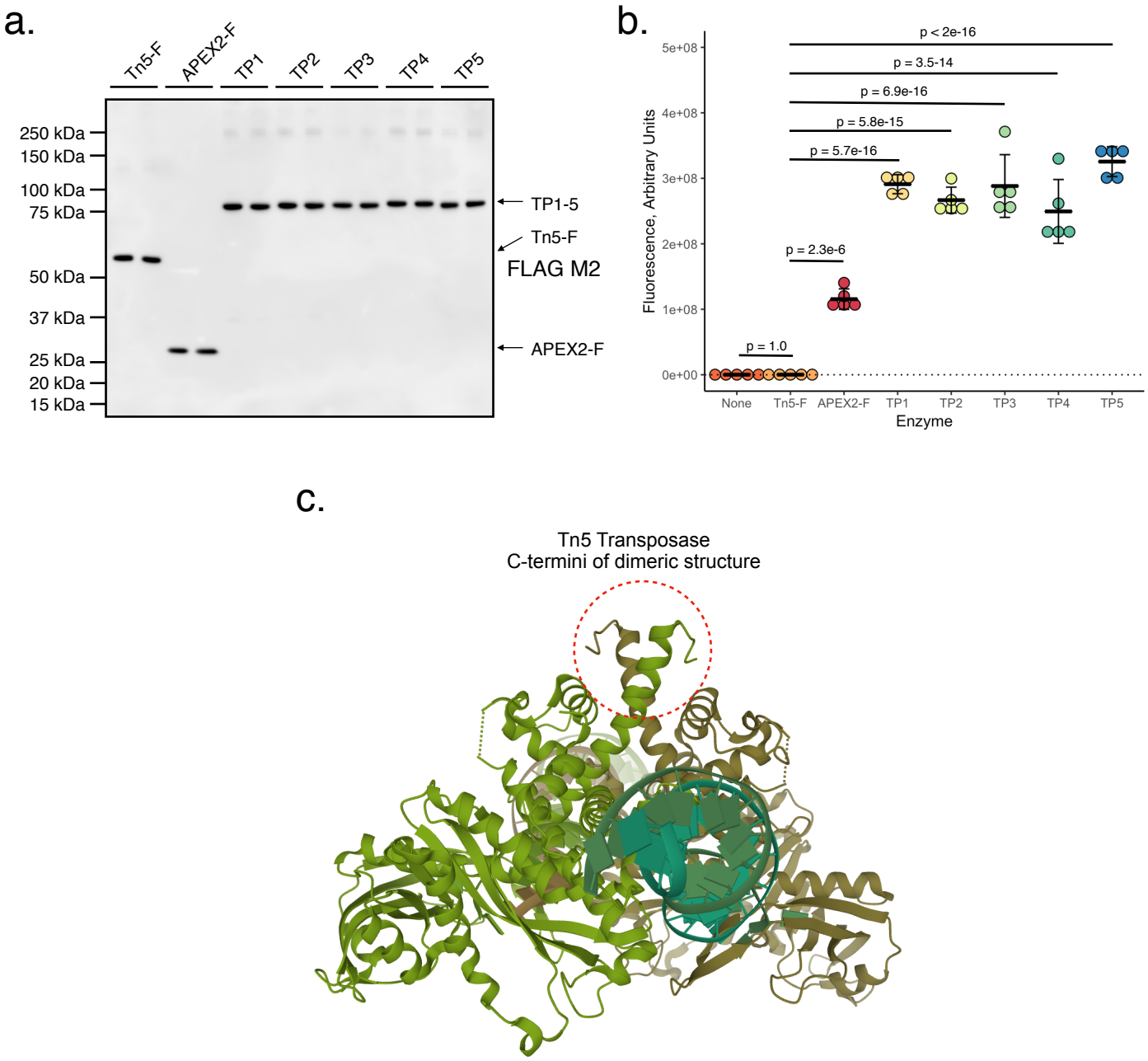
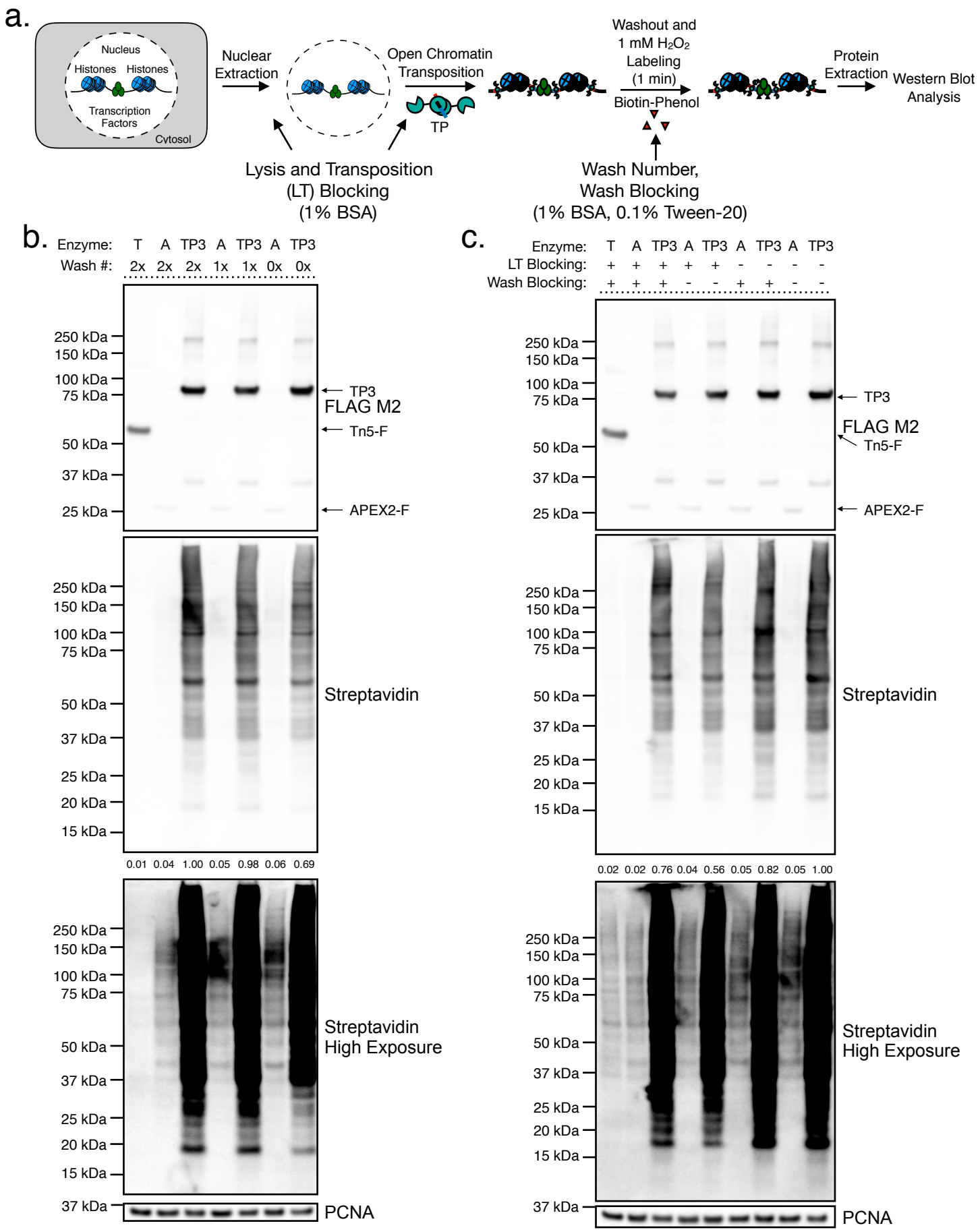
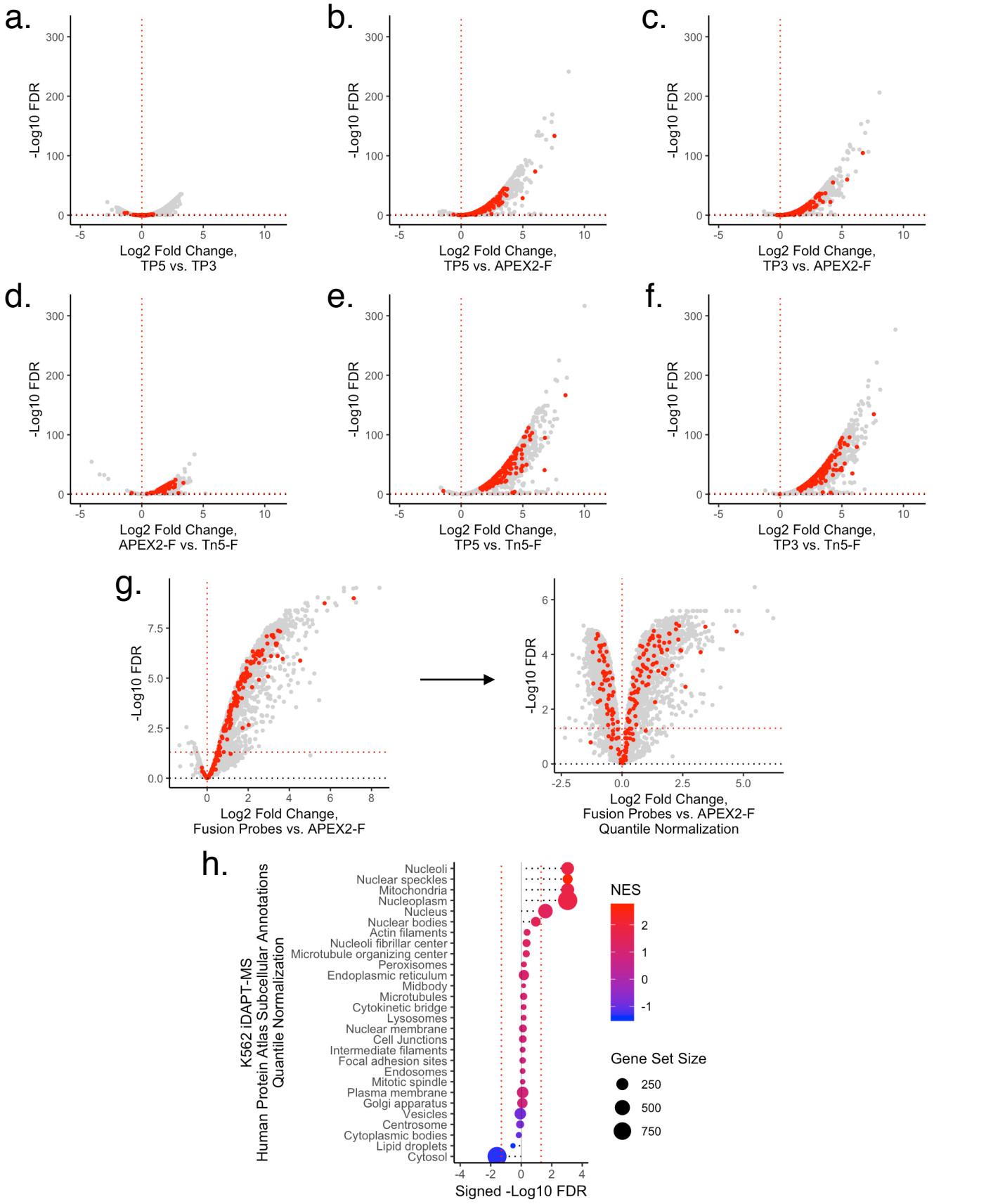
Dual DNA and Protein Tagging of Open Chromatin Unveils Dynamics of Epigenomic Landscapes in Leukemia



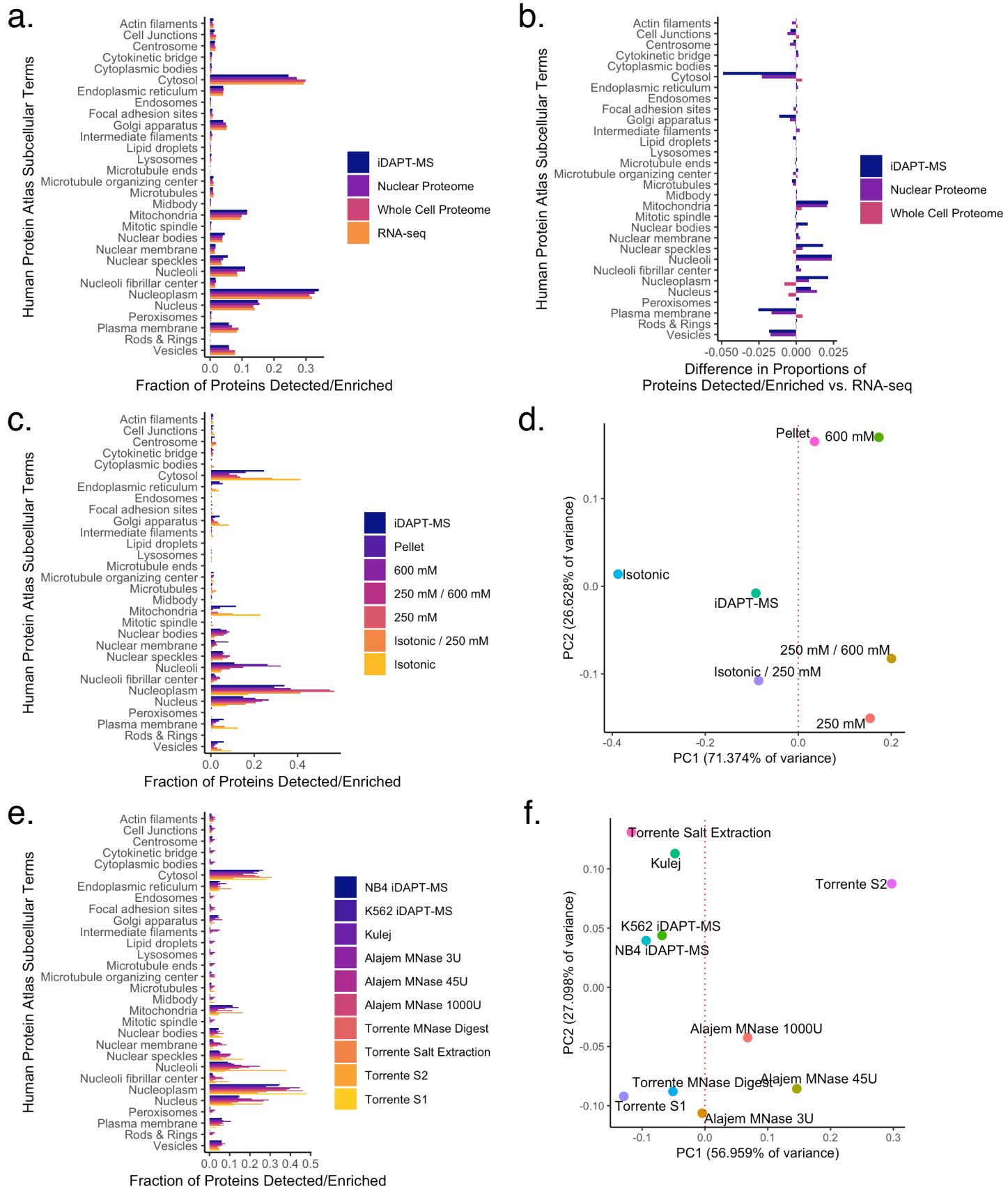
Supplementary Fig. 1. Assessment of peroxidase activity of transposase/peroxidase (TP) fusion probes. (a) Western blot of relative purified enzyme inputs (FLAG M2). The image is representative of two independent experiments. (b) Peroxidase activity assessment of purified recombinant enzymes measured by Amplex UltraRed fluorescence in the presence of 1 mM hydrogen peroxide for one minute (mean ± s.e.m.; *n* = 5 distinct samples for each condition, single protein purification batch per enzyme). Pairwise two-tailed t-tests with pooled variance were performed, using Holm p-value adjustment to control for family-wise error rate. (c) Crystal structure of dimeric Tn5 transposase from ref. [23] (PDB: 1MUH). Visualization was performed using Mol*[1,2].



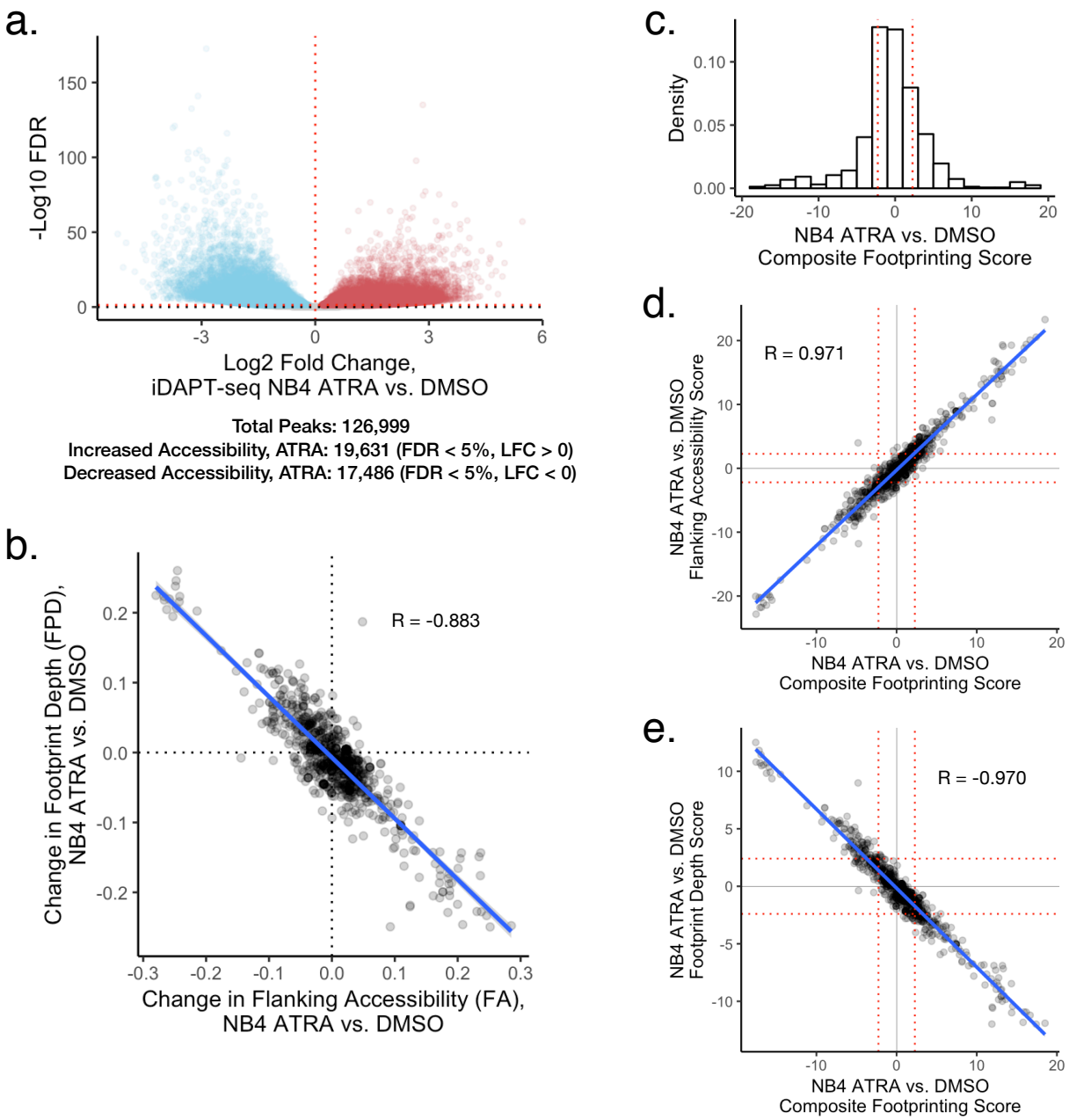
Supplementary Fig. 2. Optimization of iDAPT protein labeling in the HEK293T cell line. (a) Schematic of iDAPT protein labeling, with points of protocol optimization demarcated. (b and c) Western blot of labeled nuclear lysates with varying numbers of post-transposition washes (b) and buffer adjustments (c). Images are representative of two independent experiments. Ratios, relative total streptavidin intensities normalized by corresponding PCNA intensities. T, Tn5-F; A, APEX2-F. LT, lysis and transposition.



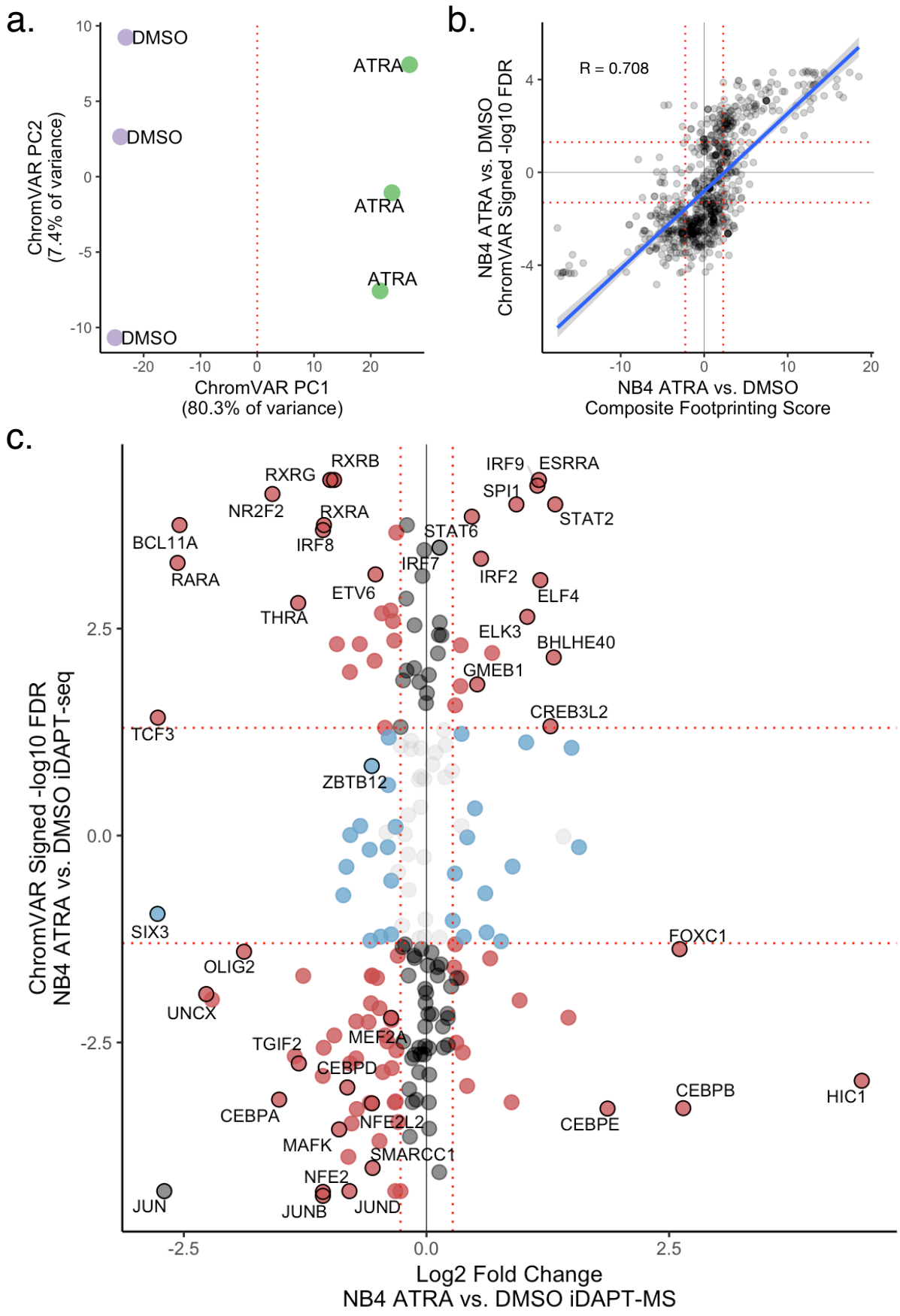
Supplementary Fig. 3. Binary comparison of K562 iDAPT-MS profiles enriched via recombinant fusion and negative control probes. (a-f) Volcano plots of pairwise comparisons of K562 iDAPT-MS profiles from recombinant fusion and negative control probes. Red points, CisBP sequence-specific transcription factors. (g) Volcano plots of K562 iDAPT-MS profiles from fusion probes versus APEX2-F, with profiles subjected to either bait (streptavidin/trypsin) peptide normalization or quantile normalization. Red points, CisBP sequence-specific transcription factors. (h) Subcellular enrichment of quantile-normalized K562 iDAPT-MS profiles as in (g), using annotations from the Human Protein Atlas. NES (normalized enrichment score) and FDR (false discovery rate), gene set enrichment analysis.



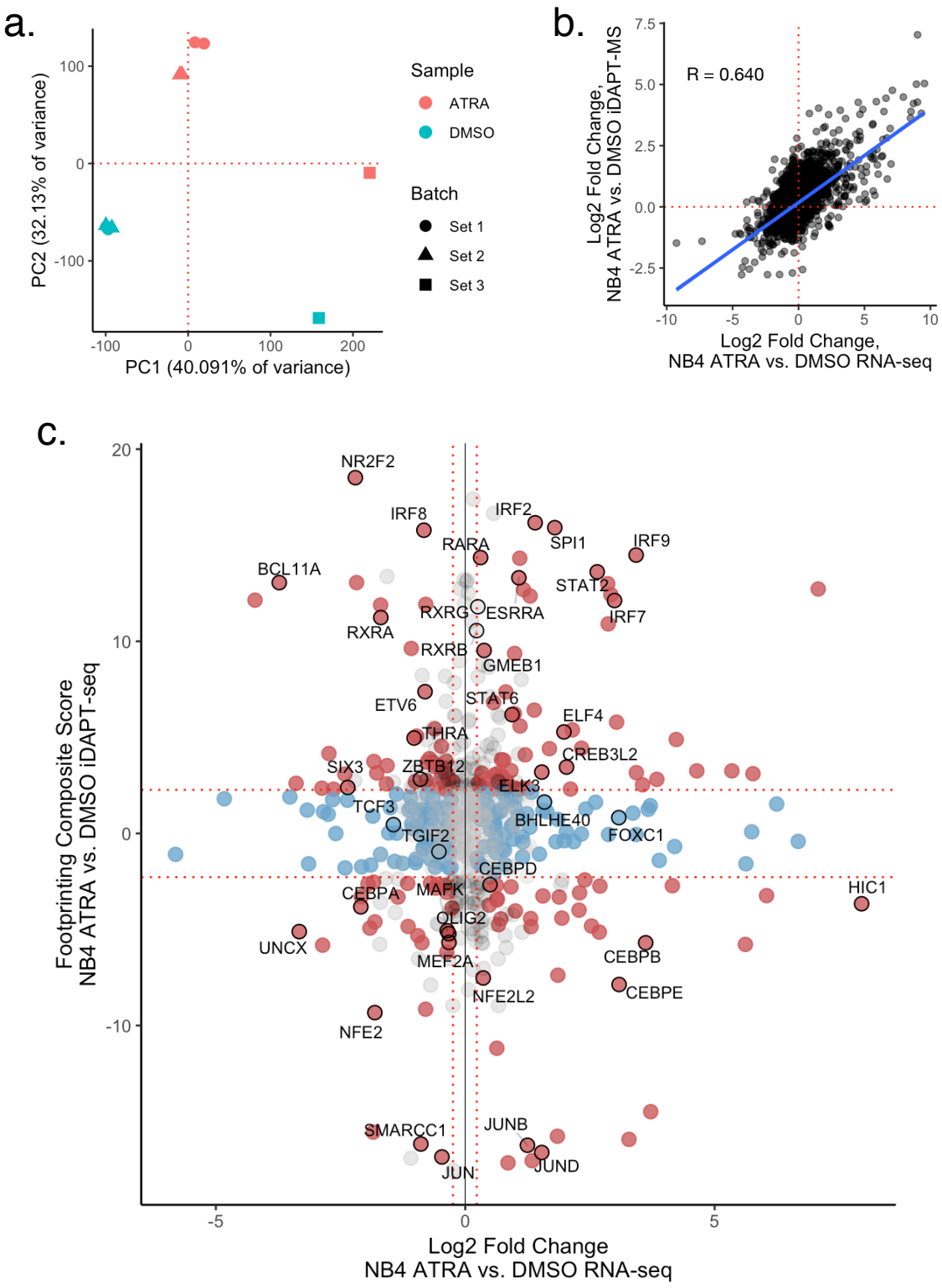
Supplementary Fig. 4. Analysis of published open chromatin proteome enrichment by iDAPT-MS. (a and b) Fraction of proteins detected or enriched (a) and differences in proportions relative to RNA-seq (b) of K562 iDAPT-MS, nuclear proteome, whole cell proteome, or RNA-seq datasets among annotated proteins by the Human Protein Atlas. (c and d) Fraction of proteins detected or enriched (c) and principal component analysis (d) of K562 iDAPT-MS and K562 differential salt extraction proteomic datasets among annotated proteins by the Human Protein Atlas. (e and f) Fraction of proteins detected or enriched (e) and principal component analysis (f) of iDAPT-MS and published differential MNase digestion or salt extraction proteomic datasets among annotated proteins by the Human Protein Atlas.



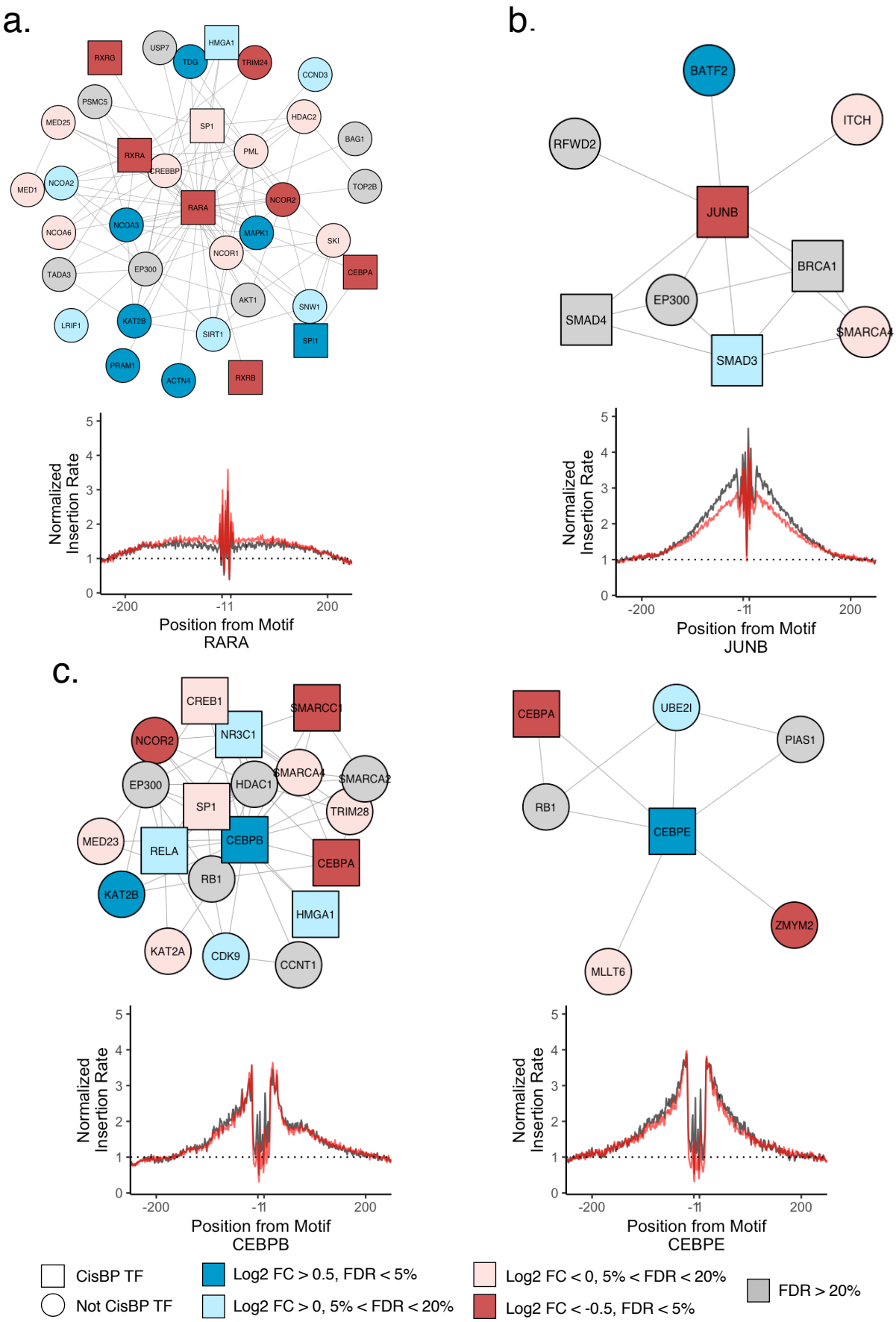
Supplementary Fig. 5. Analysis of NB4 iDAPT-seq profiles upon treatment with ATRA. (a) Volcano plot of NB4 iDAPT-seq profiles upon either ATRA or DMSO treatment as analyzed with DESeq2. Peak statistics are listed below. FDR, false discovery rate; LFC, log2 fold change. (b) Bivariate footprinting analysis of iDAPT-seq from the NB4 cell line treated with ATRA versus DMSO. R, Pearson correlation coefficient. (c) Distribution of composite footprinting scores from NB4 ATRA versus DMSO iDAPT-seq datasets. Thresholds were assigned based on false discovery rate < 5%. (d-e) Scatterplots of flanking accessibility (d) and footprint depth (e) versus composite footprinting score. R, Pearson correlation coefficient.



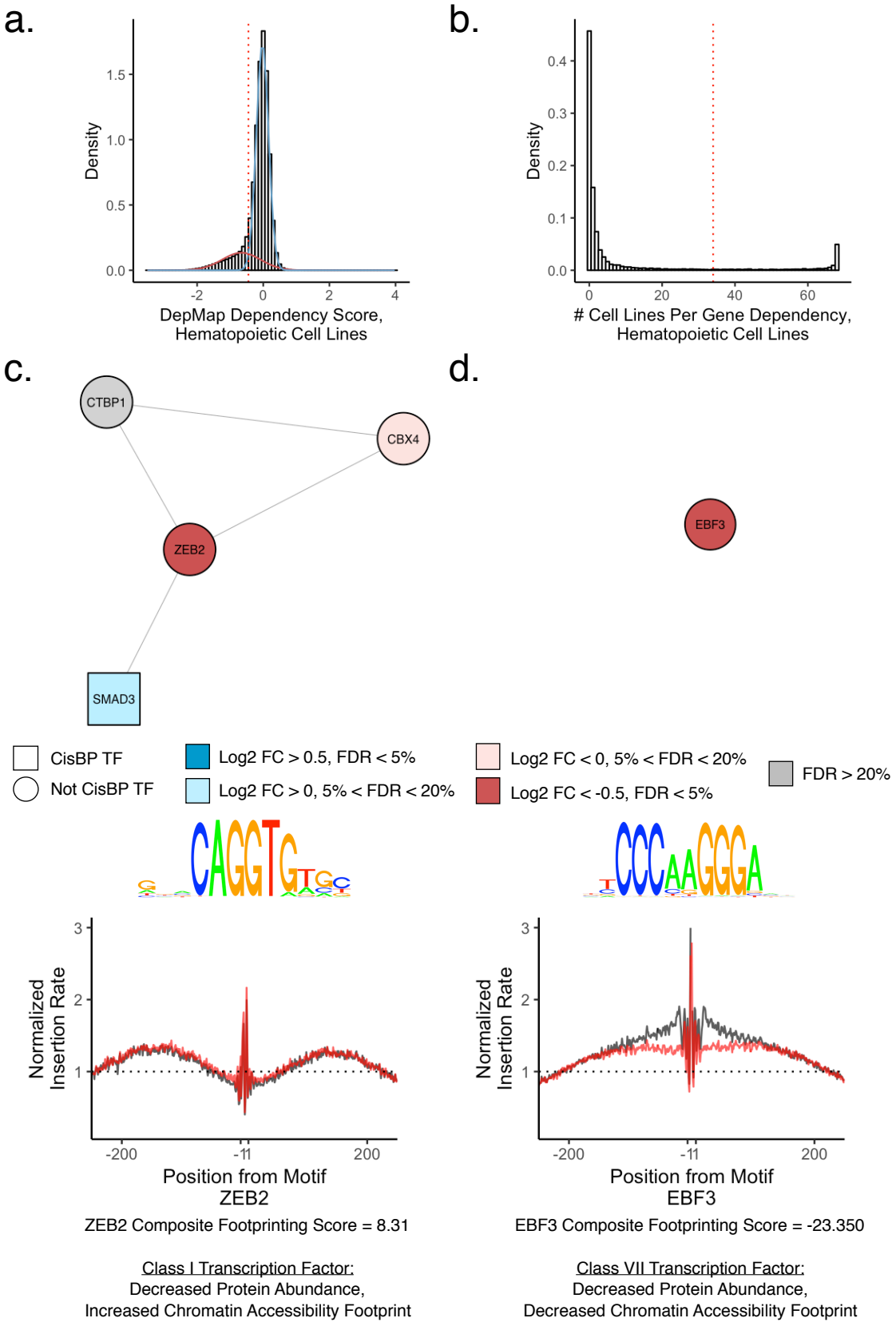
Supplementary Fig. 6. Assessment of iDAPT-seq footprinting versus motif enrichment analyses upon NB4 treatment with ATRA. (a) Principal component analysis of ChromVAR motif enrichment scores from iDAPT-seq profiles of ATRA- and DMSO-treated NB4 cells (b) Scatterplot of signed -log10 false discovery rates (FDR) of ChromVAR motif enrichment versus composite footprinting scores from iDAPT-seq upon ATRA treatment in the NB4 cell line. R, Pearson correlation coefficient. (c) Comparison of CisBP sequence-specific transcription factor enrichment by iDAPT-MS (log2 fold change) versus ChromVAR motif enrichment (signed -log10 FDR) in the NB4 cell line upon treatment with either ATRA or DMSO.



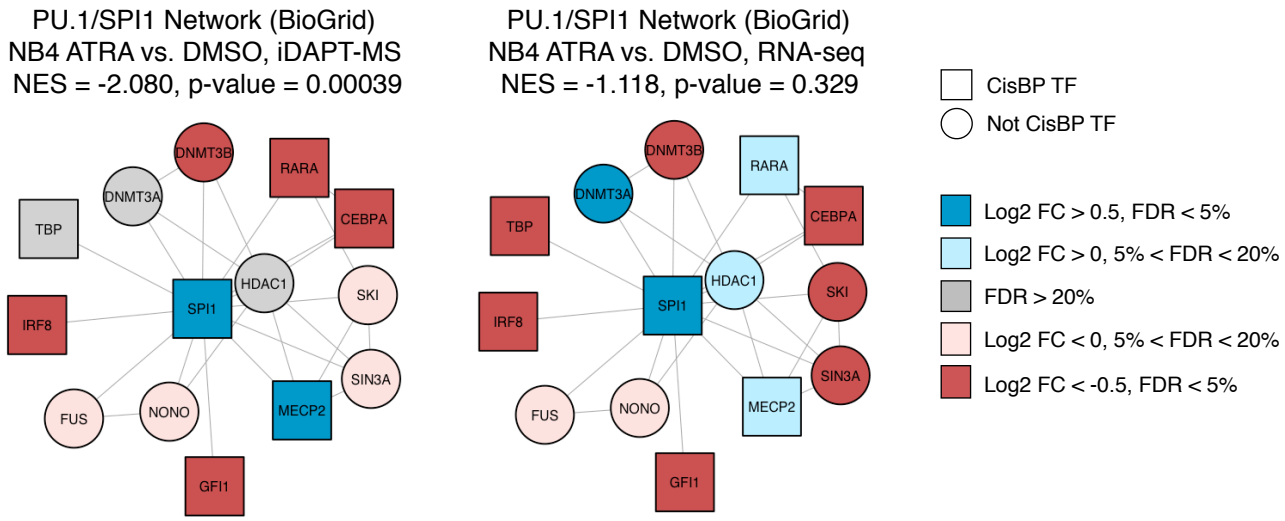
Supplementary Fig. 7. Assessment of iDAPT-MS versus RNA-seq datasets upon NB4 treatment with ATRA. (a) Principal component analysis of publicly available RNA-seq profiles of ATRA- and DMSO-treated NB4 cells (GSM1288651, GSM1288652, GSM1288653, GSM1288654, GSM1288659, GSM1288660, GSM1288661, GSM1288662, GSM2464389, GSM2464392). (b) Scatterplot of log2 fold changes of protein abundances versus transcript abundances from iDAPT-MS and RNA-seq, respectively, upon ATRA treatment in the NB4 cell line. R, Pearson correlation coefficient. (c) Comparison of CisBP sequence-specific transcription factor enrichment by RNA-seq (log2 fold change) versus iDAPT-seq footprinting analysis (composite footprinting score) in the NB4 cell line upon treatment with either ATRA or DMSO.



Supplementary Fig. 8. Integrative analysis of representative transcription factor abundances, activities, and protein complex dynamics. (a-c) Inference of transcription factor complex dynamics (top) and footprinting activities (bottom) of representative class I (a), class VII (b), and class IX (c) transcription factors upon treatment with either ATRA (red line) or DMSO (black line) in the NB4 cell line. Legend, individual protein-level iDAPT-MS enrichment.

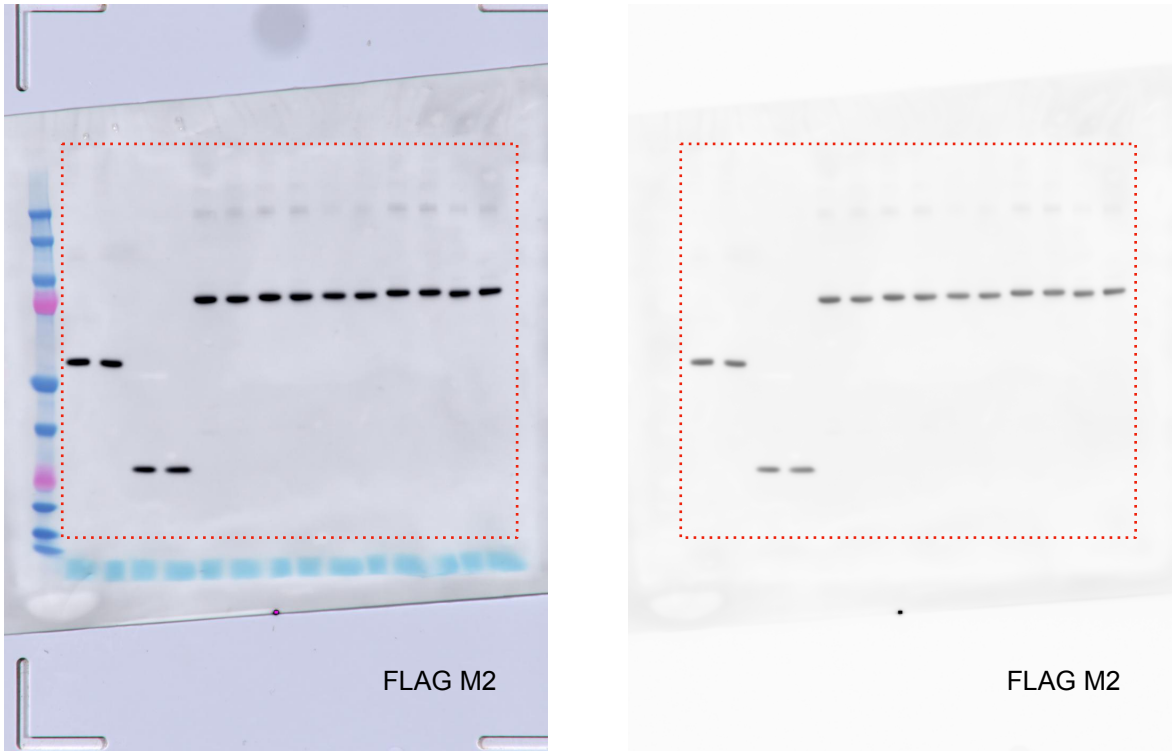


Supplementary Fig. 9. Integration of genetic dependency maps and iDAPT datasets. (a) Distribution of genetic dependency scores across all hematopoietic cancer cell lines assayed in the CRISPR (Avana) 19Q3 dataset. The DepMap score threshold for hematopoietic cell line dependency was determined by a two-state Gaussian mixture model. (b) Distribution of the number of cancer cell lines dependent on a given gene as determined in (a). Genes classified as dependencies in at least half of all hematopoietic cell lines were demarcated as essential genes. (c-d) Inference of transcription factor complex dynamics (top) and footprinting activities (bottom) of ZEB2 (c) and EBF3 (d) upon treatment with either ATRA (red line) or DMSO (black line) in the NB4 cell line. Cognate sequence motifs are displayed above the corresponding footprinting profiles. Legend, individual protein-level iDAPT-MS enrichment.



Supplementary Fig. 10. Analysis of PU.1/SPI1 transcription factor complex dynamics inferred by iDAPT-MS versus RNA-seq. PU.1/SPI1 BioGrid first-order protein interaction network enrichment by iDAPT-MS (left) or RNA-seq (right) in the NB4 cell line upon treatment with ATRA. NES (normalized enrichment score) and p-value, gene set enrichment analysis. Legend, individual protein-level iDAPT-MS or transcript-level RNA-seq enrichment.

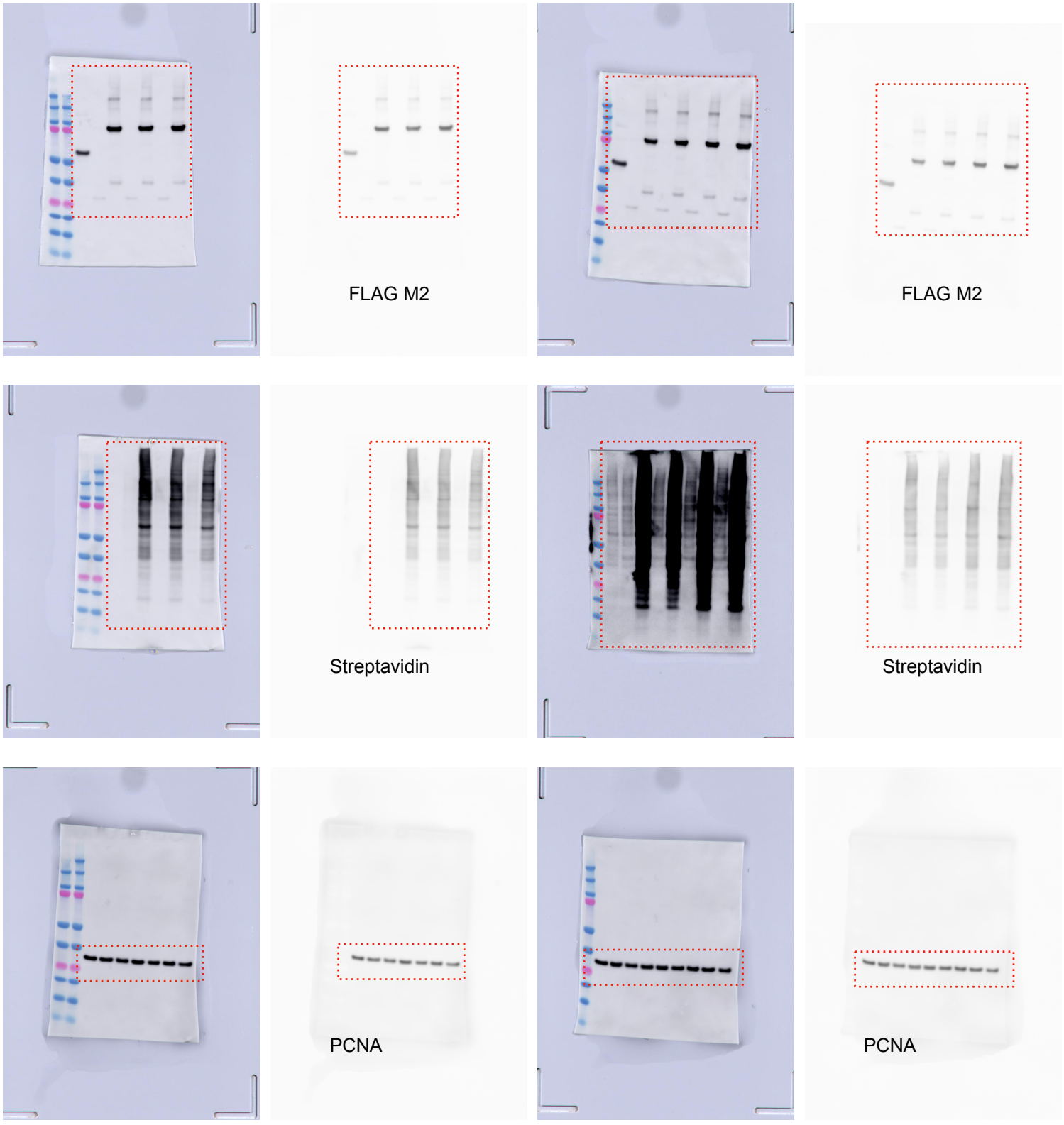
Supplementary Fig. 1a



Supplementary Fig. 11. Unprocessed blot images for Supplementary Fig. 1.

Supplementary Fig. 2b

Supplementary Fig. 2c



Supplementary Fig. 12. Unprocessed blot images for Supplementary Fig. 2.

1. Sehnal, D., Rose, A., Koča, J., Burley, S. & Velankar, S. Mol*: towards a common library and tools for web molecular graphics. *Proc. Work. Mol. Graph. Vis. Anal. Mol. data* 29–33 (2018). doi:10.2312/molva.20181103
2. Berman, H. M. *et al.* The Protein Data Bank. *Nucleic Acids Res.* **28**, 235–242 (2000).

iDAPT Step-By-Step Protocol Rev: 9/10/2020

Jonathan D. Lee

Protein purification

Plasmid Name	Addgene Catalog	Insert
pTXB1-Tn5	#60240	Tn5 transposase
pTXB1-Tn5-F	#160081 (this manuscript)	Tn5-FLAG
pTXB1-APEX2-F	#160083 (this manuscript)	APEX2-FLAG
pTXB1-TP1	#160084 (this manuscript)	Tn5-APEX2, no linker
pTXB1-TP2	#160085 (this manuscript)	Tn5-APEX2, PAPAP linker
pTXB1-TP3	#160086 (this manuscript)	Tn5-APEX2, AEAAAKEAAAKA linker
pTXB1-TP4	#160087 (this manuscript)	Tn5-APEX2, $(GGGGS)_4$ linker
pTXB1-TP5	#160088 (this manuscript)	Tn5-APEX2, GSGAGA linker

Note: protein purification follows previous reports (PMIDs 25079858 and 27749837) with a few modifications.
Note: pTXB1 constructs contain an in-frame C-terminal self-cleaving intein sequence sensitive to thiols and a chitin binding domain

Day 1

1. Transform pTXB1 plasmids into Rosetta2 bacteria (EMD Millipore #71400-3)
2. Plate on LB agar plates with ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL) overnight (37 °C)

Day 2

1. Inoculate bacterial clone in 10 mL LB broth with ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL)
2. Culture at 37 °C overnight with shaking (225 rpm)

Day 3

1. Add overnight culture into 500 mL LB with ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL)
2. Incubate 2 hrs at 37 °C with shaking (225 rpm)
3. Lower temperature to 30 °C and incubate for 1-2 hrs until OD600 is approximately 0.9
4. *Optional: harvest 50 µL culture as "uninduced"*
5. Add 250 µL 0.5 M IPTG (Gold Bio #I2481C5) to liquid culture
6. Incubate 2 hrs 30 °C with shaking
7. *Optional: harvest 50* µL *culture as "induced"*
8. Pellet bacteria (4,000 x g, 5 min) and store at -80 °C
9. *Optional: run an SDS-PAGE gel of "uninduced" and "induced" aliquots to assess protein expression*
    a. *Add 50 µL 4x LDS sample buffer (Thermo #NP0007) to each 50 µL sample aliquot*
    b. *Boil for 30 min at 95 °C*
    c. *Spin for 30 min at 13,000 x g*
    d. *Load 20 µL of supernatant in 15-well SDS-PAGE gel*
    e. *Stain gel with Coomassie Blue (Expedeon #ISB1L) and/or run Western blotting with murine anti-CBD (chitin binding domain) antibody (NEB #E8034S; 1:1,000 dilution)*

Day 4

1. Prepare HEGX buffer: 1 L buffer is good for 5 samples with 5 mL chitin resin
    a. 20 mL 1 M HEPES Buffer, pH 7.5 (final: 20 mM HEPES Buffer, pH 7.5; Boston BioProducts #BBH-75)
    b. 200 mL 5 M NaCl (final: 1 M NaCl; Fisher Scientific #S271-10)
    c. 2 mL 0.5 M EDTA (final: 1 mM EDTA; Thermo #15575020)
    d. 100 mL 100% glycerol (final: 10% glycerol; Fisher Scientific #BP229-1)

   e. 2 mL 100% Triton X-100 (final: 0.2% Triton X-100; Sigma #X100-500 mL)
   f. 20 µL 1 M PMSF (final: 20 µM PMSF; Thermo #36978)
   g. 674 mL ddH2O (prechilled at 4 °C)
2. Resuspend bacterial pellet in 40 mL ice-cold HEGX buffer
3. Sonicate (Fisher Scientific, Sonic Dismembrator 100) at setting 7 for 30 sec on/off for 5 cycles with resting on ice in cold room
4. *Optional: harvest 50 µL culture for analysis as "crude" lysate*
5. Centrifuge lysate at 15,000 x g for 30 min at 4 °C
6. Prepare 10% PEI solution: add 1 g 50% PEI (Sigma #03880-100ML) w/v to 4 mL HEGX buffer, mix to resuspend
7. Add 10 mL 50% chitin resin slurry (NEB #S6651S) to an Econo-Pac column (Bio-Rad #7321010)
8. Wash resin with 50 mL HEGX buffer (10x column volumes)
9. After lysate centrifugation, transfer supernatant to new tube
10. *Optional: harvest 50 µL supernatant for analysis as "supernatant"*
11. Add 1 mL 10% PEI dropwise to supernatant with mixing
12. Centrifuge lysate at 15,000 x g for 15 min at 4 °C
13. *Optional: harvest 50 µL supernatant for analysis as "post-PEI"*
14. Resuspend chitin resin in clarified lysate; transfer to 50 mL falcon tube
15. Incubate at 4 °C for 1 hr with rocking
16. Add lysate/slurry mix to Econo-Pac column
17. *Optional: harvest 50 µL flowthrough for analysis as "flowthrough"*
18. Wash with 100 mL HEGX buffer (20x column volumes)
19. Add 2.5 mL 1 M DTT (final: 50 mM DTT; Thermo #R0861) to 50 mL HEGX buffer
20. *Optional: harvest 50 µL flowthrough for analysis as "DTT wash"*
21. Add 5 mL HEGX + 50 mM DTT to column
22. Close the column bottom and add an additional 5 mL HEGX + 50 mM DTT
23. Cap column top and incubate at 4 °C for two days

Day 6

1. Prepare 500 mL 2x Tn5 dialysis buffer (2xDB)
   a. 50 mL 1 M HEPES Buffer, pH 7.5 (final: 100 mM HEPES Buffer, pH 7.5; Boston BioProducts #BBH-75)
   b. 20 mL 5 M NaCl (final: 0.2 M NaCl; Fisher Scientific #S271-10)
   c. 0.2 mL 0.5 M EDTA (final: 0.2 mM EDTA; Thermo #15575020)
   d. 100 mL 100% glycerol (final: 20% glycerol; Fisher Scientific #BP229-1)
   e. 1 mL 100% Triton X-100 (final: 0.2% Triton X-100; Sigma #X100-500 mL)
   f. 1 mL 1 M DTT (final: 2 mM DTT; Thermo #R0861)
   g. 328 mL ddH2O (prechilled at 4 °C)
2. Elute protein from Econo-Pac column (~5 mL) into 15 mL 10k MWCO Amicon tube (Millipore #UFC901024)
3. Further rinse Econo-Pac column with 5 mL 2xDB and elute into 15 mL Amicon tube
4. *Optional: harvest 50 µL combined eluate for analysis*
5. Spin sample down for 45 min at 4,000 x g 4 °C (~1.5 mL residual volume)
6. Wash PD-10 buffer exchange columns (GE Healthcare #17085101) 5x with 5 mL 2xDB buffer each wash
7. Apply 1.5 mL protein sample retentate to PD-10 columns
8. Add additional 1 mL 2xDB to PD-10 columns
9. Add 3 mL 2xDB buffer and collect subsequent flowthrough
10. Concentrate protein with 15 mL 10k MWCO Amicon tube (~500 µL)
11. Transfer retentate to 2 mL tubes

12. Spin samples at 15,000 x g 10 min 4 °C to remove protein aggregates
13. Transfer supernatant to new 2 mL tubes and keep on ice
14. Determine protein concentrations with Detergent Compatible Bradford (Thermo #23246)
15. Aliquot protein into individual tubes
16. Snap freeze protein suspensions with liquid nitrogen
17. Store aliquots at -80 °C
18. *Optional: run an SDS-PAGE gel of aliquots to assess protein solubility and purity (refer to above for protocol)*

iDAPT Step-By-Step Protocol Rev: 9/10/2020

Jonathan D. Lee

iDAPT protocol

Day 1

1. Transposon (MEDS) preparation
    a. Order oligos (e.g., Thermo Fisher or IDT) and resuspend each in 200 µM ddH2O

Oligo Name	Sequence
Tn5ME-A	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
Tn5ME-B	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'
Tn5MErev	5'-[phos]CTGTCTCTTATACACATCT-3'

    b. Add 1:1 volumes (250 µL each) of Tn5ME-A/Tn5MErev and Tn5ME-B/Tn5MErev (final: 100 µM of annealed dsDNA) in 1.5 mL DNA Lo-Bind tubes (Eppendorf #022431021)
    c. Boil at 95 °C for 10 min on a heat block
    d. Remove heat block with tubes and place on bench to cool down to room temperature
    e. Aliquot and store at -20 °C
2. Thaw enzyme and MEDS stocks on ice
3. Prepare 500 µM hemin chloride stock: add 10 µL 5 mM hemin chloride (Cayman Chemical #16487) in DMSO (Sigma #D2650) to 90 µL ddH2O
4. Incubate enzymes and cofactors (MEDS, hemin chloride) for 1 hr at room temperature
    a. Per sample, combine 2000 pmol enzyme, 2500 pmol MEDS, and 1000 pmol hemin chloride in a 1.5 mL DNA Lo-Bind tube
    b. Normalize enzyme volumes with 2xDB buffer

	MW (kDa)	Enzyme	100 µM MEDS	500 µM hemin chloride
Tn5-F	54.3	2000 pmol	25 µL	2 µL
APEX2-F	27.9	2000 pmol	25 µL	2 µL
TP3	81.6	2000 pmol	25 µL	2 µL
TP5	81.2	2000 pmol	25 µL	2 µL

5. Prepare stock solutions
    a. 1% digitonin (Sigma #D141-100MG) – resuspend as a 2% w/v solution in DMSO; dilute 1:1 with ddH2O for a working 1% stock solution (store at -20 °C)
    b. 10% Tween-20 (Sigma #P2287-500ML) - dilute 1:10 in ddH2O (store at 4 °C)
    c. 10% NP-40 (Sigma #I3021-500ML) - dilute 1:10 in ddH2O (store at 4 °C)
    d. 10% BSA (Sigma #A7906-100G) – resuspend as a 10% w/v solution in ddH2O (store at -20 °C)
    e. 50x protease inhibitor (Sigma #11873580001) – resuspend one tablet in 1 mL ddH2O
    f. 50 mM biotin phenol (ApexBio #A8011) – resuspend 100 mg in 5.5 mL DMSO (store at -20 °C)
    g. 1 M sodium azide (Sigma #S2002-5G) – resuspend in ddH2O (store at -20 °C)
    h. 20 mg/mL Trolox (Sigma #238813-1G) – resuspend in DMSO (store at -80 °C)
    i. 1 M sodium ascorbate (Sigma #A7631-25G) – resuspend in ddH2O (store at -80 °C)
    j. 100 mM H_2O_2 (Sigma #H1009-500ML) – add 4 µL 30% H_2O_2 to 388 µL DPBS
6. Harvest 1e7 cells in 1xDPBS (Thermo #14190144) per sample in 2 mL tubes
7. Spin 500 x g 5 min at 4 °C
8. Remove supernatant

9. Prepare ATAC RSB-BSA (volumes representative for 8x samples)
    a. 100 µL 1 M Tris-HCl, pH 7.5 (final: 10 mM Tris-HCl, pH 7.5; Thermo #15567027)
    b. 20 µL 5 M NaCl (final: 10 mM NaCl; Fisher Scientific #S271-10)
    c. 30 µL 1 M $MgCl_2$ (final: 3 mM $MgCl_2$; Sigma #63020-1L)
    d. 1 mL 10% BSA (final: 1% BSA)
    e. 8.85 mL ddH2O
10. Prepare LB1 buffer (100 µL/sample; volumes representative for 8.5x samples)
    a. 8.5 µL 10% NP-40 (final: 0.1% NP-40)
    b. 8.5 µL 10% Tween-20 (final: 0.1% Tween-20)
    c. 8.5 µL 1% digitonin (final: 0.01% digitonin)
    d. 17 µL 50x protease inhibitor
    e. 850 µL RSB-BSA
11. Add 100 µL LB1 to each tube and triturate 3x
12. Incubate on ice for 3 min
13. Prepare LB2 buffer (1 mL/sample; volumes representative for 8.5x samples)
    a. 8.5 mL RSB-BSA
    b. 85 µL 10% Tween-20 (final: 0.1% Tween-20)
    c. 170 µL 50x protease inhibitor
14. Spin 500 x g 10 min 4 °C
15. Remove supernatant carefully with a pipet tip
16. Prepare tagmentation mix (500 µL/sample; volumes representative for 9x samples)
    a. 900 µL 100% N,N-dimethylformamide (final: 20% DMF; Sigma #D4551-250ML)
    b. 1.485 mL DPBS (final: 33% DBPS)
    c. (1.305 - 9x enzyme volume) mL ddH2O
        - *E.g., if enzyme:MEDS:hemin chloride volume per sample is 50 µL, then add 855 µL ddH2O*
    d. 90 µL 1 M Tris-HCl, pH 7.5 (final: 20 mM Tris-HCl, pH 7.5)
    e. 45 µL 1 M $MgCl_2$ (final: 10 mM $MgCl_2$)
    f. 45 µL 1% digitonin (final: 0.01% digitonin)
    g. 45 µL 10% Tween-20 (final: 0.1% Tween-20)
    h. 90 µL 50x protease inhibitor
    i. 45 µL 50 mM biotin phenol (final: 500 µM biotin phenol)
    j. 450 µL 10% BSA (final: 1% BSA; add last)
17. Combine enzyme:MEDS:hemin chloride solution with tagmentation mix (final volume 500 µL/sample)
18. Resuspend pelleted nuclei in 500 µL tagmentation mix
19. Incubate nuclei at 37 °C with 1,000 rpm shaking on a thermomixer for 30 min
20. *Optional: analyze tagmentation efficiency by library qPCR and next-generation sequencing (iDAPT-seq/ATAC-seq)*
    a. Transfer 5 µL nuclear suspension into new DNA lo-bind tubes
    b. Add 45 µL DPBS and mix well
    c. Purify DNA with Zymo Clean and Concentrator-5 (Zymo #D4014)
    d. Resulting DNA libraries can be assessed as described in PMID 28846090
21. Prepare wash buffer (volumes representative for 8x samples)
    a. 8 mL DPBS
    b. 80 µL 50 mM biotin phenol (final: 500 µM biotin phenol)
    c. 80 µL 10% BSA (final: 0.1% BSA)
    d. 80 µL 10% Tween-20 (final: 0.1% Tween-20)
    e. 160 µL 50x protease inhibitor
22. Prepare resuspension buffer (volumes representative for 8x samples)
    a. 2 mL DPBS

   b. 20 µL 50 mM biotin phenol (final: 500 µM biotin phenol)
   c. 40 µL 50x protease inhibitor
23. Prepare 2x labeling solution (volumes representative for 8x samples)
   a. 2 mL DPBS
   b. 20 µL 50 mM biotin phenol (final: 500 µM biotin phenol)
   c. 40 µL 50x protease inhibitor
   d. 40 µL 100 mM H_2O_2 (final: 2 mM H_2O_2, added right before labeling step)
24. Prepare 2x quenching buffer (volumes representative for 9x samples)
   a. 5.5 mL DBPS
   b. 135 µL 1 M sodium ascorbate (final: 20 mM sodium ascorbate)
   c. 844.5 µL 20 mg/mL Trolox (final: 10 mM Trolox)
   d. 135 µL 1 M sodium azide (final: 20 mM sodium azide)
   e. 135 µL 50x protease inhibitor
25. Spin down tagmented nuclei at 5 min 3,000 x g 4 °C
26. Remove supernatant and resuspend in 500 µL wash buffer
27. Spin down nuclei 5 min 3,000 x g 4 °C
28. Repeat steps 22-23
29. Remove supernatant and resuspend pellet in 250 µL resuspension buffer
30. Add 250 µL 2x labeling solution
31. Incubate for 1 min
32. Add 500 µL 2x quenching buffer
33. Spin down nuclei 5 min 3,000 x g 4 °C and aspirate supernatant
34. Resuspend nuclear pellet in 250 µL 2x quenching buffer and 250 µL DBPS
35. Spin down nuclei 5 min 3,000 x g 4 °C and aspirate supernatant
36. Resuspend pellet in 500 µL RIPA (Boston BioProducts #BP-115) with 1x protease inhibitor
37. Freeze nuclear suspensions at -80 °C for storage

Day 2

38. Thaw nuclear suspensions on ice
39. Sonicate (Fisher Scientific, Sonic Dismembrator 100) at setting 3, four pulses for 15 sec each on ice
40. Add 1 µL benzonase (Millipore #70746-3) to each suspension
41. Incubate nuclear suspensions for 30 min on ice
42. Spin 15,000 x g 20 min 4 °C
43. Transfer supernatant to new 1.5 mL tubes
44. Measure concentrations with Detergent Compatible Bradford (Thermo #23246), using BSA as standards
45. *Optional: assess labeling by Western Blot*
   a. *Load 5-10 µg/lane in 1x LDS sample buffer (Thermo #NP0007) and 1x Sample reducing agent (Thermo #NP0009) for SDS-PAGE*
   b. *Assess protein loading by Ponceau S staining (Sigma #P7170-1L) of post-transfer membrane and anti-PCNA (Santa Cruz #sc-56; clone PC10; 1:1,000) staining*
   c. *Assess biotin labeling with streptavidin-HRP (Cell Signaling Technology #3999S; 1:1,000)*
46. For each sample, wash 60 µL Pierce streptavidin magnetic bead slurry (Thermo #88816) per sample twice with 1 mL RIPA buffer
47. Add 150-250 µg lysate in 500 µL RIPA buffer supplemented with 5 mM DTT (2.5 µL 1 M DTT)
48. Incubate bead/lysate suspension overnight at 4 °C with end-to-end rotation
49. Magnetize beads and discard flowthrough
50. Resuspend beads with 1 mL RIPA
51. Transfer bead suspension to new 1.5 mL tube
52. Magnetize beads and discard flowthrough

53. Resuspend beads with 1 mL RIPA and rotate beads for 5 min
54. Magnetize beads and discard flowthrough

NOTE: the following steps are representative of on-bead peptide digestion and downstream SL-TMT/SPS-MS3 analysis. Depending on the mass spectrometry analysis, these steps may need to be modified accordingly.

55. Wash beads 3x with 1 mL 200 mM EPPS pH 8.5 (Sigma #E9502-25G; pH adjusted with NaOH) as in steps 16-17
56. Add 1 µL 1 µg/uL mass spectrometry-grade Lys-C (Fisher Scientific #NC9223464) to each tube
    a. NOTE: resuspend 20 µg Lys-C aliquots with 20 µL ddH2O, store at -20 °C
57. Incubate 37 °C 3 hrs on a thermomixer with 600 rpm mixing
58. Add 1 µL 1 µg/uL mass spectrometry-grade trypsin (Thermo #90305) to each tube
59. Incubate at 37 °C overnight on a thermomixer with 600 rpm mixing

Day 3

60. The next day, spin tubes, magnetize beads, and collect eluate (~100 µL). Samples are ready for SL-TMT labeling and SPS-MS3 analysis as described in PMID 29734811.