BRWD1 orchestrates epigenetic landscape of late B lymphopoiesis

Malay Mandal1, Mark Maienschein-Cline2, Patrick Maffucci3,4, Margaret Veselits1, Domenick E. Kennedy1, Kaitlin C. McLean1, Michael K. Okoreeh1, Sophiya Karki5, Charlotte Cunningham-Rundles3,4 & Marcus R. Clark1

Transcription factor (TF) networks determine cell fate in hematopoiesis. However, how TFs cooperate with other regulatory mechanisms to instruct transcription remains poorly understood. Here we show that in small pre-B cells, the lineage restricted epigenetic reader BRWD1 closes early development enhancers and opens the enhancers of late B lymphopoiesis to TF binding. BRWD1 regulates over 7000 genes to repress proliferative and induce differentiation programs. However, BRWD1 does not regulate the expression of TFs required for B lymphopoiesis. Hypogammaglobulinemia patients with BRWD1 mutations have B-cell transcriptional profiles and enhancer landscapes similar to those observed in Brwd1−/− mice. These data indicate that, in both mice and humans, BRWD1 is a master orchestrator of enhancer accessibility that cooperates with TF networks to drive late B-cell development.
B-cell development consists of sequential and mutually exclusive states of proliferation and immunoglobulin gene recombination1-2. Following in-frame Igμ recombination, the expressed immunoglobulin μ-chain assembles with surrogate light chain (λ5 and VpreB), Igα, and Igκ to form the pre-B cell receptor complex (pre-BCR). The pre-BCR, in concert with cell extrinsic (IL-7R)3 and intrinsic4 cues direct large pre-B-cell proliferation, followed by cell cycle exit and Igk recombination in small pre-B cells5. Aberrations in the mechanisms that segregate proliferation from Igk recombination can lead to either immunodeficiency or genomic instability and leukemic transformation6-8.

Many of the signaling mechanisms that coordinate proliferation and Igk recombination in pre-B cells have recently been elucidated. Downstream of the pre-BCR, E2A, and the interferon-regulatory factor family (IRF) members IRF4 and IRF8, direct cell cycle exit and open the Igk locus for recombination5-10. Igk recombination also requires escape from IL-7R signaling, which results in loss of STAT5 activation, downregulation of cyclin D3, and derepression of the Igk locus11,12. Coordinate loss of IL-7R-dependent PI-3K activation derepresses FOXP1 and FOXP3, which then induces RAG1 and RAG210,13,14. Feedback inhibition is provided by RAG-mediated dsDNA breaks which activate NF-kB2 that then represses expression of pre-BCR signaling intermediates4. Through these and other feed-forward and backward loops1, transcription factor (TF) networks enforce context-appropriate cell-fate decisions.

The primary targets of these TFs are enhancers, which, following TF binding, productively interact with cognate gene promoters to regulate specific gene expression programs15. Indeed, differential activation of enhancers appears to determine most cell type-specific gene expression16. Regulation of enhancer accessibility to TFs is an area of intense interest. Some TFs, such as PU.1, IKZF1 (Ikaros), and EBF1, can invade enhancers occupied by nucleosomes and, either singly or in combination, open the enhancers to subsequent TFs17-19. In some cases, these pioneering TFs open enhancers by recruiting histone methyltransferases and acetyltransferases19.

Active enhancers are typically marked by histone H3 lysine 27 acetylation (H3K27ac) and histone H3 lysine 4 monomethylation (H3K4me1), whereas silent enhancers are usually enriched for histone H3 lysine 27 trimethylation (H3K27me3)20,21. These post-translational modifications form recognition motifs for epigenetic reader complexes22,23 containing chromatin remodelers such as SWI/SNF (switching defective/sucrose non-fermenting)24. However, not all enhancers are opened by pioneer TFs19. Furthermore, it is unclear how molecular mechanisms downstream of TFs and histone methyltransferases and acetyltransferases are regulated and how these regulatory mechanisms determine enhancer landscapes.

Recent studies of B lymphopoiesis have identified critical TFs and some of their targets1,8,9,11-13,25. However, remarkably little is known regarding the epigenetic regulation of B lymphopoiesis. Some TFs important for B lymphopoiesis, including E2A, Pax, and STAT5, can modulate enhancer accessibility through the recruitment of histone modifying and remodeling complexes11,26,27. Furthermore, EZH2, which generates H3K27me3, plays important roles in both repressing Igκ accessibility11 and regulating p5328. Recruitment of the RAG proteins, and assembly of the recombination center2, requires H3K4me3. Downstream of histone post-translational modifications, mutations in humans have implicated epigenetic readers such as the bromodomain and extraternal (BET) domain protein BRD4 in peripheral leukemias29,30. However, the role of epigenetic readers in normal B lymphopoiesis is poorly understood.

We have previously demonstrated that the BROMO and WD40 domain containing epigenetic reader BRWD1 is necessary for opening the Jk genes, assembly of the RAG recombination center, and subsequent Igk recombination31. The expression of BRWD1 is lineage and stage specific and thereby contributes to restricting Igκ recombination to the small pre-B-cell stage. However, BRWD1 binds to numerous sites across the genome31, suggesting that it could play additional roles in B lymphopoiesis. Here we demonstrate that BRWD1 orchestrates a genome-wide reordering of enhancer accessibility by both silencing early developmental enhancers and opening those critical for late B lymphopoiesis to TF binding. Additionally, BRWD1 inhibits proliferation by coordinately repressing Myc and MYC’s downstream targets. Interestingly, BRWD1 mutations are relatively common in patients with idiopathic hypogammaglobulinemia. Furthermore, analyses of cells from patients with BRWD1 mutations reveal a similar transcriptional and epigenetic program as that observed in Brwd1-/- mice including the activation of MYC and MYC-dependent pathways. Overall, this study identifies a previously unrecognized mechanism, in both mice and humans, for remodeling the enhancer landscape of late B lymphopoiesis.

Results

BRWD1 orchestrates transcription of late B-cell development. RNA-Seq (Supplementary Table 1) of Brwd1-/- and wild-type (WT) small pre-B cells indicated that over 7000 genes were dysregulated (q < 0.05) in Brwd1-/- small pre-B cells (Fig. 1a and Supplemental data 1), with enhanced expression (3901 genes) being more common than the decreased expression (3405). For example, BRWD1 repressed Ccr9 (Fig. 1b), and CCR9 surface density were intermediate between pre- and small pre-B cells (Fig. 1c). A similar expression pattern was observed for Flt3 (Fig. 1d, e). In contrast, normal upregulation of the IL-2 receptor (Il2ra, CD25) (Fig. 1f, g) and CXCR4 (Fig. 1h, i) in pre-B cells was diminished in Brwd1-/- cells, with surface expression levels intermediate between WT pre- and small pre-B cells. These examples suggest that BRWD1 both represses early, and induces late, developmental genes.

To test this, we grouped all differentially expressed genes during B lymphopoiesis (one-way ANOVA, q < 0.05, 13387 genes, Immgen32) by their expression, before, at, and after the small pre-B-cell stage (Fig. 1j). Remarkably, most genes were clustered into two large expression groups. The first cluster (Cluster 1) contained genes that were highly expressed in early B-cell development that were then downregulated starting in small pre-B cells. The second cluster (Cluster 2) contained genes that were induced after the small pre-B-cell stage. These data indicate that the small pre-B-cell stage is a watershed between early and late B-cell development, in which there is a global reordering of transcriptional programs.

BRWD1 preferentially repressed Cluster 1 genes (p = 4.1 x 10^(-44)) and upregulated Cluster 2 (p = 4.9 x 10^(-62)) (Fig. 1k). At the small pre-B-cell stage, cells stop proliferating and initiate Igκ recombination1. Indeed, ontology analysis demonstrated that BRWD1 induced B-cell activation and differentiation transcription programs, while repressing genes involved in proliferation and metabolism (Supplementary Fig. 1a, b and Supplementary Table 2). These data indicate that BRWD1 controls the transition between early B-cell proliferative and late differentiative developmental programs.

BRWD1 regulates chromatin accessibility. We next used ATAC-Seq to examine how BRWD1 regulated chromatin accessibility (Fig. 2 and Supplementary Table 3). In WT cells, progression from the pre-pro B to small pre-B-cell stage was associated with progressive net loss of chromatin accessibility (Fig. 2a). At each stage, new accessibility sites appeared, but these
lower and higher transcript abundance in WT and 13387 differentially expressed genes (one-way ANOVA, q < 0.05) were hierarchically clustered.

Ten B-cell development and maturation stages including common lymphoid progenitor (CLP), and transitional (T1-3) and germinal center (GC). A total of pre-B cells (Fig. 2c). Comparison to other developmental stages Brwd1-/- (17,713 peaks) cells. Conversely, 2,943 sites failed to open in downregulated genes in NATURE COMMUNICATIONS | DOI: 10.1038/s41467-018-06165-6 | www.nature.com/naturecommunications

Upon transit to the immature B-cell stage (Fig. 2b), this trend decreased from 63,492 to 36,276 including 3694 new open sites. Transition from large to small pre-B cells, total accessible sites represented a minority of all changed sites. For example, in transition from large to small pre-B cells, total accessible sites decreased from 63,492 to 36,276 including 3694 new open sites. Upon transit to the immature B-cell stage (Fig. 2b), this trend reversed and there was a large net gain in accessibility including 29,314 sites not observed in immediate precursor populations. Remarkably, there were 82,295 accessible sites in Brwd1-/- small pre-B cells (Fig. 2c). Comparison to other developmental stages indicated that many of these new accessibility peaks could also be found in WT pre-pro-B (35,828), pro-B (26,127), and large pre-B (17,713) peaks. Conversely, 2,943 sites failed to open in Brwd1-/- small pre-B cells.

Many accessibility peaks in Brwd1-/- small pre-B cells were also present in WT immature B cells (Fig. 2d). However, most of these peaks were also found in WT pre-pro B cells. Furthermore, de novo accessibility peaks observed in WT immature B cells were not regulated by BRWD1. Overall, these data suggest that BRWD1 broadly represses chromatin associated with early B-cell developmental stages while opening more restricted chromatin regions associated with the small pre-B-cell stage.

We next examined the relationships between BRWD1-dependent changes in transcription and chromatin accessibility. Provided in Fig. 2e are quantile (Q-Q) plots of RNA differential statistics (Brwd1-/- vs. WT) as a function of increased (up), decreased (down), or no change (noc) in chromatin accessibility in Brwd1-/- small pre-B cells. The relationships between chromatin accessibility and transcription were then considered as a function of distance from transcription start sites (TSSs). Increased accessibility in Brwd1-/- small pre-B cells was associated with increased transcription up to 500 kb removed from TSSs. In contrast, decreased accessibility was clearly associated with diminished transcription within 5 kb of TSSs. However, this effect was largely lost beyond 5 kb. This data suggest that BRWD1 represses transcription through both long- and short-range mechanisms from TSSs, while it primarily enhances transcription through mechanisms proximate to TSSs.

**Fig. 1** BRWD1 orchestrates transcriptional programs of late B-cell development. a Heatmap of RNA-Seq results with clustering of upregulated and downregulated genes in Brwd1-/- vs. WT small pre-B cells (q < 0.05, replicates shown). b, d Representative RNA-Seq alignments for Ccr9 (b) and Flt3 (d) in WT and Brwd1-/- small pre-B cells. Corresponding surface expression of CCR9 (c) and FLT3 (e) on indicated cell populations as assessed by flow cytometry. f, i. Representative RNA-Seq alignments for Il2ra (f) and Cxcr4 (h) in WT and Brwd1-/- small pre-B cells. Corresponding surface expression of CD25 (g) and CXC4 (i) on indicated cell populations as determined by flow cytometry. j Gene expression microarray data from ImmGen (10) covering ten B-cell development and maturation stages including common lymphoid progenitor (CLP), and transitional (T1-T3) and germinal center (GC). A total of 13387 differentially expressed genes (one-way ANOVA, q < 0.05) were hierarchically clustered. k Log2 enrichment of Cluster 1 and Cluster 2 genes with lower and higher transcript abundance in Brwd1-/- small pre-B cells than in WT small pre-B cells. Corresponding P-values (unpaired t test) indicated
To determine the relationship between BRWD1 binding and gene expression (RNA-Seq), we first analyzed BRWD1 ChIP-Seq peaks using two calling methods, irreproducible discovery rate (idr, q < 10⁻⁶), or reproducible peaks with a cut-off of p < 10⁻⁵ significance. The idr was more rigorous providing 1843 peaks, while p < 10⁻⁵ identified 9474 BRWD1 chromatin-bound sites. We then compared the relationship between BRWD1-bound sites and distance to TSSs. Remarkably, irrespective of peak calling method, the distance relationship between BRWD1 binding and distance to TSSs was remarkably similar (Fig. 2f). These data suggest that the method of peak calling does not affect the apparent relationship between where BRWD1 is bound and the distance to potential gene targets. For subsequent analysis, we used idr called ChIP-Seq peaks.

We next examined the distance relationships between BRWD1-bound sites and genes differentially regulated by BRWD1. Interestingly, the TSSs of only 566 differentially regulated genes (7.7%) were within 5 kb of a BRWD1-bound site (Fig. 2g). Even at distances of up to 500 kb from a BRWD1-bound site, only 4615 (63.2%) of differentially expressed genes were captured. Distances of up to 5 mb from BRWD1-bound sites captured essentially all differentially expressed genes. A similar pattern was seen when examining ∆ATAC-Seq peaks. However, the effect on accessibility changed as a function of distance from
BRWD1-bound sites. Shown in Fig. 2h (Supplementary Table 4) is the differential probability distribution of accessibility peaks in \textit{Brwd1}−/− vs. WT small pre-B cells as a function of distance from BRWD1-bound sites in WT small pre-B cells. At sites of BRWD1 binding (0 bp), there is an over-representation of diminished accessibility peaks in \textit{Brwd1}−/− cells which persisted to about 5 kb. However, at distances beyond 100 kb, there was an over-representation of open peaks in \textit{Brwd1}−/− small pre-B cells that persisted up to 5 mb. The same relationships between distance from BRWD1-bound sites and BRWD1-dependent modulation of chromatin accessibility was evident when the data were displayed as Q–Q plots (Fig. 2i). These data indicate that BRWD1 enhances the accessibility and transcription locally, but can repress both at distances up to 5 mb away.

**BRWD1 acts at enhancers.** Comparison of ATAC-Seq with relevant H3K27Ac and H3K4me1 ChIP-Seq data sets\textsuperscript{26,33} revealed that BRWD1 preferentially regulated the enhancers (Fig. 3a, b). Each unique area in the accessibility Venn diagram for WT Pro-B, WT small pre-B, and \textit{Brwd1}−/− small pre-B cells was given a letter (Fig. 3a), which corresponded to bar graphs (Fig. 3b) demonstrating percent of peaks at enhancers and the number of these enhancer peaks that were within 5 kb of pro-moters or were within intragenic or intergenic regions. Enhancers repressed by BRWD1 (areas ‘D’ and ‘G’) were primarily intragenic or intergenic, with only 5% and 2% lying near the TSSs. In contrast, 11% of BRWD1-dependent small pre-B cell accessibility peaks (‘C’) were near the promotors. This is consistent with BRWD1 preferentially increasing the accessibility at enhancers within 5 kb of TSSs (Fig. 2e, g). Examples of this included \textit{Cd79b} (encoding Igβ, Fig. 3c, d) and \textit{Cxcr4} (Supplementary Fig. 2a, b), in which increased expression was associated with opening of a TSS proximate enhancer.

Examples of BRWD1-mediated enhancer silencing included \textit{Mpeg1}, \textit{Dtx4}, \textit{Fam111a}, \textit{Left1}, and \textit{Ccr9} (Supplementary Fig. 2c–j), and \textit{Myc} (Fig. 3e). Myc has 14 known enhancers including four
ChIP-Seq peaks in WT and Data are from combination (d) of independent experiments (Supplementary Fig. 3c) of independent experiments (n = 2) or are presented as average ± s.d. (n = 2) (b).

BRWD1 determines genomic targets accessible to TFs. We next examined if those enhancers activated and repressed by BRWD1 contained common DNA motifs. As shown in Fig. 4a, the pro-B-cell accessibility peaks repressed by BRWD1 ('D' peaks) were enriched in motifs for TFs involved in early B-cell development (EBF) and hematopoietic development (RUNX, CEBPA). In contrast, BRWD1-dependent small pre-B accessibility peaks ('B' and 'C' peaks) were enriched for ETS (e.g., PU.1), IRF4, and YY1 binding sites, which are TFs (Fig. 4a) required for late B-cell development.

While BRWD1 modulated the accessibility of TF binding sites, expression of TF genes critical for lymphopoiesis and myelopoiesis9,11,26,36–38, including Ebf1, Runx1, Irf4, Sfpi1, and general upregulation of Myc-dependent metabolic pathways in Brwd1/- small pre-B cells (Supplementary Fig. 3g).
sites were generally lost in enriched (Fig. 4e). Furthermore, in all peak subsets, IRF4 sites were apparent. In all peak subsets, IRF4 binding sites were (Fig. 4f, g). Examination of IRF4 sites in the preferentially bound to the open and nucleosome-free sites binding at the proximal Jk binding in Brwd1-/− in WT, but only 4983 sites in Brwd1-/− WT and the expressions of TFs.

accessible to TFs critical for B-cell fate decisions without changing data suggest that BRWD1 determines the genomic targets

and WT small pre-B cells (Fig. 4c). These data suggest that BRWD1 determines the genomic targets accessible to TFs critical for B-cell fate decisions without changing the expressions of TFs.

To examine this possibility, we performed IRF4 ChIP-Seq from WT and Brwd1+/− small pre-B cells. We chose IRF4, as it cannot bind DNA occupied by nucleosomes17. IRF4 bound 10,668 sites in Brwd1-/− and WT small pre-B cells (Fig. 4b). For IRF4, normal mRNA levels were associated with normal protein expression as assessed by intracellular flow cytometry of Brwd1+/− and WT small pre-B cells (Fig. 4c). These data suggest that BRWD1 determines the genomic targets accessible to TFs critical for B-cell fate decisions without changing the expressions of TFs.

To understand the importance of BRWD1 in human immunity, we examined 50 patients with hypogammaglobulinemia who had undergone whole-exome sequencing and for which no known mutations associated with B-cell deficiency had been found. Remarkably, four of these patients (clinical characteristics in Supplementary Table 5) had mutations in the BRWD1 gene. The distribution of these mutations is provided in Fig. 5a, b and family trees in Fig. 5c. Two patients (P1 and P2) were brothers and had a mutation predicted to affect splicing 3′ of exon 22. Patient 3 had an indel mutation in exon 21 that led to a frameshift and premature stop codon, while P4 had a missense mutation in exon 17 encoding the WD40 repeat domain predicted to change an asparagine to a serine. Patient 4 had no detectable peripheral B cells or serum immunoglobulins while the three other patients had severely diminished, but detectable B cells and immunoglobulin levels (Supplementary Table 5).

BRWD1 mutations in human hypogammaglobulinemia. To understand the importance of BRWD1 in human immunity, we examined 50 patients with hypogammaglobulinemia who had undergone whole-exome sequencing and for which no known mutations associated with B-cell deficiency had been found. Remarkably, four of these patients (clinical characteristics in Supplementary Table 5) had mutations in the BRWD1 gene. The distribution of these mutations is provided in Fig. 5a, b and family trees in Fig. 5c. Two patients (P1 and P2) were brothers and had a mutation predicted to affect splicing 3′ of exon 22. Patient 3 had an indel mutation in exon 21 that led to a frameshift and premature stop codon, while P4 had a missense mutation in exon 17 encoding the WD40 repeat domain predicted to change an asparagine to a serine. Patient 4 had no detectable peripheral B cells or serum immunoglobulins while the three other patients had severely diminished, but detectable B cells and immunoglobulin levels (Supplementary Table 5).
We next performed RNA-Seq on EBV-transformed peripheral blood B cells from two patients (P1 and P2), their mother (PM) who carried the same BRWD1 mutation but was healthy, five healthy controls (C1–C5), and four unrelated common variable immunodeficiency disease (CVID) patients (CVID1–4). Hierarchical clustering of gene expression (Fig. 5d) revealed that P1 and P2 had similar expression patterns and that these were very different from that observed in healthy controls. Expression in PM was intermediate between P1/2 and healthy controls. In contrast, CVID patients had heterogeneous distributions of expression likely reflecting different underlying causal mutations. Alignment of genes that were differentially expressed in P1/P2 and Brwd1−/− mice (Supplementary Figs. 4, 5) revealed similar patterns of expression that were different from those observed in either healthy controls or CVID patients.

BRWD1 represses proliferation in human B cells. Pairwise analysis between patient group (P1 and P2) and indicated controls (Fig. 5e) revealed that at least 1100 genes were differentially expressed in any given comparison (q < 0.05). In contrast, 732 genes were differentially expressed between BRWD1 mutant patients and their mother. Principal component analysis (Fig. 6a) confirmed that mRNA expression in P1 and P2 was similar and closely related to PM. In contrast, expression in the healthy controls and CVID patients were widely distributed. As seen in mice, BRWD1 appeared to repress the expression of metabolic and cell cycle pathways while inducing expression of molecular pathways of activation and differentiation (Fig. 6b, c). Indeed, there was a similar enrichment in expression of MYC/Myc-induced genes (Fig. 6d, e) and E2F pathway enriched in BRWD1-Mut patient group relative to controls (f) and Brwd1−/− small B cells compared to WT small B cells (g).

Human BRWD1 modulates accessibility at specific enhancers. Examination of chromatin accessibility revealed that P1–2 had 25,691 accessible peaks, while healthy controls had less with 19,038 peaks (Fig. 7a and Supplementary Fig. 6a). Accessible peaks were significantly enriched in P1–2 at enhancers of MYC/Myc-induced genes (Fig. 7b). Indeed, there was a similar enrichment in expression of MYC/Myc-induced genes (Fig. 6d, e) and E2F pathway enriched in BRWD1-Mut patient group relative to controls (f) and Brwd1−/− small B cells compared to WT small B cells (g).
Fig. 7 Human BRWD1 determines the genomic targets accessible to TFs. a Overlap of open chromatin peaks (ATAC-Seq) in EBV-transformed cells of patient group (P1 and P2), control group (C1-5), unrelated common variable immunodeficiency group (CVID1-4) and patients’ mother (PM). b Overlap of open chromatin peaks (ATAC-Seq) in cells from indicated populations. For (a) and (b) total number of peaks for each population shown in parentheses with number in each Venn region indicated. c De novo DNA sequence motifs enriched in indicated areas of Venn diagram. d, f Average accessibility at transcription factors IRF4 and E2A (e) and LEF1 and SOX (f). Accessibility at indicated binding sites for patients (red) and control (blue) cells. g Average accessibility of IRF4 and E2A transcription factor binding sites in open chromatin regions of all the groups described in (a). h Quantitative normalized mRNA expression of transcription factors EBF1, RUNX1, LEF1, CEBPA, STAT5A, STAT5B, PAX5, IRF4, SPI1, TCF3, E2A, SOX1, SOX2 and SOX3 in EBV-transformed cells of BRWD1-mut hypogammaglobulinaemia patients (P1 and P2), controls (C1-5), unrelated common variable immunodeficiency patients (CVID1-4) and PM along with Individual expression. Data are presented as average ± s.d. (n = 2). i Comparison of accessible enhancers (open chromatin + H3K4me1 + H3K27Ac+) between controls and BRWD1-Mut patients groups.
were shown clustering of differentially expressed genes identified (Fig. 7i), as was observed in Additionally, BRWD1 preferentially regulated the enhancers TFs critical for either lymphopoiesis or myelopoiesis (Fig. 7h).

were not associated with substantial differences in expression of diminished in P1/P2 B cells (Fig. 7e), while accessibility at LEF1, Fig. 6b). Genome-wide accessibility at IRF and E2A motifs was

IRF4 and E2A sites genome-wide in all groups demonstrated that accessibility was lowest in P1–2 and highest in the CVID patients (Fig. 7g). As was observed in Brwd1−/− mice, BRWD1 mutations were not associated with substantial differences in expression of TFs critical for either lymphopoiesis or myelopoiesis (Fig. 7h). Additionally, BRWD1 preferentially regulated the enhancers (Fig. 7i), as was observed in Brwd1−/− mice (Fig. 3b). Examination of genes derepressed in P1–2 cells, and Brwd1−/− small pre-B cells, revealed that increased expression was associated with increased accessibility at specific enhancers (Supplementary Fig. 7a–f). The converse relationship between enhancer accessibility and gene expression was observed in genes induced by BRWD1 (Supplementary Fig. 8a–f).

The human MYC locus has 31 putative enhancers (Fig. 8a). Comparison with accessibility across the locus revealed that BRWD1 mutations were specifically associated, in both P1–2 and PM, with enhanced accessibility at E7–8, E19–20, and E28 and increased MYC expression (Fig. 8b). Overall, these data indicate that in humans, BRWD1 mutations are associated with transcriptional and epigenetic signatures similar to those observed in Brwd1−/− mice.

**BRWD1 is required for normal human B-cell transcription.** The above data indicate that mutations in BRWD1 are associated with human B-cell immunodeficiency. Furthermore, these mutations appear to have an autosomal dominant pattern of inheritance with incomplete penetrance (Fig. 5). Therefore, to independently confirm that BRWD1 is biologically active in human B cells, we used two different short hairpin RNAs (shRNA40-RFP and shRNA197-RFP, Supplementary Table 6) targeting BRWD1. Retrovirus expressing each shRNA, or empty vector, was used to infect EBV-transformed peripheral blood B cells from three normal individuals (C2, C3, and C4). Infected cells were then sorted by flow cytometry and subjected to RNA-Seq. Initial analysis of the RNA-Seq data revealed that shRNA40 and shRNA197 diminished BRWD1 expression by 58% and 64%, respectively. Principal component analysis confirmed that mRNA

peaks in PM and CVID patients were slightly reduced compared with controls (17,971 and 16,039, respectively), with 10,360 peaks being shared between all groups (Fig. 7a and Supplementary Fig. 6a). In those accessibility peaks found in control but not P1–2 cells (Fig. 6b), there was an enrichment for accessibility of EBF1, IRF, and E2A TF binding sites (Fig. 7c and Supplementary Fig. 6b). In contrast, at those accessibility sites specific to P1–2 cells (Fig. 7b), there was an enrichment of LEF1/2, RUNX, CEBP, STAT5, and SOX binding sites (Fig. 7d and Supplementary Fig. 6b). Genome-wide accessibility at IRF and E2A motifs was diminished in P1/P2 B cells (Fig. 7e), while accessibility at LEF1, RUNX1, and SOX sites was increased (Fig. 7f). Comparison with IRF4 and E2A sites genome-wide in all groups demonstrated that accessibility was lowest in P1–2 and highest in the CVID patients (Fig. 7g). As was observed in Brwd1−/− mice, BRWD1 mutations were not associated with substantial differences in expression of TFs critical for either lymphopoiesis or myelopoiesis (Fig. 7h). Additionally, BRWD1 preferentially regulated the enhancers (Fig. 7i), as was observed in Brwd1−/− mice (Fig. 3b). Examination of genes derepressed in P1–2 cells, and Brwd1−/− small pre-B cells, revealed that increased expression was associated with increased accessibility at specific enhancers (Supplementary Fig. 7a–f). The converse relationship between enhancer accessibility and gene expression was observed in genes induced by BRWD1 (Supplementary Fig. 8a–f).
expression in patients (P1 and P2, red) was similar and closely related to BRWD1-specific shRNA-expressing controls (blue and purple). In contrast, expression in all the above populations was very different than either mock infected or healthy controls (Fig. 8c, green). Hierarchical clustering of gene expression (2786 genes by multi-group differential analysis between control, BRWD1-Mut, mock, shRNA40, and shRNA197 groups, and filtered the genes on q-value < 0.05) revealed that mock-transfected cells have similar expression patterns to C2, C3, and C4. However, control cells expressing BRWD1-specific shRNAs have similar expression patterns to P1 and P2, and these were very different from that observed in healthy controls or mock-transfected controls (Fig. 8d). The correlation between BRWD1 shRNA and BRWD1-Mut cells was most striking for genes that were downregulated compared with controls. In toto, these data demonstrate that BRWD1 plays a critical role in normal human humoral immunity.

Discussion

Our studies demonstrate that TF networks are not sufficient to regulate developmental programs of late B lymphopoiesis. In addition, expression and recruitment of BRWD1 to chromatin is critical for determining the accessibility of enhancers to TFs, such as IRF4. Together, regulated TF networks and BRWD1 both silence the transcriptional programs of early development and induce those required for Igk recombination and subsequent B-cell development. In humans, BRWD1 appeared to have similar critical functions highlighting the central and conserved function of BRWD1 in B lymphocytes.

Like the important TFs of B lymphopoiesis, the expression and recruitment of BRWD1 is dependent on stage-specific signals that restrict the activities of BRWD1 to a precise developmental context. The expression of Brwd1 is repressed by STAT5 and therefore escape from IL-7R signaling is necessary for BRWD1 induction. Subsequently, upon expression of pre-BCR, signals are activated that lead to the deposition of the histone acetyl and phosphorylation marks (H3K9acS10pK14ac) that recruit BRWD1 to chromatin. At Jk, the necessary histone acetylation marks require E2A, which is downstream of the pre-BCR, and binds the Igk intronic enhancer (iEk). However, in many cases, BRWD1 is recruited to sites that are not enhancers, suggesting that recruitment is not solely dependent on pioneer TF binding. Furthermore, independent of histone acetylation, H3S10 can be directly phosphorylated by ERK and p38. These observations suggest that the epigenetic landscape that recruits BRWD1 results from integration of multiple developmentally restricted signaling pathways.

One of the remarkable features of BRWD1 is that it regulated the expression of MYC and MYC target genes. At the Myc/Myc locus, in both mice and humans, BRWD1 specifically repressed distal enhancers, which are associated with lineage-specific expression. This is in contrast to both BRD4 and Brg1, which induce MYC expression by opening specific enhancers. Indeed, MYC is thought to be a main oncogenic target of BRD4 and provides much of the rationale for targeting BRD4 therapeutically in various cancers. Together, our studies and these published observations reveal another level of complexity in MYC regulation, in which functionally antagonistic epigenetic readers determine the expression. BRWD1 has a predicted molecular weight of 263 kD and contains tandem BROMO domains and WD40 repeats. However, it does not have strong homology with known catalytic domains. Therefore, it likely assembles a multimeric complex that then either opens or closes the chromatin. One component is likely SMARCA4 (Brg1), which co-immunoprecipitates with BRWD1 and is a component of the SWI/SNF chromatin remodeling complex. The BRWD1 complex likely contains histone acetyltransferases as at Jks, BRWD1 binding is associated with H4K16ac which plays a direct role in opening the chromatin. Intriguingly, in regions opened by BRWD1, nucleosomes are positioned relative to repetitive GAGA DNA motifs, suggesting that the BRWD1 complex functions similarly to the GAGA factor Trithorax expressed in Drosophila. Interestingly, the sites repressed by BRWD1 are also enriched for GAGA motifs, suggesting some commonality in the mechanisms by which the BRWD1 complex both opens and closes the enhancers.

One the remarkable features of BRWD1 is that it regulated enhancer accessibility over genomic distances of up to several mb. It is possible that this simply reflected an inability to detect small, biologically important, BRWD1–chromatin binding events. However, relaxing peak calling stringency did not change the fundamental relationship between BRWD1-bound sites and potential target genes. Therefore, our observations are not likely a consequence of experimental artifact. The mechanism by which BRWD1 regulates enhancer accessibility at a distance is unclear, although it likely requires three-dimensional interactions similar to those that enable the regulation of promoters by distal enhancers. Further work is required to determine the interdependency of BRWD1 and chromatin three-dimensional structure in small pre-B cells.

In addition to determining the functions and importance of BRWD1, our studies reveal the extent of the transcriptional and epigenetic rewiring that occurs in small pre-B cells. This molecular reordering includes transition from proliferation, which selects for cells expressing competent immunoglobulin heavy chains, to a state conducive to Igk recombination. However, thousands of genes are differentially expressed indicating that the small pre-B cell transition involves more than just repressing cell cycle genes and inducing the core recombination machinery.

One aspect of early B lymphopoiesis, and the pre-B cell transition in particular, is the silencing of early developmental and even multipotential enhancers. Transit from the large pre-B cell to small pre-B-cell stage is associated with a loss of almost 20,000 accessibility sites, with most of these being enhancers. In the absence of BRWD1, many of these enhancers become open. Furthermore, some enhancers associated with the multipotential pre-pro B-cell stage opened including those targeted by the myeloid TF CEBP. This suggests a role for BRWD1 in lineage specification. It is not clear if derepression of pre-pro B-cell enhancers reflects silencing in pre-B cells or a role for BRWD1 earlier in development where it is expressed at low levels. However, the first defect in B-cell progenitor numbers in Brwd1-/- mice is at the small pre-B-cell stage. Therefore, if BRWD1 plays a role in early lineage specification, it is not critical for early B-cell development.

Interestingly, many of the genes silenced by BRWD1 in small pre-B cells are re-expressed in germinal centers. Likewise, many BRWD1-induced genes are repressed. This suggests a general repurposing of developmental genes for the rapid proliferation and selection that occurs in germinal centers. It is possible that BRWD1 might play a role in modulating these GC transcriptional programs, as it is highly expressed in late GC B cells (M.R.C. and M.M., unpublished observation).

In humans, we identified three different BRWD1 mutations associated with severe B lymphopenia and hypogammaglobulinemia. All three mutations are predicted to lead to expression of a truncated or mutant protein that could act as a dominant negative. This could explain why the B-cell deficiency observed in some patients was more severe than that observed in Brwd1-/- mice. BRWD1 mutations occurred in 8% of screened patients.
indicating that if is both a novel and relatively common cause of human immunodeficiency.

Recent large-scale whole-genome sequencing studies, including the 1000 genomes project (http://www.internationalgenome.org), demonstrate that healthy individuals can have large numbers of genome-associated genetic variants without manifesting disease. Most monogenic, and essentially all polygenic, human diseases display reduced penetrance\(^{46}\). Several factors contribute to reduced penetrance including gender, environment, and the presence of modifier genes. In the family with the BRWD1 mutation, the two affected individuals were both male and had grown up with similar environmental exposures. All three BRWD1 mutant individuals we studied only shared 50% genetic relatedness, which means one or more might have different modifier genes. Furthermore, the mother did not manifest disease, she did share some molecular features associated with the BRWD1 mutation in the brothers and manifest in Brwd1\(^{-/-}\) mice. Therefore, the reduced disease penetrance in mother of the patients is not unexpected. Furthermore, our shRNA studies directly demonstrate that BRWD1 is required for normal transcription in human peripheral B cells. Our data provides the first example of a human immunodeficiency associated with mutations of an epigenetic reader gene\(^{42}\).

There was remarkable concordance in the genes dysregulated in murine Brwd1\(^{-/-}\) small pre-B cells and BRWD1 mutant human transformed peripheral B cell lines. We postulate, this reflects the strong and central role that BRWD1 plays in B-cell function. It also suggests that BRWD1 might play similar roles in both the bone marrow and periphery. Indeed, BRWD1 is highly expressed in follicular B cells and splenic follicular architecture is disrupted in Brwd1\(^{-/-}\) mice (M.C. and M.M., unpublished observations). Further studies are needed to determine how BRWD1 contributes to peripheral humoral immunity.

Our studies provide an integrated model of late B lymphopoiesis. As B-cell progenitors transit to the small pre-B-cell stage, early developmental TFs are repressed and those critical for late development induced. However, for these TFs to direct late lymphopoiesis, critical enhancers must be available for binding. This is the function of BRWD1. Both TF network expression and BRWD1 function are dependent upon a complex integration of intrinsic and extrinsic developmental signals that ensure that only those cells most fit to express a functional BCR proceed along the developmental pathway.

Methods

Mice. WT and Brwd1\(^{-/-}\) mice were housed in the animal facilities of the University of Chicago. Mice were used at 6–12 weeks of age, and experiments were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Chicago.

Analysis of bone marrow B-cell progenitors. Bone marrow (BM) was collected from WT or Brwd1\(^{-/-}\) mice, and cells were resuspended in the staining buffer (3% (vol/vol) FBS in PBS). Erythrocytes were lysed, and cells were stained with anti-CD11c (HL3), anti-NK1.1 (PK136), anti-CD43 (S7), IgM (R6-60.2), IgD (11-36), anti-CD19 (1D3), anti-B220 (RA3-6B21), anti-CD44 (7D4), anti-CD45 (30-F11), anti-CD49d (29F1-A12), anti-CD122 (2B8), anti-CD161 (2G4), and fragment bias correction was performed. UCSC gene annotations for mm9 were used for characterization.

RNA-sequencing and analysis. RNA was isolated from the Qiagen. Concentration and purity was analyzed on a Nanodrop 1000 Spectrophotometer. mRNA was isolated by oligo-dT beads and library was prepared using standard Illumina library protocol (Kit: RS-122-2101 TruSeq® small pre-B cells and mutant human bone marrow as described previously\(^{36}\). Briefly, WT and Brwd1\(^{-/-}\) small preB cells were fixed and permeabilized using True-Nuclear® Transcription Factor Buffer Set (Biolegend, 424401). Cells were then stained intracellularly with anti-IRF4-AP647 (Biolegend, clone IRF4.3E4, 646408) or isotype control (Biolegend, 400418), and analyzed by flow cytometry.

ChIP-seq analysis. Chromatin from flow sorted small pre-B cells (20 × 10^6 for low DNA samples from WT and Brwd1\(^{-/-}\) mice) was used for each ChIP experiment with anti-IRF4 (M-17, Santa Cruz Biotechnology, sc-6059), anti-B220 (RA3-6B21) and anti-BRD1 (E-15, Santa Cruz Biotechnology, sc-83317, lot 11508) antibodies described above. DNA libraries were prepared from the sheared chromatin (200–600 bp). Libraries were sequenced on the Illumina HiSeq2500. The sequences were aligned to the mm9 reference genome (National Center for Biotechnology Information build mm9_GenBank_37.1) with Bowtie alignment software\(^{48}\) and only reads with unique matches were retained.

ChIP-Seq peak calling and motif analysis. Peaks for ChIP-Seq samples were called using MACSA2 at a p-value threshold of 10^-5 or 10^-3 as done previously\(^{36}\). Normalized bedgraph tracks were generated using the SPMR flank, and converted to BigWig using UCSC bedGraphToBigWig. Peaks with a score > 5 were retained. Peak groups were generated by considering overlapping peak regions by at least 10 bp. HOMER software (hypergeometric optimization of motif enrichment) for de novo motif discovery and next-generation sequencing analysis was used for new prediction of motifs in the peaks. Additionally, de novo motif searches were performed independently on each peak group using MEME asking for the top 10 motifs.

For further motif analysis and DNA footprinting, peaks were recalled at a p-value threshold of 10^-7. We then searched for the identified de novo motifs in each of these peak groups using MAST counting both the total number of hits for the motif, and the fraction of sequences with at least one hit for the motif.

RNA-sequencing and analysis. RNA was isolated with the Qiagen RNeasy kit (Qiagen). Concentration and purity was analyzed on an Agilent 1000 Spectrophotometer. miRNA was isolated by oligo-dT beads and library was prepared using standard Illumina library protocol (Kit: RS-122-2101 TruSeq® Stranded mRNA LT-SetA). Libraries were sequenced on the Illumina HiSeq2500. All raw sequencing data was quality trimmed to a minimum phred score of 20 using trimmomatic\(^{49}\). QC-passed reads were filtered against a reference of mouse RNA sequences using bowtie2 (v2.2.4)\(^{50}\) with default parameters, then aligned to the mm9 reference genome in a splice-aware manner using the STAR aligner. Gene expression was quantified using cuffdiff, differential expression statistics (fold-change and p-value) were computed using edgeR, on raw expression counts obtained from quantification. P-values were adjusted for multiple testing using the false discovery rate (FDR) correction of Benjamini and Hochberg.

Assay of transposase-accessible chromatin using sequencing. ATAC-Seq was performed as recently described\(^{41}\). Briefly, to prepare nuclei, respective mouse or human cells (1 × 10^6) were centrifuged at 500 g for 5 min, which was followed by a wash with ice-cold PBS and centrifugation at 500 g for 5 min. Cells were lysed using cold lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl\(_2\), and 0.1% v/v Nonidet P-40). Immediately after lysis, nuclei were spun at 500 g for 10 min at 4°C and supernatant removed. The nuclei pellet was resuspended in the transposase reaction mix (25 μl 2X Tagment buffer, 2.5 μl Tagment DNA enzyme
and used as the nucleosome signal. The resulting BED regions were analyzed using mononucleosome, dinucleosome, and trinucleosome regions were concatenated with two BED regions, each spanning half the overall fragment length; and sizes between 315 and 473 bp were considered dinucleosomes and replaced with a single BED region, and used as a background. Sizes between 180 and 247 bp were considered mononucleosome and replaced with a single BED region for ChIP-Seq, like the human reference genome (hg19) using Maximum Exact Matches algorithm in bedtools genomcov, then converted to bigWig using UCSC tool bigWigToWig. The start position track was generated by taking just the strand alignments or the last base of the alignment for strand alignments; the latter was included to prevent regions of very high enrichment from dominating the correlation. Whole-exome sequencing. Genomic DNA from peripheral blood mononuclear cells was extracted and sequenced with a Covaris S2 Ultrasonicator. The data generated during and/or analyzed during the current study are available from the corresponding author(s) on reasonable request. GEO accession codes for publicly available data sets: Small pre-B BRWD1 ChIP-Seq (GSE63302); Pro-B c-Myc ChIP-seq (GSE40173; Pro-B H3K4me1 ChIP-seq (GSE20178), Pro-B (CD19+/-) H3K27ac; (GSE24164), CHI2 H3K4me4 ChIP-seq (ENCRR001DHQ); CH2 K3H2K4ac ChIP-seq (GSE23352); BRWD1 ChIP-seq (GSE31039), Bone Marrow H3K4me1 (GSE31039), Bone Marrow H3K4me3 (GSE31039), Bone Marrow H3K27ac (GSE31039). Human B cell, T cell and Monocyte ATAC-Seq, H3K4me1, and H3K27ac ChIP-seq data used were shared by the McGill Epigenomics Mapping Centre and it is available from the European Genome-phenome Archive of the European Bioinformatics Institute (http://epigenomesportal.ca/ihec/) and International Human Epigenome Consortium (IHEC) (http://epigenomesportal.ca/ihec/). The sequences, ATAC-Seq for WT pre-proB, WT pre-B, WT large pre-B, WT small pre-B, Brwd1/-/- small pre-B and Immature B, RNA-Seq for WT pre-B, WT small pre-B and Brwd1/-/- small pre-B, IRF4 ChIP-seq for WT and Brwd1/-/- small pre-B, RNA-Seq and ATAC-Seq for human EBV-transformed peripheral blood B cells from patients (P1 and P2), patient mother (PM), healthy controls (C1–5), four unrelated Combined Immunodeficiency patients (CVID1-4) and RNA-Seq for mock-shRNA and human BRWD1-specific shRNA40 and shRNA197 expressing healthy controls C2, C3 and C4 reported in this paper has been deposited in the GenBank database (accession no. GSE103657).
2. Schatz, D. G. & Ji, Y. Recombination centers and the orchestration of V(D)J recombination. *Nat. Rev. Immunol.* 11, 251–263 (2011).

3. Herzog, S., Reth, M. & Jumaa, H. Regulation of B-cell proliferation and differentiation by pre-B-cell receptor signalling. *Nat. Rev. Immunol.* 9, 195–205 (2009).

4. Bednarski, J. J. et al. RAG-mediated DNA double-strand breaks activate a cell type-specific checkpoint to inhibit pre-B-cell receptor signalling. *J. Exp. Med.* 213, 1203–1216 (2016).

5. Durandy, A., Kracker, S. & Fischer, A. Primary antibody deficiencies. *Nat. Rev. Immunol.* 13, 519–533 (2013).

6. Zhang, L., Reynolds, T. L., Shan, S. & Desiderio, S. Coupling of V(D)J recombination centers and the orchestration of V(D)J recombination to cell cycle suppresses genomic instability and lymphoid tumorigenesis. *Immunity* 34, 163–174 (2011).

7. Mandal, M. et al. Ras orchestrates exit from the cell cycle and light-chain recombination during early B cell development. *Nat. Immunol.* 10, 1110–1117 (2009).

8. Beck, K., Peak, M. M., Ota, T., Nemazee, D. & Murre, C. Distinct roles for E12 and E47 in B cell specification and the sequential rearrangement of immunoglobulin light chain loci. *J. Exp. Med.* 206, 2271–2284 (2009).

9. Johnson, K. et al. Regulation of immunoglobulin light-chain recombination by the transcription factor IRF-4 and the attenuation of interleukin-7 signalling. *Immunity* 28, 335–345 (2008).

10. Chovanec, K. et al. A self-reinforcing regulatory network triggered by limiting IL-7 activates pre-B-cell signalling and differentiation. *Nat. Immunol.* 13, 300–307 (2012).

11. Mandal, M. et al. Epigenetic repression of the Ig-kappa locus by STAT5 to the Ig-kappa locus. *Nat. Immunol.* 16, 1094–1103 (2015).

12. Heng, T. S. & Painter, M. W., Consortium, I.G.P. The immunological genome project: networks of gene expression in immune cells. *Nat. Immunol.* 9, 2093–2104 (2008).

13. Haberle, V. et al. Two independent transcription initiation codes overlap on vertebrate core promoters. *Nature* 507, 381–385 (2014).

14. Bossen, C. et al. The chromatin remodeler Brh1 activates enhancer repertoires to establish B cell identity and modulate cell growth. *Nat. Immunol.* 16, 775–784 (2015).

15. Kleinman, E., Jia, H., Loguercio, S., Su, A. I. & Feeney, A. J. YY1 plays an essential role at all stages of B-cell differentiation. *Proc. Natl Acad. Sci. USA* 113, E911–E920 (2016).

16. Coohara, C., Schebesta, A., Delougo, A. & Busslinger, M. Pax5: the guardian of B cell identity and function. *Nat. Immunol.* 8, 463–470 (2007).

17. Shimizu, K. et al. Divergent functions of hematopoietic transcription factors in lineage priming and differentiation during erythro-megakaryopoiesis. *Genome Res.* 24, 1932–1944 (2014).

18. Ebina, W. & Rossi, D. J. Transcription factor-mediated reprogramming toward hematopoietic stem cells. *EMBO J.* 34, 694–709 (2015).

19. Maffucci, P. et al. Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. *Front. Immunol.* 7, 220 (2016).

20. Dunn, K. L. & Davie, J. R. Stimulation of the Ras-MAPK pathway leads to independent phosphorylation of histone H3 on serine 10 and 28. *Oncogene* 24, 3492–3502 (2005).

21. Delmore, J. E. et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 146, 904–917 (2011).

22. Perez-Salvia, M. & Esteller, M. Bromodomain inhibitors and cancer therapy: from structures to applications. *Epigenetics* 12, 323–339 (2017).

23. Huang, H., Rambaldi, L., Daniels, E. & Featherstone, M. Expression of the Wdr9 gene and protein products during mouse development. *Dev. Dyn.* 227, 608–614 (2003).

24. Shogren-Knaak, M. et al. Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* 311, 844–847 (2006).

25. Farkas, G. et al. The trithorax-like gene encodes the Drosophila GAGA factor. *Nature* 371, 806–808 (1994).

26. Cooper, D. N., Krawczak, M., Polychronakos, C., Tyler-Smith, C. & Kehrer-Sawatzki, H. Where genotype is not predictive of phenotype: towards an understanding of the molecular basis of reduced penetration in human inherited disease. *Hum. Genet.* 132, 1077–1130 (2013).

27. Picard, C. et al. International union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. *J. Clin. Immunol.* 38, 96–128 (2018).

28. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359 (2012).

29. Boy, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 4172–4173 (2014).

30. Dawson, M. A. et al. Recurrent mutations, including NPM1c, activate a BRD4-dependent core transcriptional program in acute myeloid leukemia. *Leukemia* 28, 311–320 (2014).

31. Mandal, M. et al. Histone reader BRWD1 targets and restricts recombination to the Ig-kappa locus. *Nat. Immunol.* 16, 1094–1103 (2015).

32. Kieffer-Kwon, K. R. et al. Interactome maps of mouse gene regulatory networks reveal basic principles of transcriptional regulation. *Cell* 155, 1507–1520 (2013).

33. Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. & Greenleaf, W. J. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat. Methods* 10, 1213–1218 (2013).

34. Heinz, S. et al. Chip-seq combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol. Cell* 28, 576–589 (2010).

35. Choukroun, S. et al. Comparison of open chromatin, DNA-binding proteins and nucleosome position. *Nat. Methods* 10, 1213–1218 (2013).

36. Heinz, S. et al. Chip-seq combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol. Cell* 28, 576–589 (2010).

37. Shogren-Knaak, M. et al. Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* 311, 844–847 (2006).

38. Farkas, G. et al. The trithorax-like gene encodes the Drosophila GAGA factor. *Nature* 371, 806–808 (1994).

39. Cooper, D. N., Krawczak, M., Polychronakos, C., Tyler-Smith, C. & Kehrer-Sawatzki, H. Where genotype is not predictive of phenotype: towards an understanding of the molecular basis of reduced penetration in human inherited disease. *Hum. Genet.* 132, 1077–1130 (2013).

40. Picard, C. et al. International union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. *J. Clin. Immunol.* 38, 96–128 (2018).

41. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359 (2012).

42. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120 (2014).

43. Trapnell, C. et al. Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nat. Biotechnol.* 31, 46–53 (2013).

44. Li, H. & Durbin, R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 26, 589–595 (2010).

45. Chen, K. et al. DANPOS: dynamic analysis of nucleosome position and occupancy by sequencing. *Genome Res.* 23, 341–351 (2013).

46. DePristo, M. A. et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat. Genet.* 43, 491–498 (2011).

47. Bujold, D. et al. The international human epigenome consortium data portal. *Cell Syst.* 3, 496–499 (2016).
assisted M.M. in analyzing some data. M.M. and M.R.C. oversaw the entire project and wrote the final paper.

**Additional information**

Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-06165-6.

**Competing interests:** The authors declare no competing interests.

**Reprints and permission** information is available online at http://npg.nature.com/reprintsandpermissions/

**Publisher’s note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018