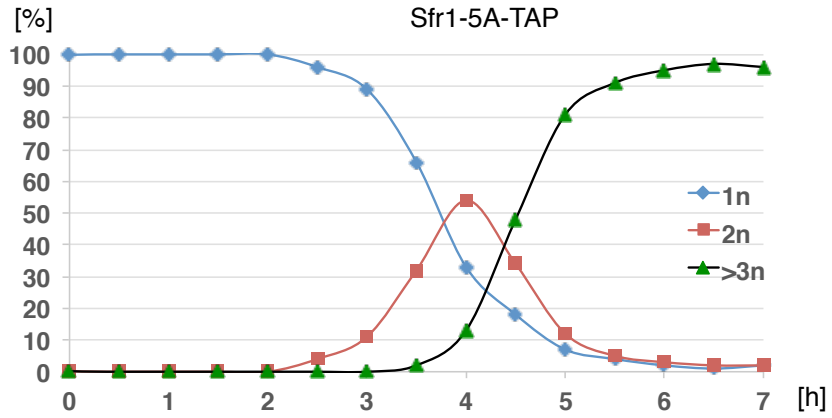
Figure S1

A



B

Sfr1-5A-TAP	unique peptides	coverage	phosphorylated residues
Sfr1-5A (299 amino acids)	24	82%	S52 (or S48), T73, S135, S147 (or T146), T152, S175, S253
Swi5 (85 amino acids)	7	60%	

Figure S1. Sfr1 phosphorylation sites identified by mass spectrometry.
(A) Haploid *pat1-114* cells expressing Sfr1-5A-TAP were arrested by nitrogen starvation and released into meiosis at 34°C by inactivation of Pat1. Small aliquots of the cell culture were harvested at the indicated time points (hours). Fixed cells were stained with DAPI and nuclei were counted in 100 cells per time point. Shown are the fractions of cells that contained one nucleus (1n), two nuclei (2n) or more than two nuclei (>3n) at the indicated time points.
(B) The cells were harvested around 3 hours after meiosis induction and Sfr1-5A-TAP was isolated by tandem affinity purification. Purified proteins were analyzed by mass spectrometry. Phosphorylation sites identified on Sfr1 by mass spectrometry are shown. For the full list of identified proteins see Table S1.

Figure S2

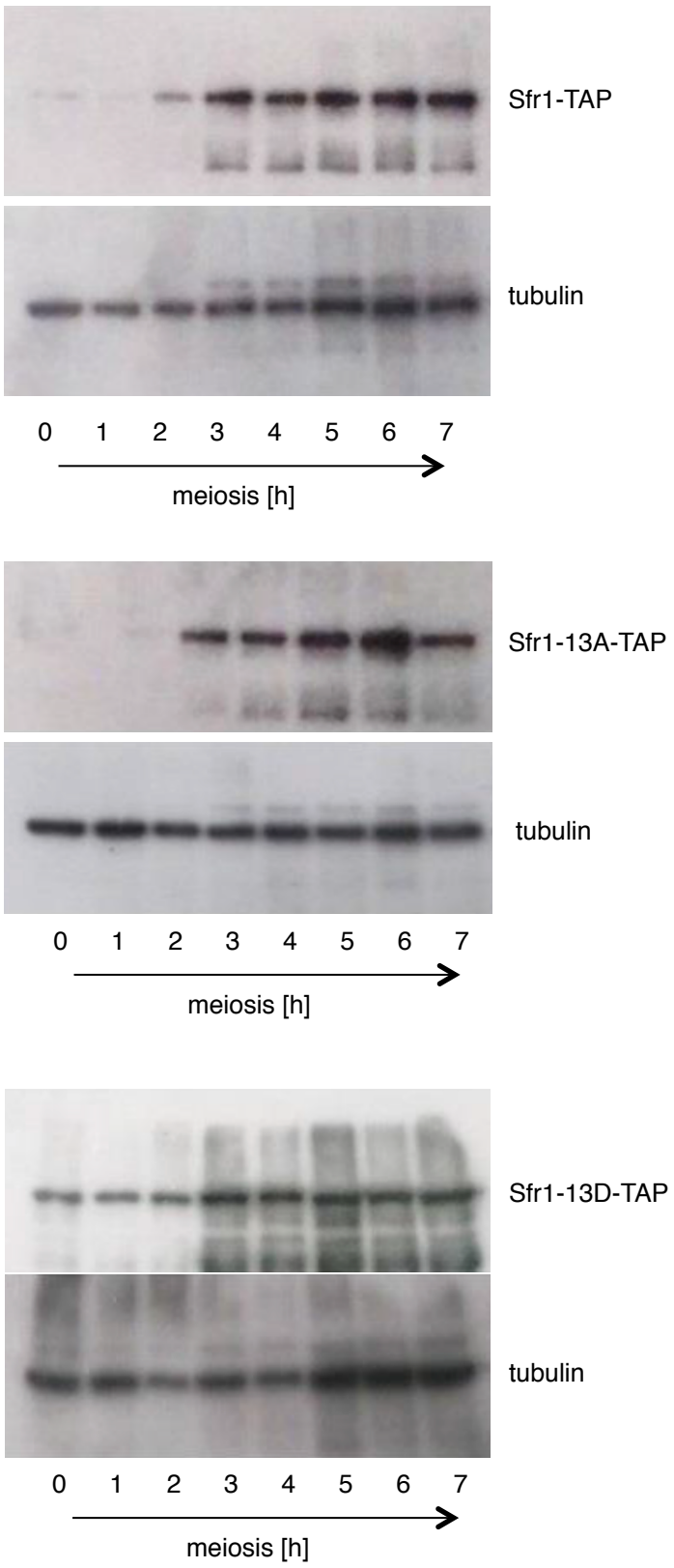


Figure S2. Expression of Sfr1-TAP, Sfr1-13A-TAP and Sfr1-13D-TAP proteins during meiosis.
pat1-114 cells expressing Sfr1-TAP, Sfr1-13A-TAP and Sfr1-13D-TAP were arrested by nitrogen starvation and released into meiosis at 34°C by inactivation of Pat1. Cells were harvested at the indicated time points (hours). Proteins extracted from meiotic cells were analyzed by gel electrophoresis and Western blotting using anti-tubulin antibodies. The TAP epitope was detected using PAP antibodies (rabbit antiperoxidase antibody linked to peroxidase).

Figure S3

A Swi5

```
Sc (Sae3) ------------------------------------------------------------    0
Sp        ------------------------------------------------------------    0
Mm        ------------------------------------------------------------    0
Hs        MQRRGQRDLWRHNKSCARNRCPRPPRERGGAGFPWVRAQLSVRQFTLRVRVPGPVHLRGR    60

Sc (Sae3) ------------------------------------------------------------    0
Sp        ------------------------------------------------------------    0
Mm        ------------------------------------------------------------    0
Hs        SPTPALDPLAPLNPLIRGPRTPGLRRWIQSLALLLPNCSSSRIPTVPRPHSGLWVQSDFP    120

Sc (Sae3) -------------------------------MNYLETQLNKKQKQIQEYE-SMNGNLIKM    28
Sp        ---------------------------------MEKSQLESRVHLLEQQKEQLESSLQDA    27
Mm        ----------------------------MIDENNDVSEEALSSDIKKLKEKHDMLDKEISQL    34
Hs        LGFLSRTEPRLTRSCRGAFRSPRPLPKSGQADGTSEESLHLDIQKLKEKRDMLDKEISQF    180
                                              :  *    : ::: .  :: .: .

Sc (Sae3) FEQLSKEKKNDETPKKISSTYIKELKEYNELRDAGLRLAQIIADEKQCKIKDVFEEIGYS    88
Sp        LAKLKNRD-----AKQTVQKHIDLLHTYNEIRDIALGMIGKVAEHEKCTSVELFDRFGVN    82
Mm        ---IAEGY-----RVIELEKHISLLHEYNDIKDVSQMLLGKLAVTRGVTTKELYPDFDLN    86
Hs        ---VSEGY-----SVDELEDHITQLHEYNDIKDVGQMLMGKLAVIRGVTTKELYPEFGLD    232
             : :           . :*  *: **:::* .  :   :*  .  .  :::  :. .

Sc (Sae3) MKD     91
Sp        GSE     85
Mm        LND     89
Hs        MND     235
           .:
```

Sfr1

```
Sc (Mei5) ------------------------------------------------------------    0
Sp        MSQTINSELNENATSQCKEDLKVSLSESDLRDSQGQLGIENPPKCNNS---GNHSDNLGF    57
Mm        ----MAEEGNQEFTSKMENSS-----DSASTSPDAPQPSENPPSPPTSPAAPQTSENPPS    51
Hs        ---MAEGEKNQDFTFKME-------------------------------------------    15

Sc (Mei5) ------------------------------------------------------------    0
Sp        IEQSETVHPENEKA-------LTPDLRDT-------------KIHTSLPITT--------    89
Mm        PPTSPAVPQTRENPPSPPTSPAAPQPRENPPSPPTSPAAPQPRENPPSPPTSPAAPQPRE    111
Hs        ----------------------------------------SPSDSAVVLPSTPQASA    32

Sc (Mei5) ------------------------------------------------------------    0
Sp        -PFSKKRAREAKNILLKPFKSPLRQTASPQVADTNLKPSLAVTNLNSDETNTSSEPVTSP    148
Mm        NPPSPHSNSSGKQPLSGTPKERLKKARSSSHSFCSVVKRMKVENDENN--ETLSEPGESS    169
Hs        NPSSPYTNSSRKQPMSATLRERLRKTRFSFNSSYNVVKRLKVESEEND--QTFSEKPASS    90

Sc (Mei5) -----------MHNQEEWLDKDKTL--VNEEENTCINHSYTKKDTNNYRVGKSGIKD-LK    46
Sp        LRT----TPNSIKRQKRL------F---KSPISNCLNPKSDP-----------EITQLLS    184
Mm        KEENCSKAQESLKNKDSEPGEKS-----SEEKNTCESKSSDTGSSNALPKES-E-NAIIR    222
Hs        TEENCLEFQESFKHIDSEFEENTNLKNTLKNLNVCESQSLDSGSCSALQNEF-VSEKLPK    149
                     ::. .             .  . * . .

Sc (Mei5) KPTNQKEIAIKNRELTKQLTLLRQENNHLQQACKILSENKIIENRKSIEKWRTICEMELS    106
Sp        R-----R-----LKLEKEVRNLQEQLITAETARKVEAKNEDKDLQTLIQKWKNAAQQAAE    234
Mm        EKLKQEK-----IRLIRQVEEKEDLLRRLKLVKMYRIKNDVTELENLIKKWRKCGQRLLC    277
Hs        QRLNAEK-----AKLVKQVQEKEDLLRRLKLVKMYRSKNDLSQLQLLIKKWRSCSQLLLY    204
          .     .      .* :::   .:     : .     :*.  : .  *:**:.  :

Sc (Mei5) FILNSTLIKINRMGGYKDFLEKEMEAKKRR---------L-EYQIDNGMEDQICEIKESD    156
Sp        VLFKPMAERIRLAGGVTQSFRIEEGENKGQIQEVRTEFTMSMFLNQFGVPVHLMSFDE--    292
Mm        ELQSIMSED------------------------EDEKLTLTELIDFYGIDDNLLHYNR--    311
Hs        ELQSAVSE-------------------------ENKKLSLTQLIDHYGLDDKLLHYNR--    237
           : .                                  :       *:  ::   ..

Sc (Mei5) DFRQLSEVEKQEWESQMNEQLKELEKKKIAELEKLNKVLHDSEGKDFGMAELCTRLKLDY    216
Sp        --------ENGDWKS-------------------------------------------    299
Mm        --------SEEEFTGV-----------------------------------------    319
Hs        --------SEEEFIDV-----------------------------------------    245
                 .: :: .

Sc (Mei5) SLIFPQ  222
Sp        ------  299
Mm        ------  319
Hs        ------  245
```

B

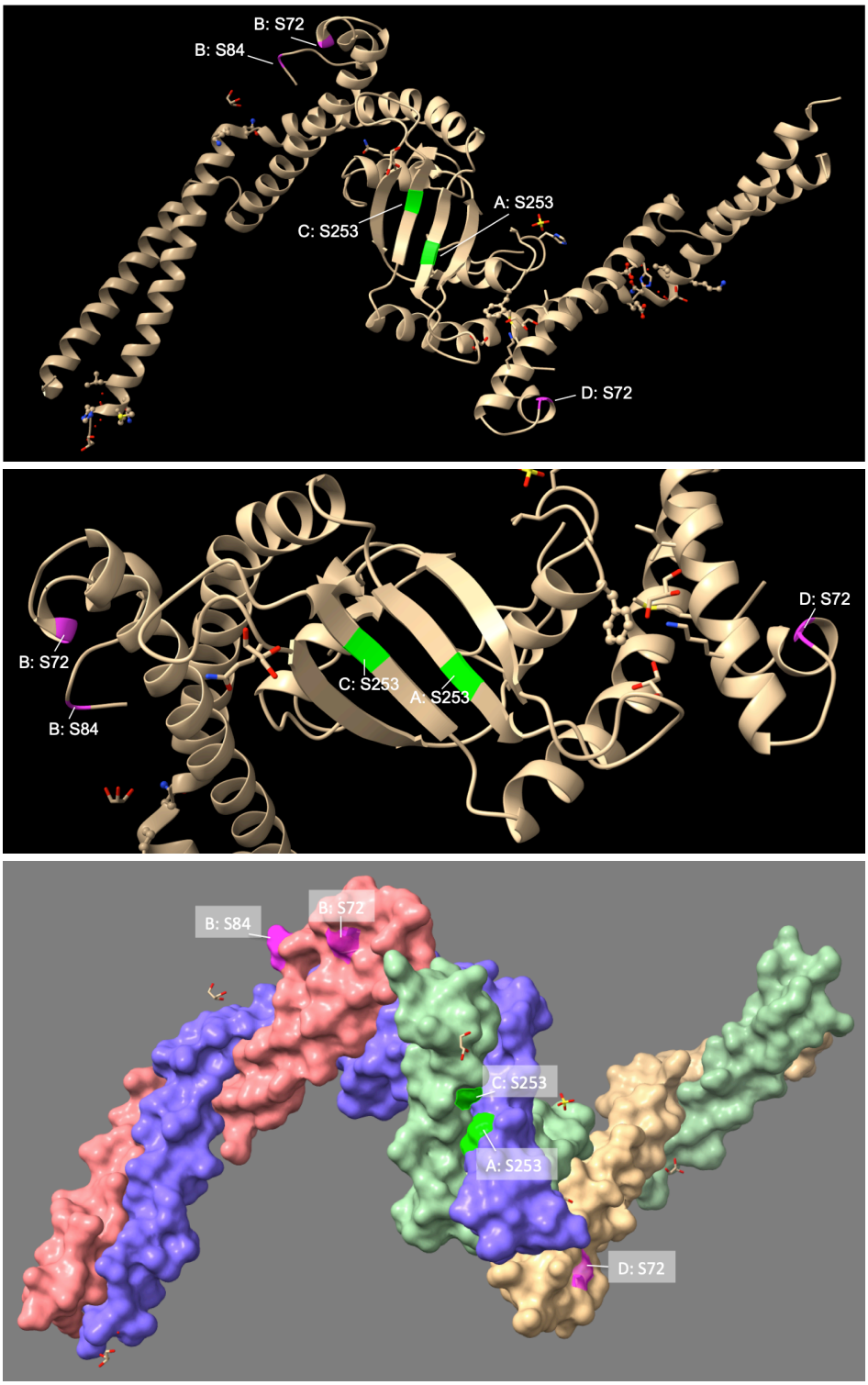

B: S72
B: S84
A: S253
C: S253
D: S72
D: S72
B: S72
C: S253
A: S253
B: S84
B: S84
B: S72
C: S253
A: S253
D: S72

Figure S3. Visualization of Swi5 and Sfr1 phosphorylation sites.
(A) Swi5 and Sfr1 protein sequences from *Saccharomyces cerevisiae* (Sc), *Schizosaccharomyces pombe* (Sp), *Mus musculus* (Mm) and *Homo sapiens* (Hs) were aligned using Clustal Omega (Sievers et al., 2017; https://pubmed.ncbi.nlm.nih.gov/21988835). An asterisk (*) indicates positions of fully conserved residues, a colon (:) indicates conservation between residues of strongly similar properties and a period (.) indicates conservation between residues of weakly similar properties. Swi5 and Sfr1 phosphorylation sites are highlighted in red.
(B) Crystal structure of fission yeast Swi5 and its complex with the Sfr1C (C-terminal domain, residues 181-299) was retrieved from the RCSB Protein Data Bank (PDB ID: 3VIQ) (Kuwabara et al., 2012; https://pubmed.ncbi.nlm.nih.gov/22405003) and visualized using ChimeraX software (Pettersen et al., 2021; https://pubmed.ncbi.nlm.nih.gov/32881101). Presented are the two Swi5-Sfr1C heterodimers in the asymmetric unit. Swi5 and Sfr1 phosphorylation sites located on individual protein chains (denoted A, B, C and D) are indicated in magenta and green, respectively. Upper and middle figures: ribbon diagram of Swi5-Sfr1C, lower figure: surface representation of Swi5-Sfr1C.

Figure S4

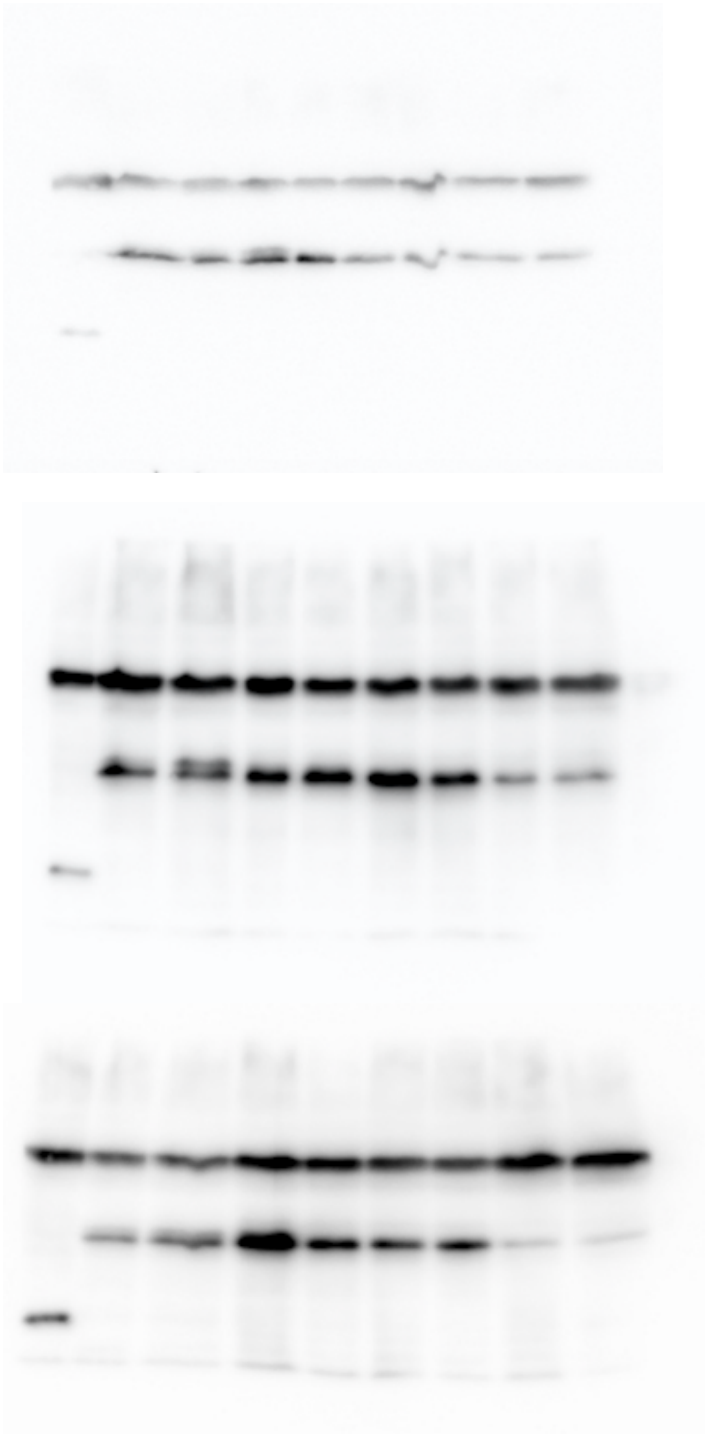


Figure S4. Full original images of Western blots shown in Figure 3.