The Impact of the Tumor Microbiome on Anti-tumor Immunity in Mucosal Melanoma

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Martin D. McCarter[2], Theresa M. Medina, Catherine Lozupone[1], William A. Robinson[1], Kasey L. Couts[1]

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Introduction

Mucosal melanoma is a rare, but lethal, form of melanoma

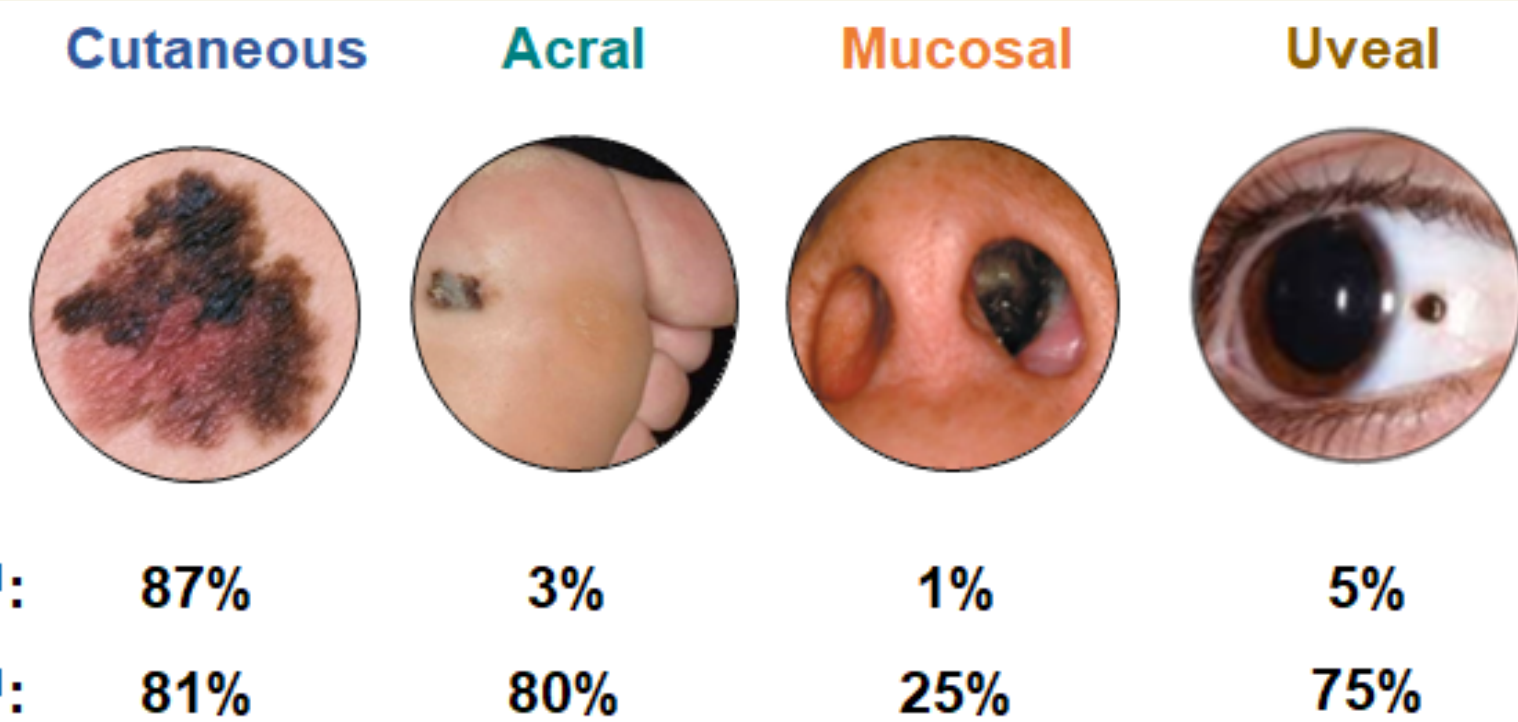|	Cutaneous	Acral	Mucosal	Uveal
% of US Cases[1]:	87%	3%	1%	5%
5-year survival rate[1]:	81%	80%	25%	75%

Figure 1. Cutaneous melanoma and different rare, non-sun related melanoma subtypes. Mucosal melanomas are typically not detected until late stage, are challenging to surgically resect, and respond poorly to standard melanoma treatments *including immune checkpoint blockade (ICB) therapy.*[2-4]

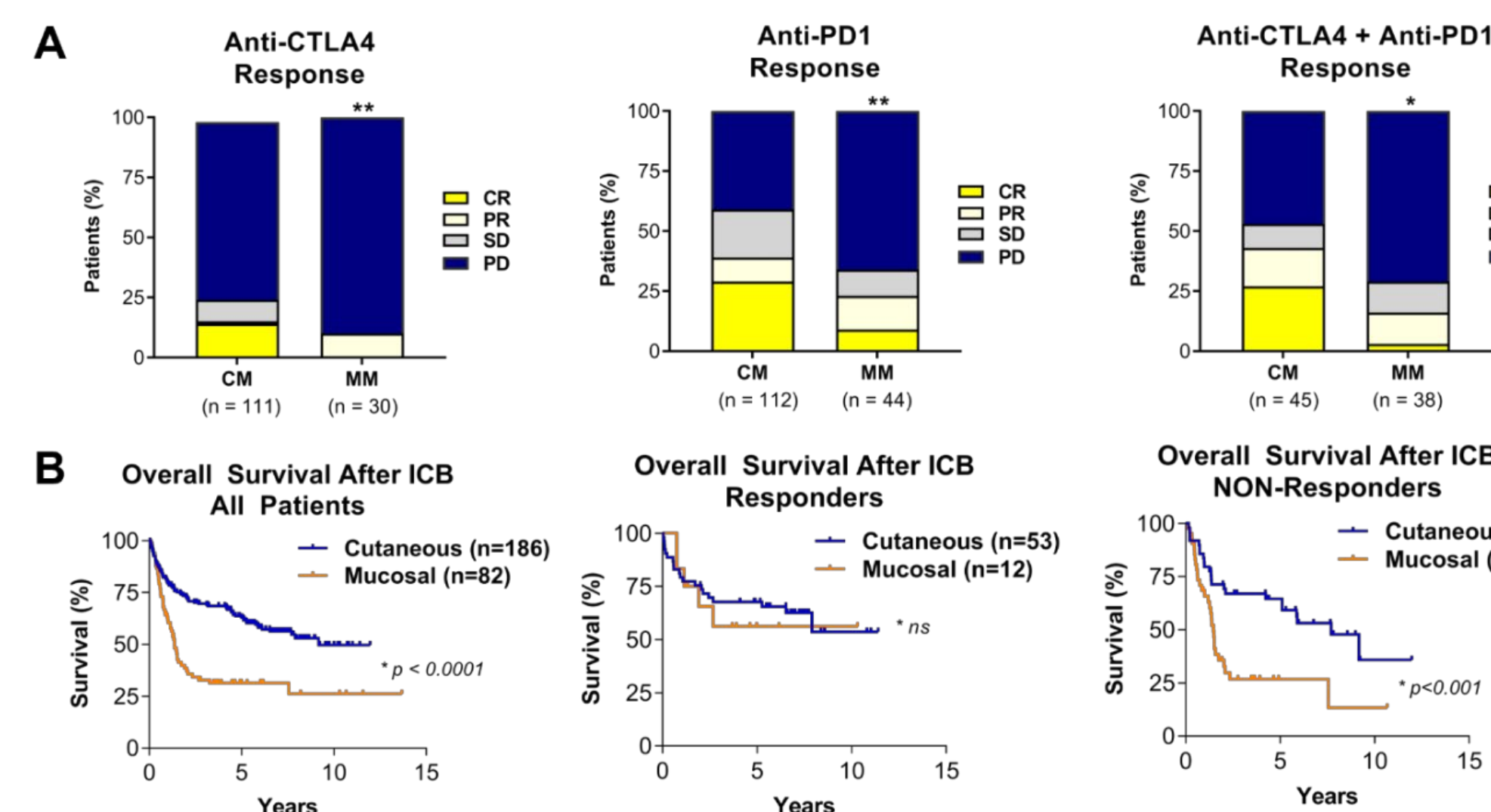### Mucosal melanoma responds poorly to immune checkpoint blockade (ICB) compared to cutaneous melanoma

Figure 2. Immune checkpoint blockade therapies are less effective in mucosal melanoma. (A) Percent of CU melanoma clinic patients with complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). *p<0.01, **p<0.001. (B) Overall survival from the date of first ICB treatment for patient cohort indicated.

Hypothesis

Given the role of the microbiome in tumor immunity and ICB response, we *hypothesize* distinct mucosal tissue microbiomes contribute to poor anti-tumor immunity and ICB resistance in mucosal melanoma.
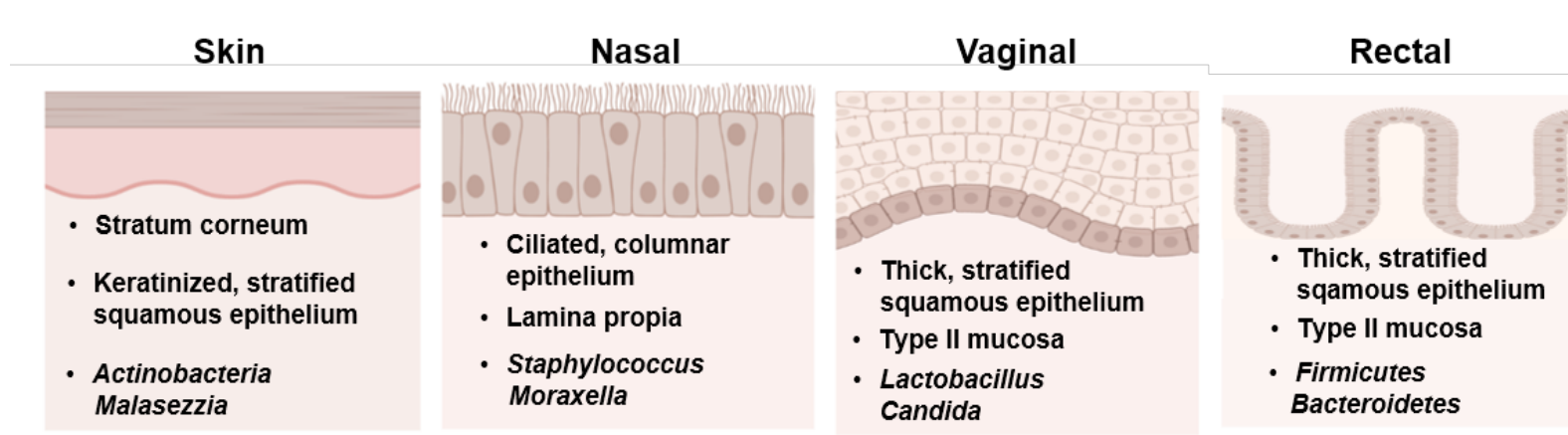

Figure 4. Structural and microbial differences in skin *versus* mucosal tissues.

Methods

Patient gDNA specimens

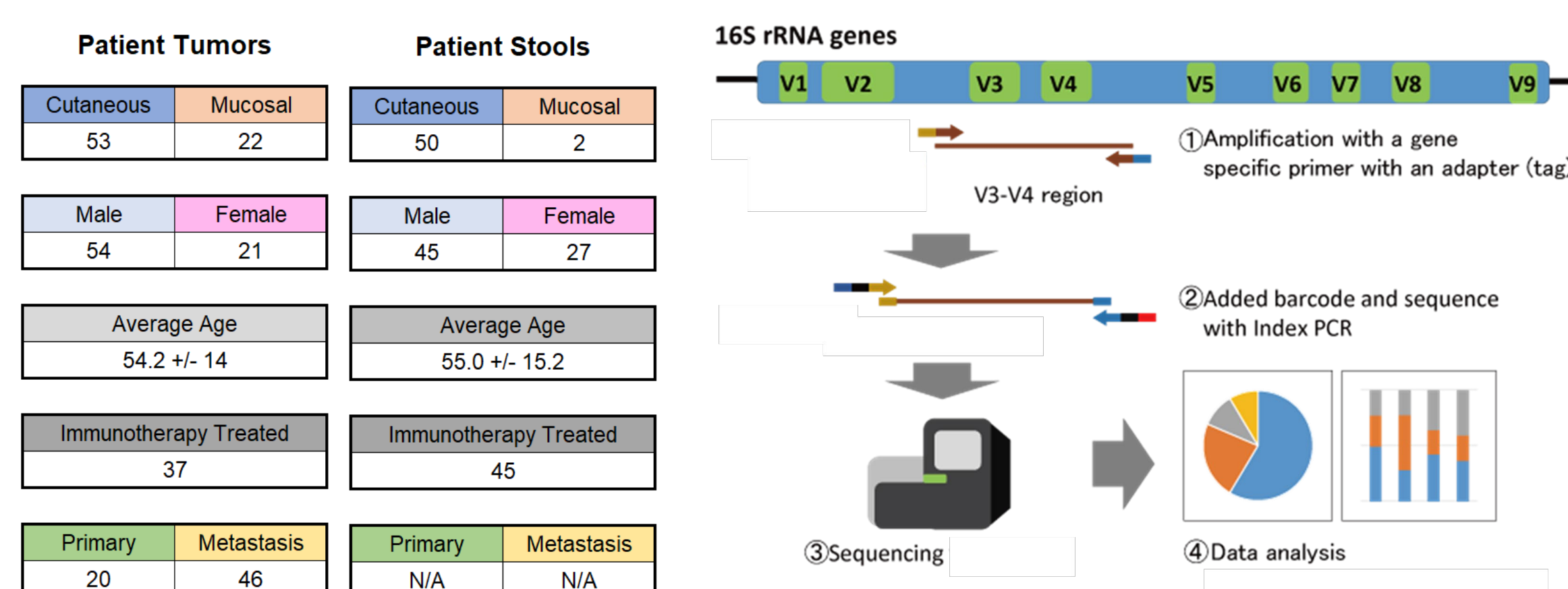### 16S rRNA bacterial sequencing

Figure 5. 16S rRNA sequencing of melanoma patient specimens. (A) Specimens were collected by the CU Melanoma Biorepository under COMIRB #05-0309. Genomic DNA was purified using the Qiagen DNeasy UltraClean Microbial Kit (tumors) or Dneasy PowerSoil Pro Kit (stool). (B) Diagram of 16S rRNA sequencing process.

Results

~50% of tumors are "positive" for bacteria

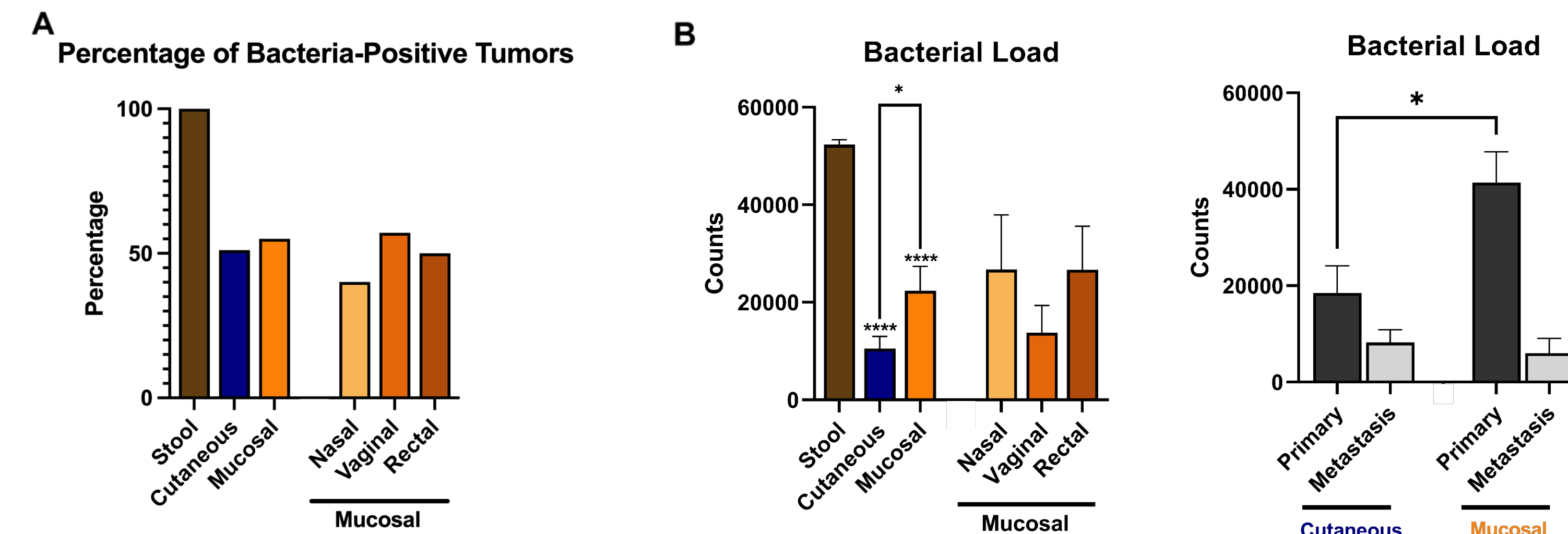### Primary mucosal melanoma tumors have higher bacterial load *vs* cutaneous melanomas

Figure 5. Bacteria positivity and total bacterial load in patient specimens. (A) Percentage of tumors identified as "positive" for the presence of bacteria. (B) Bacterial load in specimens shown by subtype/anatomic site and by primary or metastatic tumor status.

Mucosal melanoma tumors have more bacteria species *vs* cutaneous melanomas

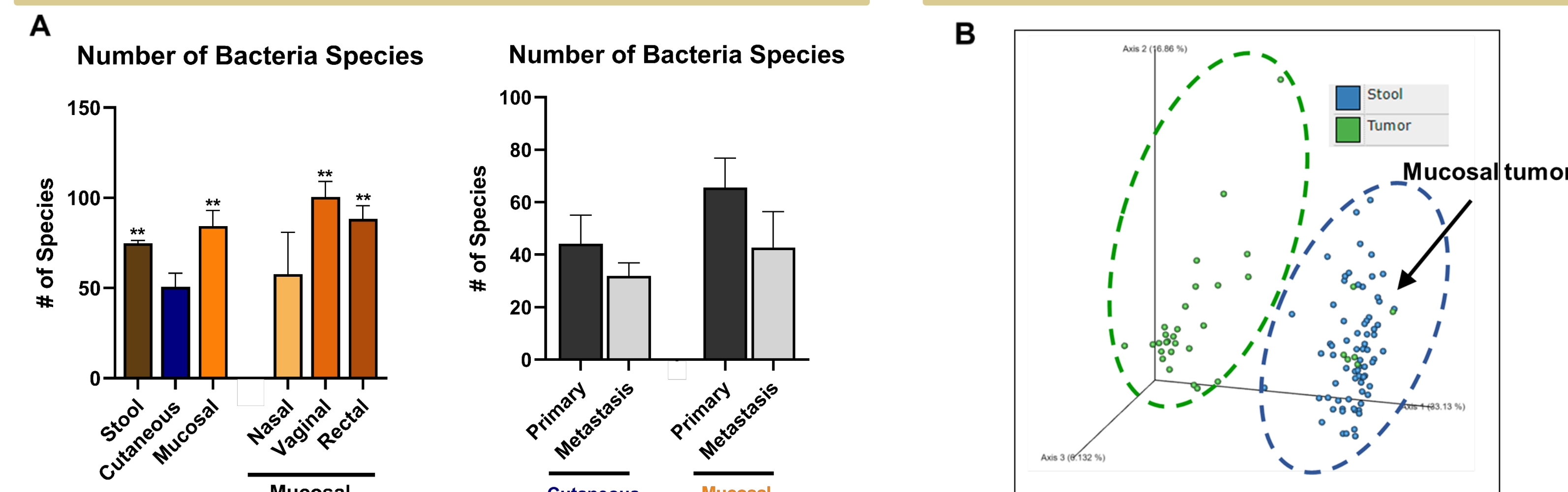### Tumors clustering with stools are mucosal melanoma tumors

Figure 6. Bacteria species counts and principal component analysis (PCA). (A) Total number of bacteria species shown by subtype/anatomic site and by primary or metastatic tumor status. (B) Principal component analysis of data using QIME 2.0 software.

Mucosal melanoma tumor microbiomes more closely resemble stool than cutaneous melanoma tumors

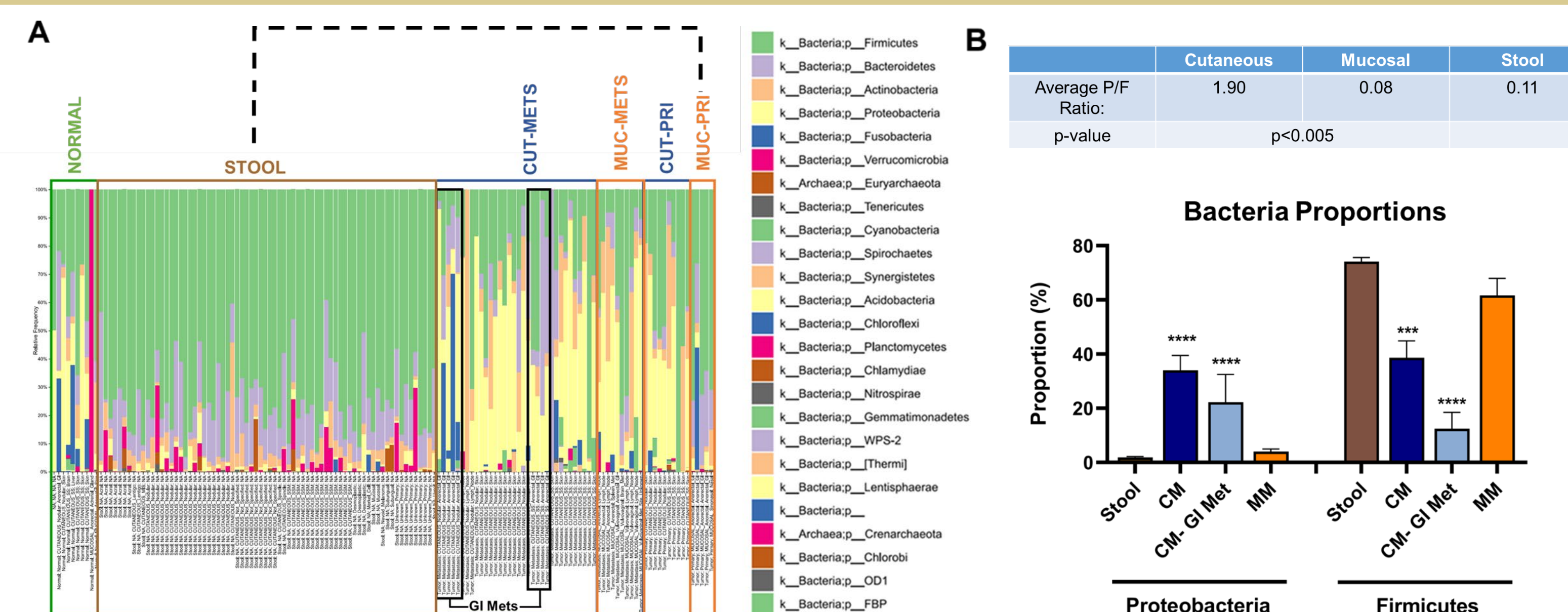|	Cutaneous	Mucosal	Stool
Average P/F Ratio:	1.90	0.08	0.11
p-value		p<0.005	

Figure 7. Taxa plot and P:F ratio analysis. (A) Taxa plot for specimens generated with Qime 2 software. (B) Analysis of the Proteobacteria to Firmicutes ratio (P:F), which is a common metric used to generally characterize microbiome composition.

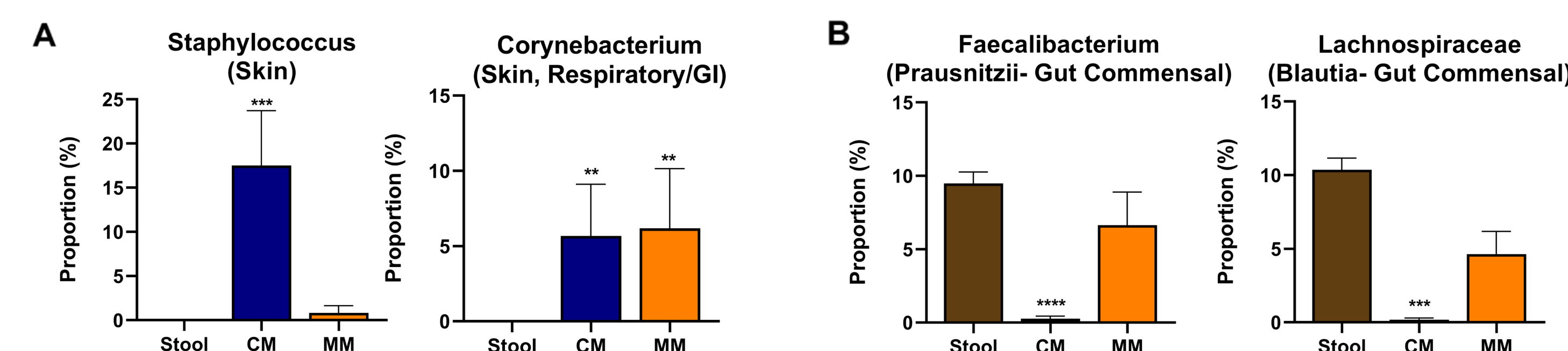### Specimens have expected proportions of known skin and gut commensal bacteria

Figure 8. Levels of known skin vs gut commensal bacteria across specimens. (A,B) Average proportion of species per total bacteria for know (A) skin or skin/respiratory/GI commensals, or (B) known gut commensals.

Results (Cont.)

Mucosal melanoma tumors have proportions of bacteria consistent with poor anti-tumor immunity

HIGHER levels of immunosuppressive bacteria in mucosal melanomas vs stool

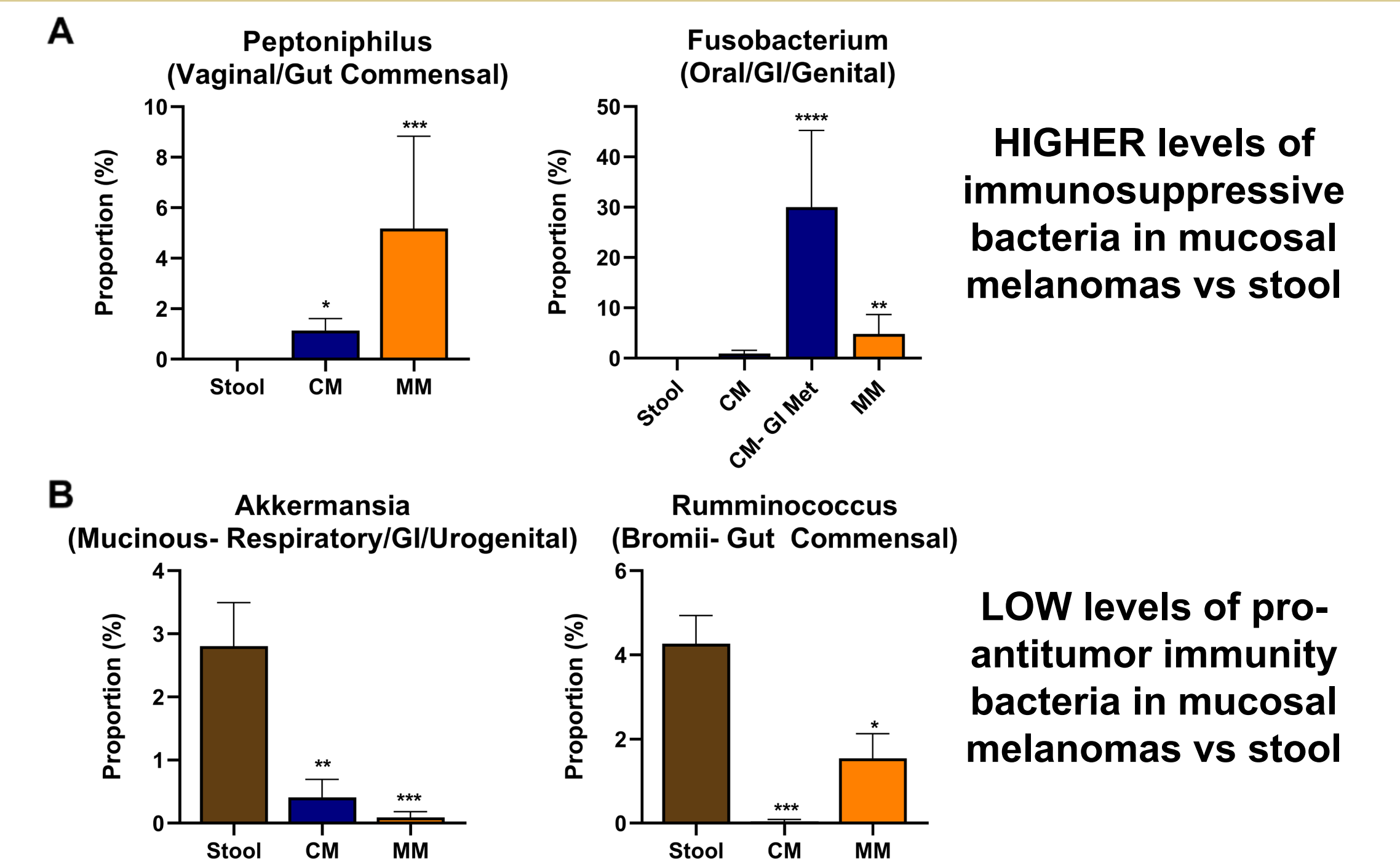**LOW levels of pro-antitumor immunity bacteria in mucosal melanomas vs stool**

Figure 9. Levels of known immunosuppressive and pro-antitumor immunity bacteria species across specimens. (A,B) Bacteria species known to be (A) immunosuppressive and pro-tumorigenic, or (B) tumor suppressive and pro-antitumor immunity/inflammation.

Conclusions

- Mucosal melanoma is a rare, but particularly lethal, form of melanoma which responds poorly to ICB compared to cutaneous melanomas.
- The mucosal melanoma tumor microbiome is significantly different from cutaneous melanoma, and it more closely resembles the gut microbiome.
- MM tumors have unfavorable proportions of bacteria species controlling tumorigenicity and immunity.

Future Directions

- Multiomics analysis with RNAseq and ATACseq to determine the correlation between the microbiome and epigenetic-mediated immune gene silencing in mucosal melanoma.
- Mucosal melanoma microbiome (gut and tumor) reconstitution in mice to determine effect on tumor epigenetics, immunity, and ICB response.
- ITS sequencing of specimens for fungal species analysis.

References

1. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. Cancer 1998;83:1664-78.
2. Kuk D, Shoushtari AN, Barker CA, et al. Prognosis of Mucosal, Uveal, Acral, Nonacral Cutaneous, and Unknown Primary Melanoma From the Time of First Metastasis. Oncologist 2016;21:848-54.
3. D'Angelo SP, Larkin J, Sosman JA, et al. Efficacy and Safety of Nivolumab Alone or in Combination With Ipilimumab in Patients With Mucosal Melanoma: A Pooled Analysis. J Clin Oncol 2017;35:226-35.
4. Shoushtari AN, Munhoz RR, Kuk D, et al. The efficacy of anti-PD-1 agents in acral and mucosal melanoma. Cancer 2016;122:3354-62.

Acknowledgements