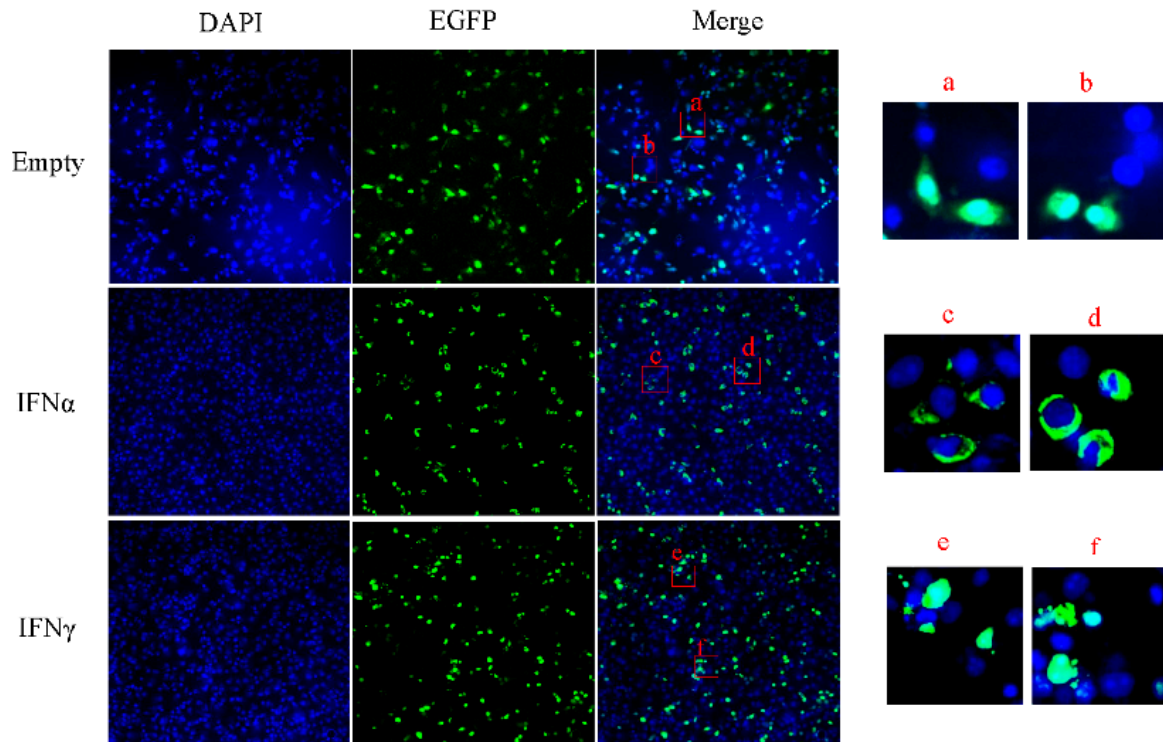
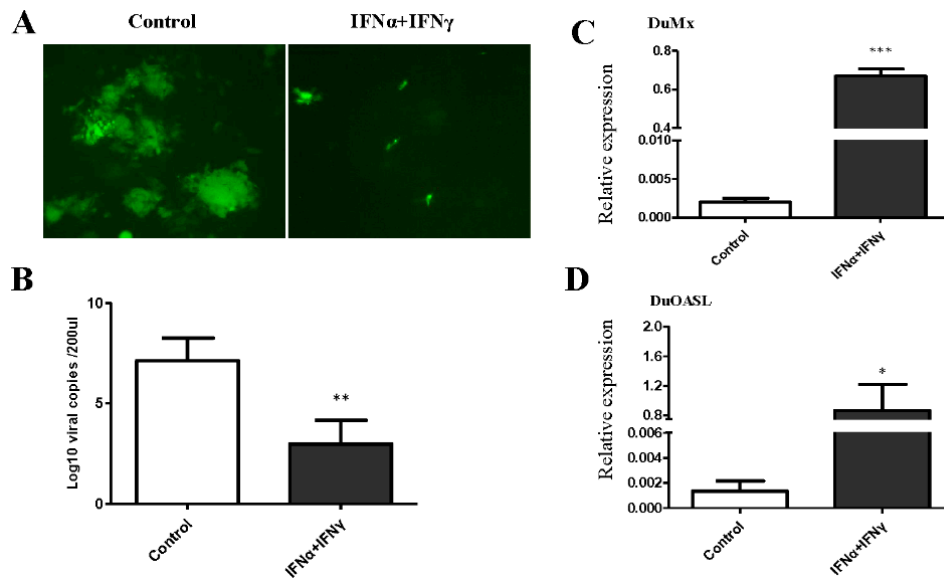


# Supplementary Materials: Cross-Species Antiviral Activity of Goose Interferons against Duck Plague Virus Is Related to Its Positive Self-Feedback Regulation and Subsequent Interferon Stimulated Genes Induction

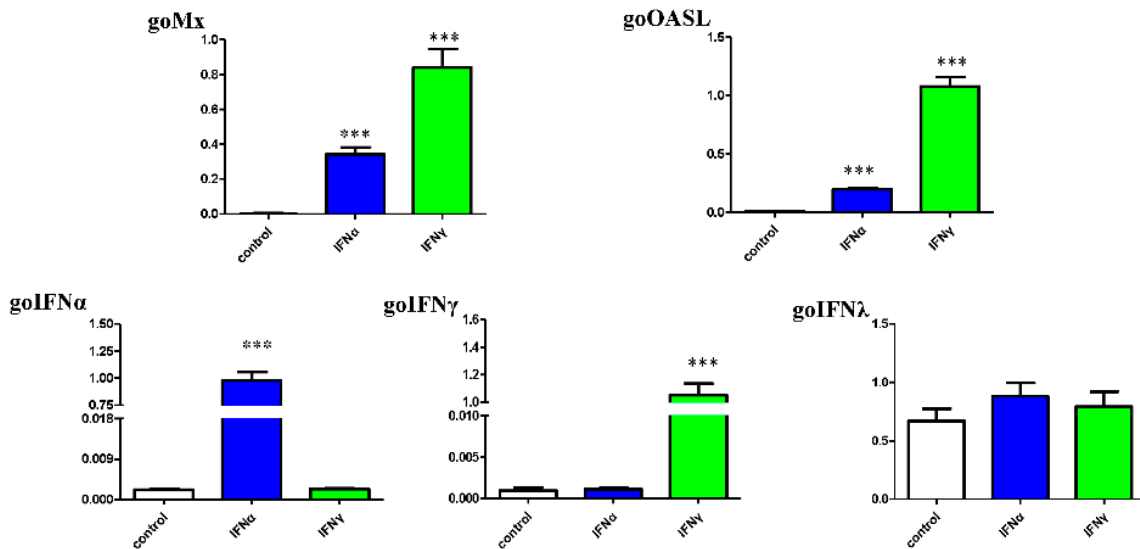
Hao Zhou, Shun Chen, Qin Zhou, Yunan Wei, Mingshu Wang, Renyong Jia, Dekang Zhu, Mafeng Liu, Fei Liu, Qiao Yang, Ying Wu, Kunfeng Sun, Xiaoyue Chen and Anchun Cheng



**Figure S1.** Subcellular localization of goose IFN $\alpha$  and IFN $\gamma$  protein in BHK21 cells. BHK21 cells plated onto glass coverslips and incubated overnight in 6-well plates were transfected with 2  $\mu$ g pEGFP-IFN $\alpha$  or pEGFP-IFN $\gamma$ . At 24 h post transfection, the cells were fixed for immunofluorescence analysis (original magnification  $\times$  400). The nuclei were stained with DAPI (blue). Higher magnification views of the area indicated by the box are shown on the right.



**Figure S2.** The synergistic effect of both goose IFN $\alpha$  and IFN $\gamma$  proteins on DPV replication. (A) Inhibition of DPV replication by both goose IFN $\alpha$  and IFN $\gamma$  proteins is shown by green fluorescence (Magnification 400 $\times$ ). DEF cells were seeded in 6-well plates, 12 h later, treated with indicated both IFN $\alpha$  and IFN $\gamma$ , at respective 60  $\mu$ g per well or left control; 12 h later, the cells were then infected by DPV-EGFP ( $4 \times 10^4$  TCID<sub>50</sub>/well). At 36 hpi, green fluorescence was examined via fluorescence microscopy; (B) The effect of co-treatment of both IFN $\alpha$  and IFN $\gamma$  protein on the viral copies of DPV (\*\*  $p < 0.01$ ); (C,D) The levels of DuMx and DuOASL gene expression in GEFs after treatment with the co-treatment of both goIFN $\alpha$  and goIFN $\gamma$  proteins were detected by qRT-PCR. The data represent the mean  $\pm$  SEM per group ( $n = 3$ ). Significant differences compared with cells without goIFN $\alpha$  or goIFN $\gamma$  treatment are denoted by (\*  $p < 0.05$ ) and (\*\*\*)  $p < 0.001$ ).



**Figure S3.** Effect of goose interferons on goose IFNs and ISGs in GEFs. The levels of goMx, goOASL, goIFN $\alpha$ , goIFN $\gamma$ , and goIFN $\lambda$  gene expression in GEFs after treatment with the goIFN $\alpha$  and goIFN $\gamma$  proteins (60  $\mu$ g/well) were detected by qRT-PCR. Total RNA was extracted from GEF cells after incubation with interferon for 24 h. The internal control was  $\beta$ -actin. The data represent the mean  $\pm$  SEM from four replicates per group. Significant differences compared with cells without treatment goIFN $\alpha$  or goIFN $\gamma$  treatment are denoted by \*\*  $p < 0.01$ .

