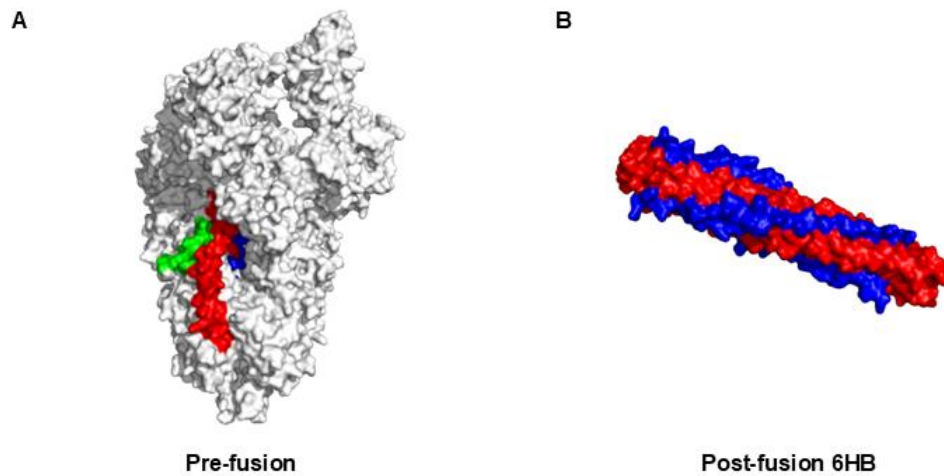


Supplemental Figures

Supplementary Figure 1

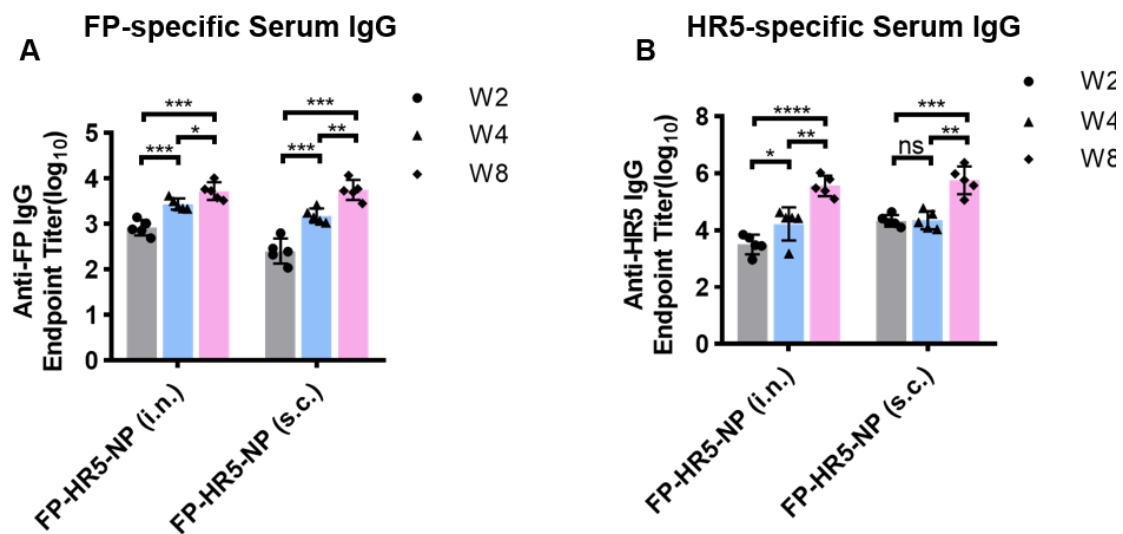


Supplementary Figure 1. The pre-fusion and post-fusion structures of FP, HR1, and HR2.

(A) Pre-fusion structures of FP (green), HR1 (red), and HR2 (blue) are shown in the SARS-CoV-2 spike with an electrostatic surface (PDB: 6VSB).

(B) The post-fusion structure six-helix bundle (6HB) structure formed by HR1 and HR2 is depicted with an electrostatic surface representation (PDB: 7RZQ).

Supplementary Figure 2



Supplementary Figure 2. FP and HR5-specific IgG titers of immunized C57 mice at each time point were detected by ELISA.

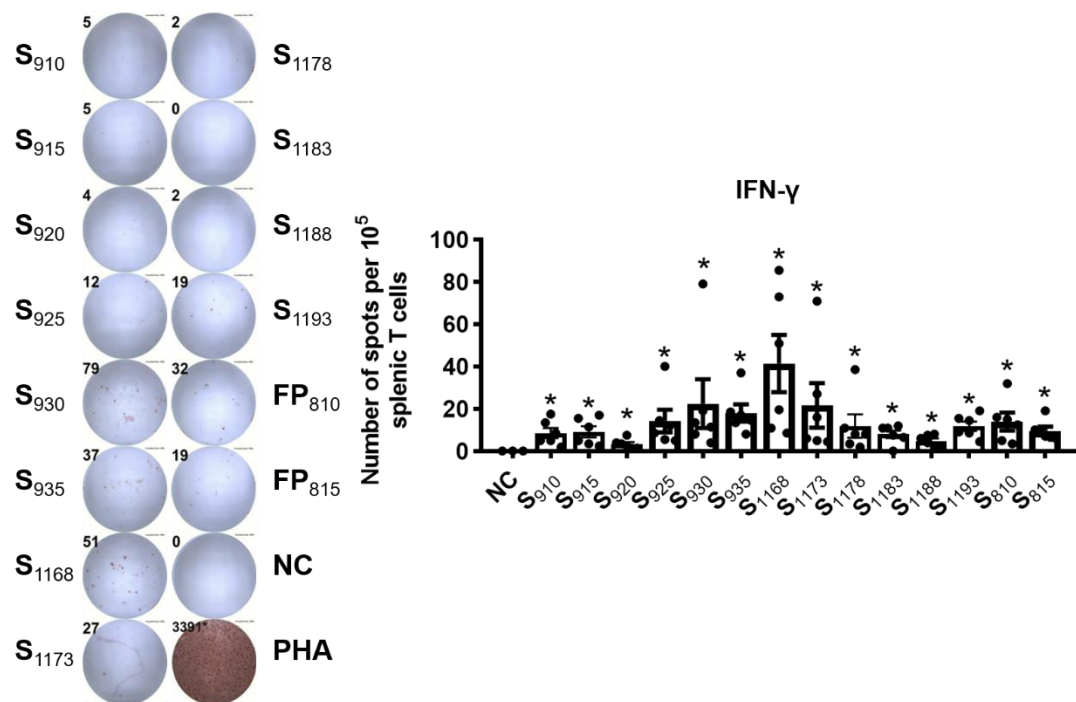
C57 mice were immunized with either 10 µg FP-HR5-NP intranasally or subcutaneously. Five mice per group were prime-boost vaccinated at weeks 0, 3, and 6. Serum was collected every two weeks, and all mice were euthanized at week 8.

(A) FP-specific IgG levels in the serum of different vaccine groups were measured by ELISA at weeks 2, 4, and 8.

(B) HR5-specific IgG levels in the serum of different vaccine groups were measured by ELISA at weeks 2, 4, and 8.

Data are represented as mean (SD). Adjusted p values were calculated by one-way ANOVA with Tukey's multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant.

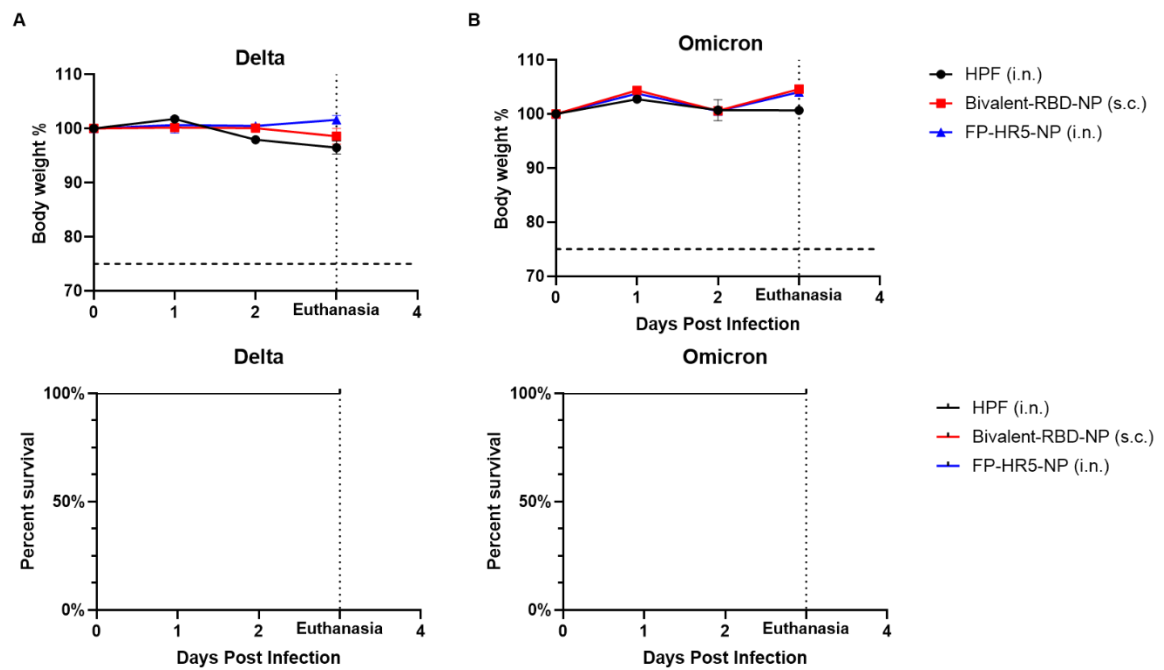
Supplementary Figure 3



Supplementary Figure 3. ELISpot assays measuring IFN- γ secretion from splenic T cells of C57BL/6 mice were performed with the addition of SARS-CoV-2 S2 peptide pools as a stimulus.

Splenic T cells of C57BL/6 mice were stimulated by SARS-CoV-2 S2 peptide pools, HR1(S₉₁₀-S₉₃₅), HR2(S₁₁₆₈-S₁₁₉₃), FP(S₈₁₀-S₈₁₅).

Supplementary Figure 4



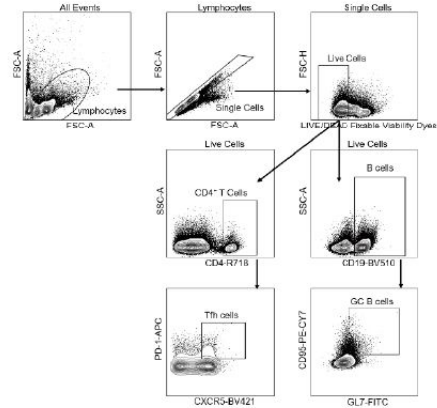
Supplementary Figure 4. Weight loss and survival curves of hACE2 mice

(A) weight loss and survival curves of hACE2 mice after Delta challenge.

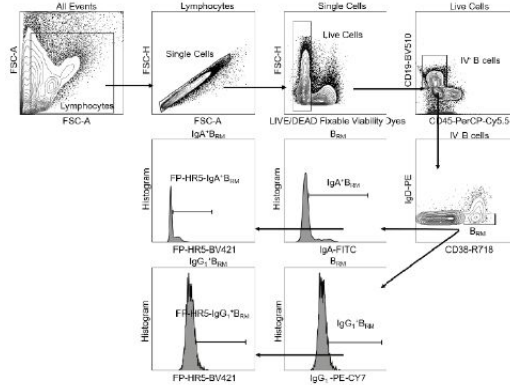
(B) weight loss and survival curves of hACE2 mice after Omicron challenge.

Supplementary figure 5

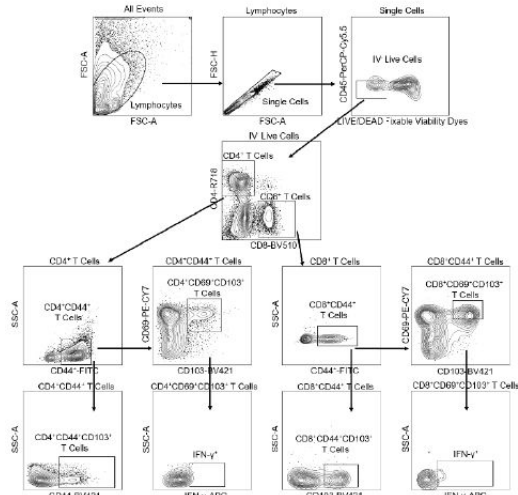
A



B



C



Supplementary Figure 5. Gating strategies for analysis of Immune Responses by FP-HR5 nanoparticle vaccines after intranasal immunization in mice.

(A) Gating strategies of germinal center B (GCB) cells (CD19⁺CD95⁺GL7⁺) and T follicular helper (Tfh) cells (CD4⁺CXCR5⁺PD-1⁺) from mediastinal lymph nodes.

(B) Gating strategies of B cell subsets, including IgG1⁺CD38⁺ B_{RM} cells, IgG1⁺CD38⁺ FP-HR5-specific B_{RM} cells, IgA⁺CD38⁺ B_{RM} cells, and IgA⁺CD38⁺ FP-HR5-specific B_{RM} cells from lung tissues.

(C) Gating strategies of T cell subsets, including IV⁻CD44⁺CD4⁺ T cells, IV⁻CD44⁺CD4⁺CD103⁺ T cells, IV⁻CD44⁺CD4⁺CD69⁺CD103⁺ T_{RM} cells, IV⁻CD44⁺CD8⁺ T cells, IV⁻CD44⁺CD8⁺CD103⁺ T cells, and IV⁻CD44⁺CD8⁺CD69⁺CD103⁺ T_{RM} cells from lung tissue. IFN-γ secreting CD4⁺CD69⁺CD103⁺ T_{RM} cells and CD8⁺CD69⁺CD103⁺ T_{RM} cells indicated the elicitation of an antigen-specific T_{RM} response.

Supplementary Table 1.

S2 peptides of T-cell epitope screening were used in this study

Region	Peptide	Sequence
HR1	910-924	GVTQNVLYENQKLIA
	915-929	VLYENQKLIANQFNS
	920-934	QKLIANQFNSAIGKI
	925-939	NQFNSAIGKIQDSL
	930-944	AIGKIQDSLSTASA
	935-949	QDSLSTASALGKLQ
HR2	1168-1182	DISGINASVVNIQKE
	1173-1187	NASVVNIQKEIDRLN
	1178-1192	NIQKEIDRLNEVAKN
	1183-1197	IDRLNEVAKNLNESL
	1188-1202	EVAKNLNESLIDLQE
	1193-1207	LNESLIDLQELGKYE
FP	810-824	SKPSKRSEFIEDLLFN
	815-829	RSFIEDLLFNKVTLA