

**Supplemental Data for accompanying manuscript entitled:**

***Saccharomyces cerevisiae* as a toolkit for COP9  
signalosome research**

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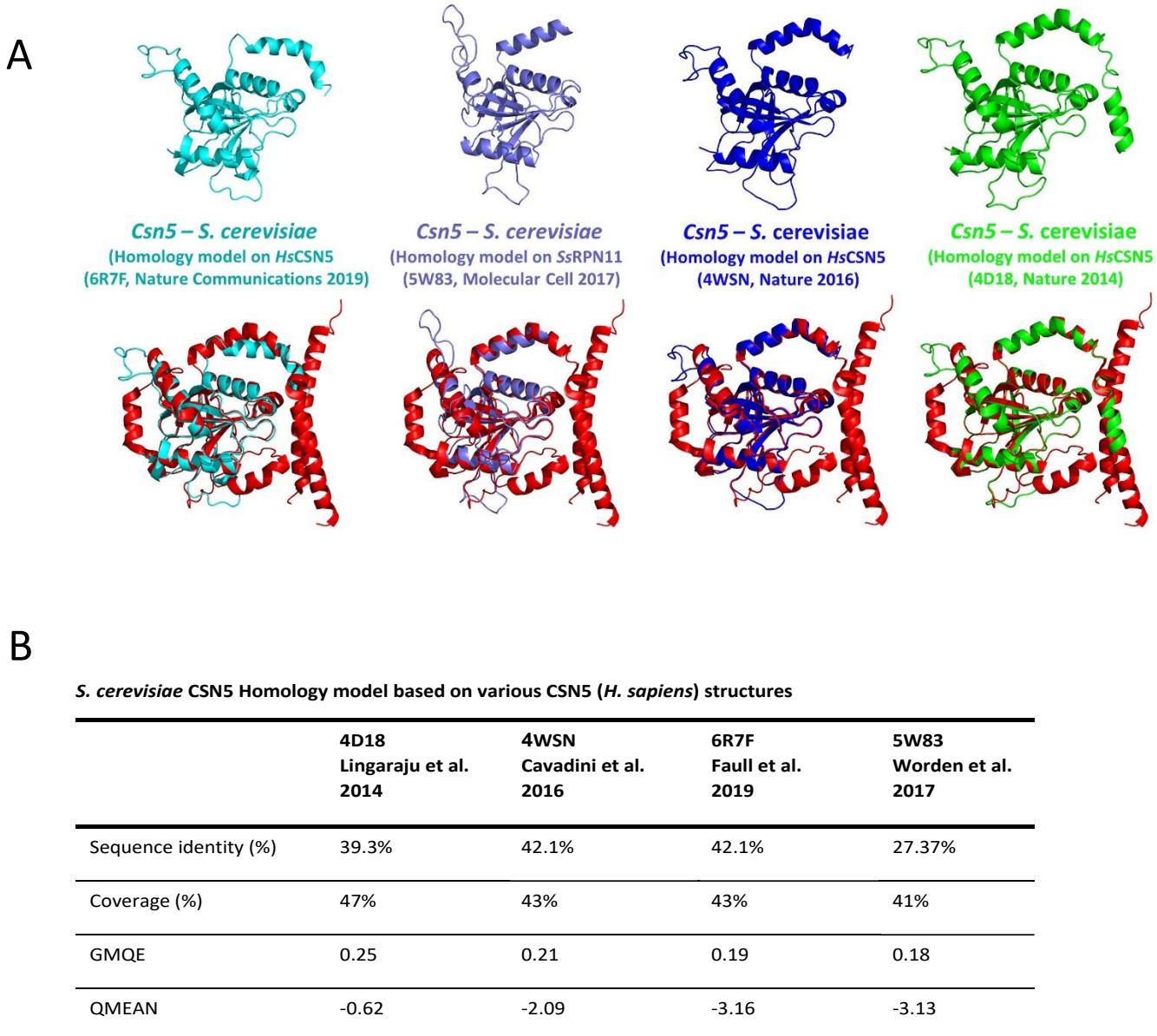
# Shared co-first authorship

\*To whom correspondence should be addressed.

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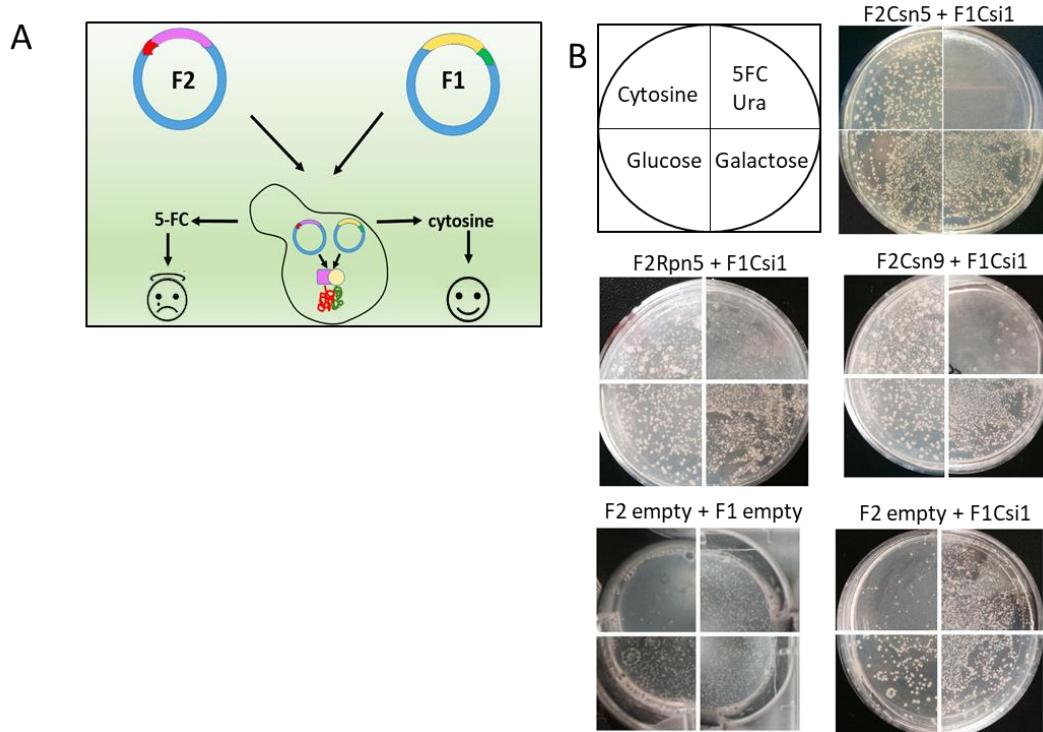
**Fig S1**



**Supplemental Figure S1 (for Figure 1C): Structural comparison between *Homo sapiens* and *Saccharomyces cerevisiae* orthologues of CSN5.**

**(A)** *S. cerevisiae* Csn5 homology model comparison suggest that a highly conserved architecture between the *Homo sapiens* (Red) and *Saccharomyces cerevisiae* (other colors) orthologues. CSN5 Homology models were built using the template of four different available CSN5 crystal structures (PDB IDs: 6R7F, 5W83, 4WSN and 4D18), and were superimposed on the *H. sapiens* CSN5 crystal structure (PDB ID: 4D18). **(B)** Query coverage and percentage of identity of the sequences used in A. \* References for Figure S1: [1-4]. GMQE (Global Model Quality Estimation) > 0 is an estimator for good accuracy of a model built with that alignment and template structure [5]. The QMEAN (Qualitative Model Energy ANalysis) > (-4) provides reliability estimation for the "degree of nativeness" of the structural features in the model [6].

**Fig S2**

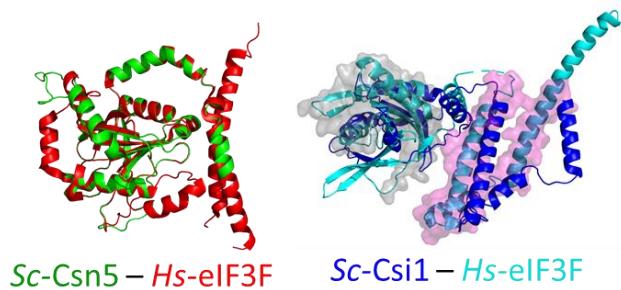


**Supplemental Figure S2 (for Figure 2): Evaluating interactions between CSN subunits.**

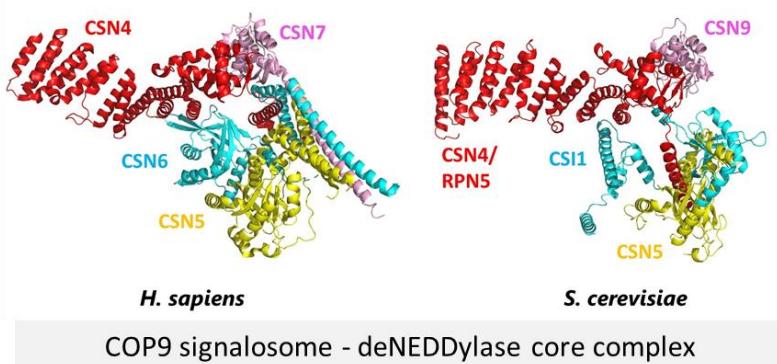
Protein-fragment Complementation Assay (PCA) was approached to detect proximal interactions between CSN subunits. The assay is based on an optimized *S. cerevisiae* cytosine deaminase (FCY1) as a reporter that allows death and survival assay by deaminating cytosine to the essential uracil (survival assay) but also deaminates 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU), which forms a toxic compound that causes death (death assay). If the candidate proteins interact with each other, the 2 distinct parts of the FCY1 enzyme (A, green and red) are reconstituted and the enzyme becomes active. Accordingly, yeast cells can grow in selection medium lacking uracil, but contain cytosine or no longer grow in medium containing uracil but complemented with 5-FC (B, top and middle). The lack of interaction between the two reporter parts, leads to opposite results (B, bottom).

**Fig S3**

A



B

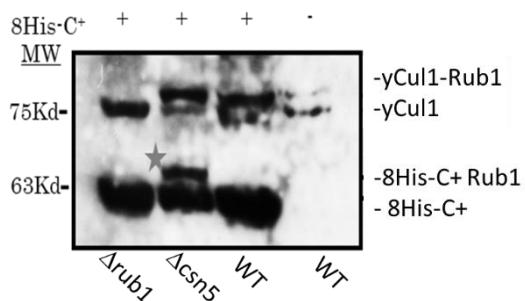


**Supplemental Figure 3 (for Figure 2C, D): Superimposition of the *S. cerevisiae* CSN deNEDDylase core complex with the corresponding *H. sapiens* CSN**

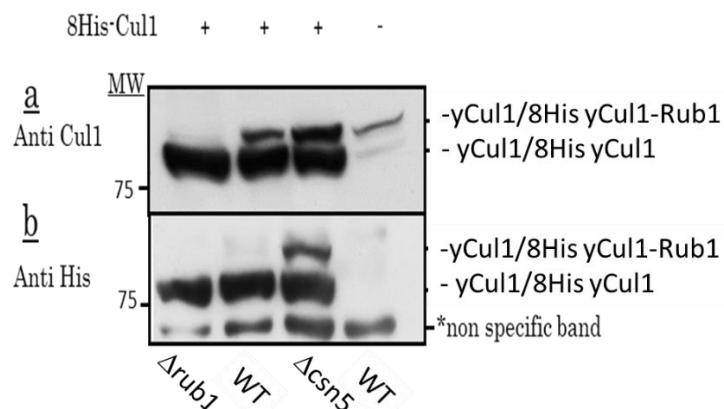
(A) Superimposition of the *S. cerevisiae* Csn5 (green) and Csi1 (blue) homology models with their corresponding *H. sapiens* Csn5 and CSN6 template structures (Red and cyan respectively, PDB ID 4D18 for Csn5 and eIF3F PDB ID A5T5 for Csi1). Grey and pink surfaces representation is shown for the CSN6/Csi1 MPN and S6CD domains, respectively. (D) Empirical 3D structure of the human and *S. cerevisiae* CSN deNEDDylase core complexes. *S. cerevisiae* homology models were built using Swiss-Model for Csn5, CSN4/Rpn5 and CSN7/Csn9, or Phyre<sup>2</sup> for Csi1 (using eIF3F as a template). Note that residues that are not aligned with the sequence of the template are not shown in this predicted comparative model.

## Fig S4

A



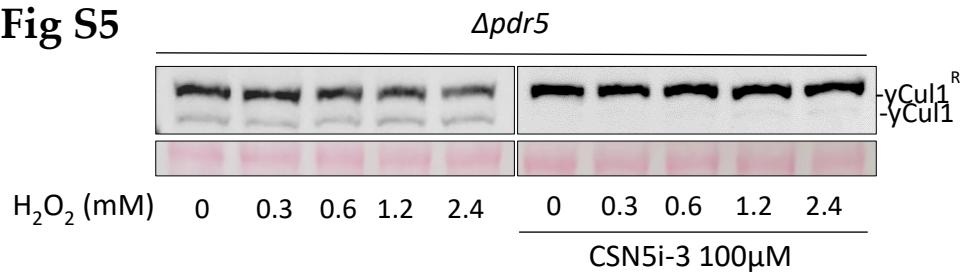
B



**Supplemental Figure 4 (for Figure 3).**

- (A) **Neddylation status of the 8His-C548 truncation mutant.** Total cell extract of logarithmic wildtype,  $\Delta_{csn5}$  and  $\Delta_{nedd8}$  mutant strains, expressing 8His-C548 used for immunoblotting with anti-yCul1 antibody.
- (B) **Neddylation status of ectopic 8His-Cul1.** Total cell extract of logarithmic wildtype,  $\Delta_{csn5}$  and  $\Delta_{nedd8}$  mutant strains, expressing 8His-yCul1 (FL815) approached for immunoblotting with (a) anti-yCul1 antibody and (b) anti-His antibody.

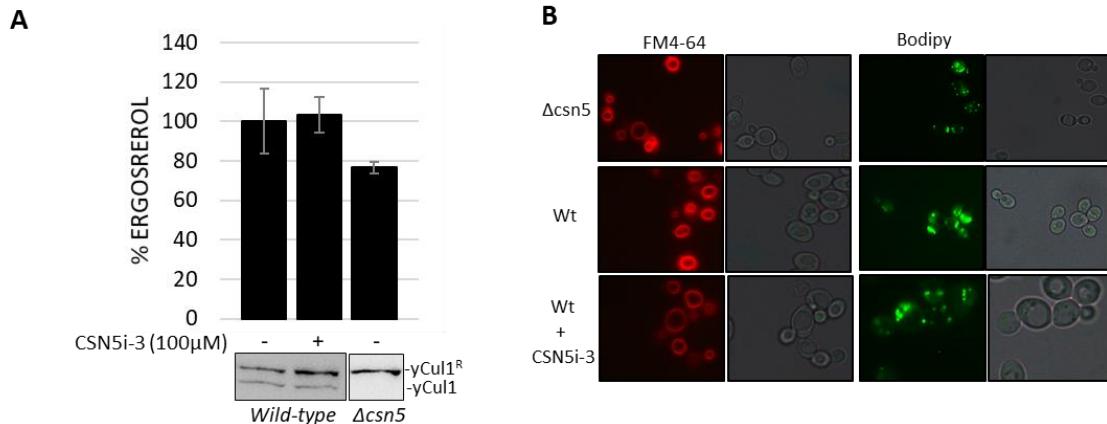
**Fig S5**



**Supplemental Figure 5 (for Figure 6): Cullin neddylation status in *Δpdr5* cells is not affected by  $\text{H}_2\text{O}_2$ .**

Logarithmic *Δpdr5* cells were treated with  $\text{H}_2\text{O}_2$  as indicated. With or without the addition of 100  $\mu\text{M}$  CSN5i-3. Representative immunoblots of anti-yCul1 (7.5% SDS PAGE) show yCul1neddylation status. The results show that unlike the wildtype strain [7], the addition of  $\text{H}_2\text{O}_2$  has neither led to alterations in cullin neddylation status (left), nor inhibited CSN activity (right).

## Fig S6



**Supplemental Figure 6 (for Figure 6): Testing the phenotype of *S. cerevisiae* cells pre-treated with CSN5i-3.**

*S. cerevisiae* wildtype and  $\Delta$ csn5 mutant cells were diluted to 0.5 OD<sub>600</sub> and grown 4 hours to the log phase. Samples were treated with the CSN5i-3 inhibitors for 2 hours. The percentage of ergosterol upon treatment with the inhibitor (200  $\mu$ M) in each strain was determined according to Sinha et al. 2020 [8] (A, top). Inhibition of yCul1 deneddylation by the inhibitor is shown below the graph (A, bottom). The same culture was used to evaluate if the Csn5i-3 treatment confer to wildtype cells phenotypes of  $\Delta$ csn5 mutant cells (B). Accordingly, vacuole morphology was assessed by FM46-4 (16  $\mu$ M) and natural lipids by treatment with BODIPY493/503 (1  $\mu$ M). No changes in wildtype morphology was observed. Intensities were observed with a NIKON Eclipse E600 fluorescent microscope. Representative images are shown.

**Table S1 (Plasmids)**

Number	Name	Description	Source
EP53	Empty vector	(Leu) Yeplac181 including ADH1 <i>p</i> promoter and terminator	[9]
EP115	Rub1-GG	(Leu) Yeplac181, ADH <i>p</i> , RGS-Rub1 $\Delta$ N77	This study
EP153	8HIS-Ub	(Leu) Yeplac181, ADH <i>p</i> , RGS-8HIS-Ub (k0)	[10]
EP172	$\Delta$ N623	(Leu) Yeplac181, ADH1 <i>p</i> , RGS-8His -Cul1 (623-815aa)	This study
EP181	6His-Flag-Rub1	(Leu) Yeplac181, ADH <i>p</i> , 6His-Flag-Rub1(intron-free)	This study
EP233	GFP-Rub1	(Ura) pYES2, GAL1 <i>p</i> , ADH <i>p</i> , GFP-Rub1	This study
EP237	8His-yCul1	(Leu) Yeplac181, ADH <i>p</i> , Cul1 (1-815aa) (Full length)	This study
EP238	8His-C <sup>+</sup>	(Leu) Yeplac181, ADH <i>p</i> , Cul1 (267-815aa)	This study
EP247	$\Delta$ N438	(Leu) Yeplac181, ADH <i>p</i> , 8HIS- Cul1 (438-815aa)	This study
EP248	$\Delta$ N522	(Leu) Yeplac181, ADH <i>p</i> , 8His-Cul1 (522-815aa) under	This study
EP250	PCA F1	(His) GAL <i>p</i> , F1 yeast cytosine deaminase	
EP251	PCA F2	(Leu) GAL <i>p</i> , F2 yeast cytosine deaminase	
EP252	PCA F2 CSN5	(Leu) GAL <i>p</i> , F2 yeast cytosine deaminase CSN5	This study
EP258	ADH-GFP-Nedd8	(Ura) ADH <i>p</i> , GFP-Nedd8	This study
EP259	PCA F2 CSN9	(Leu) GAL <i>p</i> , F2 yeast cytosine deaminase CSN9	This study
EP260	PCA F1 CSI1	(His) GAL <i>p</i> , F1 yeast cytosine deaminase CSI1	This study

**Table S2 (*S. cerevisiae* strains)**

Name	Strains	Genotype	Source
YP65	wildtype	BY4741; ( his3ko; leu2ko; met15ko; ura3ko); Mat a	Open Biosystems
YP193	wildtype	W303; (Ura3-52,Lys2-807,ade2-101,trp1-^36, his3-^200, leu2-^1); Mat a	[8]
YP333	$\Delta csn5$	W303; $\Delta$ YDL216C::G418, Mat a	[8]
YP146	$\Delta csn5$	<del>BY4742; His3ko1; leu2ko0; lys2ko0; ura3ko0; YDL216C::G418; mat alpha</del>	Open Biosystems
YP142	$\Delta rub1$	BY4742; YDR139C::G418; mat alpha	Open Biosystems
YP67	$\Delta pdr5$	W303; pdr5::hisG, Mat a	
YP 147	$\Delta csn5$	BY4741; his3ko; leu2ko; met15ko; ura3ko YDL216C::G418	Open Biosystems
YP 331	$\Delta rub1$	W303; YDR139C::G418, Mat a	[8]
YP 421	$\Delta csn5 \Delta nedd8$	BY4741; YDL216C::hisMX6 YDR139c:kanMX4 Mat a	This study
YP 485	CSN9-MYC Csn10-CBP	CSN9 and CSN10 tagged with MYC and CBP respectively	[9]
YP480	PCA parental strain ( $\Delta fcy1$ )	BY4741; YPR062W: kanMX4 Mat a	Open Biosystems

**Table S3 (DNA primers)****3.1.1 Primers**

<b>Num.</b>	<b>Description</b>	<b>Sequence</b>
<b>Pr48</b>	8His-Cul1 NTD (forward) designed for ligation with plasmid EP153	GATCGCATCACCACCATCACCATCACCAT CACGGATCCGAGACTCTGCCTAGATCT
<b>Pr49</b>	8His-C <sup>+</sup> NTD (forward) designed for ligation with plasmid EP153	GATCGCATCACCACCATCACCATCACCAT CACGGATCCACAATATATTGGGATGATCAT
<b>Pr50</b>	ΔN438 (forward) designed for ligation with plasmid EP153	GATCGCATCACCACCATCACCATCACCAT CACGGATCCCTAGCTAAGTACAGTGATAT
<b>Pr51</b>	ΔN522 NTD (forward) designed for ligation with plasmid EP153	GATCGCATCACCACCATCACCATCACCAT CACGGATCCTTCAAGATATTAGACTTTCC
<b>Pr52</b>	ΔN623 NTD (forward) designed for ligation with plasmid EP153	GATCGCATCACCACCATCACCATCACCAT CACGGATCCTTCAACTTACGGTAACACT
<b>Pr54</b>	Cul1 (reverse) designed for ligation with plasmid EP153	GCTTATTAGAAAGTGTCTAGAGG ATCCCTGCAGTTAACAGCAAGGTAAGC ATACG
<b>Pr55</b>	GFP-Nedd8 (forward) designed for ligation with plasmid EP222	ATTACACATGGCATGGATGAACATAT ACAAAATTGTTAAAGTGAAGACACTG
<b>Pr56</b>	Nedd8 (reverse) designed for ligation with plasmid EP222	ATAACTAATTACATGATGCCCTC TAGACTAACCTCTTAGTGTAA

**Table S4** (complementary for Figure 1).

<i>H. sapiens</i>	NEDD8 E1-activating enzyme	CSN5 with CSN5i-3
VS		
<i>S. cerevisiae</i>	UBA3 <sup>[*1]</sup>	CSN5 with CSN5i-3 <sup>[*2]</sup>
Sequence identity (%)	47%	27.07%
Coverage (%)	96%	44%
GMQE	0.78	0.18
QMEAN	-2.16	-2.85

[\*1] Built on the human NEDD8-activating enzyme in complex with NEDD8 and MLN4924 crystal structure (PDB ID:3GZN, [11]). [\*2] Built on PDB ID 5JOG [12].

## Supplemental Alignment

### Sequences with a high confidence alignment -

Oligo-State ⓘ  
Monomer

Ligands ⓘ  
1 x ZN ⓘ  
1 x ZINC ION ⓘ  
ZN.2: 4 residues within 4A: ⓘ  
4 PLIP interactions: ⓘ

GMQE ⓘ QMEAN ⓘ  
0.78 -2.16 ⓘ

Model 03 ▾  
Structure Assessment

Global Quality Estimate Local Quality Estimate Comparison

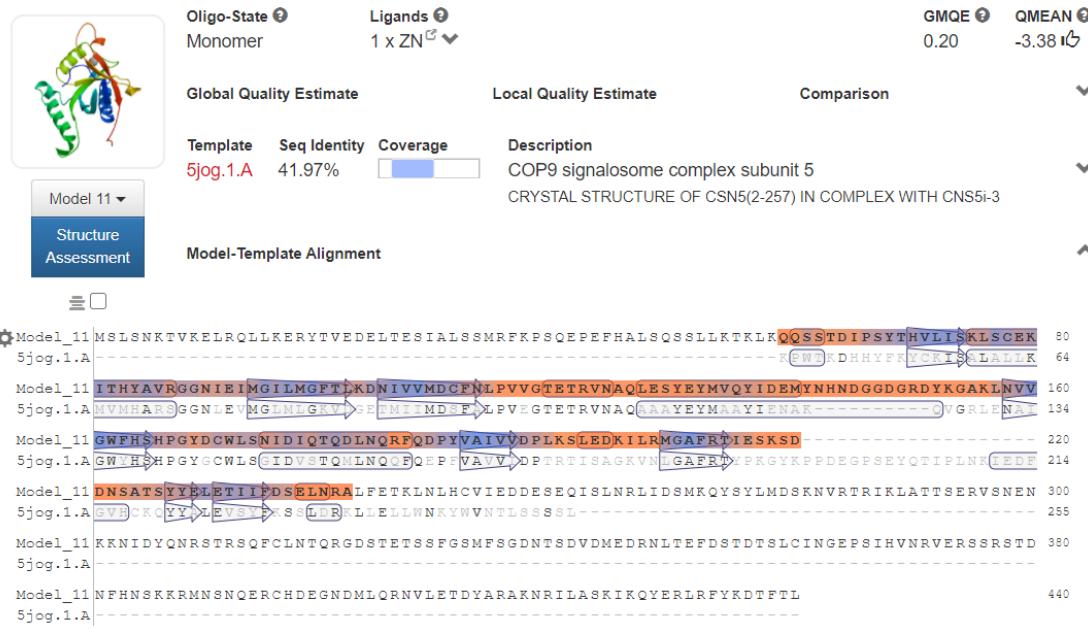
Template Seq Identity Coverage Description  
3gzn.1.B 47.04% NEDD8-activating enzyme E1 catalytic subunit  
Structure of NEDD8-activating enzyme in complex with NEDD8 and MLN4924

Model-Template Alignment

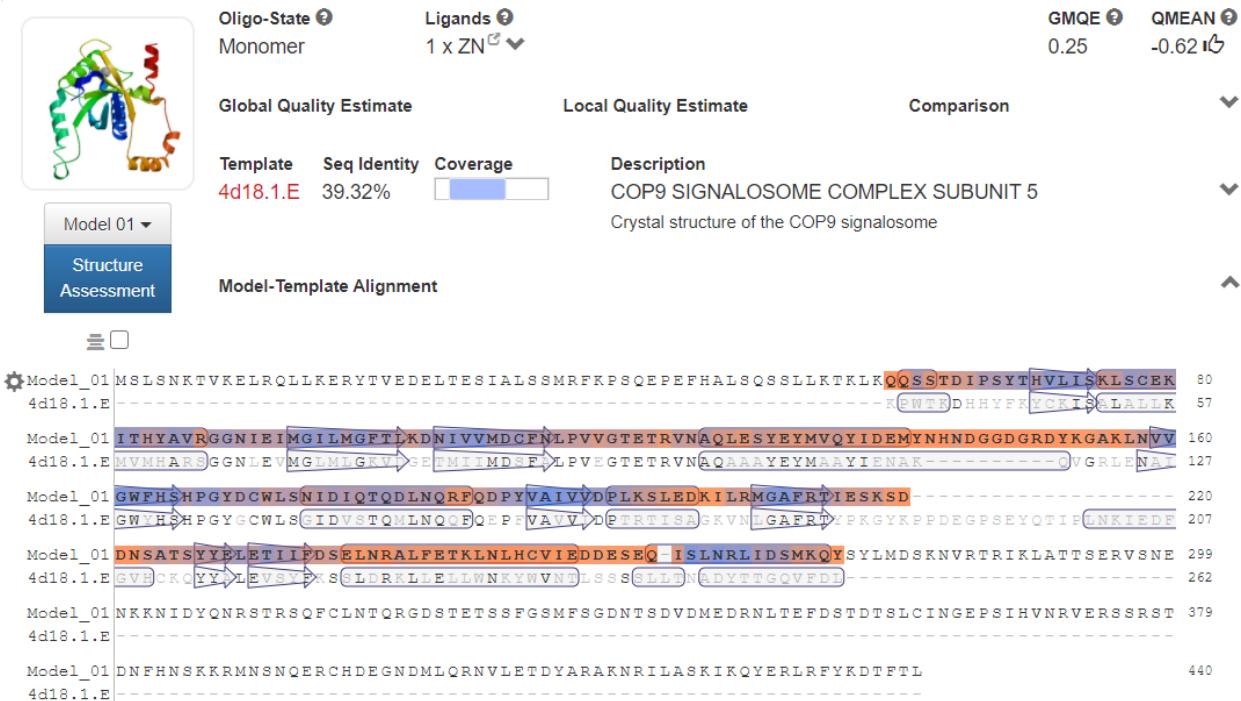
Model_03	3gzn.1.B	Length
MDCKILVLGAGGLGCIELKNLTM	CKVLVSGAGSLGCE	80
LSFVKOVHIVDIDDIETLN	LLKNA	146
NRQFLCDKDIGKPKAQVA	QFLFREKDGPKAEVAA	
QYVNTRFPQLEVVVAH	FLNDRVPNQNVVDFN	
YQ	DFN	
Model_03	DLTTLPPSFYKDFQFIISGLDAIEP	153
3gzn.1.B	RQFHIVVLDSTIARRWINGML	223
TE	LL	
YVSTI	SSIVPLIDGGT	
QYPD	FGKGNARVLPGMTAC	
LNIE	EC	
STADMEFLLKECCERA	PGMTAC	
SISTEK	EC	
LS	EC	
TS	EC	
Model_03	SIDTLPSQQDTVP	224
3gzn.1.B	MCTIAFPQVN-FPMCTIASMPRI	300
YV	QPPGEGVPLDGDD	
PD	FEHQWIFQKSLERAS	
LN	YINI--RGV	
LE	YR	
LS	YR	
TS	YR	
TS	YR	
Model_03	FILGI	299
3gzn.1.B	IKSIIIPSV	373
ST	ST	
TNA	AV	
MA	CATEVFKI	
AT	YAYIPL	
CC	NN	
QMV	DN	
KIYND	LVFDV	
LIDLEN	GL	
GNNFTLINCSEGC	Y	
FMYS	DA	
PKFERLPDCTVCSNSNSN	ERKENC	
TS	CPACSQL	
TS	YR	

For figure 1B: *S. cerevisiae* Uba3, built on the structure of NEDD8-activating enzyme in complex with NEDD8 and MLN4924 (PDB ID: 3GZN).

GMQE = 0.78. QMEAN = -2.16. Sequence identity = 47.04%. Coverage = 96%.



For figure 1C: *S. cerevisiae* Csn5, based on the structure of the human Csn5 in complex with CSN5i-3 (PDB ID: 5JOG) GMQE = 0.20, QMEAN = -3.38. Seq identity = 41.97%. Coverage = 44%.



For figures 2C (left), 2D and S1A: *S. cerevisiae* Csn5, built on the template of the human CSN5 crystal structure (PDB ID: 4D18).

GMQE = 0.25. QMEAN = -0.62. Sequence identity = 39.32%. Coverage = 47%.

Oligo-State: Monomer

Ligands: 1 x ZN 1 x ZINC ION ZN.1: 4 residues within 4Å: 5 PLIP interactions:

GMQE: 0.21 QMEAN: -2.09

**Model 02 ▾**

**Structure Assessment**

Template	Seq Identity	Coverage	Description
4wsn.1.E	42.11%		COP9 signalosome complex subunit 5 Crystal structure of the COP9 signalosome, a P1 crystal form

**Model-Template Alignment**

```

Model_02|MSLSNRTVKELRQLLKERYTVEDELTESIALSSMRFKPSQEPFHALSQSSLLTKLKQQSSTDIPSYTHVLISKLSCEK| 80
4wsn.1.E|-----KRWTKDHHYFKYCKGCLALDK| 55

Model_02|I THYAVRGNGNIEIMGILMGFTIKDNIVVMDCFNLPVVGTETRVNAQLESYEMVQYIDEMYNHNDGGDGRDYKGAKLNVV| 160
4wsn.1.E|MVMHARSGNLEVMGMLMGKVGETMTIMDSFLPVEGETDVNAQARAYEYMAARYENAK| 125

Model_02|GWPHSHPGYDCWLSSNIDIQTQDLNQRFQDPYVAIVVDPLKSLEDKILRMGAFFDIESKSD| 220
4wsn.1.E|GWPHSHPGYGCWLSSIDVSTQMLNQDFQEPFVAVVDPFRFISAGKVN| 205

Model_02|DNSATSVYPLETIDFDSBLNRALFETKLNLHCVIEDDESEQISLNRLIDS MKQYSYLMDSKNVRTRIKLATTSERVSNN| 300
4wsn.1.E|GVHCKQYYLEVDFKSLDRKLELLWNRYWVN| 243

Model_02|KKNIDYQNRSTRSQFCINTQRGDSETSSFGSMFSGDNTSDVDMEDRNLTEFDSTDTSCLINGEPSIHVNVERSSRSTD| 380
4wsn.1.E|NFHNSKKRMNSNQERCHDEGNDMLQRNVLETDYARAKNRILASKIKQYERLRFYKDTFTL| 440

```

For figure S1: *S. cerevisiae* Csn5, built on the template of the human CSN5 crystal structure (PDB ID: 4WSN).

**GMQE = 0.21. QMEAN = -2.09. Sequence identity = 42.11%. Coverage = 43%.**

**Oligo-State** Monomer    **Ligands** None    **GMQE** 0.19    **QMEAN** -3.16

**Global Quality Estimate**    **Local Quality Estimate**    **Comparison**

**Template** 6r7f.1.E    **Seq Identity** 42.11%    **Coverage**

**Description** COP9 signalosome complex subunit 5  
Structural basis of Cullin-2 RING E3 ligase regulation by the COP9 signalosome

**Model-Template Alignment**

```

Model_08 MSLSNKTVKELRQLLKERYTVEDELTESIALSSMRFKPSQEPEFHALSQSSLLKTKLQQSSTDIPSYTHVLSKLSCEK 90
6r7f.1.E -KEWWDHHDHYFRYCPGSKALLK 41

Model_08 ITHYAVRGGNIEIMGILMGFTLKDNDIIVVMDCFNLPVVGTETRVNAQLESYEYMVQYIDEMYNHNDDGGDGRDYKGAKLNVV 160
6r7f.1.E MVVMHARSAGGNLEV MGLMLGKVNSGEMIIMDSFLPVEGTETRVNAQAAAYEYMAVYIENAK-----QVGRLEN 111

Model_08 GWFHSHPGYDCWLNSNIDIQTDLNQRFQDPYVAIVVDPLKSLEDKILRMGAFTIESKSD 221
6r7f.1.E GWIHDHPGYGCWLSDIDVSTQMLNQCFQEPFVAVVDPTRTISAGKVNNGAFRDYPKGYKPPDEGPSEYQTIPLNKREDF 191

Model_08 NSATSYYELETIDFSELNRALFETKLNHLHCVIEDDESEQISLNRLIDSMSKQYSYLMDSKNVRTRIKLATTSERVSNE 300
6r7f.1.E GVHCKQYYALEVYIFKSSLRKLLELLWNKYWNTLSS 229

Model_08 KKNIDYQNRSTRSQFCCLNTQRGDSTETSSFGSMFSGDNTSDVDMDRNLTEFDSTDTSLCINGEPSIHVNRRVERSSRSTD 380
6r7f.1.E ----

Model_08 NFHNSKKRMNSNQERCHDEGNDMLQRNVLETDYARAKNRILASKIKQYERLRFYKDTFTL 440
6r7f.1.E -----

```

For figure S1: *S. cerevisiae* Csn5, built on the template of the human CSN5 crystal structure (PDB ID: 6R7F).

GMQE = 0.19. QMEAN = -3.16. Sequence identity = 42.11%. Coverage = 43%.

**Oligo-State** Monomer    **Ligands** 1 x ZN    **GMQE** 0.18    **QMEAN** -3.13

**Global Quality Estimate**    **Local Quality Estimate**    **Comparison**

**Template** 5w83.1.B    **Seq Identity** 27.37%    **Coverage**

**Description** Ubiquitin carboxyl-terminal hydrolase RPN11  
Rpn8/Rpn11 dimer complex

**Model-Template Alignment**

```

Model_12 MSLSNKTVKELRQLLKERYTVEDELTESIALSSMRFKPSQEPEFHALSQSSLLKTKLQQSSTDIPSYTHVLSKLSCEK 80
5w83.1.B -TGRDDTKEAVYVLSKALLK 36

Model_12 ITHYAVRGGNIEIMGILMGFTLKDNDIIVVMDCFNLPVVGTETRVNAQLESYEYMVQYIDEMYNHNDDGGDGRDYKGAKLNVV 159
5w83.1.B MLIKHGGRAGVPMEV MGLMLGKFVYDVNVVVDVFEYMPQSGTGVSVEAVDQVFQ---AKMMMDML-----KOTGRDQMV 104

Model_12 GWFHSHPGYDCWLNSNIDIQTDLNQRFQDPYVAIVVDPLKSLEDKILRMGAFTIESKSD 220
5w83.1.B GVWVHSHPGFGCWLS SVDVNTQKSFETQINSRAVAVVVDPIQSVB-GKVIDAFR-----IDTGALINNLEPRQTTSNGLNKA 183

Model_12 DNSATSYYELETIDFSELNRALFETKLNHLHCVIEDDESEQISLNRLIDSMSKQYSYLMDSKNVRTRIKLATTSE 294
5w83.1.B NIQALIHIQLNRHYYSLNIDYKXAKETKMLMLNLHKEQWQ----- 222

Model_12 RVSNEENKKNIDYQNRSTRSQFCCLNTQRGDSTETSSFGSMFSGDNTSDVDMDRNLTEFDSTDTSLCINGEPSIHVNRRVER 374
5w83.1.B ----

Model_12 SSRSTDNFHNSKKRMNSNQERCHDEGNDMLQRNVLETDYARAKNRILASKIKQYERLRFYKDTFTL 440
5w83.1.B -----

```

For figure S1: *S. cerevisiae* Csn5, built on the template of the human CSN5 crystal structure (PDB ID: 5W83).

GMQE = 0.18. QMEAN = -3.13. Sequence identity = 27.37%. Coverage = 43%.

**Oligo-State** Monomer    **Ligands** None    **GMQE** 0.76    **QMEAN** -2.61

**Global Quality Estimate**    **Local Quality Estimate**    **Comparison**

**Template** 5mpd.1.I    **Seq Identity** 100.00%    **Coverage**

**Description**  
26S proteasome regulatory subunit RPN5  
26S proteasome in presence of ATP (s1)

**Model-Template Alignment**

Model\_01 | M S R D A P I K A D K D Y S Q I L K E E F P K I D S L A Q N D C N S A L D Q L L V L E K T R Q A S D L A S S K E V L A K I V D L L A S R N K W D D L N E Q L T | 80  
 5mpd.1.I | M S R D A P I K A D K D Y S Q I L K E E F P K I D S L A Q N D C N S A L D Q L L V L E K T R Q A S D L A S S K E V L A K I V D L L A S R N K W D D L N E Q L T | 80

Model\_01 | L L S K K H G Q L K L S I Q Y M I Q K V M E Y L K S S K S L D L N T R I S V I E T I R V V T E N K I F V E V E R A R V T K D L V E I K K E E G K I D E A A D I L | 160  
 5mpd.1.I | L L S K K H G Q L K L S I Q Y M I Q K V M E Y L K S S K S L D L N T R I S V I E T I R V V T E N K I F V E V E R A R V T K D L V E I K K E E G K I D E A A D I L | 160

Model\_01 | C E L Q V E T Y G S M E M S E K I Q F I L E Q M E L S I L K G D Y S Q A T V L S R K I L K K T F K N P K Y E S L K L E Y Y N L L V K I S L H K R E Y L E V A Q Y | 240  
 5mpd.1.I | C E L Q V E T Y G S M E M S E K I Q F I L E Q M E L S I L K G D Y S Q A T V L S R K I L K K T F K N P K Y E S L K L E Y Y N L L V K I S L H K R E Y L E V A Q Y | 240

Model\_01 | L Q E I Y Q T D A I K S D E A K W K P V L S H I V Y F L V L S P Y G N L Q N D L I H K I Q N D N N L K K L E S Q E S L V K L F T T N E L M R W P I V Q K T Y E P | 320  
 5mpd.1.I | L Q E I Y Q T D A I K S D E A K W K P V L S H I V Y F L V L S P Y G N L Q N D L I H K I Q N D N N L K K L E S Q E S L V K L F T T N E L M R W P I V Q K T Y E P | 320

Model\_01 | V L N E D D L A F G G E A N K H H W E D L Q K R V I E H N L R V I S E Y Y S R I T L L R L N E L L D L T E S Q T E T Y I S D L V N Q G I I Y A K V N R P A K I V | 400  
 5mpd.1.I | V L N E D D L A F G G E A N K H H W E D L Q K R V I E H N L R V I S E Y Y S R I T L L R L N E L L D L T E S Q T E T Y I S D L V N Q G I I Y A K V N R P A K I V | 400

Model\_01 | N F E K P K N S S Q L L N E W S H N V D E L L E H I E T I G H L I T K E E I M H G L Q A K | 445  
 5mpd.1.I | N F E K P K N S S Q L L N E W S H N V D E L L E H I E T I G H L I T K E E I M H G L Q A K | 445

For figure 2D: *S. cerevisiae* Rpn5, built on the template of the *S. cerevisiae* 26S proteasome Rpn5 crystal structure (PDB ID: 5MPD).

GMQE = 0.76. QMEAN = -2.61. Sequence identity = 100%. Coverage = 100%.

**Oligo-State** Monomer    **Ligands** None    **GMQE** 0.39    **QMEAN** -1.20

**Global Quality Estimate**    **Local Quality Estimate**    **Comparison**

**Template** 3chm.1.A    **Seq Identity** 23.39%    **Coverage**

**Description**  
COP9 signalosome complex subunit 7  
Crystal structure of PCI domain from *A. thaliana* COP9 signalosome subunit 7 (CSN7)

**Model-Template Alignment**

Model\_01 | M V M R E E T I K S L E D P Y K Y H Y K E E W L | 68  
 3chm.1.A | -- S P L I T E A T H P S I L F A P S E I T I A L E N V A Q L E G T T D S V Y L D L L R L F A R G T W G D Y K C O N A T H P L S E D Q I L K L R Q L T V I T L | 103

Model\_01 | S E I Y N E L S Y E L I K E E C Q I E D D G I I I E S H L I | 145  
 3chm.1.A | A R E S N K V L Y D T D L M V E D V S N V R E L E D F L I N E C M Y P G I V R G K L D Q L K R C F E V P F | 165

Model\_01 | V Q N L R S W E T K L K Q N I L E | 162  
 3chm.1.A | -----

For figure 2D: *S. cerevisiae* Csn9, built on the template of the *A. thaliana* CSN7 crystal structure (PDB ID: 3CHM).

GMQE = 0.39. QMEAN = -1.2. Sequence identity = 23.39%. Coverage = 77%.

In three cases the QMEAN parameters were < (-4). For these cases Phyre2 confidence was calculated as well:

Oligo-State	Ligands	GMQE	QMEAN
Monomer	None	0.50	-4.08 ↗

Global Quality Estimate      Local Quality Estimate      Comparison

Template    Seq Identity    Coverage      Description

**Model 02 ▾**

**Structure Assessment**

**Model-Template Alignment**

Model\_02      4d10.2.F

74      106      154      176      234      250      295      306

Model\_02      4d10.2.F

Model\_02      4d10.2.F

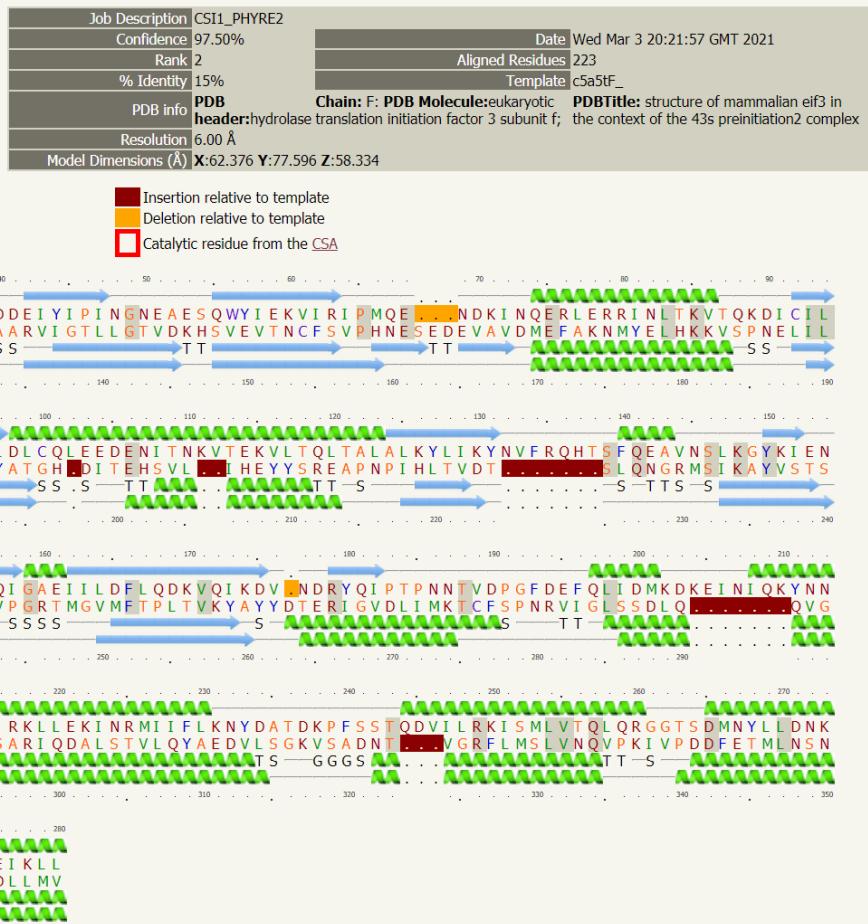
Model\_02      4d10.2.F

Model\_02      4d10.2.F

For figure 2C (left), 2D: *S. cerevisiae* Csi1, built on the template of the human CSN6 crystal structure (PDB ID: 4D10).

GMQE = 0.5. QMEAN = -4.08 (Low quality homology model). Sequence identity = 12.84%. Coverage = 87%.

# Phyre<sup>2</sup>



For figure 2C (left), 2D: *S. cerevisiae* Csi1, built on the template of the *Oryctolagus cuniculus* eIF3F crystal structure (PDB ID: 5A5T).

**Confidence = 97.5%. Sequence identity = 15%. Coverage = 82%.**

Model 01 ▾

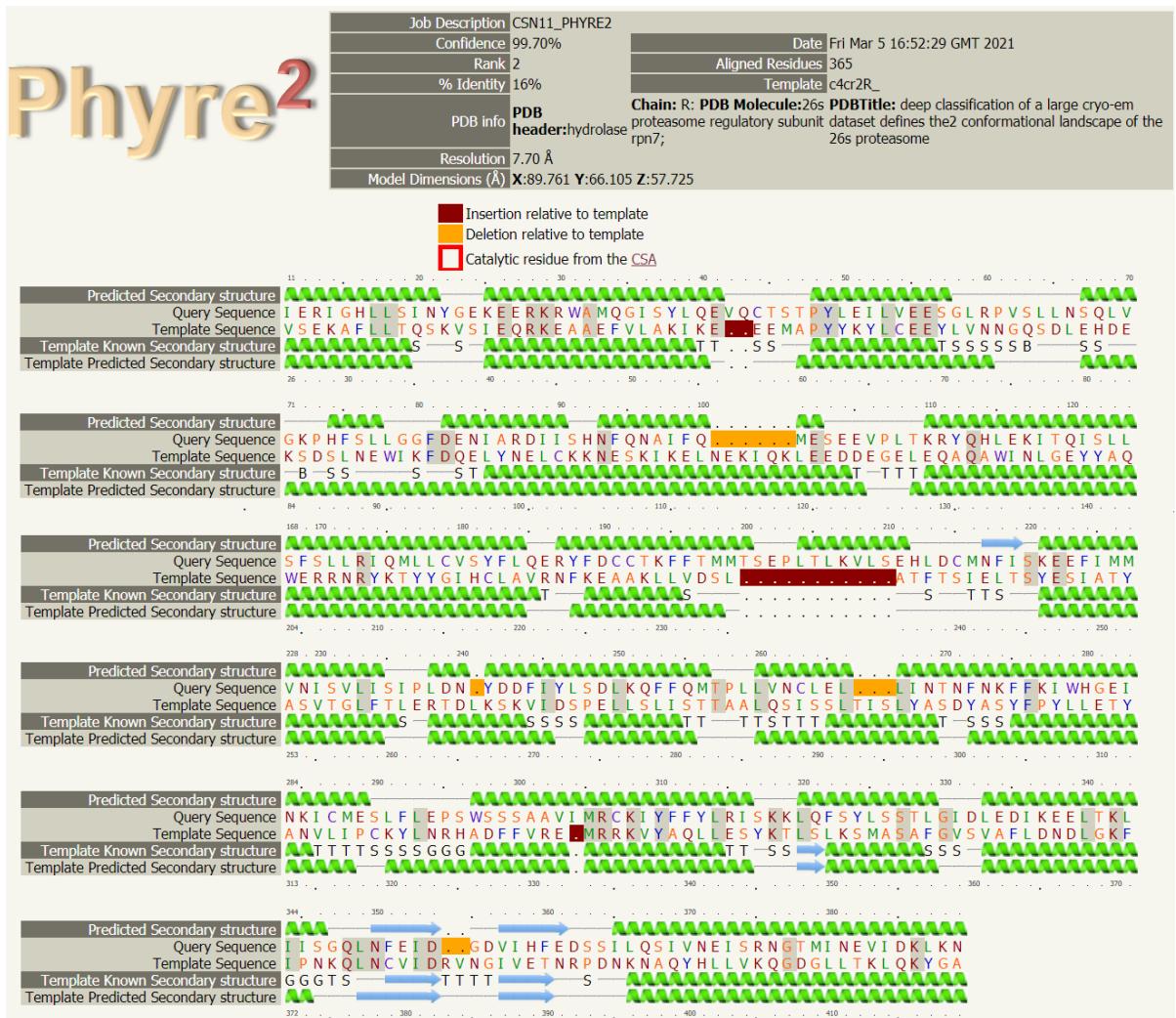
Structure Assessment

Oligo-State: Monomer, Ligands: None, GMQE: 0.38, QMEAN: -5.57

Global Quality Estimate			Local Quality Estimate		Comparison
Template	Seq Identity	Coverage			Description
6fvw.1.c	14.66%	<div style="width: 78%;">78%</div>			26S proteasome regulatory subunit RPN7 26S proteasome, s4 state
Model-Template Alignment					
<pre> Model_01 MFHGAKGPLLIERIGHLLSINYGEKEERKRWAMQGISYLYQEVQCTSTPYLEILVEESGLRPVSSLNSQLVGKPHFSLLGG 80 6fvw.1.c -----NYEVSEKAFLLTQSRSVSIEQRKAAEFVLAKIKEEE--MAPYYKYLCEEYLVNNNGQSDEHDERSDSINEWIK 74  Model_01 FDENIARDIISH-----NFQNAIFQMESEEVPLTKRYQHLE---K 117 6fvw.1.c FDQELYNELCKKNESKIKELNEKIQKLEEDDEGELEQAQAWINLGEYAAQIGDKDNAEKTLGKSLSKAISTGAKIDVMLT 154  Model_01 ITQISLLCKNFKGIEEIEYNVKNIQQGRKNFDMLNSMEKDRISHEVVQDDSFSSLRIQMLLCVSYFLQERYFDCCTKFFT 197 6fvw.1.c IARLGFFYNDQLYVKEKLEAVNSMIEKGG-----DWERRNRYKTYYGIHCLAVRNFKEAKLLVD 214  Model_01 MMTSEPLTLKVLSEHLCMNFIKEFIMMVNISVLISIPLDNYDD-FIYLSDLKQFFQMTPLL---VNCLELLINTNFN 273 6fvw.1.c SLAT-----F---TSIELTSYESIATYASVTGLFTLERTDLKSKVIDSPILLSLISTTAALQSISSLTISLYASDYA 283  Model_01 KFFKIWHGEINKICMESLFLFLEPSWSSSAAVIMRCKIYFFYRISKKLOFSYLSSTLGEDIKEELTKLIISGQLNFE 353 6fvw.1.c SYFPYLLETYANVLIPCKYLNRRHADF-FVREMRRKVYAQILESYKTLSLKSMASAFCGVSVAFLDNLGKFIPNKQLNCVI 362  Model_01 D--GDVHDFEDSSILQSIVNEISRNGTMINEVIDKLKNENTDLKDIIQGNPLMYSGNNNTATIINNESSDDMDIDEVNDR 431 6fvw.1.c DRVNGIVETNRPDNKNAQYHLLVVKQGDGLLTKLQKYGA----- 400  Model_01 SDISDSEGGLFEC 444 6fvw.1.c ----- </pre>					

For table 1: *S. cerevisiae* Csn11, built on the template of the *S. cerevisiae* CSN1 crystal structure (PDB ID: 6FVW).

**GMQE = 0.38. QMEAN = -5.57 (Low quality homology model). Sequence identity = 14.66%. Coverage = 78%.**



For table 2, 3: *S. cerevisiae* Csn11, built on the template of the *S. cerevisiae* Rpn7 crystal structure (PDB ID: 4CR2).

Confidence = 99.7%. Sequence identity = 16%. Coverage = 85%.

Model 03 ▾

**Structure Assessment**

Oligo-State: Monomer   Ligands: None   GMQE: 0.12   QMEAN: -4.74 ⓘ

Global Quality Estimate   Local Quality Estimate   Comparison

Template: 4d18.2.B   Seq Identity: 17.30%   Coverage: [progress bar]

Description: COP9 SIGNALOSOME COMPLEX SUBUNIT 2  
Crystal structure of the COP9 signalosome

Model-Template Alignment

```

Model_03 MSDEDNNYDDFMLSDEGMESIEMEEETDDEDKQNIEINEDNSQDDQDRGAARHKQHEQGTFEKHDRVEDICERIFEQGQ 80
4d18.2.B ----

Model_03 ALKEDERYKEARDLFLKIVYYKEEFSSDESIERLMTWKFKSLIEILRLRALQLYFQKNGAQDQLVLQILEDTATMSVFLQRI 160
4d18.2.B ----

Model_03 DDFQIDGNIFELLSDTDFEVLAFLKWERVFLFDIEKVDRRENICKIDFQKNFMDQFQWILRKPGKDCKLQNLQRITRKKIFIA 240
4d18.2.B ----

Model_03 VVWYQRLTMGNVFTPEISSQIEILVKDNECSSFEENNNDLESVSMLLQQYILEYMNTRINRRLFKCIDFFEMLISKSL 320
4d18.2.B ----

Model_03 TFSQESGLMVILYTSKIVFILDSDSENDLSFALMRYYDRKEELKNMFYILKHLEEMGKLRRERDITSLFHKFILSGIFT 400
4d18.2.B ---HPLAMGVIRECGGKMH-----RGECEKAHTLFFEARFKNYDESGSFR-----RTTCALKYLVLA 287

Model_03 SMILEAISTDKINPFGFEQVKIALGSPIVNVLEDVYRCFAQLELRQLNASISLIP-----LSVVLSGIIQDIYVLAQTLKL 477
4d18.2.B NMIMKSNMKS----GINPFDSQEAQPYRNDEPIEAMTNLV SAYQNNDITEFEKILKANHSNINODPFIHEHIEELLRNIRTQVL 363

Model_03 WRKIARLYSCISIISDIIISMLQISDDNEMTRDDLLTILMRSIMKNRSVVYFKLDLTSIDLVYFGDENKVMLPRCSKEEFLRM 557
4d18.2.B IFLIDRP-YTRIHPFISKEIDNID-----VADVESLLVQCIDNT--IHGRDQVNCLLBDQHQRKG----- 421

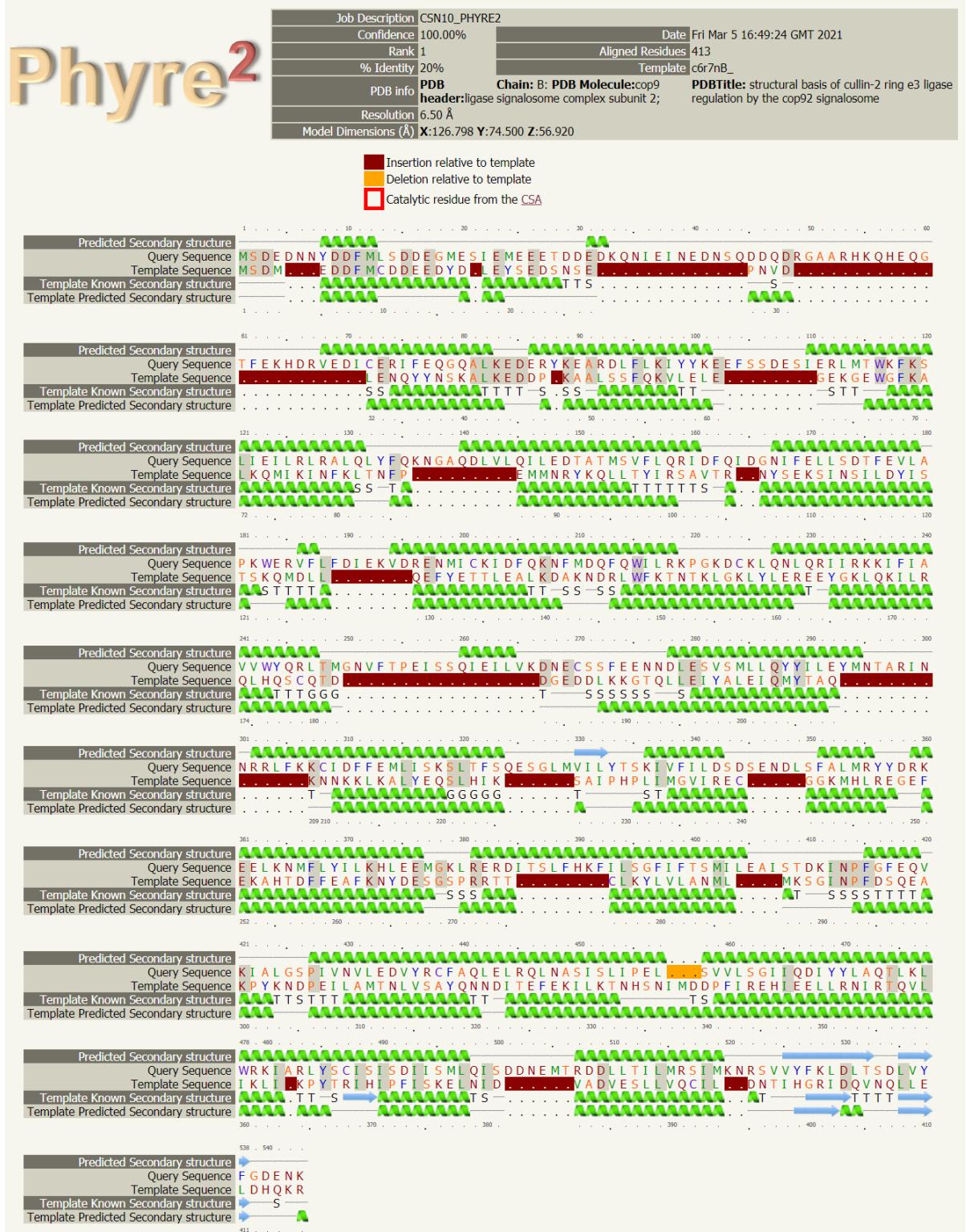
Model_03 ISPKDEETTEKARLIDFEYVNDVAIYNNTTRIRTKSSKEFFNTLRLKSRETVKLPRVSNQSNETDFTLPSYMKFSNKYLELC 637
4d18.2.B ----

Model_03 KLASNNLE 645
4d18.2.B -----

```

For table 1: *S. cerevisiae* Csn10, built on the template of the human CSN2 crystal structure (PDB ID: 4D18).

**GMQE = 0.12 = -4.74 (Low quality homology model). Sequence identity = 17.3%. Coverage = 29%.**



For tables 2, 3: *S. cerevisiae* Csn10, built on the template of the human Cullin-2 RING E3 ligase (CSN2) crystal structure (PDB ID: 6R7N).

Confidence = 100%. Sequence identity = 20%. Coverage = 84%.

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