

Supplemental Material

Redox requirements for ubiquitin-like urmylation of Ahp1, a 2-Cys peroxiredoxin from yeast

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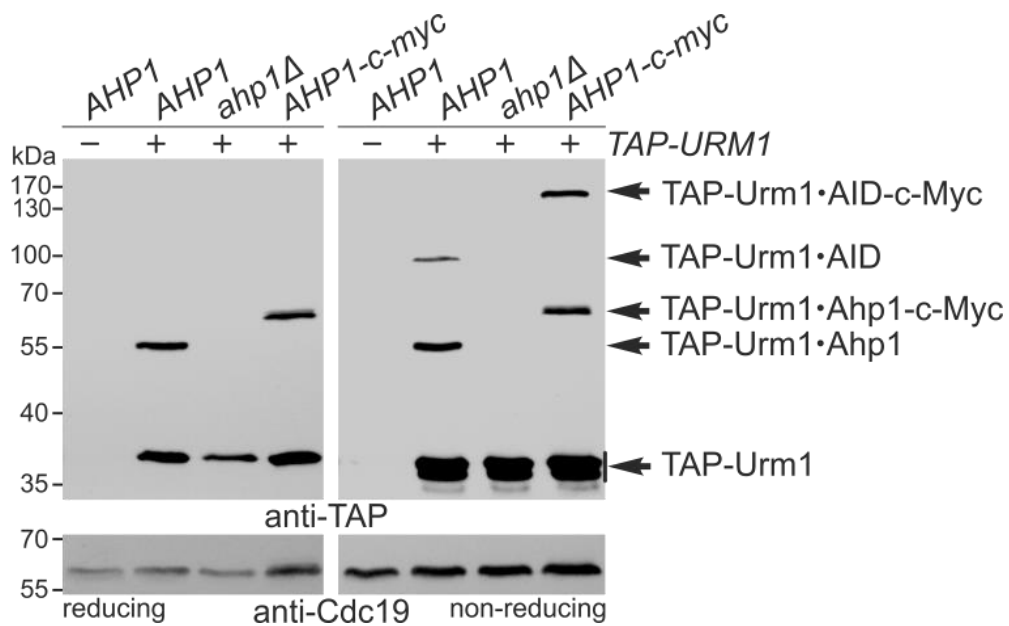
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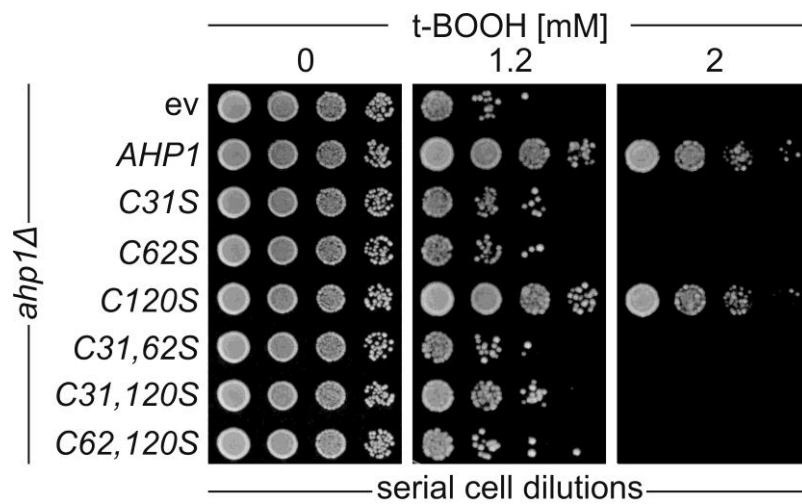
1) Supplemental Figures

Fig. S1.



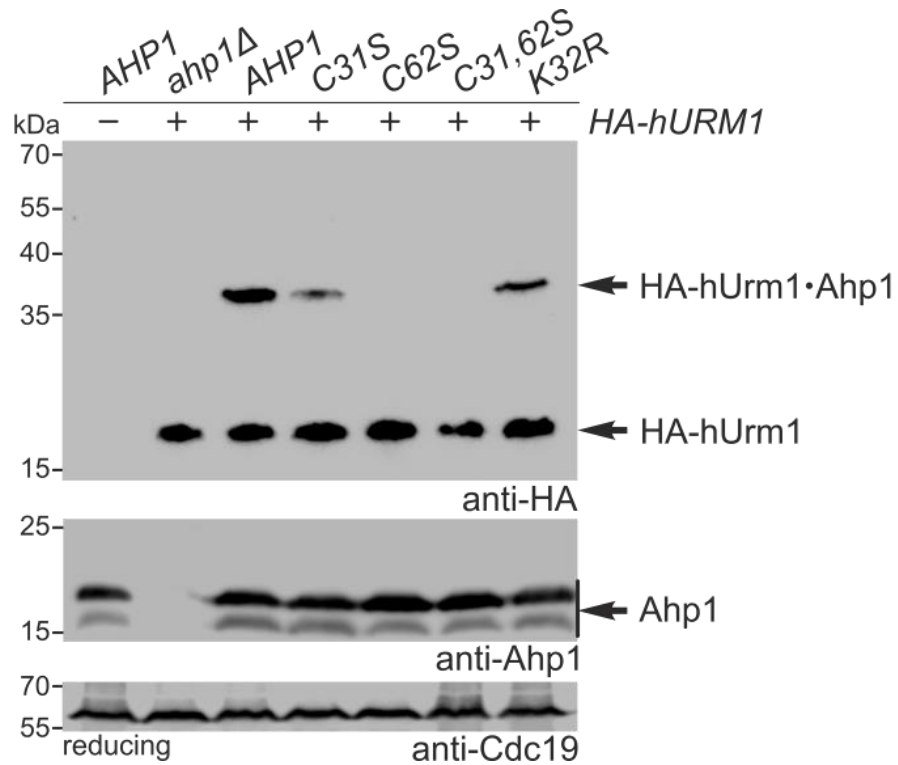
Supplemental Fig. S1. TAP-Urm1·Ahp1 conjugation *in vivo*. Shown are EMSAs under reducing (left panels) and non-reducing (right panels) conditions with protein extracts from indicated strains expressing *TAP-URM1* (+) or not (-). NEM-stabilized urmylation was studied with anti-TAP (top panels) diagnostic for free TAP-Urm1 (~37 kDa), urmylated Ahp1 (~55 kDa) and Ahp1 intersubunit disulfide (AID ~100 kDa) forms as well as Urm1-modified forms of c-Myc tagged Ahp1 (~65 kDa) and AIDs (~135 kDa). anti-Cdc19 blots (bottom panels) served as loading control. Reproduced in part from permission by Jüdes *et al.* (2016); *Microb Cell* 3: 554-564 (doi: 10.15698/mic2016.11.539).

Fig. S2.



Supplemental Fig. S2. t-BOOH cytotoxicity assay *in vivo*. Using ten-fold serial cell dilutions, growth of an *ahp1Δ* yeast strain carrying empty vector (*ev*), wild-type peroxiredoxin (*AHP1*) gene or indicated Cys substitutions of Ahp1 (C31S; C62S; C120S; C31,62S; C31,120S; C62,120S) was monitored at 30°C in the presence of the denoted t-BOOH doses.

Fig. S3.



Supplemental Fig. S3. Ahp1 urmylation by the human homolog of yeast Urm1, hURM1, requires peroxidatic Cys-62. Shown are EMSA under reducing conditions from indicated strains expressing human HA-tagged hURM1 (+) or not (-). NEM-stabilized HA-hURM1 conjugation was studied by anti-HA blot (top panel) diagnostic for free HA-hURM1 and urmylated Ahp1 (~36 kDa). anti-Ahp1 (middle panel) detects unmodified Ahp1 (~19 kDa) and anti-Cdc19 (bottom panel) served as internal standard.

2) Supplemental Tables

Supplemental Table S1. Yeast strains used in this study.

Strain	Genotype	Source
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Euroscarf
Y01400	BY4741, <i>urm1Δ::kanMX4</i>	Euroscarf
Y02720	BY4741, <i>ahp1Δ::kanMX4</i>	Euroscarf
FEY14	BY4741, <i>urm1Δ::kanMX4 AHP1-c-myc::ScHIS3</i>	[20]
FEY16	BY4741, <i>ahp1Δ::kanMX4 urm1Δ::ScHIS3</i>	[20]
FEY18	BY4741, <i>yap1Δ::kanMX4 ahp1Δ::SpHIS5</i>	This study
FEY42	BY4741, <i>urm1Δ::kanMX4 trx1Δ::SpHIS5</i>	This study
FEY43	BY4741, <i>trx2Δ::kanMX4 urm1Δ::KIURA3</i>	This study
FEY47	BY4741, <i>urm1Δ::kanMX4 trx1Δ::SpHIS5 trx2Δ::KILEU2</i>	This study
RK101	BY4741, <i>urm1Δ::kanMX4 trr1Δ::SpHIS5</i>	This study
RK53	BY4741, <i>yap1Δ::kanMX4 urm1Δ::SpHIS5</i>	This study

Supplemental Table S2. Primers used in this study.

Primer	Sequence (5'-3')	Application
AHP1_C31S_FW	CAGTGAATCTAGTAAGATGCCACAAAC	AHP1 SM *
AHP1_C31S_RV	GTTTGTGGCATCTTACTAGATTCCTG	AHP1 SM
AHP1_C62S_FW	CTTTCTCCCAACCAGTACTGTCAGCCATATTC	AHP1 SM
AHP1_C62S_RV	GAATATGGCTGACAGTACTGGTTGGGGAGAAA	AHP1 SM
AHP1_C120S_FW	CGACCCAGGCAGTGCTTTCACCAAATC	AHP1 SM
AHP1_C120S_RV	GATTTGGTGAAAGCACTGCCTGGGTCG	AHP1 SM
AHP1_F58A_FW	CCGGTGCTCCAGCTGCTGCTTCCCAACCTGTACTG	AHP1 SM
AHP1_F58A_RV	CAGTACAGGTTGGGGAAGCAGCAGCTGGAGCACCGG	AHP1 SM
AHP1_F95A_FW	GTTACTGTTGACAACCCGGCTGCTAACCAAGCGTGGGC	AHP1 SM
AHP1_F95A_RV	GCCCACGCTTGTTAGCAGCCGGTGTCAACAGTAAC	AHP1 SM
AHP1_K32R_FW	CAGTGAATCTTGTAGGATGCCACAAAC	AHP1 SM
AHP1_K32R_RV	GTTTGTGGCATCCTACAAGATTCCTG	AHP1 SM
AHP1_K156R_FW	CTTACGCTGCCAGGGAAACCAACC	AHP1 SM
AHP1_K156R_RV	GGTTGGTTTCCCTGGCAGCGTAAG	AHP1 SM
AHP1_FW_HindIII	GGGAAGCTTCCTTGGCCTCGATCTATTGC	Sequencing
AHP1_RV_EcoRI	GGGGAATTCCTGCTCCAACCTACTCTGTC	Sequencing
KO_URM1_FW	CAATACTGATTTCTGATACTAAAACGAGATAGGTTAATAGCA AAATCGGGCAGCTGAAGCTTCGTACGC	URM1 KO **
KO_URM1_RV	CTTTATATATATATATGTAGCTGCTTCTTAAAAATTATTTGCT GCTATTTGCATAGGCCACTAGTGGATCTG	URM1 KO
AHP1KOF	ATTTCAACAAACCAGAACAACACAAGTACTACCAATAACCAC AACAAAACCAGCTGAAGCTTCGTACGC	AHP1 KO
AHP1KOR	TTTTGAATTTTTTTTATATAAACATGGTTTTATTGTCTATTACA TAGCATGCATAGGCCACTAGTGGATCTG	AHP1 KO
KO_TRX1_FW	CCCTGAAACTGCATTAGTGAATAGAAGACTAGACACCTCG ATACAAATACAGCTGAAGCTTCGTACGC	TRX1 KO
KO_TRX1_RV	TATATAACAAACACAGTATAGAAACACAATATATCGGTCATT GGGTGAGTGCATAGGCCACTAGTGGATCTG	TRX1 KO
KO_TRX2_FW	CACGCACACATACACGAGAGTCTACGATATCTTTAAATAACA CATCAATACAGCTGAAGCTTCGTACGC	TRX2 KO
KO_TRX2_RV	TTTAAACTGGTAAACATGATGTACTTTACGTAGCGTTAATAT ACCGGCAAGCATAGGCCACTAGTGGATCTG	TRX2 KO
KO_TRR1_FW	CTATACAGCAAATAGCGAACAGTACGAAAGTAAACATCATAT TATCAATACAGCTGAAGCTTCGTACGC	TRR1 KO
KO_TRR1_RV	GATAAGTATACAAAAATTTGAGTGTATCTATTTTATAATGGAA AATTCATGCATAGGCCACTAGTGGATCTG	TRR1 KO

* SM: site-directed mutagenesis ** KO: gene knock-out generation

Supplemental Table S3. Plasmids used in this study.

Plasmid	Description	Reference
pRS426	2 μ ori, <i>ScURA3</i>	[81]
YCplac33	<i>ARS1-CEN4</i> , <i>ScURA3</i>	[80]
YCplac111	<i>ARS1-CEN4</i> , <i>ScLEU2</i>	[80]
pHA-URM1	<i>HA-URM1</i> cloned into pRS426 _{<i>Sma</i>I}	[17]
pCB45	ρ _{<i>GAL1-TAP-URM1</i>} cloned into YCplac33 _{<i>Hind</i>III/<i>Sal</i>I}	[20]
pAJ46	ρ _{<i>ADH1-(HA)3-hURM1-TCYC1</i>} cloned into YCplac33 _{<i>Hind</i>III/<i>Sal</i>I}	[20]
pAJ31	<i>AHP1</i> cloned into YCplac111 _{<i>Hind</i>III/<i>Eco</i>RI}	This study
pAJ33	<i>C31S</i> in pAJ31 by SM	This study
pAJ35	<i>K32R</i> in pAJ31 by SM	This study
pAJ39	<i>C62S</i> in pAJ31 by SM	This study
pAJ55	<i>K156R</i> in pAJ31 by SM	This study
pAJ57	<i>K32, 156R</i> in pAJ31 by SM	This study
pAJ67	<i>C31, 62S</i> in pAJ31 by SM	This study
pLK1	<i>C120S</i> in pAJ31 by SM	This study
pLK2	<i>C31, 120S</i> in pAJ33 by SM	This study
pLK3	<i>C62, 120S</i> in pAJ39 by SM	This study
pLK90	<i>C62S</i> in pAJ35 by SM	This study
pLK91	<i>C62S</i> in pAJ55 by SM	This study
pLK92	<i>C31S</i> in pAJ55 by SM	This study
pLK93	<i>C62S</i> in pAJ57 by SM	This study
pCiB40	<i>F58A</i> in pAJ31 by SM	This study
pCiB19	<i>F95A</i> in pAJ31 by SM	This study
pCiB39	<i>F58, 95A</i> in pCiB19 by SM	This study
pMT173	ScUrm1-COSH in Intein system	[29]

3) Supplemental References

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