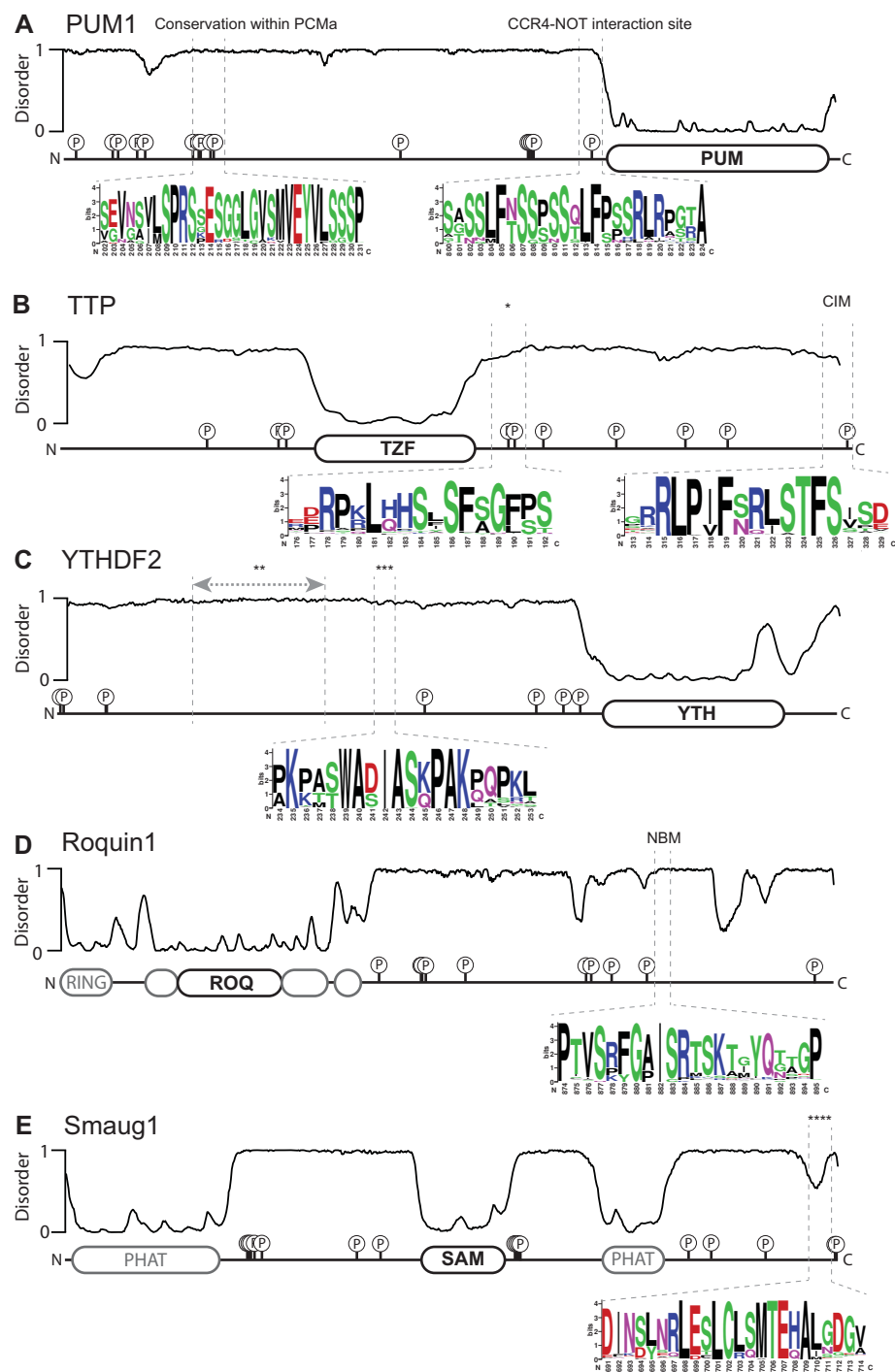


Phosphorylation-dependent tuning of mRNA deadenylation rates

In the format provided by the
authors and unedited

<i>S. pombe</i>	1	MFTAVNSNPNASESISGNSAFNFP	SAPVSSSLDTNNY	GQRRPS	L	SGTSP	TSS	52																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
<i>S. octosporus</i>	1	MYATVKSNAKLSDGLNENVPFQFSSG	-----	YADGRPS	L	ISSGSP	--	40																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
<i>S. cryophilus</i>	1	MYATVKSNAKVSDGMS	ENAPFNFS	SSG	-----	YTDGRPS	L	40																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
<i>S. pombe</i>	53	FFNSSMISSNYTFPHG	SNKQASLES	SPVSYSNPI	PSLTWLSLDGD	SPDSL	SVST	104																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
<i>S. octosporus</i>	41	--RENKLP	SLSPYSQSS	SGSDHSDHST	SFFNPFPS	FQKL	SLNQDITDGG--	87																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
<i>S. cryophilus</i>	41	--RENMI	SSLSPYSQSS	SGSDHSDTSS	SFLNPFPS	FHRLSMNQET	ADTV--	87																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
<i>S. pombe</i>	105	PTAPSANHHGNPFP	NGKQSIKAMP	SLVN	LQEDSVISK	FPNSLEV	PFRKRS--	154																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
<i>S. octosporus</i>	88	-----	SLNNSHSFRNK	HLPKPP	LSLTLNLQ-	DPVF	SNYFGGVEPP	FHSSKSFV	133																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
<i>S. cryophilus</i>	88	-----	SLNSAHPFRNK	HLA	KPP	LSLTLNLQ-	DPVF	THYPANIES	P	FHSSKGLV	133																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
<i>S. pombe</i>	155	ESTSSSL	SGLHSDLR	P	LKTEL	Y	GQLNSE	CGARFP	QTLK	SPLT	P	IGGD	SARTV	206																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
<i>S. octosporus</i>	134	DSNSRC	NALSQDATT	P	SKVN	MYGQL	GSDSA	IRFPH	SLQNS	VPP	LQTD	FGRTL	L	185																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
<i>S. cryophilus</i>	134	DS	SNKSNALNQDT	SS	P	SKVN	LYGQL	GSDSG	VRFPQ	SLQNS	VPP	L	KADL	GLRTL	185																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
<i>S. pombe</i>	207	SASTART-	SDKFFP	RHTRAHS	DFW	I	PATSKP	SRHAS	SHSS	IGDLTT	ITQSS	I	S	257																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
<i>S. octosporus</i>	186	STGISHTP	SSGILP	RHTRAHS	DFWK	P	VNRR	AVRHM	SHSS	SVGDMT	SITQA	APL	S	237																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
<i>S. cryophilus</i>	186	SSGLPQTP	SSRILS	RHTRAHS	DFWK	P	VNRR	AVRHT	SHSS	IGDMA	SITQA	APL	S	237																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
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<i>S. pombe</i>	258	SGSGSFK	P	SWDGS	F	DSS	SLMAHQ	S	-----	YGTSP	A	FANGN	S	P	T	L	K	N	D	S	S	-	300																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
<i>S. octosporus</i>	238	-GT--	-	L	P	N	Y	K	E	N	L	D	A	N	F	L	S	P	P	S	K	V	N	E	R	N	S	I	S	V	T	S	P	T	L	A	S	D	I	S	P	T	A	K	S	D	R	T	C	285																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
<i>S. cryophilus</i>	238	SGT--	-	L	P	N	Y	K	D	K	L	D	V	N	F	L	S	P	P	N	V	N	E	R	N	S	I	S	A	T	S	P	T	L	A	S	D	I	S	S	S	S	K	S	D	L	T	S	286																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
<i>S. pombe</i>	301	--FFGSA	SVRPT	V	S	P	I	G	T	S	F	R	Q	S	L	P	D	I	S	A	F	G	I	P	K	T	E	T	N	P	S	E	V	V	A	P	G	T	I	P	I	S	V	350																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
<i>S. octosporus</i>	286	VPFVSSQ	SFFP	I	P	N	S	-	-	-	S	V	K	R	N	V	S	D	-	S	A	R	P	Y	V	S	V	S	P	D	F	S	P	A	D	G	F	G	S	I	S	F	D	G	333																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
<i>S. cryophilus</i>	287	MPLVSNQ	SVYP	I	A	N	P	-	-	-	T	V	K	H	N	A	L	D	-	S	S	R	S	Y	A	N	I	N	Q	D	L	A	P	V	G	G	F	G	S	I	P	I	D	G	334																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
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<i>S. pombe</i>	351	LPTSNF	S	A	A	T	P	A	N	P	S	L	I	N	Q	N	G	E	F	L	Q	Q	S	R	V	L	Y	L	F	H	A	N	K	Q	R	H	F	E	L	S	D	I	L	G	N	V	V	402																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
<i>S. octosporus</i>	334	NPTP-	Y	S	T	L	S	V	E	D	Q	F	P	K	E	N	H	G	S	A	Y	I	S	H	S	K	L	L	Q	I	F	H	N	N	K	K	R	F	E	L	S	D	I	L	G	N	V	V	384																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
<i>S. cryophilus</i>	335	I	S	N	P	-	Y	P	T	L	N	V	E	D	Q	L	L	K	G	S	H	G	H	N	Y	F	S	Q	S	K	L	L	H	V	F	H	N	N	K	K	R	F	E	L	S	D	I	L	G	N	V	V	385																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
<i>S. pombe</i>	403	LFST	DQHG	S	R	F	I	Q	Q	K	L	A	T	A	T	E	E	E	R	E	A	V	F	Q	E	I	A	S	T	S	C	L	Q	L	M	M	D	I	F	G	N	Y	V	V	Q	K	Y	454																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
<i>S. octosporus</i>	385	LFSS	DDQY	G	S	R	F	I	Q	Q	K	L	A	T	C	T	E	E	E	K	E	A	V	F	E	E	I	V	S	D	N	C	L	Q	L	M	M	D	I	F	G	N	Y	V	I	Q	K	F	436																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
<i>S. cryophilus</i>	386	LFSS	DDQY	G	S	R	F	I	Q	Q	K	L	A	T	A	T	E	E	E	K	S	A	V	F	E	E	I	I	S	N	C	L	Q	L	M	M	D	I	F	G	N	Y	V	I	Q	K	F	437																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
<i>S. pombe</i>	455	FEFG	NEKQ	K	Q	I	L	L	S	Q	I	K	G	H	V	F	S	L	S	L	Q	M	Y	G	C	R	V	V	Q	K	A	I	E	Y	I	S	P	E	H	Q	V	L	I	Q	E	L	506																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
<i>S. octosporus</i>	437	LEYG	NAEQ	K	Q	L	L	V	S	K	I	K	G	H	T	F	I	L	A	V	H	M	Y	G	C	R	V	V	Q	K	A	I	E	H	I	S	A	E	Q	Q	I	E	I	S	E	L	488																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
<i>S. cryophilus</i>	438	LEYG	TDKQ	K	T	L	L	V	S	K	I	K	G	H	T	F	V	L	A	V	H	M	Y	G	C	R	V	V	Q	K	A	I	E	H	I	A	T	E	Q	Q	V	E	I	V	S	E	L	489																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
<i>S. pombe</i>	507	DGHV	LDCV	CDQ	NG	NH	V	I	Q	K	A	I	E	C	I	D	T	G	H	L	Q	F	I	L	R	A	L	R	P	Q	I	H	V	L	S	A	H	P	Y	G	C	R	V	558																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
<i>S. octosporus</i>	489	DSHV	LEC	V	CDQ	NG	NH	V	I	Q	K	S	I	E	C	I	D	S	E	R	L	D	F	I	F	D	A	L	R	P	Q	I	H	V	L	S	A	H	P	Y	G	C	R	V	540																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
<i>S. cryophilus</i>	490	DGHV	LEC	V	CDQ	NG	NH	V	I	Q	K	S	I	E	C	I	D	S	K	K	L	G	F	V	F	E	A	L	R	P	Q	I	H	V	L	S	A	H	P	Y	G	C	R	V	541																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
<i>S. pombe</i>	559	IQRA	I	E	H	C	H	S	E	R	K	L	I	E	E	L	L	P	H	I	L	K	L	T	Q	D	Q	Y	G	N	Y	V	V	Q	H	I	L	R	T	G	S	E	S	D	K	K	Y	I	F	610																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
<i>S. octosporus</i>	541	IQR	I	I	E	K	F	T	Y	K	R	Q	L	I	I	K	D	L	L	P	Y	S	L	Q	L	T	Q	G	Q	Y	G	N	Y	V	V	Q	H	I	L	K	E	G	S	E	K	D	K	F	V	F	592																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
<i>S. cryophilus</i>	542	IQRV	I	E	K	F	T	Y	E	R	Q	I	I	I	D	D	L	L	P	Y	S	L	Q	L	T	Q	G	Q	Y	G	N	Y	V	V	Q	H	I	L	K	K	G	T	N	K	D	K	N	F	V	F	593																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
<i>S. pombe</i>	611	DLM	I	D	H	L	L	F	L	S	C	H	K	F	A	S	N	V	V	E	R	C	I	S	Y	I	S	D	V	D	R	R	R	I	L	N	K	I	I	S	E	K	A	E	N	C	S	I	L	M	L	662																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<i>S. octosporus</i>	593	NLI	A	K	N	L	L	Y	L	S	C	H	K	F	A	S	N	V	V	E	R	C	I	S	Y	I	S	E	D	D	R	A	Y	I	I	Q	K	T	L	T	E	K	V	D	N	V	S	I	L	M	L	644																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<i>S. cryophilus</i>	594	KLI	T	K	N	L	L	Y	L	S	C	H	K	F	A	S	N	V	V	E	R	C	I	S	Y	I	S	E	N	D	R	A	Y	I	I	Q	K	V	L	T	E	K	V	D	N	V	S	I	L	M	L	645																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<i>S. pombe</i>	663	MMK	D	K	Y	A	N	Y	V	I	Q	K	L	L	D	A	S	P	E	E	E	R	D	L	L	I	S	Y	I	Y	P	H	I	S	V	L	K	K	F	T	Y	G	K	H	L	I	M	S	V	E	R	714																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<i>S. octosporus</i>	645	MMK	D	K	Y	A	N	Y	V	V	Q	K	L	L	E	A	S	N	D	K	E	R	D	L	L	I	S	Y	I	Y	P	H	I	A	T	L	K	K	Y	T	Y	G	K	H	L	I	M	S	V	E	R	696																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<i>S. cryophilus</i>	646	MMK	D	K	Y	A	N	Y	V	V	Q	K	L	L	E	A	S	N	E	K	E	R	E	L	L	I	S	Y	I	Y	P	H	I	S	T	L	K	K	Y	T	Y	G	K	H	L	I	M	S	V	E	R	697																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<i>S. pombe</i>	715	FRQ	K	S	I	S	A	V	P	K	L	A	S	K	E	C	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Supplementary Figure 1: Sequence alignments of Puf3. Puf3 protein homologues within the *Schizosaccharomyces* genus. IDR and Pumilio RNA binding domain (RBD) boundaries are indicated. Residues in the DFW motif are in bold (black) and residues mutated in [Extended Data Fig. 3](#) are in bold magenta. Phosphorylation sites characterized in this study are indicated with blue dots.



Supplementary Figure 2: Schematics of human CCR4-NOT interacting RNA-binding proteins.

Disorder prediction and domain diagrams of known *H. sapiens* RNA-binding proteins that recruit the CCR4-NOT complex. Structured domains are depicted as rounded rectangles, with known RNA-binding domains in bold, with IDRs as lines. IDR boundaries are defined based on Metapredict¹. Phosphorylation sites are annotated using data curated in PhosphoSitePlus². Sequence logos show patches of conservation within IDRs which could be important for protein interactions and/or post-translational modifications, often containing conserved serines, aromatic and hydrophobic residues. Sequence logos were generated using Clustal omega alignments of 500-1000 homologous sequences from Chordates, Molluscs and Arthropods. (A) Pumilio homolog 1 (PUM1) contains a ~1000 amino acid IDR N-terminal to

the Pumilio RNA-binding domain. Several regions within the IDR have been implicated in interaction with CCR4-NOT. Two conserved regions of interest have been highlighted (sequence logos). The autonomously repressive Pumilio conserved motif a (PCMa) region contains several conserved phosphosites ³. A separate region proximal to the RBD has been shown to interact with several subunits of the CCR4-NOT complex ⁴. **(B)** Tristetraprolin (TTP) contains both N- and C-terminal IDRs relative to the RBD. Both have been shown to interact with CCR4-NOT. The major CCR4-NOT-interacting motif (CIM) lies close to the C-terminus of the protein ⁵. Other regions within the C-terminal IDR contain both conserved aromatic and serine residues (which can be phosphorylated), indicated by an asterisk (*). **(C)** YTH domain-containing family protein 2 (YTHDF2) can directly bind the CCR4-NOT complex. A region of the N-terminal IDR spanning residues 100-200 (denoted by **) is sufficient for the interaction with the C-terminus of the NOT1 scaffold ⁶. This region lies outside of the most conserved region in the IDR that contains a W-containing SLiM (shown in inset ***). This could be important for interaction with CCR4-NOT but equally could be involved in binding distinct protein partners or subcellular localization, e.g. via membrane binding. **(D)** Roquin contains multiple CCR4-NOT interacting motifs within its C-terminal IDR. Roquin has been shown to interact with both the NOT-module and CNOT9. Sequence logo shows conservation within the NOT-binding motif (NBM). **(E)** Smaug contains multiple IDRs with putative phosphoregulatory sites. *Drosophila* Smaug binds CCR4-NOT through an unknown interaction with the NOT module ⁷. One possible candidate is a conserved C-terminal SLiM (****). This SLiM is within 10 amino acids of several C-terminal phosphosites (outside of sequence logo shown).

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