

**iScience, Volume 24**

**Supplemental information**

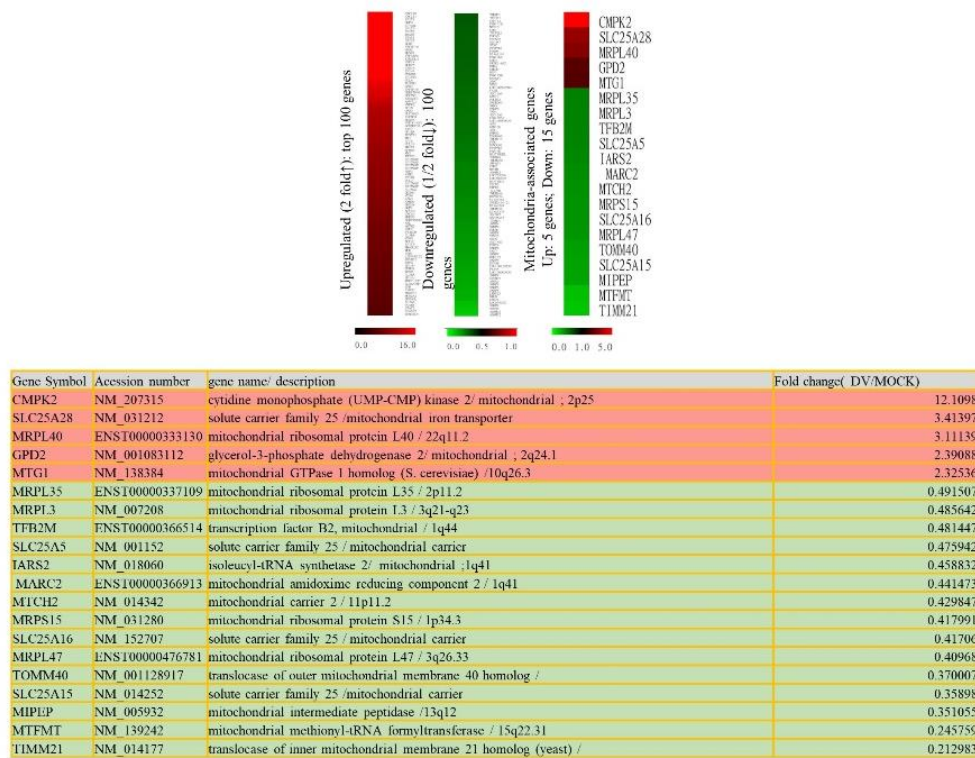
**Mitochondrial CMPK2 mediates immunomodulatory  
and antiviral activities through IFN-dependent  
and IFN-independent pathways**

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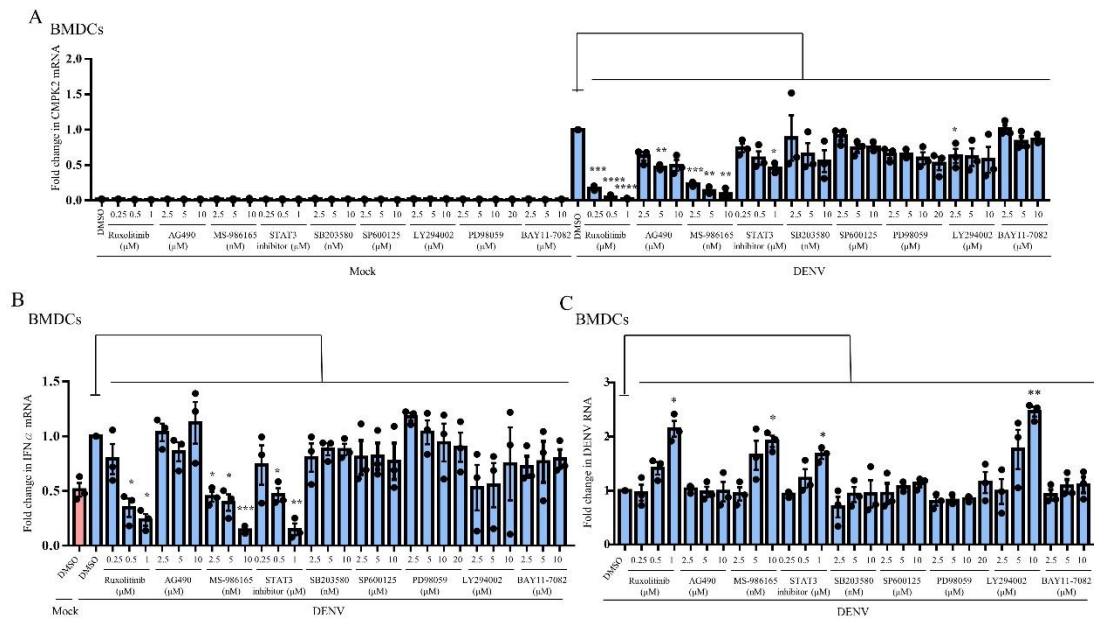
## **Supplemental information**

**Table S1. Summary of primer sequences used in the study. Related to Figure 1, 2, 3, 4, 6, 7 and Figure S1, S5, S6.**

<b>Gene name (<i>Homo sapiens</i>)</b>	<b>Accession number</b>	<b>Forward</b>	<b>Reverse</b>
CMPK2	NM_207315.4	AGGTGAAGGTCGGAGTCAAC	CCATGTAGTTGAGGTCAATG AAGG
GAPDH	NM_001289746.1	AGGTGAAGGTCGGAGTCAAC	CCATGTAGTTGAGGTCAATGAAGG
TLR9	NM_017442.3	ACTTCTTCCAAGGCCTGAGC	GGCCAGGTAATTGTCACGGA
IFN- $\alpha$	NM_024013.3	TGGAAGCCTGTGTGA T	ATGATTTCTGCTCTGACA
IFN- $\lambda$ 1	NM_172140.1	GAGGCCCCCAAAAAGGAGTC	AGGTTCCCATCGGCCACATA
mtDNA-16S	KY399206.1	TAACCCAAGTCAATAGAAGCC	CTAGAGGGATATGAAGCACC
B2M	ENSG00000166710	CTCACGTCATCCAGCAGAGA	CGGCAGGCATACTCATCTTT
mtDNA-ND5	KY399206.1	TTCATCCCTGTAGCATTGTTTCG	GTTGGAATAGGTTGTTAGCGGTA
TERT	NM_198253.3	CTTCCTCTACTCCTCAGGCG	CAAGCAGCTCCAGAAACAGG
<b>(<i>Mus musculus</i>)</b>	<b>Accession number</b>	<b>Forward</b>	<b>Reverse</b>
CMPK2	NM_020557.4	GGAACCTCATCTGCACCCAT	GTGGTCTTACCAGTGGCATCC
GAPDH	NM_001289726.1	TGGTGAAGGTCGGTGTGAAC	CCATGTAGTTGAGGTCAATGAAGG
TLR9	NM_031178.2	GAATCCTCCATCTCCCAACAT	CCAGAGTCTCAGCCAGCACT
IFN- $\alpha$	NM_010503.2	AAGGACAGGCAGGACTTTGGATTC	GATCTCGCAGCACAGGGATGG
IFN- $\lambda$ 2/ $\lambda$ 3	NM_001024673.2; NM_177396.1	AGCTGCAGGTCCAAGAGCG	TGGGAGTGAATGTGGCTCAG
TNF- $\alpha$	NM_013693.3	CTGAACTTCGGGGTGATCGG	GGGAGTAGACAAGGTACAACCC
12S	KY018919.1	ACCGCGGTCATACGATTAAC	CCCAGTTTGGGTCTTAGCTG
Actin	NC_000071.6	AAAGCCGTAT TAGGTCCATCTTGA	GGCCATTGAGGCGTGATC
D-loop	KY018919.1	GCCCATGACCAACATAACTG	CCTTGACGGCTATGTTGATG
Albumin	NC_000071.6	TGAAACATATGTCCCAAAAGAGTTT	TTCTCCTTCTCTGGAAGTGTGCAGAT
CCR7	NM_007719.2	AAAGCACAGCCTTCCTGTGT	AGTCCACCGTGGTATTCTCG
<b>Others</b>			
DENV2	KY586699.1	CTCTCAGTGAAGTCCCGGAGACC	CGTACCATAGGAGGATGCTAGCCG
Flag		GAAAAGTGCCACCTGACGC	GCCCCGATTTAGAGCTTGA
Lenti-pLKO_AS3w.puro-mCmpk2-DYK		ATGACGTACCAGCGTATGGAGAA	TCCTTGTAATCACGACCAGAAGCTC

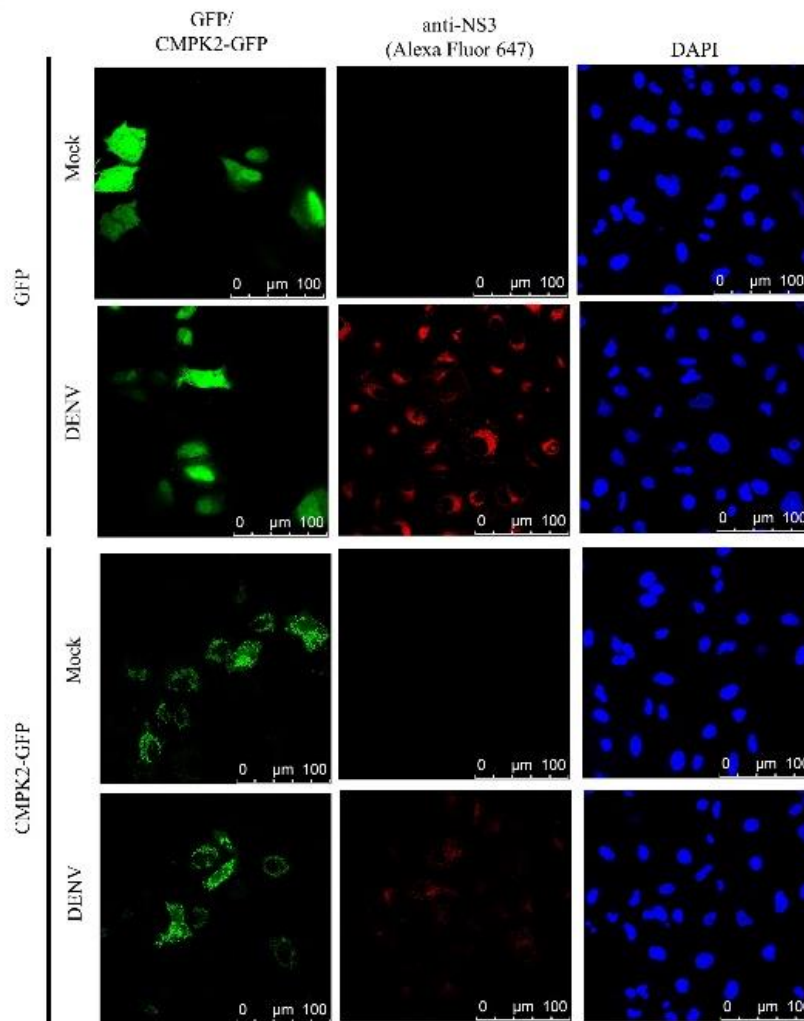


**Figure S1. Genes regulated in human dendritic cells infected by DENV. Related to Figure 1.** Human dendritic cells (DCs) were infected with DENV (MOI=5) or mock infected for 24 h, and microarray analysis was conducted. Among the 20 listed mitochondria-associated genes, CMPK2 was the one most highly induced by DENV infection.

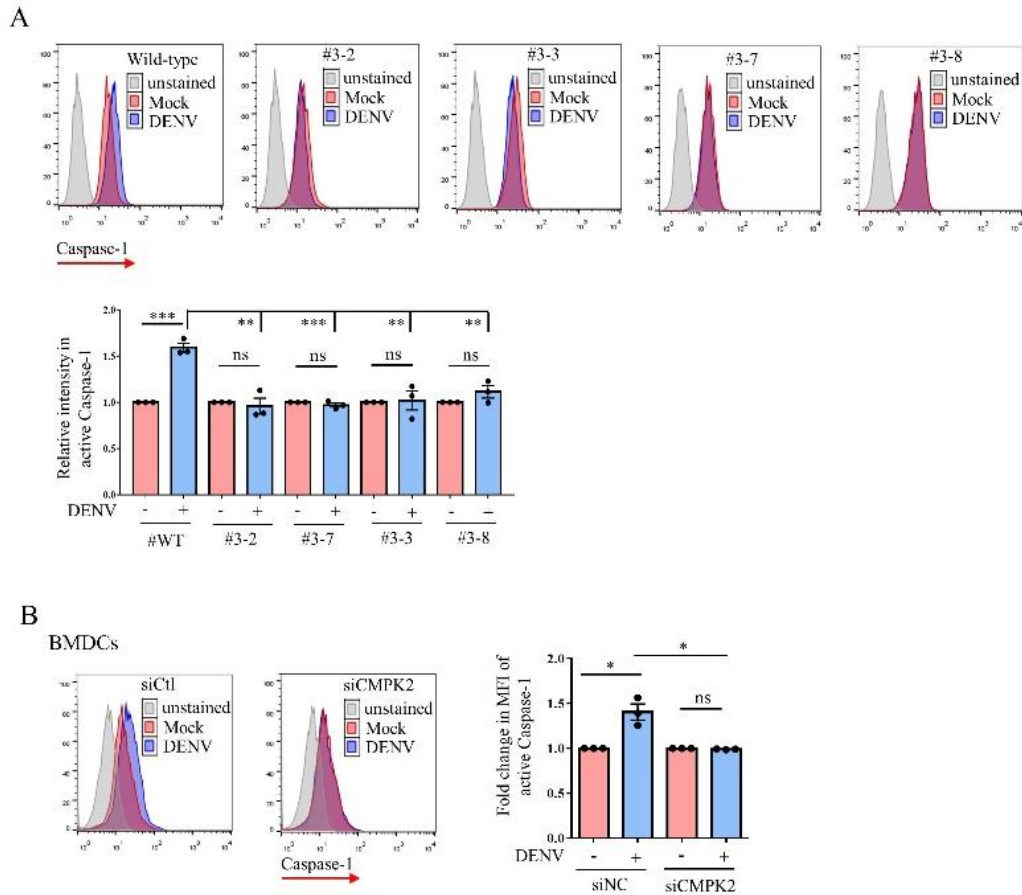


**Figure S2. Potential signaling involved in DENV-induced CMPK2. Related to Figure 1.** BMDCs were pretreated with several chemical compounds at different doses and infected with DENV (MOI=1), and the mRNA expression of CMPK2 was measured by qPCR (A). The expression of IFN- $\alpha$  mRNA and DENV RNA was measured by qPCR and is shown in (B) and (C), respectively. Values represent the mean of the individual measurements in each sample  $\pm$  SEM. \*P < 0.05, and \*\*P < 0.01. P-value was calculated by the Student's T-test.

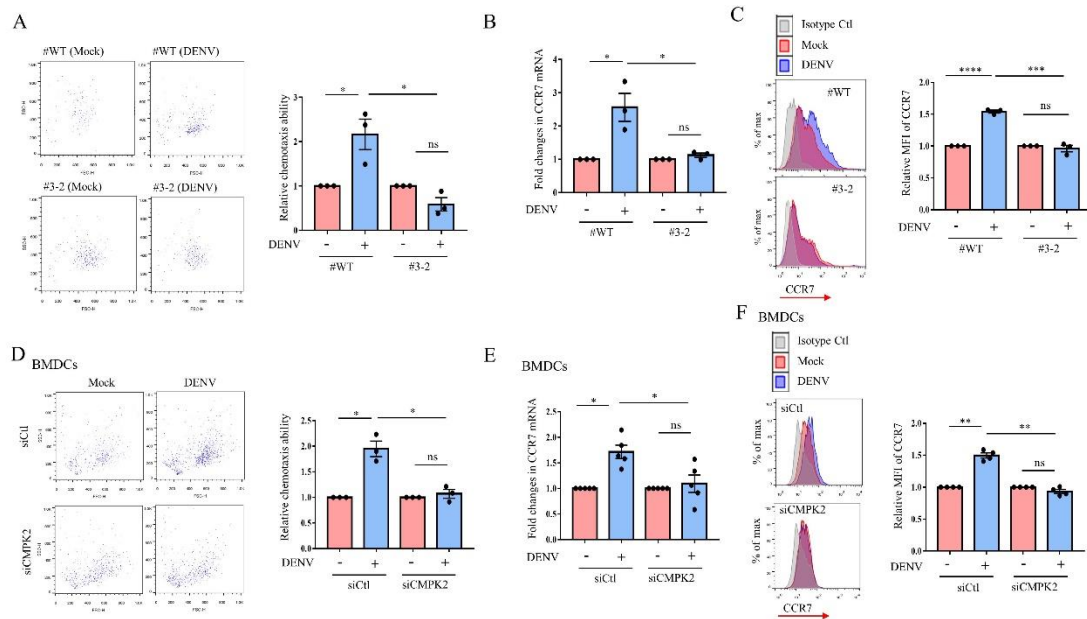
A549 cells



**Figure S3. Overexpression of CMPK2 inhibited viral production. Related to Figure 2.** A549 cells were transfected with CMPK2-GFP or a GFP control and then infected with DENV, and the expression of CMPK2 and viral NS3 was measured by confocal microscopy. DAPI staining was also included. Representative results from more than 3 independent experiments are shown.



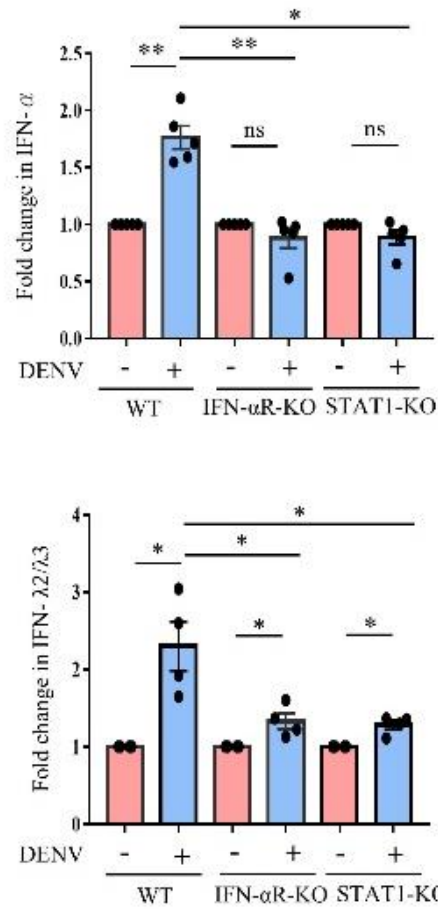
**Figure S4. Effects of CMPK2 KO or KD on DENV infection-induced caspase 1 activity. Related to Figure 5.** THP-1 cells and THP-1 CMPK2-KO clones were infected with DENV (MOI=5), and the levels of active caspase1 were determined by flow cytometry (A). Similarly, the activity of active caspase1 in mock- or DENV-infected BMDCs with or without CMPK2 KD was measured by flow cytometry (B). Values represent the mean of the individual measurements in each sample  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ . P-value was calculated by the Student's T-test.



**Figure S5. Effects of CMPK2 KO or KD on DENV infection-induced cell migration. Related to Figure 3.** THP-1 cells and THP-1 CMPK2-KO clone #3-2 were infected with DENV (MOI=5), and a chemotaxis assay was carried out (A). CCR7 mRNA expression was evaluated by qPCR (B), and CCR7 protein expression was assessed by flow cytometry (C). In parallel, the effects of CMPK2 knockdown on DENV infection (MOI=1)-induced BMDC migration were examined, and chemotaxis (D), CCR7 mRNA expression (E) and CCR7 protein expression (F) were measured by flow cytometry and are shown individually. Values represent the mean of the individual measurements in each sample  $\pm$  SEM. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and \*\*\*\*P < 0.0001. P-value was calculated by the Student's T-test.

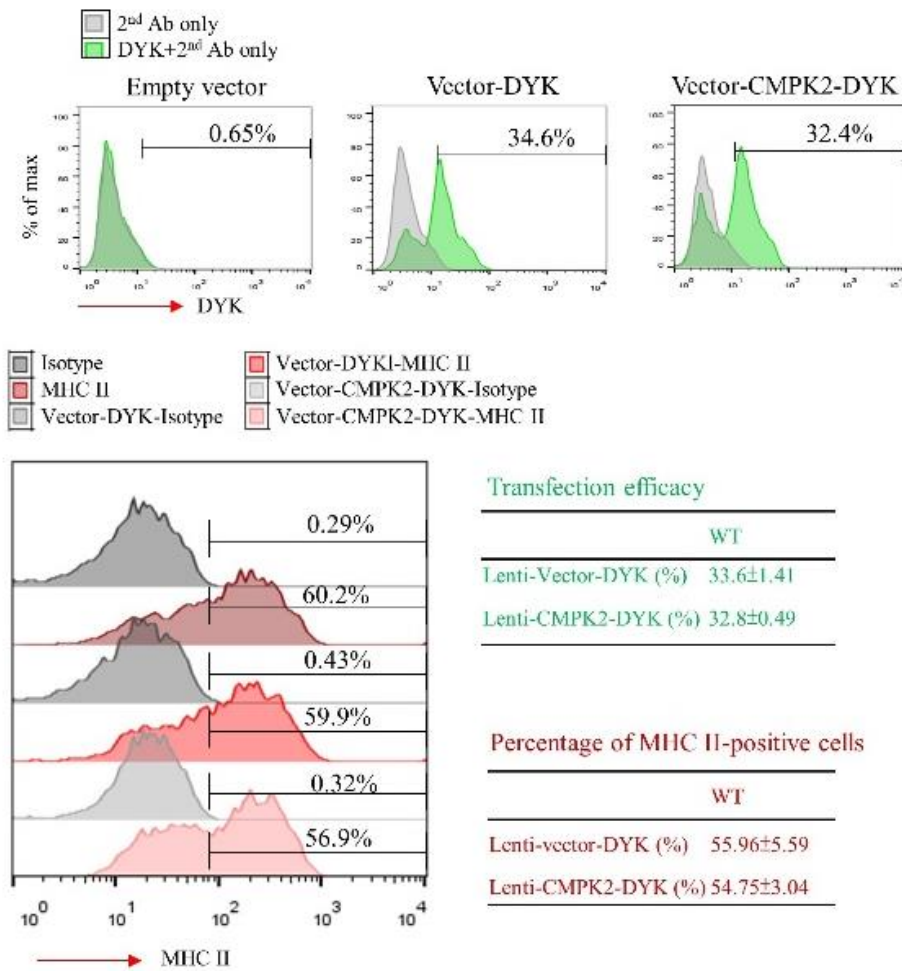


BMDCs

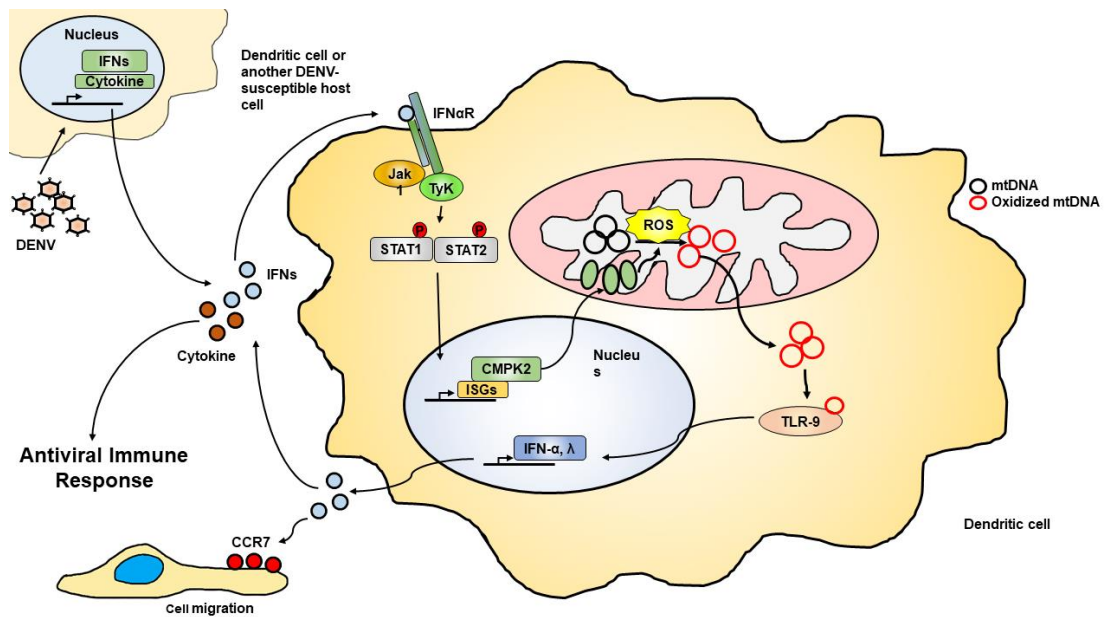


**Figure S6. The effects of IFN- $\alpha$  receptor or STAT1-KO on DENV-induced IFNs mRNA expression. Related to Figure 6.** BMDCs were prepared from mice with KO of IFN- $\alpha$ R or STAT1 or control mice. Cells were then infected with DENV (MOI=1), and the expression of IFNs mRNA was measured by qPCR. Values represent the mean of the individual measurements in each sample  $\pm$  SEM. \*P < 0.05, \*\*P < 0.01, and \*\*\*P < 0.001. P-value was calculated by the Student's T-test.

BMDCs



**Figure S7. Overexpression of CMPK2 did not affect MHC II expression of BMDCs. Related to Figure 7.** BMDCs were treated as described in Figure 7D. The percentages of MHC II-positive cells after transfection with DYK or CMPK2-DYK were measured by flow cytometry. The results from two independent experiments were shown.



**Figure S8. CMPK2 is extensively involved in the DENV infection-mediated immune response. Related to Figures 1-7.** DENV infection induces the production of type I and type III IFNs, which are antiviral cytokines. Through the JAK2/TyK2-STAT1 signaling pathway, CMPK2 is induced, and subsequently, it moves to and localizes in the mitochondria. Then, a series of events, including the activation of mtROS production and release of oxidized mtDNA into the cytosol, occur. Binding of mtDNA to TLR9 as well as the associated immune responses then cause several events, such as the induction of IFN release, increased expression of CCR7 mediating cell migration and suppression of viral replication. Both autocrine and paracrine effects were likely to be present.