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

Stephen J. Popper[a], Fiona R. Strouts[a*], Janet C. Lindow[b*#], Henry K. Cheng[a], Magelda Montoya[c], Angel Balmaseda[d], Anna P. Durbin[e], Stephen S. Whitehead[f], Eva Harris[c], Beth D. Kirkpatrick[b], David A. Relman[a,g,h]

[a] Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA

[b] Vaccine Testing Center, University of Vermont College of Medicine, Burlington, VT 05401, USA

[c] Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA 94720-3370, USA

[d] Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua

[e] Center for Immunization Research (CIR), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA

[f] Laboratory of Infectious Diseases, National Institute for Allergy and Infectious Diseases, Bethesda, MD 20892, USA

[g] Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA

[h] Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304, USA

* These two authors contributed equally to the work

Correspondence: D. A. Relman, VA Palo Alto Health Care System 154T, 3801 Miranda Avenue, Palo Alto, CA 94304; 650-736-6822 tel; 650-852-3291 fax; relman@stanford.edu

[#] Current address: Center for Mental Health Research and Recovery, Montana State University, Bozeman, MT 59717, USA

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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

66 **BACKGROUND**

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

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

84 Little is known about the role of early innate immune responses in enhancing NAb
85 production and promoting protective immune memory against dengue. Studies of innate
86 immunity have been hampered by the difficulty inherent in identifying individuals with early
87 infection, when innate immune responses are most active, particularly those with mild or
88 subclinical infections. Trials of LAVs provide a unique opportunity to examine early immune
89 responses in a setting where the time, dose, and viral serotype are known. Genome-wide
90 transcript responses to vaccines have provided important clues about early steps in the
91 generation of humoral and 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 the temporal course of changes in transcript abundance and identified early signatures correlated with NAb titers measured six weeks post-vaccination. We also compared these results with the 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 enrolled and 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 (PAXgene, Preanalytix) were collected immediately prior to vaccination and on days 2, 5, 6, 8, 9, 12,14, 20, 29, 42 and 180, and stored at -80°C until used. 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

118 (viremia) were measured using a standard plaque assay as described previously [24]. Serum
119 NAb titer was determined by 60% plaque reduction ($PRNT_{60}$) [25]. Seroconversion was defined
120 by a $\geq$4-fold increase in $PRNT_{60}$ on study day 28 or 42 relative to day 0 and corresponds to a
121 post-vaccination titer >10 [23].
122
123 **Dengue patient population.** Patients presenting with fever and suspected dengue during the
124 2010 dengue season were enrolled at the Hospital Infantil Manuel de Jesús Rivera (HIMJR) in
125 Managua, Nicaragua. Inclusion criteria, recruitment, and laboratory testing have been described
126 previously [26]; a full description is available in the Supplementary Information. Blood samples
127 from healthy subjects were collected as part of a separate prospective cohort study in which
128 healthy children in the same general population were enrolled without regard to dengue status
129 [27].
130
131 **Ethics statement.** The trial of rDEN3Δ30/31 was approved by the Committee for Human
132 Research at the University of Vermont, and written informed consent was obtained from all
133 subjects following a review of risks and benefits and a comprehension assessment. The study in
134 Nicaragua was approved by the Institutional Review Boards of the University of California,
135 Berkeley, and the Nicaraguan Ministry of Health, and by the Stanford University Administrative
136 Panel on Human Subjects in Medical Research. All clinical research followed human
137 experimentation guidelines of the United States Department of Health and Human Services
138 and/or those of the authors' institutions. Parents or legal guardians of all subjects provided
139 written informed consent, and subjects 6 years of age and older provided assent.
140
141 **RNA sample processing and transcriptome analysis.** PAXgene RNA was amplified and
142 hybridized to Human Exonic Evidence Based Oligonucleotide (HEEBO) microarrays [14].
143 Microarray data were submitted to the Princeton University MicroArray (PUMA) database for

144 normalization and gene filtering and are deposited at Gene Expression Omnibus
145 (http://www.ncbi.nlm.nih.gov/geo/; accession numbers GSE96656 and GSE98053). Data
146 analysis was carried out using packages cited in the main text; a full description of both sample
147 processing and analysis steps is available in the Supplementary Information.
148
149 **RESULTS**
150
151 **Temporal patterns of the transcriptional responses to live dengue vaccination**
152 To identify the temporal pattern of the early human transcriptional response to dengue
153 vaccination, we examined changes in genome-wide transcript abundance in serial whole blood
154 samples from 10 volunteers infected with 1,000 plaque forming units (pfu) of rDEN3Δ30/31, the
155 dose included in TV003, and four volunteers inoculated with placebo (L-15 medium). Nine of ten
156 vaccinees seroconverted 28 days post-vaccination, defined as a 60% plaque reduction
157 neutralization titer ($PRNT_{60}$) >10 (Table 1). Four of the vaccinees had low-level viremia on one
158 or more days within the first 10 days post-vaccination, five developed a mild maculopapular
159 rash, and none were febrile. The four placebo recipients remained seronegative for DENV
160 serotypes.
161 We collected whole blood for isolation of RNA immediately before vaccination (day 0),
162 and on days 2, 5, 6, 8, 9, 12, 14, 20, 29, 42 and 180 post-vaccination from all volunteers and
163 measured genome-wide transcript abundance levels. Data were available for eight of the nine
164 participants who seroconverted. For each of these eight subjects, we compared transcript
165 abundances for each post-vaccination day with those for the matched pre-vaccination sample
166 (see Supplementary Information). Almost all significant changes in transcript abundance
167 occurred 5-20 days after vaccination, with a peak of 161 and 156 transcripts changing in
168 abundance (days 8 and 9, respectively), and 286 transcripts with a significant change in
169 abundance on at least one day (Figure 1). Fewer transcripts met criteria for significance when

170 comparing vaccinees to placebo recipients (n=131), but the direction of change for 271 of the
171 286 transcripts from vaccinees was the same whether the comparison was with day-matched
172 placebo recipients or each subject's baseline sample (Supplementary Figure 1).
173 To infer the functional implications of these changes in transcript abundance, we used
174 hierarchical clustering to organize the transcripts and compared gene membership in Gene
175 Ontology and the KEGG pathways using the DAVID bioinformatics resource [28]. Gene
176 transcripts were grouped in three clusters (Figure 2 and Supplementary Figure 2). Transcripts in
177 Cluster 1 were more abundant after vaccination (Figure 2C), peaked on days 8 and 9 post
178 vaccination, and included canonical interferon-stimulated gene (ISG) transcripts; IFI44, IFI44L,
179 IFI27, HERC5, IFIT1, USP18, and ISG15 transcripts all increased 10- to 22-fold compared to
180 baseline. Cluster 1 was strongly enriched for genes involved in the innate immune response to
181 viruses and highly enriched for genes we previously found to be expressed after treatment of
182 PBMCs with type I interferon (p<1E-36) [29].
183 Gene transcripts in Clusters 2 and 3 showed maximal changes on day 14, with Cluster 2
184 transcripts increasing and Cluster 3 transcripts decreasing in abundance from baseline (Figure
185 2A and 2B). Cluster 2 included TYMS, CEP55, CCNA2, and NEK2, whose genes products are
186 involved in DNA replication and cell division, and other genes associated with mitosis (p<2E-9,
187 Figure 2C). Genes in Cluster 3 were enriched in both reticulocytes (p=1E-20) and neutrophils
188 (p=2E-7) [30]. We did not measure reticulocyte counts, but we did measure neutrophils and the
189 relative neutrophil abundance in vaccinees did not change significantly with time (p=0.55, paired
190 t-test), suggesting that decreased expression of these genes was not due to decreased
191 neutrophil abundance.
192
193 **Changes observed after vaccination are a subset of those observed in natural**
194 **symptomatic DENV-3 infection**

To establish which features of the early response to vaccination are shared with the response to natural symptomatic infection, we examined transcript responses in Nicaraguan children hospitalized with acute dengue. We previously demonstrated that a history of previous DENV exposure is the most prominent source of variation in gene expression in dengue patients [14]. To ensure that DENV immune status, as well as serotype, did not confound our analysis, we identified 30 children diagnosed with acute primary DENV-3 infection during a single year (Supplementary Table 1), and compared transcript abundance in whole blood with measurements from 9 healthy individuals. Principal components analysis confirmed previous findings that there are significant day-to-day changes in the transcript response to natural infection [14,31] (Supplementary Figure 4); thus, we subsequently performed analyses stratified by day of fever.

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

Despite differences in response magnitude and number, the response following natural symptomatic infection included 90% (250/278) of transcripts that changed after vaccination, and the direction of change was the same for 96% of these transcripts (240/250) (Figure 3C). The

221 transcripts that changed the most post-vaccination (IFI44, IFI44L, IFI27 and HERC5) were
222 among the 20 transcripts with the biggest differences in abundance following natural infection,
223 and relative increases in transcript abundance were strongly correlated across the two groups
224 (Spearman $r^2 = 0.75$).
225
226 **Responses to dengue vaccination and symptomatic natural infection share a common**
227 **temporal sequence**
228 We used gene set enrichment analysis and information from all measured transcripts to
229 identify 141 blood transcript gene modules that changed in abundance following either
230 immunization or infection [8] (FDR<1%). Many of these modules demonstrated similar changes
231 in both vaccinees and patients (Figure 4A). Modules enriched for ISG expression were elevated
232 on days 5-14 post-vaccination and were also persistently elevated after natural DENV infection.
233 Modules representing monocyte-associated transcripts were elevated on days 1-3 of natural
234 infection and on days 8-9 post-vaccination, while modules associated with the mitotic cell cycle
235 were elevated on later days in both groups, with the highest levels on day 5 of natural infection
236 and on day 14 post-vaccination. When we compared the overall profiles of the gene modules in
237 the two groups, we found that the responses to natural infection on fever days 1-3 were most
238 similar to responses to vaccination on days 8-9 (Pearson's r ≥0.60; peak on day 9), while fever
239 day 4 was most similar to vaccination day 12 (r>0.75, peak on day 12), and fever day 5 was
240 most similar to vaccination day 14 and subsequent time-points (r≥0.70, peak on 14) (Figure 4B,
241 Supplementary Dataset 1). Thus, the enrichment of common modules in the same sequence
242 indicates a similar progression in the early host response to vaccination and to natural infection.
243 We note there was also a cluster of 16 gene modules, six associated with platelet
244 activation and cytoskeletal remodeling, that were elevated in natural infection but not vaccinees
245 (Figure 4A and Supplementary Dataset 1). Previous studies have demonstrated that platelet

246 activation and TGFβ expression are elevated in DENV infection and higher in patients with more
247 severe disease [32]. TGFβ, which is expressed at high levels in platelets [33], was elevated on
248 fever days 1-2 in dengue patients but was never elevated post-vaccination (Supplementary
249 Figure 6).
250
251 **Early transcriptional responses linked to neutralizing antibody production**
252 DENV-specific NAbs are the primary endpoint for assessing vaccine responses in clinical trials
253 and are associated with protection from both symptomatic infection and severe disease [3–5].
254 To determine whether changes in host transcript patterns predicted differences in NAb titer we
255 calculated the correlation between the change in abundance of each transcript on each day and
256 the NAb titer on post-vaccination day 42, when NAbs are generally at peak titer (Table 1,
257 Supplementary Figure 7). During the first 6 days post-vaccination, we found no significant
258 correlations with NAb titer, but by day 8, expression of the ISGs in Cluster 1 positively correlated
259 with the day 42 NAb titer ($p<0.01$; Figure 5). This correlation was equally strong on day 9, and
260 131 transcripts were significantly correlated with day 42 NAb titer on both days. Among the
261 individual ISG transcripts most strongly correlated with day 42 NAb titer on both days 8 and 9
262 ($r>0.8$) was IFI44, the transcript whose abundance changed the most post-vaccination. IFI44
263 was also elevated at one time-point in each of two placebo recipients, but the timing of elevated
264 expression was different and correlated with unrelated respiratory viral infections in each
265 instance (Supplementary Figure 8). Twelve of the 131 transcripts were also associated with
266 subsequent development of a rash, which was the only significant correlate with positive NAb
267 titer in a clinical trial of TV003 [21] (Supplementary Figure 9). Interestingly, the one vaccinee
268 who failed to develop neutralizing antibodies showed little evidence of increased abundance in
269 Cluster 1 genes (Supplementary Figure 3). The association of interferon-related transcript
270 abundance and later NAb titer diminished on days 12 and 14, but BUB1 ($r=0.9$) and other

271 transcripts associated with the mitotic cell cycle were correlated with subsequent NAb titers on
272 day 14 (Figure 5).

273 When we performed similar comparisons for naturally infected patients, we found no
274 transcript clusters significantly correlated with either convalescent or three month NAb titer
275 (Supplementary Figure 5B and 5C). However, the pattern of blood transcript module
276 enrichment indicated a similar relationship between day-specific gene expression and later
277 production of NAb; gene enrichment for both interferon-stimulated and cell cycle-associated
278 gene modules was associated with higher NAb titer in both vaccinees and patients (Figure 6),
279 albeit more weakly in patients, and cell cycle-associated modules were correlated with NAb titer
280 later in both groups.

281 There are at least three subpopulations of monocytes with distinct transcript profiles [34];
282 Kwissa et al. identified an increase in $CD14^{+}CD16^{+}$ intermediate-phenotype population after
283 secondary DENV infection, and showed that in vitro these cells stimulated formation of the
284 plasmablasts that secrete antibodies weeks after infection, mediated in part by secretion of the
285 ISG cytokine BAFF [19]. In our study, gene set enrichment analysis indicated enrichment of
286 transcripts for both intermediate and nonclassical monocytes at multiple time-points in both
287 vaccinees and patients, while BAFF transcripts were most abundant on fever days 1 and 2 in
288 the patients and days 8 and 9 in the vaccinees (Supplementary Figure 10).

289

290 **DISCUSSION**

291 In this study, we used intensive longitudinal sampling to characterize the transcriptional
292 response to dengue vaccination, compared results with those from natural infection with the
293 same DENV serotype, and identified early features that may predict a protective immune
294 response. We found that vaccination and natural infection induced common gene expression
295 programs, and the abundance of individual interferon-stimulated transcripts 8 days post-

296 vaccination was correlated with NAb titers measured five weeks later, representing the earliest
297 identified correlates of a protective adaptive immune response following dengue vaccination.
298 An interferon response signature has been observed in other studies profiling viral
299 vaccine transcriptional responses. Inactivated influenza and meningococcal vaccines both
300 induce a mild interferon response during the first week post-vaccination, but the response is
301 particularly strong after vaccination with live attenuated vaccines [9,12,35]. We reported that
302 ISG expression was much stronger in cynomolgous macaques infected with wild-type DENV
303 compared to live attenuated virus [35]. Here, we found that ISG expression was much stronger
304 in symptomatic dengue patients than vaccinees, presumably due to higher viral load after
305 infection with wild-type virus. Expression of ISGs was correlated with viral load in the patients,
306 as seen in other studies [19,36]. However, this association did not persist when patients were
307 stratified by day of fever, highlighting the importance of temporal variation in the innate immune
308 response and in viral load, and suggesting that factors in addition to viral replication influence
309 ISG expression. Several studies have found stable inter-individual differences in the response to
310 interferon, suggesting that genetic and environmental features may affect the relationship
311 between viral infection and the interferon response [37,38].
312 The links between type I interferon production and NAb production are likely to 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 (Supplementary Figure 10C).
317 Gene module analysis also suggested that T cells were responsible for the increase in cell
318 cycle-associated transcripts two weeks after vaccination that was linked to NAb titers. Future
319 targeted studies of pDCs, monocytes, and T cell populations during the first two weeks post-
320 vaccination will help clarify their role in establishing long-lasting antibody responses. In addition,
321 the link between an early interferon response and later NAb titer was only apparent in natural

322 infection when we used a module analysis approach. This may indicate a plateau, or saturation
323 effect, in the relationship between ISG expression and antibody titer. Alternatively, it may reflect
324 the variability in pathogen dose, prior health status and/or days of infection absent in clinical
325 trials but inherent in 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 should be validated using a tetravalent dengue vaccine formulation. We
345 previously studied transcript-based responses to a different tetravalent dengue vaccine in
346 nonhuman primates and found that the interferon response was associated with antibody
347 formation [35]. We believe it is likely that the same relationship between early transcriptional

responses and neutralizing antibodies will exist in humans immunized with tetravalent LAV dengue vaccines. The initiation of Phase 3 clinical trials of TetraVax-DV-TV003 provides the opportunity to establish whether specific transcriptional profiles can be used as early surrogate markers of both immunogenicity and protection.

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502

503 **Figure Legends**

504

505 Figure 1. Significant differences in transcript abundance post-vaccination (FDR<1%; minimum
506 2-fold change compared to pre-vaccination sample).

507

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

515

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

524

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

529 clustering of each day post-vaccination or post-infection using the NES from (A). Days in
530 bold italics represent days of fever for infected patients; days preceded by "v" represent
531 days post-vaccination.
532
533 Figure 5. Correlation of transcript abundance and day 42 $PRNT_{60}$ among vaccine recipients. A)
534 Average fold change in abundance by day for all transcripts with significant differences
535 from baseline post-vaccination. Transcripts are ordered and clusters labeled as in Figure
536 2. Asterisk marks IFI44. B) Spearman correlation of each transcript and day 42 $PRNT_{60}$
537 using a moving average of window size 9. Solid lines indicate days post-vaccination on
538 which a significant correlation was identified ($p<0.01$, indicated by vertical dotted grey line).
539
540 Figure 6. Gene modules correlated with subsequent neutralizing antibody response. A) Blood
541 Transcript Modules that were significantly enriched for transcripts positively correlated with
542 day 42 $PRNT_{60}$ (vaccinees) or convalescent NT_{50} (patients) on at least one day (FDR<1%)
543 were hierarchically clustered. NES; normalized enrichment score. Vertical lines delineate
544 module clusters described in the text. B) Significant modules (FDR<1%) are marked in red.
545 Modules and samples are organized as in (A). C) Hierarchical clustering of gene module
546 expression from each day post-vaccination or post-infection using the NES from (A). Day
547 labels in bold italics represent fever day for infected patients; day labels preceded by "v"
548 represent day post-vaccination.
549

550 Table 1. Characteristics of subjects in vaccine trial

551

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

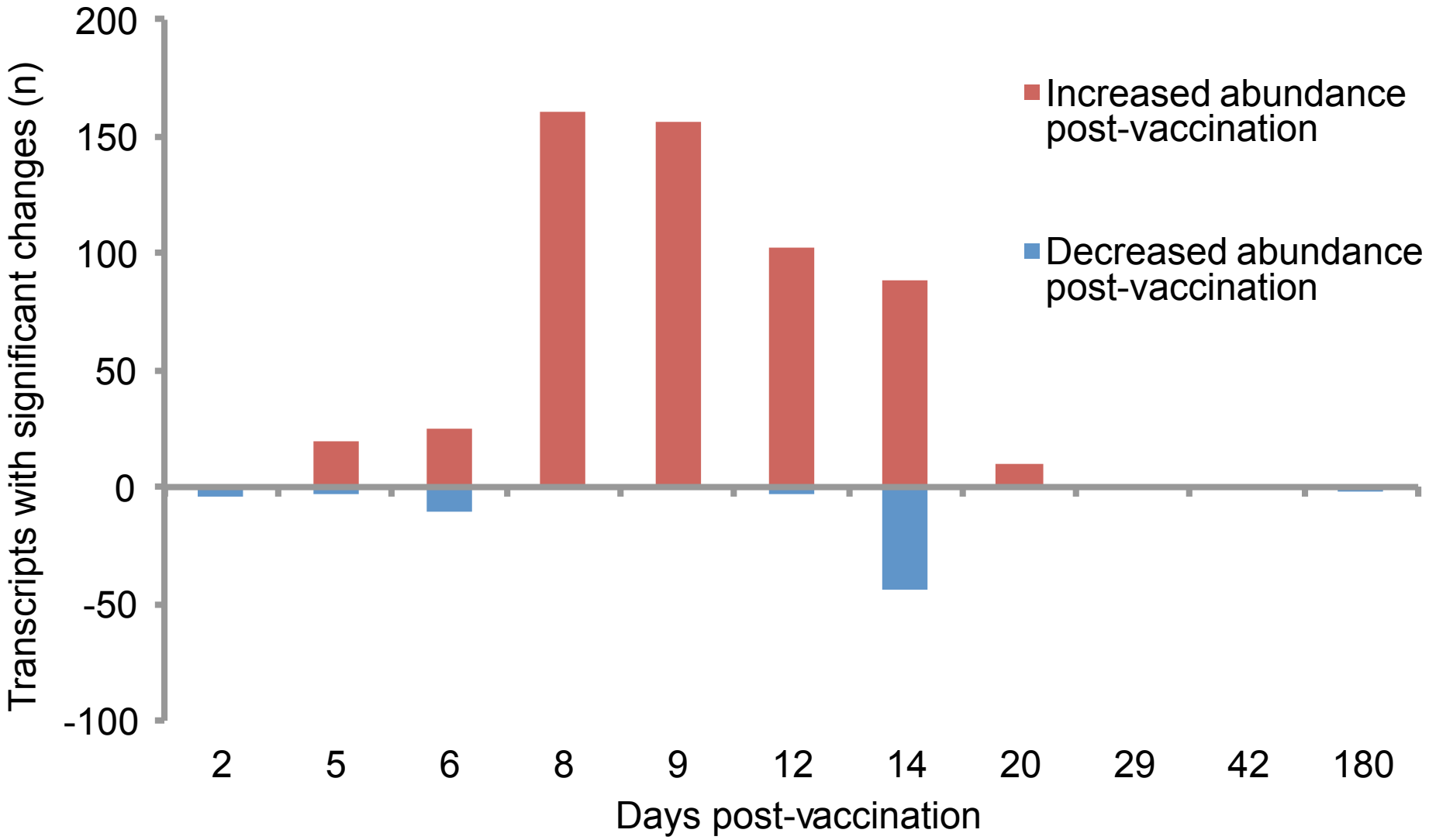
552

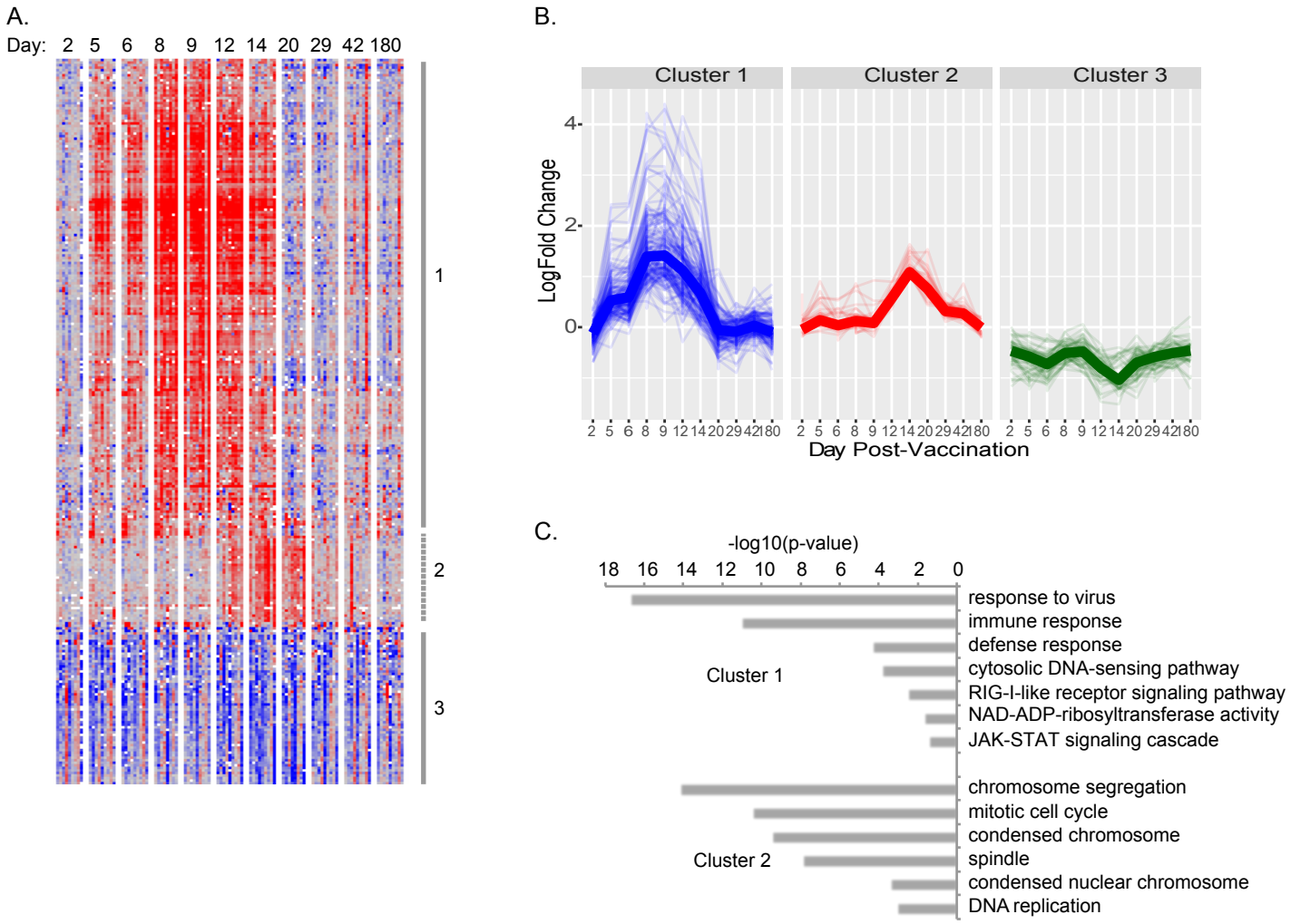
553 [a] Virus detected in serum from tissue culture plaque formation assay

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

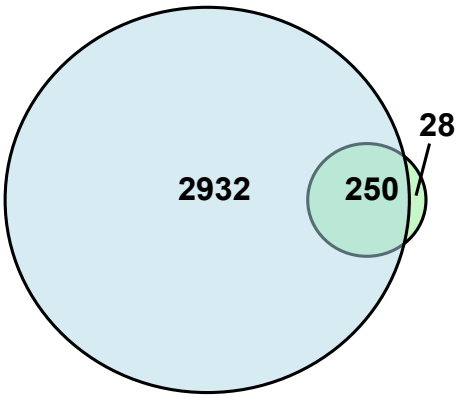
555 [c] Reciprocal serum dilution providing 60% reduction in plaque formation

556

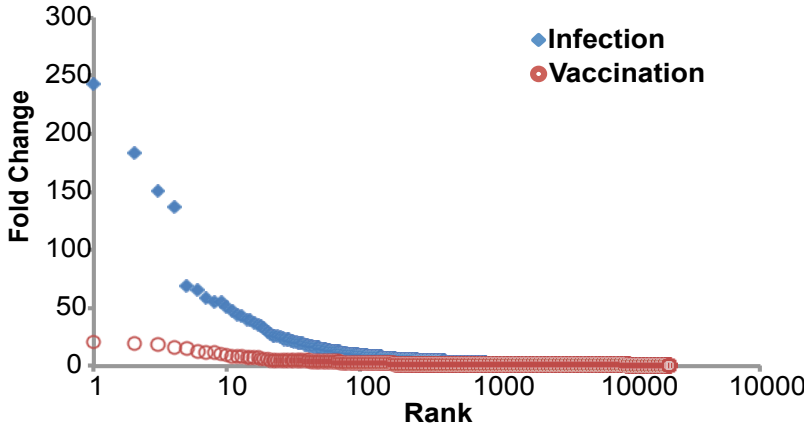

200
150
100
50
0
-50
-100
Transcripts with significant changes (n)
Increased abundance post-vaccination
Decreased abundance post-vaccination
2
5
6
8
9
12
14
20
29
42
180
Days post-vaccination


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

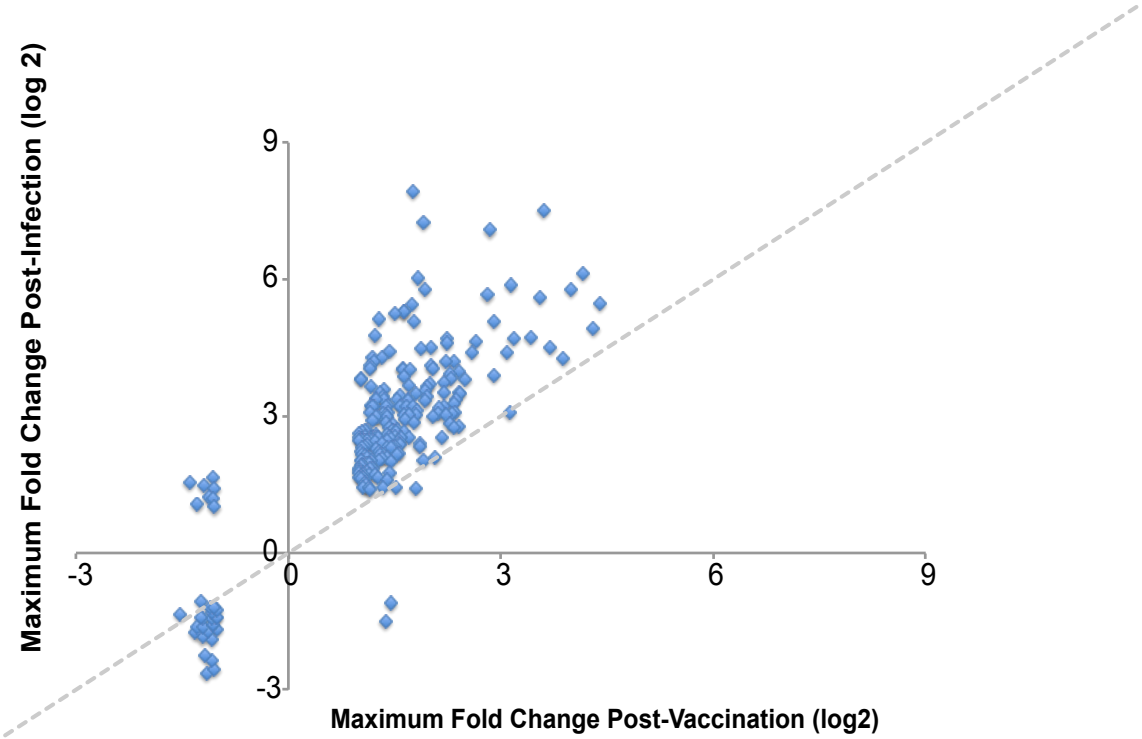
A.

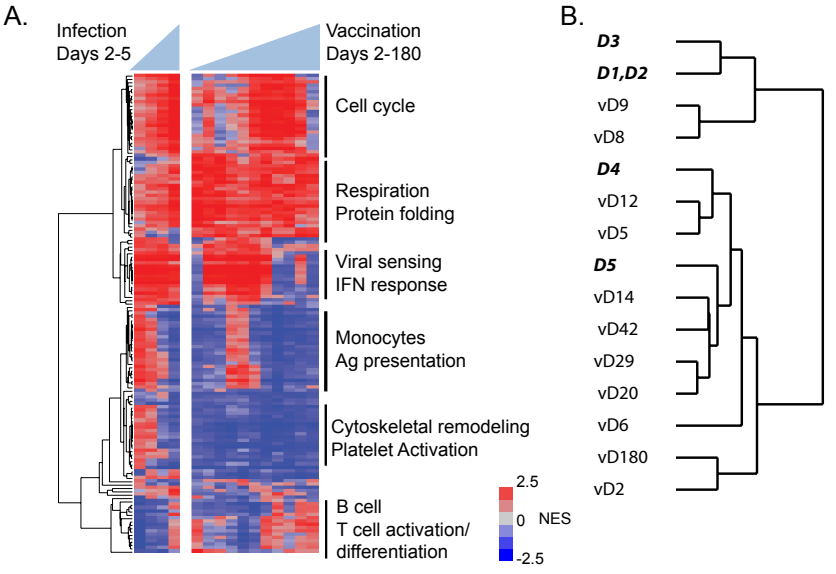

2932
250
28

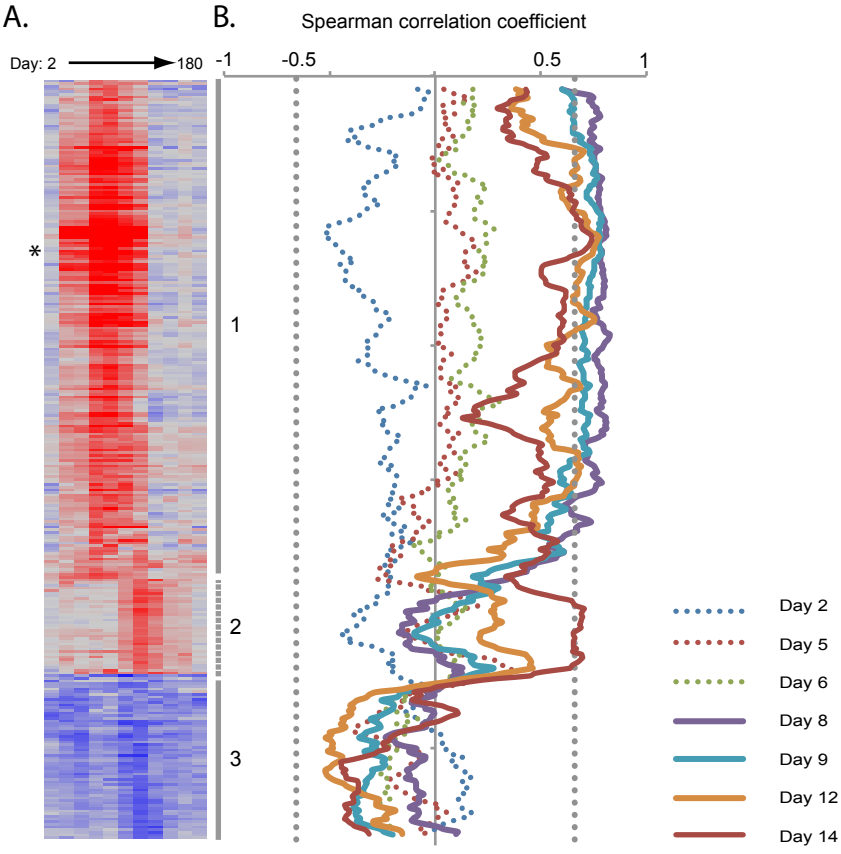
B.

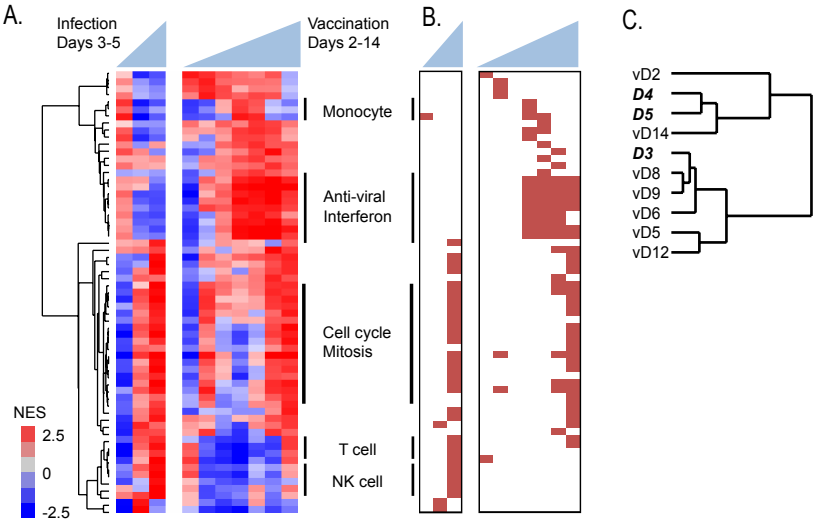

Infection
Vaccination
Fold Change
300
250
200
150
100
50
0
1
10
100
1000
10000
10000
Rank

C.


Maximum Fold Change Post-Infection (log 2)
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