AWARD NUMBER: W81XWH-21-1-0577

TITLE: Antibiotic-Induced Dysbiosis Promotes Lung Metastasis

PRINCIPAL INVESTIGATOR: Ruud Dings, Ph.D.

CONTRACTING ORGANIZATION: University of Arkansas for Medical Sciences

REPORT DATE: OCTOBER 2023

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE		*Form Approved OMB No. 0704-0188*
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1. REPORT DATE OCTOBER 2023	**2. REPORT TYPE** ANNUAL	**3. DATES COVERED** 30-Sep-2022 – 29-Sep-2023
4. TITLE AND SUBTITLE Antibiotic-Induced Dysbiosis Promotes Lung Metastasis Concept Award		**5a. CONTRACT NUMBER** W81XWH-21-1-0577 **5b. GRANT NUMBER** **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S) Ruud P.M. Dings, Ph.D. E-Mail: rpmdings@uams.edu		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES) University of Arkansas for Medical Sciences 4301 W MARKHAM ST SLOT 812 BIO-MED BLDG 1 STE LITTLE ROCK AR 72205-7101		**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)** **11. SPONSOR/MONITOR'S NUMBER(S)**

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Recent studies by various groups have revealed that antibiotics (ABX)-induced dysbiosis has systemic consequences including changes in vasculature beds at distal sites, and ultimately acceleration of lung carcinogenesis. These observations and their clinical implications motivated us to investigate whether dysbiosis also has an influence on lung metastasis. The **long-term goal** of our work is to define the systemic effects of ABX-induced dysbiosis, to develop strategies to quell metastasis. The **overall objective** of this proposal is to determine the influence of ABX on lung metastasis progression and dissemination. Attaining this objective will be a major step in understanding the processes of lung metastasis and off-target effects of ABX. In this concept award, we test our **central hypothesis** is that ABX-induced dysbiosis increases fibronectin in the perivasculature stroma, creating a favorable pre-metastatic niche.
None of the tasks of the original approved Statement of Work (SOW) were changed.

15. SUBJECT TERMS

Lung metastasis, antibiotics, dysbiosis, cooperative human tissue network (CHTN), lung cancer biospecimen resource network (LCBRN), stroma.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	16	**19b. TELEPHONE NUMBER**

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. Introduction

Antibiotic-induced microbial imbalance, or dysbiosis, has systemic and long-lasting deleterious effects on the host. Although they fight infections, antibiotics (ABX) severely alter microbiomes by disrupting commensal bacteria crucial for maintaining various host homeostatic mechanisms. The use of ABX has significantly increased by 30% in recent years, yet the influence of ABX-induced dysbiosis on metastasis is largely unknown. Metastasis, the successful spreading of primary tumor cells to distant organs is the primary cause of cancer morbidity and mortality, and is estimated to account for 90% of the cancer death. Importantly, military personnel are at a higher risk of developing lung cancer and metastasis than the general population due to increased rates of smoking as well as an increased likelihood of being exposed to environmental carcinogens during their service. Thus, there is a pressing need to reach a deeper understanding of the biological consequences of ABX-induced dysbiosis on lung metastasis to uncover strategies to intervene and reverse by restoring and promoting beneficial microbiota.

2. Keywords

Lung metastasis, antibiotics, dysbiosis, cooperative human tissue network (CHTN), lung cancer biospecimen resource network (LCBRN), stroma.

3. Accomplishments

Please note: we were granted a second 12-months no-cost extension (NCE) due to unforeseen challenges. There were no significant changes in the project or its direction. Please see more detail under **Section 5**. Accomplishments and approximate percentage of completion, as outlined in the approved SOW:

- **What were the major goals of the project and what was accomplished under these goals?**

Major Goal 1: Identify ABX primarily responsible for stromal modulation and metastasis dissemination.

- Last period we identified that the cocktail of antibiotics (ABX), ampicillin, and vancomycin affected the microbiota the most, as compared to broad-spectrum neomycin, anaerobic targeting metronidazole, and ciprofloxacin and bactrim - antibiotics prescribed in high frequency in the clinic. This was determined by the changes by 16S rRNA gene exact sequence variants (ESVs) in fecal matter, and cecum weights on the host side.

Update Subtasks 1.1-1.4 and Milestone 1 and Milestones 1-5:

- This period we investigated the effects of oral antibiotics, i.e. ampicillin, neomycin, vancomycin, metronidazole, ciprofloxacin and bactrim and their effect on the lung microbiota (**Figure 1** – end of the document). We found that oral ABX does not induce significant changes in microbial community composition of the lungs. Since our main interest is the microbe-host changes induced by oral ABX on distal lung carcinogenesis and progression of metastatic melanoma, we induced dysbiosis by a cocktail of 4 antibiotics (i.e., ampicillin; neomycin; vancomycin; metronidazole) in C57BL/6J mice (as described in Jenkins, *et al.* PMC6891208) and quantified and analyzed the microbial taxa changes by 16S rRNA amplicon sequence variants (ASVs) in lung samples and compare these to untreated orthobiotic conditions. We found no significant changes in the microbial community composition of the lungs after 14 days of exposure to the cocktail of ABX in their drinking water (**Figure 1**). Namely, their phylum taxonomy and ASVs did not significantly change (**Figure 1A and B**). This lack of significant changes was also reflected in the comparable alpha diversity (observed species) and the *unweighted* (qualitative) and weighted (quantitative) *UniFrac* analysis (**Figure 1D** and **E**). The *unweighted UniFrac* only considers their presence or absence, whereas the weighted UniFrac reflects the abundance of observed organisms. We are now further analyzing the ASVs by the Quantitative Insights Into Microbial Ecology (QIIME 2) pipeline. On the host side, we also did not see any obvious changes seen in the gross pathology of the lungs. This lack of significant changes was, at least in part, due to the variability between subjects (**Figure 1**), similar as seen in the clinic. In the past we have observed enhanced acceleration of experimental metastasis in the lung after oral ABX and we can now tentatively conclude that this is not likely due to direct changes of the lung microbiome, rather through changes in the GI-

tract microbiome, as we reported on in our last period/report. This is an important finding in the sense that the effects we are seeing in lung carcinogenesis and dissemination is thus mediated by systemic effects of the induced dysbiosis in the GI-tract, rather than local changes in the lung microbiota.

- Previously we also reported that broad-spectrum ampicillin and Gram-positive targeting vancomycin affected the GI-tract microbiota the most. In order to address this further we are also analyzing possible changes in 45 cytokine levels in paired serum samples before and after ABX, as well as before and after Lewis Lung Carcinoma induction (LKTM015 - murine XL cytokine multiplex panel; Luminex^{tm}).
- Identified that some stromal adhesion molecules change during dysbiosis (e.g. CD54, CD106, MECA79, whereas others do not e.g. CD146). E.g., stromal ICAM-1, as defined as CD54+ of the CD45- CD31+ population, was suppressed under dysbiotic conditions (**Figure 2**). As quantified and expressed as total amount of cells these CD54+ / [CD45- CD31+] were significantly less prominent as compared in the orthotopic controls. Same trends were seen based on percentage, yet did not reach statistical significance (**Figure 2**). We are currently finalizing the analysis on potential other changes in extra cellular matrix proteins, i.e. collagen and fibronectin, rather than cellular stromal changes alone.
- Last period we showed that indeed dysbiosis accelerated the morality rate due to succumbing of lung metastasis in female mice. Namely, all the mice under dysbiotic conditions succumbed by 25 days, whereas the orthobiotic controls lived until day 29. We are currently repeating these studies in male mice in order to address possible sex differences (sex as a variable) and rigor and reproducibility.
- Last period we identified that Gram-positive clostridial cluster IV (*Ruminococcaceae*) and XIVa (*Lachnospiraceae*), are important for stromal homeostasis, as dysbiosis induced by ampicillin and vancomycin induced the greater changes in microbiota, and consequently accelerated mortality by increasing and altering the micro-metastasis dissemination. We are having our microbiome bioinformatics specialist, Michael Robeson, Ph.D., further analyze the ASVs by the Quantitative Insights Into Microbial Ecology (QIIME 2), to verify these finding and possibly expand on the bacteria strains involved.

Please see **Table 1** for our subtasks and milestones and our interim achievements and key outcomes as updates below it.

Table 1. Interim achievements and key outcomes of major goal 1.

Major Goal 1: Identify ABX primarily responsible for stromal modulation and metastasis dissemination.	**Timeline (months)**	**Completion (%)**
Major Task 1	Months	
Subtask 1.1 – Isolate lung and GI tract bacteria and perform 16S rRNA analysis by Argonne National Library.	1-3	100%
Subtask 1.2 – Define the ABX-induced stromal changes as determined by IHC and IF.	1-6	80%
Subtask 1.3 – Quantify experimental (iv injected) and spontaneous (from sc implanted) LLC and B16-F10 lung and kidney metastases (nodules and overall burden), by microscopic, macroscopic and whole animal imaging (IVIS). Mice obtained from JAX and cell lines from ATCC.	5-11	80%
Subtask 1.4 – Correlate bacteria taxa changes with stromal changes and subsequent lung metastasis progression and dissemination.	10-12	80%
Milestones: 1. identify which antibiotic causes which changes in bacteria taxa. 2. identify which antibiotic(s) is/are responsible for stromal changes. 3. determine which stromal changes occur due to antibiotic-induced dysbiosis. 4. identify which bacteria are important for stromal homeostasis. 5. determine the increase in the number of micrometastases, dissemination and accelerated mortality rate due to ABX-induced dysbiosis per tumor model.		

Major Goal 2: Determine differences in bacteria taxa in Bronchoalveolar lavage (BAL) fluids and stromal changes in matched NSCLC specimen.

- BAL fluids and matched NSCLC specimens – a mix of adenocarcinoma and squamous cell carcinoma along with their respective adjacent normal tissue were obtained from the lung cancer biospecimen resource network (LCBRN), now integrated into the cooperative human tissue network (CHTN). Cryo-tissue sections are being generated (5 μm thickness) and mounted on glass slides for histopathology and immunohistochemistry (IHC). Stromal and extracellular matrix changes (i.e. alpha smooth muscle actin, desmin, NG2, PDGFRs and fibronection, collagens, laminins), lipids (Oil Red O, BODIPY) and mucins/glycogen (Periodic Acid Schiff) were identified. Bacterial metabolites were identified in matched serum samples by Liquid Chromatography - Mass Spectrometry (LC-MS), to correlate the serum bacterial metabolites with the changes in stromal tissue signatures.

Update Subtasks 2.1-2.4, and Milestone 6-8:

- We obtained 20 matched NSCLC specimen from LCBRN/CHTN. However, due the lack of availability of BAL specimen, esp. as these are collected prospectively, we first obtained murine samples. We are still pursuing BAL fluids, but decided to broaden this subtask to also include bacterial metabolites in the serum and fecal matter. In part this is because we now know the bacteria in the bronchoalveolar space are not significantly affected by oral antibiotics (**Fig. 1.**) Additionally, obtaining serum and fecal specimen is a relative less invasive strategy and clinically more pragmatic for patients with advanced lung cancer. Effectively the bacterial metabolites are the effector molecules which dictate the systemic effect on lung carcinogenesis. While the epithelial barrier ensures that bacteria are largely confined to the lung alveolar space, microbial metabolites cross-over the epithelial barrier, allowing the metabolites to enter the host circulatory system maintaining various processes of cell homeostasis. In order to detect both targeted and nontargeted metabolomics, specifically in the context of SCFA, we have started a local collaboration with Renny S. Lan, Ph.D., director of the metabolomics core at the Arkansas Children's Nutrition Center (**Figure 3**). Although preclinical experiments often have the advantage to dissect causal consequences after one single permutation, for example ABX usage only, clinically this is often not an option. E.g. lifestyle, diet, and other medications, to name a few external factors, might influence metabolite levels as well. Thus, we are establishing and optimizing a new targeted and untargeted Liquid Chromatography - Mass Spectrometry (LC-MS) acquisition work flow to analyze bacterial derived SCFA in both fecal matter as well as plasma (**Figure 3A-C**). We elucidated bacterial metabolites in serum and fecal matter from murine origin as these signatures of SCFA are the same in both murine and human species. We found that depending on the specific SCFA some were affected more than others by particular antibiotics (**Figure 3E-J**). Particularly we observed that an inverse profile of dysbiosis induction (**Figure 3D**) was reflected in the butyric acid serum levels (**Figure 3G**). Namely, oral administration of either ampicillin, vancomycin, or the cocktail of ABX, induced dysbiosis as measured by cecum size, and consequently reduced the amount of butyric acid in the serum ($p < 0.05$). This is in line with our previous findings that the bacteria families producing metabolite butyrate, i.e. Gram-positive clostridial cluster IV (*Ruminococcaceae*) and XIVa (*Lachnospiraceae*), are reduced during antibiotic-induced dysbiosis (Jenkins *et al.* Cancer Research; 2019 PMCID: PMC6891208). Subsequent measurements will include comparisons of fecal and plasma derived SCFA of lung cancer patients and we will repeat the murine studies (rigor and reproducibility). A technical manuscript describing the LC-MS approach is in final preparation for submission.
- Considering, the the association of butyrate and SFCAs in general are correlative we followed up by mechanistic studies. We were able to confirm that butyrate increases ICAM-1 (**Figure 4**). Namely, butyrate increased ICAM-1 (CD54) expression in a dose-dependent manner on tumor endothelial cells up to 6-fold (**Fig. 4A**). Other SCFA bacterial metabolites: acetate, propionate and beta-hydroxy butyrate, did not increase ICAM-1 expression (**Fig. 4B**).
- Update Subtask 2.4 and Milestone 8: This subtask integrates the previous 3 subtasks. As we finalize those subtasks in our NCE, we preliminary conclude that ABX-induced dysbiosis causes significant changes in bacterial metabolites in the serum, i.e. short chain fatty acids. Particularly, butyrate (C4) showed the inverse profile of dysbiosis induction by ampicillin and vancomycin (**Fig. 3**).

Please see **Table 2** for our subtasks and milestones and our interim achievements and key outcomes as updates below it.

Table 2. Interim achievements and key outcomes of major goal 2.

Major Goal 2: Determine differences in bacteria taxa in BAL fluids and stromal changes in matched NSCLC specimen.	**Timeline (months)**	**Completion (%)**
Major Task 2		
Subtask 2.1 – Acquisition of 30 BAL fluids and their matched NSCLC specimen from LCBRN/CHTN.	1-3	66%
Subtask 2.2 – Isolate bacteria in bronchoalveolar lavage fluids and perform 16S rRNA analysis by Argonne National Library.	2-10	30%
Subtask 2.3 – Stain for proteins, lipids and mucins in human specimen.	2-10	30%
Subtask 2.4 – Correlate changes in bacteria taxa, stroma, medical history and clinical outcomes.	9-12	50%
Milestones achieved: 1. defined bacteria in BAL fluids. 2. defined the stromal differences in NSCLC specimen as compared to normal tissues. 3. correlated bacteria in BAL, ABX use, medical history and clinical outcomes with stromal signatures in NSCLC specimen.		

- **What opportunities for training and professional development has the project provided?**

Although not a major objective of this project, last year it did provided training for a High School student (Kinsey Garofalo), two Undergraduate Research Students (Savannah Huyvaert and Hailey Campbell), a Research Assistant (Amir Mortazavi) and Junior Faculty fellow (Samir Jenkins, Ph.D.). This period we were able to expand on that for Research Assistants Hailey Campbell (graduated), Amir Mortazavi, and Junior Faculty fellow (Samir Jenkins, Ph.D.). Additionally, we were able to host a Little Rock Central High School student Ross Davis, a Summer Research Intern trough the Division for Diversity, Equity and Inclusion (DDEI).

- **How were the results disseminated to communities of interest?**

We have one manuscript under final review (federal support acknowledged) and the results were presented at local and international symposia and scientific meetings.

Conferences (poster presentation):
American Association for Cancer Research (AACR)
Location: Orlando, FL; **Date(s):** 04/14-19, 2023

Society of Thermal Medicine
Location: San Diego, CA; **Date(s):** 04/23-27, 2023

Annual Central Arkansas Undergraduate Summer Research Symposium (CAUSRS)
Location: Little Rock, AR; **Date(s):** 07/26, 2023

International Congress for Radiation Research (ICRR)
Location: Montreal, Canada; **Date(s):** 08/26-30, 2023

Metabolomics Association of North America
Location: Alberta, Canada; Date(s): 09/16-19, 2023
Title: Delineating dysbiosis-induced metabolomics signatures to optimize precision medicine
Authors: Renny Lan, Hailemariam Abrha Assress, Samir V. Jenkins, Ruud P.M. Dings.
*won 1st place award for highly meritorious research in metabolomics by the society

Invited talks:
The UAMS annual Cancer Institute Retreat
Location: Little Rock, AR; Date(s): 05/16/2023
Title: Improvement of immunotherapy during dysbiosis by modulating the tumor microenvironment.

Southeast Regional IDeA Conference
Location: Columbia, SC; **Date(s):** 09/15-17, 2023
Title: Improvement of cellular immunotherapy during dysbiosis by modulating the tumor microenvironment.

- **What do you plan to do during the next reporting period to accomplish the goals?**

During our NCE we will continue and finalized our subtasks as described in our originally approved SOW and address the comments we received on our submitted manuscript.

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

  At least in mice, we preliminary conclude that certain antibiotics have the potential to accelerated mortality by increasing and altering lung micro-metastasis dissemination. Particularly, broad-based ampicillin and Gram-positive targeting vancomycin are potential harmful as Gram-positive clostridial cluster IV (*Ruminococcaceae*) and XIVa (*Lachnospiraceae*) produce short-chain fatty acid butyrate important for stromal homeostasis.

- **What was the impact on other disciplines?**

  This study generated a foundation for future large-scale studies aimed at intervening or even preventing metastatic spread, and clinical decision-making regarding the use of certain antibiotics in individuals at high risk for developing lung cancer, i.e. veterans. Additionally, although further studies are warranted, we envision that bacterial metabolites in specimens can be used as a supplemental screen for lung cancer prognosis. Of note, the society of Metabolomics Association of North America recognized our work with an award and deemed the work described in this report highly meritorious research in metabolomics.

- **What was the impact on technology transfer?**

  Nothing to report at this interim. We are investigating if it is feasible to intellectually protect a signature of the microbiota metabolites as a prognosticator.

- **What was the impact on society beyond science and technology?**

  We now have a better understanding of the potential negative consequences of antibiotic use. Many of the antibiotics, although effective and necessary, have undisclosed 'off target' effects.

5. Changes/Problems

- **Changes in approach and reasons for change**

  Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  There were no significant changes in the project or its direction. We did encounter some challenges, which were all practical in nature. We were granted another 12-months no-cost extension (NCE) as progress under this award was delayed by the back tail of SARS-CoV-2 pandemic and supply chain issues, causing challenges on multiple levels, including

  - An initial hiring-freeze: the animal-use certified technician ended up not available anymore and there was a freeze on hiring someone else.
  - Suppressed clinical trial recruitment: the prospective collection of human specimens has taken more time than anticipated.
  - Additionally, as was much needed, our whole Institute (clinical and academic) transitioned to a cloud-based enterprise management platform, Workday. This included all of our financial and human resources management systems. Consequently, all our vendors and the appropriate paperwork have/had to be manually added to the new system, including the federal Cooperative Human Tissue Network (CHTN), essential for our proposed work.

  Update and resolve: We have overcome these challenges: although supply chain issues are persistent, they seem less frequent for now; hiring freeze was lifted; hired and trained a new technician but came with a steep learning curve; clinical trial recruitment has commenced again, although slow due to staffing issues. However, unfortunately and unforeseen we are still transitioning over to Workday, a year after its implementation. Although still not everything is functional, e.g. vendor integration, ordering, timely budget reports, these should be resolved in the next couple of weeks/months. All the tasks in the original approved

Statement of Work (SOW) are unchanged and moving forward. However, extra time was requested to use the originally awarded funds as wisely and efficiently as possible to complete the repeats of the animal studies (SOW Aim 1, subtasks 3 and 4), acquire additional human specimen from the federal Cooperative Human Tissue Network (CHTN) and analyze the acquired data further (SOW Aim 2, subtasks 1-4).

- **Changes that had a significant impact on expenditures**

  Please see previous sub-heading.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  Nothing to report.

- **Significant changes in use or care of human subjects**

  Nothing to report.

- **Significant changes in use or care of vertebrate animals.**

  Nothing to report.

- **Significant changes in use of biohazards and/or select agents**

  Nothing to report.

6. Products

- **Publications, conference papers, and presentations**

  - **Journal publications.**

    Authors: Jenkins SV, Shah S, Jamshidi-Parsian A, Mortazavi A, Boysen G, Vang KB, Griffin RJ, Rajaram N, Dings RPM.
    Title: Acquired radiation resistance induces thiol-dependent cisplatin cross-resistance in lung cancer.
    Under final review at the journal for Radiation Research. Acknowledgement of federal support (yes), but not directly associated with this award.

  - **Books or other non-periodical, one-time publications.**

    Nothing to report.

  - **Other publications, conference papers, and presentations.**

    Nothing to report.

- **Website(s) or other Internet site(s)**

  Nothing to report.

- **Technologies or techniques**

  Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

  Nothing to report.

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name	Ruud P.M. Dings
Project Role:	PI
Researcher Identifier:	0000 0001 7686 1331
Nearest person month worked:	2 – no change
Contribution to Project	Dr. Dings is the PI of the project and has overseen all elements.
Funding Support	Nothing to Report

Name	Amir Mortazavi
Project Role:	Research Assistant
Researcher Identifier:	
Nearest person month worked:	2
Contribution to Project	Mr. Mortazavi has performed animal work – to collect and analyze samples for 16S rRNA.
Funding Support	Nothing to Report

Name	Hailey Campbell
Project Role:	Research Assistant
Researcher Identifier:	
Nearest person month worked:	2
Contribution to Project	Ms. Campbell has performed work in the area of flow cytometry and immunohisto chemistry and fluorescence to analyze the stromal changes.
Funding Support	Nothing to Report

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Nothing to report.

- **What other organizations were involved as partners?**

  Nothing to report.

8. Special Reporting Requirements

Nothing to report.

9. Appendices

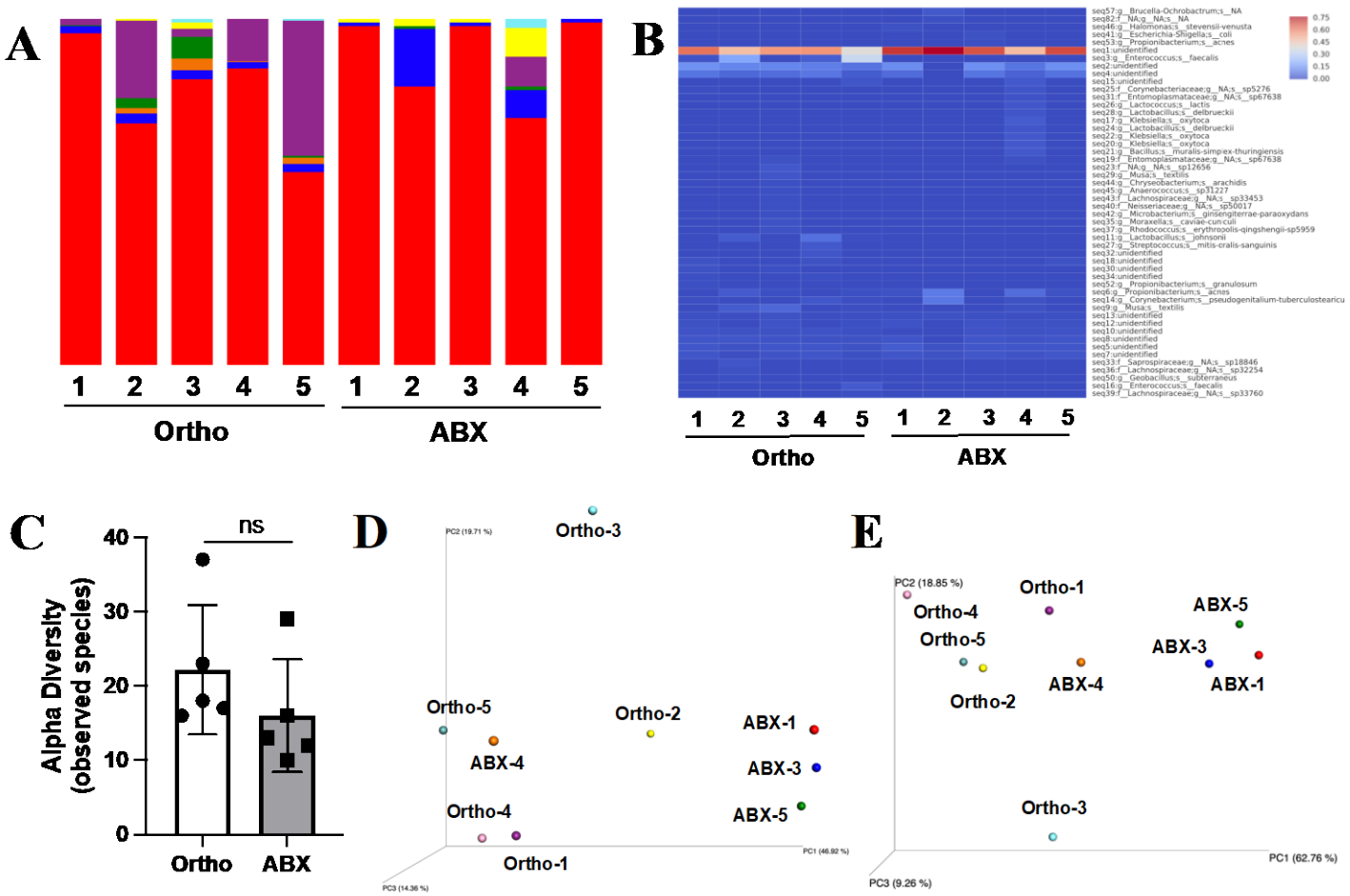


Figure 1, Oral ABX does not induce significant changes in microbial community composition of the lungs. **(A)** Phylum taxonomy bar plots of microbial composition in the lungs obtained from 16S rRNA sequence analysis in healthy controls (Ortho) and ABX-exposed mice. **(B)** 16S rRNA amplicon sequence variants (ASV) heatmap of lung microbial composition Ortho and ABX-exposed mice. **(C)** The alpha diversity of the amount of observed microbial species did not significantly change in the lung after ABX. **(D)** Beta diversity comparisons unweighted (qualitative) UniFrac. **(E)** Beta diversity comparisons weighted (quantitative) UniFrac.
Biological independent samples per group (n = 5 each). Ortho = healthy control (water); Dysbiosis was induced by adding ABX to their drinking water for 2 weeks: Ampi = ampicillin [250 mg/L]; Neo = Neomycin [250 mg/L]; Metro = metronidazole [250 mg/L]; Vanco = vancomycin [125 mg/L]. Data presented as mean ± SEM (n=4/5 group). ns = not significant as determined by two-sided t-test.

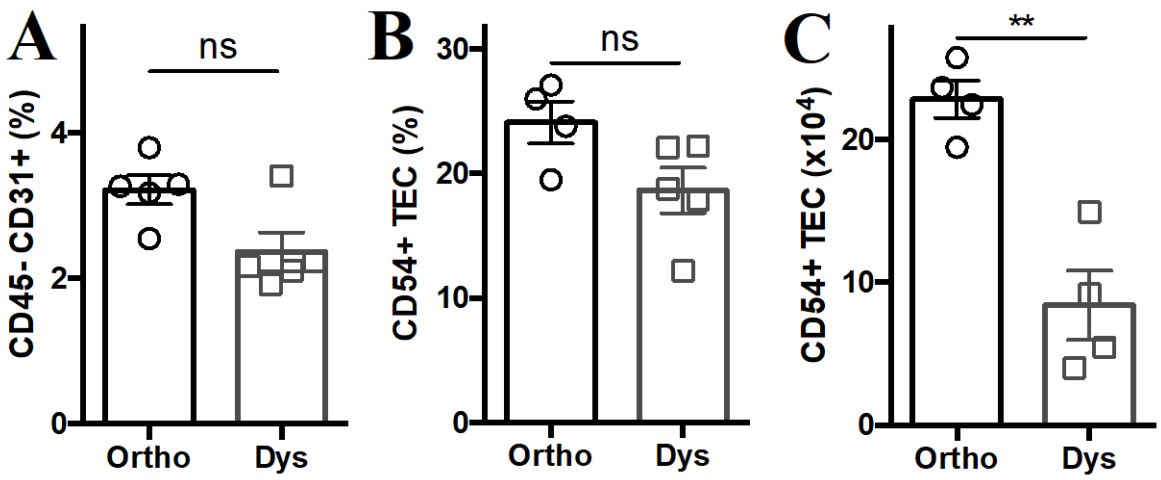
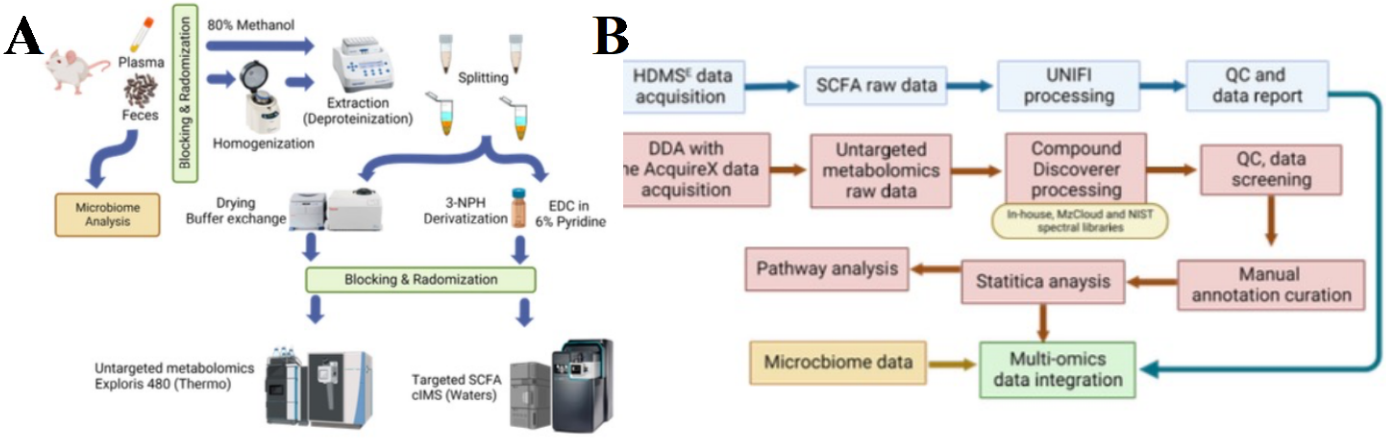


Figure 2. ICAM-1 expression is suppressed under dysbiotic conditions in the LLC model. (**A**) Tumor endothelial cells (TEC; CD45- CD31+) are not reduced by antibiotic-induced dysbiosis. (**B**) Quantification in percentage and (**C**) absolute number of ICAM-1 (CD54) TEC. Data are the mean ± SEM (n = 4-5 LLC-bearing mice per group). $^{**}P < 0.01$, two-sided t-test. ns = not significant.
-▯ - orthobiotic (ortho) and -□- dysbiotic (dys) mice



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SCFA	feces mean (ng/mL)	feces % CV	Plasma mean (ng/mL)	Plasma % CV
Acetic acid	284446.2	3.7	27999.7	6.8
Propionic acid	43460.0	1.4	199.1	18.2
Isobutyric acid	3527.3	1.8	126.6	5.7
Butyric acid	92580.6	5.3	129.2	4.2
2-Methylbutanoic acid	3122.5	3.6	19.5	5.3
Isovaleric acid	4674.2	1.1	120.4	8.2
Valeric acid	5744.9	0.4	69.6	3.9
3-Methylvaleric acid	11.9	11.9	16.0	9.4
Isocaproic acid	88.9	3.8	27.2	6.3
Caproic acid	1027.5	1.7	293.6	0.9
Enanthic acid	190.4	10.7	123.8	4.0
Caprylic acid	426.3	6.6	205.2	3.9

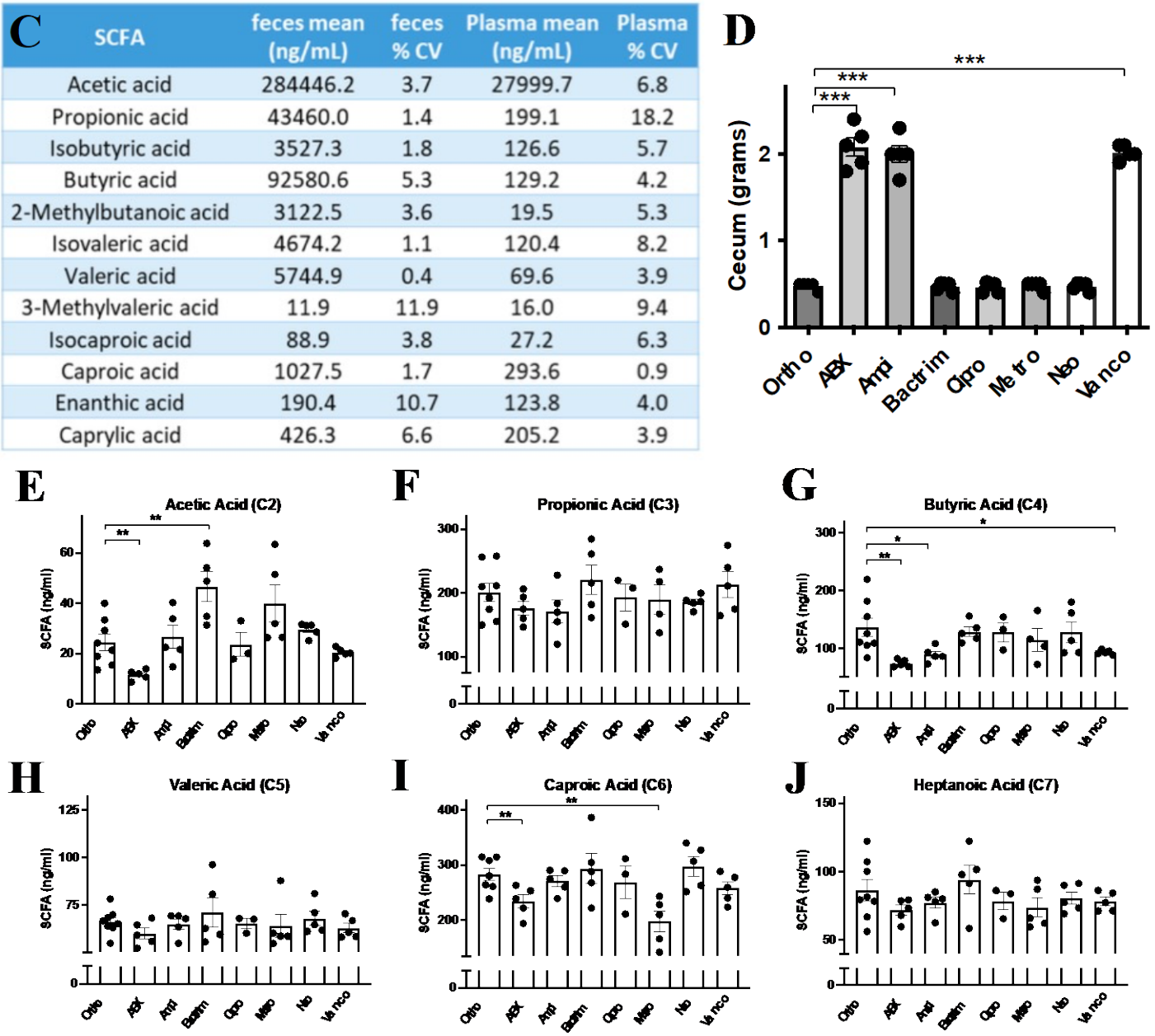

Figure 3. Optimization of SFCA detection in fecal matter and plasma. (**A**) Targeted and untargeted Liquid Chromatography - Mass Spectrometry (LC-MS) workflow. (**B**) Data acquisition and processing workflow. (**C**) Mean concentrations of SCFA in pooled C57BL/6 murine feces and plasma samples. Data presented as mean ± %CV (n=5 / group) from female littermate C57BL/6 mice.

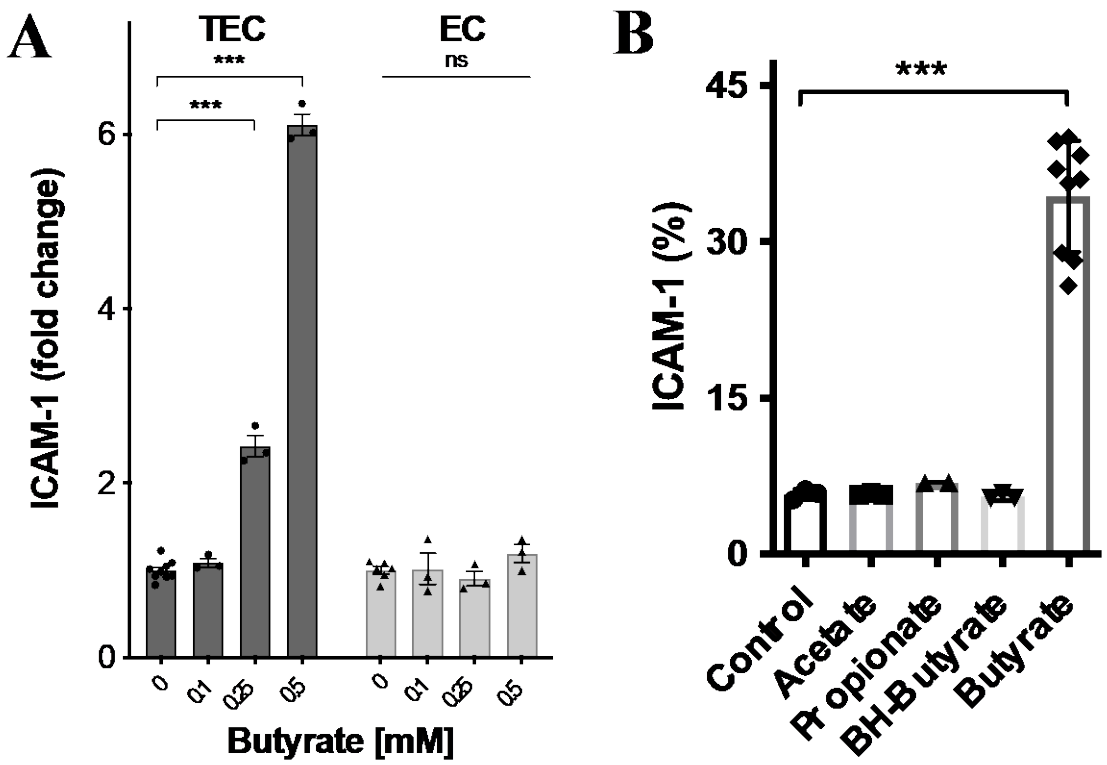

Figure 4. Butyrate induces ICAM-1 on tumor endothelial cells (TEC), whereas normal EC are not affected. (A) Dose response rate of ICAM-1 (CD54) induction by butyrate. **SCFAs other than butyrate do not affect ICAM-1 expression.** ICAM-1 induction on TEC is butyrate specific.
Data presented as mean ± SEM. Determined by FACS, pre-gated on viable single cells. All SCFA 1 mM for 18hrs. ***$P < 0.001$ two-sided t-test.