Herbal medicine and drug addition: Role of the enteric microbiome and its metabolites

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Two Medicines in the U.S.

- Conventional medicine (or Western medicine)
- Complementary and alternative medicine (CAM)
- U.S. NIH: National Center for Complementary and Alternative Medicine (NCCAM)
- In 2014, changed to National Center for Complementary and Integrative Health (NCCIH)
- Currently, some major medical centers are using limited CAM therapies for integrative health

Western Medicine (WM) vs. Traditional Chinese Medicine (TCM)

- WM - body systems and specific diseases
- TCM has a more *holistic view* of the human body, but also emphasizes *individualization*
- Herbal formulations often composed of a number of botanicals
- The formulation is complicated, but it is an essential part of TCM – quality control issue
- Acting on multi-pathways and multi-targets

Two Ways of Herbal Drug Usage

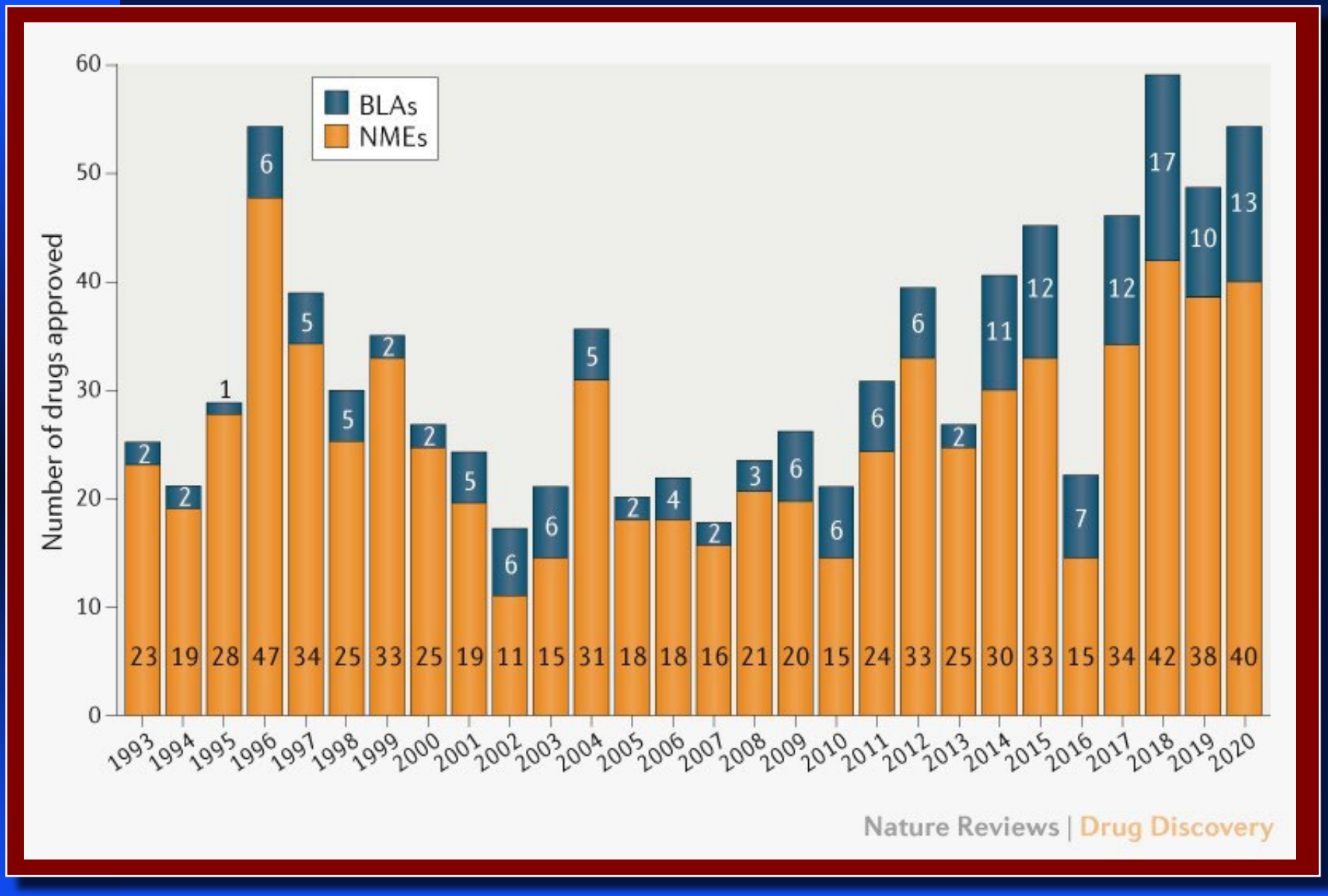
One Herb (West) vs. Multiple Herbs (East)

- Single herb/drug use in the West for medical conditions
- TCM uses multiple herbs in combination to treat diseases for multiple activities
- However, complicated botanical analysis
- Difficult for quality control
- Single compound for new drug development

Two Types of Medications or Drugs

- Prescription drugs – NDA
- Generic drugs – sNDA

- Botanical drugs (or herbal medicines, dietary supplements, or natural products)
- U.S. FDA regulated?
- Status of botanical drug approval



BLAs = Biologics license applications
NMEs = New molecular entities

FDA Approved Botanical Drugs

- **Two FDA-approved plant extracts**
  - Veregen, from green tea, for treating genital and anal warts
  - Fulyzaq/Mytesi (crofelemer) for treating non-infectious diarrhea in HIV/AIDS pts on anti-retroviral therapy
- **Cost in U.S.** (Unlike European Union, also WHO)
  - Veregen (approved 2006): 15% topical ointment is $1,400 for 30 grams; Then, generic Sinecatechins, $250/tube
  - Mytesi (approved 2012): Oral delayed release tablet $2,300/60 tablets (>$30 each; No generic version)
- **Many candidates**
  - Many obtained INDs, in different stages of trials for NDAs
  - As always, important to closely communicate with FDA

Discover/Develop New Drugs

- Natural products as candidates for new drugs
- New compounds and patent protections
- Preclinical studies
  - Pharmacology
  - Toxicology - based on proposed usage
  - Potential effect prior to human use
- Clinical trials: Testing in humans; IND

Drugs, from discovery to marketing

PHASE		
Discovery	2-10 YEARS	Searching for an experimental drug.
Preclinical Testing	1-3 YEARS	Lab and animal testing.
Phase I Is it safe?	Up to 1 year	Drug used in 20-100 healthy people to test safety and to determine the proper dosage.
Phase II Does it work?	Up to 2 years	Drug used in 100-300 patients to study its effectiveness and side effects.
Phase III Are there side effects?	1-4 YEARS	Drug used on 1,000-3,000 patients to learn how it works long-term, and continue to determine dosage and identify side effects.
FDA Review	2 MONTHS-7 YEARS	Federal regulations analyze data from the laboratory and clinical tests to determine whether a drug is safe and effective.

>$800 M

YEARS 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

New drug development, based on my own experience:

Methylnaltrexone (MNTX)

- University of Chicago licensed MNTX to U.S. pharmaceutical industry in 2001
- In December 2005, Wyeth and Progenics announced the joint development MNTX for over $500 million
- In April 2008, the FDA approved subQ MNTX for opioid-induced constipation in advanced illness patients
- In September 2014, the FDA approved MNTX for opioid-induced constipation in non-cancer pain patients
- In July 2016, the FDA approved MNTX oral tablets
- By 2023, this new drug was available in over 60 countries

TANG CENTER
for HERBAL MEDICINE RESEARCH

UCTC

At the forefront of research on herbal therapies

One out of three Americans uses herbal therapies. Yet, less than one out of 3,000 scientific studies focuses on this increasingly popular therapy. Investigators at the Tang Center are changing that.

Researchers at the Tang Center continue the long tradition of academic medicine at the University of Chicago, a place that has produced more than 70 Nobel laureates. Led by world-renowned University of Chicago researchers, the Tang Center is applying advanced science to uncover the advantages and possible dangers of herbal therapies.

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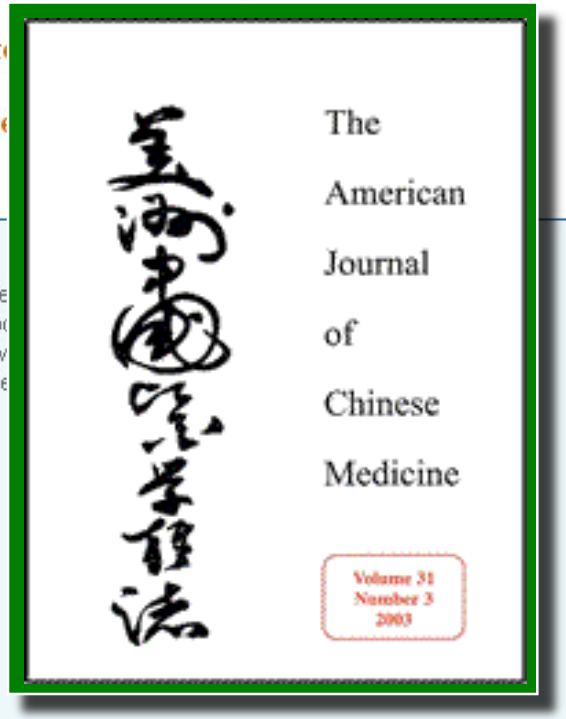

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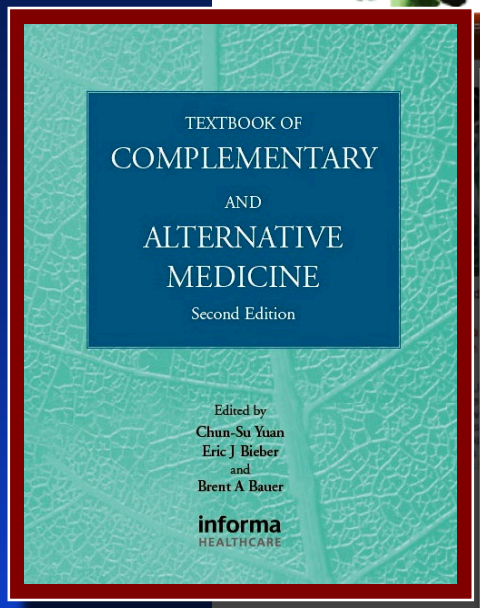
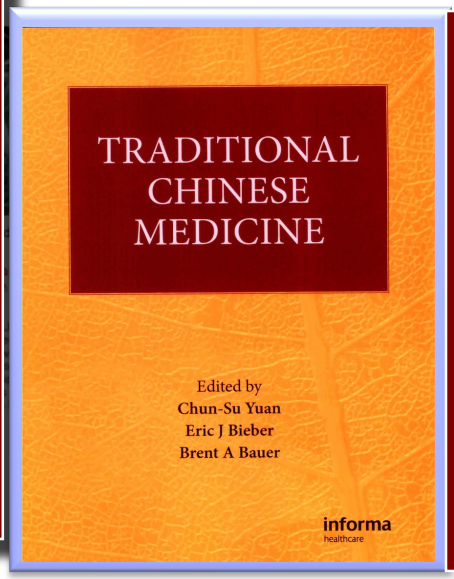
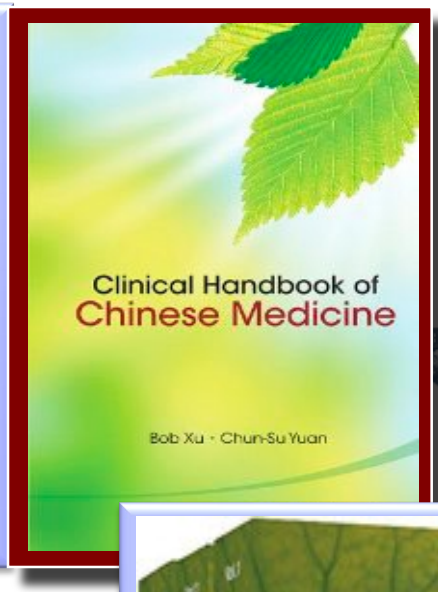
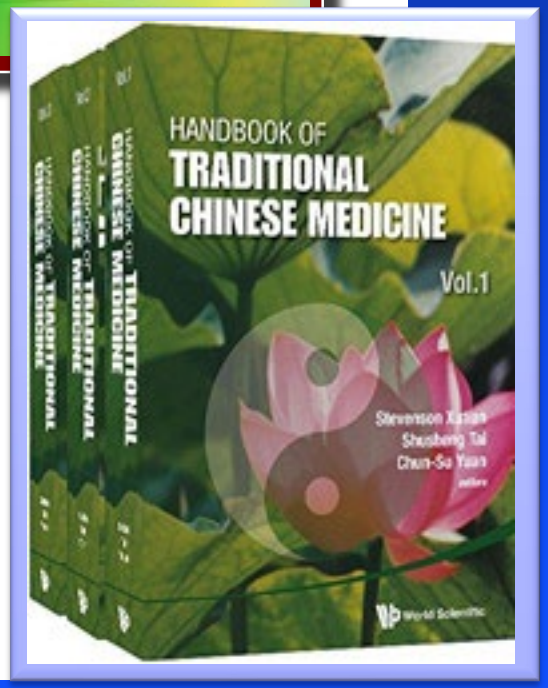








Website: Tangcenter.uchicago.edu

http://tangcenter.uchicago.edu/

Our Pharmacological Studies of American Ginseng

- Multiple constituents and multiple pharmacological activities
- Central nervous system
- Cardiovascular system - antioxidant
- Type 2 diabetes - using ginseng berry
- Chemo-induced side effects
- Potential anti-cancer effects:

  Such as colorectal cancer, a leading cause of cancer related death

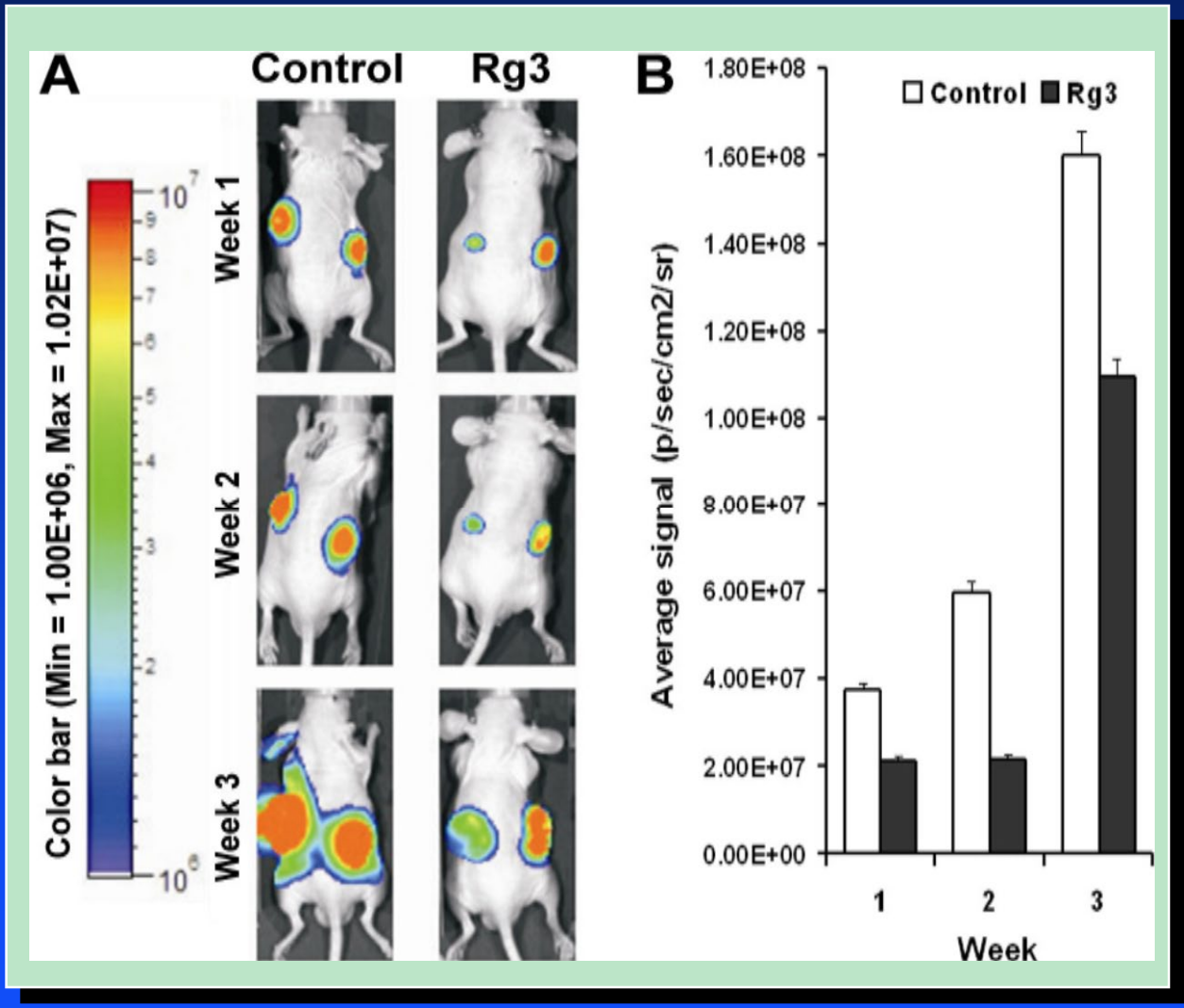
Ginseng on Cancer
- A Reported Human Trial

- Case-control studies on >1,000 Korean subjects
- Long-term ginseng consumption decreased risk for many different cancers
- Ginseng has a non-organ specific cancer preventive effect

Yun and Choi, 1995 and 1998; Yun, 2003

Issues of Previous Studies

- Whole herb –
  Fractions –
  Compounds –
  In vitro –
  In vivo –
- Botanical bioavailability overlooked

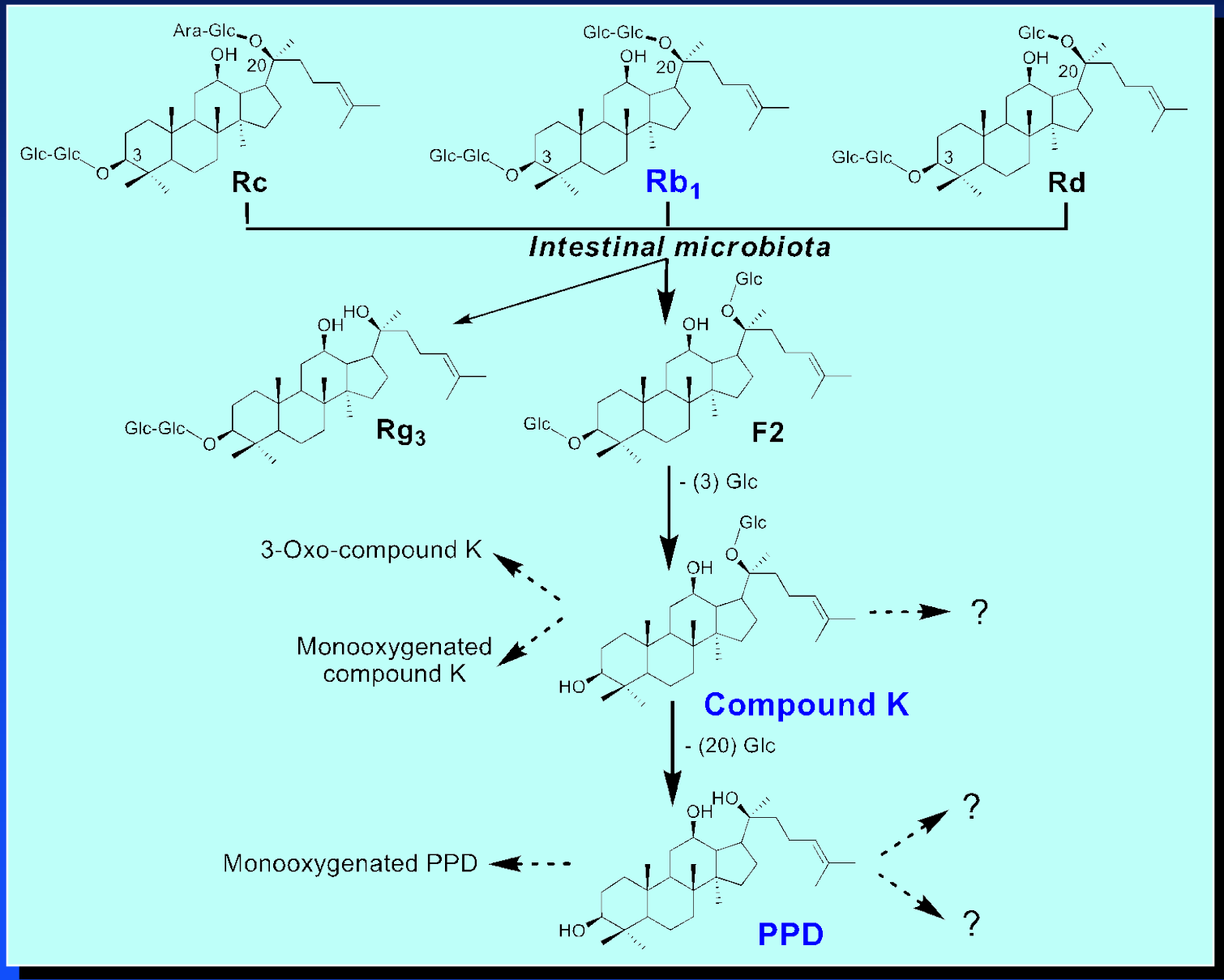


After HCT-116-Luc cell inoculation
Whole body bioluminescence imaging; $P < 0.01$

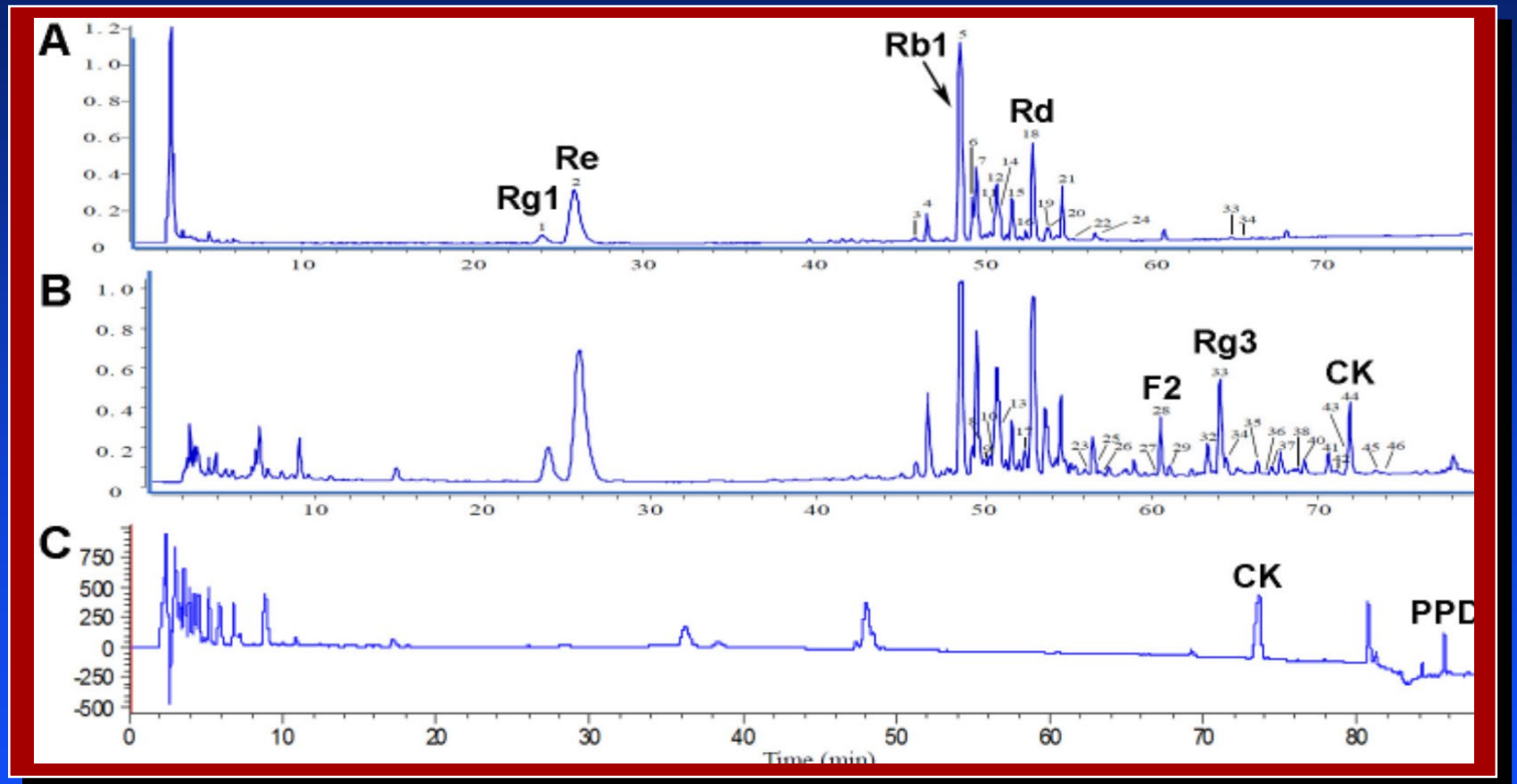
In the Real World

- Herbal medicines almost always taken orally
- Same for American ginseng
- Low absorption of parent compounds
- Biotransformation to metabolites by the enteric microbiome
- Other substances too, e.g. melamine (三聚氰胺 in Chinese)

Biotransformation Pathways



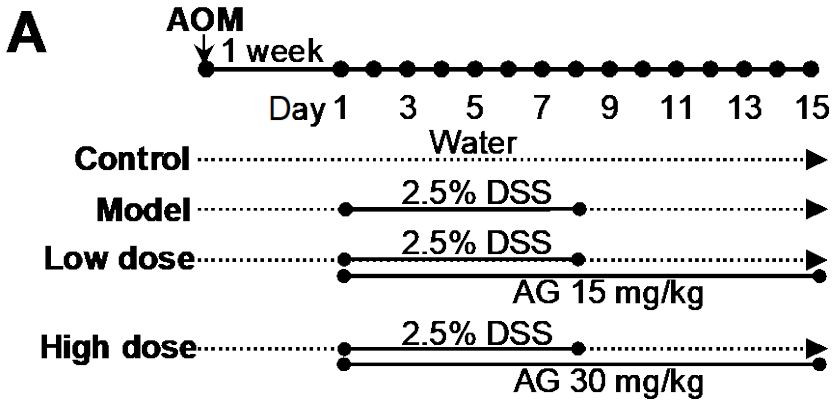
Human Gut Microbiota Converted Ginsenosides to Metabolites

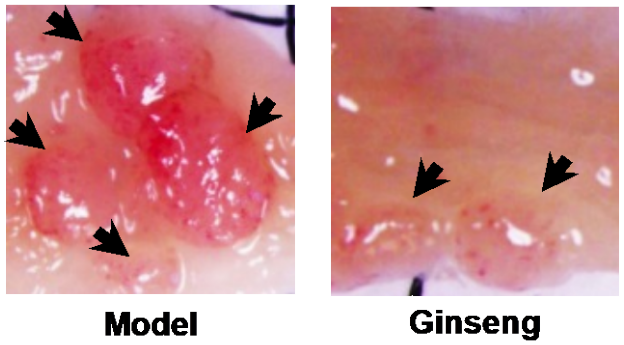


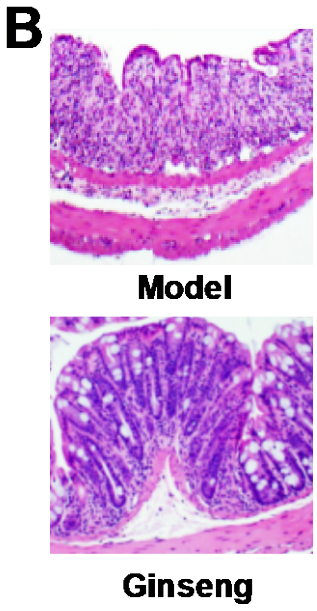
A. Chromatograms of American ginseng extract
B. The transformed compounds
C. Compound K (CK) further converted to PPD

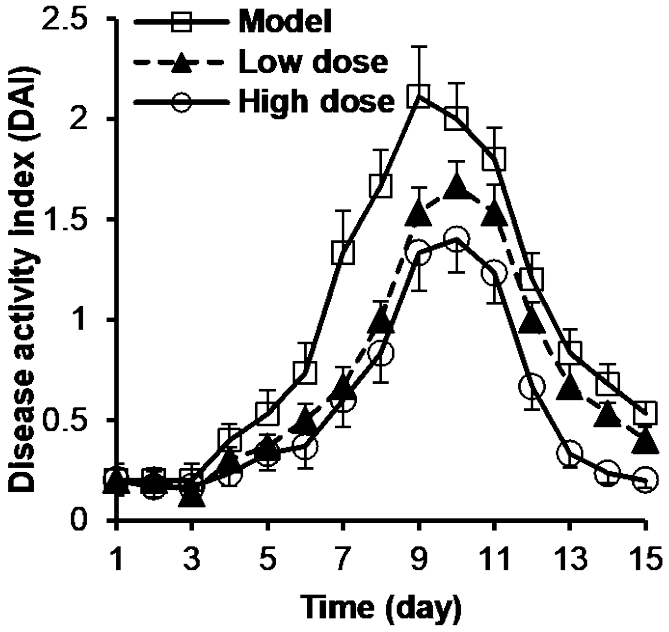
Wan et al., *J. Chromatogr. A* 1286: 83-92, 2013.

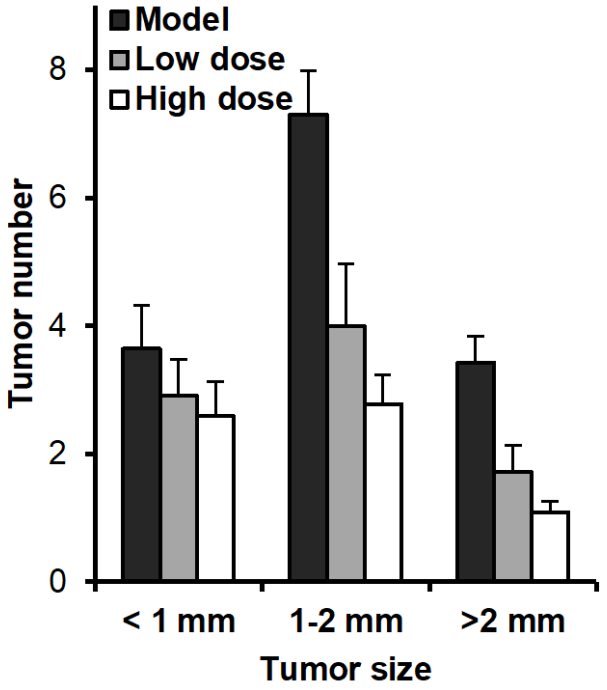
Oral Ginseng on AOM/DSS Mice





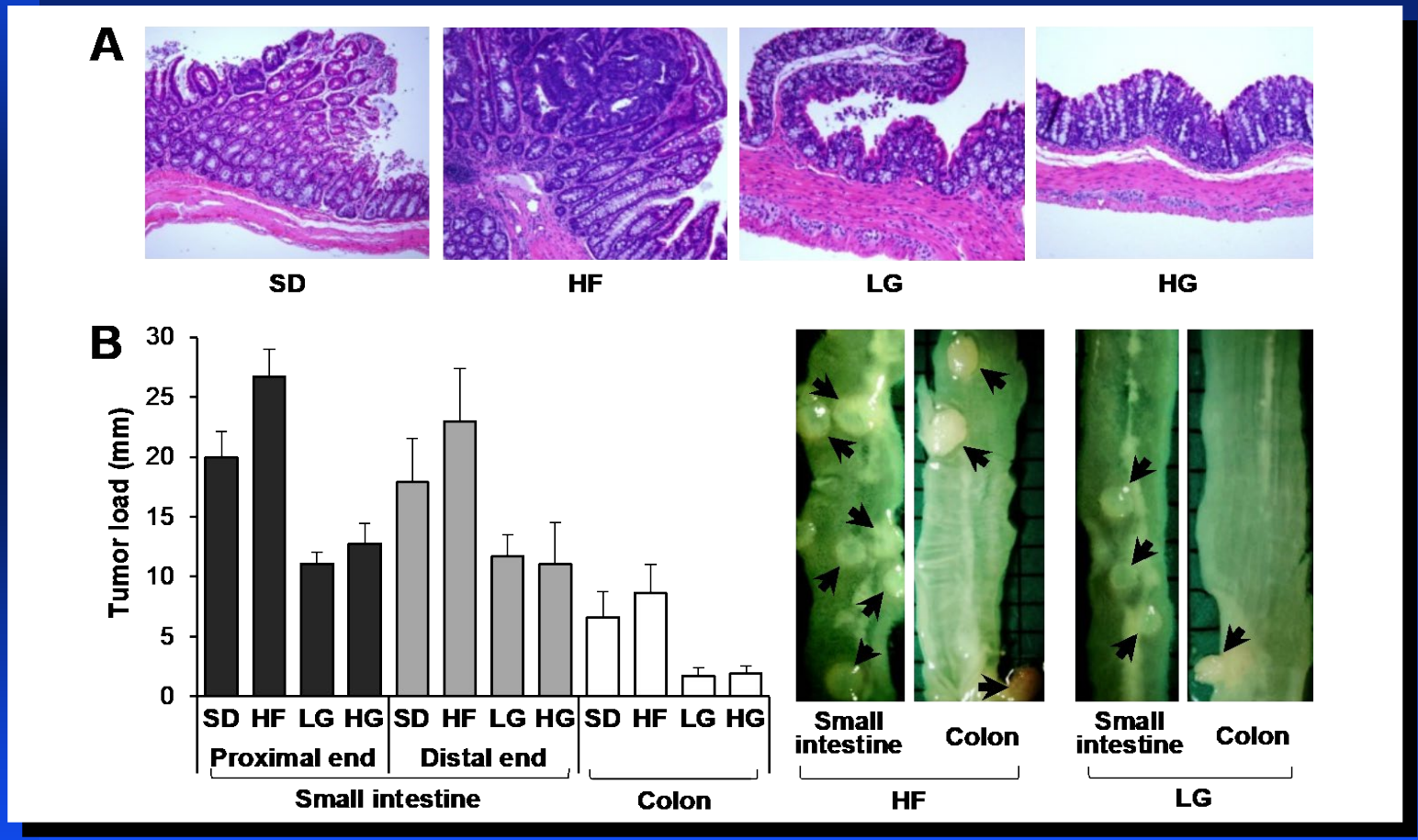






Ginseng Effects using *Apc*$^{Min/+}$ Mice

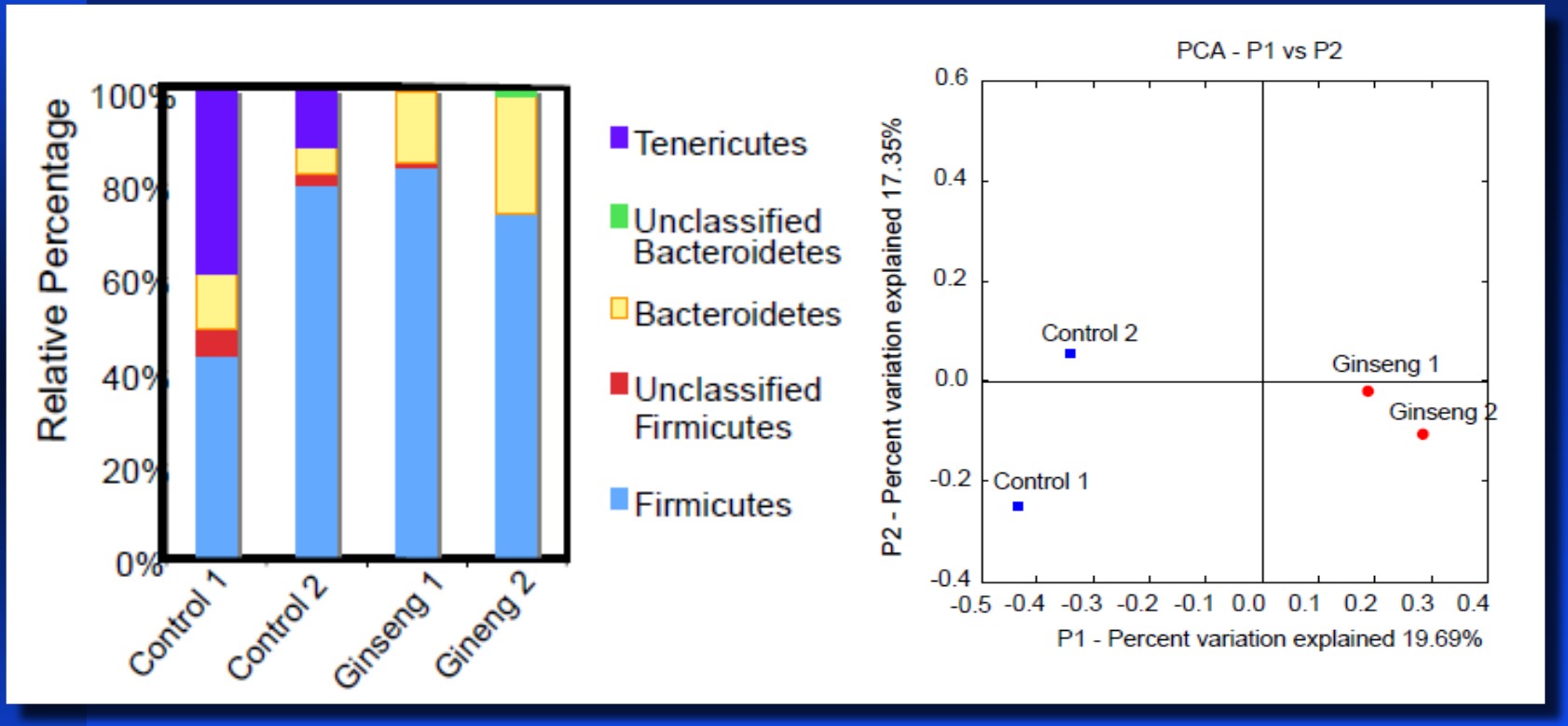
- Supported by our gut microbiome and metabolomic analyses



A. H&E staining gut sections; B. Gut tumor load changes.

SD, standard diet; HF, high fat (20%) diet; LG, low-dose ginseng; HG, high-dose ginseng

Effects of Ginseng on Species Diversity of Enteric Microbiota

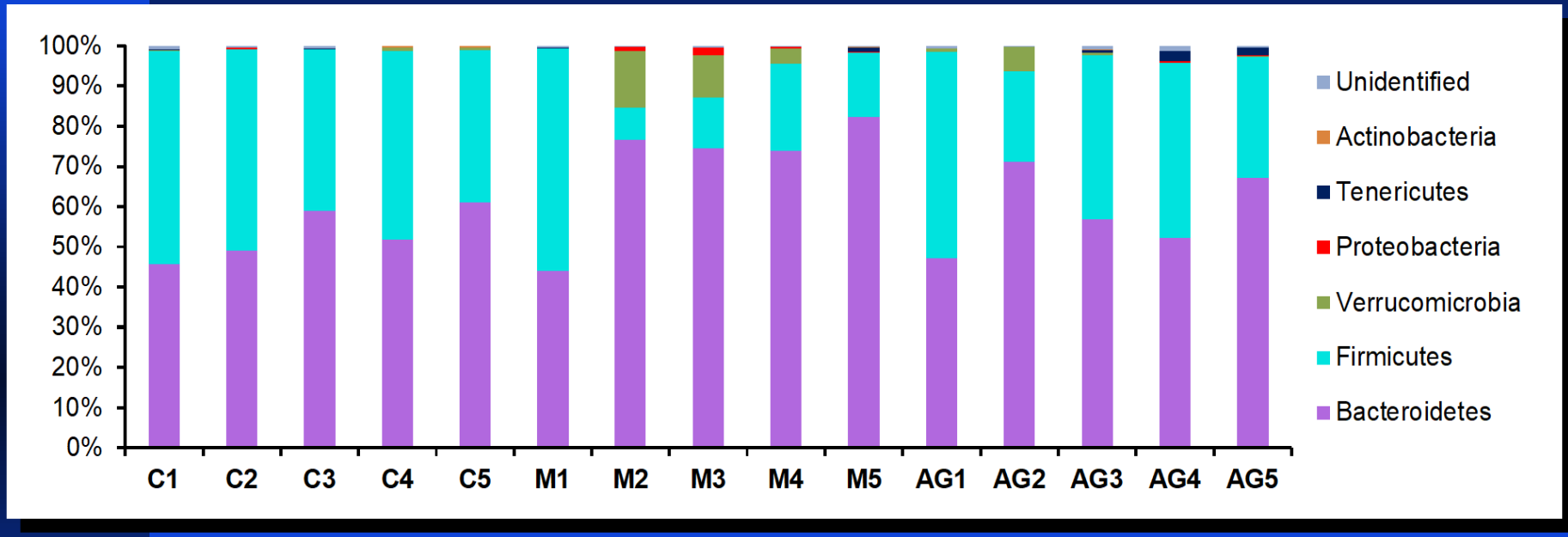


A. Phylotype composition of two mice cecal microbiota

B. PCoA shows microbiota community similarities in stool

Control, AOM/DSS model control; **Ginseng,** ginseng treatment group

Effects of Ginseng on Enteric Microbiota Diversity



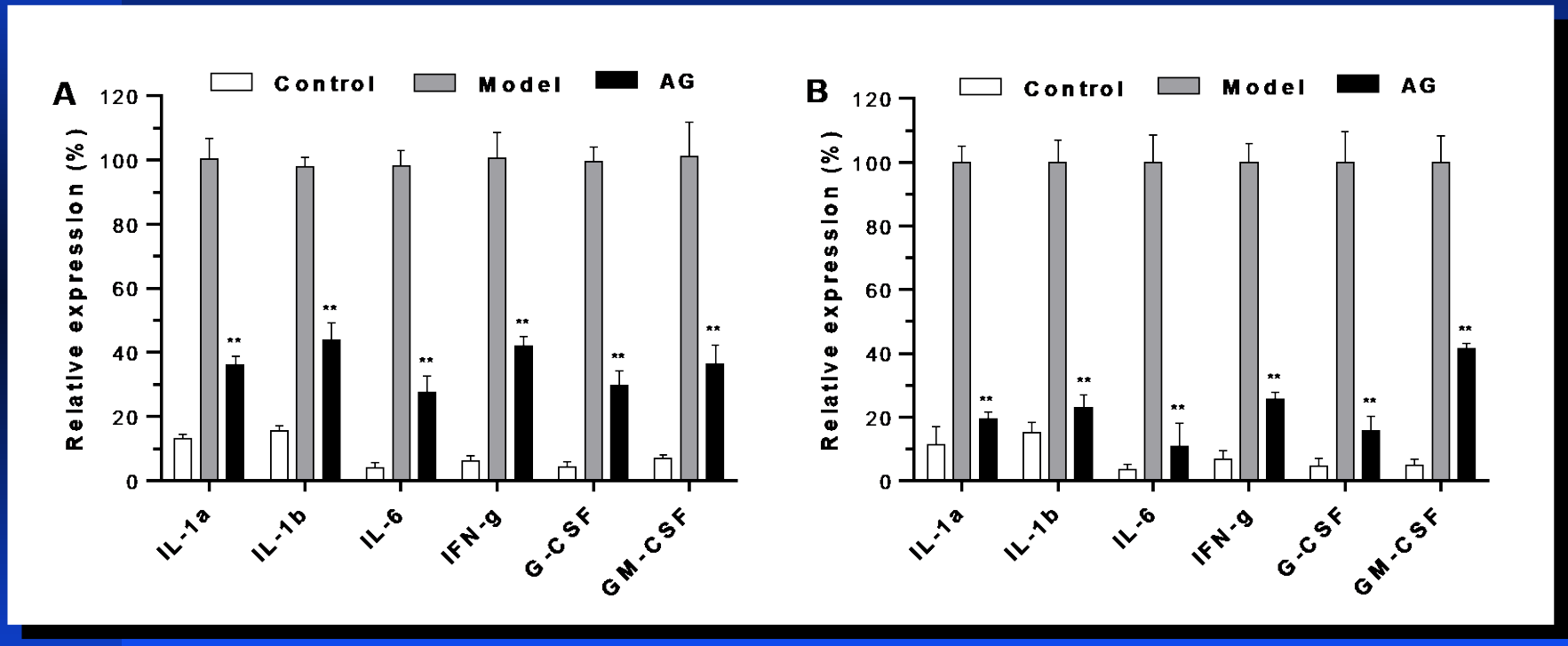
Taxonomic composition of the microbiota at phylum level identified with16S rRNA gene sequencing

C, control; M, AOM/DSS model; AG, ginseng treatment

1-5: stool samples collected at weeks 0, 3, 6, 9 and 14 (Each microbiota in taxology)

Consistent with the data of inflammatory cytokine genes expression in colon tissue

Expression of Inflammatory Cytokine Genes in Colon Tissue

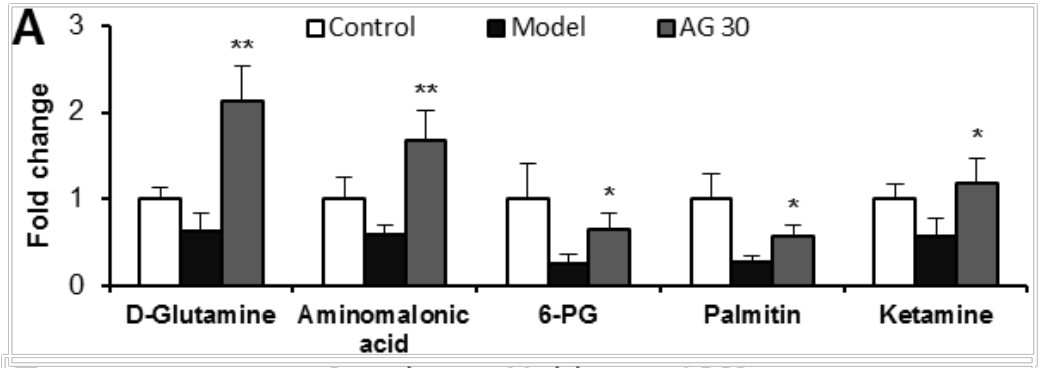


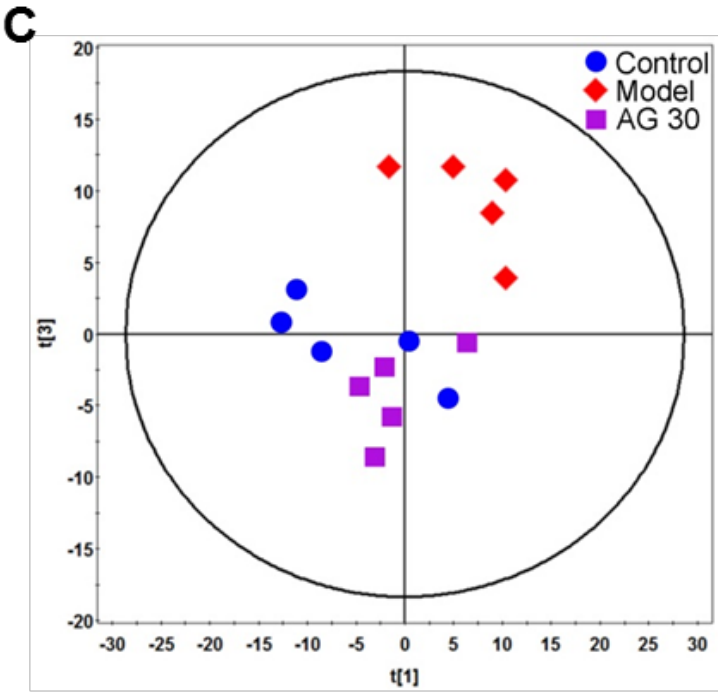
Total RNA isolated from mice colon tissue for qPCR

A. In acute phase at 2 weeks

B. In chronic phase at 13 weeks

Metabolomic Analysis





Mice plasma samples at week 13

A and B. Top-five up-regulated and down-regulated metabolites

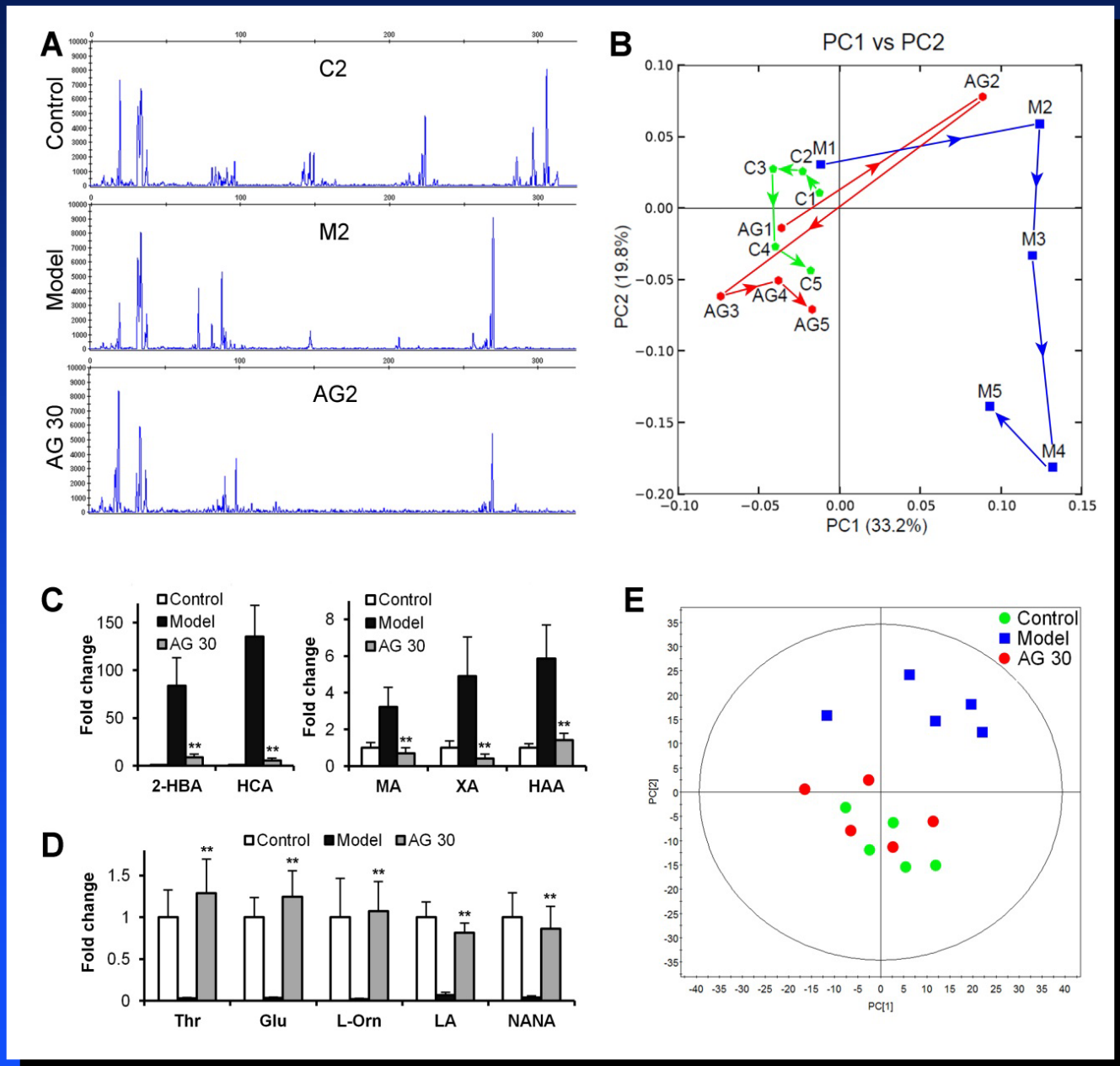
C. Principal components analysis (PCA) score plot

Microbiota and Metabolomics: Stool Sample

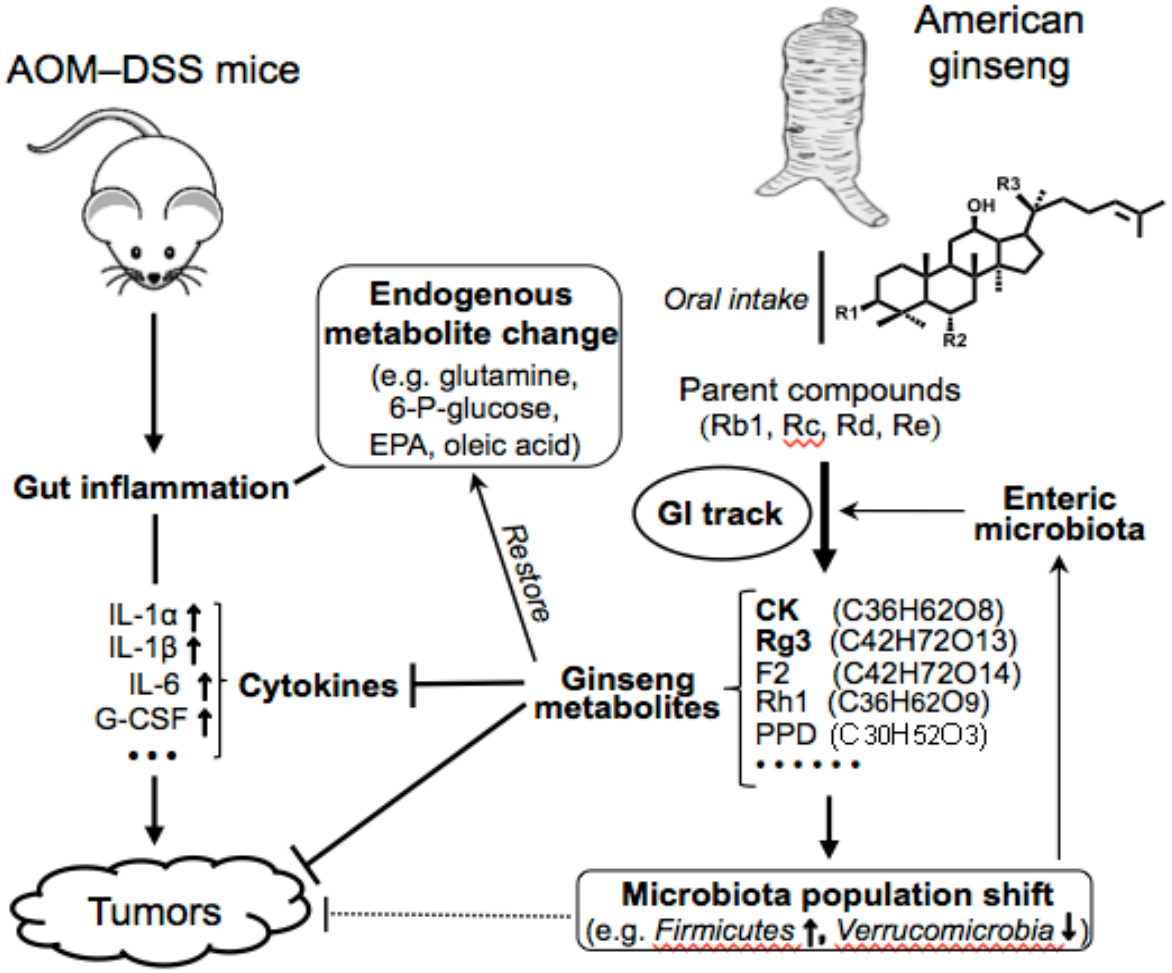
A, B: T-RFLP and PCA showing microbiota profile differences over 13 weeks

C, D: The top 10 metabolites

E. Metabolic profiling

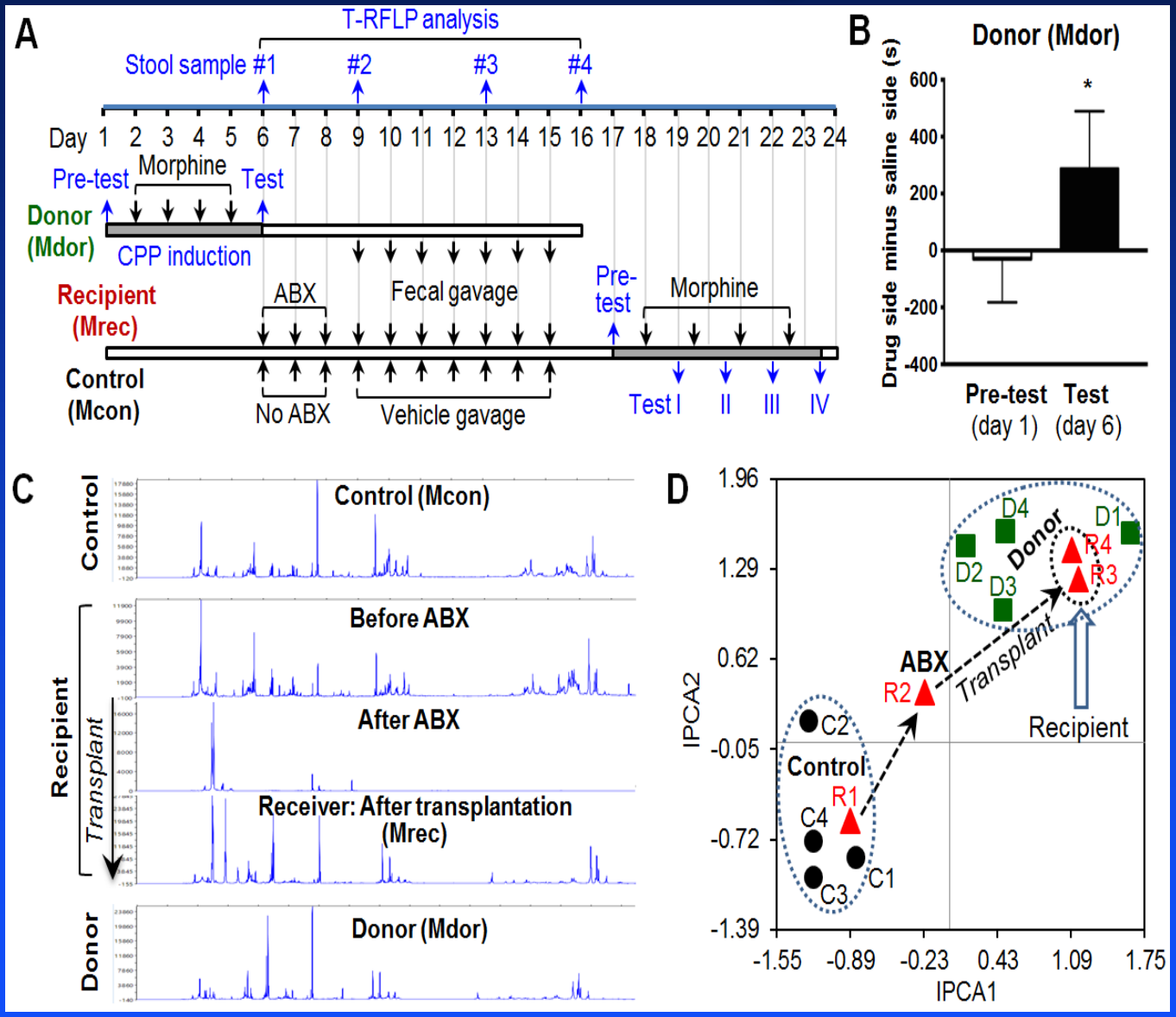


Role of Enteric Microbiota in Ginseng's Activities



Role of Gut Microbiome in Drug Addition

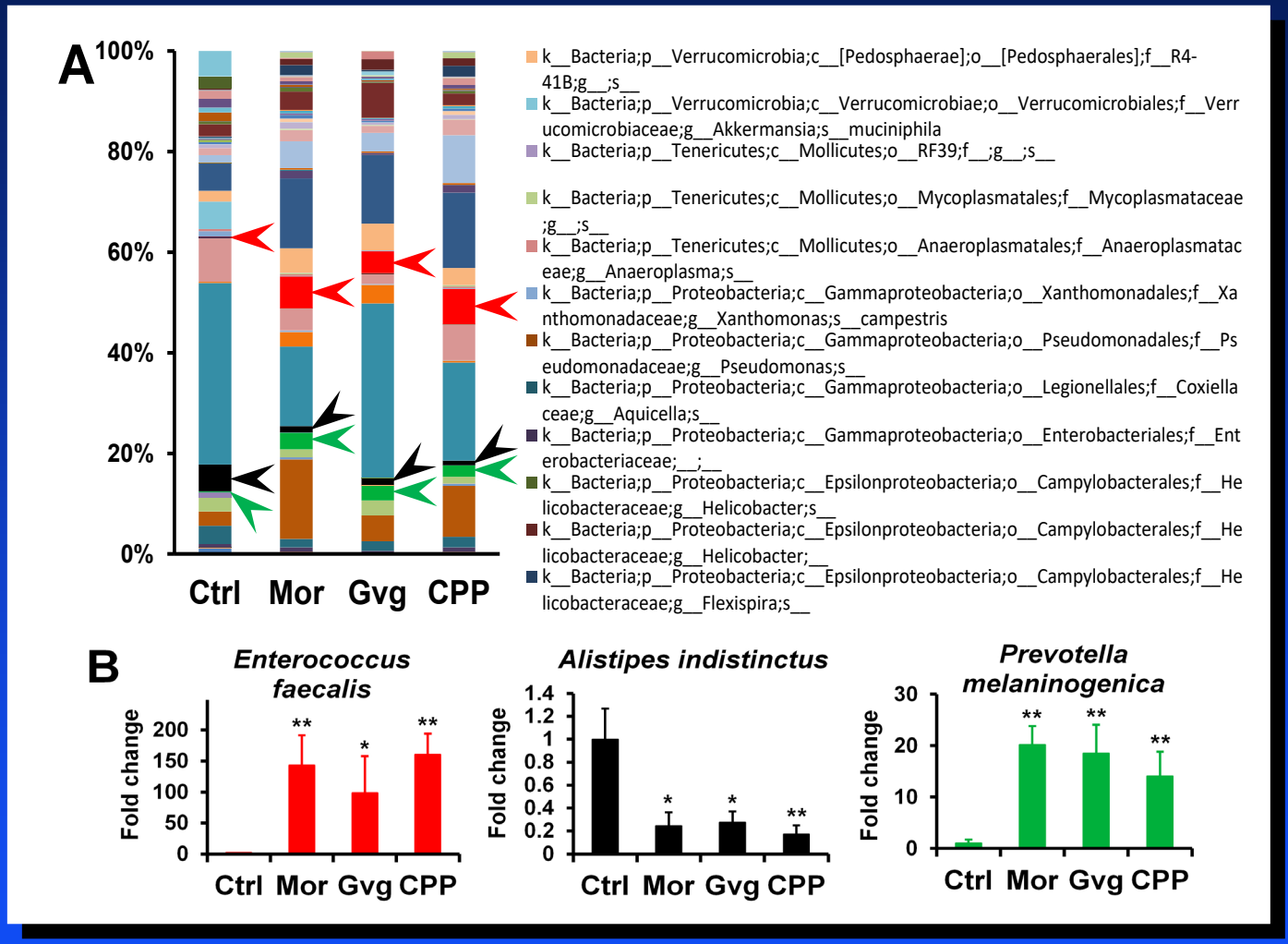
- Opioid abuse is a significant problem
- The gut, its microbes and the brain are connected
- via a complex communication/regulation system
- After repeated morphine (MS), composition of the gut microbiomes changed
- Using MS-induced conditioned place preference (CPP) mice model



(A) Timeline for the study. (B) Morphine CPP induction in Mdor mice. (C) Different-sized bacterioplankton populations using T-RFLP analysis. (D) PCA profiles showing differences in the microbiota profile.

Gut Microbiome Profile after Morphine (MS)

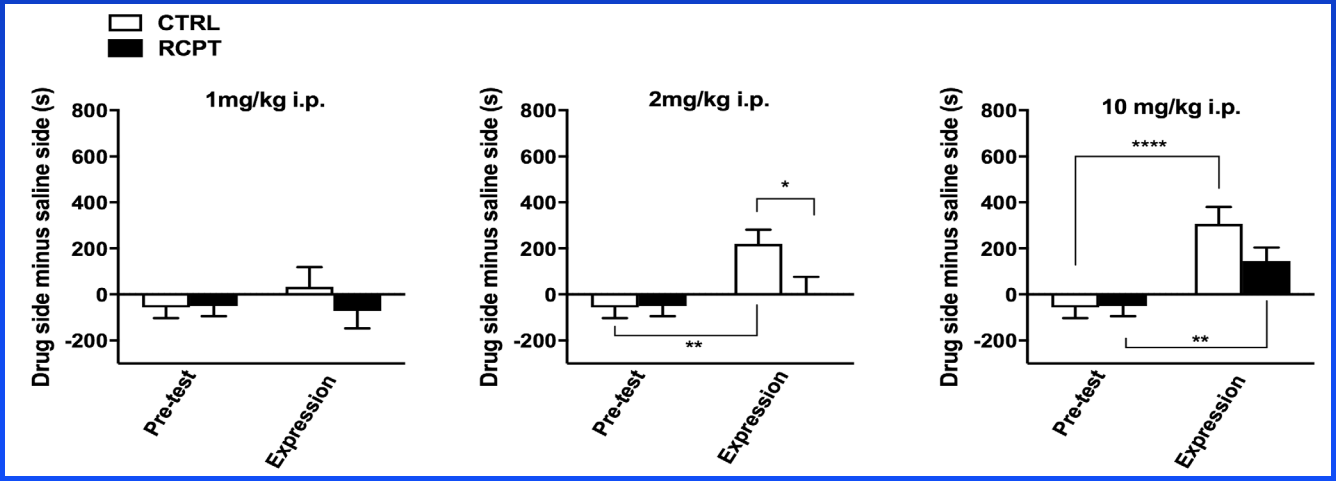
- Increase in *Enterococcus faecalis*
- Consistent with literature
- Observed changes correlated with gut dysbiosis



MS exposure on enteric microbiome composition changes. (A) Taxonomic compositions from Mcon (Ctrl), Mdor after MS (day 6, Mor), microbiome gavaged Mrec mice (day 16, Gvg), and Mrec mice after MS induced CPP (day 24, CPP).

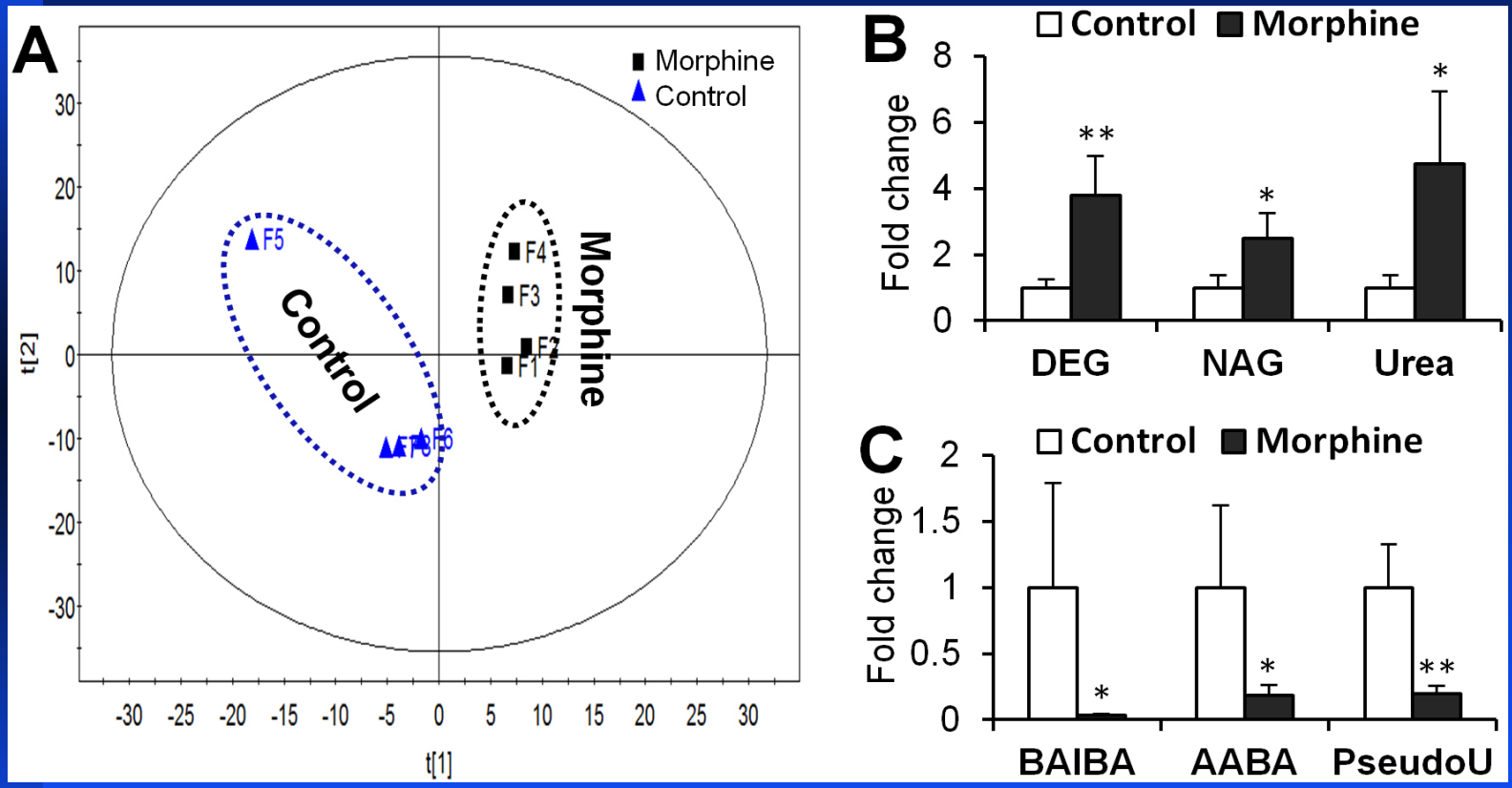
Active Role of Gut Microbiome in Opioid Abuse

- After transplanted microbiome fecal sample from MS-CPP donor (Mdor) to gut germ-free MS-naïve recipient (Mrec)
- The recipient mice showed reduced CPP induction by MS, suggesting that gut microbiomes from donor mice decreased the recipient mice in developing MS-induced reward behaviors
- The recipient mice resistant to MS-induced drug addition



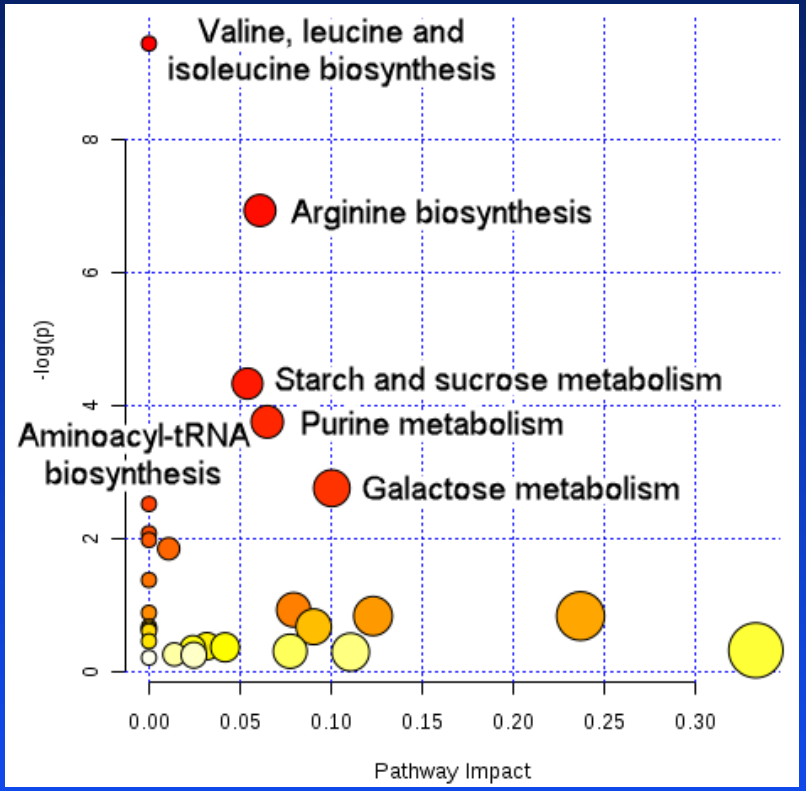
Microbial oral gavaged Mrec mice exhibit resistance to CPP induction by MS compared to Mcon mice

Morphine-induced Changes in Metabolomics Profiles in Fecal Samples



(A) PCA plot of fecal samples showing metabolic profiling from morphine (f1-f4) and control (f5-f8). (B) Three upregulated metabolites: DEG, diethylene glycol; NAG, N-acetyl-L-glutamic acid; urea. (C) Three downregulated metabolites: BAIBA, 3-aminoisobutyric acid; AABA, 2-aminobutyric acid; PseudoU, pseudouridine.

Metabolomics Studies for Metabolic Pathway Analysis



Metabolic pathways	*P* value
Valine, leucine and isoleucine biosynthesis	8.00E-05
Arginine biosynthesis	9.80E-04
Starch and sucrose metabolism	0.013176
Purine metabolism	0.023393
Galactose metabolism	0.063352
Aminoacyl-tRNA biosynthesis	0.080279

- MS induced changes in heatmap
- Metabolic pathways affected by MS
- Metabolic pathways with their *P* values

Future Studies

- In depth analysis of the composition of enteric microbiome in different groups
- Metabolomics profiles changes
- Mechanisms of the contribution of fecal extracts to morphine abuse
- Explore the brain-gut-microbiome axis
- For providing new treatment targets for opioid abuse

Summary

- Prescription meds vs. herbal medicines
- Our ginseng studies on colon cancer
- Enteric microbiome plays a critical role in parent compound metabolism
- Role of enteric microbiome in opioid addiction for future therapeutics
- Metabolomics for biol. signature/markers and metabolic pathways
- Need controlled clinical trials