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Supplemental information

Custom affinity probes reveal DNA-damage-induced, ssDNA-independent chromatin SUMOylation in budding yeast

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A

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Name (selection ID)                          N-terminal capping AR          internal AR module            internal
                                  8xHis        α1              α2             βt      α1       α2          βt     α1
E3_5                     MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNATDNDGYTPLHLAASNGHLEIVEVLLKNGADVNASDLTGITPLHLAAT
E10  (006-698-2025_D1)   MRGSHHHHHHHHGSDLGKKLLEAALYGHLDEVRILMANGADVNATDHYGVTPLHLAAQNGHLEIVEVLQKTGADVNAVDFHGRTPLHLAADN
E11  (006-698-2027_D7)   MRGSHHHHHHHHGSDLGKKLLEAATHGQLDEVRILMANGADVNA---------------------------------VDNTGMTPLHLAAYE
D10  (006-698-2025_A7)   MRGSHHHHHHHHGSDLGKKLLEAAYHGQDDEVRILMANGADVNAYDVSGMTPLHLAAQKGHLEIVEVLLKTGADVNANDMIGATPLHLAAHS
C10  (006-698-2025_B11)  MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNA---------------------------------QDTQGRTPLHLAAQR
F11  (006-698-2027_E7)   MRGSHHHHHHHHGSDLGKKLLEAANVGHLDEVRILMANGADVNAEDKDGATPLHLAAHQGHLEIVEVLLKTGADVNATDNEGHTPLHLAAEH
B12  (006-698-2028_F12)  MRGSHHHHHHHHGSDLCKKLLEAARAGQDDEVRILMANGADVNAVDQWGLTPLHLAAHQGHLEIVEVLLKTGADVNATDNEGHTPLHLAAEH
G10  (006-698-2026_H4)   MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNAKDQYGATPLHLAAHQGHLEIVEVLLKTGADVNATDNEGHTPLHLAAEH
G11  (006-698-2027_D12)  MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNASDSDGATPLHLAAHQGHLEIVEVLLKTGADVNATDNEGHTPLHLAAEH
A10  (006-698-2025_E5)   MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNAEDLRGFTPLHLAANSGHLEIVEVLLKTGADVNAQDNYGWTPLHLAAVI
H10  (006-698-2026_D3)   MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNAEDLRGFTPLHLAANSGHLEIVEVLLKTGADVNAQDNYGWTPLHLAAVI
F10  (006-698-2026_G11)  MRGSHHHHHHHHGSDLGKKLLEAANNGQDDEVRILMANGADVNA---------------------------------QDWAGWTPLHLATTI
C11  (006-698-2026_B12)  MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNAQDTQGRTPLHLAAQRGHLEIVEVLLKTGADVNAFDIVGWTPLHLAANR
B10  (006-698-2025_H2)   MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNA---------------------------------MDKWGITPLHLAAQH
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C12  (006-698-2028_H5)   MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNA---------------------------------TDKIGRTPLHLAAWV
H11  (006-698-2027_G2)   MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNASDVVGRTPLHLAASAGHLEIVEVLLKTGADVNAIDHWGKTPLHLAAAD
D11  (006-698-2027_C1)   MRGSHHHHHHHHGSDLGKKLLEAAYHGQDDEVRILMANGADVNAYDVSGMTPLHLAAAAGHLEIVEILLKTGADVNAHDNMGATPLHLAAET
B11  (006-698-2026_C10)  MRGSHHHHHHHHGSDLGKKLLEAAHIGQDDEVRILMANGADVNAMDRYGTTPLHLAAMQGHLEIVEVLLKTGADVNAFDNVGVTPLHLAAET
A11  (006-698-2026_F8)   MRGSHHHHHHHHGSDLGKKLLEAARAGQDDEVRILMANGADVNAIDLVGWTPLHLAAANGHLEIVEVLLKTGADVNANDHMGATPLHLAAET
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Name (selection ID)      AR module            internal AR module            C-terminal capping AR
                             α2            βt     α1       α2            βt     α1      α2            FLAG
E3_5                     GHLEIVEVLLKHGADVNAYDNDGHTPLHLAAKY-GHLEIVEVLLKHGADVNAQDKFGKTAFDISIDNGNEDLAEILQ---KLNDYKDDDDK
E10  (006-698-2025_D1)   GHLEIVEVLLKTGADVNAEDEYGYTPLHLAARH-GHLEIVEVLLKHGADVNAQDFVGQTPFDLAAMVGNEDIAEVLQKAAKLNDYKDDDDK
E11  (006-698-2027_D7)   GHLEIVEVLLKHGADVNALDVHGWTPLHLAAQE-GHLEIVEVLLKHGADVNAQDHAGDTPFDLAAMYGNEDIAEVLQKAAKLNDYKDDDDK
D10  (006-698-2025_A7)   GHLEIVEVLLKAGADVNAHDQYGWTPLHLAAHANGHLEIVEVLLKHGADVNAQDWIGVTPFDLAVDDGNEDIAEVLQKAAKLNDYKDDDDK
C10  (006-698-2025_B11)  GHLEIVEVLLKAGADVNAHDQYGWTPLHLAAHANGHLEIVEVLLKHGADVNAQDWIGVTPFDLAVDDGNEDIAEVLQKAAKLNDYKDDDDK
F11  (006-698-2027_E7)   GHLEIVEVLLKAGADVNAHDQYGWTPLHLAAHANGHLEIVEVLLKHGADVNAQDWIGVTPFDLAADDGNEDIAEVLQKAAKLNDYKDDDDK
B12  (006-698-2028_F12)  GHLEIVEVLLKAGADVNAHDQYGWTPLHLAAHANGHLEIVEVLLKHGADVNAQDWIGVTPFDLAADDGNEDIAEVLQKAAKLNDYKDDDDK
G10  (006-698-2026_H4)   GHLEIVEVLLKAGADVNAHDQYGWTPLHLAAHANGHLEIVEVLLKHGADVNAQDWIGVTPFDLAVDDGNEDIAEVLQKAAKLNDYKDDDDK
G11  (006-698-2027_D12)  GHLEIVEVLLKAGADVNAHDQYGWTPLHLAAHANGHLEIVEVLLKHGADVNAQDWIGVTPFDLAVDDGNEDIAEVLQKAAKLNDYKDDDDK
A10  (006-698-2025_E5)   GHLEIVEVLLKHGADVNAKDNFGSTPLHLAARL-GHLEIVEVLLKHGADVNAQDKWGETPFDLAIDNGNEDIAEVLQKAAKLNDYKDDDDK
H10  (006-698-2026_D3)   GHLEIVEVLLKHGADVNAKDNFGSTPLHLAARL-GHLEIVEVLLKHGADVNAQDKWGETPFDLAIDNGNEDIAEVLQKAAKLNDYKDDDDK
F10  (006-698-2026_G11)  GHLEIVEVLLKTGADVNASDQYGTTPLHLAARL-GHLEIVEVLLKHGADVNAQDKWGETPFDLAIDIGNEDIAEVLQKAAKLNDYKDDDDK
C11  (006-698-2026_B12)  GHLEIVEVLLKAGADVNANDHIGKTPLHLAAWR-GHLEIVEVLLKHGADVNAQDKFGKTAFDISIDNGNEDIAEVLQKAAKLNDYKDDDDK
B10  (006-698-2025_H2)   GHLEIVEVLLKTGADVNAADITGWTPLHLAAEF-GHLEIVEVLLKHGADVNAQDNIGDTPFDLAIDNGNEDIAEVLQKAAKLNDYKDDDDK
A12  (006-698-2028_A11)  GHLEIVEVLLKAGADVNATDDWGQTPLHLAAVF-GHLEIVEVLLKHGADVNAQDKFGKTPFDLAIDNGNEDIAEVLQKAAKLNDYKDDDDK
C12  (006-698-2028_H5)   GHLEIVEVLLKAGADVNAWDHWGKTPLHLAAYK-GHLEIVEVLLKHGADVNAQDEWGMTPFDLAAHDGNEDIAEVLQKAAKLNDYKDDDDK
H11  (006-698-2027_G2)   GHLEIVEVLLKAGADVNAQDEWGNTPLHLAAYF-GHLEIVEVLLKHGADVN------------------EDIAEVLQKAAKLNDYKDDDDK
D11  (006-698-2027_C1)   GHLEIVEVLLKTGADVNAWDWAGWTPLHLAAHE-GHLEIVEVLLKHGADVNAQDEWGKTPFDLAIDNGNEDIAEVLQKAAKLNDYKDDDDK
B11  (006-698-2026_C10)  GHLEIVEVLLKAGADVNASDHAGWTPLHLAAWE-GHLEIVEVLLKHGADVNVQDKFGKTPFDLAIDNGNEDIAEVLQKAAKLNDYKDDDDK
A11  (006-698-2026_F8)   GHLEIVEVLLKAGADVNASDHAGWTPLHLAAWE-GHLEIVEVLLKHGADVNAQDKFGKTPFDLAIDNGNEDIAEVLQKAAKLNDYKDDDDK
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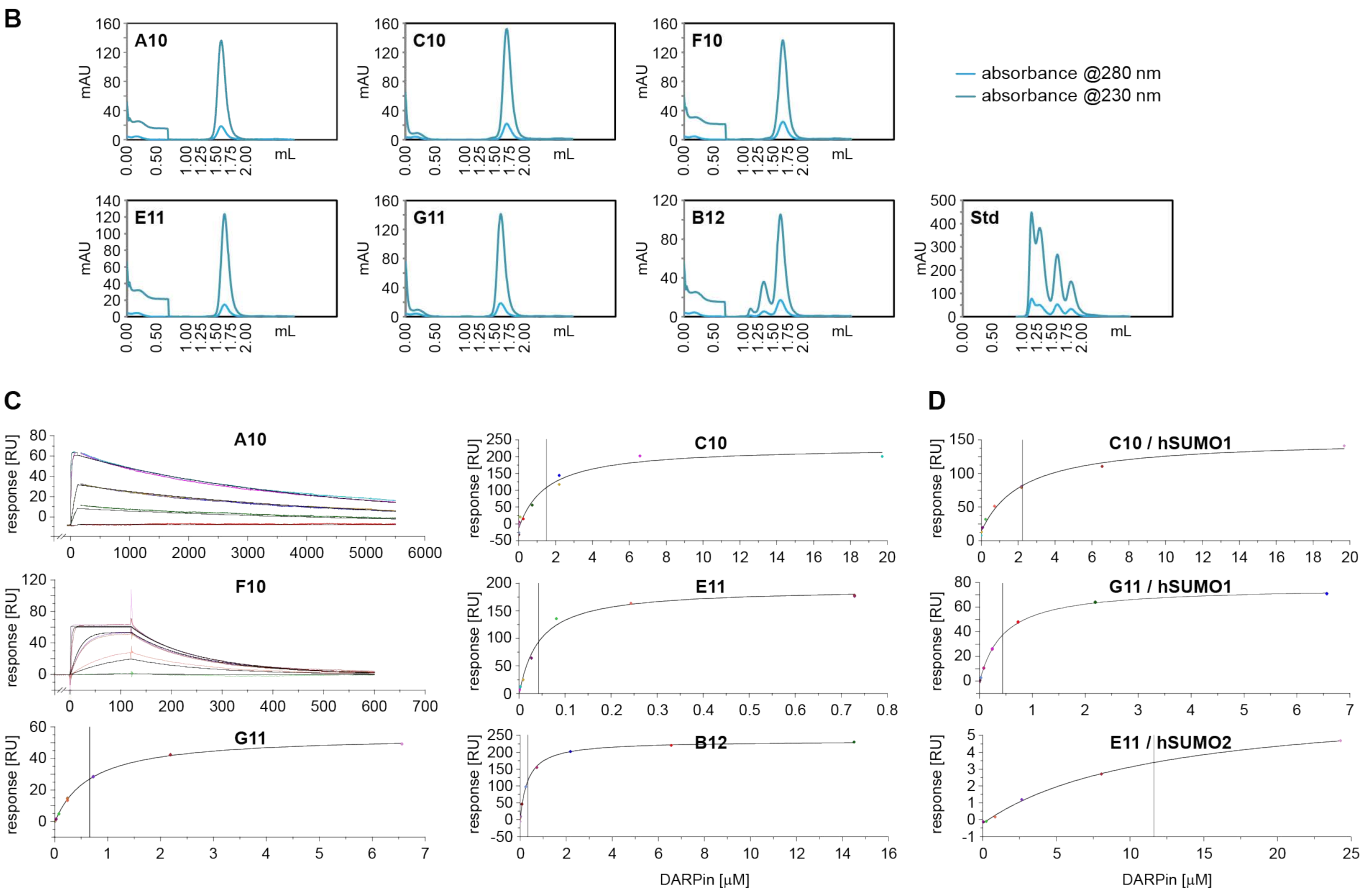


Figure S1. Sequences and properties of Smt3-specific DARPins. Related to Figure 1.

(**A**) Amino acid sequence alignment of DARPins selected against Smt3. An unselected DARPin, E3_5, is shown for comparison. Annotations illustrate secondary structure motifs such as α-helices (α), β-turns (βt) and the capping as well as internal ankyrin repeat (AR) modules with randomized residues. Randomized residues are shown in red; positions randomized with a smaller set of variants according to Binz et al. [S1] are colored in orange, the N-terminal His_8-tag in green and the C-terminal FLAG-tag in blue. Additional residues randomized during the affinity maturation inherent in the ribosome display are shown in purple.

(**B**) Analytical gel filtration indicates monodisperse behavior of most Smt3-specific DARPins, except for B12, which exhibits a tendency to dimerize. Absorbance units (AU) were recorded at 280 and 230 nm. Std: size standard.

(**C**) Determination of binding affinities of DARPins for Smt3 by surface plasmon resonance, using biotinylated Smt3 immobilized on a Streptavidin chip. For DARPins A10 and F10, dissociation constants (K_D) were determined by fitting to a 1:1 binding model. Plots show representative examples from a total of three independent measurements. For DARPins C10, E11, and B12, equilibrium K_D values were determined by fitting the steady-state plateau values to a 1:1 binding model. Plots show representative examples from a total of four independent measurements.

(**D**) Determination of dissociation constants of selected DARPins by surface plasmon resonance, using biotinylated human SUMO1 (hSUMO1) or human SUMO2 (hSUMO2) immobilized on a Streptavidin chip. Equilibrium K_D values were determined by fitting the steady-state plateau values to a 1:1 binding model.

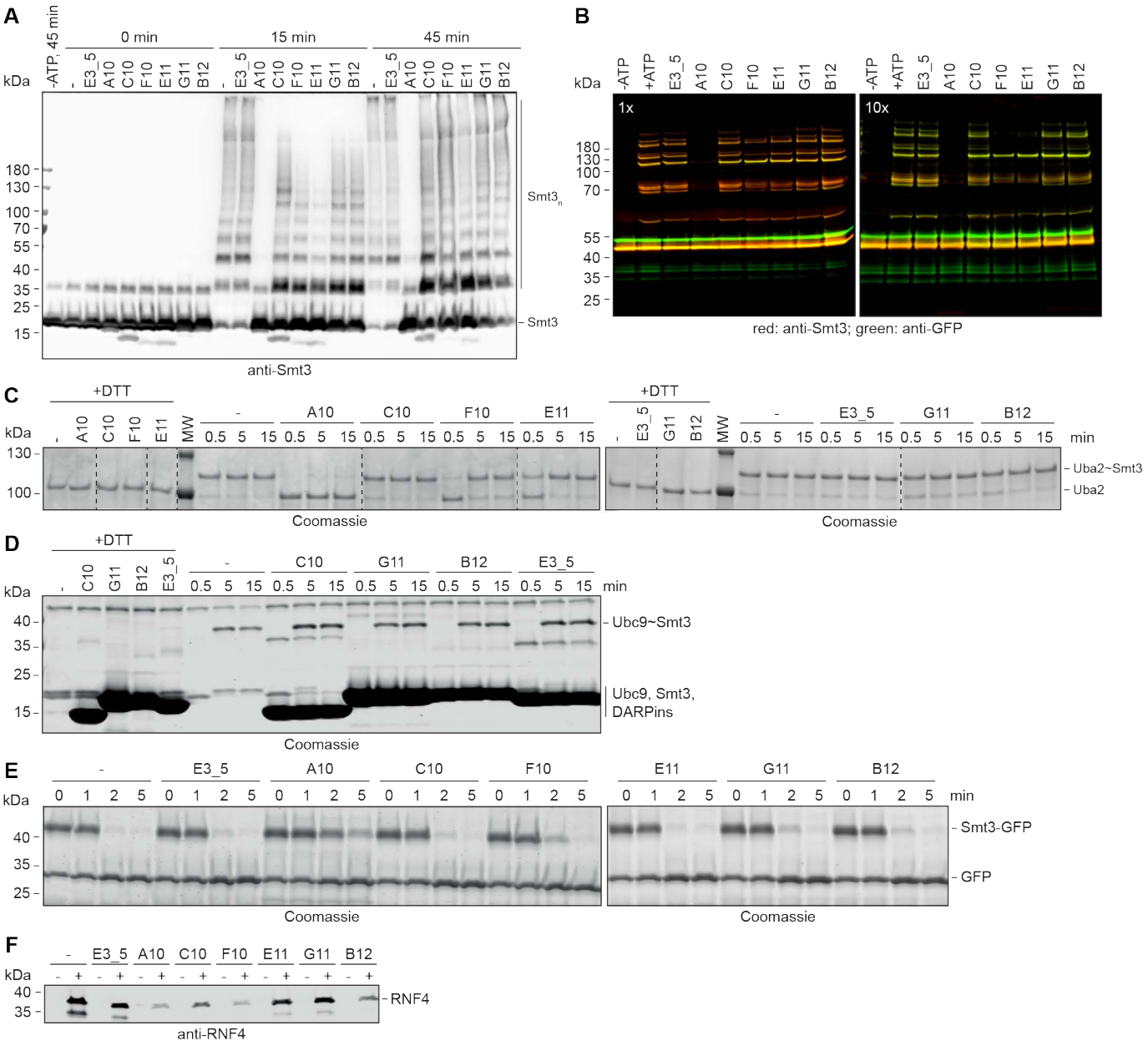


Figure S2. Effects of Smt3-specific DARPins on Smt3 conjugation, deconjugation, and SIM interaction. Related to Figure 2.

(**A**) Smt3-specific DARPins inhibit the formation of unanchored Smt3 chains to varying degrees. *In vitro* SUMOylation reactions were set up and analyzed as in Figure 2A, but reactions included 50 nM ^{His}Siz1(1-508).

(**B**) Western blots show the end products of *in vitro* $^{His\text{-}CFP}$GAPtail SUMOylation reactions shown in Figure 2B. Smt3 and the substrate were detected by anti-Smt3 and anti-GFP antibodies, and visualized by means of fluorescent secondary antibodies.

(**C**) Representative images of Coomassie-stained gels used for E1 thioester formation assays shown in Figure 2C (MW: molecular weight markers). Dashed lines indicate splicing of lanes from the same gel.

(**D**) Selected Smt3-specific DARPins do not interfere with E2 thioester formation. Reactions containing 500 nM Aos1·Uba2, 750 nM Ubc9 (K153R), 5 µM Smt3(3R) and a 5-fold molar excess of the indicated DARPins were analyzed in a non-reducing gel-based assay. Quantification was not possible because of overlap between the DARPin signal with free E2 and unconjugated Smt3.

(**E**) Representative images of Coomassie-stained gels used for Ulp1 activity assays shown in Figure 2D.

(**F**) Representative image of an anti-RNF4 western blot used for analysis of the interference by DARPins with the interaction between RNF4 and Smt3 (-/+ refers to the presence of Smt3 on the affinity beads).

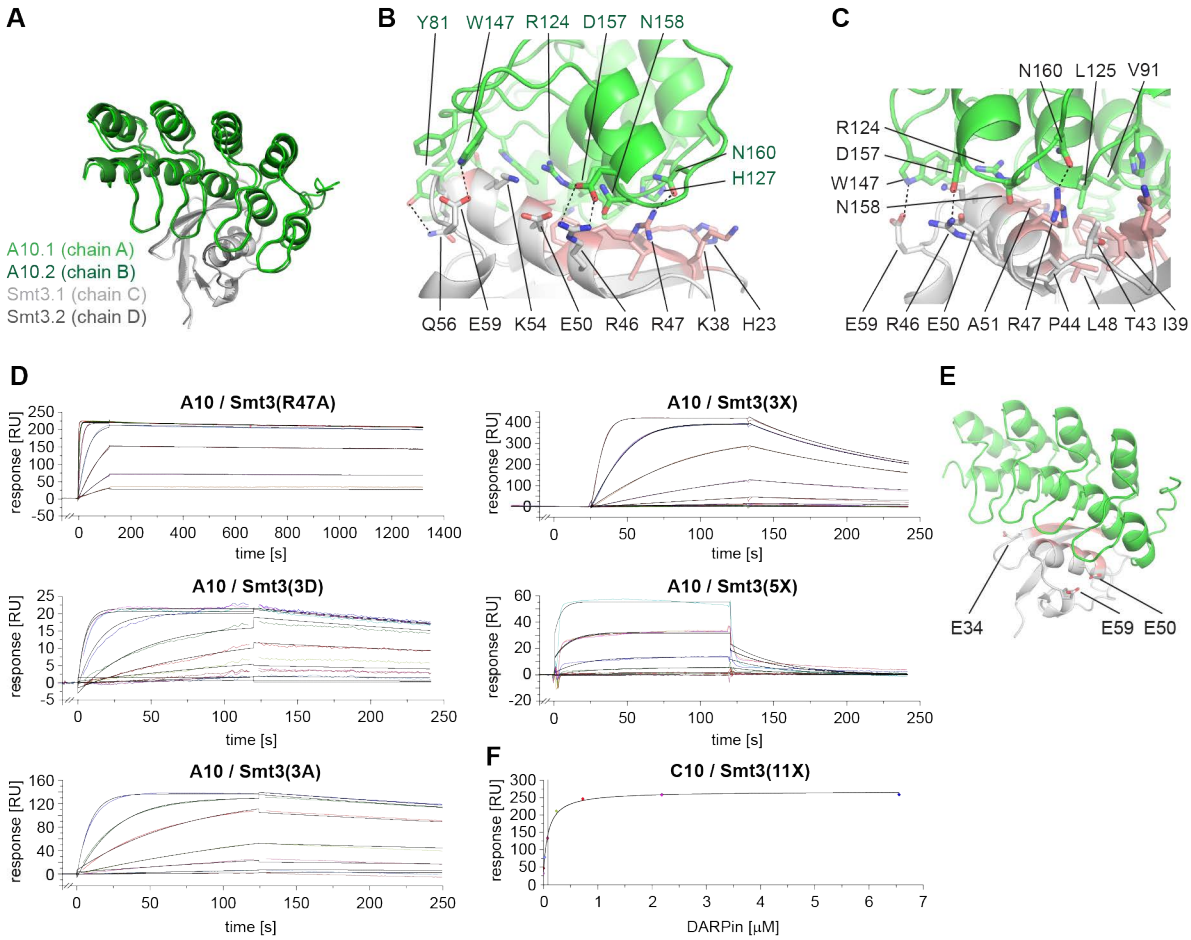


Figure S3. Structural details of the A10·Smt3 complex. Related to Figure 3.

(**A**) Alignment of the two A10·Smt3 complexes in the asymmetric unit of the crystal, generated by superposition of the Smt3 structures.

(**B**) Structural detail of the A10·Smt3 interface reveals a series of charged and polar residues. Potential hydrogen bonds and salt bridges are indicated by dashed lines. Residues involved in SIM binding are colored pink.

(**C**) Structural detail of the region surrounding R47 at the A10·Smt3 interface.

(**D**) Determination of association and dissociation rate constants of DARPin A10 by surface plasmon resonance, using biotinylated Smt3 mutants immobilized on a Streptavidin chip. Dissociation constants (K_D) were determined by fitting to a 1:1 binding model. Each plot shows a representative example from a minimum of three independent measurements.

(**E**) Cartoon model of the A10·Smt3 complex illustrating the position of three acidic residues on Smt3 close to the interface with A10.

(**F**) Determination of equilibrium dissociation constant of DARPin C10 by surface plasmon resonance, using biotinylated Smt3(11X) mutant immobilized on a Streptavidin chip. The equilibrium K_D value was determined by fitting the steady-state values to a 1:1 binding model. The plot shows a representative example from a total of three independent measurements.

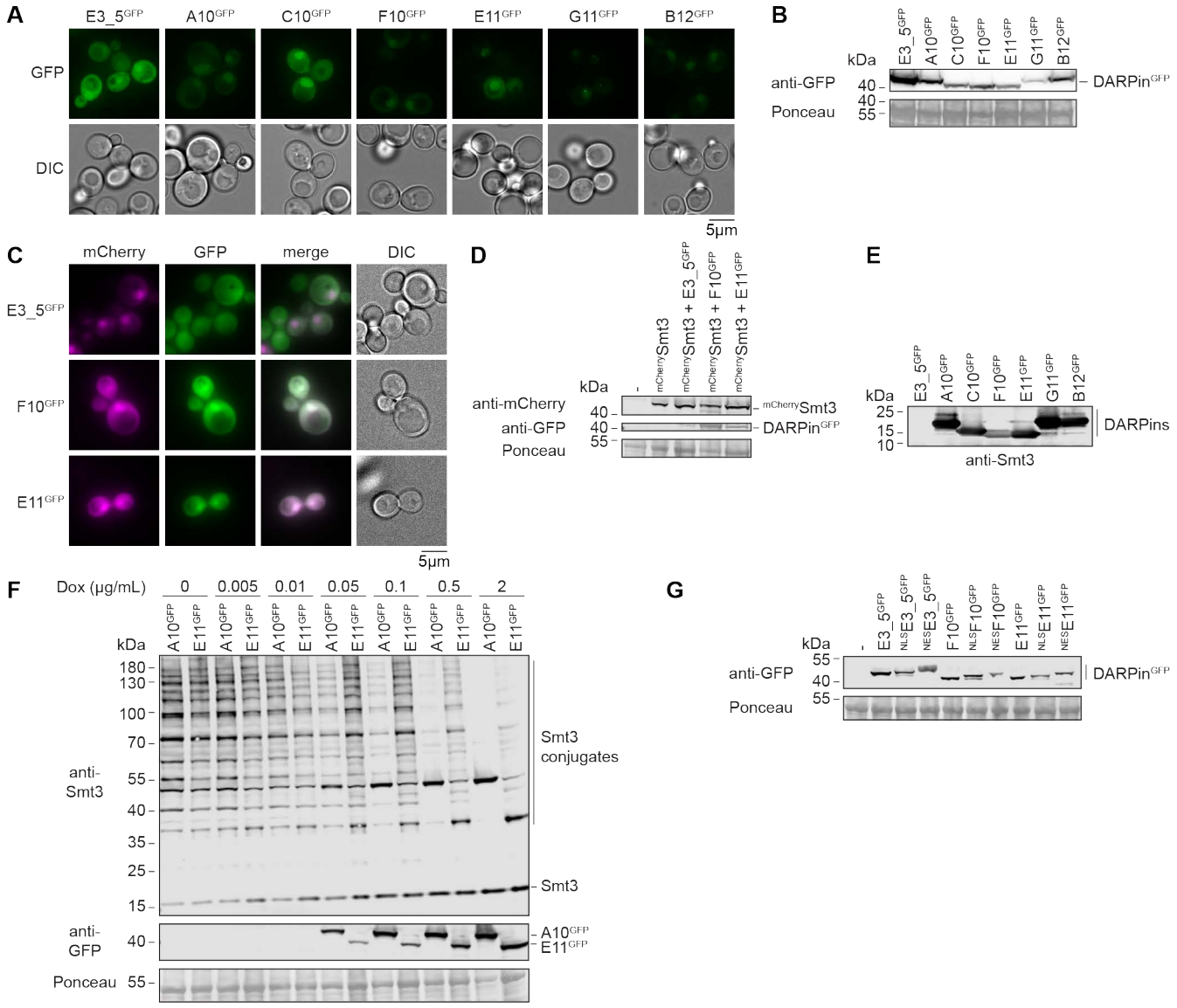


Figure S4. Effects of Smt3-specific DARPins in yeast. Related to Figure 4.

(**A**) Smt3-specific DARPins localize predominantly to the yeast nucleus. Expression of DARPins was induced for 20 h with 0.5 µg/mL doxycycline. DARPin E3_5^{GFP} served as a non-interacting control and does not exhibit a preferential nuclear localization.

(**B**) Control blots for panel A showing expression of GFP-tagged DARPins. Ponceau S staining served as loading control.

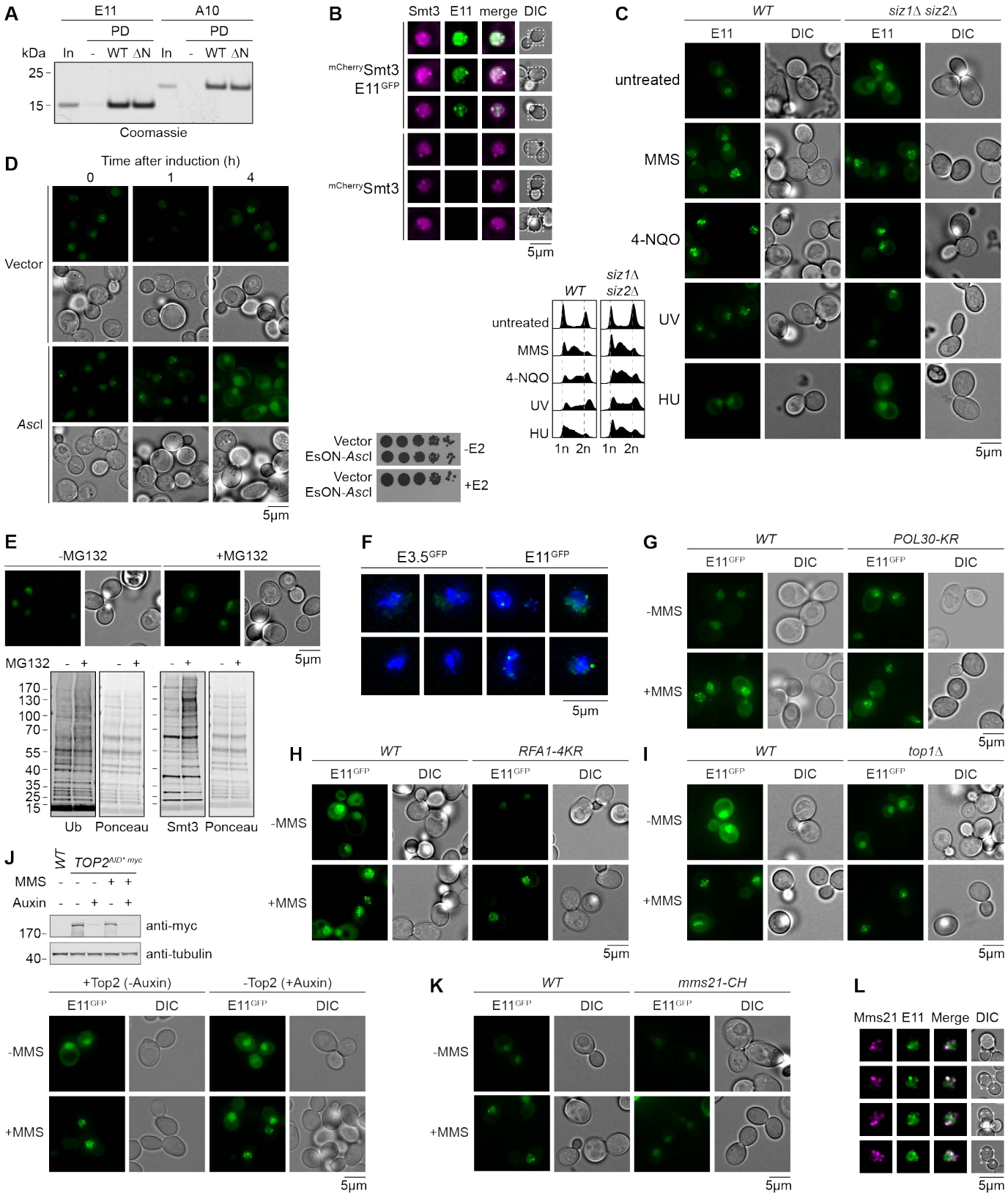
(**C**) DARPins F10^{GFP} and E11^{GFP} co-localize with Smt3 under unperturbed conditions. Expression of DARPins was induced as in panel A in strains constitutively expressing ^{mCherry}Smt3.

(**D**) Control blots for panel C showing expression of ^{mCherry}Smt3 and GFP-tagged DARPins. Ponceau S staining served as loading control.

(**E**) Refolded DARPins immobilized on western blot membranes trap solubilized Smt3, thus generating cross-reactivity towards anti-Smt3 antibody. A standard western blot of a gel loaded with purified DARPins (450 ng each) was incubated with 6.25 µg of purified recombinant ^{His}Smt3 after the blocking step, followed by washing of the membrane and detection with anti-Smt3 antibody.

(**F**) Smt3-specific DARPins interfere with SUMOylation in a concentration-dependent manner. Expression of A10^{GFP} or E11^{GFP} was induced in exponential cultures by treatment with the indicated concentrations of doxycycline for 20 h, and DARPins as well as free and conjugated Smt3 were detected in total lysates by western blotting. Ponceau S staining served as loading control.

(**G**) Control blot for Figure 4F showing expression of the indicated DARPin^{GFP} constructs. Ponceau S staining served as loading control.


A
E11
A10
PD
PD
kDa
In
-
WT
ΔN
In
-
WT
ΔN
25
15
Coomassie
B
Smt3
E11
merge
DIC
mCherrySmt3
E11GFP
mCherrySmt3
5μm
C
WT
siz1Δ siz2Δ
E11
DIC
E11
DIC
untreated
MMS
4-NQO
UV
HU
5μm
D
Time after induction (h)
0
1
4
Vector
AscI
5μm
WT
siz1Δ
siz2Δ
untreated
MMS
4-NQO
UV
HU
1n 2n 1n 2n
Vector
EsON-AscI
-E2
Vector
EsON-AscI
+E2
E
-MG132
+MG132
5μm
MG132
- + - + - + - +
170
130
100
70
55
40
35
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15
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Ponceau
Smt3
Ponceau
F
E3.5GFP
E11GFP
5μm
G
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DIC
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+MMS
5μm
H
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I
WT
top1Δ
E11GFP
DIC
E11GFP
DIC
-MMS
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J
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MMS
- - + +
Auxin
- - + +
170
anti-myc
40
anti-tubulin
+Top2 (-Auxin)
-Top2 (+Auxin)
E11GFP
DIC
E11GFP
DIC
-MMS
+MMS
5μm
K
WT
mms21-CH
E11GFP
DIC
E11GFP
DIC
-MMS
+MMS
5μm
L
Mms21
E11
Merge
DIC
5μm

Figure S5. Cellular features governing the detection of DNA damage-induced Smt3 foci by DARPin E11. Related to Figure 5.

(**A**) Interaction of DARPins A11 and E11 with Smt3 is not affected by Smt3's extended N-terminal region. Pull-down assays were performed with the indicated Smt3 variants (WT or ΔN, lacking amino acids 1-19) as in Figure 1B.

(**B**) MMS-induced Smt3 foci are detected by $E11^{GFP}$, but their formation does not require the presence of the DARPin. Induction, damage treatment, and fluorescence microscopy were performed as in Figure 5C in strains harboring either ^{mCherry}Smt3 alone or ^{mCherry}Smt3 and $E11^{GFP}$.

(**C**) Smt3 foci are induced by a range of DNA-damaging agents. Wildtype (*WT*) and *siz1Δ siz2Δ* mutant cells harboring DARPin $E11^{GFP}$ were treated with the indicated agents and subjected to microscopy as in Figure 5C (0.02% MMS, 0.05 µg/mL 4-NQO or 30 mM HU for 90 min, or 40 J/m^2 UV irradiation with 90 min recovery). The panel on the bottom left indicates cell cycle profiles of the same cultures obtained by flow cytometry.

(**D**) Expression of a restriction endonuclease in the yeast nucleus does not induce $E11^{GFP}$ foci. *Asc*I expression was induced by treatment with 2 µM β-estradiol for the indicated times before imaging. The panel on the right shows growth assays of the same strains in the presence and absence of 2 µM β-estradiol (E2) as a control for efficient DSB induction by *Asc*I.

(**E**) Proteotoxic stress does not induce $E11^{GFP}$ foci. DARPin $E11^{GFP}$ was expressed in a *pdr5Δ* background, and proteasome inhibition was induced by treatment with 50 µM MG132 for 90 min before imaging. The panels on the bottom show control blots indicating the accumulation of ubiquitin and Smt3 conjugates in total lysates after MG132 treatment. Ponceau S staining served as loading control.

(**F**) $E11^{GFP}$ foci are associated with chromatin. Foci were detected on chromatin spreads of cells expressing DARPin $E11^{GFP}$ and treated with MMS as in Figure 5C (blue: DAPI; green: GFP). Out of 60 and 27 spreads imaged for E3_5 and E11, GFP foci were detected in 2.5% and 26% of spreads, respectively.

(**G**) DARPin $E11^{GFP}$ forms MMS-induced foci in cells independently of PCNA SUMOylation. Induction, damage treatment, and fluorescence microscopy were performed as in Figure 5C in a *pol30(K127/164R)* mutant and an isogenic wildtype (*WT*) strain.

(**H**) DARPin $E11^{GFP}$ forms MMS-induced foci in cells independently of Rfa1 SUMOylation. Induction, damage treatment, and fluorescence microscopy were performed as in Figure 5C in a *rfa1(K170/180/411/427)* mutant and an isogenic wildtype (*WT*) strain.

(I) DARPin $E11^{GFP}$ forms MMS-induced foci in cells independently of the presence or absence of topoisomerase I. Induction, damage treatment, and fluorescence microscopy were performed as in Figure 5C in a *top1Δ* mutant and an isogenic wildtype (*WT*) strain.

(**J**) DARPin $E11^{GFP}$ forms MMS-induced foci in cells independently of the presence or absence of topoisomerase II. Induction, damage treatment, and fluorescence microscopy were performed as in Figure 5C in a strain harboring an auxin-inducible degron-tagged allele of *TOP2* (*TOP2$^{AID*\text{-}myc}$*). Degradation of Top2 was induced by addition of 1 mM auxin for 30 min prior to MMS treatment. The panel on the right shows control blots of total lysates, probed for the presence of $Top2^{AID*\text{-}myc}$ via an anti-myc antibody. Blotting against α-tubulin served as loading control.

(K) Accumulation of MMS-induced Smt3 foci depends on the catalytic activity of Mms21. Fluorescence images were obtained as in Figure 5C, using a strain expressing harboring a catalytically inactive allele of *MMS21* (*mms21-CH*) and its isogenic wildtype (*WT*).

(**L**) MMS-induced DARPin $E11^{GFP}$ foci overlap with Mms21. Induction, damage treatment, and fluorescence microscopy were performed as in Figure 5C in a strain expressing $E11^{GFP}$ in a background harboring endogenously tagged $Mms21^{mCherry}$.

Table S1. X-ray crystallographic data collection and refinement statistics. Related to Figure 3.

	A10-Smt3(20-98) – PDB: 9G8I	**C10-Smt3(20-98) – PDB: 9GAU**
Data collection		
Beamline	SLS X06DA	SLS X06SA
Wavelength (Å)	1.00003	0.9999
Resolution range (Å)	47.37–2.51 (2.61–2.51)	47.05-2.64 (2.77–2.64)
Space group	P 21 21 21	C 1 2 1
a, b, c (Å)	42.97, 94.75, 120.96	90.46, 44.69, 47.60
α, β, γ (°)	90, 90, 90	90, 98.76, 90
Unique reflections	17608 (1948)	5539 (695)
Multiplicity	13.1 (12.8)	5.0 (5.2)
Completeness (%)	99.9 (100)	98.5 (95.8)
Mean I/σ(I)	17.7 (1.7)	6.5 (1.6)
$CC_{1/2}$	1.000 (0.897)	0.974 (0.401)
R_{merge}	0.097 (1.496)	0.293 (1.614)
Refinement		
Protein atoms	3417	1506
Water molecules	56	31
R_{work}	0.2413	0.2330
R_{free}	0.3000	0.2883
RMSD bonds (Å)	0.0023	0.0022
RMSD angles (°)	0.6283	0.5891
B-factors overall	76.82	53.27
B-factors	76.91 (A10-Smt3), 70.99 (H_2O)	53.38 (C10-Smt3), 47.76 (H_2O)
Favored (%)	95.98	96.41
Allowed (%)	4.02	3.08
Outliers (%)	0	0.51

Table S2. Yeast strains used in this study. Related to STAR Methods.

Name	ID	Genotype	Source
DF5α alpha (WT α)	002	Matα, *his3-Δ200, leu2-3,2-112, lys2-801, trp1-1(am), ura3-52*	[S2]
DF5a (WT a)	003	Mata, *his3-Δ200, leu2-3,2-112, lys2-801, trp1-1(am), ura3-52*	[S2]
ubc9^{ts}	584	DF5α *ubc9::TRP1, leu2-3,2-112::ubc9-1 (LEU2)*	[S3]
TetR-SSN6	5144	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3)*	This study
TetR-SSN6 ^{His}POL30 bar1	4984	DF5a *hisG::pol30, leu2-3,2-112::YIp128-P30-^{His}POL30 (LEU2), bar1::HISMX6, trp1-1::YIp204-SpADH-SSN6 (TRP1)*	This study
TetR-SSN6 SHS1^{mCherry}	5235	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), SHS2^{mCherry}::natNT2*	This study
TetR-SSN6 ^{His}POL30 bar1 SPC42^{mCherry}	5408	DF5a *hisG::pol30, leu2-3,2-112::YIp128-P30-^{His}POL30 (LEU2), bar1::HISMX6, trp1-1::YIp204-SpADH-SSN6 (TRP1), SPC42^{mCherry}::natNT2*	This study
TetR-SSN6 ^{His}POL30 bar1 KRE28^{mCherry}	5412	DF5a *hisG::pol30, leu2-3,2-112::YIp128-P30-^{His}POL30 (LEU2), bar1::HISMX6, trp1-1::YIp204-SpADH-SSN6 (TRP1), KRE28^{mCherry}::natNT2*	This study
TetR-SSN6 E11^{GFP}	5376	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2)*	This study
W303 E11^{GFP}	5913	W303 *fpr1::natMX, RPL13A-2xFKBP12::TRP1, tor1-1::HIS3MX, RAD5, hmlΔ, hmrΔ, ura3-1::YIp211-TetO7-DARPin-E11-FLAG-GFP (URA3)*	[S4]
W303 EsON-AscI E11^{GFP}	5914	W303 *fpr1::natMX, RPL13A-2xFKBP12::TRP1, tor1-1::HIS3MX, RAD5, hmlΔ, hmrΔ, leu2::P_lexO-AscI-T_CYC1-P_ACT1-LexA-ER-B112-T_CYC1 (LEU2MX), ura3-1::YIp211-TetO7-DARPin-E11-FLAG-GFP (URA3)*	[S4]
TetR-SSN6 E11^{GFP} siz2	5457	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), siz2::hphNT1*	This study
TetR-SSN6 E11^{GFP} siz1	5456	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), siz1::hphNT1*	This study
TetR-SSN6 E11^{GFP} siz1 siz2	5814	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), siz1::hphNT1, siz2::natNT2*	This study
TetR-SSN6 E11^{GFP} POL30	5736	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), ura3-52::YIp211-P30-POL30 (URA3), pol30::natNT2*	This study
TetR-SSN6 E11^{GFP} pol30(K164R)	5737	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), ura3-52::YIp211-P30-POL30(K127R/K164R) (URA3), pol30::natNT2*	This study
TetR-SSN6 E11^{GFP} rfa1(4KR)	5740	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), rfa1(4KR)::kanMX*	This study
TetR-SSN6 E11^{GFP} top1	5775	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), top1::CaURA3*	This study
TetR-SSN6 E11^{GFP} pdr5	5910	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), pdr5::NatNT2*	This study
TetR-SSN6 E11^{GFP} apn1 apn2	5945	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), apn1::natNT2, apn2::hphNT1*	This study
TetR-SSN6 E11^{GFP} rad51	5931	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), rad51::NatNT2*	This study
TetR-SSN6 E11^{GFP} mms21-CH	5946	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), mms21-CH::KanMX*	This study
TetR-SSN6 E11^{GFP} AFB2^{FLAG} TOP2-AID-myc*	5783	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), trp1-1::YIp204-ADH-AFB2-FLAG (TRP1), TOP2-AID*-9myc::natNT2*	This study
TetR-SSN6 E11^{GFP} AFB2^{FLAG} MMS21-AID-myc*	5915	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), trp1-1::YIp204-ADH-AFB2-FLAG (TRP1), TOP2-AID*-9myc::natNT2*	This study

TetR-SSN6 E11^{GFP} RFA1^{mRuby2}	5474	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), RFA1-mRuby2::kanMX*	This study
TetR-SSN6 E11^{GFP} MCM4^{mRuby2}	5779	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), MCM4-mRuby2::CaURA3*	This study
TetR-SSN6 E11^{GFP} ^{mRuby2}POL30	5801	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), ura3-52::YIp211-pPOL30-mRuby2-POL30 (URA3)*	This study
TetR-SSN6 E11^{GFP} CMR1^{mCherry}	5800	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), CMR1-mCherry::natNT2*	This study
TetR-SSN6 E11^{GFP} NIC96^{mCherry}	5811	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), NIC96-mCherry::natNT2*	This study
TetR-SSN6 E11^{GFP} MMS21^{mCherry}	5932	DF5a *Δhis3-200::pRS303-TetR-SpADH-SSN6 (HIS3), leu2-3,2-112::YIp128-TetO7-DARPin-E11-FLAG-GFP (LEU2), MMS21-mCherry::hphNT1*	This study

Table S3. Oligonucleotides used in this study. Related to STAR Methods.

Name	ID	Sequence
Cloning & mutagenesis		
UBC9-f	711	GAGATATACATATGAGTAGTTTGTGTCTACAGCG
UBC9K-153R-r	713	GGTGGTACCCTTTAGAATACTGTCGAGCTTGAAGCAAAACTTTCTTG
SMT3-(GG-STOP)-r	1329	ATCGGATCCTTAACCACCAATCTGTT
SMT3-NdeI-f	1437	GAGGGATCCATATGTCGGACTCAGAAG
SMT3-BamHI-f	1793	CCTGGGATCCAAAATGTCGGACTCAGAAGTC
KpnI-yeGFP-f	2746	CTCTGCTGGTACCGGATCCGGAGCAGGTGCTGGTG
yeGFP-SalI-NotI-r	2757	AAATATGGCGGCCGCGGTCGACTTATTTGTACAATTCATCCAT
GFP-BamHI-f	3018	GGATGGATCCATGTCTAAAGGTGAAGAATTATTCACTGGTGTTGT
AVI-SMT3-f	4451	ACGATATCTTCGAAGCTCAGAAAATCGAATGGCACGAAATGTCGGACTCAGAAGTC
His-AVI-SMT3-r	4452	ATTCGATTTTCTGAGCTTCGAAGATATCGTTCAGACCACCATGGTGATGATGGTG
SMT3(20-98)-NdeI-r	4851	GCAACATATGCCTGAGACTCACATCAATTTAAAGG
BglII-2NLS-DARPin-f	4995	GCAAAGATCTGCAAATGCCCAAGAAAAAGCGTAAGGTCCCCAAGAAAAAGCGTAAGGTCATG GGATCCGACCTGGGTAAGAAAC
KpnI-DARPin-FLAG-r	4996	GCAAGGTACCGCACTTGTCGTCGTCATCCTTGTAG
BglII-DARPin-f	4997	GCAAAGATCTGCAAATGGGATCCGACCTGGGTAAGAAAC
SMT3-E34A-f	5108	GGTGTCCGATGGATCTTCAGCCATCTTCTTCAAGATCAAAAAGACC
SMT3-E34A-r	5109	GGTCTTTTTGATCTTGAAGAAGATGGCTGAAGATCCATCGGACACC
SMT3-R47A-f	5110	GACCACTCCTTTAAGAGCACTGATGGAAGCGTTCGC
SMT3-R47A-r	5111	GCGAACGCTTCCATCAGTGCTCTTAAAGGAGTGGTC
SMT3-E50A-f	5112	CTCCTTTAAGAAGGCTGATGGCCGCGTTCGCTAAAAGACAGG
SMT3-E50A-r	5113	CCTGTCTTTTAGCGAACGCGGCCATCAGCCTTCTTAAAGGAG
SMT3-E59A-f	5114	CGCTAAAAGACAGGGTAAGGCCATGGACTCCTTAAGATTCTTG
SMT3-E59A-r	5115	CAAGAATCTTAAGGAGTCCATGGCCTTACCCTGTCTTTTAGCG
DARPin-FLAG-GFP-r	5117	CCTGCTCCGGATCCGGTACCCTTAAGCTTGTCGTCGTCATCCTTGTAGTC
SMT3-E59P-f	5410	CGCTAAAAGACAGGGTAAGCCAATGGACTCCTTAAGATTCTTG
SMT3-E59P-r	5411	CAAGAATCTTAAGGAGTCCATTGGCTTACCCTGTCTTTTAGCG
SMT3-F36H-r	5412	GTGGTCTTTTTGATCTTGAAGTGGATCTCTGAAGATCCATCGG
SMT3-F36H-f	5413	CCGATGGATCTTCAGAGATCCACTTCAAGATCAAAAAGACCAC
SMT3-R47K-f	5414	GACCACTCCTTTAAGAAAGCTGATGGAAGCGTTCGC
SMT3-R47K-r	5415	GCGAACGCTTCCATCAGCTTTCTTAAAGGAGTGGTC
hsSUMO1-NdeI-f	3872	GATATACATATGTCTGACCAGGAGGC
hsSUMO1-AVI-XhoI-r	3873	GTGGTGCTCGAGTTCGTGCCATTCGATTTTCTGAGCTTCGAAGATATCGTTCAGACCACCCCCC GTTTGTTCCTG
hsSUMO2-NdeI-f	3874	GATATACATATGGCCGACGAAAAGC
hsSUMO2-AVI-XhoI-r	3875	GTGGTGCTCGAGTTCGTGCCATTCGATTTTCTGAGCTTCGAAGATATCGTTCAGACCACCTCCC GTCTGCTGTTG
BamHI-mCherry-f	5432	CGCGGATCCTTAATTAACATGGTGAGCAAGGGCGAGG
NotI-r	5451	ATAGTTTAGCGGCCGC
BglII-2xNES-EcoRI-DARPin-f	5604	GATCAGATCTGTCATGCTGCCTCCCCTGGAGCGCCTGACCCTGCTGCCTCCCCTGGAGCGCCTG ACCCTGGATCGAATTCGGATCGGATCCGACCTGGGT
BglII-FLAG-r	5605	GATCCAGATCTGATCTTGTCGTCGTCATCCTTG
NdeI-3His-f	5852	GGAATTCCATATGTCGTACTACCATCACCATC
NdeI-mVenus-r	5853	GGAATTCCATATGACGGATCCCTTTGTACAGCTCG
BamHI-mCherrry-noSTOP-r	5889	GCGCGGATCCGCGTTTGTACAGCTCGTCCATGCCG
AmpR 3′ pBirAcm-f	cf83	CCTCACTGATTAAGCATTGGTAATTTTTTTAAGGCAGTTATTGGTGC
AmpR 3′ pBirAcm-r	cf82	GCACCAATAACTGCCTTAAAAAAATTACCAATGCTTAATCAGTGAGG

AmpR 5´ pBirAcm-f	cf81	CTTTTGGCGAAAATGAGACGTCGCGGAACCCCTATTTG
AmpR 5´ pBirAcm-r	cf80	CAAATAGGGGTTCCGCGACGTCTCATTTTCGCCAAAAG
lacI pBirAcm-r	cf79	GGCTGTGCAGGTCGTAAATCAC
lacI pBirAcm-f	cf78	GTGATTTACGACCTGCACAGCC
KanR 3´ pBirAcm-f	cf77	GATGCTCGATGAGTTTTTCTAATTTTTTTAAGGCAGTTATTGGTGC
KanR 3´ pBirAcm-r	cf76	GCACCAATAACTGCCTTAAAAAAATTAGAAAAACTCATCGAGCATC
KanR 5´ pBirAcm-f	cf75	CTTTTGGCGAAAATGAGACGTGAACAATAAAACTGTCTGCTTAC
KanR 5´ pBirAcm-r	cf74	GTAAGCAGACAGTTTTATTGTTCACGTCTCATTTTCGCCAAAAG
PCR knockout & tagging		
POL30-knockout-f	481	TCACAGCAACAAGCAGCAAGCACTAAGTACGCAGTCAAAAGAGAGAAAAACGTACGCTGCAG GTCGAC
POL30-knockout-r	367	TTTTTTTTTGTTTATTATTTTTAGTATACAACTATATAGATAATTTACATATCGATGAATTCGAGCT CG
TOP1-knockout-f	5701	ACTTGATGCGTGAATGTATTTGCTTCTCCCCTATGCTGCGTTTCTTTGCGATCGATGAATTCGAG CTCG
TOP1-knockout-r	5700	TAAAAAAAATCTAAAGGGAGGGCAGAGCTCGAAACTTGAAACGCGTAAAACGTACGCTGCAG GTCGAC
SIZ1-knockout-f	453	AAGAAGACTCCAACTCAAACAGTTGAGTGTTCCATATACATTCTGTTTCACGTACGCTGCAGGT CGAC
SIZ1-knockout-r	454	AAATATTTCATGAAAGAGCTGGACGGAACCGTCCAATTTTAGCCTCGTTTATCGATGAATTCGA GCTCG
SIZ2-knockout-f	1022	CCACAAACGATACACTGATAATCAAGAAACGTATAAGGGAAAAGAGCACGCGTACGCTGCAG GTCGAC
SIZ2-knockout-r	1023	AAATAAAAATAGAATACAATCGGAAAGGAAAGAAATCAAAAGACGGTTAAATCGATGAATTC GAGCTCG
PDR5-knockout-f	1556	AAGACCCTTTTAAGTTTTCGTATCCGCTCGTTCGAAAGACTTTAGACAAAACGTACGCTGCAGG TCGAC
PDR5-knockout-r	1557	AAAAAGTCCATCTTGGTAAGTTTCTTTTCTTAACCAAATTCAAAATTCTAATCGATGAATTCGAG CTCG
APN1-knockout-f	3030	GTCGACCTGCAGCGTACGTACGATGGTTCCGATATGCCAAAAGCTTATTAATGTTGCGTTTTGT GTTT
APN1-knockout-r	3031	CGAGCTCGAATTCATCGATTGAGAAGCGAGAAGAATTTTAAATACGTAATCAATTTTTGTAGAT TATCT
APN2-knockout-f	7713	AGAAGCTATTTCACCGTAAAGAAAATCCCTTTCCTTGTCAGGACACTATGCGTACGCTGCAGGT CGAC
APN2-knockout-r	7714	GAAGAAAGTGTTTTATTCTCCCAAAATATCAGCTGACGTTTTCATATTTAATCGATGAATTCGAG CTCG
RAD51-knockout-f	621	AAGAGCAGACGTAGTTATTTGTTAAAGGCCTACTAATTTGTTATCGTCATCGTACGCTGCAGGT CGAC
RAD51-knockout-r	315	TGAAAGTAAACCTGTGTAAATAAATAGAGACAAGAGACCAAATACATCGATGAATTCGAGCTC G
RFA1-tag-r	312	TCTCATATGTTACATAGATTAAATAGTACTTGATTATTTGATACAATCGATGAATTCGAGCTCG
RFA1-mRuby2-tag-f	4261	GAAGCCGACTATCTTGCCGATGAGTTATCCAAGGCTTTGTTAGCTGGTGACGGTGCTGGTTTA
SPC42-tag-f	3019	CACAGAACGCTTTAAGAATGCGCCATACTCCTTAACTGCTTTTTAAATCAATCGATGAATTCGAG CTCG
SPC42-tag-r	3020	CTGAAAATAATATGTCAGAAACATTCGCAACTCCCACTCCCAATAATCGACGTACGCTGCAGGT CGAC
KRE28-tag-f	3021	CTTTTTTTTTGGCTAGTAATATTACATACATCTTTATCTAGATAATTAATATCGATGAATTCGAGC TCG
KRE28-tag-r	3022	TGCAAATCTTTGAGGTAATGGATGACATTATAAGCGAGCTAACAAACGAACGTACGCTGCAGG TCGAC
CDC48-tag-f	4673	CAGGTGCTGCATTTGGTTCTAATGCGGAGGAAGATGATGATTTGTATAGTCGTACGCTGCAGG TCGAC
CDC48-tag-r	4675	AGAAATGACTTGAATTTACGATTTAAAATAAAAATATACCTGGCATATAAATCGATGAATTCGA GCTCG
SHS1-tag-f	5403	AATGACACGTATACTGATTTAGCCTCTATTGCATCGGGTAGAGATCGTACGCTGCAGGTCGAC
SHS1-tag-r	5404	TTTATTTATTTATTTGCTCAGCTTTGGATTTTGTACAGATACAACATCGATGAATTCGAGCTCG
TOP2-tag-f	7197	ATGATGAAGAGGAAAACCAAGGATCAGATGTTTCGTTCAATGAAGAGGATCGTACGCTGCAG GTCGAC

TOP2-tag-r	7196	TCTGATATAAACATATAAAAAGAATGGCGCTTTCTCTGGATAAATATTATATCGATGAATTCGAGCTCG
MCM4-mRuby2-tag-f	4998	TTGTCCTTGGCGAGGGTGTAAGGAGATCAGTTCGCCTGAATAACCGTGTCGGTGACGGTGCTGGTTTA
MCM4-tag-r	1803	TAATTAGTATTTATTAATTGTTACGCAGGGAATGATTGTAGTAGACAGCAATCGATGAATTCGAGCTCG
CMR1-tag-f	4323	AAAAAGCTGCCAAGATTGGTGGGTTATTAAAACCTTCCGAACTTGATGATCGTACGCTGCAGGTCGAC
CMR1-tag-r	4322	AATAAAAGGGAAAAAATATTGGAAATTTAAAGAATGATACGCCACCGCCTATCGATGAATTCGAGCTCG
NIC96-tag-f	3831	AGGGAAACGTACAGCACTTTAATTAATATAGACGTCTCTCTACGTACGCTGCAGGTCGAC
NIC96-tag-r	3832	CGCATACTGATATATAGATATAAACAAAAATATACAATATTTAAAACATCGATGAATTCGAGCTCG
MMS21-tag-f	7672	AAAGAATCTCAGGAACAGGATAAAAGAAGTAGTCAAGCCATCGATGTTTTACGTACGCTGCAGGTCGAC
MMS21-tag-r	7673	GAACTTCGGGCCGAAGGGCTCGGATAAGAGAAACAATAATTTTGTTTTCAATCGATGAATTCGAGCTCG
MMS21-test-fwd	7674	CCTTTCTACCTGGGATAAATATCGT
MMS21-test-rev	7724	ATTTTTGTAAAAGTTGAGCGAAGTG

Table S4. Plasmids used in this study. Related to STAR Methods.

Name	ID	Use	Source
***E. coli* expression vectors for production of recombinant proteins**			
pBluescript SK+	4	PCNA sumoylation assays	Stratagene
pET-UBA2	733	$Uba2^{His}$	[S5]
pET-AOS1	734	$^{His}Aos1$	[S5]
pET-UBC9	736	$Ubc9^{His}$	[S6]
pET-ubc9(K153R)	1059	$Ubc9(K153R)^{His}$	This study
pQE32-SIZ1(1-508)	1539	$^{His}Siz1(1-508)$	[S7]
pET28-His6-ULP1	2731	$^{His}Ulp1(403-621)$	[S3]
pLou3-RNF4-wt	3762	$^{His\text{-}MBP\text{-}TEV}$RNF4 (rat)	[S8]
pET11a-SMT3(3R)	1640	Smt3(3R) (untagged)	This study
pET21a-His6-cys2-SMT3-eGFP	3545	$^{His\text{-}cys2}$Smt3-GFP	This study
pET21a-His6-cys2-SMT3	3546	$^{His\text{-}cys2}$Smt3	This study
pET21a-His6-cys2-SMT3 Δ N	6451	$^{His\text{-}cys2}Smt3(20-89)$	This study
pET3a-SMT3ΔN	3942	Smt3(20-98) (untagged)	This study
pET21a-His6-AVI-SMT3	3670	$^{His\text{-}AVI}$Smt3 & biotinylated Smt3	This study
pET21a-His6-AVI-SMT3(R47A)	4495	$^{His\text{-}AVI}Smt3(R47A)$	This study
pET21a-His6-AVI-SMT3(E34A-E50A-E59A)	4502	$^{His\text{-}AVI}Smt3(3A)$	This study
pET21a-His6-AVI-SMT3(F36H-R47K-E59P)	4730	$^{His\text{-}AVI}Smt3(3X)$	This study
pET30-His6-AVI-SMT3(I35D-F36D-F37D)	4731	$^{His\text{-}AVI}Smt3(3D)$	This study
pET30-His6-AVI-Smt3(H23Y-N25K-F36H-I39V-K41M-R46K-R47K-A51S-K54Q-K58V-E59P)	4914	$^{His\text{-}AVI}Smt3(11X)$	This study
pET30-His6-AVI-Smt3(T22A-I35S-F37S-K38E-K40E-T43A-L48S-R55E-N86A)	5059	$^{His\text{-}AVI}Smt3(SIMX)$	This study
pET30-His6-AVI-Smt3(K41M-R46K-K54Q-K58V-E59P)	5118	$^{His\text{-}AVI}Smt3(5X)$	This study
pET11a-His6-TEV-YFP-SMT3(3R)	5210	$^{His\text{-}TEV\text{-}YFP}Smt3(3R)$	This study
pET30-SUMO1-AVI-His6	3240	$hSUMO1^{AVI\text{-}HIS}$	This study
pET30-SUMO2-AVI-His6	3241	$hSUMO2^{AVI\text{-}HIS}$	This study
pQE30-POL30	637	$^{His}PCNA$	[S9]
pBL481-RFC	1086	RFC	[S10]
pET30a-His-TEV-ECFP-GAPtail1(RanGAP1)	4876	RanGAP1 tail	[S11]
pBirAcm	3613	BirA (for *in vivo* biotinylation)	Avidity LLC
pBirA-Amp	4513	BirA (for *in vivo* biotinylation)	This study
pBirA-Kan	4514	BirA (for *in vivo* biotinylation)	This study
pQIq-MA-His6-TwinStrep-Smt3	N/A	$^{His\text{-}TwinStrep}$Smt3	This study
pQIq-MRGS-His8-control DARPin E_5-FLAG	3522	$^{His}E3\_5^{FLAG}$	This study
pQIq-MRGS-His8-anti SMT3 DARPin A10-FLAG	3523	$^{His}A10^{FLAG}$ (006-698-2025_E5)	This study
pQIq-MRGS-His8-anti SMT3 DARPin B10-FLAG	3524	$^{His}B10^{FLAG}$ (006-698-2025_H2)	This study
pQIq-MRGS-His8-anti SMT3 DARPin C10-FLAG	3525	$^{His}C10^{FLAG}$ (006-698-2025_B11)	This study
pQIq-MRGS-His8-anti SMT3 DARPin D10-FLAG	3526	$^{His}D10^{FLAG}$ (006-698-2025_A7)	This study
pQIq-MRGS-His8-anti SMT3 DARPin E10-FLAG	3527	$^{His}E10^{FLAG}$ (006-698-2025_D1)	This study
pQIq-MRGS-His8-anti SMT3 DARPin F10-FLAG	3528	$^{His}F10^{FLAG}$ (006-698-2026_G11)	This study
pQIq-MRGS-His8-anti SMT3 DARPin G10-FLAG	3529	$^{His}G10^{FLAG}$ (006-698-2026_H4)	This study
pQIq-MRGS-His8-anti SMT3 DARPin H10-FLAG	3530	$^{His}H10^{FLAG}$ (006-698-2026_D3)	This study
pQIq-MRGS-His8-anti SMT3 DARPin A11-FLAG	3531	$^{His}A11^{FLAG}$ (006-698-2026_F8)	This study
pQIq-MRGS-His8-anti SMT3 DARPin B11-FLAG	3532	$^{His}B11^{FLAG}$ (006-698-2026_C10)	This study
pQIq-MRGS-His8-anti SMT3 DARPin C11-FLAG	3533	$^{His}C11^{FLAG}$ (006-698-2026_B12)	This study
pQIq-MRGS-His8-anti SMT3 DARPin D11-FLAG	3534	$^{His}D11^{FLAG}$ (006-698-2027_C1)	This study

pQIq-MRGS-His8-anti SMT3 DARPin E11-FLAG	3535	$^{His}E11^{FLAG}$ (006-698-2027_D7)	This study
pQIq-MRGS-His8-anti SMT3 DARPin F11-FLAG	3536	$^{His}F11^{FLAG}$ (006-698-2027_E7)	This study
pQIq-MRGS-His8-anti SMT3 DARPin G11-FLAG	3537	$^{His}G11^{FLAG}$ (006-698-2027_D12)	This study
pQIq-MRGS-His8-anti SMT3 DARPin H11-FLAG	3538	$^{His}H11^{FLAG}$ (006-698-2027_G2)	This study
pQIq-MRGS-His8-anti SMT3 DARPin A12-FLAG	3539	$^{His}A12^{FLAG}$ (006-698-2028_A11)	This study
pQIq-MRGS-His8-anti SMT3 DARPin B12-FLAG	3540	$^{His}B12^{FLAG}$ (006-698-2028_F12)	This study
pQIq-MRGS-His8-anti SMT3 DARPin C12-FLAG	3541	$^{His}C12^{FLAG}$ (006-698-2028_H5)	This study
pQIq-MRS-His6-GCCG-antiSMT3-DARPin-A10	3673	^{His}A10 without FLAG-tag	This study
pQIq-MRS-His6-GCCG-antiSMT3-DARPin-C10	3674	^{His}C10 without FLAG-tag	This study
pQIq-MRS-His6-GCCG-antiSMT3-DARPin-E10	3675	^{His}E10 without FLAG-tag	This study
pQIq-MRS-His6-GCCG-antiSMT3-DARPin-F10	3676	^{His}F10 without FLAG-tag	This study
pQIq-MRS-His6-GCCG-antiSMT3-DARPin-E11	3677	^{His}E11 without FLAG-tag	This study
pQIq-MRS-His6-GCCG-antiSMT3-DARPin-G11	3678	^{His}G11 without FLAG-tag	This study
pQIq-MRS-His6-GCCG-antiSMT3-DARPin-B12	3679	^{His}B12 without FLAG-tag	This study
Yeast expression vectors			
YIp211-P30-POL30	1389	PCNA	[S12]
YIp211-P30-POL30(K127R/K164R)	718	PCNA(K127R/K164R)	[S12]
YIp128-P30-His-POL30	732	^{His}PCNA	[S12]
pCM252	1375	tTA‘ vector (Tet-regulated expression)	[S13]
pRS303-SpADH-TetR-SSN6	2393	TetR-SSN6 (Tet-regulated expression)	This study
YIp204-SpADH-TetR-SSN6	4631	TetR-SSN6 (Tet-regulated expression)	This study
YIp211-TetO7-GFP	4486	Tet-inducible yeGFP	This study
YEp195-CUP1-mCherry-His-SMT3	5237	$^{mCherry\text{-}His}$Smt3	This study
YIp211-TetO7-DARPinF10-FLAG-GFP	4479	Tet-inducible $F10^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-DARPinA10-FLAG-GFP	4480	Tet-inducible $A10^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-DARPinC10-FLAG-GFP	4481	Tet-inducible $C10^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-DARPinE11-FLAG-GFP	4482	Tet-inducible $E11^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-DARPinG11-FLAG-GFP	4483	Tet-inducible $G11^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-DARPinB12-FLAG-GFP	4484	Tet-inducible $B12^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-DARPinE3_5-FLAG-GFP	4485	Tet-inducible $E3\_5^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-2xNES-DARPin-E3.5-FLAG-GFP	4965	Tet-inducible $^{NES}E3\_5^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-2xNES-DARPin-F10-FLAG-GFP	4966	Tet-inducible $^{NES}F10^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-2xNES-DARPin-E11-FLAG-GFP	4967	Tet-inducible $^{NES}E11^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-2xNLS-DARPin-E11-FLAG-GFP	4968	Tet-inducible $^{NLS}E11^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-2xNLS-DARPin-F10-FLAG-GFP	4969	Tet-inducible $^{NLS}F10^{FLAG\text{-}GFP}$	This study
YIp211-TetO7-2xNLS-DARPin-E3.5-FLAG-GFP	4970	Tet-inducible $^{NLS}E3\_5^{FLAG\text{-}GFP}$	This study
YIp128-TetO7-DARPin-E11-FLAG-GFP	5119	Tet-inducible $E11^{FLAG\text{-}GFP}$	This study
YIp128-TetO7-DARPin-F10-FLAG-GFP	5120	Tet-inducible $F10^{FLAG\text{-}GFP}$	This study
YIp128-TetO7-DARPin-E3_5-FLAG-GFP	5121	Tet-inducible $E3\_5^{FLAG\text{-}GFP}$	This study
YIp204-PADH-AFB2-FLAG	5198	AFB2-FLAG	This study
YIp211-P30-mRuby2-POL30	6229	^{mRuby2}PCNA	This study
Yeast tagging & deletion constructs			
pFA6a-natNT2	1633	Deletion cassette	[S14]
pFA6a-hphNT1	1634	Deletion cassette	[S14]
pNat-AID*-9myc	2189	Auxin-inducible degron cassette	[S15]
pFA6a-link-yomRuby2-CaUra3	2402	mRuby2 (C-terminal tagging)	[S16]
pYM-mCherry-natNT2	2622	mCherry (C-terminal tagging)	This study
pFA6a-CaURA3	4761	Deletion cassette	This study
pRS416-RFA1(4KR)-KanMX	pXZ623	Introduction of RFA1(4KR)	[S17]

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