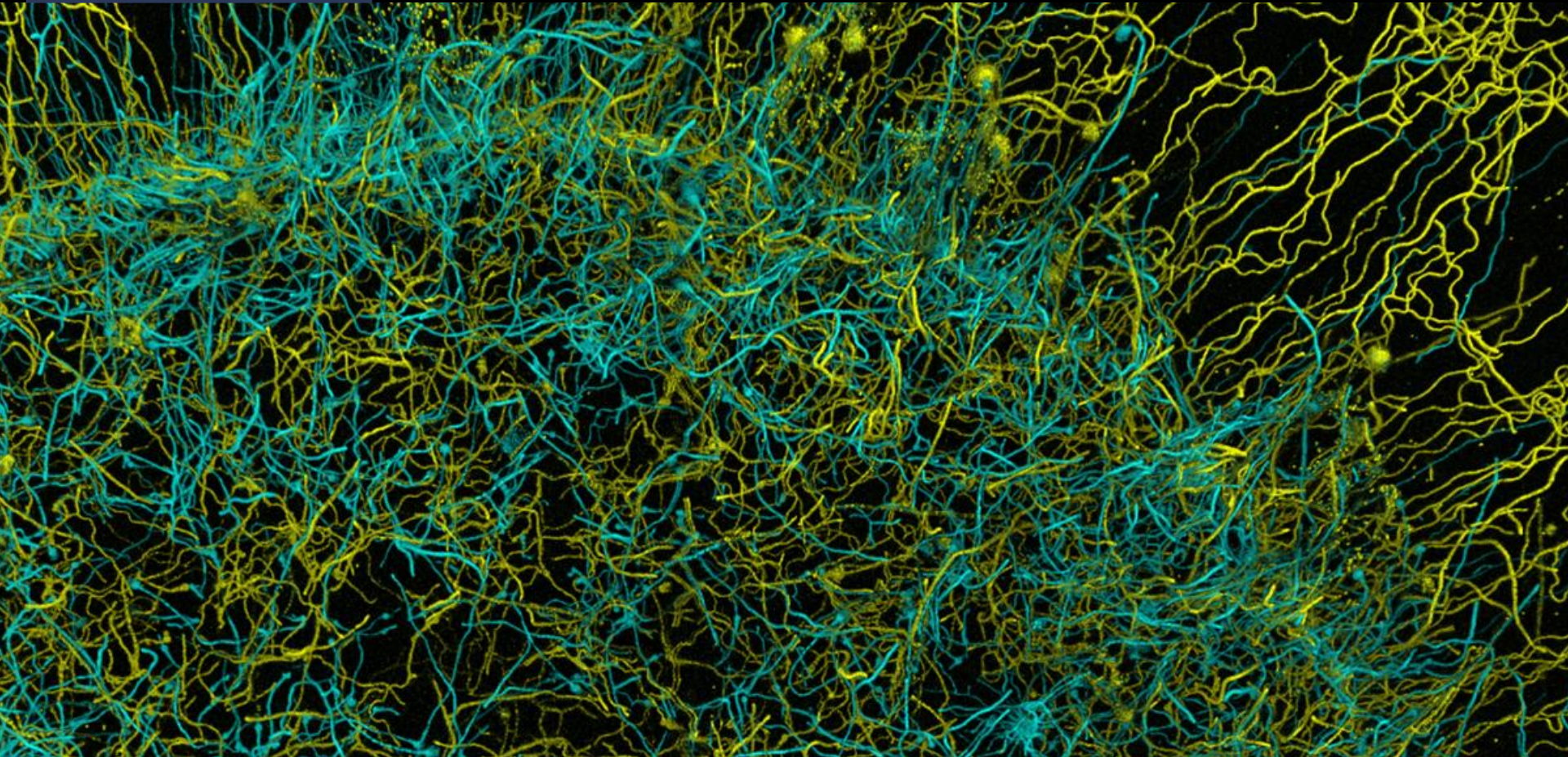




Manchester Fungal Infection Group

COFUN: An update on the construction of the genome wide knockout library in *A. fumigatus*.



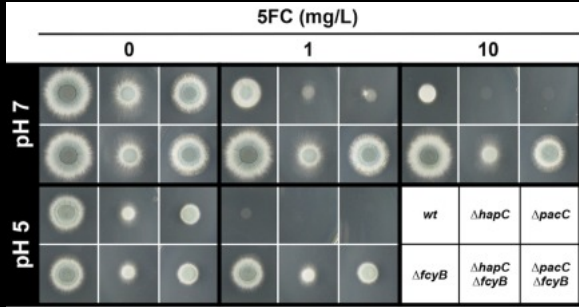
Mike Bromley

EFGC:Feb2020

@UoMCOFUN

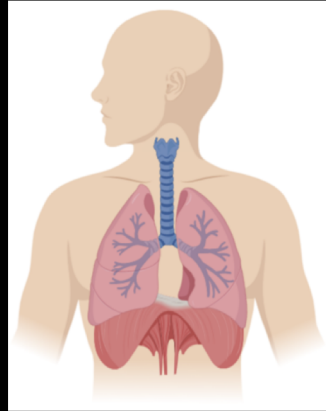
Why do we need another genome-wide KO library?

Drug resistance

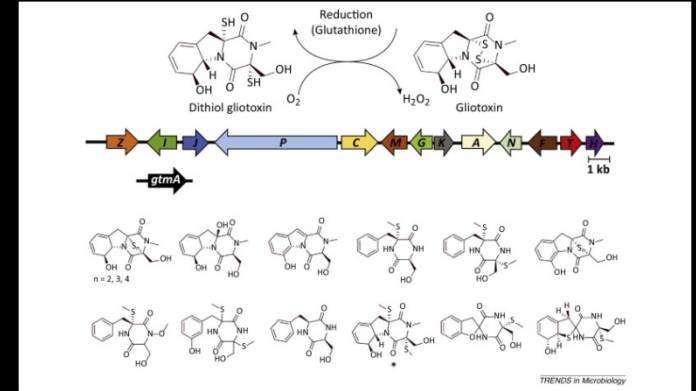


Gsaller et al 2018, AAC.

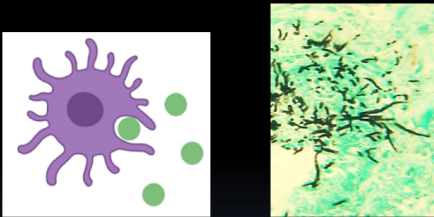
Pathogenic niche



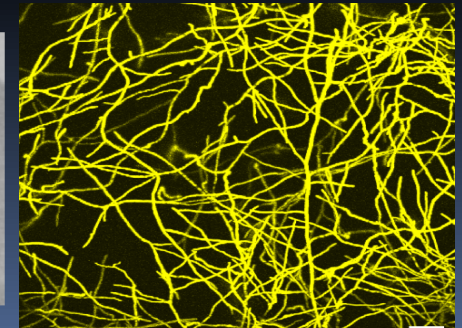
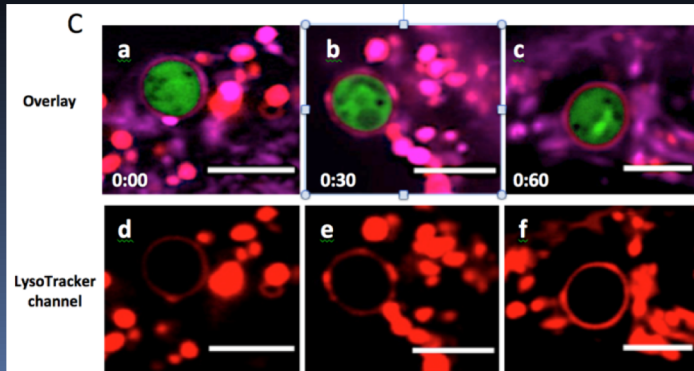
Immunogenic modulators and effectors



Host pathogen interactions



Dimorphic nature and cellular structure

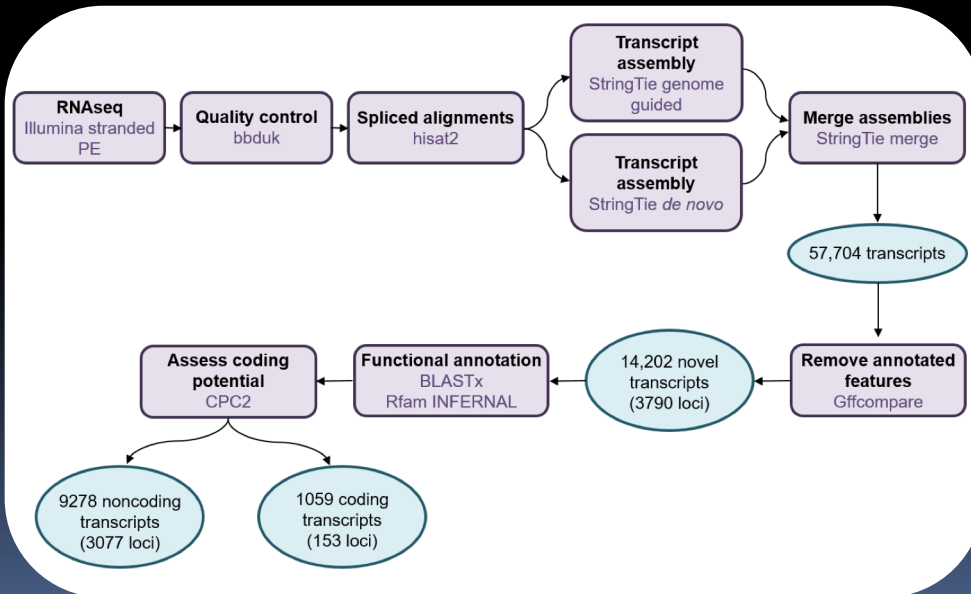


Reannotation of the *A. fumigatus* (A1163) genome

Putative coding transcripts identified: 10,322

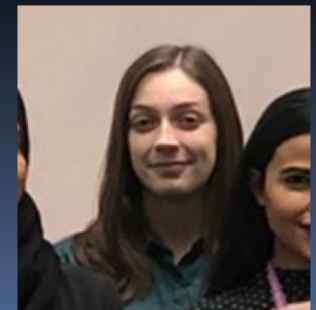
New coding transcripts: 486

Re-annotated genes: ~2,800

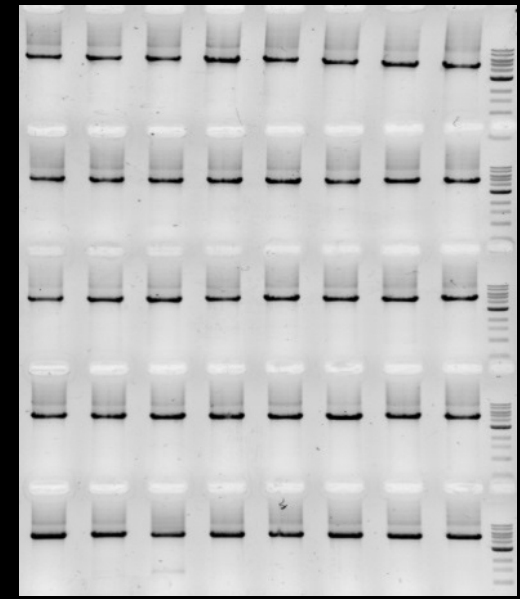
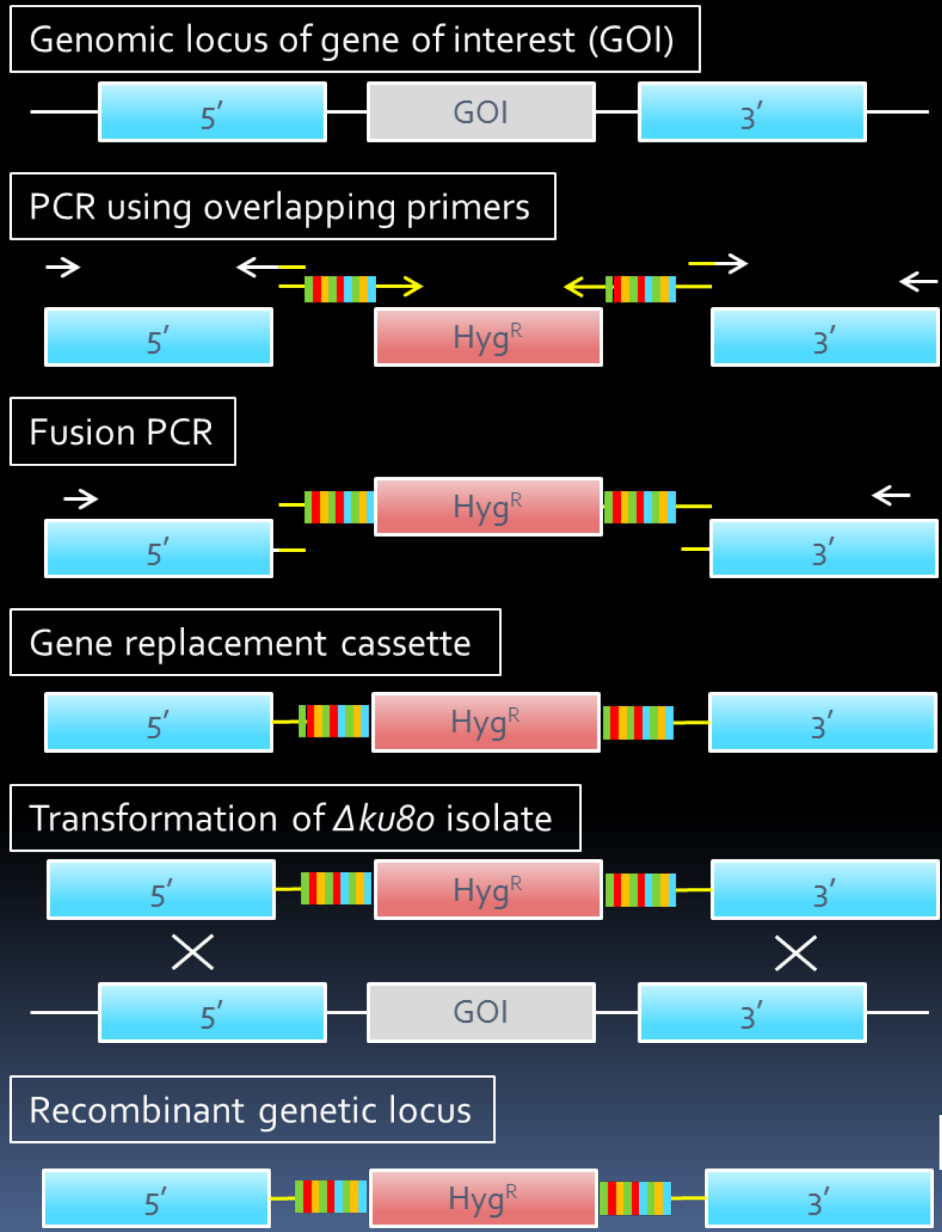


9278 non-coding transcripts

3077 non-coding loci



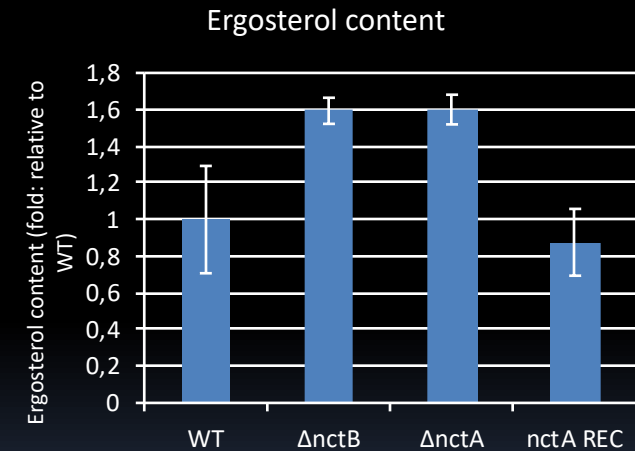
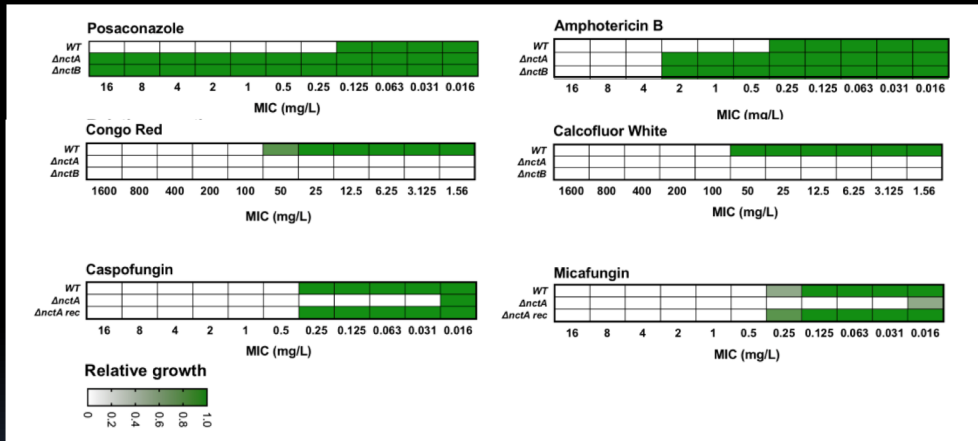
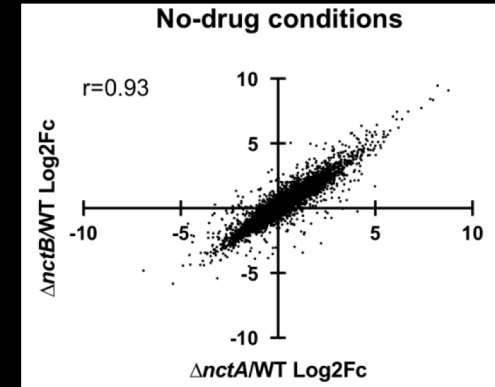
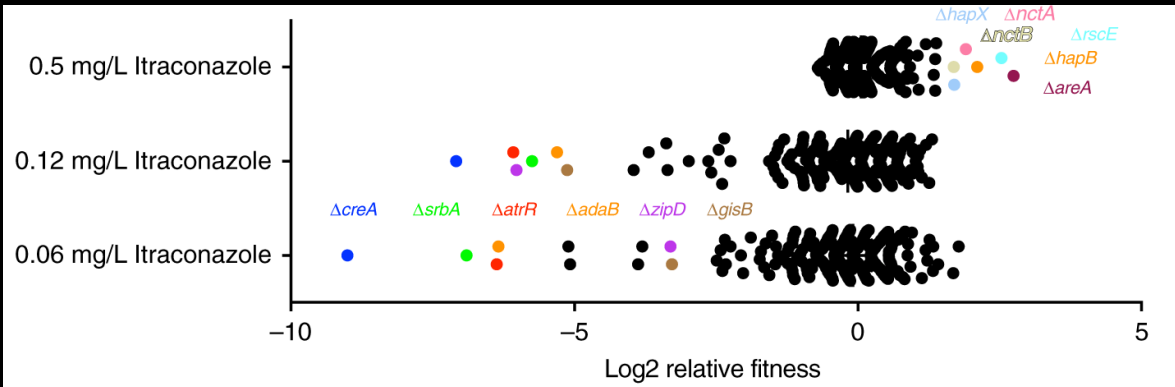
Generating a library of KO mutants



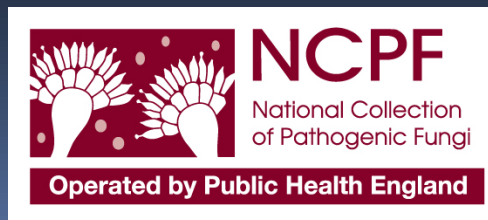
Final summary AFKO9												
	1	2	3	4	5	6	7	8	9	10	11	12
A	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/-	+/-	+/+
B	-/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/-	+/+	+/+	+/+
C	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/-	+/+	+/+	+/+	+/-
D	+/+	+/+	-/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-
E	+/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-
F	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/-	+/+	-/-	+/-
G	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/-	-/-	-/-	-/+
H	+/+	+/+	+/+	+/+	+/+	-/-	+/+	+/+	+/+	-/+	+/+	+/+

Fitness profiling of strains

Drug resistance: AF transcription factors



The TFKO library can be accessed from Public Health England: National Collection of Pathogenic Fungi (NCPF)



nature COMMUNICATIONS
ARTICLE
<https://doi.org/10.1038/s41467-019-14191-1> OPEN

The negative cofactor 2 complex is a key regulator of drug resistance in *Aspergillus fumigatus*

Takanori Furukawa^{1,2,7}, Norman van Rhijn^{1,2,7}, Marcin Fraczek¹, Fabio Gsaller¹, Emma Davies¹, Paul Carr¹, Sara Gago^{1,2}, Rachael Fortune-Grant^{1,2}, Sayema Rahman^{1,2}, Jane Mabey Gilsean¹, Emma Houlder², Caitlin H. Kowalski³, Shriya Raj⁴, Sanjoy Paul⁵, Peter Cook², Josie E. Parker⁶, Steve Kelly⁶, Robert A. Cramer³, Jean-Paul Latgé⁴, Scott Moye-Rowley⁵, Elaine Bignell^{1,2}, Paul Bowyer^{1,2,8*} & Michael J. Bromley^{1,2,8*}

The library has been used successfully by a number of groups

PLOS PATHOGENS

RESEARCH ARTICLE

Filamentous fungal carbon catabolite repression supports metabolic plasticity and stress responses essential for disease progression

Sarah R. Beattie¹, Kenneth M. K. Mark², Arsa Thammahong¹, Laure Nicolas Annick Ries³, Sourabh Dhingra¹, Alayna K. Caffrey-Carr^{1,4}, Chao Cheng^{2,5,6}, Candice C. Black⁷, Paul Bowyer⁸, Michael J. Bromley⁹, Joshua J. Obar¹, Gustavo H. Goldman³, Robert A. Cramer^{1*}

AMERICAN SOCIETY FOR MICROBIOLOGY **mBio**

RESEARCH ARTICLE
Molecular Biology and Physiology

The *Aspergillus fumigatus* Phosphoproteome Reveals Roles of High-Osmolarity Glycerol Mitogen-Activated Protein Kinases in Promoting Cell Wall Damage and Caspofungin Tolerance

Eliciane Cevolani Mattos,^a Lilian Pereira Silva,^a Clara Valero,^a Patrícia Alves de Castro,^a Thaila Fernanda dos Reis,^a Liliane F. C. Ribeiro,^b Mark R. Marten,^b Rafael Silva-Rocha,^c Cauã Westmann,^c Carlos Henrique Tomich de Paula da Silva,^{a,j} Carlton Anthony Taft,^d Narjes Al-Furajji,^e Michael Bromley,^a Uffe H. Mortensen,^f J. Philipp Benz,^{g,h} Neil Andrew Brown,ⁱ Gustavo H. Goldman^{a,h}

PLOS GENETICS

RESEARCH ARTICLE

Aspergillus fumigatus calcium-responsive transcription factors regulate cell wall architecture promoting stress tolerance, virulence and caspofungin resistance

Patrícia Alves de Castro^{1*}, Ana Cristina Colabardini^{1*}, Adriana Oliveira Manfiolli¹, Jéssica Chiaratto¹, Lilian Pereira Silva¹, Eliciane Cevolani Mattos¹, Giuseppe Palmisano², Fausto Almeida³, Gabriela Felix Persinoti⁴, Laure Nicolas Annick Ries³, Laura Mellado⁵, Marina Campos Rocha⁶, Michael Bromley⁶, Roberto Nascimento Silva³, Gabriel Scalini de Souza⁷, Flávio Vieira Loures⁷, Iran Malavazi⁵, Neil Andrew Brown^{8*}, Gustavo H. Goldman^{1*}

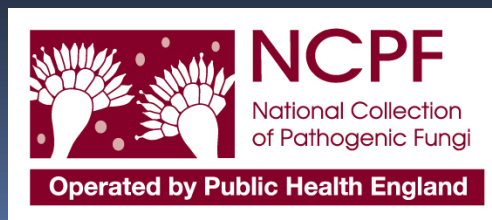
AMERICAN SOCIETY FOR MICROBIOLOGY **mSphere**

RESEARCH ARTICLE
Molecular Biology and Physiology

Weak Acid Resistance A (WarA), a Novel Transcription Factor Required for Regulation of Weak-Acid Resistance and Spore-Spore Heterogeneity in *Aspergillus niger*

Ivey A. Geoghegan,^a Malcolm Stratford,^a Mike Bromley,^b David B. Archer,^a Simon V. Avery^a

The TFKO library can be accessed from
Public Health England:
National Collection of Pathogenic Fungi
(NCPF)



Competitive fitness profiling (Bar-seq)

REPORTS

Functional Characterization of the *S. cerevisiae* Genome by Gene Deletion and Parallel Analysis

Elizabeth A. Winzeler,^{1*} Daniel D. Shoemaker,^{2*} Anna Astromoff,^{1*} Hong Liang,^{1*} Keith Anderson,¹ Bruno Andre,³ Rhonda Bangham,⁴ Rocio Benito,⁵ Jef D. Boeke,⁶ Howard Bussey,⁷ Angela M. Chu,¹ Carla Connelly,⁶ Karen Davis,¹ Fred Dietrich,⁸ Sally Whelen Dow,² Mohamed El Bakkoury,⁹ Françoise Foury,¹⁰ Stephen H. Friend,² Erik Gentalen,¹¹ Guri Giaever,¹ Johannes H. Hegemann,¹² Ted Jones,¹ Michael Laub,¹ Hong Liao,⁴ Nicole Liebundguth,⁸ David J. Lockhart,¹¹ Anca Lucau-Danila,¹⁰ Marc Lussier,⁷ Nasiha M'Rabet,³ Patrice Menard,⁷ Michael Mittmann,¹¹ Chai Pai,¹ Corinne Rebischung,⁸ Jose L. Revuelta,⁵ Linda Riles,¹³ Christopher J. Roberts,² Petra Ross-MacDonald,⁴ Bart Scherens,⁹ Michael Snyder,⁴ Sharon Sookhai-Mahadeo,⁶ Reginald K. Storms,⁷ Steeve Véronneau,⁷ Marleen Voet,¹⁴ Guido Volckaert,¹⁴ Teresa R. Ward,² Robert Wysocki,¹⁰ Grace S. Yen,¹ Kexin Yu,⁶ Katja Zimmermann,¹² Peter Philippsen,⁸ Mark Johnston,¹³ Ronald W. Davis^{1†}

Science v285 1999

OPEN ACCESS Freely available online

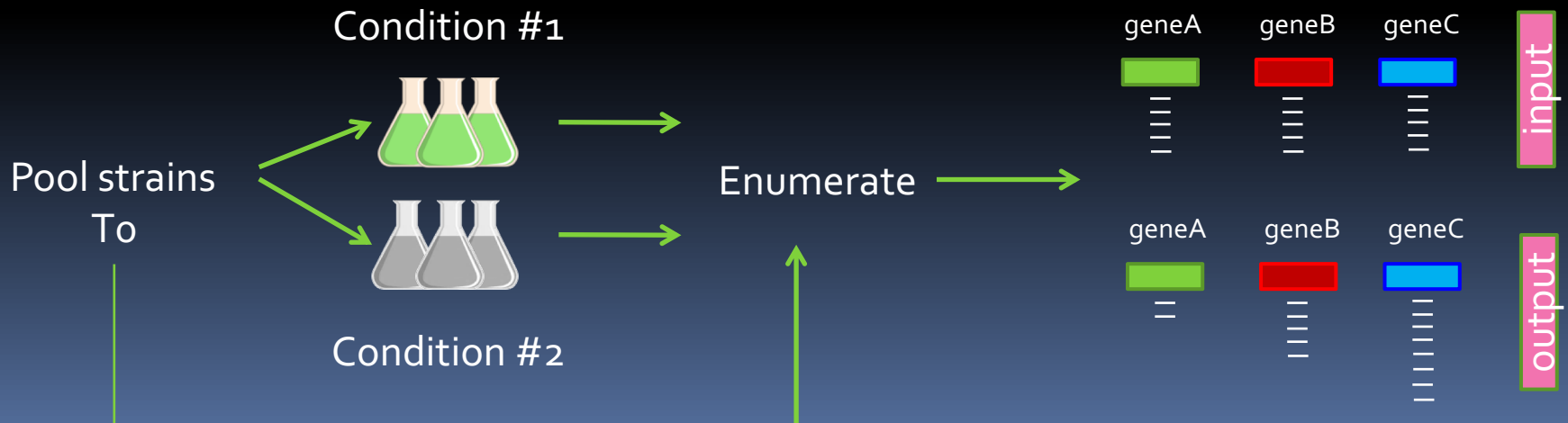
PLoS PATHOGENS

Genome-Wide Fitness Test and Mechanism-of-Action Studies of Inhibitory Compounds in *Candida albicans*

Deming Xu¹*, Bo Jiang¹*, Troy Ketela², Sebastien Lemieux³, Karynn Veillette¹, Nick Martel¹, John Davison¹, Susan Sillaots¹, Steve Trosok¹, Catherine Bachewich⁴, Howard Bussey⁵, Phil Youngman⁶, Terry Roemer^{1*}

1 Center of Fungal Genetics, Merck Frost Canada Ltd., Montreal, Quebec, Canada, **2** Infinity Pharmaceuticals, Cambridge, Massachusetts, United States of America, **3** Institute of Research in Immunology and Cancer, University of Montreal, Montreal, Quebec, Canada, **4** Department of Biology, Concordia University, Montreal, Quebec, Canada, **5** Department of Biology, McGill University, Montreal, Quebec, Canada, **6** Department of Infectious Disease, Merck & Co., Inc., Rahway, New Jersey, United States of America

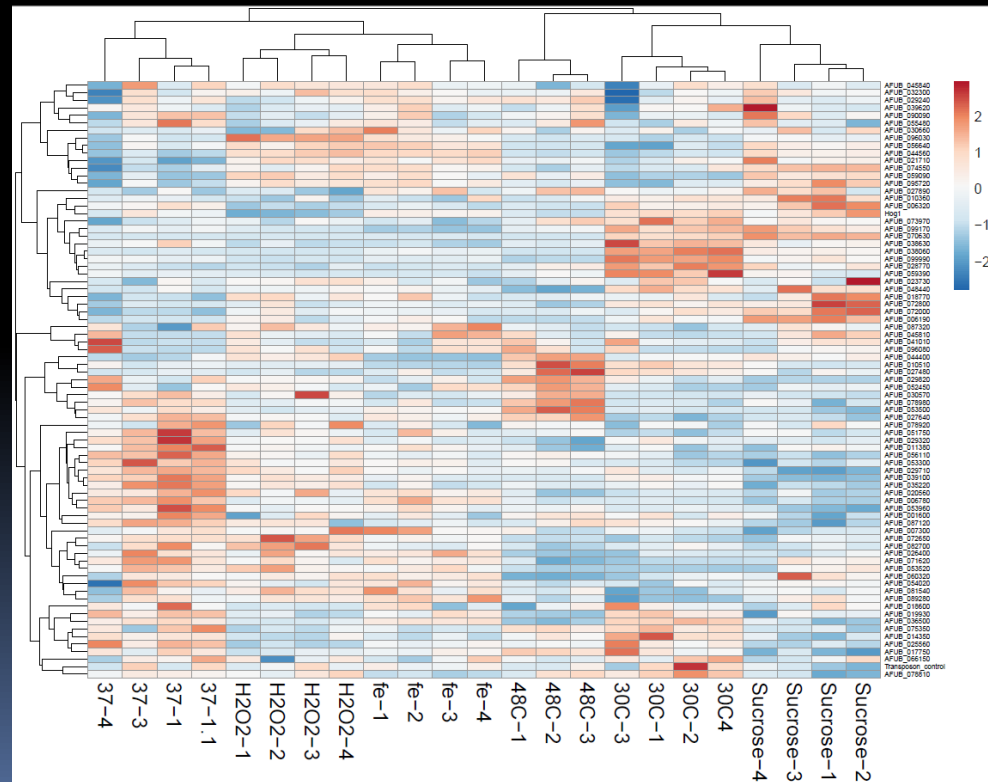
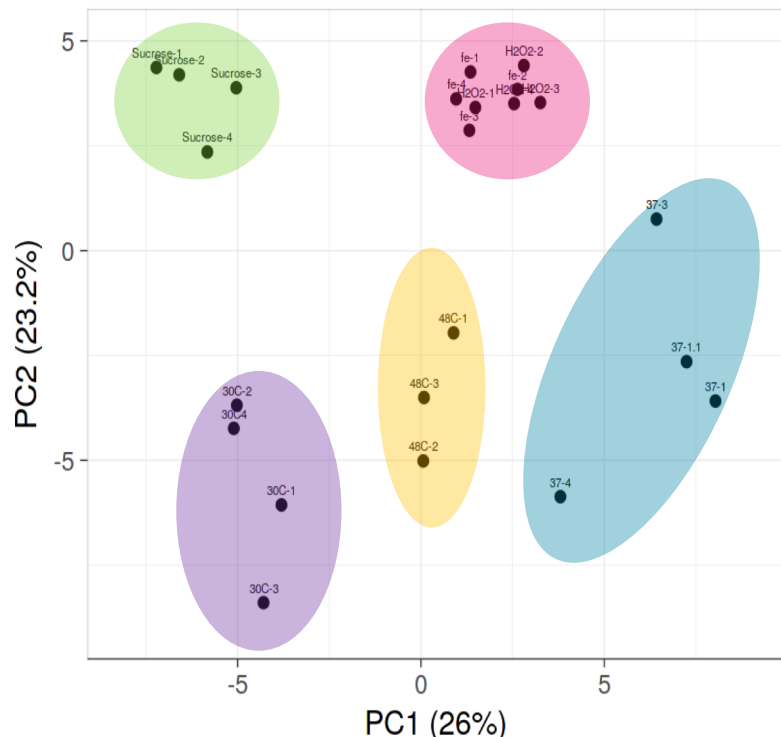
PLoS Pathog 3(6): e92 2007



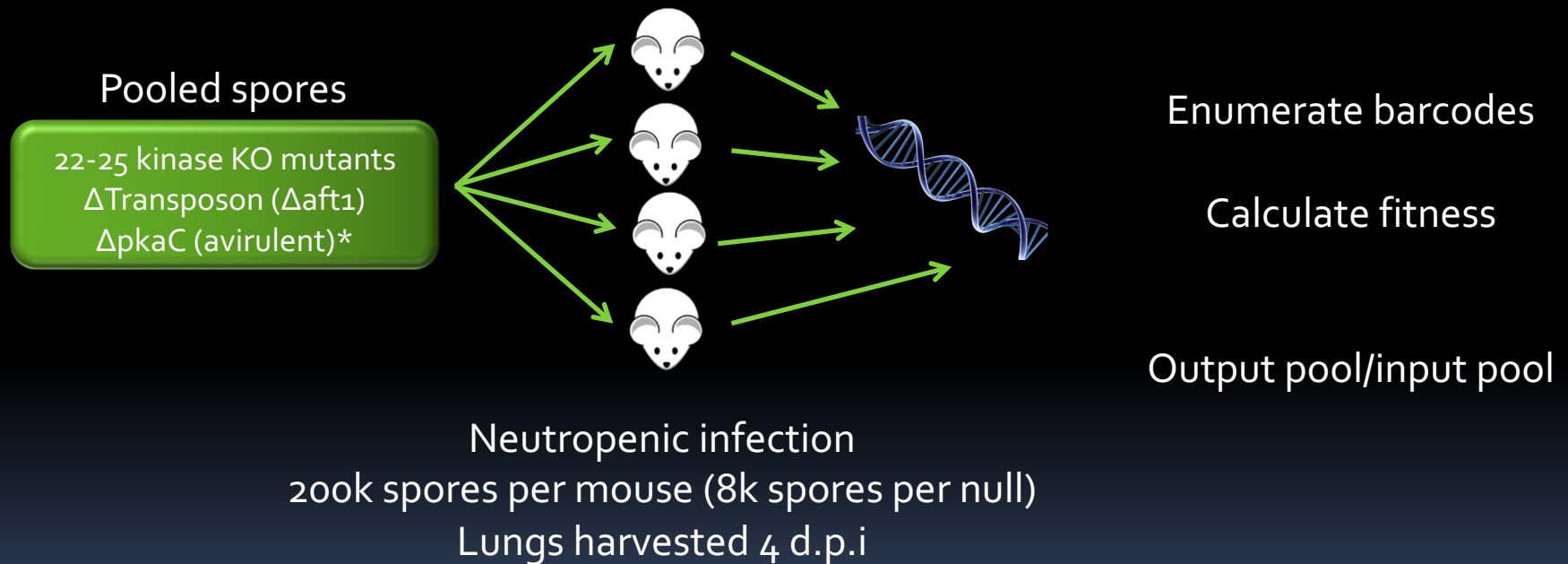
Bar-seq is highly reproducible in *A. fumigatus*

Kinase KO library (n=120 isolates):
Competitive fitness profiles for 6 conditions

RPMI: 30 °C, 37 °C, 42 °C ; oxidative stress (H₂O₂), low iron, high osmolarity

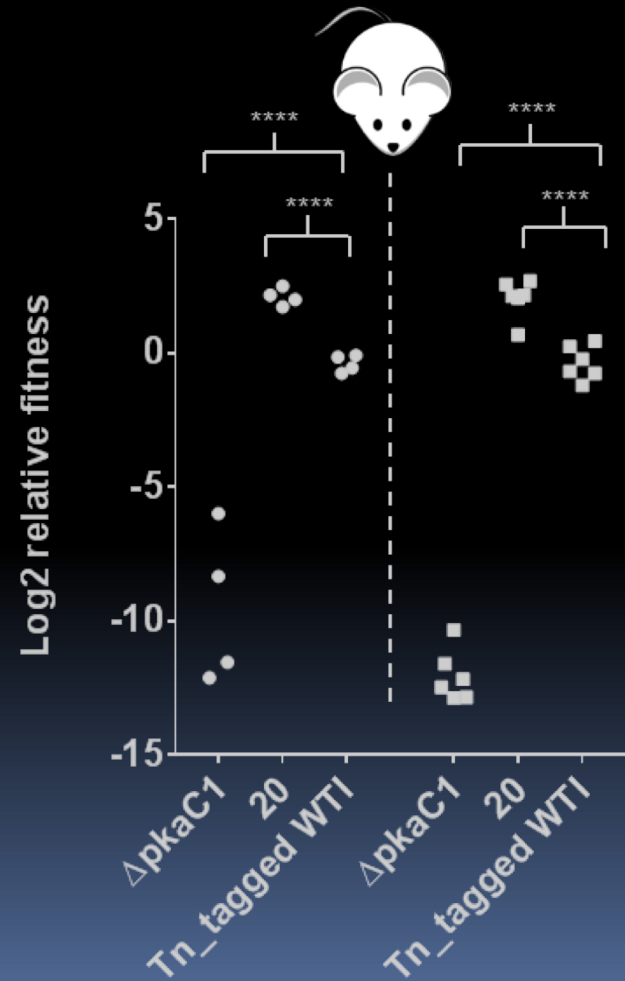
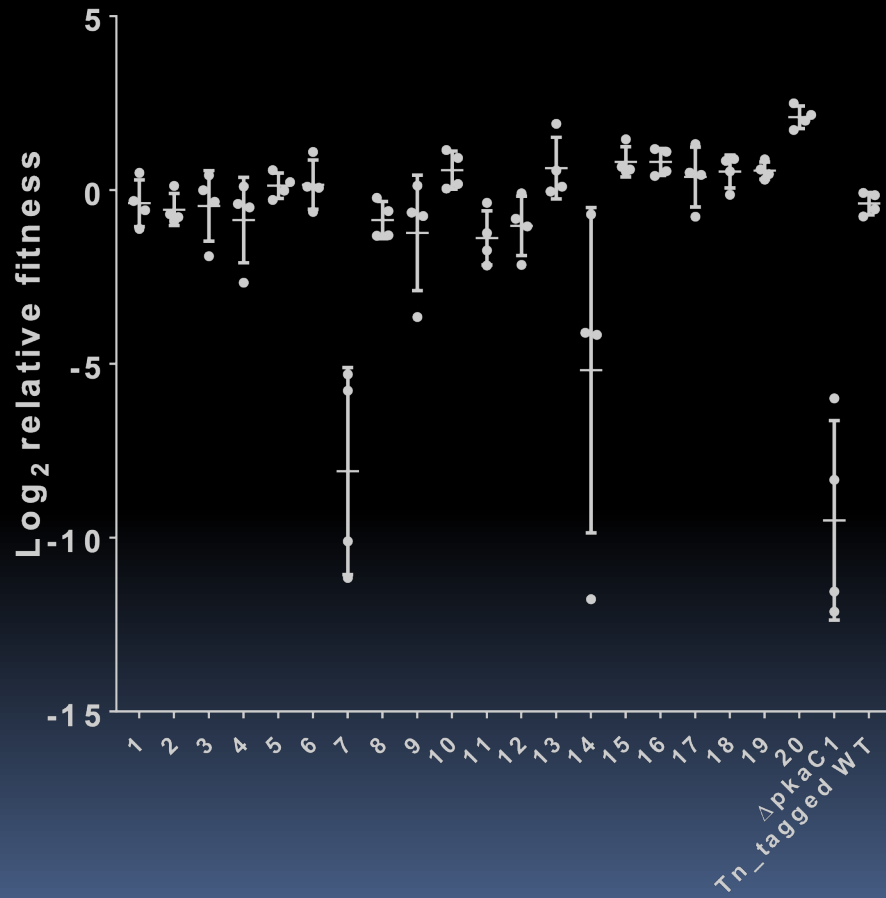


Competitive fitness in a mouse model using barcoded kinase KO strains: experimental setup

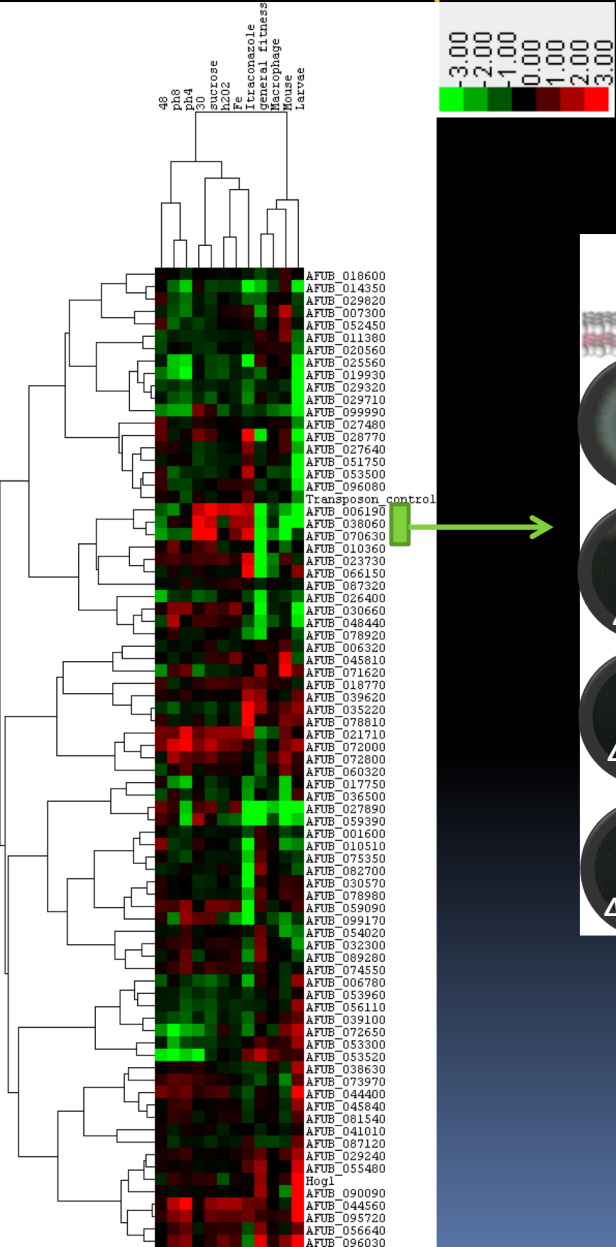


*Liebmann et al Infect Immun 2004 Sep; 72(9): 5193–5203.

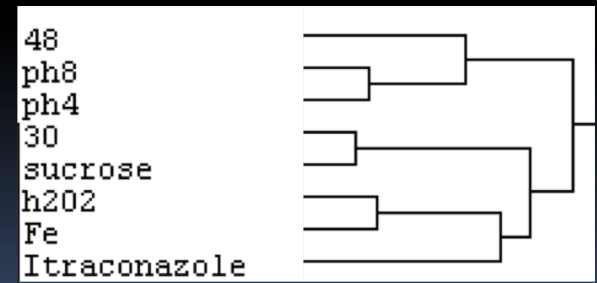
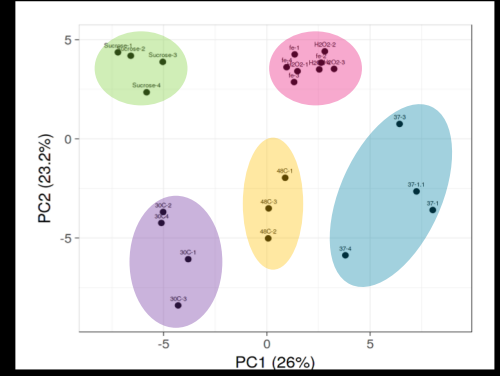
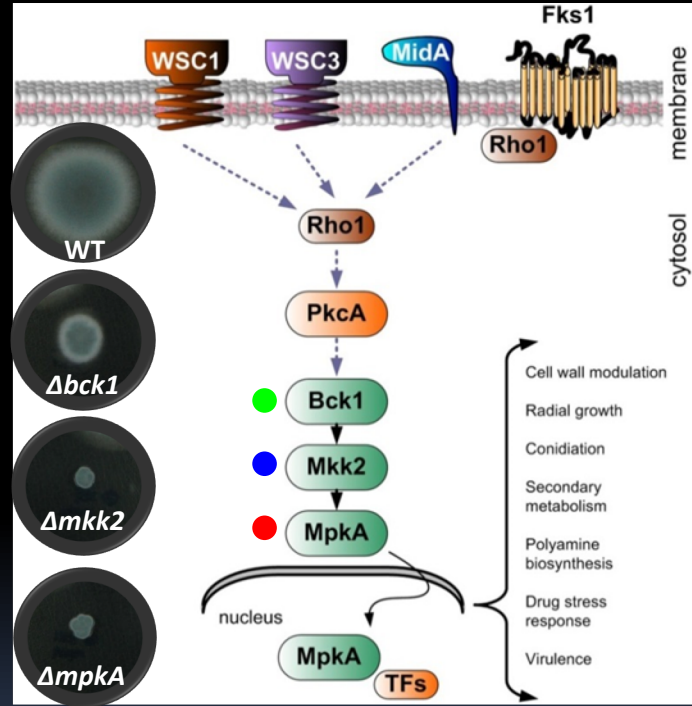
Competitive fitness in a mouse model using barcoded kinase KO strains



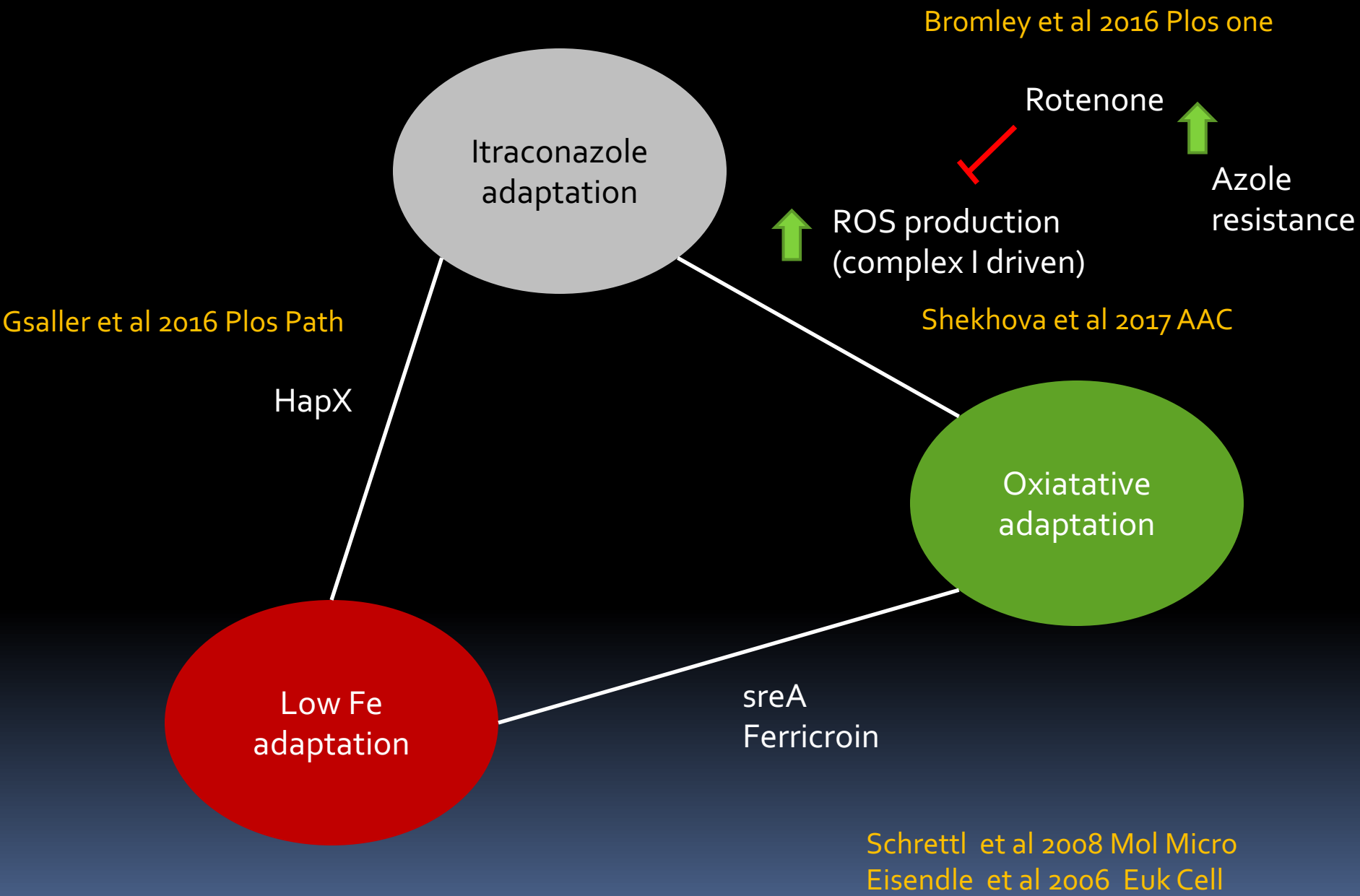
Fitness profiles can be used to identify functional groups of kinases and provide an environmental sensor



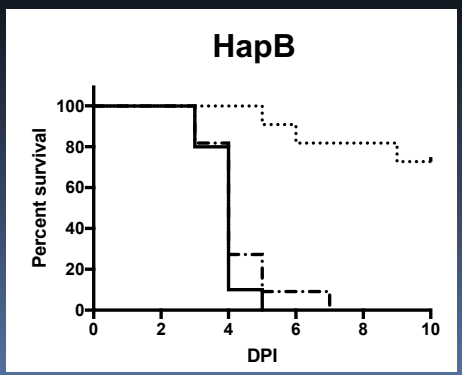
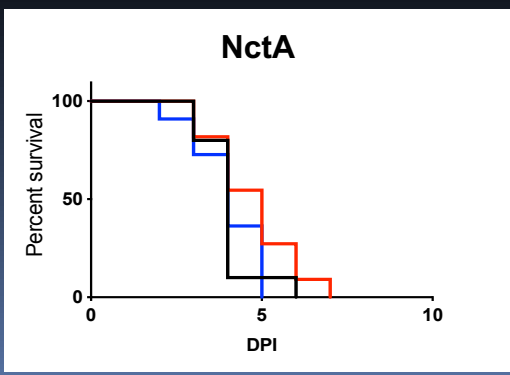
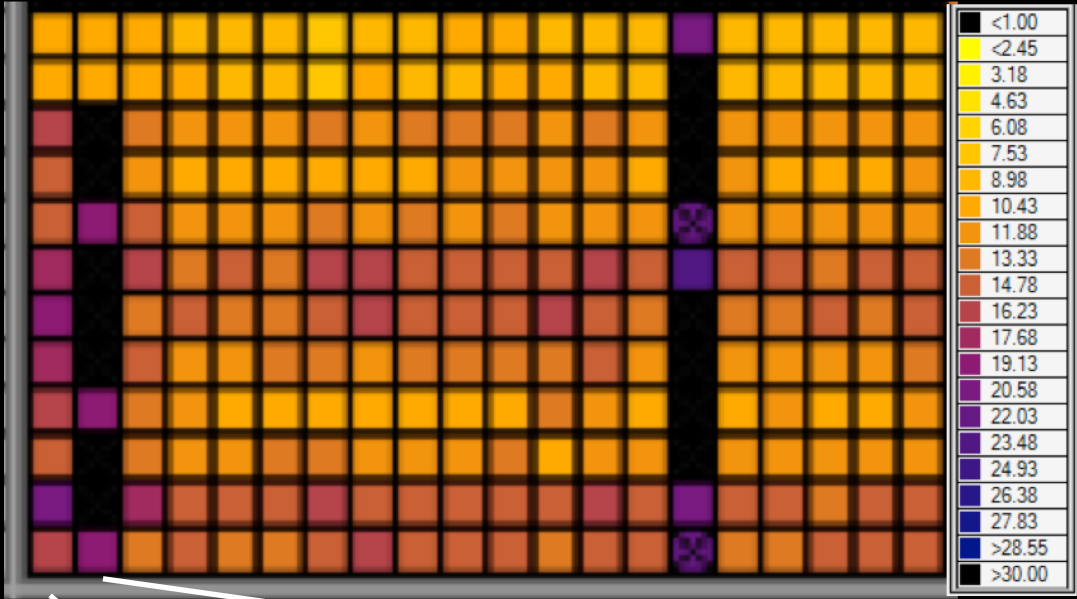
Cell wall integrity pathway



Valiante et al 2015
Frontiers in Microbiology



Competitive fitness profiling is possible in a mouse model using non-barcoded isolates



Current status of genome wide KO collection

- The first passage of gene knockouts for 10,322 coding genes has been completed.

Coding genes	10,322
Genes with viable transformants	8,463 (82%)
Number of strains completed validation	6,358 (62%)
Number of validated null mutants	5,150 (50%)

- Completion of the library is expected by the end of 2020.

Accessing KO collection: COFUN project



Culture Collections

You are here: Home > Products > Fungi Search > COFUN - *Aspergillus fumigatus* gene-wide knock out collection

Menu

- About Us - Culture Collections
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 - LENTICULE Discs
 - Cell Lines and Hybridomas
 - ECACC DNA Products
 - RNA from Cell Lines
 - cDNA
 - Fungi Search
 - COFUN - *Aspergillus fumigatus* gene-wide knock out collection
 - Virus Search
- Services
 - Technical Support
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 - Forms
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COFUN - *Aspergillus fumigatus* gene-wide knock out collection



COFUN is a Wellcome Trust funded project undertaken by the University of Manchester that aims to generate knockout mutants for all of the coding genes in the human pathogen *Aspergillus fumigatus*. In total around 10,000 strains will be generated for which Culture Collections will be the sole distributor.

The first sub-library consists of >400 transcription factor null mutants in the form of a panel (frozen). The transcription factor knockout strains incorporate a class III restriction site on one flank providing the capability to perform rapid quantitative parallel fitness analysis (the methodologies for this can be supplied on request).

Related Links

- About NCPF
- Search Fungi
- Browse Fungi
- Culture Collections News
- Fungi Identification Course
- NCPF News

NCPF No	Gene Knocked Out	Format	Price
NCPF 7922	Transcription Factor	DNA panel (frozen) (5x96 well)	£1000

Acknowledgements

MANCHESTER
1824

The University of Manchester

Narjes Al-Furaji
Anna Johns
Norman Van Rhijn
Hajer Alshammri
Takanori Furukawa

@UoMCOFUN

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Daniela Delneri
Can Zhao
Lauren Dineen
Marcin Fraczek
Isabelle Storer



Axel Brakhage
Thorsten Heinekamp
Juliane Macheleidt
Vito Valiante

Peter Hortschansky



Hubertus Haas
Fabio Gsaller



Swansea University
Prifysgol Abertawe

Josie Parker
Steve Kelly



Scott Moye-Rowley

Elaine Bignell
Lydia Tabernero
Ben Thornton

tRNA null collection: Poster #C2-14
LncRNA annotation: Poster #C2-31

MRC

Medical
Research
Council



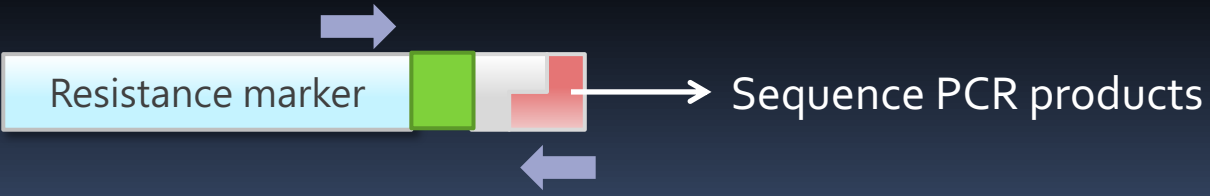
wellcome trust



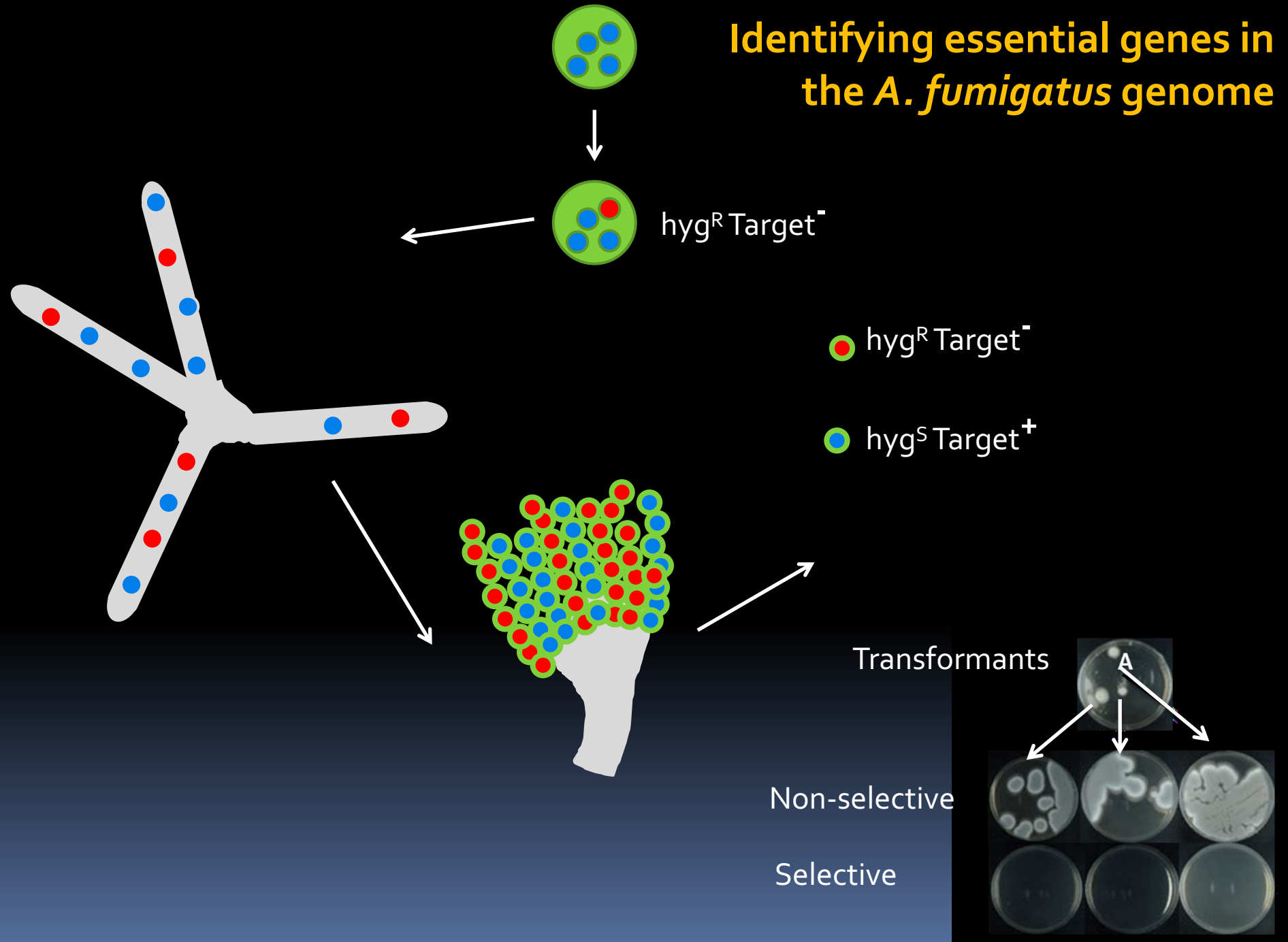
Shear, polish and A-tail



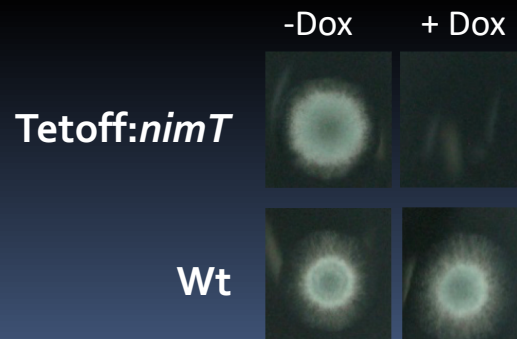
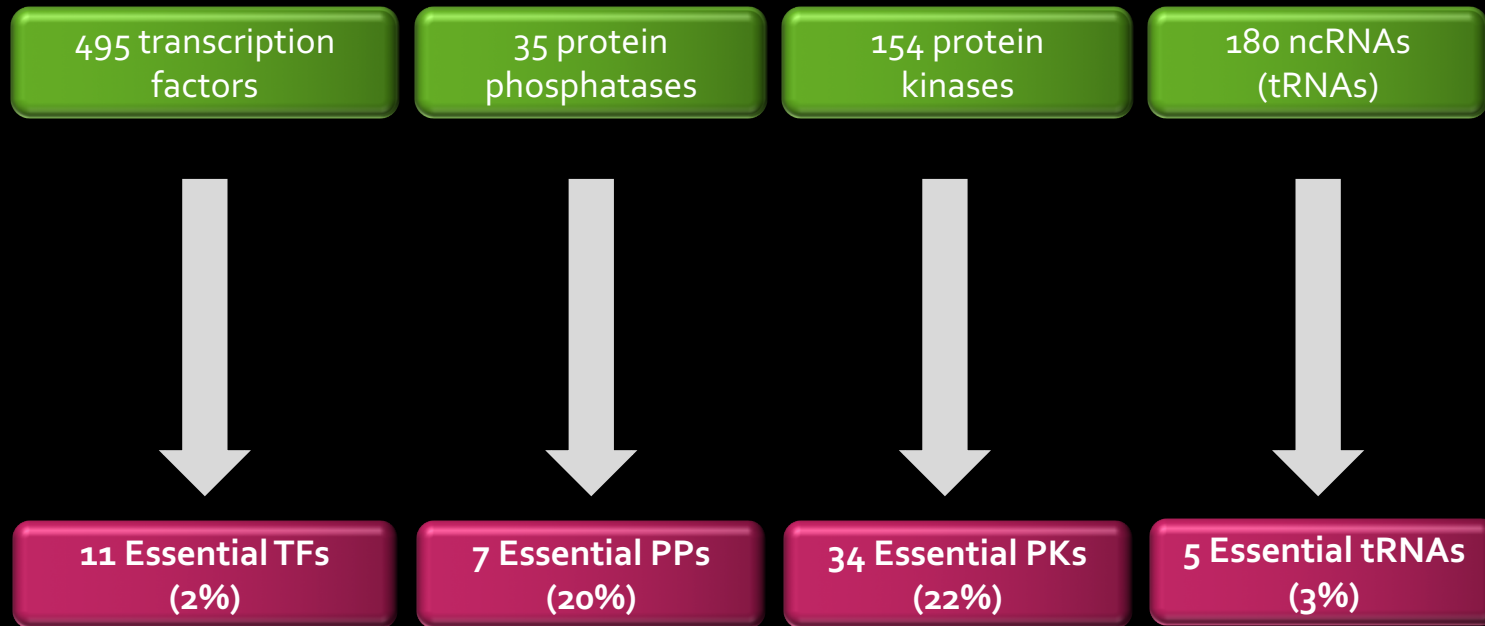
Ligate asymmetric adaptors



Identifying essential genes in the *A. fumigatus* genome

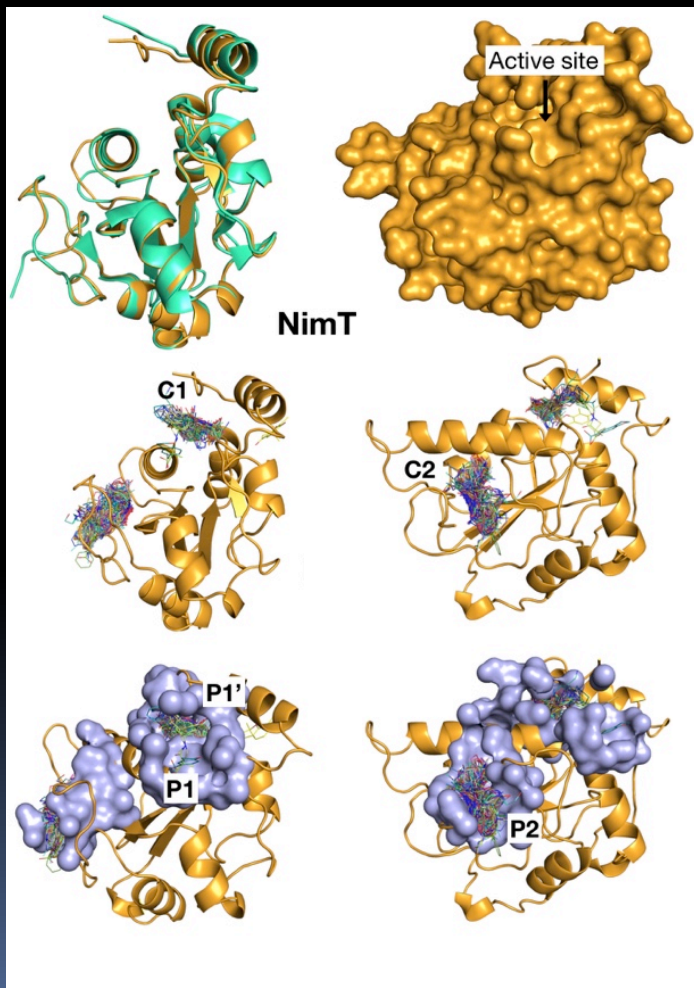


The *A. fumigatus* essential gene complement



The *A. fumigatus* essential gene cohort: potential drug targets

Assessing druggable space within protein phosphatases (NimT *aka*: CDC25)



Structural model


Identification of discrete druggable pockets

VSpine and PockDrug reveal overlapping druggable sites

International Journal of
Molecular Sciences

Article

Identification of Functional and Druggable Sites in *Aspergillus fumigatus* Essential Phosphatases by Virtual Screening

Benjamin P. Thornton, Anna Johns, Reem Al-Shidhani, Sandra Álvarez-Carretero †, Isabelle S. R. Storer, Michael J. Bromley and Lydia Tabernero * 

School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester M13 9PT, UK

* Correspondence: Lydia.Tabernero@manchester.ac.uk; Tel.: +44-(0)-161-275-7794

† Current address: School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, UK.





Virulence

AFUB_017750

AFUB_036500

AFUB_010510

AFUB_099170

AFUB_059390

AFUB_027890

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AFUB_070630

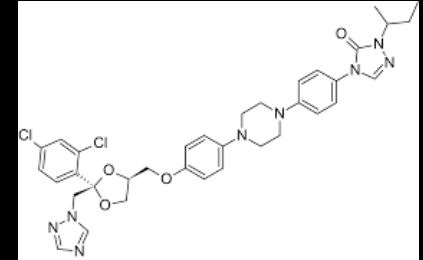
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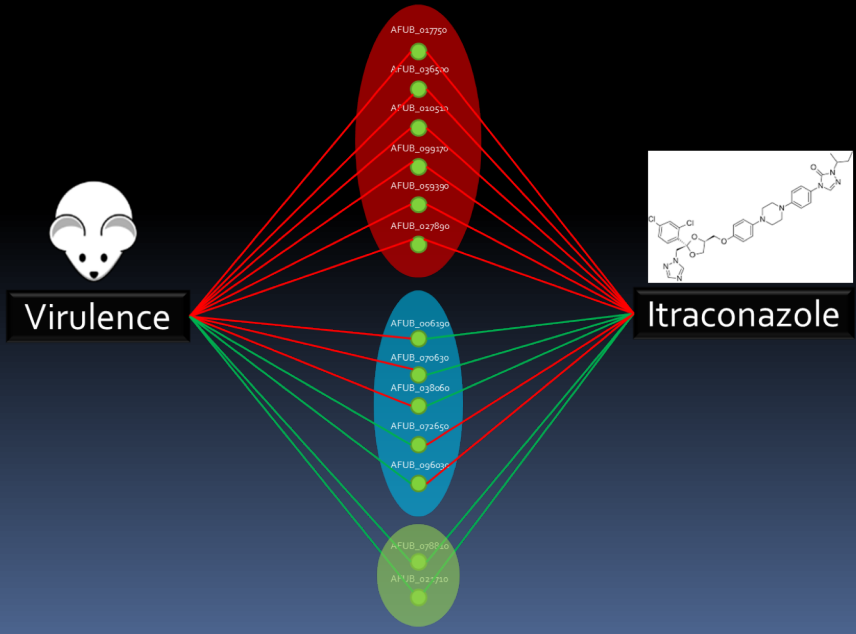
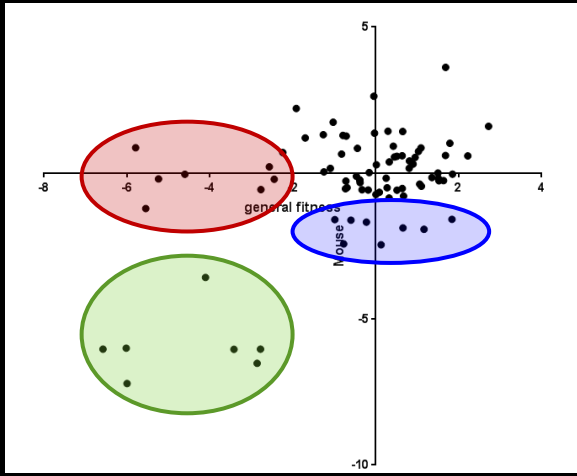
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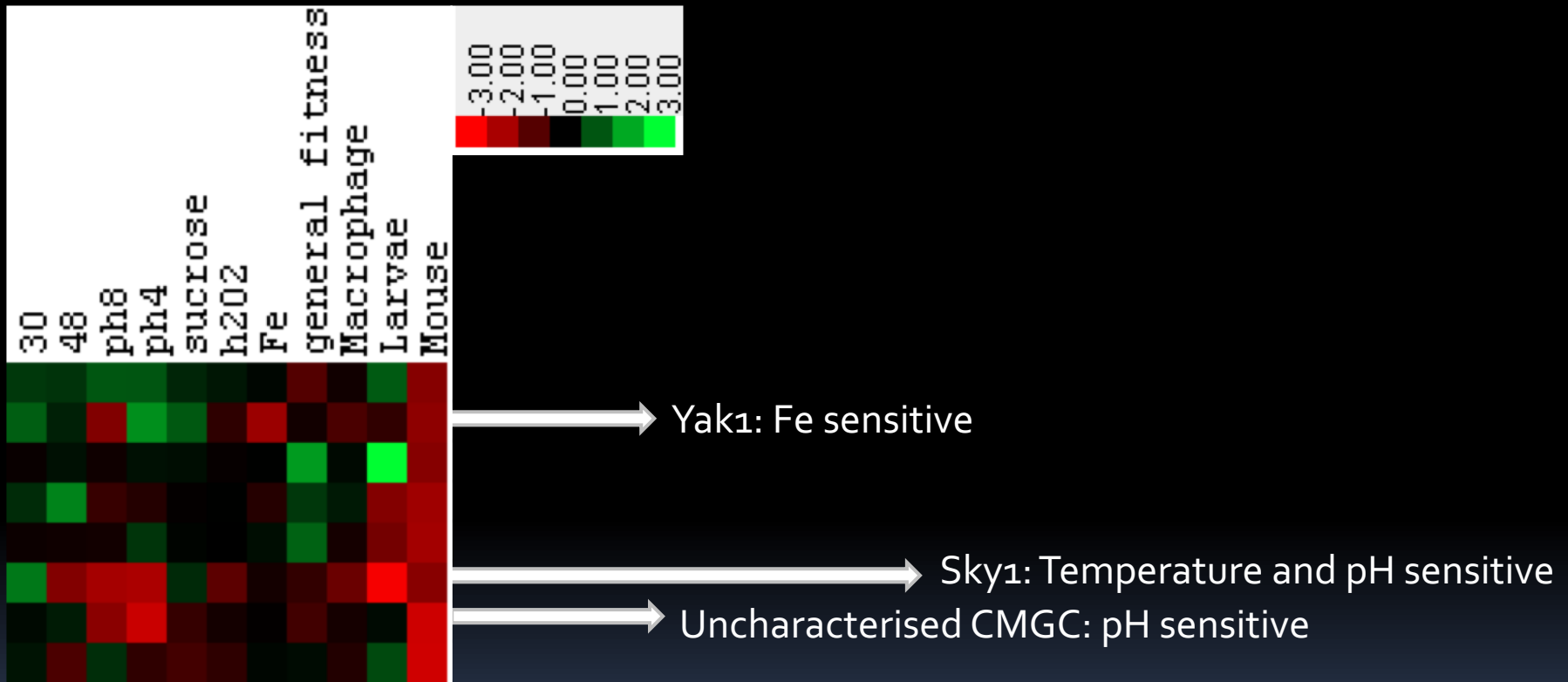
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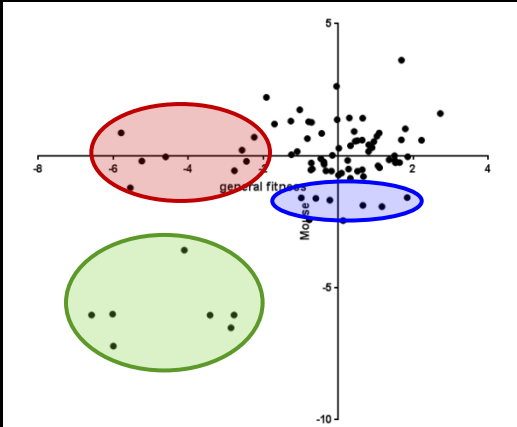


Itraconazole

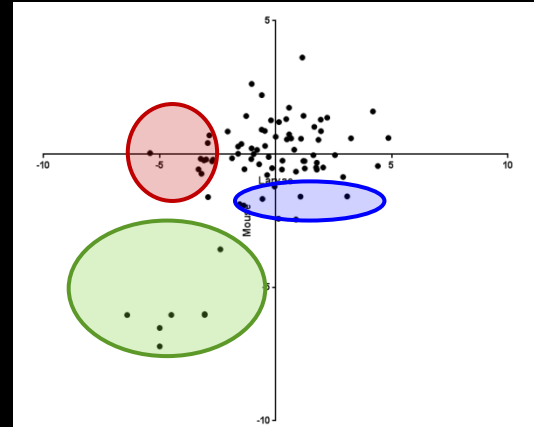


What could be the drivers for in vivo fitness reduction





RPM1640 vs Neutropenic infection



Larval infection vs Neutropenic infection

Generating a library of KO mutants

Genomic locus of gene of interest (GOI)



PCR using overlapping primers



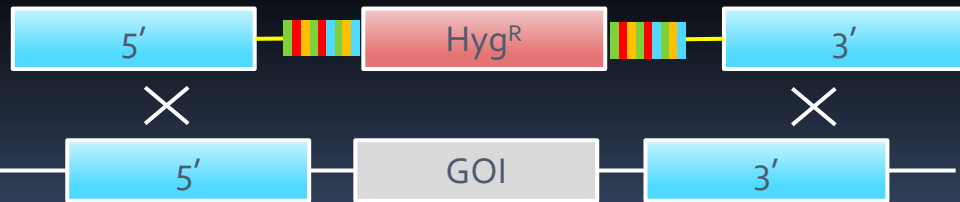
Fusion PCR



Gene replacement cassette



Transformation of $\Delta ku80$ isolate



Recombinant genetic locus

