Supplementary Information

Fig.S1

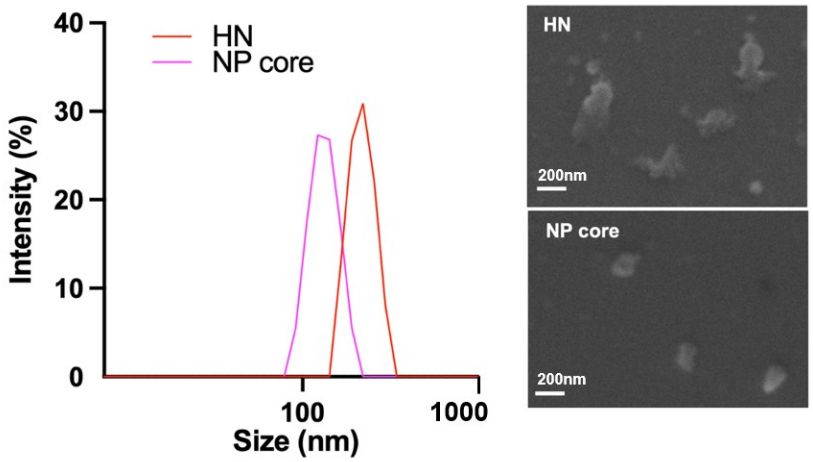

Figure S1. Characterization of the NP core and HN Protein Nanoparticle. Particle size distribution and SEM images of NP core and HN protein nanoparticles.

Fig.S2

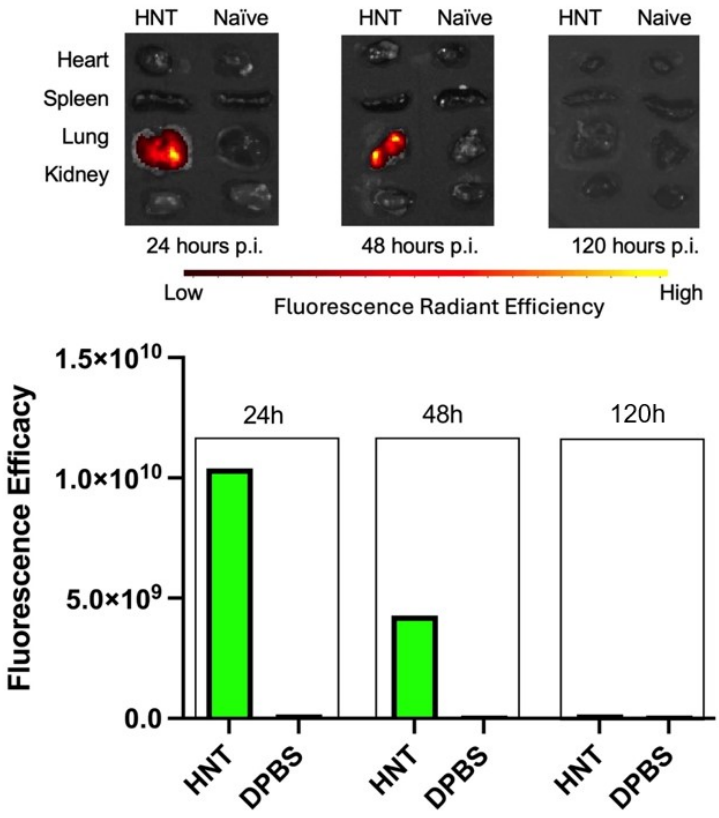

Figure S2. Biodistribution of protein nanoparticles in mouse organs. To study the distribution of intranasally administered fluorescent nanoparticles in mice, we used an in vivo imaging system (IVIS). Mice were sacrificed at 24-, 48-, and 120 hours post-administration, and organs (heart, lungs, kidneys, spleen) were collected for ex vivo imaging on Petri dishes. The Perkin-Elmer IVIS Spectrum system captured images, and Living Image software quantified the fluorescent intensity in the tissues.

Fig.S3

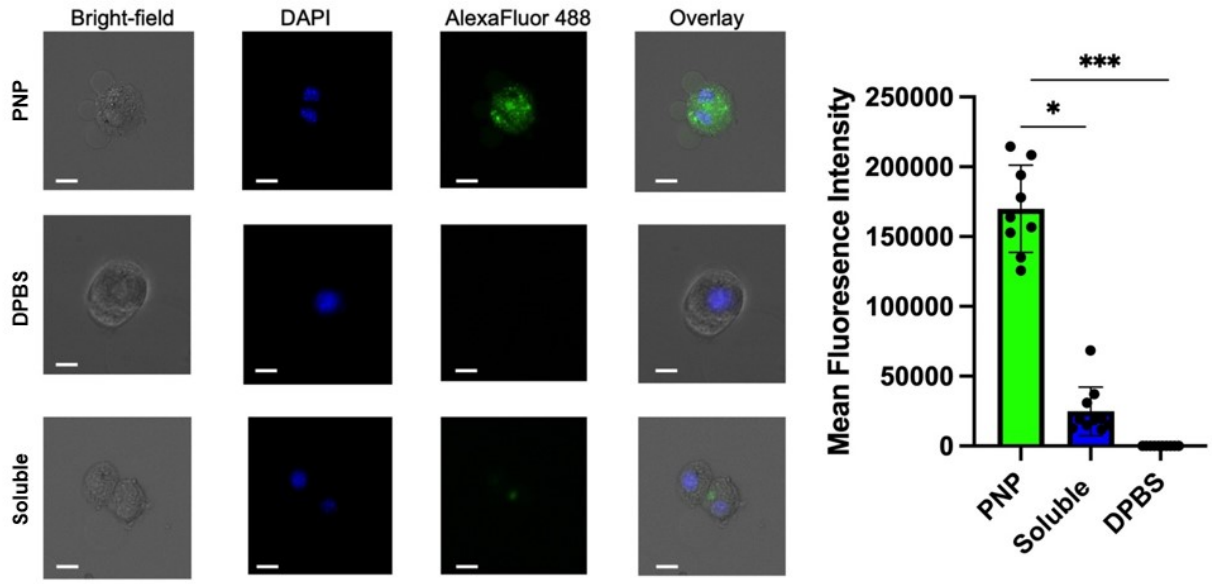

Figure S3. The cellular uptake of protein nanoparticles in JAWSII cells. JAWS II cells were exposed to Alexa Fluor 488 conjugated protein nanoparticles (PNP) or soluble HA3 protein (Soluble), respectively. DPBS was added as a control. After 24-hour incubation, the attached cells were stained with DAPI and observed under a fluorescent microscope (BZ-X710, Keyence) with 40x magnification (Scale bars, 10μm). The fluorescence intensity of Alexa Fluor 488 was quantitatively analyzed using the BZ-X analysis software (Keyence).

Fig.S4

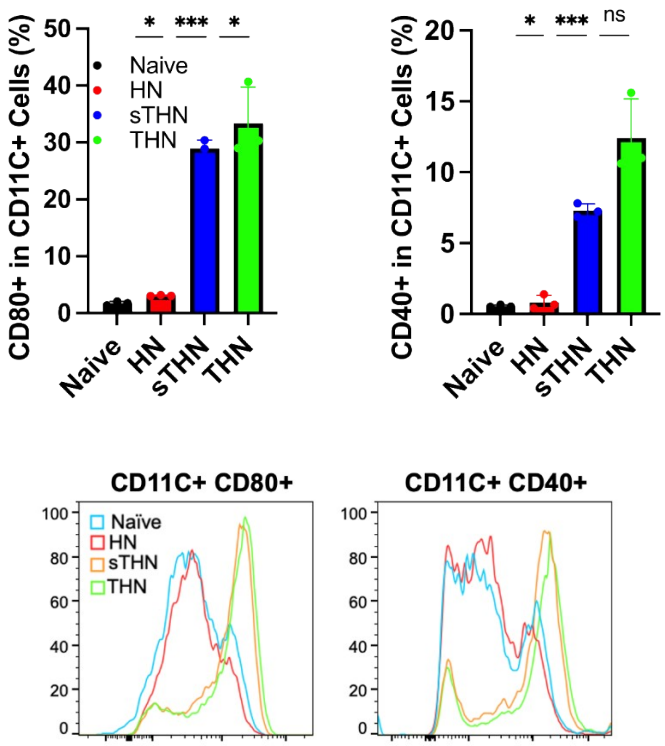

Figure S4. The expression of maturation markers on BMDCs. Cultured BMDCs were stimulated overnight with 4μg/ml of protein nanoparticles with and without tFliC adjuvant. The expression of CD80 and CD40 on CD11c+ BMDCs was determined by flow cytometry.

Fig.S5

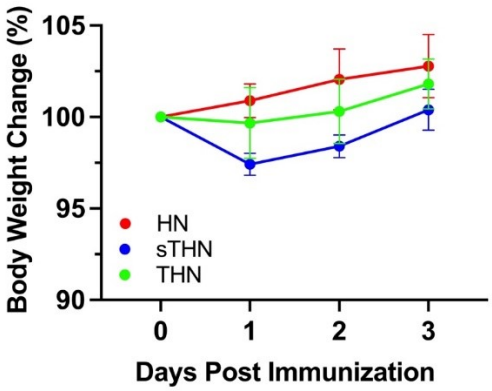

Figure S5. Body weight changes post intranasal immunization. Mice's body weights were monitored in the early days after intranasal immunization.

Fig.S6

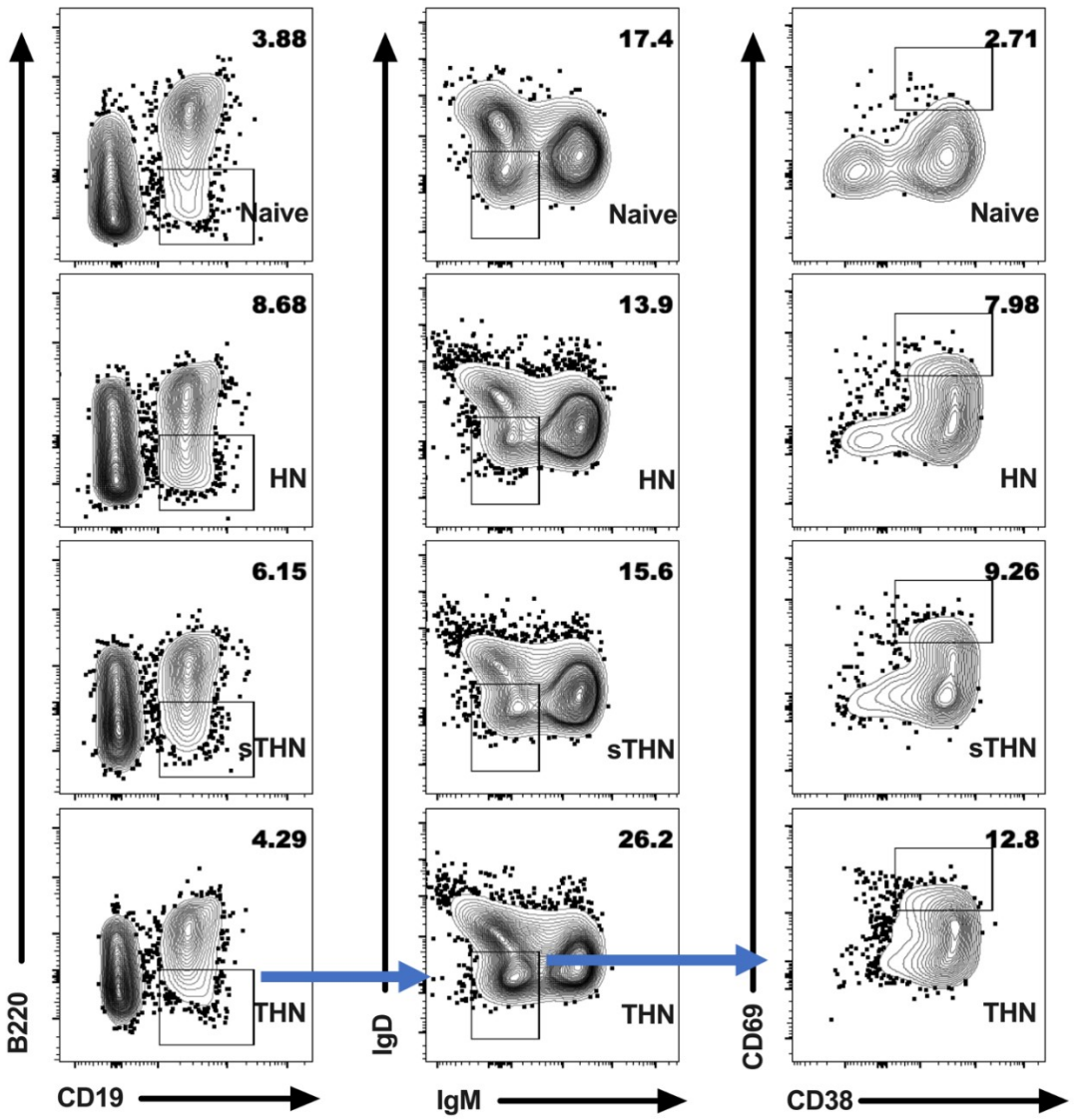

Figure S6. The gating method for tissue-resident memory B cells in the lung. Cells were isolated from lung tissues four weeks post-boosting immunization and stained for lung tissue-resident memory B cells, designated as CD19+B220-IgD-IgM-CD69+CD38+ populations.