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

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**Supplementary Information**

Background pattern

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Figure 1. Confocal images of 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 20µm.

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Figure 2. Confocal images of cells expressing recombinant ASFV proteins B646L/p72, B602L 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 20µm.

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Figure 3. Western blot detection of recombinant Nluc-tagged ASFV proteins. Nluc-tagged ASFV proteins from transfected 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.

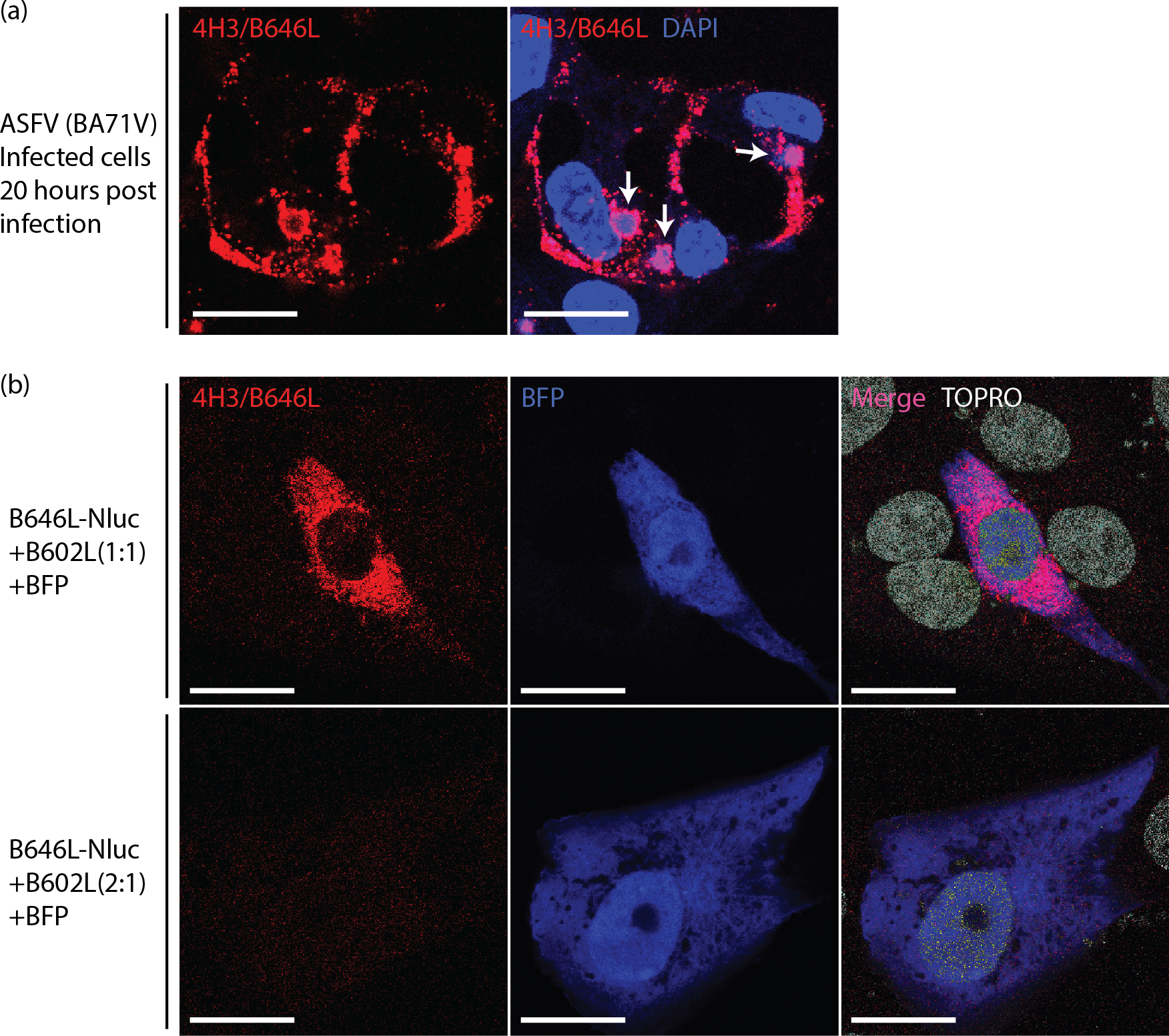


Figure 4. Determination of B646L/p72 and chaperone B602L transfection ratio. (a) ASFV (BA71V) infected Vero cells were used as a positive control for the 4H3 antibody that binds to a conformational epitope on B646L/p72 (red). Nuclear and viral DNA were stained with DAPI (blue). White arrows indicate 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 20µm.

Chart

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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 °C. The negative cutoff for temperature was set at 40.5°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.

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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 °C. The negative cutoff for temperature was set at 40.5°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.

Table

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

Table

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

Text

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

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

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

Table

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

Table

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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 cells were used for confocal imaging. Briefly, cells were seeded at a density of 5 x 104 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. 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 |
| AAGCTTgccacc**atg**gtaccagcatctggcggagccttttgtctgatcgccaacgacggaaaggccgacaagatcattctggcccaggacctgctgaacagccgcatcagcaacatcaagaacgtgaacaagagctacggcaagcccgatccagagccaacactgagccagatcgaagagacacacctggtgcacttcaacgcccacttcaagccctacgtgccagtgggcttcgagtacaacaaagtgcgcccacacaccggcacacccacactgggaaacaagctgaccttcggcatccctcagtacggcgacttcttccacgacatggtcggacaccacatcctgggagcctgtcacagctcttggcaggatgccccaatccagggaacctctcagatgggagcacacggccagctgcagacattccctagaaacggctacgactgggacaaccagacaccactggaaggcgccgtgtacacactggtggacccattcggaaggccaatcgtgccaggcaccaaaaacgcctaccgcaacctggtgtactactgcgagtacccaggcgagcgcctgtacgagaacgtgcgcttcgacgtgaacggcaacagcctggacgagtacagcagcgacgtgacaaccctcgtgcgcaagttctgtatccccggcgacaagatgaccggctacaagcacctcgtgggccaagaggtgtcagtggaaggcacatctggacccctgctgtgcaacatccacgacctgcacaaaccccaccagagcaagccaatcctgaccgacgagaacgacacccagagaacctgcagccacactaaccccaagtttctgagccagcacttccccgagaacagccacaacatccagacagccggcaagcaggacatcaccccaatcaccgatgccacctacctggacatcaggcgcaacgtgcactacagctgcaacggcccacagacacccaagtactaccagccacctctggctctgtggatcaagctgcgcttctggttcaacgagaatgtgaacctggctatcccctccgtgtctatccctttcggcgagcgcttcatcaccatcaaactggccagccagaaggacctggtcaacgagttcccaggactgttcgtccgccagagccgctttattgctggacgaccaagccgccgcaacatccgcttcaagccatggtttatcccaggcgtgatcaacgagatcagcctgaccaacaacgagctgtacatcaacaacctgttcgtgaccccagagatccacaacctcttcgtcaagcgcgtgcggttctctctgatccgcgtgcacaaaacccaagtgacccacaccaacaacaaccaccacgatgagaagctgatgagcgccctgaagtggcccatcgagtacatgttcatcggcctgaagccaacctggaacatctccgaccagaatccacaccagcaccgcgactggcacaagttcggacacgtggtcaacgccatcatgcagccaacacaccacgccgaaatcagcttccaggacagggatacagccctgccagacgcctgtagctccatcagcgatatcagccccgtgacataccccatcacactgcccatcatcaaaaacatcagcgtgacagcccacggcatcaacctgatcgacaagttcccatccaagttctgcagcagctacatccccttccactacggcggcaatgccatcaagacaccagatgacccaggcgccatgatgatcaccttcgctctgaagccccgcgaggaataccagccaagcggacacatcaacgtgtcccgcgccagagagttctacatcagctgggacacagactacgtgggcagcatcacaacagccgacctggtcgtgtctgccagcgccatcaattttctgctgctccagaacggcagcgccgtgctgagatattctacacggcgcgcc |
| M1249L |
| AAGCTTgccaccatggaggaggtcatcaccatcgcccagatcgtgcacagaggcacggatatcctgagtctgaacaatgaagaaatcgaggccctggtggacgagatttactccacgttaaaagggagcaatgatataaagaatatacggctgatcgacttcctgtttactctgaaagattttgtgaaccatgtcagagcggagcagagcaagctgcctgacctcagcatgcccatagaggcctacatccggcagttattagtagatcctgatgtggttccaattgtctccgagaagaagaaagagttgagggtgcggcctagcacccgaaaggaaatatttcttatcaatggcactcatcttgcggtcccagccgaggcgcccatcgagatttatgggctgaaactccgtctgaagactttcagtccccagtgcttcatgagaatggctgaaattggcagcttttcccctgaaactttaggctatgtggcctctggggccaatctgactaatttcattcgtgtattcatgaagtgtgtggaccaggagacctggaagaaaaatggggaaggggtcgtcgtgaccacaaaggaaaatatcatacagttcacgcaccagtatatcgaactttataagtttctccgatcaggcggccatagctggctcattaaccgacttgcagaggagatggtgcatcgcaagctggatcgagaggaccagggctcgcacatcagcaatattgtggagacggaagaaatcgagcccgaggagaacattaaaagagtgatcttttttttaaaagagctatctacaatgtacagtgtttcacccgtgtttacttctggctacatgcctctattgtatgatctttaccgggccggatacctggaggttctgtggaaccccgtcgagcaaaaattcctgcaacatgcagagcagagagaaaaggagcagatgatcctgcaacaggtggacatgaaactgactgaagtcattacgcaggccaggcagtattttaagatcatggaggaaaaaattggccgcgtccagtctgatgccattcgggagatcctcaccatggaaggaaaggtggatgatcccaactccatactgcaggaagtaatcaaggcctgtggcaagcaagaagctgagttgataaccacagaatacctcaatattaagaaacaatgggaactgcaagaaaagaacgcctgtgcccacctaaagttagttaagcaattgcgctcaggcctgcagtatgccgaactccttaaggttctggaatctatcagagtgctctataaggaaaaaaataatacaaccaactggaatctgtgcaaagcctgtggatttaagctactgtgcccgcacgtggacatgctgattcagctgcaggctgccgaagcttcctacgacaccatgaggaccaaactgatgaagttctctggtatcaataaagagaaagaaaacaaccagggactgatatacagttatttttgcaagatttgtggagaggagttagctcatttcatccaggaggaccgcacagcagatgtaggaatcatcggggacctaaacagcaagctgcgggtgttcatctggcaggagaccatgaaggcatgcacattcattcacttcggcaaactcgtagatgtcaagcagtttgccaacattgcggtgaatgtatgcctgccgctggtgtattcaatagagaatataaaaaaagaggaagattatgacccccttactcagctctacgcagtgatctacatttatgcctatattctgaacctgatctacagctctcagaagaacaaggaatttcttactatcacgatacatggcatgaaagccgactcctccctcaacgcttacgtaacattcttgctggagaaaatgatgcagcagtacagcgggattatcaatcaactttcggagattacagatcagtggatagcaaataacttccgggaggccttcaaaaaaatcattcaccaaaacggtcttcaaggactcagtgtacaggacgacacaaaggttctcttaactgagattctgctcgacccgatgtatgattacgctgctaccgtcgccagaatagacggctccatccccatgcacaaacccaggacacctaaagaggccgagtatgagtttaagactgtgattggaaggactcccgctgagttattgtcccagaaagaattttatgataagatctacacgtcgaagtacagaccagattttacccaactgacgaggttaaatgacatctattttcaggaagaaagcctgcgggtgtggtggggtggaagggatgaggagaaaacatcaactctgatctacttgcgggcatatgagctctttctgaaatacctccagaacgccccaaatttcaacagtgaattggctgagttcaaaacatatgagaacgcttacggcgaacaaaaggccctgttggctcagcagggtttctacaacatctttgacccgaatactgggagggcagaccagcgcactcgcctctttgaatacaaaaggctacccatctccaccctttatgatgaacggggactgccacacaagtggaccatctatgtttacaaagcagttgacagttctcagaagccagcagaaatagaggtcaccagaaaggatgttattaaaaagatagacaaccactacgcgctggctgaccttagatgctcggtgtgtcacgtcttacagcacgaggtgggtcagctgaatatcaaaaaagtgcagaccgctttgaaggctagtctggagttcaacacattctatgcgttctatgagtcacgctgccccaaaggaggacttcatgacttccaggacaagaagtgtgtcaaatgcggtctcttcacttacatcatatatgaccacctgagccagcctgagctggtacacgactactacaacaactacaaggaccagtacgataaggagaagatgagcattcgaagcatacaaattaagaaggatatgacgacgccaagtaccgaaacacagcccaagcctcctcaggaaccctggacatttgactacgggaaaatcattaagaccgccaagattttggatatctccccagccgtcattgaggcaatcggagccatggaagggcgttcctatgcagacatccgcgaggggcagggtgcaccgccaccaccgaccagtatggacgatccacgcctcatggccgttgacagcgcagtaagaattttcctttataattacaactgtctccgtcatgtctcgaccttcaataaaccccctattcatgtggaaaggttggtcaaacatctctcatatgaagagaaagaggacctggagaaagtcttgcctaacgtggtgaatgagtaccacaccacattcaagcacttgagggttacagaccctgccagcgcactactctactccatcgagtttctctgcatcagctttctaacactgtacgaaatcaaggagccatcatgggtggtgaacattgtgagggagtttgcgctcacagagctgaataccatcatccaatctgaaaagctcctgtccaaacccggggcttttaactttatgattttcggtgaagatttcgtgtgttctggcgaagattcttccatggatgatatatcagcttactcttccccgggccttttcggcgaggacattattgatagactggacgaccctttctccattgaagatgtggacatcagtttagatgtgttggataacttggcccctcaagggcgcgcc |
| D117L |
| AAGCTTgccaccatggacacagaaacttcacctttgctcagtcacaacctctctacaagggaaggaatcaagcagtctactcaaggtcttctagctcataccattgccaagtacccagggaccacagccatccttctgggcatcctcatcctgttgatcatcattttaataattgtggctattgtttactacaaccgcacgattgactgcaaatcctccatacccaagcctccacccagctattatgtgcagcagccagagccccaccatcattttcctgtcttcttccggaaaagaaaaaattcaacctccctgcagagccacatcccgagtgatgagcagctggcagagctggcgcacagcgggcgcgcc |
| B438L |
| AAGCTtgccacc**atg**taccatgactatgcaagcaagctcctcgcggattacaggagtgatccaccactgtgggagtcagatttacccagacataatcgctacagcgacaacatcctgaacagcaggtactgtggaaataaaaatggcgctgcgcctgtctacaatgaatataccaatagccctgagaaggctgaaaaagggctgcagctgtctgacttgcgtaacttcagtttcatgctcaacccacaacacaagaatattggatatggggacgcccaggatttagagccgtattctagtatccctaagaacaaactcttcaatcacttcaaaaaccatcggccggccttctccactcatactgaaaatttgatccggcggaatgttgtaagaaccgagaagaagaccttccctcaggtggccagtctgaagggcacgcagaagaactgcctgactcagccctcttcgcttccttccctaaaaaaccccaagaactcctcagttccgtcaacacgcttcagcgagcacaccaaatttttttcttacgaggaccttccaaagttaagaacgaaaggaacaataaaacatgagcagcacttgggtgaccagatgccaggccagcattataacggctatatcccgcacaaagatgtctacaatatcctctgtcttgcacacaacctgcccgcatcagtggaaaaagggattgctgggcgaggaataccactgggcaaccctcacgtgaagccaaatattgaacaagagctgataaagagcacatcaacatatacagatgtccccatgctaggccccctccctcccaaagacagccaacacggccgggagtaccaggaattttccgctaatagacacatgttgcaggtgagcaatatcctgcactccgtgtttgcaaatcattccatcaagccgcagattctggaggatattccagtgcttaacgctcagctcacgtcgataaaacctgtaagccccttcctgaacaaggcctatcaaacgcactacatggagaacattgtcaccctggtgcccaggtttaaatccattgcgaactattcttcccccatccccaactactccaagaggaacagtggacaggccgaatactttgatacttcaaagcagaccatcagtcgacataacaattatatacctaagtacaccgggggcattggtgattctaagctggacagcaccttccctaaagactttaatgcctcatctgttcccctgacttccgccgaaaaggaccacagcctccgcggtgacaattctgcctgctgcatcagcagtatctccccatctttggggcgcgcc |
| E120R |
| AAGCTTgccacc**atg**gccgacttcaacagccccatccagtacctgaaagaagactcgagagacaggacatctatagggagtttggaatatgacgagaatgctgataccatgatcccttcctttgctgcaggactggaggaatttgagcccattccagattatgatcctacgacttcaacatccctctacagccagctgactcacaacatggagaagattgccgaggaggaggacagcaacttcctgcatgacacccgggagttcaccagcttagtgcctgatgaggcggacaataaaccagaagatgatgaagaaagtggcgccaagcccaagaaaaagaagcatctctttccgaaactttcatctcacaagtccaaagggcgcgcc |
| B602L |
| AAGCTTgccacc**atg**ggtaccgccgagttcaacatcgacgagctgctgaagaacgtgctggaagatcccagcaccgagatcagcgaggaaaccctgaagcagctgtaccagcgcacaaacccctacaagcagttcaagaacgacagccgcgtggccttctgcagcttcaccaatctgcgcgagcagtacatccgccgcctgatcatgaccagcttcatcggctacgtgttcaaggccctgcaagagtggatgcccagctacagcaagcccacacacaccaccaagacactgctgagcgagctgatcaccctggtggacaccctgaaacaagagacaaacgacgtgcccagcgagagcgtggtcaacaccatcctgtctatcgccgacagctgcaagacccagacacagaagtccaaagaggccaagacaacaatcgacagcttcctgcgcgaacacttcgtgttcgacccaaacctgcacgcccagagcgcctacacatgtgccgacacaaacgtggacacctgtgccagcatgtgcgccgacaccaatgtggatacttgcgcctctatgtgtgccgatacgaatgtcgacacatgcgccagcacctgtacctccaccgagtacacagatctggccgatccagagcgcatcccactgcacatcatgcagaaaacactgaacgtgccaaacgagctgcaggccgacatcgacgccatcacacagacaccacagggatatagagccgccgctcacatcctccagaacatcgagctgcaccagagcatcaagcacatgctcgaaaaccccagggccttcaagcccatcctgttcaacaccaagatcacccgctacctgagccagcacatcccaccacaggacaccttctacaagtggaactactacatcgaggacaactacgaggaactgagggccgccaccgagagcatctacccagagaaacccgacctggaattcgccttcatcatctacgacgtggtggacagcagcaaccagcagaaggtggacgagttctactacaagtacaaggaccagatcttcagcgaggtgtccagcatccagctcggcaattggactctgctgggcagcttcaaagccaaccgcgagcgctacaactacttcaaccagaacaacgagatcatcaaacgcatcctggaccgccacgaagaggacctgaagatcggcaaagagatcctgaggaacaccatctaccacaagaaggccaagaacatccaagagacaggcccagacgctcccggcctgtccatctacaacagcaccttccacaccgacagcggcatcaagggactgctgtccttcaaagagctgaaaaacctggaaaaggccagcggcaacatcaagaaagcccgcgagtacgacttcatcgacgattgcgaggaaaagatcaagcagctcctgagcaaagagaatctgaccccagacgaggaaagcgaactgatcaagaccaagaagcagctcgacaacgccctggaaatgctgaatgtgcccgacgacacaatcagagtggatatgtgggtcaacaacaacaacaagctggaaaaagagatcctctacaccaaggccgagctggggcgcgcc |

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

|  |
| --- |
| MCS-Nluc |
| AAGCTTgccacc**atg**gtaccaggttctcggcgcgccgtgttcacactcgaagatttcgttggggactggcgacagacagccggctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgtttcagaatctcggggtgtccgtaactccgatccaaaggattgtcctgagcggtgaaaatgggctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcggcgaccaaatgggccagatcgaaaaaatttttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgcactatggcacactggtaatcgacggggttacgccgaacatgatcgactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcaaccccgacggctccctgctgttccgagtaaccatcaacggagtgaccggctggcggctgtgcgaacgcattctggcgcgcgccaagcaccaccatcaccatcactaa |

**References**

1. Goatley, L. C.; Nash, R. H.; Andrews, C.; Hargreaves, Z.; Tng, P.; Reis, A. L.; Graham, S. P.; Netherton, C. L., Cellular and Humoral Immune Responses after Immunisation with Low Virulent African Swine Fever Virus in the Large White Inbred Babraham Line and Outbred Domestic Pigs. *Viruses* **2022,** 14, (7).