Microbiome, Cancer and Probiotics

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1 December 2016

Gut Microbiome & Colon Cancer

- **1970~** *H. pylori* → gastritis, stomach ulcers, risk factor of stomach cancer
- **2000s** *Citrobacter rodentium* spur colon tumor in mice
  H. hepaticus cause colon tumor in immunocompromised mice

- **2013** Alternating mice microbiome → Change tumor development
- **2014** Use gut microbiota to screen colon cancer
  US Study: 90 people
  Europe Study: 156 people + 335 from different countries

J.V. Newman et al. J Infect Dis, 184:227-30, 2001.
J.P. Zackular et al.mBio, 4:e00692-13, 2013.
S.E. Erdman et al. Am J Pathol, 162:691-702, 2003.
J.P. Zackular et al. Cancer Prev Res, 7:1112-21, 2014.
G. Zeller et al. Mol Syst Biol, 10:766, 2014.

Systemic effects

- **2006** *H. hepaticus* → colon cancer **+ mammary/prostate cancer**
- **2013** Intact gut microbiome mice have more efficient treatment & improved survival
- **2015** Melanoma tumor in Taconic mice > Jackson mice

1. Gut microbiome regulate inflammation and other immune pathway
2. Influence the effectiveness of cancer therapies

V.P. Rao et al. Cancer Res, 66:7395, 2006.
N. Iida et al. Science, 342:967-70, 2013.
S. Viaud et al. Science, 342:971-76, 2013.
A. Sivan et al. Science, 350:1084-89, 2015.

Gut Microbiome Modification

- Manipulate a patient's resident microbial communities → Improve prognosis and treatment
- **Problem**: Some bacteria not present in every cancer patient
- **Combination of microbes or antibiotics**

Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice

Jun Li[a,1], Cecilia Ying Ju Sung[b,1], Nikki Lee[c], Yueqiong Ni[a], Jussi Pihlajamäki[d,e], Gianni Panagiotou[a,2], and Hani El-Nezami[b,d,2]

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Probiotics: health-beneficial bacteria,

1. Inducing regulatory T-cells in gut to regulate inflammation
2. Suppressing Th17 differentiation to alleviate the severity of some inflammatory diseases

Possible mechanisms:

1. Suppress pathogenic microorganisms
2. Interact with mucosal system → affect systemic immunity

J. Li et al. PNAS, doi:10.1073/pnas.1518189113, 2016.

Pilot study: Three commercial probiotics

Escherichia coli
Nissle 1917 (EcN)

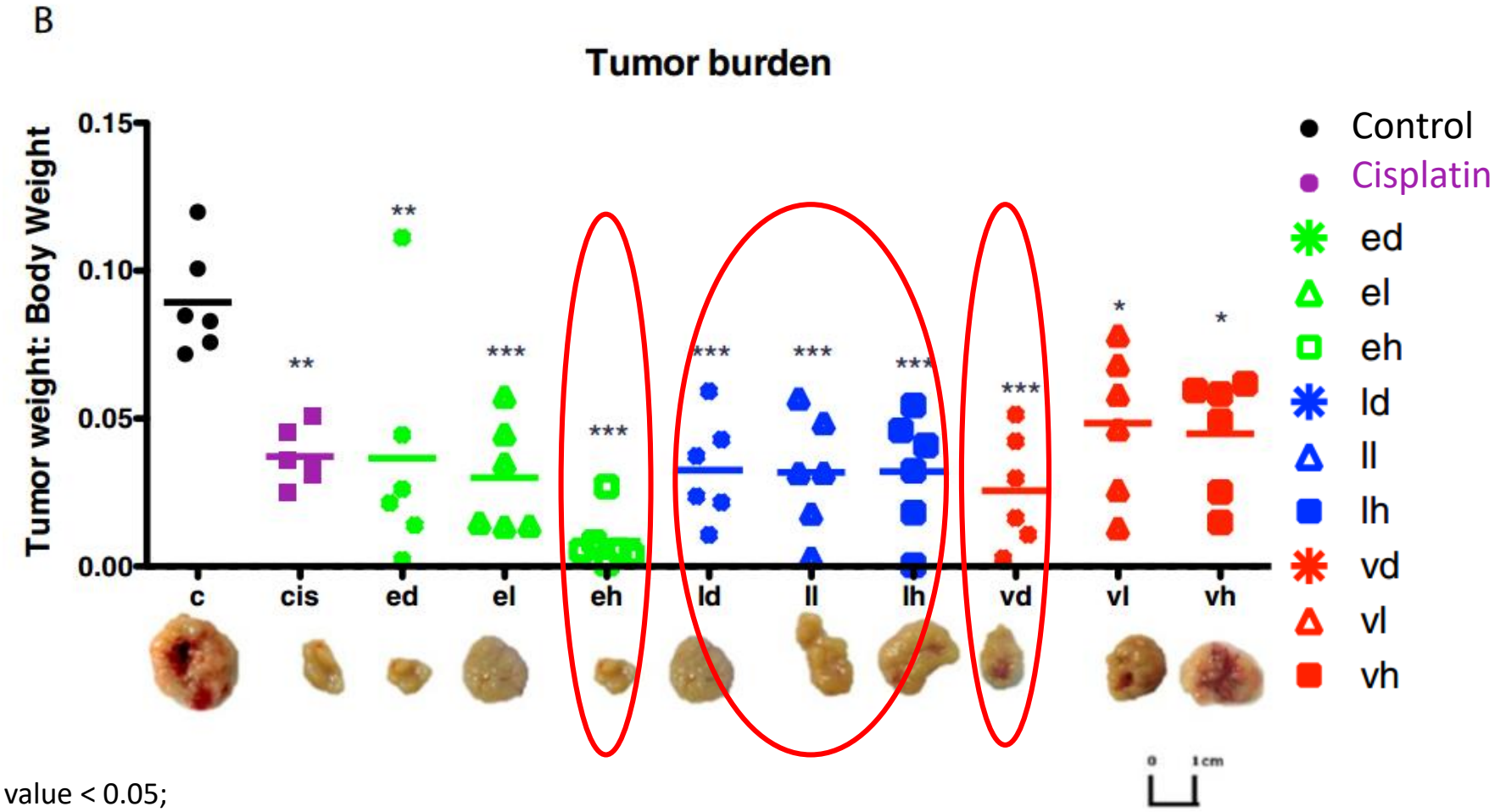
Lactobacillus rhamnosus GG (LGG)

VSL #3

8 strains of 3 genus:

Streptococcus ,
Bifidobacterium,
Lactobacillus

Three probiotics can relieve tumor burden



Prohep

EcN;LGG;

heat-inactivated

VSL#3:

1:1:1

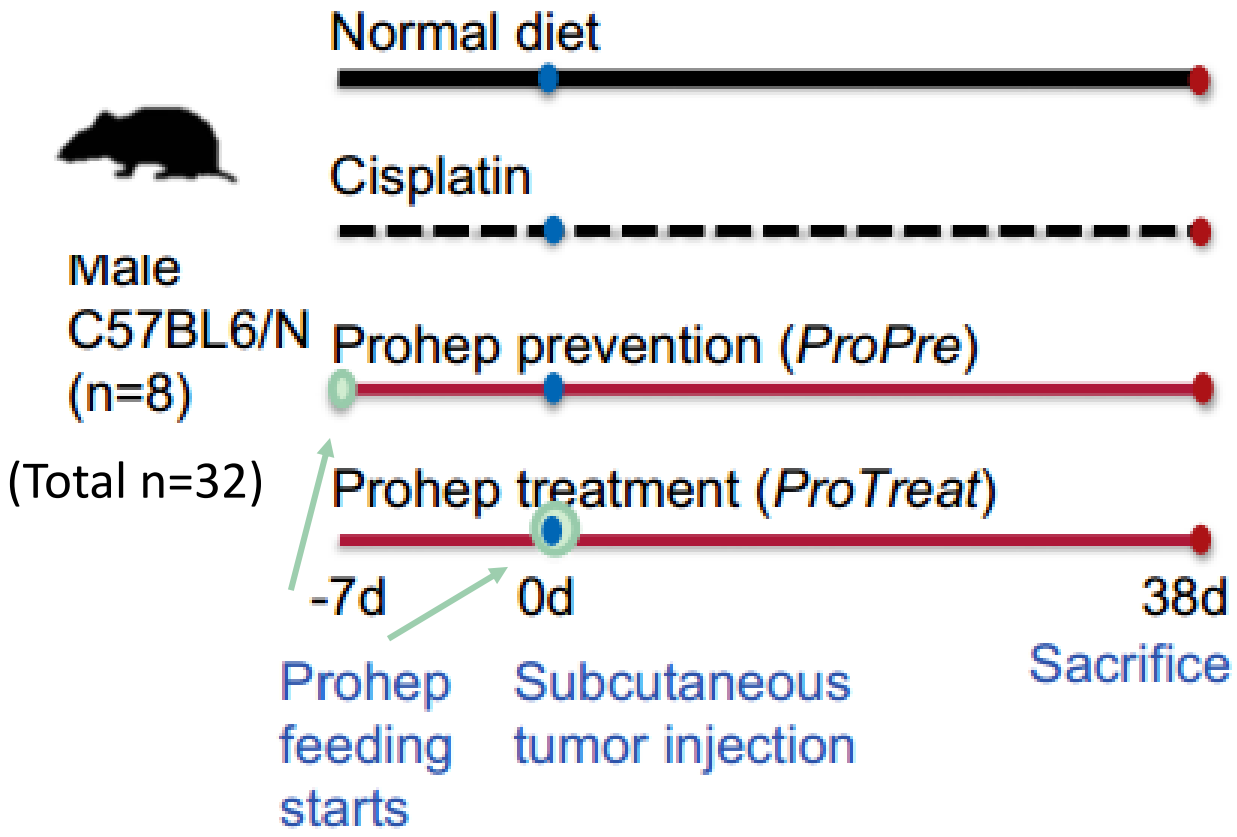
*0.01 < P value < 0.05;
**0.001 < P value < 0.01;
***P value < 0.001

eh: EcN in high dose

ld: LGG

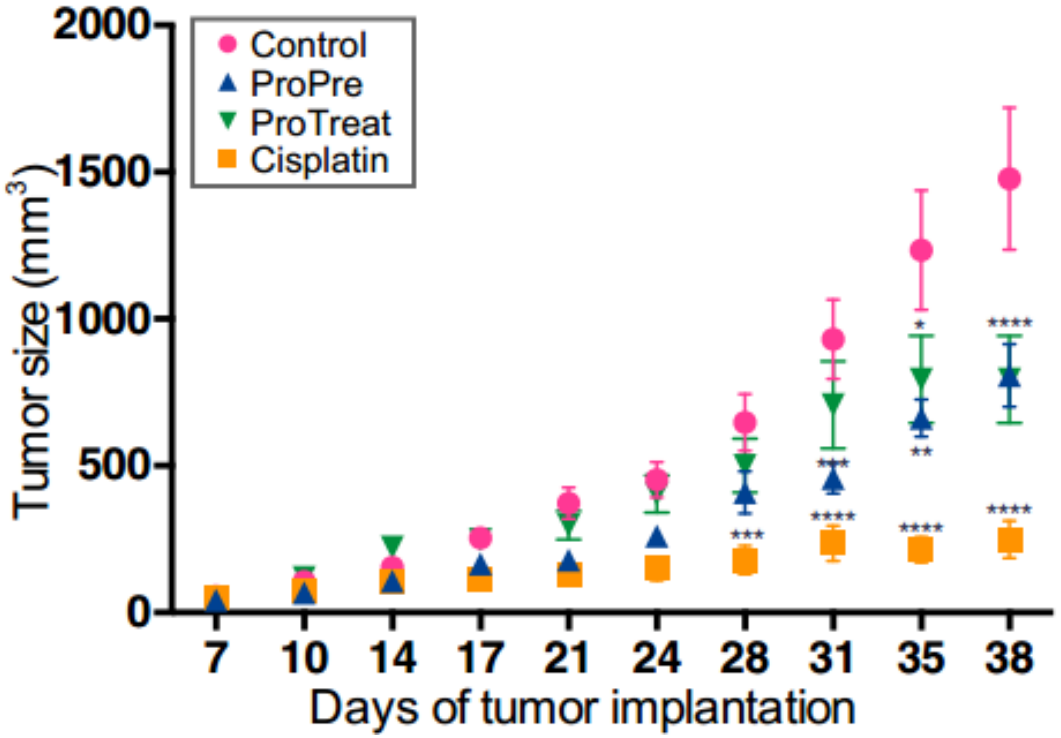
vd: heat-inactivated VSL#3

Effect of **Prohep** in mice study

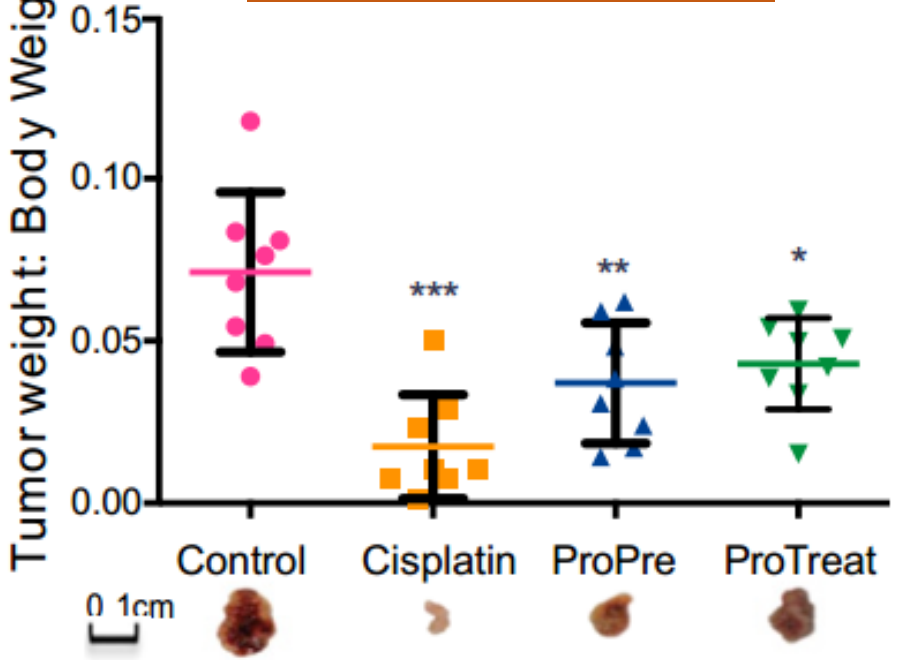


Prohep suppress tumor development

Change in tumor size over time



Change in tumor burden



Compared with Control

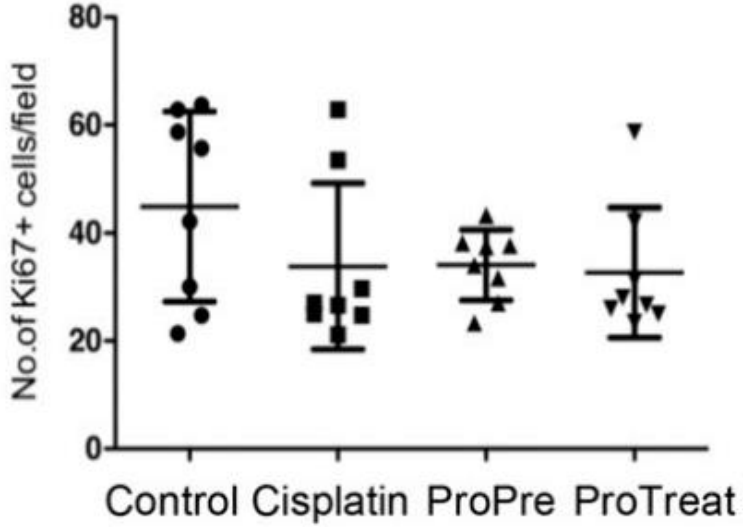
*0.01 < P value < 0.05;

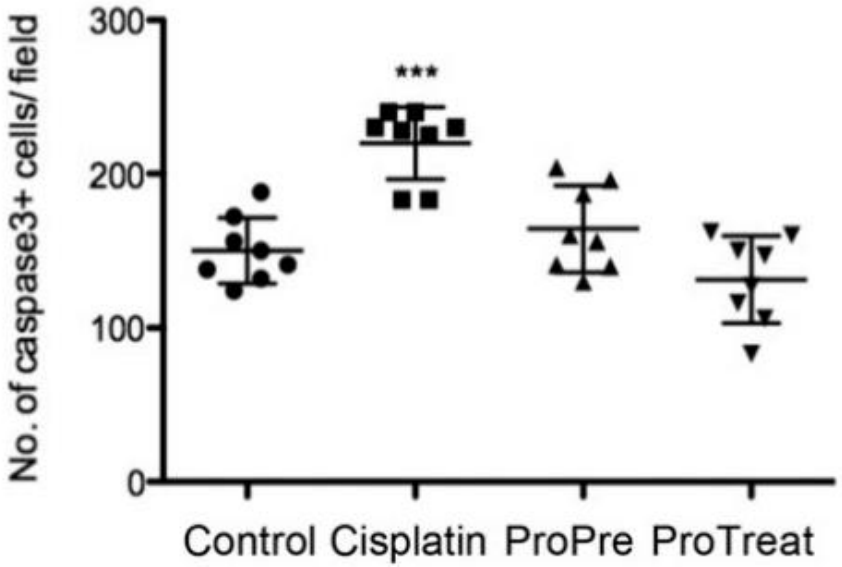
**0.001 < P value < 0.01;

***P value < 0.001

Prohep inhibit tumor growth by Increased hypoxia

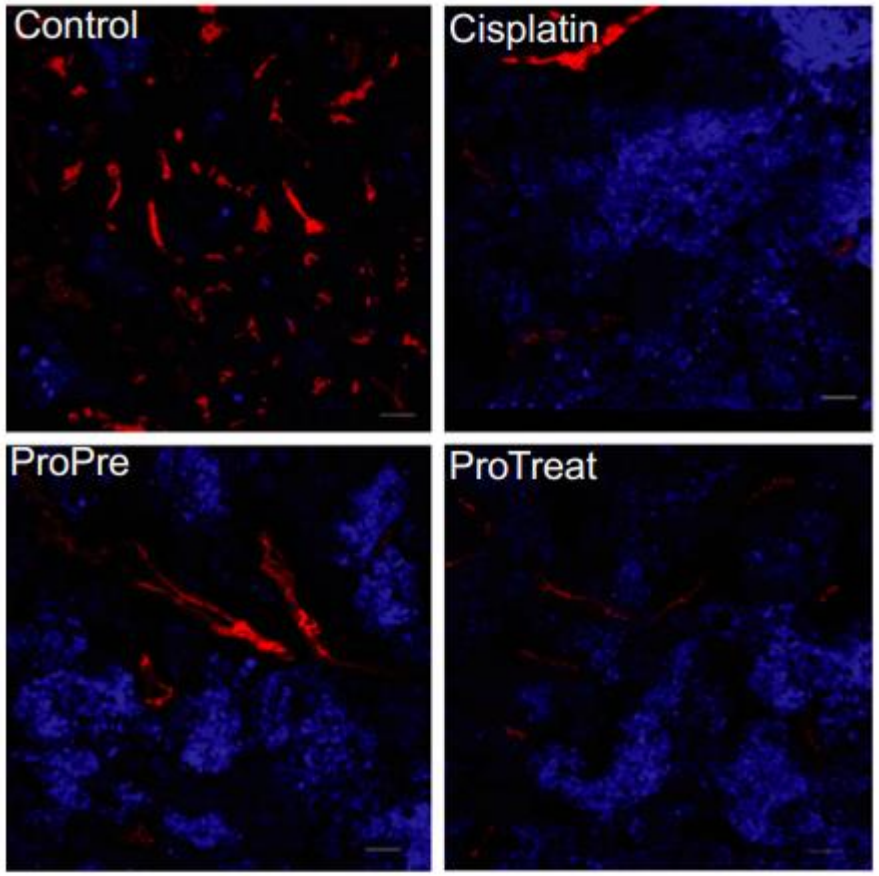
- Decreased cell proliferation ✗
- Increased cell death ✗
- Increased hypoxia ✓



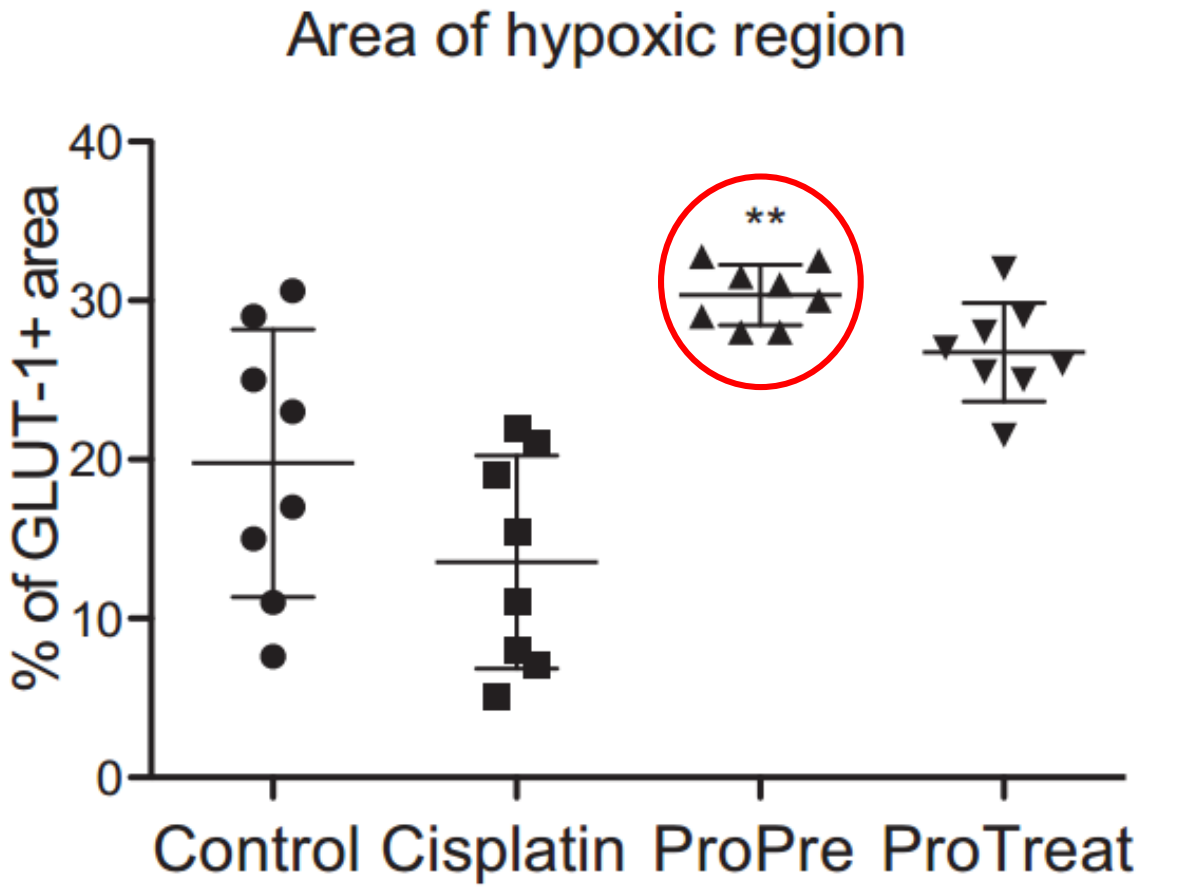


Proliferative cell (Ki67+) Apoptotic cell (caspase 3+)

Increased hypoxia: Increased hypoxic region

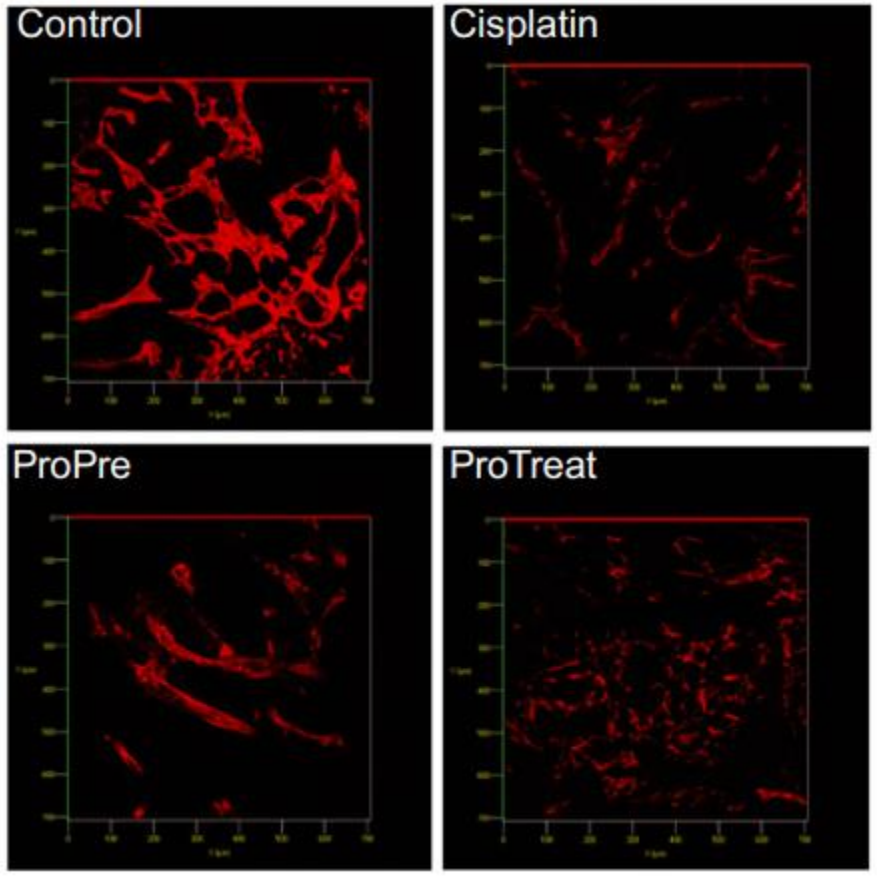


Hypoxic marker staining (GLUT-1+)

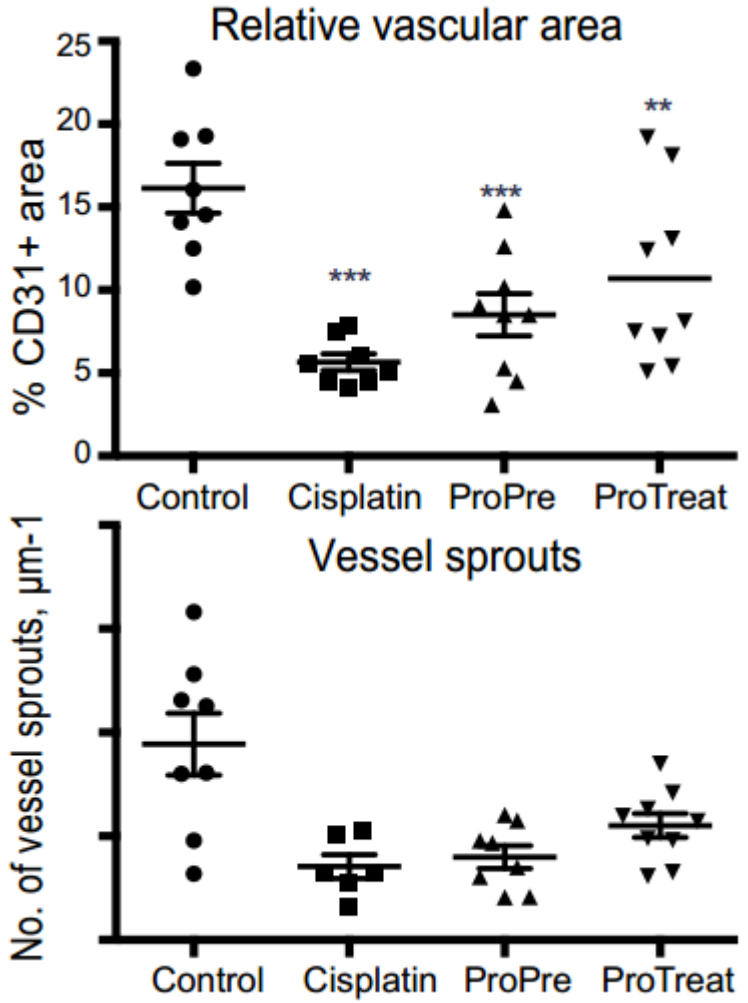


**0.001 < *P* value < 0.01 compared with the control.

Weakened Angiogenesis relates to hypoxia



Microvessel Density (MVD)



**0.001 < P value < 0.01;
***P value < 0.001

Mechanism research (in brief)

62 genes (angiogenesis / immuno-regulation) : Downregulation of IL-17

↓

IL-17 release (T cells, macrophages, neutrophils): T cells

↓

T cells (CD 4+, CD8+, NK cells): CD4+ cells express IL-17

↓

CD4+ cells (Th1, Th2, Th17, Treg, Tr1): Th17 release IL-17 less in ProPre groups

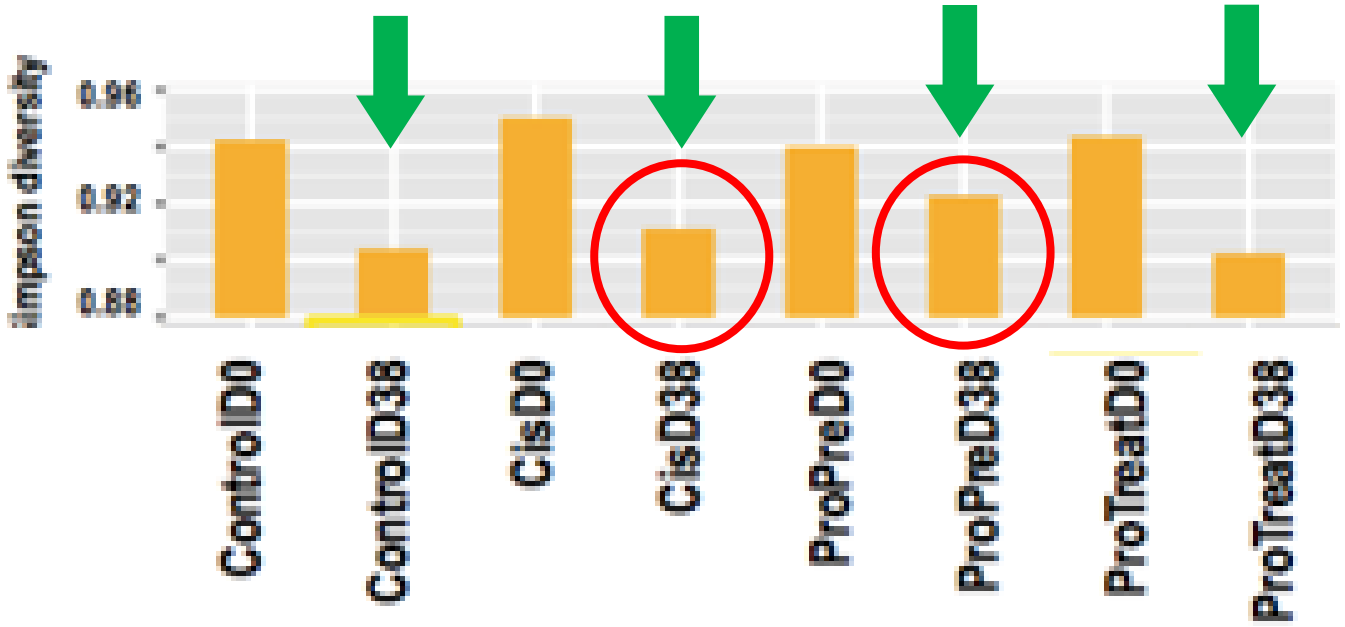
↓

Prohep groups: 1. Repressed recruitment of IL-17 in tumor.
2. Th17 distribution less in S.int and blood

CONCLUSION: **Prohep** feeding may reduce the Th17 frequency in intestine, and thus reduce the recruited Th17 in the tumor microenvironment. The reduced Th17 cells in the tumor could impede the inflammation and angiogenesis and limit the tumor growth

Prohep & Gut Microbiome

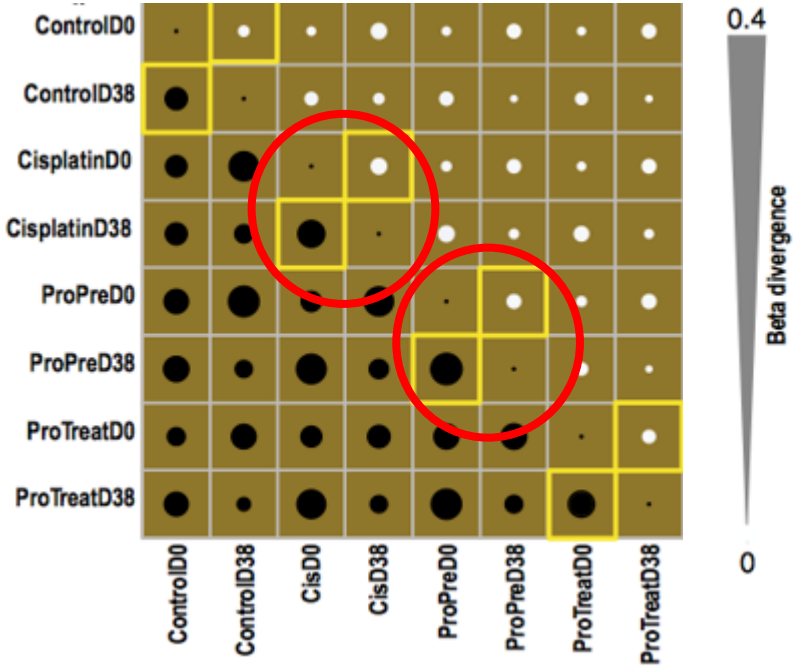
ProPre group rebalances gut microbiota



α diversity (Simpson diversity)

- ProPre and Cisplatin groups are significantly higher after 38 days
- Rebalancing Microbiota

ProPre group reshapes gut microbiota

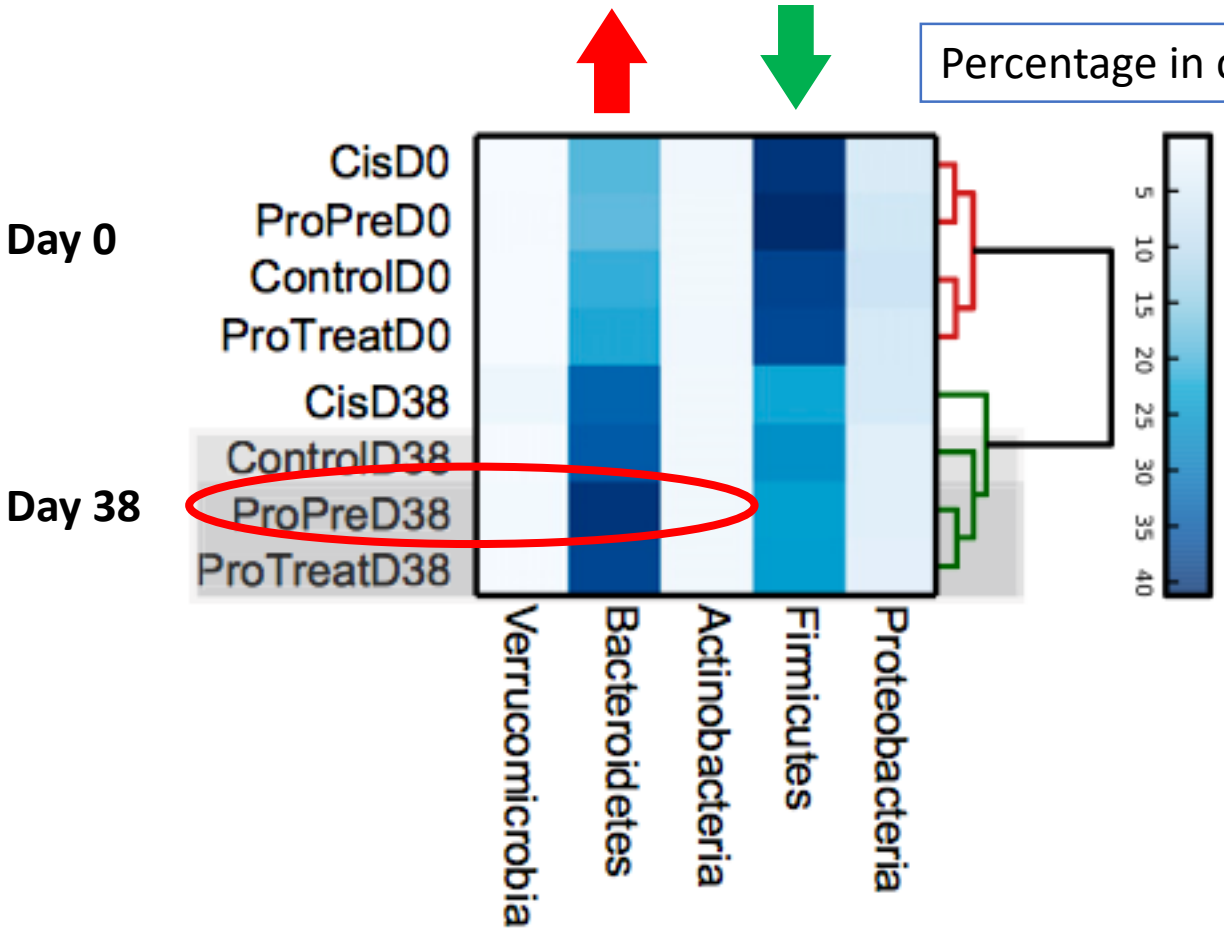


- functional beta diversity
- taxonomic beta diversity

β diversity

- ProPre and Cisplatin groups can drastically shifted the community
- Reshaping community structure

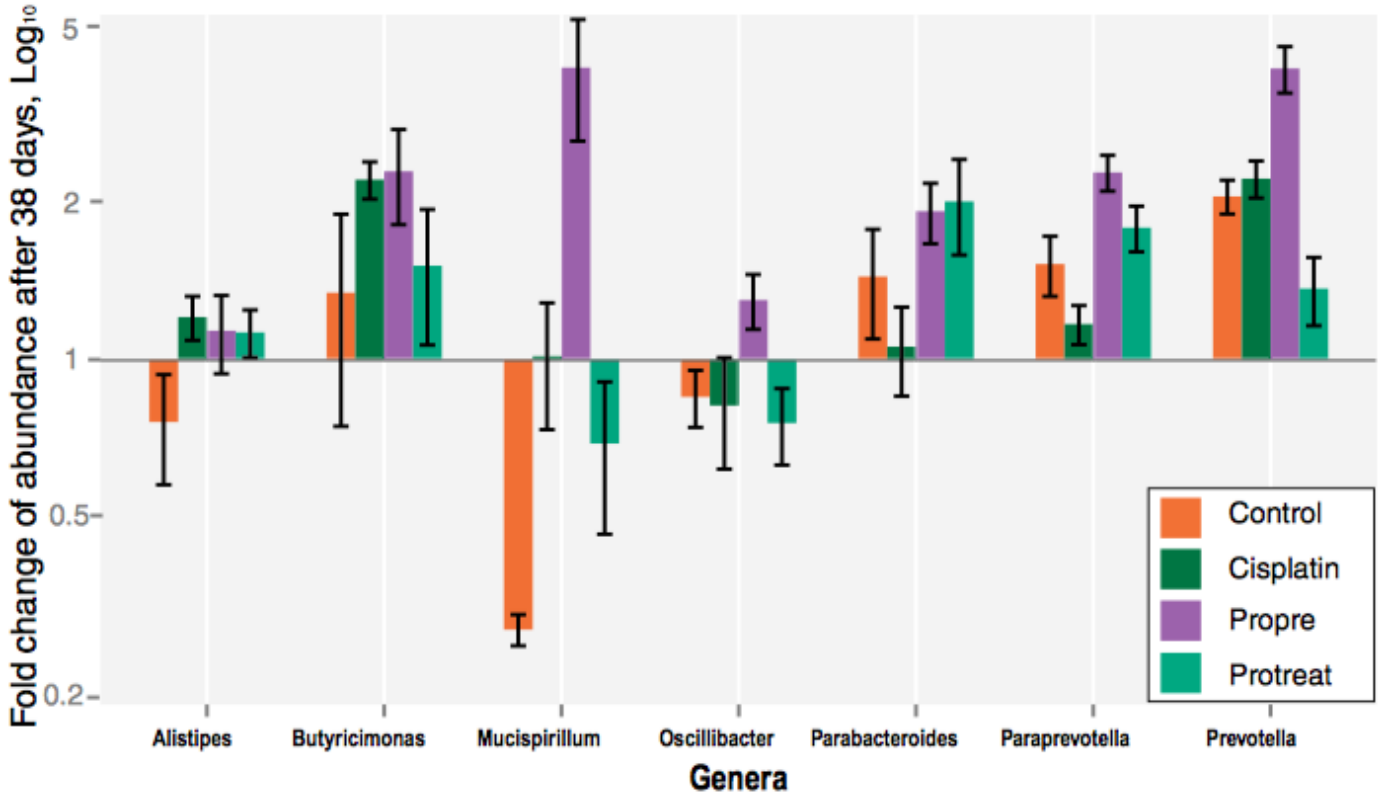
Phylum level change



- Similar community shift before & after tumor development
- Two dominant phylum: *Bacteroidetes* & *Firmicutes*
- *Bacteroidetes*: produce anti-inflammatory molecules

Hierarchical clustering and taxonomy profiling of 8 samples at phylum level.

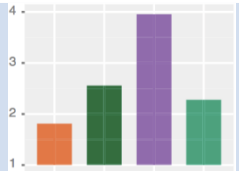
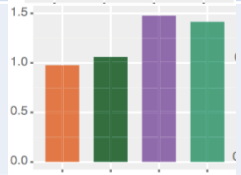
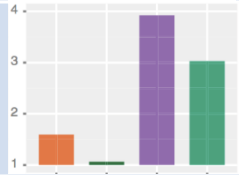
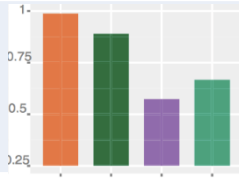
Genus level change: 7 significantly* enriched genera in **ProPre** group



- 3 related with short-chain fatty acids (SCFAs) producing: **anti-inflammatory**
- 2 related with **T-cell differentiation**
- 2 related with **anti-inflammatory**

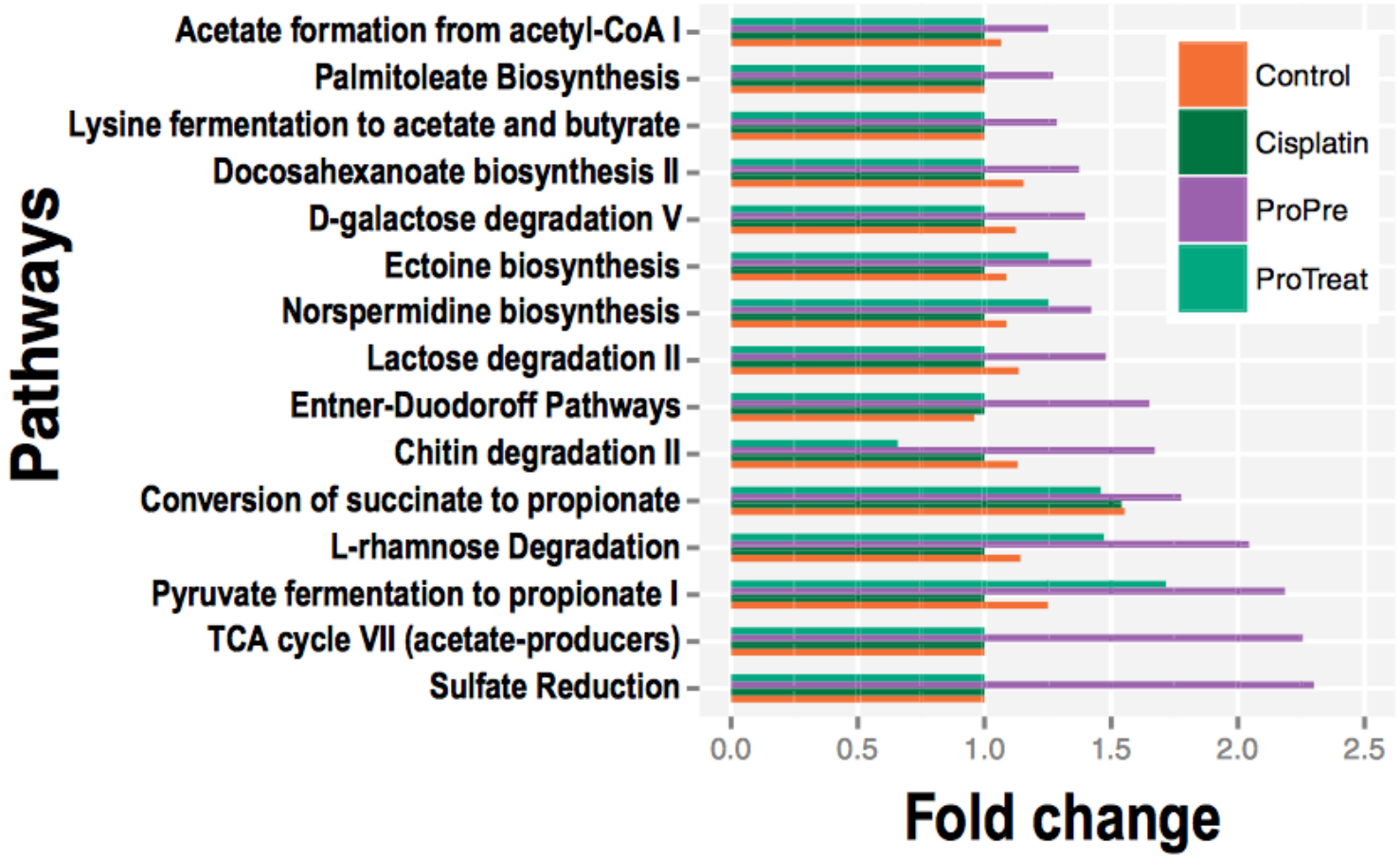
* Bonferroni adjusted P value <0.05 in Wilcoxon rank-sum test using 100 bootstraps for each sample

Species level change: 4 significantly* enriched species in **ProPre** group

Species	Function	Change in Propre
Bacteroides fragilis	Gut immunoregulatory	Increase 
Alistipes shahii	Modulator in the suppression of tumor growth	Increase 
Parabacteroides distasonis	Antiinflammatory	Increase 
Segmented filamentous bacteria (SFB)	Th17-inducing	Decrease 

* Bonferroni adjusted P value <0.05 in Wilcoxon rank-sum test using 100 bootstraps for each sample

Metabolic Pathway: Top 15 enriched in *ProPre group*



- 6 are related to SCFAs
- 2 are long-chain fatty acids: reduce the pro-inflammation cytokines in endothelial cells
- Others: not mentioned

Conclusion: **Prohep** & Gut Microbiome

- Taking **Prohep** preventively increased abundance of many beneficially anti-inflammatory bacteria and decreasing the Th17-inducing bacteria

Summary

- **Prohep**, a new probiotic combination treatment, is non-invasive yet beneficial to cancer patients.
- **Prohep** therapy can serve as a prophylaxis to hepatocellular cancer.
- Study offered insight into the mechanism of probiotics modulate the microbiota and immuno-regulatory effects.
- Gut microbiome modification can be explored for non-invasive and non-toxic anti-cancer treatment.

Thanks