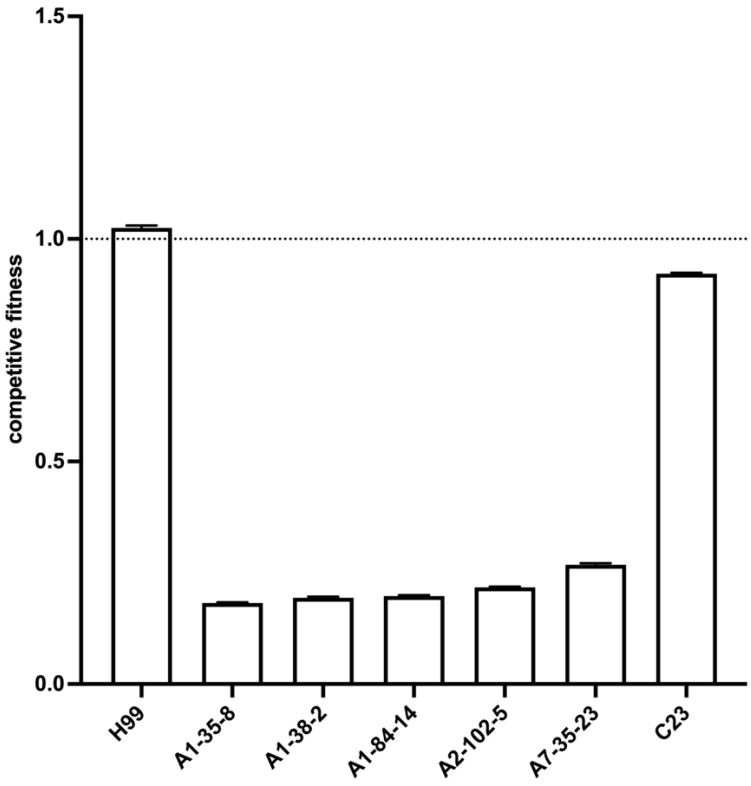

Figure S1

Competition assay replicates phenotypes of CO_2-sensitive strains

Overnight cultures of mNeon-Green labeled H99 and CO_2-tolerant or CO_2-sensitive strains were combined in a 1:1 ratio and incubated at 30°C for 24 hrs in ambient air or with 5% CO_2. Cell populations were characterized by flow cytometry and the percentage of mNeonGreen negative cells in 5% CO_2 normalized to those in ambient conditions to determine a competitive fitness score for each mutant strain as indicated.

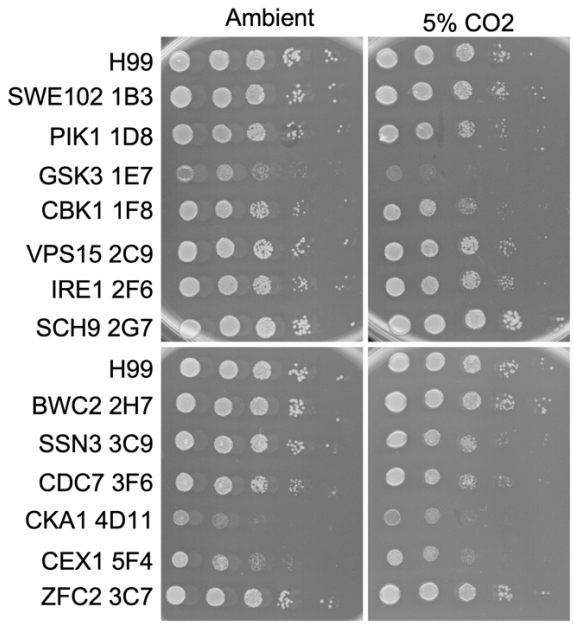

Figure S2

Spot dilution assay confirms competition assay phenotypes

Ten-fold serial dilutions from overnight cultures of indicated strains (plate location from kinase or transcription factor deletion library indicated) were spotted on solid RPMI medium with 165 mM MOPS, pH 7 at 30°C in ambient air or at 5% CO_2 for 48 hrs before images were acquired.

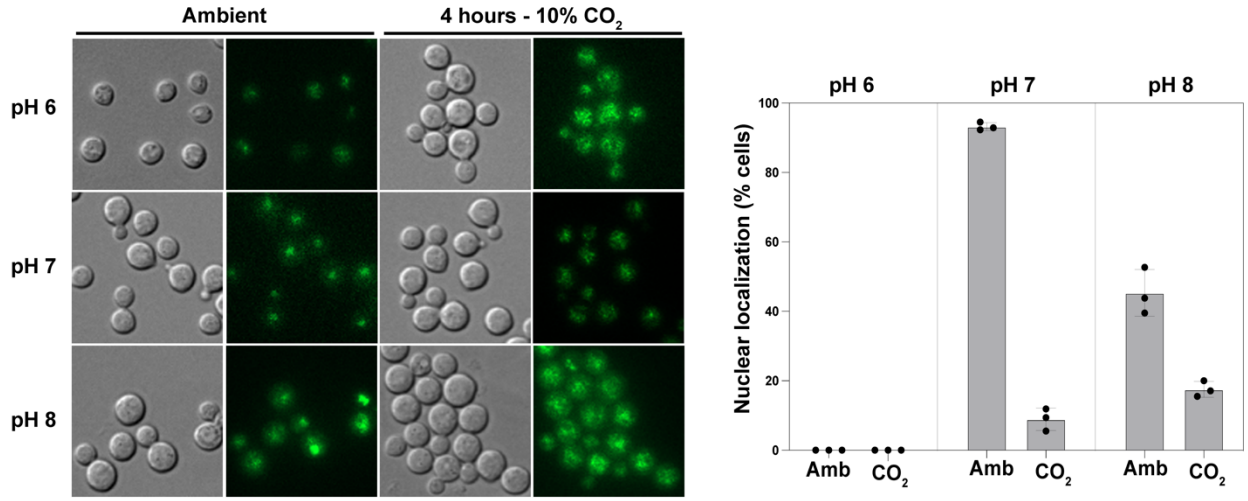

Figure S3

Rim101 delocalized under elevated CO_2 concentrations

Overnight cultures of H99 + RIM101-GFP were washed twice with water before plating ~2x10^8 cells on RPMI solid medium buffered with MOPS to pH 6, 7, and 8, and incubated for 4 hrs with or without 10% CO_2. Cells were swabbed onto a slide before imaging. A minimum of 200 cells were scored for each condition for enriched nuclear localization or diffuse fluorescence throughout the cell.

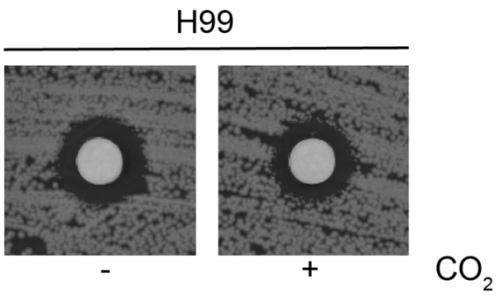

Figure S4

CO_2 exposure does not affect sensitivity to the IPC targeting antifungal aureobasidin

Cells from overnight cultures of H99 were spread on RPMI 1640 medium with 165 mM MOPS, pH 7. Sterile disks were placed on plates and 100 µg aureobasidin was added to each disk. Cells were incubated at 30°C for 48 hrs in ambient air or 5% CO_2 before images were acquired.

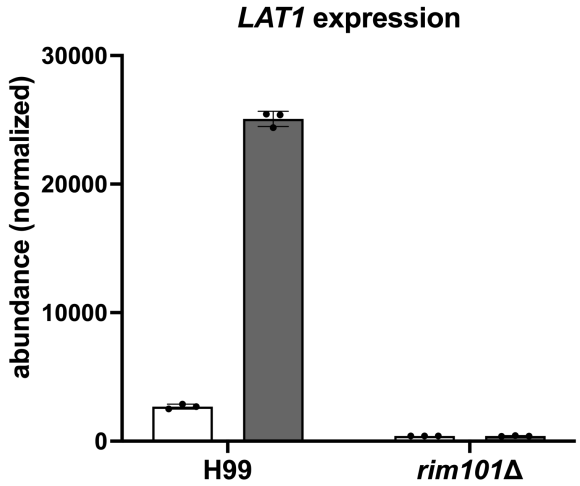

Figure S5

LAT1* expression reduced under ambient air and CO_2 conditions in CO_2 resistant mutant *rim101Δ

H99 and *rim101Δ* strains were cultured in RPMI 1640 medium with 165 mM MOPS, pH 7 for 24 hrs at 37°C in ambient air or 5% CO_2. Total RNA was isolated from harvested cells, 100 ng of RNA was hybridized to a custom Nanostring probe set and quantified on a Nanostring Sprint nCounter. Normalized Nanostring counts from total RNA for LAT1 in ambient (white bars) or 5% CO_2 conditions (gray bars) displayed.

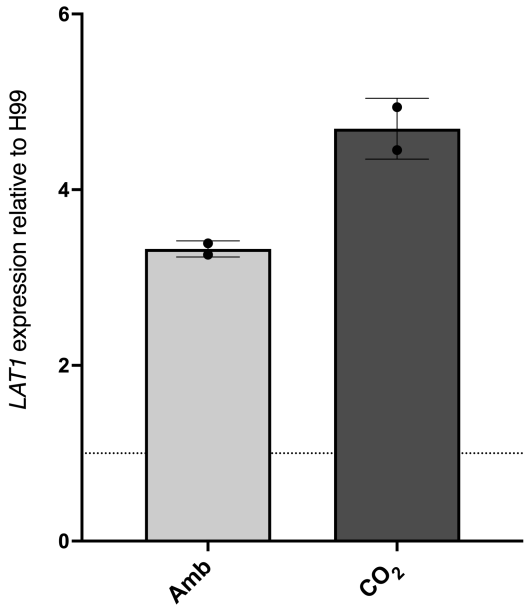

Figure S6

Basal expression of *LAT1* is increased under histone 3 promoter compared to H99

Overnight cultures of H99 and P_{H3}:*LAT1* were washed and diluted to $7.5x10^5$ cells/ml and cultured in RPMI 1640 medium with 165 mM MOPS, pH 7 for 4 hr at 37°C in ambient air or 5% CO_2. *LAT1* gene expression was measured from total RNA and normalized to actin expression. P_{H3}:*LAT1* normalized expression is displayed relative to H99 for each condition. Measurements were performed in technical duplicates with biological triplicates.

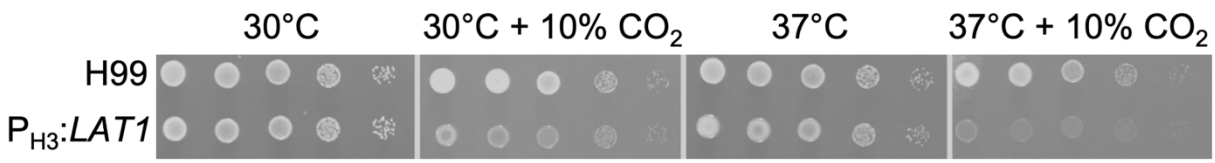

Figure S7

Constitutive expression of *LAT1* increases sensitivity to CO_2

Ten-fold serial dilutions from overnight cultures of indicated strains were spotted on solid RPMI medium with 165 mM MOPS, pH 7 at 30°C or 37°C in ambient air or at 10% CO_2 for 48 hrs before images were acquired.

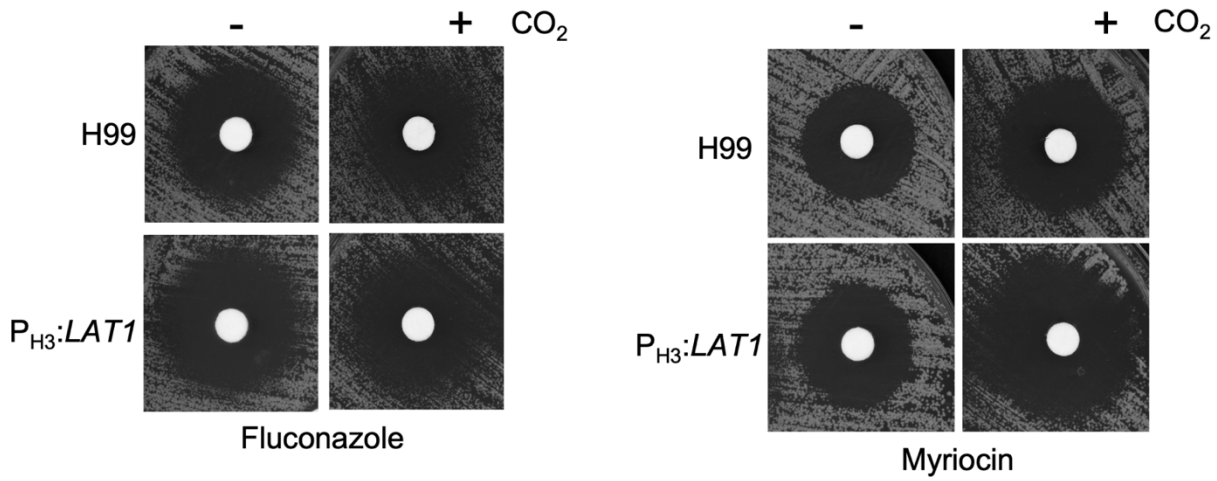

Figure S8

Constitutive expression of *LAT1* increases sensitivity to myriocin, not fluconazole

Cells from overnight cultures of H99 and P_{H3}:*LAT1* were spread on RPMI 1640 medium with 165 mM MOPS, pH 7. Sterile disks were placed on plates and 40 µg fluconazole or 8 µg myriocin was added to each disk. Cells were incubated at 30°C for 48 hrs in ambient air or 5% CO_2 before images were acquired.

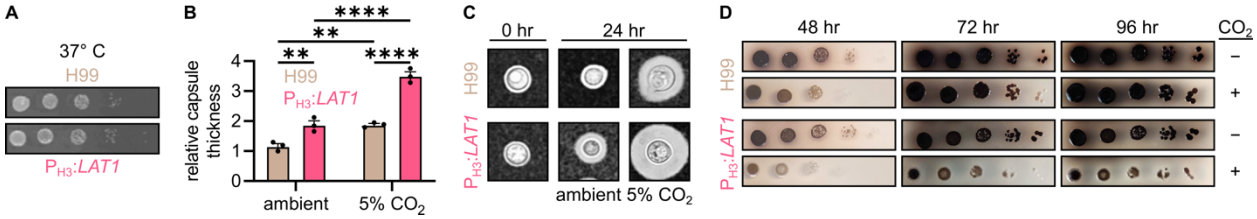

Figure S9

Constitutive expression of *LAT1* does not induce defects in survival at host temperature, ability to form capsule, or melanin production

A) Ten-fold serial dilutions from overnight cultures of indicated strains were spotted on solid RPMI medium with 165 mM MOPS, pH 7 at 37°C in ambient air or at 5% CO_2 for 48 hrs before images were acquired. B&C) Indicated strains were grown in RPMI 1640 +165 mM MOPS for 24 hours at 37°C in ambient air or 5% CO_2, then prepared for microscopy by counterstaining with India ink. At least 50 cells were quantified per condition and processed in ImageJ software to measure capsule. Capsule thickness at 24 hours was normalized to 0 hour. Two-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests was performed in GraphPad Prism. **, $P < 0.01$, ****, $P < 0.0001$. D) Washed cells from overnight cultures were spotted on melanin-inducing agar medium and incubated at 30°C in ambient air or 5% CO_2 before imaging at indicated time points.