Supplementary information

Title

Pwp2 mediates UTP-B assembly via two structurally independent domains

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SUPPLEMENTAL DATA

Table S1: Oligonucleotides used in this study. Oligonucleotides used for different purposes are described. Database numbers, sequence and usage are indicated. Oligonucleotides marked with "***#***" belong to the Department of Biochemistry III at the University of Regensburg. Oligonucleotides marked with "***o***" belong to the collection of the UTP-Lab.

Col. N.	Name	5'-3'-sequence	Purpose
#1203	RPA135-pYM-for	CTATCCGCAATGGGTATAAGATTG CGTTATAATGTAGAGCCCAAACGT ACGCTGCAGGTCGAC	Forward oligo for the mCherry tagging of Rpa135 using the plasmid pFA6a:1xmCHERRY-Hph
#1204	RPA135-pYM-rev	CCTTCATTTACCATTCTATATCAATT TGGAAAGAAGGGTATTTCTATCGA TGAATTCGAGCTC	Reverse oligo for the mCherry tagging of Rpa135 using the plasmid pFA6a:1xmCHERRY-Hph
#3942	SalI_Pwp2_5	GTCGACGCGGTTCAATCTGCGGTC TGCTC	Used to eliminate the PWP2 coding region and insert a SalI site instead.
#3943	PstI_Pwp2_3	TGACTGCAGAAAATATTCACTGTCC TCTA	Used to eliminate the PWP2 coding region and insert a PstI site instead.
#3949	5'SLIC_PWP2	GTCACGACGTTGTAAAACGACGGC CAGTGAATTCGAGCTCGACTCCAA GATGCGTAAGGC	Cloning of PWP2 locus containing 5' and 3' regulatory sequences.
#3950	3'SLIC_PWP2	TCACACAGGAAACAGCTATGACCA TGATTACGCCAAGCTTTGATAGTAT GAAACGGTAGTCG	Cloning of PWP2 locus containing 5' and 3' regulatory sequences.

Col. N.	Name	5'-3'-sequence	Purpose
#3950	3'SLIC_PWP2	TCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTTGATAGTATGAAACGGTAGTCG	5'UTR_PWP23'UTR cloning
#3951	SalI_PWP2Fo	GTAGACAGGAGAGCAGACCGCAGATTGAACCGCGTCGACATGAAATCCG	Cloning of *PWP2* ORF containing a SalI site for subcloning
#3952	PstI_Pwp2Re	TGATGTGAATGTGCATAAATAGAGGACAGTGAACTGCAGTCAAGGAAGCTC	Cloning of *PWP2* ORF containing a PstI site for subcloning
#3953	PstI_Pwp2_corr	TGACTGCAGTTCACTGTCCTCT	Fow primer to clone truncations
#3162	PWP2_XmaI_fw	TTGTTGCCCGGGATGAAATCCGATTTCAAGTTCTCT	forward primer for sequencing of mutated pwp2 on pJPF19 and pCJPF39
#3185	PWP2_seq_fw2	GAGGCCATCCGATGACGA	forward primer for sequencing of mutated pwp2 on pJPF19 and pCJPF39
#3186	PWP2_seq_rev2	CCCATCAGTTGATACCAAAAATCT	reverse primer for sequencing of mutated pwp2 on pJPF19 and pCJPF39
#3187	PWP2_seq_rev3	TATCAATACGATCCTCCAAATCTGA	reverse primer for sequencing of mutated pwp2 on pJPF19 and pCJPF39
#3999	Pwp2_103C_SLIC	GTGCATAAATAGAGGACAGTGAACTGCAGTCA_AGCCGTCGAGAAGAGATATTTG	Cloning of DC103 truncation together with #3951
#4000	Pwp2_206C_SLIC	GTGCATAAATAGAGGACAGTGAACTGCAGTCA_CAGATCAAAGGGATCAAATAATATC	Cloning of DC206 truncation cloning together with #3951
#4001	Pwp2_309C_SLIC	GTGCATAAATAGAGGACAGTGAACTGCAGTCA_TCTTTTTAACAAGACTTCATTTGG	Cloning of DC309 truncation cloning together with #3951
o1	Utp21_S3	GTGAAGTTTTGCATGGGAGTGGCAGCTTTTGTGACCACCGCG_CGTACGCTGCAGGTCGAC	TAP tagging of Utp21 using the pYM collection
o2	Utp21_S2	CAATCTGCCTTTGTTACTAATATACTTGTTCTATATAATGC_ATCGATGAATTCGAGCTCG	TAP tagging o Utp21 using the pYM collection
o13	PWP2_61XD_re	TTCTTCTAACAAGACTTCATTTGG	reverse primer used to introduce mutations into *PWP2*; as a result Lys at position 613 and Arg at position 614 are replaced by Asp
o14	PWP2_61XD_fo	CCAAATGAAGTCTTGTTAGAAGAATTCATTGTGTCCAGAAACATGG	forward primer used to introduce mutations into *PWP2*; as a result Lys at position 613 and Arg at position 614 are replaced by Asp
o16	PWP1_32C	CTCGTCGTCAGATTCCATGAC	Cloning of DC32 truncation cloning together with #3953
o17	PWP2_66C	TTCCATGGACCCATCAGTTG	Cloning of DC66 truncation cloning together with #3953
o26	R140-fo2	CTTCGTCCGCCATGACGTACATGCGGGACACTTTCAAGAC	forward primer used to introduce mutations into *PWP2*; as a result Arg at position 140 is replaced by Asp

Col. N.	Name	5'-3'-sequence	Purpose
o27	R140-re2	GTGTCCCGCATGTACGTCATGGCG GACGAAGGGAGCAAAC	reverse primer used to introduce mutations into *PWP2*; as a result Arg at position 140 is replaced by Asp
o32	Utp21_2302fo	GGGGATGAAGCCTCTGTCAGAG	Movilization of C-terminal tagging coding sequences at *UTP21* locus
o33	Utp21_3302re	GGATTCGGAGATCCCTATCCAC	Movilization of C-terminal tagging coding sequences at *UTP21* locus
o45	Mut_129-30E_Fo	CAGATTTGGAAGACTCCAGATGTT AACAAACGCAGACAGTTTGCTCCC TTCGTCCG	forward primer used to introduce mutations into *PWP2* together with o47; as a result Asp at position 130 is replaced by Lys.
o46	Mut_130K_Fo	CAGATTTGGAAGACTCCAGATGTT AACAAACGCAGACAGTTTGCTCCC TTCGTCCG	reverse primer used to introduce mutations into *PWP2* together with o47; as a result Arg at position 129 and Lys at position 131 are replaced by Glu as well as Asp at position 130 is replaced by Lys
o47	Mutloop1_Re	GTTAACATCTGGAGTCTTCCAAATC TG	
o48	MtR505E_Fo	CGCATATGGTCCATATTTGGTGAA_ AGCCAACAAGTAGAACCTATAGAA GTTTATTCCG	forward primer used to introduce mutations into *PWP2*; as a result Arg at position 505 is replaced by Glu
o49	MtR505E_re	TACTTGTTGGCTTTCACCAAATATG GACCATATGCGAATTGTTTTATCCC ATGATGC	reverse primer used to introduce mutations into *PWP2*; as a result Arg at position 505 is replaced by Glu
o50	FLAG_pwp2A692	ATGGATTACAAGGATGACGACGAT AAGGGTACCGGATCCGCATTTGCC GCTGCTTCAAC	Forward primer used together with #3950 to amplify the coding region of last 232 aa of PWP2
o51	FLAG_pwp2G807	ATGGATTACAAGGATGACGACGAT AAGGGTACCGGATCC_GGTGGTTA CATAAATGAACACAAATATC	Forward primer used together with #3950 to amplify the coding region of last 117 aa of PWP2
o82	Pwp2_F4	GGTAGACAGGAGAGCAGACCGCA GATTGAACCGCATCCCA_GAATTC GAGCTCGTTTAAAC	forward primer for insertion of KANMX::GAL::HA cassette in the *PWP2* locus
o84	Pwp2_R3	GCCTGTAGACCGTACCTAAAAGGT TAGAGAACTTGAAATCGGATTTCA TGCACTGAGCAGCGTAATCTG	reverse primer for insertion of KANMX::GAL::HA cassette in the *PWP2* locus
#205	o2-18S	CATGGCTTAATCTTTGAGAC	Hybridize in the rDNA, 18S
#207	o4-A2/A3	TGTTACCTCTGGGCCC	Hybridize in the rDNA, ITS1
#2921	A0A1-Sonde	CCCACCTATTCCCTCTTGCTAG	Hybridize in the rDNA, 5'-ETS
#3468	U3-SnR17A	CCGCTAAGGATTGCGGACCAAGC	Hybridize in the U3 snoRNA
#3570	SnR128-U14	TCACTCAGACATCCTAGGAAGG	Hybridize in the U14 snoRNA
#3839	ITS1 D-A2 mid	AAGCCTAGCAAGACCGCGCA	Hybridize in the rDNA, ITS1

Table S2: Plasmids used in this study. Database numbers, name of the plasmid, contained markers for selection and the cloning procedure are indicated. Plasmids marked with "**#**" belong to the Department

of Biochemistry III at the University of Regensburg. Plasmids marked with "***p***" belong to the collection of the UTP-Lab.

#	Name	Gene	Marker	Origin	Cloning procedure
#1	pBS	Empty backbone	Amp	Biochemie III	
#49	YCplac33	empty backbone plasmid	Amp/ URA3	Biochemie III	[1]
#1225	pYM44	One-step PCR	Amp	Biochemie III	[2]
#1837	pCM182-LEU2	Empty backbone plasmid	Amp/ URA3	Biochemie III	*LEU2* marker replaces *TRP1* in pCM182 [3,4]
p107	pJPF19	*PWP2*	Amp	This study	*PWP2* locus including 5' and 3' regulatory regions cloned in pBluescriptKS (+/-).
p108	pCMS1	*pwp2-R129/K131E*	Amp	This study	PCR product obtained with oligonucleotides o45-o47 cloned in pJPF19 using the SLIC protocol
p109	pCMS2	*pwp2-D130K*	Amp	This study	PCR product obtained with oligonucleotides o46-o47 cloned in pJPF19 using the SLIC protocol
p110	pCMS3	*pwp2-E473Δnsi1*	Amp	This study	Subcloning from XX in pJPF19 using BamHI and NsiI sites.
p111	pCMS4	*pwp2-R505E*	Amp	This study	PCR product obtained with oligonucleotides o48-o49 cloned in pJPF19 using the SLIC protocol
p120	pCMS13	*pwp2-ΔC32*	Amp	This study	PCR product obtained with oligonucleotides o16-#3953 cloned in pJPF19 using blunt end ligation
p121	pCMS14	*pwp2-ΔC66*	Amp	This study	PCR product obtained with oligonucleotides o17-#3953 cloned in pJPF19 using blunt end ligation
p122	pCMS15	*pwp2-ΔC103*	Amp	This study	PCR product obtained with oligonucleotides #3999-3951 cloned in pJPF19 using the SLIC protocol
p123	pCJPF39	*PWP2*	Amp/ URA3	Pérez-Fernández	[SacI-HinDIII]-fragment form pJPF19 subcloned in YCplac33
p124	pCCMS1	*pwp2-R129/K131E*	Amp/ URA3	This study	[SalI-PstI]-fragment from pCMS1 subcloned in pCJPF39
p125	pCCMS2	*pwp2-D130K*	Amp/ URA3	This study	[SalI-PstI]-fragment from pCMS2 subcloned in pCJPF39
p126	pCCMS3	*pwp2-E473Δnsi1*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pCMS3 subcloned in pCJPF39

#	Name	Gene	Marker	Origin	Cloning procedure
p127	pCCMS4	*pwp2-R505E*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pCMS4 subcloned in pCJPF39
p128	pCCMS5	*pwp2-K613/R614E*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pCMS5 subcloned in pCJPF39
p136	pCCMS13	*pwp2-ΔC32*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pCMS13 subcloned in pCJPF39
p137	pCCMS14	*pwp2-ΔC66*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pCMS14 subcloned in pCJPF39
p138	pCCMS15	*pwp2-ΔC103*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pCMS15 subcloned in pCJPF39
p168	pJPF20	*pwp2-E335R*	Amp	This study	Subcloning from XX in pJPF19 using AflII and MscI sites.
p169	pJPF21	*pwp2-K368E*	Amp	This study	Subcloning from XX in pJPF19 using AflII and BamHI sites.
p170	pJPF22	*pwp2-R418E/R420E*	Amp	This study	Subcloning from XX in pJPF19 using AflII and BamHI sites.
p171	pJPF23	*pwp2-E473R*	Amp	This study	Subcloning from XX in pJPF19 using BamHI and NsiI sites.
p172	pCJPF40	*pwp2-E335R*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pJPF20 subcloned in pCJPF39
p173	pCJPF41	*pwp2-K368E*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pJPF21 subcloned in pCJPF39
p174	pCJPF42	*pwp2-R418E/R420E*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pJPF22 subcloned in pCJPF39
p175	pCJPF43	*pwp2-E473R*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pJPF23 subcloned in pCJPF39
p176	pCMS16	*pwp2-ΔC206*	Amp	This study	PCR product obtained with oligonucleotides #4000-3951 cloned in pJPF19 using the SLIC protocol
p177	pCMS17	*pwp2-ΔC309*	Amp	This study	PCR product obtained with oligonucleotides #4001-3951 cloned in pJPF19 using the SLIC protocol
p178	pCCMS16	*pwp2-ΔC206*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pCMS16 subcloned in pCJPF39
p179	pCCMS17	*pwp2-ΔC309*	Amp/ URA3	This study	[BamHI-PstI]-fragment from pCMS17 subcloned in pCJPF39
p180	pCCMS24	*pwp2-G807-P923*	Amp/ URA3	This study	PCR product from o51-#3950 cloned in pCM182-LEU2-FLAG at BamHI-HindIII using the SLIC protocol
p181	pFA6a-kanMX6-PGAL1::3HA	One-step PCR	Amp	This Study	[5]

#	Name	Gene	Marker	Origin	Cloning procedure
p182	pFA6a::1xmCHERRY:Hph	One-step PCR	Amp	This Study	[2] Plasmid obtained by religation of *AleI* digested plasmid pFA6-mCHERRY-Hph (kindly provided by Brigitte Pertschy).
	pET-His-Pwp2(1-716)	One-step PCR	Amp	This Study	PCR product was cloned by LIC strategy in a modified pET-15b [6]
	pET-His-Pwp2(717-923)	One-step PCR	Amp	This Study	PCR product was cloned by LIC strategy in a modified pET-15b [6]
	pEt-Utp12p-Utp13p	One-step PCR	Kan	This Study	PCR product was cloned by LIC strategy in a modified pET-28b [6]
	pCDF-Utp21(699-939)	One-step PCR	Sm	This Study	PCR product was cloned by LIC strategy in a modified pCDF [6]
	pET-Utp21p	One-step PCR	Sm	This Study	PCR product was cloned by LIC strategy in a modified pCDF [6]
	pET-His-Utp12p	One-step PCR	Amp	This Study	PCR product was cloned by LIC strategy in a modified pET-15b [6]
	pET-Utp13p	One-step PCR	Kan	This Study	PCR product was cloned by LIC strategy in a modified pET-28b [6]
	pET-Utp13p(649-817)	One-step PCR	Kan	This Study	PCR product was cloned by LIC strategy in a modified pET-28b [6]
	pET-His-Utp13p(1-648)	One-step PCR	Amp	This Study	PCR product was cloned by LIC strategy in a modified pET-15b [6]
	pET-Utp12p(1-687)	One-step PCR	Kan	This Study	PCR product was cloned by LIC strategy in a modified pET-28b [6]
	pET-HisUtp12p(1-687)	One-step PCR	Amp	This Study	PCR product was cloned by LIC strategy in a modified pET-15b [6]
	pET-His-Utp12p(687-943)	One-step PCR	Amp	This Study	PCR product was cloned by LIC strategy in a modified pET-15b [6]
	pET-His-Utp12p(688-943)-Utp13(649-817)	One-step PCR	Amp	This Study	PCR product was cloned by LIC strategy in a modified pET-15b [6]
	pET-Kan-Pwp2p(717-943)-Utp21(699-939)	One-step PCR	Kan	This Study	PCR product was cloned by LIC strategy in a modified pET-15b [6]

Table S3: Yeast strains used in this study. Database numbers, name of the plasmid, contained markers for selection and the cloning procedure are indicated. Yeast strains marked with "***#***" belong to the Department of Biochemistry III at the University of Regensburg. Plasmids marked with "***Y***" belong to the collection of the UTP-Lab.

#	Name	Genotype	Origin
#206	BY4741	MATa; *his3-1*; *leu2-0*; *met15-0*; *ura3-0*	Biochemie III
#2506	BY4741 UTP21-TAP	MATa; *his3-1*; *leu2-0*; *met15-0*; *ura3-0*; *YLR409C*-TAP::*HIS3*MX6	Biochemie III
Y287	YMJH1-1a	MATa; *his3-1*; *leu2-0*; *met15-0*; *ura3-0*; KANMX::GAL::HA-*PWP2*	This study
Y289	YCMS3-1a	MATa; *his3-1*; *leu2-0*; *met15-0*; *ura3-0*; KANMX::GAL::HA-*PWP2*; *YLR409C*-TAP::*HIS3*MX6	This Study
Y339	YCMS4-1a	MATa; *his3-1*; *leu2-0*; *met15-0*; *ura3-0*; KANMX::GAL::HA-*PWP2*; *YLR409C*-GFP::*HIS3*MX6	This Study
Y344	YCMS5-1a	MATa; *his3-1*; *leu2-0*; *met15-0*; *ura3-0*; KANMX::GAL::HA-*PWP2*; *YLR409C*-GFP::*HIS3*MX6; *RPA135*-mCHERRY::Hph	This Study

Figure Legend

Figure S1: General SSU-processome proteins.

As indicated in figure 1, Utp21-TAP-purified proteins were identified and quantitated by Mass Spectrometry. Isobaric labelling of peptides (iTRAQ, see Materials and Methods) was used to compare the protein levels in wild-type Utp21 and in Pwp2 deleted strains. The bar graphs represent the ratios for the indicated purifications represented in logarithmic scale. The average of ratios for proteins identified in tree experiments with at least two peptides is shown for SSU-processome factors that do not fit in the categories shown in figure 1D, 1E and 1F.

Figure S2: Sequence conservation and electrostatic surface potential of Pwp2p tandem repeat.

Pwp2p tandem repeat structure is displayed in **A)** under the same orientation as Figure 1A. The residue conservation has been calculated using the sequence alignment displayed in Figure S1 and using the CONSURF server interface [7](Panel A, C, E, G). Only residues conserved above 80 % are displayed in a gradient from orange (80 % conservation) to purple (identical). Panels B, D, F, and H display the

electrostatics surface potential as calculated from PyMol using APBS plugin [8](blue = positively charged, red = negatively charged). Panels C-D; E-F, and G-H were generated from the orientation A-B by iterative rotations of 90° in the x-axis as indicated. Some conserved residues are indicated, mutated residues used in this study are shown in bold.

Figure S3: Sequence alignments of Pwp2 orthologues.

Sequences were aligned using ClustalX (6) and displayed using Jalview (7). Disordered regions of Pwp2 are boxed. Numbering on top of the sequences refers to the yeast Pwp2 while numbering beneath the alignment is an absolute count. The secondary structures indicated above the sequences are deduced from the crystal structure of Pwp2p (this work) and inferred from the crystal structure of *C.t* Utp1 [9].* indicates the amino acids substituted by site directed mutagenesis. Other relevant sequences for this work are also indicated.

Figure S4: Growth assays and expression analysis of different *pwp2* mutants.

A-B) Drop assays of Pwp2 mutant strains. The yCMS3 strain was transformed with the backbone plasmid YCplac33 ("empty"), the wild-type *PWP2* containing plasmid pJPF39 ("*PWP2*") or a plasmid containing a mutation in *PWP2*. Serial dilutions of cells were plated either on SCG medium allowing for the expression of a genomic copy of an HA-tagged copy of PWP2, or in the SCD medium where only the Pwp2 copy encoded in the plasmid is expressed. Incubation temperature is indicated at the right side. **C)** Expression analysis of Pwp2 mutants. 5 ODs of cell cultures expressing the different Pwp2p mutants were obtained and proteins were obtained by TCA extraction. Samples were resolved in 8% SDS-PAGE for western blot analysis with antibodies against GFP, Pwp2p and Utp18p. **D)** Doubling time of different pwp2 mutants at 30° and 37°C. Expression of the genomic copy of PWP2 (g*PWP2* "ON") allows a similar doubling time for all strains regardless of the *PWP2* allele co-expressed at both temperatures. In contrast, *pwp2-ΔC66* mutant show a mild growth defect at both temperatures in absence of the endogenous Pwp2p (g*PWP2* "OFF") and *pwp2-K613E/R614E* mutant shows a mild growth defect only at 37°C.

Figure S5: Relevance of the CTD domain of Pwp2 for cell growth and trans-complementation analysis between tWD and CTD domains of Pwp2.

A) Drop assay of Pwp2 mutant strains. The yCMS3 strain was transformed with the backbone plasmid YCplac33 ("empty"), the wild-type *PWP2* containing plasmid pJPF39 ("*PWP2*") or a plasmid containing a mutation in *PWP2*. Serial dilutions of cells were plated either on SCG medium allowing for the expression of a genomic copy of an HA-tagged copy of PWP2, or in the SCD medium where only the Pwp2 copy encoded in the plasmid is expressed. Incubation temperature is indicated at the right side. **B)** Doubling time of the Pwp2p tWD/CTD*Δ66* mutant shows a stronger growth defect at 20 °C when compared to a strain expressing the wt Pwp2p. **C)** Trans-complementation assay. The yCMS3 strain was co-transformed with two different plasmids. On one hand, the backbone plasmid YCplac33 ("empty"), the plasmid containing the wild-type *PWP2* ("*PWP2*") or a plasmid containing one of the *Pwp2p* truncated forms (*pwp2-ΔC103*, *pwp2-ΔC206*). On the other hand, a plasmid containing the coding region of the C-terminal fragments of Pwp2 complementary to the Pwp2p-ΔC206 or Pwp2p-ΔC103, CTD_{232} or CTD117 respectively (see Fig. 3A). Expression of the CTD of Pwp2p is repressed in presence of Doxycyclin (1mg/ml). All clones grew similar on SCG medium allowing for the "overexpression" of a genomic copy of an HA-tagged copy of PWP2 (g*PWP2* "ON") and lacking Doxycyclin (CTD "ON"). In contrast, the expression of the two complementary coding regions of Pwp2p correlates with a reduction in the doubling time (compare CTD "ON" and "OFF" in absence of endogenous Pwp2p).

Figure S6: UTP-B subunits overexpression in bacteria.

A) Schematic representation of Utp21p, Utp12p, and Utp13p domain organization. Interactions observed in Figure 3E and 3F are summarized. **B-D)** In order to ensure that proteins were expressed in bacteria an assay was set-up in which the total bacterial cell content is analysed before and after induction by IPTG and revealed on SDS-PAGE and Coomassie-Blue staining. Overexpressed proteins are identified with an arrow.

Figure S7: Utp12p(688-943)-Utp13p(648-817) complex purification.

The minimal core domain necessary for Utp12p and Utp13p interaction was expressed and purify to

homogeneity. The size exclusion chromatography column profile and the corresponding SDS-PAGE analysis are shown. Additionally, a SDS-PAGE analysis displays the complex prior and after TEV cleavage.

Figure S8: Localization of Utp21p and expression level of the Utp21p-GFP.

The strain yCMS4-1a (containing *GAL::HA-PWP2* and *UTP21-GFP* at the endogenous *loci*) was transformed with plasmids containing the Pwp2p wild type allele or alleles encoding the truncated forms of Pwp2p (*pwp2-ΔC32*, p*wp2-ΔC66* or p*wp2-ΔC103*). Exponentially growing cells were shifted from galactose to glucose containing medium and further cultured for 14 h (Wt, *pwp2-ΔC32* and *pwp2-ΔC66*) or 10 h (*pwp2-ΔC103*) keeping exponential growth conditions. In both cases cells were harvested and processed for fluorescent microscopy analysis as indicated in the Material and Methods. From left to right: DAPI staining (blue), Utp21p:GFP (green) and merged images. The scale bar represents 4 μm.

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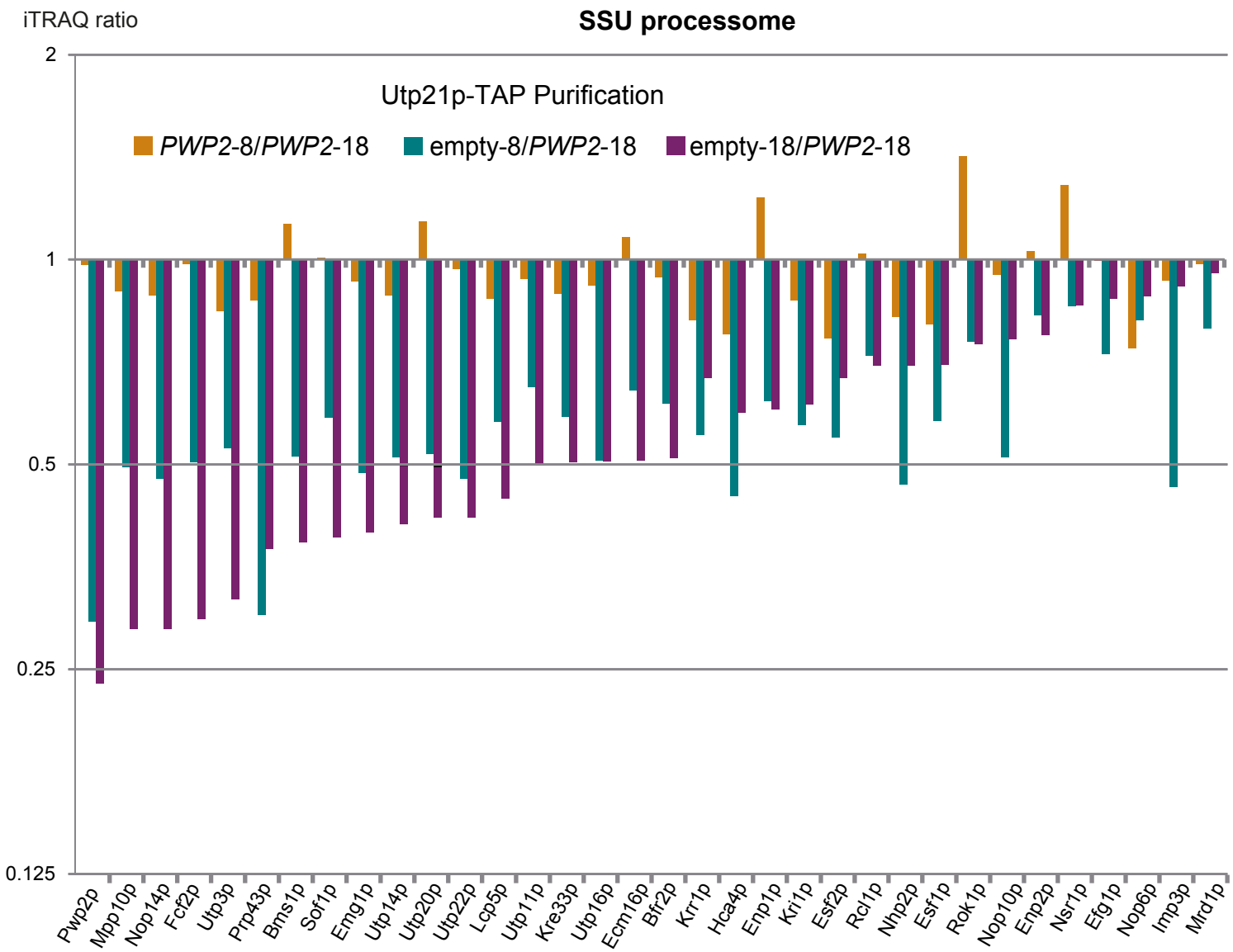
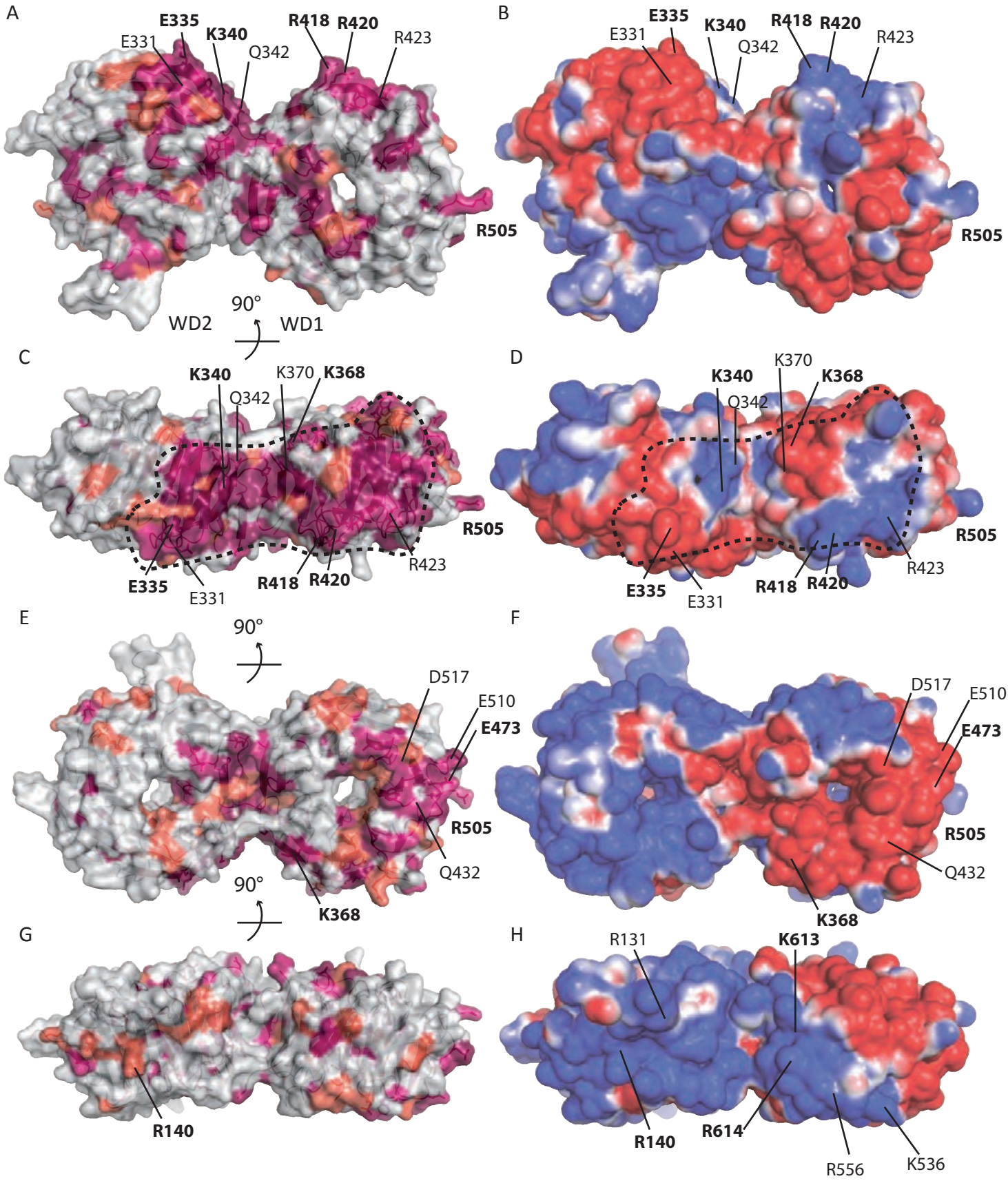
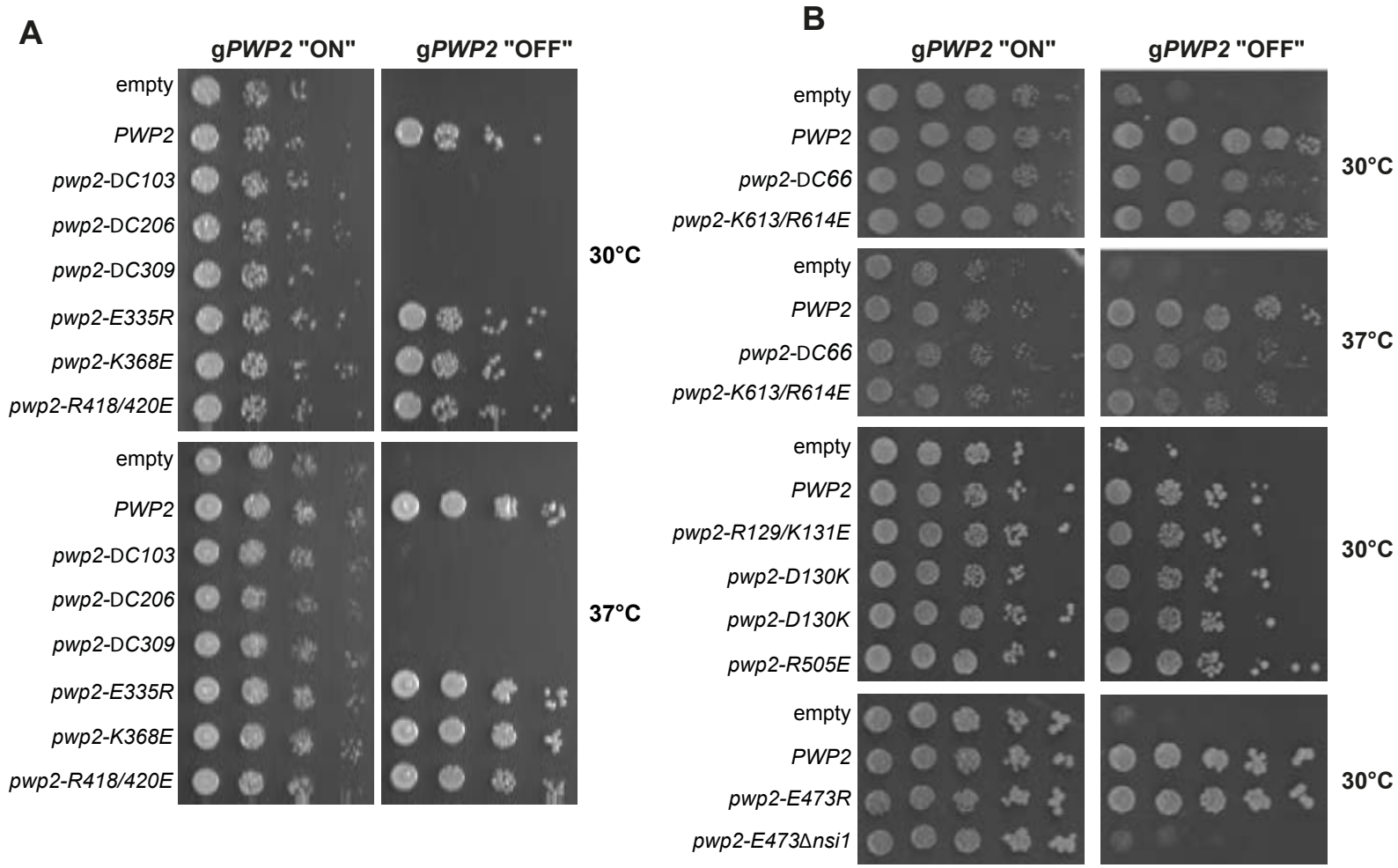
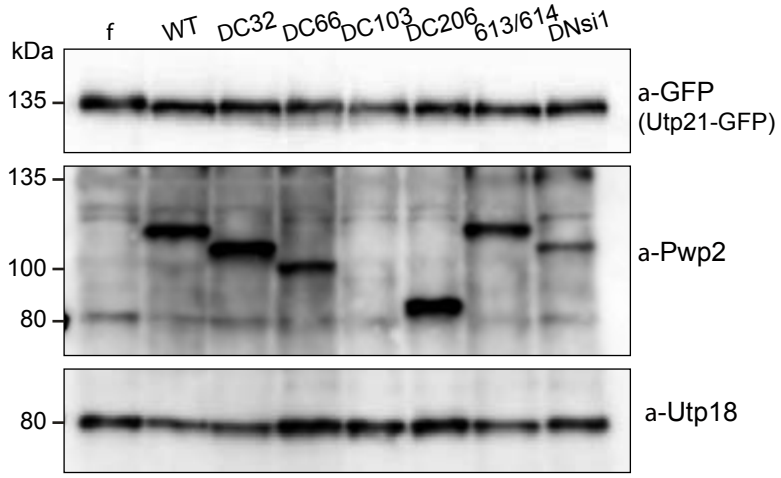
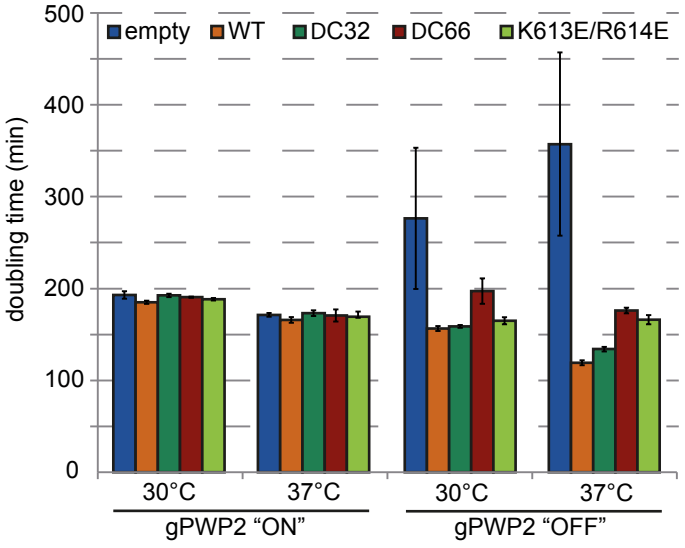

iTRAQ ratio
SSU processome
Utp21p-TAP Purification
PWP2-8/PWP2-18
empty-8/PWP2-18
empty-18/PWP2-18
2
1
0.5
0.25
0.125
Pwp2p
Mpp10p
Nop14p
Fcf2p
Utp3p
Prp43p
Bms1p
Sof1p
Emg1p
Utp14p
Utp20p
Utp22p
Lcp5p
Utp11p
Kre33p
Utp16p
Ecm16p
Bfr2p
Krr1p
Hca4p
Enp1p
Kri1p
Esf2p
Rcl1p
Nhp2p
Esf1p
Rok1p
Nop10p
Enp2p
Nsr1p
Efg1p
Nop6p
Imp3p
Mrd1p

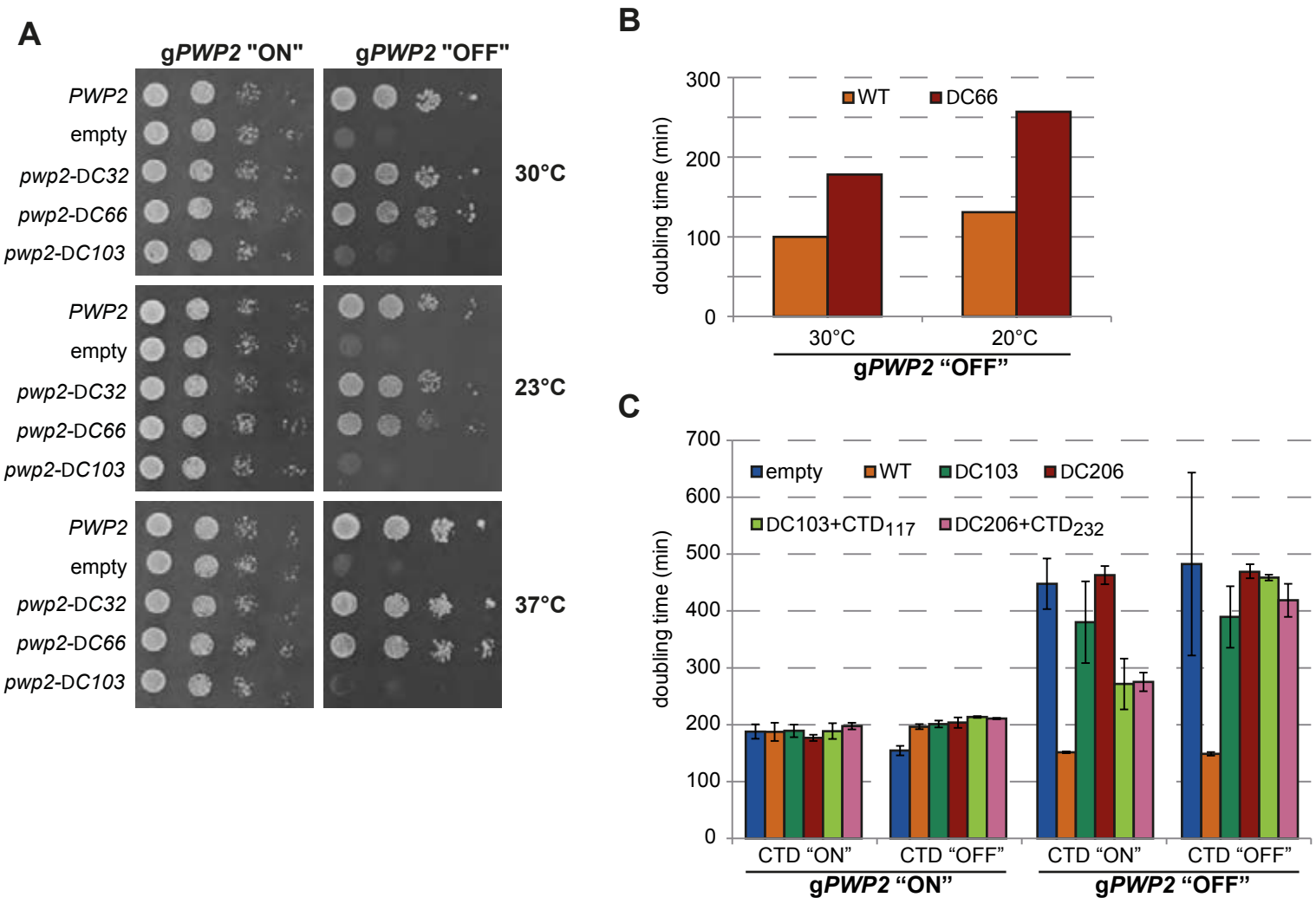

A
E335
K340
R418
R420
E331
Q342
R423
R505
90°
WD2
WD1
B
E335
K340
R418
R420
E331
Q342
R423
R505
C
K340
K370
K368
Q342
R505
E335
E331
R418
R420
R423
D
K340
K370
K368
Q342
R505
E335
E331
R418
R420
R423
E
90°
D517
E510
E473
R505
Q432
90°
K368
F
D517
E510
E473
R505
Q432
K368
G
R140
H
R131
K613
R140
R614
R556
K536

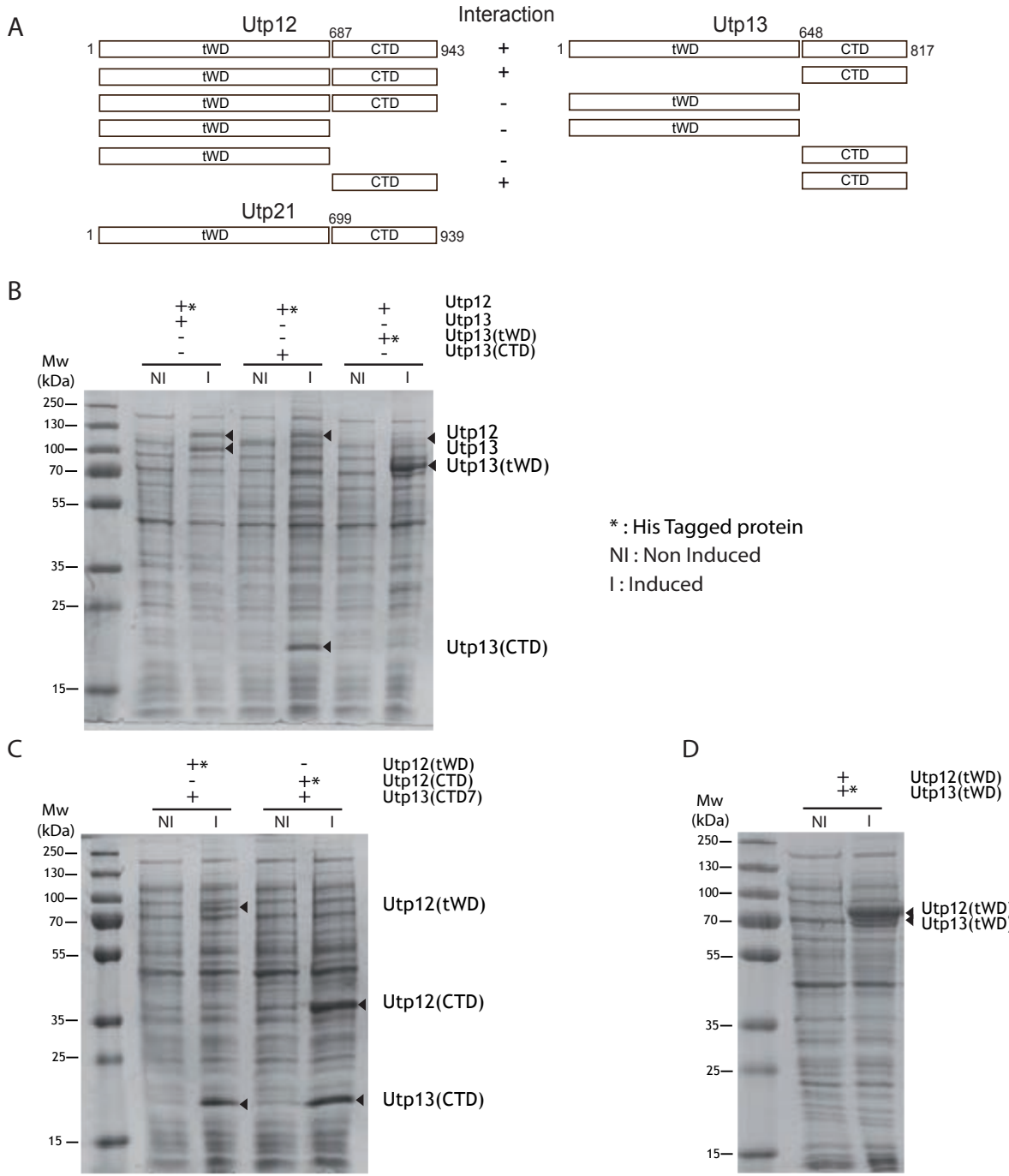

loop1
loop2
loop3
Dnsi1
Dnsi1
CTD
CTD
ΔC309
ΔC206
ΔC103
ΔC66
ΔC32


A
gPWP2 "ON"
gPWP2 "OFF"
empty
PWP2
pwp2-DC103
pwp2-DC206
pwp2-DC309
pwp2-E335R
pwp2-K368E
pwp2-R418/420E
30°C
37°C
B
gPWP2 "ON"
gPWP2 "OFF"
empty
PWP2
pwp2-DC66
pwp2-K613/R614E
pwp2-R129/K131E
pwp2-D130K
pwp2-D130K
pwp2-R505E
pwp2-E473R
pwp2-E473Δnsi1
30°C
37°C

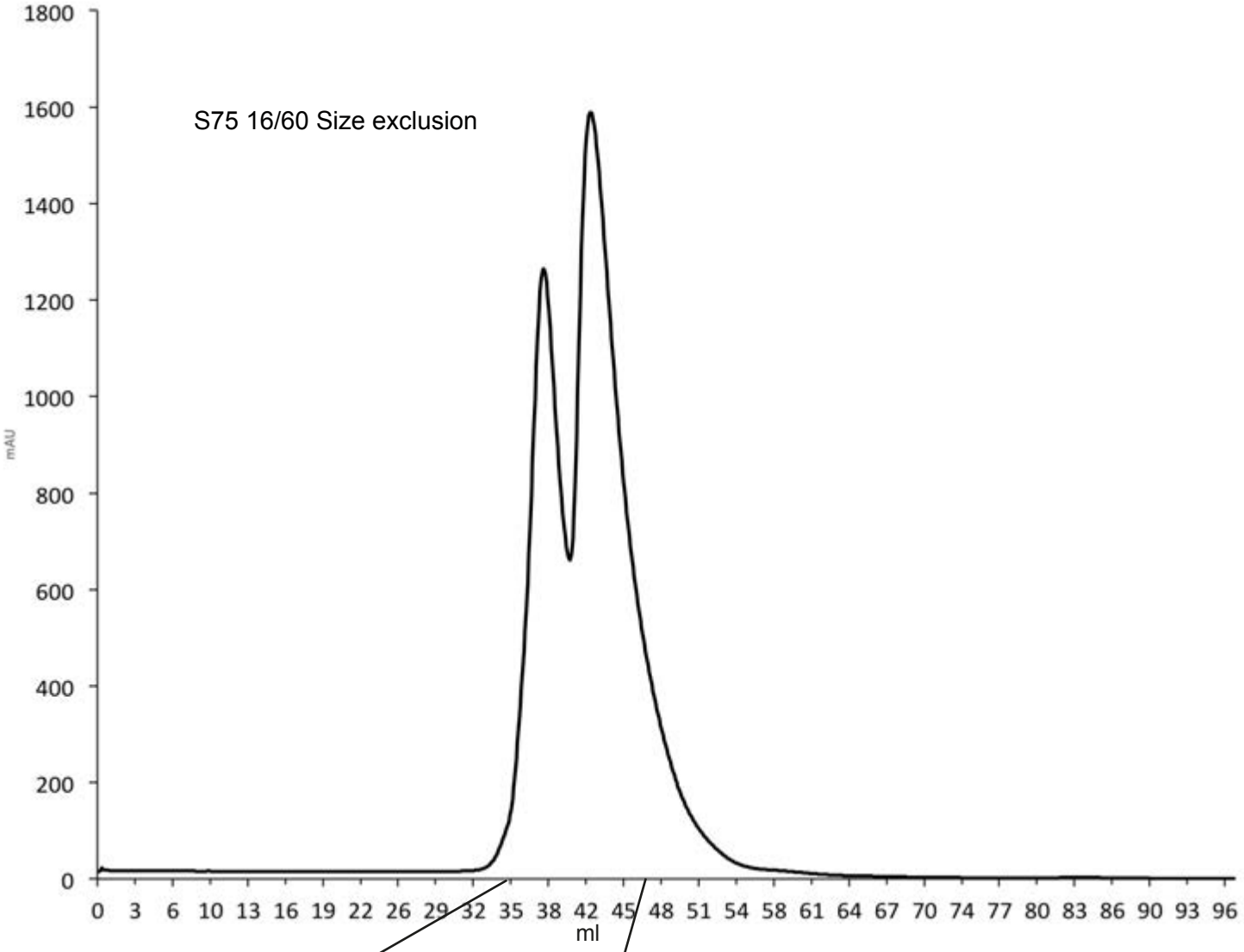

C
f
WT
DC32
DC66
DC103
DC206
613/614
DNsi1
kDa
135
135
100
80
80
a-GFP
(Utp21-GFP)
a-Pwp2
a-Utp18

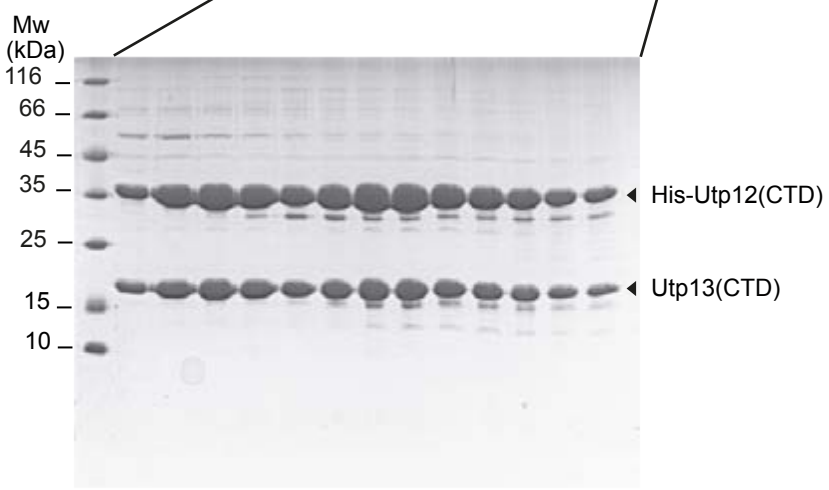

D
empty
WT
DC32
DC66
K613E/R614E
doubling time (min)
500
400
300
200
100
0
30°C
37°C
30°C
37°C
gPWP2 "ON"
gPWP2 "OFF"

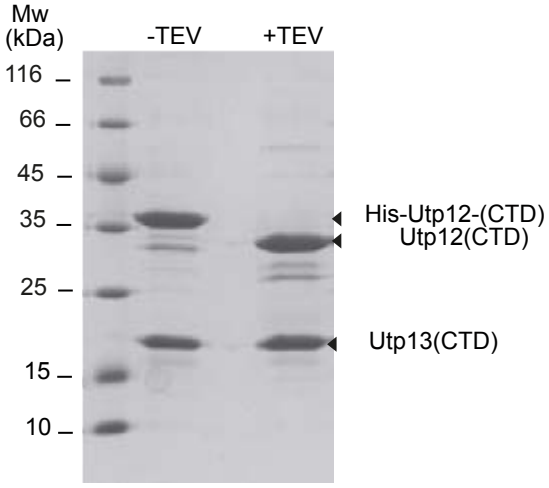

A
gPWP2 "ON"
gPWP2 "OFF"
PWP2
empty
pwp2-DC32
pwp2-DC66
pwp2-DC103
30°C
23°C
37°C
B
doubling time (min)
300
200
100
0
WT
DC66
30°C
20°C
gPWP2 "OFF"
C
doubling time (min)
700
600
500
400
300
200
100
0
empty
WT
DC103
DC206
DC103+CTD117
DC206+CTD232
CTD "ON"
CTD "OFF"
CTD "ON"
CTD "OFF"
gPWP2 "ON"
gPWP2 "OFF"

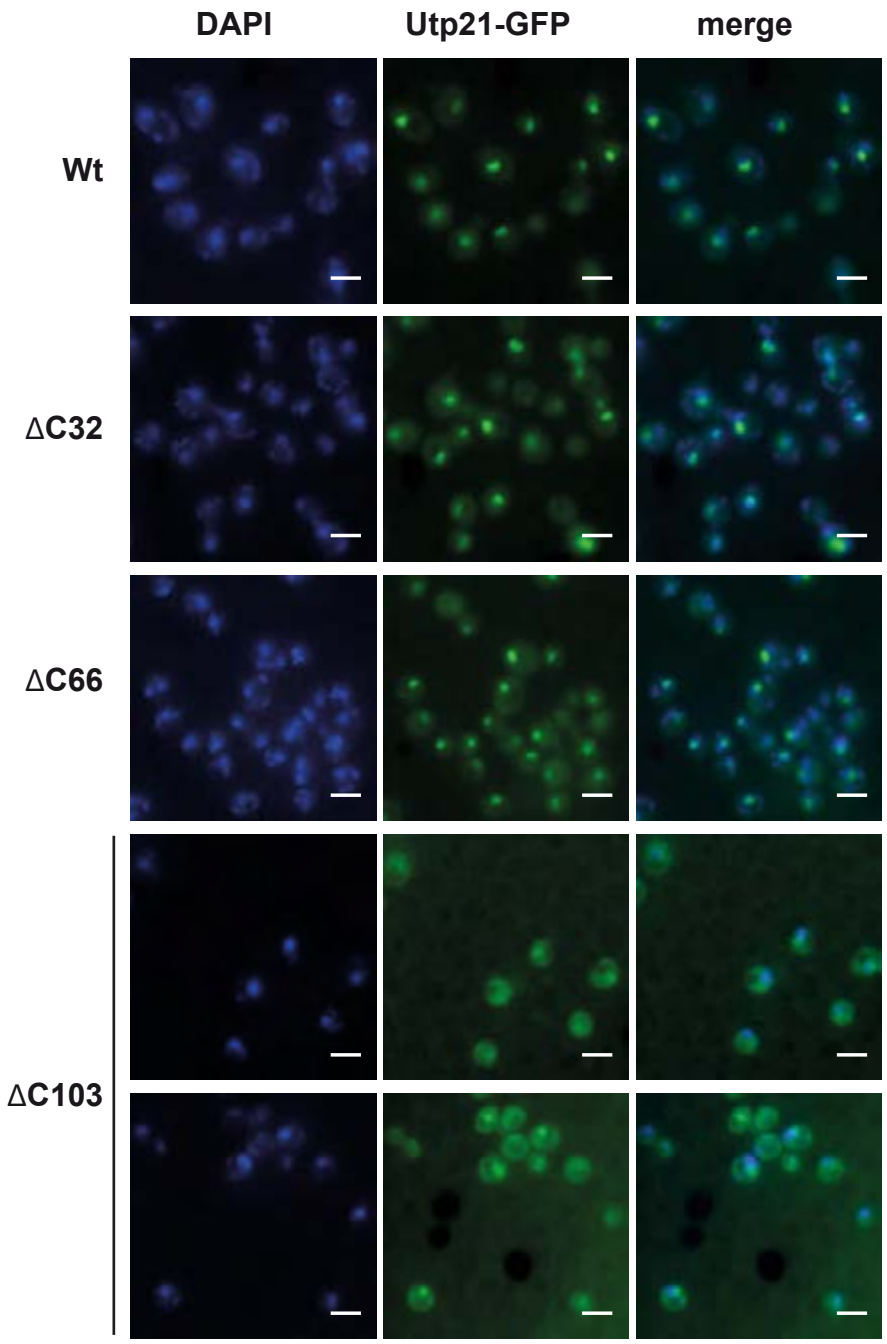

A
Utp12
687
1
tWD
CTD
943
Interaction
+
+
-
-
-
+
Utp13
648
1
tWD
CTD
817
Utp21
699
1
tWD
CTD
939
B
+*
+
-
-
Utp12
Utp13
Utp13(tWD)
Utp13(CTD)
Mw
(kDa)
NI
I
250
130
100
70
55
35
25
15
Utp12
Utp13
Utp13(tWD)
Utp13(CTD)
* : His Tagged protein
NI : Non Induced
I : Induced
C
+*
-
+
-
+*
+
Utp12(tWD)
Utp12(CTD)
Utp13(CTD7)
Mw
(kDa)
NI
I
250
130
100
70
55
35
25
15
Utp12(tWD)
Utp12(CTD)
Utp13(CTD)
D
+
+*
Utp12(tWD)
Utp13(tWD)
Mw
(kDa)
NI
I
250
130
100
70
55
35
25
15
Utp12(tWD)
Utp13(tWD)

Size exclusion chromatography of Utp12(CTD) and Utp13(CTD)








DAPI
Utp21-GFP
merge
Wt
ΔC32
ΔC66
ΔC103