ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
CENTRO INTERDIPARTIMENTALE
ALMA MATER INSTITUTE ON HEALTHY PLANET

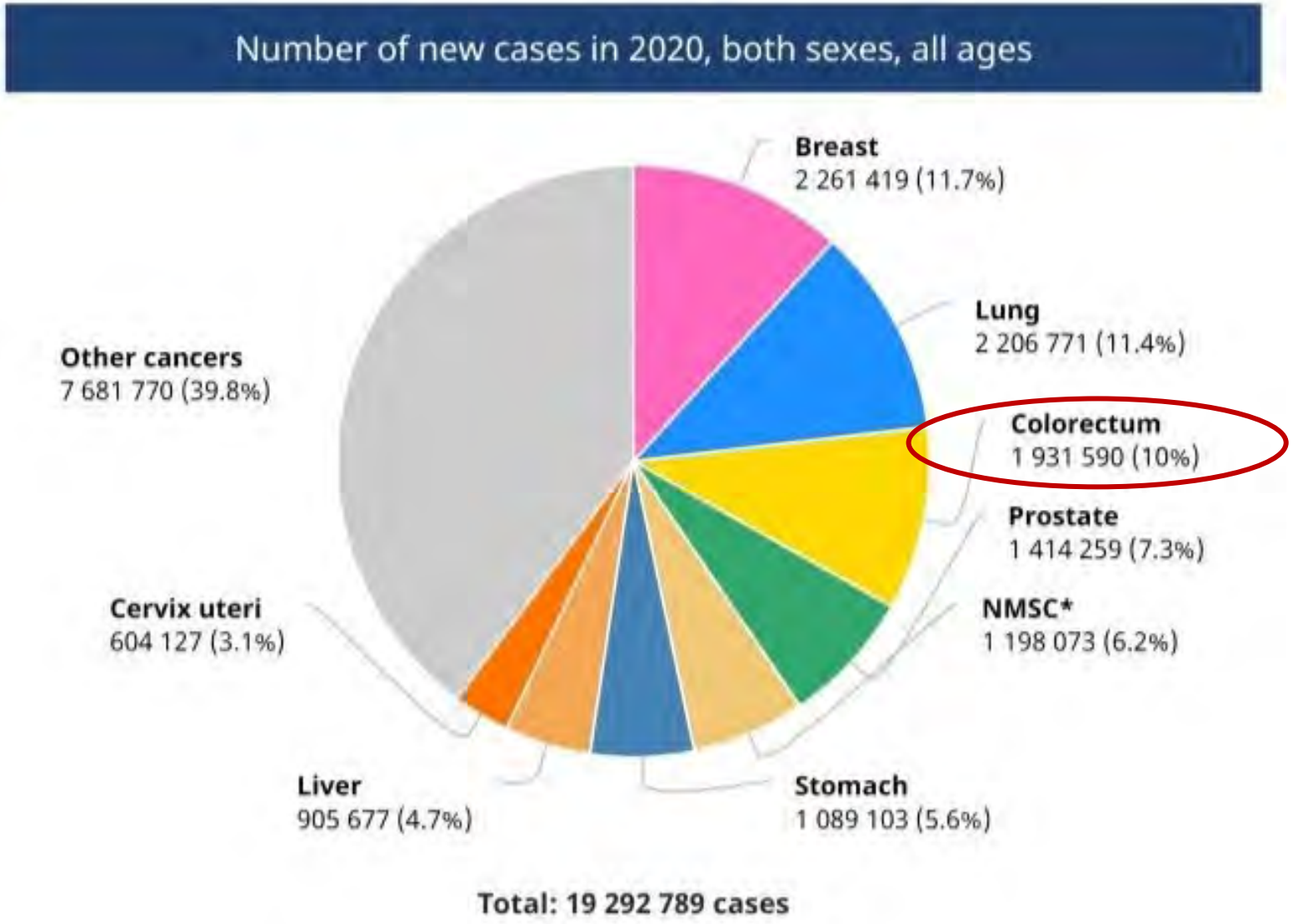
Ruolo del microbiota nella patogenesi e prevenzione delcancro colorettale

Luigi Ricciardiello, MD
University of Bologna

Disclosure

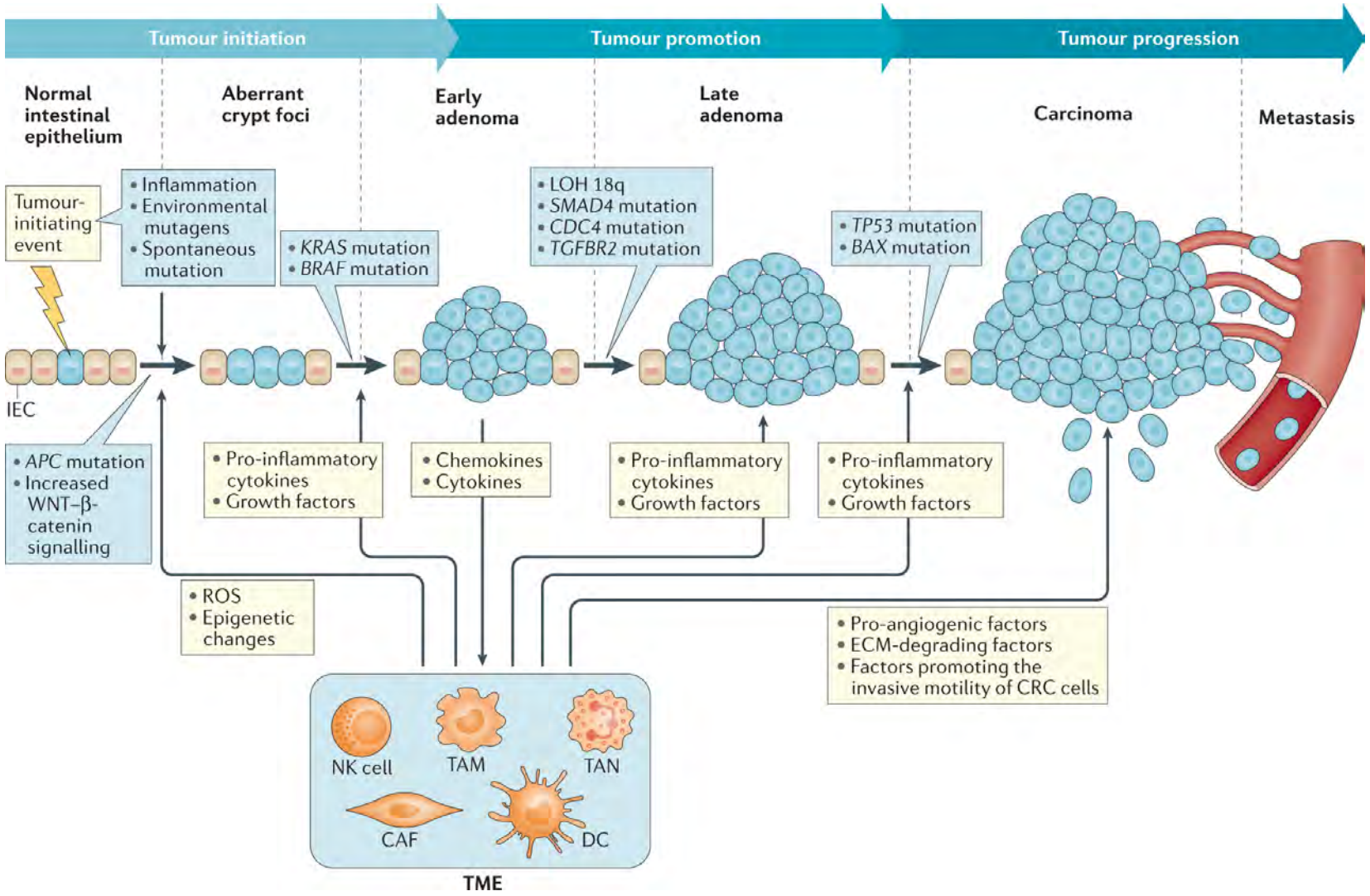
- SLA Pharma AG - Consultant

Colon cancer: Epidemiology (Worldwide)



2020 WHO GLOBOCAN Database

Mechanisms of tumour initiation and promotion in colorectal cancer



Mark Schmitt & Florian R. Greten. Nature *Reviews Immunology 2021; 21:* 653–667

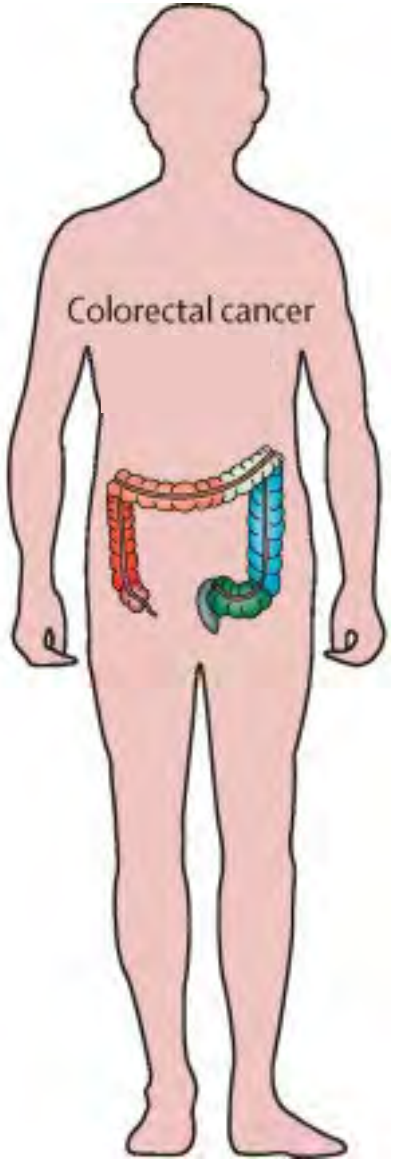
Risk Factors for CRC

Background

- Age
- Hereditary predisposition (Lynch, FAP)
- Positive family history

Co-morbidities

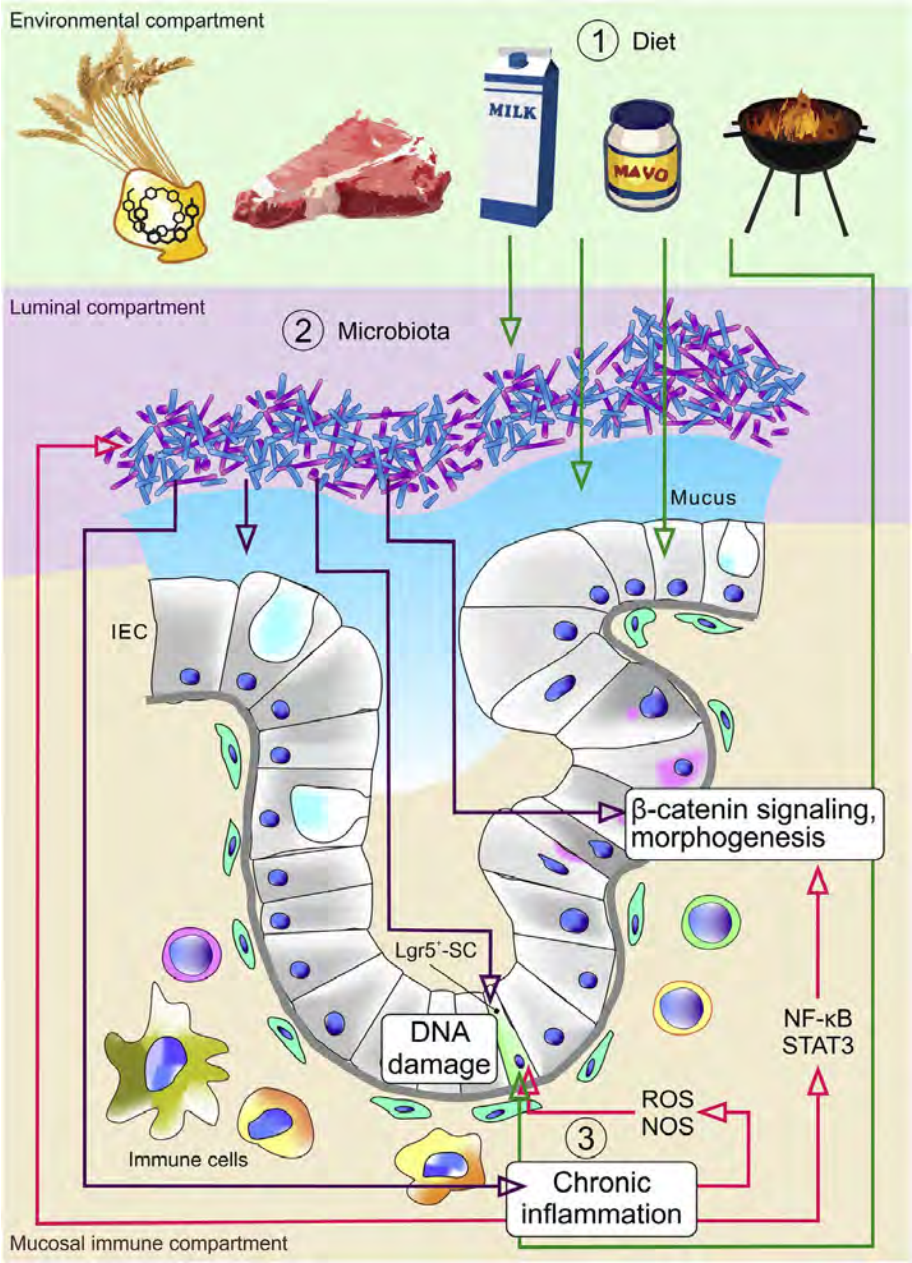
- Type II diabetes
- Inflammatory bowel disease (IBD)



Life-style

- Smoking
- Alcohol intake
- Body fat and obesity
- Ultra-processed food
- Low intake of fruits and vegetables

Multidimensional Framework of CRC Evolution



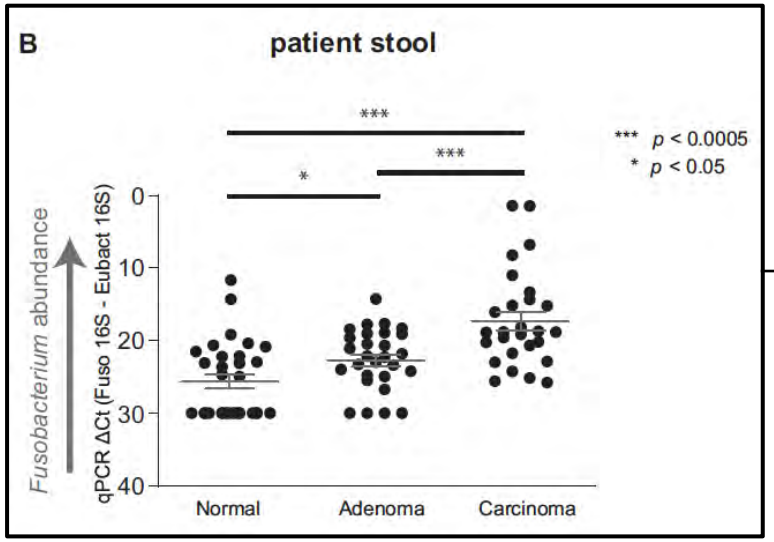
- Diet
- Microbiota
- Chronic inflammation

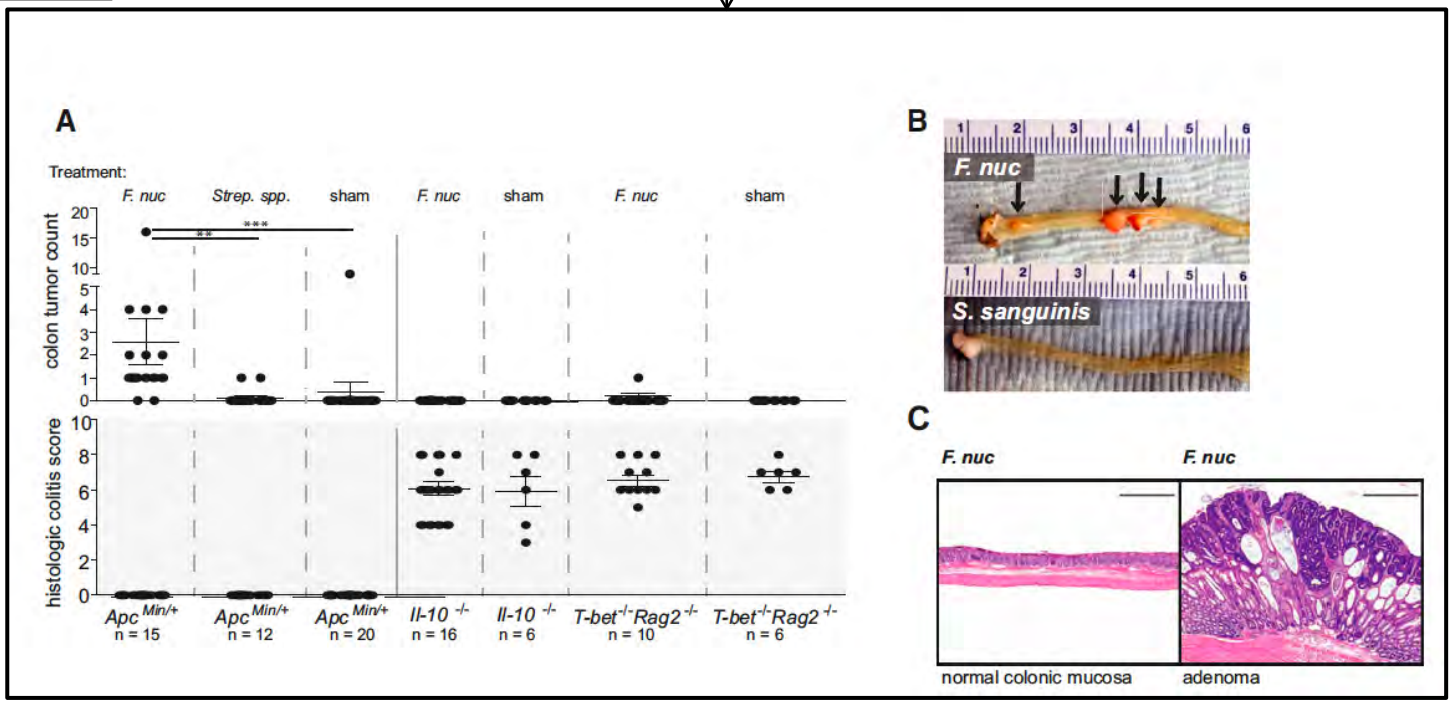
Tilg H et al., Cancer Cell 2018; 33(6):954-964

Role of Microbiota in CRC

- Effector of Colorectal Carcinogenesis
- Protector of Colorectal Carcinogenesis
- Diagnosis/screening tool for CRC
- Modulating response to treatment

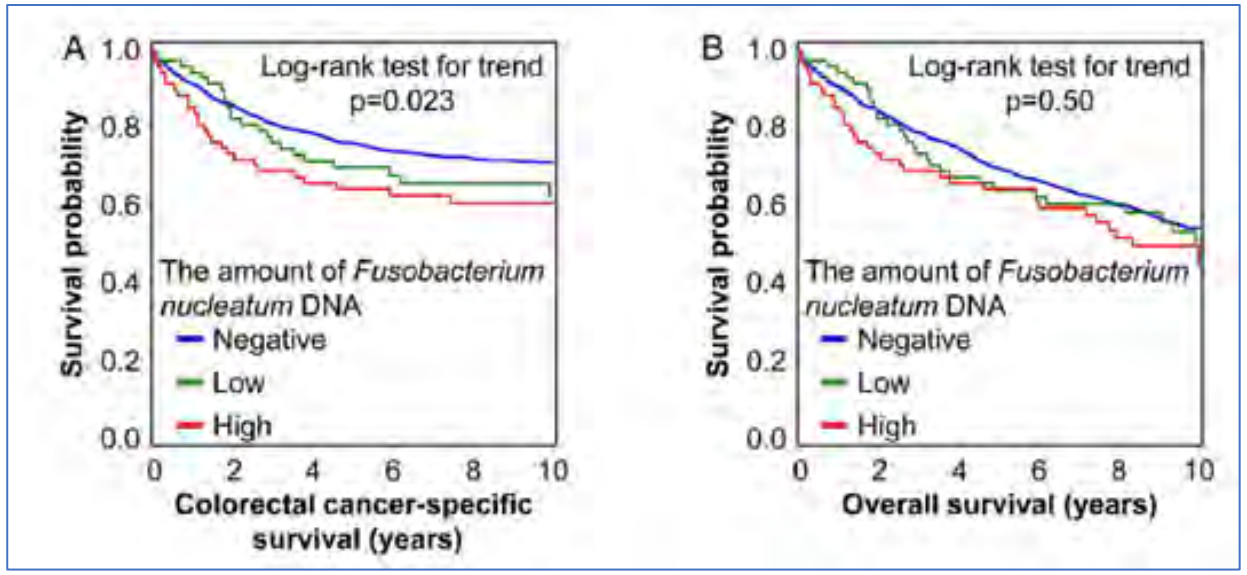
Fusobacterium Nucleatum and Colon Cancer



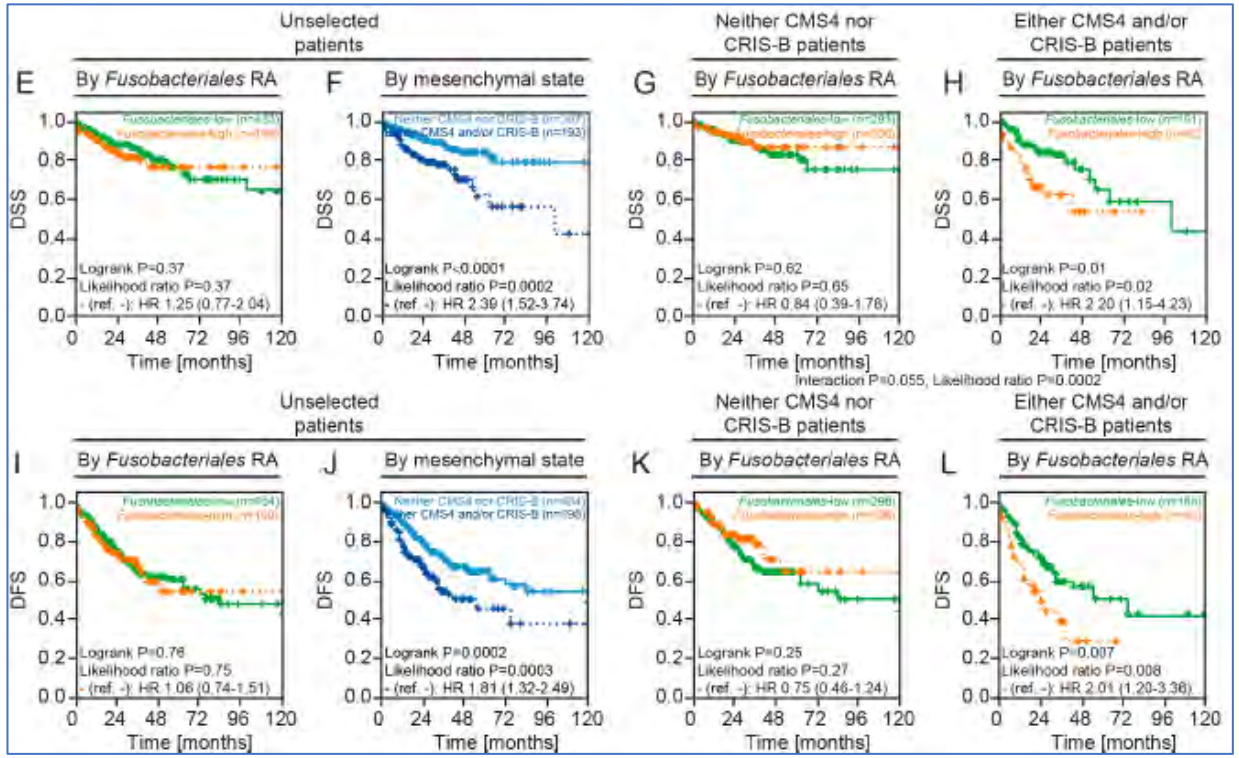


Kostic et al., Cell Host & Microbe 2013; 14 (2):207-15

Fusobacteria and CRC prognosis

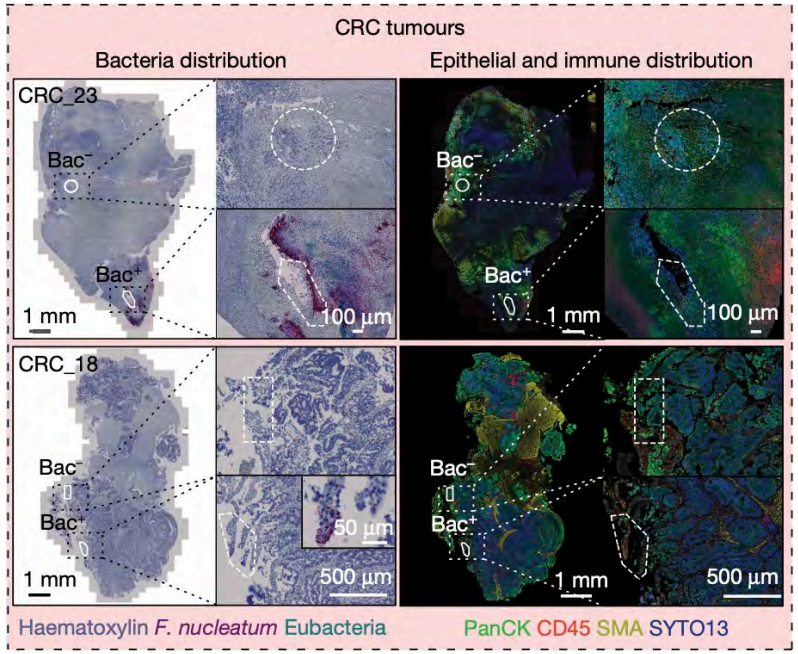


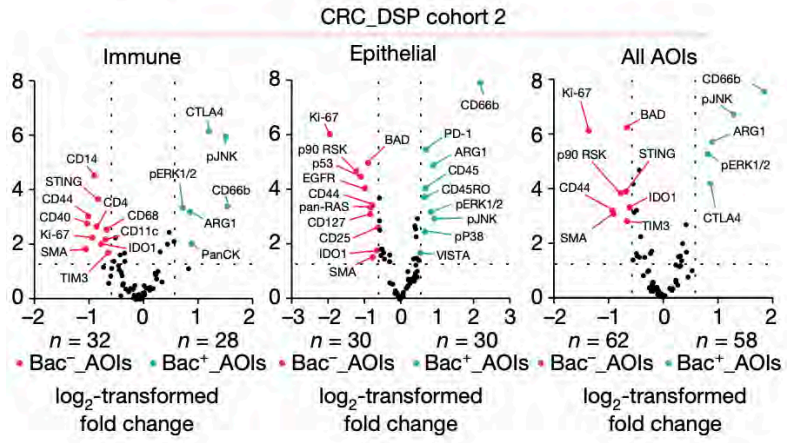
Mima K, et al. Gut 2016;65(12):1973-1980.

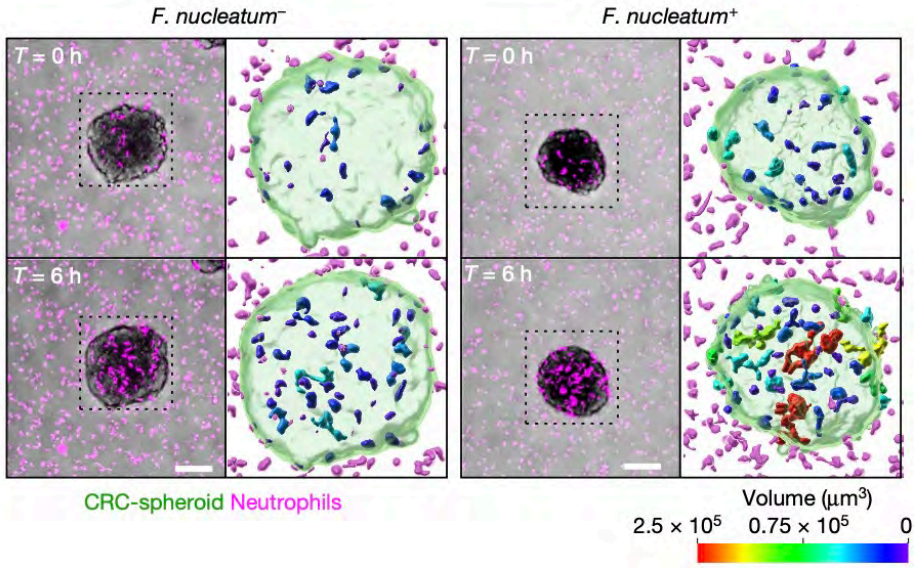


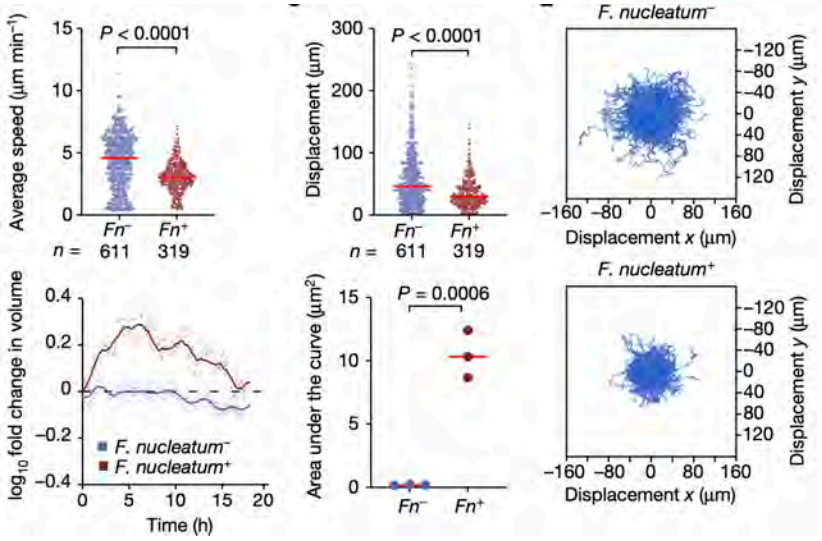
Salvucci M, Crawford N, Stott K, et al. Gut 2021

Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer



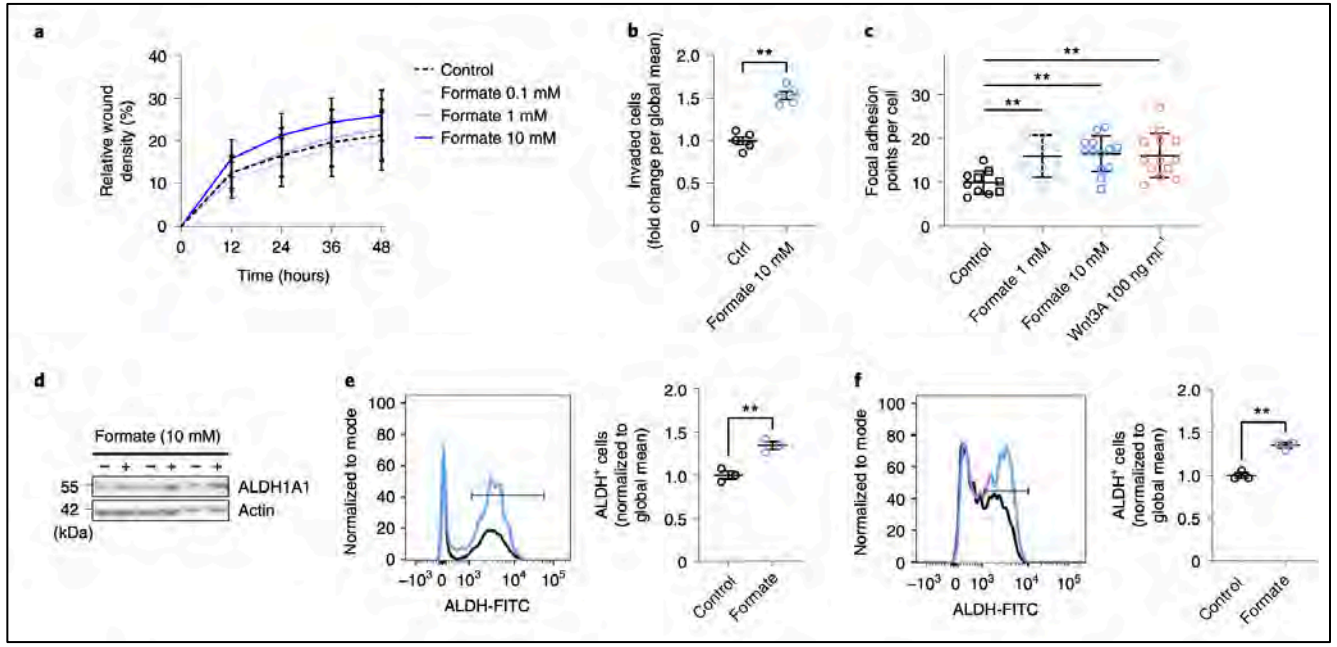


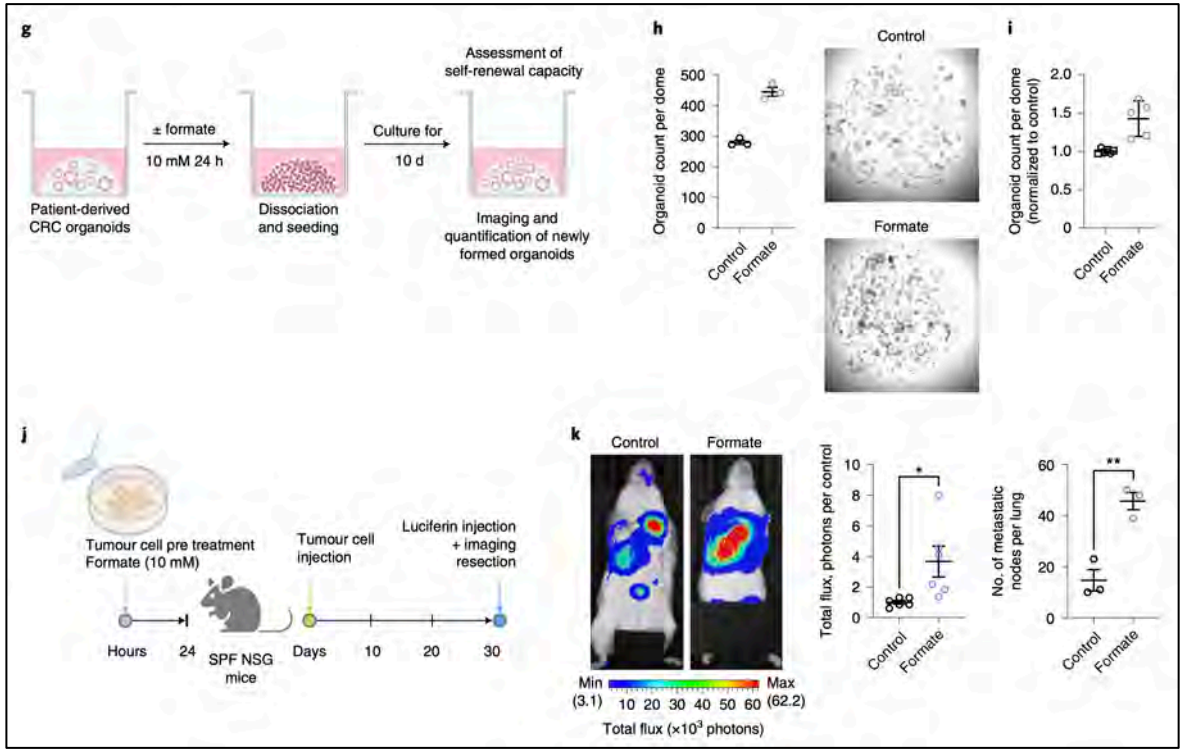




Galeano Niño et al. Nature. 2022;611(7937):810-817

The gut microbial metabolite formate exacerbates colorectal cancer progression





Ternes D et al. Nat Metab. 2022;4(4):458-475.

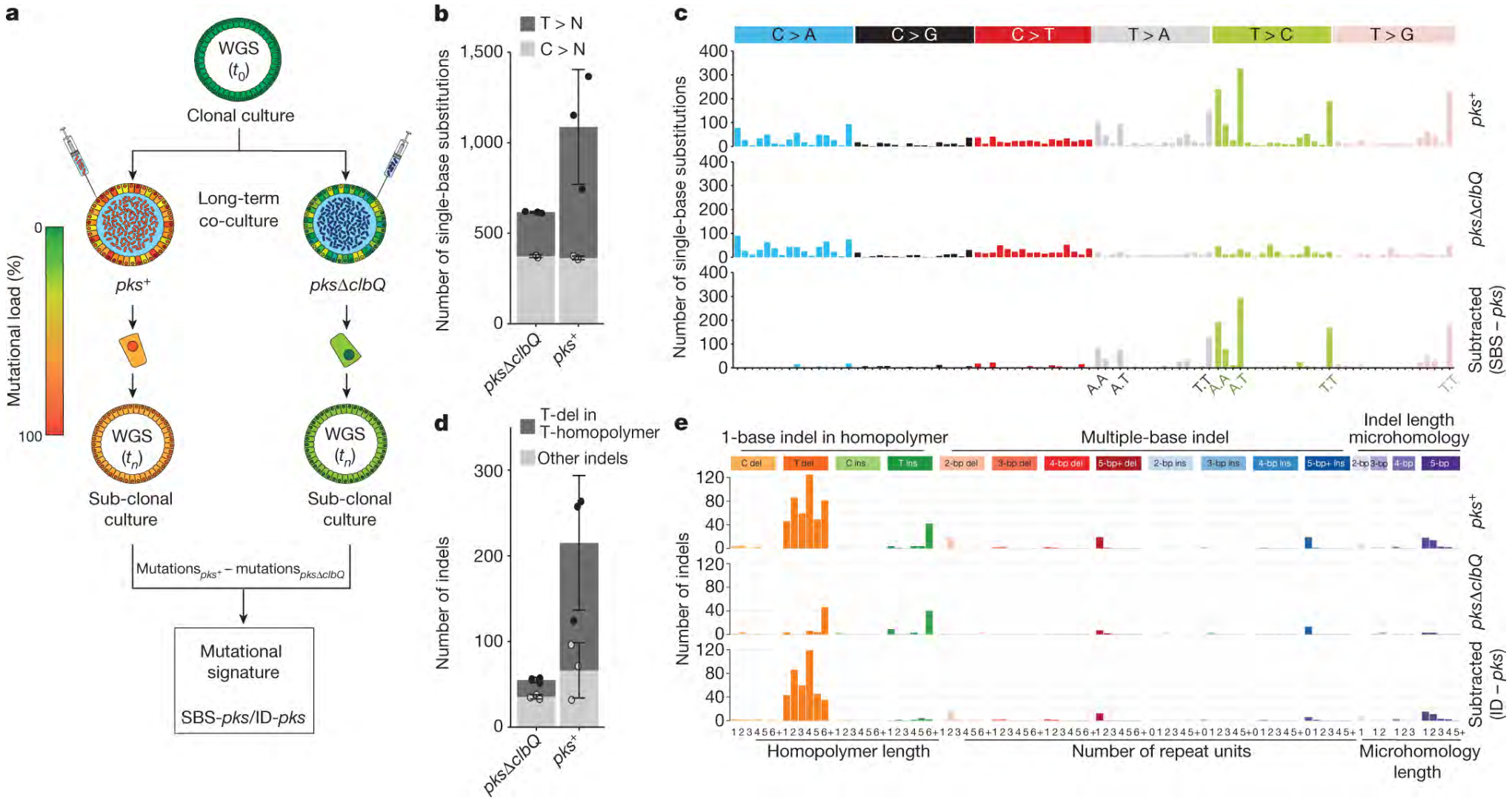
E. Coli and CRC
Evidence from pre-clinical studies

Table 2. Mouse models demonstrating cancer-inducing properties of *E. coli*.

Model	Colonization	Comparison	Result	Reference
Germfree IL^{-10} mice treated with carcinogen	Monocolonization results in tumours	Mouse strain NC101 vs. isogenic *pks*$^{-}$ mutant	More rapid and larger tumours in treatment group than in control	Arthur et al. (2012)
C57B/6J-$Apc^{-/+}$ mice (Min mice) treated with streptomycin	Colonization results in tumours	Human CRC strain 11G5 vs. K-12 strain MG1665	More colonic polyps in treatment group than in control	Bonnet et al. (2014)
Colon-loop colonization	Six-hour presence in closed loops results in DNA strand breaks	human strain SP15 vs. isogenic *pks*$^{-}$ mutant	3x more DNA strand breaks in colonocytes of treatment than in control	Cuevas-Ramos et al. (2010)
Subcutaneous injection of tumour cells	Tumour cells were infected with *E. coli* containing *pks* on pBAC	*E. coli* strain DH10B with pBAC containing *pks* from strain CCR20 vs. empty pBAC	Larger tumours in treatment than in control due to growth factors	Cougnoux et al. (2014)
Xenografts of HC116 cells	Xenograft cells were infected with *E. coli*	Unspecified *E. coli* strain with or without *pks*	Tumors are suppressed by high MOI, stimulated by low MOI, only with pks^{+} strain, *via* cytokines	Dalmasso et al. (2014)

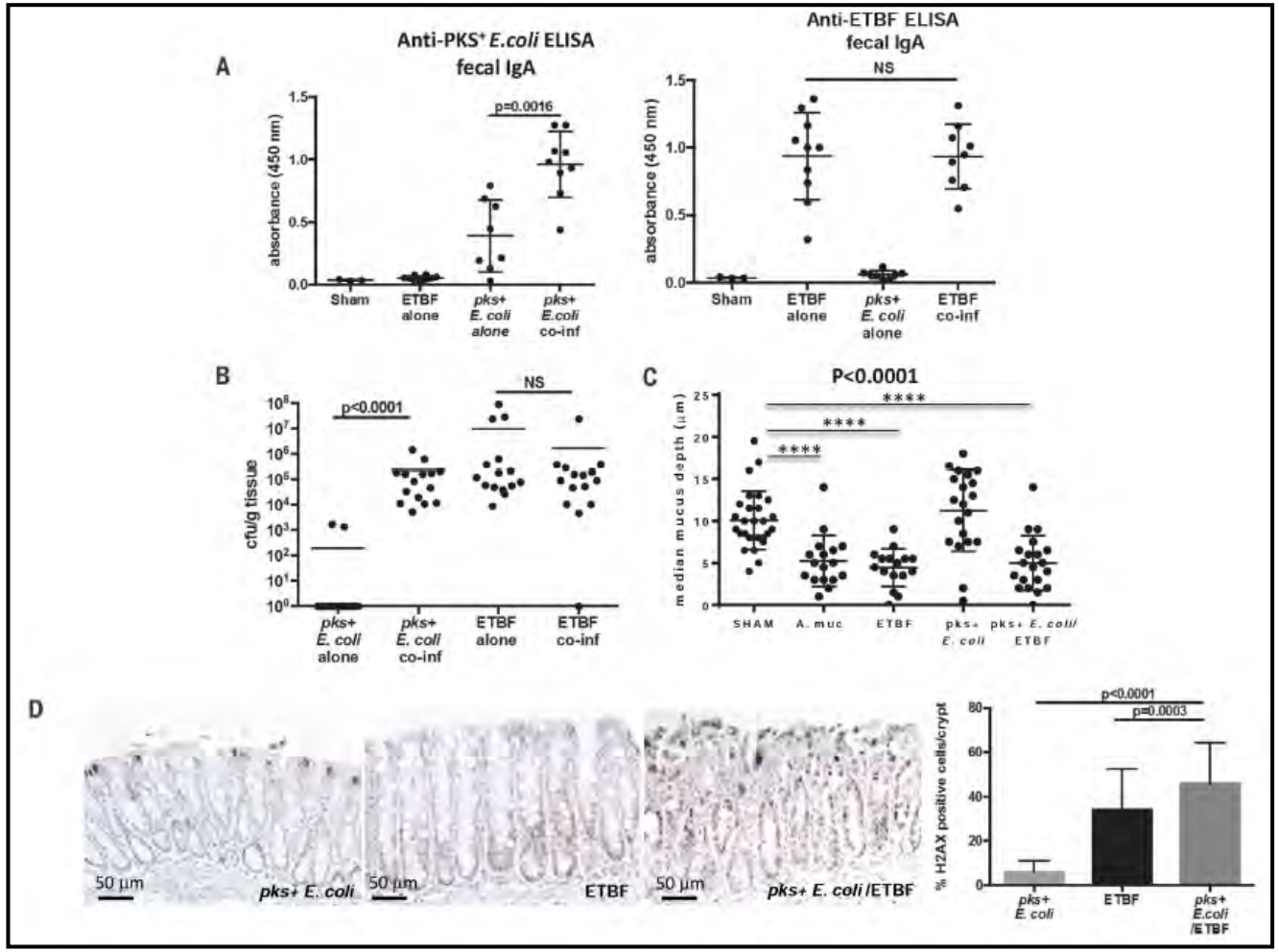
Wassenaar T. M., Critical Reviews in Microbiology 2018; 44(5):619-632

Mutational signature in colorectal cancer caused by genotoxic *pks*⁺ *E. coli*



Pleguezuelos-Manzano C et a. Nature 2020; 580: 269–273

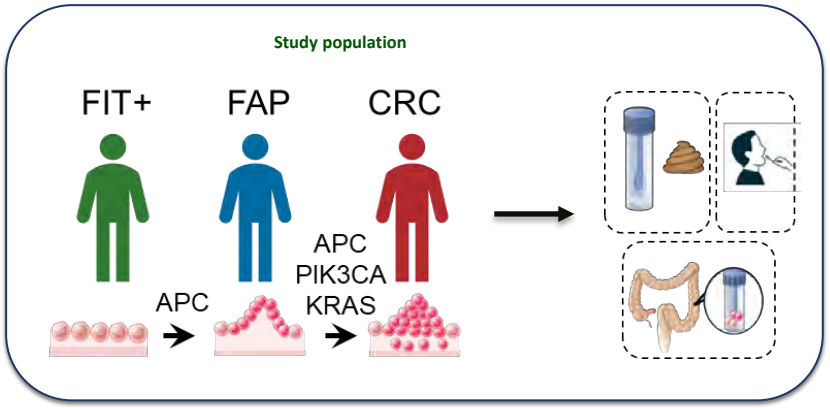
Patients with FAP disease harbor colonic biofilms containing tumorigenic bacteria



ETBF: Bacteroides fragilis
PKS+ Ecoli: polyketide synthase gene-expressing Escherichia coli

Dejea et al. Science 2018; 359:592-597

A multitarget framework for WNT signalling-driven colorectal cancer prevention and early detection



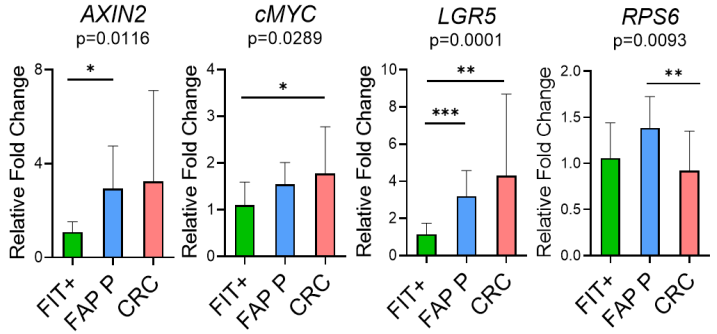


Figure 1. RNA expression levels of Wnt/β-catenin and PI3K/mTOR in FIT+, FAP and CRC tissues.

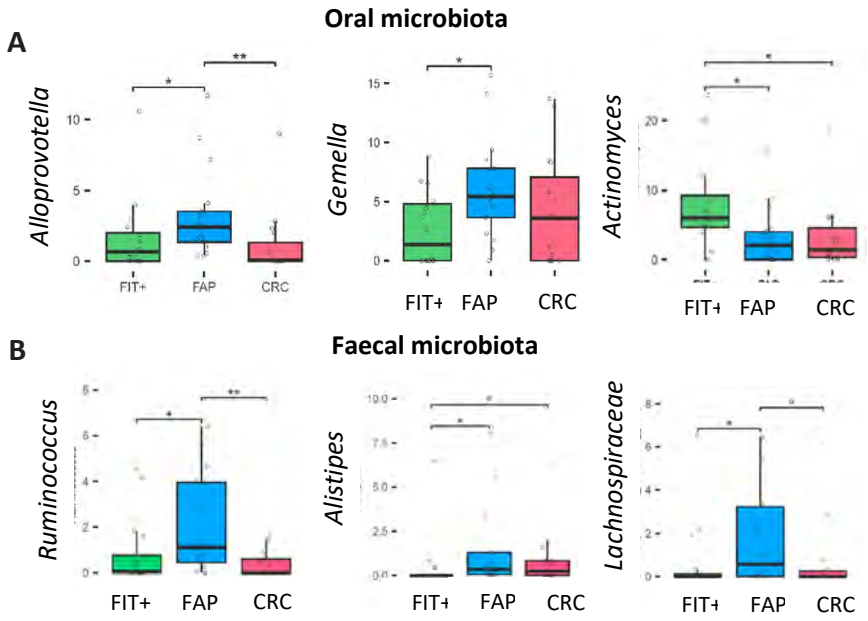


Figure 3. Oral and faecal microbiota in FIT+, FAP and CRC tissues.

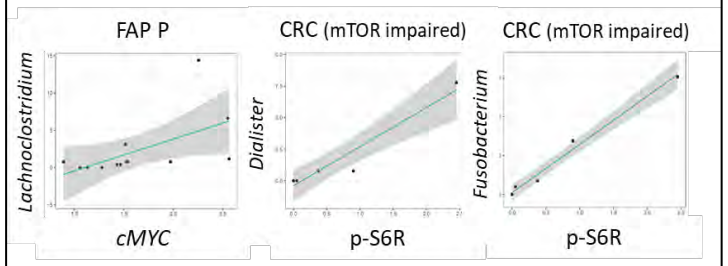


Figure 4. Correlation plot between RNA/protein levels and mucosa-associated bacteria in FAP P e CRC tissues mutated in *PIK3CA* and/or *KRAS* gene.

Di Paola, Alquati C, Conti G et al. UEG Week 2023

Global Burden of EoCRC 2000-2019

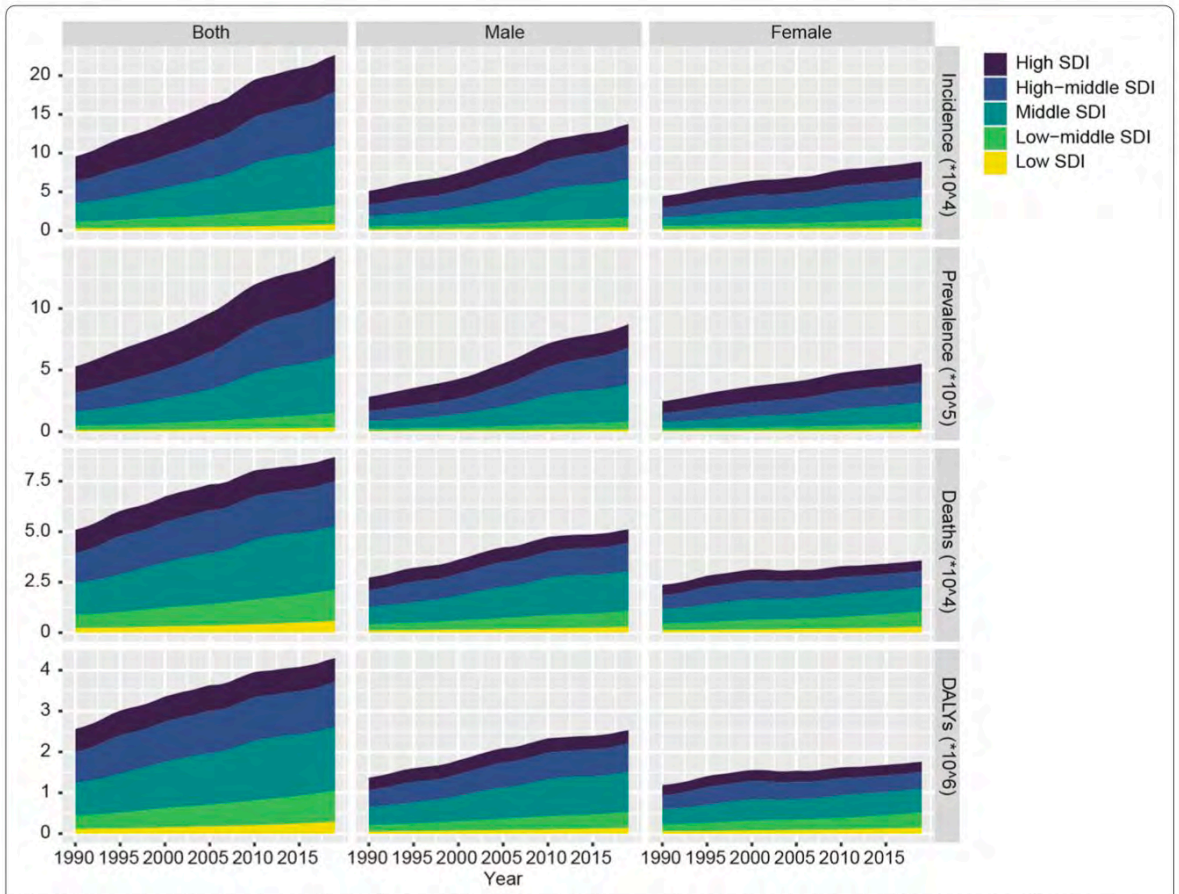


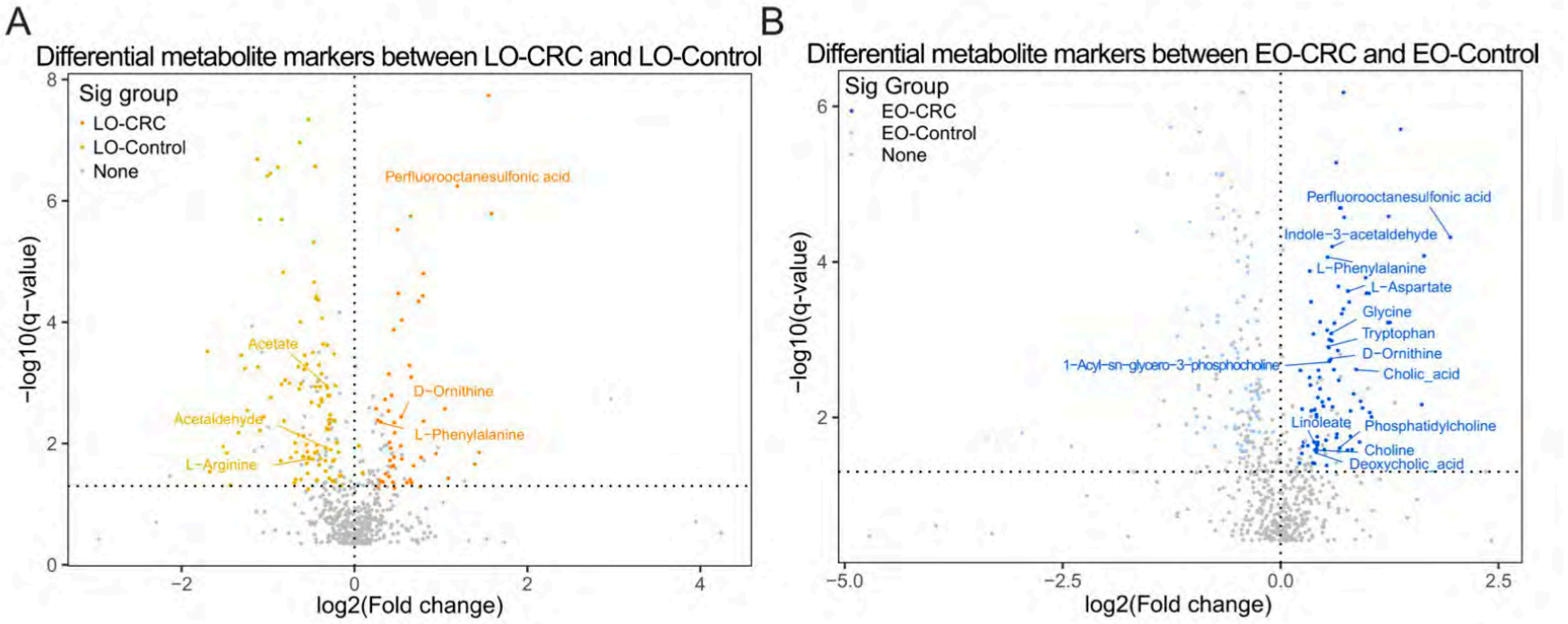
Fig. 5 The number of incidence, prevalence, deaths and DALYs due to early-onset colorectal cancer grouped by SDI quintiles for different sexes from 1990 to 2019. SDI: Socio-demographic index; DALYs, Disability-Adjusted Life Years

SEER DATA 2019: Age-Adjusted incidence 1988-2015 → from 7.9/100.000 to 12.9/100.000 an increase of 63%

EO-CRC now accounts for 10% of all CRC cases in the US

Pan *et al. BMC Public Health (2022) 22:1896*

Integrated metagenomic and metabolomic analysis reveals distinct gut-microbiome- derived phenotypes in early-onset colorectal cancer



LO-CRC → Fusobacterium nucleatum enrichment and short-chain fatty acid depletion characterise, including reduced microbial GABA biosynthesis and a shift in acetate/acetaldehyde metabolism towards acetyl-CoA production.

EO-CRC Enriched Flavonifractor plauti and elevated red-meat intake-related species, choline metabolites, KEGG orthology (KO) pldB and cbh gene axis may be potential tumour stimulators

Kong C et al. Gut. 2023;72(6):1129-1142.

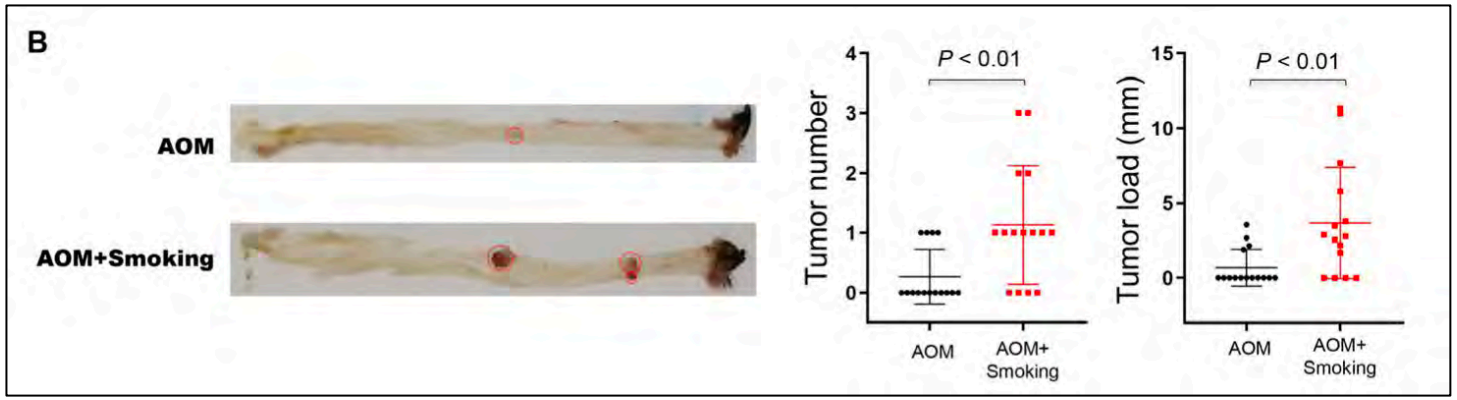
The link between Environmental Factors and CRC

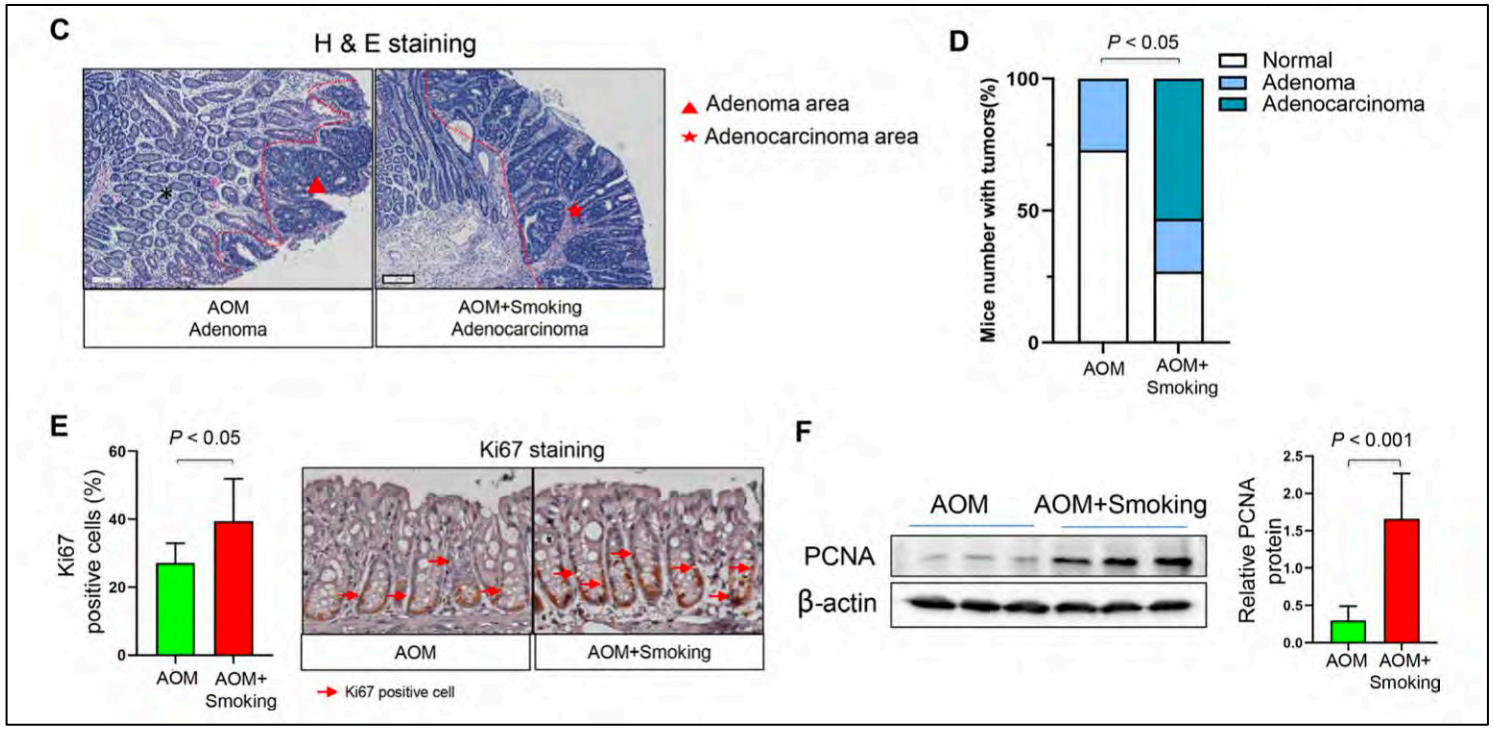
Table 1. Summary of Evidence and Potential Mechanisms Underlying the Relationship Between Environmental Factors, Gut Microbiota, and Colorectal Cancer

Risk factor	Estimated association with colorectal cancer (RR [95% CI])[a]	Associated microbes	Potential microbiota-related mechanisms
Overweight and obesity	1.05 (1.03–1.07) per 5 kg/m^2 increase in body mass index	↓Bacteroidetes/Firmicutes ratio	Increased LPS levels; epigenetic changes in the colonic epithelial cells; increased production of DNA-damaging bile acids
Physical activity	0.80 (0.72–0.88) comparing the highest with the lowest levels	↑Bacteroidetes/Firmicutes ratio; ↑SCFAs-producing bacteria	Increased production of SCFAs; changes in the metabolism of the microbiota
Smoking	1.26 (1.11–1.43) for current smokers and 1.18 (1.09–1.27) for former smokers, compared with never smokers[b]	↓Diversity ↑Firmicutes, Actinobacteria; ↓Bacteroidetes, Proteobacteria	Altered mucin composition of the mucus layer and increased inflammatory response
Diet			
Western dietary pattern	1.40 (1.26–1.56) comparing the highest with the lowest category of the Western diet score[c]	↑*Bacteroides*, *Escherichia*, and *Acinetobacter*; ↓*Prevotella*	Lower production of SCFAs from fiber deficiency
Fiber	0.93 (0.87–1.00) per 10 g/day increase in dietary consumption	↑SCFAs-producing bacteria	Epigenetic regulation and immunomodulatory benefits of SCFAs
Red and processed meat	1.12 (1.04–1.21) per 100 g/day increase in consumption	↑Mucin-degrading bacteria (eg, *Akkermansia muciniphila*)	Microbiota-dependent barrier dysfunction induced by heme iron; increased production of secondary bile acids and hydrogen sulfide by microbiota associated with high red meat consumption
Marine omega-3 fatty acid	0.76 (0.59–0.97) comparing the highest with the lowest category of biospecimen composition of marine omega-3 fatty acids[d]	↑*Lactobacillus* and *Bifidobacterium*; ↓ *Fusobacterium nucleatum* and LPS-producing bacteria	Preserved intestinal immune homeostasis caused by increased abundance of immune-protective bacteria and reduced abundance of proinflammatory bacteria, and increased production of SCFAs and lipid mediators
Alcohol	1.07 (1.05–1.08) per 10 g/day increase in consumption	Dysbiosis; ↓Bacteroidetes, Firmicutes, and butyrate-producing bacteria; ↑Proteobacteria and Actinobacteria	Intestinal bacterial overgrowth and hyperpermeability; reduced production of SCFAs; bacterial production of acetaldehyde from ethanol
Dairy products	0.87 (0.83–0.90) per 400 g/day increase in consumption	↓*Bilophila wadsworthia*	Increased production of SCFAs and decreased abundance of proinflammatory pathobionts
Vitamin D	0.92 (0.85–1.00) per 30 nmol/L increase in circulating vitamin D	Variants in the vitamin D receptor gene are among the most significant loci associated with gut microbial composition	Unclear
Metformin	0.80 (0.64–1.00) comparing users to nonusers[e]	↑*Escherichia* and *Bifidobacterium*; ↓*Intestinibacter*	Increased production of SCFAs

Song M. and Chan A.T.; CGH 2019; 17: 275-289

Cigarette smoke promotes colorectal cancer through modulation of gut microbiota and related metabolites



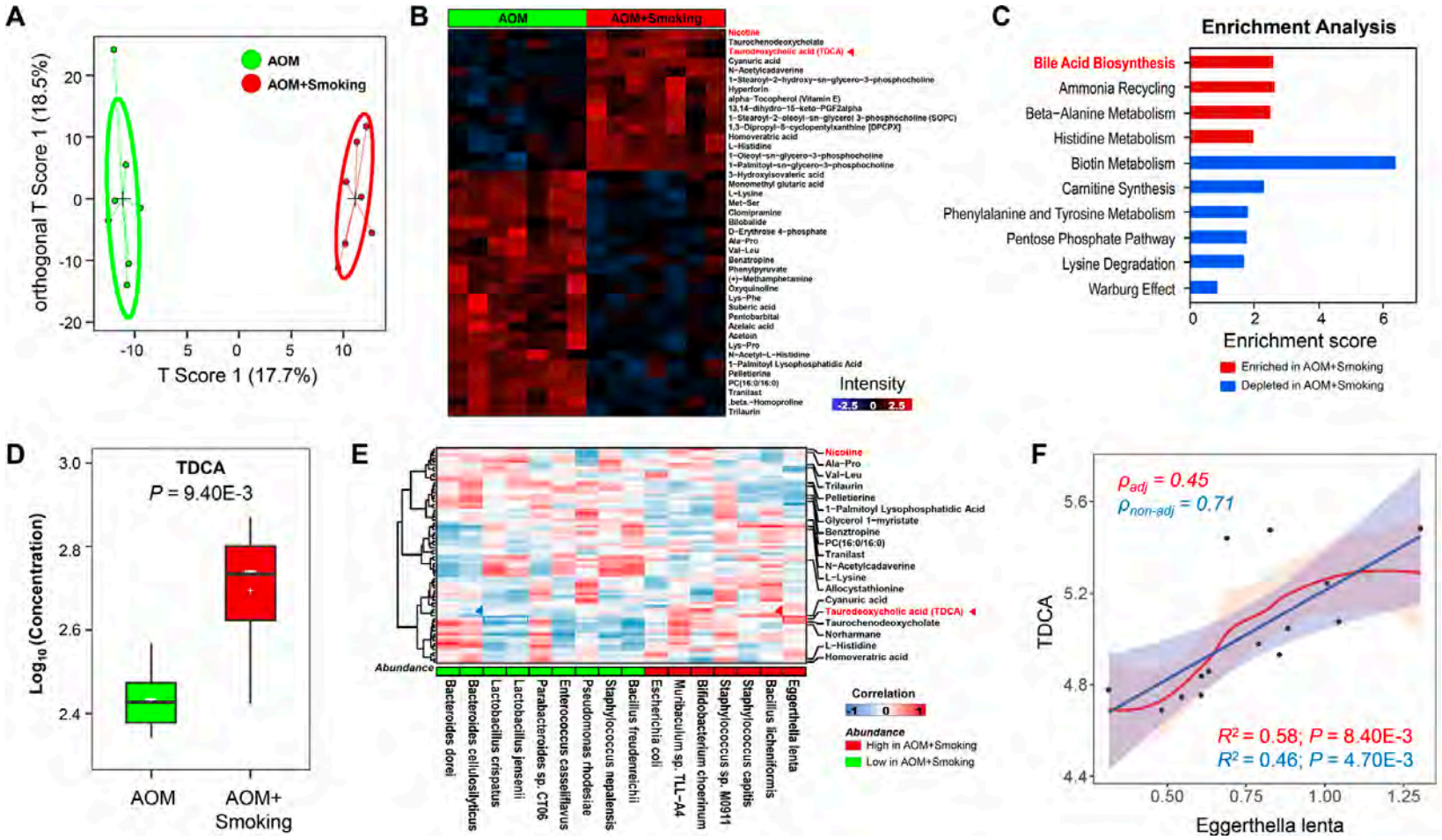


Enrichment of Eggerthella Lenta

Depletion of Parabacteroides disastonis

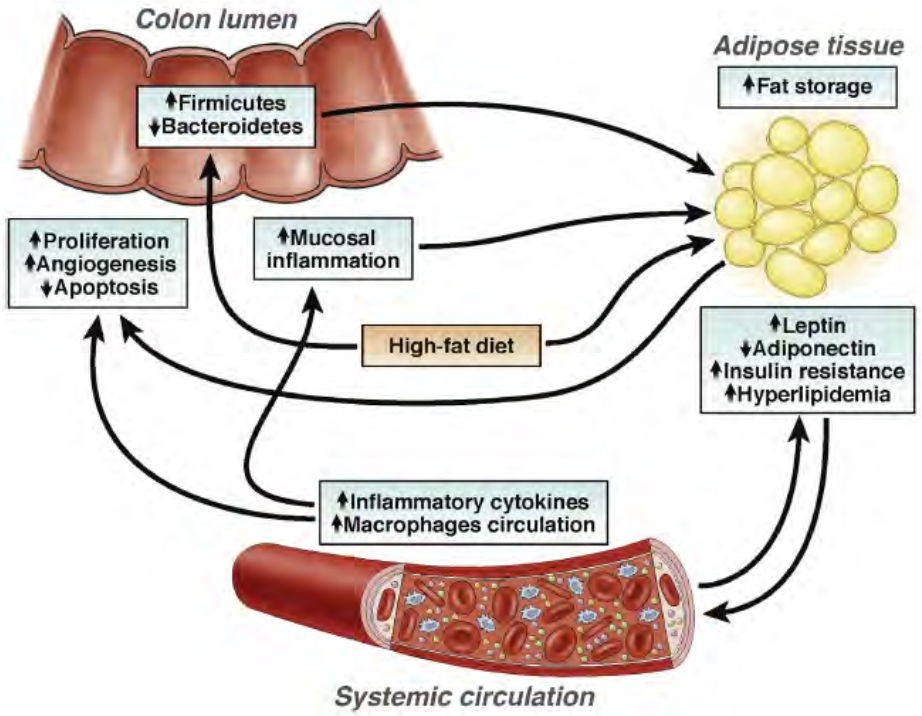
Bai X, Wei H, Liu W, et al. Gut 2022;71:2439–2450

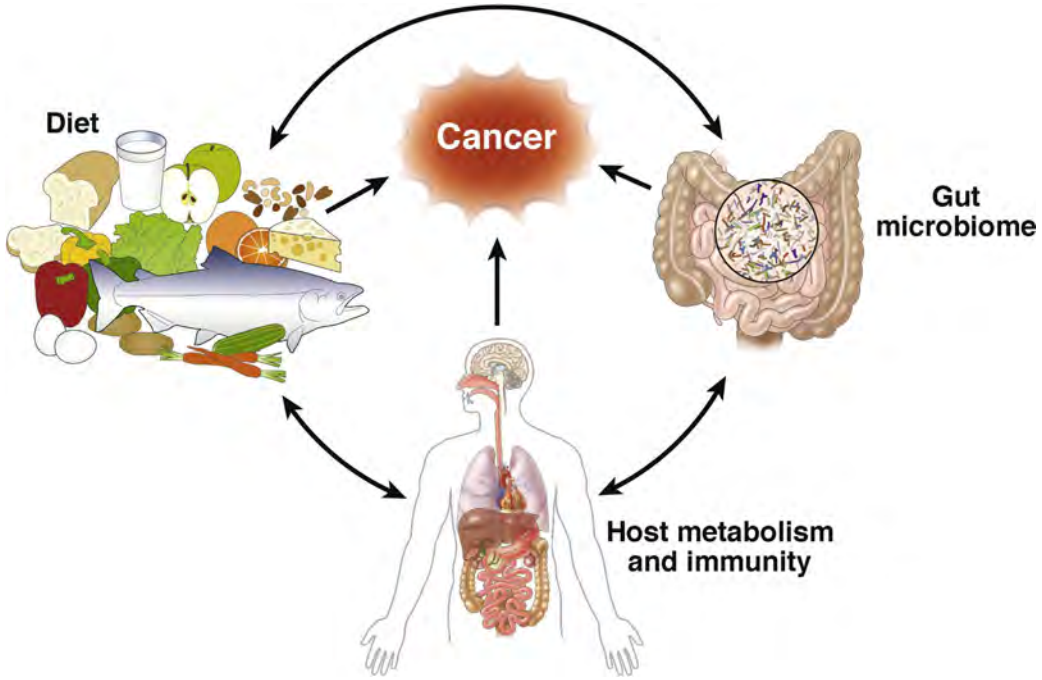
Cigarette smoke promotes colorectal cancer through modulation of gut microbiota and related metabolites



Bai X, Wei H, Liu W, et al. Gut 2022;71:2439–2450.

The link between obesity, western diet and CRC

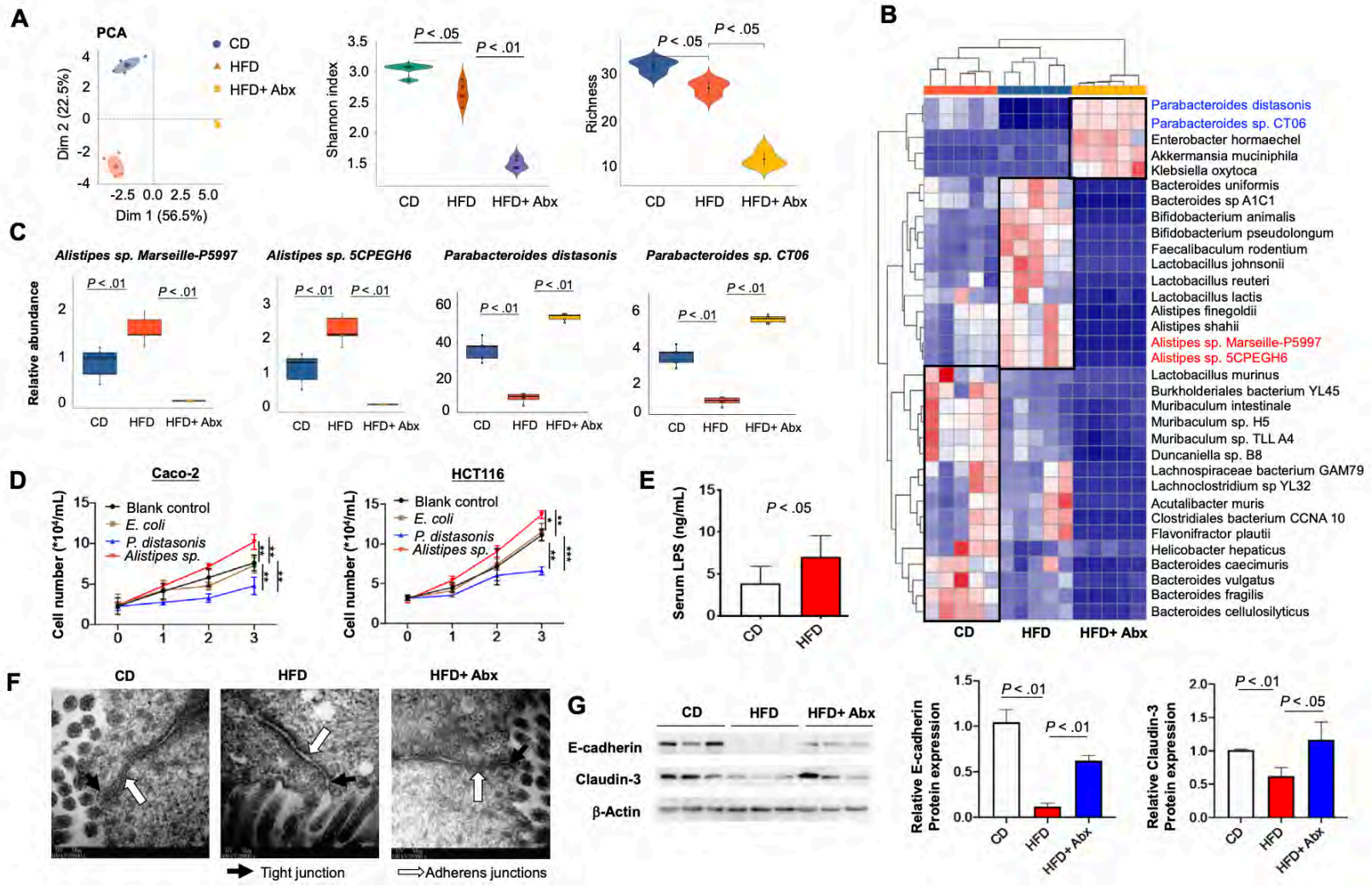




Alemán J.O., Eusebi LH, Ricciardiello L et al. Gastroenterology 2014 146 (2): 357-373

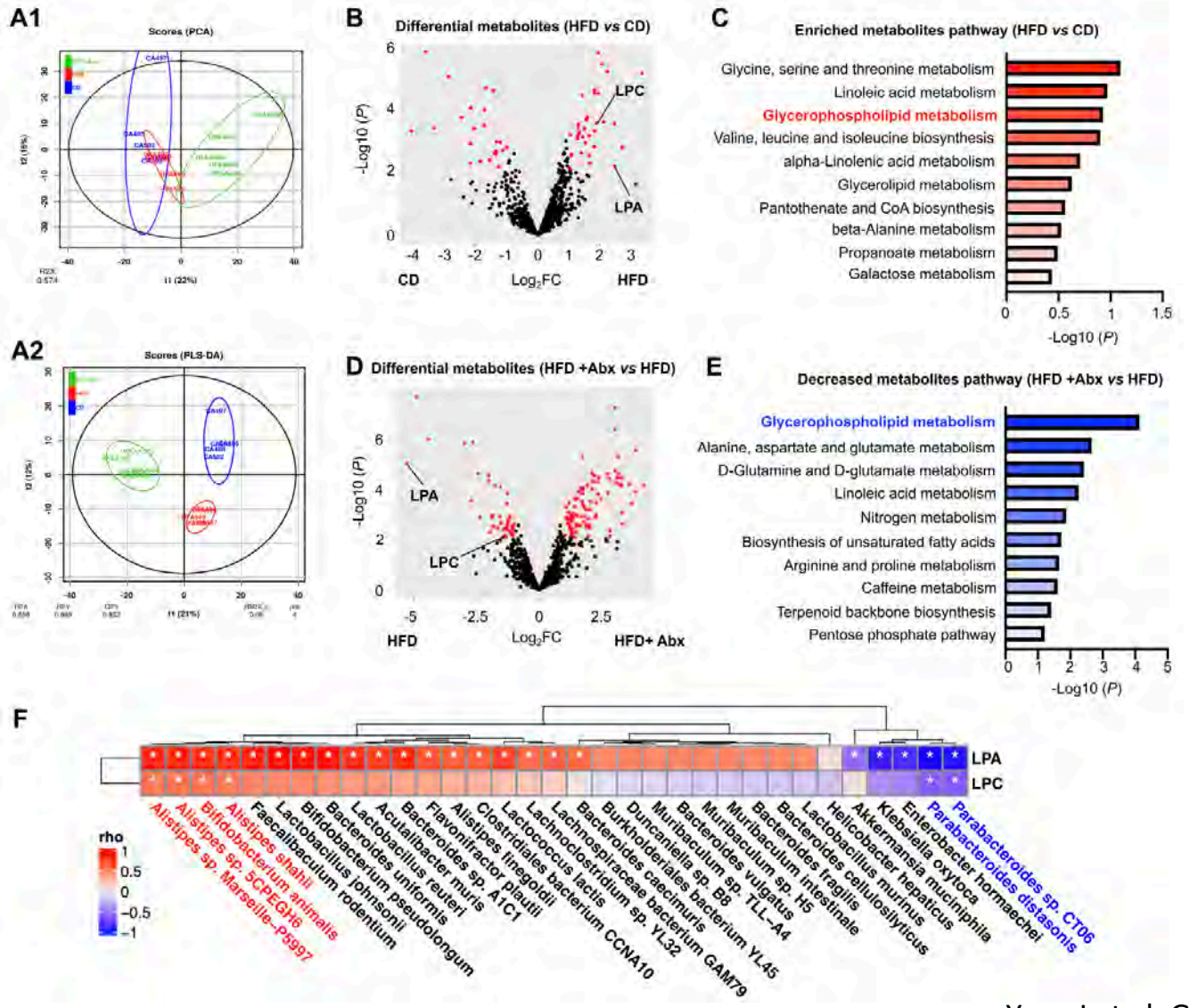
Song M. and Chan A.T.; CGH 2019; 17: 275-289

High-Fat Diet Promotes Colorectal Tumorigenesis Through Modulating Gut Microbiota and Metabolites



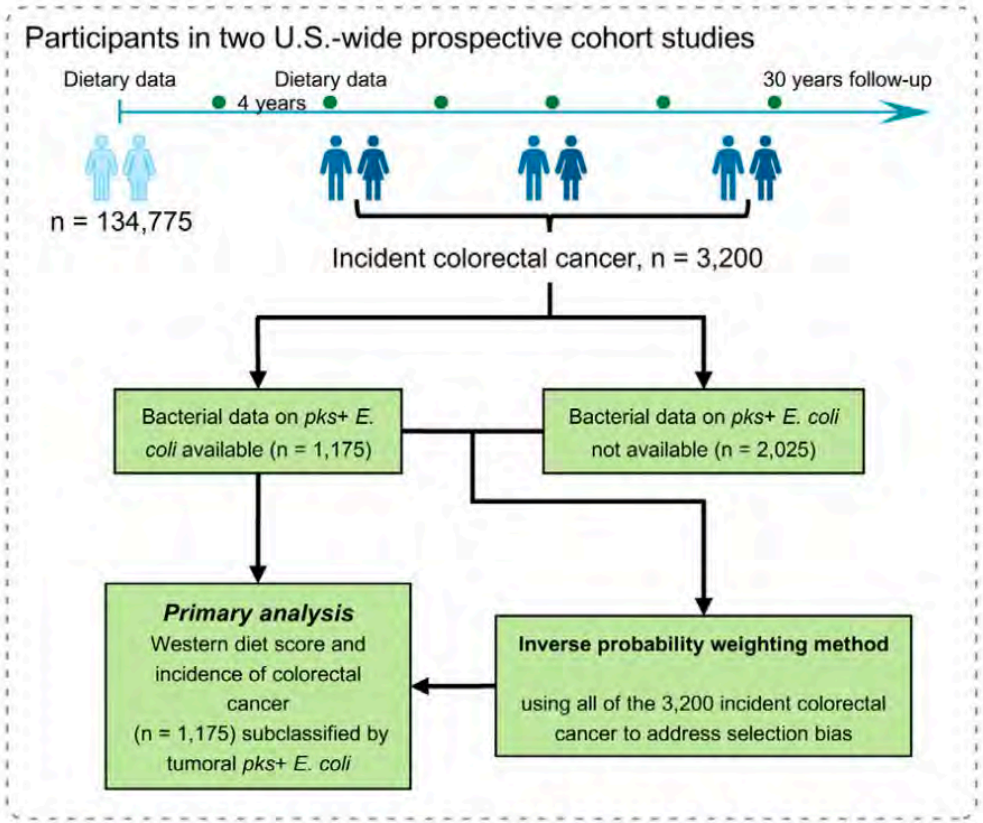
Yang J et al. Gastroenterology 2022;162:135–149

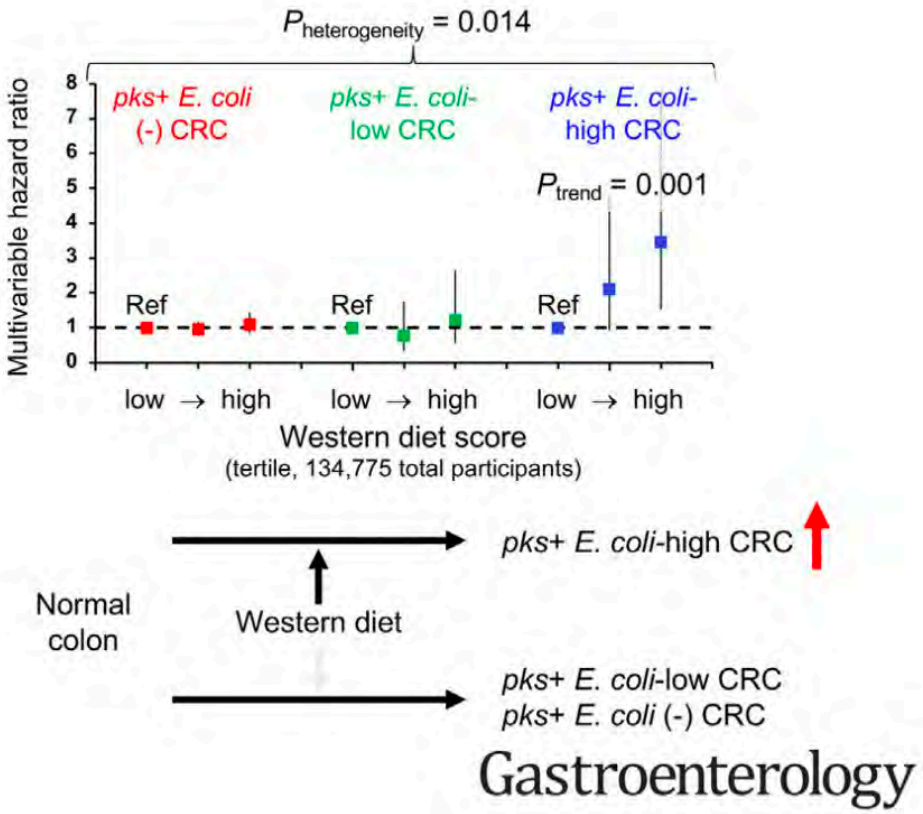
High-Fat Diet Promotes Colorectal Tumorigenesis Through Modulating Gut Microbiota and Metabolites



Yang J et al. Gastroenterology 2022;162:135–149

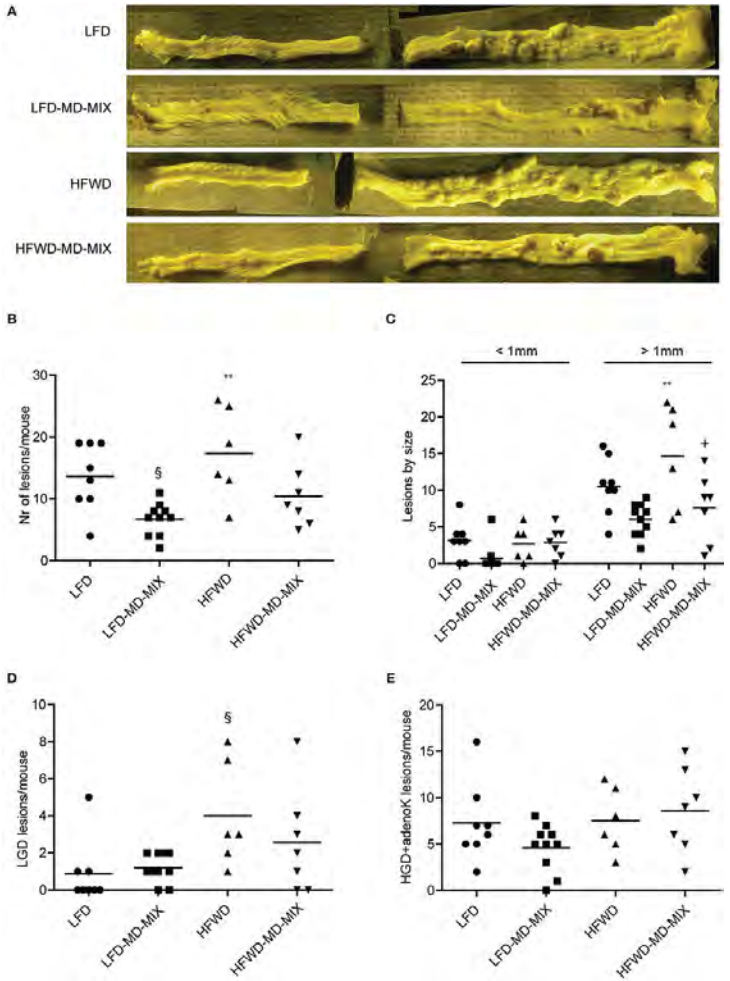
Western-Style Diet, pks Island-Carrying Escherichia coli, and Colorectal Cancer: Analyses From Two Large Prospective Cohort Studies

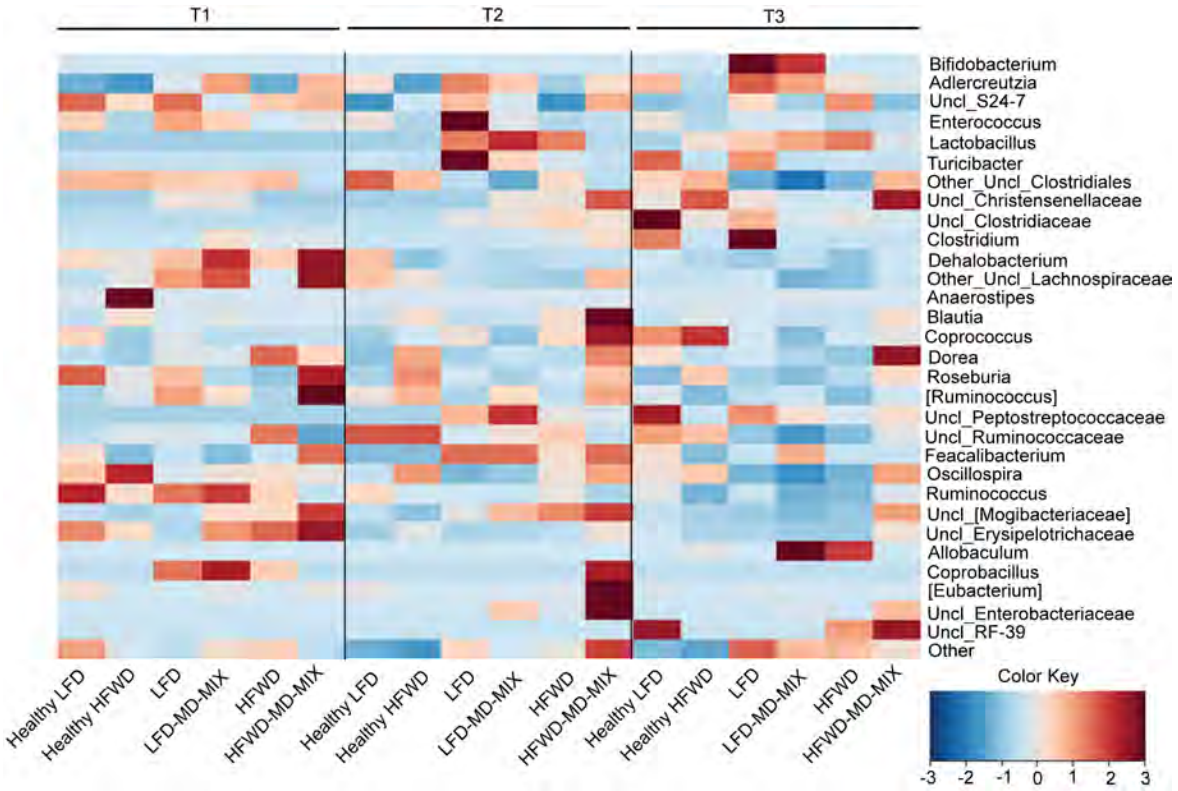




Gastroenterology 2022;163:862–874

Mediterranean Diet affects microbiota in AOM-Induced CRC

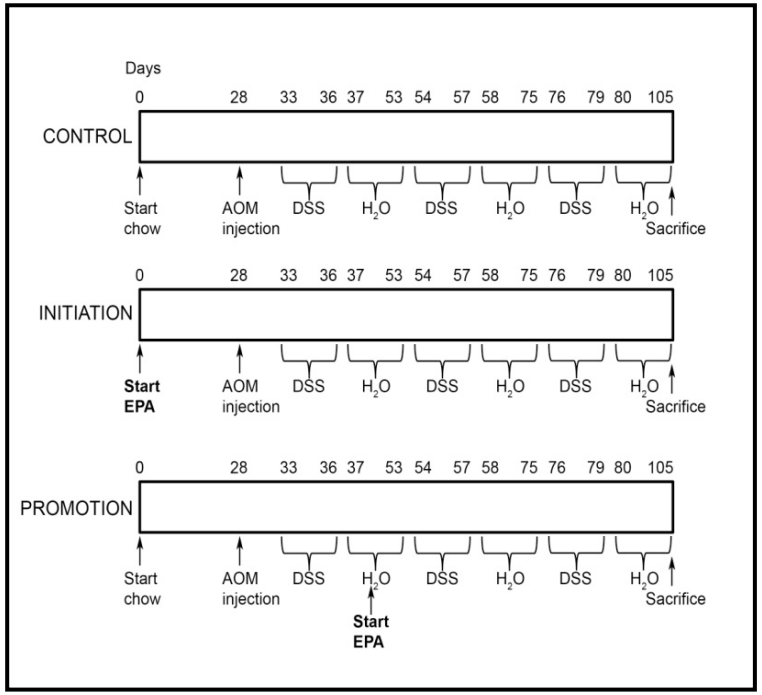


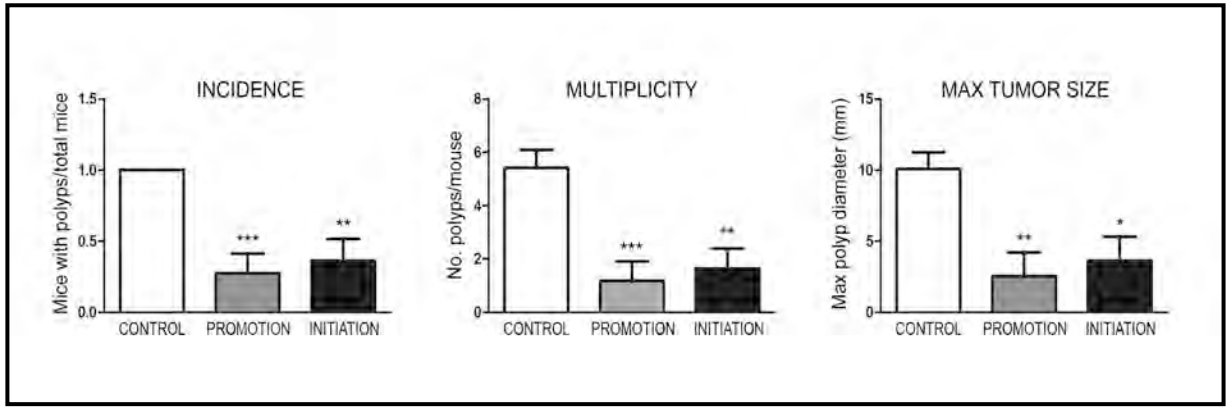


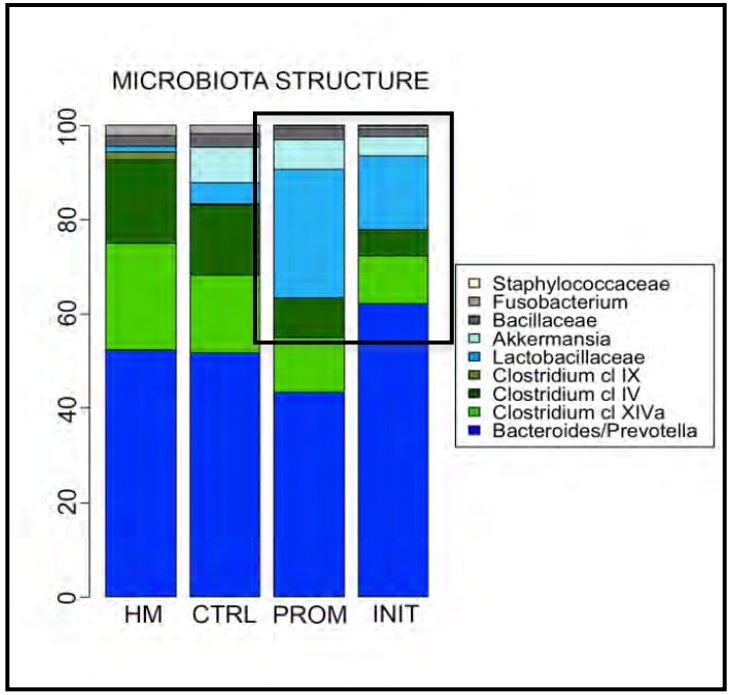
AOM → increase in lactobacillaceae in LFD e HFWD → MD-MIX in HFWD only
AOM-HFWD-MD MIX → Firmicutes profile Like Non-AOM-HFWD

Piazzi G., Prossomariti A. et al. Frontiers in Oncology 2019

EPA-FFA, microbiota and CAC

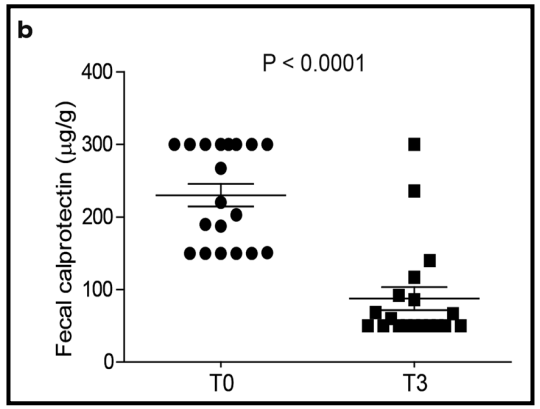


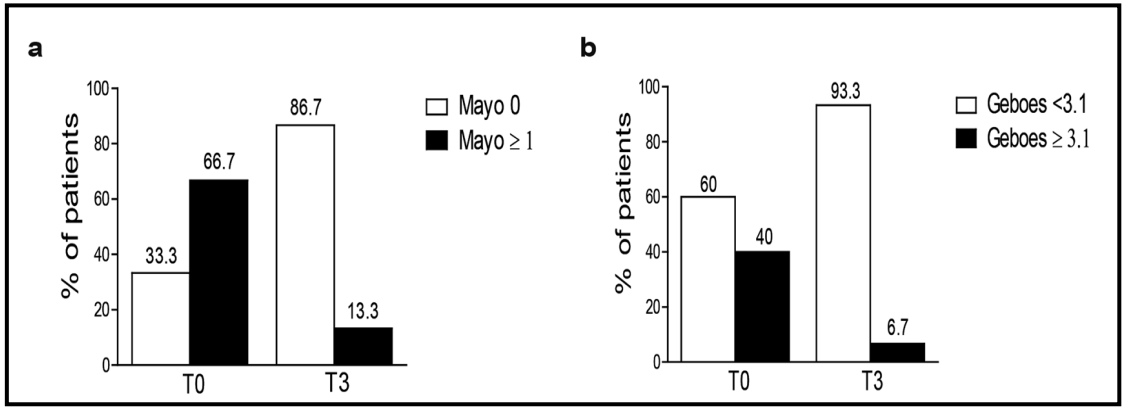


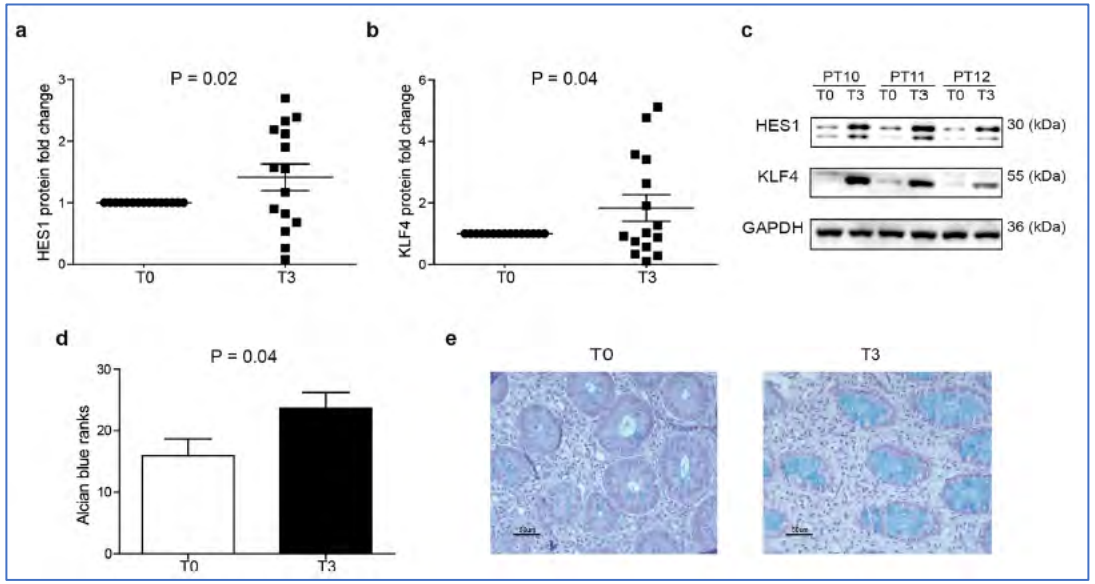


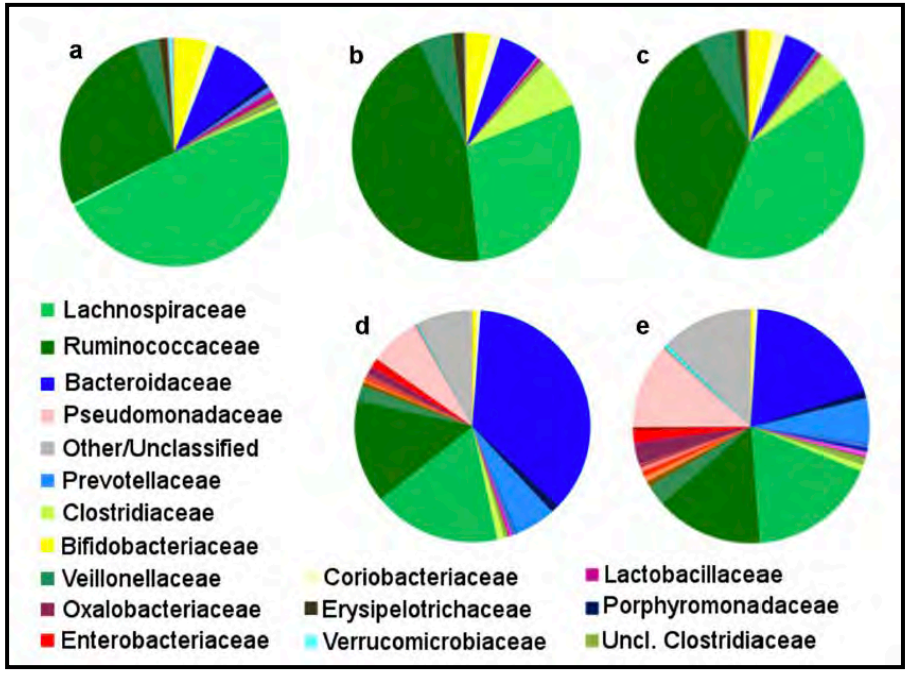
Piazzi G. et al. Intenational Journal of Cancer 2014; 135:2004-2013

EPA-FFA affects microbiota in high-risk IBD



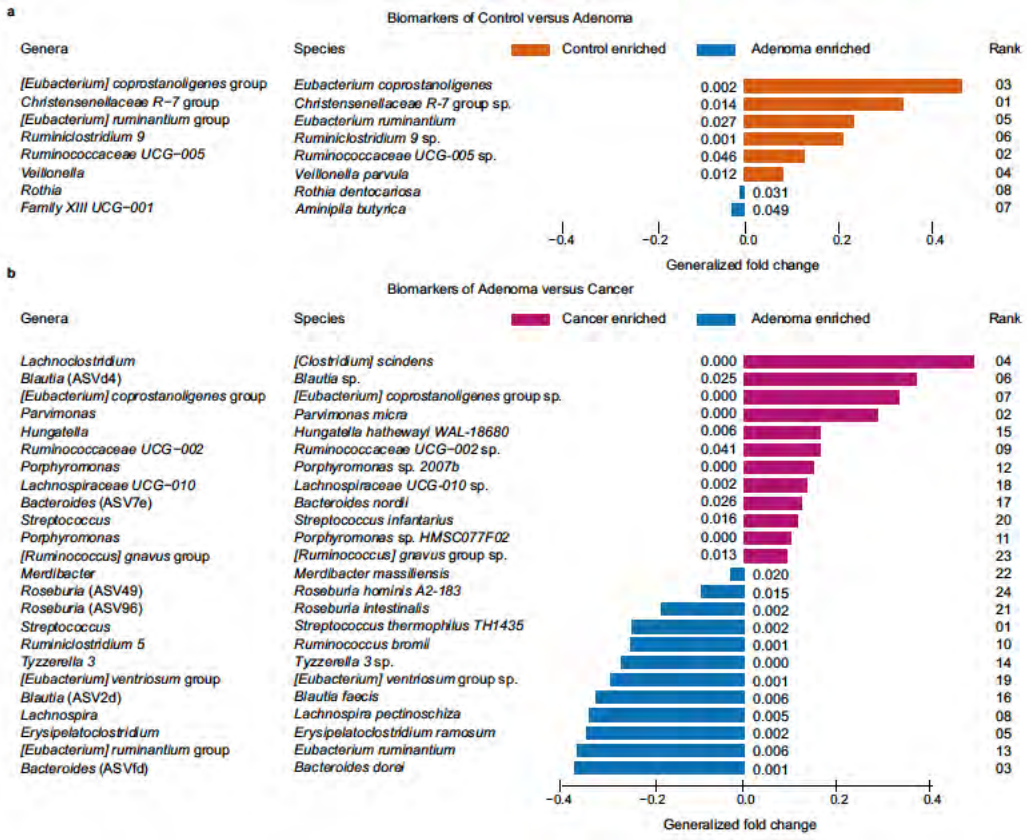


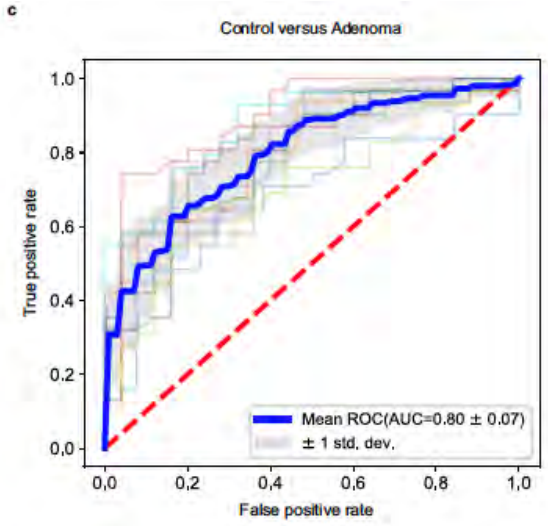


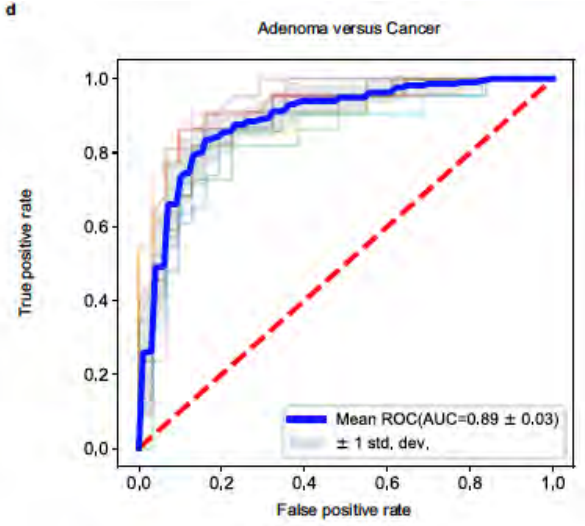


Prossomariti A., Scaioli E. et al. Sci Rep 2017; 7:7458

Microbial markers across populations in early detection of colorectal cancer







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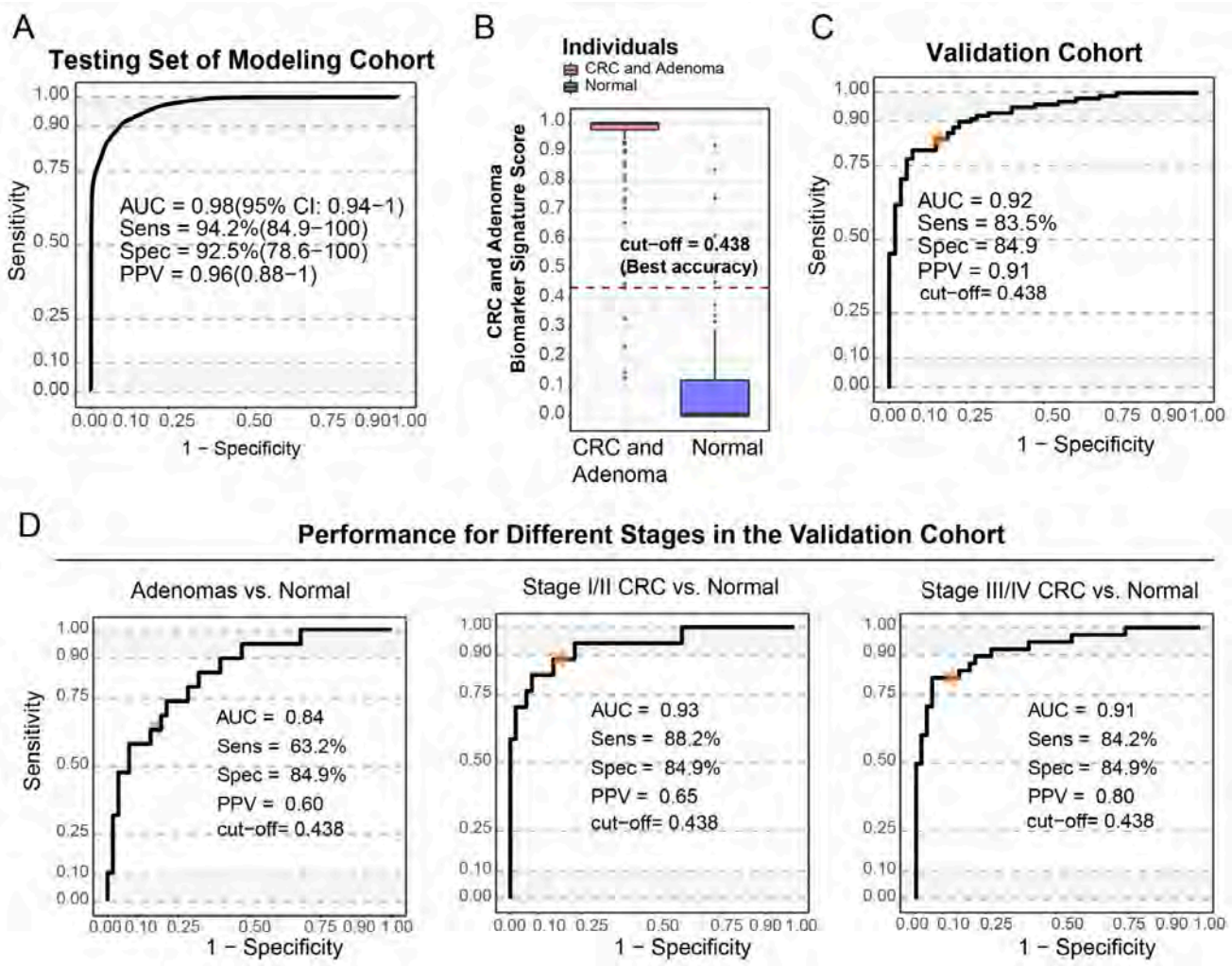

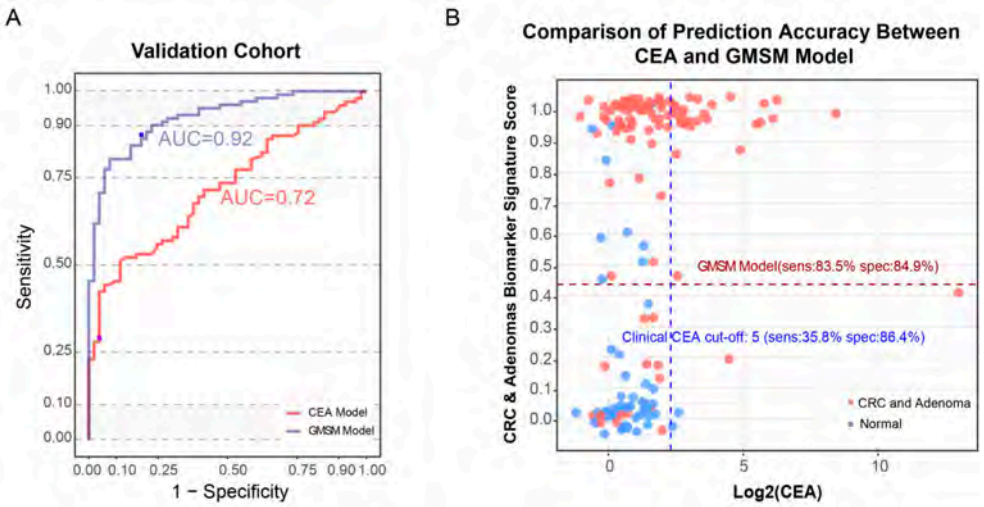

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Conclusions

- GM plays an important role in health and disease conditions, including cancer
- Data indicate that specific bacterial strains could have a critical role on the oncogenic potential
- The GM in its entirety seems to play a role in CRC etiology
- Experimental models indicate that dietary changes may have preventive effects through manipulation of GM
- New data indicate that GM could be used as next generation screening tools

PNRR Project: «Strengthening of the Biobanking and Biomolecular Resources Research Infrastructure of Italy»

- 26 million euro project coordinated by the CNR
- UNIBO 3.7 million Euros

- Implementing International Standards University Biobanks that would harmonise procedures thus widening their capacity to collect and process highly qualified samples from different centres.
- Developing a dedicated platform to collect, enrich and share clinical and genomic data, with the objective of implementing the FAIR principles, as well as ethical and legal aspects.
- Creating innovative digital tools and open-access knowledge base/exchange platforms to strengthen the ELSI extended community as a digital RRI community of practices.
- 4th generation biobanks (Biobanking 4.0) providing the opportunity to advance novel research:
  - Organoids
  - Microbiome
  - Induced pluripotent stem cells (iPSCs)
  - Adult progenitor cells

BBMRI.it
Biobanking and BioMolecular Resources Research Infrastructure of Italy

Aknowledgments

Chiara Alquati
Floriana Jessica Di Paola
Chiara Alquati
Giulia Calafato
Anna Prossomariti
Giulia Plazzi
Chiara Fazio
Nadia D'Angelo
Vincenzo Fogliano
Dajana Cuicchi

Patrizia Brigidi
Gabriele Conti
Silvia Turroni
Chiara Pierantoni
Francesco Buttitta
Sara Miccoli
Daniela Turchetti
Andrea Belluzzi
Elena Biagi
Marco Candela

AIRC

Piano Nazionale di Ripresa e Resilienza

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