

Supplementary Information for:

***Hermes* transposon mutagenesis shows [URE3] prion pathology prevented by a ubiquitin-targeting protein: evidence for carbon/nitrogen assimilation cross-talk and a second function for Ure2p**

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METHODS

Strains and media. Strains of *S. cerevisiae* are listed in Table S1. Media are as described (SHERMAN 1991), except ½ YPD is 5 g Yeast Extract, 20 g Peptone, 20 g dextrose and 20 g agar per liter; YES is 5 g yeast extract, 30 g dextrose, and 30 mg tryptophan per liter. Plasmid DNA was isolated from yeast as described (ROBZYK and KASSIR 1992).

Table S1. Strains of *Saccharomyces cerevisiae*.

YHE1265G	<i>MATa lys1 rho</i> ⁰	Not BY241
YHE1266G	<i>MATa lys1 rho</i> ⁰	Not BY241
YHE1608 = BY241	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o], [PIN+]</i>	1
YHE1609 = BY241 [URE3-1]	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1], [PIN+]</i>	2
YHE1627	<i>MATa ura3 leu2 his3::TRP1^{alb} trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1] [PIN+]</i>	3
YHE1628	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K</i>	4
YHE1630	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K ylr352w::kanMX</i>	4, 6
YHE1631	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K rho</i> ⁰	4, 5
YHE1633	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K rho</i> ⁰ ylr352w::kanMX	4, 5, 6
YHE1634	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K rho</i> ⁰ ylr352w::kanMX	7
YHE1635	<i>MATa ura3 leu2 his3::TRP1^{alb} trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [pin-]</i>	8
YHE1636	<i>MATa ura3 leu2 his3::TRP1^{alb} trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 ure2::URA3^{alb} [PIN+]</i>	9
YHE1646	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K rho</i> ⁰ rub1::kanMX	6
YHE1647	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K rho</i> ⁰ ula1::kanMX	6
YHE1648	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K rho</i> ⁰ uba3::kanMX	6
YHE1649	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K rho</i> ⁰ ubc12::kanMX	6
YHE1650	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K</i>	this work

	Cytoductant from YHE1634 → YHE1631	
YHE1651	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K rub1::kanMX</i> Cytoductant from YHE1634 → YHE1646	this work
YHE1652	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K ula1::kanMX</i> Cytoductant from YHE1634 → YHE1647	this work
YHE1653	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K uba3::kanMX</i> Cytoductant from YHE1634 → YHE1648	this work
YHE1654	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] cyh2-Q38K ubc12::kanMX</i> Cytoductant from YHE1634 → YHE1649	this work
YHE1655	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1] [PIN+] cyh2-Q38K</i> Cytoductant from YHE1627 → YHE1631	this work
YHE1656	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1] [PIN+] cyh2-Q38K rub1::kanMX</i> Cytoductant from YHE1627 → YHE1646	this work
YHE1657	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1] [PIN+] cyh2-Q38K ula1::kanMX</i> Cytoductant from YHE1627 → YHE1647	this work
YHE1658	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1] [PIN+] cyh2-Q38K uba3::kanMX</i> Cytoductant from YHE1627 → YHE1648	this work
YHE1659	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1] [PIN+] cyh2-Q38K ubc12::kanMX</i> Cytoductant from YHE1627 → YHE1649	this work
YHE1674	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1] [PIN+] ylr352w::kanMX</i> YHE1627 x YHE1633 spore 19B	this work
YHE1689	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [URE3-1] [PIN+] YHE1627 x YHE1631 spore 13C</i>	this work
YHE1697	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 ure2::URA3^{alb} [PIN+] YHE1630 x YHE1636 spore 4B</i>	this work
YHE1705	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] ylr352w::kanMX</i> YHE1630 x YHE1636 spore 7C	this work
YHE1713	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [ure-o] [PIN+] YHE1628 x YHE1634 spore 1B</i>	this work
YHE1714	<i>MATa ura3 leu2 trp1 his3::TRP1^{alb} kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 [PIN+] cyh^R rho⁰</i> YHE1628 x YHE1634 spore 1C made rho ⁰	this work
YHE1716	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 ure2::URA3^{alb} [PIN+] ylr352w::kanMX</i> YHE1630 x YHE1636 spore 1B	this work
YHE1721	<i>MATa ura3 leu2 trp1 his3::TRP1^{alb} kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 ylr352w::kanMX [PIN+] cyh2-Q38K rho⁰</i> YHE1630 x YHE1636 spore 1A made rho ⁰	this work

YHE1760	<i>MATa ura3 leu2 trp1 kar1 P_{DAL5}ADE2 P_{DAL5}CAN1 ylr352w::kanMX</i> [ure-o] YPG+ when [URE3]	this work
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All strains are isogenic with BY241 (BRACHMANN *et al.* 2005) unless stated otherwise.

- 1 (BRACHMANN *et al.* 2005)
- 2 (WICKNER *et al.* 2014)
- 3 The mating type from YHE1609 was changed from *MATa* to *MATα* using pJH298 as described (EDSKES and WICKNER 2013). The *HIS3* ORF was replaced with the *TRP1* ORF from *C. albicans* (Darlington) through transformation with a *TRP1^{alb}* PCR product containing *HIS3* 5' and 3' UTR sequences.
- 4 A cycloheximide-resistant *CYH2* allele (Q38K) was amplified by PCR from strain L2598 (TANEJA *et al.* 2007), and transformed into YHE1608 as described by Edskes et al (EDSKES *et al.* 2014).
- 5 The strain was made ρ^0 by growth on YPAD containing 30 μ g/ml ethidium bromide.
- 6 Knockout mutants were produced by PCR-amplifying the knocked-out gene from the collection strain (WINZELER *et al.* 1999), transformation of log phase cells and selecting for resistance to G418, and confirmation by PCR that the normal gene was missing and the putative mutant had been formed.
- 7 [URE3] was spontaneously lost from YHE1627.
- 8 [URE3] was removed from YHE1627 by growth on YPAD + 5mM guanidine hydrochloride to inhibit Hsp104 activity.
- 9 The *URA3* ORF was amplified from *C. albicans* (Stellatoidae) and targeted to *URE2* by incorporation of *URE2* 5' and 3' UTR sequences in the PCR primers.

Hermes transposition protocol

Cells containing pSG36 were grown as described by Gangadharan et al. (GANGADHARAN *et al.* 2010). *Hermes* integrations were scored as cells resistant to 5-FOA (5-fluoroorotic acid, 1 g/l) and NAT (nourseothricin, 0.1 g/l) resistant cells. For each culture we determined the amount of viable cells, the amount of 5FOA resistant cells (cells that have lost pSG36) and the amount of 5FOA+NAT resistant cells (cells that have lost pSG36 and have integrated the NAT cassette in their genome).

1. A starter culture was grown in glucose media lacking uracil. *Hermes* integrations were below 1 in 10^7 cells (our detection limit).
2. 50 ml of galactose medium (to induce the galactose promoter that drives expression of the *Hermes* transposase) lacking uracil was inoculated with the starter culture to an OD600 of 0.05 and grown to saturation. This culture had a detectable but low *Hermes* integration level (cells resistant to FOA and NAT over total cells).
3. Cells were transferred at an OD600 of 0.05 into 50 ml galactose medium lacking uracil. Upon reaching saturation this culture contained around 2 million cells with a *Hermes* integration. However, for some strains and growth conditions we had to add one more passage. Prolonged passage reduced the frequency of *Hermes* integrations in our hands.

Plasmid pSG36 is absent from around 10% of the cells during selective growth in minimal medium (with glucose or galactose). Growing in galactose without uracil is necessary to prevent excessive plasmid loss before transposition. Growing without uracil also prevents over-representation of early transpositions in the total population, because after the transposase excises the NAT cassette from pSG36 the remainder of the plasmid (and *URA3*) is usually lost from the cell. The unusual cases of plasmid religation forming a new replicon, or integration into the genome, cannot be readily distinguished from the starting strain. Only cells that have lost the plasmid (as determined by the *URA3* marker) and have remained NAT resistant are counted as having a *Hermes* integration.

4. After growth in galactose medium cells were inoculated into 500 ml medium containing glucose, uracil and 5FOA to an OD600 of 0.25. In our situation we needed all, or almost all, of our 50 ml galactose culture to obtain this cell density. Cells were grown to saturation which normally took two days.
5. Finally 500 ml of glucose medium containing uracil, 5FOA and NAT was inoculated with the culture from step four to an OD600 of 0.5. Cells were grown to saturation (which normally took 2 days) and harvested. This gives a much larger cell pellet than you need for the remainder of the protocol. Because we needed all the integrations from the induction culture we could not scale the volumes back.

For each integration experiment, five independent cultures were grown and each culture was independently processed. For ligation of linkers and PCR, reagents were tested and steps were optimized using pSG36 DNA.

- **Prepare genomic DNA**

Genomic DNA was prepared using either the YeaStar Genomic DNA Kit from Zymo Research or the MasterPure Yeast DNA Purification Kit from Epicentre.

- **Digest genomic DNA**

Genomic DNA (3 microgram) was digested with either MseI (TTAA) or CviQI (GTAC) in a total volume of 200 μ l. Both enzymes leave a 5' TA overhang. The enzymes were removed after digestion using the Qiagen MinElute Reaction Cleanup Kit.

- **Prepare linkers**

Linkers (see below) HE968 (20 picomol per μ l) and HE969 (20 picomol per μ l) were annealed in a thermocycler:

95 °C for 2 minutes
 95 °C to 75 °C in 13 minutes
 75 °C to 55 °C in 13 minutes
 55 °C to 35 °C in 13 minutes
 35 °C to 30 °C in 3 minutes and 30 seconds
 20 °C for 5 minutes
 Hold at 4 °C

- **Ligate linkers to genomic DNA**

Restriction endonucleases that recognize four nucleotides cut, on average, every 250 bp. 750 ng genomic DNA digested with this type of enzyme would generate 4.5 picomole of ends. Our CviQI digested DNA migrated as a smear on agarose gels with most fragments between 400 and 1000 bp. MseI digested DNA migrated between 100 and 1500 bp with a peak of fragments between 300 and 600 bp. So in 750 ng digested genomic DNA there are less than 10 picomole of ends (~1.6 pmoles and 2.5 pmoles, respectively). Annealed linkers (100 picomoles each) were ligated to 750 ng digested genomic DNA in a total volume of 20 μ l using 1 μ l of NEB ligase (400 u / μ l). The reaction was placed at 16 °C (there is only a 2 nucleotide overhang) for 3 hours. Following ligation, ligase and some of the linkers were removed using two columns from a Qiagen MinElute Reaction Cleanup Kit. Elution was done with 20 μ l of water. When analyzed on an agarose gel the CviQI and MseI digests looked similar with both showing a smear between 250 bp and 1000 bp.

- **Amplify *Hermes* insertion sequences**

For primers see below. Q5 (NEB) and Titanium Taq (Clontech) were used with and without the buffers supplied to enhance amplification of GC rich regions. As input we added between 1 and 8 μ l of genomic DNA (obtained in 40 μ l after purification of the linker ligation mixture in the previous step). Running the entire 50 μ l PCR reaction on an agarose gel we needed 20-22 cycles to actually see a faint DNA smear. The PCR reactions from the five cultures for each of the enzyme / PCR conditions were combined. Each combined pool was concentrated to 20 μ l using Qiagen MinElute Reaction Cleanup kit (two columns per pool). Titanium Taq samples were cleaned by phenol/chloroform extraction before loading onto the MinElute columns.

Titanium Taq

- 1: 94 °C / 3 minutes
- 2: 94 °C / 20 seconds
- 3: 55 °C / 20 seconds
- 4: 68 °C / 30 seconds
- 5: go to 2 for x cycles
- 6: 68 °C / 5 minutes

Q5

- 1: 98 °C / 30 seconds
- 2: 98 °C / 10 seconds
- 3: 55 °C / 20 seconds
- 4: 72 °C / 10 seconds
- 5: go to 2 for x cycles
- 6: 72 °C / 2 minutes

- **Purify *Hermes* insertion sequences**

To remove primers, small products and higher molecular weight products the PCR reactions were fractionated on a 2% agarose gel (1% SeaKem + 1% NuSieve 3:1) and 100-500 bp fragments were excised and purified using a Qiagen MinElute gel isolation kit. At this stage, DNA from the 5 cultures x two enzyme digestions x 4 PCR conditions (40 reactions) were combined. The Quant iT PicoGreen dsDNA assay kit (Molecular Probes) was used to quantitate the obtained DNA.

Fig. S1. PCR amplification and Illumina sequencing scheme.

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HE1033                                     GCTAA
Hermes RE  AAATACTTGCACTCAAAAGGCTTGACACCCAAAACACTTGTGCTTATCTATGTGGCTTA
HE970      -----AATGATACGGCGACCACCGAGATCTACACCTATGTGGCTTA
                                     *****

HE1033      CGTTTGCCTGTGGCTTGTGAAGTTCTCTG
Hermes      CGTTTGCCTGTGGCTTGTGAAGTTCTCTG
HE970      CGTTTGCCTG-----
          *****

HE967      CAAGCAGAAGACGGCATAACGAGATGCCTAAGTAATACGACTCACTATAGGGC-----
HE969      -----GTAATACGACTCACTATAGGGCTCCGCTTAAGGGAC
          *****

HE969      GTAATACGACTCACTATAGGGCTCCGCTTAAGGGAC
          |||||
HE968      [AmC7~Q]-AGGCGAATTCCTGAT-[phos]

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Oligo HE968 is blocked at 3' end and phosphorylated at 5' end.

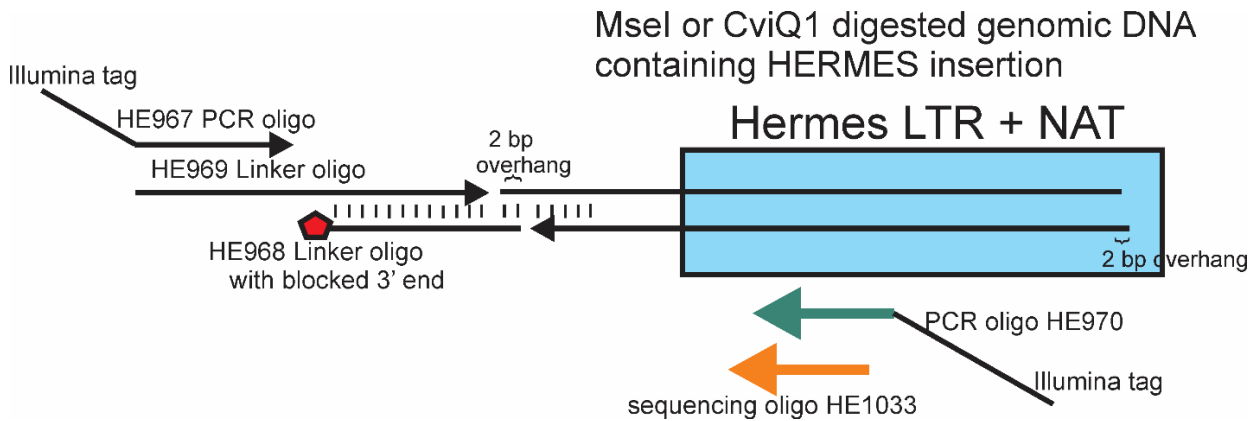


Table S2. Growth parameters of transposition cultures.**A. YHE1609 [URE3-1] -Ade**

	media	# cells plated based on OD600	Colonies counted	OD600 culture	generations
Starter culture	YES+W	10^2	89		
(glucose)	5-FOA	10^3	30		
	5-FOA+NAT	10^7	0		
Induction 1	YES+W	10^2	121		
(Gal/Raf)	5-FOA	10^3	57	Start 0.05	
	5-FOA+NAT	10^6	28	End 3.3	6
Induction 2	YES+W	10^2	107		
(Gal/Raf)	5-FOA	10^3	88	Start 0.05	
	5-FOA+NAT	10^5	197	End 3.5	6
Induction 3	YES+W	10^2			
(Gal/Raf)	5-FOA	10^3		Start	
	5-FOA+NAT	10^4		End	
5-FOA	YES+W	10^2	20		
	5-FOA	10^2	17	Start 0.312	
	5-FOA+NAT	10^4	33	End 2.28	4*
5-FOA+NAT	YES+W	10^2	54		
	5-FOA	10^2	56	Start 0.92	
	5-FOA+NAT	10^2	50	End 2.60	6*

* based on the number of live cells.

Generations : $\ln(N/N_0)/\ln(2)$. Together the five independent cultures had 17×10^6 FOA^r NAT^r cells.

B. YHE1608 [ure-o] +Ade

	media	# cells plated based on OD600	Colonies counted	OD600 culture	generations
Starter culture	YES+W	10^2	103		
(glucose)	5-FOA	10^3	31		
	5-FOA+NAT	10^7	0		
Induction 1	YES+W	10^2	136		
(Gal/Raf)	5-FOA	10^3	15	Start 0.05	
	5-FOA+NAT	10^5	30	End 5.9	7
Induction 2	YES+W	10^2	108		
(Gal/Raf)	5-FOA	10^3	30	Start 0.05	
	5-FOA+NAT	10^4	123	End 5.2	6.7
Induction 3	YES+W	10^2	113		
(Gal/Raf)	5-FOA	10^3	157	Start 0.05	
	5-FOA+NAT	10^4	90	End 5.0	6.7
5-FOA	YES+W	10^2	29		
	5-FOA	10^2	28	Start 0.453	
	5-FOA+NAT	10^4	184	End 2.47	3.5*
5-FOA+NAT	YES+W	10^2	77		
	5-FOA	10^2	69	Start 0.46	
	5-FOA+NAT	10^2	63	End 3.05	6*

* based on the number of live cells.

Generations : $\ln(N/N_0)/\ln(2)$. Five cultures were grown at the same time = 113×10^6 FOA^r NAT^r clones total (assuming similar number of transpositions).

C. YHE1609 [URE3-1] +Ade (1st culture)

	Media	# cells plated based on OD600	Colonies counted	OD600 culture	generations
Starter culture	YES+W	10 ²	77		
(glucose)	5-FOA	10 ³	40		
	5-FOA+NAT	10 ⁷	0		
Induction 1	YES+W	10 ²	114		
(Gal/Raf)	5-FOA	10 ³	13	Start 0.05	
	5-FOA+NAT	10 ⁶	7	End 4.5	6.5
Induction 2	YES+W	10 ²	101		
(Gal/Raf)	5-FOA	10 ³	15	Start 0.05	
	5-FOA+NAT	10 ⁵	27	End 3.92	6.4
5-FOA	YES+W	10 ²	33 (5% sectored)		
	5-FOA	10 ²	32	Start 0.301	
	5-FOA+NAT	10 ⁴	83	End 2.15	7.2*
5-FOA+NAT	YES+W	10 ²	13		
		10 ³	232 (27% loss)		
	5-FOA	10 ²	18	Start 0.480	
	5-FOA+NAT	10 ²	24	End 3.71	6.4*

Loss refers to cells no longer [URE3]

* based on the number of live cells. "27% loss" means 27% of colonies were [ure-o].

Generations : $\ln(N/N_0)/\ln(2)$. Together the five cultures had 2.5×10^6 FOA^r NAT^r cells.

D. YHE1609 [URE3-1] +Ade (2nd culture)

	media	# cells plated based on OD600	Colonies counted	OD600 culture	generations
Starter culture	YES+W	10 ²	99		
(glucose)	5-FOA	10 ³	40		
	5-FOA+NAT	10 ⁷	3		
Induction 1	YES+W	10 ²	Lost		
(Gal/Raf)	5-FOA	10 ³	Lost	Start	
	5-FOA+NAT	10 ⁵	lost	End	
Induction 2	YES+W	10 ²	103		
(Gal/Raf)	5-FOA	10 ³	32	Start 0.05	
	5-FOA+NAT	10 ⁵	112	End 3.30	6.1
Induction 3	YES+W	10 ²	94		
(Gal/Raf)	5-FOA	10 ³	56	Start 0.05	
	5-FOA+NAT	10 ⁵	553	End 3.8	6.2
5-FOA	YES+W	10 ²	31 (5% loss)		
	5-FOA	10 ²	37	Start 0.335	
	5-FOA+NAT	10 ³	22	End 2.27	5.4*
5-FOA+NAT	YES+W	10 ²	84 (37% loss)		
	5-FOA	10 ²	80	Start 0.458	
	5-FOA+NAT	10 ²	86	End 3.78	7*

Loss refers to cells no longer [URE3]

* based on the number of live cells. "37% loss" means 37% of colonies were [ure-o].

Generations : $\ln(N/N_0)/\ln(2)$. Together, the five cultures had 9.5×10^7 FOA⁺ NAT⁺ cells.

Sequencing and sequence analysis.

PCR products were sequenced by the NIDDK genomics core facility using an Illumina platform.

Sequence reads were aligned to the *Saccharomyces cerevisiae* genome using HISAT2 (KIM *et al.* 2015)

<https://ccb.jhu.edu/software/hisat2/index.shtml>. Sequencing data supplied as fastq files was subjected to quality control by FASTQC, and aligned to the yeast genome using HISAT2 (KIM *et al.* 2015) producing a .bam file. The .bam file is converted to a .sam file using, e.g., BAM-to-SAM, and the latter is input for visualization of the distribution of inserts using IGV (ROBINSON *et al.* 2011) or a program counting insertions per gene.

The program counting insertions per gene reads the chromosome number, nucleotide location and transposon orientation. This information, along with a file listing the locations of *S. cerevisiae* open reading frames (sac_cer3_genes.bed), is used to place each insertion within a particular ORF or between ORFs. The Python program used for counting insertions is in a separate file, "Count Insertions in ORFs and Introns.txt".

Table S3. Summary of sequence reads

Each sample was sequenced twice and the data from the two reactions were merged and the merged data was used for further analysis.

	YHE1609	YHE1608	YHE1609	YHE1609
	[URE3-1]	[ure-o]	[URE3-1]	[URE3-1]
	-Ade	+Ade	+Ade 1st	+Ade 2nd
Total reads aligned to chromosome	177,480,190	135,537,475	142,773,189	97,650,609
Reads in ORFs including introns	57,461,286	45,909,571	55,692,869	37,670,600
Reads in introns	419,085	478,369	424,422	402,152
Reads in ORFs not including introns	57,042,201	45,431,202	55,268,447	37,268,448
Total unique insertions	303,010	710,420	627,085	406,801
Unique insertions in ORFs including introns	124,912	325,205	288,760	177,670
Unique insertions in introns	1,150	3,082	2,694	1,833
Unique insertions in ORFs lacking introns	123,762	322,123	286,066	175,837

For insertions into the ORFs see Excel file “Exon Intron Counts.xlsx”, which includes a brief description of each gene. IGV screen shots of genes having more insertions in the [ure-o]+Ade culture than in the [URE3]-Ade culture are shown in “LUGsIGV-InsertDistributions.pptx”. Insertions in introns excludes tRNA genes with introns.

Table S3. Genes rarely mutated by *Hermes* if carrying [URE3] and reported to produce negative genetic interactions with *ure2Δ* [summarized at <https://www.yeastgenome.org/locus/S000005173/interaction#annotations>].

Systematic name	Functional name	Description
<i>YAR015W</i>	<i>ADE1</i>	adenine biosynthetic enzyme
<i>YMR120C</i>	<i>ADE17</i>	adenine biosynthetic enzyme
<i>YBL008W</i>	<i>HIR1</i>	nucleosome assembly component
<i>YOR038C</i>	<i>HIR2</i>	nucleosome assembly component
<i>YOR123C</i>	<i>LEO1</i>	Paf1 complex; histone methylation; RNAP assoc.
<i>YNL097C</i>	<i>PHO23</i>	Rpd3L histone deacetylase complex; PHO5, snoRNA
<i>YCR033W</i>	<i>SNT1</i>	Subunit of the Set3C histone deacetylase complex
<i>YDR392W</i>	<i>SPT3</i>	subunit of SAGA: histone acetylase
<i>YPL139C</i>	<i>UME1</i>	In both Rpd3S and Rpd3L histone deacetylase complexes
<i>YPL202C</i>	<i>AFT2</i>	Iron-regulated transcriptional activator
<i>YKR099W</i>	<i>BAS1</i>	transcription factor; adenine & his biosynt
<i>YNL027W</i>	<i>CRZ1</i>	Transcription factor for stress response genes
<i>YGL166W</i>	<i>CUP2</i>	Copper-binding transcription factor for CUP1
<i>YLR451W</i>	<i>LEU3</i>	transcription factor; branched chain aa biosynth
<i>YMR070W</i>	<i>MOT3</i>	transcription factor; anoxia, hyperosm, erg
<i>YDL020C</i>	<i>RPN4</i>	Transcription factor -> incr expr of proteasome genes
<i>YBL066C</i>	<i>SEF1</i>	Putative transcription factor
<i>YDR463W</i>	<i>STP1</i>	transcription factor,
<i>YML076C</i>	<i>WAR1</i>	transcription factor, resistance to weak acid
<i>YLR119W</i>	<i>SRN2</i>	IN ESCRT-I complex; ubiquitin-dep sorting into endosome;
<i>YMR275C</i>	<i>BUL1</i>	Ubi-BP of the Rsp5p E3-ubiquitin ligase complex
<i>YGL066C</i>	<i>SGF73</i>	anchors deubiquitination module in SAGA & SLIK complexes
<i>YDL190C</i>	<i>UFD2</i>	Ubi chain assembly factor (E4) works w/ E1,E2, E3s
<i>YNL064C</i>	<i>YDJ1</i>	Hsp40, helps Rsp5p E3-ubiquitin ligase complex
<i>YLR392C</i>	<i>ART10</i>	fcn unknown; ubiquitinated by Rsp5p
<i>YFR021W</i>	<i>ATG18</i>	Phosphoinositide BP; vesicle formation in autophagy
<i>YPL069C</i>	<i>BTS1</i>	Geranylgeranyl diphosphate synthase
<i>YFL001W</i>	<i>DEG1</i>	tRNA:pseudouridine synthase
<i>YBI051C</i>	<i>PIN4</i>	G2/M phase progression, response to DNA damage
<i>YER139C</i>	<i>RTR1</i>	CTD phosphatase; dephosphorylates S5-P of Rpo21p CTD
<i>YMR216C</i>	<i>SKY1</i>	SR protein kinase. regs RNA metab, cation metab
<i>YPL042C</i>	<i>SSN3</i>	Cycin-dep PK, RNAPol II CTD kinase, glucose repression
<i>YPR074C</i>	<i>TKL1</i>	transketolase, pentose PO4 pathway
<i>YPL158C</i>	<i>AIM44</i>	regulator of Cdc42p & Rho1p: cell separation
<i>YLR386W</i>	<i>VAC14</i>	regulates synthesis of phosphatidylinositol 3,5-bisphosphate
<i>YLR352W</i>	<i>LUG1</i>	'lets [URE3]/ure2 grow' on glycerol [this work]; F-box protein, ubiquitination

Fig. S2. *Hermes* insertions in Ty sequences. Each bar represents a different Ty at a different genome location. The data is in Supplemental file “TY gag-pol Counts.xlsx”. Ty2s are marked with an “X” above the data bar. The frequency observed in different cultures is indicated by the colored bar segment according to the key. Ty2s were targeted far more often than other Tys (A, C), but the ratios of insertions remained relatively constant in the different cultures (B, D). The gene names beneath each panel are the systematic names for the Ty element ORF, in alphabetical order. Only half of the names are shown.

Fig S2A

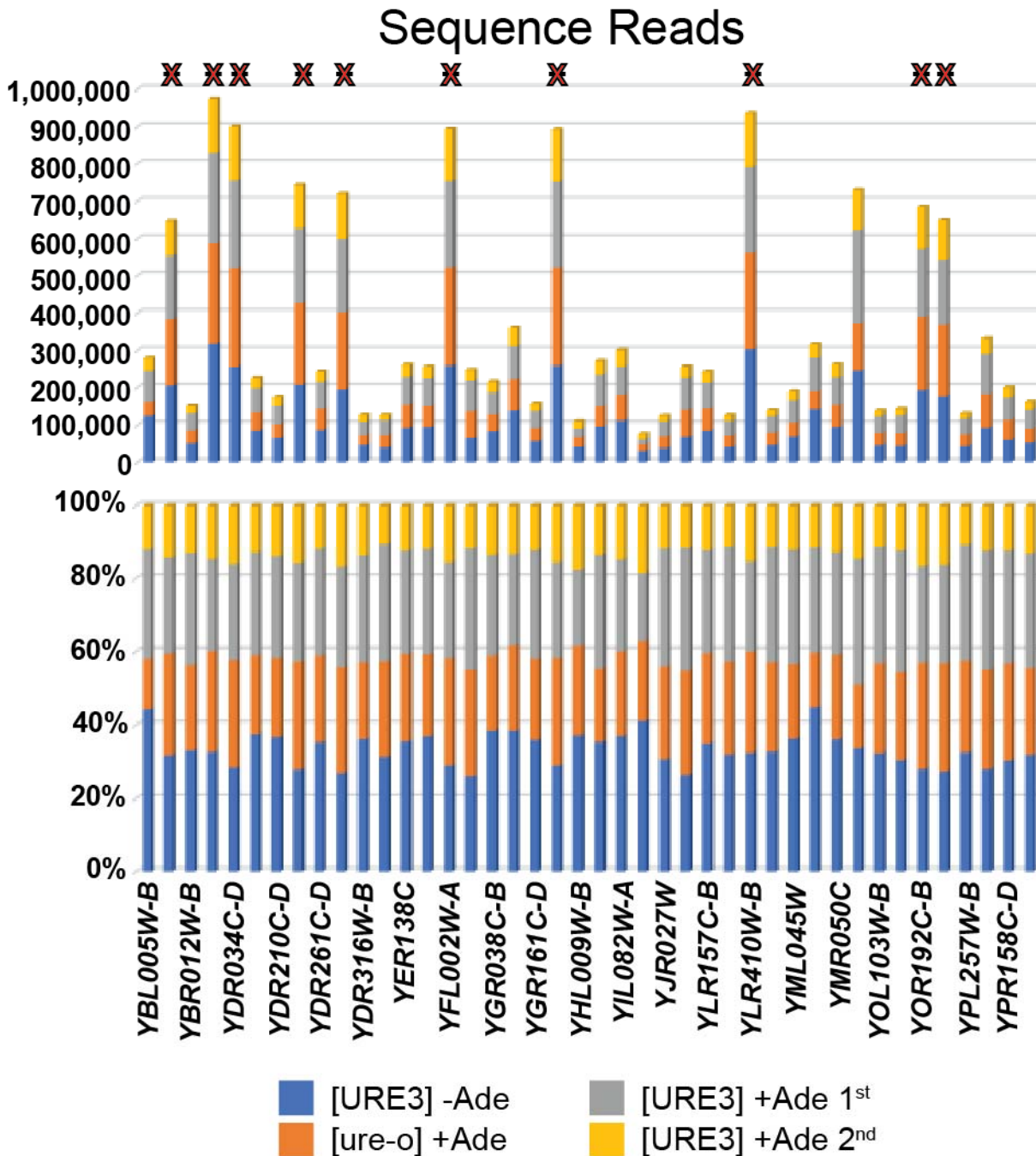
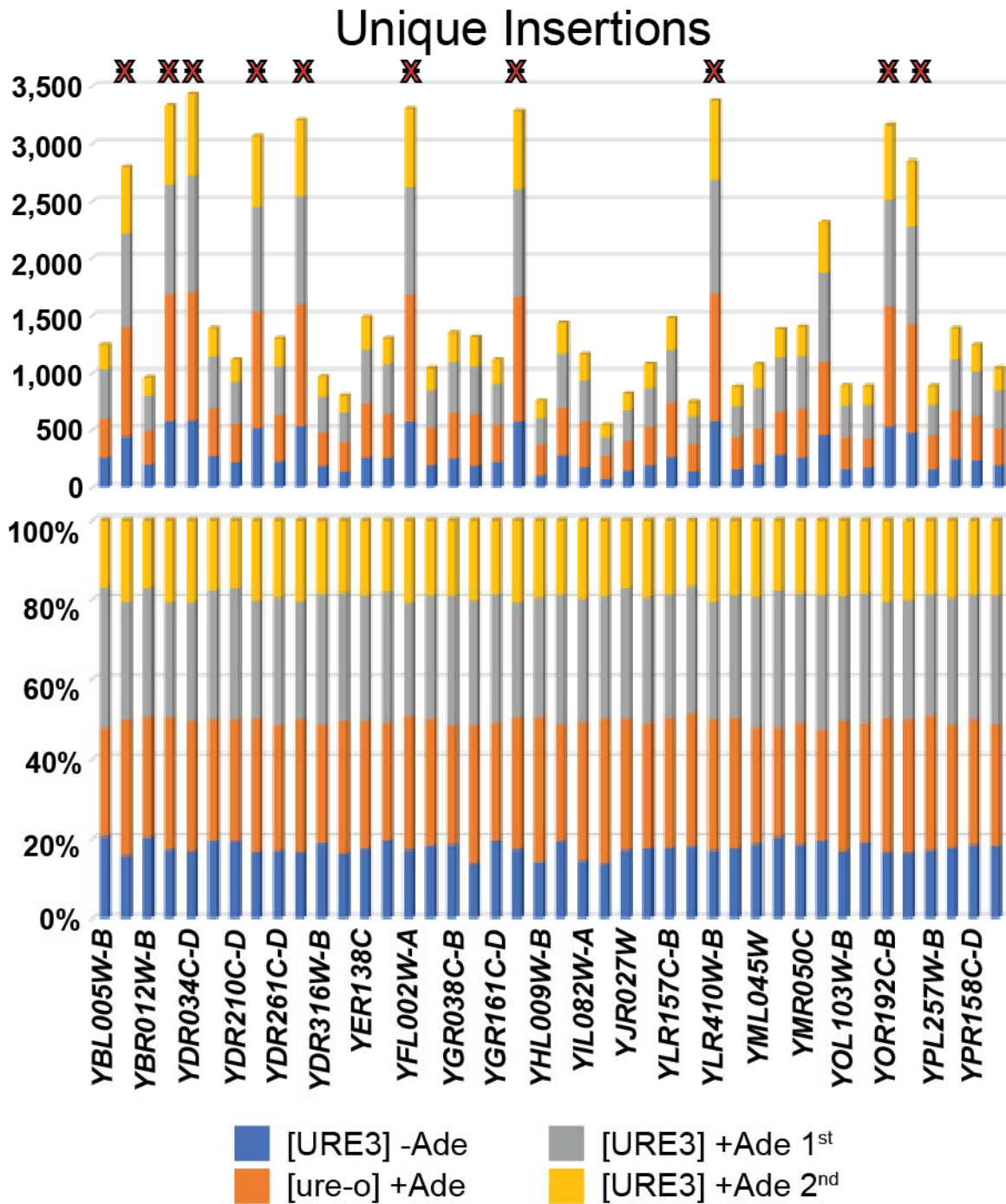


Fig S2B

A. Total sequence reads in Ty elements at 42 different locations in the genome. The vertical scale is total sequence reads.

B. Fraction of total sequence reads at each location in the different cultures.

Fig S2C



C. Total unique insertions in Ty sequences at each of 42 locations in the genome.

D. Fraction of unique inserts at each location in the different cultures.

Fig S3A

Hermes amplified insertion profile of chromosome I

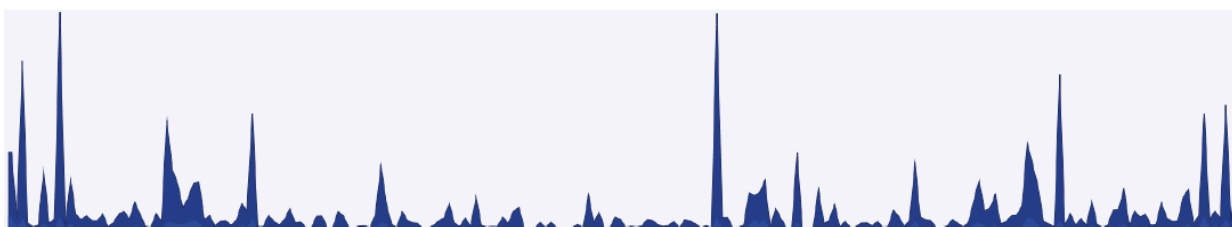
YHE1609 ([URE3]) grown without adenine



YHE1608 ([ure-o]) grown with adenine



YHE1609 ([URE3]) grown with adenine (1st culture)



YHE1609 ([URE3]) grown with adenine (2nd culture)

Hermes unique insertion profile of chromosome I



YHE1609 ([URE3]) grown without adenine



YHE1608 ([ure-o]) grown with adenine



YHE1609 ([URE3]) grown with adenine (1st culture)



YHE1609 ([URE3]) grown with adenine (2nd culture)

Fig. S3. *Hermes* insertion profile for chromosome I. A. Total sequence reads after growth and PCR amplification. **B.** Unique insertion profile. The vertical axis is density of insertions and the horizontal axis shows position along the chromosome. The figure was prepared using the CLC Genomics Workbench (Qiagen).

Fig S4

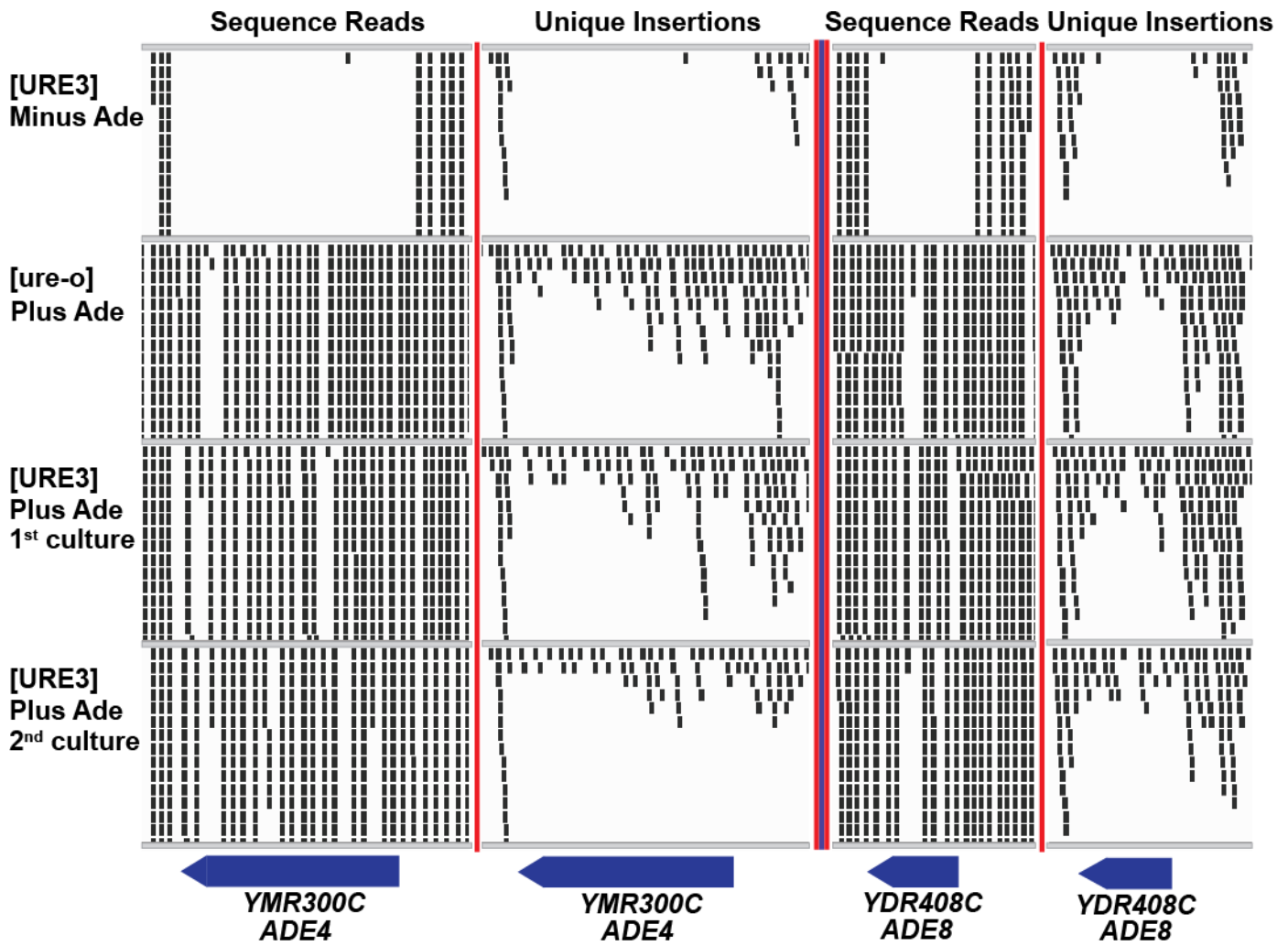
Fig. S4. Distribution of *Hermes* insertions in *ADE4* and *ADE8*.

Fig S5

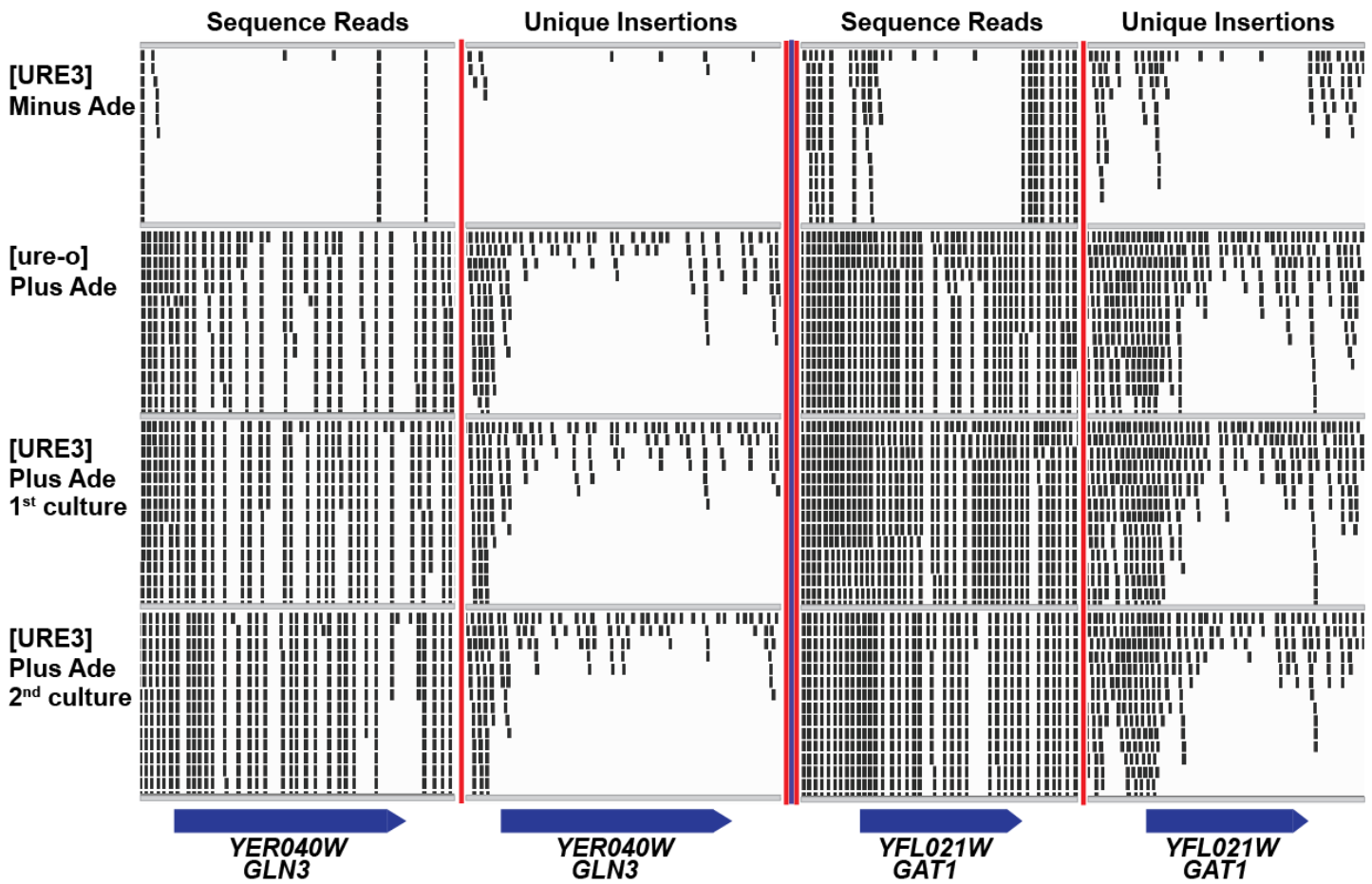


Fig. S5. Distribution of *Hermes* insertions in *GLN3* and *GAT1*, genes necessary for expression of genes (like *DAL5*) controlled by nitrogen catabolite repression.

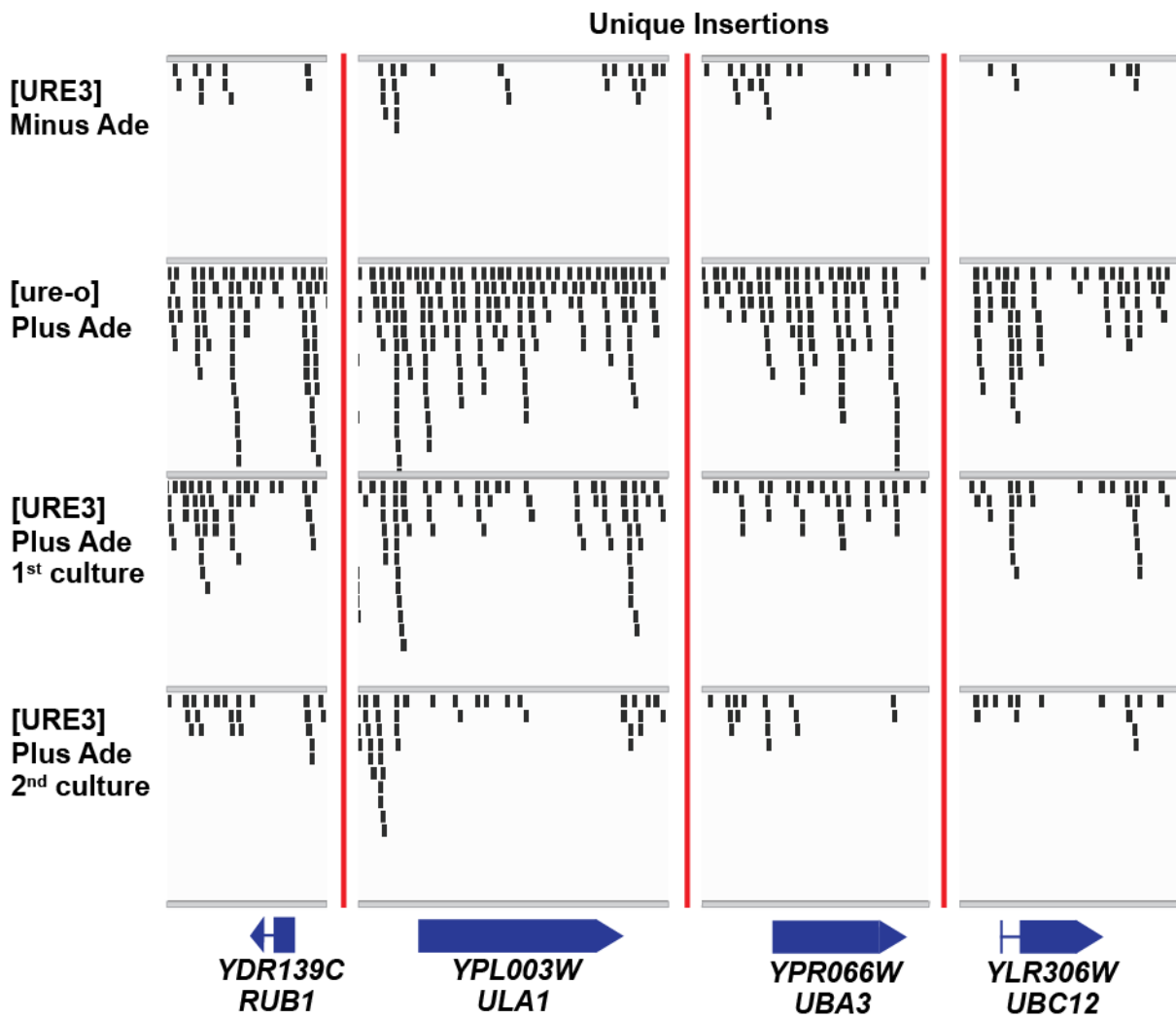


Fig. S6. Unique insertions of *Hermes* in NEDDylation genes.

Fig S7

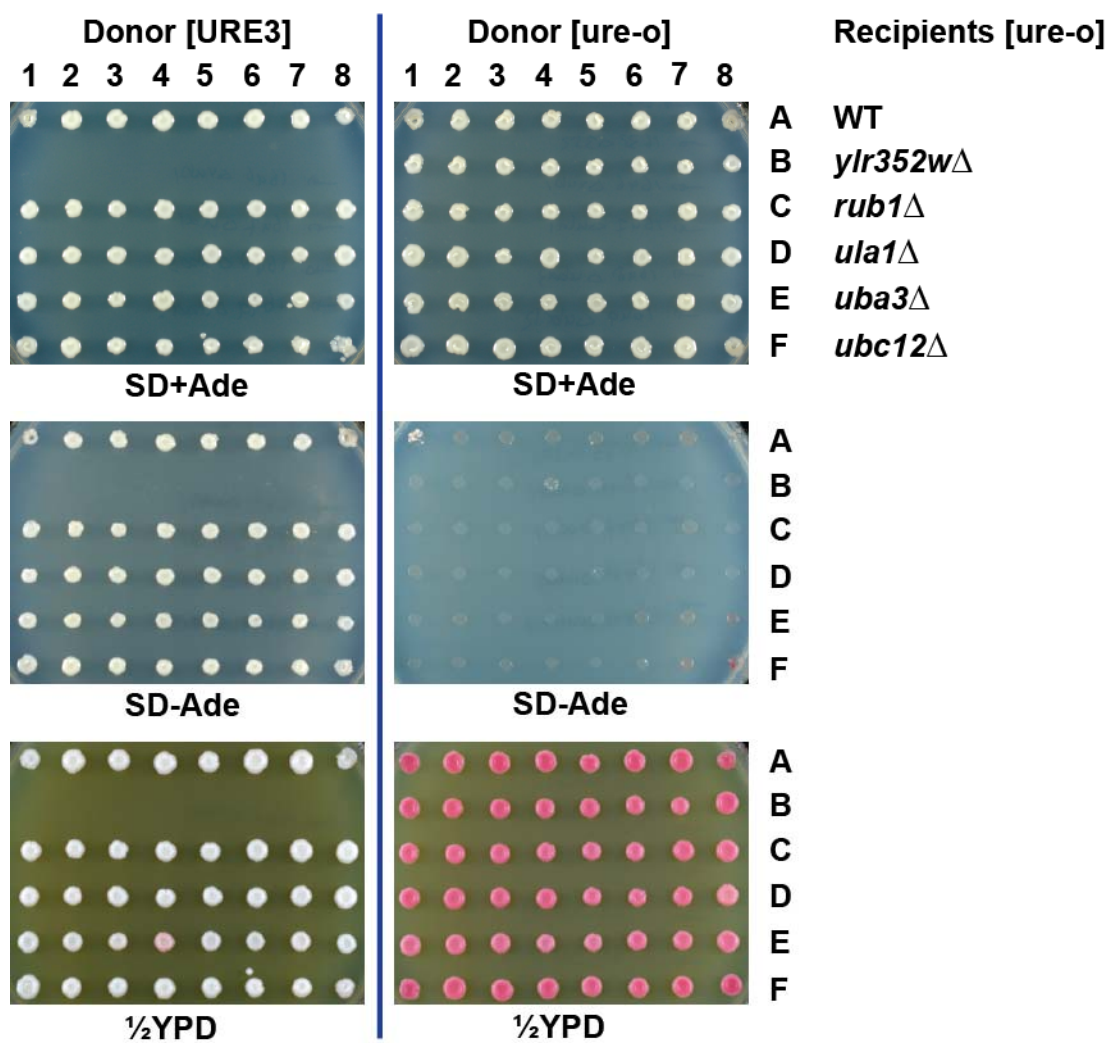
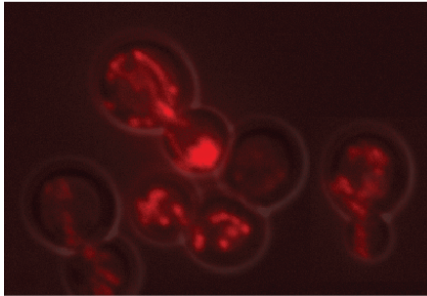


Fig. S7. Acquisition of Ade⁺ phenotype by NEDDylation mutants on cytoduction from a [URE3] donor. Cytoductants into *ylr352w*Δ could not be recovered (see main text). 1 - 8 are individual cytoductants.

Fig S8



YHE1713 WT

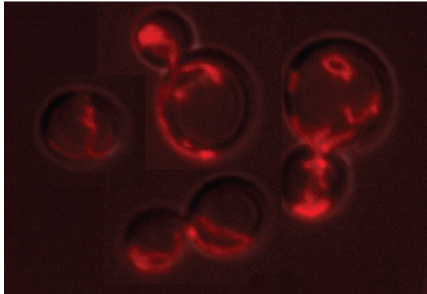
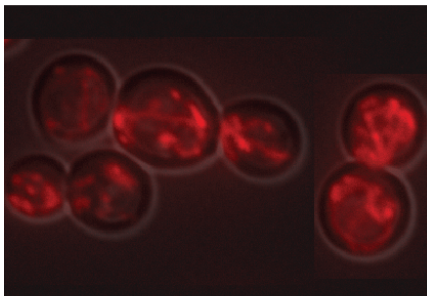
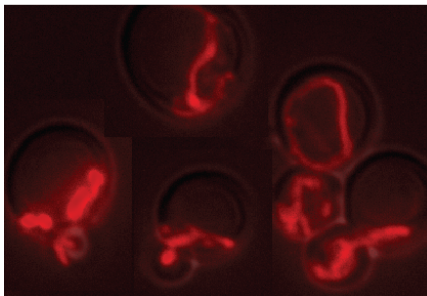
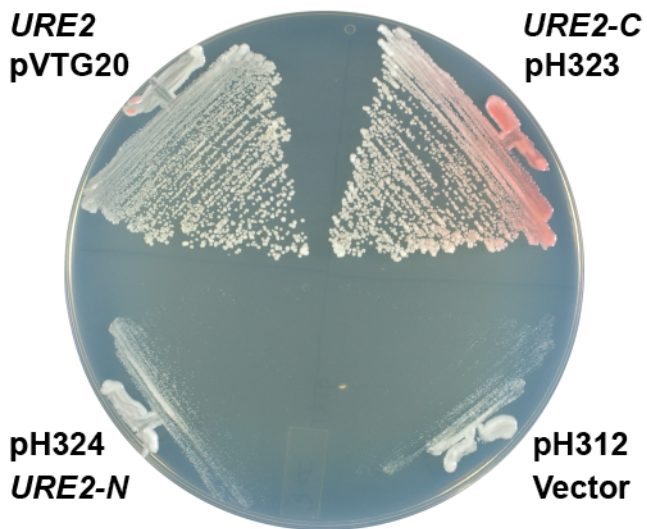
YHE1697 *ure2*ΔYHE1705 *ylr352w*ΔYHE1716 *ure2*Δ *ylr352w*Δ

Fig. S8. MitoDsRed staining of *ylr352w*Δ *ure2*Δ cells. Cells carrying p mitoDsRed expressing mitoDsRed (WESTERMANN and NEUPERT 2000) were grown to log phase in SD liquid medium, washed with 2% glucose and 0.5% ammonium sulfate and directly imaged in the same buffer. The figure shows a composition of red channel and Nomarski images.

Fig S9



YHE1716 (*ure2* Δ *ylr352w* Δ)

Plasmid: *LEU2* CEN *URE2* promoter

SD+TRP+Ade

3 days 25 °C

Fig. S9. Complementation of YPG-neg phenotype of *ylr352w* Δ *ure2* Δ cells by *URE2C*.

Fig S10

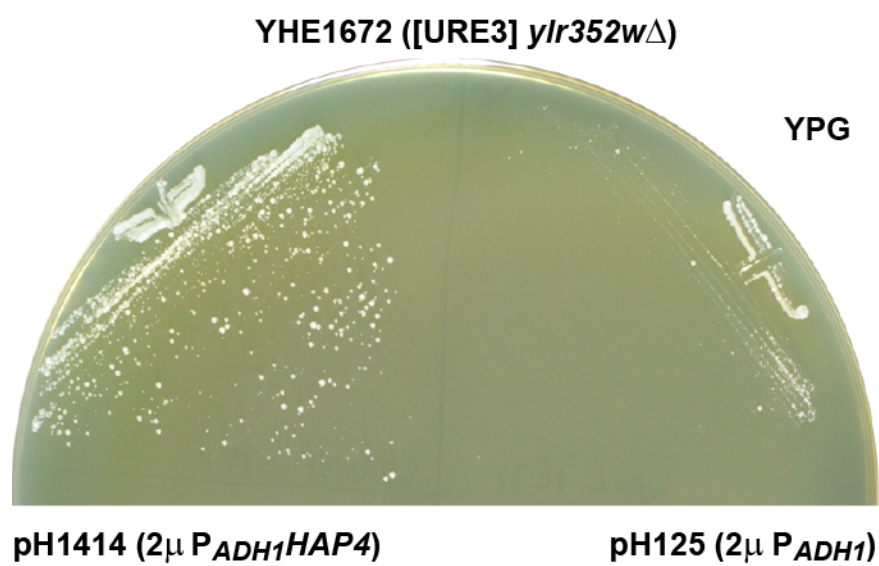


Fig. S10. Overexpression of *HAP4* is sufficient to allow growth of *ylr352w*Δ *ure2*Δ cells on YPG.

Fig S11

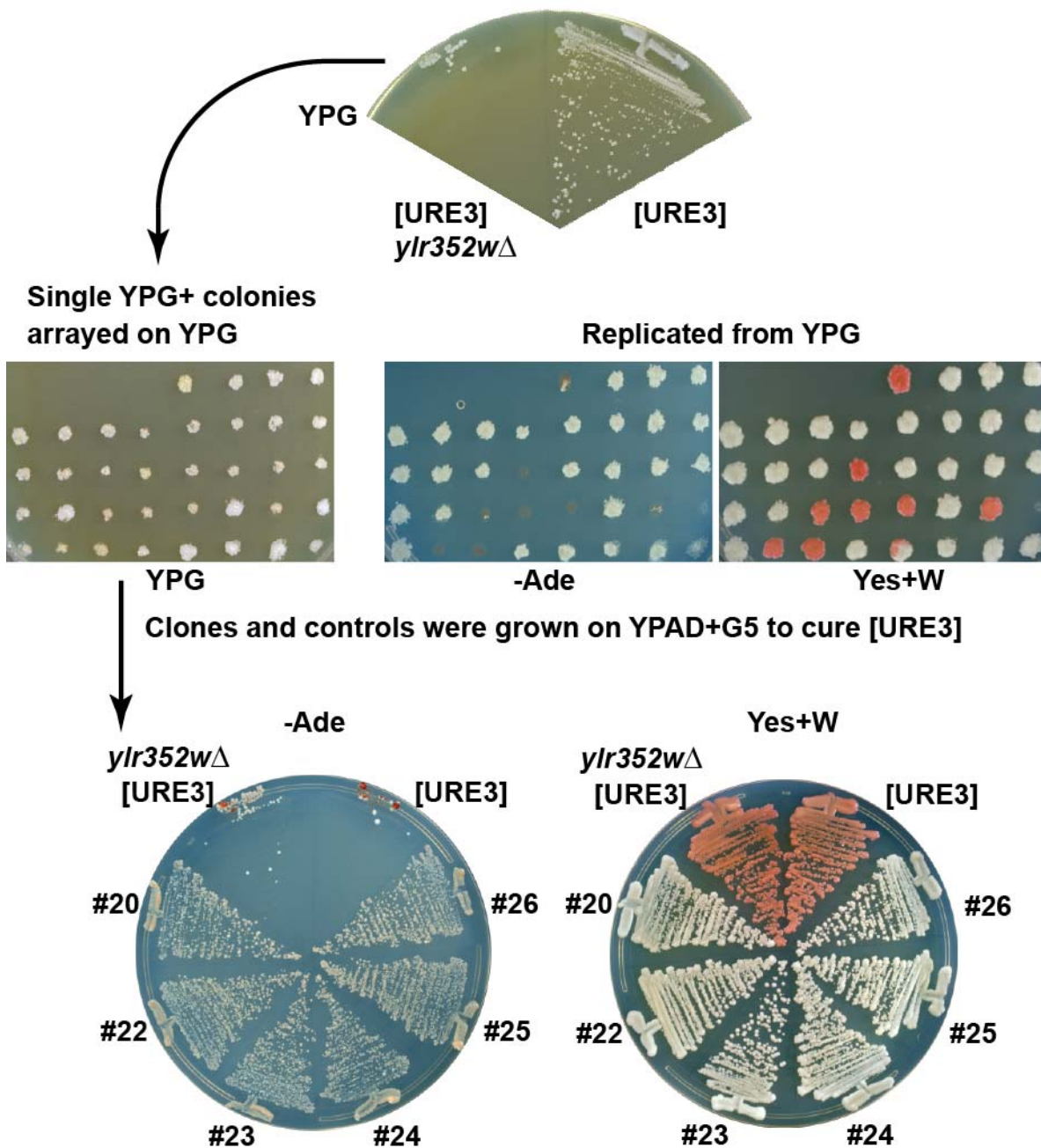


Fig. S11. Isolation and analysis of spontaneous suppressors of the YPG-neg phenotype of *ylr352wΔ ure2Δ* cells. Rare YPG-pos clones derived from *ylr352wΔ [URE3]* cells (strain YHE1674) were selected and processed as shown. Note that control w.t. *[URE3]* (strain YHE1689) and *ylr352wΔ [URE3]* (YHE1674) cells became Ade- by growth on guanidine, but most YPG-pos suppressor-containing clones remained Ade+ after guanidine, although their *[URE3]* had been cured (see text).

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