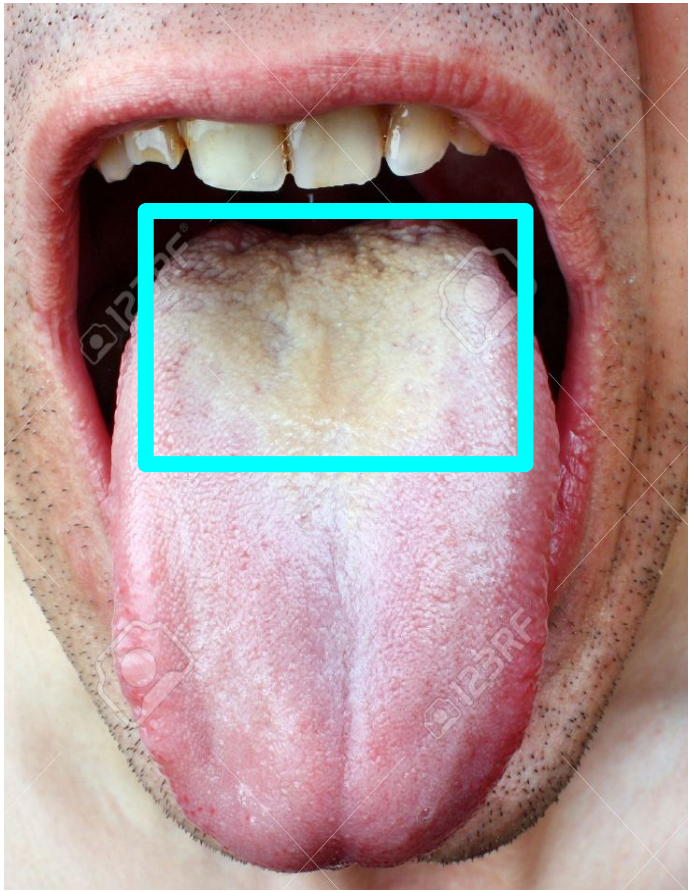
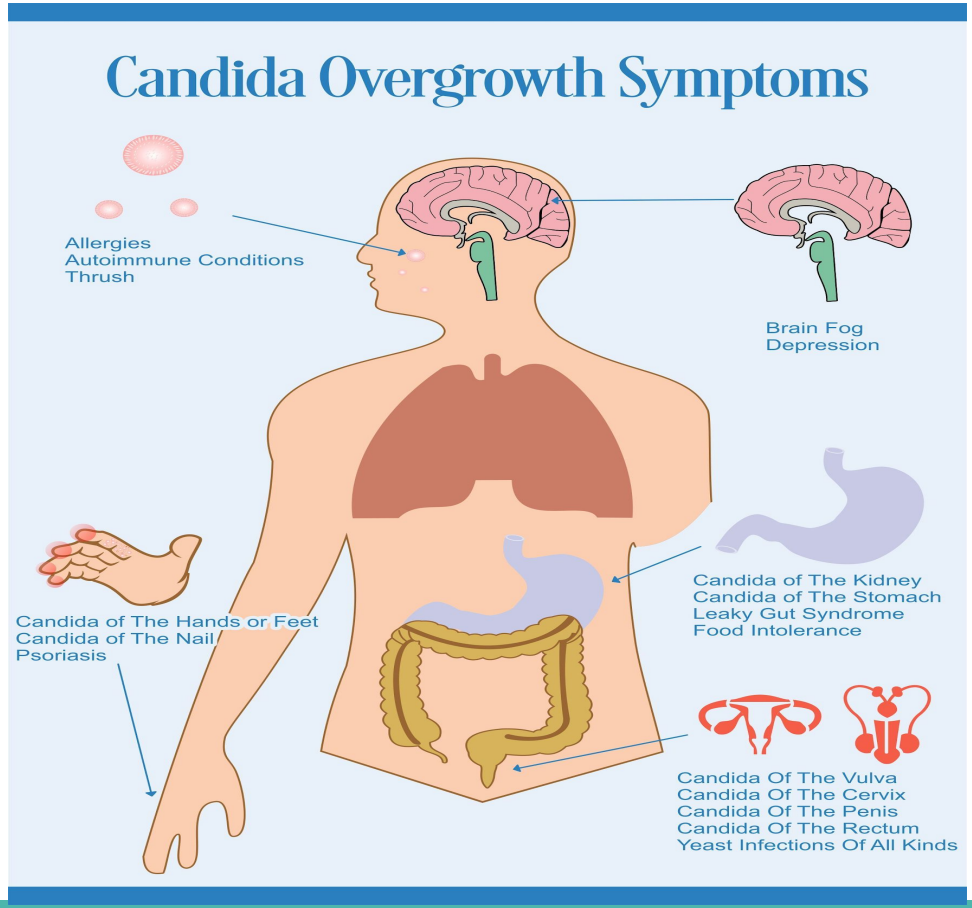
A Synthetic System That Senses *Candida albicans* and Inhibits Virulence Factors

Michael Tscherner, Tobias W. Giessen, Laura Markey, Carol A. Kumamoto, and Pamela A. Silver

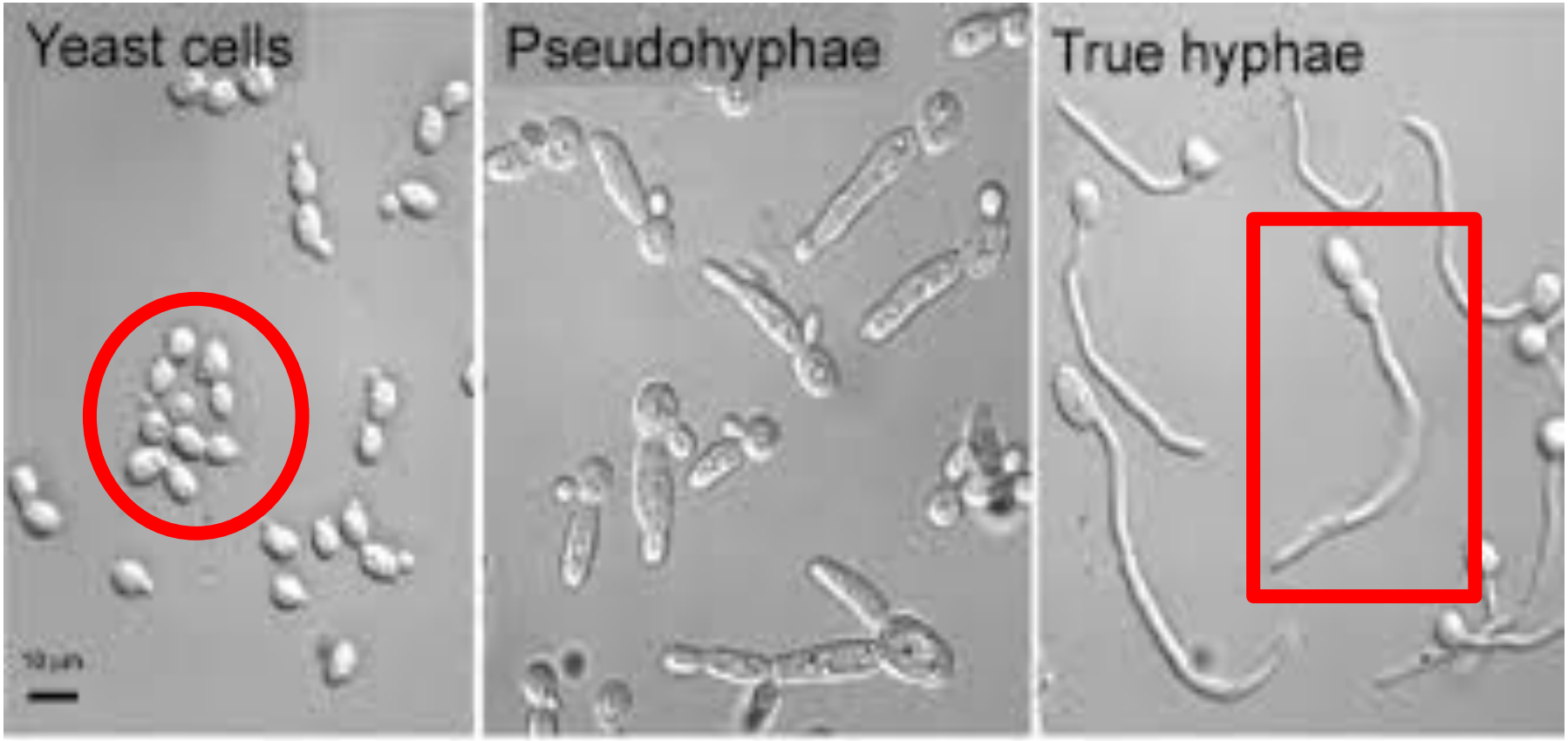
Presented By: Yogindra Raghav and Matthew Eckelmeyer

Candida Albicans Has Serious Health Implications

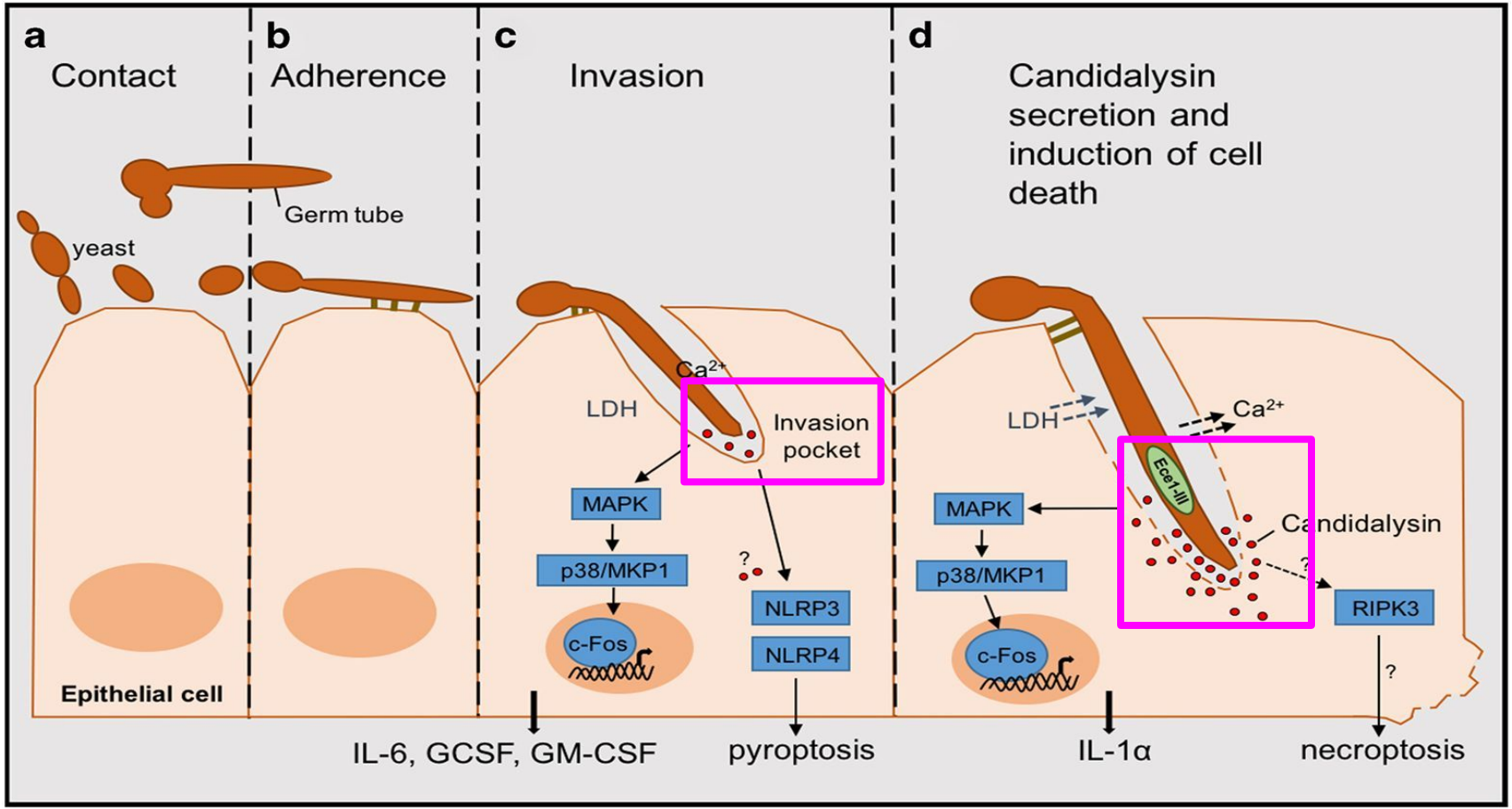




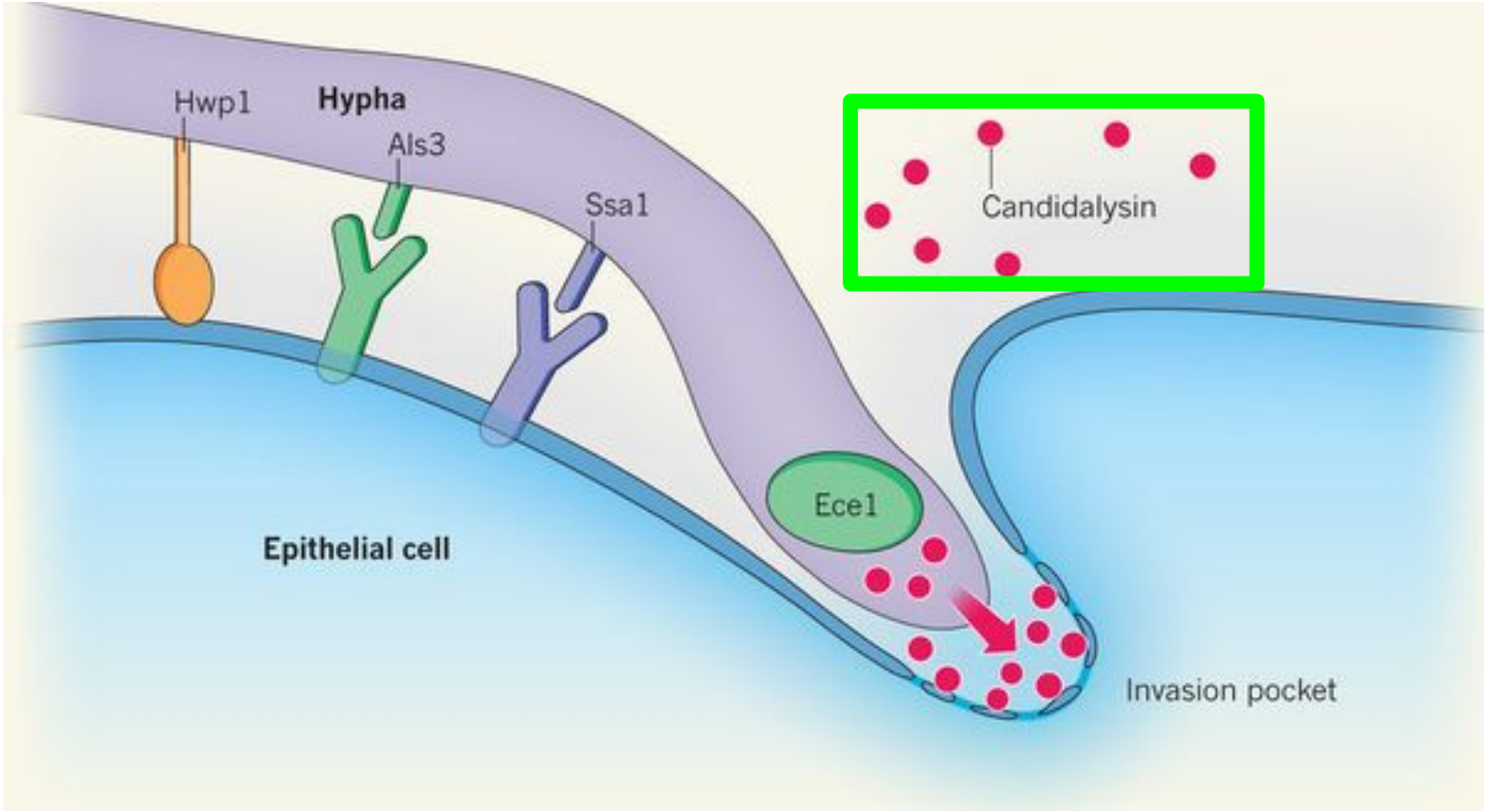
Candida Albicans Exhibits Morphological Plasticity



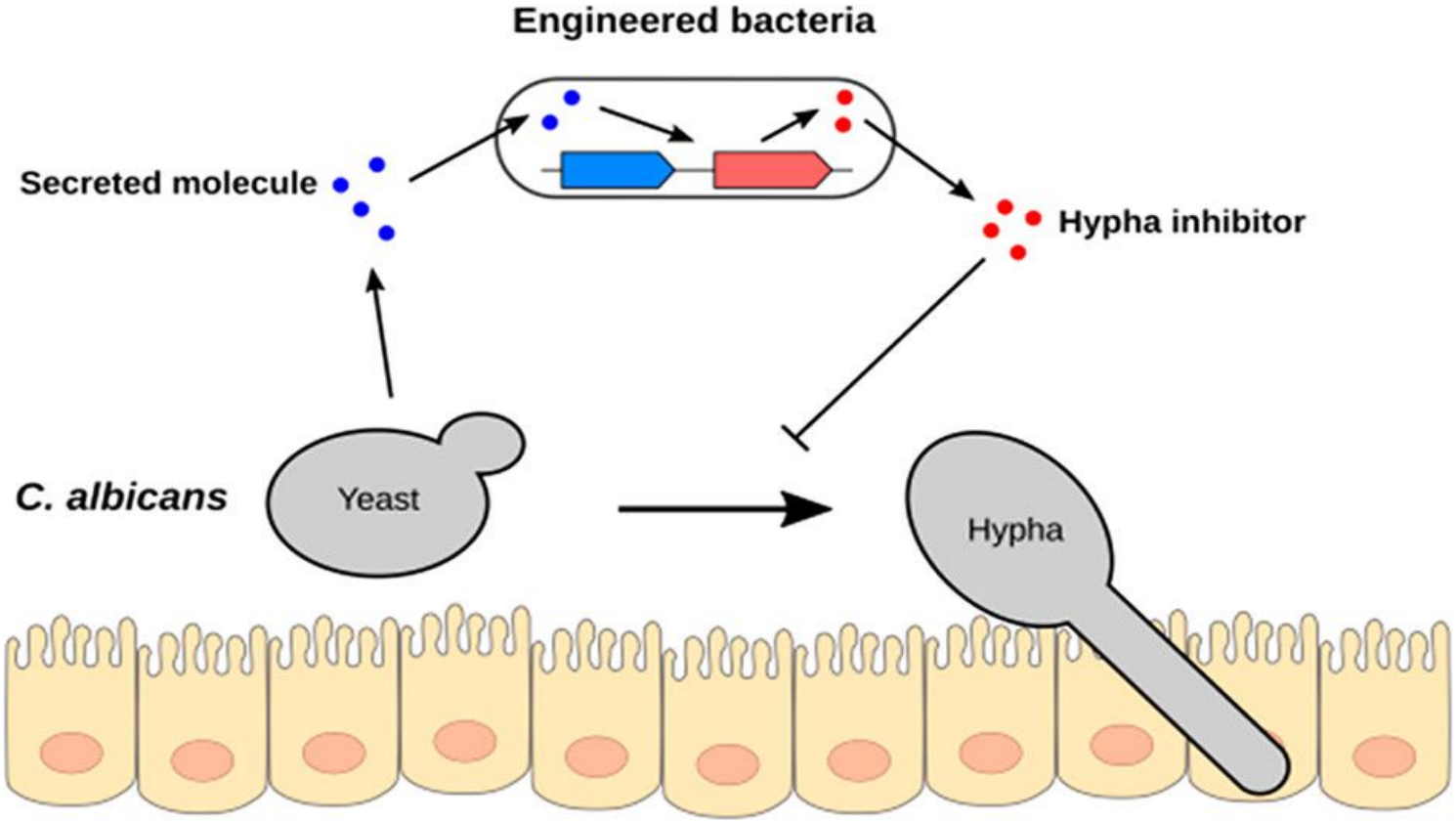
Hypha *C. Albicans* Causes Epithelial Cell Death



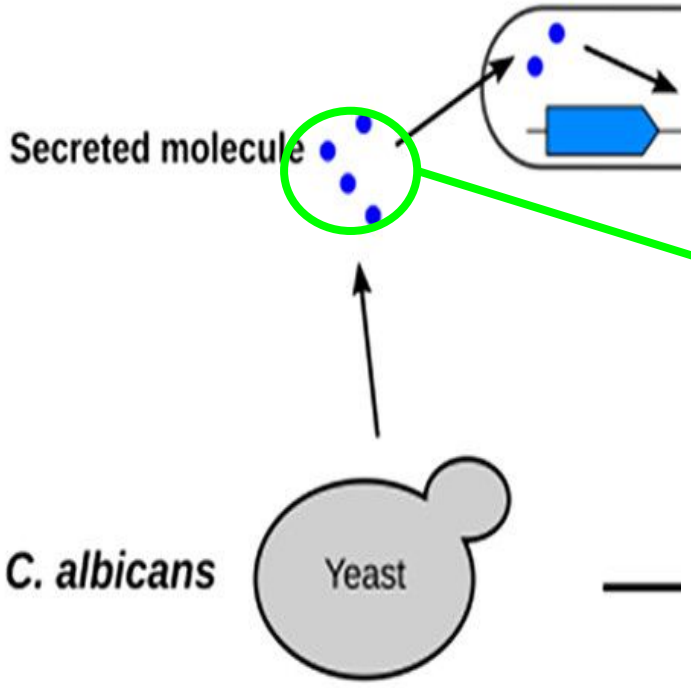
Candidalysin Causes Epithelial Cell Death

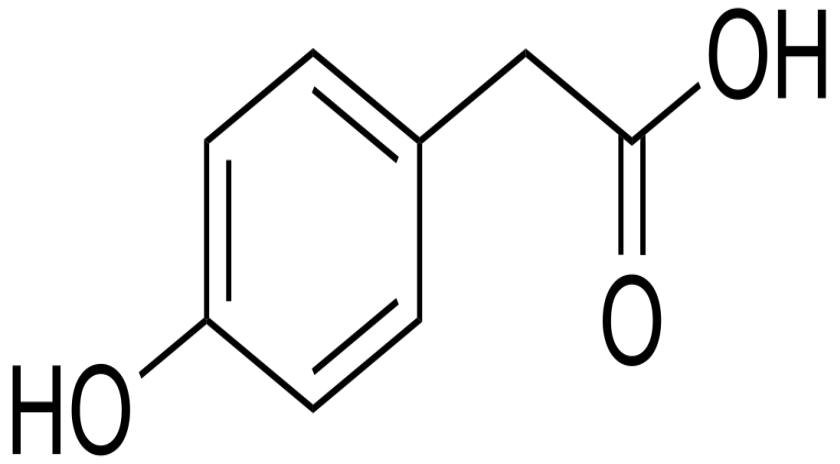


Synthetic *E. coli* Can Sense and Inhibit Yeast Hypha Formation

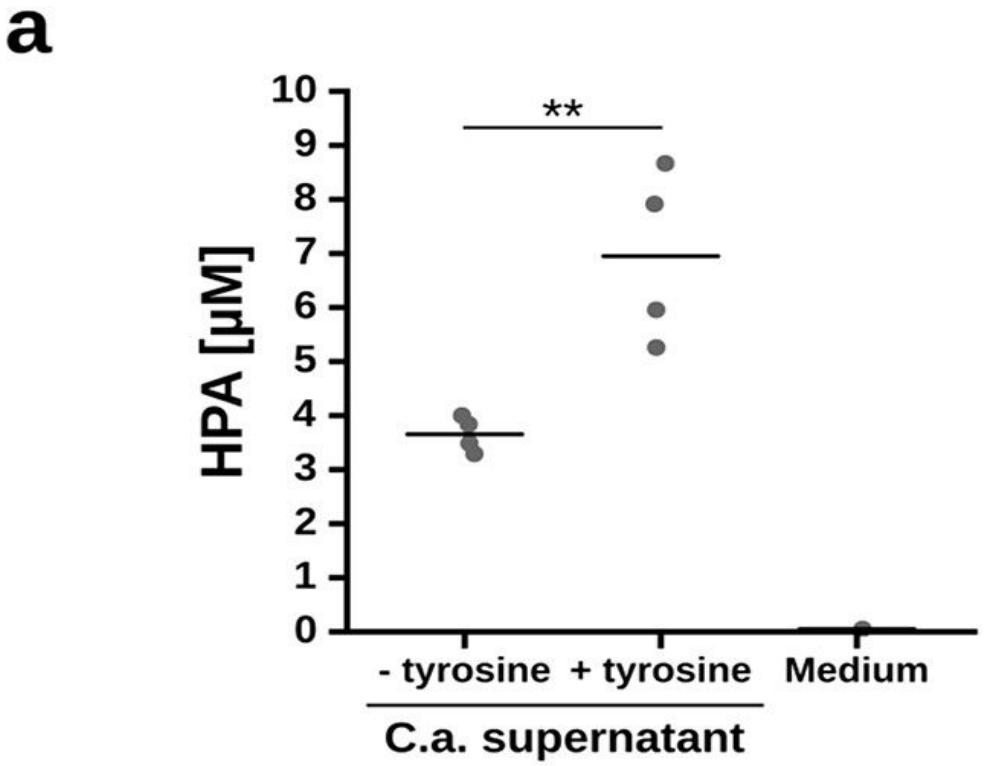


E. Coli (W) Uses 4-Hydroxyphenylacetic Acid (HPA) to Sense Presence of *Candida Albicans*

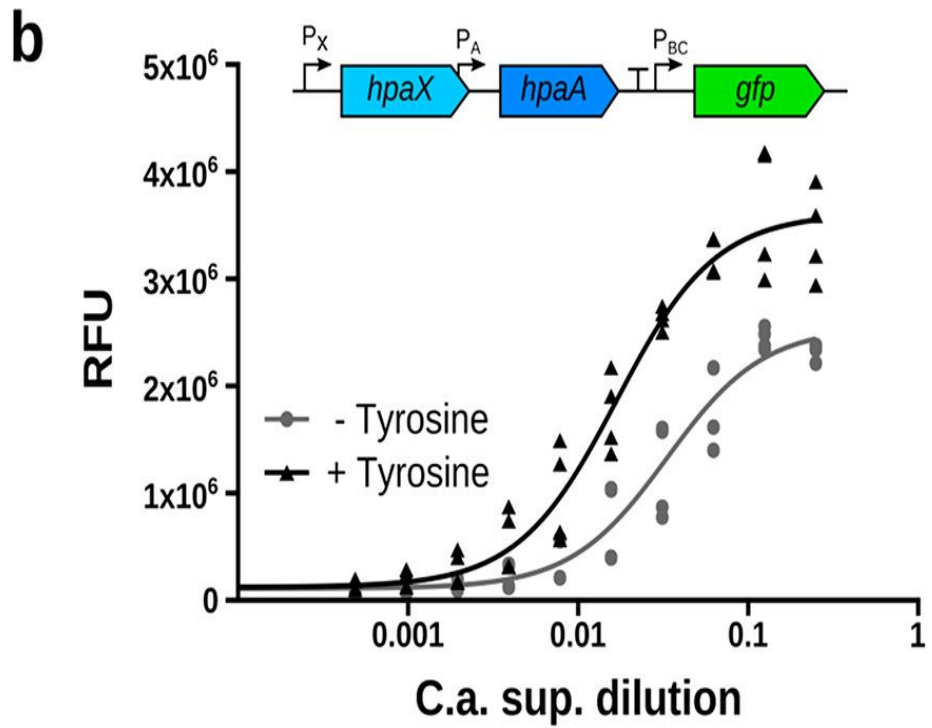


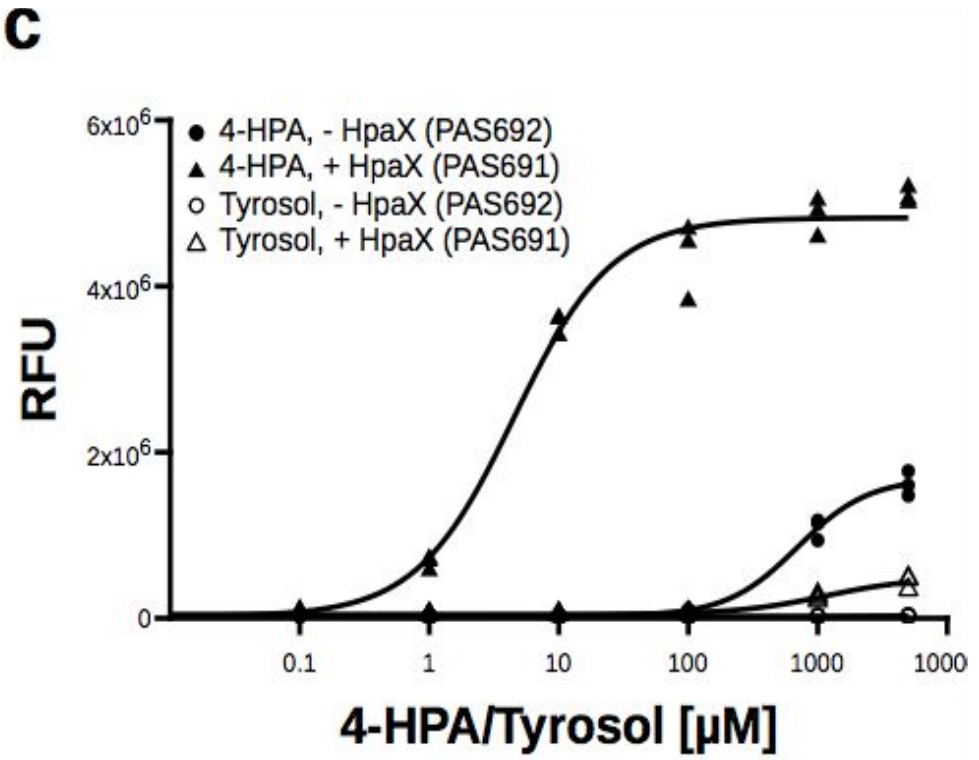


HPA is Produced in *C. albicans* and Increases With Tyrosine Supplementation

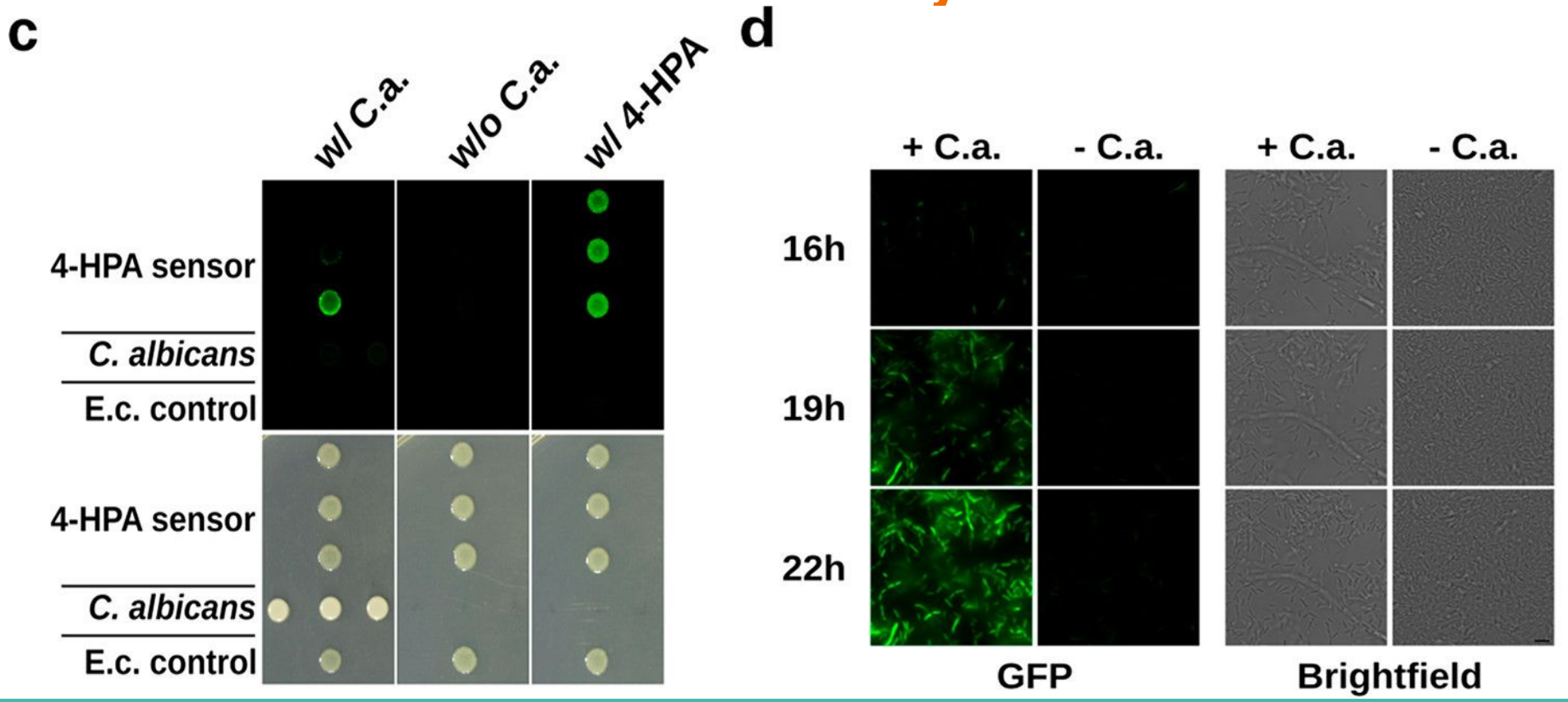


E. coli W HPA Transporter and Transcription Factor are Sufficient for Creation of HPA Sensor

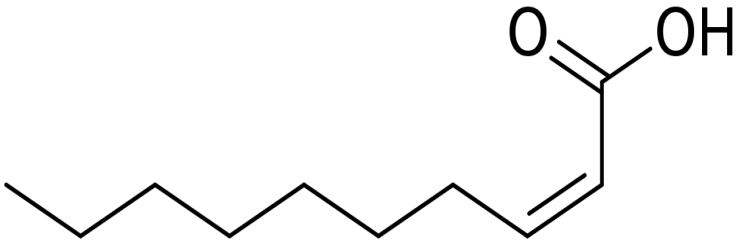


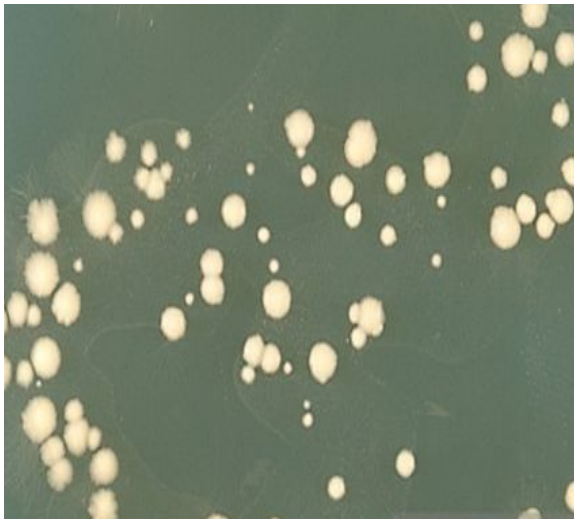


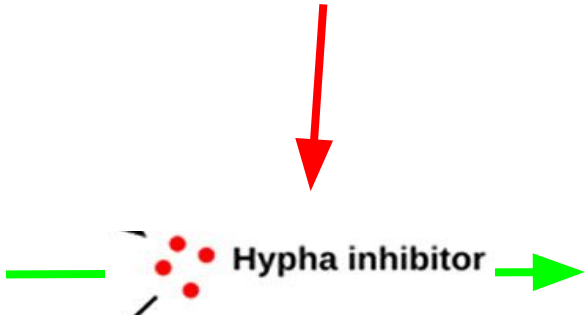
HPA Sensor Plasmid Exhibits Increased Fluorescence in Close Proximity to *C. albicans*



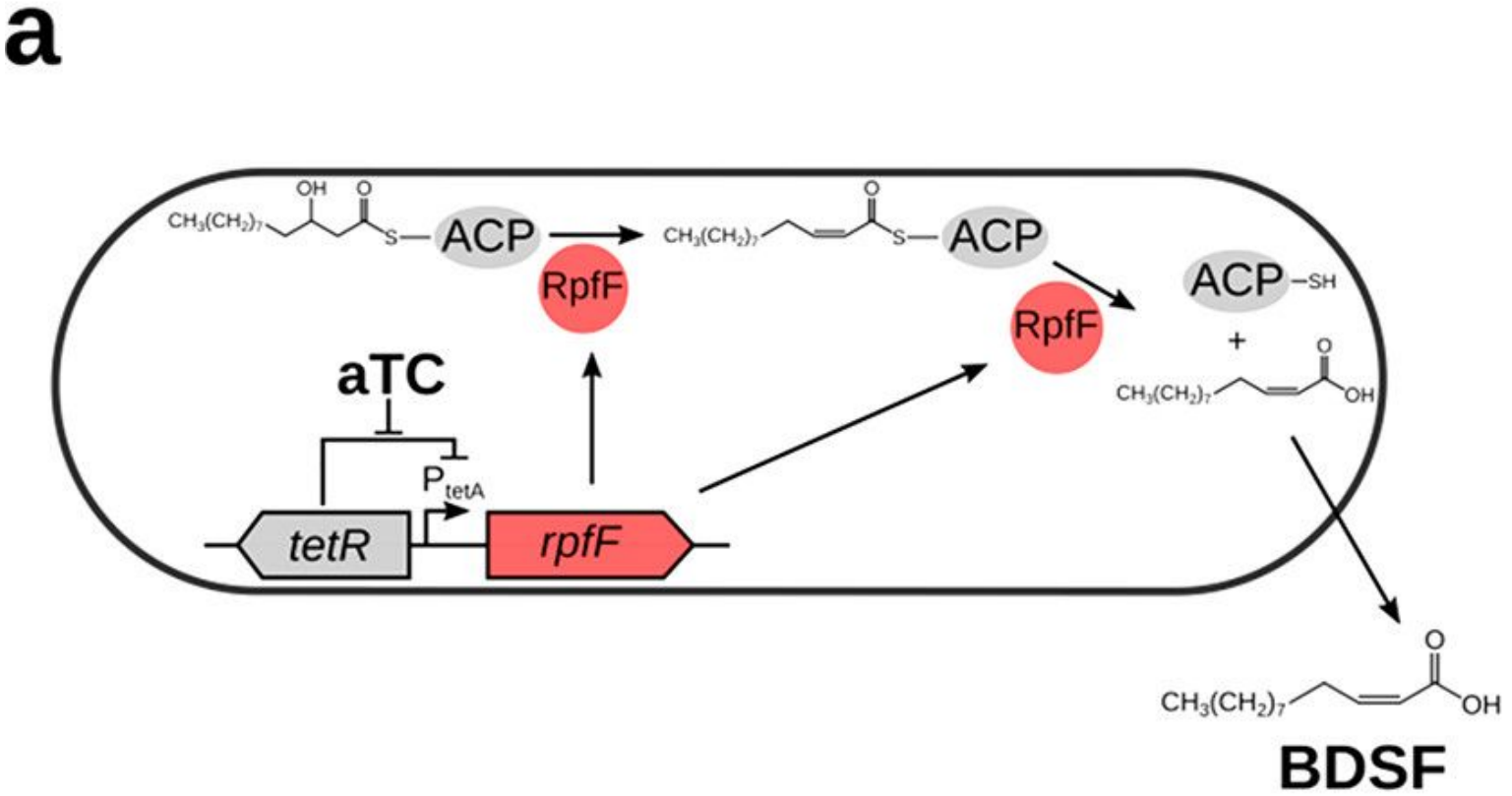
Cis-2-Dodecenoic Acid (BDSF) Inhibits Hypha Formation



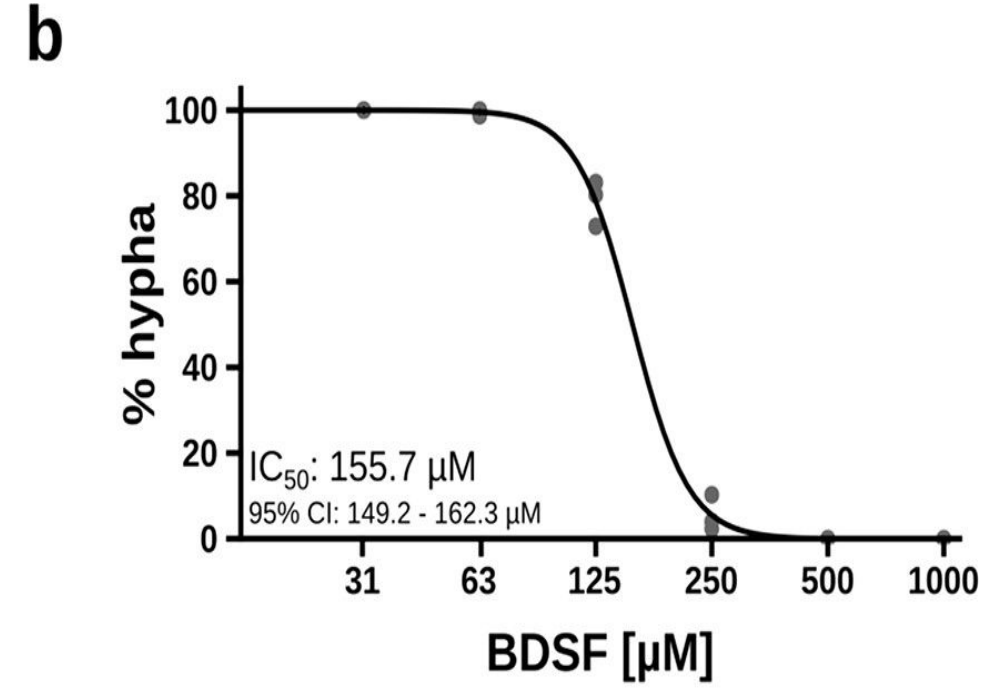


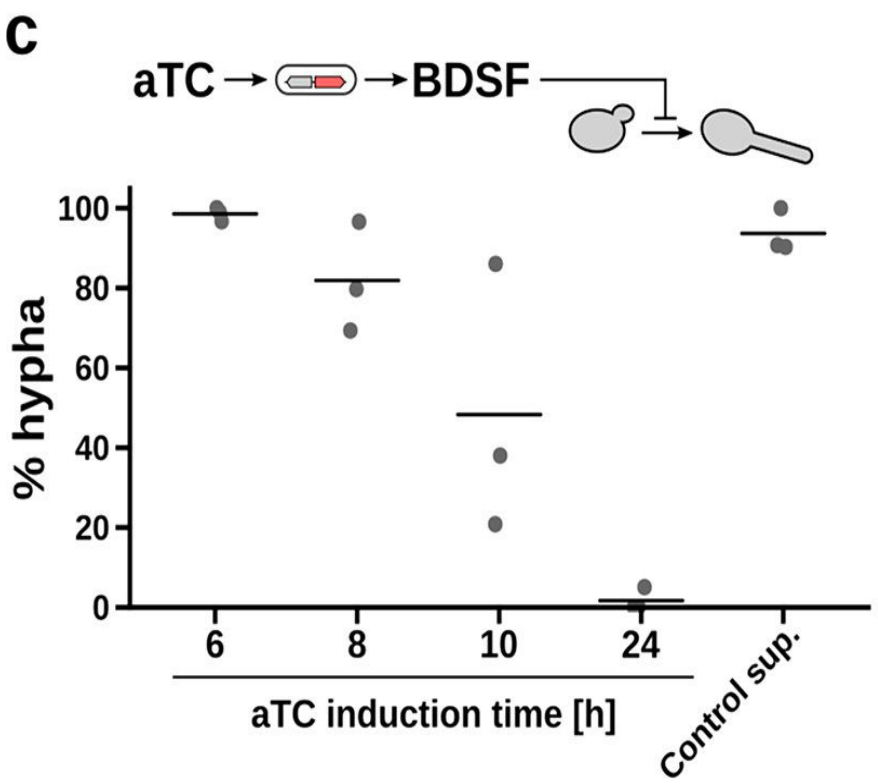


Expression of *rpfF* in *E. coli* Results in Production of Hypha Inhibitor

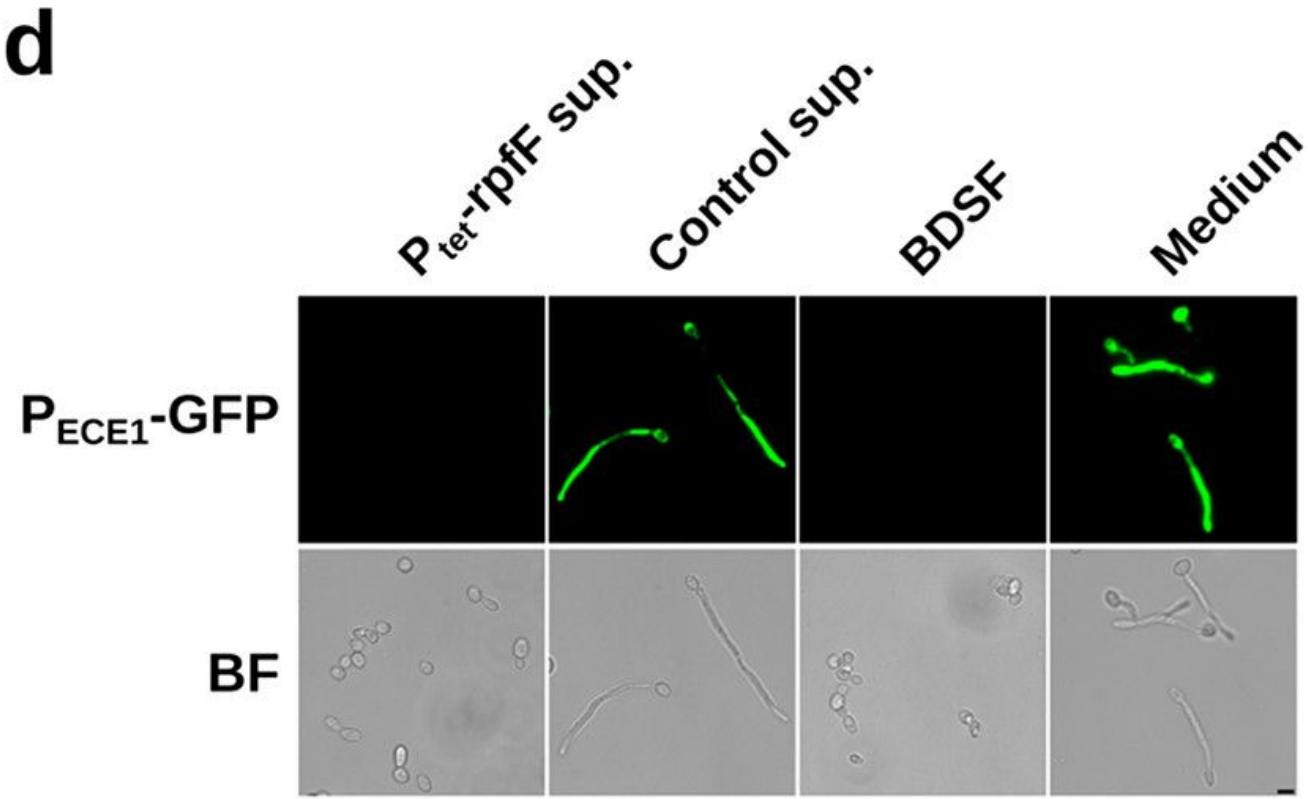


Expression of BDSF in *E. coli* is Sufficient to Inhibit Hypha Formation

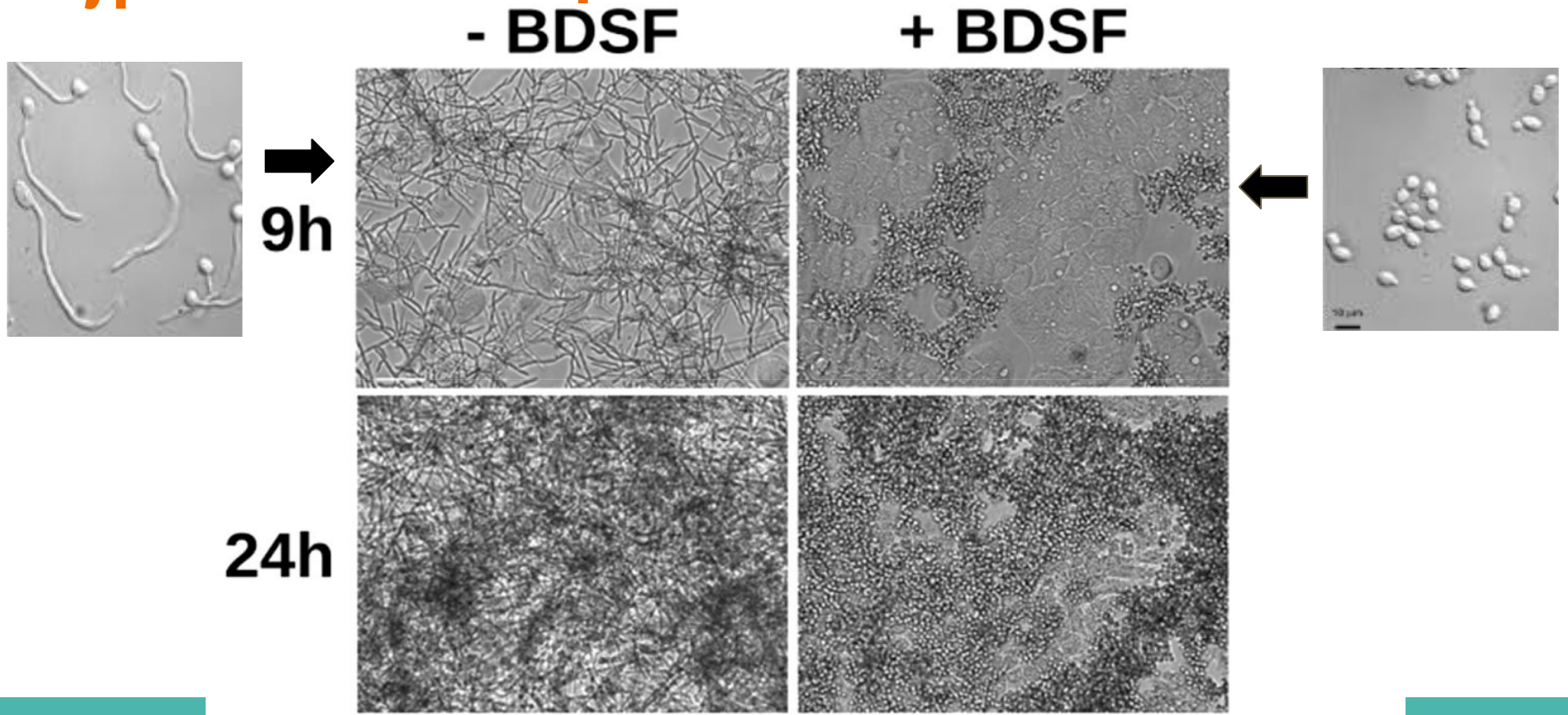




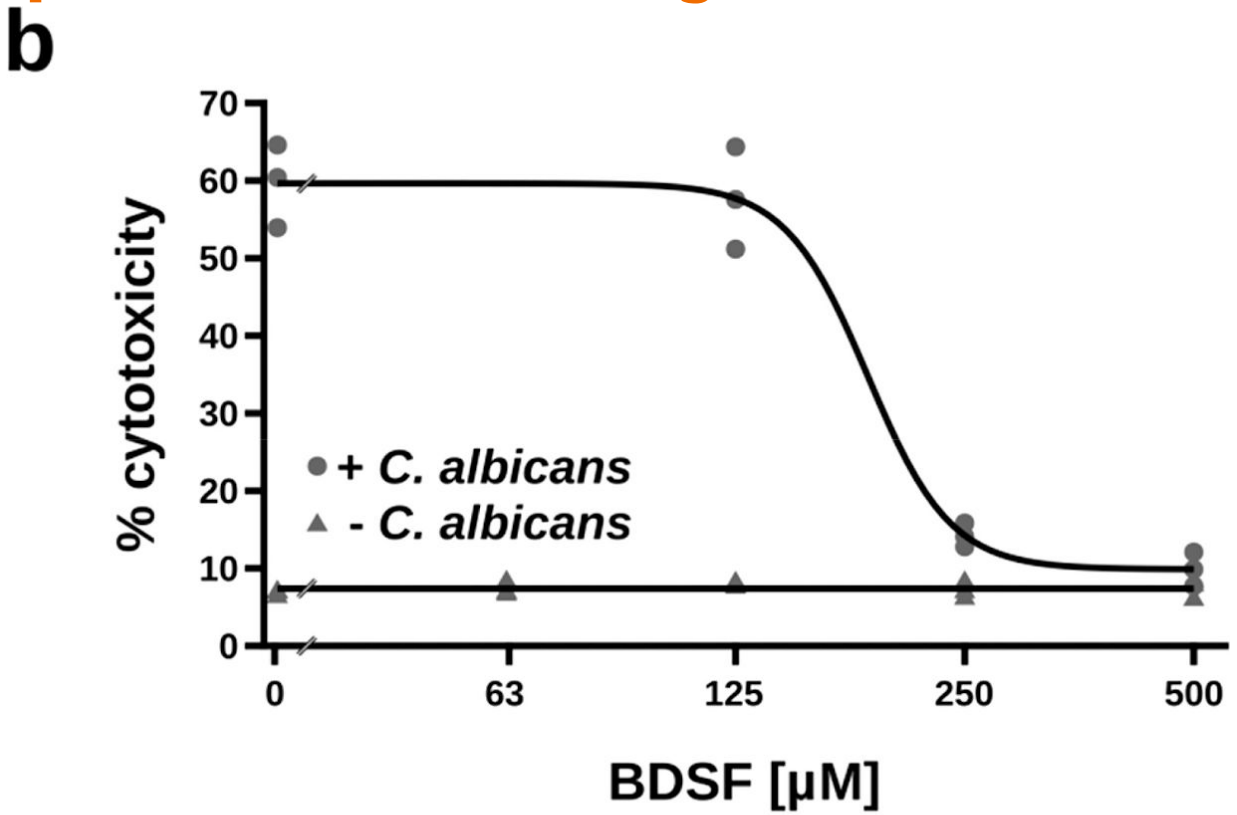
BDSF Inhibition of Hypha Formation Reduces Expression of Virulence Factor Candidalysin



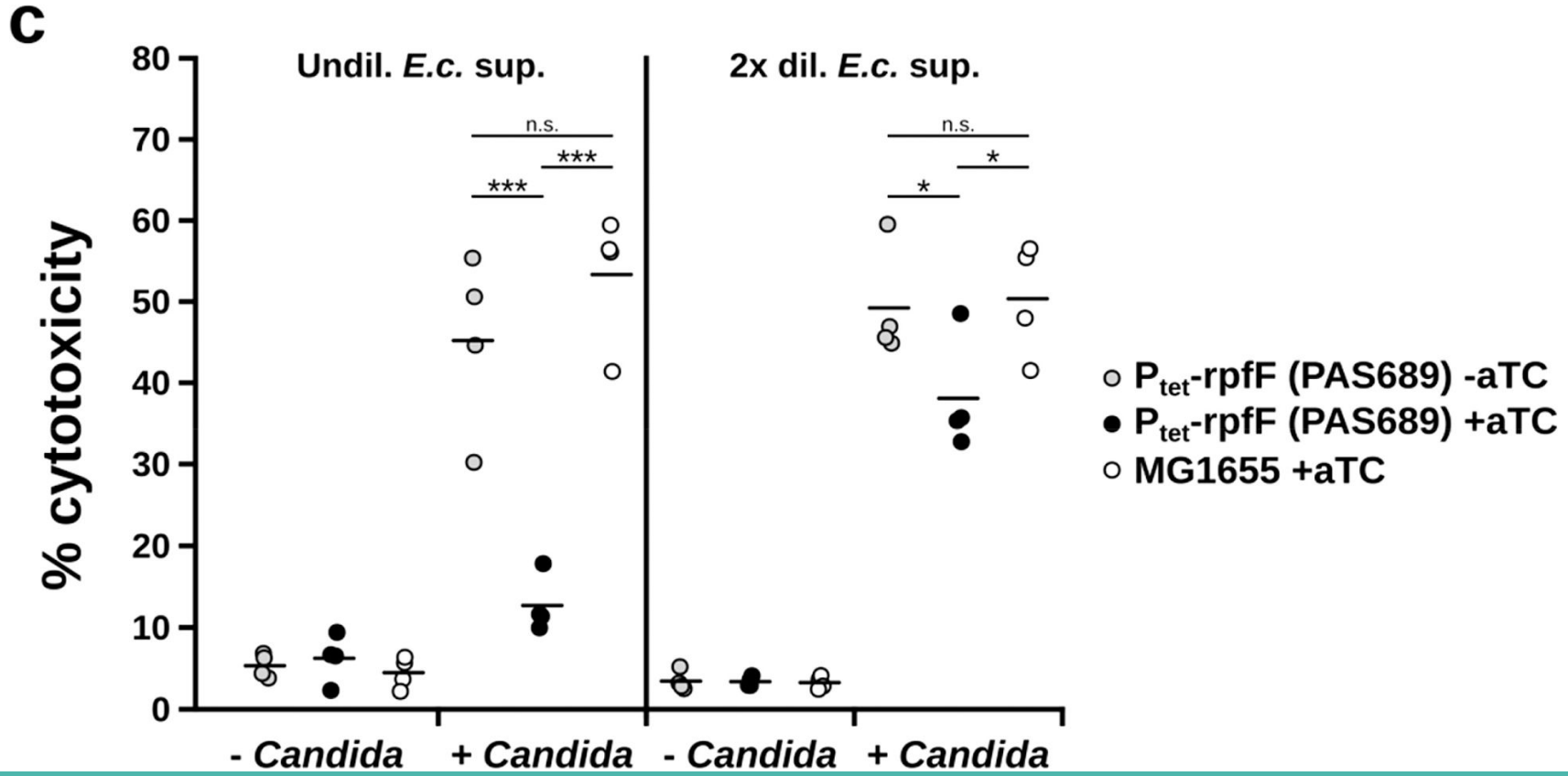
BDSF Production Prevents Attack of *C. albicans* Hypha on *Caco-2* Epithelial Cells



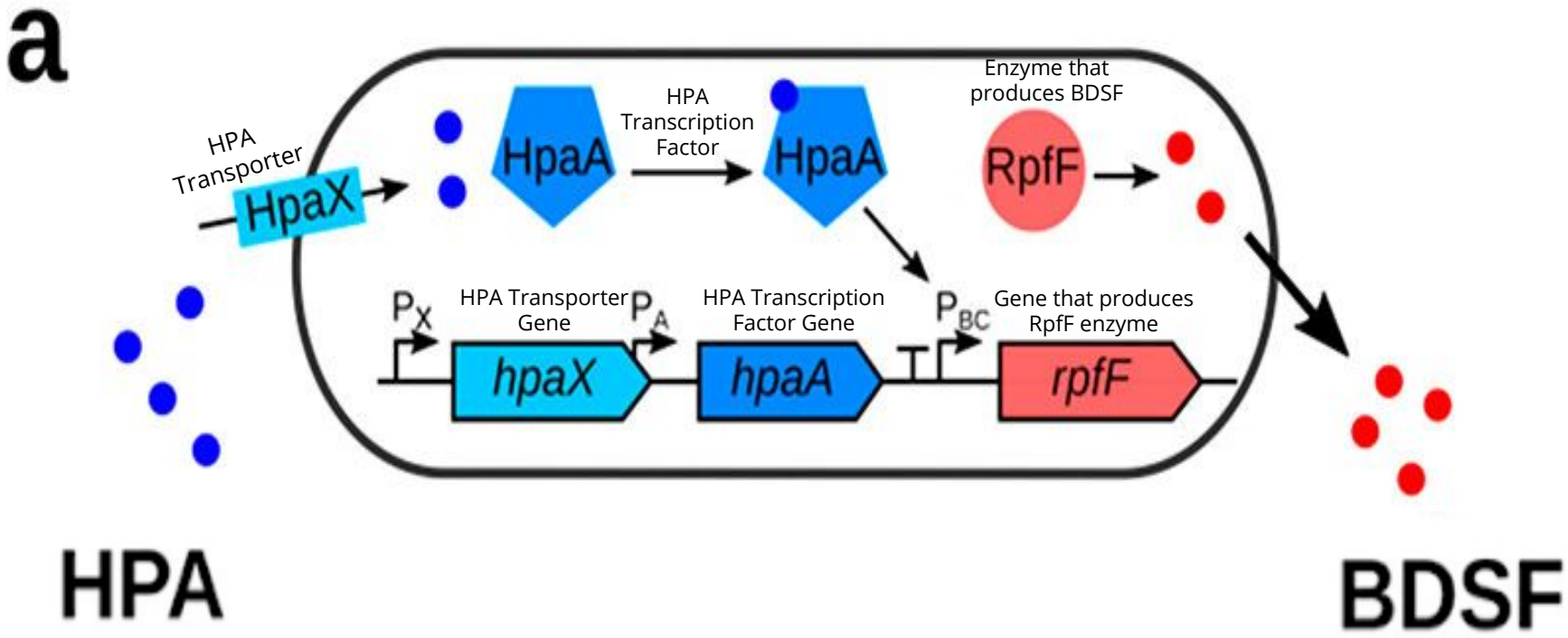
Increasing BDSF Supplementation Decreases *Caco-2* Epithelial Cell Damage



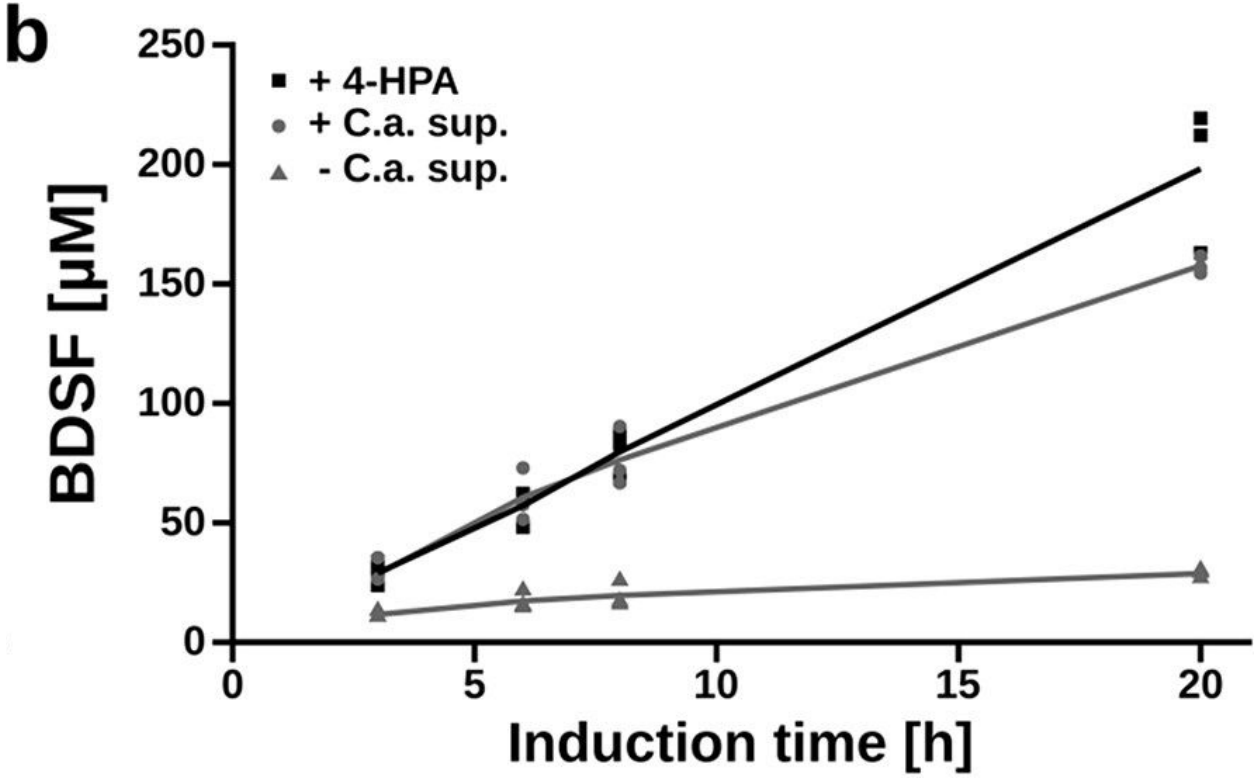
C. albicans Induces *Caco-2* Epithelial Cell Damage



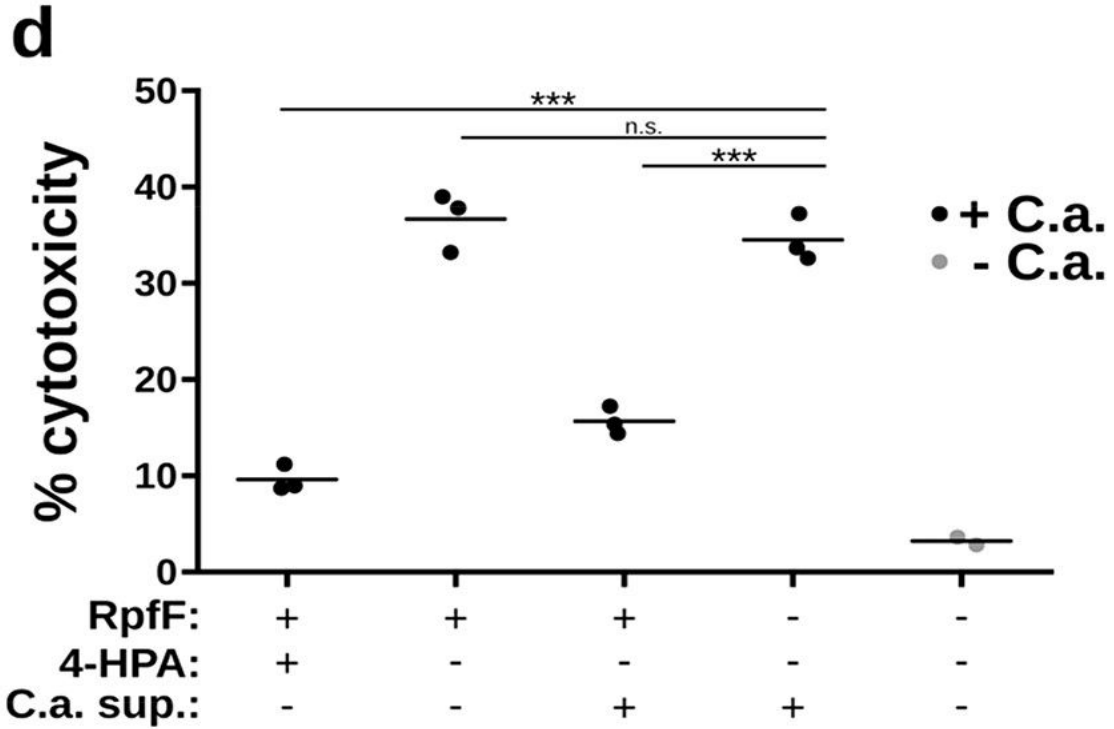
E. Coli Can Be Engineered to Combine HPA Sensor and *rpfF* Plasmid



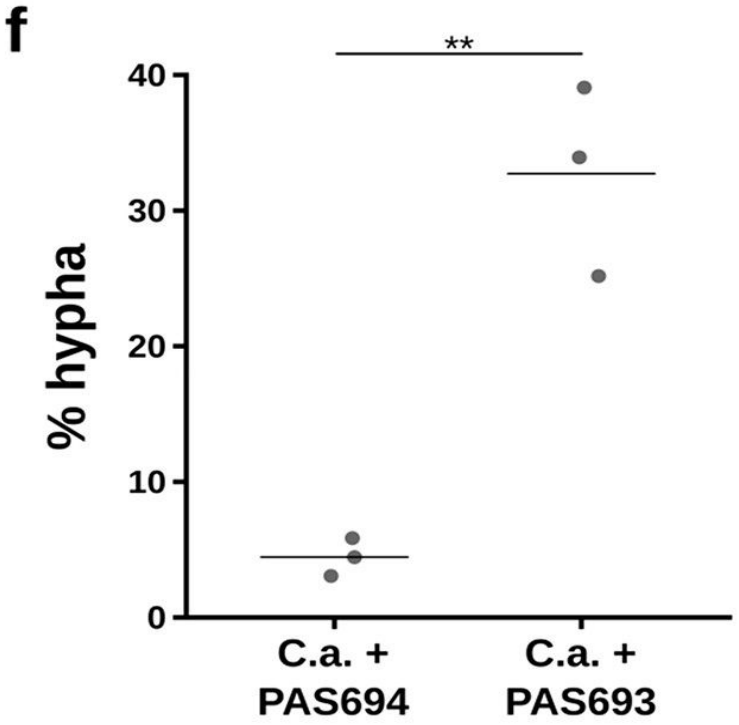
Induction With HPA Stimulates Production of BDSF in Mouse *E. Coli* Strain NGF-1



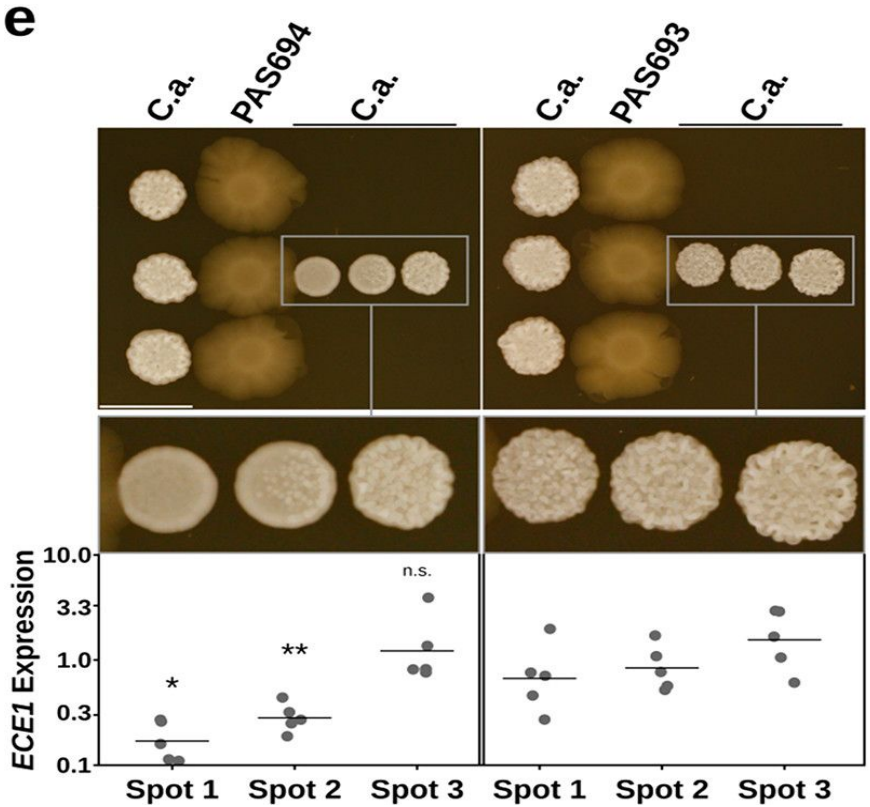
Sense-and-Respond System Decreases *C. albicans*-Mediated Damage of Mouse Epithelial Cells



Sense-and-Respond System Causes Significant Reduction in Hypha Formation vs. Control



The Functional System Reduces Expression of Virulence Factor Candidalysin



Journal Club Discussion Points

- Why is targeting virulence factors potentially better than using antifungals?
  You can avoid “superbug” resistance strains.

- Is HPA actually unique molecule secreted by C. albicans? What does this mean for specificity?
  Made by humans and plants

- What is the purpose of lactate dehydrogenase? How could it be used to measure cell lysis?
  Converts pyruvate to lactate and is generally abundant. Converts NAD+ to NADH and H+ which then the H+ can react to form a red color with enzyme in colorimetric assay

- What is the most common disease caused by C. albicans infection?
  Candidemia

- What other important E. coli strain contains a HPA transporter?
  E. coli HS (Human strain)

- Could such a system prevent conditions like oral thrush?
  No. This only inhibits hyphal formation. This does not control the existence and abundance of yeast.

- What other organisms could be inhibited using this system?
  Pseudomonas aeruginosa

Figure 1

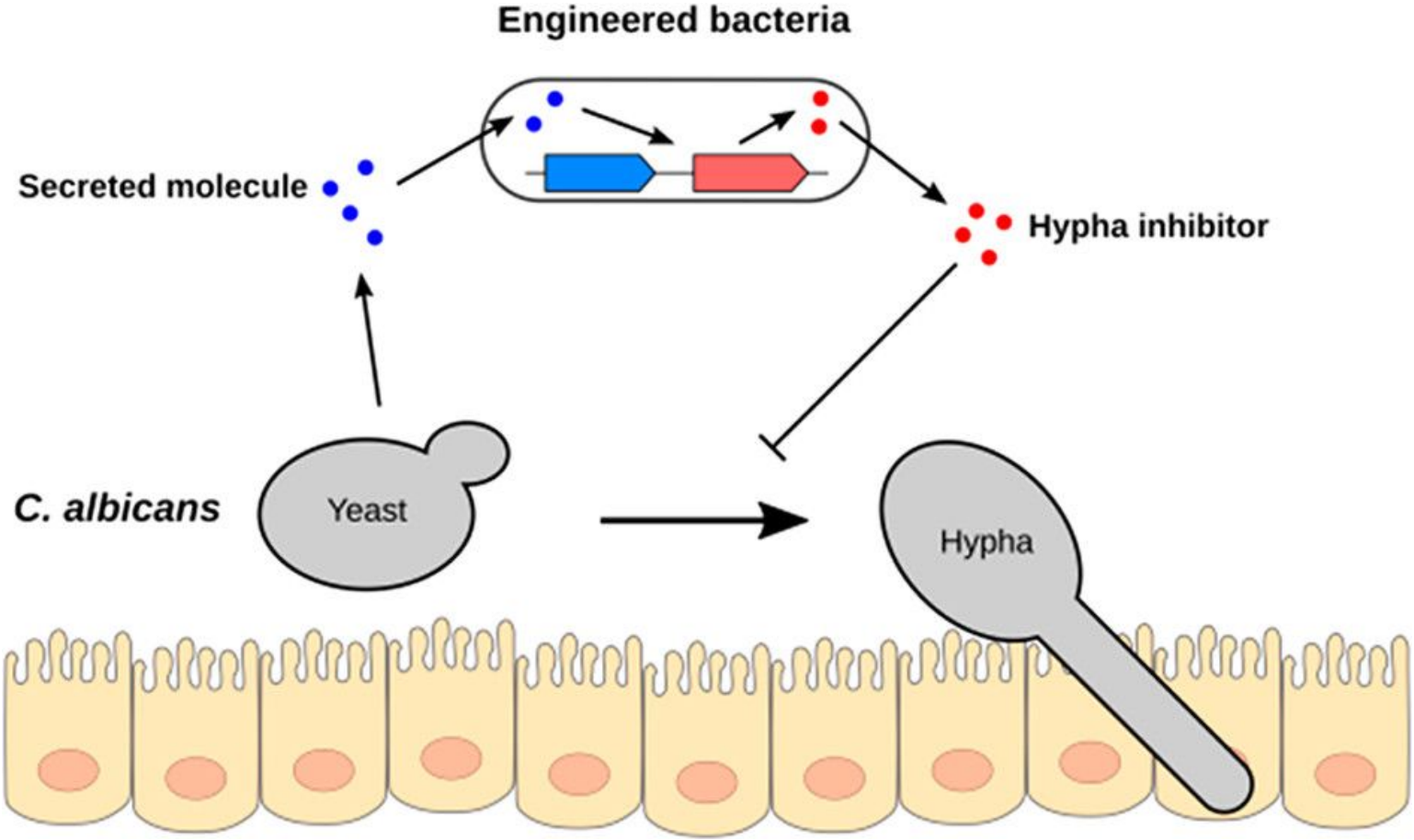

Engineered bacteria
Secreted molecule
Hypha inhibitor
C. albicans
Yeast
Hypha

Figure 2

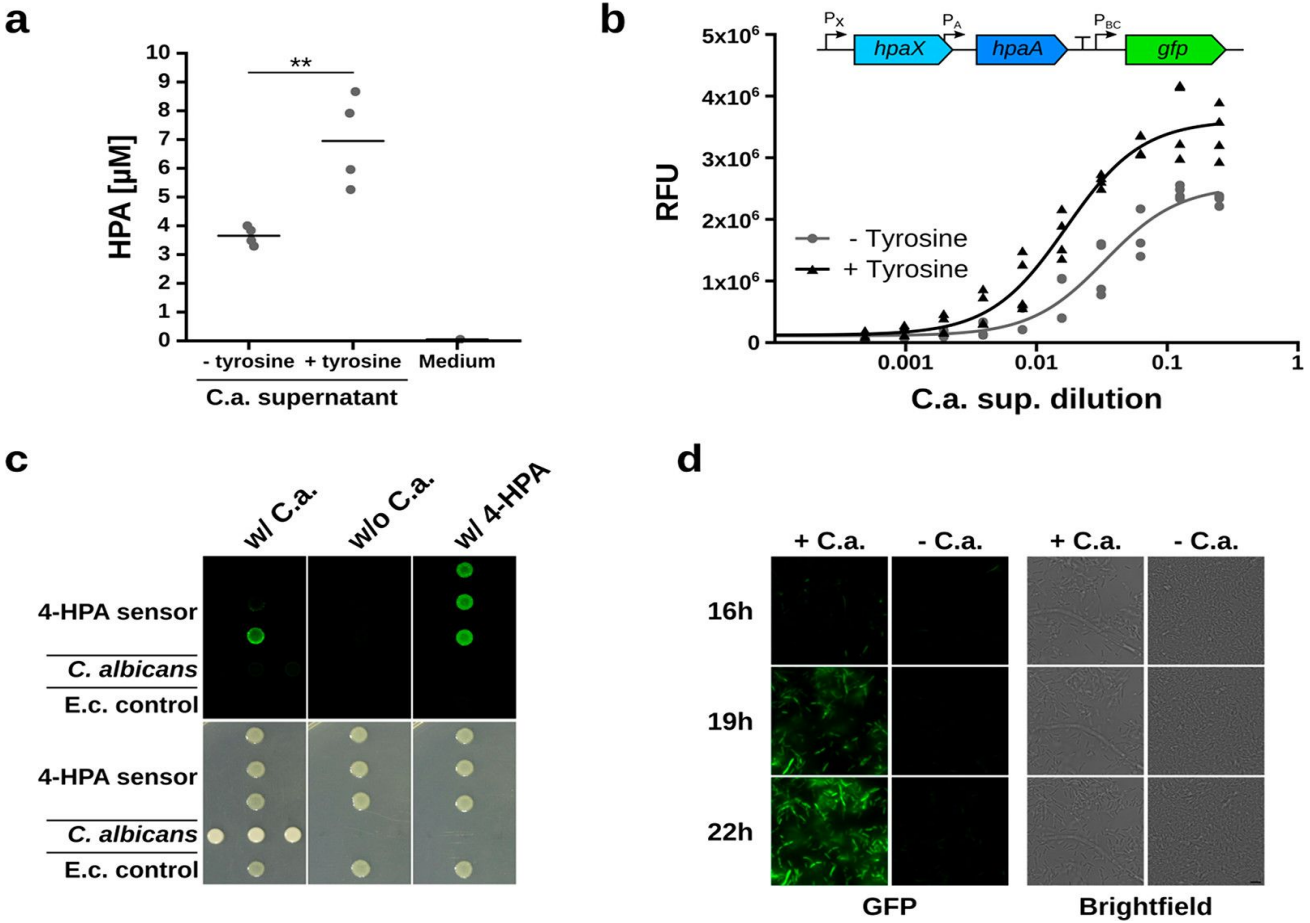

a
HPA [µM]
10
9
8
7
6
5
4
3
2
1
0
**
- tyrosine
+ tyrosine
Medium
C.a. supernatant
b
RFU
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
PX
PA
PBC
hpaX
hpaA
gfp
- Tyrosine
+ Tyrosine
0.001
0.01
0.1
1
C.a. sup. dilution
c
w/ C.a.
w/o C.a.
w/ 4-HPA
4-HPA sensor
C. albicans
E.c. control
4-HPA sensor
C. albicans
E.c. control
d
+ C.a.
- C.a.
+ C.a.
- C.a.
16h
19h
22h
GFP
Brightfield

Figure 3

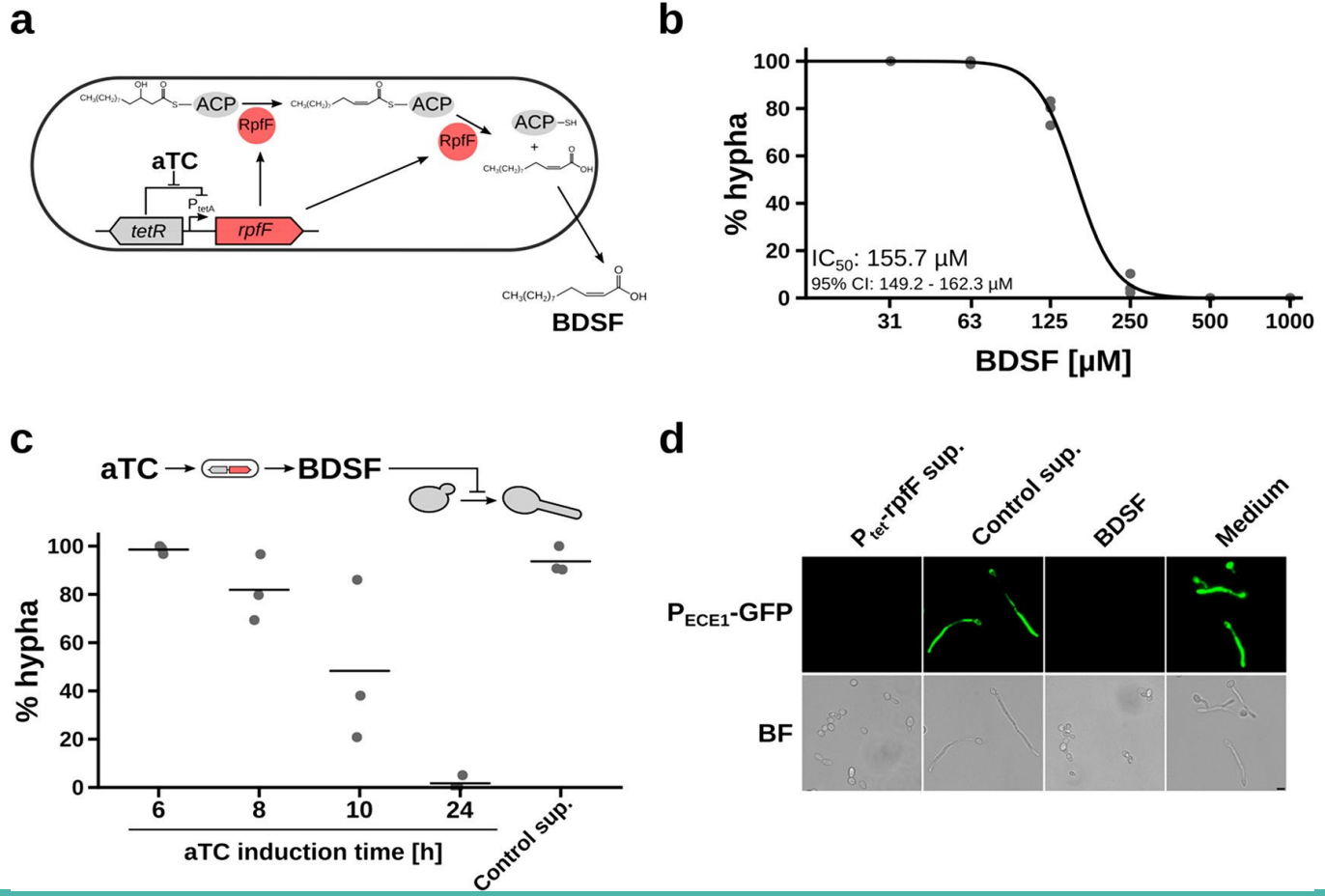

a
ACP
RpfF
ACP
RpfF
ACP
aTC
P_{tetA}
tetR
rpfF
BDSF
b
% hypha
100
80
60
40
20
0
IC_{50}: 155.7 µM
95% CI: 149.2 - 162.3 µM
31
63
125
250
500
1000
BDSF [µM]
c
aTC
BDSF
% hypha
100
80
60
40
20
0
6
8
10
24
Control sup.
aTC induction time [h]
d
P_{tet}-rpfF sup.
Control sup.
BDSF
Medium
P_{ECE1}-GFP
BF

Figure 4

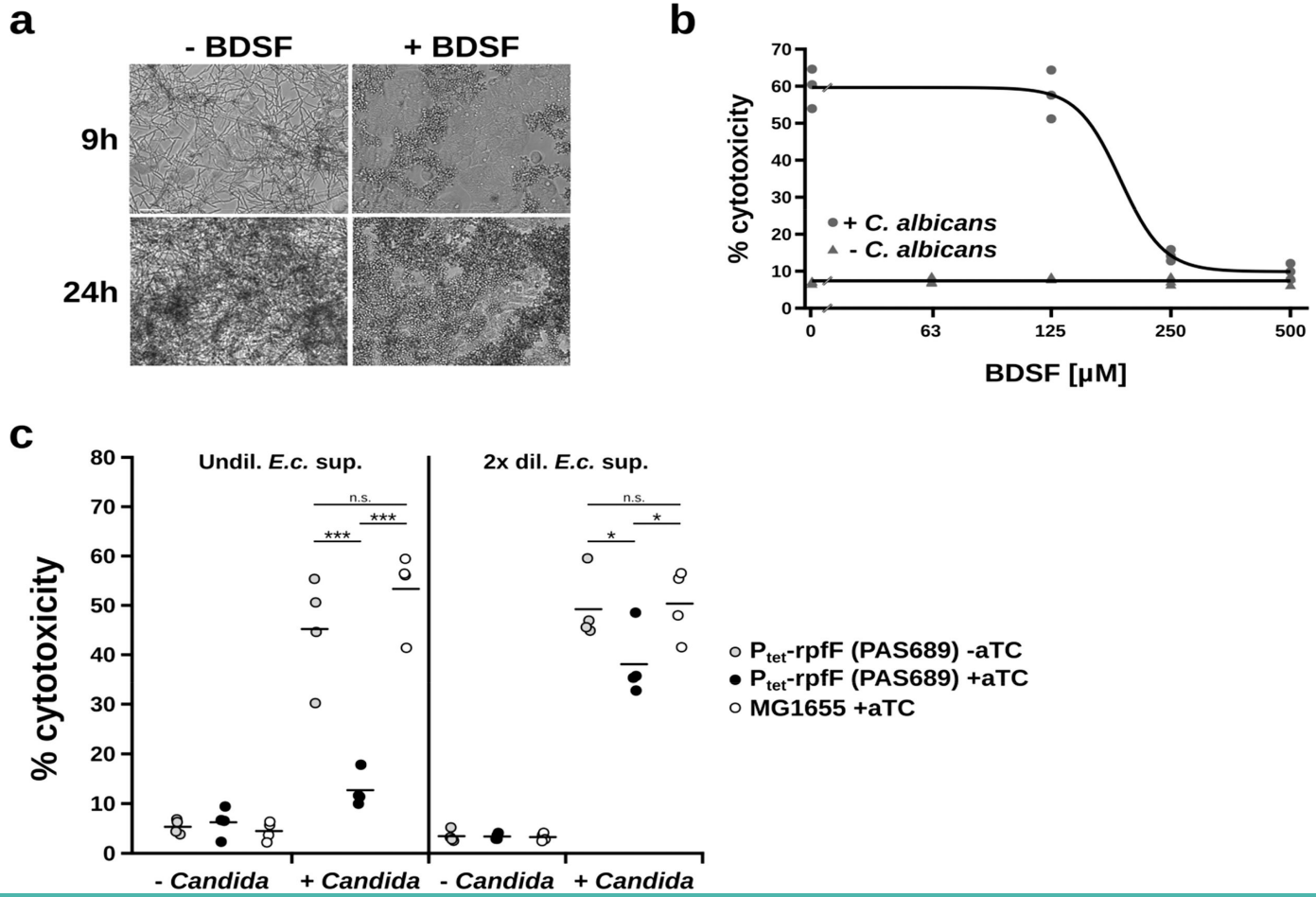

a
- BDSF
+ BDSF
9h
24h
b
% cytotoxicity
+ C. albicans
- C. albicans
BDSF [µM]
c
Undil. E.c. sup.
2x dil. E.c. sup.
% cytotoxicity
n.s.
***
*
- Candida
+ Candida
Ptet-rpfF (PAS689) -aTC
Ptet-rpfF (PAS689) +aTC
MG1655 +aTC

Figure 5

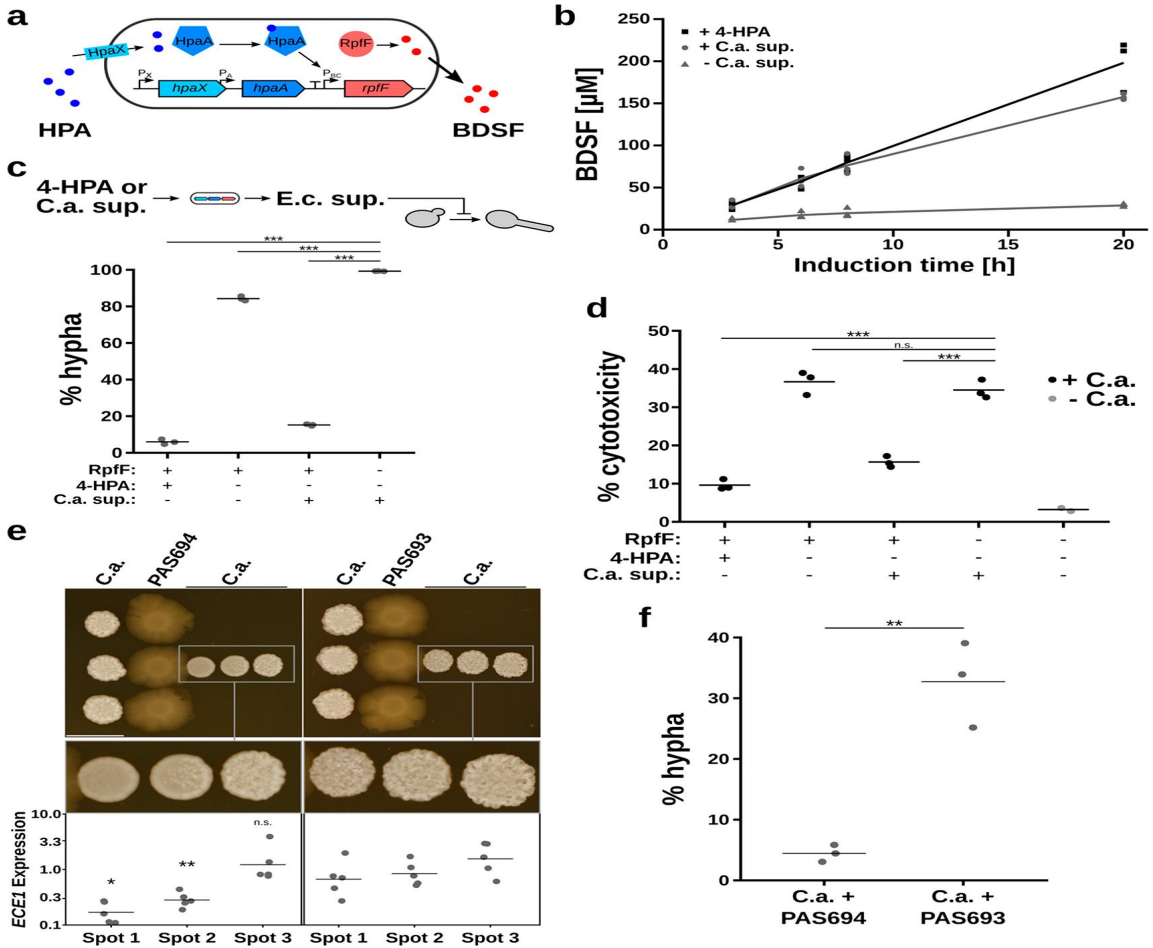
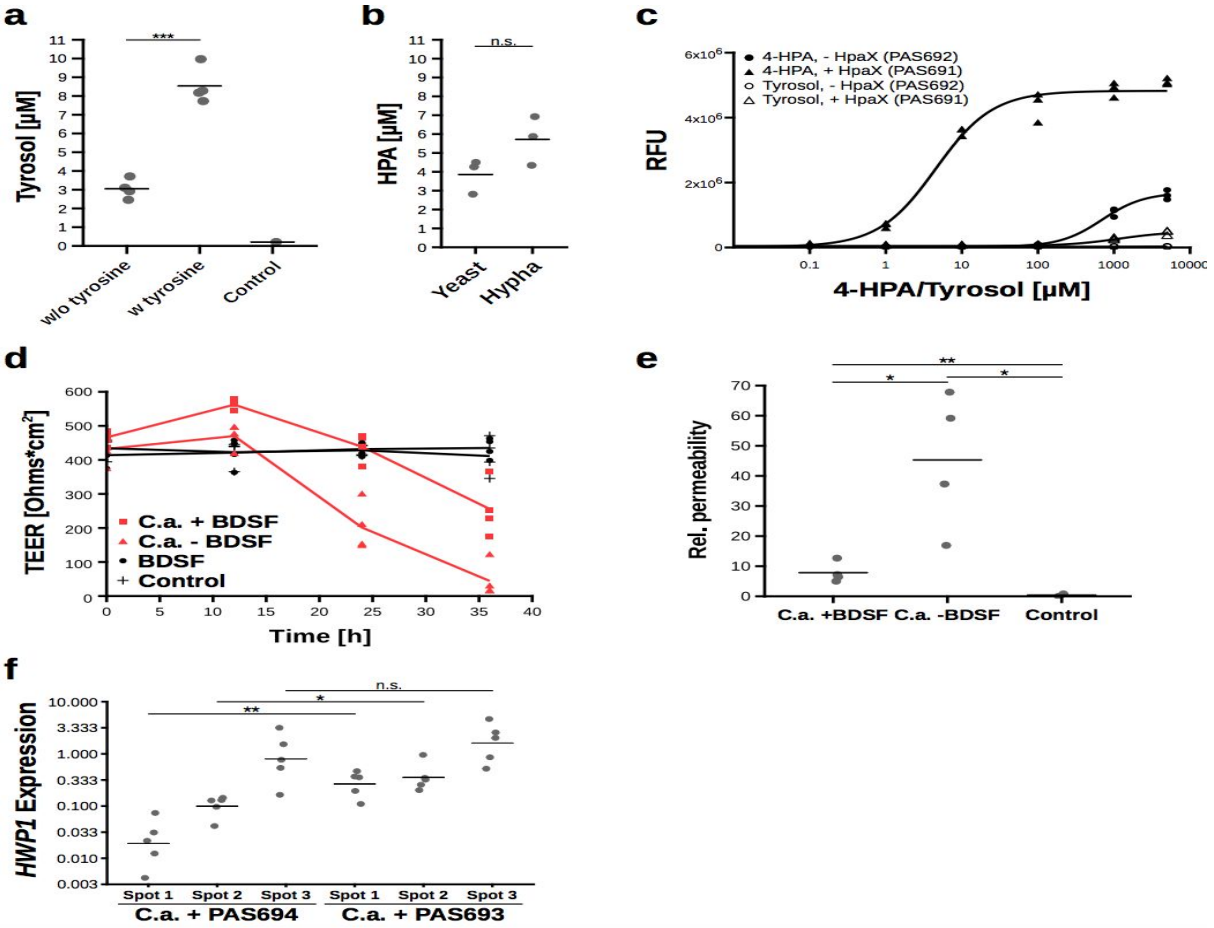

a
HpaX
HpaA
HpaA
RpfF
hpaX
hpaA
rpfF
HPA
BDSF
b
+ 4-HPA
+ C.a. sup.
- C.a. sup.
BDSF [µM]
Induction time [h]
c
4-HPA or C.a. sup.
E.c. sup.
% hypha
RpfF:
4-HPA:
C.a. sup.:
d
% cytotoxicity
+ C.a.
- C.a.
RpfF:
4-HPA:
C.a. sup.:
e
C.a.
PAS694
C.a.
C.a.
PAS693
C.a.
ECE1 Expression
Spot 1
Spot 2
Spot 3
Spot 1
Spot 2
Spot 3
f
% hypha
C.a. + PAS694
C.a. + PAS693

Figure 1S


a
Tyrosol [µM]
***
wlo tyrosine
w tyrosine
Control
b
HPA [µM]
n.s.
Yeast
Hypha
c
RFU
4-HPA, - HpaX (PAS692)
4-HPA, + HpaX (PAS691)
Tyrosol, - HpaX (PAS692)
Tyrosol, + HpaX (PAS691)
4-HPA/Tyrosol [µM]
d
TEER [Ohms*cm²]
C.a. + BDSF
C.a. - BDSF
BDSF
Control
Time [h]
e
Rel. permeability
C.a. +BDSF
C.a. -BDSF
Control
f
HWP1 Expression
Spot 1
Spot 2
Spot 3
C.a. + PAS694
C.a. + PAS693