Supplemental Information

To accompany Jakobson[*], Hartl[*], *et al.*

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Key Resources Table

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Key Resources Table

Reagent or resource	Source	Identifier
Chemicals		
Yeast nitrogen base	RPI	Y20040
Glucose	Fisher	D16-3
Uracil	Sigma	U0750
Agar	IBI	IB49170
Fluconazole	TCI	F0677
Ethanol (95%)	Fisher	04-355-226
Water (LC-MS grade)	Fisher Scientific	Cat 10509404; CAS: 7732-18-5
Acetonitrile (LC-MS grade)	Fisher Scientific	Cat 10489553; CAS: 75-05-8
Methanol (LC-MS grade)	Fisher Scientific	Cat 10767665; CAS: 67-56-1
Formic acid (LC-MS grade)	Fisher Scientific	Cat 5.33002; CAS: 64-18-6
Dithiothreitol (≥99.5%)	Sigma Aldrich	Cat 43815; CAS: 3483-12-3
Iodoacetamide (≥99%)	Sigma Aldrich	Cat I1149; CAS: 144-48-9
Ammonium Bicarbonate	Sigma Aldrich	Cat 40867; CAS: 1066-33-7
Urea	Sigma Aldrich	Cat 33247
Trypsin/Lys-C (Mass Spec Grade)	Promega	V5072
Solid-glass beads (borosilicate, diam. 4 mm)	Sigma Aldrich	Cat Z143936
Deposited data		
Mass spectrometry data	This study	Will be made available on the PRIDE archive upon acceptance
Experimental models: Strains		
RM11 haploid	[34]	YDJ6649

YJM975 haploid	[34]	YDJ6635
YJM975 *ERG11*$^{\text{122014T>C}}$	[34]	YDJ8281
YJM975 *ERG11*$^{\text{Asn433Lys}}$	[34]	YDJ8436
YJM975 *ERG11*$^{\text{122014T>C; Asn433Lys}}$	[34]	YDJ8437
RM11 *MCR1*$^{\text{G>A}}$	This study	YDJ8524
RM11 *NCP1*$^{\text{A>T}}$	This study	YDJ8525
RM11 *SER2*$^{\text{G>A}}$	This study	YDJ8526
RM11 *AAT2*$^{\text{G>A}}$	This study	YDJ8527
YJM975 *GCS1*$^{\text{C>T}}$	This study	YDJ8528
RM11 *IRA2*$^{\text{G>A}}$	This study	YDJ8578
YJM975 *IRA2*$^{\text{A>G}}$	This study	YDJ8529
Oligonucleotides		
MCR1 CRISPEY editing oligo GAGTTACTGTCTGTTTTCCTGTTACTTACTTTGTTGACGACAAGCAAGATGACCAAGACTTTGATGGTGAAATTAGTTTCATCTCCAAAGATTTTATTCAGGAGCATGTTCCAGGTCCAAAGGAAACCCGTTTCTTCTGACGTAAGGGTGCGCACAAGACTTTGATGGTGAAATGTTTCAGAGCTATGCTGGAA	This study	CMJP697
NCP1 CRISPEY editing oligo GAGTTACTGTCTGTTTTCCTGGTCAACCCGCTATTGTTCTCCAGCCAGCTTTATCGTTTTGCATTTTTTTTTCGGGCTGCTTTTCGTTCTTCGAGGACAAACGCACCTGTAAAGCTCAGAGGAA	This study	CMJP703

ACCCGTTTCTTCTGACGTAAGGGTGCGCAATCGTTTTGCATATTTTTTTGTTTCAGAGCTATGCTGGAA		
SER2 CRISPEY editing oligo GAGTTACTGTCTGTTTTCCTTTCTTGGCTACACCGATGATGAAATATACAATAGACAATGAAGAAAATAATGATAGATAGATGTAATAGAGTTTCTTTTTAAAATTGTTTATTTAAACTGAGGAAACCCGTTTCTTCTGACGTAAGGGTGCGCAGACAATGAAGAAAATAATGAGTTTCAGAGCTATGCTGGAA	This study	CMJP721
AAT2 CRISPEY editing oligo GAGTTACTGTCTGTTTTCCTAACTGCGTGGGTTTCTTCAAGTCGTTTAACCATTTGAGGAGTCAATCCTGTAAAGGAGAACATCCCGCATTGATTTACTATATGATCCCAGTTGCCAGGAAGGAAACCCGTTTCTTCTGACGTAAGGGTGCGCATTGAGGAGTCAACCCTGTAAGTTTCAGAGCTATGCTGGAA	This study	CMJP707
GCS1 CRISPEY editing oligo GAGTTACTGTCTGTTTTCCTAATCCATACATTTCTTATTTGCACCAATCTTTTGCAATTGCAAAAGACGCCTACGGGTATCTGGGTCCACTTTCCAATCTGACATGCTCTATAATCCGCGAGGAAACCCGTTTCTTCTGACGTAAGGGTGCGCAGCAATTGCAAAAGACGCCTGGTTTCAGAGCTATGCTGGAA	This study	CMJP706

IRA2 CRISPEY editing oligo (RM>YJM) GAGTTACTGTCTGTTTCCTAAGTTCAATACAAGAACTTTGCAAATTTTACAAAATATGATCAGTCATGTTCATGGAAACATTCTAACGACTTTGAGTTCCTCGATTCTTCCCCGCCACAAGGAAACCCGTTTCTTCTGACGTAAGGGTGCGCAAGTATGATCAGTCATGTTCAGTTTCAGAGCTATGCTGGAA	This study	CMJP709
IRA2 CRISPEY editing oligo (YJM>RM) GAGTTACTGTCTGTTTCCTAAGTTCAATACAAGAACTTTGCAAATTTTACAAAGTATGATCAGTCATGTTCATGGAAACATTCTAACGACTTTGAGTTCCTCGATTCTTCCCCGCCACAAGGAAACCCGTTTCTTCTGACGTAAGGGTGCGCAAATATGATCAGTCATGTTCAGTTTCAGAGCTATGCTGGAA	This study	CMJP710
Recombinant DNA		
CRISPEY editing plasmid: PDJ2318	[34]	PDJ2318
Software and algorithms		
DSSP	[84]	https://swift.cmbi.umcn.nl/gv/dssp/index.html
DIA-NN	[26]	https://github.com/vdemichev/DiaNN
maxLFQ	[82]	https://rdrr.io/cran/iq/man/maxLFQ.html
Other		

Custom genetic mapping code	This study	https://github.com/cjakobson/pqtl-mapping
Genetic mapping dependencies	This study	https://www.dropbox.com/scl/fo/3xbcbe9ivwz8aahrlk137/APGxHor01S7jnNX3a1Yk3Og?rlkey=yx81ckrtaq8eb5pu80ggprjhs&dl=0
Custom protein structure analysis code	This study	https://github.com/cjakobson/pop-gen-structure
Protein structure analysis dependencies	This study	https://www.dropbox.com/scl/fo/le2voq9djr79p2ehxqvs3/AGecke84_fLbzrsis2Ncpjk?rlkey=g5etvtwhay27j4y0sh6dh8dh7&dl=0

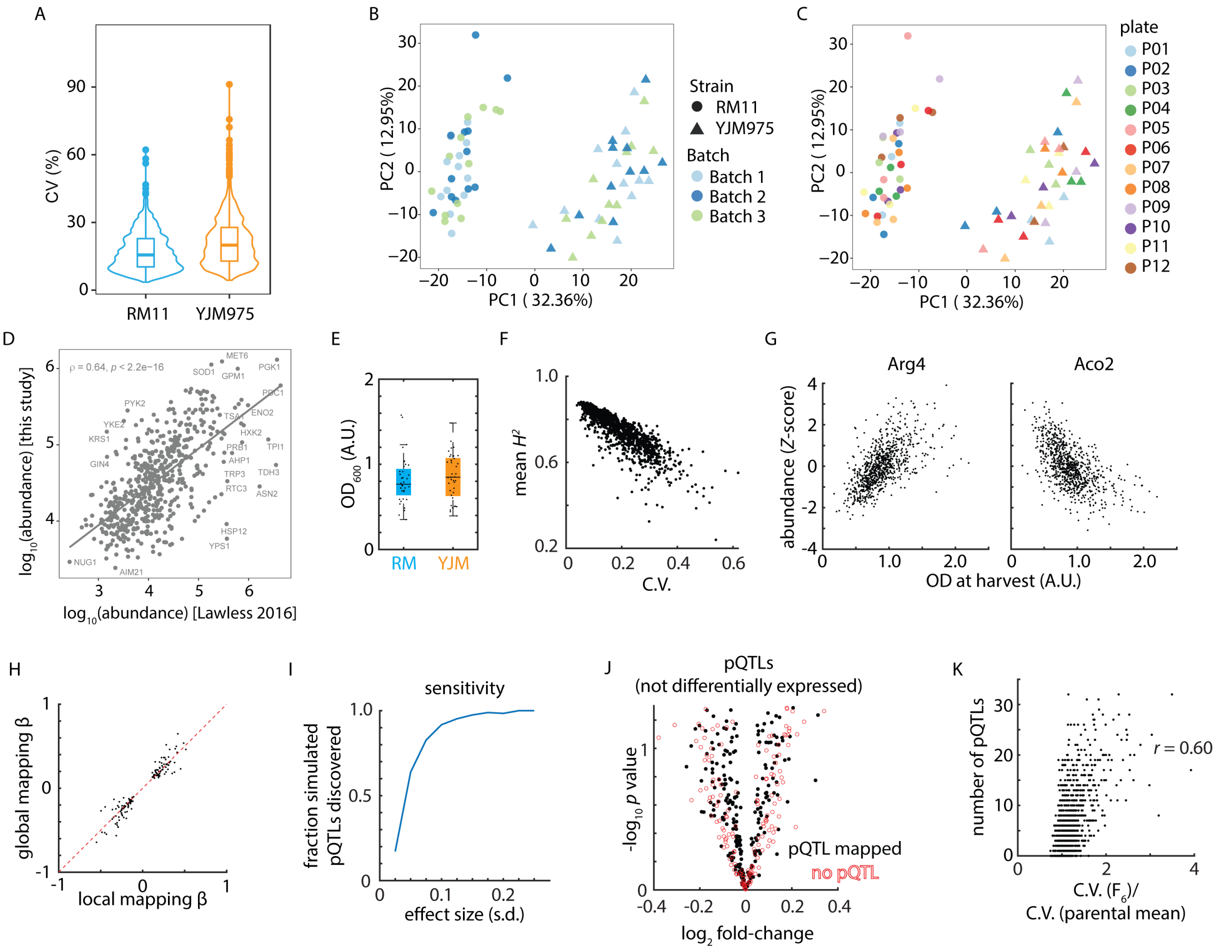

Figure S1. To accompany Figure 1. (A) Distribution of coefficients of variation (CVs) of all analyzed proteins (n=1225) from biological replicates of RM11 (n=36) and YJM975 (n=34) distributed and processed in 12 plates and measured in 3 LC-MS batches. (B-C) Variation according to principal components 1 and 2 of data described in (A) with only proteins without missing values (n=850) colored according to MS batch (B) or processing plate (C). (D) Median protein abundances from n=851 F_6 strains (ordinate) plotted and spearman correlated against absolute quantitative protein data from Lawless[85] (abscissa) for matching proteins (n=538). (E) Harvest OD_{600} of RM and YJM biological replicate samples. (F) Mean broad-sense heritability (ordinate) as a function of technical variability (C.V.; abscissa). (G) Abundance of Arg4 (left) and Aco2 (right) as a function of harvest OD_{600} amongst the F_6 progeny. (H) Genetic mapping coefficient (beta; units of st. dev.) from global (ordinate) and local (abscissa) approaches. Line of parity is shown in a red dashed line. (I) Genetic mapping sensitivity (fraction of simulated pQTLs discovered; ordinate) as a function of effect size (st. dev.; abscissa). Shown is the mean of $N = 100$ simulations of protein traits with 50 pQTLs. (J) Volcano plot illustrating $\log_2$ fold-change in protein abundance (abscissa) and Benjamini-Hochberg-corrected *t* test *p* value (ordinate) between the vineyard (RM) and clinical (YJM) parents highlighting proteins not differentially expressed between the parents. Closed symbols have a mapped pQTL; open symbols have no identified pQTLs. $n = 36 - 39$. (K) Number of pQTLs discovered (ordinate) as a function of normalized C.V. amongst the F6 progeny as compared to the mean C.V. in the parental isolates (abscissa).

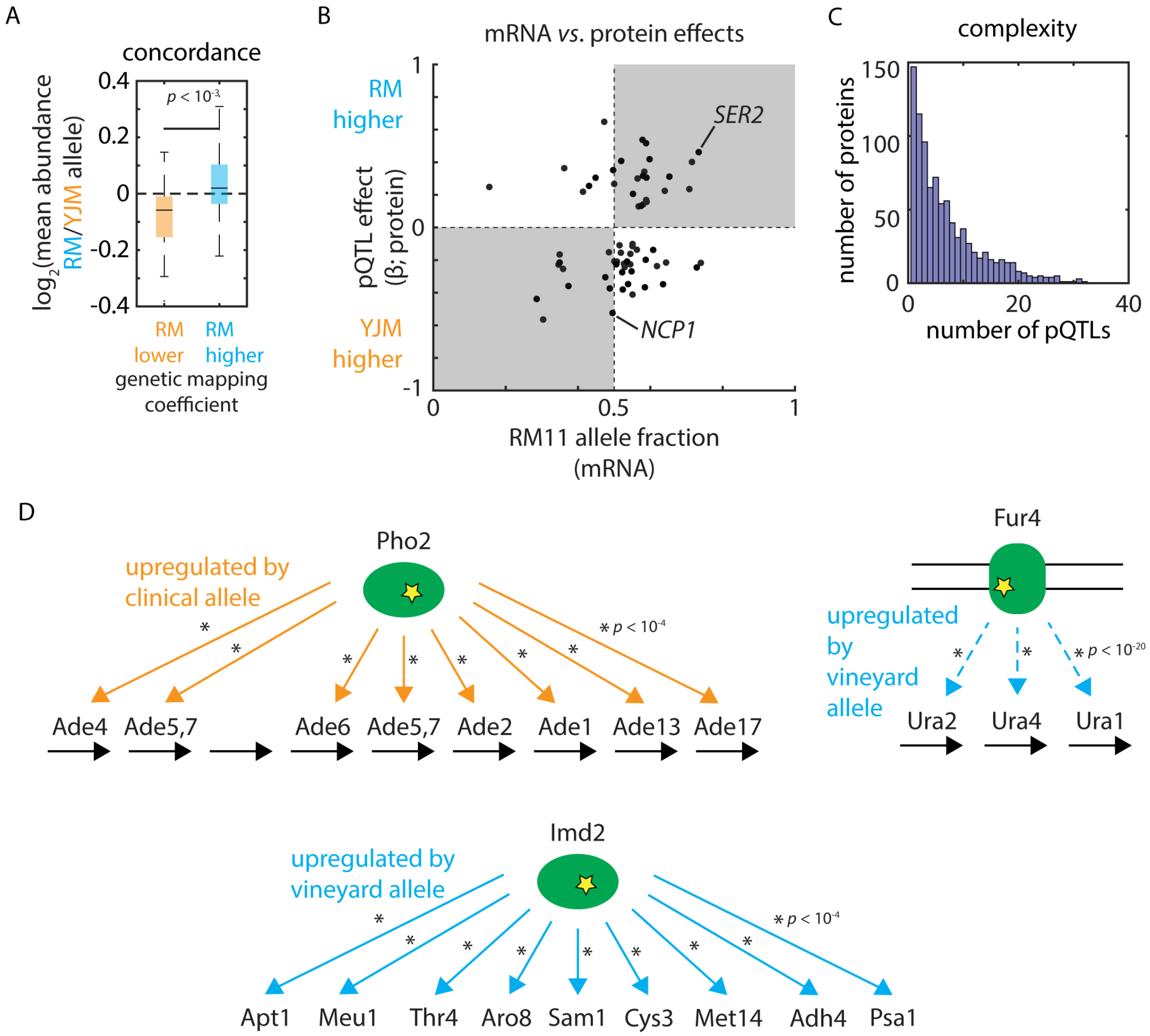

Figure S2. To accompany Figure 2. (A) Summary of replication across all *cis*-acting pQTLs we discovered; shown is $\log_2$ fold-change in mean protein abundance between 1,002 Yeast Genomes strains bearing the RM and YJM alleles in *cis*, divided by whether genetic mapping predicted the YJM or RM allele to exhibit higher protein level. p value by two-sided t test. (B) Predicted effect on protein level from genetic mapping (ordinate) and measured effect on mRNA level from allele-specific expression analysis (abscissa) for regulatory *cis*-pQTLs. Highlighted are *cis*-pQTNs selected for reconstruction. (C) Frequency of proteins (ordinate) as a function of the number of controlling pQTLs (abscissa). (D) Schematic of adenine biosynthetic pathway enzymes controlled by Pho2, uracil biosynthetic pathway enzymes controlled by Fur4, and metabolic enzymes controlled by Imd2.

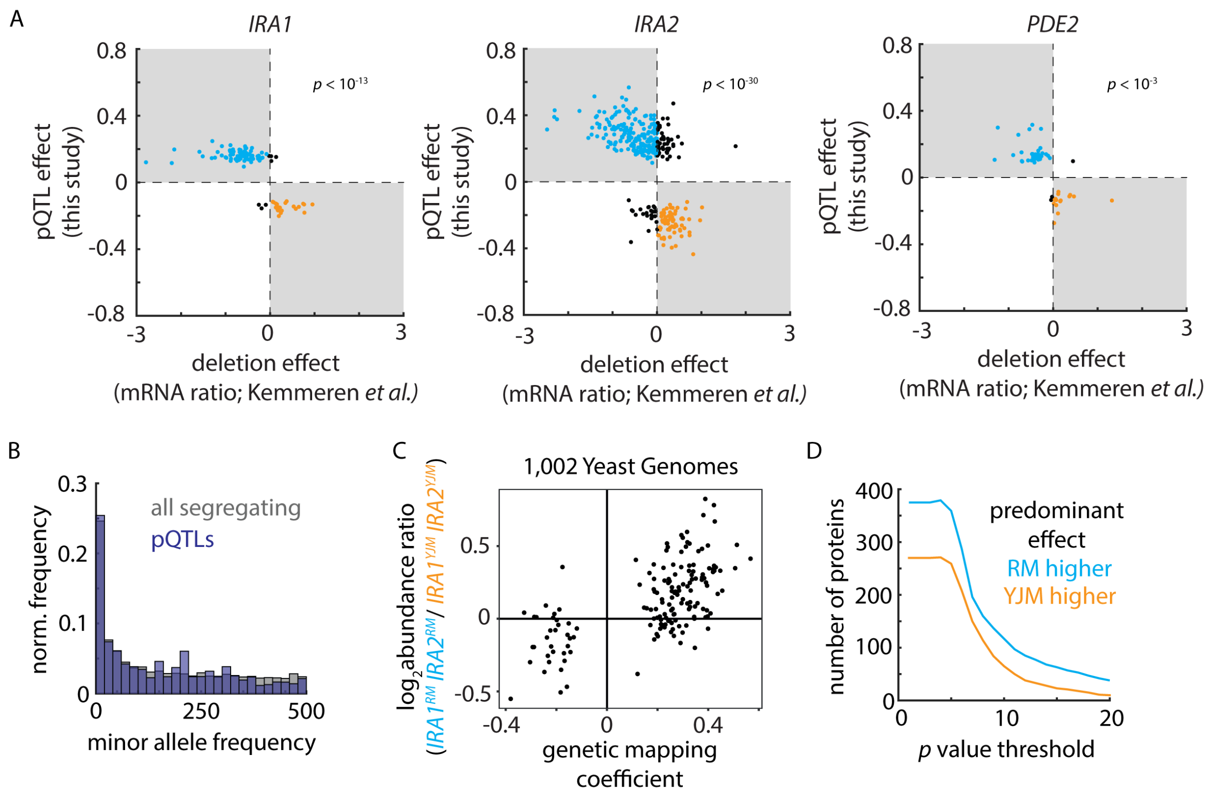

Figure S3. To accompany Figure 3. (A) Predicted effects of *trans* pQTNs from genetic mapping (ordinate) as a function of measured mRNA effects of deleting the corresponding gene (abscissa) [44] for *IRA1*, *IRA2*, and *PDE2*, as indicated. *p* values by *t* statistic. (B) Normalized frequency (ordinate) of minor allele frequencies amongst the 1,002 Genomes collection (absicssa) for pQTL variants (blue) and all segregating variation amongst the F_6 progeny (grey). (C) Relative abundance of Ira1 and Ira2 targets (ordinate; identified by pQTL mapping) in wild yeast proteomes bearing $IRA1^{RM}$ and $IRA2^{RM}$ alleles (n=5) as compared to strains with $IRA1^{YJM}$ and $IRA2^{YJM}$ (n=371) as a function of predicted effect from pQTL mapping (abscissa). (D) Number of proteins (ordinate) predominantly controlled by RM-higher *trans* pQTL alleles (blue) or YJM-higher *trans* pQTL alleles (orange) as a function of genetic mapping *p* value (abscissa).

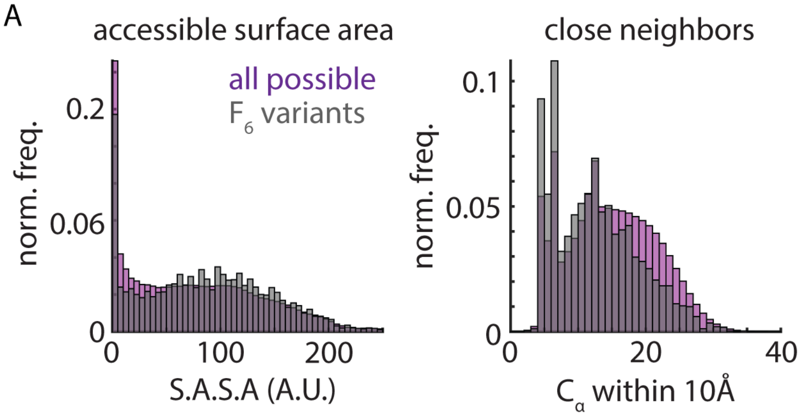

Figure S4. To accompany Figure 4. (A) Normalized frequencies of solvent-accessible surface area (left) and number of C_α within 10Å (right) for all possible missense SNPs (purple) and all missense variants segregating in the F_6 mapping panel (grey).

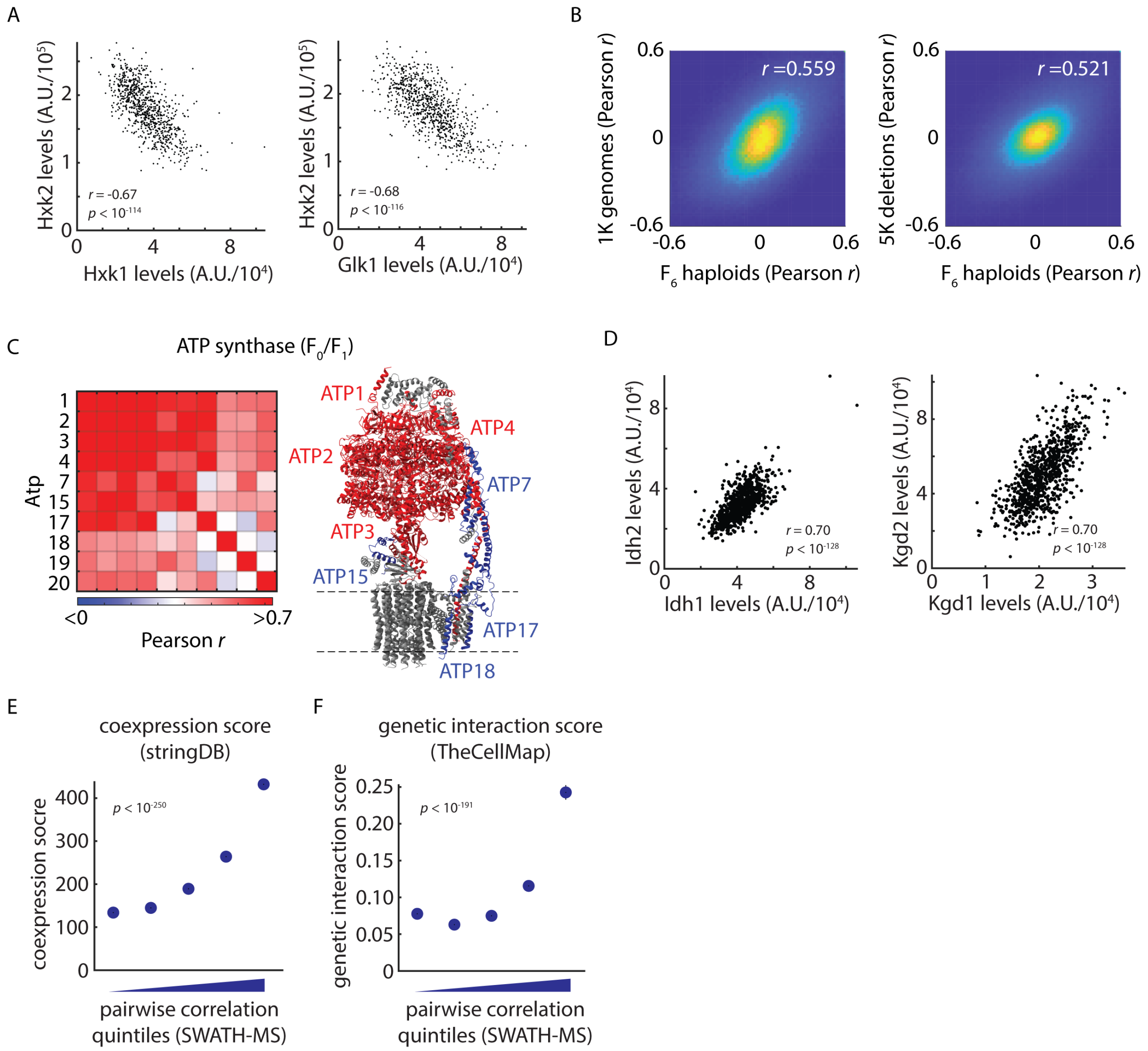

Figure S5. To accompany Figure 5. (A) Top: Hxk2 levels (ordinate) as a function of Hxk1 levels (abscissa) amongst F_6 progeny. Bottom: As above, but for Hxk2 and Glk1. (B) Left: Correlation between protein-protein correlations amongst 1,002 Genomes strains (ordinate) [32] and the same statistic amongst the F_6 progeny (abscissa). Shown is Pearson's *r*. Right: As on the left, for protein-protein correlations amongst precise deletions[30]. (C) Pairwise correlations of observed ATP synthase components (left) and cryo-EM structure of the yeast ATP synthase (6CP6)[86] with subunits highlighted as indicated (right). (D) Left: Idh2 levels (ordinate) as a function of Idh1 levels (abscissa) amongst F_6 progeny. Right: As left, but for Kgd2 and Kgd1. (E) Coexpression score from stringDB for ascending quintiles of protein pairs sorted by SWATH-MS abundance correlation. (F) As in (E) for the genetic interaction score from TheCellMap.

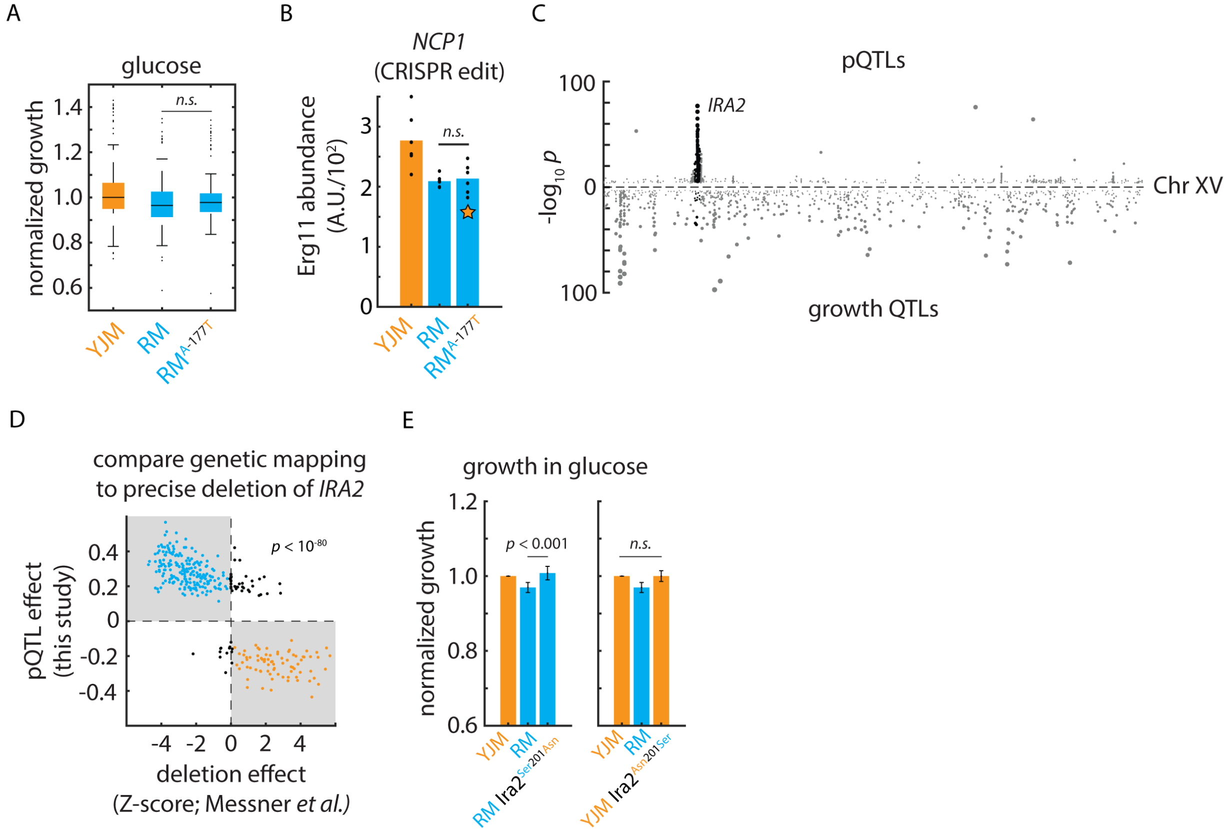

Figure S6. To accompany Figure 6. (A) Growth in glucose of clinical (YJM), vineyard (RM), and CRISPR-edited RM $NCP1^{A\text{-}177T}$ mutant strains in glucose. $n = 96$; p value by Student's t test. (B) CRISPR reconstruction and mass spectrometry to test the effect of the $NCP1^{\mathrm{A\text{-}177T}}$ variant on Erg11 levels. $n = 6$; p value by two-sided t test. (C) Miami plot of pQTLs (top) and growth QTLs (bottom) on Chromosome XV. *IRA2* pQTLs and QTLs are highlighted in black. (D) Predicted *IRA2* pQTL effects from genetic mapping (this study; ordinate) as compared to measured effects of *IRA2* precise deletion (Z-scored by protein; [30]; abscissa) for all proteins predicted to be controlled by *IRA2*. p value from t statistic. Estimated abundances normalized to wild-type in each case. (E) Growth of clinical (YJM), vineyard (RM), and CRISPR-edited RM $\mathrm{Ira2}^{\mathrm{Ser201Asn}}$ mutant (left) and YJM $\mathrm{Ira2}^{\mathrm{Asn210Ser}}$ mutant (right) in glucose. $n = 96$; p values by Student's t test.

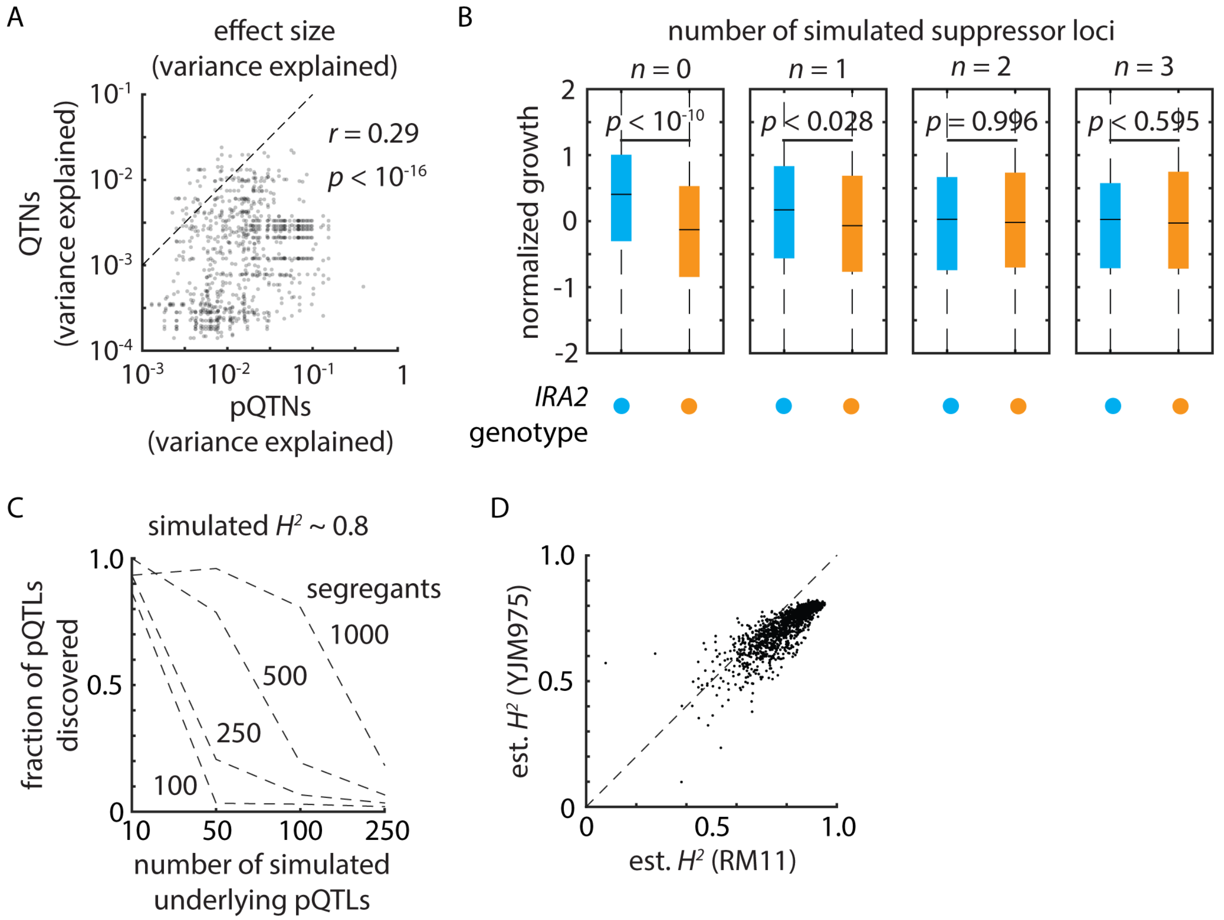

Figure S7. To accompany Figure 7.(A) Variance explained by phenotypic QTNs (ordinate) as a function of variance explained by pQTNs mapped to the same variant (abscissa). r by Pearson's correlation; p value from t statistic. (B) *In silico* simulations of the apparent effect of a linear QTL at *IRA2* with the number of segregating suppressing alleles indicated. p value by t test. (C) Fraction of simulated pQTLs discovered (ordinate) as a function of the number of true underlying simulated pQTLs (abscissa) for hypothetical pQTL mapping panels with the number of segregants indicated. N = 3 simulations for each parameter combination. (D) Estimated broad-sense heritability H^2 in YJM (ordinate) as a function of estimated H^2 in RM (abscissa) for all measured proteins.

Upregulated in YJM975		***Upregulated in RM11***	
TF	***p* value**	**TF**	***p* value**
Sfp1	$< 10^{-22}$	Sut1	$< 10^{-11}$
Stb3	$< 10^{-16}$	Msn2	$< 10^{-11}$
Abf1	$< 10^{-10}$	Msn4	$< 10^{-11}$
Sum1	$< 10^{-10}$	Hap3	$< 10^{-9}$
Gcn4	$< 10^{-8}$	Abf1	$< 10^{-9}$
Tod6	$< 10^{-7}$	Hap5	$< 10^{-9}$
Dot6	$< 10^{-6}$	Gis1	$< 10^{-9}$
Arg81	$< 10^{-6}$		
Swi4	$< 10^{-4}$		

Supplemental Table S6. PSCAN transcription factor target enrichments and *p* values for proteins up- and down-regulated in the YJM975 and RM11 parents.