

Supplemental Materials

Molecular Biology of the Cell

Feng et al.

Supporting Information

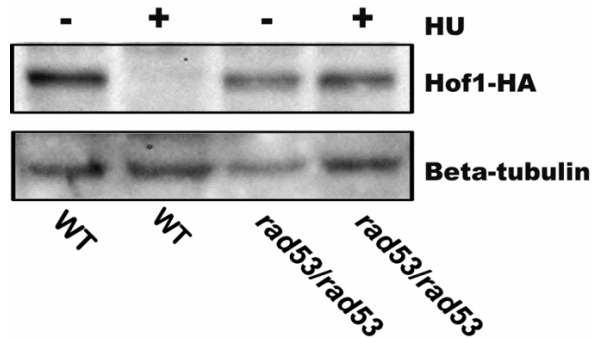


Figure S1. Loss of *RAD53* stabilizes Hof1 levels in cells exposed to HU. Hof1-HA levels in wild type (WT) and *rad53/rad53* strains were assessed by Western blot with an anti-HA antibody, with Beta tubulin used as a loading control. Cells were grown in YPD to log phase were either sampled (- HU) or treated with 40mM HU for 90 mins prior to harvesting (+ HU).

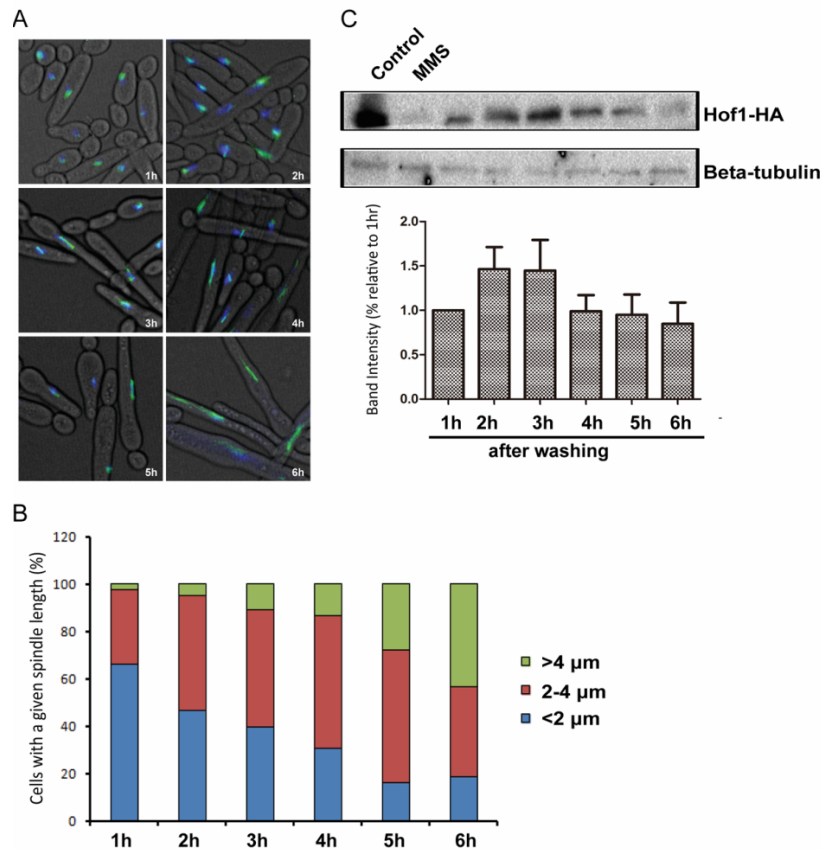


Figure S2. Tracking the levels of Hof1 through the cell cycle in a population of synchronized cells, using spindle length as an indication of cell cycle progression. Cells expressing Tub2-GFP and Hof1-HA were synchronized by exposure to 0.02% MMS for 6 h, washed, released into YPD and tracked through reinitiation of the cell cycle. (A) At the indicated time points cells were imaged for morphology (DIC, gray), nuclei (DAPI, blue) and spindles (Tub2-GFP, green). (B) The length of spindles in the images was also measured. ≥ 80 spindles/time. (C) Hof1-HA levels were assessed at the same time points by Western blot.

Name	Oligos	Description
P7	ATCTCATTAGATTTGGAACCTGTGGGTT	CAS9 gene
P8	TTCGAGCGTCCAAAACCTTCT	CAS9 gene
P1	AAGAAAGAAAGAAAACCAGGAGTGAA	sgRNA
P4	ACAAATATTTAACTCGGGACCTGG	sgRNA
P5	GCGGCCGCAAGTGATTAGACT	sgRNA
P6	GCAGCTCAGTGATTAAGAGTAAAGATGG	sgRNA
HOF1-sg-F	ATTTGatagccaaataaacctgacaG	sgRNA for <i>HOF1</i>
HOF1-sg-R	AAAACGTGCAGGTTTATTTGGCTATC	sgRNA for <i>HOF1</i>
HOF1-Re-F	accctatttgaataacttttgggtaactccccagagaaaag ttttgaaatcgtcacatccaatacctcgccagaac	repair DNA for <i>HOF1</i>
HOF1-Re-R	AAGCATATTTGTTGGGAATGGTAGATCTAATGG CAAGTGTCTTCTGTAAAAAGATGAAACTCACTATA GGGAGACCG	repair DNA for <i>HOF1</i>
HOF1-Te-F	gcgagtatctaataccacaac	genotype confirmation for <i>HOF1</i>
HOF1-Te-R	GAATGTGGGTCGGAAGAG	genotype confirmation for <i>HOF1</i>
HOF1-F	CGGGGTACCGgacaacaacaatggaactgg	<i>HOF1</i> gene cloning
HOF1-R	CGGGGTACCGAATGTGGGTCGGAAGAG	<i>HOF1</i> gene cloning
RAD23-sg-F	aatggattagtttggtatcGTTTAGAGCTAGAAATAG CAAGTTAAA	sgRNA for <i>RAD23</i>
RAD23-sg-R	GATAACCAAATAATCCATTCAAATTAATAATAGT TTACGCAAGTC	sgRNA for <i>RAD23</i>
RAD23-Re-F1	tgtgtgtgtgcccgttc	repair DNA for <i>RAD23</i>
RAD23-Re-R1	GTTCTGGCGAGGTATTGGACCTGTTGTTATTGTG GAAGTC	repair DNA for <i>RAD23</i>
RAD23-Re-F2	cggctcccctatagtgaggcttttcatcccgttc	repair DNA for <i>RAD23</i>
RAD23-Re-R2	CGGGGTACCATCTGGTTCAATAAAGGC	repair DNA for <i>RAD23</i>
RAD23-Te-new F	tagggtaggttctgtattg	genotype confirmation for <i>RAD23</i>
RAD23-Te-new R	TGACAACACTACAGCCAAAACAG	genotype confirmation for <i>RAD23</i>
SIZ1-sg-F	gaattaaagacacttttcgGTTTAGAGCTAGAAATAG CAAGTTAAA	sgRNA for <i>SIZ1</i>
SIZ1-sg-R	CGAAAAGTGTCTTTAAATCCAAATTAATAATAGT TTACGCAAGTC	sgRNA for <i>SIZ1</i>
SIZ1-Re-F1	gatgtctgtcttcttga	repair DNA for <i>SIZ1</i>
SIZ1-Re-R1	GTTCTGGCGAGGTATTGGAGTCCCCTGTAAACG CAT	repair DNA for <i>SIZ1</i>
SIZ1-Re-F2	cggctcccctatagtgaggtaactcgccaaggaagg	repair DNA for <i>SIZ1</i>
SIZ1-Re-R2	GCCTCTATCAGTTCAGCC	repair DNA for <i>SIZ1</i>
SIZ1-Te-F	acgcgtcgacgttgacgaggcattgagat	genotype confirmation for <i>SIZ1</i>
SIZ1-Te-R	acgcgtcgacGATTACGCCATAGGAAGC	genotype confirmation for <i>SIZ1</i>
Rad4-sg-F	agaatcaggtaatgatacatGTTTAGAGCTAGAAATA GCAAGTTAAA	sgRNA for <i>RAD4</i>
Rad4-sg-R	ATGTATCATTACCTGATTCTCAAATTAATAATAGTT TACGCAAGTC	sgRNA for <i>RAD4</i>
Rad4-Re-F	cгааagctattgaatgatgtaataataagagaagaactgccact gagaаagaagcgggatccaatacctcgccagaac	repair DNA for <i>RAD4</i>
Rad4-Re-R	GTAACATCTATATTTCCATATTGATTTTAGGAAC AATGCCATCAGTCACGGGTGGAGGCTCACTATAG GGAGACCG	repair DNA for <i>RAD4</i>
Rad4-Te-F	caatgagcaaccagagcc	genotype confirmation for <i>RAD4</i>

Rad4-Te-R	GGTCTTCCTCCTCTTGTTCC	genotype confirmation for <i>RAD4</i>
Rad52-sg-F	aatttgctaataaatattGTTTTAGAGCTAGAAATAGCAAGTTAAA	sgRNA for <i>RAD52</i>
Rad52-sg-R	AATATTTTCATTAGCCAAATTCAAATTAATAATAGTTTACGCAAGTC	sgRNA for <i>RAD52</i>
Rad52-Re-F	cagcaacctcaaccaaccaacagaggcttcctttcgaccagacgagtcacgagctcgtccaatacctcgcagaac	repair DNA for <i>RAD52</i>
Rad52-Re-R	CATAGCTGACCACTTTGGTTGGCAGTTTTGGTGTAAACAAAAGCTGCTGCATGGTGGGAACTCACTATAGGGAGACCG	repair DNA for <i>RAD52</i>
Rad52-Te-new F	acaaacccttccaccac	genotype confirmation for <i>RAD52</i>
Rad52-Te-new R	ATCAACTGCCATAGACTCG	genotype confirmation for <i>RAD52</i>
MMS22-sg-F	actaaaaaaaaaggtatccaaGTTTTAGAGCTAGAAATAGCAAGTTAAA	sgRNA for <i>MMS22</i>
MMS22-sg-R	TTGGATACCTTTTTTTTAGTCAAATTAATAATAGTTTACGCAAGTC	sgRNA for <i>MMS22</i>
MMS22-Re-F	tacctgaagaccaagaaaaagaaatcatttgcacatattgaaataccacgaactcatccaatacctcgcagaac	repair DNA for <i>MMS22</i>
MMS22-Re-R	TGTACCAGTAAAGCAATTGCATGAATCGTTCAAAAATGAATCATCAGGGATATCAAATCTCACTATAGGGAGACCG	repair DNA for <i>MMS22</i>
MMS22-Te-F	gacgattcagattcaaaccc	genotype confirmation for <i>MMS22</i>
MMS22-Te-R	CCACTTTACATACCAGCGC	genotype confirmation for <i>MMS22</i>
Rad18-sg-F	gcctcattattacgattatGTTTTAGAGCTAGAAATAGCAAGTTAAA	sgRNA for <i>RAD18</i>
Rad18-sg-R	ATAATCGTAATAAATGAGGCCAAATTAATAATAGTTTACGCAAGTC	sgRNA for <i>RAD18</i>
Rad18-Re-F1	gctgtcgtaccatctgtc	repair DNA for <i>RAD18</i>
Rad18-Re-R1	GTTCTGGCGAGGTATTGGAGCGGACATCTATTATCTCG	repair DNA for <i>RAD18</i>
Rad18-Re-F2	cggctccctatagtgaggaagagcagcgaagaggac	repair DNA for <i>RAD18</i>
Rad18-Re-R2	CTGGGGATGGAGATGAGAC	repair DNA for <i>RAD18</i>
Rad18-Te-F	cgctcctcatcaatcac	genotype confirmation for <i>RAD18</i>
Rad18-Te-R	GCTTGCCCTTGGAAGTGG	genotype confirmation for <i>RAD18</i>
Rad14-sg-F	agatcgacagaatagactaGTTTTAGAGCTAGAAATAGCAAGTTAAA	sgRNA for <i>RAD14</i>
Rad14-sg-R	TAGTCTATTCTGTTTCGATCTCAAATTAATAATAGTTTACGCAAGTC	sgRNA for <i>RAD14</i>
Rad14-Re-F1	ctgacaaactaacacacg	repair DNA for <i>RAD14</i>
Rad14-Re-R1	GTTCTGGCGAGGTATTGGATTGTCGTGGTGGTGGAGGTG	repair DNA for <i>RAD14</i>
Rad14-Re-F2	cggctccctatagtgagcgaatagagaaccaacc	repair DNA for <i>RAD14</i>
Rad14-Re-R2	TCACGGGCGAAGACCAATCA	repair DNA for <i>RAD14</i>
Rad14-Te-F	gagtttgggtagtattggc	genotype confirmation for <i>RAD14</i>
Rad14-Te-R	CCACTTCTCTGTCTCAATACC	genotype confirmation for <i>RAD14</i>
HOF1-Nsg-F	tgaacaactcaataatgtGTTTTAGAGCTAGAAATAGCAAGTTAAA	sgRNA for the N terminal of <i>HOF1</i>
HOF1-Nsg-R	ACATTATTTGAGTTGTTTCACAAATTAATAATAGTTTACGCAAGTC	sgRNA for the N terminal of <i>HOF1</i>
HOF1-N-ReF	atgcgagtatctaaccacaactgtcaaaaaccctatttggatgaacttttgggtaactccggatcc	repair DNA for the N terminal of <i>HOF1</i>

HOF1-N-ReR	TCCAATCTCGTTGTAGATTCGTTTATTTCTTTGT AATTTACTAATGCCATATGATAGTGGGATCCGGAG TTACCCCAAAAAG	repair DNA for the N terminal of <i>HOF1</i>
HOF1-C-sg-F	atagcgatgaagttgcaaaaGTTTtagagctagaaata GCAAGTTAAA	sgRNA for the C terminal of <i>HOF1</i>
HOF1-C-sg-R	TTTGCCAACCTTCATCGCTATCAAATAAAAATAGT TTACGCAAGTC	sgRNA for the C terminal of <i>HOF1</i>
HOF1-C-re-F	gatctaatagcaggtgtaatggagacgtaatcaatttagacg atcattgagaatggaggatcc	repair DNA for the C terminal of <i>HOF1</i>
HOF1-C-re-R	TAAATCATCTCCTTCAACAATATAGTTGCCAGGAA CTAAGCCAATCATACTGATGACTGGGATCCTCCA TTTCTCAATG	repair DNA for the C terminal of <i>HOF1</i>
HOF1-tag-F	tcatcaggtatgattggcttagttcctggcaactatattgtgaag gagatgatttattcctgctgcaggtcgacggatc	tag for <i>HOF1</i>
HOF1-tag-SH3 F	ccaaatcaacagtgccaagaagatcaataaaacttaccctcag caacaaaaaccattgttTAGTAAGctgcaggtcgacggat c	tag for C terminal of <i>HOF1</i>
Rad53-sg-F	tatcaaaatccaccaaataatGTTTTAGAGCTAGAAATAG CAAGTTAAA	sgRNA for <i>RAD53</i>
Rad53-sg-R	ATATTTGGTGGATTTTGATACAAATAAAAATAGT TTACGCAAGTC	sgRNA for <i>RAD53</i>
Rad53-TagF	caaacctcagaagaaatgaatatttacccttatttcgggttta agtagtataagttcagctgcaggtcgacggatc	tag for Rad53
Rad53-TagR	AATCAAATATCCGATCCTATCCAAAAACAAATGTTT CAGGACAAGTACATGGGTAAACTACTATAGGG AGACCGGC	tag for Rad53
Rad53-TF	ttgcttagaggaggggag	genotype confirmation for <i>RAD53</i>
Rad53-TR	ATGTGGAATCTCAACGACC	genotype confirmation for <i>RAD53</i>
Rad53-Re-F	ATGGAAGTAACACAACGGACGCAGAGTCAGACA CAACCAACACAACAGTCACCGACAACCTTCCAATA CCTCGCCAGAAC	repair DNA for <i>RAD53</i>
Rad53-New-TF	ACATTCAGTAACAACACCCT	genotype confirmation for <i>RAD53</i>
sgRad53-prom oter-F	aaagattgttcaaaattgaGTTTTAGAGCTAGAAATAG CAAGTTAAA	sgRNA for the promoter of <i>RAD53</i>
sgRad53-prom oter-R	TCAATTTGAACAAATCTTCAAATAAAAATAGT TTACGCAAGTC	sgRNA for the promoter of <i>RAD53</i>
Rad53-promot er-Re-F	gagctgtatatacatatagttatttacgaaatcgcatacgt tagacgaataccggatcggtatgataaacggaac	repair DNA for the promoter of <i>RAD53</i>
Rad53-promot er-Re-R	CTGAGTTGTCGGTGACTGTTGTGTTGGTTGTGTC TGACTCTGCGTCCGTTGTGTTACTTCCATGTTTT TGGGGAGGGTA	repair DNA for the promoter of <i>RAD53</i>
Rad53-promot er-Te-F	cgcttatgccagtttcttc	genotype confirmation for the promoter of <i>RAD53</i>
Rad53-promot er-Te-R	GCGTCCGTTGTGTTACTTCC	genotype confirmation for the promoter of <i>RAD53</i>
Tub2-GFP-F	attgatgaagaagaattagaatgccgatgaaatcccattaga agatgcccatggaaaagcttcgtacgctcgag	construct Tub2-GFP strain
Tub2-GFP-R	GTATATTACATATACATATTCTTATTATTAACATT AATCACCCCTTATATTAACAAGCTCACTATAGGG AGACCG	construct Tub2-GFP strain

Strains	Genotype	Source
SN148	<i>arg4/arg4 leu2/leu2 his1/his1</i> <i>ura3::imm434/ura3::imm434 iro1::imm434/iro1::imm43</i>	Lab stock
JR7	RM1000 <i>pph3::hisG/pph3::HIS1</i>	Feng, 2013
	<i>mms21::HIS1/mms21::URA3</i>	Islam, 2019
JC1	<i>hof1::HIS1/hof1::HIS1</i>	This study
JC2	<i>SN148 +CIP10</i>	This study
JC3	<i>hof1::HIS1/hof1::HIS1 +CIP10</i>	This study
JC4	<i>hof1::HIS1/hof1::HIS1 +CIP10-HOF1</i>	This study
JC5	<i>Hof1::myc-URA3/Hof1::myc-URA3</i>	This study
JC6	<i>Hof1-N^Δ/Hof1-N^Δ</i>	This study
JC7	<i>Hof1-SH3^Δ/Hof1-SH3^Δ</i>	This study
JC8	<i>Hof1-N^Δ-myc/Hof1-N^Δ-myc</i>	This study
JC9	<i>rad14::ARG4/rad14::ARG4</i>	This study
JC10	<i>hof1::HIS1/hof1::HIS1 rad14::ARG4/rad14::ARG4</i>	This study
JC11	<i>hof1::HIS1/hof1::HIS1 mms21::HIS1/mms21::URA3</i>	This study
JC12	<i>hof1::URA3/hof1::URA3 pph3::HIS1/pph3::hisG</i>	This study
JC13	<i>rad52::ARG4/rad52::ARG4</i>	This study
JC14	<i>hof1::HIS1/hof1::HIS1 rad52::ARG4/rad52::ARG4</i>	This study
JC15	<i>siz1::ARG4/siz1::ARG4</i>	This study
JC16	<i>hof1::HIS1/hof1::HIS1 siz1::ARG4/siz1::ARG4</i>	This study
JC17	<i>mms22::ARG4/mms22::ARG4</i>	This study
JC18	<i>hof1::HIS1/hof1::HIS1 mms22::ARG4/mms22::ARG4</i>	This study
JC19	<i>rad4::ARG4/rad4::ARG4</i>	This study
JC20	<i>hof1::HIS1/hof1::HIS1 rad4::ARG4/rad4::ARG4</i>	This study
JC21	<i>rad18::ARG4/rad18::ARG4</i>	This study
JC22	<i>hof1::HIS1/hof1::HIS1 rad18::ARG4/rad18::ARG4</i>	This study
JC23	<i>rad53::HIS1/rad53::HIS1</i>	This study
JC24	<i>rad53::HIS1/rad53::HIS1 hof1::ARG4/hof1::ARG4</i>	This study
JC25	<i>Hof1::HA-HIS1/Hof1::HA-HIS1</i>	This study
JC26	<i>rad23::ARG4/rad23::ARG4</i>	This study
JC27	<i>hof1::HIS1/hof1::HIS1 rad23::ARG4/rad23::ARG4</i>	This study
JC28	<i>hof1::HIS1/hof1::HIS1</i>	This study
JC29	<i>rad53::HIS1/rad53::HIS1 Hof1::HA-HIS1/Hof1::HA-HIS1</i>	This study

JC30	<i>RAD53/rad53::HA-HIS1</i>	This study
JC31	<i>hof1::HIS1/hof1::HIS1 RAD53/rad53::HA-ARG4</i>	This study
JC32	<i>rad23::ARG4/rad23::ARG4 RAD53/rad53::HA-HIS1</i>	This study
JC33	<i>mms21::HIS1/mms21::URA3 RAD53/rad53::HA-ARG4</i>	This study
JC34	<i>hof1::HIS1/hof1::HIS1 rad23::ARG4/rad23::ARG4 RAD53/rad53::HA-URA3</i>	This study
JC35	<i>hof1::HIS1/hof1::HIS1 RAD53/pMET3-RAD53</i>	This study
JC36	<i>Hof1::HA-HIS1/Hof1::HA-HIS1 TUB2/Tub2::GFP-URA3</i>	This study