

Figure 1

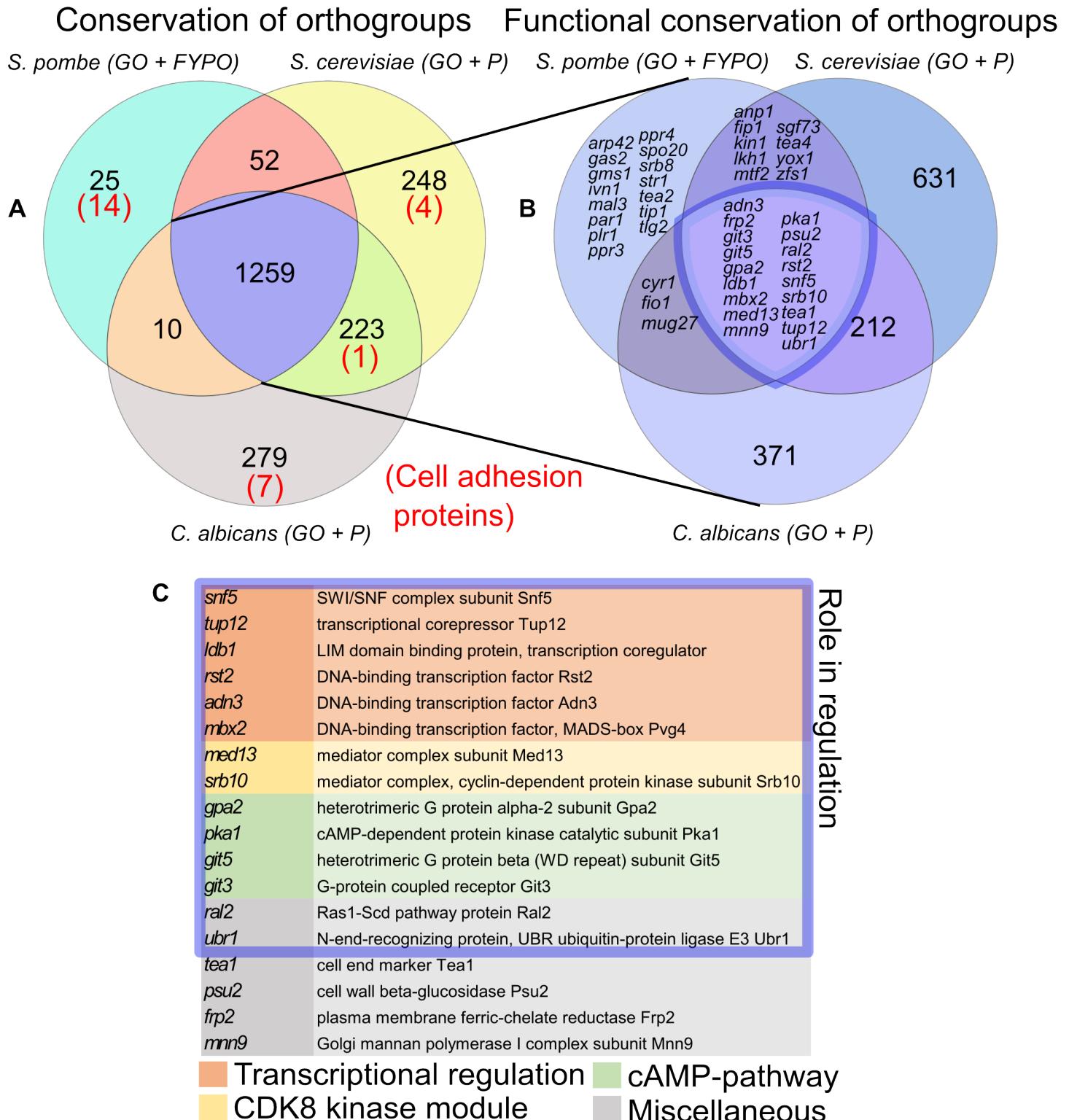


Figure 1: Several regulators of MLP formation are conserved between fission and budding yeast, but cell-adhesion effector proteins are not. A: Venn diagram of numbers of orthogroups, in which at least one gene in the orthogroup is annotated in GO-terms or phenotypic data related to MLP formation in either *S. pombe*, *S. cerevisiae* or *C. albicans*. Red numbers indicate cell-adhesion proteins. B: Venn diagram of orthogroups that are conserved across the 3 species (i.e. the middle subset on A) asking whether they are also functionally conserved, i.e. contain at least one gene that is annotated in GO-terms or phenotypic data related to MLP formation in all three species. C: Functionally conserved genes coloured by their broad functional category as indicated. GO: Gene Ontology, FYPO: Fission Yeast Phenotype Ontology, P: Phenotype annotations. Venn diagrams were made using matplotlib-venn in Python (118).

Figure 2

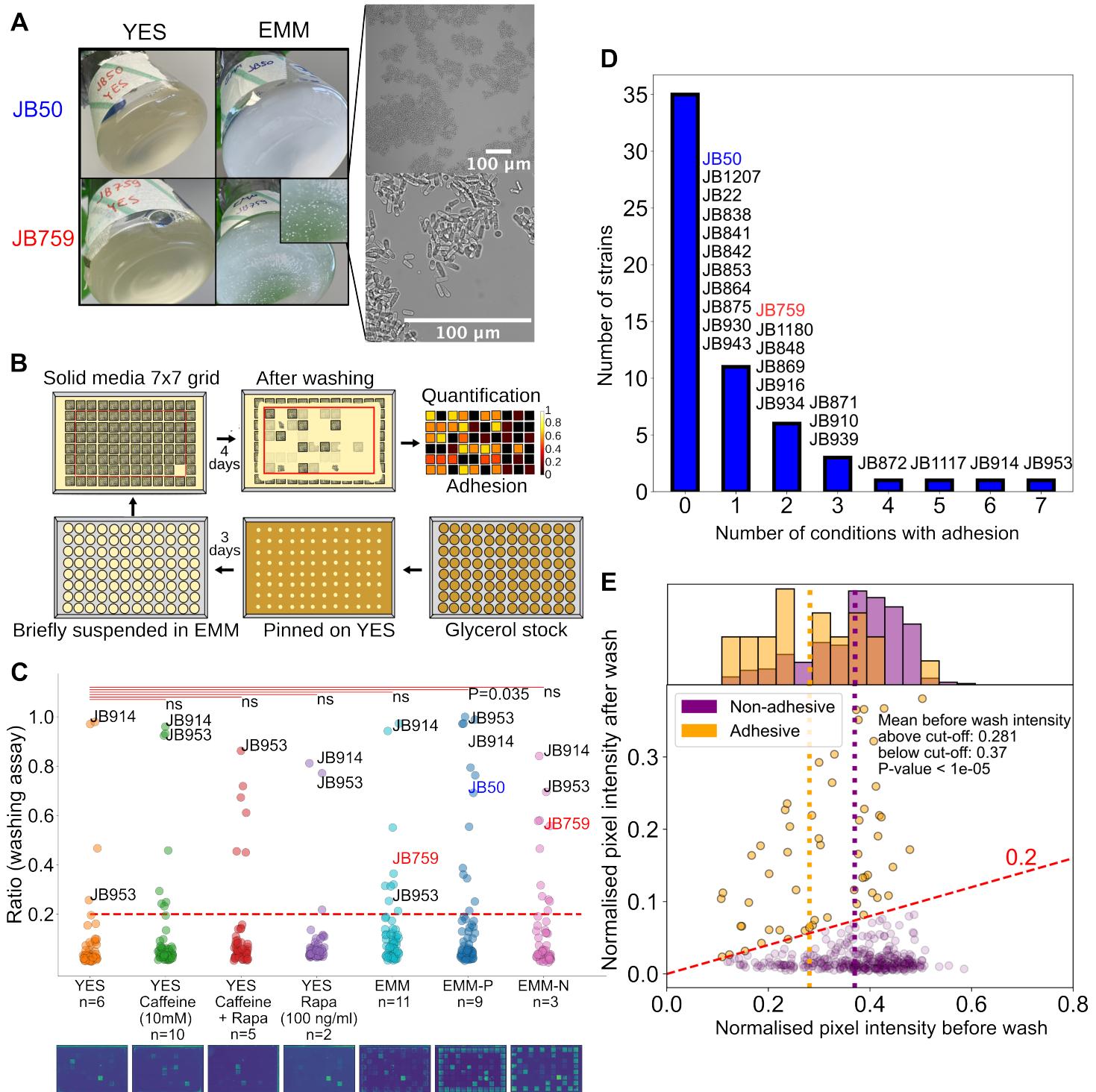


Figure 2: MLP formation in *S. pombe* natural isolates varies with nutrient conditions and is associated with decreased growth. A: Left: Images of our initial observations on the standard laboratory strain JB50 and the natural isolate JB759 showing MLP formation of JB759 in EMM. Right: Microscopy images at two magnifications of the JB759 strain grown in EMM for 2 days. B: Scheme of the high-throughput adhesion assay used to assess MLP formation in *S. pombe*. C: Strip-plot of adhesion to agar across different conditions in the natural isolate library, with images of representative post-wash agar plates below. Each dot represents the mean adhesion value for a given strain in a specific condition. The red dashed line shows a cut-off for strong phenotypes (intensity after wash >0.2 times than before wash). Each condition was compared to rich media (YES) with the null hypothesis that they do not increase MLP formation. P-values were obtained using a one-sided permutation-based T-test and Bonferroni correction. Comparisons were marked not significant (ns.) where the null hypothesis could not be rejected at significance threshold 0.05. Strains around the edges were not taken into account for any statistical analysis (see Methods). The lab strain JB50 and natural isolate JB759 used in panel A are highlighted with colour. D: Histogram showing the number of unique strains forming MLPs in a given number of conditions. E: Scatterplot of mean cell densities before and after washing. Each dot represents one strain in one condition, orange dots represent adhesive data points (ratio of before wash to after wash intensity >0.2, dashed red line) and purple dots represent non-adhesive data points. The histogram shows the distribution of cell densities before washing, as a proxy for growth. The vertical dotted orange and purple lines mark the mean pre-wash densities for the two populations.

Figure 3

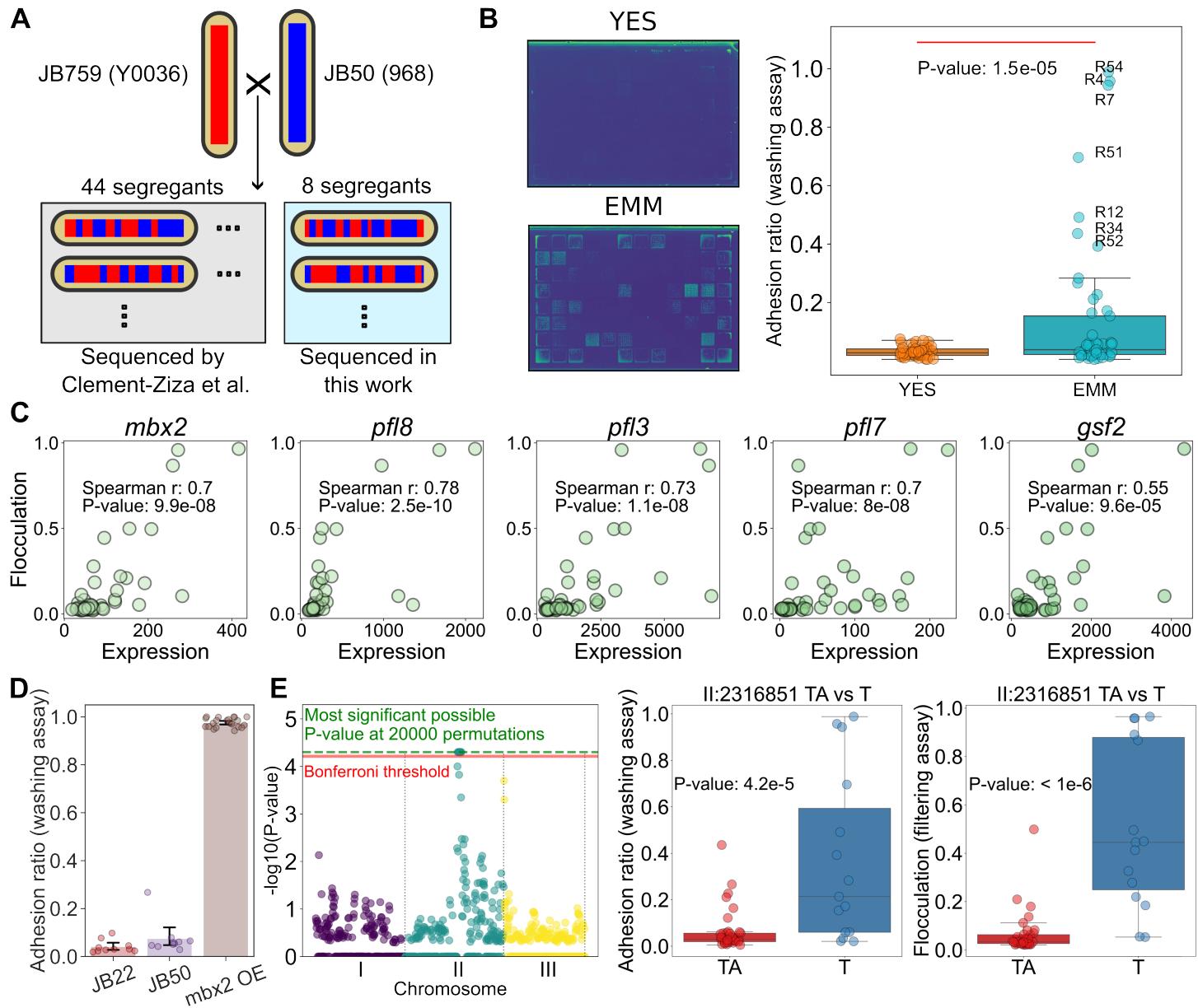


Figure 3: In the JB50-JB759 segregant library, MLP formation on EMM is driven by *mbx2* expression and is associated with a single-nucleotide deletion on chromosome II. A: Scheme for the segregant library. Red and blue stripes indicate genomic recombination resulting from meiosis. SNPs for the strains inside the grey box were previously identified (46) and genome sequencing data for the strains in the light blue box were generated in this work (Methods). B: (Left) Example plates of segregants grown on EMM or YES after washing, shown in viridis colormap. (Right) Adhesion to agar of segregant strains on EMM (mean of 10 replicates) compared to YES (mean of 2 replicates), along with significance of the difference obtained using permutation-based T-test. C: Correlations of *mbx2* and flocculin gene expression with flocculation in EMM. Each dot represents a strain from the segregant library. D: Barplot with measurements overlaid comparing adhesion measurements from standard laboratory strains JB22 and JB50, and the *mbx2* overexpression strain generated in this work (Methods). Error bars represent the 95% confidence interval. E: (Left) Manhattan plot of QTL analysis results for flocculation in EMM. The red line shows the Bonferroni threshold, while the green dashed line shows the highest possible significance achievable using 20,000 permutations. (Middle, Right) Candidate variant is associated with both increased adhesion to agar (mean of 10 replicates) and increased flocculation in EMM (filtering assay, mean of 3 replicates). P-values were determined using a permutation-based T-test with 1E+6 permutations.

Figure 4

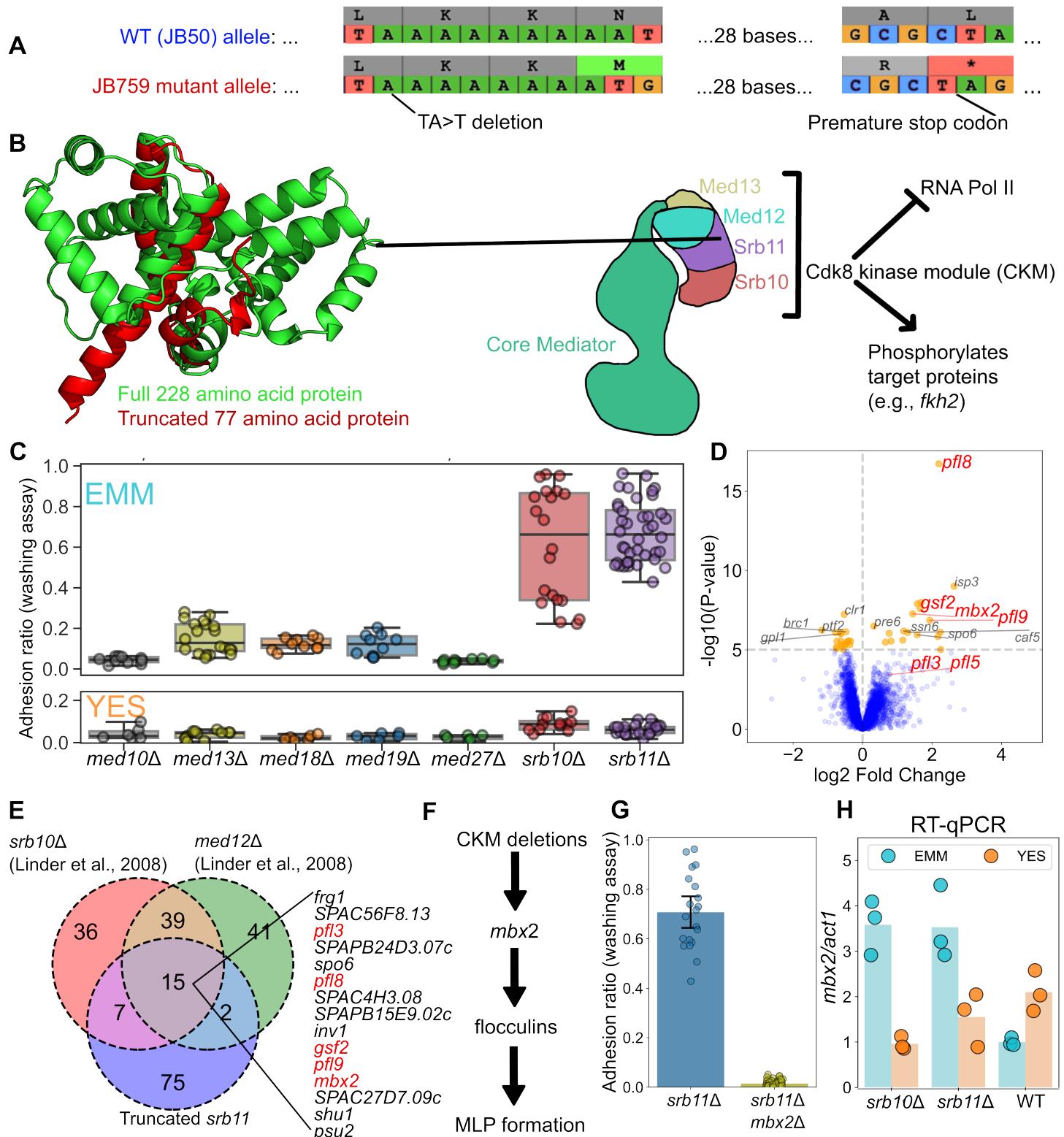


Figure 4: Cdk8 kinase module deletions upregulate *mbx2* in EMM, but not in YES, and lead to MLP formation. A: Scheme showing how a single nucleotide deletion leads to frameshift and premature stop-codon in *sr11*. B: Full Srb11 structure (green) compared with the truncated Srb11 structure (red) as predicted using Colabfold (119). Scheme on the right shows Srb11 in the context of the Cdk8 kinase module of the Mediator complex. The structure was sketched based on structural data (77), and functional roles were summarized based on (77,79). C: Strip-boxplot showing adhesion values from Mediator gene deletion strains on EMM and YES as indicated. Each dot represents a replicate. D: Differential expression analysis of segregant strains split on the II:2316851 TA>A single-nucleotide deletion. Fold-change values and P-values were obtained from DESeq2 (59). E: Overlap of upregulated genes in three CKM mutants based on our *sr11* data and data from Linder et al. (60). F: Simplified model for how CKM deletions lead to MLP formation. See main text for details. G: Adhesion measurements for the *sr11Δ* strain obtained from the deletion collection, and its derived strain after *mbx2* knock-out with CRISPR. Each dot represents a replicate measurement. Error bars represent the 95% confidence interval. H: RT-qPCR showing *mbx2* expression of *sr10Δ::Kan*, *sr11Δ::Kan* and wild-type (WT) strains in EMM or YES. Height of each bar reflects the mean of three biological replicates which are indicated by dots.

Figure 5

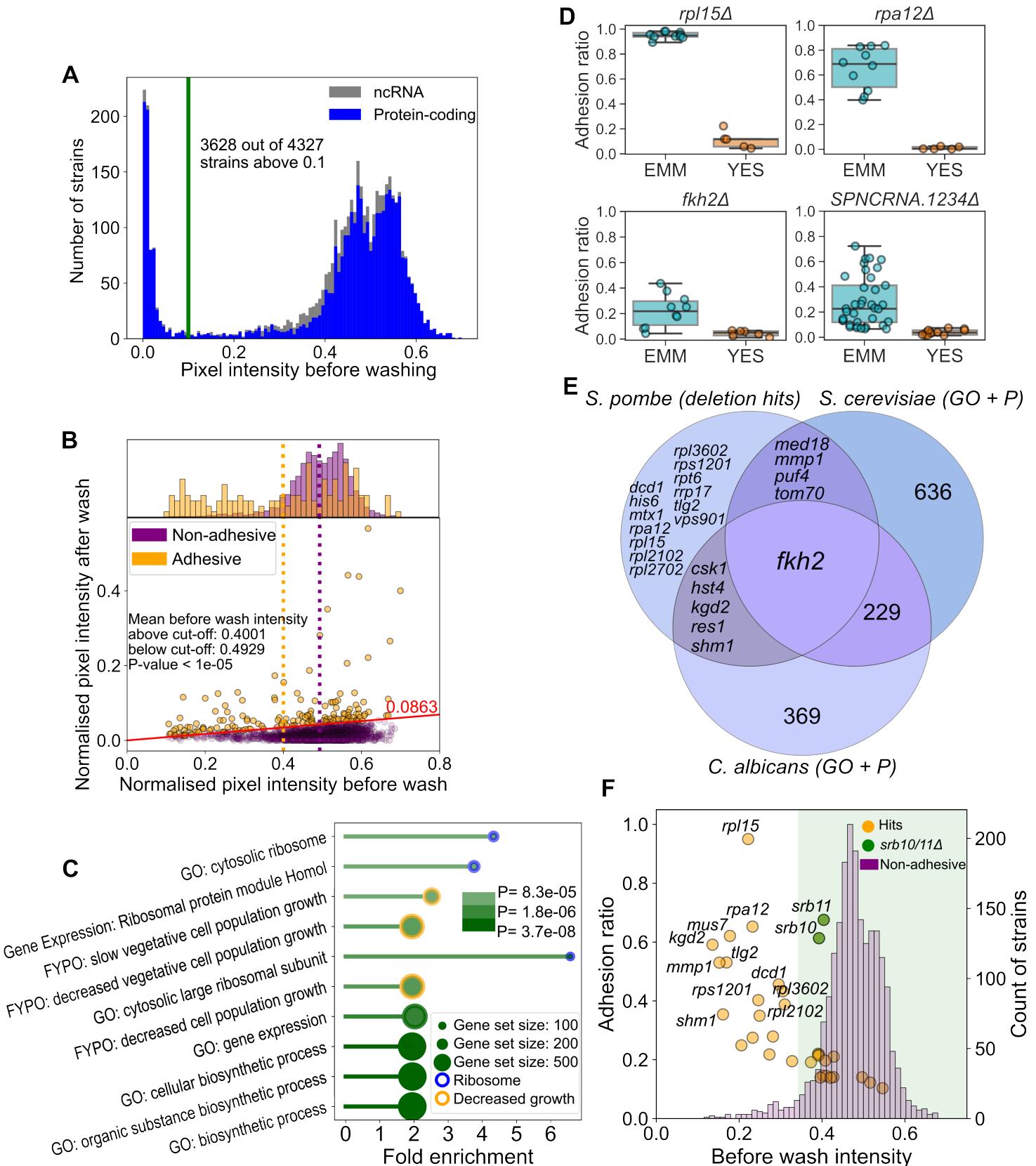


Figure 5: Deletion library screen identified 31 genes associated with MLP formation on EMM. A: Histogram showing cell densities of strains in our deletion library screen before washing. B: Scatterplot of mean cell densities before and after washing. Each dot represents a deletion strain, and colors represent adhesive and non-adhesive strains as indicated. The red line represents the cut-off at the 95th percentile of adhesion values, also shown on Supp Fig 9. The histogram shows the distribution of cell densities before washing as a proxy for growth. The dotted orange and purple lines mark the mean pre-wash intensity values for the two populations. The P-value was determined using a permutation-based T-test with 1E+5 permutations. C: Barplot showing the fold enrichment of the top-10 most significantly enriched processes, with blue circles for terms associated with ribosomes and orange circles for terms associated with decreased growth. Terms were sorted based on P-values and increasing color intensity represents increasing $-\log_{10}(P\text{-value})$. The size of the circle at the end of each bar represents the size of the gene set. D: Strip-boxplots of adhesion ratios obtained with the washing assay for 4 of the 31 verified hits on EMM (light blue) vs YES (orange). Each dot is an independent observation. E: Venn diagram showing the functional conservation of the genetically conserved hits from our screen. Only *fkh2* is annotated as being involved in MLP formation in all three species. Venn diagram was made using matplotlib-venn in Python (118). F: Scatterplot of adhesion ratios and before-wash colony intensities overlaid by a histogram showing before-wash colony intensities of non-adhesive deletion strains which were assayed in the middle 60 spots during the original screen. The green shaded area marks strains that are above the 5th percentile of colony intensities in the non-adhesive strains. The *srp10/11 Δ* strains, highlighted with green, are the most adhesive strains from those with growth values above the 5th percentile.

Figure 6

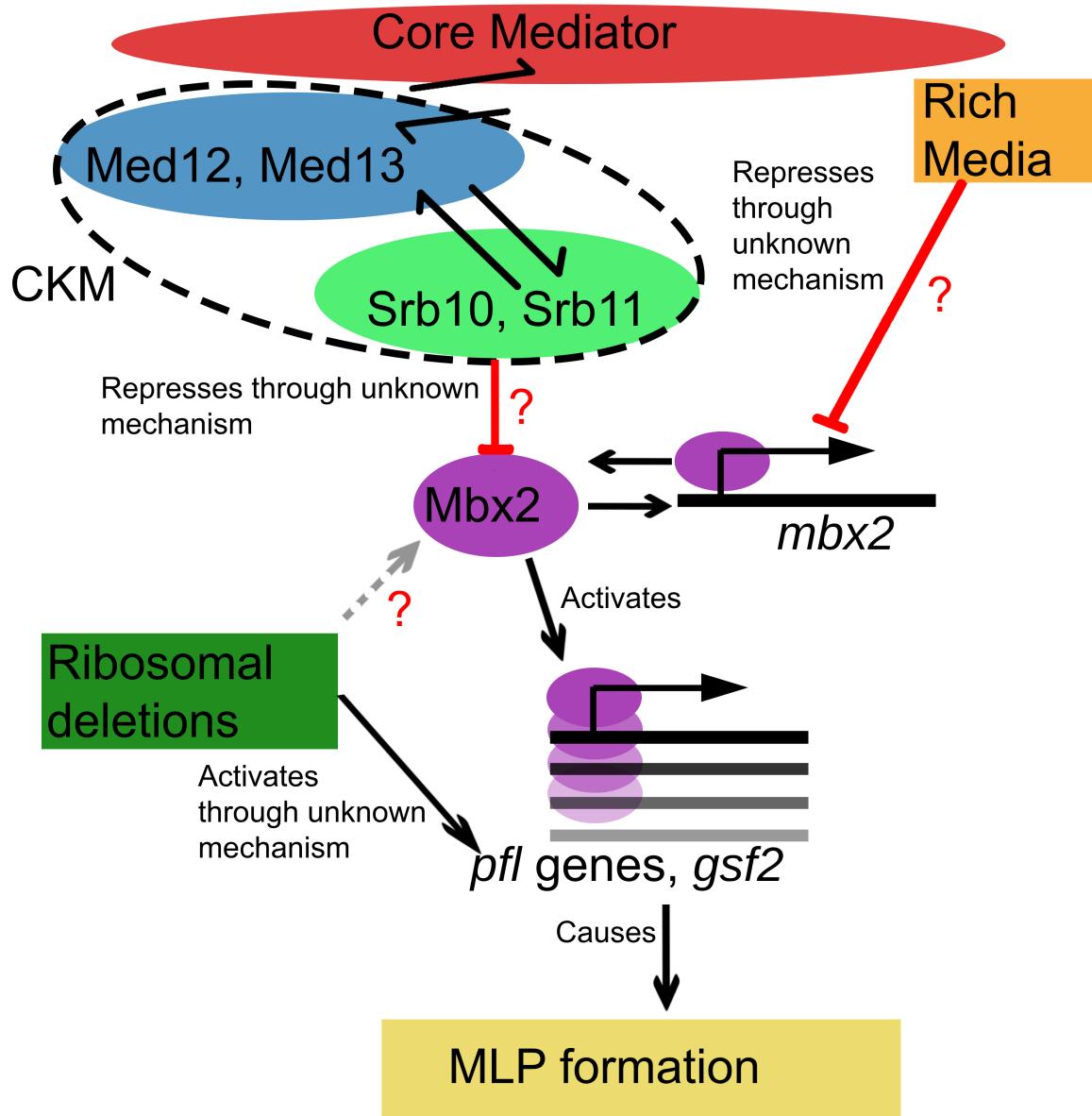


Figure 6: Model for EMM-dependent MLP formation of CKM mutants. We propose that the CKM phosphorylates Mbx2 and targets it for degradation, based on similar observations in *C. albicans* (81) and *S. cerevisiae* (83). Additionally, the *mbx2* transcript is repressed through an unknown mechanism in YES as we observed using RT-qPCR. In minimal media, if members of the CKM are deleted, *mbx2* becomes upregulated, triggering the expression of flocculin genes, which in turn cause MLP formation. Deletion of ribosomal genes also triggers MLP formation, although it is unclear whether this occurs through upregulation of *mbx2* or directly through the flocculin genes. The coloured boxes show our main pathway of interest. The red arrows show our main findings, while red question marks show the main outstanding mechanistic questions.