

Capsid Specific Antibody Responses of Domestic Pigs Immunized with Low Virulent African swine fever virus

Priscilla Y. L. Tng ^{1,*}, Laila Al-Adwani ¹, Egle Pauletto ^{1,2}, Joshua Hui ¹, and Christopher L. Netherton ^{1,*}

¹ The Pirbright Institute, Ash Road, Pirbright, Woking GU24 0NF, UK

² The Institute of Medical Sciences, Foresterhill, University of Aberdeen, Aberdeen AB25 2ZD, UK

* Correspondence: priscilla.tng@pirbright.com (P.Y.T.); christopher.netherton@pirbright.ac.uk (C.L.N.)

Supplementary Information

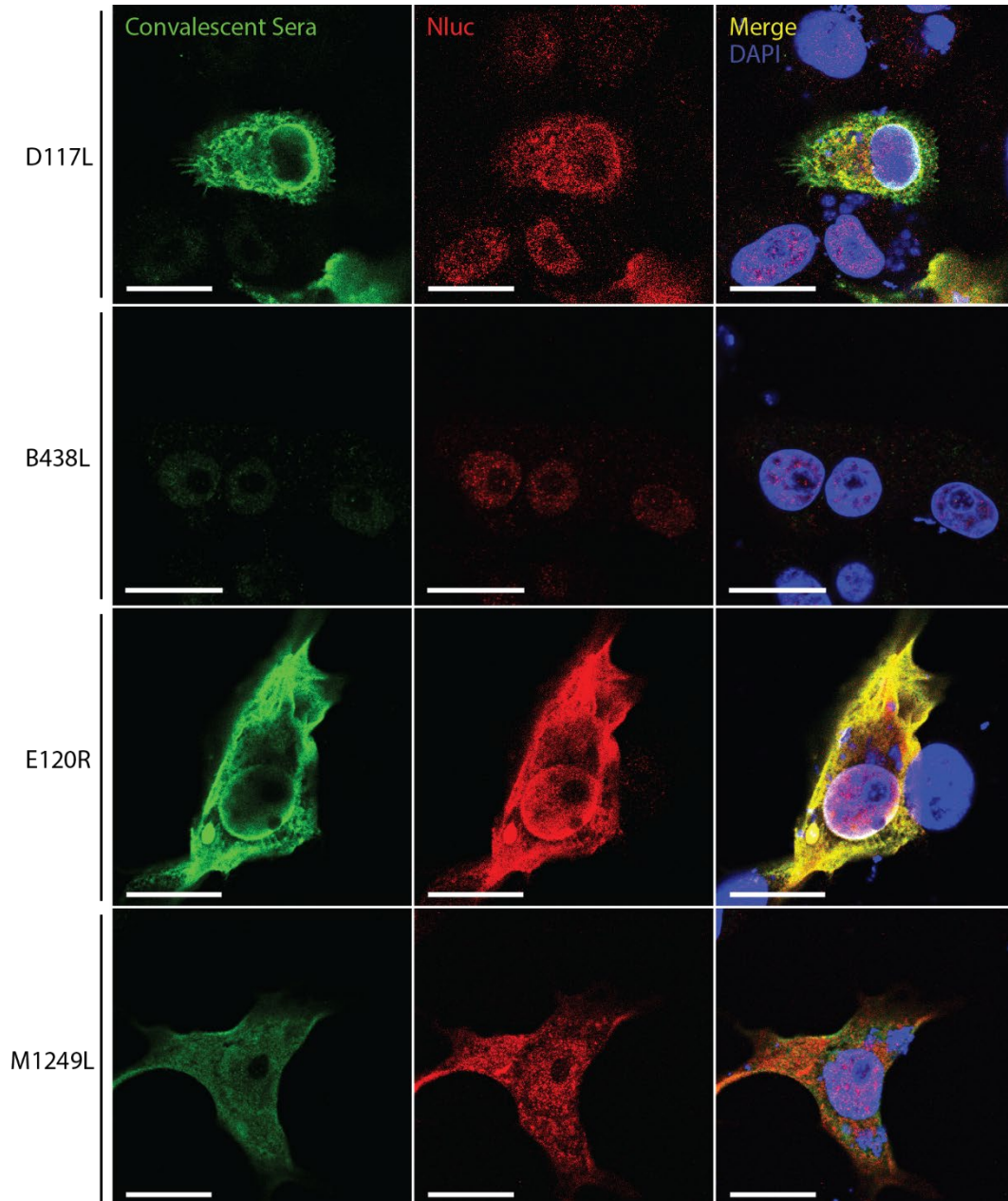


Figure 1. Confocal images of Vero cells expressing recombinant ASFV proteins D117L, B438L, E120R and M1249L. Detection of expression of recombinant nanoluciferase (Nluc)-tagged ASFV proteins in transfected Vero cells was achieved with the monoclonal anti-Nluc antibody (red, Promega) and with convalescent outbred pig sera from our previous study [1] (green). Nuclear DNA was stained with DAPI (blue). White bar denotes 20 μ m.

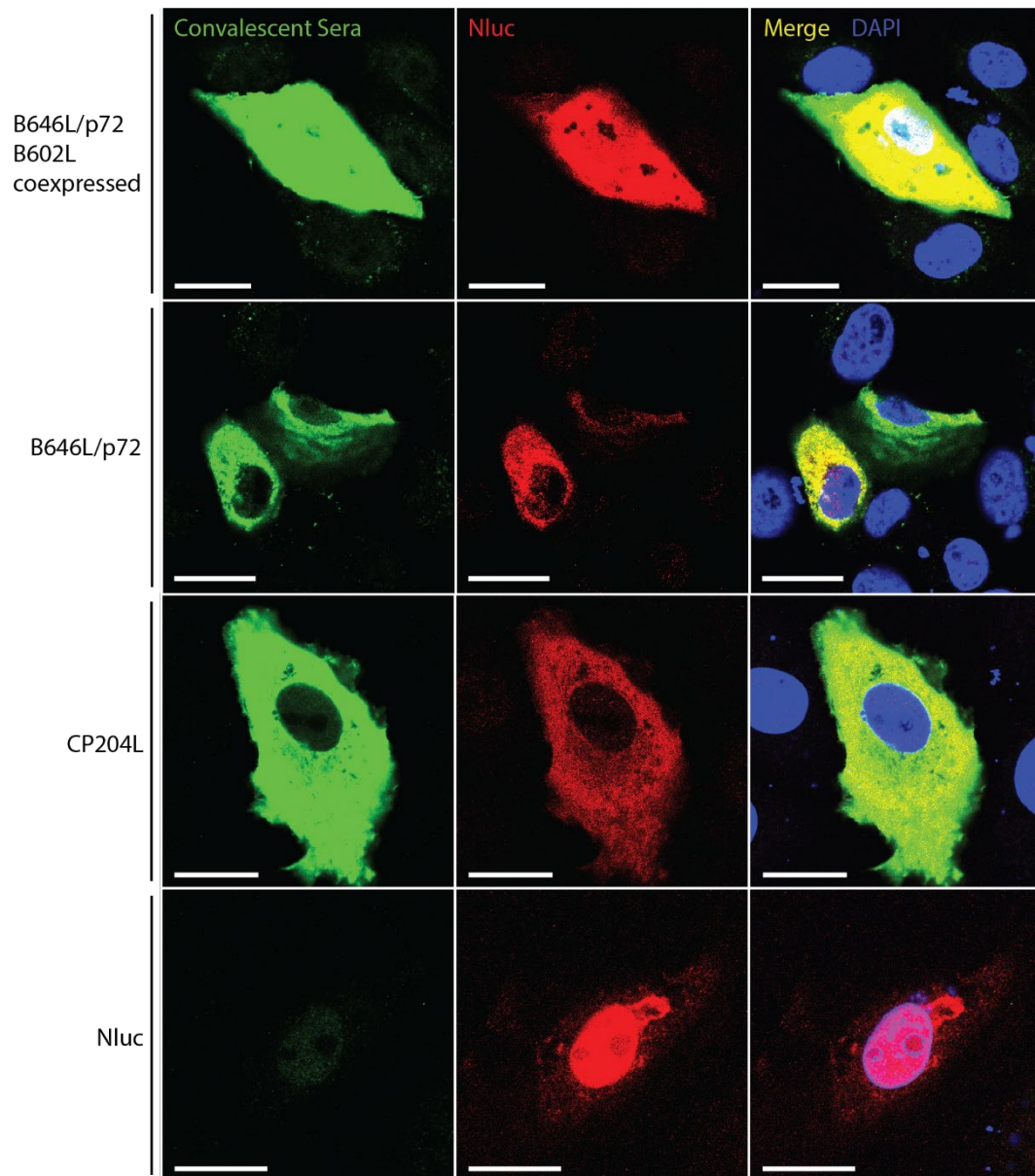


Figure 2. Confocal images of Vero cells expressing recombinant ASFV proteins B646L/p72, B602L, CP204L/p30 and Nluc. B646L/p72 was expressed alone or with chaperone B602L. Detection of expression of recombinant nanoluciferase (Nluc)-tagged ASFV proteins in transfected Vero cells was achieved with the monoclonal anti-Nluc antibody (red, Promega) and with convalescent outbred pig sera from our previous study [1] (green). Nuclear DNA was stained with DAPI (blue). White bar denotes 20 μ m.

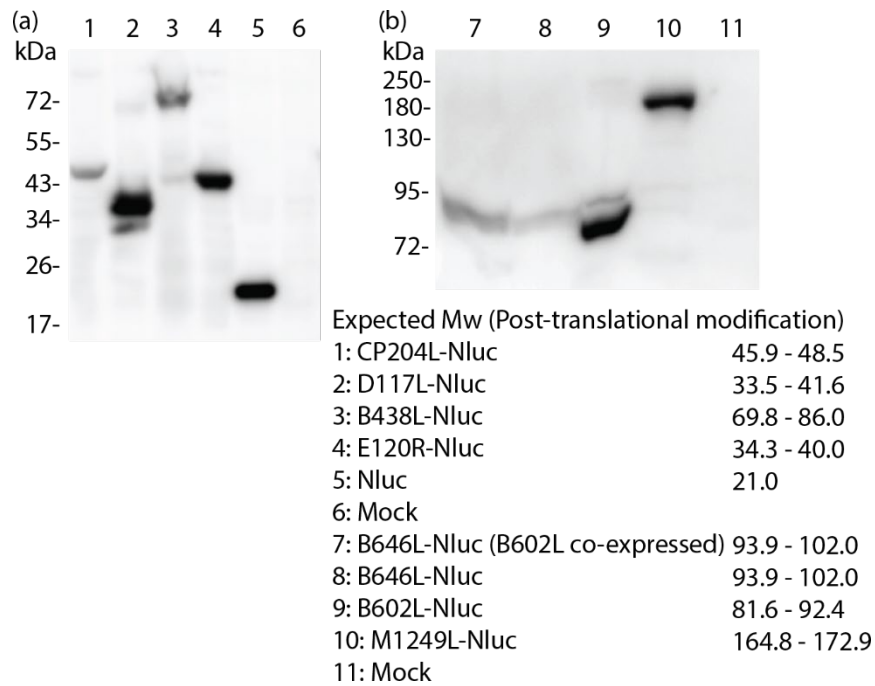


Figure 3. Western blot detection of recombinant Nluc-tagged ASFV proteins. Nluc-tagged ASFV proteins from transfected HEK293T cell lysates were detected with the monoclonal anti-Nluc antibody (Promega). The expected protein molecular weights (Mw) are listed as a range that takes post-translational modifications into account.

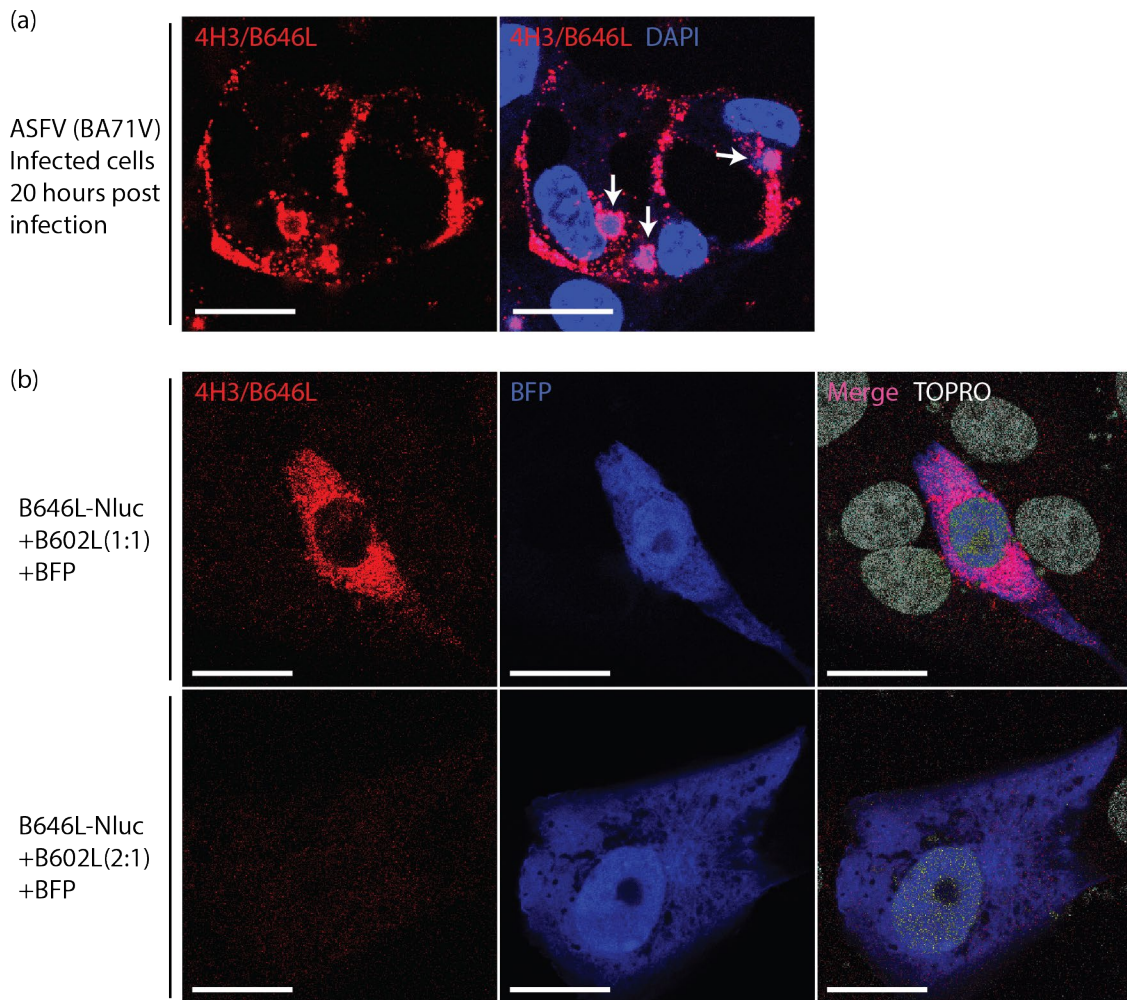


Figure 4. Determination of B646L/p72 and chaperone B602L transfection ratio. (a) ASFV (BA71V) infected Vero cells were used as a positive control for the 4H3 antibody that binds to a conformational epitope on B646L/p72 (red). Nuclear and viral DNA were

stained with DAPI (blue). White arrows indicate ASFV viral factories. (b) Representative confocal images of Vero cells transfected with different ratios of Nluc-tagged B646L/p72 to the chaperone B602L. The amount of B646L-Nluc expressing plasmid was held constant and different amounts of B602L-expressing plasmid were used. A blue fluorescent protein (BFP, blue) expressing plasmid was co-transfected as a transfection control. The 4H3 anti-B646L/p72 antibody (red) was used to detect for expression of recombinant Nluc-tagged B646L/p72 with its native conformation. Nuclear DNA was stained with TOPRO (white). White bar denotes 20 μ m.

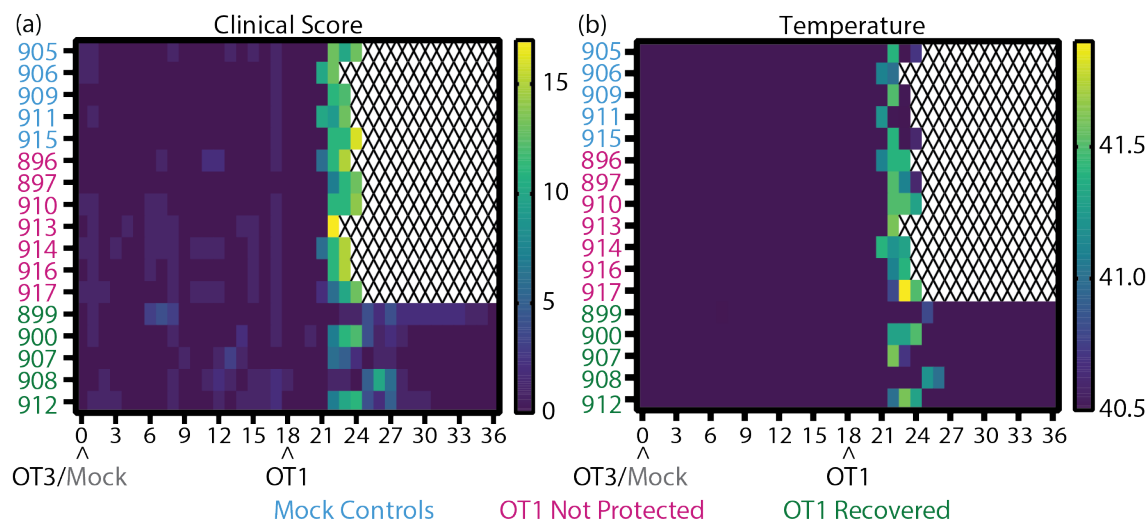


Figure 5. Heatmaps of (a) clinical scores and (b) temperatures of inbred Babraham animals. Data was obtained from our previous study [1]. (b) Data plotted as $^{\circ}$ C. The negative cutoff for temperature was set at 40.5 $^{\circ}$ C as this is highest value that does not count towards a humane endpoint [1]. Each row denotes the responses of a single animal. Crosses indicate data that were not available for analysis. Arrowheads denote the immunization and ASFV challenge time points. Blue, Mock: mock control animals immunized with PBS, Pink, NP: OURT88/3 immunized animals that were not protected from OURT88/1, Green, R: OURT88/3 immunized animals that recovered from OURT88/1.

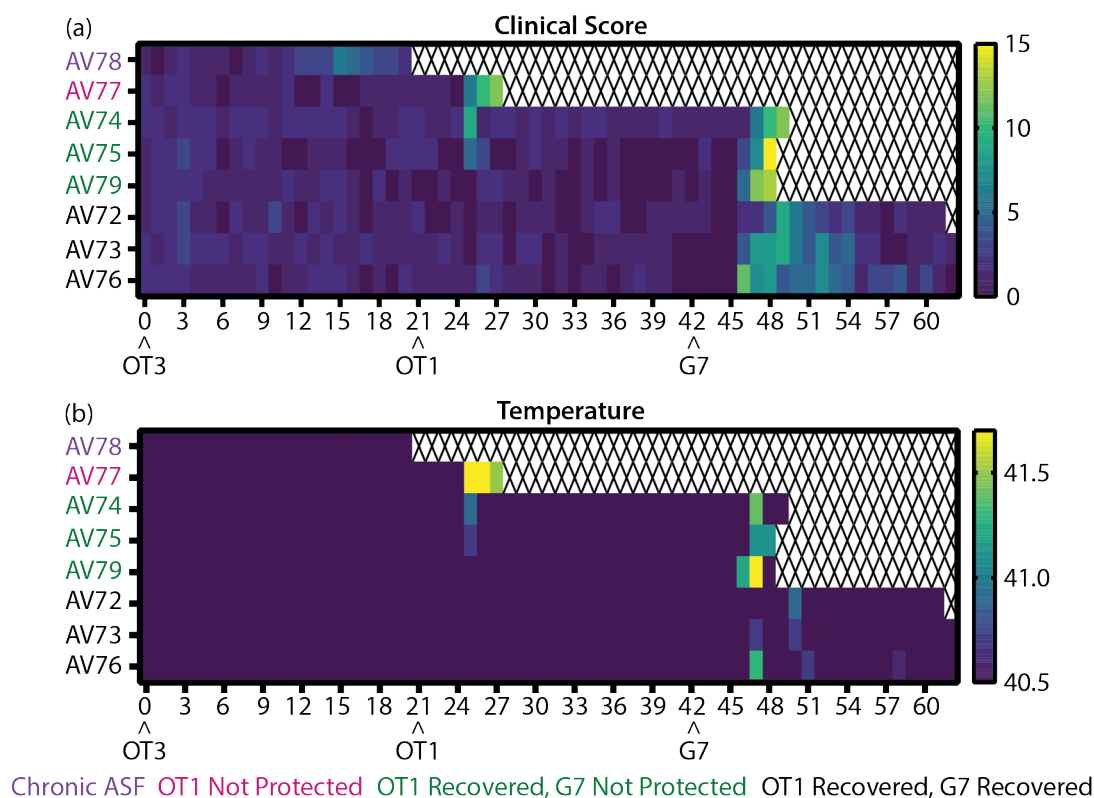


Figure 6. Heatmaps of (a) clinical scores and (b) temperatures of outbred domestic pigs. Data was obtained from our previous study [1]. (b) Data plotted as $^{\circ}$ C. The negative cutoff for temperature was set at 40.5 $^{\circ}$ C as this is highest value that does not count

towards a humane endpoint [1]. Each row denotes the responses of a single animal. Crosses indicate data that were not available for analysis. Arrowheads denote the immunization and ASFV challenge time points. Purple: OURT88/3 immunized animal that suffered from chronic ASF, Pink: OURT88/3 immunized animal that was not protected from OURT88/1, Green: OURT88/3 immunized animals that recovered from OURT88/1, but were not protected from Georgia 2007/1, Black: OURT88/3 immunized animals that recovered from OURT88/1 and Georgia 2007/1.

B646L_Georgia2007_1 (NC_044959)	MASGGAFCLIAN DK GKADKIILAQDLLNSRISNIKNVNKSY	40
B646L_OURT88_3 (NC_044957)	MASGGAFCLIAN DK GKADKIILAQDLLNSRISNIKNVNKSY	40
B646L_Georgia2007_1 (NC_044959)	GKPDPEPTLSQIEETHLVHFNAHF KPY VPVGF EYN KVRPH	80
B646L_OURT88_3 (NC_044957)	GKPDPEPTLSQIEETHLVHFNAHF KPY VPVGF EYN KVRPH	80
B646L_Georgia2007_1 (NC_044959)	TGTPTLG NK LTFGI PQY G DF FHDMVGH HIL GACHSSWQDA	120
B646L_OURT88_3 (NC_044957)	TGTPTLG NK LTFGI PQY G DF FHDMVGH HIL GACHSSWQDA	120
B646L_Georgia2007_1 (NC_044959)	PIQGT S QMGAHGQLOT F PRNGYDWDNQT P LEGAVYTLVDP	160
B646L_OURT88_3 (NC_044957)	PIQGT A QMGAHGQLOT F PRNGYDWDNQT P LEGAVYTLVDP	160
B646L_Georgia2007_1 (NC_044959)	FGRPIVPGTKNAYRN L VYYCEYPGERLYENVRFDVNGNSL	200
B646L_OURT88_3 (NC_044957)	FGRPIVPGTKNAYRN L VYYCEYPGERLYENVRFDVNGNSL	200
B646L_Georgia2007_1 (NC_044959)	DEYSSDVTTLV RK FCIPGDKMTGYKHLVGQEVSV E GTSGP	240
B646L_OURT88_3 (NC_044957)	DEYSSDVTTLV RK FCIPGDKMTGYKHLVGQEVSV E GTSGP	240
B646L_Georgia2007_1 (NC_044959)	LLCNIHDLHKPHQSKPILTDENDTQRTCSHTNP K FLSQHF	280
B646L_OURT88_3 (NC_044957)	LLCNIHDLHKPHQSKPILTDENDTQRTCSHTNP K FLSQHF	280
B646L_Georgia2007_1 (NC_044959)	PENSHNIQTAGKQDITPITDATYLDIRRVH Y SCNGPQTP	320
B646L_OURT88_3 (NC_044957)	PENSHNIQTAGKQDITPITDATYLDIRRVH Y SCNGPQTP	320
B646L_Georgia2007_1 (NC_044959)	KYYQPPLALWIKLRFWFNENVNLAIPSVSIPFGERFITIK	360
B646L_OURT88_3 (NC_044957)	KYYQPPLALWIKLRFWFNENVNLAIPSVSIPFGERFITIK	360
B646L_Georgia2007_1 (NC_044959)	LASQKDLVNEFPGLF V RQSRFI A GRPSRRNIRFKPWFIPG	400
B646L_OURT88_3 (NC_044957)	LASQKDLVNEFPGLF I RQSRFI P GRPSRRNIRFKPWFIPG	400
B646L_Georgia2007_1 (NC_044959)	VINEISLTNNELYINNLFVTPEIHNLFV K RVRFSLIRVHK	440
B646L_OURT88_3 (NC_044957)	VINEISLTNNELYINNLFVTPEIHNLFV K RVRFSLIRVHK	440
B646L_Georgia2007_1 (NC_044959)	TQVTH T NNHHDEKLMSALKWPIEYMF I GLKPTWNISDQN	480
B646L_OURT88_3 (NC_044957)	TQVTH T NNHHDEKLMSALKWPIEYMF I GLKPTWNISDQN	480
B646L_Georgia2007_1 (NC_044959)	PHQHRDWHKFGHVVN A IMQPTHAEISFQDRDTALPDACS	520
B646L_OURT88_3 (NC_044957)	PHQHRDWHKFGHVVN A IMQPTHAEISFQDRDTALPDACS	520
B646L_Georgia2007_1 (NC_044959)	SISDISPVTYPITLPIIKNISVTAHGINLIDKFPSKFCSS	560
B646L_OURT88_3 (NC_044957)	SISDISPVTYPITLPIIKNISVTAHGINLIDKFPSKFCSS	560
B646L_Georgia2007_1 (NC_044959)	YIPFHYGGNAIKTPDDPGAMMITFALKPREEYQPSGHINV	600
B646L_OURT88_3 (NC_044957)	YIPFHYGGNAIKTPDDPGAMMITFALKPREEYQPSGHINV	600
B646L_Georgia2007_1 (NC_044959)	SRAREFYISWDTDYVGSITTADLVVSASAINFLLLONGSA	640
B646L_OURT88_3 (NC_044957)	SRAREFYISWDTDYVGSITTADLVVSASAINFLLLONGSA	640
B646L_Georgia2007_1 (NC_044959)	VLRYS T	646
B646L_OURT88_3 (NC_044957)	VLRYS T	646

Figure 7. Multiple sequence alignment of B646L/p72 amino acid sequences from Georgia2007/1 and OURT88/3. These sequences have 99.5% identity.

B602L_Georgia2007_1 (NC_044959)	MAEFNIDELLKNVLEDPSTEISEETLKQLYQRTNPKQFK	40
B602L_OURT88_3 (NC_044957)	MAEFNIDELLKNVLEDPSTEISEETLKQLYQRTNPKQFK	40
B602L_Georgia2007_1 (NC_044959)	NDSRVAFCSTNLREQYIRRLIMTSFIGYVFKALQEWMP	80
B602L_OURT88_3 (NC_044957)	NDSRVAFCSTNLREQYIRRLIMTSFIGYVFKALQEWMP	80
B602L_Georgia2007_1 (NC_044959)	YSKPTHHTKTLSELITLVDTLKQETNDVPSESVNTILS	120
B602L_OURT88_3 (NC_044957)	YSKPTHHTKTLSELITLVDTLKQETNDVPSESVNTILS	120
B602L_Georgia2007_1 (NC_044959)	IADSKTQTQKSKEAKTTIDSFLREHFVFDPNLHAQSAY-	159
B602L_OURT88_3 (NC_044957)	IADSKTQTQKSKEAKTTIDSFLREHFVFDPNLHAQSAYT	160
B602L_Georgia2007_1 (NC_044959)	-----	159
B602L_OURT88_3 (NC_044957)	CASTCADTNVDTCASTCASTCASTCASTCASTCAST	200
B602L_Georgia2007_1 (NC_044959)	-----	159
B602L_OURT88_3 (NC_044957)	CASTCASTCASTCASTCASTCASTGASTGASTCADTNVDT	240
B602L_Georgia2007_1 (NC_044959)	-----	159
B602L_OURT88_3 (NC_044957)	CASTCADTNVDTCASTCASTCASTCASTCASTGASTCADT	280
B602L_Georgia2007_1 (NC_044959)	-----TCADTNVDTCASMCADTNVDT	180
B602L_OURT88_3 (NC_044957)	NVDTCASTCADTNVDTCASTTCADTNVDTCASTCADTNVNT	320
B602L_Georgia2007_1 (NC_044959)	CASMCADTNVDTCAS---TCTSTEYTDLADPERIPLHIM	216
B602L_OURT88_3 (NC_044957)	CASMCADTNVDTCASTCANTCSTEYTDLADPERIPLHIM	360
B602L_Georgia2007_1 (NC_044959)	QKTLNVPNELQADIDAITQTPQGYRAAAHILQNIELHQSI	256
B602L_OURT88_3 (NC_044957)	QKTLNVPNELQADIDAITQTPQGYRAAAHILQNIELHQSI	400
B602L_Georgia2007_1 (NC_044959)	KHMLNPRAFKPILFNTKITRYLSQHIPPQDTFYKWNYYI	296
B602L_OURT88_3 (NC_044957)	KHMLNPRAFKPILFNTKITRYLSQHIPPQDTFYKWNYYI	440
B602L_Georgia2007_1 (NC_044959)	EDNYEELRAATESIYPEKPDLEFAFIIYDVVDSSNQQKVD	336
B602L_OURT88_3 (NC_044957)	EDNYEELRAATESIYPEKPDLEFAFIIYDVVDSSNQQKVD	480
B602L_Georgia2007_1 (NC_044959)	EFYYKYKDQIFSEVSSIQLGNWTLGSGFKANRERYNYFNQ	376
B602L_OURT88_3 (NC_044957)	EFYYKYKDQIFSEVSSIQLGNWTLGSGFKANRERYNYFNQ	520
B602L_Georgia2007_1 (NC_044959)	NNEIIKRILDRHEEDLKIGKEILRNTIYHKKAKNIQETGP	416
B602L_OURT88_3 (NC_044957)	NNEIIKRILDRHEEDLKIGKEILRNTIYHKKAKNIQETGP	560
B602L_Georgia2007_1 (NC_044959)	DAPGLSIYNSTFHTDSGIKGLLSFKELKNLEKASGNIKKA	456
B602L_OURT88_3 (NC_044957)	DAPGLSIYNSTFHTDSGIKGLLSFKELKNLEKASGNIKKA	600
B602L_Georgia2007_1 (NC_044959)	REYDFIDDCEEKIKQLLSKENLTPDEESELIKTKKQLDNA	496
B602L_OURT88_3 (NC_044957)	REYDFIDDCEEKIKQLLSKENLTPDEESELIKTKKQLNNA	640
B602L_Georgia2007_1 (NC_044959)	LEMLNVPDDTIRVDMWVNNNNKLEKEILYTKAEL	530
B602L_OURT88_3 (NC_044957)	LEMLNVPDDTIRVDMWVNNNNKLEKEILYTKAEL	674

Figure 8. Multiple sequence alignment of B602L amino acid sequences from Georgia2007/1 and OURT88/3. These sequences have 78.0% identity.

D117L_Georgia2007_1 (NC_044959)	MDTETSPLLSHNLSTREGIKQSTQGLLAHTIAKYPGTTAI	40
D117L_OURT88_3 (NC_044957)	MDTETSPLLSHNLSTREGIKQSTQGLLAHTIARYPGTTAI	40
D117L_Georgia2007_1 (NC_044959)	LLGILILLIIILIIVAIVYYNRTIDCKSSI PKPPPSYYVQ	80
D117L_OURT88_3 (NC_044957)	LLGILILLVIILIIVAIVYYNRSVDCKSSMPKPPPSYYVQ	80
D117L_Georgia2007_1 (NC_044959)	QPEPHHHFPVFFRKRKNSTSLQSHIPSEQLAELAHS	117
D117L_OURT88_3 (NC_044957)	QPEPHHHFPVFFRKRKNSTSLQSHIPSEQLAELAHS	117

Figure 9. Multiple sequence alignment of D117L/p17 amino acid sequences from Georgia2007/1 and OURT88/3. These sequences have 95.7% identity.

B438L_Georgia2007_1 (NC_044959)	MYHDYASKLLADYRSDPPLWESDLPRHNRYSDNILNSRYC	40
B438L_OURT88_3 (NC_044957)	MYHDYASKLLADYRSDPPLWESDLPRHNRYSDNILNSRYC	40
B438L_Georgia2007_1 (NC_044959)	GNKNGAAPVYNEYTNSPEKAEGQLQLSDLRNFSFMLNPQH	80
B438L_OURT88_3 (NC_044957)	GNKNGAAPVYNEYTNSPEKAEGQLQLSDLRNFSFMLNPQH	80
B438L_Georgia2007_1 (NC_044959)	KNIGYGDAQDLEPYSSIPKNKLFNHFKNHRPAFSTHTENL	120
B438L_OURT88_3 (NC_044957)	KNIGYGDAQDLEPYSSIPKNKLFNHFKNHRPAFSTHTENL	120
B438L_Georgia2007_1 (NC_044959)	IRRNVRTEKKTFFQVASLKGTQKNCLTQPSSLPSLKNPK	160
B438L_OURT88_3 (NC_044957)	IRRNVRTEKKTFFQVASLKGTQKNCLTQPSSLPSLKNPK	160
B438L_Georgia2007_1 (NC_044959)	NSSVPSTRFSEHTKFFSYEDLPKLRTKGTIKHEQHLGDQM	200
B438L_OURT88_3 (NC_044957)	NSSVPSTRFSEHTKFFSYEDLPKLRTKGTIKHEQHLGDQM	200
B438L_Georgia2007_1 (NC_044959)	PGQHYNGYIPHKDVYNILCLAHNLPASVEKGIAGRGIPLG	240
B438L_OURT88_3 (NC_044957)	PGQHYNGYIPHKDVYNILCLAHNLPASVEKGIAGRGIPLG	240
B438L_Georgia2007_1 (NC_044959)	NPHVKPNIEQELIKSTSTYTGVPM LGPLPPKDSQHGREYQ	280
B438L_OURT88_3 (NC_044957)	NPHVKPNIEQELIKSTSTYTGVPM LGPLPPKDSQHGREYQ	280
B438L_Georgia2007_1 (NC_044959)	EFSANRHMLQVSNILHSVFANHSIKPQILEDIPVLNAQLT	320
B438L_OURT88_3 (NC_044957)	EFSANRHMLQVANILHSVFANHSIKPQILEDIPVLNAQLT	320
B438L_Georgia2007_1 (NC_044959)	SIKPVSPFLNKAYQTHYMENIVTLVPRFKSIANYSSPIPN	360
B438L_OURT88_3 (NC_044957)	SIKPVSPFLNKAYQTHYMENIVTLVPRFKSIANYSSPIPN	360
B438L_Georgia2007_1 (NC_044959)	YSKRNSGQAEYFDTSTKQTSRHNHYIPKYTGGIGDSKLD	400
B438L_OURT88_3 (NC_044957)	YSKRNSGQAEYFDTSTKQTSRHNHYIPKYTGGIGDSKLD	400
B438L_Georgia2007_1 (NC_044959)	TFPKDFNASSVPLTSAEKDHSLRGDNSACCISSISPSL	438
B438L_OURT88_3 (NC_044957)	TFPKDFNASSVPLTSAEKDHSLRGDNSACCISSISPSL	438

Figure 10. Multiple sequence alignment of B438L/p49 amino acid sequences from Georgia2007/1 and OURT88/3. These sequences have 99.1% identity.

E120R_Georgia2007_1 (NC_044959)	MADFNSPIQYLKEDSRDRTSIGSLEYDENADTMIPSFAAG	40
E120R_OURT88_3 (NC_044957)	MADFNSPIQYLKEDSRDRTSIGSLEYDENSDTIIPSFAAG	40
E120R_Georgia2007_1 (NC_044959)	LEEFEPIDYDPTTSTSLYSQ LTHNMEKIAEEEDSNFLHD	80
E120R_OURT88_3 (NC_044957)	LEDFEPIP--SPTTSTSLYSQ LTHNMEKIAEEEDIINFLHD	78
E120R_Georgia2007_1 (NC_044959)	TREFTSLVPDEADNKPEDDEESGAKPKKKKHLF PKLSSHK	120
E120R_OURT88_3 (NC_044957)	TREFTSLVPDEADNKPEDDEESGAKPKKKKR L FPKLSSHK	118
E120R_Georgia2007_1 (NC_044959)	SK	122
E120R_OURT88_3 (NC_044957)	SK	120

Figure 11. Multiple sequence alignment of E120R/p14.5 amino acid sequences from Georgia2007/1 and OURT88/3. These sequences have 93.4% identity.

M1249L_Georgia2007_1 (NC_044959)	MEEVITIAQIVHRGTDILSLNNEEIEALVDEIYSTLKGSN	40
M1249L_OURT88_3 (NC_044957)	MEEVITIAQIVHRGTDILSLNNEEIEALVDEIYSTLKGSN	40
M1249L_Georgia2007_1 (NC_044959)	DIKNIRLIDFLFTLKDFVNHVRAEQSKLPDLSMPIEAYIR	80
M1249L_OURT88_3 (NC_044957)	DIKNIRLIDFLFTLKDFVNHVRAEQSKLPDLSMPMEAYIR	80
M1249L_Georgia2007_1 (NC_044959)	QLLVDPDVVPIVSEKKKELRVRPSTRKEIFLINGTHLAVP	120
M1249L_OURT88_3 (NC_044957)	QLLVDPDVVPIVSEKKKELRVRPSTRKEIFLINGTHLAVP	120
M1249L_Georgia2007_1 (NC_044959)	AEAPIEIIYGLKLRKLTFSPOCFMRMAEIGSFSPETLGYYA	160
M1249L_OURT88_3 (NC_044957)	AEAPIEIIYGLKLRKLTFSPOCFMRMAEIGSFSPETLGYYA	160
M1249L_Georgia2007_1 (NC_044959)	SGANLTNFIRVFMKCVDQETWKKNGEGVVVTTKENIIQFT	200
M1249L_OURT88_3 (NC_044957)	SGANLTNFIRVFMKCVDQETWKKNGEGVVVTTKENIIQFT	200
M1249L_Georgia2007_1 (NC_044959)	HQYIELYKFLRSGGHSWLINRLAEEMVHRKLDREDQGSNI	240
M1249L_OURT88_3 (NC_044957)	HQYIELYKFLRSGGHSWLINRLAEEMVHRKLDREDQGSNI	240
M1249L_Georgia2007_1 (NC_044959)	SNIVETEEIEPEENIKRVIFFLKLSTMYSVSPVFTSGYM	280
M1249L_OURT88_3 (NC_044957)	SNIVETEEIEPEENIKRVIFFLKLSTMYSVSPVFTSGYM	280
M1249L_Georgia2007_1 (NC_044959)	PLLYDLRAGYLEVLWNPVEQKFLOHAEQREKEQMILQOV	320
M1249L_OURT88_3 (NC_044957)	PLLYDLRAGYLEVLWNPVEQKFLOHAEQREKEQMILQOV	320
M1249L_Georgia2007_1 (NC_044959)	DMKLTEVITQARQYFKIMEEKIGRVQSDAIREILTMEGKV	360
M1249L_OURT88_3 (NC_044957)	DMKLTEVITQARQYFKIMEEKIGRVQSDAIREILTMEGKV	360
M1249L_Georgia2007_1 (NC_044959)	DDPNSILOEVIKACGKQEAELITTEYLNIAKKQWELQEKNA	400
M1249L_OURT88_3 (NC_044957)	DDPNSILOEVIKACGKQEAELITTEYLNIAKKQWELQEKNA	400
M1249L_Georgia2007_1 (NC_044959)	CAHLKLVKQLRSGLOQAELLKVLESIRVLYKEKNNTTNWN	440
M1249L_OURT88_3 (NC_044957)	CAHLKLVKQLRSGLOQAELLKVLESIRVLYKEKNNTTNWN	440
M1249L_Georgia2007_1 (NC_044959)	LCKACGFKLLCPHVDMLIQQAEEASYDTMRTKLMKFSGI	480
M1249L_OURT88_3 (NC_044957)	LCKACGFKLLCPHVDMLIQQAEEASYDTMRTKLMKFSGI	480
M1249L_Georgia2007_1 (NC_044959)	NKEKENNQGLIYSYFCKICGEELAHFIQEDRTADVGIIGD	520
M1249L_OURT88_3 (NC_044957)	NKEKENNQGLIYSYFCKICGEELAHFIQEDRTADVGVIGD	520
M1249L_Georgia2007_1 (NC_044959)	LNSKLRVFIWQETMKACTFIHFGKLVQFANIAVNVCL	560
M1249L_OURT88_3 (NC_044957)	LNSKLRVFIWQETMKACTFIHFGKLVQFANIAVNVCL	560
M1249L_Georgia2007_1 (NC_044959)	PLVYSIENIKKEEDYDPLTQLYAVIYIYAYILNLIYSSQK	600
M1249L_OURT88_3 (NC_044957)	PLVYSIENIKKEEDYDPLTQLYAVIYIYAYILNLIYSSQK	600
M1249L_Georgia2007_1 (NC_044959)	NKEFLTITIHGMKADSSLNAYVTFLEKMMQQYSGIINQL	640
M1249L_OURT88_3 (NC_044957)	NKEFLTITIHGMKADSSLNAYVTFLEKMMQQYSGIINQL	640
M1249L_Georgia2007_1 (NC_044959)	SEITDQWIANNFREAFFKKIIHQNGLQGLSVQDDTKVLLTE	680
M1249L_OURT88_3 (NC_044957)	SEITDQWIANNFREAFFKKIIHQNGLQGLSVQDDTKVLLTE	680
M1249L_Georgia2007_1 (NC_044959)	ILLDPMYDYAATVARIDGSIPMHKPRTPKEAEYEFKTVIG	720
M1249L_OURT88_3 (NC_044957)	ILLDPMYDYAATVARIDGSIPMHKPRTPKEAEYEFKTVIG	720

Figure 12. Multiple sequence alignment of M1249L amino acid sequences (1-720) from Georgia2007/1 and OURT88/3. These sequences have 99.4% identity.

M1249L_Georgia2007_1 (NC_044959)	RTPAELLSQKEFYDKIYTSKYRPDFTQLT	RLNDIYFQEE	760
M1249L_OURT88_3 (NC_044957)	RTPAELLSQKEFYDKIYTSKYRPDFTQLA	RLNDIYFQEE	760
M1249L_Georgia2007_1 (NC_044959)	LRVWVGGRDEEKTSTLIYLRAYELFLKYLQNAPNFNSEL		800
M1249L_OURT88_3 (NC_044957)	LRVWVGGRDEEKTSTLIYLRAYELFLKYLQNAPNFNSEL		800
M1249L_Georgia2007_1 (NC_044959)	EFKTYENAYGEQKALLAQQGFYNIFDPNTGRADQRTLFE		840
M1249L_OURT88_3 (NC_044957)	EFKTYENAYGEQKALLAQQGFYNIFDPNTGRADQRTLFE		840
M1249L_Georgia2007_1 (NC_044959)	YKRLPISTLYDERGLPHKWTIYVYKAVDSSQKPAEIEVTR		880
M1249L_OURT88_3 (NC_044957)	YKRLPISTLYDERGLPHKWTIYVYKAVDSSQKPAEIEVTR		880
M1249L_Georgia2007_1 (NC_044959)	KDVIKKIDNHYALADLRCSVCHVLQHEVGQLNIKKVQTAL		920
M1249L_OURT88_3 (NC_044957)	KDVIKKIDNHYALADLRCSVCHVLQHEVGQLNIKKVQTAL		920
M1249L_Georgia2007_1 (NC_044959)	KASLEFNTFYAFYESRCPKGGHDFQDKKCVKCGLFTYII		960
M1249L_OURT88_3 (NC_044957)	KASLEFNTFYAFYESRCPKGGHDFQDKKCVKCGLFTYII		960
M1249L_Georgia2007_1 (NC_044959)	YDHLSQPELVHDYNNYKDQYDKEKMSIRSIQIKKDMTTP		1000
M1249L_OURT88_3 (NC_044957)	YDHLSQPELVHDYNNYKDQYDKEKMSIRSIQIKKDMTTP		1000
M1249L_Georgia2007_1 (NC_044959)	STETQPKPPQEPWTFDYGKIIKTAKILDISPAVIEAIGAM		1040
M1249L_OURT88_3 (NC_044957)	SSETQPKPPQEPWTFDYGKIIKTAKILDISPAVIEAIGAM		1040
M1249L_Georgia2007_1 (NC_044959)	EGRSYADIREGQGAPPPPTSMDDPRLMAVDSAVRIFLYNY		1080
M1249L_OURT88_3 (NC_044957)	EGRSYADIREGQGAPPPPTSMDDPRLMAVDSAVRIFLYNY		1080
M1249L_Georgia2007_1 (NC_044959)	NCLRHVSTFNKPPTHVERLVKHLSEEEKEDLEKVLPNVVN		1120
M1249L_OURT88_3 (NC_044957)	NCLRHVSTFNKPPMHVERLVKHLSEEEKEDLEKVLPNVVN		1120
M1249L_Georgia2007_1 (NC_044959)	EYHTTFKHLRVTDPASALLYSEFLCTISFLTLYEIKEPSW		1160
M1249L_OURT88_3 (NC_044957)	EYHTTFKHLRVTDPASALLYSEFLCVSFLTLYEIKEPSW		1160
M1249L_Georgia2007_1 (NC_044959)	VVNIVREFALTELNTIIQSEKLLSKPGAFNFMIFGEDFVC		1200
M1249L_OURT88_3 (NC_044957)	VVNIVREFALTELNTIIQSEKLLSKPGAFNFMIFGEDFVC		1200
M1249L_Georgia2007_1 (NC_044959)	SGEDSSMDDISAYSSPGLFGEDIIDRLDDPFSIEDVDISL		1240
M1249L_OURT88_3 (NC_044957)	SGEDSSMDDISAYSSPGLFGEDIIDRLDDPFSIEDVDISL		1240
M1249L_Georgia2007_1 (NC_044959)	DVLDNLAPQ		1249
M1249L_OURT88_3 (NC_044957)	DVLDNLAPQ		1249

Figure 13. Multiple sequence alignment of M1249L amino acid sequences (721-1249) from Georgia2007/1 and OURT88/3. These sequences have 99.4% identity.

Supplementary Materials and Methods

Confocal staining and imaging

Vero African green monkey cells were used for confocal imaging as these enable better demarcation of cell morphology and have been used previously in our group (add DOI: 10.1099/jgv.0.001637). Briefly, cells were seeded at a density of 5×10^4 cells before transfection with plasmids and TransIT-LT1. After 24 hours incubation post-transfection, cells were fixed in 4% paraformaldehyde for 20 min before three washes with 1xPBS. For cells that were infected with ASFV (BA71V), cells were infected at an multiplicity of infection of 2, before washing and fixing 20 hours after infection. Cells were permeabilized with 0.2% (v/v) Triton-X in 1x PBS, then blocked with block buffer consisting of 10% (v/v) goat sera, 0.2% (v/v) NaN₃ and 0.2% (v/v) fish skin gelatin (Sigma-Aldrich, USA) in 1x PBS. Primary antibodies from pig sera were first pre-adsorbed on untransfected cells that were treated in the same manner. Coverslips were incubated for 1h with the following primary antibodies: pre-adsorbed pig sera (1:800) and mouse anti-nanoluciferase (1:200, Promega, USA) or anti-B646L/p72 (1:10, 4H3, in-house hybridoma supernatant) diluted in block buffer. After 3 washes with 1x PBS, coverslips were incubated with the appropriate secondary antibodies: anti-pig IgG Alexa Flour 488 (1:250, Southern Biotech, USA) and/or anti-mouse IgG Alexa Flour 568 (1:200, Invitrogen, USA) diluted in block buffer. Coverslips were washed another three times with 1x PBS before DNA staining with DAPI or TOPRO. Coverslips were mounted with Vectashield and imaging on a Leica SP8 confocal microscope.

Western blot analysis

Protein expression was confirmed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blot analysis. Human embryonic kidney cells (HEK293T) were used as these have a high transfection efficiency that is suitable for transient expression of exogenous proteins. Briefly, cell lysates from plasmid transfected HEK293T cells were prepared for separation on 4-12% NuPAGE Bis-Tris protein gels (Invitrogen, USA) with NuPAGE LDS sample buffer and reducing agent. Proteins were then transferred onto PVDF membranes (Cyvita, USA). Mouse anti-nanoluciferase antibodies (1:400, Promega, USA) were used for protein detection. Membranes were blocked with 5% skim milk (w/v) in 0.5% (v/v) PBS-Tween20 (PBS-T). Primary antibodies were diluted in 5% bovine serum albumin (BSA, w/v) in PBS-T and incubated with the blocked membranes for 1h with agitation. Membranes were washed three times with PBS-T before detection of primary antibodies with goat anti-mouse IgG HRP-conjugated antibodies (1:2000, BioRad, USA) diluted in 5% BSA in PBS-T. Protein visualization was achieved with SuperSignal West Pico PLUS chemiluminescent substrate (Thermo Scientific, USA) on the G:BOX XX6 (Syngene, USA).

Multiple alignments of ASFV protein sequences

Multiple sequences alignments generated from the selected ASFV capsid protein amino acid sequences derived from Georgia 2007/1 (GenBank No. NC_044959) and OURT88/3 (GenBank No. NC_044957) were aligned with EMBOSS Needle (EMBL-EBI). The FASTA output was shaded with the Multiple Align Show (http://www.bioinformatics.org/SMS/multi_align.html).

Table 1. DNA sequences of recombinant Nluc tagged ASFV protein open reading frames. Open reading frames were inserted into the pNeoSec vector containing a Nluc tag with a multiple cloning site containing a *HindIII* (Capitalised) and *AscI* cloning site (underlined). Start codon highlighted in bold.

B646L/p72
<p>AAGCTTgccaccatggtaccagcatctggcggagcctttgtctgatcgccaacgacggaaaggccgacaagatcattctggcccaggacctgctgaacagccgcatcagcaacatcaagaacgtgaacaagagctacggcaagcccgatccagagccaacactgagccagatcgaaagacacacctggtgcacttcaacgcccacttcaagccctacgtgccagtgggcttcgagtacaacaaagtgcgcccacacacgggcacacccacactgggaacaagctgaccttcggcatccctcagtagcggcacttctccacgacatggtcggacaccacatcctggagcctgtcacagctctggcaggatgcccaatccagggaacctctcagatgggagcacacggccagctgcagacattccctagaaacggctacgactgggacaaccagacaccactggaaggcgccgtgtacacactggtgacccattcggaaggccaatcgtgccaggcaccaaaaacgctacgcgaacctggtgtactactgcgagtaccaggcgagcgctgtacgagaacgtgcgcttcgacgtgaacggcaacagcctggagcagtagacagcagcgtgacaacctcgtgcgcaagtctgtatccccggcgacaagatgaccggctacaagcacctcgtgggccaagaggtgtcagtggaaggcacatctggaccctgctgtgcaacatccacgacctgcacaaacccaccagagacaagccaatcctgaccgacgagaacgacaccagagaacctgcagccacactaaccccaagtttctgagccagcacttccccgagaacagccacaacatccagacagccggcaagcaggacatcacccaatcacctgatgccactacctggacatcaggcgcaacgtgactacagctgcacggcccacagacaccaagtactaccagccacctgtgctctgtggatcaagctgcgcttctggttcaacgagaatgtgaacctggctatccccctcgtgtctatcccttctggcgagcgcttcatcacatcaaatggccagccagaaggacctggtcaacgagttcccaggactgttctcgcgacgagccgcttattgtctggacgaccaagccgcccgaacatccgcttcaagccatggttatcccaggcgtgatcaacgagatcagcctgaccaacaacgagctgtatcatcaaacctgttctgtgacccagagatccacaacctcttctgaagcgcggtgcggttctctctgatccgctgcacaaaacccaagtgaaccacaccaacaacaccacagatgagaagctgatgagcgccctgaagtggccatcgagtacatgttcatcgccctgaagccaacctggaacatctccgaccagaatccacaccagcacggcagctggcacaagttcggacacgtggtcaacgccaatcatgcaccaacacaccacgcccgaatcagcttccaggacagggatagacgctgcagacgcctgtagctccatcagcgatatcagcccgtgacatacccatcacactgccatcatcaaaaacatcagcgtgacagcccacggcatcaacctgatcagaagttcccatccaagttctgcagcagctacatcccccttccactacggcggaatgccatcaagacaccagatgaccaggcgccatgatgatcacttgcctgaagccccgaggaataccagccaagcgacacatcaacgtgtcccgcgacgagagattctacatcagctgggacacagactacgtgggcagcatcacaacagccgacctggtcgtgtctgccagcgccatcaatttctgctctccagaacggcagcgccgtgctgagatattctacacggcgccgc</p>
M1249L
<p>AAGCTTgccaccatggaggaggtcatcaccatgcccagatcgtgcacagaggcacggatatcctgagctgaacaatgaagaatcgaggccctggtgacgagatttactccagcttaaaaggagcaatgatataaagaatatacggctgatcgacttctgtttactctgaagattttgtgaacctgtcagagcggagcagagcaagctgcctgacctcagatgccatagaggcctacatccggcagttattagtagatcctgatgtggttccaattgtctccgagaagaagaagagttgagggtgcgacctagcaccgaaaggaaatatttctatcaatggcactcatcttcggtccacgcccagggcgccatcgagatttatgggctgaactcctgtgaagacttctcagctccagctgctcatgagaatggtgaaattggcagcttccccctgaaactttaggctatgtgacctctggggccaatctgactaatttcattctgtattcatgaagtgtgtggaccaggagacctggaagaaaaatggggaaggggtcgtcgtgaccacaaaggaaaatacatagttcacgcaccagtatatcgaactttataagtttctccgatcaggcgccatagctggtccttaaccgacttgcagaggagatggtgcatcgcaagctggatcgagaggaccagggtcgcacatcagcaatattgtggagacggaagaaatcgagcccaggagagaacattaaagagtgatcttttttaaaagagctatcacaatgtacagtggttccacctgtttacttctggctacatgcctctattgtatgatctttaccgggcccagatacctggagggttctgtggaacccctgcagcaaaaatctctgcaacatgcagagcagagagaaaaggagcagatgatcctgcaacaggtggacatgaactgactgaagtcattacgcaggccaggcagttatttaagatcatggaggaaaaaattggcgcgctccagctgatgcattcgggagatctccacctggaaggaaaggtggatgatccaactccatctgcaggaagtaatacaggcctgtggcaagcaagaagctgagttgataaccacagaatacctcaatattaagaacaatgggaactgcaagaaaagacgcctgtgccaactaaagtttagtaagcaattgcgtcaggcctgcagatgccgaactccttaaggttctggaatctatcagagtgtctataaggaaaaaataatacaaccaactggaatctgtgcaagcctgttggtgatttaagctactgtcccgcagctggacatgctgattcagctgcaggctgcggaagcttctacgacacatgaggaccaactgatgaagtctctggatcaataaagagaaagaaacaaccagggactgatatacagttattttgcaagatttgggagaggagttagctcatttaccaggaggacgcacagcagatgtaggaatcatcggggacctaaacagcaagctgcgggtgttcatctggcaggagacatgaaggcatgcacattcattccttgcgcaaacctctagatgtcaagcagtttccaacattgcggtgaatgtatcctgcgctggtgtattcaatagagaatataaaaaaaggaggaagattatgaccccttactcagctctacgcagtgatctacattatgctctatattctgaacctgatctacagctctcagaagaacaaggaaatttctactatcacgatacatggcatgaaagccgactcctcctcaacgcttactgtaacattctgtctggagaaaatgatgcagcagtagcgggattatcaatcaacttccggagattacagatcagtggaatagcaataacttccgggaggccttcaaaaaatcattcaccaaaacggcttcaaggactcaggttacaggacgacacaaaggcttcttaactgagattctgctgcacccgatgtatgattacgctgtaccgtcgcgaataagacggctccatccccatgcacaaacccaggacactaaagaggccgagtagtgaatttaagactgtgattggaaggactccgctgagttattgtcccagaaagaattttatgataagatctacagctgaagtagacagacgattttaccaactgcagagggttaaatgacatctattttcaggaagaaagcctgcgggtgtgtgtgggtggaagggtgaggagaaaacatcaactctgatctacttgcgggcataatgactcttctgaataactccagaacgccccaaatttcaacagtgaattggctgagttcaaaacatagagaacgcttacggcgcaacaaaaggccctgttggtcagcagggttttacaacatctttgaccgaatactgggagggcagaccagcgactgccttcttgaatacaaaaaggctaccatctccacctttatgatgaacggggactgccacaaagtggaccatctatgtttacaagcagttgacagttctcagaagccagcagaaaatagaggtcaccagaaaaggatgttataaaaagatagacaaccactacgcgtggctgaccttagatgctcgggtgtgtcacgctttacagcacgaggtgggtcagctgaatatcaaaaagtgcagaccgcttgaaggctagtctggagttcaacacattctatgcgttctatgagtcagctgccccaaaggaggacttcatgacttccaggacaagaagtgtgtcaaatgcggtcttctactacatcatatgaccacctgagccagcctgagctggtacagactactacaacactacaaggacagtagacgataaggagaagatgagcattcgaagcacaataaagaaggatatgacgacgcaagtagccaaacacagcccaagcctcctcaggaaacctggacatttgactacgggaaatcattaaagaccgccaagatttggatatctcccagccgtcattgaggcaatcgagccatggaaggcgcttctatgcagacatccgcgaggggcagggtgcaccgccaccaccgaccaatgatggacgatccacgcctcatggcgttgacagcgagtaagaattttctttataattacaactgtctccgtatgtctgcacttcaataaaacccctattcatgttgaaagggttggtcaacaatctctatgaagagaagaggacctgggaaagtcttgcctaactgtgtgaatgagtaccacaccacattcaagcacttgagggttacagacctgcagcgactactctactccatcgagtttctctcatcagcttctaaactgtacgaaatcaaggagccatcatgggtgtgaacattgtgaggagtttgcgtcacagagctgaataccatcatcaatctgaaaagctctgtccaacccgggcttttaactttatgatttccggtgaagatttctgtgttctggcgaagatttctcatgatgatataatcagcttacttccccgggcttttccggcaggacattattgatagactgacgaccttctcattgaagatgtggacatcagtttagatgtgttgataacttggccctcaagggcgccgc</p>
D117L
<p>AAGCTTgccaccatggacacagaaacttcacctttgctcagtcacaacctcttacaagggaaggaaatcaagcagctactcaaggtcttctagctataccattgccaagtaccagggaacacagccatccttctgggcatcctcatcctgttgatcatcattttaataattgtggctattgtttactacaaccgcagctgactgcaaatcctccataaccaagcctccaccagctattatgtcagcagccagagccccaccatcatcttctgtcttctccggaaaaagaaaaattcaacctcctgcagagccacatcccagtgatgagcagctggcagagctggcgacagcggcgccgc</p>

B438L
AAGCTTgccac cat gtaccatgactatgcaagcaagctcctcgcgattacaggagtgatccaccactgtgggagtcagatttaccagacataatcgctacagcgacaacatcctgaacagcaggctactgtggaaataaaatggcgctgcgcctgtctacaatgaatataccaatagccctgagaaggctgaaaaagggtgcagctgtctgacttgcgtaacttcagtttcatgctcaaccacacacacaagaatattggatatggggacgcccaggatftagagccgtattctagatccctaaagaacaaactctcaatcacttcaaaaaccatcgccggccttccctcactatctgaaaaattgatccggcggaatgttgaagaaccgagaagaagaccttccctcagggtggccagctgaaggggcacgcagaagaactgcctgactcagccctctcgcttcttccctaaaaaaccccaa
E120R
AAGCTTgccac cat ggccgacttcaacagccccatccagtacctaagaagactcgagagacaggacatctatagggagtttgaatatgacgagaatgctgataccatgatcccttcttctgctcaggactggaggaaatttgagccattccagattatgactctacgacttcaacatcccttacagccagctgactcacaacatggagaagattgcccaggaggaggacagcaactcctgcatgacacccgggagttcaccagcttagtgctgatgagggcgacaataaacagaagatgatgaagaagtggcgccaagccaagaaaaagaagcatcttctccgaaacttca
B602L
AAGCTTgccac cat gggtaccgcccaggttcaacatcgacgagctgtgaagaacgtgctggaagatccagcaccgagatcagcgaggaaacctgaagcagctgtaccagcgcaacacccctacaagcagttcaagaacgacagccgctggtgcttctgcagcttccaatctgcgcgagcagctacatccgcccgtgatcatgaccagcttcatcggtactgttcaaggccctgaagagtggtatgccagctacagcaagcccacacaccaccaagacactgctgagcgagctgatcacctggtggacacccctgaacaagagacaaacgactgtcccagcgagagcgtggtcaacacatctgtctatcgccgacagctgcaagaccagacagagaagtcgaagaggccaagacaacatcgacagcttctgcgcgaacacttctgttctgacccaaacctgca

Table 2. DNA sequence of recombinant Nluc with a 5' multiple cloning site (MCS) containing a *HindIII* (Capitalised) and *Ascl* cloning site (underlined). Start codon highlighted in bold.

MCS-Nluc
AAGCTTgccac cat gggtaccaggttctcgcgccggtgttcaactcgaagatttcttggggactggcgacagacagccggctacaacctggaccaagtcttgaacaggagggtgtgtccagtttgttcagaatctcggggtgtccgttaactccgatccaaaggattgtcctgagcgggtgaaaatgggctgaagatcgacatccatgtcatctccgtatgaagggtctgagcggcgacaaatgggcccagatcgaaaaattttaagggtgtgtacctgtggatgatcatcactttaagggtgatcctgcactatggcacactggtaatcgacggggttacccgaacatgatcactat

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