cell free RNAs as cancer biomarkers

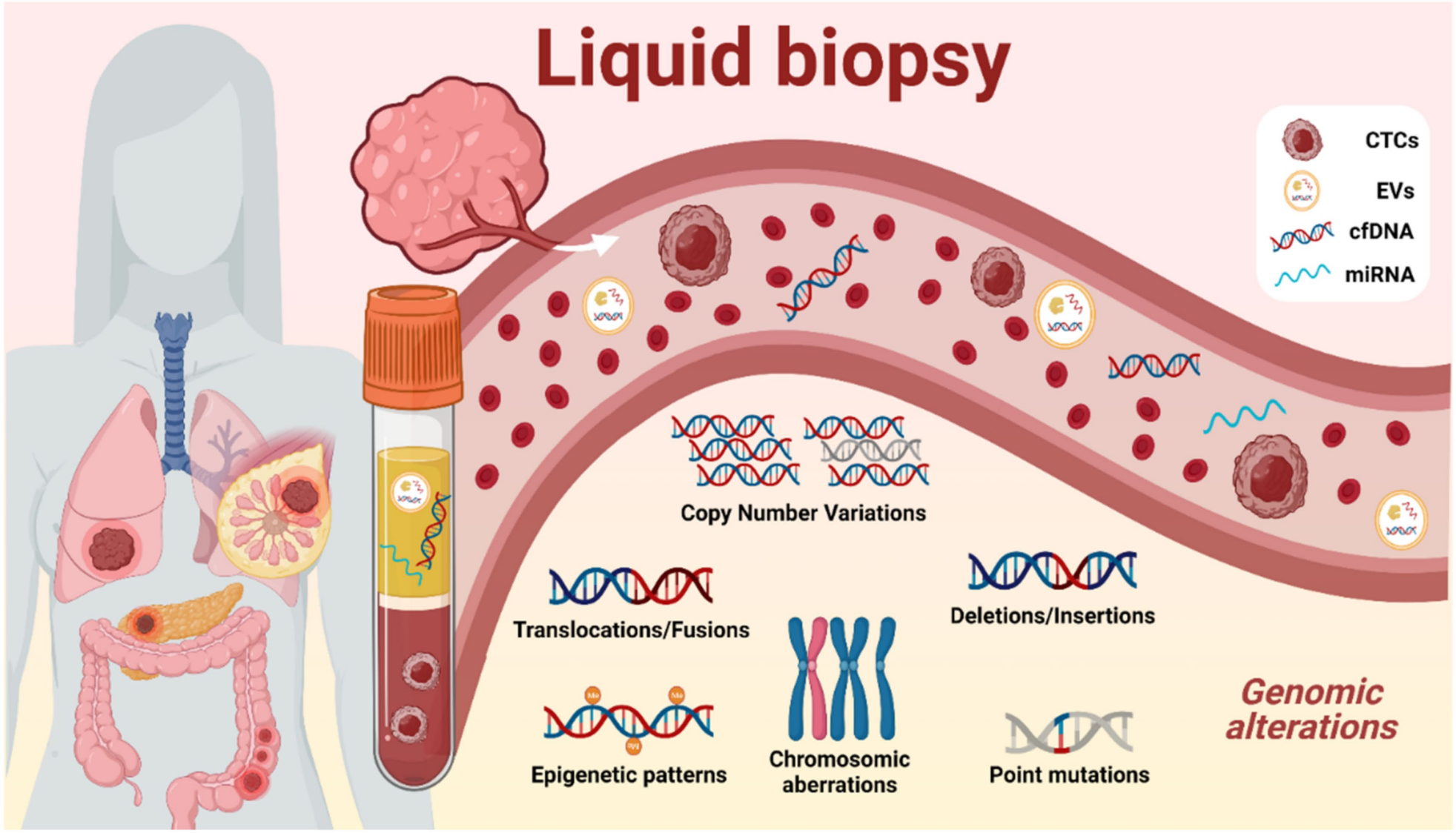
Pieter Mestdagh
30/06/2023

GHENT UNIVERSITY

OncoRNALab

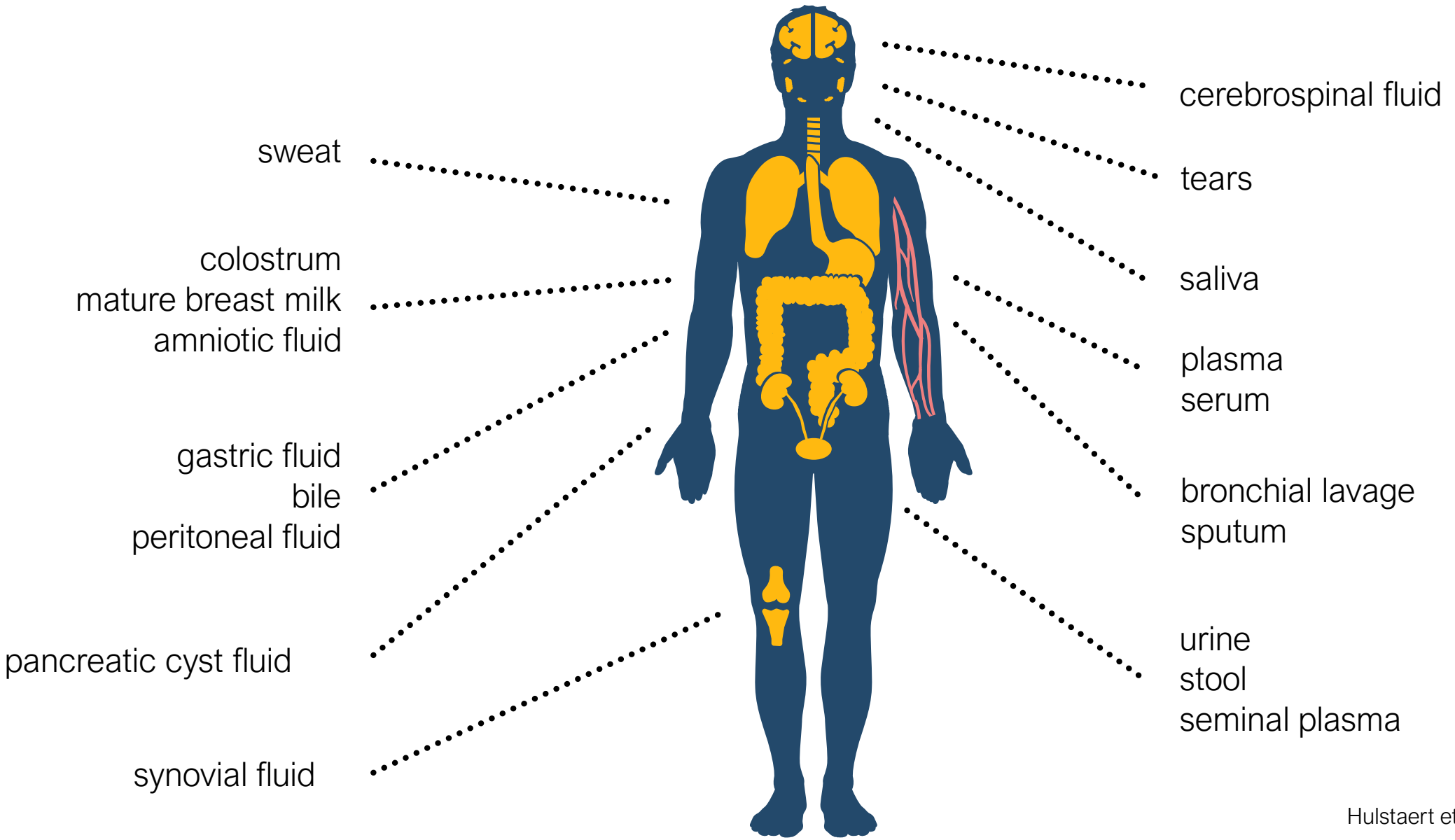
CRIG

Liquid biopsies for minimally invasive cancer diagnosis or monitoring



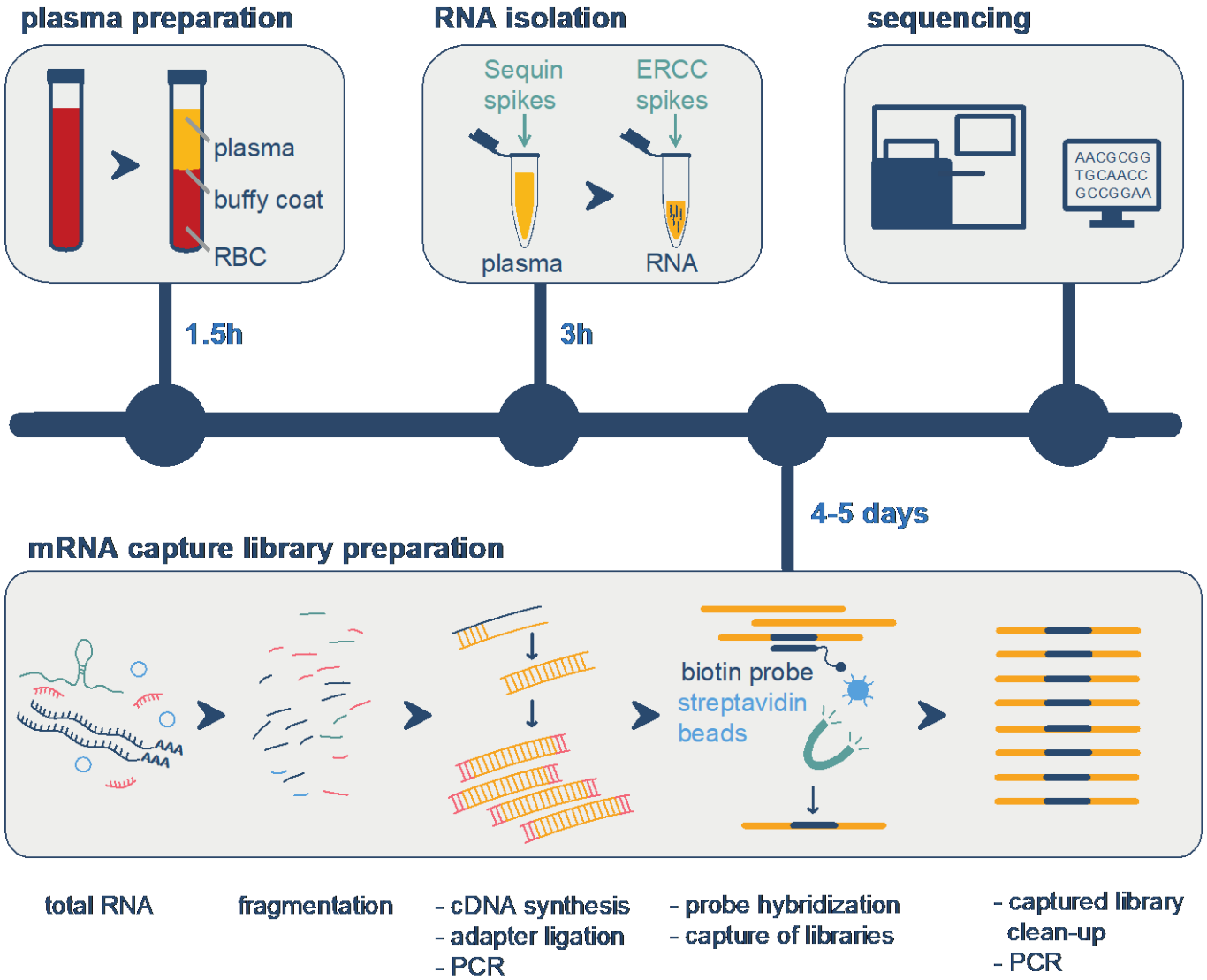
Palacín-Aliana et al., Biomedicines, 2021

Expanding the liquid biopsy field beyond the bloodstream



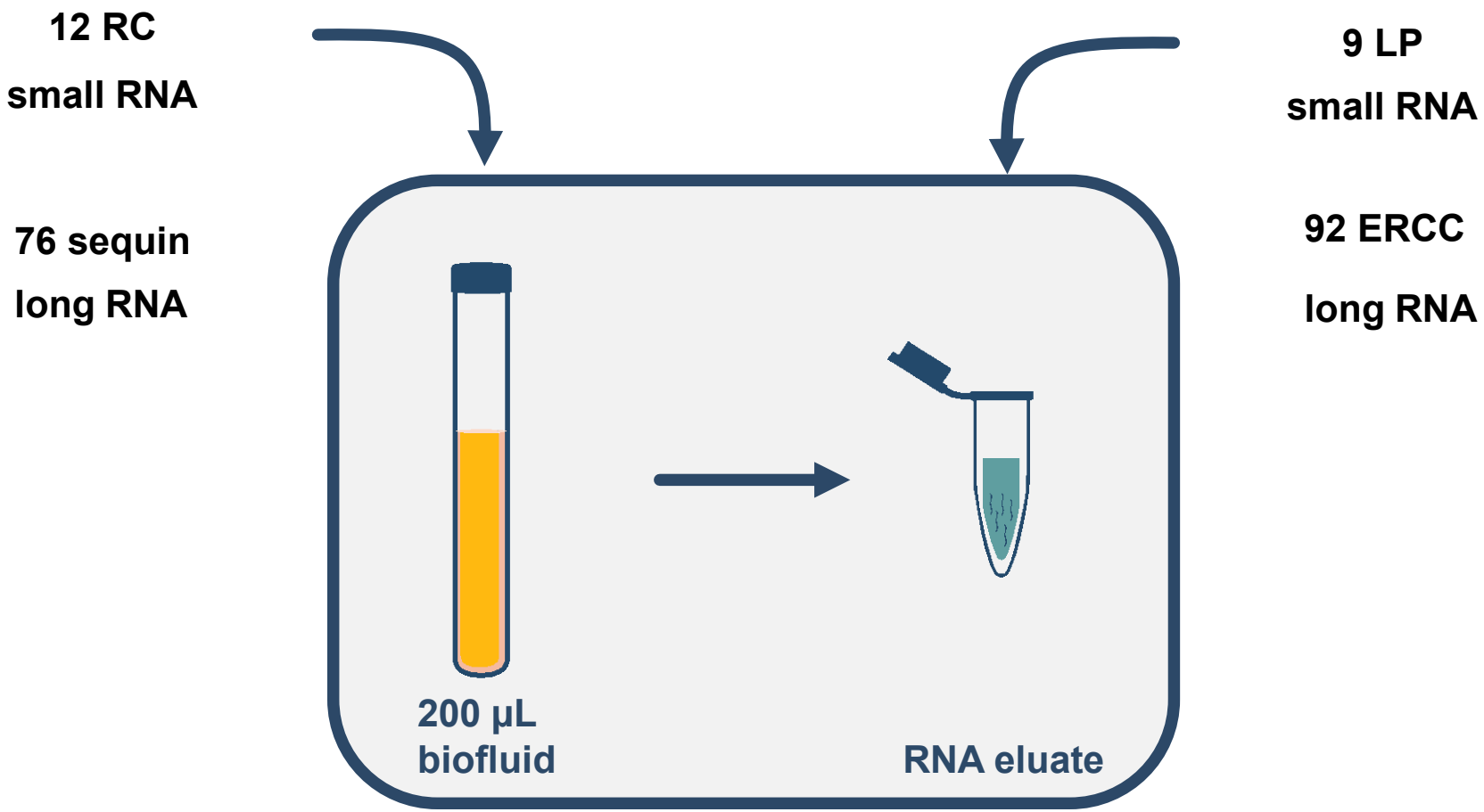
Hulstaert et al, Cell Reports, 2020

mRNA capture sequencing for cfRNA profiling in liquid biopsies



Hulstaert et al, Cell Reports, 2020
Hulstaert et al, STAR protocols, 2021

Spike-in RNAs as processing and normalization controls

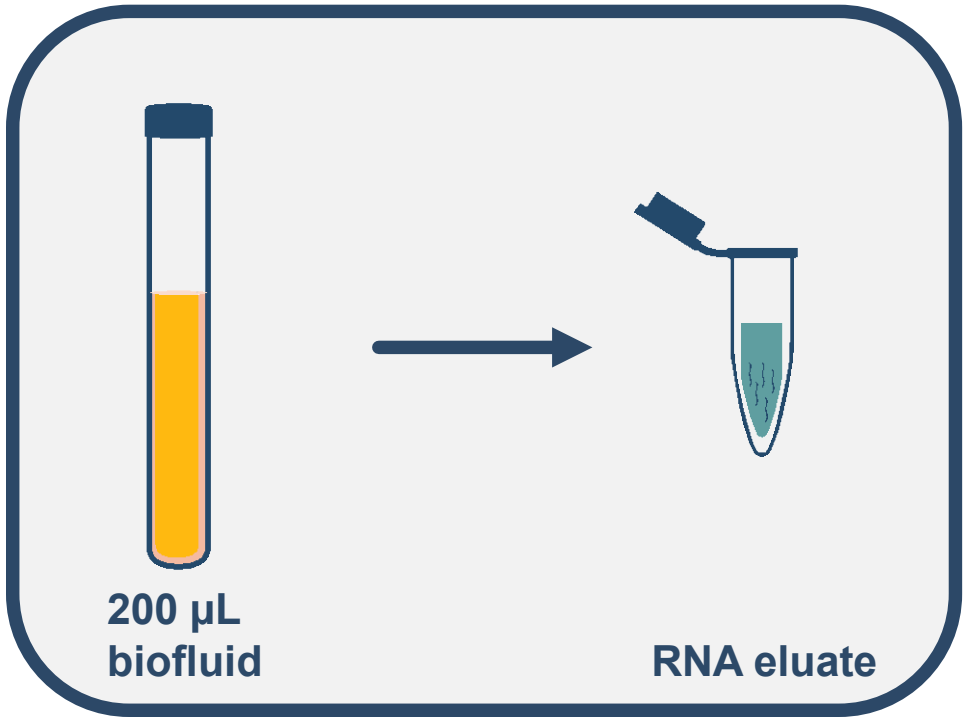


Hulstaert et al, Cell Reports, 2020
Hulstaert et al, STAR protocols, 2021

Spike-in RNAs as processing and normalization controls

$$\frac{\text{fluid spike}}{\text{eluate spike}} = \text{RNA extraction efficiency}$$

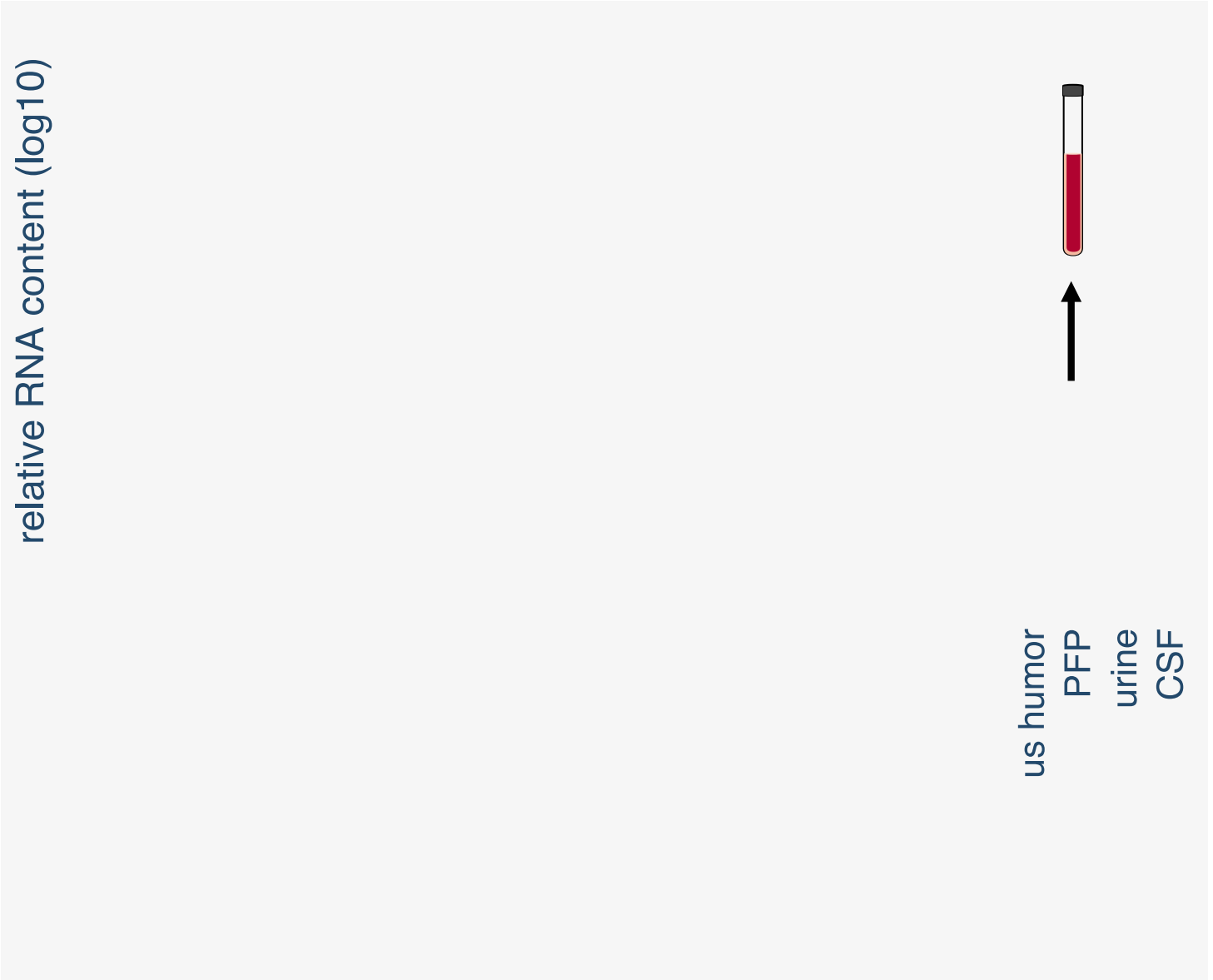
$$\frac{\text{human RNA}}{\text{fluid spike}} = \text{relative RNA concentration in fluid}$$



$$\frac{\text{human RNA}}{\text{eluate spike}} = \text{relative RNA concentration in eluate}$$

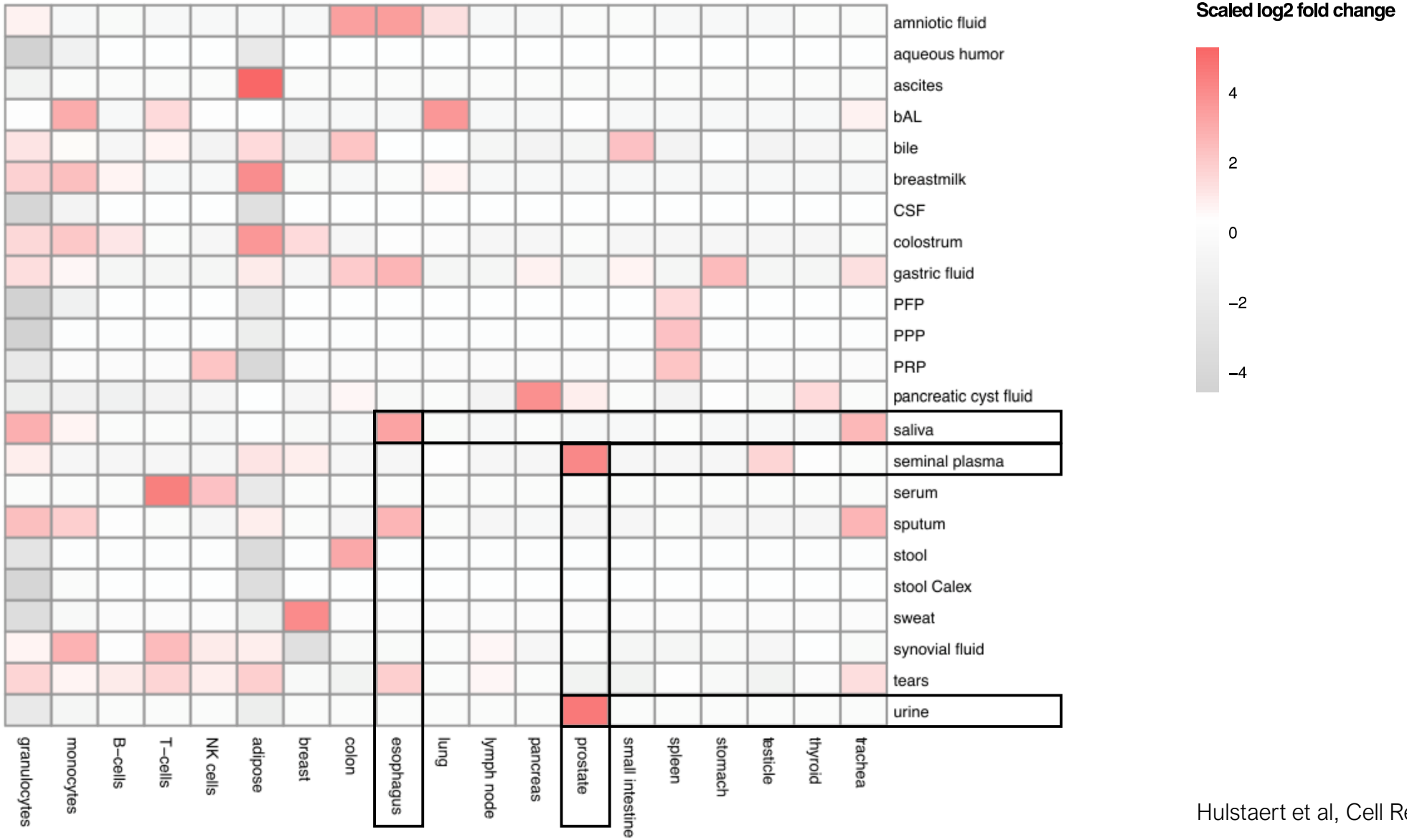
Hulstaert et al, Cell Reports, 2020
Hulstaert et al, STAR protocols, 2021

Highly variable RNA concentrations amongst different biofluids



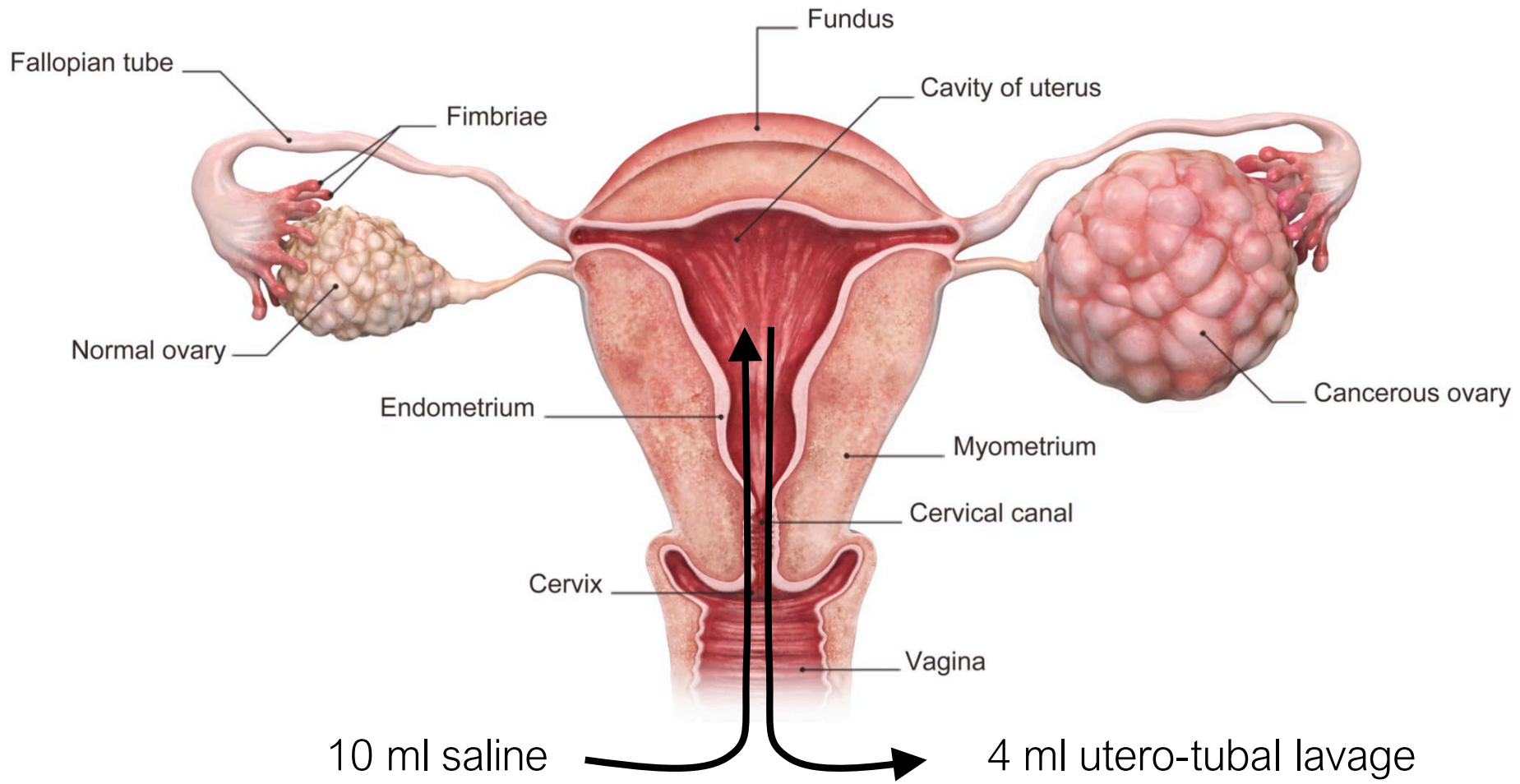
Hulstaert et al, Cell Reports, 2020

Tissue-specific RNAs are enriched in proximal fluids



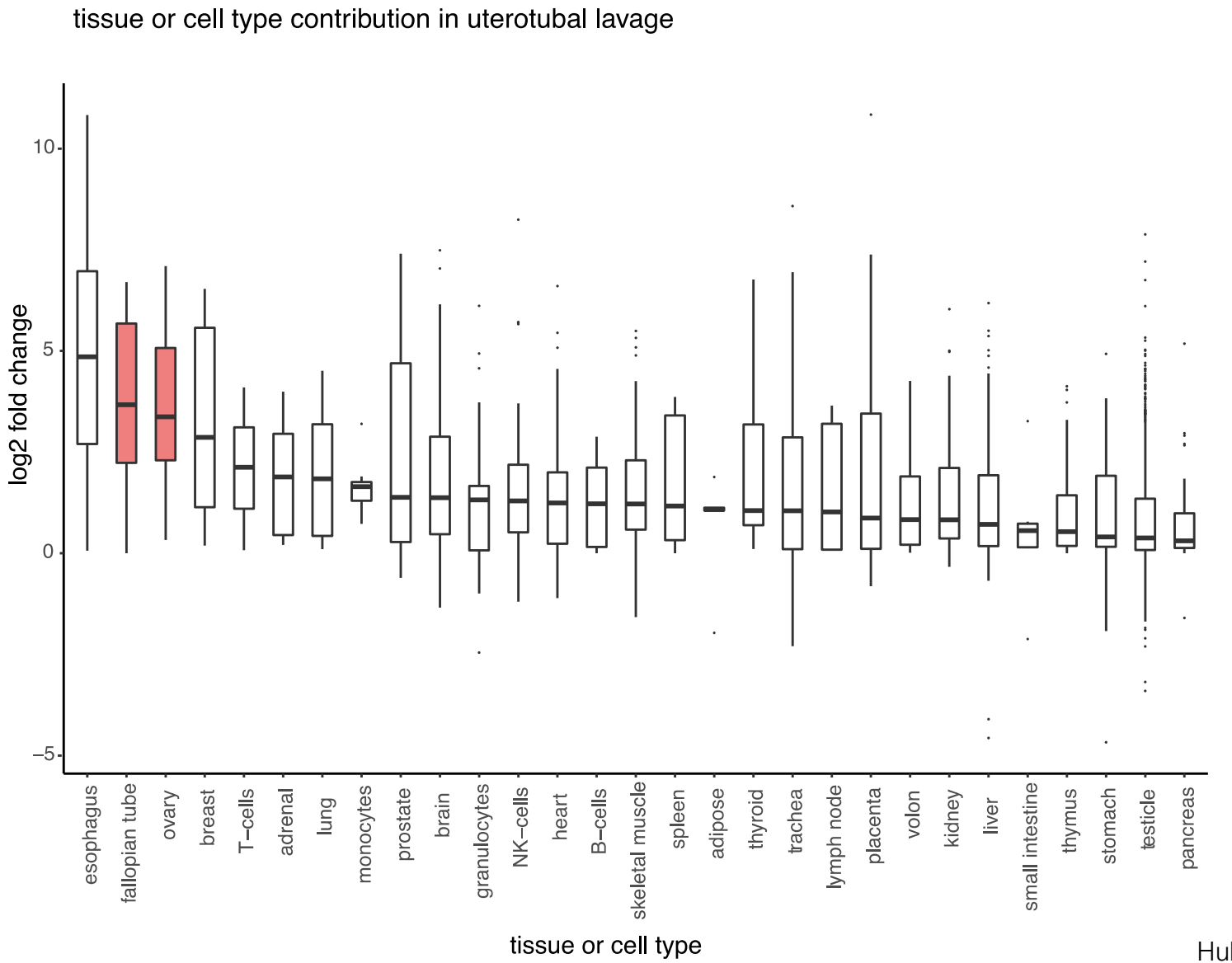
Hulstaert et al, Cell Reports, 2020

Utero-tubal lavage as a proximal liquid biopsy for ovarian cancer



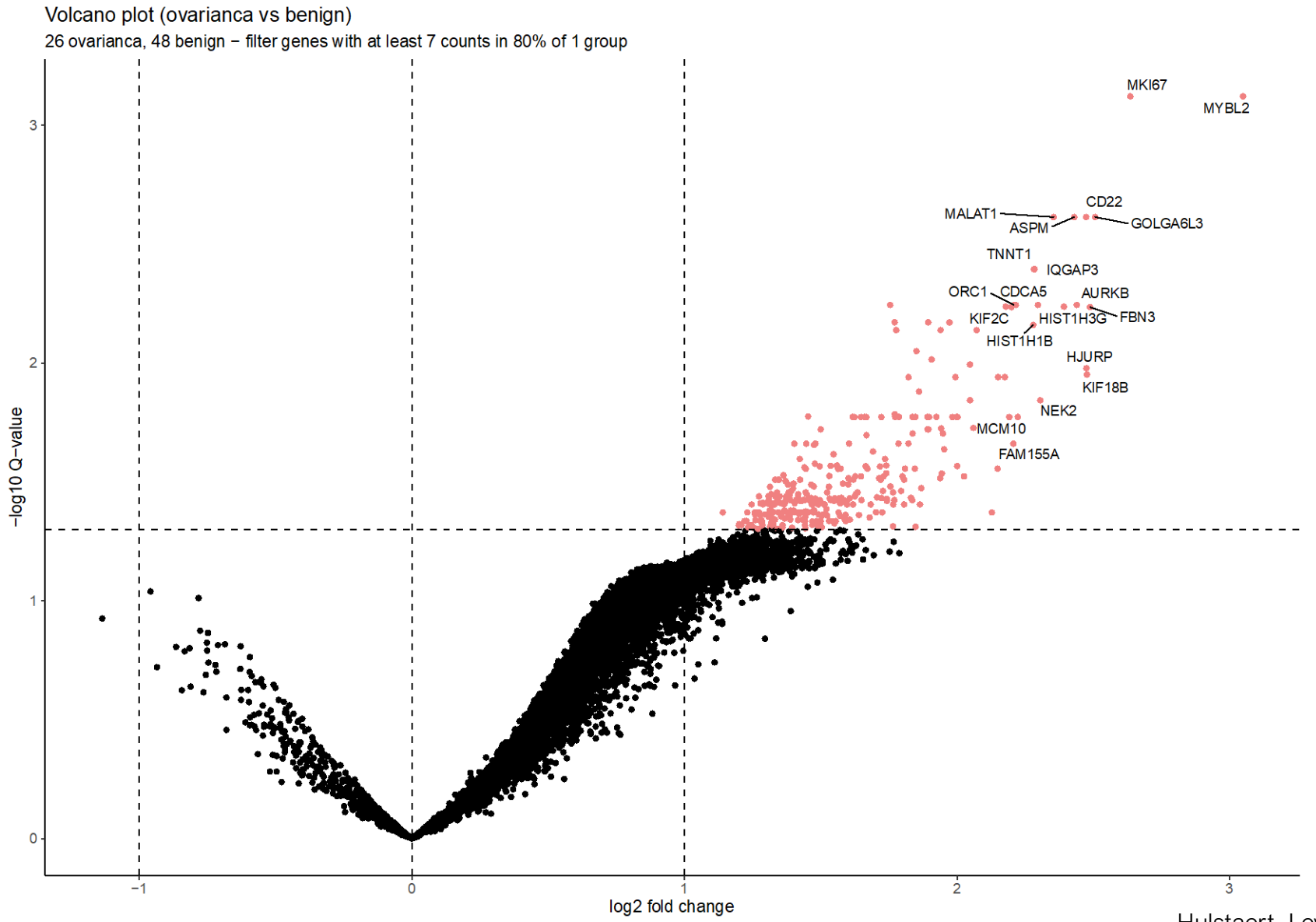
Hulstaert, Levanon et al, Neoplasia, 2022

Fallopian tube and ovary specific mRNAs overrepresented in utero-tubal lavage



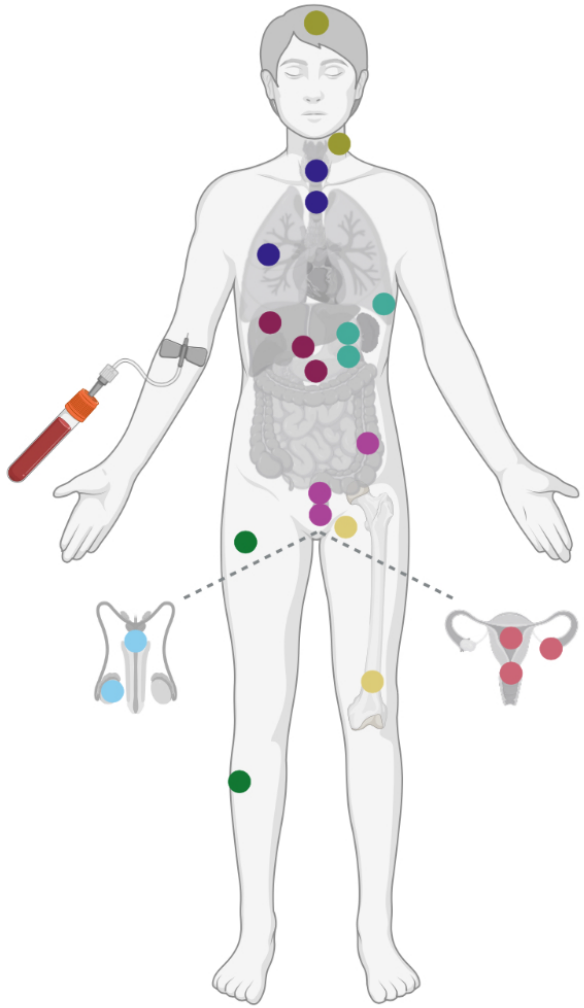
Hulstaert, Levanon et al, Neoplasia, 2022

Higher abundance of proliferation mRNAs in cancer versus control



Hulstaert, Levanon et al, Neoplasia, 2022

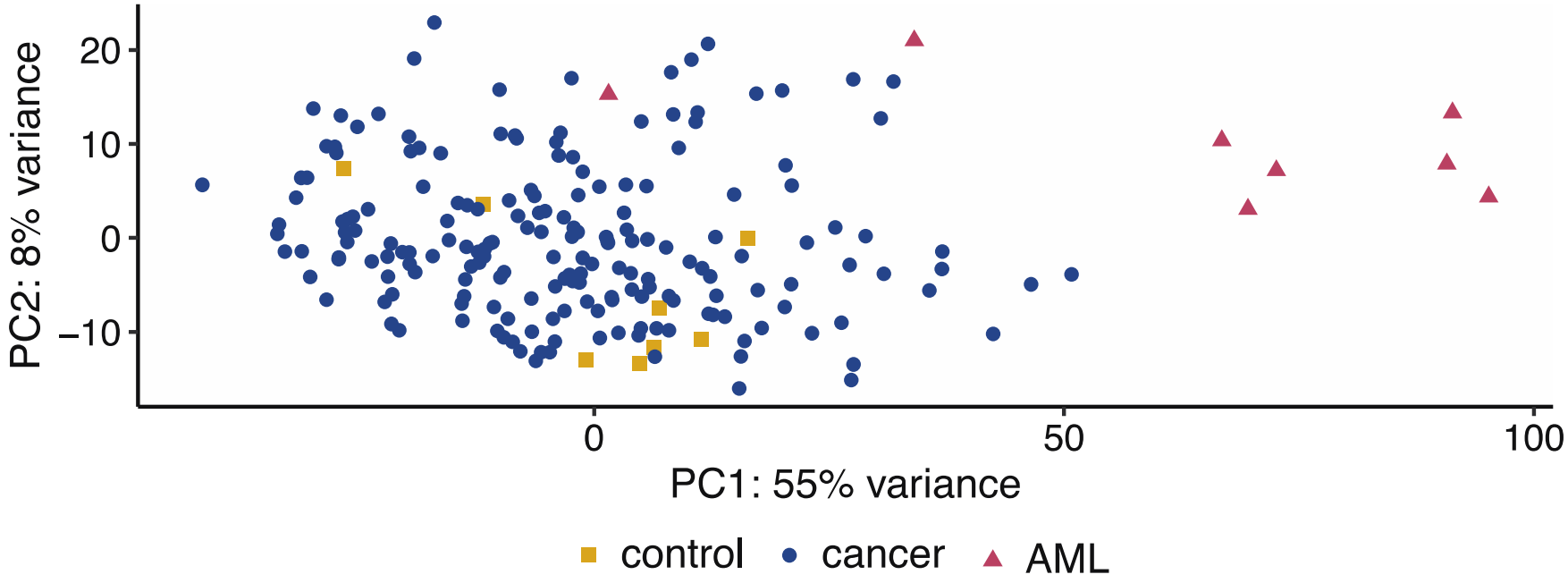
Pan-cancer plasma cfRNA profiles



- brain tumor (GBM & ANA)
- head and neck cancer (HNSC)
- thyroid cancer (THCA)
- esophageal cancer (ESCA)
- lung cancer (LUAD & LUSC)
- breast cancer (BRCA)
- stomach cancer (STAD)
- kidney cancer (KIRC)
- liver cancer (LIHC)
- cholangiocarcinoma (CHOL)
- pancreas cancer (PAAD)
- colon cancer (COAD)
- bladder cancer (BLCA)
- rectal cancer (READ)
- lymphoma (DLBCL)
- leukemia (AML)
- sarcoma (SARC)
- melanoma (SKCM)
- prostate cancer (PRAD)
- testicular cancer (TGCT)
- ovarian cancer (OV)
- cervical cancer (CESC)
- uterine cancer (UCEC)

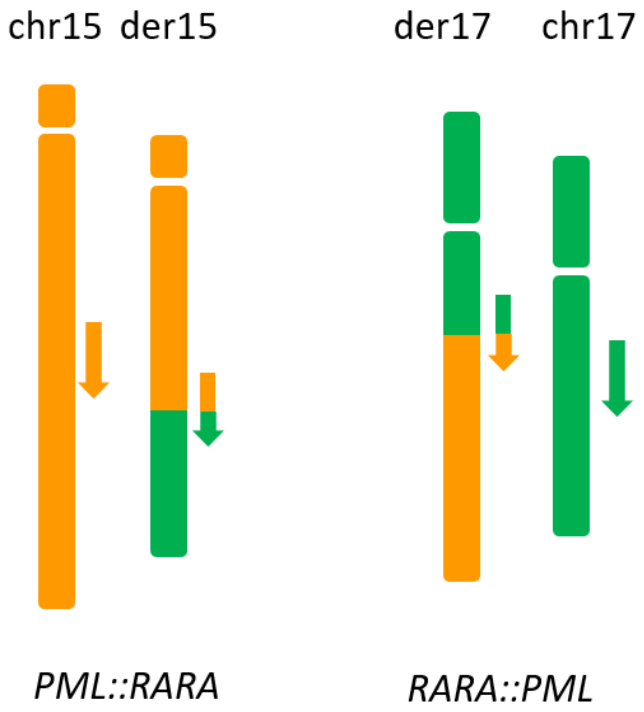
Morlion et al, in review
Morlion et al, 2023 medRxiv

AML cfRNA profiles are distinct from solid tumors and controls

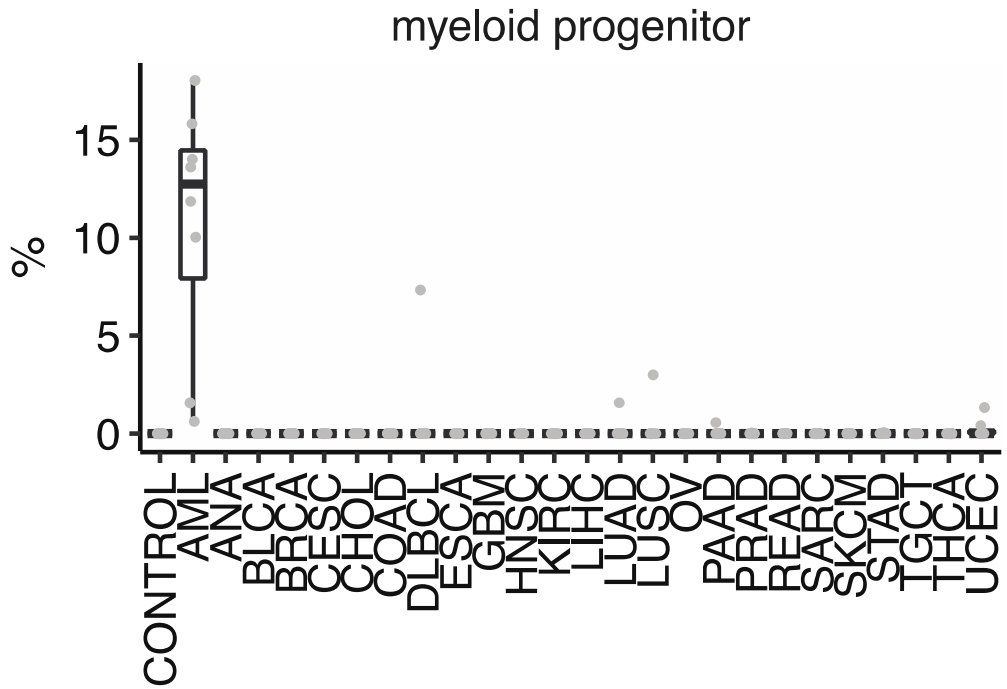


Morlion et al, in review
Morlion et al, 2023 medRxiv

Evidence of tumor derived RNA in cfRNA profiles from AML patients

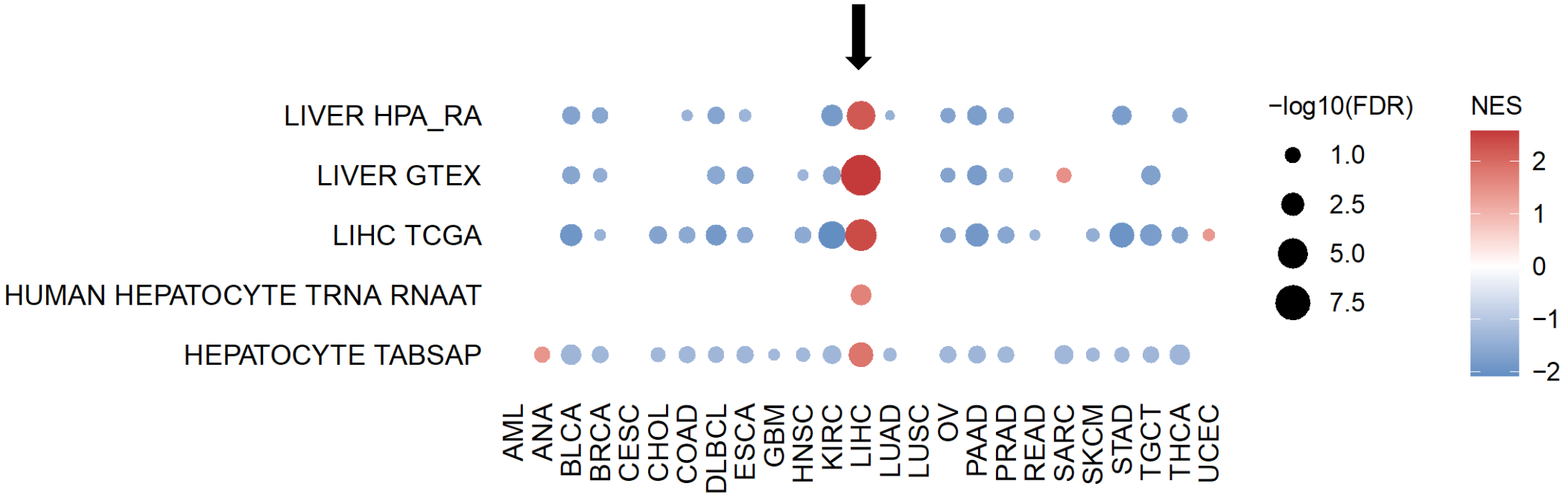


http://cancergeneticslab.ca



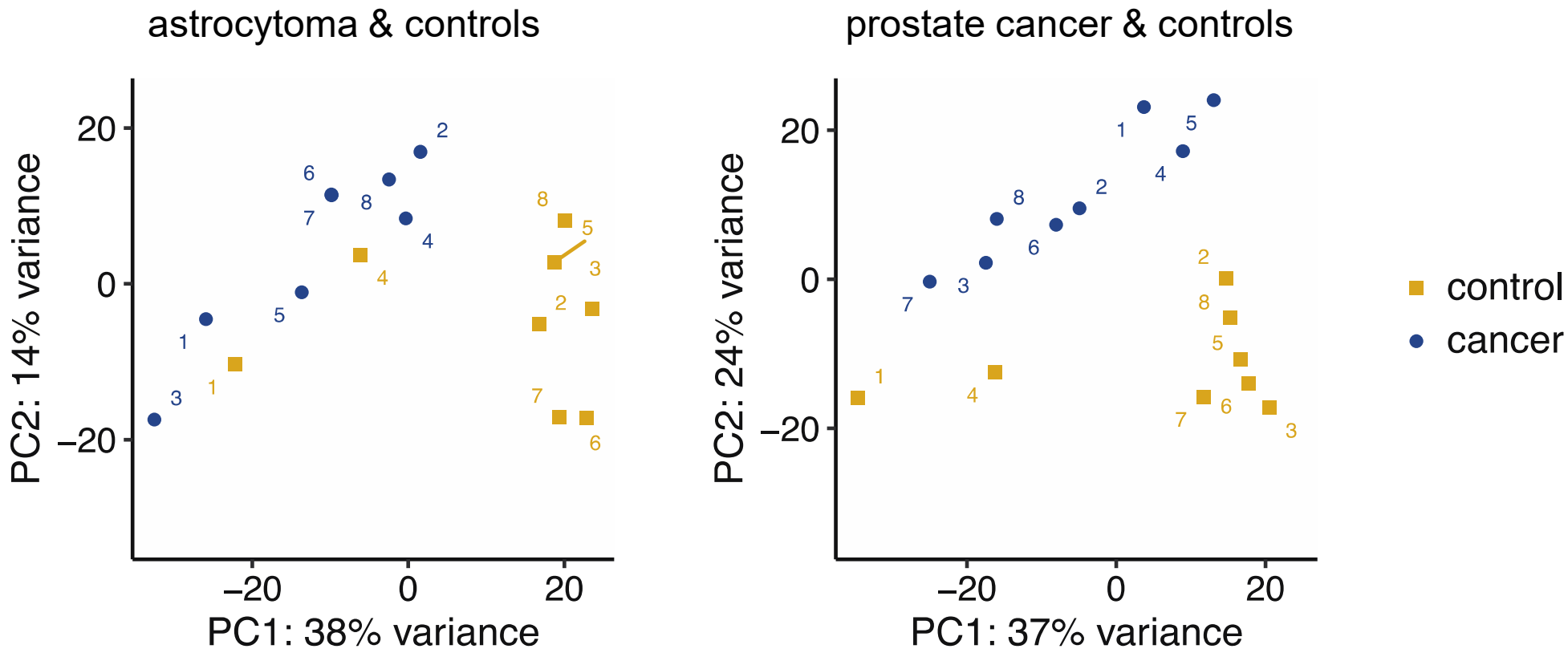
Morlion et al, in review
Morlion et al, 2023 medRxiv

Tissue of origin signal in plasma of liver cancer patients



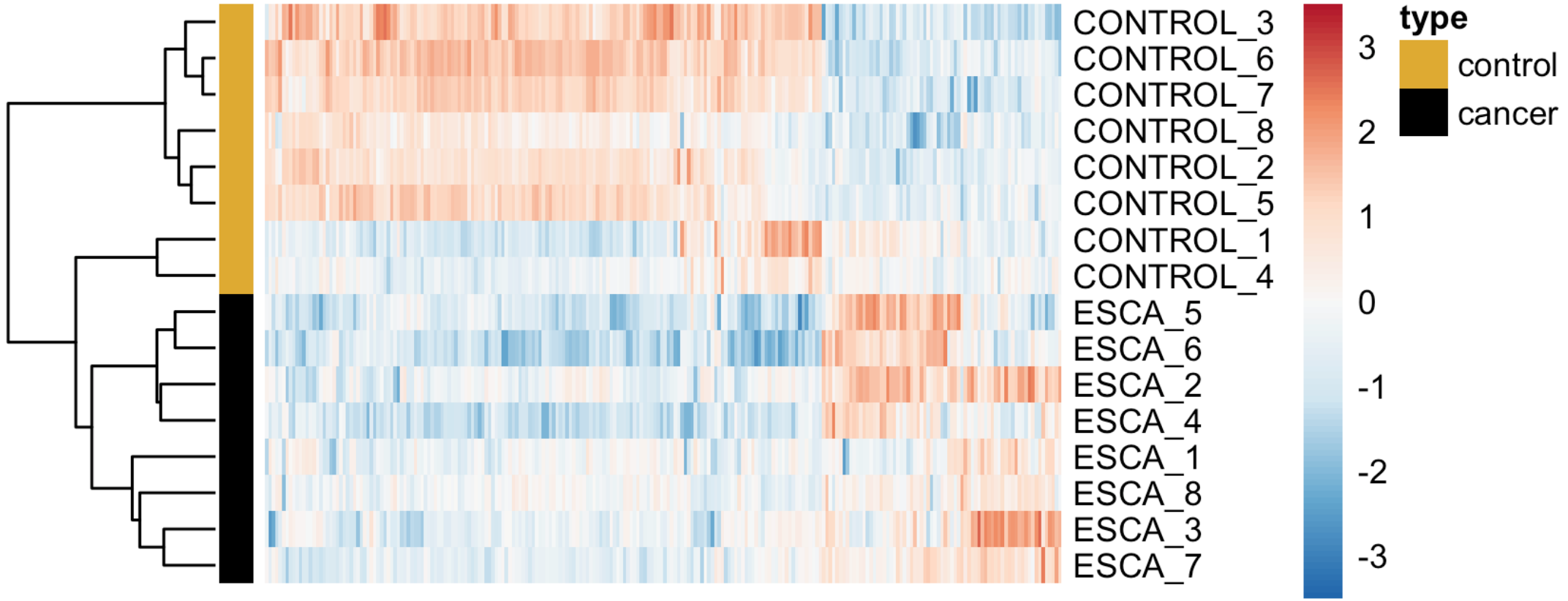
Morlion et al, in review
Morlion et al, 2023 medRxiv

Different cfRNA profiles between solid tumor and controls



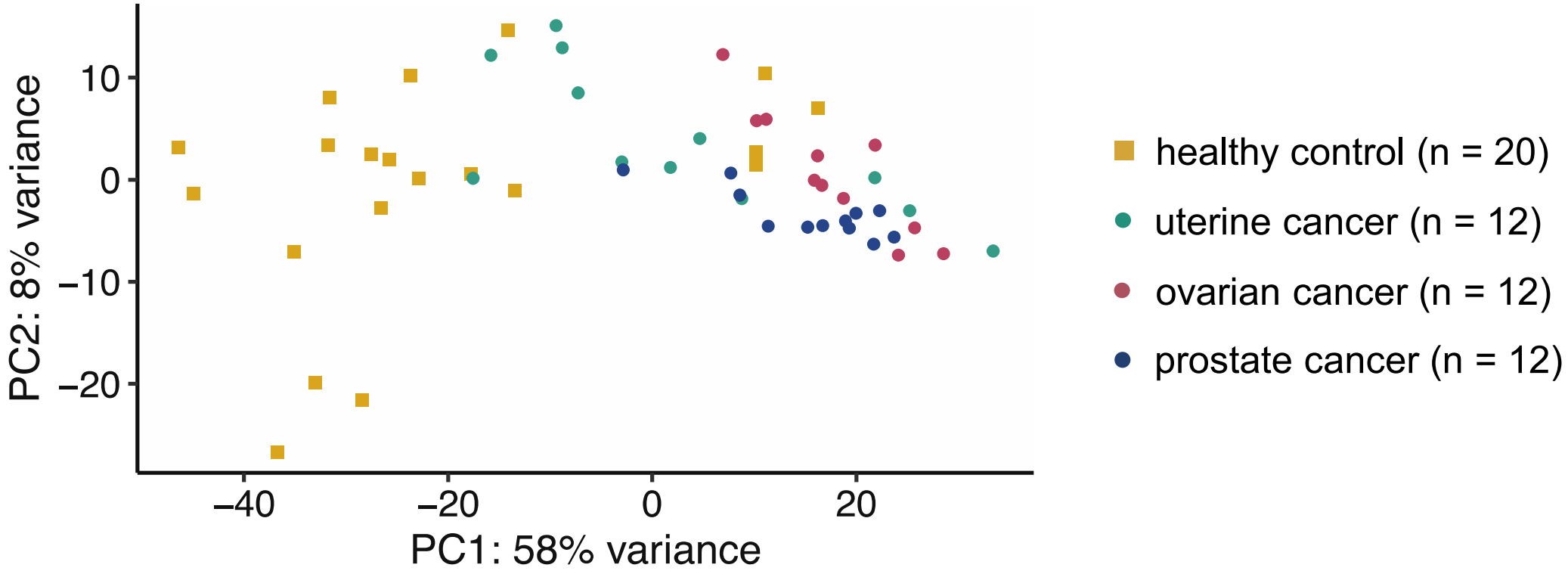
Morlion et al, in review
Morlion et al, 2023 medRxiv

Heterogeneity in cfRNA profiles between patients with same cancer type



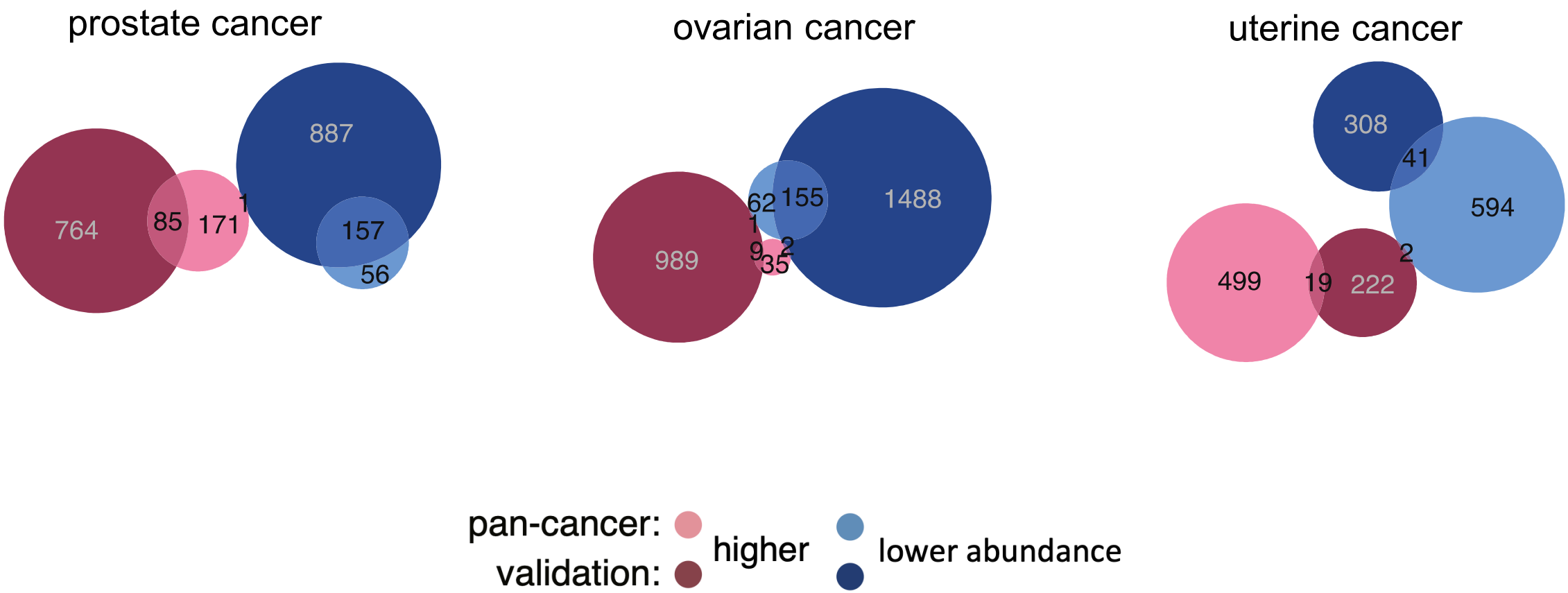
Morlion et al, in review
Morlion et al, 2023 medRxiv

Independent validation cohort confirms different cfRNA profiles between solid tumor and controls



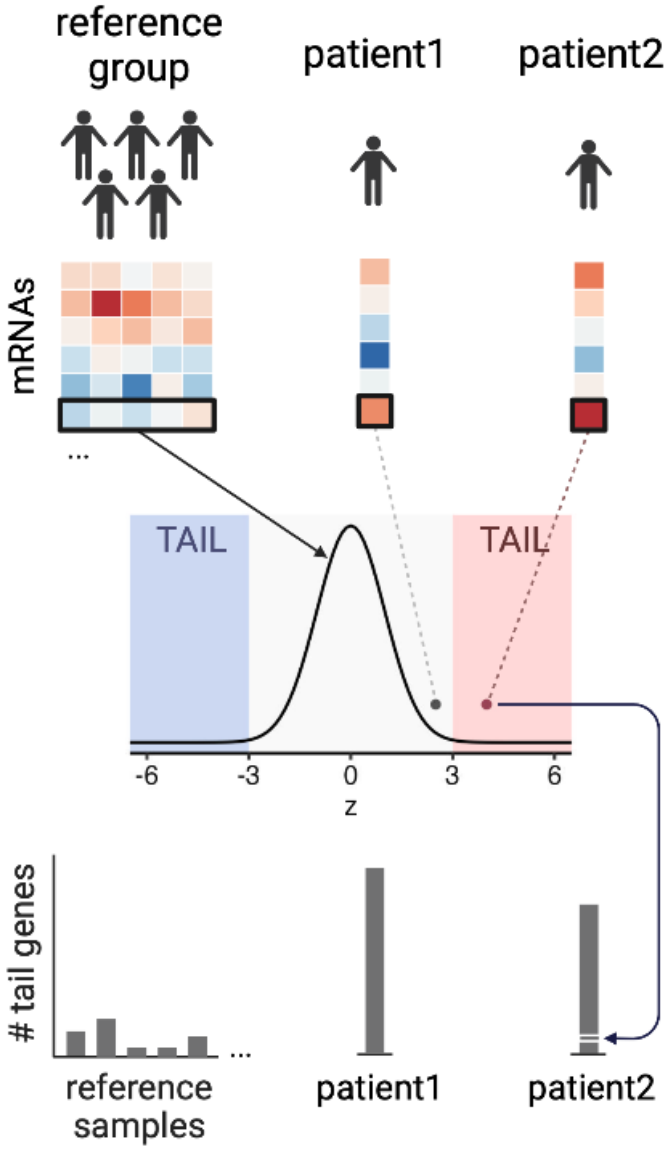
Morlion et al, in review
Morlion et al, 2023 medRxiv

Significant but limited overlap of differentially abundant mRNAs between cohorts



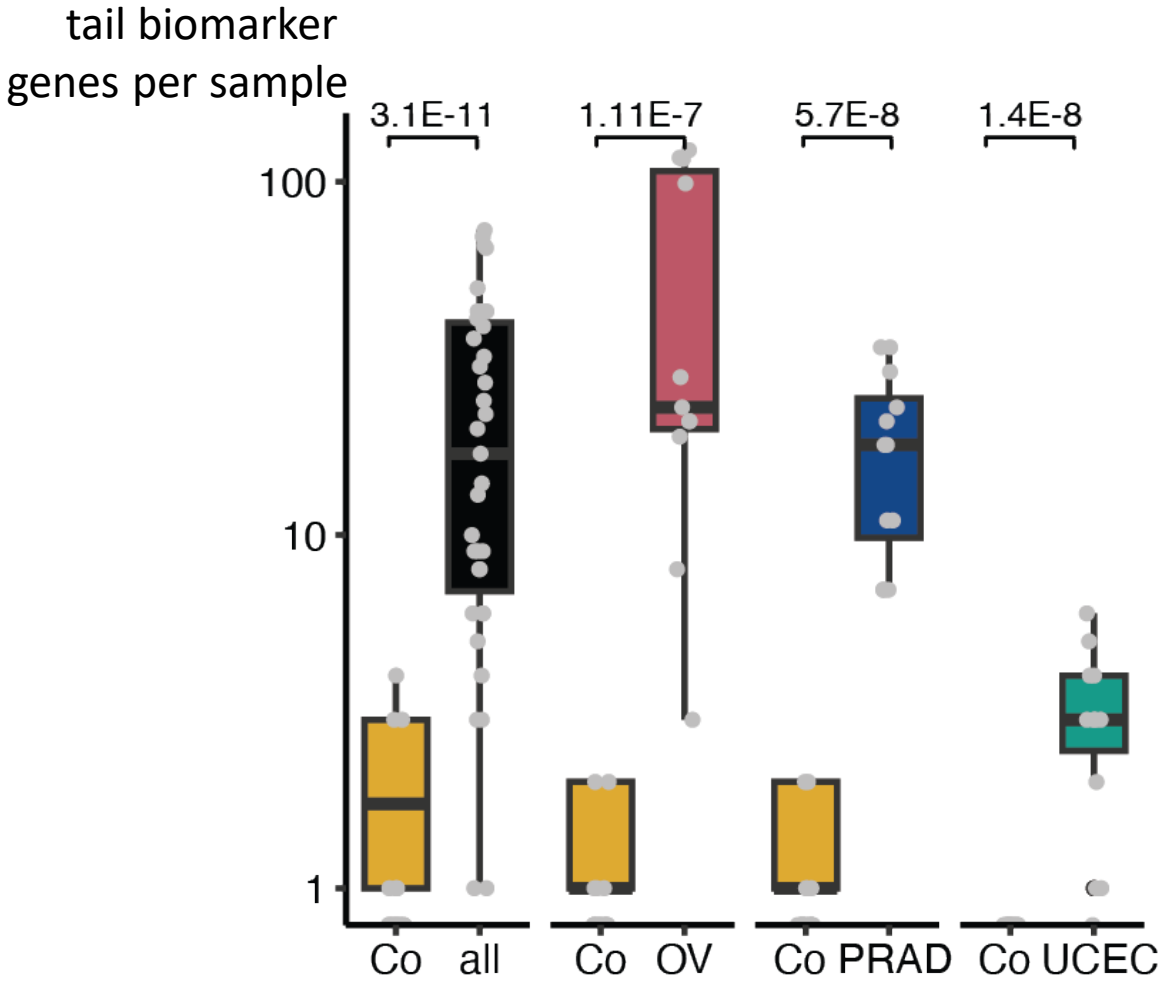
Morlion et al, in review
Morlion et al, 2023 medRxiv

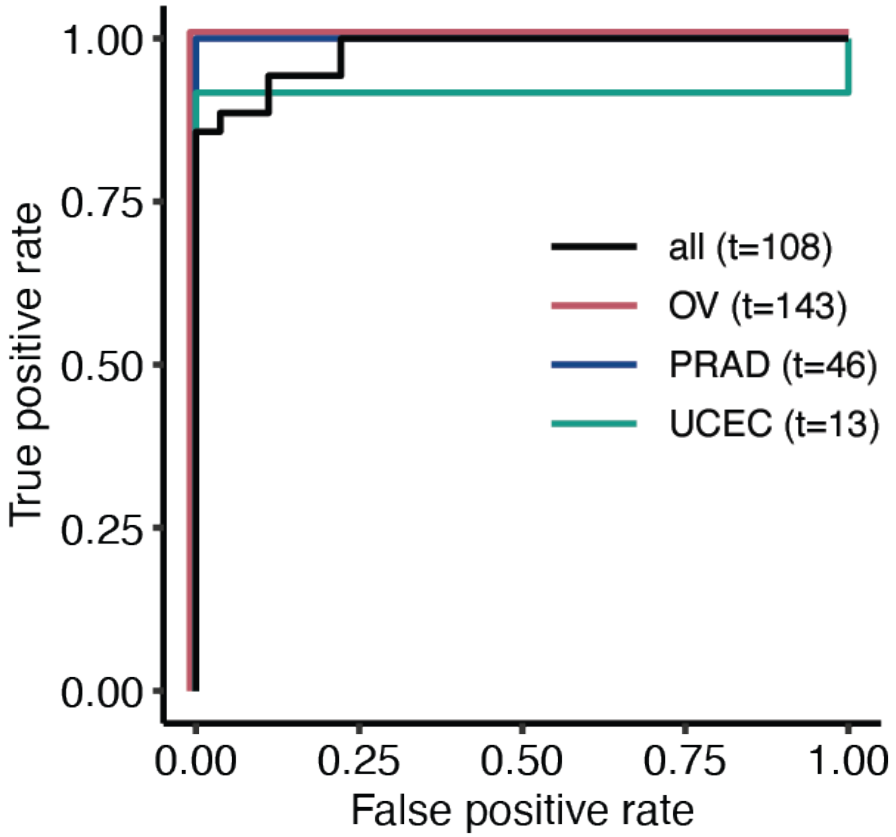
Variability calls for an alternative approach



Morlion et al, in review
Morlion et al, 2023 medRxiv

Accurate classification of cancer and controls based on the number of tail biomarker genes

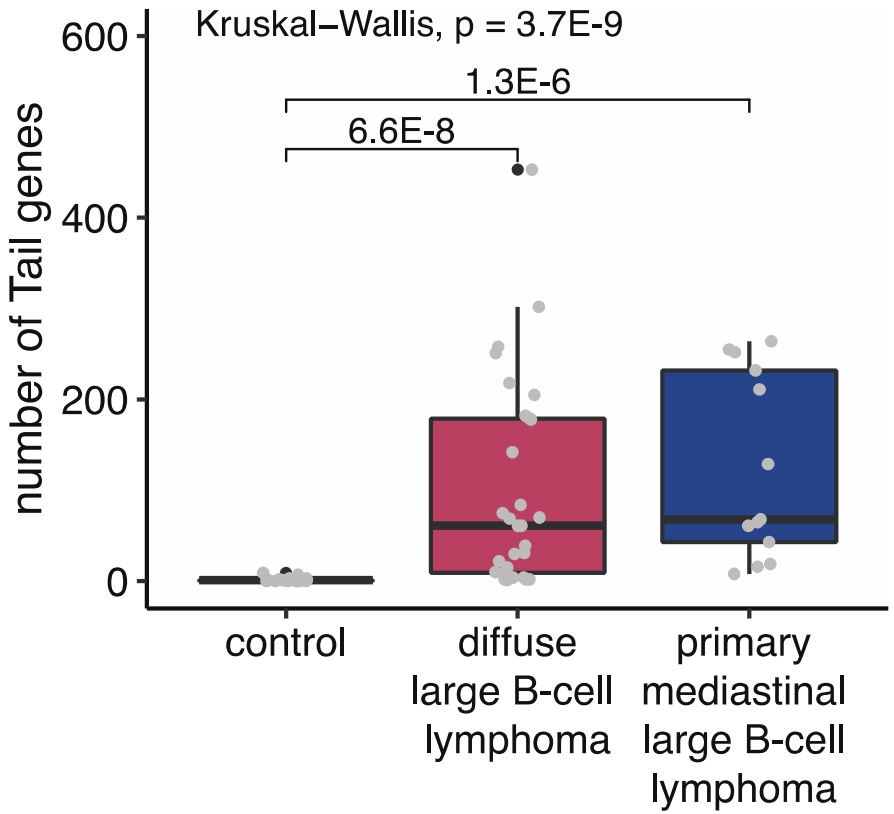


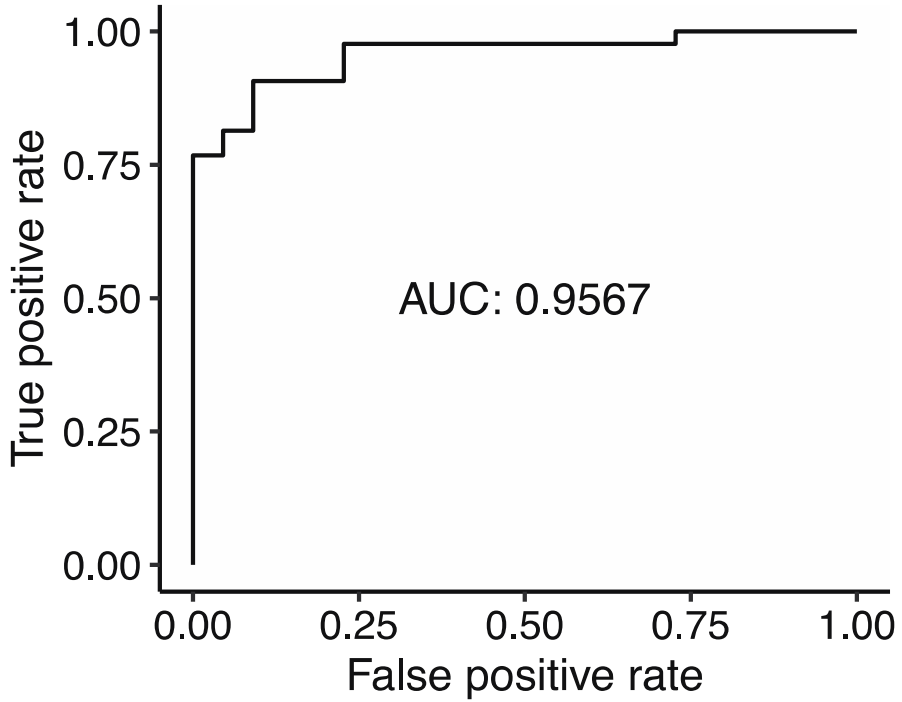


Morlion et al, in review
Morlion et al, 2023 medRxiv

Accurate classification of cancer and controls based on the number of tail biomarker genes in plasma of DLBCL

plasma
total RNA seq

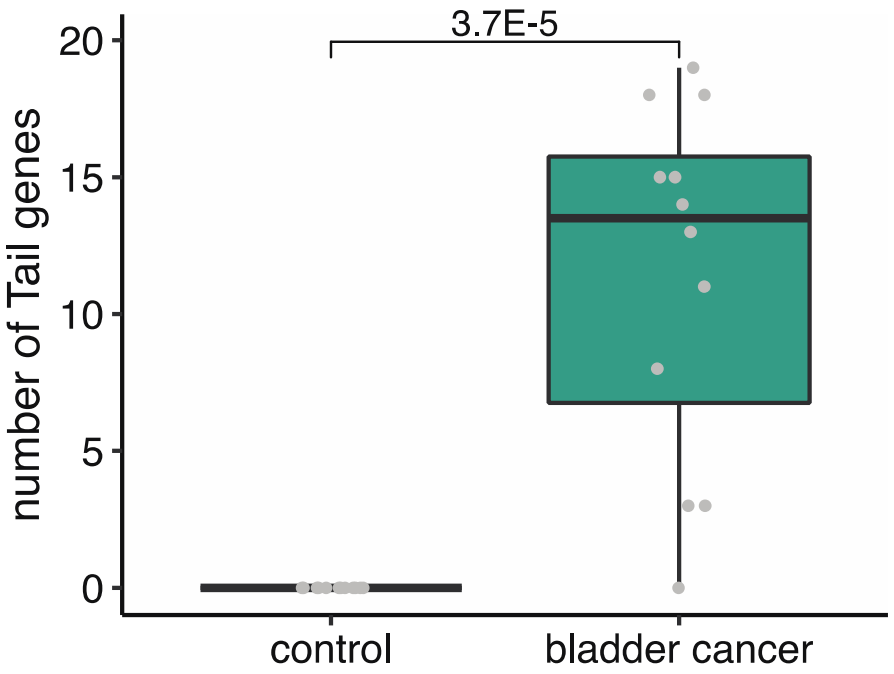


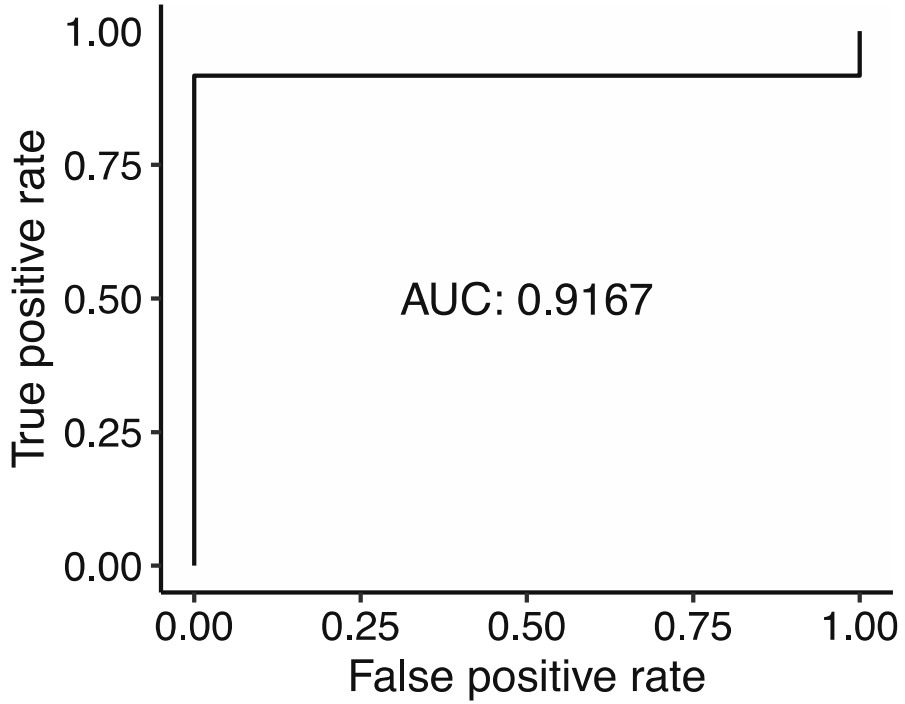


Morlion et al, in review
Morlion et al, 2023 medRxiv

Accurate classification of cancer and controls based on the number of tail biomarker genes in urine of bladder cancer patients

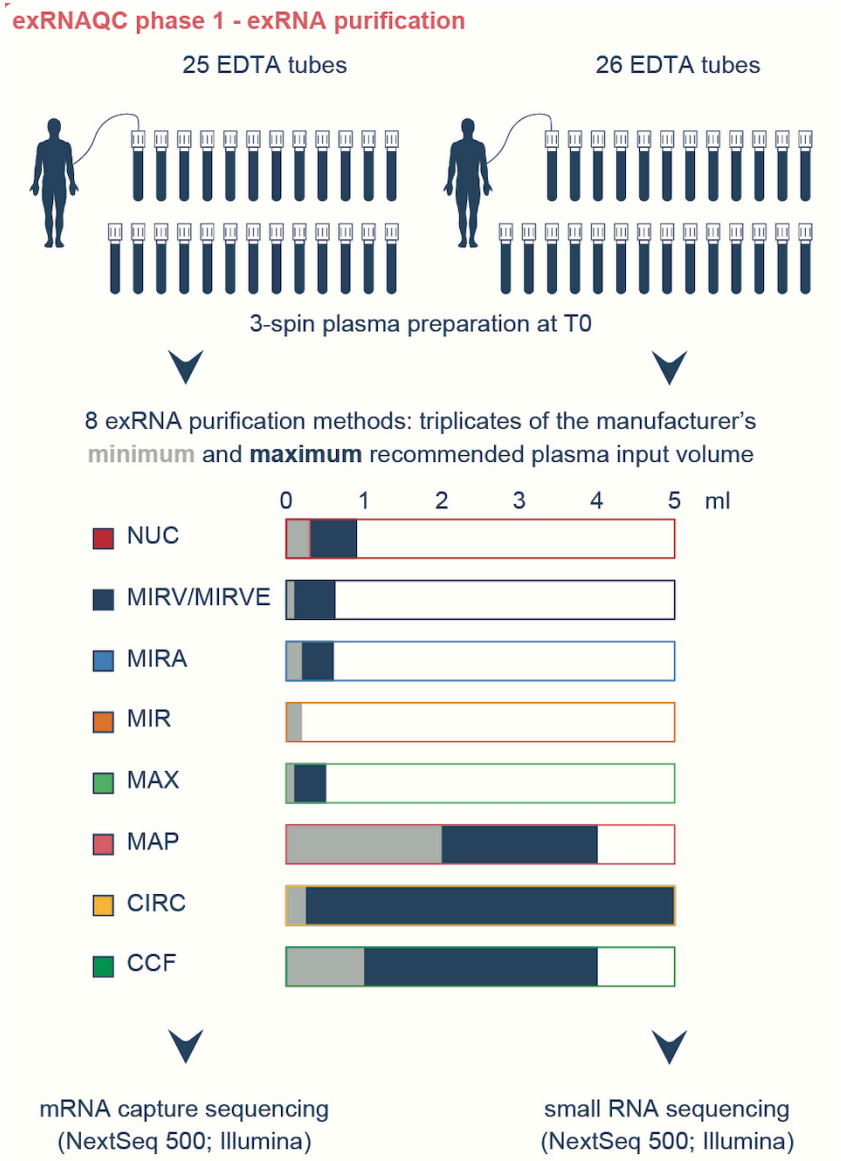
urine
mRNA-capture seq

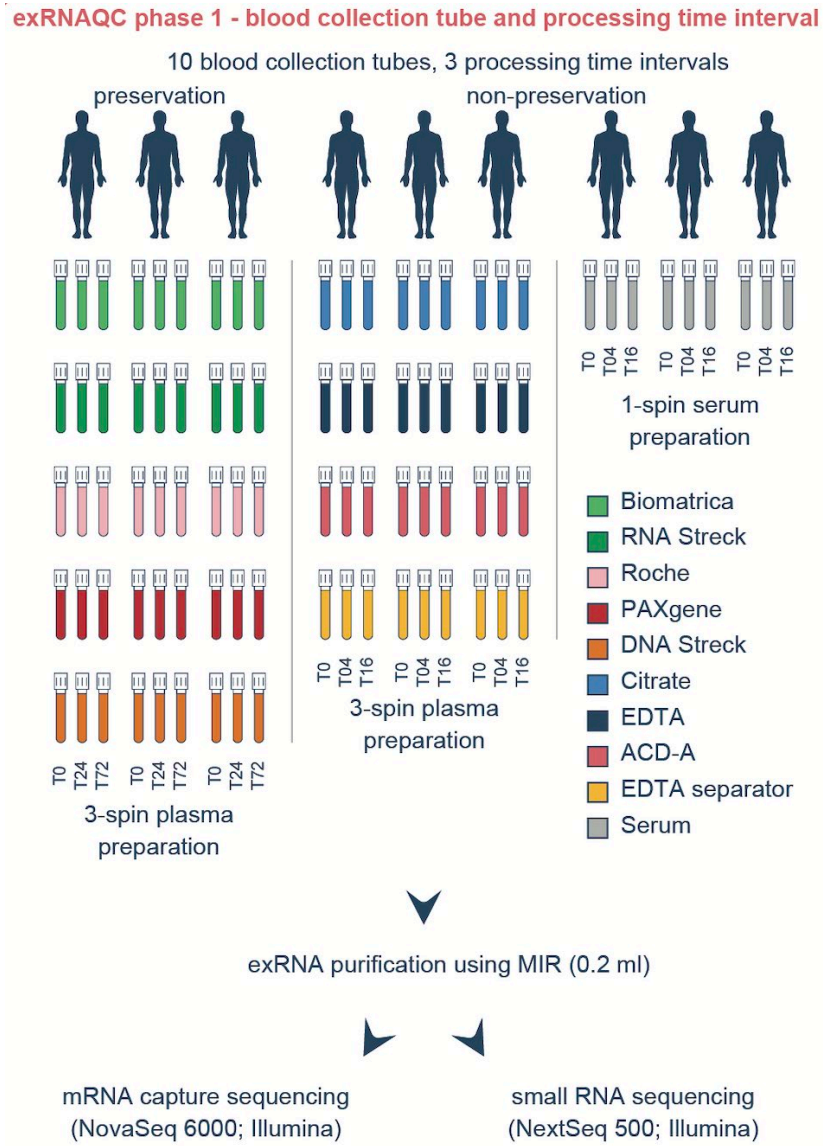




Morlion et al, in review
Morlion et al, 2023 medRxiv

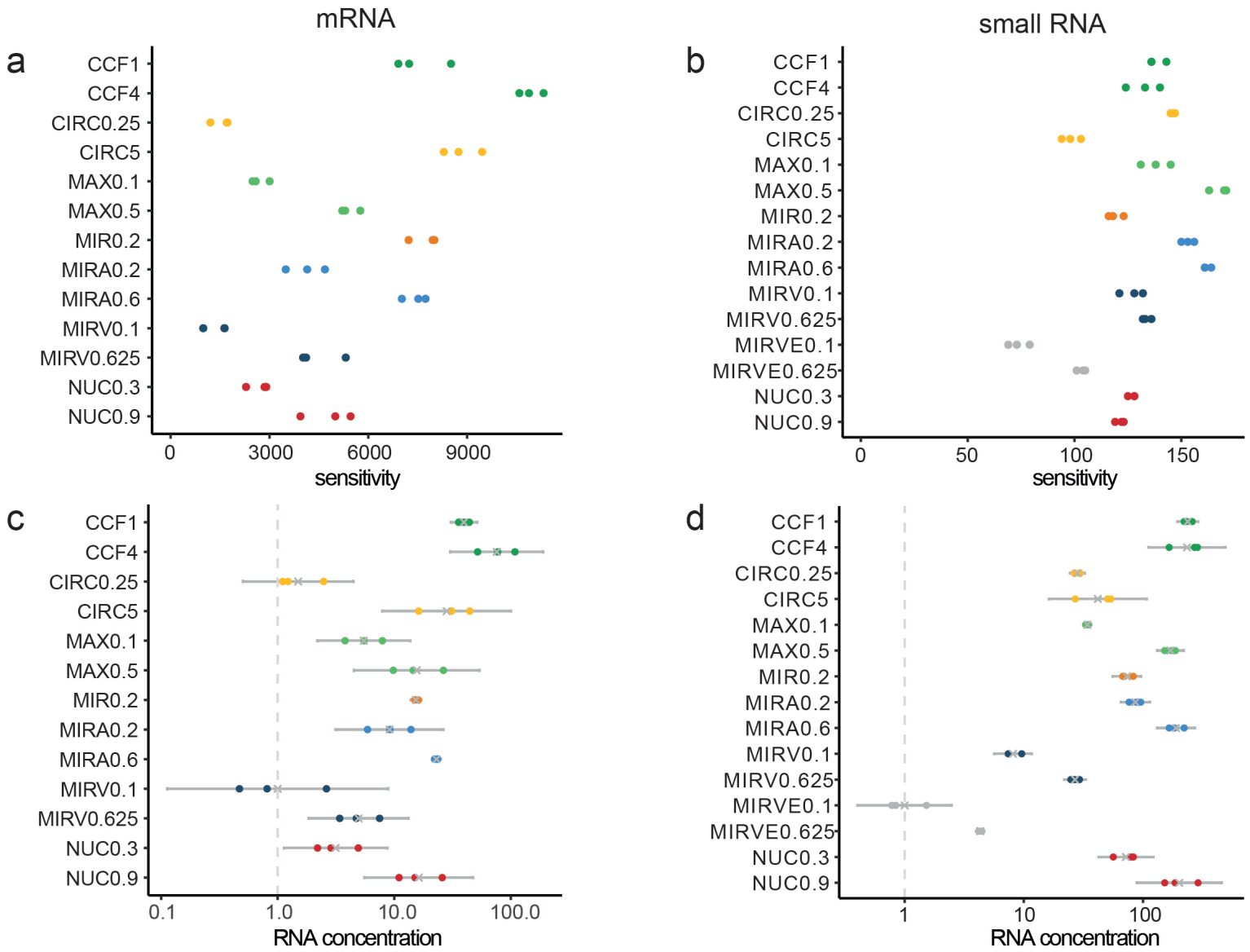
Impact of preanalytical variables on cfRNA profiles insights from the exRNAQC study





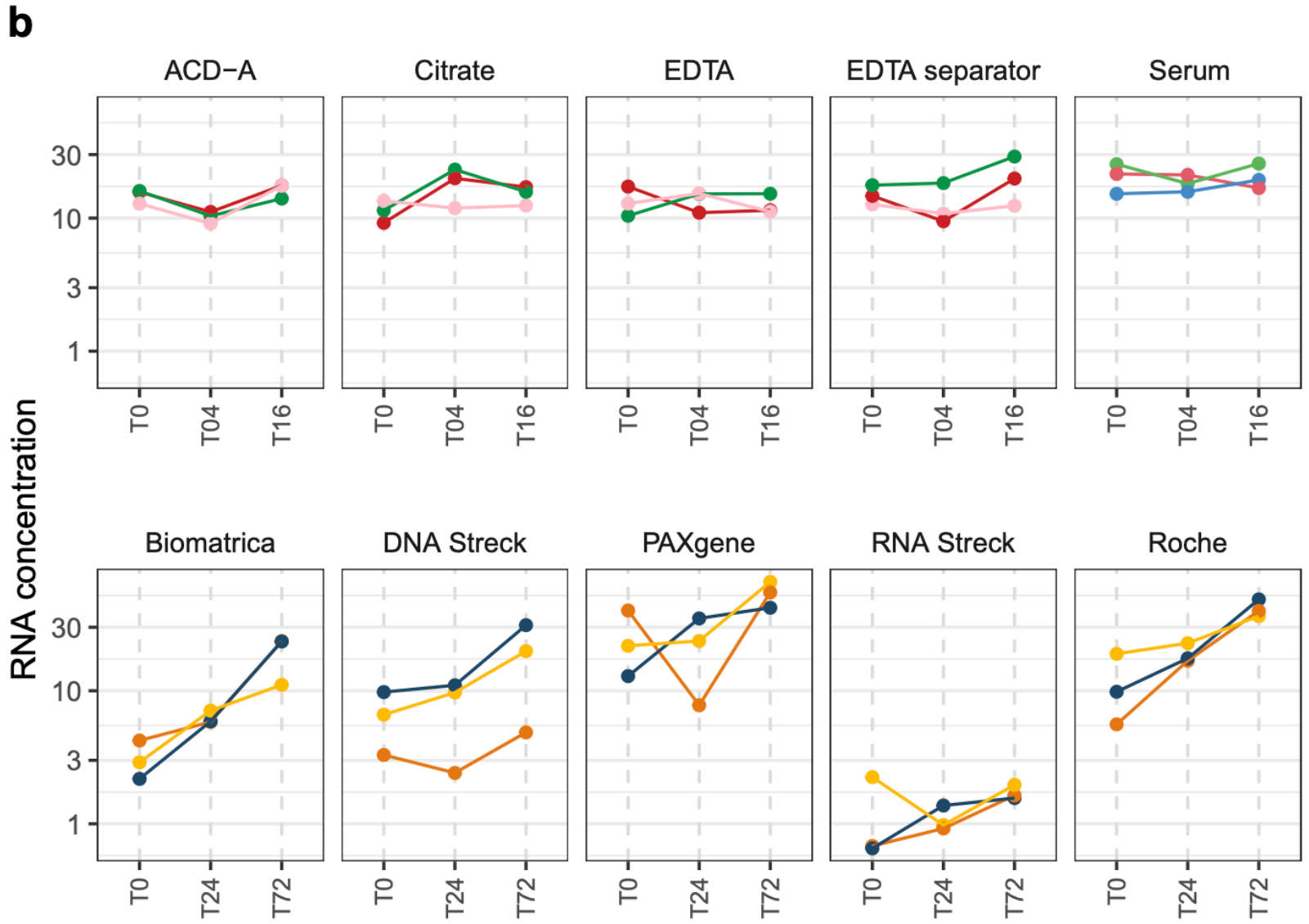
exRNAQC, 2022 bioRxiv

Large differences in RNA eluate concentration between kits



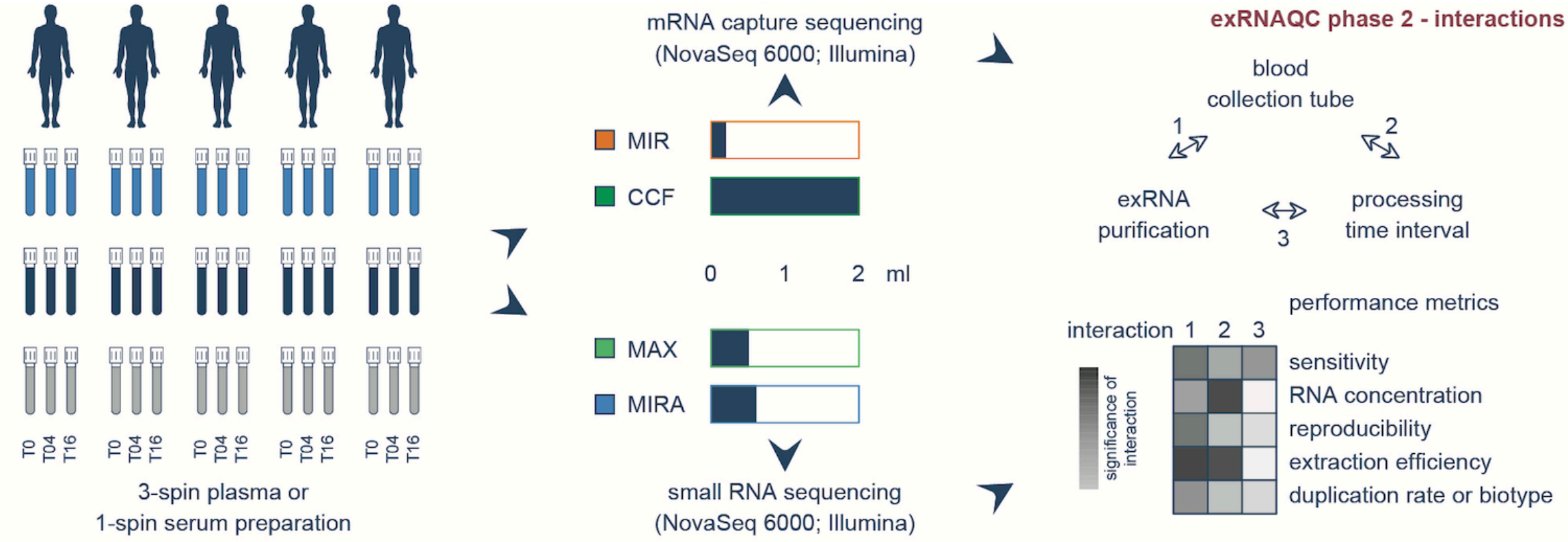
exRNAQC, 2022 bioRxiv

Preservation tubes do not preserve or stabilize cfRNA



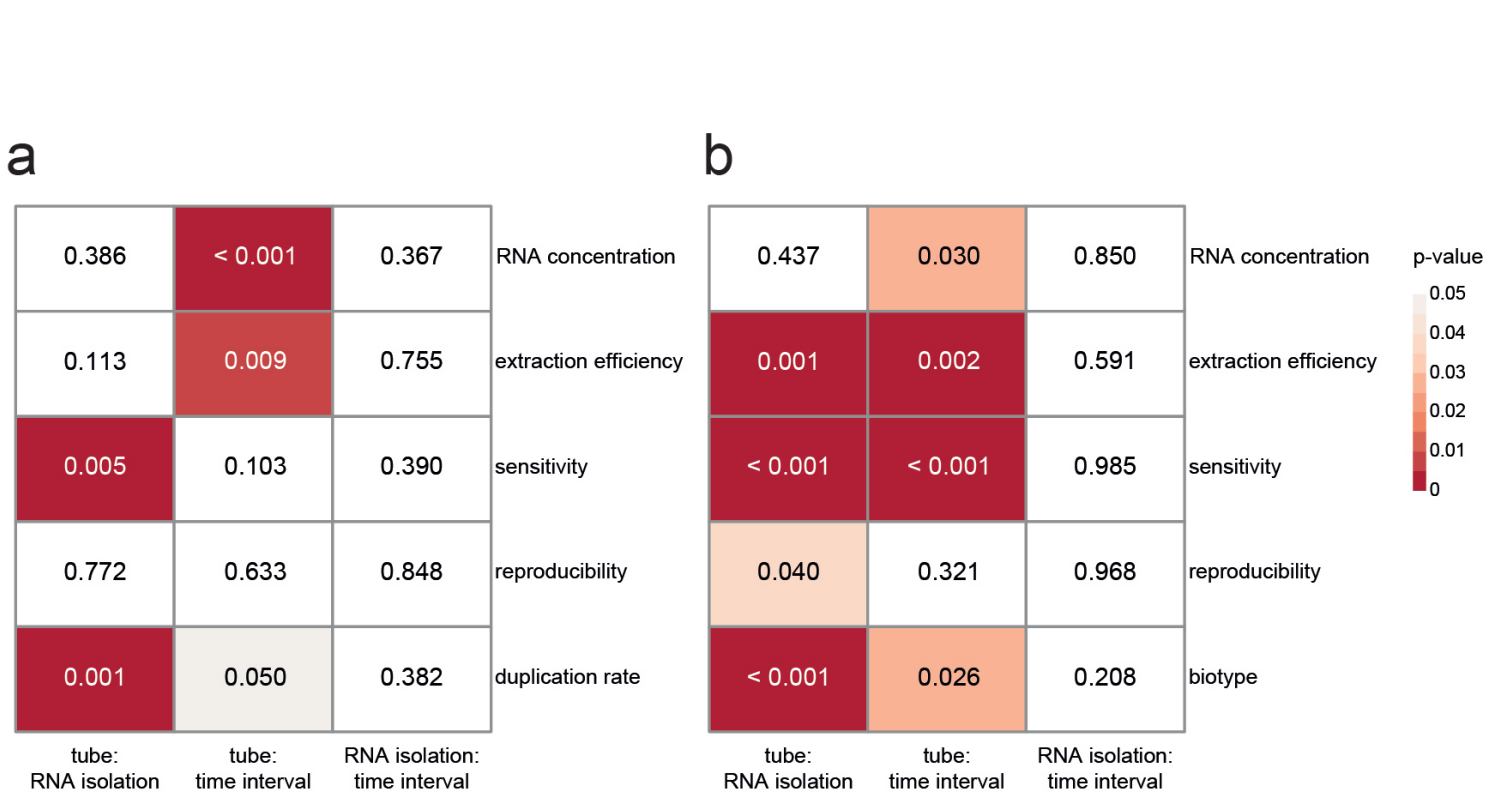
exRNAQC, 2022 bioRxiv

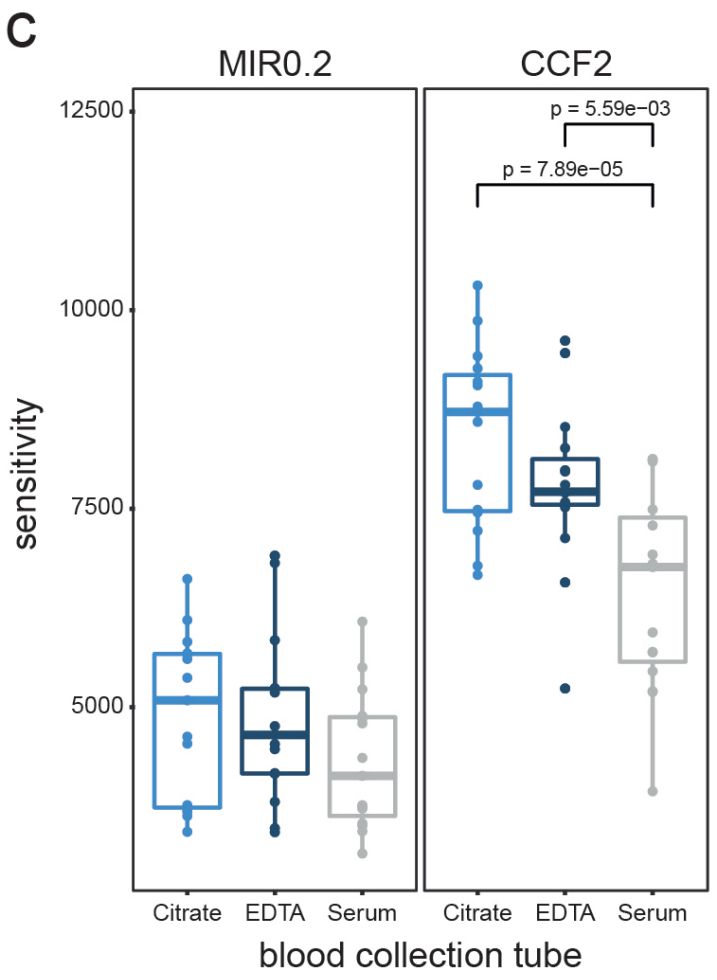
Interactions between preanalytical variables



exRNAQC, 2022 bioRxiv

Interactions between preanalytical variables





exRNAQC, 2022 bioRxiv

Conclusions and take home messages

- Standardize preanalytical variables for cfRNA research
- Use spike-in controls for workflow control

- Proximal liquid biopsies are enriched for proximal tissue RNA and may prove valuable for cancer biomarker detection

- Cancer plasma cfRNA profiles are heterogeneous and tail genes may serve as a cancer biomarker

Human biofluid atlas
Hulstaert E

Utero-tubal lavage cfRNA study
Hulstaert E
Levanon K (Sheba Cancer Center, Israel)

Cancer plasma cfRNA study
Morlion A

exRNAQC study
Avila-Cobos F, Decock A, Decruyenaere P, Deleu J, Dewilde J, Everaert C, Helsmoortel H, Hulstaert E, Morlion A, Poma Soto FA, Schoofs K, Vandesompele J, Van Paemel R, Verwilt J

Illumina
Gary Schroth
Scott Kuersten

CRIG

fwo

Kom op tegen Kanker