# Combinatorial profiling of chromatin-binding modules reveals multi-site discrimination

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## Supplementary Methods

### Library Construction

The combinatorial histone H3 peptide library was synthesized on TentaGel $NH_2$ resin (90 $\mu$m, 0.26 mmol/g loading, 2.86 x $10^6$ beads/g) using the split-pool approach [1] for sites of posttranslational modification. Standard amino acid building blocks were purchased from Peptides International. All modified amino acids are commercially available from Novabiochem and peptide reagents were obtained from Sigma Aldrich. A linker composed of methionine, arginine and a polyethylene glycol (PEG) segment was installed with standard Fmoc/tBu chemistry [2]. Sites of modification included positions 2 (R, Rme1, Rme2s, Rme2a, Cit), 3 (T, Tph), 4 (K, Kme1, Kme2, Kme3, Kac), 6 (T, Tph), 8 (R, Rme1, Rme2s, Rme2a, Cit), 9 (K, Kme1, Kme2, Kme3, Kac) and 10 (S, Sph). Varying amounts of capping reagents were added to the coupling reactions at lysine and arginine residues to generate a mass ladder for peptide sequencing (Supplementary Fig. 1) [3]. The capping scheme was as follows: 2.5 % Ac-Ala for K, Kme1, Kme2 and Kme3 and 5 % Boc-Ala for Kac at position 9; 1.5 % Ac-Ala for R, Rme1, Rme2a and 1.5 %

Ac-Ala + 3 % Boc-Ala for Rme2s and Cit at position 8; 2.5 % Ac-Ala for K, Kme1, Kme2 and Kme3 and 5 % Boc-Ala for Kac at position 4; 1.5 % Ac-Ala for R, Rme1, Rme2a and 1.5 % Ac-Ala + 3 % Boc-Ala for Rme2s and citrulline at position 8. A 5 mg portion of the library was deprotected 2 x 2 hours with Reagent K (TFA/EDT/thioanisole/water/phenol: 82.5%, 2.5%, 5%, 5%, 5%) [4] prior to use.

**Immunoblotting and Immunoabsorption Assay**

Lyophilized untreated histones purified by acid extraction from HeLa cells (Millipore 17-306) were reconstituted in 1x NEBuffer 3 (50 mM Tris-HCl, 10 mM MgCl$_2$, 100 mM NaCl, 1 mM DTT, pH 7.9) and phosphatase inhibitors (10 mM NaF and 1 mM NaVO$_4$). Histones (10 µg, in duplicate) were separated by SDS-PAGE (15%) and transferred to PVDF. Membranes were blocked overnight in 5% BSA-TBST (0.05%) at 4°C. Membranes were incubated for 3 hours at room temperature with 1:7,500 anti-histone H3 phospho T6 (Abcam ab14102) in blocking solution +/- 1 µM histone H3 (residues 1-10) phospho T6 peptide (peptide was preincubated with antibody solution for 1 hour prior to application to membranes, and all antibody solutions were pre-equilibrated in batch to insure uniform solutions across membranes). Membranes were washed 5 minutes in TBST (0.05%) three times. Membranes were incubated for 1 hour at room temperature with 1:5,000 HRP-conjugated goat anti-rabbit (Santa Cruz) in blocking solution. Membranes were then washed 5 minutes in TBST (0.05%) four times. Detection was performed using 1:1 SuperSignal West Dura Substrate (Pierce) and membranes were exposed to film.

Following detection with anti-histone H3 phospho T6, the membranes were stripped in 0.8 M Tris, 2% SDS, 0.01 M 2-mercaptoethanol for 30 minutes at 42°C, followed by extensive washing in TBST (0.05%). Membranes were blocked overnight at 4°C in 5% BSA-TBST (0.05%). Membranes were incubated for 1 hour with 1:10,000 anti-histone H3 (Abcam ab46765) in blocking solution, followed by three five-minute washes with TBST (0.05%). Membranes were incubated with 1:600 HRP-conjugated goat anti-rabbit (Pierce) in blocking solution, followed by three five-minute washes. Detection was performed using 1:1 SuperSignal West Dura Substrate (Pierce) and membranes were exposed to film. All antibody solutions were pre-equilibrated in batch to insure uniformity.

**Mass Spectrometry for H3T6ph identification**

Acid extracted histones from HeLa cells were separated by reverse-phase HPLC using a C18 column (2.1 mm i.d. ×250 mm, Vydac, Hesperia, CA) on an Beckman Coulter System Gold HPLC (Fullerton, CA). The gradient consisted of 30-60% B in 100min, followed by 60-100%B in 20min (A = 5% MeCN in 0.2% TFA, B = 90% acetonitrile in 0.188% TFA). Fractions corresponding to histone H3 were pooled and dried by speed vacuum. The H3 sample was then diluted in deionized water. An aliquot corresponding to approximately 10 μg was then taken and treated with propionic anhydride reagent as previously described[5]. The sample was then digested by trypsin at a protein:enzyme ratio of 10:1 for 6 hours at 37°C, with the reaction being quenched by addition of glacial acetic acid to drop the pH to ~3. The H3 sample was then subjected to methyl esterification and immobilized metal affinity chromatography (IMAC) as

previously described[6]. H3 peptides were desalted using STAGE tips as previously reported[7] and loaded onto a C18 packed fused silica microcapillary column (75um) constructed with an integrated ESI tip through an Eksigent AS-2 autosampler (Eksigent Technologies Inc., Dublin, CA) for nanoLC-MS/MS on an Orbitrap mass spectrometer (ThermoFisher Scientific, San Jose, CA). A full mass spectrum at 30,000 resolution was acquired in the Orbitrap prior to obtaining 10 data-dependent MS/MS spectra in the ion trap. All spectra were manually inspected.

**Isothermal Titration Calorimetry (ITC)**

Peptides for ITC analysis were synthesized using solid phase peptide synthesis (SPPS) on the Intavis ResPep SL robot (Intavis, Koeln, Germany). Standard Fmoc/tBu amino acid couplings were used and premodified amino acid building blocks (Tph, Kme3, Rme2s; Novabiochem) were incorporated at positions 2,3,4 and 6 depending on the peptide. Peptide concentration was determined by weight of purified dry product. All H3 peptides were normalized to each other by HPLC analysis. In order to confirm the concentration of several peptide stock solutions amino acid analysis was performed (UC-Davis; Proteomics Core Facility). Thermodynamic analyses were performed at 25 °C using a VP-ITC isothermal titration calorimeter (MicroCal LLC, Northampton, MA, USA). Histone peptides (0.5- 2.8 mM) were injected into solutions containing GST-fusion protein (20-350 μM) . In a typical experiment 37 injections of peptide were delivered at 200s intervals to a 1.4 mL solution of protein. Different volumes were injected during the course of the experiment ($1 \times 1 \, \mu L$ , $6 \times 4 \, \mu L$, and $33 \times 8 \, \mu L$). For the titration of H3K4me3T3ph into the JMJD2A-DTD injections were: $1 \times 1 \, \mu L$ , $6 \times 4 \, \mu L$,

$15 \times 8\,\mu\text{L}$, and $9 \times 16\,\mu\text{L}$. The initial point was routinely discarded, a common practice in ITC. Curve fitting was done by using Origin 7.0 with a standard one-site model provided by MicroCal Lavenberg-Marquardt nonlinear regression. A peptide into buffer control corrected for dilution heats of injection in each experiment. All experiments were performed in 25mM Tris buffer (pH 7.2) containing 50mM NaCl and 2mM BME.

**Supplementary Results**

Supplementary Dataset 1

**SI Table 1. Sequences of 100 randomly chosen beads from the H3 library**

**SI Table 2. Screening results for BHC80-PHD finger**

**SI Table 3. Screening results for AIRE-PHD finger 1**

**SI Table 4. Screening results for ING2-PHD finger**

**SI Table 5. Screening results for RAG2-PHD finger**

**SI Table 6. Screening results for for JMJD2A DTD**

**Supplementary Table 1**

Random Beads

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | Rme2s | Tph | Kme1 | Q | T | A | Rme2a | Kme2 | Sph |
| 2 | A | Cit | Tph | K | Q | Tph | A | Rme2a | Kme3 | S |
| 3 | A | R | Tph | Kme1 | Q | T | A | Cit | Kme3 | Sph |
| 4 | A | R | Tph | Kme1 | Q | Tph | A | R | Kme1 | S |
| 5 | A | R | Tph | Kme3 | Q | T | A | Rme1 | Kme1 | S |
| 6 | A | Rme1 | Tph | Kme3 | Q | T | A | Rme2a | Kme3 | Sph |
| 7 | A | Rme2a | Tph | Kac | Q | T | A | Rme2a | Kme3 | S |
| 8 | A | R | Tph | Kme2 | Q | T | A | Cit | K | Sph |
| 9 | A | Rme2a | Tph | Kme1 | Q | T | A | Rme2a | K | S |
| 10 | A | Rme2a | T | Kme1 | Q | T | A | R | Kme2 | S |
| 11 | A | Rme2s | T | K | Q | Tph | A | Rme2s | Kme1 | Sph |
| 12 | A | Rme2s | T | Kme2 | Q | Tph | A | Rme2s | Kme1 | Sph |
| 13 | A | Cit | T | Kac | Q | T | A | Rme2s | Kme3 | Sph |
| 14 | A | Rme2s | T | Kac | Q | Tph | A | Rme2a | K | Sph |
| 15 | A | Rme2s | T | Kme3 | Q | T | A | Rme2s | Kme2 | Sph |
| 16 | A | Cit | Tph | Kme1 | Q | T | A | Rme2s | Kme2 | Sph |
| 17 | A | Cit | T | Kac | Q | Tph | A | Cit | Kme1 | S |
| 18 | A | R | T | Kme1 | Q | T | A | Cit | Kac | Sph |
| 19 | A | Rme2s | T | K | Q | T | A | R | K | S |
| 20 | A | Rme1 | T | Kme2 | Q | T | A | Rme2s | K | Sph |
| 21 | A | Cit | Tph | Kme3 | Q | T | A | Rme1 | Kme2 | Sph |
| 22 | A | R | T | Kme3 | Q | Tph | A | Rme2a | Kme1 | S |
| 23 | A | Rme2s | T | Kme3 | Q | Tph | A | Rme2s | Kme1 | S |
| 24 | A | Rme2s | Tph | Kme3 | Q | T | A | Rme2a | Kme3 | S |
| 25 | A | Rme2s | T | Kme1 | Q | T | A | Rme1 | Kme2 | Sph |
| 26 | A | Rme1 | Tph | Kme3 | Q | Tph | A | Rme2a | K | S |
| 27 | A | Rme2s | Tph | Kac | Q | Tph | A | Rme2s | Kme2 | Sph |
| 28 | A | Rme2a | Tph | Kme1 | Q | T | A | Rme1 | K | Sph |
| 29 | A | R | Tph | Kme3 | Q | T | A | Rme2a | Kme2 | Sph |
| 30 | A | Rme1 | T | Kac | Q | T | A | Rme1 | Kme2 | S |
| 31 | A | R | Tph | Kme1 | Q | Tph | A | Rme1 | Kme2 | Sph |
| 32 | A | Cit | T | Kac | Q | T | A | Rme2s | K | Sph |
| 33 | A | Rme1 | T | Kme1 | Q | Tph | A | Rme2s | K | S |
| 34 | A | Cit | T | K | Q | T | A | Cit | Kme1 | Sph |
| 35 | A | R | Tph | Kme3 | Q | Tph | A | Cit | K | S |
| 36 | A | Rme2a | Tph | Kme1 | Q | T | A | Rme2a | Kme3 | Sph |
| 37 | A | Cit | Tph | Kme2 | Q | T | A | Rme1 | Kme3 | Sph |
| 38 | A | Rme2s | Tph | K | Q | Tph | A | R | K | Sph |
| 39 | A | R | Tph | Kme1 | Q | Tph | A | Rme2a | Kme1 | S |
| 40 | A | Cit | Tph | Kme3 | Q | T | A | Cit | K | S |
| 41 | A | Rme2s | Tph | Kme1 | Q | T | A | R | Kme1 | Sph |
| 42 | A | Rme1 | Tph | Kac | Q | T | A | Cit | Kme3 | Sph |
| 43 | A | Rme2a | Tph | Kme1 | Q | Tph | A | Rme2a | K | Sph |
| 44 | A | Rme2a | T | Kme1 | Q | Tph | A | Cit | Kme1 | S |

| 45 | A | Rme2s | T | Kac | Q | T | A | Rme1 | K | Sph |
|----|---|-------|---|-----|---|---|---|------|---|-----|
| 46 | A | R | T | K | Q | Tph | A | Rme1 | Kme3 | S |
| 47 | A | R | T | Kme2 | Q | Tph | A | R | Kme1 | Sph |
| 48 | A | R | T | Kme1 | Q | T | A | Cit | K | S |
| 49 | A | Rme2s | T | Kme1 | Q | T | A | Cit | Kme3 | S |
| 50 | A | Rme2a | Tph | Kme3 | Q | T | A | R | Kac | S |
| 51 | A | Rme | T | K | Q | Tph | A | Rme | Kme3 | Sph |
| 52 | A | Rme | Tph | Kme3 | Q | Tph | A | R | Kme2 | Sph |
| 53 | A | Cit | Tph | Kme2 | Q | T | A | R | K | Sph |
| 54 | A | Rme | T | Kme2 | Q | T | A | Rme2a | Kme2 | S |
| 55 | A | R | T | Kme2 | Q | Tph | A | R | Kac | Sph |
| 56 | A | Rme | T | Kme3 | Q | Tph | A | Rme | Kme2 | S |
| 57 | A | Rme2s | Tph | Kme3 | Q | Tph | A | Rme2a | Kac | S |
| 58 | A | Cit | T | Kme2 | Q | Tph | A | Rme2s | Kme2 | Sph |
| 59 | A | R | Tph | Kme3 | Q | T | A | R | Kme3 | S |
| 60 | A | Cit | Tph | Kme | Q | Tph | A | Rme2s | Kme2 | Sph |
| 61 | A | Cit | T | Kme2 | Q | T | A | Rme2s | Kac | S |
| 62 | A | R | T | K | Q | Tph | A | R | Kme2 | S |
| 63 | A | Rme2a | T | K | Q | T | A | Rme | Kac | S |
| 64 | A | Rme2s | Tph | Kac | Q | T | A | Cit | Kme3 | Sph |
| 65 | A | Rme2s | Tph | Kac | Q | Tph | A | Cit | Kme3 | S |
| 66 | A | Rme | T | K | Q | Tph | A | R | Kme | S |
| 67 | A | Rme | T | K | Q | T | A | Rme2a | Kac | Sph |
| 68 | A | Rme | T | K | Q | T | A | R | K | S |
| 69 | A | Rme2a | T | K | Q | T | A | Rme2a | Kme2 | Sph |
| 70 | A | Rme2a | T | K | Q | T | A | Rme | Kme3 | Sph |
| 71 | A | Cit | T | Kme3 | Q | Tph | A | Rme2a | Kac | S |
| 72 | A | R | T | Kme | Q | T | A | Rme | Kme | S |
| 73 | A | Rme2s | Tph | K | Q | Tph | A | Rme2a | Kme | S |
| 74 | A | Rme2a | T | K | Q | Tph | A | Cit | Kme | Sph |
| 75 | A | Cit | T | Kac | Q | Tph | A | Rme | Kme2 | Sph |
| 76 | A | Rme2s | T | Kme3 | Q | T | A | R | K | S |
| 77 | A | Cit | Tph | Kme | Q | T | A | Rme2a | Kac | Sph |
| 78 | A | R | T | Kme2 | Q | Tph | A | R | K | S |
| 79 | A | Rme2a | T | K | Q | T | A | Cit | Kme3 | S |
| 80 | A | Cit | T | K | Q | T | A | Rme | Kme | Sph |
| 81 | A | Rme2a | Tph | Kme2 | Q | T | A | Rme2a | Kac | Sph |
| 82 | A | Rme2s | Tph | Kac | Q | T | A | Rme | Kme | S |
| 83 | A | Rme2s | Tph | Kme3 | Q | T | A | R | Kac | Sph |
| 84 | A | R | Tph | Kme3 | Q | Tph | A | Rme2a | Kac | S |
| 85 | A | Rme2s | T | Kme | Q | T | A | Rme2s | Kme3 | Sph |
| 86 | A | Rme2s | T | Kme3 | Q | T | A | Rme | Kme2 | Sph |
| 87 | A | Rme | Tph | Kme | Q | T | A | Cit | K | Sph |
| 88 | A | R | T | K | Q | Tph | A | Rme | Kac | S |
| 89 | A | Rme | T | Kac | Q | Tph | A | Rme2a | Kme2 | Sph |
| 90 | A | R | T | Kac | Q | T | A | Cit | Kac | S |
| 91 | A | Rme2a | Tph | K | Q | Tph | A | R | Kme2 | Sph |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 92 | A | Rme | Tph | Kme2 | Q | Tph | A | Rme2s | K | Sph |
| 93 | A | Rme2s | Tph | Kac | Q | T | A | Rme2s | Kme3 | Sph |
| 94 | A | Rme2a | Tph | Kme | Q | Tph | A | Cit | Kme2 | S |
| 95 | A | Cit | Tph | Kme | Q | Tph | A | Rme | Kme3 | Sph |
| 96 | A | R | T | Kme3 | Q | Tph | A | Rme | Kme2 | Sph |
| 97 | A | Rme2a | Tph | Kme2 | Q | T | A | R | Kme | Sph |
| 98 | A | Rme2a | T | Kme2 | Q | Tph | A | Rme2s | Kme | S |
| 99 | A | Rme2s | Tph | K | Q | T | A | Cit | Kme | S |
| 100 | A | Cit | T | Kme2 | Q | Tph | A | Rme2a | Kme3 | Sph |

**Totals**

| R= 22 | T=52 | K= 21 | | T= 56 | R= 19 | K= 21 | S= 45 |
|---|---|---|---|---|---|---|---|
| Rme1= 16 | Tph=48 | Kme1= 25 | | Tph = 44 | Rme1= 21 | Kme1 = 21 | Sph = 55 |
| Rme2s= 25 | | Kme2= 16 | | | Rme2s= 17 | Kme2 = 23 | |
| Rme2a= 18 | | Kme3= 22 | | | Rme2a = 24 | Kme3 = 21 | |
| Cit=19 | | Kac= 16 | | | Cit= 19 | Kac = 14 | |

## Supplementary Table 2

BHC80 Positive Hits

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | R | T | K | Q | T | A | R | Kme3 | Sph |
| 2 | A | R | T | K | Q | T | A | Rme1 | Kac | S |
| 3 | A | R | T | K | Q | T | A | Rme1 | Kac | Sph |
| 4 | A | R | T | K | Q | T | A | Rme1 | Kac | Sph |
| 5 | A | R | T | K | Q | T | A | Rme1 | Kme2 | S |
| 6 | A | R | T | K | Q | T | A | Rme2s | Kac | Sph |
| 7 | A | R | T | K | Q | T | A | Cit | K | S |
| 8 | A | R | T | K | Q | T | A | Cit | K | S |
| 9 | A | R | T | K | Q | T | A | Cit | K | S |
| 10 | A | R | T | K | Q | T | A | Cit | Kme1 | S |
| 11 | A | R | T | K | Q | T | A | Cit | Kme1 | S |
| 12 | A | R | T | K | Q | T | A | Cit | Kme1 | Sph |
| 13 | A | R | T | K | Q | T | A | Cit | Kme2 | S |
| 14 | A | R | T | K | Q | T | A | Cit | Kme3 | Sph |
| 15 | A | R | T | K | Q | T | A | Cit | Kme3 | Sph |
| 16 | A | Rme1 | T | K | Q | T | A | R | Kac | S |
| 17 | A | Rme1 | T | K | Q | T | A | R | Kac | Sph |
| 18 | A | Rme1 | T | K | Q | T | A | Rme1 | K | S |
| 19 | A | Rme1 | T | K | Q | T | A | Rme1 | Kme2 | S |
| 20 | A | Rme1 | T | K | Q | T | A | Rme1 | Kme2 | S |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 21 | A | Rme1 | T | K | Q | T | A | Rme1 | Kme3 | Sph |
| 22 | A | Rme1 | T | K | Q | T | A | Rme1 | Kme3 | Sph |
| 23 | A | Rme1 | T | K | Q | T | A | Rme2s | Kac | Sph |
| 24 | A | Rme1 | T | K | Q | T | A | Rme2a | Kme1 | Sph |
| 25 | A | Rme1 | T | K | Q | T | A | Rme2a | Kac | Sph |
| 26 | A | Rme1 | T | K | Q | T | A | Cit | K | Sph |
| 27 | A | Rme1 | T | K | Q | T | A | Cit | Kac | Sph |
| 28 | A | Rme2s | T | K | Q | T | A | Rme2a | Kac | Sph |
| 29 | A | Rme2a | T | K | Q | T | A | R | Kme2 | Sph |
| 30 | A | Rme2a | T | K | Q | T | A | Rme1 | Kme1 | Sph |
| 31 | A | Rme2a | T | K | Q | T | A | Rme2a | Kac | Sph |
| 32 | A | Cit | T | K | Q | T | A | Rme1 | K | S |
| 33 | A | Rme2a | T | Kme2 | Q | T | A | Rme2s | K | Sph |

BHC80 Negative Hits

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | Rme1 | T | K | Q | Tph | A | Rme2a | Kme3 | Sph |
| 2 | A | Rme2s | T | K | Q | T | A | Rme1 | Kme1 | Sph |
| 3 | A | Rme2s | Tph | K | Q | T | A | R | Kme2 | S |
| 4 | A | Rme2s | Tph | K | Q | T | A | Cit | Kme3 | Sph |
| 5 | A | Rme2s | Tph | K | Q | Tph | A | Rme2s | Kme2 | Sph |
| 6 | A | Cit | T | K | Q | T | A | R | Kac | Sph |
| 7 | A | Cit | T | K | Q | Tph | A | Rme1 | Kme2 | Sph |
| 8 | A | R | T | Kme1 | Q | T | A | Rme1 | Kac | S |
| 9 | A | Rme2a | T | Kme1 | Q | Tph | A | Rme2a | K | Sph |
| 10 | A | Cit | T | Kme1 | Q | Tph | A | R | Kme3 | S |
| 11 | A | Rme2a | Tph | Kme1 | Q | T | A | R | Kme3 | Sph |
| 12 | A | Rme1 | Tph | Kme1 | Q | Tph | A | Rme2s | Kac | Sph |
| 13 | A | R | Tph | Kme2 | Q | Tph | A | R | Kme2 | Sph |
| 14 | A | R | Tph | Kme2 | Q | Tph | A | Rme1 | Kme1 | Sph |
| 15 | A | Rme2s | T | Kme2 | Q | T | A | R | Kac | Sph |
| 16 | A | Rme2a | Tph | Kme2 | Q | Tph | A | Rme2s | Kac | S |
| 17 | A | Cit | Tph | Kme2 | Q | Tph | A | Rme2a | Kme1 | S |
| 18 | A | R | T | Kme3 | Q | Tph | A | Rme2a | Kme3 | Sph |
| 19 | A | R | Tph | Kme3 | Q | Tph | A | Rme2a | Kme1 | S |
| 20 | A | Rme1 | T | Kme3 | Q | Tph | A | Rme2a | K | Sph |
| 21 | A | Rme1 | Tph | Kme3 | Q | T | A | Rme2a | Kme1 | S |
| 22 | A | Rme2s | Tph | Kme3 | Q | Tph | A | R | K | Sph |
| 23 | A | Rme2s | Tph | Kme3 | Q | Tph | A | R | Kac | S |
| 24 | A | Rme2s | Tph | Kme3 | Q | Tph | A | R | Kac | S |
| 25 | A | Rme1 | Tph | Kme3 | Q | Tph | A | Rme2a | Kac | S |
| 26 | A | R | Tph | Kac | Q | Tph | A | R | Kme2 | Sph |
| 27 | A | Rme2s | Tph | Kac | Q | T | A | Rme2s | Kme2 | S |
| 28 | A | Rme2a | Tph | Kac | Q | Tph | A | Rme1 | Kme1 | S |

**Supplementary Table 3**

AIRE-PHD1 Positive Hits

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | R | T | K | Q | T | A | Rme1 | Kme1 | S |
| 2 | A | R | T | K | Q | T | A | Rme1 | Kme2 | S |
| 3 | A | R | T | K | Q | T | A | Rme2a | Kme3 | S |
| 4 | A | R | T | K | Q | T | A | Cit | Kme2 | Sph |
| 5 | A | R | T | K | Q | T | A | Cit | Kme3 | S |
| 6 | A | Rme1 | T | K | Q | T | A | R | Kme1 | Sph |
| 7 | A | Rme1 | T | K | Q | T | A | R | Kme2 | S |
| 8 | A | Rme1 | T | K | Q | T | A | Rme1 | Kme1 | Sph |
| 9 | A | Rme1 | T | K | Q | T | A | Rme1 | Kac | S |
| 10 | A | Rme1 | T | K | Q | T | A | Rme2s | K | Sph |
| 11 | A | Rme1 | T | K | Q | T | A | Rme2a | Kme3 | S |
| 12 | A | Rme1 | Tph | K | Q | T | A | R | Kme2 | Sph |
| 13 | A | Rme2s | T | K | Q | T | A | Rme1 | Kme3 | S |
| 14 | A | Rme2a | T | K | Q | T | A | R | K | Sph |
| 15 | A | Rme2a | T | K | Q | T | A | Rme1 | Kme1 | S |
| 16 | A | Rme2a | T | K | Q | T | A | Rme1 | Kme1 | S |
| 17 | A | Rme2a | T | K | Q | T | A | Rme1 | Kme3 | S |
| 18 | A | Cit | T | K | Q | T | A | R | Kme2 | S |
| 19 | A | R | T | Kme1 | Q | T | A | R | Kme1 | S |
| 20 | A | R | T | Kme1 | Q | T | A | Rme1 | K | S |
| 21 | A | R | T | Kme1 | Q | T | A | Rme1 | K | Sph |
| 22 | A | R | T | Kme1 | Q | T | A | Rme1 | Kac | S |
| 23 | A | R | T | Kme1 | Q | T | A | Rme2s | K | Sph |
| 24 | A | R | T | Kme1 | Q | T | A | Rme2s | K | Sph |
| 25 | A | R | T | Kme1 | Q | T | A | Rme2s | Kme1 | S |
| 26 | A | R | T | Kme1 | Q | T | A | Rme2a | Kme3 | S |
| 27 | A | R | T | Kme1 | Q | T | A | Rme2a | Kac | S |
| 28 | A | R | T | Kme1 | Q | T | A | Cit | K | S |
| 29 | A | Rme1 | T | Kme1 | Q | T | A | Rme2a | K | S |
| 30 | A | Rme2a | T | Kme1 | Q | T | A | Rme2a | K | S |

AIRE-PHD1 Negative Hits

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | R | Tph | K | Q | T | A | Rme2s | Kme3 | Sph |
| 2 | A | R | Tph | K | Q | T | A | Cit | Kme1 | Sph |
| 3 | A | Rme2s | Tph | K | Q | T | A | R | Kme3 | Sph |
| 4 | A | Rme2s | Tph | K | Q | T | A | Rme2a | Kme1 | Sph |
| 5 | A | Rme2s | Tph | K | Q | Tph | A | Rme2s | Kme2 | Sph |
| 6 | A | Rme2a | Tph | K | Q | T | A | Rme2s | Kme1 | S |
| 7 | A | Cit | Tph | K | Q | Tph | A | Rme1 | Kme1 | Sph |
| 8 | A | R | Tph | Kme1 | Q | Tph | A | Rme2a | Kme3 | Sph |
| 9 | A | Rme1 | Tph | Kme1 | Q | T | A | Cit | K | Sph |
| 10 | A | Rme2s | T | Kme1 | Q | Tph | A | Rme2a | Kme1 | S |
| 11 | A | Rme2s | Tph | Kme1 | Q | T | A | Rme2s | Kme3 | Sph |
| 12 | A | R | Tph | Kme2 | Q | Tph | A | Rme1 | Kac | Sph |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 13 | A | R | Tph | Kme2 | Q | Tph | A | Cit | Kme1 | Sph |
| 14 | A | Rme1 | T | Kme2 | Q | Tph | A | Rme2s | K | S |
| 15 | A | Rme2s | T | Kme2 | Q | Tph | A | Rme2s | Kac | S |
| 16 | A | Cit | Tph | Kme2 | Q | Tph | A | Rme2a | Kme2 | Sph |
| 17 | A | Cit | Tph | Kme2 | Q | Tph | A | Rme2s | Kac | S |
| 18 | A | R | Tph | Kme3 | Q | Tph | A | Rme2a | Kme1 | S |
| 19 | A | Rme1 | Tph | Kme3 | Q | Tph | A | R | Kme2 | Sph |
| 20 | A | Rme1 | Tph | Kme3 | Q | Tph | A | Rme2a | Kac | Sph |
| 21 | A | Cit | T | Kme3 | Q | Tph | A | Rme2a | Kme3 | S |
| 22 | A | R | Tph | Kac | Q | Tph | A | R | Kme1 | Sph |
| 23 | A | R | Tph | Kac | Q | Tph | A | Cit | K | Sph |
| 24 | A | Rme1 | T | Kac | Q | T | A | Rme2a | Kac | S |
| 25 | A | Rme1 | Tph | Kac | Q | T | A | Rme1 | Kme2 | Sph |
| 26 | A | Rme1 | Tph | Kac | Q | Tph | A | R | K | Sph |
| 27 | A | Rme2s | Tph | Kac | Q | T | A | Rme2s | Kme2 | Sph |
| 28 | A | Rme2s | Tph | Kac | Q | T | A | Rme2s | Kme3 | Sph |
| 29 | A | Rme2s | Tph | Kac | Q | Tph | A | R | Kac | S |
| 30 | A | Rme2s | Tph | Kac | Q | Tph | A | Cit | Kme2 | Sph |
| 31 | A | Cit | Tph | Kac | Q | T | A | Rme2a | K | Sph |
| 32 | A | Cit | Tph | Kac | Q | Tph | A | Rme1 | K | Sph |
| 33 | A | Cit | Tph | Kac | Q | Tph | A | Rme2s | Kme3 | Sph |

**Supplementary Table 4**

ING2-PHD Positive Hits

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | R | T | Kme3 | Q | T | A | R | Kme2 | S |
| 2 | A | R | T | Kme3 | Q | T | A | Rme1 | K | Sph |
| 3 | A | R | T | Kme3 | Q | T | A | Rme1 | Kme2 | S |
| 4 | A | R | T | Kme3 | Q | T | A | Rme1 | Kac | Sph |
| 5 | A | R | T | Kme3 | Q | T | A | Rme2s | K | S |
| 6 | A | R | T | Kme3 | Q | T | A | Rme2s | Kme3 | Sph |
| 7 | A | R | T | Kme3 | Q | T | A | Rme2a | Kme2 | S |
| 8 | A | R | T | Kme3 | Q | T | A | Rme2a | Kme2 | Sph |
| 9 | A | R | T | Kme3 | Q | T | A | Rme2a | Kac | S |
| 10 | A | R | T | Kme3 | Q | T | A | Cit | Kme1 | Sph |
| 11 | A | R | T | Kme3 | Q | T | A | Cit | Kme2 | S |
| 12 | A | R | T | Kme3 | Q | Tph | A | R | Kme3 | S |
| 13 | A | R | T | Kme3 | Q | Tph | A | Rme1 | K | S |
| 14 | A | R | T | Kme3 | Q | Tph | A | Rme2s | Kme1 | S |
| 15 | A | R | T | Kme3 | Q | Tph | A | Rme2s | K | Sph |
| 16 | A | R | T | Kme3 | Q | Tph | A | Rme2a | K | S |
| 17 | A | R | T | Kme3 | Q | Tph | A | Rme2a | Kme2 | S |
| 18 | A | R | Tph | Kme3 | Q | T | A | R | Kme2 | Sph |
| 19 | A | R | Tph | Kme3 | Q | T | A | Rme2s | Kme2 | S |
| 20 | A | Rme1 | T | Kme3 | Q | T | A | Rme2s | Kme1 | S |

| 21 | A | Rme1 | T | Kme3 | Q | T | A | Rme2a | Kme1 | Sph |
| 22 | A | Rme1 | T | Kme3 | Q | T | A | Rme2a | Kme2 | S |
| 23 | A | Rme1 | T | Kme3 | Q | T | A | Cit | Kme2 | Sph |
| 24 | A | Rme1 | T | Kme3 | Q | T | A | Cit | Kme2 | Sph |
| 25 | A | Rme1 | T | Kme3 | Q | T | A | Cit | K | Sph |
| 26 | A | Rme2s | T | Kme3 | Q | T | A | R | Kme3 | Sph |
| 27 | A | Rme2s | T | Kme3 | Q | T | A | Rme1 | Kme2 | Sph |
| 28 | A | Rme2a | T | Kme3 | Q | T | A | Rme1 | Kme2 | S |
| 29 | A | Rme2a | T | Kme3 | Q | T | A | Cit | Kme3 | Sph |
| 30 | A | R | T | Kme2 | Q | T | A | Rme1 | Kac | S |
| 31 | A | R | T | Kme2 | Q | Tph | A | Cit | K | S |
| 32 | A | Rme1 | Tph | Kme2 | Q | T | A | R | Kme2 | S |

ING2-PHD Negative Hits

| 1 | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | A | R | Tph | Kme3 | Q | Tph | A | Cit | K | Sph |
| 3 | A | Rme1 | Tph | Kme3 | Q | Tph | A | Cit | K | Sph |
| 4 | A | Cit | Tph | Kme3 | Q | Tph | A | Rme2s | Kme1 | Sph |
| 5 | A | R | Tph | Kme2 | Q | Tph | A | Rme2s | Kac | Sph |
| 6 | A | Rme1 | Tph | Kme2 | Q | Tph | A | Cit | K | S |
| 7 | A | Rme2s | Tph | Kme2 | Q | T | A | Rme2a | Kac | Sph |
| 8 | A | Rme2s | Tph | Kme2 | Q | Tph | A | Rme2s | K | Sph |
| 9 | A | Cit | T | Kme2 | Q | T | A | Rme2a | Kac | Sph |
| 10 | A | Cit | Tph | Kme2 | Q | Tph | A | R | Kme1 | Sph |
| 11 | A | R | Tph | Kme1 | Q | Tph | A | Cit | Kme3 | Sph |
| 12 | A | Rme1 | Tph | Kme1 | Q | T | A | Cit | Kac | Sph |
| 13 | A | Rme1 | Tph | Kme1 | Q | Tph | A | Cit | Kme1 | Sph |
| 14 | A | Rme2s | Tph | Kme1 | Q | Tph | A | R | Kac | Sph |
| 15 | A | Rme2a | Tph | Kme1 | Q | Tph | A | R | Kac | S |
| 16 | A | Cit | Tph | Kme1 | Q | T | A | R | Kme2 | Sph |
| 17 | A | Cit | Tph | Kme1 | Q | Tph | A | R | Kac | S |
| 18 | A | Cit | Tph | Kme1 | Q | Tph | A | Rme2a | Kac | S |
| 19 | A | Rme1 | T | K | Q | Tph | A | Rme1 | Kme2 | Sph |
| 20 | A | R | Tph | Kac | Q | Tph | A | Rme2s | Kac | Sph |
| 21 | A | Rme1 | T | Kac | Q | Tph | A | Cit | Kme2 | Sph |
| 22 | A | Rme1 | Tph | Kac | Q | Tph | A | Cit | Kme2 | S |
| 23 | A | Rme2s | Tph | Kac | Q | Tph | A | Rme2s | Kme2 | Sph |
| 24 | A | Rme2s | Tph | Kac | Q | Tph | A | Cit | Kme3 | Sph |
| 25 | A | Cit | T | Kac | Q | Tph | A | Rme1 | Kme1 | S |
| 26 | A | Cit | T | Kac | Q | Tph | A | Rme1 | Kme2 | Sph |
| 27 | A | Cit | T | Kac | Q | Tph | A | Cit | Kac | Sph |
| 28 | A | Cit | Tph | Kac | Q | T | A | Rme2a | Kac | Sph |
| 29 | A | Cit | Tph | Kac | Q | Tph | A | Rme1 | Kme1 | Sph |
| 30 | A | Cit | Tph | Kac | Q | Tph | A | Rme2s | Kac | S |
| 31 | A | Cit | Tph | Kac | Q | Tph | A | Rme2s | Kac | Sph |

**Supplementary Table 5**

RAG2-PHD Positive Hits

|    | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|----|---|---|---|---|---|---|---|---|---|-----|
| 1  | A | R | T | Kme3 | Q | T | A | Rme1 | Kme3 | Sph |
| 2  | A | R | T | Kme3 | Q | T | A | Cit | Kme1 | S |
| 3  | A | R | Tph | Kme3 | Q | Tph | A | Rme2a | Kme3 | Sph |
| 4  | A | Rme1 | T | Kme3 | Q | T | A | R | K | S |
| 5  | A | Rme1 | T | Kme3 | Q | T | A | R | Kme1 | S |
| 6  | A | Rme1 | T | Kme3 | Q | T | A | R | Kme1 | S |
| 7  | A | Rme1 | T | Kme3 | Q | T | A | R | Kme2 | S |
| 8  | A | Rme1 | T | Kme3 | Q | T | A | R | Kac | S |
| 9  | A | Rme1 | T | Kme3 | Q | T | A | Rme1 | K | S |
| 10 | A | Rme1 | T | Kme3 | Q | T | A | Rme1 | K | S |
| 11 | A | Rme1 | T | Kme3 | Q | T | A | Rme1 | Kme1 | S |
| 12 | A | Rme1 | T | Kme3 | Q | T | A | Rme1 | Kme1 | Sph |
| 13 | A | Rme1 | T | Kme3 | Q | T | A | Rme2s | Kme1 | S |
| 14 | A | Rme1 | T | Kme3 | Q | T | A | Cit | K | S |
| 15 | A | Rme2s | T | Kme3 | Q | T | A | R | Kme1 | S |
| 16 | A | Rme2s | T | Kme3 | Q | T | A | R | Kme1 | Sph |
| 17 | A | Rme2s | T | Kme3 | Q | T | A | Rme2a | Kme2 | S |
| 18 | A | Rme2a | T | Kme3 | Q | T | A | R | K | S |
| 19 | A | Rme2a | T | Kme3 | Q | T | A | R | Kme1 | S |
| 20 | A | Rme2a | T | Kme3 | Q | T | A | R | Kme2 | S |
| 21 | A | Rme2a | T | Kme3 | Q | T | A | Rme1 | Kme1 | S |
| 22 | A | Rme2a | T | Kme3 | Q | T | A | Rme1 | Kme2 | S |
| 23 | A | Rme2a | T | Kme3 | Q | T | A | Rme2s | K | S |
| 24 | A | Rme2a | T | Kme3 | Q | T | A | Rme2s | K | S |
| 25 | A | Rme2a | T | Kme3 | Q | T | A | Rme2a | K | S |
| 26 | A | Rme2a | T | Kme3 | Q | T | A | Rme2a | Kac | S |
| 27 | A | Rme2a | T | Kme3 | Q | T | A | Cit | K | S |
| 28 | A | Cit | T | Kme3 | Q | T | A | R | K | Sph |
| 29 | A | Cit | T | Kme3 | Q | T | A | R | Kme2 | Sph |
| 30 | A | Cit | T | Kme3 | Q | T | A | Rme1 | Kac | S |
| 31 | A | Cit | T | Kme3 | Q | T | A | Rme2s | Kme1 | S |
| 32 | A | Rme1 | T | Kme2 | Q | T | A | Rme1 | Kme2 | S |

RAG2-PHD Negative Hits

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|-----|
| 1 | A | R | T | Kme3 | Q | T | A | Rme2s | Kac | S |
| 2 | A | R | Tph | Kme3 | Q | Tph | A | Rme1 | Kac | Sph |
| 3 | A | Rme2s | Tph | Kme3 | Q | Tph | A | Rme2s | Kac | S |
| 4 | A | Rme2a | Tph | Kme3 | Q | T | A | Rme2s | K | Sph |
| 5 | A | Rme2a | Tph | Kme3 | Q | T | A | Cit | Kme3 | S |
| 6 | A | Cit | T | Kme3 | Q | Tph | A | Rme2a | Kac | S |
| 7 | A | R | Tph | Kme2 | Q | T | A | Rme1 | Kme1 | Sph |
| 8 | A | R | Tph | Kme2 | Q | Tph | A | Rme1 | Kac | Sph |
| 9 | A | R | Tph | Kme2 | Q | Tph | A | Cit | K | Sph |

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | A | Rme2s | Tph | Kme2 | Q | T | A | Cit | K | Sph |
| 11 | A | Rme2a | T | Kme2 | Q | Tph | A | Rme1 | Kac | S |
| 12 | A | Rme2a | Tph | Kme2 | Q | T | A | Rme2a | Kac | Sph |
| 13 | A | Cit | Tph | Kme2 | Q | Tph | A | Rme2a | Kme3 | S |
| 14 | A | R | Tph | Kme1 | Q | Tph | A | R | K | Sph |
| 15 | A | Rme2s | T | Kme1 | Q | T | A | Rme2s | K | Sph |
| 16 | A | Rme2s | Tph | Kme1 | Q | T | A | Rme2a | Kme2 | Sph |
| 17 | A | Rme2a | Tph | Kme1 | Q | Tph | A | Rme2a | Kme3 | S |
| 18 | A | Cit | Tph | Kme1 | Q | T | A | Rme2s | Kme2 | Sph |
| 19 | A | Cit | Tph | Kme1 | Q | T | A | Cit | Kac | S |
| 20 | A | R | T | K | Q | Tph | A | Cit | Kac | S |
| 21 | A | Rme1 | Tph | K | Q | T | A | Rme2s | Kac | Sph |
| 22 | A | Rme1 | Tph | K | Q | Tph | A | Rme1 | K | Sph |
| 23 | A | Rme1 | Tph | K | Q | Tph | A | Rme1 | Kac | S |
| 24 | A | Rme2a | Tph | K | Q | Tph | A | Rme2a | K | S |
| 25 | A | R | T | Kac | Q | T | A | Rme2s | Kac | Sph |
| 26 | A | Rme1 | Tph | Kac | Q | T | A | Rme1 | Kme3 | S |
| 27 | A | Rme2s | Tph | Kac | Q | Tph | A | Cit | Kme3 | Sph |
| 28 | A | Rme2a | Tph | Kac | Q | T | A | Rme2s | Kme1 | S |
| 29 | A | Rme2a | Tph | Kac | Q | Tph | A | Rme2a | Kme1 | S |
| 30 | A | Cit | T | Kac | Q | Tph | A | Rme2a | Kme1 | Sph |
| 31 | A | Cit | Tph | Kac | Q | T | A | R | Kac | S |

**Supplementary Table 6**

JMJD2A-DTD
Positive Hits

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | R | T | Kme3 | Q | T | A | R | K | S |
| 2 | A | R | T | Kme3 | Q | T | A | R | Kac | Sph |
| 3 | A | R | T | Kme3 | Q | Tph | A | R | Kme1 | Sph |
| 4 | A | R | T | Kme3 | Q | Tph | A | R | Kac | Sph |
| 5 | A | R | T | Kme3 | Q | Tph | A | Rme1 | Kme2 | Sph |
| 6 | A | R | T | Kme3 | Q | Tph | A | Rme2s | Kme2 | S |
| 7 | A | R | T | Kme3 | Q | Tph | A | Rme2s | Kme3 | Sph |
| 8 | A | R | T | Kme3 | Q | Tph | A | Rme2s | Kac | S |
| 9 | A | Rme1 | T | Kme3 | Q | T | A | R | Kme2 | Sph |
| 10 | A | Rme1 | T | Kme3 | Q | T | A | Rme1 | K | S |
| 11 | A | Rme1 | T | Kme3 | Q | T | A | Rme2a | K | Sph |
| 12 | A | Rme1 | T | Kme3 | Q | T | A | Rme2a | Kme3 | Sph |
| 13 | A | Rme1 | T | Kme3 | Q | Tph | A | R | Kme1 | Sph |
| 14 | A | Rme1 | T | Kme3 | Q | Tph | A | R | Kme2 | S |
| 15 | A | Rme1 | T | Kme3 | Q | Tph | A | Rme2s | K | Sph |
| 16 | A | Rme1 | T | Kme3 | Q | Tph | A | Rme2s | K | Sph |
| 17 | A | Rme1 | Tph | Kme3 | Q | T | A | R | Kme3 | S |
| 18 | A | Rme2s | T | Kme3 | Q | T | A | Rme1 | Kac | S |
| 19 | A | Rme2s | T | Kme3 | Q | T | A | Rme2a | Kme2 | Sph |

| 20 | A | Rme2s | T | Kme3 | Q | Tph | A | R | Kme1 | S |
|----|---|-------|---|------|---|-----|---|-------|------|---|
| 21 | A | Rme2s | T | Kme3 | Q | Tph | A | R | Kme2 | S |
| 22 | A | Rme2a | T | Kme3 | Q | Tph | A | Rme2a | Kme3 | S |
| 23 | A | Cit | T | Kme3 | Q | T | A | R | K | Sph |
| 24 | A | Cit | T | Kme3 | Q | T | A | Rme1 | Kme2 | Sph |
| 25 | A | Cit | T | Kme3 | Q | T | A | Rme1 | Kme2 | Sph |
| 26 | A | Cit | T | Kme3 | Q | Tph | A | Rme2a | Kme1 | S |
| 27 | A | R | T | Kme2 | Q | Tph | A | R | Kme3 | S |
| 28 | A | Rme2a | T | Kme2 | Q | T | A | R | Kme2 | Sph |

JMJD2A-DTD Negative Hits

|    | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|----|---|-------|-----|------|---|-----|---|-------|------|-----|
| 1 | A | Rme2s | Tph | Kme2 | Q | Tph | A | R | Kme3 | Sph |
| 2 | A | Rme2s | Tph | Kme2 | Q | Tph | A | Cit | Kme2 | S |
| 3 | A | Rme2a | T | Kme2 | Q | T | A | Cit | K | S |
| 4 | A | Rme2a | Tph | Kme2 | Q | T | A | Cit | Kme1 | S |
| 5 | A | R | Tph | Kme1 | Q | Tph | A | R | Kme3 | Sph |
| 6 | A | Rme1 | Tph | Kme1 | Q | Tph | A | R | Kme3 | Sph |
| 7 | A | Rme1 | Tph | Kme1 | Q | Tph | A | Rme2s | K | Sph |
| 8 | A | Rme2s | Tph | Kme1 | Q | T | A | Rme1 | Kme1 | Sph |
| 9 | A | Rme2s | Tph | Kme1 | Q | T | A | Cit | Kme1 | Sph |
| 10 | A | Rme2s | Tph | Kme1 | Q | Tph | A | R | Kac | Sph |
| 11 | A | Rme2a | Tph | Kme1 | Q | T | A | R | Kme1 | S |
| 12 | A | Rme2a | Tph | Kme1 | Q | Tph | A | Rme2s | Kme3 | Sph |
| 13 | A | Rme2a | Tph | Kme1 | Q | Tph | A | Cit | Kac | Sph |
| 14 | A | R | Tph | K | Q | T | A | R | Kac | S |
| 15 | A | R | Tph | K | Q | Tph | A | Rme2s | Kac | Sph |
| 16 | A | Rme1 | T | K | Q | T | A | R | Kac | Sph |
| 17 | A | Rme2s | T | K | Q | Tph | A | Rme2a | Kme2 | Sph |
| 18 | A | Rme2s | Tph | K | Q | Tph | A | R | Kme1 | Sph |
| 19 | A | Rme2s | Tph | K | Q | Tph | A | R | Kme3 | Sph |
| 20 | A | R | Tph | Kac | Q | Tph | A | Rme2a | Kme1 | Sph |
| 21 | A | Rme1 | Tph | Kac | Q | Tph | A | Rme1 | Kme1 | S |
| 22 | A | Rme2s | Tph | Kac | Q | Tph | A | Cit | Kac | Sph |
| 23 | A | Cit | T | Kac | Q | T | A | R | K | Sph |
| 24 | A | Cit | T | Kac | Q | Tph | A | R | Kac | Sph |
| 25 | A | Cit | T | Kac | Q | Tph | A | Rme2s | Kme2 | Sph |
| 26 | A | Cit | Tph | Kac | Q | T | A | Rme1 | Kme3 | Sph |
| 27 | A | Cit | Tph | Kac | Q | T | A | Rme1 | Kac | Sph |

**SI Table 7**

**$K_d$ Values for H3K4unmod Binding Modules determined by ITC**

| Peptide | BHC80- PHD | AIRE- PHD1 |
|---|---|---|
| H3K4unmod. | 19.6 ± 3.6 µM | 1.71 ± 0.2 µM |
| H3T3ph | N.D.B (480 µM)[a] | N.D.B (480 µM)[a] |
| H3T6ph | N.D.B (1 mM)[a] | N.D.B (1 mM)[a] |
| H3R2me2s | 57.2 ± 4.9 µM | |

**$K_d$ Values for H3K4me3 Binding Modules determined by ITC**

| Peptide | ING2- PHD finger | RAG2- PHD finger | JMJD2A-DTD |
|---|---|---|---|
| H3K4me3 | 0.98 ± 0.1 µM | 17.8 ± 2.1 µM | 1.1 ± 0.1 µM |
| H3K4me3T3ph | N.D.B (500 µM)[a] | N.D.B (500 µM)[a] | N.D.B (2.9 mM)[a] |
| H3K4me3T6ph | 19.9 ± 4.9 µM | N.D.B (750 µM)[a] | 1.8 ± 0.2 µM |
| H3K4me3R2me2s | 17.3 ± 2.3 µM | | |

N.D.B = No Detectable Binding
[a] highest concentration of peptide tested

**SI Fig. 1**

a
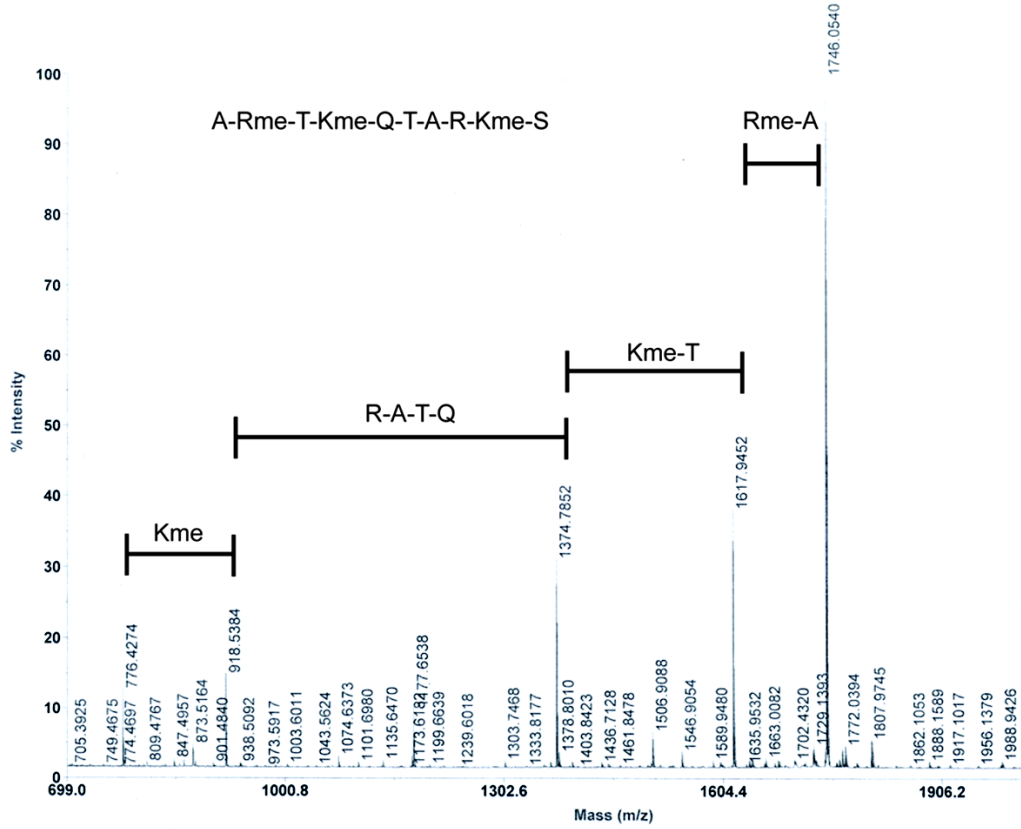


b
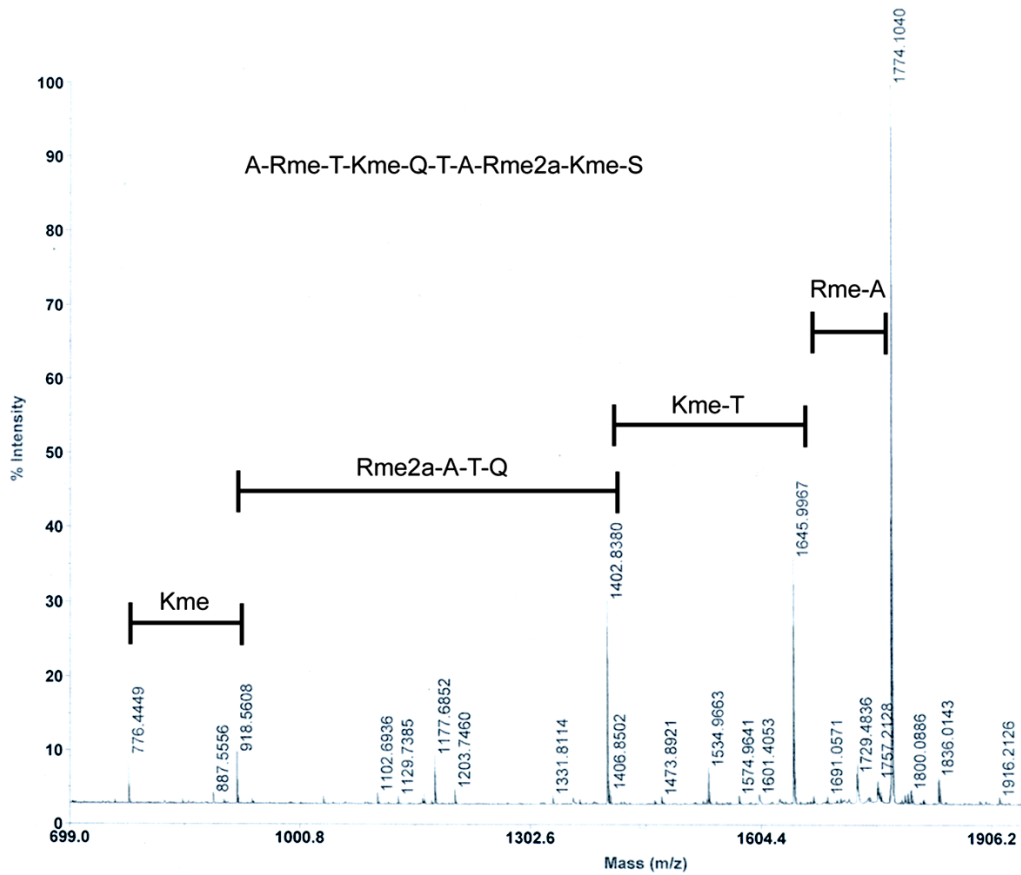
**SI Fig 1. MS mass ladder created by the library capping strategy.** (*a*) The peak at 1746.054 m/z represents the full length H3 tail from this particular bead. The difference amino acid Ala (A1) and monomethylated-arginine (R2me). The difference between 1617.95 m/z and the next capped species (1374.78 m/z) shows unphosphorylated threonine (T3) and monomethylated lysine (K4me). The next two capped species in this spectrum reveal unmodified threonine (T6), unmodified arginine (R8) and monomethylated-lysine (K9). (*b*) This spectrum represents a peptide with similar identity to that in *a* except that R8 is asymmetrically dimethylated, as opposed to unmodified. The addition of this PTM is reflected in a different m/z for two of the capped species, (1402.83 m/z and 1645.99 m/z) and a full-length product that differs in mass by two methyl groups (28.05 m/z).
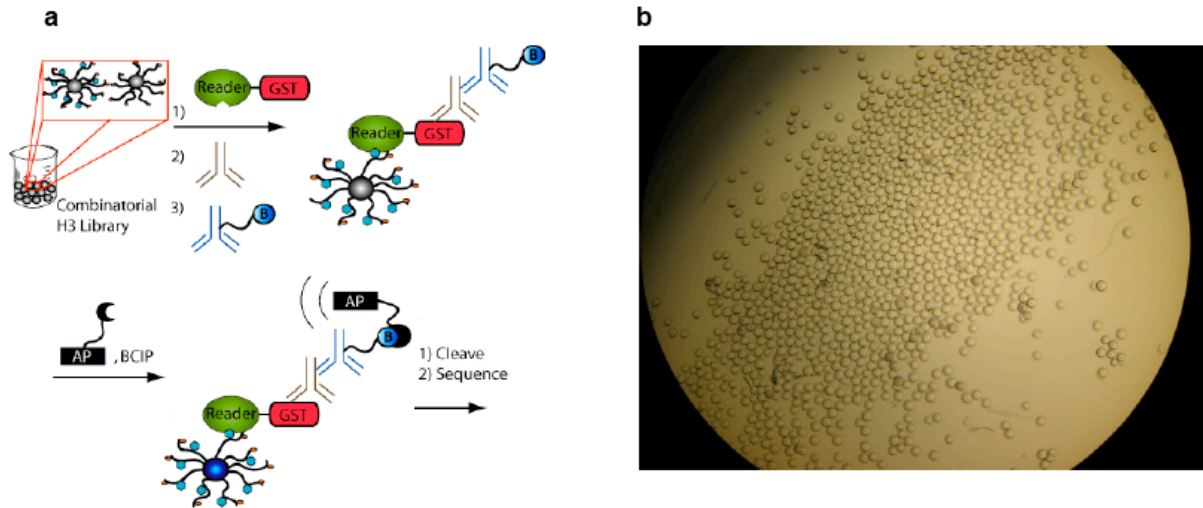
**SI Fig 2. On-bead assay for H3 library screening**. (*a*) After blocking with bovine serum albumin for nonspecific interactions, the H3 library is incubated with a GST-fusion of the protein of interest (reader). Afterwards, the library is treated with a GST-specific primary antibody followed by a biotinylated secondary antibody. Subsequent colorimetric development with SAAP and BCIP results in turquoise staining of beads bearing peptides that interact with the target protein. (*b*) A portion of the library screened with GST to ensure minimal non-specific binding incurs no color change after 40 minutes of BCIP development.

SI Fig. 3



AIRE-PHD1/H3unmod.



BHC80/H3unmod.



BHC80/H3R2me2s



RAG2/H3K4me3



ING2/H3K4me3



ING2/H3K4me3T6ph



ING2/H3K4me3R2me2s



JMJD2A/H3K4me3



JMJD2A/H3K4me3T6ph



JMJD2A/H3K4me3T3ph
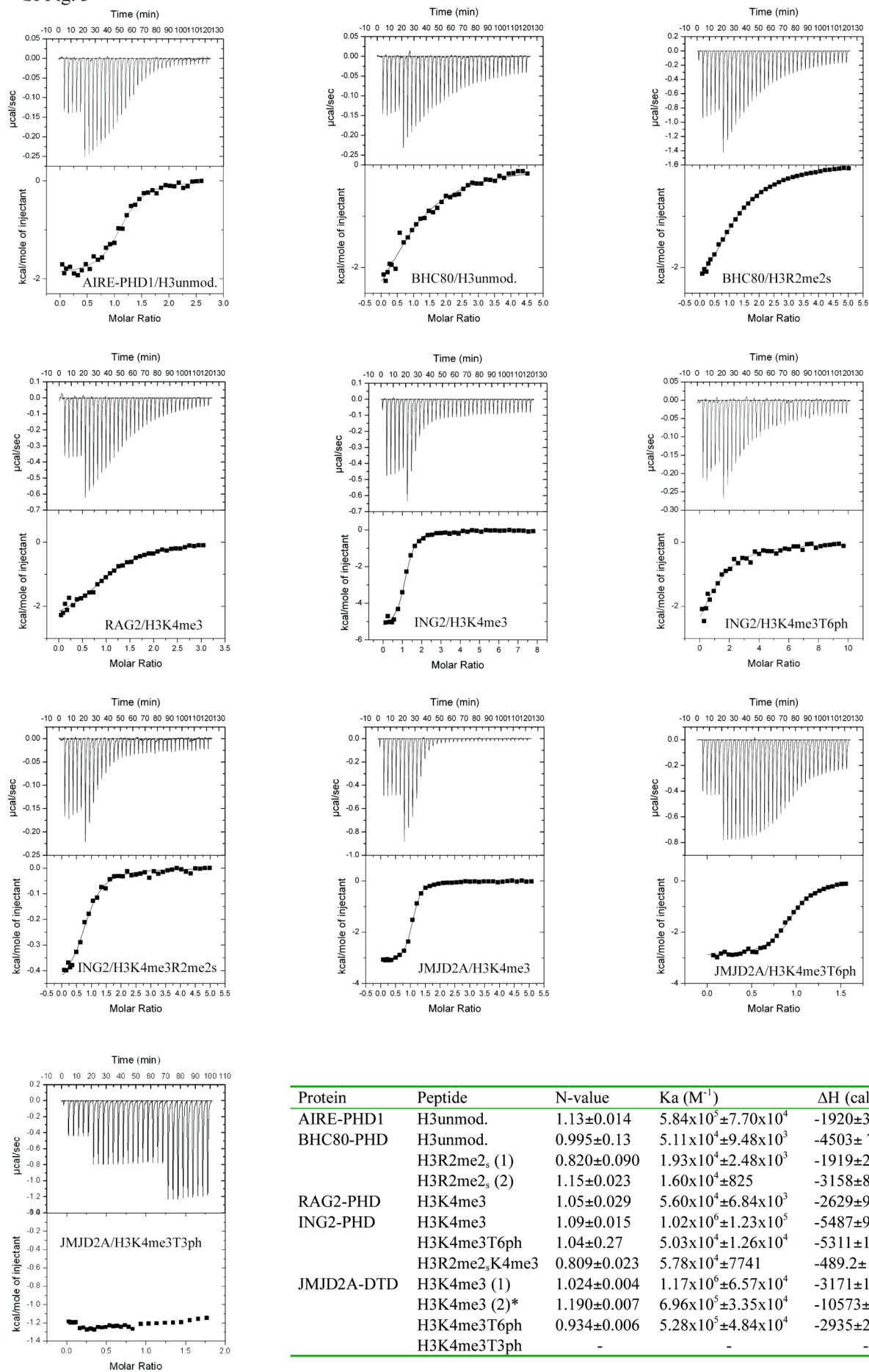
| Protein | Peptide | N-value | Ka (M$^{-1}$) | ΔH (cal/mol) |
|---|---|---|---|---|
| AIRE-PHD1 | H3unmod. | 1.13±0.014 | $5.84 \times 10^5 \pm 7.70 \times 10^4$ | -1920±31.4 |
| BHC80-PHD | H3unmod. | 0.995±0.13 | $5.11 \times 10^4 \pm 9.48 \times 10^3$ | -4503± 744.9 |
| | H3R2me2$_s$ (1) | 0.820±0.090 | $1.93 \times 10^4 \pm 2.48 \times 10^3$ | -1919±258.8 |
| | H3R2me2$_s$ (2) | 1.15±0.023 | $1.60 \times 10^4 \pm 825$ | -3158±81.7 |
| RAG2-PHD | H3K4me3 | 1.05±0.029 | $5.60 \times 10^4 \pm 6.84 \times 10^3$ | -2629±96.5 |
| ING2-PHD | H3K4me3 | 1.09±0.015 | $1.02 \times 10^6 \pm 1.23 \times 10^5$ | -5487±90.4 |
| | H3K4me3T6ph | 1.04±0.27 | $5.03 \times 10^4 \pm 1.26 \times 10^4$ | -5311±1630 |
| | H3R2me2$_s$K4me3 | 0.809±0.023 | $5.78 \times 10^4 \pm 7741$ | -489.2±17.2 |
| JMJD2A-DTD | H3K4me3 (1) | 1.024±0.004 | $1.17 \times 10^6 \pm 6.57 \times 10^4$ | -3171±14.7 |
| | H3K4me3 (2)* | 1.190±0.007 | $6.96 \times 10^5 \pm 3.35 \times 10^4$ | -10573±109.0 |
| | H3K4me3T6ph | 0.934±0.006 | $5.28 \times 10^5 \pm 4.84 \times 10^4$ | -2935±25.2 |
| | H3K4me3T3ph | - | - | - |

**SI Fig 3. Isothermal titration calorimetry (ITC) for modules bound to H3 peptides.**

The experimental curves are represented in the top panel and the corresponding enthalpy plots are shown in the bottom panel for each individual experiment. The protein and peptide are labeled in the lower panel. A summary of the thermodynamic and curve fitting parameters are listed in the bottom table. Titrations of H3R2me2s into the BHC80-PHD and the H3K4me3 peptide into JMJD2A-DTD are shown for two separate experiments (labeled as (1) and (2)) in the table and a representative curve/enthalpy plot is displayed for each. The $K_d$ values for these titrations are averaged using both experiments (Table 1 and 2 in the text). Titrations were performed at protein concentrations from 20-350 μM and peptide concentrations from 0.5-2.8 mM.

* The variability in these separate experiments is likely due to the fact that these two experiments were performed with different preparations of JMJD2A and a different synthetic preparation of H3K4me3 peptide. Although the purity was similar between preparations, we observed 3-fold higher heats of dilution with the peptide that yielded ~10,000 cal/mol, suggesting that accompanying salts/counterions may have contributed to the differences in molar heats.
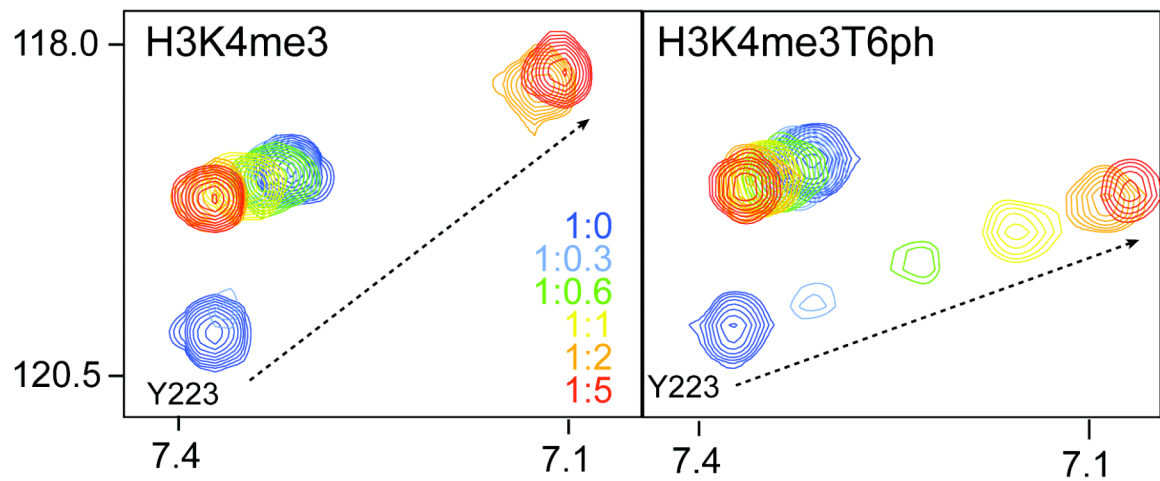
**SI Fig 4. $^1$H,$^{15}$N HSQC NMR titrations.** Six superimposed $^1$H,$^{15}$N HSQC spectra of the ING2 PHD finger (0.2 mM) collected during addition of the H3K4me3T6ph (*a*) or H3K4me3 (*b*) peptides are color-coded according to the peptide concentrations (inset).
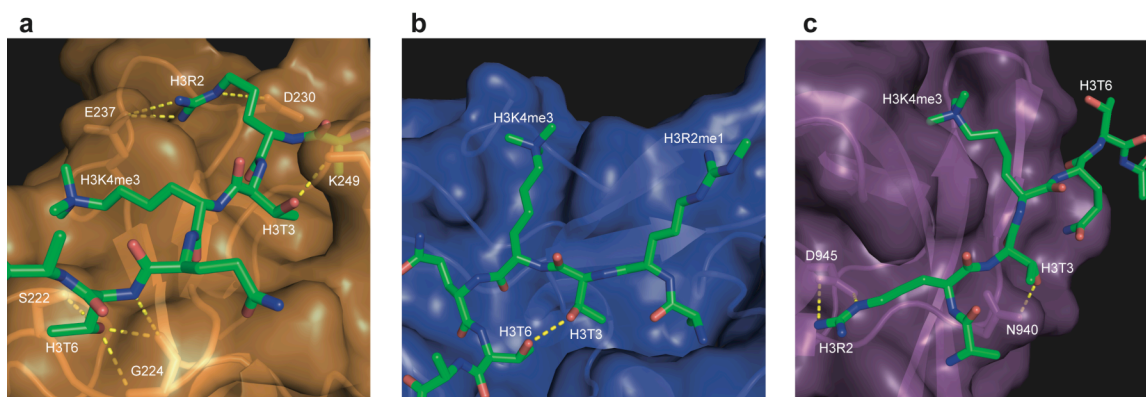
**SI Fig 5. H3 binding-module cocrystal structures**. Representations of previously solved co-crystal structures of a panel of H3K4me3 binding modules illustrate the hydrogen bonding and electrostatic contacts made between H3 side chain and the various effector domains. Space filling models of the ING2-PHD finger (A, orange, 2G6Q)[8], the RAG2-PHD finger (B, blue, 2V85)[9], and the JMJD2A DTD (C, purple, 2GFA)[10] are shown binding H3 peptides. Carbon, nitrogen and oxygen atoms are shown in green, blue and red, respectively for the H3 peptides.

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