Early transcriptional responses after dengue vaccination mirror the response to natural infection and predict neutralizing antibody titers

Running title: Early correlates of immunity to dengue

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Abstract: 199 words

Text: 3501 words (not including Acknowledgements)

Summary: Interferon- and cell cycle-associated gene transcript abundance levels in the peripheral blood of dengue vaccine recipients on days 8 and 9 post-vaccination were associated with dengue neutralizing antibody titers on day 42, and mirrored responses in primary dengue infection, suggesting the possibility of predicting protective immunity.

ABSTRACT

Background: Several promising live attenuated virus (LAV) dengue vaccines are in development, but information about innate immune responses and early correlates of protection are lacking.

Methods: We characterized human genome-wide transcripts in whole blood from 10 volunteers at 11 time-points after immunization with the dengue virus type 3 (DENV-3) component of the NIH dengue vaccine candidate TV003 and from 30 hospitalized children with acute primary DENV-3 infection. We compared day-specific gene expression patterns with subsequent neutralizing antibody (NAb) titers.

Results: The transcriptional response to vaccination was largely confined to days 5-20 and was dominated by an interferon-associated signature and a cell cycle signature that peaked on days 8 and 14, respectively. Changes in transcript abundance were much greater in magnitude and scope in symptomatic natural infection than following vaccination (maximum fold-change >200 versus 21 post-vaccination; 3,210 versus 286 transcripts with significant fold-change), but shared gene modules were induced in the same sequence. The abundance of 131 transcripts on days 8 and 9 post-vaccination was strongly correlated with NAb titers measured 6 weeks post-vaccination.

Conclusions: LAV dengue vaccination elicits early transcriptional responses that mirror those found in symptomatic natural infection and provide candidate early markers of protection against DENV infection.

Clinical Trial Registration Number: NCT00831012 (available at clinicaltrials.gov)

Keywords: dengue; vaccine; innate immune response; gene expression; microarray; correlates of protection; interferon; neutralizing antibody

63 **BACKGROUND**

64 Each year, the four dengue virus serotypes (DENV-1-4) infect an estimated 390 million
65 individuals globally [1]. While most of these infections are asymptomatic, approximately 100
66 million individuals develop clinically apparent disease, from uncomplicated fever to life-
67 threatening illness. Despite the high disease burden, there are no licensed therapeutics for
68 DENV infection. Several promising candidate dengue vaccines are in Phase III clinical trials,
69 and the live attenuated chimeric dengue vaccine Dengvaxia™ was recently licensed for use in
70 children 9 years of age and older in DENV endemic areas. However, the efficacy and duration
71 of protection were limited or uncertain, and DENV-naïve vaccine recipients were hospitalized for
72 dengue and severe dengue at a higher rate than placebo recipients, possibly due to antibody-
73 dependent enhancement (ADE) [2].

74 Studies of natural DENV infection and flavivirus LAVs have identified immune responses
75 needed for protection against dengue disease. Pre-existing neutralizing antibody (NAb) titers
76 correlate with a lack of symptomatic disease in subsequent infections [3–6] and are used as the
77 primary measure of candidate vaccine immunogenicity. However, the risk of severe disease is
78 elevated after a second infection with a heterotypic dengue virus [7]. The recognition of effective
79 homotypic immunity after natural infection has led to a common vaccine development strategy
80 of inducing homotypic NAbs to all four serotypes simultaneously.

81 Little is known about the role of early innate immune responses in enhancing NAb
82 production and promoting protective immune memory against dengue. Studies of innate
83 immunity have been hampered by the difficulty in identifying individuals with early infection,
84 when innate immune responses are most active, particularly those with mild or subclinical
85 infections. Trials of LAVs provide a unique opportunity to examine early immune responses in a
86 setting where the time, dose, and viral serotype are known. Genome-wide transcript responses
87 to vaccines have provided important clues about early steps in the generation of humoral and
88 cellular immunity [8–13]. Transcript profiling of peripheral blood also incorporates information

from cell populations that are difficult to examine in clinical settings, and has led to signatures associated with dengue disease severity, identified links between innate responses and humoral immunity in secondary DENV infection, and illustrated the dynamic nature of these responses [14–20].

In this study, we characterized the transcript response to rDEN3Δ30/31, the DENV-3 component of TV003, a tetravalent live attenuated vaccine candidate developed by NIH. TV003 is a single-dose vaccine that has proven to be both safe and immunogenic and is being evaluated in a Phase III efficacy trial [21,22]. We examined temporal changes in transcript abundance and identified early signatures correlated with NAb titers measured six weeks post-vaccination. We also compared these results with transcript patterns we observed in patients with symptomatic wild-type primary DENV-3 infection. Despite the anticipated differences in the magnitude of expression, we observed the induction of common gene expression programs in the same temporal sequence, with a similar relationship to the induction of NAb. These results reveal candidate biomarkers of early protective DENV immune responses against dengue and suggest a path towards validation and deployment.

METHODS

Vaccine study population. Samples for this study were collected from a Phase I clinical trial of the live attenuated dengue vaccine rDEN3Δ30/31-7164 (DENV-3), described previously [23]. Briefly, healthy, flavivirus-naïve adult volunteers were randomized to receive a single 0.5 ml subcutaneous dose of 1,000 PFU of DENV-3 vaccine or a placebo (0.5 ml of vaccine diluent). Blood samples including whole blood for RNA profiling were collected immediately prior to vaccination and on days 2, 5, 6, 8, 9, 12,14, 20, 29, 42 and 180. Samples from each of these time-points were available from nine of ten vaccinees and from all placebo recipients. Subject 9 had samples available for all days except days 8 and 12; 166 samples in total were used for analysis. Serum virus titers (viremia) were measured using a standard plaque assay as

115 described previously [24]. Serum NAb titer was determined by 60% plaque reduction ($PRNT_{60}$)
116 [25]. Seroconversion was defined by a $\geq$4-fold increase in $PRNT_{60}$ on study day 28 or 42
117 relative to day 0 and corresponds to a post-vaccination titer >10 [23].
118
119 **Dengue patient population.** Patients (6 months to 14 years old) presenting with fever and
120 suspected dengue during the 2010 dengue season were enrolled at the Hospital Infantil Manuel
121 de Jesús Rivera in Managua, Nicaragua. Inclusion criteria, recruitment, and laboratory testing
122 have been described previously [26] (see Supplementary Information). Blood samples from
123 healthy subjects were collected as part of a separate prospective cohort study in which healthy
124 children in the same general population were enrolled without regard to dengue status [27].
125
126 **Ethics statement.** The trial of rDEN3Δ30/31 was approved by the Committee for Human
127 Research at the University of Vermont, and written informed consent was obtained from all
128 subjects following a review of risks and benefits and a comprehension assessment. The study in
129 Nicaragua was approved by the Institutional Review Boards of the University of California,
130 Berkeley, and the Nicaraguan Ministry of Health, and by the Stanford University Administrative
131 Panel on Human Subjects in Medical Research. For further details, see Supplementary
132 Information.
133
134 **RNA sample processing and transcriptome analysis.** PAXgene RNA was amplified and
135 hybridized to Human Exonic Evidence Based Oligonucleotide microarrays [14]. Microarray data
136 were submitted to the Princeton University MicroArray database for normalization and gene
137 filtering and are deposited at Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/;
138 accession numbers GSE96656 and GSE98053). A full description of both sample processing
139 and analysis steps is available in the Supplementary Information.
140

141 **RESULTS**

142

143 **Temporal patterns of the transcriptional responses to live dengue vaccination**

144 To identify the temporal pattern of the early human transcriptional response to dengue
145 vaccination, we examined changes in genome-wide transcript abundance in serial whole blood
146 samples from 10 volunteers infected with 1,000 plaque forming units (pfu) of rDEN3Δ30/31, the
147 dose included in TV003, and four volunteers inoculated with placebo (L-15 medium). Nine of ten
148 vaccinees seroconverted 28 days post-vaccination, defined as a 60% plaque reduction
149 neutralization titer ($PRNT_{60}$) >10 (Table 1). Four of the vaccinees had low-level viremia on one
150 or more days within the first 10 days post-vaccination, five developed a mild maculopapular
151 rash, and none were febrile. The four placebo recipients remained seronegative for DENV
152 serotypes.

153 We collected whole blood for isolation of RNA immediately before vaccination (day 0),
154 and on days 2, 5, 6, 8, 9, 12, 14, 20, 29, 42 and 180 post-vaccination from all volunteers and
155 measured genome-wide transcript abundance levels. Data were available for eight of the nine
156 participants who seroconverted. For each of these eight subjects, we compared transcript
157 abundances for each post-vaccination day with those for the matched pre-vaccination sample
158 (see Supplementary Information). Almost all significant changes in transcript abundance
159 occurred 5-20 days after vaccination, with a peak of 161 and 156 transcripts changing in
160 abundance (days 8 and 9, respectively), and 286 transcripts with a significant change in
161 abundance on at least one day (Figure 1). Fewer transcripts met criteria for significance when
162 comparing vaccinees to placebo recipients (n=131), but the direction of change for 271 of the
163 286 transcripts from vaccinees was the same whether the comparison was with day-matched
164 placebo recipients or each subject's baseline sample (Supplementary Figure 1).

165 To infer the functional implications of these changes in transcript abundance, we used
166 hierarchical clustering to organize the transcripts and compared gene membership in Gene

167 Ontology and the KEGG pathways using the DAVID bioinformatics resource [28]. Gene
168 transcripts were grouped in three clusters (Figure 2 and Supplementary Figure 2). Transcripts in
169 Cluster 1 were more abundant after vaccination (Figure 2C), peaked on days 8 and 9 post
170 vaccination, and included canonical interferon-stimulated gene (ISG) transcripts; IFI44, IFI44L,
171 IFI27, HERC5, IFIT1, USP18, and ISG15 transcripts all increased 10- to 22-fold compared to
172 baseline. Cluster 1 was strongly enriched for genes involved in the innate immune response to
173 viruses and highly enriched for genes we previously found to be expressed after treatment of
174 PBMCs with type I interferon ($p<1E-36$) [29].
175 Gene transcripts in Clusters 2 and 3 showed maximal changes on day 14, with Cluster 2
176 transcripts increasing and Cluster 3 transcripts decreasing in abundance from baseline (Figure
177 2A and 2B). Cluster 2 included TYMS, CEP55, CCNA2, and NEK2, whose genes products are
178 involved in DNA replication and cell division, and other genes associated with mitosis ($p<2E-9$,
179 Figure 2C). Genes in Cluster 3 were enriched in both reticulocytes ($p=1E-20$) and neutrophils
180 ($p=2E-7$) [30]. We did not measure reticulocyte counts, but we did measure neutrophils and the
181 relative neutrophil abundance in vaccinees did not change significantly with time ($p=0.55$, paired
182 t-test), suggesting that decreased expression of these genes was not due to decreased
183 neutrophil abundance.
184
185 **Changes observed after vaccination are a subset of those observed in natural**
186 **symptomatic DENV-3 infection**
187 To establish which features of the early response to vaccination are shared with the
188 response to natural symptomatic infection, we examined transcript responses in Nicaraguan
189 children hospitalized with acute dengue. We previously demonstrated that a history of previous
190 DENV exposure is the most prominent source of variation in gene expression in dengue
191 patients [14]. To ensure that DENV immune status, as well as serotype, did not confound our
192 analysis, we identified 30 children diagnosed with acute primary DENV-3 infection during a

193 single year (24 with dengue fever and 6 with dengue hemorrhagic fever; Supplementary Table
194 1), and compared transcript abundance in whole blood with measurements from 9 healthy
195 individuals. Principal components analysis confirmed previous findings that there are significant
196 day-to-day changes in the transcript response to natural infection [14,31] (Supplementary Figure
197 4); thus, we subsequently performed analyses stratified by day of fever. There were no
198 significant differences in transcript abundance between children with dengue fever and children
199 with dengue hemorrhagic fever after matching for sex and day of fever, which is generally
200 consistent with previous studies in the same population [14,18], although the small sample size
201 could affect the results of our analysis.

202 Despite having fewer days available for comparison and lacking baseline samples for
203 each patient, we identified many more transcripts with significant changes in abundance post-
204 infection compared to those found in vaccinees: among the 20,623 transcripts measured in both
205 datasets, we identified 3,210 transcripts that differed significantly on at least one day of fever,
206 compared with 278 transcripts following vaccination (Figure 3A, Supplementary Figure 5A). The
207 magnitude of the maximum change in abundance post-infection was also nearly 10-fold greater:
208 there was a 200-fold difference post-infection compared to a maximum 21-fold difference post-
209 vaccination (Figure 3B). The transcripts with the greatest differences in relative abundance
210 during natural infection were MT2A (242-fold) and USP18 (183-fold), both of which are
211 interferon-induced; HESX1 (150-fold), which is expressed in activated dendritic cells; and
212 SPAT2SL (137-fold), which may be involved in activation and differentiation of multiple cell
213 types.

214 Despite differences in response magnitude and number, the response following natural
215 symptomatic infection included 90% (250/278) of transcripts that changed after vaccination, and
216 the direction of change was the same for 96% of these transcripts (240/250) (Figure 3C). The
217 transcripts that changed the most post-vaccination (IFI44, IFI44L, IFI27 and HERC5) were
218 among the 20 transcripts with the biggest differences in abundance following natural infection,

219 and relative increases in transcript abundance were strongly correlated across the two groups
220 (Spearman r^2 = 0.75).
221
222 **Responses to dengue vaccination and symptomatic natural infection share a common**
223 **temporal sequence**
224 We used gene set enrichment analysis and information from all measured transcripts to
225 identify 141 blood transcript gene modules that changed in abundance following either
226 immunization or infection [8] (FDR<1%). Many of these modules demonstrated similar changes
227 in both vaccinees and patients (Figure 4A). Modules enriched for ISG expression were elevated
228 on days 5-14 post-vaccination and were also persistently elevated after natural DENV infection.
229 Modules representing monocyte-associated transcripts were elevated on days 1-3 of natural
230 infection and on days 8-9 post-vaccination, while modules associated with the mitotic cell cycle
231 were elevated on later days in both groups, with the highest levels on day 5 of natural infection
232 and on day 14 post-vaccination. When we compared the overall profiles of the gene modules in
233 the two groups, we found that the responses to natural infection on fever days 1-3 were most
234 similar to responses to vaccination on days 8-9 (Pearson's r ≥0.60; peak on day 9), while fever
235 day 4 was most similar to vaccination day 12 (r>0.75, peak on day 12), and fever day 5 was
236 most similar to vaccination day 14 and subsequent time-points (r≥0.70, peak on 14) (Figure 4B,
237 Supplementary Dataset 1). Thus, the enrichment of common modules in the same sequence
238 indicates a similar progression in the early host response to vaccination and to natural infection.
239 We note there was also a cluster of 16 gene modules, six associated with platelet
240 activation and cytoskeletal remodeling, that were elevated in natural infection but not vaccinees
241 (Figure 4A, Supplementary Dataset 1). Previous studies have demonstrated that platelet
242 activation and TGFβ expression are elevated in DENV infection and higher in patients with more
243 severe disease [32]. TGFβ, which is expressed at high levels in platelets [33], was elevated on

244 fever days 1-2 in dengue patients but was never elevated post-vaccination (Supplementary
245 Figure 6).
246
247 **Early transcriptional responses linked to neutralizing antibody production**
248 DENV-specific NAbs are the primary endpoint for assessing vaccine responses in clinical trials
249 and are associated with protection from both symptomatic infection and severe disease [3–5].
250 To determine whether changes in host transcript patterns predicted differences in NAb titer we
251 calculated the correlation between the change in abundance of each transcript on each day and
252 the NAb titer on post-vaccination day 42, when NAbs are generally at peak titer (Table 1,
253 Supplementary Figure 7A). During the first 6 days post-vaccination, we found no significant
254 correlations with NAb titer, but by day 8, expression of the ISGs in Cluster 1 positively correlated
255 with the day 42 NAb titer ($p<0.01$; Figure 5). This correlation was equally strong on day 9, and
256 131 transcripts were significantly correlated with day 42 NAb titer on both days. Among the
257 individual ISG transcripts most strongly correlated with day 42 NAb titer on both days 8 and 9
258 was IFI44, the transcript whose abundance changed the most post-vaccination (Supplementary
259 Figure 7B). IFI44 was also elevated at one time-point in each of two placebo recipients, but the
260 timing of elevated expression was different and correlated with unrelated respiratory viral
261 infections in each instance (Supplementary Figure 8). Twelve of the 131 transcripts were also
262 associated with subsequent development of a rash, which was the only significant correlate with
263 positive NAb titer in a clinical trial of TV003 [21] (Supplementary Figure 9). Interestingly, the one
264 vaccinee who failed to develop neutralizing antibodies showed little evidence of increased
265 abundance in Cluster 1 genes (Supplementary Figure 3). The association of interferon-related
266 transcript abundance and later NAb titer diminished on days 12 and 14, but BUB1 ($r=0.86$) and
267 other transcripts associated with the mitotic cell cycle were correlated with subsequent NAb
268 titers on day 14 (Figure 5, Supplementary Figure 7C).

269 When we performed similar comparisons for naturally infected patients, we found no
270 transcript clusters significantly correlated with either convalescent or three month NAb titer
271 (Supplementary Figure 5B and 5C). However, the pattern of blood transcript module
272 enrichment indicated a similar relationship between day-specific gene expression and later
273 production of NAb; gene enrichment for both interferon-stimulated and cell cycle-associated
274 gene modules was associated with higher NAb titer in both vaccinees and patients (Figure 6),
275 albeit more weakly in patients, and cell cycle-associated modules were correlated with NAb titer
276 later in both groups.
277 There are at least three subpopulations of monocytes with distinct transcript profiles [34];
278 Kwissa et al. identified an increase in $CD14^{+}CD16^{+}$ intermediate-phenotype population after
279 secondary DENV infection, and showed that in vitro these cells stimulated formation of the
280 plasmablasts that secrete antibodies weeks after infection, mediated in part by secretion of the
281 ISG cytokine BAFF [19]. In our study, gene set enrichment analysis indicated enrichment of
282 transcripts for both intermediate and nonclassical monocytes at multiple time-points in both
283 vaccinees and patients, while BAFF transcripts were most abundant on fever days 1 and 2 in
284 the patients and days 8 and 9 in the vaccinees (Supplementary Figure 10).
285
286 **DISCUSSION**
287 In this study, we used intensive longitudinal sampling to characterize the transcriptional
288 response to dengue vaccination, compared results with those from natural infection with the
289 same DENV serotype, and identified early features that may predict a protective immune
290 response. We found that vaccination and natural infection induced common gene expression
291 programs, and the abundance of individual interferon-stimulated transcripts 8 days post-
292 vaccination was correlated with NAb titers measured five weeks later, representing the earliest
293 identified correlates of a protective adaptive immune response following dengue vaccination.

294 An interferon response signature has been observed in other studies profiling viral
295 vaccine transcriptional responses. Inactivated influenza and meningococcal vaccines both
296 induce a mild interferon response during the first week post-vaccination, but the response is
297 particularly strong after vaccination with live attenuated vaccines [9,12,35]. We reported that
298 ISG expression was much stronger in cynomolgous macaques infected with wild-type DENV
299 compared to live attenuated virus [35]. Here, we found that ISG expression was much stronger
300 in symptomatic dengue patients than vaccinees, presumably due to higher viral load after
301 infection with wild-type virus. Expression of ISGs was correlated with viral load in the patients,
302 as seen in other studies [19,36]. However, this association did not persist when patients were
303 stratified by day of fever, highlighting the importance of temporal variation in the innate
304 immune response and in viral load, and suggesting that factors in addition to viral replication
305 influence ISG expression. Several studies have found stable inter-individual differences in the
306 response to interferon, suggesting that genetic and environmental features may affect the
307 relationship between viral infection and the interferon response [37,38]. We were not able to
308 assess directly the impact of these features on transcriptional responses in this study, but their
309 contributions to the differences between children with symptomatic infection and vaccinated
310 adults are likely to be much less than the contribution of differences in virulence of vaccine and
311 wild type viruses (see Supplementary Information).
312 The links between type I interferon production and NAb production probably involve
313 multiple cell types. Plasmacytoid dendritic cells (pDCs) contribute to B cell differentiation and
314 antibody production after viral infection [39]. In this study, increases in monocyte-associated
315 gene expression coincided with ISG expression, and we found features related to multiple
316 monocyte phenotypes in both natural infection and vaccination. Gene module analysis also
317 suggested that T cells were responsible for the increase in cell cycle-associated transcripts two
318 weeks after vaccination that was linked to NAb titers. Future targeted studies of pDCs,
319 monocytes, and T cell populations during the first two weeks post-vaccination will help clarify

320 their role in establishing long-lasting antibody responses. In addition, the link between an early
321 interferon response and later NAb titer was only apparent in natural infection when we used a
322 module analysis approach. This may indicate a plateau, or saturation effect, in the relationship
323 between ISG expression and antibody titer. Alternatively, it may reflect the variability in
324 pathogen dose, prior health status and/or days of infection absent in clinical trials but inherent in
325 observational studies.

326 Comparison with LAV vaccination also provides a framework for identification of features
327 associated with pathogenic versus non-pathogenic infection. A recent study compared PBMC
328 gene expression in asymptomatic and clinically significant secondary DENV infection and
329 identified differences in antigen presentation and lymphocyte activation [36]. In this study
330 examining whole blood gene expression during primary infection, we found an increased
331 abundance of transcripts associated with platelet activation in natural (pathogenic) infection but
332 not vaccination (non-pathogenic infection), consistent with the hypothesis that platelet activation
333 contributes to dengue pathogenesis [40].

334 Neutralizing antibody titers were used as an endpoint for these vaccine studies because
335 many studies have shown that these antibodies play an important role in protective immunity.
336 However, recent work has demonstrated that NAbs measured in vitro are an imperfect correlate
337 of in vivo protection [37,38]. Immunity mediated by NAbs may be neither life-long nor sterilizing
338 [43,44] and will be affected by the quality as well as the quantity of NAbs [5,26,45]. Recent
339 studies also highlight a likely role for cytotoxic T cells in mediating protection against DENV
340 reinfection and severe disease [46–49]. The NIH tetravalent vaccine, of which rDEN3Δ30/31 is
341 a component, elicits $CD4^+$ T cell responses similar to those seen in natural infection [50]. It will
342 be important to establish whether the early transcript-based features we measured in this study
343 are associated with DENV-specific responses in memory T cell populations.

344 Our findings reflect the integration of data across multiple time-points and thousands of
345 transcripts, and provide a robust basis for further investigation. We previously found that early

interferon-associated transcriptional responses were associated with antibody formation in non-human primates exposed to a tetravalent dengue vaccine [35]. We believe it is likely that the same relationship will exist in humans immunized with tetravalent LAV dengue vaccines. The initiation of Phase 3 clinical trials of TetraVax-DV-TV003 provides an opportunity to validate the relationship between these early responses and NAb titers, and identify specific transcripts as early surrogate markers of both immunogenicity and protection.

Acknowledgements

We thank Cassandra Ventrone for sample collection and logistics, Chunling Wang for viral load data, Ellen Sebastian for data processing, and Elizabeth Costello for helpful suggestions. We thank past and present members of the study team based at the Hospital Infantil Manuel de Jesús Rivera, the Centro de Salud Sócrates Flores Vivas, and the National Virology Laboratory in the Centro Nacional de Diagnóstico y Referencia of the Nicaraguan Ministry of Health, as well as the Sustainable Sciences Institute in Nicaragua for their dedication and high-quality work, and we are grateful to the study participants and their families.

This work was supported by the National Institute of Allergy and Infectious Diseases Division of Intramural Research and Division of Microbiology and Infectious Diseases (U19 AI109761, D.A.R.; U54 AI065359, A.B.), the Thomas C. and Joan M. Merigan Endowment at Stanford University (D.A.R.), the Chan Zuckerburg Biohub (D.A.R.), and by a grant (VE-1) from the Pediatric Dengue Vaccine Initiative of the Bill and Melinda Gates Foundation (E.H.). For potential conflicts of interest, see ICMJE disclosure forms.

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508

509 **Figure Legends**

510

511 Figure 1. Significant differences in transcript abundance post-vaccination (FDR<1%; minimum
512 2-fold change compared to pre-vaccination sample).

513

514 Figure 2. Changes in transcript abundances over time in vaccinees. A) Hierarchical clustering
515 of the 286 transcripts whose abundance was significantly different from baseline on more
516 than one day. Lines and numbers to the right of the heatmap mark sets of co-expressed
517 genes (average cluster r>0.5). B) Change over time in abundance for each transcript in
518 each gene cluster. Heavy line indicates median expression of all genes in each cluster. C)
519 Gene ontologies associated with gene clusters described in (A) and (B). There were no
520 significant gene ontologies for Cluster 3.

521

522 Figure 3. Comparison of post-vaccination and post-infection transcript abundance changes. A)
523 Transcripts with significant changes on days 2, 3, 4, or 5 of fever in patients with primary
524 DENV-3 infection (blue circle) and on any day post-vaccination (green circle). Numbers
525 indicate transcripts unique to vaccination, infection, or shared (overlap, n=246). B)
526 Maximum fold-change in transcript abundance following vaccination (red circles) or during
527 infection (blue diamonds). C) Maximum fold-change in abundance for transcripts with
528 significant changes post-vaccination or during infection. Dotted diagonal line at equal fold
529 change included for reference.

530

531 Figure 4. Gene modules affected by DENV vaccination and natural infection. A) Blood
532 transcript modules with transcripts that were significantly up- or down-regulated on at least
533 one day (FDR <1%) were hierarchically clustered. NES; normalized enrichment score.
534 Vertical lines on right denote module clusters described in the text. B) Hierarchical

535 clustering of each day post-vaccination or post-infection using the NES from (A). Days in
536 bold italics represent days of fever for infected patients; days preceded by "v" represent
537 days post-vaccination.
538
539 Figure 5. Correlation of transcript abundance and day 42 $PRNT_{60}$ among vaccine recipients. A)
540 Average fold change in abundance by day for all transcripts with significant differences
541 from baseline post-vaccination. Transcripts are ordered and clusters labeled as in Figure
542 2. Asterisk marks IFI44. B) Spearman correlation of each transcript and day 42 $PRNT_{60}$
543 using a moving average of window size 9. Solid lines indicate days post-vaccination on
544 which a significant correlation was identified ($p<0.01$, indicated by vertical dotted grey line).
545
546 Figure 6. Gene modules correlated with subsequent neutralizing antibody response. A) Blood
547 Transcript Modules that were significantly enriched for transcripts positively correlated with
548 day 42 $PRNT_{60}$ (vaccinees) or convalescent NT_{50} (patients) on at least one day (FDR<1%)
549 were hierarchically clustered. NES; normalized enrichment score. Vertical lines delineate
550 module clusters described in the text. B) Significant modules (FDR<1%) are marked in red.
551 Modules and samples are organized as in (A). C) Hierarchical clustering of gene module
552 expression from each day post-vaccination or post-infection using the NES from (A). Day
553 labels in bold italics represent fever day for infected patients; day labels preceded by "v"
554 represent day post-vaccination.

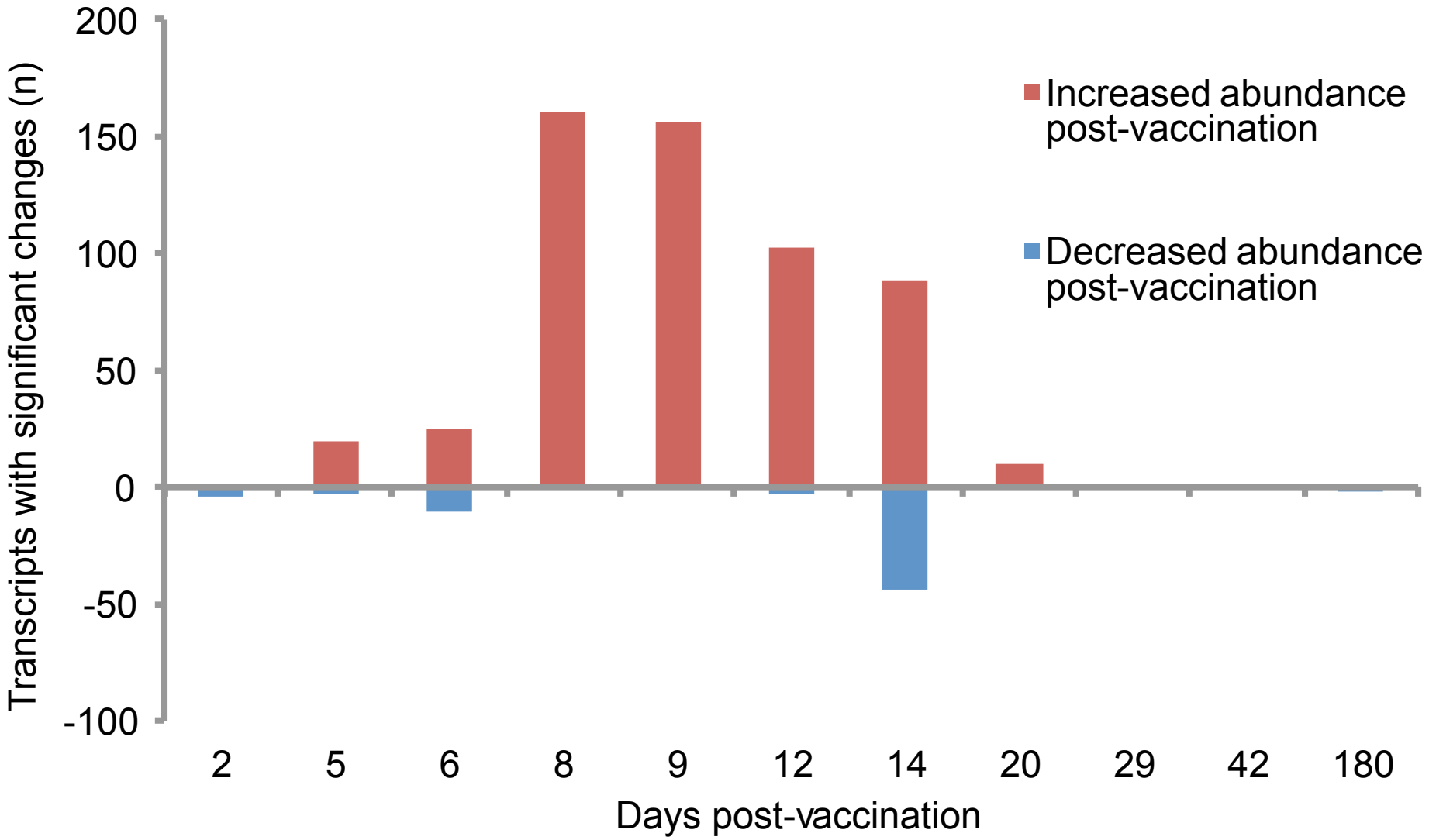
Table 1. Characteristics of subjects in vaccine trial

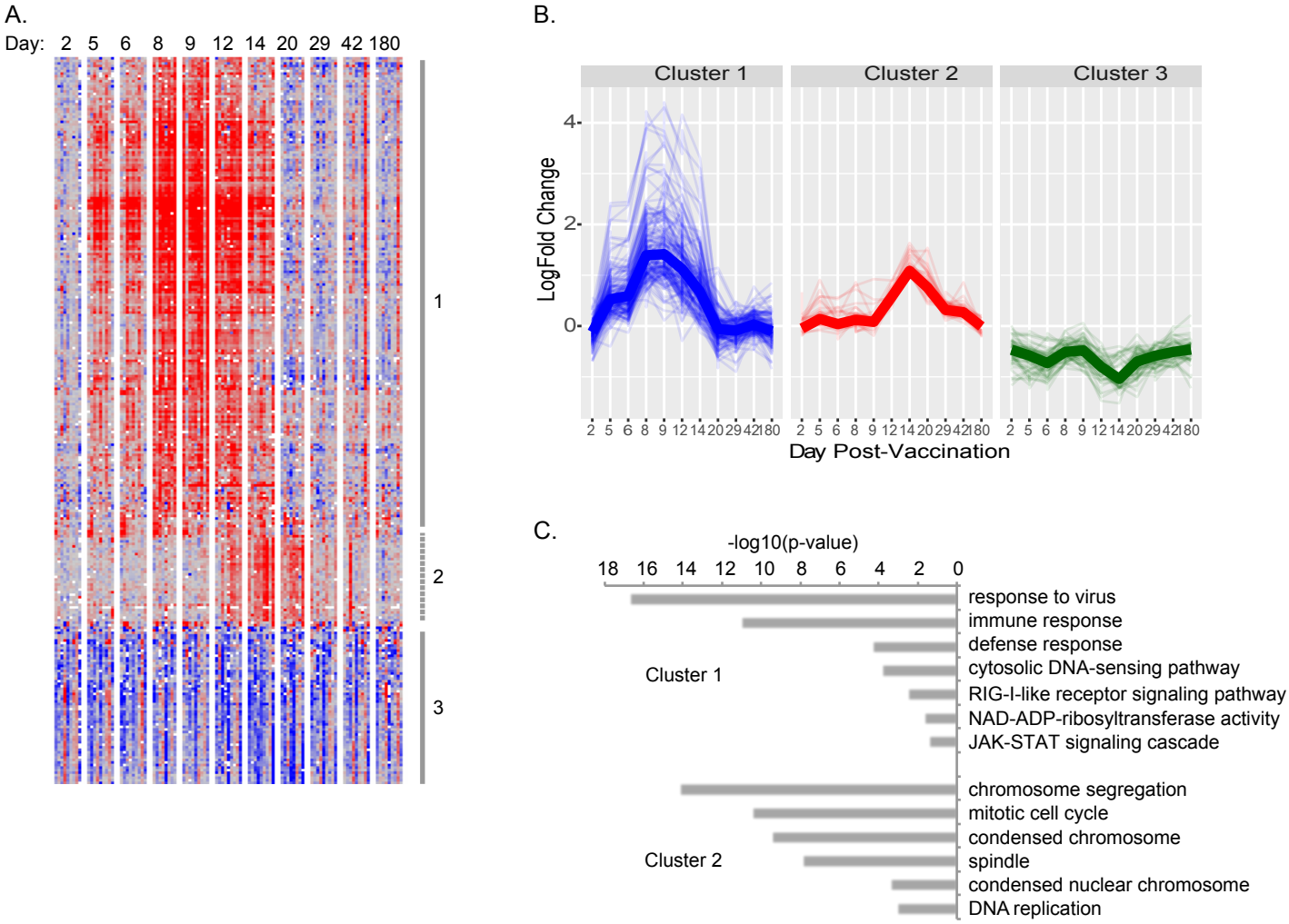
Subject	Age	Sex	Viremia[a]	Rash[b]	Day 28 $PRNT_{60}$[c]	Day 42 $PRNT_{60}$[c]	Day 180 $PRNT_{60}$[c]
1 (Vaccine)	19	F	--	Days 12-20	54	70	30
2 (Vaccine)	26	F	--	--	22	15	<5
3 (Vaccine)	25	M	Days 8-9	--	52	106	22
4 (Vaccine)	20	M	Days 8-9	Days 12-16	26	32	<5
5 (Vaccine)	20	M	Day 6	Days 12-20	33	19	<5
6 (Vaccine)	22	M	--	--	<5	<5	<5
7 (Vaccine)	19	M	--	--	18	8	8
8 (Vaccine)	22	F	Days 5-8	Days 12-20	34	29	8
9 (Vaccine)	19	F	--	--	25	33	<5
10 (Vaccine)	46	F	--	Days 12-16	70	152	64
11 (Placebo)	18	F	--	--	<5	<5	<5
12 (Placebo)	19	M	--	--	<5	<5	<5
13 (Placebo)	45	M	--	--	<5	<5	<5
14 (Placebo)	21	F	--	--	<5	<5	<5

[a] Virus detected in serum from tissue culture plaque formation assay

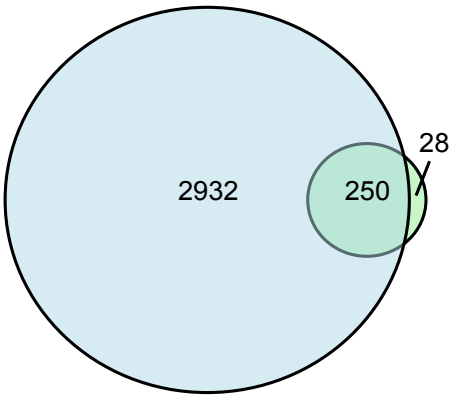
[b] First and last day on which maculopapular rash observed

[c] Reciprocal serum dilution providing 60% reduction in plaque formation

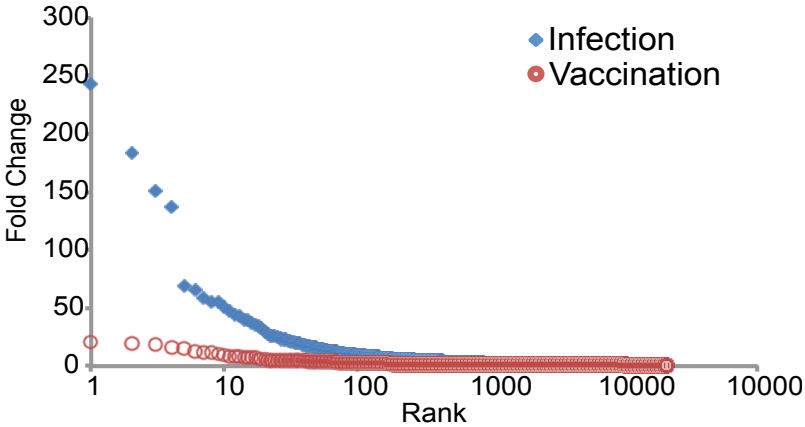

200
150
100
50
0
-50
-100
Transcripts with significant changes (n)
Increased abundance post-vaccination
Decreased abundance post-vaccination
2
5
6
8
9
12
14
20
29
42
180
Days post-vaccination


A.
Day: 2 5 6 8 9 12 14 20 29 42 180
1
2
3
B.
Cluster 1
Cluster 2
Cluster 3
LogFold Change
4
2
0
2 5 6 8 9 12 14 20 29 42 180
Day Post-Vaccination
C.
-log10(p-value)
18 16 14 12 10 8 6 4 2 0
Cluster 1
response to virus
immune response
defense response
cytosolic DNA-sensing pathway
RIG-I-like receptor signaling pathway
NAD-ADP-ribosyltransferase activity
JAK-STAT signaling cascade
Cluster 2
chromosome segregation
mitotic cell cycle
condensed chromosome
spindle
condensed nuclear chromosome
DNA replication

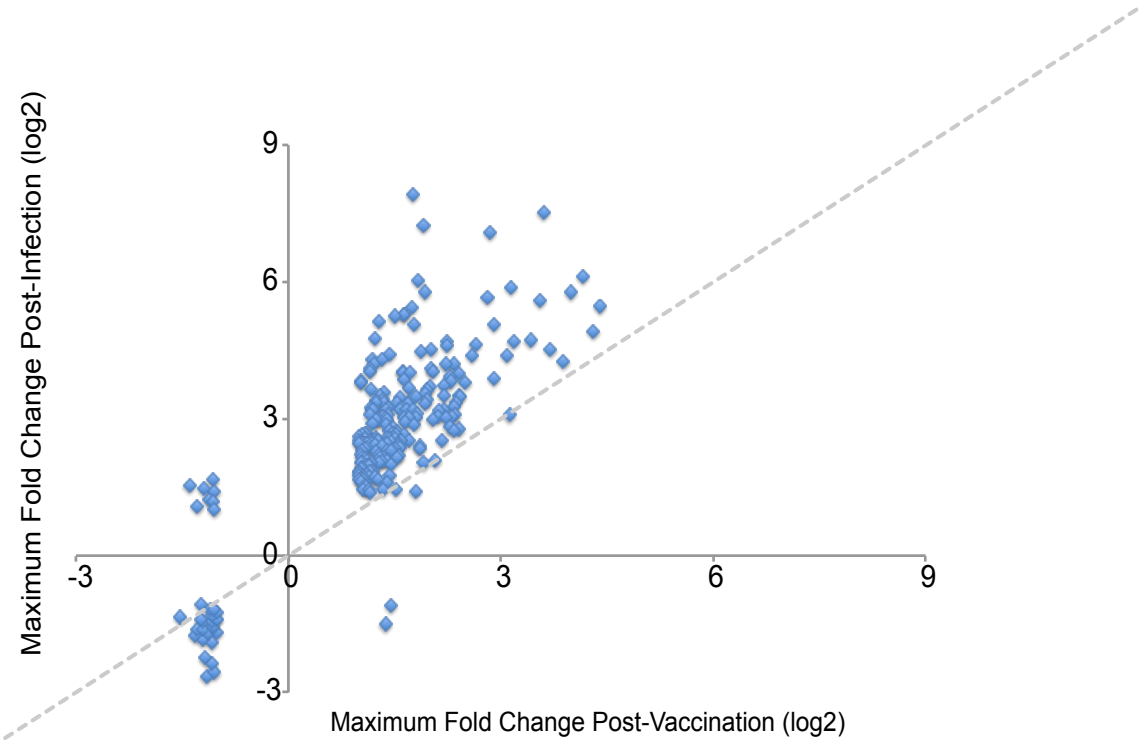
A.

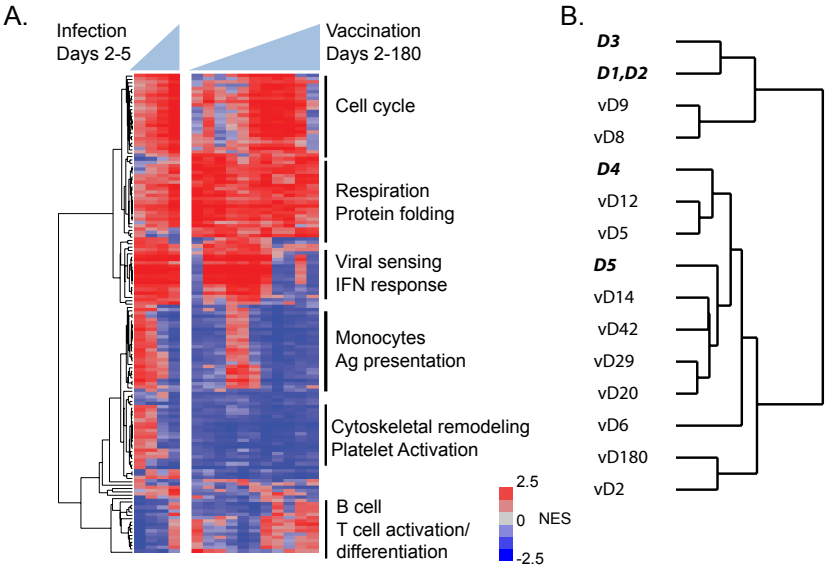

2932
250
28

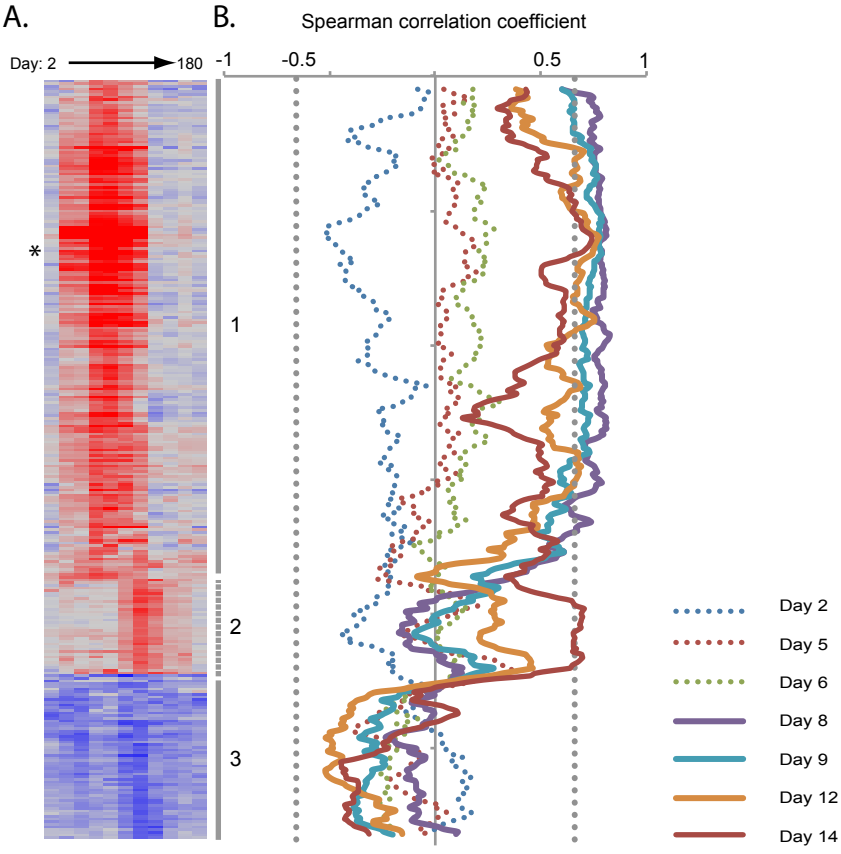
B.

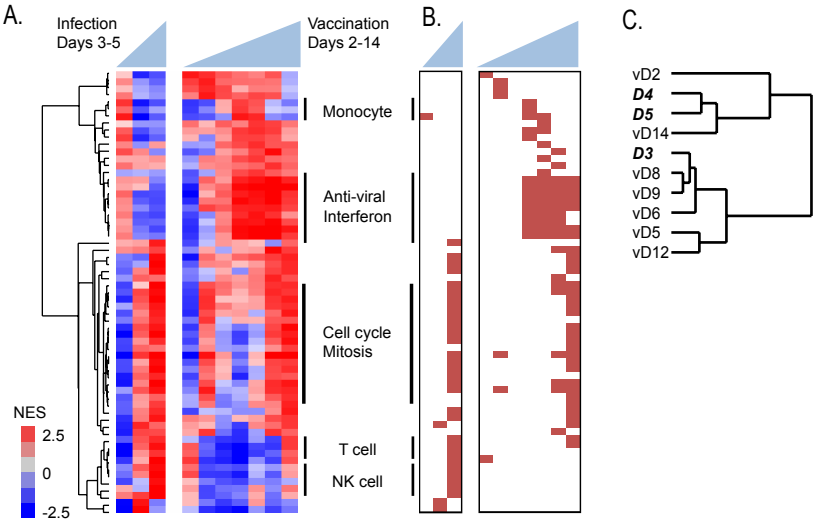

Infection
Vaccination
Fold Change
300
250
200
150
100
50
0
1
10
100
1000
10000
10000
Rank

C.


Maximum Fold Change Post-Infection (log2)
9
6
3
0
-3
-3
0
3
6
9
Maximum Fold Change Post-Vaccination (log2)


A.
Infection
Days 2-5
Vaccination
Days 2-180
Cell cycle
Respiration
Protein folding
Viral sensing
IFN response
Monocytes
Ag presentation
Cytoskeletal remodeling
Platelet Activation
B cell
T cell activation/
differentiation
2.5
0
NES
-2.5
B.
D3
D1,D2
vD9
vD8
D4
vD12
vD5
D5
vD14
vD42
vD29
vD20
vD6
vD180
vD2


A.
Day: 2
180
*
B.
Spearman correlation coefficient
-1
-0.5
0.5
1
1
2
3
Day 2
Day 5
Day 6
Day 8
Day 9
Day 12
Day 14


A.
Infection
Days 3-5
Vaccination
Days 2-14
Monocyte
Anti-viral
Interferon
Cell cycle
Mitosis
T cell
NK cell
NES
2.5
0
-2.5
B.
C.
vD2
D4
D5
vD14
D3
vD8
vD9
vD6
vD5
vD12