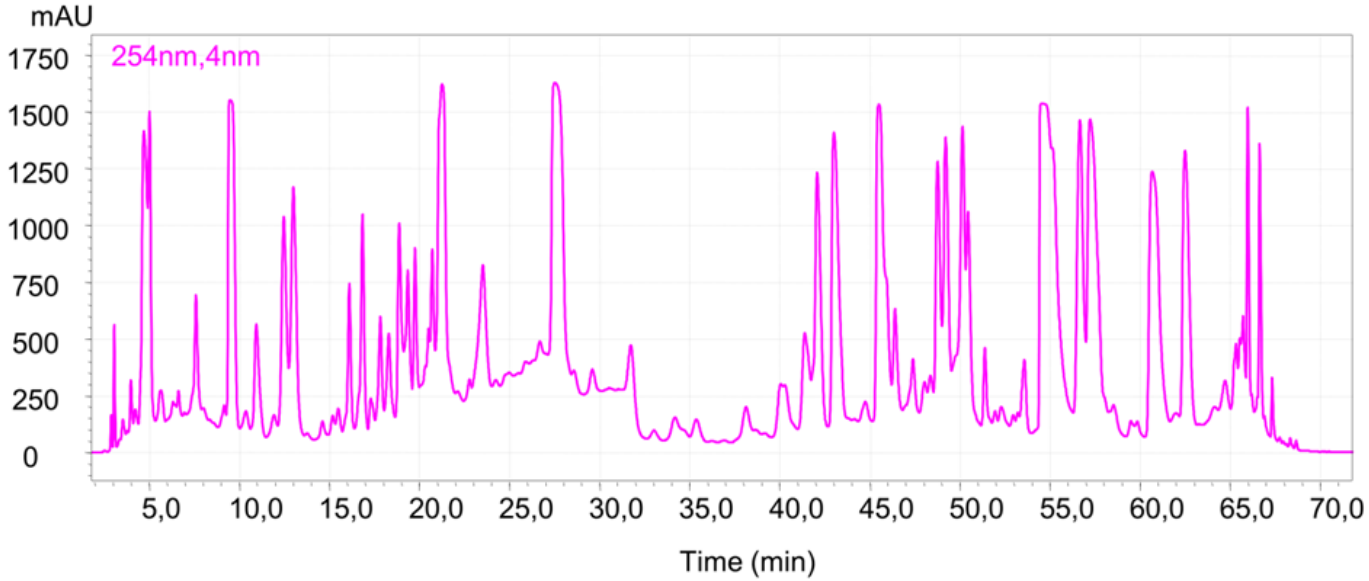
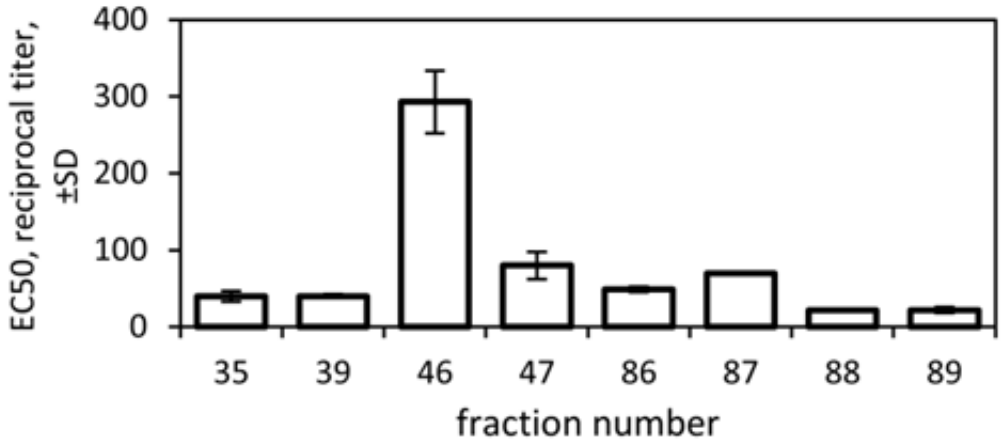
Solovarov I.S., Belskikh A.V., Oyuntsetseg N, Liapunova N.A., Danchinova G.A., Khasnatinov M.A. Identification of phytochemicals from *Terminalia chebula* extract as potential antivirals against tick-borne encephalitis virus.

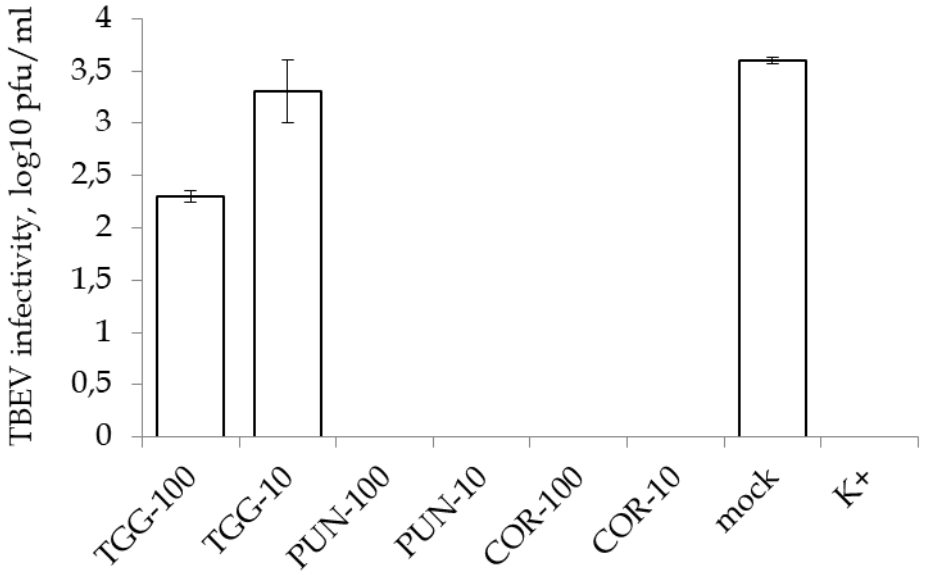
SUPPLEMENTAL FIGURES



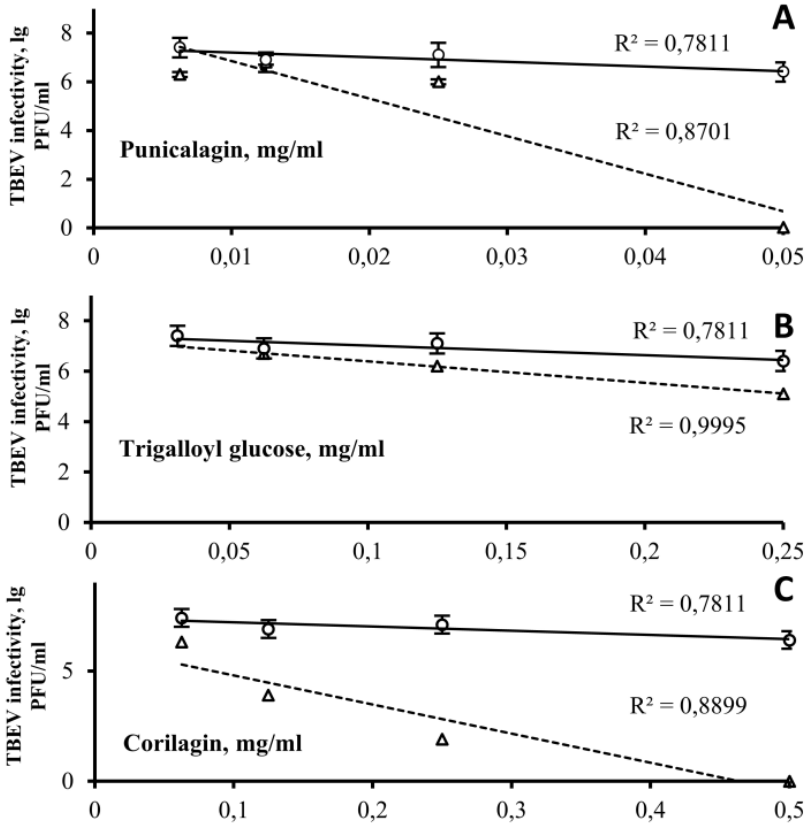

**Supplemental figure S1.** For analytical chromatography, ten microliters of 1% aqueous extract of Terminalia chebula seeds were analyzed using HPLC with Shimadzu LC30 Nexera X2 system. The UV absorbance at 254 nm is plotted on y-axis as milli-absorbance units (mAU). Time of run is plotted on x-axis. Semi-preparative HPLC and collection of fractions were conducted under the same conditions using the 50 µL volume of 1% extract.



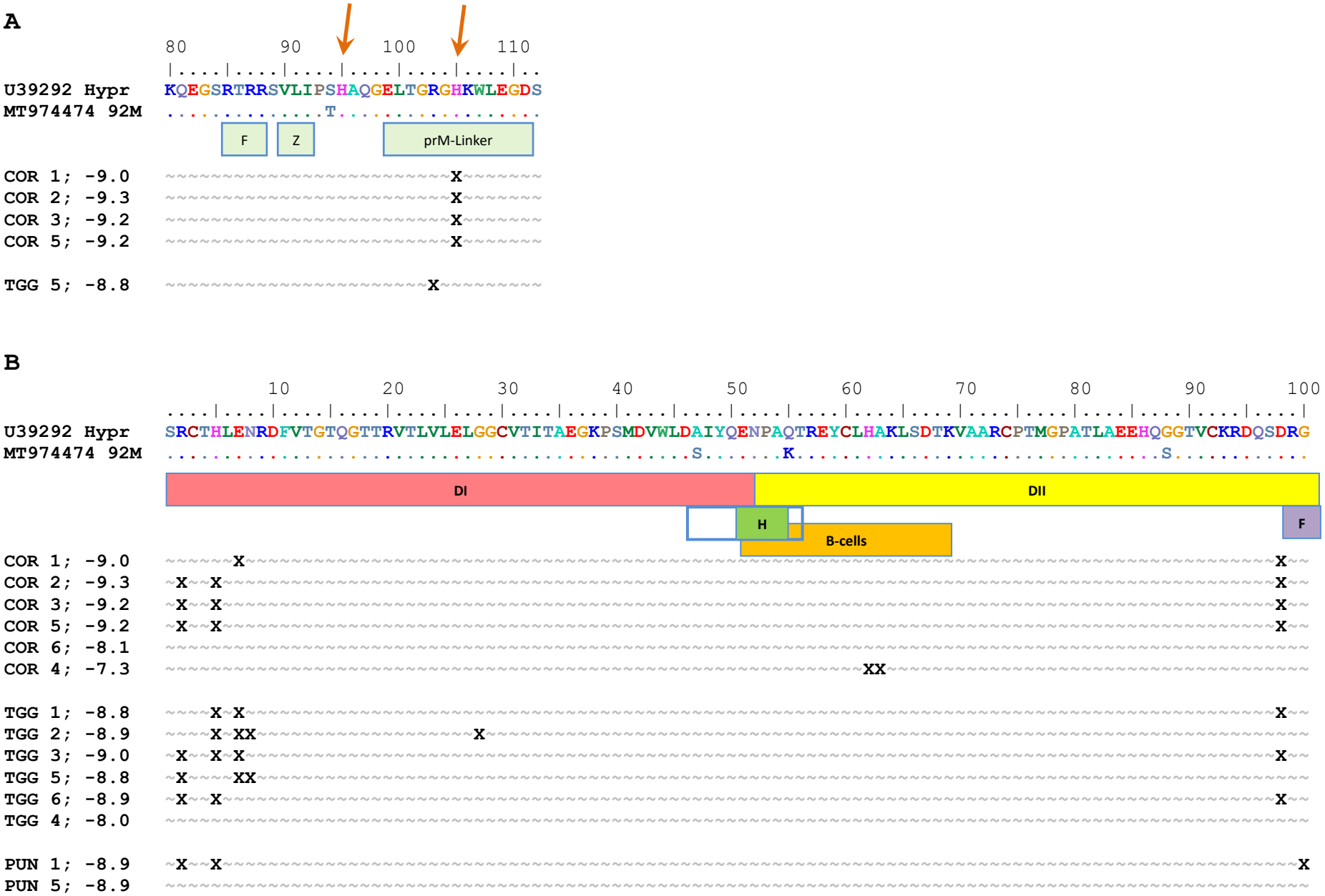

**Supplemental figure S2**. Each fraction was serially diluted two-fold and 50% effective concentration (EC50) was estimated as reciprocal titer at which the infectivity of TBEV is reduced two-fold. Fraction numbers are indicated on the x-axis. Error bars reflect standard deviation from three independent biological replications. Fraction 46 exhibited the highest antiviral activity (reciprocal EC50 titer comprised 293 ± 41) among all tested, followed by the adjacent fraction 47 (EC50 = 80 ± 18). Third active fraction appeared to be the fraction 87 (EC50 = 70 ± 2). In contrast to fraction 46, for this diapason the high antiviral activity was detected both in more mobile fraction 86 and in less mobile fractions 88 and 89 suggesting two-tailed distribution of components (Supplemental Fig. S2). Hypothetically, more than one active compound is present in the region between fractions 86 and 89. Fractions 35 and 39 exhibited average antiviral action with almost identical EC50 of 40 ± 7 and 40 ± 2. Interestingly, the adjacent fractions exhibited no significant antiviral properties. Probably, fractions 35 and 39 contain single active compounds with very uniform mobility in HPLC



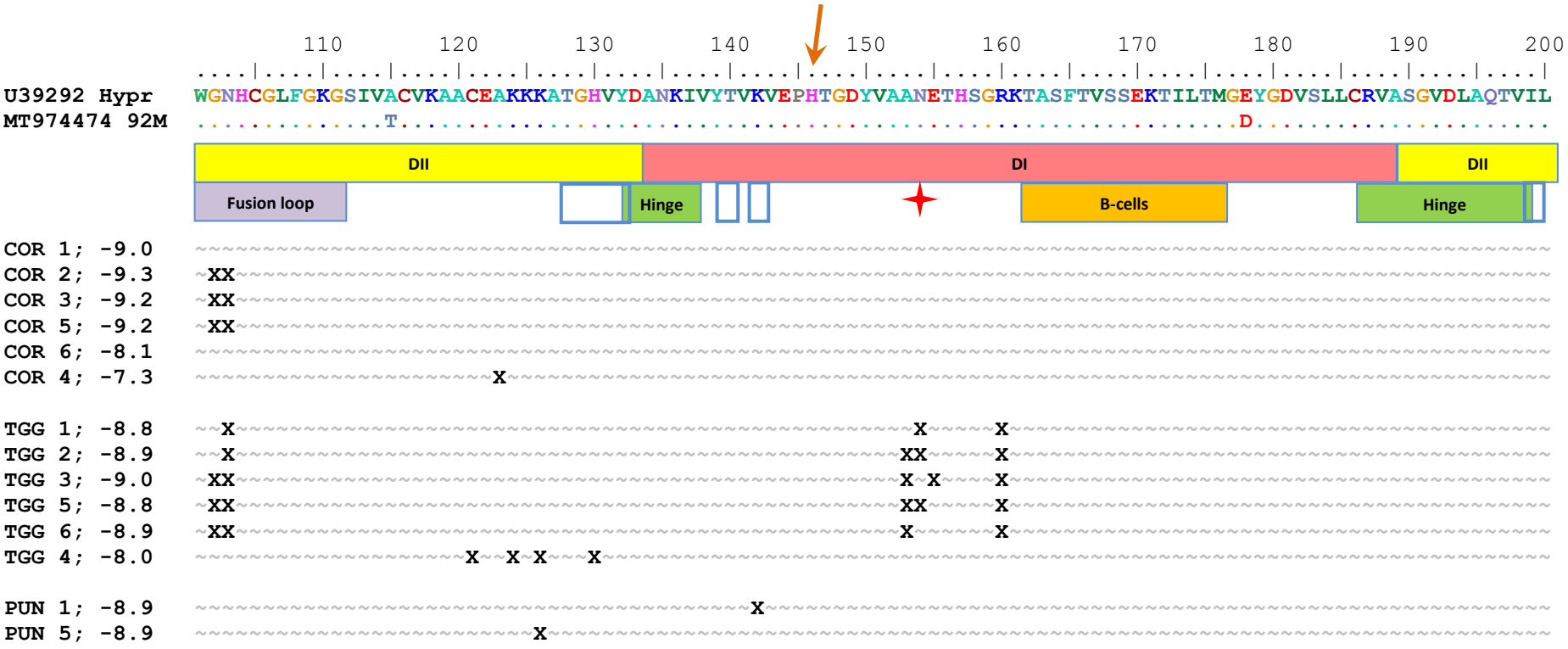

**Supplemental figure S3**. Plaque reduction test of compounds from *Terminalia chebula* extract as pure chemical substances. TGG - 1,3,6-tri-O-galloyl-β-D-glucose (CAS Number:18483-17-5); PUN - punicalagin (CAS Number:65995-63-3); COR - corilagin (CAS Number: 23094-69-1). Two concentrations were tested for each substance – 100 mkg/ml and 10 mkg/ml (indicated after the substance abbreviation on the X-axis). The TBEV residual infectivity is set up on the Y-axis. Phosphate buffered saline was used as mock-treatment; commercial human donor immunoglobulin against TBEV was used as positive control. Error bars indicate standard deviation from three replications.



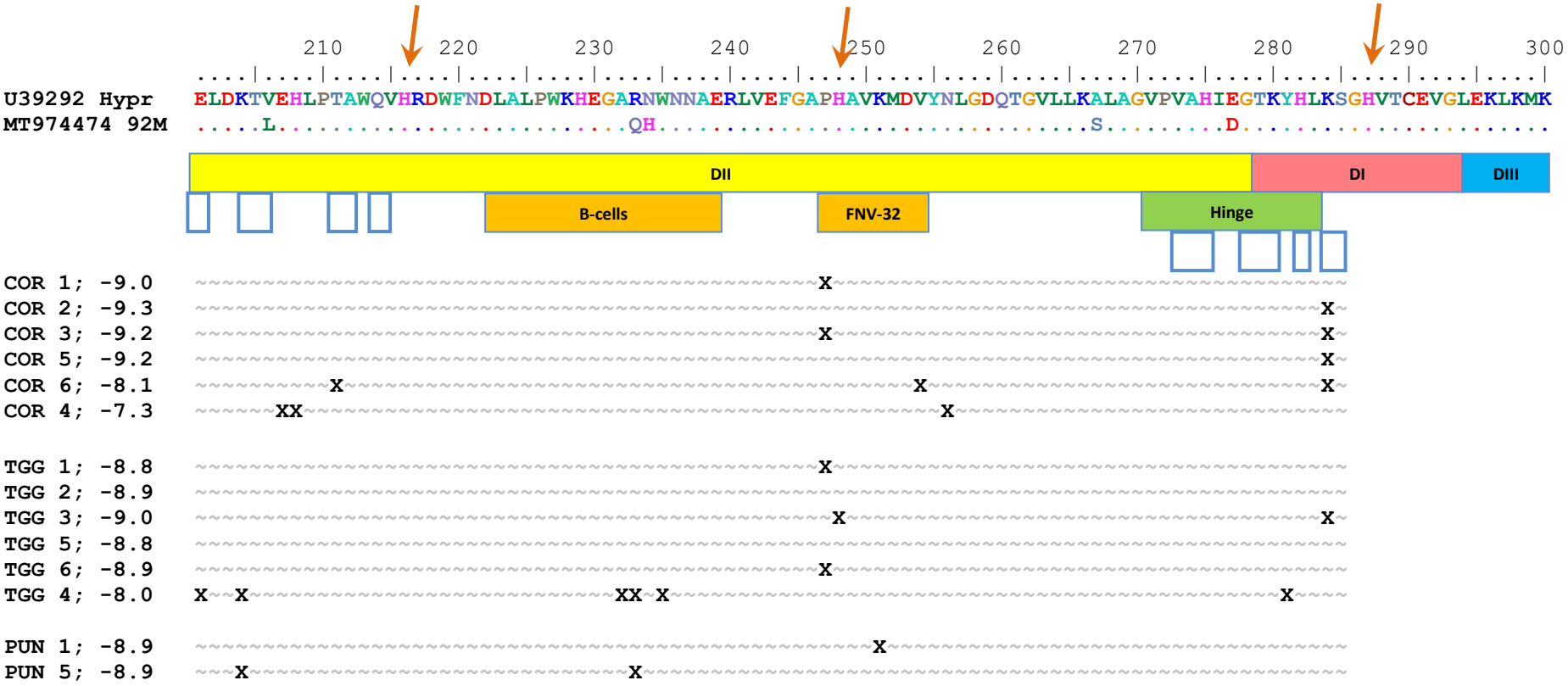

**Supplemental Figure S4.** Inhibitory effect of punicalagin (A), trigalloyl glucose (B) and corilagin (C) on TBEV reproduction in SPEV cell line. The triangles and dashed trend line indicate the values of TBEV infectivity at different concentrations of corresponding compound. The circles and solid trend line indicate the reference values of TBEV infectivity (PBS-treated). Compound concentrations are plotted on X-axis as mg/mL.




**Supplemental figure S5.** See capture below.




**Supplemental figure S5.** Continue. See capture below.




**Supplemental figure S5**. The putative in-silico binding sites for corilagin (COR), trigalloyl glucose (TGG) and punicalagin (PUN) are aligned to the aminoacid sequences of prM (A) and E (B) proteins of Hypr (EU subtype) and 92M (SIB subtype) isolates of TBEV. Amino acid sequence numbering and annotation are present according to the sequence and 3D structure of mature particle of TBEV strain Hypr (U39292 and pdb_00005o6a, respectively). The sequence of TBEV isolate 92M (MT974474) is aligned to evaluate the amino acid conservation at the involved residues. The alignments are abbreviated to the relevant residues. The numbers after compound abbreviation designate the estimated affinity of binding (in kKal/mol). The amino acid residues, involved in formation of binding sites are labeled with "X". The domains of envelope protein are indicated with boxes DI (red), DII (yellow) and DIII (blue). The functional elements are indicated by colored boxes with "F" for furin cleavage recognition site in prM, "Z" for hydrophobic "zipper" region of M-protein, "prM linker" for flexible unresolved region of M-protein, "H" for hinge region of E-protein, "FL" for fusion loop, "FNV-32" and "B-cells" for linear epitopes of monoclonal antibody FNV-32 [1] and B-cell [2] respectively. The E-protein glycosylation site is labeled with red star. Brown arrows indicate the histidines acting as pH sensors for pH-dependent conformation change of E-protein [3]. The empty blue boxes designate the hydrophobic ligand-binding pocket.

References:

[1] Matveev, A. *et al.* (2023). New Neutralizing Epitope Exposed on the Domain II of Tick-Borne Encephalitis Virus Envelope Glycoprotein E. *Viruses*. https://doi.org/10.3390/v15061256.

[2] Kuivanen, S. *et al.* (2014). Identification of linear human B-cell epitopes of tick-borne encephalitis virus. *Virology Journal*. https://doi.org/10.1186/1743-422X-11-115.

[3] Füzik, T. *et al.* (2018). Structure of tick-borne encephalitis virus and its neutralization by a monoclonal antibody. *Nature Communications*. https://doi.org/10.1038/s41467-018-02882-0.