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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.

Gene name (Homo sapiens)	Accession number	Forward	Reverse	
CMPK2	NM_207315.4	AGGTGAAGGTCGGAGTCAAC	CCATGTAGTTGAGGTCAATG AAGG	
GAPDH	NM_001289746.1	AGGTGAAGGTCGGAGTCAAC	CCATGTAGTTGAGGTCAATGAAGG	
TLR9	NM_017442.3	ACTTCTTCCAAGGCCTGAGC	GGCCAGGTAATTGTCACGGA	
IFN-α	NM_024013.3	TGGAAGCCTGTGTGA T	ATGATTTCTGCTCTGACA	
IFN-λ1	NM_172140.1	GAGGCCCCCAAAAAGGAGTC	AGGTTCCCATCGGCCACATA	
mtDNA-16S	KY399206.1	TAACCCAAGTCAATAGAAGCC	CTAGAGGGATATGAAGCACC	
B2M	ENSG00000166710	CTCACGTCATCCAGCAGAGA	CGGCAGGCATACTCATCTTT	
mtDNA-ND5	KY399206.1	TTCATCCCTGTAGCATTGTTCG	GTTGGAATAGGTTGTTAGCGGTA	
TERT	NM_198253.3	CTTCCTCTACTCCTCAGGCG	CAAGCAGCTCCAGAAACAGG	
(Mus musculus)	Accession number	Forward	Reverse	
CMPK2	NM_020557.4	GGAACCTCATCTGCACCCAT	GTGGTCTTACCAGTGGCATCC	
GAPDH	NM_001289726.1	TGGTGAAGGTCGGTGTGAAC	CCATGTAGTTGAGGTCAATGAAGG	
TLR9	NM_031178.2	GAATCCTCCATCTCCCAACAT	CCAGAGTCTCAGCCAGCACT	
IFN-α	NM_010503.2	AAGGACAGGCAGGACTTTGGATTC	GATCTCGCAGCACAGGGATGG	
IFN-λ2/λ3	NM_001024673.2; NM_177396.1	AGCTGCAGGTCCAAGAGCG	TGGGAGTGAATGTGGCTCAG	
TNF-α	NM_013693.3	CTGAACTTCGGGGGTGATCGG	GGGAGTAGACAAGGTACAACCC	
12S	KY018919.1	ACCGCGGTCATACGATTAAC	CCCAGTTTGGGTCTTAGCTG	
Actin	NC_000071.6	AAAGCCGTAT TAGGTCCATCTTGA	GGCCATTGAGGCGTGATC	
D-loop	KY018919.1	GCCCATGACCAACATAACTG	CCTTGACGGCTATGTTGATG	
Albumin	NC_000071.6	TGAAACATATGTCCCCAAAGAGTTT	TTCTCCTTCTCTGGAAGTGTGCAGA T	
CCR7	NM_007719.2	AAAGCACAGCCTTCCTGTGT	AGTCCACCGTGGTATTCTCG	
Others				
DENV2	KY586699.1	CTCTCAGTGAACTGCCGGAGACC	CGTACCATAGGAGGATGCTAGCCG	
Flag		GAAAAGTGCCACCTGACGC	GCCCCCGATTTAGAGCTTGA	
Lenti-				
pLKO_AS3w				
.puro-		ATGACGTACCAGCGTATGGAGAA	TCCTTGTAATCACGACCAGAACTC	
mCmpk2-				
DYK				

		Upregulated (2 fold): top 100 genes Upregulated (2 fold): top 100 genes MELTAD MELT	
Gene Symbol	Acession number	gene name/ description	Fold change(DV/MOCK)
CMPK2	NM_207315	cytidine monophosphate (UMP-CMP) kinase 2/ mitochondrial ; 2p25	12.1098
SLC25A28	NM_031212	solute carrier family 25 /mitochondrial iron transporter	3.41397
MRPL40	ENST00000333130	mitochondrial ribosomal protein L40 / 22q11.2	3.11139
GPD2	NM_001083112	glycerol-3-phosphate dehydrogenase 2/ mitochondrial ; 2q24.1	2.39088
MTG1	NM_138384	mitochondrial GTPase I homolog (S. cerevisiae) /10q26.3	2.32536
MRPL35	ENST00000337109	mitochondrial ribosomal protein L35 / 2p11.2	0.491507
MRPL3	NM_007208	mitochondrial ribosomal protein I.3 / 3q21-q23	0.485642
TFB2M	ENST00000366514	transcription factor B2, mitochondrial / 1q44	0.481447
SLC25A5	NM 001152	solute carrier family 25 / mitochondrial carrier	0.475942
IARS2	NM_018060	isoleucyl-tRNA synthetase 2/ mitochondrial ;1q41	0.458832
MARC2	ENS100000366913	mitochondrial amidoxime reducing component 2 / 1q41	0.441473
MTCH2	NM 014342	mitochondrial carrier 2 / 11p11.2	0.429847
MRPS15	NM_031280	mitochondrial ribosomal protein \$15 / 1p34.3	0.417991
SLC25A16	NM 152707	solute carrier family 25 / mitochondrial carrier	0.41706
MRPL47	ENST00000476781	mitochondrial ribosomal protein L47 / 3q26.33	0.40968
TOMM40	NM_001128917	translocase of outer mitochondrial membrane 40 homolog /	0.370007
SLC25A15	NM_014252	solute carrier family 25 /mitochondrial carrier	0.35898
MIPEP	NM_005932	mitochondrial intermediate peptidase /13q12	0.351055
MTFMT	NM_139242	mitochondrial methionyl-tRNA formyltransferase / 15q22.31	0.245759
TIMM21	NM_014177	translocase of inner mitochondrial membrane 21 homolog (yeast) /	0.212983

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- α 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 ± SEM. *P < 0.05, and **P < 0.01. P-value was calculated by the Student's T-test.



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 DENVinfected 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- α receptor or STAT1-KO on DENV-induced IFNs mRNA expression. Related to Figure 6. BMDCs were prepared from mice with KO of IFN- α 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 ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001. P-value was calculated by the Student's T-test.



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.