HOPE et al SUPPLEMENTARY TABLES:

**Table S1: Raw quantitative measurements for settling ratios.** Data visualized in main text Figure 1 and Supplementary Figure 4, provided as separate Excel file.

**Tab 1: Clone data rep1.** Three measurement replicates from ImageJ (columns B-H) are provided for the 60-minute settling image for each evolved clone, with a calculation of the coordinate of half of the maximum grey value (I) as in (Hope and Dunham 2014), and a final calculation of the settling ratio (J).

**Tab 2: Clone data rep2.** A second biological replicate for each original clone, with three measurement replicates per image.

**Tab 3: Segregant data.** A single measurement and ratio is provided for multiple progeny from the backcross of each original evolved clone (numbers in column A) to the laboratory strain FY4.

**Table S2: List of high quality mutations called for each evolved clone from whole genome sequencing.** Provided as separate Excel file. Raw sequencing data is available under NCBI BioProject PRJNA339148, BioSample accessions SAMN05729740-5729793 and filtering was performed as described in Materials and Methods, with additional filtering steps detailed below. Mutations verified as causal have been highlighted in green. This table lists high quality SNPs (Tab 1), indels (Tab 1), Ty element insertions (Tab 2), structural variants (SVs) (Tab 3), and copy number variants (CNVs) (Tab 4) for all evolved clones. Tabs 1-3 have the following column labels: Sample: sample number (note "EV" samples are from second evolution experiment); Chrom: chromosome; Pos: chromosome location; Ref: reference allele; Alt: alternative allele; Qual: quality score; Info: descriptive mutation information generated by bcftools, retroseq, or lumpy; Format: format of genotype information; Genotype: genotype information generated by bcftools, retroseq, or lumpy; Mutation type: coding-nonsynonymous, coding-synonymous, intergenic, or 5'-upstream; Gene: systematic gene name; AA: amino acid annotation; Gene Alias: common gene name. Tab 4 has the following additional column labels: Start: start location of copy number segment; End: end location of copy number segment; Copy Number: average copy number for segment, calculated using DNAcopy.

**Tab 1: SNPs_indels.** SNPs/indels present in 7 or more samples for the original 23 evolved clones or 4 or more samples for the 5 new evolved clones were filtered out to remove common false positives or ancestral mutations, using bcftools isec (Li and Durbin 2009; Faust and Hall 2014). Mutations were then filtered for quality (QUAL>50, DP>=10), and mutations annotated as telomeric, mitochondrial, LTR_retrotransposon, intergenic, or coding-synonymous were removed. All remaining mutations were visually confirmed in IGV. See bcftools documentation for genotype and quality annotation information.

**Tab 2: retroseq.** Ty insertions were called using the program retroseq (Keane, Wong, and Adams 2013) with non-default parameters (discover: -q 28 -id 85 -len 25 -align, call: -depth 400), annotated (97), and verified by visual inspection in IGV. Intergenic mutations were ignored, excepting FLO gene promoters, which were manually re-annotated as 5’-upstream mutations. Additionally, known Ty insertions in the FLO1 promoter were not called by retroseq in Samples 2, 4 and 8, though PCR and visual inspection indicate otherwise. Note that retroseq gives inexact breakpoints so insertion positions are approximate. See retroseq documentation for genotype and quality annotation information.

**Tab 3: lumpy.** SVs were called using the program lumpy (Layer et al. 2014) with default parameters. SVs with at least 10 supporting reads were confirmed using visual inspection in IGV. See lumpy documentation for information on SV type and quality scores.

**Tab 4: CNV.** CNVs were called as described in Material and Methods using the R program DNAcopy (Seshan and Olshen 2015), with additional visual inspection to validate the findings. To remove noise that would otherwise cause unnecessary splits, a standard deviation correction of 2 was implemented. Chromosomes or chromosome segments that had a copy number different from 1 are listed.
**Table S3: Aggregation candidate genes.** Curated list of candidate genes with known contributions to separation defects, flocculation, or other biofilm-related phenotypes and their publication(s) of origin.

| Gene | Systematic Name | Study |
|------|-----------------|-------|
| ACE2 | YLR131C         | Ratcliff 2015 |
| AGA1 | YNR044W         | Brückner and Mösch 2012 |
| AMN1 | YBR158W         | Li 2013 |
| ASH1 | YKL185W         | Brückner and Mösch 2012 |
| CDC28| YBR160W         | Lee 2011 |
| CYC8 | YBR112C         | Brückner and Mösch 2012 |
| CYR1 | YJL005W         | Granek 2013 |
| DEP1 | YAL013W         | Brückner and Mösch 2012 |
| DIA1 | YMR316W         | Palecek 2000 |
| END3 | YNL084C         | Taylor 2014 |
| FIG2 | YCR089W         | Brückner and Mösch 2012 |
| FLO1 | YAR050W         | Brem 2002 |
| FLO10| YKR102W         | Brückner and Mösch 2012 |
| FLO11| YIR019C         | Granek 2013, Ryan 2012 |
| FLO1p| YAR062W         | Vestrepen 2005 |
| FLO5 | YHR211W         | Brückner and Mösch 2012 |
| FLO8 | YER109C         | Granek 2013, Brem 2002, Taylor 2014, Ryan 2012 |
| FLO9 | YAL063C         | Roop 2013 |
| FUS3 | YBL016W         | Brückner and Mösch 2012 |
| GCN1 | YGL195W         | Granek 2013 |
| GCN2 | YDR283C         | Brückner and Mösch 2012 |
| GCN4 | YEL009C         | Brückner and Mösch 2012 |
| GLN3 | YER040W         | Brückner and Mösch 2012 |
| GBP1 | YOR371C         | Taylor 2016 |
| HAA1 | YPR008W         | Brückner and Mösch 2012 |
| HDA1 | YNL021W         | Brückner and Mösch 2012 |
| HOG1 | YLR113W         | Cullen 2015 |
| HOT1 | YMR172W         | Granek 2013 |
| IRA1 | YBR140C         | Roop 2013, Taylor 2016 |
| IRA2 | YOL081W         | Roop 2013, Taylor 2014, Taylor 2016 |
| IRC8 | YJL051W         | Taylor 2016 |
| KSS1 | YGR040W         | Brückner and Mösch 2012 |
| MFG1 | YDL233W         | Ryan 2012 |
| MGA1 | YGR249W         | Borneman 2006, Brückner and Mösch 2012 |
| MSB2 | YGR014W         | Brückner and Mösch 2012 |
| MSN2 | YMR037C         | Granek 2013 |
| MSS11| YMR164C         | Su 2009, Kim 2004, Kim 2014, Taylor 2014, Ryan 2012 |
| NRG1 | YDR043C         | Brückner and Mösch 2012 |
| Gene | Systematic Name | Study |
|------|----------------|-------|
| NRG2 | YBR066C        | Brückner and Mösch 2012 |
| PGU1 | YJR153W        | Cullen 2015 |
| PHD1 | YKL043W        | Borneman 2006, Brückner and Mösch 2012 |
| PPM1 | YDR435C        | Granek 2013 |
| PRP42| YDR235W        | Granek 2013 |
| RGA1 | YOR127W        | Li 2013 |
| RGT1 | YKL038W        | Granek 2013 |
| RIM101| YHL027W       | Brückner and Mösch 2012 |
| RME1 | YGR044C        | Brückner and Mösch 2012 |
| SFL1 | YOR140W        | Brückner and Mösch 2012, Taylor 2016 |
| SKS1 | YPL026C        | Granek 2013 |
| SLF1 | YDR515W        | Granek 2013 |
| SNF1 | YDR477W        | Bruckner and Mosch 2012 |
| SOK2 | YMR016C        | Borneman 2006, Brückner and Mösch 2012 |
| SOL3 | YHR163C        | Granek 2013 |
| SSN3 | YPL042C        | Taylor 2016 |
| SSN8 | YNL025C        | Taylor 2016 |
| STA1 | STA1           | Kim 2004, Kim 2014 |
| STE12| YHR084W        | Kim 2004, Brückner and Mösch 2012 |
| SWI5 | YDR146C        | Brückner and Mösch 2012 |
| TEC1 | YBR083W        | Kim 2004, Brückner and Mösch 2012 |
| TPK1 | YJL164C        | Brückner and Mösch 2012 |
| TPK2 | YPL203W        | Brückner and Mösch 2012 |
| TRR1 | YDR353W        | Taylor 2014 |
| TUP1 | YCR084C        | Brückner and Mösch 2012 |
| URE2 | YNL229C        | Brückner and Mösch 2012 |
| YAK1 | YJL141C        | Granek 2013, Brückner and Mösch 2012 |
| YAP1 | YML007W        | Brückner and Mösch 2012 |
| Primer   | Sequence 5'-3' | Amplicon size | Experiment                                                                 |
|----------|----------------|---------------|-----------------------------------------------------------------------------|
| EH030PF  | CAATATGCAAGCTCCTGGCA | 2.2kb         | Amplifies FLO11 repeats from S288C - matches Up776flo11 primer from Zara 2009 pair for EH030PF |
| EH030PR  | GCCAGGGTGTTTGGATGATG | 2.2kb         |                                                                                                           |
| EH045PF  | GAATTGTGCGGACGGCTCCTC | 507bp        | Amplifies HSL7 around potential secondary modifier mutation for YMD2683 pair for EH045PF                  |
| EH045PR  | GTGGAGGGCGCCAATTTAGC | 507bp        |                                                                                                           |
| EH046PF  | CTGCCAGCGCTACTATCTCA | 676bp        | Amplifies IRA1 around potential secondary modifier mutation for YMD2683                                                                 |
| EH046PR  | GCATTCACACTCGACTGCTT | 676bp        | pair for EH046PF                                                                                          |
| EH047PF  | GCTACCTGCAATTGCATCAC | 533bp        | Amplifies VTS1 around potential secondary modifier mutation for YMD2683 pair for EH047PF                 |
| EH047PR  | GACCAGCATTTAGGATCGTA | 533bp        |                                                                                                           |
| EH048PF  | GAGAGGCACACTAGAGAGTA | 596bp        | Amplifies TCP1 around potential secondary modifier mutation for YMD2683 pair for EH048PF              |
| EH048PR  | CAGAGTCAGCACCAGATGTC | 596bp        |                                                                                                           |
| CJA007F  | TCCACGGAGACATACGGTTG | 2.1kb/8.1kb  | Amplifies promoter region of FLO1 to identify Ty insertions; 2.1kb without Ty; 8.1kb with Ty pair for CJA007F |
| CJA007R  | TGTCCTCCGACAGAACCTAG | 2.1kb/8.1kb  |                                                                                                           |
| CJA009F  | TATTCGGAAAGGGCATGATGTC | 2.5kb       | Validates correct insertion of FLO1 into S288C genome for knockout construction pair for CJA009F        |
| CJA009R  | TAAGCGAACCACACTAGATC | 2.5kb        |                                                                                                           |
| EH052PF  | GCTCATCCTTATTCGACTTCC | 391bp       | Amplifies HOG1 around potential secondary modifier mutation for YMD2690 pair for EH052PF               |
| EH052PR  | GTATGGCCTGGTTACCGTAG | 391bp       |                                                                                                           |
| EH053PF  | CTACAGCTCTTATCCGGTGTC | 425bp       | Amplifies MIT1 around deletion to confirm breakpoints for YMD2694 pair for EH053PF                     |
| EH053PR  | ATTGTTCGCGTGACCAGCATAG | 425bp     |                                                                                                           |
| EH054PF  | ATCTTTTTCTGGATAGGAGGCC | 6.4kb      | Amplifies YBLWTy2-1 from S288C genome pair for EH054PF                                                |
| EH054PR  | CAAGAGGGAGCCGCTATTC | 6.4kb        |                                                                                                           |