# flowReMix

(temporary name)

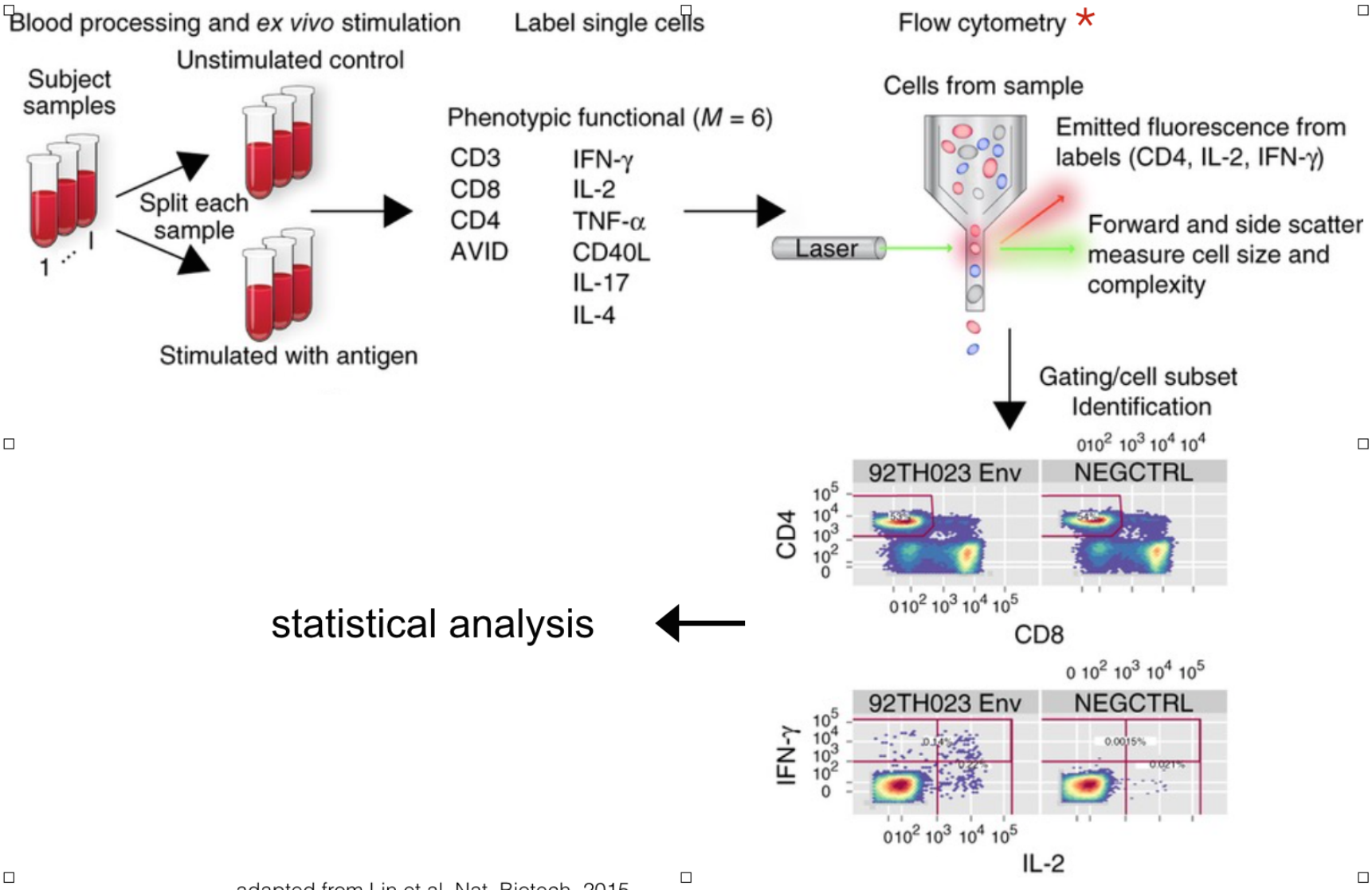
A **Mixture** of **Mixed** Beta-Binomial **Regression** Models for Analyzing **Flow**-Cytometry Count data

February 21, 2017

# Outline

1. Introduction to Flow-Cytometry
2. Motivation
3. Models:
   - A Marginal Model
   - A Joint HMRF model
4. Data analysis.
5. Computation

# Introduction to Cytometry Count Data




adapted from Lin et al, Nat. Biotech. 2015

# Introduction to Cytometry Count Data

| PTID | Subset | stim | count | parentcount |
|---|---|---|---|---|
| P1003 | CD154 | stim | 38 | 23524 |
| P1003 | CD154 | nonstim | 31 | 28099 |
| P1003 | CD154,IL17a | stim | 23 | 23524 |
| P1003 | CD154,IL17a | nonstim | 30 | 28099 |
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| P1003 | IFNg,IL2 | stim | 2 | 23524 |
| P1003 | IFNg,IL2 | nonstim | 0 | 28099 |
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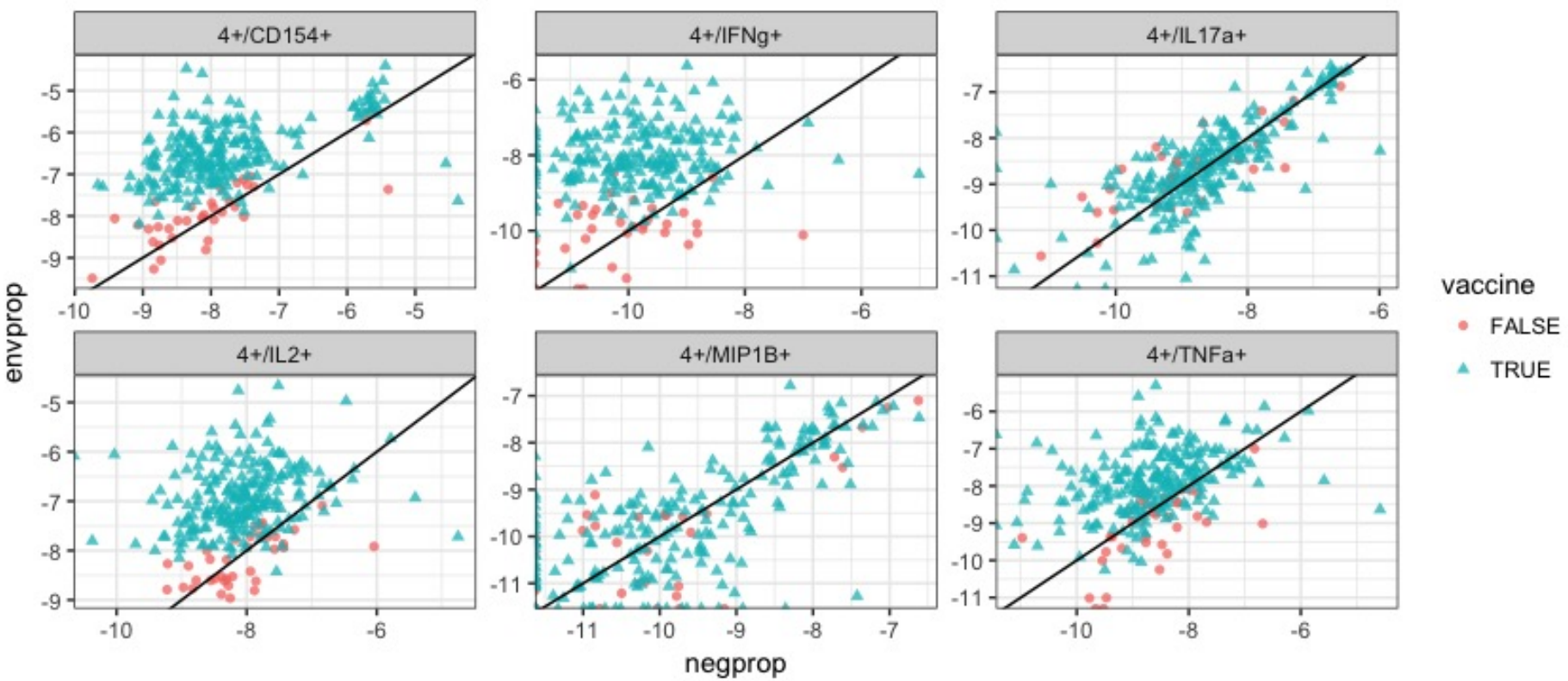
| P1003 | IFNg,IL2,CD154 | nonstim | 0 | 28099 |
| P1003 | IFNg,IL4,IL2,CD154 | stim | 0 | 23524 |
| P1003 | IFNg,IL4,IL2,CD154 | nonstim | 0 | 28099 |

# The RV144 HIV Vaccine Study

- **286 Subjects**
  - 246 Cases
  - 40 Controls
- **2 Types of stimulus**
  - HIV antigen
  - Negative control
- **6 types of cytokines.**

# Marginal Counts for RV144



# Motivation: COMPASS

ANALYSIS

_computational BIOLOGY

## COMPASS identifies T-cell subsets correlated with clinical outcomes

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**Or in general, with immune response.**

# How do current methods work? (Approximately)

Current models are baseline/stimulation models.

- Unstimulated blood sample are compared stimulated ones.

For the unstimulated sample of the $i$th subject out of $n$, we sample a count proportion:

$$p_{i0} \sim \text{Dirichlet}(\alpha_0, \beta_0),$$

$$y_{i0} \sim \text{Multinomial}(N_{i0}, p_{i0}).$$

Let $k_i \in \{0,1\}^p$ indicate in which subsets $i$ responds:

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# Controlled Human Malaria Infection Study

- 9 Tanzanian adults were infected with Malaria.
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- 113 measured cell-types divided into 8 groups.

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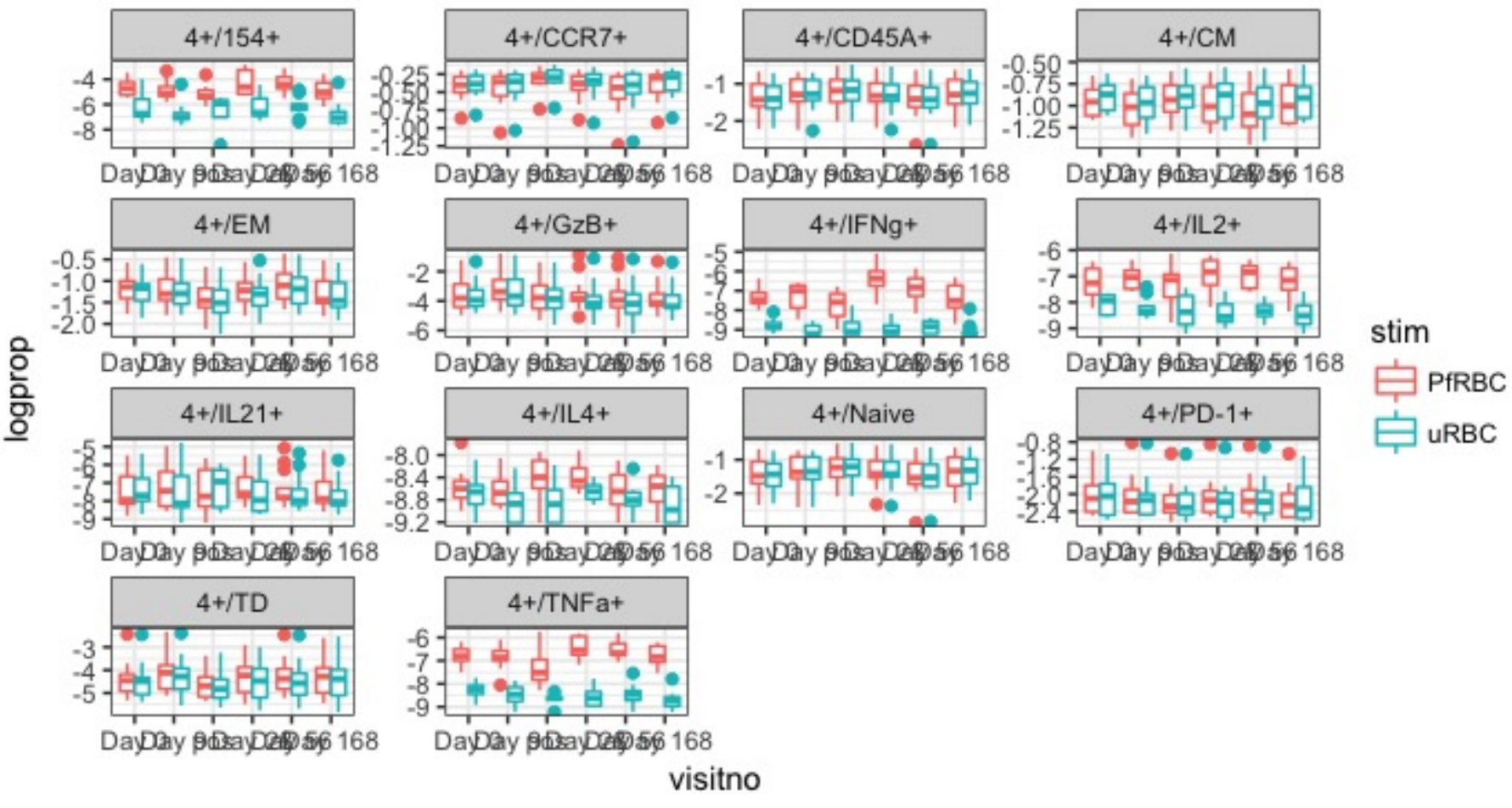

# Motivation - A Regression Model

- We want to be able to include covariates:
  - Batch effects.
  - Other covariates such as age, gender...
- Longitudal data.
- More than one stimulation.
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# Motivation - Unique Challanges

- **Dependence**
  - Within sample between cell subsets.
  - Within subject / across time.
- **Heterogenous treatment effect**
- **Over-dispersed Binomial counts**

# A Marginal Model - Single Subset

Indexing: **i**-subject, **t**- stimulation/time-point.

Overdispersion $\Rightarrow$ Beta Binomial Model.

$$\text{logit}(\mu_{it}) = X_{it}\beta,$$

$$p_{it} \sim \text{Beta}(M\mu, M(1-\mu)),$$

$$y_{it} \sim \text{Binom}(N_{it}, p_{it})$$

# A Marginal Model - Single Subset

Indexing: **i**-subject, **t**- stimulation/time-point.

Dependence $\Rightarrow$ 'random' subject baseline:

$$\nu_i \sim N(0, \sigma^2)$$

$$\text{logit}(\mu_{it}) = X_{it}\beta + \nu_i$$

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# A Marginal Model - Single Subset

Indexing: **i**-subject, **t**- stimulation, **k**- cluster.

Non-response $\Rightarrow$ Mixture-Model:

$$k \sim Ber(\pi),$$

$$\text{logit}(\mu_{itk}) = X_{it}\beta + T_{it}\tau_k + \nu_i,$$

- $T$ a matrix of covariates related to the treatment.
- $\tau_k$ equals 0 if $k = 0$ or $\tau \neq 0$ if $k = 1$.

# A Marginal Model - Recap

Indexing: **i**-subject, **t**- stimulation, **k**- cluster.

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**Model can be estimated via an EM algorithm**

# Wellness of Fit Evaluation

How do we evaluate the model?

- We fit the model without information regarding the true treatment allocation.
- The model should be able to discriminate between vaccinees and placebos.
- We use three type of figures:
  - Scatter plots w/classification information.
  - Receiver-Operator Curves.
  - False Detection Rates.

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# Marginal Model - Results

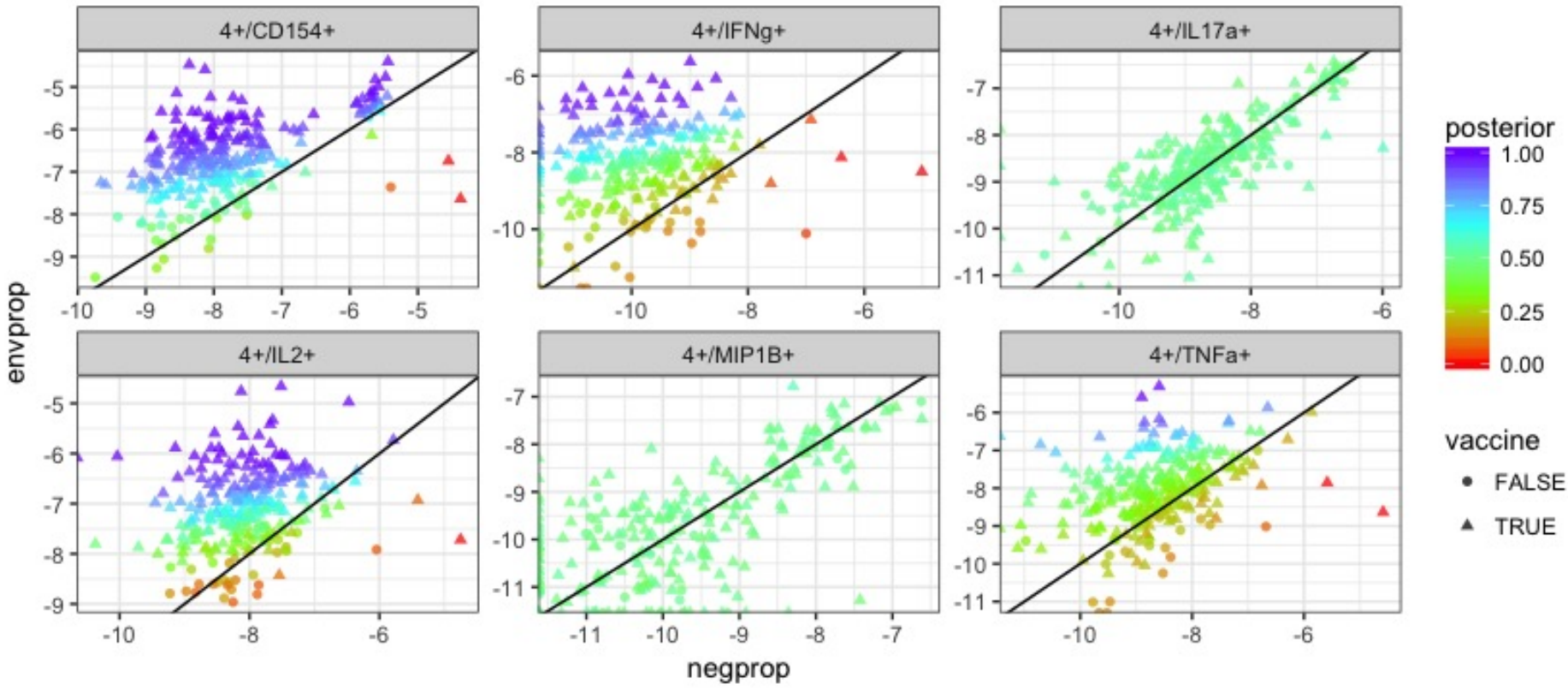


Figure: Posterior Probabilities for RV144 dataset - Independence Model

# Comparison w/ MIMOSA - Finak et al. (2013)

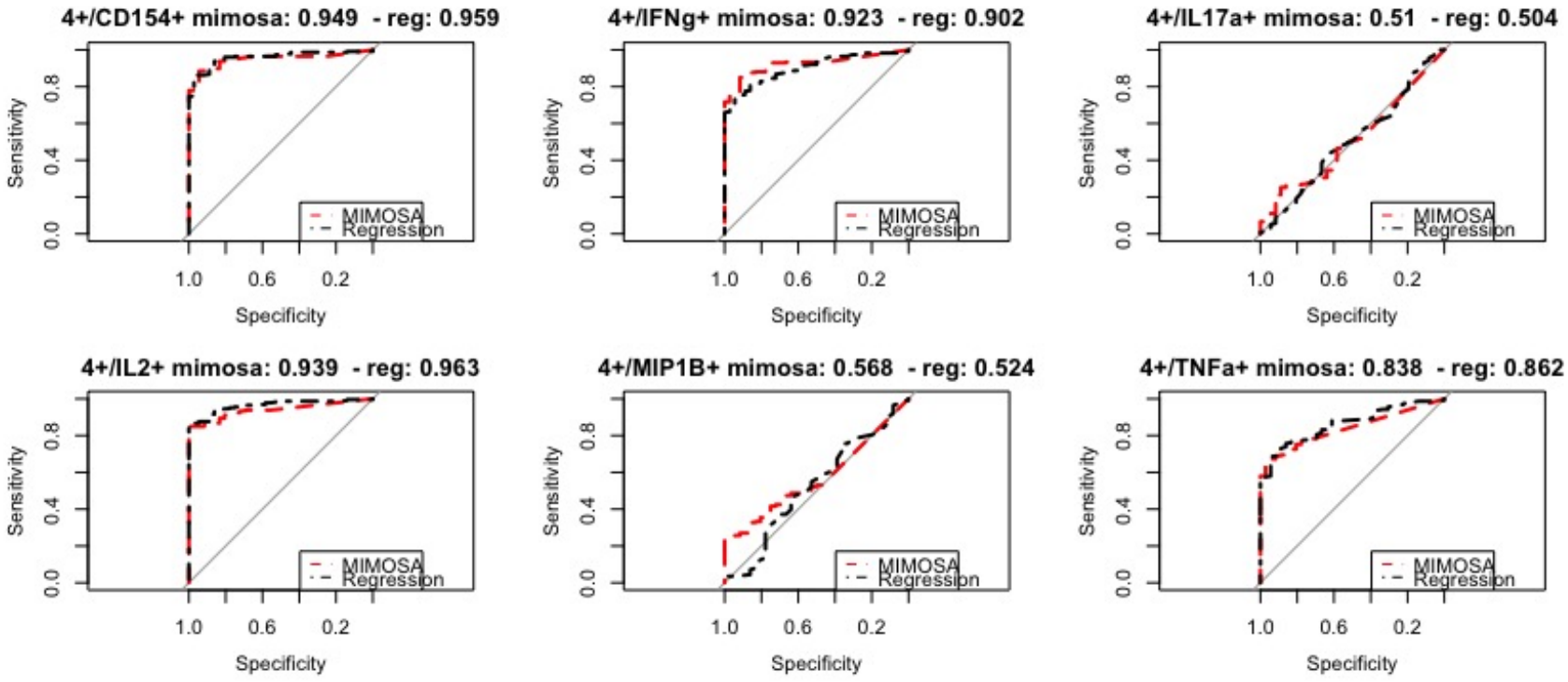


Figure: Comparison with MIMOSA (univariate COMPASS)

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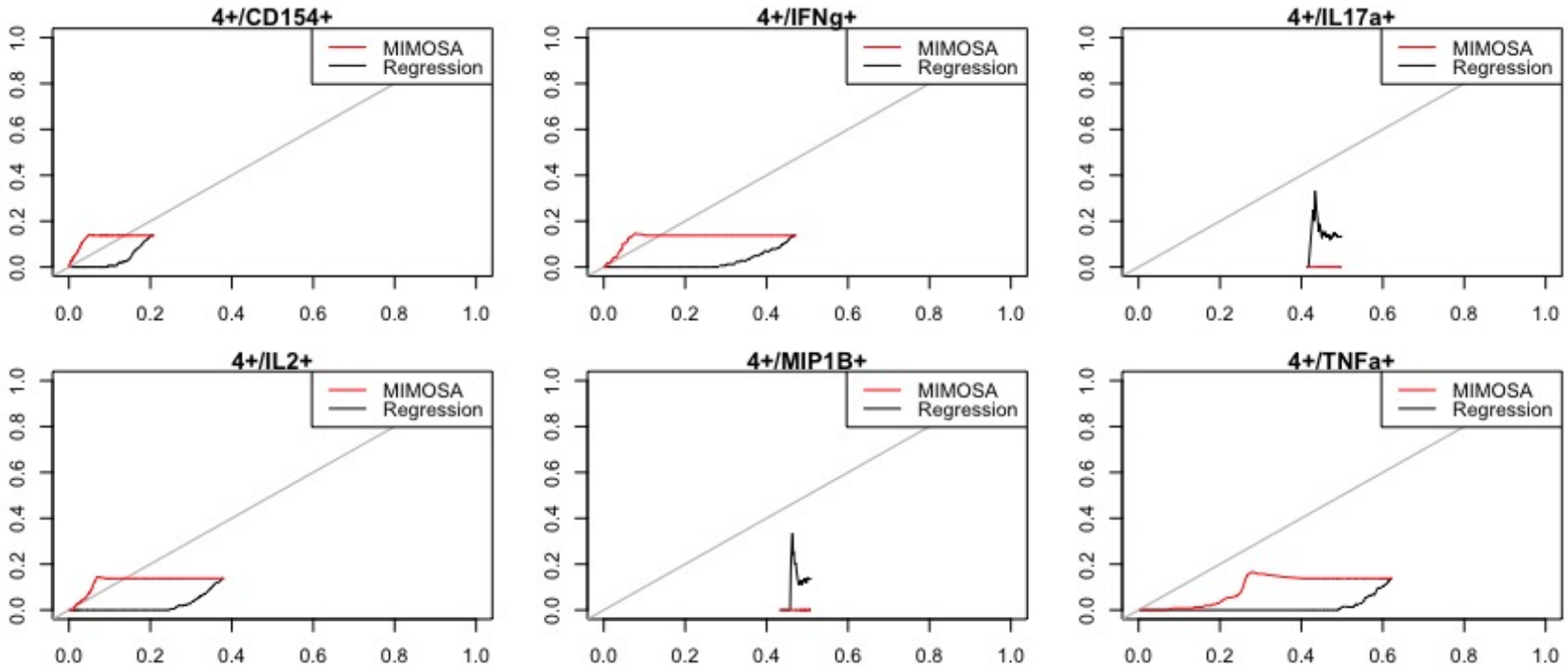


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# Subject-Response Model

Performing analysis for each cell-subset at a time doesn't use all of the information available.

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- Response is probably not independent across cell-subsets.
- We might be able to improve classification of response by looking at several cell-subsets at once.

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# A Hidden Markov Random Field Model

Indexing: **i**-subject, **t**- stimulation, **j**- subset, **k**- cluster.

Denote cluster (Response) by a $k \in \{0,1\}^p$ vector with 1 indicating a responsive subset.

We assume an Ising model for the dependence structure between subsets:

$$P(k) \propto \sum_{j=1}^{p} k_j \theta_j + \sum_{s \neq t} k_t k_s \theta_{st},$$

$$P(k_j = 1 | k_{-j}) = \theta_j + \sum_{t \neq j} k_t \theta_{tj}.$$

We can induce sparsity through an $\ell_1$ penalty.

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# HMRF Model - Results

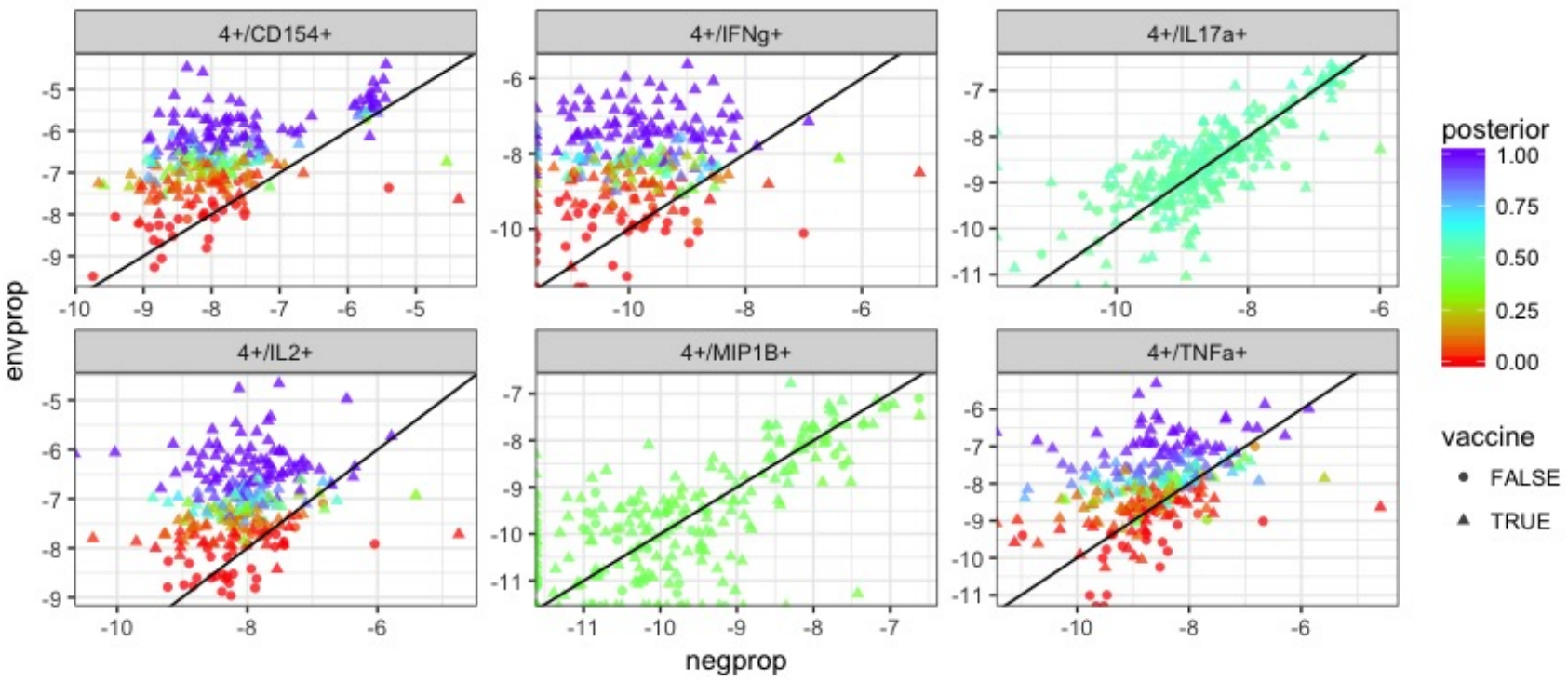


Figure: Scatter Plot for HMRF Modle Model

# Subset-Response Model - Results

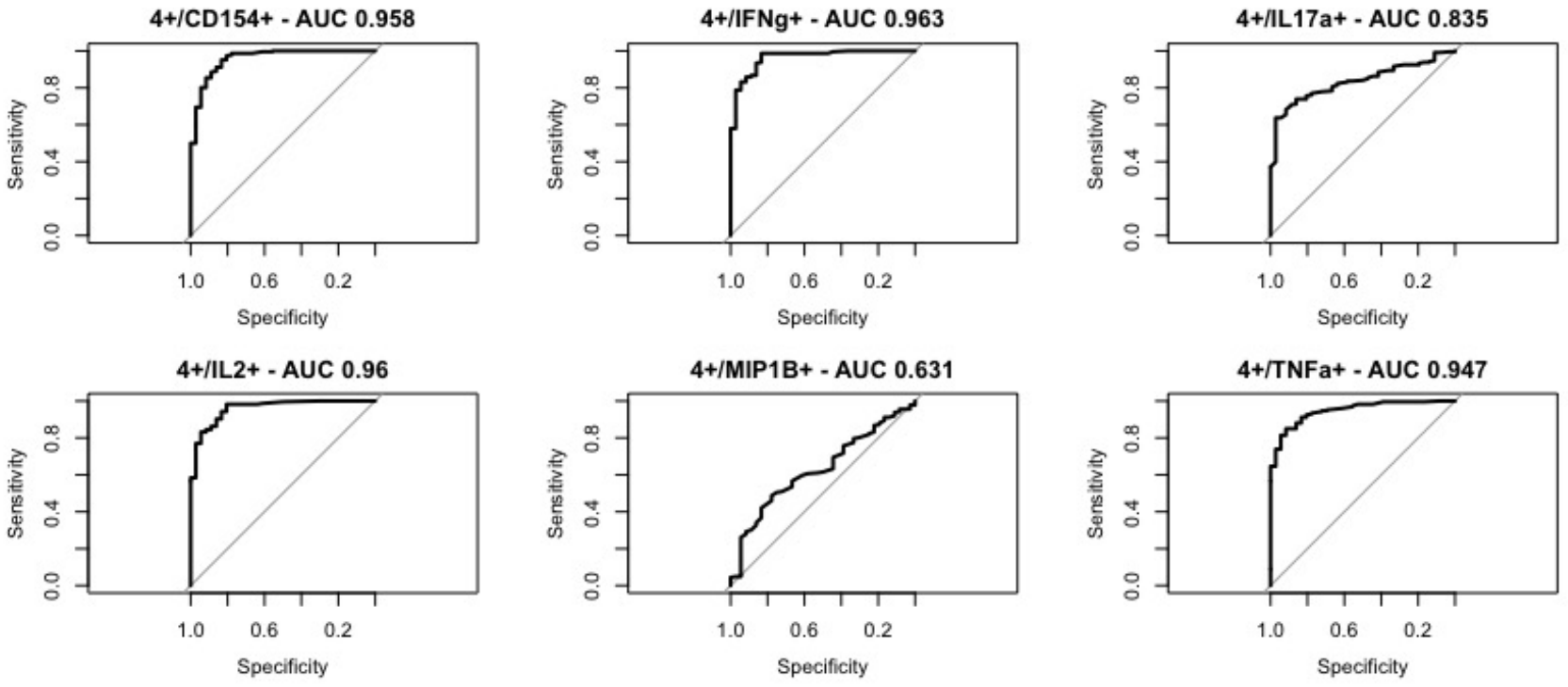


Figure: ROC for HMRF Modle

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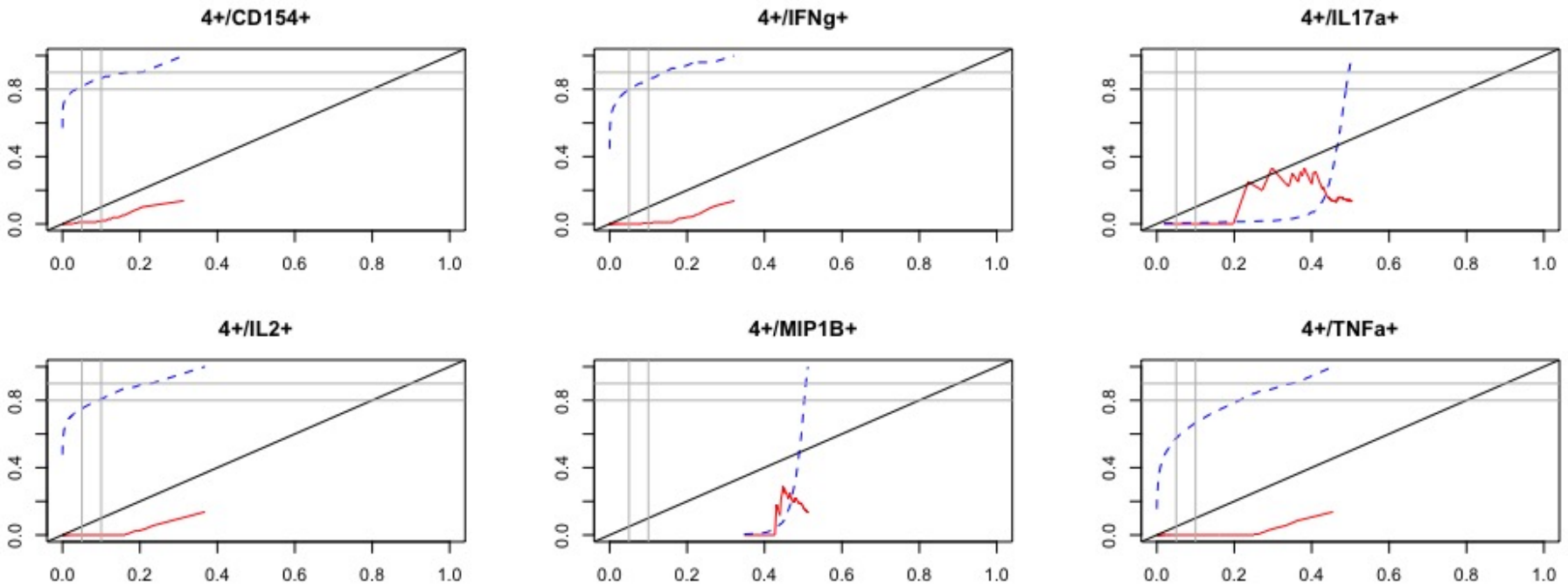


Figure: FDR for HMRF Modle

# Simulated Data Experiment

- Posterior probabilities are not well calibrated.
  - Might be due to true non-response.
- Is the optimization algorithm estimating the model properly?
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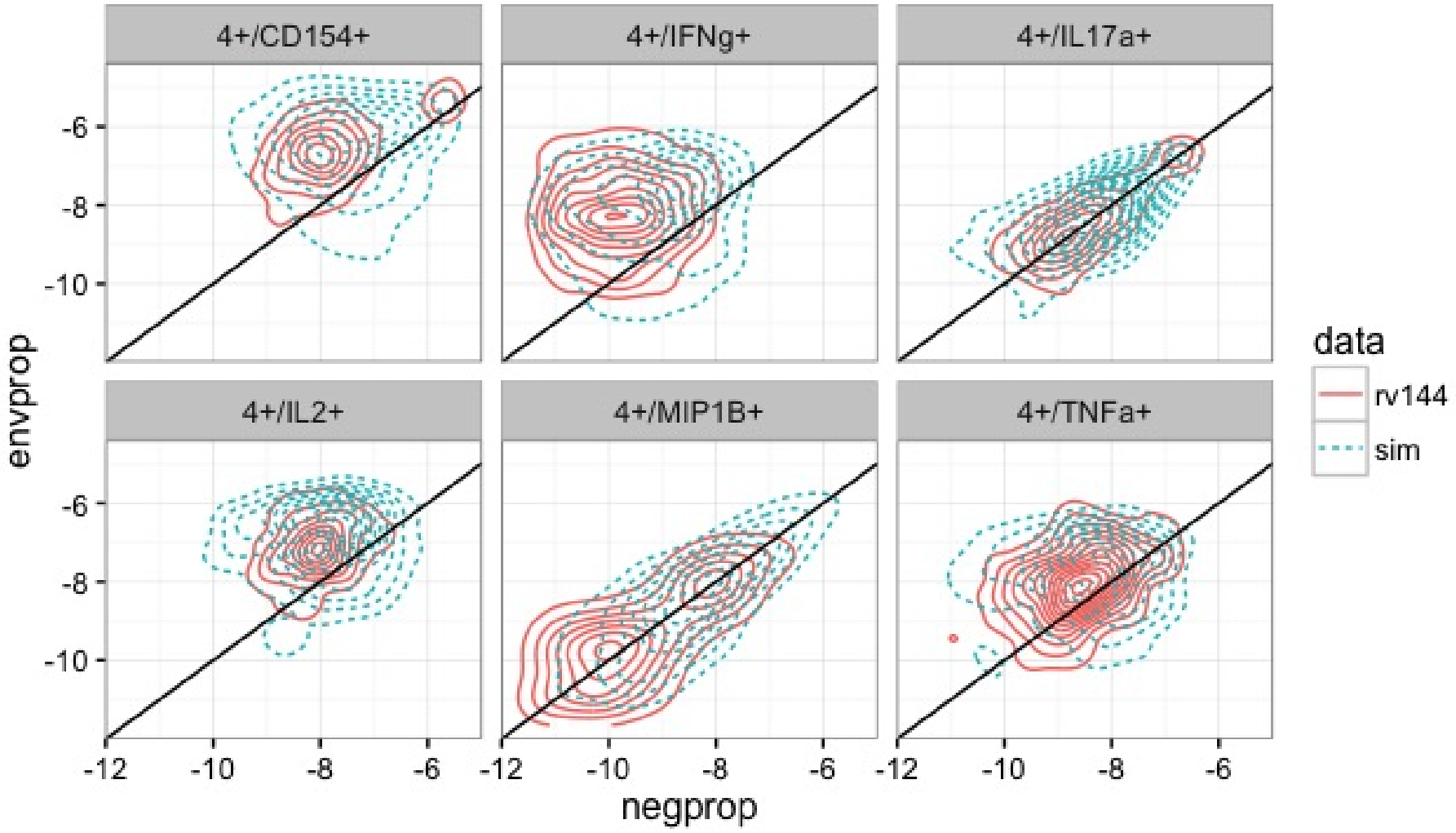
# How close are we to the distribution of the data?



# RV144 Booleans Dataset

- So far we worked with marginal counts - can be obtained from bulk assays.
- single-cell measurements enable a more comprehensive understanding of cellular functions.
- **The degree of functionality** (numbered of expressed cytokines) of responsive cell-subsets has been correlated with favorable outcomes in vaccine studies.

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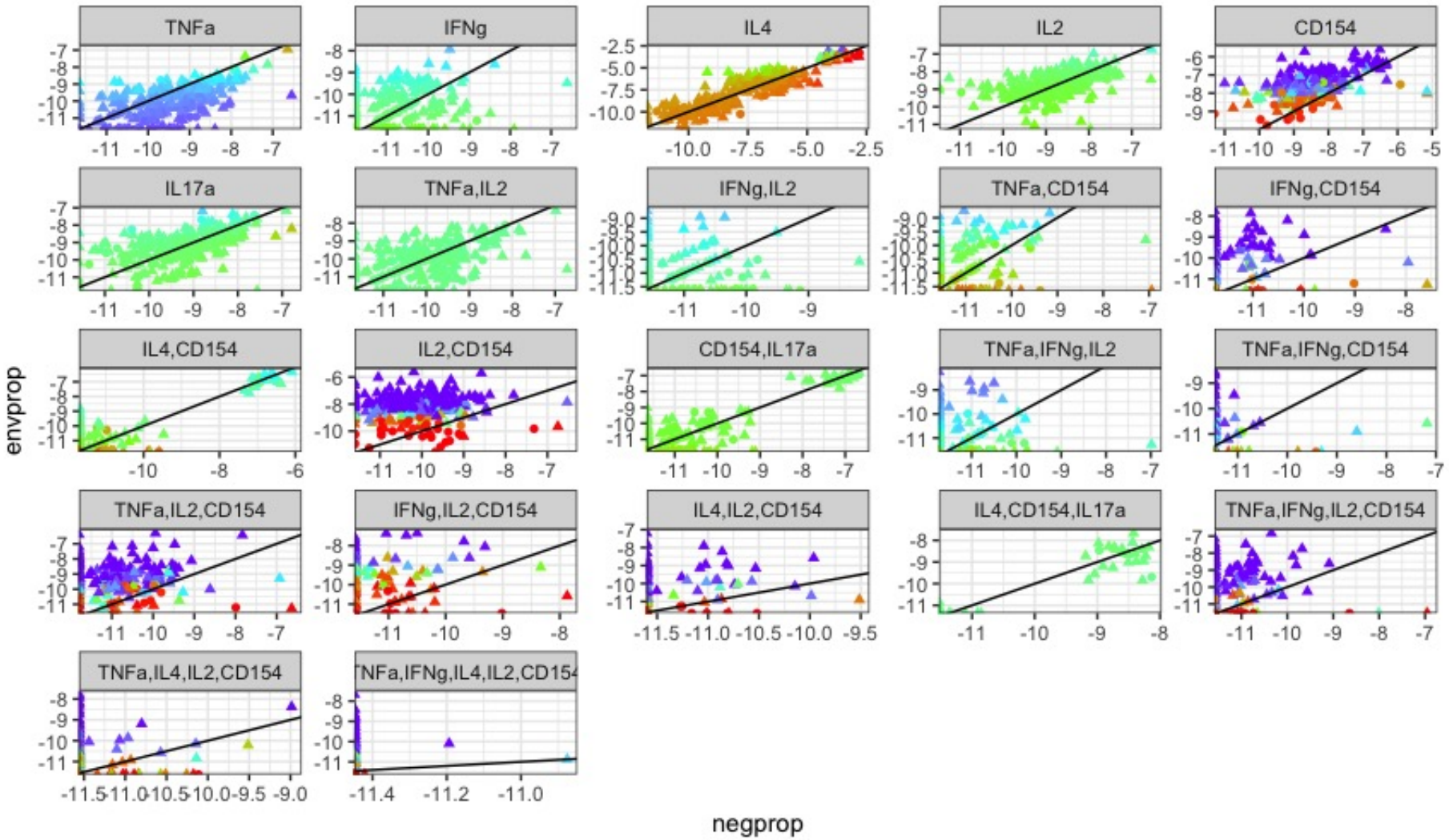
226 vaccinees, and 36 placebos, 24 cell-subsets.



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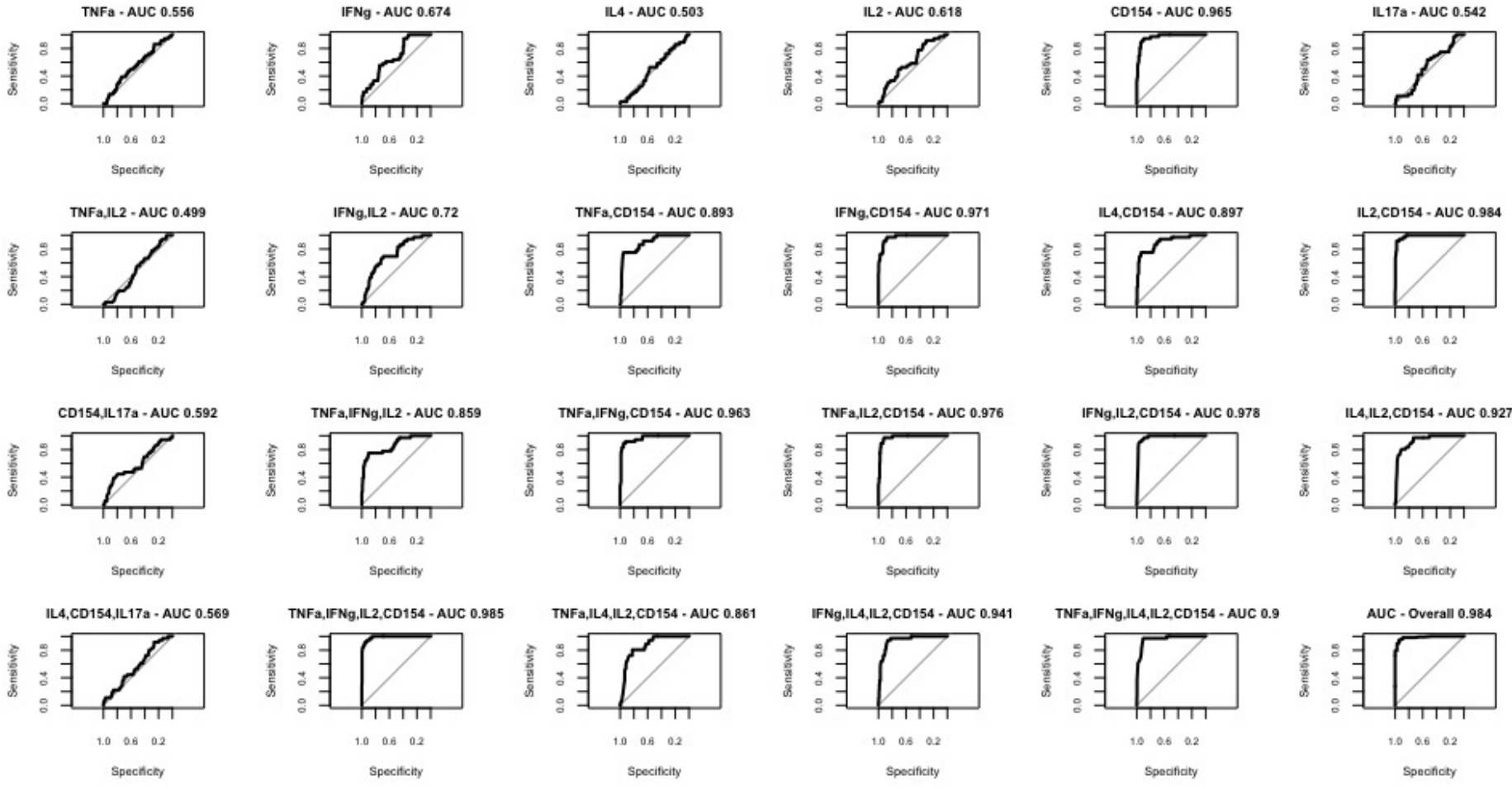


Figure: ROC for RV144 booleans dataset

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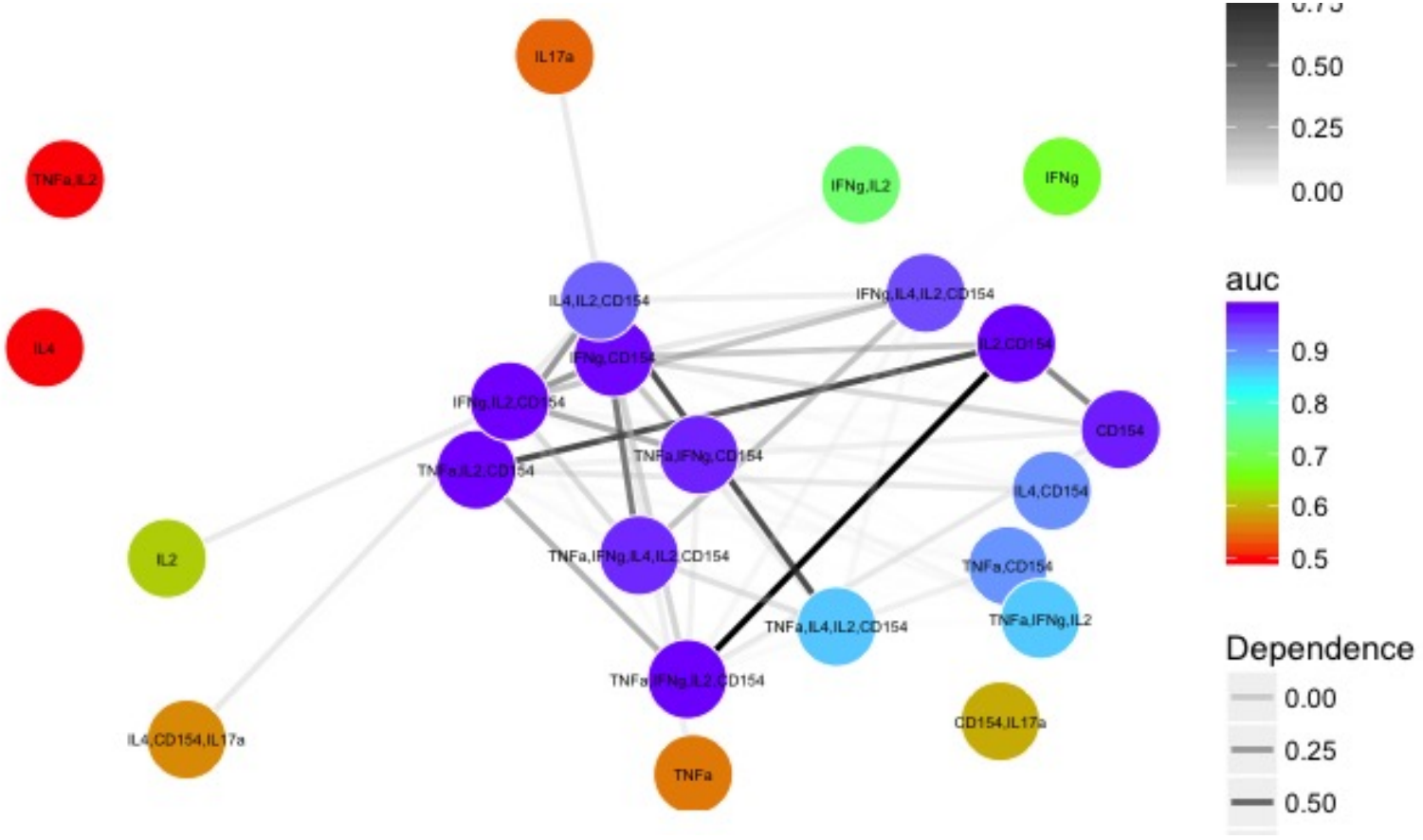


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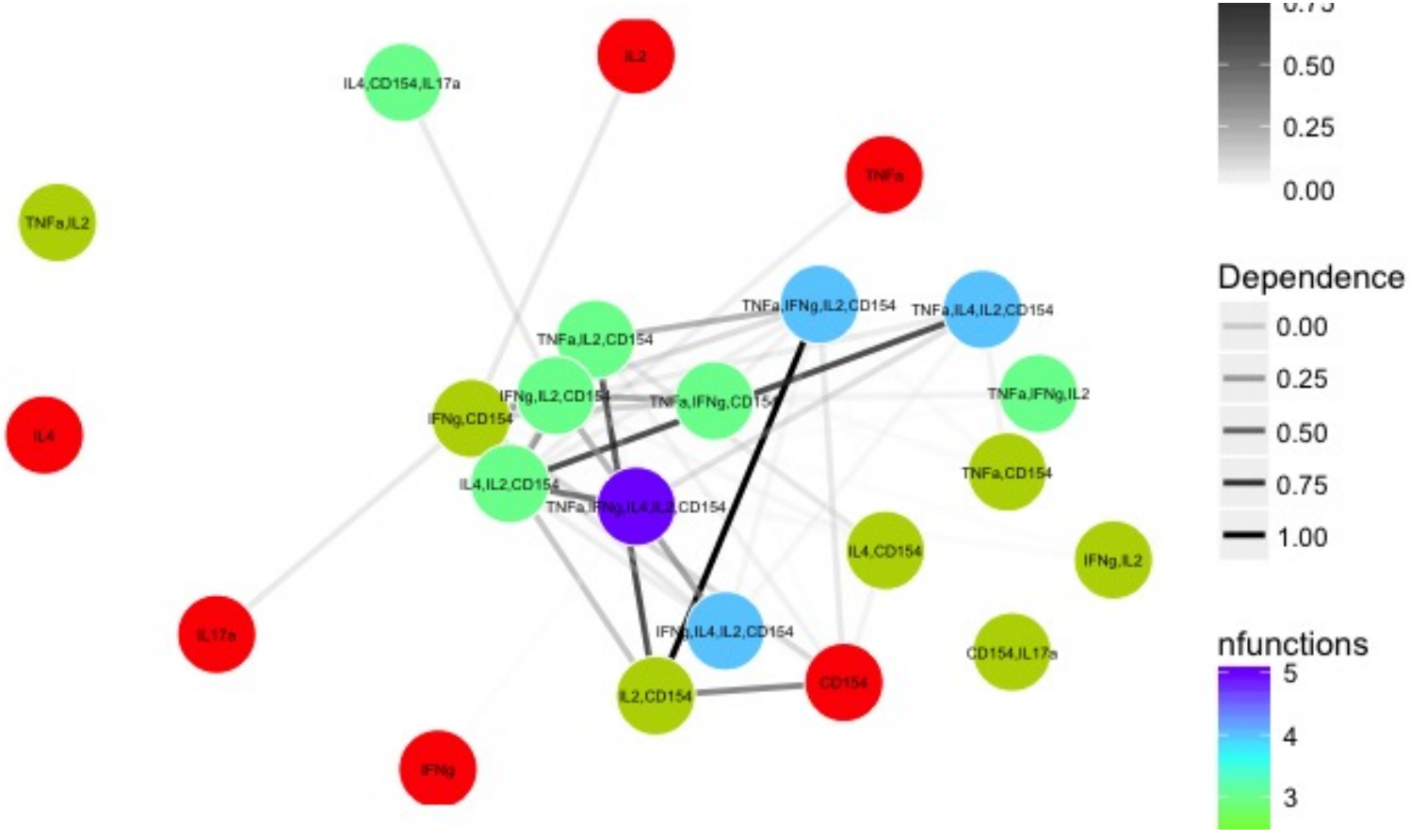


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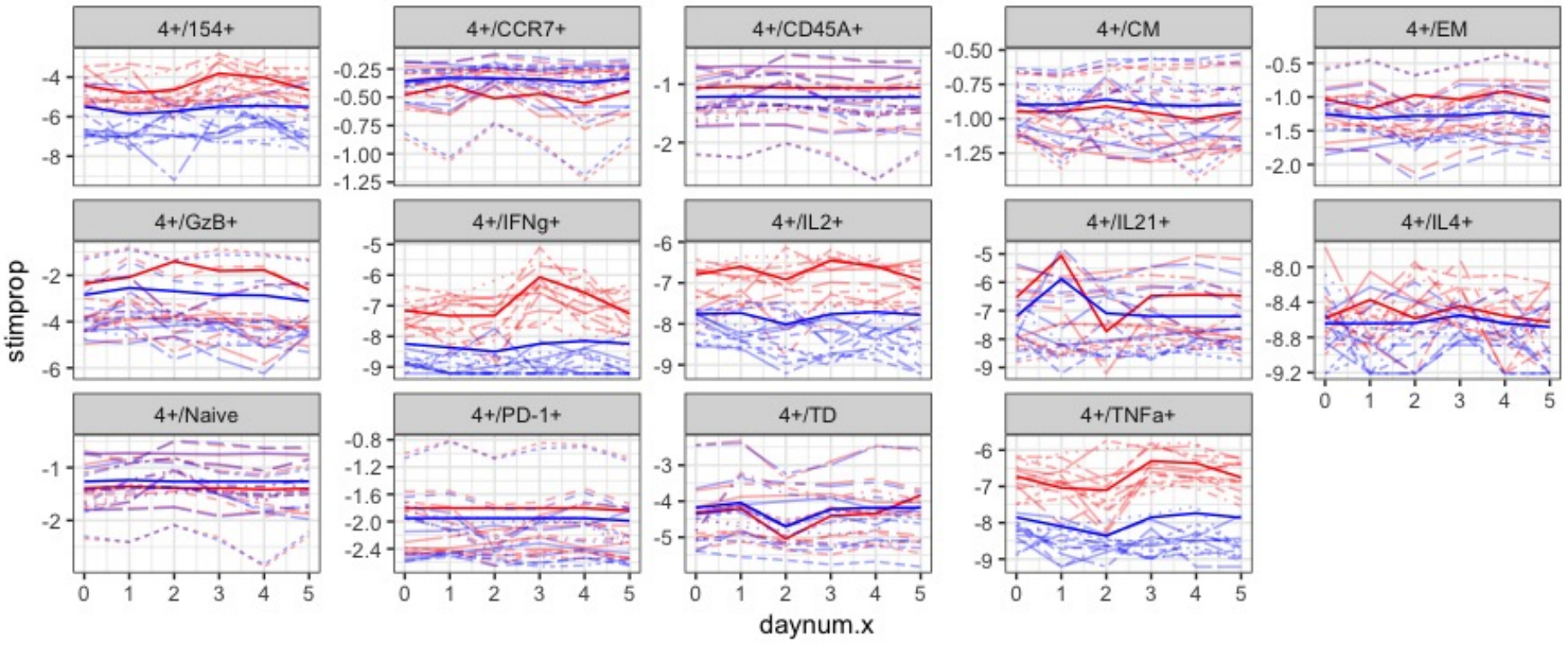


Figure: Stimulated vs. Unstimulated

# Controlled Human Malaria Infection Study

FDR adjusted p-values for Malaria Dataset

| | 4+ | 4+/CXCR5+ | 4+/CXCR5+/PD-1+ | 8+ | 8+/CXCR5+ | 56+dim | 56+hi | NK T cells |
|---|---|---|---|---|---|---|---|---|
| **154+** | 0.001 | 0.0045 | 0.001 | 0.8 | 0.7 | | | 0.001 |
| **CCR7+** | 0.97 | 1 | | 0.9 | 0.7 | | | |
| **CD45A+** | 0.54 | 0.5 | | 0.45 | 0.7 | | | |
| **CM** | 0.8 | 1 | 1 | 0.9 | 0.9 | | | |
| **EM** | 0.28 | 0.0001 | 0.00001 | 0.9 | 0.7 | | | |
| **GzB+** | 0.0345 | | | 0.11 | | 0.001 | 0.00001 | 0.21 |
| **IFNg+** | 0.0001 | 0.46 | 0.28 | 0.056 | | 0.000001 | 0.7 | 0.01 |
| **IL2+** | 0.0001 | 0.46 | 0.02 | 0.6 | 0.7 | | | 0.26 |
| **IL21+** | 0.49 | 0.46 | | 0.9 | | 0.63 | 0.14 | 0.6 |
| **IL4+** | 0.46 | 0.56 | | 0.68 | 0.7 | | 0.7 | 0.28 |
| **Naive** | 0.6 | 0.55 | 0.7 | 0.91 | 0.7 | | | |
| **PD-1+** | 0.003 | | | 0.53 | 0.7 | | | |
| **TD** | 0.4259 | 0.53 | 0.51 | 0.43 | 0.7 | | | |
| **TNFa+** | 0.0001 | 0.09 | 0.001 | 0.001 | | 0.03 | | 0.0000001 |

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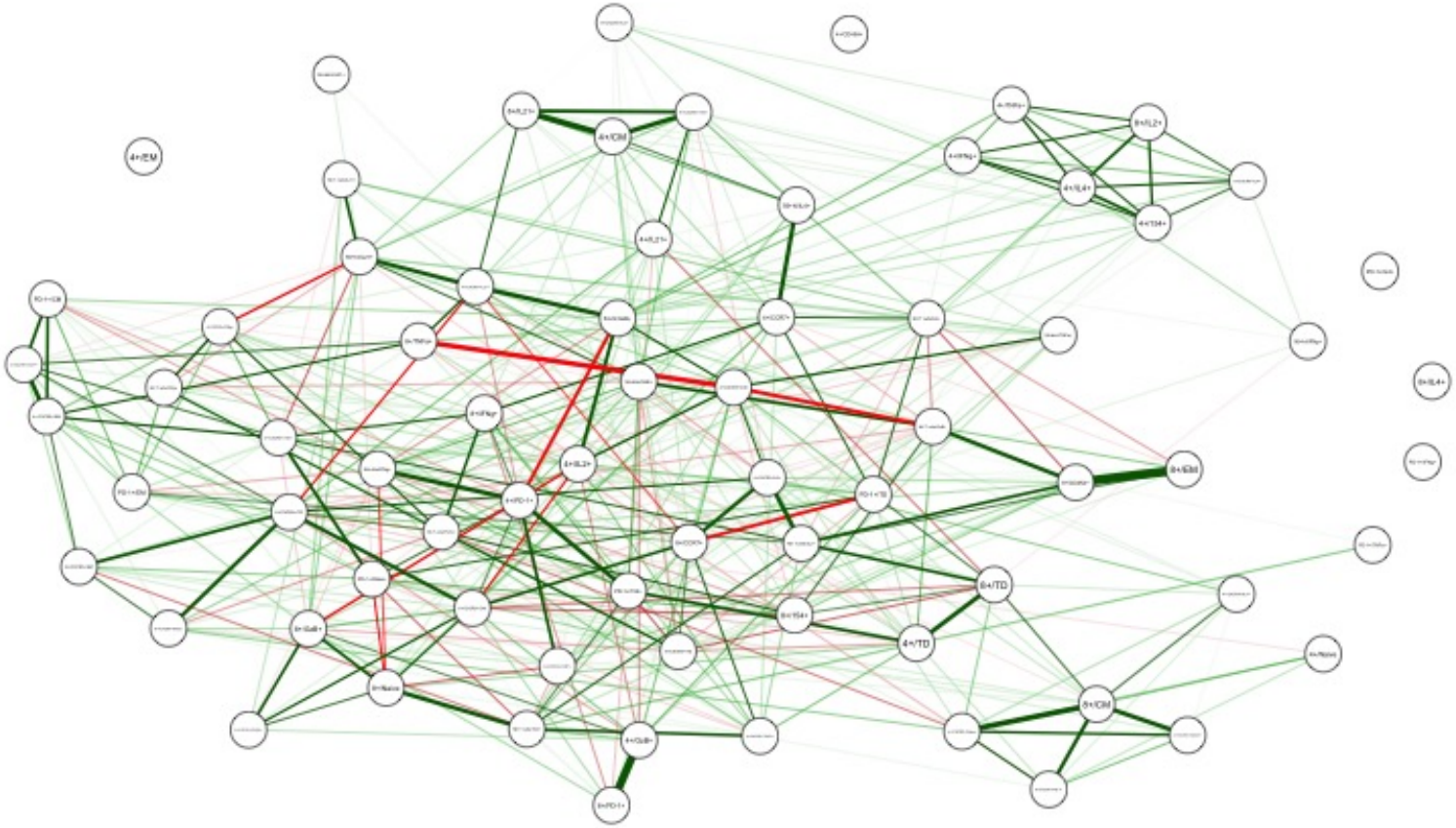

Figure: Stimulated vs. Unstimulated

# Computation

# Thank you!

Questions?

AmitMeir@uw.edu