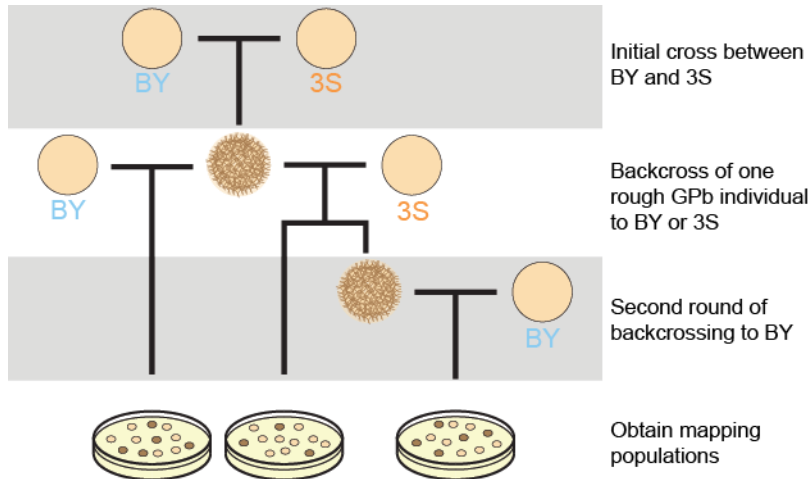**Figure S1: GPb and GPc individuals differ in their temperature sensitivities. (a)** A subset of GPb backcross segregants express rough morphology at 30 and 37°C. **(b)** GPc backcross segregants are incapable of expressing the trait at 30 and 37°C. Ninety-six backcross segregants were generated and phenotyped for each GP-backcross parent combination.

Figure S2: Scheme to generate GPb backcross mapping populations. A rough BYx3S F_2 segregant carrying GPb was backcrossed to BY and 3S. In the case of the cross to 3S, a second-generation backcross to BY was then performed, which was necessary to enrich for segregants that express the rough phenotype at 37°C.
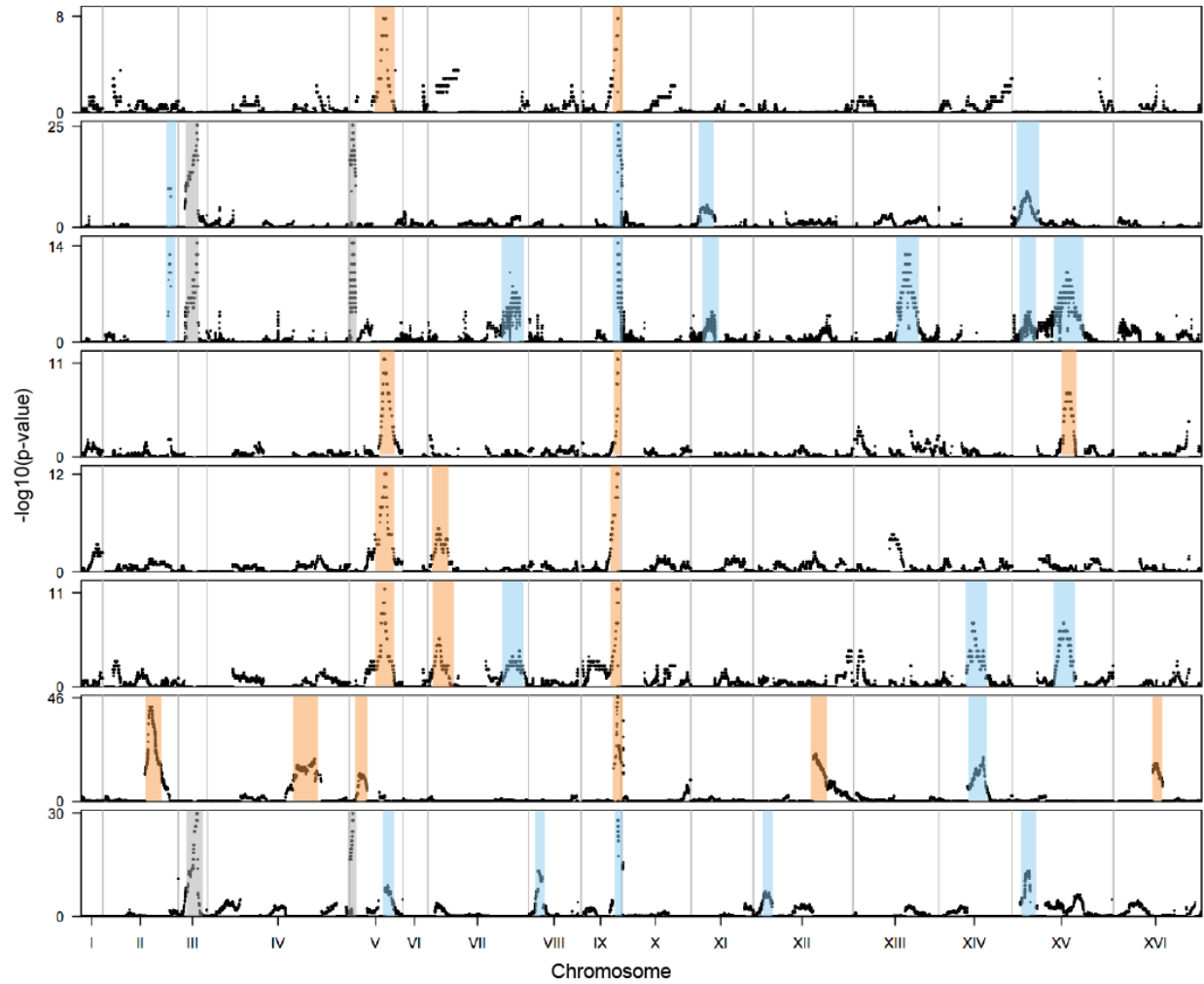
Figure S3: Genetic mapping of loci interacting with GPb and GPc. For each mapping population based on GP, backcross, and temperature sensitivity, a binomial test was performed for each SNP to identify regions of the genome associated with the rough phenotype. Results in are plotted in -$\log_{10}$(p)-values in the following order: GPb (BY backcross, 21, 30, 37°C), GPb (3S backcross, 21°C), GPb (3S backcross, 21, 30°C), GPb (2nd generation backcross, 21°C), GPb (2nd generation backcross, 21, 30°C), GPb (2nd generation backcross, 21, 30, 37°C), GPc (BY backcross, 21°C), GPc (3S backcross, 21°C). Blue bars indicate loci originating from BY, and orange bars indicate loci from 3S. Gray bars denote selectable markers used in generating mapping populations. Details regarding each locus are found in Table S1.
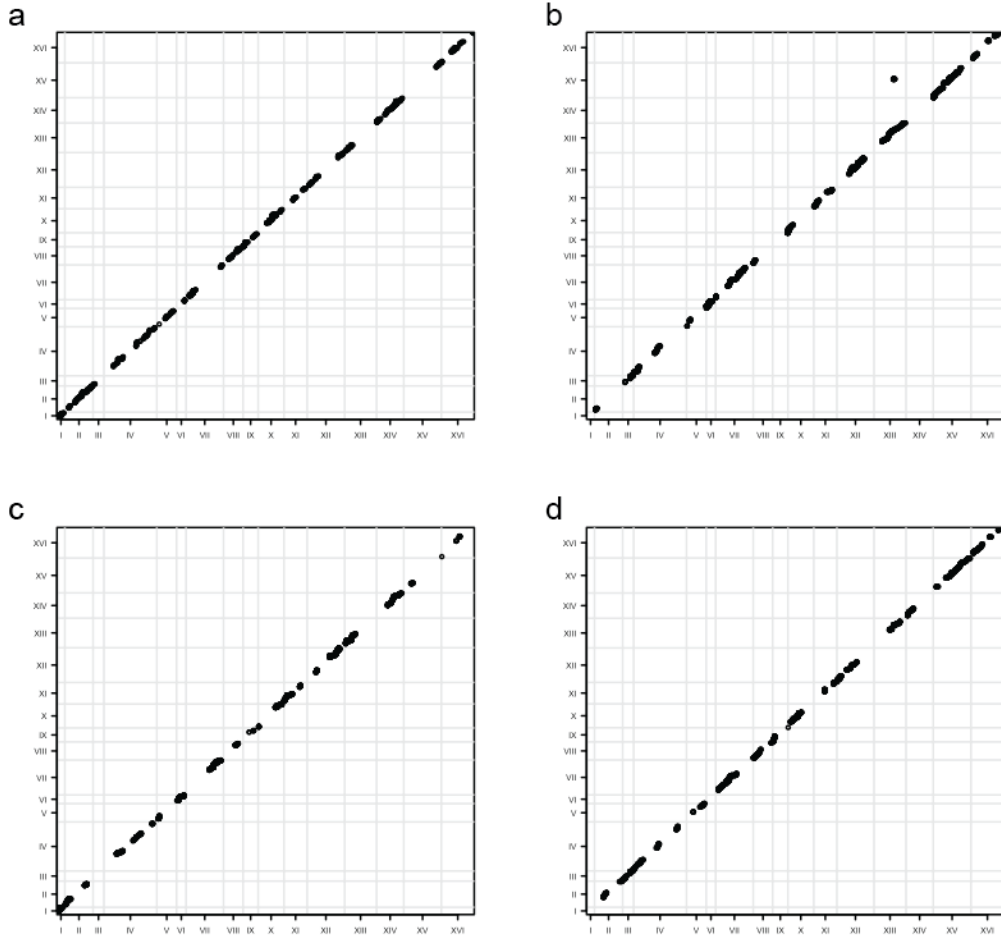
Figure S4: Scan for loci with correlated allele states. Significant p-values for χ^2 tests between each pair of uniquely segregating genomic regions: GPb – **(a)** backcross to BY, **(b)** backcross to 3S, and GPc – **(c)** backcross to BY, **(d)** backcross to 3S. Results are only plotted for the upper triangle. Significant results were only found in the GPb backcross to 3S, identifying an interaction between the genes *MSS11* and *SFL1*.
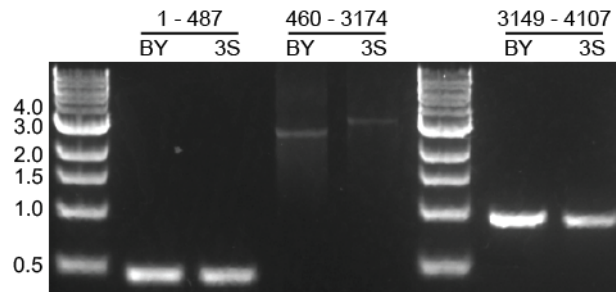
Figure S5: Variation in the *FLO11* coding region. The 3S strain carries a longer *FLO11* allele relative to BY. This is due to a ~500-600 nucleotide length polymorphism in the middle of the gene. Nucleotide positions are provided based on the BY gene sequence.
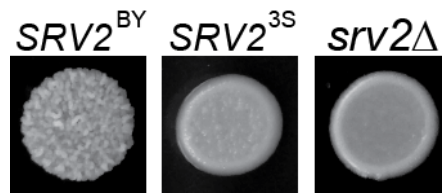
Figure S6: The *SRV2* gene underlies a locus on chromosome XIV that interacts with GPb. Replacement of the BY *SRV2* allele with the 3S version results in loss of the rough phenotype. A similar loss of phenotype is also observed when *SRV2* is deleted.
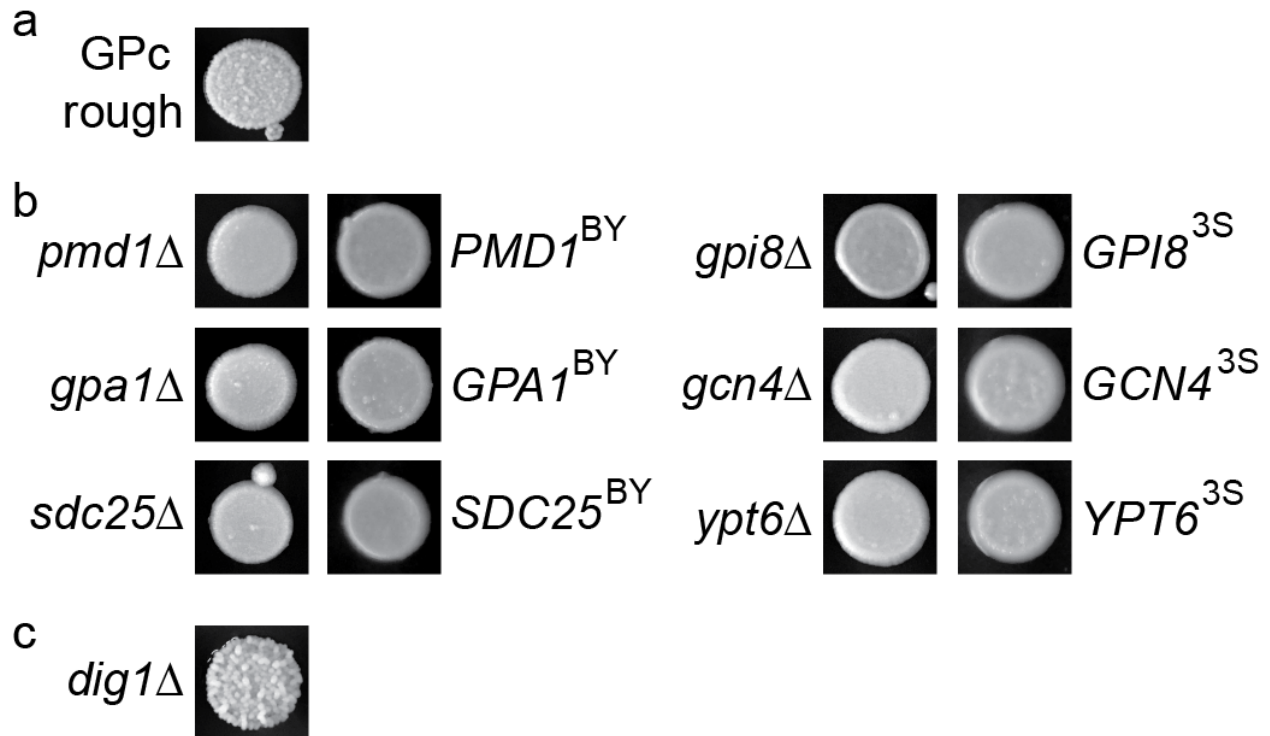
Figure S7: Genetic engineering supports interactions between GPc and identified candidate genes. Complete deletion of the candidate genes in a GPc rough segregant was performed using tailed *kanMX* cassettes. The phenotype of the GPc rough segregant is shown in **(a)**. In **(b)**, we show that deletion of six candidate genes causes loss of the phenotype. We also show that allele replacement with the non-causal allele at these genes results in no phenotypic recovery, implying these genes harbor functional genetic variation. Note, these replacements involved the entire coding region, as well as 100 to 200 bases of promoter sequence and 100 to 200 bases of downstream sequence. Control experiments were performed in parallel and resulted in restoration of the phenotype. In **(c)**, for the seventh candidate gene, deletion enhances the phenotype. Notably, *DIG1* encodes a repressor of the *FLO11* activator Ste12. Thus, the phenotypic enhancement seen upon its deletion is consistent with cryptic variation promoting transcription of *FLO11*.

Note S1: For GPb individuals expressing the rough phenotype at 21°C, we detected two multi-locus genotypes among segregants from the backcross to 3S. We also identified a third genotype in the second-generation backcross population, involving a different combination of alleles than those from the initial 3S backcross.

Note S2: We deleted each non-essential gene at the GPb chromosome II locus – *RRT2*, *HIS7*, *ARO4*, *MRPS5*, *MTC4*, *SHG1*, and *YBR259W* – and found that none of these deletions caused loss of the rough phenotype. This implies that loss-of-function in a non-essential gene is not responsible for the trait at this locus, and the causal genetic variant is either a gain-of-function polymorphism or is an essential gene. Among the essential genes at this locus, *SRB6* was determined to be the strongest candidate.