Materials and Methods

Materials and General Methods

Oligonucleotides were received from Integrated DNA Technologies. Enzymes and reagents used for cloning were purchased from New England BioLabs Inc. All other chemical reagents and solvents were obtained from chemical suppliers (Acros, Fisher Scientific, Sigma-Aldrich, Chem Impex, and P3 Biosystems) and used without further purification. DNA sequencing was performed by Genewiz. Mass spectrometry was performed using an Agilent Technologies Rapid Resolution LC-MSD system (1200 Series).

Cloning and Protein Sequences

DIDO1 and BPTF wild-type genes were graciously donated by the Strahl Lab. TAF3 wild-type gene was purchased from Integrated DNA Technologies. Wild-type genes were cloned into a pET28a vector using NdeI and XhoI restriction sites. Canonical mutations were generated using overlap PCR methods. Successful mutagenesis was confirmed by Sanger sequencing (Genewiz).

DIDO1 wild-type protein sequence:
MGSSHHHHHHSSGLVPRGSHMGSEPGDLGRPKPECEGYDPNALYCICRQPHNNRFMIC
CDRCEEWFHGDCVGISEARGRLLERNGEDYICPNCTILQVQDETHSETADQQEAKWLEH
HHHHH

DIDO1 M35A protein sequence:
MGSSHHHHHHSSGLVPRGSHMGSEPGDLGRPKPECEGYDPNALYCICRQPHNNRF**A**ICC
DRCEEWFHGDCVGISEARGRLLERNGEDYICPNCTILQVQDETHSETADQQEAKWLEHH
HHHH

DIDO1 M35C protein sequence:
MGSSHHHHHHSSGLVPRGSHMGSEPGDLGRPKPECEGYDPNALYCICRQPHNNRF**C**ICC
DRCEEWFHGDCVGISEARGRLLERNGEDYICPNCTILQVQDETHSETADQQEAKWLEHH
HHHH

DIDO1 M35F protein sequence:
MGSSHHHHHHSSGLVPRGSHMGSEPGDLGRPKPECEGYDPNALYCICRQPHNNRF**F**ICC
DRCEEWFHGDCVGISEARGRLLERNGEDYICPNCTILQVQDETHSETADQQEAKWLEHH
HHHH

DIDO1 M35Nle protein sequence:
MGSSHHHHHHSSGLVPRGSHMGSEPGDLGRPKPECEGYDPNALYCICRQPHNNRF
NleICCDRCEEWFHGDCVGISEARGRLLERNGEDYICPNCTILQVQDETHSETADQQEAK
WLEHHHHHH

DIDO1 M35SeM protein sequence:
MGSSHHHHHHSSGLVPRGSHMGSEPGDLGRPKPECEGYDPNALYCICRQPHNNRF
SeMICCDRCEEWFHGDCVGISEARGRLLERNGEDYICPNCTILQVQDETHSETADQQEAK
WLEHHHHHH

TAF3 wild-type protein sequence:
MGSSHHHHHHSSGLVPRGSHMVIRDEWGNQIWICPGCNKPDDGSPMIGCDDCDDWYH
WPCVGIMTAPPEEMQWFCPKCANKKKDKKHKKRKHRAH

TAF3 MtoQ protein sequence (used as wild-type sequence):
MGSSHHHHHHSSGLVPRGSHMVIRDEWGNQIWICPGCNKPDDGSPMIGCDDCDDWYH
WPCVGI**Q**TAPPEE**Q**QWFCPKCANKKKDKKHKKRKHRAH

TAF3 M882A protein sequence:
MGSSHHHHHHSSGLVPRGSHMVIRDEWGNQIWICPGCNKPDDGSP**A**IGCDDCDDWYH
WPCVGIQTAPPEEQQWFCPKCANKKKDKKHKKRKHRAH

TAF3 M882C protein sequence:
MGSSHHHHHHSSGLVPRGSHMVIRDEWGNQIWICPGCNKPDDGSP**C**IGCDDCDDWYH
WPCVGIQTAPPEEQQWFCPKCANKKKDKKHKKRKHRAH

TAF3 M882F protein sequence:
MGSSHHHHHHSSGLVPRGSHMVIRDEWGNQIWICPGCNKPDDGSP**F**IGCDDCDDWYH
WPCVGIQTAPPEEQQWFCPKCANKKKDKKHKKRKHRAH

TAF3 M882Nle protein sequence:
MGSSHHHHHHSSGLVPRGSHMVIRDEWGNQIWICPGCNKPDDGSP**Nle**IGCDDCDDWY
HWPCVGIQTAPPEEQQWFCPKCANKKKDKKHKKRKHRAH

TAF3 M882SeM protein sequence:
MGSSHHHHHHSSGLVPRGSHMVIRDEWGNQIWICPGCNKPDDGSP**SeM**IGCDDCDDWY
HWPCVGIQTAPPEEQQWFCPKCANKKKDKKHKKRKHRAH

BPTF wild-type protein sequence:
MGSSHHHHHHSSGLVPRGSHMDTKLYCICKTPYDESKFYIGCDRCQNWYHGRCVSILQ
SEAELIDEYVCPQCQSTEDA

BPTF Y23M protein sequence:
MGSSHHHHHHSSGLVPRGSHMDTKLYCICKTPYDESKF**M**IGCDRCQNWYHGRCVSILQ
SEAELIDEYVCPQCQSTEDA

Protein Expression and Purification

Wild-type DIDO1 and TAF3 and canonical mutant PHD domains were recombinantly expressed in E. coli BL21-DE3 Gold (Agilent). Flasks containing 1L LB-kanamycin and 50 μM $ZnSO_4$ were inoculated with 5 mL overnight cultures and incubated to an OD_{600} of ~0.6 at 37˚C with 220 RPM shaking. Expression was induced with 0.1 mM IPTG, and cultures were incubated at 18 ˚C overnight with 220 RPM shaking.

SeMet mutants were recombinantly expressed in E. coli B834 methionine auxotroph strain (Agilent). Two-5mL overnight cultures were grown in LB-kanamycin for each 1L expression flask. Overnight cultures were then pelleted at 4500 RPM for 10 minutes and each pellet was resuspended in 1mL sterile water. Flasks containing 1L M9-kanamycin media[1], 50 μM $ZnSO_4$, 10 mg/L L-Met, and 120 mg/L L-SeMet were inoculated with 2 resuspended overnight cultures (total 2mL overnight culture/flask) and incubated to an OD_{600} of ~0.6 at 37 ˚C with 220 RPM shaking. Expression was induced with 0.1 mM IPTG, and cultures were incubated at 18 ˚C overnight with 220 RPM shaking.

Nle mutants were recombinantly expressed in E. coli B834 methionine auxotroph strain (Agilent). Two-5mL overnight cultures were grown in LB-kanamycin for each 1L expression flask. Overnight cultures were then pelleted at 4500 RPM for 10 minutes and each pellet was resuspended in 1mL sterile water. Flasks containing 1L M9-kanamycin media[1], 50 μM $ZnSO_4$, and 25 mg/L L-Met were inoculated with 2 resuspended overnight cultures (total 2mL overnight culture/flask) and incubated to an OD_{600} of ~0.6 at 37 ˚C with 220 RPM shaking. Cultures were then pelleted and washed twice with 0.9% NaCl. Pellets were resuspended in 1L of fresh M9-kanamycin + 50 μM $ZnSO_4$ media and incubated for an additional 3 hours at 37 ˚C with 220 RPM shaking. 300mg/L of L-Nle was then added to the media. After 30 minutes, expression was

induced with 0.1 mM IPTG, and cultures were incubated at 18 ˚C overnight with 220 RPM shaking.

Following expression, cultures were pelleted at 4500 RPM for 10 minutes. Cell pellets were resuspended in 20mL lysis buffer (50mM Tris, pH 8, 150mM NaCl, 30mM imidazole, 1mg/mL lysozyme) for 30 min at 37 ˚C. Resuspended pellets were then sonicated for 30sec on/30sec off for four cycles, then clarified at 15,000 RPM for 1 hr.

The expressed PHD domains were first purified with Ni-affinity chromatography. Filtered lysate was loaded onto an ÄKTAPurifier UPC 10 (GE) equipped with a HisTrap- 5mL HP column (GE). His-tagged proteins were eluted at 55% Buffer B (Buffer A: 50 mM Tris, 150 mM NaCl, 30 mM imidazole, 2 mM DTT, pH 7.4; Buffer B: 50 mM Tris, 150 mM NaCl, 300 mM imidazole, 2 mM DTT, pH 7.4). Fractions were combined and concentrated using a 3 kDa Amicon Ultra-15 Centrifugal filter. The concentrated sample was purified by size exclusion chromatography using a Superdex 200 10/300 GL size exclusion column equilibrated in buffer containing 50 mM sodium phosphate, pH 8, 125 mM NaCl, 2 mM TCEP. Eluted fractions were pooled, concentrated and run on SDS-PAGE and ESI-LCMS to confirm identity.

Intact Mass Spectrometry of PHD Domains

Thermo QExactive HF Biopharm coupled with 908 Devices ZipChip CE was used for Intact Mass Spectrometry of PHD domains. Samples were prepared to 1 mg/mL.

Peptide Sequences, Synthesis, and Purification

Histone peptide sequences
H3K4me3 1-13 (Tyr for concentration determination):
ARTKme3QTARKSTGGY

H3K4tBuNle4 1-13 (Tyr for concentration determination):
ARTtBuNleQTARKSTGGY

Histone peptides were synthesized on a CEM Liberty Blue peptide synthesizer using standard Fmoc-protected amino acids and Rink Amide AM Resin. The amino acid residues were activated with Oxyma (Ethyl cyanohydroxyiminoacetate) and DIC (N,N'-Diisopropycarbodiimide). 5 equivalents of the amino acid (two equivalents for Kme3 and tBuNle), Oxyma, and 10 equivalents of DIC were used for each coupling step. Two coupling cycles of 4 minutes were performed at 90ºC in DMF (dimethylformamide) for each residue. Deprotections of Fmoc were carried out in 20% piperidine in DMF, twice for 1 minute each. The resin was washed with DMF before every deprotection and coupling cycle. Peptides were cleaved from the resin with 95:2.5:2.5 trifluoroacetic acid (TFA):water:triisopropylsilane for 3 hours. Crude peptide material were purified by reverse phase HPLC using a C18 XBridge 5 μM column (Waters) and a gradient of 0 to 100% B in 60 minutes, where solvent A was 95:5 water:acetonitrile, 0.1% TFA and solvent B was 95:5 acetonitrile:water, 0.1% TFA. Histone peptides were purified by reverse phase HPLC using a 0-20% B in 60 minute gradient. The purified peptides were lyophilized, and identity was confirmed by ESI-LCMS.

Synthesis of Lysine Analogs

Synthesis of Fmoc-Kme3-OH

Fmoc-Kme3-OH was synthesized by reductive amination of subsequent methylation using MeI and MTBD. 2 g (5 mmol) of Fmoc-Lys-OH was dissolved in 100mL 50:50 DMF and ACN, then 10 equiv (50 mmol) of STAB and formaldehyde were added. The reaction was stirred at room temperature for 24 hrs. An additional 10 equiv of STAB and formaldehyde were added and the reaction was stirred for another 24 hrs at room temperature. The reaction was dried, then redissolved in 100 mL of DMF, and 10 equiv of MeI and 2 equiv of MTBD were added. The reaction was stirred overnight, then dried and purified by reverse phase HPLC using a 0-100% B

gradient where solvent A was 95:5 water:acetonitrile, 0.1% TFA and solvent B was 95:5 acetonitrile:water, 0.1% TFA. The identity was confirmed by ESI-LCMS.

Synthesis of Fmoc-tBuNle-OH

Fmoc-tBuNle-OH was synthesized as previously reported.[2] 1 g (3 mmol) of N-Fmoc allyl glycine and 1.5 equiv (4.5 mmol) of 4,4-Dimethyl-1-Pentene were dissolved in 50 mL degassed dichloromethane. Hoveyda-Grubbs second generation catalyst (10 mol%, 0.3 mmol) was then added to the mixture before the reaction was brought to reflux under nitrogen overnight. After reaction completion, products were dried then purified by column chromatography (0 to 40 % EtOAc gradient in cyclohexane, 0.1 % AcOH). The identity was confirmed by ESI-LCMS. The metathesis product was dissolved in ethanol and the round bottom was flushed with nitrogen and then evacuated before being flushed with H_2. Palladium on carbon (50% by mass) was then added to the reaction. The reaction was then flushed with H_2 and then left stirring under a H_2 atmosphere overnight at room temperature. Upon reaction completion, the mixture was filtered through a Celite plug and washed with methanol. The filtrate was concentrated to dryness. The mixture was taken up in toluene then purified by column chromatograph (0-50% EtOAc in hexanes, 0.1% AcOH). The identity was confirmed by ESI-LCMS.

Circular Dichroism of PHD Domains

CD experiments were performed using an Applied Photophysics Chirascan. Spectra were obtained with 25 μM PHD domain in 10 mM sodium phosphate buffer, pH 7.4 at 20 °C. The mean residue ellipticity (MRE) was calculated using eq 1, where θ is MRE, signal is CD signal, l is path length, c is protein concentration, and r is the number of amino acid residues. A quartz cuvette with a path length of 0.1 cm was used to correct spectra.

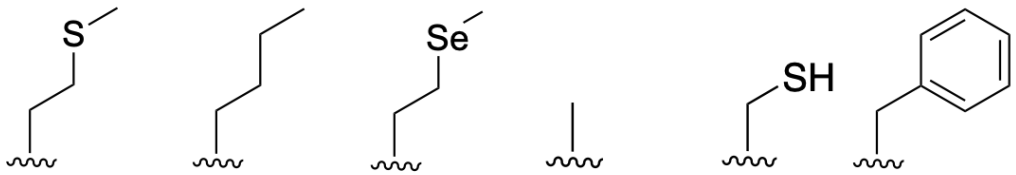
$$\theta = \frac{signal}{10 \cdot l \cdot c \cdot r} \quad (1)$$

Isothermal Titration Calorimetry (ITC) Binding Measurements with PHD Domains and Histone Peptides

ITC experiments were performed by titrating histone peptide into PHD domains in 50 mM sodium phosphate, pH 7.4, 150 mM NaCl, 2 mM TCEP at 25 °C using a MicroCal PEAQ-ITC. Peptide and protein concentrations were determined by measuring absorbance at 280 nm on a Nanodrop 2000 (Thermo). Data was modeled using the One-Site binding model supplied in MicroCal PEAQ-ITC Analysis software (version 1.1.0.1262) with the fitted offset control. Replicate ITC experiments were performed for each protein / peptide pair. Errors given for Kd, $\Delta G°_{binding}$, $\Delta H°$, and $T\Delta S°$ are calculated as the standard error of the mean.

Tables and Figures

Table S1. Sidechain LogP and polarizability values for single point mutations investigated in DIDO1 and TAF3.



AA	Met	Nle	SeMet	Ala	Cys	Phe
LogP	1.63	2.58	1.48	1.33	0.89	2.52
Polarizability (10^{-30} m^3)	68.56	61.09	75.91	42.60	46.69	47.3

Table S2. ESI-LCMS data for PHD domains. Observed masses correlate well with expected masses.

Protein	ε	Expected Mass (amu)	- Initiator Met (amu)	Observed Mass(amu)
DIDO1 WT	15700	13793.27	13662.07	13661
DIDO1 M35C	15700	13765.22	13636.02	13634
DIDO1 M35Nle	15700	13829.26	13698.06	
DIDO1 M35SeMet	15700	13887.67	13756.47	13755
TAF3 MtoQ (WT)	29730	10999.45	10868.25	10868
TAF3 M882A	29730	10939.33	10808.13	10808, 10986 (+formic acid)
TAF3 MtoQ M882F	29730	11015.43	10884.23	10883, 11061 (+formic acid)
TAF3 MtoQ M882Nle	29730	10963.27	10832.17	10832, 11010 (+formic acid)
TAF3 MtoQ M882SeMet	29730	11093.85	10962.65	10963
BPTF WT	12090	8958.00	8826.80	
BPTF Y23M	12090	8926.03	8794.83	

DIDO1 wild-type

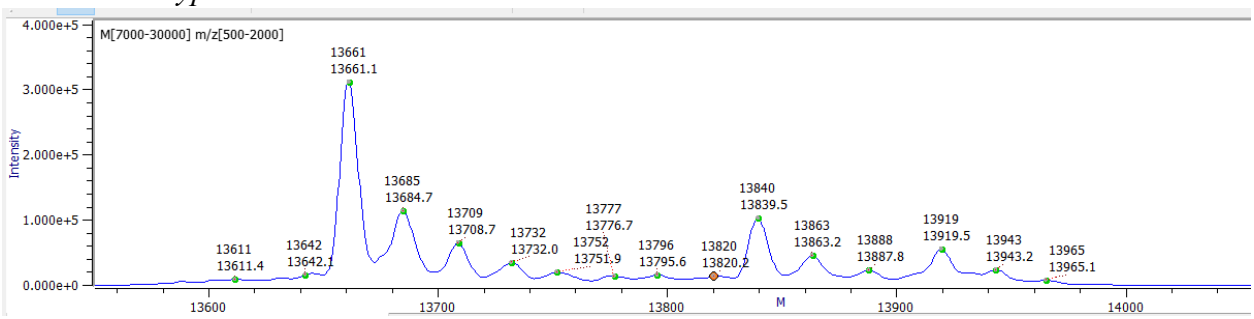


Figure S1. DIDO1 WT intact mass spectrum. Expected mass: 13793.27, 13662.07 (no initiator Met); Observed mass: 13661

DIDO1 M35C

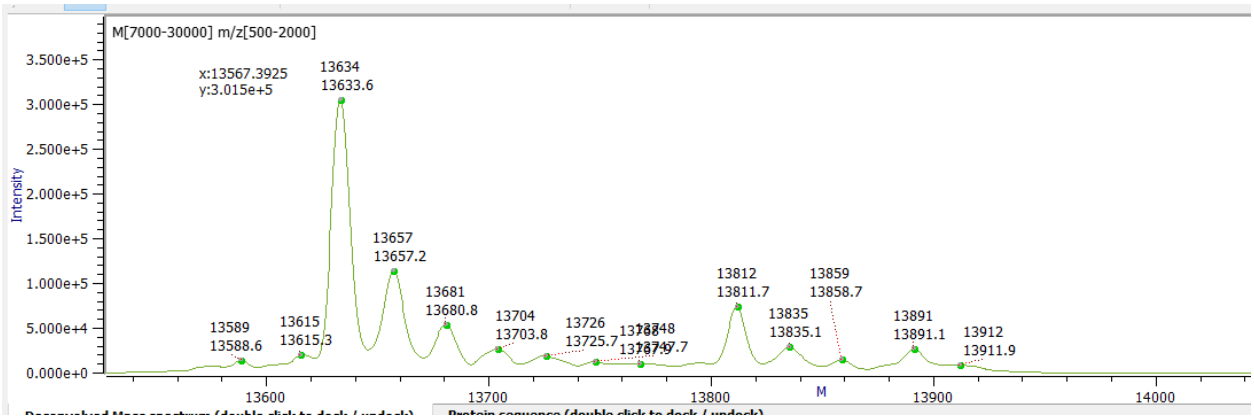


Figure S2. DIDO1 M35C intact mass spectrum. Expected mass: 13765.22, 13636.02 (no initiator Met); Observed mass: 13634

DIDO1 M35SeMet

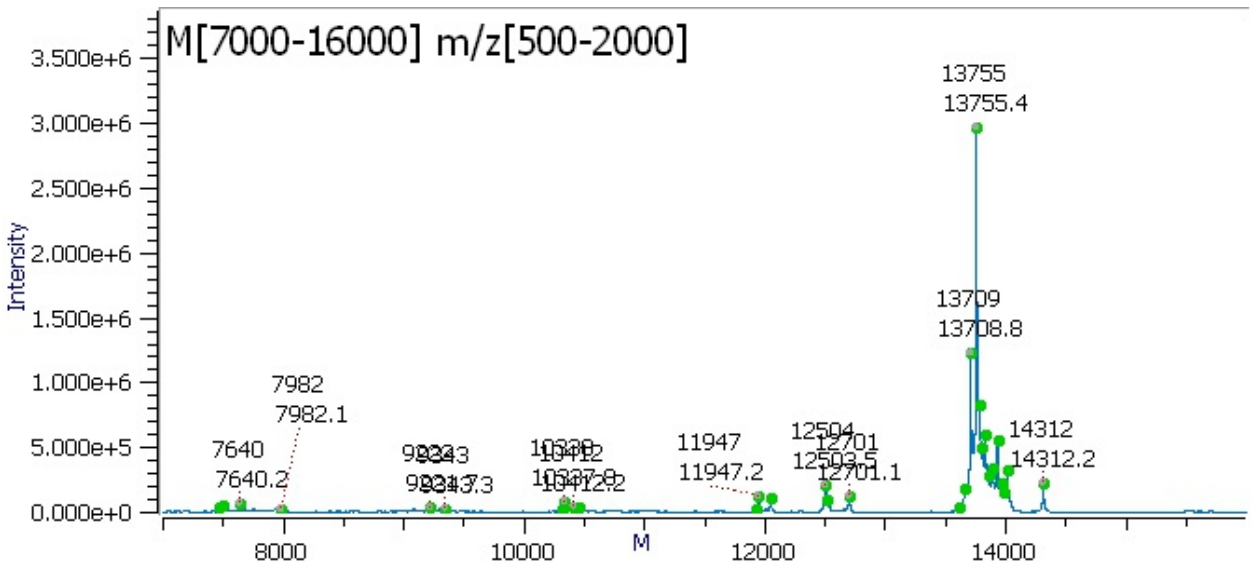


Figure S3. DIDO1 M35SeMet intact mass spectrum. Expected mass: 13887.67, 13756.47 (no initiator Met); Observed mass: 13755

TAF3 MtoQ (WT)

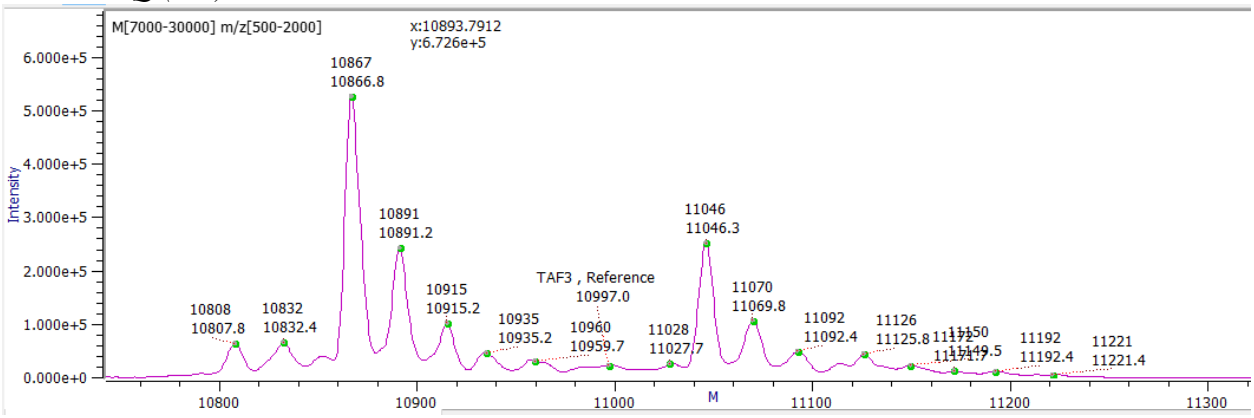


Figure S4. TAF3 MtoQ (WT) intact mass spectrum. Expected mass: 10999.45, 10868.25 (no initiator Met); Observed mass: 10867

TAF3 M882A

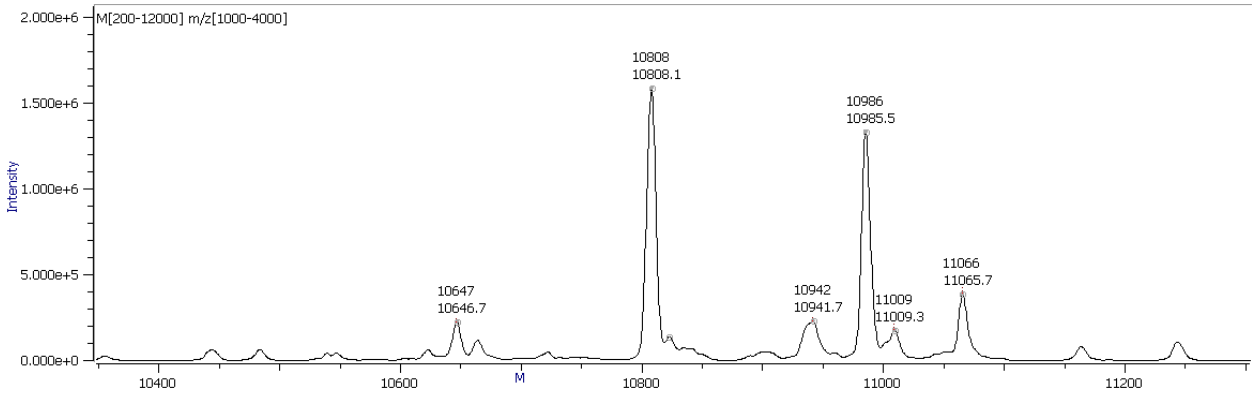


Figure S5. TAF3 M882A intact mass spectrum. Expected mass: 10939.33, 10808.13 (no initiator Met); Observed mass: 10808, 10986 (+formic acid).

TAF3 M882Nle

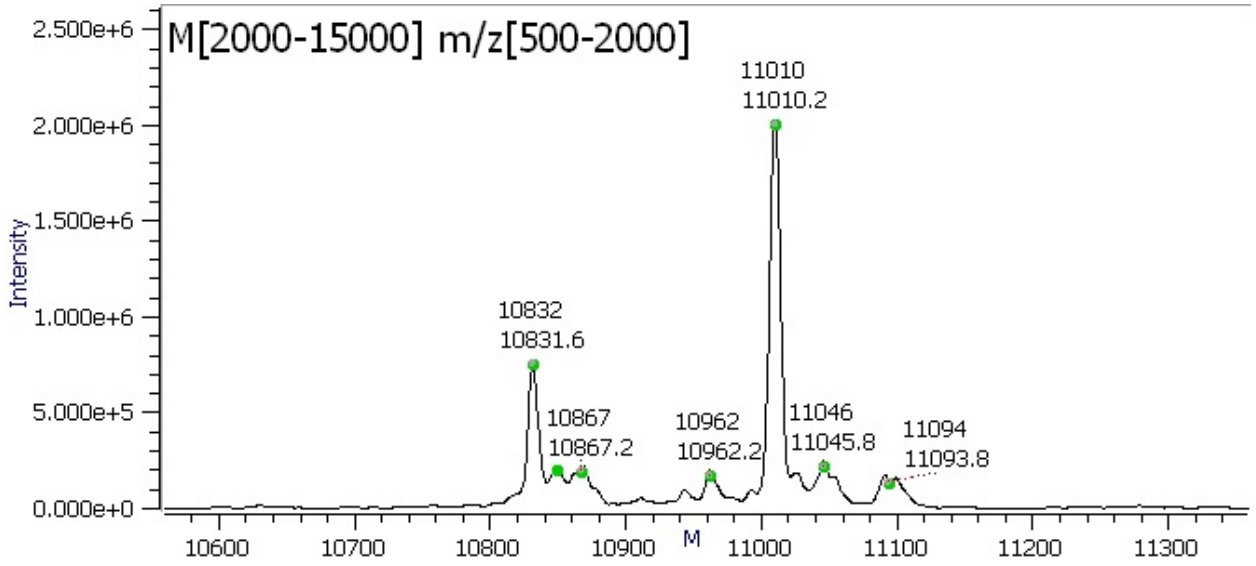


Figure S6. TAF3 M882Nle intact mass spectrum. Expected mass: 10963.27, 10832.17 (no initiator Met); Observed mass: 10832, 11010 (+formic acid)

TAF3 M882SeMet

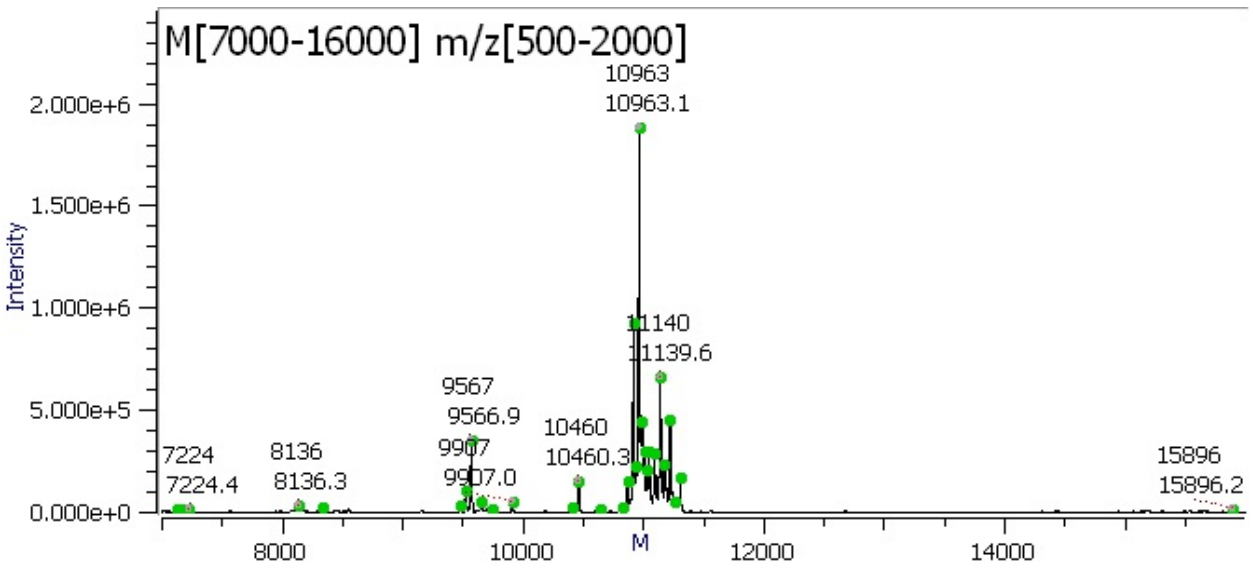


Figure S7. TAF3 M882SeMet intact mass spectrum. Expected mass: 11093.85, 10962.55 (no initiator Met); Observed mass: 10963

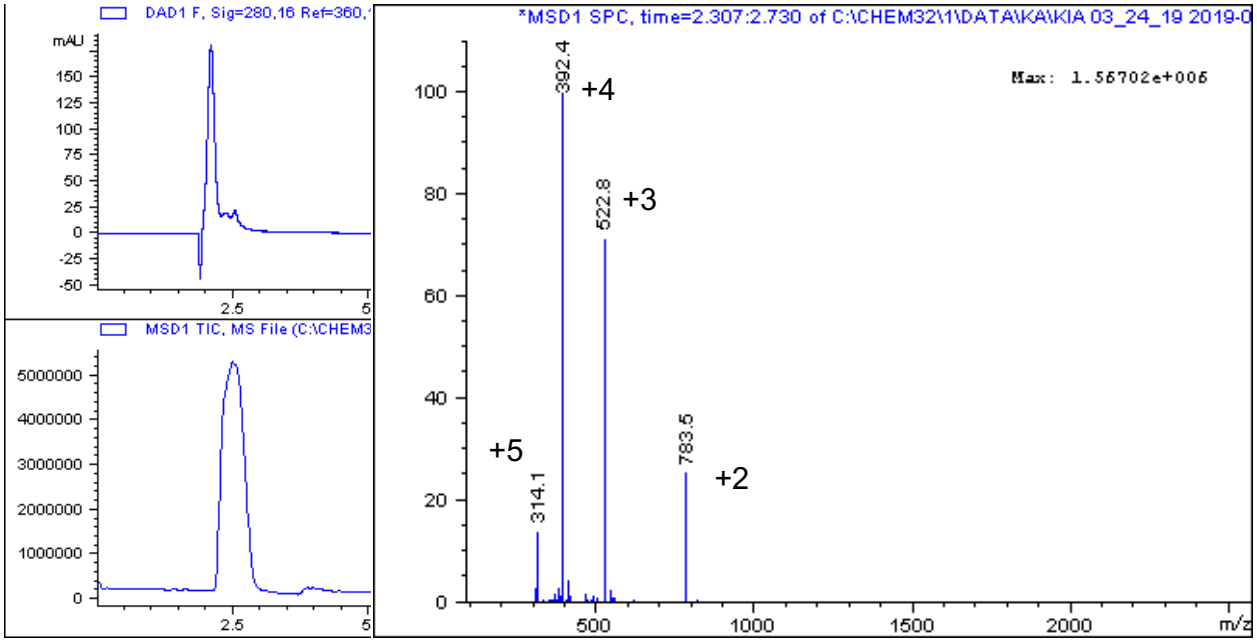


Figure S8. UV-absorbance at 280nm, TIC, and low-resolution mass spectrum of H3K4me3. Mass calculated: 1565.89 [M+H]. Mass observed: 783.5 [M+2], 522.8 [M+3], 392.4 [M+4], 314.1 [M+5].

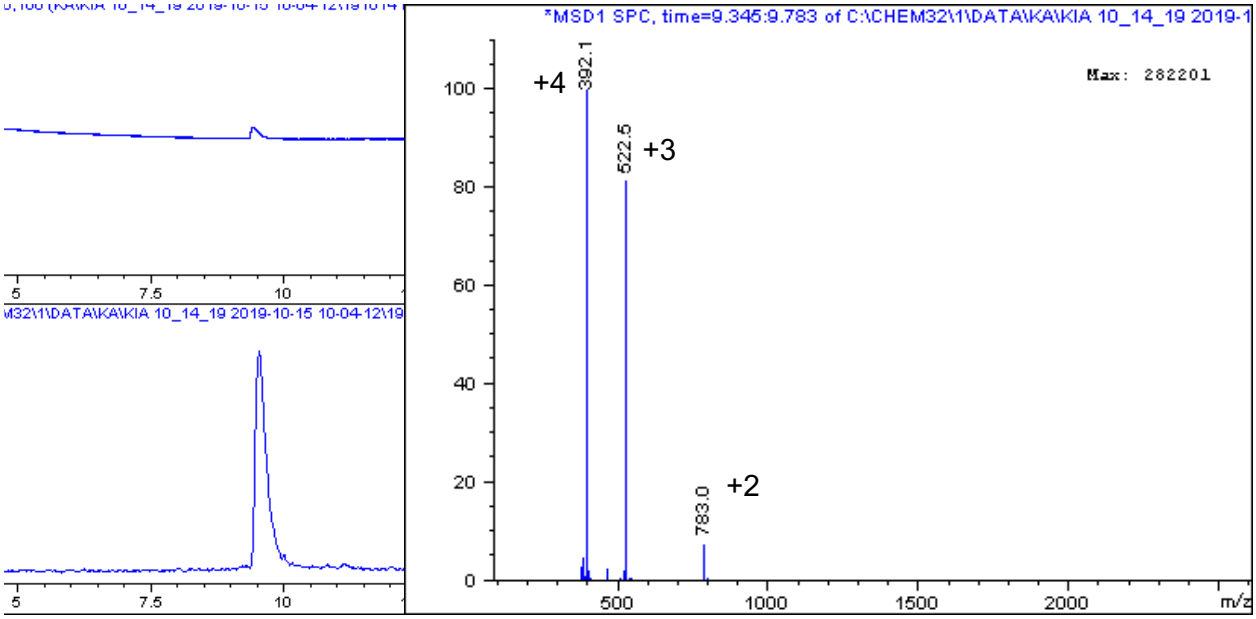


Figure S9. UV-absorbance at 280nm, TIC, and low-resolution mass spectrum of H3K4tBuNle. Mass calculated: 1564.88 [M+H]. Mass observed: 783.0 [M+2], 522.5 [M+3], 392.1 [M+4], 314.1 [M+5].

Fmoc-Kme3-OH

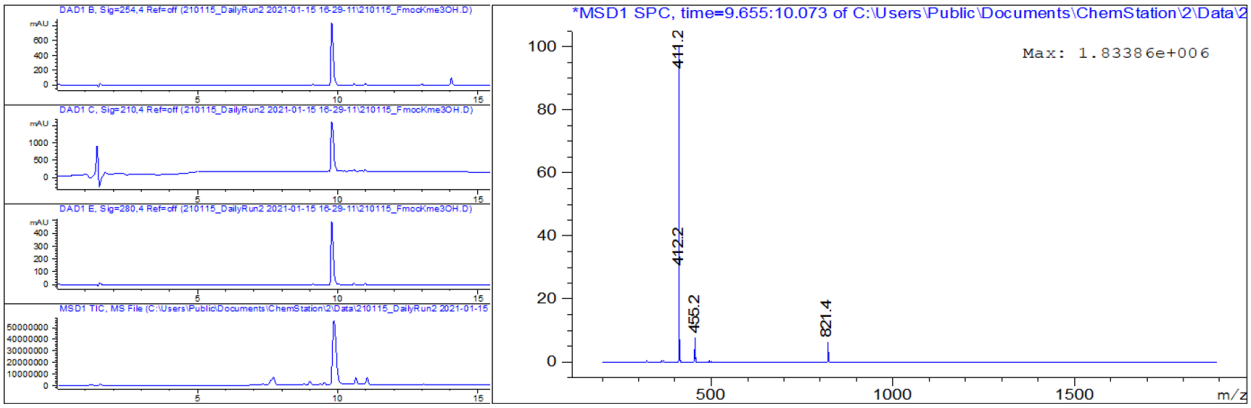


Figure S10. UV-absorbance at 280nm, TIC, and low-resolution mass spectrum of Fmoc-Kme3-OH. Mass calculated: 411.23 [M]. Mass observed: 821.4 [M+2], 411.2 [M+H].

Fmoc-tBuNle-OH

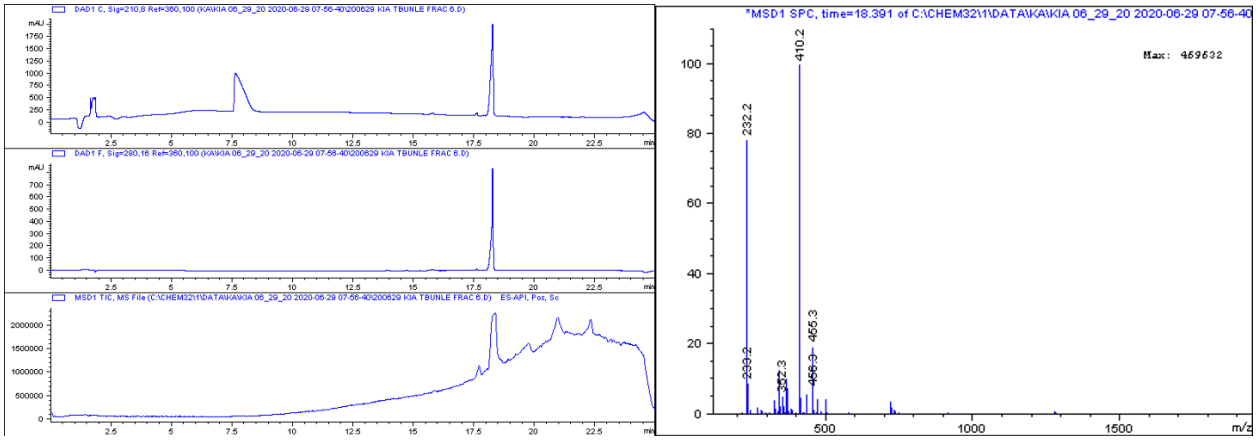


Figure S11. UV-absorbance at 280nm, TIC, and low-resolution mass spectrum of Fmoc-tBuNle-OH. Mass calculated: 409.23 [M]. Mass observed: 410.2 [M+H].

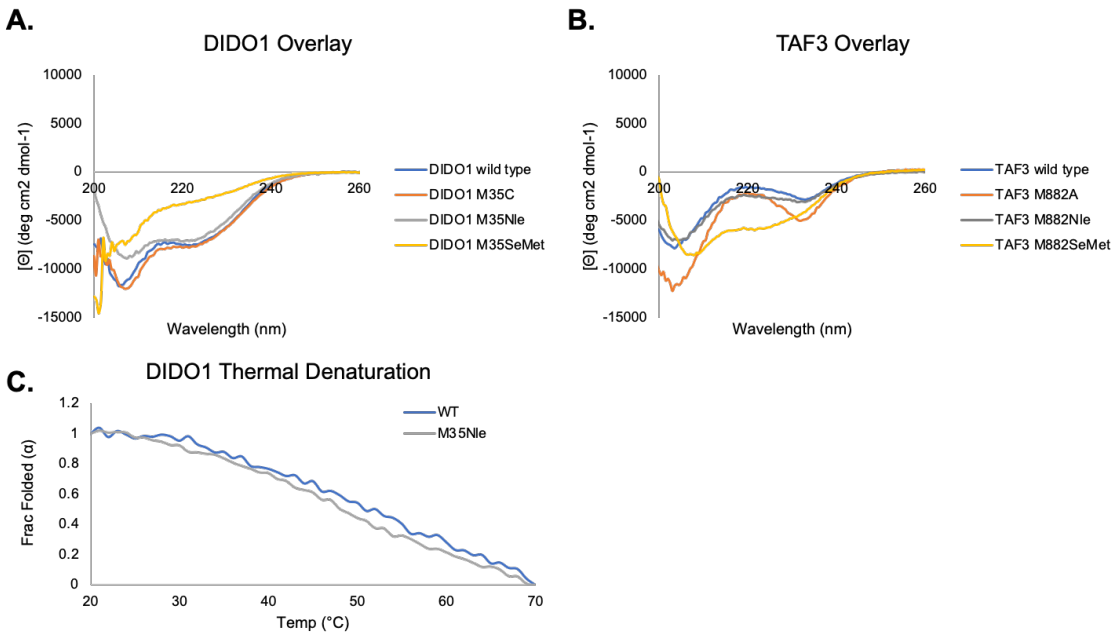


Figure S12. Circular dichroism of (A) DIDO1 and (B) TAF3 PHD domains at 25 µM in 10 mM sodium phosphate, pH 7.4. (C) DIDO1 WT and M35Nle thermal denaturation experiments at 25 µM in 10 mM sodium phosphate, pH 7.4.

Table S3. Thermodynamic parameters of canonical PHD domain mutants:H3K4me3 binding measured by ITC.

H3K4me3[a]	Kd (uM)		ΔG° (kcal/mol)		ΔH° (kcal/mol)		-TΔS° (kcal/mol)	
	DIDO1	TAF3	DIDO1	TAF3	DIDO1	TAF3	DIDO1	TAF3
Met	2.28±0.03	1.05±0.01	-7.70±0.01	-8.16±0.01	-10.7±0.4	-11.3±0.2	3.1±0.3	3.1±0.1
Ala	nd	120±50	nd	-5.4±0.3	nd	-8±2	nd	2±2
Cys	36±1	nd	-6.07±0.01	nd	-11.5±0.2	nd	5.0±0.2	nd
Phe[b]	nd	>120	nd	-5.4	nd	-4.2±0.3	nd	-1.2

[a]Experiments were performed by titrating H3K4me3 peptide (500 µM – 3 mM) into TAF3 and DIDO1 mutants (50-200 µM) in 50 mM sodium phosphate, pH 7.4, 150 mM NaCl, 2 mM TCEP at 25°C. [b] N =1. Error calculated as standard error of the mean.

Table S4. Thermodynamic parameters of Met isostere PHD domain mutants:H3K4me3 binding measured by ITC.

H3K4me3[a]	Kd (µM)		ΔG° (kcal/mol)		ΔΔG° (kcal/mol)		ΔH° (kcal/mol)		TΔS° (kcal/mol)	
	DIDO1	TAF3	DIDO1	TAF3	DIDO1	TAF3	DIDO1	TAF3	DIDO1	TAF3
Met	2.28±0.03	1.05±0.01	-7.70±0.01	-8.16±0.01	-	-	-10.2±0.4	-11.3±0.2	3.1±0.3	3.1±0.1
Nle	10±2	1.7±0.2	-6.9±0.1	-7.90±0.06	0.9±0.2	0.3±0.2	-9±1	-8.6±0.1	2±1	0.7±0.3
SeMet	4.2±0.2	0.84±0.07	-7.34±0.02	-8.29±0.05	0.36±0.05	-0.19±0.09	-9.7±0.2	-9.5±0.7	2.2±0.2	1.2±0.7

[a]Experiments were performed by titrating H3K4me3 peptide (500 µM – 1 mM) into TAF3 and DIDO1 mutants (50-200 µM) in 50 mM sodium phosphate, pH 7.4, 150 mM NaCl, 2 mM TCEP at 25°C. Error calculated as standard error of the mean.

Table S5. Thermodynamic parameters of PHD domain:H3K4tBuNle binding measured by ITC.

H3K4tBuNle[a]	Kd (uM)		ΔG° (kcal/mol)		ΔH° (kcal/mol)		-TΔS° (kcal/mol)	
	DIDO1	TAF3	DIDO1	TAF3	DIDO1	TAF3	DIDO1	TAF3
Met	2.9±0.3	10.7±0.4	-7.56±0.08	-6.78±0.02	-5.73±0.08	-5.54±0.02	-2.1±0.6	-1.25±0.02

[a]Experiments were performed by titrating H3K4me3 peptide (500 μM – 1 mM) into TAF3 and DIDO1 mutants (50-200 μM) in 50 mM sodium phosphate, pH 7.4, 150 mM NaCl, 2mM TCEP at 25°C. Error calculated as standard error of the mean.

Table S6. Binding affinities measured by ITC to H3K4me3 and H3K4tBuNle for BPTF WT and Y23M

BPTF	Kd (μM) Kme3	Kd (μM) tBuNle
WT[a]	0.49	0.76
Y23M[b]	7.5±0.4	7.48±0.07

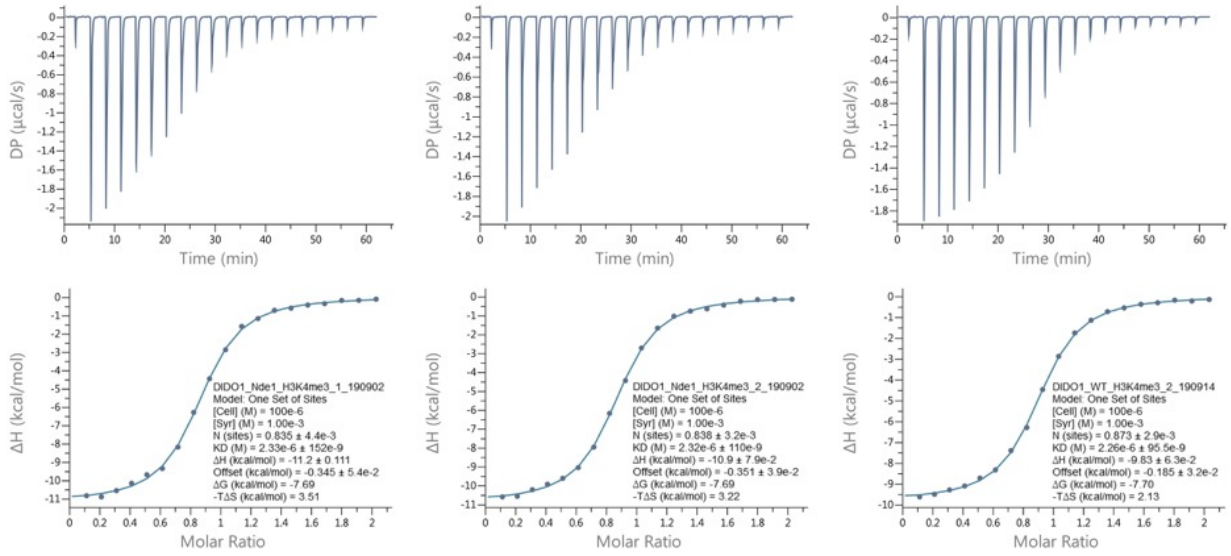
[a] Previously reported by Mecinović and coworkers.[52] [b] Experiments were performed by titrating H3K4me3 and H3K4tBuNle peptide (750μM) into BPTF (75 μM) in 50 mM sodium phosphate, pH 7.4, 150 mM NaCl, 2 mM TCEP at 25 °C. Error was determined as standard error of the mean.

Table S7. Thermodynamic parameters of Met isostere TAF3 mutants:H3K4tBuNle binding measured by ITC.

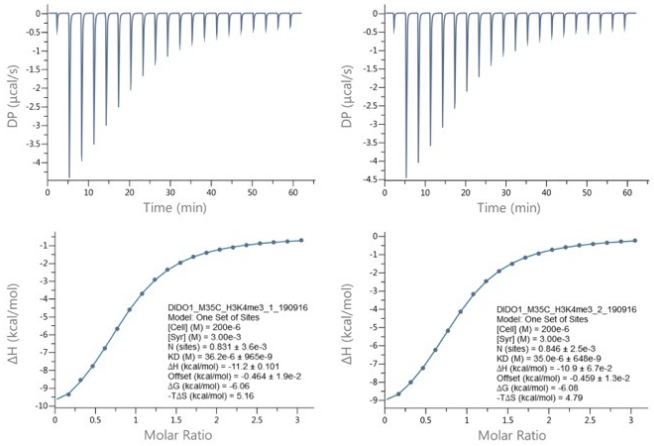
TAF3 Mutants[a]	Kd (μM)	ΔG° (kcal/mol)	ΔΔG° (kcal/mol)	ΔH° (kcal/mol)	-TΔS° (kcal/mol)
Met	10.7±0.7	-6.78±0.02	-	-5.54±0.02	-1.25±0.02
Nle	13.7±0.3	-6.64±0.01	0.15±0.04	-6.99±0.09	0.4±0.1
SeMet	8.3±0.2	-6.94±0.01	-0.15±0.04	-8.1±0.5	1.2±0.5

[a]Experiments were performed by titrating H3K4tBuNle peptide (500 μM – 1 mM) into TAF3 mutants (50-200 μM) in 50 mM sodium phosphate, pH 7.4, 150 mM NaCl, 2mM TCEP at 25°C. Error calculated as standard error of the mean.

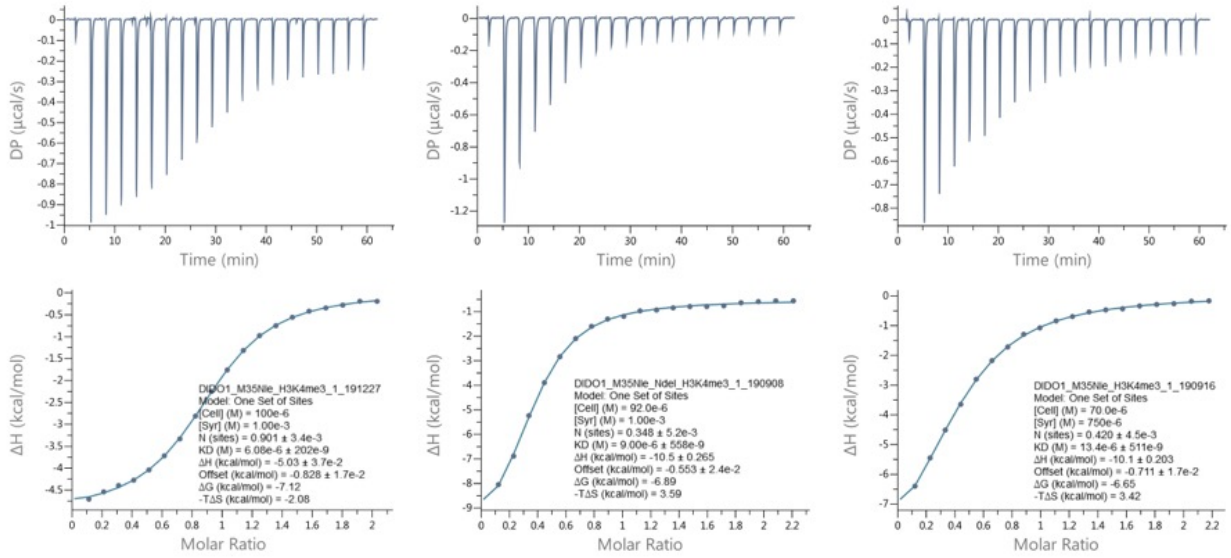
H3K4me3:
DIDO1 WT


DP (µcal/s)
Time (min)
ΔH (kcal/mol)
Molar Ratio
DIDO1_Nde1_H3K4me3_1_190902
Model: One Set of Sites
[Cell] (M) = 100e-6
[Syr] (M) = 1.00e-3
N (sites) = 0.835 ± 4.4e-3
KD (M) = 2.33e-6 ± 152e-9
ΔH (kcal/mol) = -11.2 ± 0.111
Offset (kcal/mol) = -0.345 ± 5.4e-2
ΔG (kcal/mol) = -7.69
-TΔS (kcal/mol) = 3.51
DIDO1_Nde1_H3K4me3_2_190902
Model: One Set of Sites
[Cell] (M) = 100e-6
[Syr] (M) = 1.00e-3
N (sites) = 0.838 ± 3.2e-3
KD (M) = 2.32e-6 ± 110e-9
ΔH (kcal/mol) = -10.9 ± 7.9e-2
Offset (kcal/mol) = -0.351 ± 3.9e-2
ΔG (kcal/mol) = -7.69
-TΔS (kcal/mol) = 3.22
DIDO1_WT_H3K4me3_2_190914
Model: One Set of Sites
[Cell] (M) = 100e-6
[Syr] (M) = 1.00e-3
N (sites) = 0.873 ± 2.9e-3
KD (M) = 2.26e-6 ± 95.5e-9
ΔH (kcal/mol) = -9.83 ± 6.3e-2
Offset (kcal/mol) = -0.185 ± 3.2e-2
ΔG (kcal/mol) = -7.70
-TΔS (kcal/mol) = 2.13

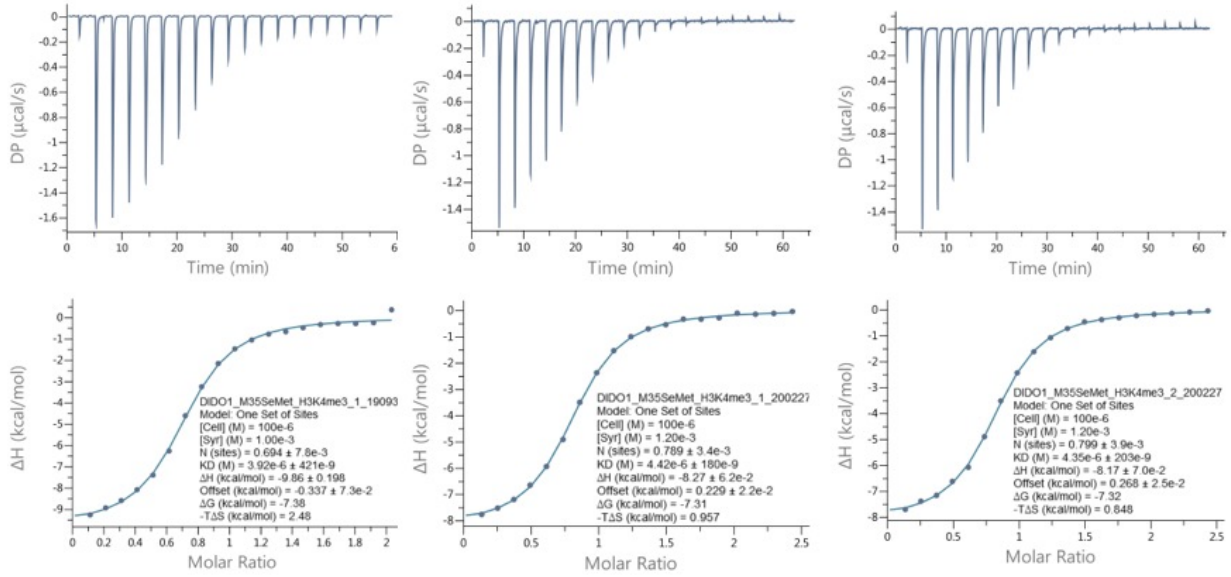
DIDO1 M35C


DP (µcal/s)
Time (min)
ΔH (kcal/mol)
Molar Ratio
DIDO1_M35C_H3K4me3_1_190916
Model: One Set of Sites
[Cell] (M) = 200e-6
[Syr] (M) = 3.00e-3
N (sites) = 0.831 ± 3.6e-3
KD (M) = 36.2e-6 ± 965e-9
ΔH (kcal/mol) = -11.2 ± 0.101
Offset (kcal/mol) = -0.464 ± 1.9e-2
ΔG (kcal/mol) = -6.06
-TΔS (kcal/mol) = 5.16
DIDO1_M35C_H3K4me3_2_190916
Model: One Set of Sites
[Cell] (M) = 200e-6
[Syr] (M) = 3.00e-3
N (sites) = 0.846 ± 2.5e-3
KD (M) = 35.0e-6 ± 648e-9
ΔH (kcal/mol) = -10.9 ± 6.7e-2
Offset (kcal/mol) = -0.459 ± 1.3e-2
ΔG (kcal/mol) = -6.08
-TΔS (kcal/mol) = 4.79

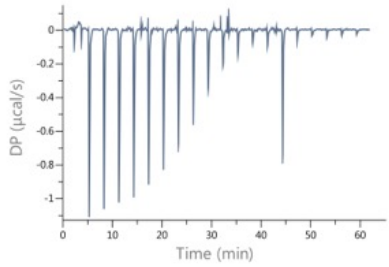
DIDO1 M35Nle

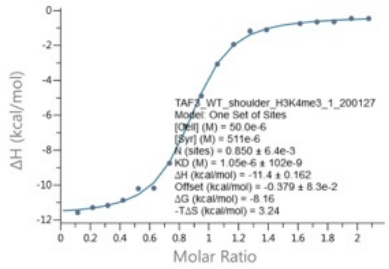


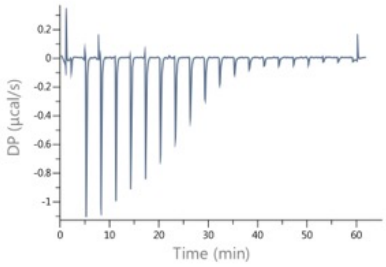
DIDO1 M35SeMet

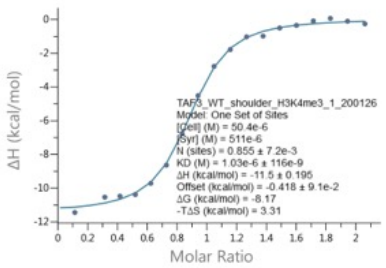


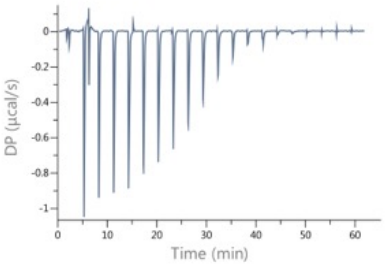
TAF3 MtoQ (WT)

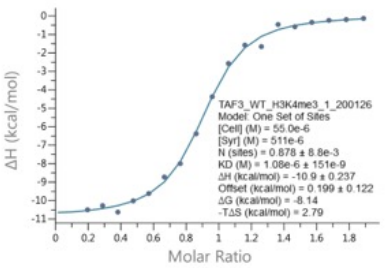




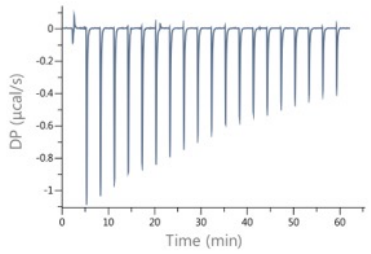


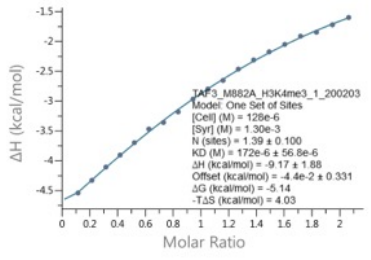


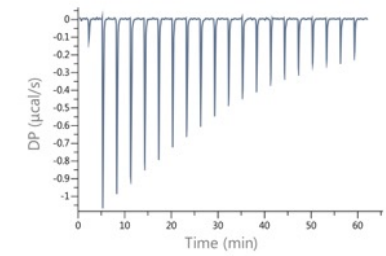


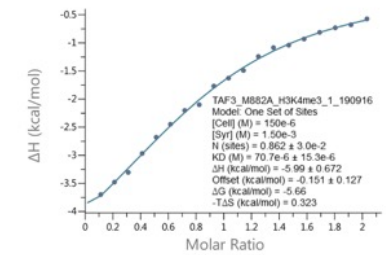


TAF3 M882A

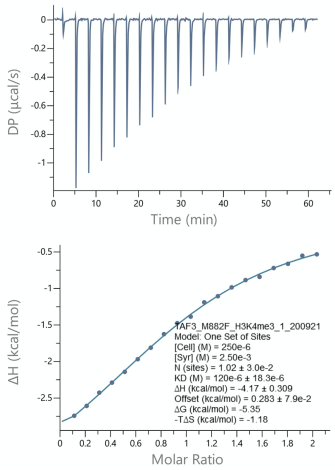




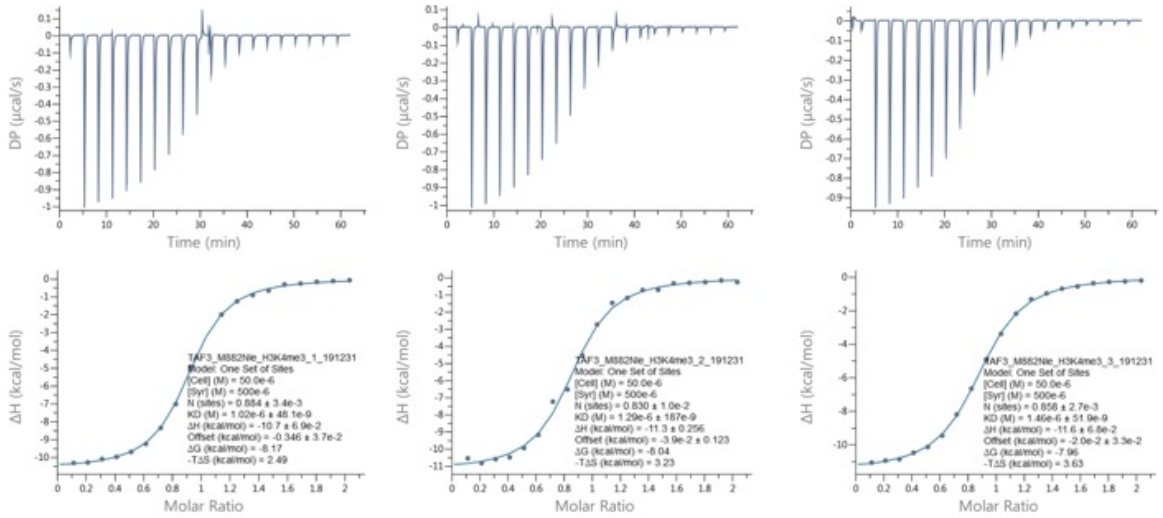




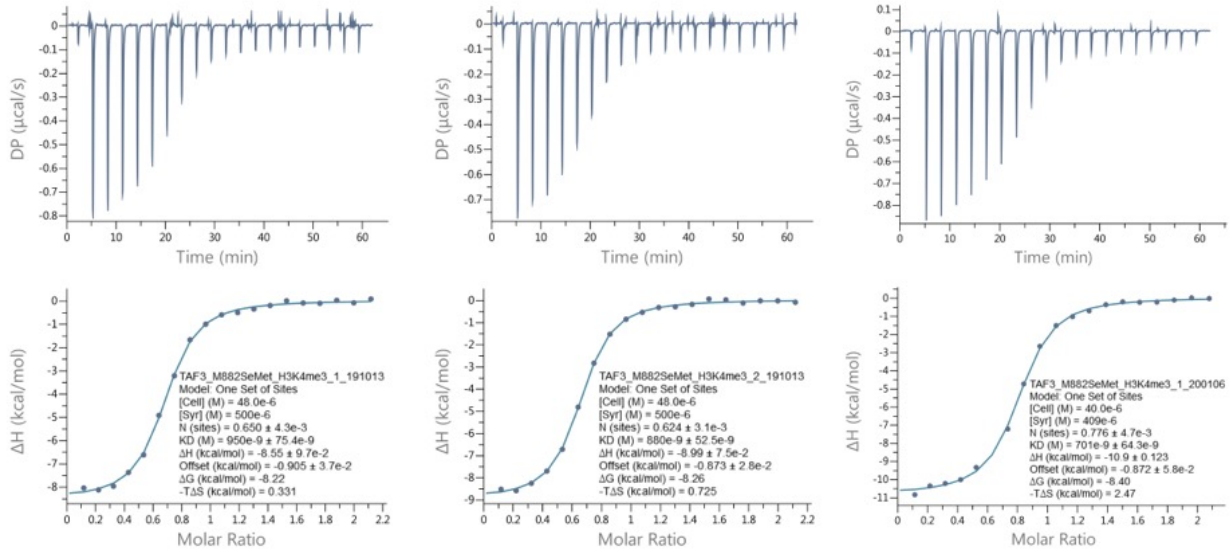
TAF3 M882F



TAF3 M882Nle



TAF3 M882SeMet



BPTF Y23M

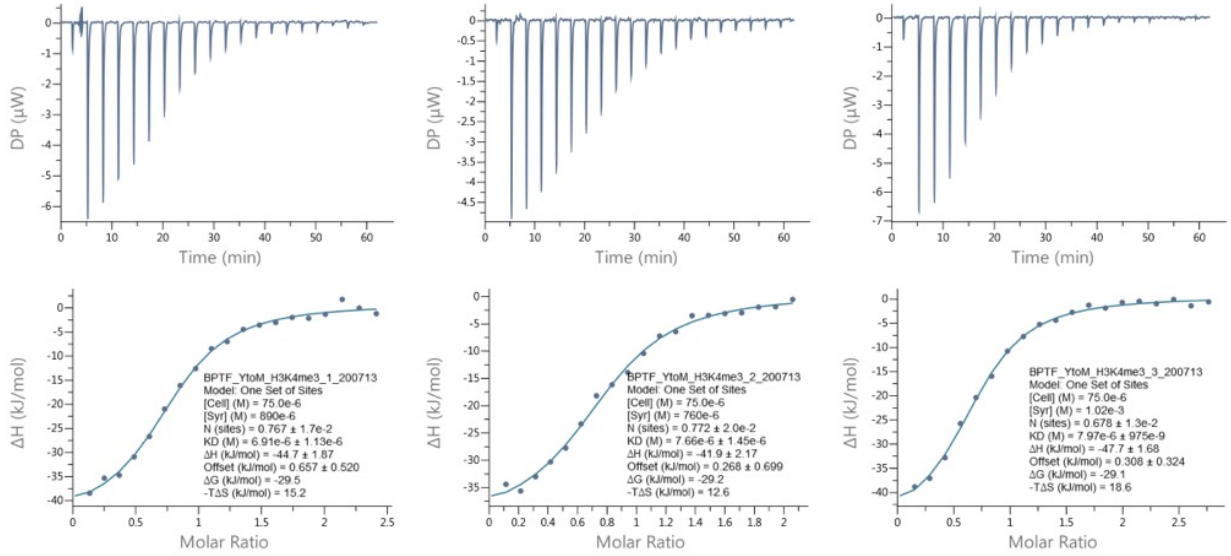
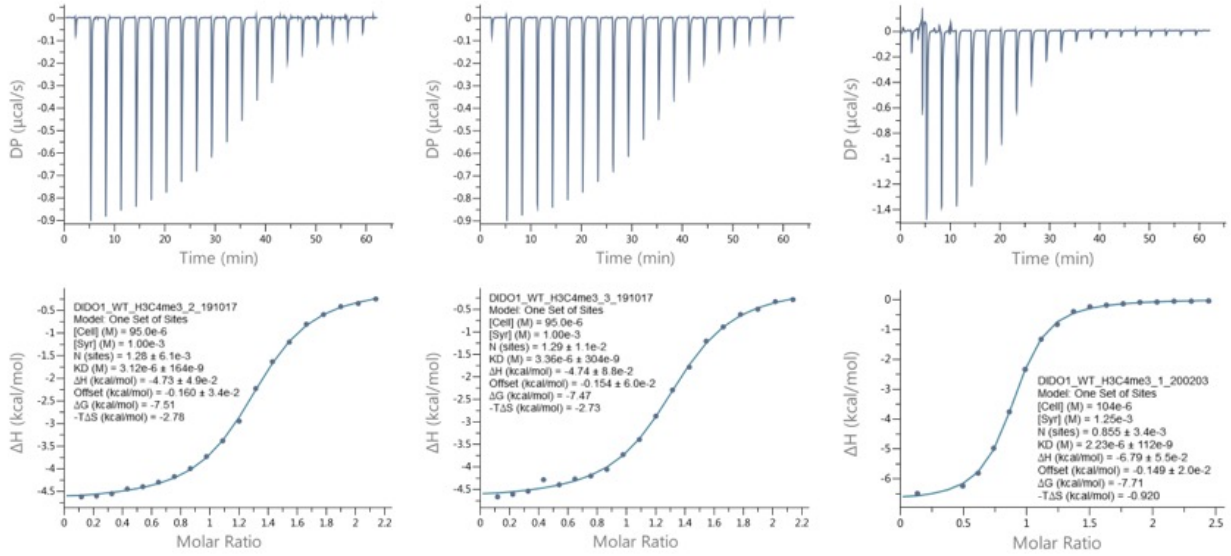


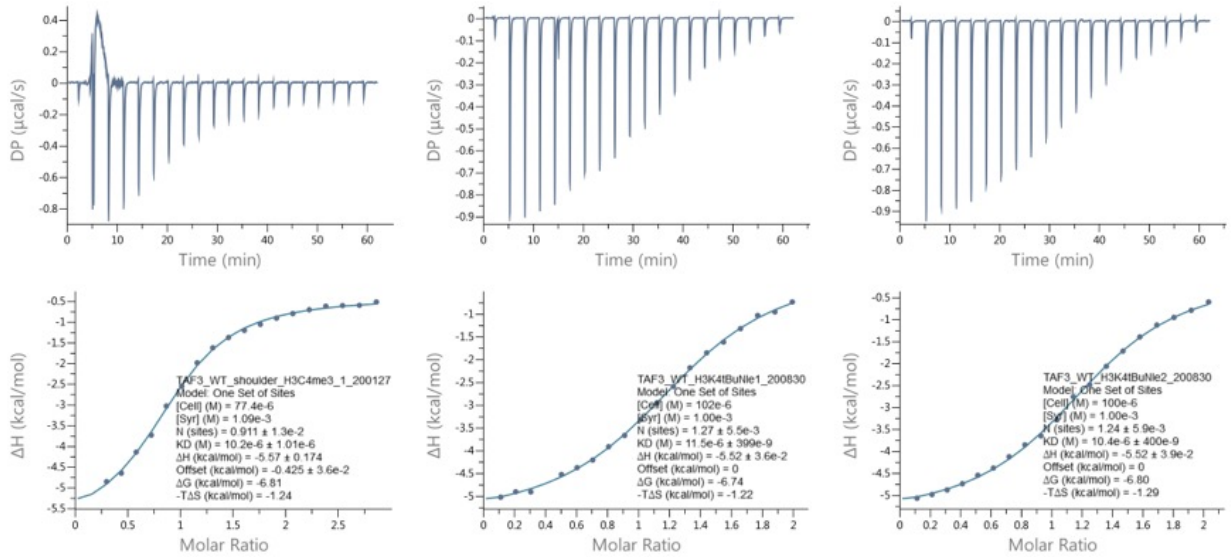
Figure S13. ITC curves of H3K4me3 binding to DIDO1, TAF3, and BPTF PHD domains

H3K4tBuNle:

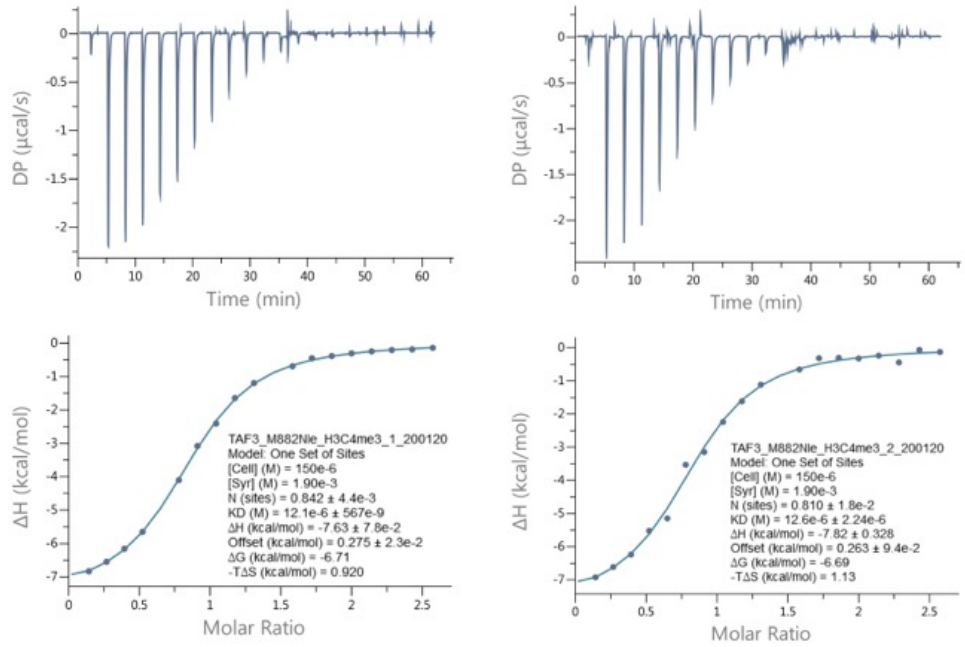
DIDO1 WT



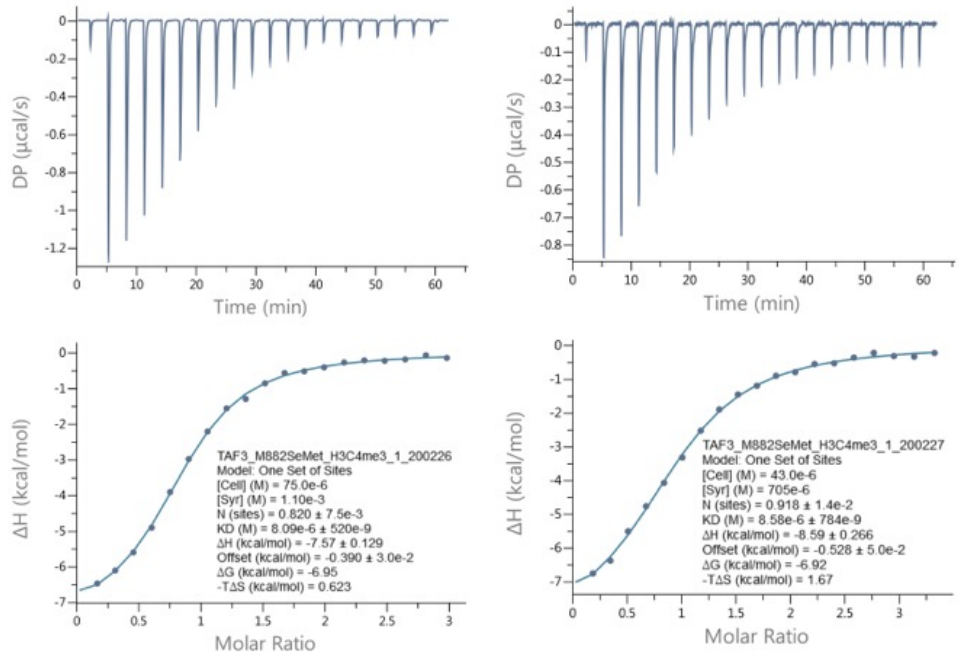
TAF3 MtoQ (WT)



TAF3 M882Nle



TAF3 M882SeMet



BPTF Y23M

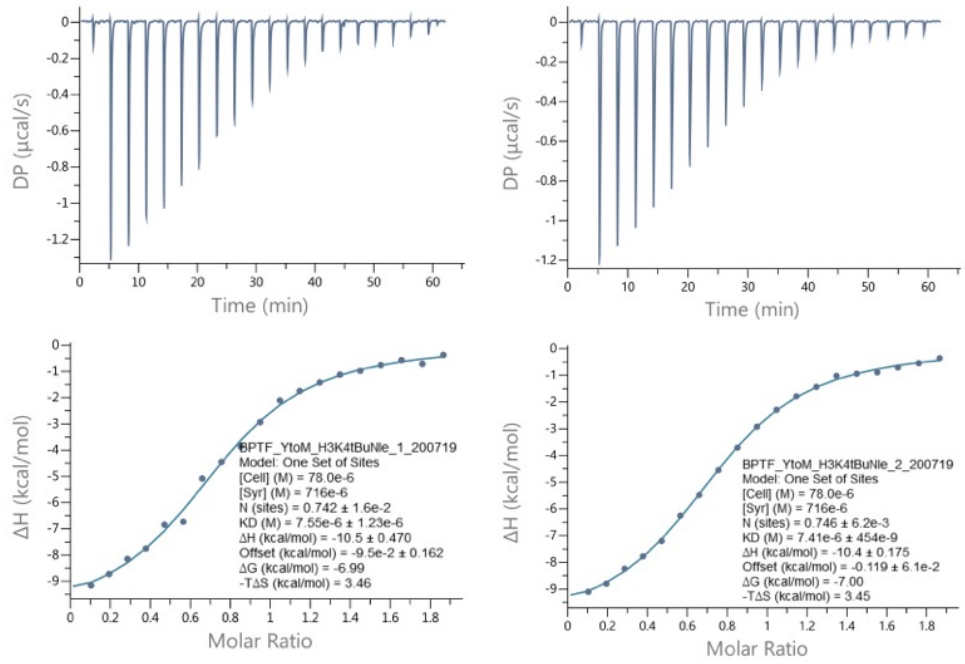


Figure S14. ITC curves of H3K4tBuNle binding to DIDO1, TAF3, and BPTF PHD domains

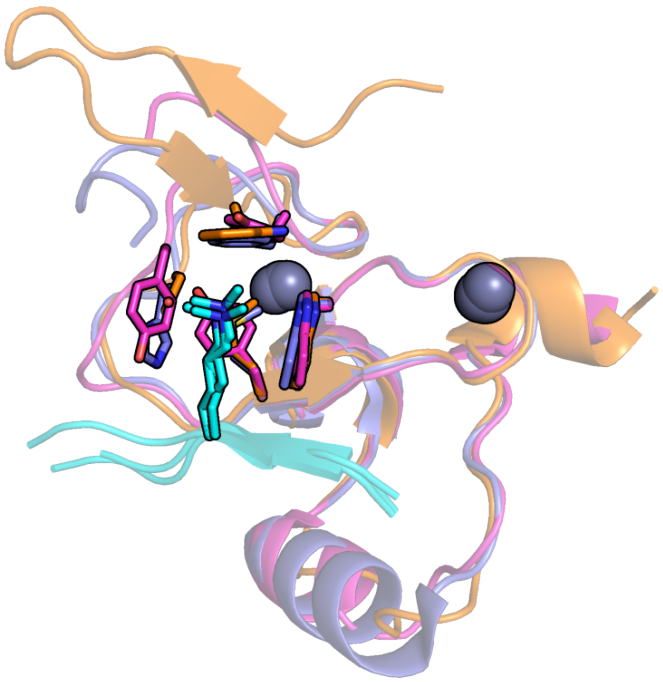


Figure S15. Overlay of TAF3 (PDB ID: 5WXH, orange). DIDO1 (PDB ID:4L7X, purple), and BPTF (PDB ID: 2F6J, magenta) bound to H3K4me3 (cyan).

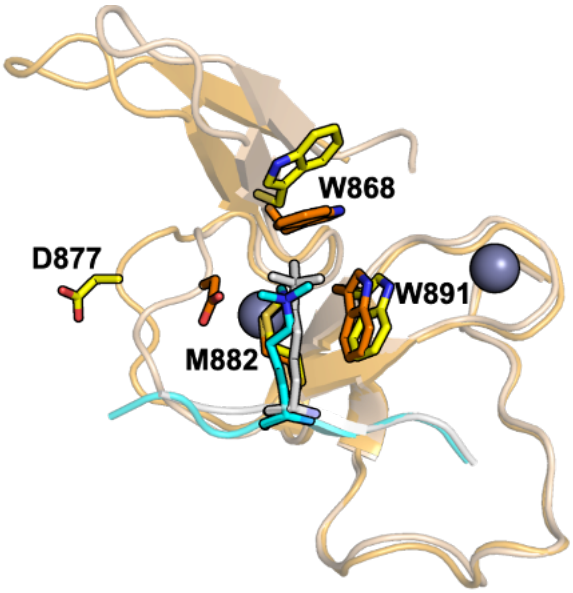


Figure S16. Overlay of TAF3 bound to H3K4me3 (PDB ID 5WXH, orange; H3K4me3, cyan) and H3K4tBuNle (PDB ID: 5C13, yellow, H3K4tBuNle, grey).

1. Studier, F. W., Protein production by auto-induction in high-density shaking cultures. *Protein expression and purification* **2005,** *41* (1), 207-234.
2. Langley, G. W.; Brinkø, A.; Münzel, M.; Walport, L. J.; Schofield, C. J.; Hopkinson, R. J., Analysis of JmjC Demethylase-Catalyzed Demethylation Using Geometrically-Constrained Lysine Analogues. *ACS Chemical Biology* **2016,** *11* (3), 755-762.