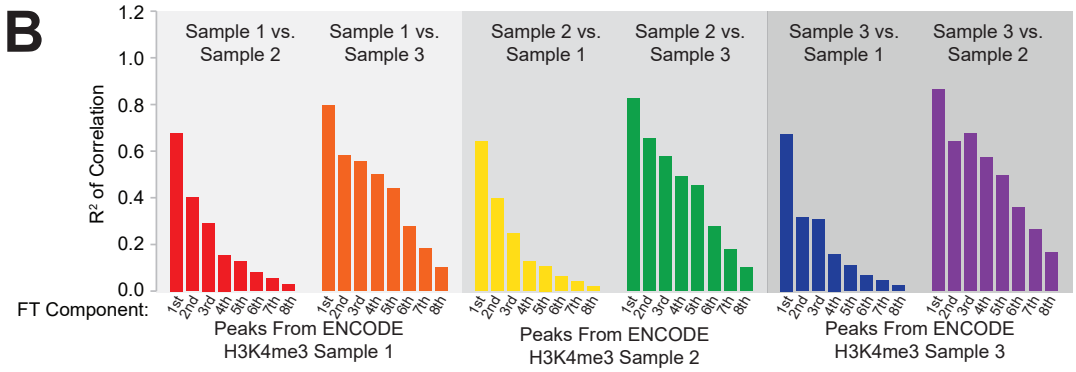
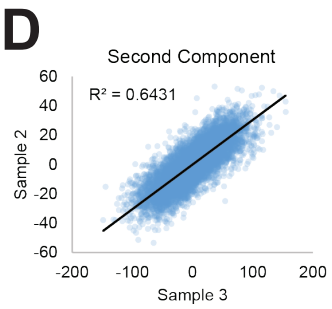
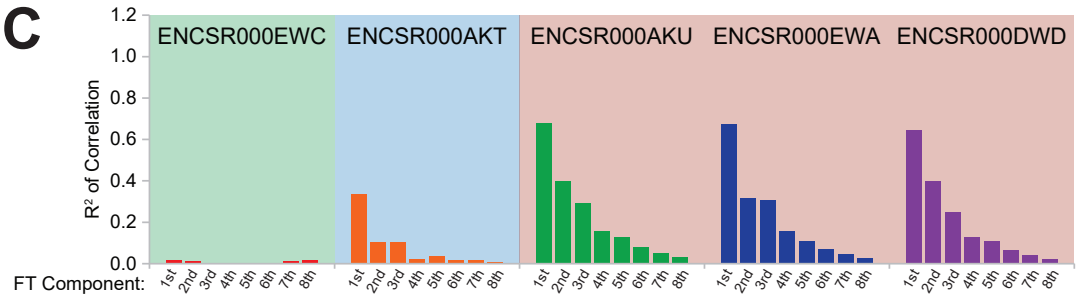
Figure S1

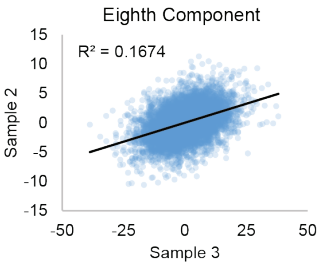
A

	EMD-07-473 ENCSR000AKU	ab8580 ENCSR668LDD	CST-9751S ENCSR000EWA	CST-9751S ENCSR000DWD
Replicated peaks	43,677	21,402	22,884	30,800
Replicated peaks intersecting replicated peaks from all other ENCODE H3K4me3 Datasets in K562	22,455 **(51.4%)**	17,230 **(80.5%)**	18,563 **(81.1%)**	20,248 **(65.7%)**









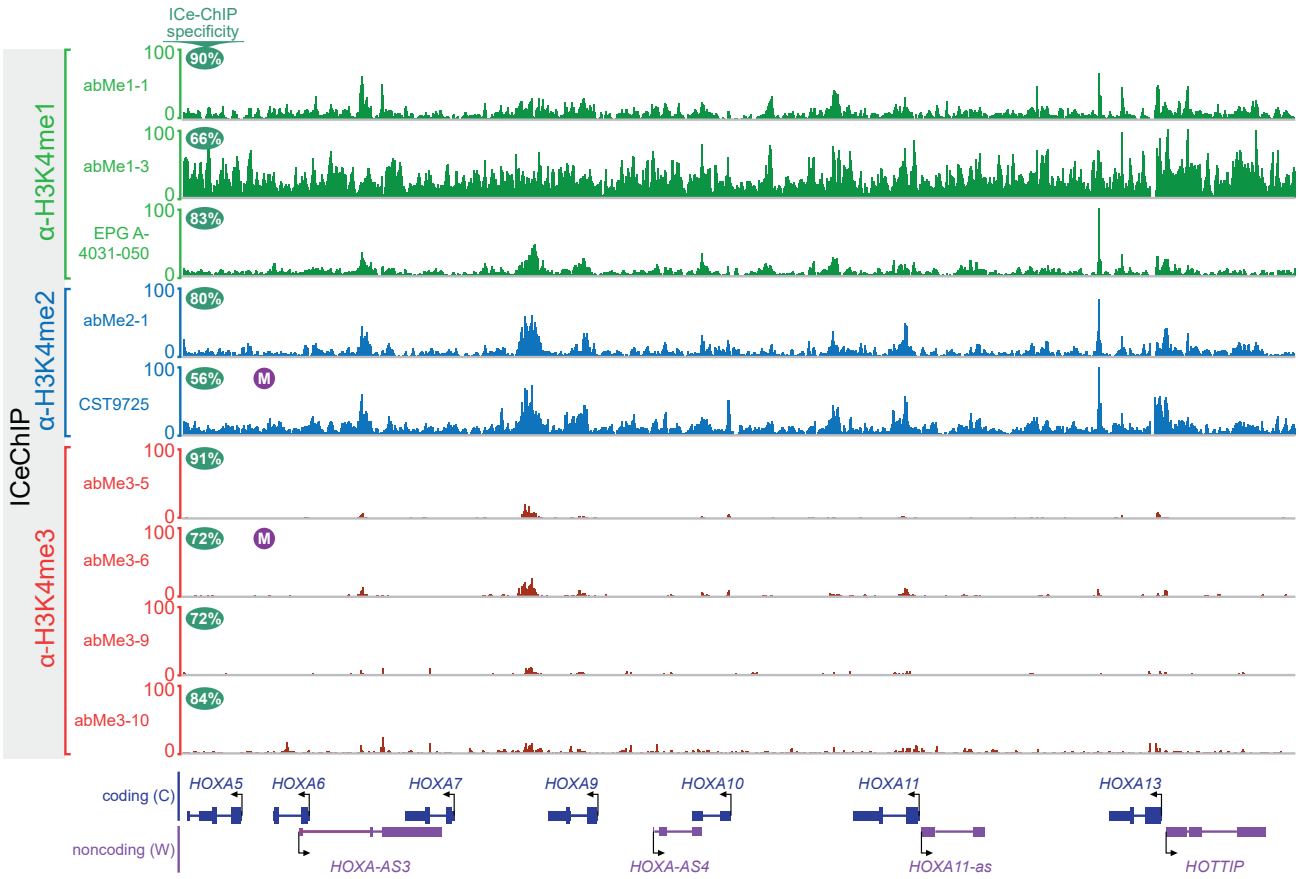


Figure S2

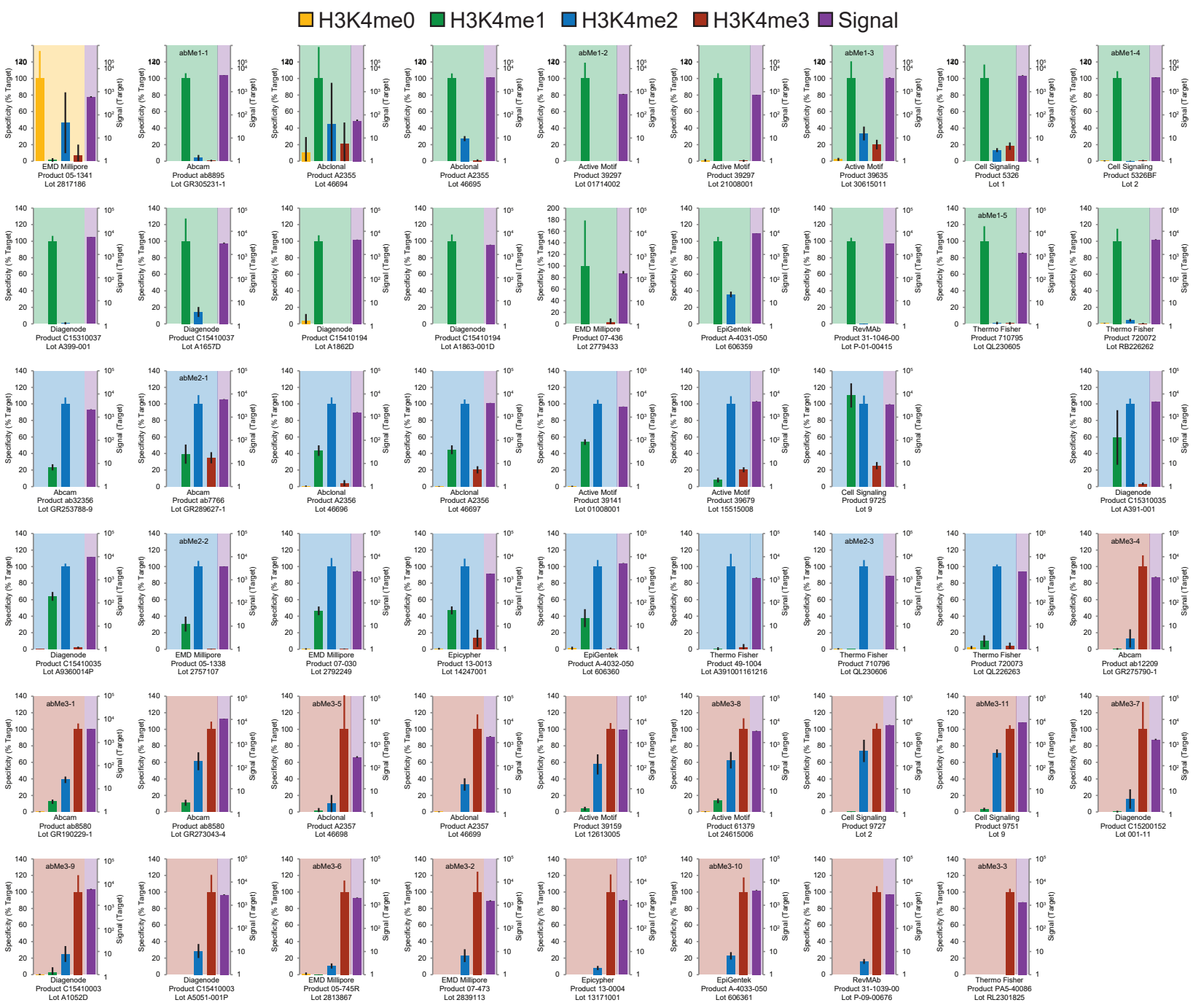

H3K4me0
H3K4me1
H3K4me2
H3K4me3
Signal
Specificity (% Target)
Signal (Target)
EMD Millipore
Product 05-1341
Lot 2817186
abMe1-1
Abcam
Product ab8895
Lot GR305231-1
Abclonal
Product A2355
Lot 46694
Abclonal
Product A2355
Lot 46695
abMe1-2
Active Motif
Product 39297
Lot 01714002
Active Motif
Product 39297
Lot 21008001
abMe1-3
Active Motif
Product 39635
Lot 30615011
Cell Signaling
Product 5326
Lot 1
abMe1-4
Cell Signaling
Product 5326BF
Lot 2
Diagenode
Product C15310037
Lot A399-001
Diagenode
Product C15410037
Lot A1657D
Diagenode
Product C15410194
Lot A1862D
Diagenode
Product C15410194
Lot A1863-001D
EMD Millipore
Product 07-436
Lot 2779433
EpiGentek
Product A-4031-050
Lot 606359
RevMAb
Product 31-1046-00
Lot P-01-00415
abMe1-5
Thermo Fisher
Product 710795
Lot QL230605
Thermo Fisher
Product 720072
Lot RB226262
Abcam
Product ab32356
Lot GR253788-9
abMe2-1
Abcam
Product ab7766
Lot GR289627-1
Abclonal
Product A2356
Lot 46696
Abclonal
Product A2356
Lot 46697
Active Motif
Product 39141
Lot 01008001
Active Motif
Product 39679
Lot 15515008
Cell Signaling
Product 9725
Lot 9
Diagenode
Product C15310035
Lot A391-001
Diagenode
Product C15410035
Lot A9360014P
abMe2-2
EMD Millipore
Product 05-1338
Lot 2757107
EMD Millipore
Product 07-030
Lot 2792249
Epicypher
Product 13-0013
Lot 14247001
EpiGentek
Product A-4032-050
Lot 606360
Thermo Fisher
Product 49-1004
Lot A39100116121 6
abMe2-3
Thermo Fisher
Product 710796
Lot QL230606
Thermo Fisher
Product 720073
Lot QL226263
abMe3-4
Abcam
Product ab12209
Lot GR275790-1
abMe3-1
Abcam
Product ab8580
Lot GR190229-1
Abcam
Product ab8580
Lot GR273043-4
abMe3-5
Abclonal
Product A2357
Lot 46698
Abclonal
Product A2357
Lot 46699
Active Motif
Product 39159
Lot 12613005
abMe3-8
Active Motif
Product 61379
Lot 24615006
Cell Signaling
Product 9727
Lot 2
abMe3-11
Cell Signaling
Product 9751
Lot 9
abMe3-7
Diagenode
Product C15200152
Lot 001-11
abMe3-9
Diagenode
Product C15410003
Lot A1052D
Diagenode
Product C15410003
Lot A5051-001P
abMe3-6
EMD Millipore
Product 05-745R
Lot 2813867
abMe3-2
EMD Millipore
Product 07-473
Lot 2839113
Epicypher
Product 13-0004
Lot 13171001
abMe3-10
EpiGentek
Product A-4033-050
Lot 606361
RevMAb
Product 31-1039-00
Lot P-09-00676
abMe3-3
Thermo Fisher
Product PA5-40086
Lot RL2301825

Figure S3

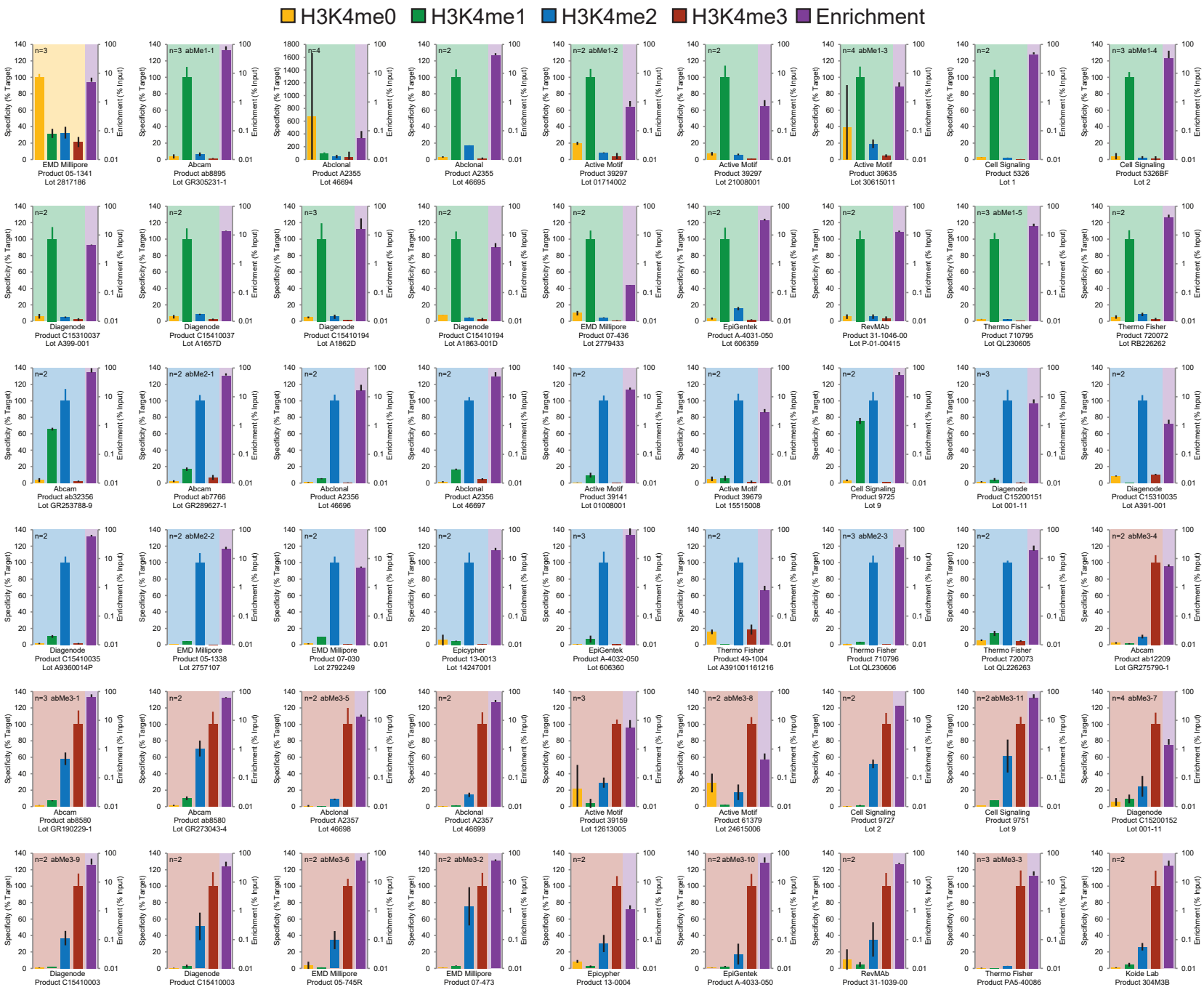
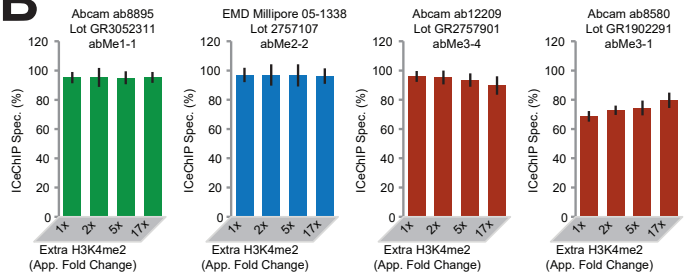

H3K4me0
H3K4me1
H3K4me2
H3K4me3
Enrichment
Specificity (% Target)
Enrichment (% Input)
n=3
EMD Millipore
Product 05-1341
Lot 2817186
n=3 abMe1-1
Abcam
Product ab8895
Lot GR305231-1
n=4
Abclonal
Product A2355
Lot 46694
n=2
Abclonal
Product A2355
Lot 46695
n=2 abMe1-2
Active Motif
Product 39297
Lot 01714002
n=2
Active Motif
Product 39297
Lot 21008001
n=4 abMe1-3
Active Motif
Product 39635
Lot 30615011
n=2
Cell Signaling
Product 5326
Lot 1
n=3 abMe1-4
Cell Signaling
Product 5326BF
Lot 2
n=2
Diagenode
Product C15310037
Lot A399-001
n=2
Diagenode
Product C15410037
Lot A1657D
n=3
Diagenode
Product C15410194
Lot A1862D
n=2
Diagenode
Product C15410194
Lot A1863-001D
n=2
EMD Millipore
Product 07-436
Lot 2779433
n=2
EpiGentek
Product A-4031-050
Lot 606359
n=2
RevMAb
Product 31-1046-00
Lot P-01-00415
n=3 abMe1-5
Thermo Fisher
Product 710795
Lot QL230605
n=2
Thermo Fisher
Product 720072
Lot RB226262
n=2
Abcam
Product ab32356
Lot GR253788-9
n=2 abMe2-1
Abcam
Product ab7766
Lot GR289627-1
n=2
Abclonal
Product A2356
Lot 46696
n=2
Abclonal
Product A2356
Lot 46697
n=2
Active Motif
Product 39141
Lot 01008001
n=2
Active Motif
Product 39679
Lot 15515008
n=2
Cell Signaling
Product 9725
Lot 9
n=3
Diagenode
Product C15200151
Lot 001-11
n=2
Diagenode
Product C15310035
Lot A391-001
n=2
Diagenode
Product C15410035
Lot A9360014P
n=2 abMe2-2
EMD Millipore
Product 05-1338
Lot 2757107
n=2
EMD Millipore
Product 07-030
Lot 2792249
n=2
Epicypher
Product 13-0013
Lot 14247001
n=3
EpiGentek
Product A-4032-050
Lot 606360
n=2
Thermo Fisher
Product 49-1004
Lot A3910011612l6
n=3 abMe2-3
Thermo Fisher
Product 710796
Lot QL230606
n=2
Thermo Fisher
Product 720073
Lot QL226263
n=2 abMe3-4
Abcam
Product ab12209
Lot GR275790-1
n=3 abMe3-1
Abcam
Product ab8580
Lot GR190229-1
n=2
Abcam
Product ab8580
Lot GR273043-4
n=2 abMe3-5
Abclonal
Product A2357
Lot 46698
n=2
Abclonal
Product A2357
Lot 46699
n=3
Active Motif
Product 39159
Lot 12613005
n=2 abMe3-8
Active Motif
Product 61379
Lot 24615006
n=2
Cell Signaling
Product 9727
Lot 2
n=2 abMe3-11
Cell Signaling
Product 9751
Lot 9
n=4 abMe3-7
Diagenode
Product C15200152
Lot 001-11
n=2 abMe3-9
Diagenode
Product C15410003
Lot A1052D
n=2
Diagenode
Product C15410003
Lot A5051-001P
n=2 abMe3-6
EMD Millipore
Product 05-745R
Lot 2813867
n=2 abMe3-2
EMD Millipore
Product 07-473
Lot 2839113
n=2
Epicypher
Product 13-0004
Lot 13171001
n=2 abMe3-10
EpiGentek
Product A-4033-050
Lot 606361
n=2
RevMAb
Product 31-1039-00
Lot P-09-00676
n=3 abMe3-3
Thermo Fisher
Product PA5-40086
Lot RL2301825
n=2
Koide Lab
Product 304M3B
Lot 040416AG

Figure S4

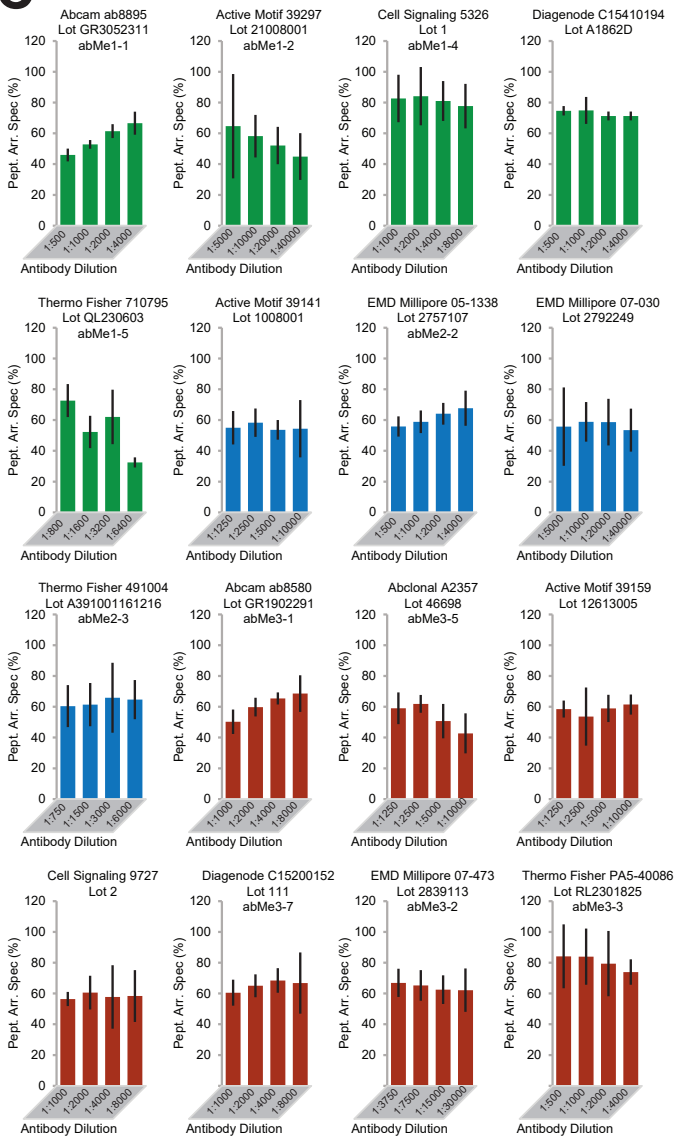
A

	ICeChIP More Specific	Approx. Equally Specific	Pept. Arrays More Specific	Total
α-H3K4me0	0	1	0	1
α-H3K4me1	1	11	5	17
α-H3K4me2	12	2	2	16
α-H3K4me3	1	11	6	18
Total	14	25	13	52

B



C



D

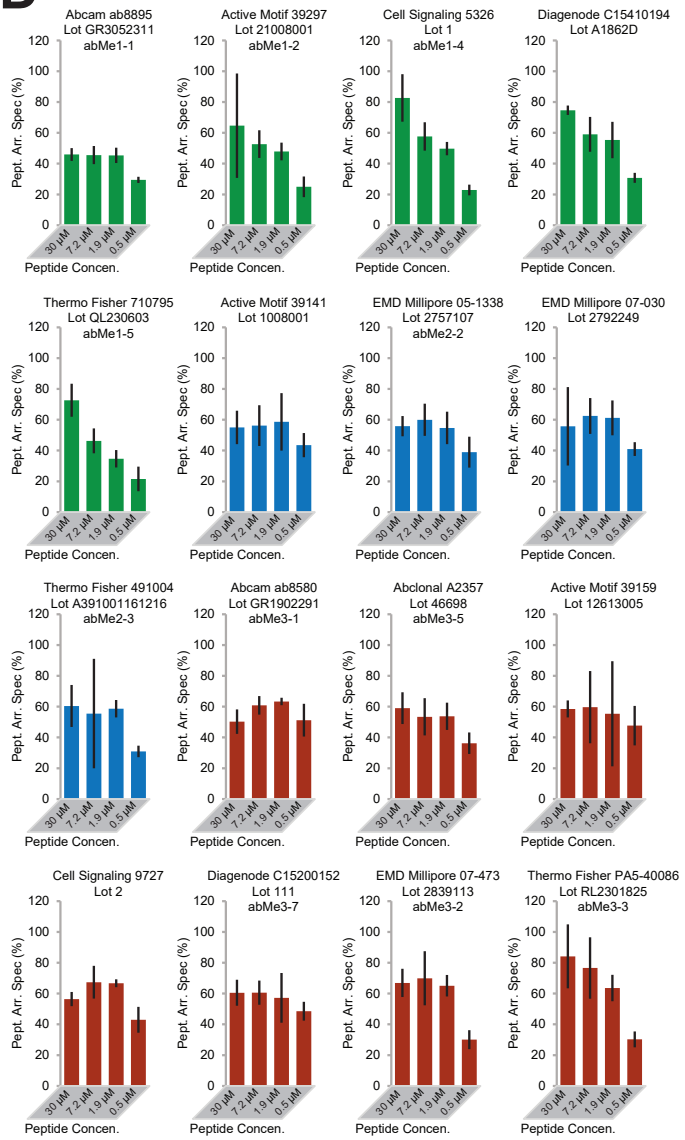


Figure S5

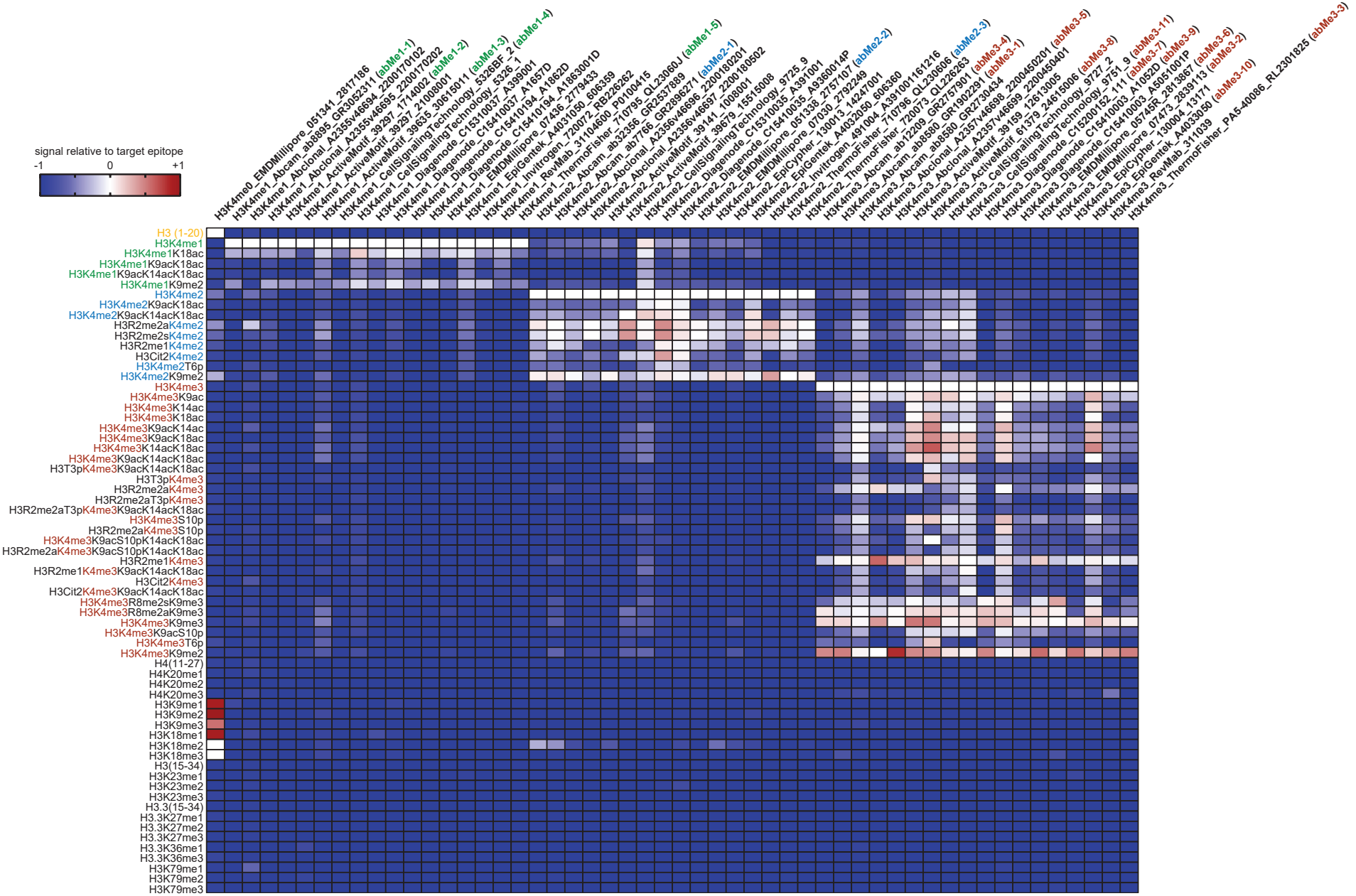

signal relative to target epitope
-1
0
+1
H3K4me0_EMDMillipore_051341_2817186
H3K4me1_Abcam_ab8895_GR3052311 (abMe1-1)
H3K4me1_Abclonal_A2355v46694_2200170102
H3K4me1_ActiveMotif_39297_1714002 (abMe1-2)
H3K4me1_CellSignalingTechnology_5326BF_2 (abMe1-4)
H3K4me1_Diagenode_C15410037_A1657D
H3K4me1_EMDMillipore_07436_2779433
H3K4me1_EpiGentek_A4031050_606359
H3K4me1_Invitrogen_720072_RB226262
H3K4me1_RevMab_31104600_P0100415
H3K4me1_ThermoFisher_710795_QL23060J (abMe1-5)
H3K4me2_Abcam_ab32356_GR2537889
H3K4me2_Abclonal_A2356v46696_2200180201
H3K4me2_ActiveMotif_39141_1008001
H3K4me2_CellSignalingTechnology_9725_9
H3K4me2_Diagenode_C15310035_A391001
H3K4me2_EMDMillipore_051338_2757107 (abMe2-2)
H3K4me2_EpiCypher_130013_14247001
H3K4me2_Invitrogen_491004_A3910011161216
H3K4me2_ThermoFisher_710796_QL230606 (abMe2-3)
H3K4me3_Abcam_ab12209_GR2757901 (abMe3-4)
H3K4me3_Abcam_ab8580_GR1902291 (abMe3-1)
H3K4me3_Abclonal_A2357v46698_2200450201 (abMe3-5)
H3K4me3_ActiveMotif_39159_12613008
H3K4me3_CellSignalingTechnology_9727_2
H3K4me3_Diagenode_C15200152_111 (abMe3-7)
H3K4me3_EMDMillipore_07473_2839113 (abMe3-6)
H3K4me3_EpiCypher_130004_13171
H3K4me3_RevMab_3110039
H3K4me3_ThermoFisher_PA5-40086_RL2301825 (abMe3-3)
H3 (1-20)
H3K4me1
H3K4me1K18ac
H3K4me1K9acK18ac
H3K4me1K9acK14acK18ac
H3K4me1K9me2
H3K4me2
H3K4me2K9acK18ac
H3K4me2K9acK14acK18ac
H3R2me2aK4me2
H3R2me2sK4me2
H3R2me1K4me2
H3Cit2K4me2
H3K4me2T6p
H3K4me2K9me2
H3K4me3
H3K4me3K9ac
H3K4me3K14ac
H3K4me3K18ac
H3K4me3K9acK14ac
H3K4me3K9acK18ac
H3K4me3K14acK18ac
H3K4me3K9acK14acK18ac
H3T3pK4me3K9acK14acK18ac
H3T3pK4me3
H3R2me2aK4me3
H3R2me2aT3pK4me3
H3R2me2aT3pK4me3K9acK14acK18ac
H3K4me3S10p
H3R2me2aK4me3S10p
H3K4me3K9acS10pK14acK18ac
H3R2me2aK4me3K9acS10pK14acK18ac
H3R2me1K4me3
H3R2me1K4me3K9acK14acK18ac
H3Cit2K4me3
H3Cit2K4me3K9acK14acK18ac
H3K4me3R8me2sK9me3
H3K4me3R8me2aK9me3
H3K4me3K9me3
H3K4me3K9acS10p
H3K4me3T6p
H3K4me3K9me2
H4(11-27)
H4K20me1
H4K20me2
H4K20me3
H3K9me1
H3K9me2
H3K9me3
H3K18me1
H3K18me2
H3K18me3
H3(15-34)
H3K23me1
H3K23me2
H3K23me3
H3.3(15-34)
H3.3K27me1
H3.3K27me2
H3.3K27me3
H3.3K36me1
H3.3K36me3
H3K79me1
H3K79me2
H3K79me3

Figure S6

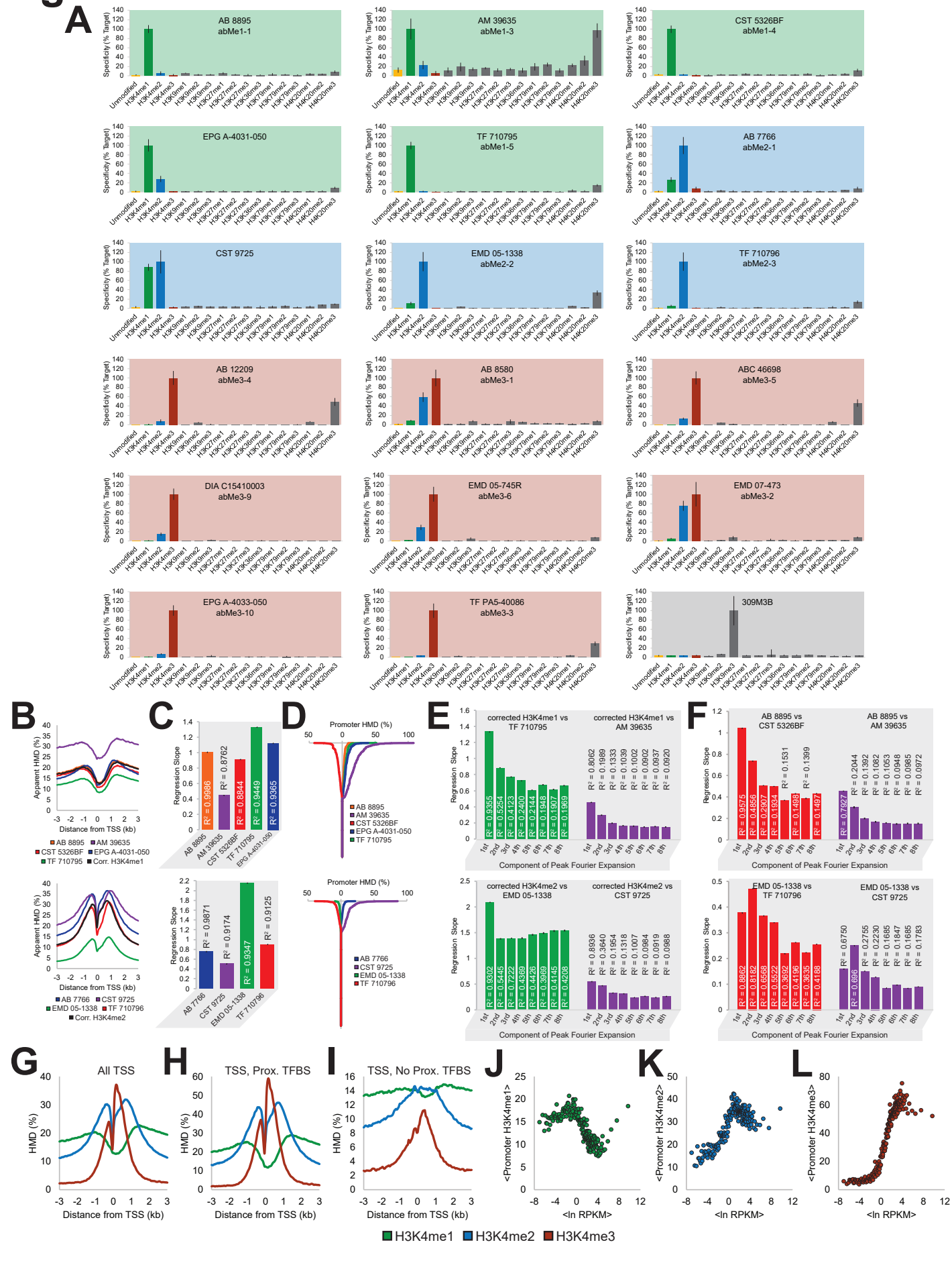

A
AB 8895
abMe1-1
AM 39635
abMe1-3
CST 5326BF
abMe1-4
EPG A-4031-050
abMe1-5
TF 710795
abMe2-1
AB 7766
abMe2-2
CST 9725
abMe2-3
EMD 05-1338
abMe2-3
TF 710796
AB 12209
abMe3-4
AB 8580
abMe3-1
ABC 46698
abMe3-5
DIA C15410003
abMe3-9
EMD 05-745R
abMe3-6
EMD 07-473
abMe3-2
EPG A-4033-050
abMe3-10
TF PA5-40086
abMe3-3
309M3B
Specificity (% Target)
Unmodified
H3K4me1
H3K4me2
H3K4me3
H3K9me1
H3K9me2
H3K9me3
H3K27me1
H3K27me2
H3K27me3
H3K36me3
H3K79me1
H3K79me2
H3K79me3
H4K20me1
H4K20me2
H4K20me3
B
Apparent HMD (%)
Distance from TSS (kb)
AB 8895
AM 39635
CST 5326BF
EPG A-4031-050
TF 710795
Corr. H3K4me1
AB 7766
CST 9725
EMD 05-1338
TF 710796
Corr. H3K4me2
C
Regression Slope
R² = 0.9986
R² = 0.8762
R² = 0.8844
R² = 0.9449
R² = 0.9365
R² = 0.9871
R² = 0.9174
R² = 0.9347
R² = 0.9125
D
Promoter HMD (%)
E
corrected H3K4me1 vs TF 710795
corrected H3K4me1 vs AM 39635
corrected H3K4me2 vs EMD 05-1338
corrected H3K4me2 vs CST 9725
Component of Peak Fourier Expansion
F
AB 8895 vs CST 5326BF
AB 8895 vs AM 39635
EMD 05-1338 vs TF 710796
EMD 05-1338 vs CST 9725
G
All TSS
H
TSS, Prox. TFBS
I
TSS, No Prox. TFBS
HMD (%)
J
<Promoter H3K4me1>
K
<Promoter H3K4me2>
L
<Promoter H3K4me3>
<ln RPKM>
H3K4me1
H3K4me2
H3K4me3

Figure S7

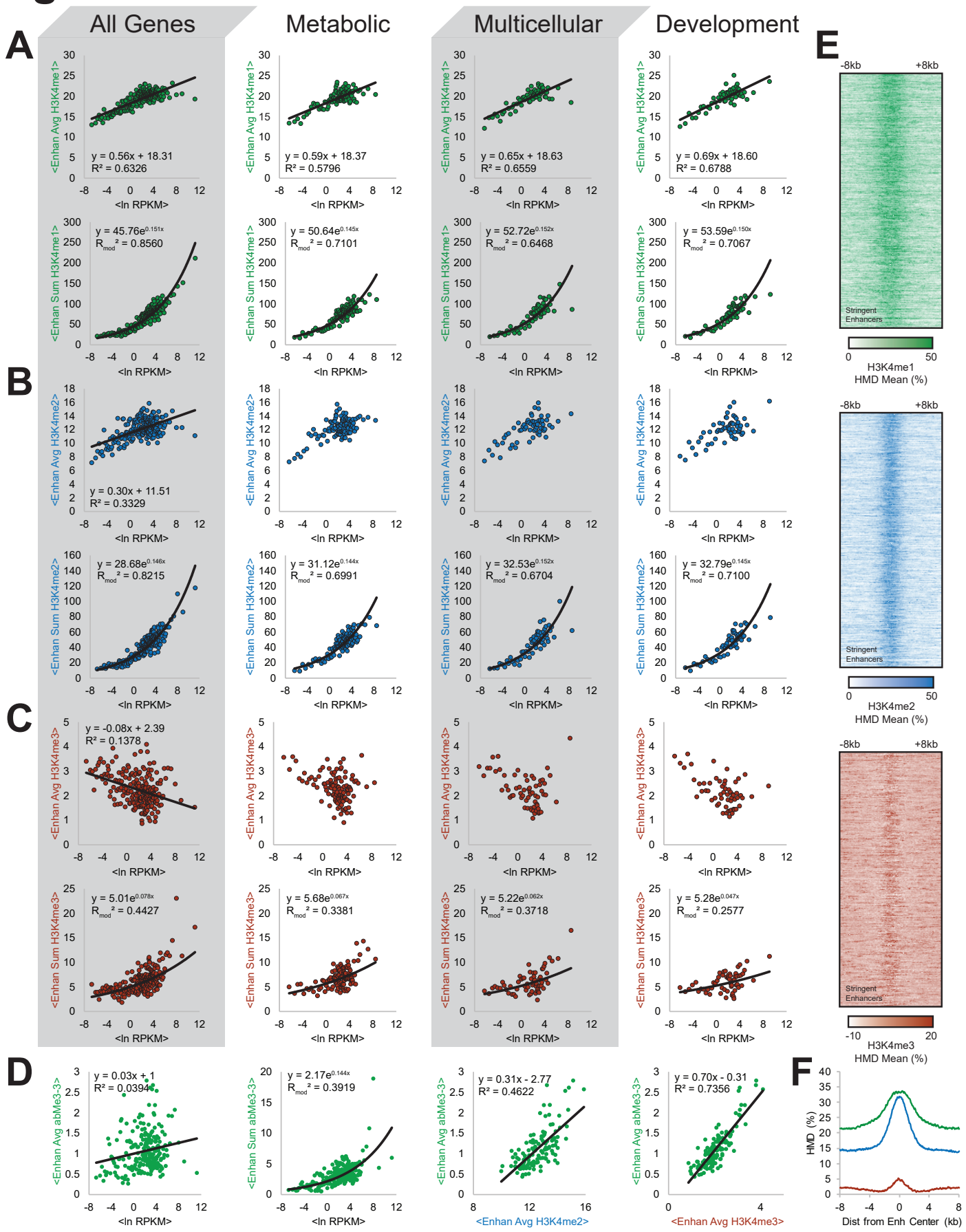

All Genes
Metabolic
Multicellular
Development
A
B
C
D
E
F
<Enhan Avg H3K4me1>
<Enhan Sum H3K4me1>
<Enhan Avg H3K4me2>
<Enhan Sum H3K4me2>
<Enhan Avg H3K4me3>
<Enhan Sum H3K4me3>
<Enhan Avg abMe3-3>
<Enhan Sum abMe3-3>
<ln RPKM>
y = 0.56x + 18.31
R² = 0.6326
y = 45.76e^0.151x
R_mod² = 0.8560
y = 0.59x + 18.37
R² = 0.5796
y = 50.64e^0.145x
R_mod² = 0.7101
y = 0.65x + 18.63
R² = 0.6559
y = 52.72e^0.152x
R_mod² = 0.6468
y = 0.69x + 18.60
R² = 0.6788
y = 53.59e^0.150x
R_mod² = 0.7067
y = 0.30x + 11.51
R² = 0.3329
y = 28.68e^0.146x
R_mod² = 0.8215
y = 31.12e^0.144x
R_mod² = 0.6991
y = 32.53e^0.152x
R_mod² = 0.6704
y = 32.79e^0.145x
R_mod² = 0.7100
y = -0.08x + 2.39
R² = 0.1378
y = 5.01e^0.078x
R_mod² = 0.4427
y = 5.68e^0.067x
R_mod² = 0.3381
y = 5.22e^0.062x
R_mod² = 0.3718
y = 5.28e^0.047x
R_mod² = 0.2577
y = 0.03x + 1
R² = 0.0394
y = 2.17e^0.144x
R_mod² = 0.3919
y = 0.31x - 2.77
R² = 0.4622
y = 0.70x - 0.31
R² = 0.7356
<Enhan Avg H3K4me2>
<Enhan Avg H3K4me3>
-8kb
+8kb
Stringent Enhancers
H3K4me1 HMD Mean (%)
H3K4me2 HMD Mean (%)
H3K4me3 HMD Mean (%)
HMD (%)
Dist from Enh Center (kb)

Table S1

Manufacturer	Product	Lot	Antibody Code	IP Methyl. Specificity (% H3K4 Pulldown)	PA Methyl. Specificity (% H3K4 Signal)	Target IP Efficiency (% Input)	Citations	ENCODE Status
EMD Millipore	05-1341	2453179	--	54 ± 5	64 ± 6	5 ± 2	2	--

Manufacturer	Product	Lot	Antibody Code	IP Methyl. Specificity (% H3K4 Pulldown)	PA Methyl. Specificity (% H3K4 Signal)	Target IP Efficiency (% Input)	Citations	ENCODE Status
Abcam	ab8895	GR305231-1	abMe1-1	90 ± 4	96 ± 3	64 ± 22	218	--
Abclonal	A2355	46694	--	37 ± 35	57 ± 19	0.06 ± 0.04	0	--
Abclonal	A2355	46695	--	82 ± 2	78 ± 2	43 ± 7	0	--
Active Motif	39297	01714002	abMe1-2	76 ± 1	100 ± 0	0.7 ± 0.4	11	--
Active Motif	39297	21008001	--	88 ± 2	99 ± 2	0.7 ± 0.4	11	Not Char Stds.
Active Motif	39635	30615011	abMe1-3	66 ± 17	64 ± 4	3 ± 1	1	--
Cell Signaling	5326	1	abMe1-6	94.4 ± 0.1	76 ± 2	46 ± 7	2	--
Cell Signaling	5326BF	2	abMe1-4	93 ± 2	99 ± 1	33 ± 27	2	Char. To Stds.
Diagenode	C15310037	A399-001	--	88 ± 4	99 ± 1	4.4 ± 0.2	0	--
Diagenode	C15410037	A1657D	--	86 ± 3	87 ± 5	14 ± 0.5	2	--
Diagenode	C15410194	A1862D	--	90 ± 3	97 ± 8	16 ± 22	7	Awaiting Char.
Diagenode	C15410194	A1863-001D	--	88 ± 2	100 ± 0	4 ± 2	7	--
EMD Millipore	07-436	DAM1687548	--	87 ± 1	97 ± 6	0.179 ± 0.003	16	--
EpiGentek	A-4031-050	606359	--	83 ± 3	74 ± 2	32 ± 5	0	--
RevMAb	31-1046-00	P-01-00415	--	87 ± 6	99.9 ± 0.2	13 ± 1	0	--
Thermo Fisher	710795	QL230603	abMe1-5	94.7 ± 0.5	98 ± 2	20 ± 5	0	--
Thermo Fisher	720072	RB226262	--	86 ± 4	95 ± 2	40 ± 11	0	--

Manufacturer	Product	Lot	Antibody Code	IP Methyl. Specificity (% H3K4 Pulldown)	PA Methyl. Specificity (% H3K4 Signal)	Target IP Efficiency (% Input)	Citations	ENCODE Status
Abcam	ab32356	GR253788-9	--	58 ± 2	81 ± 2	70 ± 29	35	--
Abcam	ab7766	GR289627-1	abMe2-1	80 ± 4	57 ± 4	55 ± 11	55	--
Abclonal	A2356	46696	--	94 ± 1	68 ± 3	16 ± 10	0	--
Abclonal	A2356	46697	--	81.3 ± 0.7	61 ± 3	51 ± 21	0	--
Active Motif	39141	01008001	--	91 ± 2	65 ± 1	18 ± 3	8	Char. To Stds.
Active Motif	39679	15515008	--	89 ± 1	78 ± 3	3 ± 1	0	--
Cell Signaling	9725	9	--	56 ± 1	42 ± 3	56 ± 16	4	--
Diagenode	C15200151	001-11	--	94 ± 2	----	6 ± 2	0	--
Diagenode	C15310035	A391-001	--	83.8 ± 0.2	62 ± 13	1.1 ± 0.4	0	--
Diagenode	C15410035	A9360014P	--	88 ± 2	60 ± 2	58 ± 8	6	--
EMD Millipore	05-1338	2757107	abMe2-2	95.6 ± 0.1	77 ± 5	21 ± 5	6	--
EMD Millipore	07-030	DAM1479603	--	89.8 ± 0.2	68 ± 2	4.8 ± 0.4	126	--
Epicypher	13-0013	14247001	--	90 ± 5	62 ± 4	19 ± 4	1	--
EpiGentek	A-4032-050	606360	--	92 ± 4	72 ± 6	66 ± 45	0	--
Thermo Fisher	49-1004	A391001161216	--	74 ± 2	97 ± 4	0.8 ± 0.4	2	--
Thermo Fisher	710796	QL230606	abMe2-3	95.3 ± 0.4	99.5 ± 0.8	25 ± 5	0	--
Thermo Fisher	720073	QL226263	--	81 ± 1	86 ± 6	19 ± 9	0	--

Manufacturer	Product	Lot	Antibody Code	IP Methyl. Specificity (% H3K4 Pulldown)	PA Methyl. Specificity (% H3K4 Signal)	Target IP Efficiency (% Input)	Citations	ENCODE Status
Abcam	ab12209	GR275790-1	abMe3-4	88 ± 3	88 ± 9	5 ± 1	6	--
Abcam	ab8580	GR190229-1	abMe3-1	60 ± 3	66 ± 2	63 ± 17	418	Char. To Stds.
Abcam	ab8580	GR273043-4	--	55 ± 4	58 ± 4	59 ± 4	418	--
Abclonal	A2357	46698	abMe3-5	91 ± 2	90 ± 9	13 ± 3	0	--
Abclonal	A2357	46699	--	86 ± 2	75 ± 4	43 ± 8	0	--
Active Motif	39159	12613005	--	66 ± 11	62 ± 5	5 ± 5	80	--
Active Motif	61379	24615006	abMe3-8	67 ± 1	57 ± 3	0.4 ± 0.3	2	--
Cell Signaling	9727	2	--	65 ± 3	58 ± 4	31 ± 0.7	11	Char. To Stds.
Cell Signaling	9751	9	abMe3-11	59 ± 7	57 ± 2	59 ± 22	24	--
Diagenode	C15200152	001-11	abMe3-7	73 ± 12	86 ± 9	1.4 ± 0.8	0	--
Diagenode	C15410003	A1052D	abMe3-9	72 ± 5	78 ± 7	40 ± 24	43	--
Diagenode	C15410003	A5051-001P	--	65 ± 8	78 ± 5	35 ± 16	43	Char. To Stds.
EMD Millipore	05-745R	2813867	abMe3-6	72 ± 8	89 ± 3	55 ± 18	10	--
EMD Millipore	07-473	DAM1623866	abMe3-2	56 ± 7	81 ± 5	54 ± 7	189	Char. To Stds.
Epicypher	13-0004	13171001	--	71 ± 5	93 ± 2	1.1 ± 0.4	1	--
EpiGentek	A-4033-050	606361	abMe3-10	84 ± 10	81 ± 3	48 ± 25	0	--
RevMAb	31-1039-00	P-09-00676	--	67 ± 5	86 ± 2	42 ± 5	0	--
Thermo Fisher	PA5-40086	RL2301825	abMe3-3	96.5 ± 0.5	100 ± 0	17 ± 7	0	--
Koide Lab	304M3B	040416AG	--	76 ± 1	----	36 ± 19	0	--

Table S2

Workflow Step	Details and Quality Control
Isolate nuclei	Cells and nuclei should not be cross-linked.
↓ Spike in nucleosomes	Spike-ins contain a pool of on- and off-target barcoded PTMs.
↓ Mnase digest	Check digestion (>95% mononucleosomes).
↓ Antibody IP	Start with supplier recommended dilution, ensure bead-antibody complexes are saturated. In **rare** cases, antibody dilutions may improve specificity.
↓ Purify DNA	DNA purification with size selection (e.g. Ampure XP beads) can be used to enrich mononucleosome-sized fragments.
↓ qPCR	**Phase I:** Check the most likely cross-reacting species (e.g. me1, me2, me3 at the same site). **Phase II:** Check all barcoded nucleosomes in the spike-in panel.
↓ **STOP**/**GO**	• Does the antibody exhibit specificity for the target? • Is antibody efficiency sufficiently high to minimize noise? • Is the variability between samples within an acceptable range?
↓ NGS	**Double check antibody specificity:** All barcodes are captured in the sequencing data. **Normalize the data:** Express read counts relative to spike-in control.

Figure S1, related to Figure 1. ENCODE ChIP-seq datasets display incongruity between each other and ICeChIP-seq datasets. (A) Concordance of replicated peaks for each H3K4me3 ENCODE dataset with indicated antibody in K562 cells. Top row shows number of called peaks replicated across the two biological replicates for each dataset, and bottom row the number and percentage of such peaks that intersect with peaks common to all four of the ENCODE H3K4me3 datasets. **(B)** Peak shape and intensity analysis by pairwise correlation between pre-cosinusoidal factors of eight-component discrete Fourier cosine transform of fold changes over control about peaks from ENCODE H3K4me3 Sample 1 (ENCSR000AKU, left), Sample 2 (ENCSR000EWA, centre), or Sample 3 (ENCSR000DWD, right). If only intensity of signal was different (i.e. same data with different scaling, the R^2 would approach 1, and scalar factor reflecting difference would be apparent from the slope. These comparisons (Movie S1) indicate very limited similarity amongst any of two ENCODE data sets, with modest large-scale similarity (early terms) decaying to negligible fine-scale similarity (late terms). **(C)** R^2 of correlation between pre-cosinusoidal factors of eight-component discrete Fourier cosine transform on antibody-measured peaks of corrected ICeChIP-seq HMD versus antibody-measured fold change over control for H3K4me1, H3K4me2, and H3K4me3 ENCODE ChIP-seq datasets in K562 cells. **(D)** Example scatterplots showing high correlation (top) and low correlation (bottom) of Fourier Transform components on peaks from ENCODE H3K4me3 Sample 3. **(E)** Genome browser view at the HOX locus for antibodies not shown in Figure 1A. Green circle represents ICeChIP aggregate specificity, and purple circle with M indicates monoclonality.

Figure S2, related to Figure 2. Anti-H3K4 methylation antibodies display a broad range of specificities in peptide arrays. The specificity of H3K4 methylform antibody binding on peptide arrays expressed relative to on-target capture. Black error bars represent SD of off-target specificity; colored error bars represent average standard error of on-target signal. Purple bar represents raw fluorescence signal from secondary axis and maps onto secondary axis. Fluorescence measurements for each antibody (n=6), independent at the level of spotting, but simultaneously measured against one antibody dilution.

Figure S3, related to Figure 2. Anti-H3K4 methylation antibodies display a broad range of specificities in ICeChIP. The specificity of H3K4 methylform antibody binding in ICeChIP relative to on-target capture. Black error bars represent SD of off-target specificity; colored error bars represent average standard error of on-target signal. Purple bar represents ChIP enrichment and maps onto secondary axis (right). ICeChIP was conducted with 3 µg of mammalian chromatin and 3 µg of each antibody (see Methods). Enrichment of each standard was measured by qPCR (compare to ICeChIP-seq in Fig. S6A); n represents independent ICeChIP experiments averaged for each antibody.

Figure S4, related to Figure 3. ICeChIP and peptide arrays have discrepancies that can be modulated. (A) Agreement of antibody of methyl-form specificity between ICeChIP and peptide arrays, to within 10 percentage points. **(B)** ICeChIP aggregate specificity for four antibodies when H3K4me2 nucleosomes bearing a different DNA sequence is added in excess of endogenous H3K4me2. One replicate per ICeChIP experiment for a total of four ICeChIPs per antibody. **(C, D)** Antibody specificity on EpiTitrate peptide arrays with varying amounts of (C) antibody and (D) modified peptide. Approximately half the antibodies tested show marginally altered specificity with increasing dilution, albeit not always in the same direction. Most antibodies show decreased specificity at the most dilute modified peptide concentration, and approximately a third show decreasing specificity with increased modified peptide dilution more broadly. Six fluorescence readings per peptide array experiment, with one independent experiment per antibody dilution.

Figure S5, related to Figure 3. Combinatorial modifications can impact antibody binding in peptide arrays. Heatmap of antibody binding to a wide range of combinatorial and off-target peptides on peptide arrays. Signal is normalized to singly-modified target epitope for a select set of H3K4-proximal combinatorial modifications.

Figure S6, related to Figure 4. Specificity of antibodies is broadly recapitulated in ICeChIP-seq and can impact measured modification profiles. (A) Specificities of antibodies in ICeChIP-seq ex-

periments are identical within experimental error to those measured by qPCR, and a broader range of off-target internal standards are sampled. Error bars represent SD of estimate based on internal variability of ladder members. **(B)** Metagene contours about Refseq TSSs of anti-H3K4me1 (upper panel) and anti-H3K4me2 (lower panel) antibodies and corresponding corrected profiles. **(C)** Correlation between antibody-measured HMD and corrected HMD at antibody called peaks for anti-H3K4me1 and anti-H3K4me2 antibodies. **(D)** Antibody-measured HMD at +1/+2 nucleosomes of genes with no measured HMD in corrected profile for anti-H3K4me1 and anti-H3K4me2 antibodies. **(E)** Correlation and magnitude analysis of pre-cosinusoidal factors for eight term Fourier series comparing corrected HMD versus antibody-measured HMD for anti-H3K4me1 and anti-H3K4me2 antibodies contoured over called peaks in the corrected. **(F)** Similar analysis of pre-cosinusoidal factors of eight-component discrete Fourier cosine transform of measured HMDs by listed antibodies versus high-specificity reference antibodies abMe1-1 (upper panel) or abMe2-2 (lower panel) on peaks from listed antibodies. **(G, H, I)** Metacontours about TSSs of H3K4me1/2/3 HMD for (G) all TSSs (58,951 TSSs), (H) TSSs with a transcription factor binding site (Wang et al. 2014) within 200bp of the TSS (32,531 TSSs), and (I) TSSs without a transcription factor binding site (Wang et al. 2014) within 200bp of the TSS (26,420 TSSs). **(J, K, L)** Average (J) H3K4me1, (K) H3K4me2, and (L) H3K4me3 corrected HMD of +1/+2 nucleosomes versus ln RPKM of genes. Error bars for all correlations represent 99.99% CI for correlation slope.

Figure S7, related to Figure 5. High-quality H3K4 methylation HMD datasets reveal quantitative relationships between enhancer H3K4 methylation and promoter activity. (A, B, C) Average (top) and sum (bottom) of (A) H3K4me1, (B) H3K4me2, and (C) H3K4me3 corrected HMD across all enhancers contacting corresponding promoter regions versus ln RPKM for all classes of genes (left), metabolic genes (centre-left), multicellular system process genes (centre-right), and developmental process genes (right). **(D)** From left to right: abMe3-3 measured HMD sum versus ln RPKM. abMe3-3 measured HMD average across enhancers versus ln RPKM, average corrected H3K4me2 enhancer HMD, and average corrected H3K4me3 enhancer HMD. **(E)** Heatmaps of stringently defined enhancer HMD averages for H3K4me1, H3K4me2, and H3K4me3. All heatmaps sorted by ln RPKM of target genes. R_{mod}^2 represents R^2 of linear correlation between actual and predicted/modeled HMD. **(F)** Signal-corrected H3K4me1, H3K4me2, and H3K4me3 modification profiles contoured over stringently-defined enhancers.

Table S1. Summary of Data. Antibody information with ICeChIP and peptide array specificity, IP efficiency, ChIP citations from Google Scholar, and ENCODE validation status (none, awaiting characterization, or characterized to standards). Values represent average ± SD.

Table S2. Workflow of antibody validation for ICeChIP-seq. Pipeline offers suggested procedure for conducting ICeChIP-seq, including pause points for assessment of antibody quality prior to NGS or biological interpretation.