

## **Supplementary Material**

### **Characterization of Parrot Bornavirus-4 Infection in Immortalized and Primary Cell Lines**

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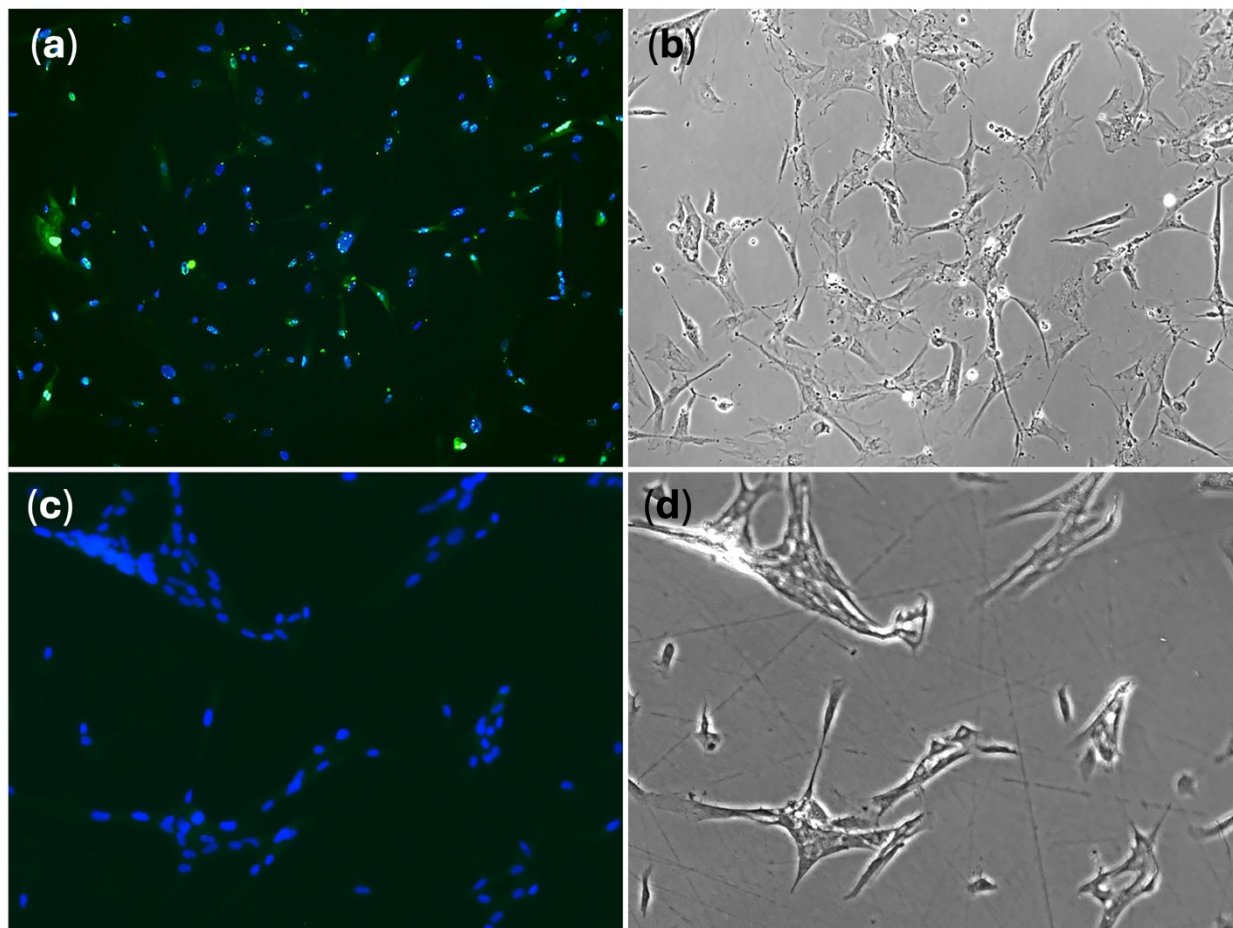
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54323.

**Table S1.** Mutations detected by Next-Generation RNA Sequencing in PaBV-4 genomes collected from DEF, DF-1, CCL-141, and QT-35 at the end of the growth curves.

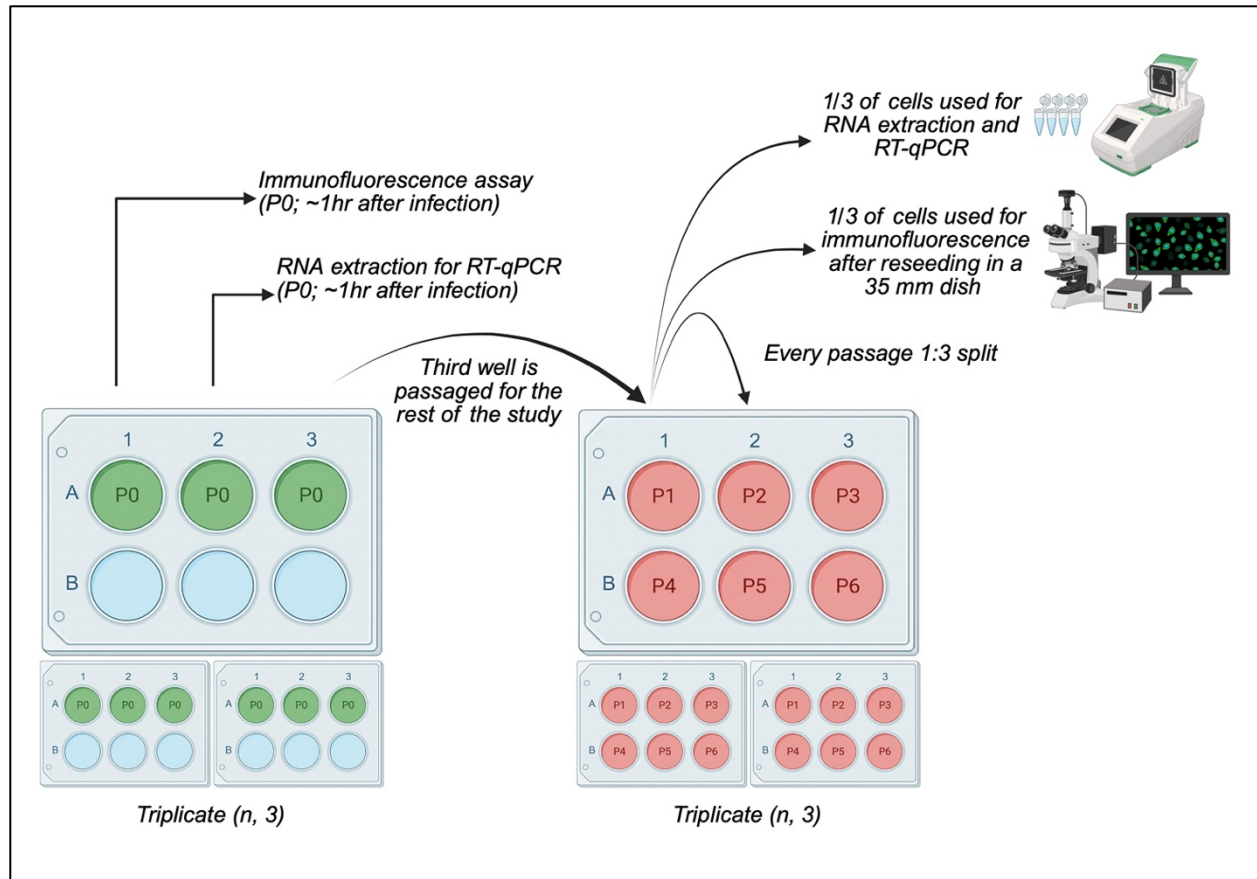
| Sample *    | Total # of Mutations ** | Mean Read Depth *** | Position in the genome | Nucleotide change (%)# | Coding / non-coding | Amino acid change    |
|-------------|-------------------------|---------------------|------------------------|------------------------|---------------------|----------------------|
| DEF-p5      | 3                       | 6270                | 1461                   | A>C (9.71%)            | P-protein           | Asn>His (missense)   |
|             |                         |                     | 1461                   | A>C (9.71%)            | X-protein           | Glu>Ala (missense)   |
|             |                         |                     | 7207                   | C>T (10.55%)           | L-protein           | Leu>Phe (missense)   |
| CCL-141-p15 | 7                       | 5569                | 1190                   | T>C (5.63%)            | non-coding          | NA                   |
|             |                         |                     | 1467                   | G>A (17.23%)           | P-protein           | Asp>Asn (missense)   |
|             |                         |                     | 1637                   | C>T (5.56%)            | P-protein           | Ser>Ser (synonymous) |
|             |                         |                     | 6935                   | T>C (18.49%)           | L-protein           | Ile>Thr (missense)   |
|             |                         |                     | 7207                   | C>T (44.11%)           | L-protein           | Leu>Phe (missense)   |
|             |                         |                     | 8234                   | C>T (7.57%)            | L-protein           | Ala>Val (missense)   |
|             |                         |                     | 8606                   | C>T (9.23%)            | L-protein           | Thr>Ile (missense)   |
| CCL-141-p24 | 9                       | 7376                | 1190                   | T>C (40.74%)           | non-coding          | NA                   |
|             |                         |                     | 1467                   | G>A (13.26%)           | P-protein           | Asp>Asn (missense)   |
|             |                         |                     | 1631                   | C>T (26.62%)           | P-protein           | Tyr>Tyr (synonymous) |
|             |                         |                     | 3675                   | T>C (7.67%)            | G-protein           | Val>Ala (missense)   |
|             |                         |                     | 3675                   | T>C (7.67%)            | L-protein           | Arg>Arg (synonymous) |
|             |                         |                     | 5280                   | T>C (5.28%)            | L-protein           | Asn>Asn (synonymous) |
|             |                         |                     | 6935                   | T>C (9.91%)            | L-protein           | Ile>Thr (missense)   |
|             |                         |                     | 7207                   | C>T (41.01%)           | L-protein           | Leu>Phe (missense)   |
|             |                         |                     | 8234                   | C>T (6.10%)            | L-protein           | Ala>Val (missense)   |
| DF-1-p21 A  | 13                      | 882                 | 33                     | A>C (5.00%)            | non-coding          | NA                   |
|             |                         |                     | 1190                   | T>C (65.80%)           | non-coding          | NA                   |
|             |                         |                     | 1198                   | C>T (6.10%)            | non-coding          | NA                   |
|             |                         |                     | 1631                   | C>T (35.58%)           | P-protein           | Tyr>Tyr (synonymous) |
|             |                         |                     | 2039                   | A>G (5.04%)            | M-protein           | Lys>Arg (missense)   |
|             |                         |                     | 2155                   | G>A (5.11%)            | M-protein           | Asp>Asn (missense)   |
|             |                         |                     | 3628                   | G>T (5.14%)            | G-protein           | Trp>Cys (missense)   |
|             |                         |                     | 3675                   | T>C (29.79%)           | G-protein           | Val>Ala (missense)   |
|             |                         |                     | 3675                   | T>C (29.79%)           | L-protein           | Arg>Arg (synonymous) |
|             |                         |                     | 4625                   | C>T (5.16%)            | L-protein           | Ala>Val (missense)   |

|             |    |      |      |              |            |                         |
|-------------|----|------|------|--------------|------------|-------------------------|
|             |    |      | 6934 | A>G (7.11%)  | L-protein  | Ile>Val<br>(missense)   |
|             |    |      | 6935 | T>C (6.39%)  | L-protein  | Ile>Thr<br>(missense)   |
|             |    |      | 7207 | C>T (34.19%) | L-protein  | Leu>Phe<br>(missense)   |
| DF-1-p21 B  | 12 | 1638 | 1190 | T>C (75.17%) | non-coding | NA                      |
|             |    |      | 1631 | C>T (43.12%) | P-protein  | Tyr>Tyr<br>(synonymous) |
|             |    |      | 2238 | G>T (8.18%)  | G-protein  | Arg>Leu<br>(missense)   |
|             |    |      | 2238 | G>T (8.18%)  | M-protein  | Ser>Ser<br>(synonymous) |
|             |    |      | 2280 | C>T (17.16%) | G-protein  | Thr>Ile<br>(missense)   |
|             |    |      | 2280 | C>T (17.16%) | M-protein  | Asp>Asp<br>(synonymous) |
|             |    |      | 2774 | C>T (6.83%)  | G-protein  | His>Tyr<br>(missense)   |
|             |    |      | 3675 | T>C (34.37%) | G-protein  | Val>Ala<br>(missense)   |
|             |    |      | 3675 | T>C (34.37%) | L-protein  | Arg>Arg<br>(synonymous) |
|             |    |      | 6934 | A>G (10.08%) | L-protein  | Ile>Val<br>(missense)   |
|             |    |      | 6935 | T>C (7.10%)  | L-protein  | Ile>Thr<br>(missense)   |
|             |    |      | 7207 | C>T (28.96%) | L-protein  | Leu>Phe<br>(missense)   |
| QT-35-p21 A | 5  | 5699 | 1467 | G>A (6.41%)  | P-protein  | Asp>Asn<br>(missense)   |
|             |    |      | 6935 | T>C (20.75%) | L-protein  | Ile>Thr<br>(missense)   |
|             |    |      | 7207 | C>T (52.11%) | L-protein  | Leu>Phe<br>(missense)   |
|             |    |      | 8234 | C>T (6.82%)  | L-protein  | Ala>Val<br>(missense)   |
|             |    |      | 8606 | C>T (6.79%)  | L-protein  | Thr>Ile<br>(missense)   |
| QT-35-p21 B | 10 | 6372 | 15   | C>G (5.56%)  | non-coding | NA                      |
|             |    |      | 1439 | G>T (5.21%)  | P-protein  | Lys>Asn<br>(missense)   |
|             |    |      | 1439 | G>T (5.21%)  | X-protein  | Asp>Tyr<br>(missense)   |
|             |    |      | 1467 | G>A (6.69%)  | P-protein  | Asp>Asn<br>(missense)   |
|             |    |      | 2280 | C>T (6.79%)  | G-protein  | Thr>Ile<br>(missense)   |
|             |    |      | 2280 | C>T (6.79%)  | M-protein  | Asp>Asp<br>(synonymous) |
|             |    |      | 6935 | T>C (17.85%) | L-protein  | Ile>Thr<br>(missense)   |
|             |    |      | 7207 | C>T (61.45%) | L-protein  | Leu>Phe<br>(missense)   |
|             |    |      | 7744 | G>A (6.31%)  | L-protein  | Glu>Lys<br>(missense)   |
|             |    |      | 8606 | C>T (6.13%)  | L-protein  | Thr>Ile<br>(missense)   |

\*Indicates the origin of the samples, with A/B indicating biological replicate. PaBV-4 was sequenced from initial isolation (DEF-p5), growth of the virus stock (CCL-141-p15), and after growth curves (DF-1 p21, QT-35-p21, CCL-141-p24). \*\* Total number of mutations in each sequenced genome, compared and aligned to the reference genome (sequenced from tissues). This includes mutations at a single position that affected two overlapping genes \*\*\*Mean read depth is rounded down to the nearest whole number. # Indicates the percentage of reads where change was identified. A 90/10 strand bias filter (based on total counts) at each position was applied to differentiate authentic low-frequency mutations from sequencing artifacts. This ensures no single sequencing direction accounts for more than 90% of the total reads at each position. A minimum read depth of 20 per position was set as the threshold, and a variant was called when the % of reads exhibiting the mutation were > 5% of the total reads.



**Figure S1.** Detection of ABBV-1 nucleoprotein in immortalized duck fibroblasts (CCL-141) by indirect immunofluorescence. Representative merged fluorescence (green and blue channels; **a** and **c**) and corresponding phase-contrast (**b** and **d**) images. The immunoreactivity for ABBV-1 nucleoprotein (N) is shown in green and nuclei are counterstained with DAPI (blue). Panels **a** and **b** represent ABBV-1-infected cells, whereas panels **c** and **d** show uninfected control cultures. Positive cells show finely granular to stippled nuclear reactivity with mild, finely diffuse cytoplasmic labelling. Negative cells show not reactivity.



**Figure S2.** Experimental design for serial passaging of PaBV-4-infected cell cultures. Three biological replicates of infected cultures were maintained in parallel. At passage 0 (P0), one well each was collected for RT-qPCR and immunofluorescence assay (IFA), while the cells from the remaining well were serially passaged (1:3 split) through six passages (P1–P6). At each passage, one-third of the cells was used for RNA extraction and RT-qPCR, one-third for IFA following reseeding in a 35-mm dish, and the remaining one-third was used to establish the subsequent passage. See Methods for additional details.