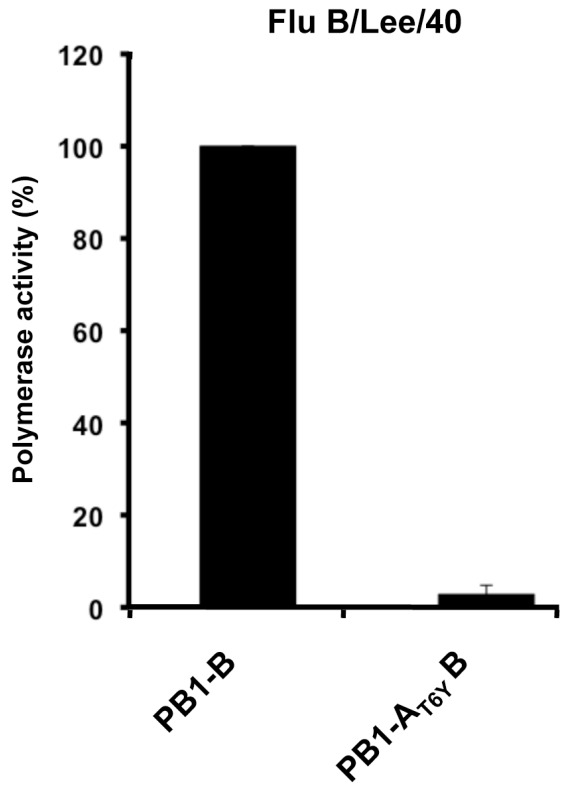

Fig. 1S:

The PB1-AT6YB variant of B/Lee/40 is strongly reduced in replication/transcription activity. Human 293T cells (5 x 10e5) were transfected with expression plasmids for the influenza B/Lee/40 virus PB1 wild-type (PB1-B) or PB1-AT6YB variant together with vectors encoding the replicative PA, PB2 (50 ng each) and NP proteins (100 ng), and pPol-B/NS-Luc (125 ng) encoding a firefly luciferase cDNA of negative polarity flanked by the non-coding regions of the viral NS segment. The constitutive expression vector pTK-RL was used to normalize for transfection efficiency (5 ng). Transfected cells were seeded in 12 well plates and analyzed after 24 hours by dual luciferase assay (Promega). Firefly luciferase activity was normalized by use of RL activity. The values for PB1-wt were arbitrarily set to 100% and compared to the PB1 mutant activity. Average results from four independent experiments are shown. The error bar represents the standard deviation.

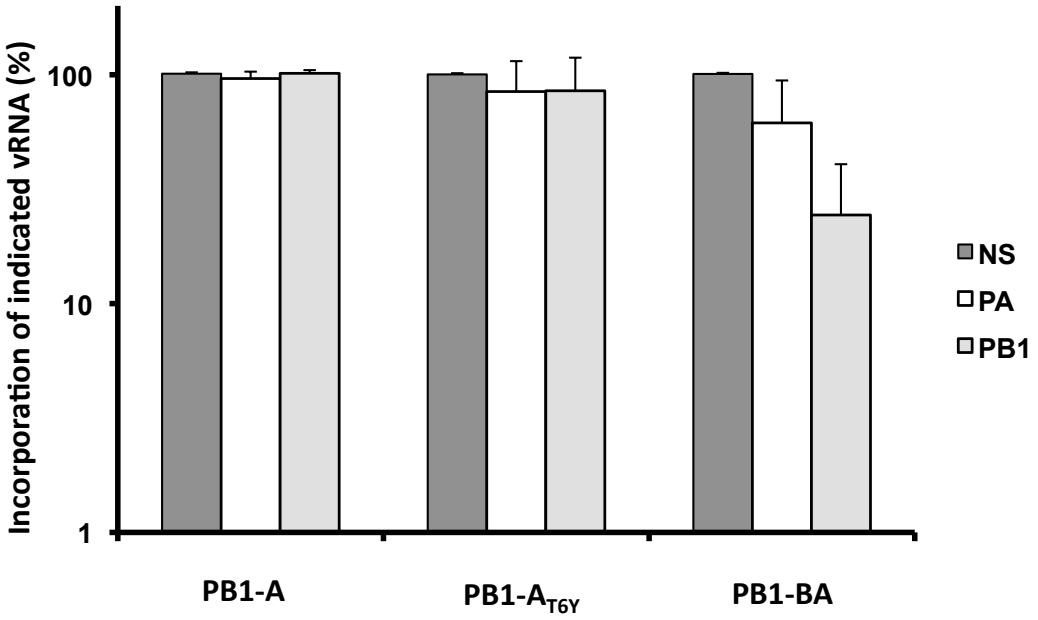

Fig. S2:
Analysis of the influence of PB1$_{1-25}$ mutations on packaging of the PB1 segments into progeny viruses by qRT-PCR. Values are given as percentages for packaging vRNA in comparison to those for the wild type virus. Quantitation required the assumption that each vRNA in a population of wt virus is present in an approximately equimolar ratio. Results from three independent experiments, with assays performed in duplicates, are given (n=6). Error bars represent standard deviation.

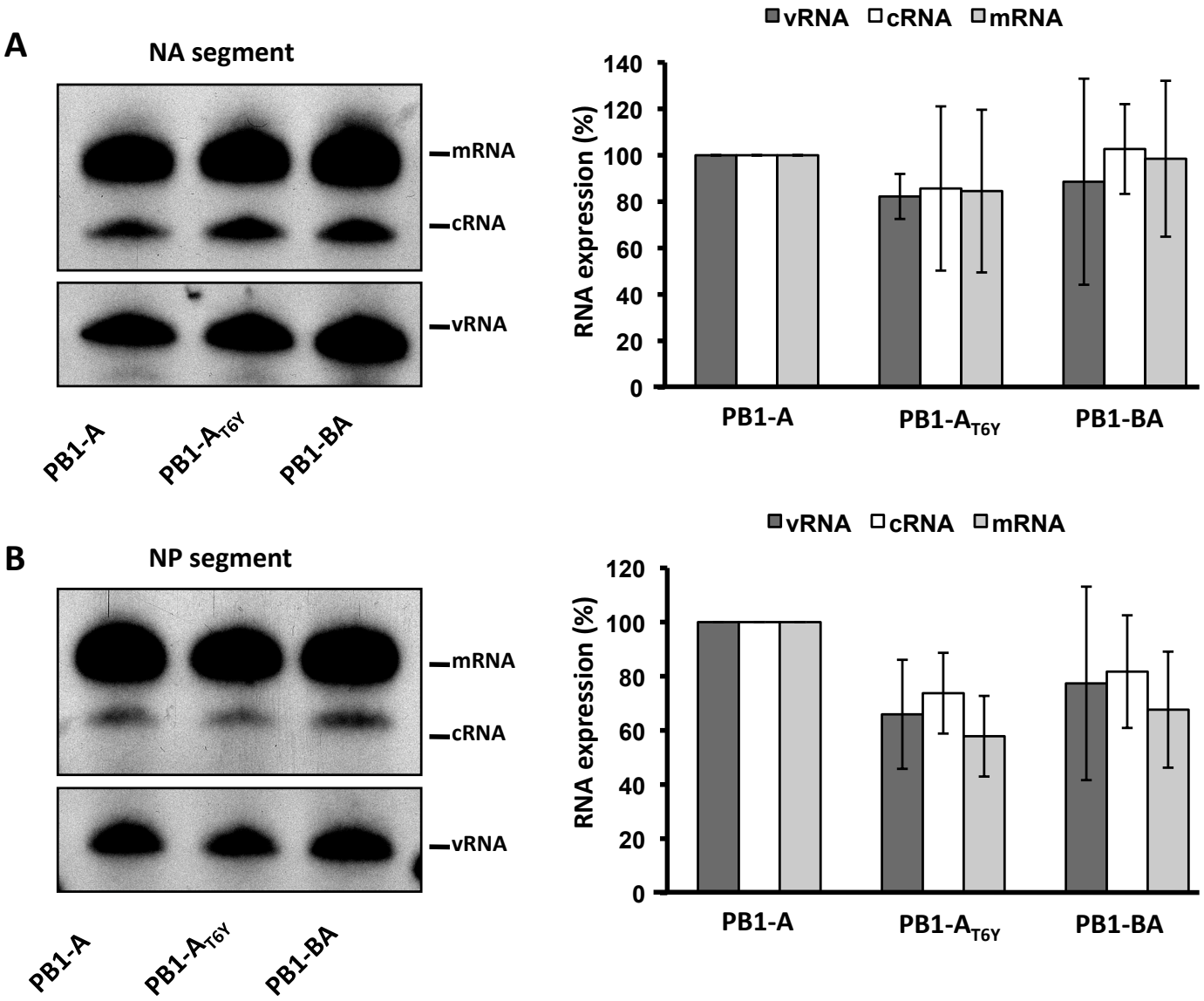

Fig. S3. Transcription and replication activities of FluA mutants in MDCKII cells. mRNA, cRNA, and vRNA levels in infected MDCKII cells were determined by primer extension for NS segment (A) and NP segment as described in (30-32) (B). Primer extension analysis was performed with ^{32}P-labeled segment-specific primer oligonuleotides and total RNA prepared from cells infected with a MOI of 2 for 6h. Transcription products of four independent experiments were quantified using Fuji MacBas v2.2 software. Error bars represent standard deviations from four independent experiments.

Table S1. Primers used for the qPCR analysis of packaged vRNA

segment	Forward primer	Reverse primer	Probe (5`FAM, 3`BHQ1)
NS	CTGGGTGATGCCCCATTC	AGAGTGCTGCCTCTTCCTCTTAGAG	TTGACCGGCTTCGCCGAGATCA
PA	AATGATCCCTGGGTTTTGCTTA	TGCCACAGCTATTTCAGTGCAT	TGCATCTTGGTTCAACTCCTTCCTCACA
PB1	CCAGACCTATGATTGGACATTAAACA	CCGTTCGATCTGAAGACCTCTATAGT	CCGGCTGCAACTGCATTGGCTAA