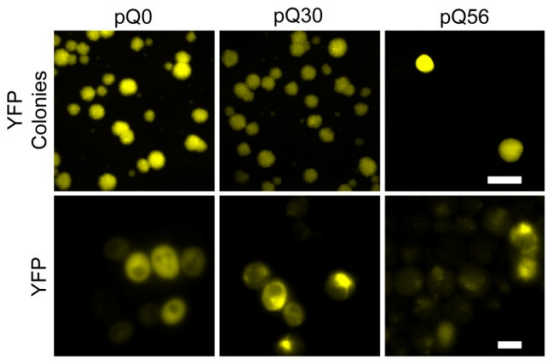
A network of genes connects polyglutamine toxicity to ploidy control in yeast

Christoph J. O. Kaiser[1], Stefan W. Grötzinger[1], Julia M. Eckl[1], Katharina Papsdorf[1], Stefan Jordan[2] and Klaus Richter[1]

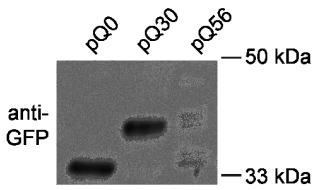
Supplementary Information

Supplementary Figures S1-S13

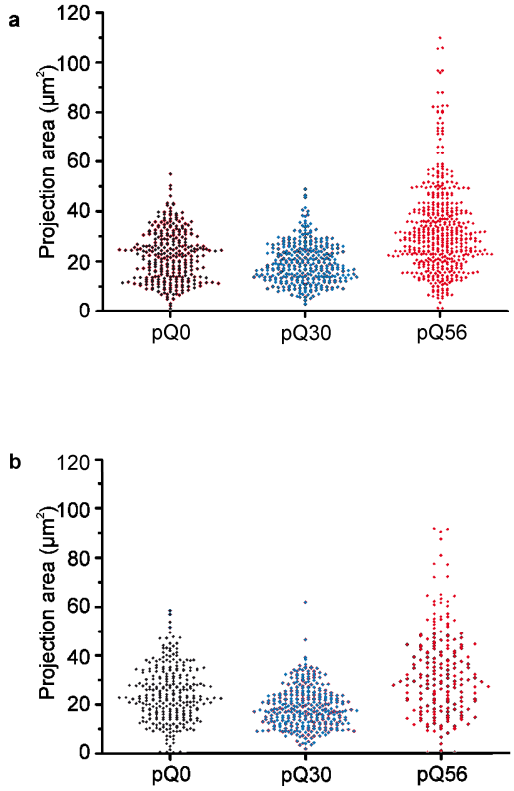
Supplementary Tables S1-S3



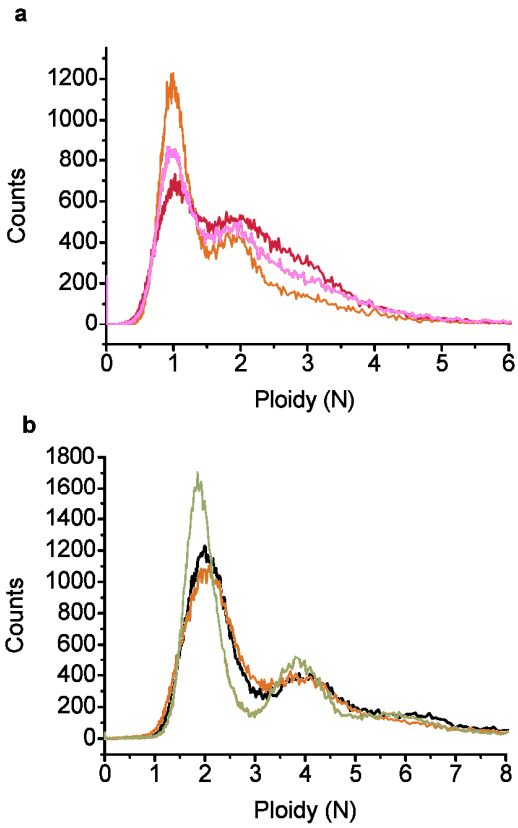
Supplementary Figure S1: Fluorescence properties of pQ0, pQ30 and pQ56 transformed yeast. All colonies of pQ0 and pQ30 transformants show similar fluorescence levels and similar sizes. Fluorescence of small colonies on pQ56-transformed plates is very weak and only a few large colonies show high fluorescence intensities. The scale bar represents 1 mm (upper row). Fluorescence micrographs of yeast cells reveal a homogeneous fluorescence distribution for Q_0-YFP. Q_{30}-YFP in contrast exhibits focal aggregates or fibrous structures. Q_{56}-YFP fluorescence is generally much lower and cells rarely exhibit punctate or focal aggregates. The scale bar represents 5 µm (lower row).



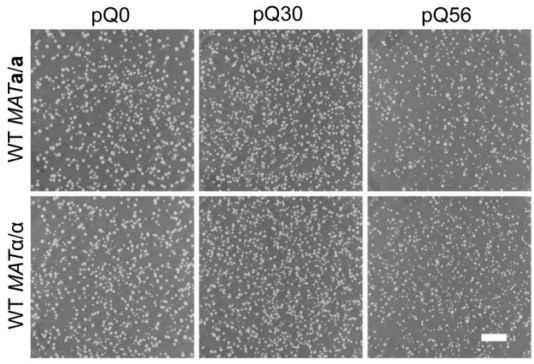
Supplementary Figure S2: Analysis of polyQ expression in WT yeast. Western blotting against YFP using an anti-GFP antibody exhibits distinct protein bands of the correct molecular weight for all three constructs as well as degradation products of Q_{56}-YFP. For pQ0 and pQ30, lysates were diluted 15-fold in comparison to pQ56 to enable simultaneous detection on a single blot.



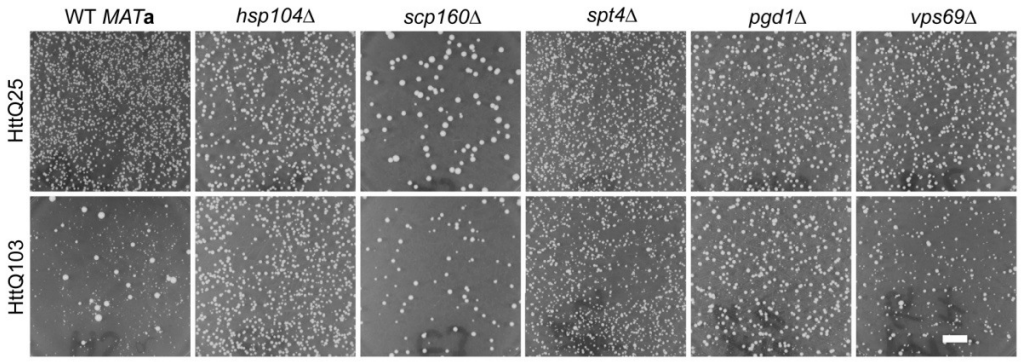
Supplementary Figure S3: Reproducibility of size analysis. To evaluate the reproducibility of the bee-swarm plots presented in Fig. 1 b, two additional biological replicates were subjected to the same assay to estimate cellular size. Both plots are derived from a mixture of small colonies, obtained from two separate inoculates on different days. Evaluated cells were (a) *n*=300, *n*=325 and *n*=499 and (b) *n*=279, *n*=316 and *n*=201 for pQ0, pQ30 and pQ56 transformants, respectively.



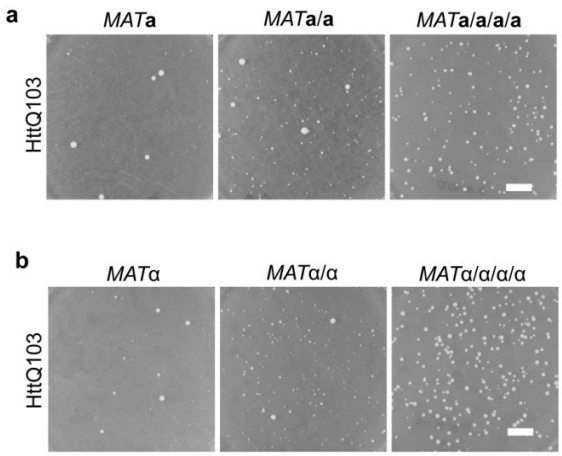
Supplementary Figure S4: FACS analysis of large survivor colonies from pQ56 yeast. (**a**) Strongly fluorescent large colonies were picked from plates of WT *MAT***a** yeast transformed with pQ56 and subjected to FACS analysis. Histograms of three representative samples are shown. All samples exhibit mainly a haploid growth pattern. (**b**) Weakly fluorescent large colonies were picked and subjected to the same analysis. Three representative histograms are shown, exhibiting a ≥2N/4N cycling behavior.



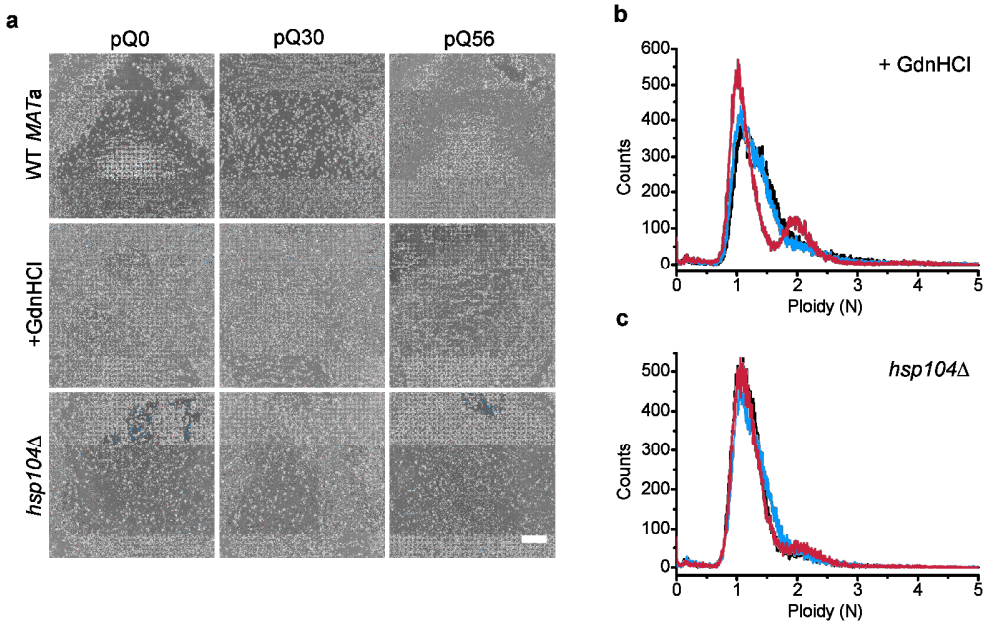
Supplementary Figure S5: *MAT*a/a and *MATα/α* yeast are resistant to *pica*. Colony patterns are homogenous after transformation with pQ56 for the yeast strains PY4995 (*MAT***a/a**, upper row) and PY4994 (*MATα*/α, lower row) indicating that no *pica* phenotype is apparent for these two strains. The scale bar represents 10 mm.



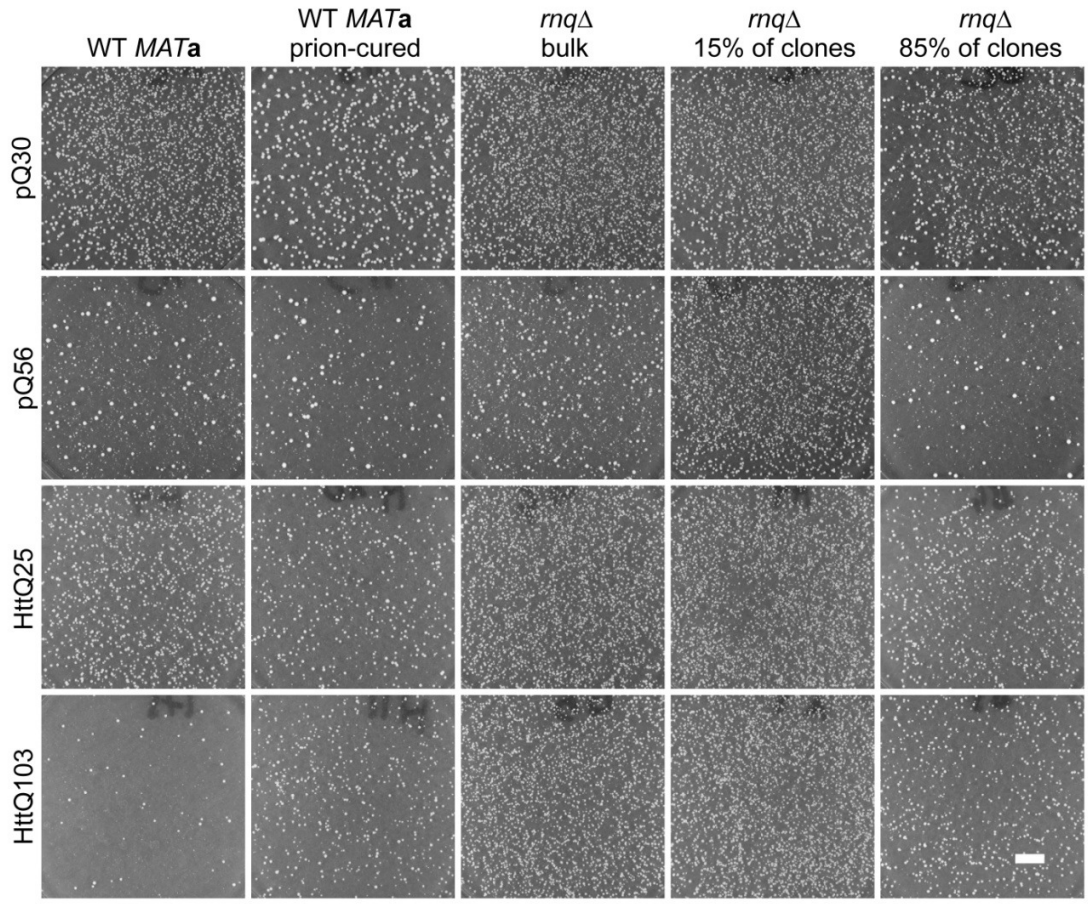
Supplementary Figure S6: Rescue strains and their phenotype for HttQ103. Identified strains were also examined regarding the cytostatic effect induced by HttQ103 expression (lower panel, left column). Some of the strains restore normal growth, while others rescue at least to some extent. Strong rescue is seen for *hsp104Δ*, *spt4Δ*, intermediate rescue for *scp160Δ*, and *pgd1Δ* while weak rescue is seen for *vps69Δ*. The images shown are representative results for these selected strains. *scp160Δ* exhibited generally less colonies in this experiment. The scale bar represents 10 mm.



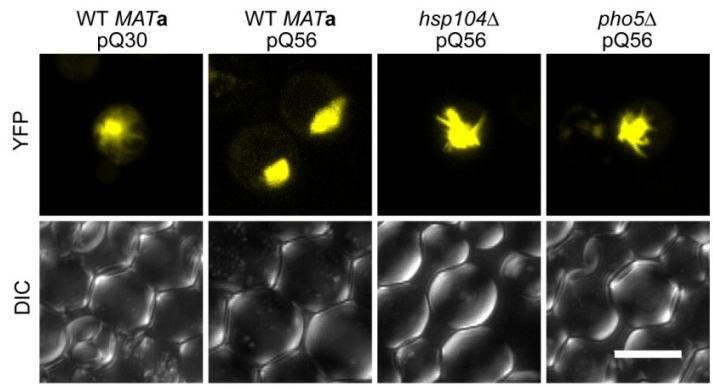
Supplementary Figure S7: Growth inhibition by HttQ103 is fully abolished in tetraploid yeast. (**a**) Toxicity of HttQ103 is evident in WT *MAT***a** cells. Slow colony growth is still observable under identical conditions in *MAT***a/a** (PY4995). Full rescue becomes evident for *MAT***a/a/a/a** (PY5006). (**b**) The same effect is observable in *MAT*α strains, where *MAT*α/α rescues partially and *MAT*α/α/α/α rescues fully. The scale bars represent 10 mm.



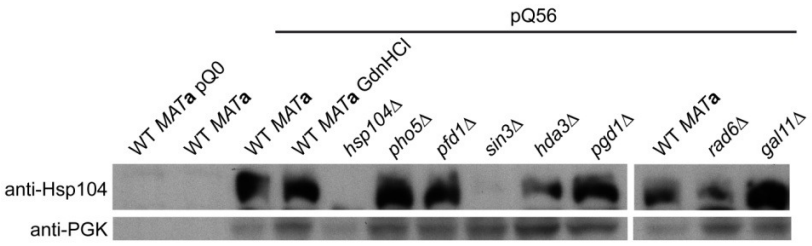
Supplementary Figure S8: GdnHCl reduces the *pica* phenotype of Q_{56}-YFP. (**a**) Addition of 4 mM GdnHCl to plates reduces the *pica* phenotypeto undetectable levels (middle row versus upper row). The effect is similarily strong as the rescue effect of a *HSP104* deletion (lower row). The scale bar represents 10 mm. (**b**) FACS histogram of GdnHCl resuced pQ56 (red) compared to pQ0 (black) and pQ30 (blue). Only small differences are observed. (**c**) Even smaller differences between the three histograms are observed for *hsp104*Δ.



Supplementary Figure S9: The *pica* phenotype caused by pQ56is independent of the prion state. Transformation assays comparing the reaction of the pQ30/pQ56 and HttQ25/HttQ103 systems in respect to endogeneous prion phenotypes. The WT *MAT***a** strain BY4741 exhibits a growth inhibiton phenotype for both systems. Curing the strain from prions by sequential passage on GdnHCl (prion-cured) abolishes the HttQ103 phenotype, while it is maintained for pQ56. Similarly, the transformation of bulk stock of an *RNQ1* deletion exhibits no phenotype for HttQ103 and a slight rescue effect for pQ56. This slight rescue is due to heterogeneity within the knockout strain, as clonally selected deletions for *RNQ1* abolish both phenotypes in 15% of transformed clones. In 85% however, *pica* through pQ56 is maintained, while it is lost for HttQ103. The scale bar applies to all photographs and represents 10 mm. All *rnq1Δ* clones (*n*= 15) were sequenced to verify their genetic identity.



Supplementary Figure S10: The deletion of *HSP104* and *PHO5* alters aggregate morphology. Confocal microscopy of WT cells transformed with pQ30 reveals compact focal aggregation as well as fibrous protein deposits. This is in marked contrast to strongly fluorescent WT cells transformed with pQ56 which exhibit mainly compact aggregation. The deletion of *HSP104* as well as *PHO5* leads to a change in this aggregation pattern, rendering pQ56 transformants morphologically more similar to WT cells transformed with the pQ30 construct. All micrographs in the panel are maximum projections of image stacks recorded simultaneously in epifluorescence and DIC modes. The scale bar represents 5 µm for all micrographs.



Supplementary Figure S11: Hsp104 levels are unaltered for most group I strains. Western blotting against Hsp104 reveals that expression of Hsp104 is reduced in some of the knockout strains. While the level of Hsp104 is unchanged for most group I strains, the protein is properly absent in *hsp104*Δ. While *hda3*Δ and *rad6*Δ exhibit only slightly compromised Hsp104 levels, the protein is barely detectable in *sin3*Δ. The lysates of pQ0 and pQ30 transformed cells were diluted 15-fold to be in the dynamic range of immunogenic detection for the polyQ proteins in comparison to pQ56 transgenic cells.

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pQ56            ATGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAA 60
pQ30            ATG---------------------------------------------------------
pQ0             ATG---------------------------------------------------------

pQ56            CAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAA 120
pQ30            ----------------------CAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAA 42
pQ0             ---------------------------------------------------------

pQ56            CAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCTGCAGCTG 180
pQ30            CAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCAACAGCTGCAGCTG 102
pQ0             ----------------------------------------------------CTGCAGCTG 12

pQ56            AAACTGCAGGCTAGCGCTAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTG 240
pQ30            AAACTGCAGGCTAGCGCTAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTG 162
pQ0             AAACTGCAGGCTAGCGCTAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTG 72

pQ56            GTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGC 300
pQ30            GTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGC 222
pQ0             GTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGC 132

pQ56            GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTG 360
pQ30            GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTG 282
pQ0             GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTG 192

pQ56            CCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCC 420
pQ30            CCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCC 342
pQ0             CCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCC 252

pQ56            GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAG 480
pQ30            GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAG 402
pQ0             GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAG 312

pQ56            CGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG 540
pQ30            CGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG 462
pQ0             CGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG 372

pQ56            GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAAC 600
pQ30            GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAAC 522
pQ0             GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAAC 432

pQ56            ATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGAC 660
pQ30            ATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGAC 582
pQ0             ATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGAC 492

pQ56            AAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGC 720
pQ30            AAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGC 642
pQ0             AAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGC 552

pQ56            GTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG 780
pQ30            GTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG 702
pQ0             GTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG 612

pQ56            CCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC 840
pQ30            CCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC 762
pQ0             CCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC 672

pQ56            GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAG 900
pQ30            GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAG 822
pQ0             GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAG 732

pQ56            CTGTACAAGTAA 912
pQ30            CTGTACAAGTAA 834
pQ0             CTGTACAAGTAA 744
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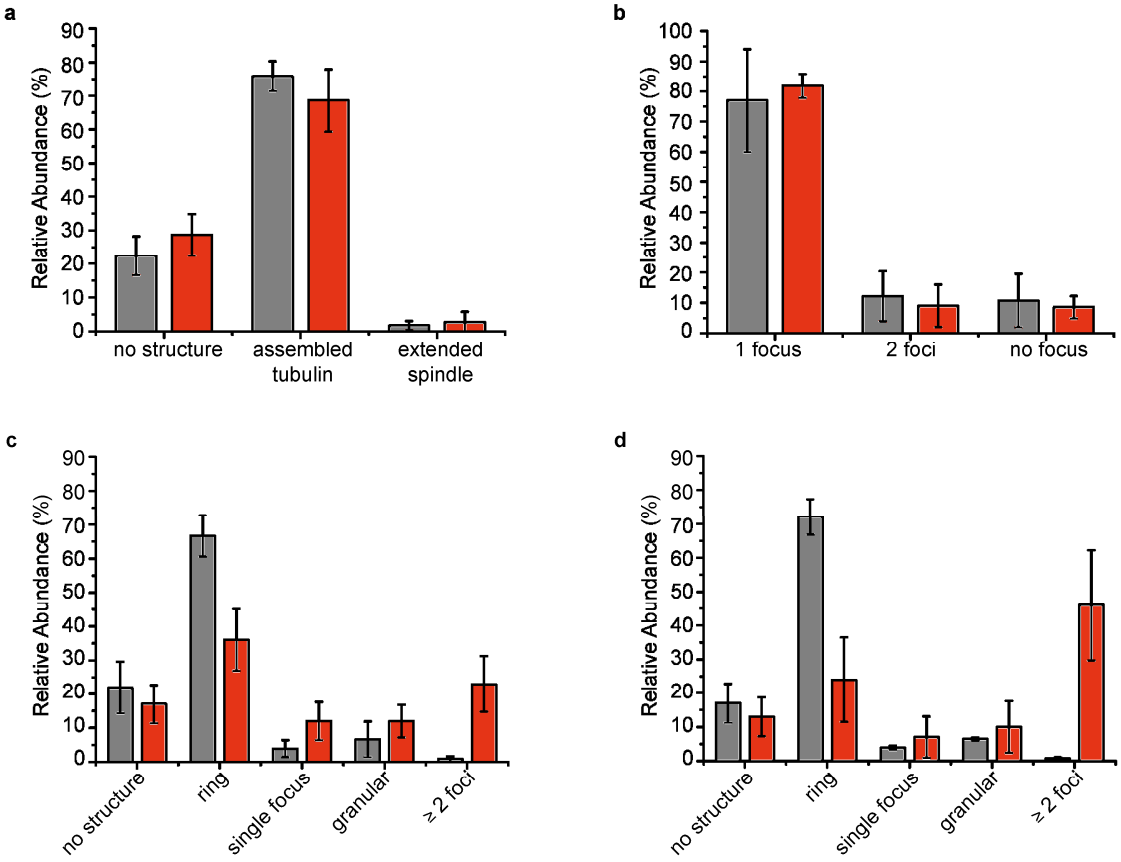
Supplementary Figure S12: Sequences coding for pQ0, pQ30, and pQ56. The coding sequences for the plasmids used in our study were synthesized as above and cloned into p425GPD via BamHI and SalI restriction sites added directly upstream and downstream of the depicted sequences, respectively.



Supplementary Figure S13: pQ56 interferes with septin assembly. Averaged morphological classification results for GFP-tagged reporter proteins transformed with the pQ0cherry construct (grey) and pQ56cherry (red) from three independent biological replicates are given. n ist the cumulated number of cells evaluated. The error bars represent the standard deviation. (a) Results for Tub1-GFP (*n*=830 for pQ0cherry and *n*=742 for pQ56cherry). (b) Results for Nud1-GFP (*n*=748 for pQ0cherry and *n*=673 for pQ56cherry). (c) Results for Cdc10-GFP (*n*=967 for pQ0cherry and *n*=1436 for pQ56cherry). (d) Results for Shs1-GFP (*n*=1058 for pQ0cherry and *n*=1305 for pQ56cherry).

Fluorescence Intensity	PolyQ Length	Ploidy State
very strong	Q8	haploid
strong	Q20	haploid
strong	Q16	haploid
strong	Q12	haploid
strong	Q20	haploid
medium	Q30	diploid and higher
medium	Q52	diploid and higher
weak	Q52	diploid and higher
weak	Q12	diploid and higher
weak	Q28	diploid

Supplementary Table S1: Analysis of spontaneously occurring large colonies. Ten large colonies of different fluorescence properties from WT *MAT***a** plates transformed with pQ56 were picked and analyzed. The length of the polyQ stretch and ploidy was determined for each clone.

Strain Number	Systematic Name	Standard Name
589***	YMR014W	*bud22Δ*
1401***	YIL009W	*faa3Δ*
1440***	YIL047C	*syg1Δ*
1442**	YIL049W	*dfg10Δ*
1484**	YIL093C	*rsm25Δ*
1666**	YOR369C	*rps12Δ*
2072	YPL180W	*tco89Δ*
2280**	YIL121W	*qdr2Δ*
2293	YIL134W	*flx1Δ*
2316	YIL157C	*coa1Δ*
2345**	YIR009W	*msl1Δ*
3223	YBR085W	*aac3Δ*
3765	YDL068W	*YDL068WΔ*
3770	YDL073W	*YDL073WΔ*
3923	YDL225W	*shs1Δ*
4278	YDR442W	*YDR442WΔ*
4296	YDR462W	*mrpl28Δ*
4401	YGL033W	*hop2Δ*
5727	YBR279W	*paf1Δ*
6421*	YHR191C	*ctf8Δ*
6422*	YHR193C	*egd2Δ*
6423*	YLL030C	*rrt7Δ*
6424*	YLL044W	*YLL044WΔ*
6426*	YLL049W	*ldb18Δ*
6797**	YJL029C	*vps53Δ*
6861**	YJR063W	*rpa12Δ*
6867	YAL047C	*spc72Δ*
7316***	YNL138W	*srv2Δ*
7395***	YPL183W-A	*rtc6Δ*
7533	YKL096c-B	*YKL096c-BΔ*

Supplementary Table S2: Growth restoring *MAT*a/α strains uncovered in the library. 68 strains of the *MAT***a** library were identified to restore growth of Q_{56}-YFP expressing yeast. For all strains genomic DNA was purified and determined by PCR, whether they are in fact *MAT***a** or *MAT***a**/α. 30 strains were found to be true diploids heterozygous at the *MAT* locus. Many of those strains were already known to be *MAT***a**/α (*) or assigned with MET15 (**) or MET+ (***) in the library manual. For strain 7533 no conclusive result could be obtained.

Gene Knockout	Strain Number	Gene function	Rescue Q_{56}-YFP	Rescue Htt103Q	Group
hsp104Δ	1514	Protein folding	+++	+++	
pho5Δ	3232	Phosphate regulation	+++	+++	
pfd1Δ	1246	Protein folding	+++	++	
hda3Δ	5594	Chromatin organization, Transcription	+++	o	
cyk3Δ	3814	Budding	+	+++	I
gal11Δ *	1742	RNA-Polymerase II regulation, subunit of Mediator Complex	+++	++	
sin3Δ	1695	Chromatin organization	++	o	
pgd1Δ *	4393	RNA-Polymerase II regulation, subunit of Mediator Complex	+++	++	
rad6Δ	4425	Protein degradation, Cell cycle control	++	+++	
bem2Δ *	7548	Budding	+++	+	
tif3Δ	5578	Translation	+++	o	II
asc1Δ	6556	Translation	++	+	
hof1Δ *	7817	Budding	++	o	
med2Δ	3701	RNA-Polymerase II regulation, subunit of Mediator Complex	+++	+++	
sin4Δ	1976	RNA-Polymerase II regulation, subunit of Mediator Complex	+++	++	
eap1Δ	7036	Translation	+++	o	
ymr185wΔ	770	Unknown	+++	o	
hsl7Δ *	3272	Budding	+++	+++	
cdc10Δ	3482	Budding	+++	o	
swa2Δ	3679	Budding / Vesicle transport COP	+++	++	
kre6Δ	5574	Cell wall biogenesis	+++	o	
hnt3Δ	2514	DNA repair	+++	++	
gpm2Δ	3717	Metabolism	+++	++	III
ldb16Δ	3413	Mitochondrion	+++	++	
ybl094cΔ	3120	Unknown	+++	++	
rox3Δ	3119	RNA-Polymerase II regulation, subunit of Mediator Complex	++	++	
spt4Δ *	6986	RNA-Polymerase I/II regulation, pre-mRNA processing	+++	+++	
scp160Δ	1343	Translation	+++	++	
bfr1Δ	2454	Translation, Nuclear segregation	+++	++	
vps69Δ	5504	Vacuolar protein sorting	+++	+	
tho2Δ	2937	Transcription	+++	o	
whi3Δ	2015	Cell cycle control	++	++	

Supplementary Table S3: Summary of the strains indentified in the genome-wide screen. Gene function was assigned based on literature reviews. Most of the genes rescued to an extent that no growth difference was evident between cells producing Q_0-YFP, Q_{30}-YFP or Q_{56}-YFP, as indicated by +++ in the fourth column. Few strains rescued to a lesser extent (+ or ++). The same scale was applied for the rescue of HttQ103-GFP induced growth inhibition. If no rescue was detectable, this is indicated by an open circle. See Figs. S6 and S7 for representative images. Genes are classified according to FACS analyses, assigning them to the groups I, II or III. Transformation assays for each strain were performed at least in six separate replicates and

FACS measurements were four separate experiments for each strain (separate inoculum for every replicate). * *hsl7Δ*: two strains in the library (7539, 3272), of which both rescue; *gal11Δ*: two strains (1741, 1742) with similar results; *pgd1*Δ: two strains (4392, 4393) with similar results; *bem2Δ*: two strains (6152, 7548) with similar results; *hof1Δ*: two strains (7817, 608) with similar results; *spt4Δ*: two strains (4694, 6986) with similar results.