



Lessons learned from perturbing the skin microbiome

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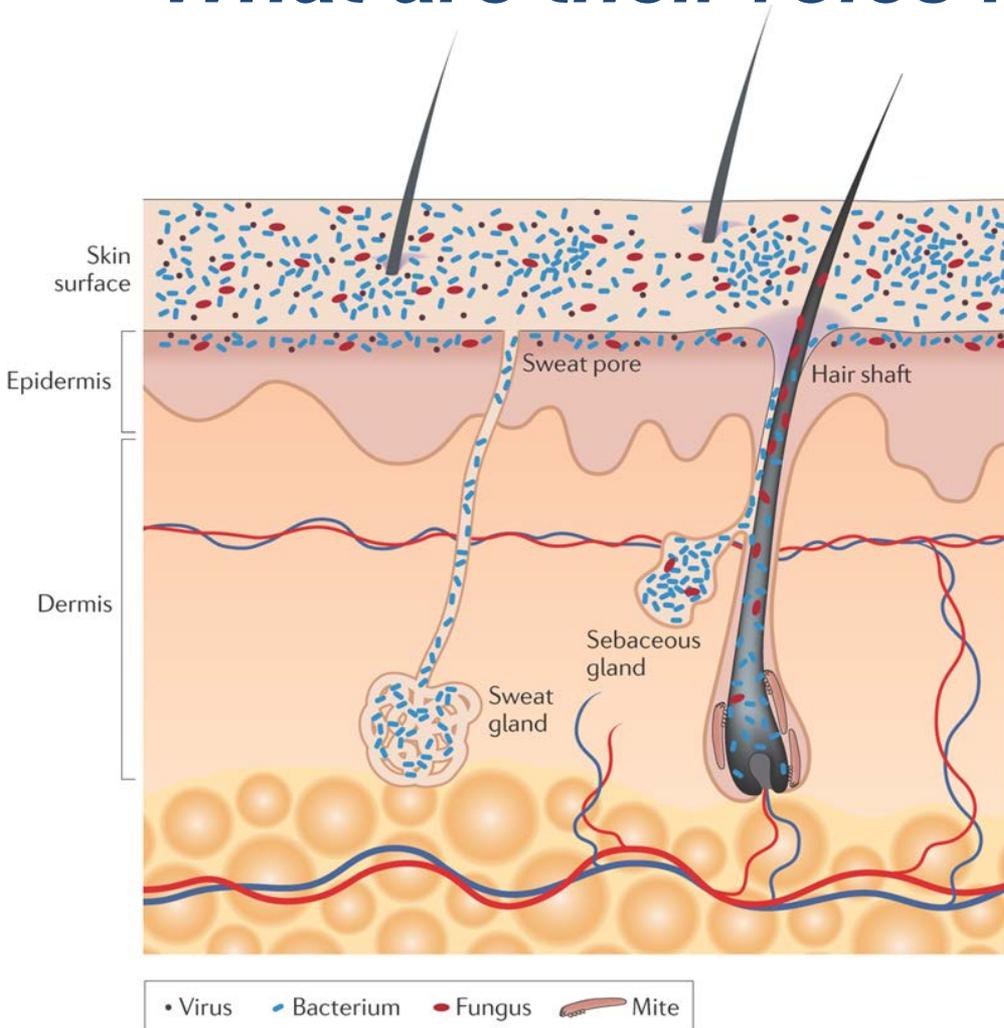
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>1 million microbes/cm² of skin:

What are their roles in health & disease?



• **Functional Significance:**

- Educates/stimulates immune responses
- Colonization resistance
- Production of secondary metabolites

• **Therapeutic and Diagnostic Applications:**

- Microbiome engineering: transplants, probiotics
- Diagnostic, prognostic tool

But, we are constantly trying to disrupt our skin microbiome!

Hand sanitizer



Hand washing



Antiseptics



Bathing



Antiseptics are efficacious against pathogens

TABLE
EFFICACY OF ANTISEPTICS AND DISINFECTANTS AS DETERMINED BY THE QUANTITATIVE SUSPENSION TEST AFTER 5 MINUTES OF CONTACT

Test Strain	No. of Isolates	Log ₁₀ Reductions of Microbial Count*				
		Savlon (1:100)	Chlorhexidine Gluconate (4)	Sodium Hypochlorite		
				1:50	1:500	1:500 [†]
<i>Pseudomonas aeruginosa</i>	20	6.24	6.24	6.20	0.19	0.37
<i>Escherichia coli</i>	17	6.26	6.26	5.90	1.25	3.24
<i>Klebsiella pneumoniae</i>	15	6.07	6.07	4.72	0.72	0.55
<i>Acinetobacter baumannii</i>	13	6.19	6.19	4.01	0.41	0.35
<i>K. oxytoca</i>	5	6.10	6.10	6.07	0.10	1.62
<i>Enterobacter cloacae</i>	3	6.17	6.17	5.85	0.24	0.24
<i>A. lwoffii</i>	2	5.62	5.62	5.62	0.17	0.18
<i>Stenotrophomonas maltophilia</i>	2	6.37	6.37	0.01	0.29	0.15

*Inoculum size: 1 to 2 × 10⁸.

[†]Sodium hypochlorite at 1:500 concentration, contact time of 15 minutes.

Ekizoglu MT, et al., 2003

What are they doing to our resident skin microbiota?



Lessons learned from perturbing the skin microbiome

- **Abundance does not equate to importance.**
 - **Small effect size can have big consequences.**
 - **The microbiome regulates host gene expression.**
- 

How do commonly used topical antiseptics affect resident skin microbial populations?



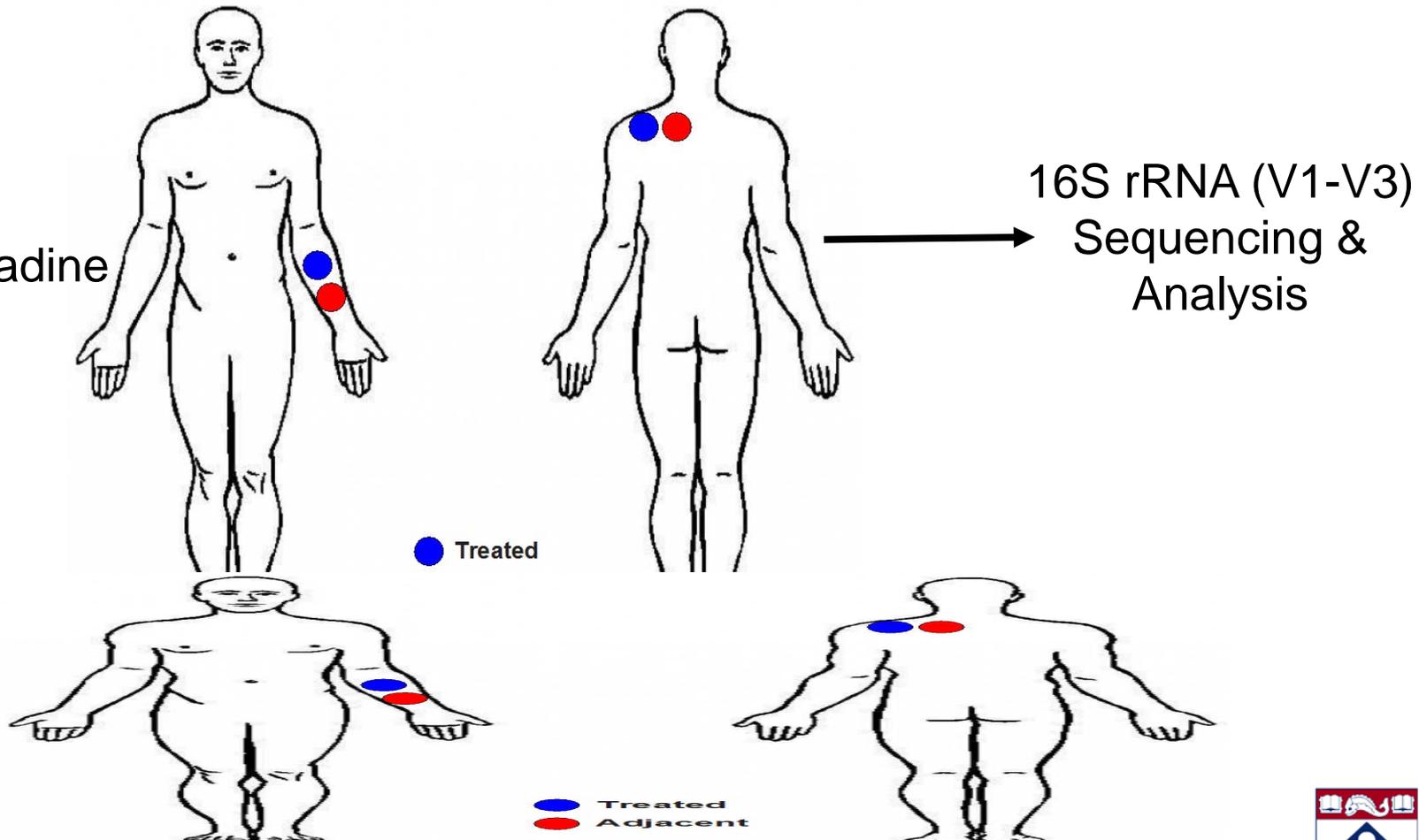
Adam SanMiguel, CAMB/MVP student



The SAME Study: Skin Antiseptic Microbiome Experiment

13 subjects, 6 male / 7 female

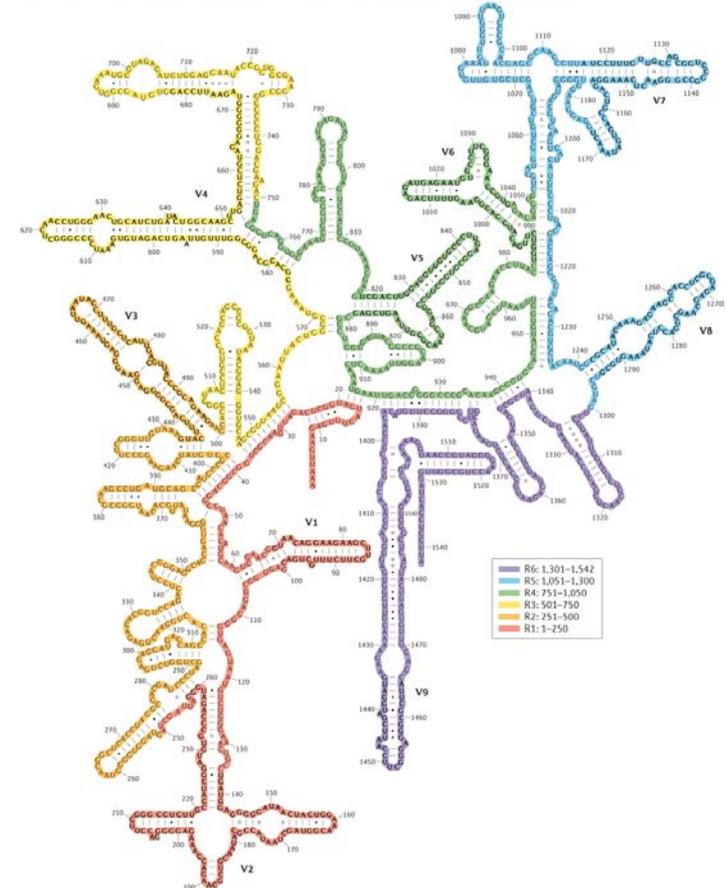
- Ethanol
- Betadine
- Chlorhexadine
- Water



DNA sequence-based identification of bacteria:

16S ribosomal RNA gene

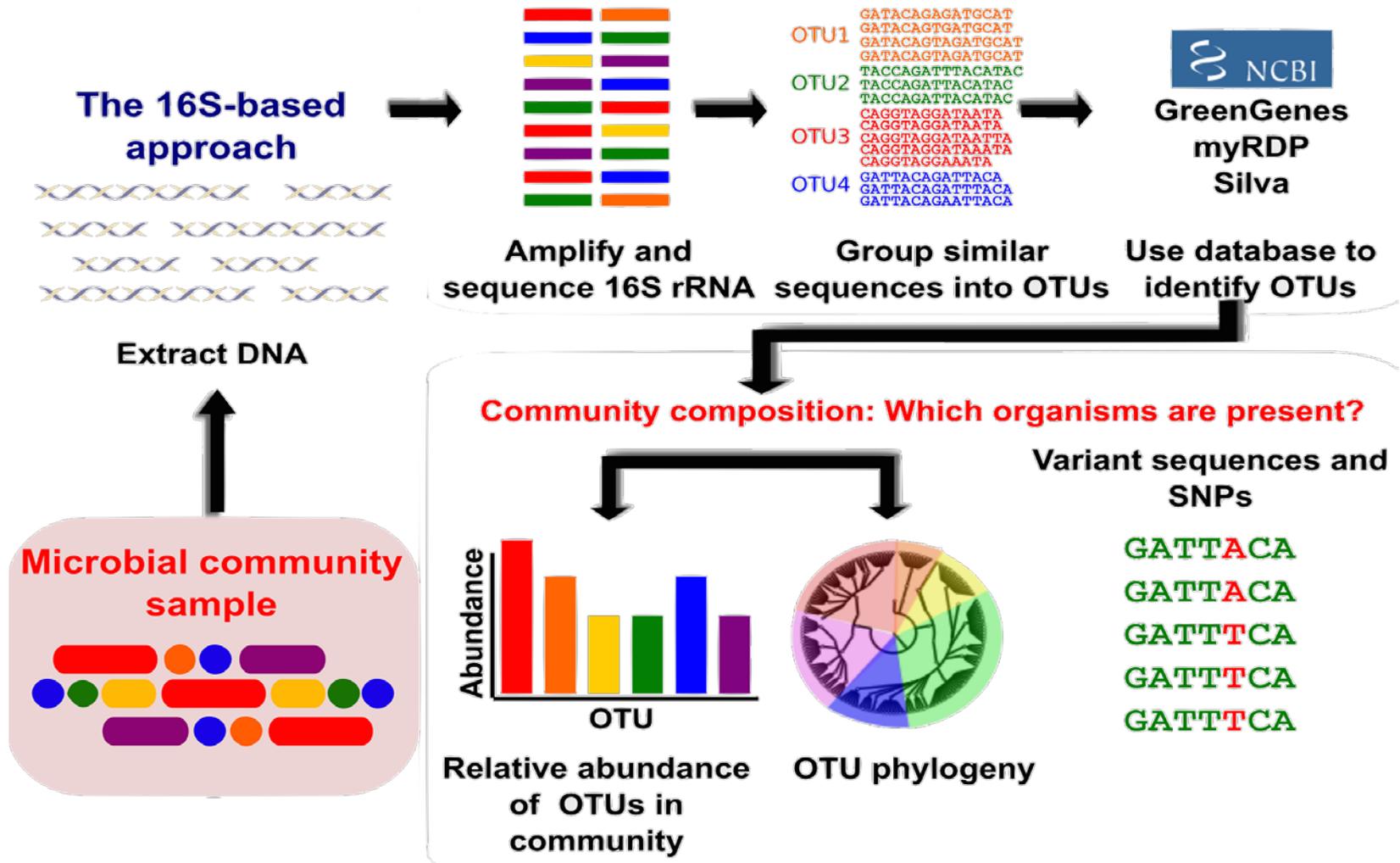
- A gene specific to prokaryotes
- Encodes a structural RNA that scaffolds proteins during translation
- Nine hypervariable regions
 - species-specific DNA sequence signatures
- Eliminates biases associated with cultures



Nature Reviews | Microbiology

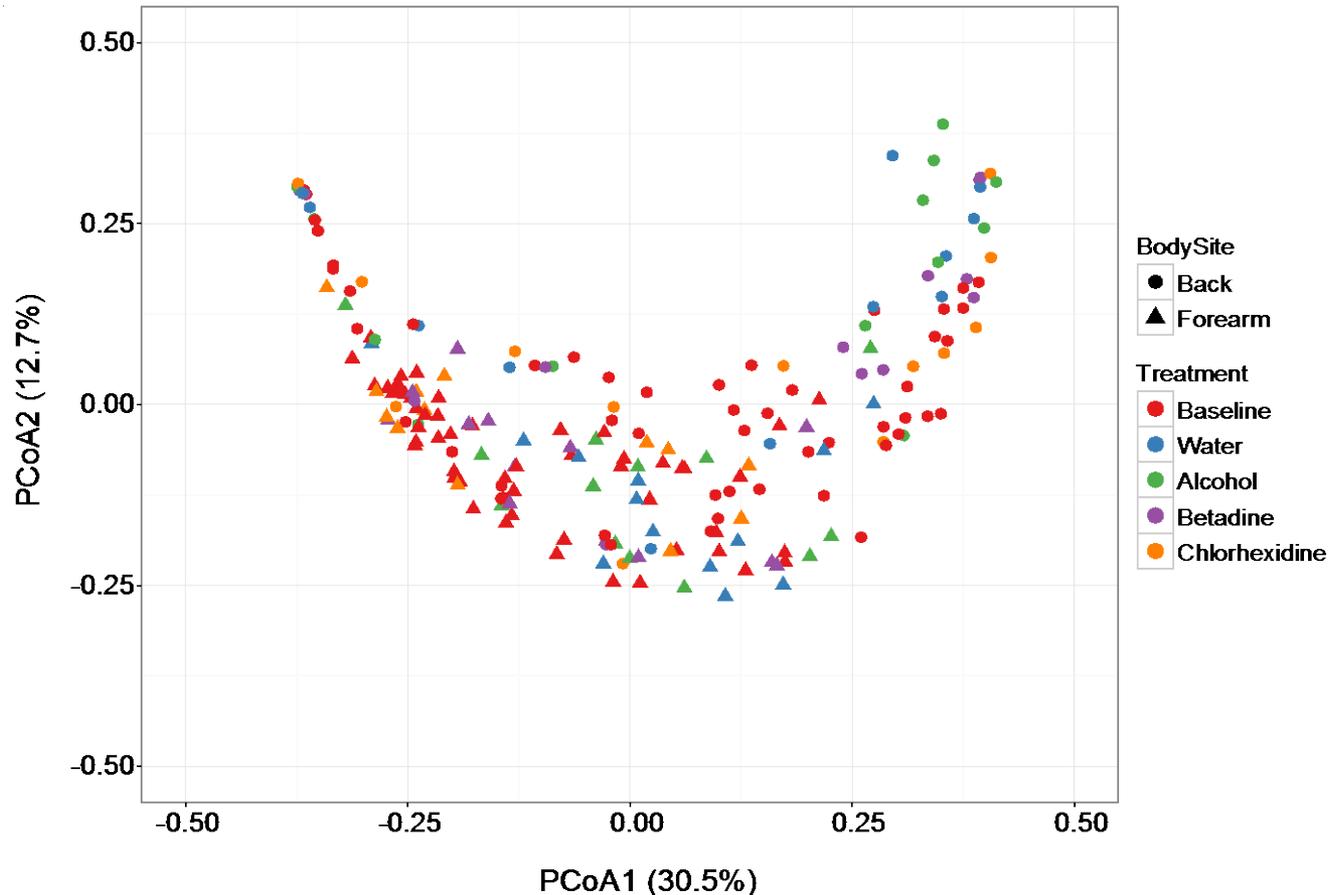


Overall Approach: 16S rRNA gene Sequencing



What are the immediate effects 1 hour post-treatment?

Weighted UniFrac distance metric: measures the “unique fraction” of phylogenetic branch length & weighs by the abundance of taxa contributing the branch length

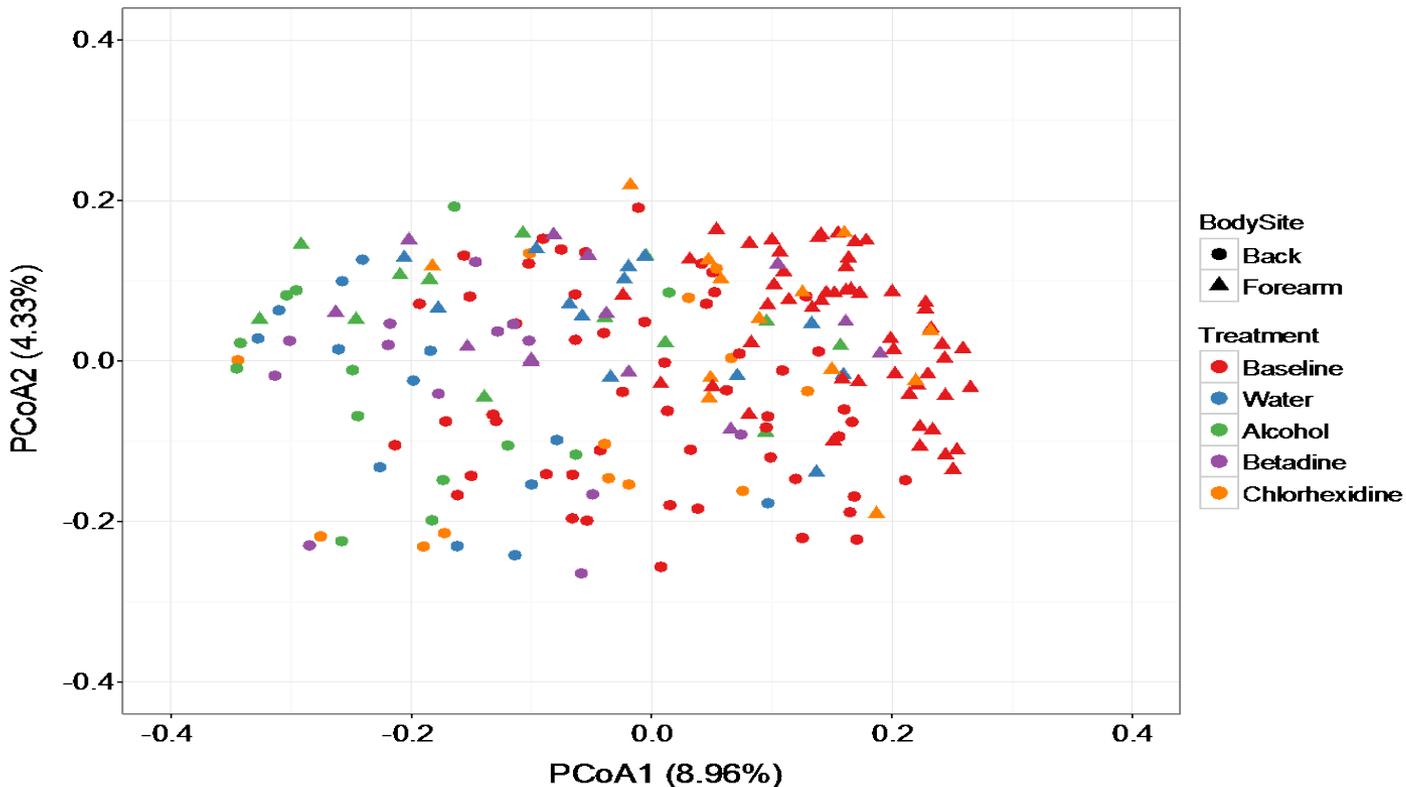


Minimal changes to bacterial community structure



Is bacterial community membership influenced by antiseptic trx?

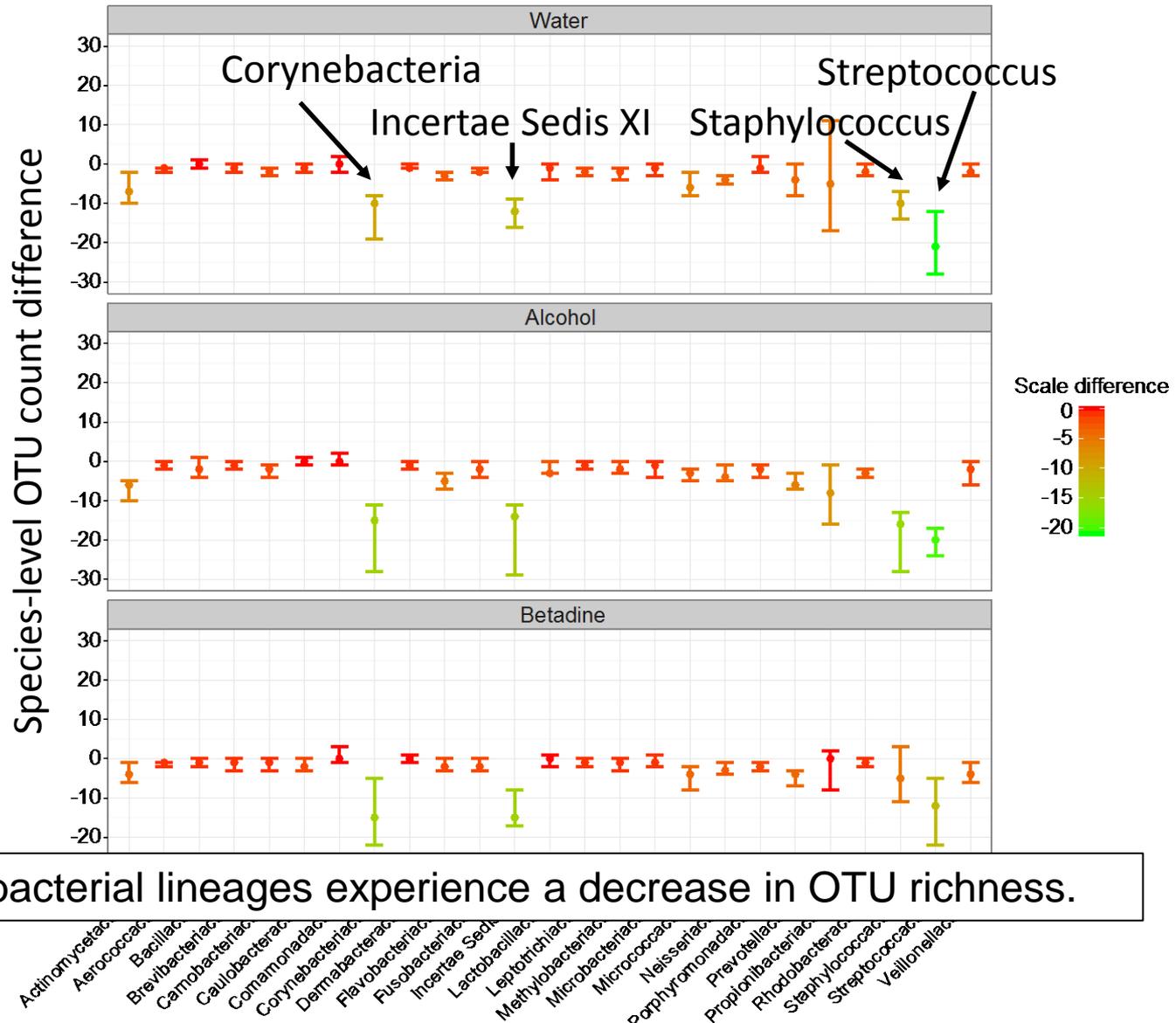
Unweighted UniFrac distance metric: measures the “unique fraction” of phylogenetic branch length without weighing for abundance



Unweighted metrics suggest that treatment eliminates less abundant taxa



Are there changes in bacterial diversity due to treatment?

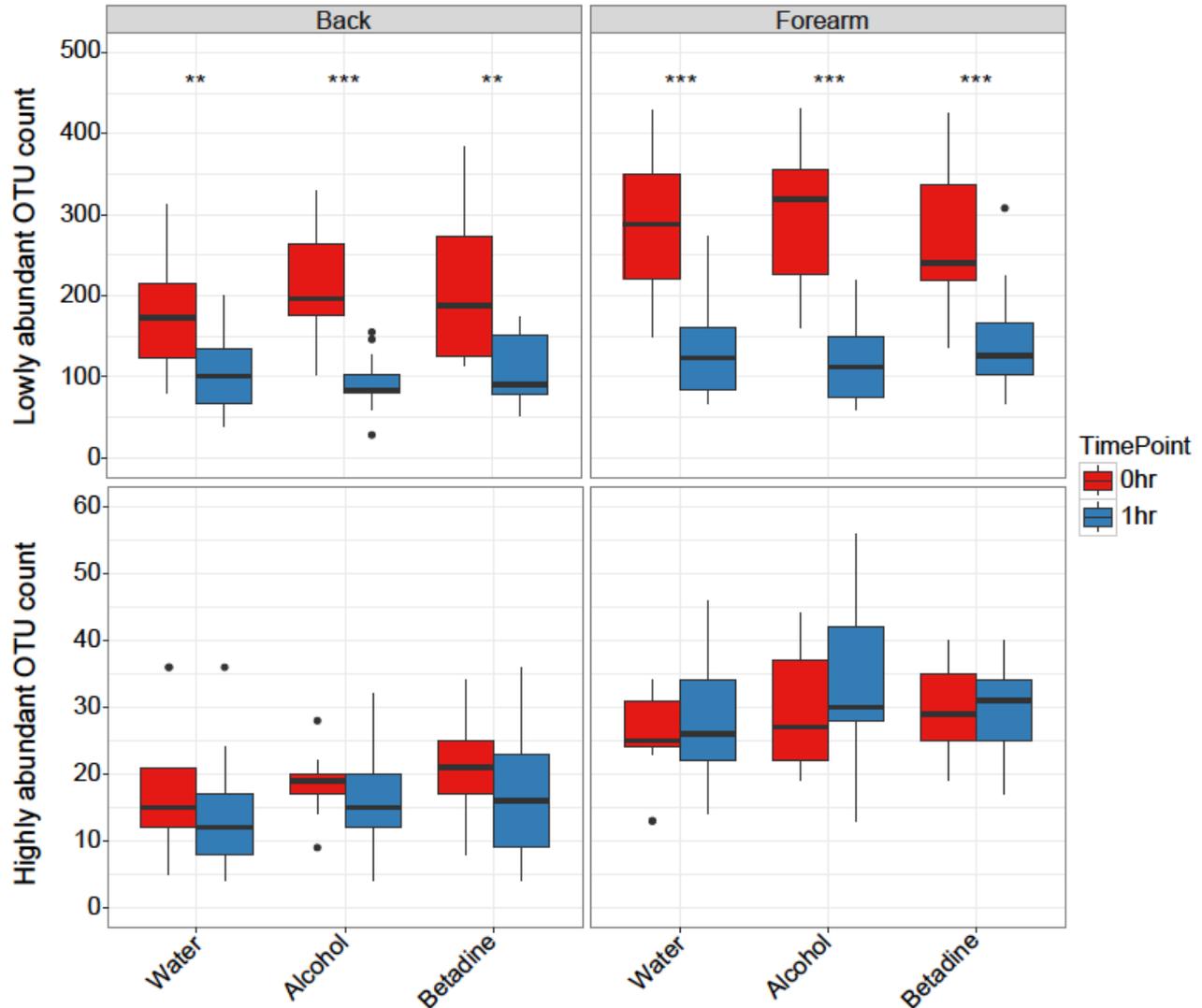


Certain bacterial lineages experience a decrease in OTU richness.



Are common and rare OTUs equally minimized by treatment?

Lowly abundant:
<0.05% relative abundance

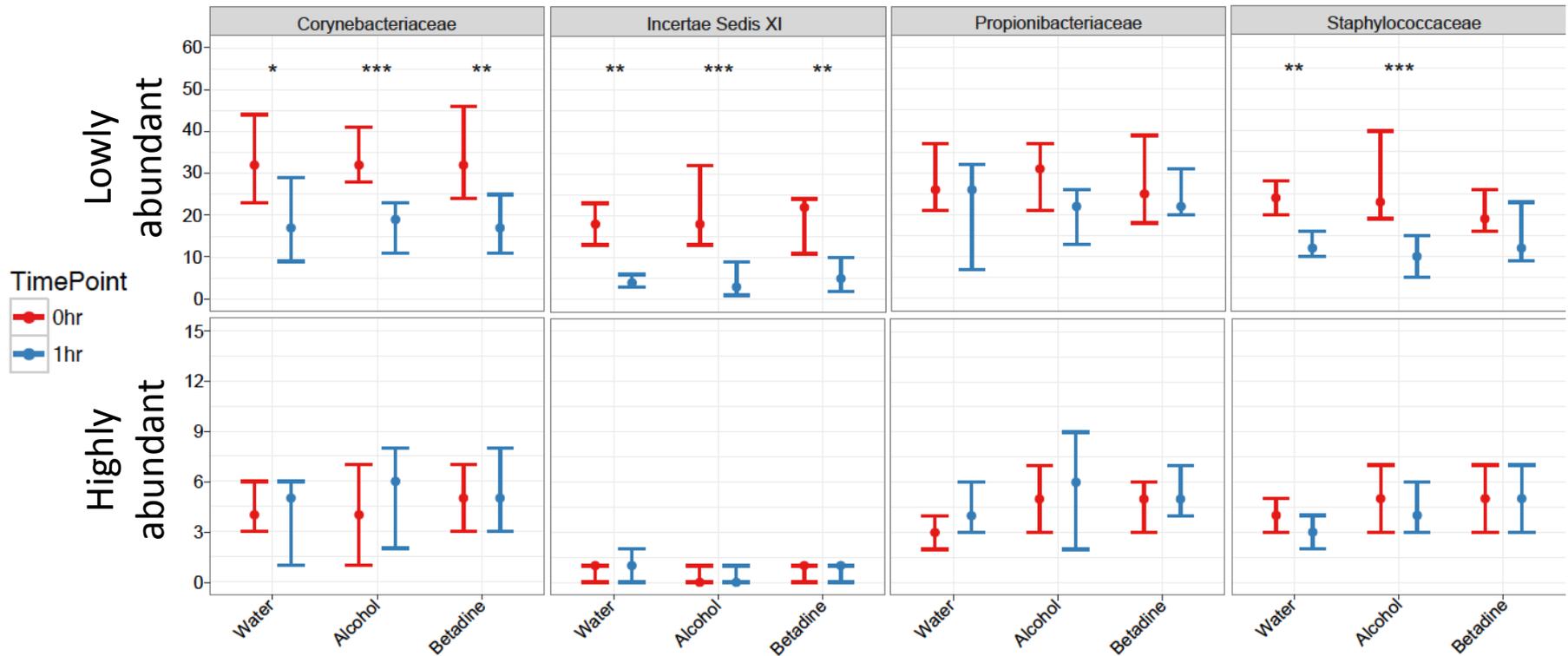


Highly abundant:
>0.05% relative abundance

** P < 0.01, *** P < 0.001 by Wilcoxon rank sum test



Are common and rare OTUs equally affected by treatment?



Rare OTUs are preferentially depleted, but an exception is *Propionibacterium*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Wilcoxon rank sum test



Lessons learned from perturbing the skin microbiome

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- 



What are the consequences for colonization resistance to pathogens?

Staphylococcus aureus: the leading cause of skin and soft tissue infection and surgical site infection.

- ~30% asymptotically colonized → risk factor for infection
- Antibiotic resistance is a major concern
- Heavy colonization/infection characterizes multiple dermatological disorders



Atopic dermatitis



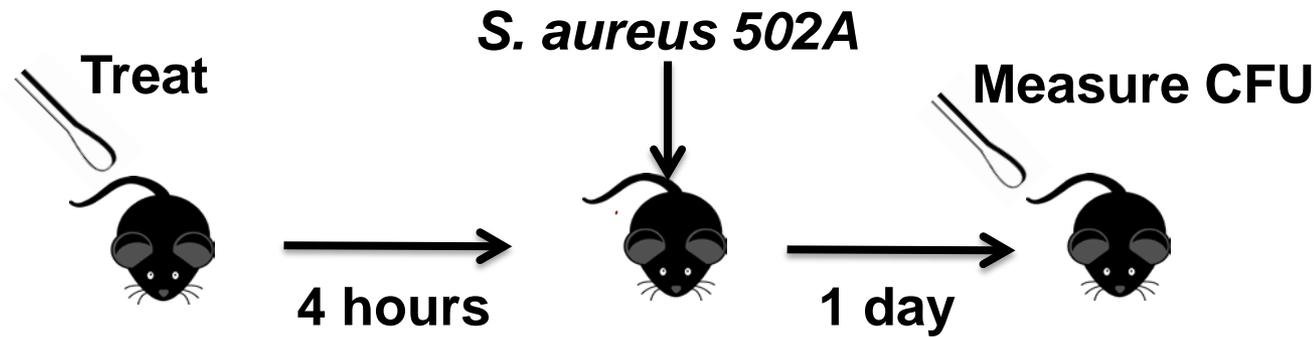
Staphylococcal scalded skin syndrome



Impetigo



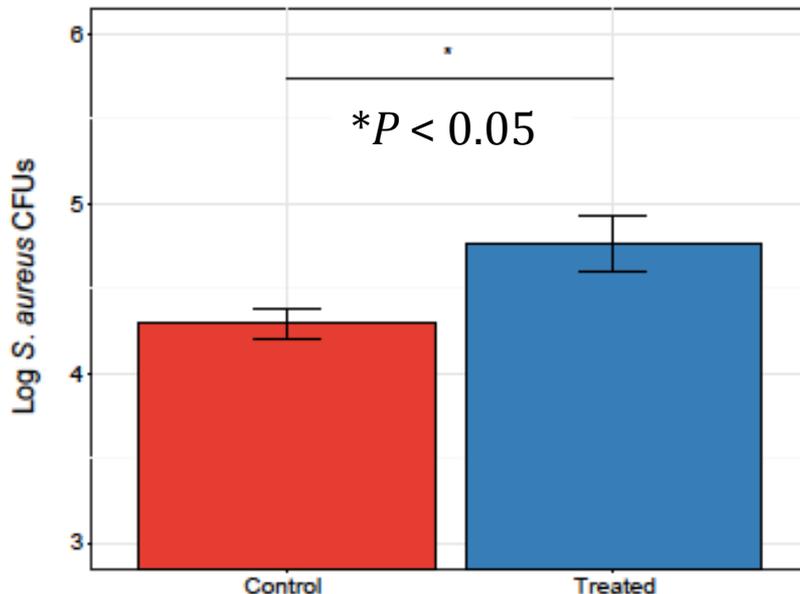
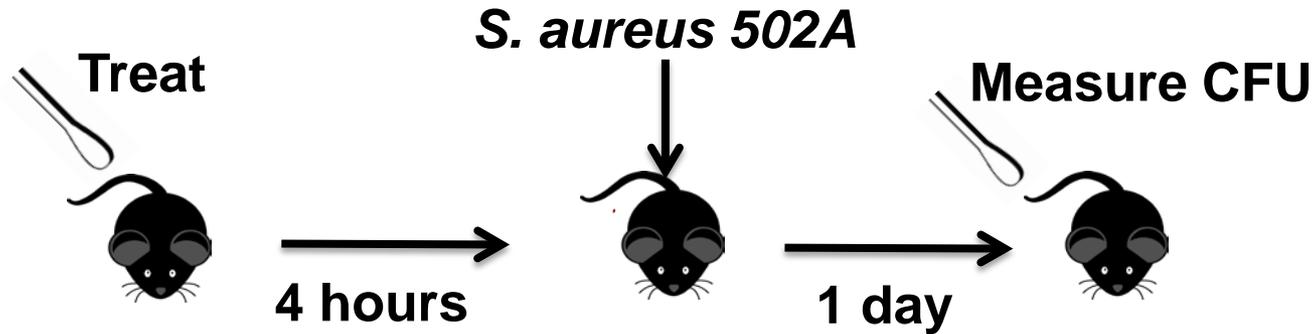
Does antimicrobial treatment disrupt colonization resistance to *S. aureus*?



SKH1-elite hairless mouse



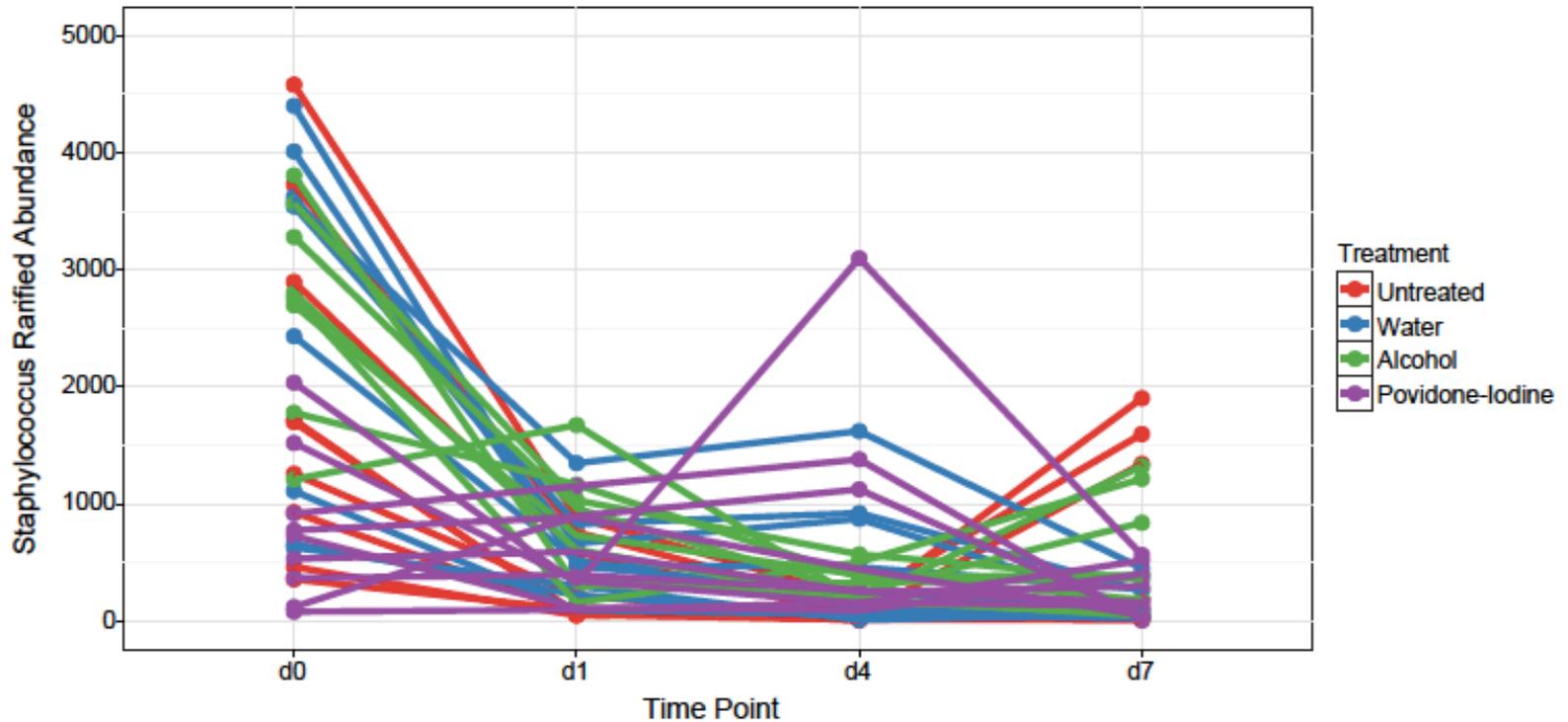
Does antimicrobial treatment disrupt colonization resistance to *S. aureus*?



Antimicrobial perturbation of skin microbiota increases colonization efficiency of *S. aureus*

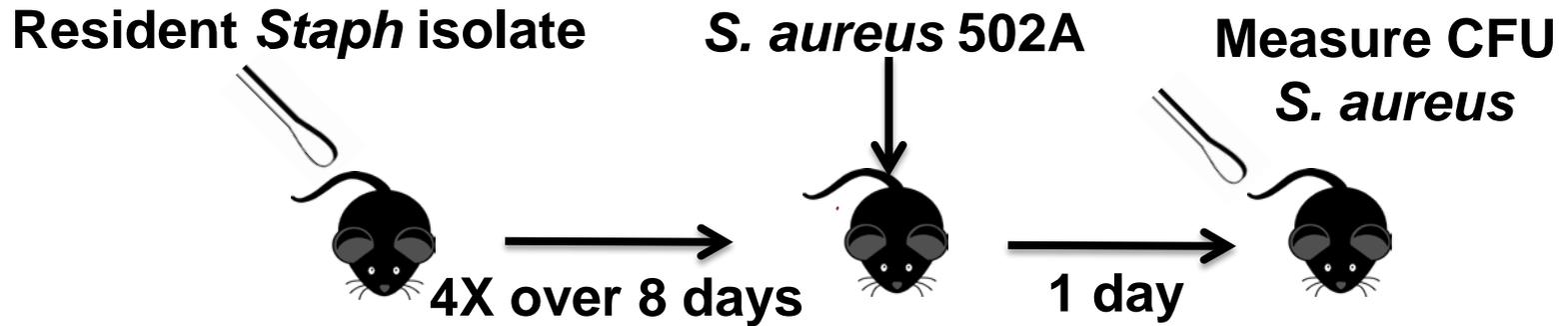


Does antimicrobial treatment remove bacteria that compete against *S. aureus*?

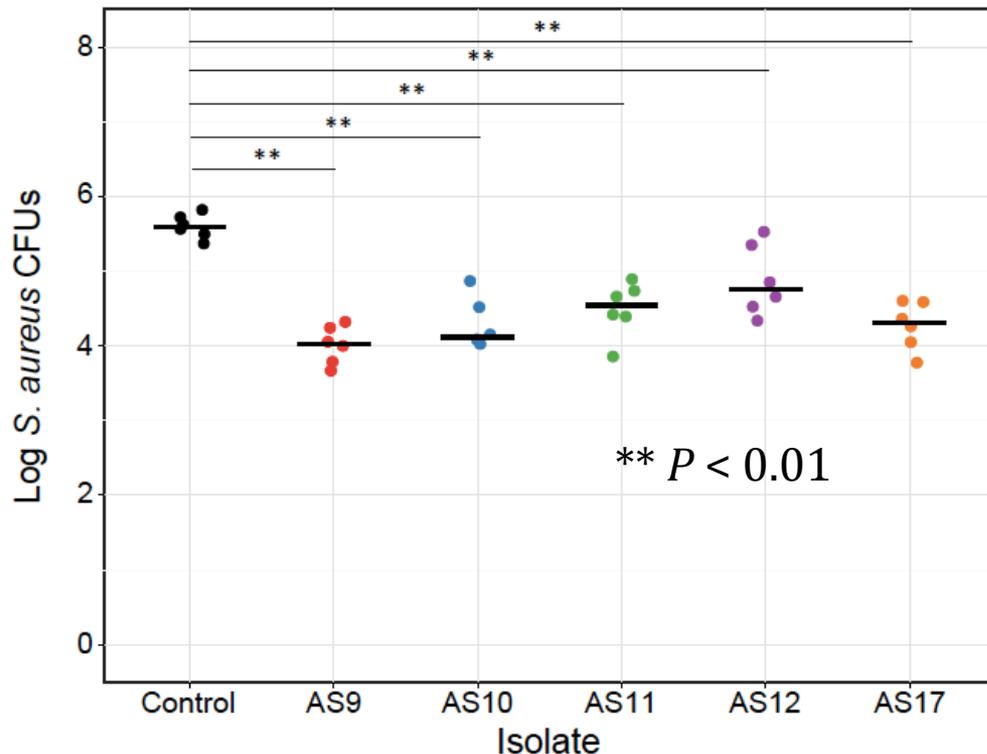
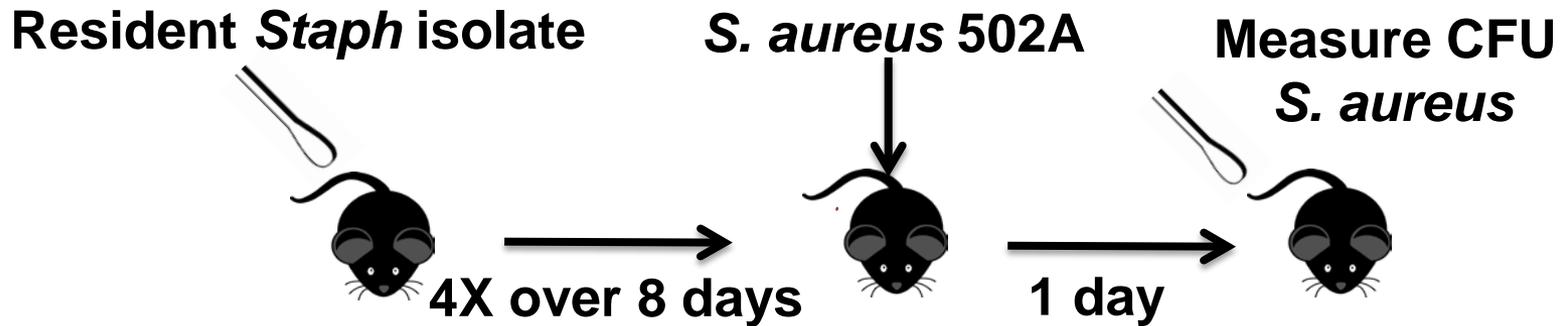


Staphylococcus spp. exhibit conserved decrease in abundance following treatment & are also readily cultured.

Do resident *Staphylococcus* spp. compete against *S. aureus*?



Do resident *Staphylococcus* spp. compete against *S. aureus*?



Resident *Staphylococcus* spp., removed by treatment, provide colonization resistance to *S. aureus*

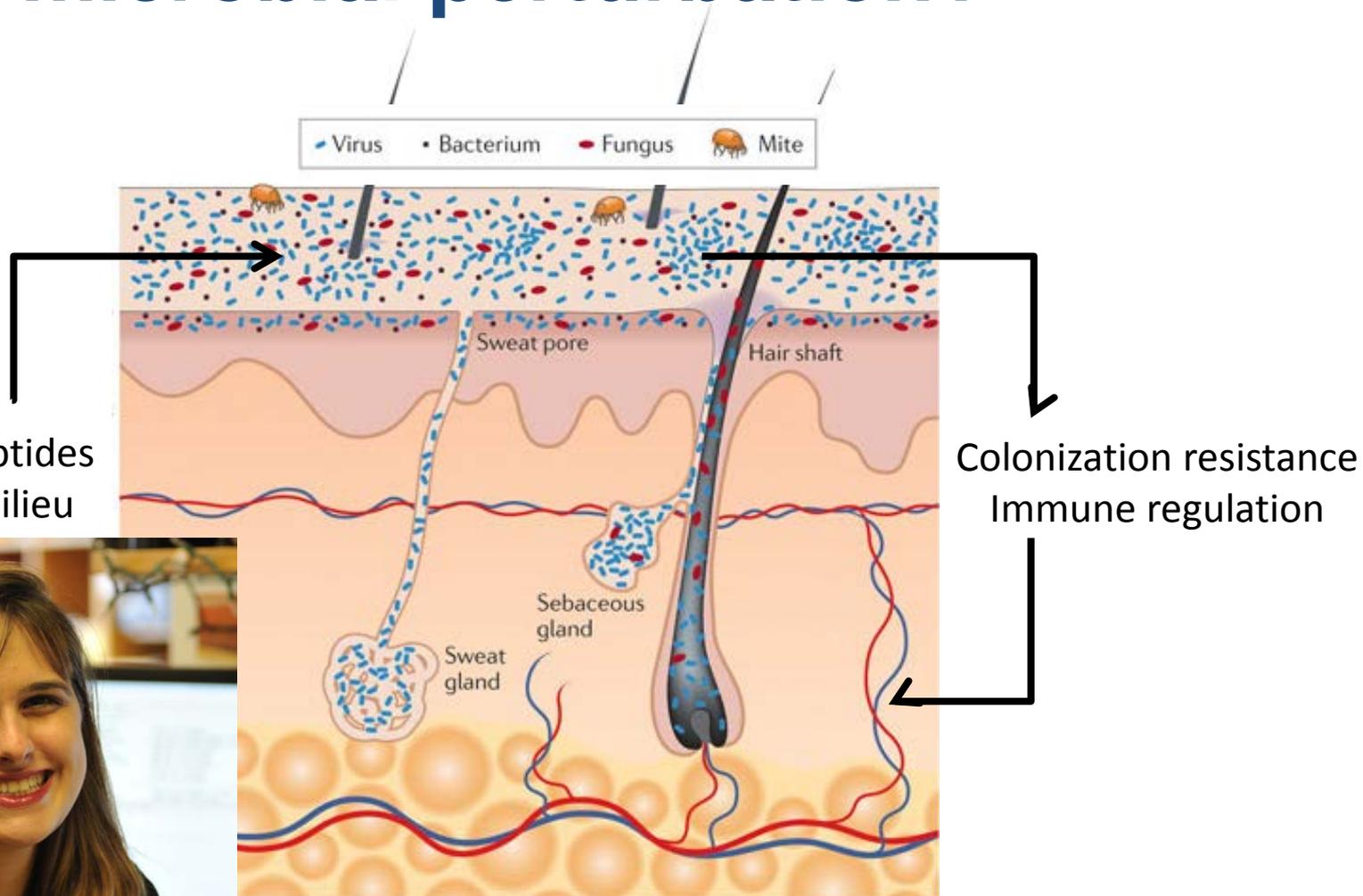


Lessons learned from perturbing the skin microbiome

- **Abundance does not equate to importance.**
- **Small effect size can have big consequences.**
- **The microbiome regulates host gene expression.**



What skin functions are affected by microbial perturbation?



Jackie Meisel



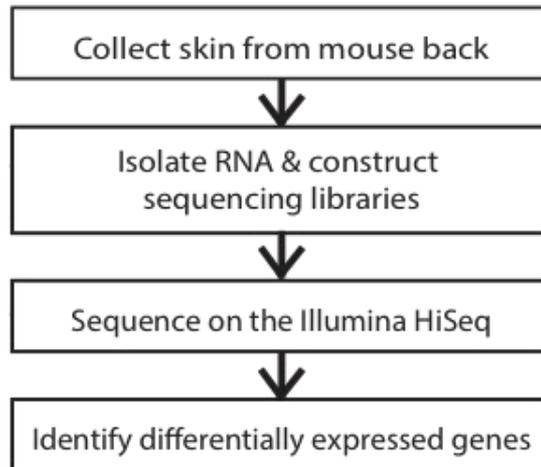
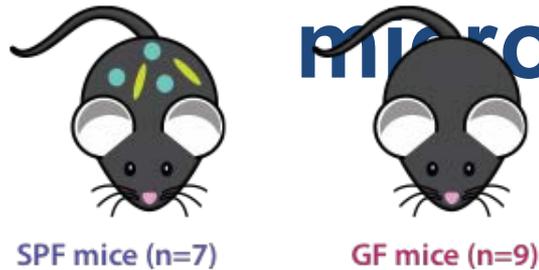
Germ free mice are a powerful tool to investigate host-microbe interactions



Penn Gnotobiotic Mouse Facility

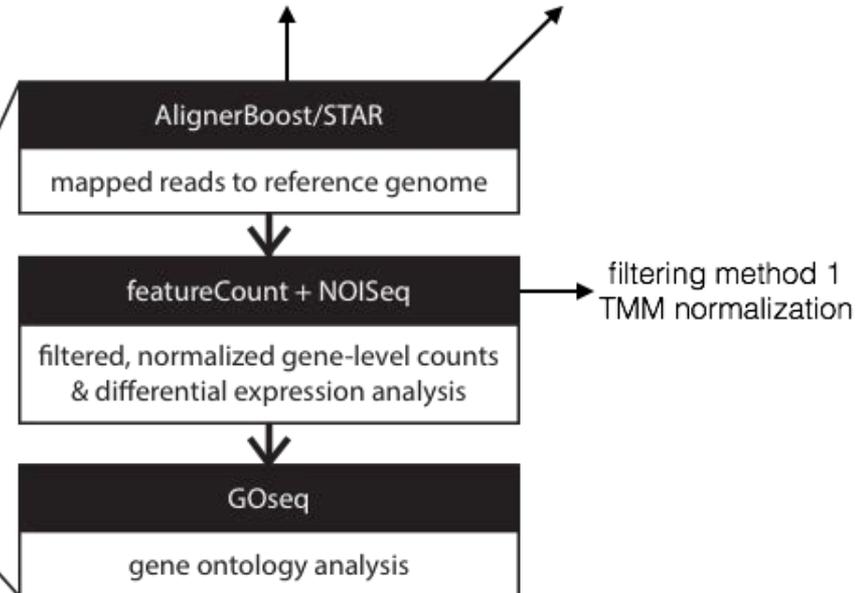


What host cutaneous pathways and functions are mediated by the microbiota?



aligned to GRCm38.p4
seed length: 25
seed mismatch %:4
seed indel %: 0
all mismatch %:8
all indel %: 3

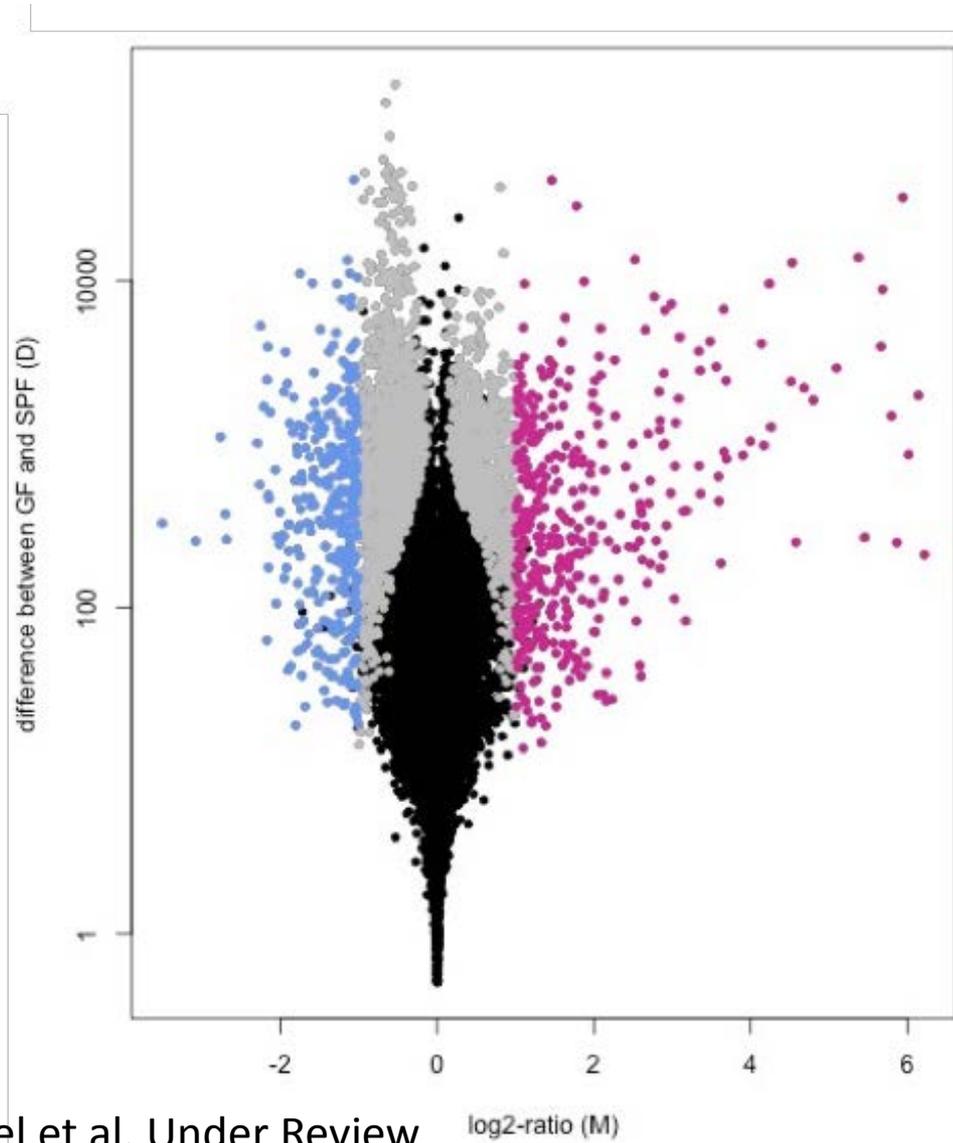
retained reads mapping to numbered & sex chromosomes
removed reads mapping to rRNA



Differential gene expression analysis will elucidate the molecular mechanisms by which the host interacts with the microbiota.



Do commensal microbes modulate gene expression in the skin?



2,280 genes significantly
differentially expressed
($q < 0.1$)

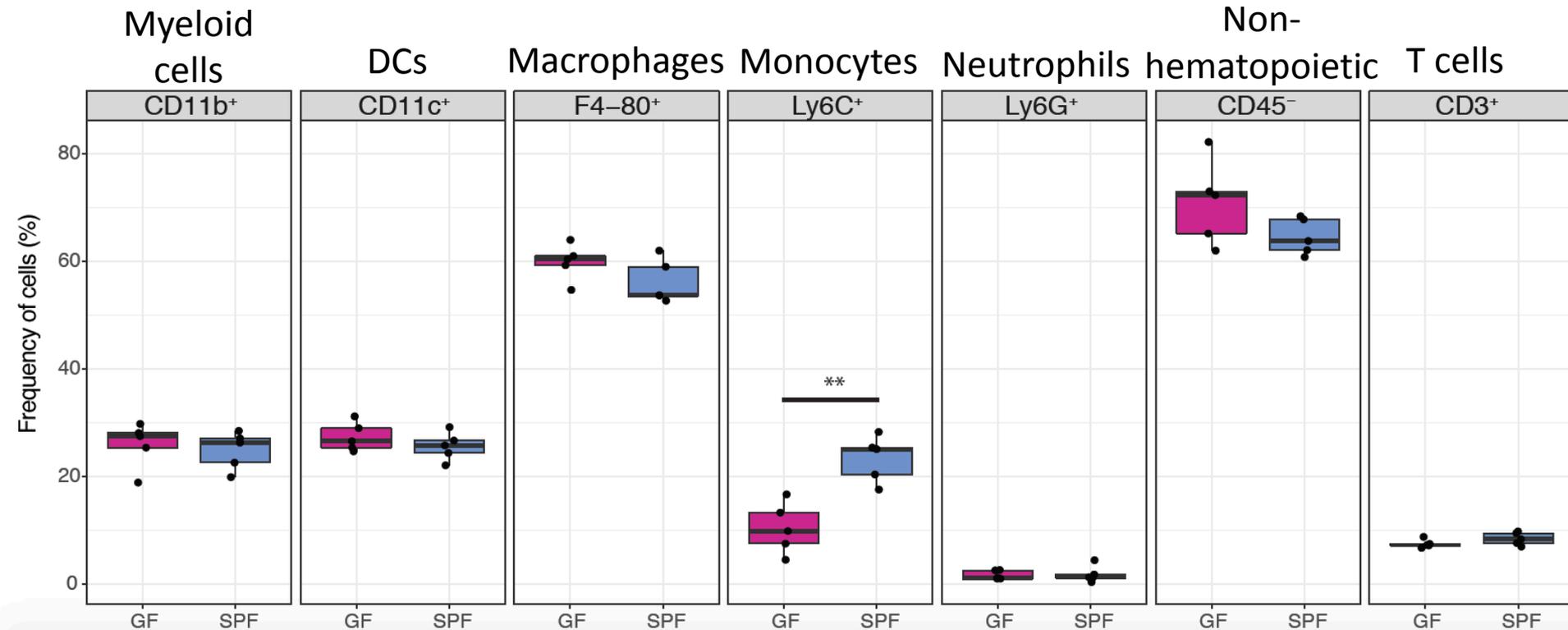
~

730 genes > 2-fold
differentially expressed

~

408 genes downregulated;
322 genes upregulated due to
microbial colonization

Are their different frequencies of immune cells in SPF vs GF skin?



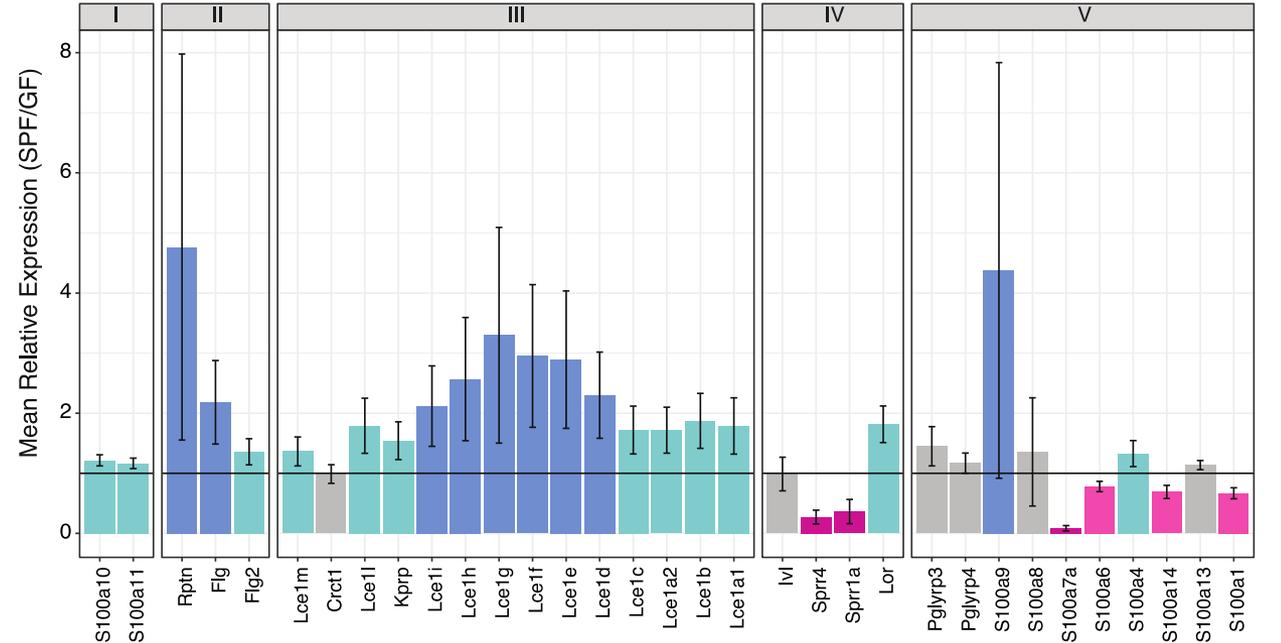
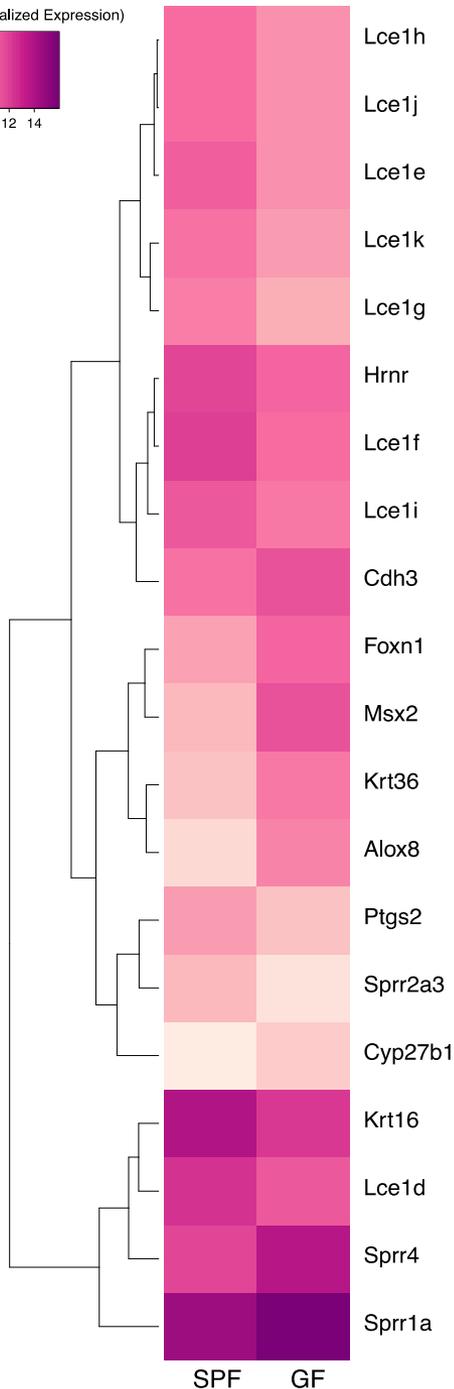
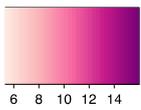
Similar frequency of broad cell types except Ly6C+ monocytes.



Keratinocyte differentiation & epidermal development genes are differentially expressed

Epidermal Differentiation Complex

log2(Mean Normalized Expression)



■ Up regulated with < 2 FC in GF
 ■ Up regulated with < 2 FC in SPF
 ■ Up regulated with > 2 FC in GF
 ■ Up regulated with > 2 FC in SPF

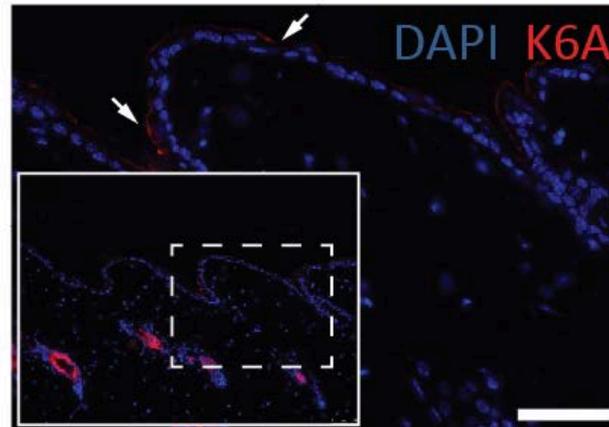
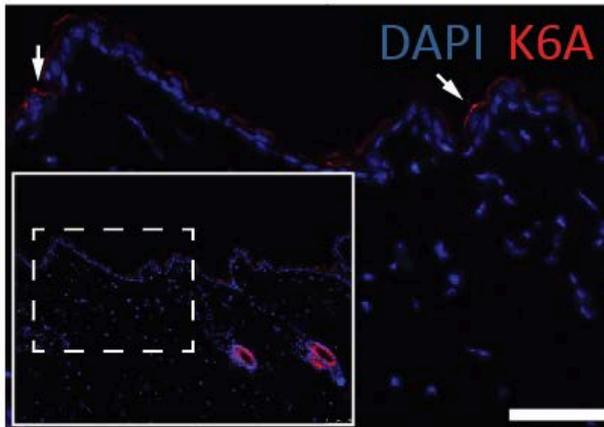
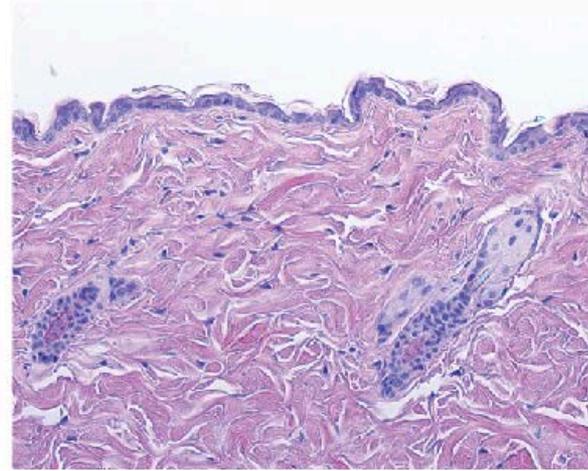


Is epidermal structure & function similar in SPF vs GF mice?

SPF



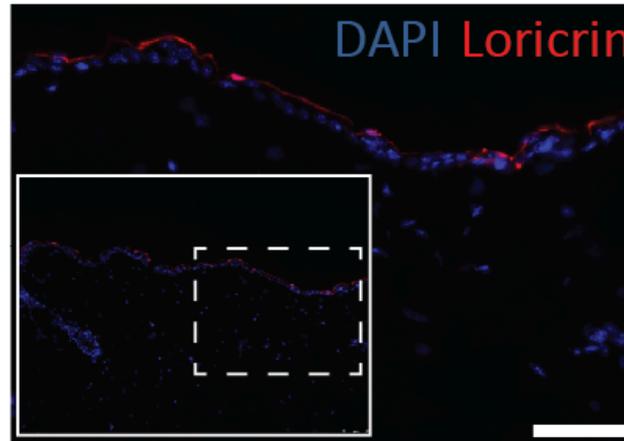
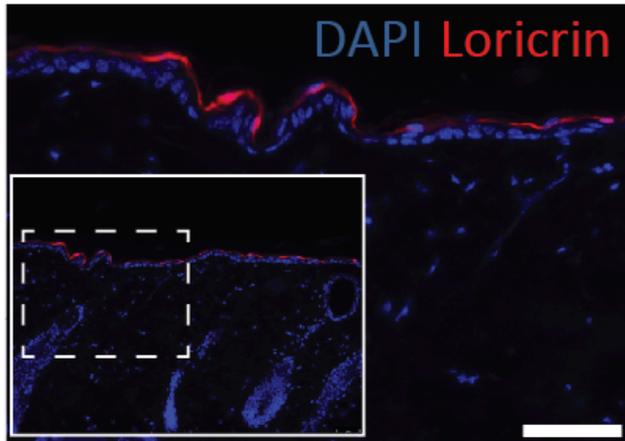
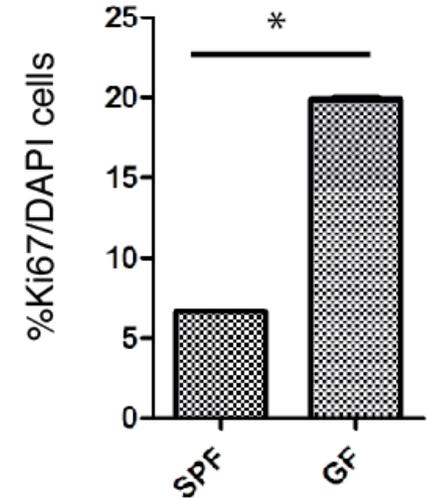
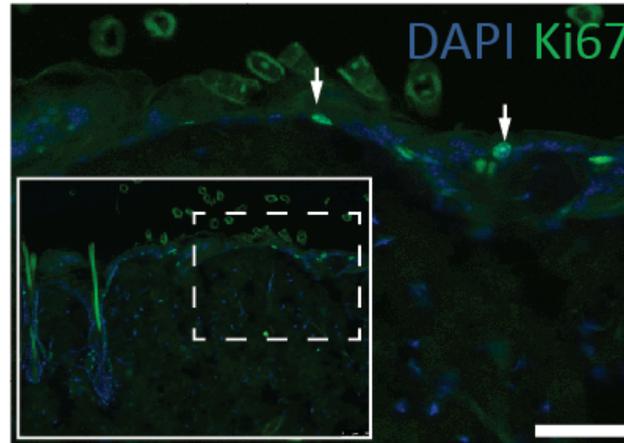
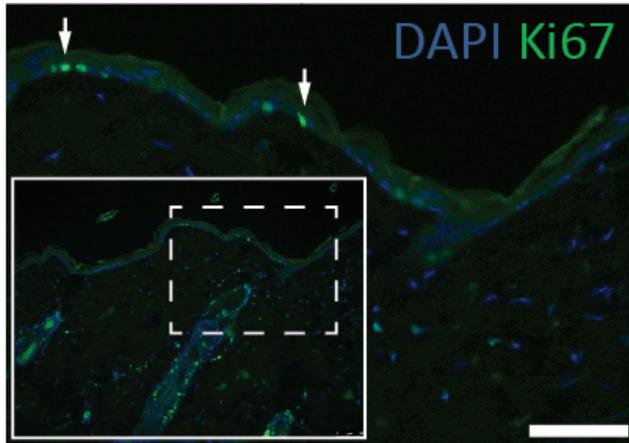
GF



Similar epidermal thickness and staining of keratin 6A suggest intact barrier.



Is proliferation and differentiation similar in SPF vs GF mice?



Differential staining of loricrin and Ki67+ cells suggests imbalanced proliferation/differentiation in GF mice.

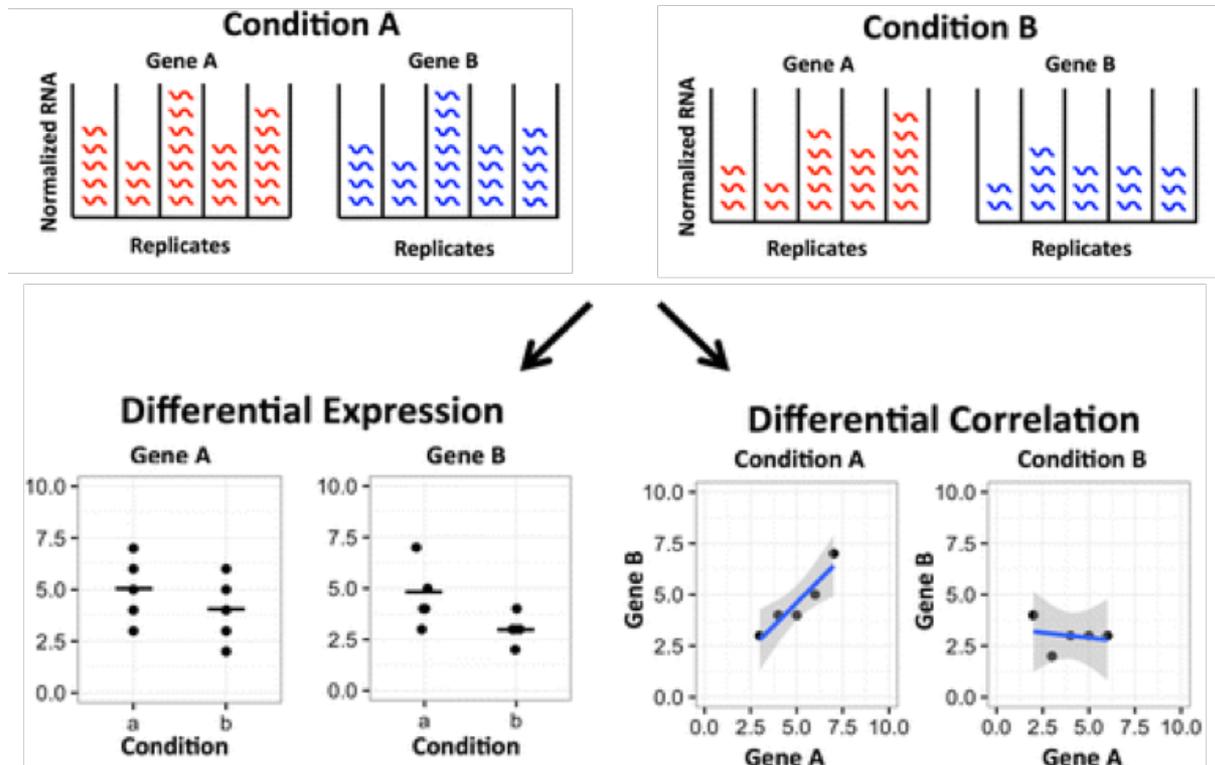




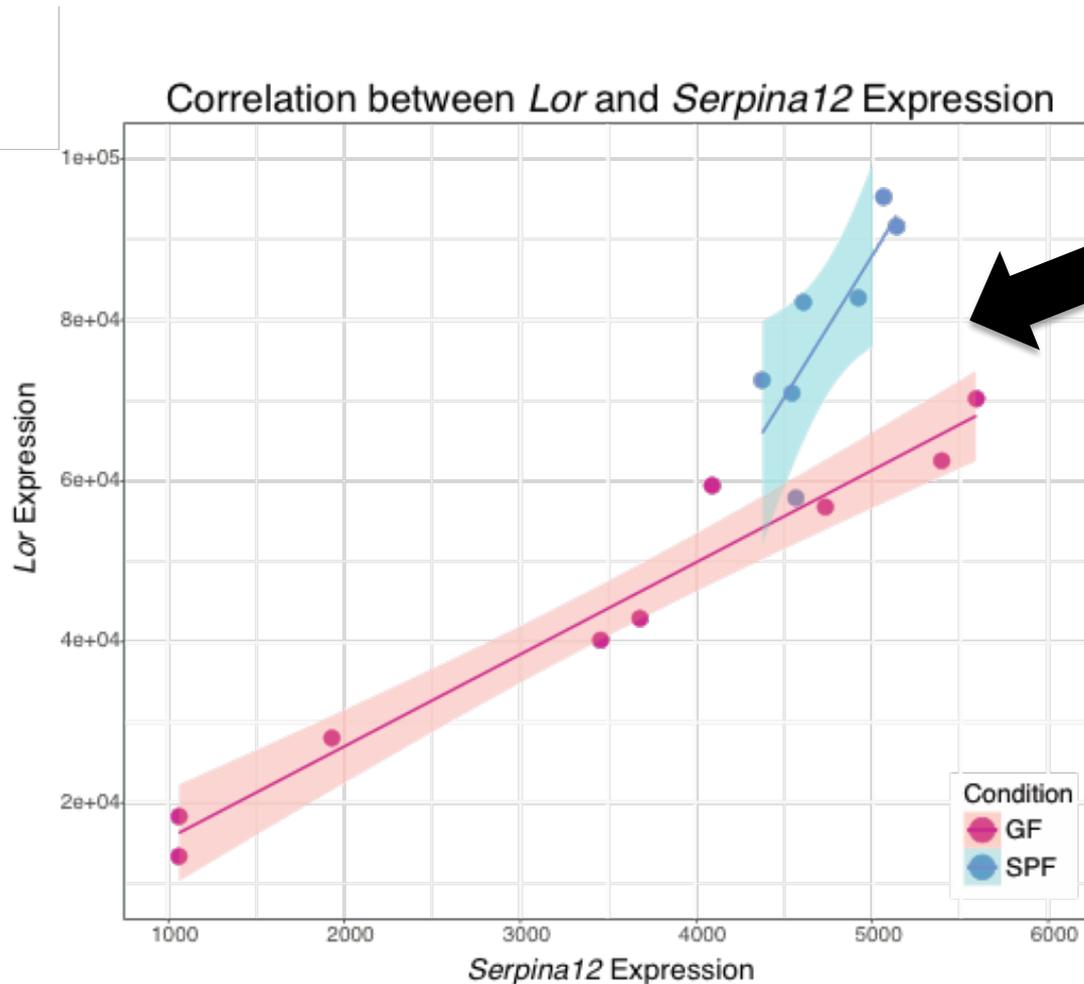
Casey Bartow-McKenney
PhD student

What are the upstream transcriptional regulatory mechanisms for differential gene expression?

DGCA: Differential Gene Correlation Analysis



Microbial-dependent alterations in gene networks controlling epidermal development



Gene Pair Correlation in SPF

	+	0	-
+	14707	68926	1368
0	5406	75921	4508
-	1544	51733	6747

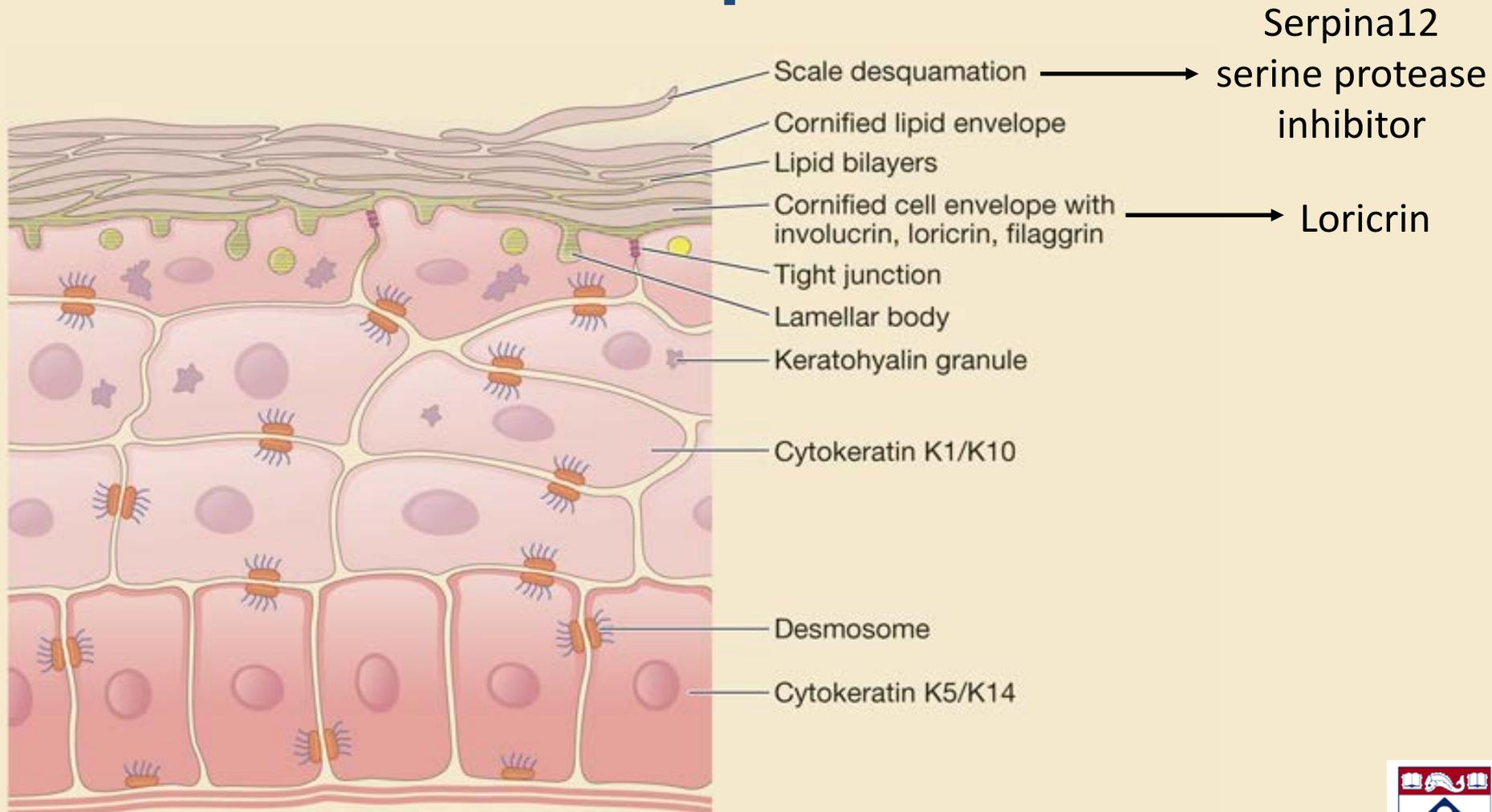
Gene Pair Correlation F

Gene Pair Correlation

- +
- 0
-

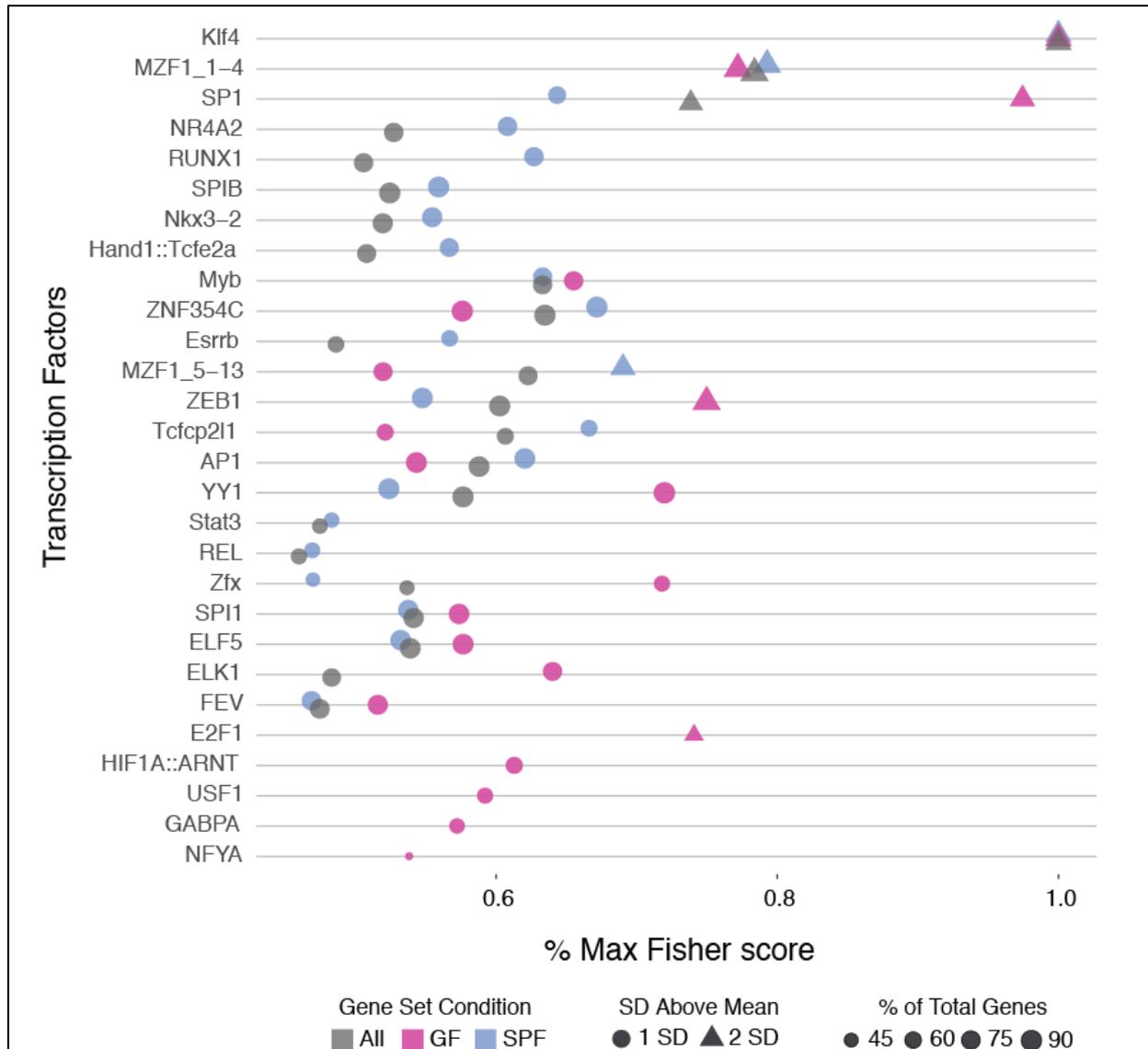


Microbial-dependent alterations in gene networks controlling epidermal development



Do correlated DEGs share TFBS?

oPOSSUM3 prediction of over-represented TFBS in positive-positive correlated genes



Lessons learned from perturbing the skin microbiome

- **Abundance does not equate to importance.**
 - *Rare OTUs of commensal skin microbes are removed by antiseptic treatment, while abundant OTUs are unaffected.*
- **Small effect size can have big consequences.**
 - *While antiseptic treatments appear to minimally influence the skin microbiome, the functional consequences for colonization resistance to *S. aureus* are potentially profound.*
- **The microbiome regulates host gene expression.**
 - *Commensal microbes prime cutaneous immune pathways and regulate epidermal differentiation and barrier formation.*



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Gnotobiotic Mouse Core

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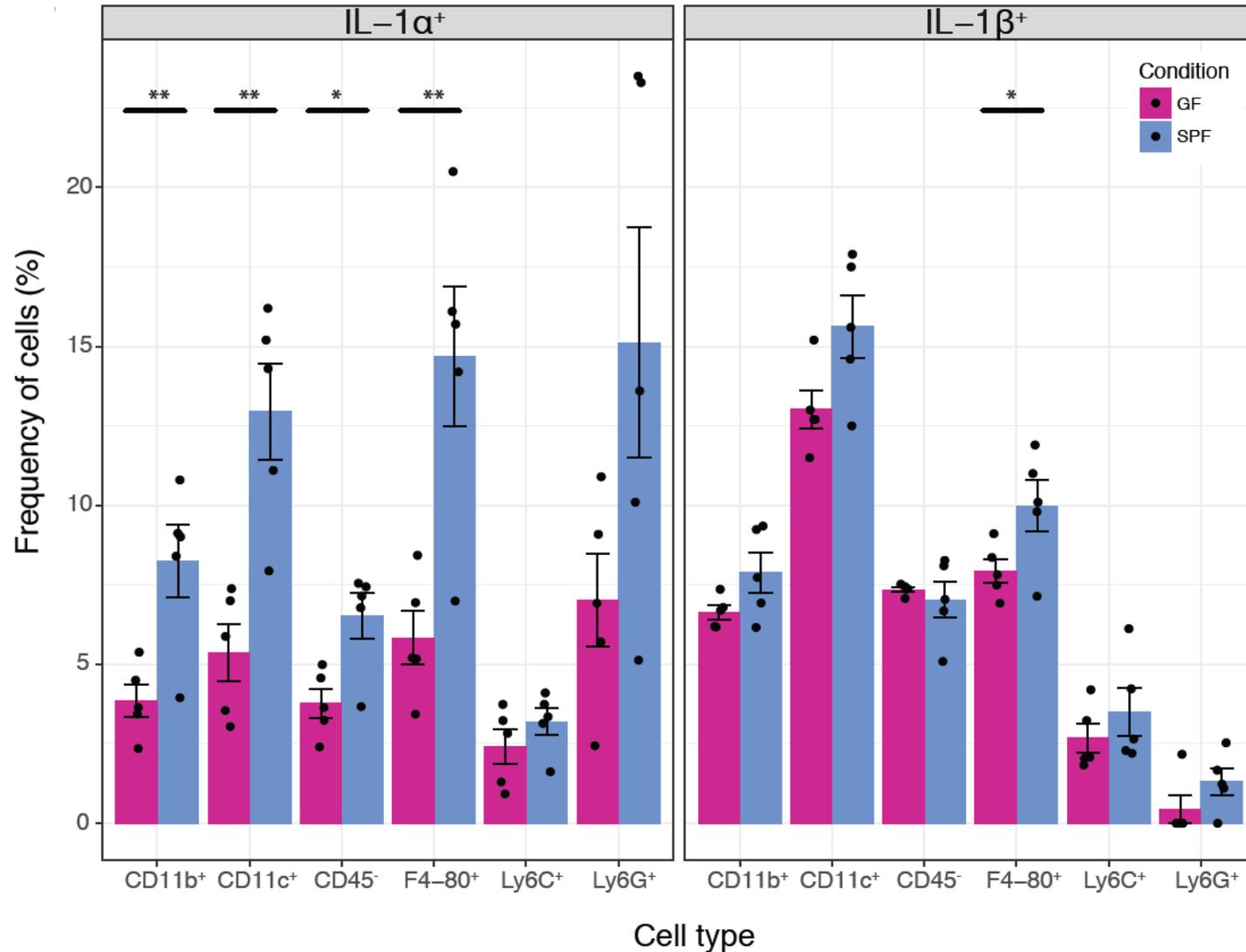
Pennsylvania Department of Health

Linda Pechenik Montague Investigator Award

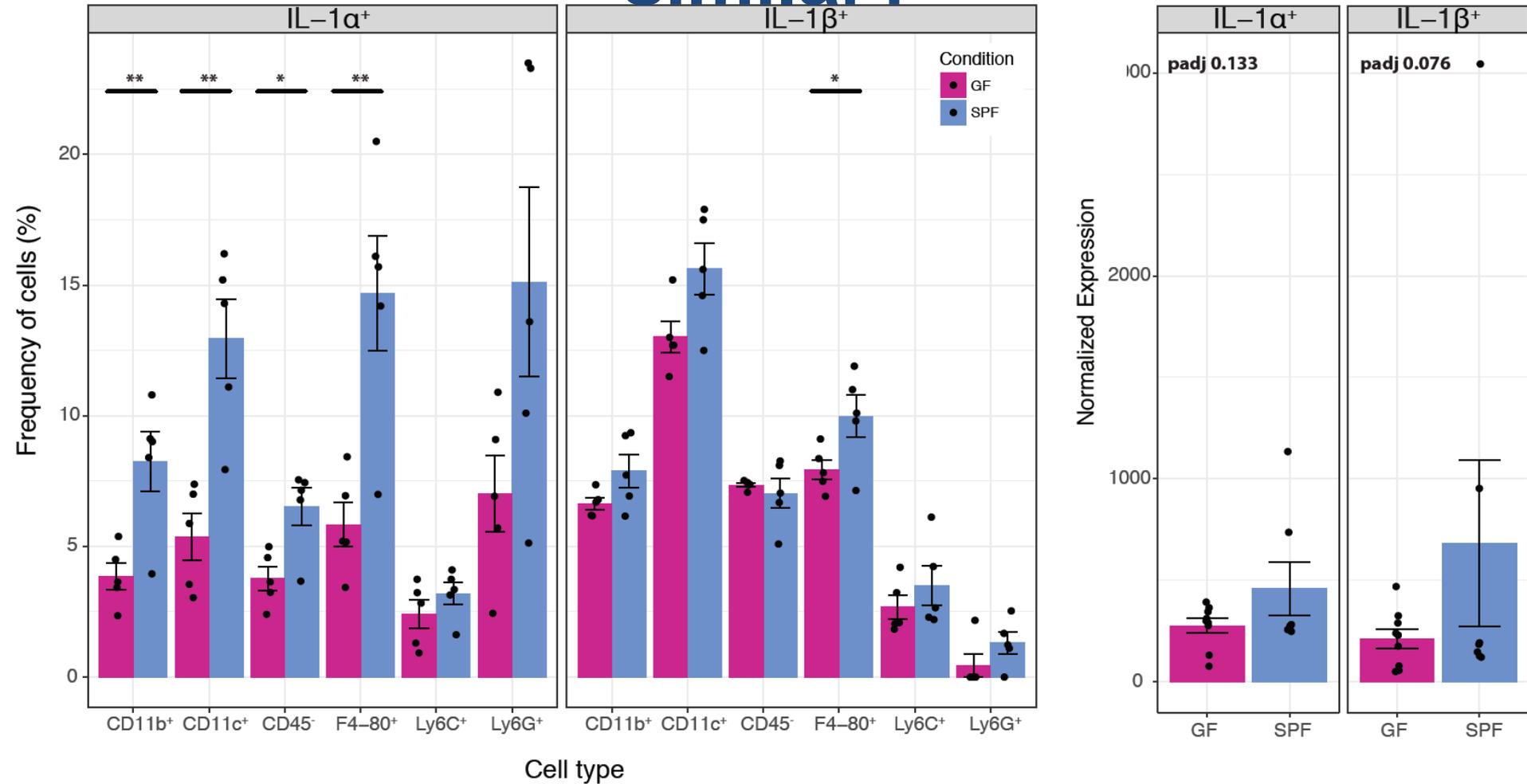


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Are SPF and GF cells functionally similar?



Are SPF and GF cells functionally similar?



Increased production of IL-1 confirms previous findings and correlates with gene expression findings.