

Title: Induction of a robust immunity response against novel duck reovirus in ducklings using a subunit vaccine of sigma C protein

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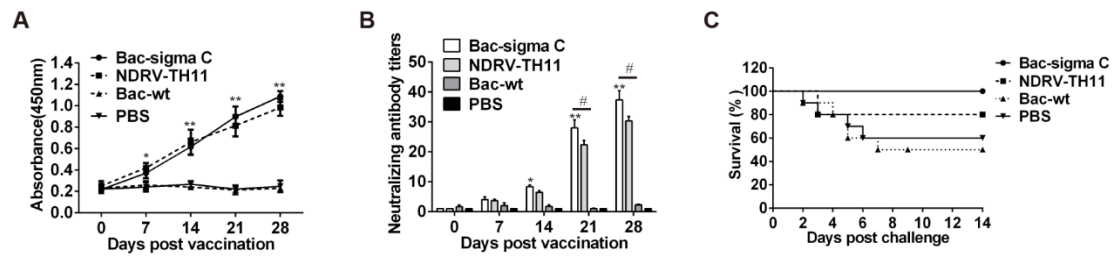
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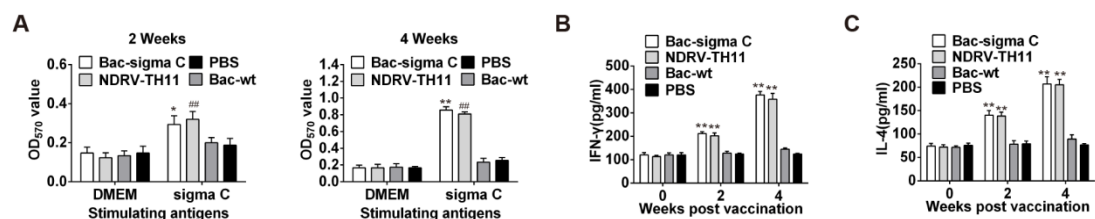
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Supplementary Materials:

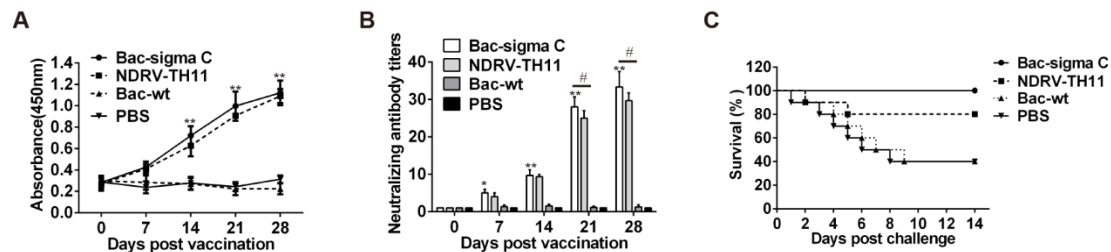


Supplementary Figure 1. Antibody response and survival rate in ducklings immunized with recombinant sigma C protein (the second replicate experiment). (A) Sigma C-specific antibody levels detected by indirect enzyme-linked immunosorbent assay (ELISA) analysis. (B) Neutralization antibody levels detected by serum neutralization assay. Each data represents the mean \pm SD. *, $p < 0.05$, **, $P < 0.01$ vs PBS group. #, $p < 0.05$ vs NDRV-TH11 group. (C) Survival curves after challenge. The statistical significance of differences in mortality between groups was determined using the Kaplan-Meier method, and analyzed with a Log-rank (Mantel-Cox) test. For Bac-sigma C vs PBS, $P < 0.05$; for Bac-sigma C vs Bac-wt, $p < 0.05$; and for Bac-wt vs NDRV-TH11, $p > 0.05$.



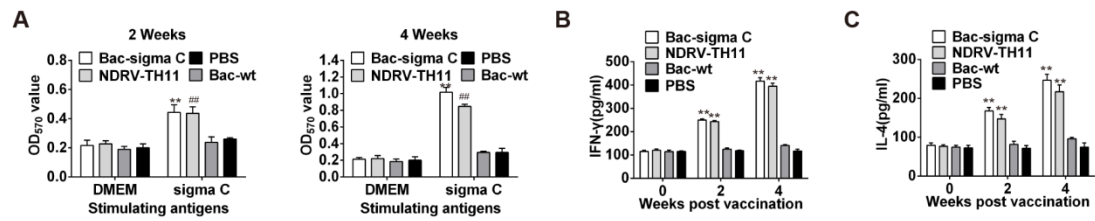
Supplementary Figure 2. Cellular immune response in ducklings immunized with sigma C (the second replicate experiment). (A) The dynamic changes of lymphocyte proliferation in immune response test (A_{570} value). The lymphocyte proliferation response was measured on 2 weeks and 4 weeks after the first

immunization. Each data represents the mean \pm SD. *, $P < 0.05$, **, $P < 0.01$ vs Bac-sigma C group stimulated with DMEM alone. ##, $p < 0.01$ vs NDRV-TH11 group stimulated with DMEM alone. (B) The interferon gamma (IFN- γ) concentration in serum harvested from immunized ducklings was measured by enzyme-linked immunosorbent assay (ELISA). (C) The interleukin 4 (IL-4) concentration in serum harvested from immunized ducklings was measured by ELISA. Data are the mean \pm SD. **, $p < 0.01$ vs PBS group.



Supplementary Figure 3. Antibody response and survival rate in ducklings immunized with recombinant sigma C protein (the third replicate experiment).

(A) Sigma C-specific antibody levels detected by indirect enzyme-linked immunosorbent assay (ELISA) analysis. (B) Neutralization antibody levels detected by serum neutralization assay. Each data represents the mean \pm SD. *, $p < 0.05$, **, $P < 0.01$ vs PBS group. #, $p < 0.05$ vs NDRV-TH11 group. (C) Survival curves after challenge. The statistical significance of differences in mortality between groups was determined using the Kaplan-Meier method, and analyzed with a Log-rank (Mantel-Cox) test. For Bac-sigma C vs PBS, $P < 0.05$; for Bac-sigma C vs Bac-wt, $p < 0.05$; and for Bac-wt vs NDRV-TH11, $p > 0.05$.



Supplementary Figure 4. Cellular immune response in ducklings immunized

with sigma C (the third replicate experiment). (A) The dynamic changes of lymphocyte proliferation in immune response test (A_{570} value). The lymphocyte proliferation response was measured on 2 weeks and 4 weeks after the first immunization. Each data represents the mean \pm SD. **, $P < 0.01$ vs Bac-sigma C group stimulated with DMEM alone. ^{###}, $p < 0.01$ vs NDRV-TH11 group stimulated with DMEM alone. (B) The interferon gamma ($IFN-\gamma$) concentration in serum harvested from immunized ducklings was measured by enzyme-linked immunosorbent assay (ELISA). (C) The interleukin 4 (IL-4) concentration in serum harvested from immunized ducklings was measured by ELISA. Data are the mean \pm SD. **, $p < 0.01$ vs PBS group.