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Supplementary Information for

**Hepatitis E virus infects brain microvascular endothelial cells, crosses blood-brain barrier, and
invades the central nervous system**

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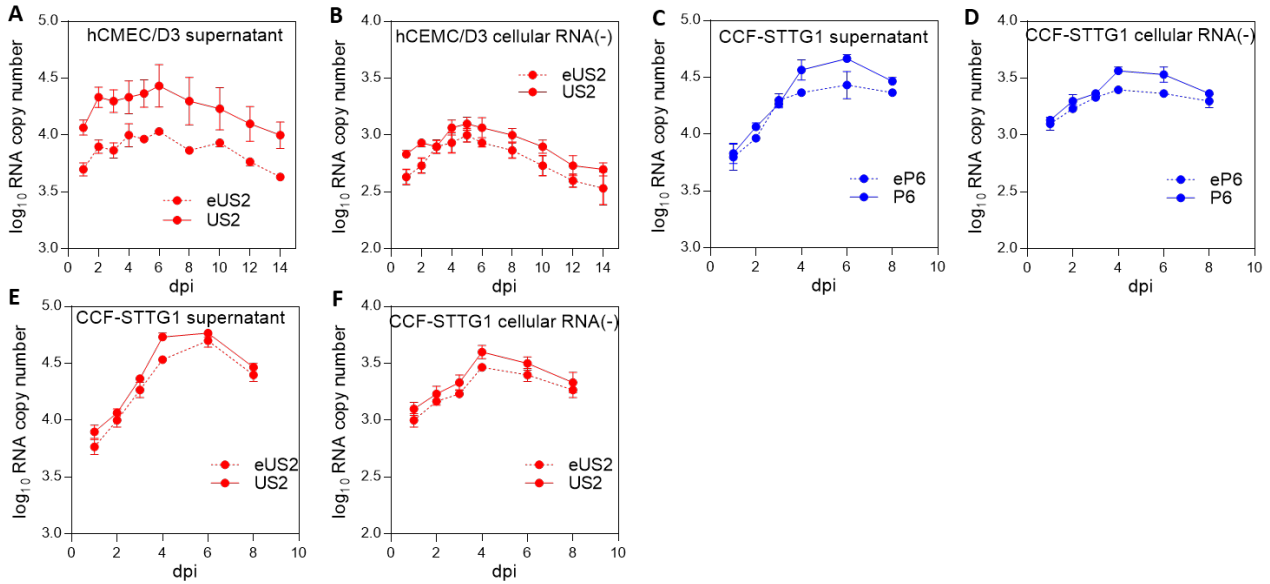
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- Figure S1
- Tables S1, S2



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21 **Figure S1.** HEV infects brain microvascular endothelial cells lining the blood-brain barrier (BBB) as

22 well as the astrocytes. The brain microvascular endothelial cells (hCMEC/D3) grown in a 12-well plate

23 were inoculated with 1.0×10^6 genomic RNA copies of quasi-enveloped eUS2 virus stock or with non-

24 enveloped US2 virus stock and incubated for 2 h. After washing the cells twice, culture medium was

25 added and the infected cells were cultured for 14 days. Culture supernatants and monolayer cells were

26 collected at indicated different time points and subjected to quantification of HEV RNAs (**A**) and

27 negative-stranded RNAs (**B**) by qRT-PCR. The astrocytes (CCF-STTG1) grown in a 12-well plate were

28 inoculated with 1.0×10^6 genomic RNA copies of HEV quasi-enveloped virus eP6 or non-enveloped

29 virus P6 (**C, D**), and with HEV quasi-enveloped virus eUS2 or non-enveloped US2 (**E, F**), respectively,

30 and incubated for 2 h. After washing twice, culture medium was added and the infected cells were

31 cultured for 8 days. Culture supernatants and monolayer cells were collected at indicated different time

32 points and subjected to quantification of HEV RNA (**C, E**) and negative-strand HEV RNA (**D, F**) by

33 qRT-PCR.

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Table S1. Oligonucleotide primers used in this study

Primer ID	Sequence (5'-3')	Used for
JVHEV F	GGTGGTTTCTGGGGTGAC	qRT-PCR, HEV
JVHEV R	AGGGGTTGGTTGGATGAA	qRT-PCR, HEV
JVHEV P	FAM-TGATTCTCAGCCCTTCGC-BHQ)	qRT-PCR probe, HEV
Tag-FP	CGGTCATGGTGGCGAATAAGGTGGTTTCTGGGGTGAC	cDNA synthesis, HEV RNA (-)
Tag	CGGTCATGGTGGCGAATAA	qRT-PCR, HEV RNA (-)
F-P6-5487	TGACAGGGTTGATTCTCAGCCCTTC	Nested PCR, HEV
R-P6-6876	ACCAGTGGCCACATTGACAAAGGT	Nested PCR, HEV
F-P6-5713	ACAGCCCCTGTACCTGATGTTGATTC	Nested PCR, HEV
R-P6-6623	TCTTGCTGCGCATTCTCCACAGATG	Nested PCR, HEV
F-P-GAPDH 341	GATGGTGAAGGTCGGAGTG	qRT-PCR, pig GAPDH
R-P-GAPDH 579	GATCTCGCTCCTGGAAGATG	qRT-PCR, pig GAPDH
F-P-Occludin 1351	TCCACCCATCACTTCAGATC	qRT-PCR, pig Occludin
R-P-Occludin 1532	ATTCATCAGCAGCAGCCATG	qRT-PCR, pig Occludin
F-P-ZO1a	ACCAGCCTCTGCTGTTAATCCT	qRT-PCR, pig ZO1a
R-P-ZO1a	GCTCTTGGTCTCTGGGCACT	qRT-PCR, pig ZO1a
F-P-VE 691	GTGCCCATGTACAAGATCG	qRT-PCR, pig VE-C
R-P-VE 889	CTGGGTCCTTGACAAACAG	qRT-PCR, pig VE-C
F-P-Claudin5 289	GTGCAAGGTGTACGACTCGG	qRT-PCR, pig Claudin 5
R-P-Claudin5 542	AGAACTCGCGGACCACGATG	qRT-PCR, pig Claudin 5

38 **Table S2.** HEV RNA loads (log₁₀ per mL or per gram of tissue) in various samples from pigs
 39 experimentally-infected with the membrane-associated quasi-enveloped eUS2 virus and the non-
 40 enveloped US2 virus
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Group	Pig no.	Serum				Feces						Bile	CSF		Spinal cord	Brain
		0 dpi	7	14	21	0	3	7	10	14	21	21	3	21	21	21
Control	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
eUS2	21	0	0	0	0	0	0	6	6.5	6.5	0	3.7	0	0	0	0
	22	0	0	3.8	0	0	0	5.9	7.1	7.7	5.7	5.8	0	0	0	4.3
	23	0	0	0	0	0	0	5.4	7.2	6.3	0	0	0	0	0	0
	24	0	0	0	0	0	0	5.8	6.7	6	0	4.6	0	0	0	0
	25	0	0	0	0	0	0	5.1	5.4	5	0	0	0	0	0	0
	51	0	0	0	0	0	0	5.8	6.4	5	0	0	0	0	0	0
	52	0	0	0	0	0	5.7	6.2	6.6	6.7	0	0	0	0	0	0
US2	1	0	3.3	0	0	0	6.8	7.5	6.9	6.2	6.4	6.8	0	0	4.9	5.0
	2	0	3.5	5	4.6	0	6.3	7.1	6	8.4	8.5	8.8	0	4.4	0	5.2
	3	0	0	4.9	0	0	5.2	7.9	8.4	8	7.7	8.5	0	0	0	0
	4	0	0	4.9	5.6	0	6.4	7.5	8.1	9.1	9.4	8.8	0	0	0	0
	5	0	0	4.8	4.8	0	6.6	7.4	8.4	8.8	9.2	9.4	0	0	0	5.1
	6	0	0	4.1	0	0	6.5	6.1	6.6	7.9	6.3	4.9	0	0	0	0
	7	0	0	0	0	0	6.8	7.9	8.4	6.7	7.3	0	0	0	0	0

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