Glucagon-like peptide-2 receptor is a receptor for tick-borne encephalitis virus to infect nerve cells

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Abstract

Tick-borne encephalitis virus (TBEV) is a tick-borne flavivirus that causes severe encephalitis disease1,2. Host proteins required for TBEV entry remain largely unknown3. Here we performed a genome-wide CRISPR-Cas9 knockout screen and identified G-protein-coupled receptor glucagon-like peptide-2 receptor (GLP2R) as a receptor for TBEV to infect nerve cells. Knockdown or knockout of GLP2R reduced TBEV infection of different nerve cells; trans supply of GLP2R restored viral infection. GLP2R directly binds to viral envelope domain III through its extracellular loop 1 (ECL1). TBEV infection can be blocked by the ECL1 peptide, a functional ligand to GLP2R, or GLP2R antibodies. GLP2R-deficient mice were generated to validate the role of GLP2R in TBEV infection and pathogenesis. Wild-type mice succumbed to TBEV infection and developed >107 TCID50 (median tissue culture infectious dose) virus per gram of brain tissue. In contrast, all GLP2R-deficient mice survived TBEV infection without detectable infectious virus in brain. Altogether, our results support GLP2R as a receptor for TBEV to infect nerve cells.

Main Text

Host factors are essential for viral replication and pathogenesis. To identify host proteins critical for TBEV infection cycles, we performed a genome-wide screen using a lentivirus based CRISPR-Cas9 system4,5. The CRISPR-Cas9 system delivered single-guide RNAs (sgRNAs) targeting 19,050 human genes. Human glioma T98G cells were transduced with pooled lentivirus and challenged with TBEV Far Eastern subtype (TBEV-FE, strain WH2012)6. Cells that survived the TBEV-FE infection were cultured for 39 days to reach about 10^8 cells (Extended Data Fig 1a-d). Genomic DNA (gDNA) was extracted from the uninfected control cells (10^8) as starting population or the cells that survived TBEV infection (10^8) for sgRNA sequencing, and the enrichment of each sgRNA was analyzed by MAGeCK7. A total of 1,085 genes (p<0.05) were statistically enriched (Extended Data Fig 2a and Supplementary Data Excel 1). GO analysis uncovered enriched genes that function in cell-matrix adhesion, membrane and vesicle formation, and cell proliferation process, implying the potential roles of the enriched genes in TBEV infection (Extended Data Fig 2b-d and Supplementary Data Excel 2-4).

Glucagon-like peptide-2 receptor (GLP2R) emerged as a top candidate from the screen (Extended Data Fig 2a & e). GLP2R belongs to G protein-coupled receptor (GPCR) superfamily B8,9. It is mainly expressed in the central nervous system and enteric neurons10-12. To examine its function in TBEV infection, we performed a GLP2R knockdown experiment in two human glioma cells (T98G and U251) (Extended Data Fig 3a & b). Knockdown of GLP2R significantly impaired the infection of both TBEV-FE and TBEV European subtype (TBEV-Eu, strain Neudoerfl) (Extended Data Fig 3c-f), as evidenced by >23-fold reduction in infectious virus yields (Extended Data Fig 3f). To confirm the knockdown results, we knocked out GLP2R (glp2r^-/-) in T98G and U251 cells using CRISPR-Cas9 with multiple sgRNAs (Supplementary Table 3 and Extended Data Fig 4a-c). The knockout of GLP2R did not affect cell viability, but significantly reduced viral replication of TBEV-FE and TBEV-Eu (Fig 1a & b and Extended Data Fig 5a-e). At 48 h post-infection, the glp2r^-/- cells produced >56-fold less infectious viruses than the parental normal cells (Fig. 1c
Trans expression of GLP2R in the \( glp2r^{-/} \) cells restored the infectious virus production (Fig 1c & d). In contrast, the knockout of GLP2R did not affect the infection of other neurotropic viruses, including Japanese encephalitis virus (JEV), Zika virus (ZIKV), vesicular stomatitis virus (VSV), human cytomegalovirus (HCMV) and herpes simplex virus (HSV) (Fig 1e). Collectively, the results indicate that GLP2R is specifically required for TBEV infection.

We validated the role of GLP2R in TBEV infection in other nerve cells, including human neuroblastoma SK-N-SH, human astrocytoma CCF-STTG1, and human primary astrocytes. Although these cells expressed different levels of GLP2R (Extended Data Fig 6a), knockdown of GLP2R decreased TBEV infection (Extended Data Fig 6b & c), confirming the function of GLP2R in TBEV infection in these nerve cells. Since TBEV can also infect non-human, non-neurogenic BHK-21 and Vero-E6 cells, we examined GLP2R expression in these cells. No and low expression of GLP2R was found in BHK-21 and Vero-E6 (Extended Data Fig 6d); consequently, treatment with GLP2R siRNAs did not affect TBEV infection of these cells (Extended Data Fig 6e); however, ectopic expression of GLP2R enhanced TBEV infection of Vero-E6 cells. As a control, TBEV infection was also enhanced when glioblastoma U251 cells (with low endogenous GLP2R) expressed exogenous GLP2R (Extended Data Fig 6f). The results imply that GLP2R is specifically required for TBEV infection of human nerve cells.

Since GLP2R is a cell membrane protein, we examined its role in TBEV entry using a lentivirus-based pseudovirus system. Knockout of GLP2R significantly reduced the transducing efficiency of TBEV envelope-pseudovirus, but not the VSV glycoprotein-pseudovirus, on T98G cells (Fig 2a). Knockout of GLP2R also reduced the binding of authentic TBEV to cells at 4 °C (which allows virus binding but not entry), whereas over expression of GLP2R increased TBEV binding (Fig 2b). To analyze TBEV internalization, we quantified intracellular viral RNA after incubating the cells with TBEV at 37 °C infection for 1 h. Compared with normal T98G cells, less viral RNA was detected in the \( glp2r^{-/} \) cells, whereas more viral RNA was detected in the GLP2R-overexpressing cells (Fig 2c). Consistent with the role of GLP2R in TBEV entry, pre-treatment of T98G cells with an anti-GLP2R mAb or a TBEV envelope mAb (A4) suppressed viral infection (Fig 2d). Furthermore, incubation of T98G cells with H33D peptide, a functional ligand to GLP2R, inhibited TBEV infection (Fig 2e & f). In contrast, incubation with D31D peptide, a non-functional H33D mutant that lacks the N-terminal two amino acids, did not affect TBEV infection (Fig 2g). The results demonstrate that GLP2R contributes to TBEV attachment and entry.

To determine whether GLP2R affects viral translation and RNA replication, we transfected a luciferase reporter subgenomic replicon RNA of TBEV into control and \( glp2r^{-/} \) T98G cells. No significant difference in luciferase activity was detected between the two transfected cell types (Fig 2h), suggesting that GLP2R does not affect viral translation and RNA synthesis.

We mapped the domains required for the GLP2R and TBEV envelope interaction. TBEV envelope could efficiently pull-down human (huGLP2R) and less efficiently mouse GLP2R (moGLP2R) (Fig 3a). Pulldown experiments with a panel of deletion GLP2R and TBEV envelope (Extended Data Fig 7a & b) mapped the interaction to the extracellular loop 1 (ECL1) of GLP2R and domain III of envelope (Fig 3b & c, Extended
Data Fig 7c & d). A synthetic peptide Y41L, representing the ECL1 amino acid sequence, efficiently competed away the GLP2R/viral envelope interaction (Fig 3d). The dissociation equilibrium constants ($K_D$) between peptide Y41L and domain III of viral envelope (G99I-lys-biotin) or purified TBEV prM-E (pre-membrane-envelope) protein were estimated to be 172 nM or 76 nM, respectively (Fig 3e & f, Extended Data Fig 8a & b). Functionally, the Y41L peptide inhibited TBEV infection of T98G cells (Extended Data Fig 8c). To further demonstrate the importance of ECL1 in viral envelope binding, we replaced different lengths of ECL1 by a linker Gly-Ser-Gly (GSG) in the context of GLP2R (Extended Data Fig 9a). These ECL1 mutant GLP2R variants remained expressed on the cytoplasmic membrane (Extended Data Fig 9b), but attenuated their binding to viral envelope by 60-66% (Extended Data Fig 9c). Consistently, the ECL1 mutant GLP2R (mutGLP2R=239GSG254) lost its ability to rescue TBEV infection of $glp2r^{-/-}$ T98G cells (Fig 3g). Besides ECL1, we also found the N-terminal extracellular domain (ECD) of GLP2R may modestly facilitate viral envelope binding (Extended Data Fig 9d); however, the ECD deletion GLP2R (GLP2R-$\Delta$ECD) could still rescue TBEV infection of $glp2r^{-/-}$ T98G cells (Fig 3g). Thus, the ECL1 of GLP2R is the key domain for interacting with TBEV envelop.

To evaluate the significance of GLP2R in TBEV infection in vivo, we generated Cas9-KO mice with a deletion of exons 2-10 in $glp2r$ gene (Extended Data Fig 10a). The $glp2r^{-/-}$ mice did not develop observable defects. After infected with TBEV-FE ($3.2\times10^7$ PFU) via an intraperitoneal route, wild-type mice developed significant weight loss (Extended Data Fig 10b); 58.8% ($n=10/17$) of them succumbed to the infection (Fig 4a); all animals developed viremia (Fig 4b) and high viral loads in brain, spleen, and intestine (Fig 4c & d). Remarkably, none of the $glp2r^{-/-}$ mice ($n=13$) developed weight loss (Extended Data Fig 10b); all survived the infection (Fig 4a); no infectious virus was detected from blood, brain, spleen, or intestine (Fig 4b-d). Only low levels of viral RNA (<$10^5$ RNA copies per g of tissue) were detected from the $glp2r^{-/-}$ brain, spleen, and intestine on day 7 post-infection (Fig 4c). Since TBEV is an encephalitis virus, it is important to note that, on day 7 post-infection, the wild-type mice developed 2.4×$10^7$ TCID$_{50}$ infectious virus per gram of brain tissue, whereas no infectious virus was detected in the $glp2r^{-/-}$ brain (Fig. 4c). The results demonstrate that GLP2R is essential for TBEV to productively infect mice.

We compared the histopathology of infected wild-type and $glp2r^{-/-}$ mice. H&E staining showed multifocal lesions to the brain, spleen, and small intestine from the wild-type mice (Fig. 4e). No such histopathology was observed in the $glp2r^{-/-}$ mice in general (Fig 4e) with only one mouse exhibiting mild pathological changes (data not shown). These findings were supported by quantitative pathological scoring (Extended Data Fig 10c). Consistent with the inflammatory cell infiltration (Fig. 4e), RNAseq analysis showed significantly higher levels of chemokines in the brain of infected wild-type mice (lost >20% body weight) than those from the infected $glp2r^{-/-}$ mice (Fig 4f). The histopathological results support the critical role of GLP2R in TBEV infection and pathogenesis.

Heparan sulfate proteoglycans (HSPGs) facilitate TBEV infection via interaction with viral envelope protein, allowing virion to engage in receptor binding$^{24}$ (Fig 4g). Our results have identified GLP2R as a host protein to mediate TBEV attachment and entry to neurogenic cells. In cell culture, TBEV infection is
diminished when GLP2R expression is knocked down or knocked out, but the infection is rescued when GLP2R is trans complemented. In a mouse model, knockout of glp2r abolished viral replication and pathogenesis, leading to no morbidity and mortality. Biochemically, the viral envelop/receptor interaction is primarily mediated by an extracellular loop ECL1 of GLP2R and domain III of viral envelope. These in vitro and in vivo evidence strongly support GLP2R as a receptor for TBEV to infect neurogenic cells. However, since GLP2R-negative non-neurogenic cells are also susceptible to TBEV infection, we speculate that an unidentified alternative host factor can mediate TBEV entry to these cells. Nevertheless, it is important to point out that, due to the neurotrophic nature of TBEV, GLP2R, which is predominantly expressed in the central nervous system and enteric neurons, could serve as a major receptor for TBEV infection in vivo. Our study has advanced the fundamental knowledge of flavivirus biology and provided a new antiviral approach through blocking the viral envelope/GLP2R interaction.

Methods

Cell lines, viruses, and virus titration. T98G (ATCC, CRL-1690) and SK-N-SH (ATCC, HTB-11) cells were cultured in Minimum Essential Medium (MEM), HEK-293T (ATCC, CRL-3216), BHK-21 (ATCC, CCL-10), Vero-E6 (ATCC, CRL-1586) and U251 (ATCC, CM-1925) were cultured in Dulbecco’s modified Eagle Medium (DMEM), CCF-STTG1 (ATCC, CRL-1718) was grown in RPMI 1640 medium. All medium was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin. The human primary astrocytes (HPAs) were purchased from ScienCell Research Laboratories and maintained on poly-lysine (Sigma-Aldrich)-coated plates in astrocyte medium. All cell lines were cultured at 37 °C in a fully humidified atmosphere containing 5% CO₂, and regularly tested for mycoplasma contamination.

The TBEV infection experiments were performed in a biosafety level 3 (BSL3) facility, approved by the biosafety committee of Wuhan Institute of Virology (WIVA05202003). TBEV Far-Eastern subtype (TBEV-FE, strain WH2012, KJ755186) was obtained from National Virus Resource Center of China, amplified in BHK-21 cells, aliquoted and stored at -80°C. The TBEV European subtype (TBEV-Eu, strain Neudoerfl, U27495) stocks were prepared by electroporation of BHK-21 cells with in vitro transcribed Neudoerfl RNA from cDNA clone and subsequent virus amplification in Vero-E6 cells. The TBEV subgenomic replicon with renillar luciferase as reporter was kindly provided by Professor Z. H. Zheng, Wuhan Institute of Virology, China.

TBEV titers were determined by 50% tissue culture infective dose (TCID₅₀) assay with 10-fold serial dilutions in BHK-21 cells. BHK-21 cells were seeded in 96-well plates at a density of 2 × 10⁴/well and inoculated with serial diluted supernatant or serum. 8 replicates were set for each dilution. The wells with cytopathic effect (CPE) were counted at 3 d post inoculation, and the TCID₅₀ was calculated by the Reed-Muench formula.
Genome-Scale CRISPR-Cas9 Knockout Screen and data analysis. A pooled GeCKO v2 library encompassing 122,411 single-guide RNAs (sgRNAs) against 19,050 human genes derived by the Zhang laboratory\textsuperscript{27} was obtained from a commercial source (Addgene, #1000000048). The lentivirus packaging, purification, titers using the GeCKO library were performed as previously described\textsuperscript{4}. To generate the knockout cell pools, approximately $8 \times 10^7$ T98G cells were transduced with the lentivirus library at a multiplicity of infection (MOI) of 0.3 and selected with puromycin (2 ug/mL) for 10 days. After selection, about $3 \times 10^8$ mutagenized cells were infected with TBEV-FE (MOI of 1.0). Few cells survived after TBEV infection. These cells were cultured for 39 d and proliferated to about $1 \times 10^8$ in total. Genomic DNA were extracted from uninfected and survived cells with Blood & Cell Culture Midi kit (Qiagen) and the inserted sgRNAs sequences were amplified from the gDNA by flanking primers and prepared for next-generation sequencing on a MiSeq platform (Illumina). sgRNA sequence were analyzed using Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout (MAGeCK)\textsuperscript{7} (see Supplementary Excels).

RNA interference (RNAi). The small interfering RNA (siRNA) duplexes specific for GLP2R were purchased from GenePharma. The sequence of siRNAs was listed in Supplementary Table 2. The siRNA duplexes were transfected using Lipofectamine RNAiMAX reagent (Invitrogen) at a final concentration of 20 nM according to the manufacturer's instructions. Nonsilencing siRNA with a scrambled sequence were used as a negative control (siNC). To keep the silencing efficiency for the duration of the test, the cells were split at 24 h post-transfection and transfected with the same siRNA again. TBEV infection was performed 20 h after the second transfection.

Cell viability assay. Cell viability was evaluated using a Cell Titer-Glo Luminescent Cell Viability Assay kit (Promega) according to the manufacturer's instructions. In brief, $1\times10^4$ cells in 100 $\mu$l culture medium were seeded into opaque-walled 96-well plates for 24 h and 100 $\mu$l of Cell Titer-Glo reagent was added to each well. After a 5 min shaking and 10 min incubation, luminescence was measured by GloMax 20/20 (TurnerBio Systems).

GLP2R knockout cell lines. The GLP2R knockout cell lines were generated based on CRISPR/Cas9. The sequence of sgRNAs target to GLP2R are listed in Supplementary Table 3. The sgRNA were cloned into the lentiCRISPRv2 plasmids (Addgene, #98290) and packaged in HEK-293T cells by cotransfection with psPAX2 (Addgene, #12260) and pMD2.G (Addgene, #12259). To generate stable knockout cell lines, T98G and U251 cells at ~50% confluence were transduced with the sgRNA containing lentivirus at a MOI of 0.3 and selected in with 2 $\mu$g ml\textsuperscript{-1} puromycin for 10 days. The knockout efficiency was analyzed using western blotting assays and sanger DNA sequencing.

Expression construct. The wild type human or mouse GLP2R and its mutants with HA-tag or egfp-tag at the C-terminus were cloned into the pcDNA3.1(-) or pLVX-puro expression vectors. Flag-tagged TBEV envelope and its mutants were cloned into the pcDNA3.1(-) expression vector. The plasmids transfection was performed using Lipofectamine 3000 (Invitrogen) transfection reagent according to the manufacturer’s protocol. A list of cloning primers is displayed in Supplementary Table 1.
qRT-PCR. Total cellular RNA was isolated with TRIzol (Invitrogen) reagent according to the manufacturer’s protocols. Viral RNA in culture supernatants was extracted with QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions. The quantification of specific gene transcripts was analyzed by one-step real-time qRT-PCR with specific primers and the HiScript II One Step qRT-PCR SYBR Green Kit (Vazyme) on the Applied Biosystems QuantStudio 6 Flex. The primer sequences for qPCR/RT-PCR were designed using Primer3 software (see Supplementary Table 4). The data were normalized to levels of β-actin mRNA in each individual sample. The relative expression level and absolute quantification were calculated by 2^{-ΔΔCt} method and standard curve line respectively.

Western blotting (WB) and Co-immunoprecipitation (Co-IP). Whole-cell lysates for both WB and Co-IP were prepared using a lysis buffer containing 50 mM Tris-base (pH 7.5), 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 150 mM NaCl, 100 µM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitors (Roche) for 30 min at 4°C. Cell lysates were centrifuged at 14,000×g for 10 min at 4 °C and quantified using the Bradford method. For WB, the supernatants were recovered and denaturized at 95 °C for 10 minutes. For Co-IP, the supernatants were collected and mixed with Protein G-agarose (Millipore) and various antibodies for 16 h at 4 °C. After six washes using ice-cold lysis buffer, protein G agarose-bound immune complexes were then eluted and subjected to WB analysis.

30 µg of total protein from each sample was resolved by SDS-PAGE and transferred to nitrocellulose. Membranes were blocked with TBST (pH 7.4, containing 0.1% Tween-20) containing 5% skimmed milk for 1 h at room temperature, followed by incubation with anti-sera containing primary antibodies overnight at 4 °C (see Supplementary Table 6). Membranes were washed and incubated for 1 h at room temperature with the HRP-conjugated secondary antibodies. Membranes were imaged using the FluorChem HD2 system (Alpha Innotech). Images were analyzed using AlphaEaseFC software (Alpha Innotech).

Pseudovirus production and transduction. Pseudoviruses were produced by co-transfection 293T cells with psPAX2, pLenti-GFP-luc, and plasmids encoding either TBEV envelope or VSV-G by Lipofectamine 2000 (Invitrogen). The supernatants were harvested at 48 h post transfection, centrifuged and then passed through 0.45 µm filter. For transduction, cell in 24-well-plate were inoculated with pseudovirus containing medium overnight. Cells were lysed with passive lysis buffer (Promega) and luciferase activity were measured to calculate the transduction efficiency at 40 hours post transduction.

Virus binding and internalization assays. Control or glp2r/- cells were washed twice with ice-cold PBS and incubated with TBEV at an MOI of 20 in cold medium supplemented with 2% FBS on ice for 1 h. For the binding assay, the supernatant was removed, and cells were washed with ice-cold PBS six times. After washing, cells were collected, and RNA was measured by qRT–PCR. For the internalization assay, the washed cells were then incubated in medium supplemented with 2% FBS and 15 mM NH₄Cl at 37 °C for 1 h. Cells were chilled on ice and treated with 500 ng/ml proteinase K on ice for 1 h. After three additional washes, cells were collected, and RNA was measured.
Blocking assays with antibodies and peptide. T98G cells were preincubated with isotype control or GLP2R antibodies at different concentration for 1 h, or serially diluted H33D, D31D and Y41L peptides for 2 h at 37 °C. After three washes, TBEV (MOI of 0.5) were added and incubated for 24 h. Cells were collected, and viral RNA was measured by qRT–PCR. The peptides used for blocking assays in this study were purchased from GLSBioChem and listed in Supplementary Table 7.

Purification of prM-E recombinant fusion protein. The DNA sequence encoding prM (residues 1 to 163), sE (residues 1 to 401), a N-terminal signal peptide (MGILPSPGMPALLSLVSLLSVLLMGCA) for secretion and a His 10-tag at the C-terminus was amplified by standard PCR techniques. The transmembrane region of prM (residue 126 to 163) was replaced by a linker containing a Tobacco Etch Virus (TEV) protease recognition site (GENLYFQG). The furin cleavage site of prM (85-RTRR-88) was mutated to (85-TTRS-88) to prevent cleavage of the recombinant protein by intracellular proteases.

The prM-E recombinant fusion protein was expressed in pExpi293 cells (Thermo Fisher Scientific) at 33 °C for 5 days and purified on a 5-ml Talon Cobalt column. After sample application, the column was washed with 50 ml of buffer A (25 mM phosphate pH 8.0, 300 mM NaCl, 7 mM imidazole), and protein was eluted with a 100-ml linear gradient to 100% buffer B (25 mM phosphate pH 8.0, 300 mM NaCl, 500 mM imidazole). Fractions containing prM-E protein were pooled, concentrated and buffer exchanged into PBS in a 30-kDa MWCO spin concentrator. Proteins were flash-frozen in liquid nitrogen and stored at −80 °C.

Octet binding assay. Octet binding experiments were performed on an Octet RED96 system (ForteBio, Fremont, CA). The purified biotinylated TBEV prM-E protein or domain III of viral envelope (E DIII, G99I-lys-biotin) was immobilized on a Streptavidin Biosensor (ForteBio) at 50 μg mL⁻¹ in PBS with 0.02% Tween 20 and 0.1% BSA. GLP2R ECL1 peptide (Y41L) was diluted to different concentrations with PBS with 0.02% Tween 20 and 0.1% BSA. 1200s and 600s association/dissociation processes were performed for Y41L binding to E DIII and prM-E respectively. The resulting data were background subtracted and from the dissociation and association curves, $k_a$, $k_d$ and $K_D$ ($k_d/k_a$) were calculated using Octet data acquisition 6.4, ForteBio data analysis software 6.4 (Refer to the data in the Extended Data Fig 8b). Additionally, the equilibrium responses were estimated from the association curves using a 2-phase exponential association equation, these responses were plotted against Y41L concentrations to obtain equilibrium binding constant $K_D$ in GraphPad Prism 6 using a single site binding model (Refer to the data in the Fig 3e&f).

Mouse experiments. The animal experiments were approved by the Animal Care and Use Committee at the Wuhan Institute of Virology, and conducted in conformity with the Guide for the Care and Use of Laboratory Animals of the Wuhan Institute of Virology in ABSL3 facility (Ethic number WIVA02202002). glp2r⁻/⁻ mice purchased from Jiangsu GemPharmatech Co., Ltd, were generated in the C57BL/6J background by depleting exons 2~10 of the glp2r using CRISPR/Cas9 technology (see Supplementary Table 5 & Extended Data Fig 10). 3.2 × 10⁷ PFU of TBEV-FE (with a volume of 50 μl of virus suspensions in PBS) was administered via an intraperitoneal route to four-week-old WT or glp2r⁻/⁻ male mice. Mice
were then monitored daily for body weight, survival and signs of pathogenesis. Mortality rate was assessed at the indicated time points. Animals were euthanized at 3 d, 7 d and 14 d post-infection, whole blood was obtained by cardiac puncture. Simultaneously, the brain, small intestine and spleen samples were collected after perfusion with PBS. The TBEV RNA and titers in serum and tissue samples were determined. The brain, small intestine and spleen samples were fixed with 4% paraformaldehyde, embedded in paraffin and cut into 3.5-mm sections. Fixed tissue samples were used for hematoxylin-eosin (H&E) staining. The image information was collected using a Pannoramic MIDI system (3DHISTECH, Budapest).

**Statistical analyses.** The data were presented as the mean ± s.d. using Prism Version 6 (GraphPad). Statistical significance between groups were evaluated using Student's t-test or an analysis of variance (ANOVA) as indicated with 95% confidence intervals.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data Availability.** The Supplementary Excel files provide data for the CRISPR–Cas9 screen and NGS gene analysis. The remainder of the data that support the findings of this study are available from the corresponding authors upon reasonable request.

**Declarations**

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**Author contributions**

Q. Y, J. Z. C, R. J. P, and X. W. C. conceived the project. Q. Y, J. Z. C, R. J. P, P.-Y. S, and X. W. C. wrote the manuscript with input from all co-authors. Q. Y. was involved in all experiments and analyses. R. J. P and J. K. C. contributed to CRISPR–Cas9 screen. J. Z. C and H. Z contributed to the mouse experiments. J. L. T, X. L. X and J. H. helped with plasmid constructs and protein purification. Y. W and M. Y help data analysis. MS. Y. Z. helped with grant applications and project management.

**Declaration of interests**

The authors declare no competing interests.
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