**Supplemental Table 1: Strains used in this study.**

| Strain | Genotype | Reference |
|---|---|---|
| SC5314 | Prototroph | (4) |
| RM1000 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG* | (8) |
| CJC5 | *ura3::λimm434/ura3:: λimm434 his1::hisG/his1::hisG* *RPS10/rps10*::*URA3*-*HIS1*-GFP | This study |
| CJC25 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG* *RPS10/rps10*::*URA3*-*HIS1*-GFP *ADH1/adh1::*yCherry-*SAT1* | This study |
| CJC26 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG* *RPS10/rps10::ACT1p*-GFP-*URA3-HIS1* *ADH1/adh1::*yCherry-*SAT1* | This study |
| CJC27 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG* *RPS10/rps10::ARG1p*-GFP-*URA3-HIS1* | This study |
| CJC28 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG* *RPS10/rps10::ARG1p*-GFP-*URA3-HIS1* *ADH1/adh1::*yCherry-*SAT1* | This study |
| CJC29 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG* *RPS10/rps10::ARG3p*-GFP-*URA3-HIS1* *ADH1/adh1::*yCherry-*SAT1* | This study |
| CJC30 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG* *RPS10/rps10::LYS1p*-GFP-*URA3-HIS1* *ADH1/adh1::*yCherry-*SAT1* | This study |
| CJC31 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG* *RPS10/rps10::LEU2p*-GFP-*URA3-HIS1* | This study |

| | | |
|---|---|---|
| CJC32 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG RPS10/rps10::ARO4p*-GFP-*URA3-HIS1* | This study |
| HZY28 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG arg81::HIS1/arg81::ARG4* | (7) |
| DSY3426-2 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG arg83::URA3/arg83::ARG4* | (7) |
| DSY3233 | *ura3::λimm434/ura3::λimm434 gcn4::hisG/gcn4::hisG* | (7) |
| CJC33 | *ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG arg81::HIS1/arg81::ARG4 RPS10/rps10::ARG3p*-GFP-*URA3-HIS1 ADH1/adh1::*yCherry-*SAT1* | This study |
| CJC34 | *ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG arg83::URA3/arg83::ARG4 RPS10/rps10::ARG3p*-GFP-*URA3-HIS1 ADH1/adh1::*yCherry-*SAT1* | This study |
| CJC35 | *ura3::λimm434/ura3::λimm434 gcn4::hisG/gcn4::hisG RPS10/rps10::ARG3p*-GFP-*URA3-HIS1 ADH1/adh1::*yCherry-*SAT1* | This study |
| OXYellow | *ura3::λimm434/ura3:: λimm434 CTA1p-GFP-URA3::hisG ADH1p-yCherry-SAT1* | (2) |
| ARGiallo | *ura3::λimm434/ura3:: λimm434 ARG3p-GFP-URA3::hisG ADH1p-yCherry-SAT1* | This study |
| JRC12 | *arg1Δ::FRT/arg1Δ::FRT* | This study |
| JRC29 | *arg1Δ::FRT/arg1Δ::FRT-ARG1* | This study |
| JRC38 | *arg3Δ::FRT/arg3Δ::FRT* | This study |

| | | |
|---|---|---|
| JRC41 | *arg3*Δ*::FRT/arg3*Δ*::FRT-ARG3* | This study |
| JC50 | *ura3/ura3, his1/his1, hog1::LoxP-ura3-LoxP/hog1::LoxP-HIS1-LoxP* CIp20 (*URA3, HIS1*) | (6) |
| JC52 | *ura3/ura3, his1/his1, hog1::LoxP-ura3-LoxP/hog1::LoxP-HIS1-LoxP* CIp20-*HOG1* (*URA3, HIS1*) | (6) |
| RGC1 | *RPS10/rps10::*CIp10-*ACT1p*-GFP-*SAT1* | This study |
| JRC47 | *arg1*Δ*::FRT/arg1*Δ*::FRT RPS10/rps10::*CIp10-*ACT1p*-GFP-*SAT1* | This study |
| JRC42 | *arg3*Δ*::FRT/arg3*Δ*::FRT RPS10/rps10::*CIp10-*ACT1p*-GFP-*SAT1* | This study |

**Supplemental Table 2. Plasmids used in this study.**

| Plasmid | Features | Reference |
|---|---|---|
| CIp10 | CaURA3-RPS10 | (5) |
| CIp20 | CIp10-HIS1 | (3) |
| pGFP | CIp10-GFP | (1) |
| pACT1-GFP | CIp10-ACT1p-GFP | (1) |
| pCJ1 | pGFP-HIS1 | This study |
| pCJ2 | pACT1-GFP-HIS1 | This study |
| pCJ4 | pCJ1-ARG3p-GFP | This study |
| pCJ5 | pCJ1-ARG1p-GFP | This study |
| pADH1-mCherry | CIp-ADH1p-mCherry-SAT1 | (2) |
| pJRC28 | ARG1:FRT-SAT1-FRT | This study |
| pJRC40 | ARG3:FRT-SAT1-FRT | This study |
| pHZ130 | CIp-ACT1p-GFP-SAT1 | This study |

**Supplemental References**


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**Figure S1. Misannotation of the *ARG1* gene.** A) A transcriptional reporter generated based on the originally annotated start codon (at position 151,359 on Chromosome R) was not expressed on arginine-deficient YNB medium (left image). In contrast, a reporter using a second, in-frame, ATG 231 nt to the 3' (position 151,128) is strongly fluorescent in the absence of arginine (right image). Both images are GFP-DIC overlays. B) 5' RACE identifies one major and two minor transcriptional start sites at -48, -44, and -35 relative to the 3' ATG. C) A ClustalW alignment of the amino termini of Arg1p homologs from related species, using the originally annotated protein sequence of *C. albicans*, showing a 77 amino acid addition not homologous to the other species.

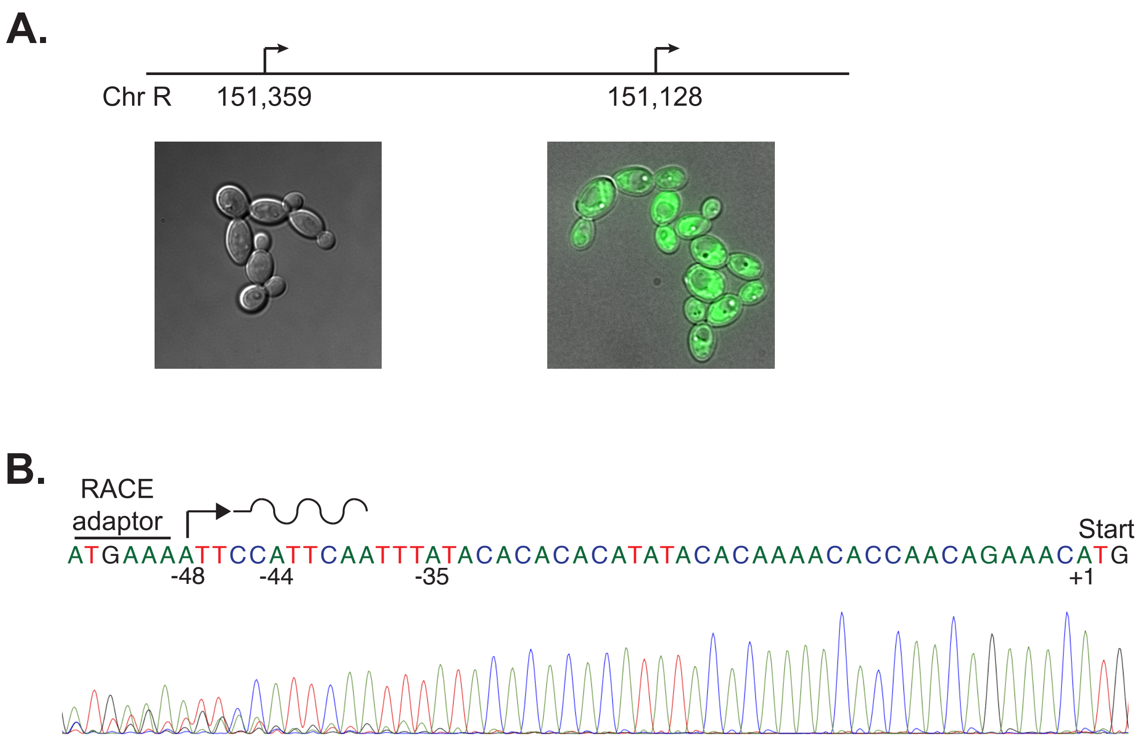
**Figure S2. *ARG1* and *ARG3* respond specifically to arginine deprivation.** *ARG1p-GFP* (CJC27) *ARG3p-GFP* (CJC29) cells were grown overnight in YPD and subculture in fresh YPD, YNB, or YNB supplemented with the indicated concentrations of L-arginine (R), L-leucine (L) and L-lysine (K). Cells were grown for 4 hours at 30°C. The images shown are GFP-DIC overlaid pictures.

**Figure S3. ARG3 is induced in phagocytosed cells in vivo.** Zebrafish were inoculated with the *ARG3-GFP/ADH1*-yCherry strain (ARGiallo) or the GFP-less control strain by microinjection into the hindbrain. At the indicated times, fish were anesthetized and immobilized in agarose prior to visualization by fluorescence microscopy. A) Representative images of phagocytosed ARGiallo cells. B) Ratiometric quantitation of GFP fluorescence intensity in intracellular versus extracellular cells over time.

**Figure S4.** ***arg1*Δ and *arg3*Δ mutants are arginine auxtrophs.** Wild-type (SC5314), *arg1*Δ (JRC12), *arg3*Δ (JRC38), and complemented (JRC29, JRC41) strains were diluted to an $OD_{600}$ of 0.1, and five-fold serial dilutions were spotted onto synthetic complete (SC) plates, synthetic dropout plates lacking arginine (SD-Arg) supplemented with or without additional arginine and grown at 30°C for 2 days.

**Figure S5.** ***arg* mutants are not sensitive to oxidative stress.** Wild-type (SC5314), *hog1*Δ/Δ (JC50), *arg1*Δ (JRC12), *arg3*Δ (JRC38), and complemented (JC52, JRC29, and JRC41, respectively) strains were diluted to an $OD_{600}$ of 0.1 and five-fold serial dilutions were spotted onto YPD plates with the indicated concentrations of $H_2O_2$ or menadione and grown at 30°C for 3 days.

**Supplemental Movie 1.** The *ARG3p*-GFP reporter strain was co-cultured with macrophages and images were collected every three minutes for 3.5 hours, beginning 15 minutes after initiation of the co-culture. The movie shows the merged GFP-DIC image.




C.

```
orf19.7469   MTNSVIGQLKNFLTALNLLSIHSSRKEIFFFLNYKRDSKNLLNENFIYLFFFSFFSFLLF 60
CD36_25720   ------------------------------------------------------------
CTRG_01005   ------------------------------------------------------------
CLUG_04362   ------------------------------------------------------------
YOL058w      ------------------------------------------------------------
CAGL0C05115g ------------------------------------------------------------

orf19.7469   LFHSIYTHTYTQNTNRNMSKGKVCLAYSGGLDTSVILAWLLEQGYEVIAFLANIGQEEDF 120
CD36_25720   -----------------MSKGKVCLAYSGGLDTSVILAWLLEQGYEVIAFLANIGQEEDF 43
CTRG_01005   -----------------MSKGKVCLAYSGGLDTSVILAWLLEQGYEVIAFLANIGQEEDF 43
CLUG_04362   -----------------MSKGKVCLAYSGGLDTSVILAWLLEQGYEVVAFLANIGQEEDF 43
YOL058w      -----------------MSKGKVCLAYSGGLDTSVILAWLLDQGYEVVAFMANVGQEEDF 43
CAGL0C05115g -----------------MSKGKVCLAYSGGLDTSIILAWLLEQGYEVVAFMADVGQEEDF 43
                              ****************:******:*****:**:*::******
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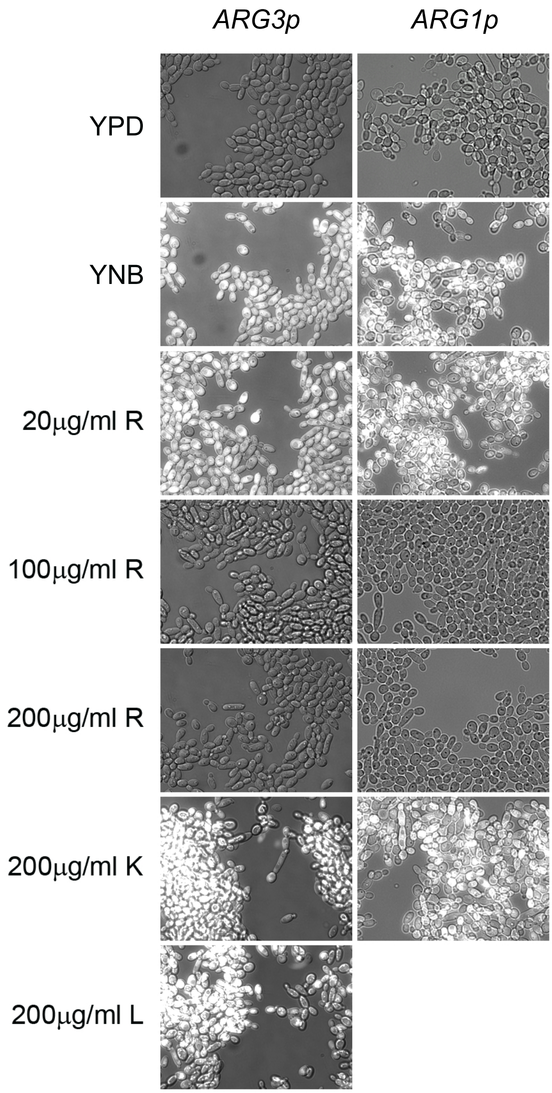
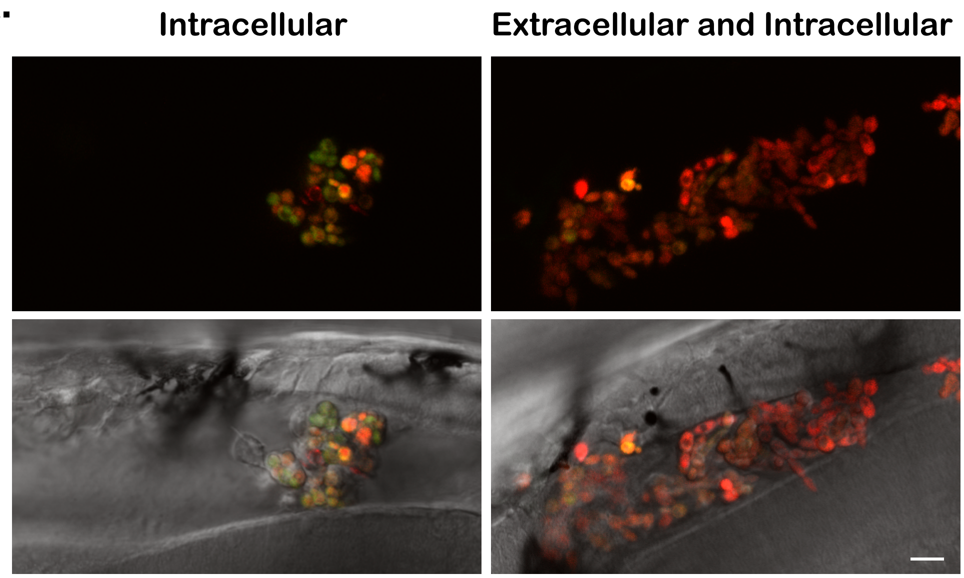


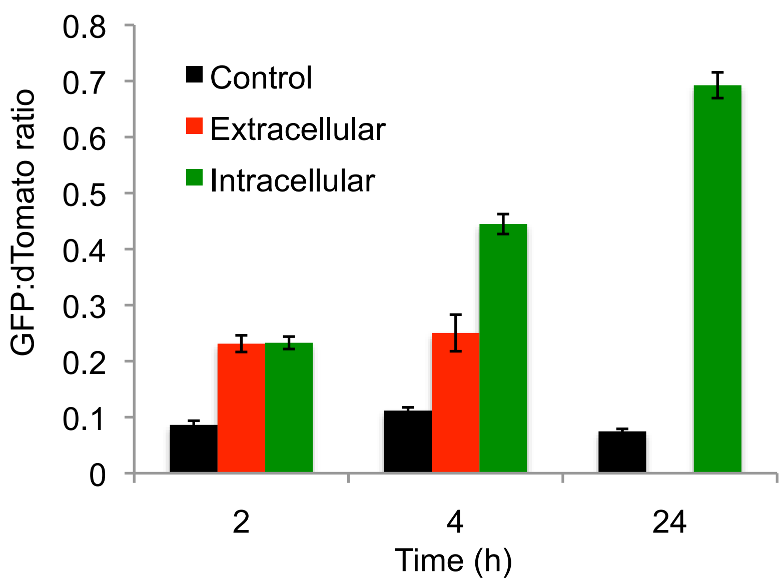
ARG3p
ARG1p
YPD
YNB
20μg/ml R
100μg/ml R
200μg/ml R
200μg/ml K
200μg/ml L

A.

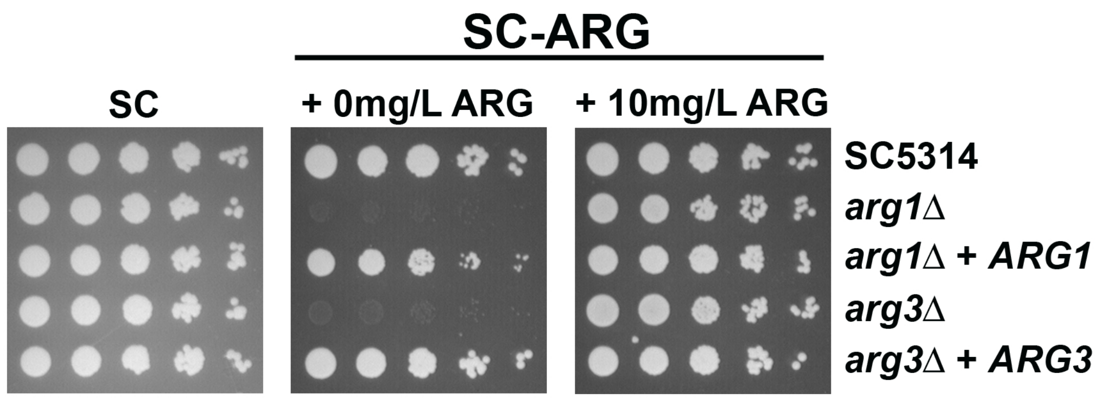


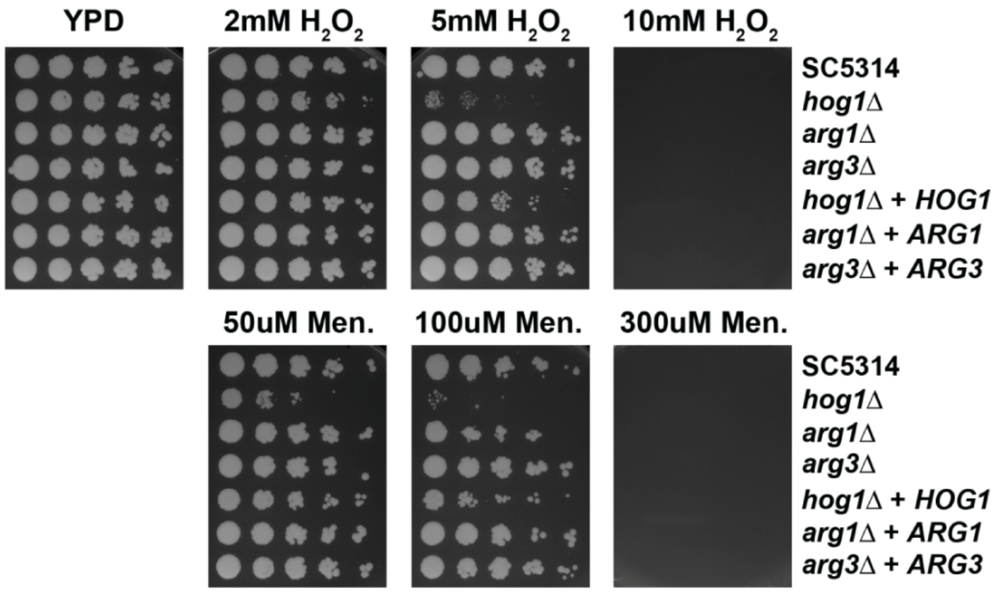
Intracellular
Extracellular and Intracellular


B.



0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
GFP:dTomato ratio
Control
Extracellular
Intracellular
2
4
24
Time (h)


SC-ARG
SC
+ 0mg/L ARG
+ 10mg/L ARG
SC5314
arg1Δ
arg1Δ + ARG1
arg3Δ
arg3Δ + ARG3


YPD
2mM $H_2O_2$
5mM $H_2O_2$
10mM $H_2O_2$
SC5314
*hog1*Δ
*arg1*Δ
*arg3*Δ
*hog1*Δ + *HOG1*
*arg1*Δ + *ARG1*
*arg3*Δ + *ARG3*
50uM Men.
100uM Men.
300uM Men.
SC5314
*hog1*Δ
*arg1*Δ
*arg3*Δ
*hog1*Δ + *HOG1*
*arg1*Δ + *ARG1*
*arg3*Δ + *ARG3*

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