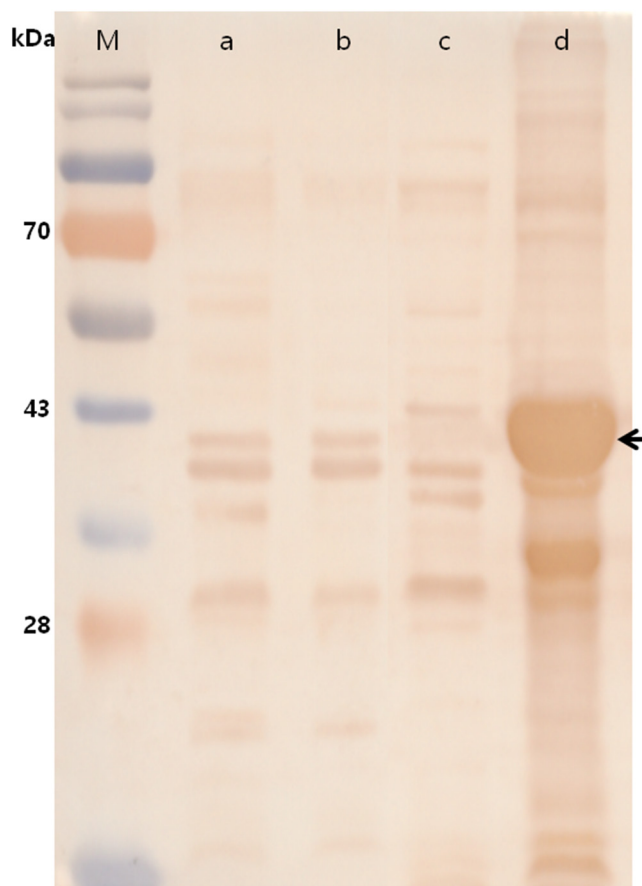
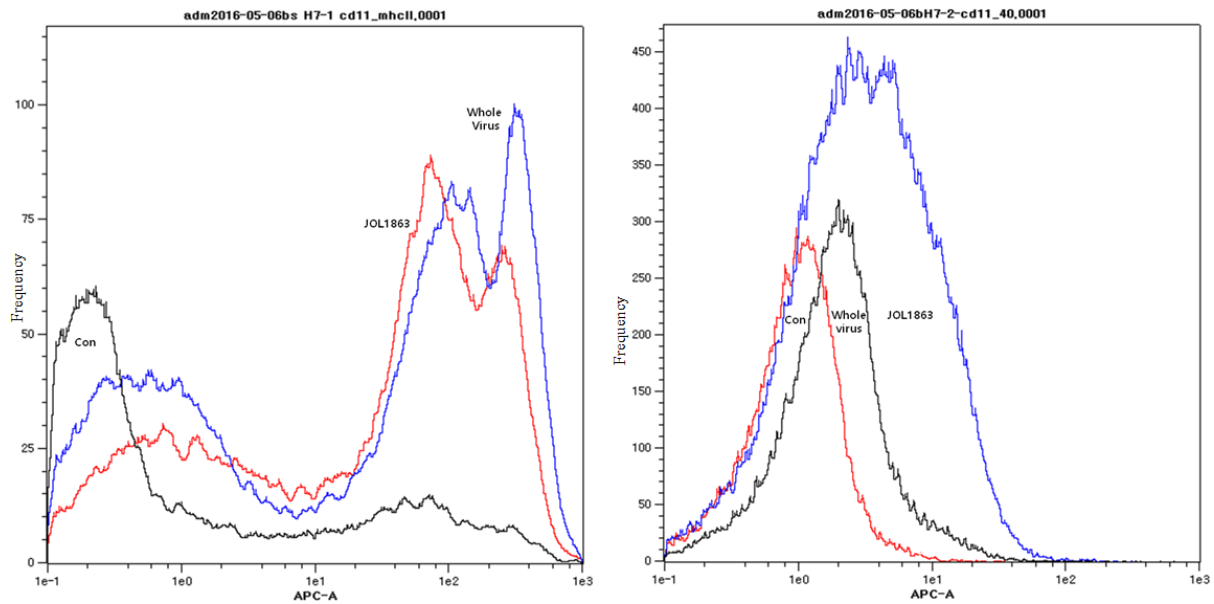


A consensus-hemagglutinin-based vaccine delivered by an attenuated *Salmonella* mutant protects chickens against heterologous H7N1 influenza virus

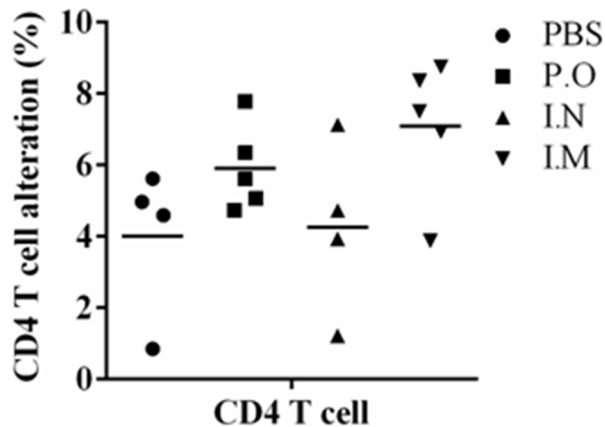
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Western blot analysis of HA expressed by JOL1863. The expression of HA protein expressed by JOL1863 was confirmed by Western blot using polyclonal HA antibody (catalog #, MBS1488599). JOL1863 bacteria harbouring pMMP65-HA plasmid and JOL912 alone (serving as control) were allowed to grow till 0.6 OD_{600 nm}. Then culture supernatant and bacterial pellet were subjected to Western blot analysis as described in material methods. Lane M, protein Marker; lane a, control supernatant; lane b control bacterial pellet; lane c, supernatant of JOL1863 showing a band of 41 kDa; and lane d, bacterial pellet of JOL1863 showing a 41 kDa band.



Supplementary Figure 2: Flow cytometric comparison of chicken immature MoDCs and mature MoDCs with regard to the maturation markers MHC-II and CD40. Cells were stained with anti-chicken monoclonal antibodies against specific cell surface markers. Immature DCs were stimulated with JOL1863 (10 particles/cell) or H7N1 virus (500 ng/ml) or left unstimulated for 48 hr. The immature DCs (Con) have shown low amounts of MHC II and CD40 while cells stimulated with either JOL1863 or H7N1 virus have shown up-regulation of MHC II and CD40. Data shown are representative of three different experiments.



Supplementary Figure 3: Flow cytometry analysis of CD4 T cell alteration after H7N1 challenge. After 5 days post challenge, CD4⁺ T cells in PBMCs were analysed by flow cytometry using anti-chicken CD4 monoclonal antibody. Each data points represent mean+ SD of five chickens per group.

Supplementary Table 1: List of primer pairs used in this study

Primer	Sequence (5' – 3')	References
Influenza H7 HA primer F	GCGAAGCTTTTTTCGGGATTTCCGGA	Designed
Influenza H7 HA primer R	CGCGGATCCGCGGACAAAATCTGCC	Designed
Influenza GRAM/7F	CTTCTAACCGAGGTCGAAACGTA	WHO
Influenza GRAM/161R	GGTGACAGGATTGGTCTTGTCTTTA	
GAPDH-F	AGAACATCATCCCAGCGTCC	[1]
GAPDH-R	CGGCAGGTCAGGTCAACA	
IFN- γ F	CAAAGCCGCACATCAAACA	[1]
IFN- γ R	TTTCACCTTCTTCACGCCATC	
IL10 F	GCTGAGGGTGAAGTTTGAGGAA	[2]
IL-10 R	TGCTGATGACTGGTGCTGGT	
IL-17 F	GAGAAGAGTGGTGGGAAAG	[3]
IL-17 R	TCTACAAACTTGTTTATCAGCAT	

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