SUPPORTING INFORMATION
Appendix

for

The CRL4-like Clr4 Complex in *S. pombe* depends on an exposed surface of Dos1 for heterochromatin silencing

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SI Materials and Methods

Strains

ZB677: h-, Delta-dos1::KanMX6, ade6-M216, ura4D18, otr1L(SphI)::ura4+, leu1-32

Plasmids

For the pairwise interaction screen and protein expression, plasmids were cloned by Sequence Ligation Independent Cloning (SLIC) using standard procedures (1). The complete list of plasmids that were used for protein expression in insect SF9 cells can be found in **Table S3**. The pairwise interaction screen was designed taking advantage of the MultiBac expression system in insect cells (2). Except for the *Clr4* gene, all the others were codon optimized for insect cell expression (Mr. Gene GmbH, Regensburg, Germany). Since there are no antibodies available against any of the CLRC components, one of each pair was cloned with a modified N-terminal One STrEP Sumo (OSS) tag (IBA) in a pFL vector (3) and the other one was cloned with either an N-terminal or a C-terminal FLAG tag in a pUCDM vector. To express the Dos1WD (213-638) and Dos1ΔN (66-638) proteins, the corresponding codon optimized cDNAs were cloned into a pFL vector with a TEV cleavage site between the OSS tag and the protein (4).

For functional assays in *S. pombe*, the prep41-HA-Dos1 plasmid was built by amplifying *dos1* cDNA from pBSdos1 (5) with primers that had the BamHI (reverse) and SalI (forward) sites. The forward ones were designed to provide in-frame cloning with the HA tag, so that the HA is N-terminal. The PCR product was then inserted into a prep41-HAN vector (6) between the SalI and BamHI restriction enzyme sites. All mutants were cloned using the SLIC method described above with primers designed to anneal to the mutation sites as well as primers

designed to anneal to the vector backbone outside of the dos1 gene. The complete list of Dos1 truncations and mutants can be found in **Table S4**.

Protein expression and purification

To express protein pairs from one virus for the pairwise interaction screen, corresponding plasmids were fused using Cre recombination, integrated into the baculovirus genome and expressed according to a published procedure (2). The initial viruses were collected after 3-4 days. $5x10^7$ cells in suspension were infected with 500ul of initial virus to co-express the proteins. The cells were collected 48 hrs after infection and flash frozen in liquid nitrogen. Full-length Rik1 is problematic due to its very low solubility. To overcome this problem, we designed a truncated version of Rik1 in which we deleted the second WD40 domain by linking Alanine 377 to Leucine 664 in Rik1 according to a homology model based on the DDB1 structure. This new version was cloned into pFL-OSS, expressed as described above and used in pull down assays with Dos1 truncations (see Fig. 2B) as described below.

For Dos1 expression and purification, the bacmid DNA was purified from pFL-OSS-Dos1 plasmid-transformed DH10MultiBAC cells with an alkaline lysis followed by isopropanol precipitation and transfected into SF-9 cells to generate viruses. Viruses were amplified by infecting SF-9 cells with a 1:1000 dilution of initial virus (V_0) from the previous stage and incubating cells at 27°C for 3-4 days (V_1). For protein expression, cells around $2x10^6$ concentration were infected with a 1:100 (v/v) ratio of V1, harvested 48-60 hr post infection, resuspended in 5-10 ml of PBS per 1 L of cells and flash frozen in liquid nitrogen.

Prior to initial purification, cells were thawed in a water bath at 25°C and lysis buffer (100 mM HEPES pH 7.6, 200 mM KCl, 5 mM DTT, 5 mM $MgCl_2$) was added at 2:1 (v/w)

supplemented with 1X protease inhibitors (16µg/ml benzamidine HCl, 10 µg/ml phenanthroline, 10 µg/ml aprotinin, 10 µg /ml leupeptin, 10 µg /ml Pepstatin A, 1 mM PMSF) and 1:50 v/v TurboNuclease (Accelagen). Cells were incubated on a shaker for 15 min for complete lysis. After centrifugation for 45 min at 142,000 x g at 4°C, the supernatant was decanted and incubated on a roller at 4°C for 1 hr with 0.5 ml lysis buffer equilibrated with Strep-Tactin resin. The beads were washed 3 times with 5 column volumes (CV) of lysis buffer supplemented with 5 mM ATP to remove chaperones and 5 times with 5 CV lysis buffer. The protein was eluted in 3 steps with 1 ml lysis buffer supplemented with 2.5 mM desthiobiotin. SUMOstar protease (LifeSensors) was used to cleave the tag. However, complete cleavage could not be achieved even with an increased amount of protease. Subsequent constructs therefore included a TEV site for tag cleavage. The protein was further purified from uncleaved fusion with anion exchange chromatography using MonoQ column with a salt gradient from 0.1-1 M KCl (MQ buffer), and size exclusion chromatography using Superdex 200 column equilibrated in GF buffer (50 mM HEPES pH 7.6, 200 mM KCl, 2 mM DTT).

Dos1WD and Dos1ΔN fragments were expressed in insect cells as described above, except that Hi5 cells were used instead of SF-9 cells, yielding ~5 times more protein per liter of cells. The protocol for selenomethionine labeled protein expression was modified from (7). The minor changes are: 1) Hi5 cells were used to express the protein; 2) The media was changed to methionine free media 6 hr after infection; 3) 200 mg/L selenomethionine was added 10 hr after infection. The initial protein purification for Dos1WD and Dos1ΔN was the same as above except that the tag was cleaved on the column by incubating the resin with 1:100 dilution of TEV protease overnight at 4°C. The untagged protein was eluted and subjected to anion exchange and

size exclusion chromatography in MQ and GF buffers supplemented with 10 mM DTT instead of 2 mM DTT.

Size exclusion chromatography analysis

To test for proper folding and stability of the Dos1 K489A/D490A mutant, we used size exclusion chromatography. Wild type and mutant proteins were expressed and purified by streptactin affinity purification as described above. 200µl of 2mg/ml protein of each construct was loaded onto a Superdex 200 16/60 (GE Healthcare) column and 0.5ml fractions were collected. The peak fractions were loaded into a NuPAGE gel and analyzed by commassie blue staining.

Pull down assays

For pull down assays using Strep-Tactin beads (IBA), SF-9 cells expressing pairs of CLRC complex subunits were lysed in 1 ml lysis buffer supplemented with 1X protease inhibitors. Cells were centrifuged at 18,000 x g for 20 min, and the supernatant was incubated with 100 µl Strep-Tactin beads equilibrated with 5 CV of lysis buffer for 1hr at 4°C. The beads were washed with 5 CV lysis buffer supplemented with 5 mM ATP to remove chaperones, and twice with 5 CV lysis buffer. The proteins were eluted in 200 µl lysis buffer supplemented with 2.5 mM desthiobiotin and subjected to western blot analysis. 10µg of total input protein were loaded into an 8% NuPAGE gel, and elutions normalized as indicated in the figures. Gels were semi-dry blotted on a nitrocellulose membrane at 150 mA for 1 hr. To prevent cross contamination of signals from secondary antibodies, sequential western blotting was used. For the first western, the membrane was incubated in 10 ml Odyssey Blocking Buffer (LI-COR

Biosciences) overnight at 4°C by shaking. This was followed by incubation with FLAG antibody (Sigma-Aldrich) at 1:5000 dilution in 20% Odyssey blocking buffer for 1hr at room temperature (RT). The membrane was then washed 3 times with 1X PBS-T with 5 min incubations. Donkey anti-rabbit IRDye 800CW (LI-COR Biosciences) with 1:10000 dilution in 20% Odyssey blocking buffer was used. The membrane was then washed 3 times with 1X PBS-T with 5 min incubations, and once in 1X PBS before scanning (Odyssey). The membrane was stripped at least twice with stripping buffer (25 mM glycine pH 2.0, 1.5% SDS) and washed with 1X PBS-T and 1X PBS. After confirming with a quick scan on the Odyssey, a second western was started with 1hr incubation in Odyssey blocking buffer. It was incubated with STrEP MAB Classic antibody (IBA) using a 1:1000 dilution and tubulin antibody at 1:5000 dilution (abcam) in 20% Odyssey blocking buffer for 1hr at room temperature (RT). The membrane was then washed 3 times with 1X PBS-T with 5 min incubations. Goat anti-mouse IRDye 800CW (LI-COR Biosciences) at a 1:10000 dilution and Goat anti-rat IRDye 680CW (LI-COR Biosciences) in 20% Odyssey blocking buffer was used as a secondary detection. The membrane was then washed 3 times with 1X PBS-T with 5 min incubations, and once in 1X PBS before scanning (Odyssey). The membrane was scanned using both 700 and 800 nm channels.

Limited proteolysis

Limited proteolysis was performed with Thermolysin (Sigma) in a 20 µl reaction volume of 10 mM Tris-HCl pH 8.0, 2 mM $CaCl_2$ and 5% glycerol containing protein:protease ratios of 12.8, 3.2, 0.8, 0.2 and 0.05. After 60 min at 37°C, the reactions were quenched in 1X SDS loading dye (2.5% (w/v) glycerol, 3% (w/v) Triethanolamine, 1% (w/v) SDS, 0.25% (w/v) Ficoll

400, 0.5 mM EDTA, 26% (w/v) Brilliant Blue G250). 5 µl of samples from each reaction were loaded onto an SDS-PAGE gel for analysis.

Crystallization and structure determination

Diffraction quality crystals were obtained by the hanging drop vapor diffusion method where 5mg/ml Dos1WD in 200 mM KCl, 50 mM HEPES pH 7.5, 2 mM DTT was mixed with an equal volume of the reservoir solution containing 50 mM Tris-HCl (pH 7.0), 0.9 M di-sodium tartrate, 50 mM $MgCl_2$. Selenomethionine derivative crystals were obtained under the same conditions. Crystals appeared within 4 days and grew to their final size in 2 weeks. Prior to data collection, selenomethionine labeled crystals were transferred into a cryopreservation solution containing mother liquor supplemented with 25% glycerol added in a stepwise manner, incubated overnight, and frozen in liquid nitrogen. Native crystals were directly transferred into a cryopreservation solution with the addition of 25% ethylene glycol and frozen in liquid nitrogen. Data were collected under cryogenic conditions at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, beamline X25 at the selenium peak wavelength (0.9788 Å). The data were processed and scaled with XDS (8). Initial heavy atom positions were identified using the SAD method implemented in Phenix (9) using reflections up to 2.5 Å resolution. The initial model was built using the Autobuild utility in Phenix which was only able to build alanine chains. The complete initial model was built in Coot (10) using this auto-build model as a guide. The structure was further refined against the native dataset up to 2 Å resolution using phenix.refine yielding a model with two molecules per asymmetric unit and excellent geometry (97.5% in favored region and 2.5% in allowed regions). 361 waters, 1 Chloride ion and

100 ethylene glycols were added. Final model building was carried out in Coot and evaluated using the MolProbity server (11). Data and refinement statistics are shown in **Table S2**.

Computational analysis

Structure similarity searches and structure-based sequence alignments were performed using DALI (12). PSI-BLAST (13) was used to identify Dos1WD orthologs using Dos1 residues 213-638. Alignments were done using ClustalW (14) and surface conservations were analyzed using ConSurf (15). PyMOL (Schrödinger) was used for comparison of Dos1WD and CRL4 and other propellers. Electrostatic surface potentials were generated using the APBS plugin in PyMOL (16).

Spotting assays, TCA precipitation and western blotting

The *S. pombe* strains transformed with the indicated mutant plasmid according to (17). Strains were grown to an exponential phase (OD 0.2-0.6) and counted under the microscope. On a sterile 96 well plate, cells were diluted to give 2 x 10^3 cells per µl of water in the first column (one row per strain) and then diluted in a serial manner over the next 4 columns to make 1:10 dilutions. Dilution range is from 2 x 10^3 to 0.2. 5ul of each dilution were spotted on each plate (-Leu, -Leu-Ura, -Leu+FOA). The concentration of FOA in the plates was 1mg/ml. The plates also contained 0.05 µM thiamine to tune down the expression level of the protein. The plates were not moved until the spots dried completely and then incubated at either 30°C or 37°C for 4 days before pictures were taken. The complete list of Dos1 truncations and mutants, and their effects in *ura4*$^+$ silencing is shown in **Table S1**.

In order to detect expression of Dos1 truncations and mutants, TCA precipitation was performed with 5ml cultures and grown in PMG –Leu medium supplemented with 0.05 μM thiamine to an OD600 of 1.0 according to a published protocol (18). Total protein concentrations were measured and 5 μg of total protein was loaded into 8% NuPAGE with addition of 1X SDS sample loading dye after boiling at 95°C for 5 min. Western blotting was done using 12CA5 anti-HA from CSHL in-house facility as a primary and ECL Anti-Mouse IgG (GE Healthcare) as a secondary. Detection was done using a Pierce ECL Western Blotting Substrate.

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Table S1: Results of comparative growth assays using Dos1 mutants.

Mutant	Phenotype	Mutant	Phenotype	Mutant	Phenotype
WT	-	Dos1 ΔLIV (Δ531-546)	++++	Dos1 F416A	+++
HA-Dos1	-	Dos1 ΔLIV-v2 (Δ535-546)	++++	Dos1 Y450A	+
HA-null	++++	Dos1 N237A/E238A	++	Dos1 L465A	++
Dos1 T495I	++	Dos1 V244A	++	Dos1 T487A/ Q488A	++
Dos1 R576A	++	Dos1 V244A/T260A	++	Dos1 K489A/ D490A	++++
Dos1 R518A	+++	Dos1 H278A	-	Dos1 I491A/ N492A	++++
Dos1WD (1-212)	++++	Dos1 T301A	+++	Dos1 H493A	-
Dos1ΔWD (213-638)	++++	Dos1 D304A	++	Dos1 T508A/ D509A	++++
Dos1ΔN (66-638)	-	Dos1 F319A	+++	Dos1 N 550A	+
Dos1 (139-638)	++++	Dos1 D320A/ N321A	N/A	Dos1 Q553A	-
Dos1 (154-638)	++++	Dos1 T340A/ D341A	++	Dos1 N559A	-
Dos1 (173-638)	N/A	Dos1 H344A/ K345A	+++	Dos1 Y594A	-
Dos1 (194-638)	N/A	Dos1 K359A	-	Dos1 E599A	-
Dos1 ΔLI (Δ261-269)	++++	Dos1 E385A	-	Dos1ΔCtail (1-618)	+++
Dos1 ΔLII (Δ289-299)	+++	Dos1 E388A/ K389A	-	Dos1 F626A/ F629A	+++
Dos1 ΔLIII (Δ415-421)	++++	Dos1 E397A	+	Dos1 E628A/ E633A	-

The categories of mutants are as follows: strong silencing defect at 30°C (++++); mild silencing defect at 30°C and strong silencing defect at 37°C (+++); strong silencing defect at 37°C (++); mild at 37°C (+); no defect in silencing, same as WT (-); not enough data (N/A).

Table S2: Data collection and refinement statistics

	Native	**Selenomethionine derivative**
Data collection		
Space group	C2	C2
Cell dimensions		
a, b, c (Å), β (º)	121.15, 102.79, 95.09, 122.35	122.28, 102.09, 94.25, 122.50
Wavelength (Å)	0.9788	0.9788
Resolution (Å)[a]	45.87-2.0 (2.05-2.00)	19.9-2.50 (2.56-2.50)
***R*merge (%)**	6.0 (34.6)	5.6 (17.2)
I/σ(I)	11.9 (3.1)	16.7 (7.5)
Completeness (%)	99.0 (99.5)	99.2 (99.8)
Redundancy	3.4 (3.2)	3.5 (3.6)
Refinement		
Resolution (Å)	45.87-2.0	
No. reflections[a]	65972 (5480)	
***Rwork/Rfree* (%)**	21.32/25.01	
No. atoms (non-hydrogen)	6470	
Protein	6084	
Water	349	
Ethylene Glycol	36	
Chloride ion	1	
<*B*-factors> (Å^2)		
Protein	33.80	
Waters	37.90	
r.m.s. deviations		
Bond lengths (Å)	0.011	
Bond angles (º)	1.22	
MolProbity Statistics		
All atom clashscore	6.24	
Ramahandran favored (%)	96.74%	
Ramachandran outliers (%)	0	
Poor rotamers (%)	1.35%	
MolProbity score	1.64	

[a] Values in parentheses are for the highest-resolution shell.

Table S3: List of plasmids that are used for insect cell expression

ID	Name	Proteins
3720	pFL_OneStrep-SumoStar-clr4_YFP	OSS-Clr4
4753	pFL_ph-OneStrepSumo-Cul4 opt_p10-YFP	OSS-Cul4
4750	pFL_ph-OneStrepSumo-Dos1 opt_p10-YFP	OSS-Dos1
4722	pFL_ph-OnestrepSumo-dos2 opt_YFP	OSS-Dos2
3810	pFL_ph_OneStrepSumo_rik1opt_p10_YFP	OSS-Rik1
5394	pFL_ph_OneStrepSumo-Stc1opt_p10_YFP	OSS-Stc1
4729	pUCDM_ph-Flag-Clr4	Flag-Clr4
4745	pUCDM_ph-Flag-Cul4 opt	Flag-Cul4
4738	pUCDM_ph-Flag-Dos1 opt	Flag-Dos1
4742	pUCDM_ph-Flag-dos2 opt	Flag-Dos2
4732	pUCDM_ph-Flag-Rik1 opt	Flag-Rik1
6977	pFL_SS-TEV-Dos1 opt_p10-YFP	OSS-TEV-Dos1
6981	pFL_SS-TEV-Dos1 opt 213F_p10-YFP	OSS-TEV-Dos1WD
6984	pFL_SS-TEV-Dos1 opt 66I_p10-YFP	OSS-TEV-Dos1ΔN
6264	pFL_ph_OneStrepSumo_rik1opt(A377-L664 del)_p10_YFP	OSS-Rik1AC
6410	pUCDM_ph-flag-Dos1 opt 213F	Flag-Dos1WD
6395	pUCDM_ph-flag-Dos1 opt 66I	Flag-Dos1ΔN
11489	pUCDM_ph-Flag-Dos1opt(del531-546)	Flag-Dos1ΔLIV
11463	pUCDM_ph-FLAG-Dos1opt(T508A,D509A)	Flag-Dos1 T508A/D509A
11457	pUCDM-ph-FLAG-Dos1opt(I491A,N492A)	Flag-Dos1 I491A/N492A
11452	pUCDM-ph-FLAG-Dos1opt(K489A,D490A)	Flag-Dos1 K489A/D490A
11488	pFL_SS-Dos1opt(del531-546)	OSS- Dos1ΔLIV
11462	pFL_SS-Dos1opt(T508A,D509A)	OSS- Dos1 T508A/D509A

11456	pFL_SS-Dos1opt(I491A,N492A)	OSS- Dos1 I491A/N492A
11449	pFL_SS-Dos1opt (K489A,D490A)	OSS- Dos1 K489A/D490A
7124	Cre fusion of 3720&4745	OSS-Clr4, Flag-Cul4
7125	Cre fusion of 3720&4738	OSS-Clr4, Flag-Dos1
7126	Cre fusion of 3720&4742	OSS-Clr4, Flag-Dos2
7127	Cre fusion of 3720&4732	OSS-Clr4, Flag-Rik1
7128	Cre fusion of 4753&4729	OSS-Cul4, Flag-Clr4
7129	Cre fusion of 4753&4738	OSS-Cul4, Flag-Dos1
7130	Cre fusion of 4753&4742	OSS-Cul4, Flag-Dos2
7131	Cre fusion of 4753&4732	OSS-Cul4, Flag-Rik1
7132	Cre fusion of 4750&4729	OSS-Dos1, Flag-Clr4
7133	Cre fusion of 4750&4745	OSS-Dos1, Flag-Cul4
7134	Cre fusion of 4750&4742	OSS-Dos1, Flag-Dos2
7135	Cre fusion of 4750&4732	OSS-Dos1, Flag-Rik1
7136	Cre fusion of 4722&4729	OSS-Dos2, Flag-Clr4
7137	Cre fusion of 4722&4745	OSS-Dos2, Flag-Cul4
7138	Cre fusion of 4722&4738	OSS-Dos2, Flag-Dos1
7139	Cre fusion of 4722&4732	OSS-Dos2, Flag-Rik1
7140	Cre fusion of 3810&4729	OSS-Rik1, Flag-Clr4
7141	Cre fusion of 3810&4745	OSS-Rik1, Flag-Cul4
7142	Cre fusion of 3810&4738	OSS-Rik1, Flag-Dos1
7143	Cre fusion of 3810&4742	OSS-Rik1, Flag-Dos2
7144	Cre fusion of 5394&4729	OSS-Stc1, Flag-Clr4
7145	Cre fusion of5394&4745	OSS-Stc1, Flag-Cul4
7146	Cre fusion of5394&4738	OSS-Stc1, Flag-Dos1
7147	Cre fusion of 5394&4742	OSS-Stc1, Flag-Dos2

7148	Cre fusion of 5394&4732	OSS-Stc1, Flag-Rik1
6745	Strep-sumo-Rik1(A377-L664 del)&Flag- Dos1 cre	OSS-Rik1AC, Flag-Dos1
6746	Strep-sumo-Rik1(A377-L664 del)&Flag-Dos1 213F	OSS-Rik1AC, Flag-Dos1WD
6747	Strep-sumo-Rik1(A377-L664 del)&Flag-Dos1 66I c	OSS-Rik1AC, Flag-Dos1ΔN
12599	OSS-Rik1opt_AC+Flag-Dos1opt(del531-546)	OSS-Rik1AC, Flag-Dos1ΔLIV
12598	OSS-Rik1opt_AC+FLAG-Dos1opt(I491A,N492A)	OSS-Rik1AC, Flag-Dos1 I491A/N492A
12597	OSS-Rik1opt_AC+FLAG-Dos1opt(T508A,D509A)	OSS-Rik1AC, Flag-Dos1 T508A/D509A
12596	OSS-Rik1opt_AC+Flag-Dos1opt(K489A,D490A)	OSS-Rik1AC, Flag-Dos1 K489A/D490A
12595	OSS-Dos1opt(I491A,N492A)+Flag-Clr4	OSS-Dos1 I491A/N492A, Flag-Clr4
12594	OSS-Dos1opt(T508A,D509A)+Flag-Clr4	OSS-Dos1 T508A/D509A, Flag-Clr4
12425	OSS-Dos1opt (K489A,D490A)+Flag-Clr4	OSS-Dos1 K489A/D490A, Flag-Clr4
12424	OSS-Dos1(delLIV)+Flag-Clr4	OSS-Dos1 ΔLIV, Flag-Clr4
11661	OSS-Dos1(del531-546)+Flag-Dos2	OSS-Dos1 ΔLIV, Flag-Dos2
11641	OSS-Dos1(T508A,D509A)+Flag-Dos2	OSS-Dos1 T508A/D509A, Flag-Dos2
11637	OSS-Dos1(I491A,N492A)+Flag-Dos2	OSS-Dos1 I491A/N492A, Flag-Dos2
11633	OSS-Dos1(K489A,D490A)+Flag-Dos2	OSS-Dos1 K489A/D490A, Flag-Dos2

Table S4: List of plasmids that were used to express Dos1 mutants in *S.pombe* strains.

ID	Name
10195	pREP41-HA-null
10246	pREP41-HA-Dos1
10249	pREP41-HA-Dos1-L II(288G300)
10252	pREP41-HA-Dos1(R518A)
10257	pREP41-HA-Dos1(R576A)
10262	pREP41-HA-Dos1(T495I)
10275	pREP41-HA-Dos1-L I(260-GG-270)
10281	pREP41-HA-Dos1-L III(414G422)
10284	pREP41-HA-Dos1-L IV-v2(530GG547)
10289	pREP41-HA-Dos1-L IV(534GG547)
10294	pREP41-HA-Dos1(1-618)
10298	pREP41-HA-Dos1(1-212)
10302	pREP41-HA-Dos1(194-638)
10349	pREP41-HA-Dos1(H344A,K345A)
10354	pREP41-HA-Dos1(E397A)
10359	pREP41-HA-Dos1(F416A)
10371	pREP41-HA-Dos1(Y450A)
10374	pREP41-HA-Dos1(H493A)
10379	pREP41-HA-Dos1(N550A)
10384	pREP41-HA-Dos1(Q553A)
10389	pREP41-HA-Dos1(N559A)
10394	pREP41-HA-Dos1(Y594A)
10399	pREP41-HA-Dos1(E599A)

10479	pREP41-HA-Dos1(N237A,E238A)
10507	pREP41-HA-Dos1(H278A)
10512	pREP41-HA-Dos1(T301A)
10516	pREP41-HA-Dos1(D304A)
10519	pREP41-HA-Dos1(T3401A,D341A)
10522	pREP41-HA-Dos1(K359A)
10528	pREP41-HA-Dos1(F626A,F629A)
10531	pREP41-HA-Dos1(E628A,E633A)
10554	pREP41-HA-Dos1(173-638)
10557	pREP41-HA-Dos1(154-638)
10560	pREP41-HA-Dos1(139-638)
10580	pREP41-HA-Dos1(F319A)
10583	pREP41-HA-Dos1(D320A,N321A)
10586	pREP41-HA-Dos1(E385A)
10589	pREP41-HA-Dos1(E388A,K389A)
10593	pREP41-HA-Dos1(T487A,Q488A)
10596	pREP41-HA-Dos1(I491A,N492A)
10599	pREP41-HA-Dos1(T508A,D509A)
10605	pREP41-HA-Dos1(K489A,D490A)
11051	pREP41-HA-Dos1(V244A)
11093	pREP41-HA-Dos1(V244A, T260A)
11272	pREP41-HA-Dos1deltaN(66-638)
11275	pREP41-HA-Dos1WD(213-638)
11364	pREP41-HA-Dos1(L465A)

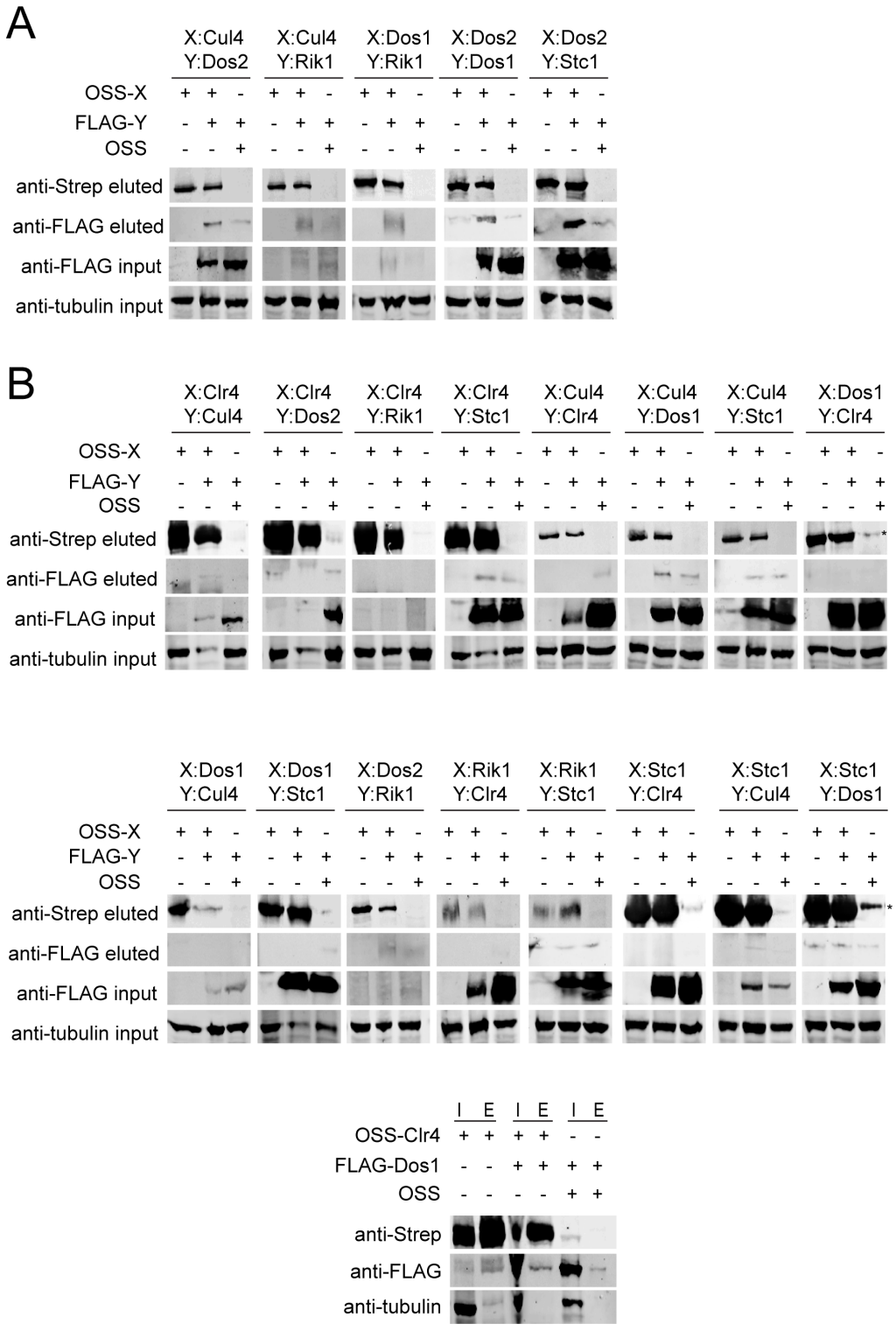


Figure S1. Additional pairwise interaction screens to those shown in Fig.1A, but used for the summary in Fig. 1B. (A) Pairs classified as interacting. (**B**) Pairs classified as non interacting. OSS and FLAG tagged single CLRC components were co-expressed and pulled down with StrepTactin beads (see SI Materials & Methods).

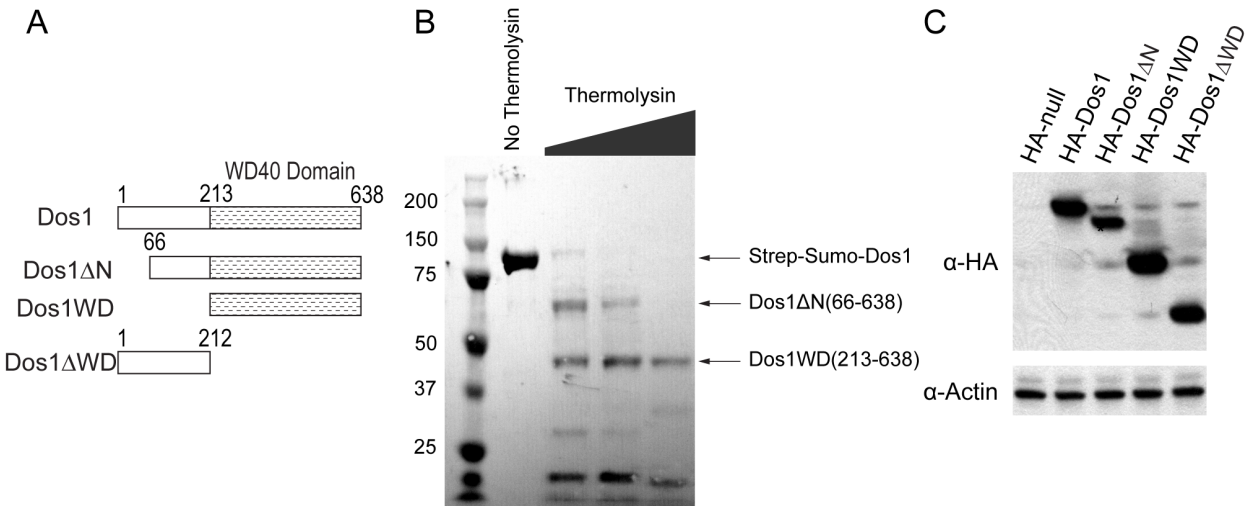


Figure S2. Limited proteolysis of Dos1 identified two distinct fragments. (**A**) Domain architecture of Dos1 fragments which were identified in limited proteolysis experiments and used in the rescue experiments. (**B**) N-terminal sequences of Dos1ΔN (66-638) and Dos1WD (213-638) were identified by Edman Sequencing at Protein Core Facility in Columbia University. (**C**) Protein levels of the Dos1 truncations as indicated detected by western using HA tag antibody and actin as a loading control. * indicates the non-specific band from HA antibody around the same size as Dos1.

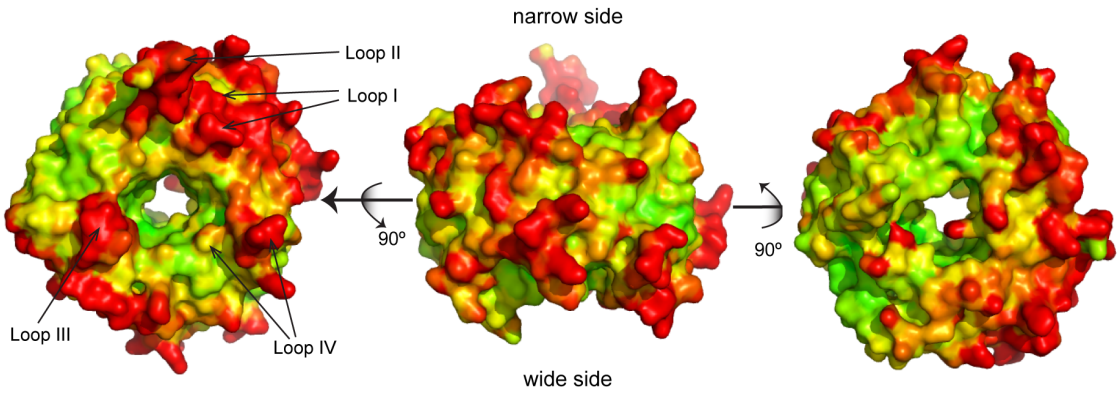
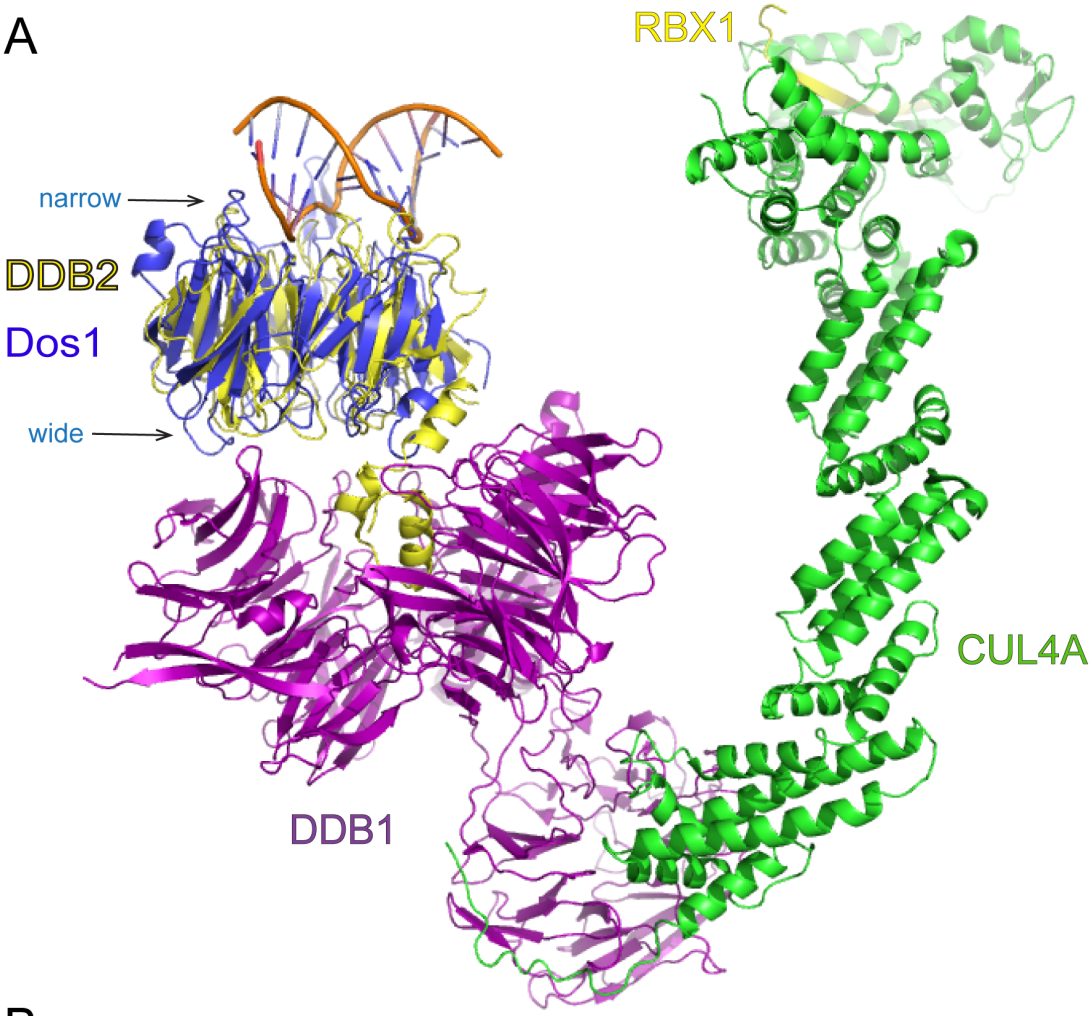


Figure S3. Flexible loops and regions on the narrow site of Dos1WD. Surface representation of Dos1WD, colored based on crystallographic *B*-factor values (yellow: low, green: mid and red: high). High B factors correlate with increased flexibility of the residues.

A



B

	CLRC	CRL4
E3 ligase	Cul4	CUL4A
3 propeller adaptor	Rik1	DDB1
DCAF	Dos1	DDB2
Methyltranferase	Clr4	-
Unknown function	Dos2	-
RITS interacting subunit	Stc1	-
Target	?	DNA

Figure S4. Superposition of Dos1WD and DDB2 in the CRL4^{DDB2} complex structure (PDB ID: 4a0k). (**A**) The alignment identifies the Rik1 binding surface and a putative target binding surface on Dos1. **(B)** Equivalent CLRC and CRL4^{DDB2} subunits.

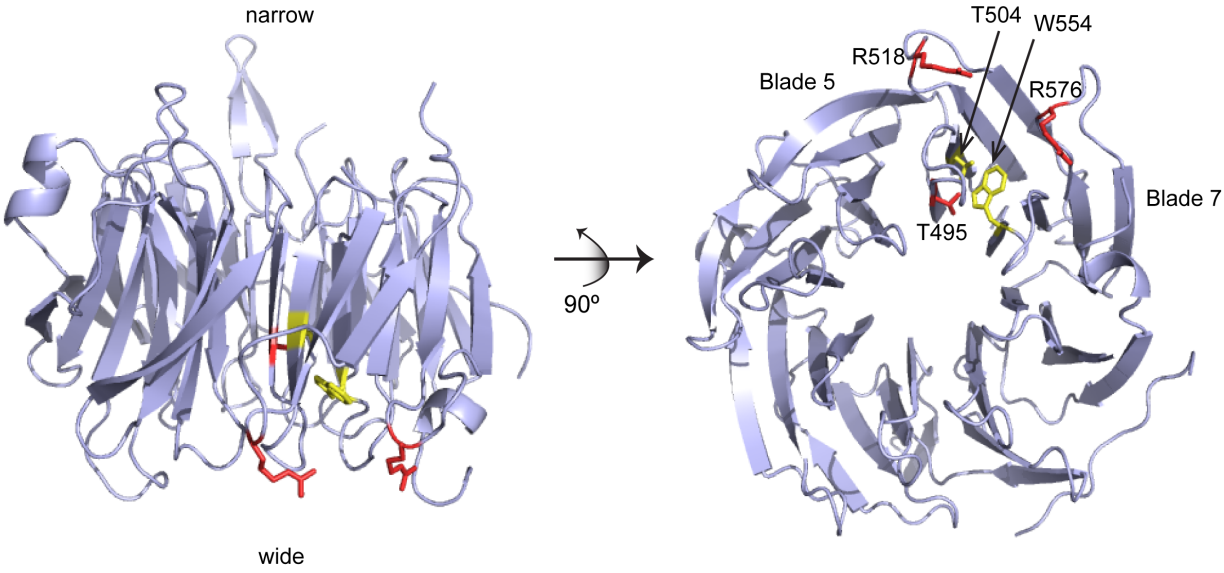


Figure S5. Location of Dos1 point mutants that affect Rik1 binding. Residues that are mutated (Thr495, Arg518, and Arg576) and previously shown to affect Rik1 binding (19) are shown in red, and residues that stabilize Thr495 conformation (Thr504 and Trp554) are shown in yellow.

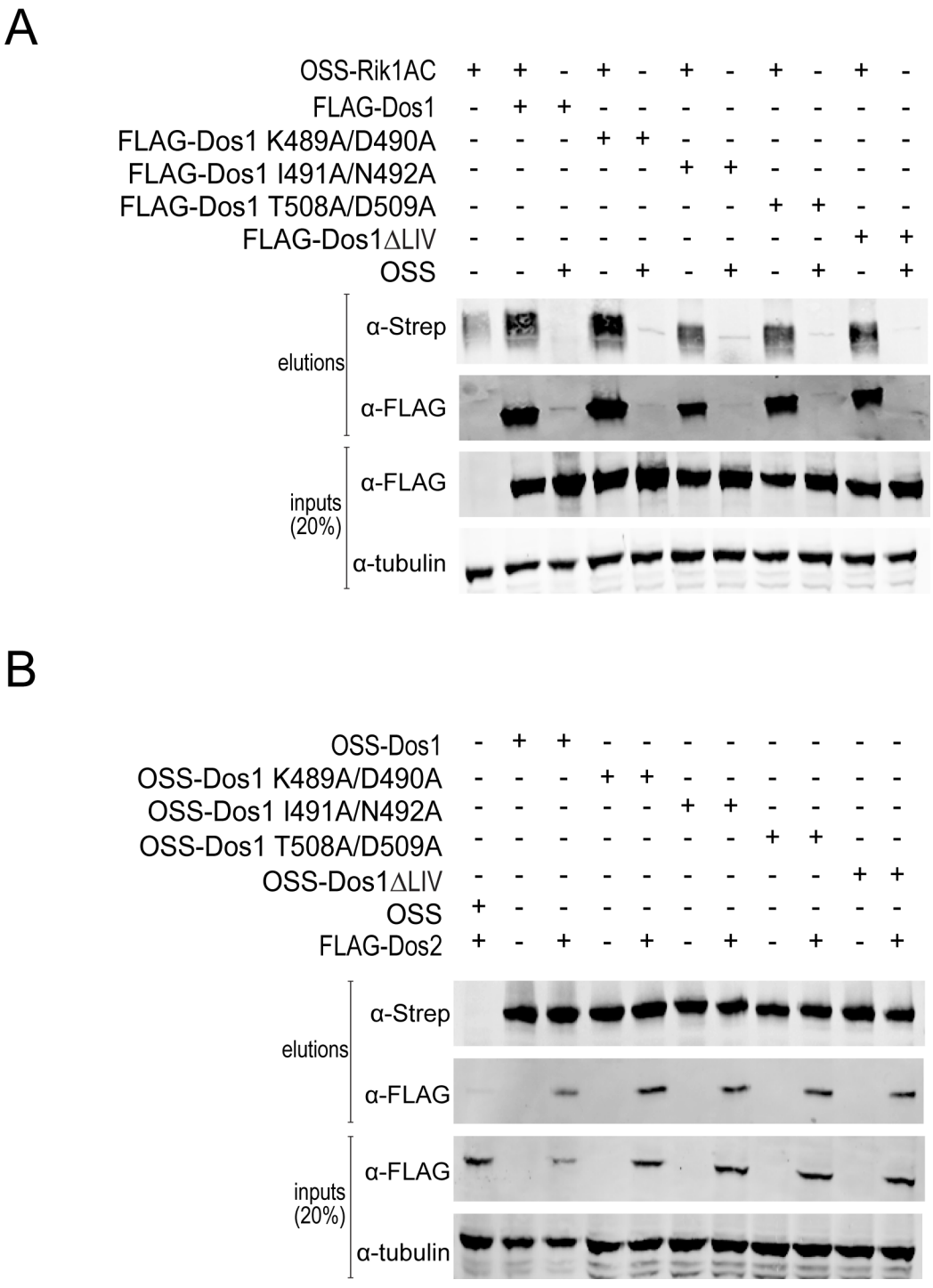


Figure S6. Dos1 mutants retain interactions with Rik1 and Dos2. Streptactin beads pulldown assays from insect-cell coexpression of **(A)** OSS-Rik1AC with various FLAG-tagged Dos1 mutants and **(B)** OSS-Dos1 mutants with FLAG-Dos2.

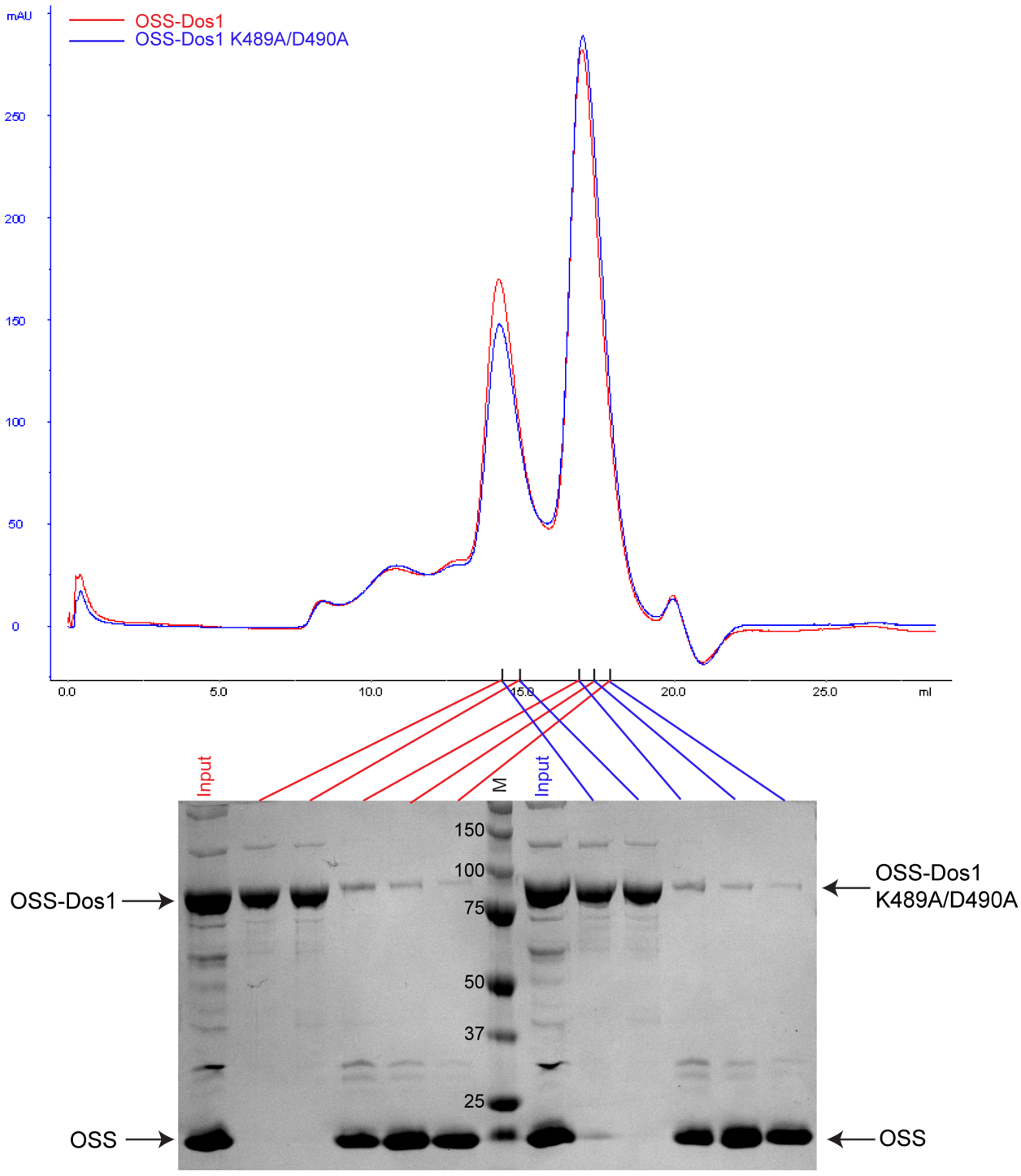


Figure S7. Size exclusion chromatography of a representative mutant and of wild type Dos1. OSS-Dos1 and OSS-Dos1 K489A/K490A behave identically on a Superdex 200 column.

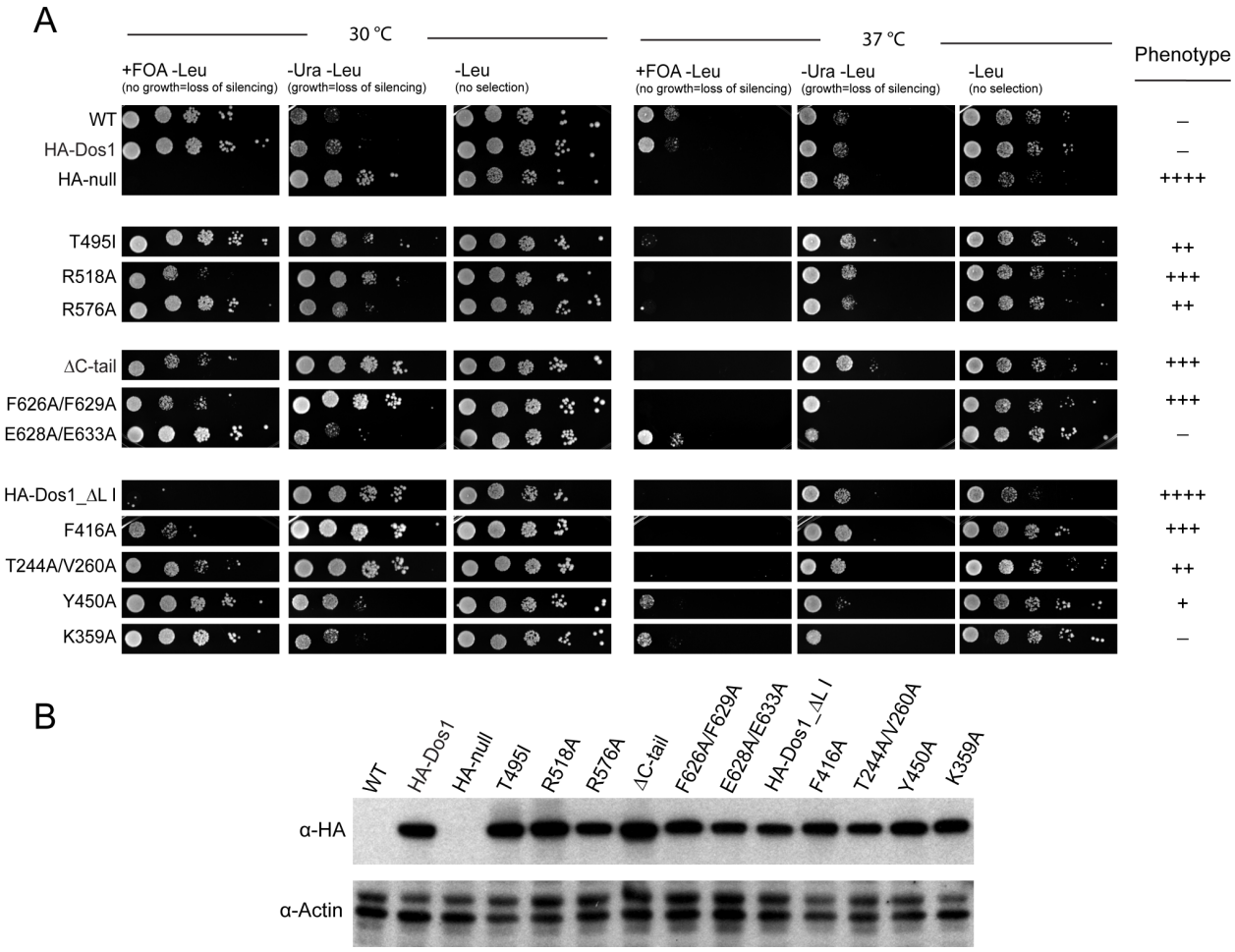


Figure S8. Classification of comparative growth assay phenotypes. (A) Comparative growth assays of serially diluted *dos1* null strain with the centromeric otr1R::ura4 reporter expressing Dos1 mutants including the those previously reported (19), the C-terminal latch and representative mutants for each class of phenotype. Strains were examined for growth on a PMG medium lacking leucine and supplemented with 5-FOA (+FOA –Leu), PMG medium lacking uracil and leucine (-Ura -Leu) and PMG medium lacking leucine (–Leu) both at 30 ºC and 37 ºC. Cells were always grown on PMG medium lacking leucine to select for a Dos1 expressing plasmid. (**B**) Protein levels of the mutants as indicated detected by western using an HA tag antibody and actin antibody as a loading control.

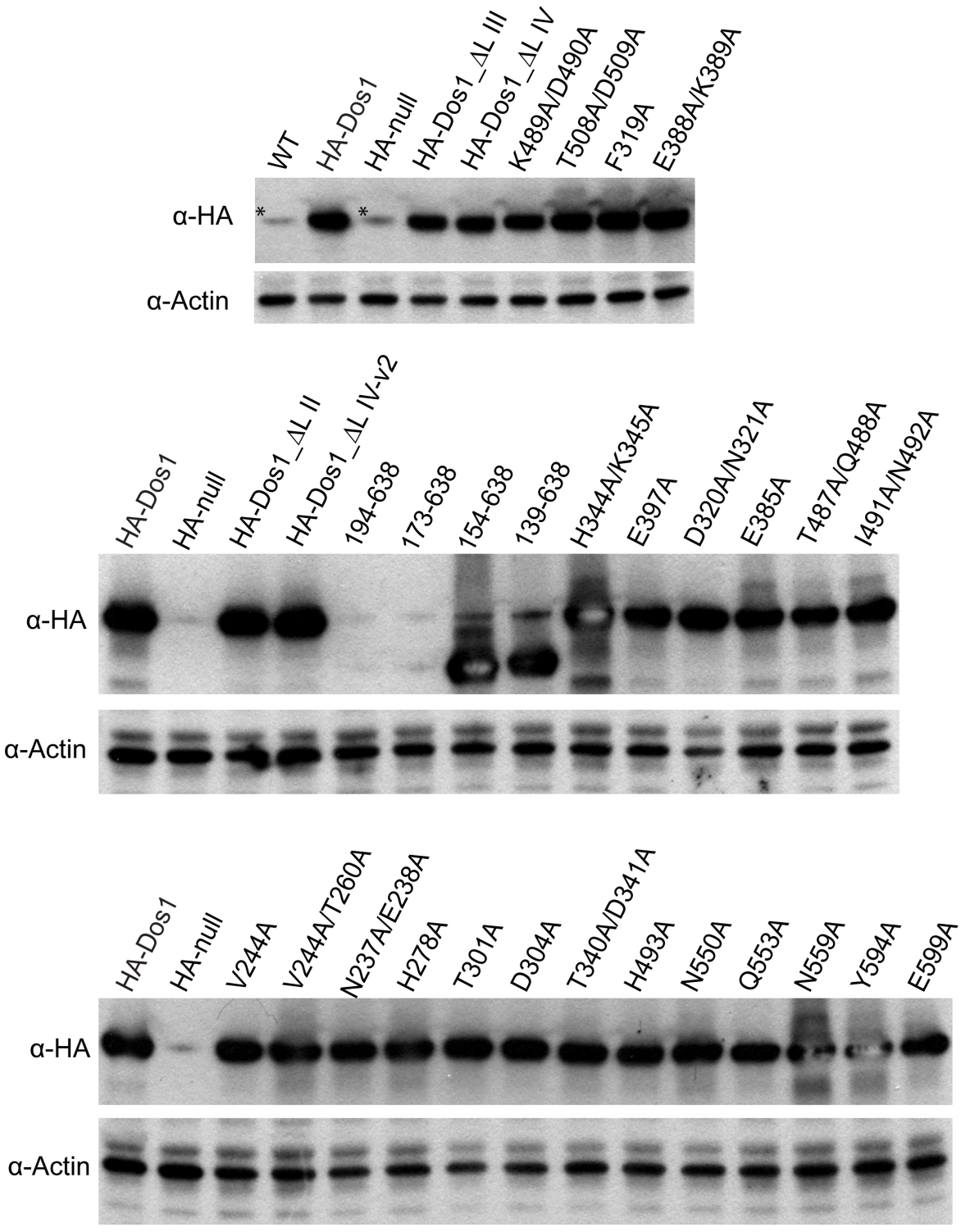


Figure S9. Expression levels of Dos1 constructs used in silencing assays in *S. pombe*. Cells expressing corresponding Dos1 mutants were TCA precipitated and subjected to western blotting using anti-HA antibody and actin antibody as a loading control.
* indicates a non-specific band from HA antibody around the same size as Dos1.

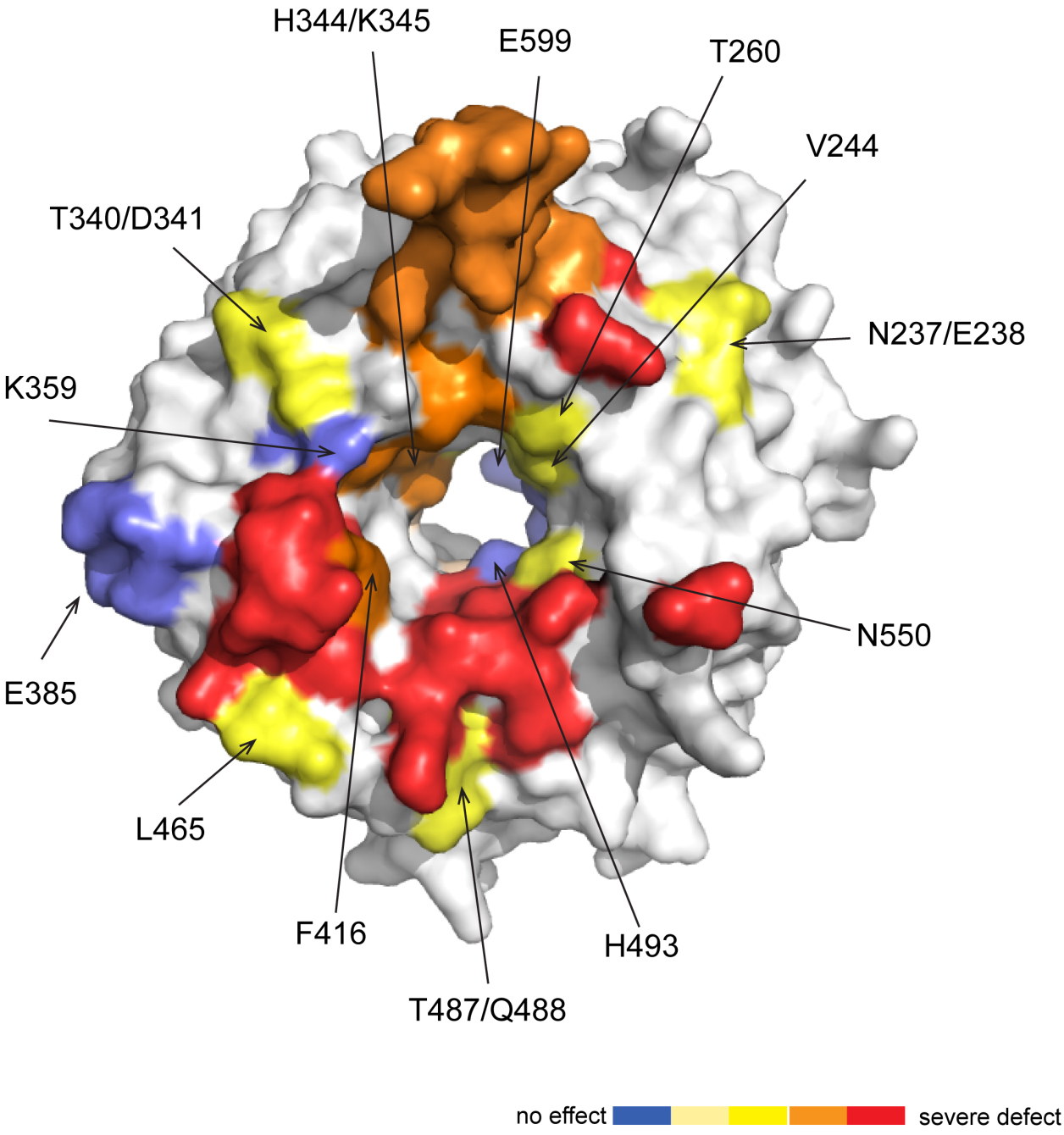


Figure S10. A surface of Dos1 is important for heterochromatic silencing, but not for interaction with known CLRC components. Surface representation of Dos1WD mapped mutations with the degree of the silencing defect (red: severe defect, orange: less severe defect, yellow: mild defect, light yellow: very mild silencing defect, blue: no effect on ura4^{+} silencing).