Fig S1

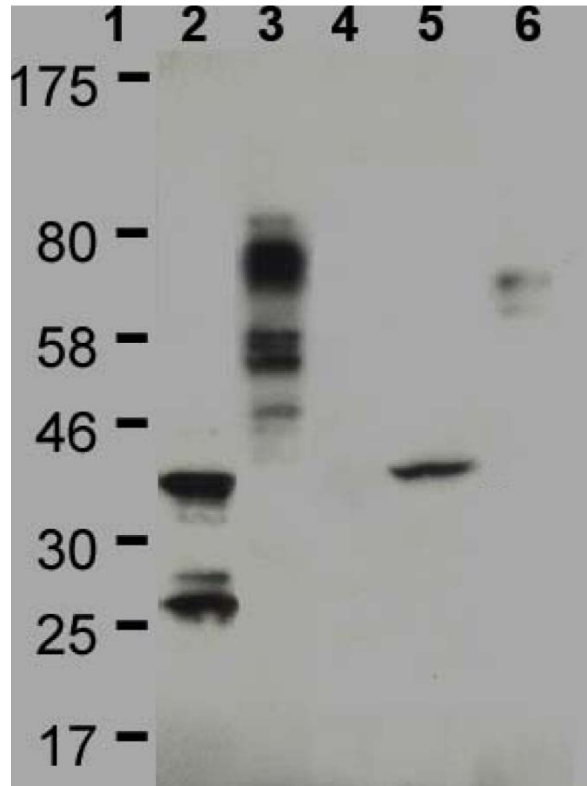

Figure S1. Immunoblot analysis of recombinant adenoviruses. Human lung epithelial cells A549 were infected with rAd5 constructs at 1000 v.p. per cell. Host cell lysates were harvested after 24 h p.i. An aliquot of the cell lysates was then resolved by SDS-PAGE and subjected to Western blot analysis by using mAb-LcrV antibody. **Lane 1:** Standard protein molecular weight markers in kilo-daltons (kDa). **Lanes 2-4:** A549 cells infected with rAd5-LcrV**,** rAd5-YFV and Ad5-empty, respectively. **Lane 5:** Purified rLcrV (50 ng). **Lane 6:** Purified rYFV (30 ng). The HRP-labeled anti-mouse secondary antibody and ECL Western blotting reagent kit (Millipore, Billerica, MA) was used for protein detection.

Fig S2

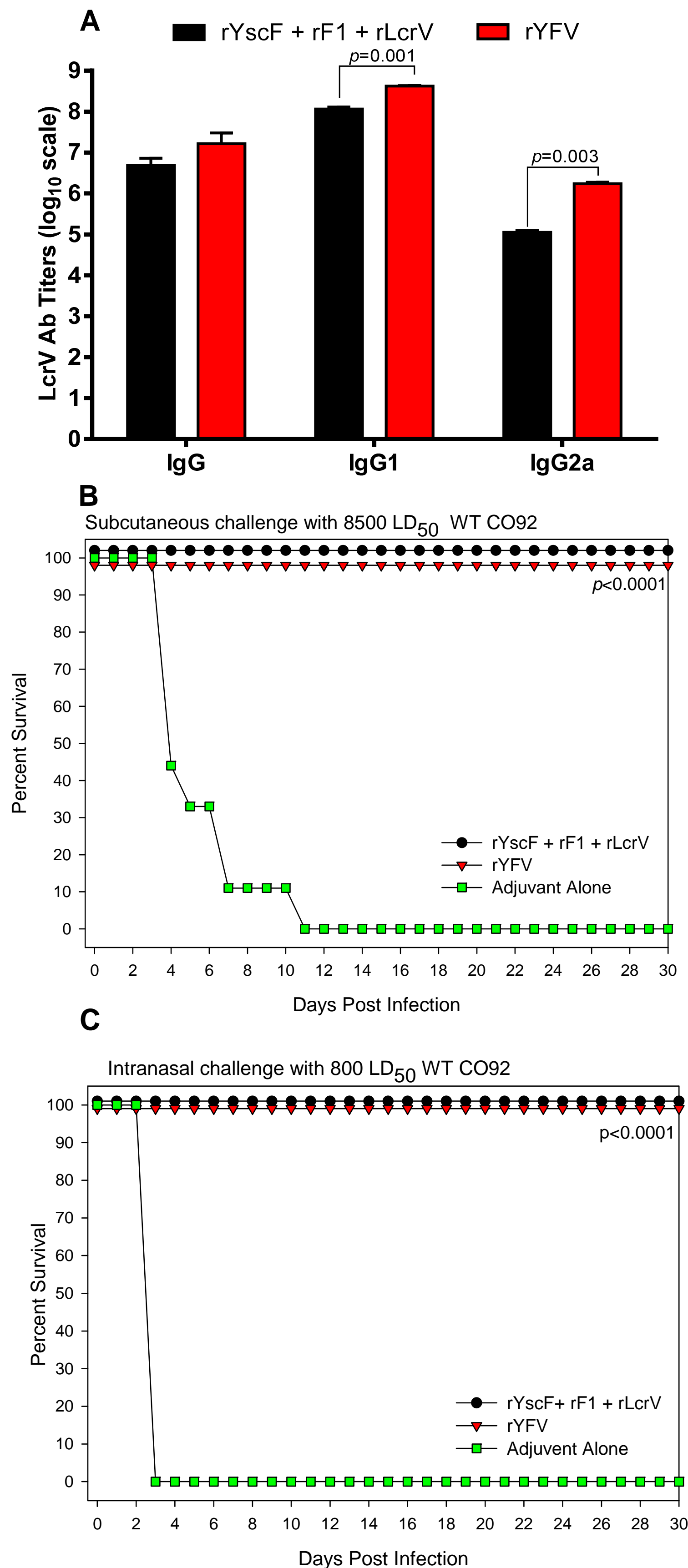

Figure S2. **Protection conferred by immunization of mice with the purified recombinant proteins**. Naïve mice (n=40) were immunized with either the mixture of three recombinant proteins (rYscF, rF1, and rLcrV, 25 μg/each) or 45 μg of the corresponding recombinant fusion protein (rYFV) *via* the i.m route. The antigens were emulsified 1:1 in Alum adjuvant. One primary immunization and two identical boosters were given on days 0, 15 and 30. Naïve mice received the adjuvant only and served as a control. Mice were bled 14 days post last immunization and an ELISA was performed to examine IgG and its isotype antibody titers to the LcrV antigen (**A**). The *P* values were in comparison to the indicated groups and were based on Two-way ANOVA (IgG1 andIgG2a) with the Tukey's *post hoc* correction. The above immunized and control mice were then split into two sets and challenged on day 15 post immunization either subcutaneously (s.c.) with 8500 LD_{50} **(B)** or intranasally (i.n.) with 800 LD_{50} **(C)** of the WT CO92. The *P* values were in comparison to the control group and were based on Kaplan-Meier Curve Analysis.

Fig S3

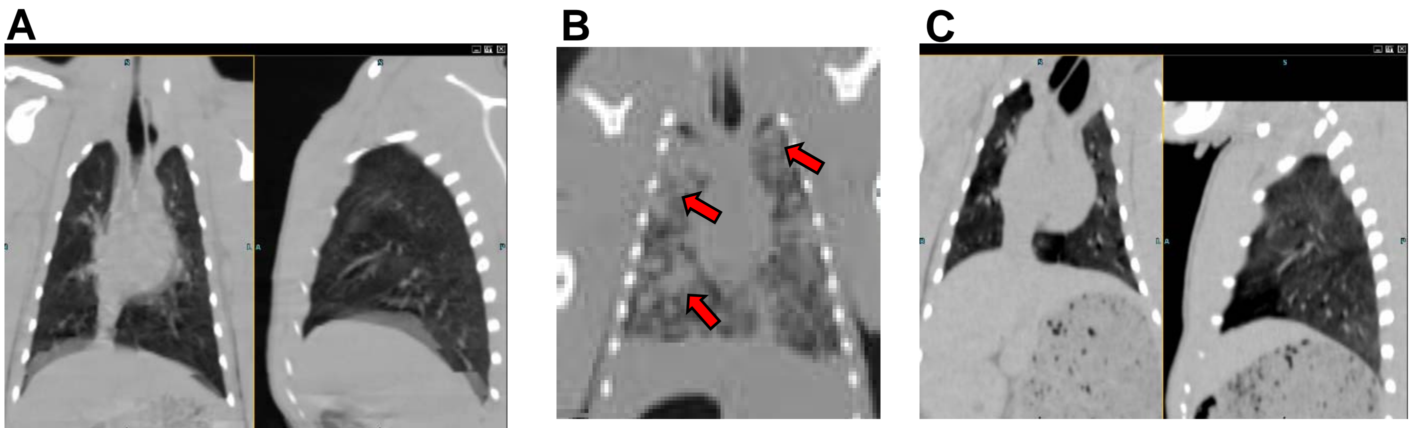

Figure S3. **CT scans.** NHPs were subjected to CT scan on day 42 (naïve and vaccinated) (**A)** and on day 88 (3 days post WT CO92 challenge) for the control NHPs (**B)** or day 167 (82 days post WT CO92 challenge) (**C)** for the immunized ones. The coronal and sagittal images of the lungs and their surrounding areas from representing NHPs were shown with the resolution of 512×512 pixels. The image sharpness was optimized to soft tissue. The arrows indicated consolidation patches in the lungs of a representative infected control NHP.

Fig S4

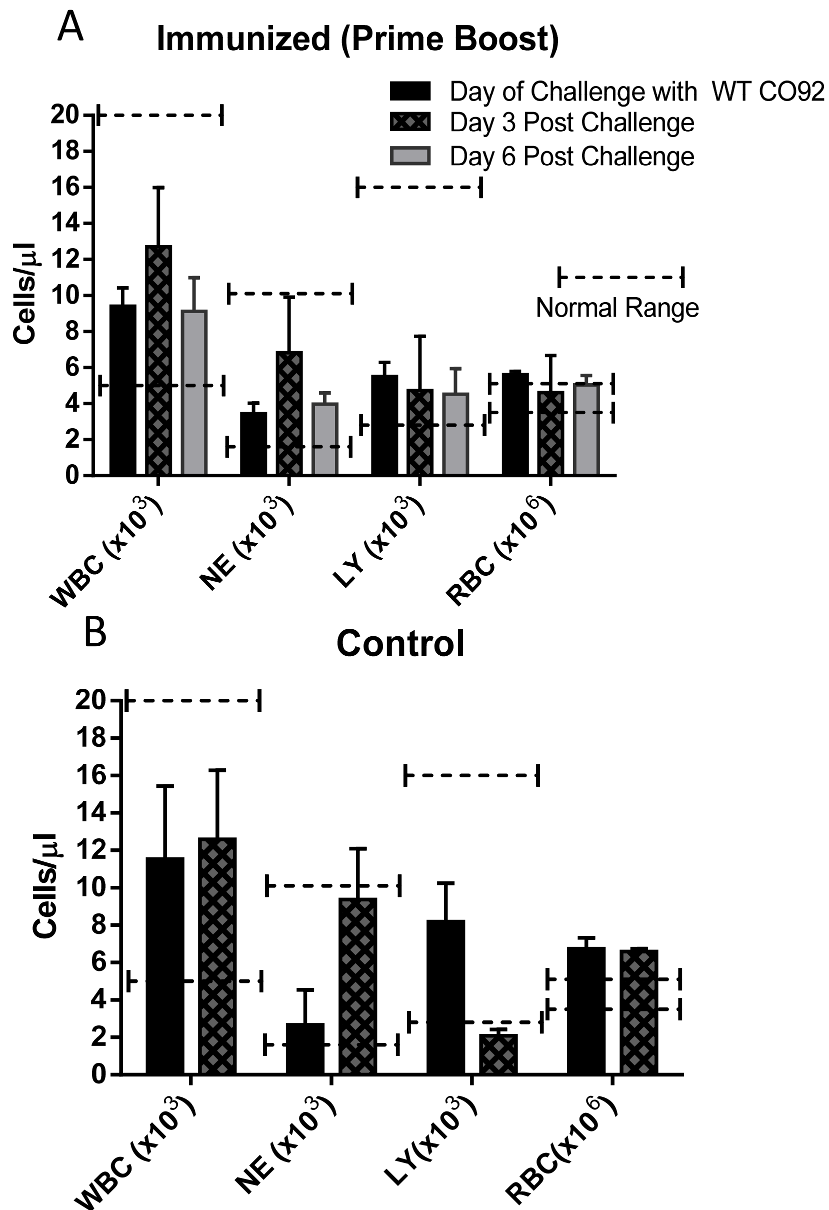

Figure S4. **Hematologic analysis**. Blood samples of immunized (**A**) and unimmunized control (**B**) NHPs were collected from the femoral veins and analyzed on the day of challenge with WT CO92 and on days 3 and 6 post challenge (days 88 and 91 post immunization and challenge) by using a Drew Scientific Hemavet 950 hematology system. WBC: white blood cells; NE: neutrophils; LY: lymphocytes. The arithmetic means ± standard deviations of the cell counts /µl are plotted. The dotted lines indicated the physiological ranges for each of the corresponding parameters measured.

Fig S5

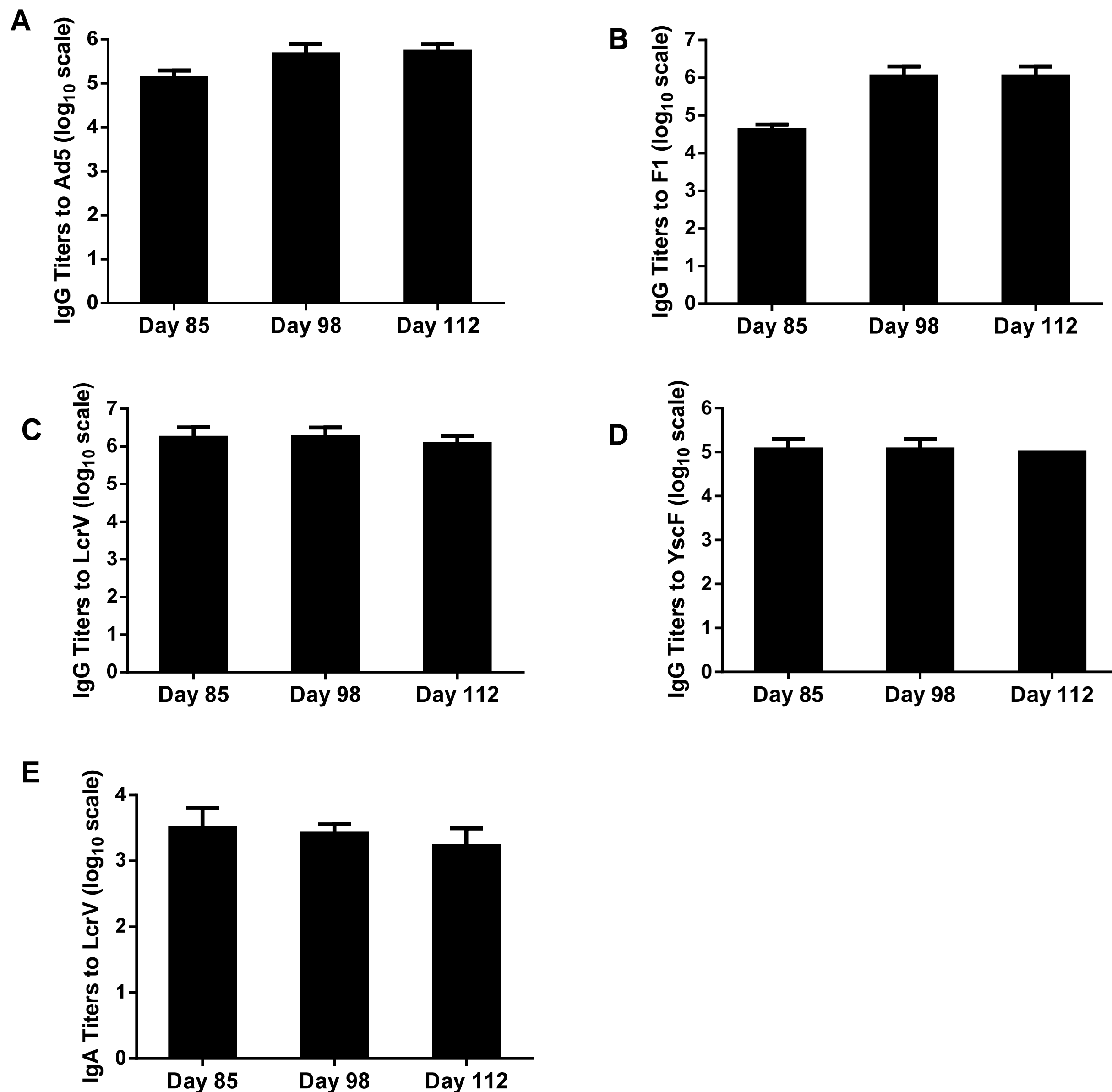

Figure S5. Antibody responses of vaccinated NHPs after WT CO92 aerosol challenge. Four randomly selected NHPs were injected in the quadriceps muscle with 5 x 10^{10} v.p. of Ad5-Empty to induce pre-existing immunity (day 0). On day 30, these NHPs were immunized by the intranasal route with 1 x 10^{11} v.p. of rAd5-YFV, followed by 50 µg of rYFV boost (emulsified 1:1 in Alum adjuvant) *via* the i.m. route on day 42. Another four NHPs received saline only (without immunization) and served as a control. On day 85, the NHPs were challenged with WT CO92 by the aerosol route. Blood samples were collected from the femoral veins of NHPs at various time points during the experiment from the immunized NHPs. The total IgG titers to Ad5 (**A**), F1(**B**), LcrV(**C**), and YscF (**D**) as well as total IgA titers to LcrV (**E**) on days 85, 98 and 112 were evaluated by ELISA. Days 98 and 112 represented 14 and 28 days post WT CO92 challenge after immunization.