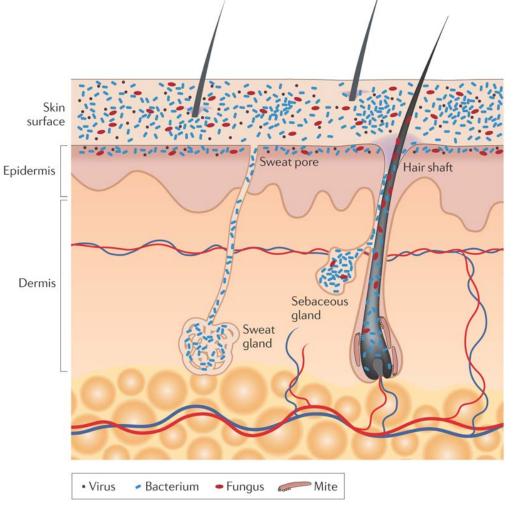
Lessons learned from perturbing the skin microbiome Elizabeth A. Grice, PhD

Assistant Professor of Dermatology & Microbiology University of Pennsylvania



>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

Grice et al. 2011 Nature Reviews Microbiology

to disrupt our skin microbiome!

Hand sanitizer



Hand washing



Bathing



Antiseptics





Antiseptics are efficacious against pathogens

TABLE

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

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



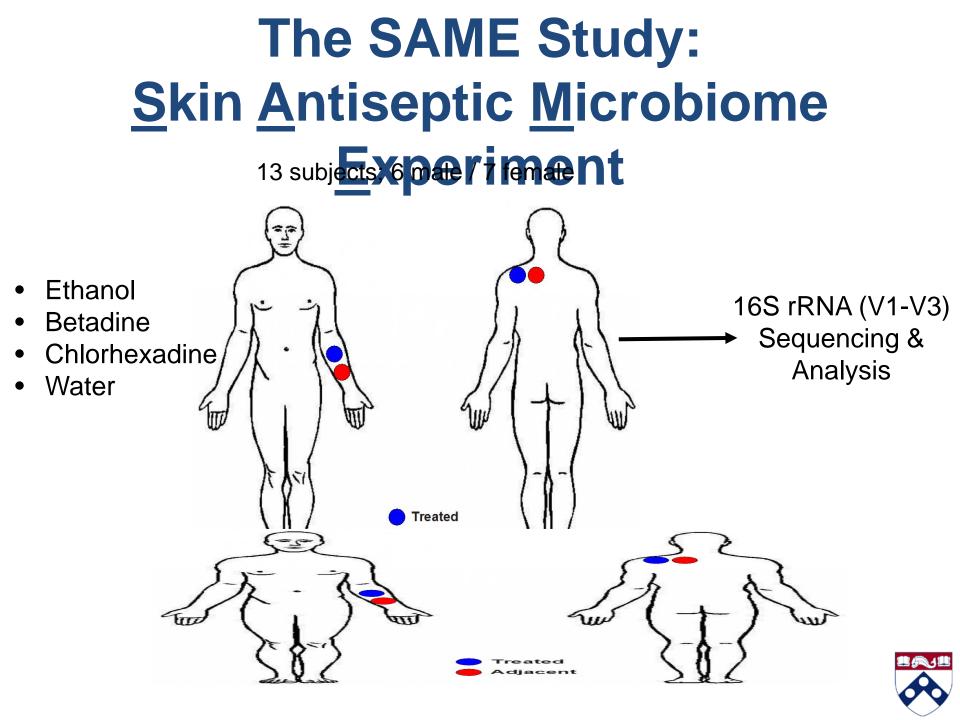








Adam SanMiguel, CAMB/MVP student



DNA sequence-based identification of bacteria: 16S ribosomal RNA none

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

V2

 Eliminates biases associated with cultures

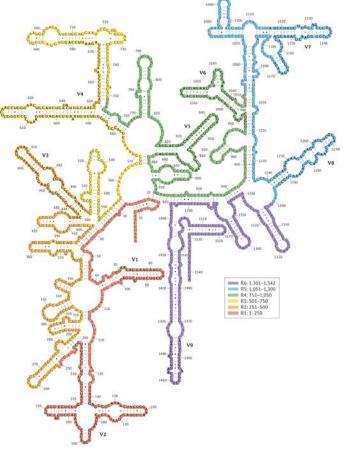
V3

V4

٧5

V6

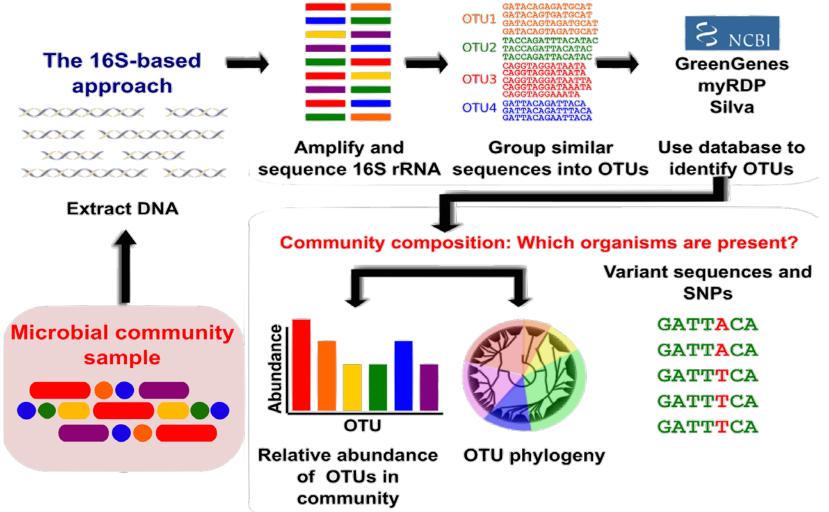
V7



Nature Reviews | Microbiology

V8

Overall Approach: 16S rRNA gene Sequencing

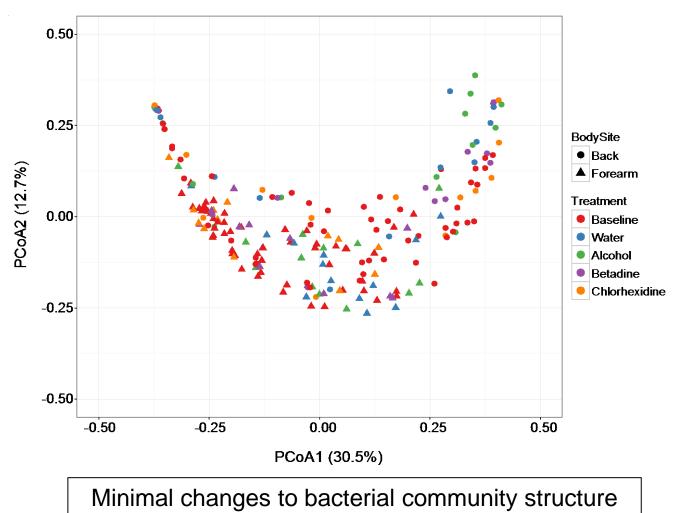




Morgan XC, Huttenhower C (2012) PLoS Comput Biol.

What are the immediate effects 1 hour post-treatment?

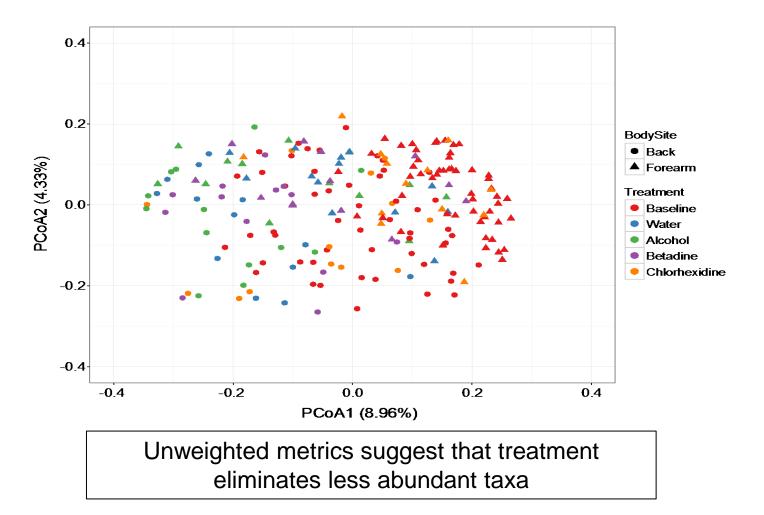
Weighted UniFrac distance metric: measures the "unique fraction" of phylogenetic branch length & weighs by the abundance of taxa contributing the branch length





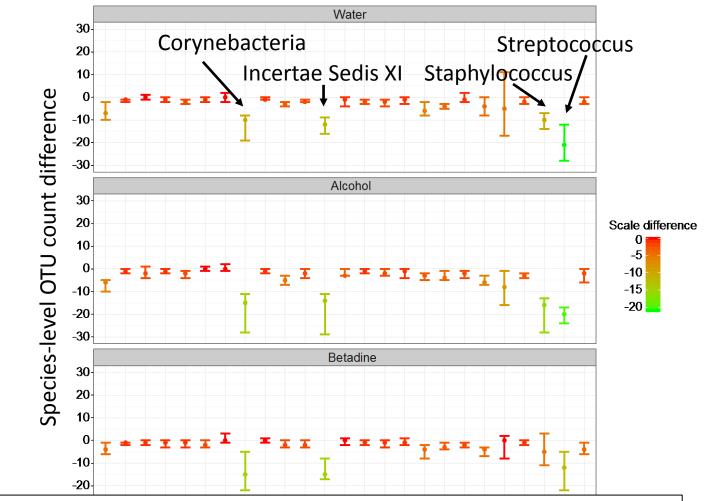
Is bacterial community membership influenced by antiseptic trx?

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





Are there changes in bacterial diversity due to treatment?



Micrococot

Staphylococcat. SHONDEDER

Certain bacterial lineages experience a decrease in OTU richness. Mathopaciatas Proportialise Convebacterias oor world a second Brevibereiler Carlobacteria Callobacterat Incertae Sed Lactobacilla Leptonichiae Microbacteriac Pilodobacterae Veillonelle

ABIOCOLO

Actinomycets

Constronadae

Damabacterat Fisvobacteriae Fusobacteria.

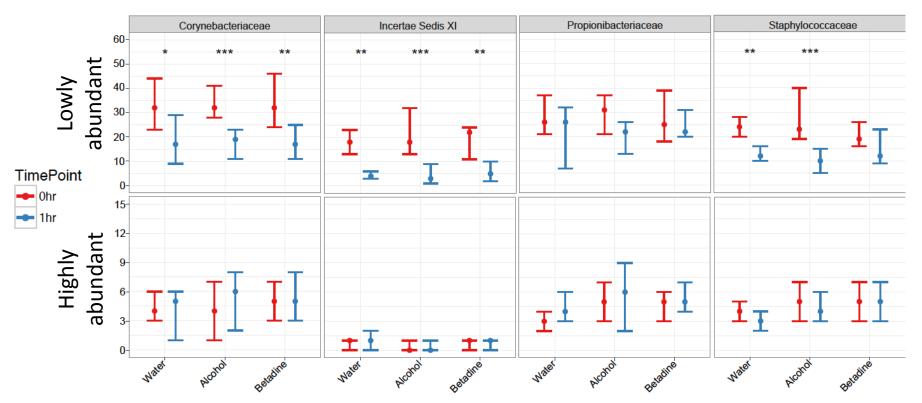


Are common and rare OTUs equally minimized by treatment?

Back Forearm 500-*** ** *** *** *** -owly abundant OTU count 400-Lowly abundant: 300-< 0.05% 200relative abundance 100-TimePoint 0 📥 Ohr 60 📥 1hr Highly abundant OTU count 50-**Highly** 40 abundant: 30->0.05% ٠ relative 20abundance 10-0 Betadine Alcohol Beladine Alcohol Water Water

** 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

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



What are the consequences for colonization resistance to pathogens?

Staphylococcus aureus: the <u>leading</u> cause of skin and soft tissue infection and surgical site infection.

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



Atopic dermatitis



Staphylococcal scalded

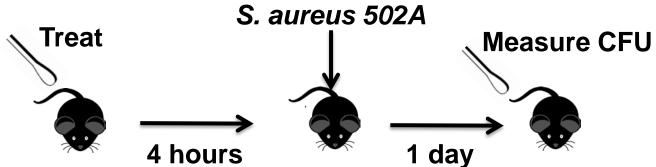
skin syndrome

Impetigo



Does antimicrobial treatment disrupt colonization resistance to

S. aureus?

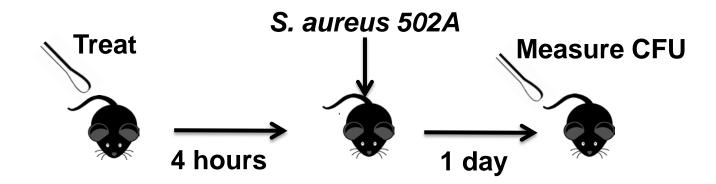


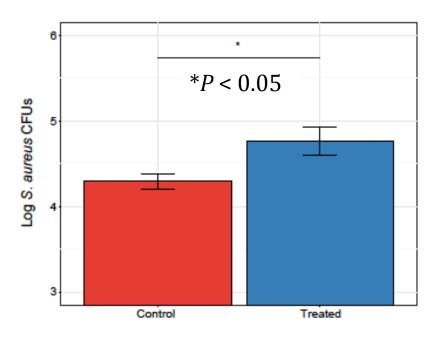


SKH1-elite hairless mouse



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



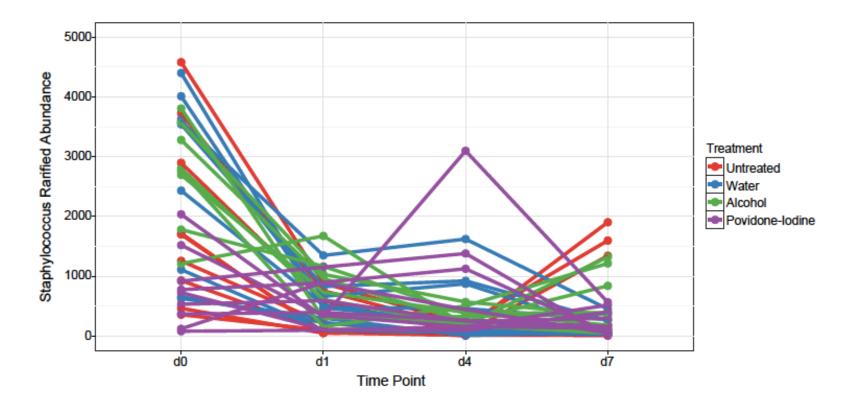


Antimicrobial perturbation of skin microbiota increases colonization efficiency of *S. aureus*



SanMiguel et al. Under revision

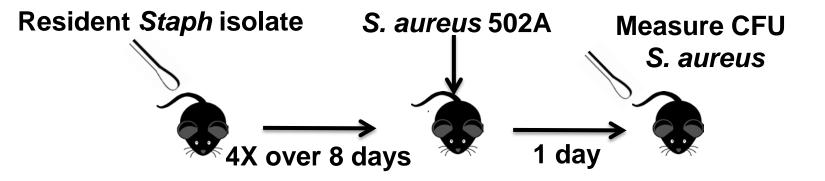
Does antimicrobial treatment remove bacteria that compete against S. aureus?



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

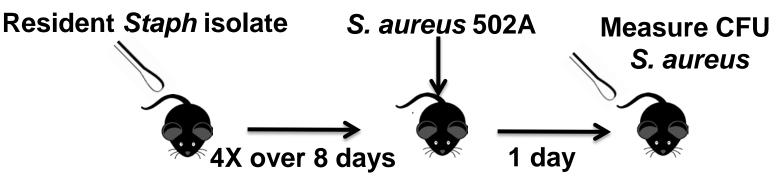
SanMiguel et al. Under revision

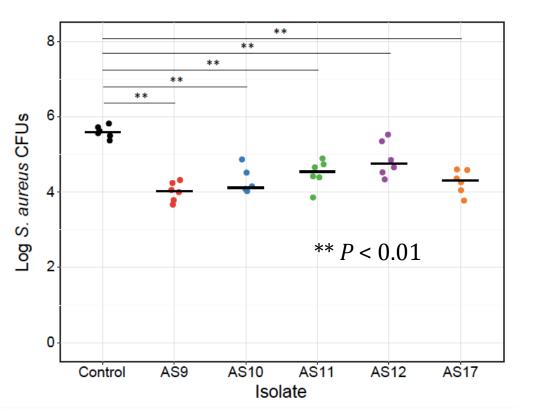
Do resident *Staphylococcus* spp. compete against *S. aureus*?





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





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



SanMiguel et al. Under revision

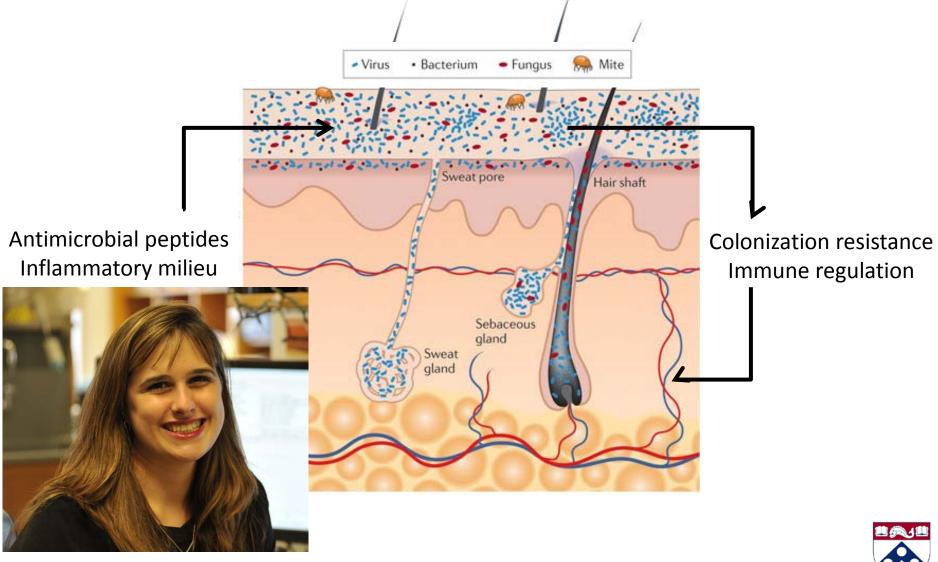
Lessons learned from perturbing the skin microbiome

- 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

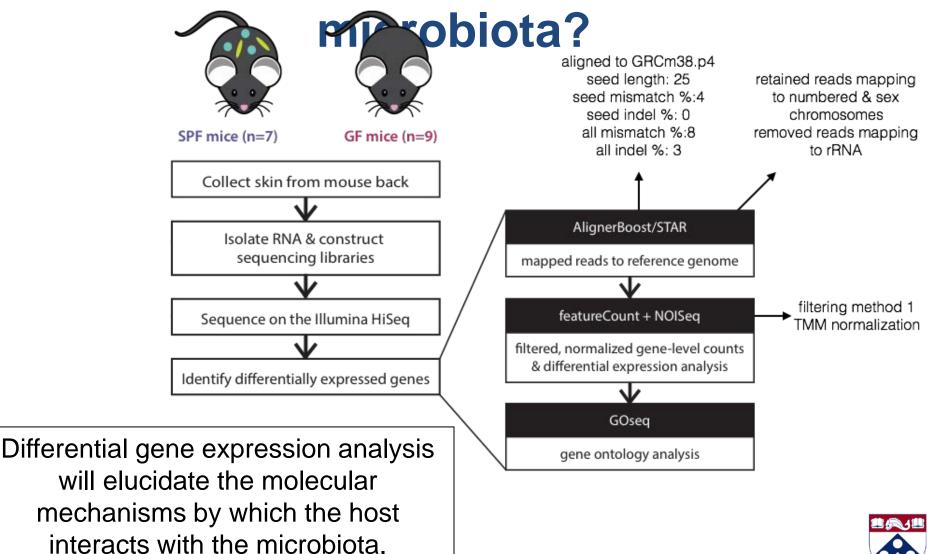
Germ free mice are a powerful tool to investigate host-microbe interactions



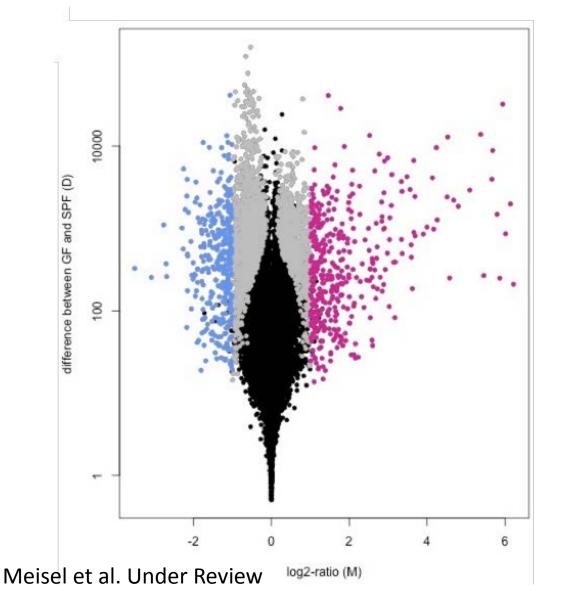
Penn Gnotobiotic Mouse Facility



What host cutaneous pathways and functions are mediated by the



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



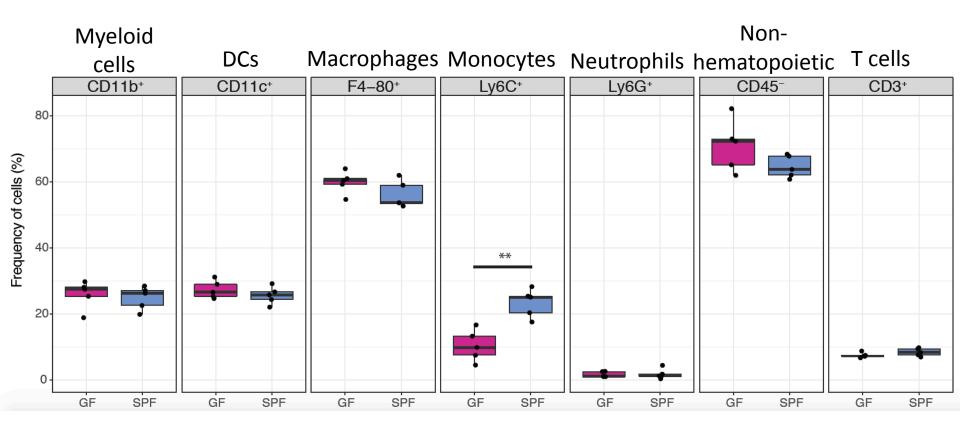
What genetic pathways/functions are influenced by the commensal

REVIGO treemap of Blog COPO to Terms Enriched in DEGs

	immune response	response to cytokine		response to other organism		cellular response to cytokine stimulus		response to external stimulus		cytokine production		ition of production	positive regulation of multicellular organismal process	epidermis development	epidern differen	itiation	
-		The second	nositive regulation		ulation of une system chemica			cellular response to chemical stimulus	regulation of multicellular		regulation of vascular endothelial egulation of kine production molting cycle		f nair cycle	dev respectitelial			
	innate immune response	response to interferon-beta	adhesion of symbiont to host	leukocyte chemotaxis	leukocyte activation	e regu n of res	sitive Iation sponse mulus	positive regulation of response to wounding	pro single-r	nismal Icess nulticellular m process	organ developmer cell differentiatio	multicel organis	ion regulation of Interferon-alpha bioxymtatic process uliar ostitular developmenta	oell differentiation planar cell polarity patheay involved in exis cloradion	epidemis developmen	narphopamesia	
	defense response	positive regulation of immune system	response to stress			MyCR8-depende 1sil-like recepto signaling pathwa	orga	ince organic substance	imm syst proc		nune		ptide peptide oss-linking	response to	multi-or	-	
		process	taxis	defense response to protozoan	to organisms	response to wounding regulation	inflamma respon inflammato response	tory cell-cell se adhesion ry hematopoietic or lymphoid					n activation cascad			ion of mail cell	
		of response to external stimulus leukocyte migration		macrophage activation	adhesion	complement	antigenk stimulus hurmoral immune esponse		divaler metal i	nt on secretio	hanamambhana itaman	bio	melanin osynthesis secondary abolic process	biological	apoptotic	stem cell	
	response to biotic stimulus	cetlular response to interferon-beta	adaptive immune response	response to protozoan	to stimulus regulation of response to external stimulus	immune system development	positive regulation locomotio regative regul of multi-orga process	a of response to stimulus ation single	transpo dival positive regulatio of NF-kapp import in nucleus	secretion	ion	ization 0	NLRP3 ammasome complex ssembly	multicellular organismal process	biological phase	atem cell prolification	

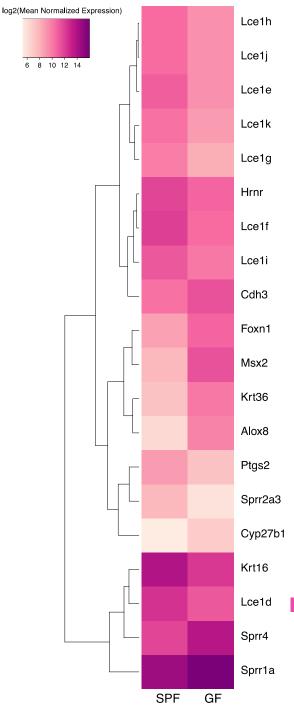
* Staphylococcus aureus infection * Complement and coagulation cascades * Cytokine-cytokine receptor interaction * Toll-like receptor signaling pathway

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

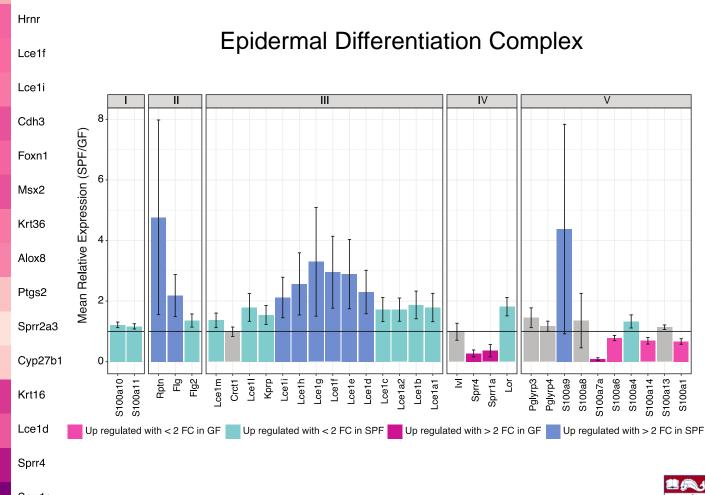


Similar frequency of broad cell types except Ly6C+ monocytes.





Keratinocyte differentiation & epidermal development genes are differentially expressed





Meisel et al. Under Review

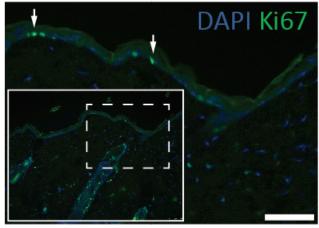
Is epidermal structure & function similar in SPF vs GF mice?

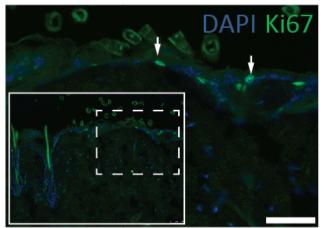
SPF GF DAPI K6A DAP

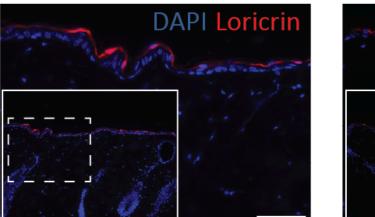
Similar epidermal thickness and staining of keratin 6A suggest intact barrier.

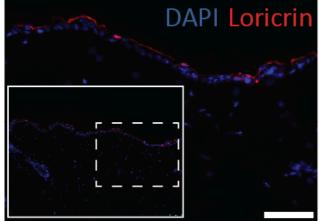


Is proliferation and differentiation similar in SPF vs GF mice?









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

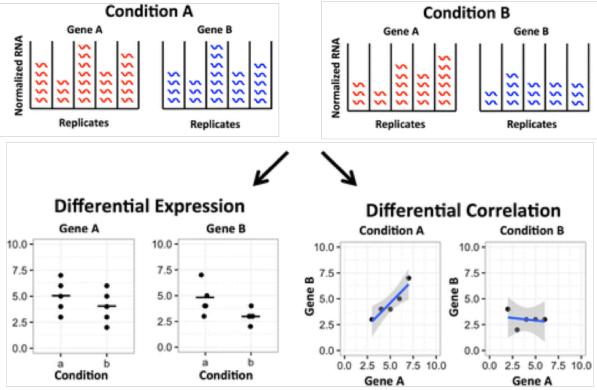




Casey Bartow-McKenney PhD student

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

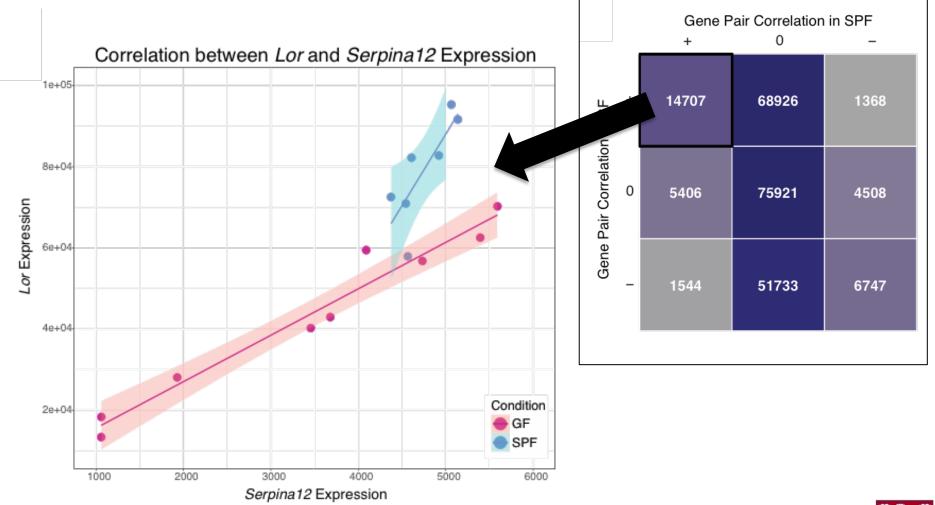
DGCA: Differential Gene Correlation Analysis





McKenzie, A.T., Katsyv, I., Song, WM. et al. BMC Syst Biol (2016) 10: 106.

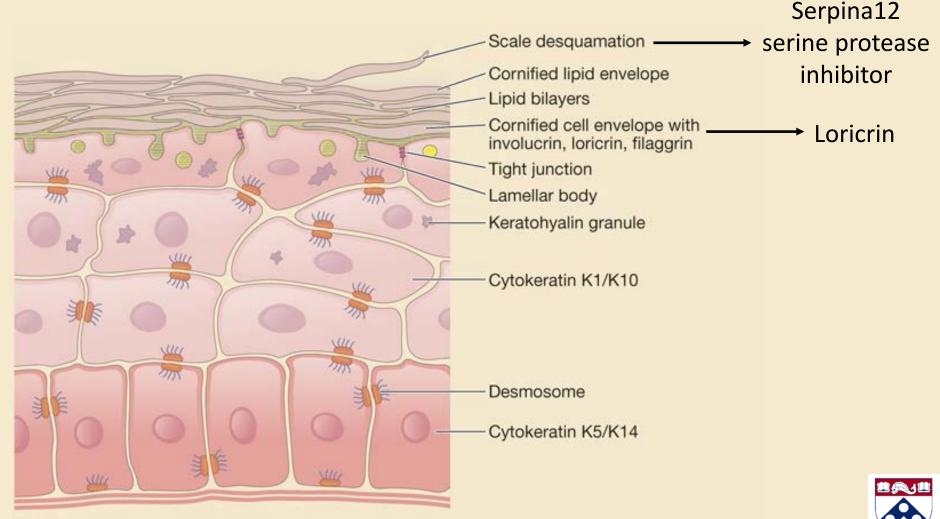
Microbial-dependent alterations in gene networks controlling epidermal development





Meisel et al. Under Review

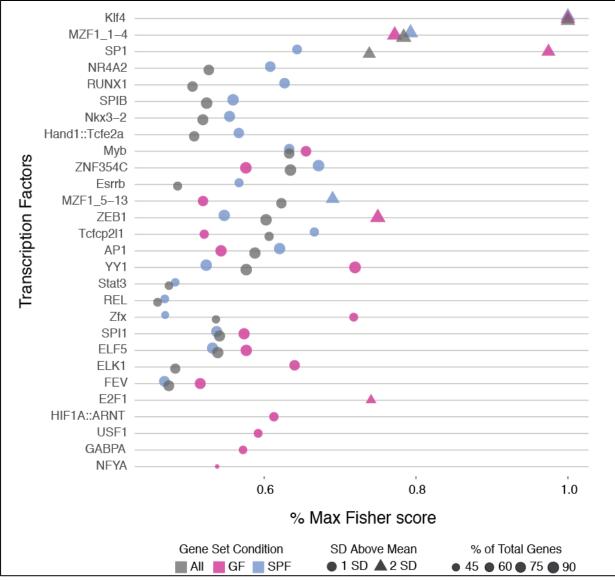
Microbial-dependent alterations in gene networks controlling epidermal development



Goldsmith et al Fitzpatrick's Dermatology in General Medicine, 8th ed.

Do correlated DEGs share TFBS?

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





Meisel et al. Under Review

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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Collaborators:

PennCHOP Microbiome Program Sequencing Core Gnotobiotic Mouse Core David Artis + lab





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NIH: NIAMS, NINR Burroughs Wellcome Fund Janssen Research and Development Pennsylvania Department of Health Linda Pechenik Montague Investigator Award

HEALTH

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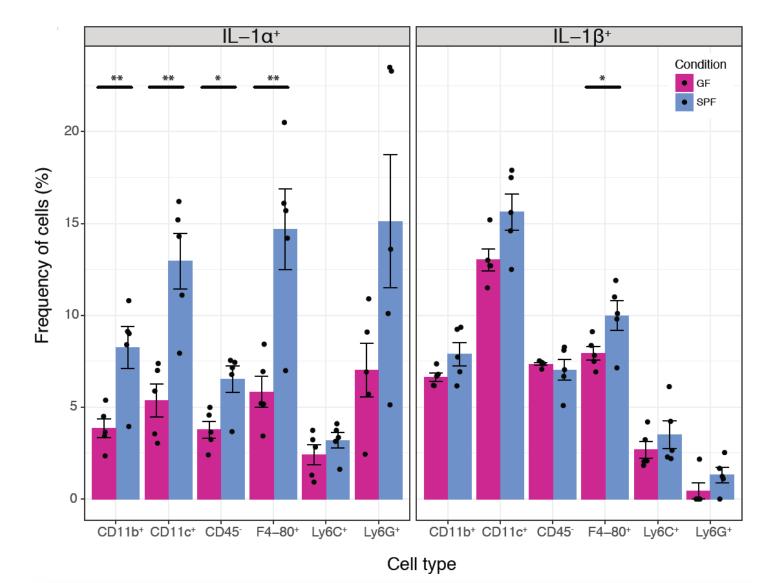


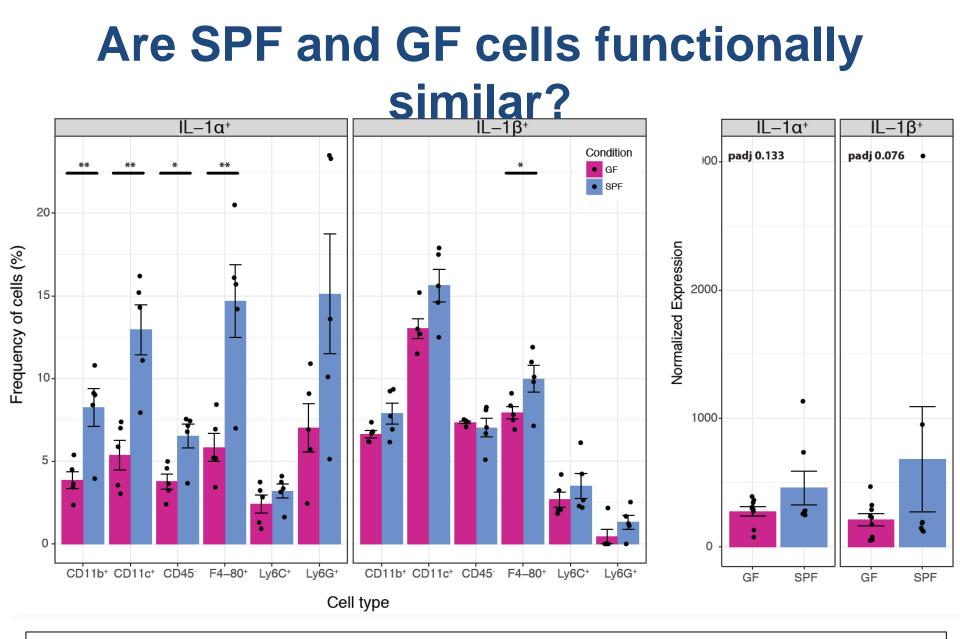
NURSING RESEARCH

BURROUGHS

WELLCOME

Are SPF and GF cells functionally similar?





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