Supplementary Information for

# RTP4 inhibits IFN-I response and enhances experimental cerebral malaria and neuropathology

Xiao He, Alison W. Ashbrook, Yang Du, Jian Wu, Hans-Heinrich Hoffmann, Cui Zhang, Lu Xia, Yu-Chih Peng, Keyla C. Tumas, Brajesh K. Singh, Chen-feng Qi, Timothy G. Myers, Carole A. Long, Chengyu Liu, Rongfu Wang, Charles M. Rice, & Xin-zhuan Su

Xin-zhuan Su

Email: xsu@niaid.nih.gov

### This PDF file includes:

Supplementary methods
Figures S1 to S8
Legends for Datasets S1 and S2
SI References

### Other supplementary materials for this manuscript include the following:

Datasets S1 and S2

## Supplementary Methods

**Generation of *Rtp4$^{-/-}$* mice.** *Rtp4$^{-/-}$* mice of C57BL/6 background were generated using the CRISPR/Cas9 method. Two single guide RNAs (sgRNAs) were designed to target the 1st coding exon of the mouse *Rtp4* gene: One (5′-ccccgatgacttcagtacttggg-3′) targeting a sequence shortly after the translation initiation codon (ATG), and the other one (5′-cctcagacccggaatacggatttt-3′) targeting a sequence near the end of Exon 2. The two sgRNA DNA constructs were obtained from OriGenes gRNA Cloning Services (Rockville, MD) and were then used as templates to synthesize sgRNAs using MEGAshortscript T7 Kit (Invitrogen). The two sgRNAs were co-microinjected with Cas9 mRNA into the cytoplasm of fertilized mouse eggs at the concentration of



www.pnas.org/cgi/doi/10.1073/pnas.2006492117

100 μg/ml of Cas9 and 20 μg/ml of each sgRNA. The injected zygotes were cultured overnight in M16 medium. When embryos reached 2-cell stage, they were implanted into the oviducts of pseudopregnant surrogate mothers. Mice born to these foster mothers were genotyped using PCR amplification of the region surrounding the CRISPR cleavage site followed by DNA sequencing. Founder mice with deletions were bred with WT C57BL/6 mice to establish independent *Rtp4* knockout mouse lines. All animal experimental protocols for KO mice were performed in accordance to the NIH Animal Care & Use Committee guidelines.

**Generation of *Rtp4*$^{-/-}$ 293T cell line.** *Rtp4*$^{-/-}$ 293T cells were generated using the CRISPR/Cas9 method in vitro. Four gRNAs (Dataset S2) were designed to target the 1st coding exon shortly after the translation initiation codon (ATG). 239T cells were transfected with lentiCRISPR plasmids containing the sgRNA sequences using calcium phosphate transfection in HEPES buffered saline (Thermo Fisher Scientific). Cells grown out of 2 μg/ml puromycin were genotyped using PCR amplification of the region surrounding the CRISPR cleavage sites (Dataset S2). The PCR products were sequenced to confirm mutations in the *Rtp4* gene. Cells were cloned using limiting dilution. Control 293T cell lines were also generated by transfection of a lentiCRISPR plasmid without the gRNAs.

**Western blot and co-immunoprecipitation (co-IP).** Western blot was performed as described previously (1). Briefly, spleen tissues from infected or uninfected mice at day 1 and day 4 pi or lysates from cultured cells were separated in 4-20% SDS-

polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a PVDF membrane (Roche Diagnostics, Basel, Switzerland), probed with indicated primary antibodies, and detected using chemiluminescent reagents (SuperSignal West Pico Chemiluminescent Substrate; Pierce). Anti-HA (batch #H6908) and anti-Flag (batch # F7425) antibodies were purchased from Sigma; anti-Myc (batch #2272), anti-V5 (batch #13202), anti-RIG-I (batch #3743), anti-MAVS (batch #4983), anti-TBK1 (batch #38066), anti-pTBK1 (batch #5483), anit-IRF3 (batch #4302), anti-pIRF3 (batch #79945), and anti-STAT1 (batch #14994) antibodies were purchased from Cell Signaling Technology; anti-pSTAT1 (batch # ab109461) and anti-pIRF3 (batch # ab76493) antibodies were purchased from Abcam (Cambridge, MA).

**Isolation of microglia.** Microglia from mouse brain was isolated using a method described previously (2). Briefly, freshly perfused mouse brains were placed in 2 mL serum-free high glucose DMEM media (Gibco Catalog number: 11960051) in a petri dish and minced using a scalpel blade. Three mL Dispase II (1.2 U/mL) solution containing 1 mg/mL papain was added to digest the brain tissue. After 20 min, 5 mL 10% FBS in DMEM was added to neutralize the Dispase II solution. Large cell clumps were broken up by pipetting, and the solution was filtered through a 40 μm cell strainer. The filtered cells are separated in 37% and 70% percoll gradient at 300 ×g (18°C) for 40 min. Microglia were collected at the interphase and washed with 1X Hanks' Balanced Salt solution (HBSS). Cell lysates from the isolated microglia were prepared for Western blot analysis and RT-qPCR.

**Immunoprecipitation.** MYC- or HA-tagged protein beads (Thermo Fisher Diagnostics, Waltham, MA) were added into cell lysates (1:10 or 1:20 [vol/vol]) and incubated at room temperature on a shaker for 30 min. After four washes, bound proteins were eluted using protein loading buffer followed by boiling for 10 min before Western blot analysis. In vitro protein translations of RTP4, TBK1, and IRF3 were performed using PURExpress® In Vitro Protein Synthesis Kit (New England BioLabs, Ipswich, MA) according to manufacturer's instructions. Proteins were pulled down and detected as described above.

**Transfection of cell lines and gene expression in vitro.** Plasmids containing target genes obtained from commercial companies (Thermo Fisher Scientific or Origene) were amplified and purified using QIAfilter Plasmid Midi Kit (QIAGEN). 293T cells ($2\times10^5$) were plated in 24-well plates and transfected with plasmids encoding the genes to be studied (200 ng or as indicated in the figures) using Lipofectamine 2000 (Invitrogen) 24 h later. IFN-β (or NF-κB) luciferase reporter (Firefly luciferase; 50 ng) and control reporter (Renilla luciferase; 20 ng) together with plasmids (100 ng) encoding MDA5, RIG-I, MAVS, STING, TRIF or TRAFs were co-transfected in various experiments. Empty pCMV14 vector was used to maintain equal amounts of DNA among wells. For stimulation of IFN-I response, cells were transfected with Poly(I:C), Poly(dA:dT), parasite RNA, or parasite DNA. Luciferase activities were measured at 24 h or at indicated time points after plasmid introduction. Relative reporter gene activity was determined by normalization of the Firefly luciferase activity to Renilla luciferase activity. Alternatively, mRNA levels were measured using qPCR as described below.

**Measurements of IFN-I.** Mouse sera were collected by centrifuging mouse blood at 6000 rpm (~4000 x g) for 5 min. Supernatants were transferred to sterile cryogenic tubes and stored at -80°C before measurements using ELISA kits according to the manufacturer's instructions (PBL Assay Science). Culture media after stimulation were also tested similarly.

**Immunofluorescence assay (IFA).** Cells transfected with plasmids expressing tagged proteins were fixed with 1% paraformaldehyde for 10 min and then 0.1% glutaraldehyde for 5 min. The samples were then incubated in PBS with 5% BSA for 30 min at 37°C. Anti-tag antibodies (HA and MYC; 1:200 dilution) were incubated at 37°C for 1 h, followed by 3 washes with PBS/BSA buffer, and then incubated with fluorophore-conjugated goat anti-rabbit IgG antibody (1:1000 dilution) for another 1 h. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen). The cells were observed under a confocal microscope (SP5/SP8 confocal microscope; Leica, Wetzlar, Germany) and processed using Huygens essential (Scientific Volume Imaging, Netherlands), Imaris X64 (Bitplane, Switzerland), and Adobe Photoshop CS (Adobe systems Inc., San Jose, CA).

**Knockdown of *Rtp4* by RNA interference.** *Rtp4*-specific and scrambled control small interfering RNAs (siRNAs) were designed and purchased from Origene (Dataset S2). The RNAi and control sequences were cloned into the pRS plasmid and transfected into 293T cells ($1.0\times10^{5}$) along with the pCMV-*Rtp4* plasmids or internal control plasmid. The cells

were lysed 48 h later, and protein levels of RTP4 or control protein (β-actin) were detected by Western blot. The 293T cells were also transfected with plasmids expressing siRNA #1, Firefly luciferase reporter driven by IFN-β promoter, and Renilla luciferase. The cells were then stimulated with Poly(I:C) or Poly(dA:dT) 24 h later. Luciferase activities were measured 48 h after stimulation.

**Real-time quantitative polymerase chain reaction (qPCR).** Total RNA was isolated with Trizol reagent (Invitrogen) and reverse-transcribed using SuperScript™ First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. qPCR was performed using iQ SYBR Green Supermix (Bio-Rad), with a typical 3- step real-time PCR protocol in a Bio-Rad CFX qPCR instrument. The PCR cycles included initial denaturation at 95°C for 2 min, denaturing at 95°C for 15 s, annealing at 60°C for 20 s, extension at 72°C for 30 seconds for 40 cycles. The primers for the qPCR are listed in Dataset S2.

**Virus infection of mice.** West Nile virus (WNV, strain NY99) was generated from an infectious plasmid provided by Pei-Yong Shi (University of Texas Medical Branch). Plasmid was linearized and transcribed in vitro using a mMessage mMachine T7 transcription kit (Ambion). Huh7.5 cells were electroporated with viral RNA and incubated at 37°C for 48 h. Supernatants were collected from electroporated cells, clarified by filtration through a 0.45 µm filter, and stored at -80°C. Viral titers were determined by plaque assay using Huh7.5 cells. WT and *Rtp4*$^{-/-}$ C57BL/6 female mice (3 to 4 weeks old) were inoculated subcutaneously in the right rear footpad with 1,000 PFUs

of WNV in PBS in a 10-µl volume. Mice were monitored for clinical signs of disease and weighed at 24-h intervals. At 7 days post infection, mice were euthanized by CO2 overdose. For analysis of viral replication, tissues were collected in PBS, weighed, homogenized with a MagNA lyser (Roche Diagnostics), and stored at -80°C. Viral titers in tissue homogenates were determined by plaque assay using Huh7.5 cells. Experiments with virus were performed using biosafety level 3 conditions.

**Histology.** Tissue samples from mice at day 6 pi with P. berghei ANKA were fixed in 10% buffered formalin and embedded in paraffin as described (3). Tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope with various magnifications (2X, 10X, 20X, and 40X). Immunostaining was performed using Vectastain Elite ABC kit (Vector, Burlingame, CA) following manufacturer's instruction. Anti-IBA1 antibodies were obtained from Bio-Care Inc (Holt, MI; Cat: CP290A).

**Statistics.** Statistical analyses were performed using GraphPad Prism 7.0 software (GraphPad Software). Values are presented as mean ± SD unless otherwise indicated. The statistical differences were assessed using Mann-Whitney test for pair tests, ANOVA for multiple comparisons, or Log-rank test for survival rates.

## Supplementary Figures

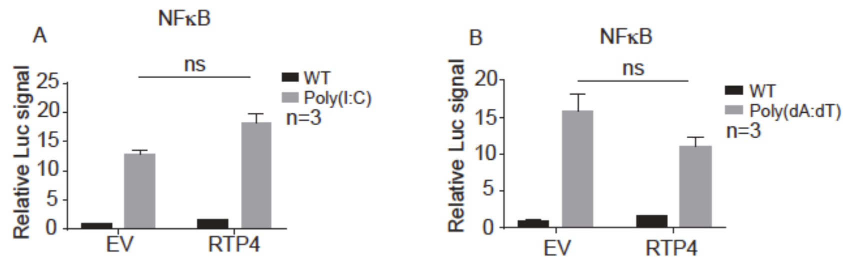


**Fig. S1.** RTP4 does not significantly affect NF-κB activity *in vitro.* Luciferase signals driven by NF-κB promoter after transfection of $2 \times 10^5$ 293T cells with 100 ng empty plasmid vector (EV) or 100 ng plasmid expressing RTP4 after stimulation with Poly(I:C) (A, 500 ng)) or Poly(dA:dT) (B, 125 ng). Mann-Whitney test; ns, not significant.

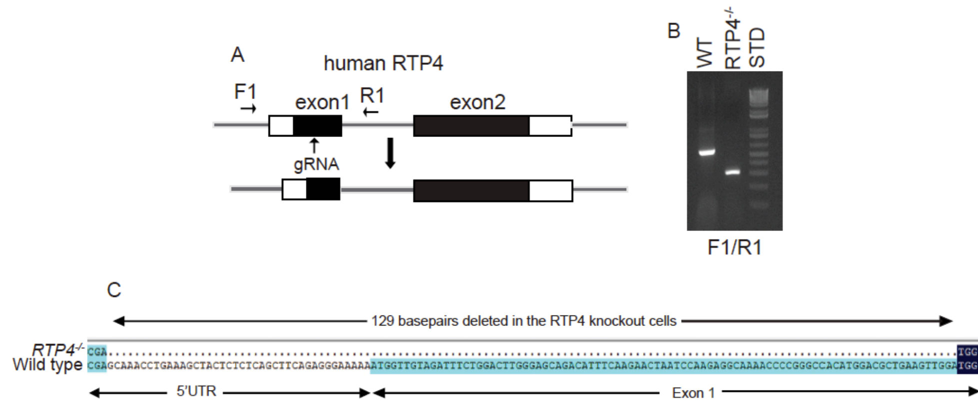


**Fig. S2.** Generation and validation of *Rtp4* knock out (KO) cell lines. (A) Diagram of *Rtp4* gene structure indicating the positions of guide RNAs (gRNAs) and primers for detection of changes after CRISPR/Cas9 editing. (B) Agarose gel of PCR products using primers as indicated. STD, molecular standard markers. (C) Aligned DNA sequences indicating deleted sequence in the *Rtp4*$^{-/-}$ clonal cell line.

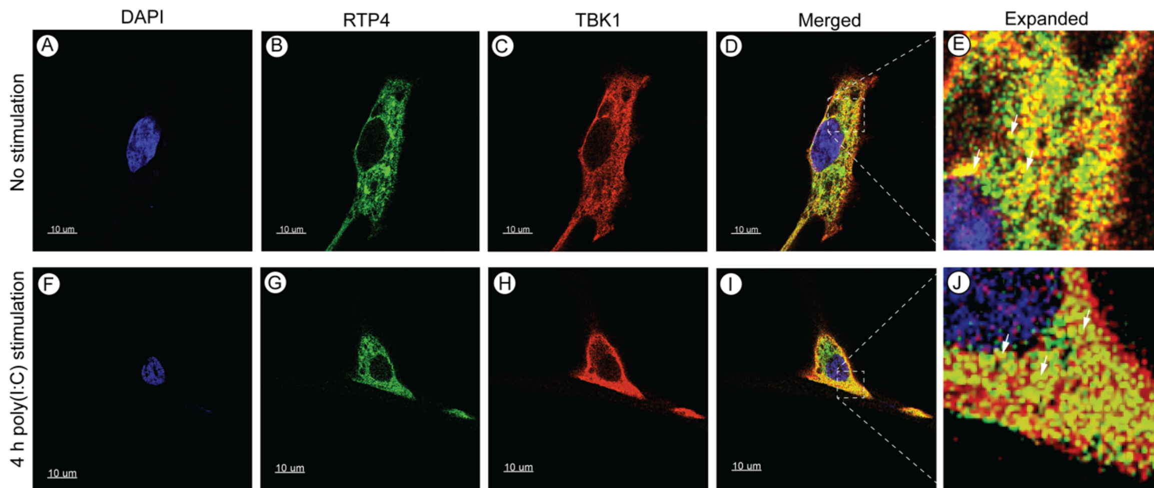


**Fig. S3.** Cytoplasmic localization of RTP4 and TBK1 in immunofluorescence assay. Cultured 293T cells were transfected with plasmids encoding TBK1-Flag (500 ng) and RTP4-HA (500 ng). After 20 h, the cells were left untreated (A-E) or stimulated with Poly(I:C) (1.5 μg) for 4 h (F-J). Cells were fixed, permeabilized, stained using anti-Flag or anti-HA fluorescent-labeled antibodies, and observed under a confocal microscope. The colors are as indicated. White arrows in (E) and (J) point to three typical yellow spots.

```
AATTAAAAAAAAAAGGAATAGCTGGTGTGAAAAGAACCTTTGGGAAGGAAGAAAGGAAGTCAGAATGGGGTCAGCCACTTAACTCTCTTAAGGGTCCCAGA
ATGTAGCAGGGCCTTTGCATAAAAGGTTTTACTAGGTGGGAAGATGTTAATATATTTGCATCCAAGGTGCAGGAGATGAAAGGAGTTGAATTATTTGCTG
CAAACACAAAAAAAAATACAAGTTTTTCAGTGTCTCAAAGAAAATACTCATATTTATTTTGTTGTGCTGTTTTAACACCAGGAACATTTGCAGTGCGGATT
TTTTTCAGGGTCACTAGGTGATTAGGAACACAACCTCTTACAGTTCTGAGATCTCCGGGCCACCAGCATCTACTGGGGCAGGCGCCTTTTCTCAGTCACT
AAGCACTCCTTTATTTCAATCCCCACACTGGTGCGTTGTTTACAGTAAAAATTATAAGAAAACAAAATGAAAAGCTTCCCACACAGGAGCCGCTAGAATG
CAAACCAGAACTCCGCAGTTTCAGCACGGCCCTTTAACACAGTGGCAGCGGCGATTTGCTGCCCTAAGGTTTCTGTGCACCTTGGAAACCGAAACTTAAA
GACAGACCGGGAAATGGAAACTGAAAGAACCGGGAAACTGAAACTGTTCTTTGTTTCCTCTCTCCCAGATTTGAGGAAGACAGTGAGCTGCTGAGTAACC
CGGGGCCTCAGCTTCCACACCTCTGAGAATGCTGTTCCCCGATGACTTCAGTACTTGGGAGCAGACATTTCAAGAACTGATGCAGGAGGAGAAGCCCGGG
                             M  L  F  P  D  D  F  S  T  W  E  Q  T  F  Q  E  L  M  Q  E  E  K  P  G
GCCAAGTGGAGCCTGCATTTGGATAAGAACATTGTACCAGATGGTGCAGCCCTGGGATGGAGGCAGCACCAGCAGACAGTGCTTGGCAGGTGAGTGGAGT
 A  K  W  S  L  H  L  D  K  N  I  V  P  D  G  A  A  L  G  W  R  Q  H  Q  Q  T  V  L  G  R
CTGGGCAGCTGGTCCCCTTAAAGACCCCACTACGTGCAGAGGCTTAACTGTGCTGATGCAGCCTCTAATACTTGTTAGAAAGAACAGGTGTGGCTAACTA
GATTTTCCCAGTCTTTTGTGATCACTAGGCTAAGATGGTTAGGGTCGCTTGTACTCCATGTCCCCATGCTGGTTTTCTTTCTTTCTTTCTTTCTTTCTTT
CTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTCTCTATCTCT
CTCTCTCTCTTTCTTTCCTTCCCCTTCCCCCCCCCTCTCTCTCTCTCTCTCTCTTTCTTTTTCTGAGACAGGTTTCTCTGTGTGGCCCTGCCTGTCCT
GAAACTCACTCTGTGGACCAGGCTGTCGAACTCAGAGATCCACCAGCCTCTGCCTCCTGAGGACTGAGAGTAAAGGCGTGCTCCATCTTGTTTAACTTAA
AAACTGCTTGCCCTGCCAGGTCTCCTTACAAAAGCATTTCAGGAGTCCTTGAAGAATGACTCCAAGTTCCTACAAAGTCTTCCCTCCTGCTCTCTGTTGC
TGGATCCCCAGCCAAAAAAAGACGTGGCATTGTATCCAGCTGCTCAGCCCTCTTATCAAGAGAAAACATAATAAAACTCTTAAAATGGAAAAATGGATTT
ATCTACAATTTGGGGATGCCACTGTTCATAGTCTGTCTCTGCCTCCCATCTAACAGTGTTATAATTCATGGAAAGGATGAGACAAGATCTGCATGTTATC
AGAAATGTTGGCATACTTGACTGTAATTGCAGATGATACCAGGGAAAGCAGGTTCCTTCTTTGGGTCCTTAATCTCCTCGCTAAAAGGATTTTAGAATGG
GGACTCAGATGGAAGTTTGAGGGCAGTTTATTAGAGTTTAAAAGAAAAGCCCCAGAGTGGGCAATACCCTAGGATTTCAGTTTCTGTGGAGCTGTGTGGT
GCTAGACAGCCACACAGCAGAGACAGGACGAGTAGAGAAGGCGGGTGTCATGGAGGAAAACATGCTTAGAGCAGAGGTTACGCTTTTTCTGAGCGTTTGG
TTTCAGACTCAGGGATGTGGGACTGAGGTGCGCGAAAACAGACCTGGCCTCCAGGTTCTCCCAGCATCCCTCAGTCCCTACCTGGCATACCTAGCTCCTA
ACCCTGAACTTTCTATCGCAGGGGCTGGGCTCCCTTCCTCCAGAGGCTCCTCCCAATATAATCCAGACATTTTGGTCTCTCCTCCTCTTCCTCCTCCTCC
TCCTCCTCTTCTTCCTCCCTTCTTCCCCCTCCTTCCCTCCCTCCATCACCTTCCCTCCTCTCTCCCACCCCCCTCTCTGCTGACACACCCTTTCTCTTCC
CCCTCCCCTCTCCCCATGCCAACTTTCCTAACCTTGGTCCTTGGGGCCAGTGAACTTGCCCAAGAGCAGCTTCGCAATAAACCTGCCTTTAATATAATCT
AATCTGGCTTGAATTGGCTCATTTCATGGGCAGAGAAGTAACCTGATGAAAAGTCATCAGACTTCTAGAGCCAGGCAAAGAGAGTTGCCATGCTGGGTCA
CATAAGAGCCTGCAATAAGGAGCAGGAGTCCTGGGAAGGAAAAGTACAAAATGGTTTAAAGGGAATTTCACCTATCTTTCTAGCATTGCTTAAGGCAAAC
GTCTTTGGAGGGGCTGTCCCTCCTTCCTGAGGGGTGGTCAATTGGTCAGGTGCATAGCCAGTCCAATTAGAAAGTTAAAAATCCACCCAGGCAATAAATT
CTATTCTCTTGACCCAAAGGAGTTTGGCCTTAATGGGCTTTTAGTGCTATTTTAAGGCTTAGATTTAGTTGTGGGATCTTTGAGATTTGACATTCTCAGG
AGATACGAACTTATGACTCAGGGGCCAGAGCTTCTACAGAAGGCCTTTCCCACTCCCAGCGTATGTCCTGTAGGGAACTAGAATTGAAGAACAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAGAAGAAGAAGAAGAAAAATTGAATTTCAAGGGAGTCCTTCCAGGCTATTCCTGACTAGCCCCATCCAGGCTGCAGCA
ACTCTCCTGGCCTGAGCCCTGCTCAGCACTAGTTCCTAAAAATTGACTAAGATCAGGCAGCCACTTTACAGAAAGCTTGTGAATAAAAAGGAGAAGGCTA
AAACCCAGGTCCTGGAGTGTCTGAGCAGTAGCCCTTTCCTATTGGCTACCTCTTCTGTAGTCTTAAACCACCTTCAGGTGACCTCCCCTTGTCAGGAGTT
TAAAGATGGCTAGCCTGGGAAGTTAAACCCTCGTTTTCCAGAACCCTAGTGACTTTTCTAGGGCTCCTAGAACCCTCAGTTCATTTTTCCCTTTTCAAGG
GTACCTGCCCACCCTTGCCCTACCTCAAACTGTGTTTTATCCCTTATGAAGCCTCAGCCATAAGTTTTTCCCATGTTAGTCATGTTGCTCATTAGAGGTG
ATGTTATGTAGTAGGTCTTTTCCTATCAAAGAGGCAGACTCAGTAACAGGAAAATATCTAAAAGAGATTGCCTCTTGCTCCTGTTTATCTGTGACCATGT
CACTCAAAACCATGTGAAGTTCTCTACACTGAAGGGGATTCTTAAATCCCTTAGCTGACCATTTCCTGTCAGGTCTGCGGCCAGCCAAGGACACAGAGGA
TTTCTTTCTTCTGTCTATAGGTTCCAGTGTTCCAGATGCTGCAGAAGTTGGACCTCTGCTCAGGTGATGATCTTGTGCCACATGTACCCGGACACTTTGA
                        F  Q  C  S  R  C  C  R  S  W  T  S  A  Q  V  M  I  L  C  H  M  Y  P  D  T  L
AATCGCAGGGCCAGGCACGCATGAGGATCTTTGGTCAGAAGTGCCAGAAGTGTTTTGGATGTCAATTTGAGACTCCCAAGTTCTCCACAGAGATCATCAA
K  S  Q  G  Q  A  R  M  R  I  F  G  Q  K  C  Q  K  C  F  G  C  Q  F  E  T  P  K  F  S  T  E  I  I  K
AAGAATTCTGAATAACCTAGTTAATTATATTCTGCAGAGATACTATGGACACAGGAAGATAGCATTGACCTCGAATGCATCTTTGGGTGAGAAGGTGACT
  R  I  L  N  N  L  V  N  Y  I  L  Q  R  Y  Y  G  H  R  K  I  A  L  T  S  N  A  S  L  G  E  K  V  T
TTGGATGGGCCCCACGACACACGCAATTGTGAGGCATGCAGTCTAAACTCTCATGGAAGATGTGCCCTTGCACACAAAGTAAAACCACCCAGATCTCCAT
 L  D  G  P  H  D  T  R  N  C  E  A  C  S  L  N  S  H  G  R  C  A  L  A  H  K  V  K  P  P  R  S  P
CTCCATTACCAAAGAGTTCCTCCCCATCAAAGAGCTGCCCTCCTCCGCCTCAGACCCGGAATACGGATTTTGGGAATAAAACTTTTCAGGATTTTGGGAA
S  P  L  P  K  S  S  S  P  S  K  S  C  P  P  P  P  Q  T  R  N  T  D  F  G  N  K  T  F  Q  D  F  G  N
TAGAACTTTTCAGGGATGCAGAGAGCCCCCCCAACGTGAAATAGAGCCACCACTATTTCTGTTTTTGTCTATTGCTGCATTTGCCCTTTTTAGTCTTTTC
  R  T  F  Q  G  C  R  E  P  P  Q  R  E  I  E  P  P  L  F  L  F  L  S  I  A  A  F  A  L  F  S  L  F
ACTAGATAATAGGTTTATCGTCTTGTTTTAATACCTTGGTGATCAATGAAATAGATGTCATTTCAATAGGACAGTGAATTCATATAGAATCAAGTAAGGT
 T  R  *
CGAGTATATAAAATAAATCTGCTTTCCTGGACATGCTCTGAAAATAGCCTAAACCATGGCCCACACATAATGGACCCAATAACAGATTTGTATTGAGTTG
CTGAACTGTCTGCACTAGCTTGCTCACGTATTGTTCTCAAGCTATGTTGATTAAATAAATCTTGATTATAACTAACAGTCACCTGTGCTATGAGATACAA
AGCGGCGGCCGGAGTAAGGGAATACCTGCAGGGCCCCTGCCAAGGTGCCCCCTGAGAAATCATGCCACGTAACAGACCTGGTGTAAGGGAGTTTATTAGT
GAGGGAAGCAGGGACCTAGAGAAGTGGGGAGCAGAGCTATGAGGACAGAGAACGGGGGACAGAGAAGGACAGAGAGAGGGGCTGGCCAGGAACACGAGGG
AACAGAGAGAGAAAGAGGAAGGAGGAGGGGGGCTGGGGAGTAGGCTGCCCCTGCACCACCTGGCTCGAACCCAGTGGGCAGCAGGTGATGACATAAGCCT
AGGTCCCTGGGAGAAGCCTAGTGGAATCTTCTGTTAACCAACAATGAGTATTATTGTAAATTTACTGTGGCAGACTGGGTGATGCTCCCCCCCCACACAC
ACCAAAAATGCCCTTCCCCATAGCCTCTAGAATTTGTGAATAAACTCTATTTGTTGGAAGAATGGACTTCGTAGACATGATTAATTATGAGATTTTGAGG
TAAATTGTCTTGCTTTATCGAAATGAATGATAACAGAAGGTCCTGTTTTGTTTTGTCTTTTCCATCAGGGTCCAACAAAAGCTCGTTGTTGAAGGTGTGG
TCTTAGGGTGGTGGTGCCCTGGGAGGTAGCAAAAAGGTTAGGATATGGGCTTAAGGGGGCATGGATCATTAGGGCAGGTTCTTGTCAGCACATTGAGGCC
CTCACCCATTCATTCCCCTCTCTTACTTCTCAGTCACAATTAGGTGAGTATCCTCTTTCTCCCTCTACAGTCAAGTCGCCACATTCATGCCTGCCACATG
```

**Fig. S4**. Murine *Rtp4* gene sequence showing positions of two gRNAs and coding region with amino acids. The two gRNA-targeting sequences are highlighted in yellow, and the PAM sequences are highlighted in green.

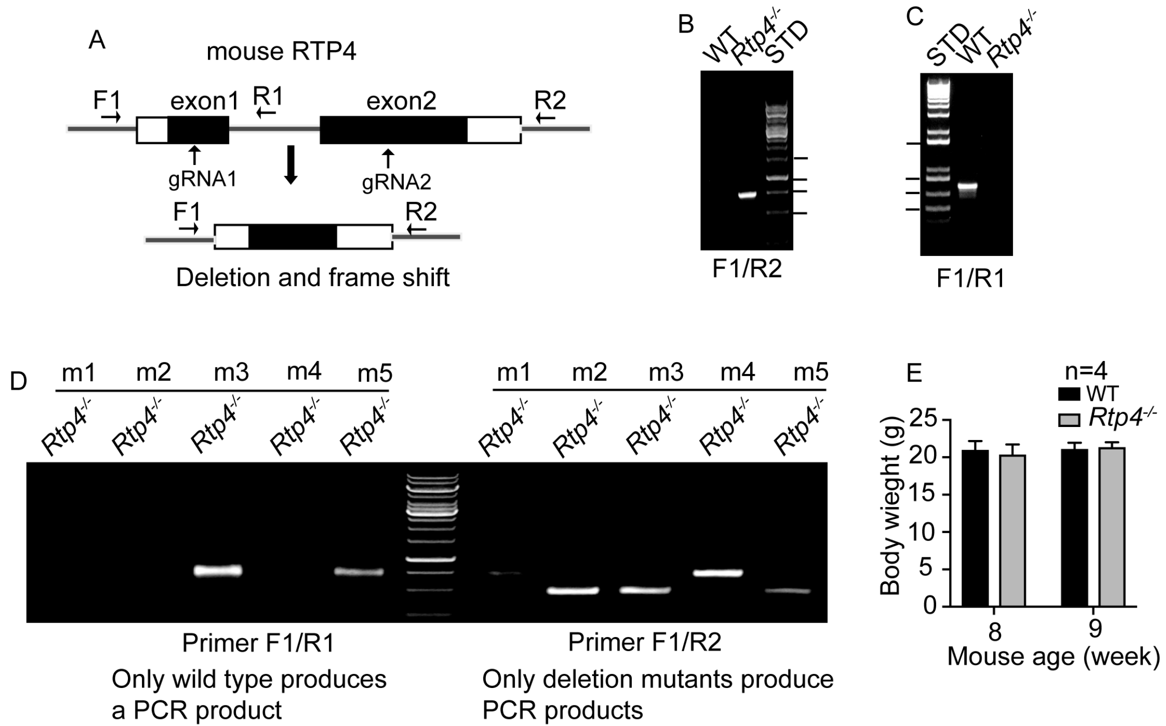


**Fig. S5.** Generation and validation of *Rtp4* knock out (KO) mice. (A) Murine *Rtp4* gene structure showing positions of the two CRISPR/Cas9 gRNAs and primers for PCR. (B and C) Agarose gels showing amplified PCR products from WT and *Rtp4*$^{-/-}$ mice. STD, molecular standard markers. (D) Agarose gel showing PCR products from individual mice with one or two *Rtp4* alleles disrupted using primers as indicated in (A). Note that the band sizes for m1 and m4 are different from those of m2, m3, and m5, suggesting two independent knockout mouse lines with different deletion sizes. (E) Body weights of WT and *Rtp4*$^{-/-}$ mice at 8 and 9 weeks after birth.

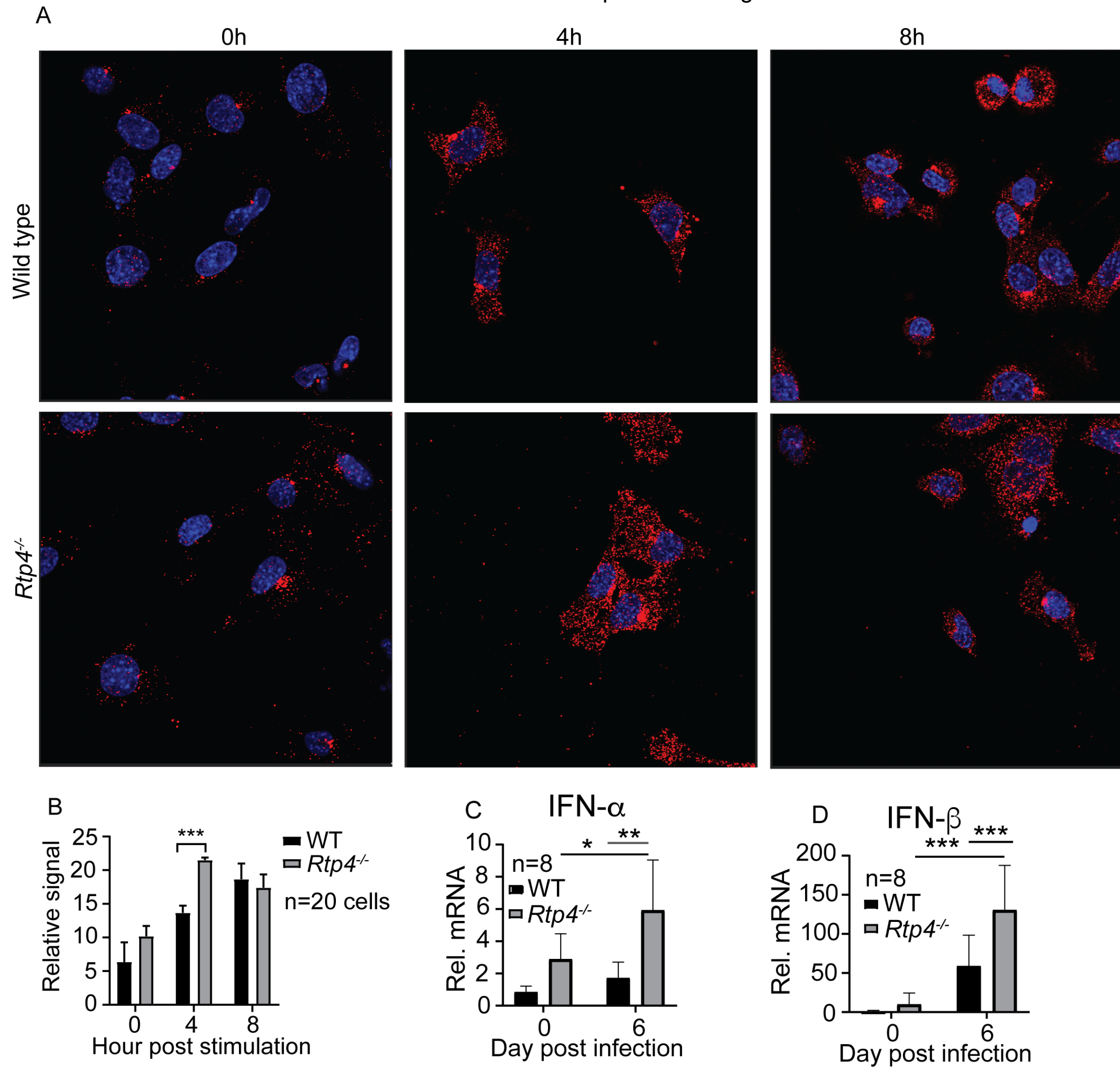


**Fig. S6.** Immunofluorescence assay detection of phosphorylated IRF3 in wild type (WT) and *Rtp4*$^{-/-}$ MEF cells. (A) MEF cells were stimulated with cGAMP (4 μg) for 0 h, 4 h and 8 h. The cells were fixed, permeabilized, and stained using anti-pIRF3 antibody (1:200). Fluorescent signal was observed under a fluorescent microscope, and signals were captured using the same camera settings. Blue, DAPI staining of nuclei; red, anti-pIRF3. (B) Plot of relative signals scanned from individual cells. Means and SD from replicates (n) as indicated; two-way ANOVA: ***, $P < 0.001$. (C and D) Relative mRNA levels of IFN-α (C) and IFN-β (D). mRNA levels from microglia of *P. berghei* ANKA-infected WT and *Rtp4*$^{-/-}$ mice were measured using RT-qPCR as described in the Methods. Two-way ANOVA, means and SD from replicates (n) as indicated; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

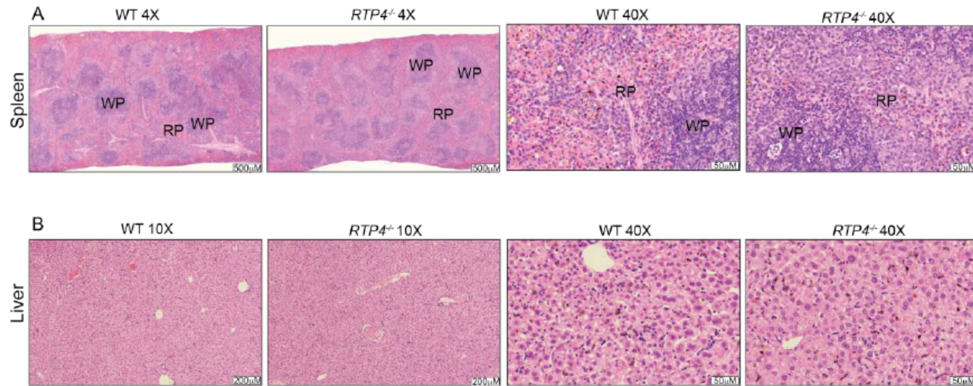


**Fig. S7.** Images of hematoxylin and eosin (H&E) stained spleen and liver sections from infected wild type (WT) and *Rtp4*$^{-/-}$ mice. Tissue samples from mice day 6 post infection were fixed in 10% buffered formalin, embedded in paraffin, and were stained with H&E as described in the Methods. The tissue sections were then examined under a light microscope at magnifications of 4X, 10X and 40X. (A) Representative images from the whole spleen. RP, red pulp; WP, white pulp. (B) Representative images from the whole liver. Mouse origins and magnifications are as indicated.

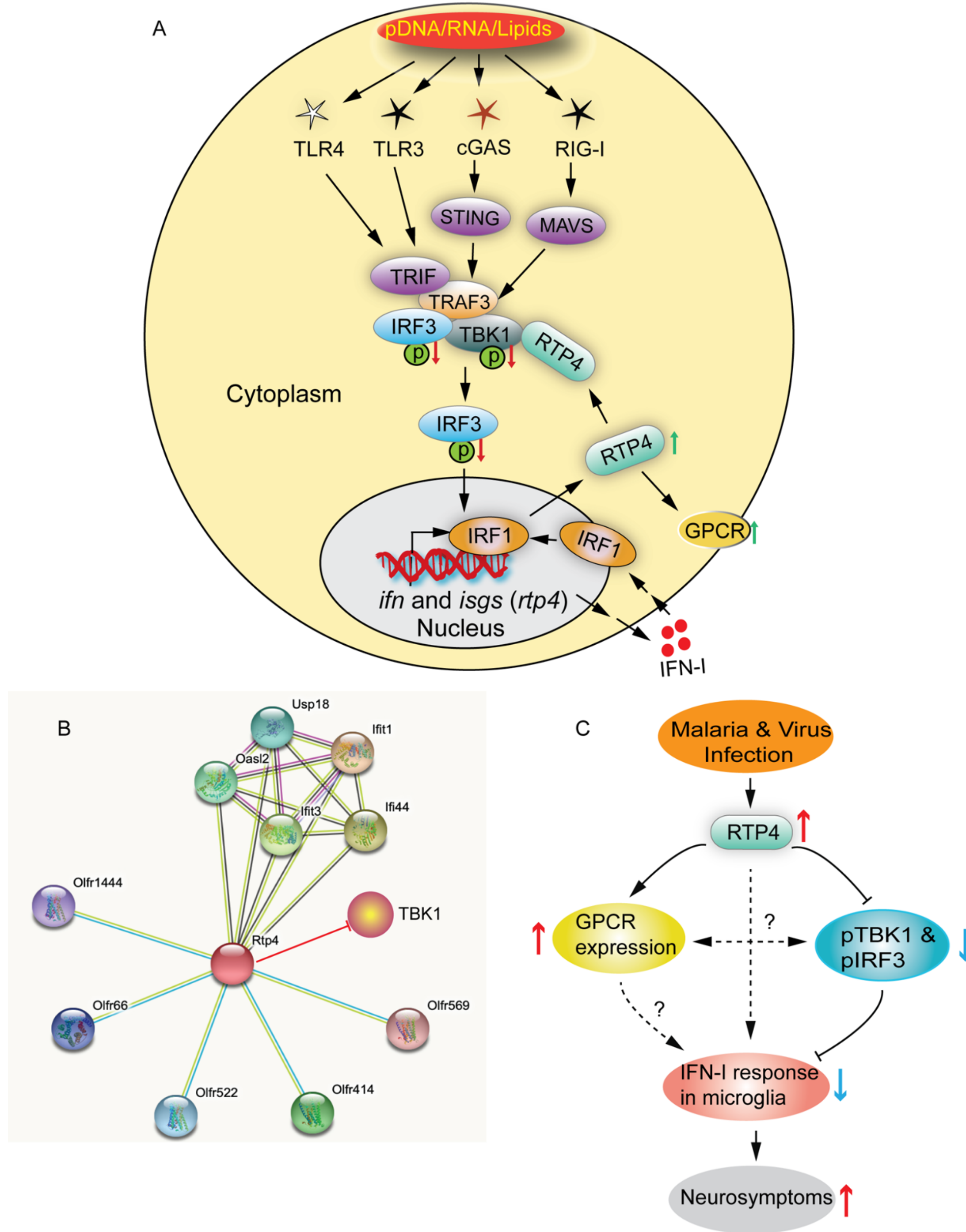


**Fig. S8.** Summary of putative mechanisms of RTP4-mediated inhibition of type I interferon (IFN-I) pathways and responses to infections. (A) Recognition of parasite DNA/RNA/lipids or damage-associated molecular patterns (DAMPs) after parasite infection by TLRs, RIG-I, MDA5, or cGAS-STING activates IFN-I pathways that signal through the TRIF/TRAF3/TBK1 complex, leading to production of IFN-I and increased expression of ISGs, including RTP4*,* possibly through IRF1 binding to its promoter. Increased expression of RTP4 in the cytoplasm binds to TBK1, interferes with TBK1 and IRF3 expression and/or phosphorylation, and inhibits IFN-I response. Higher levels of RTP4 expression may also enhance expression of various G-protein coupled receptors (GPCRs) and the subsequent signaling pathways. Disruption of RTP4 expression may increase

TBK1/IRF3 phosphorylation and IFN-I expression that helps reduce symptoms of cerebral malaria or control viral replication in the brain. Red arrows indicate decreased phosphorylation, and green arrows indicate increased RTP4 and GPCR protein levels. (B) Diagram showing interaction of RTP4 with ISGs and olfactory receptor proteins, adapted from STRING functional protein association networks (https://string-db.org). RTP4 is experimentally shown to bind TBK1 in this study. (C) Effects of RTP4 on GPCR expression, IFN-I production, and brain related symptoms after malaria or viral infections. The improvement in brain symptoms and reduced viral load upon RTP4 deletion could be due to increased IFN-I production and/or reduced specific GPCR presentation on cell surface. Red arrow, increased protein expression; blue arrow, reduced TBK1/IRF3 phosphorylation and IFN-I production; dash lines, mechanisms or interactions to be determined.

**Dataset S1**. A cluster of host genes with similar patterns of genome-wide LOD scores after Trans-species Expression Quantitative Trait Locus (Ts-eQTL) analysis.

**Dataset S2.** Oligonucleotide sequences for PCR primers, RNAi probes, and gRNAs used in this study.

## SI References


1. J. Wu *et al.*, Genome-wide Analysis of Host-*Plasmodium yoelii* Interactions Reveals Regulators of the Type I Interferon Response. *Cell Rep* **12**, 661-672 (2015).
2. J. K. Lee, M. G. Tansey, Microglia isolation from adult mouse brain. *Methods Mol Biol* **1041**, 17-23 (2013).
3. C. F. Qi *et al.*, Genomic organisation and expression of BCL6 in murine B-cell lymphomas. *Leuk Res* **24**, 719-732 (2000).