

# Epigenetically programmed resistance to tuberculosis

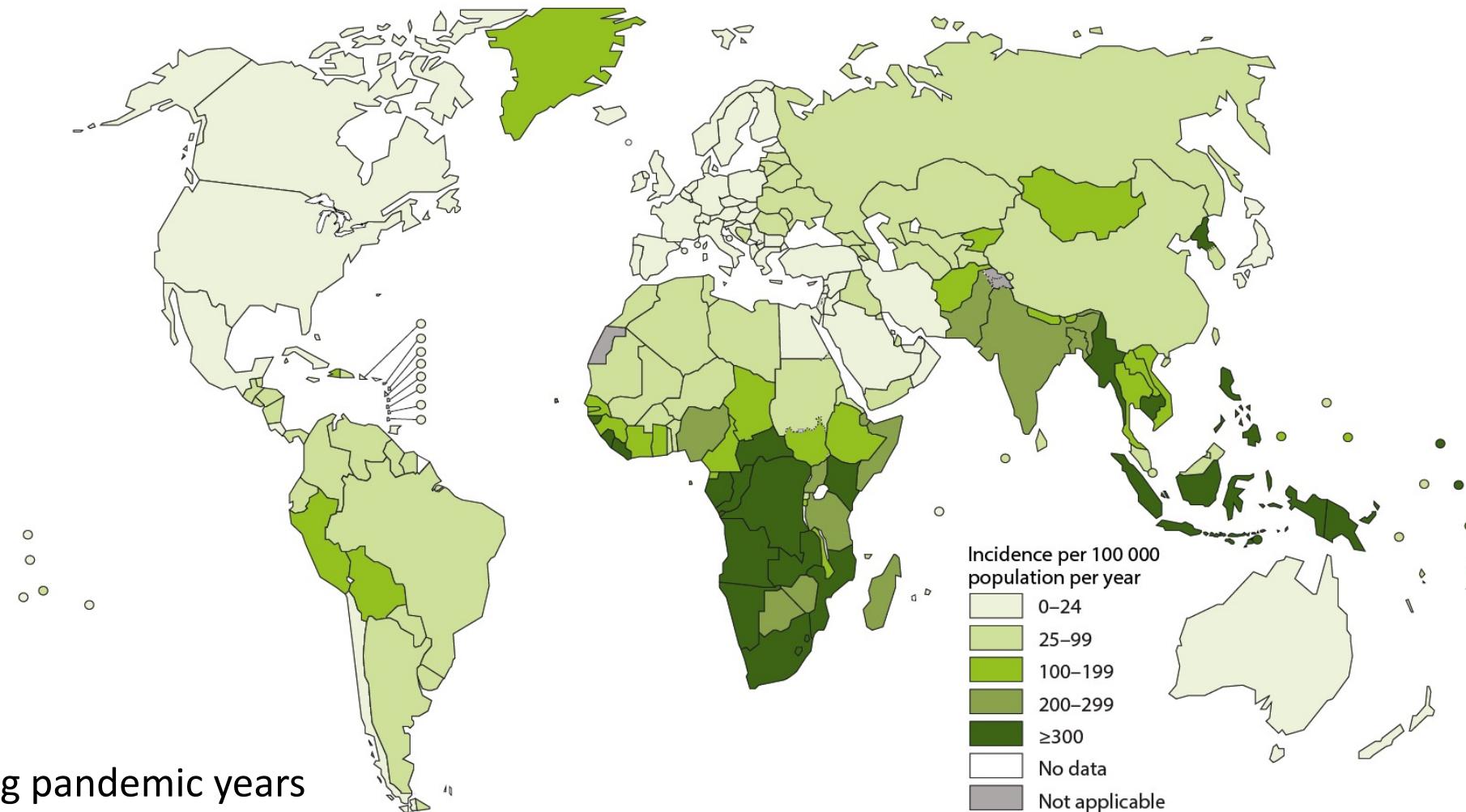
Kim Dill-McFarland

2021.06.07

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*M. tuberculosis* is the leading cause of single agent infectious disease death... and has been for 100s of years.\*

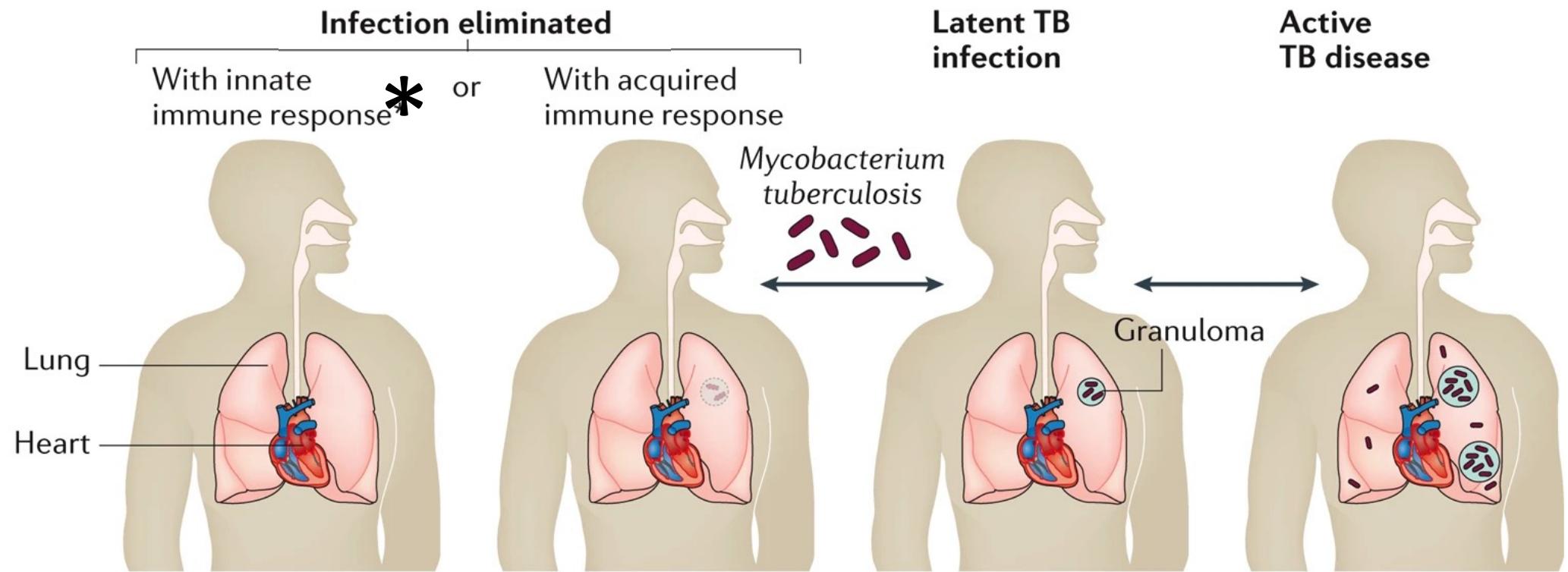


\*Except during pandemic years such as 2019-2020 SARS-CoV-2

Image: World Health Org

A subset of the population appears to be  
resistant\* to *M. tuberculosis* infection.

TST: tuberculin skin test  
IGRA: interferon gamma release assay



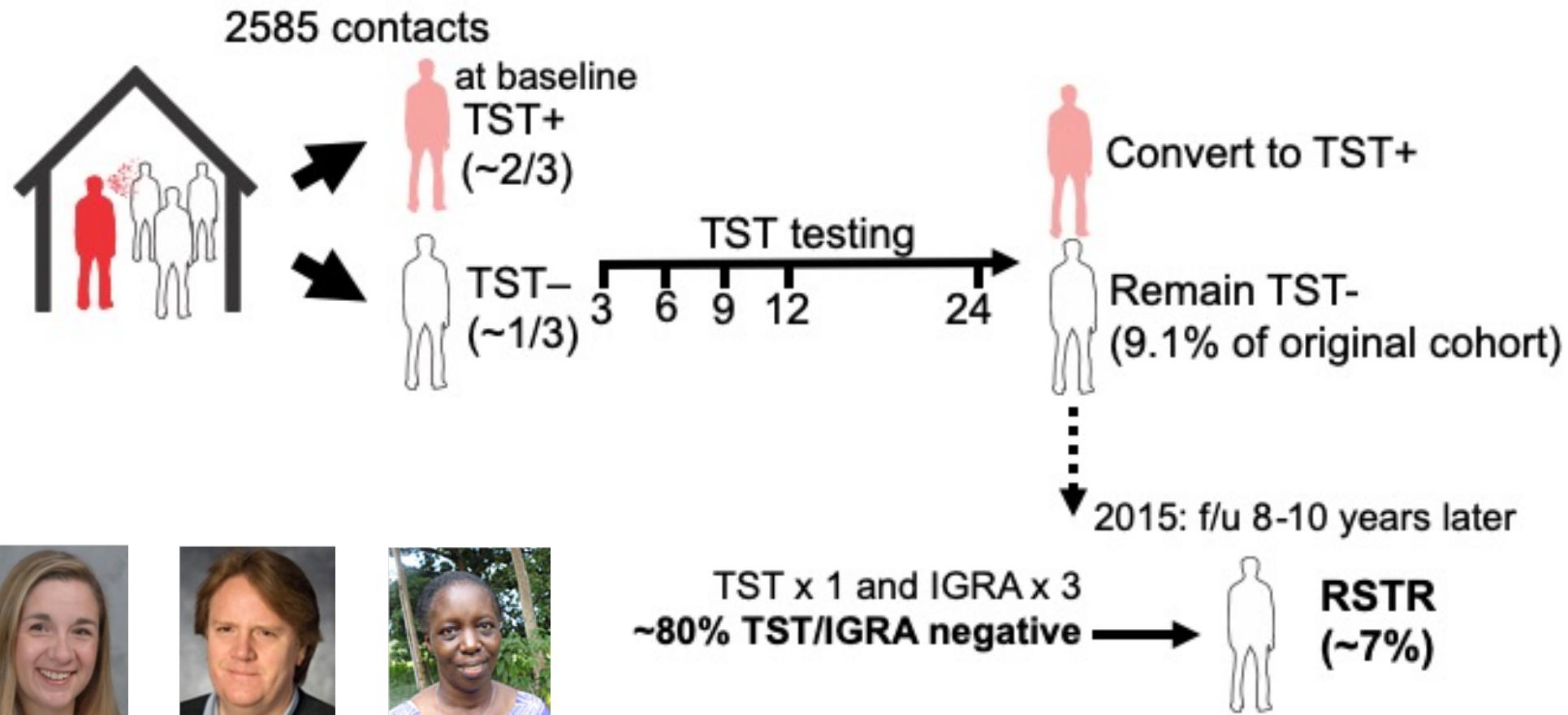
TST	Negative	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Positive

\* Resister (RSTR): successfully clear *Mtb* infection through innate immune responses or acquired immune responses without T cell priming or memory

# Study design

TST: tuberculin skin test  
IGRA: interferon gamma release assay

# Uganda household contact study



Tom Hawn  
UW



Cathy Stein  
Case Western

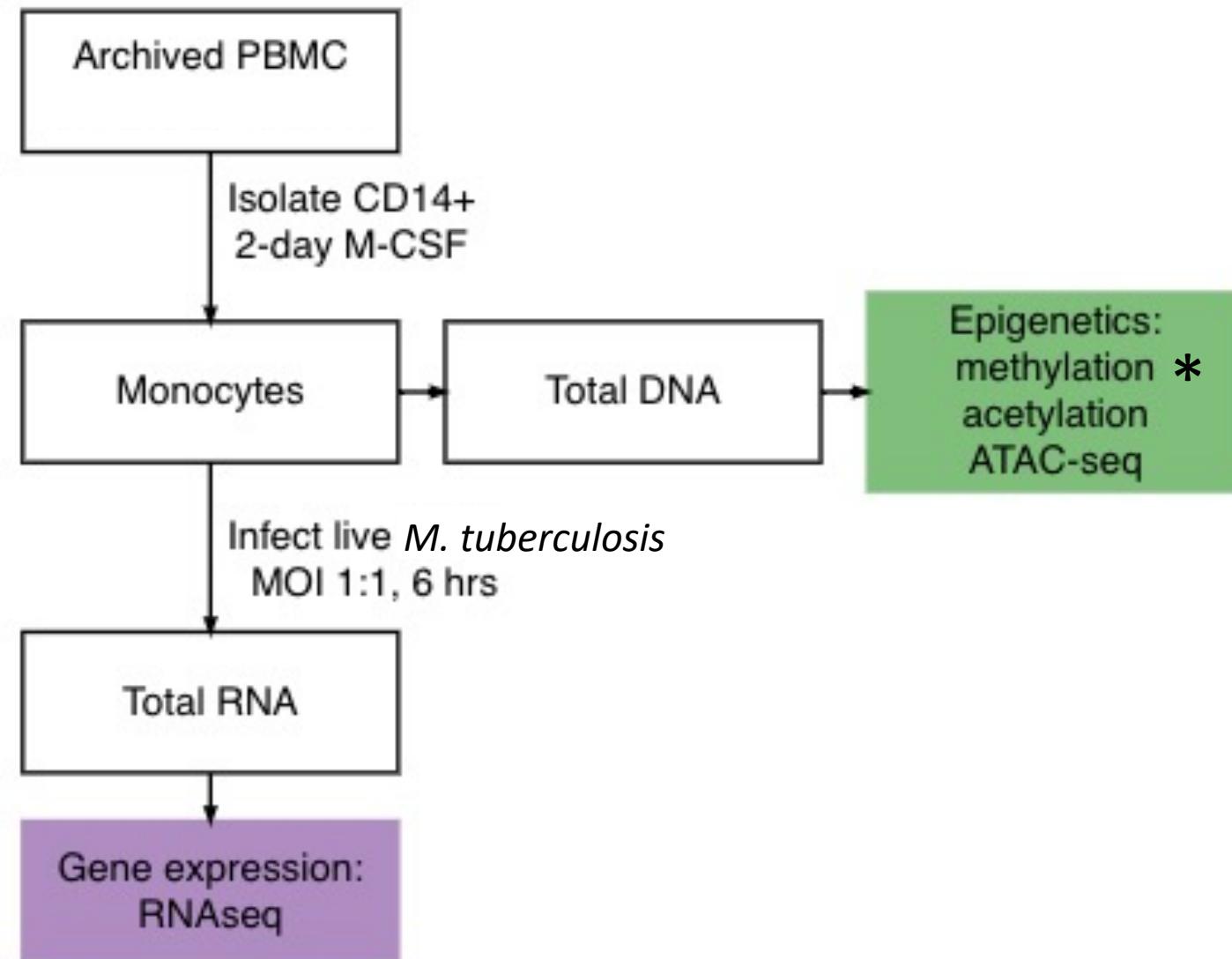


Henry Boom  
Mayanja-Kizza  
Makerere U



# Design

PBMC: peripheral blood mononuclear cells  
MOI: multiplicity of infection

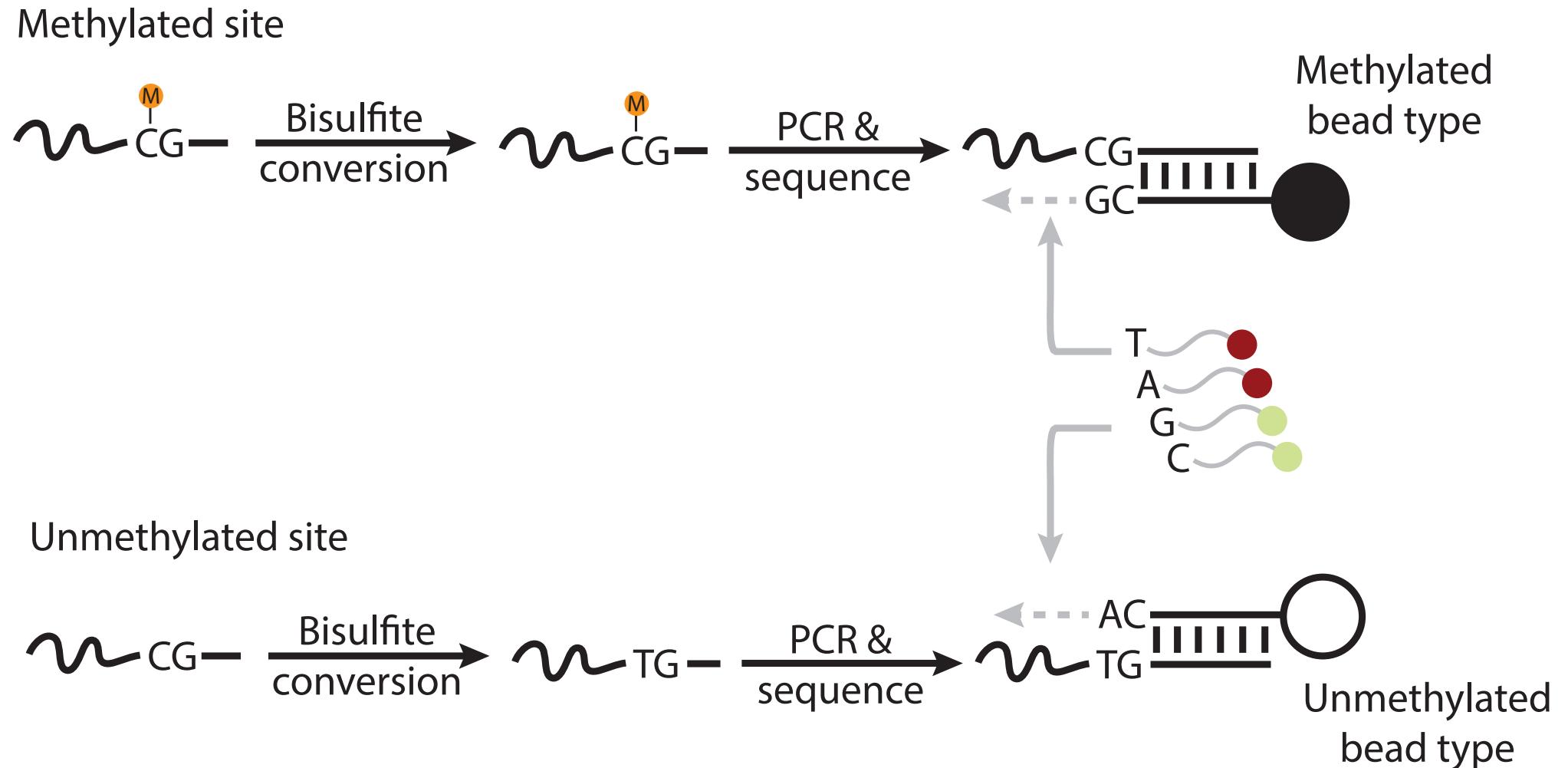


RSTR: resister  
LTBI: latent TB infection

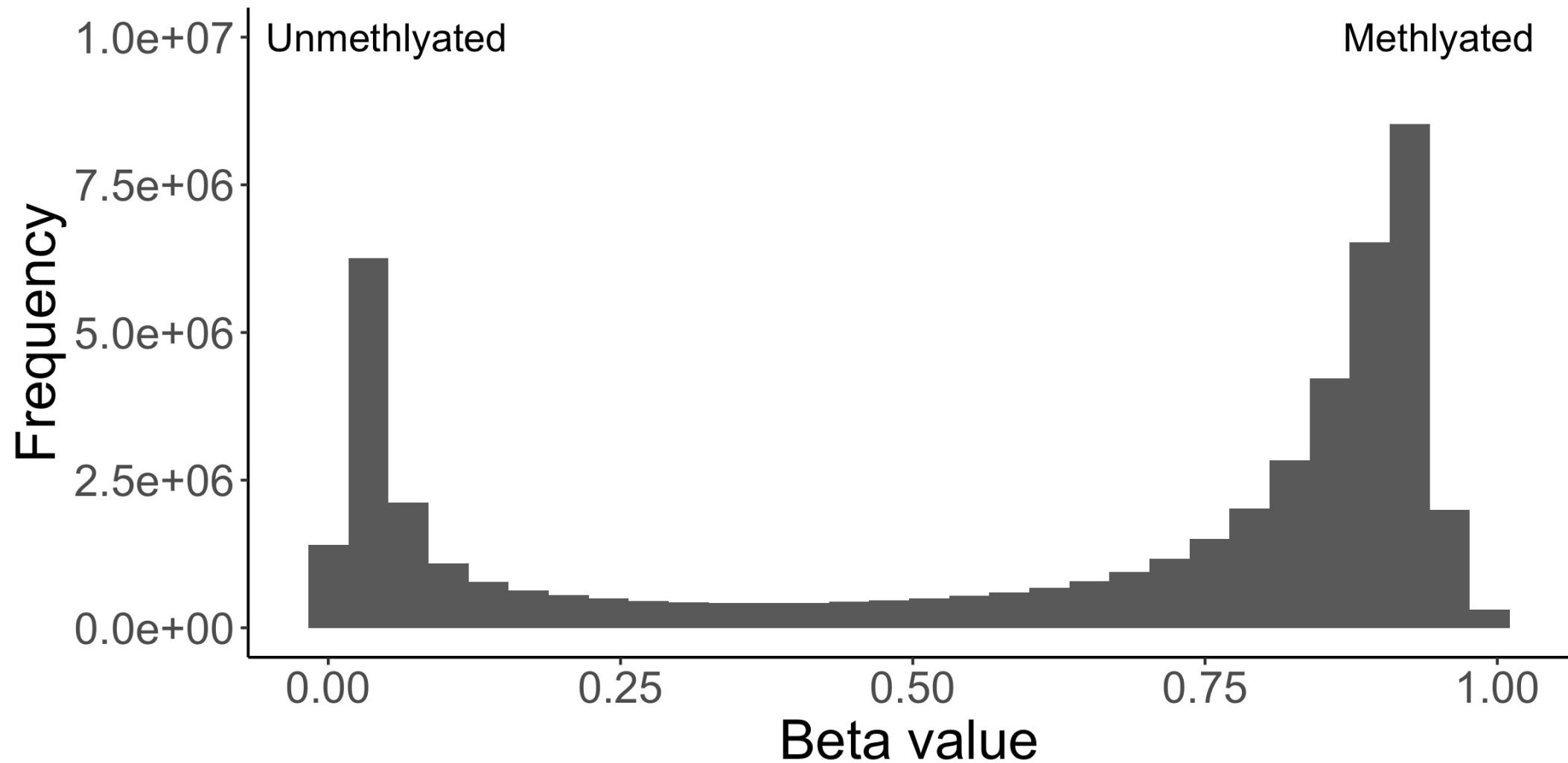
# Demographics

	Methylation	RSTR vs LTBI	p-value
Donors with kinship	RSTR	29	
	LTBI	29	
Female, %	50.0		1
Age at enrollment, yrs	$17.2 \pm 12.6$		0.439
Age at sampling, yrs	$25.8 \pm 12.3$		0.318

# Infinium MethylationEPIC array: Bisulfite sequencing



# Methylation data



# Data cleaning

# Methylation data cleaning (R::ChAMP)

## 1. Quality filtering

- Probes ≤ 3 beads in ≥ 95% of samples
- Probes failed ≥ 10% of samples
- Non-CpG, near SNP<sup>a</sup>, multi-alignment<sup>b</sup>, XY

## 2. Normalization

- Functional normalization<sup>c</sup>
  - Quantile normalization based on control probes to estimate technical variation
- ComBat batch correction
- Log2 transformation

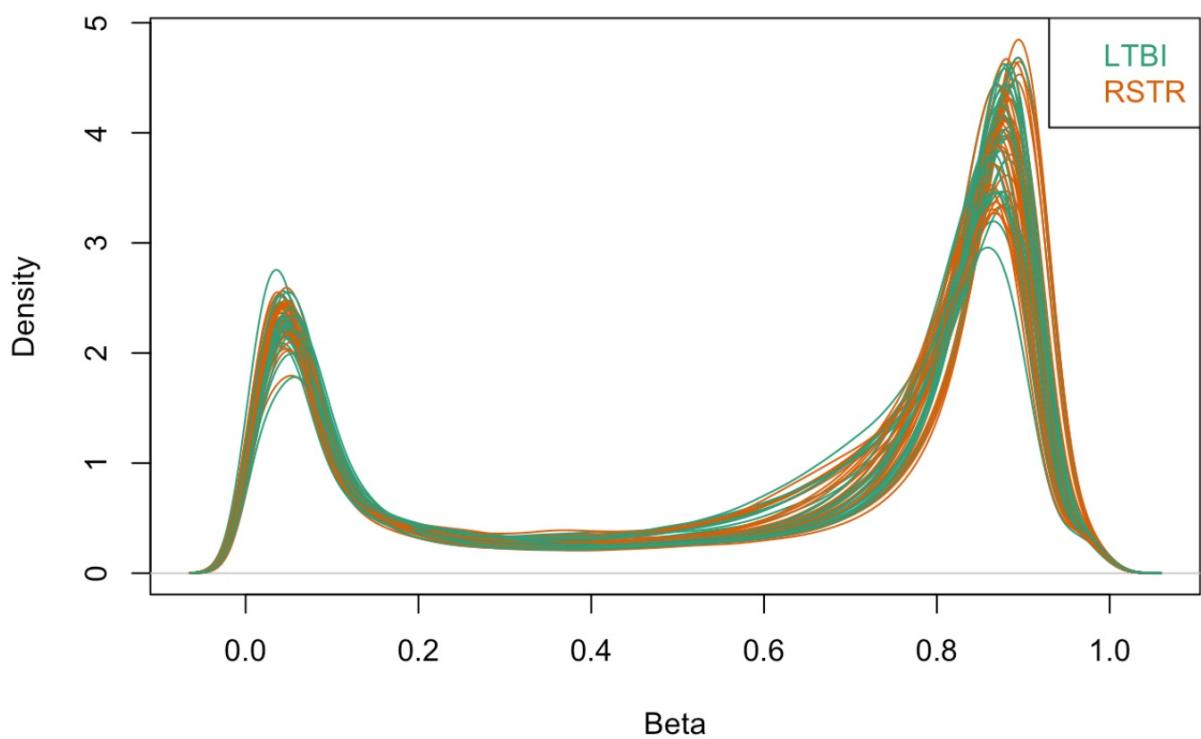
<sup>a</sup>Zhou *et al* 2017 doi: [10.1093/nar/gkw967](https://doi.org/10.1093/nar/gkw967)

<sup>b</sup>Nordlund *et al* 2013 doi: [10.1186/gb-2013-14-9-r105](https://doi.org/10.1186/gb-2013-14-9-r105)

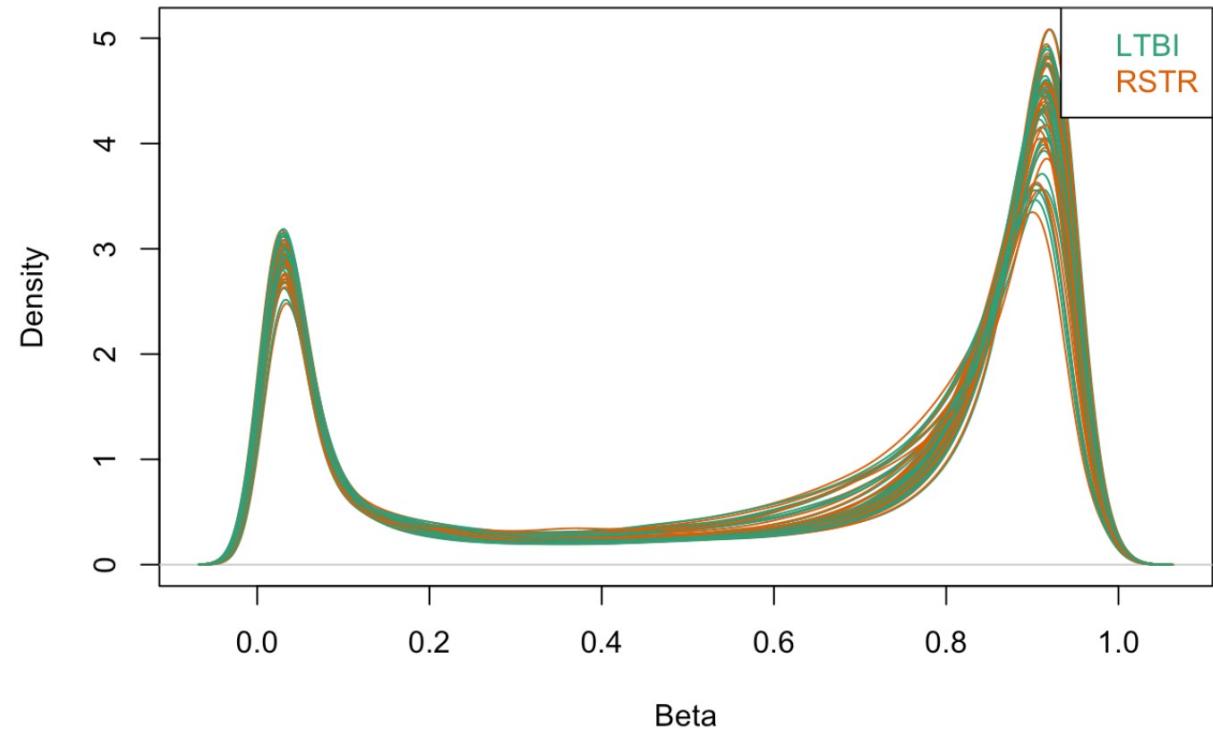
<sup>c</sup>Fortin *et al* 2014 doi: [10.1186/s13059-014-0503-2](https://doi.org/10.1186/s13059-014-0503-2)

# Functional normalization

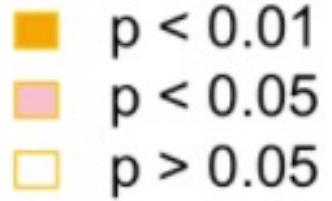
Before



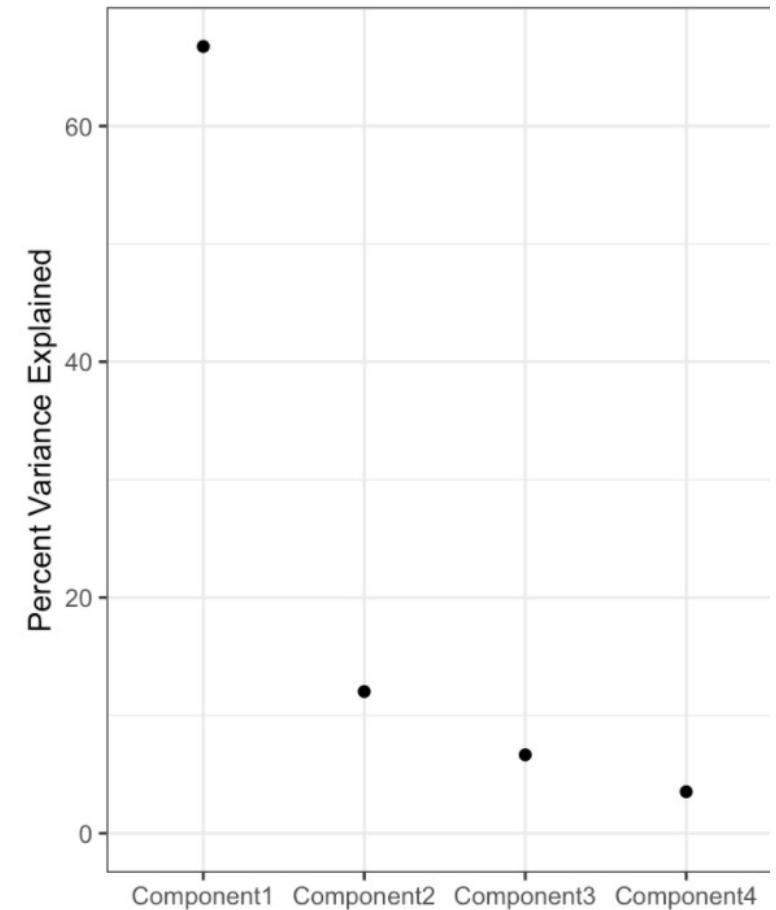
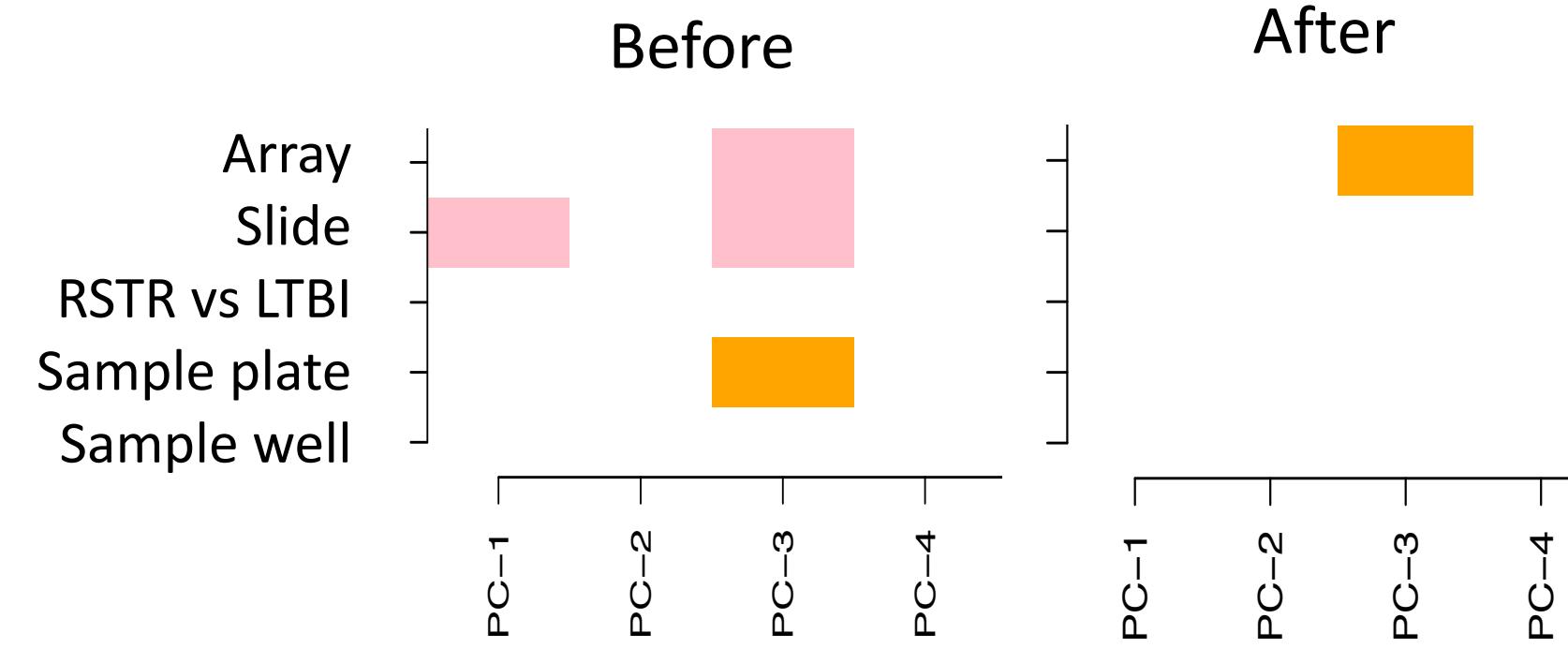
After



# ComBat batch correction



- Singular value decomposition (SVD)
- PCs up to 80% total variation explained



# Methylation in RSTR vs LTBI

# *De novo* differentially methylated regions (R :: DMRcate)

1. Linear model probe-level data and define significant probes
2. Apply Gaussian smoothing to probe test statistics using a given bandwidth ( $\lambda$ ) and scaling factor (C) where  $\lambda/C = \text{sigma}$
3. Model smoothed test statistics using Satterthwaite approximation of sample variance
4. Estimate p-values and apply false discovery rate (FDR) correction
5. Re-define significant probes and collapse nearby probes using  $\lambda$

Peters *et al* 2015 doi: [10.1186/1756-8935-8-6](https://doi.org/10.1186/1756-8935-8-6)

# Probe model fitting (R::lmeKin)

## Main variable

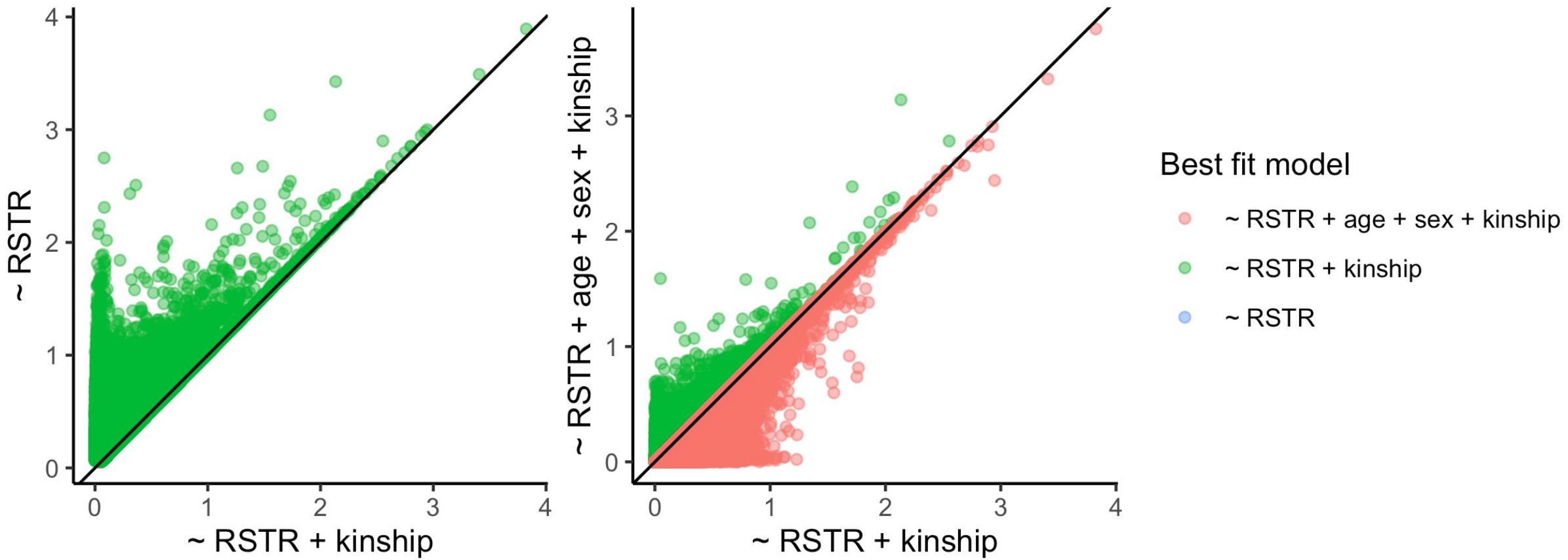
- RSTR vs LTBI

## Potential co-variates

- Genetic relatedness (family blocks, kinship)
- Age
- Biological sex
- Body mass index (BMI)
- Composite TB risk score
- BCG vaccination

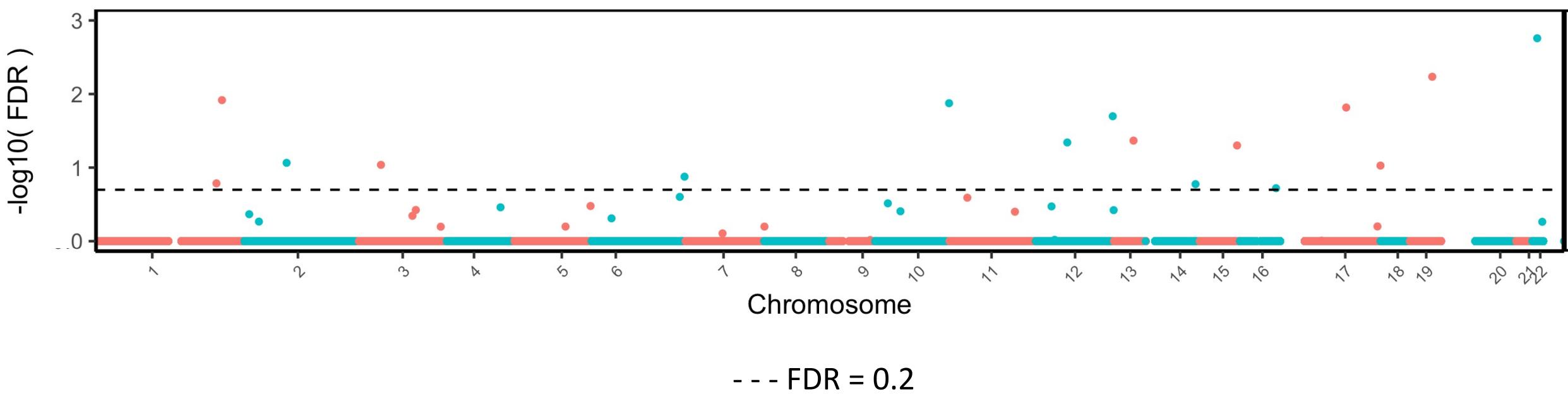
# Probe model fitting

Mean residual standard deviation (sigma)



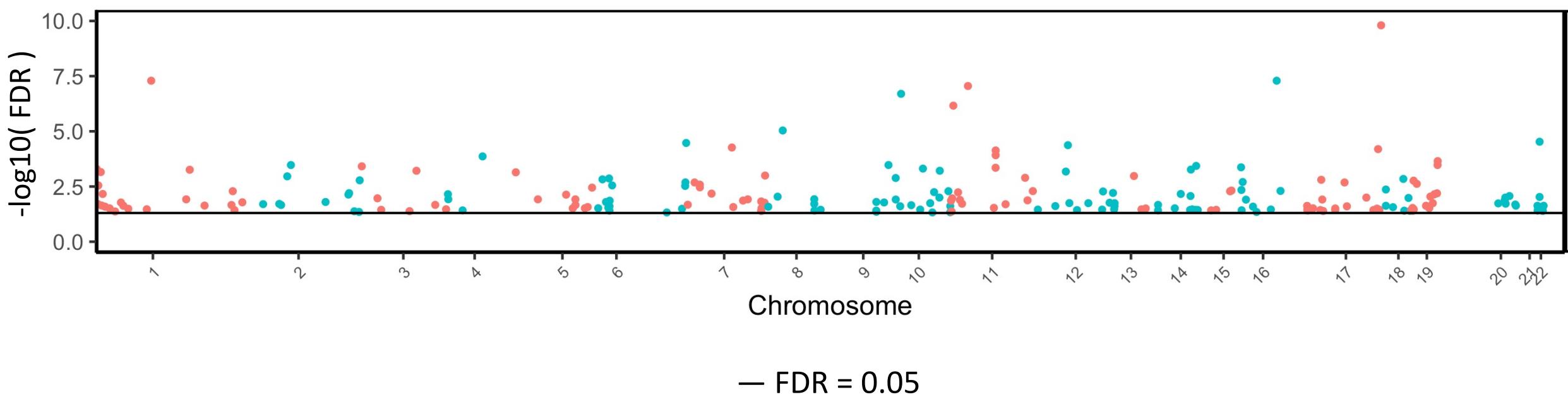
# Differentially methylated probes (DMP)

~ RSTR + age + sex + kinship

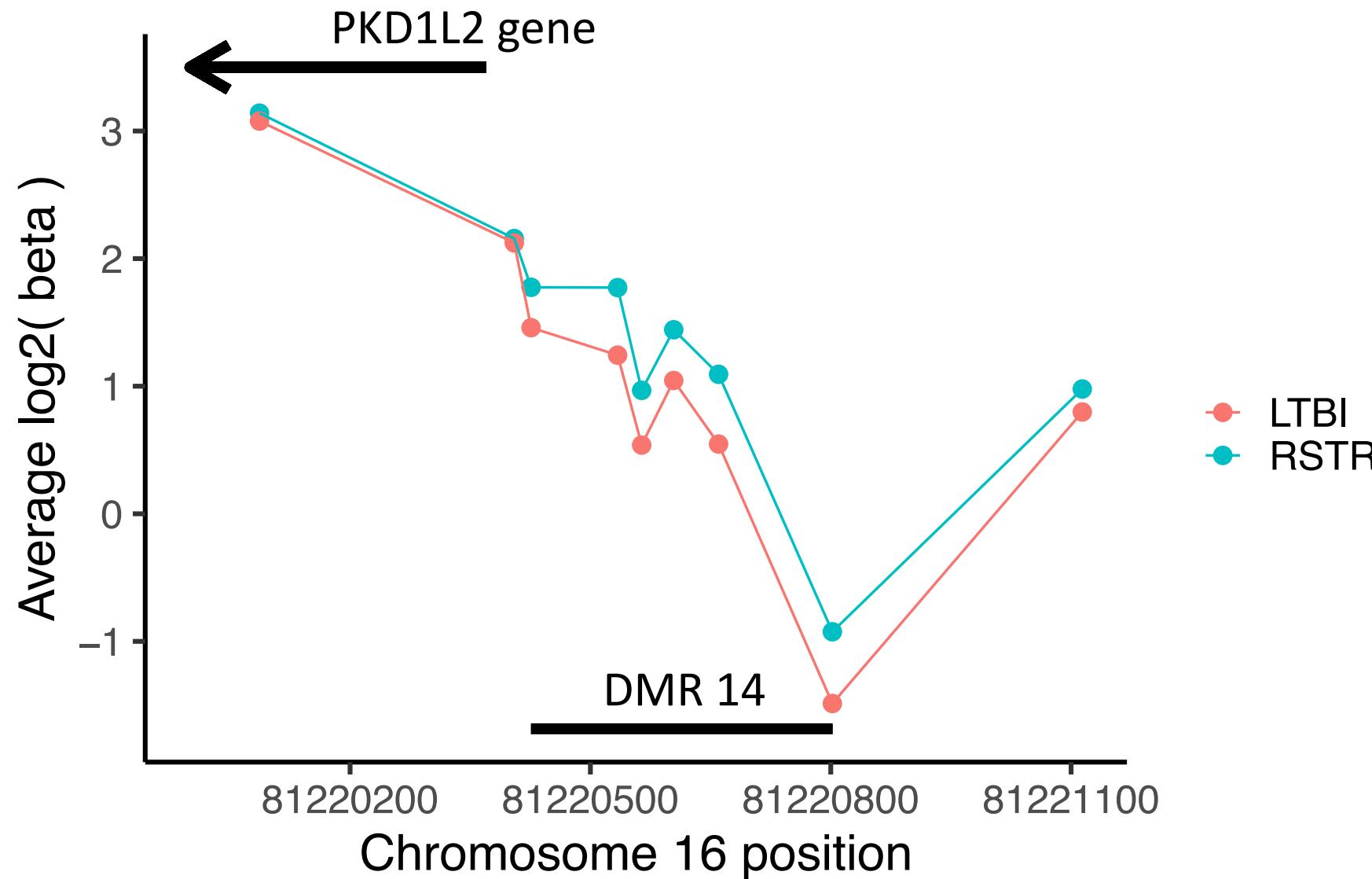


# Differentially methylated regions (DMR)

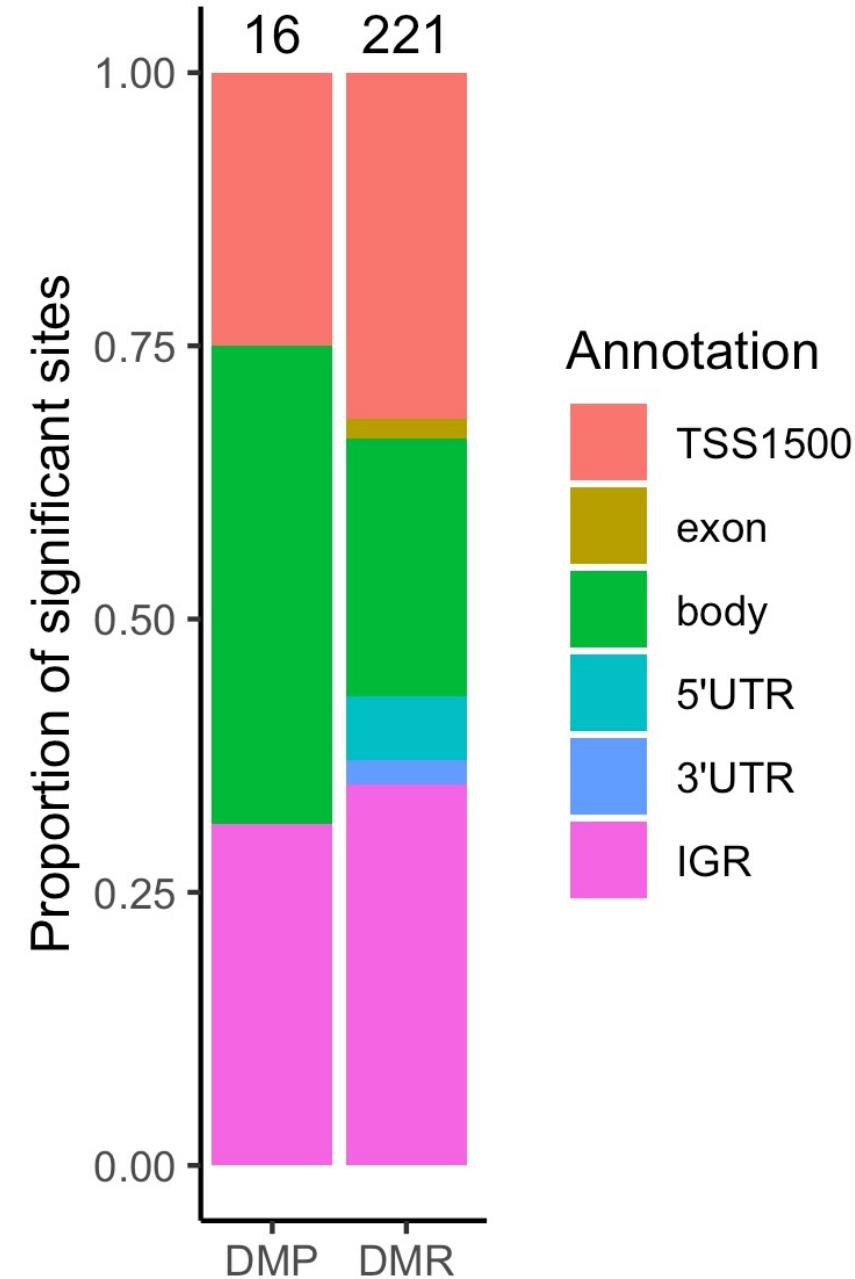
~ RSTR + age + sex + kinship



# Example



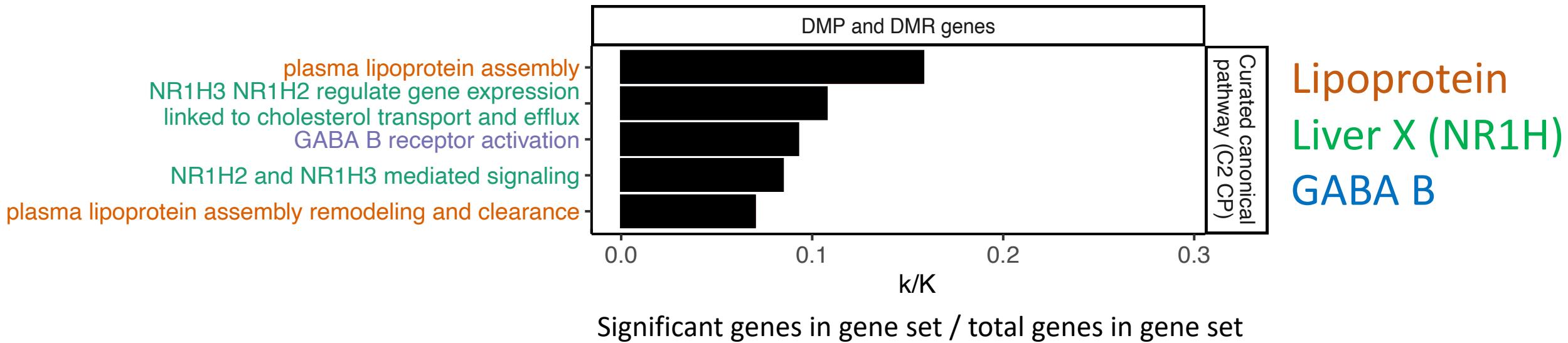
# Annotation types



# Genes with differential methylation (R::clusterProfiler)

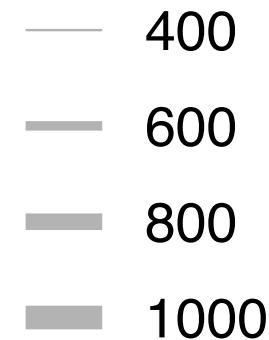
Enrichment of Broad gene sets (MSigDB)

FDR < 0.1

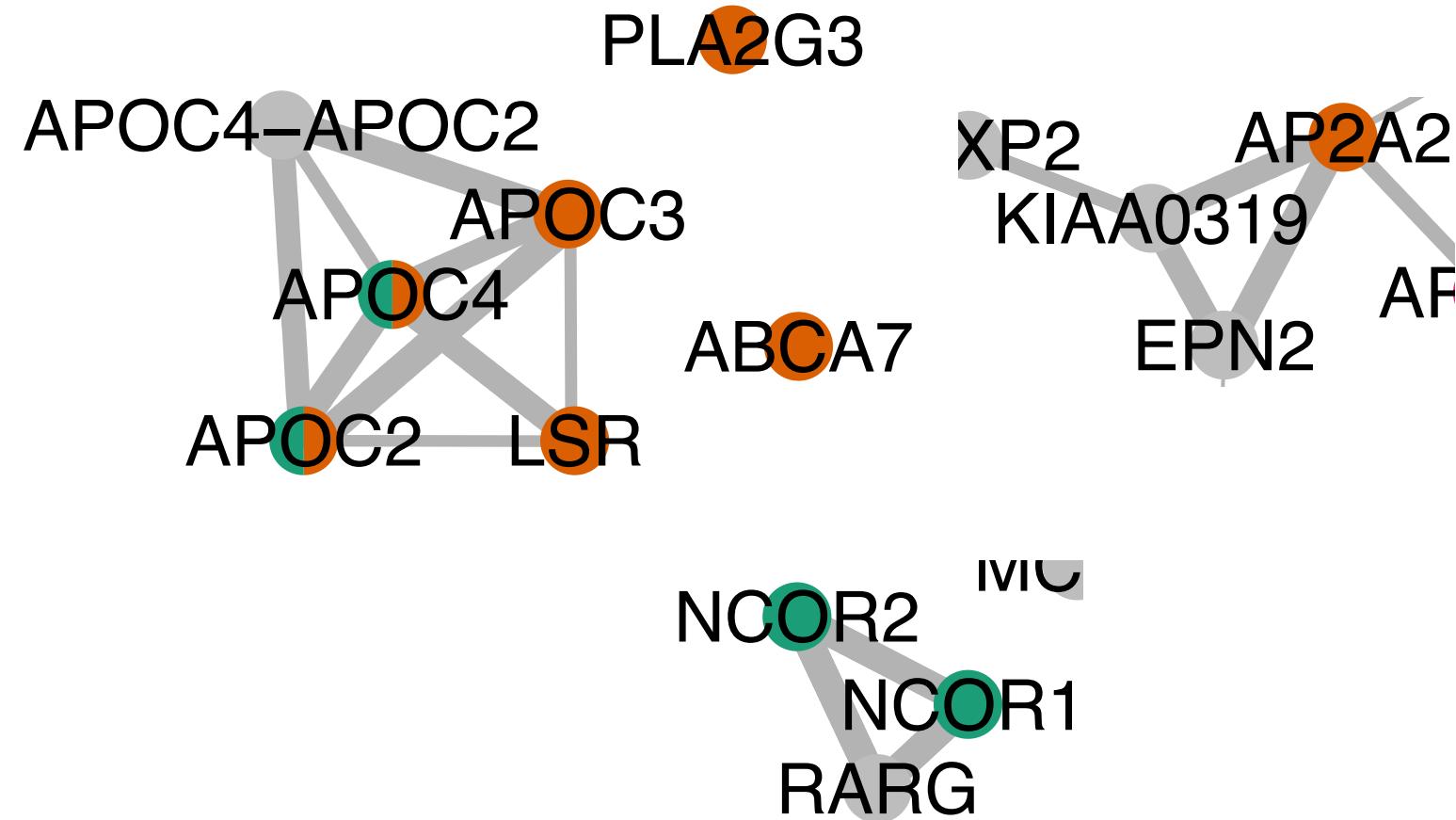


# Genes with differential methylation

STRING score



Enrichment



# Implications for TB

- *Mtb* utilizes cholesterol and fatty acids as major carbon sources
- *Mtb* promotes lipid accumulation in infected cells
- Lipid-laden (foamy) macrophages associated with TB granulomas
  - Cholestryl esters and triglycerides

## Hypothesis

- RSTR are epigenetically programmed to better control excess cholesterol and lipids
- This prevents foam cell formation, thus “starving” *Mtb* and facilitating successful clearance of infection

# Next steps

- Serum proteomics and lipidomics of high-density lipoproteins (HDL)
- Serum cholesterol efflux capacity



Tomas Vaisar  
UW

- Expression quantitative trait methylation (eQTM)
  - Associate methylation with gene expression in response to *Mtb* infection *ex vivo*



BILL &  
MELINDA  
GATES  
*foundation*



Thomas Hawn, MD

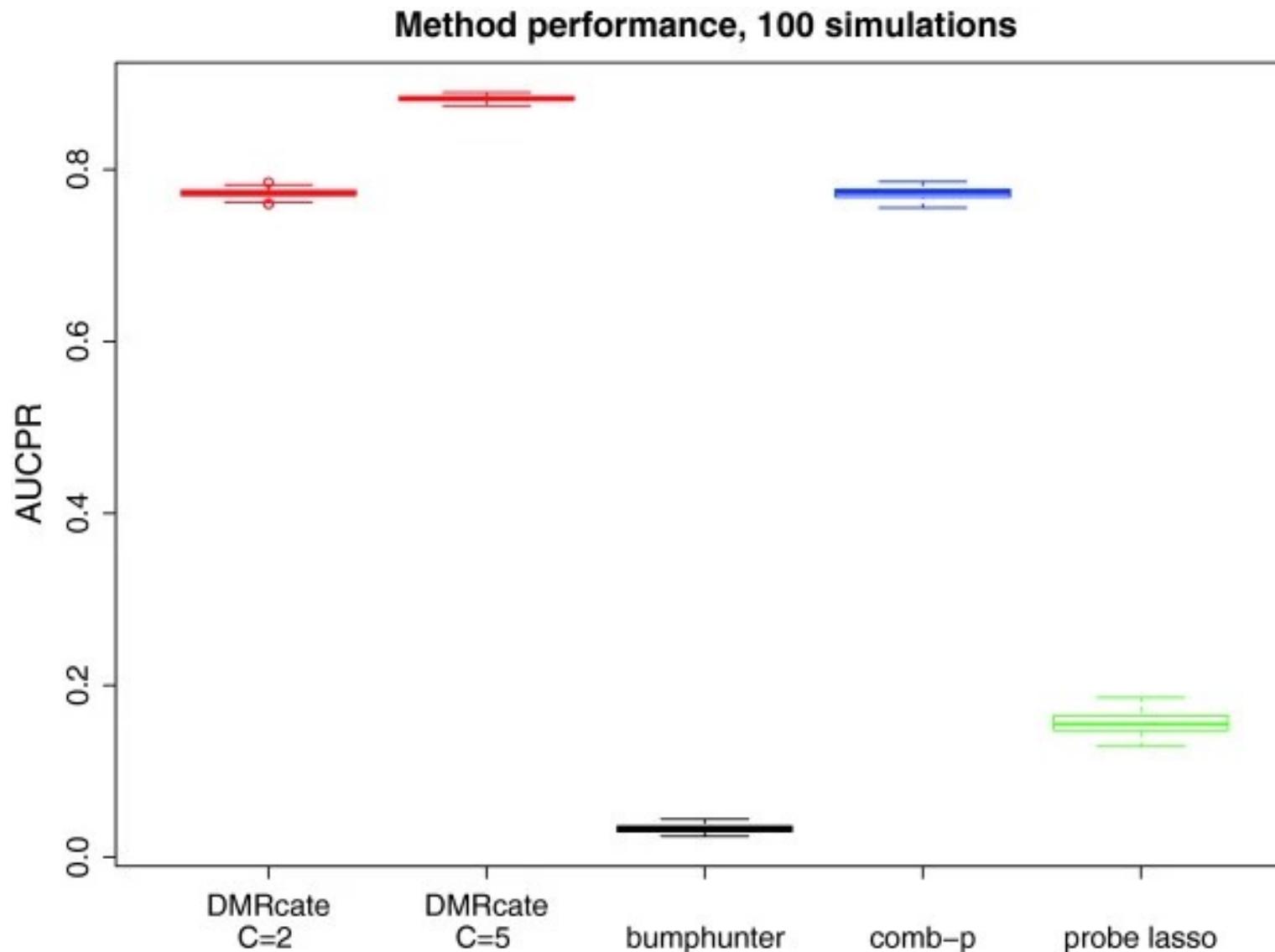
- **Felicia Nguyen**
- **Glenna Peterson**
- **Jason Simmons, MD**
- Max Segnitz, PhD
- Monica Campo, MD

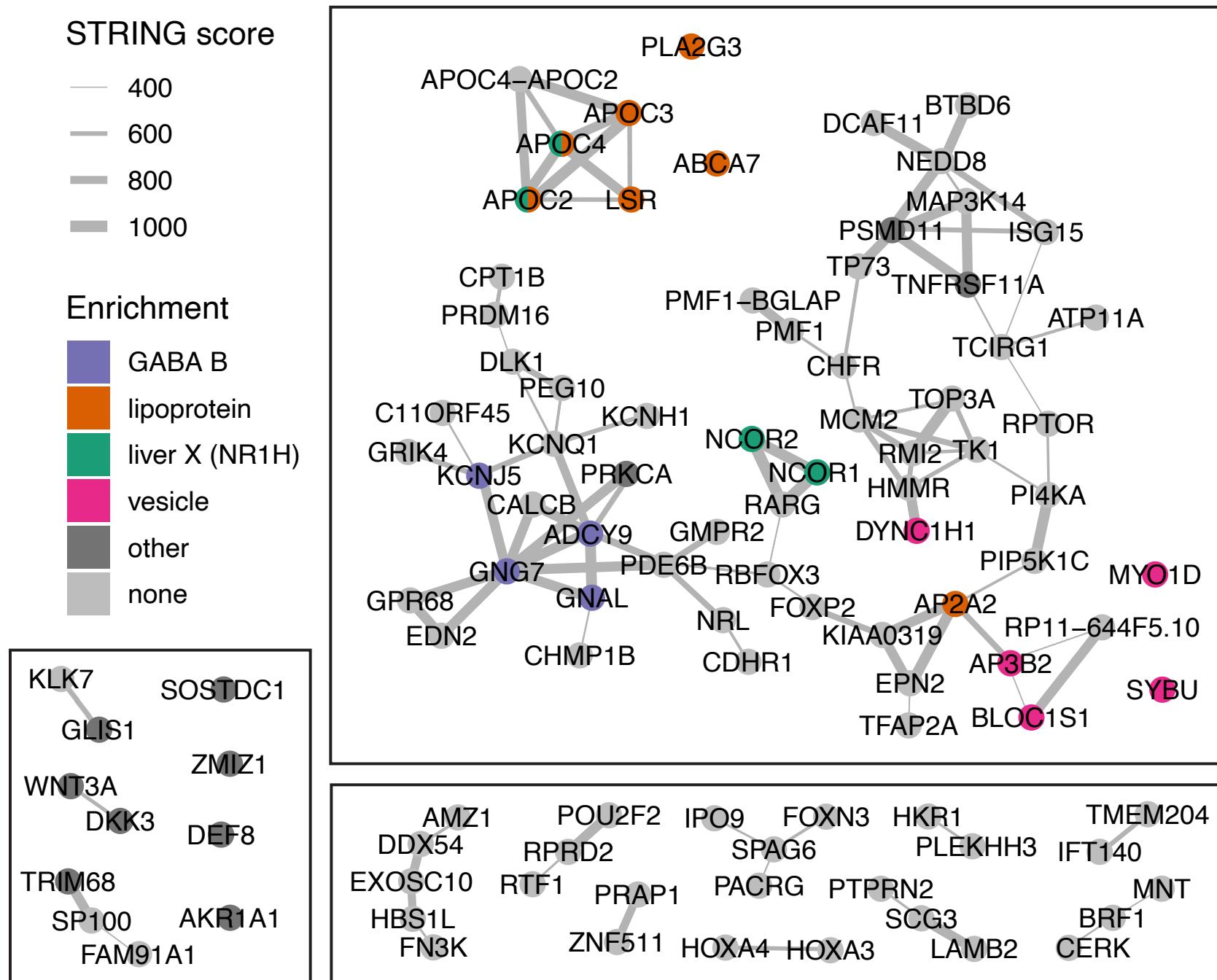
RSTR project

- Cathy Stein, PhD, Case Western
- Henry Boom, MD, Case Western
- Harriet Mayanja-Kizza, MD, Makerere University
- Chetan Seshadri, MD, UW

# DMRcate

Area under the  
precision-recall curve





# RSTR SNP data

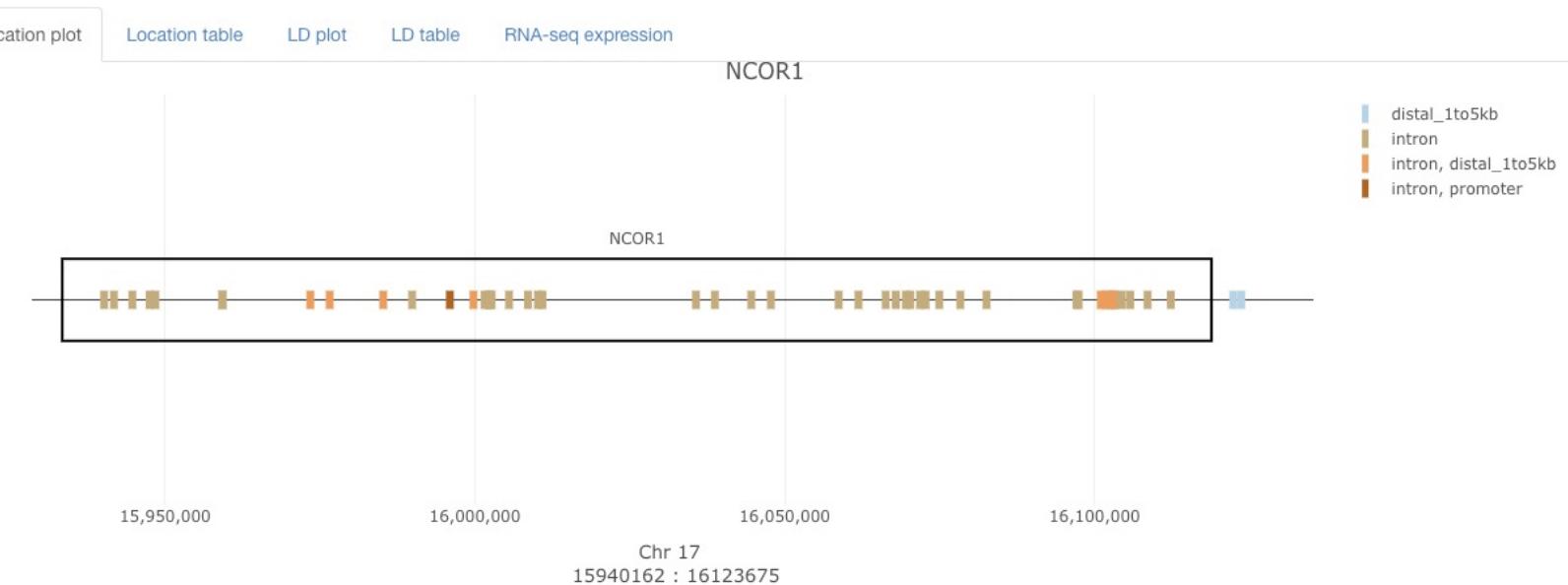
---

Select gene by Entrez symbol  
NCOR1

Select SNP annotations  
 intron  exon  promoter  5UTR  3UTR  
 distal\_1to5kb

Expand to include genes within \_\_ kb  
0

Plot options  
 Annotate LD plot\*  
\*Can increase load time by several minutes. Recommend using only once all input parameters are confirmed

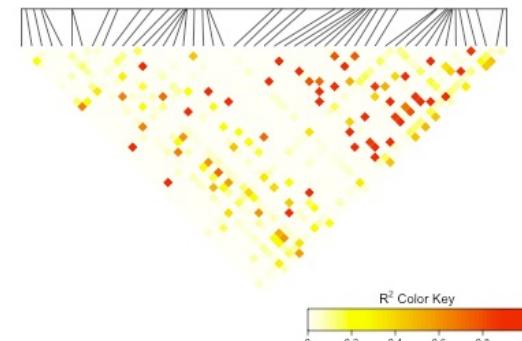


Location plot Location table LD plot LD table RNA-seq expression

Linkage disequilibrium calculated from all samples, regardless of selection on left.

Pairwise LD

Physical Length: 183.5kb



All samples included if none selected on left.

Show 10 entries

Search: 

libID	RSID	FULLIDNO	condition	Sample_Group	KCHCA_AGE_YR_CURRENT	M0_KCVSEX	experiment	NCOR1
1	RS102052_MEDIA	RS102052	84224-1-13	MEDIA	RSTR	18	F	validation 8.63350120592372
2	RS102052_TB	RS102052	84224-1-13	TB	RSTR	18	F	validation 8.26732205932194
3	RS102053_MEDIA	RS102053	84275-1-03	MEDIA	LTBI	24	M	validation 8.59187811433173
4	RS102053_TB	RS102053	84275-1-03	TB	LTBI	24	M	validation 8.59456774420368
5	RS102054_MEDIA	RS102054	84275-1-04	MEDIA	LTBI	22	M	original 8.7252137267155
6	RS102054_TB	RS102054	84275-1-04	TB	LTBI	22	M	original 8.24949554270164
7	RS102057_MEDIA	RS102057	84222-1-17	MEDIA	LTBI	22	M	original 8.67898110679159
8	RS102057_TB	RS102057	84222-1-17	TB	LTBI	22	M	original 8.62398713597264
9	RS102059_MEDIA	RS102059	84441-1-02	MEDIA	LTBI	37	F	validation 8.68402153188275
10	RS102059_TB	RS102059	84441-1-02	TB	LTBI	37	F	validation 8.38517471404199

Showing 1 to 10 of 160 entries

Previous 1 2 3 4 5 ... 16 Next