Epigenetically programmed resistance to tuberculosis

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2021.05

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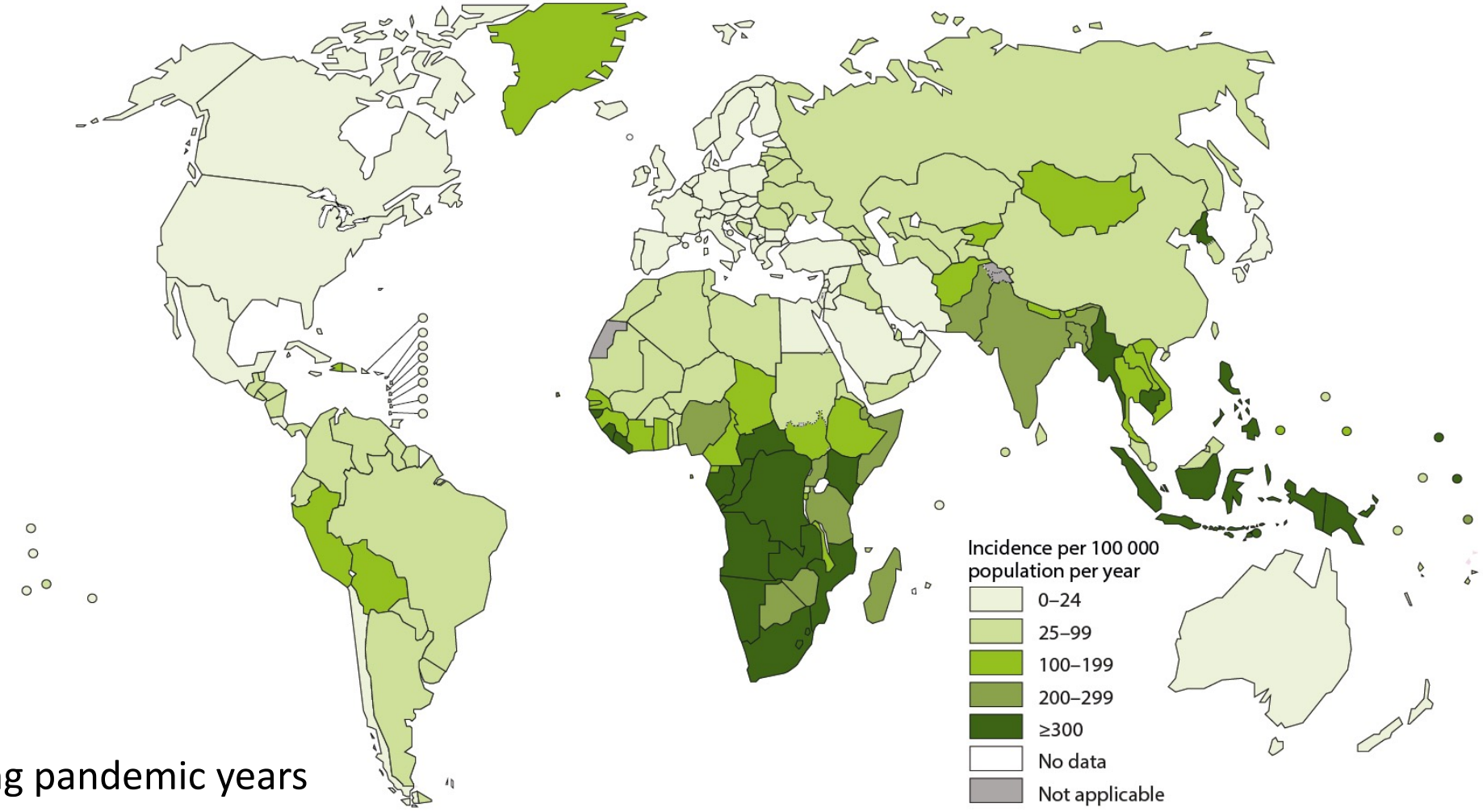
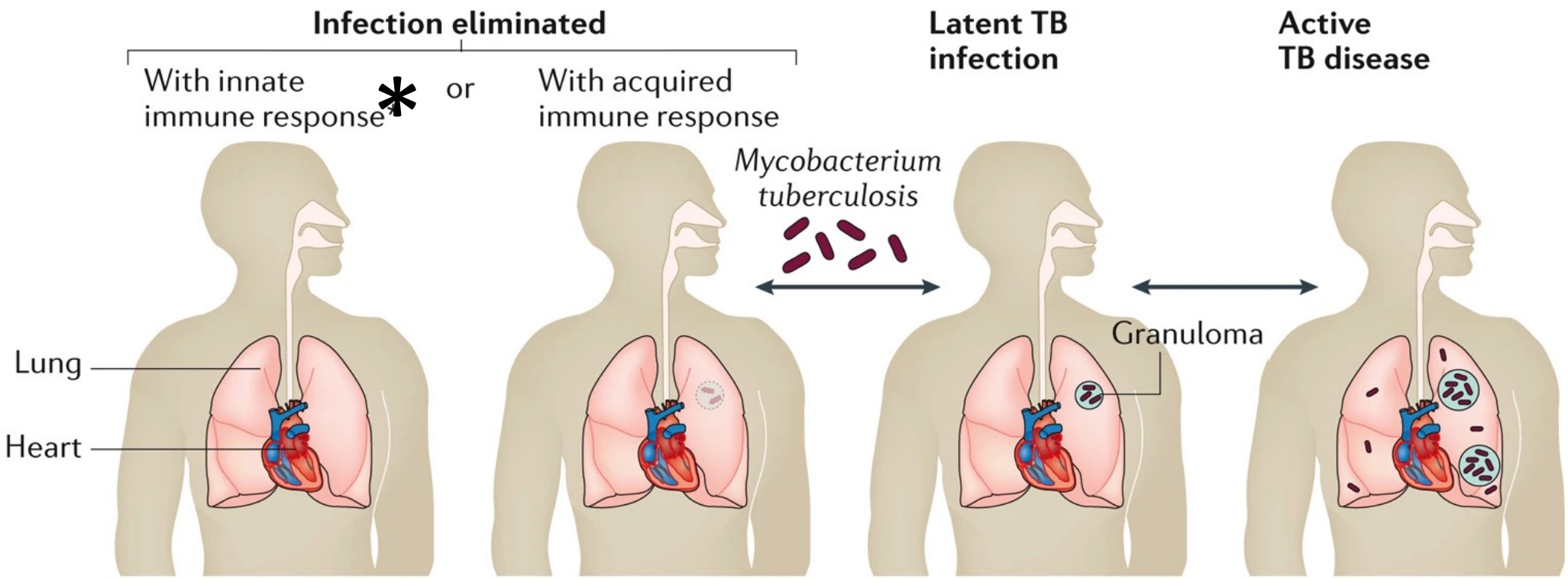
M. tuberculosis is the leading cause of single agent infectious disease death… and has been for 100s of years.*



*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	Negative	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Positive

TST: tuberculin skin test
IGRA: interferon gamma release assay

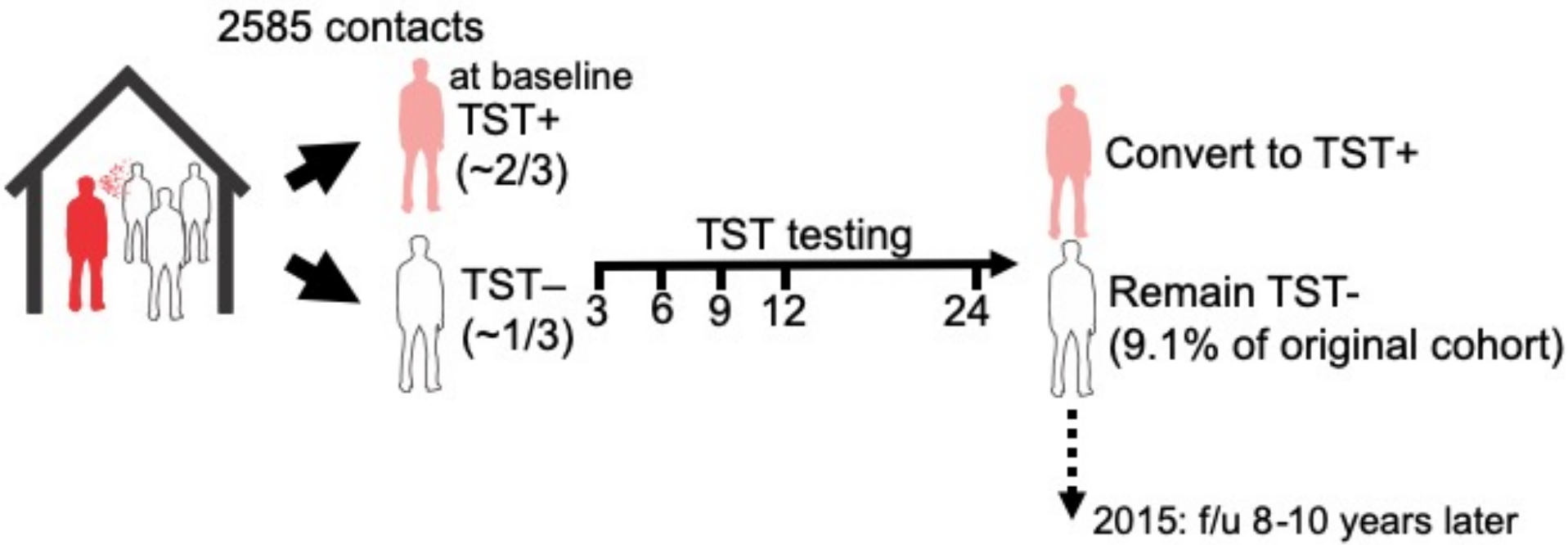
Pai *et al* 2016 doi: 10.1038/nrdp.2016.76

* 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
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Cathy Stein
Case Western

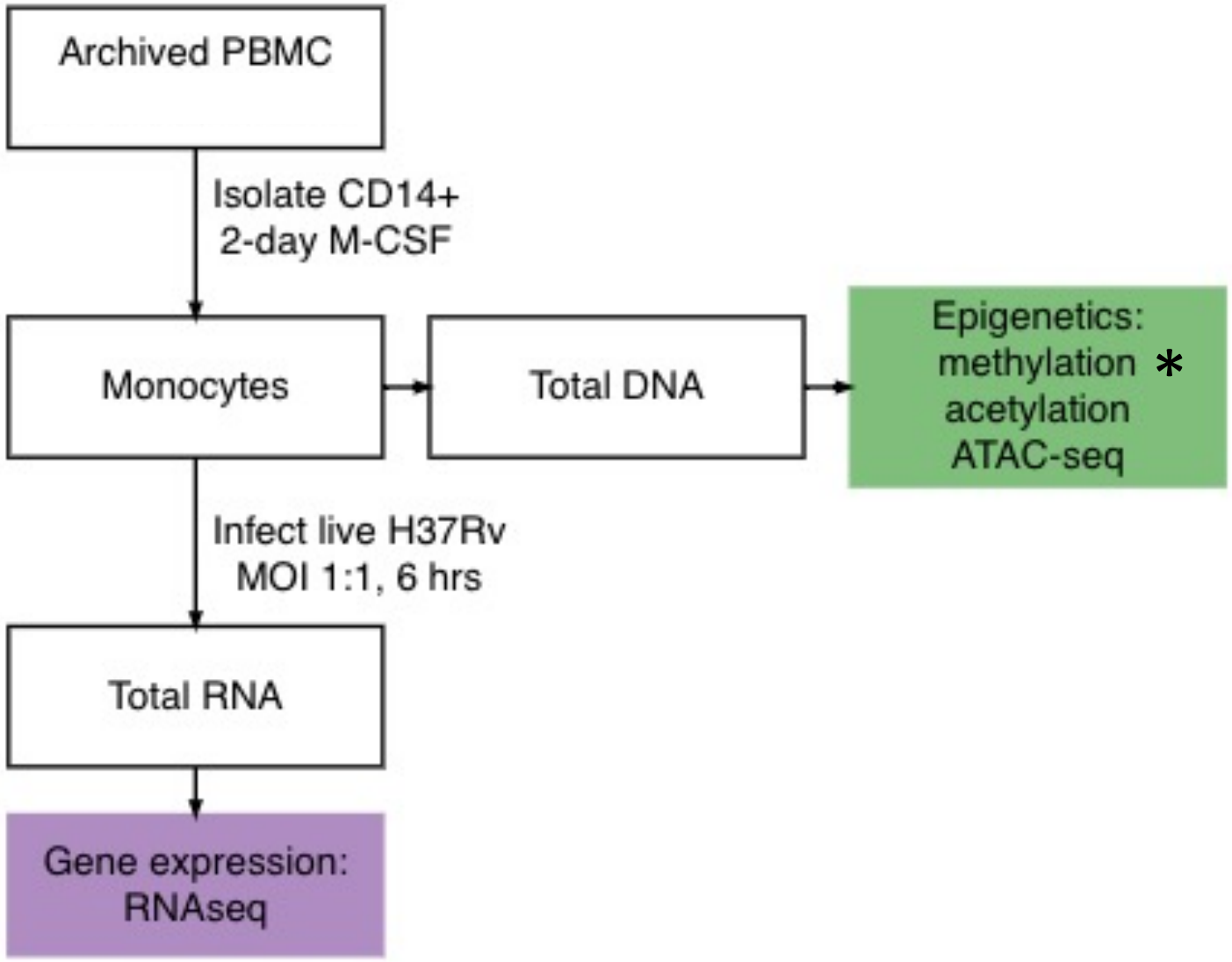
Henry Boom

Harriet Mayanja-Kizza
Makerere U

Stein *et al* 2018 doi: 10.1093/cid/ciy751

PBMC: peripheral blood mononuclear cells

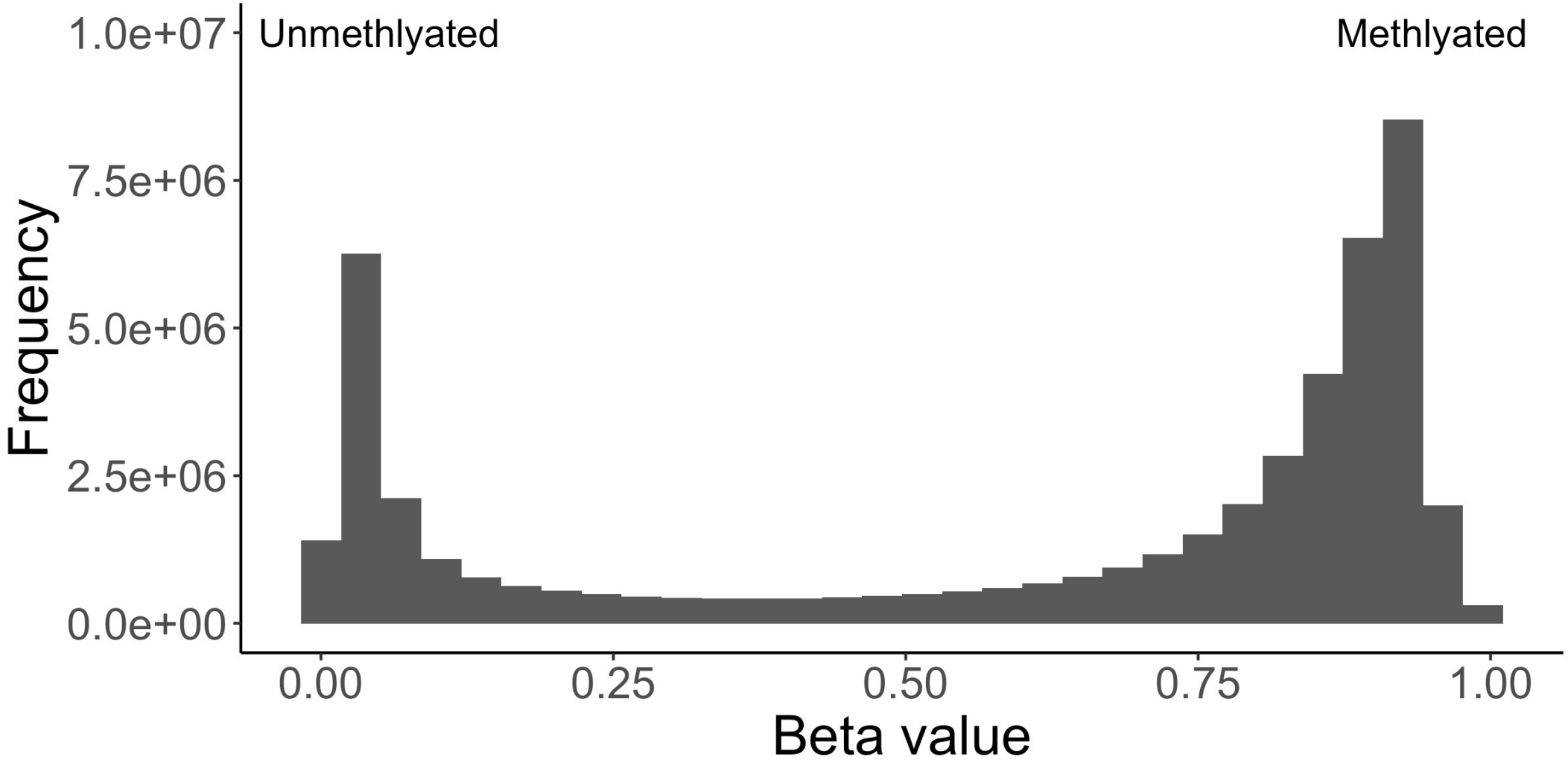
Design



Demographics

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

Infinium MethylationEPIC array (bisulfite sequencing)



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[a], multi-alignment[b], XY
2. Normalization
   - Functional normalization[c]
     - Quantile normalization based on control probes to estimate technical variation
   - ComBat batch correction
   - Log2 transformation

[a]Zhou *et al* 2017 doi: 10.1093/nar/gkw967
[b]Nordlund *et al* 2013 doi: 10.1186/gb-2013-14-9-r105
[c]Fortin *et al* 2014 doi: 10.1186/s13059-014-0503-2

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 (ƛ) and scaling factor (C) where ƛ/C = 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 ƛ

Peters *et al* 2015 doi: 10.1186/1756-8935-8-6

Probe model fitting (`R::lmekin`)

Main variable

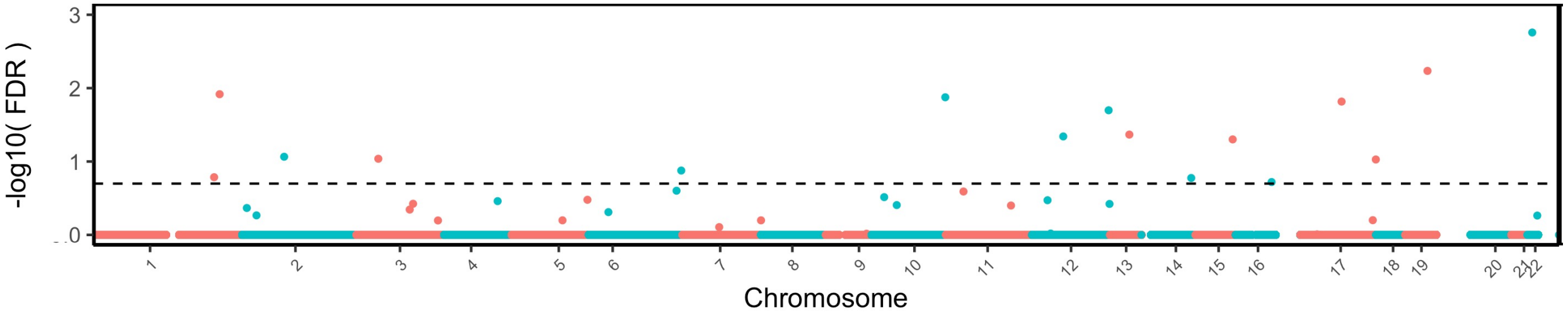
- RSTR vs LTBI

Potential co-variates

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

Differentially methylated probes (DMP)

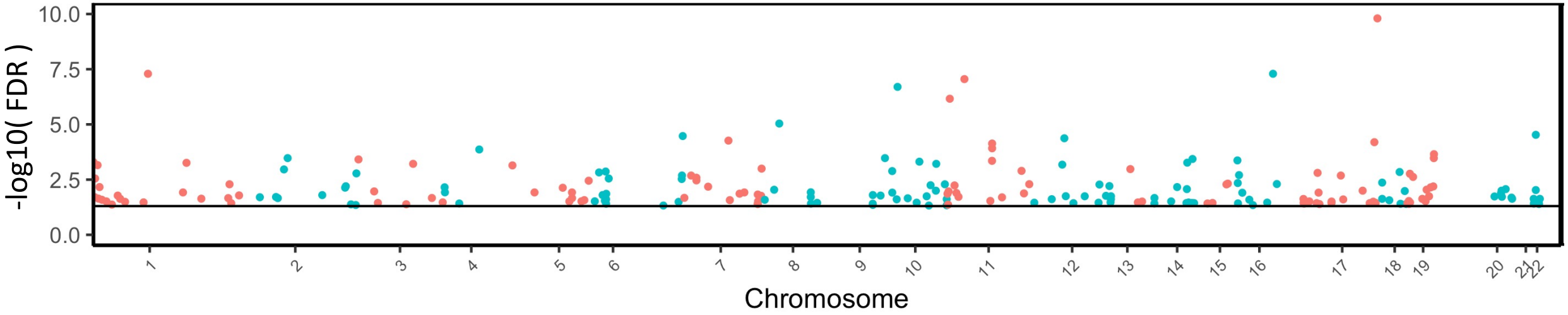
```
~ RSTR + age + sex + kinship
```



- - - FDR = 0.2

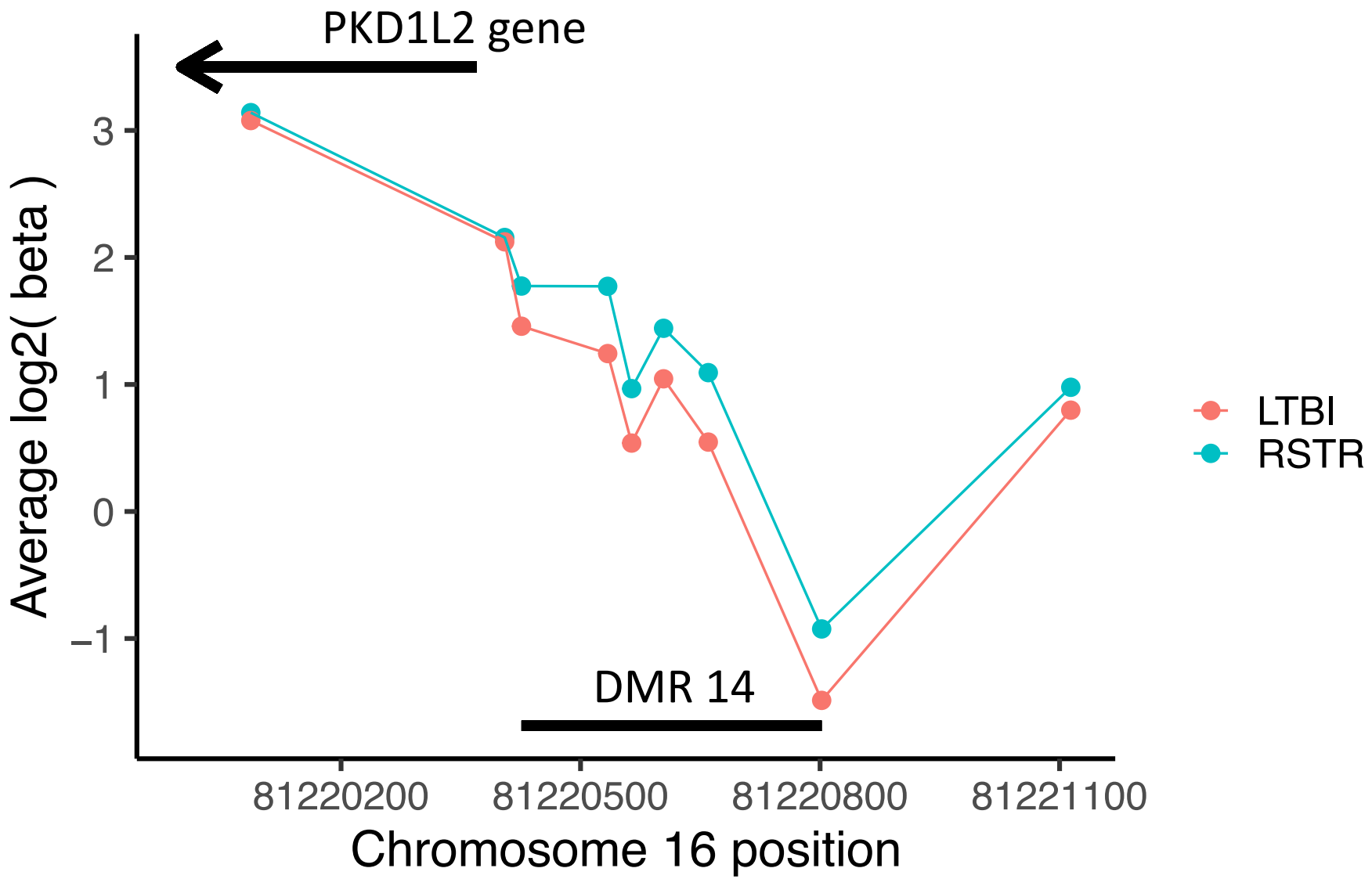
Differentially methylated regions (DMR)

```
~ RSTR + age + sex + kinship
```



— FDR = 0.05

Example

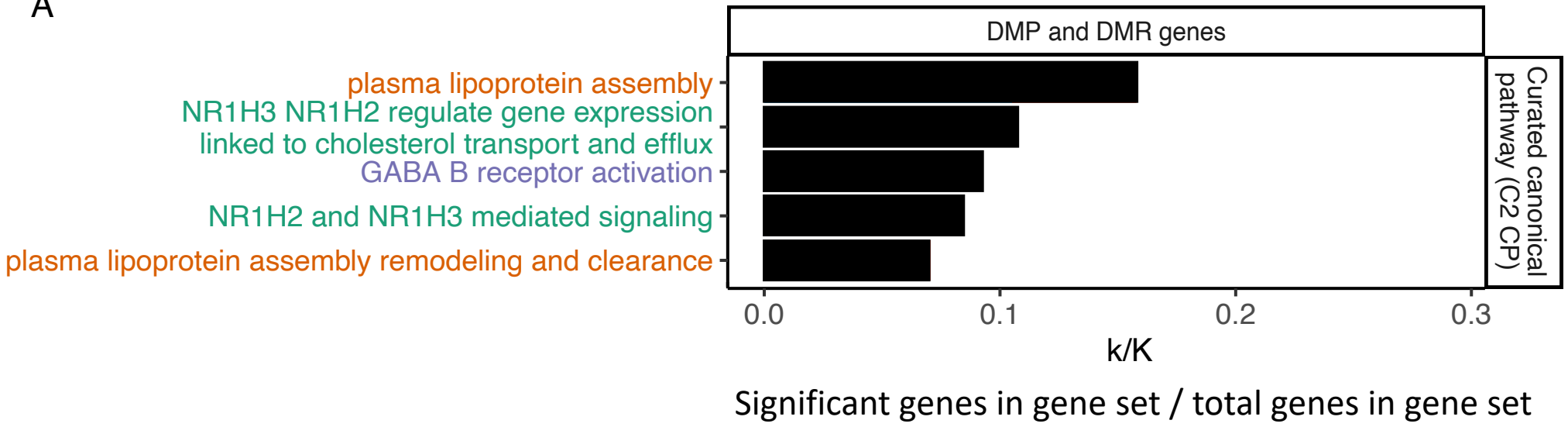

PKD1L2 gene
DMR 14
Average log2(beta)
3
2
1
0
−1
81220200
81220500
81220800
81221100
Chromosome 16 position
LTBI
RSTR

Genes with differential methylation

Enrichment of Broad gene sets (MSigDB)

FDR < 0.1

A



Lipoprotein

Liver X (NR1H)

GABA B

Genes with differential methylation

STRING score

400

600

800

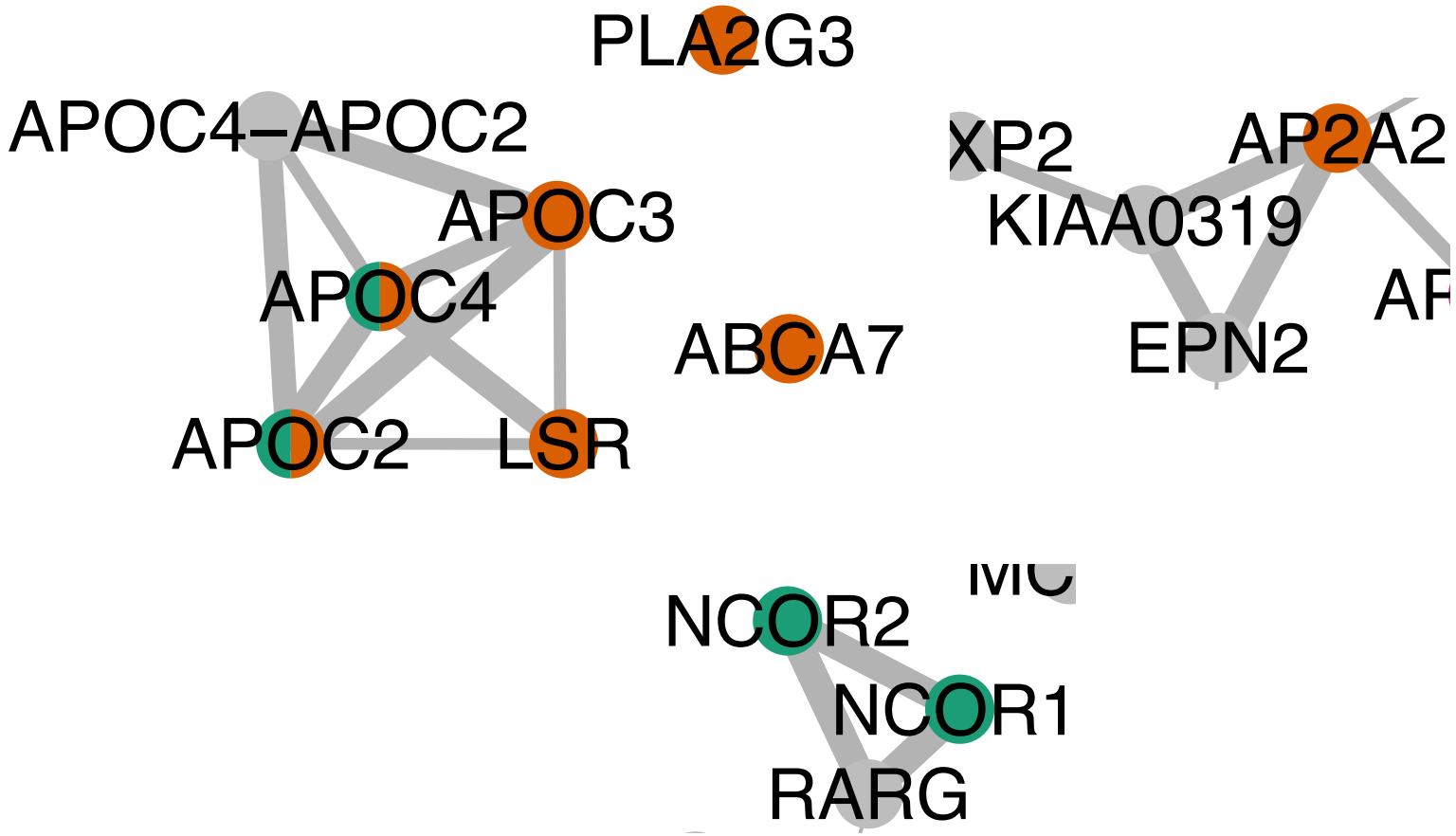
1000

Enrichment

~~GABA B~~

lipoprotein

liver X (NR1H)



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
  - Cholesteryl 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*

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

NATIONAL INSTITUTES OF HEALTH

BILL & MELINDA GATES foundation

MRC SOUTH AFRICA

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL

Tuberculosis prevention and treatment

1908: Bacillus Calmette-Guérin (BCG) vaccine

- Up to 70% prevention of death due to TB
- Less effective in adults
- Many permissive infections that lead to transmission

1943: Streptomycin discovered

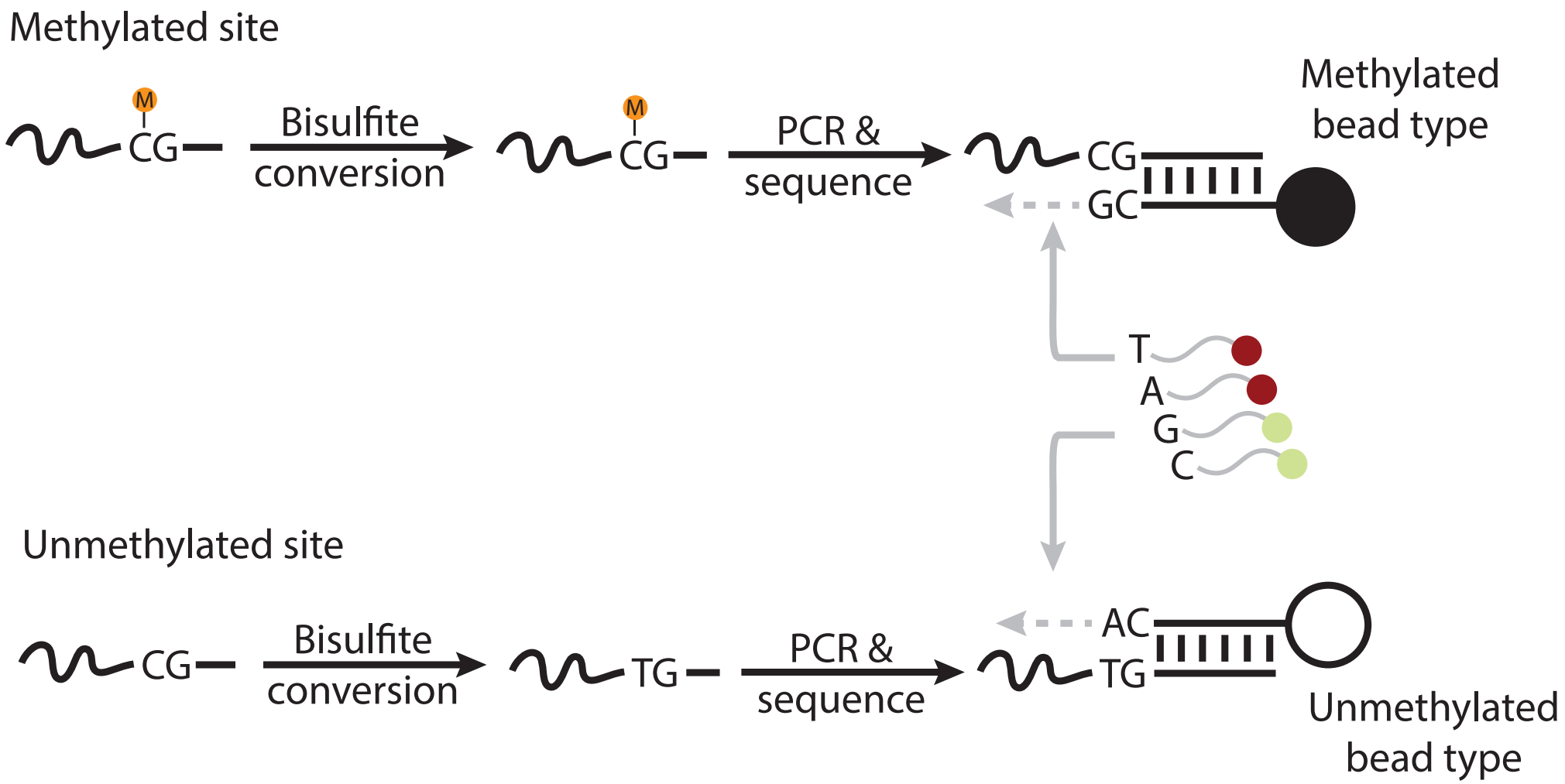
1950-1970: Further antibiotic discovery leading to 4-drug cocktail

- Resistance seen as early as 1956

Today

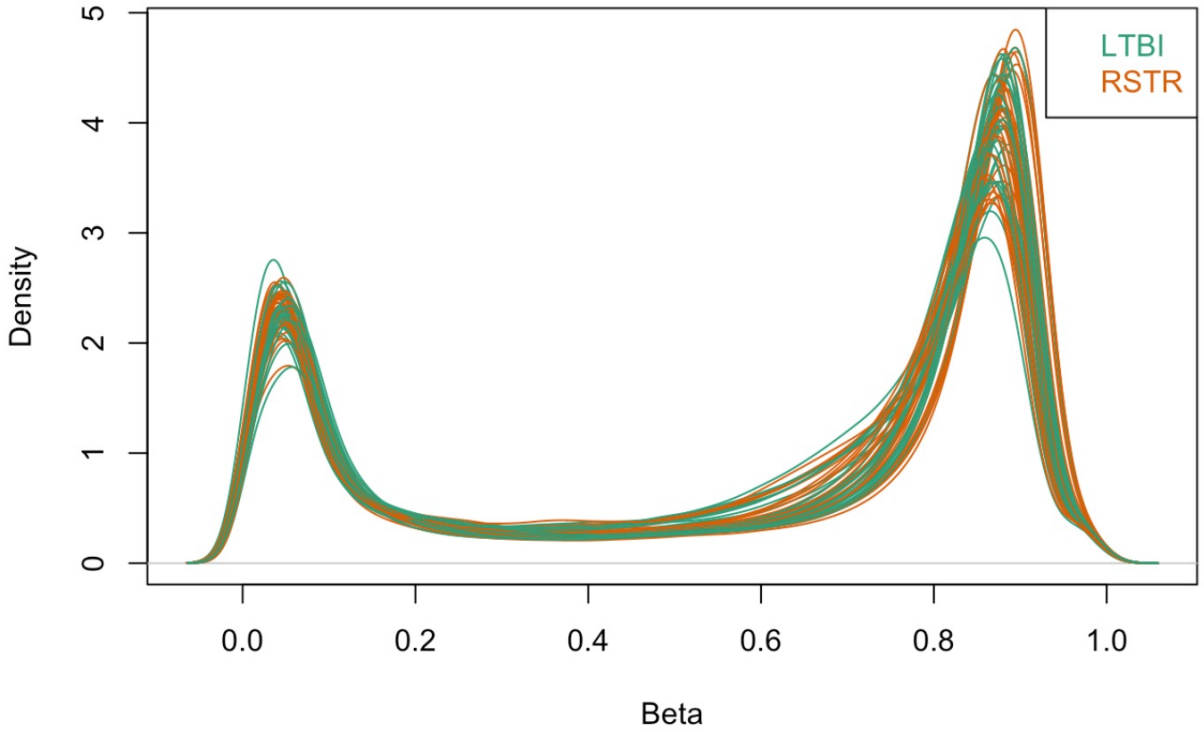
- 6 to 9-month treatment courses of 4-drug
- >10 on-going trials of new treatments, particularly 3-month short courses

Infinium MethylationEPIC array: Bisulfite sequencing

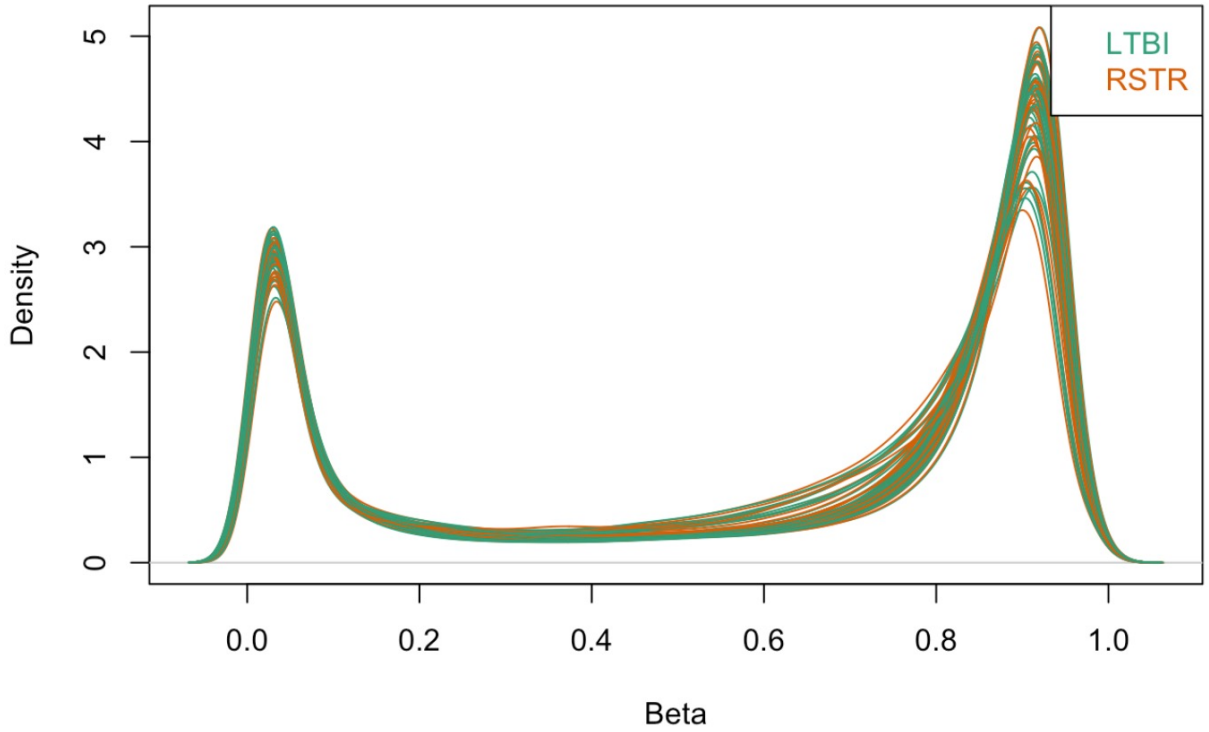


Functional normalization

Before

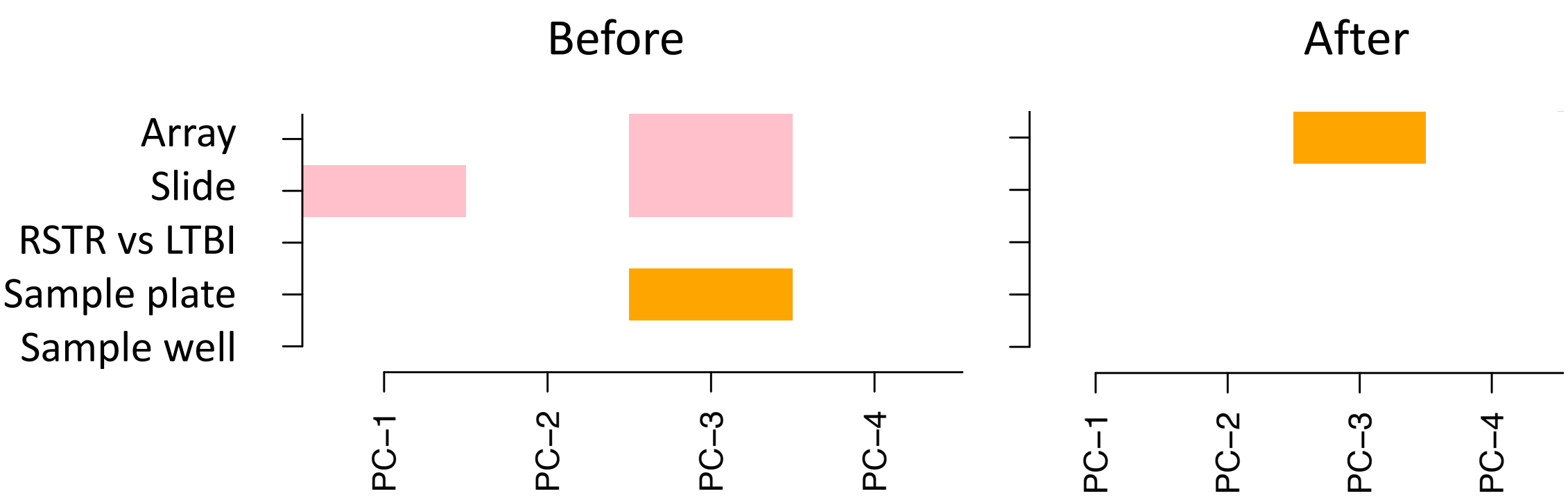


After



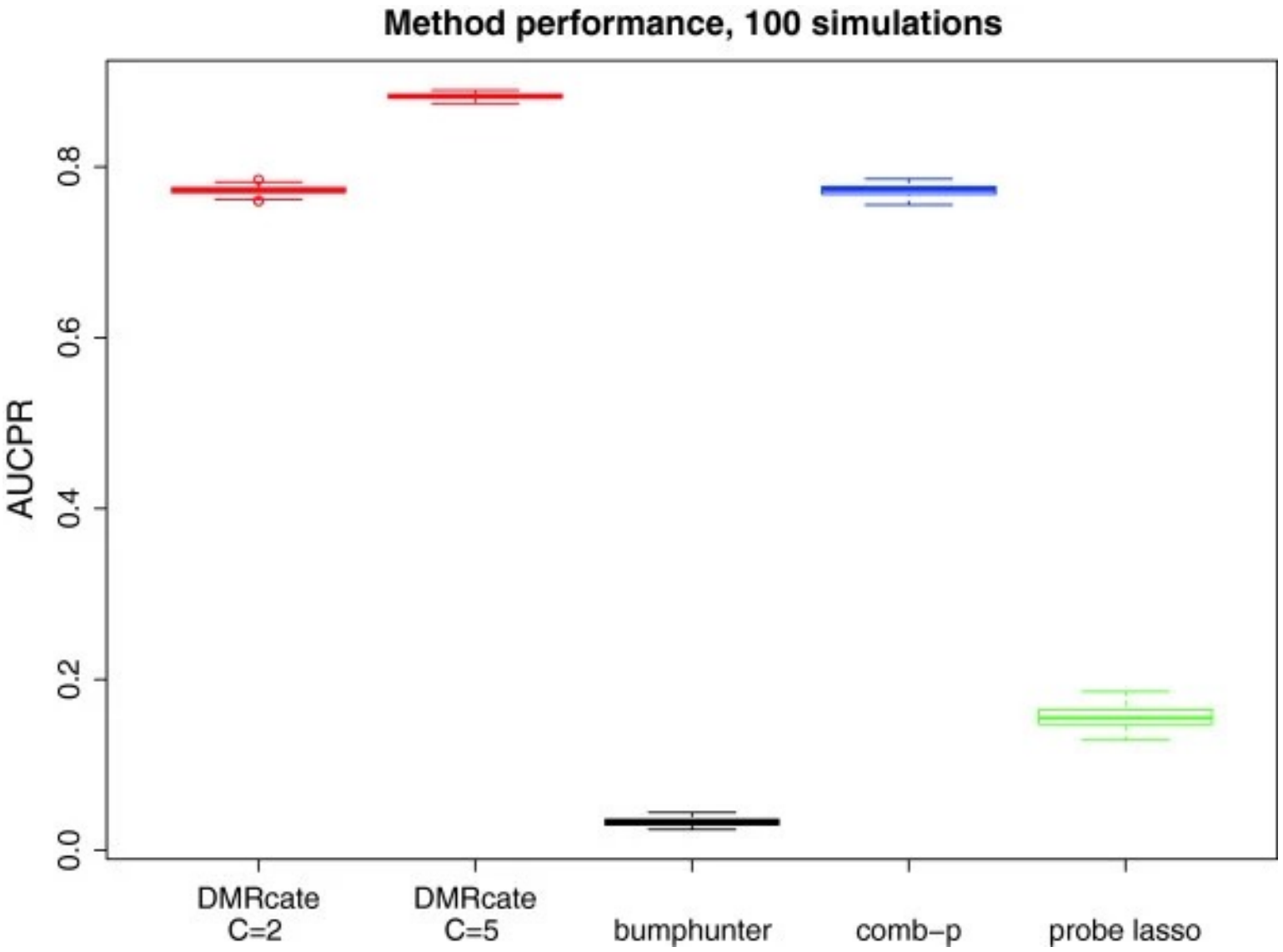
ComBat batch correction

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



DMRcate

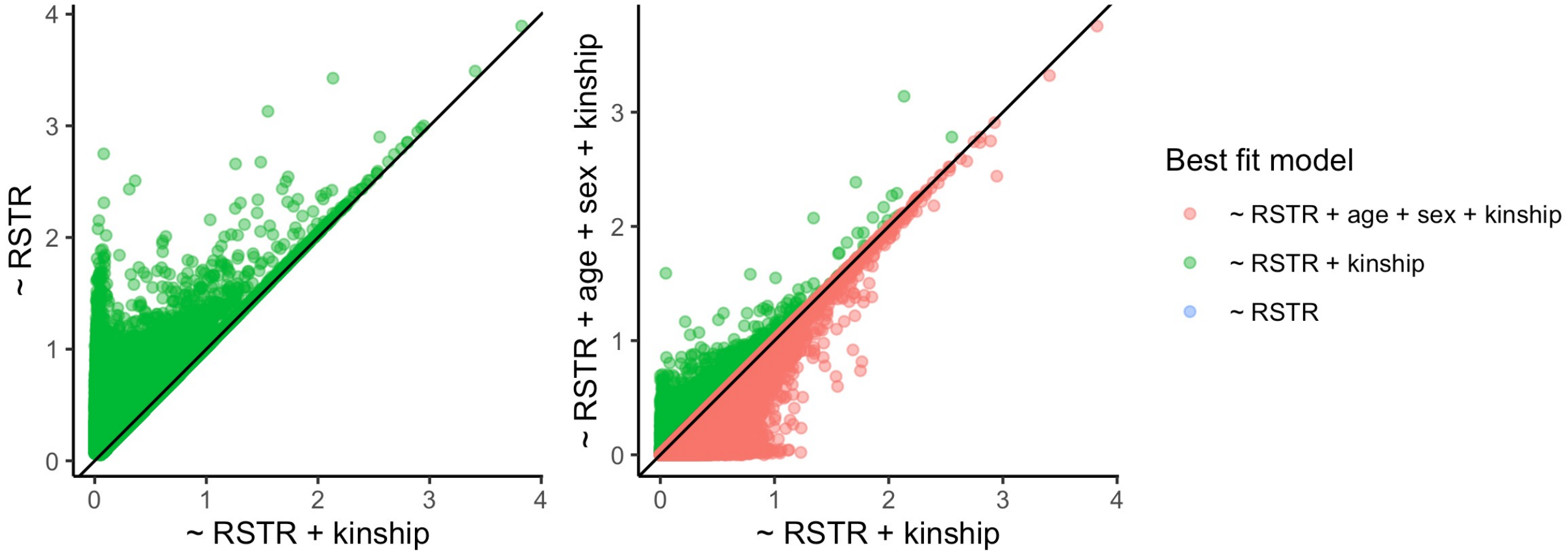
Area under the precision-recall curve



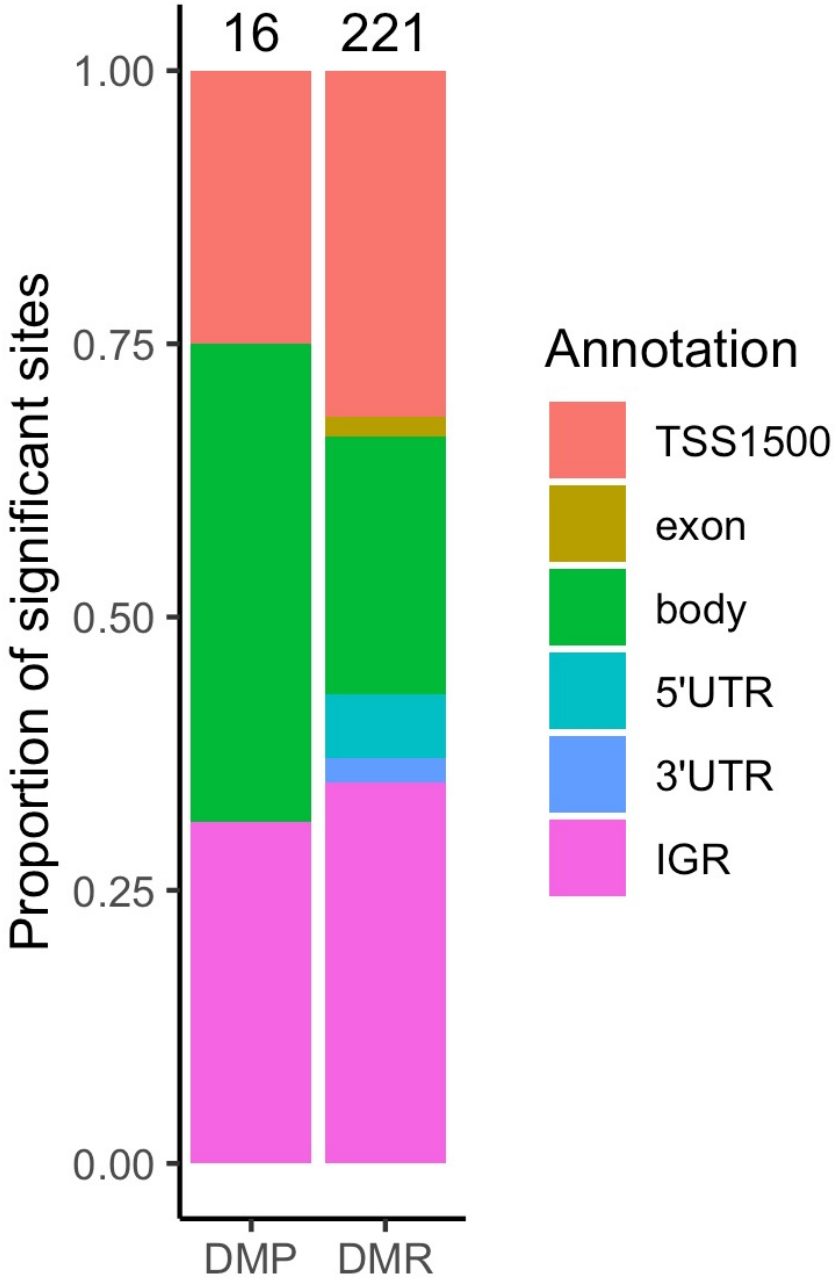
Peters *et al* 2015 doi: 10.1186/1756-8935-8-6

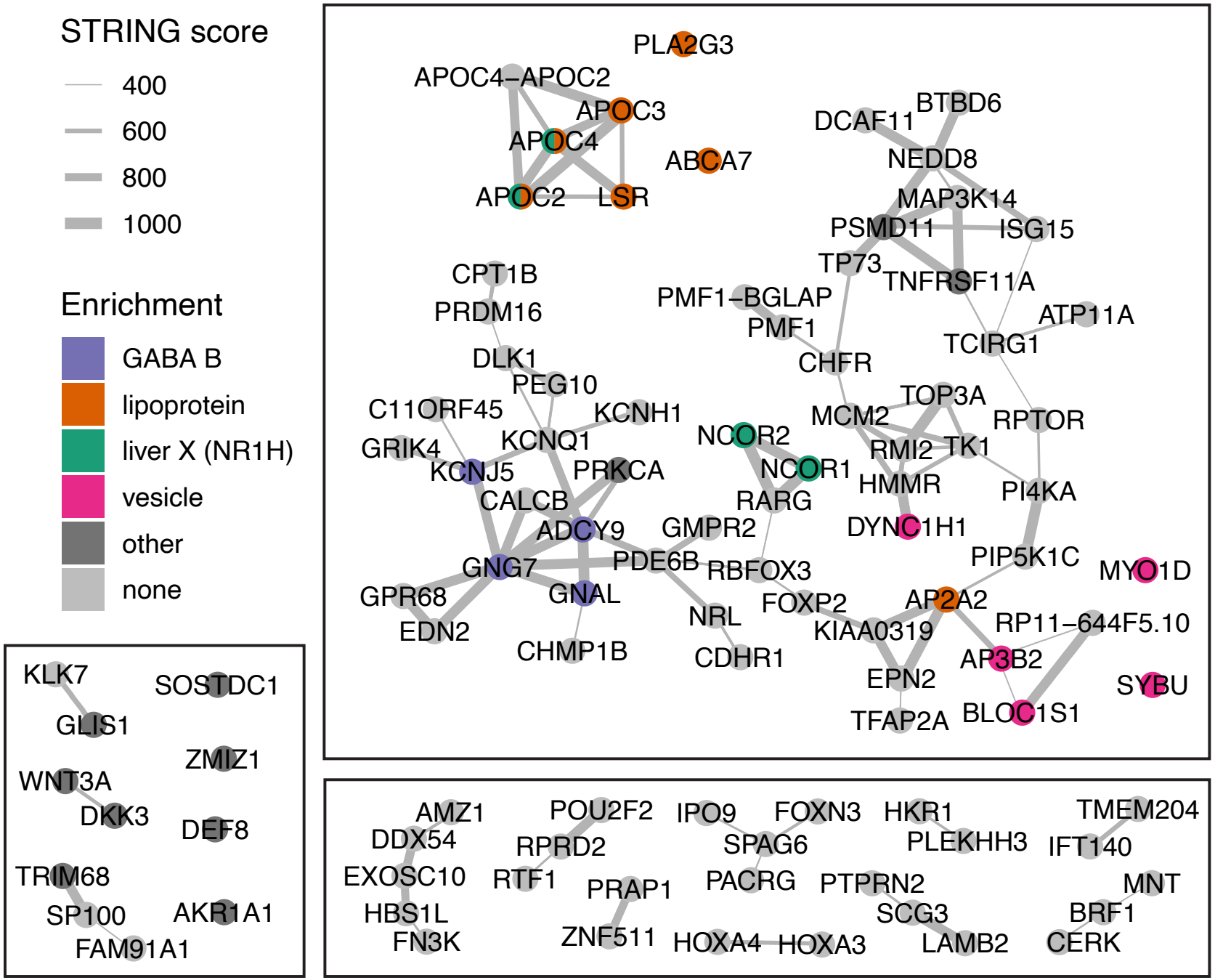
Probe model fitting

Mean residual standard deviation (sigma)



Annotation types




STRING score
400
600
800
1000
Enrichment
GABA B
lipoprotein
liver X (NR1H)
vesicle
other
none
PLA2G3
APOC4–APOC2
APOC3
APOC4
ABCA7
APOC2
LSR
DCAF11
BTBD6
NEDD8
MAP3K14
PSMD11
ISG15
TP73
TNFRSF11A
CPT1B
PRDM16
PMF1–BGLAP
PMF1
ATP11A
TCIRG1
CHFR
DLK1
PEG10
C11ORF45
KCNH1
TOP3A
MCM2
RPTOR
GRIK4
KCNQ1
NCOR2
RMI2
TK1
KCNJ5
PRKCA
NCOR1
HMMR
PI4KA
CALCB
RARG
ADCY9
GMPR2
DYNC1H1
PIP5K1C
GNG7
PDE6B
RBFOX3
MYO1D
GPR68
GNAL
FOXP2
AP2A2
NRL
RP11–644F5.10
EDN2
KIAA0319
CHMP1B
CDHR1
AP3B2
EPN2
SYBU
TFAP2A
BLOC1S1
KLK7
SOSTDC1
GLIS1
ZMIZ1
WNT3A
DKK3
DEF8
TRIM68
SP100
AKR1A1
FAM91A1
AMZ1
POU2F2
IPO9
FOXN3
HKR1
TMEM204
DDX54
RPRD2
SPAG6
PLEKHH3
IFT140
EXOSC10
RTF1
PRAP1
PACRG
PTPRN2
MNT
HBS1L
SCG3
BRF1
FN3K
ZNF511
HOXA4
HOXA3
LAMB2
CERK

Potential outcomes

Assumption: Higher methylation = lower expression

- Higher methylation in NCOR1/2 results in less repression of
  - NR1H pathways → more cholesterol efflux
  - PPAR pathways → more free fatty acids release
- Lower methylation in APOC4/2 results in more activation of
  - Lipases → more free fatty acids release

Potential for differences in cholesterol and/or lipid availability in monocytes