Supplemental Data

Table S1. Validation of differentially expressed genes identified via microarray analysis by QPCR.

Gene	Fold Change Microarray	Fold Change QPCR	P-value	Expression
*AQR1**	-10.22	-2.19	0.002	Down
*BAG7**	13.68	2.19	< 0.001	Up
*DAK2**	25.27	1.86	< 0.001	Up
HSP26	5.80	0.57	0.102	No change
HXT2	-5.52	1.55	0.008	Up
PHM6	-9.53	1.31	0.676	No change
PHO84	-4.80	1.22	0.261	No change
PRM7	-5.71	-1.31	0.238	No change
*SPL2**	-4.74	-1.52	0.021	Down
*SRX1**	8.58	2.51	0.001	Up
*TMA10**	9.76	2.01	< 0.001	Up
*YDR034W-B**	11.72	3.61	0.001	Up
YGR079W	-5.03	1.00	0.964	No change
*YLR194C**	15.02	3.07	< 0.001	Up
YOR338W	-6.02	1.54	0.055	No change
YPK2	4.28	1.27	0.223	No change
*YPR077C**	4.17	8.73	< 0.001	Up

The majority of DEGs identified by microarray analysis were validated by QPCR (9/17; 53%, indicated by astericks). An additional 3 genes were found to be differentially expressed via QPCR analysis in the same direction as observed by mRNA profiling, but failed to reach statistical significance.

Table S2. Gene ontology groups significantly up-regulated in Htt103Q versus Htt25Q expressing yeast cells.

Gene Ontology Group	Count	%	P-value	Enrichment
response to unfolded protein	11	5.0	6.2E-06	6.3
response to stimulus	42	18.9	1.9E-04	1.7
response to stress	31	14.0	2.1E-04	2.0
response to chemical stimulus	27	12.2	2.9E-04	2.1
protein folding	13	5.9	7.8E-04	3.1
ubiquitin cycle	12	5.4	1.7E-03	3.1
cell wall organization and biogenesis	15	6.8	2.1E-03	2.5
protein metabolic process	58	26.1	2.9E-03	1.4
protein ubiquitination	8	3.6	3.0E-03	4.1
cellular macromolecule metabolic process	58	26.1	3.2E-03	1.4
cellular protein metabolic process	55	24.8	6.2E-03	1.4
post-translational protein modification	24	10.8	6.5E-03	1.8
chitin- and beta-glucan-cell wall biogenesis	6	2.7	8.6E-03	4.6
protein modification by small protein conjugation	8	3.6	1.1E-02	3.2
cellular carbohydrate metabolic process	14	6.3	2.2E-02	2.0
catabolic process	23	10.4	2.4E-02	1.6
protein refolding	3	1.4	2.9E-02	10.9
cotranslational protein targeting to membrane	3	1.4	3.5E-02	9.9
hexose metabolic process	8	3.6	3.7E-02	2.5
glucose metabolic process	7	3.2	3.7E-02	2.8
carbohydrate metabolic process	15	6.8	3.9E-02	1.8
signal transduction	14	6.3	4.0E-02	1.8
vacuolar protein catabolic process	3	1.4	4.1E-02	9.1
monosaccharide metabolic process	8	3.6	4.8E-02	2.4

Gene ontology groups determined by the DAVID Functional Annotation tool. Count refers to number of genes within input gene list that fall into the specified GO group. % denotes what percentage of the total input genes analyzed fall within the specific GO group. P-value represents the threshold of EASE Score, a modified Fisher Exact P-Value, for gene-enrichment analysis. Enrichment category denotes the overall enrichment of the particular GO group over background.

Table S3. Gene ontology groups significantly down-regulated in Htt103Q versus Htt25Q expressing expressing yeast cells.

Gene Ontology Group	Count	%	P-Value	Enrichment
ribosome biogenesis and assembly	74	31.2	7.5E-40	5.9
ribonucleoprotein complex biogenesis	75	31.7	3.6E-35	5.0
rRNA metabolic process	42	17.7	2.2E-21	5.9
rRNA processing	41	17.3	5.1E-21	6.0
RNA processing	54	22.8	6.9E-16	3.3
organelle organization and biogenesis	95	40.1	5.0E-13	1.9
ribosomal large subunit biogenesis and assembly	19	8.0	2.2E-12	8.5
RNA metabolic process	73	30.8	6.4E-08	1.8
ribosome assembly	14	5.9	2.9E-07	6.1
maturation of 5.8S rRNA from tricistronic rRNA	10	4.2	1.0E-06	8.8
maturation of SSU-rRNA from tricistronic rRNA	11	4.6	1.2E-06	7.4
maturation of SSU-rRNA	12	5.1	1.5E-06	6.4
maturation of 5.8S rRNA	10	4.2	1.7E-06	8.3
cellular component organization and biogenesis	109	46.0	8.2E-06	1.4
ribosomal large subunit assembly	9	3.8	7.1E-05	6.2
ribosomal subunit assembly	10	4.2	1.2E-04	5.1
protein-RNA complex assembly	15	6.3	3.7E-04	3.0
cleavages during rRNA processing	6	2.5	5.8E-04	8.3
nucleobase, nucleoside, nucleotide metabolism	85	35.9	8.2E-04	1.3
maturation of LSU-rRNA	5	2.1	1.0E-03	10.4
gene expression	73	30.8	1.1E-03	1.4
endonucleolytic cleavage mature 5'-end of SSU-rRNA	5	2.1	2.8E-03	8.1
establishment of organelle localization	8	3.4	3.4E-03	4.0
endonucleolytic cleavage of tricistronic rRNA	5	2.1	3.5E-03	7.6
RNA modification	9	3.8	3.5E-03	3.5
transcription from RNA polymerase I promoter	6	2.5	6.3E-03	5.0
positive regulation of RNA pol I transcription	3	1.3	6.7E-03	21.8
tRNA processing	10	4.2	6.9E-03	2.9
processing of 27S pre-rRNA	4	1.7	8.8E-03	8.9
biopolymer metabolic process	96	40.5	9.4E-03	1.2
tRNA modification	7	3.0	1.2E-02	3.6
tRNA methylation	4	1.7	1.3E-02	7.7
biopolymer methylation	6	2.5	1.5E-02	4.0
ribosome export from nucleus	5	2.1	1.8E-02	4.8
endonucleolytic cleavage 5'-ETS of tricistronic rRNA	4	1.7	1.9E-02	6.8
RNA methylation	4	1.7	2.2E-02	6.4
tRNA metabolic process	11	4.6	3.0E-02	2.2
organelle localization	8	3.4	3.2E-02	2.6

Gene ontology groups determined by the DAVID Functional Annotation tool. Count refers to number of genes within input gene list that fall into the specified GO group. % denotes what percentage of the total input genes analyzed fall within the specific GO group. P-value represents the threshold of EASE Score, a modified Fisher Exact P-Value, for gene-enrichment analysis. Enrichment category denotes the overall enrichment of the particular GO group over background.

Table S4. Gene ontology groups significantly differentially expressed in *bna4*Δ Htt103Q-expressing yeast versus wild-type Htt103Q expressing cell.

Gene Ontology Group	Count	%	P-Value	Enrichment
carboxylic acid metabolic process	23	11.7	2.6E-05	2.7
translational elongation	8	4.1	3.1E-04	6.0
amino acid catabolic process	6	3.1	3.2E-04	9.6
nitrogen compound metabolic process	18	9.2	3.3E-04	2.6
amino acid and derivative metabolism	16	8.2	3.6E-04	2.9
organic acid transport	8	4.1	4.1E-04	5.7
amine catabolic process	6	3.1	5.9E-04	8.5
amino acid metabolic process	15	7.7	6.0E-04	2.9
amine metabolic process	16	8.2	1.1E-03	2.6
carboxylic acid transport	7	3.6	2.2E-03	5.1
glutamine family amino acid metabolism	6	3.1	6.4E-03	5.0
monocarboxylic acid metabolic process	10	5.1	6.5E-03	2.9
biopolymer biosynthetic process	8	4.1	8.6E-03	3.4
water-soluble vitamin metabolic process	7	3.6	2.2E-02	3.2
water-soluble vitamin biosynthesis	5	2.6	3.1E-02	4.2
alcohol metabolic process	10	5.1	3.2E-02	2.2
urea cycle intermediate metabolism	3	1.5	3.9E-02	9.3
sterol transport	3	1.5	3.9E-02	9.3
arginine metabolic process	3	1.5	3.9E-02	9.3
tyrosine catabolic process	2	1.0	4.2E-02	46.5
L-phenylalanine catabolic process	2	1.0	4.2E-02	46.5
vesicle organization and biogenesis	3	1.5	4.4E-02	8.7
cellular biosynthetic process	27	13.8	4.5E-02	1.4
catabolic process	18	9.2	4.7E-02	1.6

Gene ontology groups determined by the DAVID Functional Annotation tool. Count refers to number of genes within input gene list that fall into the specified GO group. % denotes what percentage of the total input genes analyzed fall within the specific GO group. P-value represents the threshold of EASE Score, a modified Fisher Exact P-Value, for gene-enrichment analysis. Enrichment category denotes the overall enrichment of the particular GO group over background.

Table S5. Gene ontology groups significantly differentially expressed in *mbf1*Δ Htt103Q-expressing yeast versus wild-type Htt103Q expressing cells.

Gene Ontology Group	Count	%	P-Value	Enrichment
amino acid metabolic process	28	14.0	2.7E-12	5.0
nitrogen compound metabolic process	29	14.5	2.4E-10	4.0
lysine metabolic process	8	4.0	3.1E-10	34.7
lysine biosynthetic process via aminoadipic acid	7	3.5	3.4E-09	38.0
carboxylic acid metabolic process	30	15.0	1.2E-08	3.3
aspartate family amino acid biosynthesis	10	5.0	3.0E-07	10.3
aspartate family amino acid metabolic process	11	5.5	6.5E-07	8.1
amine biosynthetic process	16	8.0	1.6E-06	4.5
amine catabolic process	8	4.0	7.1E-06	12.0
urea cycle intermediate metabolic process	6	3.0	1.4E-05	17.4
nonprotein amino acid metabolic process	5	2.5	4.9E-05	21.7
glutamine family amino acid metabolic process	8	4.0	2.5E-04	6.2
ornithine metabolic process	4	2.0	3.8E-04	24.8
cellular biosynthetic process	36	18.0	4.1E-04	1.8
arginine biosynthetic process	4	2.0	1.2E-03	17.4
translational elongation	7	3.5	2.7E-03	4.9
carboxylic acid transport	7	3.5	3.2E-03	4.7
serine family amino acid catabolic process	3	1.5	7.3E-03	21.7
amine transport	6	3.0	8.0E-03	4.7
serine family amino acid metabolic process	5	2.5	1.5E-02	5.2
glutamine family amino acid catabolic process	3	1.5	2.1E-02	13.0
glycine metabolic process	3	1.5	2.1E-02	13.0
amino acid transport	5	2.5	2.2E-02	4.6
biosynthetic process	37	18.5	2.3E-02	1.4
polyamine transport	3	1.5	2.9E-02	10.8
glutamine family amino acid biosynthesis	4	2.0	3.3E-02	5.6
biopolymer biosynthetic process	7	3.5	3.9E-02	2.8
tyrosine catabolic process	2	1.0	4.5E-02	43.4
L-phenylalanine catabolic process	2	1.0	4.5E-02	43.4
arginine catabolic process	2	1.0	4.5E-02	43.4
translation	17	8.5	4.9E-02	1.6

Gene ontology groups determined by the DAVID Functional Annotation tool. Count refers to number of genes within input gene list that fall into the specified GO group. % denotes what percentage of the total input genes analyzed fall within the specific GO group. P-value represents the threshold of EASE Score, a modified Fisher Exact P-Value, for gene-enrichment analysis. Enrichment category denotes the overall enrichment of the particular GO group over background.

Table S6. Gene ontology groups significantly differentially expressed in *ume1*Δ Htt103Q-expressing yeast versus wild-type Htt103Q expressing cells.

Gene Ontology Group	Count	%	P-Value	Enrichment
water-soluble vitamin biosynthetic process	8	4.0	1.2E-03	4.8
NAD biosynthetic process	4	2.0	1.9E-03	14.8
nitrogen compound metabolic process	20	10.1	2.6E-03	2.1
monocarboxylic acid metabolic process	13	6.5	2.6E-03	2.7
response to pheromone	10	5.0	3.9E-03	3.2
G-protein coupled receptor protein signaling	6	3.0	5.0E-03	5.3
response to chemical stimulus	25	12.6	5.2E-03	1.8
pyridine nucleotide biosynthetic process	4	2.0	7.4E-03	9.5
response to pheromone / conjugation	7	3.5	8.5E-03	3.9
regulation of catalytic activity	7	3.5	8.5E-03	3.9
water-soluble vitamin metabolic process	9	4.5	1.1E-02	2.9
signal transduction during conjugation	5	2.5	1.1E-02	5.6
carboxylic acid transport	7	3.5	1.2E-02	3.6
ammonium transport	3	1.5	1.2E-02	16.7
glutamate metabolic process	4	2.0	1.3E-02	7.8
regulation of conjugation with cellular fusion	5	2.5	1.4E-02	5.2
carboxylic acid metabolic process	21	10.6	1.5E-02	1.7
regulation of cyclin-dependent protein kinases	4	2.0	1.5E-02	7.4
transport	49	24.6	1.6E-02	1.3
amine transport	6	3.0	2.3E-02	3.6
localization	50	25.1	2.4E-02	1.3
amine metabolic process	16	8.0	2.5E-02	1.8
NAD metabolic process	4	2.0	2.6E-02	6.1
cell surface receptor linked signal transduction	6	3.0	2.7E-02	3.5
tricarboxylic acid cycle metabolism	4	2.0	3.0E-02	5.8
conjugation with cellular fusion	9	4.5	3.4E-02	2.4
glyoxylate cycle	3	1.5	3.4E-02	10.0
response to unfolded protein	6	3.0	3.9E-02	3.2
glyoxylate metabolic process	3	1.5	4.1E-02	9.1

Gene ontology groups determined by the DAVID Functional Annotation tool. Count refers to number of genes within input gene list that fall into the specified GO group. % denotes what percentage of the total input genes analyzed fall within the specific GO group. P-value represents the threshold of EASE Score, a modified Fisher Exact P-Value, for gene-enrichment analysis. Enrichment category denotes the overall enrichment of the particular GO group over background.

Table S7. Validation of differentially expressed genes identified via microarray analysis in suppressor strains by QPCR.

	*bna4*Δ103Q vs WT103Q			*mbf1*Δ103Q vs WT103Q			*ume1*Δ103Q vs WT103Q		
Gene	**Microarray**	**Fold Change**	**P-value**	**Microarray**	**Fold Change**	**P-value**	**Microarray**	**Fold Change**	**P value**
AQR1	Up*	7.86	<0.001	Up	-3.04	0.088	Up*	2.216	0.002
DAK2	Down	1.984	0.097	Down*	-2.72	0.024	Down	1.606	0.01
YGR035C	Up*	6.543	0.008	Up†	3.087	0.197	Up*	5.044	<0.001
YMC2	Up†	1.974	0.099	Up*	2.83	0.031	Up*	4.31	<0.001

Four DEGs (*AQR1*, *DAK2*, *YGR035C*, and *YMC2*) that were identified by microarray comparison of the three deletion suppressor strains (*bna4*Δ, *mbf1*Δ, *ume1*Δ) expressing Htt103Q versus WT expressing Htt103Q were validated by QPCR. Of the 12 comparisons, 7 were significantly confirmed by QPCR analysis (7/12; 58%, indicated by astericks). An additional 2 genes were found to be differentially expressed via QPCR analysis in the same direction as observed by mRNA profiling, but failed to reach statistical significance (indicated by †). Fold changes and P-values refer to QPCR data.

Experimental Procedures

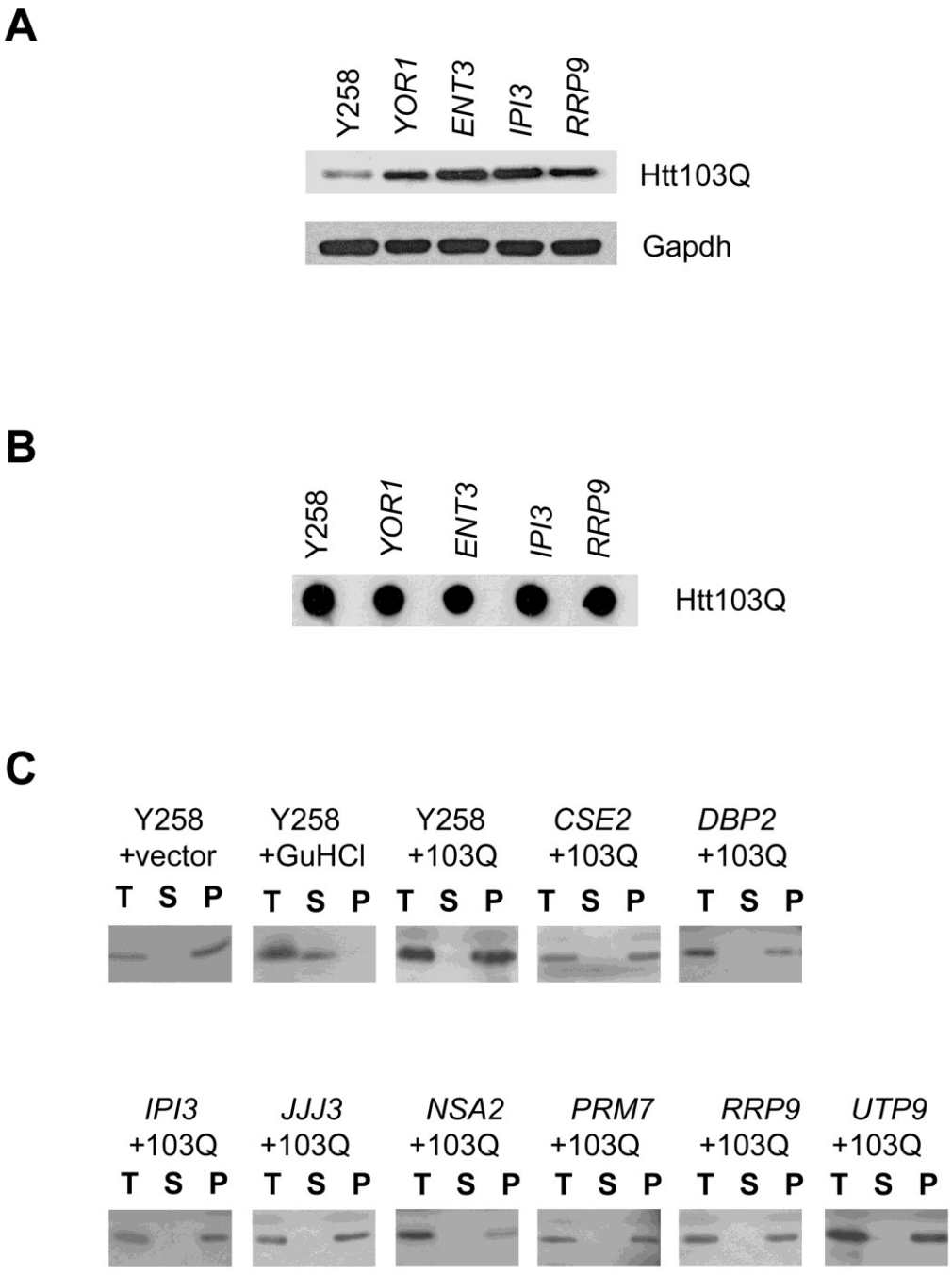
Htt103Q Expression Levels

In order to analyze the level of Htt103Q protein expression in the yeast strains, protein extracts were analyzed by immunoblotting. SC –Ura -Leu GAL yeast cultures were inoculated from overnight SC –Ura -Leu raffinose (RAF) cultures to an OD_{600} ~0.4, and were grown under inducing conditions for 5-6 hours. Cells were lysed by mechanical disruption with acid washed glass beads (425-600 μm, Sigma, St. Louis, MO) and lysis buffer (50 mM Tris pH 7.5; 1X protease inhibitor cocktail, Roche), vortexing seven times for 30 sec each, with 1 min incubations on ice in between. Glass beads and cell debris were removed by centrifugation and protein extracts were quantified using the BioRad Protein Assay (BioRad, Hercules, CA). For SDS-PAGE, extracts were mixed with 4x Protein Sample Buffer (200mM Tris-HCl, 8% SDS, 40% glycerol, 0.4% bromophenol blue, 6% β-mercaptoethanol), boiled 10 min, and subjected to electrophoresis in 12% SDS-polyacrylamide gel. The proteins were transferred to nitrocellulose membranes. For dot blots, extracts were mixed with 2% SDS and captured in nitrocellulose membrane by vacuum. Htt103Q-GFP was identified by a monoclonal α-GFP antibody (Santa Cruz Biotechnology, Inc), used at a 1:1000 dilution, followed by an α-Mouse Ig horseradish peroxidase conjugated antibody (Amersham Bioscience, Piscataway, NJ) at 1:1000 dilution. The Gapdh loading control was detected using Gapdh antibodies (Ambion) at 1:4000 dilution. Detection was done by enhanced chemiluminescence (ECL) (Amersham Bioscience, Piscataway, NJ) and exposure to X-ray film.

Figure Legend

Fig. S1. Expression of Htt103Q and Rnq1 in suppressor strains. (A) Expression levels of soluble Htt103Q-GFP were analyzed in the parental Y258 strain expressing *YOR1*, *ENT3*, *IPI3*, *RRP9*, or an empty vector control by immunoblotting with GFP antibodies. Gapdh levels were detected with by immunoblotting with Gapdh antibodies and used as a loading control for the Western blot. An increase in soluble Htt103Q-GFP is observed in suppressor strains, suggesting decreased levels of aggregated Htt103Q-GFP (B) Equal amounts of the extracts in panel A were analysed by dot immunoblotting to quantify total levels of Htt103Q-GFP (soluble and aggregated), which showed no change in total Htt103Q-GFP expression levels in the suppressor strains (C) Rnq1 prion status with ORF suppressors (Y258 parental strain) expressing Htt103Q was determined by a combination of high-speed centrifugation and immunoblotting. "T" indicates total extract for each yeast strain, while "S" indicates supernatant fraction (soluble form of Rnq1), and "P" indicates pellet fraction (prion form of Rnq1). Immunoblotting with α-Rnq1 antibody showed that Rnq1 is found in the pellet fraction for all suppressors, indicating the protein is in the prion conformation. The Y258 strain carrying empty vector (p425) or Htt103Q serve as a postive control, and treatment of cells carrying p425 with guanidine hydrochloride (GuHCL) cures Rnq1 prion, shifting this protein from the pellet fraction to the supernatant fraction.

Figure S1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Y258
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ENT3
IPI3
RRP9
Htt103Q
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B
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