

## **Supplementary information for**

### **A meiosis-specific Spt5 homolog involved in non-coding transcription**

Julita Gruchota, Cyril Denby Wilkes, Olivier Arnaiz, Linda Sperling  
and Jacek K. Nowak

**Supplementary Figures**

**Supplementary Tables**

**Supplementary References**

## Supplementary Figures

**A**

Pt_Spt5m	1	-----
Pt_Spt5v	1	-----
Tt_Spt5	1	-----
Ot_Spt5-g19	1	-----
Ot_Spt5-g12	1	MICNLIKLCFDIQIKQEEGAQPQQNQKIKSKKQQQPKDEDESSEQLKRRMRGKKRLSINDYLDVDEDQQA-----
At_SPT5-1	1	-----
At_SPT5-2	1	-----
At_KTF1	1	-----
Dm_Spt5	1	-----
Hs_Spt5	1	-----
Tb_Spt5	1	-----
Sc_Spt5	1	-----MSDNSDTNVSMQDHD-----QQFADPVVVPQSTDTKDENT
Pf_Spt5	1	-----

Pt_Spt5m	1	-----
Pt_Spt5v	1	-----MSQKNDDYRS
Tt_Spt5	1	-----MSDD-----FLEEDNNSYNY
Ot_Spt5-g19	1	-----
Ot_Spt5-g12	73	ADTNYARKGSKTLSKEFDKKLNTVDDKAPTQDSQLLKKRSKPQTSSTSELEQRVKKEYQRNQNRNDSADQMKMKENE-CNS
At_SPT5-1	1	-----MSQY-----S-----DDDYSHEDD-SEM
At_SPT5-2	1	-----MPRS-----RDEDELDGDYEALDL-EEE
At_KTF1	1	-----
Dm_Spt5	1	-----MSSEV-----SNMSDSGSEDGSISNKSQRSAR-SKS
Hs_Spt5	1	-----MSSED-----SNFSEED-----
Tb_Spt5	1	-----MS-----
Sc_Spt5	36	SDKDITVDSGNVTTTESTE-RAES-TSNIPPLDGEEKEAKSEPQQPDNAETAATEQVS--S-SNGPATDDAQTALN-TDS
Pf_Spt5	1	-----

Pt_Spt5m	1	-----
Pt_Spt5v	11	DQD-----DKSQSS-----ISIE-LPDSS-SEQKQSKSK-----
Tt_Spt5	17	DDE-----E-----DGSYGEVSNAKRK-----
Ot_Spt5-g19	1	-----MKKEVRYE-DHYDE-EDDVSY-EDYKNNKRS--KQTK--
Ot_Spt5-g12	152	DTDEGGIINTKSNAAKSQEAKQSSQLNKQPVK-----DENLKKRREA--KQDD--
At_SPT5-1	18	EDDE-----EDEYEPSSRKGRS--GKKRGRSNSD-----
At_SPT5-2	24	EEDE-----EEEEGRGGGS--RRKGRSNFIDDYA-EDSQ-EDD--DEEYGSSEG--
At_KTF1	1	-----MDR--KKGKQV-----AGSDSYGGQKRKNSVEF--
Dm_Spt5	32	RSRSRSGSRGSRVSRSRSRSQSGRSRSSESPPRRDNRGKSDS-GEE-EEPPGEI-I--TSEYDEEN-----
Hs_Spt5	15	-----SESSD-EEAEVDEER-SSAAGSE-KEE-PEDE-EE-EEFYDEEEE-----
Tb_Spt5	4	---ELGVKELA-----DLLADHEPM-----DLVVPSPDPSEVGSYDEVYKKEQQEREESRKHKR
Sc_Spt5	110	SEANIVKKEEGSDERKRPREEDTNSDC-----DTKDEGDNK-EDDD-EDDD-EDDD-EDD--
Pf_Spt5	1	-----

Pt_Spt5m	1	-----MKTTCQFIEQEAASEYSSEEDDDP-----KFEINRKE-----AERQ-
Pt_Spt5v	42	-----KLLKKQKNQKKTR-LNKQFIITEASESDNESDDN-S--VGEITKG-----
Tt_Spt5	35	-----G--KTLKQNKKKK-QKKNQFEETAEESNGEEEEVNQ--EKRLRNQ-----
Ot_Spt5-g19	35	-----KDKRSSKQKID-ARNFVEVEAAESDILSDLDL--DENEKIFRTQ-----Q----
Ot_Spt5-g12	201	-----KLQRLKQKQKQK-ASFEFEACEGDEDEEDD--EKPNRKIKESEFYTEEQLRVKTQRLDKNF
At_SPT5-1	47	-----SLGRGSKKKSS-GSAFIDWEVEVDVDDVDDDDVDVDELGKQQLKF-G-----DFSLCF
At_SPT5-2	75	-----GKGAASKKKKPS-ASIFLDREAHQVDDEDEEE-----D-----EAEDDF
At_KTF1	29	-----RDEGLRIKKKKNPE-VLQFFEESAIVYYGGSSDED--DDGL-----GF
Dm_Spt5	99	-----DHPRRKKKKKPR-FGGFIIIEAEVDDVDEDEDEW--EEGANEIGIVG-----NE
Hs_Spt5	60	-----EDDDRPPKKPR-HGGFIIIEAEVDDVDEDEDEW--EDGAEDILEKE-----EIEASN
Tb_Spt5	55	RKRSRHGAKSRIKHSGGKSDGVNPEVRSKFVIAAESGET-ESDDG-----F
Sc_Spt5	165	-----DEAPTKKRRR-QERNRFLDIEAEVSDDEDEDEDE--E--DSELVRE-----GF
Pf_Spt5	1	-----

		<b>Acidic_region</b>	
Pt_Spt5m	39	-LKE-----IQSRRAQAK-E-----R-----LNKRLOE-----LMEQD--	
Pt_Spt5v	85	-----KQEMYNELA-----LKKKHR-D	
Tt_Spt5	77	-----GDANLY-----RQKKAR-D	
Ot_Spt5-g19	80	-----HESYYTNDQ-----LRHQQG-I	
Ot_Spt5-g12	265	-LDNMMKRYDNVKSQGDEGEEDDYDDEDAGYYSQDEEDQQRHRRKEEKDQPLYNRDKR-DFIQKYQEAYEKHALKTH	
At_SPT5-1	99	IVSGEAD-----LPNEDSDHRRYYQRGF-----HPH-----E-E	
At_SPT5-2	114	IVDNGTD-----LPDERGDRR--YERRF-----LPRDND-E	
At_KTF1	70	-LNDMED-----EPEVEESSAGK-----GE-----KGS-SFV	
Dm_Spt5	145	-IDELGP-----TARDIEIRRRGT--N-----LWDTQKE-D	
Hs_Spt5	110	-IDNVV-----LDEDRSGARRLQ--N-----LWRDQRE-E	
Tb_Spt5	102	VVGSDED-----SESDVYGGPMA-----MEEGGRHIF	
Sc_Spt5	208	ITHGDD-----ED-DEAS--APGARRDDR-----LARQLDQ--	
Pf_Spt5	1	-----	

Pt_Spt5m	65	-----AID-----ETQN-----NSEYSSNVIQ-----
Pt_Spt5v	103	-----VVKQIEHYQDGDQDIQIGDD-----
Tt_Spt5	90	-----SEVIHLEQRGDQTPPMDD-----
Ot_Spt5-g19	98	-----NMNML-----QSMEE-----RYRDTVQDDNEFEDEDOEGLIGE-----
Ot_Spt5-g12	343	GREGLTGQSQLQNSRKARESSDPILKLIANKQKLVKVKVHQKQVKKEVKDENDADTDDQVVKMEVDE-----EQ-----
At_SPT5-1	128	-----DVD-----ELEKR-TLERLSTNY-----AKD-----
At_SPT5-2	143	-----DVE-----DLERR-QERFSSRH-----HE-----
At_KTF1	97	-----FPKEEDLNEEFDRIMEERYKPGSGFLR-----
Dm_Spt5	172	-----EIE-----EYLRKKYADESIAR-----HFG-----
Hs_Spt5	136	-----ELG-----EYMKKYAKSSVGET-----VYG-----
Tb_Spt5	130	-----LEGEEDMTAEFVARATEERYRSGRKKANRTEA-----
Sc_Spt5	236	-----D-----LNKTSFEAQRALAKELRERY-----GRSSSK-----
Pf_Spt5	1	-----

Pt_Spt5m	83	-----DDEDOINDDDDTLKGGPKNDPKWRFKCKGRSILEVQRLEISNLIP-----KNSHIVSVFVTPNVKGF
Pt_Spt5v	124	VDKS-----EIEIEKPGI-----RDPKFWRVSCNKGKEQEAITSMEKHNH-----LIDTNPEIISVFAIKKFPAA
Tt_Spt5	111	LDFDDID-MEHMPENIRKYGIQQRDQNIHSPVVKTKREEVVTCOLLNKIAQ-----LNPEQNYGIYSISYVPSVESL
Ot_Spt5-g19	132	-----YEGDDMIDKEKLPSVNDPFLWQVVRGRCERQATLQLMNKSIDF-----ARFGKHISILSVTCTEKVEGE
Ot_Spt5-g12	413	-----ARHSLTQQEVASHGQLPSVNDPDKLWKNINCWGMVQLVQOLLKKAEDF-----LNHEKPFMISINFCNDKTQCC
At_SPT5-1	148	-----DYELDVNDVDQALLPSVDPKLWVKCAIGREREVAVCLMOKIIVD-----FGSEPKIRSAIDLHKNY
At_SPT5-2	162	-----EY-DEEATEVEQALLPSVDPKLWVKCAIGREREVAVCLMOKIFID-----FGADLQIRSVVADLHKNF
At_KTF1	125	-----YADDIKDAEMDALAPTSNDPPIWVKVCAIGRERSVFCMLHKEVEL-----RKIGTKELISVFSVDHVKGF
Dm_Spt5	193	-----DGGHEMSDEITQQTLLPGLDPNLWVKCIGEEATATILMRKYITY-----LNTDDPQIKSILAPEVGVKY
Hs_Spt5	157	-----GSELSDDITQQQLLPGLDPNLWTVKCAIGEEATATILMRKYIAY-----QFTDTPQIKSVVAPPHVKGY
Tb_Spt5	162	LLSSGIPKGLSSLRVASHLLPQDTDPKVEAVKCPMAKRVLVARLVNKCYPFRIGRNYEYKVDIGTISVFCFHDVKEY
Sc_Spt5	263	-----QYRAAQDGYIPQRFLLPSVDATITWVFCAPGKEHEILIRKLKKKFNLD-----RAMGKKKIKLISIFORDNYTCR
Pf_Spt5	1	-----MAGKIEAVRVTHGQFETAKLITYSKVRT-----YNIPIYALAPSIVKGY

Pt_Spt5m	148	IFEFSEFFENDVKEFMRSIM--YG--QPIYIQPEOCDSLLEKK-N--NNQVQGVVRFKHNKNY--GKDLKVIKRVN
Pt_Spt5v	186	IFIEANFEQHVMAIEGTTIVRQ--QPPELVESEQCPNIFKPA--EVEQDIEEGQWVRVQHNLY--SGDLARVQVD
Tt_Spt5	183	IYIEGVNKRAVINFIDKYPDCNC--NKIELVNIIEFOAFEDQGRQESYNAEVGQVVRSEKARNSDYINDIGQHFUK
Ot_Spt5-g19	198	IYIEADKEHIVKEAIVGILSVILCGK--CILLIQKEEMPGLYQNDK-QT--INLQHOWVRPQGLIY--GGDLGLVEA--
Ot_Spt5-g12	482	IYIEAMNMSHVKSIQGMTGIYK--QKIDMIPYKEMTQLLKVCES--BINETTLOAHQWVRVKNKNG-PY--AGDLGFVEMIE
At_SPT5-1	214	IYIEADMEAHVKEAIGVRNIYA-NQKILLVPIKEMTAVLSVES-KA--DLSRDSWVRVRLKLG-IY--KGDLAQVVDVD
At_SPT5-2	227	IYIEADKEAHVKEAIGVRNIYA-NQKILLVPIEMTDVLSVES-KA--DLSRDTWVRVRLKIG-TY--KGDLAQVVDVD
At_KTF1	194	IYIEADKEHIVKEAIVGILSVILCGIYA--TRMVLIPKAEIPNLLITVQK-KT--KKVSEGTWVRVKNKNG-KY--KGDLAQVAVS
Dm_Spt5	262	IYIEADKEHIVKEAIVGILSVILCGIYA--TRMVLIPKAEIPNLLITVQK-KT--KKVSEGTWVRVKNKNG-KY--KGDLAQVAVS
Hs_Spt5	225	IYIEADKEHIVKEAIVGILSVILCGIYA--TRMVLIPKAEIPNLLITVQK-KT--KKVSEGTWVRVKNKNG-KY--KGDLAQVAVS
Tb_Spt5	242	IYIEADKEHIVKEAIVGILSVILCGIYA--TRMVLIPKAEIPNLLITVQK-KT--KKVSEGTWVRVKNKNG-KY--KGDLAQVAVS
Sc_Spt5	335	IYIEADKEHIVKEAIVGILSVILCGIYA--TRMVLIPKAEIPNLLITVQK-KT--KKVSEGTWVRVKNKNG-KY--KGDLAQVAVS
Pf_Spt5	46	IFVEAPNKGVVDEAIGIRHARG--VLPGEVVPFKEIEHFIEEKP-AV--SGLEPGDLVEITAG-PA--KQOKAKVVKID

Pt_Spt5m	216	LIONFAIKVIKRNKQ-----GQKEPIS-----WORTR-----
Pt_Spt5v	259	QEBKLKIKVPRQKLLMKSLEEENKKNKSKNNANADQSIFQMKEEEGEPIEDNEPFORGSKFKFLKRAKYLQEQR
Tt_Spt5	261	KN-NRVVVKLIPRIE--DMRKKYEQVKNNSKKR--NQDSFVQVEGDAE--DDQ-ANKKKGFPYN-----
Ot_Spt5-g19	267	TVDDIVYLLIPRIE--DSKQDQ-----LNGKGRDD-----KAKN-----
Ot_Spt5-g12	555	G-GDRAIVKLIPIRVTQNELGONVLELY-----HK--A-----WOKKE-----
At_SPT5-1	286	NVRKRVTVKLIPIRIE--DLQALAN-----KLEGTE-----NVKR-----
At_SPT5-2	299	NVRKRVTVKLIPIRIE--DLQALAS-----KLDGEE-----VSKK-----
At_KTF1	265	DTNNALIKLIPIRIE--DIQALTO-----KYGGGV-----
Dm_Spt5	335	LAQNOVHKLIPRIE--DYTRVRG-----ALRTTATES-----DDSKR-----
Hs_Spt5	298	PSQNTISIKLIPIRIE--DYDRIKA-----RSLKQ-----WFAKR-----
Tb_Spt5	313	SASREVTIVKIVPRE--DIVGKT-----
Sc_Spt5	407	ENNLEVMKIVPRIE--DYGKFD-----EIDPTT-----QQRKS-----
Pf_Spt5	117	ESDEVVVQFIDAVPIPVTIKGVYRLIS--KLQKEE-----

Pt_Spt5m	244	-----QQTNQIETTNOSEFIYSDEKEY-----KCAKIDGFNLLRCPLKNIEH-----
Pt_Spt5v	339	KYIRGPKIPIQMTKKSINDNDNETSQFFY-TTIDEWTSAKK-----DGEEIILPVHQVLTG-----
Tt_Spt5	319	KFGNQPVVPTRTVDEIL-----KTRPEQOFFSLDEYEKDWSECYKAV--SEE-YRPNESTNIFQHCQVYKIFNCKDLIFN
Ot_Spt5-g19	300	-----FSNQRPBORIFNKSLVQYS--LEHRQIPALNKTFIVYKKQYL-KNGFLYKSEPSKQLOID
Ot_Spt5-g12	591	-----FGGVAGQNVQORLFPQNVNNECT--K-DREFEPLQ-KNFFIWKEQMF-RNGFLYIFKINKLID
At_SPT5-1	317	-----KAF-APPRFMNIDEARELHIRVEHRRDPMTG-DYFENIGGMF-KDGFHYKQVSLKSTIVQ
At_SPT5-2	330	-----KAF-VPPRFRMIDEARELHIRVEHRRDPMTG-DYFENIGGMF-KDGFHYKQVSLKSTIVQ
At_KTF1	292	-----TVQKGQTPARLISSEIEEFRPLIQVRDRDTC-ITFEHLDSLIL-KDGFHYKQVSLKSTIVQ
Dm_Spt5	370	-----KKKRRPAAFPDPEAVRAIG--GEVHSDG-DFLLEFECNRYR-RKGFHYKQVSLKSTIVQ
Hs_Spt5	330	-----KKFKRPPQRLDAEKIRSLG--GDVASDG-DFLIFECNRYR-RKGFHYKQVSLKSTIVQ
Tb_Spt5	333	-----CNKPEVRMPQRLVFNLAAGAHN--R--GEMYAWGDLFDGEGHLSVLSQVISG
Sc_Spt5	438	-----RRPTFAHRAFPQLFNPTMALRQDQ--ANLYKRD-DRHFTYKNEDV-IDGFLYKQVSLKSTIVQ
Pf_Spt5		-----

**KOW 2**

Pt Spt5m 287 --NITITDEELQMFDPDVDRKI--VQ-----Q-AKREILRRVDVQFKEGQKVRITGEDDINKGPKFKIMHIFD  
Pt Spt5v 397 --NIKPTTEILQYFYPDVQDYRLQLQK-----LHSSLKQVVEQKSQIQGDIYITLTHDQAK--SSRYKVSQILQ  
Tt Spt5 393 --EQLKSIIEELKKFYPHLELDIINQN-----G-RFLKMANNQTTFFVGGDPVLIDNTLK-----GKIISLIGK  
Ot Spt5-g19 359 --NVPPTTEEVQQTSYMKSSK-DDINQI--DEITGEELIRKTFMGSSSDINKGDKIRVVKGDIN--GLYGGQVVTIEG  
Ot Spt5-g12 651 --KYBPRAEWKRFQKDQTHSTDYLSDDQDEWDMDDATVMKTIKNDGLQOLEVGDREVEVINKNQK--GKGTILKIDN  
At SPT5-1 376 --NVTPTTELEERFKRPNENGE-IDFV--DES-----TLFANRKKGHFMKGDAVIVIKGDLK--NLKGWIEKVD  
At SPT5-2 389 --NVTPTTELEERFKRPNENGE-IDFV--GLS-----TLFANRKKGHFMKGDAVIVIKGDLK--NLKGWIEKVD  
At KTF1 354 --GVPTPKDELLEKTPVDRKET-GDVEWI--SEIYGEERKKKI-----KDD-PTMAHSFSMGDNVEVCVGDLK--NQKIKIADIG  
Dm Spt5 425 --GVPTTELEERFEESPEVN-LEIM--GTV-----T-G-KEREHNFQPGDNVEVCCEGLI--NQKIKISVDG  
Hs Spt5 385 --GVPTTELEERFEESPEVN-LEIM--TES-----T-G-KEREHNFQPGDNVEVCCEGLI--NQKIKISVDG  
Tb Spt5 386 VKMEKPTVEELAAEFSSDLNRV-R-----EAASHFASNGRGSAGLRGDMVRVWVGQLK--ETVGTIENIFL  
Sc Spt5 497 --NQPTTEELAREGSKGAVDLTSVS--QS-----IKKAAAKVTEFQPGDRFEVINGEQK--GSKGIWTETTK  
Pf Spt5 -----

**KOW 3**

Pt Spt5m 352 DQM-I-E-LM----CKKNREYTYLVHASEIRLAKKLYQEAQVIDGPHKGIVGVIIICIKQGVVLSNQHGTFKVPHSVL-  
Pt Spt5v 462 DENKLIISTVKNNKNNKRNNEYKDISEAKLAFKLYQQVSIIVSGPNTGLSGTILKMDLTAQISSEA---GRIDALI  
Tt Spt5 452 ETAKVLIKK--KK---ISIEEYPIKQIVKYFEDGSRVKVISCITSEGITGTIVISTKGVDCEVITDN---NNTLEVRT  
Ot Spt5-g19 432 SDV----LFG----PTIEGFEDDLRPMDFVVKHFEPPGQVRFVIDGKFKGTGIVISSETQFANVALTQ--NNEIRIFA  
Ot Spt5-g12 727 EEF----ILT---KTLDKPYELKKQSEVIKYFECGSESVRVINGTHSGESGITATAEKHAVVLMGATTSENKILL  
At SPT5-1 439 ENV----LIR---SEMKDLPNPLAVNGRELCKYFEPGNGVVKVSGIHEGSGTGMIVKVDQHMILISDT--TKEHGVFA  
At SPT5-2 452 ENV----LIR---SEVKGLPDPPLANERELCKYFEPGNGVVKVSGIHEGSGTGMIVKVDQHMILISDT--TKEHGVFA  
At KTF1 392 -----L---PT-----CR--EGCKGEGSGGCKGEGSGGCKG--EGSR-----G  
Dm Spt5 487 TMT----TVM---PKHQDLKDPILFKASELRKYKTKGDHARVLACRYEGTGLIRVEPTRVVIVSDL--TNFEVPLP  
Hs Spt5 446 NKI----TLM---PKHDLKDMLEFPAPQLRKYFKMGQHVKVIAGRFGTGLIIVRVENFVILISDL--TMHELVPL  
Tb Spt5 450 DTNTVALSCR---VPRGETIKLRVETPLCVKHEFTEGTHVVIDGVHAGESGTVVVKGVDVHVEPSDRATRELVVRA  
Sc Spt5 560 DIA-----T---IKINGFTTPEFPLSLRKIEFPGDHVTIVINGEHQGAAGLVIMVEQGQVTFMSTQ--TSRETTITA  
Pf Spt5 -----

Pt Spt5m 424 ---QFGHKNFQ-----TDNSLVKFG-----NNDFOIGCVIQQKL--QSAVV  
Pt Spt5v 539 SDLQSQKNVIKKVDIENPDAGAGQKPGKRNLDLVKFG-----LIDNDIGGILN--SN--NEVSV  
Tt Spt5 521 KDLAIIGEAS--QDAEKIVVNESSDRNLGLKKDLVKLT-----GF-NNVGLILD--SK--DYIKI  
Ot Spt5-g19 502 NSLKLKSEIDQCVLGTGY-IDKKN-FSKYSANDLIMYN-----TKYVGVIQVQD--DYIKV  
Ot Spt5-g12 799 SNLKSKEDEMEHV-KRDY-IQKSVIEIKNAGEMIMYQSTHCSQLSTSSSSCVNASSTVSGSICGIIQNP--DYIKV  
At SPT5-1 509 DHVAKSAEVTKGVTKI-----GDYELHDLVILS-----DFSFGVILKLS--FAIQI  
At SPT5-2 510 DHVSESSEVTGVTKI-----GDYELHDLVILS-----NLSFGVILRLN--FAFQV  
At KTF1 423 ---GKG---EGS---SDF---KSESSYELYNLVCFS-----RKDFGLIVGVEDKDGQYKV  
Dm Spt5 557 RDLQLCSVATGVDCI-----GQVQWGDVQLD-----SQNVGVIVRLR--BNFHV  
Hs Spt5 516 RDLQLCSVATGVDCI-----GQVQWGDVQLD-----PQTVGVIVRLR--BTFOV  
Tb Spt5 526 DDCHRSNLVGSF-----GHTSGSKLFDLVMLPD-----SSSVLCVVRINR--NDVCV  
Sc Spt5 628 NNLSKSID-----TTATSSSEVALHDLVELS-----AKNVLCIIQAGH--DIFKV  
Pf Spt5 -----

**KOW 4**

Pt Spt5m 460 LDINNEVQEIRNEKE-----ILTINGVEINEQGETFRNDNVIIIT---SGQY-ANKFGLVKKHCINGKLILNHLF--  
Pt Spt5v 597 LDLTNQIKNNKLALR---NSINIRNNVKNMYGNDIRQODLVVIL---LGFY-KNNKATVLLHYEDYLFENG-KFDN  
Tt Spt5 576 LDQNGKIKNVSSFSIN---TKIDIRKYICKNAEGNNTNNSNIILK---QGQY-SGYQCOVILHYKSLFLNRP-KFRD  
Ot Spt5-g19 556 INNREGDILQNLKLSIN--KKIDFRKRTTTVDSHRNTLYADNVVRIT---NGKY-KGKKGQVVKYITIKITLFLMDK-EFYQ  
Ot Spt5-g12 875 INSHNQVMVKQASIT--KKIFPRNTFAIDRNHNTIASISIVAIY---DGSQNLGKCEVKGFGKQDLVFLYKGLQVLQ  
At SPT5-1 554 LKGVDPSSSEVSIVKAS-EIKYIKWIKINVQDRYKNNVAVDVVRVI---EGPS-KGKQGPVVQYKGVLFTHDR--NLE  
At SPT5-2 555 LKGVDPDRPEVALVKLR-EIKCKLEKINQVDRYKNNVAVDVVRVI---EGPS-KGKQGPVKKHLYKGVLFTHDR--NLE  
At KTF1 466 LKEGIDGPVVVTVGKREMONGPFDSKFTAILDLNKKQISNDVVRIS---KGPS-BGKQGPVVQYKGVLFTHDR--NLE  
Dm Spt5 602 LGMNKCKIECKPTALH---KRKENRHTVAIDADQNOIRRDVVKVM---EGPH-AGRSGETKHLIYRSIAFLHCR-MYTE  
Hs Spt5 561 LNMVGVVTVVRHQAVT---KKKDNFAVAIDSEQNNIHVDLVKVI---EGPH-SGREGETRHLFRSFAFLHCK--NLE  
Tb Spt5 572 LTDRMTRYSTTQIK---PVLGFRQTTDQLANMITRGSEVITQNDSSPYHLDGOTGRVEQVENITLFRVRK-TVKE  
Sc Spt5 670 IDETGKVSITTKGSILSK-INTARAVSSVDANGNEIKKGTIVEK---VG---SRREGQVLYQTQQFVVS--NIVE  
Pf Spt5 -----

Pt Spt5m 527 PYQIIENANNCKLVFSKQN---KP-----DEGANT-----ISQMPN-----KQN  
Pt Spt5v 668 TQGVIIERANNCGLVSSSKP-----DEGANT-----ISQMPN-----E  
Tt Spt5 647 TYTVE--NINNVSLQTTNPVIQR--SEA---KIAYDMKYGK-----QDNLKP-----D  
Ot Spt5-g19 628 SNGIFVENARNVVLGGDELM-KQNGGA-----VANSNRR  
Ot Spt5-g12 949 TNGIYSILTRNVLAGQDFV-KNVQENHKGFI-----GQQDRKQ  
At SPT5-1 627 HTGFICTCSSCLAGG---NFRTP-----ALVPPSPRRFQRADMGYNPGAGGRHQGGRGR  
At SPT5-2 628 HAFICAKCTSCIVVGGSSS-GANRNGDLSRYGNFKAP-----APVPPSPGRFQGRGGGYNSSGGRHGGGRG-R  
At KTF1 540 NGGYFCCKSQSCQKVKLFTEESNEKTCGFDGTAFEDFVSSPK-----SPVSPKEWQPRERYNS--SNQGDI  
Dm Spt5 673 NGGFVCKTRHLQLAGGSIT-TVSLAGIVGGLGFMSPRIQ-----SPVHPSGGR-----GARGGARGGRGGRV-T  
Hs Spt5 632 NGGFVCKTRHLQLAGGSIP-RDVTNFTVGGFAPMSPRIS-----SPVHPSAGG-QRGGFGSPGGSGGMSRGRG-R  
Tb Spt5 647 NSGTVAVDASCVLGGRTTTKQIPAKQLPTVNRNPHASRADLSVNPRTSEDW-----A  
Sc Spt5 741 NAGVFNPNPSNVEAVASKDN---MLSNKMDLSKMNBEIISK-----MGFPSSSKTFQ-----PIQSRG  
Pf Spt5 -----

**KOW 5**

Pt Spt5m 552 DQMGLTGGICQLKIGQMGQYRGQIVQIKSGYLIVQISANNTKVKVSEKDILLI-----  
Pt Spt5v 702 DWKNLRGQMVITIRKQMQSYRGMVQEVTSRVATQLSAKNIVVKVPL-ECIKSESSNSH-----LQVGKTPQHHPGM  
Tt Spt5 688 DMKQYVGRQMRIIGGKYKGLQCTVTDIRNDQIKVEINSKFTTVFIPKCDIATDKGQTE-----LEYGKTPSYNAQS  
Ot Spt5-g19 662 FRDVGAKKEEITKGEKMGYRGVRCRADRQAIVELSSKCRKIPLEISLKEVDVGKSGTTRGD---MTYGGQT---VY  
Ot Spt5-g12 989 KDRKILRSYVAITAGEYKGLKGRVIFADELICKVELLAKDTKVKQIPRGMYME--IRDPTKPMEI---RDLGVVEF---MS  
At SPT5-1 681 GDDHLVGTYYKIRLGPFGKYSGRLEVEVKIKLVRVELEAKIVTGKHFERKAISDM-----TDN-----  
At SPT5-2 698 GDDSLLGTTTVKIRLGPFGKYRGVVEVKGNSVEVELEMKIVTVD---RGAISDNVA-----TTF-----  
At KTF1 605 GSTYSIGQKLRIRVGPLKGYLCRVIALRYSDVTVKLDSSQHKIFTVKSEHIAEVRDRN--TVLST-----SGDAG-----  
Dm Spt5 737 RDRELLGKTKIKISGGPYKGAVGIVKDATESTARVELHTSCQTISSVDNRHIAIVGVGTGKEG--SV---STYGTPPARTPGY  
Hs Spt5 701 RDNEELIGQTVRISQGPYKGYIGVVKDATESTARVELHSTCQTISSVDNRITTVGSRPPGG--MT---STYGTP---MY  
Tb Spt5 705 GNSEWYEMDTE-----  
Sc Spt5 796 GREVALGKTVIRSGGYKQQLGIVKDVNGDKATVELHSKNKHITDKHKITYYNREGGEGITYDELVNRRGIVE-----  
Pf Spt5 -----

Pt Spt5m -----  
Pt Spt5v 773 S--TR-----VWDDIEGVQQSAMRGGYQSPYITYQTPEMND-----  
Tt Spt5 760 IYQ-----PNNHENINSPSY-YAN--N-----  
Ot Spt5-g19 735 E---AGKTPMQYNTESYYPHSFHWGA-NQSPGYGTDYDYNNGMSFGFSRAGSEHYENKEPOTPRNPNWV--KHE-----  
Ot Spt5-g12 1060 FDD-AQKRDMGQEDD-----EMMIDTTAR-GQNEMLQONFANQLKYDCFGGEAQIATP  
At SPT5-1 739 VVAEPQYN-MGSQTPMHPSTRPLHPC-MTPMRHSGATPIIDGMRTPMRGR--AWNPEYPMSPPRDNWE-----  
At SPT5-2 754 FRDTSRYS-MGSETPMHPSTRPLHPY-MTPMRDSGATPIIDGMRTPMRDR--AWNPEYPMSPPRDNWE-----  
At KTF1 672 ---TGSFQPFQM---LGT-----ESSTGDWAGAGTSSEGGNWNIGGSTDSHSLNIERN---MVQL  
Dm Spt5 812 GAQTPSYTAAGSKTPLVGSQLENWDT-DTRTPYGTMTFSDGSMTPR-HG---AWDETANTTPARNN-D--FDYSLE-EP  
Hs Spt5 772 GSQTPMYG-SGSRTPMYGSQLENLQDG-SRTPHYGSQTPLEDGSRTPAQSG---AWDPNNENTPSAEEE--YEYAFDDEP  
Tb Spt5 -----  
Sc Spt5 870 -----QAR-MG---PSYVSAPRNMATGGIAAGAAATSSGLSGGMPFGWSSFDGC-----KTPAVNAHG-----GSG  
Pf Spt5 -----

Pt Spt5m -----  
Pt Spt5v -----  
Tt Spt5 -----  
Ot Spt5-g19 -----  
Ot Spt5-g12 1112 RGSDDKDWGDDQNLGGLHREGRDTQISQNFR-----DCDSQFDLDIAAD-----  
At SPT5-1 803 DGNPGSWGTSF-----YEAATPGSDMGSSSTPGRSSYRDAGTPINNG  
At SPT5-2 818 DGNPGSWGTSF-----QYQPGSPPSR-----AYEAPTPGSGVASTPG--GSYSDAGTPRHDG  
At KTF1 726 CREKNPWGSKPTSD-----VSPTVADD--NTSAWANAANAENKPASASDQPGGNPWGKTP--ASEAGTVSGWG  
Dm Spt5 883 SPSPG-----YNPSTPGY-----QMTSQFAPQTPGTL--YGSDRSYSP-----PNPSPSP-----  
Hs Spt5 845 TPSPQAYGGTPNPQTPGYDPDSS-PQVNPQYNPQTPGTAMPYNTD-QFSPYAAPSPQGSQPSPSQ-SYHQV-APSPAG  
Tb Spt5 -----  
Sc Spt5 927 GGGVSSWGGAS-----TWG  
Pf Spt5 -----

Pt Spt5m -----  
Pt Spt5v -----  
Tt Spt5 -----  
Ot Spt5-g19 -----  
Ot Spt5-g12 -----  
At SPT5-1 844 FVYYLL---CLNANA---PSPMTPSSTSYPSTPGGQA-----MTPGTDLDVMSLDIVNIF---QFTDYVSL-----  
At SPT5-2 868 SAYANAPSPYLPSTP---GQPMTPSSASYLPSTPGGQP-----MTPGTGLDVMSPVIGGDAEAWFMPDILVDIHKAGE  
At KTF1 791 --DT-----SASNVEASSWEKQ--GA-----STSNVADLGSWGTHGGSSGGNKQD-  
Dm Spt5 926 -----APSPYPVGYM--NTPSPSTYSPNTPGGIPQSPYNPQTPCASLD-----SSMGDWCTTDIEVRIHT-HD  
Hs Spt5 921 YQNTHPASYPHTPSPMAYQASPSPPVGYSPMPGAPSPGGYNPHTPESGIE-----QNSSDWVTTDIQVKVRDTYL  
Tb Spt5 -----  
Sc Spt5 941 -----  
Pf Spt5 -----

Pt Spt5m -----  
Pt Spt5v -----  
Tt Spt5 -----  
Ot Spt5-g19 -----  
Ot Spt5-g12 -----  
At SPT5-1 902 -----FFLCGHHQDGSVVALGHRGEGE-----TIRATQNKVSLVCPKKE-----RVKILGGK  
At SPT5-2 938 DTD---VGVIDRVDGTCVKVSLGSSGEGD-----TIMALPSELEIIPPRKSD-----RVKIVGGQ  
At KTF1 832 -----EDSVWGKLCASESSQKKESSWGKKGSDGESSWGNKDGNSASKKDGVSWGQDKGSDESKGGSAWSN  
Dm Spt5 986 DTDLVGQTGIIRTVSNGVSVFLRQEDRSV-----SIVSEHLAPVLPNGD-----EFKIYGD  
Hs Spt5 994 DTQVVGQTGVIIRSVTGGMCVSYLKDSEKVV-----SISSEHLEPIPTPKNN-----KVKVILGE  
Tb Spt5 -----  
Sc Spt5 941 -----GQGNGGA-----  
Pf Spt5 -----

```

Pt_Spt5m -----
Pt_Spt5v -----
Tt_Spt5 -----
Ot_Spt5-g19 -----
Ot_Spt5-g12 -----
At_SPT5-1 951 YCGSTAKVIGEDGQDGIVLDESLDIKILKTILAKLVHE-----
At_SPT5-2 990 YRGSTGKLLIGIDGSDGIVLIDNLDVKILDLALLAKFVQP-----
At_KTF1 902 QCGDFGSGKKKDGSSGWNKSAEDSNANSKGW---PDWGQPNDGSSWGKKGDGAASWGKKDDGG--SWG-----KKD---
Dm_Spt5 1040 DRESVGRVLSKDGDFVFCR--INEEIKLLPINFCLKMKSID-----
Hs_Spt5 1048 DREATGVLLSIDGEDGIVLMDLDEQLKILNIRFLGKLLEA-----
Tb_Spt5 -----
Sc_Spt5 948 -----SAWGGAGGGASAWGGQGTGATSTWGGASAWGNKSSWG
Pf_Spt5 -----

```

```

Pt_Spt5m -----
Pt_Spt5v -----
Tt_Spt5 -----
Ot_Spt5-g19 -----
Ot_Spt5-g12 -----
At_SPT5-1 -----
At_SPT5-2 -----
At_KTF1 968 -----DGNKDDGGSSWGKKDDGQKDDGGSSWEKKFDGGSSWGKKDDGGSSWGKKDDGGSLWGKKDDGGSSWGKEDDGG
Dm_Spt5 -----
Hs_Spt5 -----
Tb_Spt5 -----
Sc_Spt5 985 GASTWASGGESNGAMSTWGGTGDR-----SAYGGASTWGGNNNN-----
Pf_Spt5 -----

```

```

Pt_Spt5m -----
Pt_Spt5v -----
Tt_Spt5 -----
Ot_Spt5-g19 -----
Ot_Spt5-g12 -----
At_SPT5-1 -----
At_SPT5-2 -----
At_KTF1 1041 SLWGKKDDGESSWGKKDDGESSWGKKDDGGSSWGKKDEGGYSEQTFDRGGRGFGGRRGGRRGGRDQFGRGSSFNGSEDP
Dm_Spt5 -----
Hs_Spt5 -----
Tb_Spt5 -----
Sc_Spt5 1024 -----
Pf_Spt5 -----

```

```

Pt_Spt5m -----
Pt_Spt5v -----
Tt_Spt5 -----
Ot_Spt5-g19 -----
Ot_Spt5-g12 -----
At_SPT5-1 -----
At_SPT5-2 -----
At_KTF1 1121 APWSKPSGGSSWGKQDGDGGGSSWGKENDAGGGSSWGKQDNGVGSSWGKQNDG--SGGGSSWGKQNDAGGGSSWGKQDSG
Dm_Spt5 -----
Hs_Spt5 -----
Tb_Spt5 -----
Sc_Spt5 1024 -----KSTRDGGASAWGNQDDGNRSAWNNQGNKSNYGGNSTWGGH-----
Pf_Spt5 -----

```

```

Pt_Spt5m -----
Pt_Spt5v -----
Tt_Spt5 -----
Ot_Spt5-g19 -----
Ot_Spt5-g12 -----
At_SPT5-1 -----
At_SPT5-2 -----
At_KTF1 1199 GDGSSWGKQDGGGDSGSAGWKQNNNTSGGSSWGKQSDAGGGSSWGKQDGGGGSSWGKQDGGGGSGSAGWKQNETSNGSSW
Dm_Spt5 -----
Hs_Spt5 -----
Tb_Spt5 -----
Sc_Spt5 -----
Pf_Spt5 -----

```

Pt_Spt5m	-----
Pt_Spt5v	-----
Tt_Spt5	-----
Ot_Spt5-g19	-----
Ot_Spt5-g12	-----
At_SPT5-1	-----
At_SPT5-2	-----
At_KTF1	1279 GKQNDSGGGSSWGKQDGGGGSSWGKQNDGGGGSSWGKQGDGGSKPWNEHSGGGRGFGERRGGGGFRGGRNQSGRGGRSF
Dm_Spt5	-----
Hs_Spt5	-----
Tb_Spt5	-----
Sc_Spt5	-----
Pf_Spt5	-----

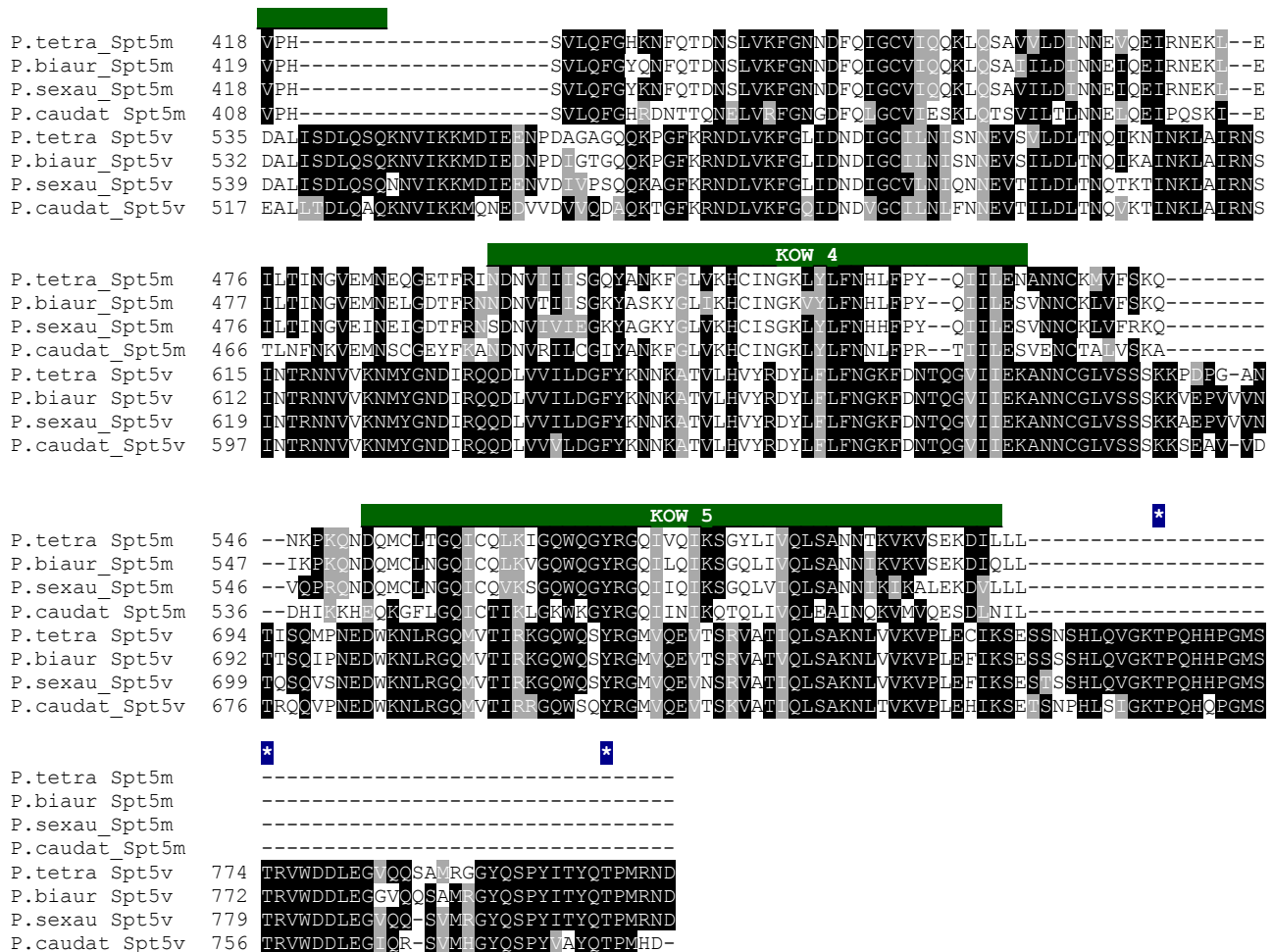
Pt_Spt5m	-----
Pt_Spt5v	-----
Tt_Spt5	-----
Ot_Spt5-g19	-----
Ot_Spt5-g12	-----
At_SPT5-1	-----
At_SPT5-2	-----
At_KTF1	1359 DGGRSSWKTDNQENTWKSDQSGGSDWKKGWGEDSNNSKPSGSSAGGCAGNWPSWDTNSKKETNDKPGDDSKSAWGTSND
Dm_Spt5	-----
Hs_Spt5	-----
Tb_Spt5	-----
Sc_Spt5	-----
Pf_Spt5	-----

Pt_Spt5m	-----
Pt_Spt5v	-----
Tt_Spt5	-----
Ot_Spt5-g19	-----
Ot_Spt5-g12	-----
At_SPT5-1	-----
At_SPT5-2	-----
At_KTF1	1439 QVNTDNNNDSWNKKPNNDVGTSGEADNAWGGKTNAVAPSPSGSAAWGTGDKKKTGW
Dm_Spt5	-----
Hs_Spt5	-----
Tb_Spt5	-----
Sc_Spt5	-----
Pf_Spt5	-----

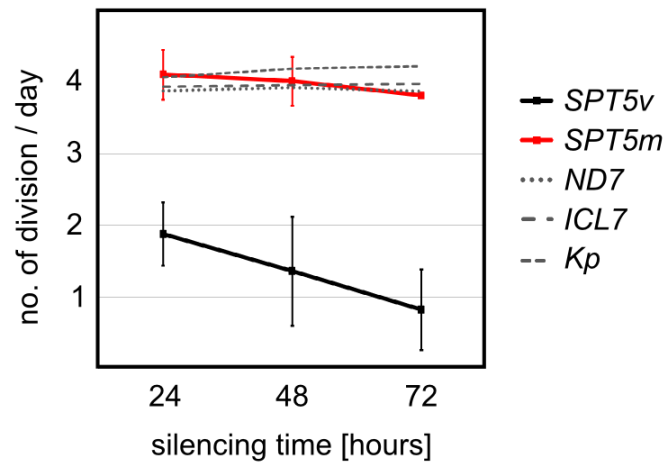
# B

P.tetra_Spt5m	1	-----MRKRTKQCFIEQEASEGYSSEE-DDDPKF
P.biaur_Spt5m	1	-----MRKRTKQCFIEQEASEGYSSEE-DDDPKF
P.sexau_Spt5m	1	-----MRKRTKQCFIEQEASEGYSSEE-DDDPKF
P.caudat_Spt5m	1	-----MRKKMRNMFIEDQASEGYSSQEEYEEQIQ
P.tetra_Spt5v	1	MSQKNDDYRSDDDKSQSSIDSDIEDPDSSESEDFKQSKSKLLKKQKNQKKTFLNKQKFIDTEASETSDNESIDN----
P.biaur_Spt5v	1	MSAKNDDYRSDDDKSQSSIDSDIEDPDSSESEDFKQSKSKLLKKQKNQKKTFLNKQKFIDTEASETE-NESEID----
P.sexau_Spt5v	1	MSAKNDDYRSDDDKSQSSIDSDIEDPDSSESEDFKQSKSKLLKKQKNQKKTFLNKQKFIDTEASETSENSEDENRKD
P.caudat_Spt5v	1	MSANNDDYRSDDDM-SQSSISFEDEE--FSSQQFVATKNKKDLKKQKNLKKKLTQKQFIEEASET-----
		<b>Acidic region</b>
P.tetra_Spt5m	29	-----EINKEAEERQLKEIQSRAQAKERLNKRLQELMEQDAIDETONNSEYSSNVIDQDDDEQDNDDDDDTLKGGPKLN
P.biaur_Spt5m	30	-----EINKKEAEERQLKEIQSRAKAKERLNKRLQELMEQDAIDETENNSNTSRNENQDNDQDDDDDDSLKGGPKLN
P.sexau_Spt5m	29	-----EINKEAEERQLKEIQSRAKAKERLNKRLQELMEQDAIDETANSSDKSKNENLERDYDDQDDDDTSIKGGPKLN
P.caudat_Spt5m	30	-----KVEDKKLDKHVCEMLVROKKEERIKQFEELIRNEVIGNTSSSSSHE-----QES-----YYNDPTEGPRLE
P.tetra_Spt5v	77	---SVGEITKKGQEQMYNELALARKHHRIVV-----KQLEERY-----CDGDQDIQIGDDVKSEDELEKPGLR
P.biaur_Spt5v	76	---VGGEITKKGQEQIYKEIDLARKHTNNVV-----EITEORY-----CDGDNIN---VEEQRSDDLEKPGLR
P.sexau_Spt5v	81	EVVKTEITKKNQEQEYKAMDARKYNNIVV-----EDLENRY-----CNGEGLN---VEEVKTDDLEKPGLR
P.caudat_Spt5v	66	---VGEISKKRDEIYDTNLLRRNQNNVV-----TEENRY-----KDGDLVV-DEAQOTDDQVEPPGWR
		<b>β1 α1 β2 β3 α2 β4</b>
P.tetra_Spt5m	104	DPKVVRFKCGRSILEVQ--R-LEISNLLP--KNSPIVSVF TPNVKGCHIFFBSSEFEEDVKEFVRST---MYGQPIYIQP
P.biaur_Spt5m	105	DPKVVRFKCGRSICEVQ--R-LEISNLLP--KNSPIVSVF TPNVKGCHIFFBSYFEKDVKEMLKST---MYGQPIYIQP
P.sexau_Spt5m	104	DPKIWRFKCGRSIFEVQ--R-LDLSNQLP--KNSPIVSVF TPNVKGCHIFFBSYFEKDVKEFVRQT---MYGQPIYIQP
P.caudat_Spt5m	94	DPKVVRFKCGRSKVVE--L-LQQIVKLP--ENCKIVS FENPNLKCLIFFEAYFEQDVKTINKDI---MYGQPTYLEP
P.tetra_Spt5v	138	DPKFWRVSCNKGKEQEAFTSI FKHNLHLLDT-NPLEIVSVFAKKFPFAIFIEANFEQHVMRAREGTTIVRQCPPELVBS
P.biaur_Spt5v	135	DPKFWRVSCNKGKEQEAFTSI FKHNLHLLDT-NPLEIVSVFAKKFPFAIFIEANFEQHVMRAREGTTIVRQSPPELVBS
P.sexau_Spt5v	142	DPKFWRVSCNKGKEQEAFTSI FKHNLHLLDT-NPLEIVSVFAKKFPFAIFIEAYFEQHVMAAREGTTIVRQYBPELVBS
P.caudat_Spt5v	125	DPKFWRVSCNKGKEHEAVTSI FKHSHLLDTETPLEIVSVFTKKFPCVIFFEAYFTHVIKAREGAIVRQTPPELVBP
		<b>α3 KOW 1</b>
P.tetra_Spt5m	176	EDCDSLEIKK--NNNIQVGQWVRFKNHKNYKDLGIVLRVNLQNFALIKVLKRNKQGQK-----
P.biaur_Spt5m	177	EDCDSLEIKK--NNNIQVGQWVRFKNHKNYKDLGIVLRNLMQNFALIKVLKRNKQGQK-----
P.sexau_Spt5m	176	EDCYSLEIKK--NNNIQVGQWVRFKNHKNYKDLGIVLRNLIQNFALIKVLKRNKQGQK-----
P.caudat_Spt5m	166	DKCPSELEVQR--SINIQLGQWVR RHHRTYGSDLGRVMNRITNITILKVLKRNKNGQK-----
P.tetra_Spt5v	217	EQCPNFKFPFAEVEQIDIEEGQWVRVRCHNIYSGDLARVMQVDQERKLIKLVPRQKLLMKSLSEEEENKKKSKKNKSN
P.biaur_Spt5v	214	EQCPNFKFPFAEVEQIDIEEGQWVRVRCHNIYSGDLARVMQVDQERKLIKLVPRQKLLMKSLSEEEENKKKSKKNKSN
P.sexau_Spt5v	221	EQCPNFKFPFAEVEQIDIEEGQWVRVRCHNIYSGDLARVMSVDQERKLIKLVPRQKLLMKSLSEEEENKKKSKKNKSN
P.caudat_Spt5v	205	ETCLOKFPFAEVEQVDIEGQWVRVRCHNIYSGDLARVMSVDQERKLIKLVPRQKLLMKSLSEEEENKKKSKK-----N
		<b>Linker 1</b>
P.tetra_Spt5m	235	-----EP-----ISWQRTQQTNQIETTQSEFIYSDIEKENK
P.biaur_Spt5m	236	-----EP-----ISWQRTQQTNQIETKNESEFIYSDIEKENK
P.sexau_Spt5m	235	-----EP-----ISWQRTQQTSEIIKNDSSEFIYSDIEKENK
P.caudat_Spt5m	225	-----EP-----ISWQRENEGEINFSKEQSEFIYSDIEKENK
P.tetra_Spt5v	297	ADQSRFQMKEEEGEPPEDNEPFGQSGKFLKRAKYLQEQRYIRGPKIPIQMTKKSINDNDDEPTSOFFYTIRDEMT
P.biaur_Spt5v	294	ADQSRFQMKEEEGEPPEDNEPFGQSGKFLKRAKYLQEQRYIRGPKIPIQMTKKSINDNDDEPTSOFFYTIRDEMT
P.sexau_Spt5v	301	ADQSRFQMKEEEGEPPEDNEPFGQSGKFLKRAKYLQEQRYIRGPKIPIQMTKKSINDNDDEPTSOFFYTIRDEMT
P.caudat_Spt5v	282	TDQIRFQMKEEEGEPLVE--ETQFGSGKFMFLKRAKYLQEQRYIRGPKIYAVQIQKKQINDNDDEPTSOFFYSIRDEMT
		<b>KOW 1</b>
P.tetra_Spt5m	268	CAKIDGNLLKCPKNI-EHNITITDEELQMFEPDVDRKILVQQAkreILRRVDVQ--FKEGQKVRLLIGEDDLNKGPKF
P.biaur_Spt5m	269	CSIIDGNLLKCPKNI-EHNITITDEELQMFEPDVDRKILVQQAkreILRRVDVQ--FKEGQKVRLLIGEDDLNKGPKF
P.sexau_Spt5m	268	CAKIDGNLLKCPKNI-EHNITITDEELQMFEPDVDRKILVQQAkreILRRVDVQ--FKEGQKVRLLIGEDDLNKGPKF
P.caudat_Spt5m	258	CAKIDGFALLKCNVKNIEHNITITDEELQMFEPDVDRKILVQQAkreILRRVDVQ--FKEGQKVRLLIGEDDMNSGPQF
P.tetra_Spt5v	377	SAKKDGEFIITLPVHQVLTGNIKPTVEILOFYFDPDVQYRLLIQKLHSSSLKQVVEQKSKIQIGDYITITN--QDQTKPSRY
P.biaur_Spt5v	374	SAKKDGEFIITLPVHQVLTGNIKPTVEILOFYFDPDVQYRLLIQKLHSSSLKQVVEQKSKIQIGDYITITN--QDQTKPSRY
P.sexau_Spt5v	381	SAKKDGEFIITLPVHQVLTGNIKPTVEILOFYFDPDVQYRLLIQKLHSSSLKQVVEQKSKIQIGDYITITN--QDQAKPARY
P.caudat_Spt5v	360	SAKKDGEFIITLPVHQVLTGNIKPTVEILOFYFDPDVQYRLLIQKMYQSLKQVVDQKOKIQVGDYITITD--QDQYRSKAF
		<b>KOW 2 KOW 3</b>
P.tetra_Spt5m	345	KIMKIEDDQMIELM-----CKKDNREYTYLVHASETRLAFKLYQEAQVIDGPHKGQVGVIIQIKQGYVVLSNQHGTFK
P.biaur_Spt5m	346	QIIKIEDDQMIELM-----CKKDNREYTYLVHASETRLAFKLYQEAQVIDGPHKGQVGVIIQIKQGYVVLSNQHGTFK
P.sexau_Spt5m	345	KIIKIEDDQMIELM-----CKKDNREYTYLVHASETRLAFKLYQEAQVIDGPHKGQVGVIIQIKQGYVVLSNQHGTFK
P.caudat_Spt5m	335	KIMKIEDDNMELM-----CKKDNREYTYLVHASETRLAFKLYQEAQVVGPHKGQVGVIIQIKQGYVVISTYGSYK
P.tetra_Spt5v	455	KVSAQILQDENKLIIVTKTVKNKNNERNYEEKQISAKLAFKLYQGVSVISGPNTELSGTIIKIMDDLTAAQISTEAGRIV
P.biaur_Spt5v	452	KVAQILQDENKLIIVTKTVKNKNNERNYEEKQISAKLAFKLYQGVSVISGPNTELSGTIIKIMDDLTAAQISTEAGRIV
P.sexau_Spt5v	459	KVAQILQDENKLIIVTKTVKNKNTNERNYEEKQISAKLAFKLYQGVSVISGANQGLSGTIIKIMDDLNASISTEAGRIV
P.caudat_Spt5v	438	KVVQILQDENKLIIVTKNVKDKS-GEVNNYEEKQISEARLAFKLYQGVSVISGPNTELSGTIIKIMDDKTAQISTEASRIV

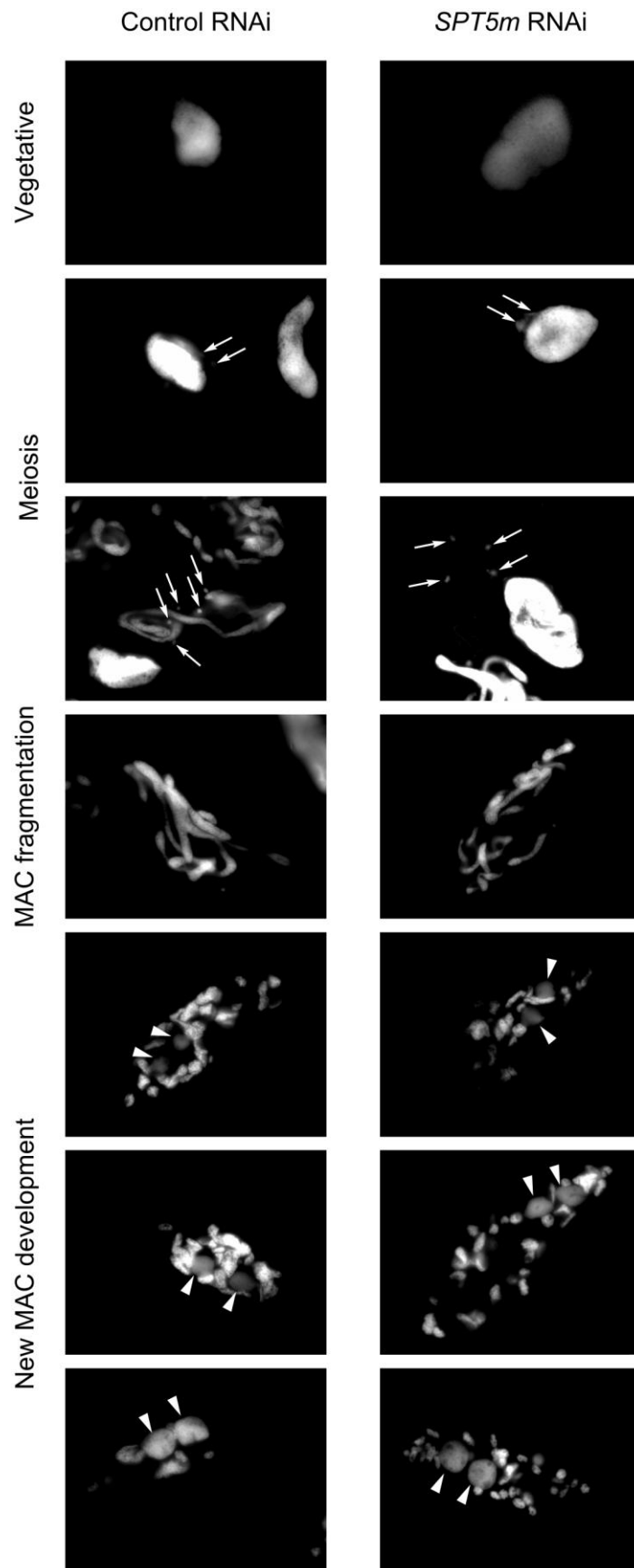




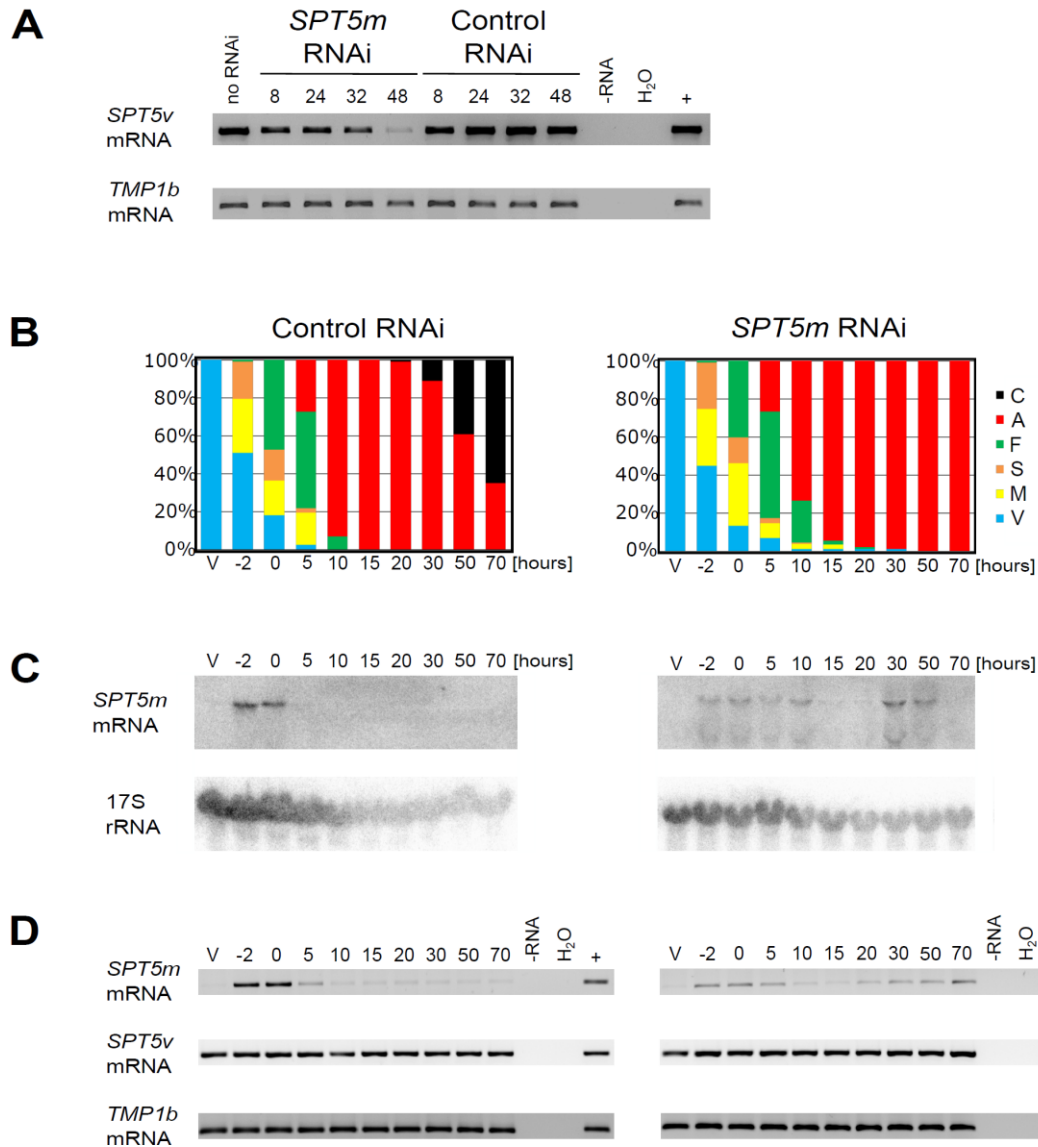
**Figure S1. Alignment and conservation of predicted structural domains for *P. tetraurelia* Spt5 proteins.** A. Complete protein sequences from different organisms were aligned as described in the legend of Figure 1. The structural annotation above the alignment is based on (65) and (23). The Glu residue important for interaction with Spt4 (66) is conserved in both *Paramecium* Spt5 proteins and is highlighted in red. Abbreviations and accession numbers are as follows: Pt, *Paramecium tetraurelia*, Spt5v: GSPATG00013468001, Spt5m: GSPATG00023145001; Tt, *Tetrahymena thermophila*, XP\_976854.1; Ot, *Oxytricha trifallax* Spt5-g19: EJY83850.1, Spt5-g12: Contig19613.0.g12(protein) (OxyDB); Tb, *Trypanosoma brucei*, Q7YUT3; Hs, *Homo sapiens*, NP\_001124296.1; At, *Arabidopsis thaliana*, KOW1: AED90724.1, SPT5-1: NP\_180968.2, Spt5-2: NP\_192575.2; Dm, *Drosophila melanogaster*, NP\_652610.1; Sc, *Saccharomyces cerevisiae*, NP\_013703.1; Pf, *Pyrococcus furiosus*, Q8TZK1.1. B. Alignment of Spt5m and Spt5v proteins from four *Paramecium* species.



**Figure S2. Silencing of *SPT5v* and *SPT5m* during vegetative growth.** The average division rate observed upon RNAi against *SPT5v*, *SPT5m* and control non-essential genes *ND7* and *ICL7* or without silencing (*Kp*) is shown. The data summarizes results obtained for more than 36 cell lines (*SPT5v*: n=93; *SPT5m*: n=36; *ND7*: n=36; *ICL7*: n=57; *Kp*: n=87). Vertical bars show standard deviation. Cells grown in *SPT5m* silencing medium grew as control cells, while for *SPT5v*-RNAi we observed slow-growth phenotype.

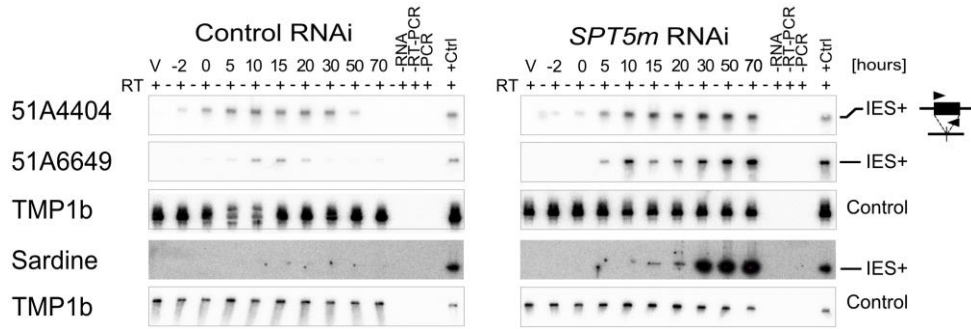


**Figure S3. Cytological observation of DAPI-stained cells silenced for *SPT5m* or the control gene (*ND7*). Arrows point to micronuclei, arrowheads indicate new macronuclei.**

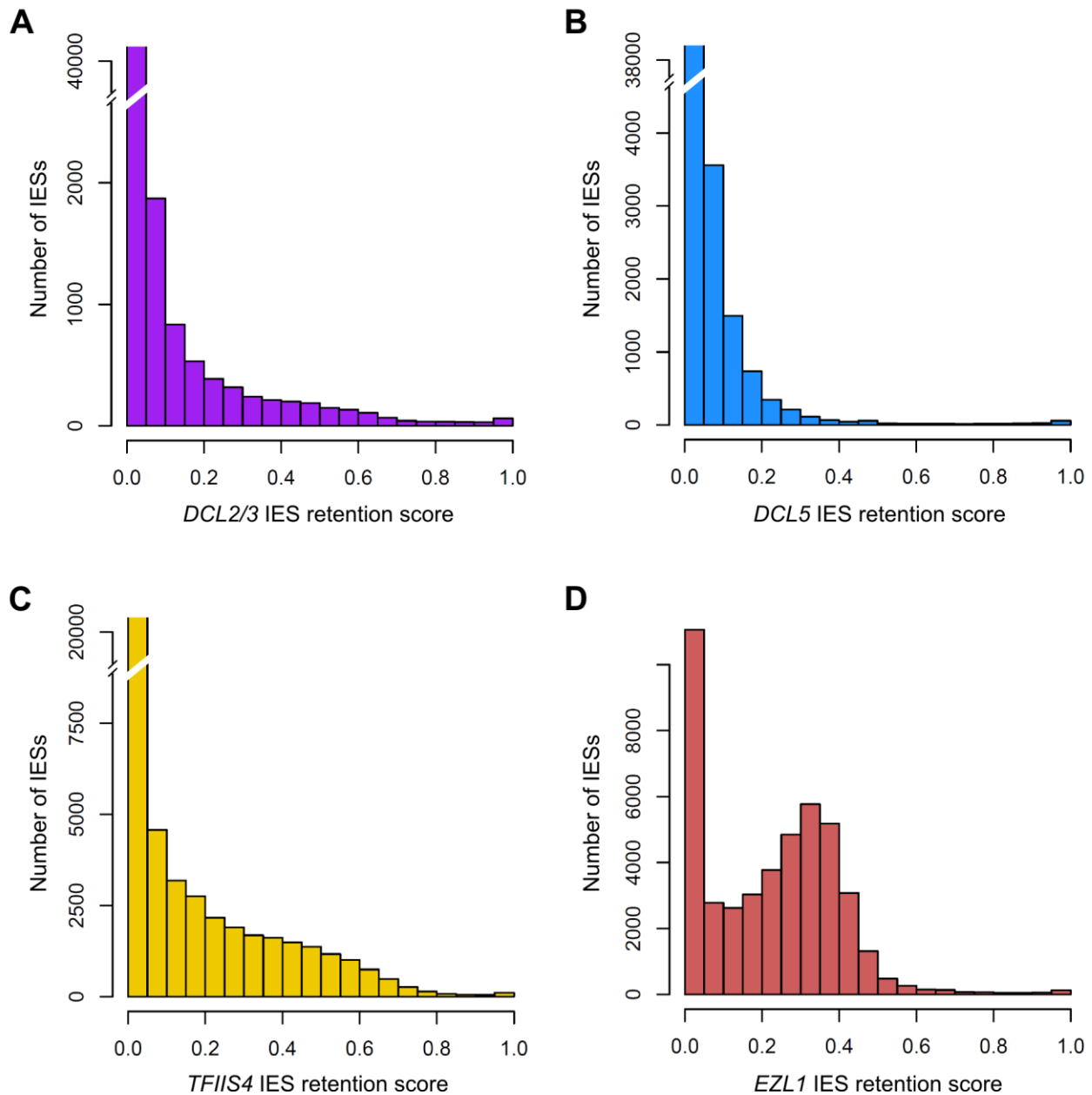


**Figure S4. Evaluation of silencing efficiency for *SPT5v* and *SPT5m*-RNAi.** A. RT-PCR analysis of the expression level of *SPT5v* after silencing of this gene. RNA samples were obtained from cultures without RNAi treatment (noRNAi) and after 8, 24, 32 and 48 hours of silencing from *SPT5v*-silenced and control RNAi cultures (*ND7* RNAi). Reverse transcription was performed using oligo dT. In order not to saturate the PCR reactions, only 25 PCR cycles were performed. Control RT-PCR obtained for the constitutively expressed *TMP1b* gene encoding a trichocyst matrix protein are shown below. A large decrease of *SPT5v* mRNA level is visible after 32-48 hours. B. Histograms show the progression of autogamy in strain 51mt7  $\Delta A$ . As a control we used cells silenced for the unrelated *ND7* gene. For each time-point (V: vegetative culture; -2: meiosis and early MAC fragmentation; 0: around 50% of cells with fragmented MAC; 5 to 70: 5 to 70 hours following time 0, respectively), cells were stained with DAPI to visualize old and new MACs. V: vegetative parental MAC; M: meiosis; S: skein formation; F: fragmented old MAC but no detectable developing new MACs; A: fragmented old MAC + 2 visible anlagen, C: post-karyonidal cells. C. Northern blot validation of *SPT5m*

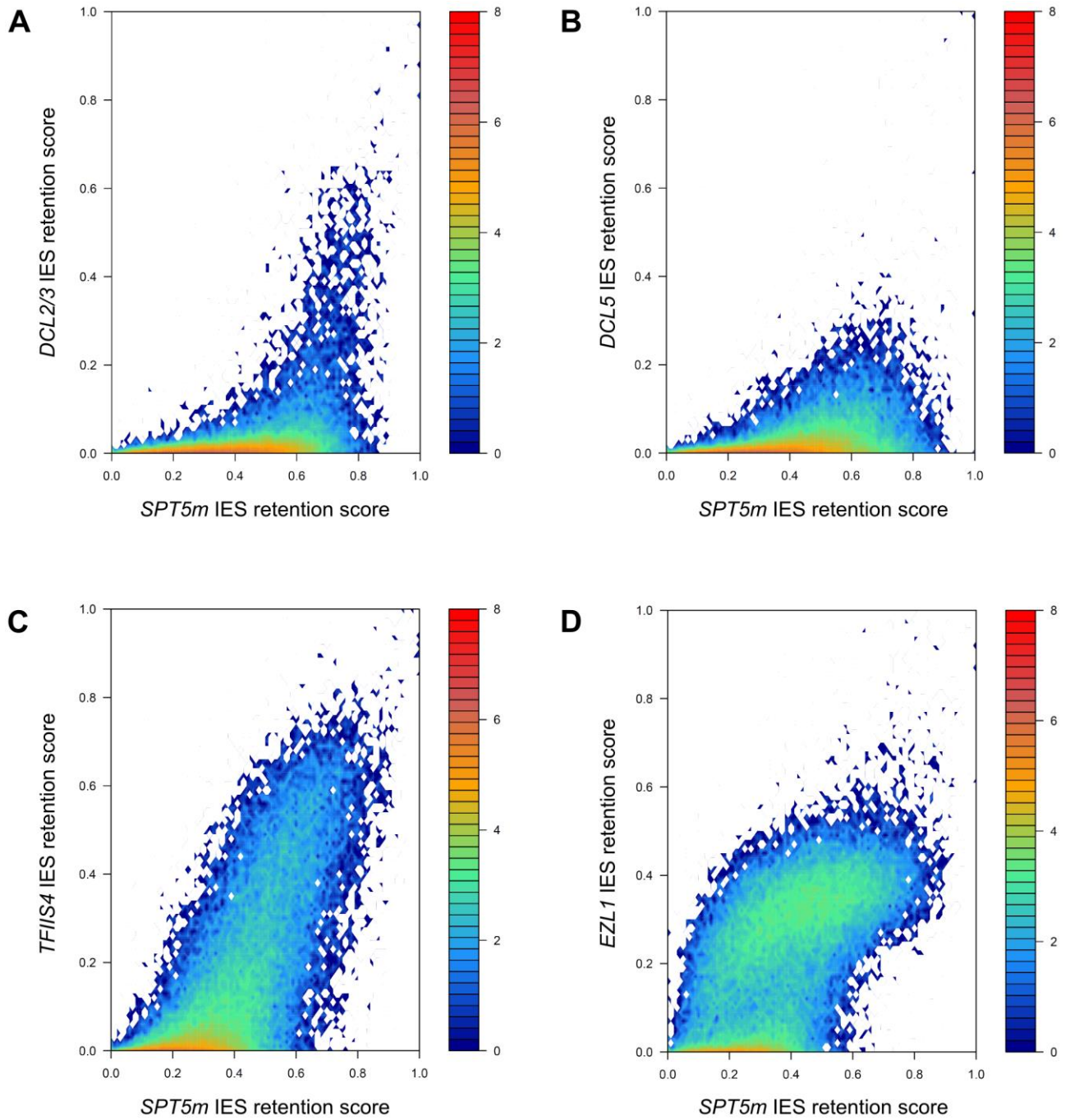
silencing. The blot was hybridized with an *SPT5m* probe and, subsequently, with a 17S rRNA probe as an RNA loading control. Additional band and smear visible in *SPT5m*-RNAi panel most probably correspond to some degradation products of *SPT5m* mRNA that comigrates with 18s and 28s rRNA (compare with Figure S3 in (41)) . Appearance of *SPT5m* mRNA signal in late time points corresponds most probably to unusual late expression of *SPT5m* due to weak silencing at that time (siRNA level decreases with time). D. RT-PCR analysis of expression level of *SPT5m* and *SPT5v* after silencing of *SPT5m*. RNA samples were treated as described in panel A. *SPT5m* expression is lower after RNAi than in the control, while *SPT5v* mRNA level is not altered, as expected



**Figure S5. Detection of transcriptional activity of the new MAC in *SPT5m*-silenced cells during autogamy.** RT-PCR and Southern blot detection of IES-containing transcripts (IES+) as well as transposon-derived transcripts in a control culture (cells silenced for *ND7* gene expression) and in *SPT5m*-silenced cells. Autogamy stages are described in detail in **Figure S4B**: V – vegetative cells, -2 – cells during meiosis/early MAC fragmentation, 0 to 70 – autogamy stages in hours. PCR primers were located within tested IESs: 51A4404, 51A6649 (34) and a conserved region of the *Sardine* transposon common to eight copies of this element (27) (primers within ORF1 of the transposon, SardinUp: GAACACCTCCTATCCTTTAATCATC, SardinLo: GACCTAATCGCAATCATTTATGGATTTC). Control RT-PCR obtained for the constitutively expressed *TMP1b* gene encoding a trichocyst matrix protein are shown below. Early appearance and accumulation of IES+ signal toward the end of autogamy was observed in *SPT5m* silencing for all tested loci, while in control cells IES+ products appeared only at earlier time-points. Results obtained for *SPT5m* were similar to those observed when genome rearrangements were totally blocked by *PGM*-RNAi (34). These results suggest that *SPT5m*-RNAi inhibits IES excision and elimination of *Sardine* transposons, which would result in amplification of non-rearranged DNA and, in consequence, increase of IES+ transcript production.

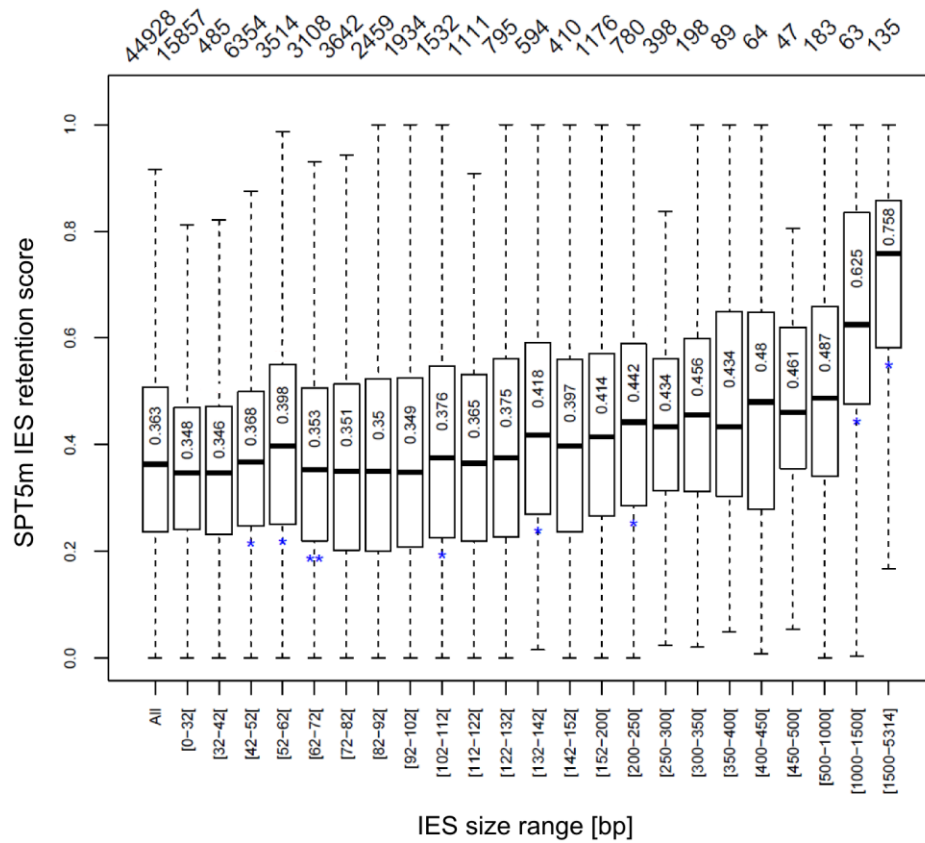


**Figure S6. Analysis of IES excision in cells silenced for expression of *DCL2/3*, *DCL5*, *TFIS4* and *EZL1*.** Retention score distribution determined by re-sequencing of DNA extracted from a cell fraction enriched in new MACs, after silencing of each factor. IES retention scores for silencing of *DCL2/3*, *DCL5*, *TFIS4* or *EZL1* were determined using published data (34, 37, 38). A. *DCL2/3*-KD retention scores; B. *DCL5*-KD retention scores; C. *TFIS4*-KD retention scores; D. *EZL1*-KD retention scores.



**Figure S7. Relation between *SPT5m*-KD retention scores and *DCL2/3*, *TFIIIS4*, *EZL1* and *DCL5* retention scores.** Heatmaps show the relation between *SPT5m* retention scores for all IESs and retention scores obtained for other factors. The color represents the number of IESs (in log2 scale) according to the legend on the right. *SPT5m*-KD retention scores were compared to: A. *DCL/DCL3*-KD retention scores; B. *DCL5*-KD retention scores; C. *TFIIIS4*-KD retention scores; D. *EZL1*-KD retention scores.





**Figure S8. Relation between IES size and *SPT5m* IES retention score.** The box plot displays the *SPT5m* IES retention score distribution for each group of IESs corresponding to a peak in the periodic IES size distribution (27). The median retention score (horizontal line inside the box) and the first (top of box) and third (bottom of box) quartiles are shown. Blue stars indicate that the retention score distribution of a given group is significantly different from the retention score distribution of the previous group according to a Mann-Whitney test.

## Supplementary Tables

Targeted gene	<i>SPT5m</i>	<i>ND7</i>	<i>ICL7</i>	none *
% wild type	1%	94%	97%	90%
% sick	2%	3%	0%	5%
% death	98%	3%	3%	5%
Total cells	480	288	192	240
No. of experiments	10	6	4	5

\* Control grown in standard *K. pneumoniae* medium.

### Table S1. *SPT5m* silencing in autogamy.

Survival test of post-autogamous cells submitted to RNAi against *SPT5m* gene and control non-essential genes - *ICL7* and *ND7*. For each condition, the number of replicate experiments is indicated in the last line.

Targeted gene	<i>SPT5m</i>	<i>ND7</i>	none *
% wild type	3%	100%	97%
% sick	8%	0%	0%
% death	89%	0%	3%
Total cells	36	34	38

\* *K. pneumoniae*

### Table S2. *SPT5m* silencing in conjugation.

Survival test of post-conjugation cells submitted to RNAi against *SPT5m* gene and control non-essential *ND7* gene.

Sample	Gene	23 nt	25 nt (scnRNA)	26-29 nt (iesRNA)	25 nt /23 nt	26-29 nt /23 nt
Early	<i>ND7</i>	19.03	361.27	4.23	<b>18.98</b>	0.22
	<i>SPT5m</i>	148.22	66.61	1.90	<b>0.45</b>	0.01
	<i>DCL2/3</i>	100.25	14.34	1.58	<b>0.14</b>	0.02
Late	<i>ND7</i>	63.70	83.51	238.04	1.31	<b>3.74</b>
	<i>SPT5m</i>	199.53	39.83	156.35	0.20	<b>0.78</b>
	<i>DCL2/3</i>	171.14	23.80	122.34	0.14	<b>0.71</b>

**Table S3. Reduction in scnRNA after *SPT5m* RNAi.**

The sRNA-seq Illumina sequencing samples for RNAi of the indicated gene(s) (first two columns) were mapped and normalized as described in Materials and Methods. The table presents the normalized counts for 23 nt sRNAs, 25 nt sRNAs presumed to be scnRNAs and the sum of the counts for 26- 29 nt sRNAs, presumed to be iesRNAs (next three columns). Finally, count ratios are presented in the last two columns, first of the scnRNA count divided by the 23 nt count and then of the iesRNA count divided by the 23 nt count. Striking differences in the ratios between *ND7* RNAi and *SPT5m* or *DCL2/DCL3* RNAi (boldface type) were found at early and late times for the scnRNA and iesRNA ratios, respectively.

	<i>DCL2/3</i> - sensitive IESs	<i>DCL5</i> - sensitive IESs	<i>TFIIIS4</i> - sensitive IESs	<i>EZL1</i> - sensitive IESs	<i>SPT5m</i> - sensitive IESs	All IESs
Number of IESs	3 020	2 475	20 524	30 892	41 380	44 928
Average IES length [nt]	208	54	94	94	78	79
Number of <i>SPT5m</i> -sensitive IESs	2 999	2 452	20 366	29 996	41 380	41 380
Percentage of <i>SPT5m</i> -sensitive IESs	99%	99%	99%	97%	100%	92%
Average <i>SPT5m</i> retention score	0.68	0.61	0.50	0.43	0.40	0.38

**Table S4. Analysis of IES retention shows a gradation of effects after RNAi of different factors.**

IES retention was determined for each sequencing sample as described in Materials and Methods, and significantly retained IESs according to the statistical test were considered to be sensitive to the factor that had been depleted by RNAi. The table provides the number, average length and average retention scores for the sensitive IESs, and shows that the IESs sensitive to each of the factors represents a subset of the *SPT5m*-sensitive IESs, the *DCL2/3*-sensitive IESs being the largest and most highly retained after *SPT5m*-RNAi.

Sample	Acc. no.	Reference
sRNA sequencing, <i>SPT5m</i> KD, T0 timepoint	SAMN04413729	This study
sRNA sequencing, <i>SPT5m</i> KD, T15 timepoint	SAMN04413730	This study
sRNA sequencing, <i>ND7</i> KD, T0 timepoint	SAMN04413731	This study
sRNA sequencing, <i>ND7</i> KD, T15 timepoint	SAMN04413732	This study
sRNA sequencing, <i>DCL2/3</i> KD, early timepoint	SRR907876	(30)
sRNA sequencing, <i>DCL2/3</i> KD, late timepoint	SRR907877	(30)
sRNA sequencing, control, early timepoint	SRR907874	(30)
sRNA sequencing, control, late timepoint	SRR907875	(30)
DNA from developing MAC, <i>SPT5m</i> KD	SAMN04358097	This study
DNA from developing MAC, control	ERX466735	(37)
DNA from developing MAC, <i>PGM</i> KD	ERA137444	(27)
DNA from developing MAC, <i>DCL2/3</i> KD	SRR2015146	(30)
DNA from developing MAC, <i>DCL5</i> KD	SRR2015148	(30)
DNA from developing MAC, <i>TFIIS4</i> KD	SRX710531	(34)
DNA from developing MAC, <i>EZL1</i> KD	ERX466734	(37)

**Table S5. List of all sequencing samples with their accession numbers.**

### Supplementary References

64. Guo,M., Xu,F., Yamada,J., Egelhofer,T., Gao,Y., Hartzog,G.A., Teng,M. and Niu,L. (2008) Core structure of the yeast spt4-spt5 complex: a conserved module for regulation of transcription elongation. *Structure*, 16, 1649–1658.
65. Martinez-Rucobo,F.W., Sainsbury,S., Cheung,A.C. and Cramer,P. (2011) Architecture of the RNA polymerase-Spt4/5 complex and basis of universal transcription processivity. *EMBO J*, 30, 1302–1310.