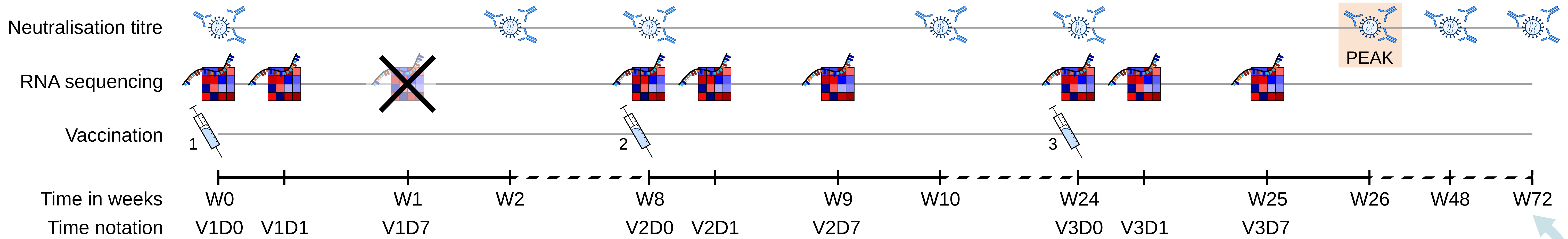


# Gene expression correlates of neutralisation differ between females and males after prophylactic HIV-1 vaccination

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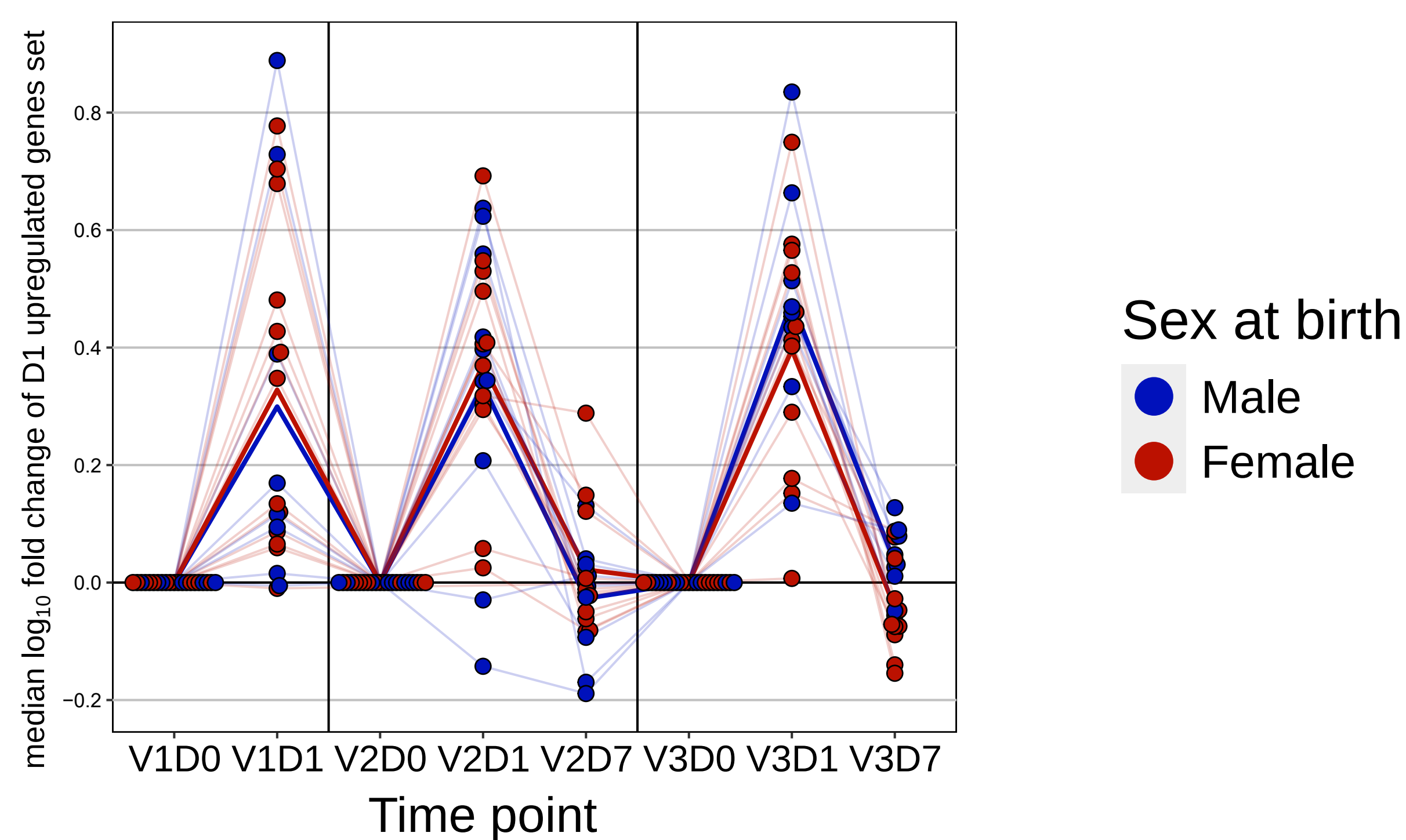


## 1 Introduction

- HIV still causes over 1 million new infections annually
- However, an effective prophylactic vaccine is still not available
- Major challenge: high genetic diversity of the HIV-1 Envelope surface antigen
- ConM SOSIP: artificial consensus Env construct of group M HIV-1 isolates
- Phase I trial: serial vaccination with MPLA-adjuvanted ConM
- Females showed 6.3-fold higher peak neutralisation titres on average
  - There was also significant variation between individuals

**Aim:** use blood bulk RNA sequencing to:

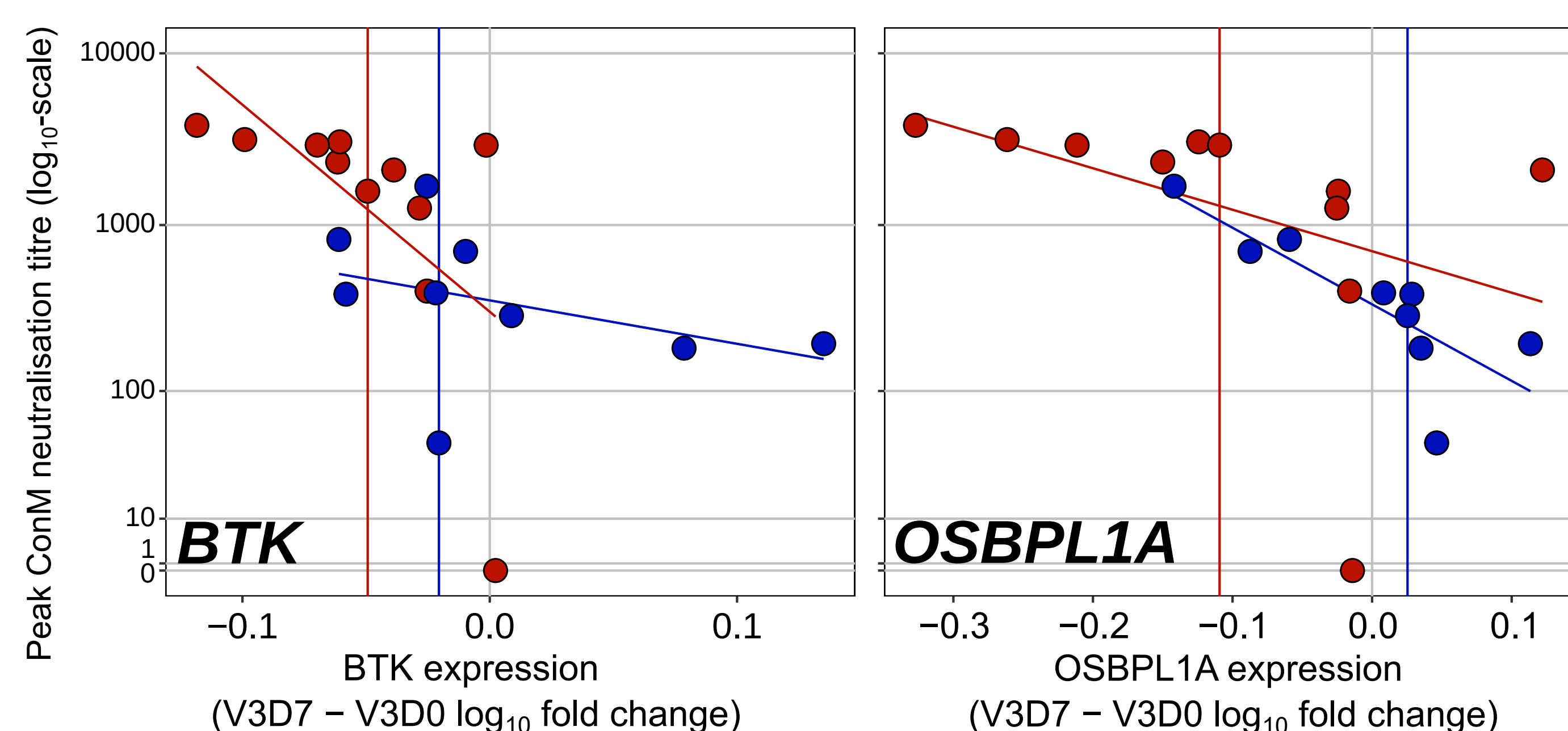
- Find gene expression patterns correlating with peak neutralisation titres
- Study patterns underlying sex-based differences



**Innate immune induction after vaccination.** Median log fold change of a day one-upregulated gene set per participant over time.

## 3 Innate-like immune response to vaccination

- This vaccine was adjuvanted with a TLR4 agonist (MPLA)
- We expected this to induce an innate immune reaction
- **Potent innate-like immune gene induction at day one after each vaccination**
- This occurred in almost all participants after each vaccination
- Most significantly induced genes were innate-related *FCGR1A*, *PI3*
- The innate immune response had abated in most cases by day seven
- **No significant difference between females and males in magnitude of induction**
- Magnitude of induction was not a strong correlate of peak neutralisation titre



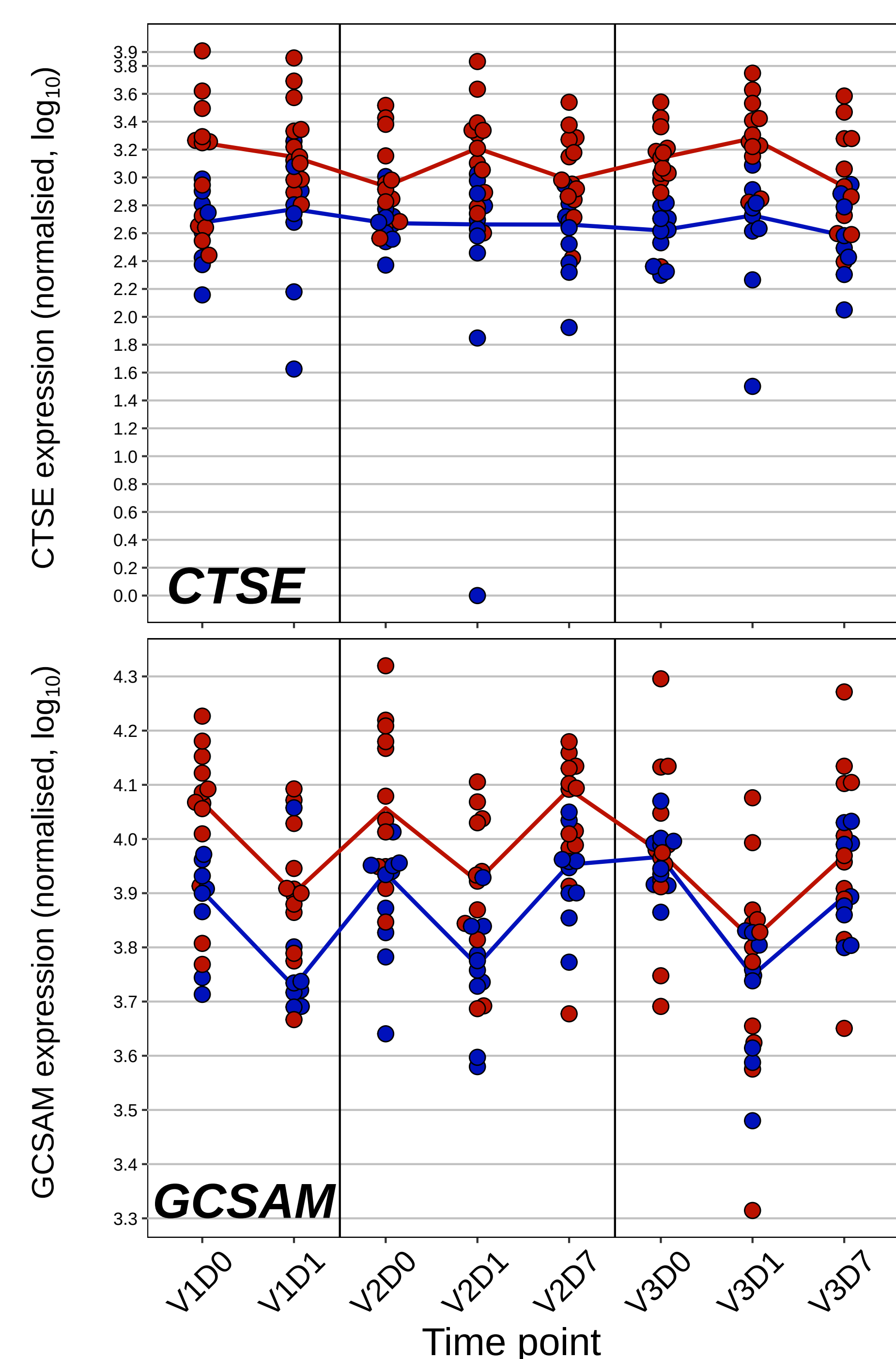
**OSBPL1A and BTK show both titre correlation and sex difference.** Log fold change of gene expression between third vaccination day zero and day seven for each participant (x-axis). Peak (week 26) neutralisation titre in log-scale (y-axis).

## 5 Gene expression correlates of neutralisation

- So far, no clear explanation for differences in peak neutralisation titres
- Broad screen of ~18k expressed genes to find correlates of neutralisation
- Most correlation signal at days one and seven after the third (last) vaccination
- **Correlates included known key immune genes:**
  - *PTPRC* (leukocyte common antigen/CD45) positive correlation at day one
  - Negative correlations at day seven, such as *TLR4*, *BCL10* and *TRAF6*
- **Key genes OSBPL1A and BTK show both:**
  - Negative correlation at day seven
  - Sex-based difference in expression levels (male-skewed)
- Interpretation:
  - Certain genes could correlate with peak neutralisation titre
  - A subset of these may show a sex-based difference in expression levels

## 2 Methods

- 23 healthy participants (13 female, 9 male + 1 MTF transgender)
- 3 vaccinations with MPLA-adjuvanted ConM SOSIP trimer
- Pseudovirus neutralisation titre measurements: peak at week 26
- **Whole blood bulk RNA sequencing** over eight time points for all participants
  - **Measures expression levels of all transcribed genes**
- Samples for days zero, one and seven after each vaccination
- Custom bioinformatics analyses, including sex-aware correlation analysis



**CTSE and GCSAM show female-skewed expression at baselines and other time points.** Log-transformed normalised expression levels (y-axis) over time (x-axis) for each participant.

## 4 Baseline sex differences in immune state

- Females and males differ in immune responses to infection and vaccination
- We screened known immune genes for sex-based differential expression at baselines, i.e. day zero after each vaccination
- **Several genes differed between females and males at baselines for each dose**, including female-skewed expression in genes of interest:
  - *CTSE*, an MHC-II antigen processing protease
  - *GCSAM*, affects germinal centre B cell residency time
- Differences in baseline immune state could affect immune processes, potentially influencing the generation of neutralising antibodies
- These genes did not directly correlate with peak neutralisation titres

## 6 Conclusions & perspectives

We set out to:

- Find blood gene expression correlates of peak neutralisation titre
- Identify patterns related to the observed sex-based difference in titres

**Key results:**

- Vaccination induced a potent innate-like immune response
- Baseline immune gene expression state may differ between sexes
- We identified several genes showing correlation with peak neutralisation titre
- *BTK* and *OSBPL1A* also show possible sex-related differential expression

These findings contribute to our understanding of the immune processes leading to protective immunity after vaccination, and in particular, regarding the contribution of biological sex to these processes. Given the limited sample size, further insights may be gained from large-scale integrative analysis of post-vaccination omics datasets.