## Supplementary materials and methods

### *Strains used in this study*

All strains used are of the SK1 strain background (Kane and Roth, 1974). For the transcriptome analyses, we used the following diploid strains : ORD6822 (*MATa/MATα lys2/lys2 ho::LYS2/ho::LYS2 ura3/ura3*), ORD6830 (same as ORD6822 but *trp1/trp1 clb5Δ::KanMX/clb5Δ::KanMX clb6Δ ::TRP1/clb6Δ ::TRP1*) and ORD6847 (same as ORD6822 but *spo11Δ::hisG/spo11Δ::hisG trp1::spo11Y135F-TRP1/trp1::spo11Y135F-TRP1*). For chromatin immunoprecipitation (ChIP) studies, the wild-type strain was ORD7339 (same as ORD6822, but *arg4-nsp,bgl/arg4-nsp,bgl leu2-K/leu2-R*), the *clb5Δ clb6Δ* strain was ORD6830 and the *spo11Y135F* strain was ORD7341 (same as ORD6822, but *arg4-bgl/arg4-RV his4/his4 leu2/leu2 trp1/trp1 nuc1Δ::LEU2/nuc1Δ::LEU2 spo11Δ::hisG-URA3-hisG/spo11Δ::hisG-URA3-hisG trp1::spo11Y135F-TRP1/trp1::spo11Y135F-TRP1)*. The *dmc1Δ* strain was ORD7354 (Robine et al., 2007) and the *set1Δ dmc1Δ* strain was ORD9624 (same as ORD6822, but *arg4/arg4 leu2-K/leu2-K set1Δ::KanMX/set1Δ::KanMX dmc1Δ::LEU2/dmc1Δ::LEU2*)

### *Culture conditions*

For sporulation, cells were grown in rich medium (YPD) for 24h, transferred into SPS pre-sporulation medium and grown overnight to ~$5.10^7$ cells/ml ($4.10^7$ in the ChIP studies). Cells were then harvested by centrifugation, washed with one volume 1% potassium acetate and resuspended into sporulation medium (1% potassium acetate, 1/1000 polypropylene glycol 2000, required supplements) at a density of $3.10^7$ cells/ml ($2.10^7$ in the ChIP studies) (time 0 h). For ChIP-chip in exponentially growing cells, cells were at a $2.10^7$ cells/ml density.

### *mRNA isolation*

Cell samples for RNA extraction were taken by filtration of 40 ml culture on nitrocellulose membranes and immediately frozen in liquid nitrogen. Frozen cells stored at –80°C were re-suspended in 1.5 ml TES buffer (10 mM Tris pH 7.5, 10 mM EDTA, 0.5% SDS), 1.5 ml acid phenol/chloroform (5:1) and 0.5 g glass beads. The cells were vortexed vigorously for 1 min, incubated in a 65°C water bath for 1 h with

20 s vortexing every 10 min, placed on ice for 5 min and centrifuged at 1500 g for 5 min. The aqueous phase was removed and 2 to 3 successive acid phenol/chloroform extractions were performed, followed by washing with 1.5 ml chloroform/isoamyl alcohol (25:1). RNA was precipitated in 2 volumes of 95% ethanol and 1/10 volume 3M sodium acetate, re-suspended in DEPC-treated H2O and stored at –80°C. 500 to 1000 µg of total RNA for each sample were used for Poly(A)+ RNA isolation with the Micro-Fast Track 2.0 kit (Invitrogen) following the manufacturer's protocol for yeast mRNA.

***Meiotic DNA extraction and Southern blot analysis***

Genomic DNA was prepared in agarose plugs, digested with restriction enzymes and analyzed by one dimensional agarose gels and Southern blot as described (Borde et al., 2000). Restriction digests and probes were as follows: For DSBs at the *YCR047C* promoter, DNA was digested with *Ase*I and probed with a PCR fragment corresponding to the *YCR048W* ORF. For DSB at *FTR1*, digest was *BspE*I and probe *YER143C*. For DSB at *SET4*, digest was *Stu*I and probe *YJL101C* and for *PES4* DSB, digest was *Stu*I and probe *YFR021W*. DSB frequency was detected and quantified using a Storm Phosphorimager and the ImageQuant software (Molecular Dynamics).

***ChIP quantification by qPCR***

Enrichment of DNA bound by H3K4me3 was estimated by quantitative PCR using an Applied Biosystems 7500 Real-Time PCR system using 1/15000 of the immunoprecipitate or 1/4500000 of the whole cell extract with 0.4 µM primers, SYBR Green PCR master mix (Applied Biosystems) and the PCR program: 95 ˚C for 15 sec; 60 ˚C for 1 min; 40 cycles. A dilution series of genomic DNA from the MAT$\alpha$ parent of ORD7339 was used to establish a standard curve from 0.5 to $5*10^{-4}$ ng. For H3K4me3 ChIP, the relative enrichment was calculated as the ratio between the target locus and a negative control locus, which is the nt1389 to nt1470 after ATG codon region internal of the *NFT1* gene (*NFT1*int), whose expression does not vary during meiosis, and which shows very low trimethylation during meiosis.

***ChIP-chip data normalization***

For H3K4me3 and H3Cter ChIP-chip analyses, we calculated the log2 ratio of background-subtracted intensities, corrected the spatial bias with the algorithm MANOR (Neuvial et al., 2006) and centered the log2 ratios such that the mean of all log2 ratios of an array is 0. Next we normalized the data within each time-course in order to compensate for slight variations in ChIP efficiency from one time-point to another. For H3Cter, we normalized for each array the standard deviation to its average value across the time-course (t=0h, 1h, 2h). For H3K4me3, for the same purpose, we normalized the height of the peak at the beginning of the ORF: we calculated for each time-point the average value of the log2 ratios for probes located between 0 and 500 bp of all the ORFs represented on the array. Next, we calculated the average value of this peak during the whole time-course, and normalized the log2 ratios of each array such that each has now this average peak value.

For DSB ChIP-chip, we normalized with a different strategy. Since we anticipated a quantitative difference in the Ip signal between the two *SET1* and *set1*$\Delta$ strains, we used for an accurate comparison of the two strains the background-based normalization procedure previously described (Buhler et al., 2007). The set of probes considered for background on our microarrays is in Table S9. DSB peaks were defined similarly as in Buhler et al (2007), by smoothing the ratio twice with a seven elements sliding average: running the sliding window twice avoids spurious peaks that could occur if the signal is not sufficiently de-noised. DSB peaks are detected using the first-derivative of this de-noised profile and were considered as significant if the corresponding ratio was higher than 5-fold over background.

## Supplementary tables

**Table S1** Average ratios of the RPA, total H3 and H3K4me3 ChIP-chip experiments. For DSB ratios, average ratios were calculated from background-normalized ratios of two independent experiments for each strain. For the other ChIP-chip experiments, the ratios were normalized as described in Supplementary Materials and Methods and the values are from one experiment, except for H3K4me3 in WT, which is the average of two independent experiments. Reference number refers to the array manufacturer element identifer.

**Table S2** List of the 1013 *dmc1*Δ meiotic DSB hotspots. Each DSB peak was attributed the coordinate of one array element where the ratio is maximum after running a sliding window of seven consecutive probes as described in the supplemental Experimental Procedures. The average DSB ratio was calculated from background-normalized ratios of two independent experiments. The star indicates DSB peaks for which the value at the indicated position was missing, and then the value of the closest probe is indicated.

**Table S3** List of the 317 *set1*Δ *dmc1*Δ meiotic DSB hotspots. Same legend as Table S1.

**Table S4** List of the 1074 genes upregulated during wild-type meiosis. The table contains the SGD ID, Gene name, upregulated group (from 1 to 11 and X for unclassified genes) as defined in the text, Process, Function and Component annotations from Gene Ontology data (Ashburner et al., 2000) provided on the Saccharomyces Genome Database website (Cherry et al., 1998) (GO Consortium: http://www.geneontology.org; SGD: http://www.yeastgenome.org).

**Table S5** List of the 723 genes downregulated during wild-type meiosis. Same legend as Table S4.

**Table S6** Microarray data for wild-type, *spo11Y135F* and *clb5*Δ *clb6*Δ transcriptomes and H3K4 trimethylation. For each gene, the up- or down-regulated group is indicated, when relevant. For each strain, columns whose title contains “Rel. exp.” are the transcriptome data relative to time 0 h. Wild-type and *spo11Y135F* transcriptome data are the average of three and two independent experiments, respectively. Other columns refer to data from the ChIP-chip experiments against H3K4me3 (“Tri”) or total histone H3 (“H3”): for each gene, the values of the probes covering the 0-500 bp regions of each ORF were averaged.

**Table S7** List of the 22 DSBs sites increased more than two fold in *set1*Δ *dmc1*Δ. For each DSB site, the chromosome number, coordinate and name of the nearest gene is indicated, as well as the background-normalized DSB ratio calculated from two independent experiments for each *dmc1*Δ and *set1*Δ *dmc1*Δ.

**Table S8** List of the primers used for qPCR.

**Table S9** Array elements used for background normalization of the DSB mapping experiments microarray data.

## Supplementary figures legends

**Figure S1** Comparison of DSB Signals in *dmc1*Δ and in *set1*Δ *dmc1*Δ. Distribution of background-normalized RPA ChIP ratios from *dmc1*Δ (blue) and *set1*Δ *dmc1*Δ (red). For each strain, data are the average of two independent experiments. Black triangle indicates the centromere. Blue and red circles indicate the position of DSB peaks determined, after smoothing with a sliding window, in *dmc1*Δ and *set1*Δ *dmc1*Δ, respectively. Green circles indicate a DSB peak higher in *set1*Δ.

**Figure S2** Comparison of our DSBs sites mapped in *dmc1*Δ by RPA ChIp-chip with two previous genome-wide studies of DSB sites mapped in *dmc1*Δ. The Venn diagram compares the DSBs sites defined in the three different studies, and considers two DSB sites being common between two experiments if they are separated by less than 1 kb, which is approximately the resolution of the DSB mapping approach used in the three studies. From the Buhler et al study (Buhler et al., 2007), we considered the 1027 DSB sites defined by the authors as enriched more than 5 fold over background after BND cellulose purification of ssDNA at the ends of *dmc1*Δ DSBs; from the Blitzblau et al study (Blitzblau et al., 2007), we considered the 258 sites defined by the authors as a cluster of three adjacent sites enriched in at least two individual experiments, after BND cellulose purification of ssDNA at the ends of *dmc1*Δ DSBs.

**Figure S3** Relative amounts of H3K4me3 during meiosis. **(A)** Total cell extracts of a wild-type strain (ORD7339) were taken at the indicated times during meiosis and the same samples probed after Western blotting with an antibody against the C terminal part of H3 or against H3K4me3. **(B)** Graph showing the relative amounts of H3K4me3 relative to total H3. The signal was quantified after densitometric scanning of the autoradiography shown in **(A)**.

**Figure S4** Comparison of wild-type meiotic gene expression regulation in the present and three previous reports. (**A**) Venn diagram comparing the genes defined with the same criteria as upregulated (left) and downregulated (right) during meiosis from the datasets of (Chu et al., 1998; Friedlander et al., 2006) and in the present work. The data of the two previous reports (Chu et al., 1998; Friedlander et al., 2006) were re-analyzed with the same criteria as ours: regulated genes were defined as having at least one time-point with a variation of at least two fold compared with time 0h. (**B**) Similar comparison between the datasets from Chu et al. (1998), Primig et al. (2000) and this study. To re-analyze the data from Primig et al, for each gene, absolute transcriptomic levels from each time-point were divided by the level at time t=0 h. Genes were considered as regulated when they vary more than 4 fold in at least one time-point (relative log2ratio above 2 or below -2). Chu et al. data were re-analyzed as in (A).
These results show that a large proportion of the genes defined in our study as regulated were also found in the previous studies. The fact that we defined fewer up- or downregulated genes and that most are common with the other studies is most likely due to the fact that our experiment was carried out in three independent replicates and suggests a more solid definition, with less false positive.

**Figure S5** H3K4me3 and total histone H3 levels among the upregulated or downregulated gene-expression clusters defined from the wild-type transcriptome, in the WT, *spo11Y135F* and *clb5Δ clb6Δ* strains. In each cluster, for relative expression, the log2 of the change of expression relative to time 0h is plotted as a function of time. For H3K4me3 and total H3, the average log2 ChIp ratio observed on the 0-500 bp of each gene is represented.

**Figure S6** Total H3 levels at the proximity of DSB sites. The non-regulated genes were divided into four quartiles according to their average transcript levels during meiosis (Primig et al., 2000). Then DSB sites were examined in each category, both for expression levels of the adjacent genes (top panel), for total H3 levels (mid panel) and for H3K4 trimethylation levels normalized to total H3 (bottom panels). Profiles of control DSB-poor regions were also calculated for each quartile (dotted lines), as described in Experimental Procedures.

**Figure S7** Meiotic transcriptome of the *spo11Y135F* and *clb5*Δ *clb6*Δ strains. (**A**) Meiotic progression of the *spo11Y135F* (ORD6847) and *clb5Δ clb6Δ* (ORD6830) strains used for the transcriptome analyses, monitored by fluorescence microscopy of 4',6'-diamidino-2-phenylindole (DAPI)-stained cells. For ORD6847, the values presented are the average of the two independent time-courses used to generate the transcriptome data. (**B**) Pair-wise average linkage clustering analysis and visualization of gene expression maps were done using the programs Cluster and Treeview (Eisen et al., 1998). We used the centered Pearson correlation as the similarity metric to classify the genes according to their temporal patterns. The regulation profile of the 1074 upregulated genes (left) and the 723 downregulated genes (right) is shown according to the clusters defined in the wild-type strain. The name of each up- or down-regulated cluster is indicated along each panel. The list of the genes of each cluster (up1-11 and down 1-8) is in Tables S3 and S4, respectively.

**Figure S8** Comparison of the H3K4me3 levels in DSB regions and in DSB-free regions. The DSB-poor control regions category was separated in two categories: DSB-mild regions (close to a DSB site showing an enrichment between 2 and 5 fold after detection using a sliding window approach as described in Supplementary Materials and Methods) and the remaining regions (DSB-free). H3K4me3 levels measured in exponentially growing cells were calculated close to each region. The statistical differences were calculated using the Wilcoxon signed-rank test.

## Supplementary references


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**Table S8: primers used for quantitative PCR analyzes**

| Gene | ORF length | Forward primer | Reverse primer | Coordinates/start |
|---|---|---|---|---|
| *NFT1* | 3656 | CATGACAGTTACGGCCCTAATG | CATGACAGTTACGGCCCTAATG | 1389-1470 |
| *MSH4* | 2636 | CTAGGACCGCAACCTCCTTAAG | GTATATGCAACAAAGAACACAGTCAATACT | 245-330 |
| *NDT80* | 1883 | AGTGGGACCTCCATTTGAATTAGT | GAACCCTCTGTCAATTCTTGGAA | 192-297 |
| *YPR078C* | 1119 | TGCACAATTAGGTGTCAGAGTTGA | GAACAAGATCGGAAAAAAGTATCACTT | 150-266 |
| *RPS0A* | 1213[a] | GATTGAAAACAGTTCGAGTGAAAGAA | TCCCAGCATTCCGTTTGGT | 133-201[b] |
| *rDNA* | NA | CTGATGTCTTCGGATGGATTTGAG | TTTCCTCTGGCTTCACCCTATTC | NA[c] |
| *YCR047C* | 827 | TATGTCGTCCACCTGGTCGTCG | TCCTAAACAGCGGTTGATGAGG | 396-507[c] |

[a] This includes the intron, from nt 91 to nt 545
[b] This is in the intron part of the *RPS0A* gene
[c] From Buhler et al, 2007

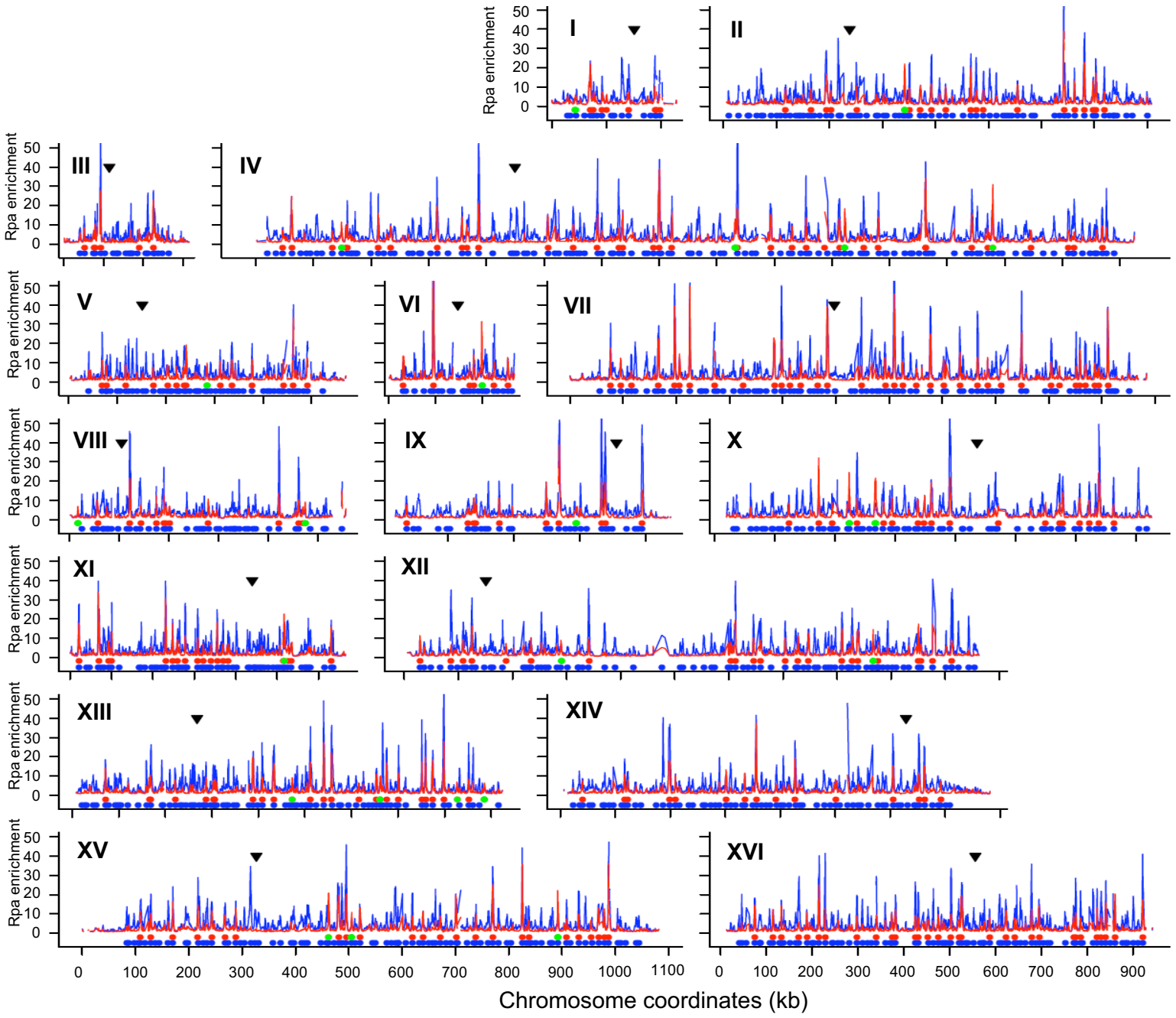

I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
Rpa enrichment
0
10
20
30
40
50
0
100
200
300
400
500
600
700
800
900
1000
1100
Chromosome coordinates (kb)

**Figure S1**

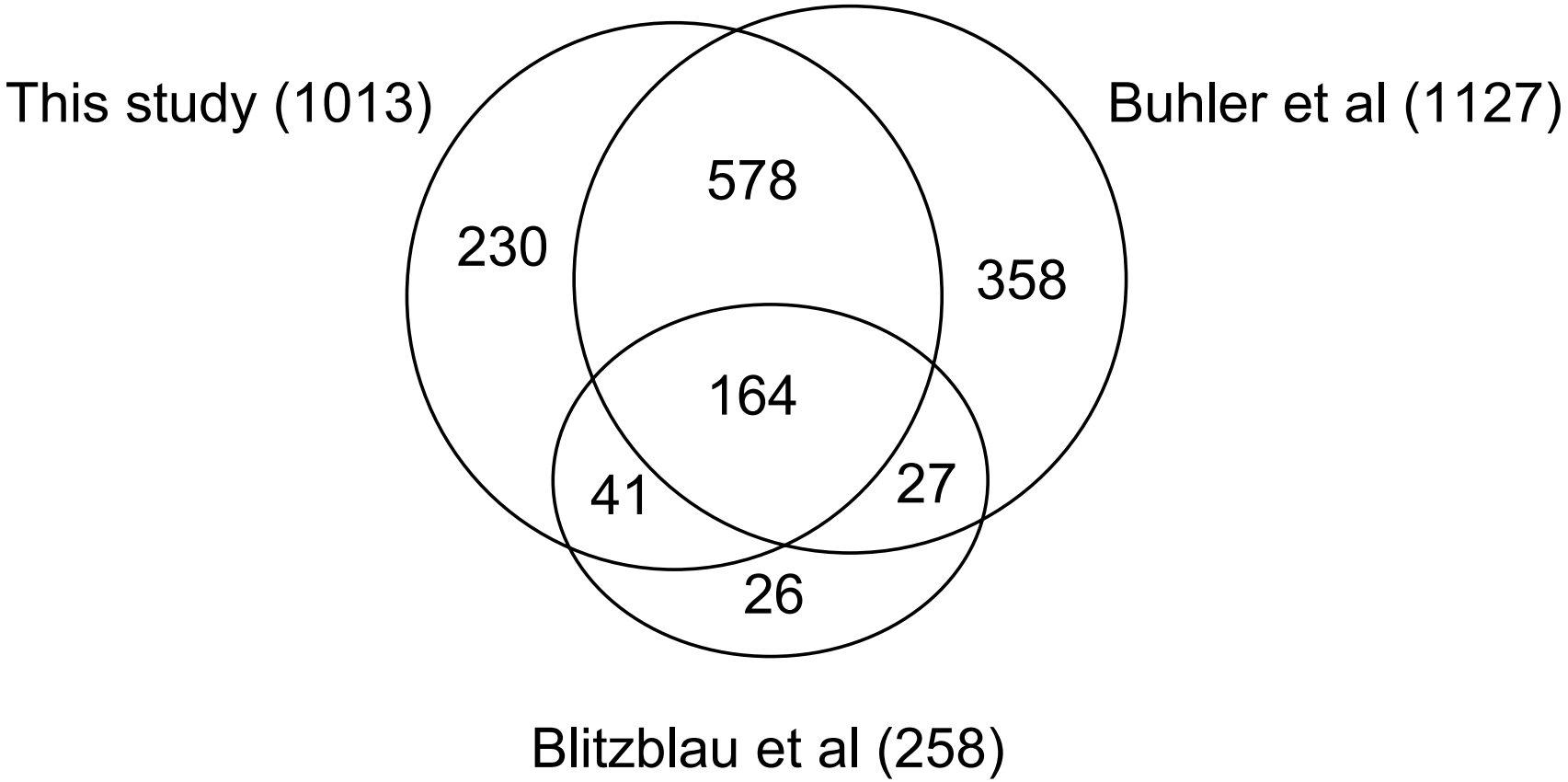

This study (1013)
Buhler et al (1127)
Blitzblau et al (258)
230
578
358
164
41
27
26

**Figure S2**

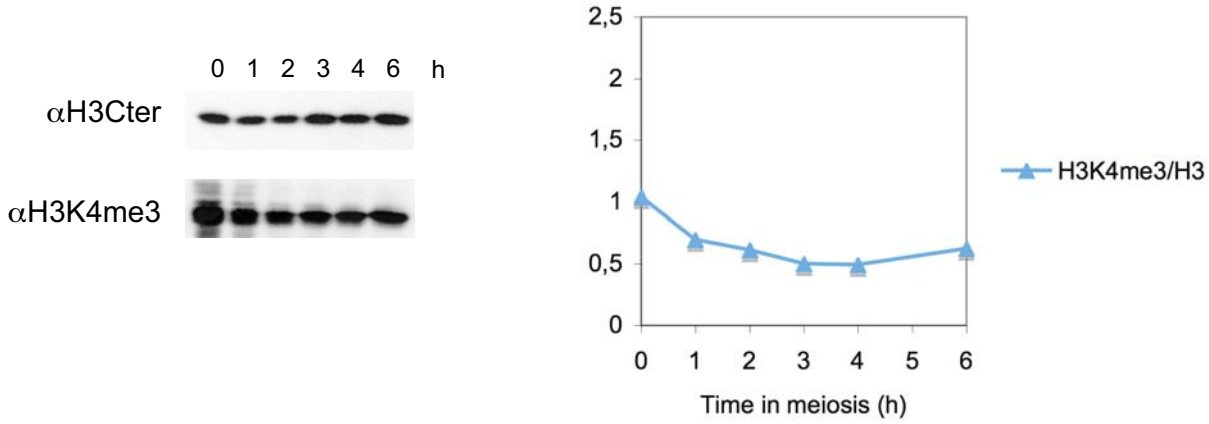
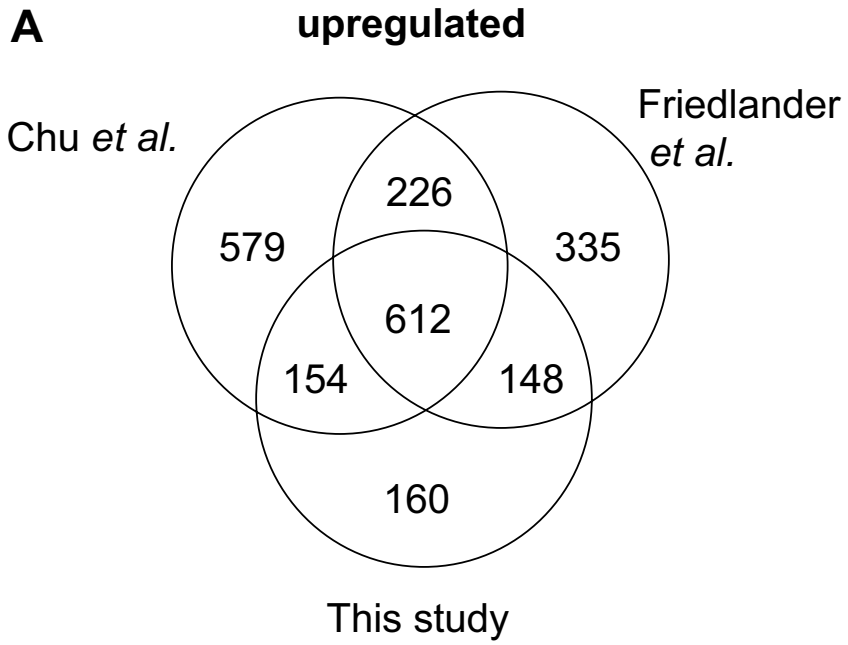
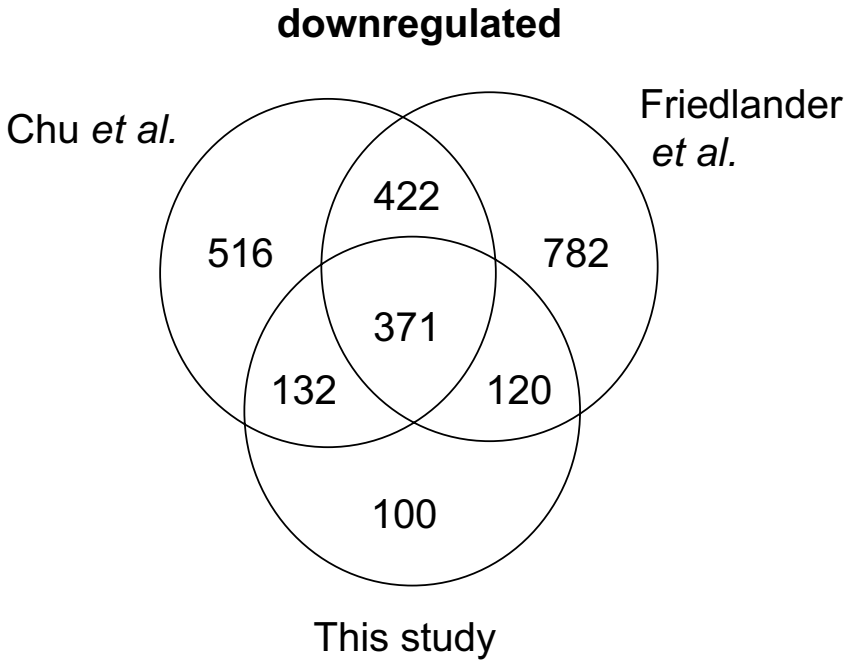

0 1 2 3 4 6 h
αH3Cter
αH3K4me3
2,5
2
1,5
1
0,5
0
0 1 2 3 4 5 6
Time in meiosis (h)
H3K4me3/H3

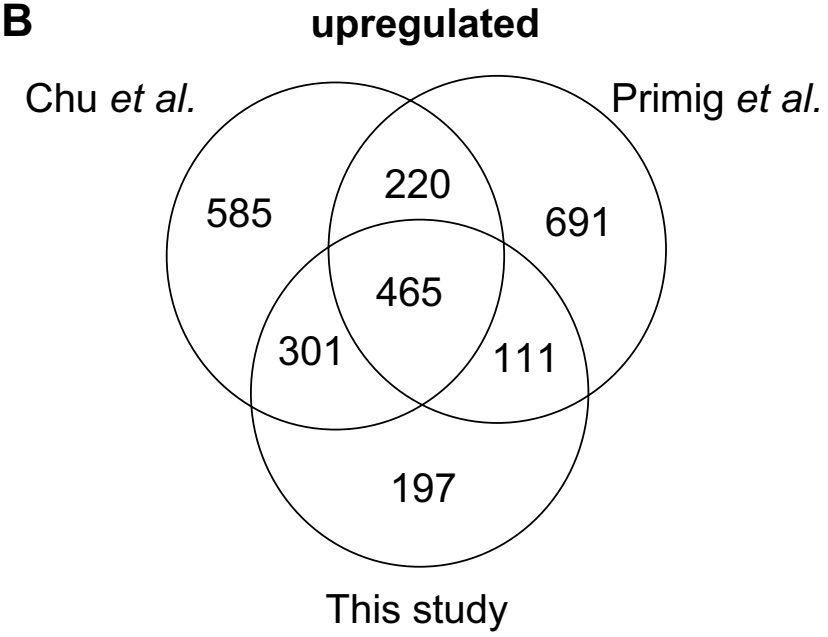
**Figure S3**

**A**


upregulated
Chu *et al.*
Friedlander *et al.*
This study
579
226
335
612
154
148
160


downregulated
Chu *et al.*
Friedlander *et al.*
This study
516
422
782
371
132
120
100

**B**


upregulated
Chu *et al.*
Primig *et al.*
This study
585
220
691
465
301
111
197

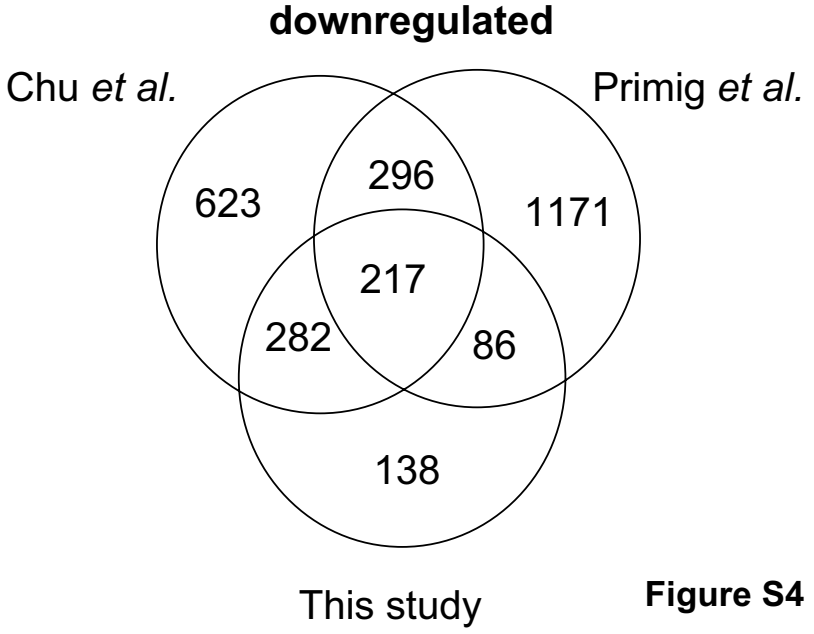

downregulated
Chu *et al.*
Primig *et al.*
This study
623
296
1171
217
282
86
138

**Figure S4**

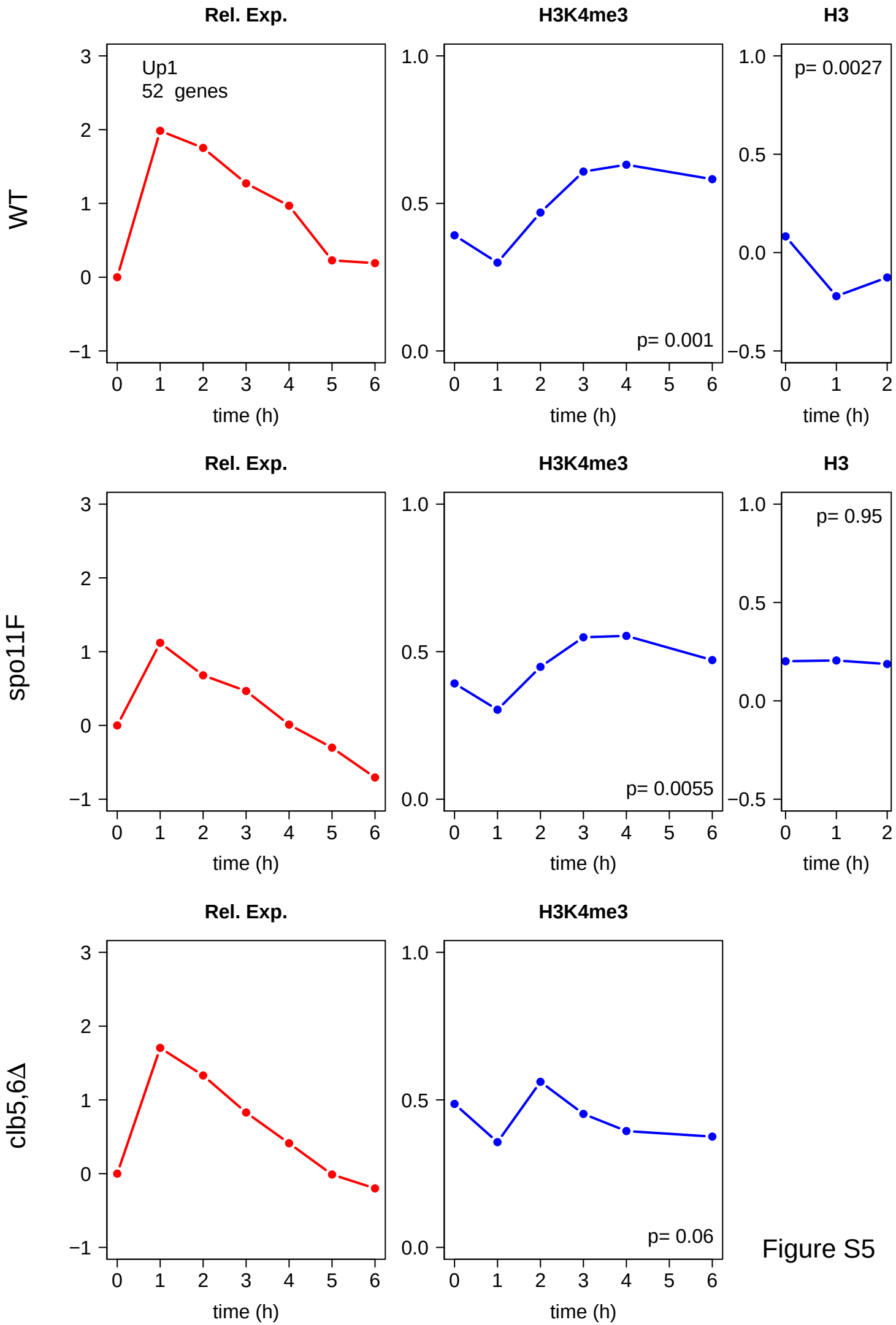
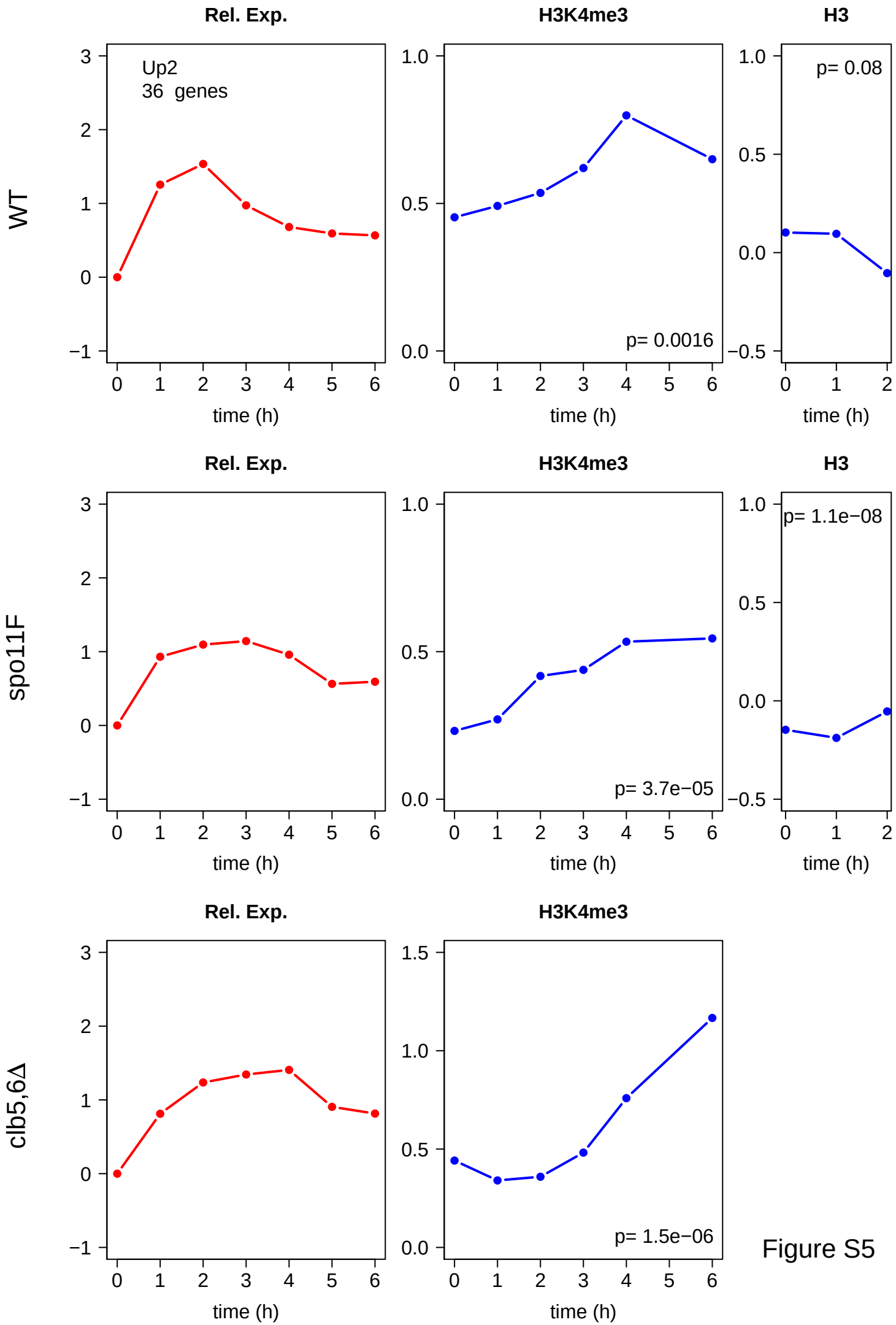

Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Up1
52 genes
p= 0.001
p= 0.0027
p= 0.0055
p= 0.95
p= 0.06
time (h)

Figure S5


Rel. Exp.
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H3
WT
spo11F
clb5,6Δ
Up2
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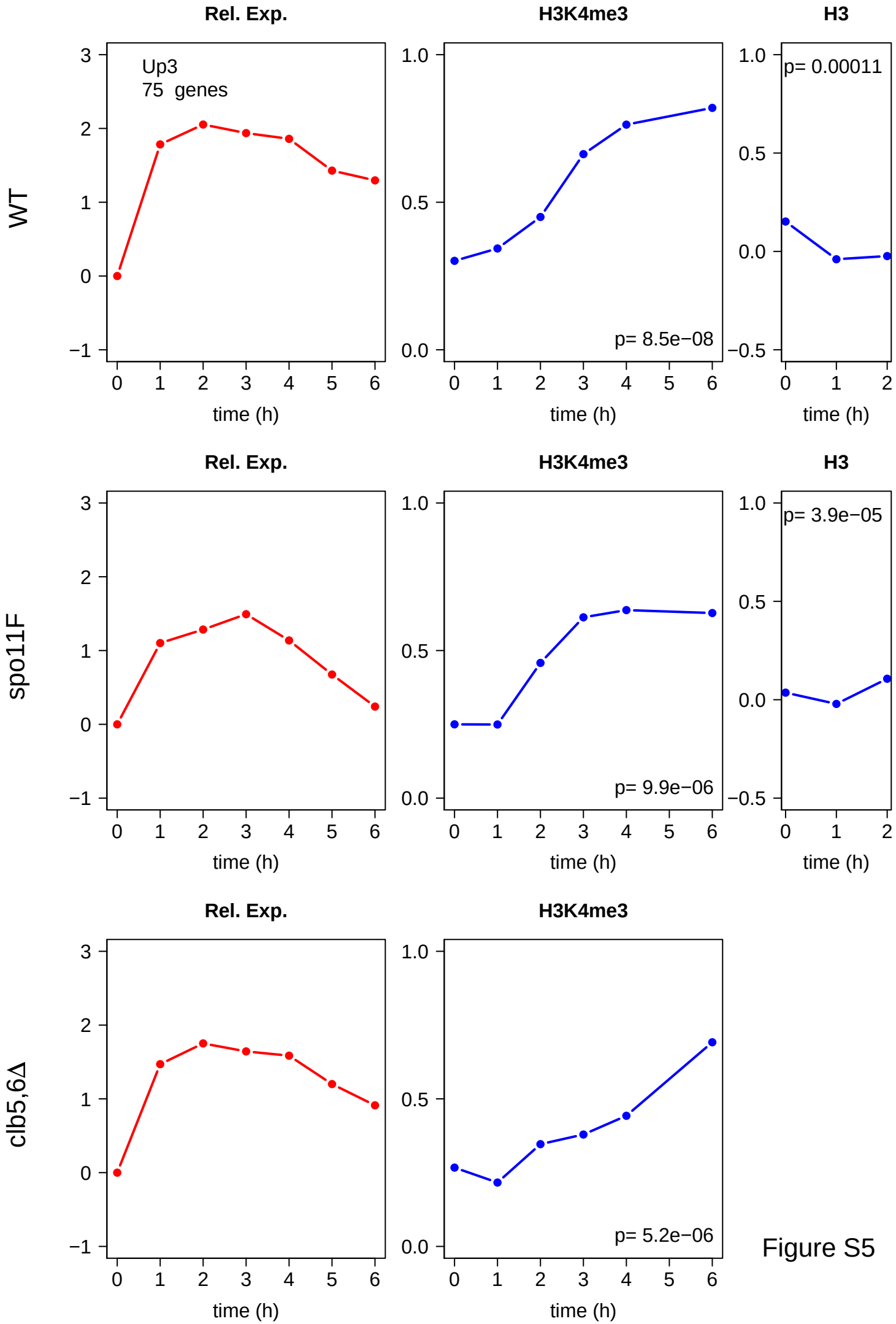
p= 1.1e−08
p= 1.5e−06
time (h)

Figure S5


Rel. Exp.
H3K4me3
H3
WT
Up3
75 genes
p= 8.5e−08
p= 0.00011
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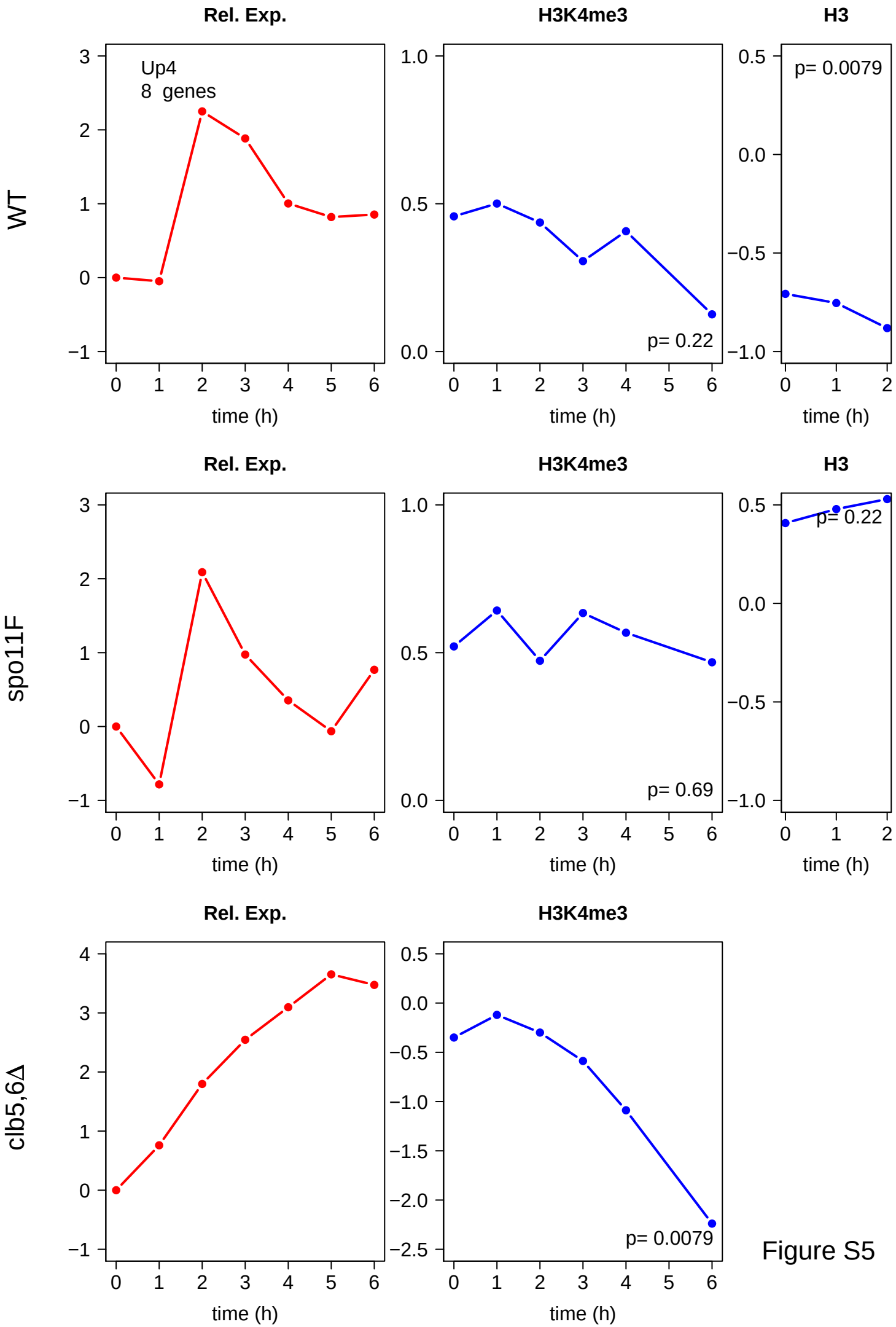
p= 3.9e−05
clb5,6Δ
p= 5.2e−06
time (h)

Figure S5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H3K4me3
H3
WT
spo11F
clb5,6Δ
Up4
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p= 0.22
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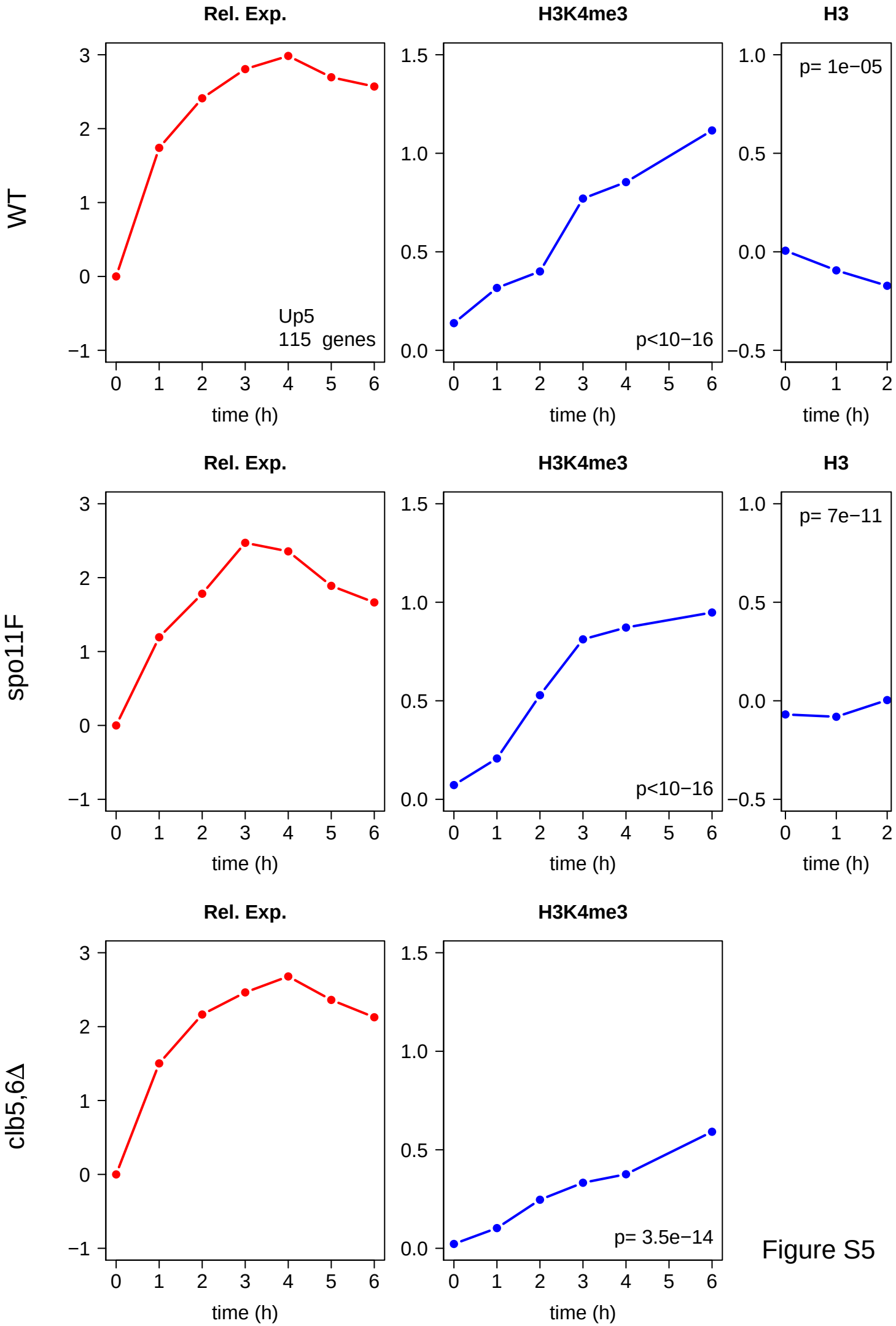
p= 0.69
p= 0.22
p= 0.0079
time (h)

Figure S5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WT
spo11F
clb5,6Δ
Up5
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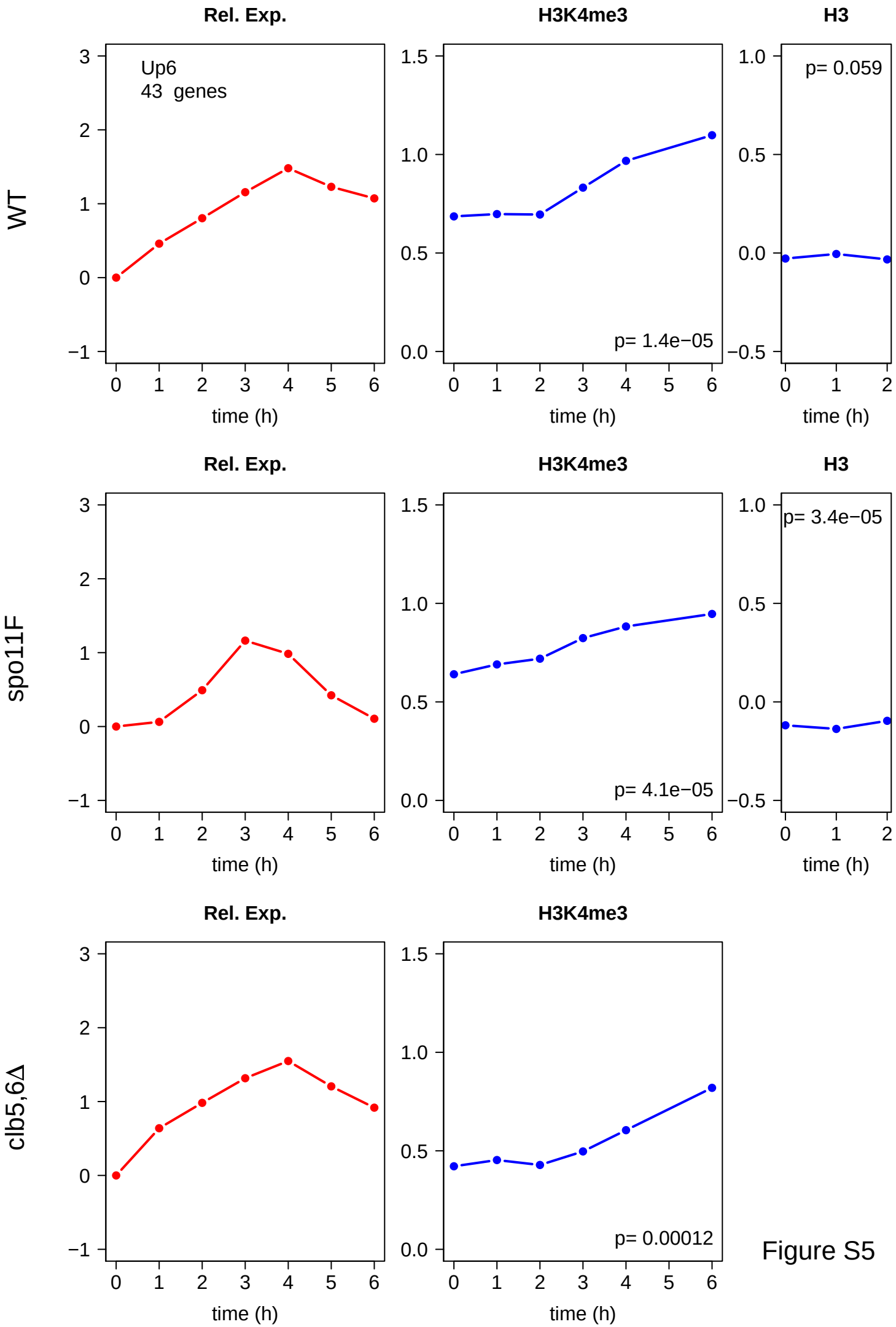
p= 1e−05
p= 7e−11
p= 3.5e−14
time (h)

Figure S5


Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Up6
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p= 0.059
p= 4.1e−05
p= 3.4e−05
p= 0.00012
time (h)

Figure S5

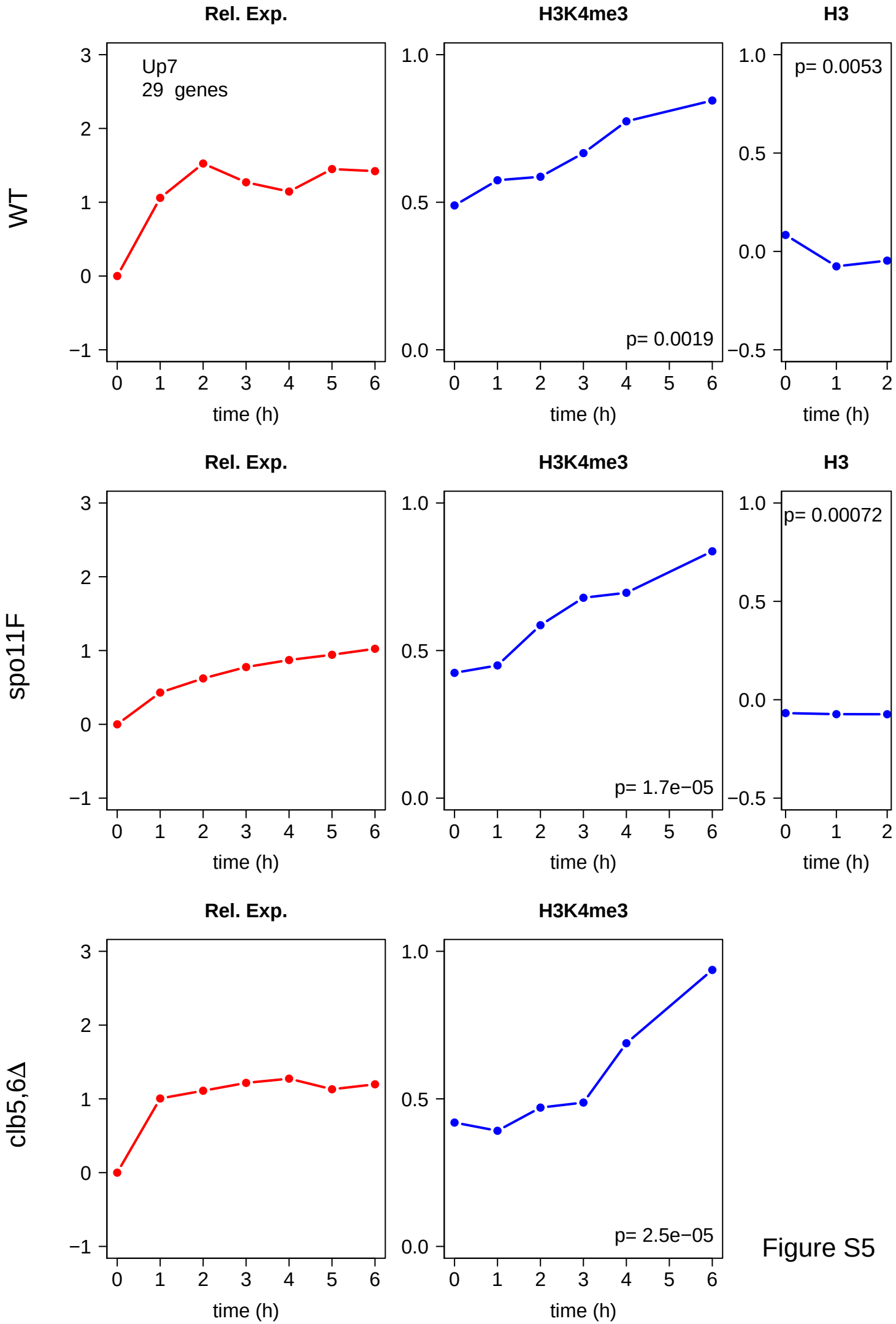
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Up7
29 genes
p= 0.0053
p= 0.0019
p= 0.00072
p= 1.7e−05
p= 2.5e−05
time (h)

Figure S5

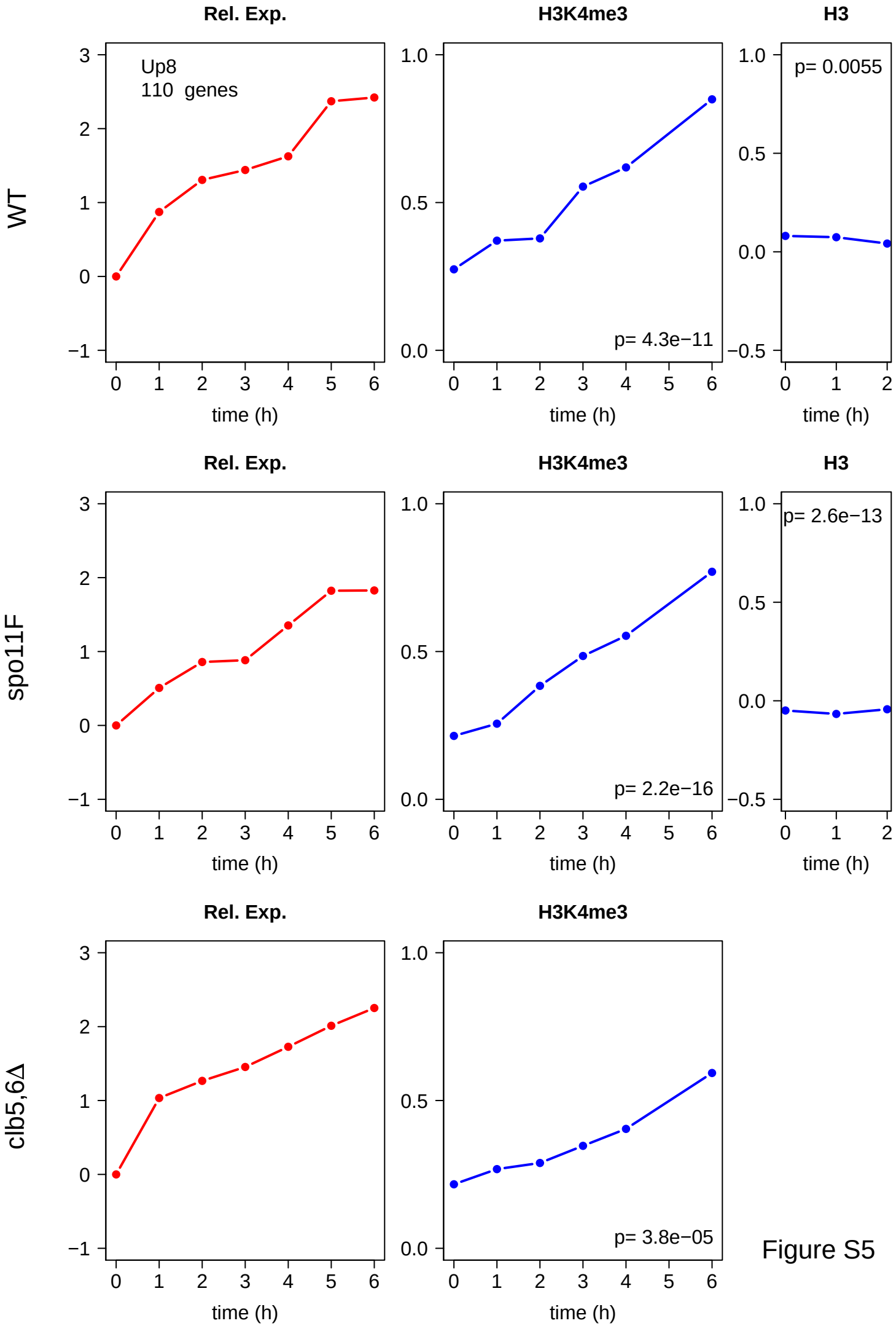
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Up8
110 genes
p= 4.3e−11
p= 0.0055
p= 2.6e−13
p= 2.2e−16
p= 3.8e−05
time (h)

Figure S5

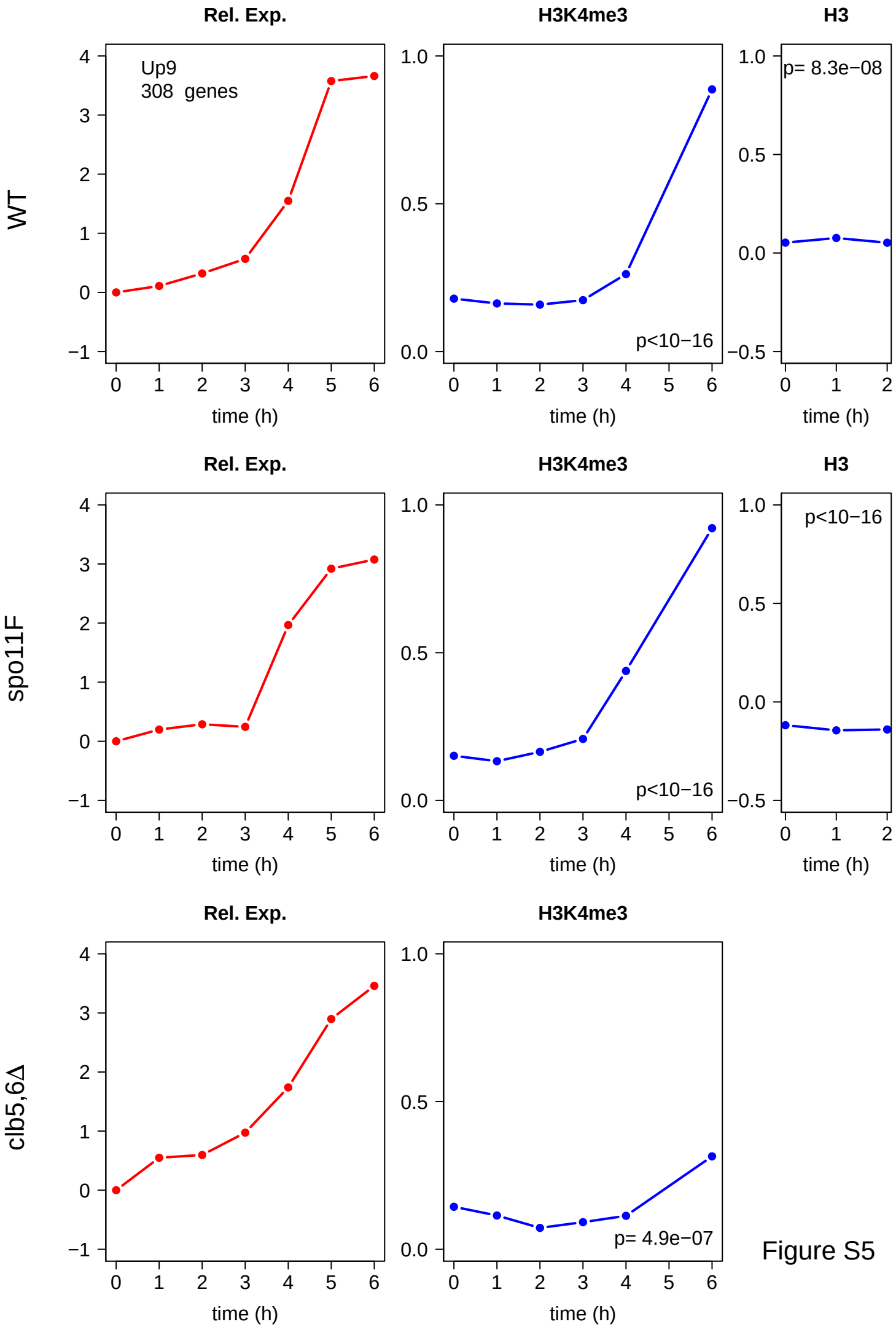
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Up9
308 genes
p<10−16
p= 8.3e−08
p= 4.9e−07
time (h)

Figure S5

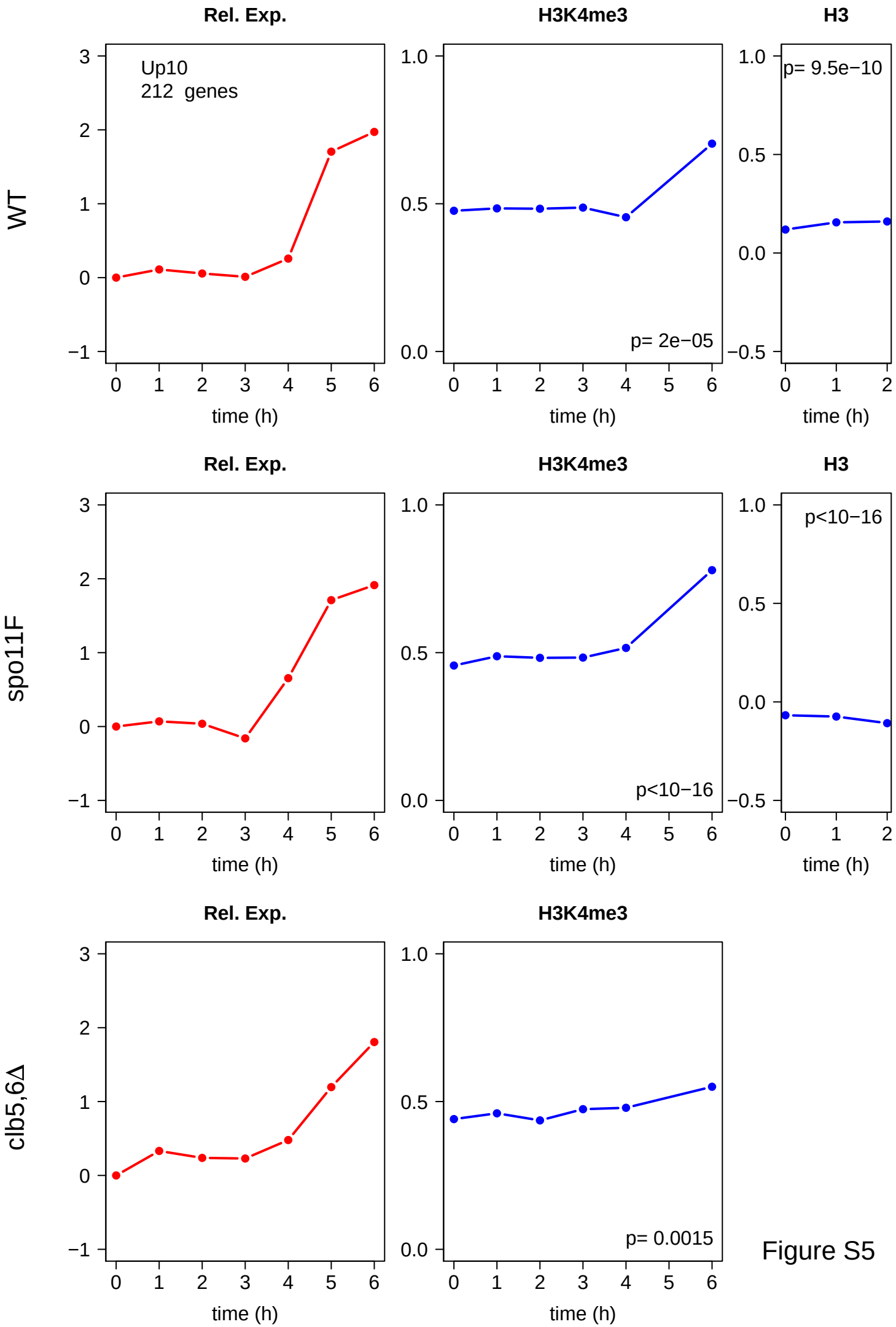
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Up10
212 genes
p= 2e−05
p= 9.5e−10
p<10−16
p<10−16
p= 0.0015
time (h)

Figure S5

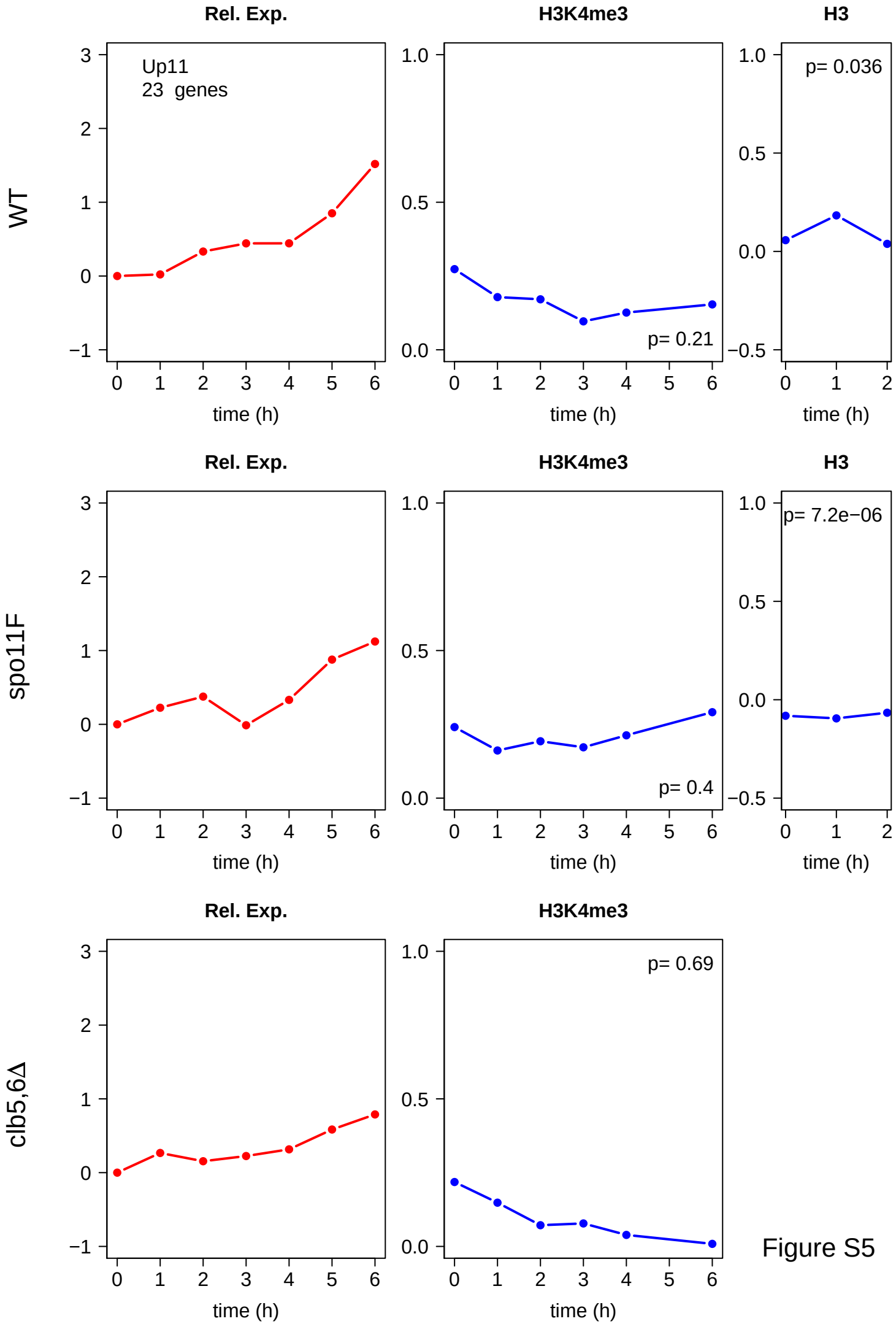
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Up11
23 genes
p= 0.036
p= 0.21
p= 7.2e−06
p= 0.4
p= 0.69
time (h)

Figure S5

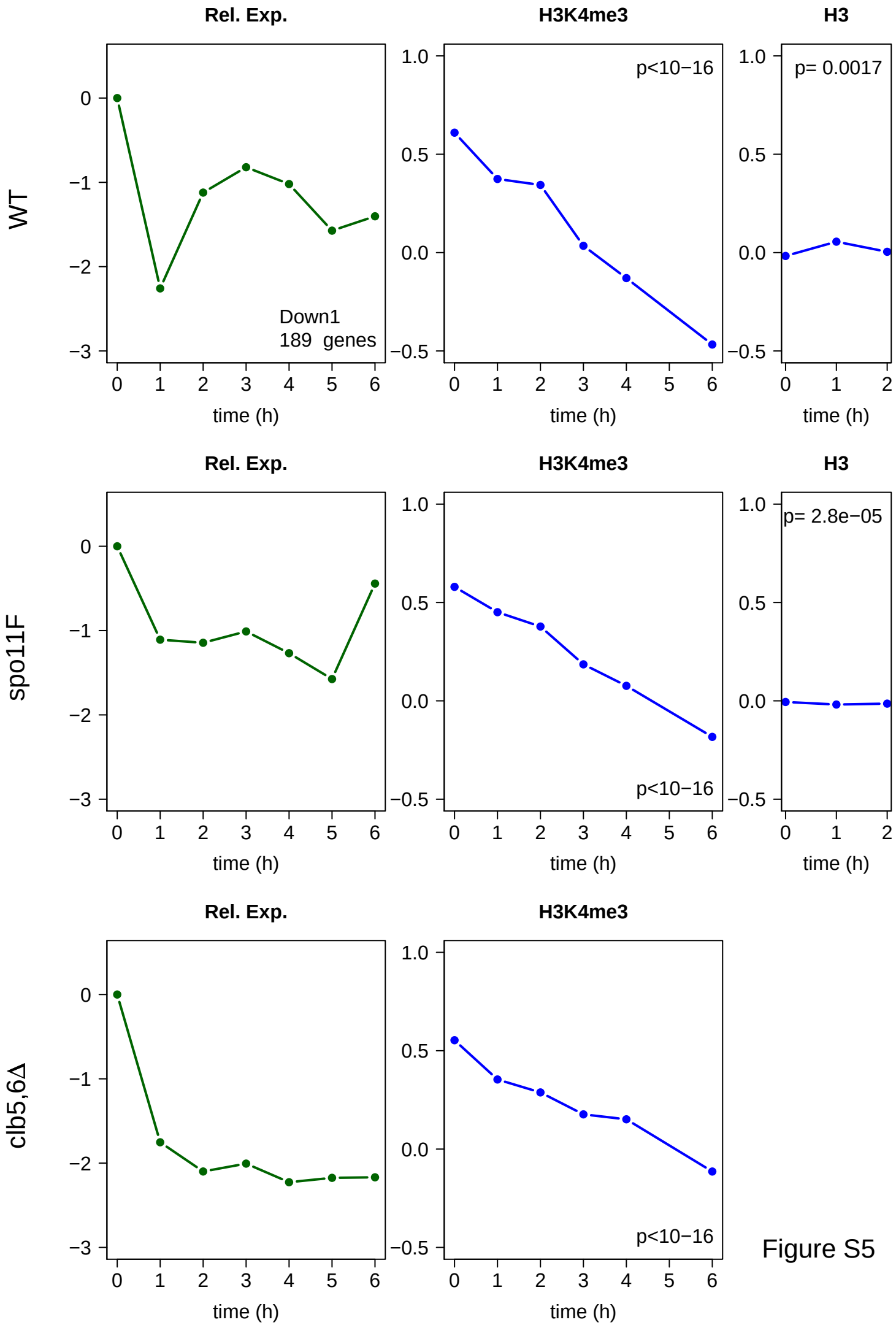
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Down1
189 genes
p<10−16
p= 0.0017
p= 2.8e−05
time (h)

Figure S5

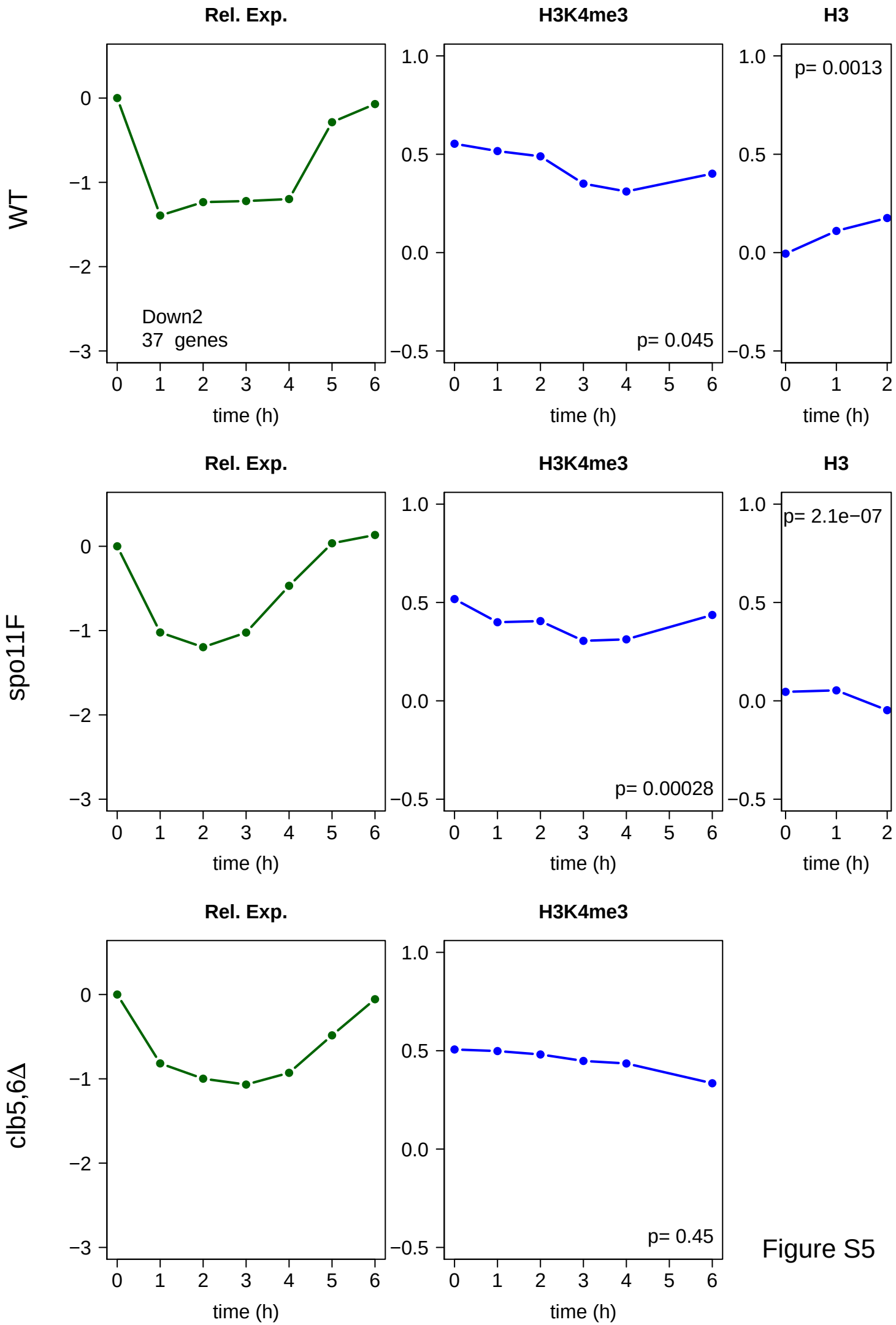
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Down2
37 genes
p= 0.045
p= 0.0013
p= 0.00028
p= 2.1e−07
p= 0.45
time (h)

Figure S5

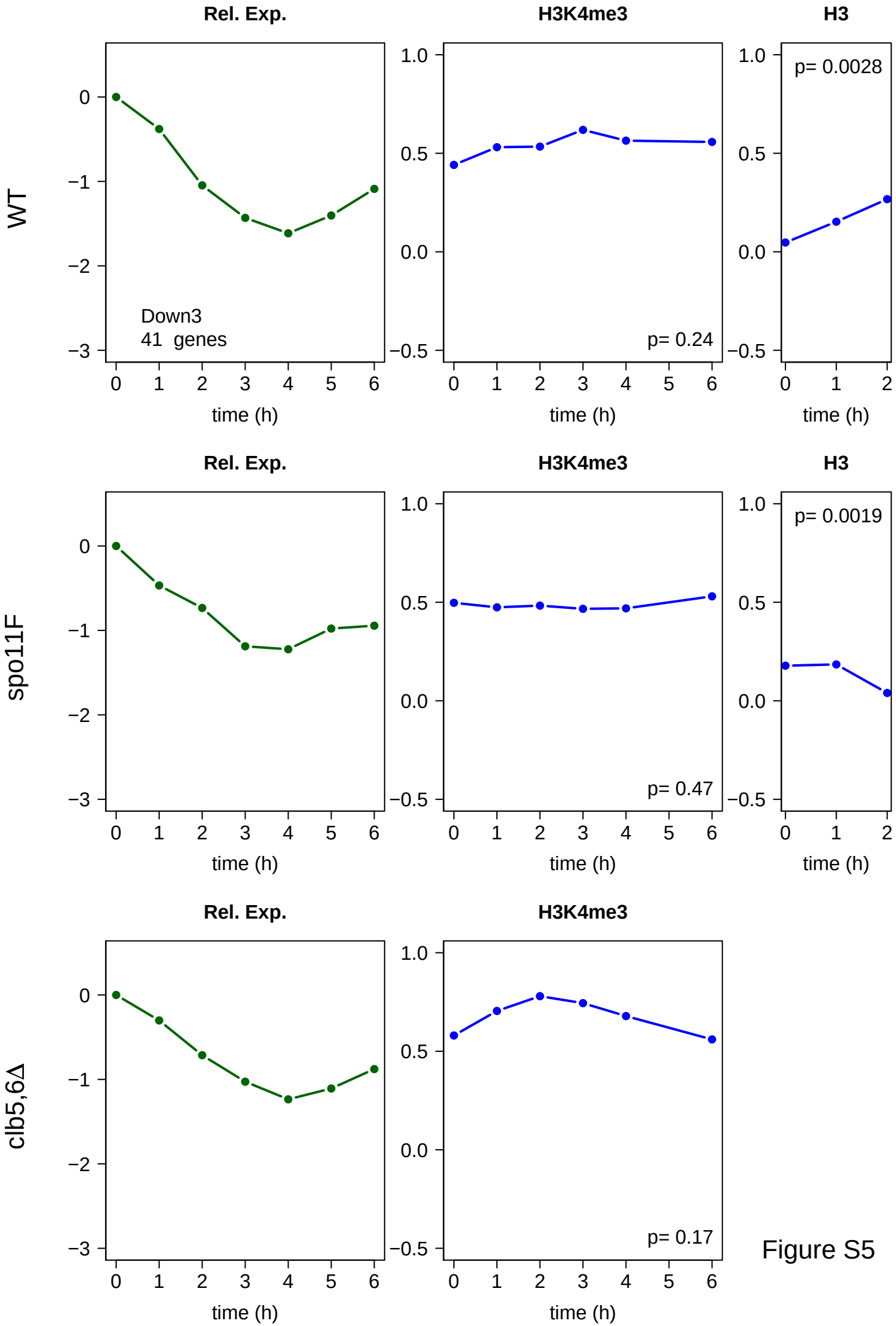
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Down3
41 genes
p= 0.24
p= 0.0028
p= 0.47
p= 0.0019
p= 0.17
time (h)

Figure S5

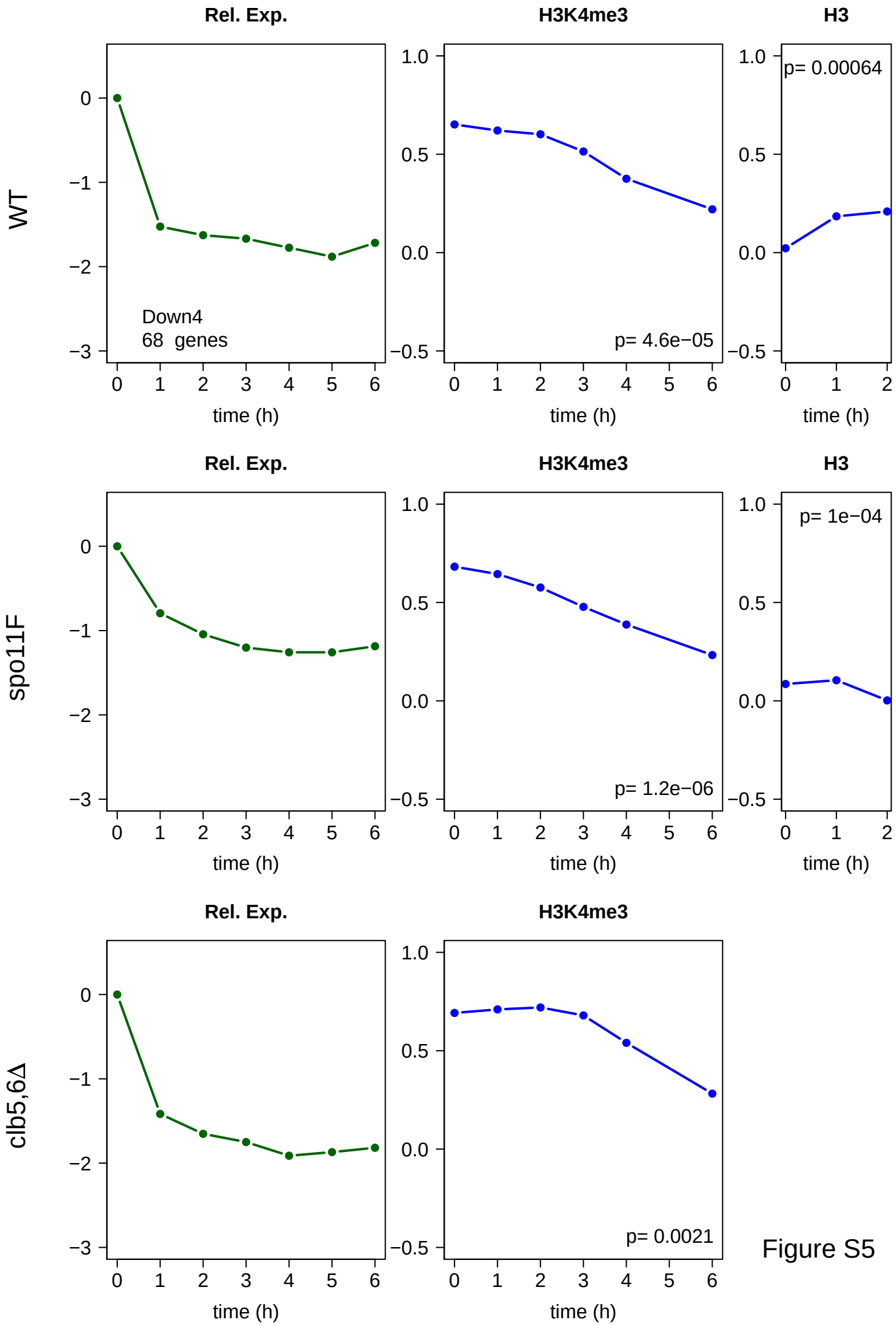
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Down4
68 genes
p= 4.6e−05
p= 0.00064
p= 1.2e−06
p= 1e−04
p= 0.0021
time (h)

Figure S5

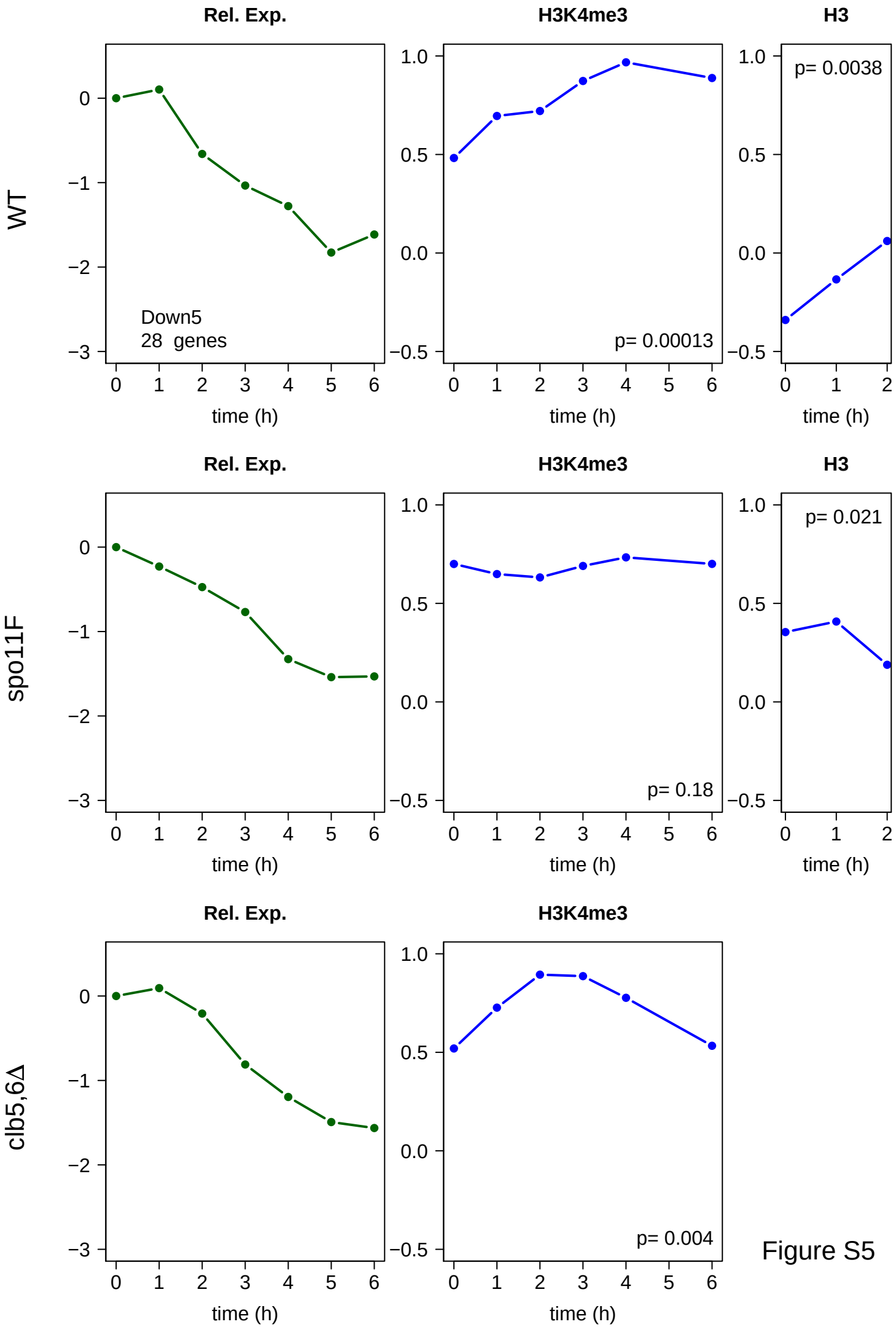
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Down5
28 genes
p= 0.00013
p= 0.0038
p= 0.18
p= 0.021
p= 0.004
time (h)

Figure S5

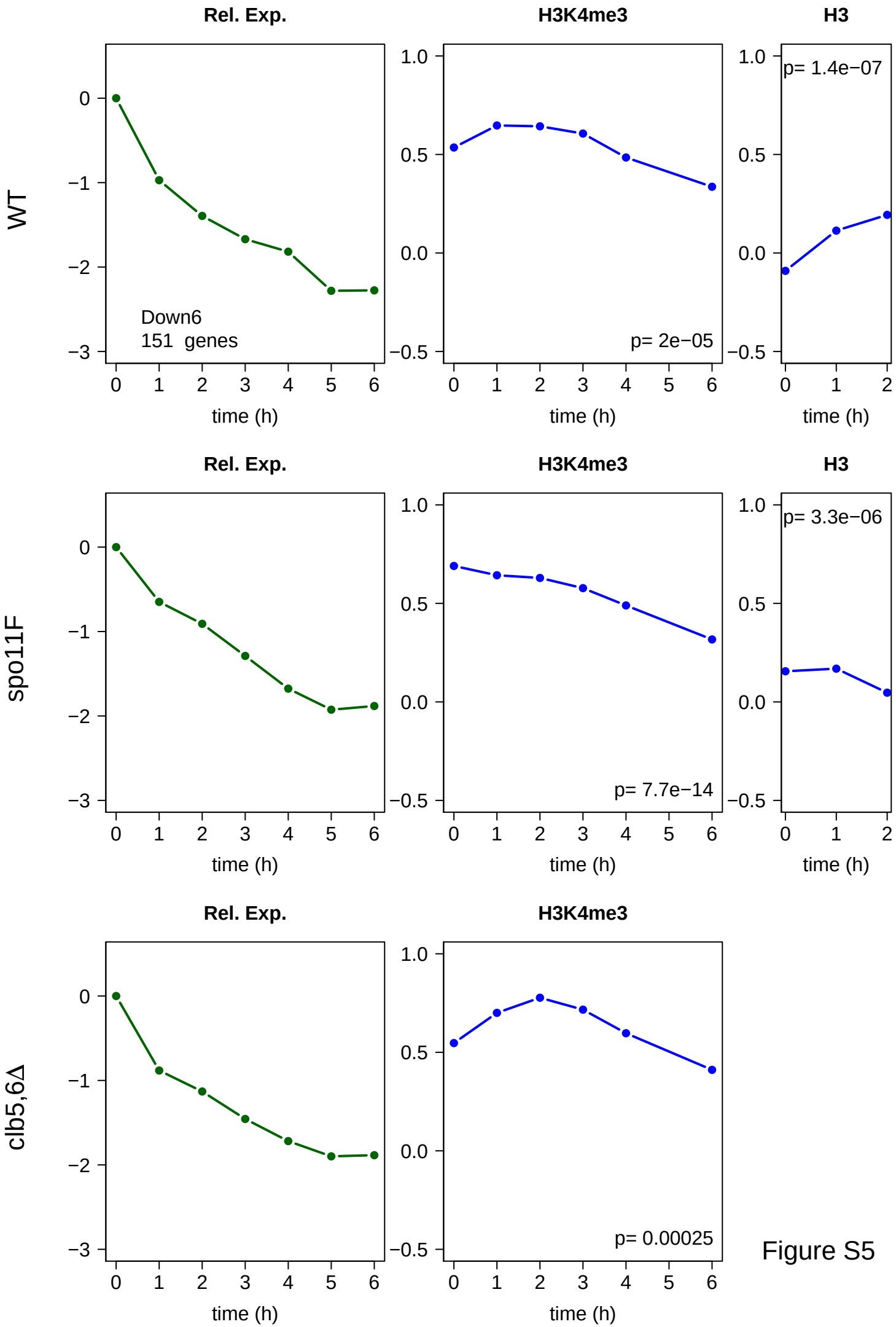
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Down6
151 genes
p= 2e−05
p= 1.4e−07
p= 7.7e−14
p= 3.3e−06
p= 0.00025
time (h)

Figure S5

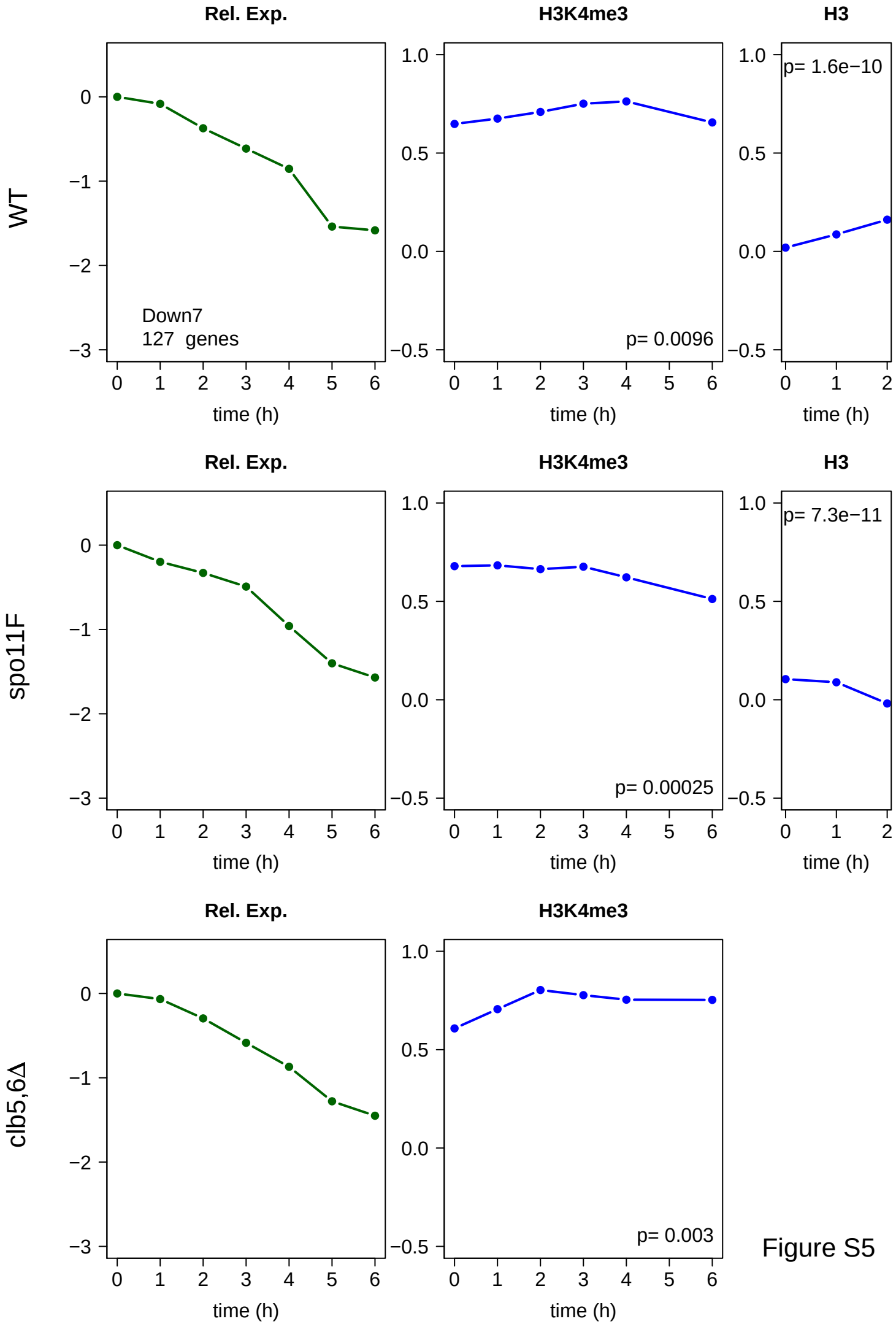
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Down7
127 genes
p= 0.0096
p= 1.6e−10
p= 0.00025
p= 7.3e−11
p= 0.003
time (h)

Figure S5

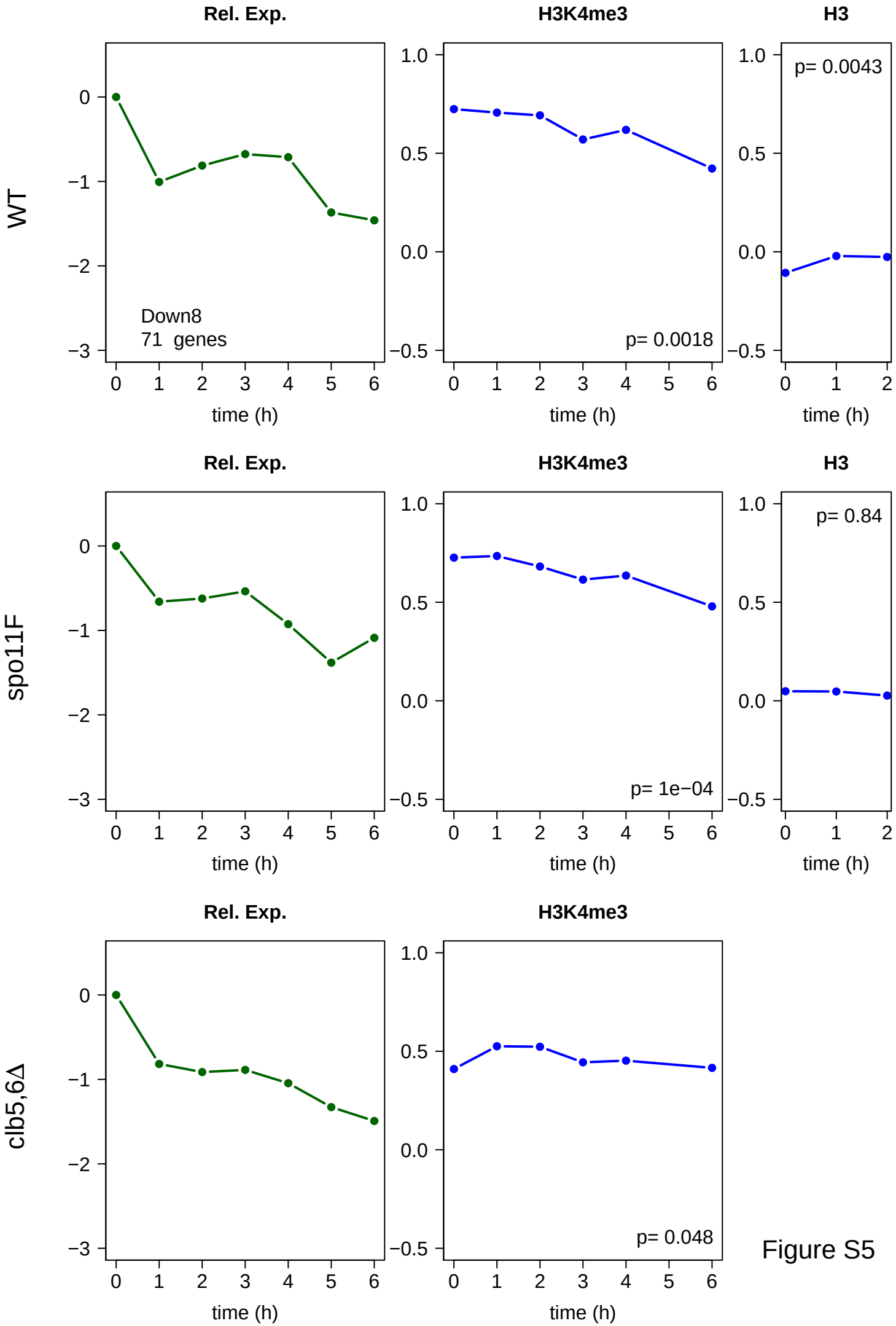
Rel. Exp.
H3K4me3
H3
WT
spo11F
clb5,6Δ
Down8
71 genes
p= 0.0018
p= 0.0043
p= 1e−04
p= 0.84
p= 0.048
time (h)

Figure S5

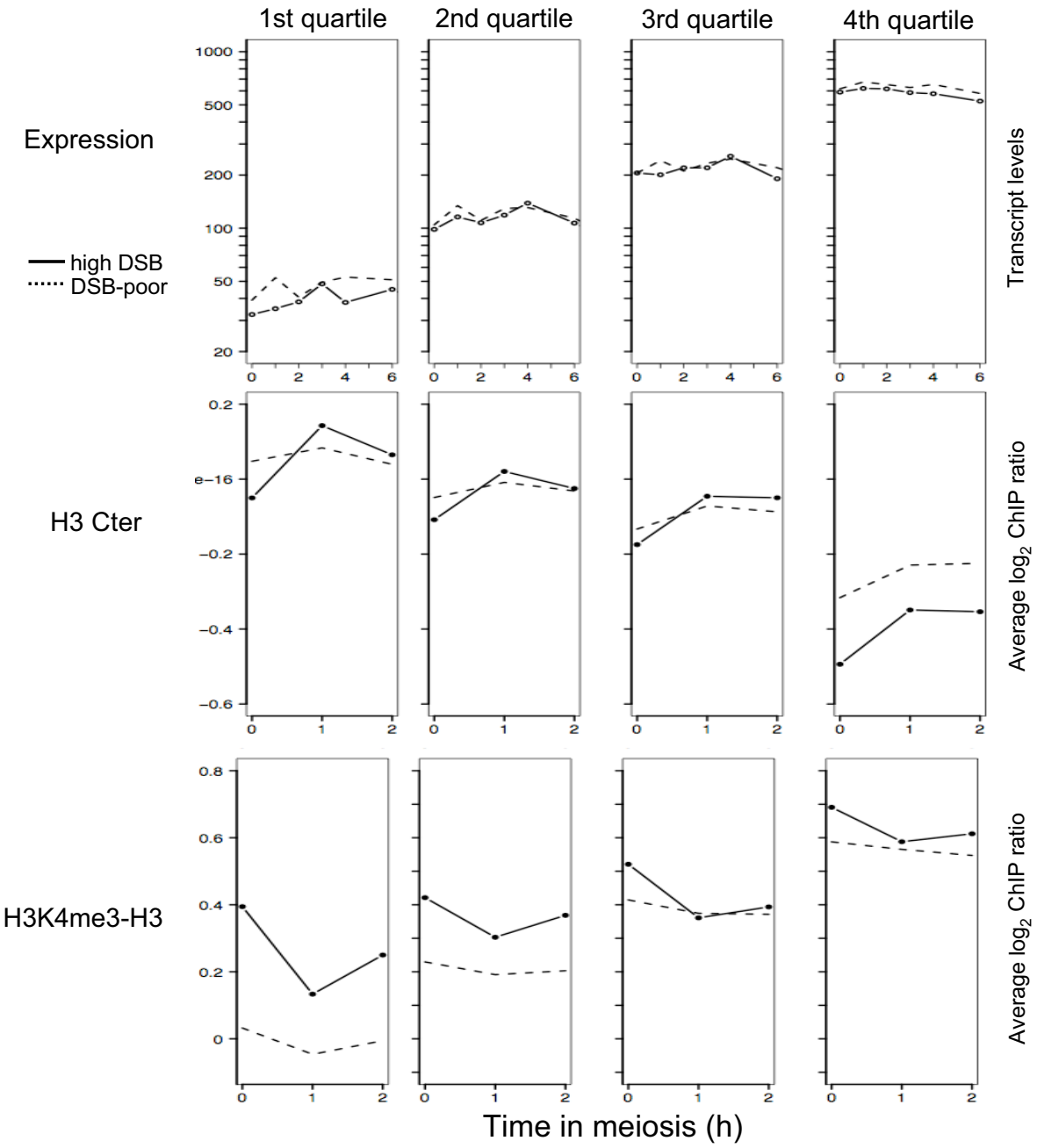
1st quartile
2nd quartile
3rd quartile
4th quartile
Expression
high DSB
DSB-poor
Transcript levels
H3 Cter
Average log2 ChIP ratio
H3K4me3-H3
Average log2 ChIP ratio
Time in meiosis (h)

**Figure S6**

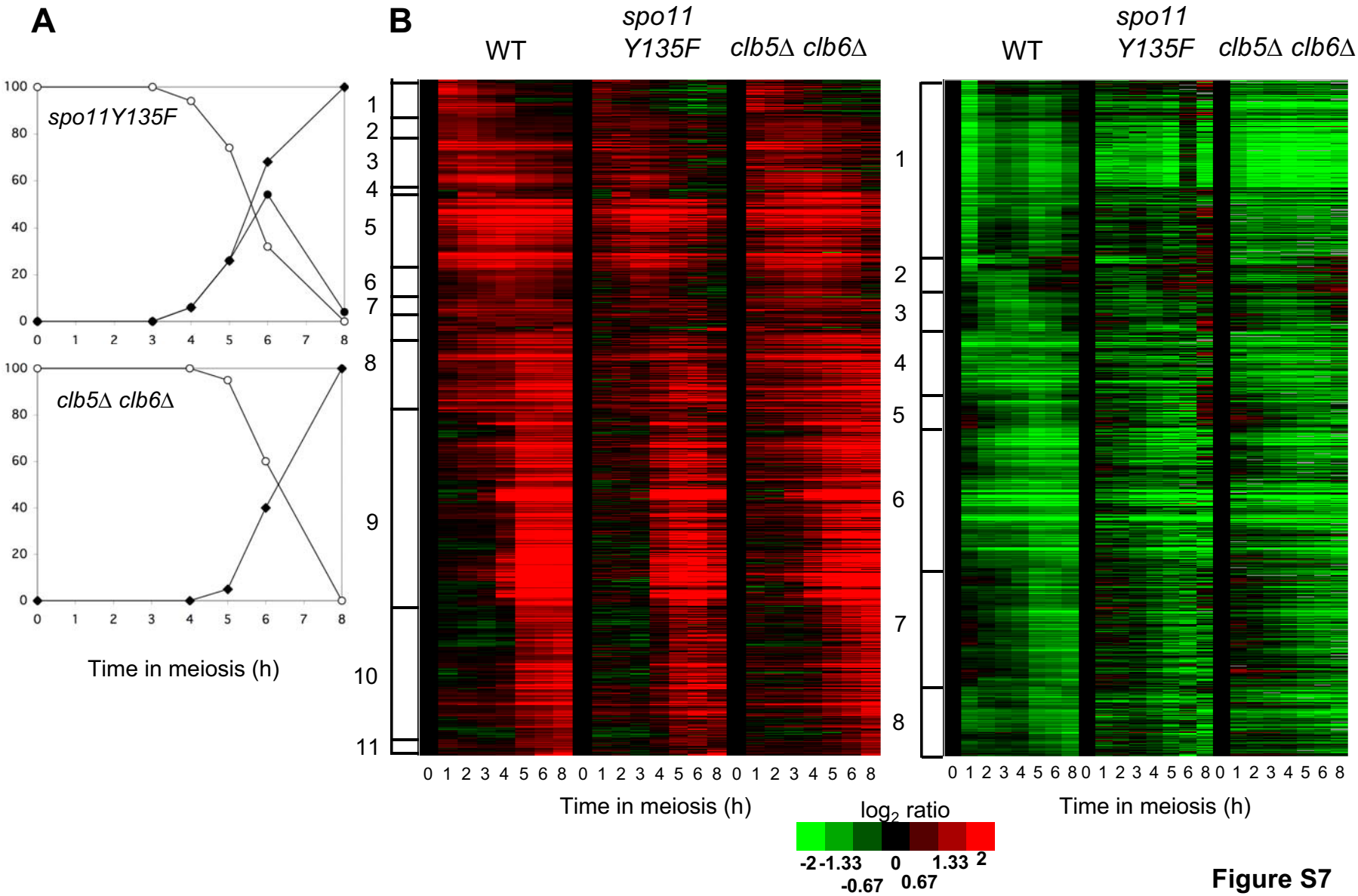
A
B
spo11Y135F
clb5Δ clb6Δ
Time in meiosis (h)
WT
spo11 Y135F
clb5Δ clb6Δ
Time in meiosis (h)
log2 ratio
-2 -1.33 -0.67 0 0.67 1.33 2

**Figure S7**

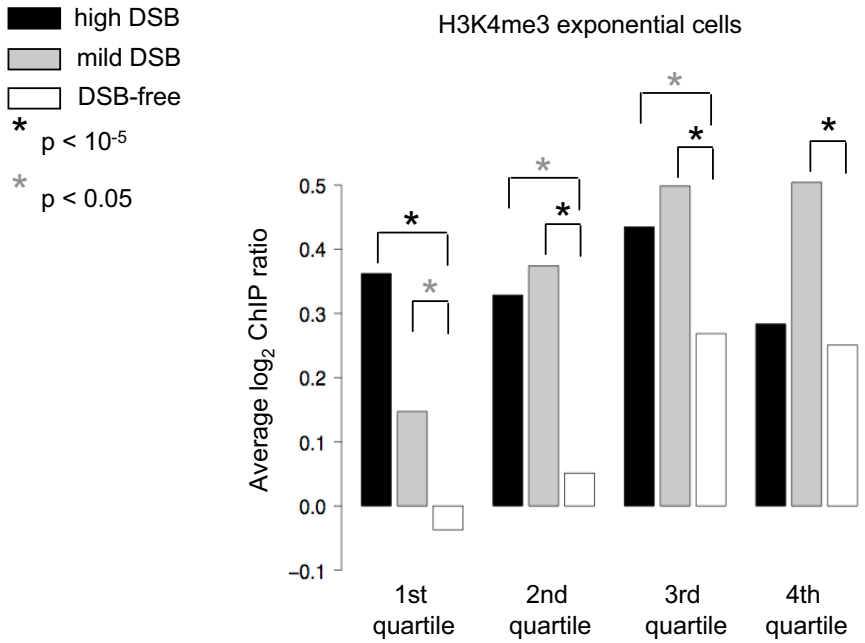
high DSB
mild DSB
DSB-free
* p < 10-5
* p < 0.05
H3K4me3 exponential cells
Average log2 ChIP ratio
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
1st quartile
2nd quartile
3rd quartile
4th quartile

**Figure S8**