

Supplemental Material

Supplemental Figure S1. Protein Sequence alignment of human EXOSC2 and EXOSC3. Protein Sequence alignment of the human RNA exosome cap subunits EXOSC2 and EXOSC3, *S. cerevisiae* Rrp4 and Rrp40, including other EXOSC2/Rrp4 and EXOSC3/Rrp40 orthologs. Conserved EXOSC2 amino acids substituted in patients with SHRF (DI DONATO *et al.* 2016) and EXOSC3 amino acids substituted in patients with PCH1b disease (WAN *et al.* 2012) are highlighted in green. Identical residues (in red) and similar residues (in blue) are indicated. The different species aligned are as follows: Hs: *Homo sapiens*, Mm: *Mus musculus*, Dr: *Danio rerio*, Dm: *Drosophila melanogaster*, Ce: *Caenorhabditis elegans*, Sc: *Saccharomyces cerevisiae*, Sp: *Schizosaccharomyces pombe*, Cn: *Cryptococcus neoformans*, At: *Arabidopsis thaliana*, Os: *Oryza sativa*, Ss: *Sulfolobus solfataricus* (archaea).

Supplemental Figure S2. Modeling of the human EXOSC2-EXOSC4 and yeast Rrp4-Rrp41 interface show structural conservation. (A) Zoomed-in representations of the interface between the RNA exosome cap subunit EXOSC2 (teal blue) and core subunit EXOSC4 (light gray). EXOSC2 G30 facilitates a β turn that positions EXOSC2 Asp31 (D31) near an arginine in EXOSC4, R232. Structural modeling shows a salt bridge that forms between EXOSC2 D31 and EXOSC4 R232, represented by the red dashed lines. (B) Zoomed-in representation of the interface between the yeast RNA exosome cap subunit Rrp4 (teal blue) and core subunit Rrp41 (light gray). Rrp4 G58, which corresponds to EXOSC2 G30, facilitates a β turn that positions Rrp4 Glu59 (E59) near an arginine in the EXOSC4 yeast ortholog, Rrp41 (R233). Structural modeling shows salt bridge forms between Rrp4 E59 and Rrp41 R233, represented by the red dashed lines. Structural modeling in (A) was performed with the human RNA exosome structure (PDB 6D6R) (WEICK *et al.* 2018) and in (B) with the yeast RNA exosome structure (PDB 6FSZ) (SCHULLER *et al.* 2018) using PyMOL (PyMOL).

Supplemental Figure S3. Volcano plot of autophagy transcripts differentially expressed in the *rrp4*-

G226D RNA-Seq. A total of 18 autophagy transcripts show ± 1.5 -fold change (*p*-value adjusted < 0.05) in *rrp4*-*G226D* cells compared to *RRP4* cells. Of those, 2 transcripts are increased 1.5-2 fold (*NFT1* and *SNO4* (blue)) and 16 transcripts are decreased 1.5-2-fold (*TEF4*, *RPL14A*, *RAS2*, *EGDI SEC4*, *TPS1*, *TMA19*, *PEP4*, *SEC14*, *MSS51*, *RPL15B*, *CAPI*, *FRQ1*, *PNP1* (blue)) with two transcripts decreased > 2 -fold (*ASC1* and *SOD1* (orange)).

Table S1. Yeast Strains and Plasmids used in this study.

Table S2. DNA Oligonucleotides employed for RT-qPCR

Table S3. Summary of *in silico* predictions for pathogenic amino acid substitutions in EXOSC2 and Rrp4.

Supplemental Table S1

Sterrett Enyenihi et al.

Strain/Plasmid	Description	Source
<i>rrp4Δ</i> (yAV1103)	MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 rrp4Δ::NEO [RRP4, URA3]	(SCHAEFFER et al. 2009)
<i>rrp40Δ</i> (yAV1107)	MATa his3Δ1 leu2Δ0 ura3Δ0 rrp40Δ::NEO [RRP40, URA3]	(SCHAEFFER et al. 2009)
<i>rrp4Δ mpp6Δ</i> (ACY2471)	<i>MATα, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ0, rrp4Δ::NEO, mpp6Δ::natMX4, [RRP4, URA3]</i>	This study
<i>rrp4Δ rrp47Δ</i> (ACY2474)	<i>MATα, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ0, rrp4Δ::NEO, rrp47Δ::natMX4, [RRP4, URA3]</i>	This study
<i>rrp4Δ rrp6Δ</i> (ACY2478)	<i>MATα, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ0, rrp4Δ::NEO, rrp6Δ::natMX4, [RRP4, URA3]</i>	This study
<i>rrp40Δ mpp6Δ</i> (ACY2638)	<i>MATa; ura3Δ0, leu2Δ0, his3Δ1, rrp40Δ::NEO, mpp6Δ::natMX4, [RRP40, URA3]</i>	This study
<i>rrp40Δ rrp47Δ</i> (ACY2462)	<i>MATa; ura3Δ0, leu2Δ0, his3Δ1, rrp40Δ::NEO, rrp47Δ::natMX4, [RRP40, URA3]</i>	This study
<i>rrp40Δ rrp6Δ</i> (ACY2466)	<i>MATa; ura3Δ0, leu2Δ0, his3Δ1, rrp40Δ::NEO, rrp6Δ::natMX4, [RRP40, URA3]</i>	This study (SIKORSKI AND HIETER 1989)
pRS315	CEN6l LEU2, amp ^R	
pAC3161	RRP40-2xMyc in pRS315, CEN6, LEU2, amp ^R	(FASKEN et al. 2017)
pAC3162	rrp40-G8A-2xMyc in pRS315, CEN6, LEU2, amp ^R	(FASKEN et al. 2017)
pAC3259	rrp40-W195R-2xMyc in pRS315, CEN6, LEU2, amp ^R	(FASKEN et al. 2017)
pAC3652	RRP40-Native 3'UTR in pRS315, CEN6, LEU2, amp ^R	This study
pAC3655	rrp40-W195R-Native 3'UTR in pRS315, CEN6, LEU2, amp ^R	This study
pAC3474	RRP4-2xMyc in pRS315, CEN6, LEU2, amp ^R	This study
pAC3476	rrp4-G58V-2xMyc in pRS315, CEN6, LEU2, amp ^R	This study
pAC3477	rrp4-G226D-2xMyc in pRS315, CEN6, LEU2, amp ^R	This study
pAC3656	RRP4-Native 3'UTR in pRS315, CEN6, LEU2, amp ^R	This study
pAC3659	rrp4-G226D-Native 3'UTR in pRS315, CEN6, LEU2, amp ^R	This study
pAC3669	RRP4-2xMyc-Native 3'UTR in pRS315, CEN6, LEU2, amp ^R	This study
pAC3672	rrp4-G226D-2xMyc-Native 3'UTR in pRS315, CEN6, LEU2, amp ^R	This study

Supplemental Table S2

Sterrett Enyenihi et al.

Description	Sequence (5'-3')	Name
pre-U4 snRNA Fwd	ATCCTTATGCACGGGAAATACG	AC5723
pre-U4 snRNA Rev	AAAGAATGAATATCGGTAAATG	AC5722
pre-U6 snRNA Fwd	GAAGTATCGTGACTTGTACATTG	AC7748
pre-U6 snRNA Rev	TCTTCTATGTTCAACCGATAGC	AC7749
U14 snoRNA (snR128) Fwd	GATCACGGTGATGAAAGACTGG	AC5397
U14 snoRNA (snR128) Rev	CTACAGTATACGATCACTCAGACATCCTA	AC5398
snR44 snoRNA Fwd	GCATTCCACATGGGATTAAA	AC6272
snR44 snoRNA Rev	ATGGTGTGATCGGGCAGTAT	AC6270
TLC1 ncRNA Fwd	AAGGCAAGGGTGTCCCTTCT	AC6420
TLC1 ncRNA Rev	TTCCGCTTGGAAAATAATGC	AC6421
RPS3 mRNA Fwd	TCCAACCAAGACCGAACGTTATC	AC9226
RPS3 mRNA Rev	GTACCTGGAGCGTACTTGAATC	AC9227
RPL15A mRNA Fwd	CCAGACAAGGCTAGAAGATTGG	AC9309
RPL15A mRNA Rev	CCGTAAGTAGCACCCCTTGG	AC9308
INO1 mRNA Fwd	TTGGACTGCAAATACTGAGAGG	AC9303
INO1 mRNA Rev	AAGATCGTGGAAAGGAGCAATC	AC9302
PTH4 mRNA Fwd	ACTGTGCTTGGATTCTTCAG	AC9248
PTH4 mRNA Rev	CTATAGAACATCGCTGCCCTTAC	AC9249
HXK2 mRNA Fwd	TACTGGTGTCAATGGTCTTAC	AC9307
HXK2 mRNA Rev	TTGGAGCAGATGGTCCAATG	AC9306
TDH1 mRNA Fwd	GGTAGATACAAGGGTACTGTTCC	AC9230
TDH1 mRNA Rev	TGAGCGGTGTCCAATTCC	AC9232
CUT501 Fwd	GGTTCAACGTTGCAGGATCT	AC9254
CUT501 Rev	GCTAGCACCTGTTGCTGTAAT	AC9255
CUT770 Fwd	AAACAACCCGCTAGTGTGAC	AC9262
CUT770 Rev	AGAGCAACTCACTGCAAAGG	AC9263
CUT896 Fwd	ATCAGCAGGTGTCAATTACAG	AC9256
CUT896 Rev	CCCAGAGGCAAAGATGTTAAGT	AC9257
NRD1 mRNA Fwd	CAAGCAGAGGTCGAAACAAATC	AC9244
NRD1 mRNA Rev	GCTGGATCTGTGGAAGTC	AC9245
NAB3 mRNA Fwd	ACAGTCGGTAGGCTCAGATAG	AC9246
NAB3 mRNA Rev	GGCGAAGTTCGACCTCTTATC	AC9247
ALG9 mRNA Fwd	CACGGATAGTGGCTTGGTAACATTAC	AC5067
ALG9 mRNA Rev	TATGATTATCTGGCAGCAGGAAAGAACTGGG	AC5068

Supplemental Table S3

Sterrett Enyenihi et al.

	<i>Hs EXOSC2</i>		<i>Sc Rrp4</i>	
Exosomopathy amino acid substitution	G30V	G198D	G58V	G226D
Missense 3D* (Phyre2)	Neutral	Damaging	Neutral	Damaging
PolyPhen-2** (HumDiv)	Possibly Damaging (Score: 1.000)	Probably Damaging (Score: 1.000)	Probably Damaging (Score: 1.000)	Probably Damaging (Score: 1.000)
Provean†	Deleterious (-7.938)	Deleterious (-6.35)	Deleterious (-8.981)	Deleterious (-6.517)
SNAP2‡	Effect (Score: 91)	Effect (Score: 94)	Effect (Score: 63)	Effect (Score: 92)

*Missense 3D part of tool suite **Phyre2** (Protein Homology/analogY Recognition Engine) v2.0; predicts structural changes introduced by an amino acid substitution through three-dimensional protein modeling.

**PolyPhen-2 (Polymorphism Phenotyping) v2 HumDiv trained model; Naïve Bayes posterior probability that mutation is damaging and qualitative classification as benign, possibly damaging, or probably damaging based on 5%/10% false positive rate (FPR), the chance the mutation is classified as damaging when it is non-damaging.

†PROVEAN (Protein Variation Effect Analyzer) v1.1; delta alignment scores equal to or below predefined threshold (-2.5), protein variant predicted to have "deleterious" effect on function.

‡SNAP2 predicted score for functional effects of mutations; scores range from -100 strong neutral prediction to +100 strong effect prediction.

Supplemental Figure S1

Sterrett Enyenih*i et al.*

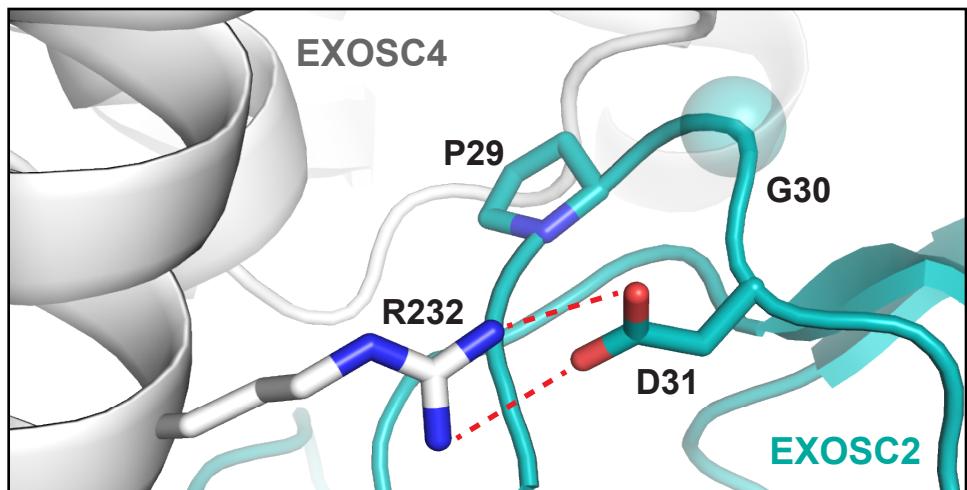
HS	Exon	Protein	Sequence	Color	Annotation	Color	Annotation
hsExoSc2			M-A-M-E-R-L-P-V-A-K--P-L-S-E-R-L-G-R-T-C-K-H-L-L-V-P--G-T-T-T-D	black	T-G-F-M-R-G-H-C-T-Y-M-E-E-K-E--	black	L-I-A-S-V-A-G-S-V-E-R-V-N-K-L--I-C-V-K-A-L
MmRrp40			M-A-L-E-R-M-L-P-K-A-R-K--P-L-S-E-S-L-G-R--D-S-K-X-V-H-V-P-G-D-T-T-T-D	black	T-G-F-M-R-G-H-C-T-Y-M-E-E-K-E--	black	L-I-A-S-V-A-G-S-V-E-R-V-N-K-L--I-C-V-K-A-L
DrRrp4			M-A-V-E-M-R-L-P-V-A-H-K--H-V-S-L-K-T-Q-S-S-E-R-D-K-H-V-P-G-D-V-T-S-D	black	T-G-F-M-R-G-H-C-T-Y-M-E-D-E-R-D--	black	L-I-T-A-S-V-A-G-Q-V-E-R-V-N-K-L--I-C-V-K-P-L
DmRrp4			M-S-T-N-A-A-I-D-L-A-L-D--R-V-D-W-R-D-L-A-Q-T-E-Q-P-R-V-Y-P-T-G-E-V-L-M-P-E	black	A-G-E-M-R-G-H-C-T-V-F-E-D-E-N-E--	black	I-K-S-V-A-G-V-I-Q-K-V-N-K-L--I-S-V-R-P-L
CeRrp4			-M-S-F-E-V-T-G-P-P--L-V-S-P-H-M-M-L-S-M-S-E-A-R-N-D-I-E-T-K-I-V-P-G-H-S-V-C-D-A-P	black	Q-Q-F-M-R-G-H-G-T-Y-V-R-D-G-E--	black	I-V-S-S-L-S-G-V-Q-Q-L-N-R-L--I-M-V-K-T-I
Skrp4			M-S-E-V-I-T-T-K-R-N-G-A-F-Q-N-S-S-L-N-I-N-T-G-I-S--D-D-E-N-D-E-D-I-Y--M-H-D-V-N-S-A-K-S-S-E-D-S-Q-V-T-P-G-E-L-V-T-D-D	black	P-I-W-M-R-G-H-C-T-Y-F-L-D-N-M--	black	T-Y-S-S-V-A-G-T-V-E-R-V-N-K-L--I-S-L-V-I-P-L
Skrp4			--M-V-T-I-L-K-P-E-E-F-Y-V-S-S-E-A-D-I-V-N-S-T-M-E-T-E-D-I-M-E-D-Q-C-M-V-L-G-D-E-V-L-E-F-D-K-S-I-H-Q-N-L-V-P-Q-L-V-T-D-D	black	P-Q-F-M-R-G-H-C-T-Y-F-E-D-G-G--	black	T-Y-S-S-V-A-G-S-V-Q-V-E-R-V-N-K-L--I-S-V-S-K-L
CnRrp4			--M-F-S-V-K-A-V---A-P-S-I-S-Q-A-T-E-V-P-S-T-H-R-K--H-Q-A-E-L-D-A-M-D-L-B-E-D-E-F-G-G-A-P-S-K-R-S-I-V-S-P-G-E-V-I-T-S-S	black	K-E-Y-M-R-G-H-C-T-Y-V-E-E-S-N--	black	T-V-V-S-A-G-T-V-E-R-V-N-K-L--I-S-V-R-P-L
AtRrp4			--M-V-M-R-K-L-Q-L-P-L-S-T-Q-O--K-V-R-F-E-A-R-E--R-L-Q-S-L-S-S-S-A-N-D-S-A-V-T-D-S-V-P-N-H-D	black	D-A-P-L-K-H-C-T-S-E-V-D-G-E--	black	L-L-A-T-V-G-V-E-R-V-D-K-L--V-Y-V-R-T-L
OsrRp40			-M-R-D-L-Q-L-S-L-N-Q-T-O--V-R-V-L-E-A-L-H--E-L-Q-T-V-A-P--A-A-V-V-T-I-P-V-N-D	black	D-N-L-K-H-G-T-O-D-Q-D-G-E--	black	V-V-A-T-L-C-G-V-E-R-V-E-R-N-K-L--V-Y-V-T-R-L
hsExoSc3			-M-A-E-P-S-A-V-A-E-S-L-A-G-S-R-A-A-R-A-T-V-L-C-Q-V-L-P--E-L-L-P-E-Q-E-D-A-E-C-P-G-G-A-V-E-R-P-L-S-I-N-A-R-A-C-S-V-R-P-L-S-V-C-P-G-C-L-R-C-D-G-R	black	L-U-T-V-K-C-G-L-H-K-E-P-P-G-S-S-G-G-Y-W-V-D-S	black	T-Y-S-S-V-A-G-T-V-E-R-V-N-K-L--I-S-V-R-P-L
MmRrp40			-M-A-E-V-L-S-A-G-P-S-V-A-G-C-R-A-R-A-V-H-V-L-N-Q-V-L-P-G-E-L-V-L-D-H-E-D-V-L-G-G-A-G-E-Q-P-L-R-N-A-G-P-R-L-V-P-W-V-C-P-G-C-L-R-C-D-G-R	black	L-U-T-V-K-C-G-L-H-K-E-P-P-G-S-S-G-G-Y-W-V-D-S	black	T-Y-S-S-V-A-G-T-V-E-R-V-N-K-L--I-S-V-R-P-L
DrRrp40			-M-D-S-V-S-T-T-S-L-L-E-R-G-D-V-L-P-G-D-L-L-F-S-S-P-E--	black	A-G-D-A-N-K-P-A-D-L-R-C-L-V-R-S-G-E--	black	I-R-V-C-R-A-G-L-H-K-Q-P-Q-N--M-Y-W-V-N-C
DmRrp40			-M-S-A-T-T-I-V-M-P-G-E-R-I-A-A--I-E-E-L-A-K-S-K-V-L-P-G-L-R-L-D-D-T	black	F-T-W-F-V-D-N--	black	V-V-A-S-K-A-G-L-H-K-E-P-C--
CeRrp40			-M-T-V-Y-L-P-G-D-V-I-N-B-P-S-S--S-D-S-S-I-C-Y-G-I-S-V-R-Q-C-T--	black	R-I-A-T-Q-P-G-F-H--N-D-D-K-G-W-L-V-L-N-V	black	
Skrp40			-M-S-T-F-I-F-P-G-D-S-F-P-V-D-P--T-T-P-V-K-L-G-P-G-I-Y-C-D-P-N-T-Q-E--	black	E-I-R-V-N-T-G-V-L-H-S-V-A-K-G--K-S-G-V-Q-T-A-Y-I-D-Y	black	
Sprp40			-M-A-E-V-E-E-K-G-L-L-S-Y-P-G-E-R-I-P-D-S-I-S--Q-D-G-S-I-L-R-G-P-L-V-F-Q-K-D-K-R-E--	black		black	
CnRrp40			-M-P-T-L-L-P-G-E-T-V-P-I-P-S--T-S-K-A-V-L-G-P-Q-I-S-Q-T-P-L-Q-A-S-S-T-A-S-G-S-S-A-Q-Q-Y-I-A-T-R-L-C-M-L-H--S-G-K-G-K-E-R-S-Q-L-K-L-W-E-G	black		black	
AtRrp40			-M-A-A-K-V-S-P-S-T-L-Q-I-V-V-P-G-D-V-V-L-L-S-N-M--N-N-Q-T-K-L-G-S-L-L-Q-Q-N-D-A--	black	I-S-A-M-P-A-K-L-Y-S-K-S-P-N--K-Y-W-E-S	black	
OsrRp40			-M-E-S-R-K-P-P-S-A-L-V-H-N-V-P-G-D-V-V-L-L-D-A-E-M--T-N-Q-T-K-L-G-A-G-L-R-Q-P-Q-C-D-T--	black	I-Q-A-T-S-G-A-L-R-L-S-K-P-S--K-Y-W-V-E-S	black	
Ssrp4			-M-N-M-S-Q-S-Q-K-V-L-Q-P-R-S-I-V-P-G-E-L-L-A-E-G-E--F-Q-P-S-P-Y-S-P-L-K-I-N-S-K--	black	Y-Y-S-T-V-G-L-F-D-V-K-D-T-Q--F-E-V-I-P-L	black	
consensus				black		black	

HsEXOSC2	PTPEHKE-	EE-AGGFIANL-EPV-SIADREVISRRLRNCIISLVTPRMMLYDTSILYCYEASLPHQIKDI-----	-LKPEIME-EIVMELTRQLR-----	-LEQEGL-
MmRrp4	PTTPEHKK-	ED-AGGFIANL-EPV-AALSREVISRRLRNCIISLVTPRMMLYDTSILYCYEASLHQIKDI-----	-LKPEVME-EIMLETQRQL-----	-LDQEG-
DrRrp4	PTFALEQ-	EQ-AGGFYTSM-EPV-SIISDRREVSRRLRNCLIAALANQKFLPDTSILYCYEASLHQIKDI-----	-LKPEIME-EIVLLETQRQL-----	-MEHEG-
DmRrp4	PTKGQEE-	EGEGGFAQNLNEHPR-PRADIREVIAARLNSRQYAEVYDLSLQHQIKDI-----	-LKPEVME-EIMLETQRQL-----	-RDAD-
CeRrp4	SPFLPETTLE	EDGS-----	-HVN-EFQIVPPDPRVMVR1IAACRVLRDYSISYIYLNTSCTCYEMSYEPIKEELAEGQDTSSRLAYL-----	-QQK-
ScRrp4	TKTSQMDLARDTSPANSNNSSIKSTG	PTGAVSLNPSTIRLEEEASISQYIISDENPSISNRRQAKICRYANVIALKAFCIGITQDVRVSAYEASMVY-----	-SNVGL-EISGSDILTAEK-----	-MRGNNG-
SprRrp4	KHNENQH-----	-SSVSITRLEEEASISQYIISDENNEI-DGYTRLNRSVSICIGKLASSRPLQTASINFYEESLVE-----	-SNLQDL-----	-TVPKNMQ-DQIAAMEAMQ-----
CnRrp4	NGTSQARRE-----	-GGEGFDSEGVSQNNDIE-PGPGREASLVLANIAKALASEGVPLTETLIGESWYAEEAKNVPSSGSPFDSETERKMLNIEIVGLEFLEFA-----	-	-
AtRrp4	EHVVERDPMALIDD-----	-QKDEMEMISS-STGKEQSHPLEPTQTCIRQ1NAIRVLNSLNGFTV1LEVIMETVNLNSRNKHID-----	-	-DMLGSEFHVVAAEAERRTRKRKK-----
OsRrp4	EHVVGGENANMME-----	-NKL-----	-N-LSAEVENFTPLETRKHHICRANAVRVLASLGFTLTVELLIIETAESVSSNIEIN-----	-NMLGAEVYVQTAEREVKRADLLRKSGAR-----
HsEXOSC3	AKTIQTLILANIILEACEHTMSDQRKQ-----	-	-IFSRILAES-----	-
MmRrp40	AKTIQTLILANIILEACEHTTEQRKQ-----	-	-IFARLAE-----	-
DrRrp40	ARTVQHTLILNSLNLIECENNMASTQAERT-----	-	-LFRRVADGDDGI-----	-
DmRrp40	AHSLKETVALANAIISALEQSGCAEIJK-----	-	-ICGNLQDPLQA-----	-
CeRrp40	ASTSSDDI1H2D1INKSFETTDEDELIT-----	LVQNNITRNSVSD-----	-	-
ScRrp40	CEELSNLTLACYRTIMECCQKNDTAAFKD-----	-	-TAKRQFKEI LTVEKE-----	-
SprRrp40	SENLLTTVLICTRNCFMSDEEQKY-----	-	-CKDLTIKLI-----	-
CnRrp40	AETVGETLAMKRLIESVDDHIIPEVEKA-----	-	-IKEKLREYLS-----	-
AtRrp40	ATAPARIVIVIANAMINSESSLGQTQRI-----	-	-MVEKLLK1IISD-----	-
OsRrp40	APSPSPNVVUNNSAIKSESLSGICQRS-----	-	-MVESSLERLS-----	-
SsRrp40	CPSRFSEEILILIAI-----	-RKI-----	-ENESH-----	-IKGLTDRIKQFIEEKLGERNASSGETKTNS-----

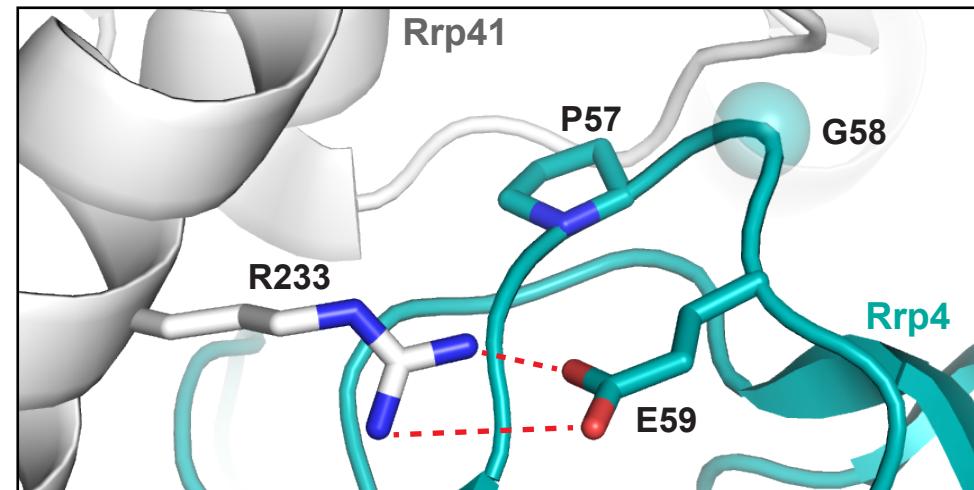
Supplemental Figure S2

Sterrett Enyenihi et al.

A



B



Supplemental Figure S3

Sterrett Enyenihi et al.

