

Supporting Information

An expanded toolbox for the auxin-inducible degron system in budding yeast

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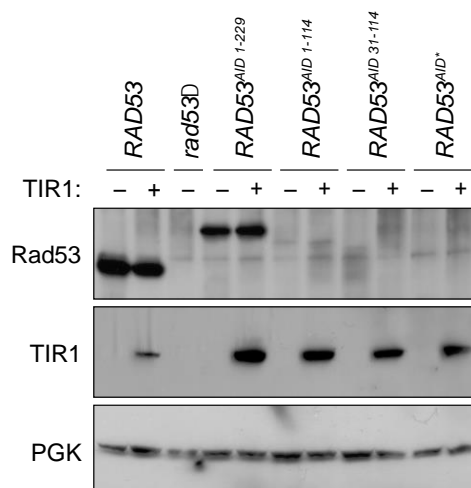


Figure S1. TIR1 is not responsible for the auxin-independent destabilisation of Rad53 by the AID-tags. Protein levels of Rad53 carrying variations of the AID-tag in comparison with the native protein were examined in strains either containing or lacking TIR1 as indicated. All cultures were grown in the absence of auxin before preparation of the lysates, and proteins were detected by Western blotting with the indicated antibodies.

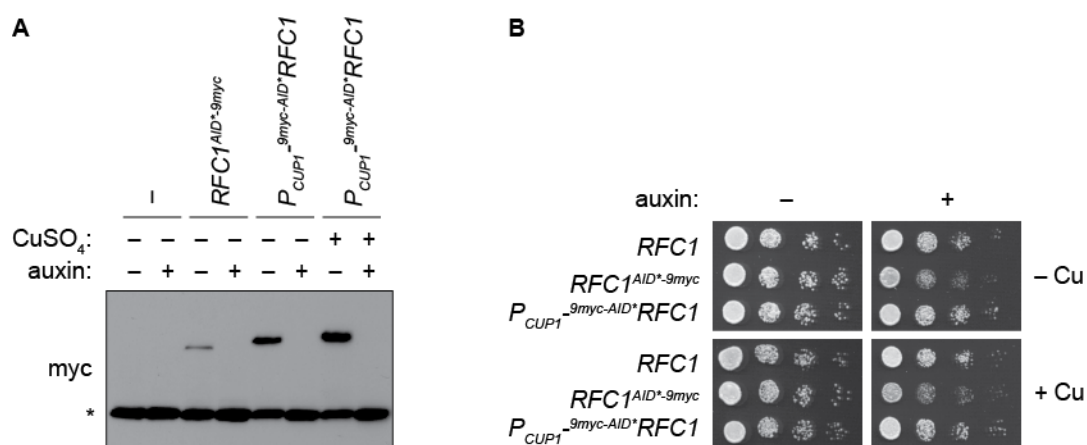


Figure S2. The position of the AID*-tag has little influence on degradation efficiency in the case of *RFC1*. (A) Western blot analysis showing protein levels of C- and N-terminally AID*-tagged Rfc1 in the presence or absence of 1 mM auxin. 1 mM CuSO₄ was added where indicated in order to enhance expression of *P_{CUP1}^{9myc-AID*}RFC1*. (B) Auxin sensitivity assays of the indicated strains in the presence or absence of 1 mM auxin and 1 mM CuSO₄ (Cu).

Table S1. Sequences of oligonucleotides used in this study

Primer	Name	Sequence (5' to 3')
97	MMS2 tag down	CCAGCGAACAAGAAATTGCGTCAACCGAAGGAAGGAGAAACCTTT CGTACGCTGCAGGTCGAC
98	MMS2 tag up	AGTGGCTTGGAAATGCTGCAAATACTGTTTAGGAAAAAGTAGATAAA TCGATGAATTCGAGCTCG
119	UBC13 tag down	AAGGCTCGCGAATGGACGAAATGTATGCAAAGAAGAAACCCGAG CGTACGCTGCAGGTCGAC
120	UBC13 tag up	GATATATATTTATATATTCAGTTGAGAAAACCTTATACAGAAATGAA TCGATGAATTCGAGCTCG
311	RFA1 tag down	GAAGCCGACTATCTTGCCGATGAGTTATCCAAGGCTTTGTTAGCTCG TACGCTGCAGGTCGAC
312	RFA1 tag up	TCTCATATGTTACATAGATTAATAGTACTTGATTATTTGATACAAT CGATGAATTCGAGCTCG
592	RAD53 ko down	TCTCATATGTTACATAGATTAATAGTACTTGATTATTTGATACAAT CGATGAATTCGAGCTCG
593	RAD53 ko up	ATCTTCTCTCTTAAAAAGGGGCAGCATTTTCTATGGGTATTTGTCCT TGGATCGATGAATTCGAGCTCG
1135	RFC1 tag down	GACTGCCACCAGTAAACCTGGTGGTAGCAAAAAAAGGAAAACGAA AGCACGTACGCTGCAGGTCGAC
1136	RFC1 tag up	CAATGAGAAGAAAAGTGTAATTATAATCTTAGTGTATGAATAAATC AATCGATGAATTCGAGCTCG
1150	POL1 tag down	GGACGTCGCTACGTTGATATGACTAGCATATTTGATTTTCATGCTAAA TCGTACGCTGCAGGTCGAC
1151	POL1 tag up	CTATATAGAATATTCATGAGATCACACAACACATACAAAATACTTA CCTAATCGATGAATTCGAGCTCG
1194	ORC6 tag down	ATATTTGGAAGAAAAGAATTGAAATGGATTGGCATTAAACAGAACC TTTACGTACGCTGCAGGTCGAC
1195	ORC6 tag up	ATGTCAGGTATTTGGTCAAATATATACTTTTAGTTAATACTGGATATG TTAATCGATGAATTCGAGCTCG
1335	POL31 tag down	TATTACTGGATTTGGAAACGTTAGAAGCAGAGACGGTCAAGATTGA CATTCTGACGCTGCAGGTCGAC
1336	POL31 tag up	ACATATAAACTTAATTGCATATCTATGTGGAAATTATTCCTTGTTT TATATCGATGAATTCGAGCTCG
1633	POL2 tag up	TTTTTCATGGTAAAGAGGCCATTGAACCTCGCGTTATATACTGCTTA CATCGATGAATTCGAGCTCG
1772	AID KpnI down	AGGTACCATGATGGGCAGTGTCG
1773	AID(114-STOP) XmaI up	CTCCCCCGGGTGATCACTTCACGAACGCCGC
1774	AID(114) XmaI up	CTCCCCCGGGTGATCACTTCACGAACGCCGC
1787	AID* KpnI down	TGGTACCCTAAAGATCCAGCCAAACC
1788	AID(31) KpnI down	CCGTACCAAGAGAGGGTTCTCAGAGACG
1790	9myc-STOP up	GATGGCGCGCCTTTTAGCTAGTGGATCCGTTT
1792	Rad53 tag down	GGGCAAAATTGGACCAAACCTCAAAGGCCCCGAGAATTTGCAATT TTCGCTACGCTGCAGGTCGAC
1795	9myc XmaI down	CGGCCCGGGGCTAGTGGTGAACAAAAG
1798	POL2 tag down	AGTATTACGGTTTTGATATATTATTGAGTTGTATTGCTGATTTGACC ATACGTACGCTGCAGGTCGAC
1992	9myc up	TTCTTCTCGAGGCTAGTGGATCCGTTCAA
1993	AID* XhoI up	GGTTCCTCGAGCATTGATACCTTCACGAACGCCGC
1996	AID*-ATG EcoRI down	GAAGGAATTCATGCCTAAAGATCCAGCCAAACC
2016	RFC1 tag(N) down	TAATTTATAAATCAAGTCACCTCATAATAAACTAAGCTGAAGAA AATGCGTACGCTGCAGGTCGAC
2039	N-AID-NheI down	CCGCTCTAGAACTAGTGCTAGCCATTACCGACATTTGG
2040	N-AID-NheI up	CCAAATGTCGGTAATGGCTAGCACTAGTTCTAGAGCGG
2064	9myc EcoRI down	AATCGAATTCATGTCGGTTCTGCTGCTAGTGG
2065	9myc EcoRI up	GATCGTTCCTACTGAATTCCTAGTGGATCCGTTCAAGTCTTC
2066	RFA1 tag(N) down	ACGGTAAAGGCGAAACCAGCAAGAAGACCAGATTATACTTACAAG AGCGTACGCTGCAGGTCGAC
2067	RFA1 tag(N) up	TTATTGGTGAAGATGCTATGAAAATCGCCCCTCGAAAGTTGAACAC TGCTAAGCTGGTACCGACTCTGG
2068	RFA1 tag(N)+ATG up	TTATTGGTGAAGATGCTATGAAAATCGCCCCTCGAAAGTTGAACAC TGCTCATAAGCTGGTACCGAGCTCTGG
2069	6HA down	CGGCCCGGGAAATACCCATACGATGTTCTGACTATGC
2070	6HA up	GATGGCGCGCCTTTTAAAGCGTAATCTGGAACGTCATATGG

2071	6FLAG down	CCGCCCCGGGATACATTATACGAAGTTATGCATGCTCACT
2072	6FLAG up	ATTTAGAAGTGGCGCGCC
2073	yEGFP down	CGGCCCGGGAAAGTGAGCAAGGGCGAGGAGCTGTTACC
2074	yEGFP up	GATGGCGCGCCTTTTACTTGTACAGCTCGTCCATGCC
2185	N-AID-PmlI down	GATATTAAGAAAAACAACTGTACACGTGTCATGTCCGGTCTGC
2186	N-AID-PmlI up	GCAGAACCGGACATGACACGTGTACAGTTTGTCTTCTTAATATC
2189	P _{REF1} up	CTGCACGTGTCTTGTAAAGTATAATCTGGTCTTCTTGC
2190	P _{REF1} down	AAAAGCTAGCTCGCCTTTTGCCGTTCTTTTGC
2276	YEN1 tag up	TCGGCGCGATCAACTGTGGTGGCGGATTTTTGACGCTGTGCCCGTT AACATCGATGAATTCGAGCTCG
2388	ASK1 tag down	AAGAAGTACCAAAGCCTGGGACCATCATTCAATTTTTCTACGAATAG ACGTACGCTGCAGGTCGAC
2389	ASK1 tag up	CCTGCGTTCTGATATTCATCACTAGTAAAAATTGTATGTACTTATT ATTATCGATGAATTCGAGCTCG
2394	RFC1 tag(N) up	GACGATCTTACGGATTTCTTATTTTTACCAAAGAAATCAGAAATATT GACAAGCTGGTACCGAGCTCTGG
2419	SEC14 tag down	AGTATATTGGACCGGAAGGTGAAGCTCCGGAAGCCTTTTCGATGAA ACGTACGCTGCAGGTCGAC
2420	SEC14 tag up	TTAGAACTCCTCTTTTCTCTCTCGAAAAAAAAAATGTCTTAAAAATA ATAATCGATGAATTCGAGCTCG
2424	YEN1 tag down	CAGTCGACCGTTTGTAGCCTGTGACAGTGATAGCAGTAGCACTAT TGAACGTACGCTGCAGGTCGAC

Oligonucleotides labelled “tag” or “ko” are used for PCR-mediated epitope-tagging or gene deletion, respectively.

Table S2. Yeast Strains used in this study

Strain	Genotype	Reference
<i>WT (DF5)</i>	<i>Mata his3-A200 leu2-3,2-112 lys2-801 trp1-1 ura3-52</i>	(Finley <i>et al.</i> , 1987)
<i>TIR1 Tet-RAD18 BrdU</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc}</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1 rad53</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG rad53Δ::HIS3MX</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1 RAD53^{AID 1-229}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG RAD53^{AID 1-229}::hphB</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1 RAD53^{AID 1-114}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG RAD53^{AID 1-114}::hphB</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1 RAD53^{AID 31-114}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG RAD53^{AID 31-114}::hphB</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1 RAD53^{AID*}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG RAD53^{AID*}::hphB</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1 RAD53^{AID 1-114-8myc}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG RAD53^{AID 1-114-8myc}::hphB</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1 RAD53^{AID 31-114-9myc}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG RAD53^{AID 31-114-9myc}::hphB</i>	This study
<i>TIR1 Tet-Rad18 BrdU sml1 RAD53^{AID*-9myc}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} sml1Δ::hisG-URA3-hisG RAD53^{AID*-9myc}::hphB</i>	This study
<i>TIR1</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc}</i>	This study
<i>TIR1 RFAI^{AID 1-229}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} RFAI^{AID 1-229}::hphB</i>	This study
<i>TIR1 RFAI^{AID*-9myc}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} RFAI^{AID*-9myc}::hphB</i>	This study
<i>TIR1 RFAI^{AID*-6HA}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} RFAI^{AID*-6HA}::hphB</i>	This study
<i>TIR1 RFAI^{AID*-6FLAG}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} RFAI^{AID*-6FLAG}::hphB</i>	This study
<i>TIR1 RFAI^{AID*-GFP}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} RFAI^{AID*-GFP}::hphB</i>	This study
<i>TIR1 RFAI^{AID*-9myc (Kan)}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} RFAI^{AID*-9myc}::KanMX</i>	This study
<i>TIR1 RFAI^{AID*-9myc (Nat)}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} RFAI^{AID*-9myc}::NatNT</i>	This study
<i>TIR1 RFAI^{AID*-9myc (His)}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} RFAI^{AID*-9myc}::HIS3MX</i>	This study
<i>TIR1 P_{CUP1}^{AID*-9myc} RFAI</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} P_{CUP1}^{AID*-9myc} RFAI::KanMX</i>	This study
<i>TIR1 P_{CUP1}^{9myc-AID*(ATG)} RFAI</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} P_{CUP1}^{9myc-AID*(ATG)} RFAI::KanMX</i>	This study
<i>TIR1 P_{CUP1}^{9myc-AID*} RFAI</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} P_{CUP1}^{9myc-AID*} RFAI::KanMX</i>	This study
<i>TIR1 P_{CUP1}^{9myc-AID*} RFAI</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} P_{CUP1}^{9myc-AID*} RFAI::KanMX</i>	This study
<i>TIR1 Tet-RAD18 BrdU ORC6^{AID*-9myc}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} ORC6^{AID*-9myc}::hphB</i>	This study
<i>TIR1 Tet-RAD18 BrdU POL1^{AID*-9myc}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} POL1^{AID*-9myc}::hphB</i>	This study
<i>TIR1 Tet-RAD18 BrdU POL2^{AID*-9myc}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} POL2^{AID*-9myc}::hphB</i>	This study
<i>TIR1 Tet-RAD18 BrdU POL31^{AID*-9myc}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} POL31^{AID*-9myc}::hphB</i>	This study
<i>TIR1 Tet-RAD18 BrdU RFC1^{AID*-9myc}</i>	<i>DF5 KanMX::TetO7-RAD18 LEU2::TetR'-SSN6 TRP::BrdU-inc URA3::ADH1-AtTIR1^{9myc} RFC1^{AID*-9myc}::hphB</i>	This study
<i>TIR1 P_{CUP1}^{9myc-AID*} RFC1</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} P_{CUP1}^{9myc-AID*} RFC1::KanMX</i>	This study
<i>TIR1 ASK1^{AID*-9myc}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} ASK1^{AID*-9myc}::hphB</i>	This study
<i>TIR1 SEC14^{AID*-9myc}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} SEC14^{AID*-9myc}::hphB</i>	This study
<i>TIR1 MMS2^{AID*-9myc}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} MMS2^{AID*-9myc}::hphB</i>	This study
<i>TIR1 UBC13^{AID*-9myc}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} UBC13^{AID*-9myc}::hphB</i>	This study
<i>TIR1 YEN1^{AID*-9myc}</i>	<i>DF5 URA3:: ADH1-AtTIR1^{9myc} YEN1^{AID*-9myc}::hphB</i>	This study

Note that those strains harbouring *Tet-Rad18 BrdU* were constructed from strains described in Daigaku *et al.*, *Nature* **465**: 951-955 (2010). The presence of these features is irrelevant to this study.