Supplemental Data for accompanying manuscript entitled:

Saccharomyces cerevisiae as a toolkit for COP9 signalosome research

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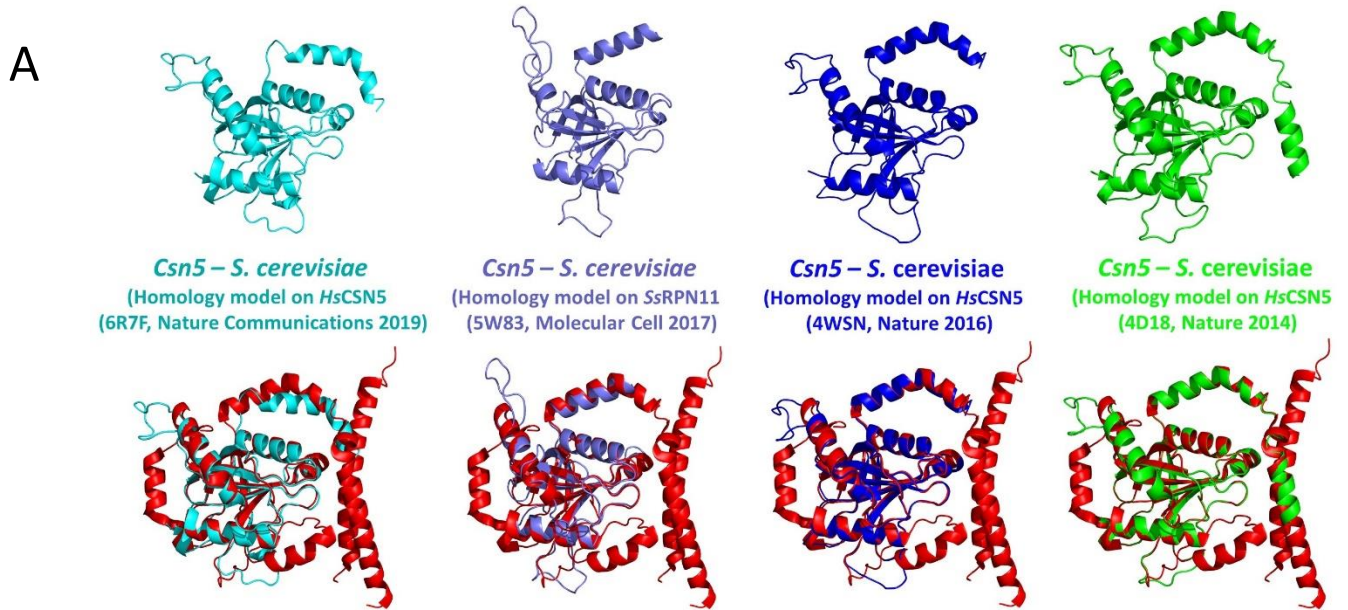
\# Shared co-first authorship

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This File contains:

Fig S1

A

Csn5 – *S. cerevisiae*
(Homology model on *Hs*CSN5
(6R7F, Nature Communications 2019)

Csn5 – *S. cerevisiae*
(Homology model on *Ss*RPN11
(5W83, Molecular Cell 2017)

Csn5 – *S. cerevisiae*
(Homology model on *Hs*CSN5
(4WSN, Nature 2016)

Csn5 – *S. cerevisiae*
(Homology model on *Hs*CSN5
(4D18, Nature 2014)

B

S. cerevisiae CSN5 Homology model based on various CSN5 (H. sapiens) structures

	4D18 Lingaraju et al. 2014	4WSN Cavadini et al. 2016	6R7F Faull et al. 2019	5W83 Worden et al. 2017
Sequence identity (%)	39.3%	42.1%	42.1%	27.37%
Coverage (%)	47%	43%	43%	41%
GMQE	0.25	0.21	0.19	0.18
QMEAN	-0.62	-2.09	-3.16	-3.13

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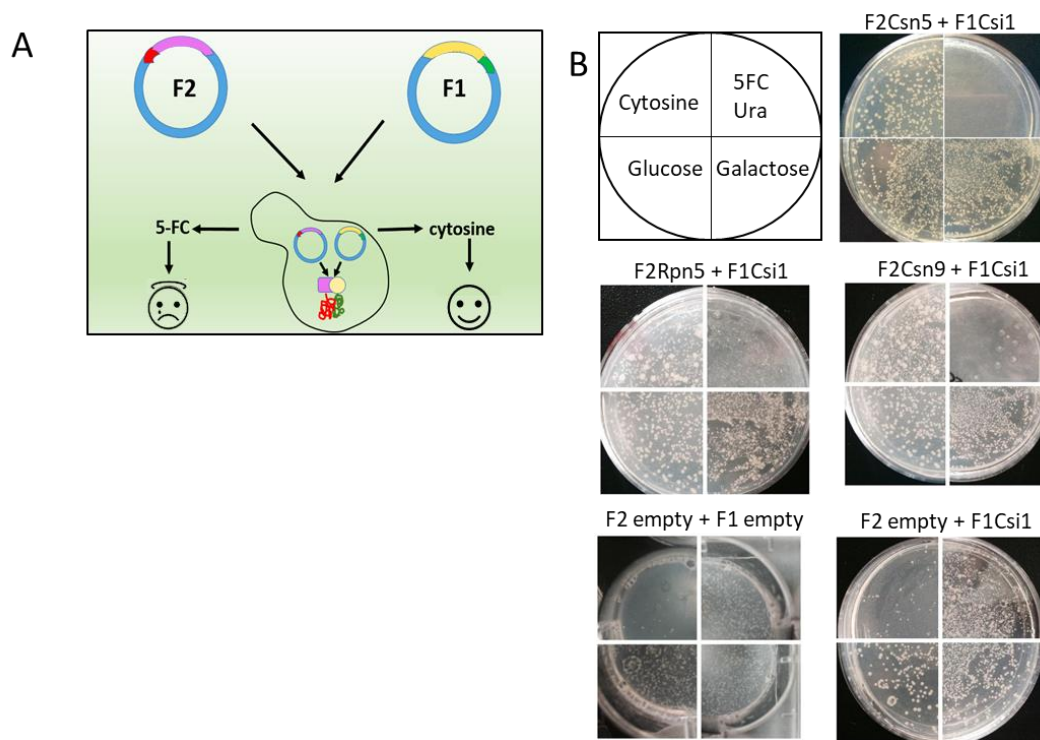
Supplemental Figure S2 (for Figure 2): Evaluating interactions between CSN subunits.

Protein-fragment Complementation Assay (PCA) was approached to detects 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

Sc-Csn5 – *Hs*-eIF3F   *Sc*-Csi1 – *Hs*-eIF3F

B

COP9 signalosome - deNEDDylase core complex
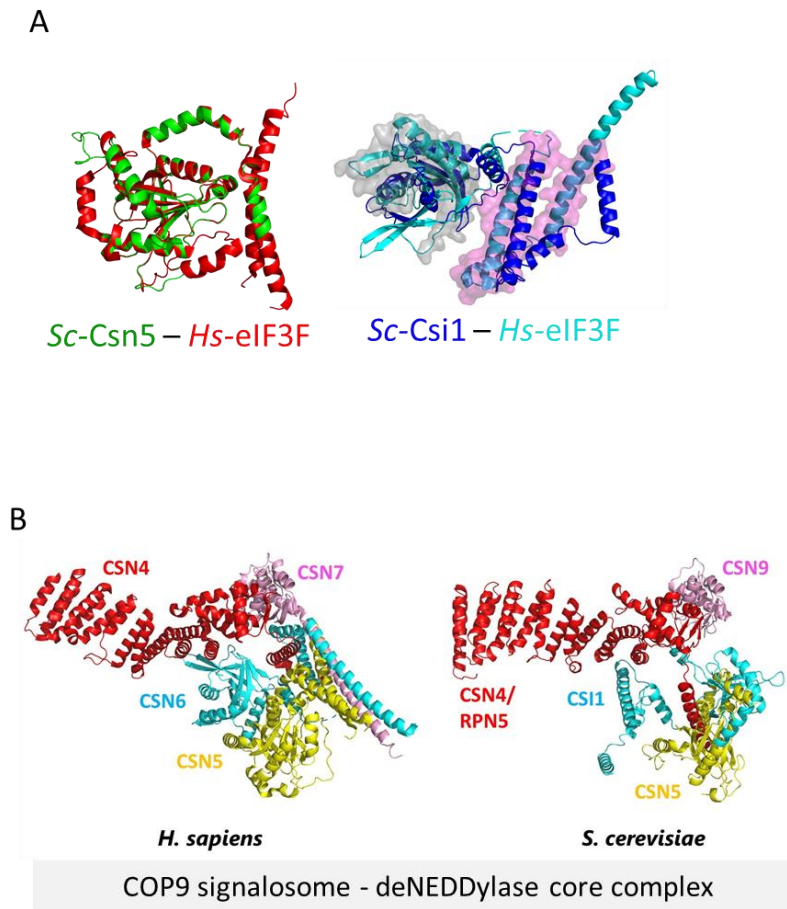
Supplemental Figure 3 (for Figure 2C, D): Superimposition of the *S. cerevisiae* CSN deNEDDylase core complex with the corresponding *H. sapiens* CSN

(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[2] 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.

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Fig S4

A

B
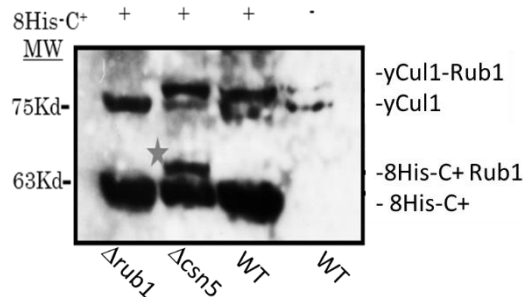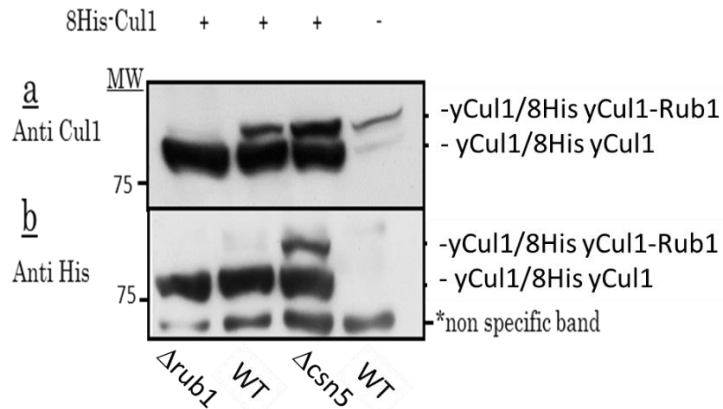
Supplemental Figure 4 (for Figure 3).

(A) Neddylation status of the 8His-C548 truncation mutant. Total cell extract of logarithmic wildtype, *Δcsn5* and *Δnedd8* 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, *Δcsn5* and *Δnedd8* mutant strains, expressing 8His-yCul1 (FL815) approached for immunoblotting with (a) anti-yCul1 antibody and (b) anti-His antibody.

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Fig S5

$\Delta pdr5$

-yCul1^{R}
-yCul1

| H_2O_2 (mM) | 0 | 0.3 | 0.6 | 1.2 | 2.4 | 0 | 0.3 | 0.6 | 1.2 | 2.4 |
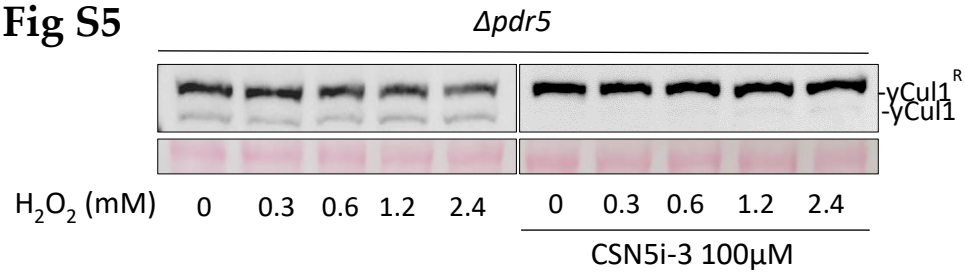
CSN5i-3 100µM

Supplemental Figure 5 (for Figure 6): Cullin neddylation status in $\Delta pdr5$ cells is not affected by H₂O₂.

Logarithmic $\Delta pdr5$ cells were treated with H_2O_2 as indicated. With or without the addition of 100 µM CSN5i-3. Representative immunoblots of anti-yCul1 (7.5% SDS PAGE) show yCul1neddylaiton status. The results show that unlike the wildtype strain [7], the addition of H_2O_2 has neither led to alterations in cullin neddylation status (left), nor inhibited CSN activity (right).

Fig S6

A

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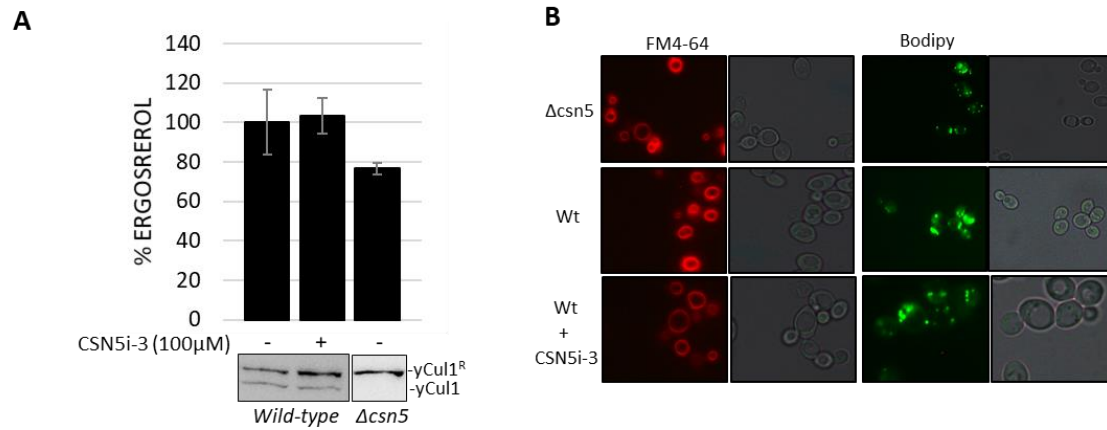
Supplemental Figure 6 (for Figure 6): Testing the phenotype of *S. cerevisiae* cells pre-treated with CSN5i-3.

S. cerevisiae wildtype and *Δcsn5* mutant cells were diluted to 0.5 OD_{600} 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 μM) 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 *Δcsn5* mutant cells (B). Accordingly, vacuole morphology was assessed by FM46-4 (16 μM) and natural lipids by treatment with BODIPY493/503 (1 μM). 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 ADH1p promoter and terminator	[9]
EP115	Rub1-GG	(Leu) Yeplac181, ADHp, RGS-Rub1ΔN77	This study
EP153	8HIS-Ub	(Leu) Yeplac181, ADHp, RGS-8HIS-Ub (k0)	[10]
EP172	ΔN623	(Leu) Yeplac181, ADH1p, RGS-8His -Cul1 (623-815aa)	This study
EP181	6His-Flag-Rub1	(Leu) Yeplac181, ADHp, 6His-Flag-Rub1(intron-free)	This study
EP233	GFP-Rub1	(Ura) pYES2, GAL1p, ADHp, GFP-Rub1	This study
EP237	8His-yCul1	(Leu) Yeplac181, ADHp, Cul1 (1-815aa) (Full length)	This study
Ep238	8His-C$^+$	(Leu) Yeplac181, ADHp, Cul1 (267-815aa)	This study
EP247	ΔN438	(Leu) Yeplac181, ADHp, 8HIS- Cul1 (438-815aa)	This study
EP248	ΔN522	(Leu) Yeplac181, ADHp, 8His-Cul1 (522-815aa) under	This study
EP250	PCA F1	(His) GALp, F1 yeast cytosine deaminase	
EP251	PCA F2	(Leu) GALp, F2 yeast cytosine deaminase	
EP252	PCA F2 CSN5	(Leu) GALp, F2 yeast cytosine deaminase *CSN5*	This study
EP258	ADH-GFP-Nedd8	(Ura) ADHp, GFP-Nedd8	This study
EP259	PCA F2 CSN9	(Leu) GALp, F2 yeast cytosine deaminase *CSN9*	This study
EP260	PCA F1 CSI1	(His) GALp, 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	*Δcsn5*	W303; ΔYDL216C::G418, Mat a	[8]
YP146	*Δcsn5*	BY4742; (his3ko1; leu2ko0; lys2ko0; ura3ko0;): YDL216C::G418; mat alpha	Open Biosystems
YP142	*Δrub1*	BY4742; YDR139C::G418; mat alpha	Open Biosystems
YP67	*Δpdr5*	W303; pdr5::hisG, Mat a	
YP 147	*Δcsn5*	BY4741; his3ko; leu2ko; met15ko; ura3ko YDL216C::G418	Open Biosystems
YP 331	*Δ rub1*	W303; YDR139C::G418, Mat a	[8]
YP 421	*Δcsn5 Δnedd8*	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 (*Δ fcy1*)	BY4741; YPR062W: kanMX4 Mat a	Open Biosystems

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Table S3 (DNA primers)

3.1.1 Primers

Num.	Description	Sequence
Pr48	8His-Cul1 NTD (forward) designed for ligation with plasmid EP153	GATCGCATCACCATCACCATCACCAT CACGGATCCGAGACTCTGCCTAGATC T
Pr49	8His-C⁺ NTD (forward) designed for ligation with plasmid EP153	GATCGCATCACCATCACCATCACCAT CACGGATCCACAATATATTGGGATG ATCAT
Pr50	ΔN438 (forward) designed for ligation with plasmid EP153	GATCGCATCACCATCACCATCACCAT CACGGATCCCTAGCTAAGTACAGTG ATAT
Pr51	ΔN522 NTD (forward) designed for ligation with plasmid EP153	GATCGCATCACCATCACCATCACCAT CACGGATCCTTTCAAGATATTAGACT TTCC
Pr52	ΔN623 NTD (forward) designed for ligation with plasmid EP153	GATCGCATCACCATCACCATCACCAT CACGGATCCTTCAACTTTACGGTAAC ACT
Pr54	Cul1 (reverse) designed for ligation with plasmid EP153	GCTTATTTAGAAGTGTCATCTAGAGG ATCCCTGCAGTTAAGCAAGGTAAGC ATACG
Pr55	GFP-Nedd8 (forward) designed for ligation with plasmid EP222	ATTACACATGGCATGGATGAACTAT ACAAAATTGTTAAAGTGAAGACACT G
Pr56	Nedd8 (reverse) designed for ligation with plasmid EP222	ATAACTAATTACATGATGCGGCCCTC TAGACTAACCACCTCTTAGTGTTA

Table S4 (complementary for Figure 1).

H. sapiens VS *S. cerevisiae*	NEDD8 E1-activating enzyme UBA3 [*1]	CSN5 with CSN5i-3 CSN5 with CSN5i-3 [*2]
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 -
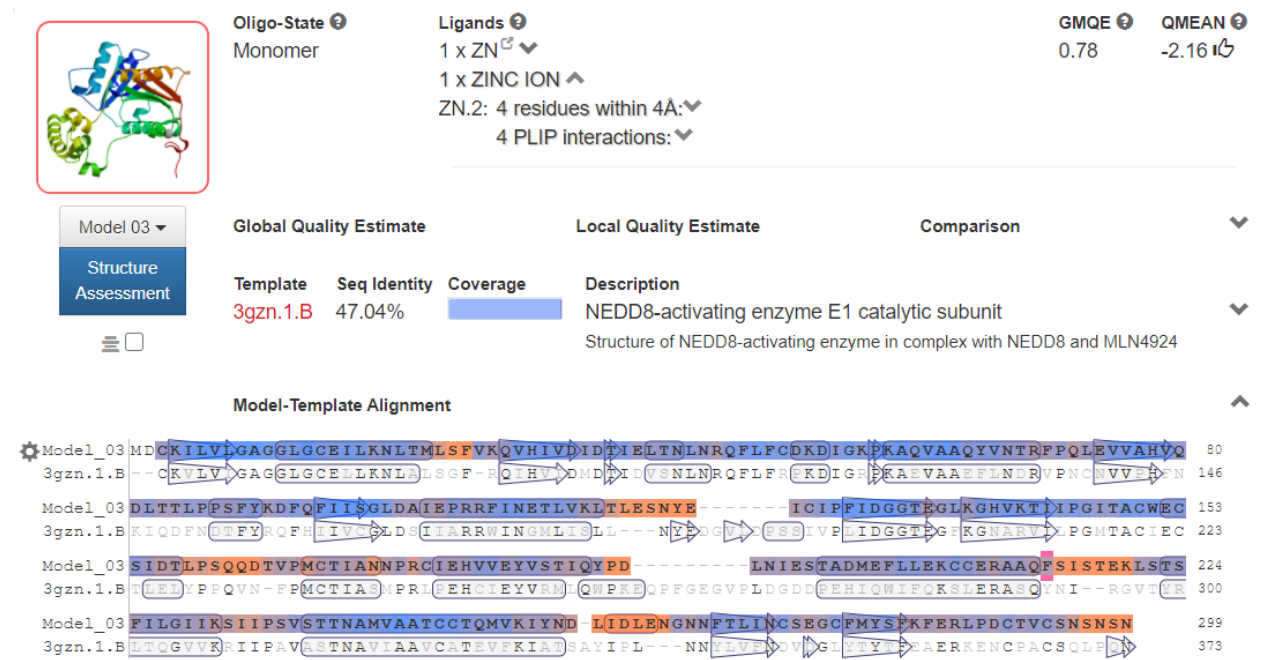
For figure 1B: *S. cerevisiae* Uba3, built on the structure of NEDD8-activating enzyme in complex with NEDD8 and MLN4924 (PDB ID: 3GZN).

GMQE = 0.78. **QMEAN** = -2.16. **Sequence identity** = 47.04%. **Coverage** = 96%.
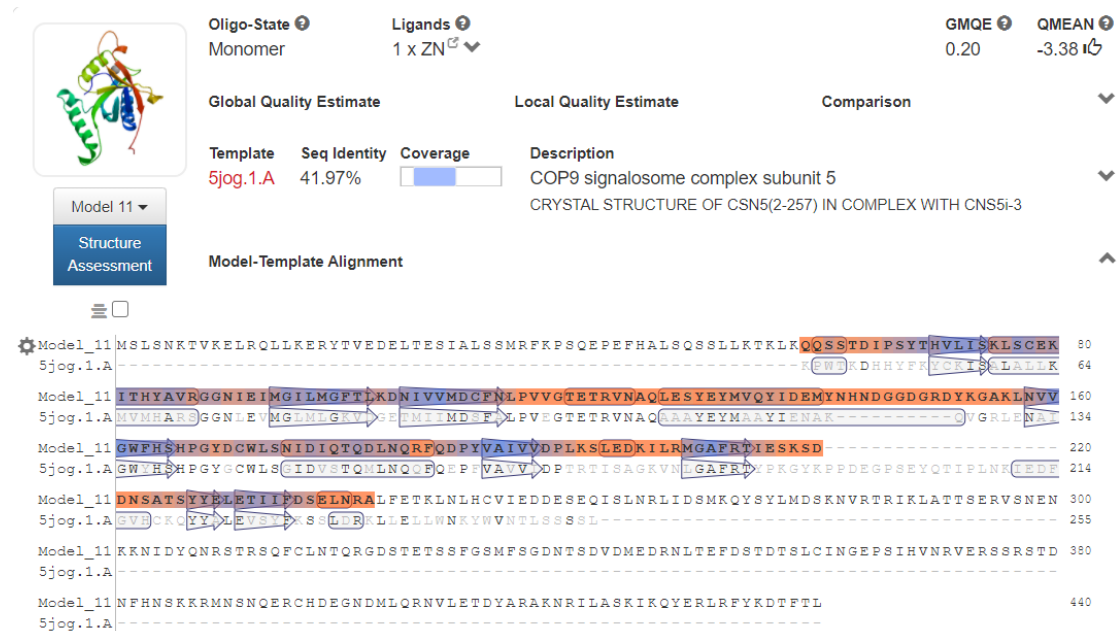
For figure 1C: *S. cerevisiae* Csn5, based on the structure of the human Csn5 in complex with CSN5i-3 (PDB ID: 5JOG) GMQE = 0.20, QMEAN = -3.38. Seq identity = 41.97%. Coverage = 44%.
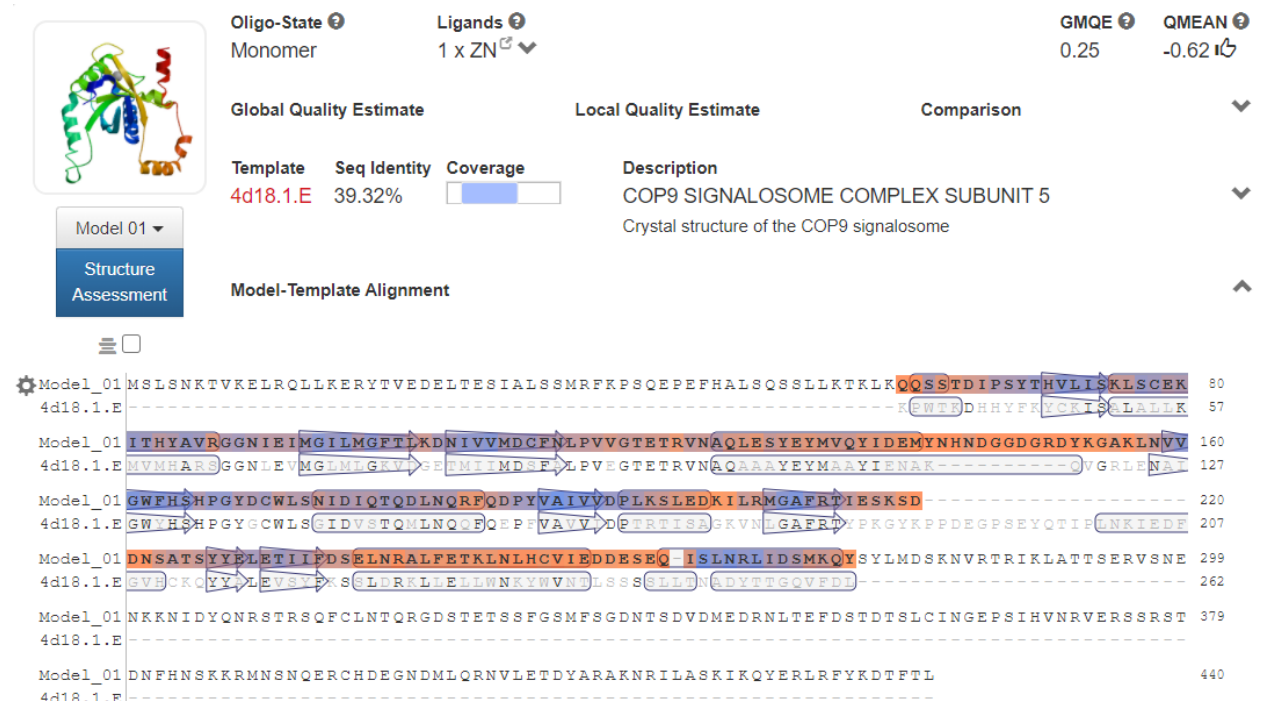
For figures 2C (left) , 2D and S1A: *S. cerevisiae* Csn5, built on the template of the human CSN5 crystal structure (PDB ID: 4D18).

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

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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%.

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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 08

Structure Assessment

Model-Template Alignment

```
Model_08 MSLSNKTVKELRQLLKERYTVEDELTESIALSSMRFKPSQEPEFHALSQSSLLKTKLKQQSSTDIPSYTHVLISKLSCEK 80
6r7f.1.E ----------------------------------------------------KPWTKDHHYFKYCKSALALLK 41

Model_08 ITHYAVRGGNIEIMGILMGFTLKDNIVVMDCFNLPVVGTETRVNAQLESYEYMVQYIDEMYNHNDGGDGRDYKGAKLNVV 160
6r7f.1.E MVMHARSGGNLEVMGLMLGKVGETMIIMDSFLPVEGTETRVNAQAAAYEYMAAYIENAK----------QVGRLENAI 111

Model_08 GWFHSHPGYDCWLSNIDIQTQDLNQRFQDPYVAIVVDPLKSLEDKILRMGAFRTIESKSDD 221
6r7f.1.E GWYHSHPGYGCWLSGIDVSTQMLNQQFQEPFVAVVDPTRTISAGKVNLGAFRYPKGYKPPPDEGPSEYQTIPLNKIEDF 191

Model_08 -NSATSYYELETIIFDSELNRALFETKLNLHCVIEDDESEQISLNRLIDSMKQYSYLMDSKNVRTRIKLATTSERVSNEN 300
6r7f.1.E GVHCKOYYALEVYFKSSLDRKLLELLWNKYWVNTLSS--------------------------------------- 229

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

Model_08 NFHNSKKRMNSNQERCHDEGNDMLQRNVLETDYARAKNRILASKIKQYERLRFYKDTFTL 440
6r7f.1.E ----------------------------------------------------------
```
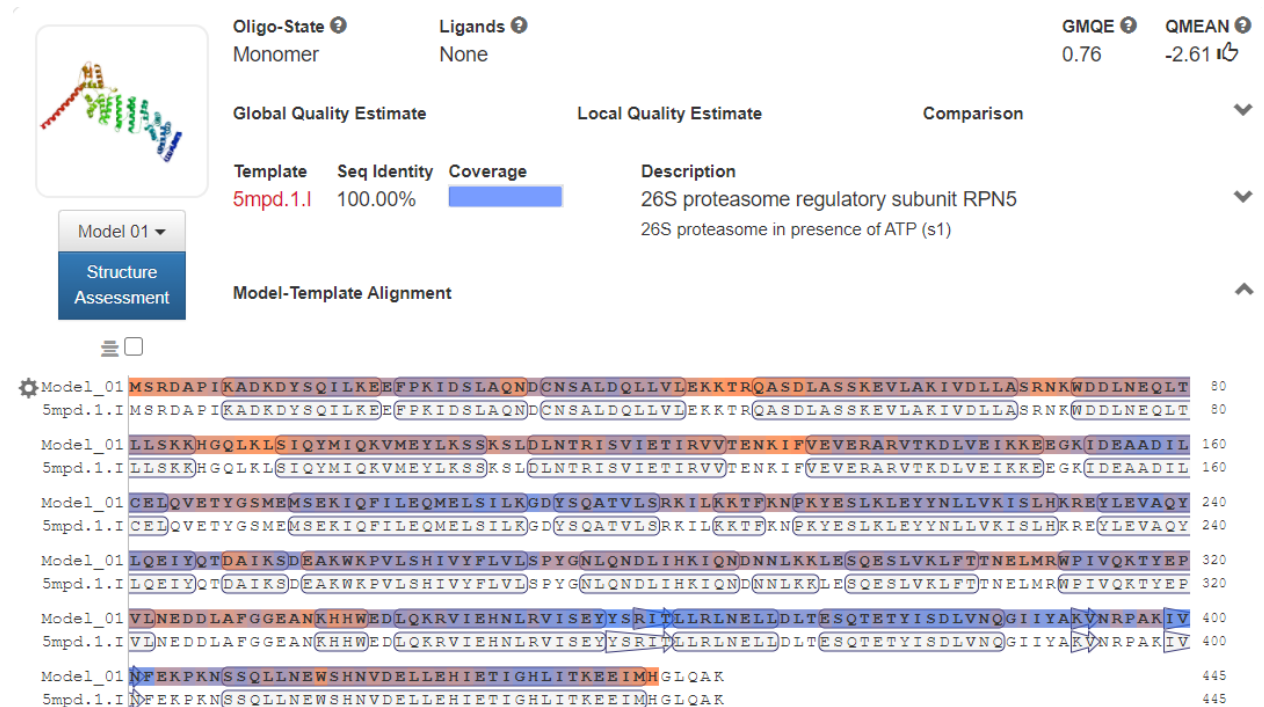
For figure S1: *S. cerevisiae* Csn5, built on the template of the human CSN5 crystal structure (PDB ID: 6R7F).

**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 12

Structure Assessment

Model-Template Alignment

```
Model_12 MSLSNKTVKELRQLLKERYTVEDELTESIALSSMRFKPSQEPEFHALSQSSLLKTKLKQQSSTDIPSYTHVLISKLSCEK 80
5w83.1.B ----------------------------------------------------TGRDDTKETVYISSIALLK 36

Model_12 ITHYAVRGGNIEIMGILMGFTLK-DNIVVMDCFNLPVVGTETRVNAQLESYEYMVQYIDEMYNHNDGGDGRDYKGAKLNV 159
5w83.1.B MLKHGRAGVPMEVMGLMLGEFVYTVNVVDVFMPQSGTGVSVEAVDDVFQ--AKMMDML----------KOTGRDQMV 104

Model_12 VGWFHSHPGYDCWLSNIDIQTQDLNQRFQDPYVAIVVDPLKSLEDKILRMGAFRTIESKSD 220
5w83.1.B VGWYHSHPGFGCWLSBVDVNTQKSFEQINSRAVAVVVDPIOSVK-GKVVIDAFRYIDTGALINNLEPRQTTSNTGLLNRA 183

Model_12 ------DNSATSYYELETIIFDSELNRALFETKLNLHCVIEDDESEQISLNRLIDSMKQYSYLMDSKNVRTRIKLATTSE 294
5w83.1.B NIQALIHGLNRHYYSLNIDYTAKETKMLMNLHKEQWQ--------------------------------------- 222

Model_12 RVSNENKKNIDYQNRSTRSQFCLNTQRGDSTETSSFGSMFSGDNTSDVDMEDRNLTEFDSTDTSLCINGEPSIHVNRVER 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%.

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%.
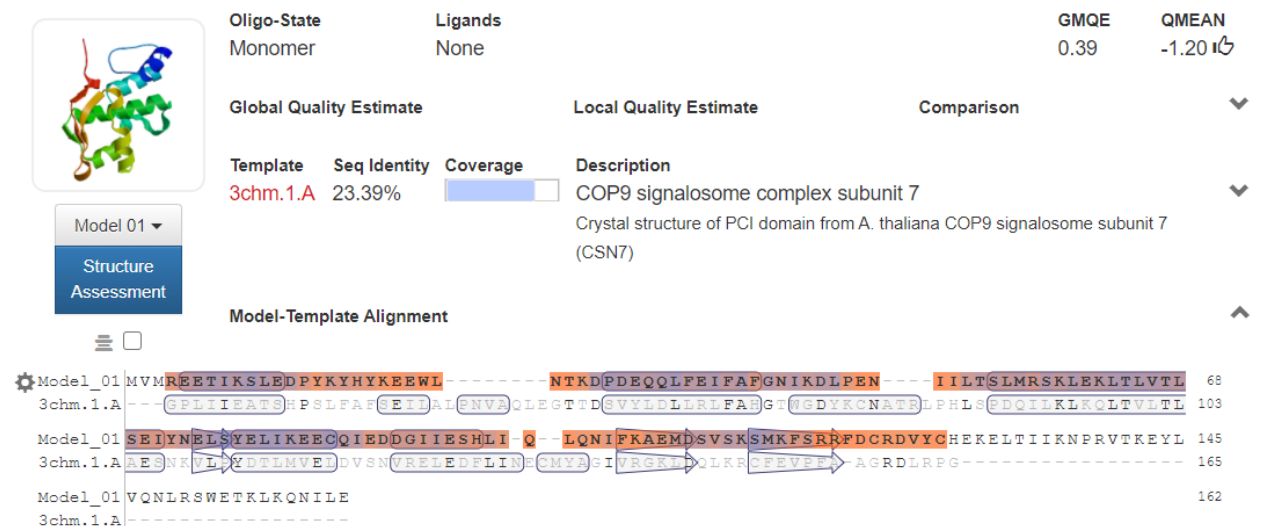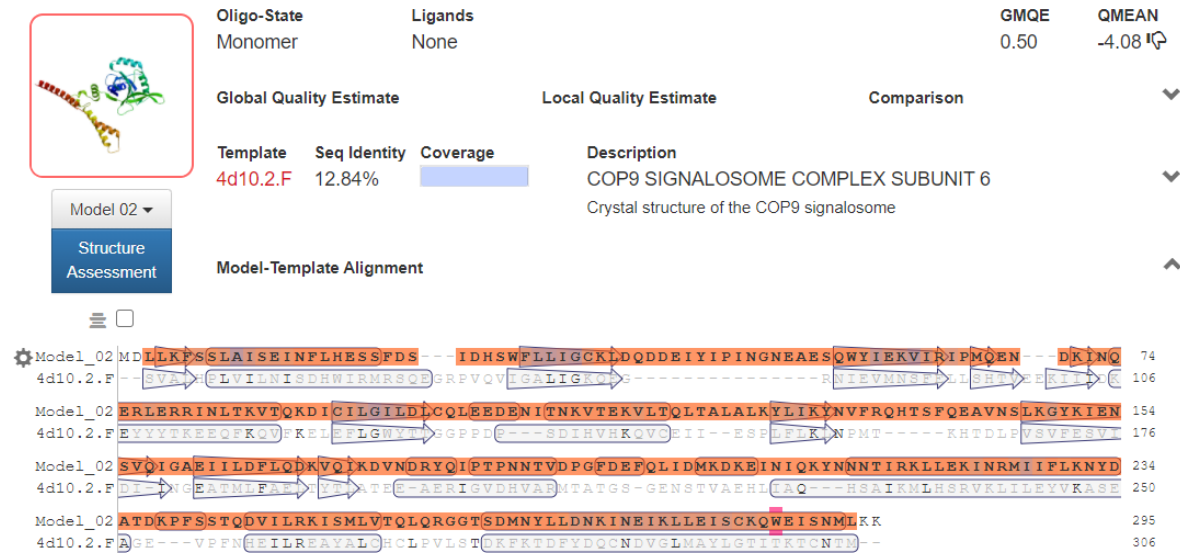

For figure 2D: *S. cerevisiae* Csn9, built on the template of the *A. thaliana* CSN7 crystal structure (PDB ID: 3CHM).

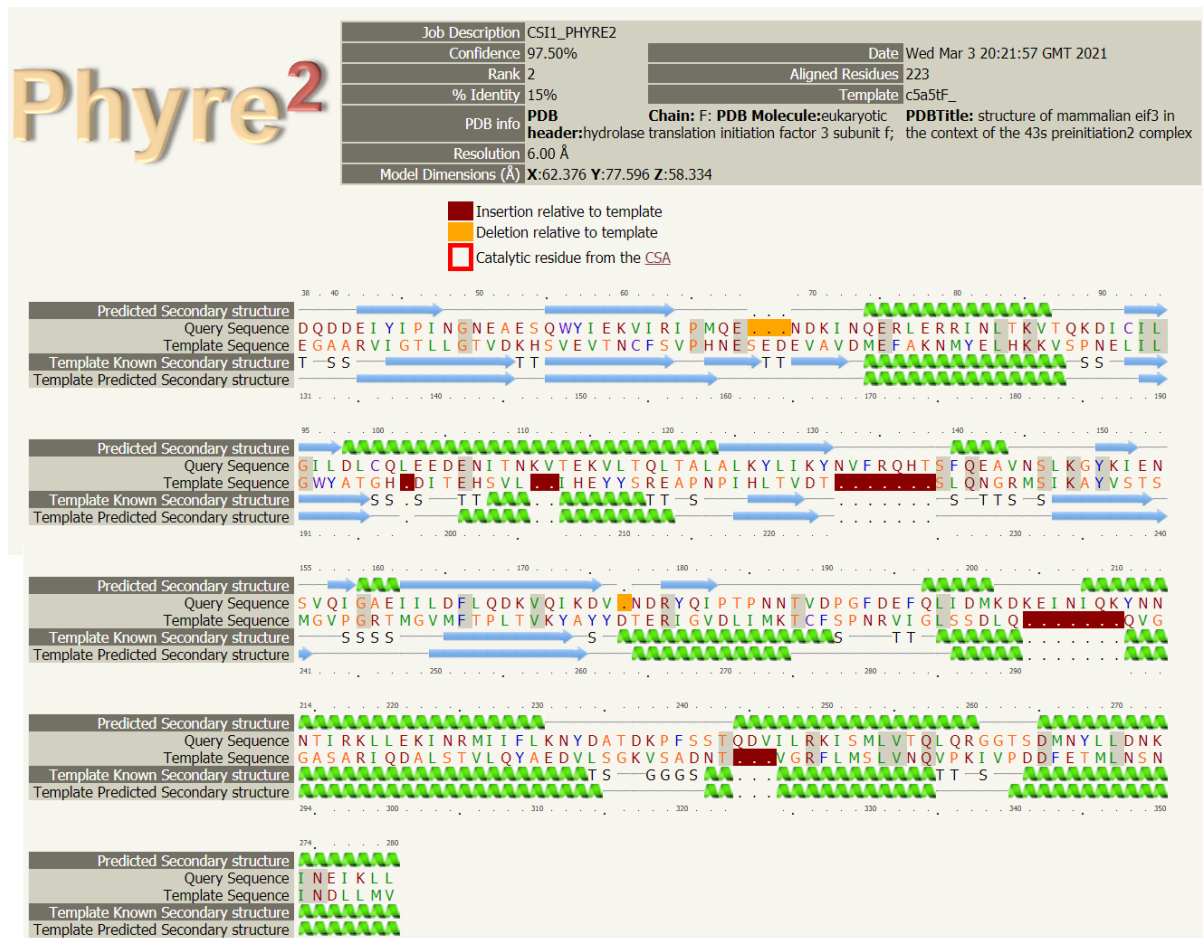**GMQE** = 0.39. **QMEAN** = -1.2. **Sequence identity** = 23.39%. **Coverage** = 77%.

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

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%.

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%.

	Oligo-State	Ligands		GMQE	QMEAN
	Monomer	None		0.38	-5.57

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

Template	Seq Identity	Coverage	Description
6fvw.1.c	14.66%		26S proteasome regulatory subunit RPN7
			26S proteasome, s4 state

Model-Template Alignment

```
Model_01  MFHGAKGPLLIERIGHLLSINYGEKEERKRWAMQGISYLQEVQCTSTPYLEILVEESGLRPVSLLNSQLVGKPHFSLLGG   80
6fvw.1.c  ------NYEVSEKAFLLTQSKVSIEQRKEAAEFVLAKIKEEE--MAPYYKYLCEEYLVNNGQSDLEHDEKSDSLNEWIK   74

Model_01  FDENIARDIISH-------------------------------------NFQNAIFQMESEEVPLTKRYQHLE---K   117
6fvw.1.c  FDQELYNELCKKNESKIKELNEKIQKLEEDDEGELEQAQAWINLGEYYAQIGDKDNAEKTLGKSLSKAISTGAKIDVMLT   154

Model_01  ITQISLLCKNFKGIEEIEYNVKNIIQGRKNFDMLNSMEKDRISHEVVQDDSFSLLRIQMLLCVSYFLQERYFDCCTKFFT   197
6fvw.1.c  IARLGFFYNDQLYVKEKLEAVNSMIEKGG------------------DWERRNRYKTYYGIHCLAVRNFKEAAKLLVD   214

Model_01  MMTSEPLTLKVLSEHLDCMNFISKEEFIMMVNISVLISIPLDNYDD-FIYLSDLKQFFQMTPLL---VNCLELLINTNFN   273
6fvw.1.c  SLAT------F----TSIELTSYESIATYASVTGLFTLERTDLKSKVIDSPELLSLISTTAALQSISSLTISLYASDYA   283

Model_01  KFFKIWHGEINKICMESLFLEPSWSSSAAVIMRCKIYFFYLRISKKLQFSYLSSTLGIDLEDIKEELTKLIISGQLNFE   353
6fvw.1.c  SYFPYLLETYANVLIPCKYLNRHADF-FVREMRRKVYAQLLESYKTLSLKSMASAFGVSVAFLDNDLGKFIPNKQLNCVI   362

Model_01  D--GDVIHFEDSSILQSIVNEISRNGTMINEVIDKLKNENTDLKDIIQGNPLMYSGGNNTATIINNESSDDMDIDEVNDR   431
6fvw.1.c  DRVNGIVETNRPDNKNAQYHLLVKQGDGLLTKLQKYGA-------------------------------------   400

Model_01  SDISDSEGGLFEC   444
6fvw.1.c  ------------
```

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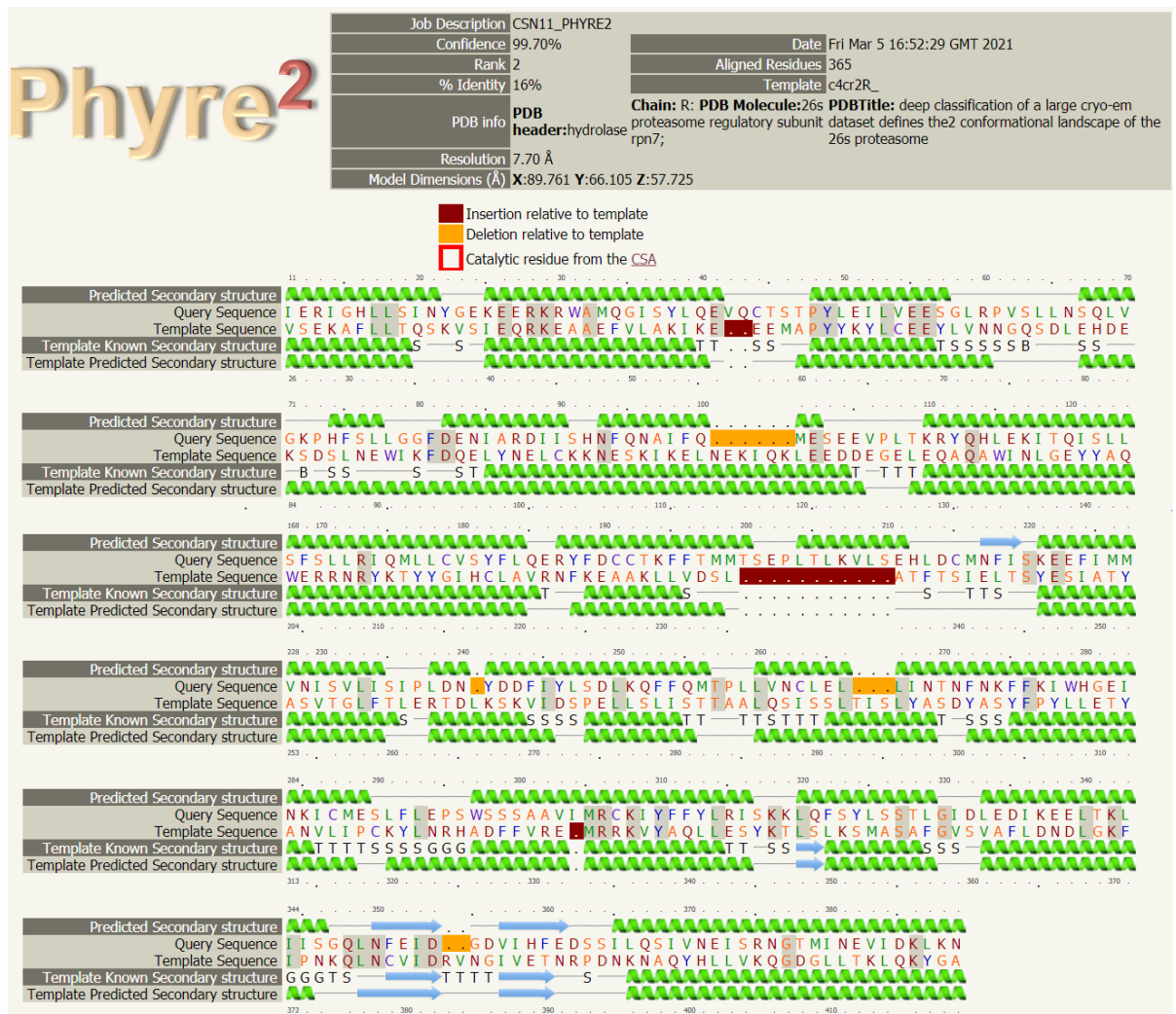
For table 2, 3: *S. cerevisiae* Csn11, built on the template of the *S. cerevisiae* Rpn7 crystal structure (PDB ID: 4CR2).

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

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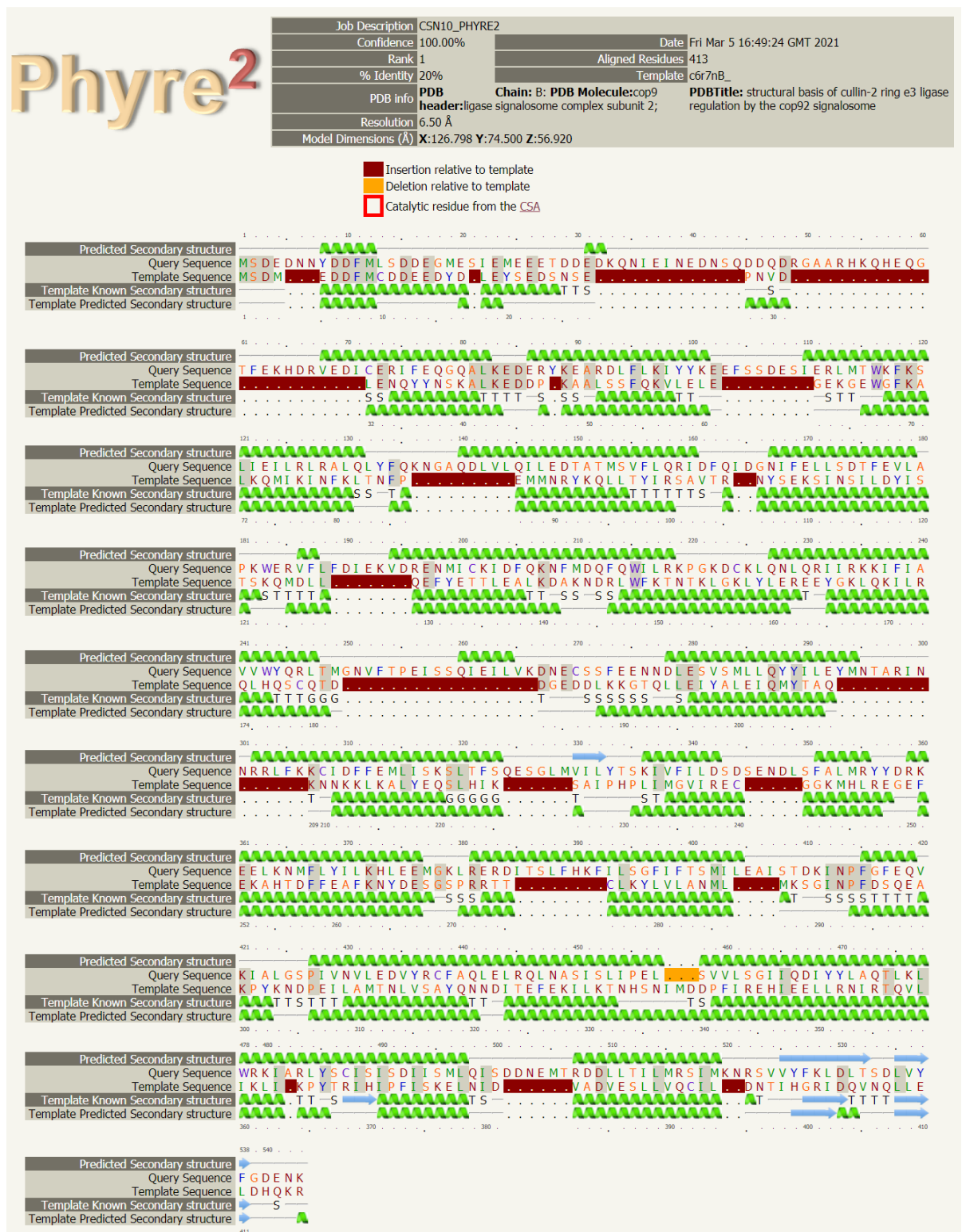
For tables 2, 3: *S. cerevisiae* Csn10, built on the template of the human Cullin-2 RING E3 ligase (CSN2) crystal structure (PDB ID: 6R7N).

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

Bibliography

1.	Lingaraju, G.M., et al., *Crystal structure of the human COP9 signalosome.* Nature, 2014. **512**(7513): p. 161-5.
2.	Eisele, M.R., et al., *Expanded Coverage of the 26S Proteasome Conformational Landscape Reveals Mechanisms of Peptidase Gating.* Cell Rep, 2018. **24**(5): p. 1301-1315 e5.
3.	Wehmer, M., et al., *Structural insights into the functional cycle of the ATPase module of the 26S proteasome.* Proc Natl Acad Sci U S A, 2017. **114**(6): p. 1305-1310.
4.	Dessau, M., et al., *The Arabidopsis COP9 signalosome subunit 7 is a model PCI domain protein with subdomains involved in COP9 signalosome assembly.* Plant Cell, 2008. **20**(10): p. 2815-34.
5.	Biasini, M., et al., *SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information.* Nucleic Acids Res, 2014. **42**(Web Server issue): p. W252-8.
6.	Benkert, P., S.C. Tosatto, and D. Schomburg, *QMEAN: A comprehensive scoring function for model quality assessment.* Proteins, 2008. **71**(1): p. 261-77.
7.	Bramasole, L., et al., *Proteasome lid bridges mitochondrial stress with Cdc53/Cullin1 NEDDylation status.* Redox Biol, 2019. **20**: p. 533-543.
8.	Sinha, A., et al., *The COP9 signalosome mediates the Spt23 regulated fatty acid desaturation and ergosterol biosynthesis.* FASEB J, 2020.
9.	Yu, Z., et al., *Dual function of Rpn5 in two PCI complexes, the 26S proteasome and COP9 signalosome.* Mol Biol Cell, 2011. **22**(7): p. 911-20.
10.	Ziv, I., et al., *A perturbed ubiquitin landscape distinguishes between ubiquitin in trafficking and in proteolysis.* Mol Cell Proteomics, 2011. **10**(5): p. M111 009753.
11.	Brownell, J.E., et al., *Substrate-assisted inhibition of ubiquitin-like protein-activating enzymes: the NEDD8 E1 inhibitor MLN4924 forms a NEDD8-AMP mimetic in situ.* Mol Cell, 2010. **37**(1): p. 102-11.
12.	Schlierf, A., et al., *Targeted inhibition of the COP9 signalosome for treatment of cancer.* Nat Commun, 2016. **7**: p. 13166.