

Table S1. List of primers

Purpose	Primer	Sequence (5 → 3', Adaptors used for Gibson assembly are labeled in red)
<i>PARS11</i> knockout	Pars11_5UTRf1199_Not1	AGTTCTAGAGCGGCCGCCTTCTTTGCATTTATAAGATAACAAG
	Pars11_5UTRr1862_N4	GTCAGGTGCCTGGTACCCCTTCTTACTTCTGATTTTTTTAATC
	Pars11_3UTRf3159_N4	CTGACGTCGCACCATCCCTTTTTAGTTATATTTTTGTGGATTG
	Pars11_3UTRr3801_Not1	ACCGCGGTGGCGGCCGCTTACAAAGCTATTAATTGTGATTC
Pars11 C-terminal HA tagging	Pars11_CDSf2468_Not1	AGTTCTAGAGCGGCCGCATCAAGGCTAAATCATCCTAATC
	Pars11_CDSr3125_HA	GTCGGGGACGTCGTAGGGGTATCCATTATTTAAGTTTAGGTTGCCTTT
	Pars11_3UTRf3126_HA	CCCTACGACGTCCCCGACTACGCCCTGAATTTAATATCAATTATTTTTT
	Pars11_3UTRr3569_N4	GTCAGGTGCCTGGTACCCAATTCATATTAATAAAGGAAATTTG
	Pars11_3UTRf3564_N4	CTGACGTCGCACCATCCCTGAATTATAAATTCTAGATTTCTAAT
	Pars11_3UTRr4151_Not1	ACCGCGGTGGCGGCCGCAATAGCATTCAATATACTTTTTG
<i>PARS11</i> knockout validation	Pars11_Cdsqf2409	CAAGAAAGTAAGGTCTCTTAATTTG
	Pars11_Cdsqr2745	TAATTACATTGCTCTAGAAAAAGTG
<i>ATR1</i> RNAi	ATR1_N5if7625_5F	TAAACTTAAACATCCCGGGGGATCCATGAGCTAGATTAACACTTTTTAG
	ATR1_N5ir8282_5R	TTGCATATCCGTTACTTACGGATCCCTTAATTAGTTTCATTATGATCCTTG
	ATR1_N5if7625_3F	GCTGACCGATTTCAGTTCGCCTGCAGATGAGCTAGATTAACACTTTTTAG
	ATR1_N5ir8282_3R	TAAAAGAAGAATTCAAAGGCTGCAGTTAATTAGTTTCATTATGATCCTTG
Pars11-16A construct	Pars11_16AQ_5UTRr912	CTTCTTATTGATATATGTTTTTCAGACTCAT
	Pars11_16AQ_IDTf	ATGAGTCTGAAAACATATATCAATAAGAAG
	Pars11_16AQ_IDTr	TAAGCAATTTGTCCAACCATCAA
	Pars11_16AQ_CDSf1514	CTTGATGGTTGGACAAATTGCT
RT-PCR	18s F	CCTGGGAAGGTACGGGTAAT
	18s R	AAGGTTCCACCAGACCATTTCG

RT-PCR

Spo11_cds2f2319

GCTAAAGGTTTAGTAACTGGTAATG

Spo11_cds2r2668

TTTCCCAGTAATCATTATACAATTC

Sequence for *PARS11* 1-603 bp mutation

atg agt ctg aaa aca tat atc aat aag aag aaa gct taa aac tta ggt tct gct tag tca tcg
gct gca gct ctc aca tca ctt tag tca aat tgc ttt aca gca aag gga taa atg gtt gct taa
aat gct tag aac gct cag aat acc att caa atg cag tgc aat gct caa gct cag tag ata ctc
gct caa ggt att tat ttt aat agt tca att tgt aat taa ttt aaa tat tta atc tct cag ctg ctt aat
ttc ctg tgc cta aaa act cta gcc tta tga ttg aag aac caa ata tct ttg gat aaa ctg gat
aca gct cat tca aaa ata aga acc taa att ctg ctt aga ata cat cct ttt taa att agt tag ctt
aag acg aag cta atg att ctt aca att aaa ttc tac atg aga ata att tta ttt aaa atc cta atc
tga act aaa gct ttc taa att aaa gct atc aac ttc ttg ctt agc ata acc taa agt aag ctc
aat tgt ttc ttc cag cga tcc aag aaa gta agg tcg ctt aat ttg atg gta agt ctg ctt aga tat
tta atc ctt ctg aat caa ggc taa atc atc cta atc tga tat aaa gta gct tga tgg ttg gac
aaa ttg ct

Table S2

Frequency distribution of cytological stages in samples taken from *atr1i* cultures for PFGE from t=5 h, 6 h and 7 h after meiosis induction. Post-meiotic stages were largely absent at these timepoints. Therefore, post-meiotic DSBs cannot account for the DNA fragmentation seen.

t	Stages %	Pre-meiotic	Early prophase	Mid prophase	Late prophase -metaphase I	Attempted Anaphase I	Post-anaphase	RNAi escapers (WT-like stages)	Sample size (no. of nuclei)
5 h		15.5	50	35.5	5	0	0	3	200
6 h		10.5	10	11.5	58	8	0.5	1.5	200
7 h		11	3	6	50	7	21	2	250

Prophase stages were classified as early mid and late according to the increasing degree of chromosome condensation, since the length of nuclei does not change in the absence of ATR.

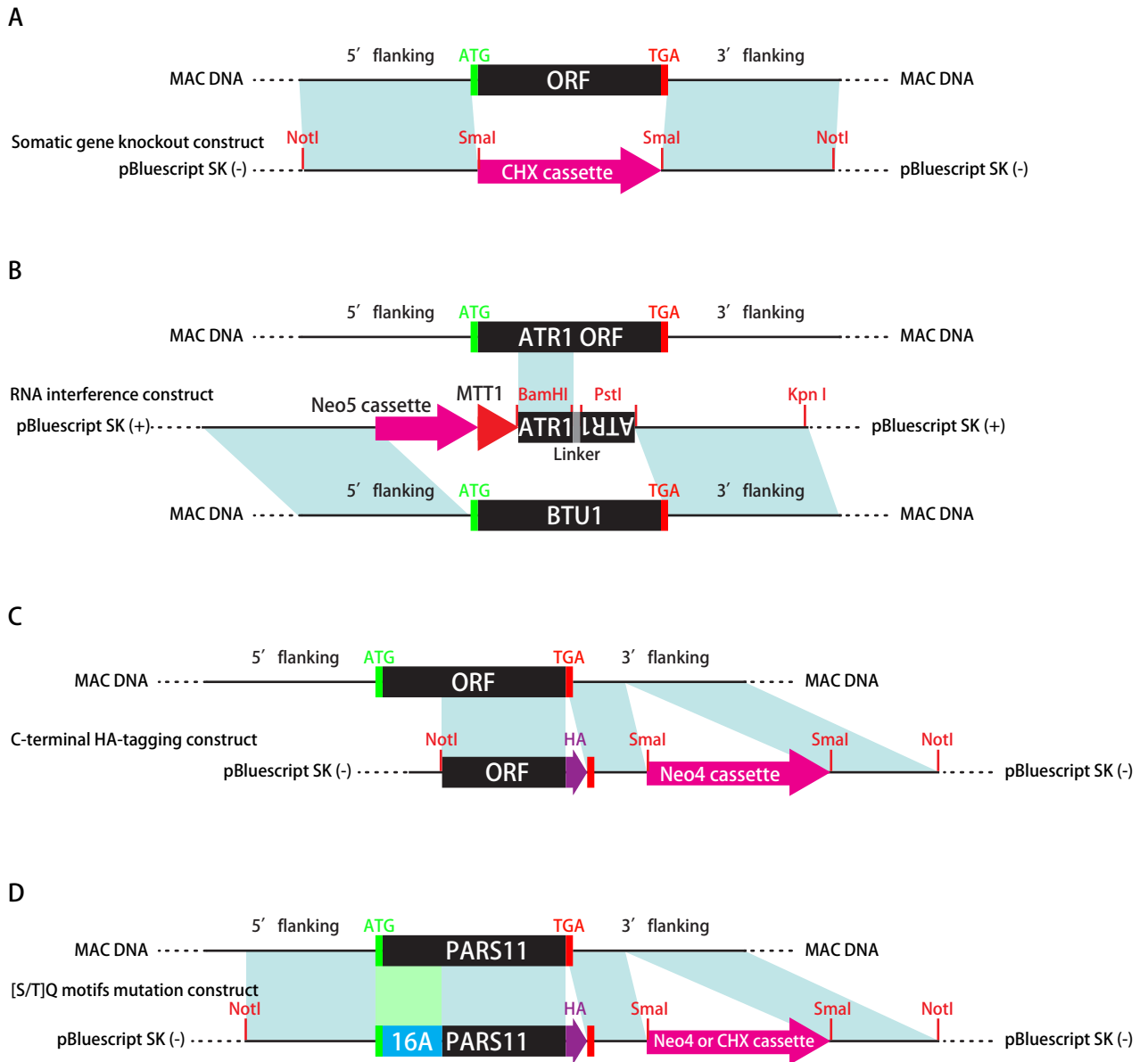
Table S3

BLAST search for Pars11 protein homologs

Group	Species	Protein ID	BLASTP E-value*	
Multicellular animals	<i>Homo sapiens</i>	sp Q9Y4D8-4 HECD4_HUMAN	0.21	
	<i>Mus musculus</i>	sp Q8CAQ8-5 MIC60_MOUSE	0.12	
	<i>Danio rerio</i>	ENSDARP00000122296	0.021	
	<i>Drosophila melanogaster</i>	FBpp0082802	0.42	
	<i>Caenorhabditis elegans</i>	ZK430.1	3.3	
Land plant	<i>Arabidopsis thaliana</i>	AT5G45610.1	0.95	
Fungi	<i>Saccharomyces cerevisiae</i>	YOR237W	0.12	
	<i>Schizosaccharomyces pombe</i>	SPAC664.14.1:pep	0.52	
Protist	Tetrahymena	<i>Tetrahymena borealis</i>	EI9_05736.1	2.31E-56
		<i>Tetrahymena ellioti</i>	EI7_06775.1	1.79E-58
		<i>Tetrahymena malaccensis</i>	EIA_05075.1	0
	Other ciliates	<i>Paramecium tetraurelia</i>	GSPATP00036354001	0.065
		<i>Ichthyophthirius multifiliis</i>	IMG5_094660	0.17
		<i>Oxytricha trifallax</i>	Contig20793.0.g60 Guanylate-binding	0.21
	Other protists	<i>Stylonychia lemnae</i>	Contig10830.g11578	0.001
		<i>Plasmodium falciparum</i>	PF3D7_1227500	0.45
		<i>Toxoplasma gondii</i>	TGME49_206430	0.61
		<i>Trypanosoma brucei</i>	Tbg972.7.3660	0.57
	<i>Giardia lamblia</i>	GL50581_1183	1.9	

*BLASTP e-value threshold was set to 10 (default).

Figure S1



A. Diagram of the somatic gene knockout construct. DNA fragments used for homologous recombination were amplified from wild-type *Tetrahymena* genomic DNA using PCR. The NEO4 (paramomycin resistance) or CHX (cycloheximide resistance) cassette was removed from the respective plasmids by *SmaI* digestion (Gao et al. 2013, *Genes Dev.* 27: 1662-1679). DNA fragments and drug resistance cassette were then cloned into the linearized pBluescript SK(-) vector by Gibson assembly. DNA fragments in the knockout construct and their corresponding loci in the somatic genome are indicated by light blue fields.

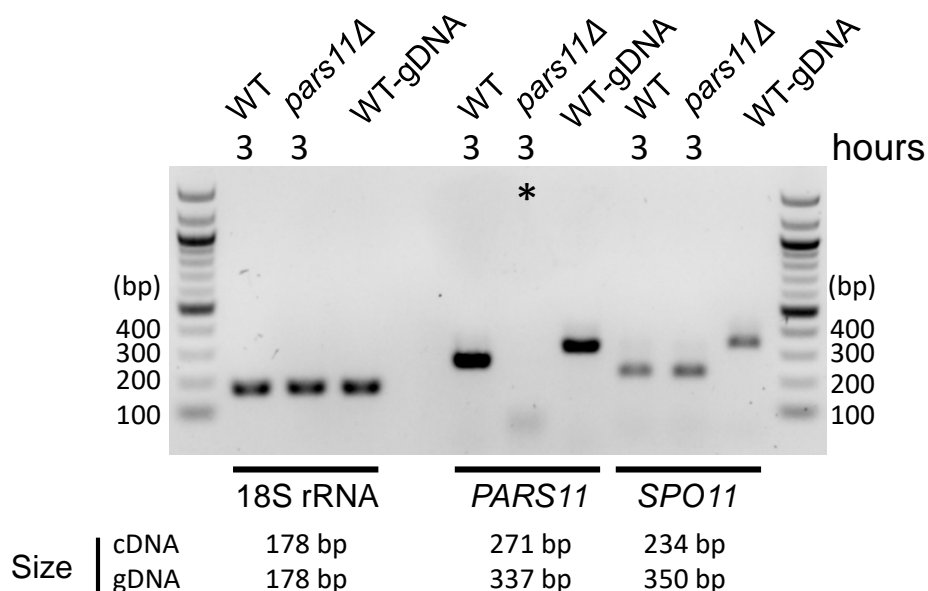
B. Diagram of the ATR1 RNA-interference construct. A fragment of the ATR1 ORF (from 1295 bp to 1952 bp) was amplified from genomic DNA using PCR. Two copies of this fragment were inserted end-to-end behind a Cd²⁺-inducible MTT1 metallothionein promoter (red arrowhead) in plasmid pNeo5-RNAi (Akematsu et al. 2017, *eLife* 6: e26176). After shooting the linearized plasmid DNA into *Tetrahymena* cells, the NEO5 (paramomycin resistance) cassette and ATR1 RNA-interference cassette is integrated into the nonessential BTU1 gene locus due to flanking homologies.

C. Diagram of the C-terminal epitope tagging construct. The Pars11 C-terminal HA tagging construct was generated in a similar way to the PARS11 somatic knockout construct.

D. Diagram of the construct for the substitution of Pars11 N-terminal S/T-Q sites. This construct was generated in a similar way as the PARS11 somatic knockout construct. In this construct, all 16 serine and threonine codons within the 603 bp 5' PARS11 coding sequence were replaced by alanine codons (16A, blue box).

Figure S2

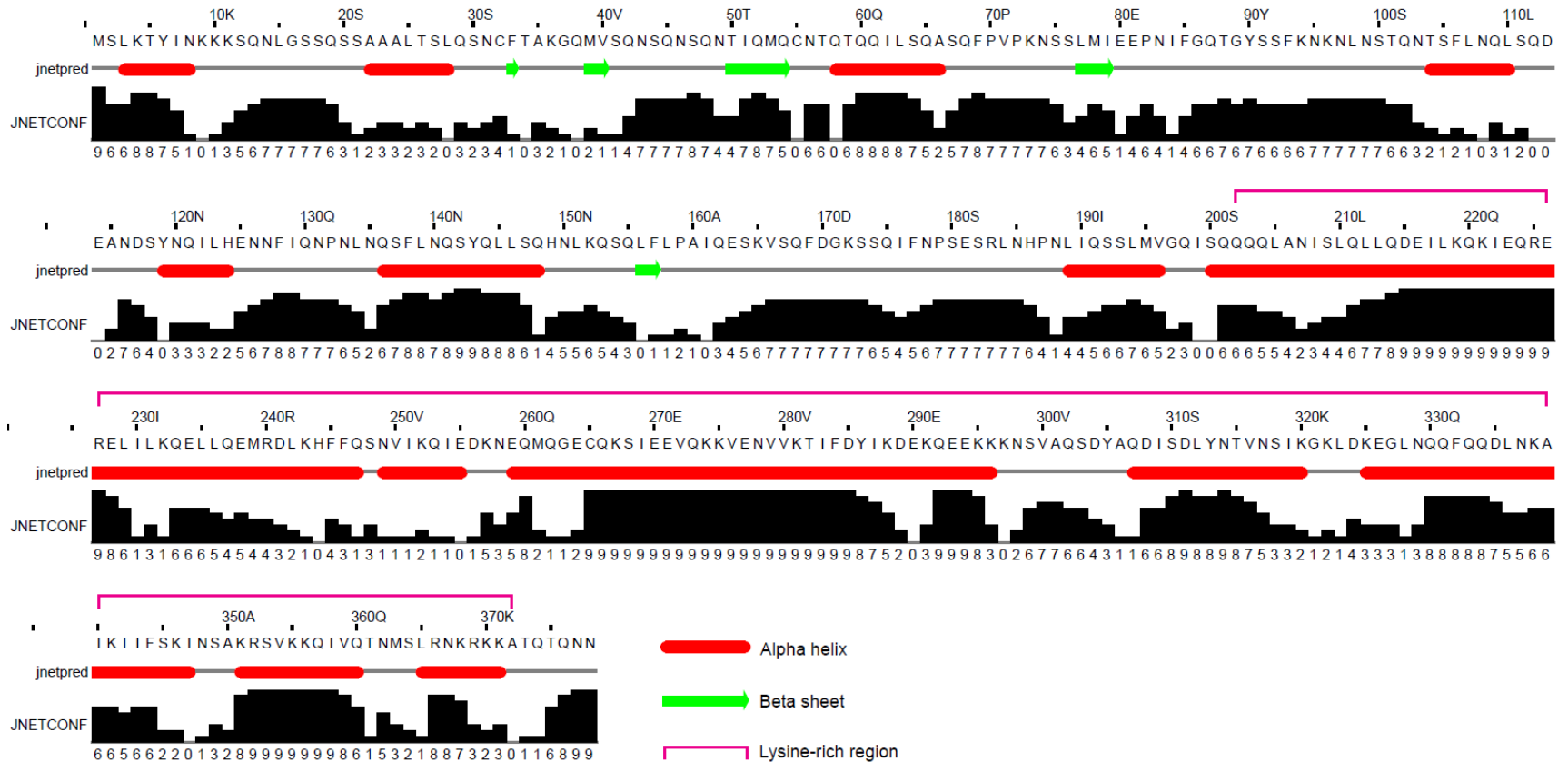
PARS11 knockout confirmation using RT-PCR



To confirm the deletion of the somatic *PARS11* gene in *pars11Δ* strains, mutants of different mating types were starved and mixed to induce conjugation. Total RNA of *pars11Δ* mating pairs was isolated at 3 hours after induction of meiosis using the TriFast reagent (PeqLab, Erlangen, Germany). cDNA was then synthesized using 1 µg of DNase I treated total RNA with the RevertAid H Minus Reverse Transcriptase kit (Thermo Fisher Scientific, Waltham, MA, USA) and random hexamers (Integrated DNA Technologies, Leuven, Belgium). cDNA synthesized from total RNA isolated from wild type 3 h after induction of meiosis, and genomic DNA (gDNA) extracted from vegetative cells of wild-type strain B2086 were used as controls. *PARS11* fragments were amplified from WT cDNA and gDNA samples using the *PARS11*-specific intron-spanning primers whereas this fragment was not amplified using *pars11Δ* cDNA (see asterisk). This result, together with the gDNA qPCR result (not shown), indicates the knockout of the *PARS11* gene from the MAC genome of *pars11Δ* mutants. *18S* rRNA/rDNA and *SPO11* specific fragments were amplified as loading control and indicator of meiosis, respectively. Primers used for PCR reactions are listed in Table S3.

Figure S3

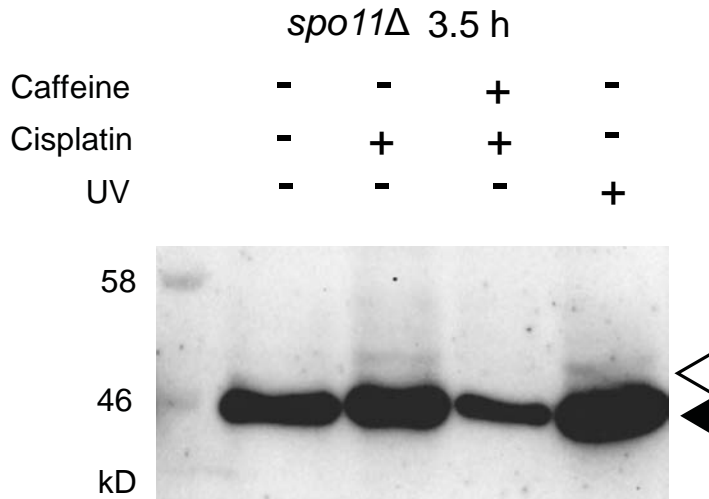
Predicted secondary structure of the Pars11 protein



The N-terminus of Pars11 is poor in secondary structural elements, but many alpha helices are found in its C-terminal lysine-rich region. The secondary structure of the *T. thermophila* Pars11 protein was predicted by JPred4 with default settings (Drozdetskiy et al. 2015, Nucl. Acids Res. 43: W389-W394). Putative alpha helices and beta sheets are annotated as red bars and green arrows, respectively. The confidence estimate for the prediction is indicated by both the height of the black bars below the structures and the number. High values mean high confidence levels.

Figure S4

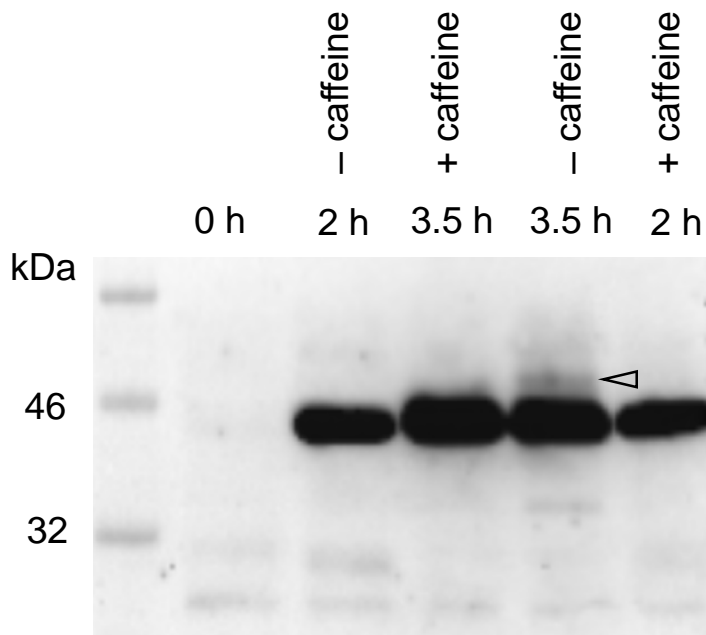
a



Pars11 remains nonphosphorylated (solid arrowhead) in *spo11*Δ, but phosphorylation (open arrowhead) is induced by DNA damaging agents cisplatin and UV, and is suppressed by the ATR inhibitor caffeine

b

Original version of the gel shown in Figure 4c



Western blot of HA-tagged Pars11 (~44 kDa). A caffeine-sensitive (i.e. possibly ATR-dependent) modified Pars11 form (open arrowhead) is present during late prophase (3.5 h after induction of meiosis).

Figure S5

MEME search for shared motifs in Pars11 and the Rec114 family

>Tetra_Pars11

MSLKTYINKKKSQNLGSSQSSAAALTSLQSNCF TAKGQMV SQNSQNSQNTIQMQCNTQTQQI
LSQASQFPVPKNSSLMIEEPNIFGQTYSSFKNKLNSTQNTSFLNQLSQDEANDSYNQILH
ENNFIQNPNLNQSFNLQSYQLLSQHNLKQSQFLPAIQESKVSQFDGKSSQIFNPSESRLNH
PNLIQSSLMVGQISQQQQLANISLQLLQDEILKQKIEQRERELILKQELLQEMRDLKHFFQS
NVIKQIEDKNEQMQGECQKSIIEEVQKKVENVVKTI FDYIKDEKQEEKKKNSVAQSDYAQDIS
DLYNTVNSIKGKLDKEGLNQQFQQDLNKAIKIIFSKINS AKRSVKKQIVQTNMSLRNKRKKA
TQTQNN

>Yeast_Rec114

MYEYCSVVIKKYSKYTIPSFAPNGFQSMLEPPQIDKWQHLSANCTLQFRVLLMDSRQILINV
VLNNSTLLENIRLPLGDNQDLIQFSCKSPIISCKYISEEFGPRMLRRFQMNL PNDVEFNRTV
VSLKNLNFVLR TARTSIAQSTITSQVQGNNGTKVCFTEGPKVSSYTNPNTQFQTQNMIMDF
SQRYQEESERESNNR SNITLPHDSIQIAQQIWPNTDLNVVQSSQDLNTPMATQTVLGRPESL
IVQPLEVVSQSPNTTCLPNAENKKKKVDTTSDFTSRKEIALCKTGLLETIHIPKERESQMQ
SVTGLDATPTIIWSPGKDNTAKKNTSNKKNIDDKLTNPQKSGNTHTPDRNKEVLPNGTLNET
RKEASPSEGLTIRVKNVNRNASRKISKRLIKEKLDKDEEFMKWVNKVETVLNKMFEK

>Elegans_DSB-2

MSARGLKVMYYKLAEKNEAETS DVSILIDGHKRKFAVICNGIGREMVDLHYDSKKFPRIFS
RERHLYVMFSESTSDGFRLTFGATEREQFLRIMHSLGCLLDTAIVKFSQQSSSSQPPMRRD
STAVFNNSYNQYTSFQQASQPRVFSTIKLELSGDSCNSAFQPSQPTGFDPNQLLTIKKSSSN
QISLSVEVADKCIQTEDLVDLLADDEYCMRKALRKMIENPSFIKAVAAAENTLQYIPQAEQE
GLEDVTEPPINDIFFDRFHQPQLEQHDSVPED

>pombe_rec7

MNIFPIVKYSVAEDSYTTDSSHINWSHYSDGGFTLSLTSGLLQIRQHEELIQSINLLDLWHQ
LIPGTKEKCLTLLSRAPCMNIRAFTNNVMKRFQVKFPSDVHYMKAKVEFEKLGVLVKDAKSS
SEKKQFNNSQSQSNNSQELSLMNNAYNKSSAQQPNNLLQPSYIPMTQTATTAVNNSTNYVNP
APLQHVMPNAEIFSNTPLKRFRGDAGMTQMPLRSDTSIESITASQQPTWDENVVITSSPFN
PNRNAYSYGANSQYPIIAATPLNSQTQASWVAQPENQAYANLIPSPPTTSQILPTELTEEEK
QLRSKVLFYLKQDSFIQLCQSLERVWNKM*

>Mus_rec114

MSEAGNVASGLGLPGEVSQWSLKRYGRFMLLDNVGSPPGSSSEAAAAGSPTWKVFESSEESGS
LVLTIVVSGHFFISQGTLLLEGFSLIGSKNWLKIVRRMDCLLFGTTIKNKSRMFRVQFSGES
KEEALERCCGCVQTLAQYVTVQEPDSTTQELQQSQGPREGESQGKDPLQQGPSLTLEQHVC
MAAGAGVLQERTSVTHRAQSILAPEKLTLAYEGSSWGTEELGPFLRLCLMDQNFPAFVEEVE
KELKKITGLRN

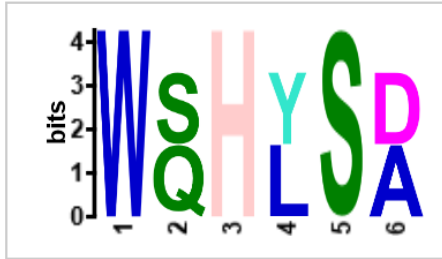
>Maize_Ph1

MRGVHRHQGTWIPASCPASLCVCHPSLPSAVPVLTISIGDVVFEHFVSILNFSWPQVTCVT
QCPIRGSRVVFVSFCDFKQIQKFAVRFPQPCDAESFLSCVECSGSSGTMDIIPFGSDYVC
EDSSASEYIVSNGLHHRLLDDASNLEEQCFDHTIDEPPMNYHEETDQHVLEPLSASNTSNNSA
FPPSFNQMLKSCSIDYDQEEPCLASNHVLQEVYVLDTSHDERTAGKGMDAAGVDASILT
YDLMARIKTYMADES FNDMLLKLDKAIDELGGMSL

DISCOVERED MOTIFS

1.

E-value: 7.6e+001 [?](#) Site Count: 2 [?](#) Width: 6 [?](#)



Log Likelihood Ratio: 35 [?](#) Information Content: 22.9 [?](#) Relative Entropy: 25.3 [?](#) Bayes Threshold: 9.91961 [?](#)

Name ?	Start ?	p-value ?	Sites ?
4. pombe_rec7	25	2.86e-9	SYTDDSSHIN WS YSD GGFTLSLTSG
2. Yeast_Rec114	37	3.64e-8	SMLEPPQIDK WQ LSA NCTLQFRVLL

2.

E-value: 8.5e+001 [?](#) Site Count: 2 [?](#) Width: 9 [?](#)



Log Likelihood Ratio: 50 [?](#) Information Content: 33.9 [?](#) Relative Entropy: 36.2 [?](#) Bayes Threshold: 9.90614 [?](#)

Name ?	Start ?	p-value ?	Sites ?
6. Maize_Phs1	5	1.34e-13	MRGV HR QGTWIP ASCPASLCVC
5. Mus_rec114	202	1.11e-9	GVLQERTSVT HRAQSILAP EKLTLAYEGS

3.

E-value: 8.8e+001 [?](#) Site Count: 2 [?](#) Width: 6 [?](#)



Log Likelihood Ratio: 35 [?](#) Information Content: 22.9 [?](#) Relative Entropy: 25.1 [?](#) Bayes Threshold: 9.91961 [?](#)

Name ?	Start ?	p-value ?	Sites ?
4. pombe_rec7	101	4.26e-9	MKRFQVKFPS DV YMK AKVEFEKLG
3. Elegans_DSB-2	49	3.17e-8	ICNGIGREMV DL YDS KKFPRIFSRE

4.

E-value: 1.7e+002 [?](#) Site Count: 2 [?](#) Width: 8 [?](#)



Log Likelihood Ratio: 45 [?](#) Information Content: 31.6 [?](#) Relative Entropy: 32.7 [?](#) Bayes Threshold: 9.91064 [?](#)

Name ?	Start ?	p-value ?	Sites ?
3. Elegans_DSB-2	262	9.08e-12	DVTEPPINDI FFDRF QP QLEQHDSVPE
6. Maize_Phs1	86	1.86e-10	FCDKFKQIQK FAVRF QP CDAESFLSCV

5.

E-value: 1.7e+002 Site Count: 2 Width: 8



Log Likelihood Ratio: 45 Information Content: 30.6 Relative Entropy: 32.6 Bayes Threshold: 9.91064

Name	Start	p-value	Sites
5. Mus_rec114	186	6.14e-11	GPSLTLEQHV CMAAGAGV LQERTSVTHR
6. Maize_Ph1	235	8.22e-11	TSHDERTAGK GMDAAGEV DASILTIDLM

6.

E-value: 2.0e+002 Site Count: 2 Width: 14

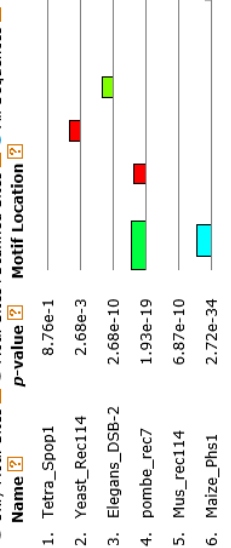


Log Likelihood Ratio: 74 Information Content: 52.5 Relative Entropy: 53.6 Bayes Threshold: 9.88341

Name	Start	p-value	Sites
6. Maize_Ph1	113	1.39e-18	VECSGSSGT MDIIPFGSDYVCE SSASEYIVSN
4. pombe_rec7	1	4.31e-15	MNIPPIVKYSVAED SYTTDSSHIN

MOTIF LOCATIONS

Only Motif Sites Motif Sites+Scanned Sites All Sequences



MEME search for shared motifs shared in Pars11 and the Mei4 family

>Tetra_Pars11

MSLKTYINKKKSQNLGSSQSSAAALTSLQSNCF TAKGQMV SQNSQNSQNTIQMCNTQTQQILSQ
ASQFPVPKNSSLMIEEPNIFGQTGYSSFKNKNLNSTQNTSFLNQLSQDEANDSYNQILHENNFIQ
NPNLNQSF LNQSYQLLSQHNLKQS QLFPAIQESKVSQFDGKSSQIFNPSESR LNHPLIQSSLM
VGQISQQQLANISLQLLQDEILKQKIEQRERELILKQELLQEMRDLKHFFQSNVIKQIEDKNEQ
MQGECQKSI EEVQKKVENNVKTI FDYIKDEKQEEKKNSVAQSDYAQDISDLYNTVNSIKGKLDK
EGLNQQFQQDLNKAIKIIFSKINSAKRSVKKQIVQTNMSLRNKRKKATQTQNN

>Yeast_Mei4

MSRGKLEDMEQKETSEVDWIIICFALIQSRNPTLWKRALSRKKGDVEDVGALKSEKNLKNPRENS
KHIIYKWVAPFENGFLNNKSLFAHLEPIYNFLCQNKYKSFEDAVGLKELQSFSDVSTADINWFL
PRYKILLKILSLKTKEIDFRGLSQVFQTLQILLVSHYSHRIDSDSSFKRTLIDVHVFNFIKFLF
NRILLKKNQNDPKWLQNFYDQGDGKHLCDKVDYKRLCSLHFTLIYSIINIQLIKIKTNQTFEPQI
LKYVSVLKLIEHILIIIESLIHVLRVSKHKLICINRKKAYCRVYLERELSLKKTYLKNFYSVI
SGVPEKELGGLLKILKIVILSLETFESIEWQHLPFLEKFP AHEISLQKKRKYIQAALLITAER
NLIARFRLSRWFNETENI

>pombe_rec24

MNGTNTEDNSKQILIQTMYTYDSSGETLKIAIAWKIILKKPKGKNIKDYIEALRKGIEDQEHCEK
YASTLLEPRPKTKKDVVLKNSNVTECVALKAKPFSKKIEDMDIFLLTNVHENLQEKRQTSGLAH
LDIEYTFNGLFRFLKCTADIKLKQTKVYEGADFLRIKTLFEEIFMFLKRDCCKSPLVLRVLDLGD
YVLDLIIITQSIMQNNANNGTGVISRKAFLEFYVFLQELIFNKLSFASVEQLEKLLDQIVKRMKI
CFTYCKNDNPSIRLLYSECFFSYAEIYFPC LHSFDAQLSSAASKCVQILRDIITNEELQTDKQEL
SKSAYSAPSILLIGLKDMLFPEDIS

>Mus_MEI4

MDIQPWYLKTSKLLALALAIHSPADRSSREYTEYLASLVTQKESTWKSLEALEAEVLQLRQKL
LLSRISSGLFKNGPDVLP T L S D Q E P T S S E N T L T L M D D S G C V L S N E Q R N E P A E L S Q H F V E S T D P P L
L P L P L E K R P R T T L E N P L S S H M Q F F Q H L L E L K K W T E S S L K V Y L T H F E K D S S T V S D S V S Q L L D A L I
T F Y R N P K L P F S S F W T E A V G T L A R L A S D F N L S N H I F K R C S K K L E E F E K T L L Q A I L E N N S I N R F Q V Q
R Y V S Q S L V T L G S C S L L R K S I I S L L L S E V N S F V D D L G A I D Q D Q G I Y D V T R Y E N I F S L F W I L E Q V L Q
Q A P Q G D R T A H M D H S I P E M Q T F L Q K H D E V I F R L S D A F P L F A F Y L W R L G V L L N S A E M E T V K N E S L P

>Arabidopsis_Prd2

MSSSVAEANHTEKEESLR LAI AVSLLRSKFHNHQSSSSTSRCYVSSSEDALRWKQKAKERKKEII
RLQEDLKDAESSFHRDLFPANASCKCYFFDNLGVFSGRRIGEASESRFNDVLRRLRRRFLRLARRRSR
RKLTRSSQRLQPSEPDYEEEAHLRISIDFLELSEADSNSNFSNWSHQAVDFIFASLKKLISM
GRNLESVEESISFMITQLITRMCTPFKGVKQLETSVGFYVQHLIRKLGSEPFIGQRAIFAISQ
RISILAENLLFMDPFDESFPMEDECMFILIQLIEFLICDYLLPWAENEAFDNVMFEEWIASVVHA
RKAVKALEERNGLYLLYMDRVTGELAKRVGQITSFREVEPAILDKILAYQEIE

DISCOVERED MOTIFS

1.

E-value: 1.8e+001 [?](#) Site Count: 2 [?](#) Width: 6 [?](#)



Log Likelihood Ratio: 36 [?](#) Information Content: 22.9 [?](#) Relative Entropy: 26.3 [?](#) Bayes Threshold: 9.87344 [?](#)

Name ?	Start ?	p-value ?	Sites ?
5. Arabidopsis_Prd2	87	4.46e-9	SFHRDLFPAN ASCKRY FPDNLGVPSG
2. Yeast_Mei4	301	7.31e-9	HKLICINRKK AYCRVY LERELSLKKT

2.

E-value: 1.8e+001 [?](#) Site Count: 2 [?](#) Width: 9 [?](#)



Log Likelihood Ratio: 52 [?](#) Information Content: 34.9 [?](#) Relative Entropy: 37.6 [?](#) Bayes Threshold: 9.86186 [?](#)

Name ?	Start ?	p-value ?	Sites ?
5. Arabidopsis_Prd2	274	2.46e-12	ILAENLLFMD FMDESIPEM DECMFILIQL
4. Mus_MEI4	335	2.46e-12	QQAPQGDRTA HMDHSIPEM QTFLOKHDEV

3.

E-value: 4.3e+001 [?](#) Site Count: 2 [?](#) Width: 9 [?](#)

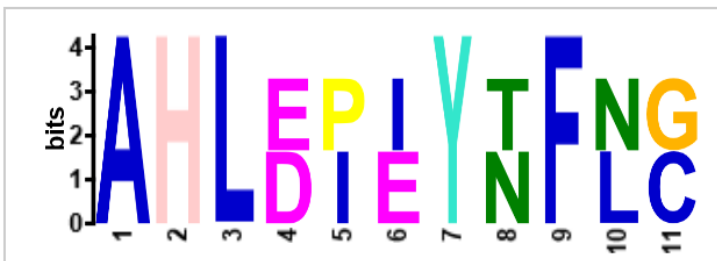


Log Likelihood Ratio: 51 [?](#) Information Content: 33.9 [?](#) Relative Entropy: 36.8 [?](#) Bayes Threshold: 9.86186 [?](#)

Name ?	Start ?	p-value ?	Sites ?
5. Arabidopsis_Prd2	218	2.37e-12	FMITQLITRM CTFKGMV KQLETSGVGY
1. Tetra_Spop1	32	6.12e-12	AAALTSLSQSN CFTAKGMV SQNSQNSQNT

4.

E-value: 1.3e+002 [?](#) Site Count: 2 [?](#) Width: 11 [?](#)



Log Likelihood Ratio: 59 [?](#) Information Content: 41.5 [?](#) Relative Entropy: 42.3 [?](#) Bayes Threshold: 9.85409 [?](#)

Name ?	Start ?	p-value ?	Sites ?
3. pombe_rec24	129	8.82e-14	QEKRTSGSL AHLDIYTFNG LFRFLKCTAD
2. Yeast_Mei4	87	8.82e-14	NGFLNNSLFL ALEPIYNFLC QNKYKSFEDA

5.

E-value: 1.6e+002 [?](#) Site Count: 2 [?](#) Width: 6 [?](#)

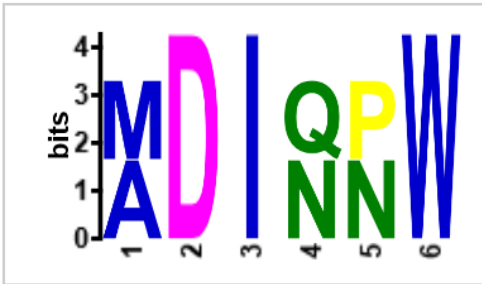


Log Likelihood Ratio: 34 [?](#) Information Content: 22.9 [?](#) Relative Entropy: 24.8 [?](#) Bayes Threshold: 9.87344 [?](#)

Name ?	Start ?	p-value ?	Sites ?
5. Arabidopsis_Prd2	234	1.37e-8	NEVKQLETSV G P Y V Q LIRKLGSEPF
3. pombe_rec24	194	2.18e-8	PLVLRRLVEL G D Y V L D LIITQSIMQ

6.

E-value: 1.5e+002 [?](#) Site Count: 2 [?](#) Width: 6 [?](#)



Log Likelihood Ratio: 36 [?](#) Information Content: 22.9 [?](#) Relative Entropy: 25.7 [?](#) Bayes Threshold: 9.87344 [?](#)

Name ?	Start ?	p-value ?	Sites ?
4. Mus_MEI4	1	1.98e-9	M D I Q P W YLKTSKLALA
2. Yeast_Mei4	123	1.82e-8	LQSFSDKVST A D I N N W FLPRYKILLK

MOTIF LOCATIONS

Only Motif Sites [?](#) Motif Sites+Scanned Sites [?](#) All Sequences [?](#)

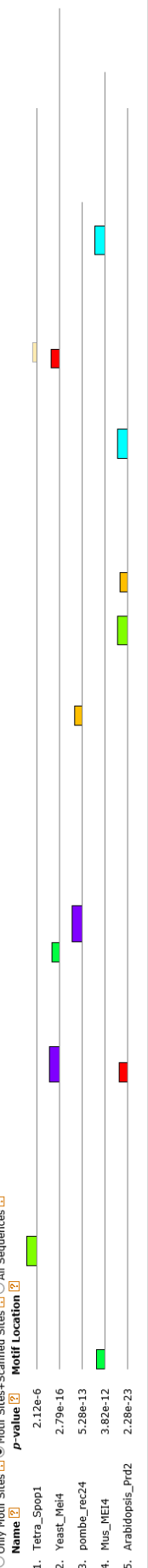
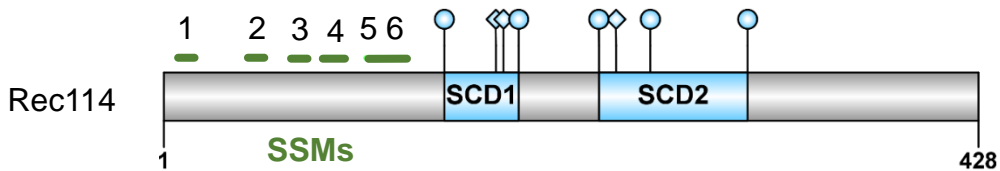
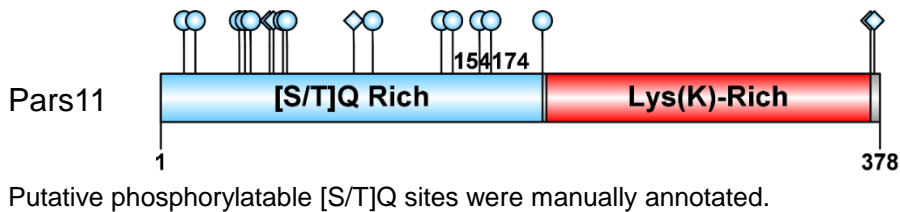
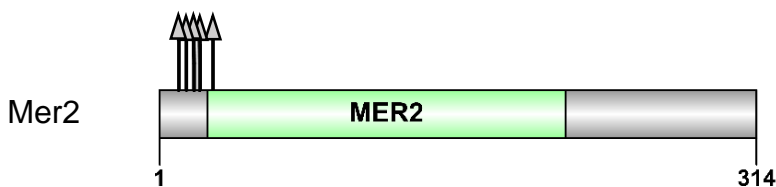


Figure S6

Structural features of *Tetrahymena* Pars11 and budding yeast RMM proteins and Hop1



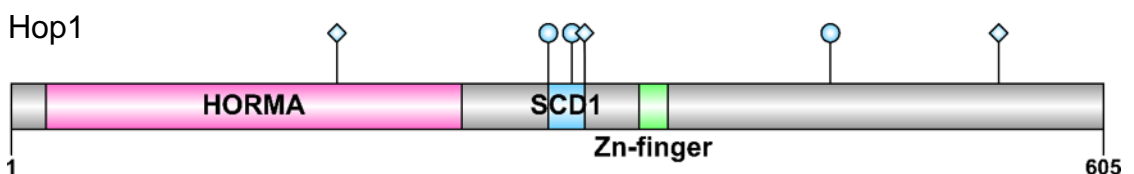
SCDs (S/T-Q cluster domains) are defined as regions where three or more SQ or TQ motifs are found within a tract of 100 residues or less (Traven and Heierhorst 2005, *BioEssays* 27: 397-407). SSMs, short signature sequence motifs (Tessé et al. 2017, *Genes Dev.* 31: 1880-1893). Phosphorylation sites (S/T-Q) annotated according to Carballo et al, 2013, *PLoS Genet.* 9, e1003545.



Cdc7-dependent Ser phosphorylation sites (Δ) according to Sasanuma et al. 2008, *Nucl. Acids Res.* 36: 984-997. Domain annotated according to the NCBI's Conserved Domain Database.



Domain annotated according to the NCBI's Conserved Domain Database.



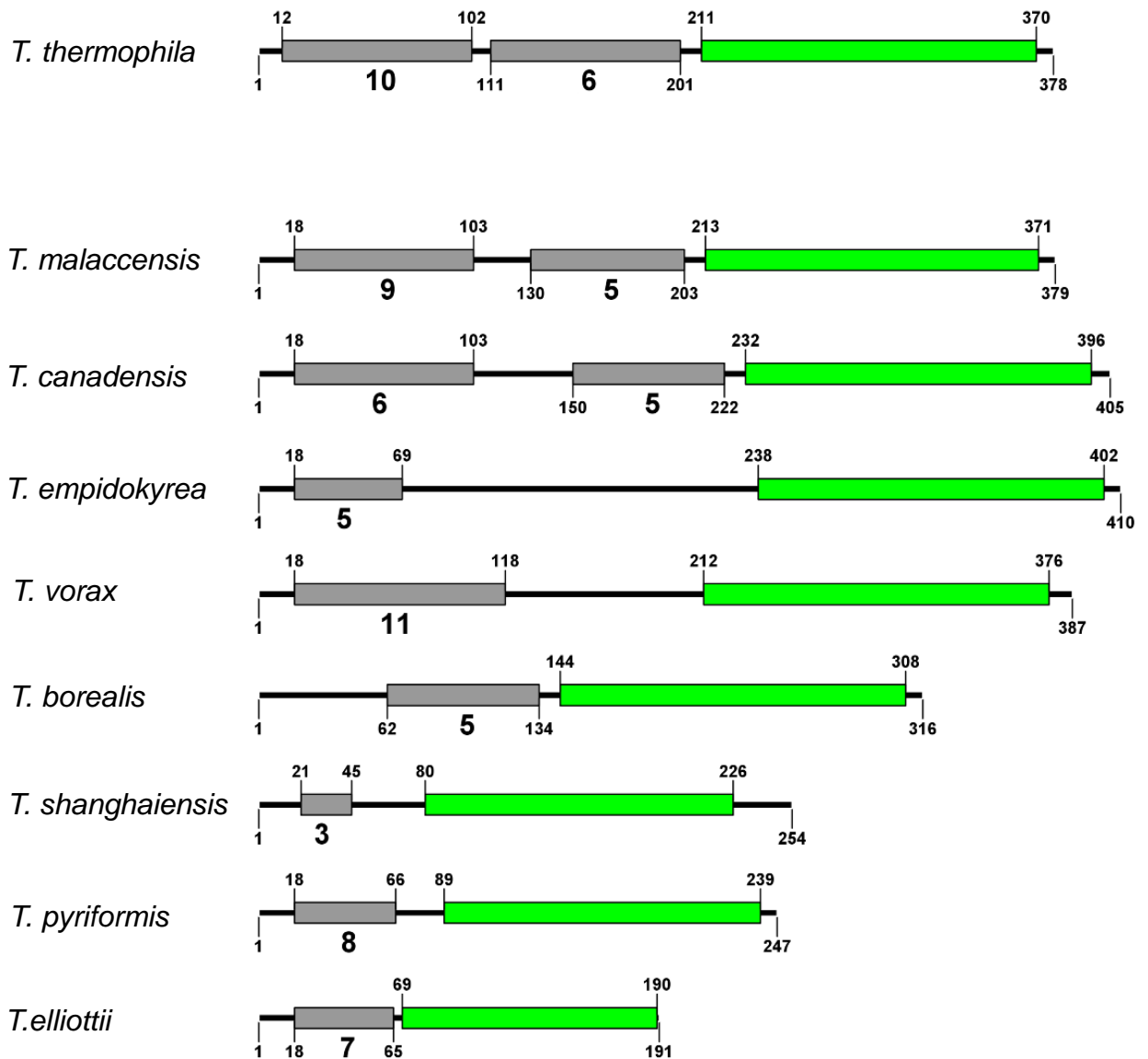
Phosphorylation sites (S/T-Q) were annotated according to Carballo et al. 2008, *Cell* 132: 758-770. The HORMA Domain was annotated according to the NCBI's Conserved Domain Database. The Zinc finger was annotated according to the Uniprot database. Domain annotated according to the NCBI's Conserved Domain Database.

Of the yeast RMM proteins, Rec114 most closely resembles Pars11 due to the presence of highly clustered S/T-Q motifs. No other similarities are found.

Diagrams were generated with the Illustrator for Biological Sequences (IBS) (Liu et al. 2015, *Bioinformatics* 31: 3359-3361).

Figure S7

Tetrahymena Pars11 alignments



The S/T-Q cluster domains are defined as regions where three or more SQ or TQ motifs are found within a tract of 100 residues or less. Gray bars indicate the N-terminal S/T-Q clusters, green bars the conserved Lys-rich C-terminus. Numbers under the S/T-Q clusters indicate the number of S/T-Q motifs. Diagrams were generated with the Illustrator for Biological sequences (Liu et al. 2015, Bioinformatics 31: 3359-3361).

Figure S7 continued

```

1      10      20      30      40      50      60      70
T.the/1-378 MSLKTYINKKK..SQNLG...SSQSSAALTSLQSNCF...AKGMV...SQNSQNSQN...TI...QM...QCNTQTQ...Q.....LTSQASQ...QFP...VPKNSSLMI
T.mal/1-379 MSLKTYINKKK..NQNLG...SSQSSAVLSSLQSNLSL...AKGMAA...QNSQNSQN...TIQ...QM...QSNTQTQ...Q.....ESTQTS...QFP...VLKNSTVMI
T.can/1-405 MSLKTFINKYK..NQNTK...SSQSSAASFSTISSTSF...APKGVV...PNSQNSQN...FSQ...TSTLFSQQCI...SFILYCTITLTF...TYFNL...TIVPSSAQKNQYTI
T.epi/1-410 MSLKNFIKGYK..QQSNK...SSQSSGAFSNI...STNSFAS...SKPQSQNNNN...SYHKV...VNSQQT...PA...SKPQSQNNNN...SYHKV...VNSQQT...PA...SFLSQNLPS...SQRNPSLQL
T.vor/1-387 MSLKTFINKYK..NQNQK...SSQSSAATFSSISANGFS...TKGQA...SSNSQNTSN...QFS...QKMNSQQT...TTQ...P...TYSQNAS...SQFSASNNN...
T.bor/1-316 KNQYTIIDEGRIASQNLN...KSYQV...NKQAS...TYSKSTSI...P...NSFLQDQ...P...TYEN...
T.sha/1-254 MSLQNFIQKYR..HSNNKQSS...SSQSS...TGS...FSNIS...NTFAS...QT...NQS...TQP...VQ...QQQQQQ...P...YFQKT...NNP...
T.pyr/1-247 MSLKTFINKYK..NQNMK...SSQSSGASFSQISTAGFS...GK...P...HG...SQNSQNSQS...PFQ...K...VNSQQT...P...VYSQSN...FL...
T.ell/1-191 MSLKTYINKKK..NQNLG...SSQSSAALFSSLQPN...NF...AKC...II...SQNSQSSQN...TL...QM...SSNTQTQ...Q...VLTQ...
consensus>60 mslktfink.k..nqn...ssqss.a.fs...n.f.k.q...qnsqn.qn...q...qtq...iy.q

```

```

80      90      100     110     120     130     140     150     160
T.the/1-379 EEPNIFGQTGYSSFKNKNLNST...QNTSFLNQLSQDEANDSY...NQILH...ENNFIQNP...LNQS...FLN...QSYQL...L.SQHNLK...QS...QL...FL...PA
T.mal/1-380 EEPSILGQNCCHSQYMNKNLNST...QNTSIFNQFPQDEANYSY...NQILN...ENNFSQIP...LNQS...YVNQ...QSYQL...L.TQHNLK...QS...QL...FL...PT
T.can/1-406 DEGRIASQNLNKSQVNVKQASTYNSKSTSI...PNSFLQDQPTYEN...QIENN...DSFFIQSQ...LNNS...AHMQ...NLNG...NLV...QNLN...QS...QL...FL...PT
T.epi/1-411 MQDEMKSPECFQSVSLKMAQPNPKQNNMNQTV...AEDINDTNTFNQLDSSNR...ESFYIQAPL...LNNS...GQTL...LSSFNNPYNQNNISNG...S...NLN...QS...QL...LQ...T
T.vor/1-388 .....NNNNSFRNNNNQPSSTYQ...TQ...SLSQ...FLQDDH...QDTQM...QS...QNDIK...ESFYIQNTS...LTNS...PNQY...QOYSNG...IYP...S...NLN...QS...QL...Y...LPT
T.bor/1-317 .....QIENN...DSFFIQSQ...LNNS...AHMQ...NLNG...NLV...QNLN...QS...QL...FL...PT
T.sha/1-255 .....NI...F...SR...QL...LV...P
T.pyr/1-248 .....NI...F...SR...QL...LV...P
T.ell/1-192 .....NI...F...SR...QL...LV...P
consensus>60 .....q.e.f.q...l.n...q...q.nl.qsq.l.lp.

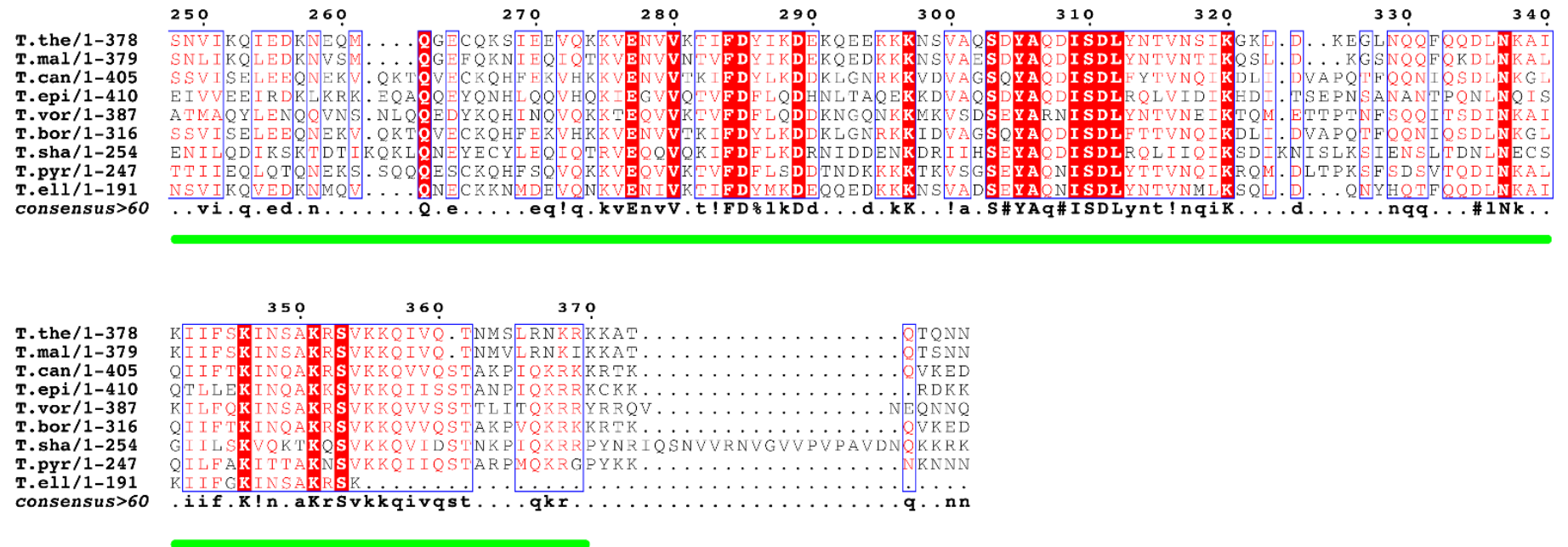
```

```

170     180     190     200     210     220     230     240
T.the/1-378 IQESKVSQFDGKSSQIFNPS...ESRLNHP...NLIQSSLMV...GQIS...QQQL...ANISL...QLLQDEIL...KQKIEQ...RE...RE...LI...LKQEL...LQE...MRDLKH...FFQ
T.mal/1-379 IQESKVSQFDGKSSQIFNPS...ESRLNNT...NLQSNLIV...GQIS...QQQL...ANFSL...QFLQDEIL...KQKIEQ...RE...RE...LI...LKQEL...LLE...MRDLKQ...FFQ
T.can/1-405 IQESKVTMFDGGQSQVYYHQ...EPRLIKP...NLQSNVYLS...QFS...QQQM...ATIQF...QILQDEIL...KQKVEQ...KER...ELL...LKQEL...LSE...MKDLKQ...YFK
T.epi/1-410 NMESSLG...YCGQMN...TQINPN...ESRLIRP...NLQSCIS...IGQLG...QH...SINGNYNCN...NYQNN...QYQ...QMLQEEM...KCRFEQ...QQR...ENH...LKI...EL...YE...MKKIK...TYFK
T.vor/1-387 IQESKVSQFDGGSQS...FFF...P...DSRI...MKP...SL...QSAI...ILG...Q...F...P...Q...SHV...SNTQF...QLIQEEL...KTKIEQ...KER...RF...Q...MKQEL...LSE...MKDLK...DYFR
T.bor/1-316 IQESKVTMFDGGSQS...VYYHQ...EPRLIKP...NLQSNVYLS...QFS...QQQM...ATIQF...QLLQDEIL...KQKVEQ...KER...ELL...LKQEL...LSE...MKDLK...QYFK
T.sha/1-254 TKNT...KQL...ANFQI...SILG...VEQ...EFL...SAT...RM...KEL...K...SYFK
T.pyr/1-247 .....KQL...ANFQI...SILG...VEQ...EFL...SAT...RM...KEL...K...SYFK
T.ell/1-191 .....KQL...ANFQI...SILG...VEQ...EFL...SAT...RM...KEL...K...SYFK
consensus>60 ..es...f...e.r...l.qs...q.q...q.lqde...k...eq.ere...lkqelL.e...MkdIK.%f.

```

Figure S7 continued



Orthologs of *T. thermophila* Pars11 proteins were identified by BLASTP or tBLASTn searches against proteomes or draft genomes of other *Tetrahymena* species in two databases (<http://ciliate.org> and <http://ciliate.ihb.ac.cn>). Protein sequences were aligned using MUSCLE (Edgar 2004, Nucl. Acids Res. 32: 1792-1797) and colored according to amino acid sequence similarity using Esript 3.0 (Robert and Gouet 2014, Nucl. Acids Res. 42 W1: W320-W324). Regions with >60% similarity are framed in blue. Identical residues are boxed in red, and similar residues are colored in red. The conserved C-terminal region was green underlined. The accession numbers of these proteins are: EIA_05075.1 (*Tetrahymena malaccensis*), TVORAX00043590 (*Tetrahymena vorax*), TSP00031430 (*Tetrahymena canadensis*), TEPIDO00188250 (*Tetrahymena empidokyrea*), TSHANG00212290 (*Tetrahymena shanghaiensis*), EI7_06775.1 (*Tetrahymena ellioti*). Pars11 coding genes in *Tetrahymena borealis* and *Tetrahymena pyriformis* were incorrectly annotated, hence we corrected their annotation manually according to their genome sequences.