

Figure S1. Schematic presentation of the construction of the recombinant HVT-NDV-HN-F. Recombination cassette contain HVT sequences (HV052-53 on the left hand side of expression cassettes and HV054 on the right hand side) for homologous recombination and two expression cassettes with AAvV-1/pigeon/332/05 F and HN genes: 1st containing HN gene (inserted under EF-1 promoter with BGH polyA) and 2nd containing F gene (inserted under ECMV promoter with SV40 polyA). Recombination was performed by transfection of HVT infected CEF with recombination cassette DNA.

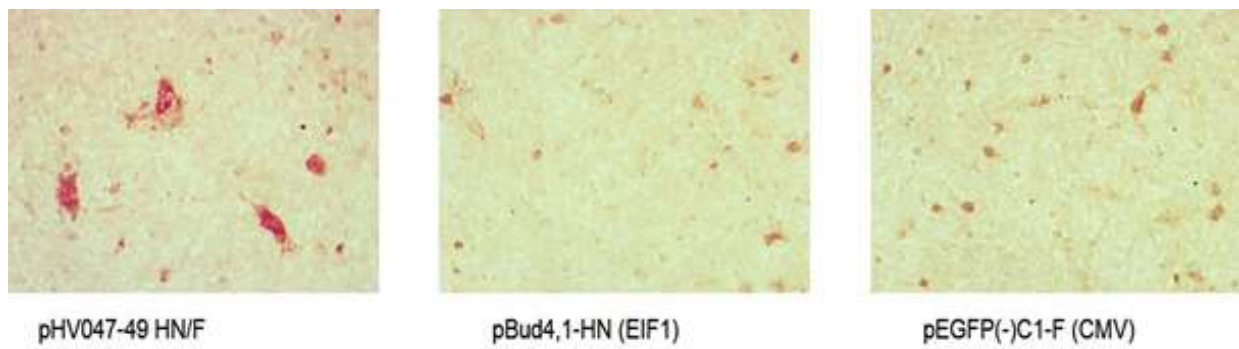


Figure S2. Control expression of NDV HN and F genes in chicken fibroblasts after transfection with expression plasmids used for obtaining recombination transfer vector pHV052-54. Immunodetection was performed by IPMA using mix of anti-NDV chicken sera. Observations were performed using confocal microscope.

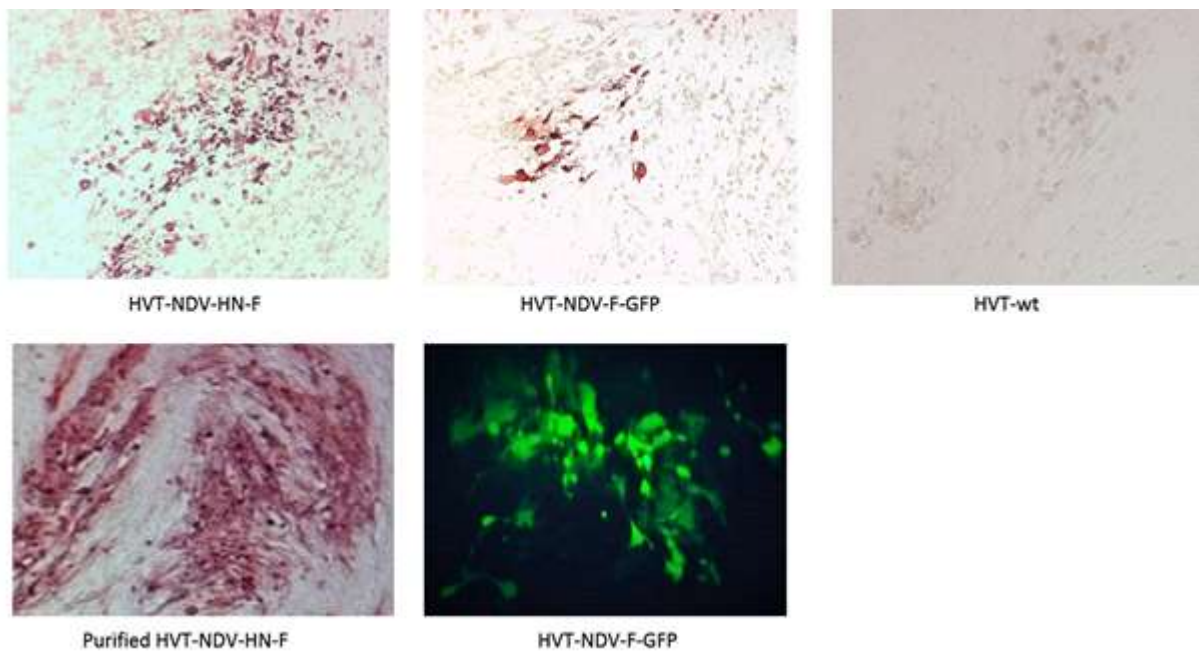


Figure S3. Detection of recombinant HVT plaques after transfection on HVT-wt infected CEF cells. Immunodetection was performed by IPMA with mix of chicken anti-NDV sera and confocal microscope observation in white light. GFP positive plaques were visualized also by UV light.

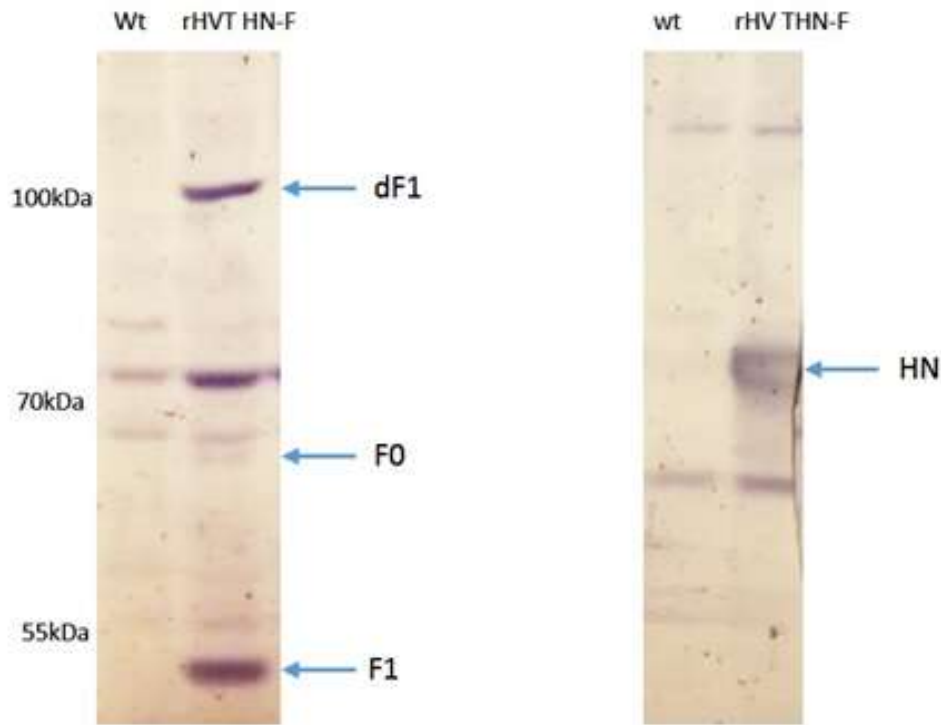


Figure S4. Western blot analyses of coexpression of the F and HN proteins in CEF cells infected with HVT-NDV-HN-F. CEF lysates in duplicate were subjected to 8% SDS-PAGE and electroblotted to PVDF membrane. Membrane was blocked and cut for separate detection of HN and F proteins with rabbit polyclonal sera specific for HN and F of NDV: AAvV-1 La Sota and AAvV-1/pigeon/332/05. Reaction was followed with incubation with alkaline phosphatase goat anti-rabbit IgG conjugate (Abcam). Protein bands were visualized by NBT/BCiP alkaline phosphatase substrate. The presence of both NDV proteins F and HN in samples is indicated (marked with arrows).

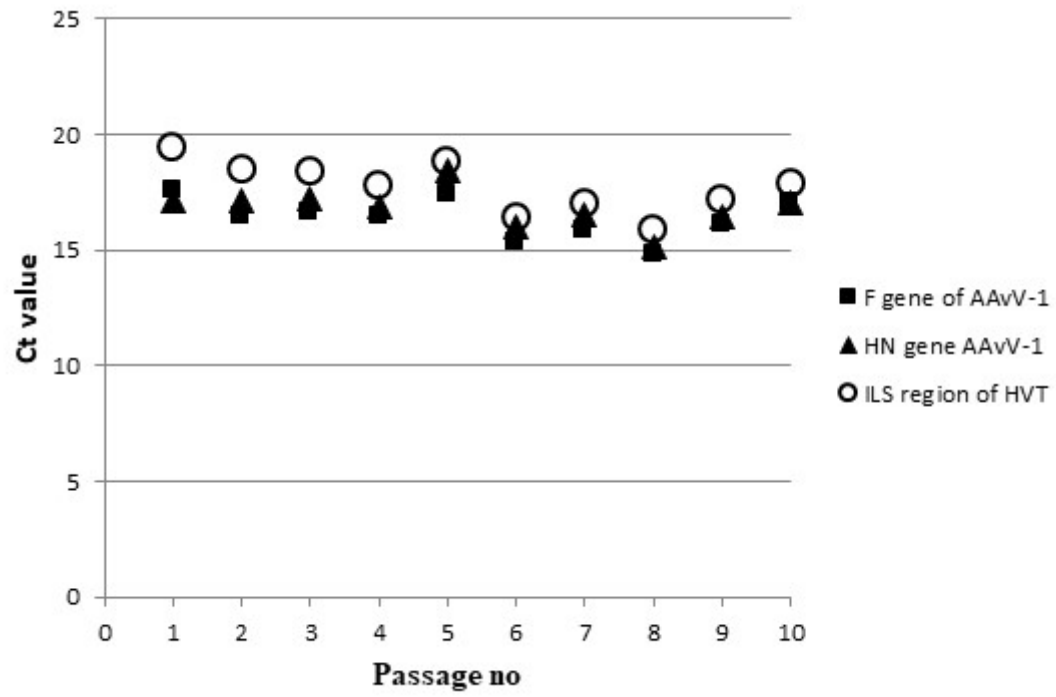
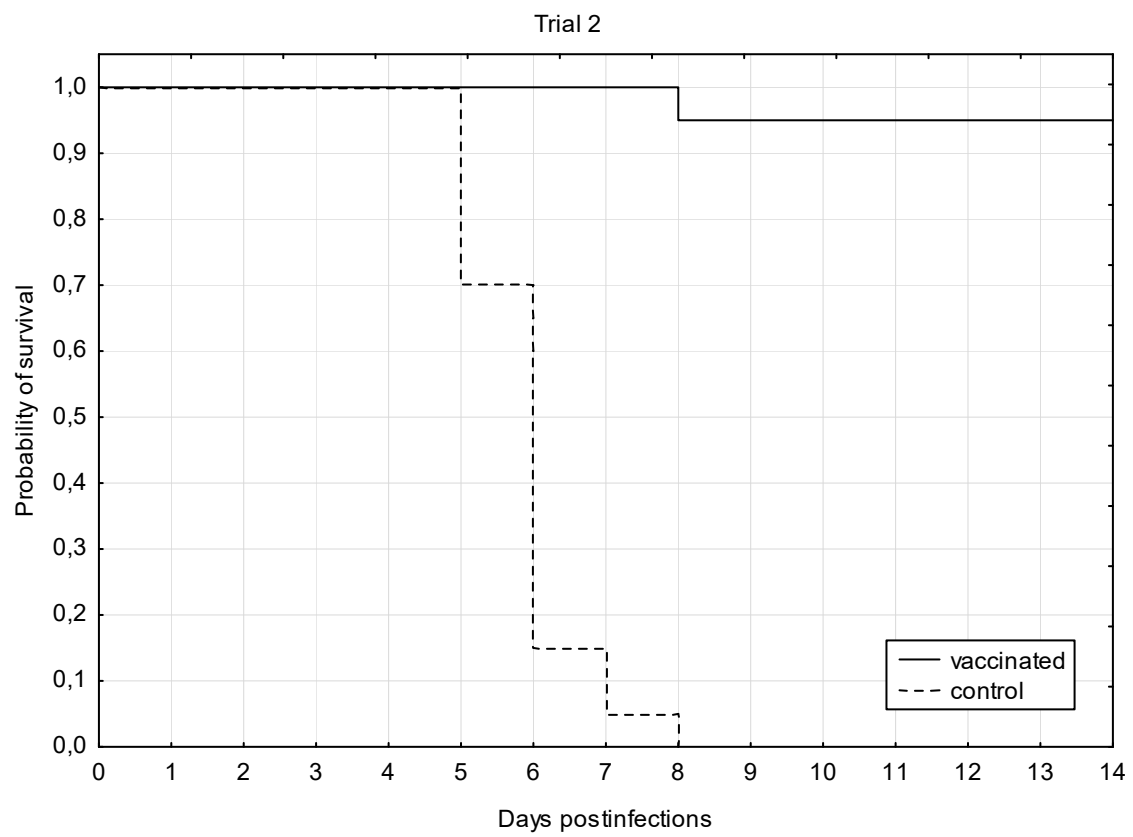
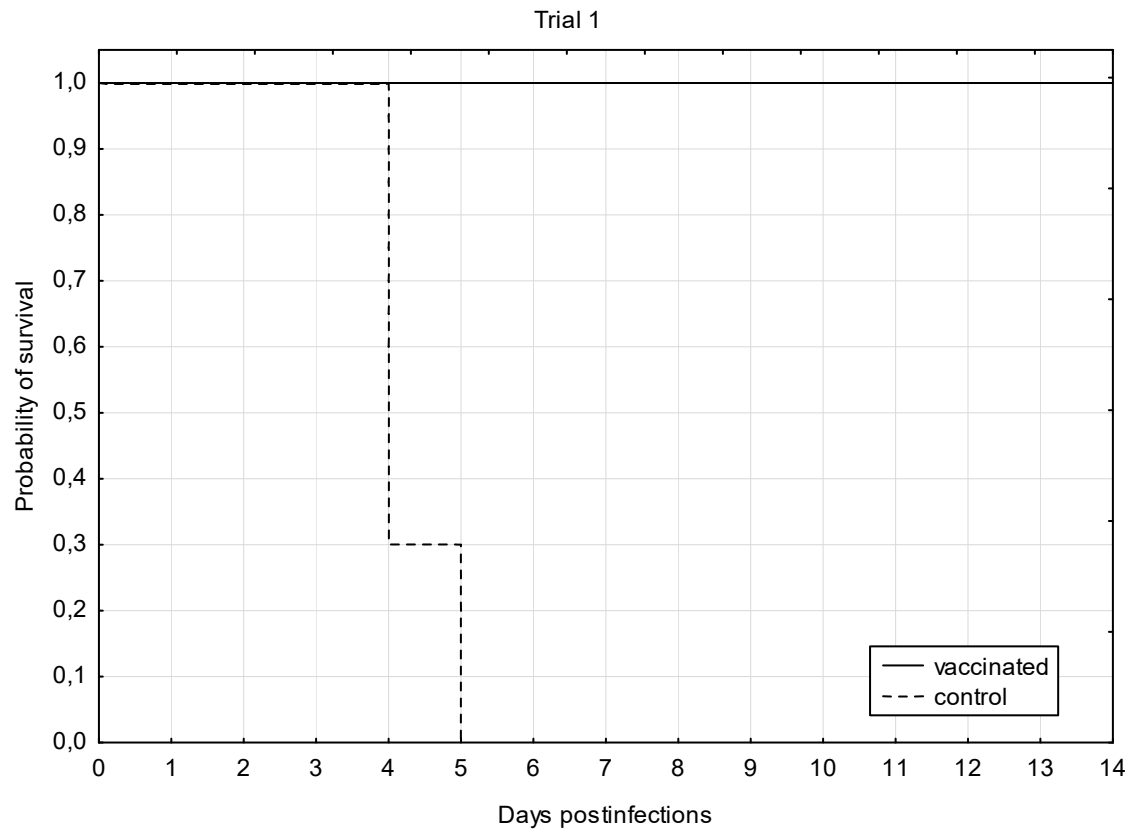


Figure S5. Effect of multiple passages on the presence of inserted genes fragments of AAVV-1 and HVT.



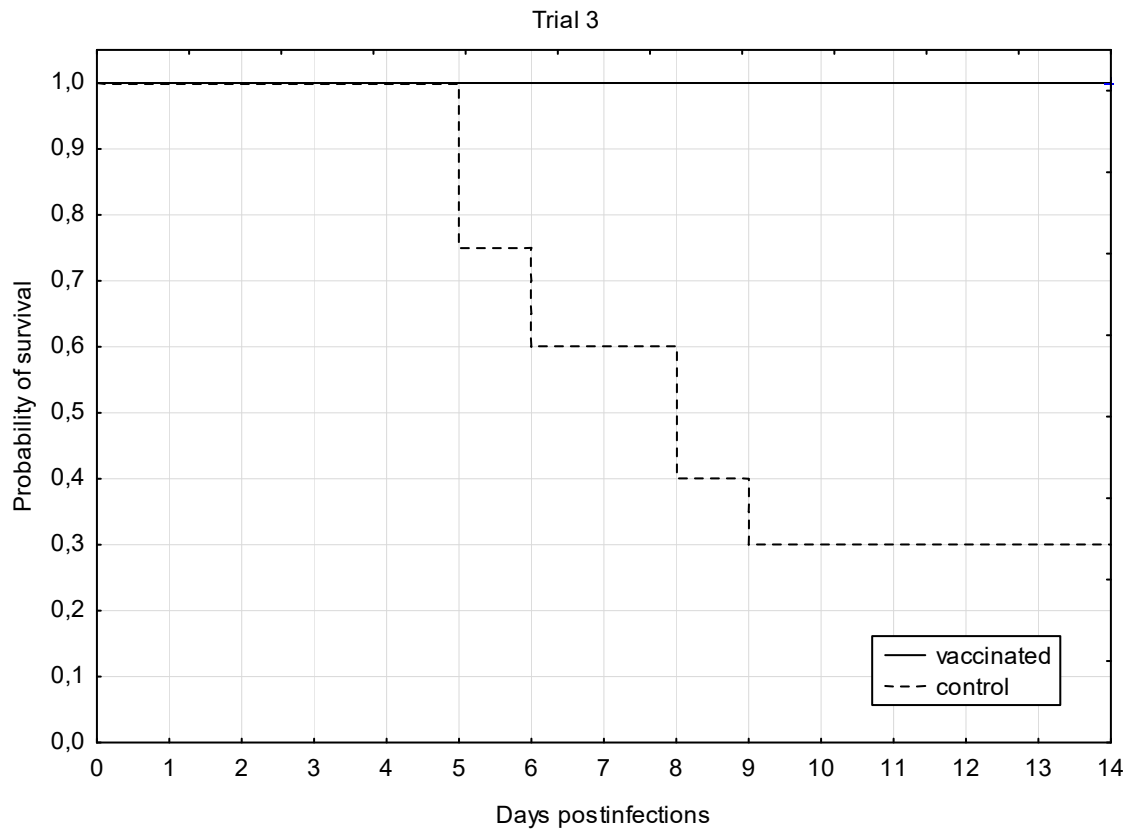


Figure S6 Survival plots for protection of chickens vaccinated with HVT-NDV-F-HN against challenge with virulent AAvV-1 genotype IV (Trial I and II) and genotype VII (Trial III) and sham-inoculated control chickens based on Kaplan-Meier analysis. In each experiment the log-rank test showed significant difference in survival rate between vaccinated and control groups ($p < 0.0001$)