Table S1. Strain list

Name	*Genotype*	
SS1(FY2002)	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+*	R.Allshire
SS40	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ set2::5FLAG::kanMX6*	This study
ss42	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ alp13::natMX6*	Our stock
ss87	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ set2::natMX6*	This study
ss117	*ade6-DN/N ura4-DS/E imr1L::ura4+ otr1R::ade6+ h- leu+*	Our stock
ss120	*ade6-DN/N ura4-DS/E imr1L::ura4+ otr1R::ade6+ clr4::kanMX6 set2::natMX6*	This study
ss123	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ set2ΔC(321-796)::5Flag::kanMX6*	This study
ss126	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ set2ΔSRI(678-796)::5Flag::kanMX6*	This study
ss129	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ dcr1::hphMX6*	Our stock
ss130	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ dcr1::hphMX6 set2::natMX6*	This study
ss134	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ rpb1CTDS2A::natMX6*	D. Hermand
ss136	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ clr4::natMX6*	This study
ss168	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ set2ΔC(321-796)::5Flag::kanMX6 clr4::natMX6*	This study
ss169	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ set2ΔSRI(678-796)::5Flag::kanMX6 clr4::natMX6*	This study
ss183	*ade6-DN/N leu-32 ura4-DS/E imr1L::ura4+ otr1R::ade6+ h+ rpb1CTDS2A::natMX6 set2:5:FLAG::kanMX6*	This study
ss216	*h+, ade6-DN/N, leu1-32, ura4-DS/E, imr1L::ura4+, otr1R::ade6+, rrp6::13myc::natMX6*	This study
ss220	*h+, ade6-DN/N, leu1-32, ura4-DS/E, imr1L::ura4+, otr1R::ade6+, rrp6::kanMX6*	Our stock
ss233	*h+, ade6-DN/N, leu1-32, ura4-DS/E, imr1L::ura4+, otr1R::ade6+, rrp6::kanMX6 set2::natMX6*	This study
ss352	*h+ his2 leu1*	Bioresouce
ss353	*h+ leu1 his2 clr6-1*	Bioresouce
ss354	*h+ his2 leu1 set2:: natMX6*	This study
ss357	*h+ his2 leu1 clr3::hphMX6*	This study
ss363	*h+ his2 leu1 clr4::natMX6*	This study
		This study

Table S2. Primer list

Primer name	Sequence	Usage
clr4 1+	TAGTCTTCATTAGCCAGCGT	Deletion
clr4 1-	TTAATTAACCCGGGGATCCGCCAAATGATGCCTGAACGAA	Deletion
clr4 2+	GTTTAAACGAGCTCGAATTCATCTGACCTGTGGGATTTGC	Deletion
clr4 2-	AAGCTCTCAGGTTTGGTGCA	Deletion
act1RT-FW	TGCCGATCGTATGCAAAAGG	RT-PCR, ChIP
act1RT-RV	CCGCTCTCATCATACTCTTG	RT-PCR, ChIP
dh-RT-3	CTCTCATCTCGACTCGTTTG	RT-PCR, ChIP
dh-RT-4	GGCATTCACGAAACATAGCG	RT-PCR, ChIP
KKO-431/ade6DN/N124a	GTAGTACGCAGTTTAGACGG	RT-PCR, ChIP
KKO-432/ade6DN/N124b	GAGCACGCTGTTGAATTGAG	RT-PCR, ChIP
KKO-433/ura4DS/E145a	GAATGGTTTGAGAAGCATACC	RT-PCR, ChIP
KKO-434/ura4DS/E145b	GAGTACGATATTGCTGTCCC	RT-PCR, ChIP
set2 1+	GTGTCTATCTTTGCGTACCA	Deletion
set2 1-	TTAATTAACCCGGGGATCCGGTAATTGCCGAAGGAAGCCA	Deletion
set2 2+	GTTTAAACGAGCTCGAATTCGCATGTCTTTAATACCTGGTG	Deletion
set2 2-	TGGATCGAGCTCATCGTATC	Deletion
set2-Δc-FL 1+	GTGTACGGACGAAGATAACG	Deletion
set2-Δc-FL 1-	TTAATTAACCCGGGGATCCGTCCGCCAATATACCCTACAC	Deletion
set2-ΔSRI domain-FL 1+	GTCAAGTTGCTCCACAGTCA	Deletion
set2-ΔSRI domain-FL 1-	TTAATTAACCCGGGGATCCGATGCAACTTTTTGGCATGTC	Deletion
set2 sequence primer 1	TGGCTTCCTTCGGCAATTAC	Sequence
set2 sequence primer 2	TTTGCGATTGTCGGCCTCATTG	Sequence
set2 sequence primer 3	ATTCGCGGTGAAGAGCTTAC	Sequence
set2 sequence primer 4	TTCGATCATTGCGTTACGTG	Sequence
set2 sequence primer 5	AAGGGATCAGGAACCACAAC	Sequence
set2 sequence primer 6	TGTTGAAGGCACAGAAGGAG	Sequence
set2 sequence primer7	GCTGAGAGTTTGGGCATTTC	Sequence
marker gene 1+	CGGATCCCCGGGTTAATTAA	amplification of marker cassette
marker gene 1-	GAATTCGAGCTCGTTTAAAC	amplification of marker cassette
KKO-586/cen siRNA A	GCGACTAAACCGAAAGCCTC	siRNA analysis
KKO-587/cen siRNA B	TACCGTGATTAGCCTTACTCCGCATT	siRNA analysis

KKO-588/cen siRNA C	TACTTATTGATGGCGAAGCTAGA	siRNA analysis
KKO-589/cen siRNA D	TACCGCTTCTCCTTAATCCA	siRNA analysis
KKO-590/cen siRNA E	ACACCTACTCTTATCACTTGT	siRNA analysis
KKO-591/cen siRNA F	GACGATAAGCAGGAGTTGCGCA	siRNA analysis
KKO-592/cen siRNA G	AGTGTGGCGCTATATCTTGTA	siRNA analysis
KKO-593/cen siRNA H	TACTGTCATTAGGATATGCTCA	siRNA analysis
KKO-594/cen siRNA I	GGGAAATGTATAAATAGGCA	siRNA analysis
KKO-595/cen siRNA J	TTTCCCAAGGACTGCTGAGGTAGA	siRNA analysis
KKO-596/cen siRNA K	TGGACACAGCATGGATATGGACACA	siRNA analysis
KKO-597/cen siRNA L	TGGCAGATATTGCAAGTTGTTTA	siRNA analysis
EOS-450/tRNA^{Asn}Rv	CGACCTCACGATTAACAGTCG	siRNA analysis
rrp6-1(Fw)	CATGGGATGGTTGCTGTTAC	Deletion
rrp6-2(Rv)	TTAATTAACCCGGGGATCCGGCTATTGTTTACCCTACTGG	Deletion
rrp6-3(Fw)	GTTTAAACGAGCTCGAATTCGAAATTGAGAATGCATGCCC	Deletion
rrp6-4(Rv)	ACAATGACGCAAACCTGTCC	Deletion
SPBPB2B2. 06c 1+	TGTATTACACGCGCCGTTAC	RT-PCR, ChIP
SPBPB2B2. 06c 1-	TCAATGCCTTCTGGGGTATC	RT-PCR, ChIP
SPBPB2B2.08 1+	TGGTGGACCTCCAAAGAAAG	RT-PCR, ChIP
SPBPB2B2.08 1-	TTTGTACCACCCACGAACAG	RT-PCR, ChIP

Table S3. Peri-centromeric non-coding RNAs showing increased expression (> 2.0-fold) in *set2* deletion mutants

	gene name	*set2Δ/wt**	*set2ΔC/wt**	*set2ΔSRI/wt**	*clr3Δ***/wt*
Chr. 1	SPNCRNA.231	2.4	3.1	1.4	44.7
	SPNCRNA.232	2.9	3.1	2.9	4.7
	SPNCRNA.234	15.0	17.9	9.5	201.8
Chr. 2	SPNCRNA.360	7.4	9.2	5.2	40.5
	SPNCRNA.371	7.2	8.0	4.6	86.5
	SPNCRNA.373	2.3	2.2	2.5	1.6
Chr. 3	SPNCRNA.484	2.7	3.0	3.0	1.4
	SPNCRNA.485	2.5	2.1	2.1	2.0

*Values obtained with *set2-FLAG* cells were used as wild type (*wt*) for *set2* mutants to avoid the possible effects of FLAG- tag that attached at C-terminal end of all mutated Set2 proteins.

** *clr3*Δ cells were used as positive control that increases peri-centromeric transcripts without affecting H3K9me level.

Figure S1

A

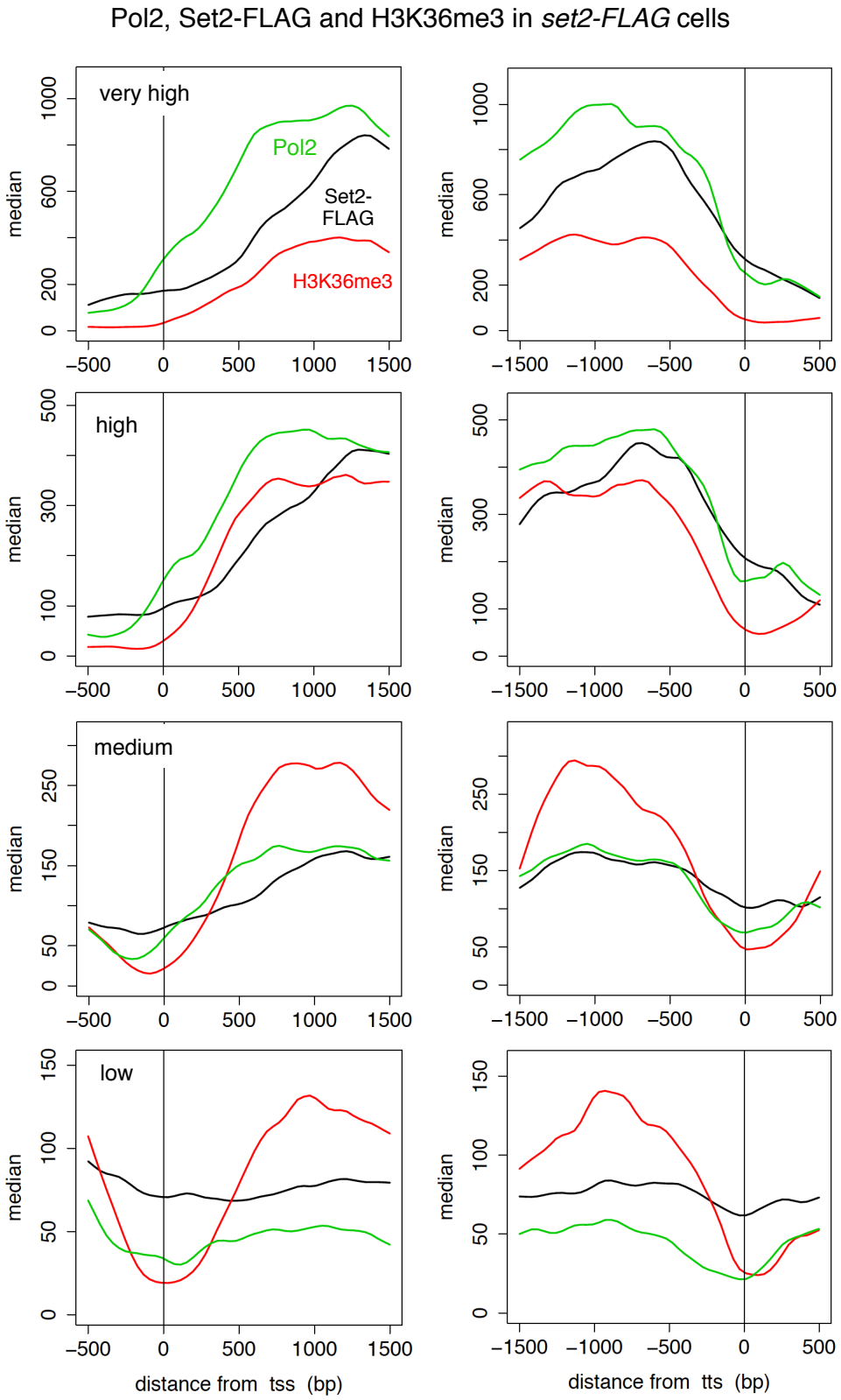
Pol2, Set2-FLAG and H3K36me3 in *set2-FLAG* cells
very high
high
medium
low
Pol2
Set2-
FLAG
H3K36me3
median
distance from tss (bp)
distance from tts (bp)

Figure S1

B

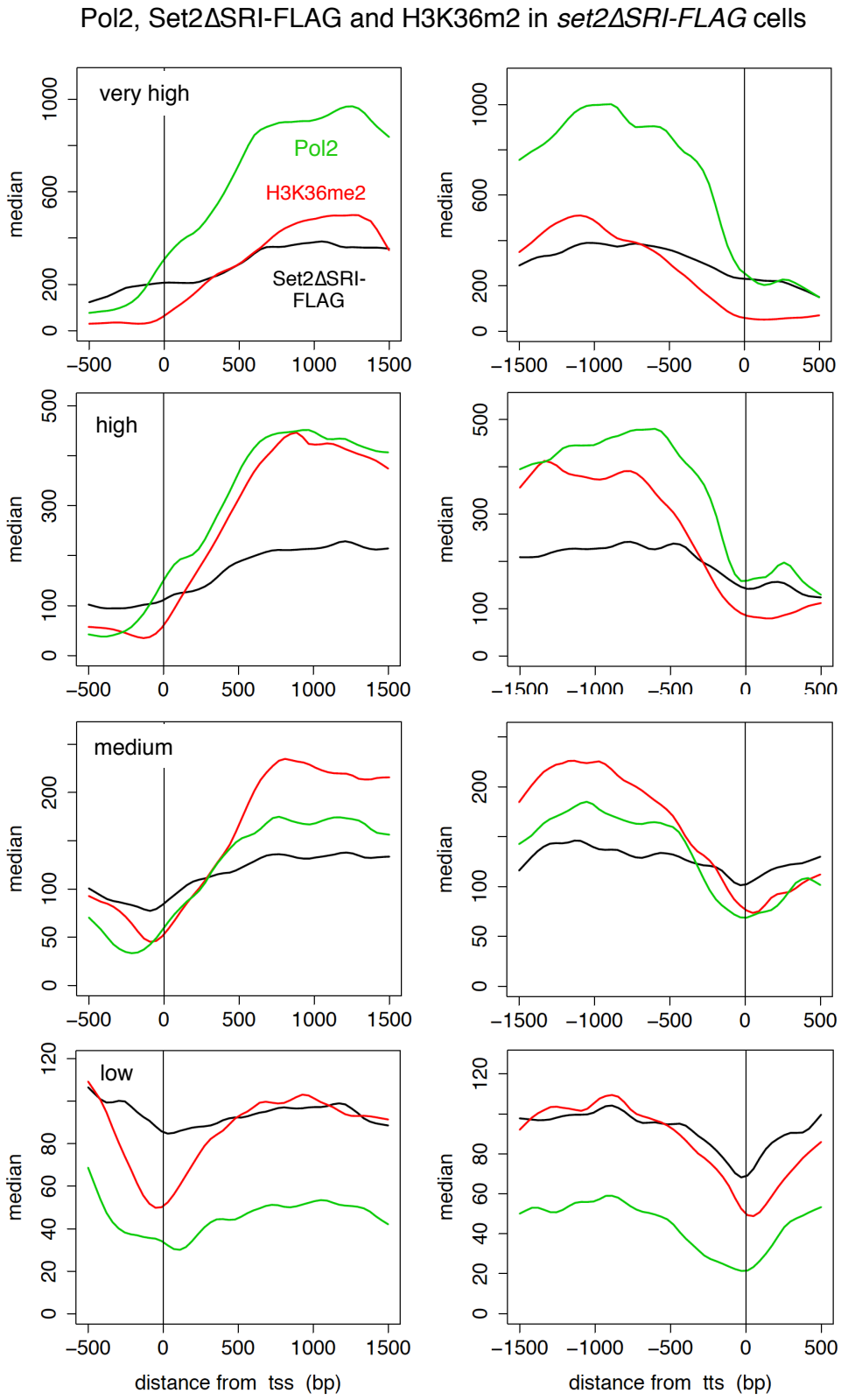
Pol2, Set2ΔSRI-FLAG and H3K36m2 in *set2ΔSRI-FLAG* cells
very high
high
medium
low
Pol2
H3K36me2
Set2ΔSRI-FLAG
median
distance from tss (bp)
distance from tts (bp)

Figure S1. Comparisons of distributions of Set2 protein and H3K36 methylation with that of Pol2. (A) Compariosns of distributions of Set2-FLAG and H3K36me3 with that of Pol2. ChIP-sequencing data of Pol2, which was analyzed using monoclonal antibody against Pol2 phosphorylated at Ser_5 (4H8), was obtained from the deposited data in the previous report {Kato:2013gj}. The data was processed by the same way as those in Figure 2 and plotted onto the same graph. (B) Compariosns of distributions of Set2ΔSRI-FLAG and H3K36me2 with that of Pol2. Distriubutions of Set2ΔSRI-FLAG and H3K36me2 were plotted with Pol2 as in Figure S1A.

Figure S2

A

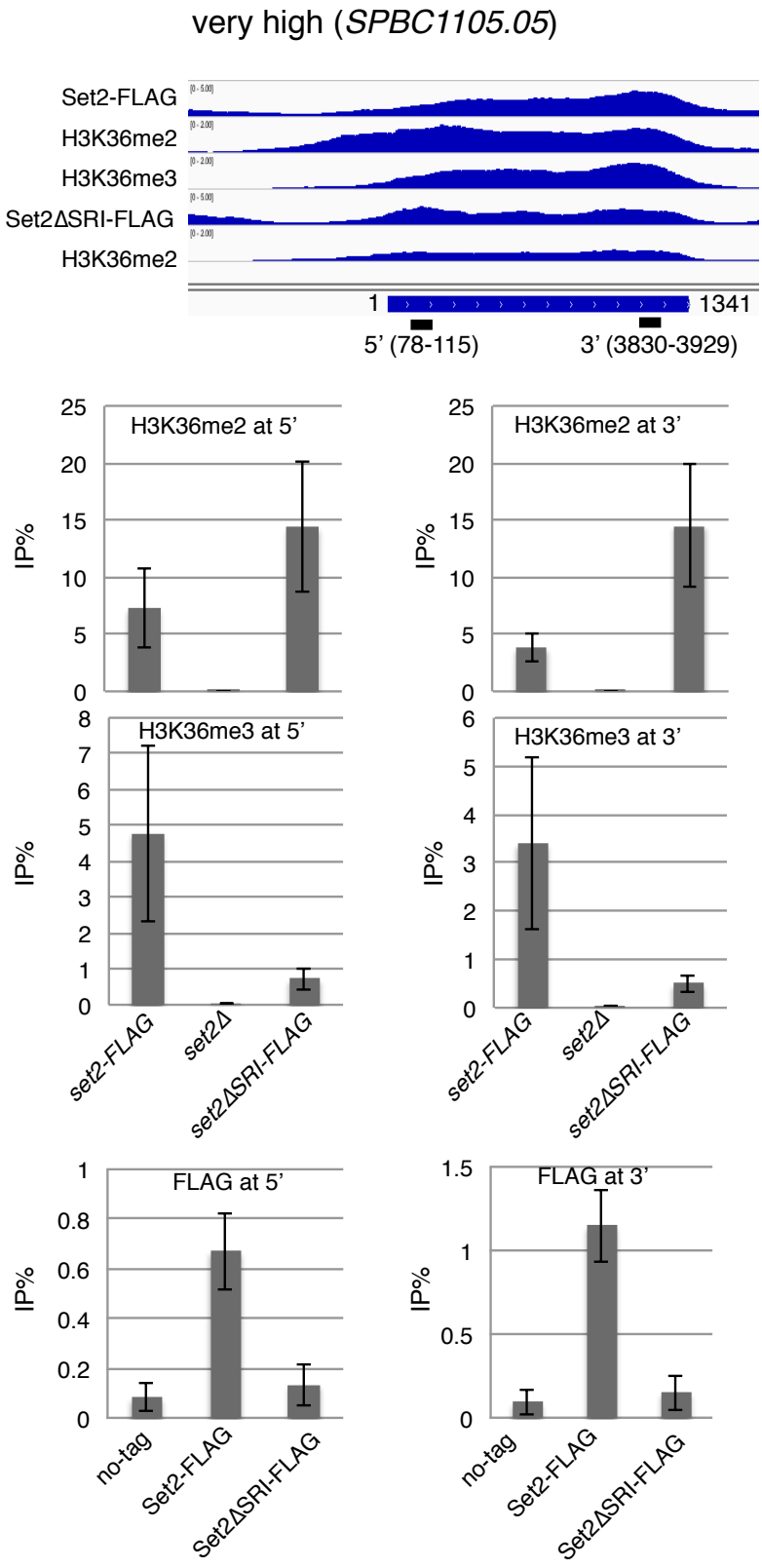
very high (*SPBC1105.05*)
Set2-FLAG
H3K36me2
H3K36me3
Set2ΔSRI-FLAG
H3K36me2
1
1341
5' (78-115)
3' (3830-3929)
H3K36me2 at 5'
H3K36me2 at 3'
H3K36me3 at 5'
H3K36me3 at 3'
FLAG at 5'
FLAG at 3'
IP%
set2-FLAG
set2Δ
set2ΔSRI-FLAG
no-tag
Set2-FLAG
Set2ΔSRI-FLAG

Figure S2

B

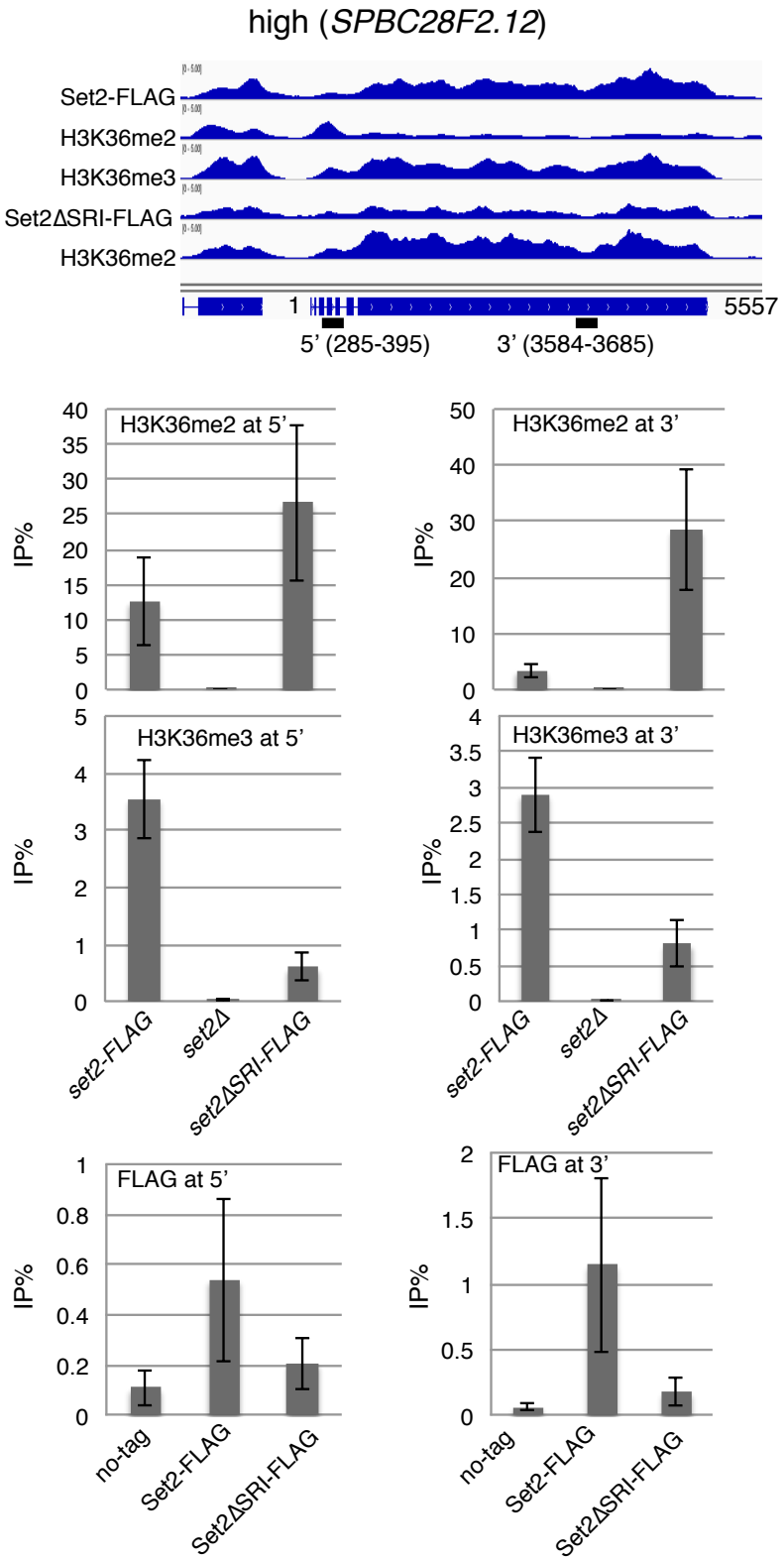
high (*SPBC28F2.12*)
Set2-FLAG
H3K36me2
H3K36me3
Set2ΔSRI-FLAG
H3K36me2
1
5557
5' (285-395)
3' (3584-3685)
H3K36me2 at 5'
H3K36me2 at 3'
H3K36me3 at 5'
H3K36me3 at 3'
FLAG at 5'
FLAG at 3'
IP%
set2-FLAG
set2Δ
set2ΔSRI-FLAG
no-tag
Set2-FLAG
Set2ΔSRI-FLAG

Figure S2

C

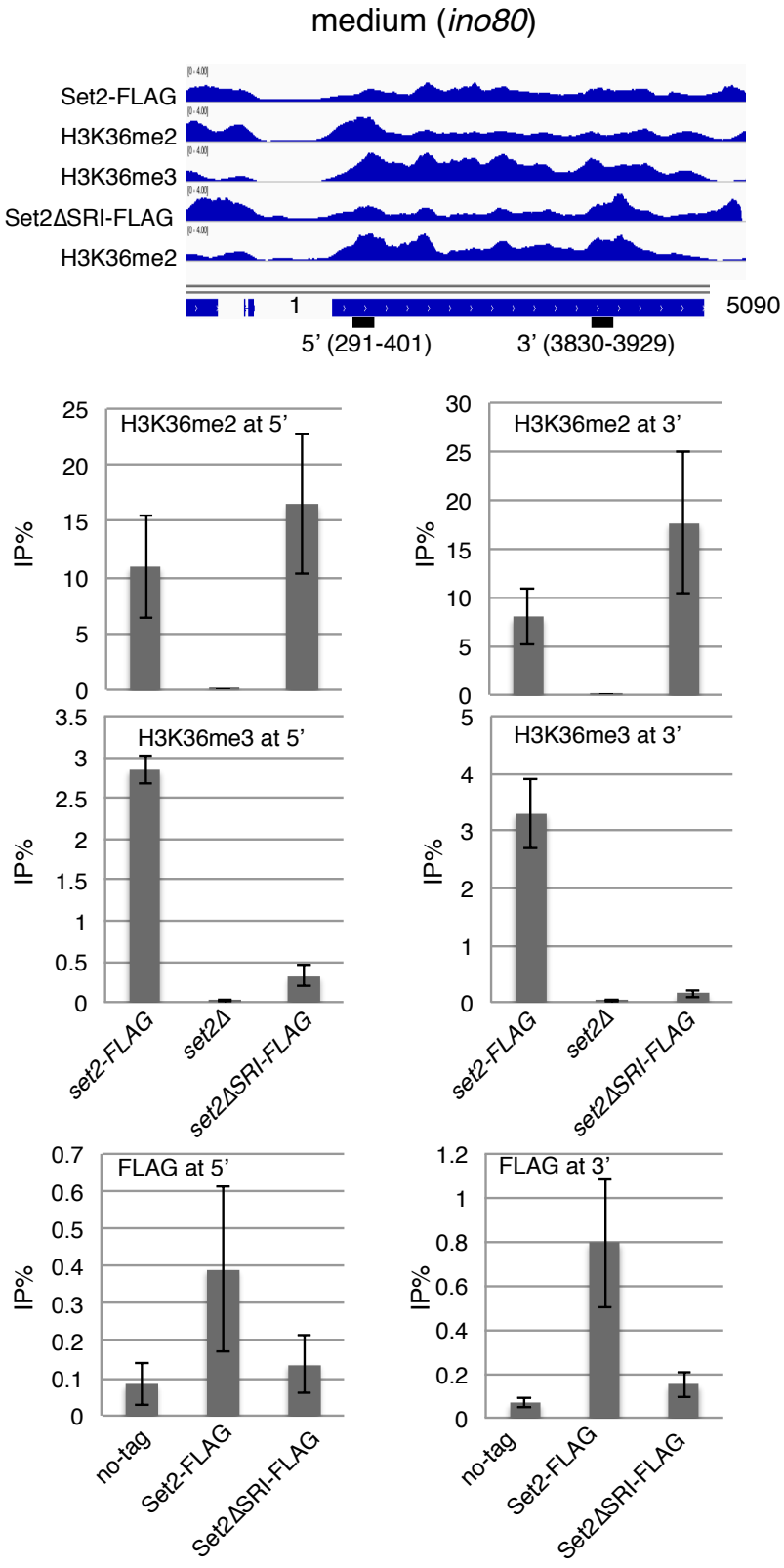
medium (*ino80*)
Set2-FLAG
H3K36me2
H3K36me3
Set2ΔSRI-FLAG
H3K36me2
1
5090
5' (291-401)
3' (3830-3929)
H3K36me2 at 5'
H3K36me2 at 3'
H3K36me3 at 5'
H3K36me3 at 3'
FLAG at 5'
FLAG at 3'
IP%
set2-FLAG
set2Δ
set2ΔSRI-FLAG
no-tag
Set2-FLAG
Set2ΔSRI-FLAG

Figure S2

D

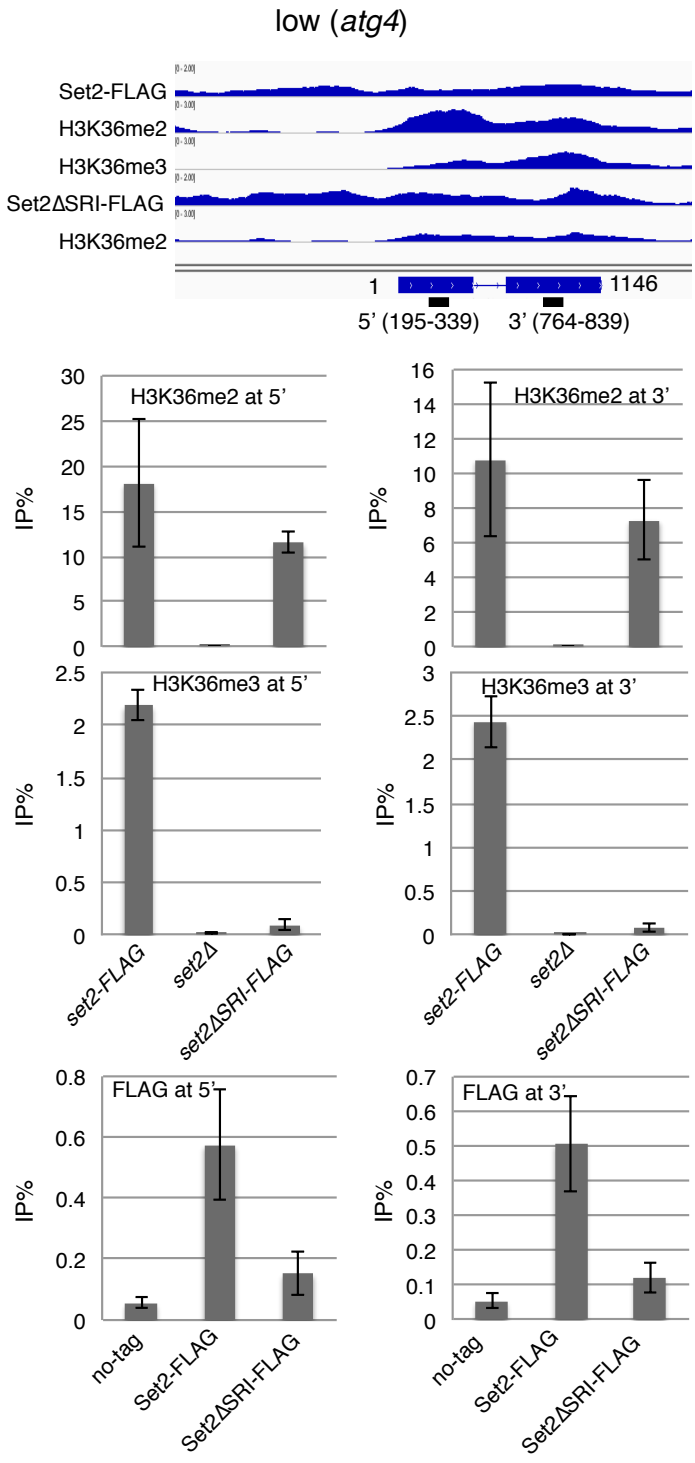
low (atg4)
Set2-FLAG
H3K36me2
H3K36me3
Set2ΔSRI-FLAG
H3K36me2
1
1146
5' (195-339)
3' (764-839)
H3K36me2 at 5'
H3K36me2 at 3'
H3K36me3 at 5'
H3K36me3 at 3'
FLAG at 5'
FLAG at 3'
IP%
set2-FLAG
set2Δ
set2ΔSRI-FLAG
no-tag
Set2-FLAG
Set2ΔSRI-FLAG

Figure S2. Effect of Set2ΔSRI mutation on H3K36 methylaiton and localization of Set2 protein. The indicated gene was chosen from each gene set as a gene showed the average expresseion level in each gene set: very high (A), high (B), medium (C) and low (D). (Upper panels) Blue box indicates each gene and blue graphs above show distributions of the indicated protein obtained from ChIP-seq analysis. Black box under each gene indicates the primer sets used for ChIP-qPCR analysis shown in lower panels. (lower panels) The amounts of H3K36me2, H3K36me3 and Flag-tagged Set2 proteins in Set2-FLAG cells or Set2ΔSRI-FLAG were measured by ChIP-qPCR. Error bars show the standard deviation of three independent experiments.

Figure S3

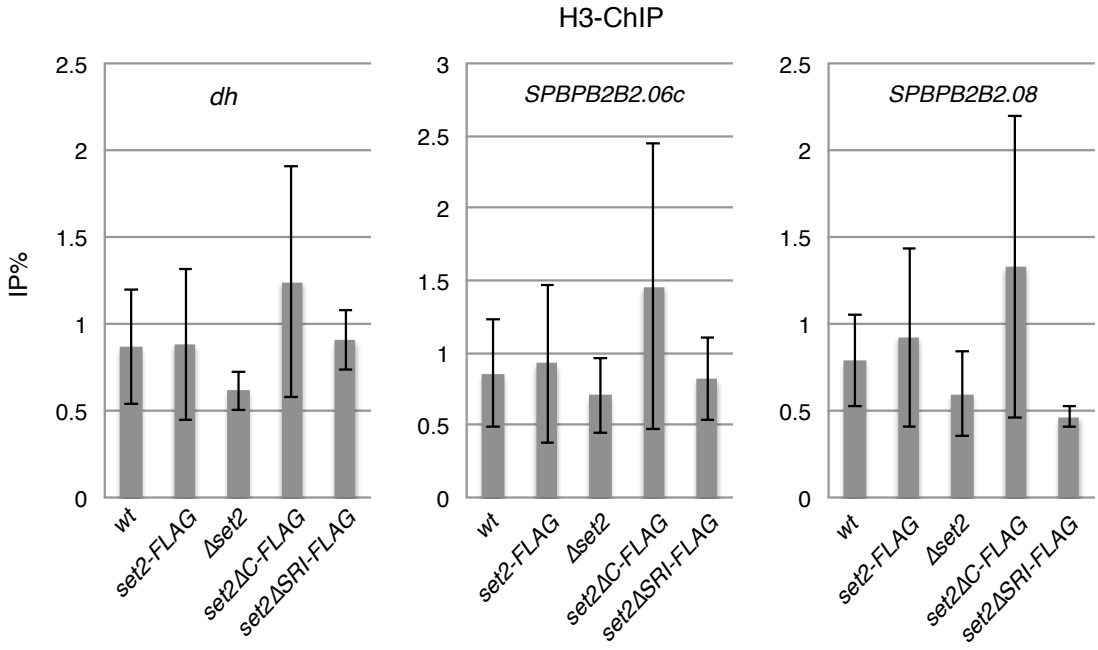

Figure S3. The levels of histone H3 were not affected by set2 mutation in heterochormatin and subtelomeric region. The levels of histone H3 at the heterochromatin (*dh*) and subtelomeric regions (*SPBPB2.06c* and *SPBPB2B2.08*) in the indicated cells were determined by ChIP-qPCR using antibody against C-terminal of histone H3. Error bars show the standard deviation of three independent experiments.

Figure S4

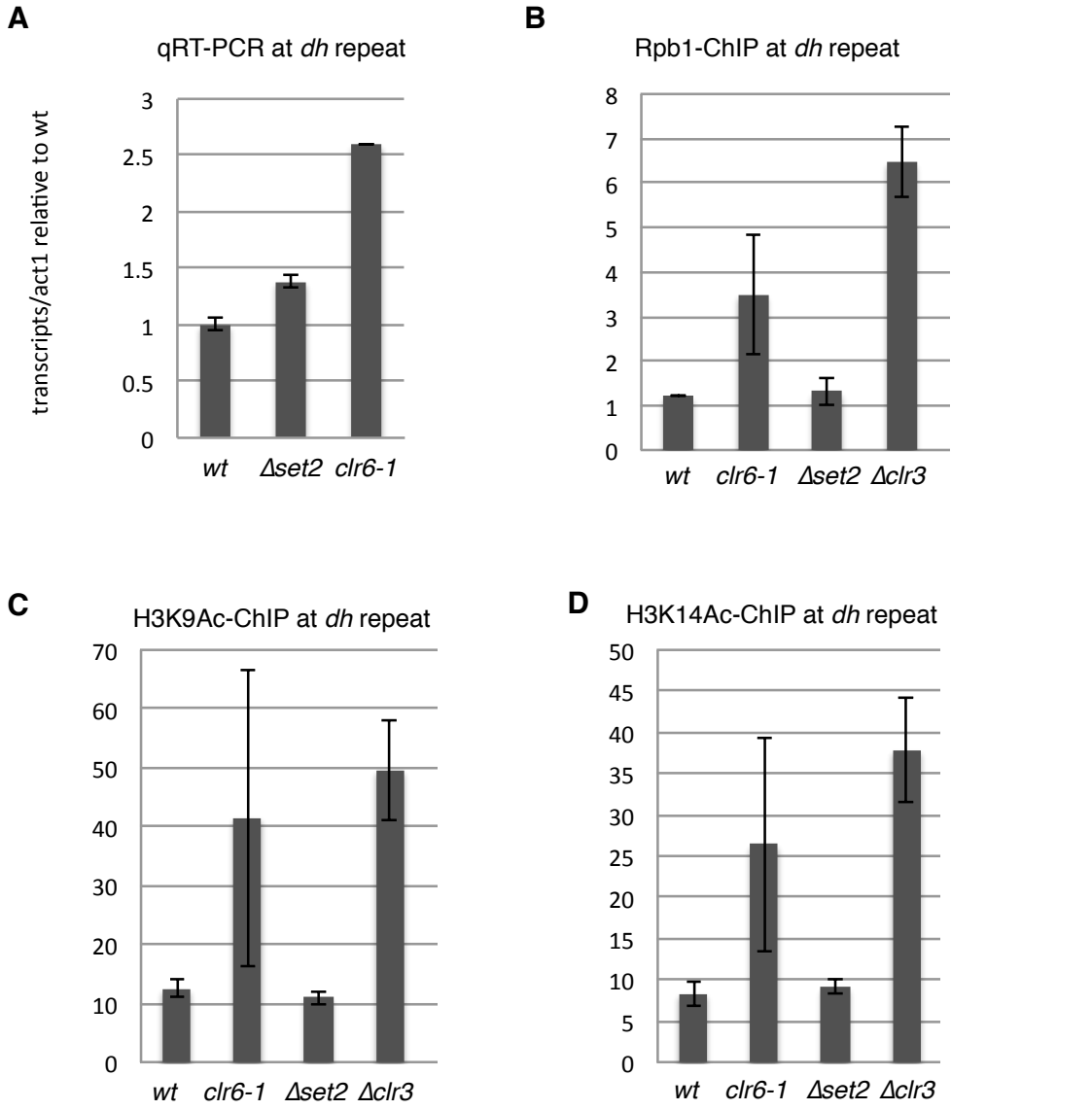

Figure S4 Clr6 mutant shows elevated levels of H3K9Ac and H3K14Ac accompanied by elevation of Pol2 and transcription at heterochromatin. (A) qPCR analysis of transcripts derived from heterochromatic *dh* repeat in the indicated strains were performed. Error bars show the standard deviation of three independent experiments. (B), (C) (D) Levels of Pol2 (C), H3K9Ac (D) and H3K14Ac (E) at heterochromatic *dh* repeats in the indicated strains were performed. Error bars show the standard deviation of three independent experiments.

Figure S5

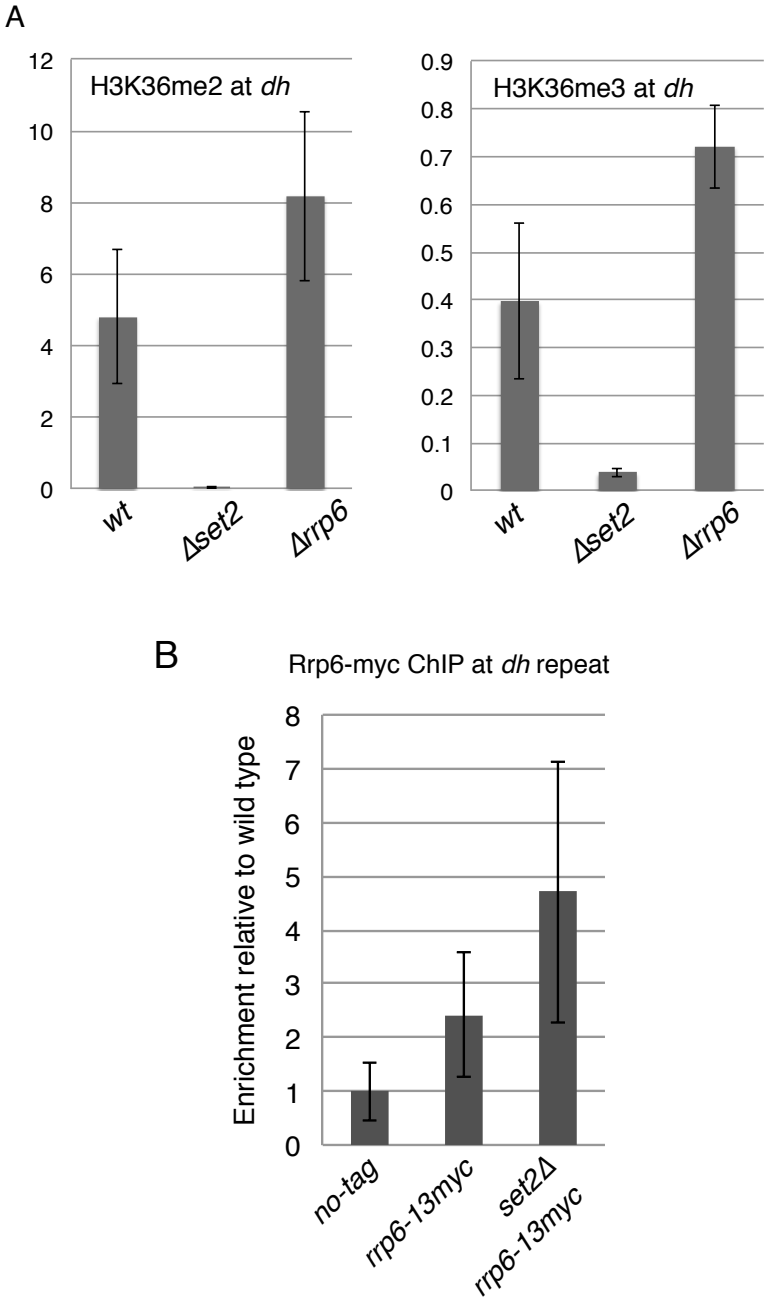

Figure S5. Genetic interaction between Set2 and nuclear exosomen. (A) Effects of *rrp6*Δ on H3K36me2 (left) and H3K36me3 (right). ChIP analyses of H3K36me2 and H3K36me3 at dh repeat in heterochromatin were performed in the indicated strans. (B) Recruitment of Rrp6-13my onto heterochromatin. ChIP analyses of Rrp6-13myc at *dh* repeat in heterochromatin were performed in the indicated strains using an anti-myc antibody.

Figure S6

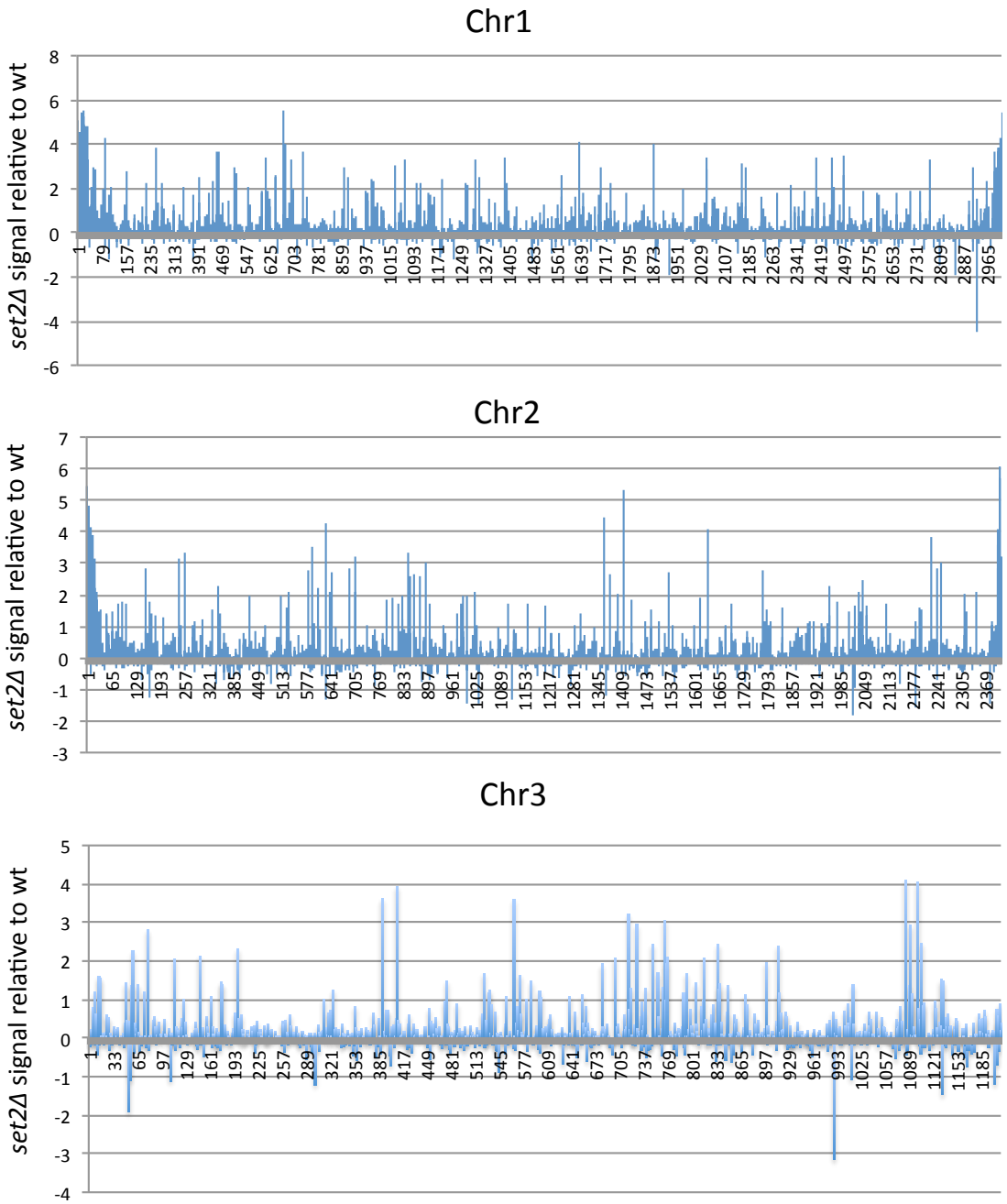

Figure S6. Distribution of genes up- or downregulated in *set2*Δ cells. Genes on each chromosome are arranged on the *y*-axis according to their order on the chromosome. The *x*-axis represents the log2 of the array signals in wild-type cells.