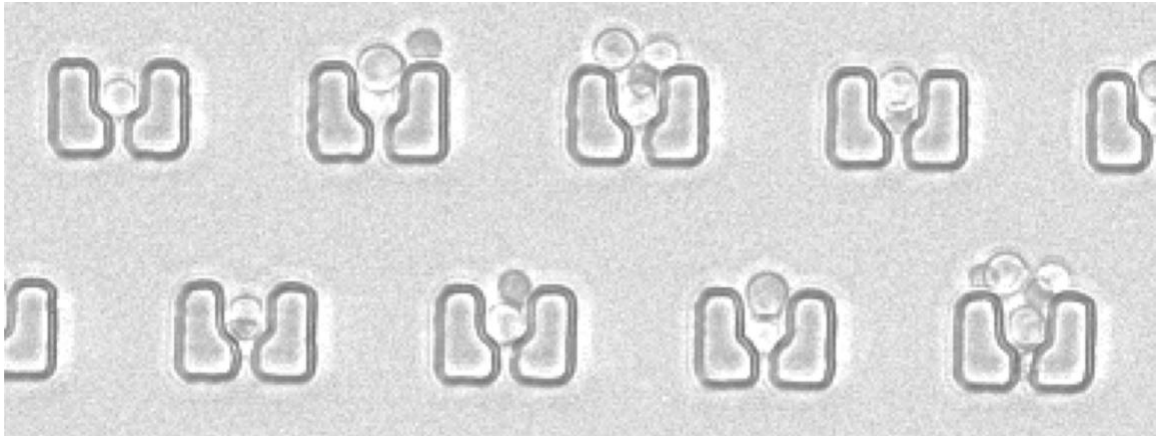


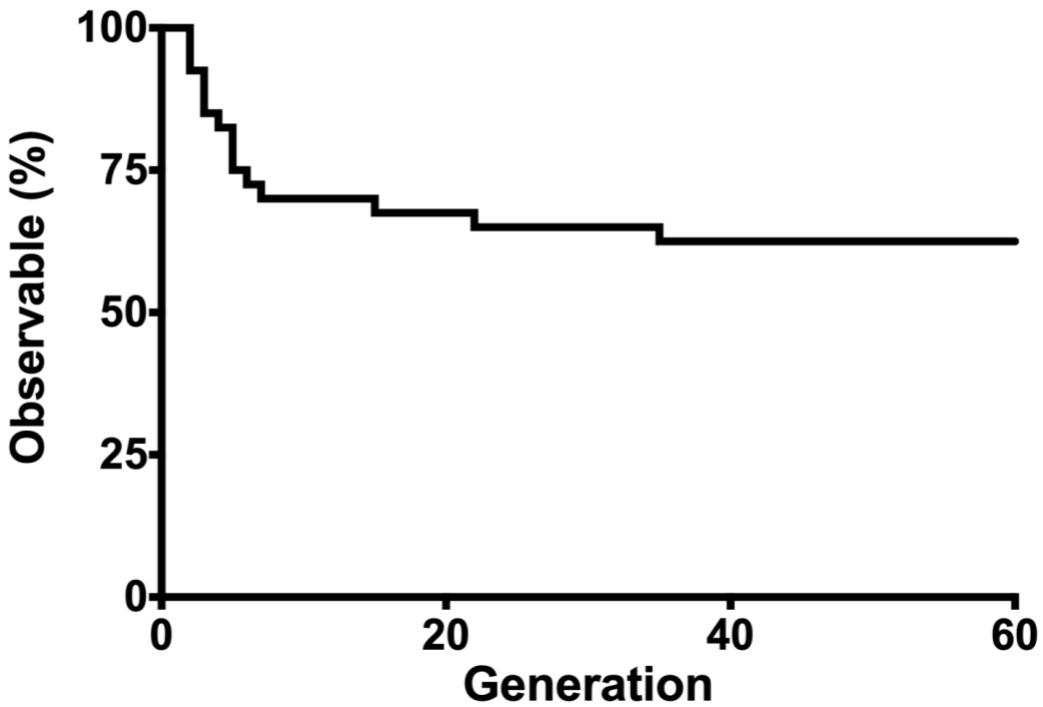
$W = 9 \text{ um}$

Supplementary Figure 1: Template outline of one HYAAC channel as designed in AutoCAD.

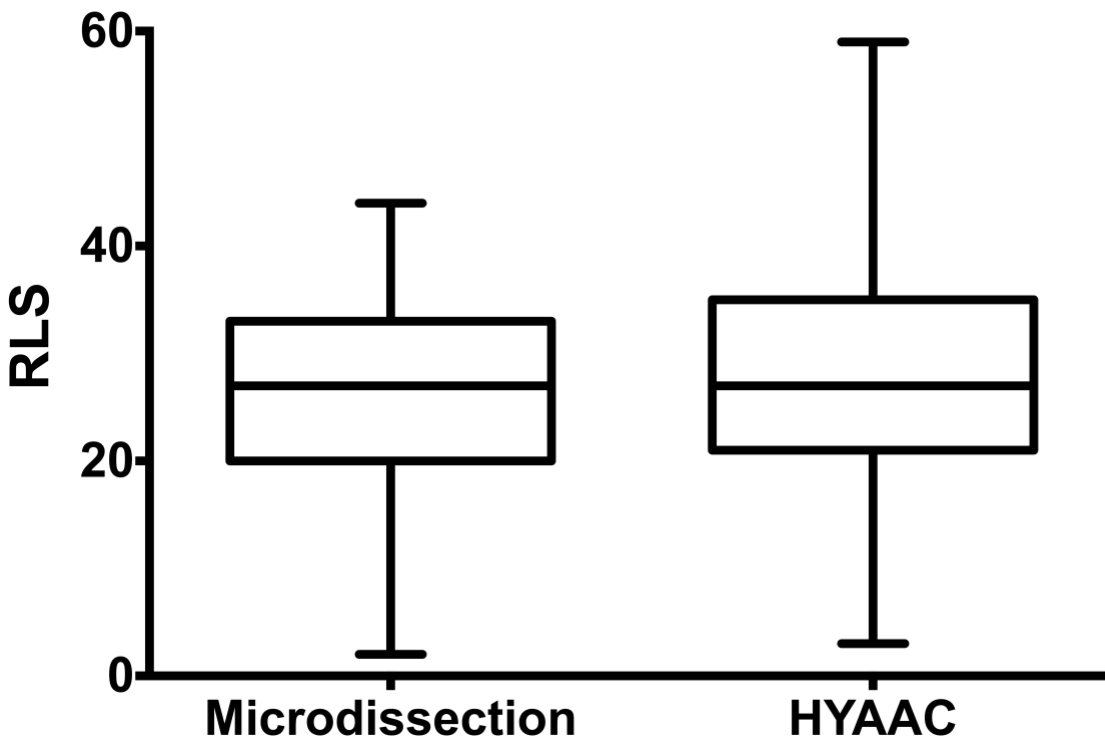


Supplementary Figure 2: Original buckets of HYAA chip filled with *C. neoformans*. Numerous issues were found with the original buckets where cells could outgrow through the top or clump and cluster together.

Retention rate in HYAAC



Supplementary Figure 3: Retention rate for cells that landed in a trap and replicated at least once. 28% of cells were lost within the first 7 generations and there was an overall retention rate of 62% for the 50 wildtype cells assessed.



Supplementary Figure 4: RLS of WT cells as measured by microdissection ($n = 30$) of in the HYAAC device ($n = 100$). $P = 0.450$ by Wilcoxon rank sum test. Center line is median, box limits are upper and lower quartiles, whiskers are minimum and maximum.

Supplementary Table 1: All strains used in this paper.

Strain	Reference
WT	H99 - Provided as a gift from Dr. John Perfect of Duke University.
RC2-ALL2:mCherry	Jain et. al, <i>Infection and Immunity</i> (2016)
H99 Δ CNAG_05431	Provided as a gift from Dr. Andrew Alspaugh of Duke University.
H99 Δ CNAG_03465	Provided as a gift from Dr. Peter Williamson of the National Institute for Health.
KN99 α Δ CNAG_00003 KN99 α Δ CNAG_04956 KN99 α Δ CNAG_00028	Purchased from the Madhani knockout collection managed by the Fungal Genetics Stock Center.
KN99 α Δ CNAG_04546 KN99 α Δ CNAG_04313 KN99 α Δ CNAG_06909	Provided as a gift from Dr. Maurizio Del Poeta at Stony Brook University who purchased from the Madhani knockout collection managed by the Fungal Genetics Stock Center.

Supplementary Table 2: Media used in this paper. Each recipe is adjusted for 1L of media in dH₂O.

Media	Recipe (in 1L dH ₂ O)
YPD broth	10 g yeast extract, 20 g peptone, 20 g dextrose (Difco)
YPD agar	10 g yeast extract, 20 g peptone, 20 g dextrose, 15 g agar (Difco)
Sabourad Dextrose (SAB) broth	5 g peptic digest of animal tissue, 5 g pancreatic digest of casein, 20 g dextrose (Difco)
RPMI 1640	10.4 g RPMI 1640 (with L-glutamine, without sodium bicarbonate), 165 ml 1M MOPS, adjusted to pH 7
SCA media	1.7 g yeast nitrogen base without amino acids, 1 g drop out mix, 0.4% ethanol, 5 g (NH ₄) ₂ SO ₄ , 3.3 g NaCl, 20 g glucose
SCA - 0.05%	1.7 g yeast nitrogen base without amino acids, 2 g drop out mix, 0.4% ethanol, 5 g (NH ₄) ₂ SO ₄ , 3.3 g NaCl, 0.5g glucose

Supplementary Table 3: Primers used in this paper.

Gene Name	Forward	Reverse
<i>CNAG_04956</i>	ACGGTATCGTCTCCACCAAC	CCAAAGCCTCGGTAGGTACA
<i>CNAG_00028</i>	AACAATGCGGGAAGTGAATC	TTCCGCCTTATCCCTTTTCT
<i>CNAG_03465</i>	TTTGGGGCCCCTTAATTATC	GGATAGGTGCATGAGGAGGA
<i>CNAG_04313</i>	GGAGCGATTCATTGGTCATT	GTCGGTACGATCGTTGGAGT
<i>CNAG_06909</i>	GCCCATAGCACTAGGAGTCG	ATGTCATCCTGCGAATAGCC
<i>CNAG_00003</i>	GTGAAACTCGGAGCTTGGTC	TACAATGGGCTCCATGAACA
<i>CNAG_04546</i>	TTATCGCATGTCTCCCCTTC	GTGGAATAGGAATGGCATGG
<i>ACT1</i>	CCCACACTGTCCCCATTTAC	AACCACGCTCCATGAGAATC