

Supplementary Materials

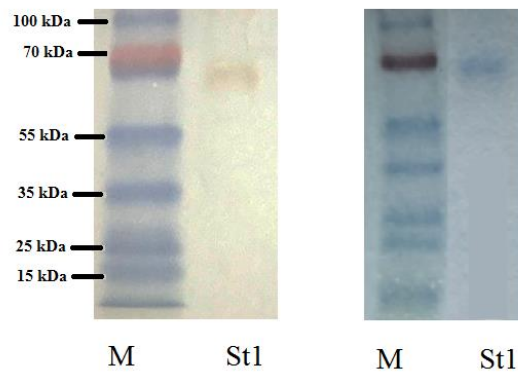
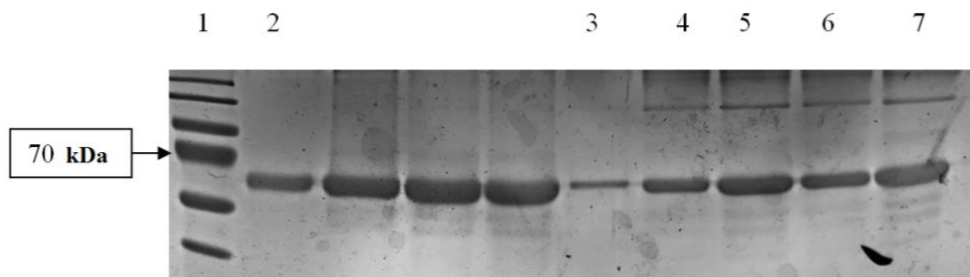
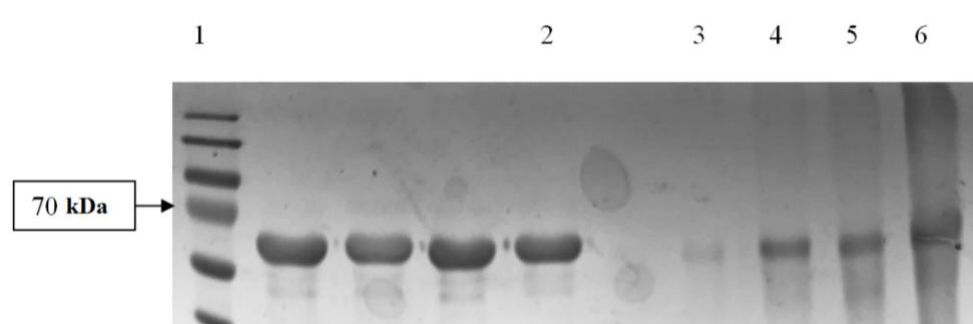


Figure S1. PAGE stained with Coomassie Blue and Western blot stained with anti-TBEV sera for standard E protein St1 and protein mass marker PageRuler (ThermoFisher).



- 1- Marker PageRuler (PageRuler™ Prestained Protein Ladder, Thermo Fisher)
- 2- Protein E used as a standard in ELISA
- 3- BSA 0.0625 mg/ml
- 4- BSA 0.125 mg/ml
- 5- BSA 0.25 mg/ml
- 6- BSA 0.5 mg/ml
- 7- BSA 1 mg/ml



- 1- Marker PageRuler (PageRuler™ Prestained Protein Ladder, Thermo Fisher)
- 2- Protein E used as a standard in ELISA
- 3- BSA 0.005 mg/ml
- 4- BSA 0.01 mg/ml
- 5- BSA 0.03 mg/ml
- 6- BSA 0.06 mg/ml

Figure S2. PAGE of different BSA concentrations and standard E protein ST1 stained with Coomassie Blue.

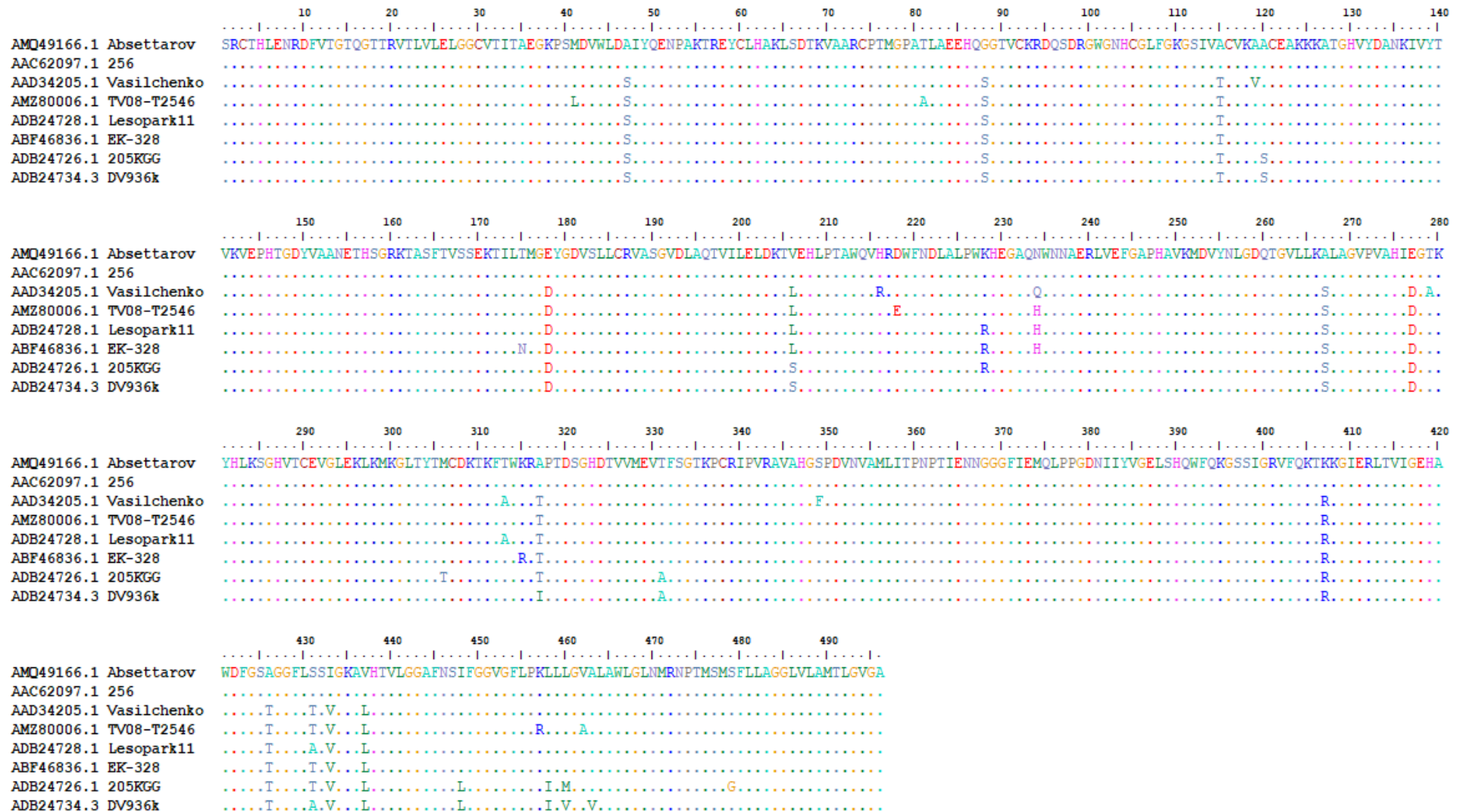


Figure S3. Multiple sequence alignment of selected for study strains. Alignment was performed on Clustal Omega web service [1, 2]. Picture was created in BioEdit [3].

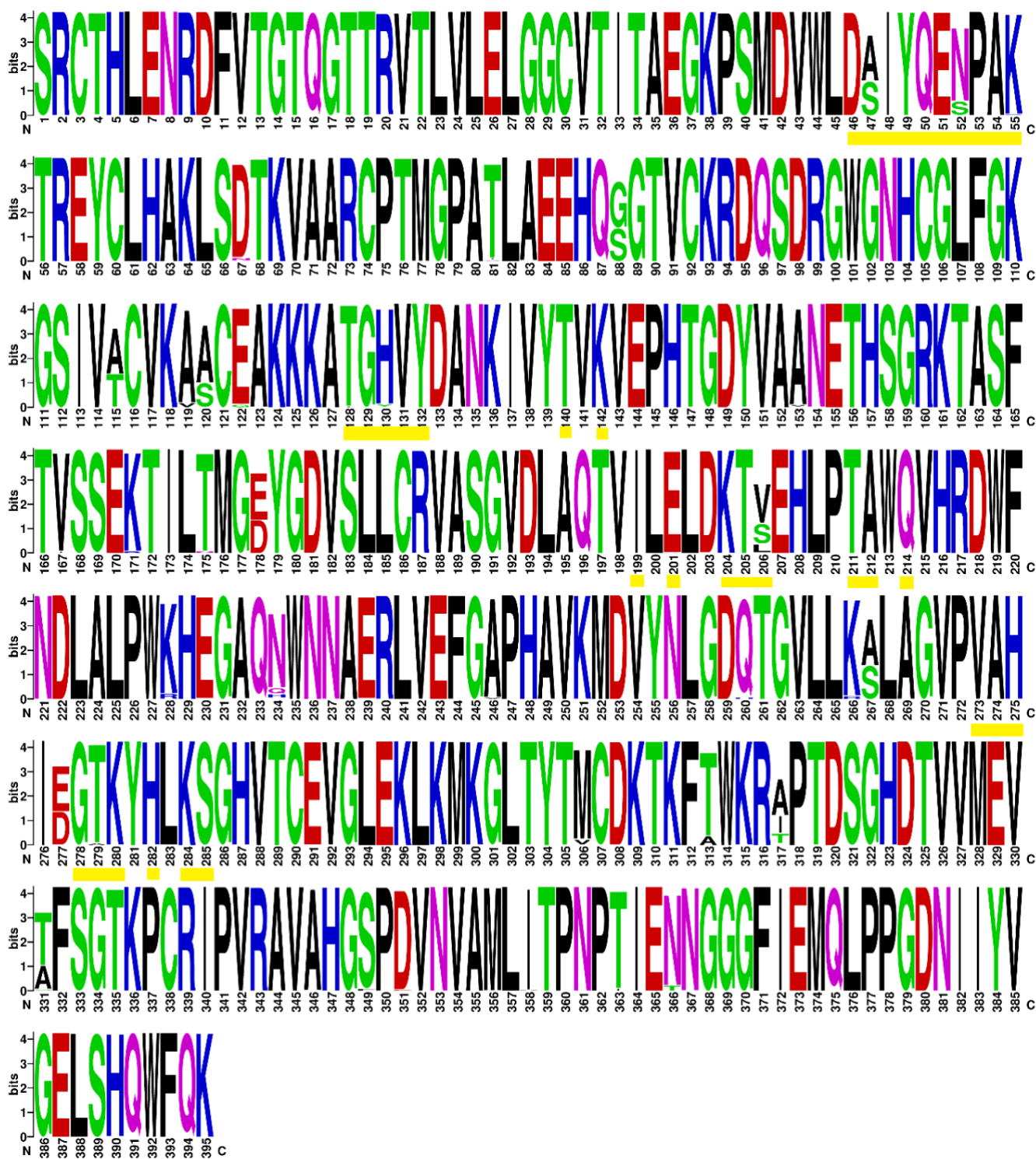


Figure S4. Sequence logo [4] of TBEV E protein amino acid sequences retrieved from GenBank. Amino acids comprising β -OG binding pocket are underlined by yellow. The overall height of the stack indicates the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino acid at that position [5]. Amino acids are coloured according to their chemical properties: polar - green, basic - blue, acidic - red, hydrophobic - black.

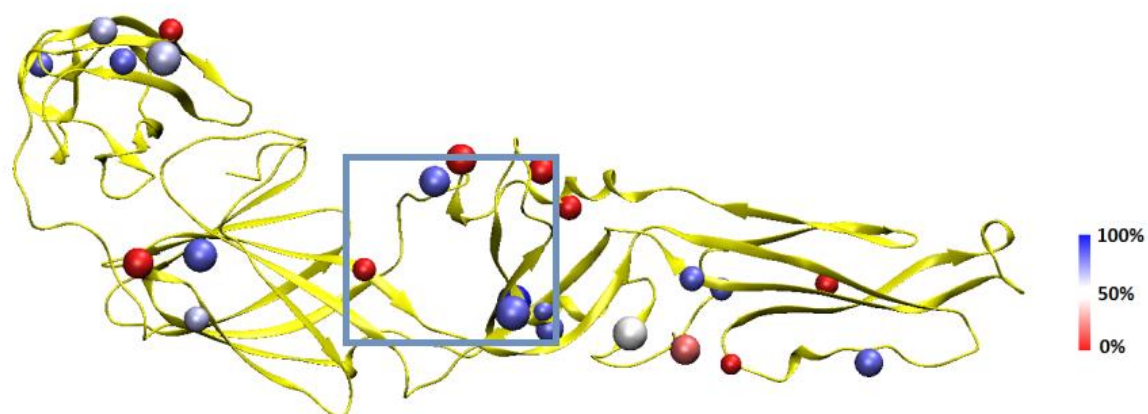


Figure S5. Overview of TBEV envelope protein soluble ectodomain (sE) homology model. Spheres correspond to the locations of amino acid substitutions in the sE protein of strains used in this study compared to strain Absettarov. Spheres are coloured from red to blue according to % of amino acid identity. Sphere size corresponds to the amino acid size in the strain Absettarov sE protein. The location of β -OG pocket is marked by rectangle.

Table S1. E protein concentrations in the strain samples calculated from infectious virions and GCP, assuming average molecular mass of TBEV E protein of 53 kDa and 180 protein molecules per virion. Data presented as mean \pm ½ 95% CI. * - corresponds to significant difference with strain 256 with $p \leq 0.05$.

Strain	E protein concentration (mg/L)	
	Estimated from infectious virions ($\times 10^{-5}$)	Estimated from GCP ($\times 10^{-2}$)
Absettarov	19 \pm 9*	0.3 \pm 0.1*
256	1.5 \pm 0.1	96 \pm 52
Vasilchenko	126 \pm 60*	3 \pm 1
TV08-T2546	41 \pm 28*	5 \pm 5
Lesopark11	6 \pm 4*	0.6 \pm 0.2*
EK-328	11 \pm 10	6 \pm 5
DV 936k	23 \pm 8*	11 \pm 2

References

- [1] Sievers F, Wilm A, Dineen D et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 2011; 7: 539.
- [2] <http://www.ebi.ac.uk/Tools/msa/clustalo/>
- [3] <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>
- [4] <http://weblogo.berkeley.edu/>
- [5] Crooks GE, Hon G, Chandonia JM, Brenner SE WebLogo: A sequence logo generator, *Genome Research*, 14:1188-1190