Supplementary Tables

Table S1. GenBank accession information for genomes utilized in comparison experiments using sourmash

Organism	GenBank acc. no.	Family
Anguillid Herpesvirus 1 (AngHV-1)	GCA_000886195.1	*Alloherpesviridae*
Cyprinid Herpesvirus 1 (CyHV-1)	GCA_000903355.1	*Alloherpesviridae*
Cyprinid Herpesvirus 2 (CyHV-2)	GCA_000900815.1	*Alloherpesviridae*
Cyprinid Herpesvirus 3 (CyHV-3)	GCA_000871465.1	*Alloherpesviridae*
Ictalurid Herpesvirus 1 (IcHV-1)	GCA_000839325.1	*Alloherpesviridae*
Ictalurid Herpesvirus 2 (IcHV-2)	GCA_008792765.1	*Alloherpesviridae*
Ranid Herpesvirus 1 (RaHV-1)	GCA_000869925.1	*Alloherpesviridae*
Ranid Herpesvirus 2 (RaHV-2)	GCA_000869245.1	*Alloherpesviridae*
Ranid Herpesvirus 3 (RaHV-3)	GCA_002158775.1	*Alloherpesviridae*
Salmonid Herpesvirus 1 (SalHV-1)†	GCA_002814575.1	*Alloherpesviridae*
Salmonid Herpesvirus 2 (SalHV-2)†	GCA_002814595.1	*Alloherpesviridae*
Salmonid Herpesvirus 3 (SalHV-3)†	GCA_003181315.1	*Alloherpesviridae*
Chenolid Herpesvirus 5 (ChHV-5)†	GCA_008792165.1	*Herpesviridae* (alphaherpesvirus)
Gallid Herpesvirus 2 (GaHV-2)	GCA_000846265.1	*Herpesviridae* (alphaherpesvirus)
Herpes Simplex Virus 1 (HSV-1)	GCA_000859985.2	*Herpesviridae* (alphaherpesvirus)
Herpes Simplex Virus 2 (HSV-2)	GCA_000858385.2	*Herpesviridae* (alphaherpesvirus)
Psittacid Herpesvirus 1 (PsHV-1)	GCA_000840765.1	*Herpesviridae* (alphaherpesvirus)
Varicella-Zoster Virus (VZV)	GCA_000858285.1	*Herpesviridae* (alphaherpesvirus)
Cytomegalovirus (CMV)	GCA_000845245.1	*Herpesviridae* (betaherpesvirus)
Elephantid Betaherpesvirus 1 (ElHV-1)	GCA_000905835.1	*Herpesviridae* (betaherpesvirus)
Human Betaherpesvirus 7 (HHV-7)	GCA_000848125.1	*Herpesviridae* (betaherpesvirus)
Murid herpesvirus 1 (MHV-1)	GCA_008792765.1	*Herpesviridae* (betaherpesvirus)
Alcelaphine Herpesvirus (AlHV-1)	GCA_000838825.1	*Herpesviridae* (gammaherpesvirus)
Epstein-Barr Virus (EBV)	GCA_002402265.1	*Herpesviridae* (gammaherpesvirus)
Equid Gammaherpesvirus 2 (EHV-2)	GCA_000843985.2	*Herpesviridae* (gammaherpesvirus)
Kaposi's Sarcoma-associated Herpesvirus (HHV-8)	GCA_000838265.1	*Herpesviridae* (gammaherpesvirus)
Abalone Herpesvirus (AbHV-1)	GCA_000900375.1	*Malacoherpesviridae*
Ostreid Herpesvirus 1 (OsHV-1)	GCA_000846065.1	*Malacoherpesviridae*

† = Partial genome

Table S2. GenBank accession information for genomes utilized in core genes comparison experiments

Organism	**Isolate**	**GenBank acc. no.**
Acipenserid Herpesvirus 2	UCD3-30	OQ626810
Acipenserid Herpesvirus 2	OCR	OQ626811
Acipenserid Herpesvirus 2	SR-WSHV	OQ626812
Acipenserid Herpesvirus 2	R20-11	OQ626813
Acipenserid Herpesvirus 2	SR-WSHV	FJ815289.2
Acipenserid herpesvirus 1	California	OK275729.1 OK275734.1 OK275725.1 OK275730.1 OK275731.1 OK275732.1 OK275728.1 OK275733.1 OK275723.1 OK275727.1 OK275726.1 OK275724.1
Lake Sturgeon Herpesvirus	Wolf River	OK485036.1
Sterlet Herpesvirus	Gen_M01	VTUV01000924.1
Silurid Herpesvirus 1	KRB14001	MH048901.1
Ictalurid Herpesvirus 1	Auburn1	M75136.2
Cyprinid Herpesvirus 1	NG-J1	JQ815363.1
Cyprinid Herpesvirus 2	ST-J1	JQ815364.1
Cyprinid Herpesvirus 3	KHV-U	DQ657948.1
Anguillid Herpesvirus 1	500138	FJ940765.3
Ranid Herpesvirus 2	Rafferty	DQ665652.1
Ranid Herpesvirus 3	FO1_2015	KX832224.1

Table S3. AciHV-2 Open Reading Frames (ORFs) Characterization using Blast

ORF	**Conserved domain**	**Accession number**	**Interval**	**E value**	**Putative identity based on homology**	**E value**
1	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
1U	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
2	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
3	RING_Ubox superfamily	cl17238	25–126	5.20×10^{-3}	Hypothetical protein	N/A
4	RING_Ubox superfamily (Incomplete conserved domain)	cl17238	25–123	7.35×10^{-3}	Hypothetical protein	N/A
5	RING_Ubox superfamily (Incomplete conserved domain)	cl17238	22–123	1.32×10^{-4}	Hypothetical protein	N/A
6	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
7	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
8	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
9	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
10	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
11	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_206667793.1)	2×10^{-117}
12	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
13	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
14	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
15	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
16	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_147187508.1)	9×10^{-129}
17	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
18	None detected	N/A	N/A	N/A	Hypothetical protein	N/A

ORF	Conserved domain	Accession number	Interval	E value	Putative identity based on homology	E value
19	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - TXC12839.1)	4×10^{-41}
20	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
21	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_147187529.1)	5×10^{-70}
22	PKc_like superfamily (incomplete catalytic domain of the serine/threonine kinases and similar kinases)	cd06629	538–1134	2.07×10^{-8}	Protein kinase (Serine/threonine or mitogen-activated protein kinase)	N/A
23	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
24	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_206667776.1)	3×10^{-67}
25	None detected	N/A	N/A	N/A	Hypothetical protein - suspected membrane protein	N/A
26	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
27	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
28	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
29	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
30	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
31	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - TXC12928.1)	9×10^{-36}
32	PRK03918 superfamily (ATPase) / PTZ00440 (Reticulocyte binding protein 2-like protein)	PRK03918 / PTZ00440	1516–3366 / 10–1983	8.57×10^{-17} / 2.69×10^{-10}	Hypothetical protein	N/A

ORF	Conserved domain	Accession number	Interval	E value	Putative identity based on homology	E value
33	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
34	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
35	PKc_like superfamily (incomplete catalytic domain of the serine/threonine kinases and similar kinases)	cl21453	157–585	4.79×10^{-16}	Protein kinase (Serine/threonine or mitogen-activated protein kinase)	N/A
36	None detected	N/A	N/A	N/A	Hypothetical protein - Putative helicase (one of the 12 core genes)	N/A
37	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
38	PHA03260 superfamily - Capsid triplex subunit 2; provisional	cl15613	262–879	2.81×10^{-3}	Putative Capsid triplex subunit 2 (one of the 12 core genes)	N/A
39	PHA03369 superfamily - capsid maturation protease; provisional	PHA03369	13–1545	6.63×10^{-45}	Putative capsid maturation protease (one of the 12 core genes)	N/A
40	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
41	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
42	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
43	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
44	PHA03336 superfamily	cl14542	4–900	1.47×10^{-86}	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
45	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
46	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A

ORF	Conserved domain	Accession number	Interval	E value	Putative identity based on homology	E value
47	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family – One of the 12 core genes: allo37	N/A
48	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
49	None detected	N/A	N/A	N/A	Putative major capsid protein (one of the 12 core genes)	N/A
50	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
51	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
52	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
53	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
54	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
55	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
56	PHA03332 superfamily - membrane glycoprotein; provisional	cl33726	310–702; 1843–3621	1.16×10^{-5}; 2.39×10^{-69}	Putative Membrane Glycoprotein	N/A
56	Glyco_hydro_18 superfamily - Glycosyl hydrolases family 18	cl38006	892–1494	9.32×10^{-10}	Putative Glycoside hydrolase	N/A
57	Peptidases_S8_S53 superfamily - peptidase domain in the S8 and S53 families	cl10459	70–741	1.20×10^{-22}	Putative Subtilisin-like proprotein convertase	N/A
58	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
59	Trimeric_dUTPase superfamily - complete domain	cl00493	37–420	3.58×10^{-19}	Putative dUTP diphosphatase	N/A
60	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A

ORF	Conserved domain	Accession number	Interval	E value	Putative identity based on homology	E value
61	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
62	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
63	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
64	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family – One of the 12 core genes: allo64	N/A
65	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
66	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family – One of the 12 core genes: allo56	N/A
67	PHA03334 superfamily - putative DNA polymerase catalytic subunit; provisional	cl33727	70–2793; 2889–4616	0; 0	Putative DNA-dependent DNA Polymerase (one of the 12 core genes)	N/A
68	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
69	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family – One of the 12 core genes: allo60	N/A
70	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
71 - First Exon	PHA03333 superfamily - putative ATPase subunit of terminase (incomplete domain)	PHA03333	23–799	6.28×10^{-78}	Putative DNA packaging terminase subunit 1 - found in various members of the *Alloherpesviridae* family (one of the 12 core genes)	N/A
71 - Second Exon	PHA03333 superfamily - putative ATPase subunit of terminase (incomplete domain) / DNA_pack_C - probable DNA packaging protein (C-terminus)	pfam02499	1–549 / 211–519	3.43×10^{-51} / 5.59×10^{-5}	Putative DNA packaging terminase - found in various members of the *Alloherpesviridae* family (one of the 12 core genes)	N/A

ORF	Conserved domain	Accession number	Interval	E value	Putative identity based on homology	E value
71 - Third Exon	PHA03333 superfamily - putative ATPase subunit of terminase (incomplete domain)	PHA03333	10–801	2.91×10^{-52}	Putative DNA packaging terminase subunit 1 - found in various members of the *Alloherpesviridae* family (one of the 12 core genes)	N/A
72	None detected	N/A	N/A	N/A	Hypothetical protein - Putative helicase-primase found in various members of the *Alloherpesviridae* family (one of the 12 core genes)	N/A
73	None detected	N/A	N/A	N/A	Hypothetical protein - Putative flap endonuclease 1 like protein (matched the flap endonuclease 1 protein of the spiny head croaker - *Collichthys lucidus)* – One of the 12 core genes: allo64	6×10^{-9}
74	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
75	Smc superfamily (Chromosome segregation ATPase - cell cycle control, cell division, chromosome partitioning) / PRK03918 superfamily (DNA double-strand break repair ATPase)	COG1196 / PRK03918	2794–5010 / 3262–4953	2.22×10^{-20} / 3.72×10^{-15}	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
76	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
76R	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_147187523.1)	N/A
77	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
78	PHA03333 superfamily - putative ATPase subunit of terminase (incomplete domain)	PHA03333	1–228	6.56×10^{-15}	Hypothetical protein - overlaps with the second exon of the putative terminase (ORF 71)	N/A
79	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A

ORF	Conserved domain	Accession number	Interval	E value	Putative identity based on homology	E value
80	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_147187551.1)	3 x 10^{-118}
81	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
82	PKc-like superfamily (Protein kinase, catalytic domain)	cl21453	1327– 1947; 1681– 2223	7.42 x 10^{-18}; 2.35 x 10^{-8}	Putative protein kinase	N/A
83	PKc-like superfamily (Protein kinase, catalytic domain)	cl21453	604– 1170; 907– 1434	1.39 x 10^{-10}; 0	Putative protein kinase	N/A
84	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
85	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
86	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
87	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
88	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
89	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
89U	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
89O	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
90	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
91	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
92	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_206667779.1)	2 x 10^{-136}

ORF	Conserved domain	Accession number	Interval	E value	Putative identity based on homology	E value
93	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_147187539.1)	9×10^{-71}
94	None detected	N/A	N/A	N/A	Hypothetical protein found in various members of the *Alloherpesviridae* family	N/A
94U	None detected	N/A	N/A	N/A	Hypothetical protein	N/A
95	NK superfamily - Nucleoside/nucleotide kinase	cl17190	643–1188	3.99×10^{-13}	Hypothetical protein - putative thymidine kinase 2 - like protein	N/A
96	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
97	None detected	N/A	N/A	N/A	Hypothetical protein (No significant match)	N/A
98	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - TXC15802.1)	2×10^{-7}
99	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - TXC15802.1)	1×10^{-95}
100	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_147187516.1)	0
101	TNFRSF14_teleost (tumor necrosis factor receptor superfamily member 14 - also known as herpes virus entry mediator)	cd13405	28–258	8.36×10^{-13}	Hypothetical protein (putative tumor necrosis factor receptor member 14 - like protein)	N/A
102	None detected	N/A	N/A	N/A	Hypothetical protein (Just one match with *Salmonella enterica* - WP_206667781.1)	0

Table S4. Nucleotide similarity of the 12 core Alloherpesvirus genes between various members of the family *Alloherpesviridae*, including the four newly sequenced Acipenserid herpesvirus 2 (AciHV-2) isolates, and the published AciHV-2 partial sequence (GenBank: FJ815289).

Virus	Isolate	GenBank	pol	ter1	allo56	hel	pri	mcp	tri2	pro	allo37	allo64	allo60	allo54
AciHV2	UCD3-30	OQ626810	99.9	99.6	100	99.9	99.9	99.9	100	99.8	100	99.9	99.6	99.9
AciHV2	OCR	OQ626811	99.7	99.7	99.9	100	99.9	99.4	100	99.7	99.7	99.9	99.5	99.8
AciHV2	SR-WSHV	OQ626812	99.9	99.9	100	100	100	99.9	100	99.9	100	100	99.9	100
AciHV2	R20-11	OQ626813	99.9	99.7	100	99.7	99.9	100	100	99.9	100	100	99.7	99.8
AciHV2	SR-WSHV	FJ815289.2	100	100	100	100	100	100	100	100	100	100	100	100
AciHV1	California	OK275729.1 OK275734.1 OK275725.1 OK275730.1 OK275731.1 OK275732.1 OK275728.1 OK275733,1 OK275723.1 OK275727.1 OK275726.1 OK275724.1	39	45.5	30.4	39.2	28.9	30.7	34.9	28.8	30.1	31.4	36.5	33.4
LSHV	Wolf River	OK485036.1	37.7	45.1	30.1	38.6	28.9	30.1	35.1	27.7	29.7	29.9	35.8	31.7
StHV	Gen_M01	VTUV01000924.1	37.1	45.8	30.1	39.8	30.1	30.3	31.9	28.2	26.4	31.0	35.4	30.8
SiHV1	KRB14001	MH048901.1	56.9	61.1	51.2	52.7	45.2	43.5	51.4	39.6	46.5	48.8	47.8	48.2
IcHV1	Auburn1	M75136.2	56.5	59.4	50.0	53.6	44.5	42.7	49.8	37.8	45.1	47.1	46.7	48.7

Virus	Isolate	GenBank	pol	ter1	allo56	hel	pri	mcp	tri2	pro	allo37	allo64	allo60	allo54
CyHV1	NG-J1	JQ815363.1	31.5	38.2	25	31.2	23.4	29.7	27.4	24.1	26.7	28	26.6	25.0
CyHV2	ST-J1	JQ815364.1	31.4	40.3	25.5	27.1	22.4	28.6	28.3	25.2	25.2	25.3	30.6	23.5
CyHV3	KHV-U	DQ657948.1	30.7	38.4	23.6	25.9	23.0	28.9	26	24.7	25.4	24.1	28.7	21.8
AngHV1	500138	FJ940765.3	31.1	40.1	25.4	34.6	23.5	28.3	27.6	27.1	28	25.7	29.8	25.0
RaHV2	Rafferty	DQ665652.1	32.5	41.2	27.2	35.5	25.9	28.1	27.6	26.4	26.8	26.6	29.9	28.9
RaHV3	FO1_2015	KX832224.1	35	41.1	33.3	37.8	27.3	30.8	30.8	27.9	27.3	30.5	29.1	31.4

[a] Cells show the percentage of bases that are identical between the isolate defined by the row and the published partial sequence of Acipenserid herpesvirus 2 (FJ815289). Isolates are color-coded by current phylogenetic grouping (teal = AciHV-2 isolates presented in this manuscript; gray = unclassified; pink = *Ictalurivirus*; purple = *Cyprinivirus*; blue = *Batrachovirus*). Abbreviations are as follows: AciHV2 = Acipenserid Herpesvirus 2; AciHV1 = Acipenserid herpesvirus 1; LSHV = Lake Sturgeon Herpesvirus; StHV = Sterlet Herpesvirus; SiHV1 = Silurid herpesvirus 1; IcHV1 = Ictalurid Herpesvirus 1; CyHV1 = Cyprinid Herpesvirus 1; CyHV2 = Cyprinid Herpesvirus 2; CyHV3 = Cyprinid herpesvirus 3; AngHV1 = Anguillid Herpesvirus 1; RaHV2 = Ranid Herpesvirus 2; RaHV3 = Ranid Herpesvirus 3; pol = DNA polymerase catalytic subunit; ter1 = DNA packaging terminase subunit 1; allo56 = Alloherpesvirus protein 56; hel = helicase-primase helicase subunit; pri = helicase-primase primase subunit; mcp = major capsid protein; tri2 = capsid triplex protein 2; pro = capsid maturation protease; allo37 = Alloherpesvirus protein 37; allo64 = Alloherpesvirus protein 64; allo60 = Alloherpesvirus protein 60; allo54 = Alloherpesvirus protein 54.

Supplementary Figures

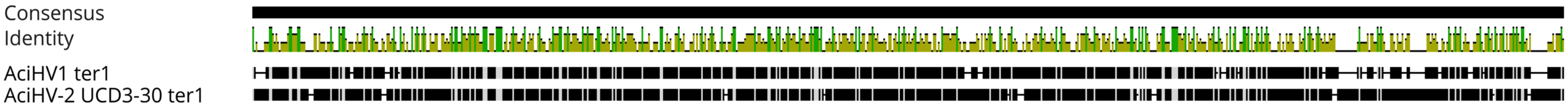

Figure S1. Terminase (ter1) sequence comparison between Acipenserid Herpesvirus 2 (AciHV-2, UCD3-30 isolate) and Acipenserid herpesvirus 1 (AciHV-1). The ter1 open reading frame was identified as a good candidate for primer design. The sequence for ter1 in both AciHV-1 and AciHV-2 were aligned using MUSCLE within Geneious Prime. The black bars represent the genomes, which are labeled on the left column. The similarity map at the top indicates the mean pairwise identity over all base pairs in the column. Green indicates 100% identity and red indicates less than 30% identity, with yellow indicating 30% ≤ identity < 100%. This depiction was made using Geneious Prime.

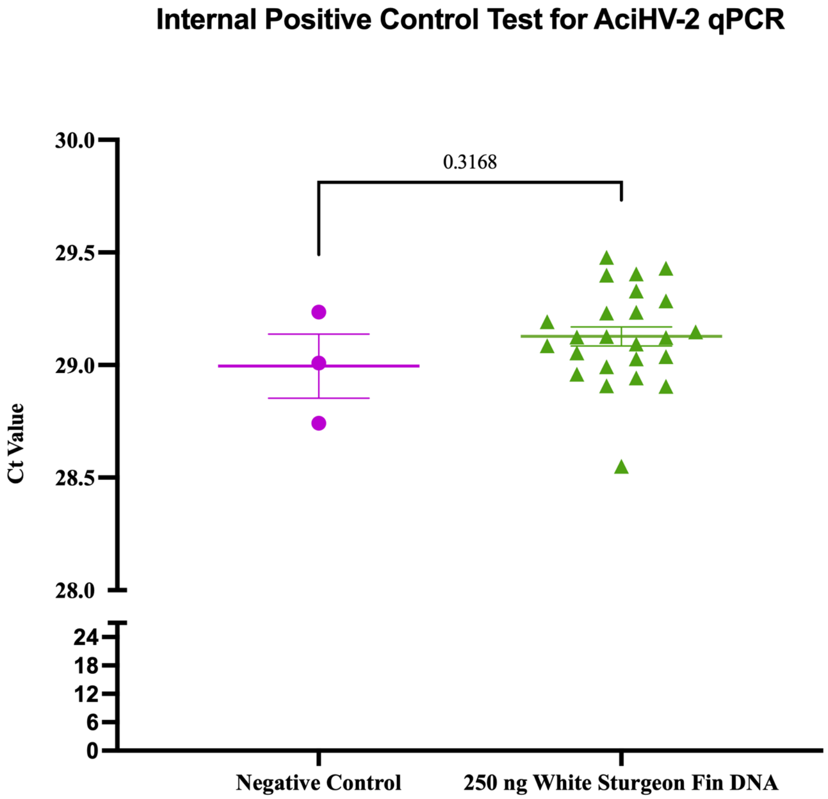

Figure S2. Internal Positive Control Assay. The TaqMan Exogenous Internal Positive Control (IPC) Reagents – VIC Probe Assay was used to assess inhibition of the PCR reaction. The experimental samples contained 83.3 ng of white sturgeon fin DNA per microliter (each in triplicate), while the negative control sample (in triplicate) received DEPC-treated sterile water. Results revealed there was no statistically significant difference in Cq values obtained between the negative control and the sample containing white sturgeon fin DNA.

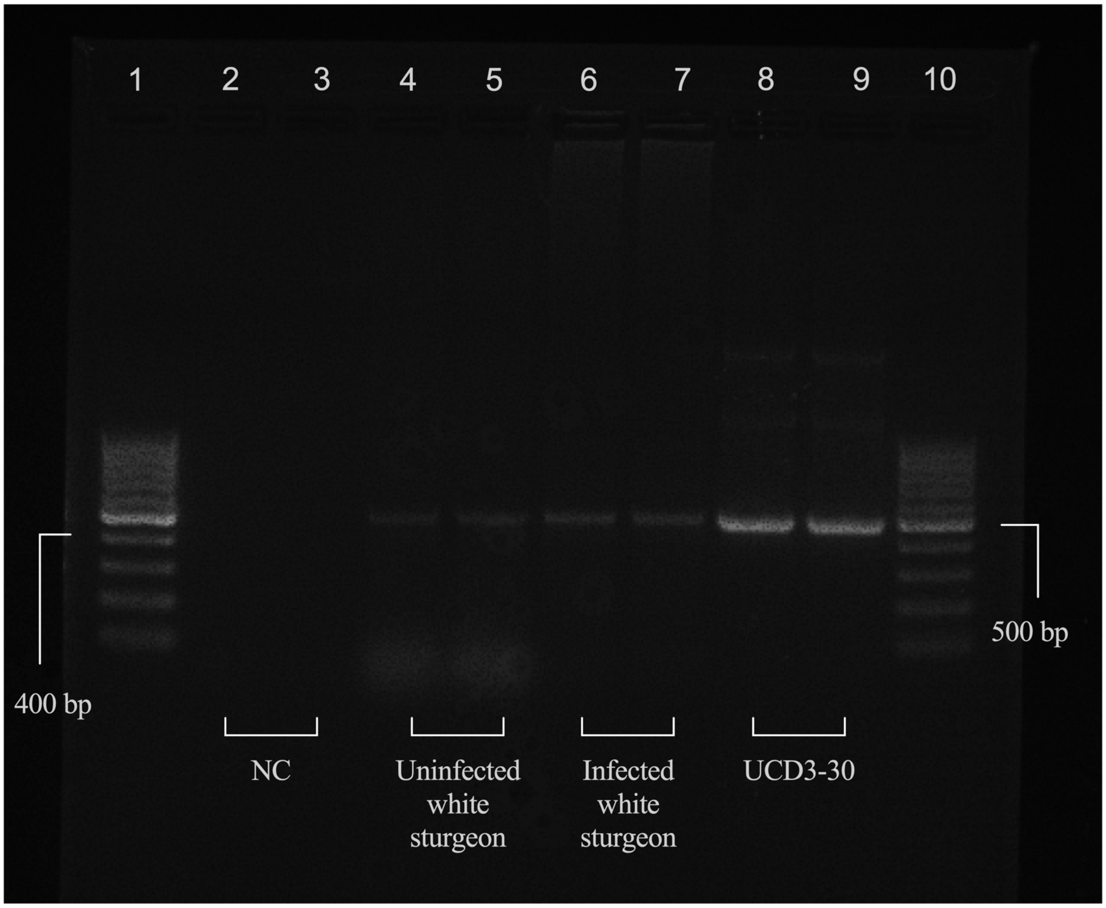

Figure S3. Degenerate primer test for Acipenserid Herspesvirus 2 (AciHV-2) in white sturgeon (*Acipenser transmontanus*). Degenerate primers and its previously published conventional Polymerase Chain Reaction (PCR) protocol (Hanson et al 2006) were used to assess the ability of this assay to detect AciHV-2 in white sturgeon fin DNA samples (300 ng of total DNA loaded per well). Gel electrophoresis with subsequent exposure to ultraviolet light were used to visualize PCR products. Lanes 1 and 10 were loaded with the GeneRuler 100 bp DNA Ladder, with the relevant bands labeled. Lanes 2-9 were loaded with the PCR products indicated underneath. A single band near the expected 465 bp length was detected for all samples, except the negative control.

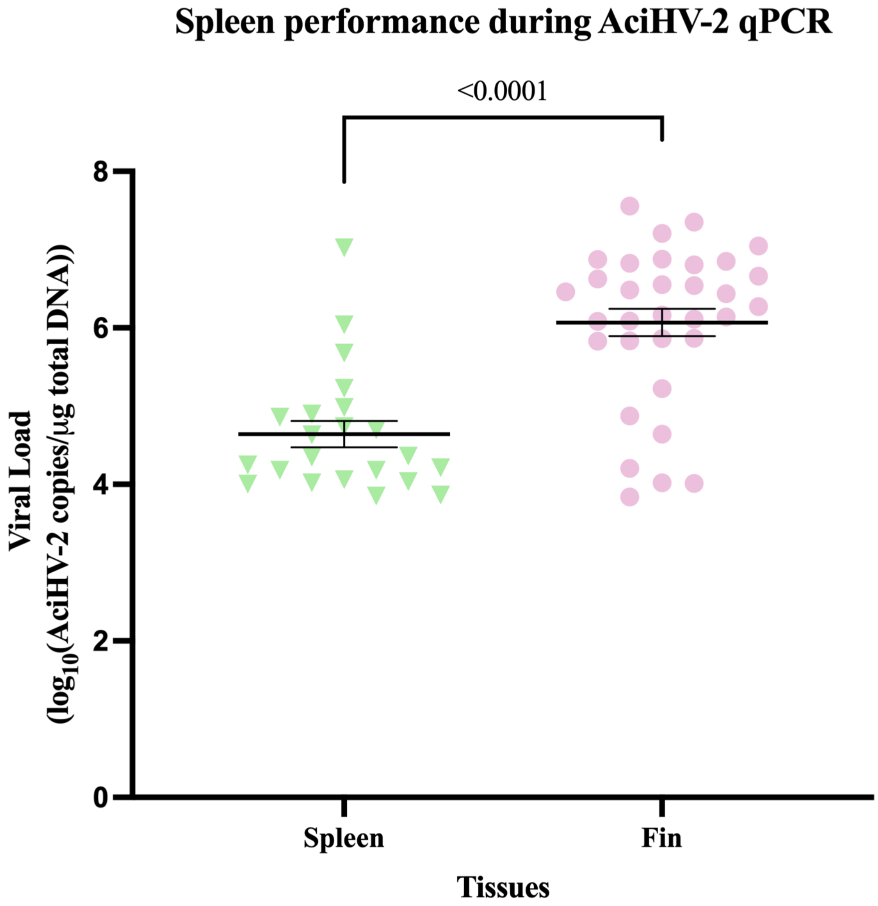

Figure S4. Spleen performance during AciHV-2 qPCR. An immersion challenge model was performed by exposing white sturgeon fingerlings to an AciHV-2 bath for 1 hour. Mortalities were tested for AciHV-2 via qPCR. The solid dots represent the mean viral load of sampled mortalities for each tissue type, with the standard error of the mean plotted. Results show a statistically significantly lower viral load in the spleen samples, with the spleen samples failing to detect positive individuals in 33.4% of the cases.