Host Response to Fungal Infections

- immunology
- immunogenetics
- fungal pathogenicity

Salomé LeibundGut-Landmann
salome.leibundgut-landmann@uzh.ch

Universität Zürich[UZH]

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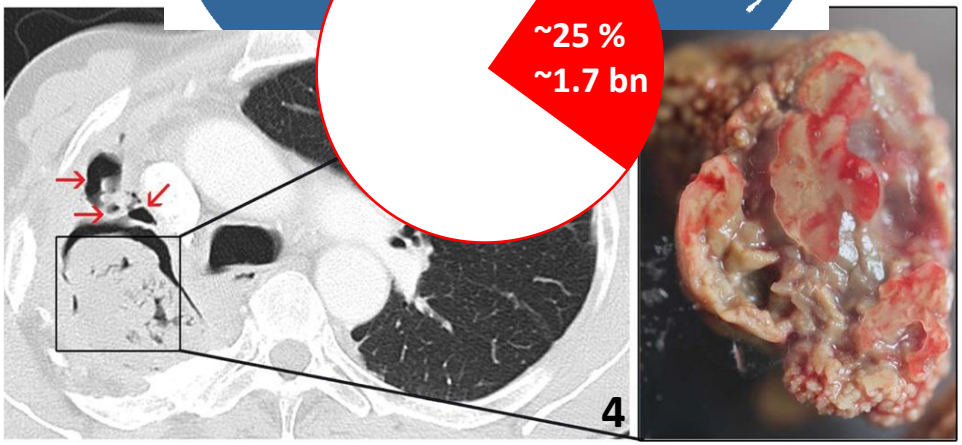
Molecular Diagnostics 2017, Zürich

Superficial fungal infections



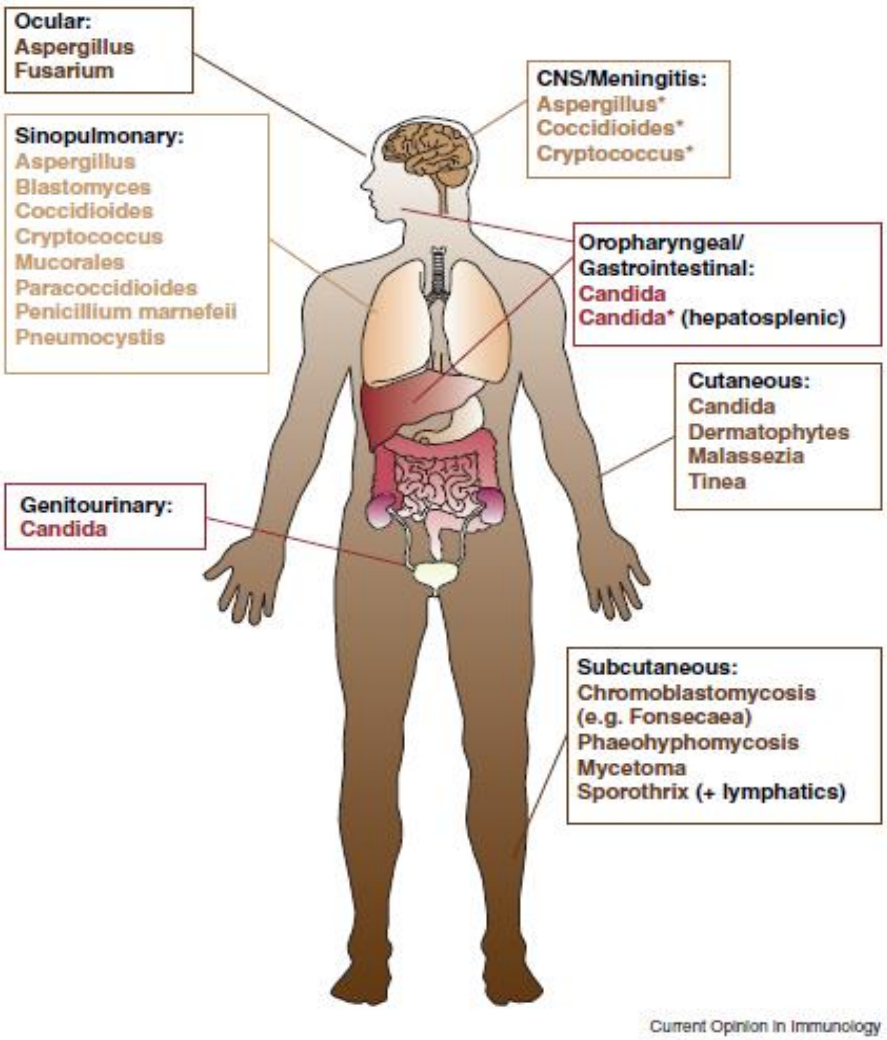
Systemic fungal infections

Mycosis	# life-threatening cases/year
Aspergillosis	>200,000
Candidiasis	>400,000
Cryptococcosis	>1,000,000
Mucormycosis	>10,000
Pneumocystosis	>400,000
Dimorphic (endemic) mycoses	~65,000

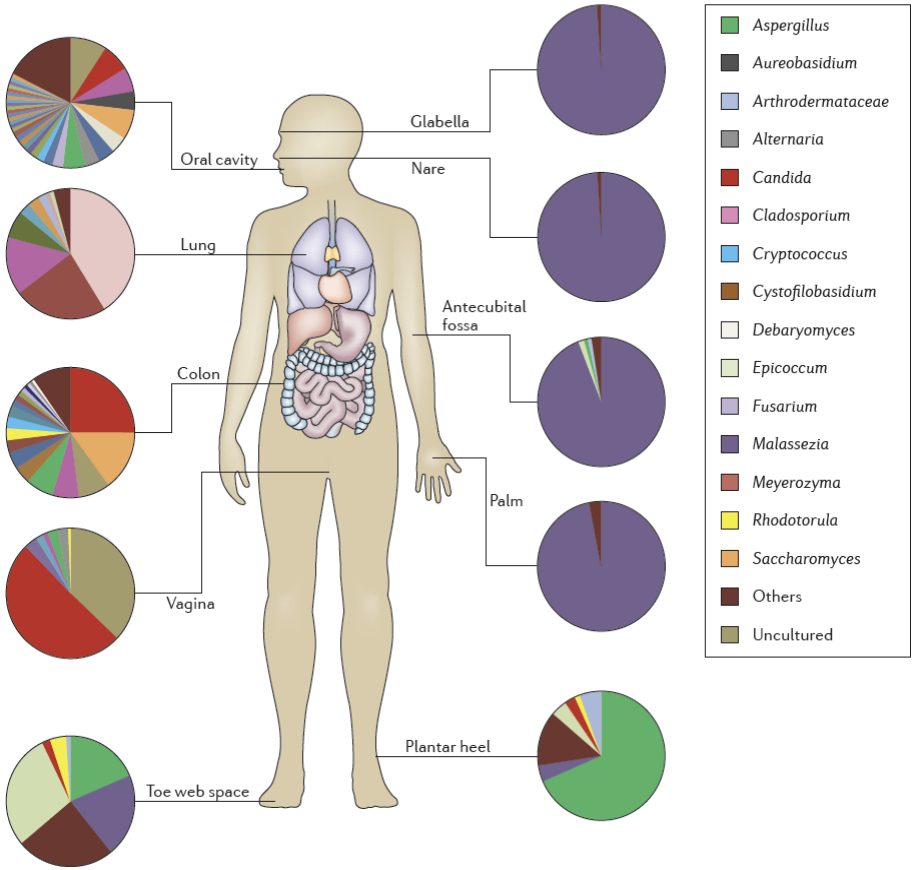
mainly nosocomial

mortality rate: 30-70%

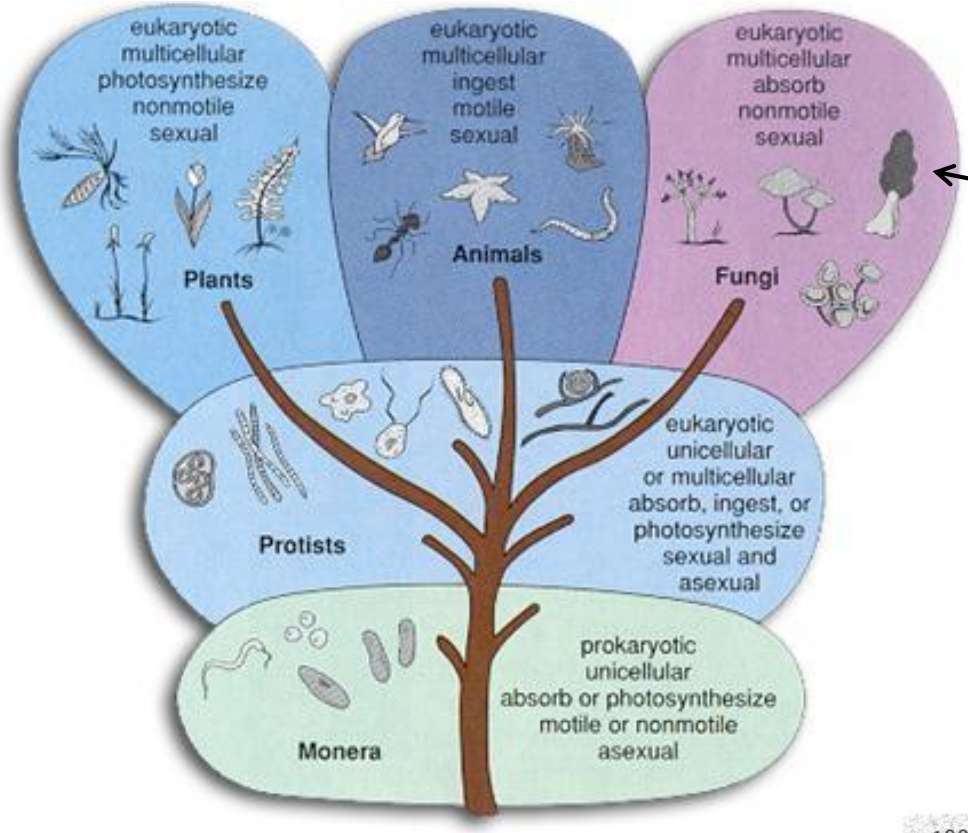
The spectrum of fungal diseases



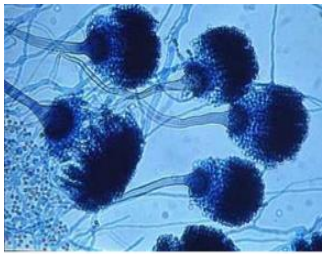
The organisms causing disease are mainly opportunistic pathogens



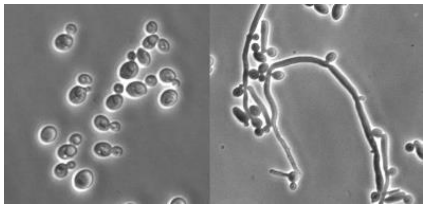
Underhill and Iliev, Nat.Rev.Immunol., 2014



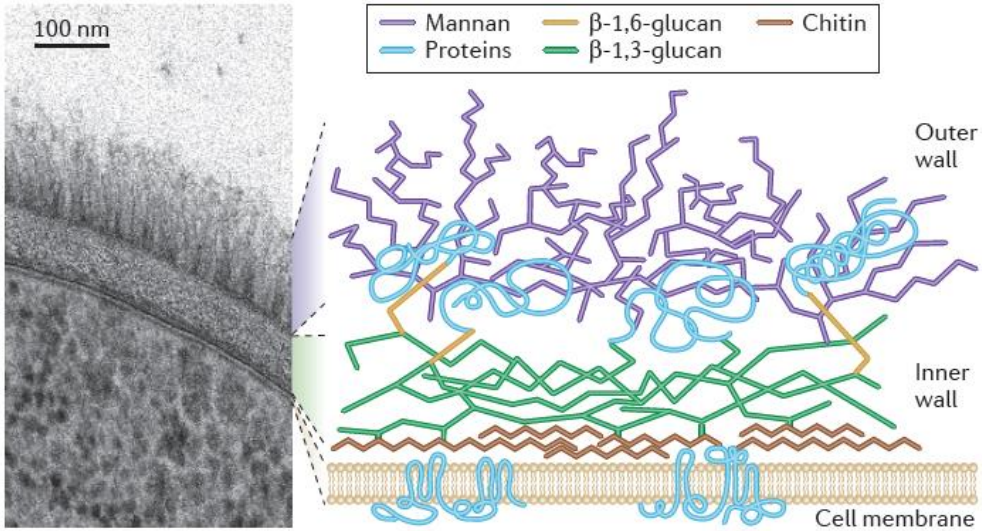
~ 10^5 fungal species
(only a few hundreds are pathogenic)



mold (*Aspergillus*)

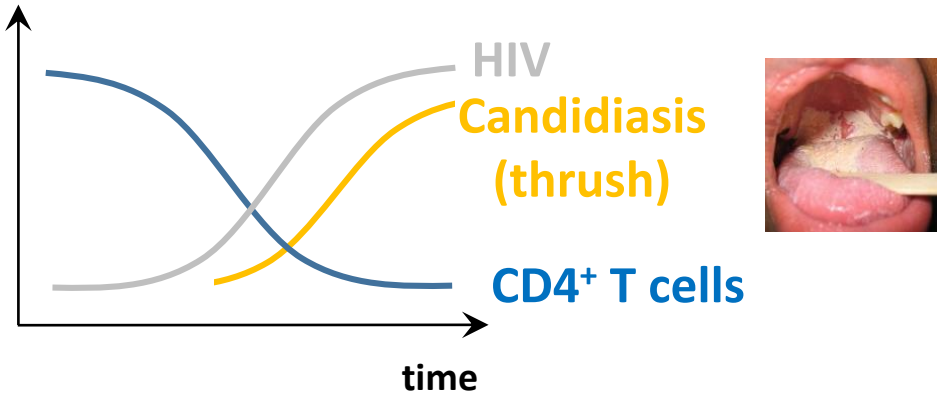


yeast (C. albicans)



Predisposing conditions for opportunistic fungal infections

- **barrier defects** e.g. severe burn wounds, cathethers
- **dysbiosis** e.g. vulvovaginal candidiasis, systemic candidiasis
- **chemotherapy** e.g. in leukemia patients
- **immunosuppression** e.g. in transplant patients
- **immune defects** - neutropenia → e.g. systemic candidiasis
  - functional neutrophil defects → e.g. aspergillosis in CGD patients
  - T cell defects → e.g. superficial candidiasis



Despite similar clinical risk factors:

some patients rapidly develop fungal infections,

while others seem to be protected and never do so.

Genetic Differences ?

1. **Immunogenetics of systemic candidiasis and aspergillosis**

2. **Congenic defects associated with superficial fungal infections**

3. **Genetic diversity of the fungus**

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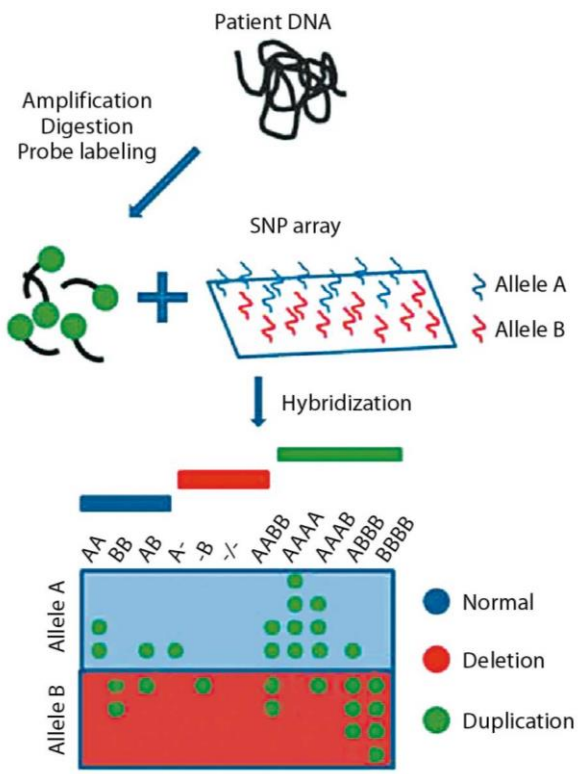
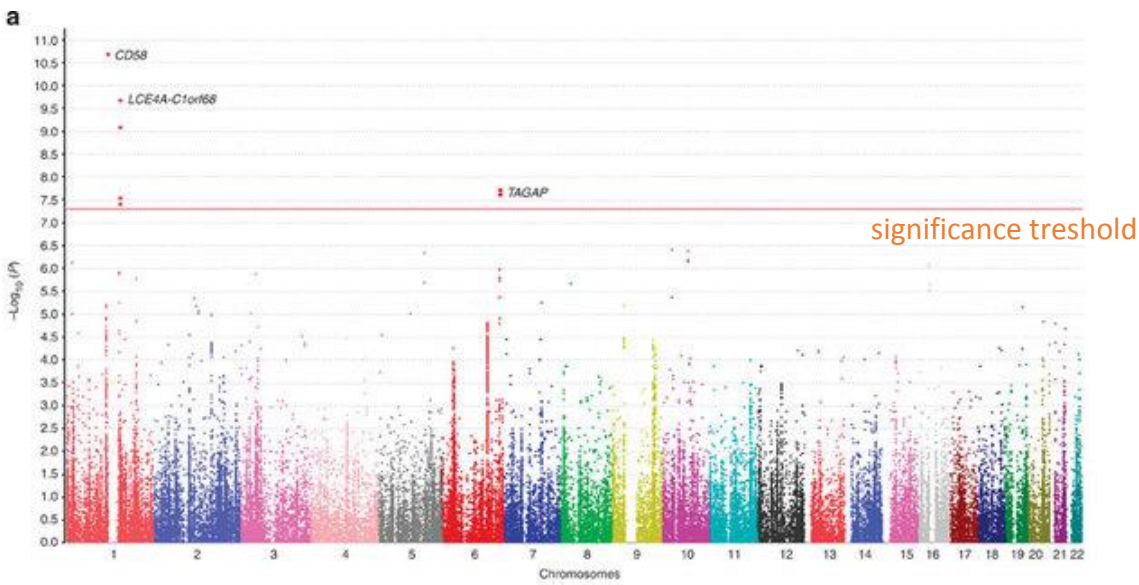
SNP array



→ limited to the SNPs on the array

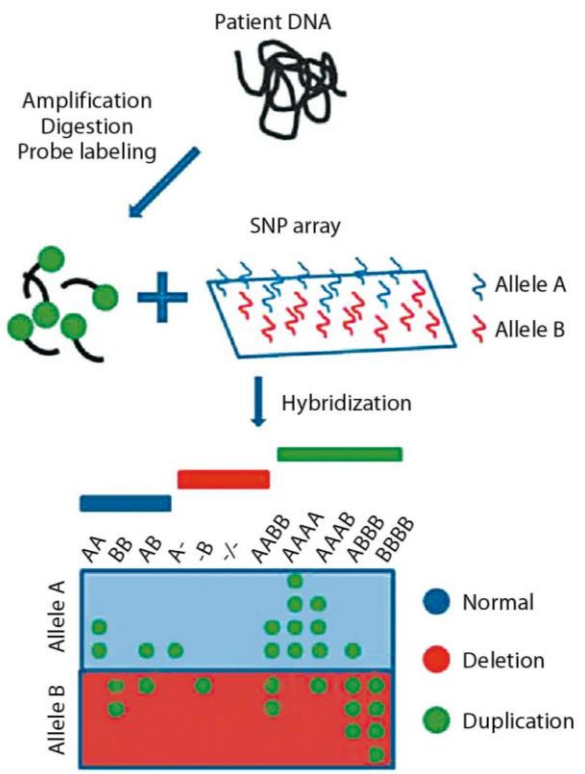
screen of 200'000 SNPs in a large candidemia cohort



→ identify novel genetic risk factors for candidaemia

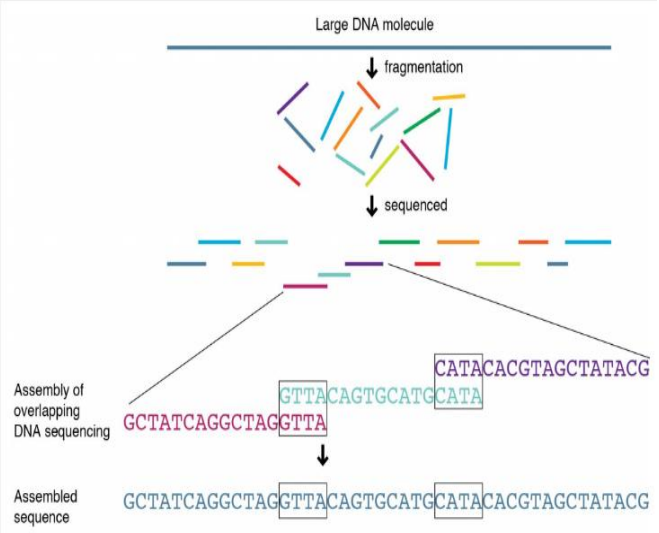
Kumar et al., Nat.Communications (2014)

SNP array

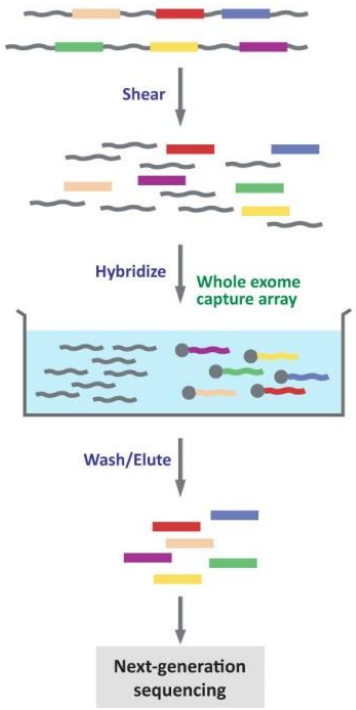


→ limited to the SNPs on the array

whole genome sequencing



whole exome sequencing

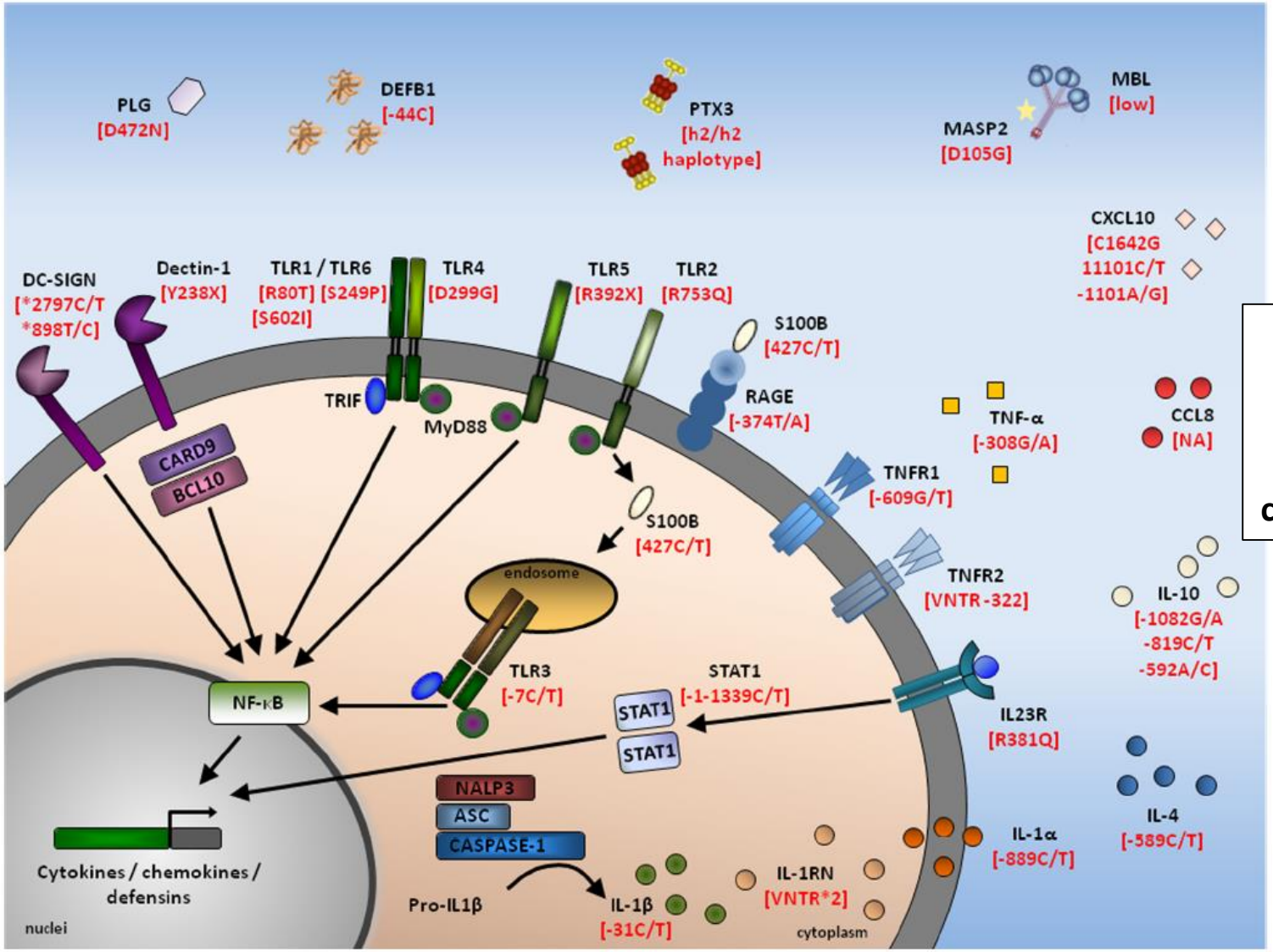


→ the majority (85%) of all disease-causing mutations occur in the exome

→ the exome is 1-2% of the entire genome

→ WES = cost effective

Candidate gene polymorphisms associated with invasive *Aspergillus* and/or *Candida* infections



PRRs
cytokines
chemokines
cytokine receptors
cytokine antagonists

Wójtowicz and Bochud, Semin Immunopathol (2015)

Caveats:

- sample size
- study design, controls
- statistical testing
- confirmation by independent cohorts
- functional evidence supporting the association

Table 1: Genetic risk factors for invasive fungal infections.

Gene	SNPs ID	Genetic association[1]		Replication	Functional evidence
		IA	IC		
Pattern recognition receptors					
TLR1	rs574361	↑	↑	Controversial	+/–
	rs4833095, rs5743618	↑	↓	Controversial	+/–
TLR6	rs5743810	↑	NA	Controversial	–
TLR4	rs4986790, rs4986791	↑	↑	Controversial	+/–
TLR2	rs5743708	NA	↑	Controversial	+
TLR3	rs3775296	↑	NS	No	+
TLR5	rs5744168	↑	NS	No	–
TLR9	rs5743836	NA	NA	No	–
CLEC7A	rs16910526	↑	NA	Controversial	+
	rs7309123, rs3901533	↑	NS	No	+
CD209	rs4804800, rs11465384, rs7248637, rs7252229	↑	NS	No	–
MBL	Low genotype	↑	↑	No	+/–
Cytokines and related genes					
IL1A	rs1800587	↑	NS	No	+
IL1B	rs1143627, rs16944	↑	NA	Controversial	+
IL1RN	82bp VNTR	↑	NS	No	+
	rs419598	↑	NS	No	+
IL4	rs2243250, rs2070874, rs2243248	NS	↑	No	–
IL8	rs2227307	↑	NS	No	–
IL10	rs1800896, rs1800871, rs1800872	↑	↑	Controversial	+/–
IL12B	rs41292470	NA	↑	No	+
	rs3212227	↓	NS	No	–
IL23R	rs11209026	↓	NS	No	+/–
TNFA	rs1800629	NA	↑	No	–
TNFR1	rs4149570	↑	NS	No	+
TNFR2	-322 VNTR	↑	NS	No	–
IFNG	rs2069705	↓	NS	Yes	+
	rs2430561	NA	NS	No	–
CCL8	1-kg-17-29697448	NS	↑	No	+
CXCL10	rs3921, rs1554013, rs4257674	↑	NS	No	+
CXCR1	rs2234671	NS	↑	No	+
CX3CR1	rs3732378	NS	↑	Yes	+

Khanna et al, Swiss Med Wkly (2016)

Caveats:

- sample size
- study design, controls
- statistical testing
- confirmation by independent cohorts
- functional evidence supporting the association

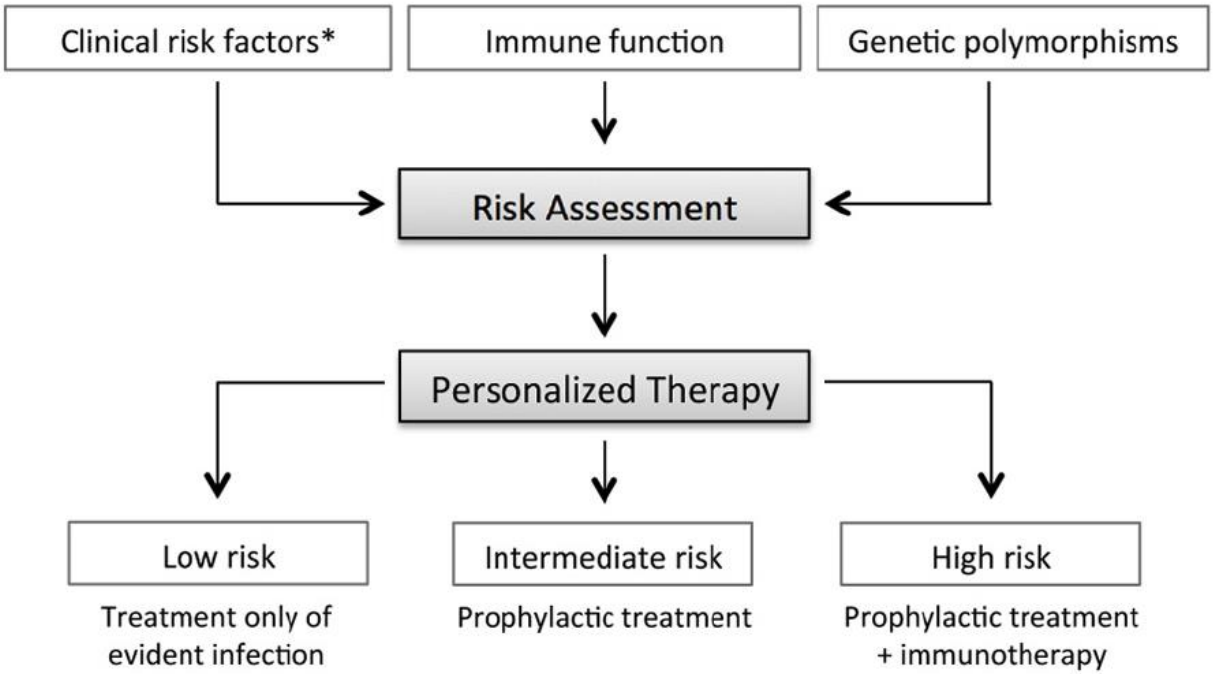
Is the identified mutation the disease-causing mutation?

→ Validation!

- synonymous vs. non-synonymous SNPs
- exclude SNPs that are frequent gene variants
- analyse the transcriptome and proteome to find the relevance of the mutation

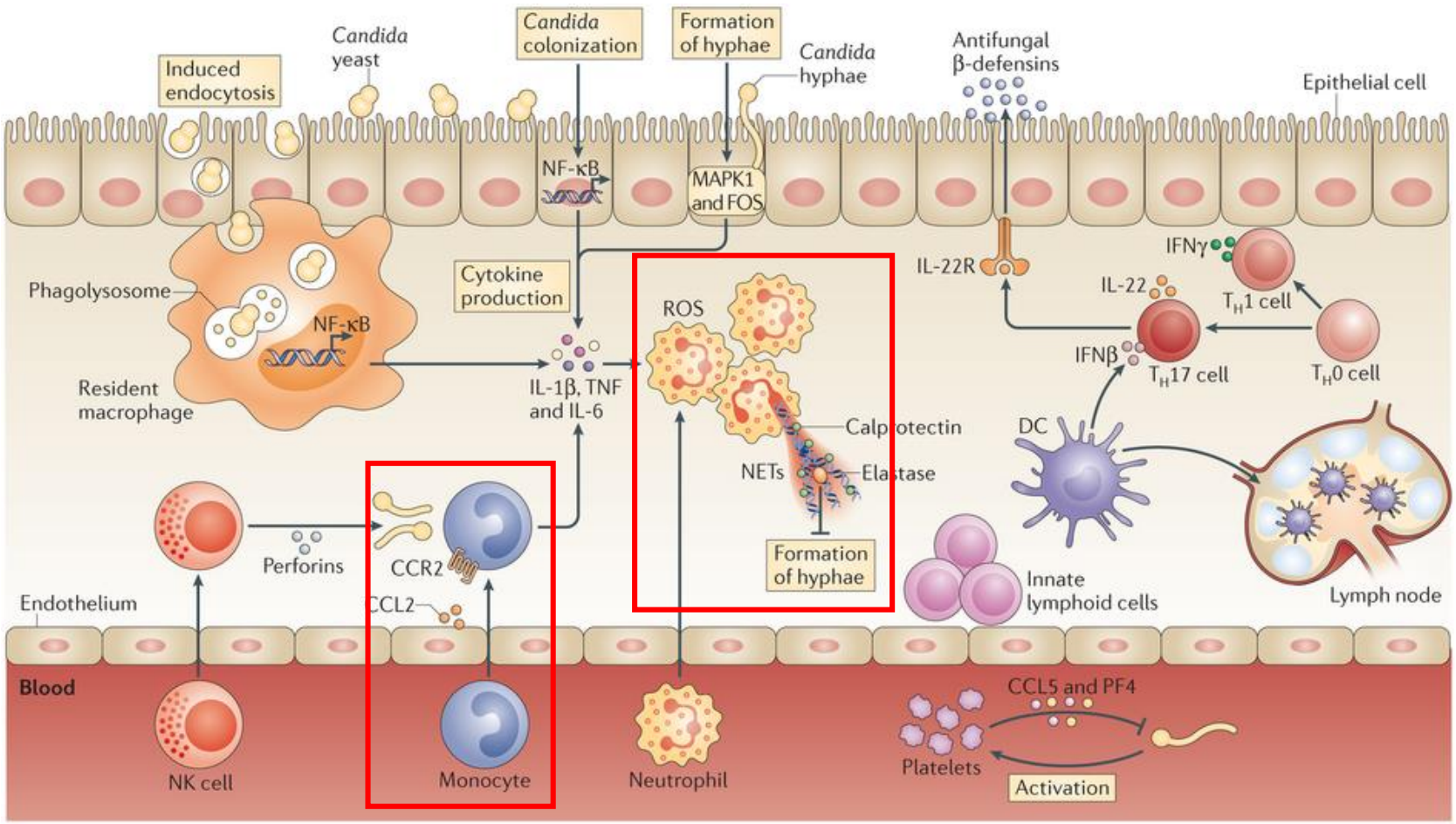
Future perspectives:

- **assign each patient an individual risk score based on his genetic background ('genetic prediction')**
- **apply personalized prophylaxis and treatment schemes (as in modern oncology)**
- **Whole-Exon-Sequencing will likely be the method of choice.**



Khanna et al., Swiss Med Wkly (2016)

Identification of novel mutations also promotes the understanding of protective mechanisms against systemic fungal infection



Nature Reviews | Immunology

1. Immunogenetics of systemic candidiasis and aspergillosis

2. **Congenic defects associated with superficial fungal infections**

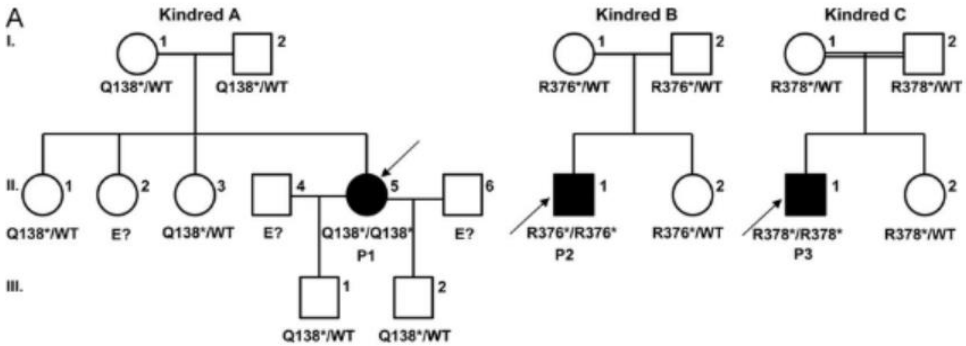
3. Genetic diversity of the fungus

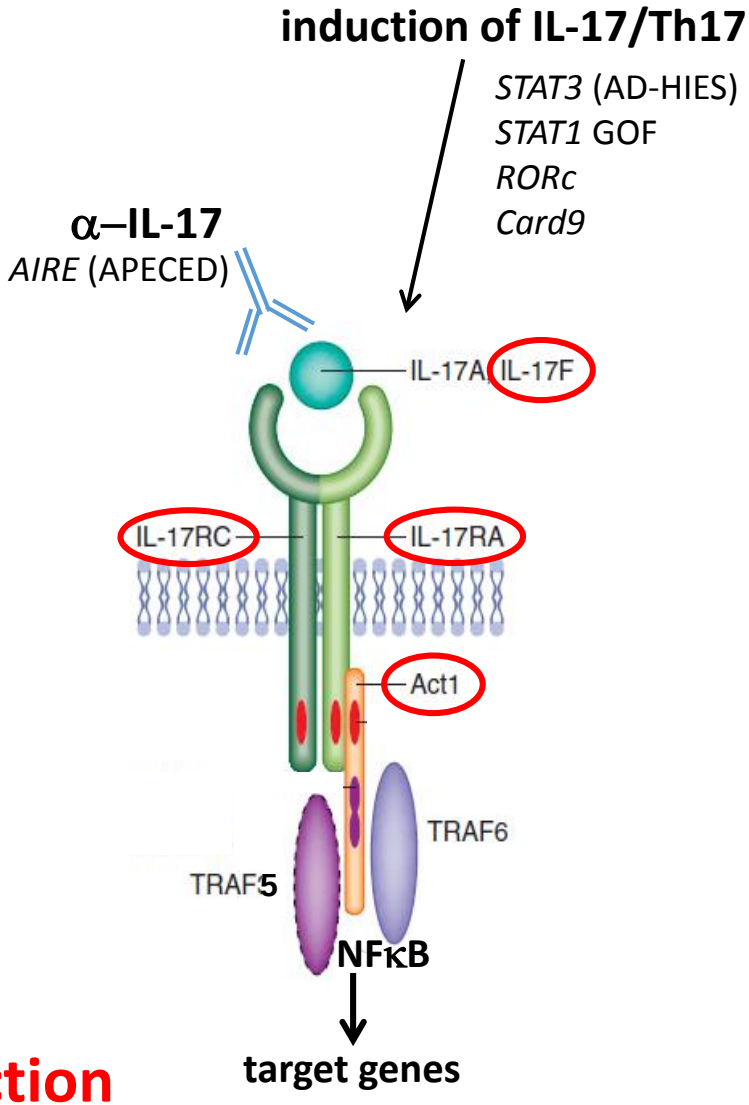
Mendelian defects in the IL-17 pathway

Chronic Mucocutaneous Candidiasis (CMC)

- oroal mucosa
- skin
- nails
- vaginal mucosa

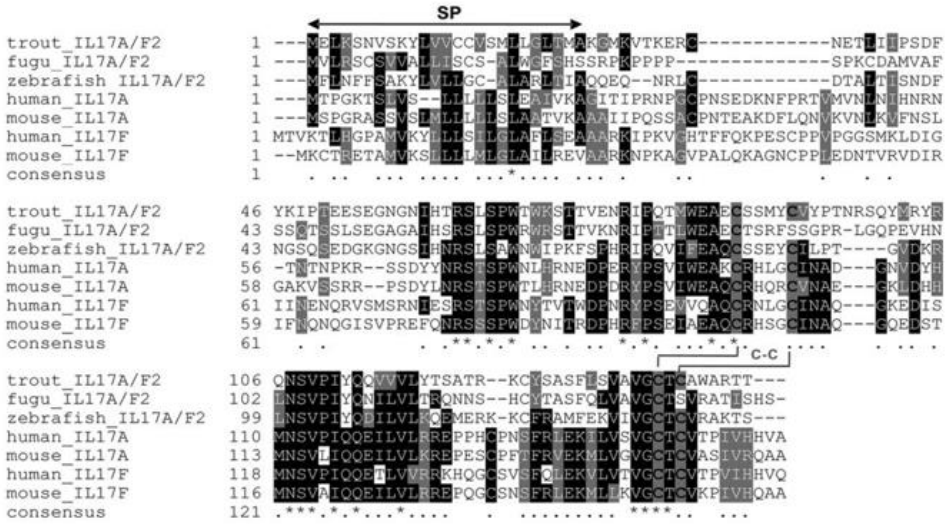
e.g. inherited IL-17RC-deficiency (Ling et al., JEM, 2015)



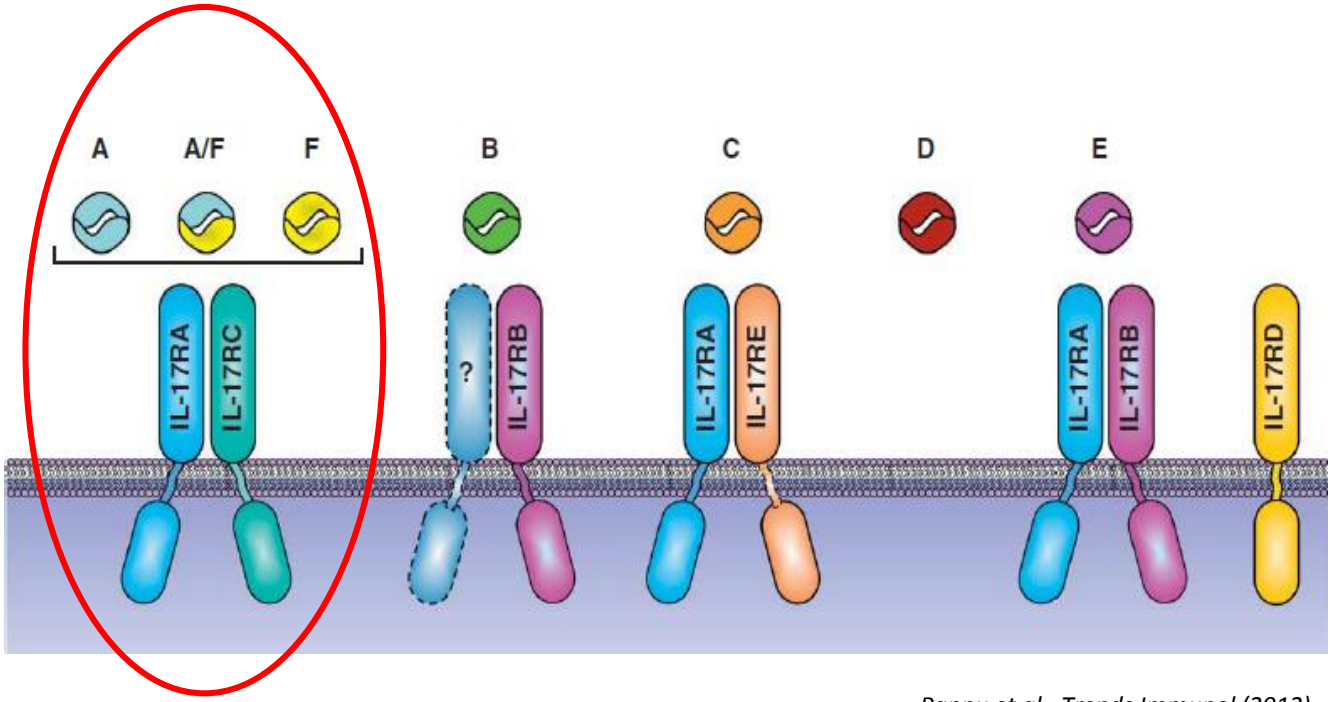


→ IL-17 plays a non-redundant role in protection from superficial *Candida* infection

The IL-17 cytokine family



Monte et al., Infect. Immun. (2013)



Pappu et al., Trends Immunol (2012)

IL-17 in autoinflammatory disease

targetting IL-17 as a therapeutical appraoch

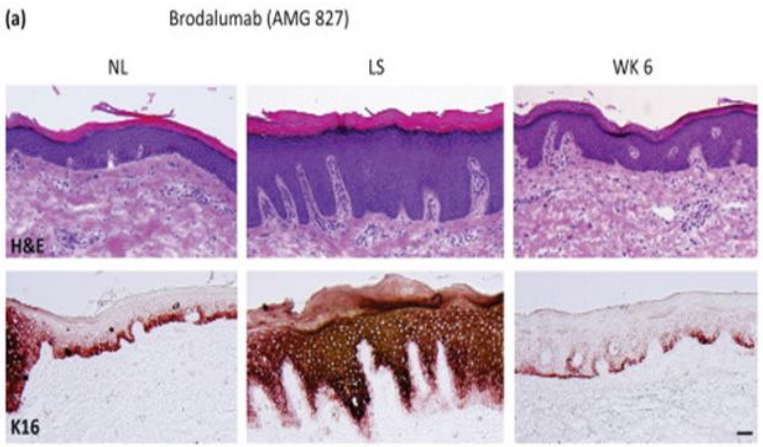
Agent	Mechanism	Clinical status[a]		
		Psoriasis	Rheumatoid arthritis	Psoriatic arthritis
Secukinumab	Anti-IL-17A			
Ixekizumab	Anti-IL-17A			
Brodalumab	Anti-IL-17RA			
SCH 900117	Anti-IL-17A			
ABT-122	Anti-IL-17A/TNF			
RG7624	Anti-IL-17A/IL-17F			

response rate:

high moderate-low moderate

[a]Based on ongoing clinical trials according to clinicaltrials.gov on 7 June 2013.
[b]Phase 2 study completed; no clinical trials in RA are ongoing.

Kirkham et al., Immunology, 2013



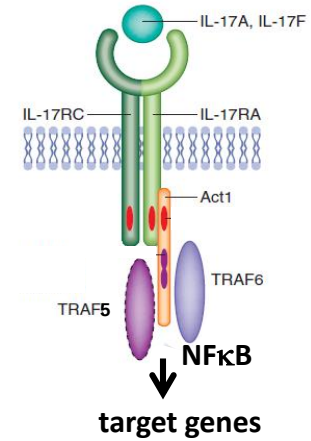
Lowes et al., TRIMM, 2013

FDA-aproved in 2015

side effects...

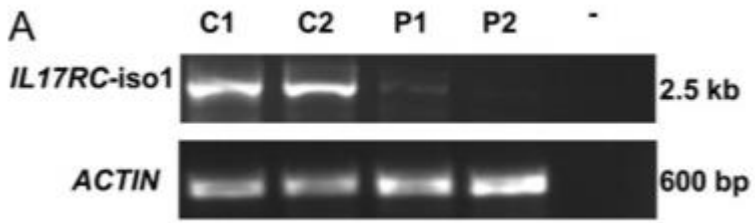
"...localized mucosal or cutaneous candidiasis, primarily oral candidiasis. These infections were mild to moderate in severity and were clinically manageable/treatable with standard therapy without discontinuation. "

Mendelian defects in the IL-17 pathway

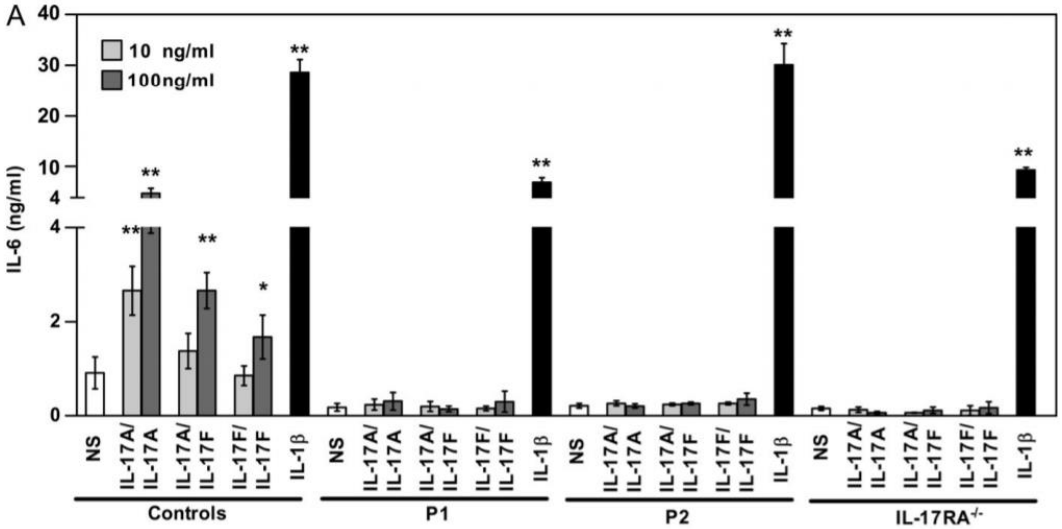


e.g. inherited IL-17RC-deficiency

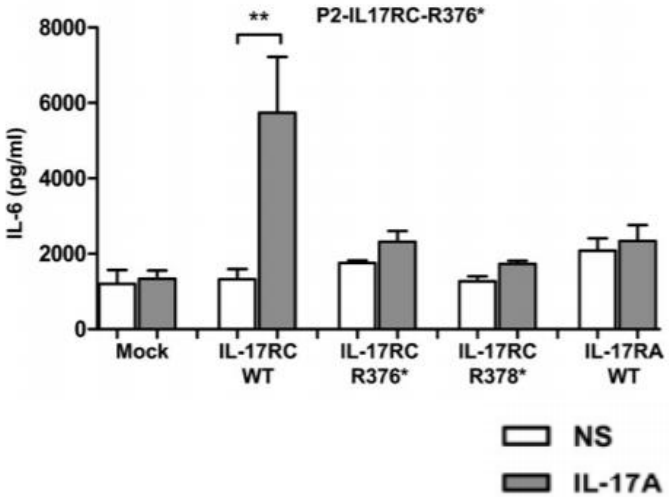
immortalized fibroblasts from patients (P1, P2) & controls (C1, C3)



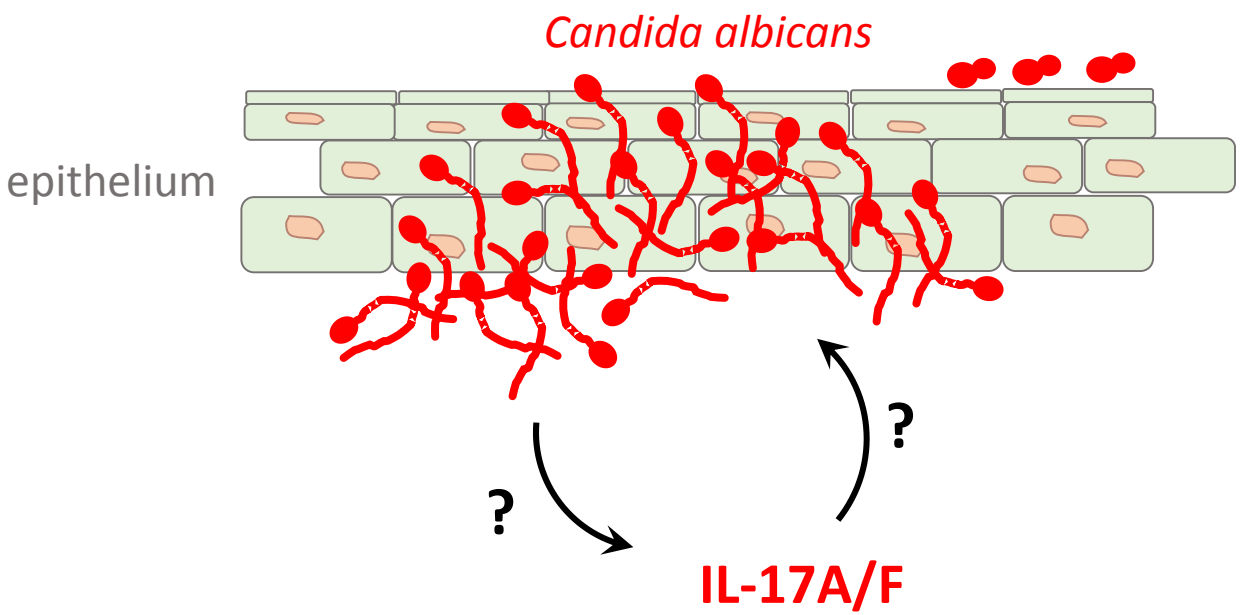
response to IL-17 stimulation



transfectants

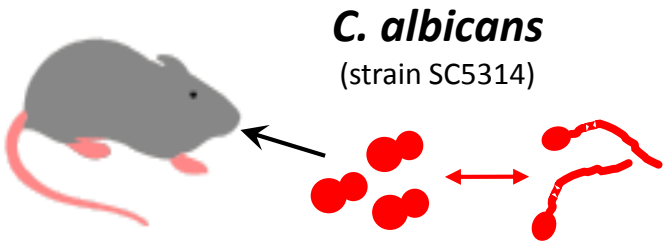


(Ling et al., JEM, 2015)



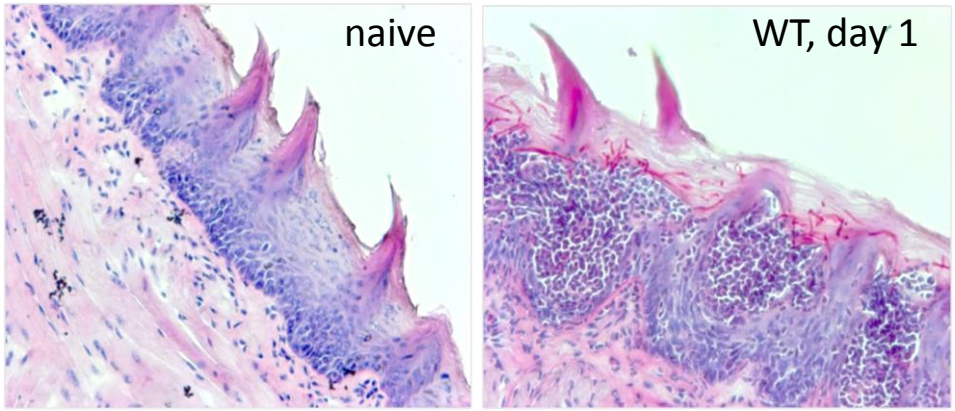
- **Regulation of IL-17 induction in response to *C. albicans***
- **IL-17-mediated effector mechanisms**
- **IL-17-independent antifungal defense**

Experimental model of oropharyngeal candidiasis (OPC)

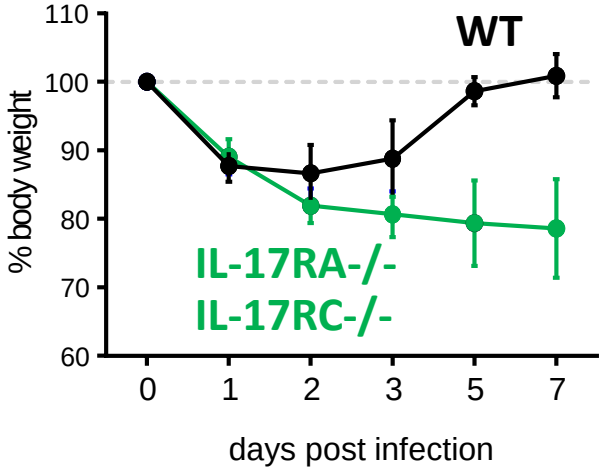


C57BL/6 (WT)

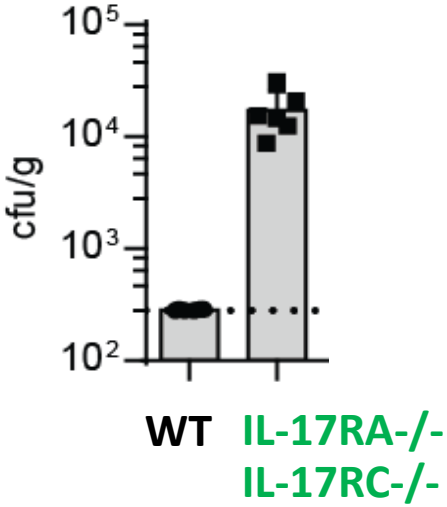
IL-17RA-/-
IL-17RC-/-



→ acute inflammatory response

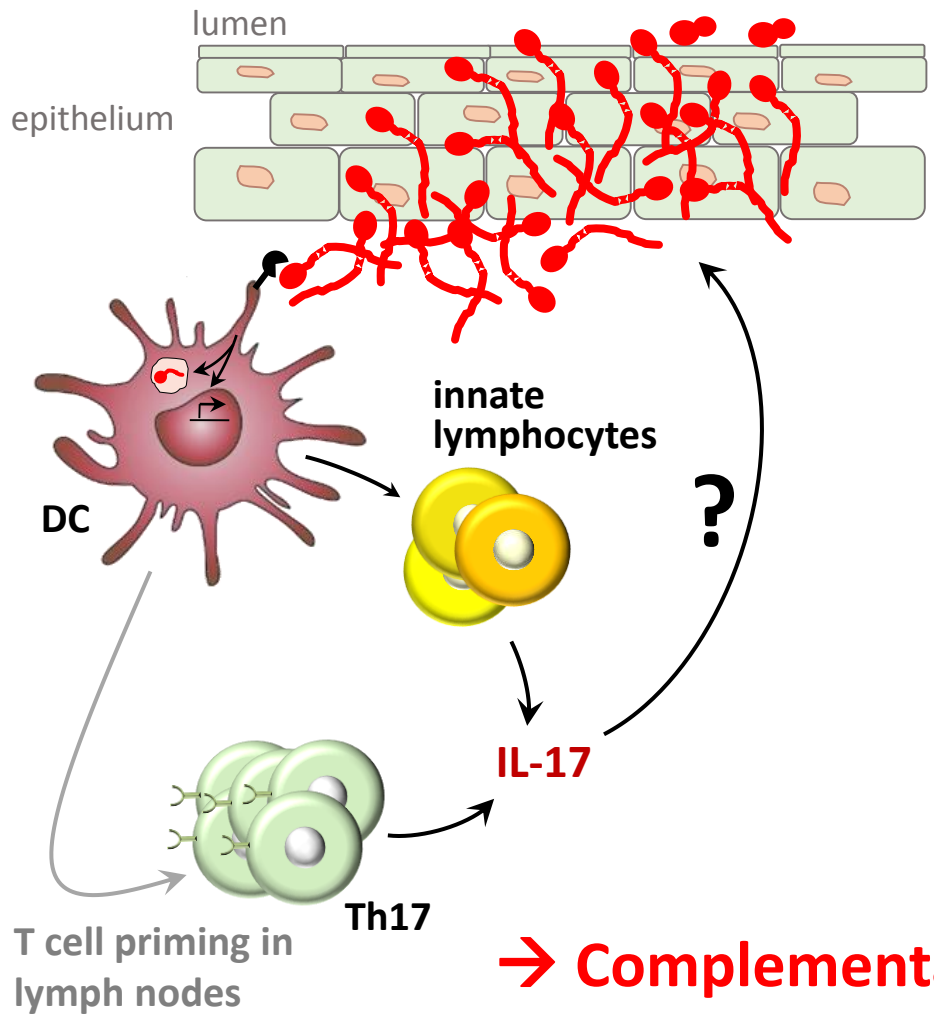


day 7 p.i.

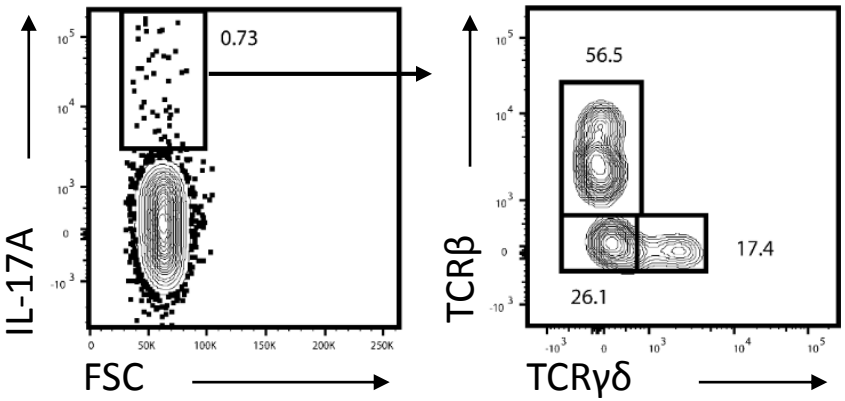


Trautwein-Kirchner et al., Mucosal Immunology 2015

Multiple sources of IL-17 during acute OPC infection



Viable CD45⁺ CD90⁺ tongue cells, 1 day post-inf.

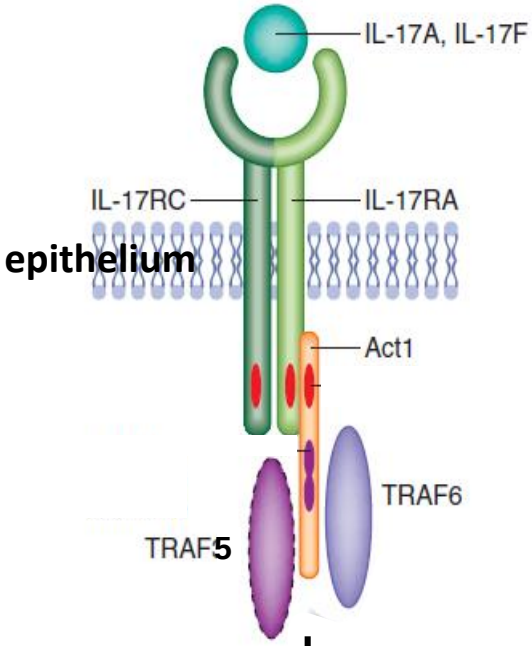


→ Complementary and redundant cellular sources for the IL-17 response during murine OPC

(in humans?)

Gloadiator et al., J.Immunol (2013)
Trautwein-Weidner et al., PLoS Pathogens (2015)
Sparber et al, in preparation

IL-17 & neutrophils



↓

NFκB
MAPK

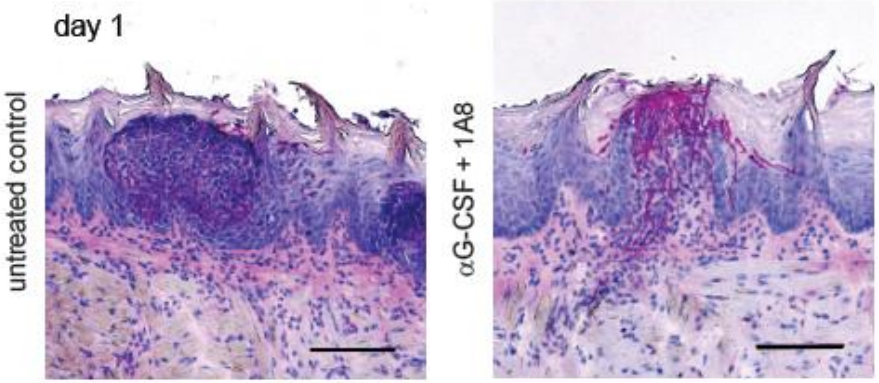
↓

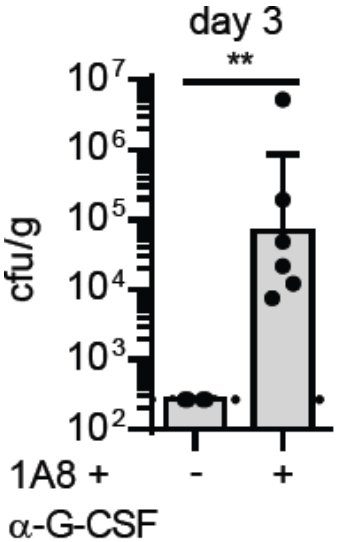
target genes
chemokines, cytokines

↓

neutrophils

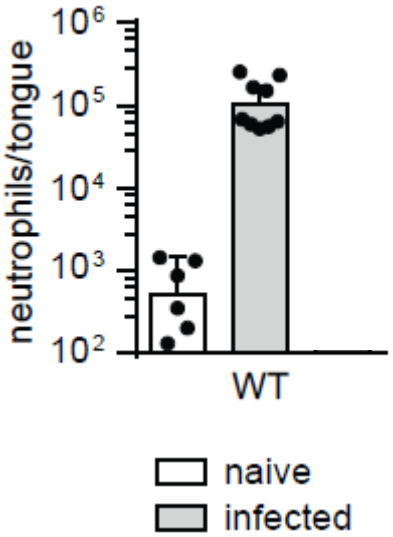
control **neutrophil-depleted**

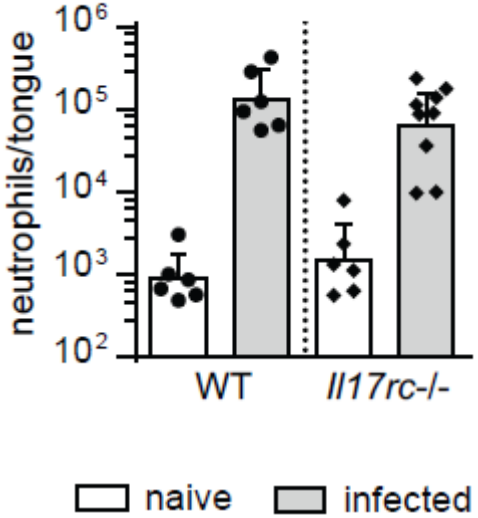


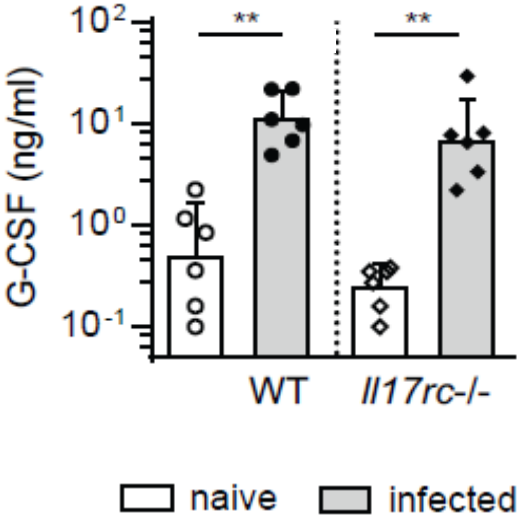


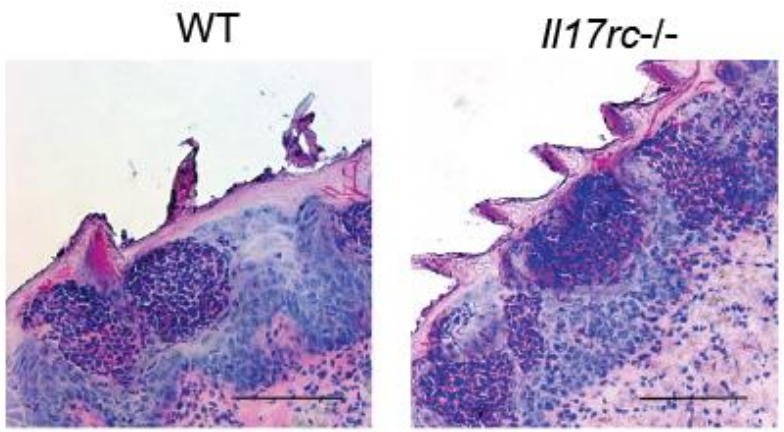
Trautwein-Weidner, Gladiator et al., Mucosal Immunology (2015)

Neutrophil recruitment during OPC is IL-17-independent

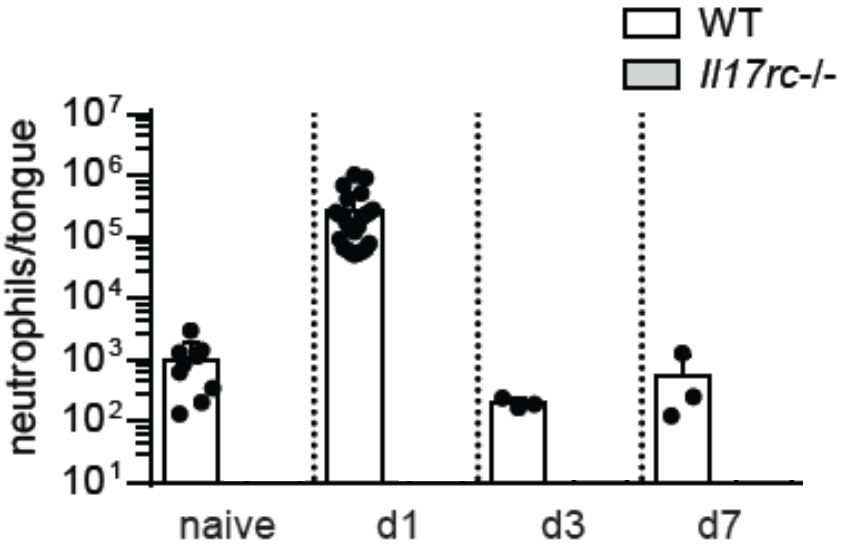






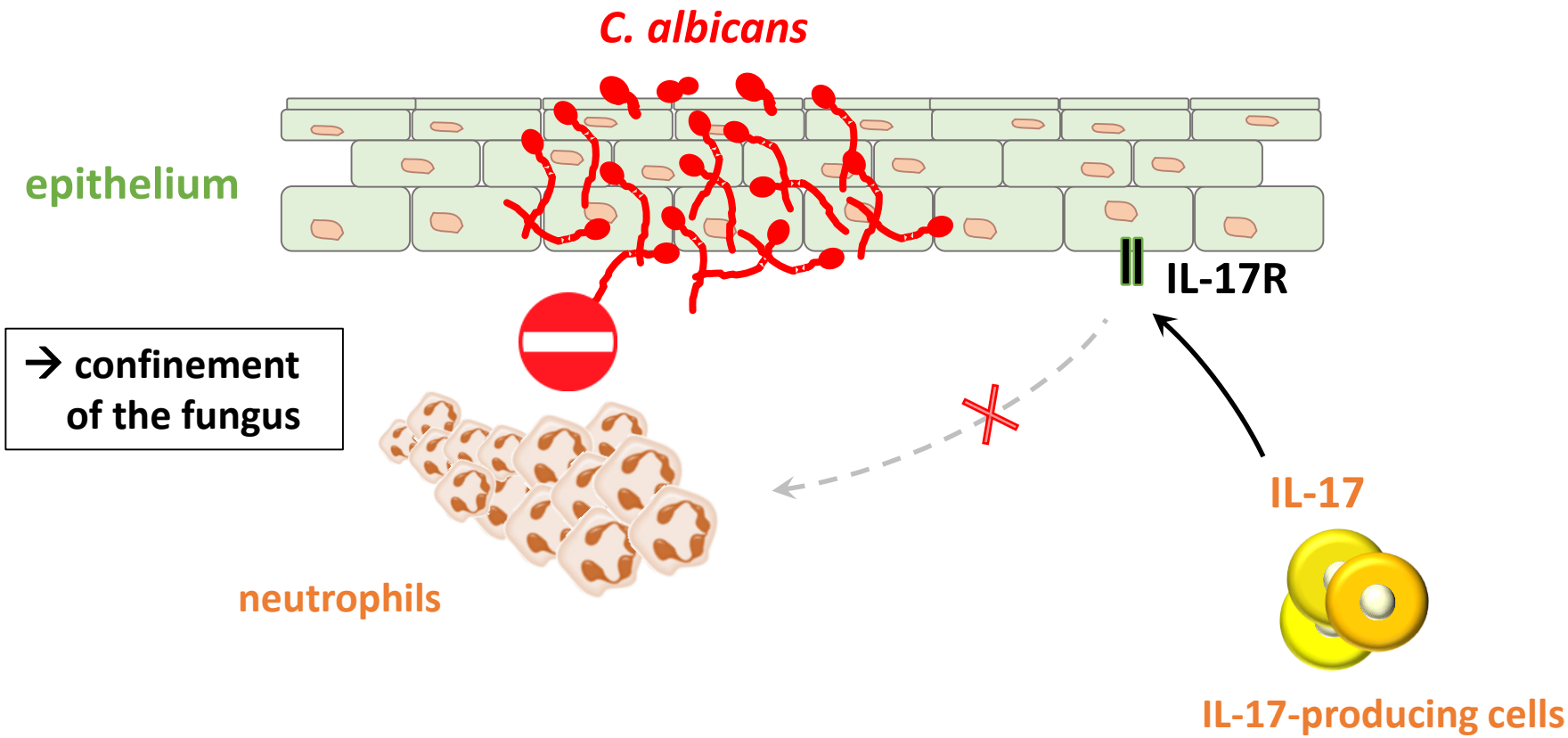


day 1 p.i.



Trautwein-Weidner, Gladiator et al., Mucosal Immunology (2015)

IL-17 immunity and neutrophil response are uncoupled and act complementary in the oral mucosa



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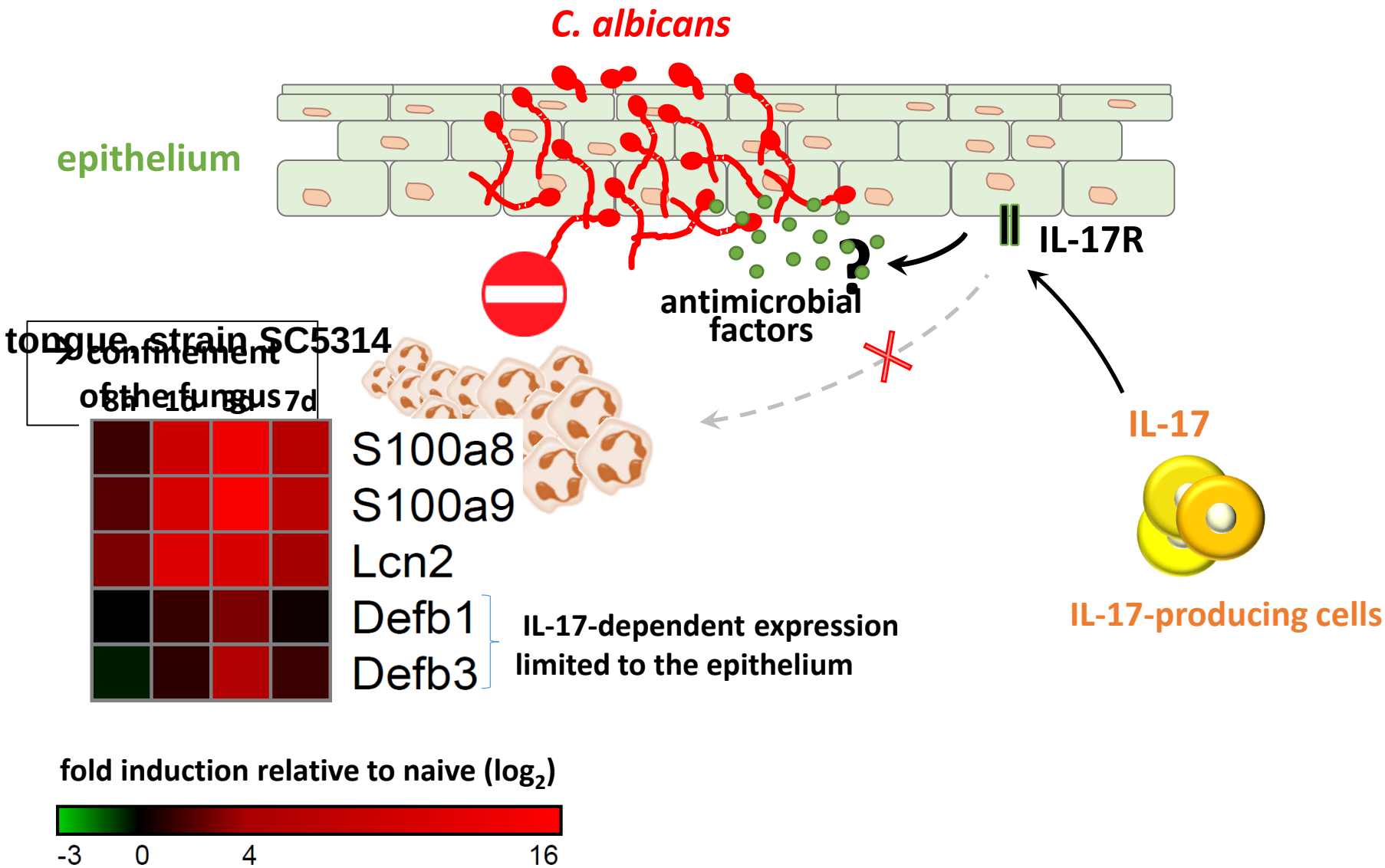


antifungal host defense research

genetic studies ↔ **experimental studies (human PBMCs, mouse models)**

Identification of genes/pathways that contribute to disease susceptibility

Generation of new hypothesis

Dissection of protective mechanisms at a cellular and molecular level

→ new therapeutic approaches, vaccines

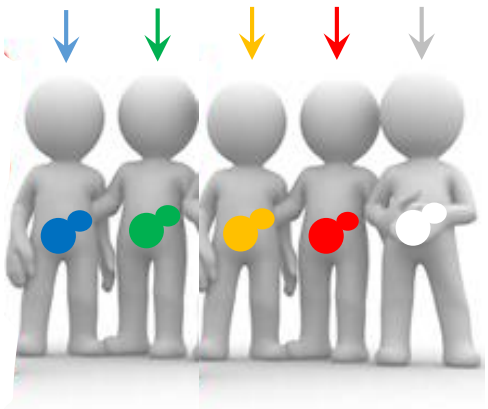
1. Immunogenetics of systemic candidiasis and aspergillosis

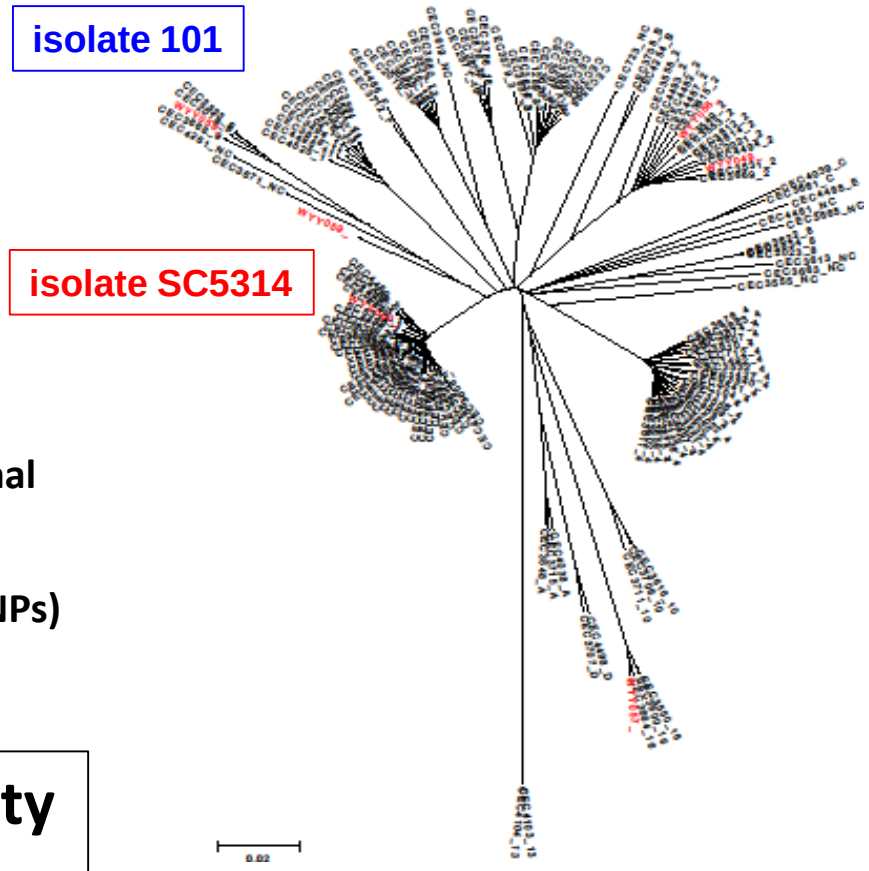
2. Congenic defects associated with superficial fungal infections

3. **Genetic diversity of the fungus**

→ **The host immune status determines susceptibility to disease**

→ **What about the fungus?**



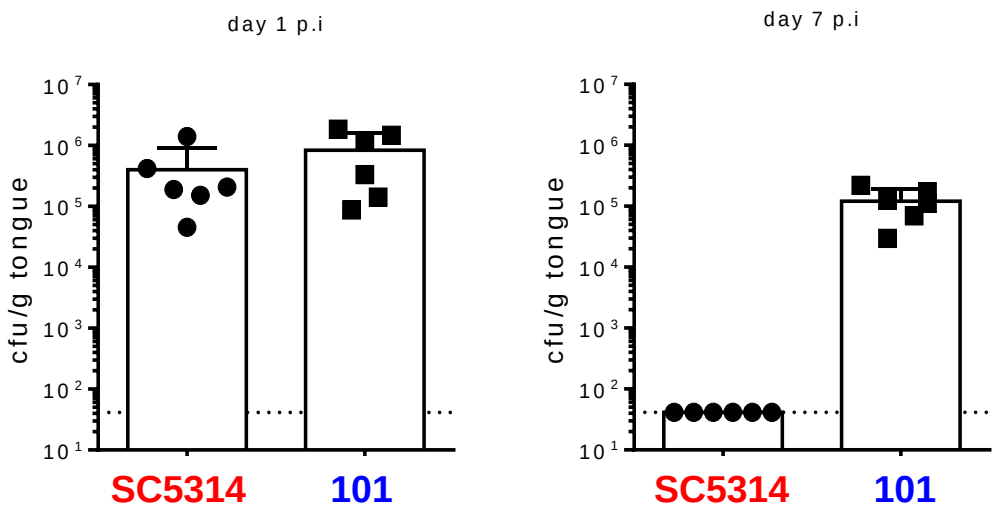


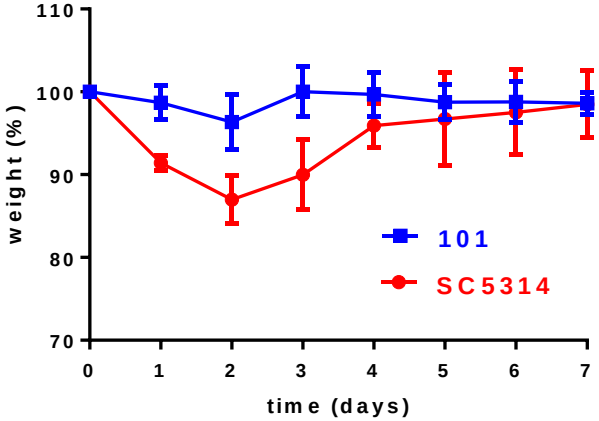
- **Colonizing population of *C. albicans* is monoclonal**
- **Vertical transmission**
- **Large intraspecies diversity (10'000 – 100'000 SNPs)**

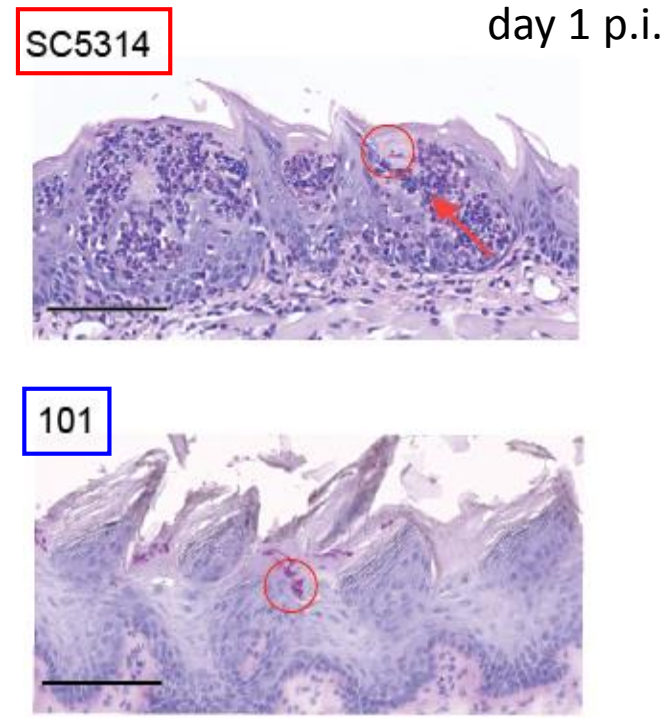
Impact of the intraspecies diversity on the outcome of infection?

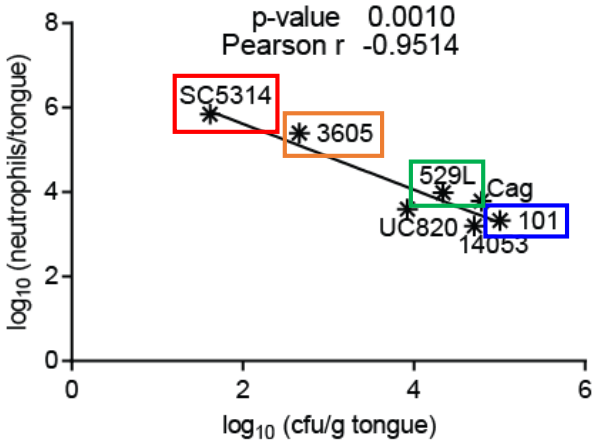
C. d'Enfert and ME. Bougnoux (Pasteur)

Very distinct outcomes of infection with different fungal isolates



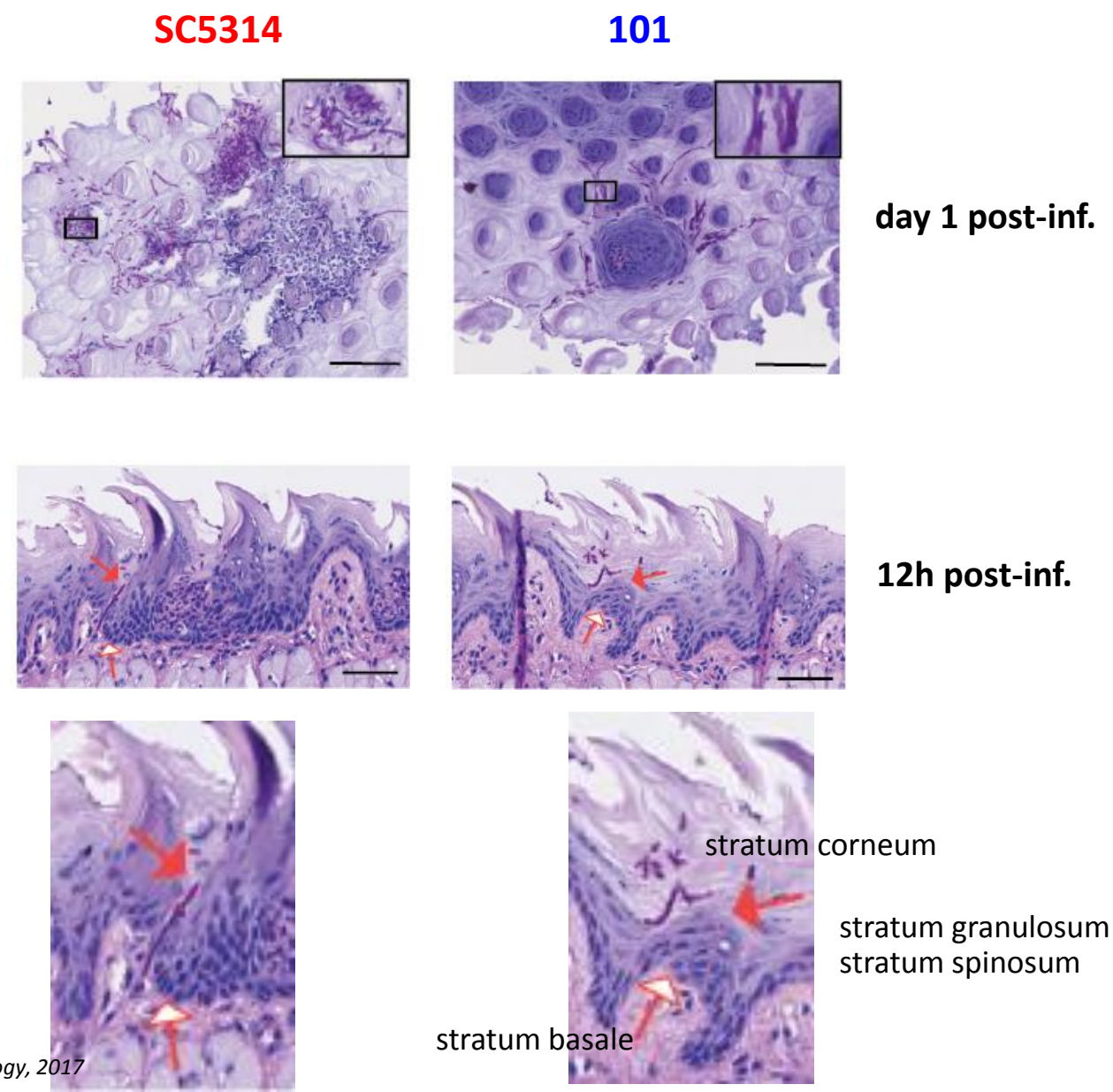






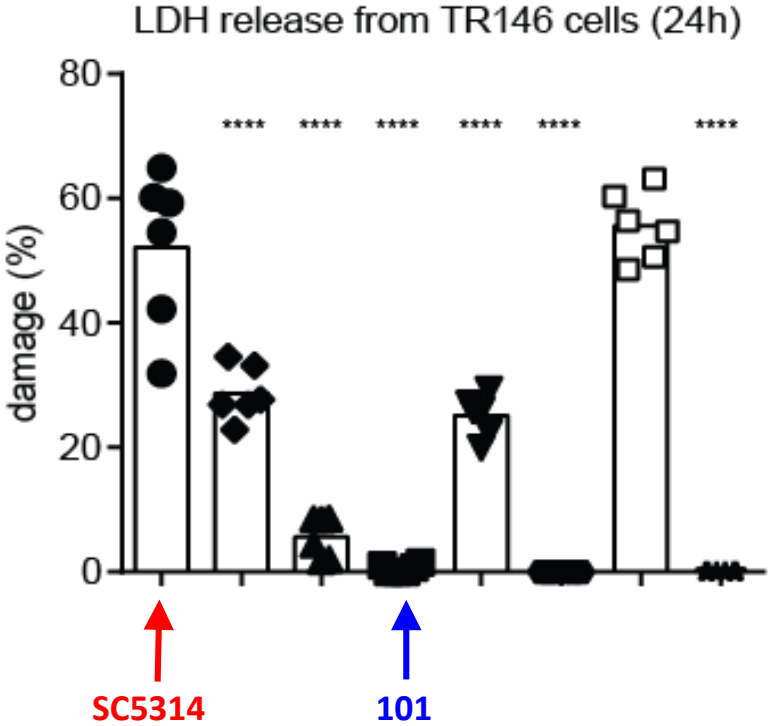
Schönherr et al., Mucosal Immunology, 2017

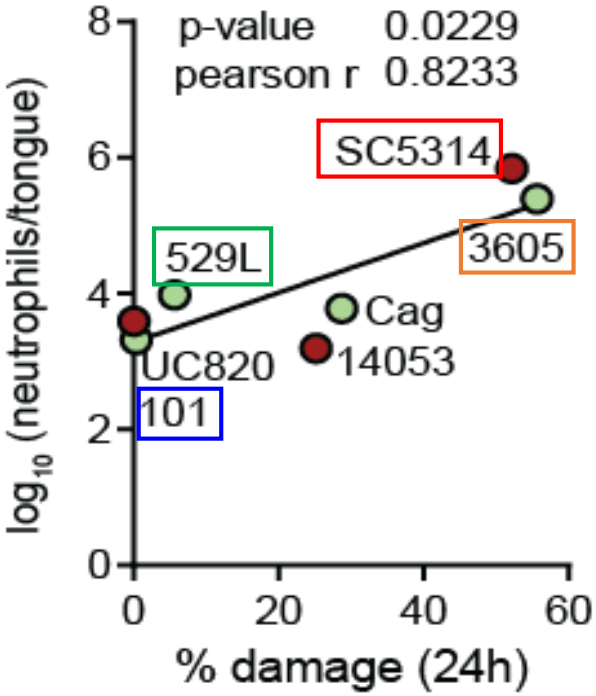
Pathogenicity correlates with the invasion depth into the epithelium



Schönherr et al., Mucosal Immunology, 2017

The epithelium senses the variation in *C. alicans* and translates it in various degrees of inflammation





Schönherr et al., Mucosal Immunology, 2017

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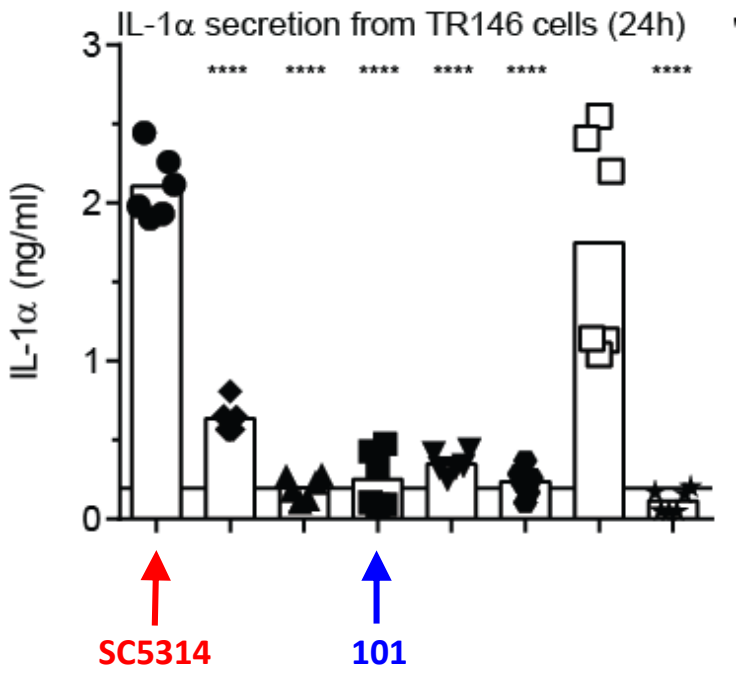
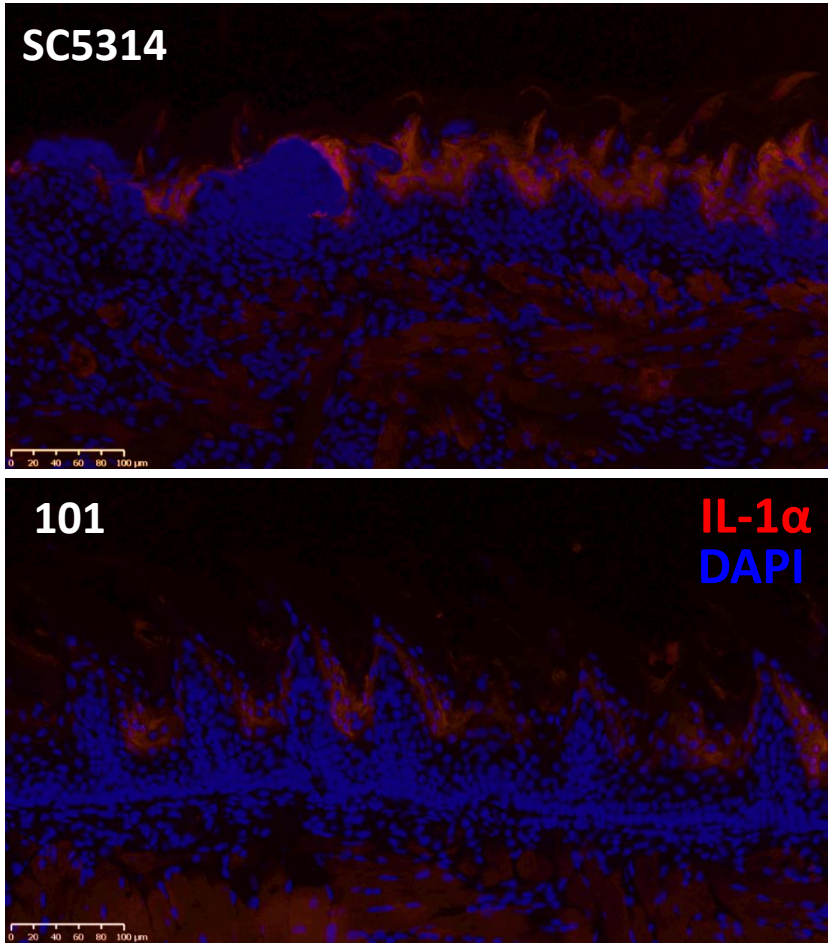


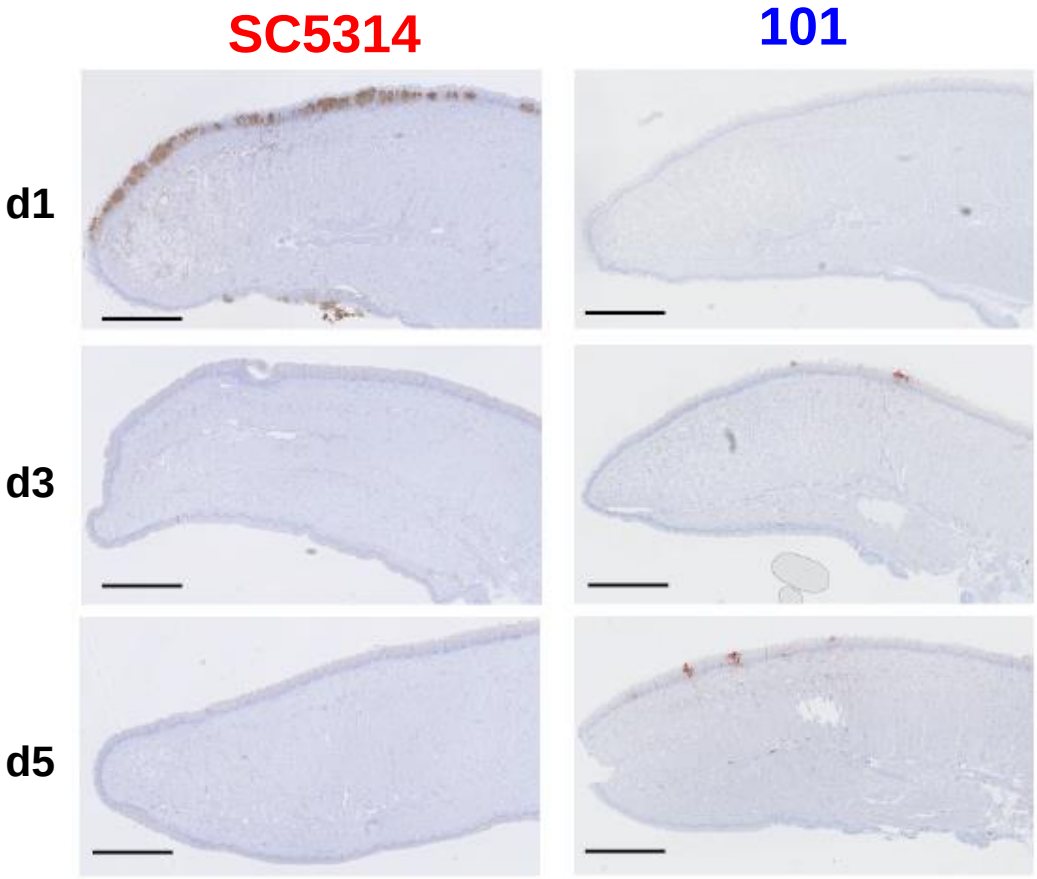
in vivo (1 day p.i.)



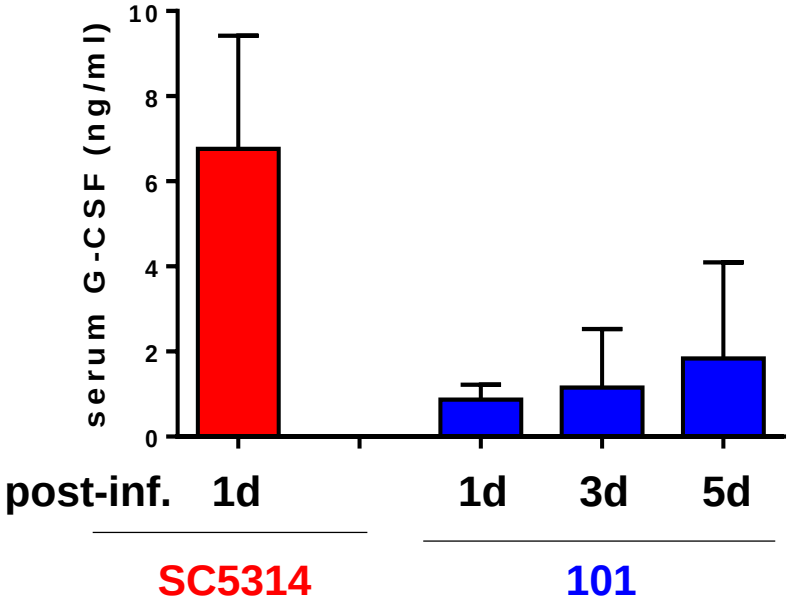
Are persistent strains 'unseen' by the immune system?

Schönherr et al., Mucosal Immunology, 2017

Persistent *C. albicans* isolates do not induce neutrophils

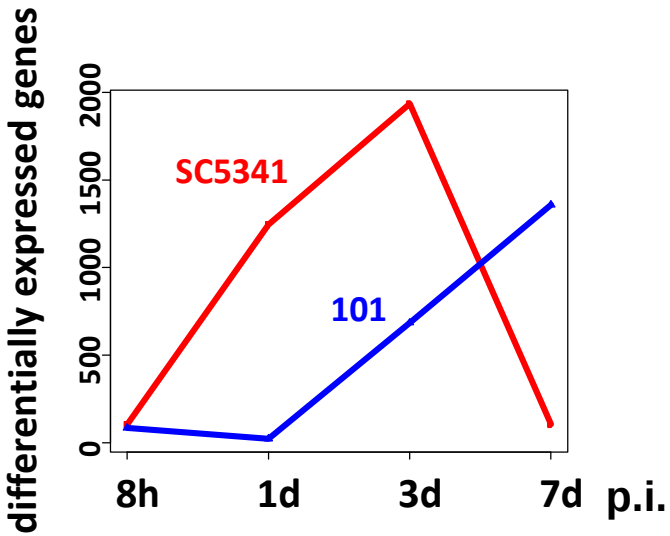


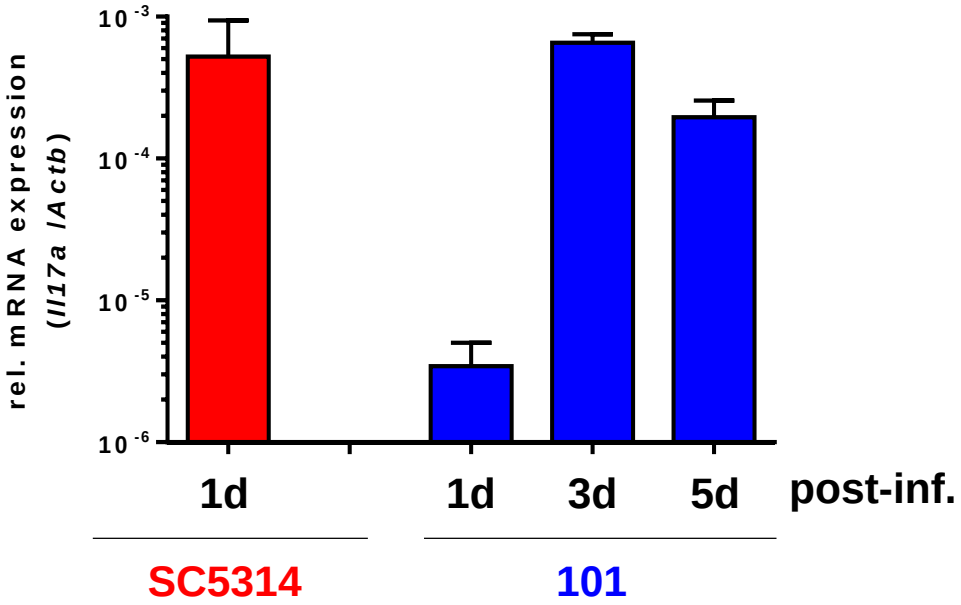
Ly6G (Neutrophils)



Schönherr et al., Mucosal Immunology, 2017

Delayed induction of the host response with non-pathogenic isolates

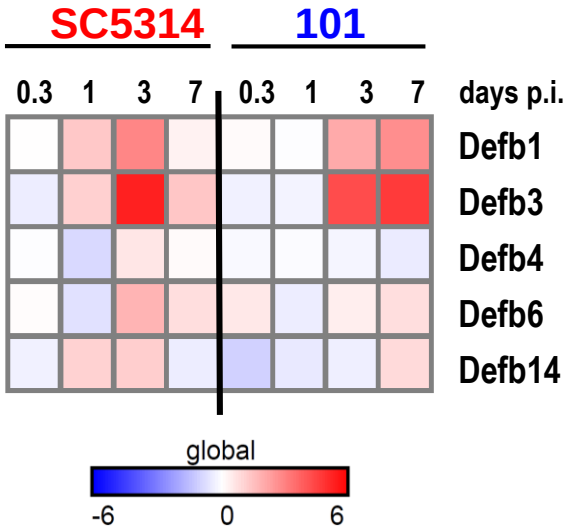


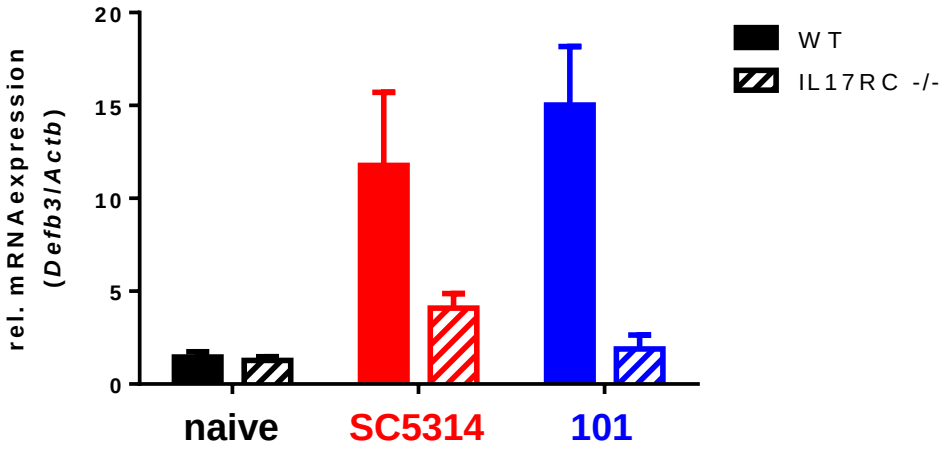


kinetic & qualitative differences in the response to different *C. albicans* isolates

Schönherr et al., Mucosal Immunology, 2017
Altmeier et al., unpublished

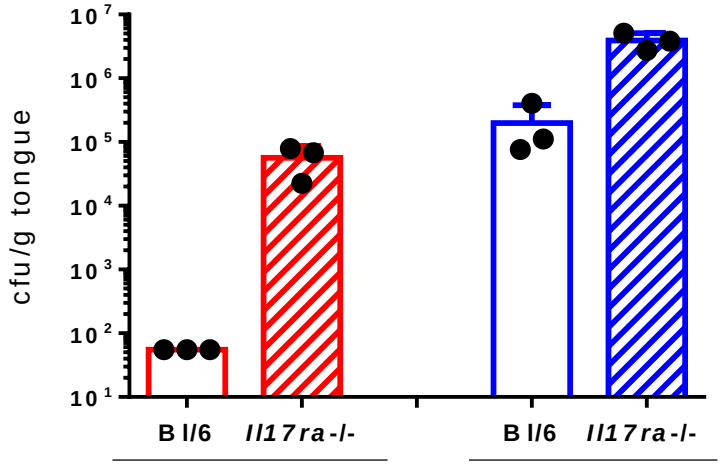
β-defensins mediate IL-17-dependent fungal control



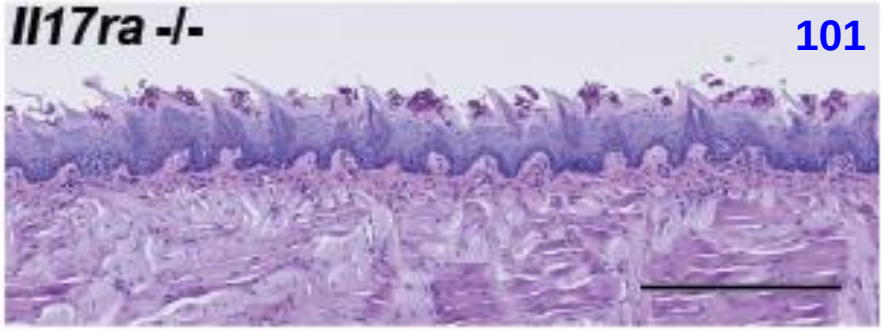


Altmeier, Mertens and LeibundGut-Landmann., unpublished

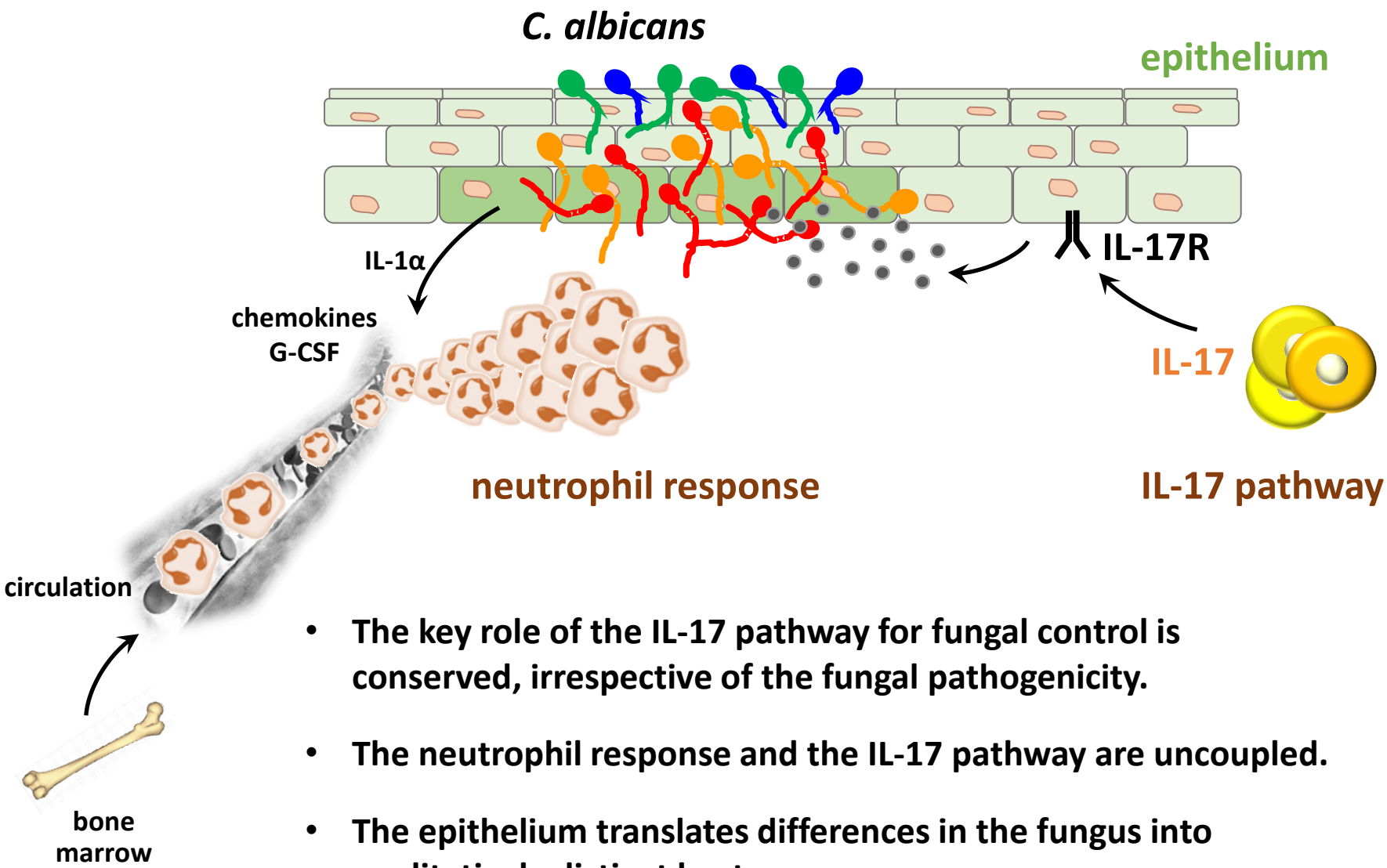
IL-17 signaling is essential for fungal control – irrespective of the degree of fungal pathogenicity



day 7 p.i. SC5314 101



Schönherr et al., Mucosal Immunology, 2017



- The key role of the IL-17 pathway for fungal control is conserved, irrespective of the fungal pathogenicity.
- The neutrophil response and the IL-17 pathway are uncoupled.
- The epithelium translates differences in the fungus into qualitatively distinct host responses.
- Natural variations in *C. albicans* determine the outcome of fungal-host interactions.

predictive value ?

Alumni:

Kerstin Trautwein-Weidner
André Gladiator
Albulena Toska
Laura Lauener
Patrizia Diethelm
Ana Chavez-Steenbock
Isabelle Zenklusen

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Norman Pavelka — SIgN
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Universität Zürich

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Swiss Federal Institute of Technology Zurich

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