A comprehensive strategy enabling high-resolution functional analysis of the yeast genome

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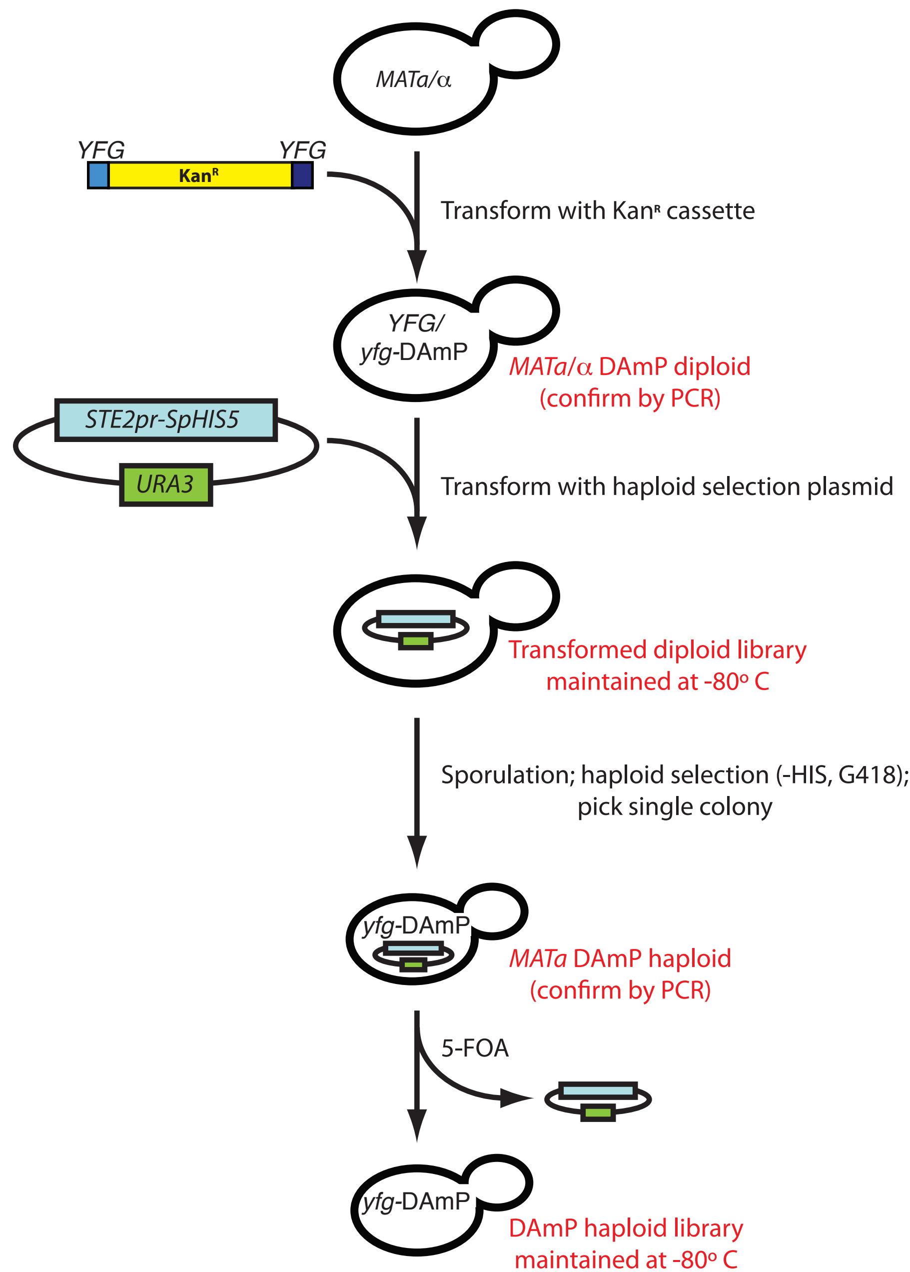
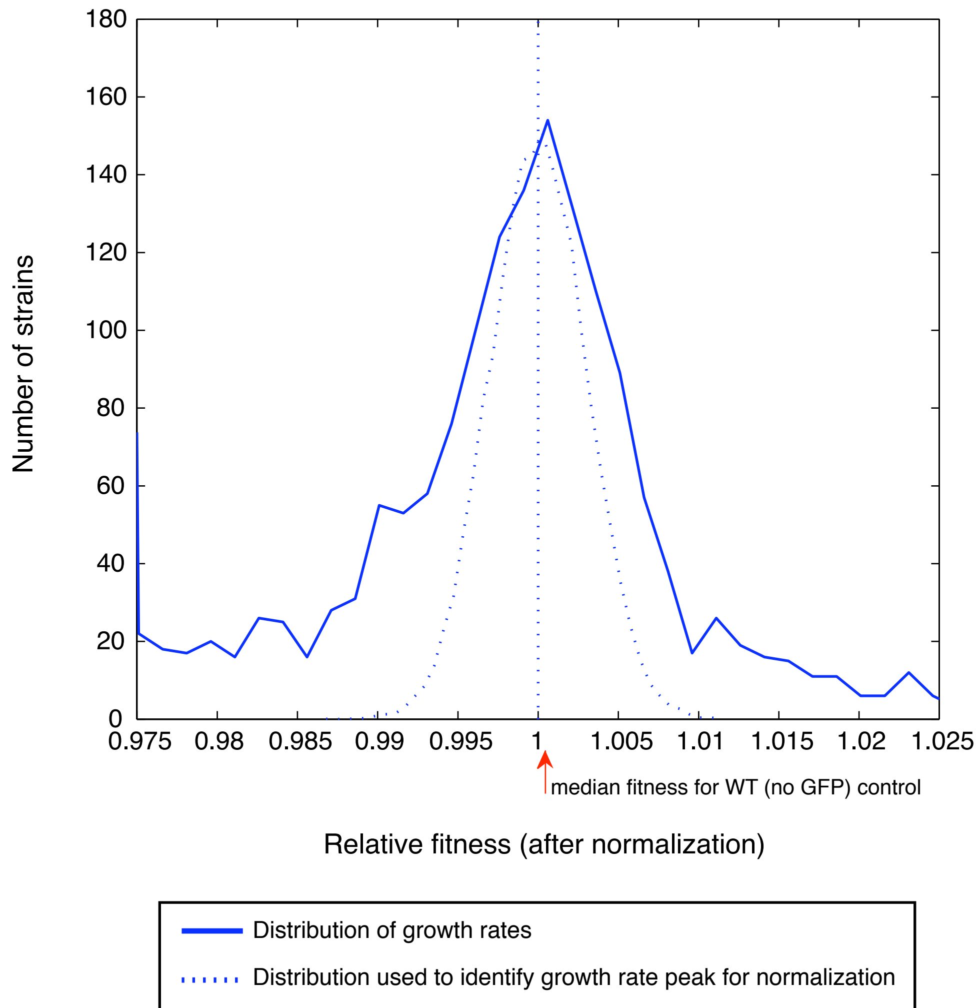
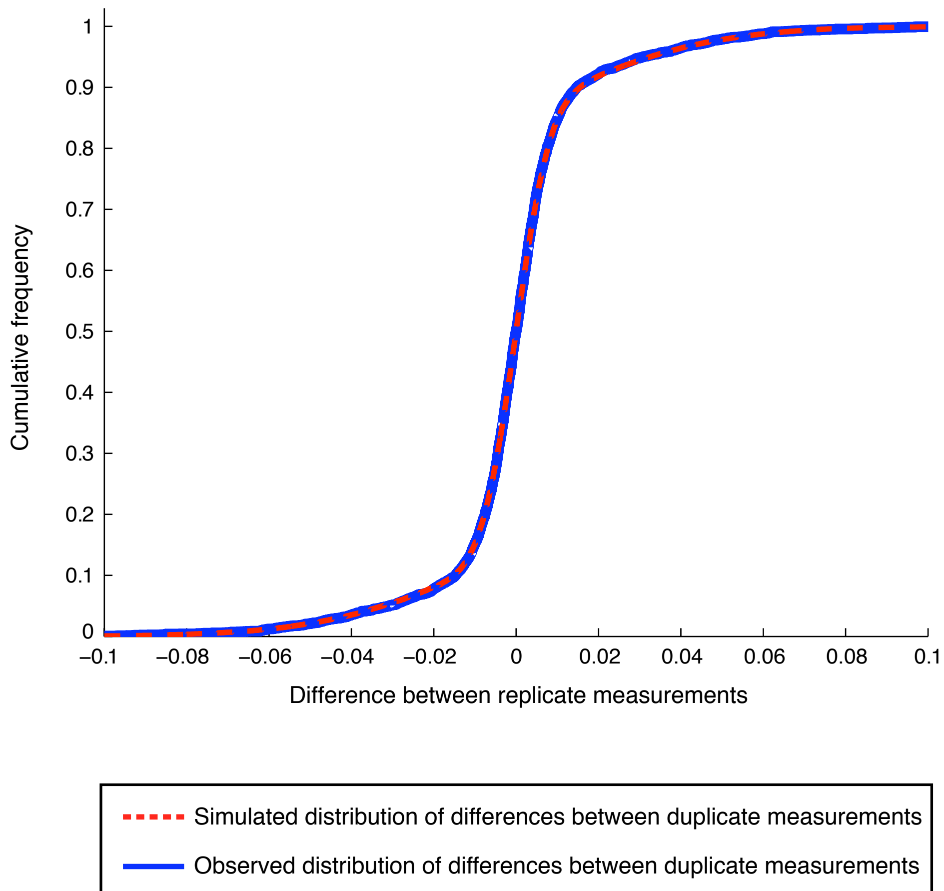
Note: Supplementary Data 1–5 are available on the Nature Methods website.



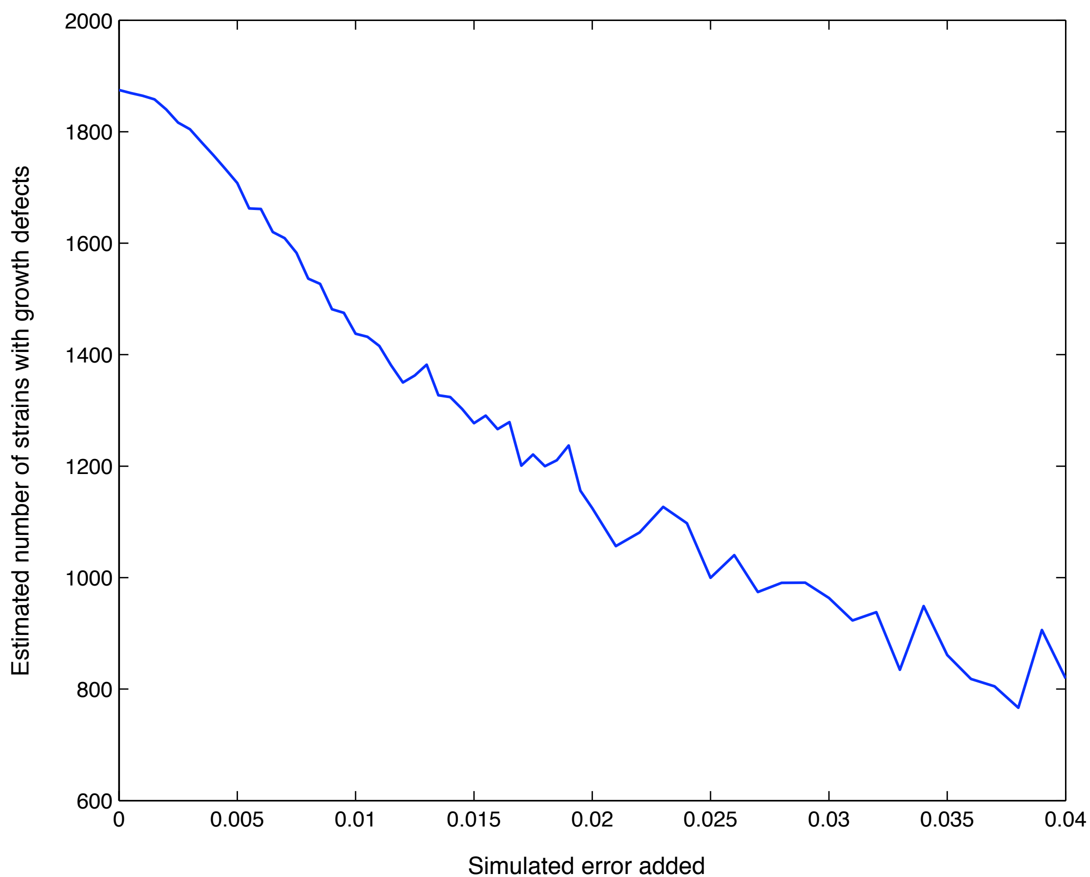
Supplementary Figure 1. Strategy used for DAmP library construction.
DAmP alleles were first constructed in diploid strains by transformation of a PCR product bearing 40 nucleotides of homology to the site of integration. DAmP diploids were confirmed by PCR then transformed with a plasmid to enable direct selection of *MAT**a*** haploids following sporulation (through the use of a *S. pombe HIS5* marker driven by the *MAT**a***-specific *STE2* promoter). Following sporulation, DAmP haploids were reconfirmed by PCR and passaged on 5-fluorouracil (5-FOA) to ensure loss of the haploid selection plasmid.



Supplementary Figure 2. Normalization of fitness measurements.
Growth rates from each flow cytometry experiment were normalized such that the peak of the distrubtion of GFP-labeled mutant strains (shown for one representative experiment above; solid line) is equal to a fitness of 1.0. The peak of the distrubution of GFP-labeled mutant cells was identified using a Parzen window smoothing strategy (dashed line; see **Supplementary Methods**). After normalization, the median fitness for a wildtype (WT) strain not expressing GFP is nearly exactly equal to 1.00 (red arrow), confirming that the expression of GFP has very little effect on growth.

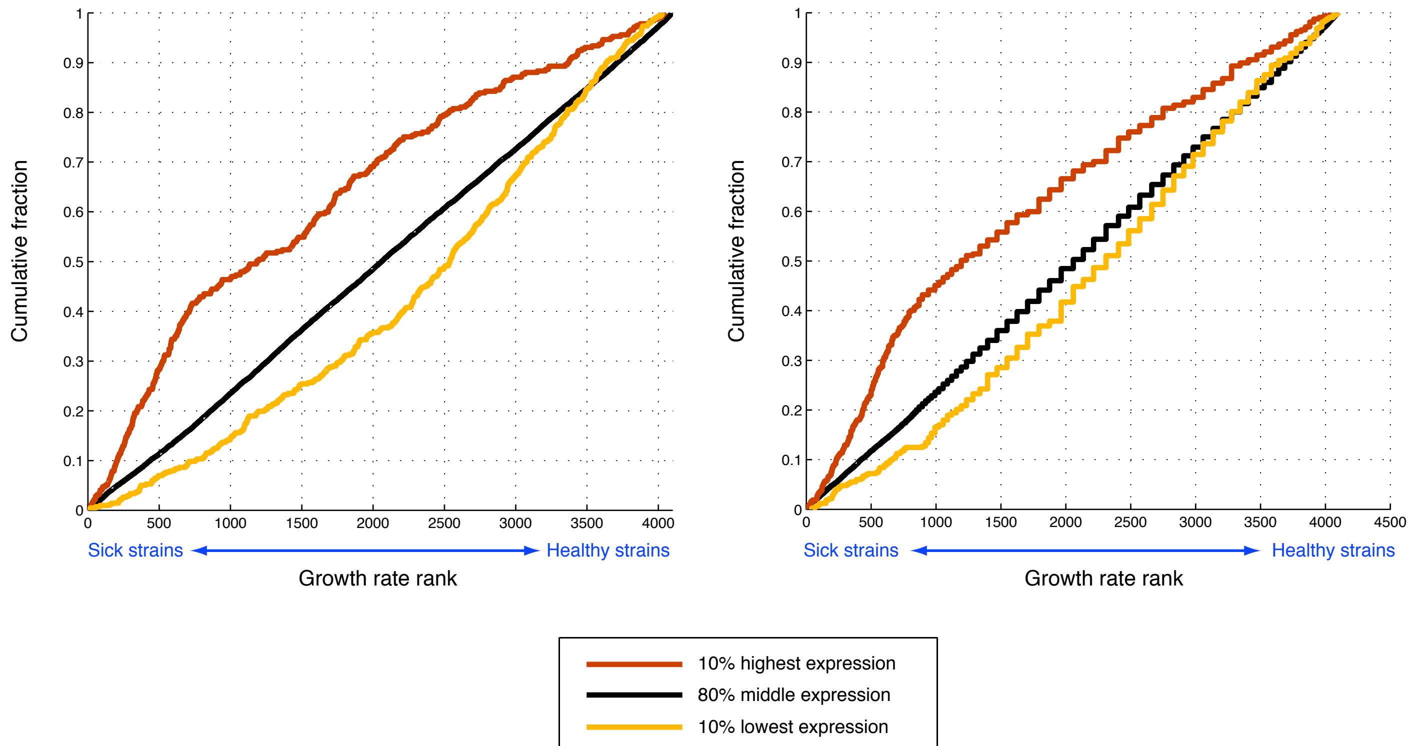


Supplementary Figure 3: Simulated versus observed distrubution of differences between duplicate growth measurements.
Our error model (dashed red line; see **Supplementary Methods**) accurately reproduces the observed distribution of differences between duplicate measurements (solid blue line).

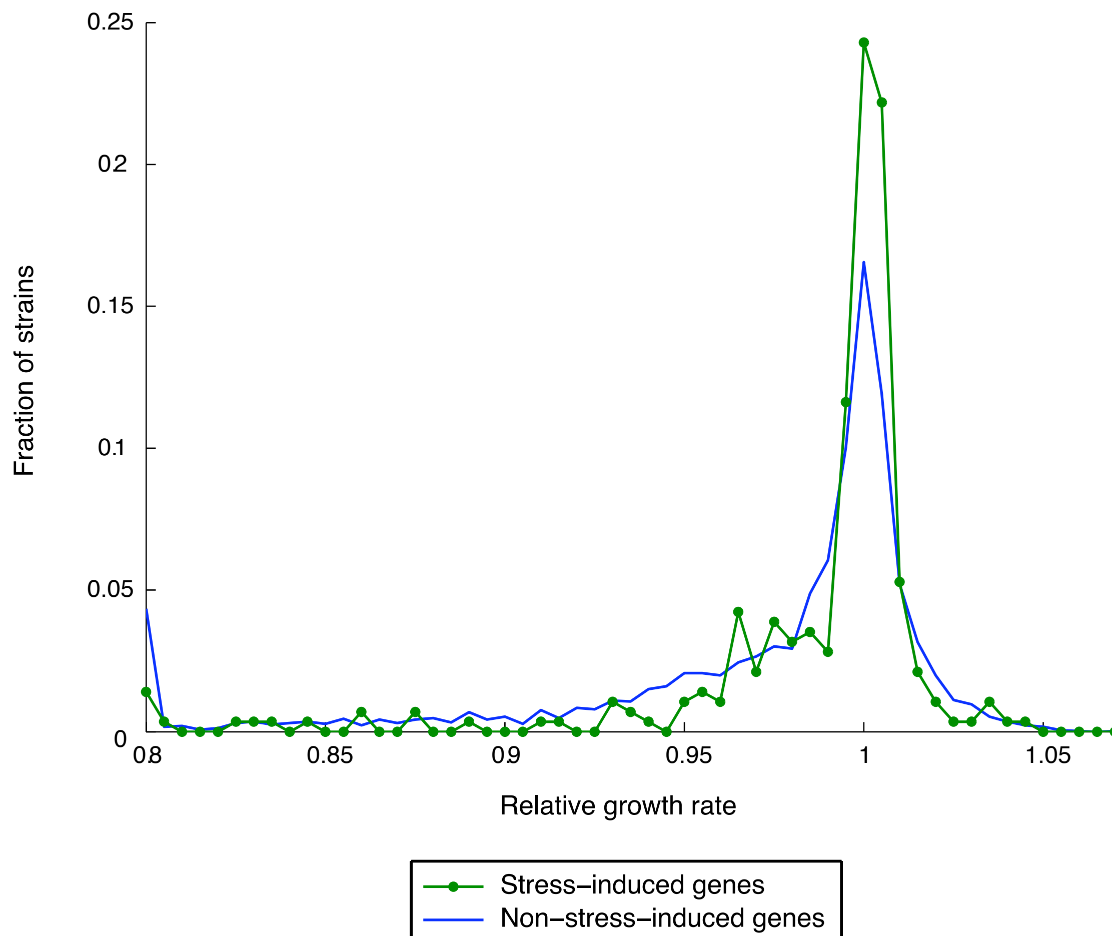


Supplementary Figure 4. Effect of simulated error on the the apparent number of deletion strains with growth defects.

Increased measurement error was simulated by the addition of normally distributed noise of a defined magnitude to our growth rate data. The error analysis described previously (see **Supplementary Methods**) was then re-applied to the simulated data to estimate a number of strains with identifiable growth defects (among the 4,204 strains measured). For each level of error added, 200 simulations were carried out and used to compute a median number of strains with growth defects (plotted above).



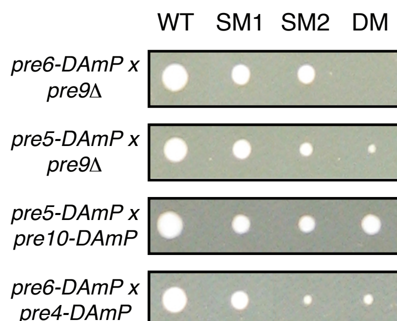
Supplementary Figure 5. Effects of gene deletions on growth are correlated with expression levels. For gene deletions measured in our dataset and in reference 11, genes were ranked according to their growth rate in each dataset. The cumulative distributions of growth rate ranks are plotted for genes with low (bottom 10%), intermediate (10th – 90th percentiles), and high (top 10%) expression levels, as measured by Wang *et al*[19]. Genes with low expression levels tend not to have growth rate defects in our dataset (*P* = 1E-7, Kolmogorov-Smirnov test), whereas genes with high expression levels are enriched for growth rate defects (*P* = 2E-16, Kolmogorov-Smirnov test). Notably, these effects are also apparent in ranked growth data from reference 11 (*P* = 0.008 for low expression levels, *P* = 1E-12 for high expression levels).



Supplementary Figure 6. Relationship between growth defect and participation in the environmental stress response.

Genes needed to respond to stress[20] have an increased likelihood of being dispensable for rapid growth in our assay. Notably, enrichment for stress-responsive genes is seen for fitness values ranging from ~0.99-1.01, but not for genes with even slight growth defects (i.e. growth from 0.97-0.99). This result suggests that small growth defects captured by our assay are functionally relevant.

a



b

α subunits: SCL1, PRE6, PRE8, PRE5, PUP2, PRE10, PRE9
β subunits: PRE1, PRE3, PRE4, PRE7, PUP1, PRE2
base: RPT1, RPT2, RPT3, RPT5, RPT6, RPN2, RPN10
lid: RPN3, RPN5, RPN6, RPN7, RPN8, RPN11, RPN12

2
1.5
1
0.5
0
−0.5

Normalized interaction score: ε_{NORM}

Supplementary Figure 7. Genetic interactions between proteasome genes.
(a) Tetrad analysis confirms negative genetic interactions between *pre9*Δ and DAmP alleles of other alpha subunits, particularly *pre6*-DAmP. In general, however, genes encoding alpha subunits exhibit positive interactions both with each other (for example, between *pre5*-DAmP and *pre10*-DAmP) and with genes encoding beta subunits (for example, *pre6*-DAmP and *pre4*-DAmP). WT = wildtype; SM1 = single mutant 1; SM2 = single mutant 2; DM = double mutant.
(b) Normalized genetic interaction scores (ε_{NORM}) between proteasome genes. ε_{NORM} was calculated as described in **Supplementary Methods**.

Supplementary Table 1. Relative growth rates for TAP, TAP-DAmP and TAP-degron+DAmP alleles.
Also shown is the fold reduction in protein abundance for the TAP-degron+DAmP strains relative to the corresponding TAP-DAmP strain. Note, growth rates slightly greater than 1.00 likely reflect the fact that SGA markers were absent from the TAP-tagged library derivatives[12], but present in the wildtype competitor strain.

GENE	TAP	TAP-DAmP	TAP-degron+DAmP	Fold protein reduction (deg. vs. DAmP)
RHO3	1.015	0.967	0.958	2.4
DIP2	1.017	0.981	1.004	3.6
CKS1	1.023	0.820	0.788	4.6
FAS2	0.972	0.947	0.771	6.1
PCM1	1.029	0.984	0.668	6.8
CCA1	1.018	0.950	0.583	8.8
YNL313C	1.024	0.952	0.696	9.2
YDR531W	1.018	0.943	0.545	21.3

Supplementary Table 2. Estimated true and false positive rates for identification of strains with growth defects.
Estimates are shown as a function of observed growth rate (for observed growth rates < 1.0) for deletion strains **(a)** and DAmP strains **(b)**.

(a) Deletion Strains:

Fitness cutoff	Cumulative # strains	Cumulative Fraction positive	Cumulative # positive	Cumulative # negative	Incremental # positive	Incremental # negative	Incremental fraction positive
0.900	438	1.000	438	0			
0.905	455	0.998	454	1	16	0	0.99
0.910	477	0.998	476	1	22	0	0.98
0.915	497	0.996	495	2	19	1	0.97
0.920	529	0.996	527	2	32	1	0.98
0.925	561	0.993	557	4	30	1	0.96
0.930	595	0.992	590	5	33	2	0.95
0.935	645	0.988	637	8	47	2	0.95
0.940	696	0.984	685	11	48	3	0.94
0.945	756	0.980	741	15	56	4	0.93
0.950	835	0.976	815	20	74	5	0.93
0.955	909	0.971	883	26	68	7	0.91
0.960	992	0.966	958	34	75	8	0.90
0.965	1081	0.959	1037	44	79	10	0.89
0.970	1200	0.954	1145	55	108	11	0.91
0.975	1309	0.948	1241	68	96	13	0.88
0.980	1446	0.943	1364	82	123	15	0.89
0.985	1610	0.939	1511	99	147	16	0.90
0.990	1820	0.930	1692	128	181	29	0.86
0.995	2141	0.865	1852	289	160	161	0.50
1.000	2728	0.687	1875	853	23	563	0.04

(b) DamP strains:

Fitness cutoff	Cumulative # strains	Cumulative Fraction positive	Cumulative # positive	Cumulative # negative	Incremental # positive	Incremental # negative	Incremental fraction positive
0.900	75	1.000	75	0			
0.905	81	1.000	81	0	6	0	1.00
0.910	87	1.000	87	0	6	0	1.00
0.915	89	1.000	89	0	2	0	1.00
0.920	93	1.000	93	0	4	0	1.00
0.925	97	1.000	97	0	4	0	1.00
0.930	104	1.000	104	0	7	0	1.00
0.935	107	1.000	107	0	3	0	1.00
0.940	113	1.000	113	0	6	0	1.00
0.945	120	1.000	120	0	7	0	1.00
0.950	128	1.000	128	0	8	0	1.00
0.955	135	1.000	135	0	7	0	1.00
0.960	144	1.000	144	0	9	0	1.00
0.965	153	1.000	153	0	9	0	1.00
0.970	160	1.000	160	0	7	0	1.00
0.975	169	1.000	169	0	9	0	1.00
0.980	186	1.000	186	0	17	0	0.98
0.985	209	0.995	208	1	22	1	0.96
0.990	252	0.976	246	6	38	4	0.90
0.995	342	0.927	317	25	71	19	0.79
1.000	526	0.688	362	164	45	139	0.24

Supplementary Note

We estimated the number of deletion strains previously reported to have growth defects in similar conditions using a variety of methods. First, among the 4,204 deletion strains we measured, growth rates for 4,098 were also measured in minimal media in reference 11. Using the authors' suggested relative growth cutoff of 0.95, 542 of 4,098 gene deletions (~13%) were found to be significantly slow-growing in this study. Second, minimal media growth data have been reported in reference 10 for 4,122 of the deletion strains in our study. Using the suggested growth defect cutoff of -0.055 (this study computed growth defects on a different scale), 710 of 4,122 gene deletions (~17%) were found to be significantly slow-growing. Combining these two studies, ~16% showed on average a significant growth defect, with 22% showing a growth defect in at least one of the two studies. Finally, to make a more direct comparison that is independent of the choice of cutoffs, we analyzed the replicate data available for growth in rich media published in reference 11 using the error model calculation procedure that we applied to our deletion and DAmP library data. With this analysis, we estimate that a total of ~1,060 of 4,146 deletion strains (~26%) have reduced fitness in reference 11.

Supplementary Methods

Growth media and Strain Construction

Details of growth media used have been described previously (Supplementary Reference 1). For growth competition experiments, we used minimal media lacking histidine, lysine and arginine and containing canavanine (50 μg/ml; SIGMA), s-Aminoethyl-cysteine (50 μg/ml, SIGMA), G418 (200 μg/ml; GIBCO) and clonNAT (200 μg/ml; Werner BioAgents).

We constructed DAmP alleles in a diploid strain derived from strain BY4741 (Supplementary Reference 2) (*MAT**a/α** his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 CYH2+/cyh2*) by transformation with a PCR product encoding the kanamycin-resistance (Kan^R) cassette flanked at either end by 40 nucleotides of homology to the site of integration (see below and **Supplementary Data** for plasmid and primer sequences). Transformants were selected on YEPD containing 200 μg/ml G418, then grown to saturation in 1 ml liquid YEPD+G418. Lysates were made by boiling cells in 20 mM NaOH for 20 minutes, vortexing briefly, then adding an equal volume of water and centrifuging at 2500*g* for 2 minutes. Correct insertion of the Kan^R cassette was confirmed by PCR using gene-specific forward primers and a universal reverse primer complementary to the promoter region of the Kan^R cassette. We then transformed correct diploid strains with the *URA3*-marked plasmid pRS316-*STE2pr-SpHIS5* encoding the *S. pombe HIS5* gene driven by the *MAT**a***-specific *STE2* promoter. Transformed diploids were sporulated and *MAT**a*** DAmP haploids selected on synthetic media lacking histidine and supplemented with cycloheximide (10 μg/ml) and G418 (200 μg/ml). Following a repeated PCR to confirm correct integration of the Kan^R cassette, DAmP haploids were passaged on 5-Fluorouracil (5-FOA) to ensure loss of the haploid-selection plasmid and then stored at -80°C in 15% glycerol.

To generate the TAP-tagged DAmP and DAmP+Degron strains, we constructed plasmids bearing the TAP sequence either with or without a C-terminal CL1 degron[16], followed by the Kan^R cassette (see below for full sequences). Because the Kan^R cassette utilizes the same downstream regulatory sequences as the *HIS3* marker contained within all strains in the TAP-tagged library[12], restriction fragments generated from these plasmids can integrate with high efficiency due to homology with both the TAP tag and the Kan^R terminator. Existing TAP-tagged strains[12] were transformed with these restriction fragments and transformants selected on YEPD+G418. We confirmed transformants by PCR as described above using primers annealing to the TAP tag and the promoter region of the Kan^R cassette.

To generate constructs suitable for GFP and RFP expression, we inserted a cassette containing the *TEF2* promoter, GFP or RFP (dTomato[34]) and the *ADH1* terminator (cloned from *Ashbya gossypii*) into the *pFA6a-NATMX4* plasmid (see below for full sequences). This construct was then integrated into either the *his3Δ1* or *leu2Δ0* locus (see **Supplementary Data** for primer sequences used for integration) of a *MATα* strain compatible with Synthetic Genetic Array (SGA) methodology (*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 can1Δ::STE2pr-SpHIS5 lyp1Δ::STE3pr-LEU2*). The resulting strain was then crossed to the deletion and DAmP libraries and SGA-based methods were used to generate haploid GFP-tagged mutant strains for use in growth rate measurements. The RFP-expressing wildtype reference strain used in the growth competitions was constructed in an analogous manner (so that it would be isogenic to the mutant query strains) and contained the Kan^R cassette at *his3Δ1* and the NAT^R-dTomato construct at the *leu2Δ0* locus. A similar strategy was used to construct a wildtype strain not expressing any fluorescent protein (to control for any possible effects of fluorophore expression on growth).

GFP-tagged double-mutant deletion/DAmP strains for genetic interaction analysis were generated by crossing BY4741-derived *MAT**a*** single-mutant Kan^R strains from the deletion/DAmP libraries (or freshly created deletion strains made by standard PCR-based gene disruption) with SGA-compatible *MAT**α*** single-mutant NAT^R strains that were independently constructed by replacement of the gene of interest with the NAT^R-GFP cassette described above. Following sporulation, double-mutant haploids were selected using standard SGA methodology. Because growth measurements of strains bearing the appropriate individual gene deletions were also required for genetic interaction calculations (see below), we generated isogenic single-mutant strains by crossing the same Kan^R and NAT^R-GFP marked single gene deletion strains with appropriate wildtype strains bearing the Kan^R or NAT^R-GFP markers integrated at the *his3Δ1* locus.

Quantification of protein abundance

The relative abundance of proteins generated from the TAP-DAmP and TAP-degron+DAmP alleles was determined by Western blot analysis as previously described[12] using a TAP-specific antibody (rabbit anti-CBPTEV-N peptide, Bethyl Laboratories, Inc.) as well as an antibody against endogenous 3-phosphoglycerate kinase (Pgk1; Molecular Probes, Inc.) to control for variation in loading. Bands were detected and quantitated using an Odyssey Infrared Imaging System (Licor Biosciences).

Drug sensitivity assays

For disk analysis, ¼ inch paper disks (Schleicher & Schuell) were immersed in the various drugs at concentrations as follows: 10 mg/ml 5-FOA (SIGMA); 10 mg/ml Clotrimazole

(SIGMA); 50 mg/ml Sulfanilamide (SIGMA). Disks were placed on SD-complete plates on which 10^6 yeast cells of the appropriate genotype were plated. Fitness measurements of DAmP strains in tunicamycin were made using the flow cytometry technique as described above except media was supplemented with 0.25 μg/ml tunicamycin (SIGMA) following the initial cytometry measurement.

Data processing and analysis

Calculation of the ratio of mutant to wildtype cells for each flow cytometry measurement

First, unreliable cell measurements were identified and removed. For example, events counted in the first 0.2s and after 5.0s were omitted from analysis, as these time segments occasionally contained aberrant fluorescence values. Additionally, to eliminate non-cellular debris, we excluded counted events if they had undefined forward or side scatter intensities (i.e. equal to 0 or the maximum value of 2^18), or if they fell in the highest or lowest 0.1 percentile of FSC values or 0.2 percentile of SSC values. Typically, ~30,000 cells remained after these filters. Next, we assigned each cell measured to one of four categories: RFP^+/GFP^- (wildtype), GFP^+/RFP^- (mutant), GFP^+/RFP^+ (rare events caused by simultaneous counting of a wildtype and mutant cell), and GFP^-/RFP^- (very rare non-fluorescent cells). For GFP^-/RFP^+ cells, it was straightforward to classify them as wildtype on the basis of their reproducibly high RFP levels (analyzed using the PE-Texas Red 'Height' parameter) and low GFP intensity (analyzed using the FITC 'Height' parameter). GFP-containing mutant cells were also clearly identifiable but occasionally exhibited minor strain-to-strain variability in GFP intensity and RFP-channel auto-fluorescence, and so were counted using a modified strategy that automatically determined appropriate boundaries for a gate drawn around the GFP-expressing mutant cells. A similar

strategy was used to identify GFP^+/RFP^+ cells, and any cells that did not fall into the first three categories were assigned as GFP^-/RFP^-. It is worth noting that the existence of rare GFP^+/RFP^+ cells (typically ~2.5% of all cells) reflects the fact that two cells can occasionally pass through the cytometer simultaneously and be recorded as a single event. Although the possible distorting effects of such events are likely quite small due to their rarity, we corrected for this issue by calculating the number of true individual mutant and wildtype cells from the raw numbers of GFP^+/RFP^-, GFP^-/RFP^+, and GFP^+/RFP^+ (assuming that any two cells have an equal probability of being mis-recorded as a single event).

Relative growth rate calculation

Relative growth rates were calculated from the rate of change of the ratio of mutant to wildtype cells over the course of the competition. Assuming a constant relative growth rate x for the mutant strain and an initial ratio of mutant to wildtype cells r_0, then r_n (the ratio of mutant to wildtype cells after a given number of generations, n) is given by the equation:

$$r_n = r_0 \times 2 \wedge [\, n \times (x - x_{WT}) \,],$$

where 1 is the growth rate of the wildtype strain. Thus, $\log_2(r_n)$ changes linearly during the competition with slope equal to $x - x_{WT}$, i.e. the relative growth defect of the mutant strain. We therefore calculated this slope for each strain using the four timepoints at which we measured the co-cultures by flow cytometry using a weighted linear least-squares fit (with weights for each timepoint determined by the expected ($\sqrt{N}$) counting error given the actual number of GFP and RFP cells recorded). Also, because n in the above equation represents the number of doublings for the wildtype strain (and not for the overall co-culture), we used the change in the ratio between each pair of timepoints to calculate the number of wildtype doublings from the number

of co-culture doublings. In addition, for very sick strains, we commonly found that after an initial large decrease in the ratio of mutant to wildtype cells, by the final timepoint the ratio had leveled out at ~2^-10 – 2^-14 (~0-40 mutant cells) and did not continue to change, suggesting that this is our lower limit of detection. We therefore only used the first timepoint with a ratio < 2^-6 and excluded all timepoints with measured ratios < 2^-9. In the event that fewer than two timepoints remained, we assigned the mutant strain a relative fitness of "<0.50".

Identification and exclusion of unreliable measurements

A number of criteria were used to identify and eliminate potentially unreliable measurements. First, strains that did not pass through the strain construction process or with extreme growth defects, as identified by an initial ratio of mutant to wildtype < 2^-6, were removed from subsequent analyses. Second, individual timepoints for a strain in which the RFP or GFP intensity fluctuated significantly during the course of reading the well were removed. Similarly, individual timepoints for a strain were excluded if > 2.5% of cells were scored as GFP^-/RFP^- (typically the result of a technical problem with the flow cytometer high-throughput sampler causing aberrant fluorescence recordings). Third, strains were removed in cases where a good linear fit of log_2(ratio of mutant to wildtype cells) versus the number of generations elapsed could not be obtained, as determined by the total sum of the square of the residuals or by the presence of an individual residual that was unusually large. Fourth, genes required for mating and for the biosynthesis of histidine, lysine and arginine were excluded from analysis. We note that the number of strains removed using these criteria was limited. Thus, the vast majority of the ~600 deletion strains not included in our final data set are absent not because they were measured and excluded but because they were not present in our deletion library.

Data normalization

Because for each experiment we inoculated all co-cultures with a wildtype RFP strain that was originally grown from a single colony, it was important to control for the possibility of slight experiment-to-experiment variability in the growth rate of the wildtype strain. We therefore normalized the growth rates measured for each experiment (typically involving the measurement of ~2,500 mutant strains) such that the most common or 'typical' mutant had relative growth = 1.0000. To find the peak of the distribution of growth rates for each experiment, we used a Parzen window smoothing strategy (Supplementary Reference 3) in which the window was a normal distribution with $\sigma = 0.0045$. As illustrated in **Supplementary Figure 2**, this process clearly identifies the peak of the distribution. Furthermore, using the peak of the distribution rather than a property related to the median allowed us to avoid imposing any artificial bias on the number of strains with growth defects and seems reasonable (or even conservative) under the assumption that the most common deletion strain will have growth that is indistinguishable from wildtype. In further support of this strategy, measurements of the growth rate of a wildtype control strain not expressing GFP ranged from 1.0004 to 1.0022 after normalization (**Supplementary Fig. 2**).

Error analysis for growth rate measurements

Estimates of the number of strains with a growth defect were calculated based on the differences between replicate measurements of identical strains. Specifically, if measurement error is symmetrically distributed about zero, then the distribution of differences between replicate measurements can be used to construct a distribution of error expected for an arbitrary number of measurements. This is possible because the distribution of differences in replicate

measurements is equal to the convolution of the overall distribution of measurement errors with itself. For measurements of the deletion library, we modeled the distribution of measurement errors as a sum of two Gaussian distributions with parameters determined by nonlinear regression: $p(x) = 0.8453 \times N(0,0.0045) + 0.1457 \times N(0,0.0334)$, where $N(\mu,\sigma)$ is a normal distribution with mean μ and standard deviation σ. Importantly, this error model accurately reproduced our observed distribution of differences between replicate measurements (**Supplementary Fig. 3**). We then estimated the distribution of measured growth rates resulting from deletion strains with true growth rate 1.00 by normalizing and translating (by 0.001) the error distribution to match the peak of the histogram of all observed growth rates (**Fig. 3d**). Confidence that a strain with a given measured growth rate grows more slowly than wildtype can then be estimated as the fraction of strains with that approximate growth rate that are not accounted for by the error model. A similar procedure was used for the DAmP strains, except that since each of the DAmP strains was measured twice, there was no need to estimate the distribution of errors that gave rise to the observed distribution of replicate differences. Instead, the observed histogram of averaged growth rates was compared to an empirically generated error model constructed directly from the set of differences between replicate measurements divided by two. In each case, since measurement error increases for substantially slow-growing strains, only strains with a median growth rate of 0.95 or higher were used to generate the error distributions.

Calculation of genetic interactions scores

Interaction scores were calculated according to the equations for ε and ε_{NORM} described above (see **Results**). One exception is that, as originally proposed[6], a different normalization procedure is used for synthetic interactions, such that synthetic lethality gives a normalized score of -1 ($\varepsilon_{NORM} = \varepsilon / (W_A \times W_B$, for $\varepsilon < 0$). For *SET2* and genes encoding components of the Rpd3 and COG complexes, Kan^R- and NAT^R-GFP-marked deletion strains for each gene were used, thereby providing two independently constructed strains for each double mutant of interest. Fitness data from each separately constructed strain were averaged; these data were typically in very high agreement and were used to calculate and normalize genetic interaction scores. This averaging was not performed for proteasome mutant strains, as the separate Kan^R- and NAT^R-GFP-marked DAmP strains did not necessarily have identical growth rates. Instead, fitnesses and raw interaction scores were separately calculated for each independently constructed strain (i.e. *yfg1*-DAmP::Kan^R x *yfg2*-DAmP::NAT^R-GFP and *yfg2*-DAmP::Kan^R x *yfg1*-DAmP::NAT^R-GFP) and then averaged to generate average raw interaction scores. Similarly, because *yfg*-DAmP::Kan^R and *yfg*-DAmP::NAT^R-GFP do not necessarily have identical growth rates, it is possible that each construction of the *yfg1*-DAmP / *yfg2*-DAmP strain should be normalized using a different normalization factor. Thus, we averaged these normalization factors and used the resulting value to normalize averaged raw interaction scores. Importantly, this averaging does not distort the normalized interaction scores obtained.

Sequences for plasmids used in this study

pFA6a-KANMX6-DAmP:

GAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGCAGGTCGACGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGT
TTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCG**ACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACG**
TGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCC
ATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCC
CCTCACAGACGCGTTGAATTGTCCCCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGT
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ACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTC
TTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGCAAAACAGCA
TTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCA
TTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATA
ACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCAT
AAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGG
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GCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAA
TTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTG
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CCACTAGTGGCCTATGCGGCCGCGGATCTGCCGGTCTCCCTATAGTGAGTCGTATTAATTTCGATAAGCCAGGTTAA
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TAP-DAmP(KAN):

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TAP-DEG+DAmP(KAN):

GGTCGACGGATCCCCGGG**TTAATTAA**TCCATGGAAGAGAAGATGGAAAAAGAATTTCATAGCCGTCTCAGCAGCCAA
CCGCTTTAAGAAAATCTCATCCTCCGGGGCACTTGATTATGATATTCCAACTACTGCTAGCGAGAATTTGTATTTTC
AGGGAGAATTCGGCCTTGCGCAACACGATGAAGCCGTGGACAACAAATTCAACAAAGAACAACAAAACGCGTTCTAT
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gcagaaagtaatatcatgcgtcaatcgtatgtgaatgctggtcgctatactgctgtcgattcgatactaacgccgcc
atccagtgtcgaaaac**gagctc**gaattcatcga

Note: The sequence encoding the CL1 degron is highlighted in yellow. Plasmids containing these cassettes were digested with PacI and SacI (restriction sites underlined) and the resulting fragments used for transformations.

pFA6a-TEF2Pr-eGFP-ADH1-NATMX4:

GAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGCA**GGTCGACGGATCCCCGGGTT**AATTAAGGCGCGCCAGATCTGT
TTAGCTTGCCTTGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACG
TGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCC
ATACATTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCC
CCTCACAGACGCGTTGAATTGTCCCCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGT
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CGGCTGACCGTCGAGGACATCGAGGTCGCCCCGGAGCACCGGGGGCACGGGGTCGGGCGCGCGTTGATGGGGCTCGC
GACGGAGTTCGCCCGCGAGCGGGGCGCCGGGCACCTCTGGCTGGAGGTCACCAACGTCAACGCACCGGCGATCCACG
CGTACCGGCGGATGGGGTTCACCCTCTGCGGCCTGGACACCGCCCTGTACGACGGCACCGCCTCGGACGGCGAGCAG
GCGCTCTACATGAGCATGCCCTGCCCCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTT
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CTAGTACCATG*AGT*AAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAAT
GGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCAC
TACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCACTTATGGTGTTCAATGCTTTTCAAGATACC
CAGATCATATGAAACGGCATGACTTTTTCAAGAGTGCCAT*GCCCGAAGGTTATGTACAGG*AAAGAACTATATTTTTC
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GGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCA
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TCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTC
TCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCT
CACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCC
GACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGC
CACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCT
ACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGA

TCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATC
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GCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGC
GGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGGACATATTGTCGTTAGAACGCGGCTACAATTAATACATAA
CCTTATGTATCATACACATACGATTTAGGTGACACTATA

pFA6a-TEF2Pr-dTomato-ADH1-NATMX4:

GAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGCA**GGTCGACGGATCCCCGGGTT**AATTAAGGCGCGCCAGATCTGT
TTAGCTTGCCTTGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTGACG
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GATTGTACTGAGAGTGCACCATATGGACATATTGTCGTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCA
TACACATACGATTTAGGTGACACTATA

Supplementary References

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