Supplementary Material

Predicting RTS,S-vaccine-mediated protection from transcriptomes in a malaria-challenge clinical trial

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1 N-PLS-DA METHODOLOGICAL DETAILS

The transcriptome data set was represented as a multiway data set (subject×probe set [gene]×time). In the N-PLS-DA, the data were transformed in to a series of components (similar to principal component analysis [PCA] (Jackson, 1991;Jolliffe, 2002)) where the first component encapsulates the most variation in the total data set that correlates with controlled human malaria infection (CHMI) outcome, and the subsequent components encapsulate progressively less variation. Together, these factors describe variations in the data set which were encapsulated in predictive mathematical models. Hence the kinetics of the changes induced by the vaccination were captured explicitly in each of the mathematical models.

Each mathematical model was generated through the iterative selection of probe sets and the selection of the minimal number of components required from the transformed data set to achieve optimal model performance. Model performance was evaluated by a double cross validation (DCV) approach. DCV resulted in 10 collections (ensembles) of 10 models of correlation, yielding a total of 100 individual models, with performance statistics. The difference in model performance was identified using the DQ^2 statistic. This statistic is based on a least-squares method for analyzing the difference between prediction and CHMI outcome (Westerhuis et al., 2008) and was more discriminatory than using the fraction of correctly classified outcomes. The consideration of two or three components was typically sufficient for optimal prediction performance. Each model typically consisted of data from 2-40 probe sets and optimal performance was typically observed after several rounds of probe set selection. Predictive performance was validated using label permutation. The worst, average and best model performance measures in a given ensemble of models were always higher than the most frequent performance measure generated by label

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2 SUPPLEMENTARY TABLE 1

Genes/probe sets selected by the data-driven modeling

Gene	Probe set ID	Frequency of use in models	Cluster
ACTG1	224585_x_at	1	D
ATL2	222700_at	2	A
ATP5I	209492_x_at	4	D
ATP6V0C[a,b]	36994_at	20	D
ATP6V0C[a]	200954_at	1	D
AURKAIP1	225555_x_at	8	D
B3GALT6	1553959_a_at	1	D
BAG1	202387_at	29	B
BAG5	202984_s_at	3	D
C11orf73	219979_s_at	1	C
C14orf64	1559097_at	3	A
CCDC59	222792_s_at	2	D
CCT6P1	227301_at	1	A
CD302	203799_at	1	C
CNPY4	227313_at	2	A
COMMD1	226024_at	1	D
CSNK1G2	202573_at	10	D
DENND4C	205684_s_at	1	A
DPP3	232510_s_at	1	D
DVL1	203230_at	1	C
EDEM2	78047_s_at	5	D
EIF4E2	213571_s_at	11	B
EMC10	224727_at	21	B
EXOSC6	227696_at	3	A
FAM21A/B /C	212370_x_at	4	B
FAM21A/B /C /D	214946_x_at	1	B
FAM53C	218023_s_at	1	B
FBXO9	212991_at	30	A
GADD45B	207574_s_at	9	B
GLIPR1	226142_at	1	D
GTF2E2	202680_at	97	B
GTF2F1	202355_s_at	45	D
HECTD1	224481_s_at	2	A

Gene	Probe set ID	Frequency of use in models	Cluster
HLA-A[a,b]	215313_x_at	71	B
HLA-A[a]	213932_x_at	1	B
HLA-B	209140_x_at	2	B
HLA-C	208812_x_at	1	B
HLA-DMB	203932_at	1	B
HSP90B1	200598_s_at	2	B
ICAM2	213620_s_at	1	D
IDH3G	202471_s_at	6	B
IL23A	217328_at	4	A
IPW	241834_at	1	A
IRF7	208436_s_at	1	B
KLHL6	1560396_at	1	B
LMLN	244881_at	3	A
LRPAP1	201186_at	11	D
LRRC14	32062_at	1	D
LSMEM1	239203_at	2	C
LYRM2	227712_at	6	D
MRFAP1	226091_s_at	1	C
MT1F	217165_x_at	2	B
MT2A	212185_x_at	1	B
MYBBP1A	219098_at	1	D
MYD88	209124_at	32	B
NAP1L5	228062_at	1	A
NAPRT1	226707_at	1	B
NCAPH2	40640_at	13	B
NCBP2-AS2	225657_at	9	D
NCF1C	214084_x_at	1	B
NOB1	223018_at	57	D
NSD1	219084_at	2	B
NUCKS1	229353_s_at	1	D
NUDT14	231914_at	2	D
OR2A9P	222290_at	10	A
ORC2	204853_at	11	A
PDCD4	212593_s_at	1	A
PDZK1	205380_at	1	A
PLA2G12A	242323_at	2	A
PLIN2	209122_at	1	D

Gene	Probe set ID	Frequency of use in models	Cluster
PMF1	202337_at	2	D
PML	211012_s_at	8	B
POLE4	1553587_a_at	1	B
PTPN6	206687_s_at	1	B
PUS7L	229751_s_at	3	A
RAD23A	201046_s_at	1	D
RAPH1	225189_s_at	1	A
RBBP6	227635_at	5	B
RHBDF2	219202_at	1	B
RIC8A	221647_s_at	2	D
RMND5A	212482_at	4	A
RNASEH2C	226453_at	2	B
RNF31	231635_x_at	58	B
RPF2	225866_at	1	D
RPL23	200888_s_at	7	C
RPS6KA3	203843_at	2	D
RRAS	212647_at	3	D
SCO2	205241_at	8	B
SELPLG	209879_at	1	D
SFXN3	217226_s_at	1	D
SHARPIN	220973_s_at	5	D
SP110[a,b]	223980_s_at	3	B
SP110[a]	209762_x_at	2	B
SRSF1	211784_s_at	1	C
STK10	40420_at	1	D
STUB1	217934_x_at	8	D
SWT1	223548_at	1	A
TAF1	227205_at	1	A
TAF10	200055_at	1	D
TAX1BP1	200977_s_at	1	B
TBCB[a,b]	211759_x_at	17	D
TBCB[a]	216194_s_at	1	D
TNIP2	48531_at	1	B
TRAPPC5	225870_s_at	1	D
TRIM26	202702_at	4	B
TRIM52	1568594_s_at	1	A
TSTA3	36936_at	1	D

Gene	Probe set ID	Frequency of use in models	Cluster
TTC33	231479_at	1	A
UBB	200633_at	2	D
UCP2[a,b]	208997_s_at	8	D
UCP2[a]	208998_at	8	D
USP40	225089_at	1	A
WAS	38964_r_at	2	B
WWP2	204022_at	6	B
ZFP36	201531_at	1	B
ZNF117	235408_x_at	9	C

[a] For genes with more than one probe set, the data from [b] the probe set that was most frequently represented in the models were considered as the representative data for that gene in the manuscript.

3 SUPPLEMENTARY TABLE 2

References to support the characterization of the immune-related genes selected by the data-driven modeling.

Gene	Cluster	Freq.	NF-κB	IFN-γ	Ubiq.	References
FBXO9	A	30	+		+	(Cenciarelli et al., 1999;Winston et al., 1999;Yang et al., 2009)
IL23A	A	4	+	+		(Carmody et al., 2007;Teng et al., 2010;Qian et al., 2011;Sheikh et al., 2011)
CNPY4	A	2				(Konno et al., 2006;Hart and Tapping, 2012)
PDCD4	A	1	+	+		(Hilliard et al., 2006;Yamanaka et al., 2009;Sheedy et al., 2010;Cohen and Prince, 2013)
HLA-A	B	71	+	+		(Hakem et al., 1991;Girdlestone et al., 1993;Min et al., 1996;Boehm et al., 1997;Girdlestone, 2000;Johnson, 2003;Shen et al., 2009;Othman et al., 2012;Norman et al., 2013;Sleiman et al., 2014)
RNF31	B	58	+		+	(Gerlach et al., 2011;Ikeda et al., 2011;Tokunaga et al., 2011;Stieglitz et al., 2013;Schaeffer et al., 2014)
MYD88	B	32	+			(Adachi et al., 1998;Burns et al., 1998;Cohen, 2014)
BAG1	B	29	+		+	(Tsukahara and Maru, 2010;Kettern et al., 2011)
NCAPH2	B	13				(Gosling et al., 2007;Gosling et al., 2008)
GADD45B	B	9	+			(Takekawa and Saito, 1998;Papa et al., 2004;Liu et al., 2005;Thyss et al., 2005)
PML	B	8	+	+	+	(Wu et al., 2003;Carracedo et al., 2011;Guo et al., 2014)
SCO2	B	8	+			(Mauro et al., 2011)
WWP2	B	6	+		+	(Chen et al., 2009;Yang et al., 2013)
SP110	B	3		+		(Pan et al., 2005;Tosh et al., 2006;Abhimanyu et al., 2011;Cai et al., 2013;Fox et al., 2014)
HLA-B	B	2		+		(Chamberlain et al., 1991;Hakem et al., 1991;Min et al., 1996;Boehm et al., 1997)
HSP90B1	B	2	+	+		(Randow and Seed, 2001;Yang et al., 2007;Liu et al., 2010;Staron et al., 2010)
MT1F	B	2		+		(Vandeghinste et al., 2000;Huang et al., 2009)
RNASEH2C	B	2				(Rice et al., 2013;Crow et al., 2015)

Gene	Cluster	Freq.	NF-κB	IFN-γ	Ubiq.	References
WAS	B	2	+			(Orange et al., 2002;Borg et al., 2004;Gismondi et al., 2004;Huang et al., 2005;Krzewski et al., 2006;Serrano-Pertierra et al., 2012;Ham et al., 2013;Catucci et al., 2014;Sarkar et al., 2014;2015)
HLA-C	B	1	+[a]	+		(Boehm et al., 1997;Boss, 1997;Johnson, 2003;Shen et al., 2009;Othman et al., 2012)
HLA-DMB	B	1	+[a]	+		(Steimle et al., 1994;Boehm et al., 1997;Boss, 1997;Westerheide et al., 1997)
IRF7	B	1	+	+		(Kawai et al., 2004;Ogawa et al., 2005;Chau et al., 2008;Beattie et al., 2011)
KLHL6	B	1				(Gupta-Rossi et al., 2003;Kroll et al., 2005)
MT2A	B	1	+	+		(Giacconi et al., 2007;Jin et al., 2010;Toh et al., 2010;Pan et al., 2013)
PTPN6	B	1	+			(Tsui et al., 2006;Orr et al., 2010;Lee et al., 2011;Orr and Lanier, 2011;Wang et al., 2012a)
RHBDF2	B	1				(Adrain et al., 2012;McIlwain et al., 2012;Issuree et al., 2013)
TAX1BP1	B	1	+		+	(Shembade et al., 2007;Shembade et al., 2010;Shembade et al., 2011;Verstrepen et al., 2011;Nakano et al., 2013)
TNIP2	B	1	+			(Papoutsopoulou et al., 2006;Leotoing et al., 2011;Callahan et al., 2013)
ZFP36	B	1	+	+		(Liang et al., 2009;Schichl et al., 2009;Bros et al., 2010;Kang et al., 2011;Kaplan et al., 2011;Qian et al., 2011;Schott et al., 2014)
CD302	C	1				(Kato et al., 2007)
STUB1	D	8	+		+	(Kettern et al., 2011;Yang et al., 2011;Chen et al., 2013)
UCP2	D	8	+	+		(Bai et al., 2005;Haschemi et al., 2011)
SHARPIN	D	5	+		+	(Gerlach et al., 2011;Ikeda et al., 2011;Tokunaga et al., 2011;Sieber et al., 2012;Wang et al., 2012b;Pouwels et al., 2013)
RRAS	D	3	+			(Li et al., 2001;Shang et al., 2011;Singh et al., 2011)
RPS6KA3	D	2	+			(Lin et al., 2008;Kakugawa et al., 2009;Peng et al., 2010;Takada, 2015;Takada et al., 2016)
COMMD1	D	1	+		+	(Maine et al., 2007;Starokadomskyy et al., 2013;Bartuzi et al., 2014;O'Hara et al., 2014)
DPP3	D	1				(Gamrekelashvili et al., 2013)

Gene	Cluster	Freq.	NF-κB	IFN-γ	Ubiq.	References
ICAM2	D	1				(Somersalo et al., 1995;Helander et al., 1996;Lehmann et al., 2003;Banerjee et al., 2007;Porter and Hall, 2009;Boscacci et al., 2010)
MYBBP1A	D	1	+			(Owen et al., 2007;Cai et al., 2013)
PLIN2	D	1				(Bougnères et al., 2009)
RAD23A	D	1			+	(Andersson et al., 2005;Fang et al., 2013)
STK10	D	1	+			(Endo et al., 2000;Tao et al., 2002;Fukumura et al., 2013)

[a] Association suggested from Ingenuity Pathway Analysis in Figure 6

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4 SUPPLEMENTARY TABLE 3

Genes/probe sets used in the IFN-driven modeling

Gene	Probe set ID	Frequency of use in models	Cluster
BAK1	203728_at	14	H
BAX[a,b]	208478_s_at	14	H
BAX[a]	211833_s_at	2	H
BCL2[a,b]	207004_at	52	G
BCL2[a]	203685_at	8	E
BCL2[a]	203684_s_at	1	E
CCL8	214038_at	11	E
CXCL10	204533_at	10	H
DDX58[a,b]	242961_x_at	24	E
DDX58[a]	218943_s_at	13	F
DDX58[a]	222793_at	5	H
EIF2AK2[a,b]	213294_at	3	H
EIF2AK2[a]	204211_x_at	2	H
GBP1[a,b]	202269_x_at	20	H
GBP1[a]	231577_s_at	9	H
GBP1[a]	202270_at	5	H
IFI16[a,b]	208965_s_at	12	F
IFI16[a]	206332_s_at	2	H
IFI16[a]	208966_x_at	2	H
IFI35	209417_s_at	3	H
IFI44[a,b]	214059_at	7	H
IFI44[a,c]	214453_s_at	0	
IFIH1[a,b]	219209_at	15	H
IFIH1[a]	1555464_at	2	H
IFIT1[c]	203153_at	0	
IFIT2	226757_at	6	H
IFIT3[a,b]	204747_at	4	H
IFIT3[a]	229450_at	2	H
IFITM1[a,b]	201601_x_at	1	F
IFITM1[a,c]	214022_s_at	0	
IFITM2	201315_x_at	3	F
IFITM3	212203_x_at	1	F
IFNGR1[a,b]	242903_at	6	E
IFNGR1[a]	202727_s_at	2	E
IFNGR1[a,c]	211676_s_at	0	
IFNGR2	201642_at	2	H

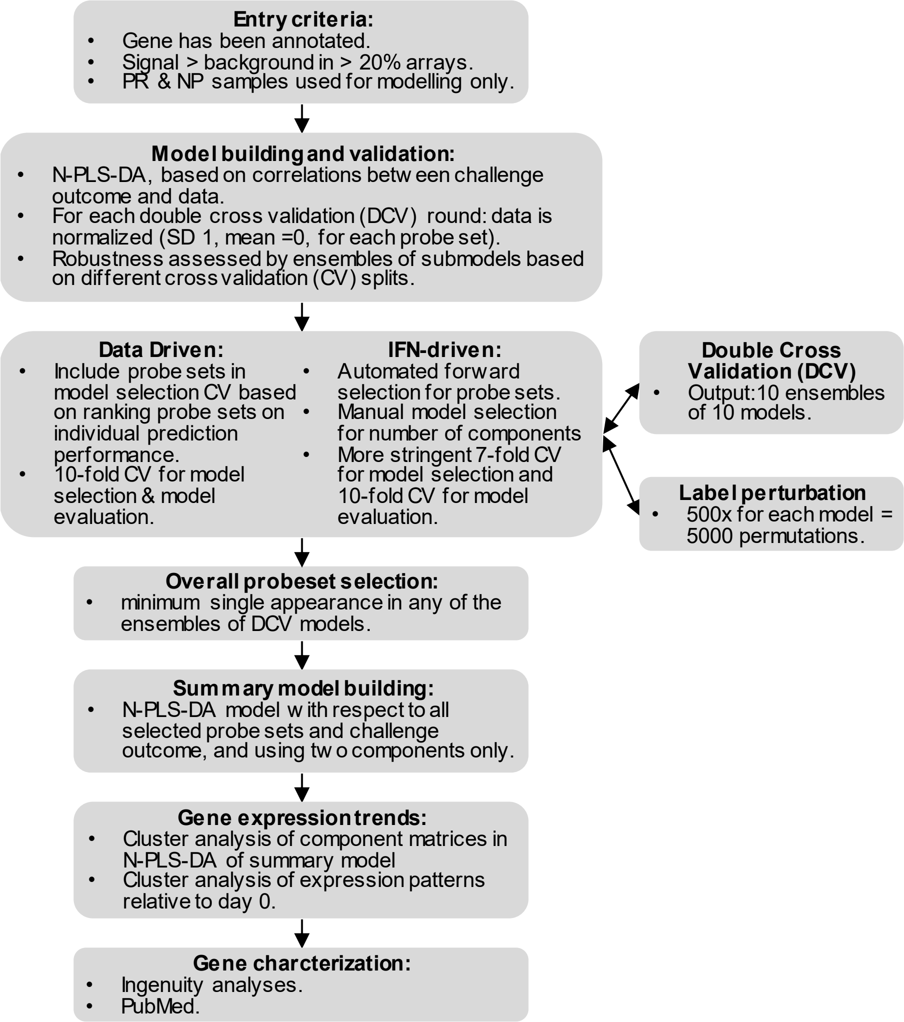
Gene	Probe set ID	Frequency of use in models	Cluster
IRF1[a,b]	238725_at	27	F
IRF1[a]	202531_at	13	F
IRF7	208436_s_at	36	F
IRF9	203882_at	9	F
ISG15	205483_s_at	30	H
ISG20[a,b]	33304_at	1	F
ISG20[a,c]	204698_at	0	
JAK1[a,b]	240613_at	67	G
JAK1[a]	201648_at	45	G
JAK1[a]	1552611_a_at	44	G
JAK1[a]	239695_at	13	E
JAK1[a]	1552610_a_at	3	G
JAK2[a,b]	205842_s_at	16	H
JAK2[a]	205841_at	11	H
JAK2[a]	1562031_at	5	H
MT1E	212859_x_at	10	F
MT1G	204745_x_at	7	F
MT2A	212185_x_at	66	F
MX1	202086_at	13	F
MX2	204994_at	2	H
OAS1[a,b]	205552_s_at	8	H
OAS1[a,c]	202869_at	0	
OAS2[a,b]	228607_at	12	F
OAS2[a]	204972_at	2	H
OAS2[a]	206553_at	1	H
OAS3	218400_at	3	H
OASL[a,b]	210797_s_at	12	H
OASL[a]	205660_at	2	H
PIAS1[a,b]	217864_s_at	24	G
PIAS1[a]	217862_at	6	E
PIAS1[a]	217863_at	1	E
PML	235508_at	8	F
PSMB8	209040_s_at	11	H
PTPN2[a,b]	241623_at	11	E
PTPN2[a]	241622_at	5	E
PTPN2[a]	241983_at	5	E
PTPN2[a,c]	213136_at	0	
PTPN2[a,c]	213137_s_at	0	
RELA[a,b]	201783_s_at	42	H
RELA[a]	230202_at	5	H

Gene	Probe set ID	Frequency of use in models	Cluster
RTP4	219684_at	15	H
SOCS1[a,b]	210001_s_at	34	H
SOCS1[a]	213337_s_at	8	H
STAT1[a,b]	200887_s_at	44	F
STAT1[a]	209969_s_at	15	F
TAP1	202307_s_at	79	F

[a] For genes with more than one probe set, the data from the [b] probe set that was most frequently represented in the models were considered as the representative data for that gene in the manuscript.

[c] Genes/probe sets that were not selected by the modeling process.

5 SUPPLEMENTARY FIGURE 1



Entry criteria:
- Gene has been annotated.
- Signal > background in > 20% arrays.
- PR & NP samples used for modelling only.

Model building and validation:
- N-PLS-DA, based on correlations between challenge outcome and data.
- For each double cross validation (DCV) round: data is normalized (SD 1, mean =0, for each probe set).
- Robustness assessed by ensembles of submodels based on different cross validation (CV) splits.

Data Driven:
- Include probe sets in model selection CV based on ranking probe sets on individual prediction performance.
- 10-fold CV for model selection & model evaluation.

IFN-driven:
- Automated forward selection for probe sets.
- Manual model selection for number of components
- More stringent 7-fold CV for model selection and 10-fold CV for model evaluation.

Double Cross Validation (DCV)
- Output:10 ensembles of 10 models.

Label perturbation
- 500x for each model = 5000 permutations.

Overall probeset selection:
- minimum single appearance in any of the ensembles of DCV models.

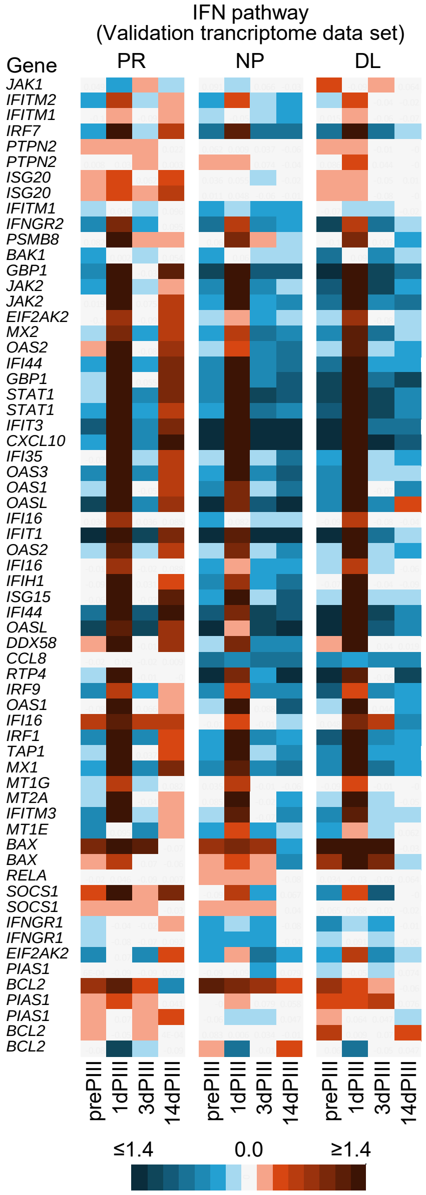
Summary model building:
- N-PLS-DA model with respect to all selected probe sets and challenge outcome, and using two components only.

Gene expression trends:
- Cluster analysis of component matrices in N-PLS-DA of summary model
- Cluster analysis of expression patterns relative to day 0.

Gene charcterization:
- Ingenuity analyses.
- PubMed.

Flow diagram describing how the N-PLS-DA was conducted.

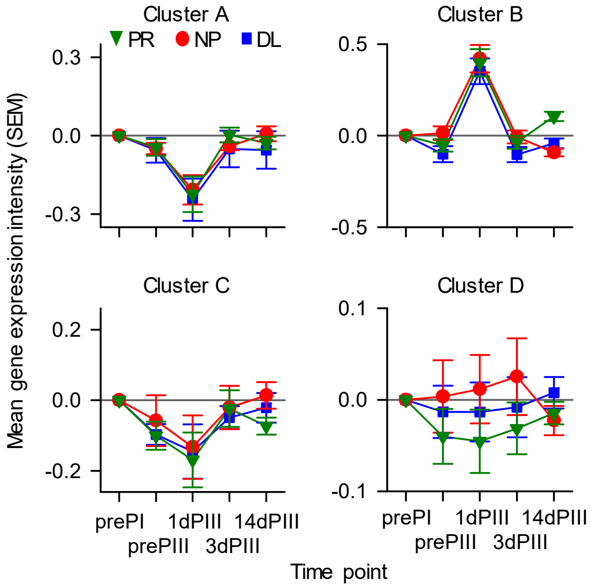
6 SUPPLEMENTARY FIGURE 2



The evaluation of IFN-pathway gene expression for a potential microarray-batch effect using the validation-transcriptome data set.

Unlike the principal transcriptome data set, the validation transcriptome data set was generated from a single kit of microarrays. The heatmap describes IFN pathway gene expression in protected (PR), non-protected (NP) and non-protected with delayed onset of parasitemia (DL) groups before (prePIII) and 1, 3 and 14 days after the third vaccine injection (1dPIII, 3dPIII and 14dPIII, respectively). Mean RNA expression relative to prePI is described in accordance with the colored scale. Certain genes are represented by more than one probe set.

7 SUPPLEMENTARY FIGURE 3



Evaluation of the expression of Clusters A to D probe sets for a potential microarray-batch effect using the validation transcriptome data set.

Unlike the principal transcriptome data set, the validation transcriptome data set was generated from a single kit of microarrays (Vahey et al., 2010). Mean RNA-expression levels relative to pre-dose 1 (prePI), at pre-dose 3 [prePIII] and 1, 3 and 14 days after dose 3 [1dPIII, 3dPIII, and 14d PIII, respectively]),, with respect to protection status of subjects (protected [PR], non-protected [NP] and non-protected with delayed parasitemia [DL]) for each of the four clusters (A–D) of probe sets among the 116 probe sets (110 genes) identified by the data-driven model. The error bars indicate the standard error of the mean (SEM). Also, simulated modeling suggested that such a batch effect (which may have confounded the effect of identifying protection status at 14dPIII) would have been mitigated by the N-PLS-DA because data from several time points were included (not shown).