SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Microarray data analysis.

We previously described how acute data analysis using ABI arrays is done (1). Raw data, after having removed the signals stemming from the different control spots as well as failed measurements, were once more median normalized in log2-space. For kinetic analyses, pairwise normalization and subtraction profile calculation in an everyone-against-everyone scheme was achieved using the NeONORM method with sensitivity parameter *k* set to 0.2 (2). These comparisons were either carried out on coefficient of variance-weighted means (3) calculated over the six animals at every time point, or animal by animal. Significance of logarithmic base two foldchanges ("$\log_2$Q", "L") were determined based on a mixture lognormal distribution hypothesis (3) of signal intensities using mixture ANOVA methodology. Multiple probes for a single gene, cross-reactivity of a single probe to several genes, as well as the resolution of probe-ID annotations was done according to the standards defined previously (4). For classification and visualization the time course data were cast gene by gene into up to fourteen distinct topographic groups. In a first step, the baseline and up to x-1 (with x being the number of time points recorded) levels of significantly differential expression are estimated using linear regression through all data-points considered for a given level. The criterion for association of any given data-point with a given expression level is based on the statistical significance of the relative foldchange to any other level ($p<0.05$). Second, a classifier, which was built using a Kohonen-Map approach on a large set of publicly available, diverse, time-course data, splits the genes into the fourteen topographic groups, including one for expressed but non-regulated genes and another for not expressed genes. Association with one of the main twelve topographic groups indicates that at least one time-point is considered statistically significantly ($p<0.05$) different from the estimated baseline. Note that due to the baseline estimation procedure the first time-point might show a foldchange different from zero, this difference, however, is never statistically significant. Heatmaps were created according to standard methods and visualized using the MultiExperiment Viewer software (TM4) (5). Gene Ontology (GO) annotations were analyzed using the Panther Protein Classification System (6) (http://www.pantherdb.org) to identify functional annotations that were significantly enriched in the different gene sets when compared to the whole set of genes present on the ABI microarray.

References

1. Jacquelin, B., Mayau, V., Brysbaert, G., Regnault, B., Diop, O.M., Arenzana-Seisdedos, F., Rogge, L., Coppee, J.Y., Barre-Sinoussi, F., Benecke, A., et al. 2007. Long oligonucleotide microarrays for African green monkey gene expression profile analysis. *FASEB J.* **21**:3262-3271.
2. Noth, S., Brysbaert, G., and Benecke, A. 2006. Normalization using weighted negative second order exponential error functions (NeONORM) provides robustness against asymmetries in comparative transcriptome profiles and avoids false calls. *Genomics Proteomics Bioinformatics* **4**:90-109.
3. Noth, S., Brysbaert, G., Pellay, F., and Benecke, A. 2006. High-sensitivity transcriptome profiles exhibit distinct signal distribution properties having significant implications for data analysis and biologic interpretation. *Genomics Proteomics Bioinformatics* **4**:212-229.
4. Noth, S., and Benecke, A. 2005. Avoiding inconsistencies over time and tracking difficulties in Applied Biosystems AB1700/Panther probe-to-gene annotations. *BMC Bioinformatics* **6**:307.
5. Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., et al. 2003. TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* **34**:374-378.
6. Thomas, P.D., Campbell, M.J., Kejariwal, A., Mi, H., Karlak, B., Daverman, R., Diemer, K., Muruganujan, A., and Narechania, A. 2003. PANTHER: A Library of Protein Families and Subfamilies Indexed by Function. *Genome Res.* **13**:2129-2141.

SUPPLEMENTAL FIGURES

Figure S1: **Timeline of Blood Drawing and LN Biopsies.** Kinetic of blood drawing (square) and lymph node biopsies (circle) in AGMs (blue) and RMs (red) throughout the course of the study. Six RMs and six AGMs were intravenously infected with, respectively, SIVmac251 and SIVagm.sab92018 on day 0.

Figure S2: **Peripheral $CD4^+$ T Cell Dynamics.** Blood $CD4^+$ T cell counts during SIV infection in the (**A**) 6 AGMs (blue) and (**B**) 6 RMs (red) measured by flow cytometry ($CD3^+$ $CD4^+$). In each graph, bold lines represent the mean from the 6 animals. The animals were infected with SIV on day 0 as indicated by the arrow. [d = days]

Figure S3: Percentage of Peripheral Proliferating T Cells during SIV Infection in AGM and RM. Percentage of proliferating Ki-67^+ cells within blood (**A** and **B**) $CD4^+$ and (**C** and **D**) $CD8^+$ T cells in the (**A** and **C**) 6 SIVagm-infected AGMs and the (**B** and **D**) 6 SIVmac-infected RMs. In each graph, bold lines represent the mean from 6 animals (blue for AGM and red for RM). All animals were SIV infected on day 0.

Figure S4: Percentage of LN Proliferating T Cells during SIV Infection in AGM and RM. Percentage of proliferating Ki-67^+ cells within LN (**A** and **B**) $CD4^+$ and (**C** and **D**) $CD8^+$ T cells in the (**A** and **C**) 6 SIVagm-infected AGMs and the (**B** and **D**) 6 SIVmac-infected RMs. In each graph, bold line represents the mean from 6 animals (blue for AGM and red for RM). All animals were SIV infected on day 0.

Figure S5: Ontology Profiling of Genes Modulated during Primary SIV Infection in AGM and RM. (**A**) The genes for which the expression was significantly modulated during the acute phase of SIV infection (between day 1 and 28) in AGM and RM blood and LN $CD4^+$ cells were grouped into biological process categories. The binomial statistics tool was used to compare classifications of multiple clusters of these genes with the list of probes present on the human ABI microarray to statistically determine over-representation of "parent" biological processes as defined in the PANTHER™ tool. The *P*-value as determined by the binomial statistic is indicated. A Bonferroni correction was applied. All parent biological processes that were significantly over-represented within at least one of the compartment are indicated. The fraction of modulated genes is represented on the Y axis (number of genes found divided by the number of genes expected in each particular biological process and present on the ABI human microarray). The immunity and defense process was significantly modulated in all the compartments and species. (**B**) The "child" categories of the immunity and defense process are represented and revealed a statistically over-representation of the Interferon-mediated immunity category.

Figure S6: Gene Expression Profiles and Systemic Levels of IL-10 and IFN-γ. Microarray gene expression profile of (**A**, **B**) *IL-10* and (**E**, **F**) *IFN-γ* in peripheral $CD4^+$ cells of (**A**, **E**) 6 AGMs and (**B**, **F**) 6 RMs are represented by box plots of the $\log_2$ of fold change (Q). BI indicates the baseline before infection (n = 24, i.e., 4 time points for each of the 6 animals) and the following boxes present the gene expression after infection (n = 6 per box). The top and the bottom of the boxes represent the 75^{th} and 25^{th} percentiles, respectively, whereas the horizontal lines between the box limits represent the median. Mean of the amounts of plasma (**C**, **D**) IL-10 and (**G**, **H**) IFN-γ levels in these (**C**, **G**) AGMs and (**D**, **H**) RMs are shown (±

standard error of the mean, SEM). Plain boxes indicate statistically significant increases or decreases relative to values found before infection (BI) ($p<0.05$).

Figure S7: Microarray Analysis of Pro- and Anti-apoptotic Genes. Heatmaps of expression profiles of pro- and anti-apoptotic genes are shown for AGM and RM peripheral $CD4^+$ cells (**A** and **B**, respectively) and LN $CD4^+$ cells (**C** and **D**, respectively). The colour scheme indicates the $\log_2Q$. Gene expressions shown in red were up-regulated and those shown in green were down-regulated. A red or green star indicates a probe found to be significantly increased or decreased, respectively when compared to before infection ($p<0.05$).

Figure S8: Microarray Validations. (**A**) Microarray data were validated by real-time RT-PCR for the *MX1* transcript. The ratios, expressed as $\log_2Q$ for both methods, were generated by comparing expression levels in $CD4^+$ cells at each time point of the kinetic for each animal to the respective mean basal levels before infection. Microarray ratios were plotted against those calculated with real-time RT-PCR, and Spearman correlations were determined. (**B**) Animal by animal correlation between the levels of plasma IP-10 and levels of *IP-10* gene transcripts in circulating $CD4^+$ cells is represented. The correlation coefficient was determined with the Spearman method. AGM and RM results are indicted, respectively, in blue and red. Best-fit lines are shown.

Figure S9: Probability of the IFN-I Pathway Involvement throughout the Kinetic. In each compartment, each species and for each time point, the set of statistically significantly regulated genes was analyzed for over-representation of the IFN-I pathway. Black boxes indicate the statistically significant enrichment ($p<0.001$) of this set of genes when compared

to random expectation using a binominal distribution hypothesis, sliding averages over directly neighbouring time-points, and a Bonferroni correction for multiple testing. [d = day]

Figure S10: Microarray Analysis of Genes Involved in the PRR and IFN-I Signaling Pathways. Heatmaps of expression profiles of the genes involved in IFN-I signaling, PRR signaling and in positive or negative feedback of those pathways are shown for AGM and RM peripheral $CD4^+$ cells (**A** and **B**, respectively) and LN $CD4^+$ cells (**C** and **D**, respectively). In each compartment, the probes found to be significantly differentially expressed ($p<0.05$) in at least one of the two species when comparing infected cells at a given time point to the baseline before infection were selected. (**E**) The colour scheme indicates the $\log_2 Q$. Gene expressions shown in red were up-regulated and those shown in green were down-regulated with respect to the baseline.

Figure S11: *TRAIL* gene expression in AGM PBMCs after IFN-α or SIVagm stimulation. Peripheral $CD4^+$ cells of healthy AGMs were cultured in presence of the $CD4^-$ cell fraction in a two chambers system with SIVagm.sab92018 or human recombinant-IFN-α for 18h. The mean of $\log_2 Q$ (±SEM) is shown. The means were calculated on 3 independent experiments.

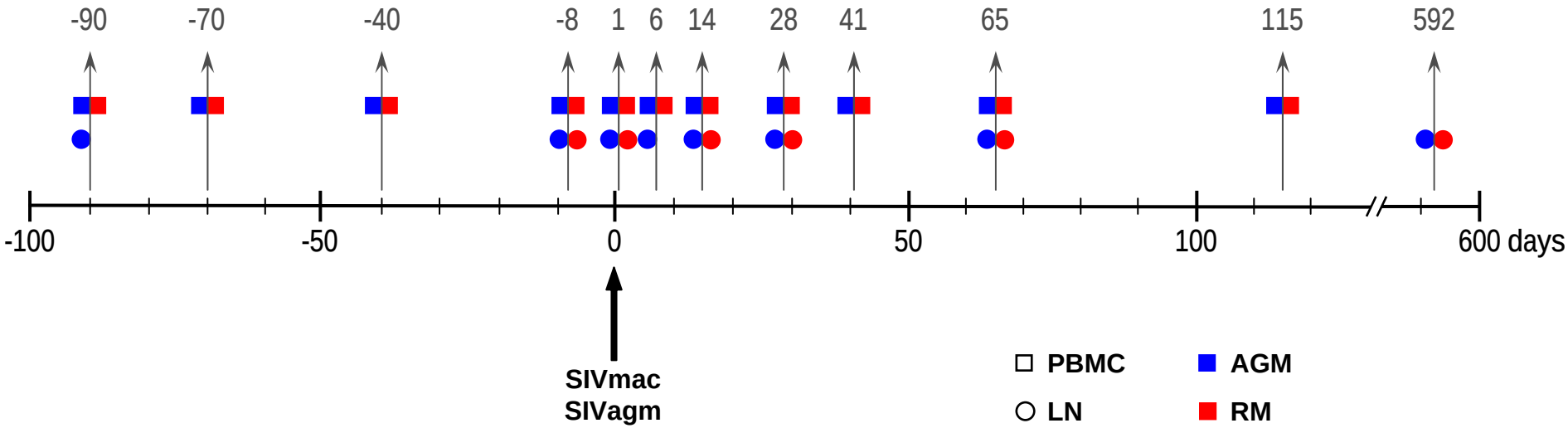
-90
-70
-40
-8
1
6
14
28
41
65
115
592
-100
-50
0
50
100
600 days
SIVmac
SIVagm
PBMC
LN
AGM
RM

Figure S1

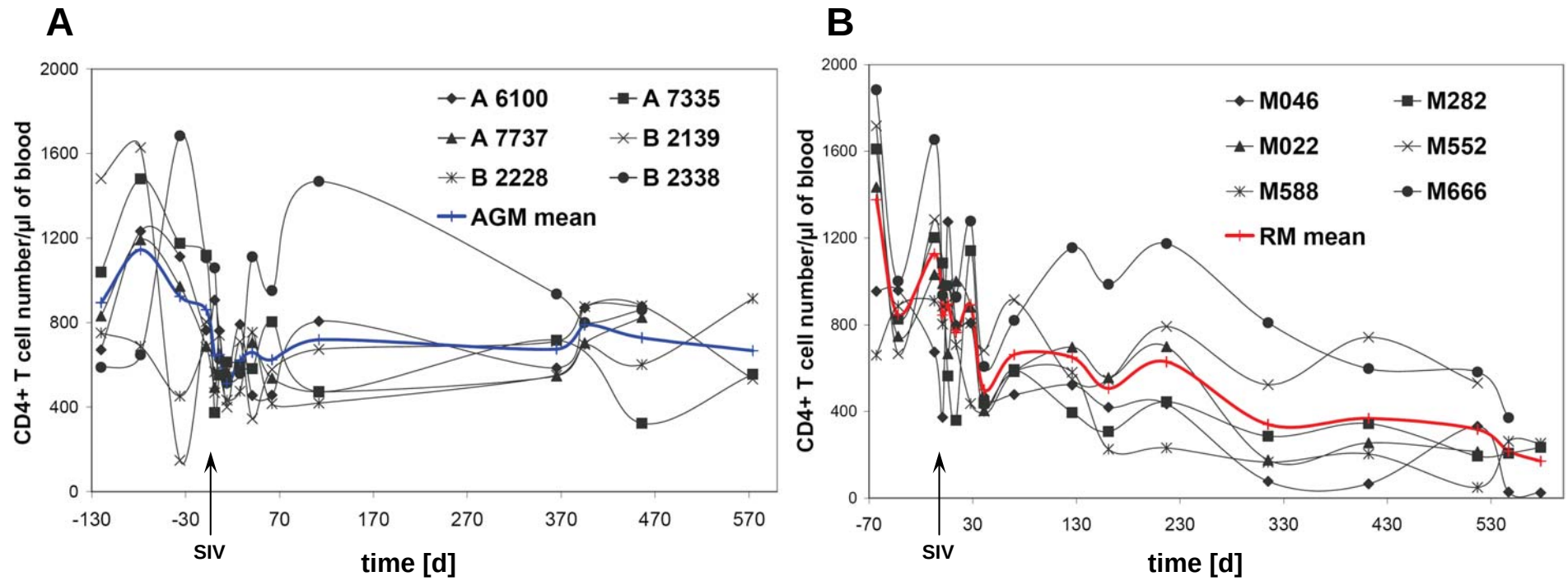
A
B
CD4+ T cell number/µl of blood
2000
1600
1200
800
400
0
A 6100
A 7335
A 7737
B 2139
B 2228
B 2338
AGM mean
-130
-30
70
170
270
370
470
570
SIV
time [d]
M046
M282
M022
M552
M588
M666
RM mean
-70
30
130
230
330
430
530

Figure S2

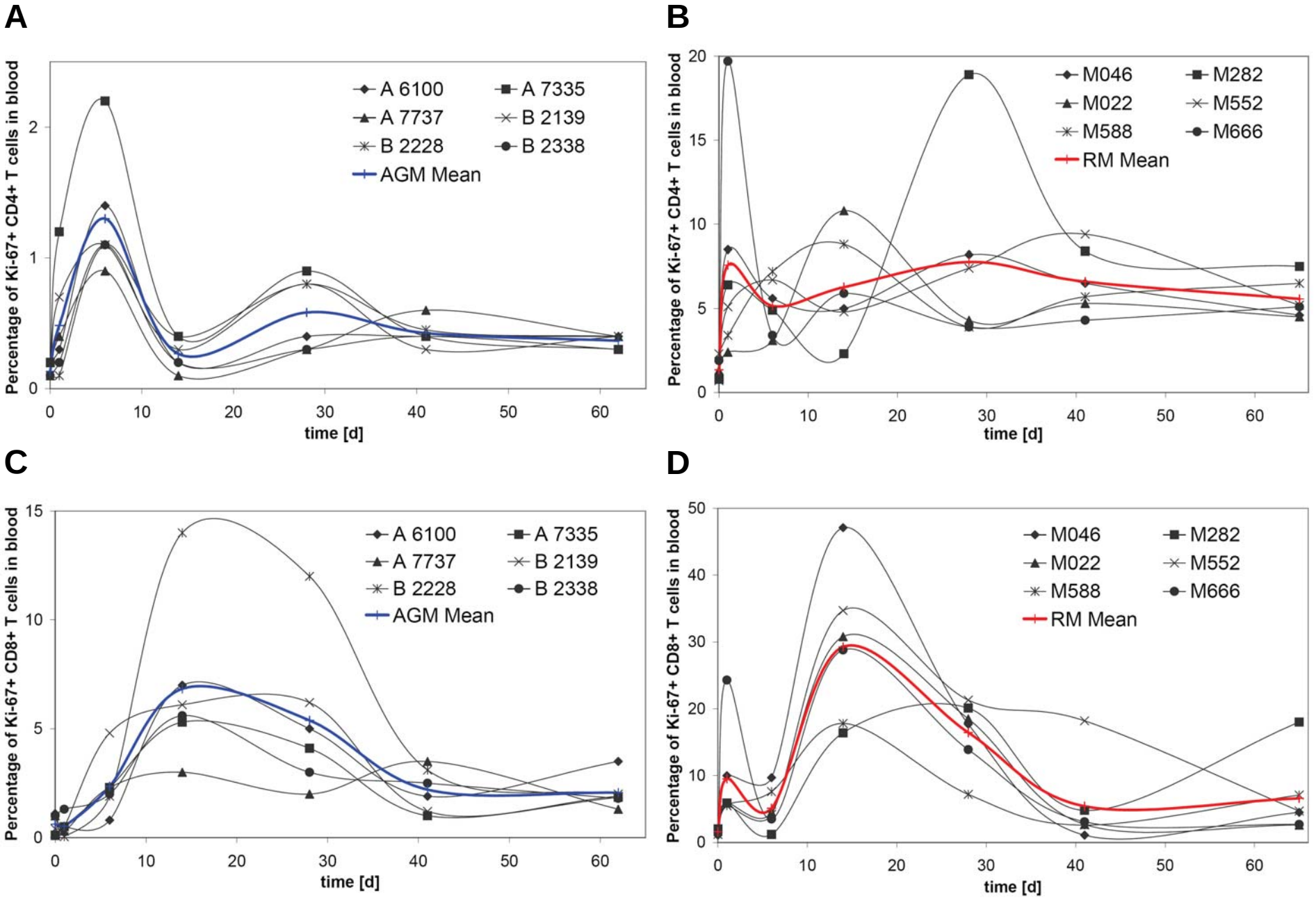
A
Percentage of Ki-67+ CD4+ T cells in blood
time [d]
A 6100
A 7335
A 7737
B 2139
B 2228
B 2338
AGM Mean
B
Percentage of Ki-67+ CD4+ T cells in blood
time [d]
M046
M282
M022
M552
M588
M666
RM Mean
C
Percentage of Ki-67+ CD8+ T cells in blood
time [d]
A 6100
A 7335
A 7737
B 2139
B 2228
B 2338
AGM Mean
D
Percentage of Ki-67+ CD8+ T cells in blood
time [d]
M046
M282
M022
M552
M588
M666
RM Mean

Figure S3

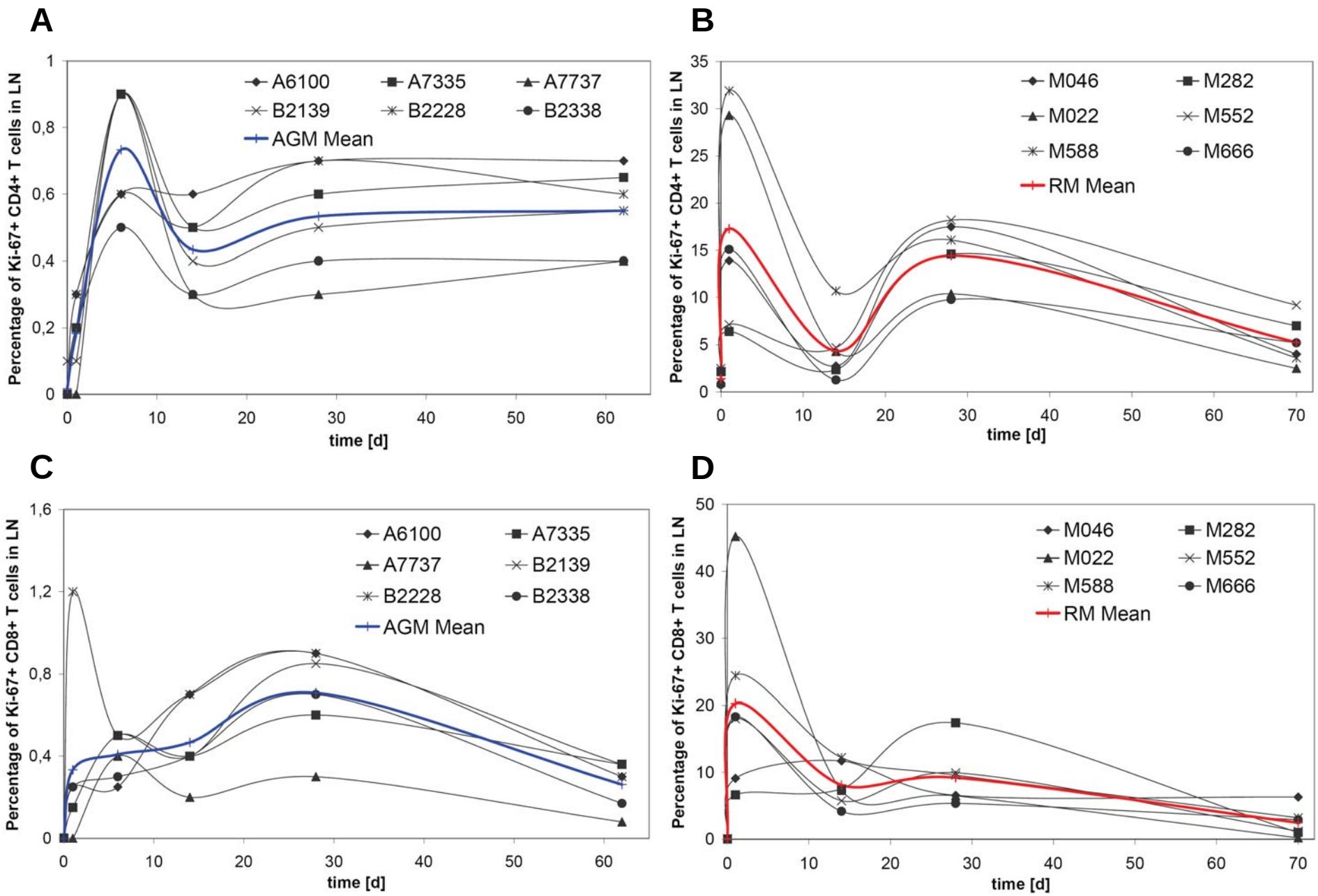
A
Percentage of Ki-67+ CD4+ T cells in LN
time [d]
A6100
A7335
A7737
B2139
B2228
B2338
AGM Mean
B
Percentage of Ki-67+ CD4+ T cells in LN
time [d]
M046
M282
M022
M552
M588
M666
RM Mean
C
Percentage of Ki-67+ CD8+ T cells in LN
time [d]
A6100
A7335
A7737
B2139
B2228
B2338
AGM Mean
D
Percentage of Ki-67+ CD8+ T cells in LN
time [d]
M046
M282
M022
M552
M588
M666
RM Mean

Figure S4

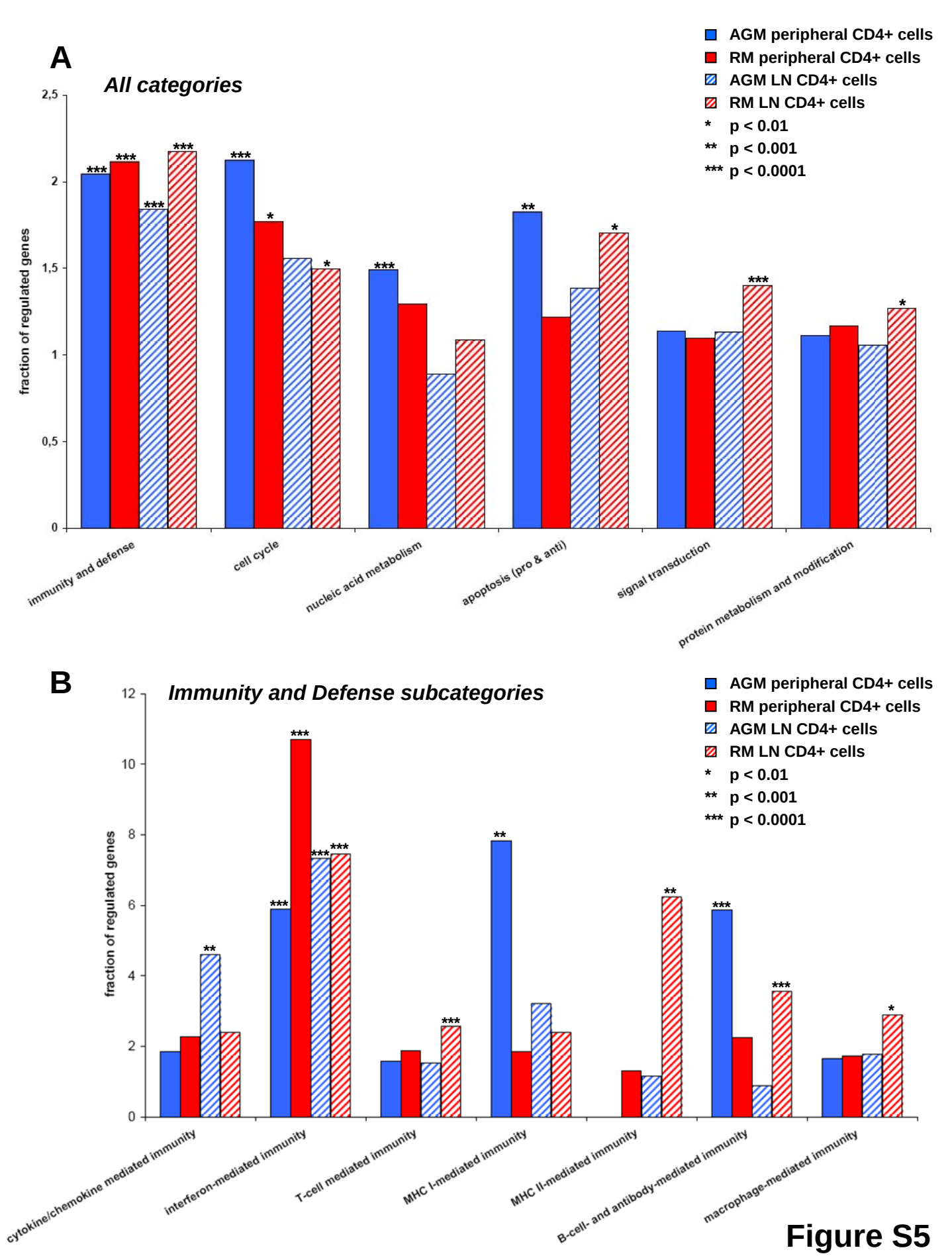
A
All categories
AGM peripheral CD4+ cells
RM peripheral CD4+ cells
AGM LN CD4+ cells
RM LN CD4+ cells
* p < 0.01
** p < 0.001
*** p < 0.0001
fraction of regulated genes
2,5
2
1,5
1
0,5
0
immunity and defense
cell cycle
nucleic acid metabolism
apoptosis (pro & anti)
signal transduction
protein metabolism and modification
B
Immunity and Defense subcategories
AGM peripheral CD4+ cells
RM peripheral CD4+ cells
AGM LN CD4+ cells
RM LN CD4+ cells
* p < 0.01
** p < 0.001
*** p < 0.0001
fraction of regulated genes
12
10
8
6
4
2
0
cytokine/chemokine mediated immunity
interferon-mediated immunity
T-cell mediated immunity
MHC I-mediated immunity
MHC II-mediated immunity
B-cell- and antibody-mediated immunity
macrophage-mediated immunity

Figure S5

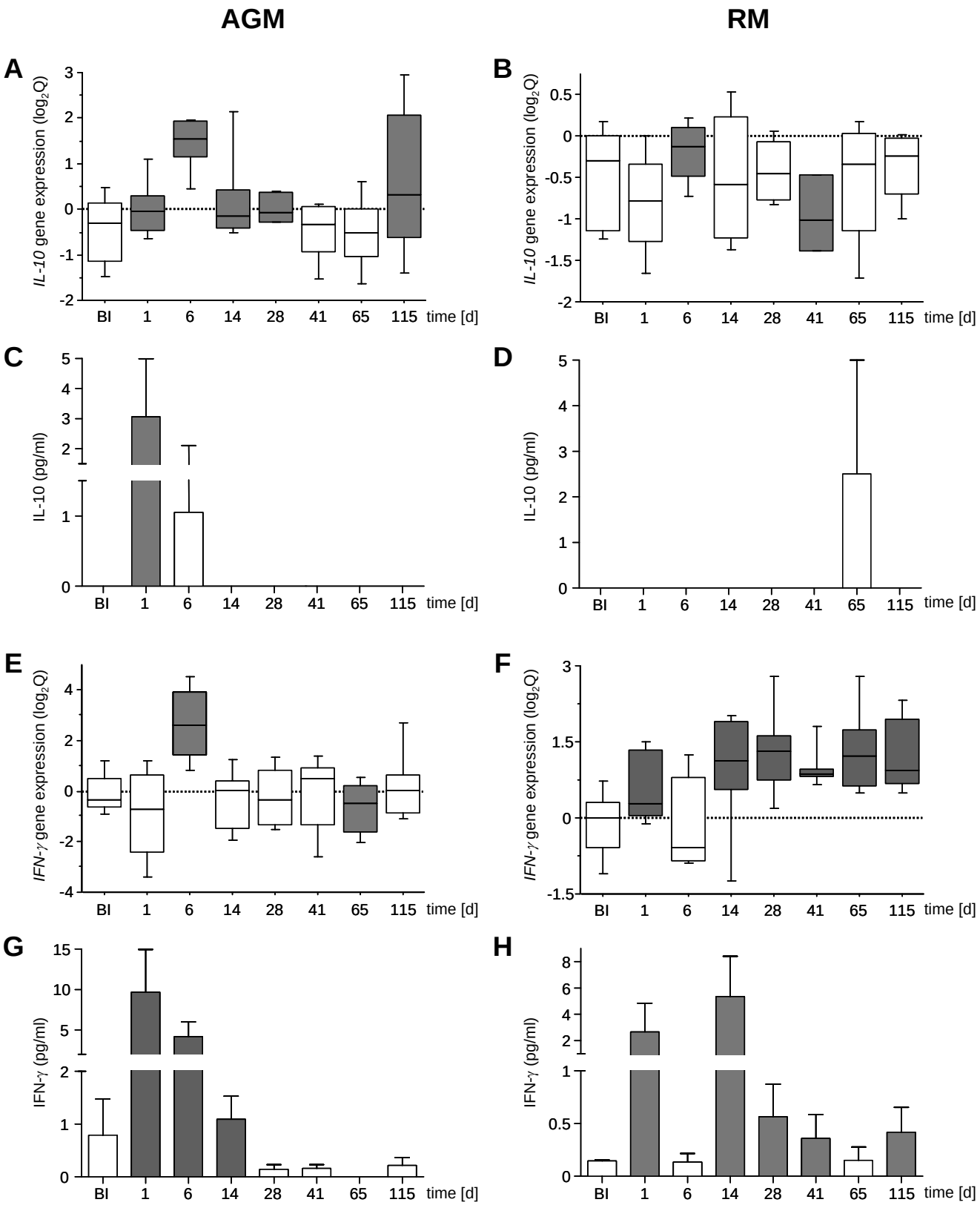
AGM
RM
A
B
C
D
E
F
G
H
IL-10 gene expression (log2Q)
IL-10 (pg/ml)
IFN-γ gene expression (log2Q)
IFN-γ (pg/ml)
BI
1
6
14
28
41
65
115
time [d]

Figure S6

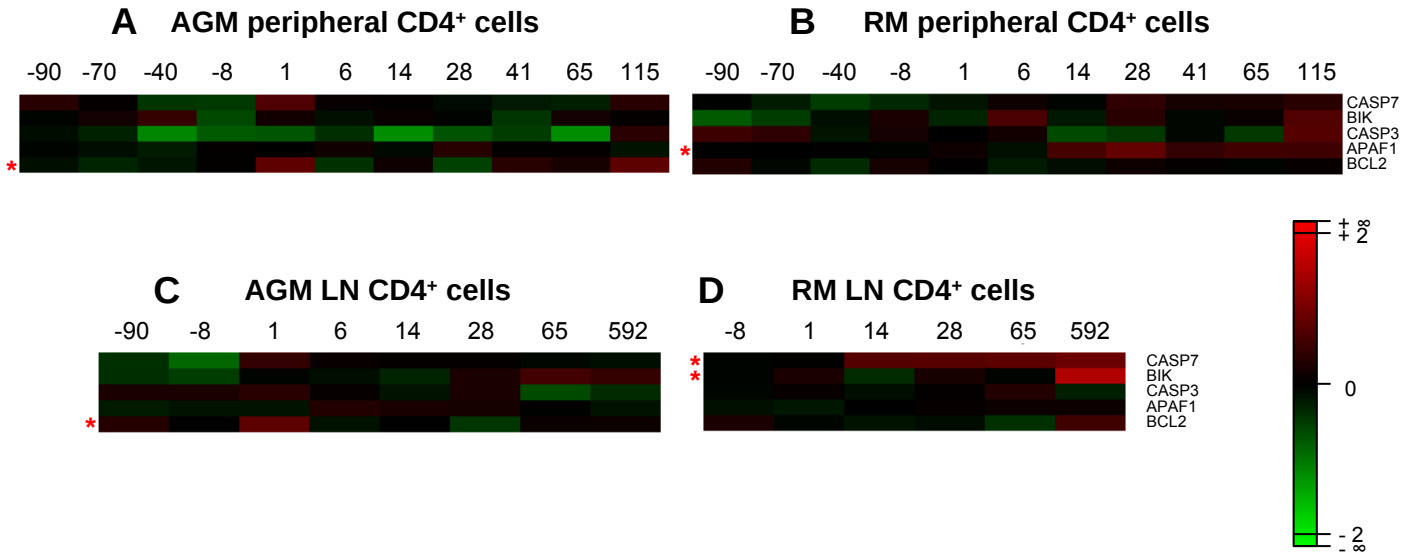
A AGM peripheral CD4+ cells
-90 -70 -40 -8 1 6 14 28 41 65 115
B RM peripheral CD4+ cells
-90 -70 -40 -8 1 6 14 28 41 65 115
CASP7
BIK
CASP3
APAF1
BCL2
C AGM LN CD4+ cells
-90 -8 1 6 14 28 65 592
D RM LN CD4+ cells
-8 1 14 28 65 592
CASP7
BIK
CASP3
APAF1
BCL2
+ ∞
+ 2
0
- 2
- ∞

Figure S7

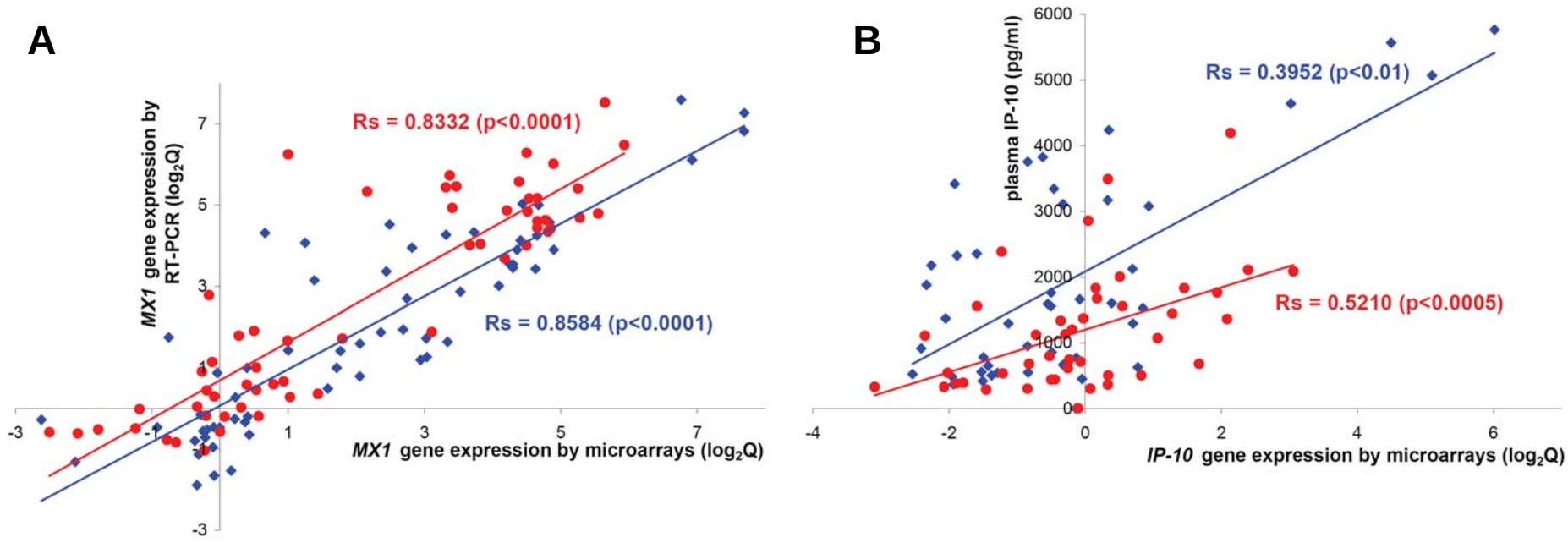
A
MX1 gene expression by RT-PCR (log2Q)
Rs = 0.8332 (p<0.0001)
Rs = 0.8584 (p<0.0001)
MX1 gene expression by microarrays (log2Q)
B
plasma IP-10 (pg/ml)
Rs = 0.3952 (p<0.01)
Rs = 0.5210 (p<0.0005)
IP-10 gene expression by microarrays (log2Q)

Figure S8

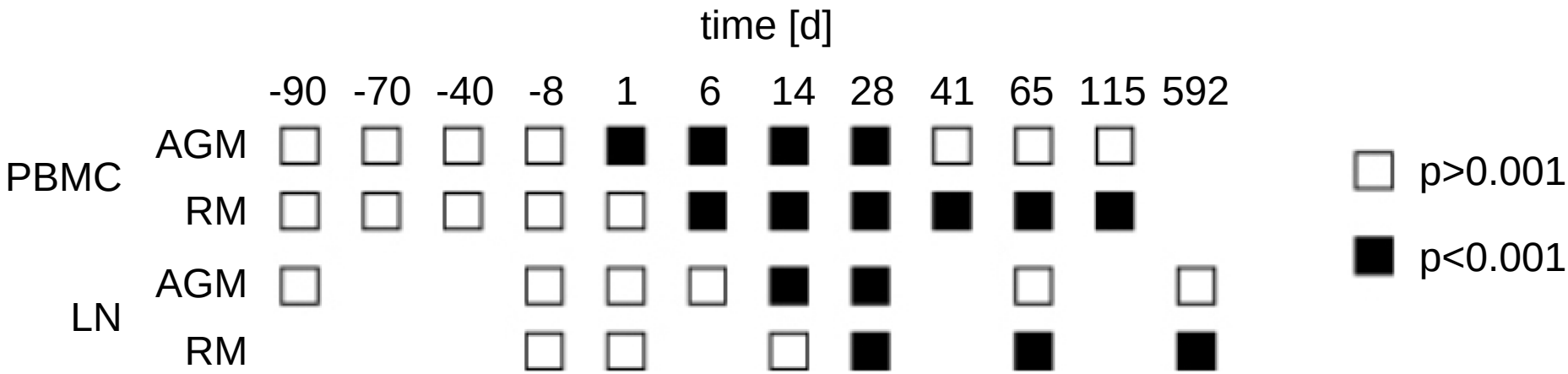
time [d]
-90
-70
-40
-8
1
6
14
28
41
65
115
592
PBMC
AGM
RM
LN
AGM
RM
p>0.001
p<0.001

Figure S9

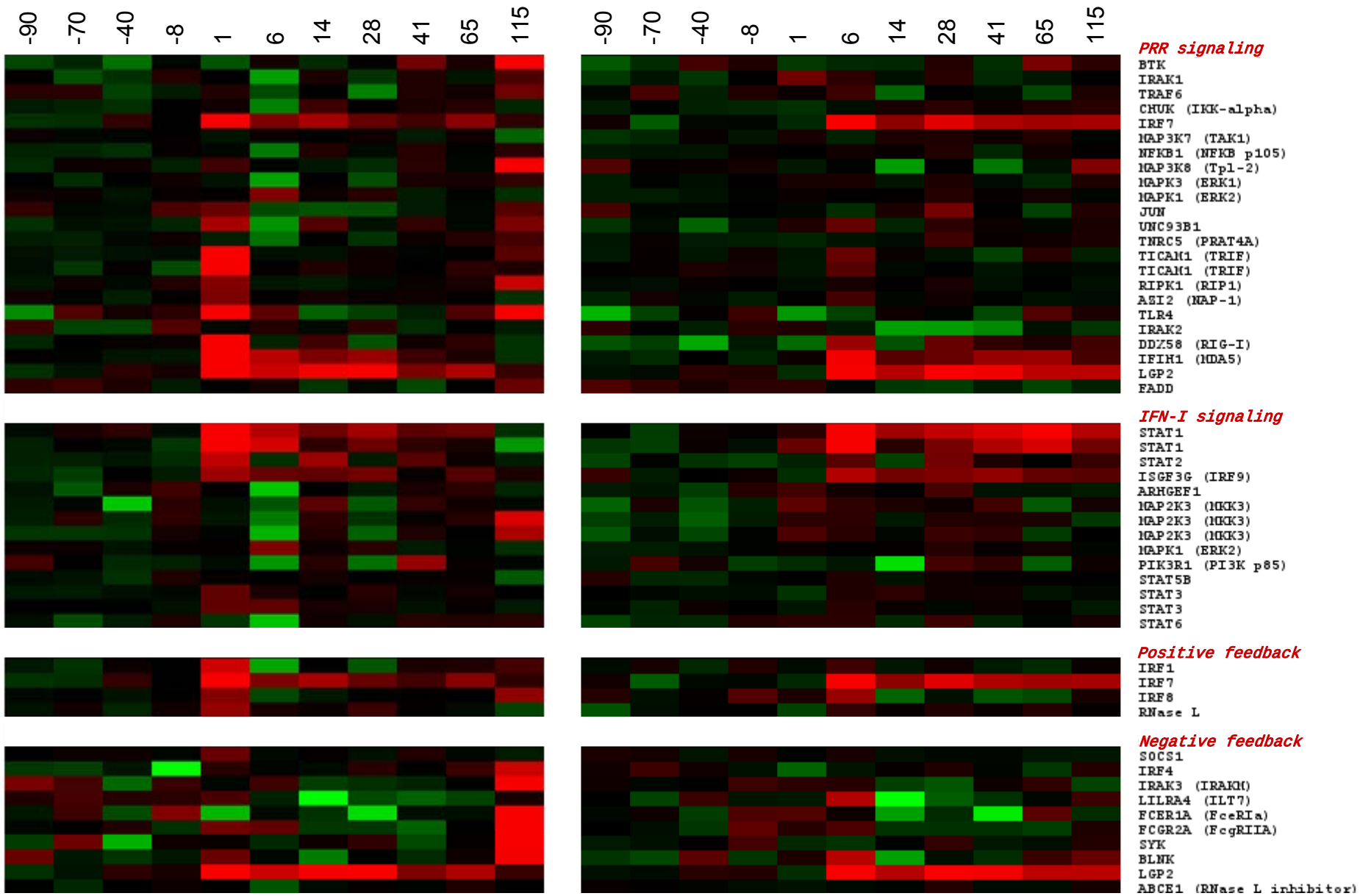
A
AGM peripheral CD4+ cells
B
RM peripheral CD4+ cells
-90
-70
-40
-8
1
6
14
28
41
65
115
PRR signaling
BTK
IRAK1
TRAF6
CHUK (IKK-alpha)
IRF7
MAP3K7 (TAK1)
NFKB1 (NFKB p105)
MAP3K8 (Tpl-2)
MAPK3 (ERK1)
MAPK1 (ERK2)
JUN
UNC93B1
TNRC5 (PRAT4A)
TICAM1 (TRIF)
TICAM1 (TRIF)
RIPK1 (RIP1)
ASI2 (MAP-1)
TLR4
IRAK2
DDX58 (RIG-I)
IFIH1 (MDA5)
LGP2
FADD
IFN-I signaling
STAT1
STAT1
STAT2
ISGF3G (IRF9)
ARHGEF1
MAP2K3 (MKK3)
MAP2K3 (MKK3)
MAP2K3 (MKK3)
MAPK1 (ERK2)
PIK3R1 (PI3K p85)
STAT5B
STAT3
STAT3
STAT6
Positive feedback
IRF1
IRF7
IRF8
RNase L
Negative feedback
SOCS1
IRF4
IRAK3 (IRAKM)
LILRA4 (ILT7)
FCER1A (FceRIa)
FCGR2A (FcgRIIA)
SYK
BLNK
LGP2
ABCE1 (RNase L inhibitor)

Figure S10

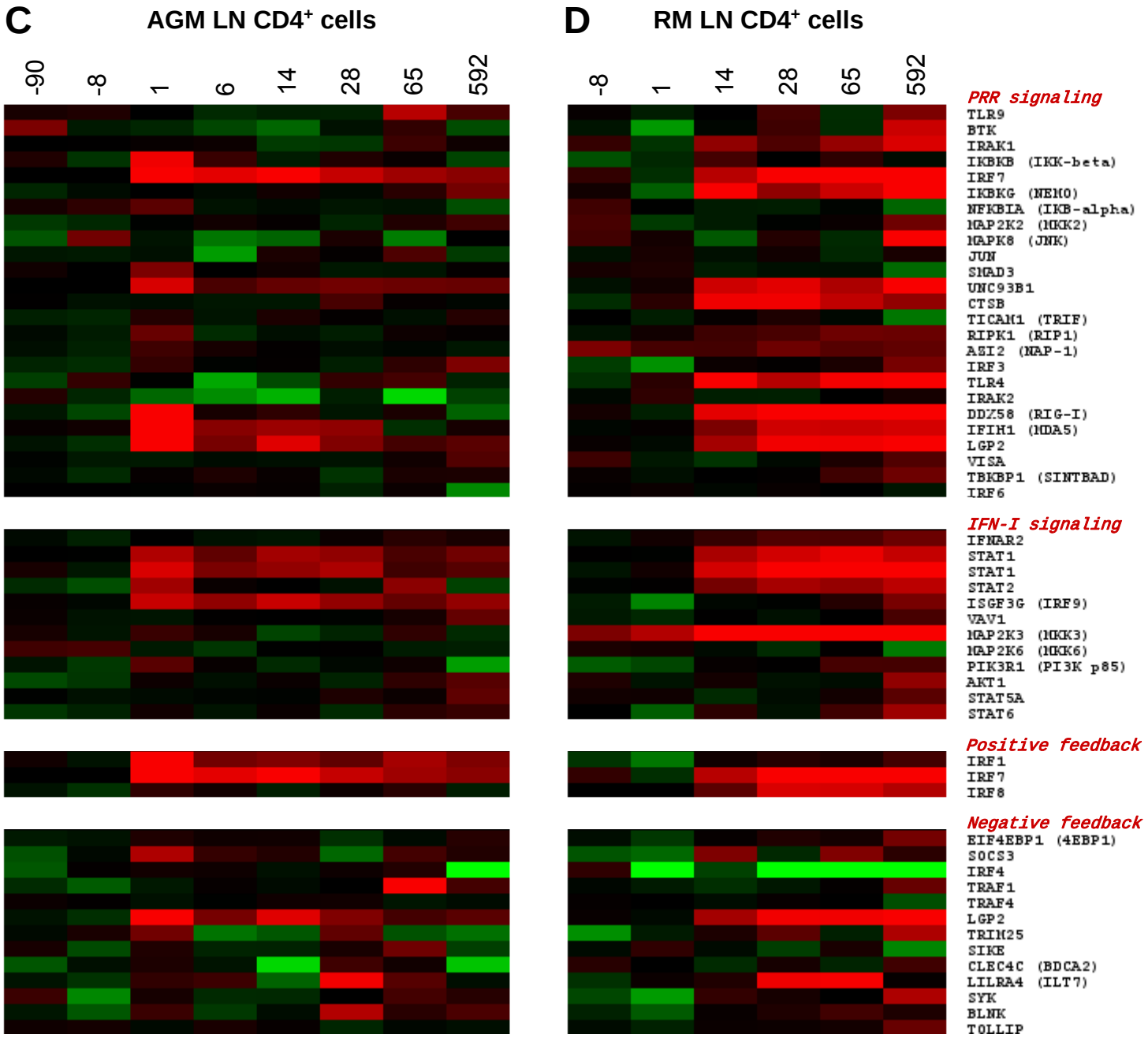

Figure S10 (cont'd)

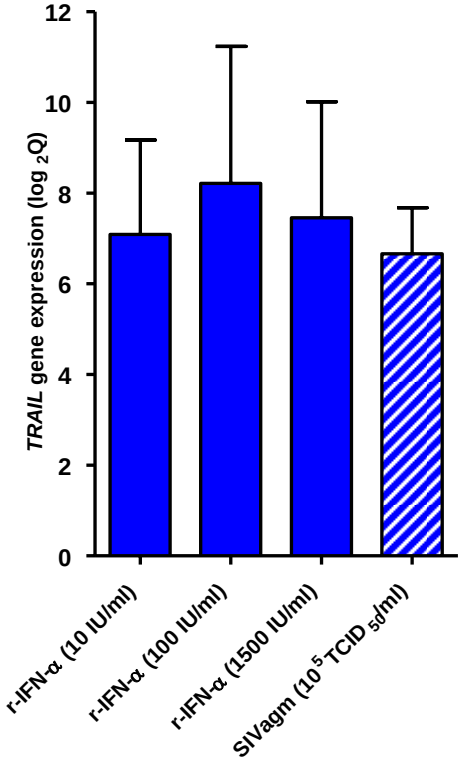
12
10
8
6
4
2
0
TRAIL gene expression (log 2Q)
r-IFN-α (10 IU/ml)
r-IFN-α (100 IU/ml)
r-IFN-α (1500 IU/ml)
SIVagm (10 5 TCID 50/ml)

Figure S11

Table S1: P-values associated with expression profiles of 11 representative type I ISG (Figure 3) calculated with the ANOVA test of statistical significance.

		Peripheral CD4+ cells											LN CD4+ cells							
	time / gene	-90	-70	-40	-8	1	6	14	28	41	65	115	-90	-8	1	6	14	28	65	592
AGM	G1P2	0.4127	0.3509	0.3989	0.382	0.039	0.0164	0.0062	0.0318	0.0814	0.0352	0.0836	0.4312	0.3493	0.0439	0.0366	0.001	0.0036	0.039	0.4522
	STAT1	0.4113	0.3687	0.3888	0.3741	0.0458	0.02	0.1249	0.0902	0.1405	0.3088	0.1097	0.376	0.3819	0.0435	0.1082	0.0262	0.0369	0.1391	0.102
	IRF7	0.3421	0.3635	0.1436	0.416	0.0136	0.015	0.0073	0.0957	0.0848	0.0328	0.1202	0.5127	0.591	0.0329	0.0385	0.0072	0.0119	0.0156	0.0094
	OAS2	0.4007	0.3298	0.1418	0.1293	0.0152	0.0003	0.0158	0.0296	0.0361	0.0098	0.4571	0.4657	0.4319	0.0451	0.0094	0.0113	0.024	0.0359	0.029
	OAS1	0.3814	0.4139	0.4145	0.4049	0.0374	0.1154	0.0499	0.0931	0.3099	0.1188	0.394	0.4221	0.4183	0.0923	0.1009	0.025	0.1122	0.1156	0.3185
	LGP2	0.3201	0.4421	0.1454	0.347	0.0111	0.0034	0.0097	0.0332	0.0298	0.031	0.0875	0.4291	0.4412	0.0307	0.0172	0.0264	0.0346	0.0767	0.0096
	STAT1	0.4367	0.3707	0.3092	0.4283	0.0228	0.014	0.0186	0.0084	0.0499	0.0242	0.3237	0.4834	0.4515	0.0484	0.0963	0.0008	0.0258	0.1086	0.0411
	STAT2	0.4604	0.4476	0.416	0.4641	0.0144	0.4393	0.0477	0.4409	0.0479	0.1392	0.4538	0.4141	0.4004	0.0455	0.0996	0.1202	0.3242	0.1301	0.443
	MX2	0.3062	0.4223	0.3302	0.1398	0.0231	0.008	0.0093	0.0336	0.0371	0.0196	0.0864	0.4711	0.4947	0.0356	0.0121	0.0006	0.0042	0.0158	0.0013
	MX1	0.4152	0.3659	0.4685	0.4721	0.0127	0.0031	0.005	0.026	0.0087	0.0009	0.0021	0.4314	0.43	0.0301	0.0185	0.0016	0.0074	0.0095	0.022
	CXCL10	0.3624	0.3653	0.3452	0.386	0.0132	0.0799	0.107	0.1175	0.3754	0.3353	0.1247	0.3666	0.3667	0.0319	0.0488	0.124	0.1263	0.3727	0.1447
RM	G1P2	0.421	0.4165	0.3496	0.413	0.4358	0.015	0.0283	0.0001	0.0038	0.0017	0.0142		0.4302	0.0991		0.0323	0.0051	0.017	0.0198
	STAT1	0.3067	0.1422	0.4085	0.4109	0.1347	0.0267	0.3162	0.0894	0.028	0.0247	0.0415		0.3941	0.3983		0.017	0.0259	0.0189	0.0176
	IRF7	0.448	0.0915	0.3711	0.3752	0.1461	0.0369	0.1066	0.0273	0.0285	0.0411	0.0285		0.3107	0.3443		0.0365	0.0001	0.0007	0.0213
	OAS2	0.361	0.4413	0.0882	0.349	0.1339	0.0023	0.0194	0.0011	0.0053	0.0006	0.0023		0.4769	0.4878		0.0213	0.0036	0.0052	0.0129
	OAS1	0.4589	0.4253	0.4238	0.3147	0.4169	0.002	0.0382	0.0121	0.0074	0.0014	0.0065		0.4495	0.4211		0.0338	0.0195	0.0044	0.0248
	LGP2	0.4675	0.4538	0.1393	0.3117	0.3548	0.0002	0.048	0.0096	0.0167	0.0036	0.0071		0.4118	0.4226		0.0457	0.0167	0.0096	0.0167
	STAT1	0.5063	0.1124	0.4551	0.4325	0.3359	0.0156	0.0393	0.0171	0.0155	0.007	0.0251		0.4527	0.4482		0.0274	0.0196	0.0187	0.0188
	STAT2	0.4009	0.3233	0.4388	0.403	0.4051	0.0276	0.4088	0.0471	0.1061	0.1477	0.0849		0.4761	0.5037		0.0401	0.0193	0.0278	0.0096
	MX2	0.1418	0.3128	0.1329	0.369	0.4162	0.0102	0.0281	0.0018	0.0068	0.0024	0.0025		0.3848	0.3663		0.036	0.0002	0.0009	0.0188
	MX1	0.1489	0.3634	0.4079	0.3674	0.3582	0.0006	0.0345	0.0046	0.0026	0.0123	0.0097		0.0973	0.312		0.0302	0.0036	0.001	0.0219
	CXCL10	0.3993	0.112	0.1317	0.3103	0.1382	0.0769	0.3637	0.1357	0.4042	0.3156	0.3704		0.3439	0.3671		0.0544	0.0077	0.0036	0.0006

Table S2: Correlation of type I ISG expression with plasma IFN-α levels in AGM during primary infection (day 1 to 28)

	AGM peripheral $CD4^+$ cells		AGM LN $CD4^+$ cells	
	Rs	p-value	Rs	p-value
IRF7	0.541	0.002	0.763	<0.0001
MDA5	0.778	<0.0001	0.749	<0.0001
LGP2	0.684	<0.0001	0.790	<0.0001
MX2	0.693	<0.0001	0.849	<0.0001
MX1	0.835	<0.0001	0.776	<0.0001
IRF9	0.529	0.003	0.698	<0.0001

Correlations were estimated with a Spearman test.