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Supplementary Materials for

Quantitative phosphoproteomic analysis identifies the critical role of JNK1 in neuroinflammation induced by Japanese encephalitis virus

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencemagsignaling.org/cgi/content/full/9/448/ra98/DC1)

Table S7 (Microsoft Excel format). Prediction of JEV-responsive kinases by using the GPS.
Table S8 (Microsoft Excel format). Overrepresented motifs in the up-regulated phosphoproteins in JEV-infected cells.

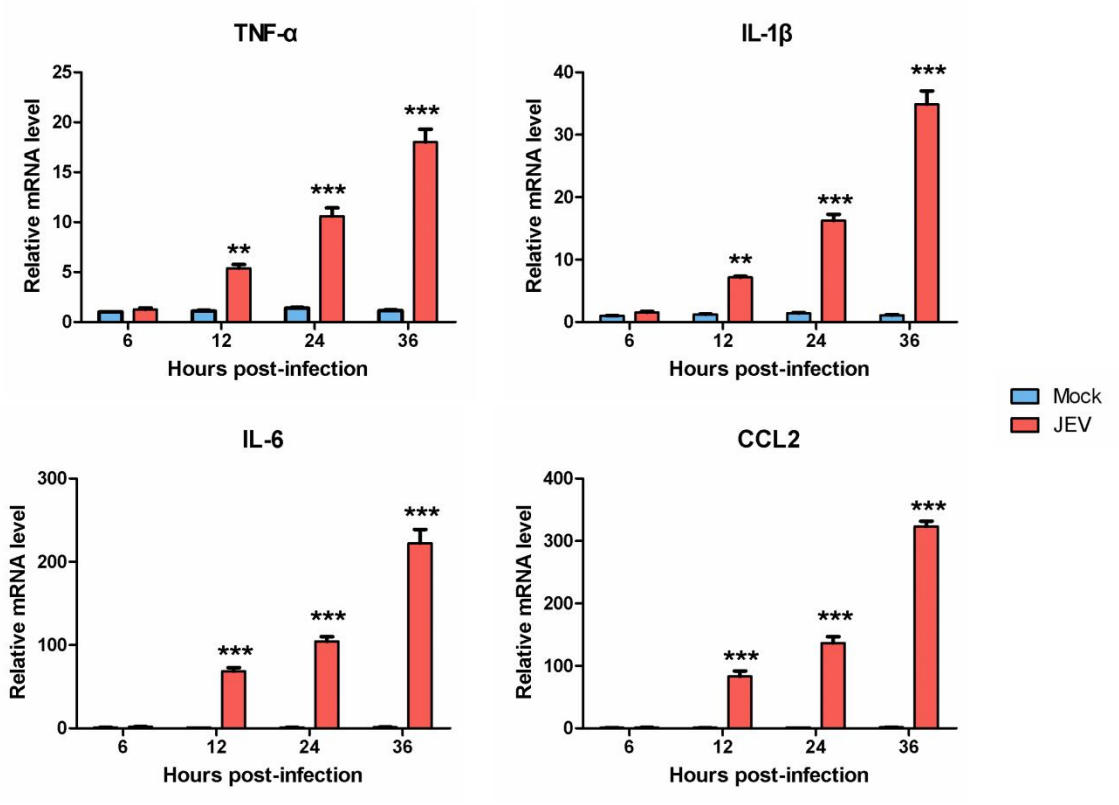

Figure S1. Expression of inflammatory cytokines in JEV-infected U251 cells. U251 cells were mock-infectd or infected with JEV at 5 MOI. The cells were harvested at 6, 12, 24 and 36 hours post-infection, respectively, and the mRNA were extracted. The relative mRNA levels of TNF-α, IL-1β, IL-6 and CCL2 were measured by RT-qPCR. Data were expressed as means ± SEM from three independent experiments. ** $p < 0.01$, *** $p < 0.001$.

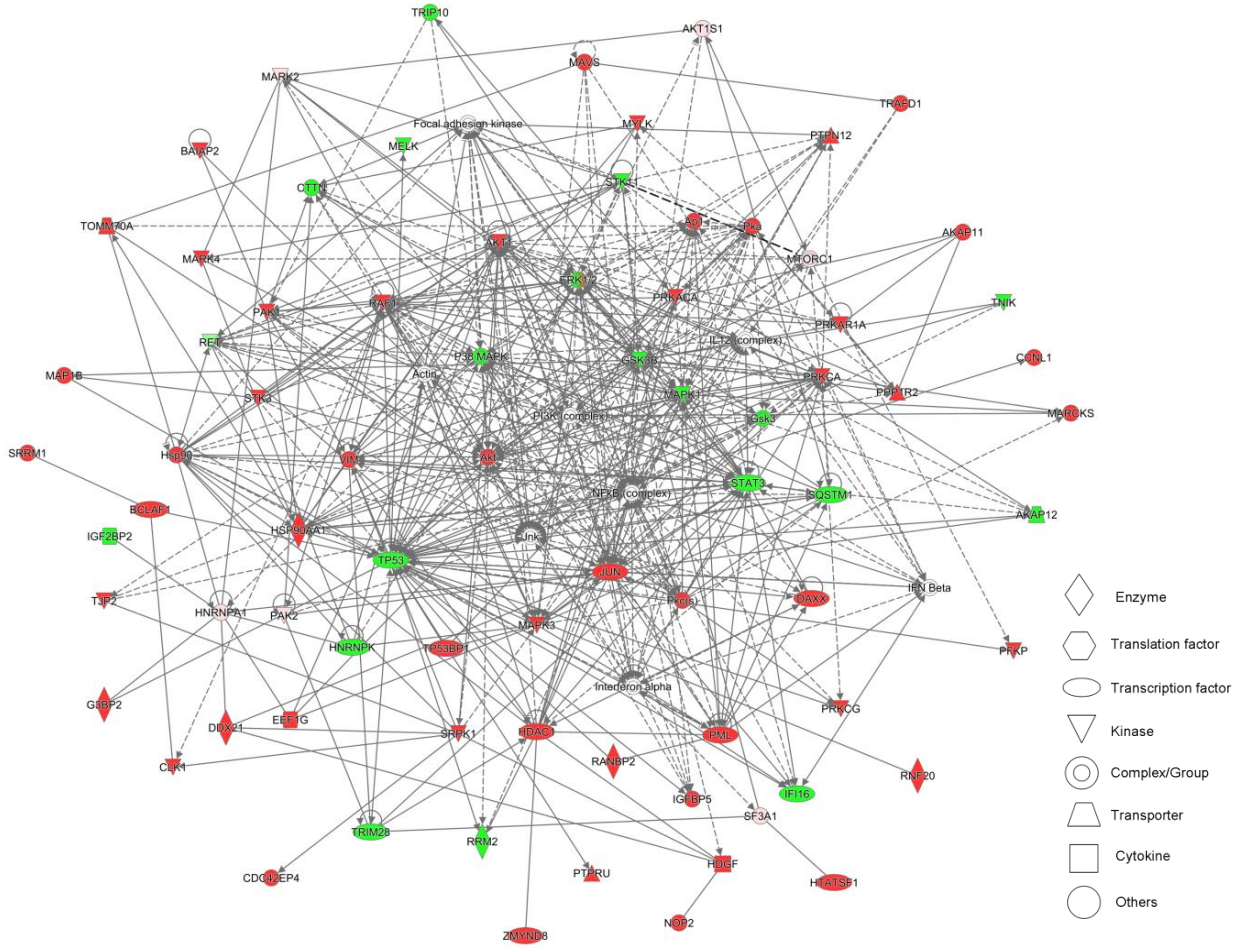

Figure S2. Protein-protein interaction network constructed from the phosphoproteins regulated by JEV infection of U251 cells. Functional or physical interactions between JEV-responsive phosphoproteins were graphically illustrated using Ingenuity Pathway Analysis (IPA, version 01-04). Up-regulated proteins are shown in red; down-regulated proteins are represented in green; proteins that were not identified in this study but involved in the networks are shown in white. The color depth indicates the magnitude of the change in protein expression. See table S5 for the identities of the proteins, and molecular function as defined by IPA.

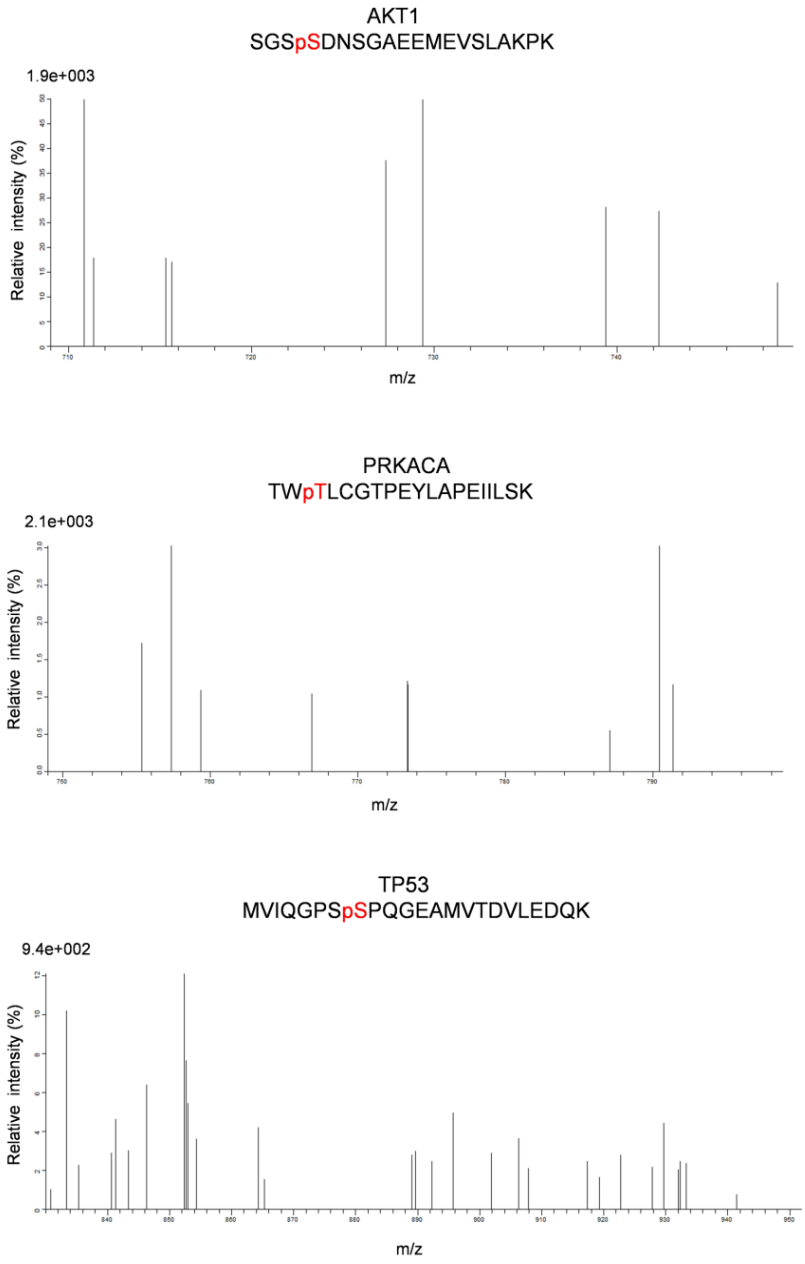

Figure S3. Representative MS spectra of phosphorylated peptides of AKT1, PRKACA, and TP53.

Table S1. Quantification of phosphopeptides detected by LC-MS/MS. If the phosphopeptide was not detected in the control (Con) or P3 group, it was assigned a value=0 for that sample, and the P3/Con ratio was defined as 9999 or 0.0001, respectively.

Table S2. Differentially regulated phosphopeptides in response to JEV infection. Both the up-regulated phosphopeptides and the down-regulated phosphopeptides are included. Protein names and gene symbols are from UniProt.

Table S3. GO and KEGG pathway analysis of the up-regulated phosphoproteome of JEV-infected U251 cells. Biological process assigned from GO, cellular component from GO, molecular function from GO, and pathways assigned from KEGG.

Table S4. GO and KEGG pathway analysis of the down-regulated phosphoproteome of JEV-infected U251 cells. Biological process assigned from GO, cellular component from GO, molecular function from GO, and pathways assigned from KEGG.

Table S5. Phosphoproteins in the interaction network of JEV-infected U251 cells. Information is derived from the protein interaction network as constructed by IPA.

Table S6. Differentially regulated phosphoproteins that are shared between cells infected with JEV or WNV. Both the up-regulated phosphopeptides and the down-regulated phosphopeptides are included. Protein names and gene symbols are from UniProt. Molecular function is defined according to DAVID (version 6.7, http://david.abcc.ncifcrf.gov/).

Table S7. Prediction of JEV-responsive kinases by using the GPS. Both the up-regulated phosphopeptides and the down-regulated phosphopeptides are included. Protein names are from UniProt. Kinase identification includes the major family and subgroups with the last term in the sequence representing the assigned individual kinase. The threshold for assigning to a kinase was a peptide score above 3.0.

Table S8. Overrepresented motifs in the up-regulated phosphoproteins in JEV-infected cells. The "motif score" is calculated by taking the sum of the negative log probabilities used to fix each position of the motif. As such, higher motif scores typically correspond to motifs that are more statistically significant, as well as more specific (that is have a greater number of fixed positions). The "foreground matches" and "background matches" statistics indicate the number of peptides containing a given motif in those respective data sets following the removal of all peptides containing previously extracted motifs. Because of this iterative "set reduction" strategy, the "foreground matches and "background matches" statistics may be less than or equal to the total number of instances of a given motif in the whole data set.