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Who regulates the regulator?

KINASE MEDIATED REGULATION OF THE MOLECULAR CHAPERONE HSP90 AND ITS ROLE IN FUNGAL VIRULENCE

Stephanie Diezmann
University of Bristol

www.yeast-genetics.co.uk

Fungal pathogens pose a serious threat to human health world-wide

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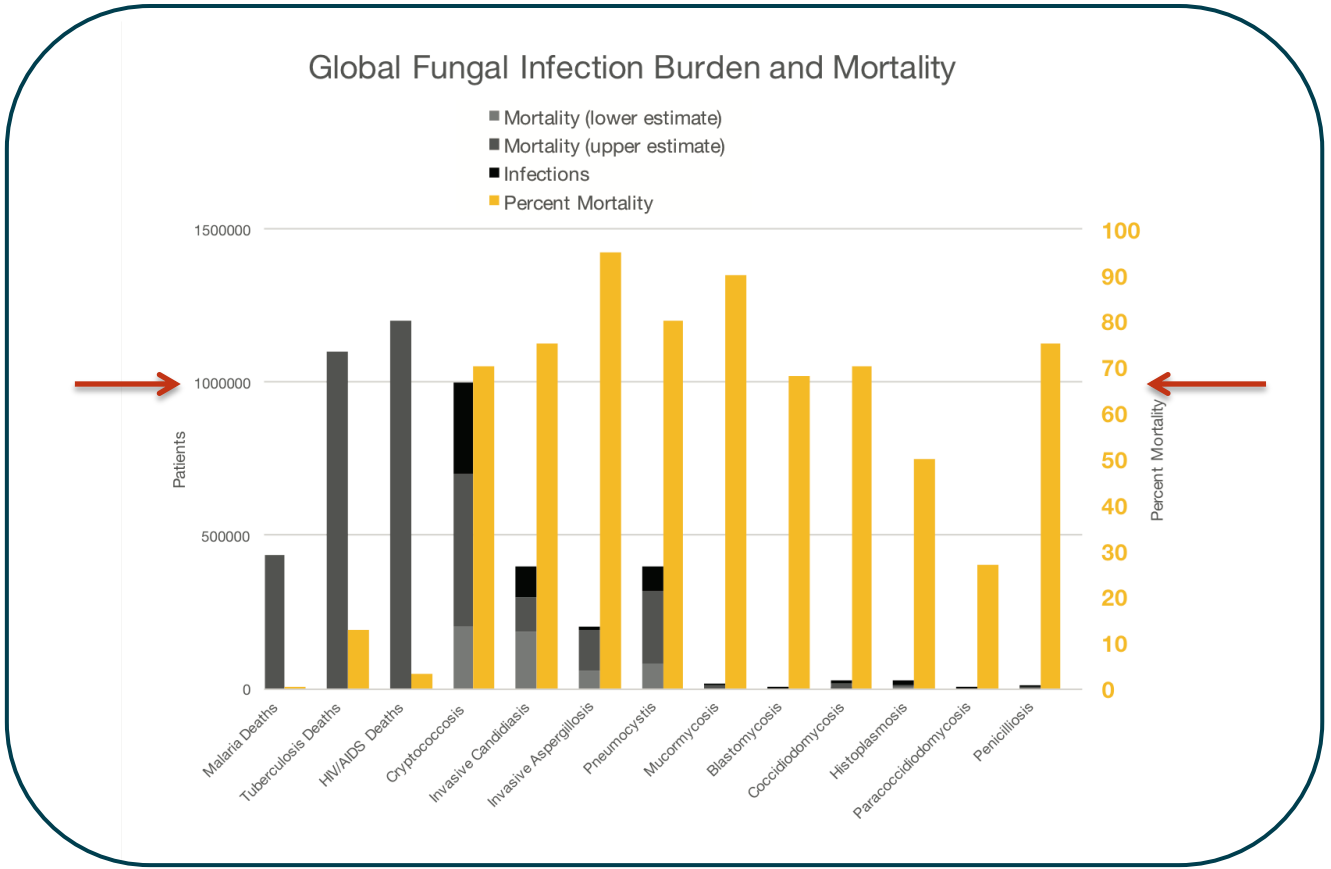
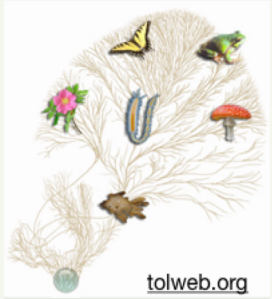


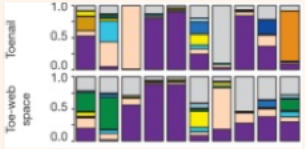
https://go.nature.com/2t4JvGh Elizabeth Ballou

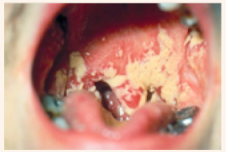
Fungal infections are difficult to treat

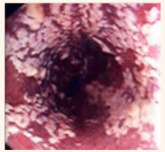
Paucity of suitable drug targets



Fungi are part of our microbiome

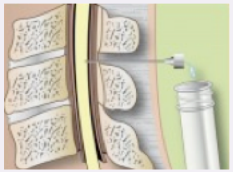




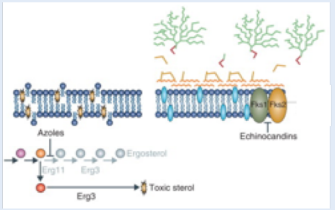


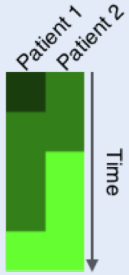
Findley et al., 2013

Poor and invasive diagnostics



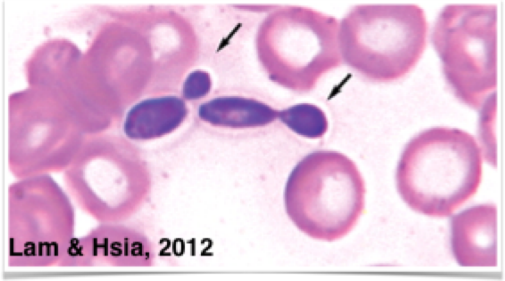
Emergence of drug resistance

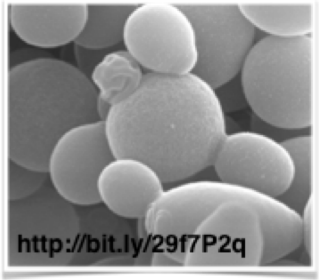


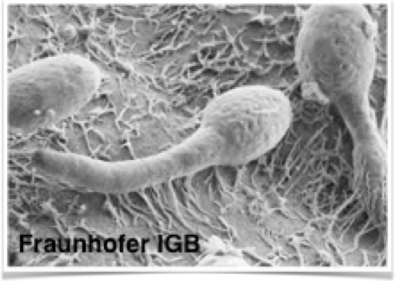


Franz et al., 1998; Cowen, 2013

Candida albicans is the leading fungal pathogen of humans



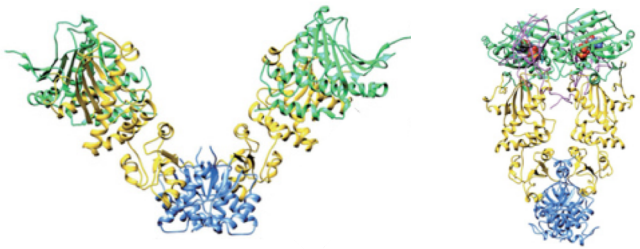




- Worldwide ~ 400,000 life-threatening infections annually
- Kills ~700 people annually in the UK alone
- Mortality rate up to 75%
- Natural member of the human microbiome - opportunistic pathogen
- Spectrum of disease ranging from oral & vaginal thrush to systemic infections
- Morphological diversity aids in distribution across the body
- Forms drug resistant biofilms on medical implant devices

The molecular chaperone Hsp90 is environmentally responsive and essential for life

Hsp90 is a highly conserved cellular signaling hub

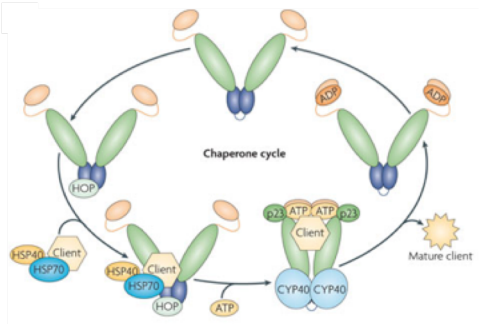


Hsp90 stabilizes proteins involved in:

- Cell growth
- Cell cycle progression
- Gene expression
- Development
- Environmental response

Saibil, 2013

Hsp90 stabilizes metastable client proteins



The Hsp90 chaperone cycle is:

- Dynamic
- Assisted by co-chaperones, eg Cdc37
- Regulated by Hsp90 phosphorylation
- Recognizes partially folded proteins

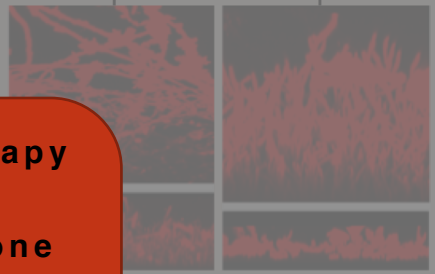
Taipale, Jarosz & Lindquist, 2010

Hsp90 regulates *C. albicans* virulence traits

Blocks morphogenetic diversity

Shapiro et al

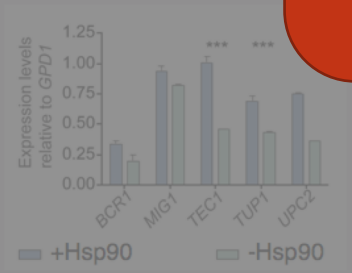
Affects biofilm architecture



Robbins et al., 2011

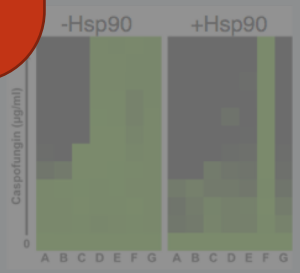
- Develop combinatorial therapy using existing Hsp90 inhibitors while targeting one of its clients.
- Identify fragile parts of fungal Hsp90 that can be specifically targeted to disrupt its function.

Regulates gene expressio
developmental s



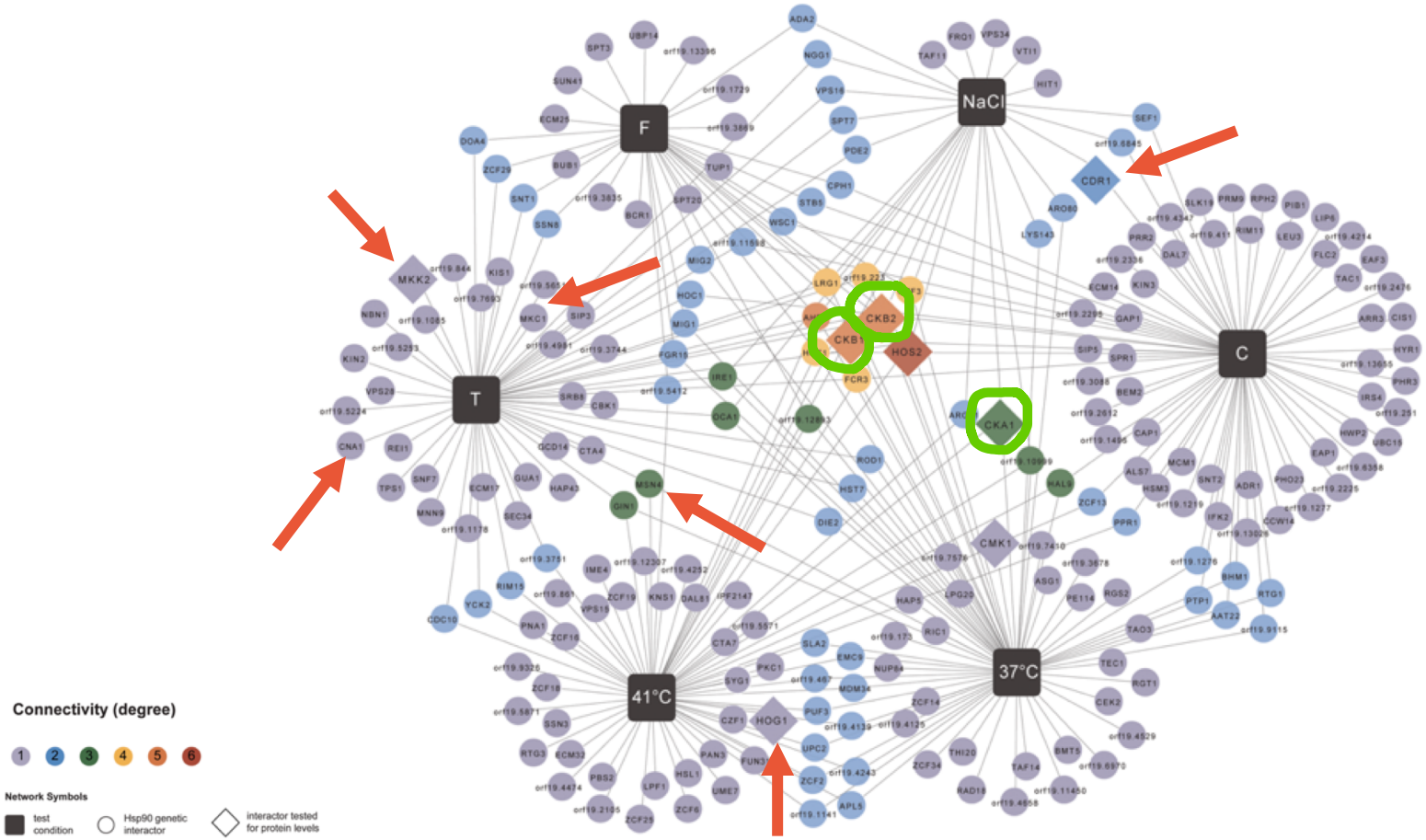
Diezmann, Leach & Cowen, 2015

resistance in *C. glabrata*



Singh-Babak et al., 20012

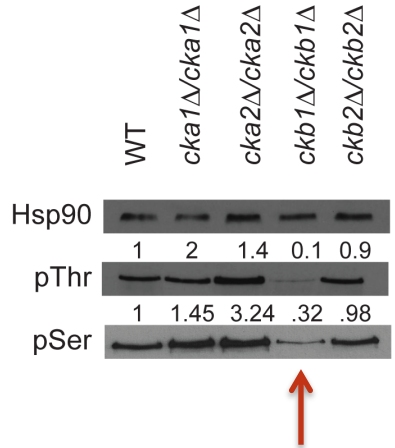
Hsp90 interacts with ~5% of the *C. albicans* genome

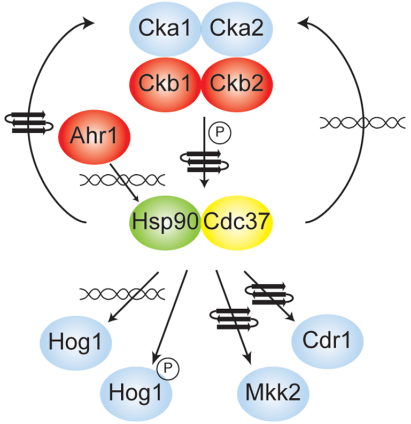


Diezmann et

Ck2 phosphorylates *C. albicans* Hsp90 thereby modulating downstream function affecting client activity and stability

Ckb1 is essential for Hsp90 phosphorylation





Interactors

High-connectivity interactor upstream of Hsp90/Cdc37

Low-connectivity interactor downstream of Hsp90/Cdc37

Effects:

gene expression

protein phosphorylation

protein levels

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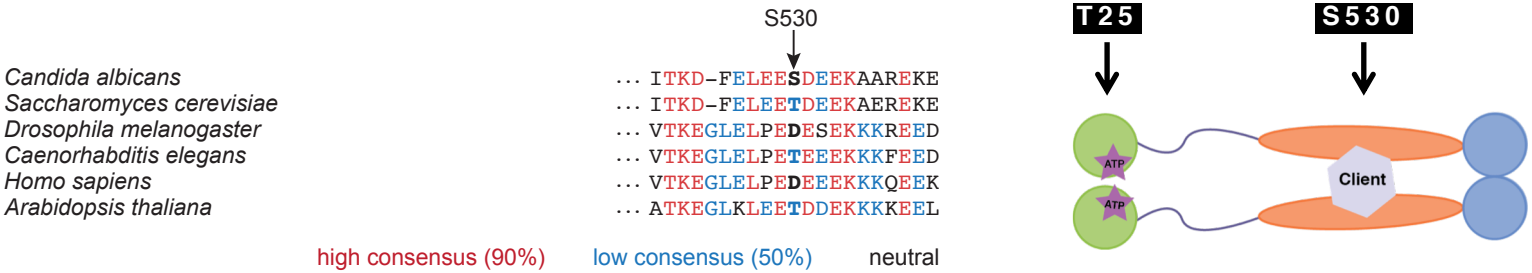
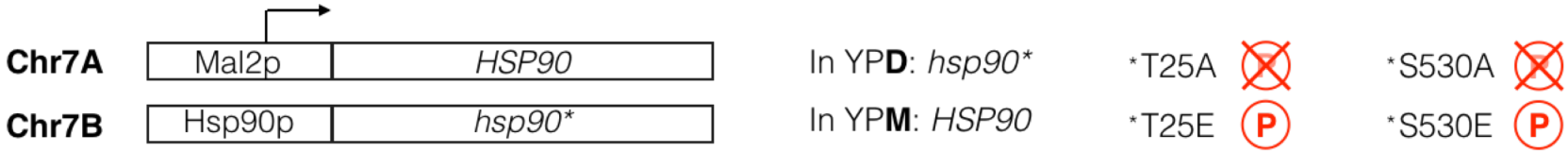
How does Ck2-mediated phosphorylation of Hsp90 affect fungal virulence?

Mass-spectrometric identification of Ck2 phosphorylation site in Hsp90

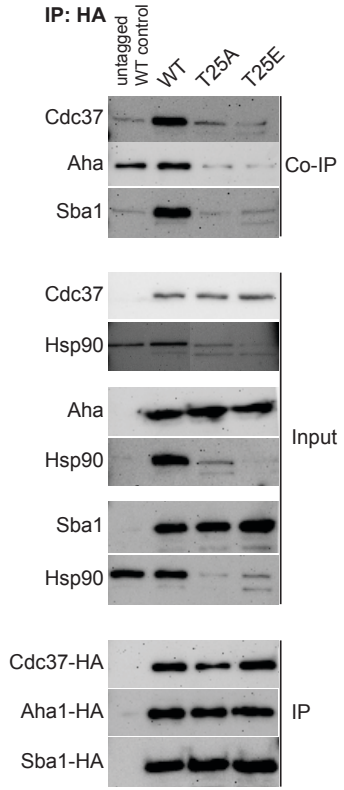


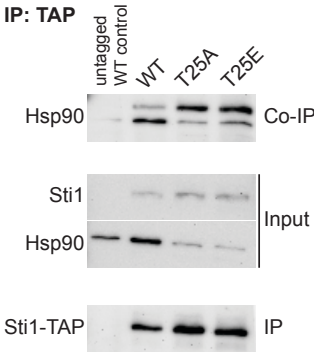
Genetic manipulation of Hsp90 phosphorylation status

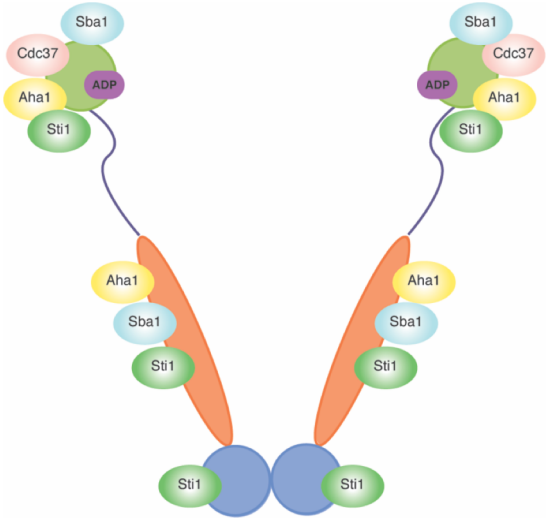


Using the maltose-inducible promoter allows for allele-specific expression of Hsp90

T25 alterations attenuate co-chaperone binding

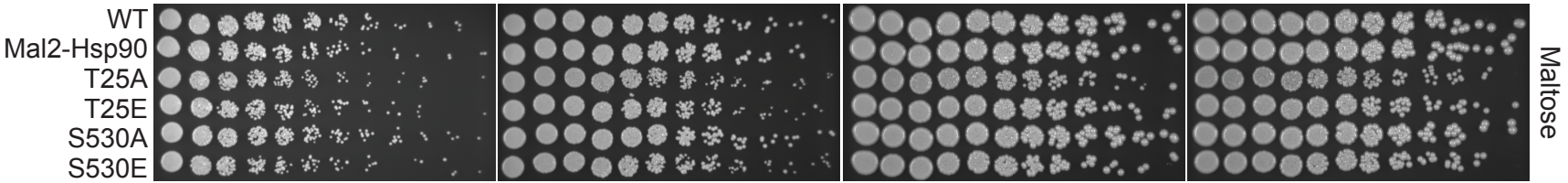






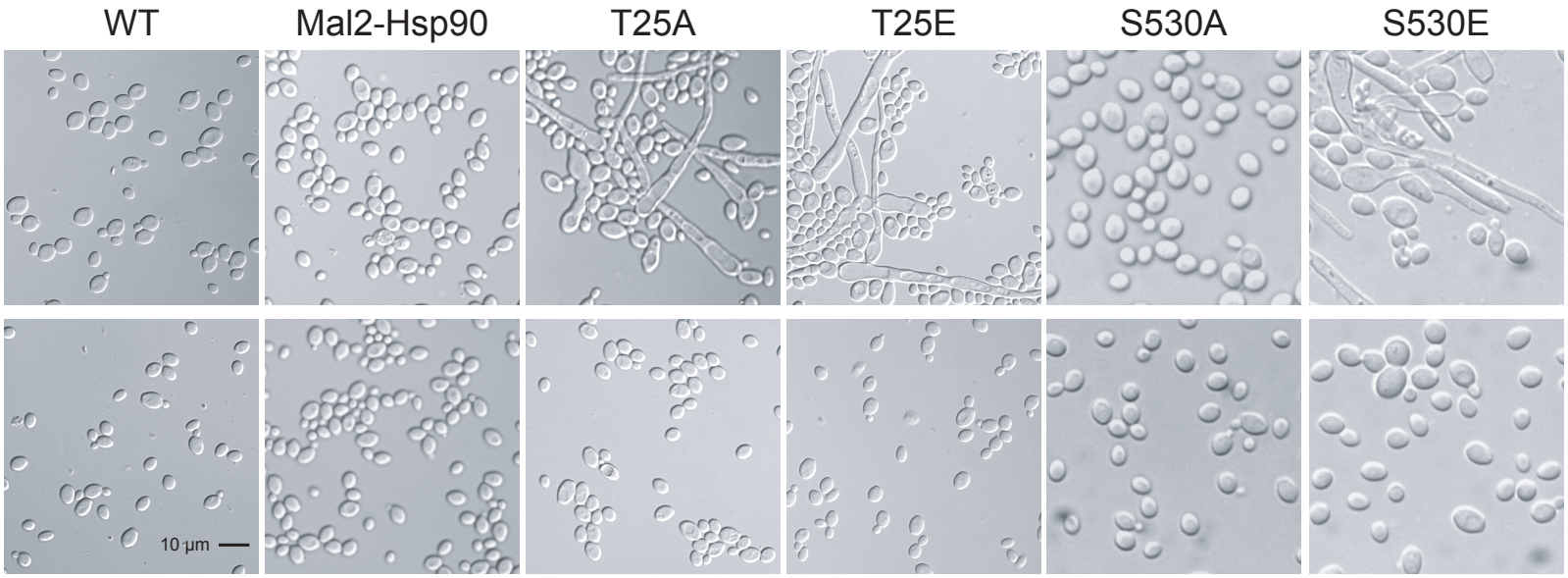
- Cdc37, Aha1, and Sba1 were HA-tagged
- Sti1 was TAP-tagged
- Tagged strains were grown to mid-log phase and Hsp90 co-immunoprecipitated with tagged co-chaperones

S530 phosphorylation abolishes survival of high temperatures



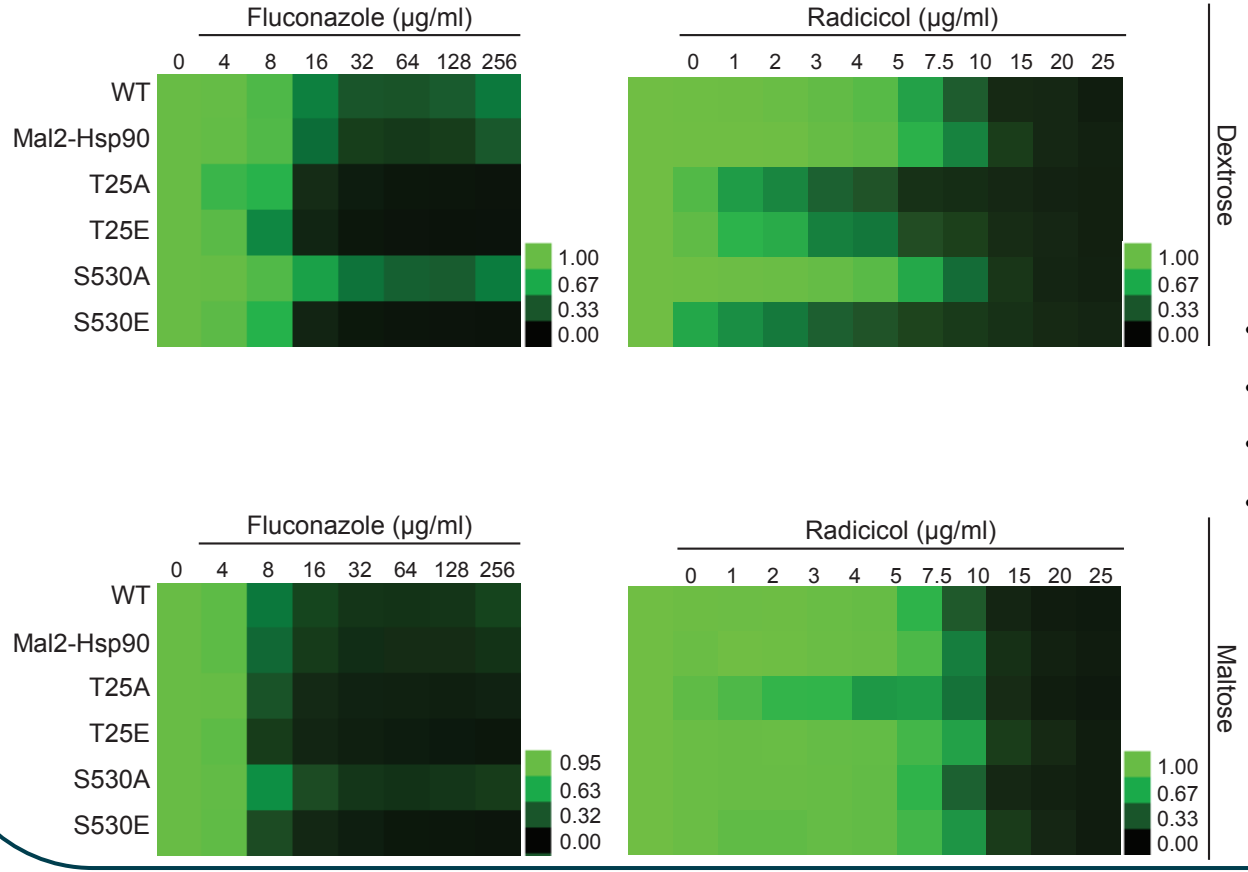
2-fold serial dilution spots on YPD or YPM were incubated at the indicated temperatures for 48 hours prior to imaging

S530 phosphorylation status determines *C. albicans* morphogenesis



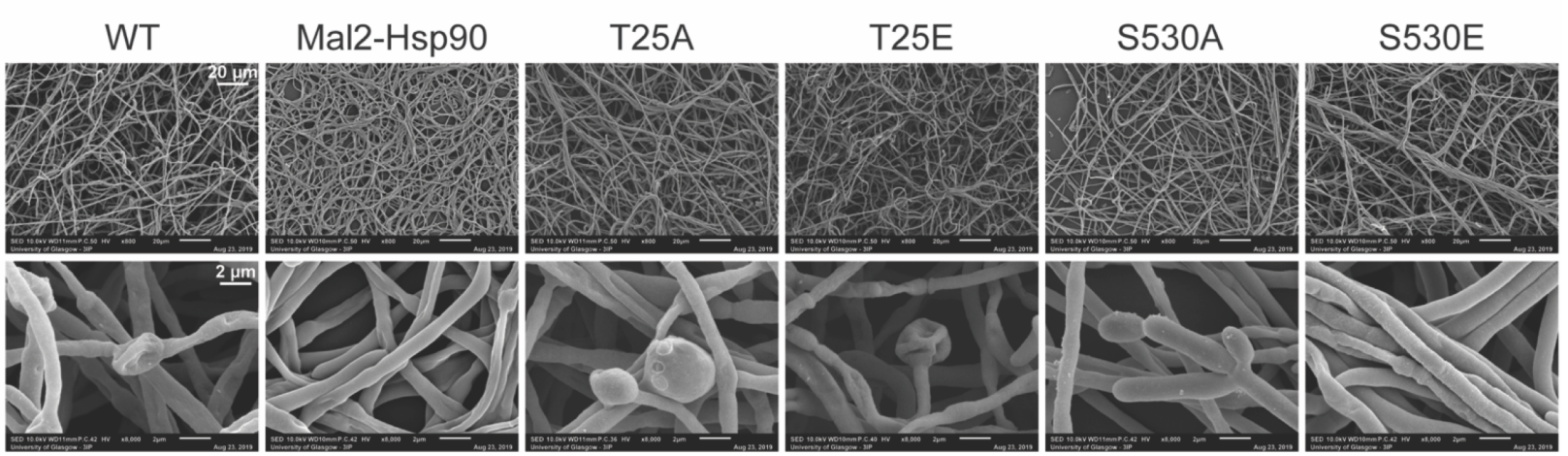
Strains were grown to mid-log phase in non filament-inducing conditions

Hsp90 phosphorylation sensitizes *C. albicans* to antifungal drugs and Hsp90 inhibitors



- MICs were set up in YPD at 25˚C
- Plates were incubated for 48 h
- Growth was measured as OD_{600}
- Samples were normalized to no drug control

Biofilm cell viability is reduced when S530 is phosphorylated



- Biofilms were grown in RPMI at 37˚C
- Cell viability is significantly decreased in strain S530E (p=0.00205)
- Biofilm biomass is not affected in any strain

Phosphorylation of S530 results in attenuated virulence in an invertebrate host model

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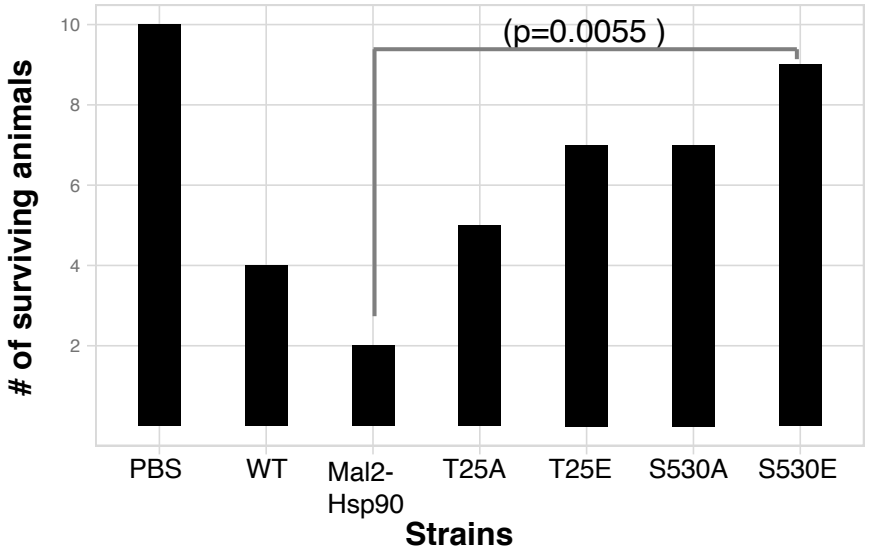
Manduca sexta caterpillars facilitate study of fungal virulence

M. sexta is susceptible to the leading yeast pathogens of humans, including *C. albicans*, *C. glabrata*, *C. auris*, and *Cryptococcus neoformans*

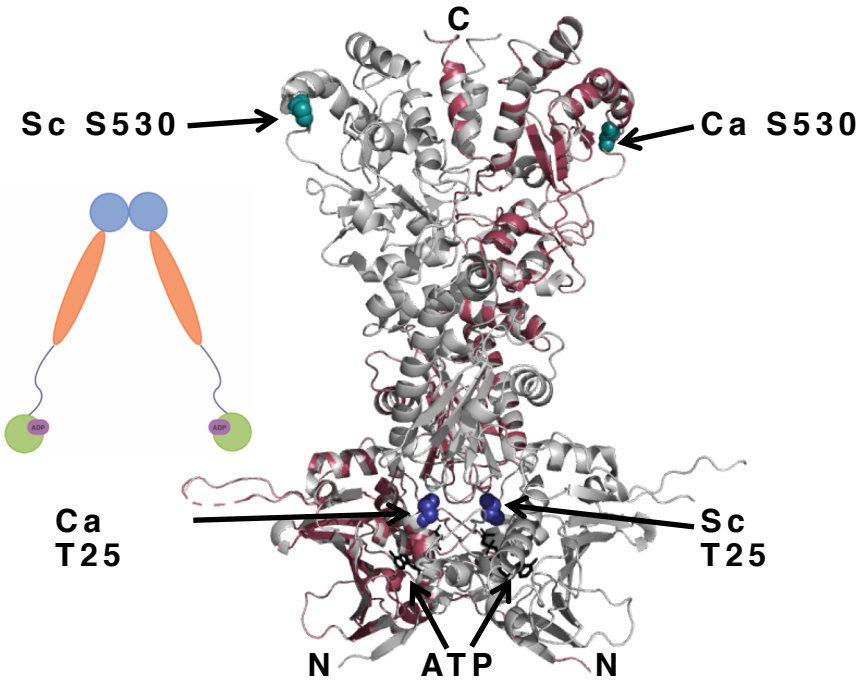
(bioRxivhttps://doi.org/10.1101/693226)

Virulence of strain S530E is significantly attenuated



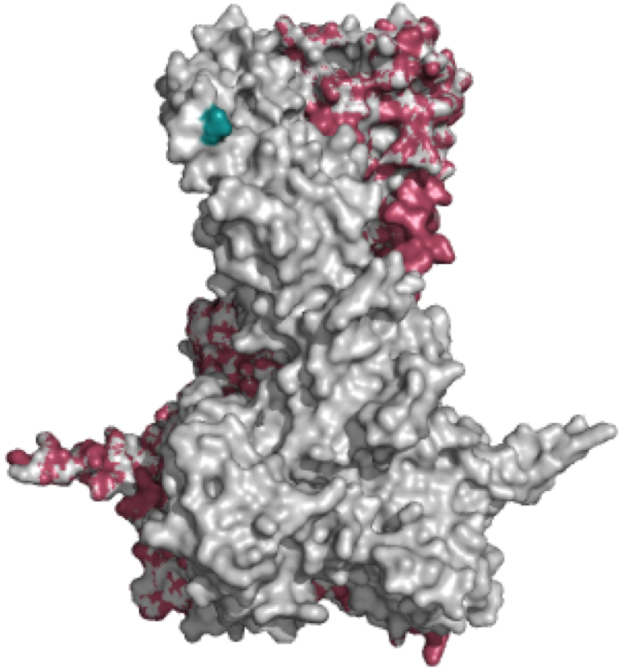
Structural modeling reveals T25 to be protected deep inside the protein

Homology model



C. albicans Hsp90 sequence is modeled on the *Saccharomyces cerevisiae* crystal structure

Surface model



Two residues that influence Hsp90 function and *C. albicans* virulence

- Characterization of two Ck2 phosphorylation residues revealed the importance of post-translational modification by phosphorylation for Hsp90 function and ultimately virulence
- Phosphorylation of S530 blocks Hsp90 function and affects a suite of *C. albicans* virulence factors (high temperature growth, cellular morphogenesis, biofilm cell viability, drug resistance and *M. sexta* weigth)
- Alterations of T25 are detrimental for Hsp90 function suggesting this residue is of importance beyond post-translational modification

Acknowledgements

Leenah Alaalm, Brown University

Julia Crunden, University of Bristol

Carolyn Williamson, University of Bath

Ulrike Obst, University of Bristol

Mark Butcher, University of Glasgow

Heath O'Brien, LivingDNA

Christiane Berger-Schaffitzel, University of Bristol

Gordon Ramage, University of Glasgow

Hsp90 levels