

Supplementary Information Guide

Supplementary Figure 1: Uncropped western blots. Format: eighteen image display items.

Table S1: Histone post translational modification mass spectrometry data in resting and stimulated BMDM. See methods for details. Format: spreadsheet and graph.

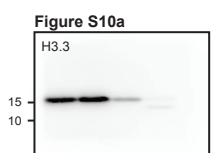
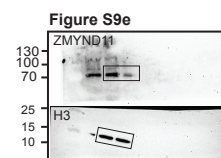
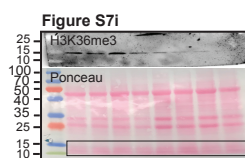
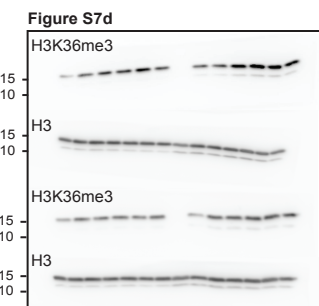
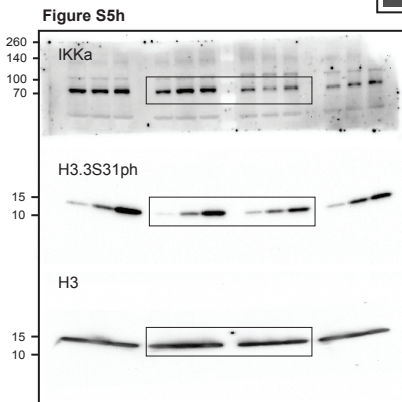
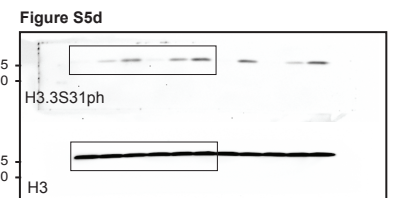
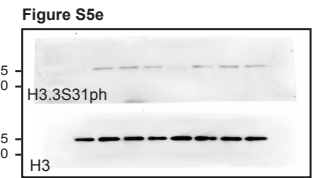
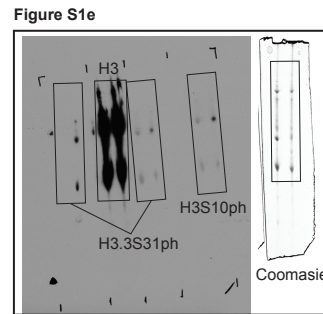
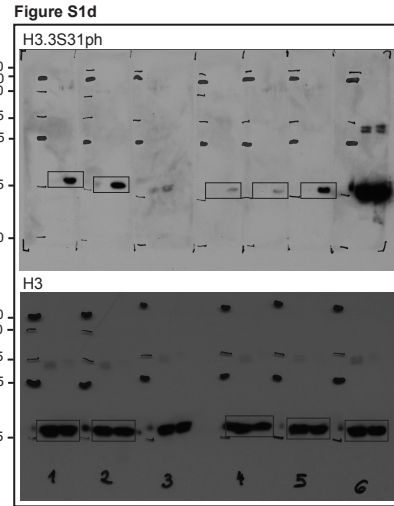
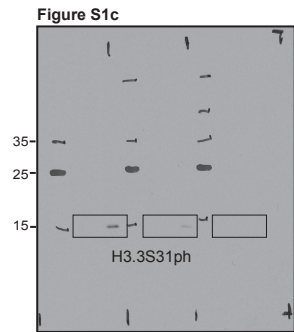
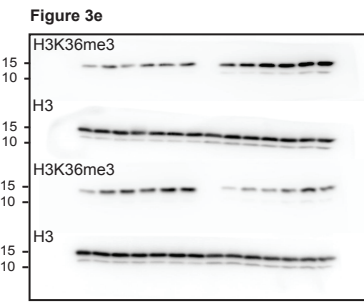
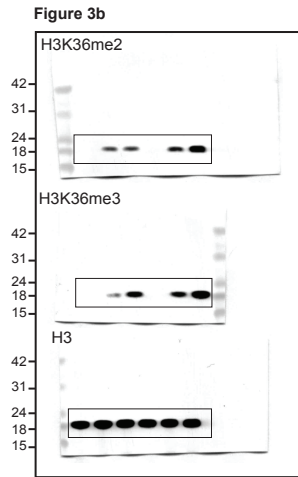
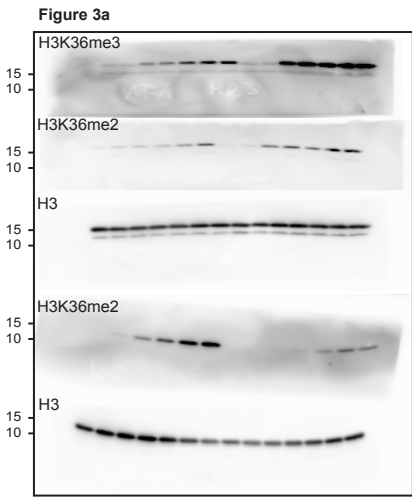
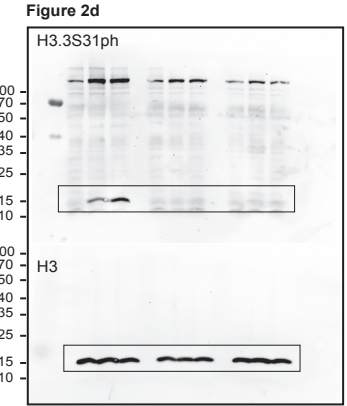
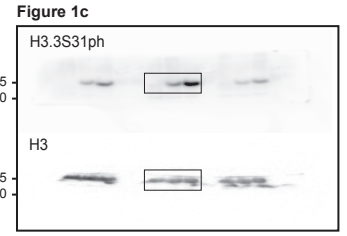
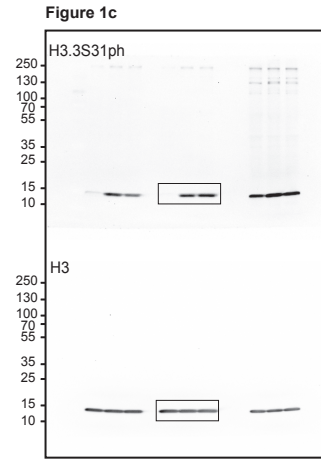
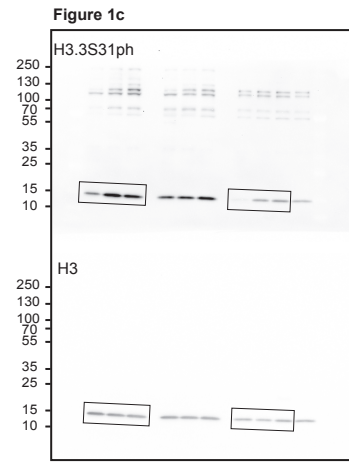
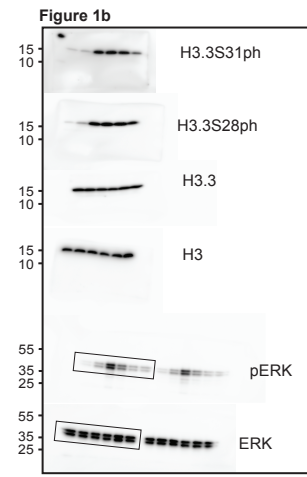
Table S2: List of top ranked H3.3S31ph peaks, BMDM, 60 min LPS. Genes were ranked by H3.3S31ph ChIPseq tag density from transcription start site to transcription end site. Format: gene list in rank order.

Table S3: SETD2:H3.3S31ph structure characteristics. Data collection and refinement statistics. Format: text table.

Table S4: Intersection of H3.3S31ph top 1% genes and H3.3S31ph peak-annotated genes. Format: gene list in alphabetical order.

Supplementary information Figure 1

Uncropped scans of Western blots

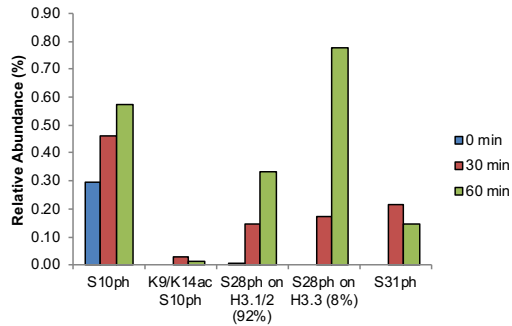


Supplemental Table 1

MS/MS quantitative analysis of H3 PTMs

H3 9-17	0	30	60	H3.1/2 27-40	0	30	60	H3.3 27-40	0	30	60
Unmod	6.93%	5.35%	3.18%	Unmod	1.12%	0.98%	0.76%	Relative H3.3 amount (out of total I	8.28%	7.44%	7.30%
K9me1K14ac	3.46%	2.66%	1.85%	K27me1	3.55%	2.85%	2.59%	Unmod	3.08%	2.92%	2.31%
K9me1	4.42%	3.13%	1.67%	K36me1	0.24%	0.28%	0.17%	K27me1	1.94%	2.34%	1.71%
K9me2	35.14%	35.53%	35.59%	K27me2	35.83%	39.27%	40.06%	K36me1	0.00%	0.08%	0.00%
K9me3K14ac	6.41%	7.11%	8.53%	K36me2	0.56%	0.41%	0.33%	K27me2	11.09%	12.33%	10.95%
K9me3	30.31%	29.64%	30.37%	K27me3	10.97%	12.34%	12.23%	K36me2	10.60%	9.90%	10.67%
K9/K14ac	4.47%	4.23%	3.34%	K27me2K36me1	16.37%	14.48%	11.34%	K27me3	6.09%	6.52%	6.64%
K9me2 K14Ac	30.89%	31.29%	35.67%	K27me1K36me2	2.00%	1.50%	1.06%	K27me2K36me1	14.06%	14.75%	13.43%
K9Ac K14Ac	0.15%	0.16%	0.14%	K27me1K36me1	1.14%	0.84%	0.70%	K27me1K36me2	13.70%	10.45%	9.75%
S10ph	0.298%	0.460%	0.575%	K27me3K36me1	7.81%	6.90%	5.37%	K27me1K36me1	1.29%	1.07%	0.39%
Within the S10ph:				K27me1K36me3	3.10%	0.86%	0.65%	K27me3K36me1	8.06%	7.81%	5.66%
S10ph	1.84%	3.14%	1.31%	K27me2K36me2	10.38%	11.24%	14.18%	K27me1K36me3	8.88%	7.32%	6.33%
K9me1S10phK14ac	0.00%	0.00%	0.00%	K27me3K36me2	6.70%	7.57%	9.77%	K27me2K36me2	5.29%	4.77%	5.36%
K9me1S10ph	0.00%	2.14%	1.10%	K27me3K36me3	0.21%	0.34%	0.44%	K27me3K36me2	15.55%	18.99%	24.98%
K9me2S10ph	28.09%	24.36%	23.88%	S28ph	0.004%	0.144%	0.332%	K27me3K36me3	0.36%	0.34%	0.91%
K9me3S10phK14ac	12.14%	10.59%	15.28%	Within the S28ph				Sph	0.000%	0.389%	0.922%
K9me3S10ph	42.19%	35.66%	34.87%	K27me2S28ph	0.00%	5.11%	1.12%	Within Sph			
K9/K14ac S10ph	0.00%	2.77%	1.60%	K27me3S28ph	0.00%	5.00%	3.42%	K27me2S28ph	0.00%	0.82%	2.15%
K9me2S10phK14Ac	15.74%	17.56%	21.19%	K27me3S28phK36me1	0.00%	1.26%	1.59%	K27me2S28phK36me1	0.00%	2.63%	0.00%
K9AcS10phK14Ac	0.00%	3.78%	0.78%	K27me1S28phK36me3	0.00%	0.11%	0.30%	K27me1S28phK36me2	0.00%	0.38%	0.00%
K9ac/K14ac S10ph	0.00%	6.55%	2.38%	K27me2S28phK36me2	0.00%	48.82%	38.80%	K27me2S31phK36me2	0.00%	55.16%	15.64%
	0.00%	0.03%	0.01%	K27me3S28phK36me2	0.00%	39.69%	54.60%	K27me3S28phK36me2	0.00%	41.01%	82.21%

Time	0 min	30 min	60 min
S10ph	0.30	0.46	0.57
K9/K14ac S10ph	0.00	0.03	0.01
S28ph on H3.1/2 (92%)	0.00	0.14	0.33
S28ph on H3.3 (8%)	0.00	0.17	0.78
S31ph	0.00	0.21	0.14
Sph on H3.3	0.00	0.39	0.92



Supplemental Table 2
Ordered top 100 H3.3S31ph peaks annotated to genes

Tnf
Cxcl2
Fbxo48
Tnfaip2
Nsd1
Rgs1
Tnfaip3
Nfkbia
Fth1
Nfkbia
Neat1
Nsd1
Mmp12
Dusp1
Zfp36
Mir221
Cnrip1
Rheb
Junb
Tnfaip3
Cd44
Gm21188
Mir222
Klf6
Cnrip1
Cd44
Mir21a
Pim1
Cd44
Mcl1
Nsd1
Ubc
Cd44
Tnfaip2
Slfn2
Lsmem1
Lgals3
Malt1
Cxcl1
Cd44
Mmp12
Icam4
4930444G20Rik
Gadd45b
Cnrip1
H3f3b
Lgals3

Lgals3
Ehd1
Malsu1
Mthfs
Mir222
Prkg2
Ftl1
Ier2
Gm20826
Irf1
Mir1938
Malsu1
Mir222
Plk2
Spp1
Ccrl2
Mthfsl
Cflar
Gm15723
Socs3
Mdga2
Klf6
Dusp1
Brd2
Vps13d
Clec2d
Ccl9
Btg1
Clec2d
Vim
Actb
Egr2
Egr2
Irf1
Gm21188
Mir5131
Nfkbiz
H2-D1
Tmem189
Gm5294
Clec2d
Gucy1a2
Mthfs
Tmsb4x
Gm21677
Cd44
Cd44
Smurf2
Mir5131
Hnrnp11

Ccl9
Il1a
Ide

Supplemental Table 3,
"Intersection of H3.3S31ph ""peak"" genes and Top 1% genes",

,gene_names

1,Abca1
2,Actb
3,Alas1
4,Anxa1
5,Bbs10
6,Bcl2a1b
7,Bcl2l11
8,Brd2
9,Btg1
10,Btg2
11,Ccl3
12,Ccl4
13,Ccl9
14,Ccnl1
15,Ccrl2
16,Cd14
17,Cd200
18,Cd274
19,Cd44
20,Cd68
21,Cd83
22,Cdkn1a
23,Cflar
24,Clec2d
25,Clec4d
26,Clic4
27,Cpeb4
28,Csrnp1
29,Ctsb
30,Ctsd
31,Ctsl
32,Cxcl1
33,Cxcl2
34,Cybb
35,Dtx4
36,Dusp1
37,Dusp4
38,Egr2
39,Ehd1
40,Eif4a1
41,Erdr1
42,Fabp4
43,Fam129b
44,Fam20c
45,Fnip2
46,Fth1
47,Ftl1

48,G530011006Rik
49,Gadd45b
50,Glipr1
51,Gna13
52,Gpnmb
53,Gpr137b
54,H2-D1
55,H2-K1
56,H3f3b
57,Hapln3
58,Hnrnp11
59,Icam1
60,Icam4
61,Id2
62,Ier2
63,Igf1
64,Igsf6
65,Il1a
66,Il1b
67,Il1bos
68,Irf1
69,Itga5
70,Junb
71,Klf6
72,Lcp1
73,Lgals3
74,Lpl
75,Lrp12
76,Lyz2
77,Malat1
78,Malt1
79,Mamdc2
80,Mapkapk2
81,Marcks
82,Mcl1
83,Mdm2
84,Met
85,Mir142b
86,Mir1894
87,Mir221
88,Mir222
89,Mir23a
90,Mir3064
91,Mir3109
92,Mir5129
93,Mir7683
94,Mmp12
95,Mpeg1
96,Mtpn
97,Neat1

98,Nfe2l2
99,Nfkbia
100,Nfkbid
101,Nfkbiz
102,Nlrp3
103,Nrp2
104,Olfr111
105,Olrl
106,Pik3r5
107,Pim1
108,Plau
109,Plekho2
110,Plk2
111,Ppp1r15a
112,Prdx1
113,Psap
114,Rab8b
115,Rassf4
116,Rhob
117,Rnf149
118,Rnf19b
119,Rtn4
120,Sdc4
121,Sirpa
122,Slamf7
123,Slc2a6
124,Slc35b2
125,Slc7a2
126,Slfn2
127,Snord89
128,Socs3
129,Spp1
130,Sqstm1
131,Syk
132,Tfec
133,Tgif1
134,Tmsb4x
135,Tnf
136,Tnfaip2
137,Tnfaip3
138,Tnfrsf1b
139,Tnip1
140,Tnip3
141,Txn1
142,Txnip
143,Ubc
144,Vim
145,Zeb2os
146,Zfp36

Supplementary Table 4: Data collection and refinement statistics

SETD2-SAH-H3.3S31phK36M	
Data collection	
Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions	
a, b, c (Å)	61.1, 77.0, 77.4
α, β, γ (°)	90, 90, 90
Resolution (Å)	50-1.78 (1.81-1.78)*
R _{merge}	7.5 (36.5)
I/σI	27(3.7)
Completeness (%)	99.8 (97)
Redundancy	6.5 (5.9)
Refinement	
Resolution (Å)	34.58 - 1.78
No. reflections	35706
R _{work} /R _{free} (%)	17.8/19.7
No. atoms	
Protein	1983
Peptide/SAH/Zn	112/26/3
Water	284
Others	12
B-factors (Å ²)	
Protein	30.5
Peptide/SAH/Zn	30.2/20.9/25.1
Water	38.2
Others	46
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.18

* Values in parentheses are for highest-resolution shell.