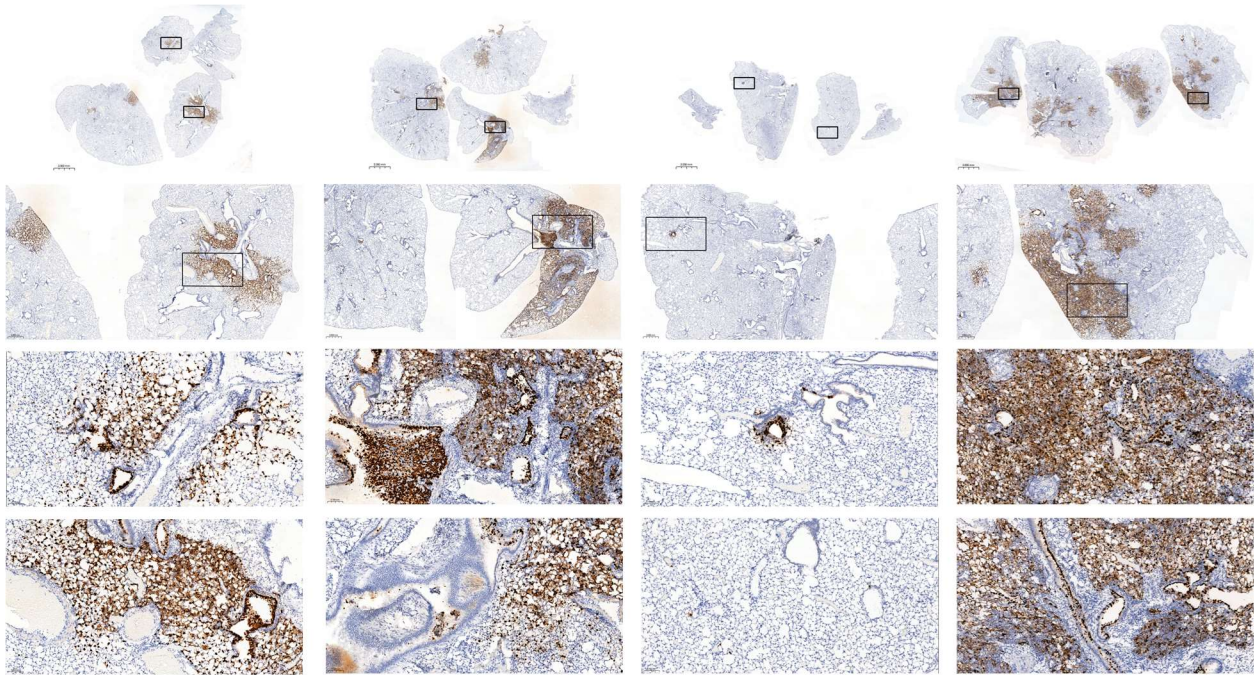
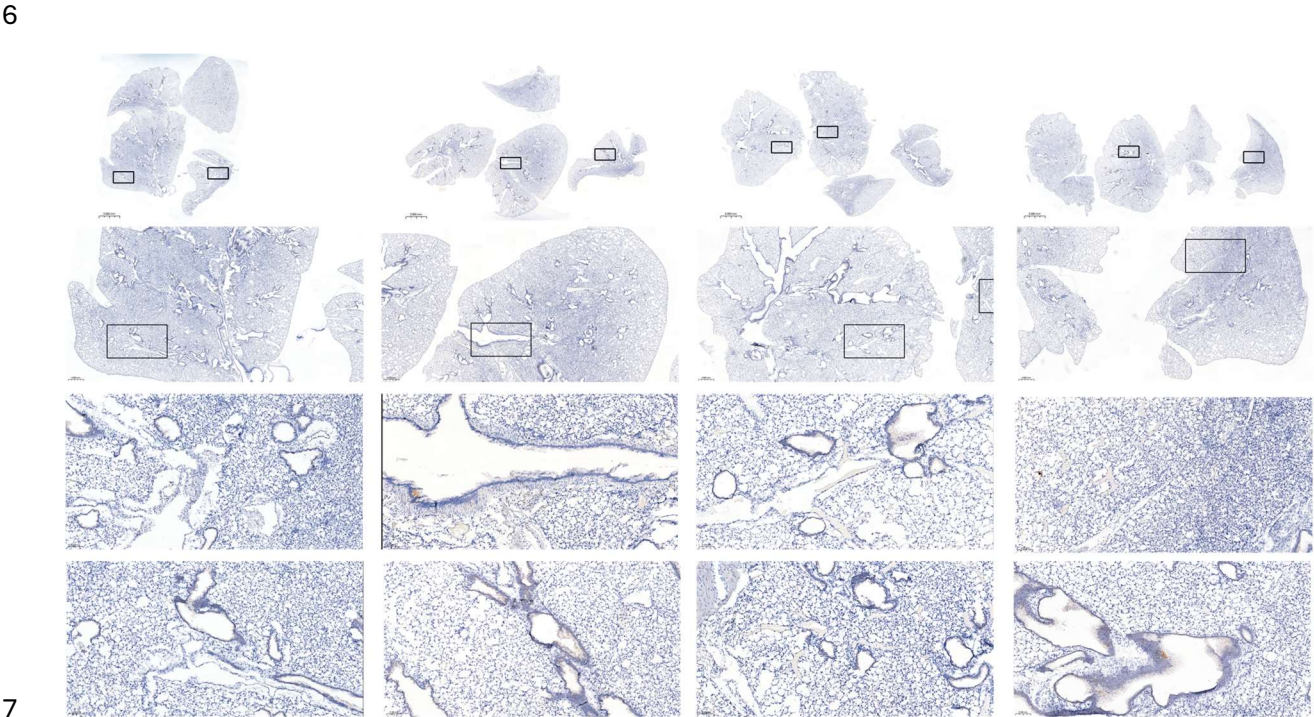


1 **SUPPLEMENTARY FIGURES**

2 **A: Placebo**



5 **B: H5-MNP (IM)**



C: H5-MNP (IN)

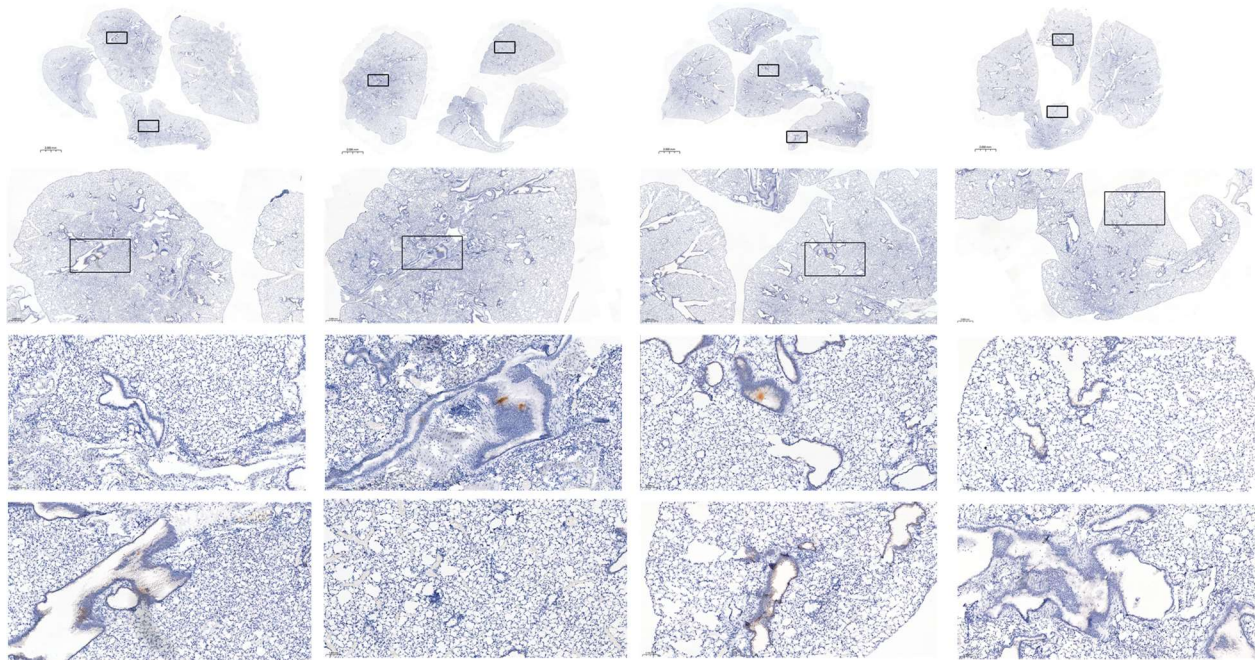


Figure S1: Immunization with H5 HA nanoparticle vaccine (H5-MNP) reduces H5N1 influenza A virus replication in the lungs. Related to Figure 1. Cohorts of naive 9-10 week-old male C57BL/6 mice (n = 4 mice per group) were immunized on days 0 and 28 via intranasal (IN) or intramuscular (IM) route with recombinant H5 HA nanoparticle (H5-MNP) influenza vaccine, or PBS as a placebo control were challenged with 200 focus forming units (FFU) of highly pathogenic H5N1 virus (A/Michigan/90/2024, clade 2.3.4.4b). The right lung lobes were collected, fixed in 10% formalin for 7 days and prepared for sectioning and RNA *in situ* hybridization detecting influenza viral NP RNA. **(A-C)** Representative images at 20x, 40x and 200x are shown for all four mice in the placebo control **(A)**, H5-MNP (IM, **(B)**), and H5-MNP (IN, **(C)**) group. Dark brown staining indicates the presence of influenza A virus nucleoprotein RNA.

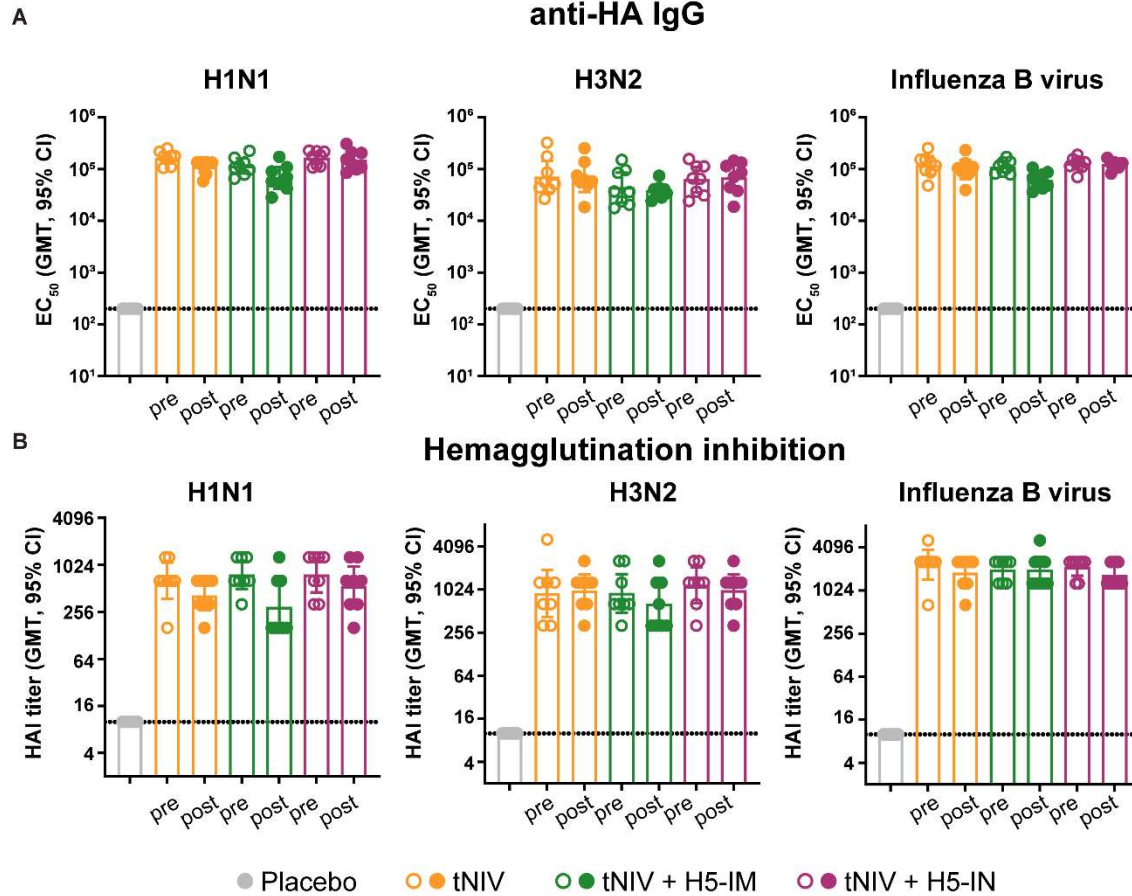


Figure S2: Trivalent Seasonal nanoparticle influenza virus (tNIV) vaccine induces high levels of H1-, H3-, and influenza B virus-specific immune responses. Related to Figure 2.

To confirm immunogenicity of tNIV and determine whether H5-MNP vaccine affects antibody responses against seasonal influenza strains, serum samples before and after H5-MNP boost were analyzed in placebo control, or mice immunized twice via the intramuscular (IM) route with a low dose (1 μ g) of recombinant tNIV vaccine (H1, H3 and influenza B virus HA) and boosted with PBS, or 1 μ g of recombinant H5-MNP vaccine via IN or IM route. **(A)** anti-H1, anti-H3, or anti-influenza B virus HA IgG titers by ELISA and **(B)** serum hemagglutination inhibition (HAI) titers against H1N1, H3N2 and influenza B virus by hemagglutination inhibition assay. Results are from two experiments ($n = 8$ mice per group), boxes illustrate geometric mean titers, error bars represent 95% confidence intervals, dotted lines show the limit of detection.

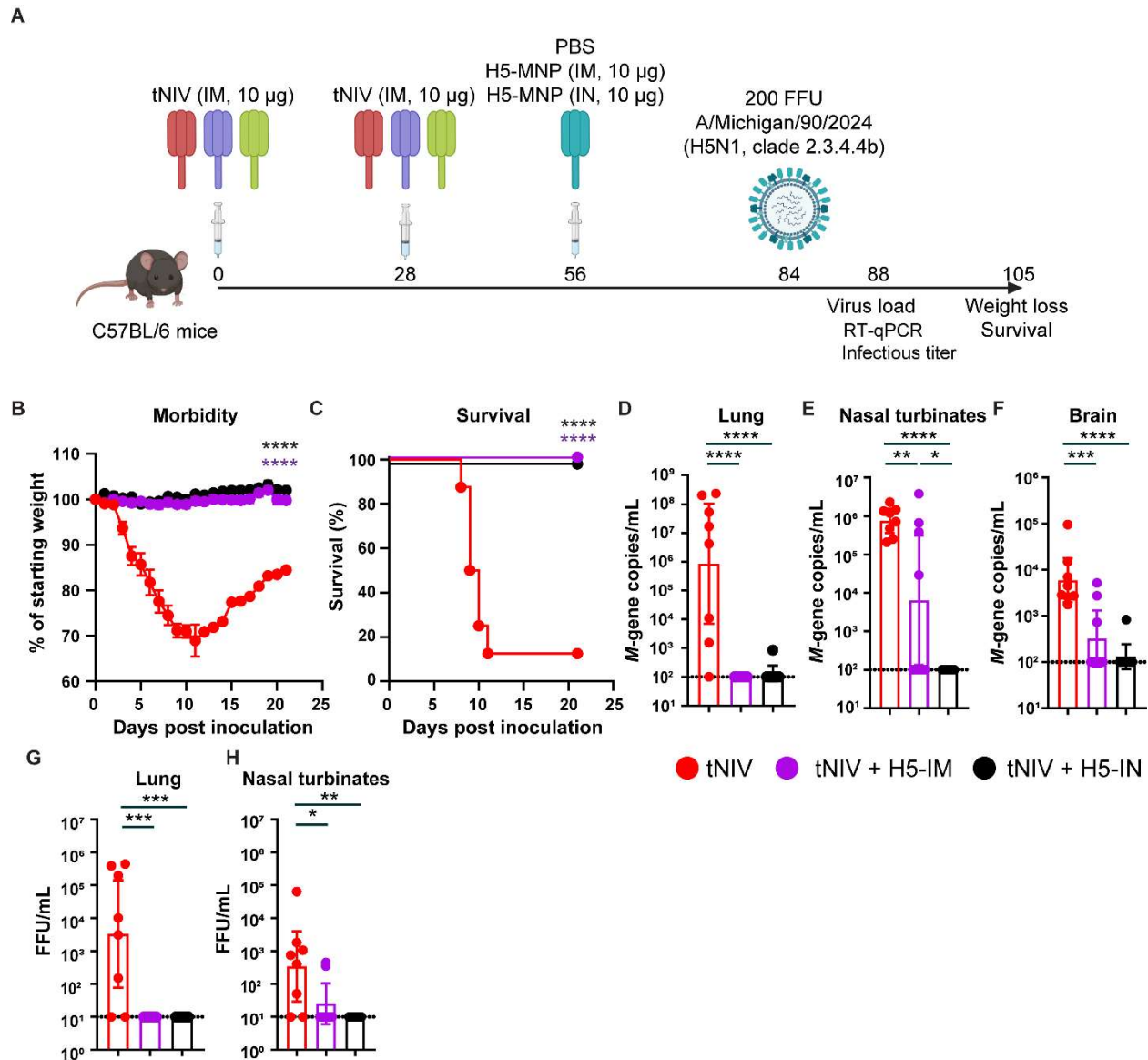


Figure S3: Efficacy of a single high dose of H5-MNP vaccine in high-dose tNIV primed mice.

Related to Figure 3. (A) Schematic of immunization and tissue collection timeline. Image created with BioRender.com. Cohorts of 7-week-old male C57BL/6 mice were immunized twice via the intramuscular (IM) route with a high dose (10 µg) of recombinant tNIV vaccine (H1, H3 and influenza B virus HA) and boosted with PBS, or 10 µg of recombinant H5-MNP vaccine via IN or IM route. Four weeks after the boost, mice were challenged with 200 focus forming units (FFU) of highly pathogenic H5N1 virus (A/Michigan/90/2024, clade 2.3.4.4b), and weight loss (**B**) and survival (**C**) were monitored for 21 days. (**D-F**) Viral RNA levels were determined at 4 days post

42 inoculation in the lung (D), nasal turbinates (E), and brains (F). (**G-H**) Infectious virus titers were
43 determined at 4 days post inoculation in the lung (G), and nasal turbinates (H) by focus forming
44 assay. Results are from two experiments (n = 8 mice per group), boxes illustrate geometric mean
45 titers with 95% confidence intervals, dotted lines show the limit of detection. Statistical analysis:
46 Lognormal ordinary one-way ANOVA with Tukey's post-test (D-F), Logrank (Mantel-Cox) test (B),
47 and ordinary one-way ANOVA with Tukey's post-test (A): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ****
48 $p < 0.0001$.