Supplementary Information

Supplementary Note 1 – Reproducibility

We performed replication experiments for 27 genes. Of these, 22 were experimental replicates where the same segregant pool was thawed, grown and subjected to X-pQTL mapping at two different times. The remaining five replicates were done from independently generated crosses for a given gene. All replicates were processed several months apart from each other. We randomly assigned one of the two replicates as the detection set, and asked to what extent the X-pQTL identified in the detection set reproduced in the validation set.

Across the 27 detection experiments, we discovered 240 X-pQTL at genome-wide significance. We first asked whether the validation experiment had an allelic effect in the same direction (i.e. with higher expression associated with either the BY or the RM allele), irrespective of significance in the validation set. The direction of effect was concordant at 234 (97.5%) of the X-pQTL. We next asked what fraction of X-pQTL was reproduced at genome-wide significance in the replication set. We found that peaks with higher LOD scores were more likely to be reproduced, ranging from 58% replication at LOD $\geq$ 4.5 to perfect replication at higher LODs (Supplementary Table 1). Genome-wide significance in both datasets is a strict criterion for the small effects detected in our study. We therefore also employed a relaxed replication criterion, where we required the validation set to show an allele frequency difference of at least 0.05 (~ half that required for genome-wide significance) and a concordant direction of allelic effect. Using this criterion, 80% of loci reproduced at detection LOD $\geq$ 4.5, rapidly approaching perfect replication at higher LOD thresholds (Supplementary Table 1).

In sum, we found that loci with strong effect virtually always reproduce. Loci of smaller effect sometimes fail to reach genome-wide significance in a replication experiment, likely due to stochastic variation in the influence of small-effect loci. Notably, even loci of small effect are still concordant in their direction of effect in the vast majority of cases. Extended Data Figure 4 illustrates these patterns for three genes.

Supplementary Table 1 – Reproducibility statistics

LOD threshold in detection set	X-pQTL	Replicated at genome-wide significance	Replicated at relaxed significance & concordant direction*
4.5	240	139 (58%)	191 (80%)
5	216	128 (59%)	176 (81%)
10	86	69 (80%)	78 (91%)
20	30	29 (97%)	30 (100%)
50	10	10 (100%)	10 (100%)

*see Supplementary Note 1 for details

Supplementary Note 2 – Influence of small effect sizes on eQTL detection

We sought to test to what extent X-pQTL that did not overlap a significant eQTL nevertheless influence mRNA levels. The analyses were restricted to the 701 X-pQTL that did not overlap a significant eQTL, were not located on chromosomes II and III, and were not located on the chromosome on which the given gene is located. For each of these X-pQTL, we extracted the published [1] mRNA levels of the given gene from segregants with the BY allele and from segregants with the RM allele. We then performed a T-test comparing these mRNA levels and recorded the p-value. The p-value distribution across all the examined positions was used to compute π_0, the fraction of true negative tests and $\pi_1 = 1 - \pi_0$, a lower bound for the fraction of true positive tests [2]. π_1 thus provides a lower bound for the fraction of X-pQTL that affect mRNA levels. We used the R package qvalue [2] for these calculations.

We obtained an estimate of $\pi_1 = 36\%$. Taken at face value, this suggests that 64% of X-pQTL are due to genetic variation that specifically influences posttranscriptional regulation, without affecting mRNA levels. We sought to explore the alternative explanation that π_1 might underestimate the fraction of true positive tests if effect sizes are small. We note that π_1 is designed to be a lower bound of the fraction of true positive tests in a multiple testing scenario [2], but we here sought to quantify this effect in more detail.

We performed simulations of the situation where a single position in a genome is tested for a difference in phenotypes (such as mRNA levels for a given gene) between haploid individuals of either of two genotypes. The test is a T-test of the phenotypes in the two groups. To form the two groups, we randomly sampled 50 phenotypes each from a normal distribution with standard deviation = 1. Individuals in the first group had mean phenotype = 0, while those in the second group had a higher mean phenotype = x. For $x << 1$, the expected variance explained by the group difference (i.e., the "eQTL" effect size) is $x^2/4$. For each x, we generated 5,000 sets of two groups (resembling 5,000 "genes"), performed a two-sided T-test in each set, recorded the p-values and calculated π_1 from the distribution of the 5,000 p-values. Importantly, these simulations probe the behavior of π_1 in the situation where *every* test is truly positive because x is never equal

to 0. If power were sufficiently high, π_1 should equal 1 irrespective of x. Any lower value of π_1 is a consequence of low power due the relatively small sample size of 100 individuals.

Extended Data Figure 7 shows that for high x, π_1 indeed approaches 1. At smaller x, however, π_1 is reduced along with the power to detect individual "genes" to be significant. For very small x, power is at chance level, and π_1 estimates are near zero. Importantly, π_1 of 0.3 – 0.4 (as seen in our actual data) is reached at values of x that correspond to an effect size of 0.5 – 1% of variance explained (center row). Thus, rather than requiring wide-spread posttranscriptional consequences of genetic variation, the observed estimate of π_1 can also be explained by many (perhaps most or even all) X-pQTL having real but small effects on mRNA levels.

References for Supplementary Note 2

1. Smith, E. N. & Kruglyak, L. Gene–Environment Interaction in Yeast Gene Expression. *PLoS Biology* **6,** e83 (2008).
2. Storey, J. D. & Tibshirani, R. Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences* **100,** 9440–9445 (2003).

Supplementary Table 2 – Local X-pQTL results

Gene	Allele frequency difference	Local LOD
YAL005C	0.06	2.4
YAL060W	0.24	23.9
YBR067C	-0.18	12.9
YCL040W	0.31	31.5
YDL126C	-0.15	13.7
YDL171C	-0.03	0.5
YEL002C	0.01	0.4
YER069W	0.11	2.6
YGL026C	-0.17	8.6
YGL195W	0.15	11.4
YGL202W	-0.23	27.7
YGL253W	0.88	84.9
YGR086C	0.02	0.5
YGR204W	0.50	101.9
YGR234W	-0.75	252.0
YHL011C	0.03	1.1
YIR022W	-0.12	7.9
YJL052W	0.11	3.8
YJL130C	-0.13	6.4
YJL201W	0.01	0.5
YJR048W	0.02	0.8
YKL029C	-0.75	184.2
YKL035W	-0.05	0.9
YKR059W	0.05	1.8
YKR080W	0.06	1.5
YLL026W	0.74	246.5
YLR075W	-0.02	0.4
YLR179C	-0.81	169.0
YLR244C	0.43	72.0
YLR325C	-0.04	1.4
YLR438W	0.05	1.0
YML024W	0.03	1.1
YMR315W	0.16	12.7
YNL044W	0.03	0.5
YNL055C	0.66	134.0
YNL061W	-0.20	20.0
YNL134C	0.49	73.8
YNR016C	0.00	0.4
YPL028W	0.01	0.7
YPL048W	-0.02	0.4
YPR156C	0.07	1.5

Supplementary Table 3 – Proteins affected by the hotspot on chromosome II

Gene	Hotspot effect	Function	translation / ribosome related?
RPS17A	0.30	ribosomal protein small subunit	yes
TIF1	0.23	translation initiation factor eIF4A	yes
PRT1	0.22	translation initiation factor subunit eIF3b	yes
TEF4	0.21	translational elongation factor	yes
RPS25A	0.19	ribosomal protein small subunit	yes
ADO1	0.18	adenosine kinase	
UTP4	0.18	Subunit of U3-containing 90S preribosome; involved in 18S rRNA production	yes
RPL9A	0.16	ribosomal protein large subunit	yes
RPL19A	0.16	ribosomal protein large subunit	yes
SUP45	0.16	Polypeptide release factor eRF1	yes
RPL21B	0.16	ribosomal protein large subunit	yes
LEU4	0.16	leucine biosynthesis	
ILS1	0.15	Isoleucine tRNA synthetase	
RPL13B	0.15	ribosomal protein large subunit	yes
CAM1	0.15	transcription factor involved in ribosome biogenesis	yes
YOP1	0.15	membrane traffic	
SUR4	0.14	elongase involved in fatty acid and sphingolipid biosynthesis	
URA5	0.14	pyrimidine biosynthesis	
RPL10	0.14	ribosomal protein large subunit	yes
ADE3	0.13	biosynthesis of purines, thymidylate, methionine, and histidine	
NOP58	0.13	pre-rRNA processing, 18S rRNA synthesis, and snoRNA synthesis	yes
ILV6	0.12	branched-chain amino acid biosynthesis	
SMI1	0.12	cell wall synthesis	
PUB1	0.11	Poly (A)+ RNA-binding protein required for mRNA stability	
TPI1	0.11	Triose phosphate isomerase involved in glycolysis	
LHP1	0.11	tRNA processing	
YIP3	0.11	ER to Golgi transport	
TIF3	0.11	Translation initiation factor eIF-4B	yes
TRX2	0.11	cell redox homeostasis	
URA2	0.11	pyrimidine biosynthesis	
ERG10	0.11	ergosterol biosynthesis	
TRX1	0.10	cell redox homeostasis	
CPR1	0.10	cellular protein metabolism	
YLR413W	0.10	unknown	
DBP3	0.10	rRNA processing	yes
YLR179C	0.10	unknown	

PFY1	0.09	cytoskeleton organization
LIA1	0.09	cytoskeleton organization
COX17	0.09	copper transport, mitochondrial respiration
PRS3	0.09	nucleotide, histidine, and tryptophan biosynthesis
TDH3	0.08	glycolysis
PDB1	0.08	pyruvate dehydrogenase
TPO1	0.08	transmembrane transporter
PHO86	0.08	ER to Golgi transport
ZWF1	0.07	pentose phosphate pathway
WBP1	0.07	protein glycosylation
CIT1	-0.10	citrate synthase, TCA cycle
HSP104	-0.13	chaperone
GPH1	-0.15	glycogen phosphorylase