Highly Parallel Quantification and Compartment Localization of Transcription Factors and Nuclear Proteins

Graphical Abstract

Highlights
- Quantification of global protein levels across different chromatin compartments
- Protein levels correspond to functional differences in euchromatin versus heterochromatin
- Method used to interrogate system-wide effects of BET degradation compounds

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In Brief
Proteins that bind DNA and regulate the expression of genes are difficult to measure accurately. In this study, we present a method that quantifies these proteins with high coverage and in high throughput, providing a system-wide view of protein dynamics on chromatin.
Highly Parallel Quantification and Compartment Localization of Transcription Factors and Nuclear Proteins

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SUMMARY

Transcription factors and other chromatin-associated proteins are difficult to quantify comprehensively. Here, we combine facile nuclear sub-fractionation with data-independent acquisition mass spectrometry to achieve rapid, sensitive, and highly parallel quantification of the nuclear proteome in human cells. We apply this approach to quantify the response to acute degradation of BET bromodomains, revealing unexpected chromatin regulatory dynamics. The method is simple and enables system-level study of previously inaccessible chromatin and genome regulators.

INTRODUCTION

The human genome encodes more than 1,600 transcription factors (TFs), along with additional cofactors, chromatin regulators, and structural proteins that work collectively to execute the regulatory instructions encoded within nuclear DNA (Lambert et al., 2018). Cell-type- or state-selective function of regulatory DNA is gated on the milieu of trans-acting regulatory factors and their relative abundance within the nucleus (Ptashne, 2011). However, we currently lack approaches for comprehensive characterization of the nuclear protein compartment and its functional sub-compartments (Wierer and Mann, 2016).

Mass spectrometry (MS)-based proteomics can be used to measure system-level protein dynamics, but fully characterizing the nuclear proteome by MS presents substantial challenges. Many chromatin proteins are expressed transiently, at low levels, or are difficult to extract from the nucleus (Shio et al., 2003; Wachter et al., 2001; Ghaemmaghami et al., 2003). Previous experimental approaches to enrich for the nuclear proteome are not comprehensive and fail to detect most expressed TFs and cofactors in single or even repeated experiments (Ji et al., 2015; Torrente et al., 2011; Alajem et al., 2015; Kustatscher et al., 2014; Kulej et al., 2017; Dutta et al., 2014; Becker et al., 2017). Moreover, sample preparation approaches typically require variable and laborious and fractionation strategies, limiting scalability and widespread adaptability (Dutta et al., 2014; Becker et al., 2017).

Compounding the problem of low TF abundance, high concentrations of structural proteins in the nucleus suppress detection of TFs in typical data-dependent acquisition (DDA) proteomics. In DDA, peptides are chosen for fragmentation and sequencing based on intensity, leading to infrequent and stochastic detection of lowly abundant peptides. This leads to incomplete peptide sampling across large-scale datasets, making it impossible to distinguish stochastic missing measurements from the true absence of a peptide. These issues can be resolved with data independent acquisition (DIA) (Venables et al., 2004; Gillet et al., 2012), in which all eluting peptides are fragmented simultaneously, resulting in complex MS spectra that are computationally deconvolved, matched with peptides, and quantified (Ting et al., 2015).

RESULTS

To quantify protein dynamics across various chromatin environments, we developed chromatin enriching salt separation coupled to DIA (CHESS-DIA) (Figure 1A). Protein-nucleic acid interactions are largely driven by electrostatic attraction (Henikoff et al., 2009; Weisbrod and Weintraub, 1979; Dignam et al., 1983; Burton et al., 1978; Hyde and Walker 1975), leading to the development of salt gradients for the physical separation of chromatin species (Berezney and Coffer, 1974; Bloom and Anderson, 1978; Sanders, 1978; Roth et al., 1984). We adapted and scaled previously reported salt gradient approaches for isolated human cell and tissue nuclei. Using a fine-scale gradient of increasing salt concentrations, we observe a bimodal disruption and release of proteins from surrounding nuclear material (Figure S1A). As previously reported, this bimodality reflects differences in the physical characteristics of “active” euchromatin versus “closed” heterochromatin in the nucleus (Henikoff et al., 2009). In brief, nuclei are isolated from cells or tissues and then subjected to a series of extraction conditions that enrich for (1) freely diffusing proteins in the nucleoplasm, (2) proteins bound in euchromatin, (3) proteins bound in heterochromatin, and (4) insoluble structural proteins. After extraction, digestion, and desalting, DIA-MS data are acquired and quantified using on-column chromatogram libraries to increase sensitivity and reproducibility across large datasets (Searle et al., 2018; Figure S1B; Table S1).
Combining this facile, scalable nuclear separation procedure with label-free DIA-MS acquisition, we assign spectra to peptides from 70.2% of the confidently expressed proteins (RNA sequencing [RNA-seq] fragments per kilobase million [FPKM] > 5) annotated to reside in the nuclear proteome (Figure 1B). Proteins detected by CHESS-DIA have high coverage, with a median value of 5.5 peptides per protein (Figure 1B). Peptide detection rates improve with increased protein expression level, and <2% of peptides detected map to genes unlikely to be expressed (Figure 1C; FPKM < 1.0).

CHESS-DIA was then benchmarked against several published nuclear proteomes datasets. First, when compared to recent data from MOLM-13 acute myeloid leukemia nuclear extracts that utilize tandem mass tagging (TMT) (Winter et al., 2015), CHESS-DIA detected 33% more TFs and cofactors, and this improvement is dependent on TF family (Figure 1D). Critical developmental regulators, such as homeodomains and forkhead proteins, which are typically present in low abundance, are detected at a 2-fold and 1.8-fold higher rate, respectively (Figure 1D).

To test the validity of CHESS-DIA sub-nuclear compartments, proteins were assigned to clusters using a k-means approach. Eight robust clusters were identified, all of which are significantly overrepresented by one or two chromatin compartments (Figure 2). Using this approach, we found that 3,699 proteins were specifically associated with the isotonic fraction, 1,329 proteins with euchromatin, 366 proteins with heterochromatin, and 290 with the insoluble fraction. CHESS-DIA clusters were then compared against enzymatically isolated chromatin fractions using MNase (Bloom and Anderson, 1978). Enzymatic chromatin preparation with MNase is another widely accepted method for crudely separating chromatin states (Burton et al., 1978). We detect 91.8% of the proteins assigned to euchromatin in this study and 90.7% assigned to heterochromatin. CHESS-DIA, however, detects 8× more protein identifications than this approach. The nuclear sub-compartments in Torrente et al. (2011) both contain a large amount of putative impurities that we find in the isotonic fraction (40%–60%), likely due to the limited nuclei washing included in this protocol. We repeated the protocol with additional washes and coupled to DIA-MS. Using this approach, we improved agreement (37% versus 71%) between euchromatin prepared by CHESS-DIA and by MNase digestion. (Figure S2).

To explore functional correlates of the isolated chromatin sub-compartmentalization, we observed the behavior of well-studied protein complexes with established roles in a defined chromatin context (Figure 1E). Nuclear export proteins and the RNA-induced silencing complex (RISC) small-RNA-processing complex are highly enriched in the nucleoplasm, consistent with their non-chromatin-related functions (Hutten and Kehlenbach, 2007; Pratt and MacRae, 2009). The mediator coactivator complex is found predominantly in the euchromatin fraction (Allen and Taatjes, 2015), as is the SwItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeler (Hodges et al., 2016), both of which are critical cofactors to enable active transcription. Centromere proteins, which comprise a canonical heterochromatin structure (Polco et al., 2008), are highly enriched in this fraction, as are proteins associated with the heterochromatin-associated nucleolus (Guetg and Santoro, 2012). Histones and nuclear lamina are predominantly insoluble, with the exception of LAP2A, which is a lamina-family protein with an established role regulating gene expression in euchromatin (Dorner et al., 2007).

Concordant with their wide range of functionalities within the nucleus, TFs are distributed across all four nuclear fractions (Figure 1F). Highly soluble nucleoplasmic TFs include conditionally activated factors such as STAT5 and NFKB, which are sequenced from chromatin until an activating phosphorylation event (Staudt, 2010). TFs segregating in euchromatin include many lineage-specific and oncogenic regulators known to dominate the transcriptional regulatory programs of early differentiating normal cells or neoplastic cells (Morgens et al., 2016). Heterochromatic TFs include proteins with known roles in nuclear organization (e.g., CTCF), interaction with methylated DNA (e.g., MECP2 and MBD1), and chromatin compaction (Ludwig et al., 2016). Insoluble TFs notably include many zinc finger TFs that constitutively suppress retrotransposon activity (Yang et al., 2017).

Gene Ontology (GO) enrichment analysis provided additional validation for the integrity of the chromatin sub-compartmentalization (Table S2). The GO term “regulation of gene expression” is most significantly enriched in euchromatin, as well as annotations related to nucleic acid binding, chromatin regulation, and nuclear localization. Heterochromatin is most uniquely enriched for rRNA metabolic processes, consistent with the close association with the nucleolus (Guetg and Santoro, 2012).

Lastly, CHESS-DIA protein clusters were queried for an increased frequency of physical protein-protein interactions (PPIs) as defined by the search tool for recurring instances of protein interactions (STRING) database (Szklarczyk et al., 2019). Across all clusters, there are significantly more intra-cluster PPIs than inter-cluster PPIs (Figure S3A), suggesting an enrichment of functional protein complexes being extracted together in CHESS-DIA. This observation also holds when observing individual clusters assigned to one specific chromatin sub-compartment.

There exists no proteome-wide gold standard to define sub-nuclear protein location, so we integrated quantitative cellular immunofluorescence (IF) microscopy data for further corroboration of the chromatin sub-compartmentalization defined with CHESS-DIA. To map proteins to subcellular structures, we utilized IF
maps produced by the Human Protein Atlas (HPA) (Thul et al., 2017). We first tested for possible protein mis-classification by CHESS-DIA where the protein is quantified but classified as non-nuclear by HPA. Mis-classification rates are low for euchromatin (11.8%) and heterochromatin (12.6%), but the isotonic fraction contains 55% extranuclear proteins (Figures S3B and S3C). Upon further inspection, these impurities originate from other membrane-bound organelles, including mitochondria and vesicles, which are likely co-isolated with nuclei. This limitation is considered for downstream applications, which are focused on proteins observed in chromatin-bound fractions in at least one experimental condition.

HPA provides some limited sub-nuclear structural identifications, so CHESS-DIA clusters were also compared to these classifications. All clusters show strong enrichment for the nuclear and nucleoplasm sub-structures. The euchromatic cluster is notably enriched within nuclear speckles, which contain splicing factors and are involved in active transcription within the nucleus (Galagan ski et al., 2017). By contrast, heterochromatic proteins were again preferentially associated with the nucleolus (Figure S3C).

We performed further validation using IF to test extraction dynamics of marker proteins for each fraction of the CHESS-DIA system. GATA1 was selected for a canonical euchromatin marker, and an analysis of GATA1 extraction kinetics show rapid and complete escape from nuclei after a 5-min extraction (Figure 3A), quantified across 100 cells per time point (Figure 3B). IF on additional factors at short timescales (10 min post-extraction) show consistent extraction patterns between IF and CHESS-DIA experiments. This includes the nuclear export protein XPO1 (CRM1) as a nucleoplasm marker and CBX5 (HP1) as a marker of constitutively repressed insoluble chromatin (Figures 3C and 3D). These extraction patterns hold for additional factors with more complex extraction patterns, including CTCF, CBP, CRD4, and SMARCA4 (Figures S3D–S3F). Collectively, these results establish CHESS-DIA as a robust method for rapidly, comprehensively, and quantitatively sampling the nuclear proteome.

We next applied CHESS-DIA to characterize the global nuclear proteome response to a rapid, chromatin-directed stimulus. Pharmacologically triggered protein degradation can deplete targeted

Figure 2. Protein Clustering to Assign Enriched Chromatin Fractions
(A) All proteins quantified across the 4 chromatin environments, row normalized, and clustered with the k-means algorithm, k = 8.
(B) Individual proteins quantified across the 4 chromatin fractions, split into clusters. Mann-Whitney tests were performed between fractions as indicated; **p < 0.0001 (Mann-Whitney). Median represented by mid-line.
proteins within minutes of applying a small-molecule stimulus (Neklesa et al., 2017). A potent exemplar is the recently-reported dBET1 molecule, which triggers rapid degradation of the BET bromodomain family chromatin “readers” BRD2, BRD3, and BRD4 (and the testes-specific BRDT), all which contain tandem bromodomains that regulate gene expression by binding acetylated lysine residues on histones and transcription factors (Winter et al., 2015).

In steady-state MOLM-13 cells, BRD2, BRD3, and BRD4 contain partially overlapping but distinct chromatin compartment distributions (Figure 4A), consistent with their partially overlapping but distinct roles in transcriptional regulation (Hsu et al., 2017; Decker et al., 2017; Roberts et al., 2017). In agreement with previously published measurements of dBET1 kinetics, CHESS-DIA confirmed the depletion of total BRD2, BRD3, and BRD4 from all chromatin compartments within 2 h of treatment with dBET1 (Figure 4B). We then looked specifically at the euchromatin protein fraction, where canonical BET bromodomain cofactors are observed. During dBET1 treatment, CDK9 (pTEFb) and the mediator complex are lost from euchromatin, consistent with previous studies showing the loss of these transcriptional cofactors upon the loss of BET bromodomains from chromatin, as measured by chromatin immunoprecipitation sequencing (ChIP-seq) (Brown et al., 2014). Protein subunits of RNA PolII are also depleted from active chromatin, consistent with a global loss in transcriptional activity (Figure 4C).

Across all fractions, we observed a striking redistribution of HDAC1 and HDAC2 peptides from euchromatin into...
Figure 4. CHESS-DIA Reveals Chromatin Reorganization during Acute BET Degradation

(A) CHESS-DIA distributions of the BET bromodomains in MOLM-13.
(B) Quantification of all peptides mapping to BET bromodomains in each fraction during the time course of dBET1 treatment.
(C) Quantification of all peptides mapping to selected BET interaction partners in each fraction during time course of dBET1 treatment.
(D) CHESS-DIA distributions of HDAC1 and HDAC2 in MOLM-13.

(legend continued on next page)
heterochromatin with the same kinetics as BET bromodomain loss (Figures 4D and 4E). HDAC1/2 cooperate as part of the NuRD complex to regulate transcription by deacetylating acetylated histones and promoting chromatin compaction, particularly at bivalent genes during development (Basta and Rauchman, 2015). Peptides for the other NuRD complex members show the same pattern of euchromatin to heterochromatin transition (Figure 4F), so we hypothesized that this phenomenon was due to chromatin compaction at sites shared by HDAC1/2 and BETs. To test for genome-wide co-occupancy of HDAC1/2 and BET bromodomains, we localized these factors on the genome using the highly sensitive and specific Cut&Run technique (Skene and Henikoff, 2017). Peaks of HDAC1/2 occupancy displayed significant overlap with BRD2 (49% of HDAC1/2 shared peaks), BRD3 (24%), and BRD4 (35%) (Figures S4A and S4B). Empirical simulations confirm that these overlaps are not driven by open chromatin as defined by DNase hypersensitivity and are not seen between HDAC1/2 and the unrelated TF CTCF (Figures S4C–S4E). These findings support a model in which steady-state HDAC1/2 activity is antagonized at active regulatory elements by BET bromodomains and associated co-factors. After BET degradation, HDAC1/2 complexes remaining at regulatory DNA promote deacetylation of nearby histones, leading to compaction of local chromatin after BET loss (Figure 4G).

**DISCUSSION**

In summary, CHESS-DIA is a facile, robust, and scalable assay to measure nuclear protein abundance across chromatin environments and can be applied to quantify protein dynamics during rapid functional processes. The chromatin compartments segregated under CHESS-DIA correspond to historically defined functional chromatin fractions and correlate with imaging-based chromatin structures defined by IF. Application of CHESS-DIA to study the consequences of rapid and selective BET bromodomain protein degradation by dBET1 provide the first description of the nuclear proteome-wide effects of pharmacological protein degradation, a powerful emerging therapeutic modality.

**STAR+METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2020.01.096.

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**AUTHOR CONTRIBUTIONS**

This work was conceptualized and planned by A.J.F., M.J.M., and J.A.S. M.J.M. and J.A.S. supervised the work. Experiments were performed by A.J.F., V.N., H.W., N.H., and T.K. Analytical tools were built by B.C.S. and L.K.P. Data analysis was performed by A.J.F., V.N., B.C.S., L.K.P., G.M., and Y.S.T. The manuscript was written by A.J.F., and all authors contributed to revision of the final version.

**DECLARATION OF INTERESTS**

The MacCoss Lab at the University of Washington has a sponsored research agreement with Thermo Fisher Scientific, the manufacturer of the instrumentation used in this research. Additionally, M.J.M. is a paid consultant for Thermo Fisher Scientific. A patent application has been filed on the methods described.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| HDAC1 antibody, rabbit polyclonal | Active Motif | Cat#40967; RRID:AB_2614948 |
| HDAC2 antibody, mouse monoclonal  | Active Motif | Cat#39533; RRID:AB_2614959 |
| BRD2 antibody, rabbit polyclonal | Active Motif | Cat#61797; RRID:AB_2793770 |
| BRD3 antibody, rabbit polyclonal | Active Motif | Cat#61489; RRID:AB_2737028 |
| BRD4 antibody, rabbit monoclonal  | Bethyl | Cat#A700-004; RRID:AB_2631885 |
| GATA1 antibody, rabbit monoclonal | Cell Signaling Technology | Cat#3535; RRID:AB_2108288 |
| BRG1 antibody, mouse monoclonal  | Santa Cruz Biotechnology | Cat#sc-17796; RRID:AB_626762 |
| CRM1 (XOP1) antibody, mouse monoclonal | Santa Cruz Biotechnology | Cat#sc-74454; RRID:AB_1122704 |
| Histone H3K27ac antibody, mouse monoclonal | Active Motif | Cat#39085; RRID:AB_2793305 |
| Histone H3K27ac antibody, rabbit polyclonal | Active Motif | Cat#39133; RRID:AB_2722569 |
| Lamin B Antibody, goat polyclonal | Santa Cruz Biotechnology | Cat#sc-6216; RRID:AB_648156 |
| CTCF antibody, rabbit polyclonal | Cell Signaling Technology | Cat#2899; RRID:AB_2086794 |
| CREBBP antibody, rabbit polyclonal | Sigma Aldrich | Cat#HPA055861; RRID:AB_2682948 |
| HP1a antibody, rabbit polyclonal | Cell Signaling Technology | Cat#2616; RRID:AB_2070987 |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| dBET1                 | Tocris | Cat#6327 |
| PPS silent surfactant | Expedeon | Cat#21011 |
| Pierce Trypsin Protease, MS Grade | Thermo Fisher | Cat#90057 |
| Pierce Peptide Retention Time Calibration Mixture | Thermo Fisher | Cat#88320 |
| **Deposited Data**   |        |            |
| Transcription Factor Annotations | Lambert et al., 2018 | https://doi.org/10.1016/j.cell.2018.01.029 |
| MOLM13 nuclear proteomics data | Winter et al., 2015 | https://doi.org/10.1126/science.aab1433 |
| ENCODE K562 data | ENCODE Encyclopedia, Version 4 | DNasel: ENCSR000EOY RNaseq: ENCSR109IQO CTCF ChIP-seq: ENCCF389ELU |
| Nuclear fraction proteomics data | Torrente et al., 2011 | N/A |
| Gene Ontology         | Panther database | version 14.0 |
| Protein-protein interaction data | BioGRID database | version 3.5.169 |
| Subcellular immunofluorescence data | The Human Protein Atlas | version 18.0 |
| **Experimental Models: Cell Lines** |        |            |
| K562                  | American Type Culture Collection | CCL-243 |
| MOLM13                | DSMZ | ACC 554 |
| **Software and Algorithms** |        |            |
| ProteoWizard          | Chambers et al., 2012 | N/A |
| Percolator            | Käll et al., 2007 | N/A |
| XCorDIA               | Unpublished (https://bitbucket.org/searleb/encyclopedia/wiki/Home) | N/A |
| EnciclopeDIA          | Searle et al., 2018 | N/A |
| BWA                   | Li and Durbin, 2009 | N/A |
| MACS2                 | Zhang et al., 2008 | N/A |
| BEDOPS                | Nep et al., 2012 | N/A |
| Bedtools              | Quinlan and Hall, 2010 | N/A |
LEAD CONTACT AND MATERIALS AVAILABILITY

This study did not generate new unique reagents. Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, John Stamatoyannopoulos (jstam@altius.org).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell Culture

K562 (ATCC) and MOLM13 (DSMZ) cells were grown in IMDM or RPMI, respectively (GIBCO), supplemented with 10% Fetal Bovine Serum (PAA), sodium pyruvate (GIBCO), L-glutamine (GIBCO), penicillin and streptomycin (GIBCO) at 37°C with 5% CO2. dBET1 (Tocris) was dissolved in DMSO (Sigma Aldrich) to a concentration of 10mM and cells were treated at a concentration of 1μM for the time indicated.

METHOD DETAILS

Protein Extraction

Nuclear extraction was performed similarly to Dorschner et al. (2004) by first washing 10^6 cells with ice cold PBS. All buffers during cell lysis and extraction were supplemented with 1x protease inhibitor solution (Roche). First, cells were resuspended in 0.05% NP-40 dissolved in buffer A (15mM Tris pH 8.0, 15mM NaCl, 60mM KCl, 1mM EDTA pH 8.0, 0.5mM EGTA pH 8). After a 5-minute incubation on ice, nuclei were pelleted at 400xg for 5 minutes. Nuclear extraction was then performed by resuspending the cell pellet first in isotonic buffer (10mM Tris pH 8.0, 15mM NaCl, 60mM KCl, 1.5 mM EDTA pH 8.0) and incubating at 4°C for 20 minutes. The soluble and insoluble fractions were separated by centrifugation at 500xg for 5 minutes. Next, cells were resuspended in euchromatin extraction buffer (10mM Tris pH 8.0, 250mM NaCl, 1 mM EDTA pH 8.0), incubated at 4°C for 20 minutes and then centrifuged at 1000xg for 5 minutes. Finally, cells were resuspended in heterochromatin extraction buffer (10mM Tris pH 8.0, 600mM NaCl, 1 mM EDTA pH 8.0), incubated at 4°C for 20 minutes and then centrifuged at 20,000xg for 10 minutes. The pellet contains the insoluble protein fraction.

Protein Preparation and Digestion

Protein samples were supplemented with 0.1% PPS silent surfactant, and the chromatin pellet was sonicated at power 5 for 10 s to solubilize proteins. All samples were then boiled at 95°C for 5 minutes, reduced with 5mM DTT at 60°C for 30 minutes and alkylated with 15mM iodoacetic acid (IAA) at room temperature for 30 minutes in the dark. Proteins were then digested with trypsin (Promega) at 37°C for 16-18 hours while shaking (1:50 trypsin:protein per sample by mass) and the pH of the sample was subsequently adjusted to < 3.0 using HCl. The digested samples were desalted using Oasis MCX 30 mg/60 μm cartridges (Waters) following the manufacturer’s protocol. Briefly, the cartridge was conditioned using 1 mL methanol, 1 mL 10% ammonium hydroxide in water, 2 mL methanol and finally 3 mL 0.1% formic acid in water. The samples were then loaded onto the cartridge and washed with 1 mL 0.1% formic acid in water and 1 mL of 90% acetonitrile in water. The peptides were eluted from the cartridge with 1 mL 10% ammonium hydroxide in methanol which was then removed by evaporation. Peptide samples were resuspended to a concentration of 1μg/3 μL in 0.1% formic acid in H2O and stored at -20°C until injected on the mass spectrometer.

Mass Spectrometry Acquisition

Peptides were separated with a Waters NanoAcquity UPLC and emitted into a Thermo Q-Exactive HF. Pulled tip columns were created from 75 μm inner diameter fused silica capillary in-house using a laser pulling device and packed with 3 μm ReproSil-Pur C18 beads (Dr. Maisch) to 300 mm. Trap columns were created from 150 μm inner diameter fused silica capillary fitted with Kasil on one end and packed with the same C18 beads to 25 mm. Solvent A was 0.1% formic acid in water, while solvent B was 0.1% formic acid in 98% acetonitrile. For each injection, 3 μL (approximately 1 μg) was loaded and eluted using a 90-minute gradient from 5% B, followed by a 40-minute washing gradient. For each chromatogram library, the Thermo QExactive HF was configured to acquire six chromatogram library acquisitions with 4 m/z DIA spectra (4 m/z precursor isolation windows at 30,000 resolution, AGC target 1e6, maximum inject time 55 ms) using an overlapping window pattern from narrow mass ranges using window placements optimized by Skyline (i.e., 396.43 to 502.48 m/z, 496.48 to 602.52 m/z, 596.52 to 702.57 m/z, 696.57 to 802.61 m/z, 796.61 to 902.66 m/z, and 896.66 to 1002.70 m/z). Two precursor spectra, a wide spectrum (400-1600 m/z at 60,000 resolution) and a narrow spectrum matching the range (i.e., 390-510 m/z, 490-610 m/z, 590-710 m/z, 690-810 m/z, 790-910 m/z, and 890-1010 m/z) using an AGC target of 3e6 and a maximum injection time of 100 ms were interspersed every 18 MS/MS spectra. For quantitative samples, the Thermo Q-Exactive HF was configured to acquire 25x 24 m/z DIA spectra (24 m/z precursor isolation windows at 30,000 resolution, AGC target 1e6, maximum inject time 55 ms) using an overlapping window pattern from 388.43 to 1012.70 m/z using window placements optimized by Skyline. Precursor spectra (385-1015 m/z at 30,000 resolution, AGC target 3e6, maximum inject time 100 ms) were interspersed every 10 MS/MS spectra.
**Immunofluorescence**

K562 cells were first washed 1x with PBS, resuspended in ice cold buffer-A (15mM Tris pH = 8, 15mM NaCl, 60mM KCl, 1mM EDTA, 0.5mM EGTA) and permeabilized with 0.025% ice cold NP40 for 4 minutes. The cell solution was then spun down at 400 g for 5 minutes, resuspended in ice cold buffer-A and subsequently treated with isotonic, euchromatin and heterochromatin extraction buffers (see Protein Extraction methods) for 5 minutes on ice. After the salt treatment, cells were seeded on Poly-L-Lysine coated cover glasses and immediately fixed with 2% PFA for 20 minutes at room temperature. Fixed cells were washed 3x with PBS in preparation for immunofluorescence labeling. Fixed cells were permeabilized with 0.25% PBS-triton for 10 minutes at room temperature, blocked for 1 hour with 3% BSA, and then incubated for 2 hours at room temperature with the relevant primary antibody (1:500 dilution of either anti-rabbit GATA1 (Cell Signaling, D52H6), anti-mouse BRG1 (Santa Cruz, G7), anti-mouse CRM1 (Santa Cruz, SC74454), and anti-rabbit BRD4 (Bethyl Labs, A301-985A50)), histones (1:500 of either anti-mouse H3K27ac (Active Motif, #39685) or anti-rabbit H3K27ac (Active Motif, #39133)), and 1:500 of anti-goat Lamin B1 (Santa Cruz, SC6216). Subsequently, cells were washed 3x3 minutes with 0.05% PBST, and then incubated for 1 hour with 1:500 dilution of either donkey anti-rabbit Cy3 (#711-166-152, Jackson Labs), donkey anti-mouse AlexaFluor 647 (#715-606-150, Jackson Labs), and donkey anti-goat AlexaFluor594 (A-11058, ThermoFisher). secondary antibodies. Lastly, cells were counterstained with DAPI (100ng/mL) for 10 minutes and washed 3x3 minutes with 0.05% PBST prior to mounting on glass slides using Prolong Gold (Molecular Probes P36930).

**Cut & Run**

Cut & Run was performed as described previously [44] with minor alterations. 100,000 K562 cells were used per condition. Briefly, cells were washed, then mixed with Concanavalin A beads and permeabilized. The primary antibody is then exposed to the cells. The following antibodies were used:

- BRD2: Active Motif 61797
- BRD3: Active Motif 61489
- BRD4: Bethyl BL-149-2H5
- HDAC1: Active Motif 40967
- HDAC2: Active Motif 39533

Cells are then incubated with protein A-MNase, which was provided by the Henikoff Lab. MNase is activated by treatment with Ca\(^{2+}\). Incubation at 37°C allows DNA fragments to diffuse out of the cell, and these fragments are collected by centrifugation underwent library preparation with ThruPLEX DNA-seq kit. Sequencing was performed on the NextSeq 500 platform (Illumina).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Details for specific statistical tests are found in the legends associated with each figure. Methods for quantification and statistical analysis for specific data types are described in each section below.

**Mass Spectrometry Data Analysis**

Thermo RAW files were converted to. mzML format using the ProteoWizard package (version 3.0.7303) where they were peak picked using vendor libraries and deconvoluted using MConvertPrism in “overlap_only” mode (Tutorial at https://skyline.ms/wiki/home/software/Skyline/page.view?name=DemultiplexingOverlappingDIAWindows). Narrow-window DIA experiments were searched with XCorDIA (manuscript in preparation, code available here: https://bitbucket.org/searleb/encyclopedia/wiki/Home) using a Uniprot protein FASTA file for *Homo sapiens* (downloaded 06/10/2017) as both the target and background database. +1H to +4H peptides were detected using default settings (10 ppm precursor and fragment tolerances, trypsin digestion with up to one missed cleavage), assuming fixed cysteine carbamidomethylation. The 6 mzML files corresponding to each gas-phase fraction and each of the 4 fractions were combined to generate a single on-column chromatogram library for each cell type. EncyclopeDIA was configured to search the wide-window DIA experiments against this chromatogram library with default settings (10 ppm precursor, fragment, and library tolerances, considering both B and Y ions, and trypsin digestion) [19]. Technical duplicates were collected for each fraction for all wide-window samples and the average was used for downstream analysis. Both XCorDIA and EncyclopeDIA were configured to use Percolator version 2.10 ([Käll et al., 2007](#)) configured for an FDR of 1%. Unless otherwise specified, default parameters were used for MConvertPrism, XCorDIA, EncyclopeDIA and Percolator.

For peptides quantified by EncyclopeDIA, any ambiguous peptides mapping to more than one parent protein was removed for downstream analysis. Heatmaps in Figures 1 and 3 were generated using peptide-level data. Peptide-level quantification is included in Table S1 (K562) and Table S3 (MOLM-13).

To assign proteins into a specific chromatin environment, we normalized a protein’s abundance in each fraction to the sum of total peptide abundance as quantified by EncyclopeDIA. Then, proteins were clustered using the k-means method (k = 8). Groups that are
identified are stable with higher values of $k$. We performed a one-way ANOVA to test for an unequal distribution of means across sub-chromatin compartments within each cluster. All clusters show significance by this test. We then used the Mann-Whitney test between the sub-compartment with the highest mean signal within the cluster versus the second-highest mean sub-compartment. This showed four clusters that are enriched for a single fraction:

- Cluster 1 = isotonic
- Cluster 2 = heterochromatin
- Cluster 3 = euchromatin
- Cluster 5 = insoluble

When that test was insignificant, we then performed a Mann-Whitney test between the second and third-highest means within the cluster. This was significant for all remaining clusters and allowed these clusters to be assigned as enriched in two nuclear sub-compartments:

- Cluster 4 = euchromatin + heterochromatin
- Cluster 6 = isotonic + heterochromatin
- Cluster 7 = isotonic + insoluble
- Cluster 8 = euchromatin + insoluble

Individual protein data across the clusters can be seen in Figure 2.

For Figure 1B, RNA-seq data was accessed from the ENCODE project (ENCSR109IQO). Expressed proteins are predicted using RNA-seq data with an FPKM > 1.0. To create a list of “expected” nuclear proteins, we created a manually curated list combining information from Uniprot, Jaspar and a recent review on transcription factors (Lambert et al., 2018). Transcription factor families used to generate Figure 1D were adapted from Lambert et al., (2018) and included as Table S4.

Protein-protein interaction analysis

Protein-protein interaction (PPI) information was sourced from the BioGrid database and limited to interactions observed in Homo sapiens, using database version 3.5.169. Proteins observed in CHESS-DIA were partitioned into clusters as defined in Figure 2. Each possible interaction between two proteins within a cluster and between two proteins in different clusters was considered, and the rate at which a PPI is observed in the BioGrid database was calculated for each cluster. Interaction rates for each protein in a cluster was plotted for the clusters representing a single chromatin fraction (Clusters 1, 2, 3 and 5), with the box representing the 25%, median and 75% and the whiskers representing the max and min interaction rate for proteins observed in each cluster. The Mann Whitney U test was used to test for differences in interaction rates for proteins within a given cluster and interaction rates for proteins between clusters.

Gene Ontology enrichment analysis

Gene ontology analysis was performed using the Panther database. Genes in each cluster were queried against all 1,787 GO terms in the Biological Processes category using the Panther Fisher overrepresentation test (version 14.0) with FDR correction. Genes with the highest FDR values for each cluster were plotted in the heatmap, with intensity mapping to the $-\log(FDR)$.

Imaging analysis

2D cell images were acquired using an inverted Nikon Eclipse Ti widefield microscope equipped with a 40x Nikon Plan Apo 0.9 NA air objective and an Andor Zyla 4.2CL10 CMOS camera with a 4.2-megapixel sensor and 6.5 $\mu$m pixel size (18.8mm diagonal FOV). Acquired images were subject to 3 rounds of iterative blind deconvolution using Autoquant software (version X3.3, Media Cybernetics, NY) to minimize the effect of out-of-focus blurring that is inherent to widefield microscopy optics. Deconvolved images were processed using custom morphometric analysis software to yield numerical estimates for nuclear size and normalized protein content in every cell nucleus.

Sequencing analysis

Reads were aligned to the human genome (hg38 build) using BWA (version 0.7.12) (Li and Durbin, 2009) and peaks called with MACS2 (Zhang et al., 2008) using a q-value cutoff of 0.01. To calculate overlaps between factors, the bedtools package was used to merge replicates for each factor (Neph et al., 2012). To identify regions with both HDAC1 and HDAC2 enriched peaks, the –intersect function was used to find overlapping peaks, followed by –element-of to subset the peaks of interest and –merge to create a merged HDAC1/2 set of peaks. The HDAC1/2 peak set was compared to each bromodomain protein using the –element-of function with a minimum overlap of 50% of the HDAC1/2 peak. To calculate statistical significance of the observed overlaps, an empirical method was used. For each factor, peaks were randomized 10,000 times across the accessible genome (as
defined by DNase-seq peaks from ENCODE dataset ENCSR000EOY). The overlaps were calculated after each randomization and plotted as a histogram (Figures S4D–S4F).

**DATA AND CODE AVAILABILITY**

Raw mass spectrometry data will be available in Chorus: 1566. (https://chorusproject.org/pages/dashboard.html#/projects/all/1566/experiments). Processed proteome-wide quantification can be found in Tables S1 and S3. Sequencing data for Cut&Run is available in the Gene Expression Omnibus: GSE140325.