**Supplementary Information**

**Table S1: Strains and primers used in this study.**

Table contains the genotypes of the applied strains and their references. Oligonucleotides for GatewayTM cloning technology (with attB sites, BAR codes and gene specific sequences) primers for real-time, colony PCR and Southern blot experiments.

**Table S2: Applied stressors and their concentrations in solid YPD media for spot assay analysis.**

**Table S3: The list of the selected genes for OE analysis.**

The table lists the selected genes (and their ortholog’s functions) for OE experiments based on preliminary results [2,3,4] and some additional genes whose othologs have known virulence or stress tolerance related functions in *C. albicans* or in *S. cerevisiae*. The table also indicates if a KO of a given gene is available and if the OE mutant showed any altered phenotype compared to the control strain in this study.

**Table S4: The results of pseudohypha formation experiments.**

The table details the results of the pseudohypha analysis of the overexpression mutant strains carried out with Amnis® FlowSight® flow cytometer. Cells were cultivated in DMEM containing 10% (*v/v*) HI FBS or in YPD for 48 hours at 37 °C in the presence of 5% (*v/v*) CO2. The shape of the non-aggregated cells was characterized as yeast or elongated.

**Table S5: The results of the antifungal susceptibility analysis.**

The table details the results of the antifungal susceptibility test of the overexpression mutant strains exposed to 3 echinocandin drugs (anidulafungin, caspofungin, micafungin). Final drug concentrations were from 8 µg/mL to 0.0156 µg/mL with 3 × 103 fungal cells in 200 µL final volume of RPMI-MOPS. Cells were incubated at 30 °C without shaking and monitored after 24 and 48 hours. MIC values for the applied drugs were defined as the lowest concentrations that resulted in at least 50% growth reduction. In addition to mCherryOE, CPRI and CLIB214 *C. parapsilosis* strains, a *C. krusei* strain with known antifungal susceptibility was also included as a control.

**Table S6: The summarized results of the OE mutant collection analysis.**

The table presents mutant strains with altered phenotypes and the comparison with former results of KO or OE mutant library analysis regarding *C. parapsilosis*, *C. albicans* and *S. cerevisiae*.

**Supplementary data**

**Figure S1:** **The schematic figure of the OE vector.**

The figure shows the structure of the expression vector used for mutant generation. Figure was modified based on the work of Németh and colleagues [48].



**Expression vector**

**7045 bp + ORF**

**Figure S2: Representative figures of** **the methods for verification of the generated OE mutant strains.**

**A)** Figure shows the schematic structure of the overexpression construction integrated into the CpNEUT5L region. Arrows show the sites of the gene specific real-time and the universal primers for rapid screen of correct integration. T: terminator. Figure was modified based on the work of Németh and colleagues [48]. **B)** Representative gel electrophoresis figure shows the verification of selected OE strains by colony PCR. Samples: 1. mCherryOE – 1900 bp; 3. CPAR2\_108840OE – 1921 bp; 5. CPAR2\_109520OE – 1722 bp; 7. CPAR2\_302400OE – 1402 bp; 9. CPAR2\_500180OE – 1902 bp; 2., 4., 6., 8., 10. samples are no-template controls. **C)** Theschematic figure represents the linearized overexpression plasmid integrated into the CpNEUT5L region and highlights the features of the Southern blot analysis. Figure was modified based on the work of Németh and colleagues [48]. **D)** Representative figure of the control, parental and OE mutant strains verified by Southern blot analysis. Samples: 1. CLIB214; 2. CPRI; 3. CPL2; 4. mCherryOE; 5. CPAR2\_108840OE; 6. CPAR2\_109520OE; 7. CPAR2\_302400OE; 8. CPAR2\_500180OE.

x bp

**PCR check product** (x+1171 bp)

CpN5L Up

WT allele

CPAR2\_303830

ReTi\_REV

ReTi\_FOR

OE allele

CpN5LUp

1171 bp

CpN5LcheckREV

CpN5L Down

CpNEUT5L

(Up+Down)

CpN5LDo

CmLEU2

PCaTDH3

CpORF

TAG

T

CpN5LUp

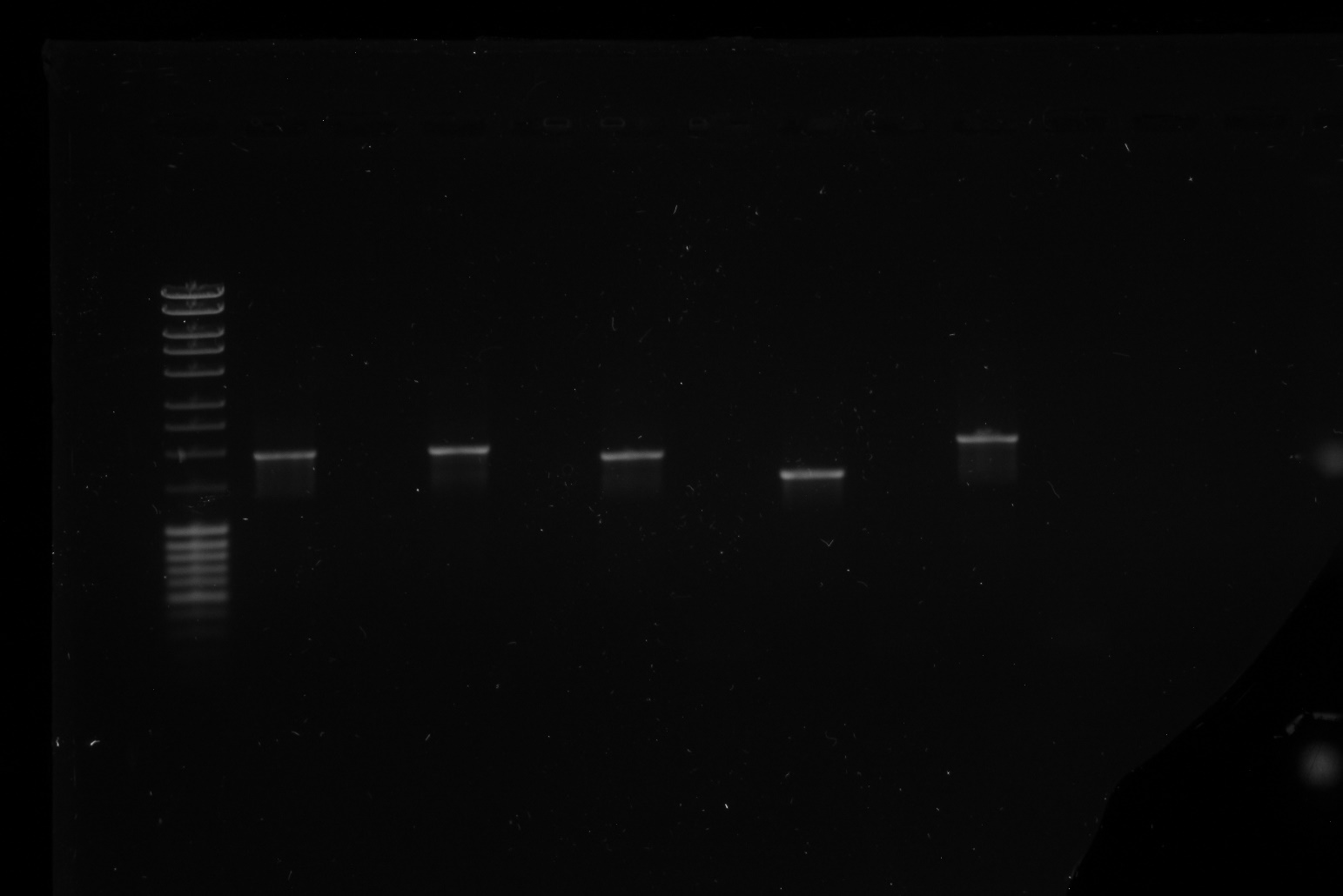
CpN5LDo

CPAR2\_303820

**A)**

**B)**

**M 1 2 3 4 5 6 7 8 9 10**



**2500 bp**

**2000 bp**

**1500 bp**

**C)**

*EcoRI* restriction sites:

DIG-labelled probe:

*EcoRI*

*EcoRI*

*EcoRI*

CpN5L Up

WT allele

CPAR2\_303830

OE allele

CpN5LUp

CpN5L Down

CpNEUT5L

(Up+Down)

CpN5LDo

CmLEU2

PCaTDH3

CpORF

TAG

T

CpN5LUp

CpN5LDo

CPAR2\_303820

7821 bp

5514 bp

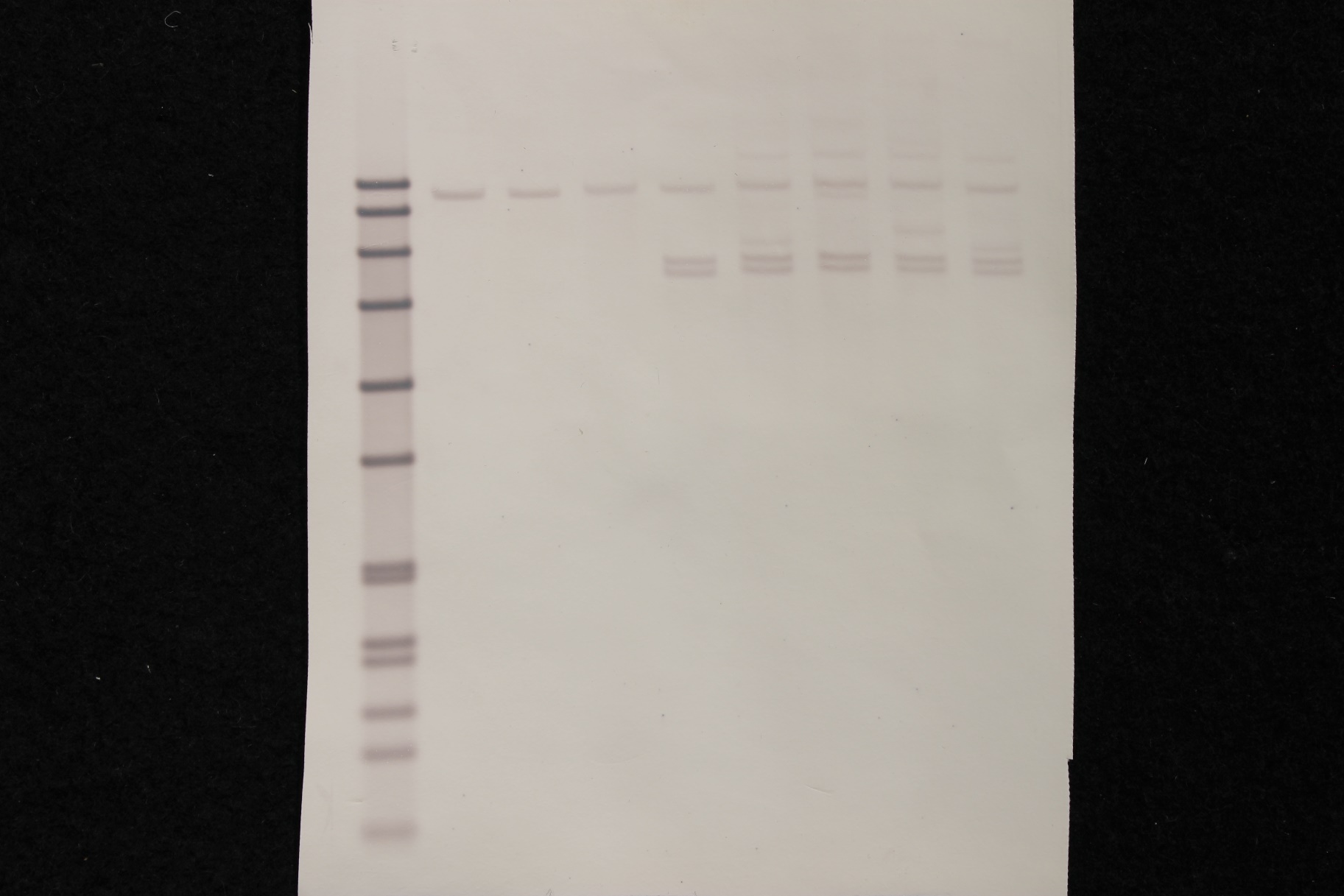
5255 bp

*EcoRI*

*EcoRI*

*EcoRI*

**D)**



M 1 2 3 4 5 6 7 8

**8576 bp**

**7427 bp**

**6106 bp**

**4899 bp**

**7821 bp**

**5514 bp**

**5255 bp**

**Figure S3:** **Results of the growth kinetic measurements in complete media (YPD).**

Graphs show the growth kinetic analyses of the OE strains in YPD liquid media at 30 °C. N=3, with 3 technical parallels per strain. All the three control strains were tested.



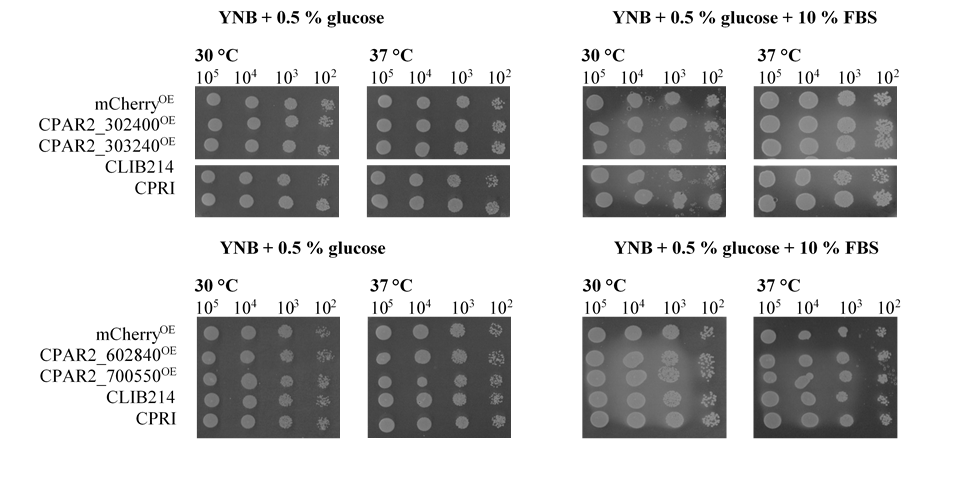
**Figure S4:** **Results of the growth kinetic measurements in minimal media (YNB + 0.5% (*m/v*)** **glucose).**

Graphs show the growth kinetic analyses of the OE strains in YNB + 0.5% (*m/v*) glucose liquid media at 30 °C. N=3, with 3 technical parallels per strain. All the three control strains were tested.



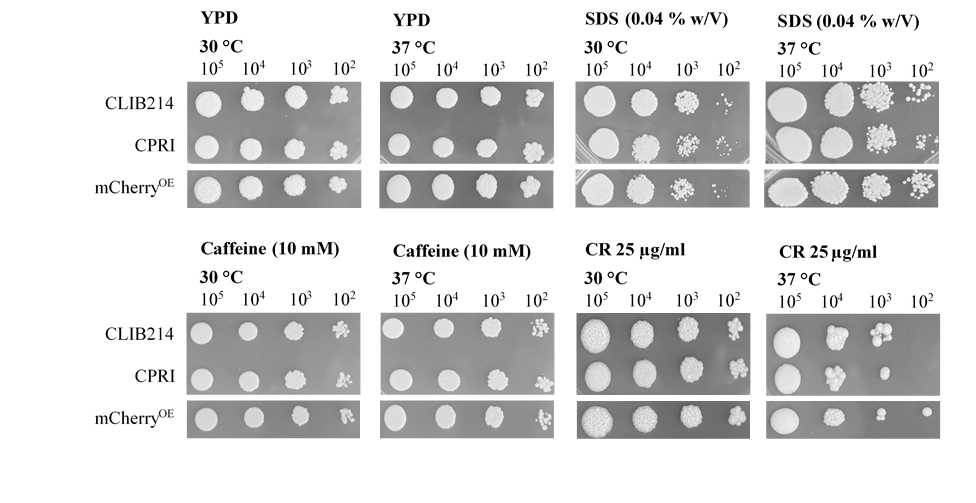
**Figure S5:** **Results of the growth analyses on solid minimal media and with additional serum.**

Representative figures show the fitness of some OE mutant and the 3 control strains on solid minimal media (0.19% YNB + 0.5% (*m/v*) glucose with or without of 10% (*v/v*) FBS) at 30 or 37 °C after 48 h incubation.

****

**Figure S6: Results of the growth analyses of the 3 control strains on solid media supplemented with different stress agents.**

Representative figures show the fitness of the 3 control strains on solid media supplemented some stressor agents at 30 or 37 °C after 48 h incubation.

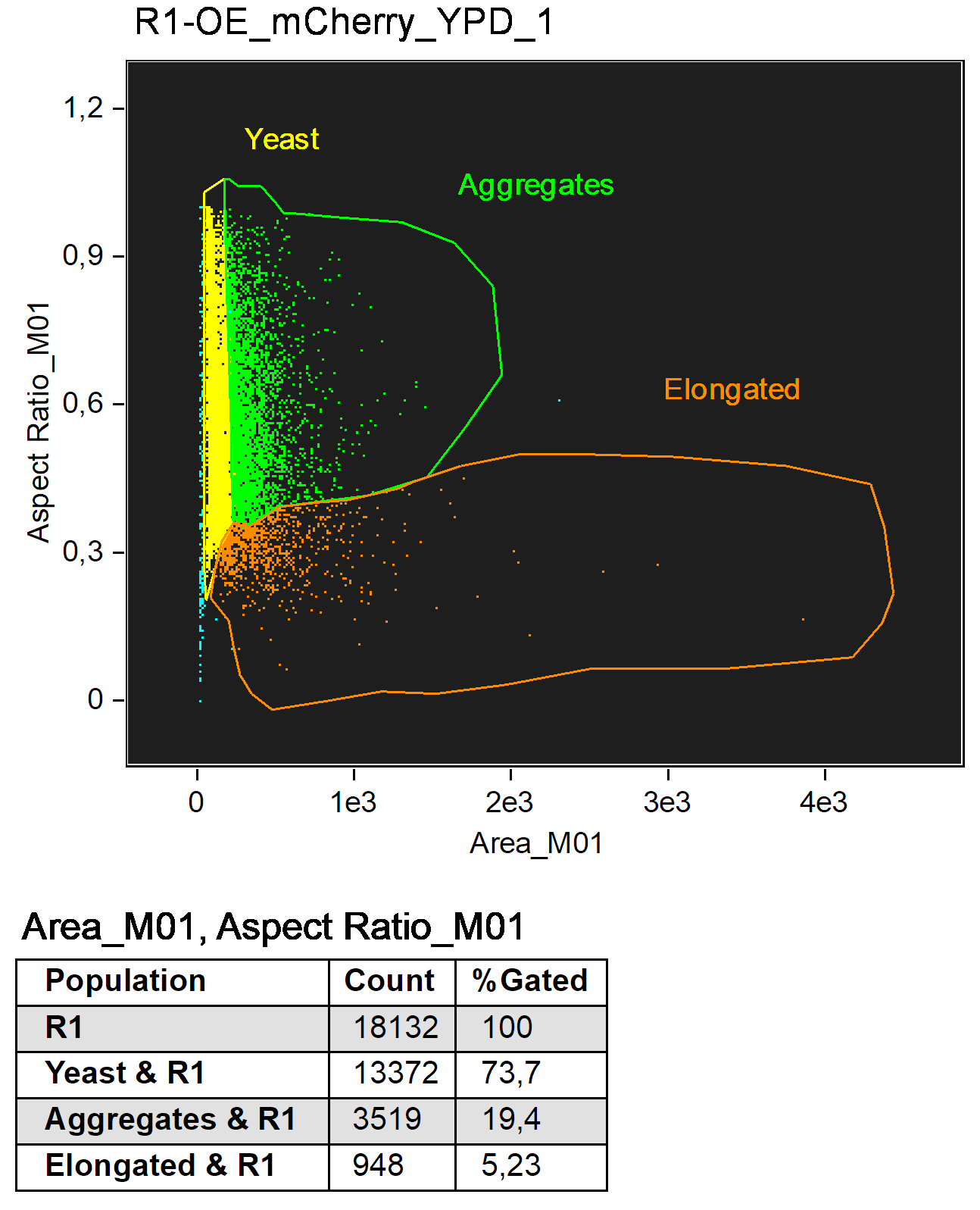
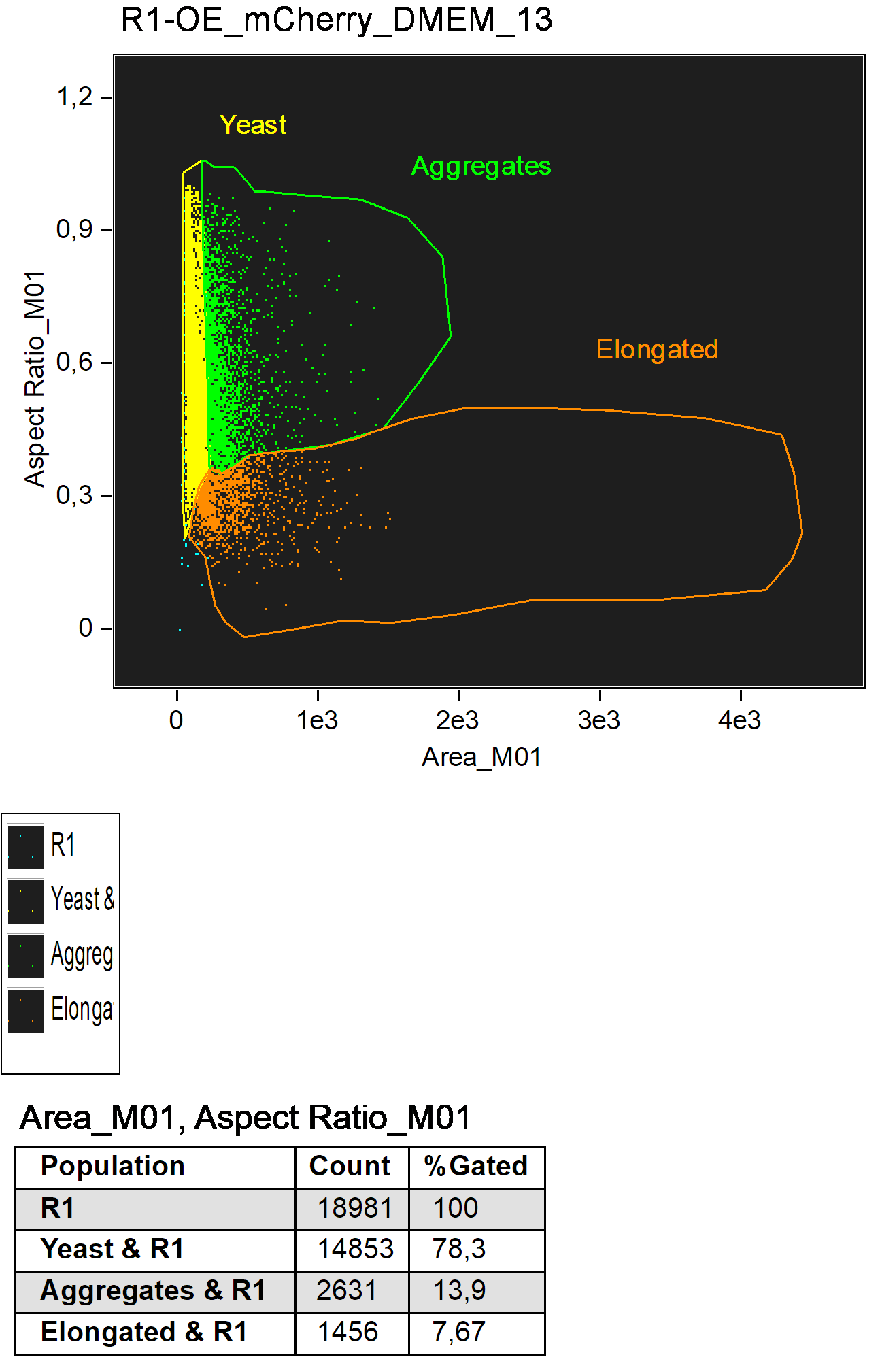
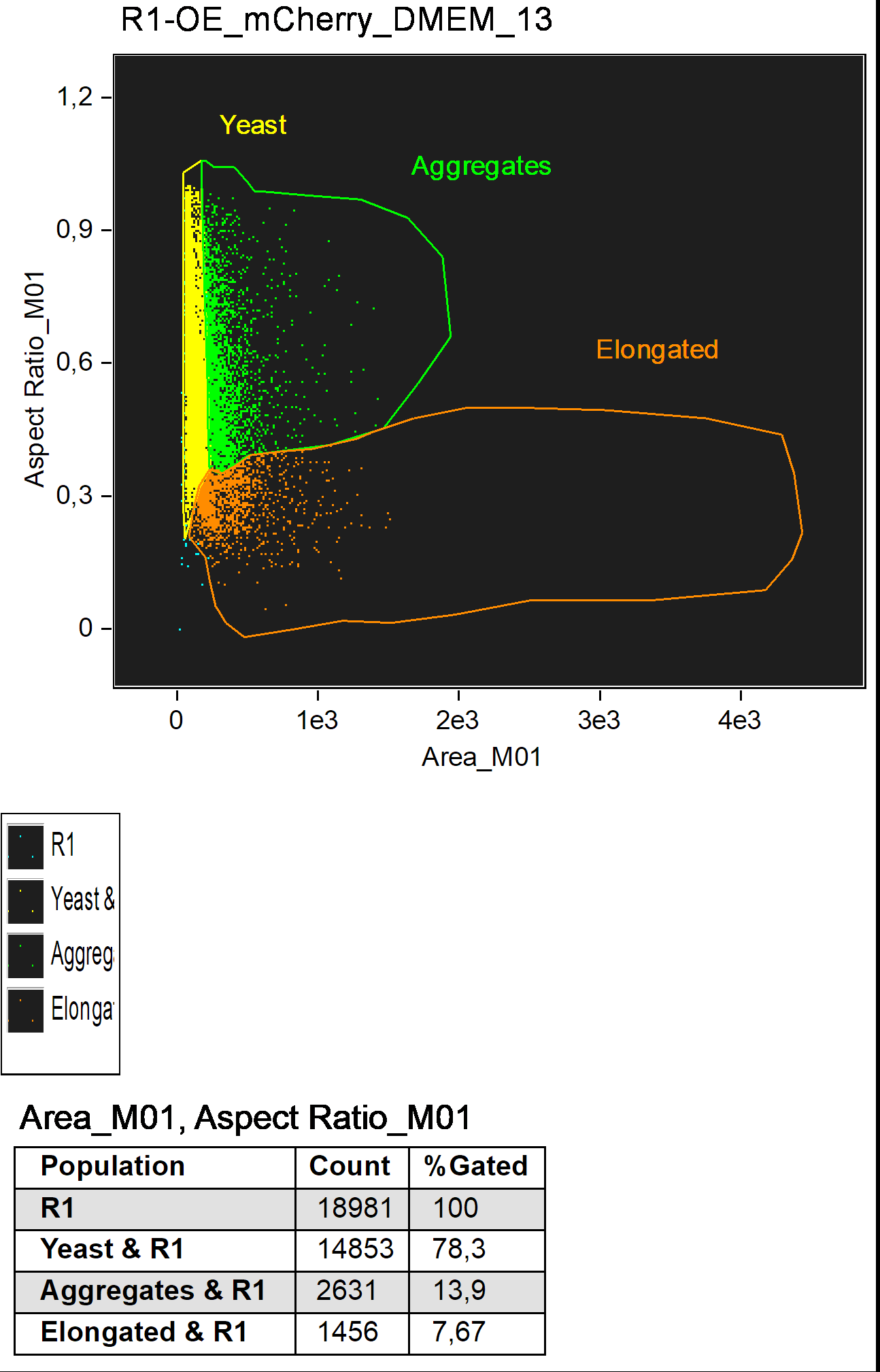
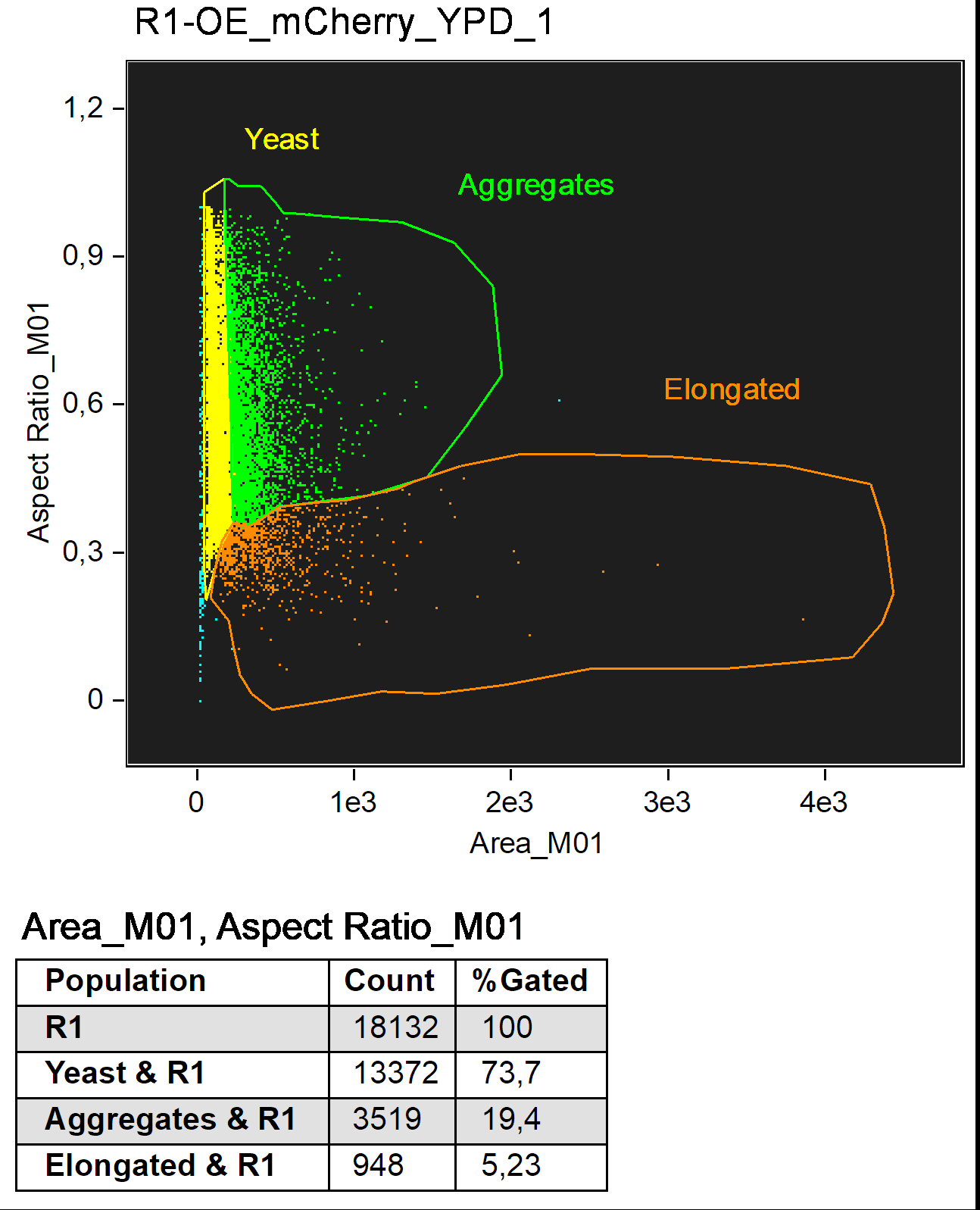


**Figure S7:** **Representative figures from pseudohypha analysis by Amnis® FlowSight® flow cytometer.**

**A)** Analysis of the CLIB214 strain in YPD and DMEM + 10% (*v/v*) FBS medium. **B)** Analysis of the CPAR2\_302400OE strain in YPD and DMEM + 10% (*v/v*) FBS medium. **C)** Representative light microscopic pictures of yeast, aggregated and elongated/filamentous cell forms. No difference was found in any of the mutants generated compared to the control.

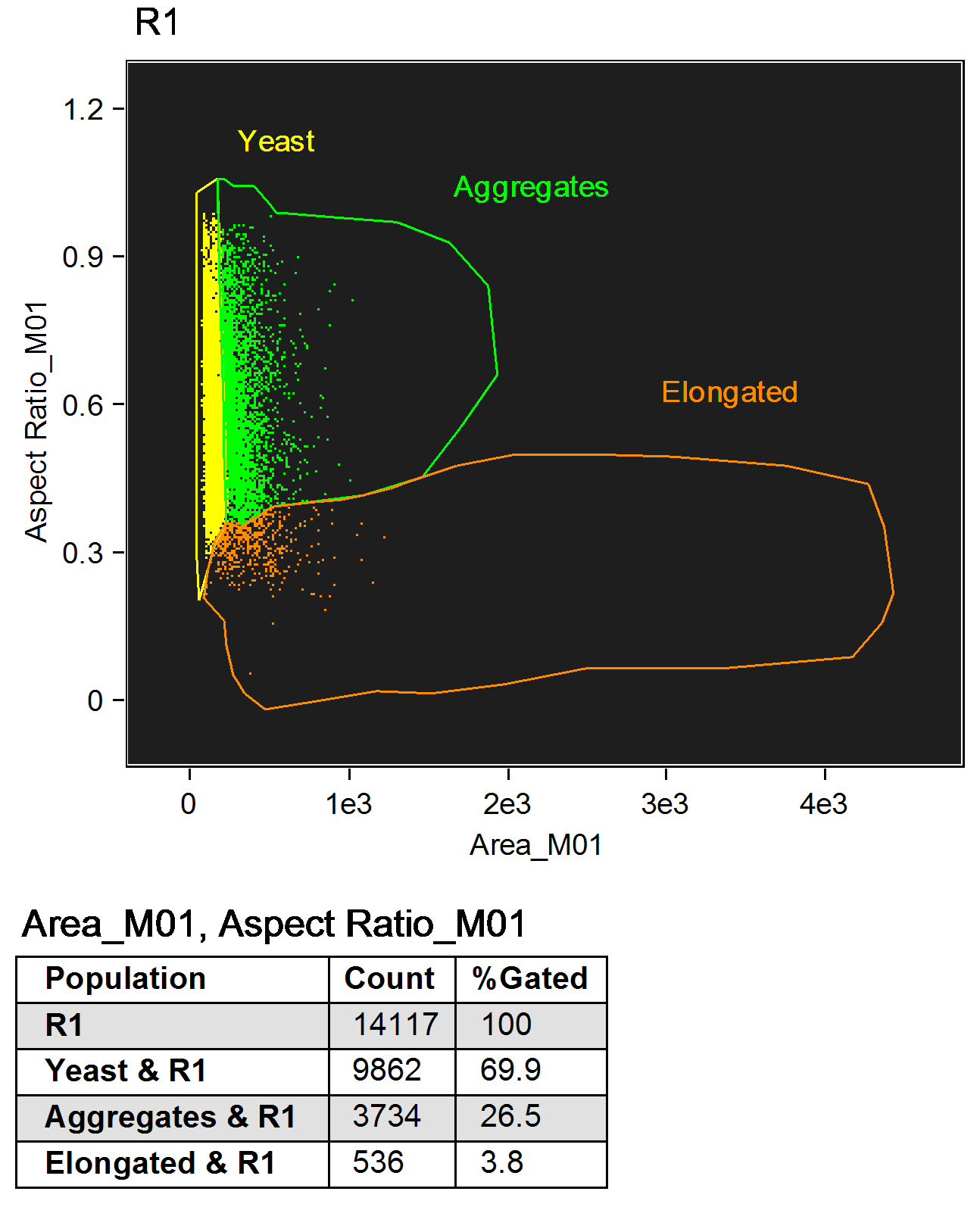
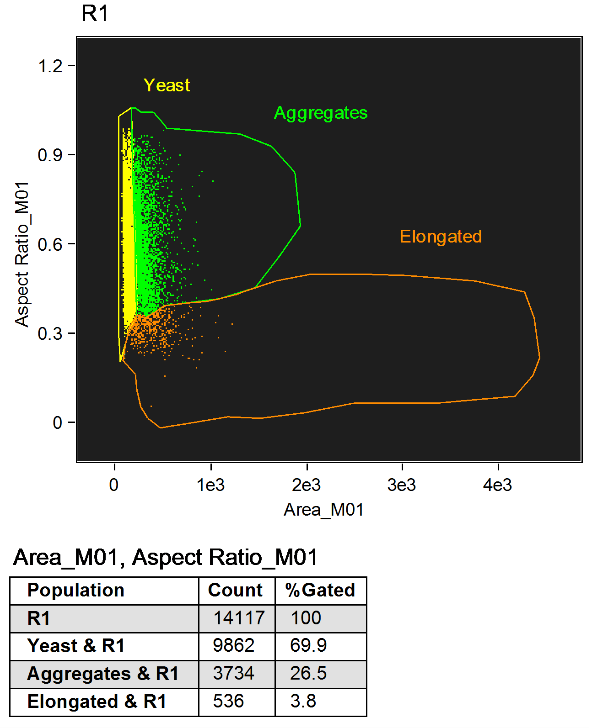
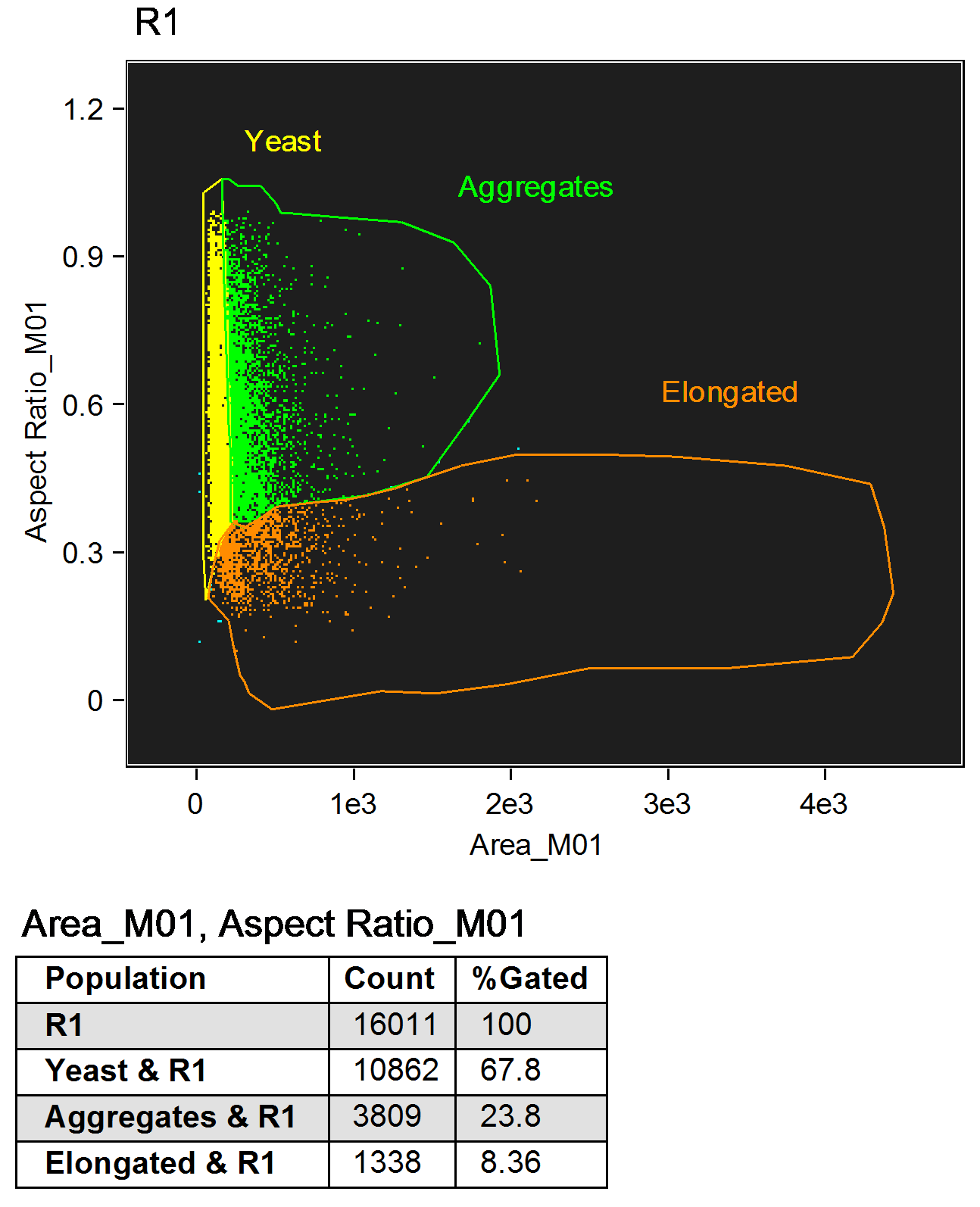
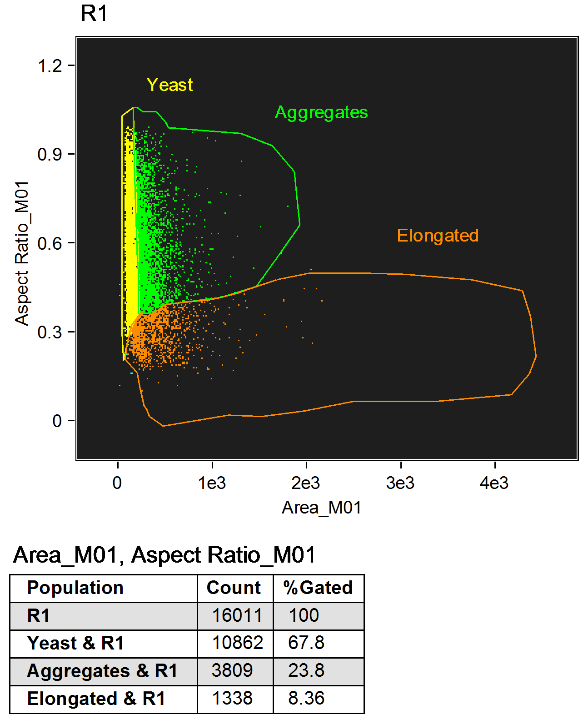
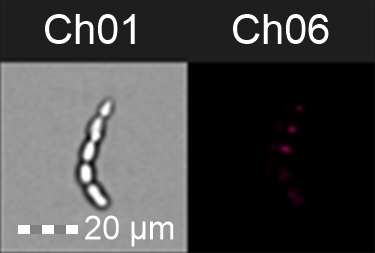
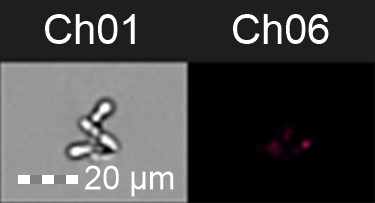
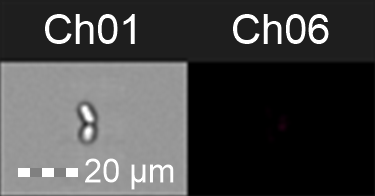
**A)**

**B)**



YPD medium

DMEM + 10% FBS medium



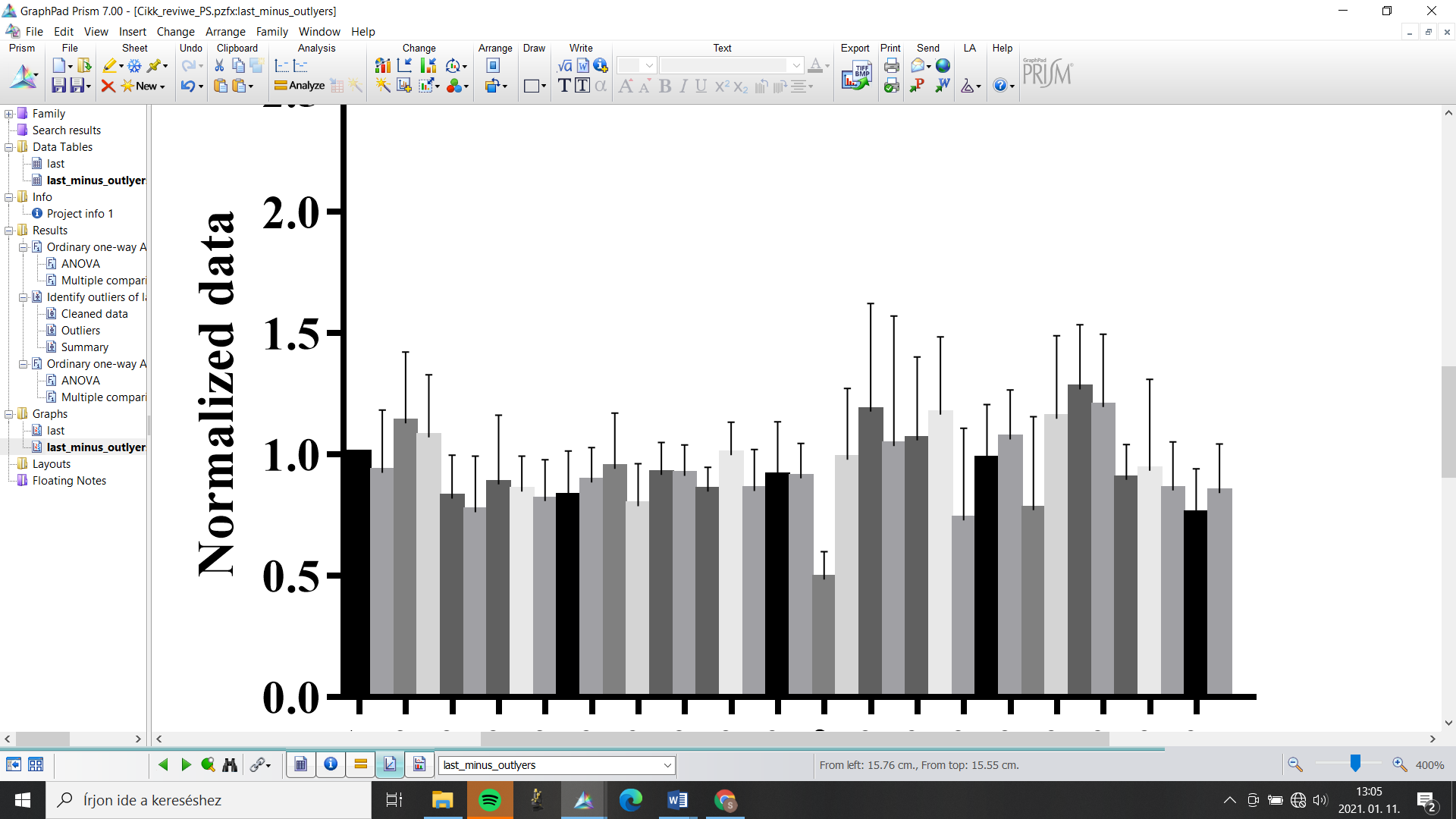
**C)**

**Figure S8:** **Biofilm forming capacity of the overexpression mutants.**

MTT assay was carried out according to the manufacturer’s instructions and OD540 values related to the OE strains were normalized to that of the CLIB214 control strain. N=3 with 8 parallel samples per experiment. Statistical analysis was performed with one-way ANOVA with Dunnett's multiple comparisons test. Only CPAR2\_302400OE showed significantly altered biofilm forming properties (\*\*\*\*p<0.0001).

CPAR2\_302400OE

**Normalized data**



\*\*\*\*

CPAR2\_303240OE

CPAR2\_303730OE

CPAR2\_400270OE

CPAR2\_406400OE

CPAR2\_500180OE

CPAR2\_500360OE

CPAR2\_501400OE

CPAR2\_503290OE

CPAR2\_503760OE

CPAR2\_602370OE

CPAR2\_602820OE

CPAR2\_602840OE

CPAR2\_700550OE

CPAR2\_703840OE

CPAR2\_804030OE

CPAR2\_805930OE

CPAR2\_806950OE

**CLIB214**

CPAR2\_100460OE

CPAR2\_100470OE

CPAR2\_100540OE

CPAR2\_104420OE

CPAR2\_105250OE

CPAR2\_107020OE

CPAR2\_107240OE

CPAR2\_108840OE

CPAR2\_109520OE

CPAR2\_200040OE

CPAR2\_200390OE

CPAR2\_201920OE

CPAR2\_204840OE

CPAR2\_205060OE

CPAR2\_208600OE

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CPAR2\_300080OE

CPAR2\_301360OE

**2.0**

**1.5**

**1.0**

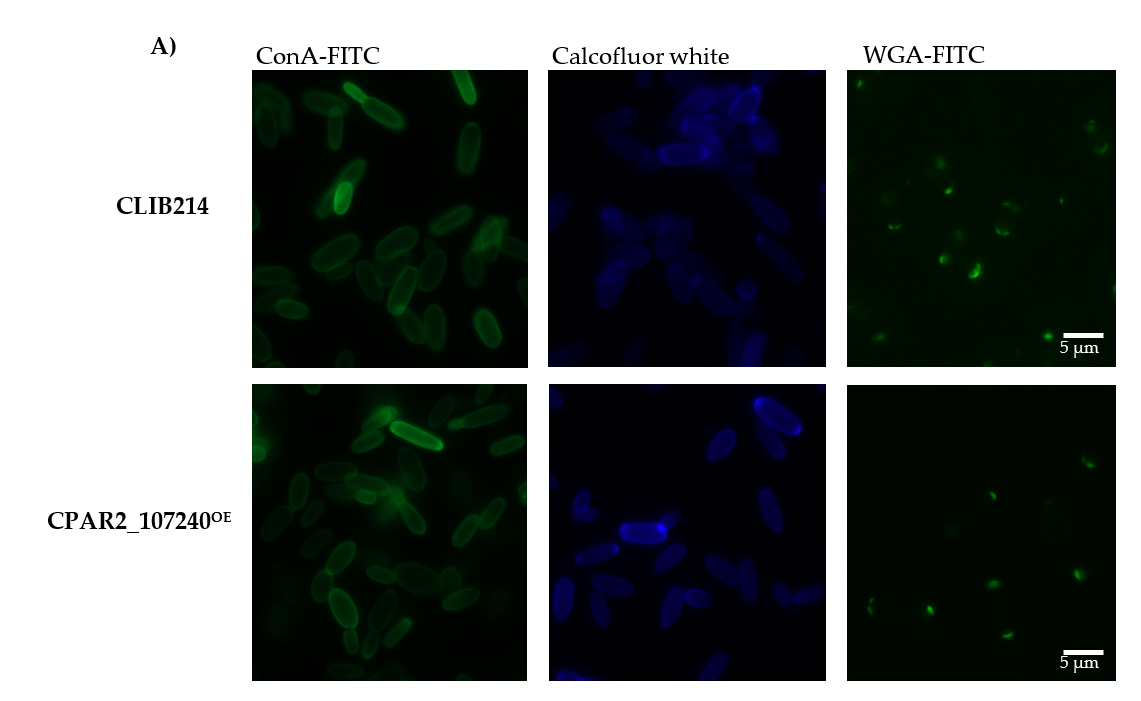
**0.5**

**0.0**

**Figure S9: Cell wall component analysis of the selected mutants.**

**A)** Representative figures present the chitin (Calcofluor white, CFW) and its oligomer (WGA-FITC) and alpha mannan (ConA-FITC) content of the CLIB214 and the CPAR2\_107240OE mutant strains. **B)** The figures give an example to the results of certain cell wall component analysis by flow cytometer (Ch-channel). No difference was found in any of the mutants generated compared to the control.

**A)**



**CLIB214**

**CPAR2\_107240OE**

**CPAR2\_107240OE**

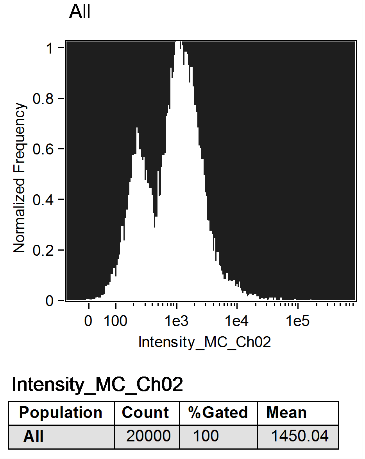
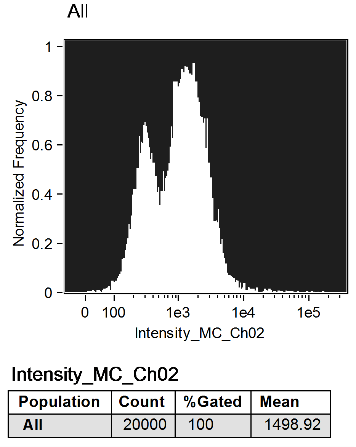
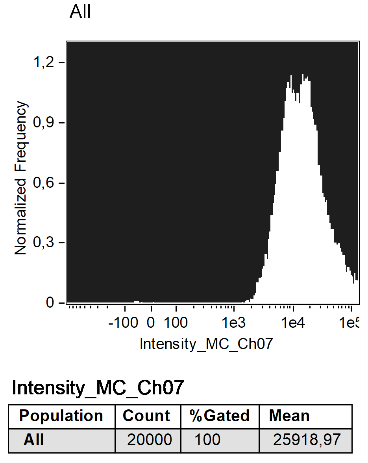
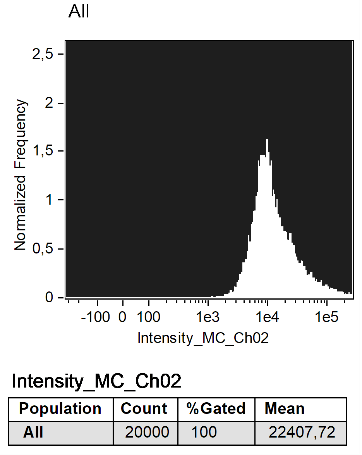
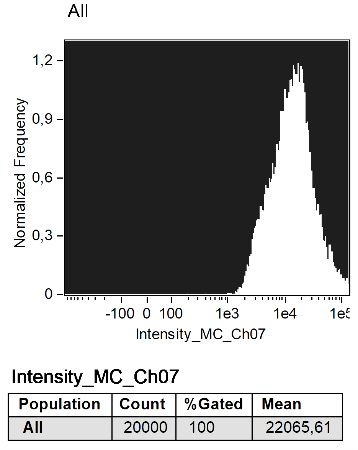
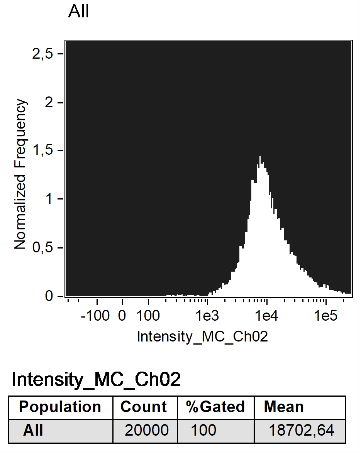
**CLIB214**

**B)**

**WGA-FITC**

**ConA-FITC**

**Calcofluor white**



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