Supplementary Table 1

SET Domain-Containing Genes Targeted by RNAi

Genes targeted in the RNAi screen are listed according to the computed gene (CG) identifier reported in the FlyBase database (http://flybase.bio.indiana.edu/). A single dsRNA was used for each gene and the protein coding region nucleotides that were targeted are listed. If multiple transcripts had been reported in FlyBase, a common element was targeted and the positions specific for different transcripts (RA, RB, RC, RD, RE) are noted. Templates for dsRNA synthesis were prepared from cDNA plasmids or CDNA obtained from the following sources: 1 = *Drosophila* Genomics Resource Center, Indiana University; 2 = BACPAC Resources Center, Children's Hospital Oakland Research Institute; 3 = cDNA generated in our lab from *Drosophila* S2 cell poly A^+ RNA. Some dsRNAs were transcribed from commercially available T7 promoter-containing dsDNA templates (4 = Open Biosystems *Drosophila* RNAi library).

Gene	Name	Region targeted by dsRNA	Source
CG1716		5439-5938	1
CG1868		RA(826-1338) RB(925-1437)	1
CG2995	G9a	1677-2196	1
CG3307	PR-Set7 (SET8)	1-700	2
CG3353		585-1078	1
CG3848	Trr	RC(666-1159) RD(729-1222)	3
CG4565		62-625	4
CG4976	Mes-4 (NSD1)	1841-2363	1
CG5249	Blimp-1	2066-2730	4
CG6476	Su(var)3-9	RA(702-1199)	3
CG6502	Enhancer of zeste	1646-2149	1
CG7759		RA(801-1366) RB(522-1087)	1
CG8378		36-511	1
CG8503		902-1377	1
CG8651	Trithorax	RB,RC,RE(2802-3425) RA,RD(3906-4529)	4
CG8887	Ash1	1429-1948	3
CG9007		8165-8903	4
CG9640		968-1390	1
CG9642		640-1147	3
CG11160		77-516	1
CG12119		357-842	1
CG13363	Suv4-20	1088-1597	1
CG13761	Bzd	607-982	4
CG14122		1279-1881	1
CG14590		1286-1781(1781 is in 3' UTR)	3
CG17086		927-1436	4
CG18136		157-753	1
CG30426		105-536	1
CG32732		135-775	4
CG33548	Msta	RA(963-1377) RB(921-1335)	1
CG40351		1248-1768	1

Supplementary Table 2

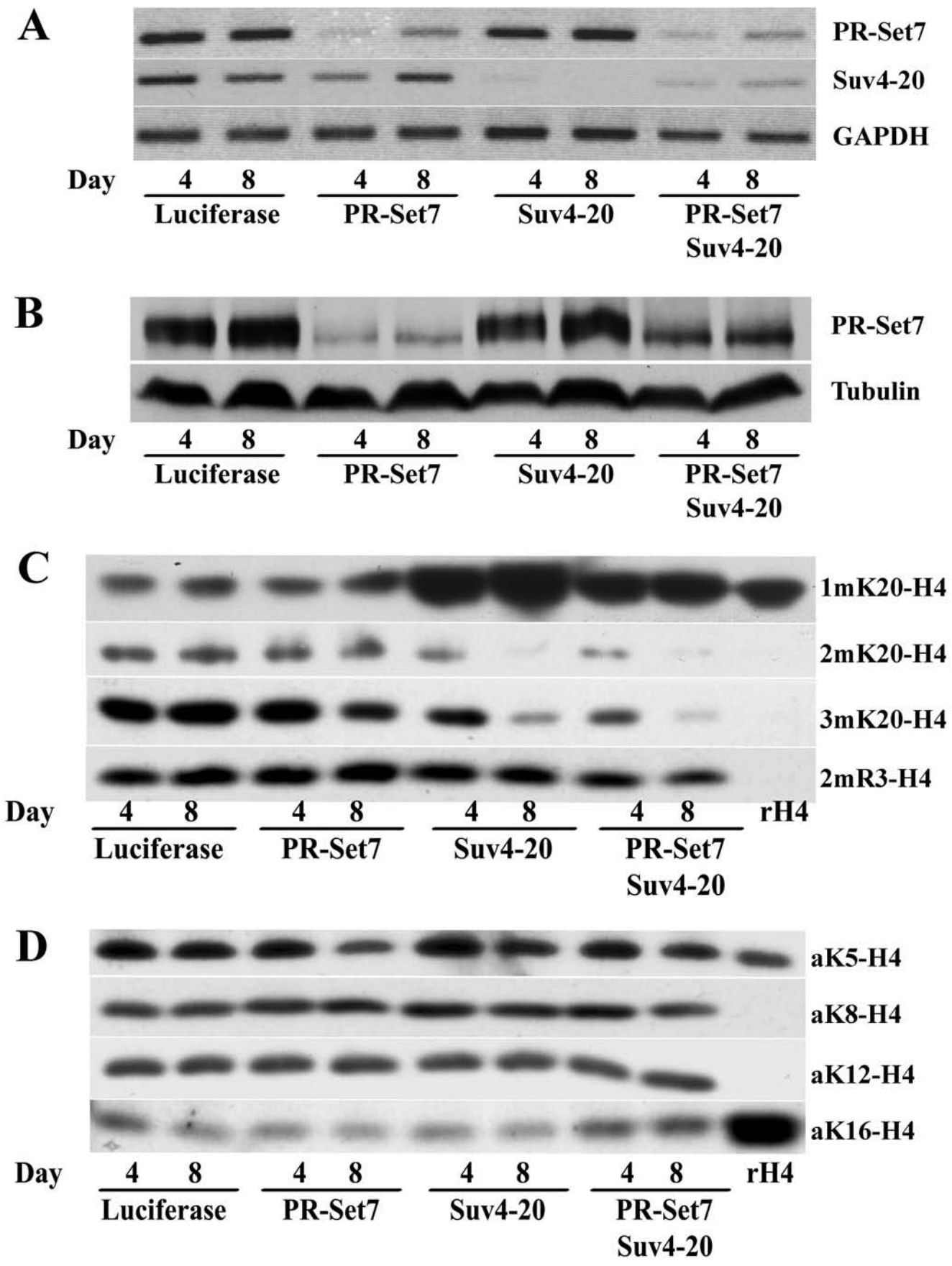
Primers for RT-PCR

The abundance of mRNAs encoded by the genes listed below was assayed using reverse transcription of total RNA followed by PCR amplification using the oligonucleotide primers shown.

Gene	5' primer	3' primer
Drosophila PR-Set7	CTGTCTCAATGGACGCTTCA	CGTCTAGCACCACGACGATA
Drosophila Suv4-20	CTCTACAACCATCAGCAATG	GATCTGCGTTGGCTGCT
Drosophila GAPDH1	CGAAGATCGGAATTAACGGA	ACATACTCGGCTCCAGCACT
Human Suv4-20h1	AACTGGTCGAGATACAGC	GACAGATTGACTGTCTGAA
Human Suv4-20h2	TCAGCAGGACTGGCACTG	TCACAGCTCTTCACCGC
Human actin	GCTCGTCGTCGACAACG	CAAACATGATCTGGGTCATC

Supplementary Fig. 1. Depletion of PR-Set7 and Suv4-20 affects H4-K20 methylation in *Drosophila* S2 cells. Typical results for days 4 and 8 of RNAi treatment to deplete firefly luciferase (negative control), PR-Set7, Suv4-20 or both PR-Set7 and Suv4-20 are shown. *A*. The relative levels of PR-Set7 and Suv4-20 mRNA were detected by RT-PCR. The levels of glyceraldehyde 3 phosphate dehydrogenase (GAPDH1) mRNA were used to ensure equivalent sample loading. *B*. The relative levels of PR-Set7 protein detected by immunoblotting with antisera raised against residues 1-100 of *Drosophila* PR-Set7. The levels of α-tubulin were used to ensure equivalent loading. *C*. The relative levels of histone H4 that was monomethylated (1mK20-H4), dimethylated (2mK20-H4) or trimethylated at K20 (3mK20-H4) were detected using K20 methylation state-specific antisera. The levels of H4 dimethylated at R3 (2mR3-H4) were used to ensure equivalent loading. Recombinant Xenopus H4 (rH4) was used as a negative control for antibody specificity. *D*. The relative levels of H4 that was acetylated at K5 (aK5-H4), K8 (aK8-H4), K12 (aK12-H4), or K16 (aK16-H4) were detected with acetylation site-specific antisera. Samples were loaded in the same relative proportions as in (*C*). Recombinant Xenopus H4 (rH4) was used as a negative control for antibody specificity.

Supplementary Fig. 2. Mass spectra of intact H4 from *Drosophila* S2 cells treated with dsRNA targeting Ash1 or NSD1 alone or in combination with PR-Set7. RNAi for Ash1 or NSD1 alone did not lead to significant changes in H4 modification profiles. Combined RNAi for Ash1 with PR-Set7 or NSD1 with PR-Set7 led to changes essentially the same as those observed following RNAi for PR-Set7 alone. Ash1 and NSD1 do not appear to mediate significant K20 methylation at the global level.


A
PR-Set7
Suv4-20
GAPDH
Day 4 8 4 8 4 8 4 8
Luciferase
PR-Set7
Suv4-20
PR-Set7 Suv4-20
B
PR-Set7
Tubulin
Day 4 8 4 8 4 8 4 8
Luciferase
PR-Set7
Suv4-20
PR-Set7 Suv4-20
C
1mK20-H4
2mK20-H4
3mK20-H4
2mR3-H4
Day 4 8 4 8 4 8 4 8 rH4
Luciferase
PR-Set7
Suv4-20
PR-Set7 Suv4-20
D
aK5-H4
aK8-H4
aK12-H4
aK16-H4
Day 4 8 4 8 4 8 4 8 rH4
Luciferase
PR-Set7
Suv4-20
PR-Set7 Suv4-20

Supplementary Figure 2