

Supplementary Materials for

A 3D human brain–like tissue model of herpes-induced Alzheimer’s disease

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Supplemental Data

Table S1. Table of qRT-PCR primer sequences.

GENE	ACCESSION #	SEQUENCE 1 (5'-->3')	SEQUENCE 2 (5'-->3')
Amyloid precursor protein (APP)	NM_000484	GTCTCTCTCCCTGCTCTACAA	GGCCAAGACGTCATCTGAATAG
Beta secretase 1 (BACE1)	NM_012104	CCATCCTTCCGCAGCAATA	CGTAGAAGCCCTCCATGATAAC
Tumor necrosis factor alpha (TNF α)	NM_000594.4	GAGGCCAAGCCCTGGTATG	CGGGCCGATTGATCTCAGC
Serpina3n (SERP3)	NM_001085.4	GCTCATCAACGACTACGTGA A	CACCATTACCCACTTTTTTCTTGC
Lipocalin 2 (LCN2)	NM_005564.5	GACAACCAATTCCAGGGGAA G	GCATACATCTTTTGCGGGTCT
Presenilin 1 (PSEN1)	NM_000021	TGGCTACCATTAAGTCAGTCA GC	CCCACAGTCTCGGTATCTTCT
Presenilin 2 (PSEN2)	NM_012486	CTGACCGCTATGTCTGTAGT GG	CTTCGCTCCGTATTTGAGGGT
Interleukin 6 (IL-6)	NM_012486	TCAATATTAGAGTCTCAACCC CC	TTGTTTTCTGCCAGTGCCTC
Interleukin 1 beta (IL1 β)	NM_000576.2	CAGAAGTACCTGAGCTCGCC	AGATTCGTAGCTGGATGCCG
Interferon gamma (IFN γ)	NM_000619	ACTGTCGCCAGCAGCTAAAA	TATTGCAGGCAGGACAACCA
Vimentin (VIM)	NM_003380.5	TCCGCACATTTCGAGCAAAGA	TGATTCAAGTCTCAGCGGGC
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	NM_002046.5	ATTGCCCTCAACGACCACT	ATGAGGTCCACCACCCTGT
Glial fibrillary acidic protein (GFAP)	NM_001131019. 2	ACTGGCAGAGCTTGTTAGTG	AGTGACAGGAAGAGGTGAGA
Human alpha-herpesvirus 1 strain MacIntyre	MN136523.1	TGGCTTTTCGGACTACACCC	TTCGAAGGCCGTGAACGTAA

Table S2. Table of antibodies used for immunostaining.

HOST	ANTIGEN	VENDOR	CATALOG #
Mouse	HSV Type1/2 gB	Thermofisher	MA1-19265
Rabbit	HSV-1	Abcam	ab9533
Rabbit	Beta III tubulin (TUJ1)	Abcam	ab18207
Mouse	Beta III tubulin (TUJ1)	Sigma	T8578
Rabbit	Cleaved Caspase 3 (CC3)	RND	AF835
Rabbit	Amyloid Fibril	Abcam	ab201062
Mouse	Phospho-Tau (Ser202, Thr205) (AT8)	Thermofisher	MN1020
Mouse	Microtubule-associated protein tau (Tau-1)	Sigma	MAB3420
Mouse	Glial fibrillary acidic protein (GFAP)	Sigma	G3893
Rabbit	Glial fibrillary acidic protein (GFAP)	Sigma	G9269
Rabbit	Tumor necrosis factor alpha (TNF α)	Cell Signaling Technology	8184S
Goat (Alexa 488 conjugated)	Rabbit IgG	Thermofisher	A-11070
Goat (Alexa 594 conjugated)	Rabbit IgG	Thermofisher	A-11072
Goat (Alexa 488 conjugated)	Mouse IgG	Thermofisher	A-11017
Goat (Alexa 594 conjugated)	Mouse IgG	Thermofisher	A-11020

Table S3. Genes upregulated in response to HSV-1 infection in 3D human brain-like tissue constructs using the Qiagen RT Profiler Array for Alzheimer's Disease.

SYMBOL	GENE NAME	ACCESSION #	FOLD UPREGULATION
CTSG	Cathepsin G	NM_001911	86.18
MPO	Myeloperoxidase	NM_000250	42.32
PRKCQ	Protein kinase C, theta	NM_006257	33.80
IL1A	Interleukin 1, alpha	NM_000575	31.45
CASP4	Caspase 4, apoptosis-related cysteine peptidase	NM_001225	28.94
SERP3	Serpin peptidase inhibitor, clade A (alpha-1 antitrypsin), member 3	NM_001085	28.77
CHAT	Choline O-acetyltransferase	NM_020985	28.29
PLAU	Plasminogen activator, urokinase	NM_002658	27.38
GNGT2	Guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2	NM_031498	22.98
INS	Insulin	NM_000207	19.45
BACE2	Beta-site APP-cleaving enzyme 2	NM_012105	19.03
GNGT1	Guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 1	NM_021955	17.63
PLG	Plasminogen	NM_000301	15.68
APOA1	Apolipoprotein A-I	NM_000039	15.49
ERN1	Endoplasmic reticulum to nucleus signaling 1	NM_001433	12.13
APBA3	Amyloid beta (A4) precursor protein-binding, family A, member 3	NM_004886	10.91
CDKL1	Cyclin-dependent kinase-like 1 (CDC2-related kinase)	NM_004196	10.74
PRKCI	Protein kinase C, iota	NM_002740	9.89
GNG11	Guanine nucleotide binding protein (G protein), gamma 11	NM_004126	8.61
NTRK1	Neurotrophic tyrosine kinase, receptor, type 1	NM_002529	7.33
BDNF	Brain-derived neurotrophic factor	NM_001709	6.44
INSR	Insulin receptor	NM_000208	6.05
GSK3B	Glycogen synthase kinase 3 beta	NM_002093	5.55
SNCB	Synuclein, beta	NM_003085	5.37
ACHE	Acetylcholinesterase	NM_000665	4.25
LRP6	Low density lipoprotein receptor-related protein 6	NM_002336	3.44
UBQLN1	Ubiquilin 1	NM_013438	3.26
A2M	Alpha-2-macroglobulin	NM_000014	2.96
HPRT1	Hypoxanthine phosphoribosyltransferase 1	NM_000194	2.90
GNB5	Guanine nucleotide binding protein (G protein), beta 5	NM_016194	2.78
PRKCE	Protein kinase C, epsilon	NM_005400	2.71
PKP4	Plakophilin 4	NM_003628	2.52
PRKCD	Protein kinase C, delta	NM_006254	2.47
PRKCZ	Protein kinase C, zeta	NM_002744	2.42
PSEN2	Presenilin 2 (Alzheimer disease 4)	NM_000447	2.37
PRKCG	Protein kinase C, gamma	NM_002739	2.23
IDE	Insulin-degrading enzyme	NM_004969	2.21
UQCRC2	Ubiquinol-cytochrome c reductase core protein II	NM_003366	2.17
PRKCA	Protein kinase C, alpha	NM_002737	2.15
BCHE	Butyrylcholinesterase	NM_000055	2.07

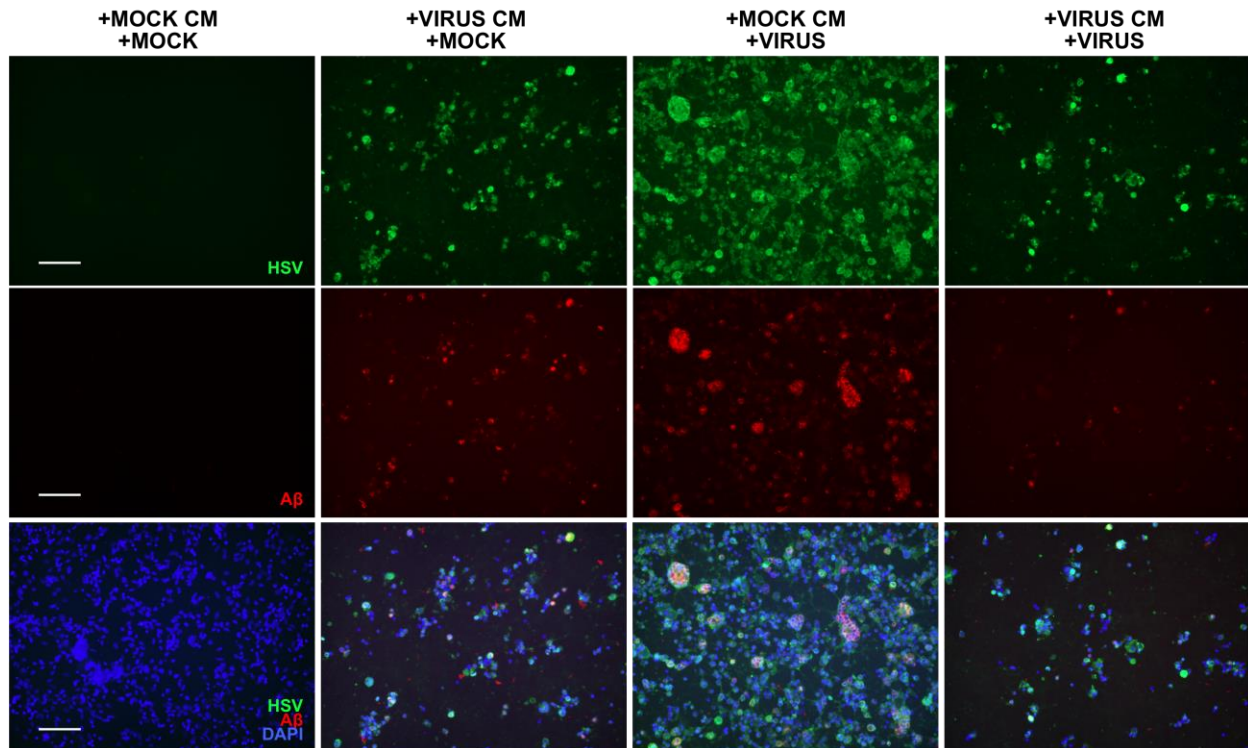


Figure S1. HSV-1-infected hiNSCs secrete HSV-1 that can infect new cells. Conditioned media (CM) was harvested and filtered from mock or HSV-1 infected hiNSCs that had been cultured for 2 days. Mock or fresh HSV-1 virus was added to CM and new hiNSCs were cultured for 4 days. Antibodies against HSV or beta amyloid fibrils (A β) were used to immunostain cells. virus CM can infect new cells suggesting that infected hiNSCs secrete HSV-1. Scale bar = 100 μ m.

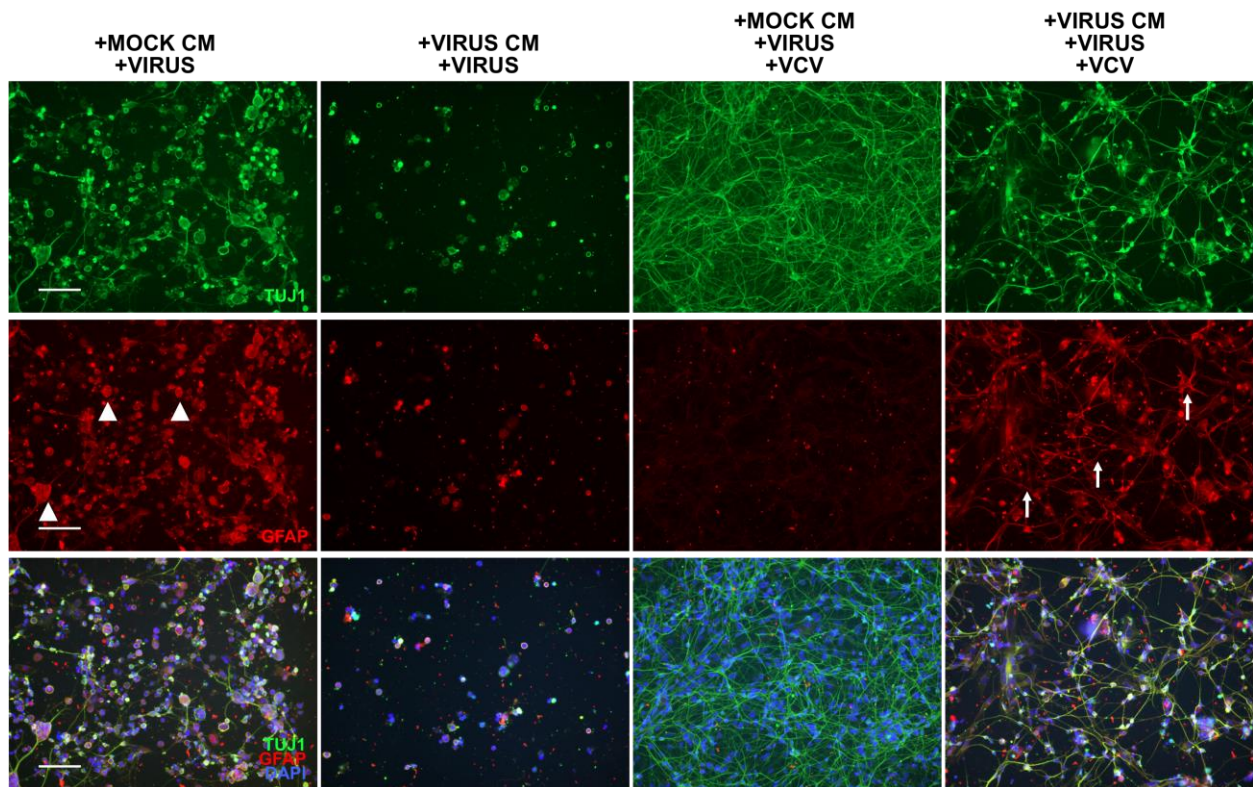


Figure S2. HSV-1-secreted factors cause gliosis even in the absence of infection.

Conditioned media (CM) was harvested and filtered from mock or HSV-1 infected hiNSCs that had been cultured for 2 days. Fresh HSV-1 virus was added to CM in the absence of presence of antiviral treatment Valacyclovir HCl (VCV) and new hiNSCs were cultured for 4 days. Beta III tubulin (TUJ1) and glial fibrillary acidic protein (GFAP) immunostaining was performed to visualize neurons and glia, respectively. Preventing HSV-1 infection using VCV in virus CM results in increased reactive glia formation suggesting that HSV-1 induced factors play a role in astrogliosis. Arrowheads = swollen activated reactive glia; arrows = intermediate reactive glia with more fibrous extensions. Scale bar = 100 μ m.

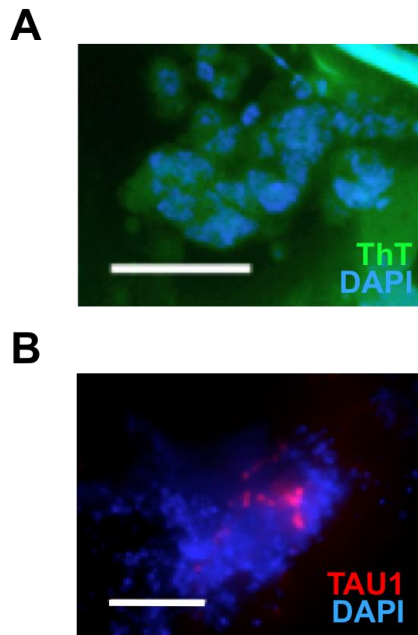


Figure S3. hiNSCs cultured in 3D human brain model then subjected to low level HSV-1 infection develop AD-like plaque formations. High magnification confocal images reveal positive Thioflavin T (ThT) (A) and TAU1 expression in plaque-like formations (PLFs) in HSV-1-infected 3D brain cultures. Scale bar = 100 μ m.

A2M	ABCA1	ACHE	ADAM10	ADAM9	APBA1	APBA3	APBB1	APBB2	APH1A	APLP1	APLP2
APOA1	APOE	APP	BACE1	BACE2	BCHE	BDNF	CAPN1	CASP3	CASP4	CDK1	CDK5
CDKL1	CHAT	CLU	CTSB	CTSC	CTSD	CTSG	CTSL	EP300	ERN1	GAP43	GNAO1
GNAZ	GNB1	GNB2	GNB4	GNB5	GNG11	GNG3	GNG4	GNGT1	GNGT2	GSK3A	GSK3B
HSD17B10	IDE	IL1A	INS	INSR	LPL	LRP1	LRP6	LRP8	MAP2	MAPT	MPO
NAE1	NCSTN	NTRK1	NTRK2	PKP4	PLAT	PLAU	PLG	PRKCA	PRKCB	PRKCD	PRKCE
PRKCG	PRKCI	PRKCQ	PRKCZ	PSEN1	PSEN2	SERP3	SNCA	SNCB	UBQLN1	UQCR1	UQCR2

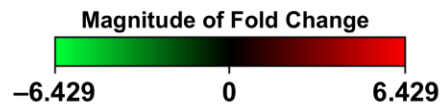


Figure S4. HSV-1 infected 3D human brain-like tissue constructs overexpress multiple AD-related factors. Heat map to visually portray relative expression levels of different genes in mock and virus-infected 3D human brain-like tissue cultures using the Qiagen RT Profiler Array for Alzheimer's Disease. Red-green color spectrum represents relative fold change of virus-infected samples as compared to mock-controls.