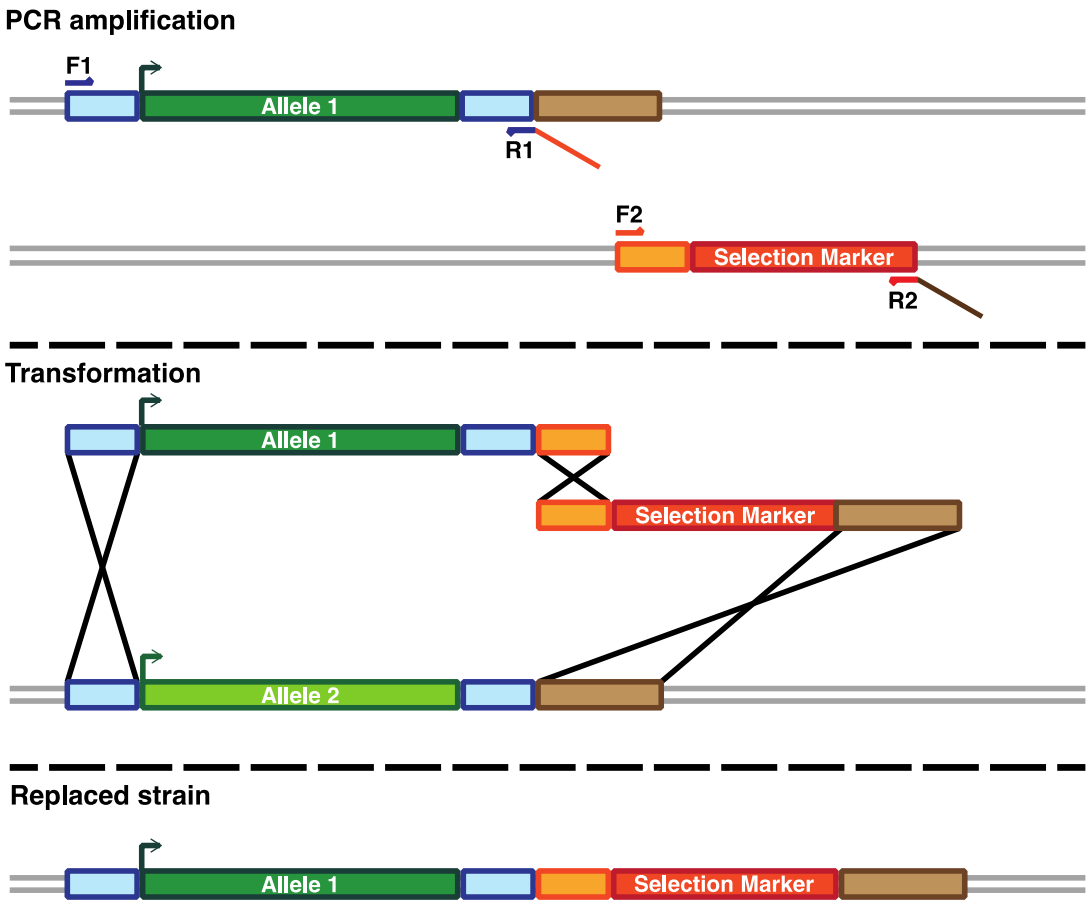

Figure S1. Construction of allele replacements. In the first step, one pair of primers (F1 and R1) was used to amplify the promoter and the coding sequence of the gene to be replaced with 60 bp overlapping the 5' end of the resistance marker attached at the 3' end of the PCR product (shown in orange). Another pair of primers (F2 and R2) was used to amplify the resistance marker with 60 bp overlapping the genomic region immediately downstream of the transcribed potion of the gene using the first primer pair attached at the 3' end of the PCR product. In the second step, the two overlapping PCR products were transformed into the strains. Integration into the genome requires recombination between the PCR products and the target locus.

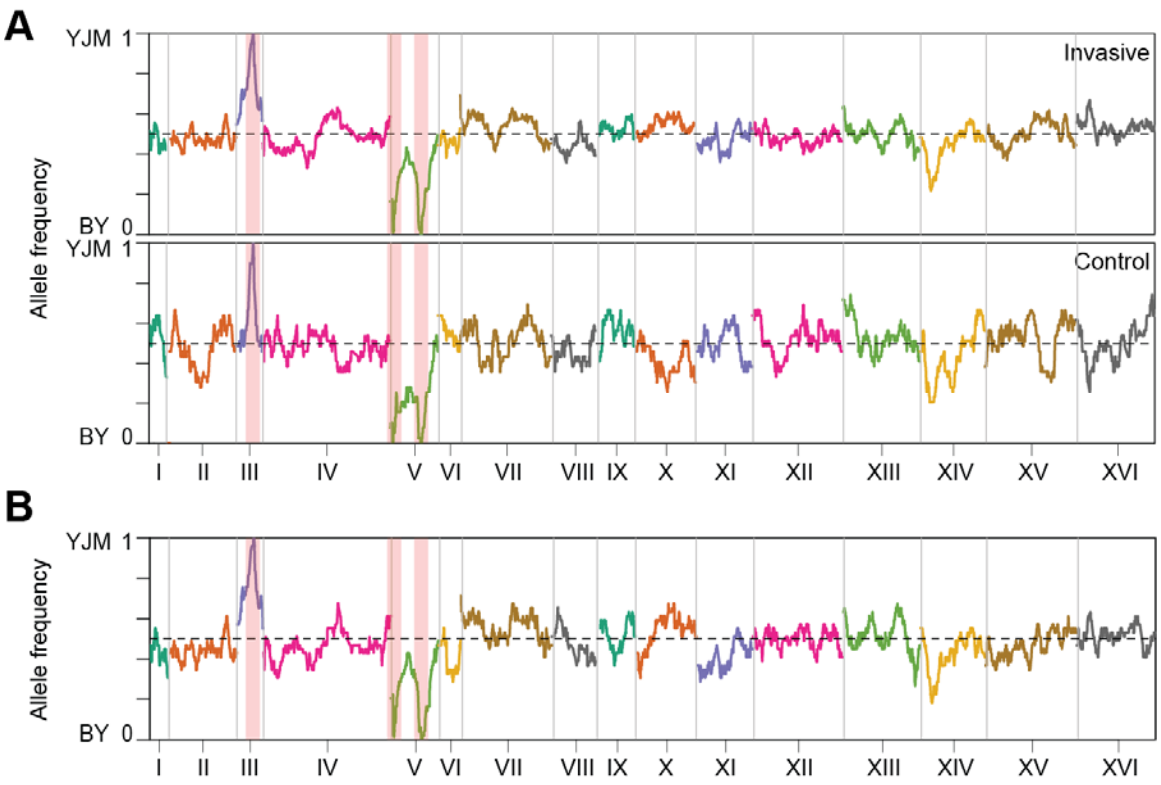

Figure S2. Initial results from selective genotyping of segregants that show *FLO8*-independent invasion. (**A**) Comparison of genome-wide relative allele frequency plot among *FLO8*^{BY} invasive progeny to a non-invasive *FLO8*^{BY} control population. (**B**) Genome-wide relative allele frequency plot among *FLO8*^{BY} segregants that invade on both glucose and ethanol.

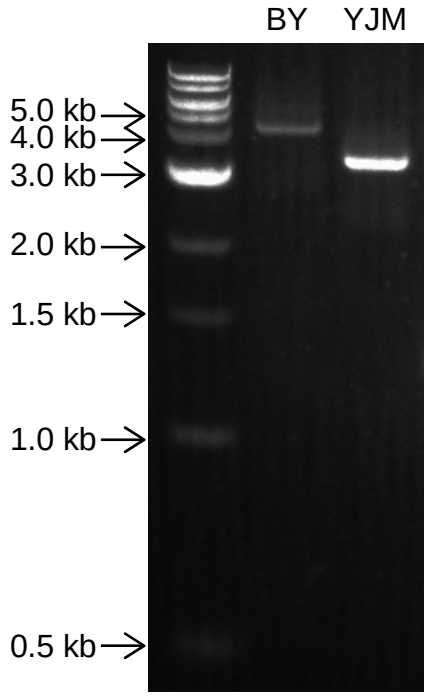

Figure S3. Differences *FLO11* coding region length between BY and YJM. PCR was used to amplify the *FLO11* coding region from the BY and YJM strains. The size of *FLO11*^{BY} was ~4.1kb, while *FLO11*^{YJM} was ~3.4kb.

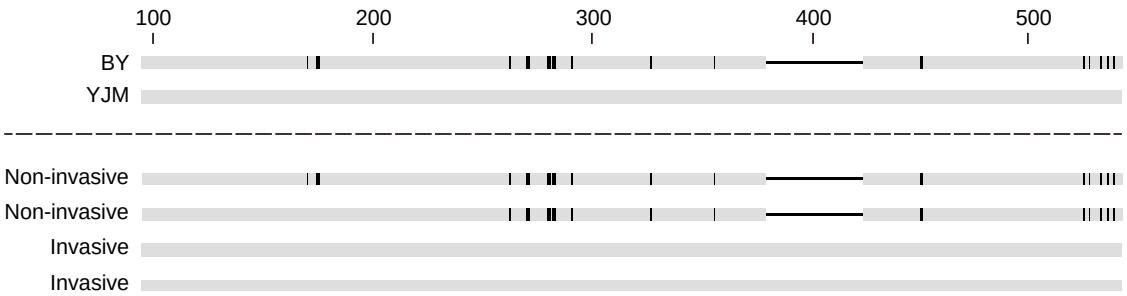

Figure S4. **Replacement of the *FLO11* coding region in segregant 2 with the BY allele causes loss of invasion.** To verify that *FLO11*^{BY} was correctly integrated and replaced using our one-step allele replacement, we PCR amplified the 5' end of the gene, and Sanger sequenced multiple invasive and non-invasive transformants. Only the transformants carrying the BY SNPs (marked in black) toward the 5' end showed loss of invasion, implying that only individuals with most of the *FLO11* gene replaced exhibited loss of invasion. Flo11 protein is comprised of three domains, which are reflected in the sequence of the *FLO11* gene. The N-terminal portion of the protein encodes a hydrophobic signal sequence, is exposed at the cell surface, and binds to ligands. The middle domain largely contains variable length tandem repeats that are enriched for serines and threonines, and is the part of the protein where heavy glycosylation occurs. The C-terminal portion of the protein is a GPI anchor that localizes Flo11 to the cell wall. The highly repetitive nature of the middle portion of *FLO11* makes it difficult to accurately determine the length and sequence of the gene using short Illumina reads. In the regions that we were able to confidently align, we identified 69 SNPs between the BY and the YJM allele, of which 31 were non-synonymous. In addition, we identified that the YJM allele of *FLO11* has a 45bp insertion in the N-terminal region between amino acid position 123 and 124. We also found that no sequencing reads from the YJM mapped to 635 base positions in comparison to BY, which is most likely due to deletions given that the YJM allele of *FLO11* was ~700 bases smaller in comparison to the BY allele (Figure S4). In particular, large stretches of the middle domains were missing from amino acid positions 207 to 315, 359 to 372, 409 to 449, 795 to 808, 824 to 845, and 881 to 899 in the YJM allele. We have not yet determined how these changes alter the functionality of Flo11. We note that this portion of the gene is known to be highly variable across yeast strains, affecting many *FLO11*-dependent traits, such as biofilm formation, flocculation, and invasion.

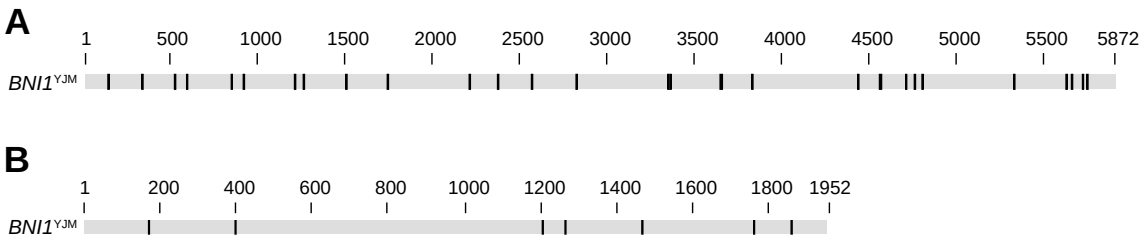

Figure S5. Alignment of the *BNI1* gene. (**A**) Alignment of the nucleotide sequences identified 31 SNPs between the BY and YJM allele of *BNI1*. (**B**) Alignment of the translated amino acid sequence revealed that 7 SNPs were nonsynonymous.

Table S1 Phenotype data and Short Read Archive identifiers for the segregants examined in the paper.

Available for download as an Excel file at
www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.180661/-/DC1

Tables S2-S6

Available for download as .txt files at
www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.180661/-/DC1

Table S2. Genotype data for the initial 127 BYxYJM segregants. Genotypes in this table, as well as the following tables, are encoded as 0 for BY and 1 for YJM.

Table S3. Genotype data for the additional 55 BYxYJM segregants that show *FLO8*-independent invasion.

Table S4. Genotype data for the backcross of Segregant 2 to BY.

Table S5. Genotype data for the backcross of Segregant 2 to YJM.

Table S6. Genotype data for the backcross of Segregant 3 to BY.

Table S7. Analysis of dissected tetrads from homozygous diploid derivatives of specific segregants. Phenotypes of spores from homozygous diploid versions of Segregants 1, 2, and 3.

Segregant	Tetrad	MATa spore 1	MATa spore 2	MATalpha spore 1	MATalpha spore 2
1	1	N	N	N	N
1	2	I	N	N	N
1	3	I	N	N	N
1	4	I	I	N	N
1	5	I	I	N	N
1	6	I	I	N	N
1	7	I	I	N	N
1	8	I	I	N	N
1	9	I	I	N	N
1	10	I	I	N	N
2	1	I	I	I	I
2	2	I	I	I	I
2	3	I	I	I	I
2	4	I	I	I	I
2	5	I	I	I	I
2	6	I	I	I	I
2	7	I	I	I	I
3	1	I	I	I	I
3	2	I	I	I	I
3	3	I	I	I	I
3	4	I	I	I	I
3	5	I	I	I	I
3	6	I	I	I	I
3	7	I	I	I	I

I = Invasive, N = Non-invasive