**Supplementary Materials for**

Intranasal HD-Ad-FS Vaccine Induces Systemic and Airway Mucosal Immunities Against SARS-CoV-2 and Systemic Immunity against SARS-CoV-2 Variants in Mice and Hamsters

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Supplementary Figure 1-8 with corresponding legends

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自動產生的描述**

**Supplementary Figure 1.** Sex-based analyses of weight change in prime-boost HD-Ad-FS vaccinated hACE2 mice. The vaccinated hACE2 mice were challenged with either Beta **(a)**, Delta **(b)**, or Omicron **(c)** strain. Weight change of the challenged hACE2 mice was monitored at 0 (pre-challenge) and 4 dpi. Dots represented individual hamsters.

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**Supplementary Figure 2. (a)** Sex-based analyses of SARS-CoV-2 variant RNA levels in the lungs of prime-boost immunized hACE2 mice at 4 dpi. The viral RNA was determined with RT-qPCR. **(b)** Sex-based analyses of infectious SARS-CoV-2 variant titers in lungs of prime-boost immunized hACE2 mice at 4 dpi. The titers of infectious virus were determined with TCID50 assay. Dots represented individual hamsters. The dotted lines represent the LOD of the assays.

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**Supplementary Figure 3.** RNA levels of SARS-CoV-2 variants in oropharyngeal swabs from prime-boost immunized hACE2 mice. Swabs were collected at 0 (post-challenge) and 4 dpi. The RNA levels were determined with RT-qPCR. Dots represent individual hACE2 mice (n=4, 5, or 6). Dots represented individual hamsters. The horizontal dotted lines represent the LOD of the assays. Statistical analysis was performed by two-way ANOVA. Bars and errors represent the geometric mean with geometric SD. ns, not significant.

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**Supplementary Figure 4. (a)** Sex-based analysis of the levels of FS-specific sIgA in BALs of prime-boost immunized hACE2 mice at 4 dpi. The starting dilution factor was 1:1. **(b)** Sex-based analysis of the levels of serum NAbs in prime-boost immunized hACE2 mice at 4 dpi. **(c)** Sex-based analysis of the levels of BAL NAbs in prime-boost immunized hACE2 mice at 4 dpi. Dots represented individual hamsters. For **(b and c)**, the dotted lines represent the LOD of the assays.

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**Supplementary Figure 5. (a)** Sex-based analysis of SARS-CoV-2 RNA levels in the lungs of single-dose immunized hamsters. The viral RNA was determined with RT-qPCR at 4 dpi. **(b)** Sex-based analysis of infectious SARS-CoV-2 titers in lungs of single-dose immunized hamsters. The titers of infectious virus were determined with TCID50 assays at 4 dpi. **(c)** Sex-based analysis of SARS-CoV-2 RNA levels in spleens and hearts of single-dose immunized hamsters. The viral RNA was determined with RT-qPCR at 4 dpi. **(d)** Sex-based analysis of serum NAb titers against SARS-CoV-2 and variants. Sera were collected from single-dose immunized hamsters at 14 dpi. Dots represented individual hamsters. The dotted lines represent the limit of detection (LOD) of the assays. Statistical analysis was performed by one-way ANOVA. Error bars represent geometric mean with geometric SD. \*p<0.05, \*\*p<0.01, and ns, not significant.

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**Supplementary Figure 6.** Sex-based analysis of weight change in prime-boost immunized hamsters. The weight was monitored at the indicated days after challenging with SARS-CoV-2 variant. The asterisk signs represent statistical analysis in boost-FS hamsters, and the pound signs represent statistical analysis in boost-control hamsters. Dots represented individual hamsters. \*/#p<0.05, \*\*/##p<0.01, ###p<0.001.

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**Supplementary Figure 7.** RNA levels of SARS-CoV-2 variants in oropharyngeal swabs from prime-boost immunized hamsters. Swabs were collected at the indicated time points. The RNA levels were determined with RT-qPCR. Dots represented individual hamsters. The dotted lines represent the LOD of the assays. Statistical analysis was performed by two-way ANOVA. Bars and errors represent the geometric mean with geometric SD. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001, ns, not significant.

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**Supplementary Figure 8. (a)** Sex-based analysis of infectious titers of the SARS-CoV-2 variants in the lungs of prime-boost immunized hamsters. The titers were determined with TCID50 assays at 4 and 14 dpi. Statistical analysis was performed by two-way ANOVA. Error bars represent geometric mean with geometric SD. \*\*\*\*, p<0.0001. **(b)** Sex-based analysis of serum NAbs against SARS-CoV-2 variants. Sera of prime-boost immunized hamsters were collected at 4 and 14 dpi. Statistical analysis was performed by one-way ANOVA. Error bars represent geometric mean with geometric SD. \*, p<0.05, ns, not significant. **(c)** Sex-based analysis of NAbs in BALs against SARS-CoV-2 variants. BALs of prime-boost immunized hamsters were collected at 4 and 14 dpi. Statistical analysis was performed by one-way ANOVA. Error bars represent geometric mean with geometric SD. \*\*\*\*p<0.0001. Dots represented individual hamsters. The dotted lines represent the LOD of the assays.