Supplementary Materials



Figure S1. PAGE stained with Coomasie Blue and Western blot stained with anti-TBEV sera for standard E protein St1and 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



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 Coomasie Blue.

	10	20	30	40	50	60	70	80	90	100	110	120	130	140
MO49166 1 Absettarow	SPOTHIENEDEVICTOR	TTPUTIVIEI	COUTTARC	KDSMDWWI DAT	VOENDARTE	VCT HART SDT	- ИЛЛ Л РС"РТМ	CDATI AFFHOC	CTVCKPDOSI	POWONHOOT F	CKCSTVACV	A CEARKY	TCHUYDANKTV	·Ι VT
AAC62097 1 256	SKOTHERKETVICIO		1000VIIIAL0	RESELVADOR.	I QUITANIN	IGHIARDODI	NVHARGE III	IOT AT BALLINGO	01VCRRDQ51	Rowonneon	GRODIVAGVI	CHROEPHINIC	ITOITVI DANKI V	
AAD34205.1 Vasilchenko				S				s			Т	v		
AMZ80006.1 TV08-T2546				T. S				A 5			т.			
ADB24728.1 Lesonark11				5				S			т.			
ABF46836 1 EK-328				s				s			т			
ADB24726.1 205KGG				S							Т	S		
ADB24734.3 DV936k											т.			
	150	160	170	180	190	200	210	220	230	240	250	260	270	280
														- I
AMQ49166.1 Absettarov	VKVEPHTGDYVAANETH	HSGRKTASFTV	/SS <mark>EK</mark> TILTMG	EYGDVSLLCR	VASGVDLAQTV	/ILELDKTVEH	LPTAWQVHR	DWFNDLALPWK	HEGAQNWNNZ	ERLVEFGAPH	AVKMDVYNLO	DQTGVLLKA	LAGVPVAHIEG	ΓK
AAC62097.1 256														
AAD34205.1 Vasilchenko				D		L	R.		Q			s	D.	Α.
AMZ80006.1 TV08-T2546				D		L		E	H			s	D.	
ADB24728.1 Lesopark11				D		L		R	H			s	D.	
ABF46836.1 EK-328			N	D		L		R	H			s	D.	
ADB24726.1 205KGG				D				R				·····s	5D.	•••
ADB24734.3 DV936k				D								······s	5D.	•••
	290	300	310	320	330	340	350	360	370	380	390	400	410	420
3MO40166 1 35							-		.	-				• 1
AMU49166.1 ADSettarov	INLESSAVICEVGLERI	LKMKGLIIIMO	DKIKLIWKRA	PIDSGRDIVVI	MEVIPSGIKP	RIPVRAVANG	SPUVNVAML	IIPNPIIEMMG	GGEIEMQLPI	GDNIIIVGEL	SHQWIQKGS:	GRVFQKIF	KGIERLIVIGE	AA.
AAC02097.1 200			л т											
AAD34203.1 VASIICHENKO			A1					•••••						
AB200000.1 1000-12340			т л					•••••						
ABE46836 1 FK-328			D T					•••••						· ·
ADB24726 1 205KGG		т	т		۵			•••••				E		
ADB24720.1 200100			т		Δ									
ADDETIONIC DVDOOR														
	430	440	450	460	470	480	490							
							. .							
AMQ49166.1 Absettarov	WDFGSAGGFLSSIGKAV	VHTVLGGAFNS	FGGVGFLPK	LLLGVALAWL	GLNMRNPTMS	ISFLLAGGLVL	AMTLGVGA							
AAC62097.1 256														
AAD34205.1 Vasilchenko	TT.VI	L												
AMZ80006.1 TV08-T2546	TT.VI	6	R	A										
ADB24728.1 Lesopark11	TA.VI	6												
ABF46836.1 EK-328	TT.VI	6												
ADB24726.1 205KGG	TT.VI	6	L	I.M		.G								
ADB24734.3 DV936k	TA.VI	6	L	I.VV										

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 $\pm \frac{1}{2}$ 95% CI. * - corresponds to significant difference with strain 256 with $p \le 0.05$.

	E protein concentration (mg/L)							
Strain	Estimated from	Estimated from GCP						
	infectious							
	virions (×10-5)	(×10-2)						
Absettarov	$19 \pm 9*$	$0.3\pm0.1*$						
256	1.5 ± 0.1	96 ± 52						
Vasilchenko	$126\pm60*$	3 ± 1						
TV08-T2546	$41 \pm 28*$	5 ± 5						
Lesopark11	$6 \pm 4^*$	$0.6\pm0.2*$						
EK-328	11 ± 10	6 ± 5						
DV 936k	$23\pm8^*$	11 ± 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