

Supplemental Material to:

Jingyi Yang, Maohua Zhong, Yan Zhang, Ejuan Zhang, Ying Sun, Yuan Cao, Yaoming Li, Dihan Zhou, Benxia He, Yaoqing Chen, Yi Yang, Jie Yu and Huimin Yan

**Antigen replacement of domains
D2 and D3 in flagellin promotes mucosal
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inflammatory response after intranasal immunization**

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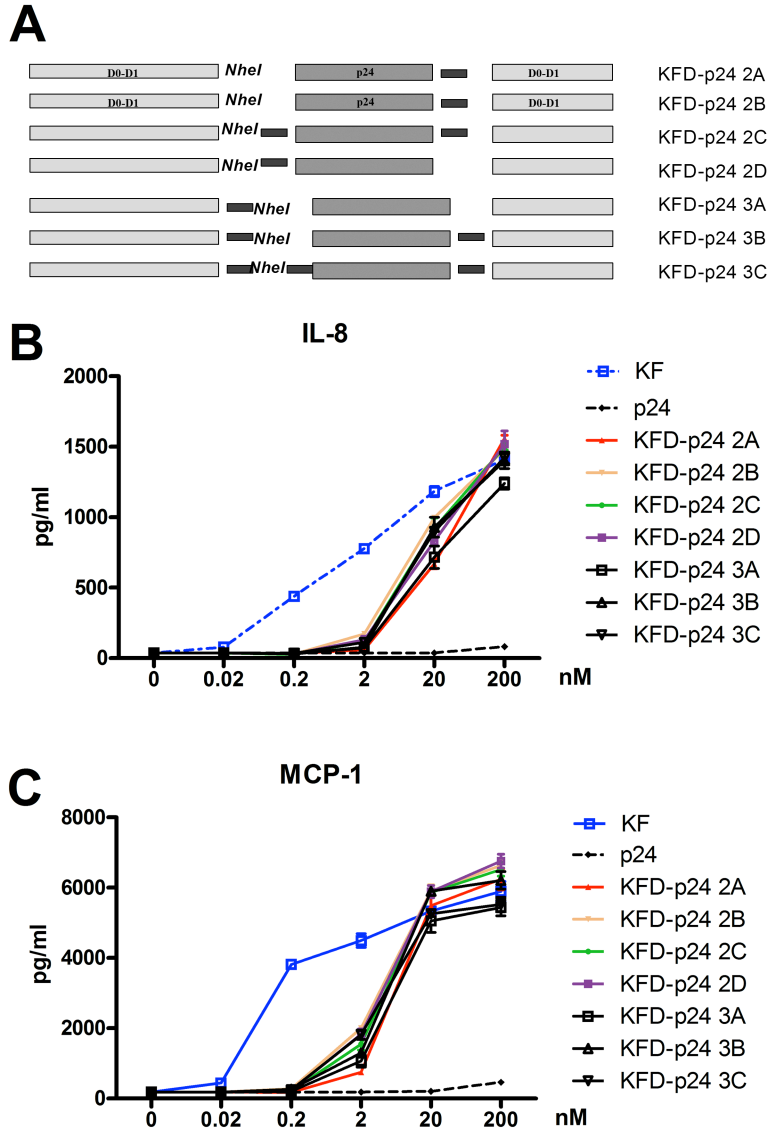


Figure S1. Flagellins with replaced D2 and D3 domains show similar TLR5 agonist efficacy. (A) Schematic diagram showing domain structures and variants. KFD-p24 2A / 2B / 2C / 2D / 3A / 3B / 3C. (B) IL-8 and (C) MCP-1 in the cell culture supernatants of Caco-2 cells 6-h post KFD-p24

stimulation were tested by ELISA. Data are presented as the means \pm SEM from triplicates of one experiment that was repeated three times.

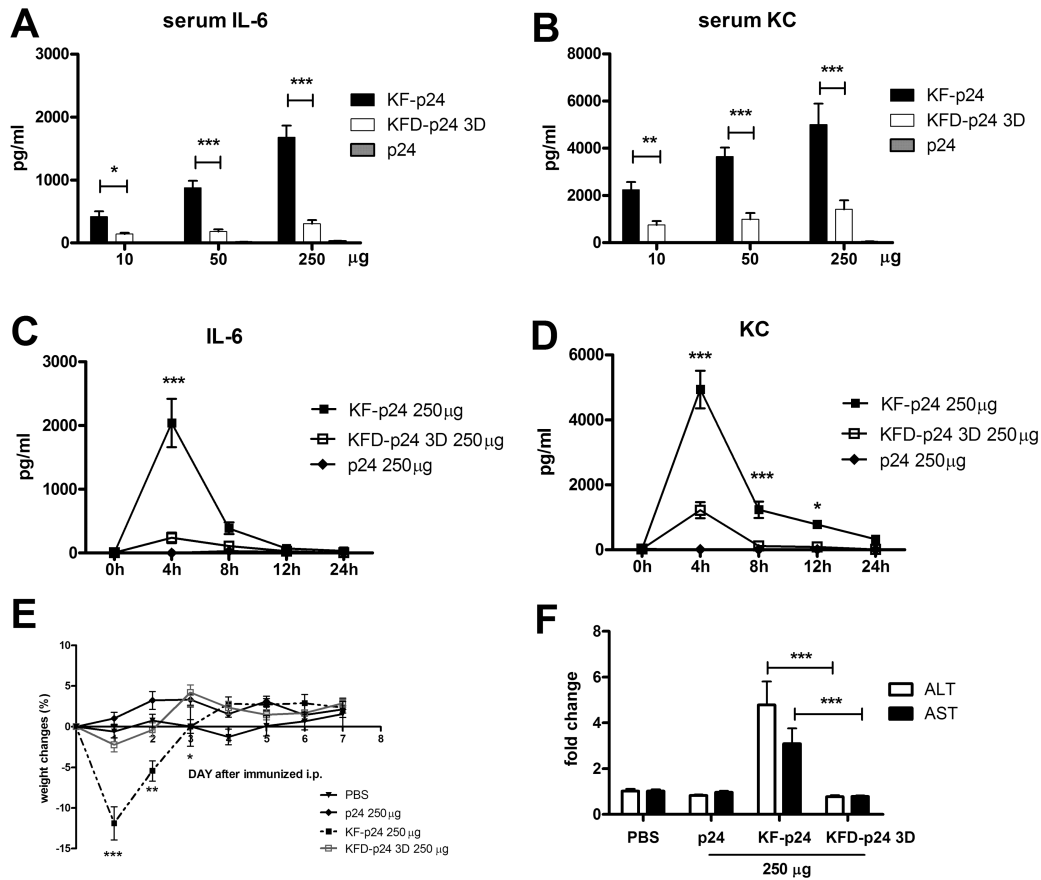


Figure S2. KFD-p24 3D induced less of a systemic inflammatory response after intra-peritoneal administration than did KF-p24. C57BL/6 mice were injected intraperitoneally with p24, KFD-p24 3D or KF-p24 in 250 μ l PBS. (A) and (B) IL-6 and KC in serum were tested by ELISA 4h after 10 μ g, 50 μ g or 250 μ g of stimulus administration. (C) and (D) Time-course of IL-6 and KC in serum were tested by ELISA after administration. (E) Time-course of body mass changes after administration. (F) Compared with the control group (250 μ g p24) the fold changes of serum transaminase level were evaluated 24 h post-administration i.p. Data are presented as the means \pm SEM from one experiment that was repeated three times (n=6 per group).

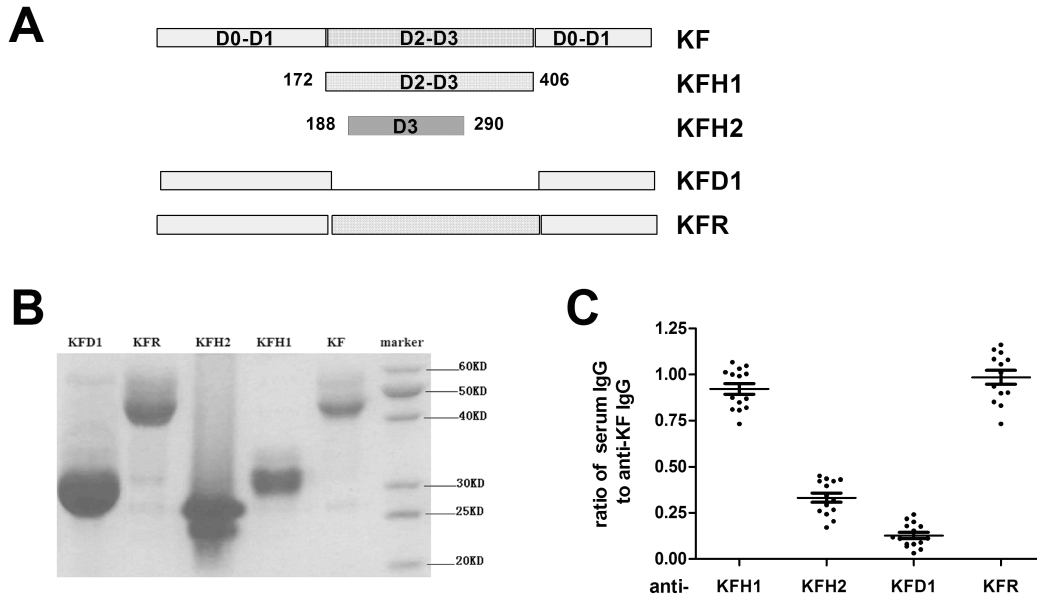


Figure S3. Domains D2 and D3 constitute the main antigenicity region of KF. A series of KF-derived flagellin was generated and coated to test the IgG titers of serum from BALB/c mice pre-immunized intranasally at 0, 4th, or 8th weeks with 5 μ g KF plus 5 μ g OVA or 5 μ g KF alone. (A) and (B) Schematic diagram and SDS-PAGE of a different KF variant. KFR: domain D2 and D3 of KF re-inserted into KFD1. (B) SDS-PAGE of a different KF variant. (C) Ratios of different KF variant-specific serum IgG titer to KF specific IgG titer. Data are presented as the means \pm SEM of one experiment that was repeated three times.