

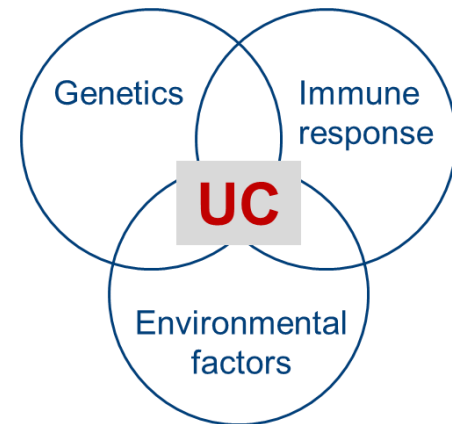
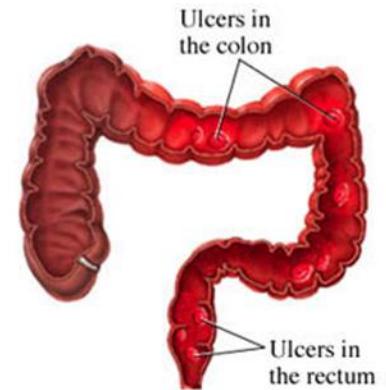


# Altered composition of colonic luminal compounds in patients with ulcerative colitis

Leen Boesmans  
27 November 2014  
16<sup>th</sup> Gut Day Symposium

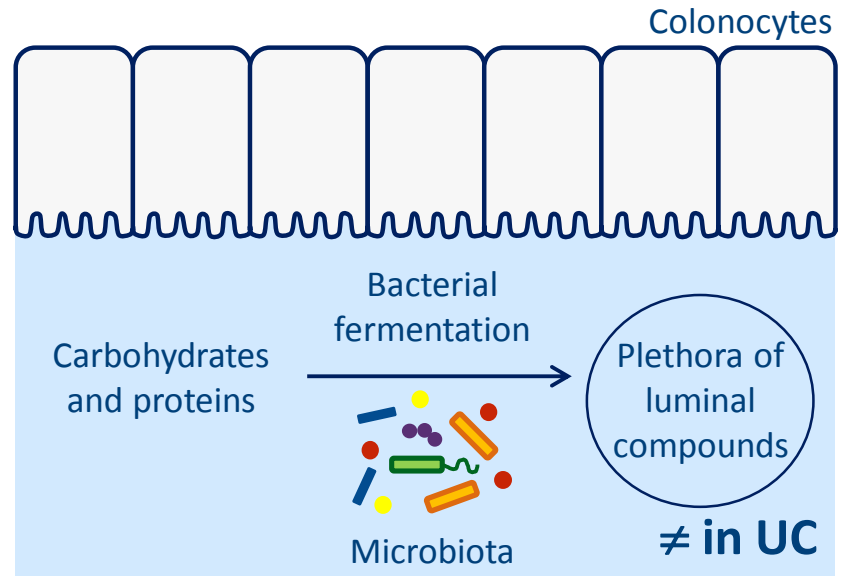
# Ulcerative colitis (UC)

- Chronic inflammation of the colonic mucosa
  - Alternating periods of active and quiescent disease
- Symptoms: - ulcers, bloody diarrhea and abdominal pain
  - increased risk of colorectal cancer (CRC) <sup>1</sup>
- Prevalence in Europe: 4.9 - 505 per 100,000 persons <sup>2</sup>
- Underlying etiology = multifactorial <sup>3</sup>
- Environmental factors
  - Smoking: protective factor
  - Diet: animal fat, high protein, alcoholic beverages
  - Microbiota



# Microbiota in health and UC

- Human colon <sup>1</sup>
  - 10<sup>14</sup> bacteria
  - Metabolic function
    - Carbohydrate fermentation
    - Protein fermentation
- Dysbiosis in UC <sup>2</sup>
  - Biodiversity ↓
  - Firmicutes ↓
  - *Lactobacillus*, *Bifidobacterium* ↓
  - *Clostridium* clusters IV & XIVa ↓



- Study aims: 1) Determine the metabolic activity in health and UC  
2) Associate the metabolome with cytotoxicity (as a CRC marker)

# Study design

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- Faecal samples were collected from:
  - Healthy controls (HC)
  - UC patients with active disease (UC-A)
  - UC patients in remission (UC-R)
- Disease activity was determined by the partial Mayo score (range 0-9):
  - Active (UC-A): pMayo  $\geq 3$
  - Remission (UC-R): pMayo  $\leq 2$
- Preparation of faecal water (FW):
  - Faecal samples were centrifuged at 50,000 g at 4 °C for 2 h
  - Supernatant was sterile filtered through a 0.22- $\mu$ m filter
- The volatile organic compounds (VOCs) were determined using GC-MS and cytotoxicity of the samples using a colorimetric method

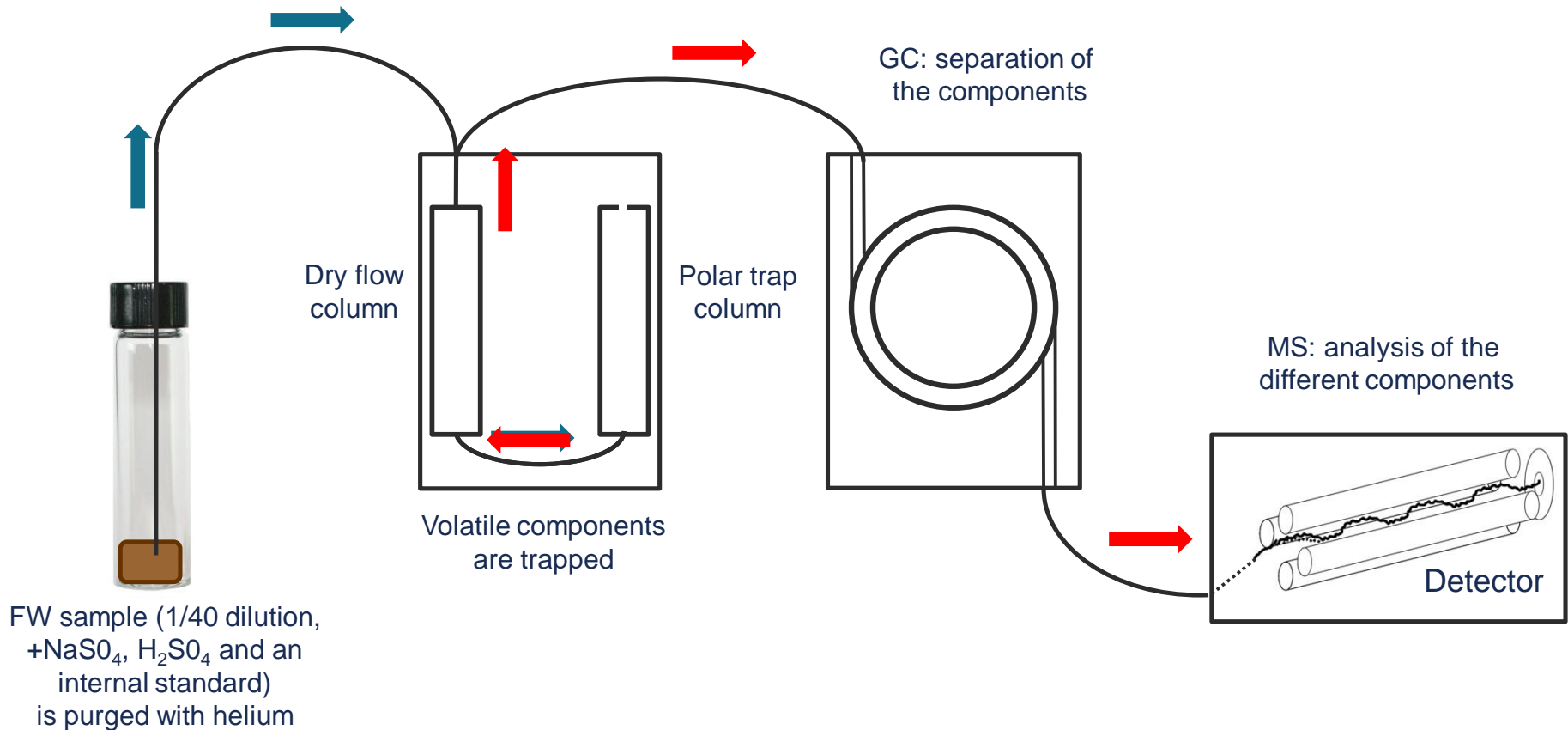
# Cohort characteristics

	HC N=52	UC-A N=58	UC-R N=60	P-value
Age (years)	38 ( $\pm$ 16)	46( $\pm$ 16)	47( $\pm$ 16)	0,001
BMI (kg/m <sup>2</sup> )	24 ( $\pm$ 3)	25 ( $\pm$ 3)	25 ( $\pm$ 3)	0,376
Male/female (%)	37/63	60/40	57/43	0,028

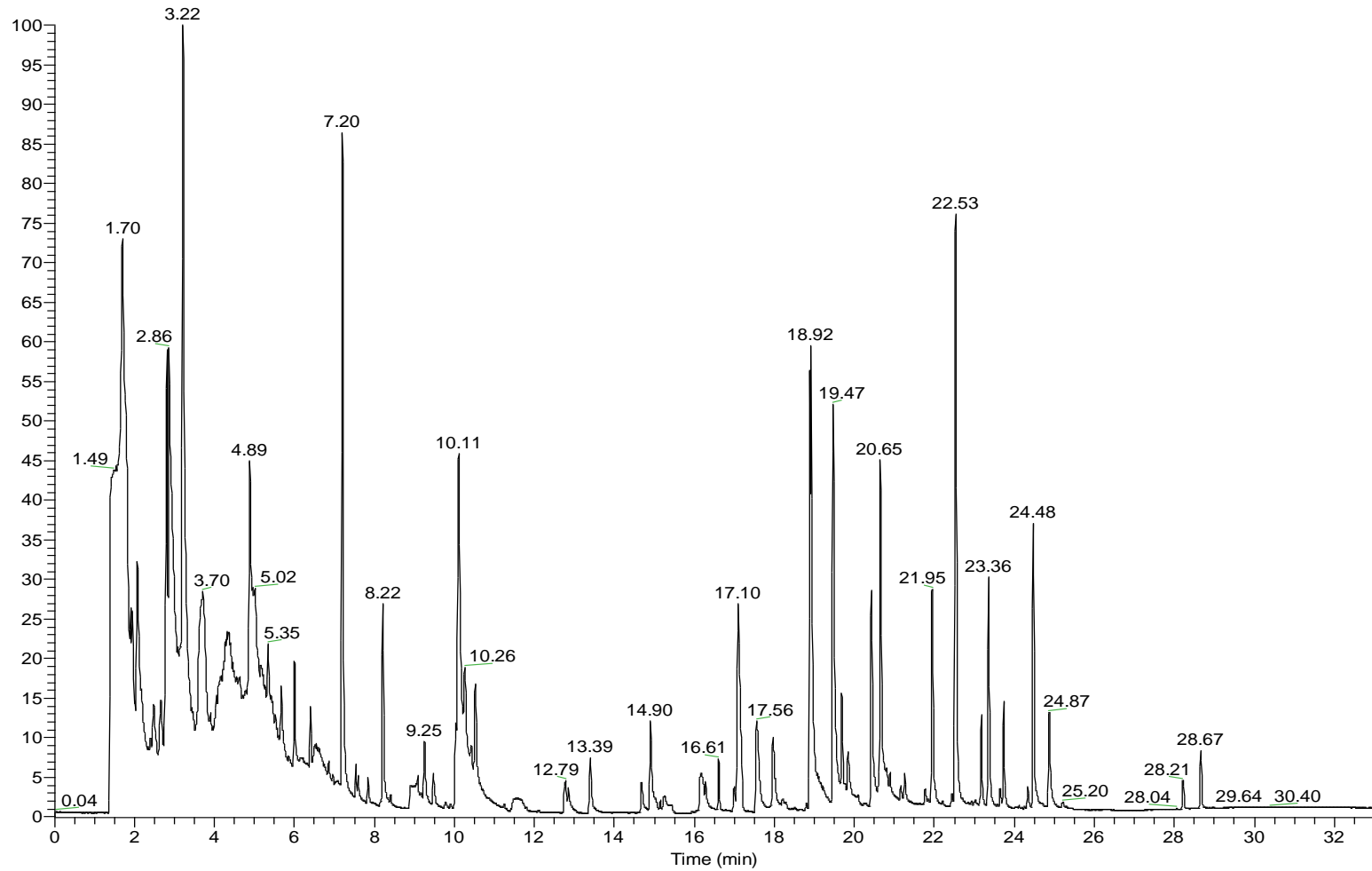
- Exclusion criteria:
  - Surgery of the gastrointestinal tract
  - Colonoscopy in the preceding month
  - Antibiotic use in the preceding month
  - UC patients: disease extent limited to the rectum

# GC-MS analysis results in metabolite profiles

- All VOCs were relatively quantified versus an internal standard and classified according to chemical class

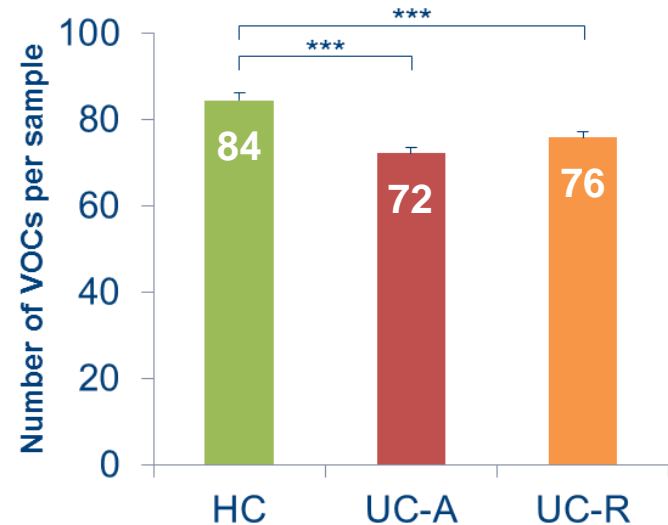


# Typical metabolite pattern of a faecal sample



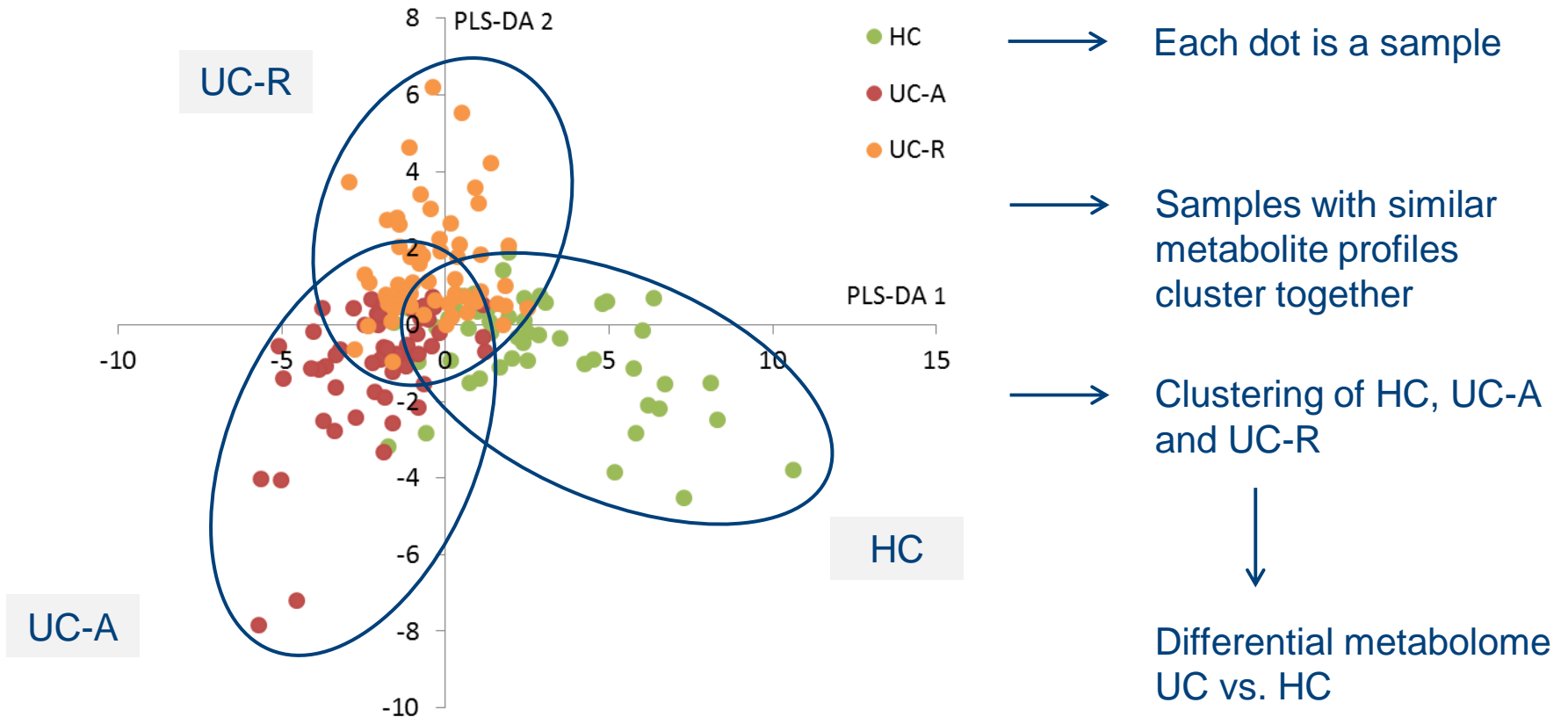
# Results metabolome analysis

- 237 VOCs were identified in the FW samples:
  - Average: 77 ( $\pm 1$ ) / sample
  - Minimum: 45 / sample
  - Maximum: 108 / sample
  - 27 sample-specific VOCs
  - 11 VOCs in all samples
- UC patients have significantly less VOCs per sample than HC

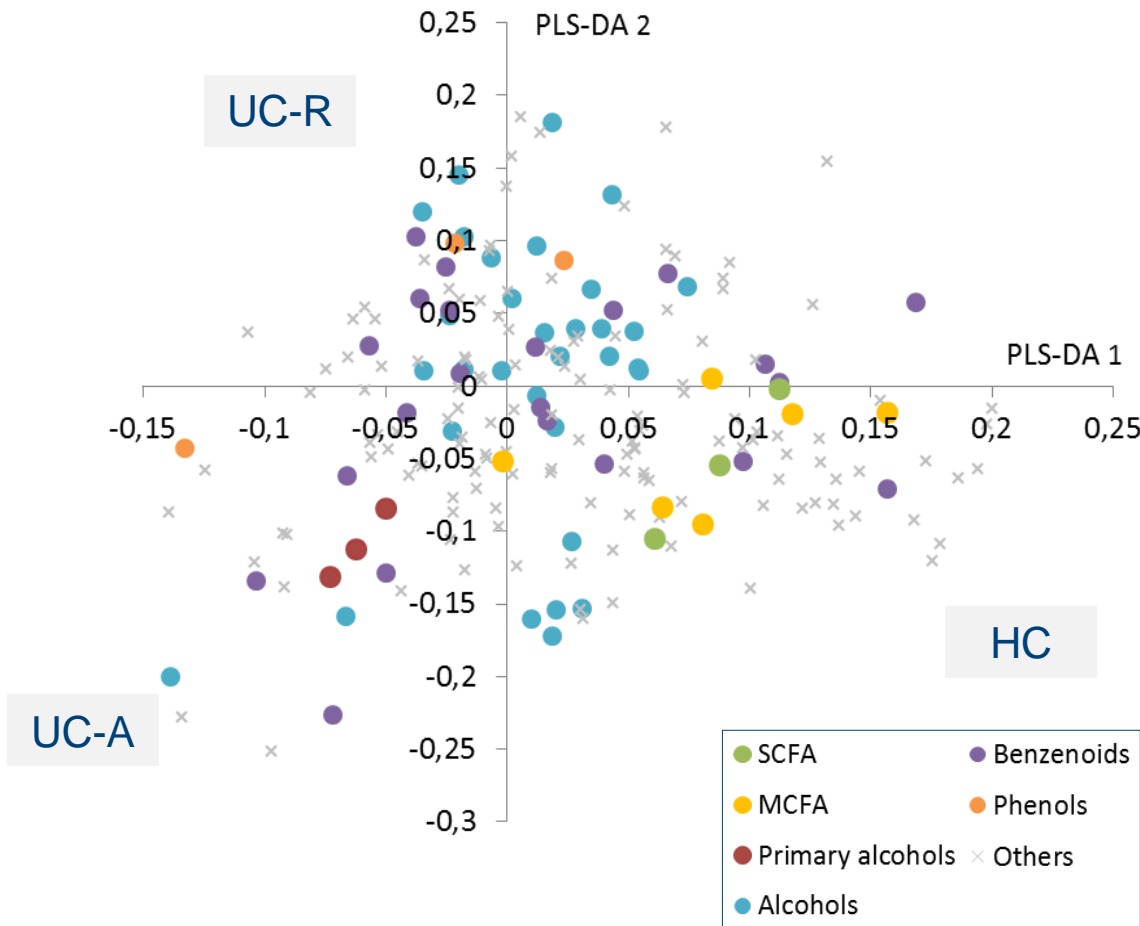




# PLS-DA score plot showing all FW samples



# PLS-DA loading plot showing all VOCs



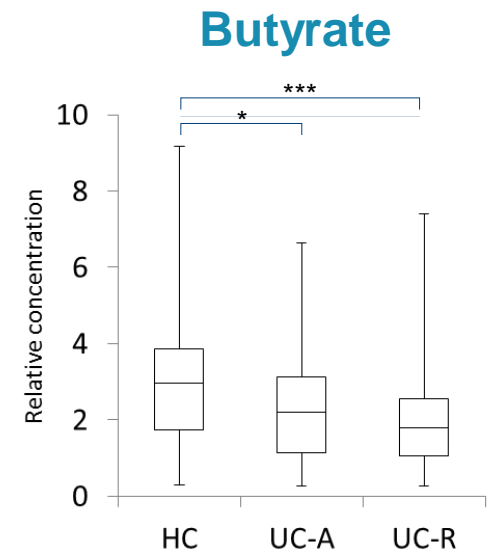
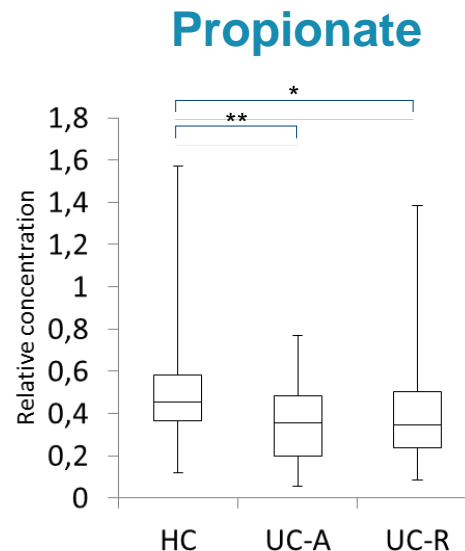
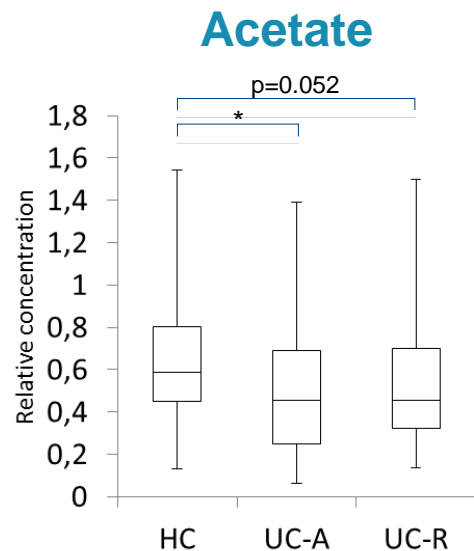
→ Each dot is a metabolite

→ Metabolites away from the center are important for the separation

→ Metabolites in the lower right quadrant are associated with HC samples

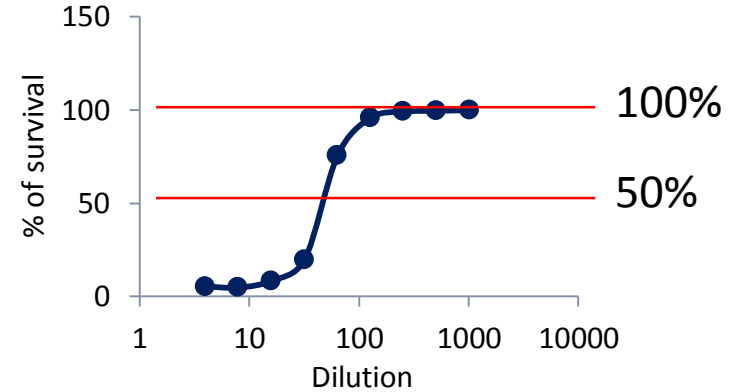
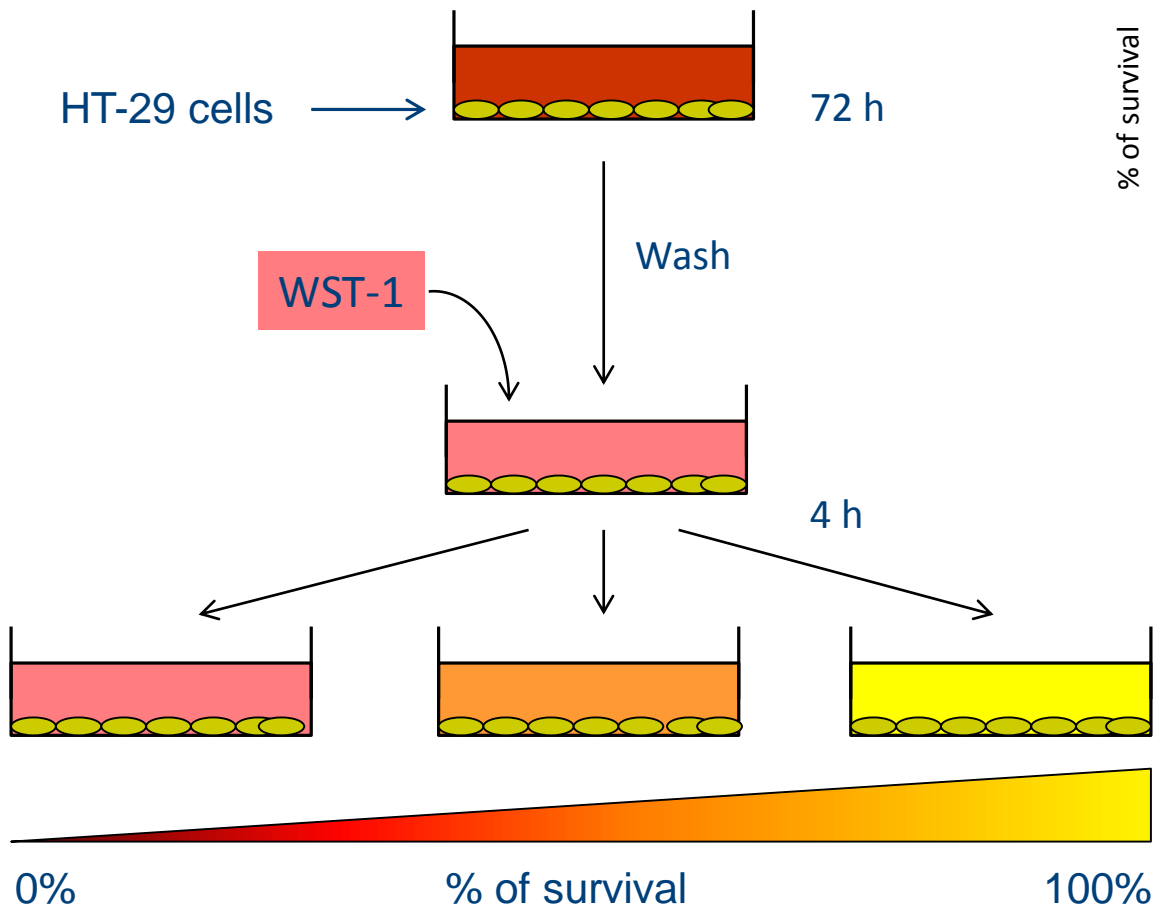
# VOCs associated with sample groups

- FW samples from UC patients were associated with:
  - Higher prevalence of:
    - Alcohols (Primary alcohols for UC-A)
    - Phenols
    - Benzene-derivatives } Protein fermentation metabolites
  - Lower prevalence of:
    - Short chain fatty acids (SCFA) } Carbohydrate fermentation metabolites
    - Medium chain fatty acids (MCFA)



# Impact of the metabolome on cytotoxicity

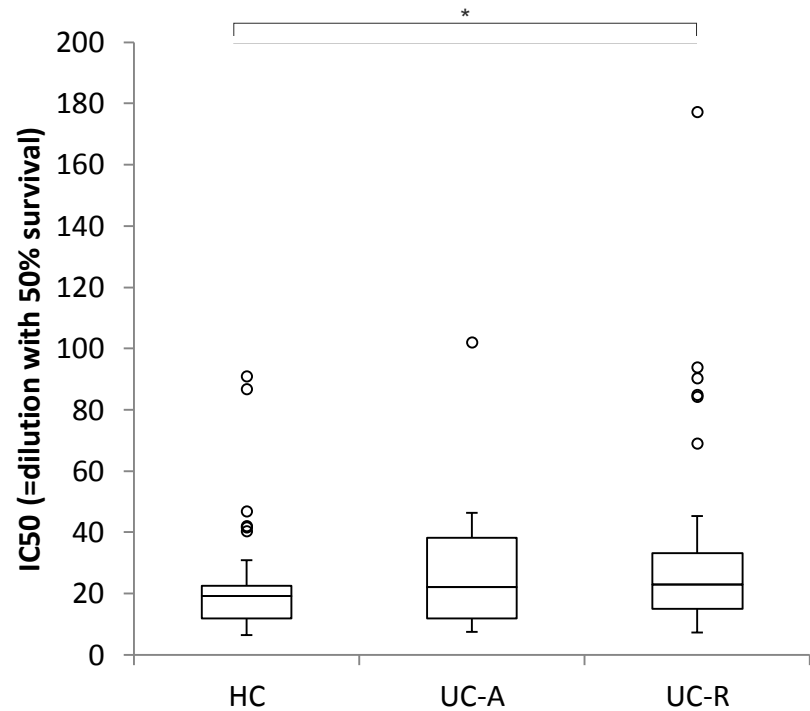
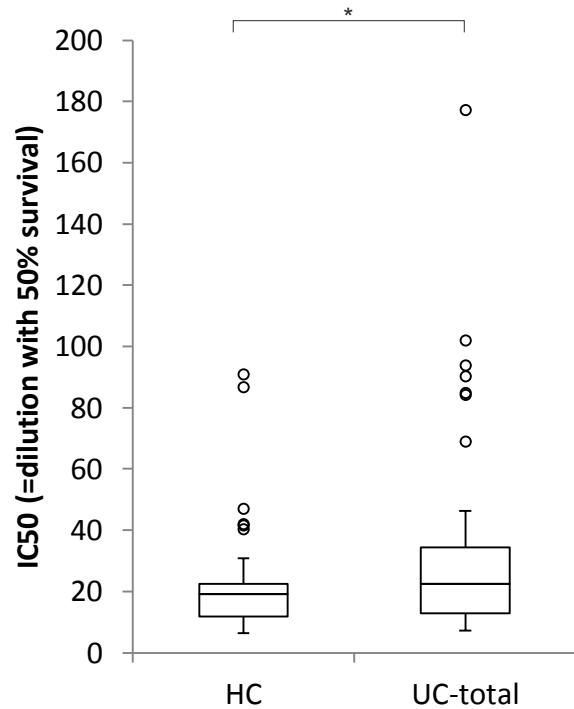
Incubation with faecal water



$IC_{50}$  =  
Dilution at which 50%  
of cells survive

High  $IC_{50}$  =  
More cytotoxic

# FW samples from UC patients are more cytotoxic



\*  $p < 0.05$

# VOCs associated with cytotoxicity

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- FW samples with higher cytotoxicity were associated with:
    - Higher prevalence of:
      - Furans
      - Aldehydes
      - Branched-chain fatty acids (BCFA)
      - S-containing compounds
    - Lower prevalence of:
      - SCFA
      - MCFA
- Protein fermentation metabolites
- Carbohydrate fermentation metabolites

# Conclusions

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- The faecal metabolite profiles allowed to differentiate HC from UC patients
- Most discriminatory metabolites:
  - For UC-A: primary alcohols
  - For HC: SCFA and MCFA
- FW samples from UC (especially UC-R) patients are more cytotoxic than HC samples
  - This could contribute to the higher CRC risk for UC patients and, through barrier disruption, to the induction and/or continuation of inflammation
- Promoting carbohydrate over protein fermentation could positively affect the colonic health in UC
  - Can be achieved by the use of prebiotics or diet adjustments

# Acknowledgements

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