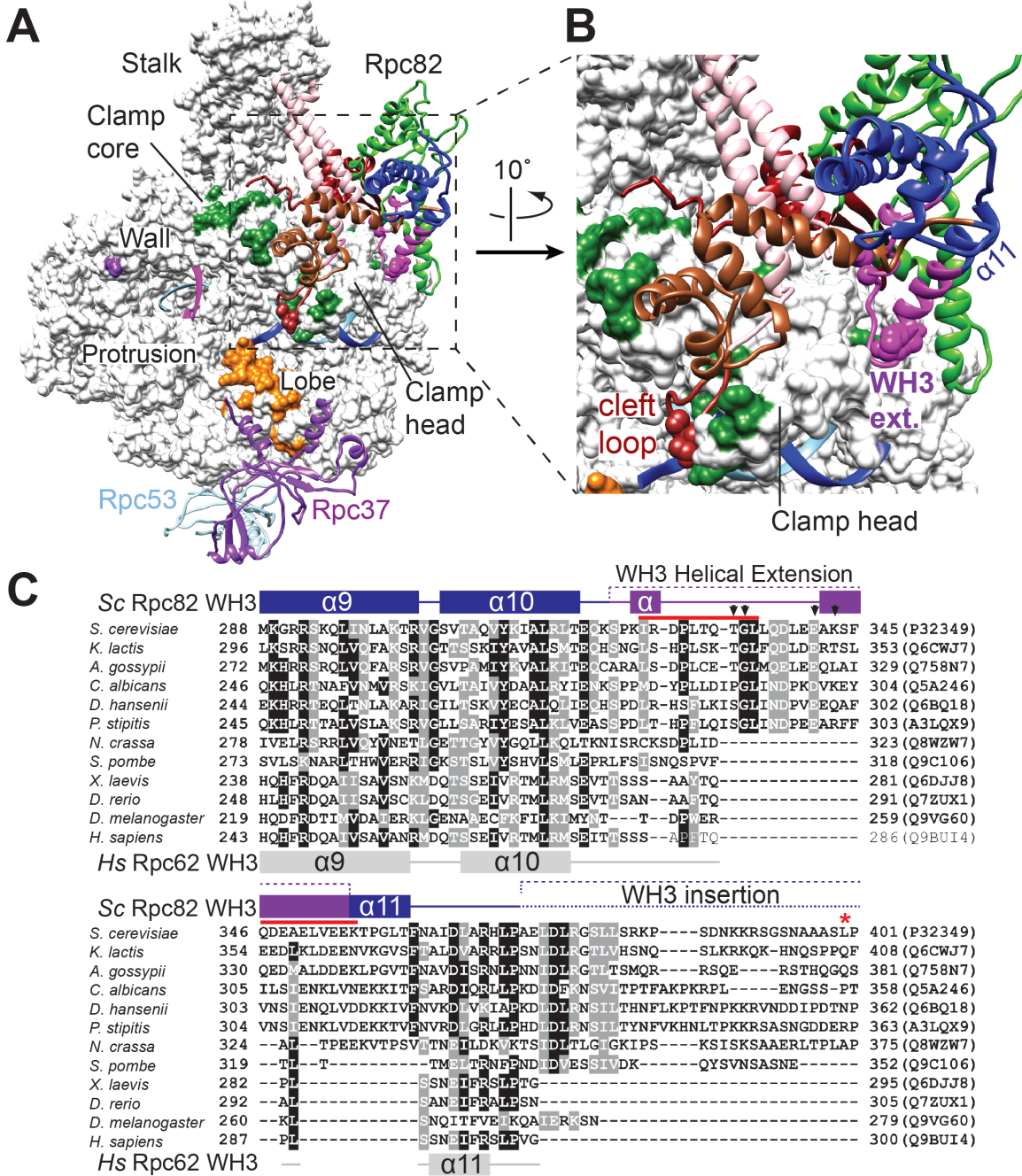


Supplementary Figure 1



Supplementary Figure 1. BPA cross-linking in the pol III cleft and multiple sequence alignment of the Rpc82 WH3 helical extension. (A) The pol III model is shown as in Figure 1B and C. Positions of BPA crosslinking with Rpc82 and Rpc37 are colored green

and orange, respectively. Rpc82- and Rpc37-crosslinking sites are distributed in the clamp and lobe domains, respectively, consistent with protein binding in the cryo-EM structure. A purple-colored position in the wall domain denotes a previously identified BPA crosslinking site with Brf1. **(B)** A view of Rpc82 interaction with the clamp head region of Rpc160. The helical extension of WH3 and the cleft loop of WH4 are indicated. Positions in the helical extension are displayed with spheres to indicate BPA crosslinking with Rpc160. Several residues in the cleft loop are highlighted with spheres to indicate BPA crosslinking with both Rpc160 and Rpc128. **(C)** Multiple sequence alignment of Rpc82 orthologs. Amino acid sequences of the Rpc82 helical extension and flanking regions are listed in the alignment. Secondary structures in *Sc*Rpc82 and *Hs*Rpc62 are indicated. Arrowheads point to residues crosslinking with Rpc160 with BPA substitutions. The Rpc160 crosslinking sites are also displayed with spheres in (B). Red bars denote Rpc82 deletions that conferred a temperature-sensitive cell growth phenotype. A red asterisk indicates the amino acid position Leu400 involved in BPA crosslinking with Brf1. Uniprot accession numbers are listed on the right (in parentheses).

Supplemental Table 1. Summary of BPA photo-crosslinking in the Rpc82 WH3 insertion (Leu370-Ala450) and the Rpc82 WH4 cleft loop (Thr554, Ala555, and Ser558)

Position	Growth (BPA)	Crosslinking	Crosslinked polypeptide
Leu370	+	No	
Leu380	+	No	
Lys390	+	No	
Ser392	+	No	
Ser394	+	Yes	Not identified
Ala396	+	Yes	Not identified
Ala398	+	No	
Leu400	+	Yes	Brf1, C34
Ser402	+	No	
Lys404	+	No	
Lys406	+	No	
Glu408	+	Yes	Not identified
Gly410	–		
Val412	+	Yes	Not identified
Pro414	+	No	
Leu416	+	No	
Ala418	+	No	
Val420	+	Yes	Not identified
Ser421	+	No	
Lys422	+	No	
Ser423	+	No	
Leu424	+	No	
Gln425	+	No	
Glu426	+	No	
Ser427	+	No	
Gly428	+	No	
Asp429	+	No	
Thr430	+	No	
Gln431	+	No	
Glu432	+	No	
Glu433	+	No	
Asp434	+	Yes	Not identified
Glu435	+	No	
Glu436	+	No	
Glu437	+	No	
Glu438	+	No	
Asp439	+	No	
Leu440	+	No	
Asp441	+	No	

Ala442	+	No	
Asp443	+	No	
Thr444	+	No	
Glu445	+	No	
Asp446	-		
Pro447	+	No	
His448	-		
Ser449	+	No	
Ala450	+	No	
Thr554	+	Yes	Rpc128
Ala555	+	Yes	Rpc160, Rpc128
Ser558	+	Yes	Rpc160, Rpc128

Positions of BPA substitution in Rpc82 are indicated and the growth of each substitution is shown as “+”: viable strain or “-”: lethal.

Supplemental Table 2. Summary of BPA photo-crosslinking in the Rpc128 protrusion (Ala413-Lys445) and wall (Thr794-Pro876) domains

Position	Growth (BPA)	Crosslinking	Crosslinked polypeptide
Ala413	+	Yes	Rpc82
Ser421	+	Yes	Rpc82
Ile422	+	Yes	Rpc82
Asn423	+	Yes	Rpc82
Val424	+	Yes	Rpc82
His425	+	Yes	Rpc82
Ser426	+	Yes	Rpc82
Asn427	+	Yes	Rpc82
Asn428	+	Yes	Rpc82
Ser431	+	Yes	Rpc82
Asn434	+	Yes	Rpc82
Arg435	+	Yes	Rpc82
Lys445	+	Yes	Rpc82
Val794	+	Yes	Rpc82
Tyr798	-		
Ala799	+	No	
Asn800	-		
His801	+	Yes	Rpc82, Brf1
Thr802	+	Yes	Rpc82
Gln803	+	Yes	Rpc82
Asp804	-		
Ile805	-		

Gly807	+	Yes	Not identified
Glu813	+	Yes	Not identified
Gly815	+	No	
Ile818	+	No	
Gln822	+	No	
Pro826	-		
Asn850	+	Yes	Rpc82
Ala852	+	Yes	Rpc160
Asp853	+	Yes	Rpc160
Asn856	+	Yes	Rpc82, Rpc160
Pro857	+	Yes	Not identified
Asn858	+	Yes	Rpc82, Rpc160
Asn859	+	Yes	Rpc82, Rpc160
Asn861	+	Yes	Rpc82, Rpc160
Val862	+	Yes	Rpc82, Rpc160
Gln863	+	Yes	Rpc82
Thr864	+	Yes	Rpc82
Tyr866	+	Yes	Rpc82
Arg867	+	Yes	Rpc82
Glu868	+	Yes	Rpc82
Ala869	+	Yes	Rpc82
Val871	+	No	
Ile872	+	Yes	Rpc82
Arg874	+	Yes	Rpc82
Pro876	+	Yes	Rpc82, Rpc160

Positions of BPA substitution in Rpc128 protrusion and wall domains are indicated. “+” : viable strain; “-”: lethal.