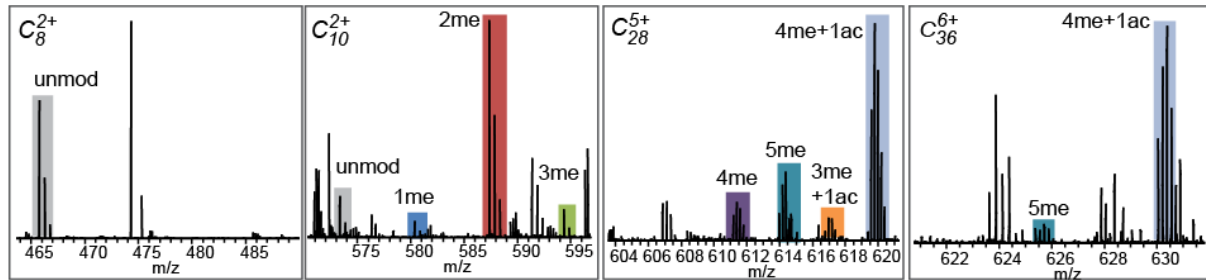


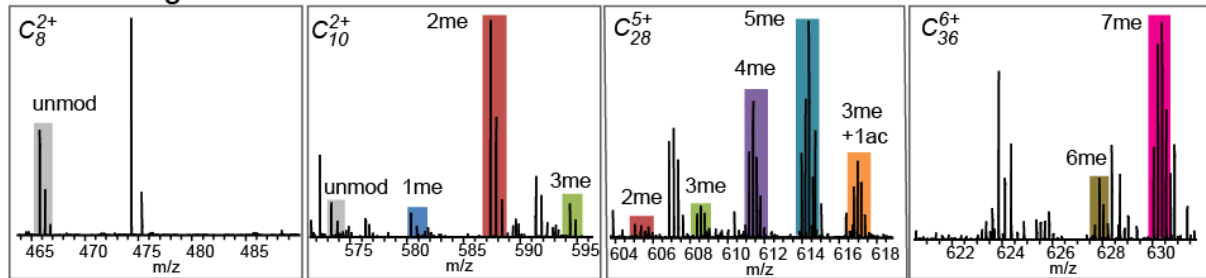
A H3.1 Methyl-Eq 7

MMSET-Low

Legend: unmod (grey), 1me (blue), 2me (red), 3me (green), 4me (purple), 5me (teal), 6me (brown), 3me+1ac (orange), 7me (pink), 4me+1ac (light blue)



MMSET-High



B Major H3.1 Proteoforms of Methyl-Eq 7 in MMSET-Low

```
N A R T K I Q T A R K S T G I G K A P R K Q L A T K A A 25
26 R K S A P A T G G V K K P H R Y R P G T V A L R E 50
51 I R R Y Q K S T E L L I R K L P F Q R L V R E I A 75
76 Q D F K T D L R F Q S S A V M A L Q E A C E A Y L 100
101 V G L F E D T N L C A I H A K R V T I M P K D I Q 125
126 L A R R I R G E R A C
```

H3.1 K9me2-K23ac-K27me2

P-Score: 2.7E-59

PCS: 666

```
N A R T K I Q T A R K S T G I G K A P R K Q L A T K A A 25
26 R K S A P A T G G V K K P H R Y R P G T V A L R E 50
51 I R R Y Q K S T E L L I R K L P F Q R L V R E I A 75
76 Q D F K T D L R F Q S S A V M A L Q E A C E A Y L 100
101 V G L F E D T N L C A I H A K R V T I M P K D I Q 125
126 L A R R I R G E R A C
```

H3.1 K9me2-K18ac-K27me2

P-Score: 1.7E-60

PCS: 682

C Major H3.1 Proteoforms of Methyl-Eq 7 in MMSET-High

```
N A R T K I Q T A R K S T G I G K A P R K Q L A T K A A 25
26 R K S A P A T G G V K K P H R Y R P G T V A L R E 50
51 I R R Y Q K S T E L L I R K L P F Q R L V R E I A 75
76 Q D F K T D L R F Q S S A V M A L Q E A C E A Y L 100
101 V G L F E D T N L C A I H A K R V T I M P K D I Q 125
126 L A R R I R G E R A C
```

H3.1 K9me3-K27me2-K36me2

P-Score: 5.3E-70

PCS: 805

```
N A R T K I Q T A R K S T G I G K A P R K Q L A T K A A 25
26 R K S A P A T G G V K K P H R Y R P G T V A L R E 50
51 I R R Y Q K S T E L L I R K L P F Q R L V R E I A 75
76 Q D F K T D L R F Q S S A V M A L Q E A C E A Y L 100
101 V G L F E D T N L C A I H A K R V T I M P K D I Q 125
126 L A R R I R G E R A C
```

H3.1 K9me2-K27me3-K36me2

P-Score: 3.8E-61

PCS: 690

```
N A R T K I Q T A R K S T G I G K A P R K Q L A T K A A 25
26 R K S A P A T G G V K K P H R Y R P G T V A L R E 50
51 I R R Y Q K S T E L L I R K L P F Q R L V R E I A 75
76 Q D F K T D L R F Q S S A V M A L Q E A C E A Y L 100
101 V G L F E D T N L C A I H A K R V T I M P K D I Q 125
126 L A R R I R G E R A C
```

H3.1 K9me1-K27me3-K36me2-K79me1

P-Score: 1.4E-37

PCS: 388

Fig. S6. Different proteoforms present in Methyl Equivalent 7 for H3.1 variant in MMSET-Low and MMSET-High cells. (A) Raw mass spectrometry data of *c* ions from ETD-MS² and their matched modifications. (B) and (C) Graphical representation of major proteoforms determined by EThcD using ProSight Lite. Green, cyan, and purple squares indicate mono-, di-, and tri-methylation respectively; red square: acetylation; red rotated-L bar: *c* ions; red L bar: *z* ions; blue rotated-L bar: *b* ions; blue L bar: *y* ions.