PNAS

Supporting Information for

Modeling extrahepatic hepatitis E virus infection in induced human primary neurons

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Other supporting materials for this manuscript include the following:

- Movies S1

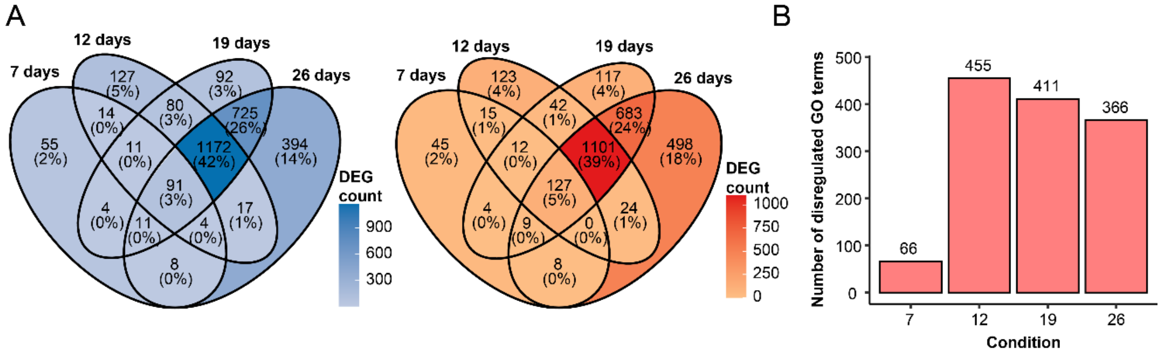

Fig. S1. **Regulation of genes during the neuron differentiation process**. **(A)** Venn diagram of significant down-regulated differentially expressed genes (DEGs, blue) and up-regulated DEGs (red) at different stages of differentiation compared to the earliest time point of 4 days. **(B)** Overview of the number of significant deregulated Gene ontology (GO) terms during the differentiation process.

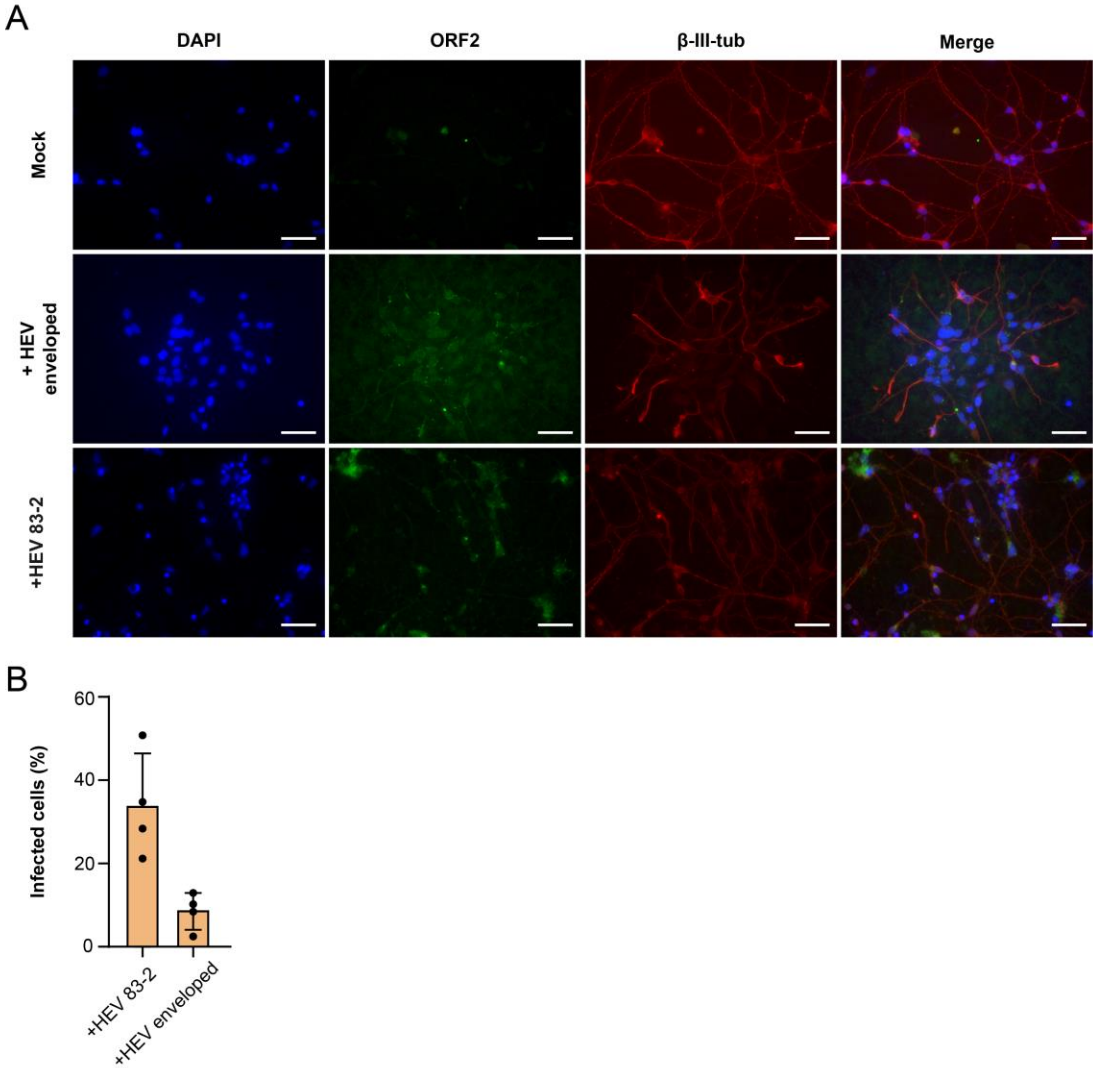

Fig. S2. Infection of human induced primary neurons with quasi-enveloped HEVcc Kernow-C1 p6 strain and wild boar HEV-3 strain 83-2. (A) Immunofluorescence staining was performed for uninfected (Mock) and infected induced primary neurons (iPNs) with quasi-enveloped Kernow-C1 p6 strain and the wild boar HEV-3 strain 83-2. 4′,6-diamidino-2-phenylindole (DAPI) was used to stain the nuclei (blue), a polyclonal rabbit anti-HEV antiserum to stain the ORF2-encoded capsid protein (green) and an anti-β-III-tubulin (β-III-Tub) antibody to stain the neuronal cytoskeleton. Scalebars represent 50 µm. (B) The susceptibility of neurons to both HEV strains was quantified by measuring the ORF2 signal using CellProfiler.

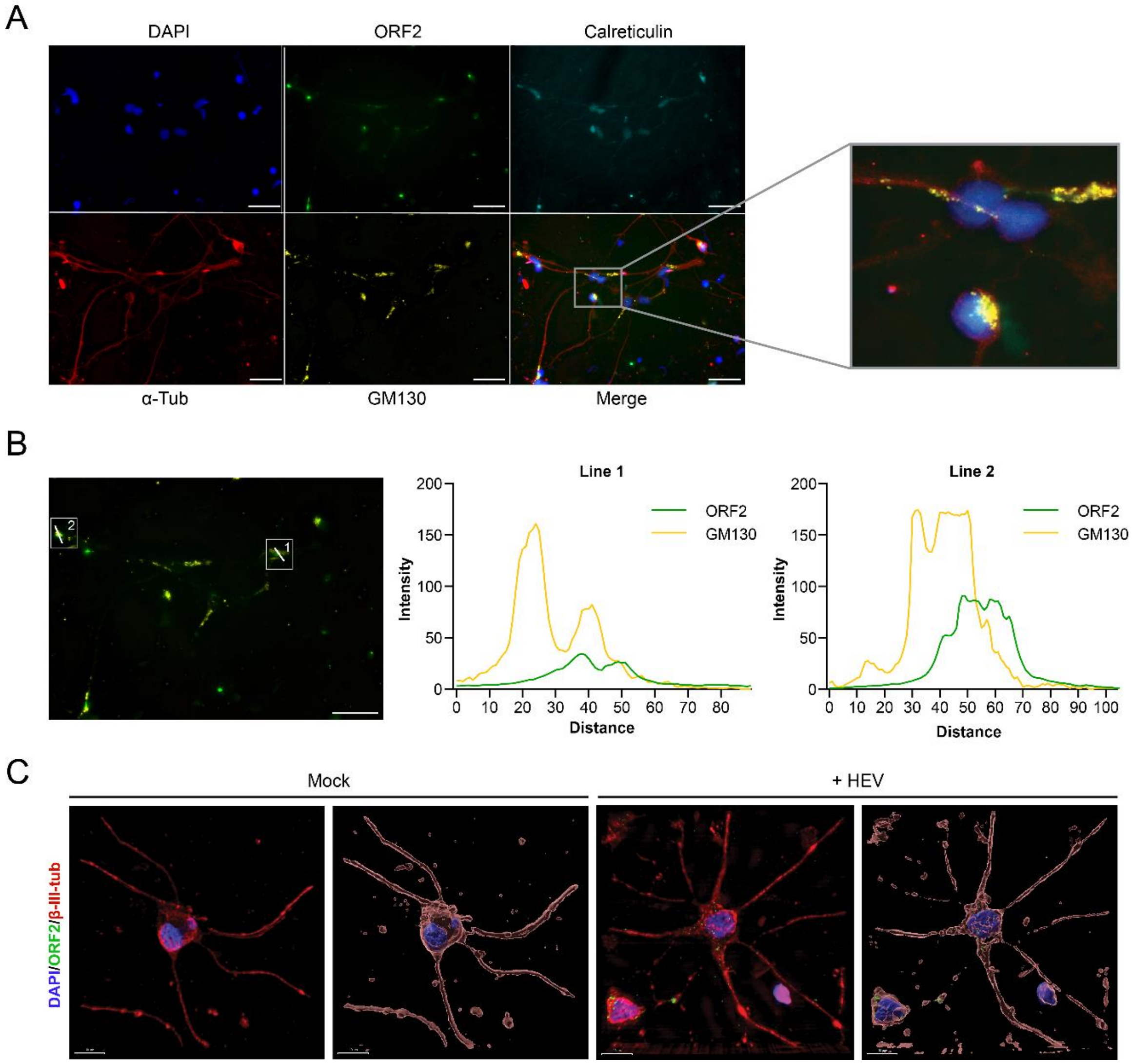

Fig. S3. The ORF2 encoded capsid protein of HEV is colocalized with the Golgi apparatus. (A) Virus localization was accessed by iterative indirect immunofluorescence imaging using multiple compartment markers. In different rounds of staining, antibodies of ORF2 (green), α-tubulin (α-Tub, red), GM130 (yellow) and calreticulin (cyan) were used to stain the HEV capsid protein, cytoskeleton, Golgi apparatus and the endoplasmic reticulum, respectively. The nucleus was stained by DAPI. White scalebar represents 50 µm **(B)** Line graphs show the fluorescence intensities of ORF2 capsid protein (green) and Golgi apparatus (GM130, yellow) measured across the regions of interest indicated by the white lines in the immunofluorescence image. White scalebar represents 50 µm. **(C)** Confocal microscope images of uninfected iPNs (Mock) and HEV-infected neurons were acquired. A 3D reconstruction of the surfaces was performed using the analysis tool Imaris 9.8.0 and a video was also generated. Cells were stained with DAPI (blue), with an antibody against ORF2-encoded capsid protein (green) and with β-III-tubulin (red). White scalebar represents 10 µm.

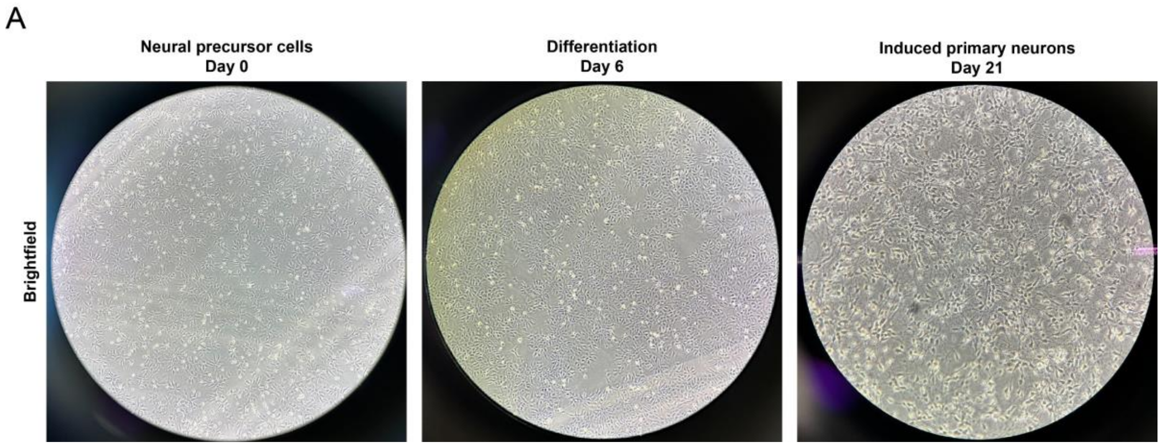

Fig. S4. Neurite development during differentiation into induced primary neurons. Brightfield images were taken at three differentiation timepoints: neural precursor cells (day 0), cells during differentiation (day 6), and induced primary neurons after 21 days of differentiation.

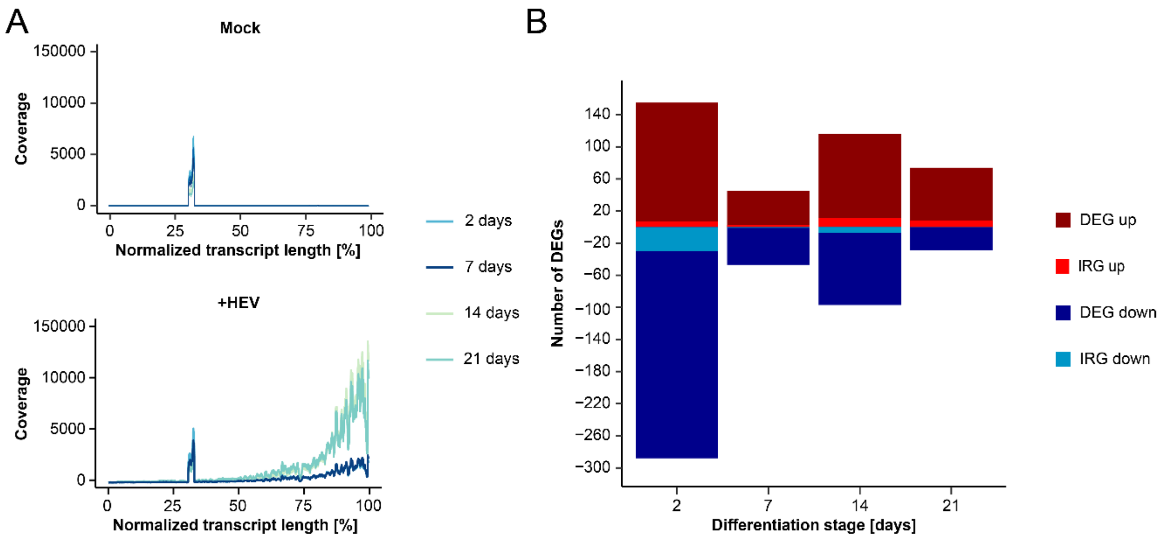

Fig. S5. General response of primary neurons to HEV infection. (A) Normalized coverage of mapped reads along the HEV genome in uninfected (Mock) and infected neurons (+HEV, five days post-infection) at the timepoints of 2, 7, 14 and 21 days of differentiation. **(B)** The number of differentially expressed genes (DEGs) by HEV infection compared to uninfected cells is shown over the course of differentiation. An online single-cell database of interferon (IFN)-regulated genes (IRGs) (http://isg.data.cvr.ac.uk/) was utilized, filtered for genes significantly up- or downregulated (FDR $\leq$ 0.05 and $\log_2$ fold change of 2 or -2) in humans, and were compared to the identified DEGs. Lighter colors indicate the number of IRGs that were significantly upregulated (red) or downregulated (blue).

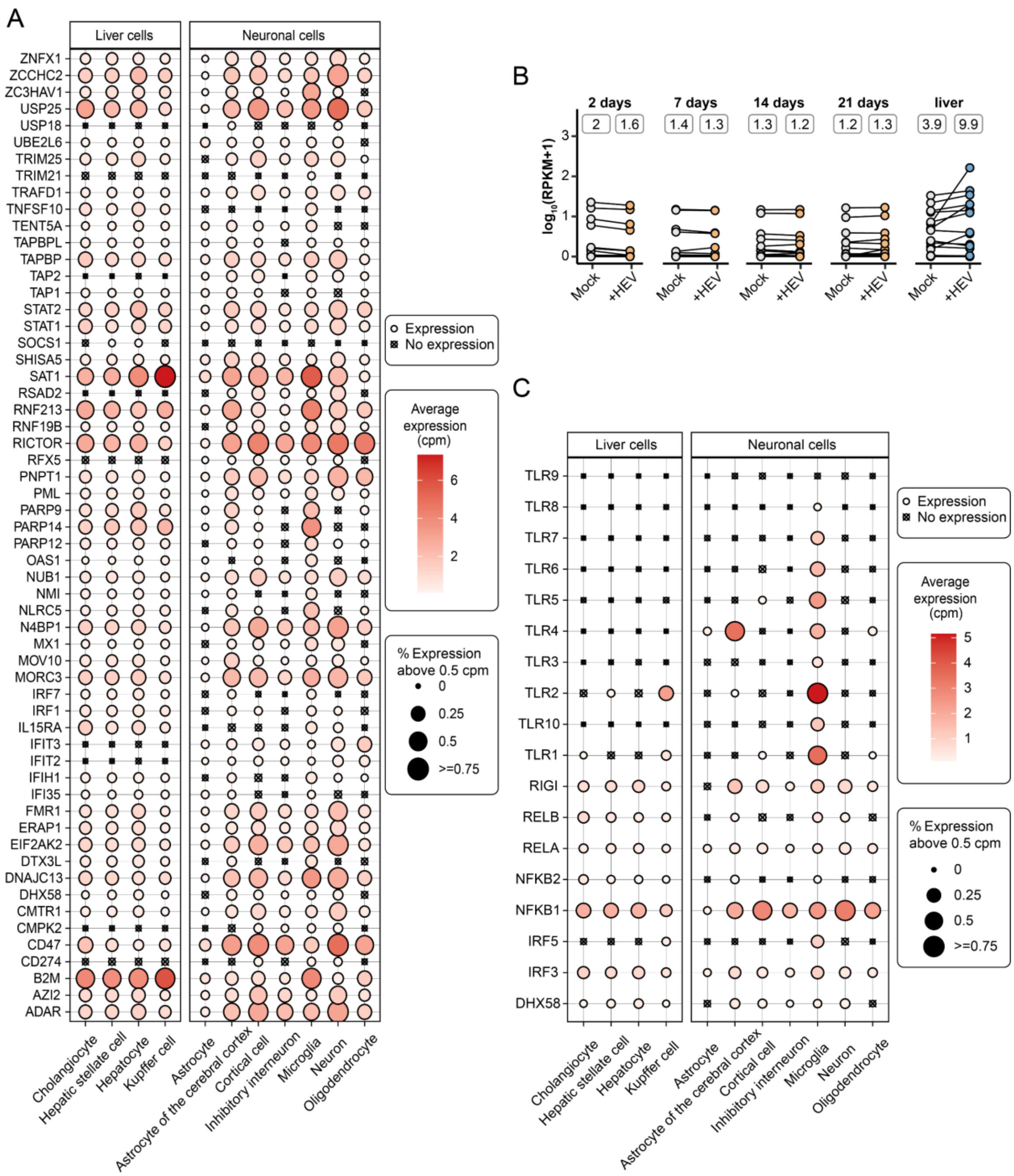

Fig. S6. Pathogen sensing in primary neurons and interferon-stimulated gene (ISG) expression across nervous system cell types. (A) Expression of ISGs in neuronal and liver cells in publicly available single-cell databases. **(B)** Overview and comparative analysis of the expression levels of pathogen-sensing genes measured in RPKM values, in both uninfected (Mock) and HEV-infected (+HEV) induced primary neurons and primary human hepatocytes (liver). The values above displayed represent the mean RPKM expression levels of all genes under each respective condition. **(C)** Expression of sensing genes in neuronal and liver cells in publicly available single-cell databases. cpm: counts per million. RPKM: Reads per kilobase of transcript per million mapped reads.

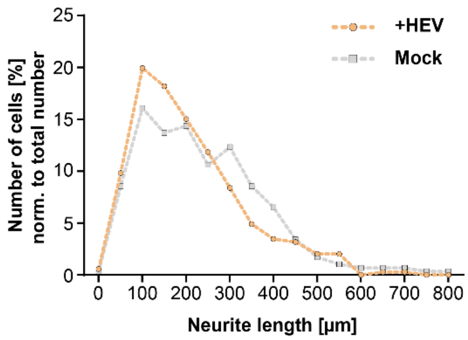

Fig. S7. Shift in neurite length after HEV inoculation. The neurite length of mock-inoculated (Mock) and HEV-inoculated (+HEV) iPNs (21-day-old cells) was examined through microscopic analysis five days post-inoculation. The frequency distribution is depicted as the percentage of cells (normalized to the total cell number) with a given neurite length.


DEG: Downregulated Unchanged Upregulated
ACSF2
ACY1
ADAMTS14
ADORA2A
ADRA2A
AHSG
ALB
ARAP3
ARHGAP11A−SCG5
ARRDC3
ATF3
ATF7−NPFF
BCAN
BOLA2B
CA2
CAV1
CCDC8
CDC42EP2
CNPY1
COL13A1
COL9A3
CSPG4
CYTL1
DIPK1C
DKKL1
ENG
FAM156A
FAM156B
FOSL1
GADD45B
GDF15
ENSG00000173867
ENSG00000254536
ENSG00000254706
ENSG00000255439
ENSG00000255872
ENSG00000258465
ENSG00000258830
ENSG00000262304
ENSG00000271741
ENSG00000272921
ENSG00000273167
ENSG00000284057
ENSG00000284292
ENSG00000284820
ENSG00000285901
ENSG00000286001
ENSG00000288640
ENSG00000289027
GGT1
GSX1
HMOX1
HSPA6
HSPE1−MOB4
ID1
ID3
LIF
MAFA
MOV10
NKX1−2
NNMT
NPAS4
NPIPA3
NPIPA8
NTF3
NUDT18
NUDT8
P3R3URF−PIK3R3
PABPC1L
PCP4L1
PENK
PHOX2A
PITX2
PLAU
PLAUR
PLD5P1
PRDM12
PRDM13
PRSS53
RENBP
RGS5
RNF135
ROBO3
RPL36A−HNRNPH2
RSC1A1
SERPINE1
SEZ6L
SLC15A3
SLC38A4
SLX1B
SNAI1
SNCB
STIMATE−MUSTN1
SULT1A4
SYNPR
TBCEL−TECTA
TGFBI
TMEM100
TNFRSF10D
TREX1
VEGFA
VMAC
ZFP91−CNTF
Log2 fold change
Differentiation stage [days]

Fig. S8: Differential regulation of genes in 21-day-old neurons relative to various stages of differentiation. The genes presented display either upregulation or downregulation in 21-day-old primary neurons following HEV infection. These differentially regulated genes (DEGs) are depicted here in comparison with their expression patterns in 2, 7, and 14-day-old cells.

Table S1. Association between upregulated human genes at 21 days post-HEV infection and neurite length. Related genes include only genes of the same family. / indicating no correlation with neurite outgrowth.

Gene name (explanation)	Involved in neurite outgrowth regulation	Reported potential function of the encoded protein	Refer-ences	Log_2 fold change	False discovery rate (FDR)	Mock: Reads per kilobase of transcripts per million mapped reads	+HEV: Reads per kilobase of transcripts per million mapped reads
MOV10 (MOV10 RISC complex RNA helicase)	yes	Knockout of *MOV10* gene in murine neuroblastoma (Neuro2a) cells lead to reduced neurite length, which could be rescued upon re-induction of *MOV10* gene	(1, 2)	1.340118	4.392000e-09	0.58869067	1.49156581
ATF3 (Activating transcription factor 3)	yes	ATF3 promotes neurite outgrowth in rat dorsal root ganglion neurons	(3, 4)	1.055239	2.749000e-06	1.73958633	3.61697248
TREX1 (Three prime repair exonuclease 1)	yes	A *TREX1* mutant (V91M) lead to a significant reduction in axonal outgrowth in mouse primary cortical neurons compared to the wildtype cells	(5)	1.654444	2.098385e-02	0.2436382	0.7727430
TGFBI (Transforming growth factor beta induced)	yes	TGFB increased neurite outgrowth in scratched primary midbrain	(6–9)	1.148161	7.208000e-14	4.5132269	10.0050609

	yes	cultures of dopaminergic cells (Wistar rats); TGFBI rescued neurite outgrowth in inhibitory environment via canonical ALK5/SMAD3 signaling in mouse N1E-115 neuron-like cells; TGF-/31 has a capability of promoting axonal regeneration in rat hippocampal neurons					
ARAP3 (ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3)	yes	Neurites outgrowth of rat pheochromocytoma (PC12) cells was affected by *ARAP3* under NGF or bFGF treatment	(10, 11)	1.355979	2.495000e-13	1.078767	2.762672
VEGFA (Vascular endothelial growth factor A)	yes	VEGF enhanced axonal/neurite outgrowth of mouse dorsal root and superior cervical ganglia, rat retinal ganglion cells and rat cerebral cortical neurons	(12–16)	1.027099	2.351000e-06	1.310088	2.671190
SERPINE1 (Serpin family E member 1)	yes	PAI-1 (plasminogen activator inhibitor-1, Serpine1), an endogenous inhibitor of tissue plasminogen activator (tPA), led to a decrease neurite length	(17, 18)	1.710986	1.403000e-09	0.54960125	1.80225088

		of rat primary neuronal progenitor cells					
CAV1 (Caveolin 1)	yes	Caveolin 1 was required for axonal outgrowth of motor neurons (from *Xenopus*); synapsin-Calveolin-1 enhanced axonal growth in differentiated NPCs derived from human iPSCs	(19, 20)	1.406676	3.374000e-04	0.3073646	0.8167771
ENG (Endoglin)	yes	Endoglin is a co-receptor of transforming growth factor β (TGF-β), thus it participates in TGF-β signaling pathway	(21)	1.130649	1.031000e-12	3.97019850	8.69529148
PLAU (Plasminogen activator, urokinase)	yes	Inhibition of uPA (another abbreviation for PLAU) gene expression reduced axonal length in Neuro2 cells and mouse dorsal root ganglia	(22, 23)	2.015380	1.463000e-10	0.44035605	1.78463341
FOSL1 (FOS like 1, AP-1 transcription factor subunit)	yes	FOSL1 siRNA injection in rats led to reduced axon growths in Schwann cells	(24)	4.547930	6.536000e-04	0.03115952	0.77415355
NPAS4 (Neuronal PAS domain protein 4)	yes	*NPAS4* gene knockdown in Neuro2a cells inhibited neurite outgrowth; In NPAS4 knockout hippocampal	(25)	1.423217	3.957000e-04	0.31114766	0.83648270

		neurons (mouse) neurite outgrowth was abolished					
NNMT (Nicotinamide N-methyltransferase)	yes	NNMT stable expression in SH-SY5Y cells reduced the neurite length compared to SH-SY5Y cells without NNMT, whereas the number of neurites and branches increased	(26)	2.016775	1.567549e-02	0.1095445	0.4487772
ROBO3 (Roundabout guidance receptor 3)	yes	The lack of ROBO3 in mouse led to a decreased axon outgrowth in spinal cord explants	(27)	1.433759	4.375000e-30	17.05178	46.07125
CSPG4 (Chondroitin sulfate proteoglycan 4)	yes	CSPG reduced neurite length in mouse cerebral cortical neurons and granule cells in a dose-dependent manner; led also in human-derived SH-SY5Y cells to reduction of neurite length; CSPG reduced neurite length in mouse cerebellar granule cells in a dose-dependent manner; NG2 (synonym for CSPG4) also inhibited neurite growth from embryonic rat	(28–32)	1.128576	3.951000e-06	0.40782702	0.89232606

		dorsal root ganglia neurons					
ACSF2 (Acyl-CoA synthetase family member 2)	yes	ACSF2-deficient Neuro2a cells showed significantly blunted neurite outgrowth in response to retinoic acid; Acyl-CoA synthetase 2 overexpression enhanced neurite outgrowth in PC12 cells	(33, 34)	1.136887	2.484000e-03	0.5538275	1.2198368
GADD45B (Growth arrest and DNA damage inducible beta)	yes	Mutated GADD45B mice showed impaired activity-dependent neurogenesis and dendritic development; GADD45G/p38 MAPK/CDC25B signaling pathway enhanced neurite outgrowth by promoting microtubule polymerization	(35–37)	2.575122	1.250000e-14	0.5593153	3.3446735
GDF15 (Growth differentiation factor 15)	yes	The neurites length of the primary culture of rat retinal ganglion cells were increased by GDF15 treatment	(38)	1.549360	2.145562e-02	0.20693118	0.60944003
PLAUR (Plasminogen activator, urokinase receptor)	yes	Exogenous uPA (PLAU) increased neurite length in mouse Neuro2a cells	(23, 39)	1.597618	8.919000e-05	0.2644716	0.8027418

		most likely via the interaction with uPAR (PLAUR); blocking of uPAR stimulated neurite formation					
CCDC8 (Coiled-coil domain containing 8)	yes	CCDC8 increased the number of neurites per cell in rat PC12 cells	(40)	1.014808	3.547000e-07	1.96235660	3.96682179
LIF (LIF interleukin 6 family cytokine)	yes	LIF promoted neurite outgrowth of rat mammalian auditory neurons in vitro; LIF promoted neurite growth of P12-E2 cells	(41, 42)	1.708004	1.181000e-03	0.14429893	0.47372902
HSPA6 (Heat shock protein family A (Hsp70) member 6)	Related genes	HSP70 alone and especially in combination with HSP40 increased neurite outgrowth in Neuro2a cells; Hsp27 is involved in GDNF-induced neurite outgrowth in rat PC12-GFRR1-RET cells; Neurite outgrowth was mediated by the heat shock protein HSP90a	(43–45)	5.420693	4.269000e-49	0.23718946	10.2284998 4
CNPY1 (Canopy FGF signaling regulator 1)	Related genes	CNPY2 (MSAP) increased the number of neurites on mouse N2-A and rat PC12 cells	(46)	1.105491	1.345000e-06	0.9920355	2.1358922

TNFRSF10D (TNF receptor superfamily member 10d)	Related genes	Tumor necrosis factor (TNF) decreased the total neurite length per cell, the axonal length and the number of branch points of primary mice hippocampal neurons cocultured with astrocyte-enriched glial cells	(47)	1.009861	6.689000e-05	0.9730365	1.9607246
CA2 (Carbonic anhydrase 2)	Related genes	CA8 induced neurite outgrowth in Neuro-2a cells but not in human SK-N-SH cells	(48)	1.173081	1.356000e-05	1.55652100	3.51307191
PRDM12 (PR/SET domain 12)	Related genes	Disruption of the PRDM14 gene resulted in axon growth defects in zebrafish	(49)	1.605972	2.794000e-13	1.303463	3.971309
PLD5P1 (Phospholipase D family member 5 pseudogene 1)	Related genes	Phospholipase D1 inhibited neurite outgrowth of rat neural stem cells via increase of synapsin 1 expression; Overexpression of PLD1 resulted in neurons to increase tissue plasminogen activator (tPA) release and therefore to tPA-dependent neurite extension	(50, 51)	1.077125	2.514724e-02	0.2453015	0.5186789

ADAMTS14 (ADAM metallopeptidase with thrombospondin type 1 motif 14)	Related genes	ADAMTS4 and ADAMTS5 led to an increase in neurite length in rat primary neurons	(52)	1.414671	3.603000e-06	0.3116316	0.8320400
SLC15A3 (Solute carrier family 15 member 3)	Related genes	SLC25A12 expression was associated with dendrites outgrowth in mouse embryonic cortical neurons	(53)	1.547206	7.039000e-03	0.1887240	0.5542340
SLC38A4 (Solute carrier family 38 member 4)	Related genes	SLC25A12 expression was associated with dendrites outgrowth in mouse embryonic cortical neurons	(53)	2.649440	2.497000e-05	0.07092644	0.44991230
RNF135 (Ring finger protein 135)	Related genes	Molluscan RING-finger protein L-TRIM is essential for neuronal outgrowth	(54)	1.374997	4.669022e-02	0.1963424	0.5120273
VMAC (Vimentin type intermediate filament associated coiled-coil protein)	Related genes	The length of astrocytic processes after entorhinal cortex lesion was shorter in GFAP-/-Vim-/- than wildtype mice (Vim=Vimentin)	(55)	1.107776	3.657000e-03	0.6299788	1.3598131
DKKL1 (Dickkopf like acrosomal protein 1)	Related genes	Dickkopf-1 protein (Dkk1) induced neurite outgrowth in TC-32 cells	(56)	1.540152	2.998727e-02	0.2884431	0.8444769
SNAI1	Related genes	SNAIL transcription factor in prostate cancer	(57)	4.665358	6.446000e-06	0.05172846	1.36721883

(Snail family transcriptional repressor 1)		cells promoted neurite outgrowth					
RSC1A1 (regulator of solute carriers 1)	/	/	/	2.927757	3.623000e-04	0.07599916	0.58922164
ID3 (Inhibitor Of DNA Binding 3)	/	/	/	1.748242	3.465000e-30	11.0787844 4	37.2322951 4
ACY1 (Aminoacylase 1)	/	/	/	1.214575	5.344000e-03	0.4597700	1.0693794
AHSG (Alpha 2-HS glycoprotein)	/	/	/	1.638499	6.277000e-04	0.4287744	1.3402270
CYTL1 (Cytokine like 1)	/	/	/	1.380700	1.976895e-02	0.5647939	1.4770756
ALB (albumin)	/	/	/	2.926996	1.716000e-22	0.45646804	3.48247791
ARRDC3 (Arrestin domain containing 3)	/	/	/	1.051950	6.683000e-14	8.266954	17.142819
PRDM13 (PR/SET domain 13)	/	/	/	1.489413	5.248000e-03	0.19102079	0.53853983
NUDT18 (Nudix hydrolase 18)	/	/	/	1.008633	7.463000e-03	0.8883395	1.7897523
COL13A1 (Collagen type XIII alpha 1 chain)	/	/	/	1.657751	1.174000e-11	0.6850415	2.1640083
NUDT8 (Nudix hydrolase 8)	/	/	/	1.076710	3.940508e-02	0.8883395	1.7897523

GSX1 (GS homeobox 1)	/	/	/	1.575707	5.544000e-03	0.2902809	0.8695302
NPIPA8 (Nuclear pore complex interacting protein family member A8)	/	/	/	1.261234	4.423000e-03	0.59556003	1.43100559
SLX1B (SLX1 homolog B, structure-specific endonuclease subunit)	/	/	/	5.257244	3.554000e-20	0.1814754	7.0508826
TMEM100 (Transmembrane protein 100)	/	/	/	1.055878	2.705273e-02	0.37648448	0.78435276
ID1 (Inhibitor Of DNA Binding 1)	/	/	/	2.002127	7.104000e-13	1.28754784	5.16763625
PABPC1L (Poly(A) binding protein cytoplasmic 1 like)	/	/	/	1.165967	2.460000e-04	0.60324994	1.35526644
COL9A3 (Collagen type IX alpha 3 chain)	/	/	/	1.177167	3.118000e-03	0.4791457	1.0854985
GGT1 (Gamma-glutamyltransferase 1)	/	/	/	1.093269	4.897000e-03	0.34972246	0.74733914
HMOX1 (Heme oxygenase 1)	/	/	/	1.390076	7.152000e-06	0.8629264	2.2650443
FAM156A	/	/	/	1.007986	1.842000e-10	2.654675	5.340031

(Family with sequence similarity 156 member A)							
RENBP (Renin binding protein)	/	/	/	1.017142	3.818000e-03	1.112195	2.253698
gene:*ENSG00000271741*	/	/	/	2.823195	1.416000e-05	0.0853795	0.6117999
gene:*ENSG00000254706*	/	/	/	1.233511	1.727228e-02	0.6527534	1.5394736
HSPE1-MOB4 Readthrough	/	/	/	2.099540	1.567000e-03	0.3633585	1.5715336
gene:*ENSG00000284820*	/	/	/	1.405860	6.634000e-05	0.2522317	0.6696288
STIMATE-MUSTN1 Readthrough	/	/	/	1.252719	4.557356e-02	0.3728846	0.8921909
gene:*ENSG00000286001*	/	/	/	1.012625	2.275000e-03	0.5374444	1.0855183
gene:*ENSG00000289027*	/	/	/	5.189919	2.232000e-12	0.02737338	1.02447209
gene:*ENSG00000284292*	/	/	/	1.213351	2.323063e-02	0.2717099	0.6319702
gene:*ENSG00000288640*	/	/	/	1.230503	4.934000e-09	1.4441618	3.3907027
gene:*ENSG00000254536*	/	/	/	1.179328	2.286894e-02	0.3346430	0.7600015
ZFP91-CNTF Readthrough	/	/	/	1.918956	8.172000e-04	0.1824514	0.6944137
gene:*ENSG00000285901*	/	/	/	2.267598	7.375000e-15	0.3936985	1.9001755

ATF7-NPFF Readthrough	/	/	/	3.141335	1.104214e-02	0.05481481	0.50224828
gene:*ENSG00000258830*	/	/	/	1.653482	2.940749e-02	0.15348320	0.48679998
gene:*ENSG00000273167*	/	/	/	1.298390	8.305000e-03	0.1909486	0.4710442
ARHGAP11A-SCG5 Readthrough	/	/	/	1.304814	6.584000e-04	0.3203187	0.7929361
gene:*ENSG00000173867*	/	/	/	1.029718	4.505890e-02	0.2787915	0.5704761
gene:*ENSG00000255439*	/	/	/	3.833119	9.724000e-09	0.07577064	1.09860765

Table S2. Association between downregulated human genes at 21 days post-HEV infection and neurite length. Related genes include only genes of the same family. / indicating no correlation with neurite outgrowth.

Gene name (explanation)	Involved in neurite outgrowth regulation	Reported potential function of the encoded protein	References	Log_2 fold change	False discovery rate (FDR)	Mock: Reads per kilobase of transcripts per million mapped reads	+HEV: Reads per kilobase of transcripts per million mapped reads
BCAN (Brevican)	yes	Brevican inhibits neurite outgrowth from rat cerebellar granule neurons; Brevican is an inhibitor of neurite outgrowth in the proteoglycan-enriched myelin fraction in cerebellar granule cells	(58, 59)	-1.358337	1.465000e-21	13.3752915	5.2167392
PCP4L1 (Purkinje cell protein 4 like 1)	yes	PCP4 enhance neurite outgrowth, while a knockdown reduces neurite outgrowth in PC12 cells	(60)	-1.344553	1.421000e-04	2.53007119	0.99478298
RGS5 (Regulator of G protein signaling 5)	yes	RGS5 leads to an inhibitory effect of neurite outgrowth by sonic hedgehog in mouse primary cortical neurons; RGS5 switched astrocytes from neuroprotective to	(61, 62)	-1.426282	8.729000e-10	1.3988044	0.5201488

		pro-inflammatory property via binding to the receptor TNFR2					
PITX2 (Paired like homeodomain 2)	yes	Loss of PITX2 disrupts axonal growth in the dorsal midbrain in mouse	(63)	-1.336801	3.957000e-04	0.60078189	0.23744417
PENK (Proenkephalin)	yes	Proenkephalin produces the peptide [Met]enkephalin and [Let]enkephalin via proteolytic cleavage; [Met]enkephalin and [Leu]enkephalin both has a neurite growth-promoting effect in rat dorsal root ganglia	(64)	-1.529499	1.205000e-10	2.83594972	0.98163152
CDC42EP2 (CDC42 effector protein 2)	yes	bind to, and negatively regulate the function of CDC42; CDC42 stimulates neurite outgrowth in primary chick spinal cord neurons ; Cdc42 plays a l role in neurite outgrowth of PC12 cells and cerebellar granule neurons	(65, 66)	-2.061407	8.766000e-05	0.9645701	0.2298073
NTF3 (Neurotrophin 3)	yes	NTF3 promotes neurite outgrowth in rat hippocampal cells and sensory neurons	(67–69)	-1.199443	3.067719e-02	1.17344583	0.50953183

ADORA2A (Adenosine A2a receptor)	yes	An ADORA2A antagonist rescued the decreased neurite length in rat primary cortical neurons from an attention deficit and hyperactivity disorder model; the suppression of ADORA2A seems to correlate with neurite outgrowth promoted by ultrasound stimulation in PC12 cells	(70, 71)	-1.614159	8.454000e-06	1.0091193	0.3289512
SNCB (Synuclein beta)	Related genes	Alpha-Synuclein reversed the SPTBN1-induced neurite over-branching in SH-SY5Y cells; Beta-synuclein is discussed as a natural negative regulator of alpha-synuclein aggregation	(72, 73)	-1.033695	2.206000e-05	4.7782508	2.3330362
PHOX2A (Paired like homeobox 2A)	Related genes	PHOX2B knockdown reduced neurite length in human motor neurons and induced short spinal neurons in zebrafish	(74)	-2.067991	3.509352e-02	0.4672003	0.1097638
SEZ6L (Seizure related 6 homolog like)	Related genes	SEZ6 suppression leads to decreased neurite length in PC12 cells; SEZ6 family member are	(75, 76)	-1.166480	2.178000e-07	1.35654181	0.60410517

		known to affect neurite length (SEZ6 and SEZ6L2)					
SYNPR (Synaptoporin)	/	/	/	-1.002154	4.107603e-02	0.66902816	0.33344849
MAFA (MAF bZIP transcription factor A)	/	/	/	-1.084093	4.790000e-07	3.8772766	1.8283201
ADRA2A (Adrenoceptor alpha 2A)	/	/	/	-1.381024	2.780000e-08	1.8795963	0.7211471
NKX1-2 (NK1 homeobox 2)	/	/	/	-1.445285	4.276000e-08	2.00510214	0.73565542
NPIPA3 (Nuclear pore complex interacting protein family member A3)	/	/	/	-1.304036	2.042396e-02	0.7830245	0.3160825
SULT1A4 (Sulfotransferase family 1A member 4)	/	/	/	-2.107897	7.278000e-06	1.7434995	0.4025545
BOLA2B (BolA family member 2B)	/	/	/	-1.307911	1.149725e-02	1.4269165	0.5746954
PRSS53 (Serine protease 53)	/	/	/	-1.794549	2.217000e-07	1.8605236	0.5351388
DIPK1C (FAM69C)	/	/	/	-1.007293	2.264083e-02	0.76087239	0.37797824

(Divergent protein kinase domain 1C)							
FAM156B (TMEM29B) (Family with sequence similarity 156 member B)	/	/	/	-1.189186	9.333000e-14	6.426085	2.817976
P3R3URF-PIK3R3 Readthrough	/	/	/	-1.833480	1.452810e-02	0.7193576	0.2001381
gene:*ENSG00000258465*	/	/	/	-1.602100	1.054000e-05	1.09259278	0.35915470
gene:*ENSG00000255872*	/	/	/	-1.477783	3.862000e-03	0.7375282	0.2639103
gene:*ENSG00000284057*	/	/	/	-1.088332	1.605000e-07	3.8209871	1.7965551
TBCEL-TECTA Readthrough	/	/	/	-1.183985	4.005257e-02	1.0880918	0.4775082
gene:*ENSG00000272921*	/	/	/	-1.046384	3.162111e-02	2.515808	1.215900
gene:*ENSG00000262304*	/	/	/	-1.626821	2.406000e-05	0.66801872	0.21579793
RPL36A-HNRNPH2 Readthrough	/	/	/	-3.273787	1.058000e-03	1.776631	0.178886

Movie S1 (separate file): The ORF2 encoded capsid protein of HEV is around the nucleus and in neurites Confocal microscope images of HEV-infected neurons were acquired. A 3D reconstruction of the surfaces was performed using the analysis tool Imaris 9.8.0. Cells were stained with DAPI (blue), with an antibody against ORF2-encoded capsid protein (green) and with β-III-tubulin (red).

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