BBA - Molecular Basis of Disease 1869 (2023) 166689

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

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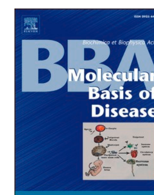

TAF1 bromodomain inhibition as a candidate epigenetic driver of congenital heart disease

Robert S. Leigh [1], Mika J. Välimäki [1], Bogac L. Kaynak [*], Heikki J. Ruskoaho [*]

Drug Research Program, Division of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

ARTICLE INFO

Keywords:
Congenital heart disease
Epigenetics
Transcription
Bromodomain

ABSTRACT

Heart formation requires transcriptional regulators that underlie congenital anomalies and the fetal gene program activated during heart failure. Attributing the effects of congenital heart disease (CHD) missense variants to disruption of specific protein domains allows for a mechanistic understanding of CHDs and improved diagnostics. A combined chemical and genetic approach was employed to identify novel CHD drivers, consisting of chemical screening during pluripotent stem cell (PSC) differentiation, gene expression analyses of native tissues and primary cell culture models, and the in vitro study of damaging missense variants from CHD patients. An epigenetic inhibitor of the TATA-Box Binding Protein Associated Factor 1 (TAF1) bromodomain was uncovered in an unbiased chemical screen for activators of atrial and ventricular fetal myosins in differentiating PSCs, leading to the development of a high affinity inhibitor (5.1 nM) of the TAF1 bromodomain, a component of the TFIID complex. TAF1 bromodomain inhibitors were tested for their effects on stem cell viability and cardiomyocyte differentiation, implicating a role for TAF1 in cardiogenesis. Damaging TAF1 missense variants from CHD patients were studied by mutational analysis of the TAF1 bromodomain, demonstrating a repressive role of TAF1 that can be abrogated by the introduction of damaging bromodomain variants or chemical TAF1 bromodomain inhibition. These results indicate that targeting the TAF1/TFIID complex with chemical compounds modulates cardiac transcription and identify an epigenetically-driven CHD mechanism due to damaging variants within the TAF1 bromodomain.

1. Introduction

Congenital heart diseases (CHDs) arise due to variants in genes controlling transcriptional networks, and many of these factors are also drivers of adult heart diseases [1–4]. As epigenetic mechanisms have been implicated in both congenital and adult heart diseases, the druggability of epigenetic targets could be used to develop novel therapies and tool compounds for disease modelling [5,6]. Indeed, BET bromodomain (BRD2, BRD3, BRD4) inhibitors that prevent the interaction between epigenetic-reader domains and acetylated lysines on histones have shown in vitro and in vivo efficacy in the prevention of pathological remodeling during heart failure [7–12]. Additionally, acetylation pathways have been shown to modulate the activity of cardiac transcription factors that mediate both congenital heart disease and the fetal gene response, such as GATA4, MEF2C, and TBX5 [13–17]. We have previously reported the successful chemical targeting of the developmental and hypertrophic transcription factor GATA4 in various models of cardiovascular disease, differentiation, and regeneration [18–21]. The identification and chemical targeting of additional cardiac transcriptional and epigenetic modifiers might have therapeutic use and provide novel chemical tools for mechanistic studies.

The TATA-binding protein associated factors (TAFs), together with TATA-binding protein (TBP), comprise the transcriptional initiation complex TFIID [22]. Of the 14 TAF family members, only TAF1 contains a double bromodomain, a tandem ‘epigenetic reader’ protein module capable of binding acetylated, butyrylated, and crotonylated lysines on histones and transcription factors [23–25]. Several recent studies describe the development of TAF1 bromodomain inhibitors from various chemical scaffolds [26–28]. The biological effects of these compounds are mostly unknown, and there are no reports of their effects on cardiac transcription or cardiovascular phenotypes. However, TAF1 and other TFIID subunits are known to control organogenesis and skeletal muscle differentiation [29–35]. Furthermore, specific TAFs and TAF-like factors (TAF5L, TAF6L, TAF9/TAF9B, TAF10, TAF12) are also components of

* Corresponding authors at: Division of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland.
E-mail addresses: bogac.kaynak@helsinki.fi (B.L. Kaynak), heikki.ruskoaho@helsinki.fi (H.J. Ruskoaho).

[1] Authors contributed equally to this work.

https://doi.org/10.1016/j.bbadis.2023.166689
Received 20 October 2022; Received in revised form 1 March 2023; Accepted 6 March 2023
Available online 21 March 2023
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the Spt-Ada-Gcn5 acetyltransferase (SAGA) complex, a multiprotein transcriptional activation complex with roles in development and disease [36,37]. Recently, damaging TAF1 missense variants were implicated in human congenital heart disease, suggesting a specific role for TFIID in heart formation [38]. Unfortunately, the requirement of the TAF1 protein for cell cycle progression has precluded detailed study of its effects during embryogenesis and adulthood [39–44]. Chemical probes that inactivate specific TAF1 protein domains without disturbing other TAF1 functions would allow for the investigation of TFIID in cardiovascular development and disease.

We here report the identification of novel inhibitors of the second bromodomain of TAF1 that induce the expression of atrial and ventricular myosins during the spontaneous differentiation of mouse embryonic stem cells (mESCs). New chemical entities were designed and synthesized with high affinity (5.1 nM) and selectivity (~172 fold) for TAF1, and novel and reference compounds were tested for the ability to modulate atrial and ventricular myosin reporter expression and inhibit activation of the natriuretic peptide B (Nppb) promoter in response to neurohormonal stimulation with endothelin-1 (ET-1). TFIID subunit composition was examined in the newly formed embryonic mouse heart, in addition to the rat heart one-week following myocardial infarction, indicating that TAFs which compose the SAGA coactivator complex are upregulated in the forming cardiac chambers. Damaging missense variants in the TAF1 double bromodomain were examined in reporter gene assays for cardiac myosin promoter activation, implicating a repressive role for TAF1 at an atrial-specific transgene that can be de-repressed by the introduction of damaging variants to the TAF1 bromodomain. Finally, transcriptome-wide effects of TAF1 bromodomain inhibition were observed by mRNA-sequencing following compound treatment in neonatal mouse ventricular cardiomyocytes, indicating genes governing gene expression (e.g. SAGA complex) are modulated by TAF1 bromodomain inhibition. Collectively, our results indicate that TAF1 bromodomain inhibition promotes epigenetic upregulation of gene subsets in immature cardiac tissue, providing a novel mechanism relevant to congenital heart diseases and regulation of fetal gene programs during adult heart disease.

2. Materials and methods

2.1. Computational modelling of protein-ligand interaction and compound synthesis

The commercial modelling package MOE 2020.0901 (Chemical Computing Group Inc., Montreal, Canada; http://www.chemcomp.com) was utilized for protein modelling and docking experiments. A large-scale protein model of TAF1, TBP, RNA polymerase II, and other TAF subunits as a part of the preinitiation complex (PIC) was compiled by superposing PIC (PDB 7EG9) [45] and AlphaFold protein of human TAF1 with two bromodomains (AF-P21675-F1). The docking protocol was carried out with a high-resolution structure of TAF1 (PDB 5I29) [46]. On-flight generated ligand conformations were placed in the cavity of the second bromodomain of TAF1 with the Triangle Matcher method and ranked with the London dG scoring function by utilizing Amber10-Extended Hückel Theory-force field. Subsequently, the 30 highest ranked poses were applied for a refinement procedure containing the energy minimization and rescoring with the Generalized-Born Volume Integral/Weighted Surface area (GBVI/WSA dG) scoring function.

Novel compounds 3i-1246, 3i-1247 and 3i-1248 were synthesized by Enamine (Kyiv, Ukraine), 3i-1103 was synthesized by the Faculty of Pharmacy, University of Helsinki [18] and reference compounds were obtained from commercial providers: CeMMEC1 (Cayman Chemical), BAY299 (Tocris), GNE371 (Biosynth/Carbosynth). All compounds conform to a minimum purity of >95 % by suppliers.

2.2. Bromodomain assays

Compounds were tested for activity against bromodomains using the Eurofins/DiscoverX bromoMAX assay against 32 bromodomains and the KdELECT assay against TAF1(2) and BRD2(1) bromodomains, specifically. Percent control was calculated using the following formula: ((test compound signal − positive control signal) / (negative control signal − positive control signal)) × 100.

2.3. Mouse embryonic stem cell culture

mESC culture and differentiation assays to determine the effects of TAF1 bromodomain inhibitors on the activation of atrial (SMyHC3-TdTomato) and ventricular (Myl2-eGFP) reporters were conducted as described [47]. In brief, mESCs were maintained in LIF-containing medium on 0.1 % gelatin in feeder-free conditions. Dual reporter (Myl2-eGFP/SMyHC3-TdTomato) mESCs in an E14 background were plated to inverted v-bottomed plates at 500 cells/well in DMEM supplemented with 20 % FBS, non-essential amino acids, glutamax, and 2-mercaptoethanol. After 2-day formation of embryoid bodies in hanging drops, medium was added with or without compounds. On day 5, embryoid bodies were transferred to a gelatin-coated 12-well plate, and medium was refreshed the following day. Compounds were diluted in DMSO and added to culture medium during D2–D10 of differentiation, and the final DMSO concentration was 0.1 %. D12 differentiation cultures were dissociated with TrypLE on a shaker, followed by inactivation of TrypLE with FBS, washing in 1×PBS, and resuspension in 1× PBS for flow cytometry analysis. A BD LSRFortessa flow cytometer was used for analysis, in addition to FlowJo (Flowjo LLC). Viability assays in mESCs were conducted following 48 h treatment with novel compounds or vehicle (DMSO) control using the Cell-titer Glo assay (Promega).

2.4. Primary mouse cardiomyocyte cell culture

Neonatal mouse ventricular cardiomyocytes (NMVCMs) were isolated using the neonatal heart dissociation kit (Miltentyi) and neonatal cardiomyocyte isolation kit (Miltentyi) and plated to gelatinized 96-well (30k cells/well) or 12-well plates (200 k cells/well). Cardiomyocytes were cultured for 24 h in DMEM/F12 supplemented with 2.5 mg/mL bovine serum albumin, insulin-transferrin-selenium, sodium pyruvate, pen-strep, and T_3 prior to ET-1 (Sigma) treatment and/or compound addition. Test compounds were diluted in DMSO, and 0.1 % DMSO was used as control for all cell culture experiments. For reporter gene assays in neonatal ventricular cardiomyocytes, cells were transfected with a BNP540-luciferase plasmid [48] using Lipofectamine 3000 in 96-well plates, and luciferase activity was detected using Neolite reagent (PerkinElmer) as previously [49]. For mRNA-sequencing, neonatal mouse ventricular cardiomyocytes were treated with DMSO or test compound with or without ET-1 for 24 h, followed by lysis and RNA isolation using Macherey-Nagel Nucleospin kit with DNAse treatment.

2.5. Embryonic tissue harvesting and qRT-PCR analysis

Embryonic tissues were dissected from wild type, C57BL/6JOlaHsd embryos and RNA was isolated using Trizol reagent (ThermoFisher Scientific) and the RNeasy MinElute cleanup kit combined with DNAse treatment (Qiagen). Biological replicates included four embryos per replicate. qRT-PCR was performed using taqman gene expression assays (Supplementary Table S1) on the Fluidigm Biomark HD. Final values were calculated by normalization to a reference gene (Actb).

2.6. Myocardial infarction sample collection and qRT-PCR analysis

Ligation of the left anterior descending (LAD) artery was used to induce myocardial infarction in rats anesthetized with ketamine (50 mg/kg, intraperitoneal) and xylazine/medetomidine (10 mg/kg,

intraperitoneal) [50,51]. Control (sham-operated) rats also underwent surgery but without ligation of the artery. Animals were sacrificed after one-week and left ventricular samples were extracted. The guanidine thiocyanate-CsCl method was used to isolate total RNA from the left ventricle lower transversal mid-section. Taqman gene expression assays (Supplementary Table S1) were used for qRT-PCR analysis on the Fluidigm Biomark HD, and final values were normalized to the reference gene Tbp [52].

2.7. Reporter gene assays with TAF1 damaging missense variants

TAF1 cDNA with predicted damaging missense variants [38] was generated using Gibson assembly. In brief, primers containing the desired variant were used to amplify a tetO-TAF1 cDNA plasmid containing the wild type sequence, and these were ligated using Gibson assembly master mix (NEB). Plasmids were sequenced to ensure fidelity and the absence of other base alterations within TAF1 cDNA. To assess effects of TAF1 overexpression on SMyHC3 activation, a SMyHC3-luciferase construct was generated by Gibson assembly in which the PCR-amplified SMyHC3 promoter was cloned into the pGL3 backbone. This construct was co-transfected into HEK cells in conjunction with FUdeltaGW-rtTA (Addgene #19780), pRL-TK-d238 [53], and novel tetO-TAF1 constructs with or without damaging variants. HEK cells were grown at 20,000 cells/well in DMEM + 10 % FBS in white-walled 96-well plates and transfected with Lipofectamine 3000. The next day, medium was changed to the same with 2 μg/mL doxycycline to induce TAF1 expression in the presence of compounds or vehicle-control (DMSO). Luciferase activity was measured after 48 h compound treatment using a dual-luciferase assay kit (Promega), and firefly/renilla luciferase was used as the final measurement to avoid effects due to altered transfection efficiency or cell viability. All luciferase measurements were performed on a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific).

2.8. mRNA-sequencing

mRNA sequencing was performed at the Biomedicum Functional Genomics Unit (University of Helsinki). RNA quality was assessed using Qubit (Thermo Fisher Scientific) and Bioanalyzer (Agilent) instruments. Samples were sequenced on an Illumina sequencer at the Biomedicum Functional Genomics Unit (University of Helsinki) using a bulk transcriptome profiling method based on Drop-seq [54,55]. Preprocessing was performed using the Drop-seq Tools package, and STAR aligner was used for alignment. PCR-bias corrected read counts were used as input for differential gene analysis in edgeR [56]. Default settings in edgeR were used for gene filtering based on count number and presence in multiple samples, and gene ontology analysis was conducted using goana (limma). For gene ontology analysis, only biological processes were considered, and FDR < 0.05 was deemed statistically significant. Each condition consisted of four biological replicates.

2.9. Statistics

Statistical analysis was performed in R. In all cases, n represents biological replicates, such as independent experiments (in vitro), pooled embryos, or experimental animals. Continuous variables are shown as the mean ± SEM, and individual data points representing biological replicates are shown (in some cases in supplementary information to complement summary figures in main body). Statistical significance was determined using *t*-test, Wilcoxon test, or ANOVA followed by Tukey's HSD or pairwise comparison with Bonferroni's correction. $p < 0.05$ was determined as statistically significant.

2.10. Role of funders

The funders (detailed in Acknowledgments) had no participation in planning, execution, or analysis of experiments.

3. Results

3.1. Identification of selective TAF1 bromodomain inhibitor and optimization of lead compound

We previously screened a structurally focused library of GATA4-targeted compounds and identified 3i-1103 as a modifier of atrial and ventricular reporter gene expression [21]. To further elucidate compound mechanism-of-action, 3i-1103 was subjected to screening against 32 bromodomains and was shown to have selective affinity for the second bromodomain of TAF1 (TAF1(2)), a component of the TFIID complex (Fig. 1, Supplementary Table S2). Subsequently, we explored whether structural modifications of 3i-1103 could potentially increase compound affinity for TAF1(2) without losing the selectivity among bromodomains. Based on the structural analysis, we introduced a bromodomain-specific warhead to the compound scaffold, leading to the discovery of TAF1(2) inhibitor 4-(*tert*-butyl)-*N*-(5-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-pyrazol-3-yl)benzamide (3i-1248), with approximately 100-fold increase in ligand affinity in comparison to screening hit 3i-1103. Our novel TAF1-inhibitor 3i-1248 also maintained selectivity within the bromodomain family, showing an excessive 170-fold preference over BRD2(1), though at higher concentrations (10 μM) multiple bromodomains were inhibited in a cell-free assay. On the contrary, the structurally similar compound 3i-1247 that lacked the warhead probe was entirely inactive up to 10 μM against all bromodomains. Indeed, off-target activity is likely not representative of bromodomain activity in cell-based assays, which would require higher concentration for target engagement. Compounds 3i-1103, 3i-1246, 3i-1247 and 3i-1248 showed comparable inhibitory activity against certain tyrosine kinase proteins, indicating the capacity of 3i-1247 to serve as a structurally similar negative control compound for anti-bromodomain activity in cell assays. Full analysis of compound activity against bromodomain and kinase protein families is shown in Supplementary Tables S3–S4.

3.2. TFIID gene expression dynamics during cardiogenesis implicates role of SAGA complex members

As compounds showed inhibitory activity against TAF1/TFIID, and TFIID subunit composition has been shown to play a role in skeletal muscle, neuronal cell, embryonic stem cell, and adipose tissue cell differentiation [31,57–59], we next sought to investigate TFIID subunit composition during cardiac formation by measuring the mRNA levels of TAF1 and other TFIID subunits in the nascent embryonic heart (Fig. 2, Supplementary Fig. S1). TFIID subunit mRNA levels were analyzed in primary (PA1) and secondary (PA2) pharyngeal arches, the embryonic atria, and embryonic ventricles of E10 mouse hearts. Notably, PA2 is known to contain Isl1+ cardiac progenitors that migrate into the forming heart, differentiate into cardiomyocytes, and populate the atrial and ventricular chambers, so these tissues allow comparison of expression changes during the native, in vivo differentiation process despite being from only a single time point [49,60]. Intriguingly, TAFs that also compose the SAGA acetylation complex (Taf9, Taf10 and Taf12) were upregulated in the embryonic ventricle vs PA2, suggesting that SAGA-complex TAFs predominate in differentiated cardiomyocytes vs cardiac progenitors. Taf6 (not reported to contribute to SAGA complex in humans) was also significantly upregulated in the ventricles vs PA2. Only Taf10 was significantly upregulated in the atria vs PA2, and downregulation of only one TFIID subunit (Taf4) was observed in embryonic ventricles vs PA2. Taf1 mRNA levels were unchanged across embryonic tissues, as were non-SAGA complex members Taf11 and Taf15. As a whole, these results suggest changes in mRNA levels of TFIID subunits that affect TFIID composition in the developing heart, and that TAFs composing the SAGA complex are upregulated in immature

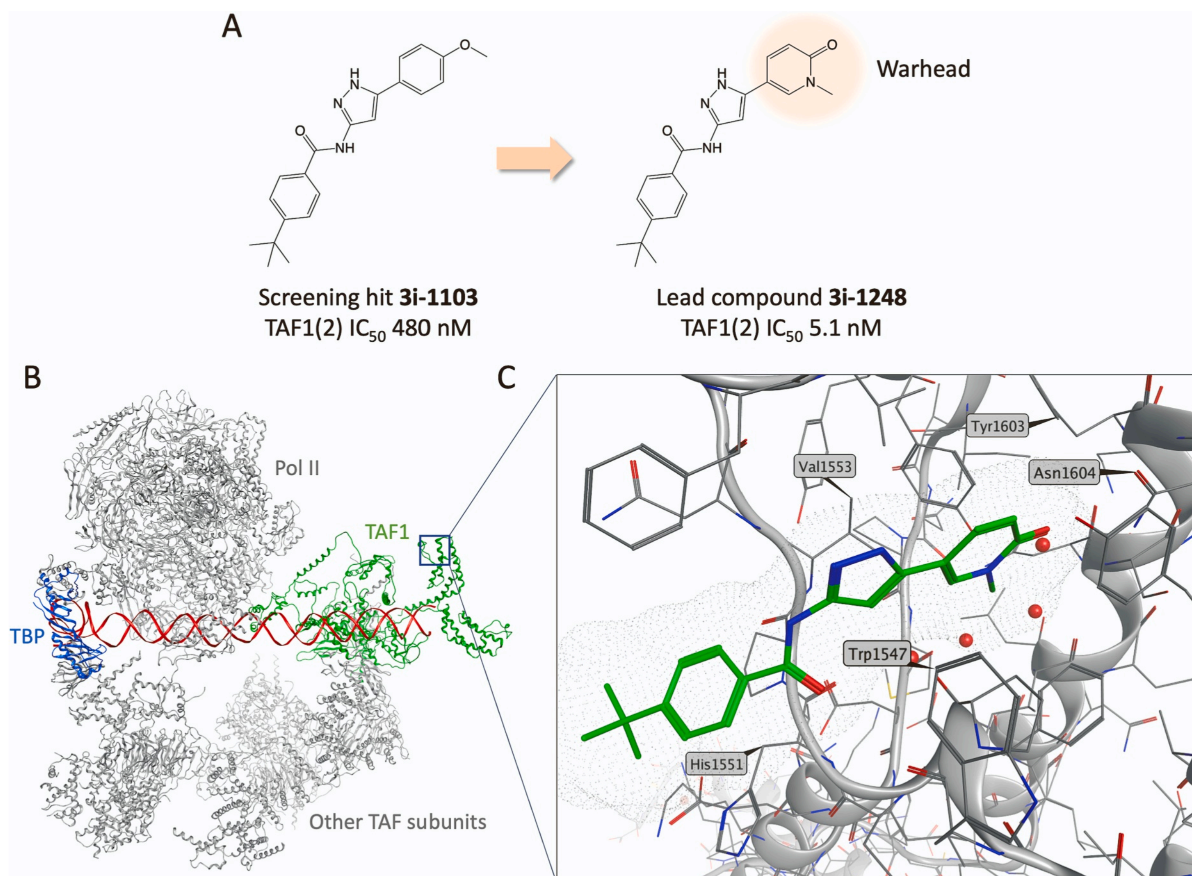


Fig. 1. Identification of TAF1/TFIID as target for cardiomyocyte differentiation by novel compounds. a Structural optimization of selective screening hit 3i-1103 into a high affinity inhibitor of the second bromodomain of TAF1 by introducing a specific warhead to lead compound 3i-1248. b Transcription factor complex TFIID recognizes core promoters and is required for initiation of RNA polymerase II (Pol II)-mediated eukaryotic transcription. Large-scale illustration of TAF1, TBP, Pol II, and other TAF subunits as a part of the preinitiation complex (PIC), compiled by superposing PIC (PDB 7EG9) and AlphaFold protein of human TAF1 (AF-P21675-F1). c Proposed binding mode of compound 3i-1248 to the second bromodomain of TAF1 presented by docking experiments (PDB 5I29). Result follows the lines of previous TAF1 experiments and confirms that ligand affinity is highly dependent on the interaction with amino acid N1604 [26,28]. Please note that the numbering is according to Uniprot P21675-2 in order to better correspond to existing human genetics studies. In this numbering scheme, N1604 corresponds to N1583 (P21675-1) observed in e.g. [28], the amino acid mediating binding of the bromodomain to acetylated histones.

chamber myocardium vs the progenitor-containing PA2.

3.3. Effects of TAF1 bromodomain inhibitors on spontaneous differentiation of mESCs

To ascertain the effects of chemical targeting of the TFIID complex on cardiogenesis, novel (3i-1103, 3i-1246, 3i-1248) and previously published TAF1 bromodomain (BAY299, CeMMEC1, GNE-371) and BET bromodomain (JQ1) inhibitors were tested for their effects on atrial and ventricular reporter expression during the spontaneous differentiation of mESCs. Viability assays were performed from 0.01 to 10 μM in mESCs in order to identify concentrations for testing that do not affect mESC viability, and also to assess the necessity of these protein domains during early embryogenesis (Fig. 3a, Supplementary Fig. S2). TAF1 inhibitors 3i-1246, 3i-1248, CeMMEC1, and GNE371 did not affect mESC proliferation up to 10 μM, indicating that the TAF1 bromodomain is dispensable for mESC viability. 3i-1103 showed anti-proliferative effects only at 10 μM, whereas BAY299 showed anti-proliferative effects at lower concentrations (0.3 μM), though this is likely due to inhibitory effects of BAY299 on BRD1, rather than TAF1 [26]. Similarly, the BET bromodomain inhibitor JQ1 showed increased anti-proliferative effects (1 μM) compared to TAF1 bromodomain inhibitors. Collectively, these results suggest that the TAF1 bromodomain is not necessary for mESC viability, in contrast to BRD1 and BET bromodomains. Additionally, they indicate that novel compounds do not likely inhibit BRD1 or BET bromodomains at tested concentrations in cells.

Subsequently, differentiation assays were conducted in a dual reporter mESC line for atrial and ventricular cardiomyocyte fates, previously used to identify teratogenic agents (Fig. 3b–e, Supplementary Figs. S3–S8) [47]. Compounds or vehicle (DMSO) were given to differentiating mESCs during D2-D10 of differentiation, and cultures were analyzed for the presence of atrial and ventricular reporter-expressing cells on D12 (Fig. 3b–e, Supplementary Figs. S3–S8). Strikingly, statistically significant increases in the % of cells expressing the atrial reporter (SMyHC3-RFP) were observed following treatment with novel TAF1 bromodomain inhibitors 3i-1103, 3i-1246, and 3i-1248 (Fig. 3e). 3i-1103 and 3i-1246 treatments also increased the % of cells expressing the ventricular reporter (Myl2-eGFP) (Supplementary Fig. S5). Though there was a trend for an increase in the % of SMyHC3-RFP+ cells upon treatment with previously identified TAF1 bromodomain inhibitors GNE371, CeMMEC1, and BAY299 (Supplementary Fig. S3), this was less pronounced, suggesting the involvement of additional targets promoting SMyHC3 upregulation. However, only the high affinity TAF1 bromodomain inhibitor GNE371 resulted in statistically significant increases in the mean single-cell levels of the atrial reporter (SMyHC3-RFP MFI), supporting the relevance of TAF1 bromodomain as a target regulating SMyHC3 expression (also non-significant increases with 3i-1103, 3i-1246, 3i-1248, and BAY299) (Supplementary Fig. S4). There were no statistically significant changes in the single-cell levels of the ventricular reporter (Myl2-GFP MFI) following compound treatments

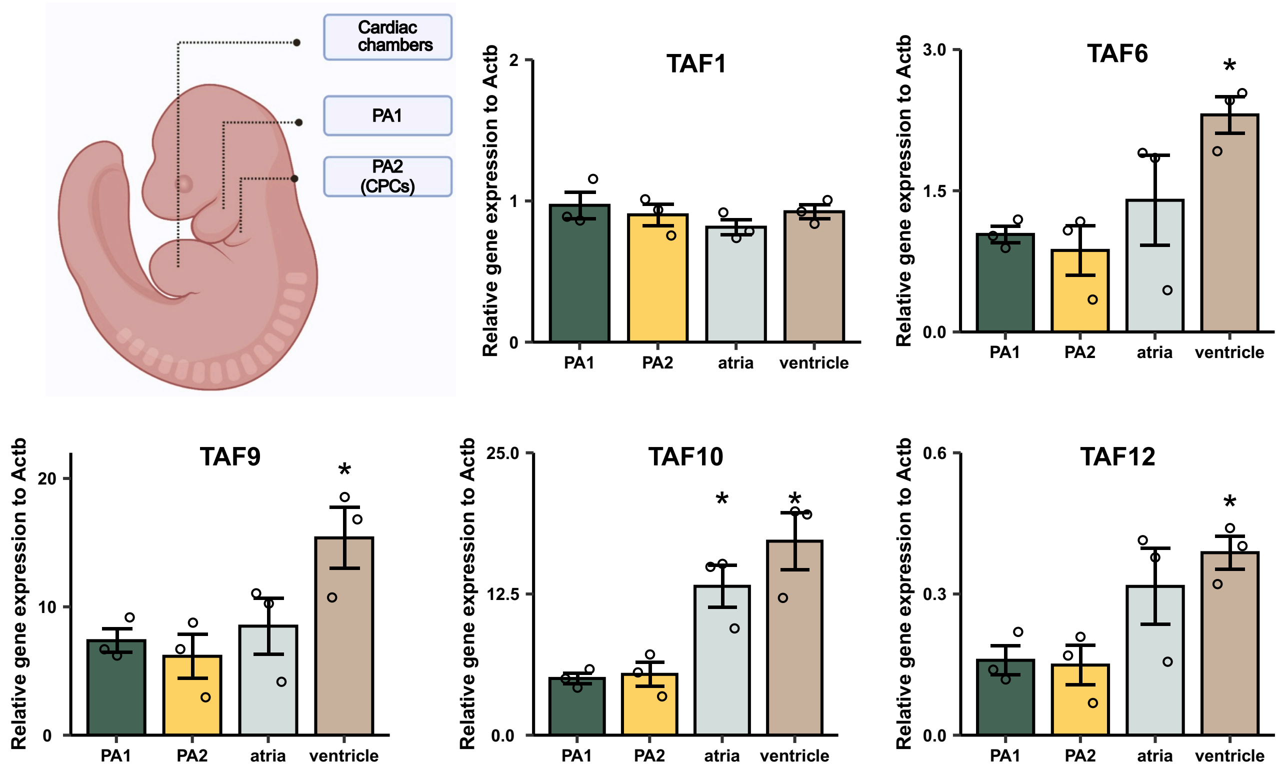


Fig. 2. TFIID subunit mRNA during in vivo mouse cardiogenesis. Cardiac tissues were harvested from E10 mouse embryos and subjected to qRT-PCR analysis to determine differential expression of TFIID subunits. Primary (PA1) and secondary pharyngeal arches (PA2) contain undifferentiated progenitors, whereas PA2 contains cardiac progenitors known to populate mature structures of the heart. Expression of TATA-box binding protein associated factors (TAF) 1, 6, 9, 10, and 12 are shown in PA1, PA2, atria, and ventricles. Data is presented as mean ± SEM (n = 3). $^*p < 0.05$ (*t*-test vs PA2).

(Supplementary Fig. S6).

To determine whether TAF1 bromodomain inhibition leads to relative increases in cardiomyocyte subtype gene expression, the ratio of atrial to ventricular cells was also analyzed. Atrial/ventricular cell ratios showed statistically significant increases upon treatment with 3i-1248, suggesting an atrializing effect of novel TAF1 bromodomain inhibition (Supplementary Fig. S7). Similarly, a statistically significant increase in the single-cell reporter levels (SMyHC3-RFP/Myl2-GFP) was observed upon treatment with GNE371, and non-significant increases when treated with 3i-1246, 3i-1248, and BAY299 (Supplementary Fig. S8). Control compound 3i-1247, which is structurally similar to other compounds but lacks bromodomain affinity, did not show comparable effects on SMyHC3 upregulation, suggesting that bromodomain modulation is the predominant mechanism governing SMyHC3 upregulation. Similarly, tyrosine kinase (TK) inhibitors ASP2215 and AC220 showed no effects on atrial and ventricular reporters (data not shown), further excluding potential off-targets among these kinase domains as the mechanism of action of 3i-1103, 3i-1246, and 3i-1248. No effects were observed upon treatment with JQ1 in differentiation assays, though JQ1 was used at very low concentrations (0.03 μM) due to toxic/anti-proliferative effects on cells during the differentiation process at higher concentrations, consistent with a recent report that BET bromodomains are necessary for epiblast formation [61]. Taken together, these results suggest that TAF1/intra-BET bromodomain inhibition affects cardiomyocyte subtype gene expression (Fig. 3c–e, Supplementary Figs. S3–S8), and indicate a potential role for the TAF1 bromodomain during cardiogenesis.

3.4. TFIID and TAF1 bromodomain in the fetal gene response in postnatal myocytes

As reactivation of fetal gene expression programs is an integral component of the pathological response, we also assessed whether TFIID subunit composition changes in the adult rat heart one-week post myocardial infarction (MI). Statistically significant increases in the mRNA levels of TFIID subunits Taf8, Taf9, Taf13, and Taf15 were observed, in addition to non-significant increases in Taf1 and Taf3 (Fig. 4). No changes in mRNA levels of other TFIID subunits were apparent. These results implicate a subset of TFIID subunits that are specifically involved in the transcriptional response post-MI. Interestingly, these do not include the SAGA complex members that were upregulated during the transition of cardiac progenitor cells to cardiomyocytes, suggesting an alternative set of TAF/TFIID subunits that regulate transcription in that context.

In order to assess whether TAF1 bromodomain inhibition with chemical compounds could affect the fetal gene expression response in postnatal cardiomyocytes, neonatal mouse ventricular cardiomyocytes were stimulated with ET-1 (100 nM), and the activity of the 540 bp Nppb promoter was measured in the presence of chemical compounds. As ET-1 also has a developmental role [62], these experiments serve as proxy for activation of fetal gene expression programs activated during embryogenesis and following neurohormonal increases after heart failure in adults. ET-1 induced ~2-fold increase in Nppb activity versus unstimulated cells, and statistically significant inhibition of this activity was observed following treatment with TAF1 bromodomain inhibitors 3i-1103, 3i-1246, and GNE371, in addition to the BET bromodomain inhibitor JQ1 (100 nM) (Fig. 5). Thus, interference with the TAF1 bromodomain using some chemical compounds can impede activation of the 540 bp Nppb promoter by ET-1, though not all TAF1 bromodomain inhibitors were able to overcome the effects of neurohormonal stimulation.

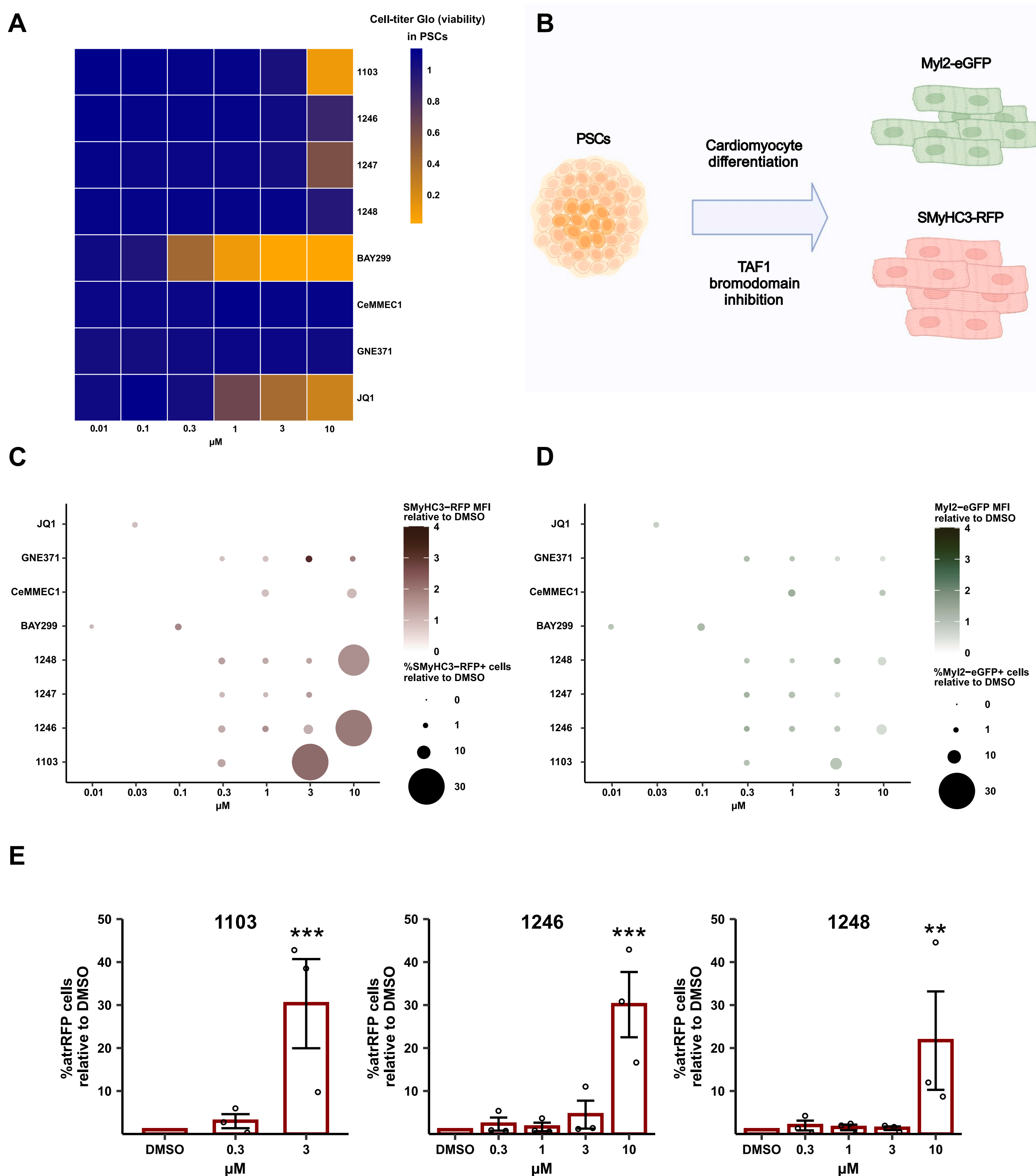


Fig. 3. Effects of chemical inhibition of TAF1 bromodomain on mESC viability and cardiomyocyte differentiation. a Effects of TAF1 bromodomain inhibition on mESC viability. E14 mESCs were treated with compounds or vehicle-control for 48 h and viability was measured using Cell-titer Glo. Data is presented as mean ± SEM (n = 3, independent experiments). Orange color depicts inhibition of mESC viability by 1103 10 μM, 1247 10 μM, BAY299 0.3 μM, and JQ1 1 μM. These were used to select concentrations for testing in the differentiation assay and reveal differential effects of TAF1 bromodomain (GNE371, 1103, 1246, 1248), BRD1 bromodomain (BAY299), and BET bromodomain (JQ1) inhibitors in mESCs. b Differentiation strategy of mESCs. Reporter mESCs were differentiated in embryoid bodies, treated with compounds during D2–D10 differentiation, and analyzed on D12 of differentiation by flow cytometry for SMyHC3-RFP and Myl2-eGFP. c Dot plot depicting effects of compounds on atrial cardiac differentiation as measured by the upregulation of SMyHC3-RFP (n = 3, independent experiments). The size of the dot corresponds to the % of cells, whereby the color intensity represents the single cell MFI. TAF1 bromodomain inhibitors 3i-1103, 3i-1246, 3i-1248 are detected as compounds promoting upregulation of the atrial-specific SMyHC3 promoter. d Dot plot depicting the effects of compounds on ventricular cardiac differentiation as measured by the upregulation of Myl2-eGFP (n = 3, independent experiments). e Effects of chemical inhibition of TAF1 bromodomain on the % of SMyHC3-RFP+ cells. Data is presented as mean ± SEM (n = 3, independent experiments). $***p < 0.001$, $**p < 0.01$, $*p < 0.05$ (ANOVA followed by pairwise comparison with Bonferroni's correction vs DMSO control, adjusted p value). Mean Fluorescent Intensity (MFI), Mouse embryonic stem cell (mESC), Myosin light chain 2 (Myl2), Slow myosin heavy chain 3 (SMyHC3).

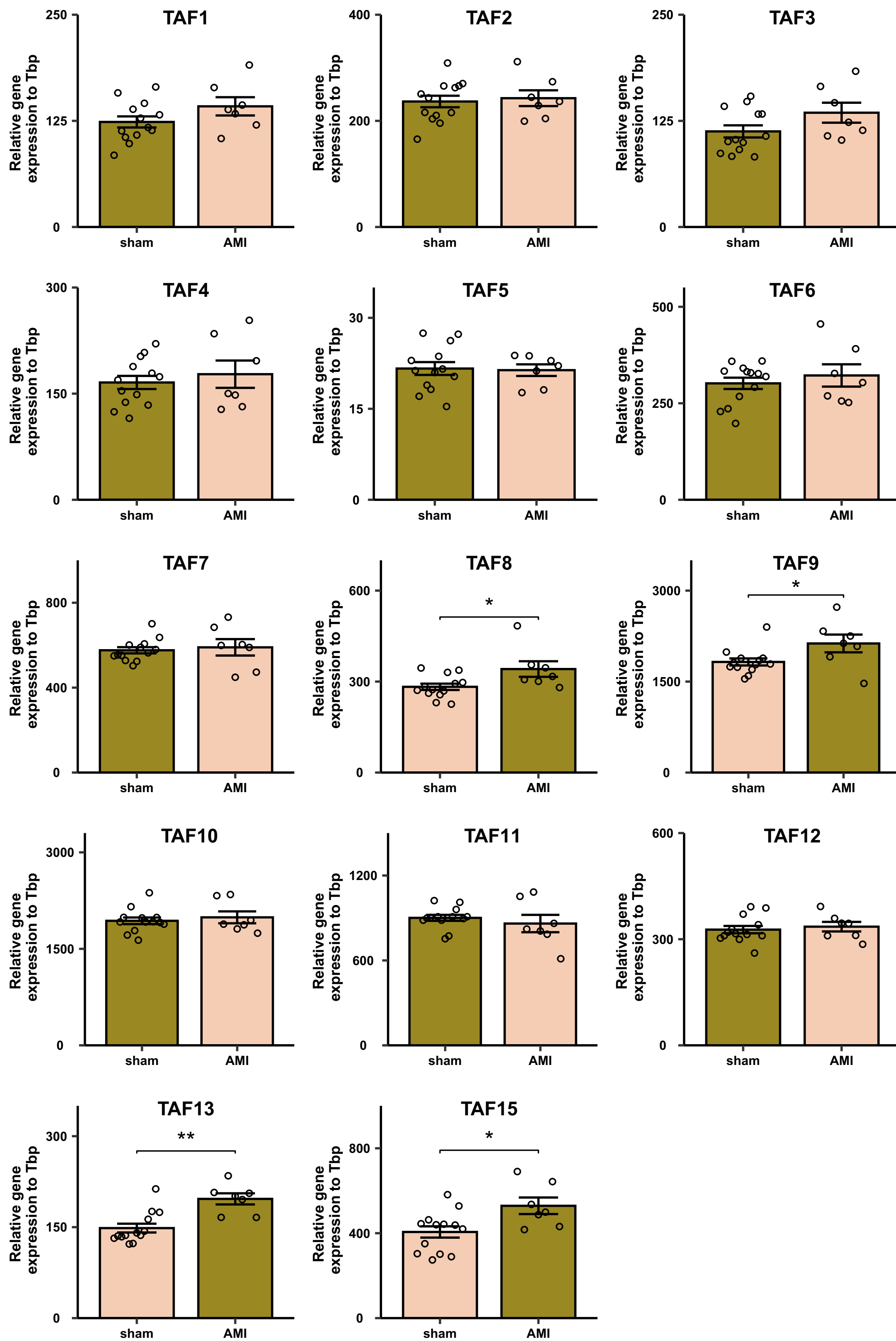


Fig. 4. Expression of TFIID subunits one-week following myocardial infarction in rats. TATA-box binding protein associated factor (TAF), TATA binding protein (TBP). Data is presented as mean ± SEM (sham, n = 13, AMI, n = 7). $^{**}p < 0.01$, $^{*}p < 0.05$ (Wilcoxon test vs sham-operated control).

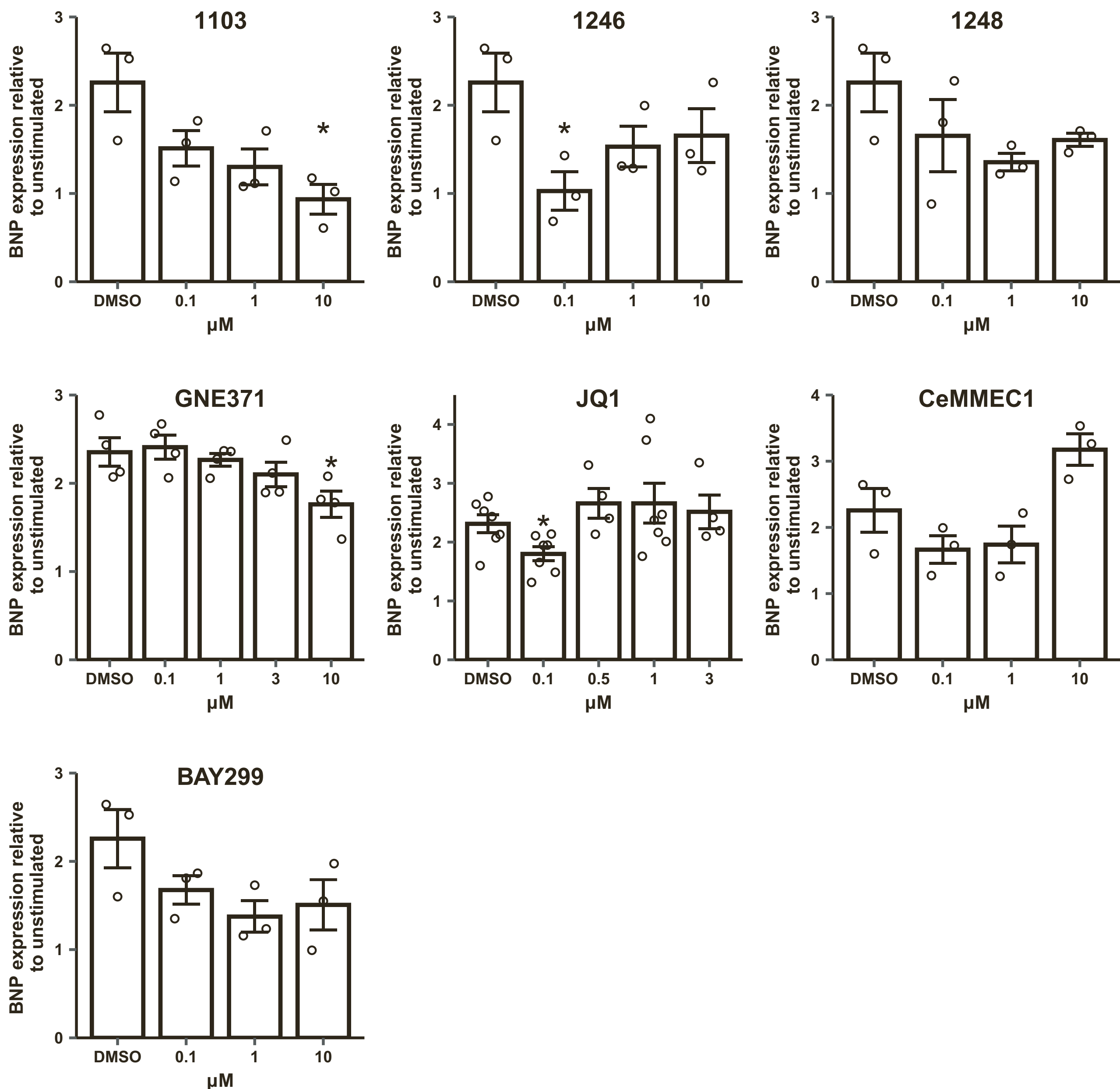
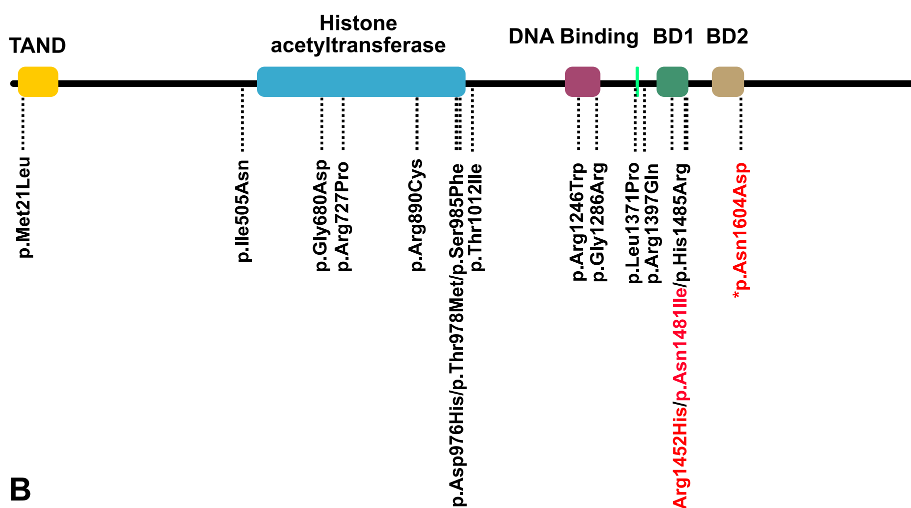


Fig. 5. Effects of TAF1 bromodomain inhibitors on endothelin-1 induced expression of the Nppb540 promoter in neonatal mouse ventricular cardiomyocytes (NMVCMs). Data is presented as mean ± SEM (n = independent experiments). n = 3 (3i-1103, 3i-1246, 3i-1248, BAY299, CeMMEC1), n = 4 (GNE371, JQ1 0.5 μM, JQ1 3 μM), n = 7 (JQ1 0.1 μM, JQ1 1 μM). *$p < 0.05$ (*t*-test vs DMSO control). Natriuretic peptide B (Nppb), TATA-box binding protein associated factor (TAF).

3.5. Effects of damaging congenital heart disease missense variants in the TAF1 bromodomain on atrial cardiac gene activation

In light of observations that chemical TAF1 bromodomain inhibition induces changes in fetal gene expression in both embryological and neonatal contexts, we next examined whether modifications to the TAF1 protein itself would lead to alterations in cardiac gene expression. Fortuitously, damaging TAF1 missense variants were recently published from patients with congenital heart disease and neurodevelopmental defects [38,63]. Upon closer analysis, it was apparent that most damaging missense variants (12/16) occur within functional protein domains of TAF1, including the TAF1 N-terminal domain (TAND), histone acetyltransferase domain, DNA binding, and bromodomain regions (Fig. 6a). In order to determine whether damaging variants affect cardiac transcription, TAF1 mutant cDNA with variants in the first bromodomain (c.4355G>A/p.Arg1452His or c.4442A>T/p.Asn1481Ile) were generated for overexpression studies. Predicted damaging variants were previously observed in patients with atrial-septal (ASD) and ventricular-septal defects (VSD), respectively. Importantly, c4442A>T/p.Asn1481Ile is located in the amino acid that mediates binding of the first bromodomain of TAF1 to acetylated lysines on histones and the acetylated p53 transcription factor [64]. As 3i-1248 and GNE-371 are high affinity inhibitors of the second TAF1 bromodomain, a c.4810A>G/p.Asn1604Asp variant in the second bromodomain was also introduced into TAF1 cDNA, in addition to the generation of double p. Asn1481Ile/p.Asn1604Asp variant cDNA containing amino acid substitutions in both bromodomains.

Activation of a luciferase construct driven by the atrial-specific SMyHC3 promoter was analyzed in HEK cells, a cell line of mesodermal (kidney) lineage with GATA gene expression but lacking other members of the cardiac gene repertoire. SMyHC3-luciferase activity was detected in unstimulated HEK cells, likely reflecting endogenous GATA4



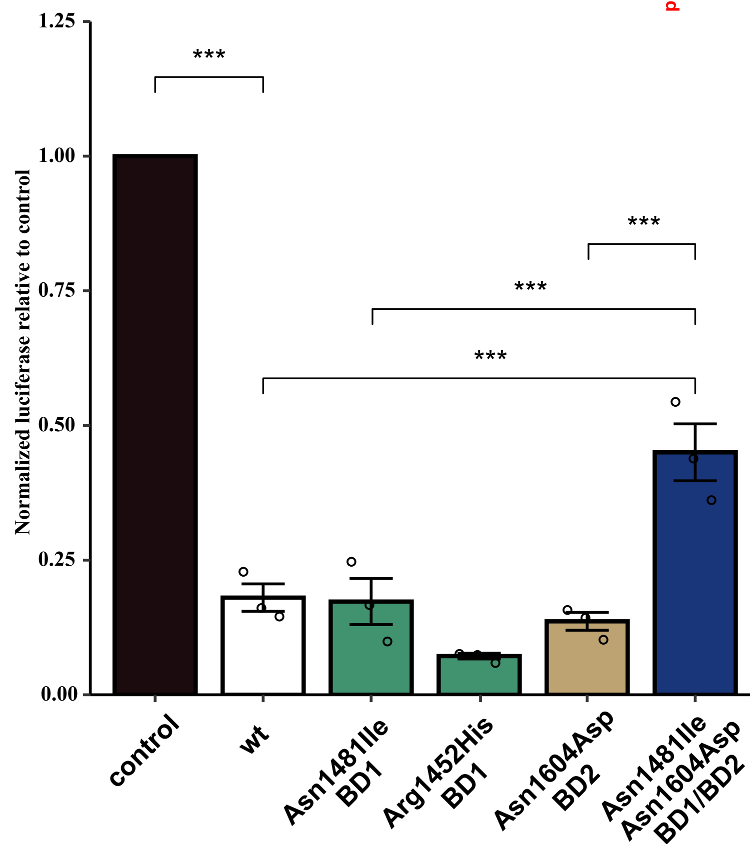


Fig. 6. Effects of damaging TAF1 missense variants from congenital heart disease patients on expression of the atrial-specific SMyHC3 promoter. a Localization of previously described damaging missense variants within TAF1 protein [38]. 12/16 damaging missense variants are localized within known functional domains of the TAF1 protein. Variants introduced into cDNA constructs for functional testing are shown in red. Please note that the numbering is according to Uniprot P21675-2 in order to better correspond to existing human genetics studies. In this numbering scheme, N1604 corresponds to N1583 (P21675-1) observed in e.g. [28], the amino acid mediating binding of the bromodomain to acetylated histones. The difference between P21675-1 and P21675-2 is a 21 amino acid addition that is unexpected to affect compound/missense variant activity. The cDNA for introduction of missense variants lacked this sequence and corresponded to the canonical isoform of TAF1, though isoform 2 numbering was maintained for clarity and better comparison to human genetics studies. *pAsn1604Asp has not been observed in CHD patients but represents the predicted binding site of 3i-1248 and GNE-371 within BD2. b Effects of overexpression of TAF1 cDNA with and without damaging variants on the expression of the atrial-specific SMyHC3 promoter. Slow myosin heavy chain 3 (SMyHC3), congenital heart disease (CHD), TAF1 N-terminal domain (TAND), bromodomain (BD). Data is presented as mean ± SEM (n = 3, independent experiments). ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (ANOVA followed by Tukey's HSD, adjusted p value). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

expression or activation by basal transcriptional machinery [65]. Strikingly, overexpression of wild type TAF1 cDNA strongly repressed SMyHC3 transcription (Fig. 6b). TAF1 cDNA containing single variants c4442A>T/p.Asn1481Ile and c.4810A>G/p.Asn1604Asp showed no difference in repression of SMyHC3 compared to wild type TAF1, but introduction of both p.Asn1481Ile and p.Asn1604Asp partially rescued SMyHC3 expression (Fig. 6b), suggesting that both bromodomains are involved in formation of the repressive TAF1 complex at the SMyHC3 promoter.

In order to examine whether inhibition of the TAF1 bromodomain can overcome TAF1-mediated repression of the SMyHC3 promoter, 3i-1248 and GNE371 were also tested in this model. Similar to ESC differentiation results, SMyHC3 was upregulated by 3i-1248 (3 μM), and there was a trend for upregulation by GNE-371 (3 μM) in HEK cells (Supplementary Fig. S9). Furthermore, 3i-1248 (3 μM) demonstrated a trend to increase SMyHC3 expression in the context of wild type TAF1 expression, and this was abrogated upon mutation of the first bromodomain (both p.Arg1452His and p.Asn1481Ile) (Supplementary Fig. S9). There was also a trend for 3i-1248-mediated activation in double bromodomain mutant TAF1 overexpression, potentially reflecting activity of the compound against endogenous, wild type TAF1 or other 3i-1248 targets (e.g., BRD2/BRD3) (Supplementary Fig. S9). Collectively, these results indicate that TAF1 participates in the repression of the atrial-specific SMyHC3 promoter under study, and that

its double bromodomain is involved in this epigenetic repression. De-repression of this cell identity gene may be induced either with damaging variants or compound treatment, representing a novel molecular mechanism by which bromodomains lead to epigenetic reprogramming of cardiac genes.

3.6. Global gene expression changes upon treatment with TAF1 bromodomain inhibitor 3i-1248 in neonatal mouse ventricular cardiomyocytes

To further characterize gene expression changes resulting from TAF1 bromodomain inhibition in immature cardiac tissue, neonatal mouse ventricular cardiomyocytes were treated with 3i-1248 (1 μM) and profiled by mRNA sequencing. 3i-1248 was chosen due to its activity in multiple assays in addition to its improved selectivity among bromodomains. In cells treated with 3i-1248, there were a total of 606 differentially expressed genes (324 downregulated, 282 upregulated) compared to DMSO/vehicle control (Fig. 7a, Supplementary Fig. S10a-b, Supplementary Table S5). Among the top 25 differentially expressed genes (Supplementary Fig. S10b) were those associated with mitochondrial function (Cox11, Atp5l, Ndufb1, Ndufs5, Acyp2), cell proliferation (Rheb, Kif20b, Ypel3), cell senescence (Ypel3), apoptosis (Pdcd5, Ypel3), cell polarity (Mpp7), cell differentiation (Nr2c2), and congenital malformations (Ccnq, Rxylt1). Intriguingly, SAGA complex

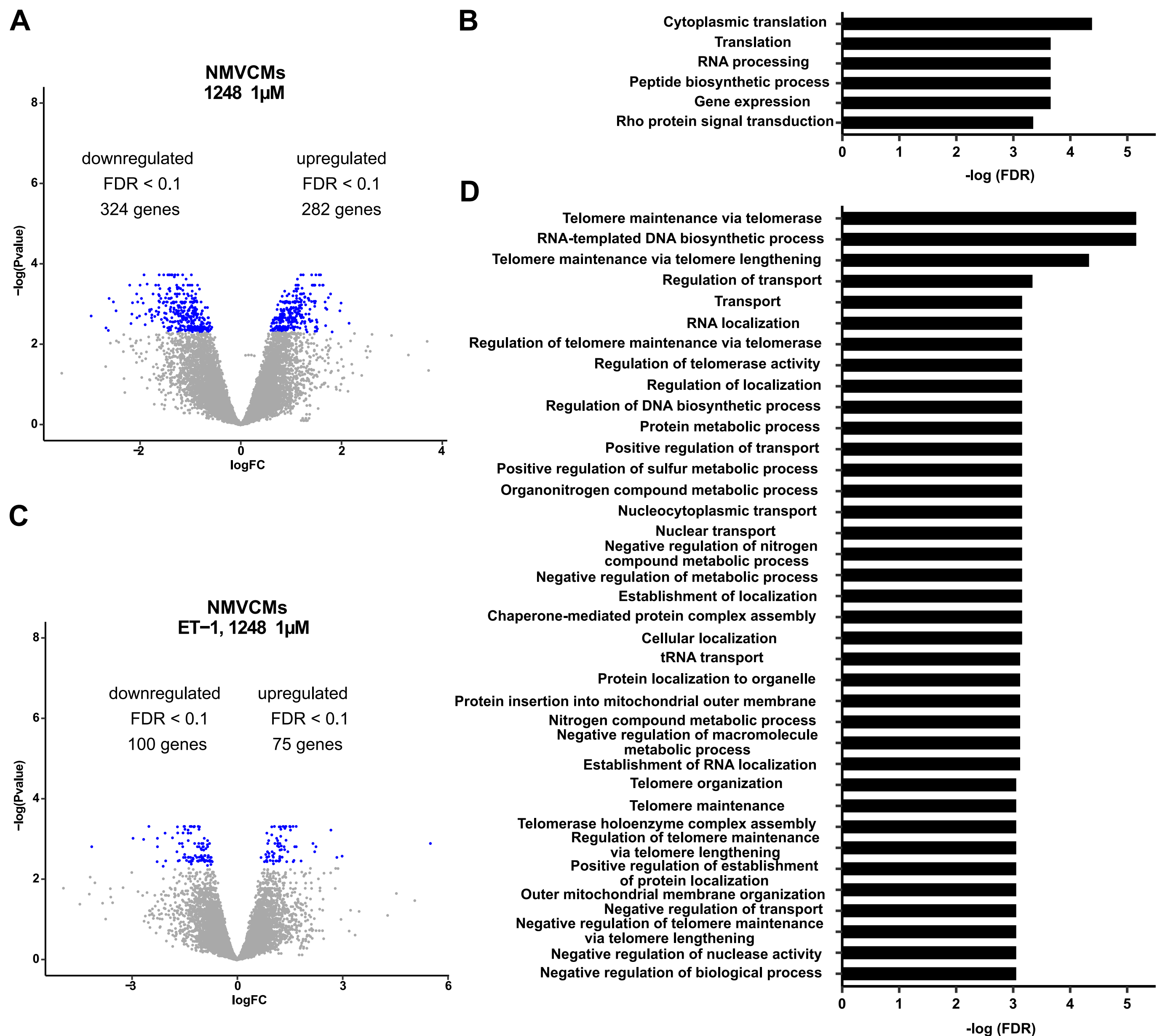


Fig. 7. Global gene expression changes upon treatment with chemical TAF1 bromodomain inhibitor in neonatal mouse ventricular cardiomyocytes (NMVCMs) with and without endothelin-1 (ET-1) treatment. Immature NMVCMs were treated with 3i-1248 for 24 h and analyzed by mRNA-sequencing. a Volcano plot depicting differentially expressed genes upon 3i-1248 1 μM treatment (compared to DMSO control). b Gene ontology (GO) analysis for biological processes comprised of differentially expressed genes following 3i-1248 treatment. Only GO terms with FDR < 0.05 are shown. All GO terms represent downregulated genes, and there were no statistically significant GO terms associated with upregulated genes. c Volcano plot depicting differentially expressed genes upon treatment with 3i-1248 1 μM + ET-1 100 nM (compared to DMSO + ET-1). d GO analysis for biological processes comprised of differentially expressed genes following treatment with 3i-1248 1 μM + ET-1 100 nM (compared to DMSO + ET-1). Only GO terms with FDR < 0.05 are shown. All GO terms represent downregulated genes, and there were no statistically significant GO terms associated with upregulated genes. n = 4 (independent experiments). TATA-box binding protein associated factor (TAF).

genes were upregulated following TAF1 bromodomain inhibition (Supplementary Table S5), including histone-fold containing subunits Taf6l, Taf12, and Tada1, as well as the histone acetyltransferase domain subunit Sgf29, suggesting feedback mechanisms governing bromodomain inhibition and transcriptional regulation of the SAGA complex. Gene ontology analyses for biological processes was also performed, and revealed that genes associated with cytoplasmic translation, translation, RNA processing, peptide biosynthetic process, and gene expression were downregulated in cardiomyocytes treated with 3i-1248, suggesting global changes in transcriptional and post-transcriptional levels of genes are altered by 3i-1248 treatment in ventricular cardiomyocytes (Fig. 7b). There was also a downregulation of Rho protein signal transduction, linking potentially cardioprotective pathways with 3i-1248 treatment. In accordance with aforementioned promoter assays, Nppb was also downregulated upon 3i-1248 treatment, though this change was not statistically significant (FDR = 0.21).

Based on a working hypothesis that bromodomain inhibition would exhibit context-dependent effects on transcription, the effects of bromodomain inhibition with 3i-1248 on global gene expression changes in the context of neurohormonal stimulation with ET-1 (100 nM) were also investigated (Fig. 7c, Supplementary Fig. S10c–d, Supplementary Table S5), revealing 175 differentially expressed genes (100 downregulated, 75 upregulated, FDR < 0.1) compared to DMSO/vehicle control. Among the top 25 differentially expressed genes (Supplementary Fig. S10d) were genes involved in chromatin/epigenetic modification (Hdac3, Hmgn2), neuronal differentiation (Itm2c), mitochondrial structure and function (Ndufb3, Cox17, Mrps36), and proteasomal degradation (Sem1, Psma4). Gene ontology analysis of 3i-1248 1 μM-treated vs DMSO control displayed a downregulation of genes associated with telomere maintenance (Fig. 7d), suggesting 3i-1248 may regulate cell senescence and/or terminal differentiation in the context of ET-1 treatment. Additionally GO terms associated with protein transport and metabolic processes were also downregulated by 3i-1248 treatment compared to DMSO + ET-1 (Fig. 7d). Intriguingly, Taf4b (non-SAGA complex member) was strongly downregulated by 3i-1248 in ET-1-treated cells (Supplementary Table S5). Collectively, these results indicate the capacity of the TAF1 bromodomain inhibitor 3i-1248 to alter genetic programs in neonatal ventricular cardiomyocytes, including gene subsets relevant to postnatal homeostasis. Additionally, they indicate a relationship between inhibition of the TAF1 bromodomain and upregulation of SAGA complex genes, an epigenetic modulator that is also upregulated in the newly formed cardiac chambers vs cardiac progenitors. These results also indicate that gene subsets regulated by 3i-1248 treatment are dependent upon the neurohormonal environment, potentially indicating context-dependent effects during exposure to neurohormonal signaling that occurs during development and/or pathological conditions.

4. Discussion

The present study utilizes a combination of chemical, genetic, and embryological methods to assign novel function to TAF1, a component of the TFIID complex previously implicated in human congenital heart disease. Novel compounds 3i-1103, 3i-1246, and 3i-1248 represent new additions to a few reported active compounds against the TAF1 bromodomain [26–28,66,67]. Interestingly, Sdelci et al. identified a TAF1 bromodomain inhibitor based on a cell-based assay in which upregulation of an RFP transgene occurs in the context of JQ1-mediated chromatin reorganization [27]. This mirrors the dual reporter cardiomyocyte differentiation assay described herein in which an atrial-specific SMyHC3-RFP transgene was upregulated in the context of chromatin reorganization during the differentiation of ESCs. These results suggest that the TAF1 bromodomain is an important target during chromatin remodeling, including during dynamic epigenetic changes which occur during cardiomyocyte differentiation [68]. Furthermore, 3i-1248 displayed high affinity (5.1 nM) and selectivity (~172 fold) for TAF1 in a cell-free assay. This compares favorably to previously published compounds BAY299 and CeMMEC1, though admittedly GNE371 displays both superior affinity and selectivity. Indeed, activity of 3i-1246 and 3i-1248 against other bromodomains or unidentified targets at higher concentrations might be required to achieve biological effects.

As the biological effects of TAF1 bromodomain inhibitors in cardiac and PSC models have not previously been described, we here investigated a set of novel (3i-1103, 3i-1246, 3i-1248) and previously published (BAY299, CeMMEC1, GNE371) TAF1 bromodomain inhibitors for their effects on differentiating mESCs and neonatal cardiomyocytes. Genetic deletion studies indicated that loss-of-function of TAF1 leads to cell cycle arrest, impeding the detailed analysis of the biological effects of TAF1 [39–42]. Intriguingly, we observed that TAF1 bromodomain inhibition does not affect mESC viability, indicating that this protein domain is dispensable for mESC cell cycle progression. This suggests that the TAF1 bromodomain might function primarily during later (post-blastocyst) development. Indeed, we observed that during the differentiation of mESCs, TAF1 bromodomain inhibition affected the activation of fetal myosin expression. To our knowledge, this is the first evidence that developmental gene regulation can be modified by treatment with chemical TAF1 bromodomain inhibitors. Furthermore, this adds to a growing body of evidence that TAF/TFIID subunits are regulators of developmental processes such as cellular differentiation and maturation [29–35,44,69].

Indeed, the precise subunit composition of the TFIID complex has been shown to play a role in cell fate determination, including the activation of muscle genes [30,31]. In the present study, we investigated changes in TAF/TFIID subunit composition in both the developing and infarcted heart. Our results indicate that TAF subunits composing the SAGA complex are upregulated in the newly formed embryonic ventricle, in contrast to a separate set of TFIID subunits that are upregulated in the rat heart one-week post myocardial infarction (Taf8–Taf9–Taf13–Taf15). Similar to our current findings, previous studies of skeletal muscle, neuronal, and ESC differentiation indicated that levels of TAF subunits differ across tissues and developmental stages, but without cell- or tissue-specific gene expression [31,57,58], potentially indicating that distinct TFIID complexes exert context-dependent effects. Intriguingly, non-SAGA complex TFIID subunits Taf2 and Taf3 were uncovered in a recent in vivo genetic screen for factors that influence cardiomyocyte maturation and the regulation of cardiac myosins [70]. In that study, the loss of Taf2 and Taf3 resulted in increased levels of Myh7, a sarcomeric myosin associated with the immature phenotype, suggesting a repressive role for the TFIID complex at myosins and cell identity genes. Though not statistically significant, TAF1 was also within the top ranked genes in those experiments [70], suggesting a general role for TAF1/TFIID in the repression of myosin gene expression.

In this line, we observed that inhibition of the TAF1 bromodomain with both chemical compounds and the introduction of damaging variants leads to epigenetic upregulation of the atrial-specific fetal myosin SMyHC3 promoter in HEK cells. This suggests that the TAF1 double bromodomain and TFIID are involved in the regulation of cell identity/myosin expression in differentiated cell types, and sheds light on potential effects of damaging missense variants in epigenetic dysregulation in congenital heart disease patients. Specifically, our data implicate a repressive role for TAF1 at the SMyHC3 promoter, as overexpression of wild type TAF1 strongly downregulated SMyHC3 in HEK cells. In previous structural and mutagenesis studies, the TAF1 N-terminal domain (TAND) was shown to mimic the structure of the TATA box, bind to TATA-binding protein (TBP), and inhibit transcriptional elongation [71–73]. Our observation that TAF1 represses the SMyHC3 promoter is thus likely mediated by displacement of TATA-binding protein from DNA via the TAF1 N-terminal domain (TAND), a phenomenon which has been described previously at other promoters [71–73]. Intriguingly, Morton et al. reported a damaging missense variant in the TAF1 TAND domain in a patient with tetralogy of fallot and syndactyly, suggesting a

role for this de-repressive mechanism in congenital heart disease [38]. Surprisingly, we observed that TAF1-mediated repression can be abrogated by the introduction of damaging missense variants within the TAF1 double bromodomain (p.Asn1481Ile/p.Asn1604Asp), similar to effects upon treatment with chemical TAF1 bromodomain inhibitors in ESC differentiation. This suggests that TAF1 missense variants outside of the TAND domain also affect the repressive activities of TAF1/TFIID, supporting a general model by which TAF1 leads to epigenetic upregulation of diverse gene programs. Admittedly, one potential limitation of the present study is that these observations were made in non-cardiac cells. However, TAF1 is ubiquitously expressed, and its repressive activities likely occur across a range of cell types. Disruption of these activities would be expected to give rise to a range of developmental phenotypes in different tissues, similar to those observed in patients with a range of defects due to altered TAF1 protein structure (e.g. cardiac, craniofacial, musculoskeletal, neurological). These findings led us to develop a working model for the effects of TAF1 perturbation in developmental defects and the functional implications of damaging variants in TAF1 protein domains (Fig. 8).

Precisely how the TAF1 double bromodomain mediates these effects is unknown, though it is conceivably via the interaction with modified histones and/or modulation of nucleosome formation at the SMyHC3 promoter. Additionally, we observed that SAGA coactivator complex members are upregulated in neonatal cardiomyocytes following TAF1 bromodomain inhibition, providing a potential mechanism through which chemical/genetic inhibition of the TAF1 bromodomain leads to increased SAGA activity and thereby increased transcriptional activation. As the SAGA complex contains multiple members, full elucidation of this mechanism would require iterative deletion of these factors in cardiac progenitors, beyond the scope of the present study. However, recent work has suggested that the SAGA complex functions in lateral plate and intermediate mesoderm, and deletion of the SAGA complex member TAF10 resulted in dysregulation of the expression of Hand2, a master regulator of heart formation [74]. Within the present study, we observed strong upregulation of TAF10 mRNA in the newly formed atrial and ventricular chambers compared to undifferentiated cardiac progenitors, and upregulation of TAF12 following treatment with the TAF1 bromodomain inhibitor 3i-1248. It is conceivable that altered levels of TAF1/TFIID/SAGA complex members could disturb TF stoichiometry of master cardiac regulators GATA4, MEF2C, TBX5, and HAND2 during cardiac septation, accounting for the appearance of ASDs and VSDs in patients with damaging TAF1 variants. Alternatively, as specific members of TFIID/SAGA complexes interact with specific developmental transcription factors [75], alterations to the subunit composition of these complexes could lead to perturbed genetic programs that phenocopy the loss of specific cardiac transcription factors. In vivo or in utero, TAF1 damaging variants could also interact with other epigenetic disturbances, such as an altered maternal epigenetic environment induced by e.g., maternal diabetes [76]. More detailed embryological studies could elucidate these mechanisms and give insight into genetic/epigenetic predisposition to congenital heart disease. Additionally, it is unknown if the mechanisms described herein extend to all TAF1-related diseases, such as X-linked Dystonia-Parkinsonism caused by aberrant splicing of TAF1 [77]. Cell-type specific introduction of TAF1 variants could help to uncover the specific role of TAF1 in different developmental contexts.

In summary, our data indicate chemical targeting of the TFIID complex as a means of modulating cardiac gene expression, including the fetal gene program in cardiomyocytes. Future studies utilizing TAF1 chemical probes and damaging variants would lead to an improved understanding of TAF1/TFIID and its role in cardiogenesis and cardiovascular diseases.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2023.166689.

Ethical approval

Animal license ESAVI-2028-041007-2014 was used for obtaining animal samples, and animal experiments were carried out following 3R principles, the EU directive 2010/63/EU, and the Finnish Act on the Protection of Animals Used for Scientific or Educational Purposes (497/2013, Government Decree on the Protection of Animals Used for Scientific or Educational Purposes (564/2013). All protocols were approved by the National Animal Experiment Board of Finland.

Funding

This work was supported by grants from Business Finland

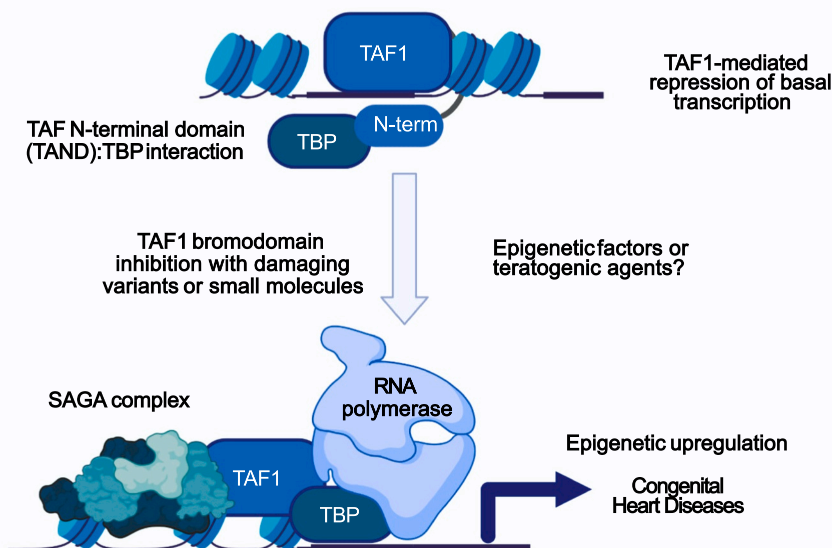


Fig. 8. Summary model for the effects of TAF1 bromodomain inhibition on cardiac gene expression. TAF1 is thought to repress gene expression by inhibiting the localization of TBP to cardiac promoters. TAF1 bromodomain inhibition by introduction of damaging variants or treatment with small molecule compounds leads to de-repression of genes, potentially by upregulation of SAGA complex genes, disruption of histone complexes, or undetermined conformational changes to the TAF1 protein. TATA-box binding protein (TBP), TATA-box binding protein associated factor (TAF).

(3iRegeneration, project 40395/13) (to H.R.), the Academy of Finland (project 2666621) (to H.R.), the Jane and Aatos Erkko Foundation (to H.R.), the Finnish Foundation for Cardiovascular Research (to H.R., R.S.L., M.J.V.), the Sigrid Juselius Foundation (to H.R.), and the Finnish Cultural Foundation (to R.S.L).

CRediT authorship contribution statement

Robert S. Leigh: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Mika J. Välimäki:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing, Visualization. **Bogac L. Kaynak:** Conceptualization, Supervision, Writing – review & editing. **Heikki J. Ruskoaho:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

All authors declare no competing interests.

Data availability

Data will be made available on request.

Acknowledgments

We are grateful to Alicia Jurado Acosta, Sirpa Rutanen, and Jaana Rysä for valuable technical assistance. We would like to acknowledge the use of the following core facilities: Biomedicum Functional Genomics Unit (University of Helsinki) and Flow Cytometry Unit (University of Helsinki). Explanatory figures were created with BioRender.com.

References

[1] B.G. Bruneau, G. Nemer, J.P. Schmitt, et al., A murine model of Holt-oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease, Cell 106 (6) (2001) 709–721.
[2] V. Garg, I.S. Kathiriya, R. Barnes, et al., GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5, Nature 424 (6947) (2003) 443–447.
[3] B. Laforest, W. Dai, L. Tyan, et al., Atrial fibrillation risk loci interact to modulate Ca2+-dependent atrial rhythm homeostasis, J. Clin. Invest. 129 (11) (2019) 4937–4950.
[4] S. Pikkarainen, H. Tokola, R. Kerkelä, H. Ruskoaho, GATA transcription factors in the developing and adult heart, Cardiovasc. Res. 63 (2) (2004) 196–207.
[5] T.G. Di Salvo, S.M. Haldar, Epigenetic mechanisms in heart failure pathogenesis, Circ Heart Fail. 7 (5) (2014) 850–863.
[6] S. Zaidi, M. Choi, H. Wakimoto, et al., De novo mutations in histone-modifying genes in congenital heart disease, Nature 498 (7453) (2013) 220–223.
[7] P. Anand, J.D. Brown, C.Y. Lin, et al., BET bromodomains mediate transcriptional pause release in heart failure, Cell 154 (3) (2013) 569–582.
[8] A. Antolic, H. Wakimoto, Z. Jiao, et al., BET bromodomain proteins regulate transcriptional reprogramming in genetic dilated cardiomyopathy, JCI Insight 5 (15) (2020), e138687.
[9] Q. Duan, S. McMahon, P. Anand, et al., BET bromodomain inhibition suppresses innate inflammatory and profibrotic transcriptional networks in heart failure, Sci. Transl. Med. 9 (390) (2017), eaah5084.
[10] P. Filippakopoulos, J. Qi, S. Picaud, et al., Selective inhibition of BET bromodomains, Nature 468 (7327) (2010) 1067–1073.
[11] H.J. Kee, I.S. Sohn, K.I. Nam, et al., Inhibition of histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding, Circulation 113 (1) (2006) 51–59.
[12] P. Li, J. Ge, H. Li, Lysine acetyltransferases and lysine deacetylases as targets for cardiovascular disease, Nat. Rev. Cardiol. 17 (2) (2020) 96–115.
[13] T.K. Ghosh, J.J. Aparicio-Sánchez, S. Buxton, et al., Acetylation of TBX5 by KAT2B and KAT2A regulates heart and limb development, J. Mol. Cell. Cardiol. 114 (2018) 185–198.
[14] M. Haberland, M.A. Arnold, J. McAnally, D. Phan, Y. Kim, E.N. Olson, Regulation of HDAC9 gene expression by MEF2 establishes a negative-feedback loop in the transcriptional circuitry of muscle differentiation, Mol. Cell. Biol. 27 (2) (2007) 518–525.
[15] T. Kawamura, K. Ono, T. Morimoto, et al., Acetylation of GATA-4 is involved in the differentiation of embryonic stem cells into cardiac myocytes, J. Biol. Chem. 280 (20) (2005) 19682–19688.
[16] J. Lu, T.A. McKinsey, R.L. Nicol, E.N. Olson, Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases, Proc. Natl. Acad. Sci. U. S. A. 97 (8) (2000) 4070–4075.
[17] C.M. Trivedi, W. Zhu, Q. Wang, et al., Hopx and Hdac2 interact to modulate Gata4 acetylation and embryonic cardiac myocyte proliferation, Dev. Cell 19 (3) (2010) 450–459.
[18] M. Jumppanen, S.M. Kinnunen, M.J. Välimäki, et al., Synthesis, identification, and structure-activity relationship analysis of GATA4 and NKX2-5 protein-protein interaction modulators, J. Med. Chem. 62 (17) (2019) 8284–8310.
[19] S.M. Kinnunen, M. Tölli, M.J. Välimäki, et al., Cardiac actions of a small molecule inhibitor targeting GATA4-NKX2-5 interaction, Sci. Rep. 8 (1) (2018) 4611.
[20] M.J. Välimäki, M.A. Tölli, S.M. Kinnunen, et al., Discovery of small molecules targeting the synergy of cardiac transcription factors GATA4 and NKX2-5, J. Med. Chem. 60 (18) (2017) 7781–7798.
[21] M.J. Välimäki, R.S. Leigh, S.M. Kinnunen, et al., GATA-targeted compounds modulate cardiac subtype cell differentiation in dual reporter stem cell line, Stem Cell Res Ther 12 (1) (2021) 190.
[22] D.A. Wassarman, F. Sauer, TAF(II)250: a transcription toolbox, J. Cell Sci. 114 (Pt 16) (2001) 2895–2902.
[23] E. Ferri, C. Petosa, C.E. McKenna, Bromodomains: structure, function and pharmacology of inhibition, Biochem. Pharmacol. 106 (2016) 1–18.
[24] E.M. Flynn, O.W. Huang, F. Poy, et al., A subset of human bromodomains recognizes butyryllysine and crotonyllysine histone peptide modifications, Structure 23 (10) (2015) 1801–1814.
[25] R.H. Jacobson, A.G. Ladurner, D.S. King, R. Tjian, Structure and function of a human TAFII250 double bromodomain module, Science 288 (5470) (2000) 1422–1425.
[26] L. Bouché, C.D. Christ, S. Siegel, et al., Benzoisoquinolinediones as potent and selective inhibitors of BRPF2 and TAF1/TAF1L bromodomains, J. Med. Chem. 60 (9) (2017) 4002–4022.
[27] S. Sdelci, C.H. Lardeau, C. Tallant, et al., Mapping the chemical chromatin reactivation landscape identifies BRD4-TAF1 cross-talk, Nat. Chem. Biol. 12 (7) (2016) 504–510.
[28] S. Wang, V. Tsui, T.D. Crawford, et al., GNE-371, a potent and selective chemical probe for the second bromodomains of human transcription-initiation-factor TFIID subunit 1 and transcription-initiation-factor TFIID subunit 1-like, J. Med. Chem. 61 (20) (2018) 9301–9315.
[29] A. Brunkhorst, M. Karlén, J. Shi, et al., A specific role for the TFIID subunit TAF4 and RanBPM in neural progenitor differentiation, Mol. Cell. Neurosci. 29 (2) (2005) 250–258.
[30] X. Chen, M. Hiller, Y. Sancak, M.T. Fuller, Tissue-specific TAFs counteract polycomb to turn on terminal differentiation, Science 310 (5749) (2005) 869–872.
[31] M.D. Deato, R. Tjian, Switching of the core transcription machinery during myogenesis, Genes Dev. 21 (17) (2007) 2137–2149.
[32] R.N. Freiman, S.R. Albright, S. Zheng, W.C. Sha, R.E. Hammer, R. Tjian, Requirement of tissue-selective TBP-associated factor TAFII105 in ovarian development, Science 293 (5537) (2001) 2084–2087.
[33] J.A. Goodrich, R. Tjian, Unexpected roles for core promoter recognition factors in cell-type-specific transcription and gene regulation, Nat Rev Genet. 11 (8) (2010) 549–558.
[34] D. Langer, I. Martianov, D. Alpern, et al., Essential role of the TFIID subunit TAF4 in murine embryogenesis and embryonic stem cell differentiation, Nat. Commun. 7 (2016) 11063.
[35] E. Scheer, J. Luo, A. Bernardini, et al., TAF8 regions important for TFIID lobe B assembly or for TAF2 interactions are required for embryonic stem cell survival, J. Biol. Chem. 297 (5) (2021), 101288.
[36] E. Koutelou, C.L. Hirsch, S.Y. Dent, Multiple faces of the SAGA complex, Curr. Opin. Cell Biol. 22 (3) (2010) 374–382.
[37] L. Wang, S.Y. Dent, Functions of SAGA in development and disease, Epigenomics 6 (3) (2014) 329–339.
[38] S.U. Morton, R. Agarwal, J.A. Madden, et al., Congenital heart defects due to TAF1 missense variants, Circ. Genom Precis. Med. 13 (3) (2020), e002843.
[39] A.M. Buchmann, J.R. Skaar, J.A. DeCaprio, Activation of a DNA damage checkpoint response in a TAF1-defective cell line, Mol. Cell. Biol. 24 (12) (2004) 5332–5339.
[40] K. Hisatake, S. Hasegawa, R. Takada, Y. Nakatani, M. Horikoshi, R.G. Roeder, The p250 subunit of native TATA box-binding factor TFIID is the cell-cycle regulatory protein CCG1, Nature 362 (6416) (1993) 179–181.
[41] J.D. Irvin, B.F. Pugh, Genome-wide transcriptional dependence on TAF1 functional domains, J. Biol. Chem. 281 (10) (2006) 6404–6412.
[42] S. Ruppert, E.H. Wang, R. Tjian, Cloning and expression of human TAFII250: a TBP-associated factor implicated in cell-cycle regulation, Nature 362 (6416) (1993) 175–179.
[43] S.S. Walker, W.C. Shen, J.C. Reese, L.M. Apone, M.R. Green, Yeast TAF(II)145 required for transcription of G1/S cyclin genes and regulated by the cellular growth state, Cell 90 (4) (1997) 607–614.
[44] D.A. Wassarman, N. Aoyagi, L.A. Pile, E.M. Schlag, TAF250 is required for multiple developmental events in drosophila, Proc. Natl. Acad. Sci. U. S. A. 97 (3) (2000) 1154–1159.
[45] X. Chen, Y. Qi, Z. Wu, et al., Structural insights into preinitiation complex assembly on core promoters, Science 372 (6541) (2021), eaba8490.
[46] T.D. Crawford, V. Tsui, E.M. Flynn, et al., Diving into the water: inducible binding conformations for BRD4, TAF1(2), BRD9, and CECR2 bromodomains, J. Med. Chem. 59 (11) (2016) 5391–5402.

[47] R.S. Leigh, H.J. Ruskoaho, B.L. Kaynak, A novel dual reporter embryonic stem cell line for toxicological assessment of teratogen-induced perturbation of anterior-posterior patterning of the heart, Arch. Toxicol. 94 (2) (2020) 631–645.
[48] S. Pikkarainen, H. Tokola, T. Majalahti-Palviainen, et al., GATA-4 is a nuclear mediator of mechanical stretch-activated hypertrophic program, J. Biol. Chem. 278 (26) (2003) 23807–23816.
[49] R.S. Leigh, H.J. Ruskoaho, B.L. Kaynak, Cholecystokinin peptide signaling is regulated by a TBX5-MEF2 axis in the heart, Peptides 136 (2021), 170459.
[50] A. Jurado Acosta, J. Rysä, Z. Szabo, et al., Transcription factor PEX1 modulates extracellular matrix turnover through regulation of MMP-9 expression, Cell Tissue Res. 367 (2) (2017) 369–385.
[51] J. Rysä, O. Tenhunen, R. Serpi, et al., GATA-4 is an angiogenic survival factor of the infarcted heart, Circ Heart Fail. 3 (3) (2010) 440–450.
[52] T. Brattelid, L.H. Winer, F.O. Levy, K. Liestøl, O.M. Sejersted, K.B. Andersson, Reference gene alternatives to gapdh in rodent and human heart failure gene expression studies, BMC Mol. Biol. 11 (2010) 22.
[53] C.K. Ho, J.F. Strauss III, Activation of the control reporter plasmids pRL-TK and pRL-SV40 by multiple GATA transcription factors can lead to aberrant normalization of transfection efficiency, BMC Biotechnol. 4 (2004) 10.
[54] E.Z. Macosko, A. Basu, R. Satija, et al., Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets, Cell 161 (5) (2015) 1202–1214.
[55] R. Vanhanen, K. Leskinen, I.P. Mattila, P. Saavalainen, T.P. Arstila, Epigenetic and transcriptional analysis supports human regulatory T cell commitment at the CD4+ CD8+ thymocyte stage, Cell. Immunol. 347 (2020), 104026.
[56] M.D. Robinson, D.J. McCarthy, G.K. Smyth, edgeR: a bioconductor package for differential expression analysis of digital gene expression data, Bioinformatics 26 (1) (2010) 139–140.
[57] F.J. Herrera, T. Yamaguchi, H. Roelink, R. Tjian, Core promoter factor TAF9B regulates neuronal gene expression, elife 3 (2014), e02559.
[58] Z. Liu, D.R. Scannell, M.B. Eisen, R. Tjian, Control of embryonic stem cell lineage commitment by core promoter factor, TAF3, Cell 146 (5) (2011) 720–731.
[59] H. Zhou, B. Wan, I. Grubisic, T. Kaplan, R. Tjian, TAF7L modulates brown adipose tissue formation, elife 3 (2014), e02811.
[60] P. Andersen, C. Kwon, Ex vivo culture of pharyngeal arches to study heart and muscle progenitors and their niche, J. Vis. Exp. 101 (2015), e52876.
[61] M. Tsume-Kajioka, C. Kimura-Yoshida, K. Mochida, Y. Ueda, I. Matsuo, BET proteins are essential for the specification and maintenance of the epiblast lineage in mouse preimplantation embryos, BMC Biol. 20 (1) (2022) 64.
[62] Y. Kurihara, H. Kurihara, H. Oda, et al., Aortic arch malformations and ventricular septal defect in mice deficient in endothelin-1, J. Clin. Invest. 96 (1) (1995) 293–300.
[63] H. Cheng, S. Capponi, E. Wakeling, et al., Missense variants in TAF1 and developmental phenotypes: challenges of determining pathogenicity, Hum. Mutat. (2019), https://doi.org/10.1002/humu.23936.
[64] A.G. Li, L.G. Piluso, X. Cai, B.J. Gadd, A.G. Ladurner, X. Liu, An acetylation switch in p53 mediates holo-TFIID recruitment, Mol. Cell 28 (3) (2007) 408–421.
[65] G.F. Wang, W. Nikovits Jr., M. Schleinitz, F.E. Stockdale, A positive GATA element and a negative vitamin D receptor-like element control atrial chamber-specific expression of a slow myosin heavy-chain gene during cardiac morphogenesis, Mol. Cell. Biol. 18 (10) (1998) 6023–6034.
[66] M.A. Clegg, N.H. Theodoulou, P. Bamborough, et al., Optimization of naphthyridones into selective TATA-binding protein associated factor 1 (TAF1) bromodomain inhibitors, ACS Med. Chem. Lett. 12 (8) (2021) 1308–1317.
[67] R.M. Karim, L. Yang, L. Chen, et al., Discovery of dual TAF1-ATR inhibitors and ligand-induced structural changes of the TAF1 tandem bromodomain, J. Med. Chem. 65 (5) (2022) 4182–4200.
[68] J.A. Wamstad, J.M. Alexander, R.M. Truty, et al., Dynamic and coordinated epigenetic regulation of developmental transitions in the cardiac lineage, Cell 151 (1) (2012) 206–220.
[69] B. Malecova, A. Dall'Agnese, L. Madaro, et al., TBP/TFIID-dependent activation of MyoD target genes in skeletal muscle cells, elife 5 (2016), e12534.
[70] N.J. VanDusen, J.Y. Lee, W. Gu, et al., Massively parallel in vivo CRISPR screening identifies RNF20/40 as epigenetic regulators of cardiomyocyte maturation, Nat. Commun. 12 (1) (2021) 4442.
[71] S.K. Burley, R.G. Roeder, TATA box mimicry by TFIID: autoinhibition of pol II transcription, Cell 94 (5) (1998) 551–553.
[72] T. Kokubo, S. Yamashita, M. Horikoshi, R.G. Roeder, Y. Nakatani, Interaction between the N-terminal domain of the 230-kDa subunit and the TATA box-binding subunit of TFIID negatively regulates TATA-box binding, Proc. Natl. Acad. Sci. U. S. A. 91 (9) (1994) 3520–3524.
[73] D. Liu, R. Ishima, K.I. Tong, et al., Solution structure of a TBP-TAF(II)230 complex: protein mimicry of the minor groove surface of the TATA box unwound by TBP, Cell 94 (5) (1998) 573–583.
[74] P. Bardot, S.D. Vincent, M. Fournier, et al., The TAF10-containing TFIID and SAGA transcriptional complexes are dispensable for early somitogenesis in the mouse embryo, Development 144 (20) (2017) 3808–3818.
[75] H. Göös, M. Kinnunen, K. Salokas, et al., Human transcription factor protein interaction networks, Nat. Commun. 13 (1) (2022) 766.
[76] S.U. Morton, A.C. Pereira, D. Quiat, et al., Genome-wide De novo variants in congenital heart disease are not associated with maternal diabetes or obesity, Circ. Genom Precis. Med. 15 (2) (2022), e003500.
[77] T. Aneichyk, W.T. Hendriks, R. Yadav, et al., Dissecting the causal mechanism of X-linked dystonia-parkinsonism by integrating genome and transcriptome assembly, Cell 172 (5) (2018) 897–909.e21.