

# The genomic basis of drug resistance and virulence in *Cryptococcus neoformans*

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 @acgerstein



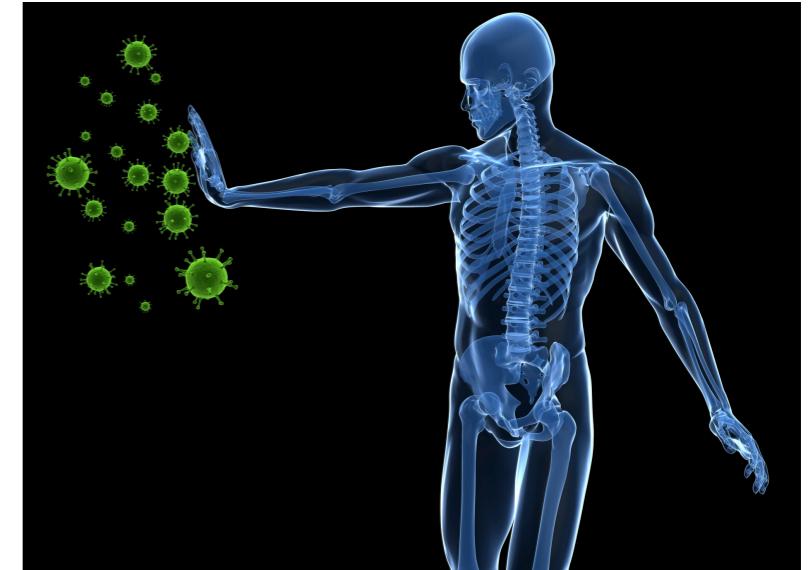
UNIVERSITY  
OF MANITOBA



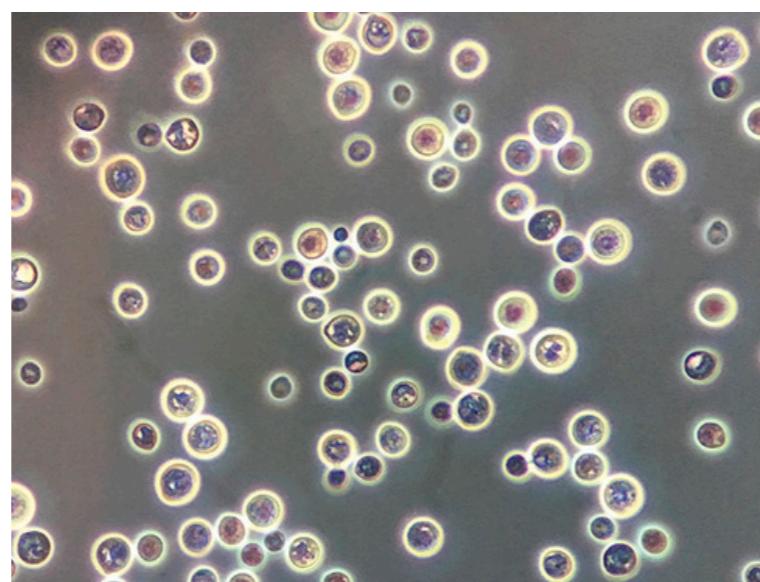
# Response to infection depends on



health center



host immunity



pathogen

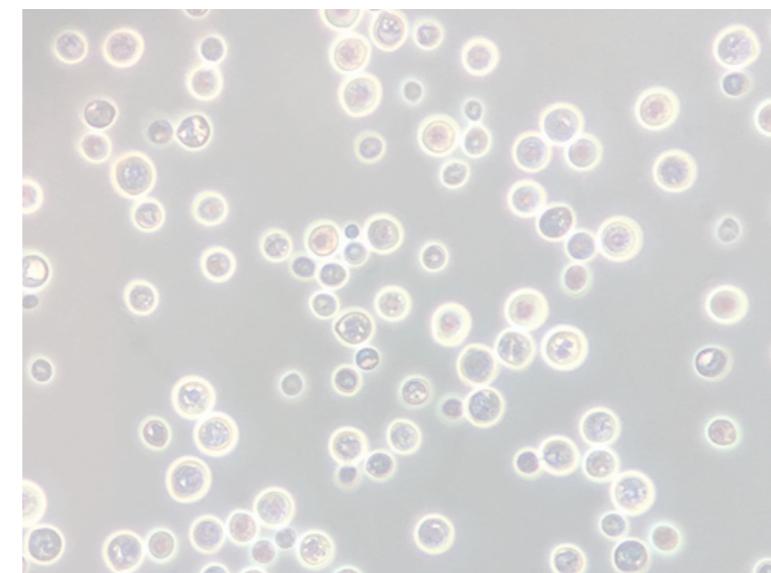
NEJM 2018: 379

# Response to infection depends on



health center

- distance to medical center
- drug availability
- capacity



pathogen

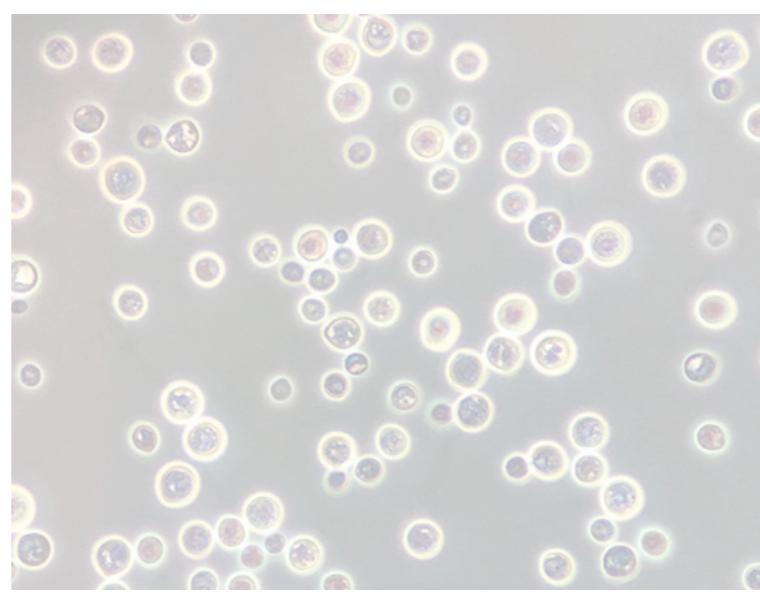


host immunity

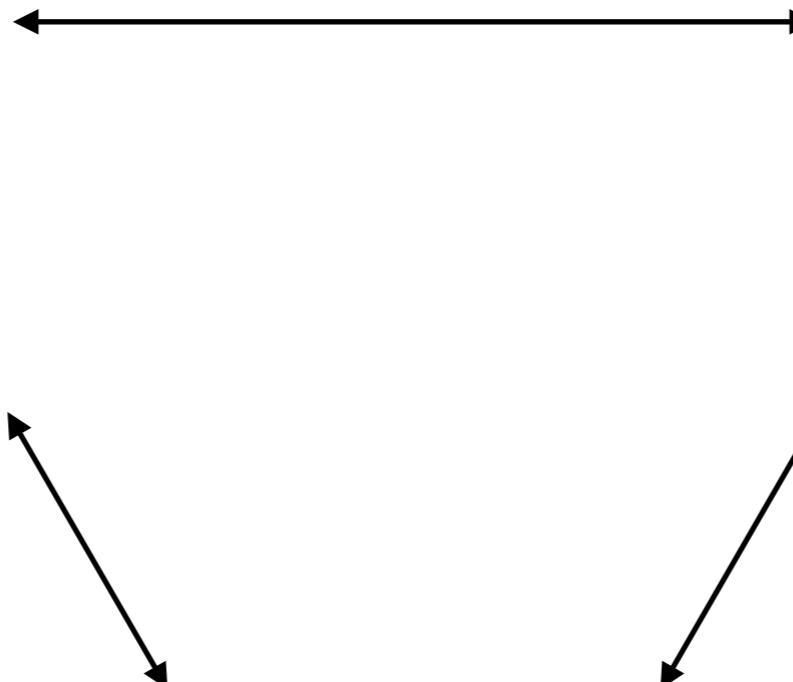
# Response to infection depends on



health center



pathogen



host immunity

- Human genetics
- HIV/AIDS
- transplant
- chemotherapy
- immunomodulatory drugs

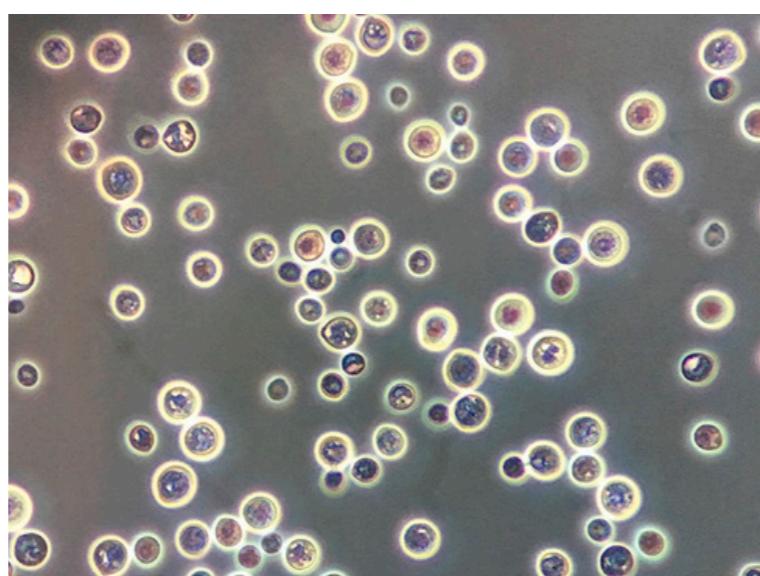
# Response to infection depends on



health center



host immunity



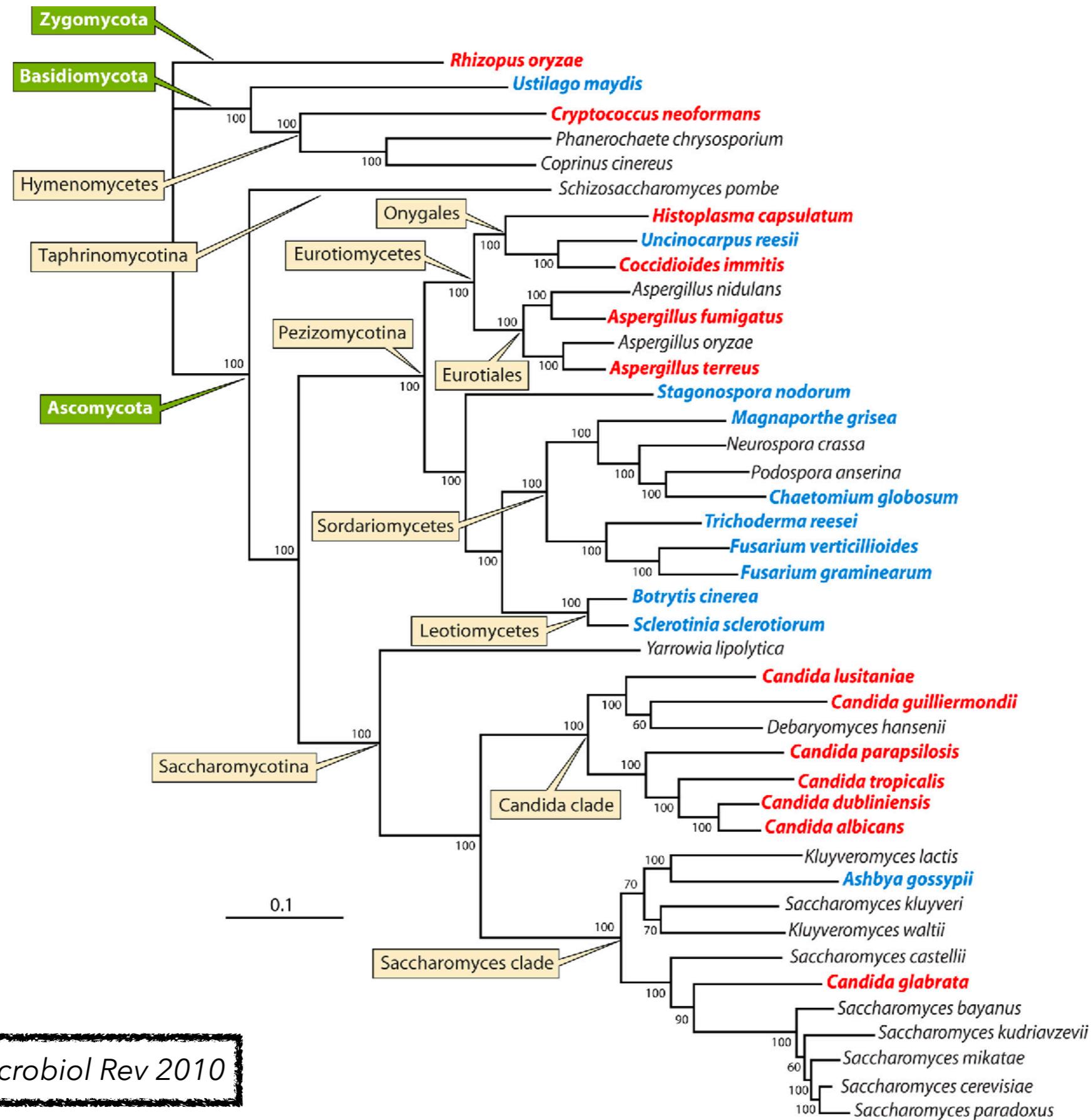
pathogen



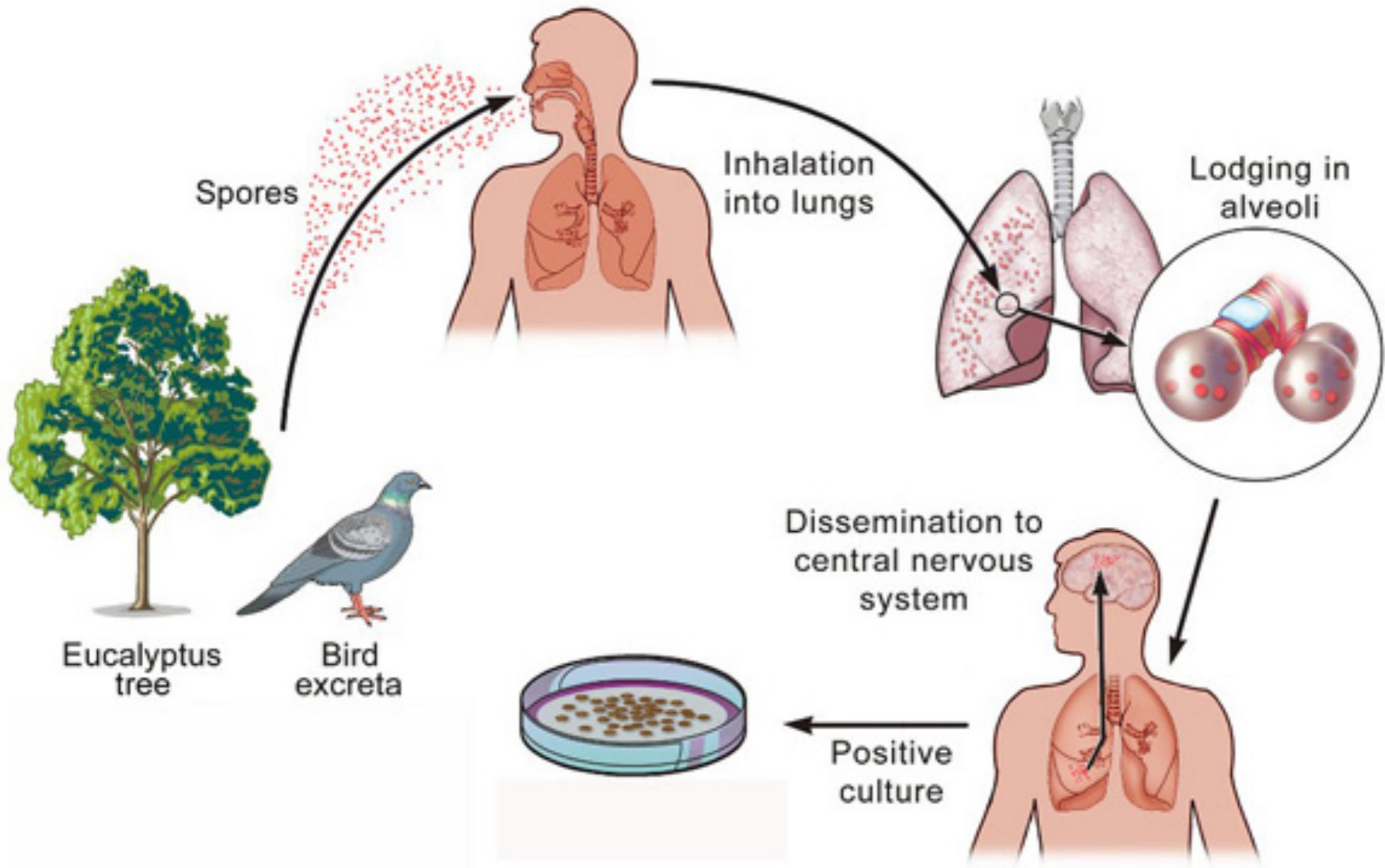
genetic variation:

- drug resistance
- virulence factors
- survival ability

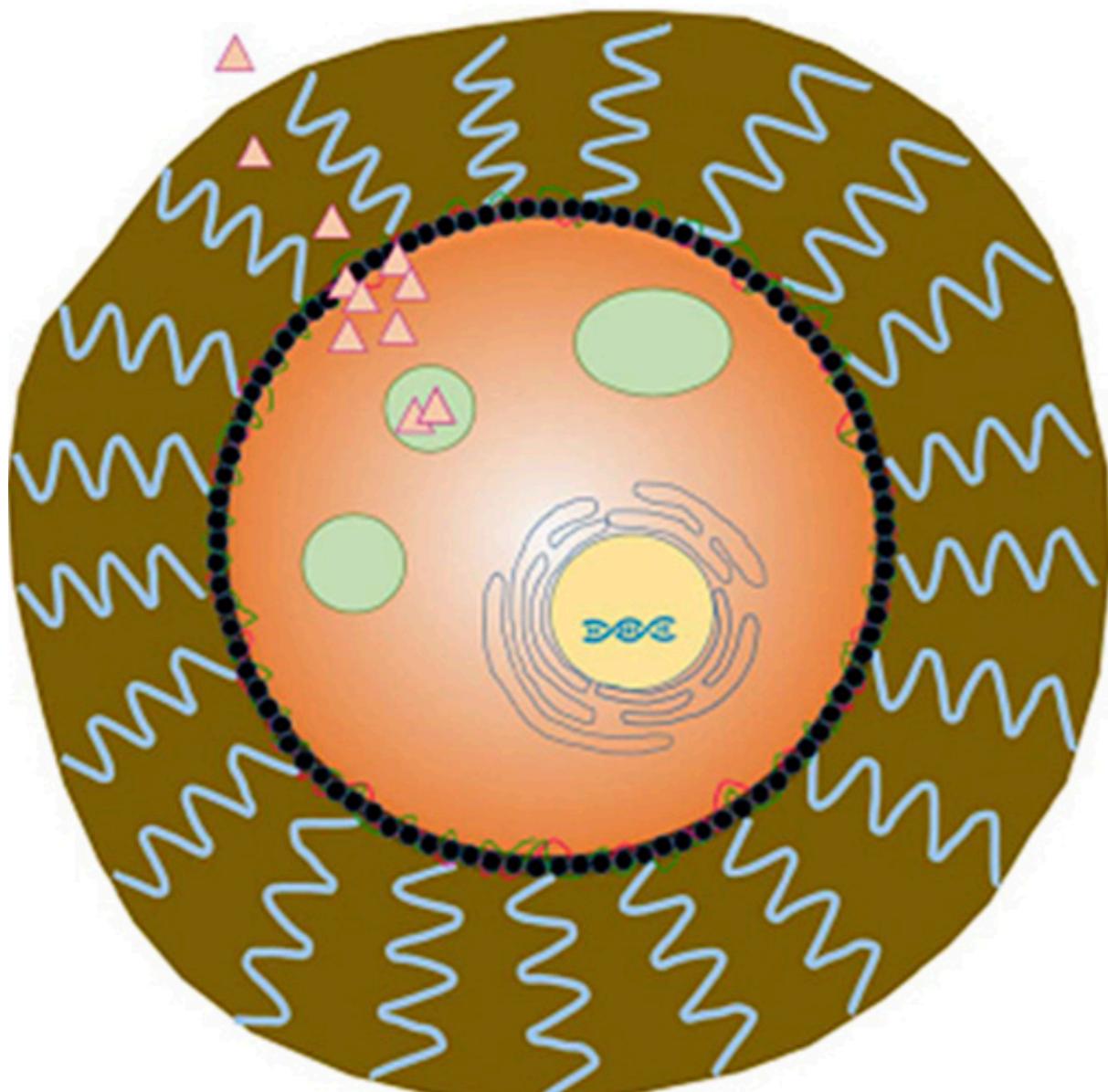
# What make fungal pathogens pathogenic?



# *Cryptococcus neoformans* life cycle



# *Cryptococcus neoformans* virulence factors

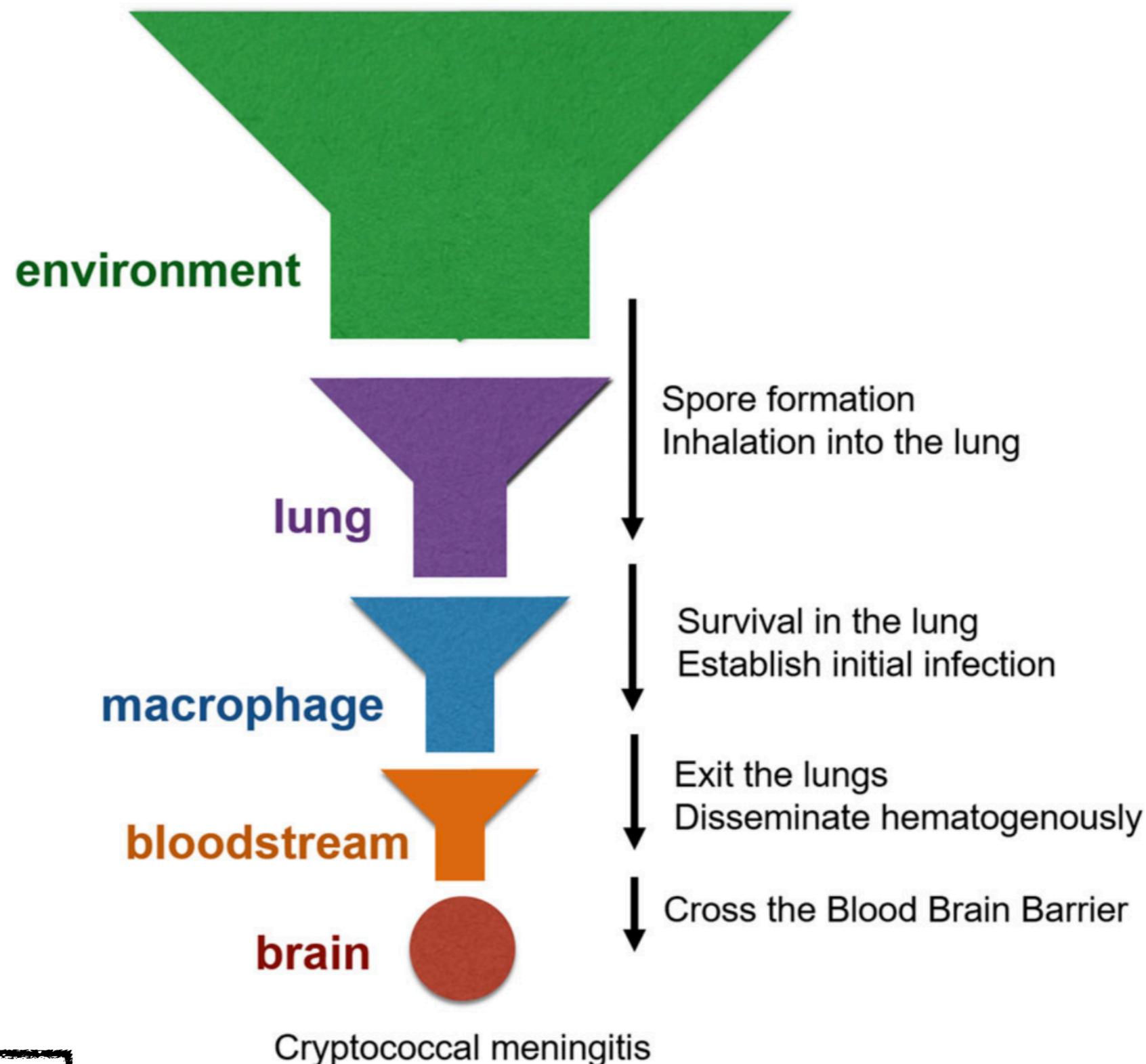


- Capsular polysaccharide
- Melanin pigment
- Urease enzyme in virulence factor delivery bag

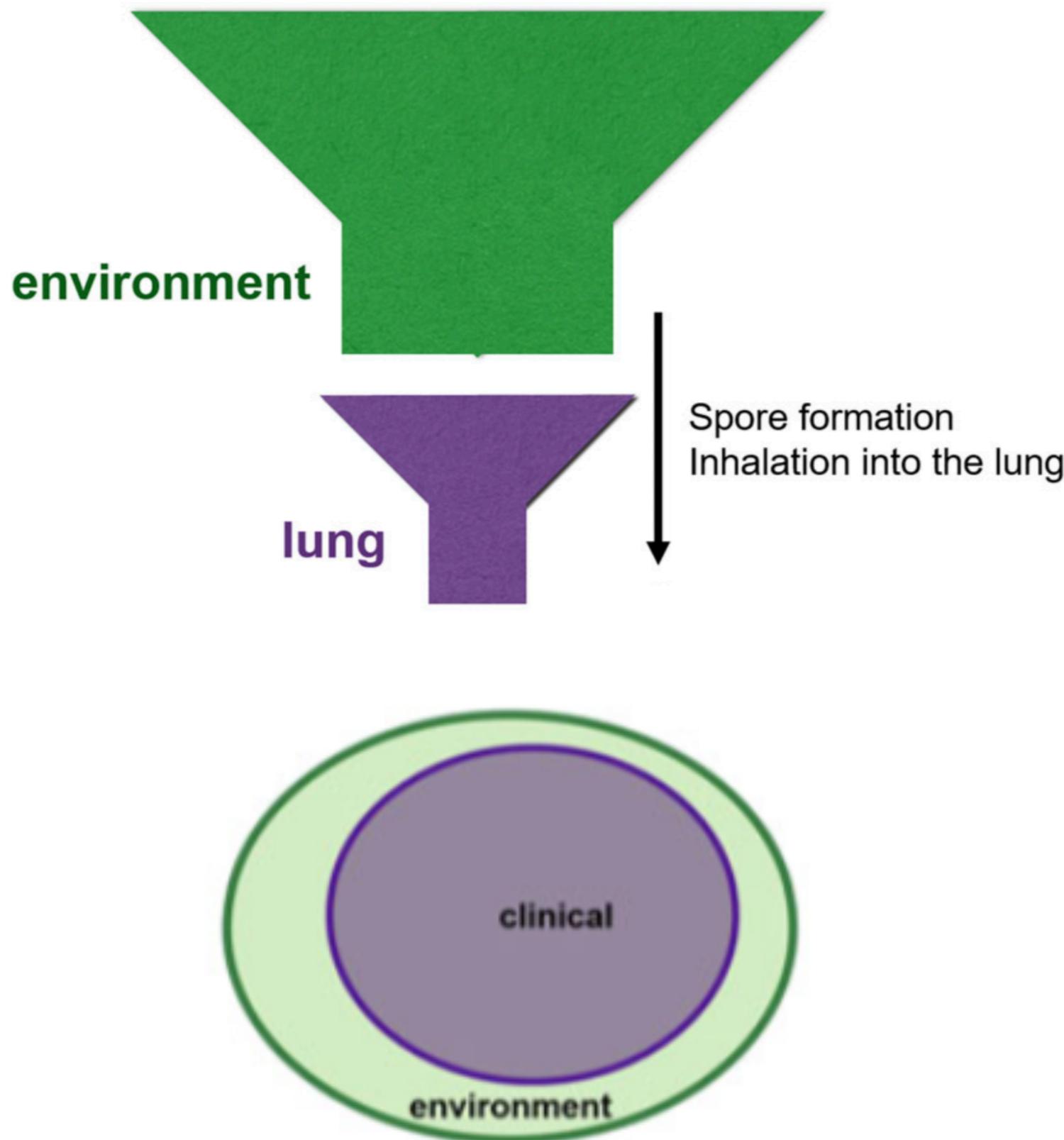


Survival  
Defense  
Regulation of host  
immune response

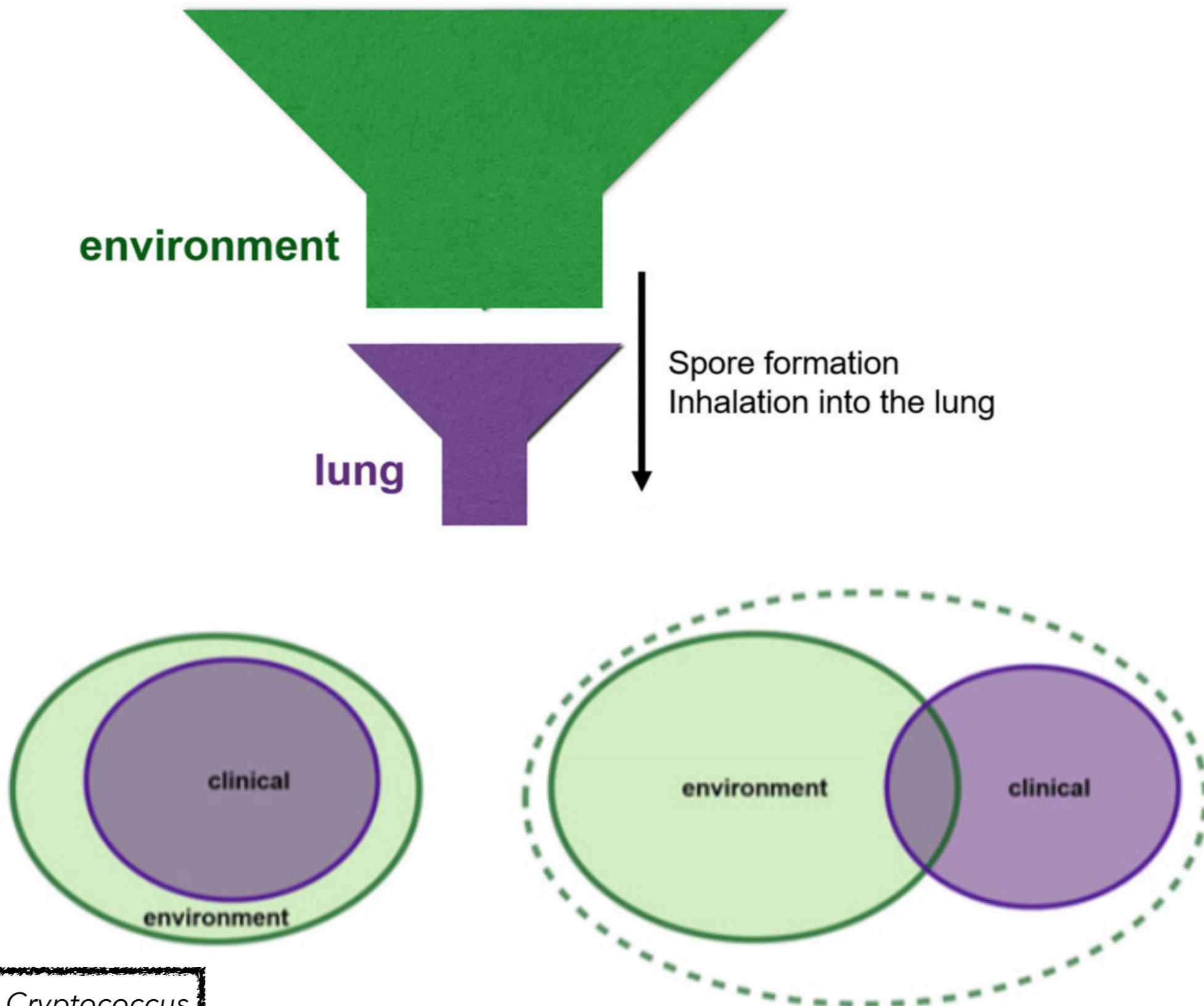
# Environmental pathogen infection sieve



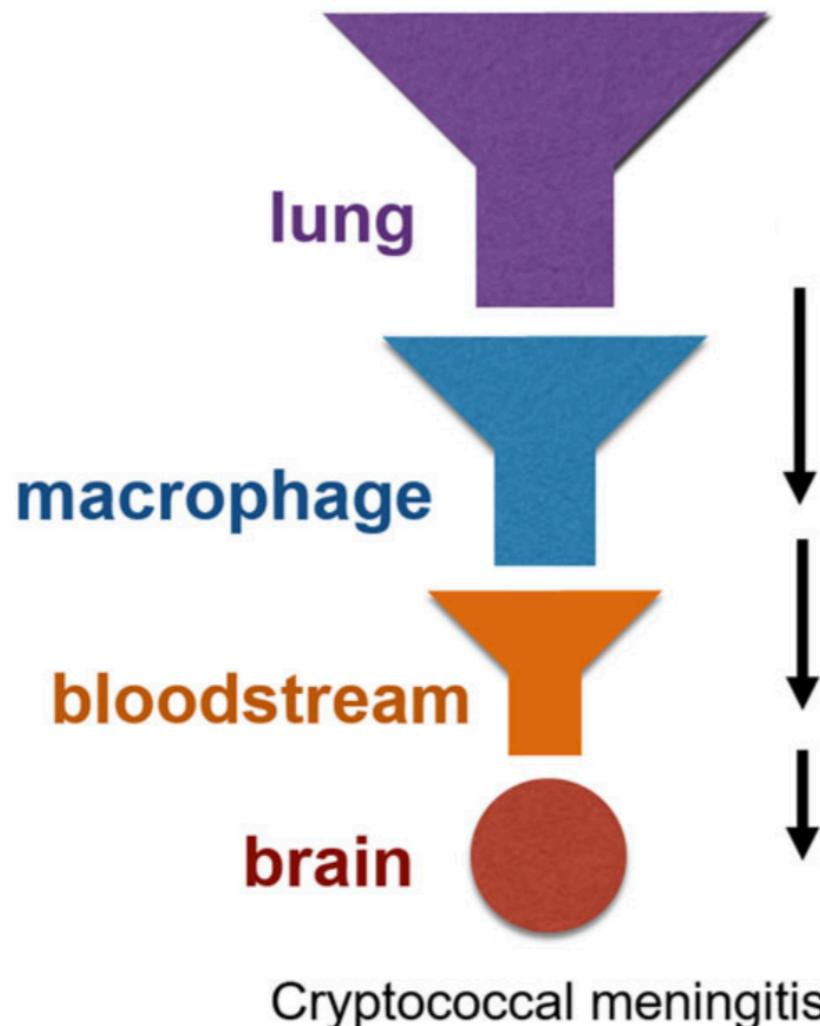
# Compare environmental to clinical isolates



# Whither the environmental diversity?



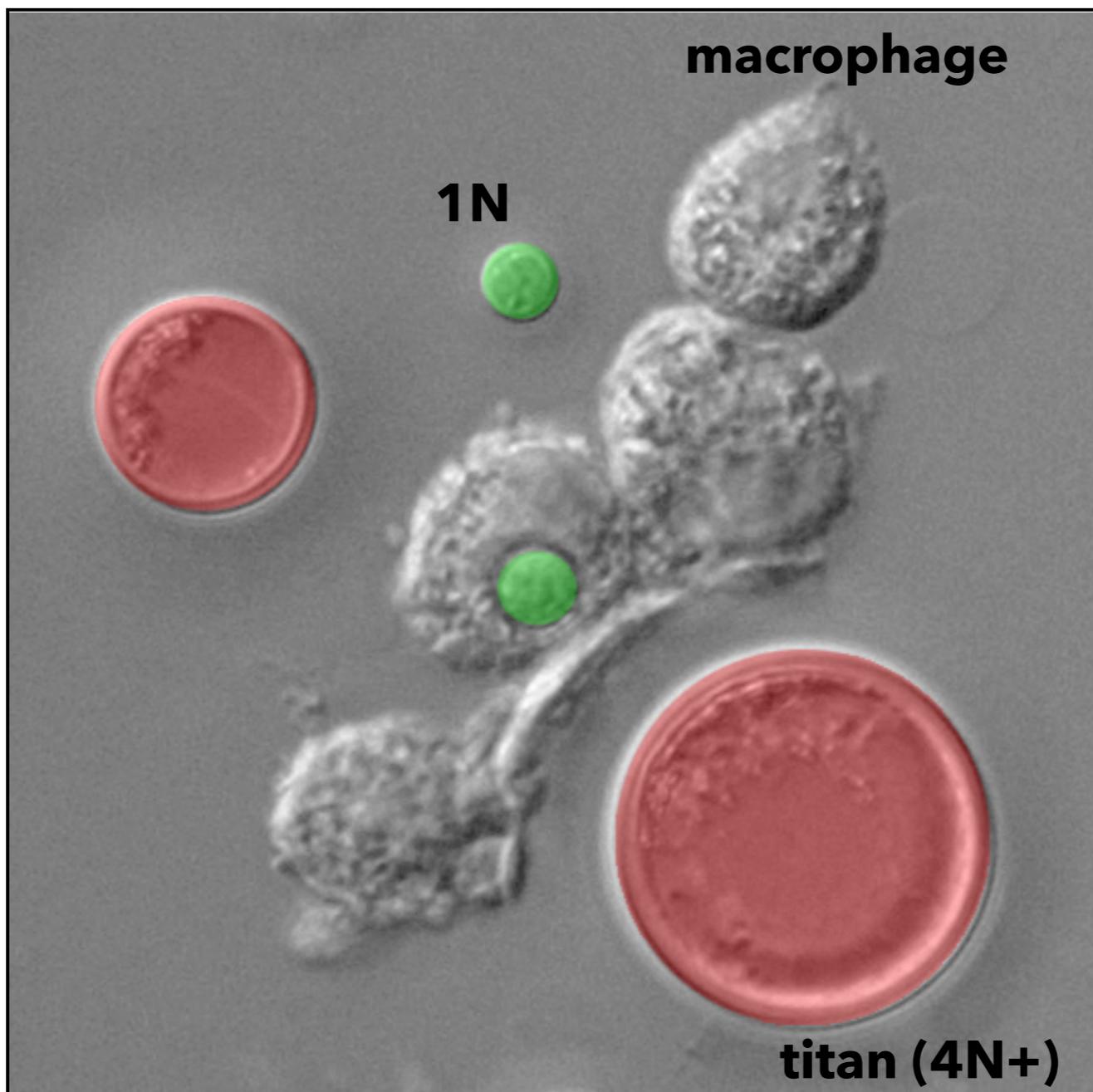
# Pathogen genotype influence on disease



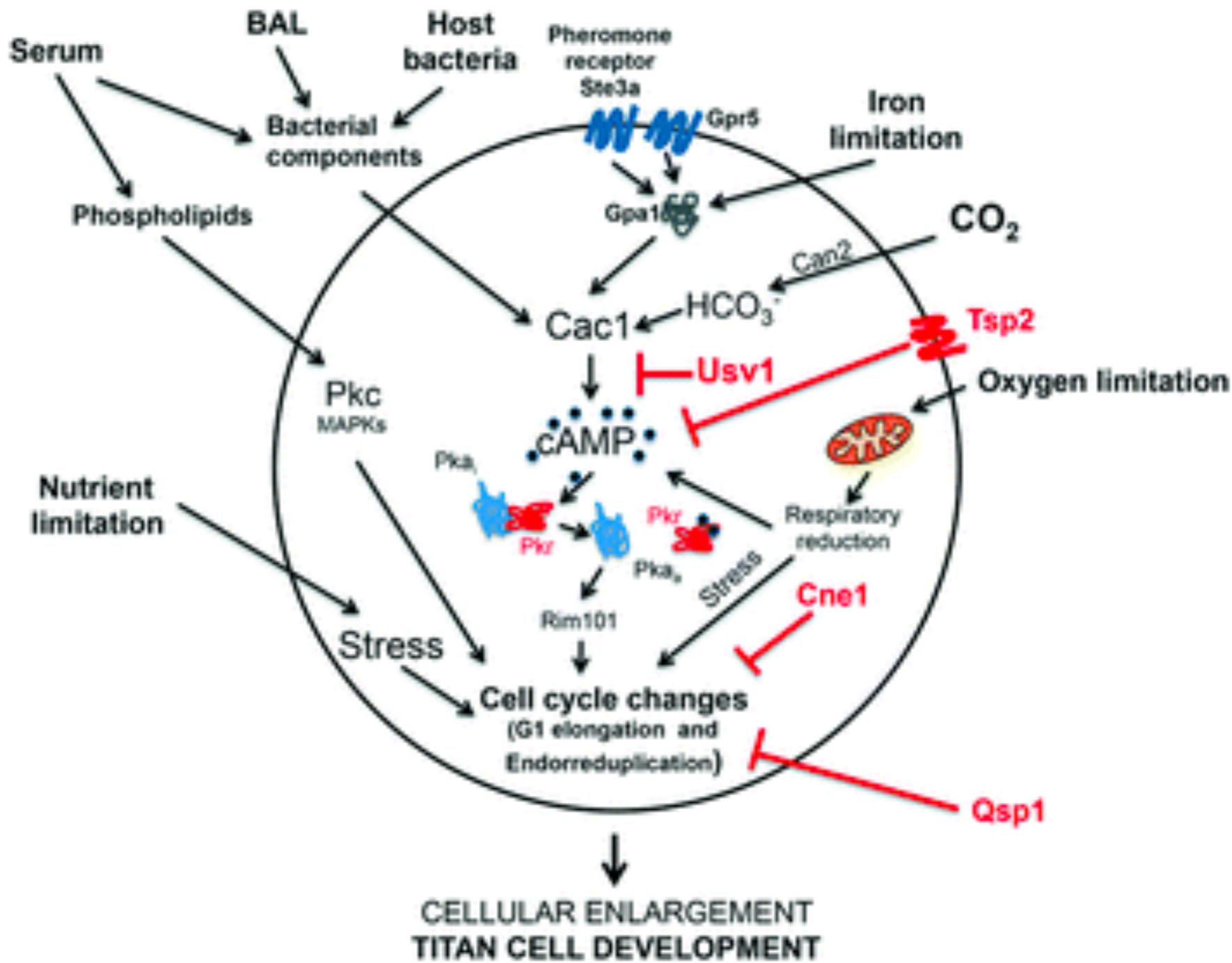
**Tactic 1:** *in vitro* experiments

**Tactic 2:** GWAS analysis for  
association between  
pathogen genotype and  
human infection phenotype

# *Cryptococcus neoformans* ploidy diversity in the lung environment

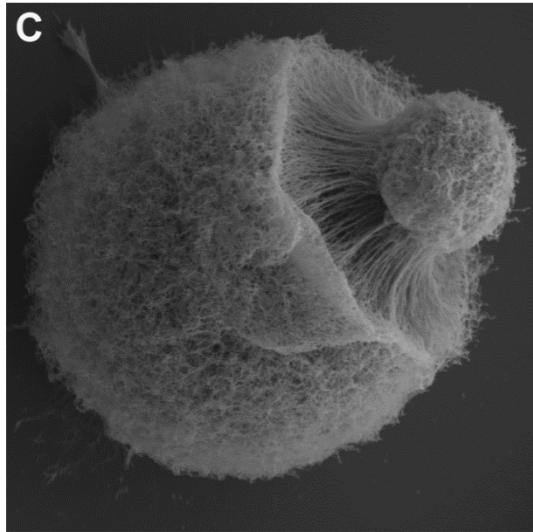


# Signals and pathways involved in titan cell formation

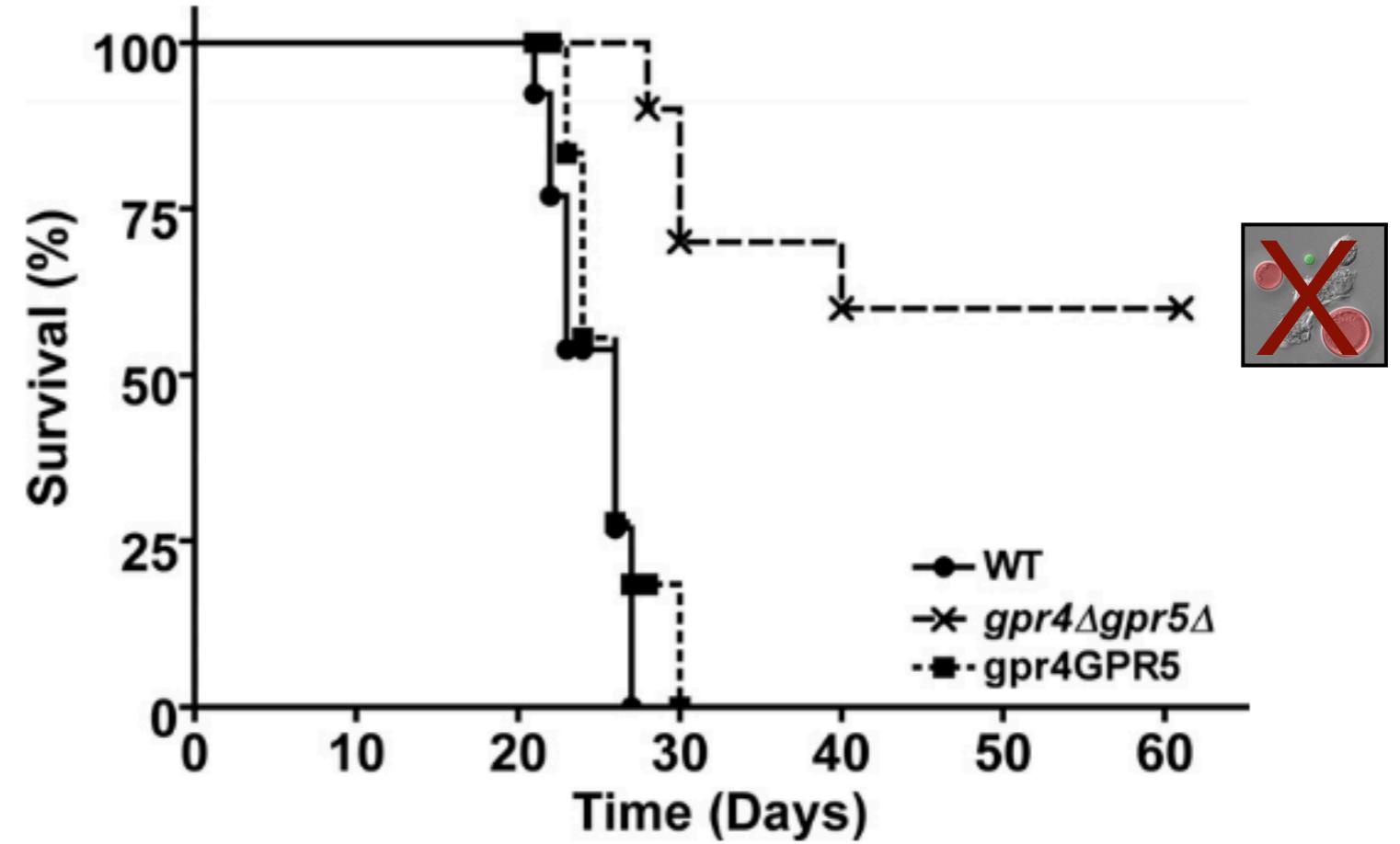
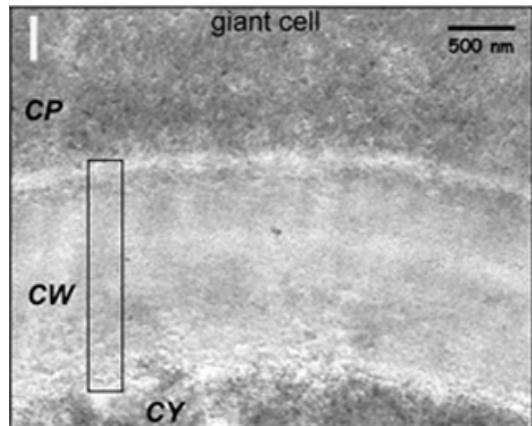


# Titan cells influence virulence

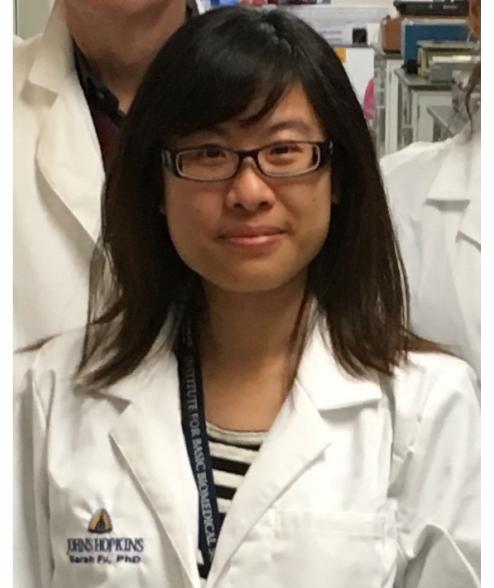
Highly cross-linked capsule



Thickened cell wall

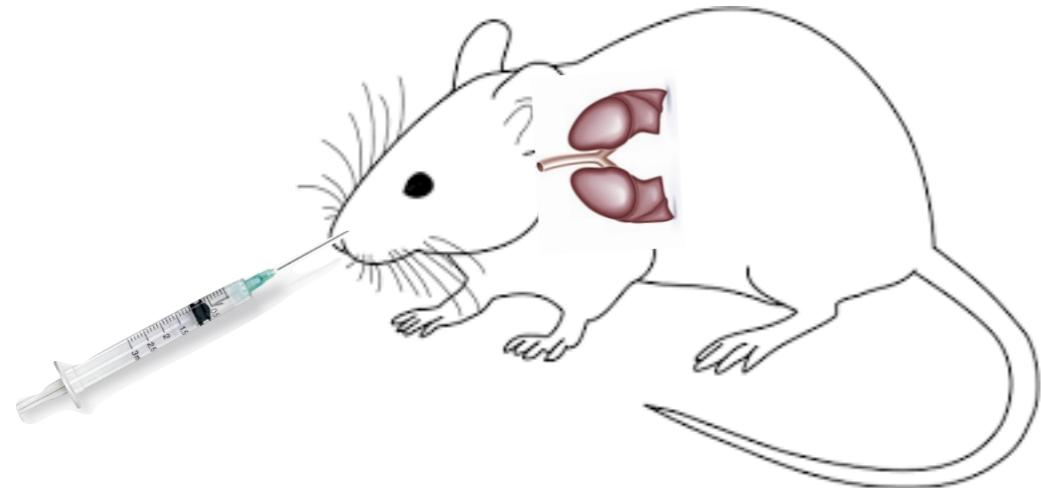


# How do titan cell populations respond to stress?



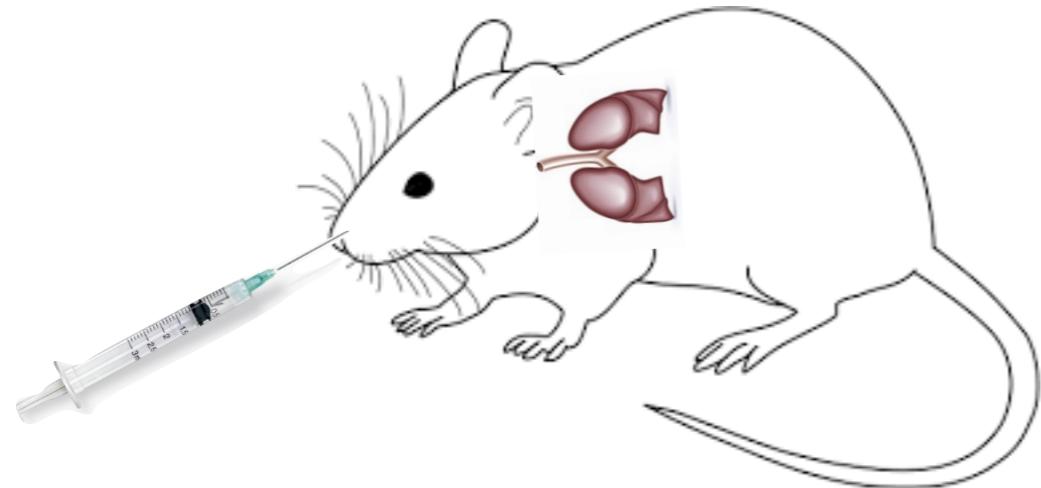
# Isolation of titan cells

Intranasal injection of typical cells

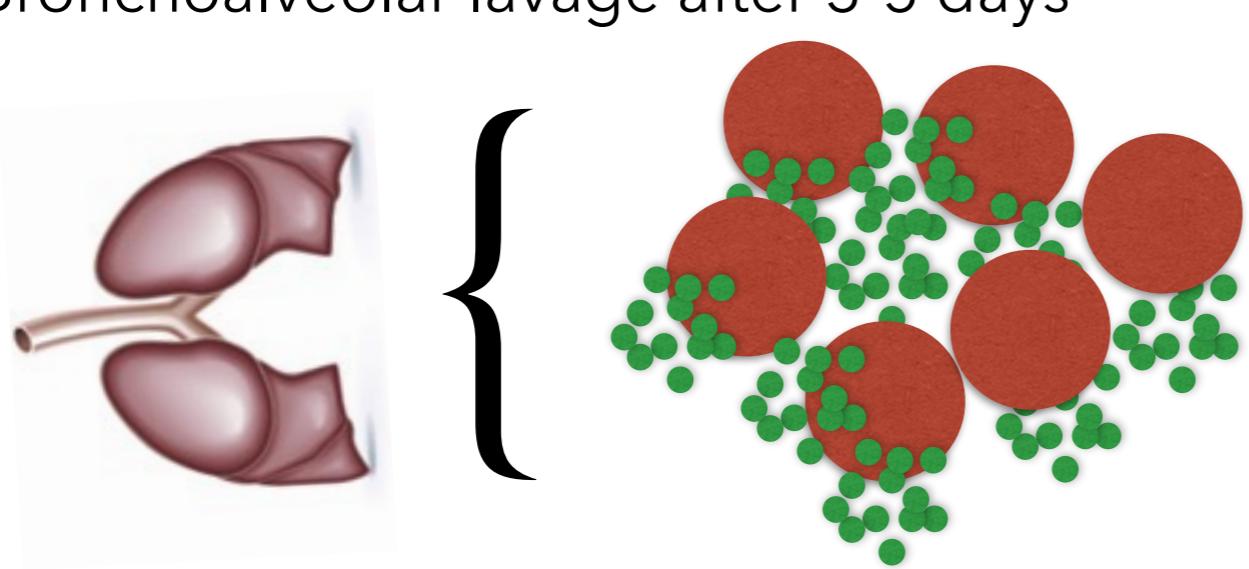


# Isolation of titan cells

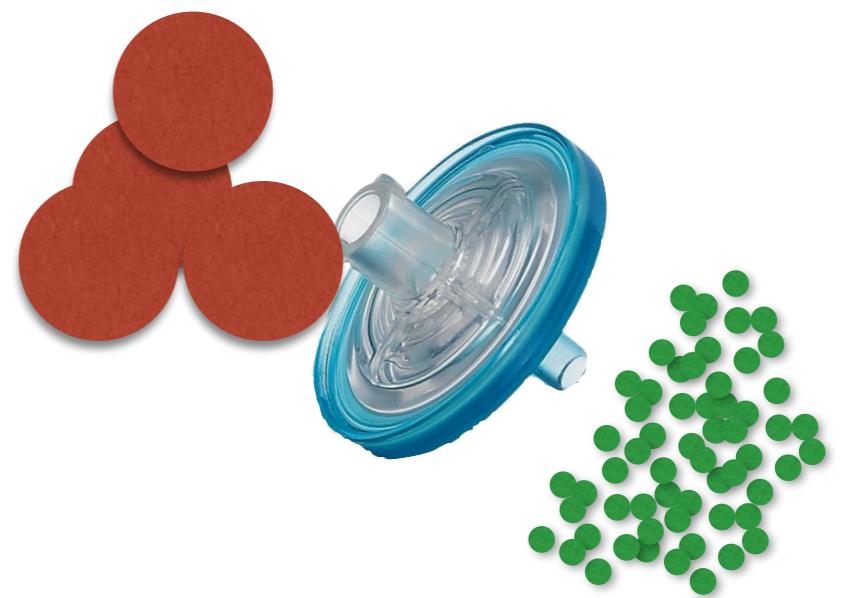
Intranasal injection of typical cells



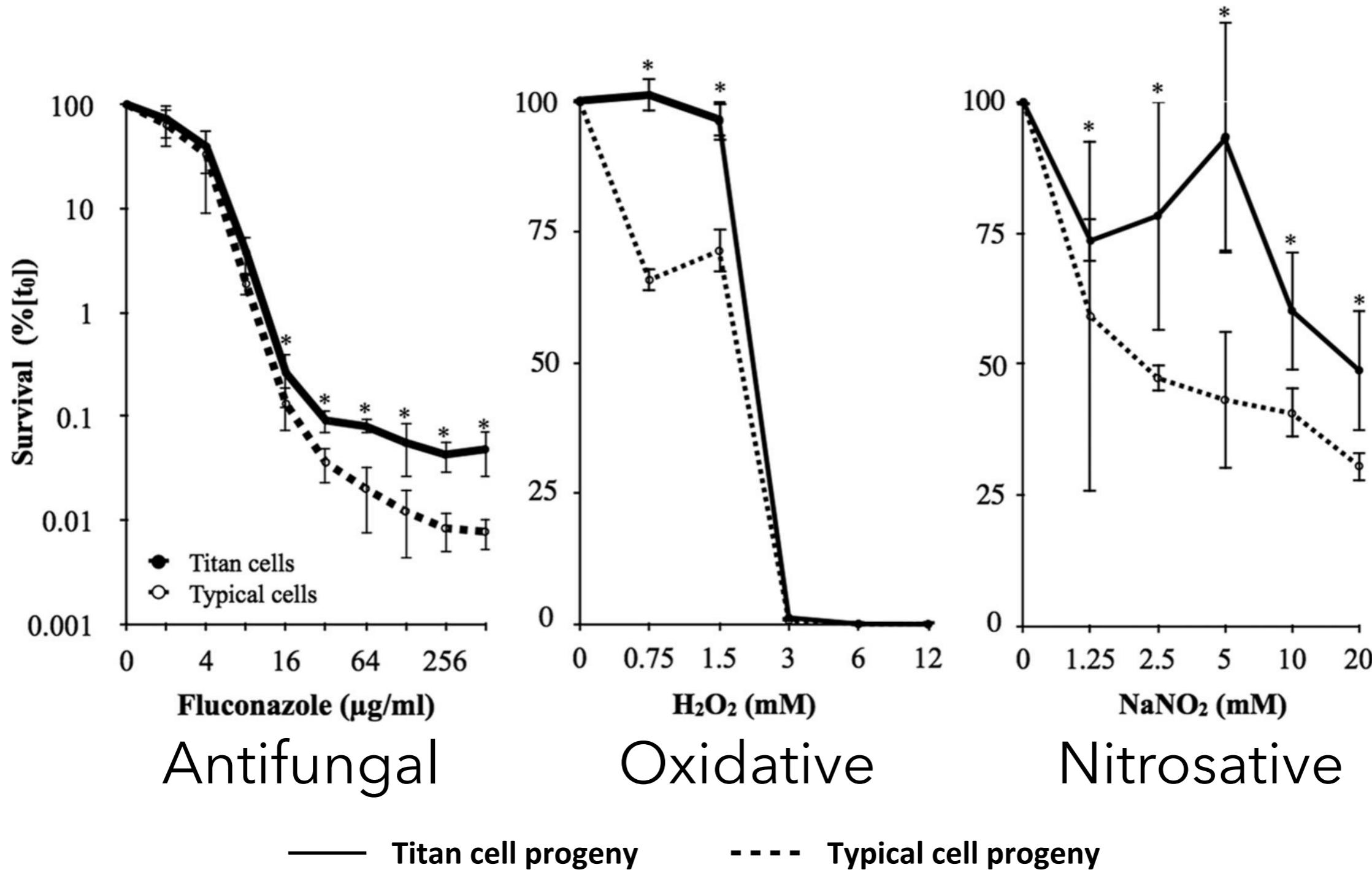
Bronchoalveolar lavage after 3-5 days



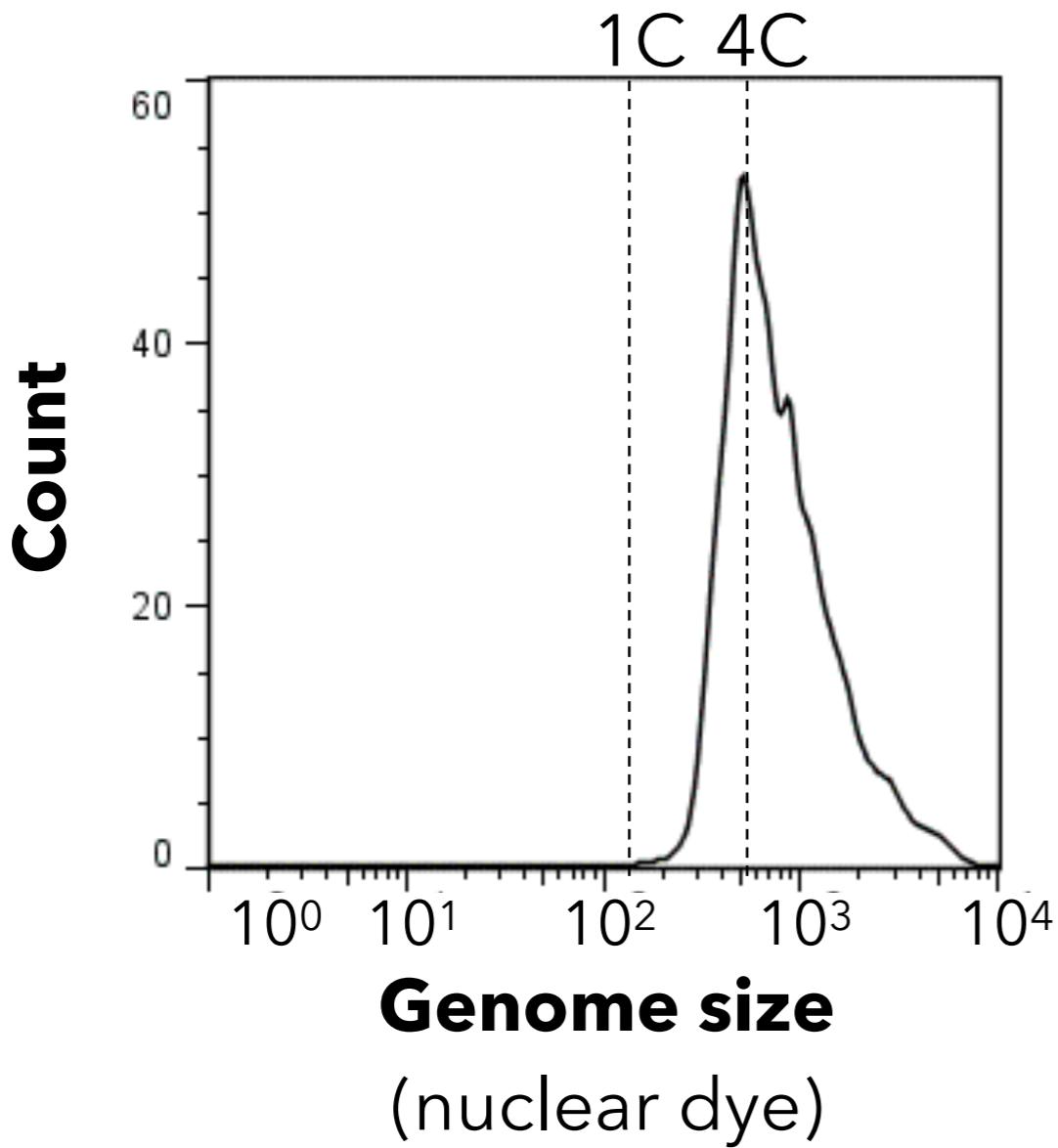
filter



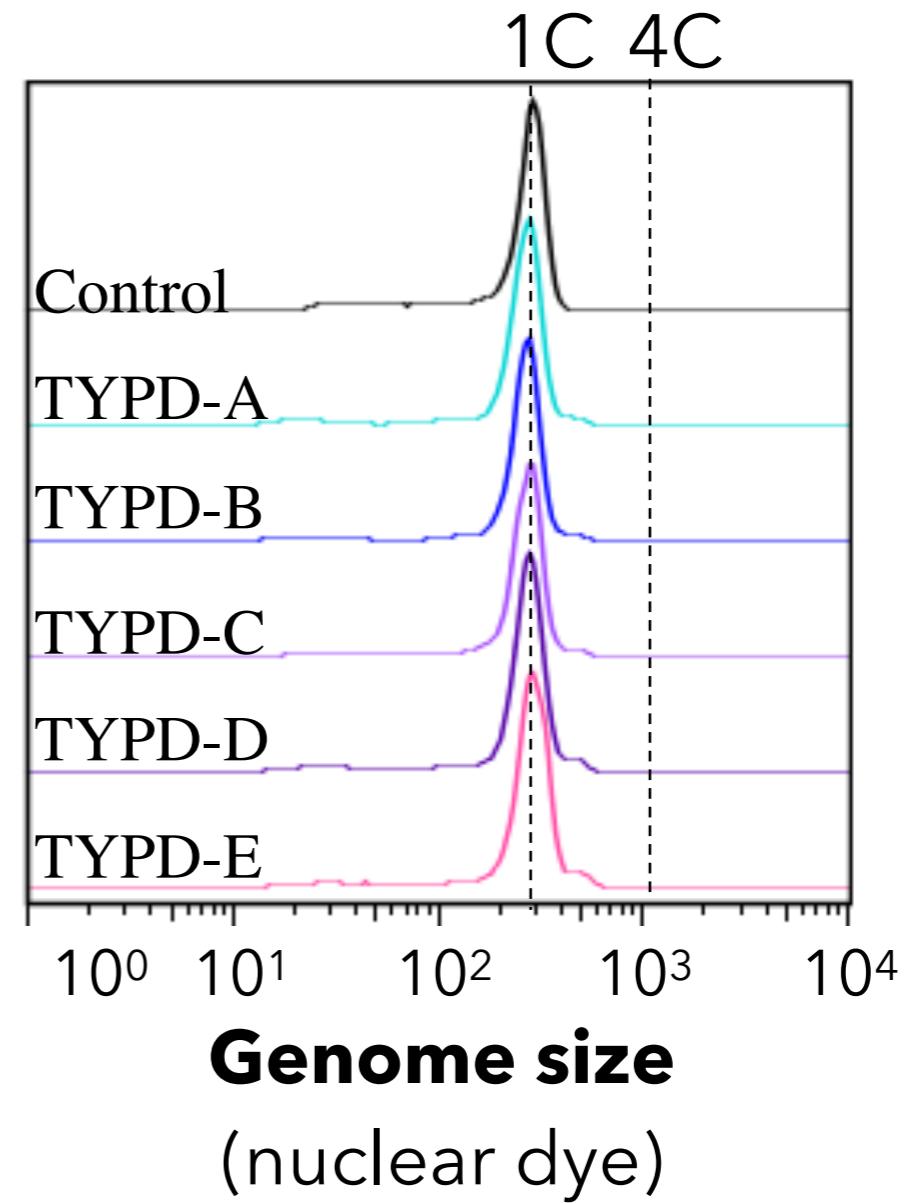
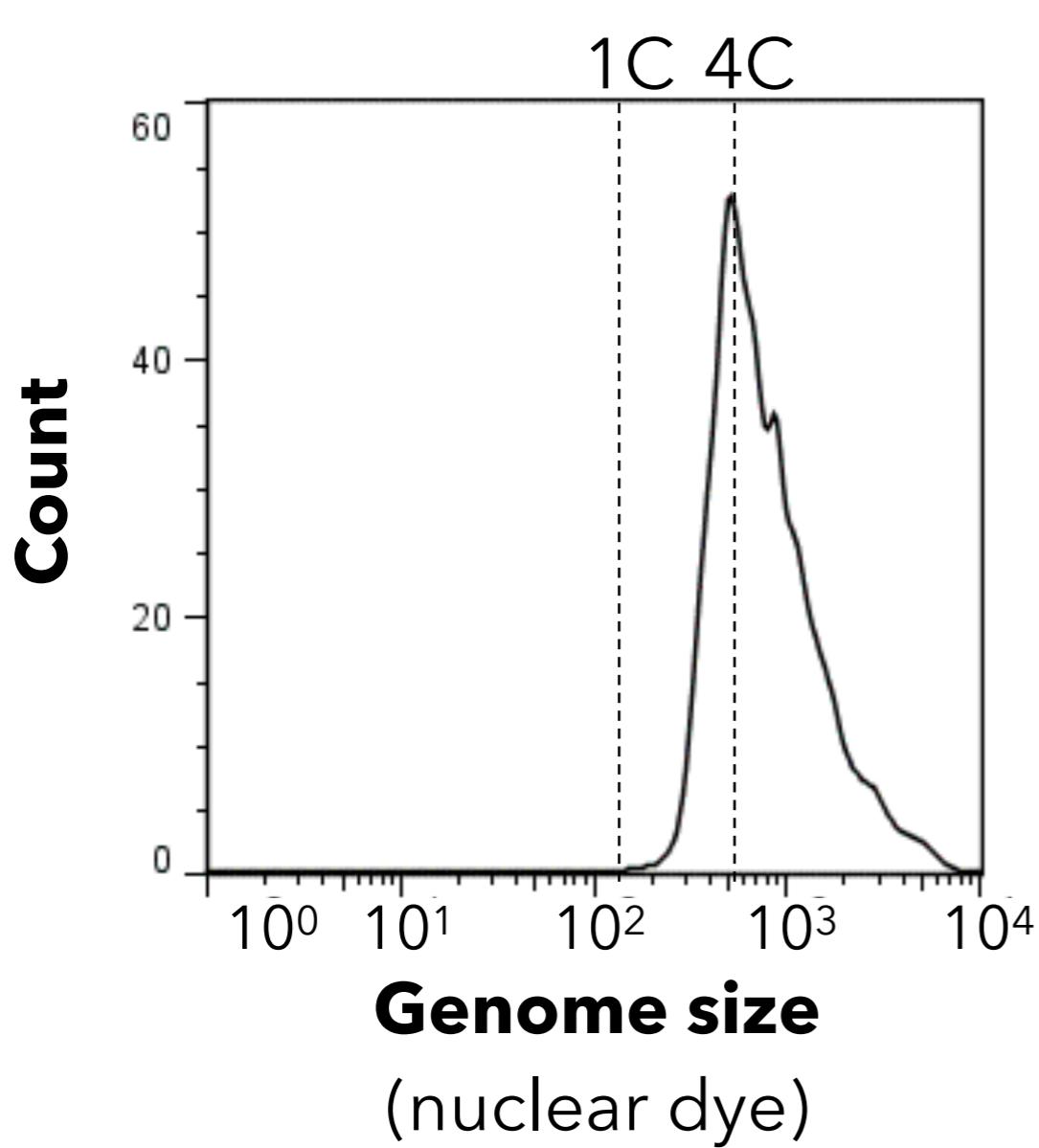
# Populations initiated with titan cells have a survival advantage in stress



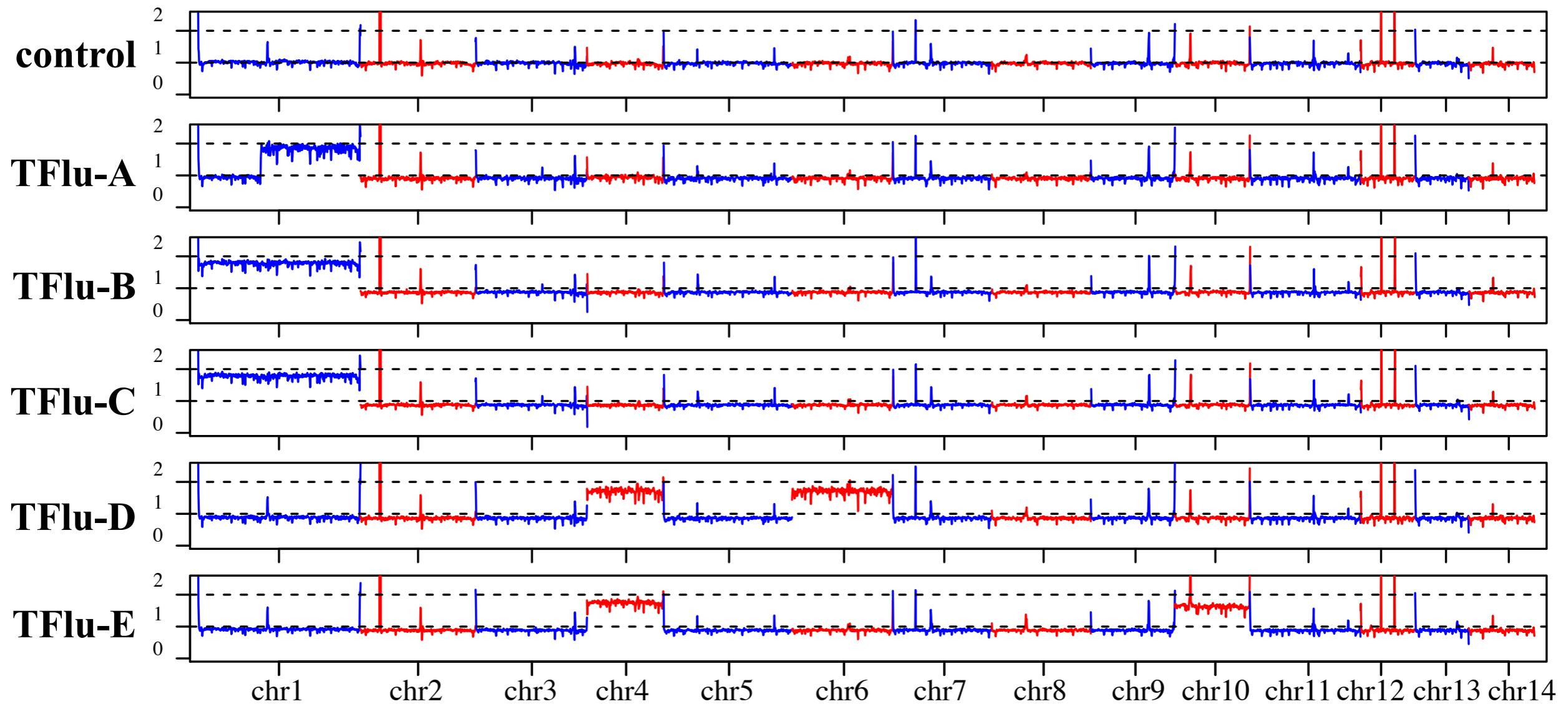
# Flow cytometry to measure genome size



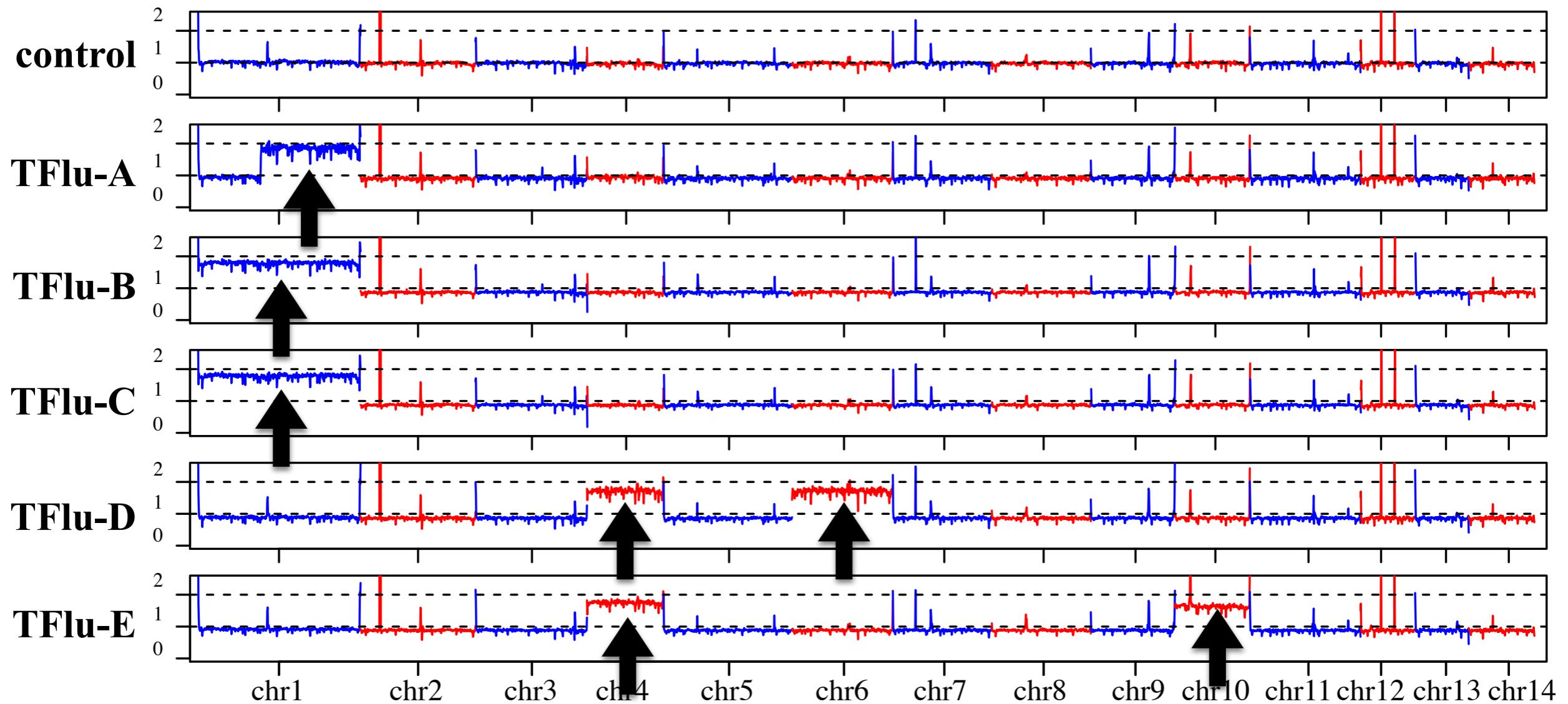
# Titan cell progeny grown in YPD become haploid



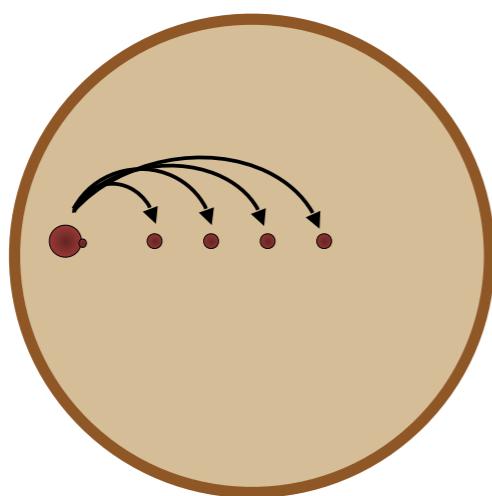
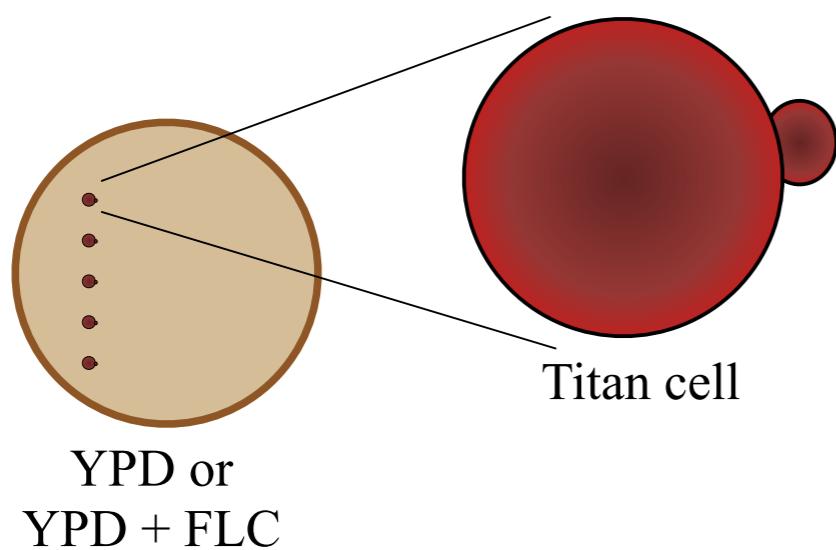
# Titan cell progeny grown in fluconazole become aneuploid



# Titan cell progeny grown in fluconazole become aneuploid

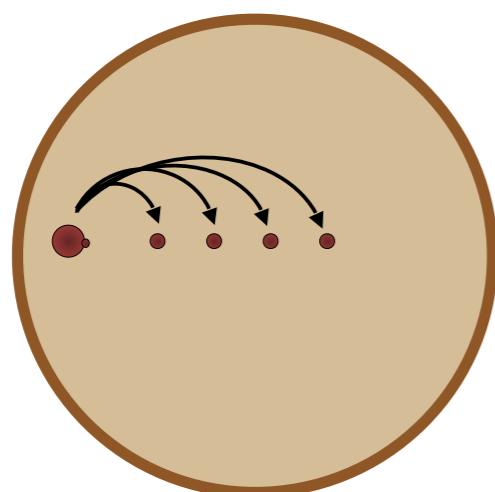
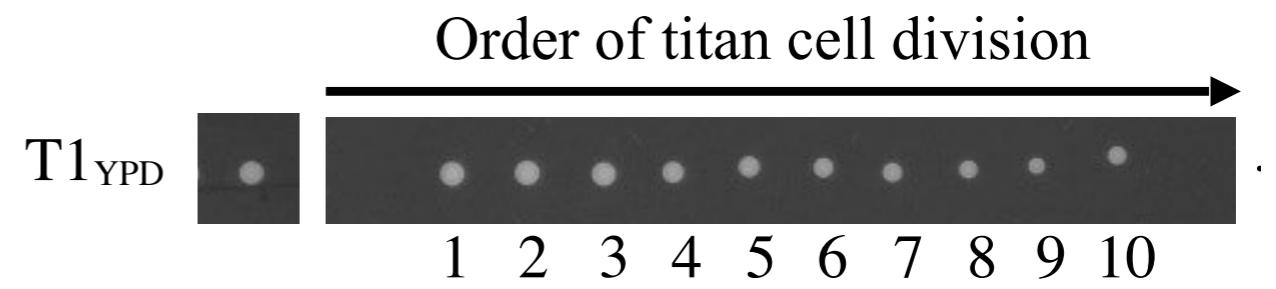
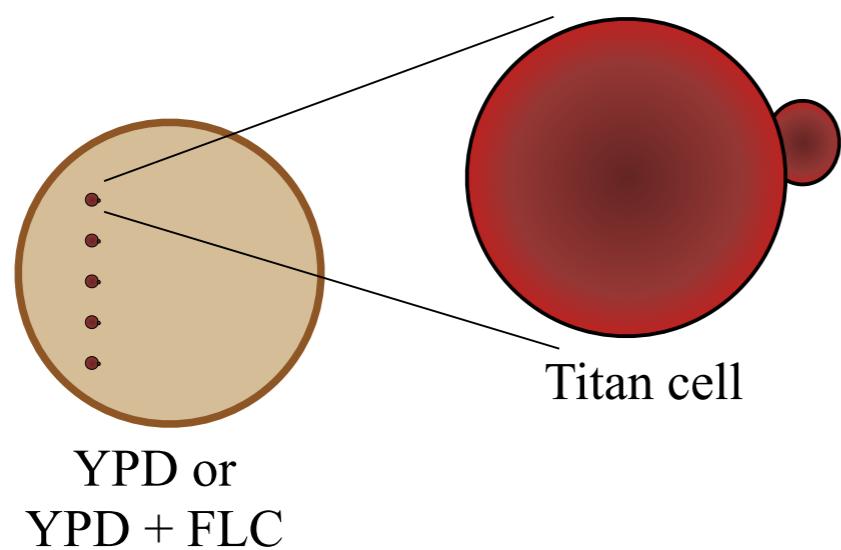


# Followed sequential titan cell progeny



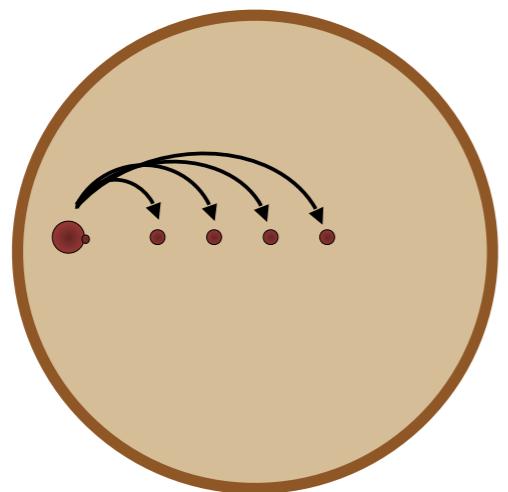
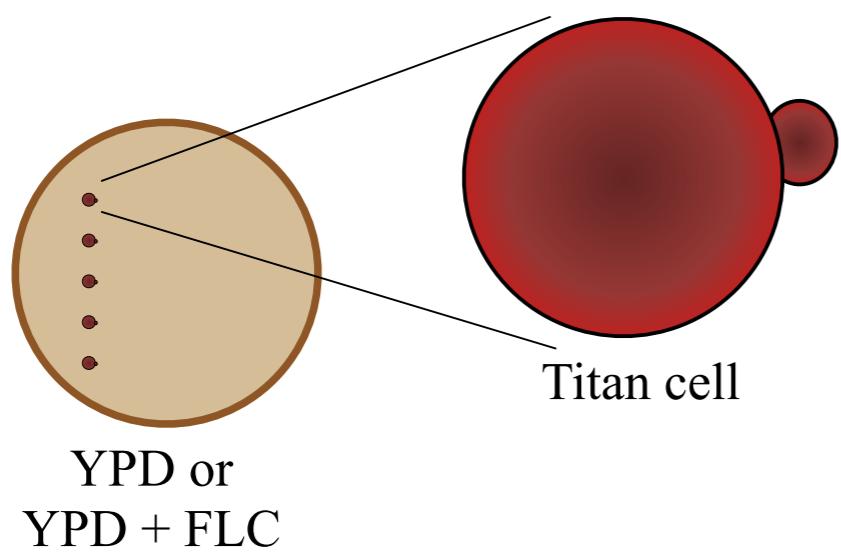
Microdissect  
sequential daughter cells

# Followed sequential titan cell progeny

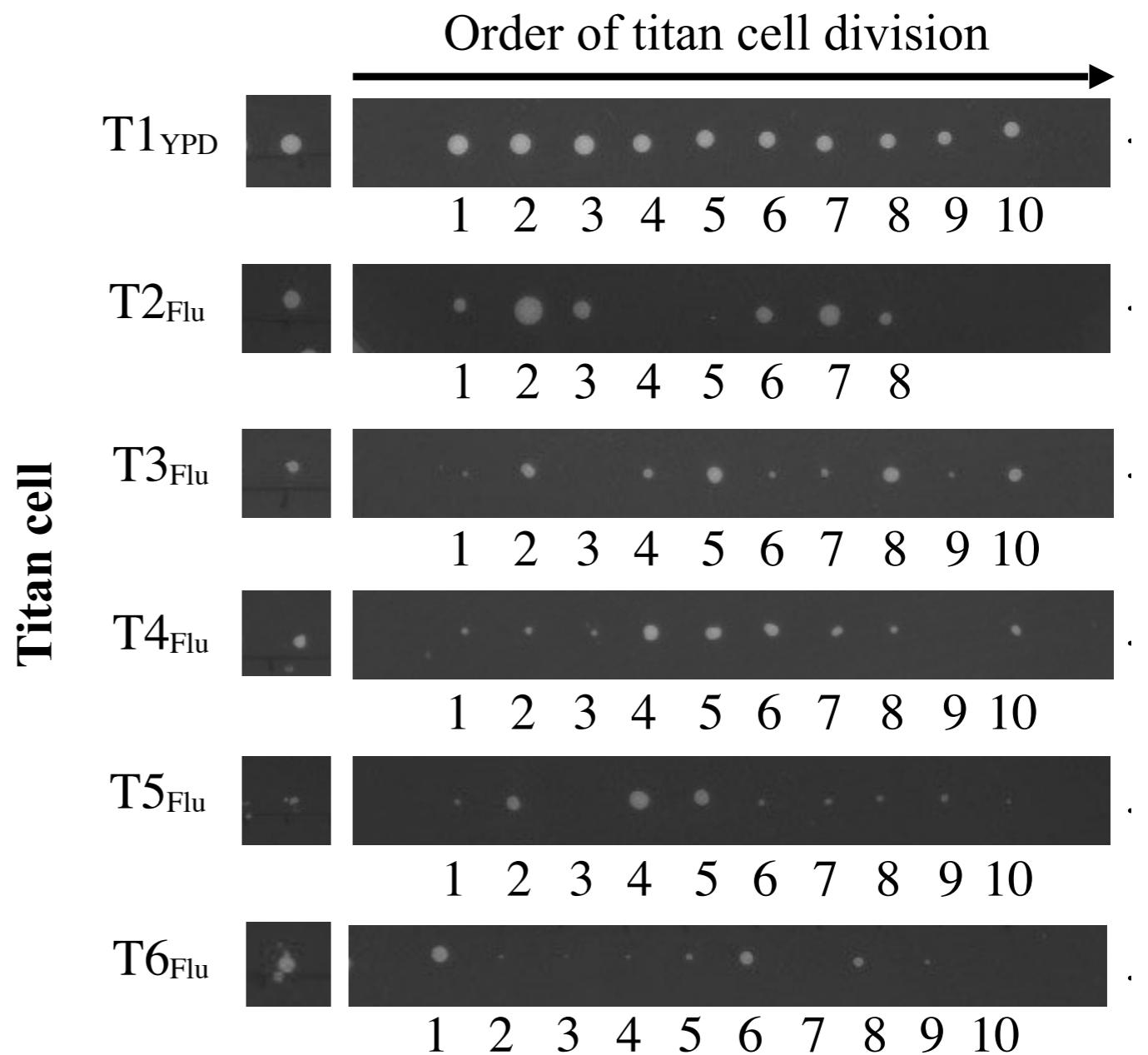


Microdissect  
sequential daughter cells

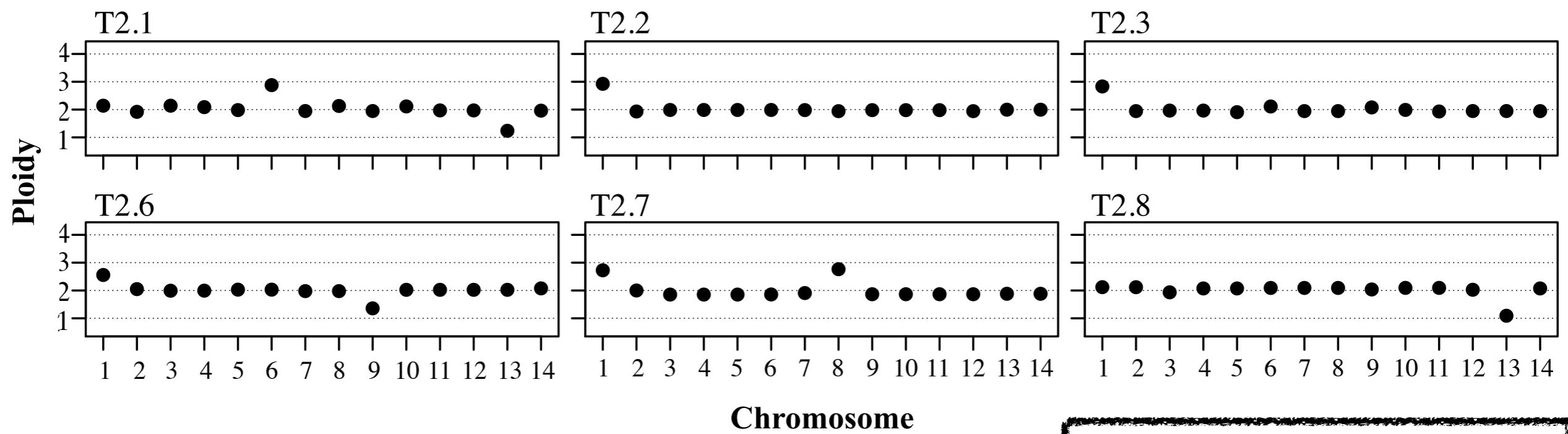
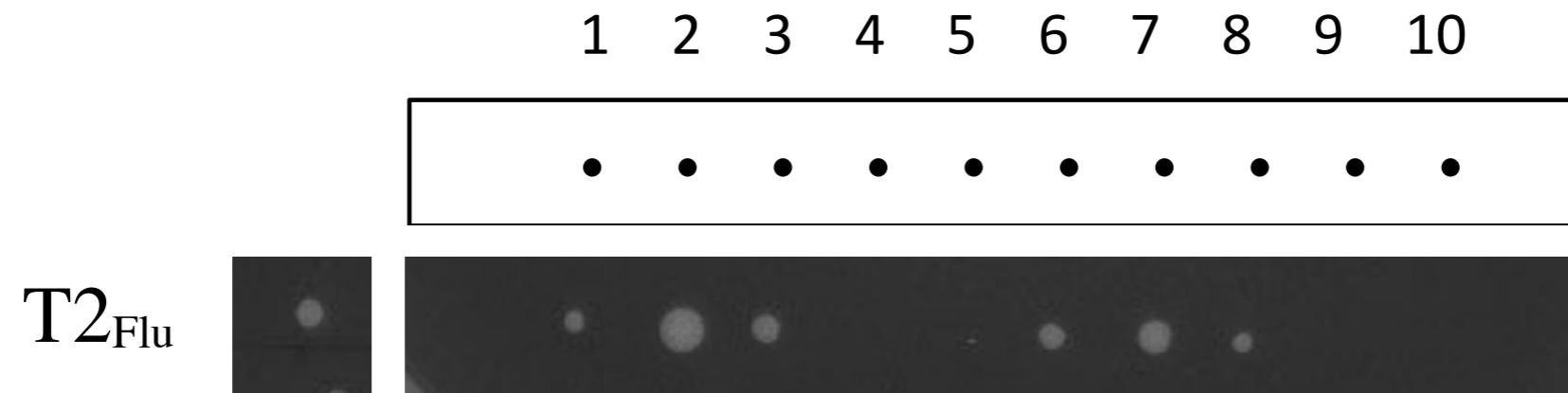
# Followed sequential titan cell progeny



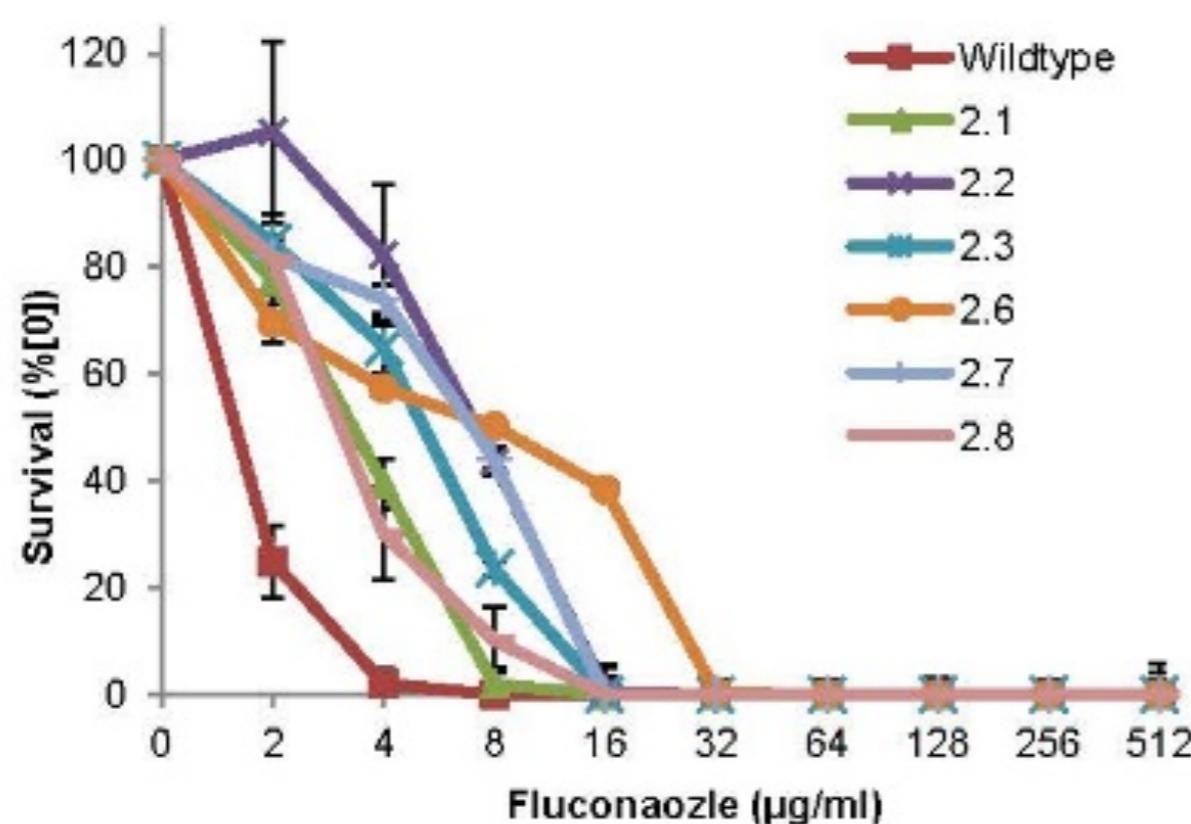
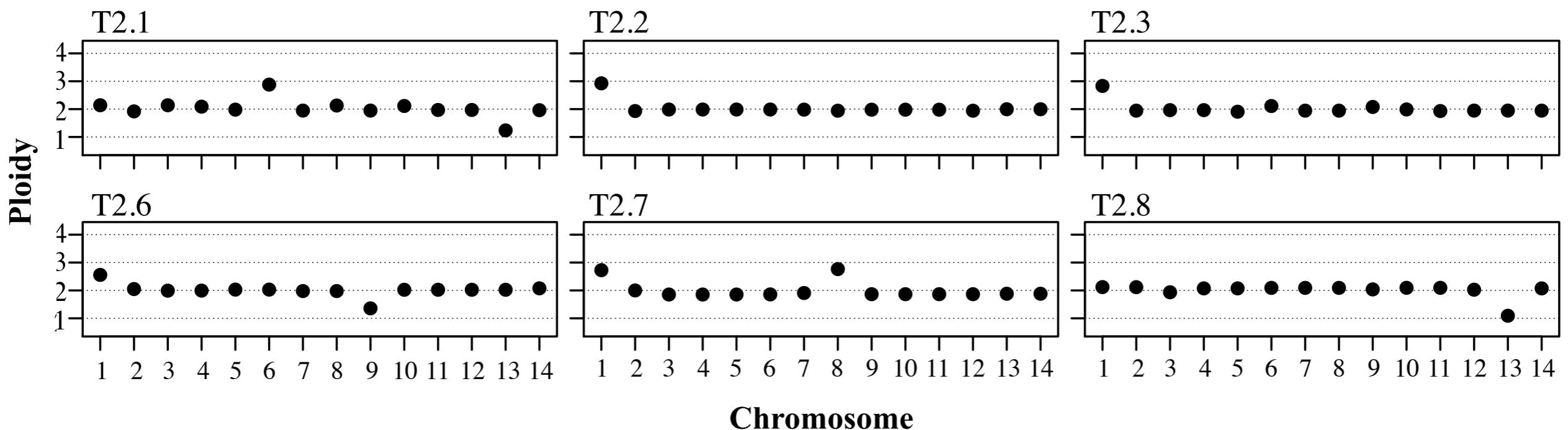
# Microdissect sequential daughter cells



# Different daughter cells contain different karyotypes

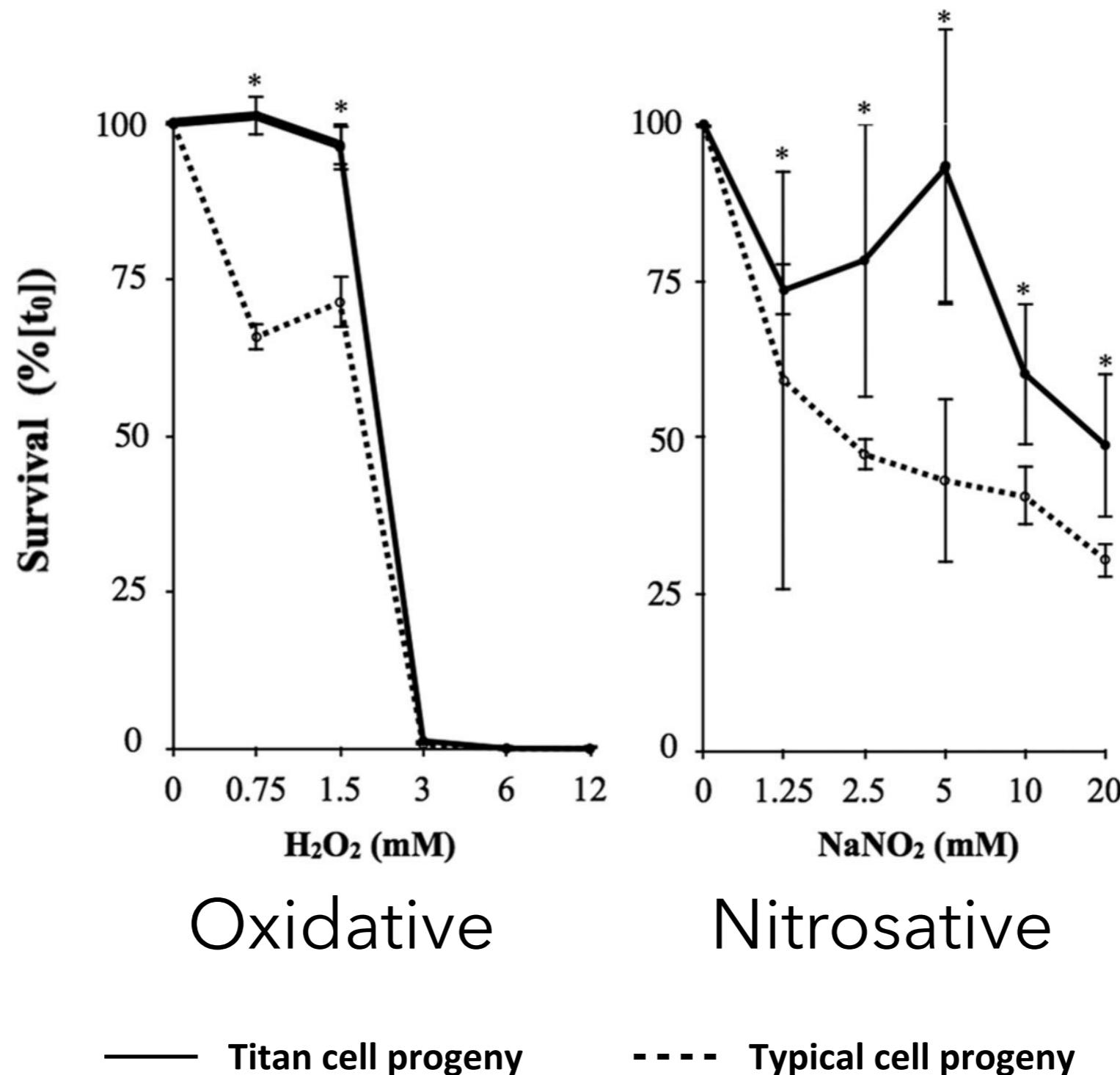


# Different karyotypic solutions lead to drug resistance

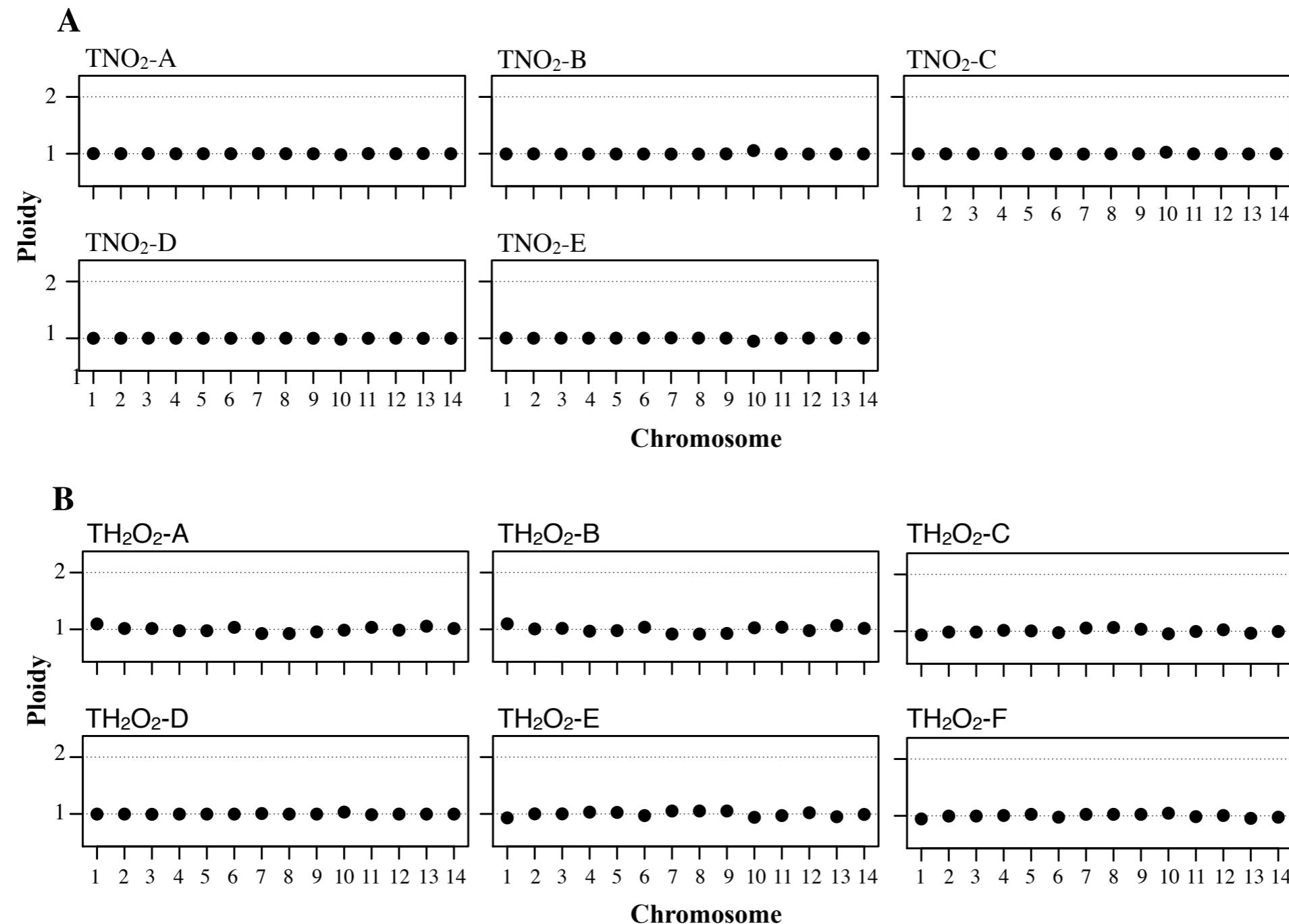


Strain	$\text{MIC}_{50}$	$\text{MIC}_{90}$
KN99a	2	4
2-1	4	8
2-2	8	16
2-3	8	16
2-6	16	32
2-7	8	16
2-8	4	8

# Biology doesn't always play along



# Titan cell progeny grown in nitrosative and oxidative stress become haploid



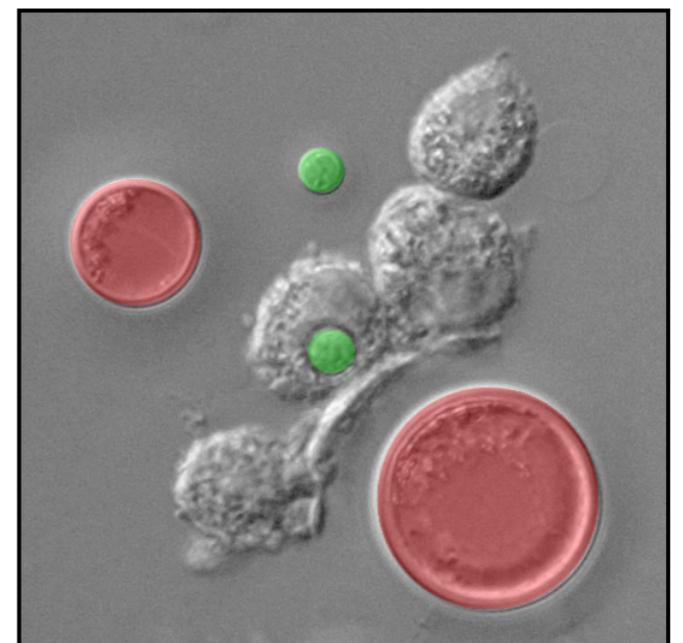
# How do titan cell populations respond to stress?

Titan cell populations have increased survival & growth under a diverse stressors

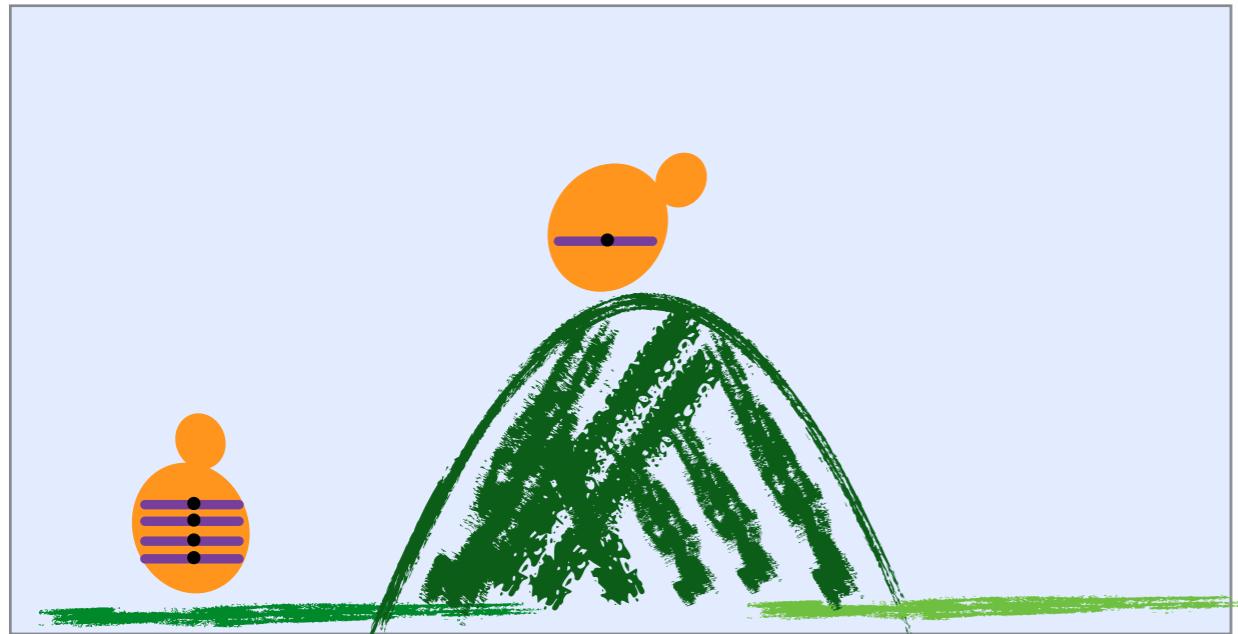
Titans rapidly haploidize in permissive environments and under nitrosative and oxidative stress

Titan progeny grown in drug form drug-resistant aneuploids

Sequential titan daughter cells have genomic diversity (!!?)



# Polypliody facilitates karyotypic diversity

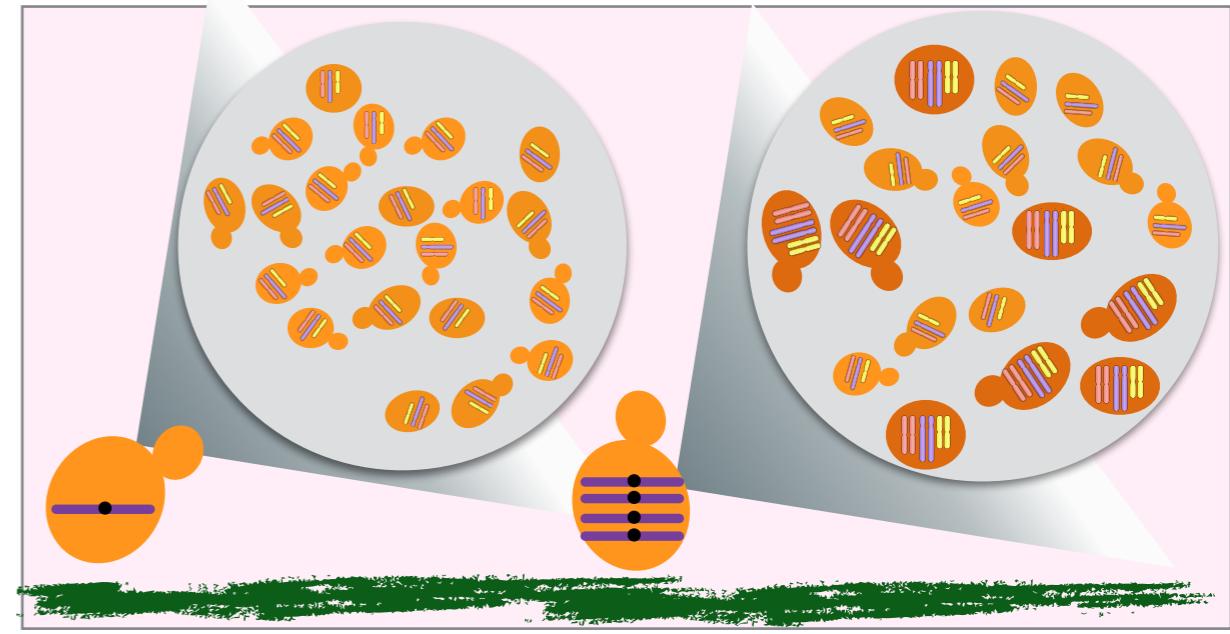


permissive environment

# Polypliody facilitates karyotypic diversity

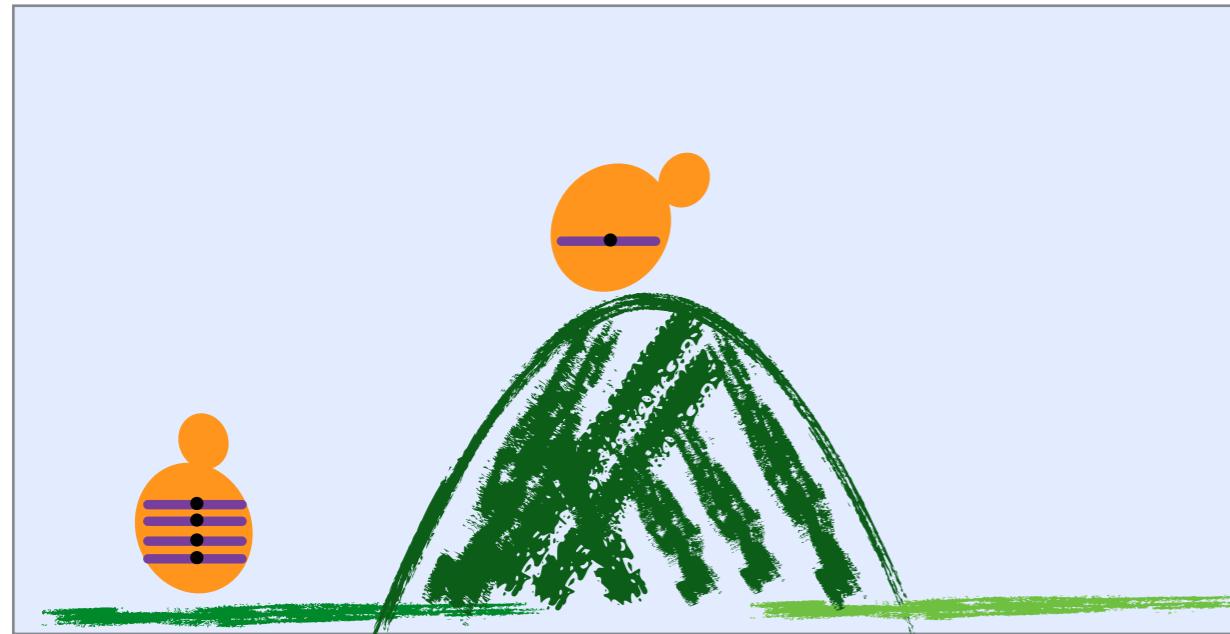


permissive environment

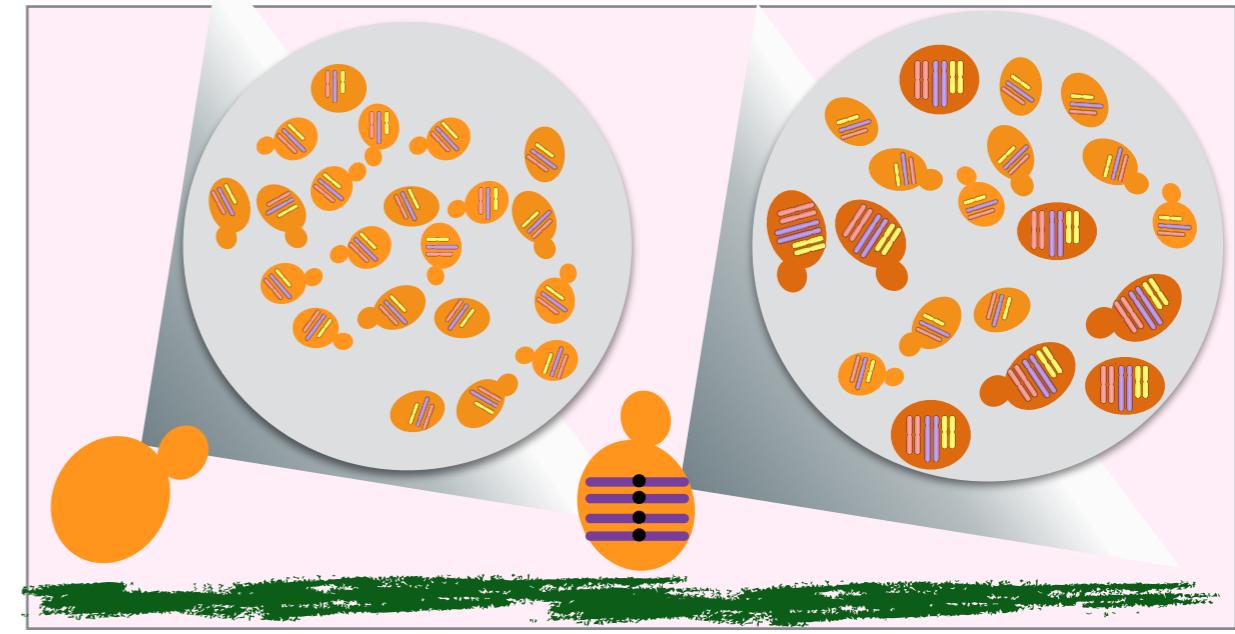


stress environment - diversification

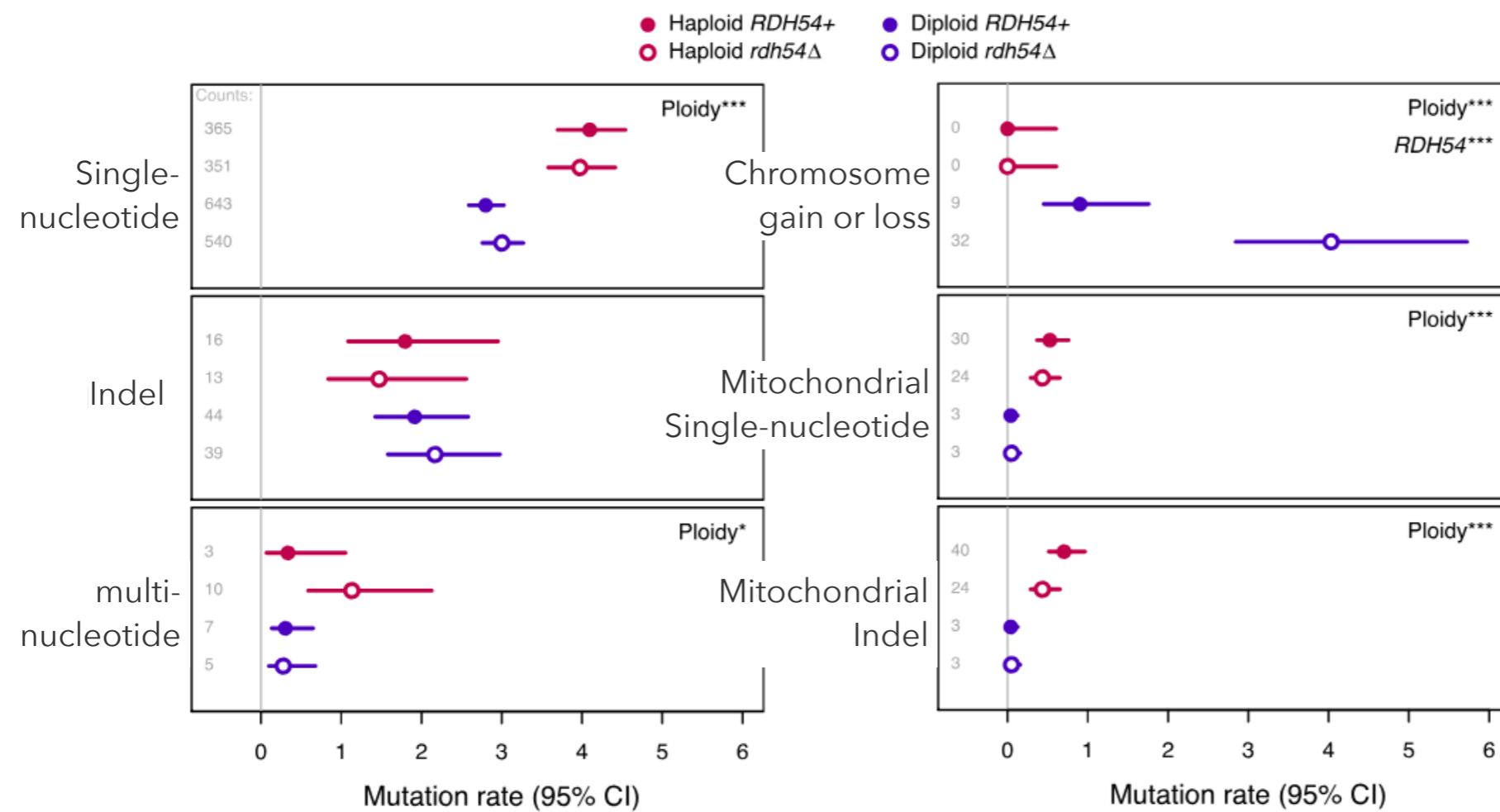
# Polyploidy facilitates karyotypic diversity



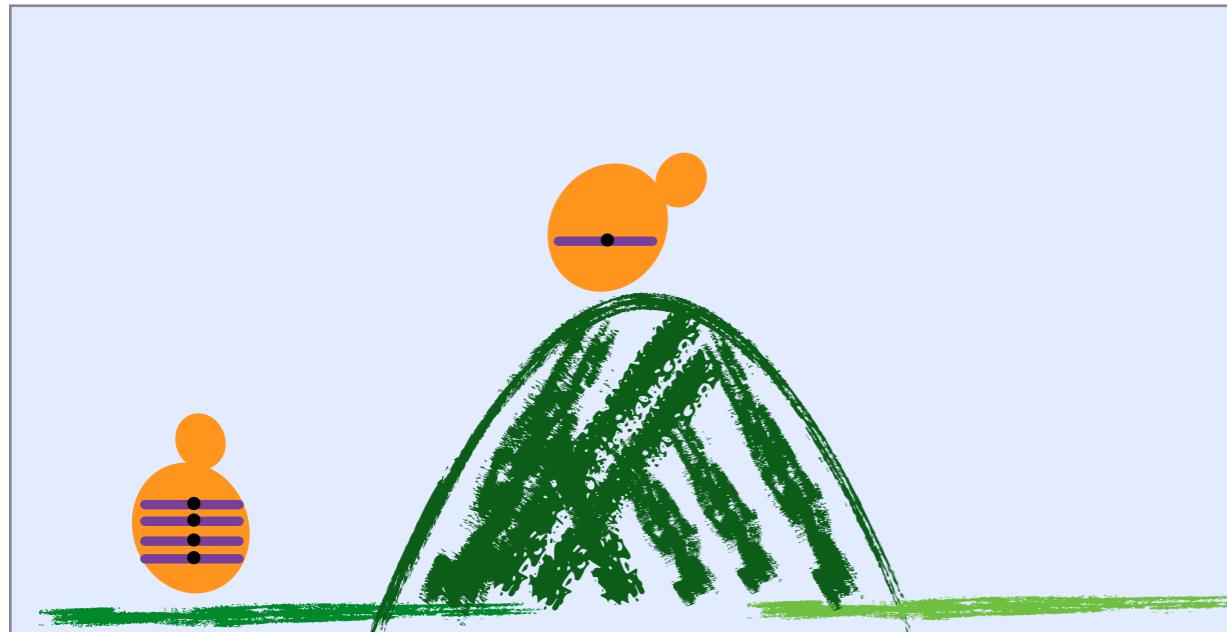
permissive environment



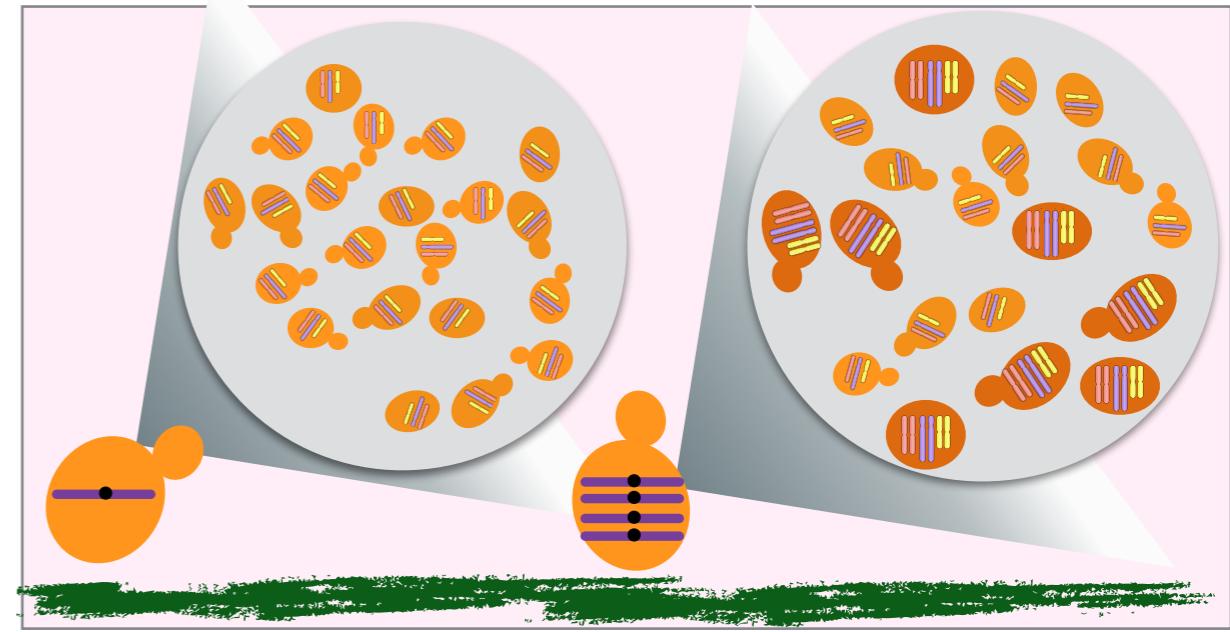
stress environment - diversification



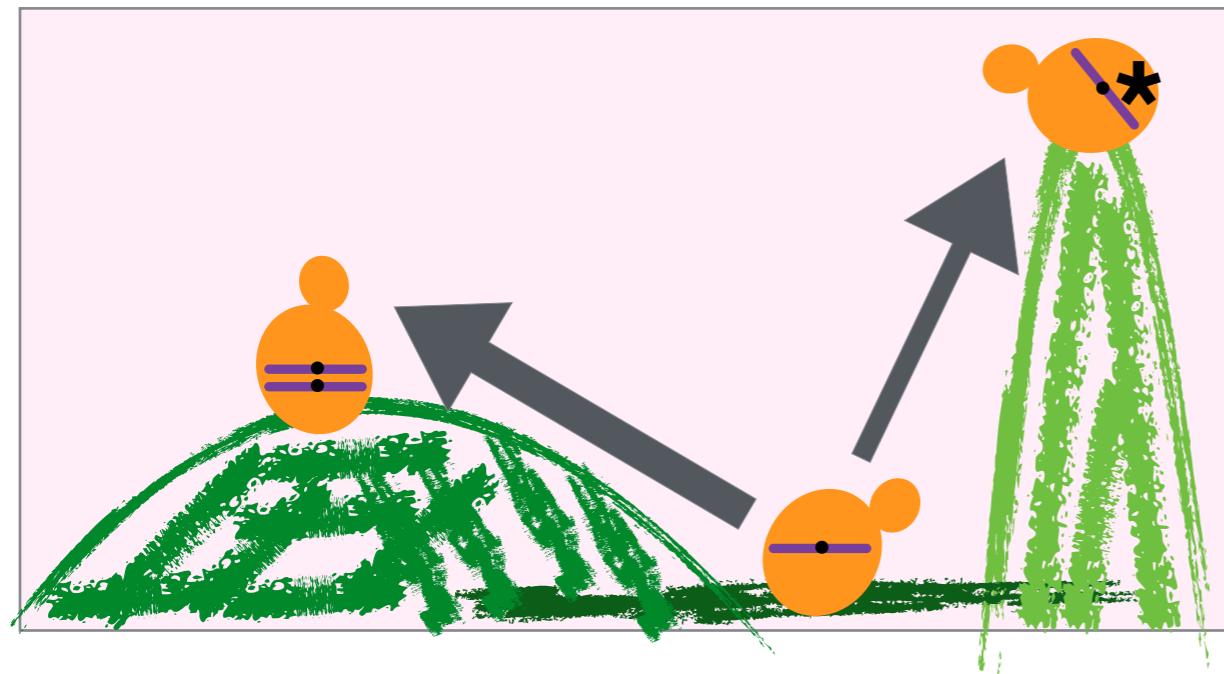
# Polypliody facilitates karyotypic diversity



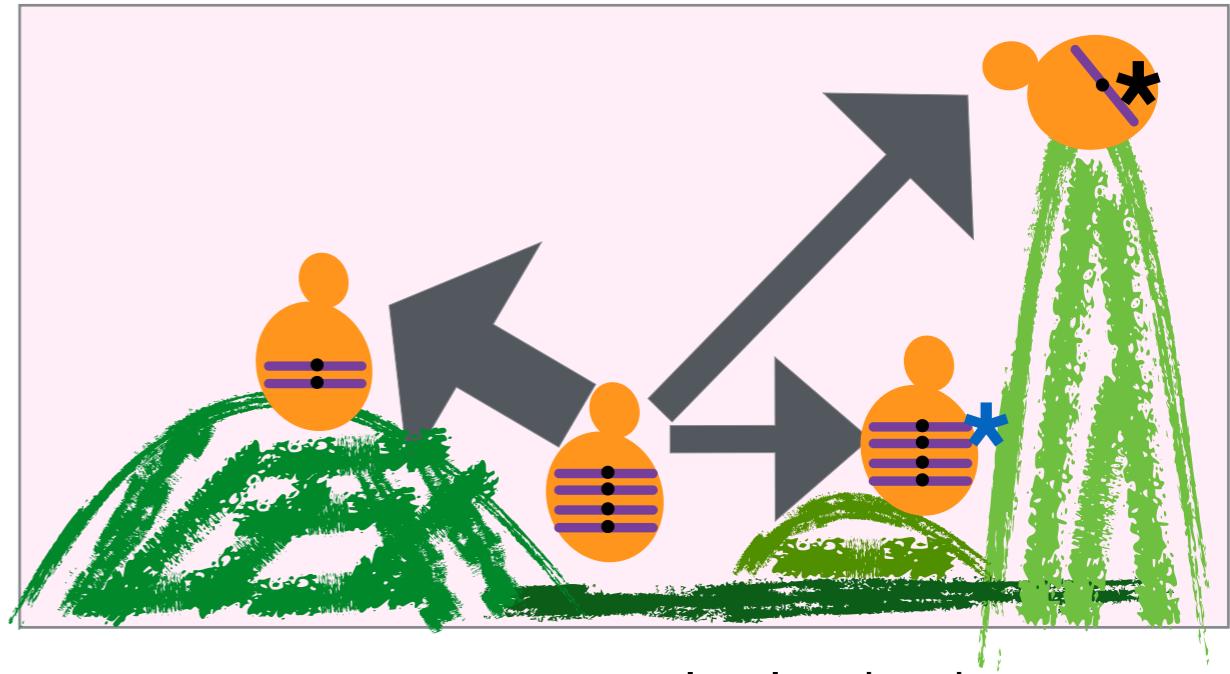
permissive environment



stress environment - diversification



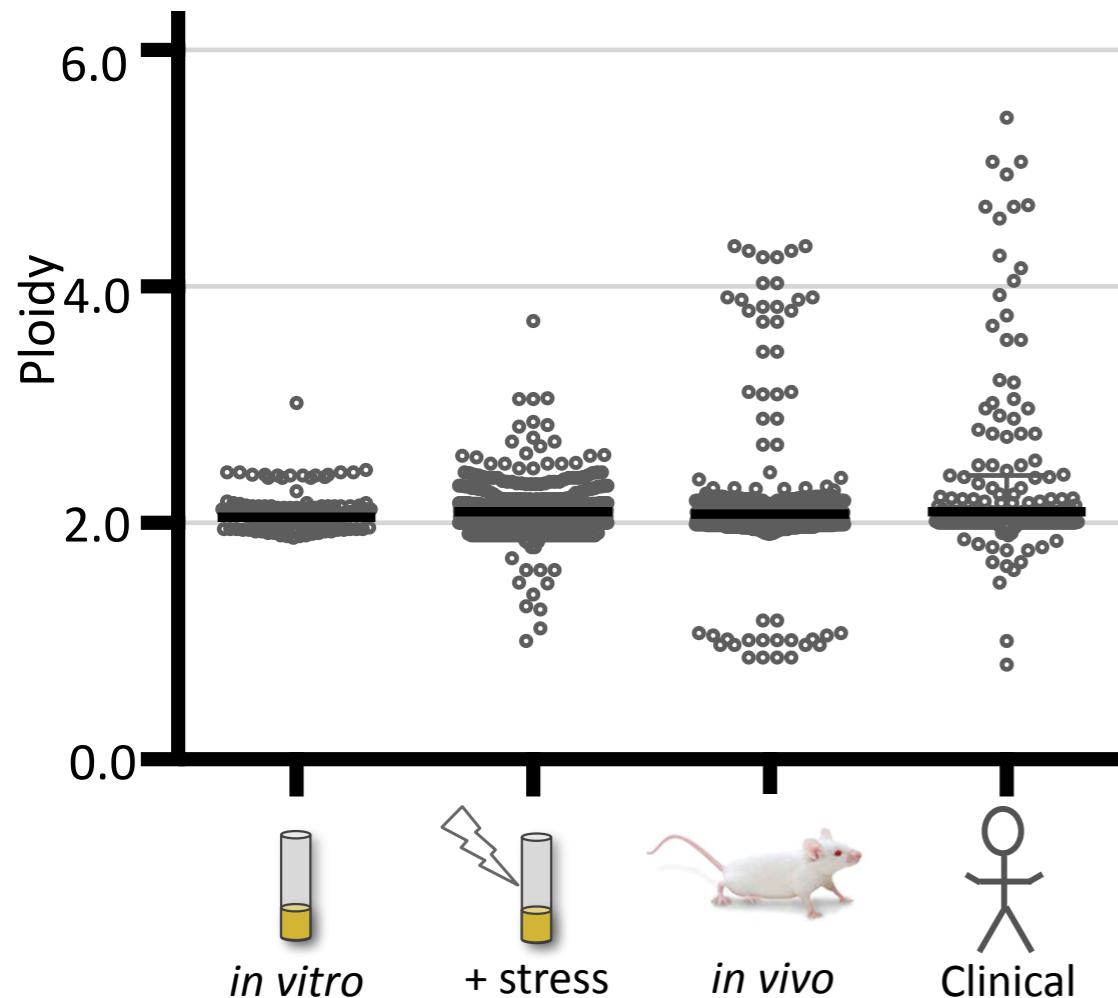
stress environment: haploid adaptation



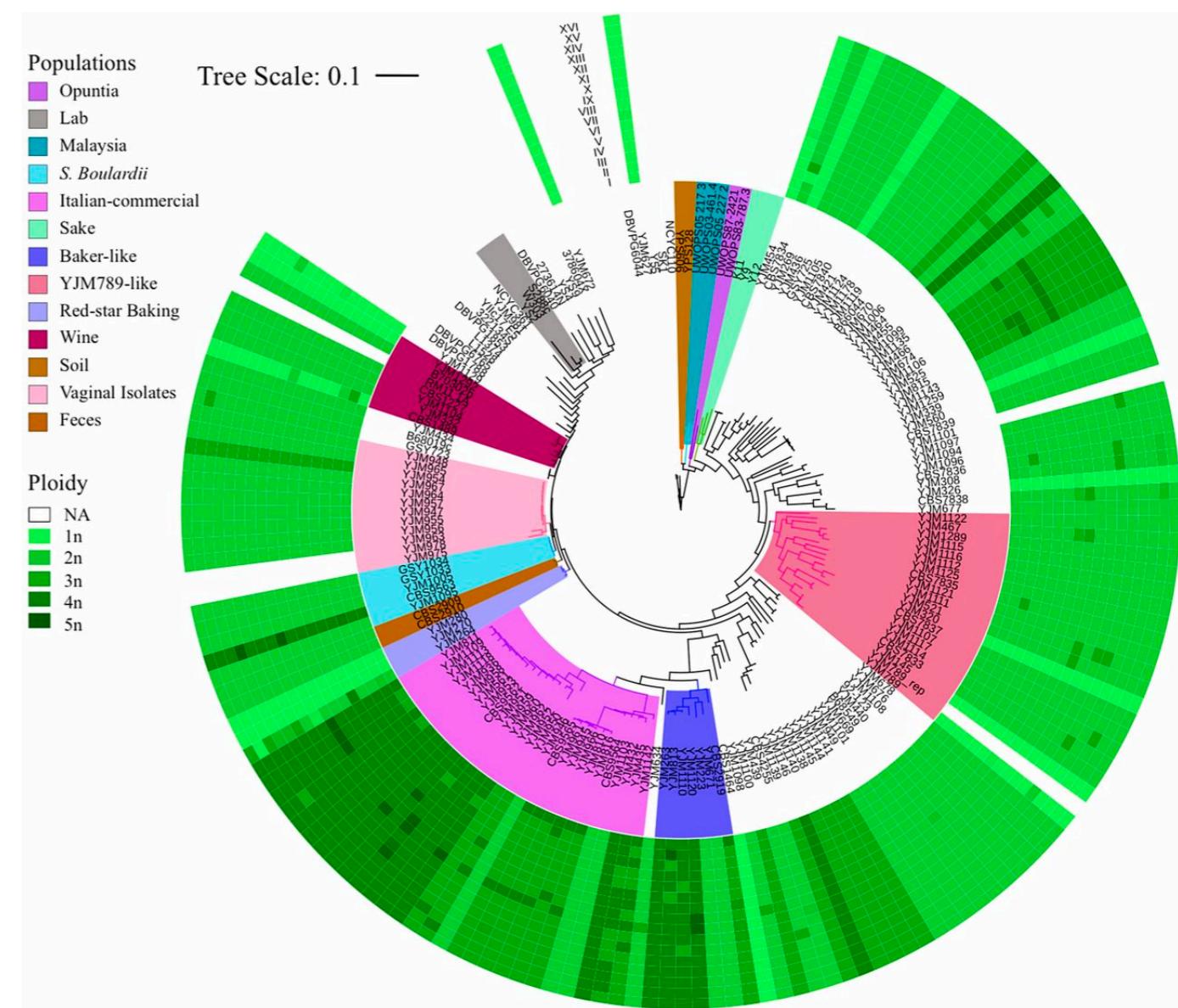
stress environment: polypliody adaptation

# Ploidy diversity exists within clinical populations

*Candida albicans*



*Saccharomyces cerevisiae*



# Ploidy transitions are rapid and prevalent in diverse fungal species (*in vitro*)

*Cryptococcus neoformans*: < 10 generations

Gerstein, Fu et al., 2015

*Candida albicans*: < 200 generations

Hickman et al., 2015, Gerstein et al. 2017

*Candida tropicalis*: < 300 generations

Seervai et al., 2013

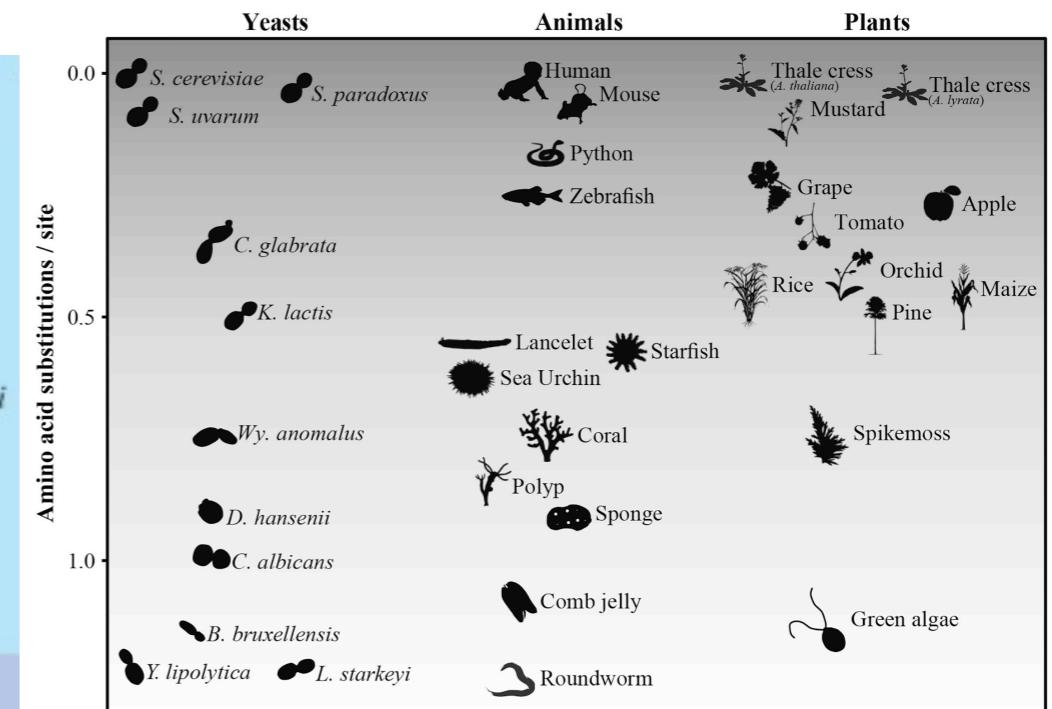
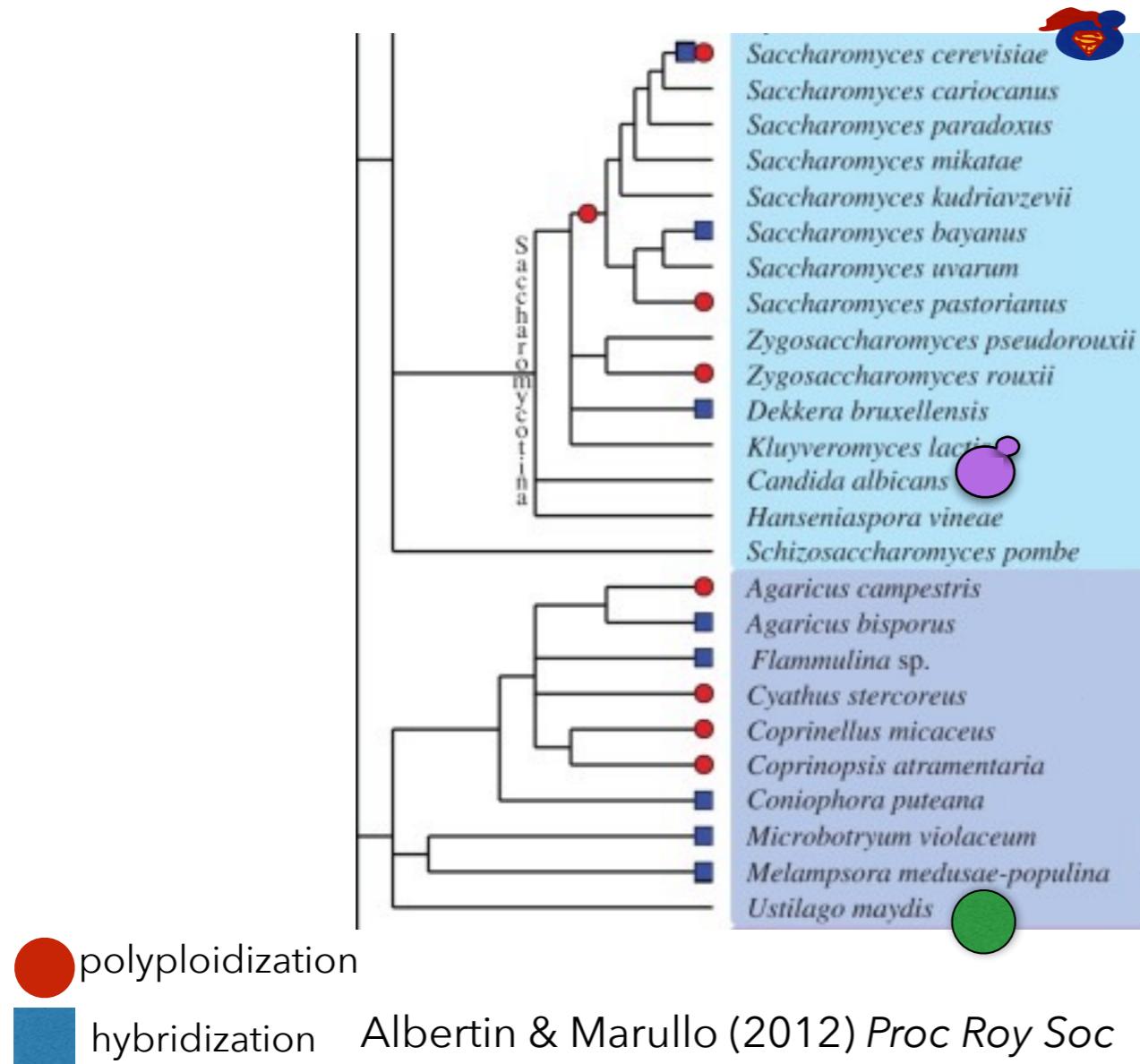
*Saccharomyces cerevisiae*: < 100-1000 generations

Gerstein et al., 2006, Gresham et al., 2008, Voordeckers et al., 2015; Venkataram et al., 2016

*Aspergillus nidulans*: < 3000 generations

Schoustra et al., 2007

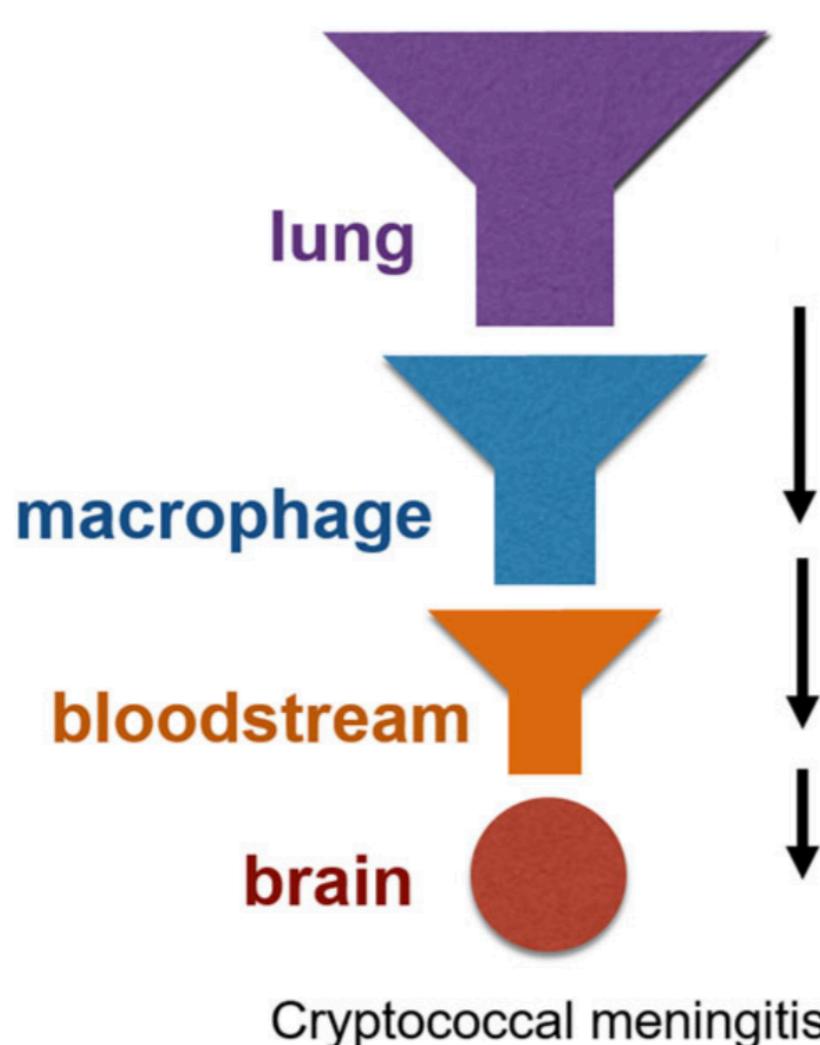
# Fungal taxa relatedness



Shen et al. (2018) Cell

Albertin & Marullo (2012) Proc Roy Soc

# Pathogen genotype influence on disease

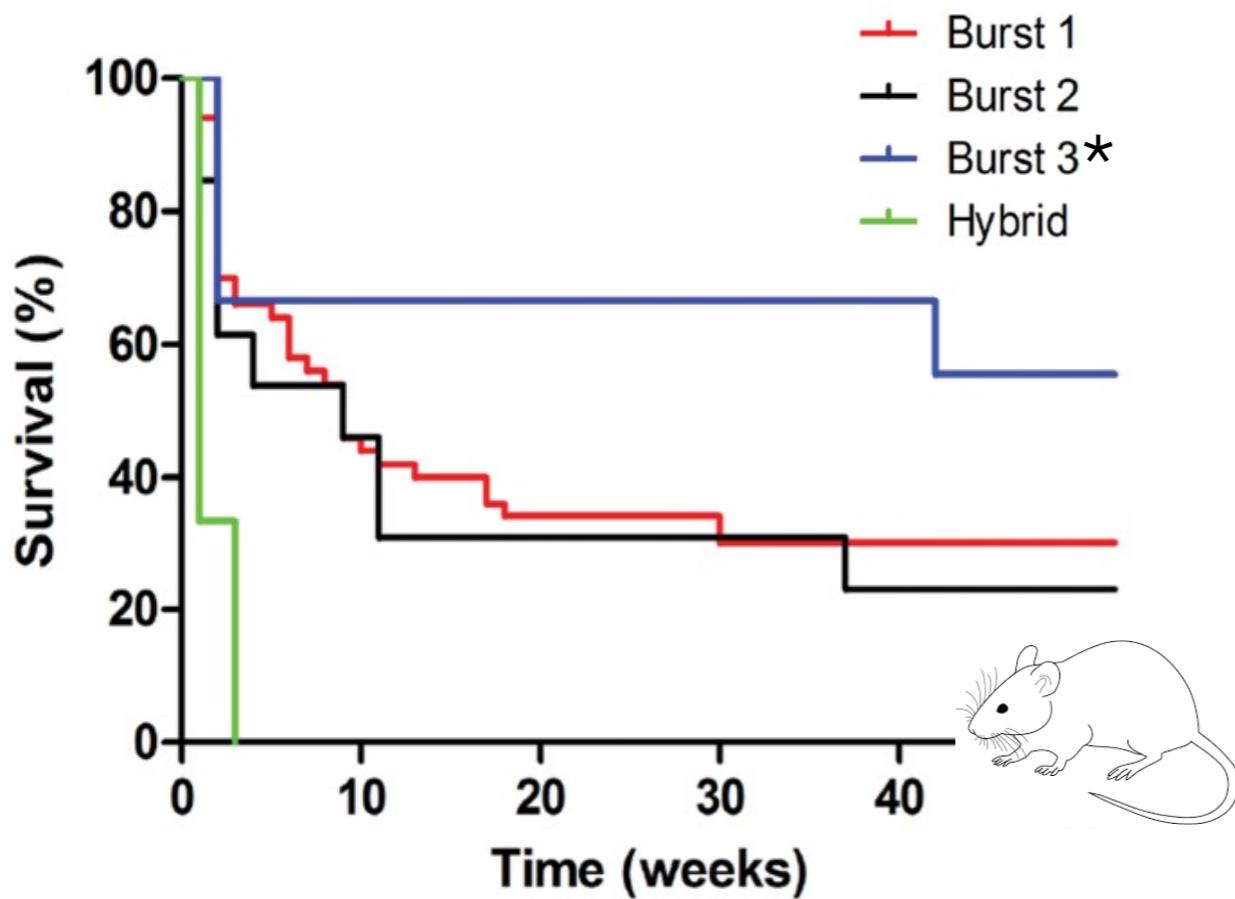


**Tactic 1:** *in vitro* adaptation experiments

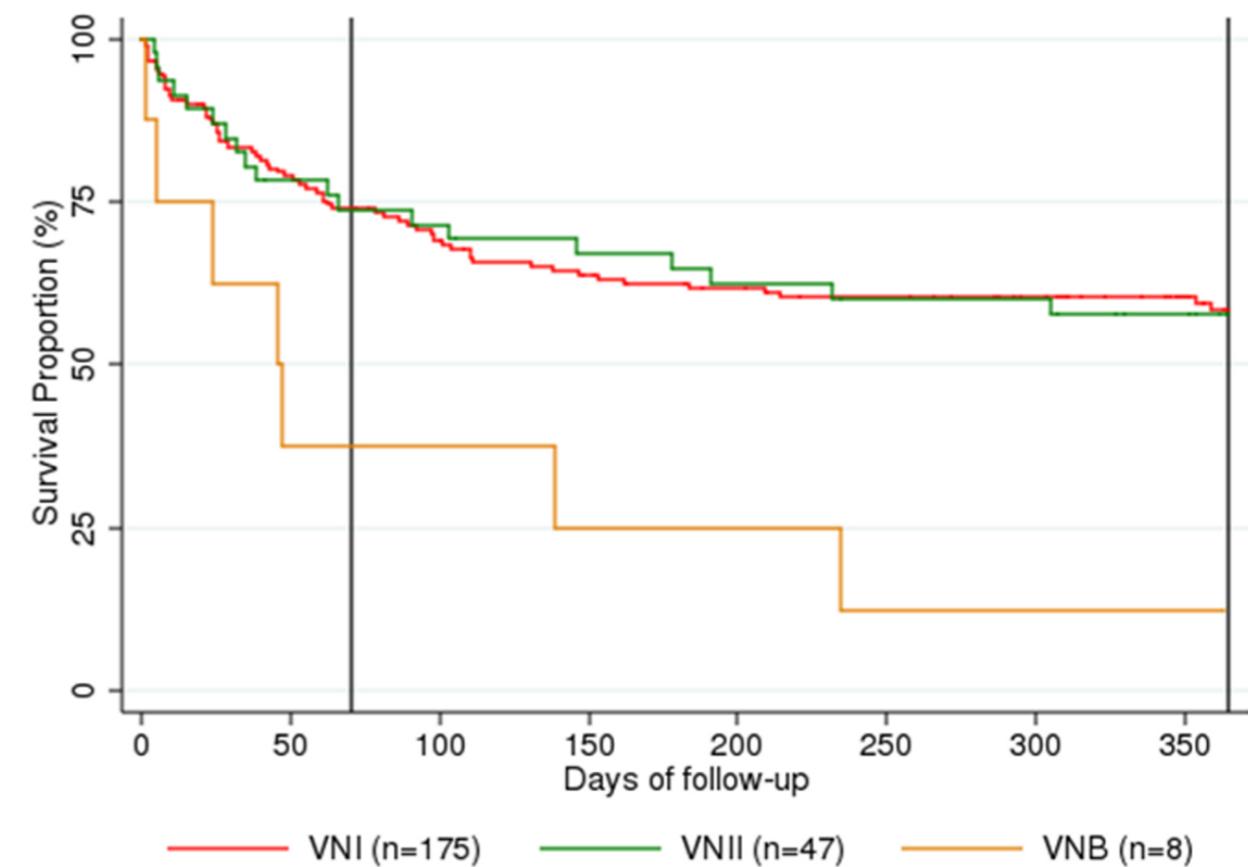
**Tactic 2:** GWAS analysis for association between pathogen genotype and human infection phenotype

# Cryptococcal genotype influences clinical outcome

140 isolates from 111 Ugandans with AIDS & CM



230 isolates from 230 HIV positive South Africans



Wiesner et al. (2012) *mBio*

Beale et al. (2015) *PLoS Negl Trop Dis*

# Look for a genomic signature of human virulence

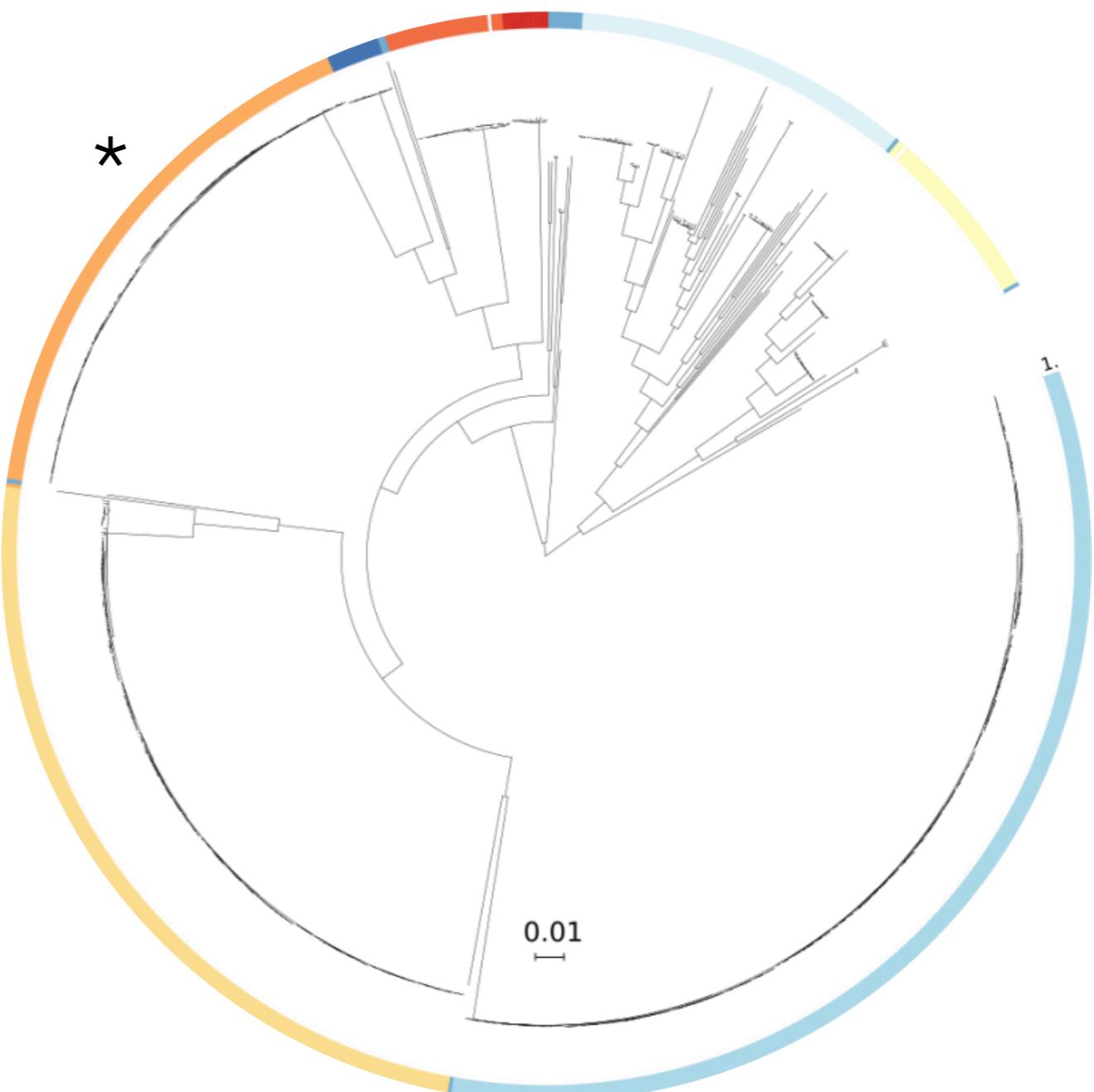
Cryptococcal Optimal ART Timing (COAT) Trial:

- Enrolled 150 patients with cryptococcal meningitis in Uganda
- Sampled Cryptococcal isolates and measured human immunological parameters prior to treatment
- Patients were treated and monitored for up to one year with standardized medical care

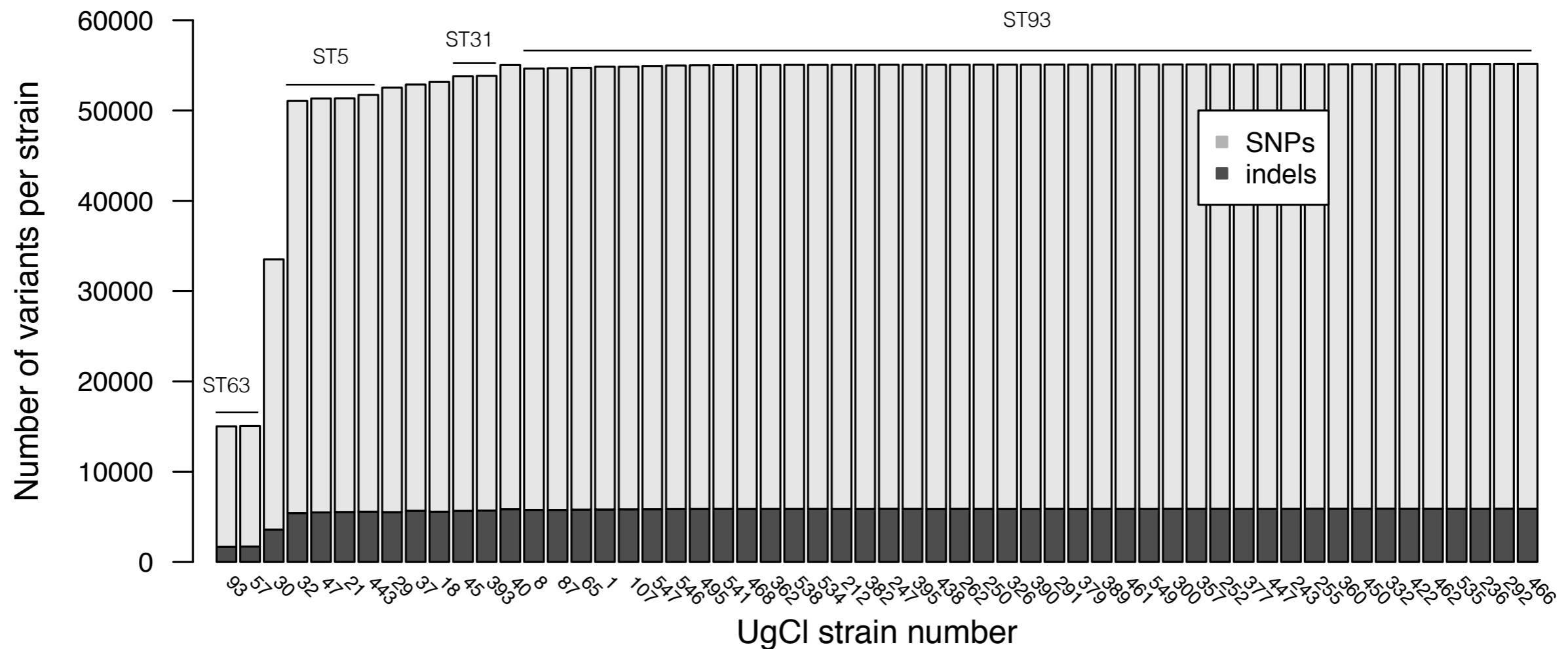


# Whole-genome sequenced closely related strains

- 21 ST93 isolates from patients that died
- 17 ST93 isolates from patients that survived
- 17 from other ST groups



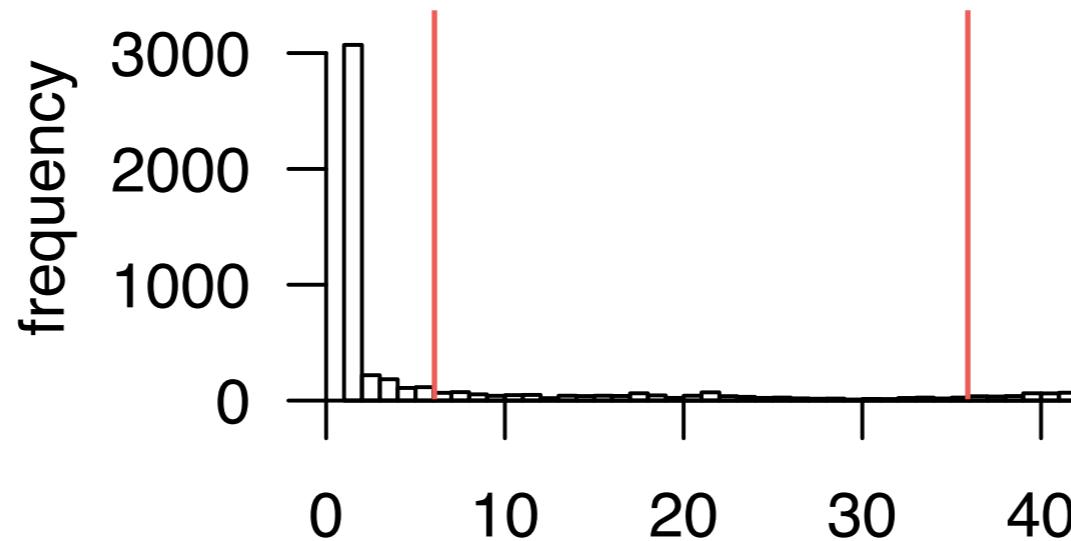
# Filter the identified variants



# Filter the identified variants

ST93 genomes: 52637 SNPs & 6452 INDELs

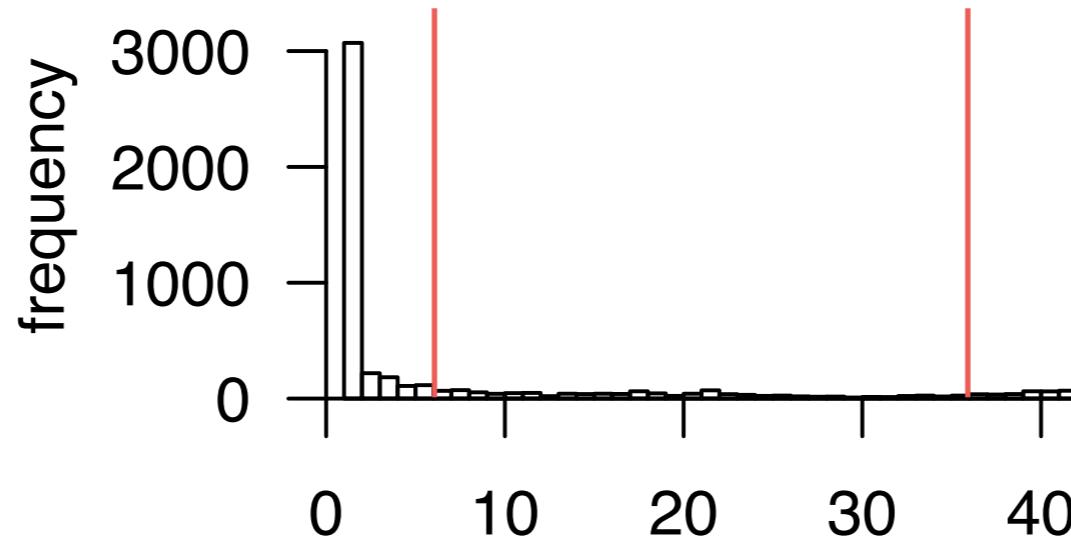
↓ Some strains: 5605



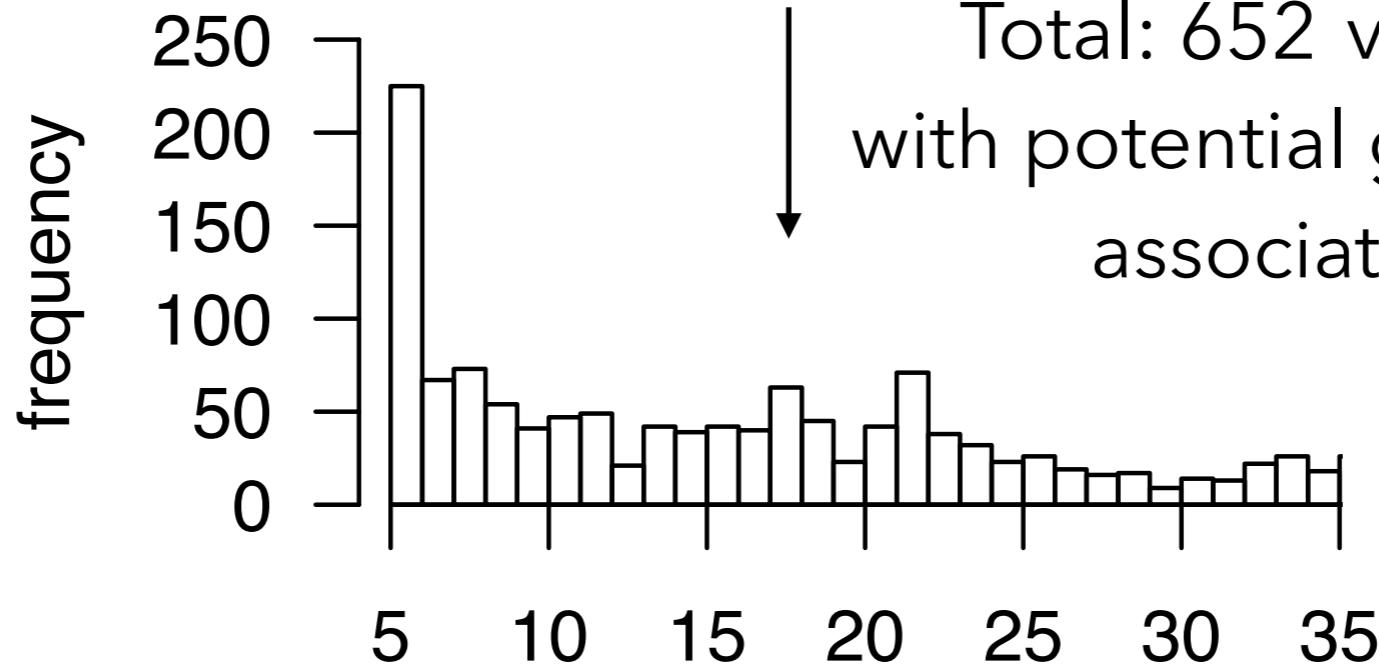
# Filter the identified variants

ST93 genomes: 52637 SNPs & 6452 INDELs

↓  
Some strains: 5605



↓  
Total: 652 variants  
with potential genotypic  
association

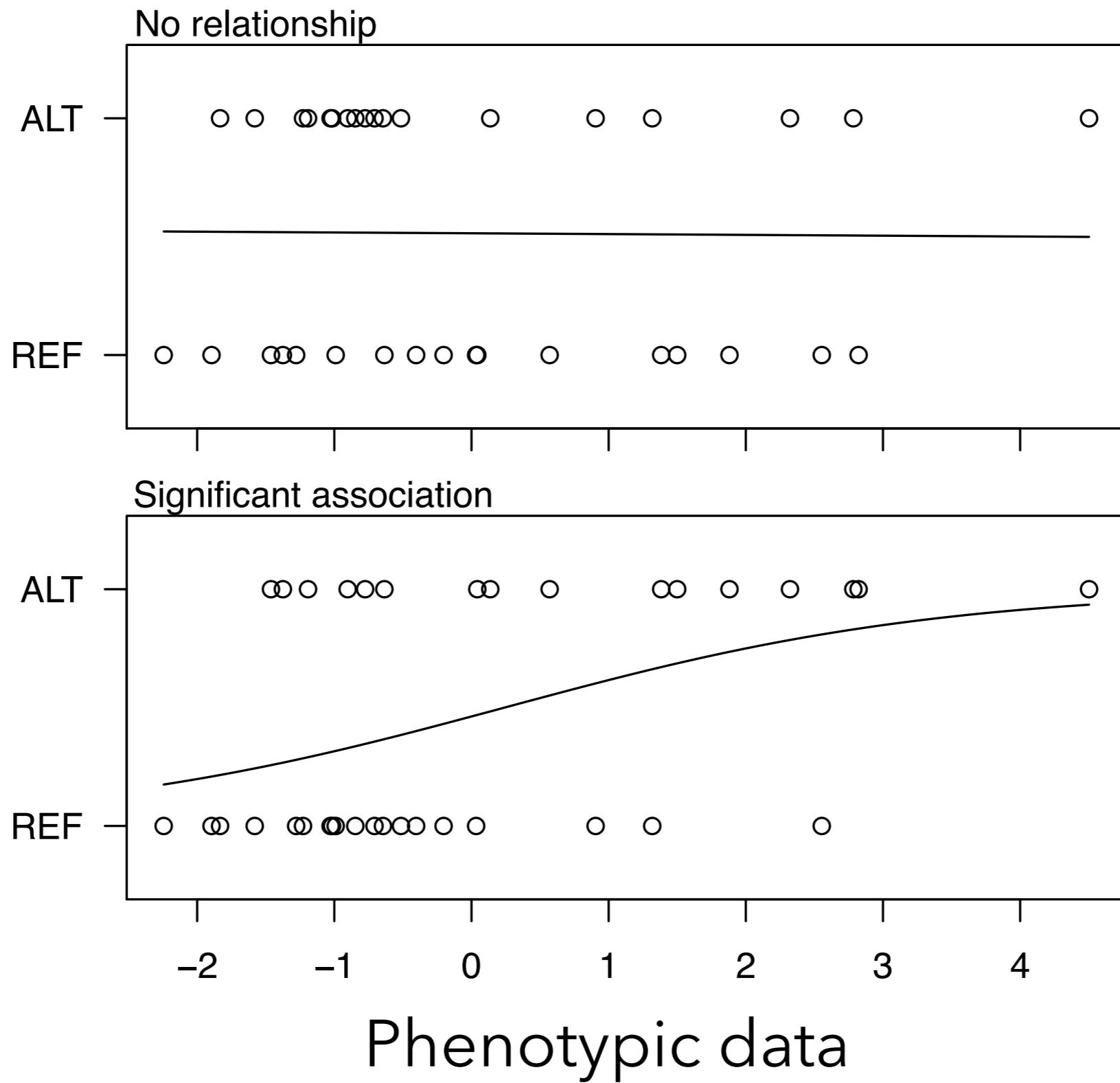


# Quantitative infection phenotypes

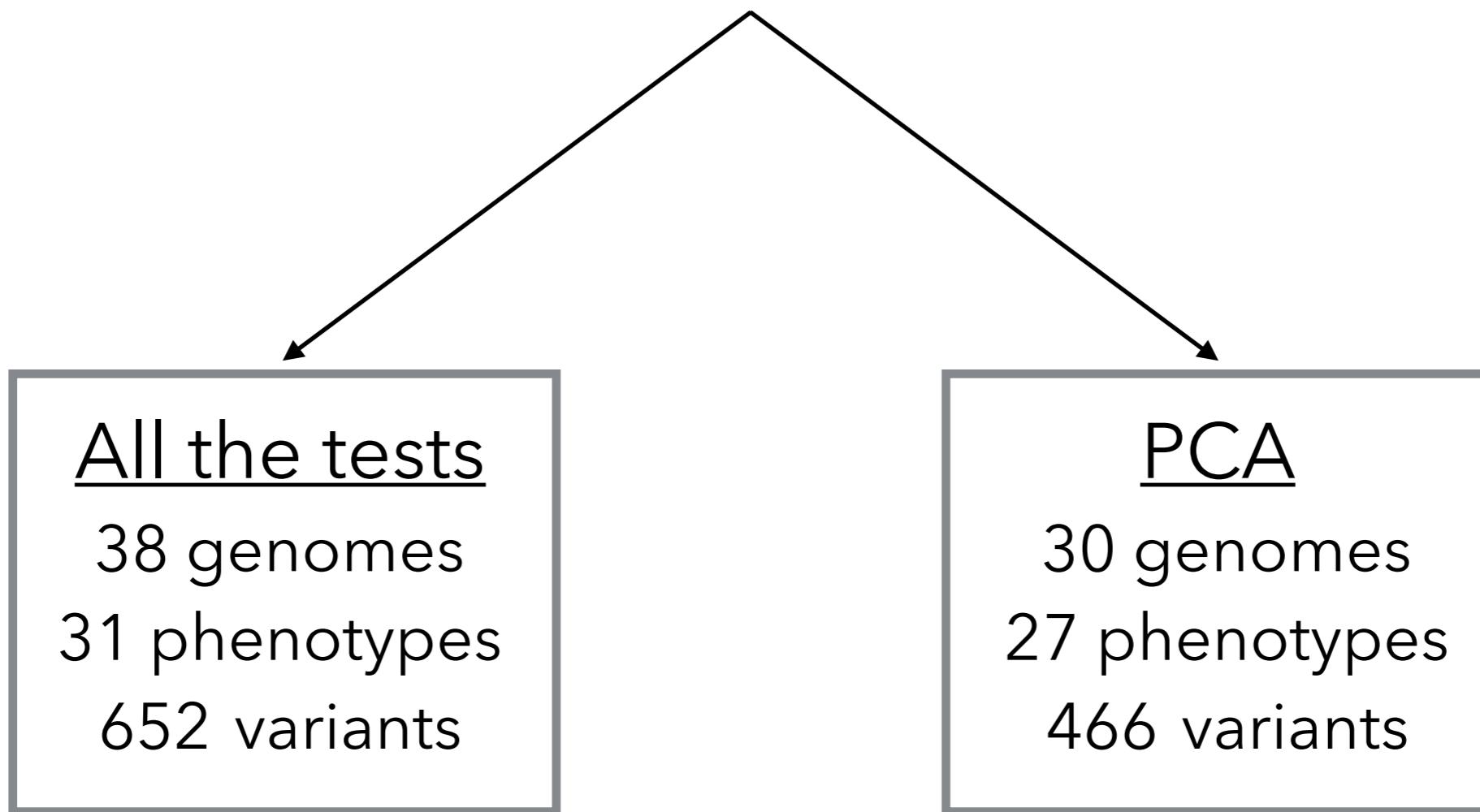
<b>Class</b>	<b>Phenotype (n strains)</b>
Immune	IL1-b (36)
Immune	IL2 (36)
Immune	IL4 (36)
Immune	IL-5 (36)
Immune	IL-6 (36)
Immune	IL-7 (36)
Immune	IL-8 (36)
Immune	IL-10 (36)
Immune	IL-12 (36)
Immune	IL-13 (36)
Immune	IL-17 (36)
Immune	GCSF (36)
Immune	GMCSF (36)
Immune	IFNg (36)
Immune	MCP1 (36)
Immune	TNF $\alpha$ (36)
Immune	MIP1b (36)

<b>Class</b>	<b>Phenotype (n strains)</b>
Mortality	Survival days (38)
Clinical	CD4 (38)
Clinical	CSF WBC (35)
Clinical	protein (31)
Clinical	HIV-viral (35)
Clinical	EFA (37)
Clinical	LFA titer (30)
<i>in vitro</i>	macrophage uptake
<i>in vitro</i>	Macrophage
<i>in vitro</i>	chitin (37)
<i>in vitro</i>	absolute growth at 30
<i>in vitro</i>	fluconazole MIC (37)
<i>in vitro</i>	amphotericin B MIC
<i>in vitro</i>	sertraline MIC (37)

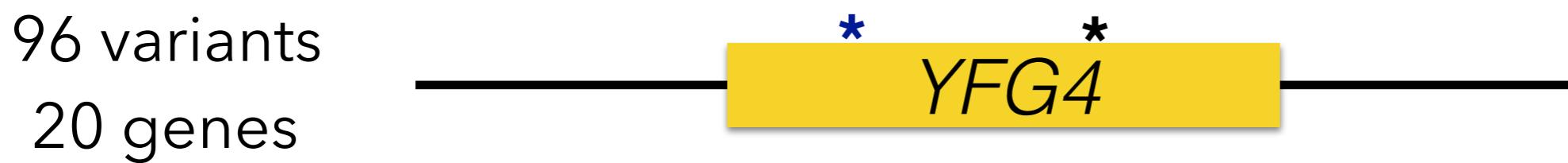
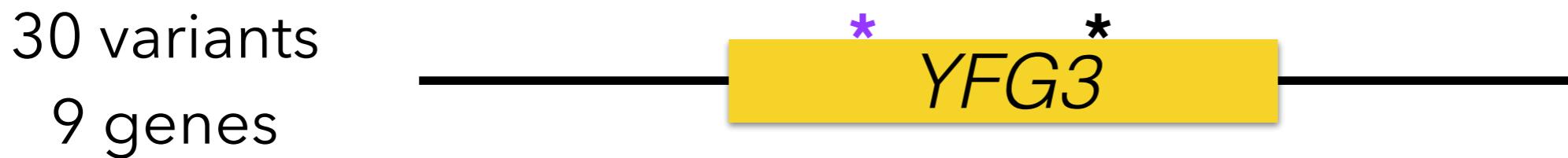
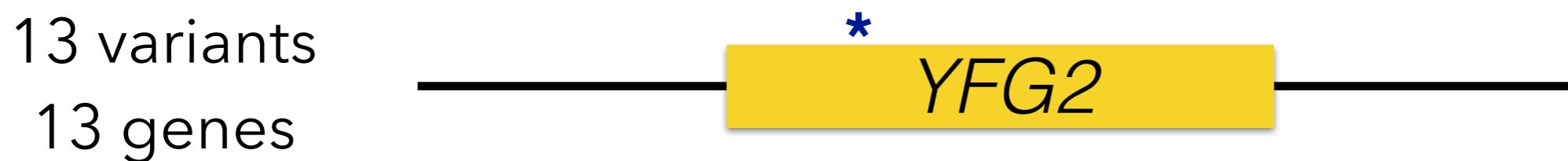
# Association by logistic regression



# Two methods to find significant variants



# Test all the things:



# PCA analysis (PC1 & PC2)

9 variants  
9 genes

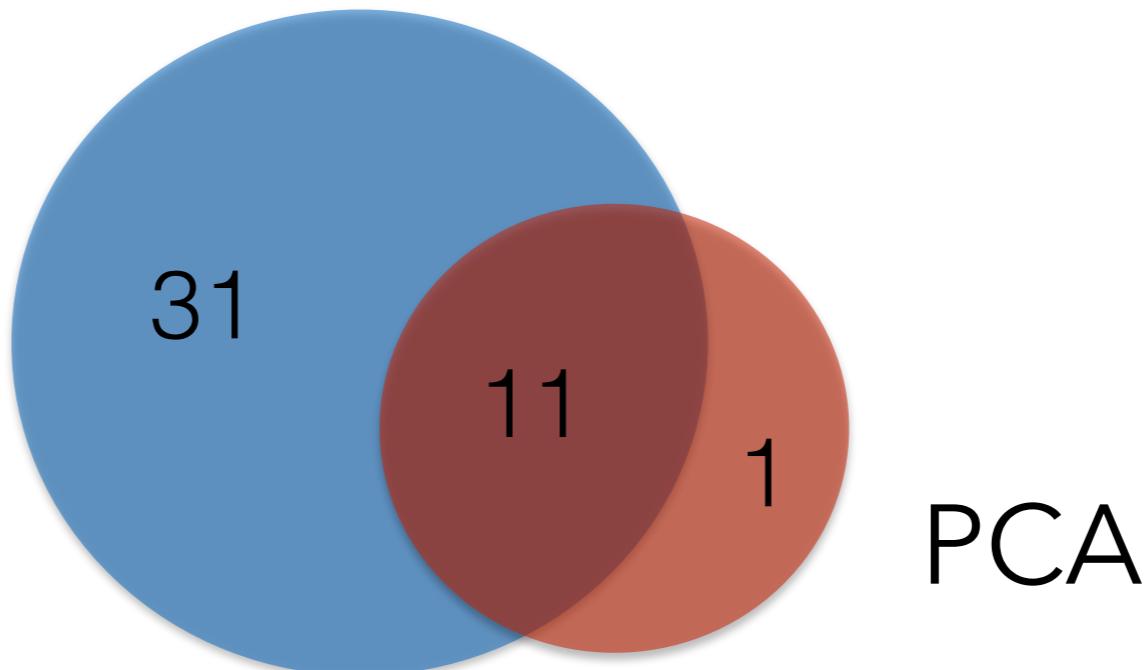


7 variants  
3 genes



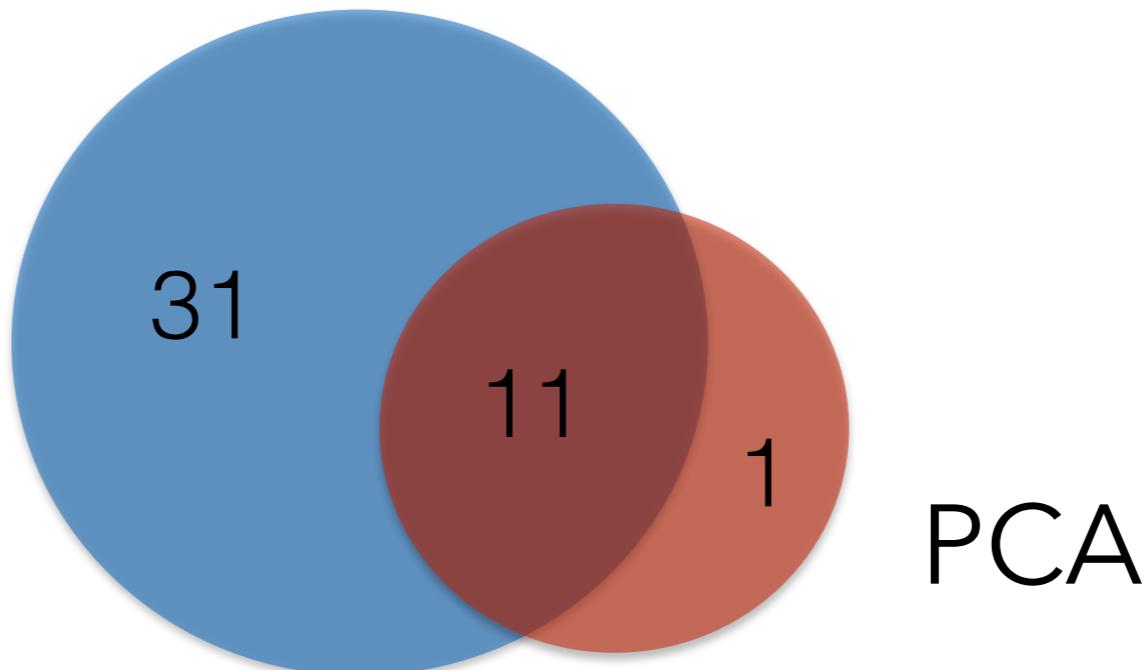
# Two methods to find significant variants

Test all  
the things



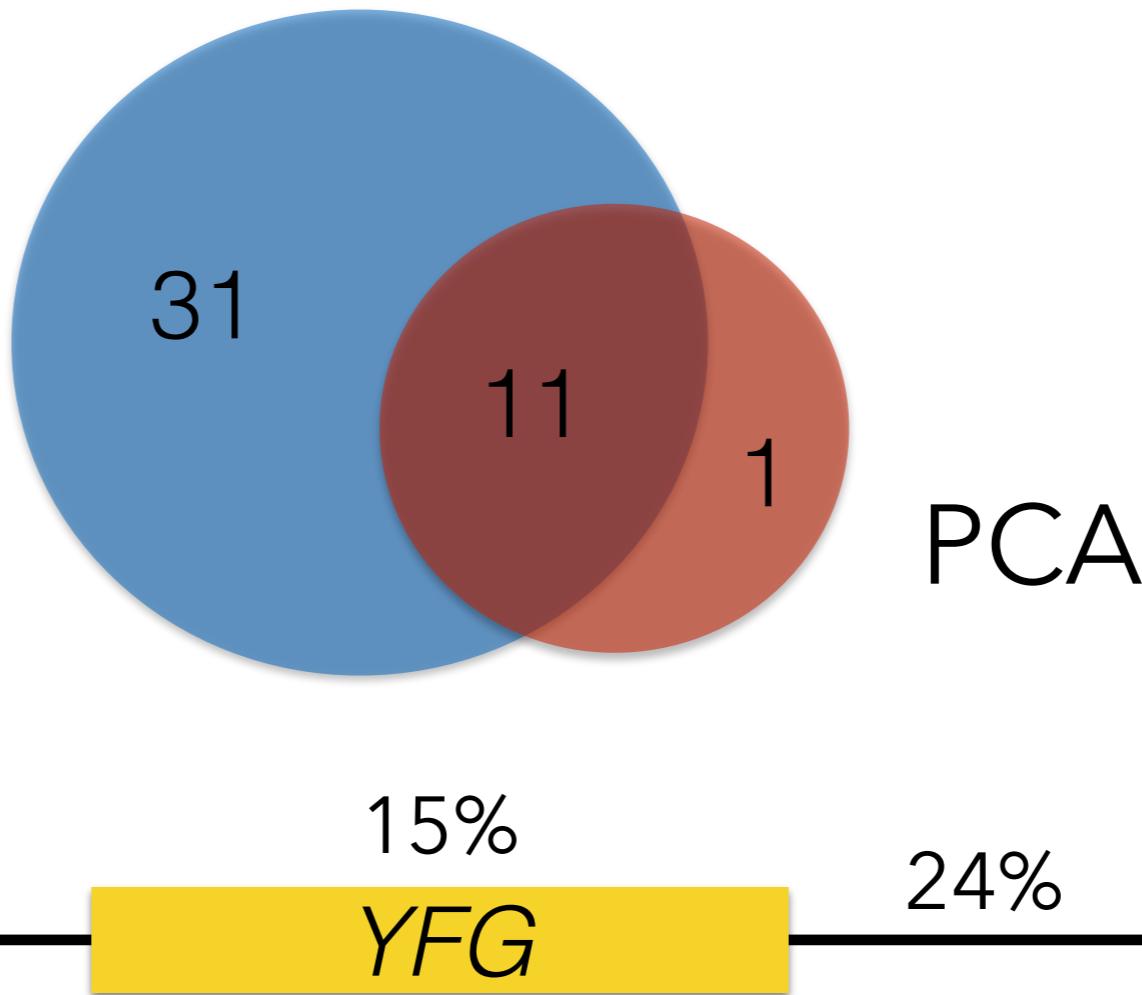
# Two methods to find significant variants

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the things



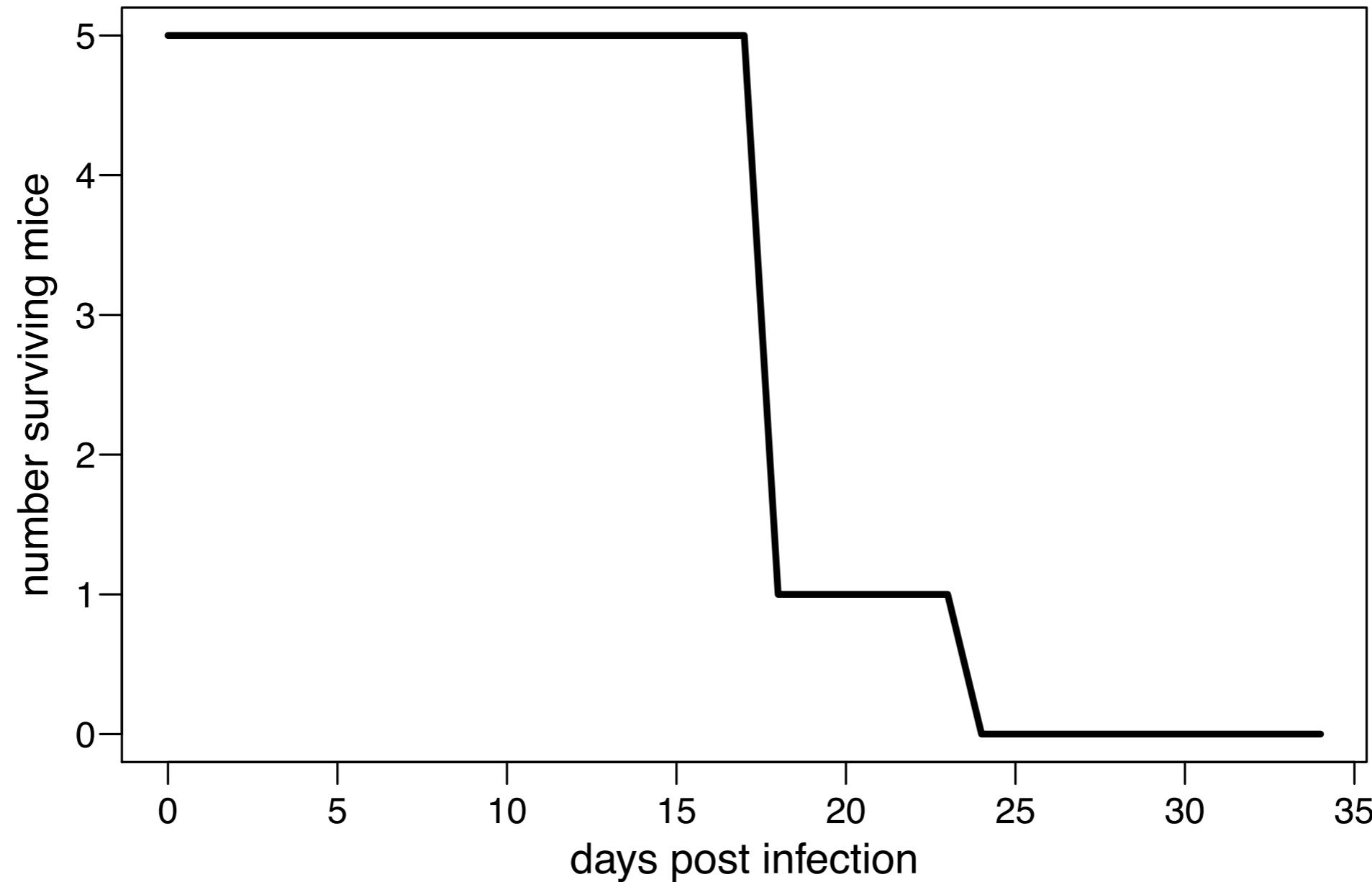
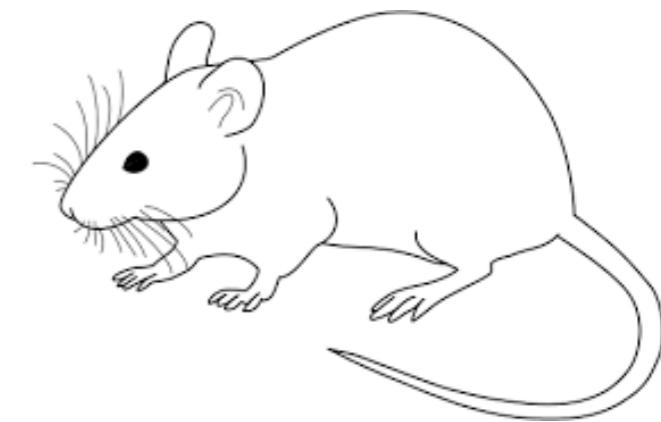
# Two methods to find significant variants

Test all  
the things

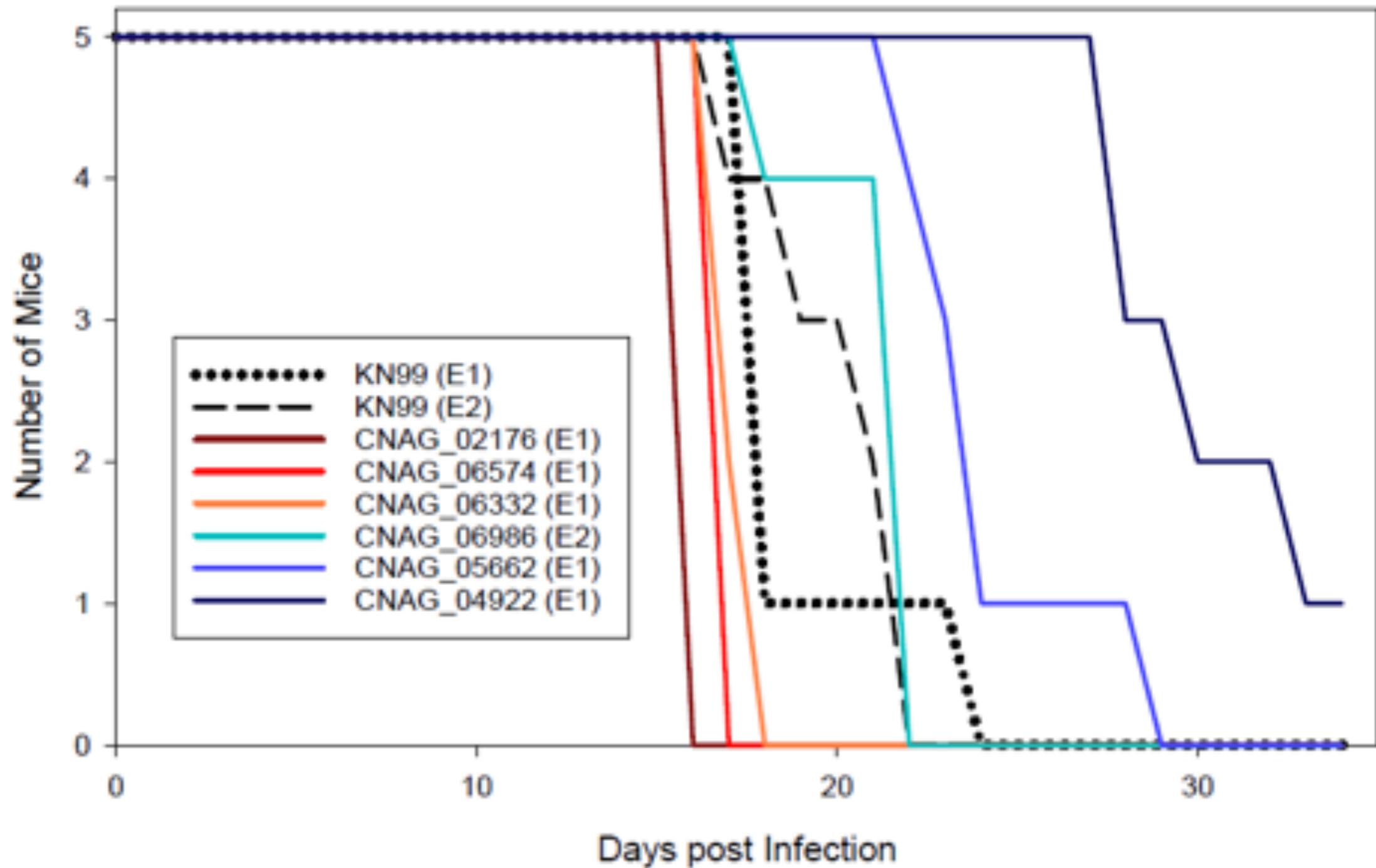


21 genes of known function (8 named)  
19 hypothetical proteins (2 conserved)  
3 hypothetical RNAs

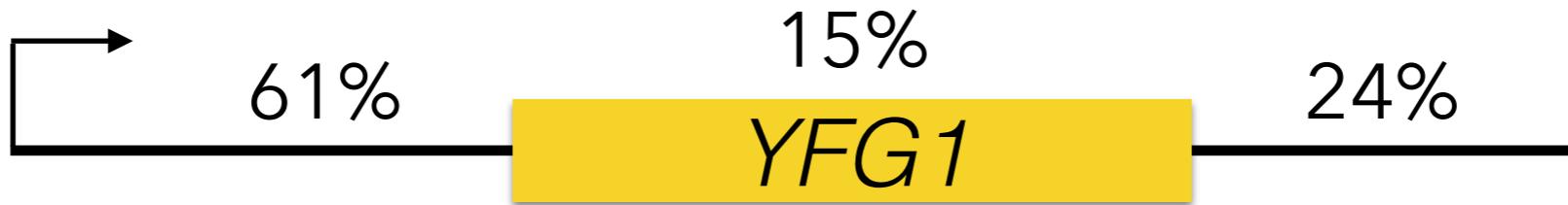
# **Test available knockout strains for virulence effect in mice**



**6 of 17 tested knockout strains have a virulence effect in mice**



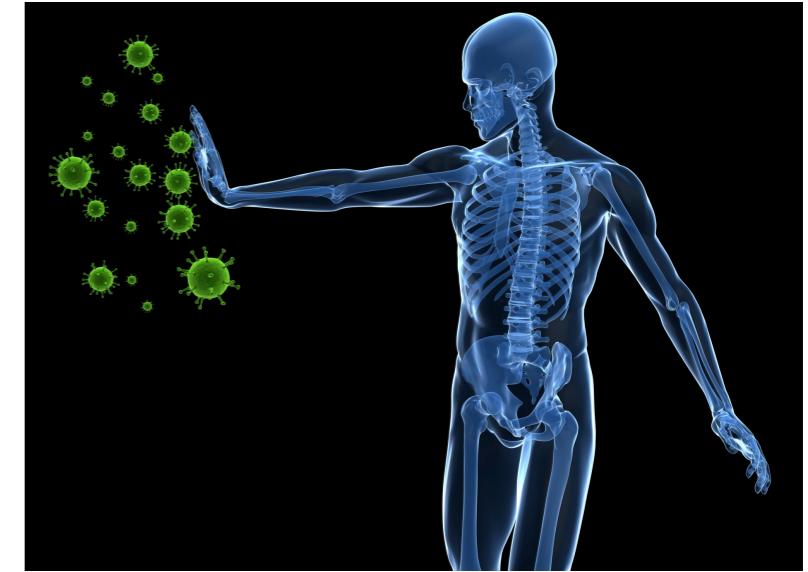
# Knockout may not recapitulate virulence



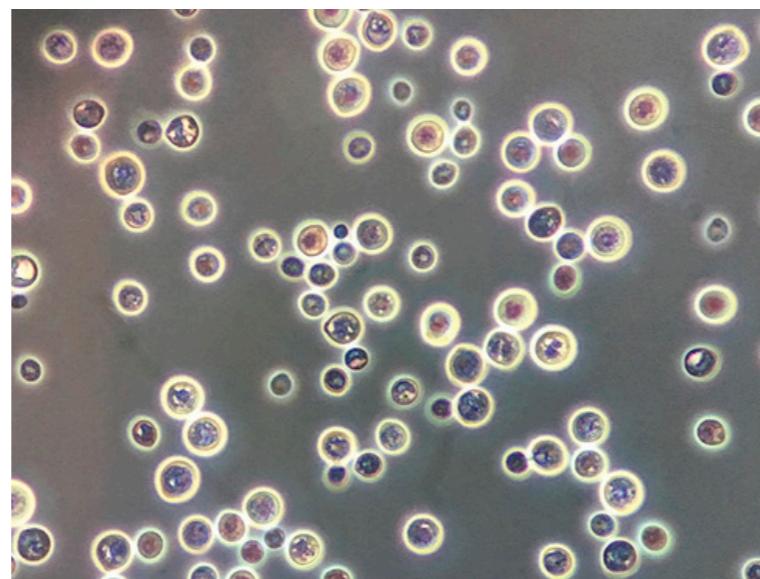
# Response to infection depends on



health center



host immunity



pathogen

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