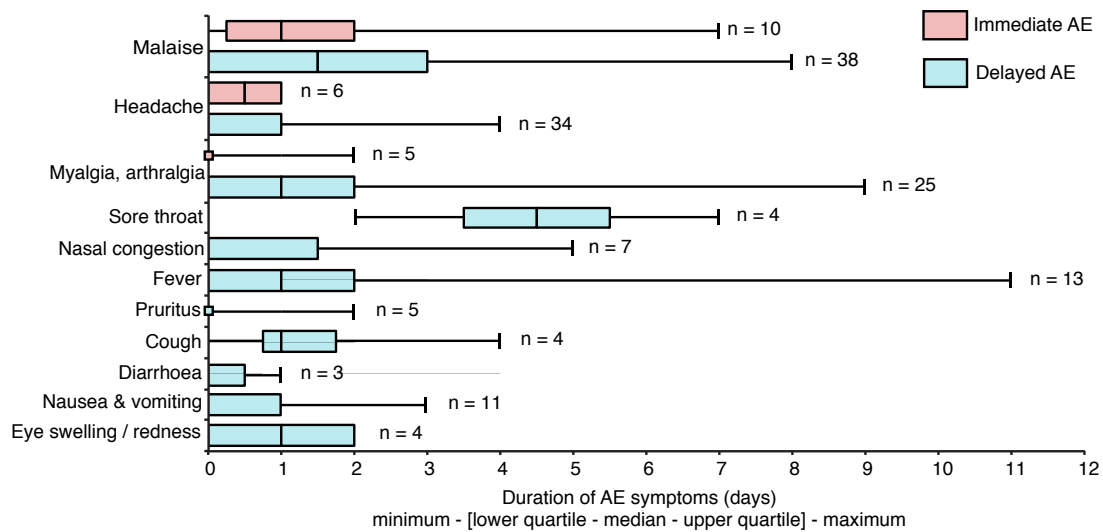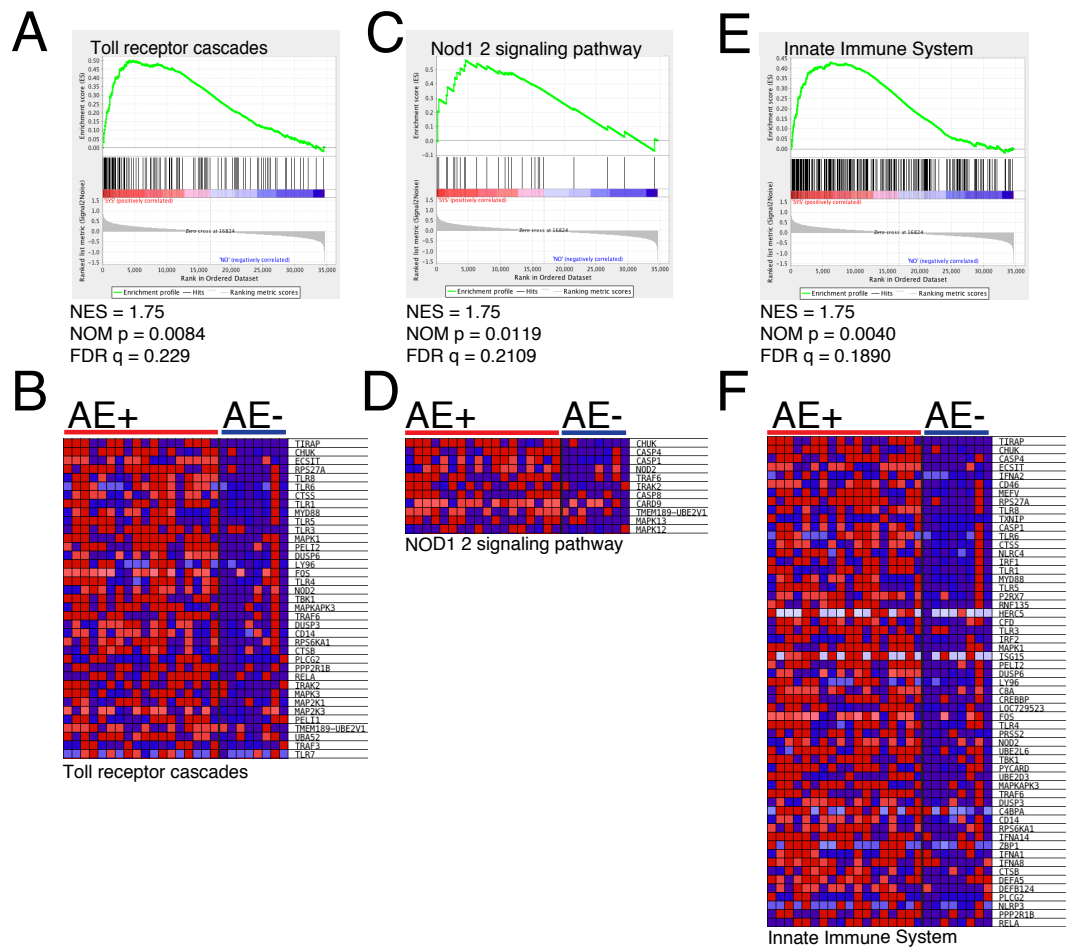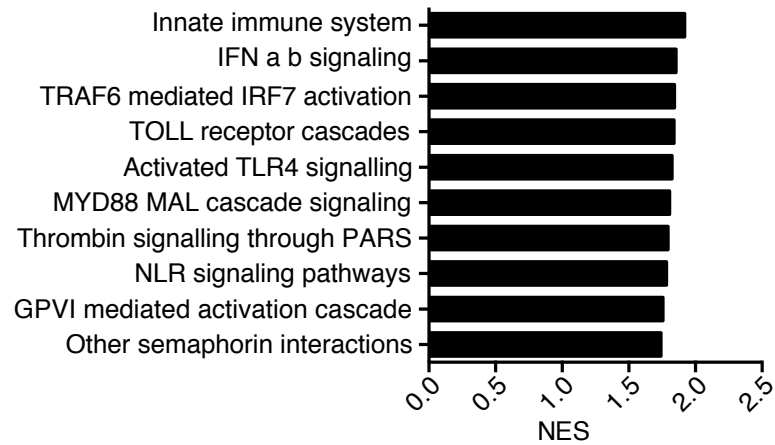1 **Supplementary Figures**

2

3 **Supplementary Figure 1. Duration of AEs.**

4 Box-and-whisker plot showing the duration of AEs following YF vaccination. Red bars

5 represent immediate AEs that $\leq$ 24 hours post-YF vaccination. Blue bars represent

6 delayed AEs that > 24 hours post-YF vaccination. The line within the box indicates the

7 median, the end of the box shows the 25th and 75th percentile. Ends of the whiskers are

8 minimum and maximum. Only events reported more than once are shown. *n* = number

9 of AEs.

A Toll receptor cascades

NES = 1.75
NOM p = 0.0084
FDR q = 0.229

C Nod1 2 signaling pathway

NES = 1.75
NOM p = 0.0119
FDR q = 0.2109

E Innate Immune System

NES = 1.75
NOM p = 0.0040
FDR q = 0.1890

B AE+ AE−

Toll receptor cascades

D AE+ AE−

NOD1 2 signaling pathway

F AE+ AE−

Innate Immune System

Supplementary Figure 2. Upregulation of innate immune response related genes in subjects with systemic AEs. GSEA analysis of AEs following YF vaccination from venous blood microarray data in 18 subjects with delayed systemic AE (AE+) compared to 8 subjects without AE (AE−) at day 1 post-YF vaccination. Enrichment plots (**A, C, E**) and blue-pink o'grams (**B, D, F**) for the leading-edge subset of genes of these top 3 gene-sets by normalized enrichment scores (NES): toll receptor cascades, NOD 1 2 signaling pathway, and innate immune system. FDR q-values and nominal (NOM) p-values are shown.

19

Supplementary Figure 3. Top 10 enriched gene-sets from venous blood microarray

data in subjects with only delayed AEs (n = 12) compared with subjects without AEs

(n = 8), ranked by normalized enrichment scores (NES), with FDR q-values < 0.25 on

day 1 post-YF vaccination.

24

Supplementary Figure 4. Comparison of innate immune signaling genes in subjects with probable, possible or without systemic AEs.

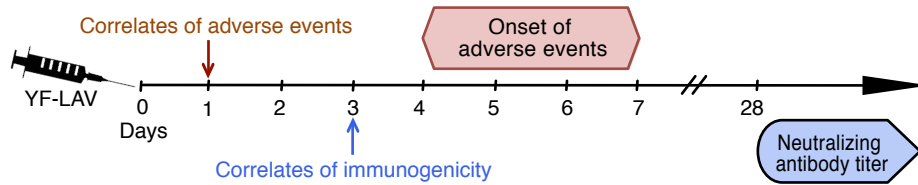(**A**) Top enriched gene-sets from venous blood microarray data in subjects with delayed probable systemic AEs ($n = 7$) compared with subjects without AEs ($n = 8$), ranked by normalized enrichment scores (NES), with FDR q-values < 0.25 on day 1 post-YFLAV. (**B**) Top 10 enriched gene-sets from venous blood microarray data ranked by NES at day 1 post-YF vaccination in subjects with only delayed possible ($n = 11$), or no AEs ($n = 8$). Dotted line represents the cut-off FDR q-value of 0.25. (**C**) **Heatmap** of microarray data showing fold-changes observed at day 1 versus day 0 for genes in the toll receptor cascades, IFN and interleukins (ILS) signaling in subjects with probable ($n = 7$), possible ($n = 11$), and without systemic AEs ($n = 8$) post-YFLAV.
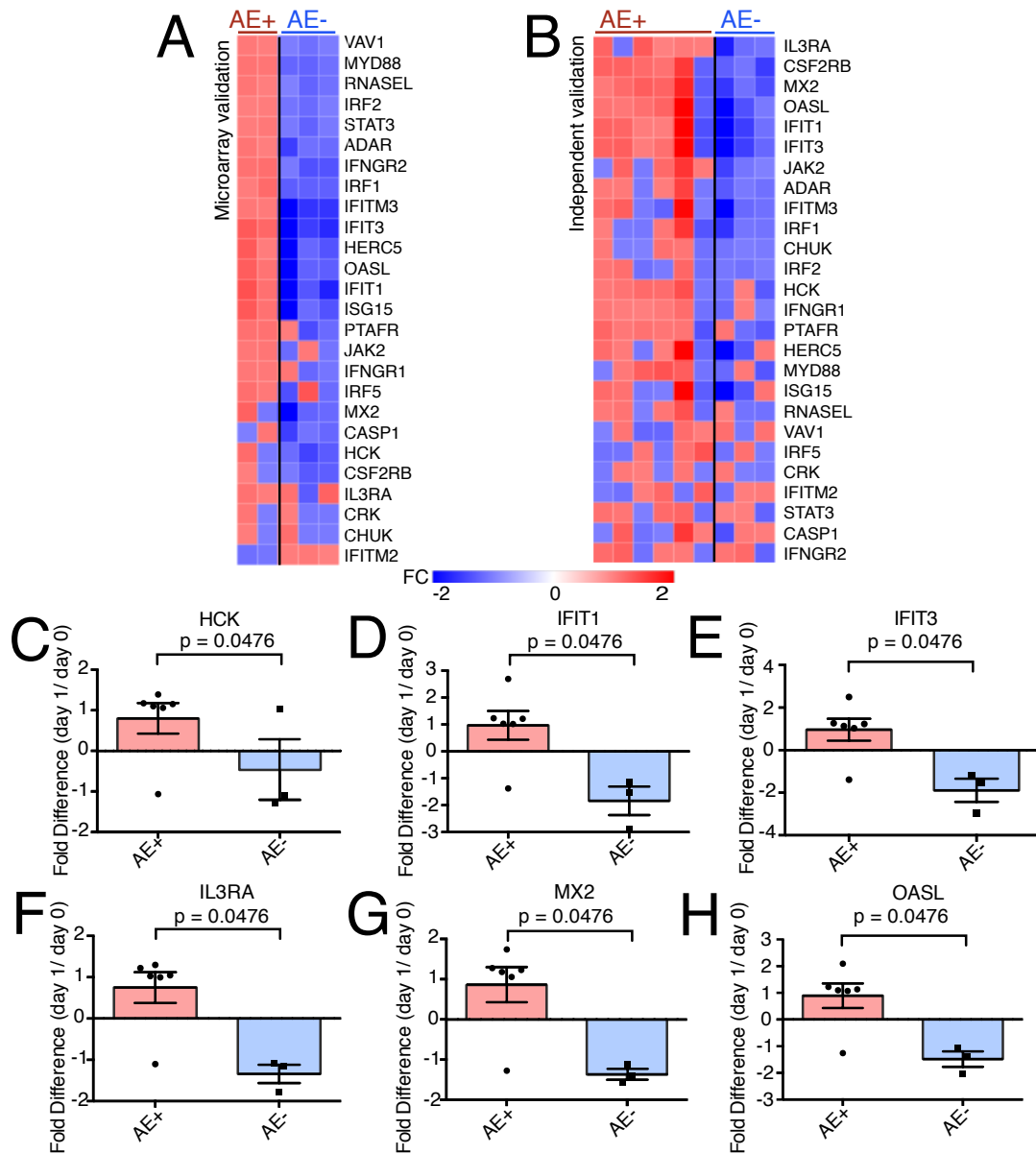
Supplementary Figure 5. NCounter validation of the microarray results. Heatmap of fold change (day 1 versus day 0) for (**A**) 2 subjects with AE (AE+) and 3 without AE (AE-), who were included in the microarray analysis and (**B**) independent validation for 6 subjects with AE (AE+) and 3 without (AE-) following vaccination. From independent validation, genes that have $p<0.05$ between the AE+ (red) and AE- (blue) groups were: (**C**) *HCK*, (**D**) *IFIT1*, (**E**) *IFIT3*, (**F**) *IL3RA*, (**G**) *MX2*, and (**H**) *OASL*. P-value: Unpaired Mann-Whitney U-test. Error bars: mean ± SEM.

Supplementary Figure 6. Expression of innate immune response related genes on day 3 versus day 0 post-YF vaccination. (**A**) Heatmap of the microarray data showing fold-changes observed at day 3 versus day 0 for genes in the toll receptor cascades, IFN and interleukins (ILS) signaling in subjects with delayed AEs (AE+) (*n* = 18) or without AEs (AE-) (*n* = 8). (**B**) Top 10 enriched gene-sets from venous blood microarray data ranked by NES at day 3 post-YF vaccination in subjects with only delayed AEs (*n* = 12) or no AEs (*n* = 8). Dotted line represents the cut-off FDR q-value of 0.25. (**C, D**) Blue-pink o'grams representing the leading-edge subset of genes of these top 2 gene-sets ranked by normalized enrichment scores (NES) shown in (**B**).

56

Supplementary Figure 7. Proposed molecular processes leading to adverse events and immunogenicity following YFLAV administration. Following YF vaccination, up-regulation of innate immune genes may mediate both YF vaccine-associated AEs and immunogenicity. However, the timings of activation of these pathways are temporally separated. Early activation on day 1 is associated with the development of systemic AEs (median day 6, interquartile range days 4–7), while responses at day 3 shape the magnitude, quality, and durability of the neutralizing antibody response (immunogenicity).