Online Methods

Crystallization and structure determination

Diffraction quality crystals of Chp1(504–960) in complex with OneStrep-SumoStar (OSS) tagged Tas3(9–83) were obtained by hanging drop vapor diffusion using microseeding\(^{45}\) in 0.95 M potassium sodium tartrate, 100 mM MES pH 6.2, 180 mM sodium thiocyanate. For data collection, crystals were cryo protected by brief transfer through the crystallization buffer supplemented with 15% ethylene glycol, and flash frozen in liquid nitrogen. Data were collected under cryo conditions at NSLS (Brookhaven National Laboratory) beamline X29A at the selenium peak wavelength (0.9792 Å). Data were processed by XDS\(^{46}\). The initial heavy atom positions were found using the Single Anomalous Dispersion method\(^{47}\) implemented in hkl2map and SHELX using reflections between 89.7–4.0 Å. Phases were refined with SHARP (Global Phasing Ltd., Cambridge UK) and solvent modified through DM\(^{48}\) providing a figure of merit of 0.49 for acentric reflections and 0.08 for centric reflections. Model building was performed in Coot\(^{49}\). No electron density was observed for residues 504–514 and residues 778–798 of Chp1. Structure refinement was performed using phenix.refine\(^{50}\), yielding a model with two heterodimers of OSS-Tas3 and Chp1 in the asymmetric unit with good geometry (90.4% in most favored, 9.4% in additionally allowed and 0.2% in generously allowed regions). Figures were produced using the PyMOL Molecular Graphics System (Schrödinger, LLC). The electrostatic surface potential was calculated using the APBS plugin in pymol\(^{51}\). Data and refinement statistics are shown in Table 1.

Structure Analysis

Structure similarity searches and structure based sequence alignments were performed using Dali\(^{52}\) and PDBFold\(^{53}\). The Chp1-Tas3 interface was analyzed using the PDBe PISA server\(^{27}\).

Generation of chp1ΔC alleles

chp1ΔC was generated via 2 step PCR: PCR fragments generated with JPO-1931 and JPO-380 and with JPO-1933 and JPO-1932 were mixed and used as template with
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oligos JPO-380 and JPO-1933 to generate a fragment extending from Chp1 promoter through to the 3'UTR, but with a stop codon replacing sequence coding for aa 809-960. This PCR product was used to transform PY258 (chp1Δ::ura4+), and the chp1 genomic locus was sequenced in the 5-FOA resistant strain (PY5335) to confirm the integrity of the chp1ΔC allele. PY5335 was then outcrossed twice and two progeny strains PY5368 and PY5369 were analyzed.

On further outcrossing of these strains to different genetic backgrounds, we noted that there was some variability in phenotype, with ~30% of chp1ΔC strains not showing accumulation of tlh transcripts. We found that tlh sequences were subject to frequent rearrangements in different strain backgrounds as has been seen previously\(^{40}\). To rule out the possibility that variability was caused by a suppressive mutation in the PY258 background that segregates independently of chp1, we made chp1ΔC strains through an alternative strategy, utilizing a completely independent chp1Δ strain background (PY 5802 [derived from PG1777\(^{54}\)]. A his3+ marked integrative plasmid for chp1ΔC was generated (plasmid JP1530), and linearized with Hpal (upstream of chp1\(^+\)). DNA was transformed into PY5802, strains were selected on media lacking histidine and correct targeting of integration verified by Southern analysis. 8 independent reintegrants (PY 5804–5813) were used for the transcript analysis in Supplementary Figure 9a and b, and all showed accumulation of tlh transcripts. A similar strategy was used to verify data obtained for point mutants of the PIN domain. Plasmids JP-1574 (encoding WT chp1) and JP-1590 (R923A, R924A chp1) were reintegrated as described above, to yield strains PY5970-5971 (WT chp1) and PY5974-5976 (R923A,R924A chp1). These were used for analyses in Supplementary Figure 9d and e. Expression of the chp1ΔC protein was confirmed by western blotting using anti-Chp1 antibody from Abcam (ab28647), which recognizes an N-terminal epitope, with the blot reprobed with anti-tubulin antibody\(^{55}\) to serve as a loading control.

**Generation of chp1 point mutated alleles**

C-terminal fragments of chp1 were synthesized with either the D904A or R923A,R924A mutations and were cloned into pBS. These plasmids were used as templates in PCR
with oligos JPO-36 and JPO-380. The wild type promoter and N-terminal sequences of Chp1 were amplified using JPO-1933 and JPO-2026. Full-length mutant chp1 sequences were amplified from a mixture of this wild type 5' PCR product and the relevant mutant 3' PCR fragment by use of JPO-380 and JPO-1933 and were transformed into PY 258 (chp1Δ::ura4+). Transformants were selected on media containing 5-FOA and proper integration was checked by PCR and sequencing through the entire chp1 locus. Strains PY5684 and PY5884 (chp1 D904A mutants) and PY5679 -PY5680 (chp1 R923AR924A mutants) were used for analysis (Figure 3E,F).

Plasmid construction
The integration plasmid for chp1ΔC (JP1530) was constructed by Gateway (Invitrogen) recombination. chp1ΔC was amplified using primers JPO-2080 (~1 kb upstream of start codon) and JPO-2081 (~600 bp after stop codon) using genomic DNA from chp1ΔC (PY5335) as template. This PCR product was recombined with pDONOR 201. The resulting BP clone (JP 1529) was recombined with JP1168 to generate the his3+ marked chp1ΔC genomic reintegration vector JP1530 which was verified by sequencing. Integration constructs for wild type chp1+ (JP 1574) and chp1 R923A, R924A (JP 1580) were generated in a similar fashion using PY42 and PY5679 genomic DNA as template, respectively.

Transcript analyses
Transcript analyses were performed as previously described. Oligos for real time PCR analysis: (dh) JPO-769 and JPO-770, (dg) JPO-986, JPO-987, (adh1) JPO-793 and JPO-794, (tlh) JPO-816 and JPO-819. RNA was prepared from duplicate cultures for every experiment, and following gene reintegration, multiple independent re-integrants were assessed. Real-time PCR was performed on an Eppendorf Mastercycler ep Realplex machine using Quantifast Sybr green (Qiagen). Data was analyzed using the ΔΔCt method, ensuring that Ct values were within the experimentally determined linear range.
Chromatin Immunoprecipitation

ChIP was performed as previously described\textsuperscript{16}, using 3\% formaldehyde fixation at 25\textdegree C, and beadbeating to lyse the cells. Following immunoprecipitation with anti-RNA pol II Ab (8WG16 Covance) and reversal of crosslinks, DNA was purified and subject to real-time PCR. Signal from primers used for centromere analysis: JPO-2184, JPO-2185, and \textit{tlh} (JPO-816 and 819) was normalized to \textit{adh1} signal (JPO-793,794).