

Supplementary Materials: Characterization of the Humoral Immune Response Induced After Infection with Atypical Porcine Pestivirus (APPV)

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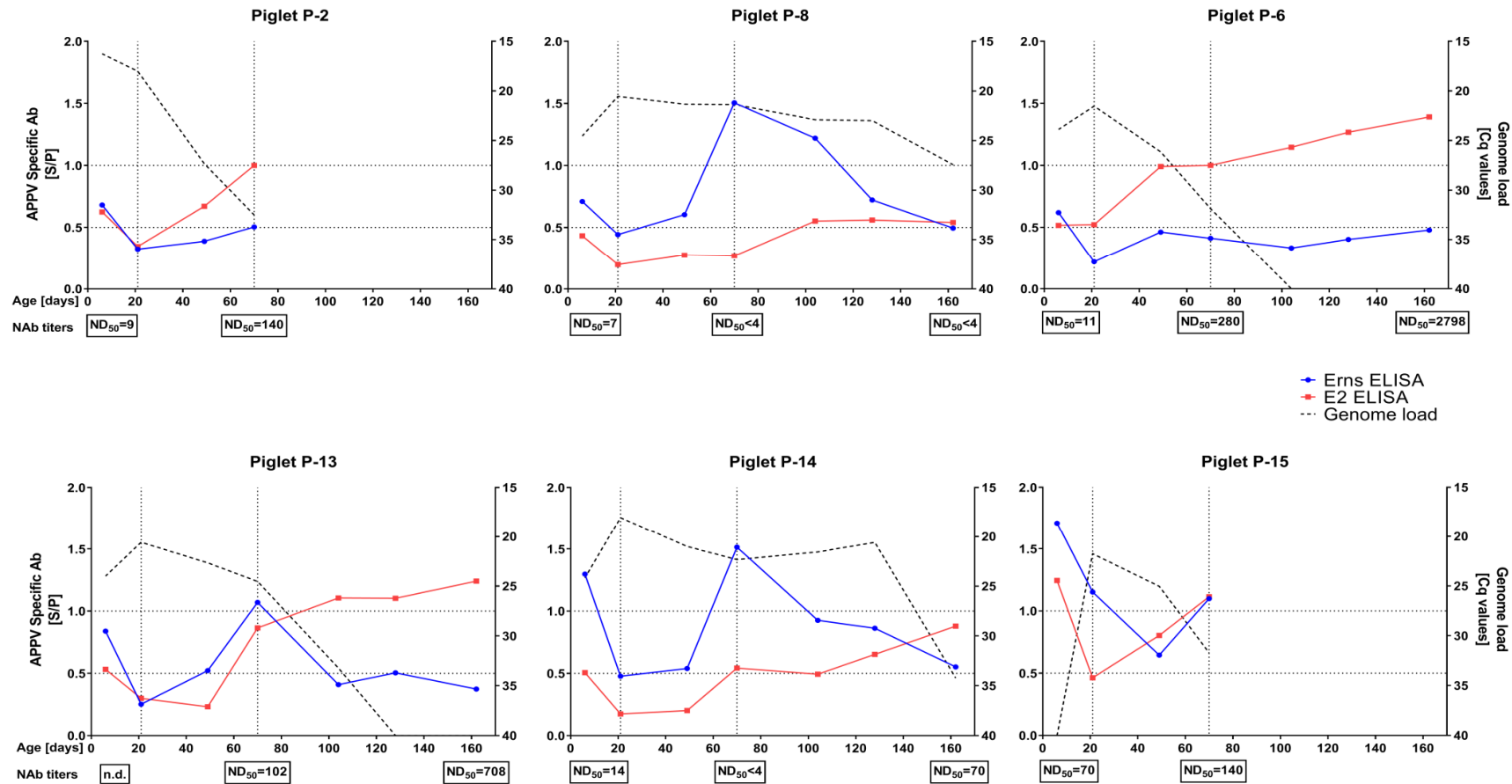
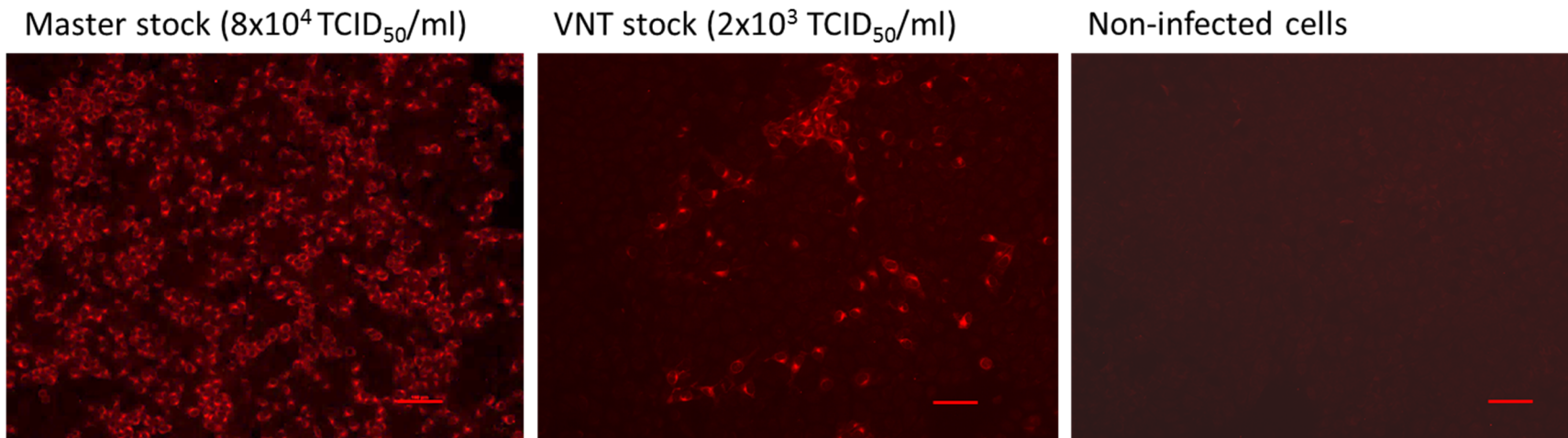


Figure S1. Course of APPV infection and antibody response in individual piglets from CT affected litters. Upper row: CT affected piglets, lower row: healthy piglets from CT affected litters. Vertical dashed lines on day 21 and day 69 indicates the time of weaning and time of transfer to a fattening farm. Horizontal dashed lines indicates the E2 and E^{ms}-specific antibody levels as low, intermediate and high, which are given as S/P values ($S/P \leq 0.5$, low; $S/P = 0.5-1.0$ intermediate; $S/P \geq 1.0$ highly reactive). Neutralizing antibody (nAb) titers are given for days six, 69 and 161. "n.d.": not done. Only the piglets, which were discussed individually within the text, are presented. Piglet P-2 is a representative for the CT affected litter 1 that was cross-fostered immediately after birth to a pluriparous sow with a healthy litter. Since various patterns of Ab response are seen in piglets from CT affected litters, two individual examples of diseased (P-6 & P-8) and healthy (P-13 & P-14) piglets of CT affected litters are shown. P-15 is the only piglet from the CT affected litters that was initially genome negative.

a) Immunofluorescence staining after *de novo* infection with virus stocks of APPV isolate “L277_{P100}” used in Virus Neutralization Test (VNT)



b) Neutralizing activity of porcine serum in dependence on the serum dilution

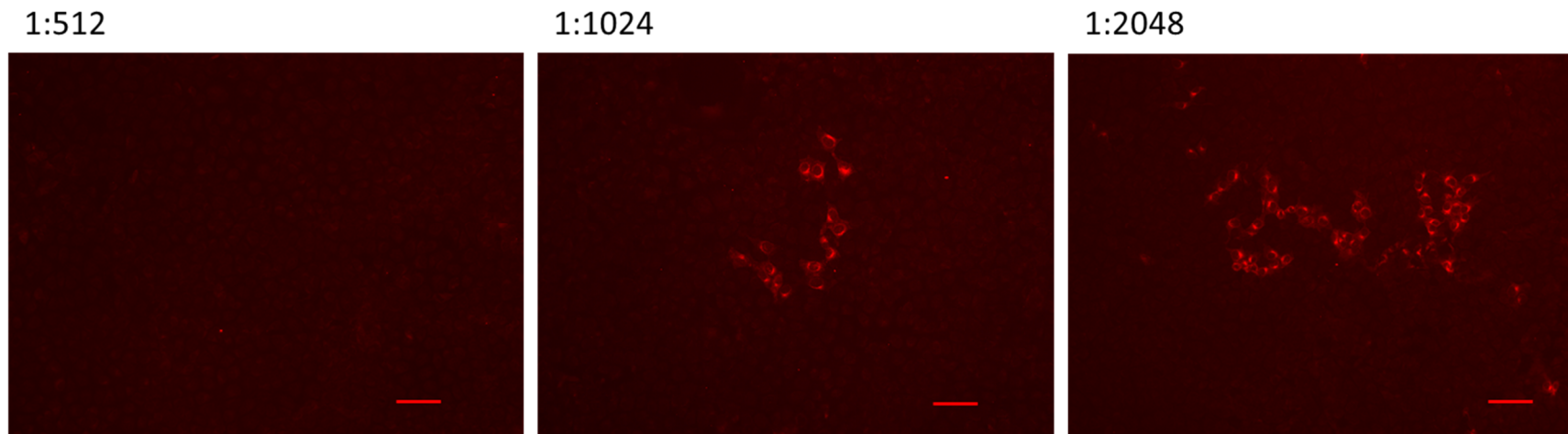


Figure S2. Development of a novel APPV Virus Neutralization Test (VNT). **Panel a)** Immunofluorescence staining after *de novo* infection with virus stocks of APPV isolate “L277_{P100}” used in VNT. SPEV cells were infected simultaneously at the time point of seeding. Cells were heat fixed 3 days post infection for immunofluorescence staining. Non infected cells served as a negative control. **Panel b)** Neutralizing activity of a porcine serum in dependence on the serum dilution. Serial dilutions of a serum sample (used as a control serum in each VNT assay) were performed using Log 2 dilution steps. VNT was performed as described within the manuscript and APPV “L277_{P100}” VNT stock (10^2 TCID₅₀/50 μ l) shown in panel a was used as a test virus. Scale bars indicate 100 μ m.