Supplementary materials accompanying this paper:

Materials included in this file:

Materials provided as separate files:

Table S1. Genes underexpressed in *meu5*Δ mutants.

List of genes whose expression levels were significantly reduced in *meu5*Δ mutants compared to wild type cells (in *pat1*-induced meiosis). Significance was determined as described in Methods.

Table S2. Meu5p-bound transcripts.

List of transcripts enriched in at least two out of three independent Meu5p RIp-chip experiments. Selection of targets was performed as described in Methods.

Table S3. Transcripts with shortened half-lives in *meu5*Δ mutants.

List of transcripts whose half-lives were significantly reduced in *meu5*Δ compared to wild type (in vegetative cells overexpressing Mei4p). Significance was determined as described in Methods.

Table S4. Classification of meiotic middle genes according to their down-regulation profiles.

Middle genes were classified into two groups ('early-decrease' and 'late-decrease') as described in Methods. A list of genes that are also regulated by the Atf21p/Atf31p transcription factors (and excluded from the classification) is also presented.

Table S5. Estimated mRNA half-lives in wild type cells.

Half-lives were estimated in wild type cells as described in Methods. The results are presented for 4sU labelling times of 15- and 30-minutes.

Dataset S1. Transcriptome analysis of *meu5*Δ cells.

Complete normalised dataset for the wild type and *meu5*Δ microarray experiments in *pat1*-diploids (except time-courses), wild type diploids and vegetative cells overexpressing Mei4p.

Dataset S2. Meu5p RIp-chip experiments.

Relative enrichment ratios in three independent RIp-chip experiments.

Dataset S3. Determination of half-lives using 4sU labelling.

Fractions of newly synthesised (4sU-labelled) and pre-existing (supernatant from purification) RNAs in wild type cells labelled for 15 or 30 minutes, fractions of newly-synthesised RNA in wild type or *meu5*Δ overexpressing Mei4p and gene expression ratios between untreated cells or cells incubated with 4sU for 30 minutes.

Dataset S4. Wild type and *meu5*Δ of *pat1*-synchronised time courses.

Complete normalised microarray dataset for the wild type and *meu5*Δ *pat1*-synchronised time courses.

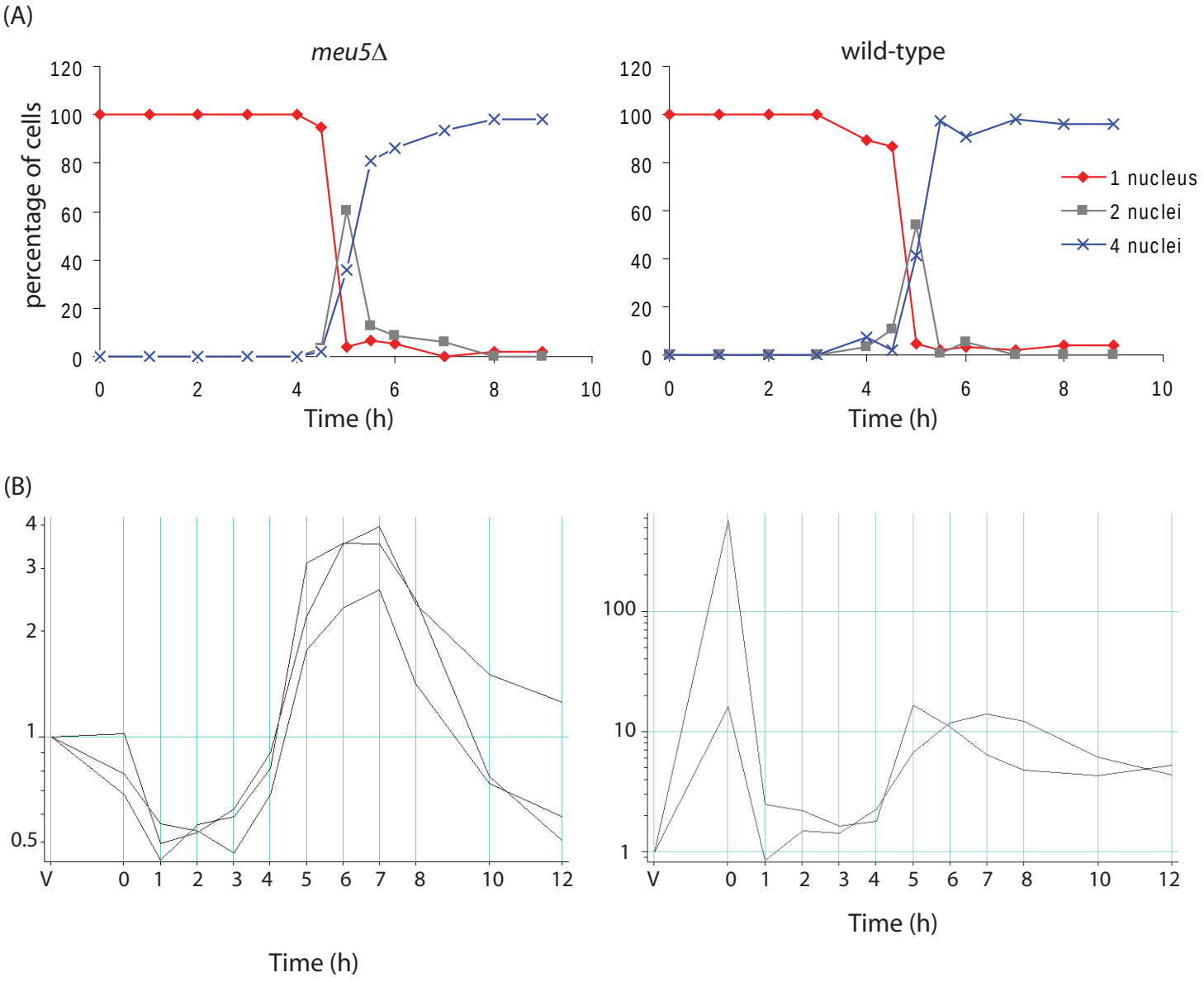


Figure S1. Characterisation of *meu5Δ* phenotype.
(A) Dynamics of the meiotic divisions in wild type and *meu5*Δ diploid cells (*pat1*-induced meiosis). Meiosis was induced as described in Figure 4. (B) Expression profiles of genes underexpressed in *meu5*Δ but not classified as middle genes. Experiments and labelling are as in Figure 1 (gene expression data are from Mata et al, 2002). (C) Overlap between genes downregulated in *meu5*Δ mutants in *pat1*-induced and wild type meiosis. Labelling of the Venn diagrams is as in Figure 1C.

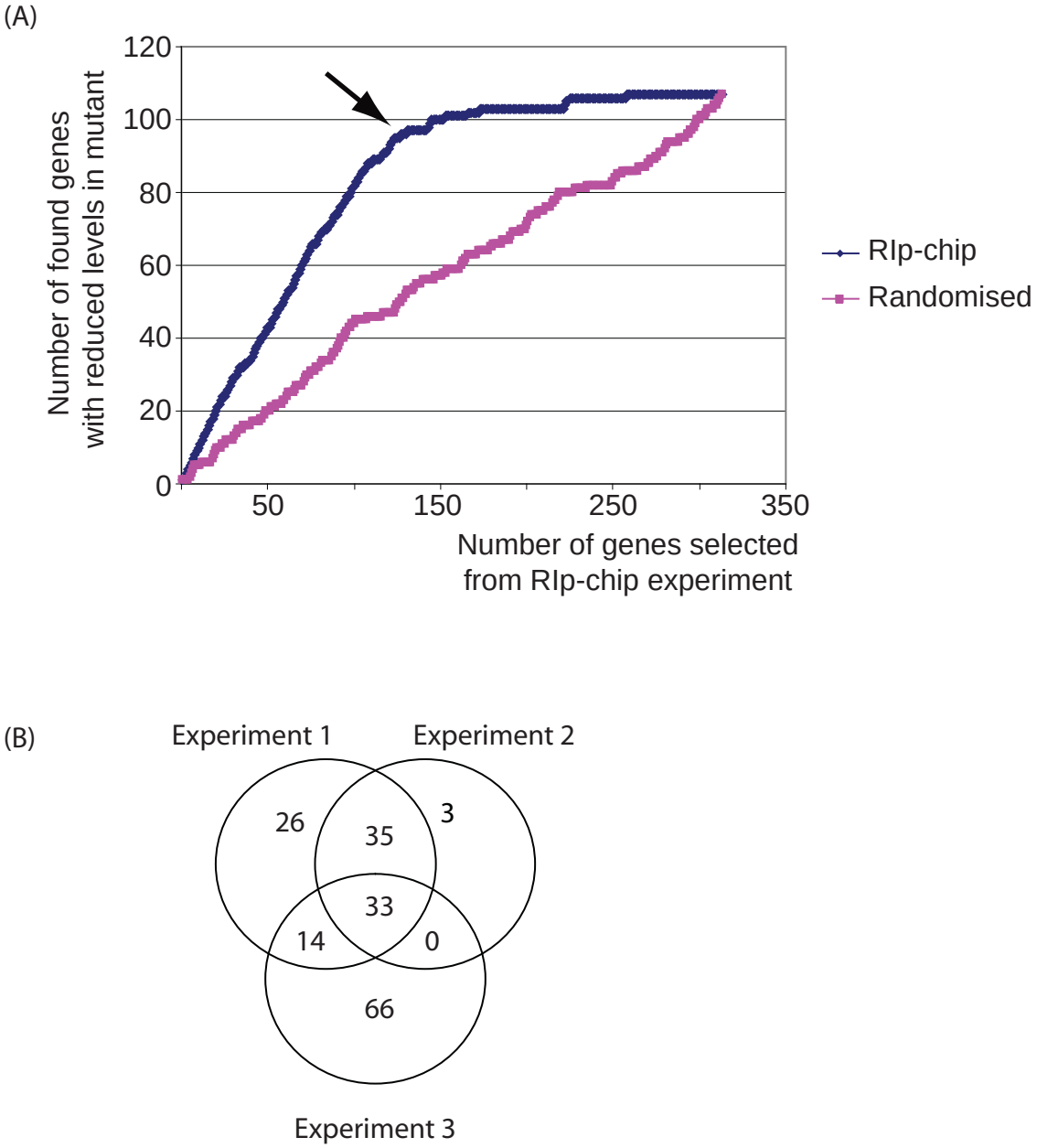


Figure S2. Identification of Meu5p targets by RIp-chip.
(A) Selection of functionally relevant Meu5p-associated transcripts (see Materials and Methods for details). The number of genes selected from a ranked list of RIp-chip enrichment is plotted against the number of genes in that list whose expression levels are reduced in *meu5*Δ mutant diploids (in *pat1*-induced meiosis) (blue line). As a control, the same analysis was performed with randomised enrichment ranks (purple). All curves eventually reach either saturation or a slope similar to that of random genes. The threshold for selection of bound mRNAs was chosen at the point of the slope change (arrow). (B) Overlap between transcripts identified in three independent RIp-chip experiments.

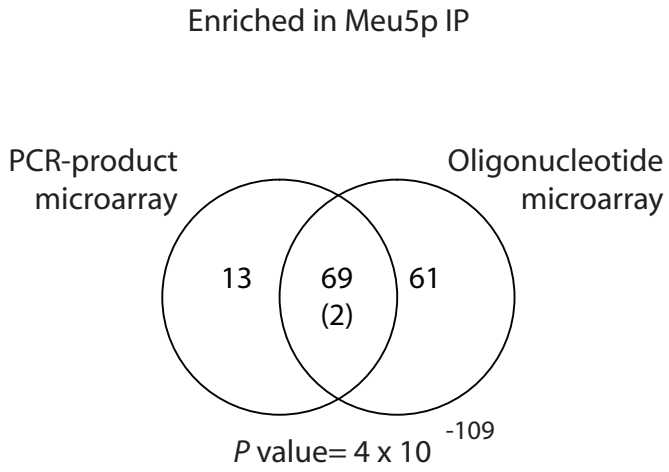


Figure S3. Comparison of RIp-chip analysed with two different microarray platforms. Overlap between Meu5p targets identified by RIp-chip using PCR-product custom-made microarrays (Lyne et al., 2003) and those analysed with a 60-mer oligonucleotide platform manufactured by Agilent. The list of Meu5p targets from the custom-made microarrays was generated from three independent biological replicates (Figure S2), and those from the oligonucleotide microarray from a fourth independent experiment. Labelling of the Venn diagrams is as in Figure 1C.

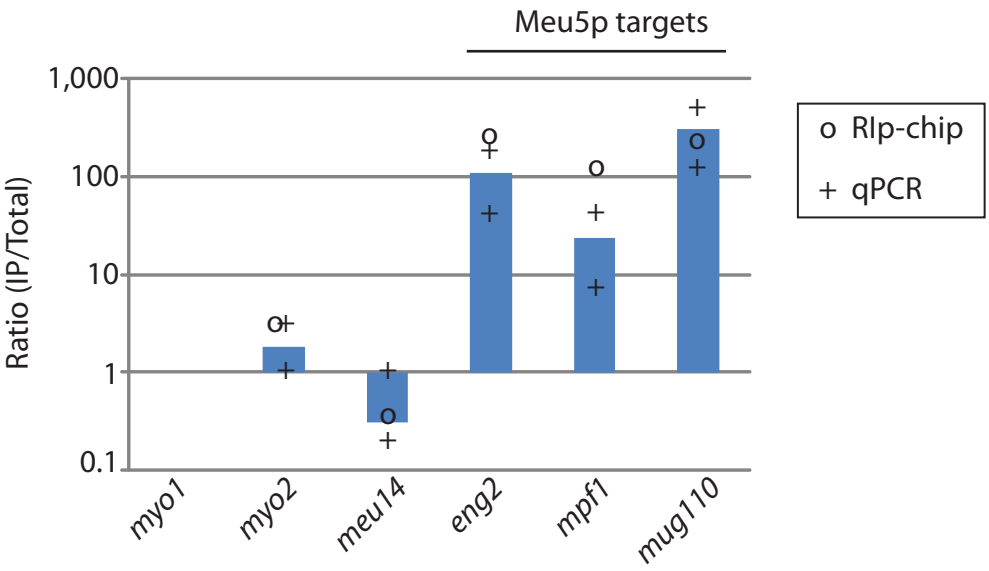


Figure S4. Real-time quantitative PCR (qPCR) validation.
Relative amounts of each mRNA in Meu5p immunoprecipitates compared to total RNA (log scale). The experiment was carried out for three Meu5p targets identified by RIp-chip (*mpf1*, *eng2* and *mug110*), a Mei4p target not enriched in Meu5p immunoprecipitates (*meu14*) and two additional transcripts (*myo1* and *myo2*). For normalisation, the level of enrichment of *myo1* was set to 1. The columns show average data for two independent biological replicates analysed by qPCR, with each of the individual data points displayed by a cross. The fold-difference was calculated as described in Materials and Methods. The circles show the enrichment levels in a RIp-chip experiment performed from the same extract used for one of the qPCR experiments and normalised in a similar way.

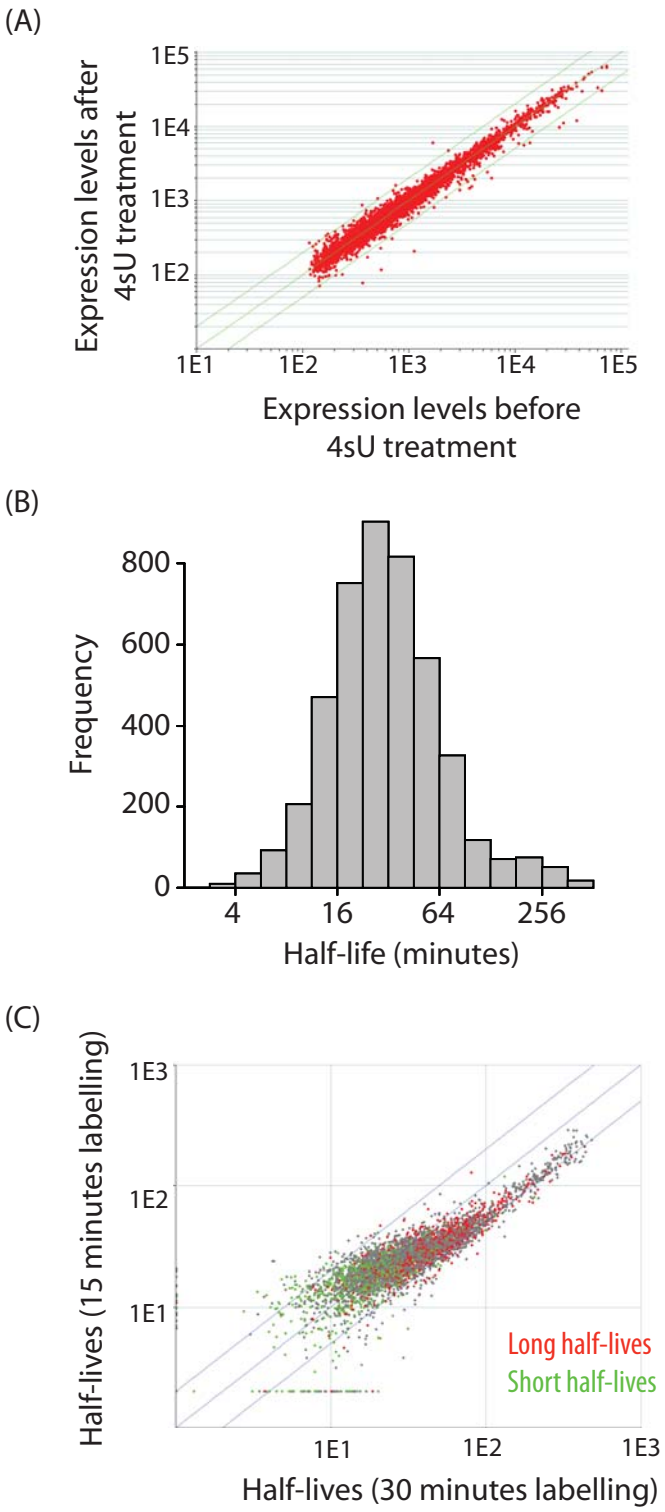


Figure S5. Genome-wide measurement of decay rates.
(A) 4sU does not cause strong changes in gene expression. Cells overexpressing Mei4p were incubated with 4sU for 30 minutes. The scatter plot shows the comparison of expression levels between cells before and after the 4sU treatment. Genes outside the green lines differ by more than two-fold in expression levels. (B) Histogram displaying all estimated transcript half-lives (using 4sU in vegetative wild type cells). (C) Scatter plot comparing the half-lives estimated with a 4sU pulse of 15 or 30 minutes. Genes classified as 'short half-lives' or 'long half-lives' in (Lackner et al, 2007) are shown in green and red respectively, while mRNAs for which half-lives were not determined are displayed in grey.

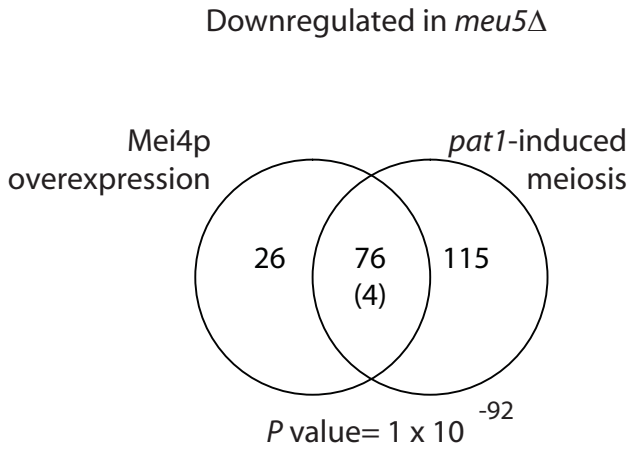


Figure S6. Overexpression of Mei4p in *meu5*Δ vegetative cells recapitulates the meiotic phenotype of *meu5*Δ mutants.

Overlap between genes downregulated in *meu5*Δ mutants in cells overexpressing Mei4p or in diploid cells undergoing meiosis (*pat1*-synchronised). Labelling of the Venn diagrams is as in Figure 1C.

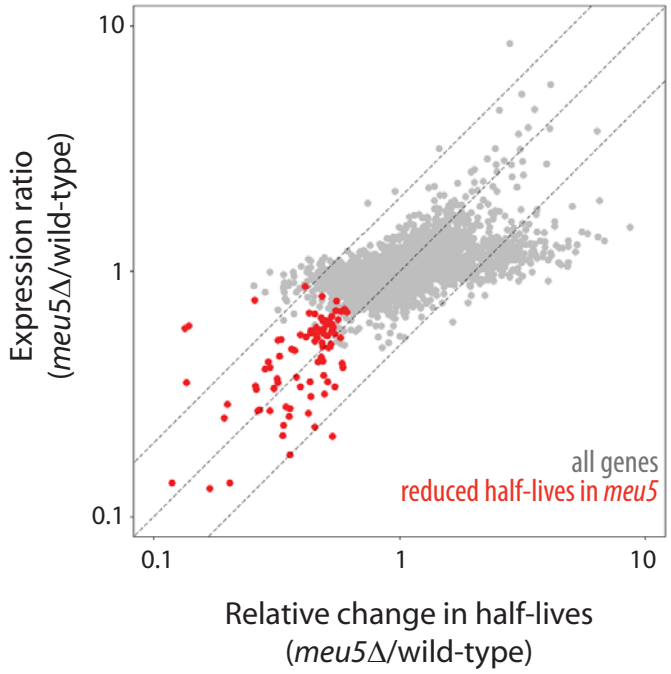


Figure S7. Correlation between changes in half-lives and gene expression levels. Scatter plot comparing changes in mRNA half lives and gene expression levels (in *meu5*Δ and wild type cells overexpressing Mei4p). Average results from three independent experiments are shown. Values outside the dashed lines differ by more than two-fold. Data for RNAs that display significantly reduced stability in *meu5*Δ mutants are shown in red. Data for other genes are presented in grey. Although the data from the 4sU-labelling experiments tend to be noisier than those from expression analysis, transcripts selected by statistical analysis as destabilised in *meu5*Δ show good correlation between changes in stability and expression levels.

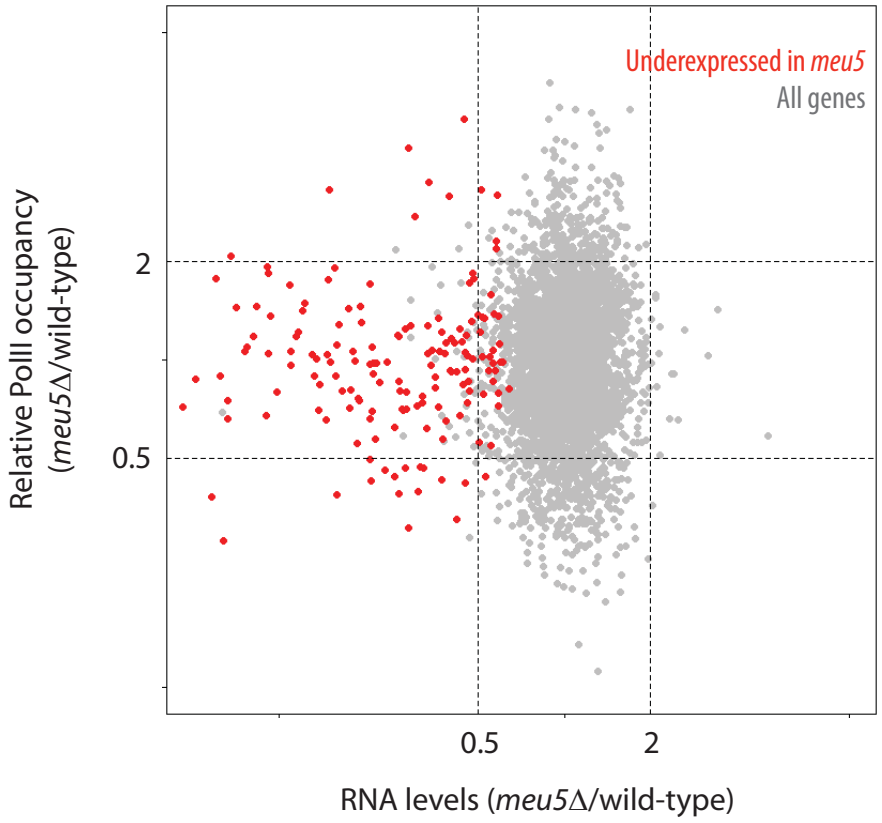


Figure S8. Transcription rates of Meu5p targets are not affected in *meu5*Δ mutants.
Scatter plot showing the ratio of RNA polymerase II occupancy between *meu5*Δ and wild type cells (y-axis) plotted against the relative RNA levels between *meu5*Δ and wild type cells (x-axis). All experiments correspond to meiotic cells. RNAs whose levels are significantly decreased in *meu5*Δ cells are shown in red. PolII ChIP-chip experiments are usually noisier than expression analysis (reflected in the wider spread of signals along the y-axis). Most Meu5p targets have similar levels in m*eu5*Δ and wild type cells.

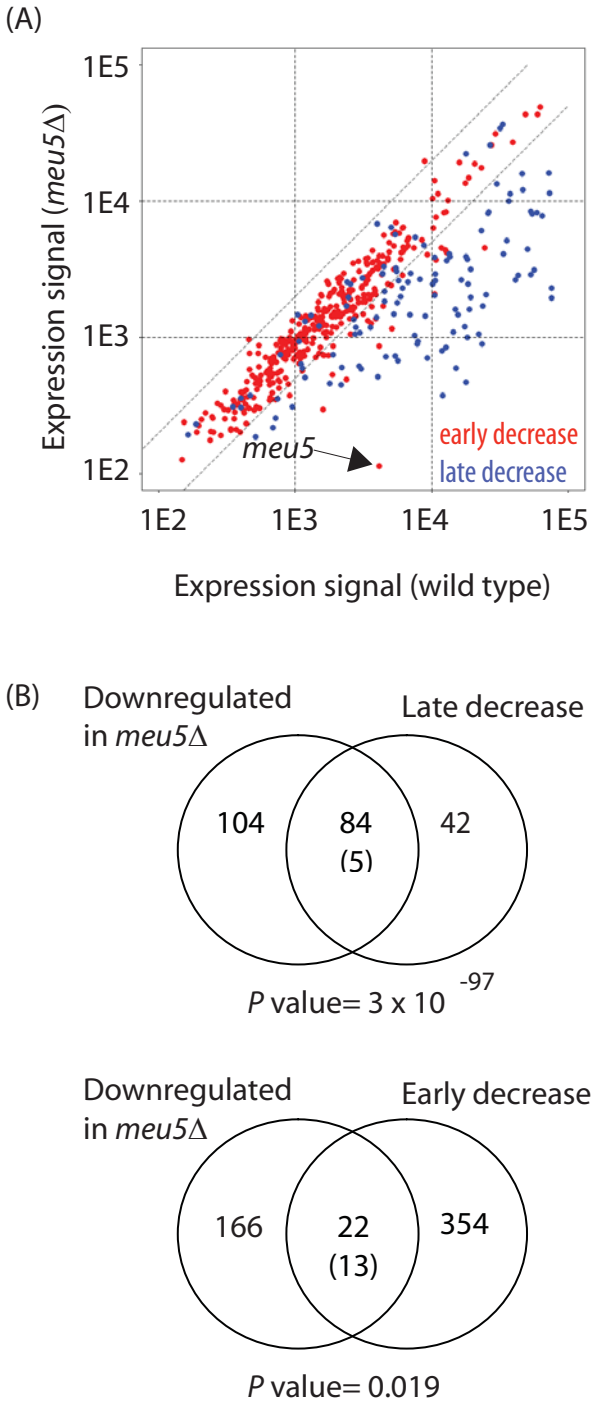
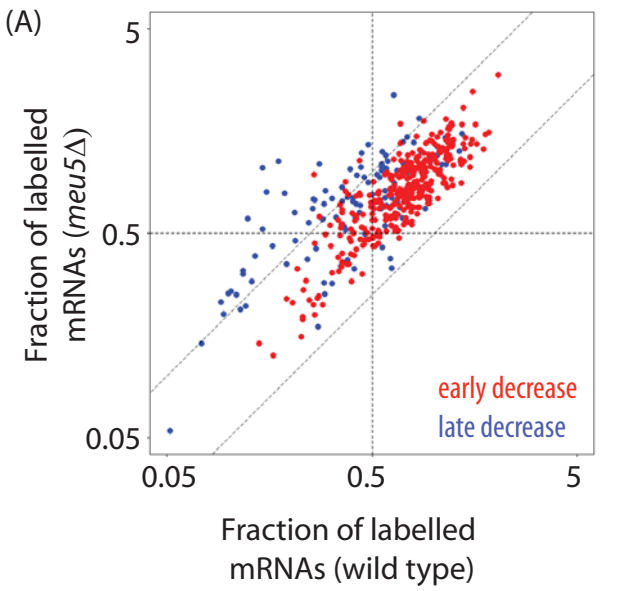


Figure S9. 'Late-decrease' genes are downregulated in *meu5Δ* cells.
(A) Comparison of expression levels between wild type and *meu5*Δ in *pat1*-synchronised meiotic diploid cells (as in Figure 1A). 'Early-decrease' genes are shown in red and 'late-decrease' genes in blue. Genes outside the green lines differ by more than two-fold in expression levels. (B) Overlap between genes downregulated in *meu5*Δ diploid cells (in *pat1*-induced meiosis) and 'late-decrease' genes (top) or 'early-decrease' (bottom). Labelling of the Venn diagrams is as in Figure 1C.



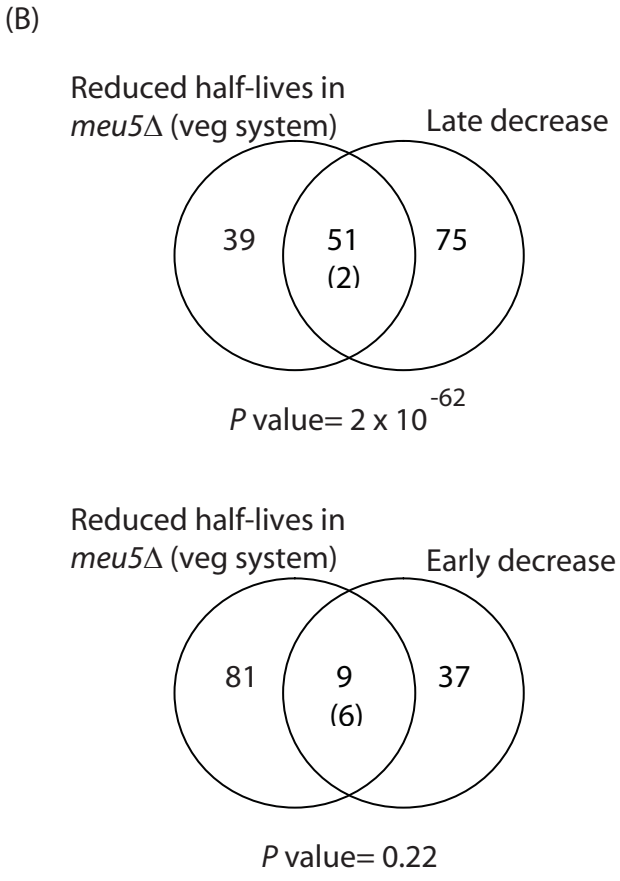


Figure S10. 'Late-decrease' genes are destabilised in *meu5*Δ cells.
(A) Comparison of the fraction of newly-synthesised RNA (4sU-labelled) between wild type and *meu5*Δ *pat1*-synchronised meiotic cells (data as in Figure 5A). 'Early-decrease' genes are shown in red and 'late-decrease' genes in blue. Genes outside the dotted lines differ by more than 2-fold in fraction levels. (B) Overlap between genes with shortened half-lives in *meu5*Δ cells and 'late-decrease' genes (top) or 'early-decrease' (bottom). Labelling of the Venn diagrams is as in Figure 1C.

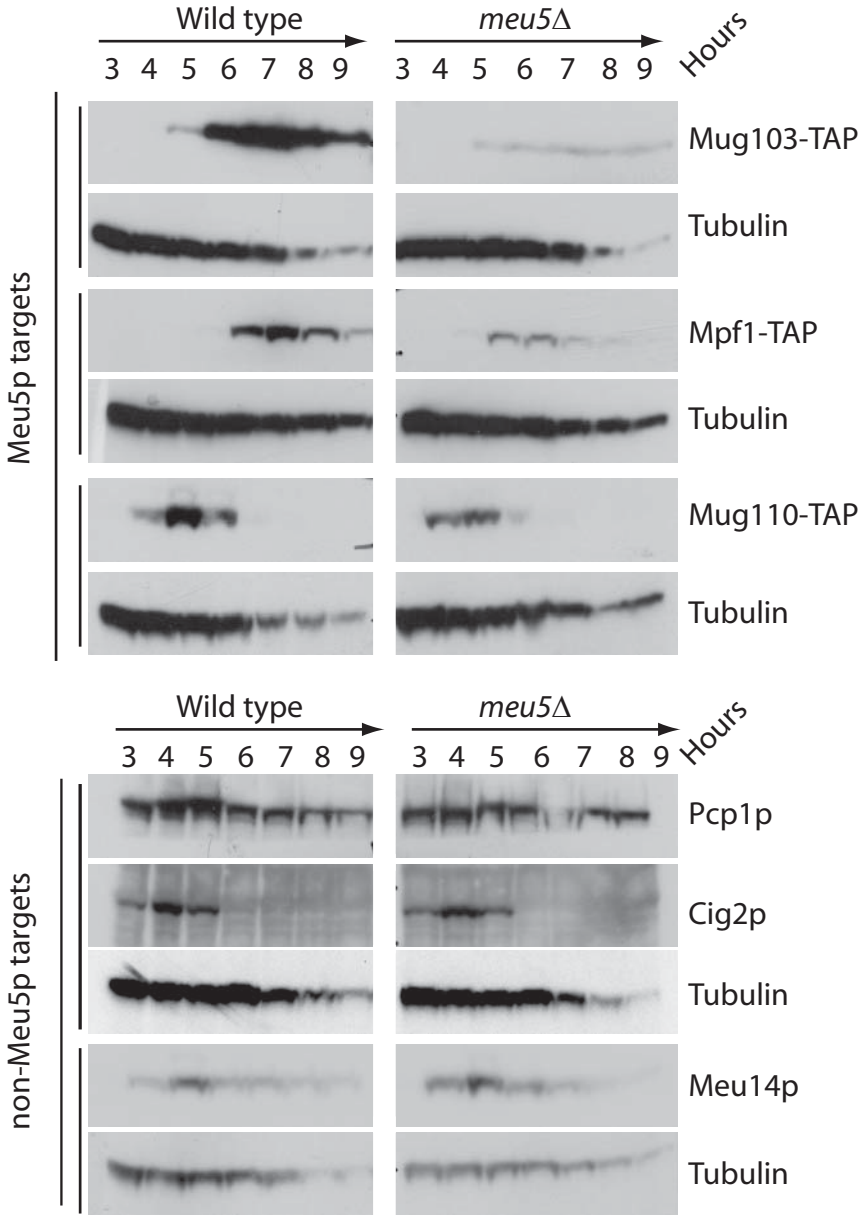


Figure S11. Correlation between mRNA and protein levels in wild type and *meu5*Δ mutant cells.

Mug110p, Mpf1p, Mug103p, Pcp1p, Cig2p and Meu14p were detected by immunoblotting in *pat1*-synchronised wild type or *meu5*Δ diploid cells (as in Figure 4). The transcripts encoding the six proteins are induced during the meiotic divisions in a Mei4p-dependent fashion (*Mata et al., 2007*). *mpf1*, *mug103* and *mug110* are Meu5p-targets, while *pcp1*, *cig2* and *meu14* are not regulated by Meu5p. Mpf1p, Mug103p, Mug110p and Meu14p were TAP-tagged (while maintaining the endogenous promoter and 5' and 3' regulatory sequences) and detected using peroxidase-anti-peroxidase complexes. Pcp1p and Cig2p were detected with specific antibodies. The inductions of *pcp1* and *cig2* mRNAs takes place in two phases (between 1-2 hours and 4-5 hours after meiotic induction; *Mata et al., 2002*), explaining why the increase in protein levels between 3 and 5 hours is less sharp. All membranes were probed with antibodies against tubulin. Although tubulin levels decrease towards the end of meiosis (*Mata et al., 2002* and unpublished observations), they allow comparison between protein levels in corresponding time points for wild type and *meu5*Δ time courses. Pcp1p and Cig2p were examined on the same samples (therefore, the tubulin control is the same for both proteins).

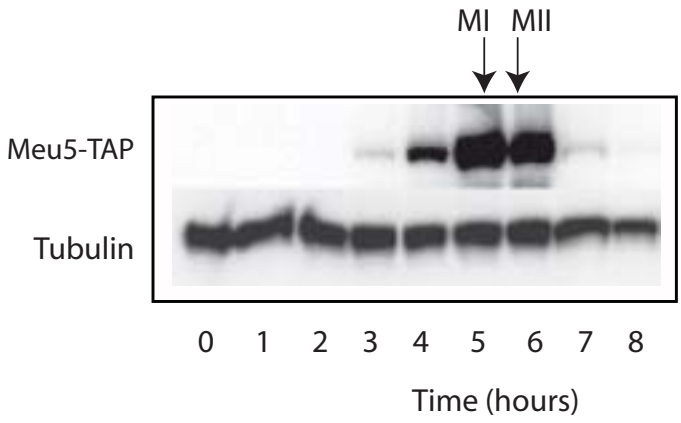


Figure S12. Meu5p is expressed transiently during meiosis.
pat1 diploids expressing Meu5-TAP were induced to enter synchronous meiosis as described in Figure 6A. The levels of Meu5-TAP and tubulin (as a loading control) were quantified by Western blot. The timing of the first (MI) and second (MII) meiotic divisions is indicated by the arrows.

Primer name	Sequence	Restriction site
mug110_ORF_F2	TCTAGTCGACAAGACCTAAAAGAACTACGG	SalI
mug110_ORF_R2	TCTACCCGGGGGTTTGATTTGCATTGTTAG	XmaI
mug110_3UTR_F	TCTAGGCGCGCCGTATCTCCTTACTTCACCGG	AscI
mug110_3UTR_R	TCTAAGATCTTAGCCGTATCTGGGTATGTG	BglII
meu14ORF_F	TCTAGTCGACGACAACGATTAATCTCAAGC	SalI
meu14ORF_R	TCTATTAATTAACAAGAAAACAGTGGATTTTGC	PacI
meu14_3UTR_F	TCTAGGCGCGCCTTATCATAACAAACTAAGAAACG	AscI
meu14_3UTR_R	TCTAAGATCTGCAACCTTATCCCATTAAGC	BglII
mug103_ORF_F	TCTAGTCGACATAATCAGAGAATCGGATGC	SalI
mug103_ORF_R	TCTATTAATTAAACTTCTACGGTAAAAACGAT	PacI
mug103_3UTR_F	TCTAGGCGCGCCTTTTTATAATTATTACCACCTGTTTCTAACTG	AscI
mug103_3UTR_R	TCTAAGATCTCAGTGAACGTAAACAAAGGC	BglII
mpf1_ORF_F	TCTAGTCGACGCAGCCTCACTATCTCTGCAACAGC	SalI
mpf1_ORF_R	TCTATTAATTAACGATTTTGATTTCTCCACTAATGCT	PacI
mpf1_3UTR_F	TCTAGGCGCGCCGTTCCGGCTTCTAAATTAATTGAAACC	AscI
mpf1_3UTR_R	TCTAAGATCTAGAAGAGGATGAGGATTATATACCACC	BglII

Table S6: Construction of TAP-tagged strains.

Primers used for the construction of TAP tagged strains. All oligonucleotides contain a 5’ tail (TCTA) followed by a restriction enzyme recognition site (underlined) and sequences specific to the corresponding gene.

Genotype	Source
968 h^{90}	Lab stock
meu5-TAP::kanMX6 h^{90}	This study
meu5Δ::kanMX6 h^{90}	This study
meu5Δ::kanMX6 leu1-32 h^{+}	This study
meu5Δ::kanMX6 ade6-M210 h^{+}	This study
meu5Δ::kanMX6 ade6-M216 h^{-}	This study
meu5Δ::kanMX6/meu5Δ::kanMX6 ade6-M210/ade6-M210 h^{+}/h^{-}	This study
meu5Δ::kanMX6 pat1-114 ade6-M210 h^{+}	This study
meu5Δ::kanMX6 pat1-114 ade6-M216 h^{+}	This study
meu5Δ::kanMX6/meu5Δ::kanMX6 pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	Lab stock
meu5-TAP::kanMX6/+ pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
mpf1-TAP-mpf1-3'UTR::natMX6/+ pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
mpf1-TAP-mpf1-3'UTR::natMX6/+ meu5Δ::kanMX6/meu5Δ::kanMX6 pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
mug103-TAP-mug103-3'UTR::natMX6/+ pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
mug103-TAP-mug103-3'UTR::natMX6/+ meu5Δ::kanMX6/meu5Δ::kanMX6 pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
mug110-TAP-mug110-3'UTR::natMX6/+ pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
mug110-TAP-mug110-3'UTR::kanMX6/+ meu5Δ::natMX6/meu5Δ::natMX6 pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
meu14-TAP-meu14-3'UTR::natMX6/+ pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study
meu14-TAP-meu14-3'UTR::natMX6/+ meu5Δ::kanMX6/meu5Δ::kanMX6 pat1-114/pat1-114 ade6-M210/ade6-M210 h^{+}/h^{+}	This study

Table S7: Strains used in this study.