

Expanded View Figures

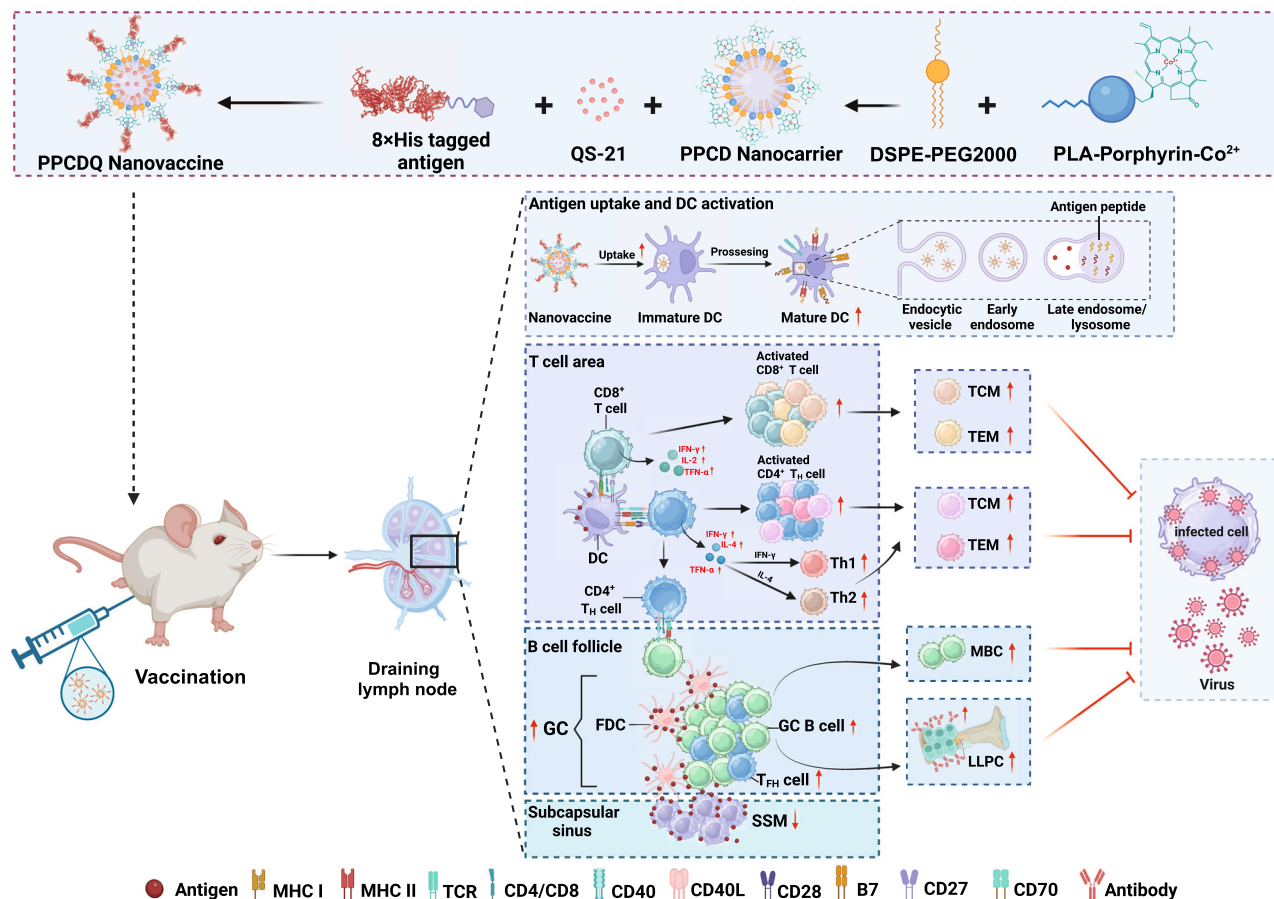


Figure EV1. Schematic illustration of the PPCDQ nanovaccine induce a humoral and cellular immune response against virus infection.

PPCDQ nanovaccine was synthesized by PLA-Porphyrin-Co²⁺, DSPE-PEG2000, QS-21, and histidine-tagged antigen. After intramuscular injection, the PPCDQ nanovaccine drained into the lymph nodes, efficiently activated DCs and then activated T cells. Furthermore, the activated CD4⁺ helper T cells (T_H cells) activated B cells to differentiate into plasma cells (PCs) to produce antibodies. Neutralizing antibodies and cytotoxic T lymphocytes (CTLs) against virus infection together. This figure was created with BioRender (<https://biorender.com/>).

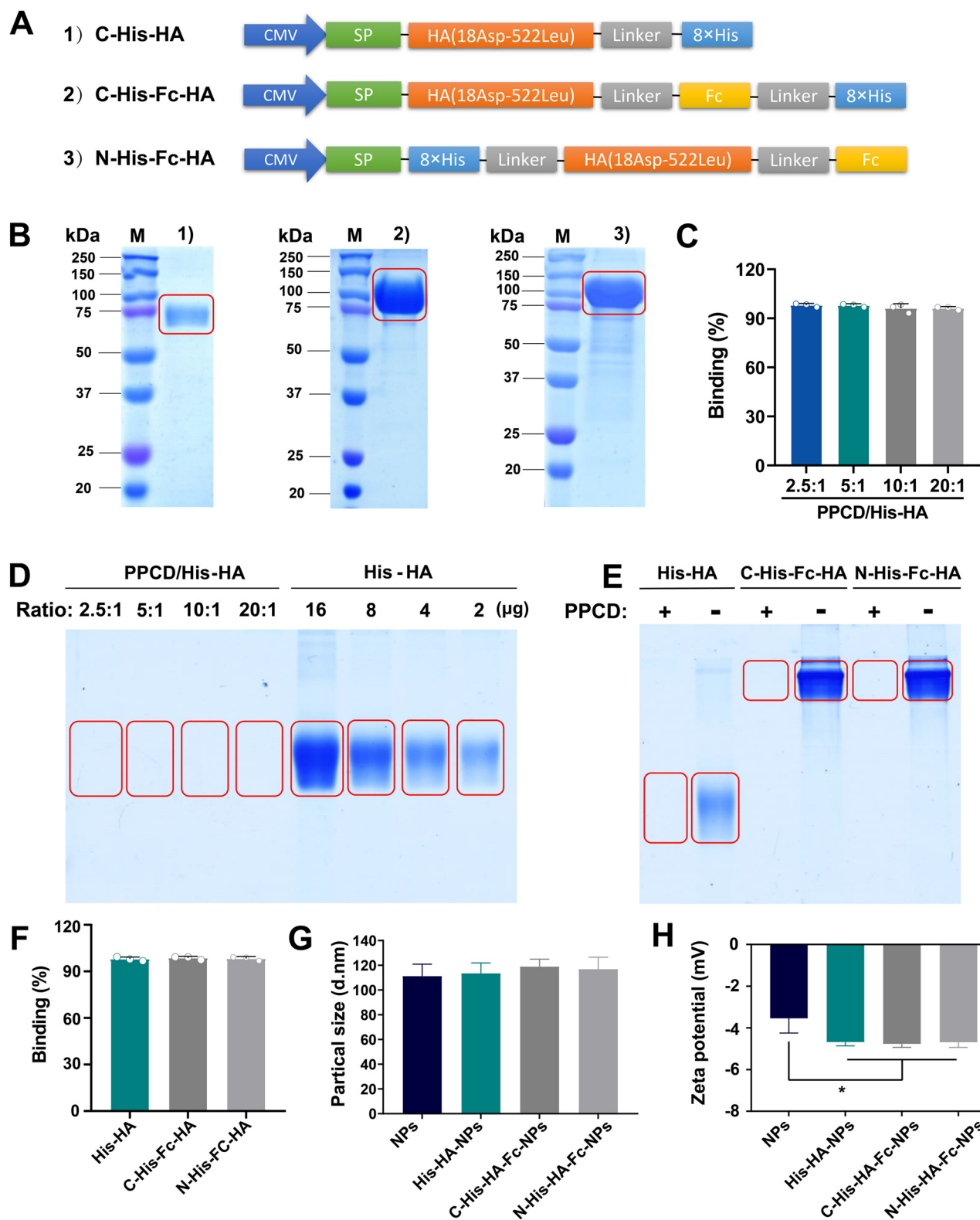
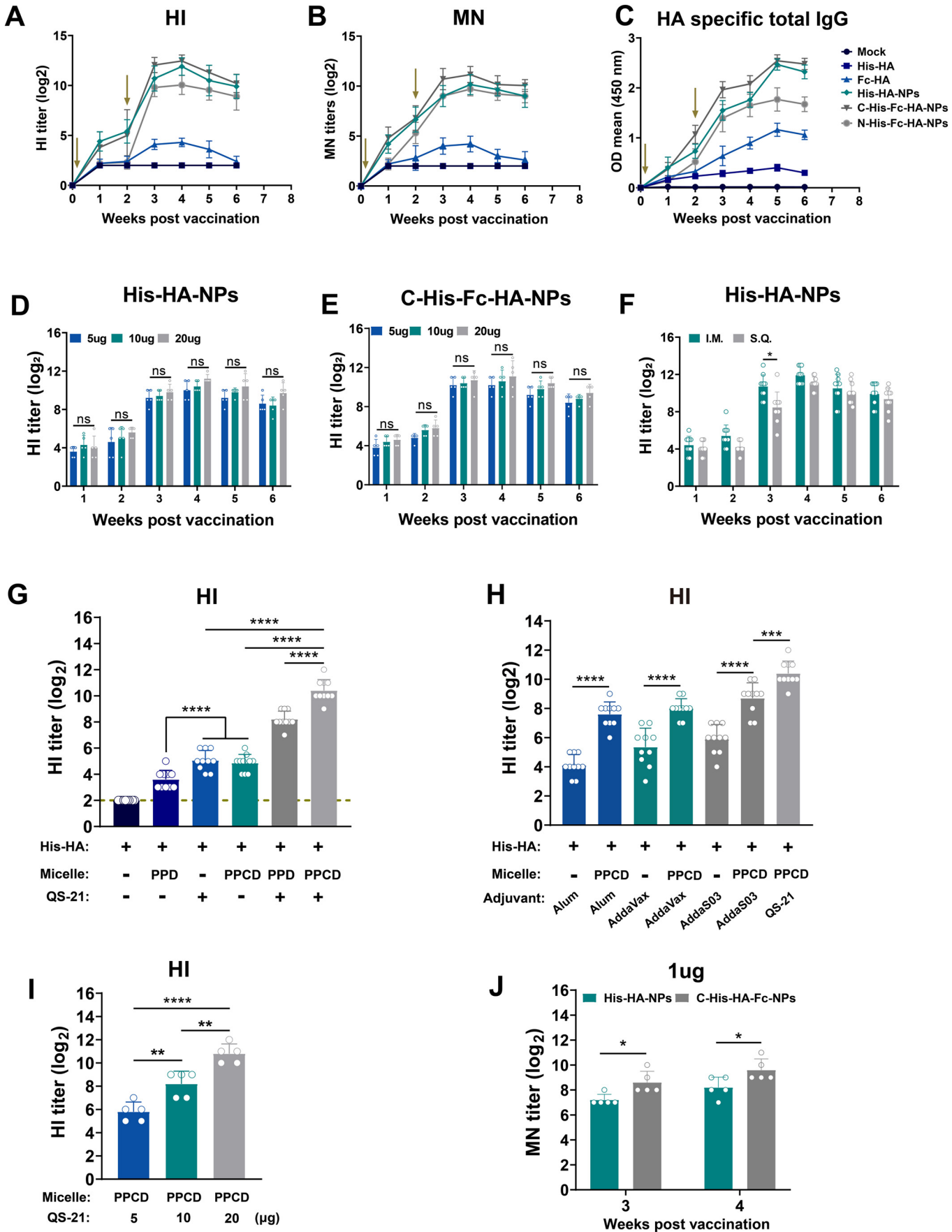


Figure EV2. Efficiency of PPCD anchoring His-tagged proteins.

(A) Construction strategy of three expression plasmids. CMV was used as a promoter, an IgG1 signal peptide (SP) was fused to the N-terminal and an 8 × His tag was fused to the N-terminal or C-terminal. Fc tag was fused to the C-terminal of HA. (B) SDS-PAGE gel of C-His-HA (1), C-His-Fc-HA (2), and N-His-Fc-HA (3). (C, D) Native PAGE gel (D) and anchoring efficiency (C) of PPCD micelles with different proportions of His-HA proteins ($n = 3$ biological replicates per group). (E, F) Native PAGE gel (E) and statistical graph of binding efficiency (F) of PPCD micelles to three kinds of HA proteins ($n = 3$ biological replicates per group). (G, H) Practical size (G) and zeta potential (H) of PPCDQ combined with or without protein ($n = 3$ biological replicates per group). Data information: Data in (C, F, G, H) are mean $\pm$ SD, statistical analysis in (H) was performed by one-way ANOVA with Tukey's multiple comparisons test. * $P < 0.05$.



◀ **Figure EV3. The synergistic effect of PPCD and QS-21 is critical for antibody production.**

(A–C) HI titers (A), MN titers (B), and HA-specific total IgG (C) of immunized C57/BL6 mice at each time point ($n = 10$ animals per group). Arrows represent the time points of vaccination. (D, E) C57/BL6 mice were immunized with different dose (5, 10, and 20 μg) of His-HA-NPs (D) or C-His-Fc-HA-NPs (E). HI titers were detected at indicated time point ($n = 5$ animals per group). (F) C57/BL6 mice were intramuscularly or subcutaneously immunized with 5 μg His-HA-NPs and HI titers of each time point were tested ($n = 10$ animals per group). (G) C57/BL6 mice were immunized with different formulations and HI titers were detected at 3 weeks post vaccination ($n = 10$ animals per group). (H) C57/BL6 mice were immunized intramuscularly with the mixture of different adjuvants (Alum, AddaVax, AddaS03, and QS-21) and His-HA or His-HA@PPCD, respectively. HI titers were detected at 3 weeks post vaccination ($n = 10$ animals per group). (I) C57/BL6 mice were immunized with His-HA@PPCDQ containing different doses of QS-21 (5, 10, and 20 μg). HI titers were detected at 3 weeks post vaccination ($n = 5$ animals per group). (J) C57/BL6 mice were immunized intramuscularly with 1 μg dose of His-HA-NPs or C-His-Fc-HA-NPs and MN titers were detected at 3 and 4 weeks post vaccination ($n = 5$ animals per group). Data information: Data are presented as mean $\pm$ SD, statistical analysis in (D–F, J) was determined by two-way ANOVA with Tukey's multiple comparisons test. statistical significance in (G–I) was determined by one-way ANOVA with Tukey's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

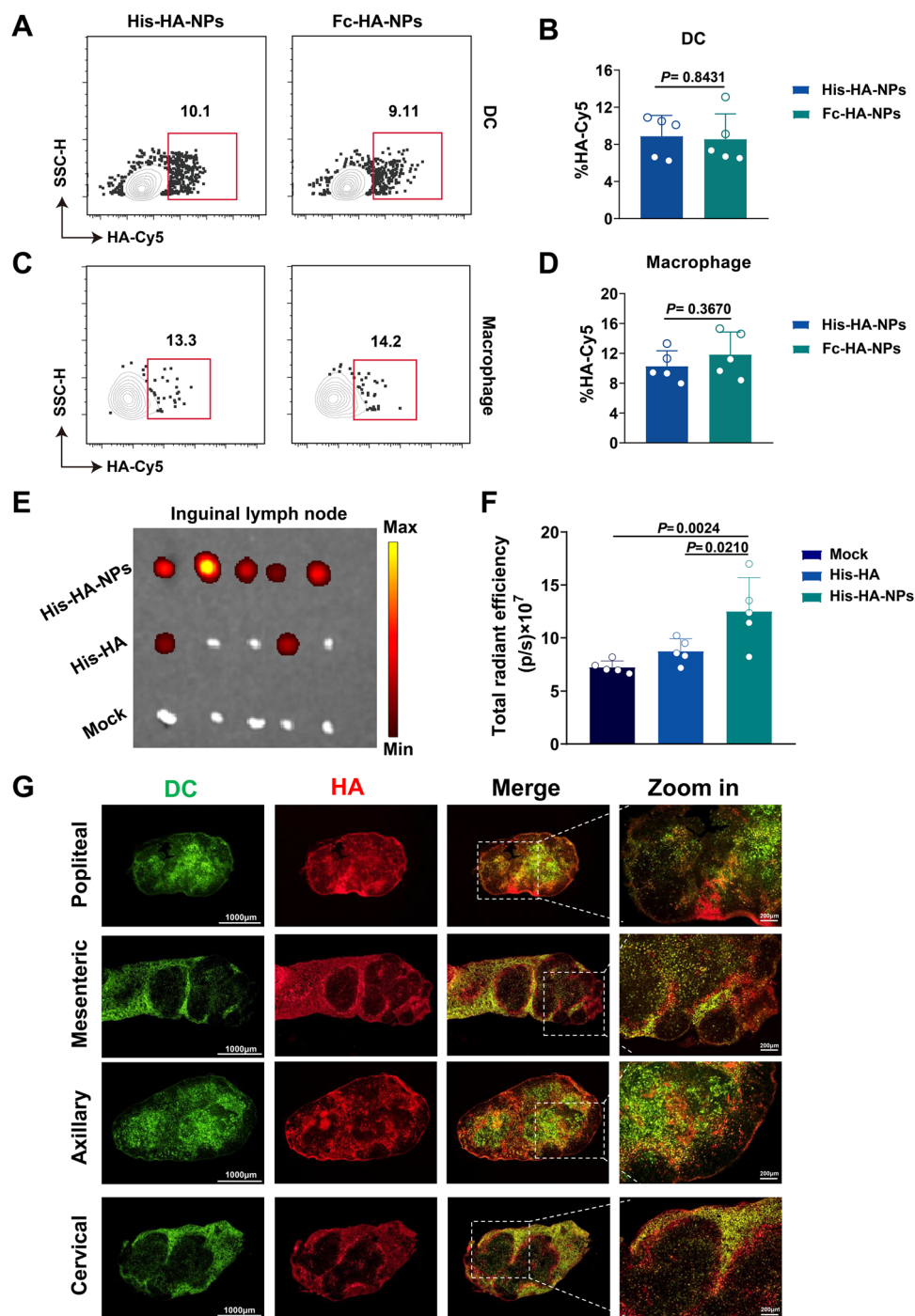


Figure EV4. His-HA-NPs rapidly accumulate in inguinal lymph nodes and flow to remote lymph nodes.

(A) Representative flow cytometric plots of Cy5 positive DCs (B220⁺CD11c⁺MHC-II⁺, top panel) in iLNs at 4 h post injection. (B) Statistical graphs of Cy5 positive DCs (n = 5 animals per group). (C) Representative flow cytometric plots of Cy5 positive macrophages (B220⁺CD11b⁺F4/80⁺). (D) Statistical graphs of Cy5 positive macrophages (n = 5 animals per group). (E) ILNs were harvested at 4 h post injection for the living image by IVIS Spectrum system. (F) Statistical graphs of the total radiant efficiency analyzed by Living Image Vision 4.4 (n = 5 animals per group). (G) Transgenic mice CD11c-EYFP were immunized with equal mass of iFlourTM 594-tagged His-HA or His-HA-NPs. Inguinal, popliteal, mesenteric, axillary, and cervical lymph nodes were obtained at 4 h post injection. Antigen distribution of these lymph nodes was shown by the cryosections. Scale bar: 1000 μm (left), 200 μm (right). Data information: Data in (B, D, F) are mean ± SD, statistical analysis was determined by one-way ANOVA with Tukey's multiple comparisons test.

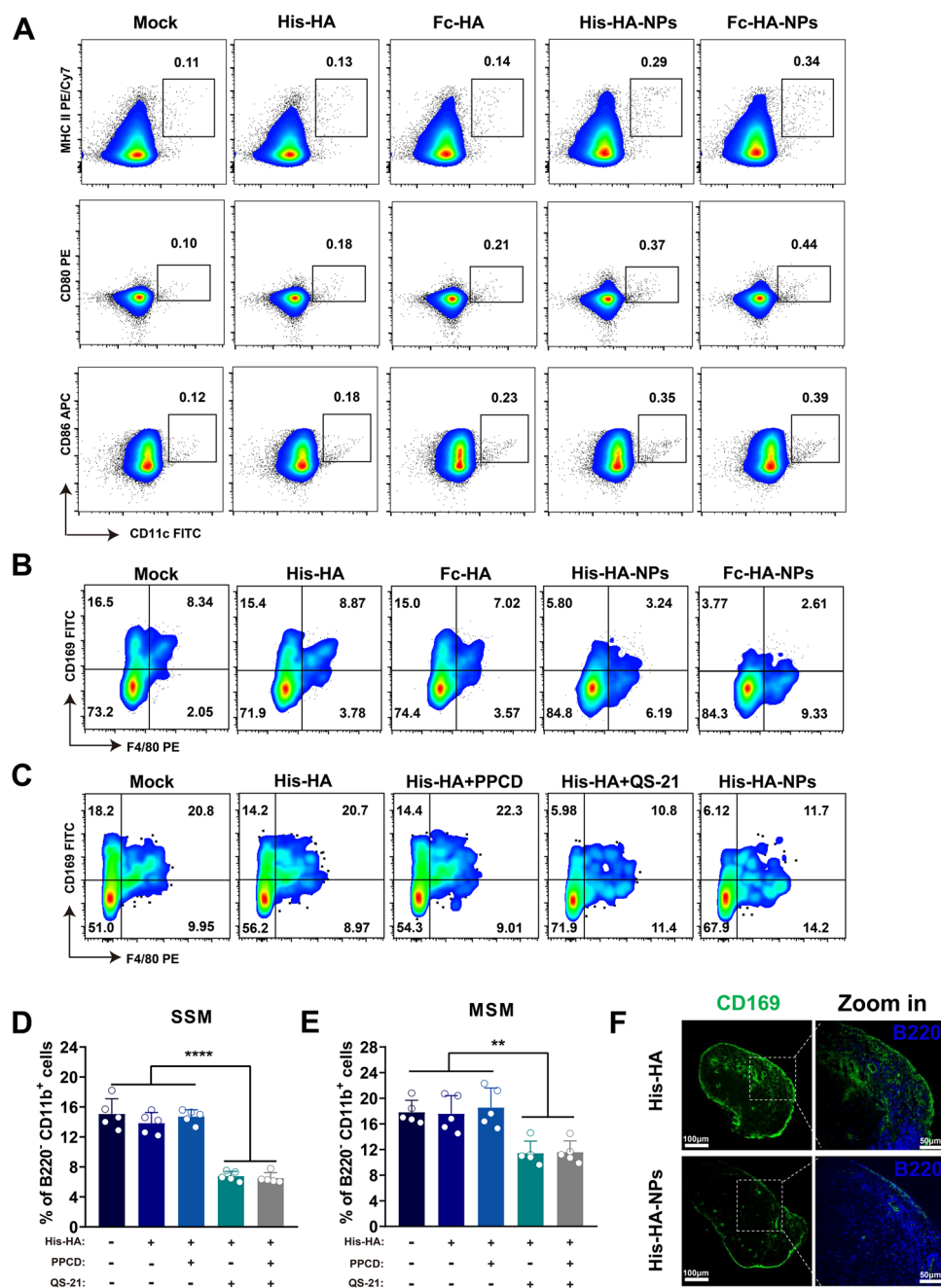


Figure EV5. HA@PPCDQ promotes DC activation and depletes CD169⁺ subcapsular sinus macrophages.

(A) Representative flow cytometric plots of CD11c⁺MHC II⁺ DCs, CD11c⁺CD80⁺ DCs, and CD11c⁺CD86⁺ DCs in iLNs at 2 days post boost immunization. (B) Representative flow cytometric plots of subcapsular sinus macrophage (SSM, B220⁺CD11b⁺F4/80⁺CD169⁺) and medullary sinus macrophage (MSM, B220⁺CD11b⁺F4/80⁺CD169⁺) in iLN. (C-E) C57/BL6 mice were immunized with different formulations, and iLNs were collected for FCM. (C) Representative flow cytometric plots of SSM and MSM. Statistical graphs of SSM (D) and MSM (E) ($n = 5$ animals per group). (F) Cryosections of inguinal lymph nodes were incubated with FITC anti-mouse CD169 (Siglec-1) and Brilliant Violet 421™ anti-mouse/human CD45R/B220. Images were taken on a Nikon super-resolution spinning-disk confocal microscopy. Scalar bar: 100 µm (left) and 50 µm (right). Data information: Data in (D), and (E) are mean $\pm$ SD, statistical analysis was determined by one-way ANOVA with Tukey's multiple comparisons test. ** $P < 0.01$, **** $P < 0.0001$.