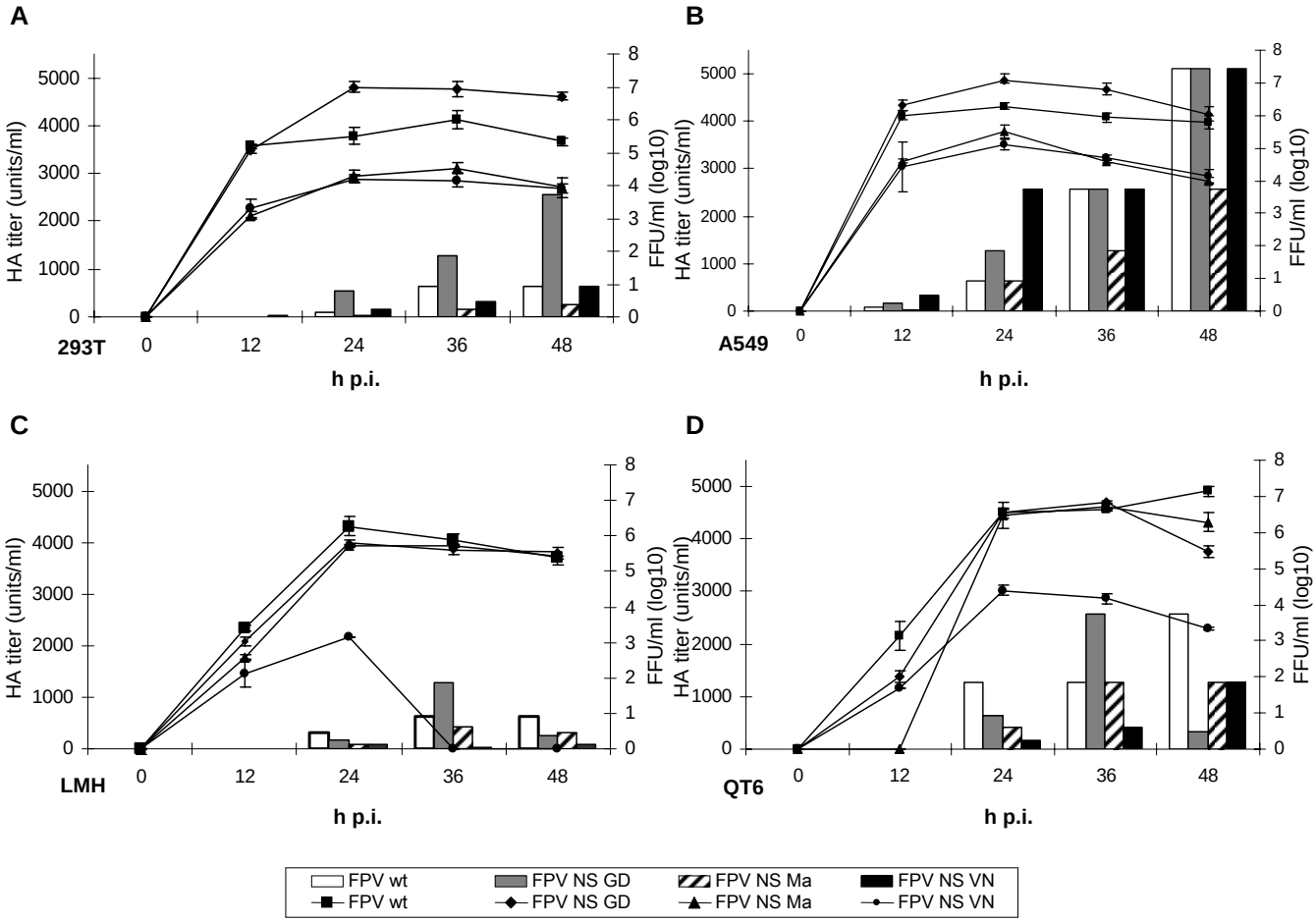
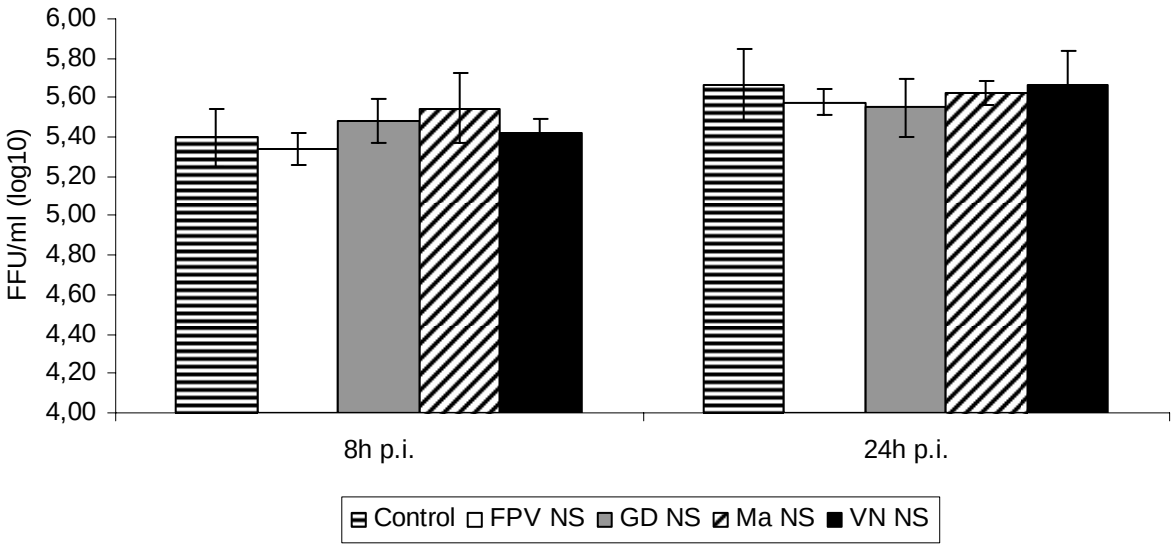
Supplementary Figure 1



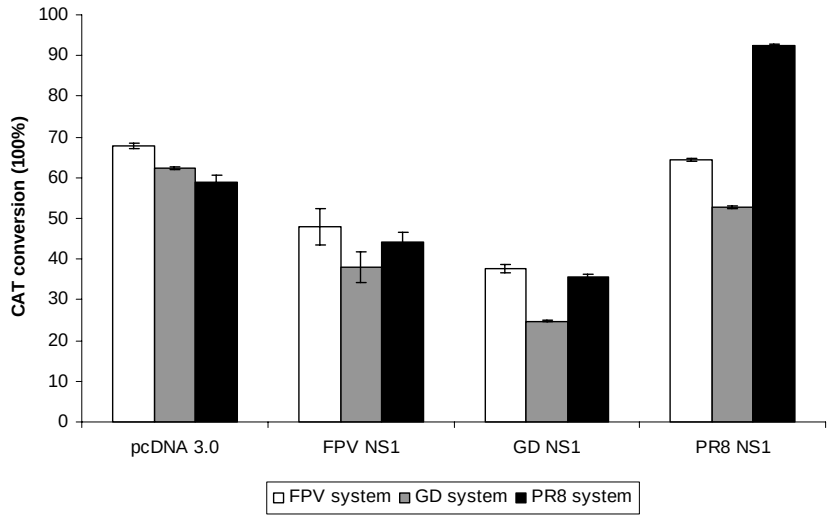
Supplementary Figure 1: Viral growth curves of recombinant virus titres in mammalian and avian cell culture systems. Confluent monolayers of cells (**A)** 293T, **B)** 549, **C)** LMH, **D)** QT6) were infected with the recombinant viruses at an MOI of 0.01. Supernatants were harvested every 12h p.i.. Virus growth curves (represented as lines) were determined by foci assays in MDCK cells and measured in FFU/ml. HA titres (represented as bars) were determined using chicken red blood cells and measured in HU/ml. Results represent the average of three independent experiments. The result is based on three independent experiments.

Supplementary Figure 2



Supplementary Figure 2: Effect of transient expressed NS2/NEP on the propagation of NS reassortants. Sub confluent MDCK cells in 3.5cm dishes were transfected with 1µg plasmids expressing FPV-, GD-, VN- and Ma-NS2/NEP, at 24h post transfection, the cells were infected with FPV wt at an MOI of 1. Supernatants were taken at 8h p.i. and 24h p.i. and virus titer were determined by Foci assay in MDCK cells. The result is derived from three independent experiments titred in duplicates.

Supplementary Figure 3



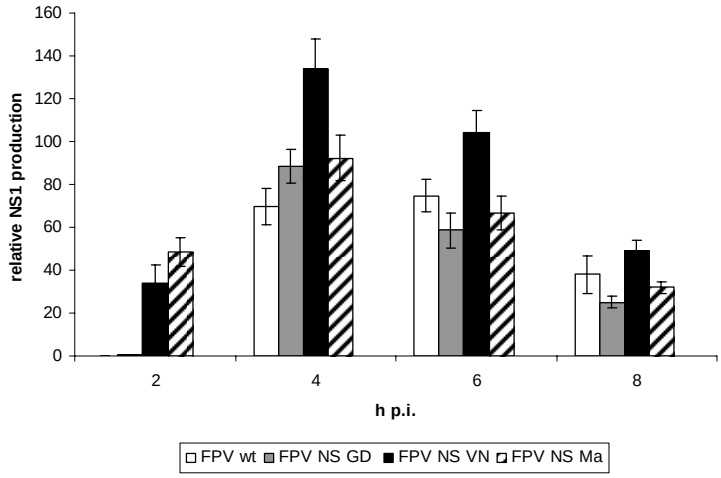
Supplementary Figure 3: CAT assay to investigate interactions between various NS1 proteins and RNP complexes. 293T cells were transfected with plasmids expressing the PB1, PB2, PA and NP proteins from FPV, GD or PR8 viruses and pPolI-CAT-RT for vRNA expression together with either empty vector or plasmids expressing FPV-NS1, GD-NS1 or PR8-NS1. At 48h post-transfection, cell extracts were prepared and tested for CAT activity. The result is based on three independent experiments.

Supplementary Figure 4

Supplementary Figure 4: Sequence comparison between different NS1 proteins.

A) Phylogenetic analysis of the NS1 proteins of A/FPV/Rostock/34 (FPV, H7N1), A/Goose/Guangdong/1/96 (GD, H5N1), A/Vietnam/1203/2004 (VN, H5N1) and A/Mallard/NL/12/2000 (Ma, H7N3) and their allele type. **B)** Alignment of the amino acid sequences of the four NS1 proteins with sequence identity colored in black. The sequence comparison was performed using Lasergene 6.0 software. **C)** Alignment of the amino acid sequences of the four NS2/NEP proteins with sequence identity colored in black.

Supplementary Figure 5



Supplementary Figure 5: NS1 protein expression measured by in-cell western blot. Confluent MDCK cells in 96-well plates were infected with the different recombinant viruses at an MOI of 1. Cells were fixed at 2 hour intervals and an “in-cell western blot” using an anti-NS1 antibody, ERK2 antibody (as a control) followed by staining with goat anti-rabbit IRDye 680 or goat anti-mouse IRDye 800 CW (both from Licor) was carried out. Infrared Imaging System and application software package (Licor) was used to quantitate the signals. For each time point six wells were analyzed.