Factors influencing the *Aspergillus fumigatus* survival into the host mediated by the calcineurin pathway

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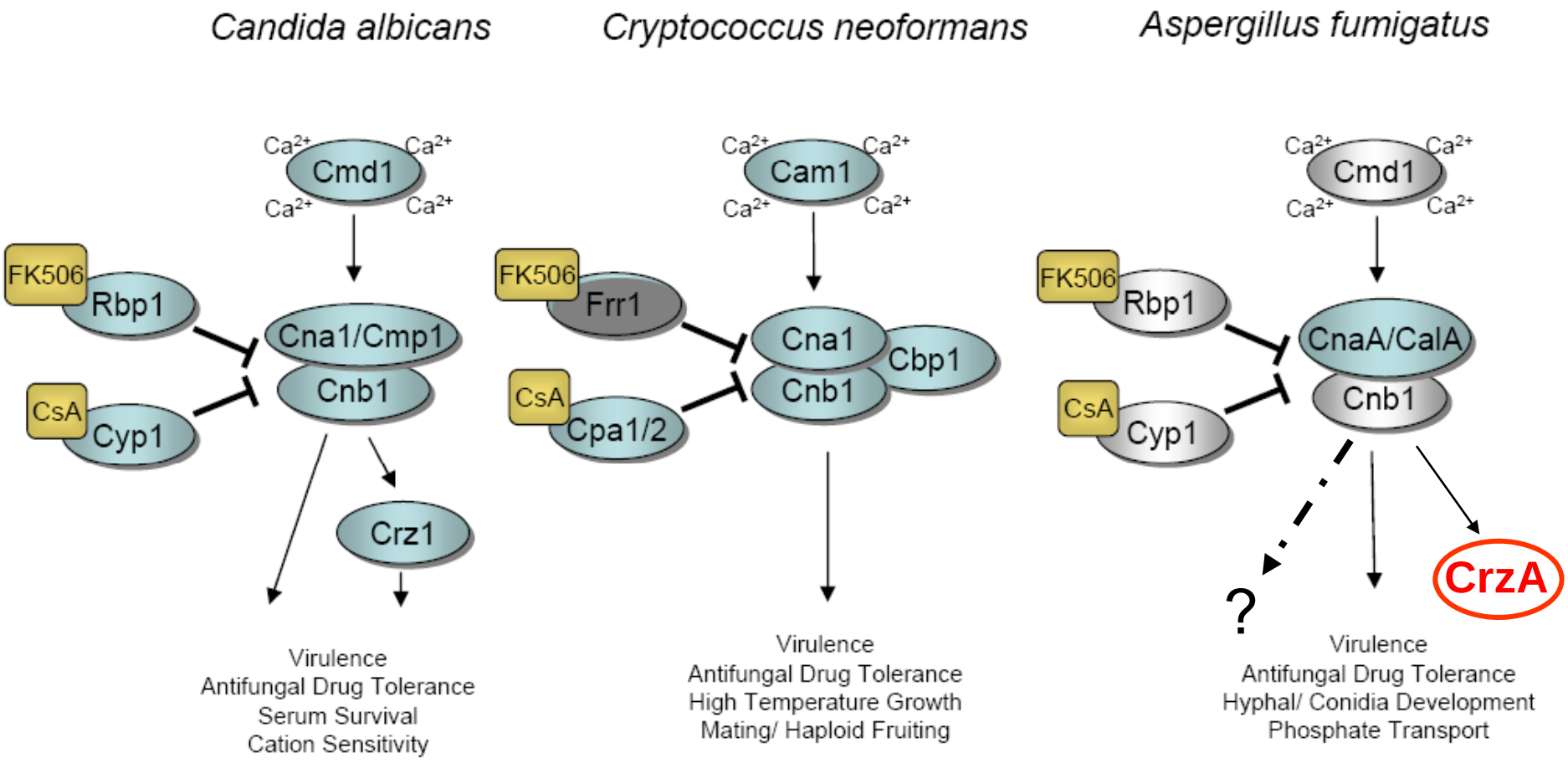
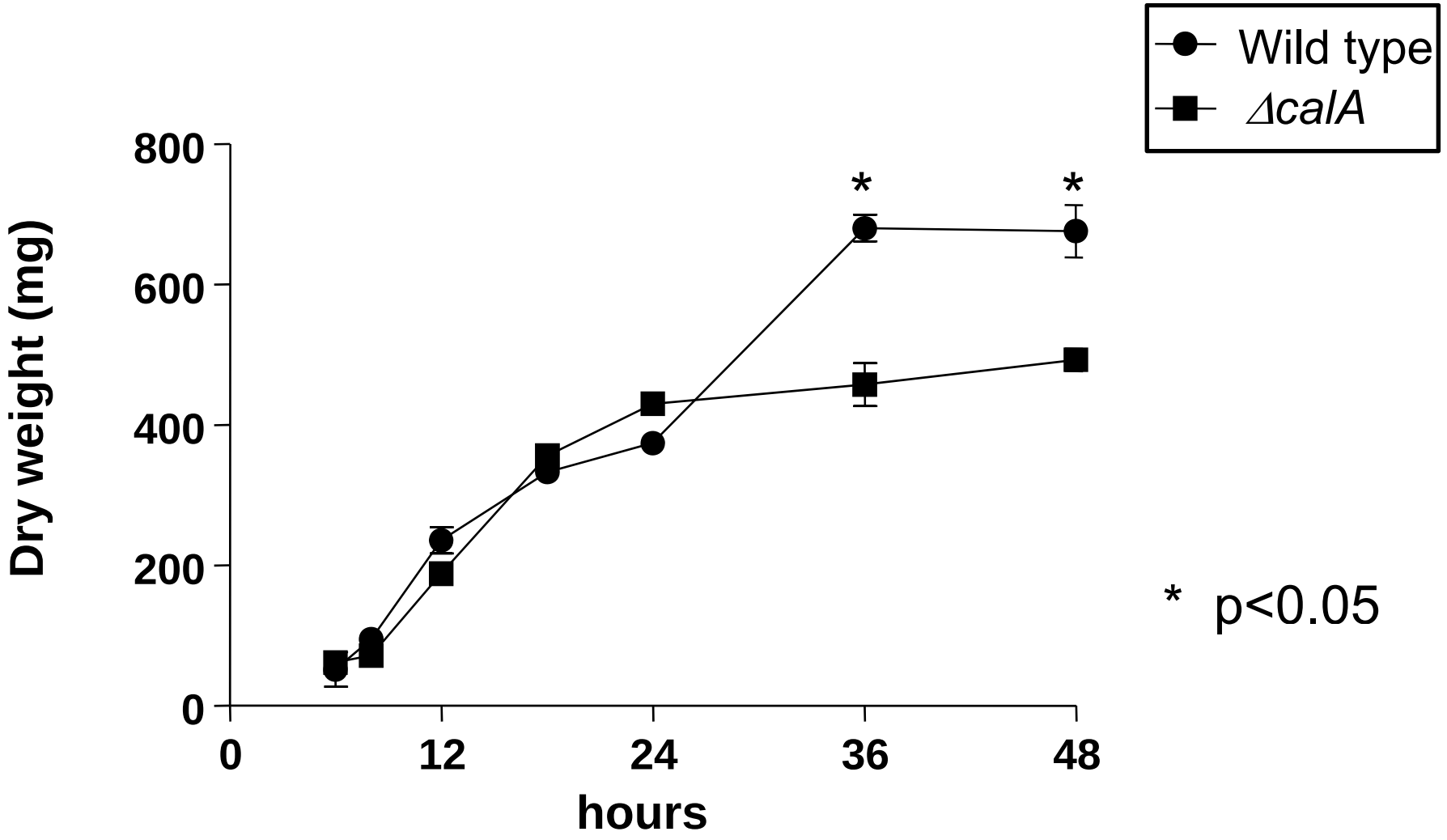
✓ **Calcineurin is a calmodulin/Ca^{+2} dependent serine/threonine phosphatase that promotes the virulence of *Cryptococcus neoformans* and *Candida albicans* through distinct mechanisms: in *C. neoformans*, calcineurin is required for growth at 37 ^{o}C, whereas in *C. albicans* it is required for survival in the serum**

The fungal calcineurin pathway



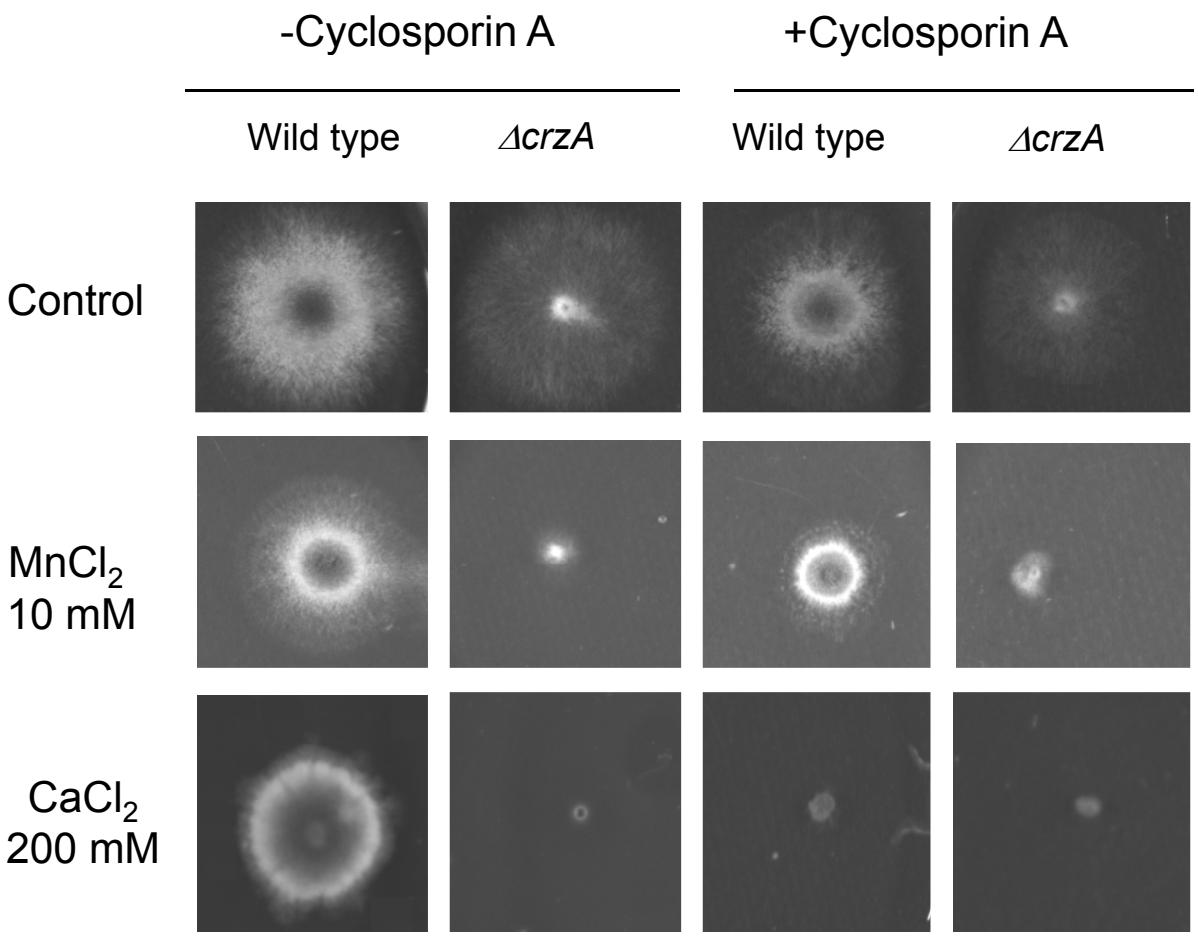
Modified from Steinbach *et al.*, Nat Rev Microbiol, 5:418-430, 2007.

Growth curve of the wild type and *ΔcalA* mutant strains grown in complete liquid medium



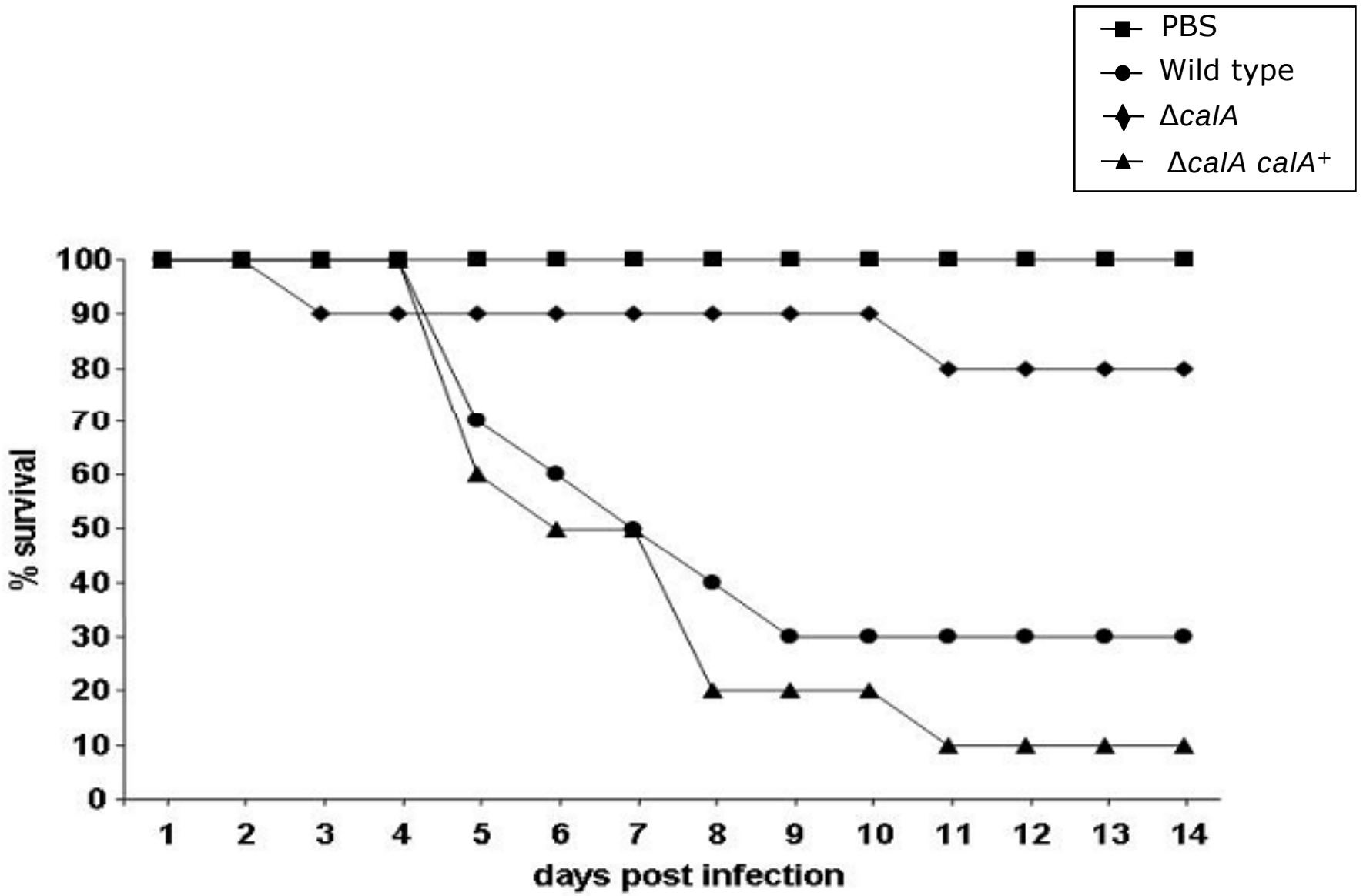
Malavazi *et al.*, submitted

Growth phenotypes of the *A. fumigatus* wild type and Δ*crzA* mutant strains



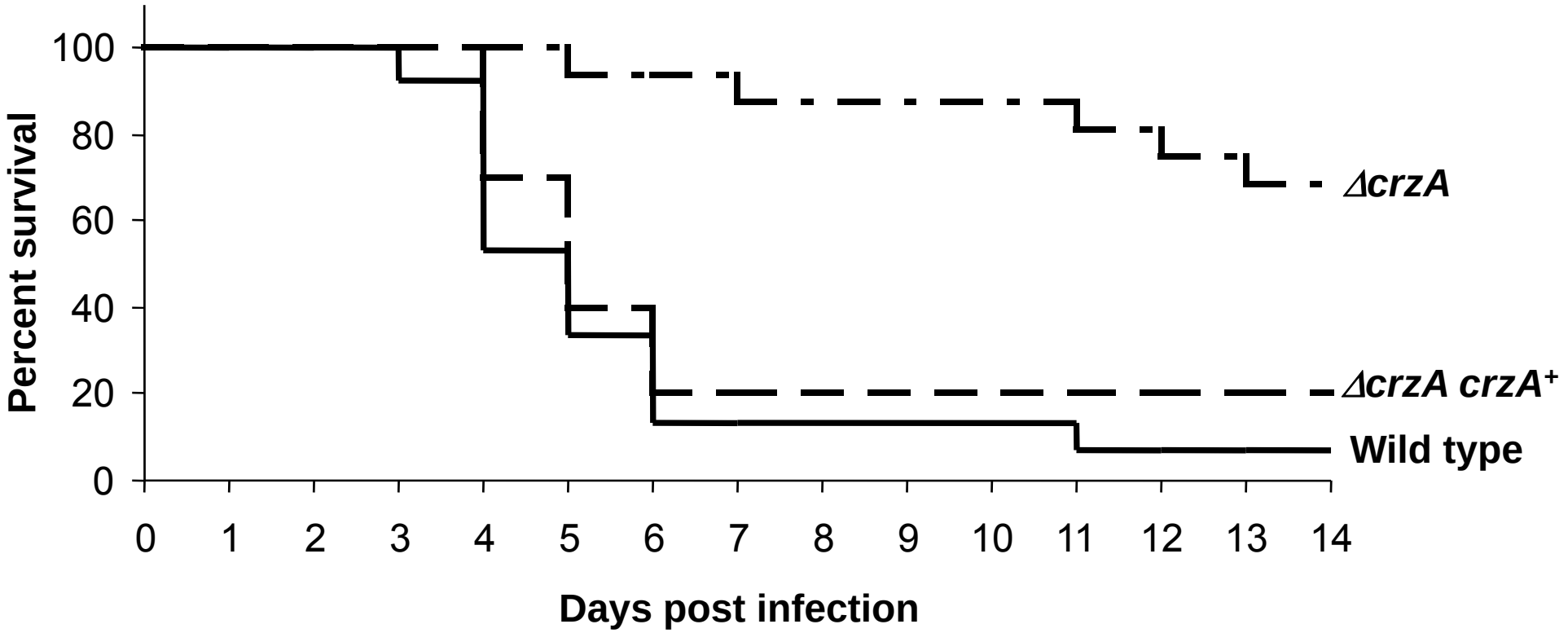
Soriani *et al.*, Molecular Microbiology 67: 1274-91 (2008)

A. fumigatus ΔcalA mutant has decreased virulence



da Silva Ferreira *et al*., Fungal Genetics and Biology, 44:219-30, 2007

The *ΔcrzA* mutant is less virulent than the wild type strain



Soriani *et al.*, Molecular Microbiology 67: 1274-91 (2008)

Key Problem

- ✓ **To characterize signaling pathways that are affected *in vitro* and *in vivo* by calcineurin-CrzA pathway**

Experimental design (dye swap analyses)

Wt 6 h X *Δcal* 6 h	
M1	M1
M2	M2

Wt 8 h X *ΔcalA* 8 h	
M1	M1
M2	M2

Wt 12 h X *ΔcalA* 12 h	
M1	M1
M2	M2

Wt 18 h X *Δcal* 18 h	
M1	M1
M2	M2

Wt 24 h X *ΔcalA* 24 h	
M1	M1
M2	M2

20 independent hybridizations,

Differentially expressed genes at the 95% confidence level were determined (1,707) using intensity-dependent *Z*-scores (with Z=1.96) as implemented in MIDAS and the union of all genes identified at each time point were considered significant in this experiment.

Experimental design (dye swap analyses)

Wt 10' $CaCl_2$ 200 mM

X

ΔcrzA 10' $CaCl_2$ 200 mM

M1 M1

M2 M2

Wt 30' $CaCl_2$ 200 mM

X

ΔcrzA 30' $CaCl_2$ 200 mM

M1 M1

M2 M2

4 independent hybridizations,

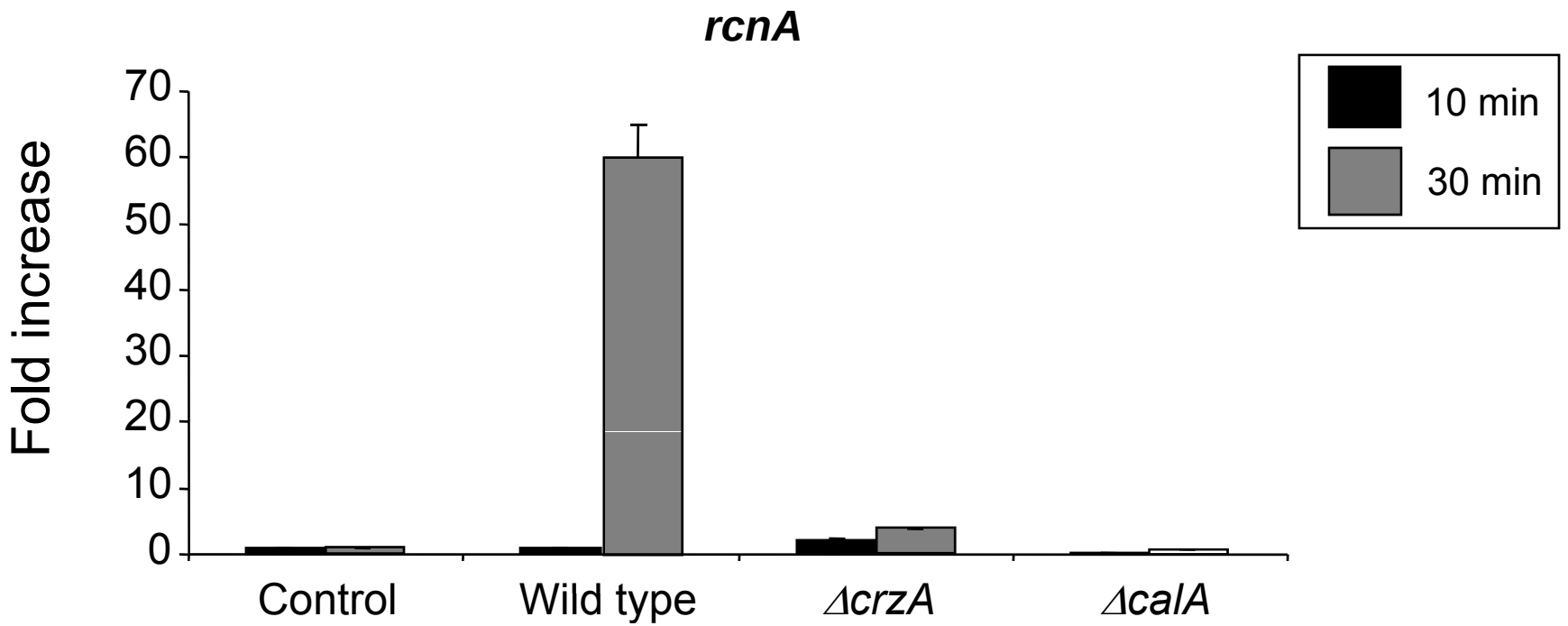
Differentially expressed genes at the 95% confidence level were determined (3,622) using intensity-dependent Z-scores (with Z=1.96) as implemented in MIDAS and the union of all genes identified at each time point were considered significant in this experiment.

The mRNA expression in both *ΔcalA* and *ΔcrzA* mutant

- Decreased mRNA expression of genes encoding proteins with mitochondrial function, involved in calcium metabolism and calcium transporters

- Increased mRNA expression of several genes encoding transcription factors and two MAP kinase kinases

The *rcnA* gene encoding a calcineurin binding protein has decreased mRNA accumulation in both *ΔcalA* and *ΔcrzA* mutants



Soriani *et al.*, submitted

RcnA is a member of the RCN family of calcineurin regulators

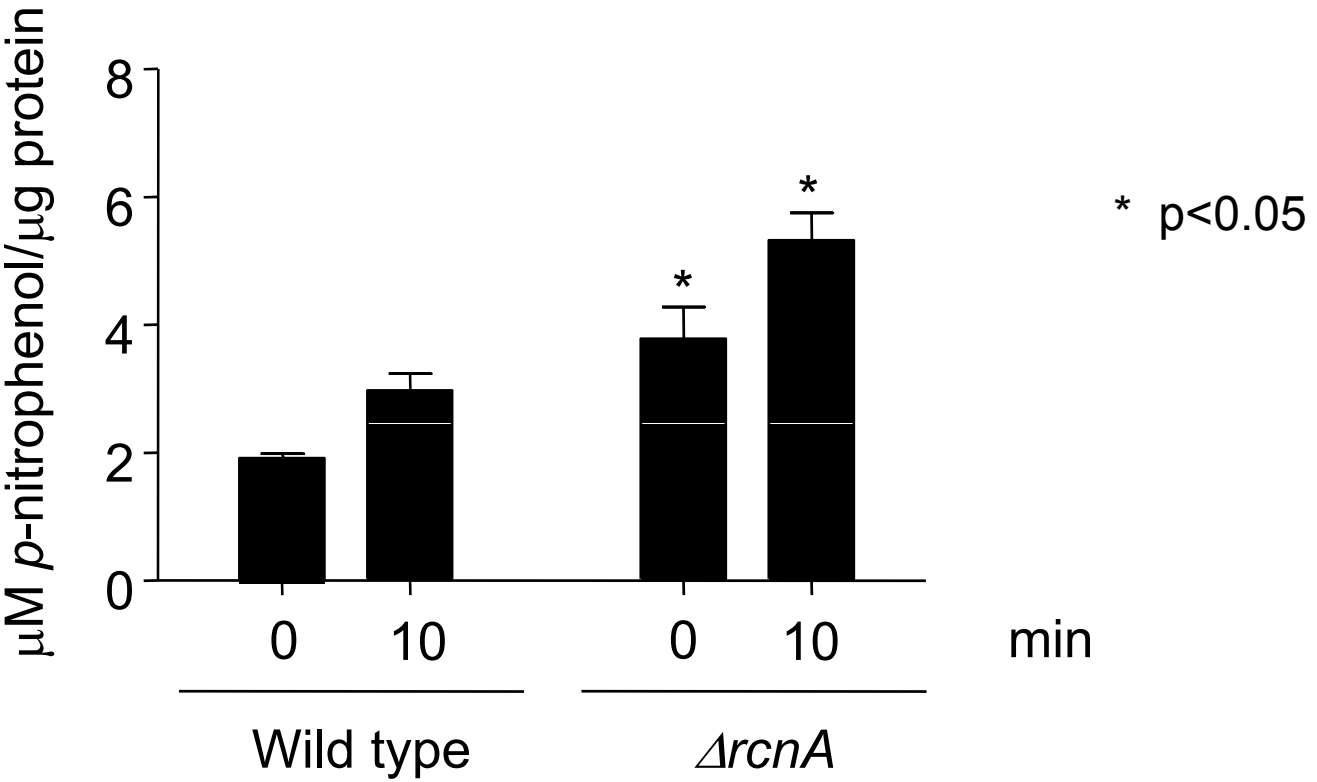
- RCNA1 and RCNA2 bind calcineurin at or near the catalytic domain of CnA

- In all experimental systems overexpression of RCNs inhibits calcineurin signaling

-Feedback inhibition of calcineurin is conserved from yeast to humans. The function of this feedback mechanism may be to fine-tune calcineurin signaling over a spectrum of intervals and conditions

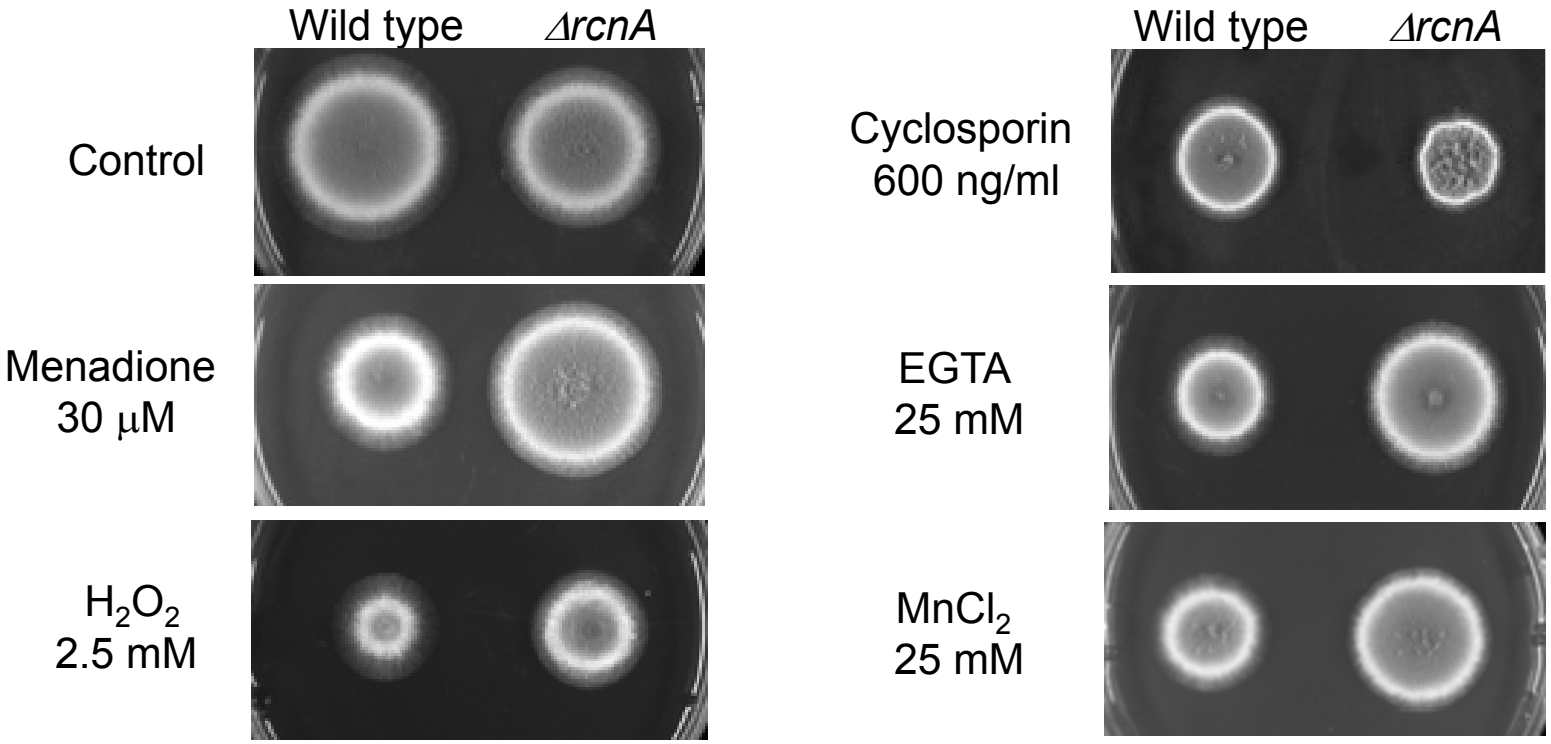
- The increased dosage of DSCR1 in trisomy-21 individuals may contribute to the neurological, cardiac, or immunological defects observed in Down Syndrome patients through inhibition of calcineurin signaling

A. fumigatus ΔrcnA has increased calcineurin activity



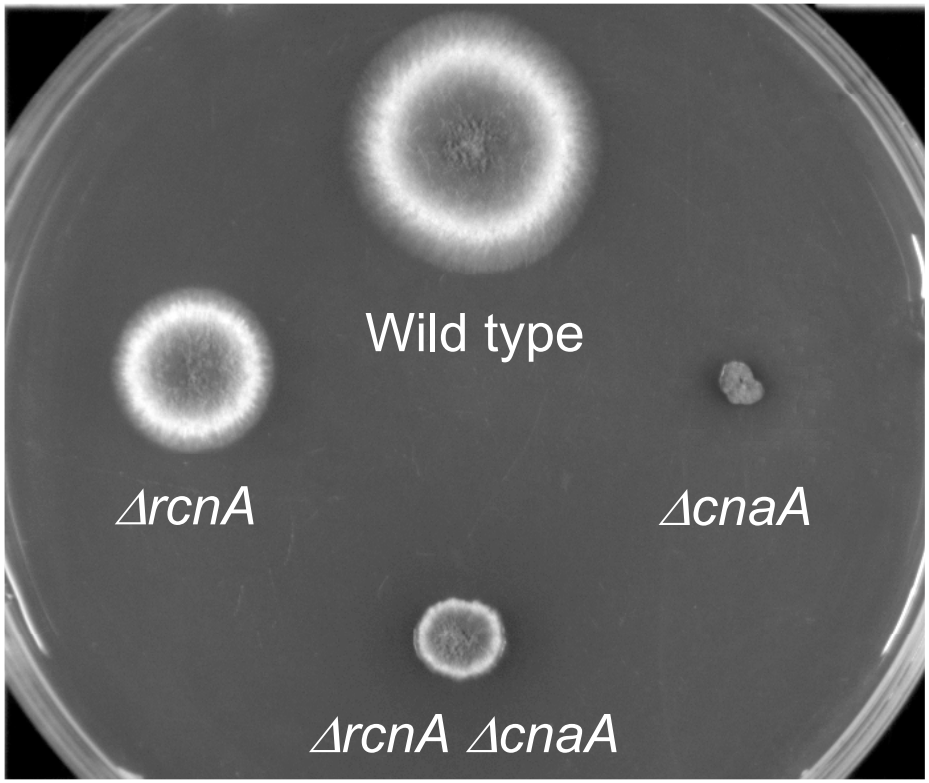
Soriani *et al.*, submitted

A. fumigatus rcnA deletion mutant has discrete phenotypes



Soriani *et al.*, submitted

Furthermore, *A. nidulans ΔrcnA* interacts genetically with *ΔcnaA*



Soriani *et al.*, submitted

Murine infections

Immunosuppressed mice

↓

infected by intranasal instillation of 10^8
conidia in 40 µl of saline

↓

Groups of infected mice were culled and
processed collectively during a 3 hours window corresponding to a
time point 11–14 hours post-infection.

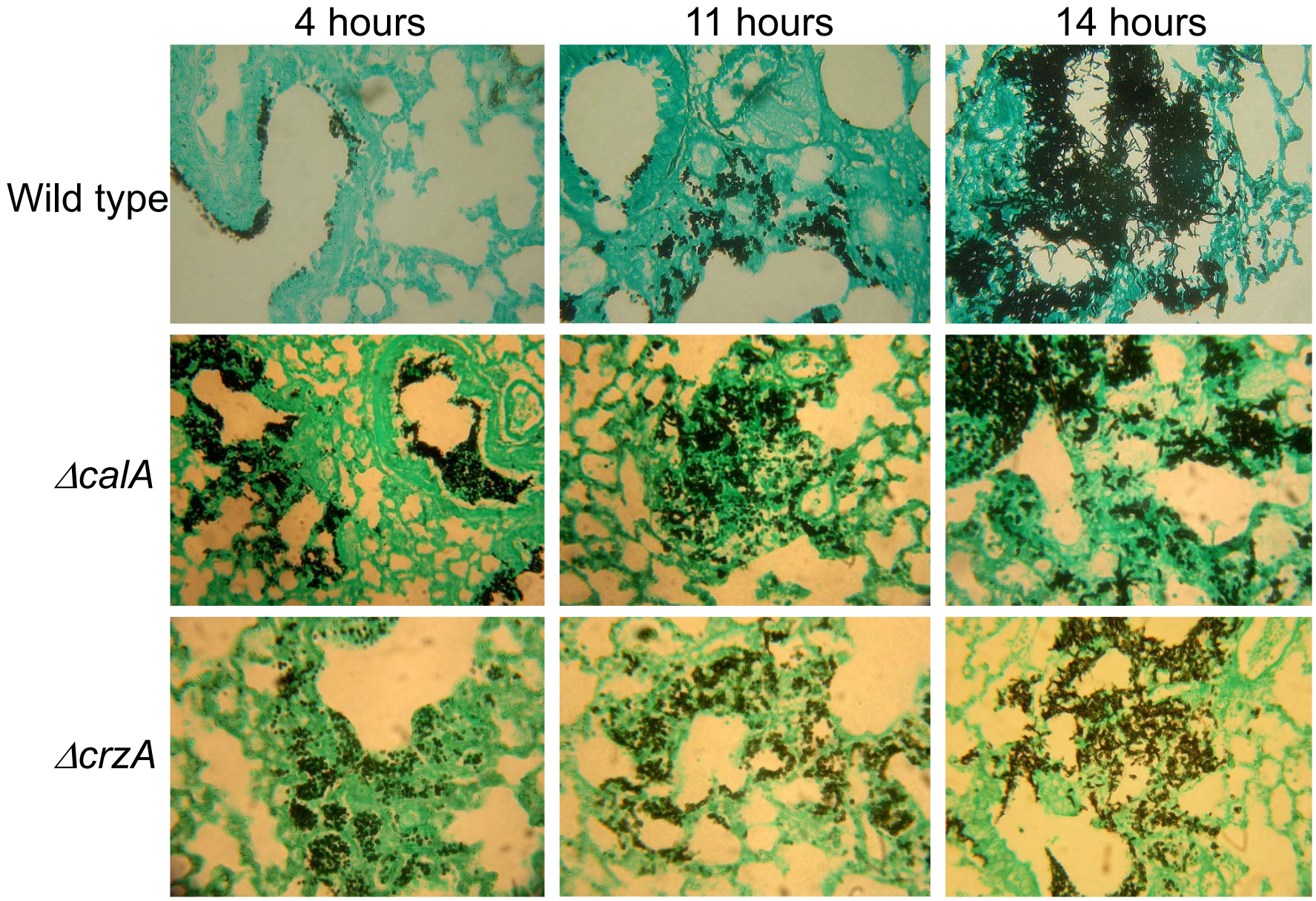
↓

Bronchoalveolar lavage (BALFs)
was performed immediately after culling using three 0.5 ml
aliquots of pre-warmed sterile saline.

↓

BALFs were snap frozen
immediately following harvest using liquid nitrogen.

Comparative time-course of A. fumigatus wild type, *ΔcalA*, and *ΔcrzA* mutant strains germination and hyphal development in the murine lung

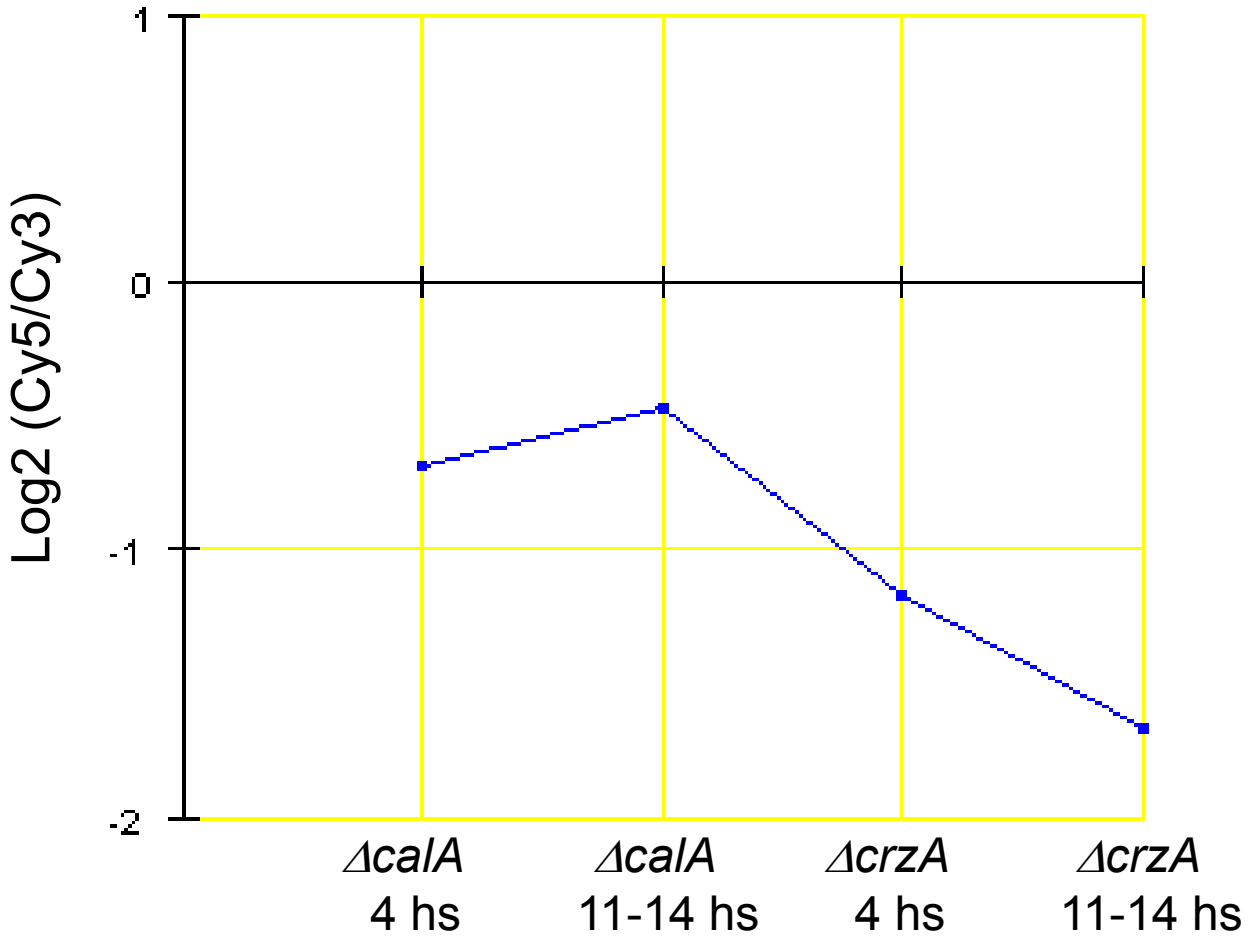


RNA Amplification

- RNA yields:
  - Unamplified 108 – 800 ng
  - Round 1 amplification: 3.7 – 19.3 µg
  - Round 2 amplification: 172.3 – 258.4 µg

- Amplification factor: $3X10^{8}$ – $4X10^{5}$

A. fumigatus rcnA has also decreased mRNA accumulation *in vivo*



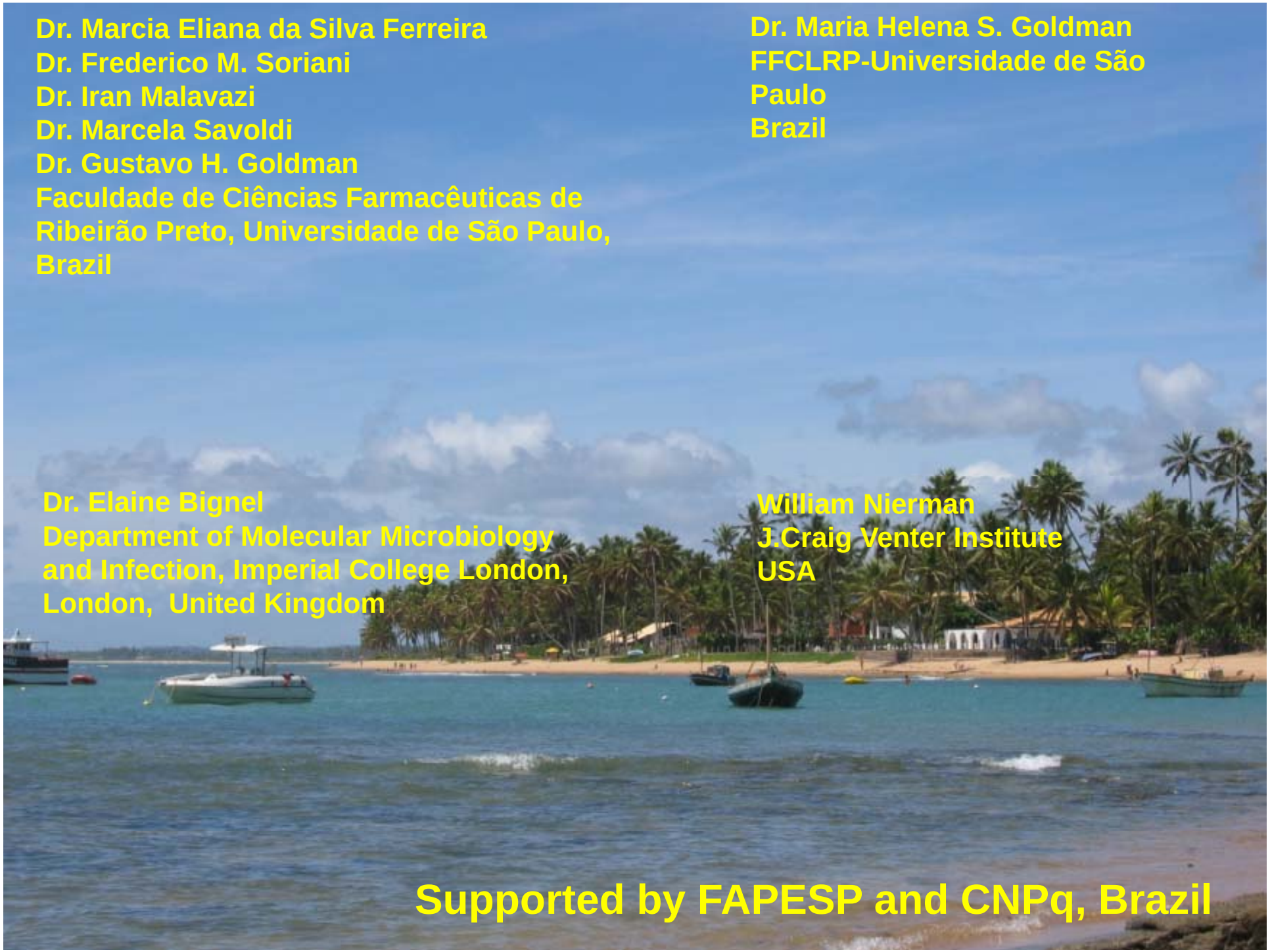
Summary

1) We use multiple expression analyses to identify genes involved directly or indirectly in the calcineurin-*CrzA* pathway

2) There is a high correlation between the *in vitro* and *in vivo* expression data

3) There is a decreased mRNA accumulation of the RcnA gene in both *ΔcalA* and *ΔcrzA* mutant

3) We have observed decreased CalA-CrzA-dependent expression of several genes encoding proteins involved in calcium metabolism and cell wall synthesis



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