

# Longitudinal changes in airway microbiome signatures and immunoregulatory cell dynamics following Bronchial Thermoplasty

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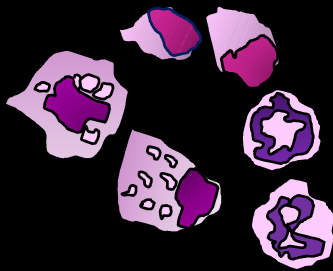
Innate Immunity Congress, Berlin

July 21-22, 2016

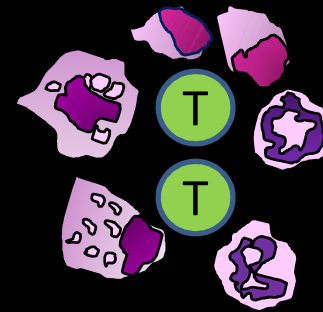
# Asthma: A Chronic Inflammatory Disease

- Asthma is a serious public health problem - affects more than 20 million people in the United States
- Reversible airway obstruction associated with airway hyper-responsiveness
- Th2 predominant inflammation
- Oxidative Stress – ROS & RNS
- Immune regulation in Asthma

# Myeloid-Derived Regulatory Cells



iNOS  
Arginase  
NADPH oxidase  
IDO  
TGF- $\beta$ , IL-10, IL-6



T cell suppression  
Induction of Tregs

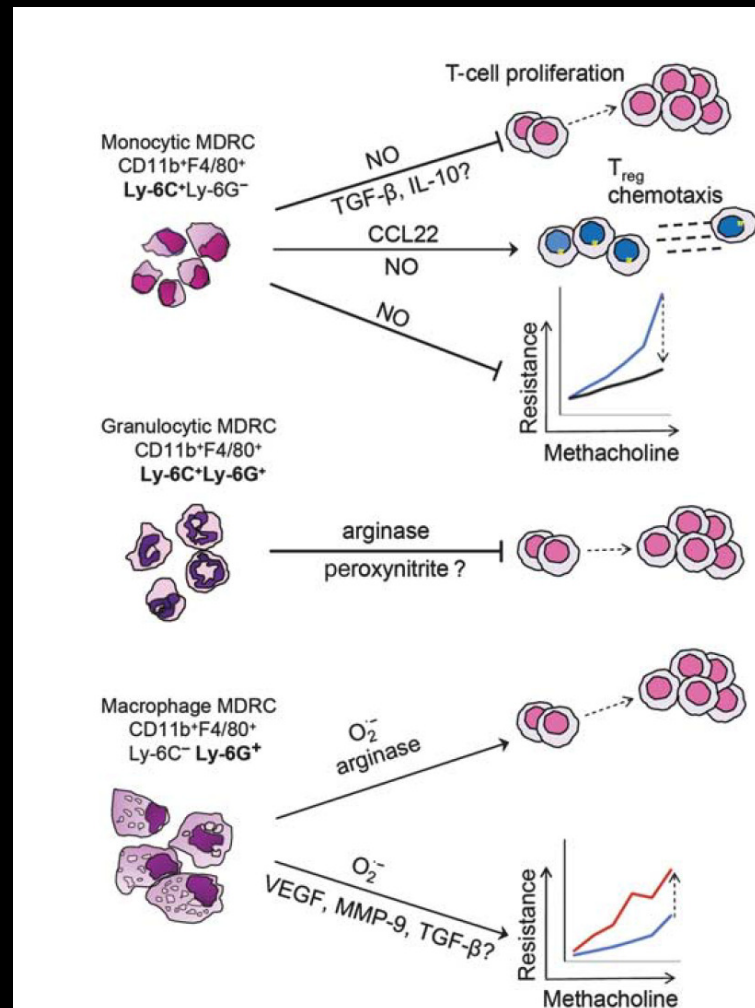
*Arora et al.*, Mucosal Immunol. 2010

Nov;3(6):578-93

*Deshane et al.*, Mucosal Immunol. 2011

Sep;4(5):503-18

# Differential regulation of airway responses by MDRC subsets



*Deshane et al*  
*Mucosal Immunol.* 2011 Sep;4(5):503-18

# MDRCs in Human Asthma

**TABLE I.** Characteristics of study subjects

Parameters	Group			P value*
	Healthy subjects (n = 10)	Asthmatic patients (n = 9)	Patients with COPD (n = 8)	
Age (y)	52.1 ± 9.4	51 ± 10.4	56.3 ± 7.9	.3752
Male/female sex	3/7	1/8§	7:1§	.0031
White/African American subjects	3/7	2/7	1/1	.5258
Never smoker	9:1†	6:3§	0:8‡§	.0002
Atopic/nonatopic	2/8†	7/2§	1/7§	.0079
Prebronchodilator FEV <sub>1</sub> (% predicted)	89 ± 13‡	83 ± 26§	55 ± 15‡§	.0006
Postbronchodilator FEV <sub>1</sub> (% predicted)	92 ± 12‡	89 ± 11§	56 ± 14‡§	.0007

Age and prebronchodilator and postbronchodilator FEV<sub>1</sub> are represented as means ± SDs.

\*For continuous variables, *P* values were determined by using the Kruskal-Wallis test to assess whether the distributions differ among the study groups. For categorical variables, *P* values were determined by using an exact Pearson  $\chi^2$  test.

†Distributions differ between the healthy and asthmatic groups by using a Bonferroni-adjusted type I error rate of 0.0167.

‡Distributions differ between the healthy and COPD groups by using a Bonferroni-adjusted type I error rate of 0.0167.

§Distributions differ between the asthmatic and COPD groups by using a Bonferroni-adjusted type I error rate of 0.0167.

***Deshane et al***  
***JACI. 2015 135:413-424***

# Phenotypic and Functional Characteristics of Airway MDRCs

CD11b<sup>+</sup>DAF-FM-DA<sup>+</sup>HLA-DR<sup>-</sup> → CD14, CD16

	Proportions of MDRCs			Modulation of T-cell response			Free radical pathway		
	Healthy subjects	Asthmatic patients	Patients with COPD	Healthy subjects	Asthmatic patients	Patients with COPD	Healthy subjects	Asthmatic patients	Patients with COPD
CD14 <sup>+</sup> CD16 <sup>+</sup>	High	High	Low	↓	↓	↓	iNOS	iNOS	iNOS
CD14 <sup>+</sup> CD16 <sup>-</sup>	Low	High	Low	ND	↓	ND	NA	iNOS ROS	NA
CD14 <sup>-</sup> CD16 <sup>+</sup>	Low	High	High	ND	None	None	NA	NA	NA

CD11b<sup>+</sup>DHE+HLA-DR<sup>+</sup>CD11c<sup>+</sup> → CD163<sup>+</sup>/CD163<sup>-</sup>

CD163 <sup>+</sup>	Low	High	Low	↑	↑	↑	ROS Arg	iNOS ROS Arg	ROS Arg
CD163 <sup>-</sup>	Low	High	High	ND	↑	↑	NA	iNOS ROS Arg	iNOS ROS Arg

# Immune regulation by MDRCs in Bronchial Thermoplasty?

- Bronchial Thermoplasty (BT) is an FDA approved therapeutic option for a subset of asthmatics who continue to be symptomatic despite high dose steroid therapy.
- Three bronchoscopic procedures in which a catheter is used to deliver 65°C thermal energy to the airways.
- BT patients experience improvements in asthma control, quality of life, and a reduction in severe exacerbations.
- Mechanisms underlying the impact of BT on airway inflammation are largely unknown.

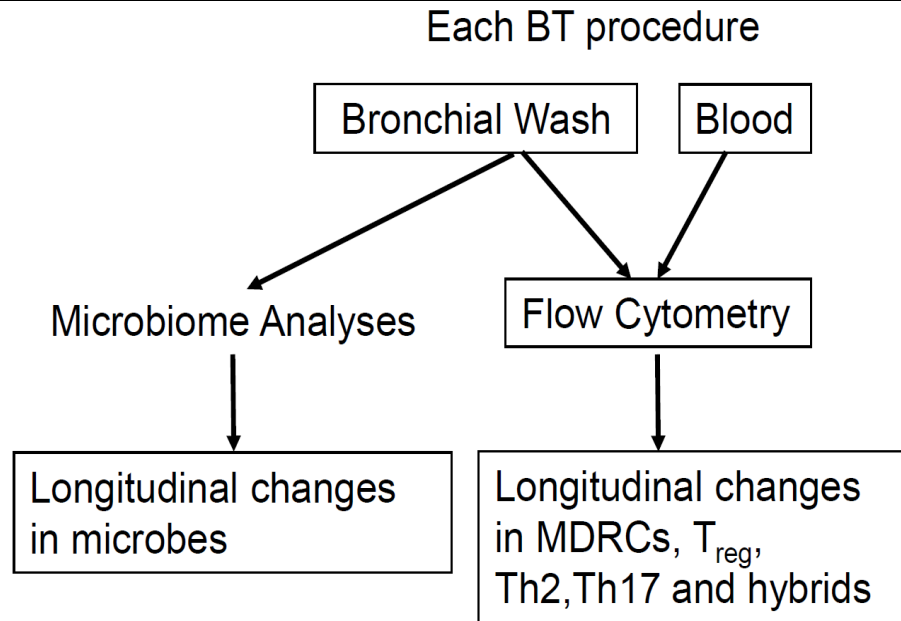
- Modulation of MDRCs in BT is unknown
- Microbiome changes in BT are unknown
- Functional relationships between MDRCs and airway microbiome are unknown



- Longitudinal changes in MDRCs and airway microbiome following BT contributes to improved outcomes

# Patient Characteristics

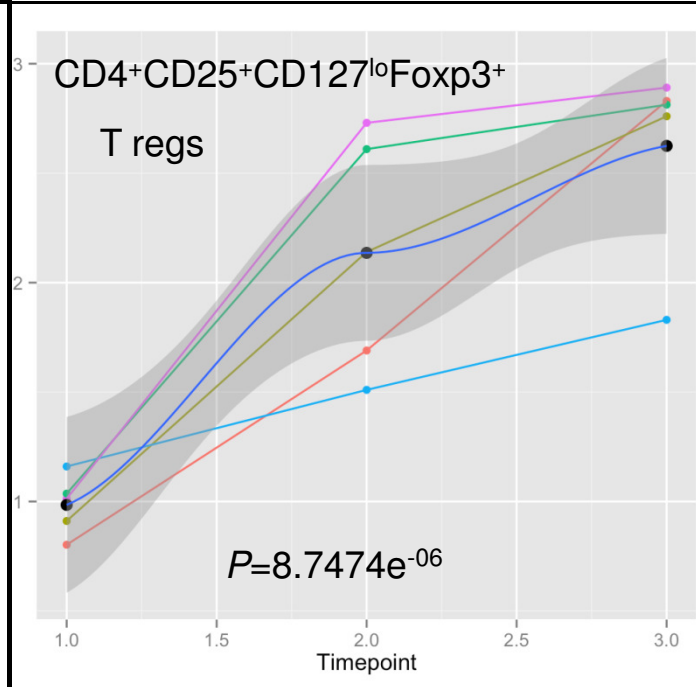
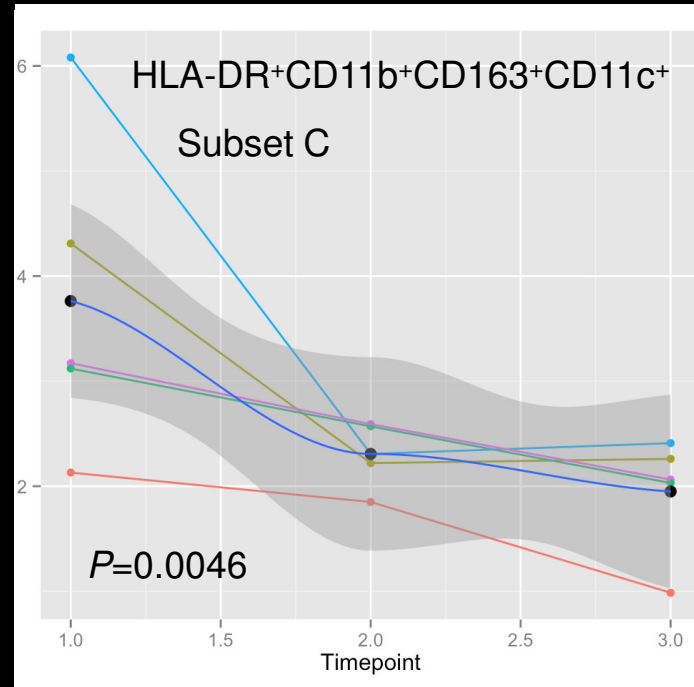
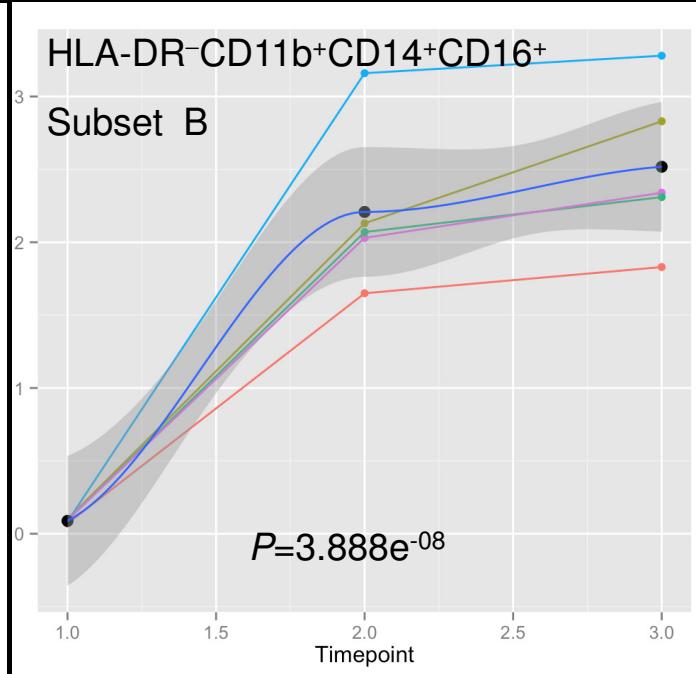
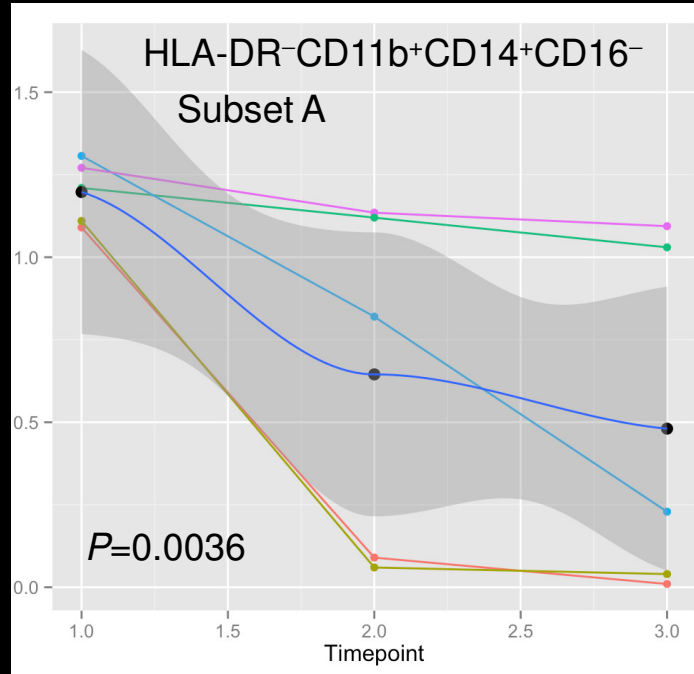
- Five patients with severe asthma managed by allergists or pulmonologists affiliated with UAB
- All five patients were poorly controlled as determined by ACT score  $<20$  despite using inhaled corticosteroids and/or daily oral steroids plus additional controller medications.
- All patients demonstrated airway hyper-responsiveness as determined by either bronchodilator reversibility or a positive methacholine challenge test



1. Longitudinal changes in individual microbial phyla and cell proportions were determined by linear mixed model regression analysis taking sample ID as a random effect.
2. Correlation between overall microbiome composition and time points was determined by PERMANOVA.
3. Microbial phyla and cell MDRC subsets were correlated by linear mixed model regressing one variable to the other adjusting time points as a fixed effect and sample ID as a random effect. All analyses were conducted in the lme4 or vegan R package.

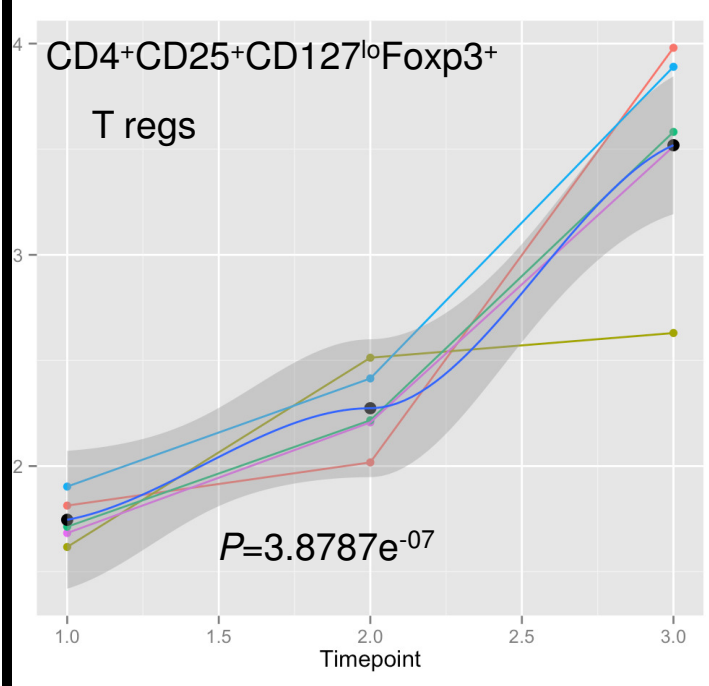
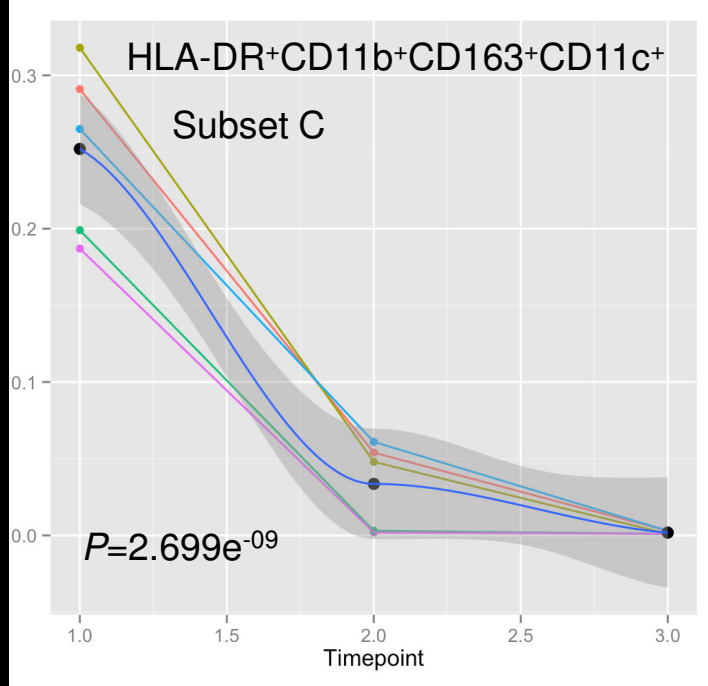
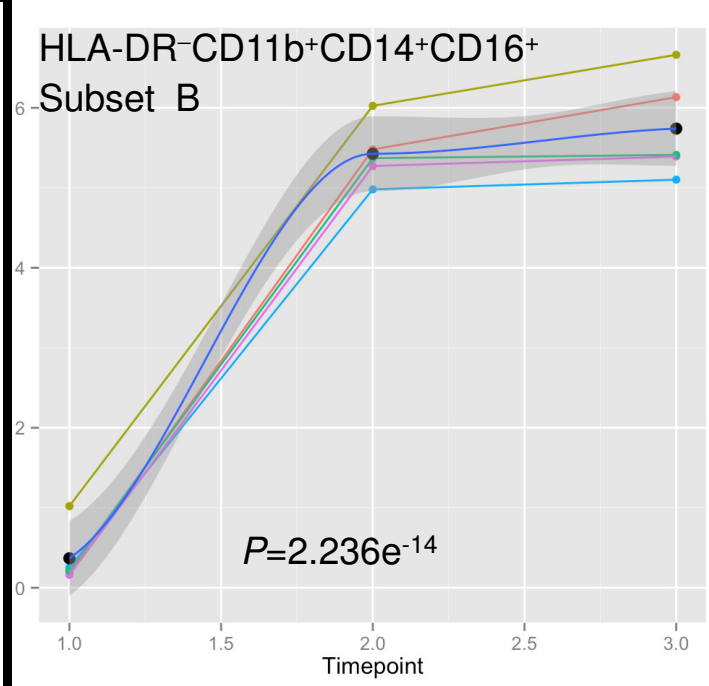
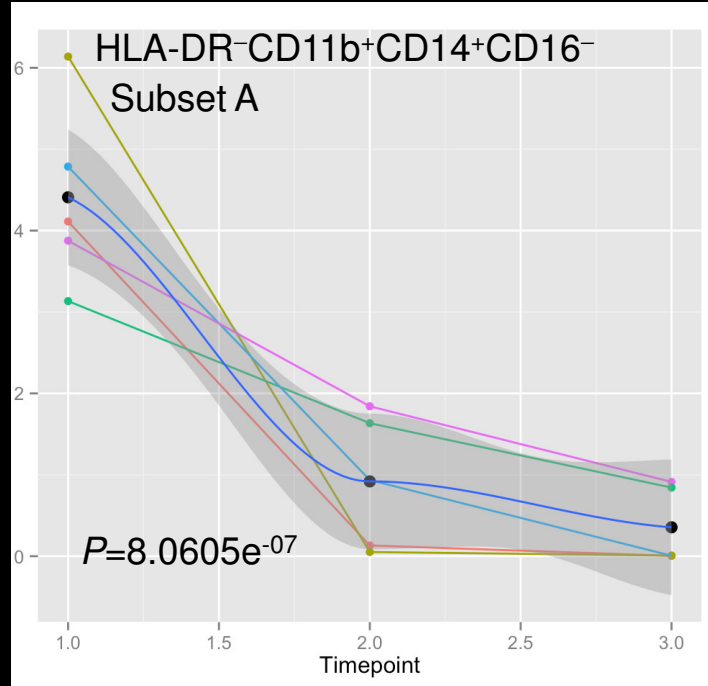
# BRONCHIAL WASH

Percent Subsets in Bronchial Wash



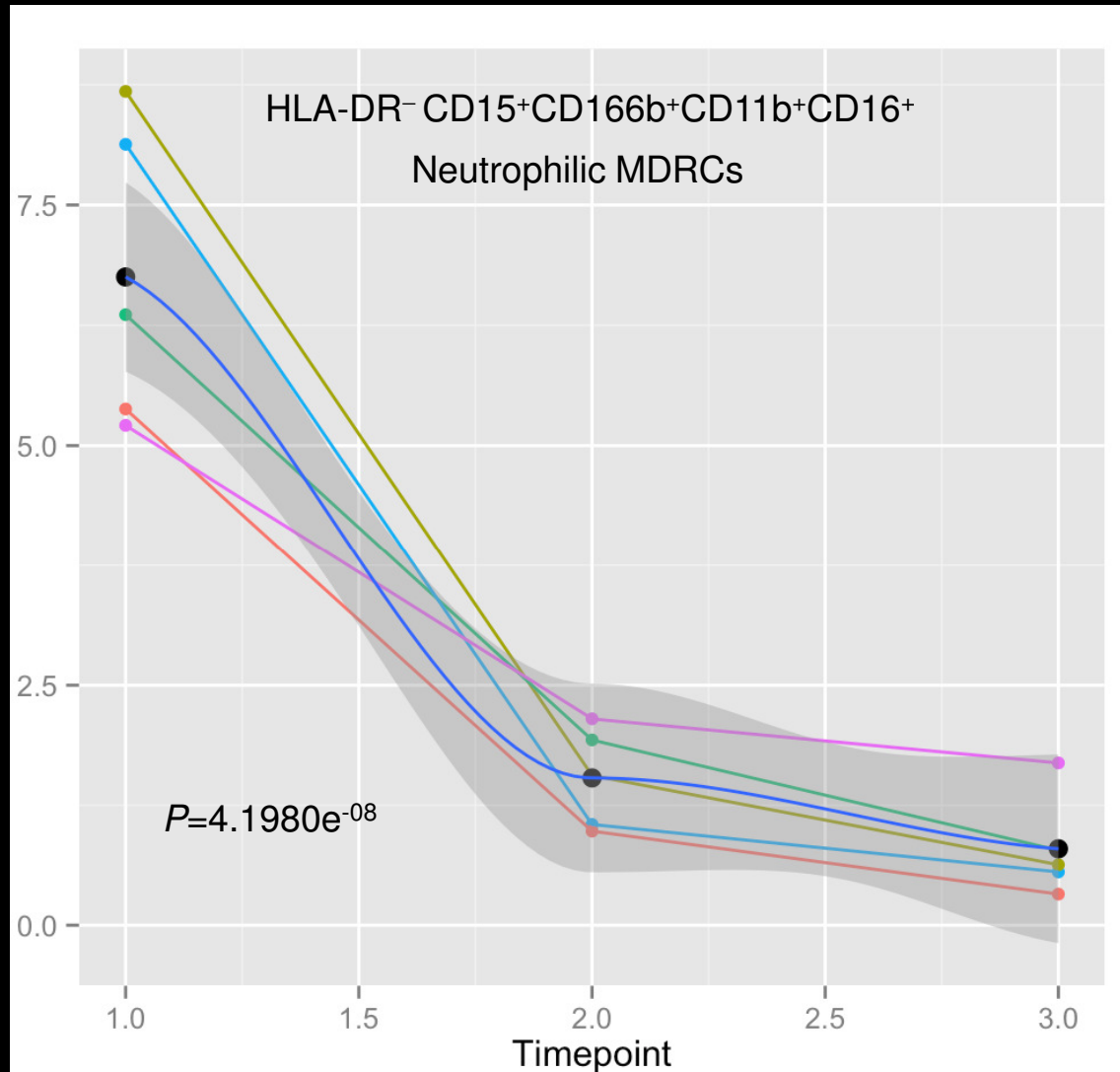
# BLOOD

Percent Subsets in Peripheral Blood



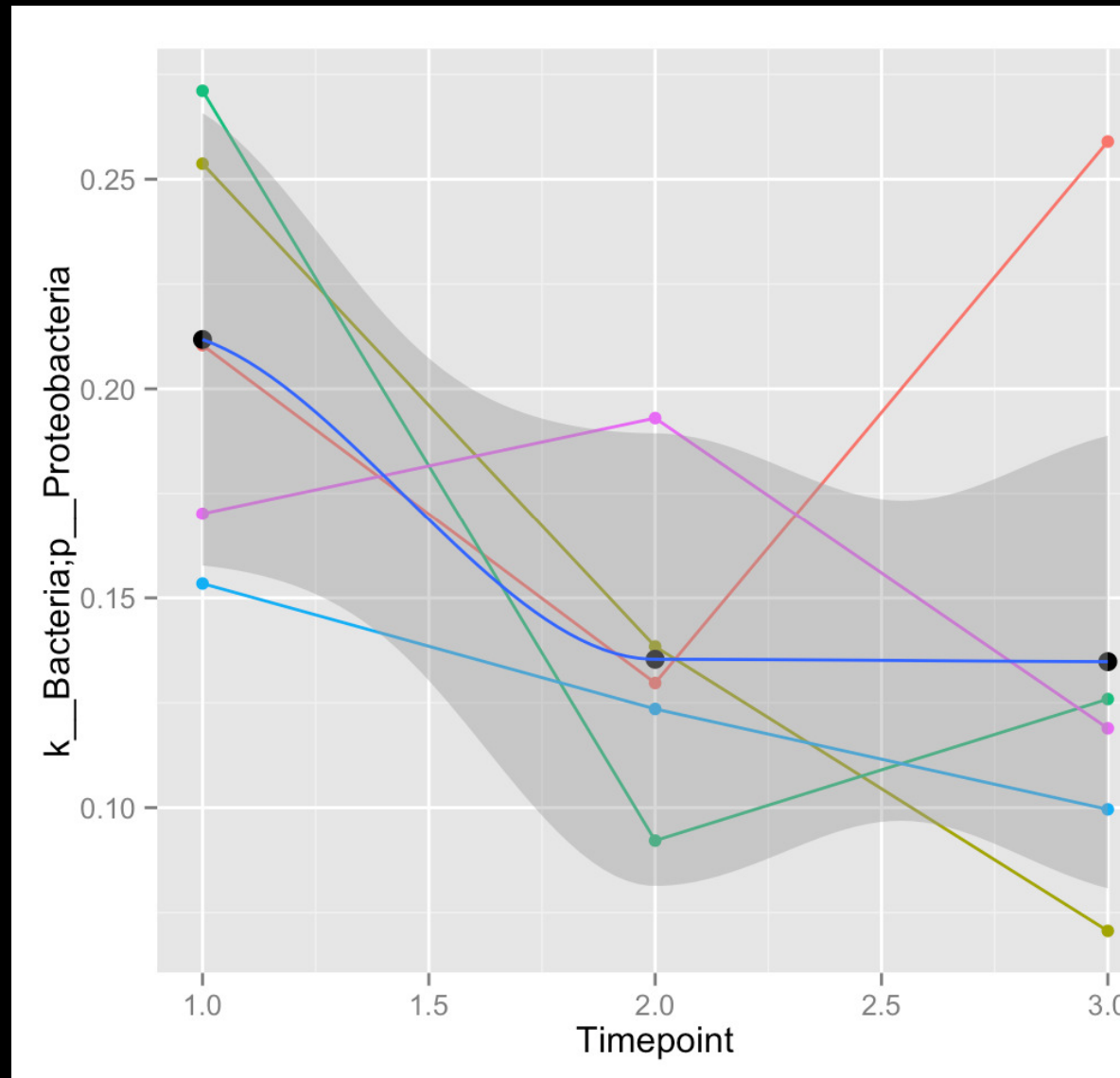
BLOOD

Percent Subsets in Peripheral Blood



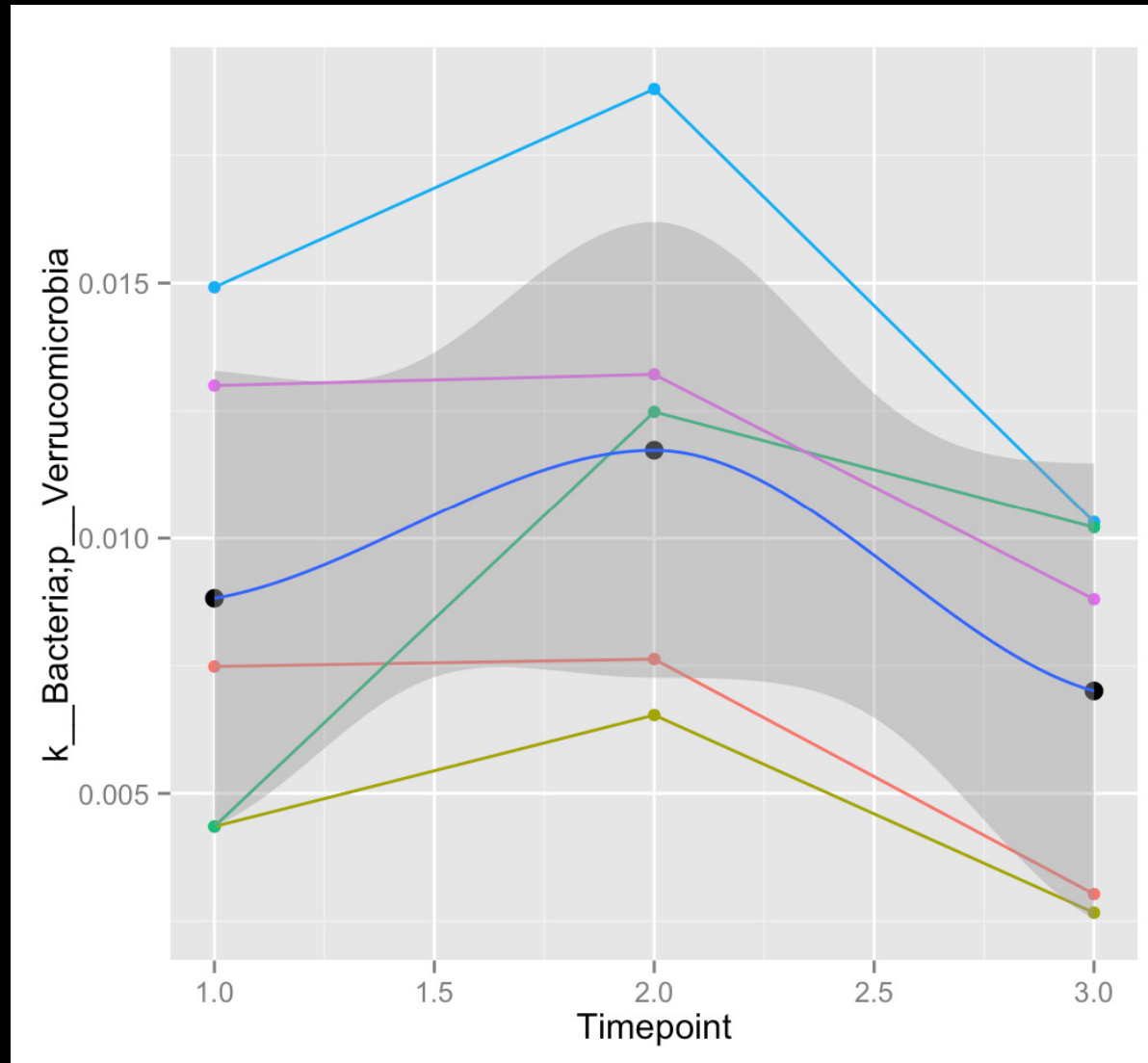
	mean abundance	Group2-1	Group3-1	p-value
k__Bacteria;Other	0.001265626	0.0004224558	-0.0001157003	0.3910571
k__Bacteria;p__Actinobacteria	0.05889575	-0.0111165	0.001884219	0.496534
k__Bacteria;p__Bacteroidetes	0.1579293	0.005732235	0.009157824	0.39593
k__Bacteria;p__Cyanobacteria	0.001442555	0.0007041906	0.0004424789	0.483212
k__Bacteria;p__Firmicutes	0.5970713	0.07983223	0.06346952	0.1458097
k__Bacteria;p__Fusobacteria	0.005077856	5.470576e-05	0.002246956	0.4060238
k__Bacteria;p__Proteobacteria	0.1606404	-0.07639408	-0.07695898	0.04095912
k__Bacteria;p__Spirochaetes	0.002110084	-0.00127247	0.001637145	0.3951153
k__Bacteria;p__Tenericutes	0.001089895	7.112489e-05	0.001380949	0.1580867
k__Bacteria;p__Verrucomicrobia	0.009186785	0.002910648	-0.001814545	0.0200815
k__Bacteria;p__[Thermi]	0.002361206	-0.0003325322	-0.0003957534	0.8506804

# Longitudinal changes in Proteobacteria





# Longitudinal changes in Verrucomicrobia

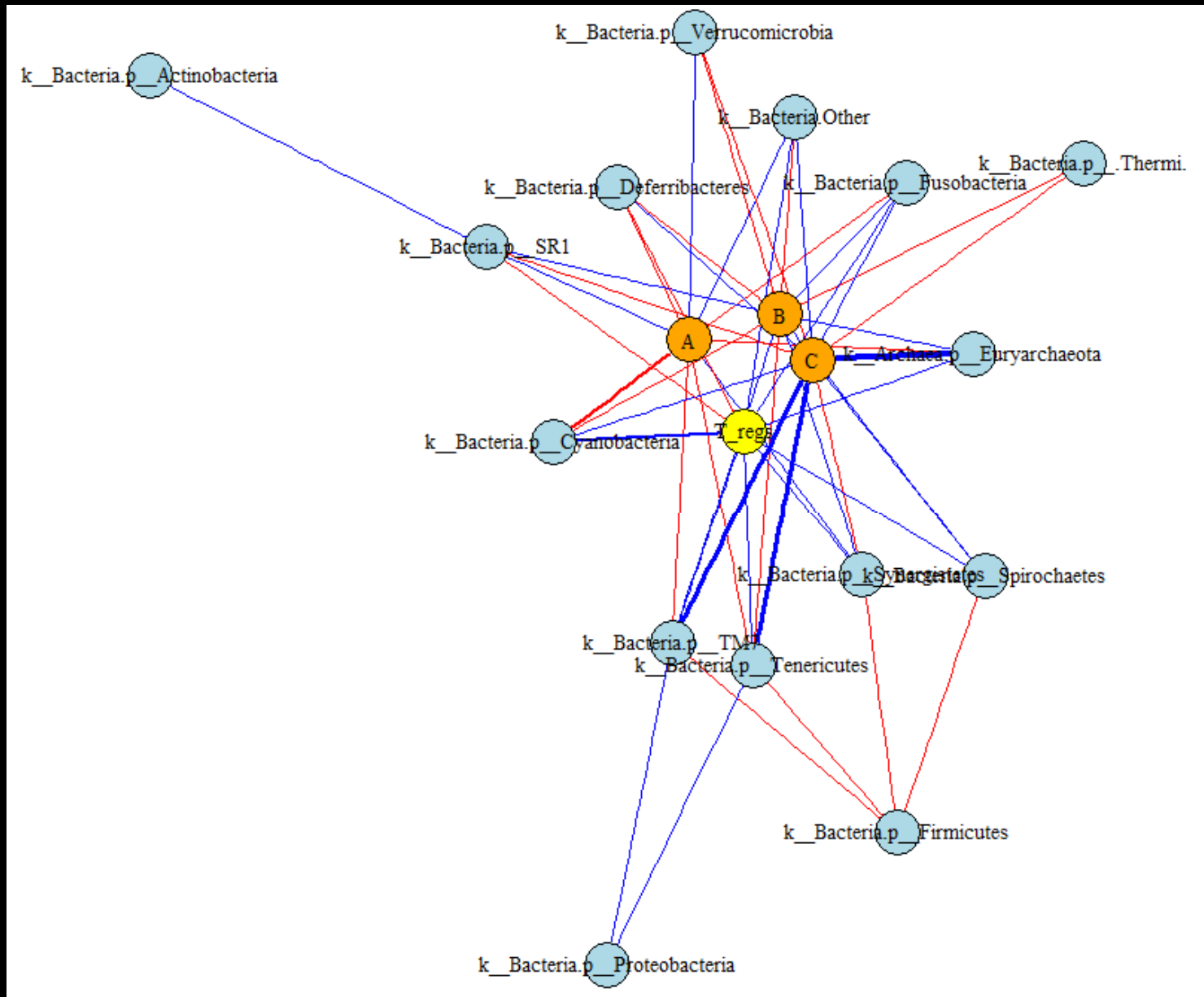


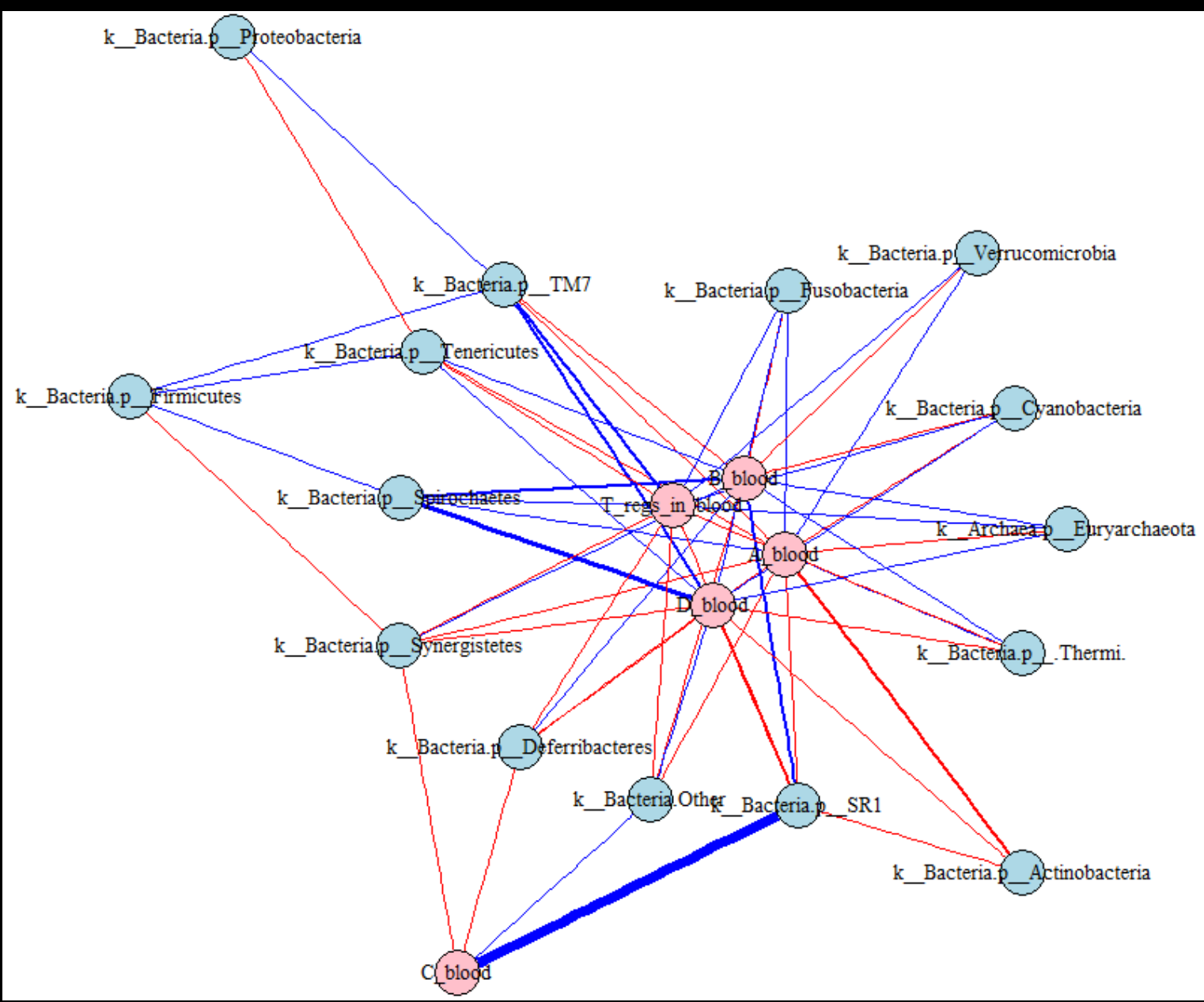
	k__Archaea;p__Euryarchaeota	k__Bacteria;Other	k__Bacteria;p__Actinobacteria	k__Bacteria;p__Bacteroidetes	k__Bacteria;p__Cyanobacteria	k__Bacteria;p__Deferribacteres	k__Bacteria;p__Firmicutes	k__Bacteria;p__Fusobacteria	k__Bacteria;p__Proteobacteria	k__Bacteria;p__SR1	k__Bacteria;p__Spirochaetes	k__Bacteria;p__Synergistetes	k__Bacteria;p__TM7	k__Bacteria;p__Tenericutes	k__Bacteria;p__Verrucomicrobia	k__Bacteria;p__[Thermi]
A blood	75.36	170.01	13.82	-1.37	-144.03	102.54	-1.90	-46.11	-0.02	-140.57	-24.84	290.31	-141.81	-157.59	49.64	-78.36
B blood	81.20	-100.86	-2.72	-7.30	-51.82	-125.94	-0.06	52.85	0.72	-186.71	26.76	-320.13	143.52	42.39	-56.25	-83.20
C blood	0.59	23.35	0.38	0.33	1.36	22.21	-0.09	-2.43	0.02	19.56	0.34	13.87	-4.57	-7.84	0.46	6.28
D blood	229.30	-42.11	14.17	-7.76	-122.66	-298.33	-0.29	-63.95	0.46	718.06	-69.01	-252.75	-197.43	-211.41	0.86	-73.93
A	32.13	276.74	1.45	4.55	20.94	296.18	-0.20	-24.78	-0.45	188.11	-4.96	282.97	-81.54	-117.45	62.41	-8.65
B	-154.77	-38.94	4.33	1.51	-96.67	125.90	0.86	-64.23	-2.43	270.44	-28.82	-462.04	-164.46	-30.72	39.16	-41.06
C	427.21	-35.77	4.81	-2.70	-77.26	-128.50	3.02	-112.69	-6.92	-676.05	-97.45	-459.42	-319.67	-122.39	76.65	-197.93
T regs in blood	32.44	13.55	-1.52	6.02	136.55	-230.27	-2.94	14.74	2.53	55.03	33.48	-144.79	92.94	151.70	21.58	19.20
T_regs	163.73	27.25	-4.29	-4.97	78.90	-68.58	0.26	34.60	1.18	-93.84	31.27	181.54	97.07	-87.42	-5.62	-4.00

Gradient of Correlation

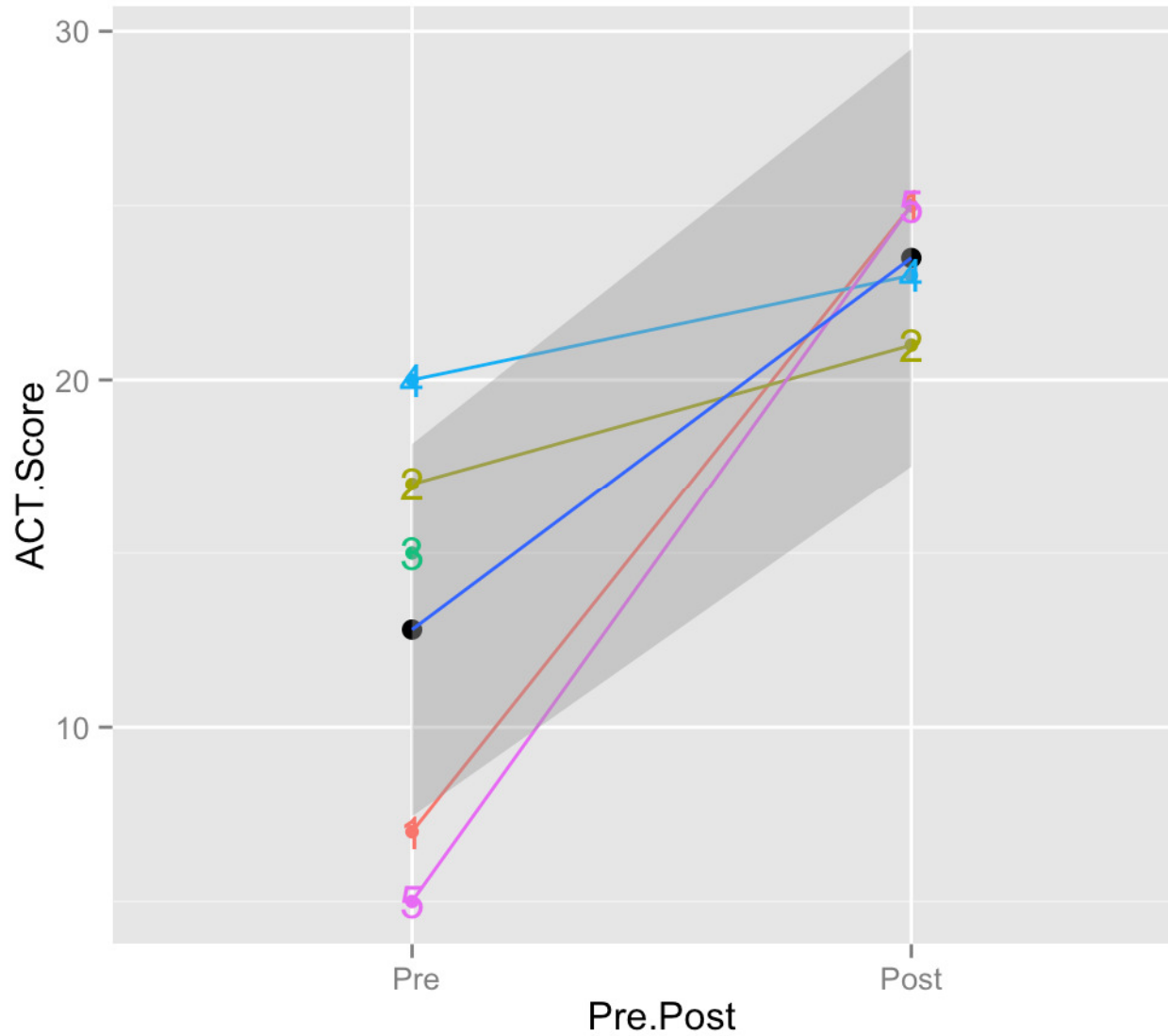


- Changes in Kingdom *Archaea*; phylum *euryarchaeota* correlated with progressive reduction of pro-inflammatory CD163<sup>+</sup>HLA-DR<sup>+</sup>CD11b<sup>+</sup>CD33<sup>+</sup>MDRCs (P=0.00003) and enhancement of both CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>lo</sup>Foxp3<sup>+</sup> T<sub>regs</sub> (P=0.0135) and CD14<sup>+</sup>CD16<sup>+</sup>HLA-DR<sup>-</sup> MDRCs (P=0.04).
- Changes in phyla *proteobacteria* and *fusobacteria* correlated (P=0.002, P=0.017) with the enhancement of CD14<sup>+</sup>CD16<sup>+</sup>HLA-DR<sup>-</sup> MDRCs in the BW.
- Alterations in phyla *tenericutes* and *verruccomicrobia* significantly correlated (P=0.039, P=0.0131) with the reduction in CD14<sup>+</sup>HLA-DR<sup>-</sup> MDRCs.

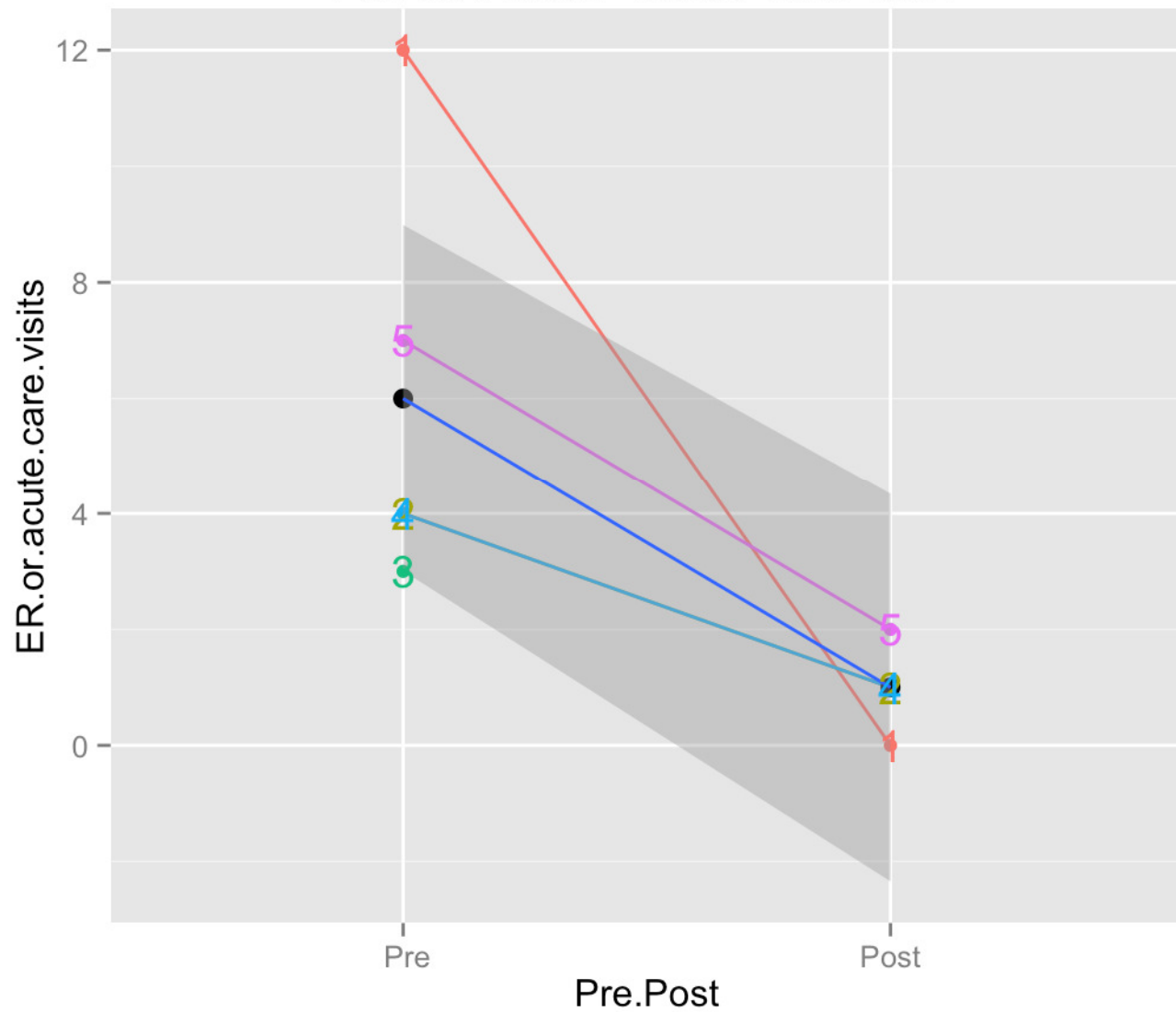




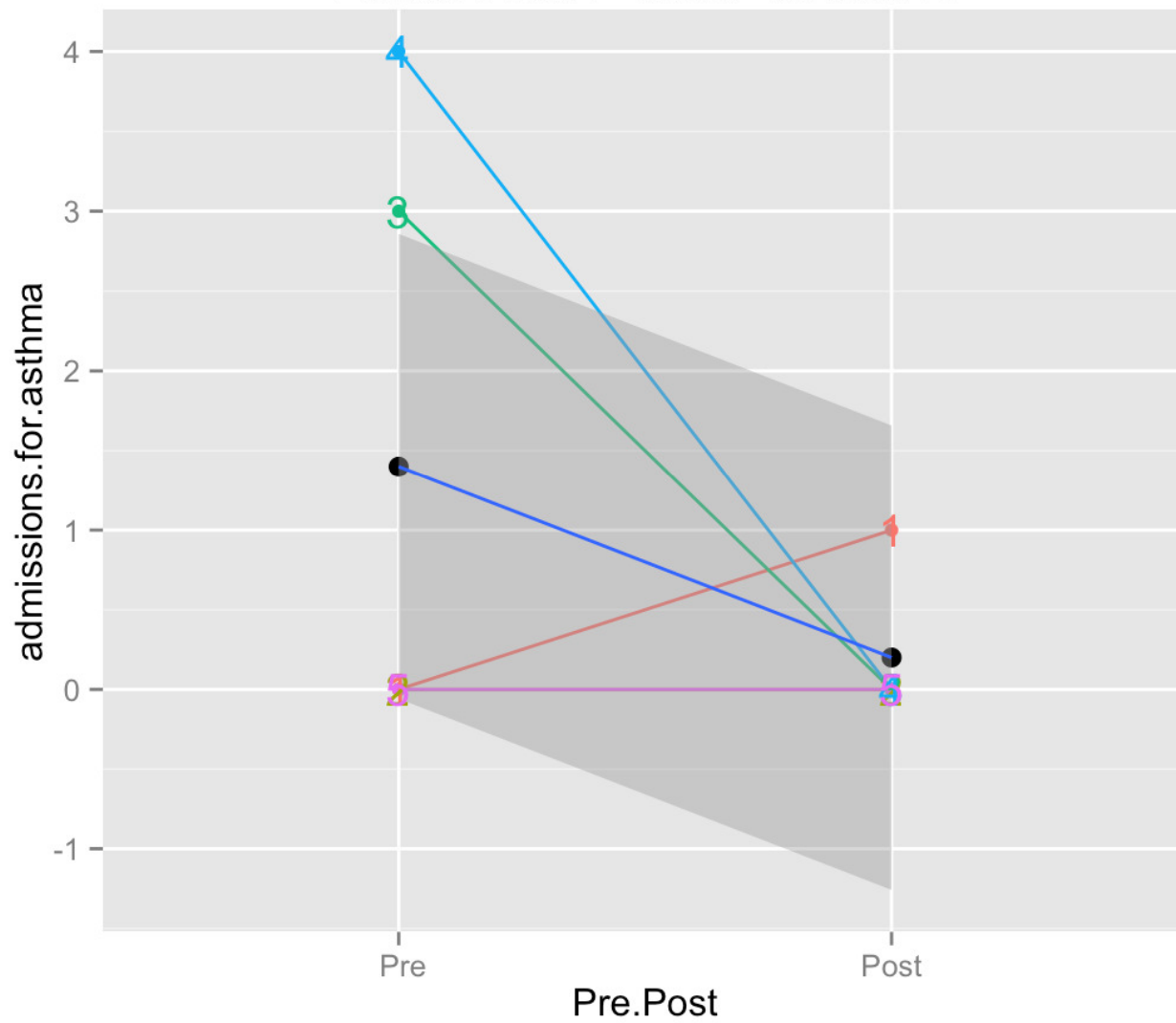
Paired t-test P-value=0.087607



Paired t-test P-value=0.074291



Paired t-test P-value=0.283511





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