subsequent experiments, we selected a representative set of three
160 ET domain mutations including a single (D571A; mET(1)), double (D571A, D573A; mET(2)) and
161 a quadrupole mutation (mET(4)) (**Fig. 1C**).
162
163 To support subsequent experiments, we integrated all *BDF1* variants and unmodified copy of
164 *BDF1* into the *TRP1* locus of a strain where endogenous Bdf1/2 carry auxin inducible degron
165 (AID) tags (Bdf1/2-AID strain) (**Figs. 1D, and Tables S1 and S2**) (*4*, *47*). In this context, *BDF1*
166 variants are constitutively expressed from ectopic locus under the control of endogenous *BDF1*
167 promoter and 3' untranslated region, while endogenous Bdf1/2 can be rapidly depleted by
168 addition of 3-indolacetic acid (IAA) (**S1C).** Importantly, additional copy of unmodified *BDF1* did
169 not change cell viability and most integrated *BDF1* variants were ectopically expressed at
170 endogenous or modestly higher levels, both with and without depletion of endogenous Bdf1/2
171 (**Fig. S1D-E**). We did not detect expression of variants containing BD2 deletion (ΔBD2, ΔBD1/2)
172 and deletion of the N-terminal unstructured region (ΔIDRs), suggesting that the antibody we
173 used recognizes epitopes in these two regions of Bdf1. Nevertheless, correct expression of
174 ΔBD2, ΔBD1/2 and ΔIDRs variants is supported by the results of experiments described below.
175
176 The complementation assay revealed which domains of BET proteins are required for viability.
177 To extend these findings, we quantified changes in growth rate of yeast cells resulting from
178 *BDF1* mutations. We cultivated yeast cells in the presence of IAA, forcing them to rely on Bdf1
179 variants as endogenous Bdf1/2 are depleted, and calculated generation time after ~15 hours of
180 growth (**Fig. 1D**). First, we observed a complete inhibition of growth of strains carrying *BDF1*
181 variants that did not support viability in the complementation assay, validating our prior results.
182 Second, we found that while a single point mutation in the ET domain was well tolerated,
183 mutation of only two of the conserved acidic residues resulted in a severe growth phenotype. As
184 a comparison, the loss or mutation of a single BD did not affect the growth of yeast cells (**Fig.
185 1E**). Third, we observed that CPS, but not NPS, mutation or deletion causes a modest delay in
186 growth. Interestingly, the NPS/CPS phosphomimetic Bdf1 variant fully supported viability,
187 suggesting that BET proteins function optimally in a fully phosphorylated state and their

188 phosphorylation is not dynamically regulated under normal growth conditions (**Fig. 1F**). Fourth,
189 a combined loss of B/BID regions strongly affected cell growth, supporting a proposed role of
190 this region in facilitating BET dimerization (*39*, *40*). Finally, we observed that truncation of Bdf1
191 IDRs does not interfere with viability, indicating that the large and unstructured N-terminal region
192 of BET proteins does not have an important biological function (**Fig. 1F**). As a validation, we
193 obtained very similar growth rates for all viable strains relying solely on *BDF1* variants
194 expressed from minichromosomal plasmids (**Fig. S2A and Table S2**). In summary, the assays
195 described so far determined the contributions of all conserved domains of BET proteins to yeast
196 viability and revealed a region of the ET domain that is required for its function.
197
198 **The ET domain is essential for genome-wide transcription.**
199 The essential role of BET proteins is sustaining global transcriptional programs (*4–7*). To
200 characterize the importance of the ET and other conserved domains for genome-wide
201 transcription we used our collection of strains expressing *BDF1* variants in the context of
202 endogenous Bdf1/2 carrying AID tags (**Fig. 1D and Table S2**). We already confirmed that the
203 additional copy of unmodified *BDF1* does not compromise cell viability and that protein levels
204 are similar when Bdf1 is expressed ectopically or from its endogenous locus (**Fig. S1D-E**). We
205 further validated that the additional copy of unmodified *BDF1* does not substantially change
206 baseline transcription and rescues most defects in transcription caused by depletion of
207 endogenous Bdf1/2 (**Figs. S3A-B**). We also tested if *BDF1* mutant variants cause dominant
208 negative growth phenotype in the presence of endogenous Bdf1/2 and found that the two
209 strains carrying variants with combined deletion or mutation of both NPS/CPS regions exhibited
210 slow-growth phenotypes (**Fig. S3C**).
211
212 In contrast to the growth assay, where cells had to rely on mutant Bdf1 variants for several
213 generations, here we depleted endogenous Bdf1/2 for 25 minutes immediately followed with 4
214 minute labeling of newly synthesized RNA for quantitation using SLAM-seq (*48*) (**Fig. 1D**). This
215 approach allowed us to track direct consequences of Bdf1 mutations, including lethal mutations,
216 with minimized bias from indirect effects that accumulate during extended reliance on adverse
217 genetic variants. We performed three replicate experiments for all strains with (IAA) or without
218 (dimethyl sulfoxide (DMSO)) depletion of endogenous Bdf1/2 (**Table S3**). The variability in RNA
219 levels between replicate experiments in our final dataset (see Materials and Methods) was
220 <30% for most genes (**Figs. S4A-B and Tables S4 and S5**). We calculated changes in
221 transcription due to perturbations in BET protein domains by comparing the results obtained for

222 strains expressing *BDF1* variants to the control strain expressing unmodified *BDF1* in the
223 context of depletion of endogenous Bdf1/2 (IAA treatment) (**Table S6**). To identify dominant
224 negative effects, we compared the results for strains expressing *BDF1* variants without
225 depletion of endogenous Bdf1/2 to the Bdf1/2-AID strain, which does not contain additional copy
226 of *BDF1* (**Fig. S4C and Table S7**). In agreement with the results of growth assay (**Fig. S3D**),
227 the two strains expressing *BDF1* variants with mutations or deletions in both NPS/CPS regions
228 (mNPS/CPS and ΔNPS/CPS) showed substantial deviations in transcription (median change >
229 2-fold) without depletion of endogenous Bdf1/2. Additionally, we observed a similar dominant
230 negative phenotype for the strain expressing CPS region deletion variant (ΔCPS).
231 Consequently, we excluded ΔCPS, mNPS/CPS and ΔNPS/CPS strains from further analysis.
232
233 We aligned the results calculated in the context of depletion of endogenous Bdf1/2 with changes
234 in transcription due to a loss of Bdf1/2 in the Bdf1/2-AID strain. Surprisingly, we found that only
235 the *BDF1* variants with the ET domain deletion or mutation of all four conserved acidic residues
236 in the ET domain (henceforth called ET domain mutation) caused substantial defects in
237 transcription (median decrease of 3.6-fold and 2.6-fold, respectively). In contrast, deletion or
238 mutation of both BDs caused relatively small changes in transcription (median decrease of 1.5-
239 fold and 1.4-fold, respectively) (**Figs. 2A and S5A**). Importantly, defects in transcription
240 observed for the loss of the ET domain function were comparable to defects due to the loss of
241 Bdf1/2 in the Bdf1/2-AID strain (median decrease of 3.7-fold). Analysis of changes in
242 transcription at the level of individual genes or previously characterized major gene classes
243 further confirmed that the loss of the ET domain function largely phenocopies the loss of BET
244 proteins (*4*) (**Figs. 2B-C and S5B)**. Perturbations in other domains had a small effect on
245 genome-wide transcription, except for the CPS mutation and the combined deletion of B/BID
246 regions, which caused modest changes (median decrease of 1.7-fold and 1.6-fold, respectively)
247 (**Fig. S5A**).
248
249 A modest change in transcription due to inactivation of BDs was surprising considering a lethal
250 phenotype associated with the loss of function of both BDs. To validate our results, we extended
251 depletion time to monitor for accumulating indirect effects on transcription. We observed that
252 after 3 hours of IAA treatment a median change in transcription in BD1/2 and ET domain
253 mutants reached 3-fold and 22.6-fold, respectively, suggesting that the loss of viability in BD1/2
254 mutant is driven primarily by indirect defects in transcription (**Fig. 2D**). Nevertheless, such
255 indirect effects on transcription are considerably stronger in case of the ET domain mutation,

reflecting the direct changes we observed after rapid depletion of endogenous Bdf1/2. In summary, we revealed a surprisingly small direct contribution of the BDs to transcription and showed that the ET domain is a dominant BET protein domain in transcription regulation.

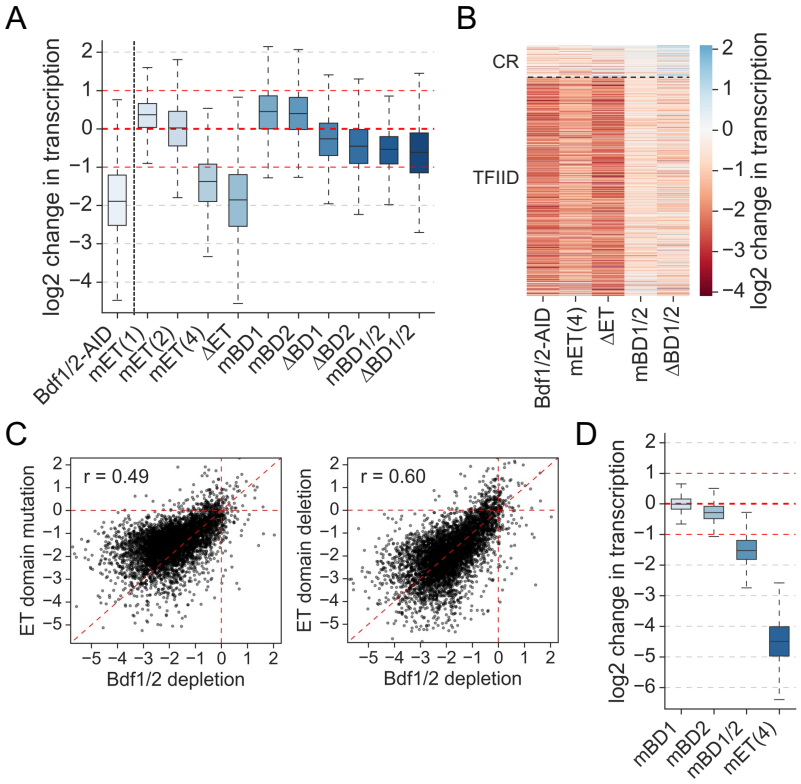


Fig. 2. **The ET domain is required for transcription of most genes.** (**A**) Boxplot showing log2 changes in transcription due to *BDF1* mutations measured by SLAM-seq after depleting endogenous Bdf1/2 for 25 min (n = 4,836). The results of depletion of Bdf1/2 in the absence of additional copy of *BDF1* are shown in the first column from the left. (**B**) Heatmap representation of selected results from (**A**). Genes are divided into previously defined major yeast gene classes (CR – coactivator-redundant, TFIID – TFIID-dependent) (*54*). (**C**) Scatter plots comparing selected results from (**A**). Pearson correlation coefficient (r) is shown. (**D**) Boxplot showing log2 changes in transcription due to *BDF1* mutations measured by SLAM-seq after depleting endogenous Bdf1/2 for 3 h (n = 4,836).

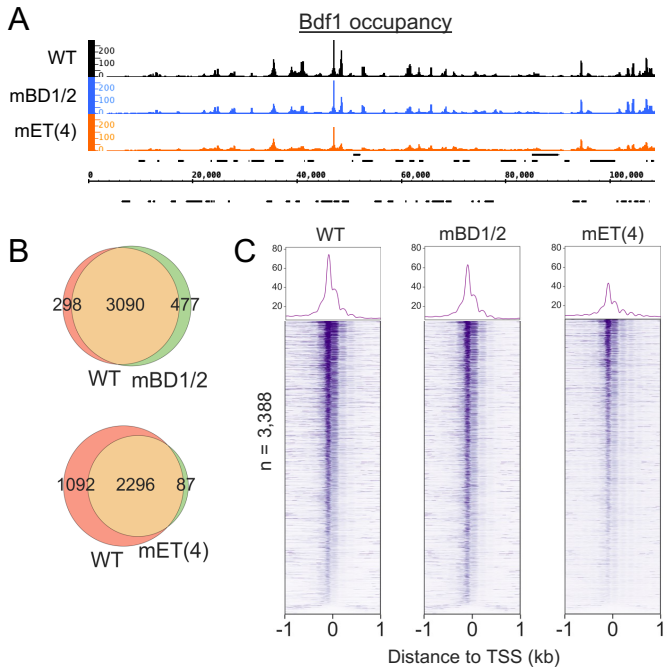
The ET domain facilitates BET protein recruitment to chromatin.

To modulate transcription, BET proteins first need to be recruited to gene regulatory elements. Experiments in both yeast and mammalian cells illustrated that the BD function does not fully explain BET protein chromatin occupancy (*4*, *6*, *22*, *24*, *31*). To identify BET protein domains contributing to Bdf1 recruitment to chromatin, we modified selected yeast strains used in SLAM-

seq experiments by inserting a micrococcal nuclease (MNase) coding sequence before the stop codon of *BDF1* variants to facilitate ChEC-seq assays (**Tables S1 and S2**) (*49*, *50*). We previously found that ChEC-seq complements and outperforms ChIP-seq in mapping BET protein chromatin occupancy in yeast cells (*4*). First, we validated that ectopically expressed Bdf1-MNase supports normal growth in the presence of AID-tagged endogenous *BDF1/2* (**Fig. S6A**). We also confirmed that ectopically expressed Bdf1-MNase shows a highly similar binding pattern as Bdf1-MNase expressed from the endogenous locus, both in the presence or absence of endogenous Bdf1/2 (**Fig. S6B**). Similarly to SLAM-seq assays, sample collection was preceded by depletion of endogenous Bdf1/2 for 25 minutes to eliminate possible confounding effects due to the presence of endogenous BET proteins. For example, endogenous Bdf1/2 could dimerize with Bdf1 variants thus facilitating recruitment. All experiments were done in three biological replicates and the results for individual replicate samples were highly similar (**Fig. S6C**).

To evaluate the impact of targeted mutations in BET protein domains on Bdf1 recruitment, we compared data collected for strains expressing *BDF1* variants to the control strain expressing unmodified *BDF1* in the context of depletion of endogenous Bdf1/2 (IAA treatment). In agreement with the major impact on transcription, we found that the ET domain broadly contributes to Bdf1 chromatin occupancy (**Figs. 3A-C**). Surprisingly, at most gene promoters, contribution of the ET domain to Bdf1 occupancy exceeds that of the BDs. We observed very similar effects in case of deletion of the BDs or the ET domain (**Fig. S7A-C**). Among other domains, we found that perturbations in the CPS domain and B/BID regions also strongly affect Bdf1 recruitment, indicating that both BET protein phosphorylation and dimerization are important factors in establishing proper BET protein occupancy genome-wide (**Fig. S7D-F**). We did not observe redistribution of Bdf1 due to mutations except for small subsets of promoters. Generally, all Bdf1 mutations decreased Bdf1 occupancy at loci bound by unmodified Bdf1, not caused Bdf1 recruitment to new loci. Finally, we found that changes in Bdf1 recruitment to gene promoters are not correlated with changes in transcription (**Fig. S7G**), in agreement with a lack of correlation between Bdf1 occupancy and transcription under normal conditions (*4*). In summary, we revealed that the ET domain, not the BDs, is a primary contributor to BET protein occupancy.

310



311

312 **Fig. 3**. **The ET domain has a major role in facilitating BET protein recruitment to**
313 **chromatin.** (**A**) Genome browser snapshot showing binding pattern of unmodified Bdf1 (WT)
314 and Bdf1 variants with mutations in the BDs or the ET domain as determined by ChEC-seq.
315 Data for the first 100,000 bases of chromosome III is shown. (**B**) Venn diagrams showing the
316 overlap of Bdf1-bound promoters for the indicated experiments. (**C**) Heatmaps showing
317 occupancy of unmodified Bdf1 and Bdf1 variants with mutations in the BDs or the ET domain
318 around the transcription start sites (TSS) of 3,388 genes, which promoters are bound by
319 unmodified Bdf1. Genes are sorted by the TSS signal (+/- 200 bp) in the WT experiment.

320

321 **The ET domain is required for Mediator and TFIID recruitment to gene promoters.**
322 The ET domain of BRD4 was proposed to modulate transcription by mediating protein-protein
323 interactions with several protein partners, but this model has not been systematically verified *in*
324 *vivo* (*2*, *31*, *32*). Both yeast and mammalian BET proteins have been linked with coactivators
325 Mediator and TFIID that regulate transcription initiation (*4*, *22*, *31*, *51*, *52*). Our prior work
326 demonstrated that Bdf1/2 are required for transcription initiation and the loss of Bdf1/2
327 decreases Mediator and TFIID promoter occupancy (*4*). Similarly, changes in Mediator
328 chromatin association were previously linked with the loss of BET protein function in mammalian
329 cells (*6*, *52*). Based on this background and our findings described so far, we hypothesized that
330 an important function of the ET domain in transcription regulation is facilitating recruitment of
331 Mediator and TFIID. To verify this hypothesis, we applied ChEC-seq to quantify changes in

332 Mediator and TFIID occupancy in cells relying solely on Bdf1 variants after rapid depletion of
333 endogenous Bdf1/2. Previously, we used MNase fusions on Mediator subunits Med8 and Med
334 17, which detect Mediator at a small number of genes and only at upstream activating
335 sequences, not promoters (*4*, *53*). Our recent work showed that by using MNase fusions to the
336 Med10 subunit we could detect Mediator directly at promoters for ~50% of genes (*53*). Thus, we
337 placed MNase coding sequence on Med10 in the Bdf1/2-AID strain and quantified changes in
338 Med10 chromatin occupancy following Bdf1/2 depletion. We observed that yeast BET proteins
339 have a strong impact on Mediator recruitment to most promoters occupied by Med10 (**Fig. S8A-**
340 **B**) (*53*).
341
342 Next, we inserted MNase coding sequence on Med10 and Taf1 (TFIID) in three strains used in
343 SLAM-seq assays – the control strain carrying unmodified additional copy of *BDF1*, and strains
344 carrying *BDF1* variants with mutations in the ET domain or the BDs (mET(4) and mBD1/2,
345 respectively). We depleted endogenous Bdf1/2 and determined changes in Med10 and Taf1
346 recruitment comparing the data collected for strains carrying *BDF1* variants to the control strain.
347 Experiments were done in three biological replicates, and the replicate results were highly
348 reproducible (**Fig. S9A**). We observed a general loss of Med10 association with promoters in
349 the context of the ET domain mutation, but not BD mutation (**Fig. 4A-B**). Importantly, changes in
350 Med10 occupancy due to the ET domain mutation or a complete loss of BET proteins were
351 comparable, indicating that the ET domain is a sole region of BET proteins implicated in
352 Mediator recruitment (**Fig. S9B**). In contrast, both the ET domain and the BDs contributed to
353 TFIID recruitment to promoters, although the contribution of the ET domain was larger at most
354 loci (**Fig. 4C-D**). Finally, we observed that changes to Mediator and TFIID recruitment in the
355 context of Bdf1 mutations did not correlate with changes in transcription (**Fig. S9C**). In
356 agreement, we previously reported that transcription was uncoupled from a baseline Mediator or
357 TFIID promoter occupancy and changes in Mediator and TFIID occupancy did not correlate with
358 changes in transcription due to a complete loss of BET proteins (*4*, *53*, *54*). In summary, we
359 showed that the ET domain facilitates Mediator recruitment to gene promoters while both the ET
360 domain and the BDs contribute to TFIID recruitment.
361
362
363
364
365

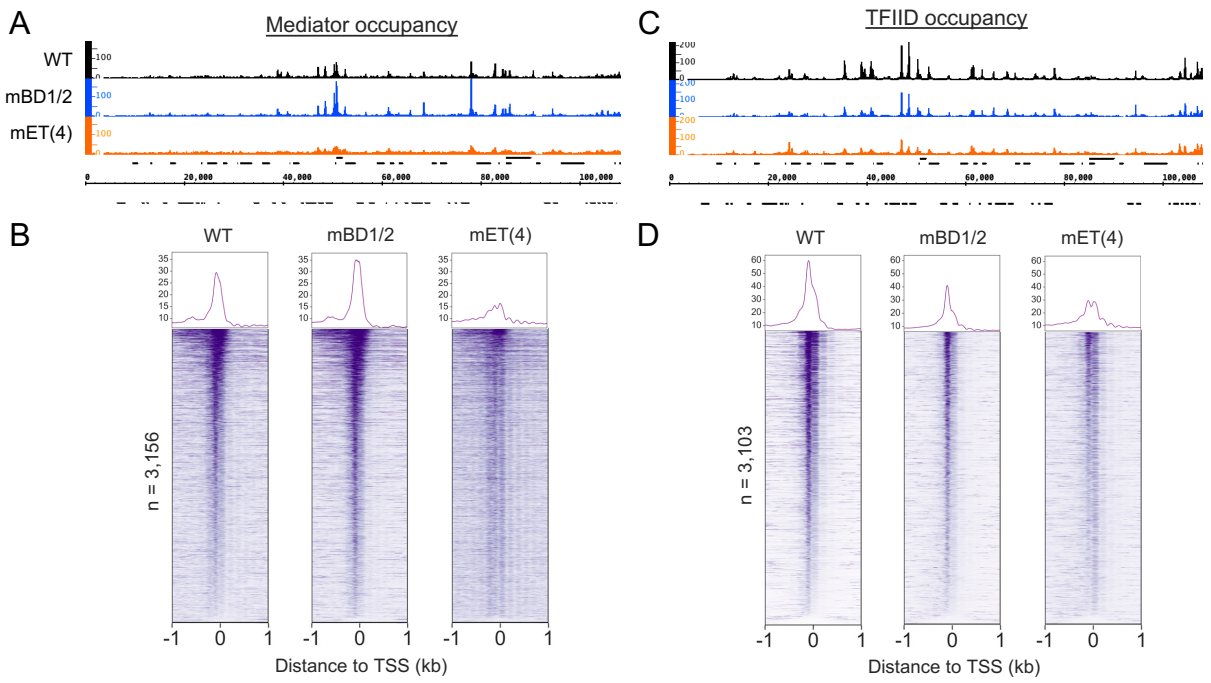


Fig. 4. **The ET domain recruits Mediator and TFIID to promoters.** (**A**) Genome browser snapshot showing Med10 (Mediator) binding pattern in the context of unmodified Bdf1 or Bdf1 variants with mutations in the BDs or the ET domain as determined by ChEC-seq. Data for the first 100,000 bases of chromosome III is shown. (**B**) Heatmaps showing Med10 occupancy around transcription start sites (TSS) of 3,156 genes, which promoters are bound by Med10 (*53*), in the context of unmodified Bdf1 or Bdf1 variants with mutations in the BDs or the ET domain. Genes are sorted by the signal around TSS (+/- 200 bp) in the WT experiment. (**C**) Genome browser snapshot showing Taf1 (TFIID) binding pattern in the context of unmodified Bdf1 or Bdf1 variants with mutations in the BDs or the ET domain as determined by ChEC-seq. Data for the first 100,000 bases of chromosome III is shown. (**D**) Heatmaps showing Taf1 occupancy around TSS of 3,103 genes, which promoters are bound by Taf1 (*4*), in the context of unmodified Bdf1 or Bdf1 variants with mutations in the BDs or the ET domain. Genes are sorted by the signal around TSS (+/- 200 bp) in the WT experiment.

The ET domain of yeast and human BET proteins uses the same mechanism to engage its targets.

Based on proteomic data, two consensus motifs recognized by the ET domain of mammalian BET proteins were proposed - Φ[+]Φ[+] or (Φ/[+])ΦΦ[+](Φ/[+]), where Φ is M, L, V, I, or F and [+] is K or R (ETM [ET-interacting motif]) (*2*, *33*, *34*). Importantly, only a few potential interactions involving the ET domain were experimentally validated. It remains to be found how many factors associate with the ET domain and what are the functional consequences of these

interactions. To begin to address these questions, we screened the annotated yeast nuclear proteome and found 974 proteins carrying at least one ETM (**Fig. S10A and Table S8**). This list included TFIID subunit Taf7, which is the only confirmed interacting partner of yeast BET proteins. A region interacting with Bdf1 was mapped to the N-terminal end (first 101 amino acids) of Taf7 where we identified four ETMs (**Fig. S10B**) (*22*). We applied a yeast two-hybrid (Y2H) system to test if Bdf1-Taf7 interaction is mediated by the ET domain (**Fig. 5**). We found that Bdf1 ET domain interacted with Taf7 and this interaction was abolished by mutating either the ET domain or by substituting basic residues to alanine in all four ETMs in the N-terminal part of Taf7. This finding provides mechanistic basis for the reported Bdf1-Taf7 interaction, suggests that the ET domain of yeast and mammalian BET proteins engages its targets using a conserved mechanism, and validates our approach to systematically identify and characterize interacting partners of the ET domain in yeast cells.

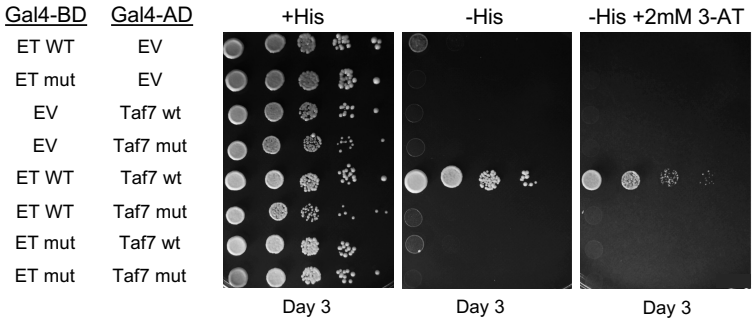


Fig. 5. **Y2H analysis of interaction between Bdf1 ET domain and Taf7.** Plasmids expressing Bdf1 ET domain, Taf7 or empty plasmids (EV) were co-transformed to a host strain as indicated. Cells were serially diluted ten-fold, plated on indicated media and imaged after three days of growth (n = 3). ET mut – ET domain with four conserved acidic residues substituted to alanine. Taf7 mut – Taf7 with all four ETMs in the N-terminal end (first 101 amino acids (*22*)) mutated by substituting basic residues to alanine.

Discussion

Discovery of specific inhibitors of BET BDs brought a promise of effective treatment of cancer and other diseases (*11*, *12*). However, the clinical application of BD inhibitors faced significant obstacles due to emerging resistance, cellular heterogeneity, and limited understanding of the biology of BET proteins (*14*, *17*, *55*). In this work we utilized a highly versatile yeast model system to reveal the contributions of all conserved domains of BET proteins to cell viability, gene transcription and BET protein chromatin occupancy. We found that the ET domain, not the

419 BDs, constitutes a primary functional module of BET proteins. Our results complement and
420 challenge the existing models of BET protein functions in transcription, explain conflicting results
421 from prior studies, and suggest directions for future research.
422
423 Many studies reported recruitment and function of BET proteins that was resistant to BD-
424 inhibition (*15*–*17*, *21*, *31*, *56*). Based on genetic analysis, we identified five domains of BET
425 proteins essential for cell viability including BDs, the ET domain, and phosphorylated regions
426 NPS/CPS. The requirement for at least one BD and NPS/CPS regions was previously reported
427 (*26*, *38*), but we are the first to show that the ET domain is essential for cell survival. In
428 agreement with changes to viability, we observed that the ET domain has major contributions to
429 global transcription and BET protein recruitment to chromatin that exceed the contributions by
430 the BDs. Additionally, guided by prior studies (*32*–*34*), we identified conserved acidic residues
431 required for the ET domain function *in vivo*. The ET domain was proposed to be a feasible drug
432 target to modulate aberrant transcription in human pathologies (*2*, *39*, *57*). An interesting
433 direction for future research is development of bivalent inhibitors capable of modulating the
434 activities of the BDs and the ET domain simultaneously (*39*). The ET domain structures with
435 peptide ligands are available and our data showed that the mechanistic basis of interactions
436 involving the ET domain are conserved between yeast and humans (*33*–*35*, *57*). As such, the
437 yeast model system can serve as valuable tool to evaluate the ability of small molecule
438 inhibitors to interfere with the function of the ET domain. In combination with prior studies, our
439 findings provide a justification and framework for the development of approaches to modulate
440 BET protein activity by targeting the ET domain in place of or in combination with BD inhibition.
441
442 Other phenotypes we observed for Bdf1 mutants also complement and extend prior studies.
443 Motif B and BID were found to facilitate BRD4 dimerization in biochemical assays and motif B
444 deletion caused dissociation of BRD2 from chromatin (*39*, *40*, *58*). We observed a significant
445 growth phenotype correlated with changes in Bdf1 recruitment and, to a lesser extent, changes
446 in transcription resulting from deletion of B/BID regions. These findings indicate that BET
447 proteins act as dimers and future studies should address the role of dimerization in modulating
448 BET protein recruitment and activity. For example, an important layer of regulation of BET
449 protein function may be based on the relative ability of BET proteins to form homo- and
450 heterodimers. Next, phosphorylation of BET proteins in NPS and CPS regions was linked with
451 resistance to BD-inhibition and BET protein their ability to associate with proteins partners (*15*,
452 *16*, *25*, *39*, *59*). Prior studies suggested that NPS/CPS phosphorylation may act as a switch to

453 modulate BET protein affinity to distinct partners and propensity for dimerization (*25*, *39*, *59*). In
454 contrast, our finding that a phosphomimetic *BDF1* variant supports normal growth and causes
455 only mild changes in transcription and Bdf1 chromatin occupancy suggests that dynamic
456 regulation of BET protein phosphorylation is not necessary for BET protein function. Further, it
457 was proposed that NPS and CPS regions may have distinct roles. For example, NPS
458 phosphorylation was linked with activity of the BDs while CPS phosphorylation was suggested
459 to modulate function of the ET domain (*15*, *25*). We observed that mutation or deletion of NPS
460 did not affect BET protein function, mutation or deletion of CPS was detrimental, while a
461 simultaneous perturbation in both regions was lethal. Based on these findings, we propose that
462 phosphorylation of NPS and CPS regions is linked to the same role or roles of BET proteins.
463 Nevertheless, the functional significance of BET protein phosphorylation remains enigmatic and
464 requires further investigation.
465
466 Two recent works provided mechanistic explanations of the BD-independent functions of
467 mammalian BET proteins in transcription (*21*, *31*). Zheng et al. (*21*) showed that the C-terminal
468 fragment specific to the long isoform of BRD4 is able to independently support the release of
469 paused Pol II by interaction with pTEFb, and rescue transcription of many genes in the context
470 of depletion of endogenous BRD4. There is no equivalent of BRD4 long isoform in yeast and
471 transcription in yeast does not involve regulated Pol II pause and release. Nevertheless, since
472 Bur1 is a yeast functional homologue of CDK9, it would be interesting to test if BET protein
473 association with Bur1/CDK9 is conserved in yeast. Since Bur1 contains two ETMs, it is possible
474 that in yeast such association is mediated by the ET domain. In such case, evolutionary gain of
475 the long BRD4 isoform could have been driven by a need to separate BET protein functions in
476 transcription elongation from other functions mediated also by a short BRD4 isoform, which
477 could be shared with BRD2 and BRD3 (*2*, *19*, *32*). Zhang et al. (*31*) reported that BRD4 is
478 recruited to estrogen receptor binding sites and activates transcription in a BD-independent
479 manner. Zhang et al. showed that association with Mediator is important for BD-independent
480 BRD4 recruitment, while BD-independent association of BRD4 with elongation factors PAF1C,
481 SPT5 and SPT6 directly supports transcription. In agreement, our findings indicate that BET
482 proteins associate with Mediator through the ET domain, not BDs, and support a model where
483 the ET domain facilitates interactions with multiple partners. Importantly, our list of putative
484 interacting partners of the ET domain in yeast includes Spt5, Spt6 and subunits of PAF1C and
485 Mediator (**Table S8**). We will focus future studies on characterizing these and other putative
486 interactions involving the ET domain. Finally, we anticipate that even though depletion of BET

487 proteins or the ET domain mutation decreases Mediator chromatin occupancy, it is possible that
488 a loss of Mediator would also affect Bdf1 recruitment. Based on the results presented here and
489 prior studies, we propose that multiple protein-protein interactions involving the ET domain
490 facilitate both recruitment of different components of the transcription machinery as well as
491 recruitment of BET proteins. Importantly, our model agrees with the current models of
492 transcription regulation based on formation of transcriptional condensates through transient
493 interactions involving multivalent protein factors (*60*, *61*), and the propensity of BET proteins to
494 support liquid-liquid phase separation at gene regulator elements (*41*).
495
496 BET proteins are the key regulators of transcription and possibly other chromatin-based
497 processes (*4–7*, *62*, *63*). Despite their fundamental and clinical relevance, many aspects of their
498 biology remain poorly understood. We revealed critical roles of BET protein ET domain in
499 sustaining global transcription and facilitating BET protein recruitment to chromatin. We
500 provided evidence that the ET domain performs these functions serving as a hub for interactions
501 with multiple protein partners, including transcription coactivators TFIID and Mediator. Our
502 findings bring another mechanistic explanation for the failure of BD inhibitors to abolish BET
503 protein chromatin occupancy and function in the clinical setting and suggest directions for future
504 investigations towards a comprehensive model of the complex roles of BET proteins in
505 transcription regulation.
506
507 **Materials and Methods**
508
509 **Yeast cell growth**
510 All *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains used in this study are
511 listed in Table S2. For strain construction, *S. cerevisiae* strains were grown in YPD medium (1%
512 yeast extract, 2% peptone, 2% glucose, 20 µg/ml adenine sulfate) or synthetic complete (SC)
513 media (0.17% yeast nitrogen base without ammonium sulfate or amino acids (BD Difco), 5 g
514 ammonium sulfate, 40 µg/ml adenine sulfate, 0.6 g amino acid dropout mix and supplemented
515 with 2 µg/m Ura and 0.01% other amino acids to complement auxotrophic markers) at 30°C.
516 Standard amino acid dropout mix contains 2 g each of Tyr, Ser, Val, Ile, Phe, Asp, Pro, and 4 g
517 each of Arg, Thr, Lys and Met. 1.5% Bacto-agar (BD Difco) was added to medium as required
518 for growth on solid media. For complementation assay, *S. cerevisiae* strains were grown in SC
519 medium lacking Leu to select for cells carrying an episomal plasmid with *BDF1* variants and
520 plated onto medium containing 5-fluoroorotic acid (5-FOA) to shuffle out the wild-type *BDF1*

521 plasmid carrying URA3 marker. For genomic and growth assays, *S. cerevisiae* cells were grown
522 in YPD medium at 30°C with shaking. *S. pombe* cells were grown in YE medium (0.5% yeast
523 extract, 3% glucose) at 30°C with shaking. In experiments involving degron depletion of target
524 proteins, cells were treated with 1 mM IAA dissolved in DMSO or with DMSO alone for 25 min
525 or 3 h to induce protein degradation followed by protocol-specific steps. In SLAM-seq and
526 ChEC-seq experiments, *S. cerevisiae* cells were grown to OD600 ~0.5. *S. pombe* cells were
527 grown to OD600 ~1.0. In growth assays, cells were grown from OD 0.0001 – 0.0005 for 15 h
528 with or without addition of IAA or DMSO. Three biological replicates were collected in all
529 experiments, except for growth assay based on expression of *BDF1* variants from episomal
530 plasmid, where 5-6 replicates were collected.
531
532 **Plasmid and strain construction**
533 DNA plasmids and *S. cerevisiae* were constructed using standard methods. Proteins were
534 chromosomally tagged by yeast transformation and homologous recombination of PCR-
535 amplified DNA. For ChEC-seq experiments, proteins were tagged with 3xFLAG-MNase::TRP1
536 using pGZ110 (*49*).
537
538 **Western blot analysis**
539 1 ml cell culture was collected and pelleted from strains after treatment with IAA or DMSO,
540 washed with 500 µl water, then resuspended in 100 µl yeast whole cell extract buffer (60 mM
541 Tris, 6.8, 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, 0.0025% bromophenol blue). After
542 heating for 5 min at 95°C, samples were centrifuged for 5 min at 21K × g and analyzed by SDS-
543 PAGE and western blot where protein signals were visualized by using the Odyssey DLx
544 scanner (Li-Cor) and quantified using Odyssey Empiria Studio software (Li-Cor). Fluorescent
545 signal for total protein content (Li-Cor Total Protein Stain workflow) was used to normalize
546 signals for target proteins.
547
548 **SLAM-seq**
549 SLAM-seq was performed as previously described (*48*). Briefly, following DMSO or IAA
550 treatment, *S. cerevisiae* cultures were treated with 5 mM 4-thiouracil (4tU) in DMSO or DMSO
551 alone for 4 min. Cells were immediately fixed in cold methanol on dry ice and stored at –80°C.
552 The OD600 at the time of collection was ~ 0.7. *S. pombe* cultures were treated with 4tU and
553 collected as described above. Three biological replicates were collected in all experiments. RNA
554 purification and DNase I treatment were performed using a Quick-RNA Fungal/Bacterial

555 Miniprep kit (Zymo Research) based on the manufacturer's recommendations under reducing
556 conditions as described (*64*). Five micrograms of total RNA was used for alkylation (*65*, *66*). A
557 total of 200 ng of alkylated RNA was used to construct 3′ mRNA-sequencing libraries. Libraries
558 were sequenced on a NovaSeq 6000 (Illumina) at the Oklahoma Medical Research Foundation
559 (OMRF) Clinical Genomics Center (Oklahoma City, OK, USA).
560
561 **SLAM-seq data analysis**
562 Data (paired-end 150 bp reads) were analyzed as previously described (*48*). Briefly, reads were
563 preprocessed using fastp (version 0.23.2) (*67*) and bbduk (BBMap version 39.06)
564 (https://sourceforge.net/projects/bbmap/). Reads were subsequently processed using SLAM-
565 DUNK (version 0.4.3) (*65*, *68*). Total read counts are defined as the number of reads remaining
566 after alignment and filtering in SLAM-DUNK. T>C read counts are defined as the number of
567 reads that have ≥2 T>C conversions. We limited the list of analyzed genes by excluding genes
568 for which there were no T>C read counts in at least one replicate sample among all collected
569 experiments based on 25 min depletion. Normalization and differential expression analysis were
570 performed on T>C read counts using DESeq2 (version 1.38.3) (*69*). Normalization factors were
571 calculated based on total read counts or spike-in read counts, depending on the experiment.
572 Spike-in read counts were used for normalization in case of 3 h depletion where major changes
573 to total read counts were expected. Adjusted p-values were calculated using IHW (version
574 1.26.0) (*70*). Genes that do not meet criteria for fold change and/or p-value calculations in
575 DESeq2 were excluded from subsequent analyses. Finally, 4836 genes were included in the
576 analysis.
577
578 **ChEC-seq**
579 ChEC-seq was performed as previously described with minor modifications (*4*, *50*, *53*). Briefly,
580 50 ml *S. cerevisiae* cultures were pelleted at 2000 × g for 3 min. Cells were resuspended in 1 ml
581 of Buffer A (15 mM Tris, 7.5, 80 mM KCl, 0.1 mM EGTA, 0.2 mM spermine (Millipore Sigma),
582 0.3 mM spermidine (Millipore Sigma), protease inhibitors mix (Millipore Sigma), transferred to a
583 1.5 ml tube, and pelleted at 1500 × g for 30 s. Cell were washed twice with 1 ml of Buffer A and
584 finally resuspended in 570 µl of Buffer A. 30 µl 2% digitonin (Millipore Sigma) was added to a
585 final concentration of 0.1%, and cells were permeabilized for 5 min at 30°C with shaking (900
586 rpm). 0.2 mM CaCl2 was added to the samples followed by incubation for another 5 min at
587 30°C. 100 µl cell suspension was mixed with 100 µl Stop Solution (400 mM NaCl, 20 mM EDTA,
588 4 mM EGTA). Samples were incubated with 0.4 mg/ml Proteinase K (Thermo Fisher Scientific)

589 for 30 min at 55°C, and the DNA was purified using ChIP DNA Clean and Concentrator kit
590 (Zymo Research). DNA was eluted in 30 µl elution buffer. 0.3 mg/ml RNase A (Thermo Fisher
591 Scientific) was added to each sample and incubated for 20 min at 37°C. 30 µl of Mag-Bind
592 reagent (Omega Biotek) was added, and the samples were incubated for 10 min at RT.
593 Supernatants were transferred to a new tube, and the volume was adjusted to 175 µl (10 mM
594 Tris, 8.0). DNA was purified using ChIP DNA Clean and Concentrator kit (Zymo Research) and
595 eluted in 30 µl of Elution Buffer.
596
597 **ChEC-seq data analysis**
598 Data (paired-end 150 bp reads) were analyzed as previously described (*4*, *50*, *53*) with
599 modifications. Briefly, reads were preprocessed using bbduk (BBMap version 39.06)
600 (https://sourceforge.net/projects/bbmap/). Reads were aligned against the *S. cerevisiae* genome
601 (version R64-3–1 using Bowtie 2 (version 2.5.0) (*71*) with the following options: --maxins 700 --
602 dovetail --no-unal --no-mixed --no-discordant. BAM files were created, sorted, and indexed
603 using Samtools (version 1.18). Genome coverage was calculated and normalized by counts per
604 million (CPM) using deepTools2 (version 3.5.4) (*72*). Average genome coverage across
605 replicates was calculated using BEDTools (version 2.30.0) (*73*) and bedGraphToBigWig (*74*).
606 SAM files for *S. cerevisiae* data were converted to tag directories with the HOMER
607 (http://homer.ucsd.edu; (*75*)) 'makeTagDirectory' tool. Peaks were called using HOMER
608 'findPeaks' tool with optional arguments set to '-o auto -C 0 L 2 F 2', with the free MNase
609 dataset used as a control (*4*). These settings use a default false discovery rate (FDR; 0.1%) and
610 require peaks to be enriched twofold over the control and twofold over the local background.
611 Resulting peak files were converted to BED files using 'pos2bed.pl' program. For each peak, the
612 peak summit was calculated as a mid-range between peak borders. For peak assignment to
613 promoters, the list of all annotated ORF sequences (excluding sequences classified as 'dubious'
614 or 'pseudogene') was downloaded from the SGD website (https://www.yeastgenome.org). Data
615 for 5888 genes were merged with TSS positions (*76*). If the TSS annotation was missing, TSS
616 was manually assigned at position −100 bp relative to the start codon. At this point, the results
617 of biological replicate experiments for each sample were averaged. Peaks were assigned to
618 promoters if their peak summit was in the range from −300 to +100 bp relative to TSS. In a few
619 cases, where more than one peak was assigned to the promoter, the one closer to TSS was
620 used. Promoters bound in at least two out of four replicate experiments were included in a final
621 list of promoters. To quantify log2 change in promoter occupancy, signal per promoter was
622 calculated as a sum of normalized reads in a 200 bp window around the promoter peak summit.

623

624 **ET-interacting motif discovery**

625 Putative ET-interacting motifs (ETMs) were identified using SLiMSearch (version 4) (*77*) to
626 search the *S. cerevisiae* proteome for proteins containing two previously described classes of
627 ET-binding motifs: Φ[+]Φ[+] and (Φ/[+])Φ�φ[+](Φ/[+]), where Φ is M, L, V, I, or F and [+] is K or R
628 (*78*). ETMs were limited to those in nuclear proteins based on evidence in AllianceMine
629 (Alliance of Genome Resources Consortium, 2024). ETMs with a disorder score ≥ 0.3 or an
630 accessibility score ≥ 0.3 were kept. ETMs below these cutoffs were assessed in specific
631 instances. Disorder and accessibility scores were calculated in SLiMSearch using IUPred3 (*79*)
632 and Alphafold2 (*80*), respectively.

633

634 **Yeast two-hybrid analysis**

635 Yeast two-hybrid experiments were performed using the Matchmaker Gold Y2H system (Takara
636 Bio) based on the manufacturer's recommendations with modifications. Briefly, the *S. cerevisiae*
637 strain Y2HGold was co-transformed with *TRP1*-marked Gal4-BD and *LEU2*-marked Gal4-AD
638 plasmids, pGBKT7 and pGADT7, respectively. Co-transformed strains were cultured in SC-Leu-
639 Trp medium at 30°C overnight, adjusted to an OD600 of 1, and serially diluted ten-fold. Five
640 microliters was spotted onto SC-Leu-Trp, SC-Leu-Trp-His, or SC-Leu-Trp-His supplemented
641 with 2 mM 3-Amino-1,2,4-triazole (3-AT, MilliporeSigma). Plates were incubated at 30°C and
642 imaged daily for a minimum of four days.

643

644

645 **Acknowledgments**

646

647 We thank Linda Thompson, Dean Dawson, Christopher Sansam and Karen Arndt for helpful
648 comments, Steven Hahn for plasmids, and Stephen Buratowski for anti-Bdf1 antibody.

649

650 **Funding**

651

652 National Science Foundation (NSF) grant 2346844 (RD).
653 Oklahoma Center for Adult Stem Cell Research (OCASCR) grant 231012 (RD).
654 National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health
655 (NIH) grant 1P20GM103636 (Linda Thompson, Project 2 to RD).

656

657 **Author contributions**

658

659 Conceptualization: JR, MD, RD

660 Methodology: JR, MD, RD

661 Investigation: IP, AM, MD, RD

662 Visualization: IP, AM, JR, MD, RD

663 Supervision: JR, MD, RD

664 Writing—original draft: RD

665 Writing—review & editing: JR, MD, RD

666

667 **Competing interests**

668

669 The authors declare that they have no competing interests.

670

671

672 **References**

673

674 1. P. Filippakopoulos, S. Picaud, M. Mangos, T. Keates, J.-P. Lambert, D. Barsyte-Lovejoy, I.
675 Felletar, R. Volkmer, S. Müller, T. Pawson, A.-C. Gingras, C. H. Arrowsmith, S. Knapp,
676 Histone Recognition and Large-Scale Structural Analysis of the Human Bromodomain
677 Family. *Cell* **149**, 214–231 (2012).

678 2. J.-P. Lambert, S. Picaud, T. Fujisawa, H. Hou, P. Savitsky, L. Uusküla-Reimand, G. D.
679 Gupta, H. Abdouni, Z.-Y. Lin, M. Tucholska, J. D. R. Knight, B. Gonzalez-Badillo, N. St-
680 Denis, J. A. Newman, M. Stucki, L. Pelletier, N. Bandeira, M. D. Wilson, P.
681 Filippakopoulos, A.-C. Gingras, Interactome Rewiring Following Pharmacological Targeting
682 of BET Bromodomains. *Mol Cell* **73**, 621-638.e17 (2019).

683 3. M. Yun, J. Wu, J. L. Workman, B. Li, Readers of histone modifications. *Cell Res* **21**, 564–
684 578 (2011).

685 4. R. Donczew, S. Hahn, BET family members Bdf1/2 modulate global transcription initiation
686 and elongation in Saccharomyces cerevisiae. *Elife* **10**, e69619 (2021).

687 5. M. Muhar, A. Ebert, T. Neumann, C. Umkehrer, J. Jude, C. Wieshofer, P. Rescheneder, J.
688 J. Lipp, V. A. Herzog, B. Reichholf, D. A. Cisneros, T. Hoffmann, M. F. Schlapansky, P.
689 Bhat, A. von Haeseler, T. Köcher, A. C. Obenauf, J. Popow, S. L. Ameres, J. Zuber,

690 SLAM-seq defines direct gene-regulatory functions of the BRD4-MYC axis. *Science* **360**,
691 800–805 (2018).
692 6. G. E. Winter, A. Mayer, D. L. Buckley, M. A. Erb, J. E. Roderick, S. Vittori, J. M. Reyes, J.
693 di Iulio, A. Souza, C. J. Ott, J. M. Roberts, R. Zeid, T. G. Scott, J. Paulk, K. Lachance, C.
694 M. Olson, S. Dastjerdi, S. Bauer, C. Y. Lin, N. S. Gray, M. A. Kelliher, L. S. Churchman, J.
695 E. Bradner, BET Bromodomain Proteins Function as Master Transcription Elongation
696 Factors Independent of CDK9 Recruitment. *Molecular Cell* **67**, 5-18.e19 (2017).
697 7. B. Zheng, Y. Aoi, A. P. Shah, M. Iwanaszko, S. Das, E. J. Rendleman, D. Zha, N. Khan, E.
698 R. Smith, A. Shilatifard, Acute perturbation strategies in interrogating RNA polymerase II
699 elongation factor function in gene expression. *Genes Dev.* **35**, 273–285 (2021).
700 8. T. Fujisawa, P. Filippakopoulos, Functions of bromodomain-containing proteins and their
701 roles in homeostasis and cancer. *Nature Reviews Molecular Cell Biology* **18**, 246–262
702 (2017).
703 9. N. Wang, R. Wu, D. Tang, R. Kang, The BET family in immunity and disease. *Signal*
704 *Transduction and Targeted Therapy* **6**, 1–22 (2021).
705 10. Y. Xu, C. R. Vakoc, Targeting Cancer Cells with BET Bromodomain Inhibitors. *Cold Spring*
706 *Harb Perspect Med* **7**, a026674 (2017).
707 11. P. Filippakopoulos, J. Qi, S. Picaud, Y. Shen, W. B. Smith, O. Fedorov, E. M. Morse, T.
708 Keates, T. T. Hickman, I. Felletar, M. Philpott, S. Munro, M. R. McKeown, Y. Wang, A. L.
709 Christie, N. West, M. J. Cameron, B. Schwartz, T. D. Heightman, N. La Thangue, C. A.
710 French, O. Wiest, A. L. Kung, S. Knapp, J. E. Bradner, Selective inhibition of BET
711 bromodomains. *Nature* **468**, 1067–1073 (2010).
712 12. E. Nicodeme, K. L. Jeffrey, U. Schaefer, S. Beinke, S. Dewell, C. Chung, R. Chandwani, I.
713 Marazzi, P. Wilson, H. Coste, J. White, J. Kirilovsky, C. M. Rice, J. M. Lora, R. K. Prinjha,
714 K. Lee, A. Tarakhovsky, Suppression of inflammation by a synthetic histone mimic. *Nature*
715 **468**, 1119–1123 (2010).
716 13. T. Shorstova, W. D. Foulkes, M. Witcher, Achieving clinical success with BET inhibitors as
717 anti-cancer agents. *Br J Cancer* **124**, 1478–1490 (2021).
718 14. Z.-Q. Wang, Z.-C. Zhang, Y.-Y. Wu, Y.-N. Pi, S.-H. Lou, T.-B. Liu, G. Lou, C. Yang,
719 Bromodomain and extraterminal (BET) proteins: biological functions, diseases and
720 targeted therapy. *Sig Transduct Target Ther* **8**, 1–26 (2023).
721 15. S. Shu, C. Y. Lin, H. H. He, R. M. Witwicki, D. P. Tabassum, J. M. Roberts, M.
722 Janiszewska, S. Jin Huh, Y. Liang, J. Ryan, E. Doherty, H. Mohammed, H. Guo, D. G.
723 Stover, M. B. Ekram, G. Peluffo, J. Brown, C. D'Santos, I. E. Krop, D. Dillon, M. McKeown,

724 C. Ott, J. Qi, M. Ni, P. K. Rao, M. Duarte, S.-Y. Wu, C.-M. Chiang, L. Anders, R. A. Young,
725 E. P. Winer, A. Letai, W. T. Barry, J. S. Carroll, H. W. Long, M. Brown, X. Shirley Liu, C. A.
726 Meyer, J. E. Bradner, K. Polyak, Response and resistance to BET bromodomain inhibitors
727 in triple-negative breast cancer. *Nature* **529**, 413–417 (2016).
728 16. J. Calder, A. Nagelberg, J. Luu, D. Lu, W. W. Lockwood, Resistance to BET inhibitors in
729 lung adenocarcinoma is mediated by casein kinase phosphorylation of BRD4.
730 *Oncogenesis* **10**, 27 (2021).
731 17. Y. Sun, Z. Zhang, K. Zhang, Y. Liu, P. Shen, M. Cai, C. Jia, W. Wang, Z. Gu, P. Ma, H. Lu,
732 L. Guan, W. Di, G. Zhuang, X. Yin, Epigenetic heterogeneity promotes acquired resistance
733 to BET bromodomain inhibition in ovarian cancer. *Am J Cancer Res* **11**, 3021–3038
734 (2021).
735 18. M. Arnold, A. Bressin, O. Jasnovidova, D. Meierhofer, A. Mayer, A BRD4-mediated
736 elongation control point primes transcribing RNA polymerase II for 3'-processing and
737 termination. *Mol Cell*, S1097-2765(21)00506–2 (2021).
738 19. O. Gilan, I. Rioja, K. Knezevic, M. J. Bell, M. M. Yeung, N. R. Harker, E. Y. N. Lam, C.-W.
739 Chung, P. Bamborough, M. Petretich, M. Urh, S. J. Atkinson, A. K. Bassil, E. J. Roberts, D.
740 Vassiliadis, M. L. Burr, A. G. S. Preston, C. Wellaway, T. Werner, J. R. Gray, A.-M.
741 Michon, T. Gobbetti, V. Kumar, P. E. Soden, A. Haynes, J. Vappiani, D. F. Tough, S.
742 Taylor, S.-J. Dawson, M. Bantscheff, M. Lindon, G. Drewes, E. H. Demont, D. L. Daniels,
743 P. Grandi, R. K. Prinjha, M. A. Dawson, Selective targeting of BD1 and BD2 of the BET
744 proteins in cancer and immunoinflammation. *Science* **368**, 387–394 (2020).
745 20. S.-K. Kim, X. Liu, J. Park, D. Um, G. Kilaru, C.-M. Chiang, M. Kang, K. M. Huber, K. Kang,
746 T.-K. Kim, Functional coordination of BET family proteins underlies altered transcription
747 associated with memory impairment in fragile X syndrome. *Science Advances* **7**, eabf7346
748 (2021).
749 21. B. Zheng, S. Gold, M. Iwanaszko, B. C. Howard, L. Wang, A. Shilatifard, Distinct layers of
750 BRD4-PTEFb reveal bromodomain-independent function in transcriptional regulation.
751 *Molecular Cell* **83**, 2896-2910.e4 (2023).
752 22. O. Matangkasombut, R. M. Buratowski, N. W. Swilling, S. Buratowski, Bromodomain factor
753 1 corresponds to a missing piece of yeast TFIID. *Genes Dev.* **14**, 951–962 (2000).
754 23. E. García-Oliver, C. Ramus, J. Perot, M. Arlotto, M. Champleboux, F. Mietton, C. Battail, A.
755 Boland, J.-F. Deleuze, M. Ferro, Y. Couté, J. Govin, Bdf1 Bromodomains Are Essential for
756 Meiosis and the Expression of Meiotic-Specific Genes. *PLOS Genetics* **13**, e1006541
757 (2017).

758 24. T. Kanno, Y. Kanno, G. LeRoy, E. Campos, H.-W. Sun, S. R. Brooks, G. Vahedi, T. D.
759 Heightman, B. A. Garcia, D. Reinberg, U. Siebenlist, J. J. O'Shea, K. Ozato, BRD4 assists
760 elongation of both coding and enhancer RNAs by interacting with acetylated histones. *Nat*
761 *Struct Mol Biol* **21**, 1047–1057 (2014).
762 25. S.-Y. Wu, A.-Y. Lee, H.-T. Lai, H. Zhang, C.-M. Chiang, Phospho switch triggers Brd4
763 chromatin binding and activator recruitment for gene-specific targeting. *Mol Cell* **49**, 843–
764 857 (2013).
765 26. O. Matangkasombut, S. Buratowski, Different sensitivities of bromodomain factors 1 and 2
766 to histone H4 acetylation. *Mol. Cell* **11**, 353–363 (2003).
767 27. M. J. Slaughter, E. K. Shanle, A. Khan, K. F. Chua, T. Hong, L. D. Boxer, C. D. Allis, S. Z.
768 Josefowicz, B. A. Garcia, S. B. Rothbart, B. D. Strahl, I. J. Davis, HDAC inhibition results in
769 widespread alteration of the histone acetylation landscape and BRD4 targeting to gene
770 bodies. *Cell Rep* **34**, 108638 (2021).
771 28. P. C. Megee, B. A. Morgan, B. A. Mittman, M. M. Smith, Genetic Analysis of Histone H4:
772 Essential Role of Lysines Subject to Reversible Acetylation. *Science* **247**, 841–845 (1990).
773 29. P. S. Kayne, U.-J. Kim, M. Han, J. R. Mullen, F. Yoshizaki, M. Grunstein, Extremely
774 conserved histone H4 N terminus is dispensable for growth but essential for repressing the
775 silent mating loci in yeast. *Cell* **55**, 27–39 (1988).
776 30. J. A. Ranish, E. C. Yi, D. M. Leslie, S. O. Purvine, D. R. Goodlett, J. Eng, R. Aebersold,
777 The study of macromolecular complexes by quantitative proteomics. *Nat. Genet.* **33**, 349–
778 355 (2003).
779 31. S. Zhang, R. G. Roeder, Resistance of estrogen receptor function to BET bromodomain
780 inhibition is mediated by transcriptional coactivator cooperativity. *Nat Struct Mol Biol*, 1–15
781 (2024).
782 32. S. Rahman, M. E. Sowa, M. Ottinger, J. A. Smith, Y. Shi, J. W. Harper, P. M. Howley, The
783 Brd4 extraterminal domain confers transcription activation independent of pTEFb by
784 recruiting multiple proteins, including NSD3. *Mol Cell Biol* **31**, 2641–2652 (2011).
785 33. Q. Zhang, L. Zeng, C. Shen, Y. Ju, T. Konuma, C. Zhao, C. R. Vakoc, M.-M. Zhou,
786 Structural Mechanism of Transcriptional Regulator NSD3 Recognition by the ET Domain of
787 BRD4. *Structure* **24**, 1201–1208 (2016).
788 34. T. Konuma, D. Yu, C. Zhao, Y. Ju, R. Sharma, C. Ren, Q. Zhang, M.-M. Zhou, L. Zeng,
789 Structural Mechanism of the Oxygenase JMJD6 Recognition by the Extraterminal (ET)
790 Domain of BRD4. *Sci Rep* **7**, 16272 (2017).

791 35. B. L. Crowe, R. C. Larue, C. Yuan, S. Hess, M. Kvaratskhelia, M. P. Foster, Structure of
792 the Brd4 ET domain bound to a C-terminal motif from γ-retroviral integrases reveals a
793 conserved mechanism of interaction. *Proceedings of the National Academy of Sciences*
794 **113**, 2086–2091 (2016).
795 36. N. Alerasool, H. Leng, Z.-Y. Lin, A.-C. Gingras, M. Taipale, Identification and functional
796 characterization of transcriptional activators in human cells. *Molecular Cell* **82**, 677-695.e7
797 (2022).
798 37. J. Trojanowski, L. Frank, A. Rademacher, N. Mücke, P. Grigaitis, K. Rippe, Transcription
799 activation is enhanced by multivalent interactions independent of phase separation.
800 *Molecular Cell* **82**, 1878-1893.e10 (2022).
801 38. C. Sawa, E. Nedea, N. Krogan, T. Wada, H. Handa, J. Greenblatt, S. Buratowski,
802 Bromodomain factor 1 (Bdf1) is phosphorylated by protein kinase CK2. *Mol. Cell. Biol.* **24**,
803 4734–4742 (2004).
804 39. F. Malvezzi, C. J. Stubbs, T. A. Jowitt, I. L. Dale, X. Guo, J. P. DeGnore, G. Degliesposti,
805 J. M. Skehel, A. J. Bannister, M. S. McAlister, Phosphorylation-dependent BRD4
806 dimerization and implications for therapeutic inhibition of BET family proteins. *Commun*
807 *Biol* **4**, 1–13 (2021).
808 40. J. D. Weissman, A. K. Singh, B. N. Devaiah, P. Schuck, R. C. LaRue, D. S. Singer, The
809 intrinsic kinase activity of BRD4 spans its BD2-B-BID domains. *Journal of Biological*
810 *Chemistry* **297**, 101326 (2021).
811 41. B. R. Sabari, A. Dall'Agnese, A. Boija, I. A. Klein, E. L. Coffey, K. Shrinivas, B. J. Abraham,
812 N. M. Hannett, A. V. Zamudio, J. C. Manteiga, C. H. Li, Y. E. Guo, D. S. Day, J. Schuijers,
813 E. Vasile, S. Malik, D. Hnisz, T. I. Lee, I. I. Cisse, R. G. Roeder, P. A. Sharp, A. K.
814 Chakraborty, R. A. Young, Coactivator condensation at super-enhancers links phase
815 separation and gene control. *Science* **361** (2018).
816 42. X. Han, D. Yu, R. Gu, Y. Jia, Q. Wang, A. Jaganathan, X. Yang, M. Yu, N. Babault, C.
817 Zhao, H. Yi, Q. Zhang, M.-M. Zhou, L. Zeng, Roles of the BRD4 short isoform in phase
818 separation and active gene transcription. *Nat Struct Mol Biol* **27**, 333–341 (2020).
819 43. A. G. Ladurner, C. Inouye, R. Jain, R. Tjian, Bromodomains Mediate an Acetyl-Histone
820 Encoded Antisilencing Function at Heterochromatin Boundaries. *Molecular Cell* **11**, 365–
821 376 (2003).
822 44. C. Dhalluin, J. E. Carlson, L. Zeng, C. He, A. K. Aggarwal, M.-M. Zhou, Structure and
823 ligand of a histone acetyltransferase bromodomain. *Nature* **399**, 491–496 (1999).

824 45. R. S. Sikorski, P. Hieter, A system of shuttle vectors and yeast host strains designed for
825 efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* **122**, 19–27 (1989).
826 46. J. D. Boeke, J. Trueheart, G. Natsoulis, G. R. Fink, "[10] 5-Fluoroorotic acid as a selective
827 agent in yeast molecular genetics" in *Methods in Enzymology* (Academic Press, 1987;
828 https://www.sciencedirect.com/science/article/pii/0076687987540769)vol. 154 of
829 *Recombinant DNA Part E*, pp. 164–175.
830 47. K. Nishimura, T. Fukagawa, H. Takisawa, T. Kakimoto, M. Kanemaki, An auxin-based
831 degron system for the rapid depletion of proteins in nonplant cells. *Nat Methods* **6**, 917–
832 922 (2009).
833 48. J. B. Ridenour, R. Donczew, An end-to-end workflow to study newly synthesized mRNA
834 following rapid protein depletion in Saccharomyces cerevisiae. *BMC Methods* **1**, 8 (2024).
835 49. G. E. Zentner, S. Kasinathan, B. Xin, R. Rohs, S. Henikoff, ChEC-seq kinetics
836 discriminates transcription factor binding sites by DNA sequence and shape in vivo. *Nature*
837 *Communications* **6**, 8733 (2015).
838 50. L. Mahendrawada, L. Warfield, R. Donczew, S. Hahn, Low overlap of transcription factor
839 DNA binding and regulatory targets. *Nature*, 1–9 (2025).
840 51. S. Sdelci, C.-H. Lardeau, C. Tallant, F. Klepsch, B. Klaiber, J. Bennett, P. Rathert, M.
841 Schuster, T. Penz, O. Fedorov, G. Superti-Furga, C. Bock, J. Zuber, K. V. M. Huber, S.
842 Knapp, S. Müller, S. Kubicek, Mapping the chemical chromatin reactivation landscape
843 identifies BRD4-TAF1 cross-talk. *Nat Chem Biol* **12**, 504–510 (2016).
844 52. A. S. Bhagwat, J.-S. Roe, B. Y. L. Mok, A. F. Hohmann, J. Shi, C. R. Vakoc, BET
845 Bromodomain Inhibition Releases the Mediator Complex from Select cis-Regulatory
846 Elements. *Cell Rep* **15**, 519–530 (2016).
847 53. L. Warfield, R. Donczew, L. Mahendrawada, S. Hahn, Yeast Mediator facilitates
848 transcription initiation at most promoters via a Tail-independent mechanism. *Molecular Cell*
849 **82**, 4033-4048.e7 (2022).
850 54. R. Donczew, L. Warfield, D. Pacheco, A. Erijman, S. Hahn, Two roles for the yeast
851 transcription coactivator SAGA and a set of genes redundantly regulated by TFIID and
852 SAGA. *eLife* **9**, e50109 (2020).
853 55. A. Alqahtani, K. Choucair, M. Ashraf, D. M. Hammouda, A. Alloghbi, T. Khan, N. Senzer, J.
854 Nemunaitis, Bromodomain and extra-terminal motif inhibitors: a review of preclinical and
855 clinical advances in cancer therapy. *Future Science OA* **5**, FSO372 (2019).
856 56. S. Shu, H.-J. Wu, J. Y. Ge, R. Zeid, I. S. Harris, B. Jovanović, K. Murphy, B. Wang, X. Qiu,
857 J. E. Endress, J. Reyes, K. Lim, A. Font-Tello, S. Syamala, T. Xiao, C. S. Reddy

858 Chilamakuri, E. K. Papachristou, C. D'Santos, J. Anand, K. Hinohara, W. Li, T. O.
859 McDonald, A. Luoma, R. J. Modiste, Q.-D. Nguyen, B. Michel, P. Cejas, C. Kadoch, J. D.
860 Jaffe, K. W. Wucherpfennig, J. Qi, X. S. Liu, H. Long, M. Brown, J. S. Carroll, J. S. Brugge,
861 J. Bradner, F. Michor, K. Polyak, Synthetic Lethal and Resistance Interactions with BET
862 Bromodomain Inhibitors in Triple-Negative Breast Cancer. *Molecular Cell* **78**, 1096-
863 1113.e8 (2020).
864 57. S. Aiyer, G. V. T. Swapna, L.-C. Ma, G. Liu, J. Hao, G. Chalmers, B. C. Jacobs, G. T.
865 Montelione, M. J. Roth, A common binding motif in the ET domain of BRD3 forms
866 polymorphic structural interfaces with host and viral proteins. *Structure* **29**, 886-898.e6
867 (2021).
868 58. P. Garcia-Gutierrez, M. Mundi, M. Garcia-Dominguez, Association of bromodomain BET
869 proteins with chromatin requires dimerization through the conserved motif B. *Journal of*
870 *Cell Science* **125**, 3671–3680 (2012).
871 59. C.-M. Chiang, Phospho-BRD4: Transcription Plasticity and Drug Targeting. *Drug Discov*
872 *Today Technol* **19**, 17–22 (2016).
873 60. M. Stortz, D. M. Presman, V. Levi, Transcriptional condensates: a blessing or a curse for
874 gene regulation? *Commun Biol* **7**, 1–10 (2024).
875 61. K. Ryu, G. Park, W.-K. Cho, Emerging insights into transcriptional condensates. *Exp Mol*
876 *Med* **56**, 820–826 (2024).
877 62. C. G. Sansam, K. Pietrzak, B. Majchrzycka, M. A. Kerlin, J. Chen, S. Rankin, C. L.
878 Sansam, A mechanism for epigenetic control of DNA replication. *Genes Dev.* **32**, 224–229
879 (2018).
880 63. J. Zhang, A. M. Dulak, M. M. Hattersley, B. S. Willis, J. Nikkilä, A. Wang, A. Lau, C.
881 Reimer, M. Zinda, S. E. Fawell, G. B. Mills, H. Chen, BRD4 facilitates replication stress-
882 induced DNA damage response. *Oncogene* **37**, 3763–3777 (2018).
883 64. H. Alalam, J. A. Zepeda-Martínez, P. Sunnerhagen, Global SLAM-seq for accurate mRNA
884 decay determination and identification of NMD targets. *RNA* **28**, 905–915 (2022).
885 65. V. A. Herzog, B. Reichholf, T. Neumann, P. Rescheneder, P. Bhat, T. R. Burkard, W.
886 Wlotzka, A. von Haeseler, J. Zuber, S. L. Ameres, Thiol-linked alkylation of RNA to assess
887 expression dynamics. *Nat Methods* **14**, 1198–1204 (2017).
888 66. A. Klein-Brill, D. Joseph-Strauss, A. Appleboim, N. Friedman, Dynamics of Chromatin and
889 Transcription during Transient Depletion of the RSC Chromatin Remodeling Complex. *Cell*
890 *Reports* **26**, 279-292.e5 (2019).

891 67. S. Chen, Y. Zhou, Y. Chen, J. Gu, fastp: an ultra-fast all-in-one FASTQ preprocessor.
892 *Bioinformatics* **34**, i884–i890 (2018).
893 68. T. Neumann, V. A. Herzog, M. Muhar, A. von Haeseler, J. Zuber, S. L. Ameres, P.
894 Rescheneder, Quantification of experimentally induced nucleotide conversions in high-
895 throughput sequencing datasets. *BMC Bioinformatics* **20**, 258 (2019).
896 69. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for
897 RNA-seq data with DESeq2. *Genome Biology* **15**, 550 (2014).
898 70. N. Ignatiadis, W. Huber, Covariate Powered Cross-Weighted Multiple Testing. *Journal of*
899 *the Royal Statistical Society Series B: Statistical Methodology* **83**, 720–751 (2021).
900 71. B. Langmead, S. L. Salzberg, Fast gapped-read alignment with Bowtie 2. *Nature Methods*
901 **9**, 357–359 (2012).
902 72. F. Ramírez, D. P. Ryan, B. Grüning, V. Bhardwaj, F. Kilpert, A. S. Richter, S. Heyne, F.
903 Dündar, T. Manke, deepTools2: a next generation web server for deep-sequencing data
904 analysis. *Nucleic Acids Research* **44**, W160–W165 (2016).
905 73. A. R. Quinlan, I. M. Hall, BEDTools: a flexible suite of utilities for comparing genomic
906 features. *Bioinformatics* **26**, 841–842 (2010).
907 74. W. J. Kent, A. S. Zweig, G. Barber, A. S. Hinrichs, D. Karolchik, BigWig and BigBed:
908 enabling browsing of large distributed datasets. *Bioinformatics* **26**, 2204–2207 (2010).
909 75. S. Heinz, C. Benner, N. Spann, E. Bertolino, Y. C. Lin, P. Laslo, J. X. Cheng, C. Murre, H.
910 Singh, C. K. Glass, Simple combinations of lineage-determining transcription factors prime
911 cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* **38**, 576–
912 589 (2010).
913 76. D. Park, A. R. Morris, A. Battenhouse, V. R. Iyer, Simultaneous mapping of transcript ends
914 at single-nucleotide resolution and identification of widespread promoter-associated non-
915 coding RNA governed by TATA elements. *Nucleic Acids Res* **42**, 3736–3749 (2014).
916 77. I. Krystkowiak, N. E. Davey, SLiMSearch: a framework for proteome-wide discovery and
917 annotation of functional modules in intrinsically disordered regions. *Nucleic Acids*
918 *Research* **45**, W464–W469 (2017).
919 78. J.-P. Lambert, S. Picaud, T. Fujisawa, H. Hou, P. Savitsky, L. Uusküla-Reimand, G. D.
920 Gupta, H. Abdouni, Z.-Y. Lin, M. Tucholska, J. D. R. Knight, B. Gonzalez-Badillo, N. St-
921 Denis, J. A. Newman, M. Stucki, L. Pelletier, N. Bandeira, M. D. Wilson, P.
922 Filippakopoulos, A.-C. Gingras, Interactome Rewiring Following Pharmacological Targeting
923 of BET Bromodomains. *Mol Cell* **73**, 621-638.e17 (2019).

79. G. Erdős, M. Pajkos, Z. Dosztányi, IUPred3: prediction of protein disorder enhanced with unambiguous experimental annotation and visualization of evolutionary conservation. *Nucleic Acids Research* **49**, W297–W303 (2021).

80. J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021).