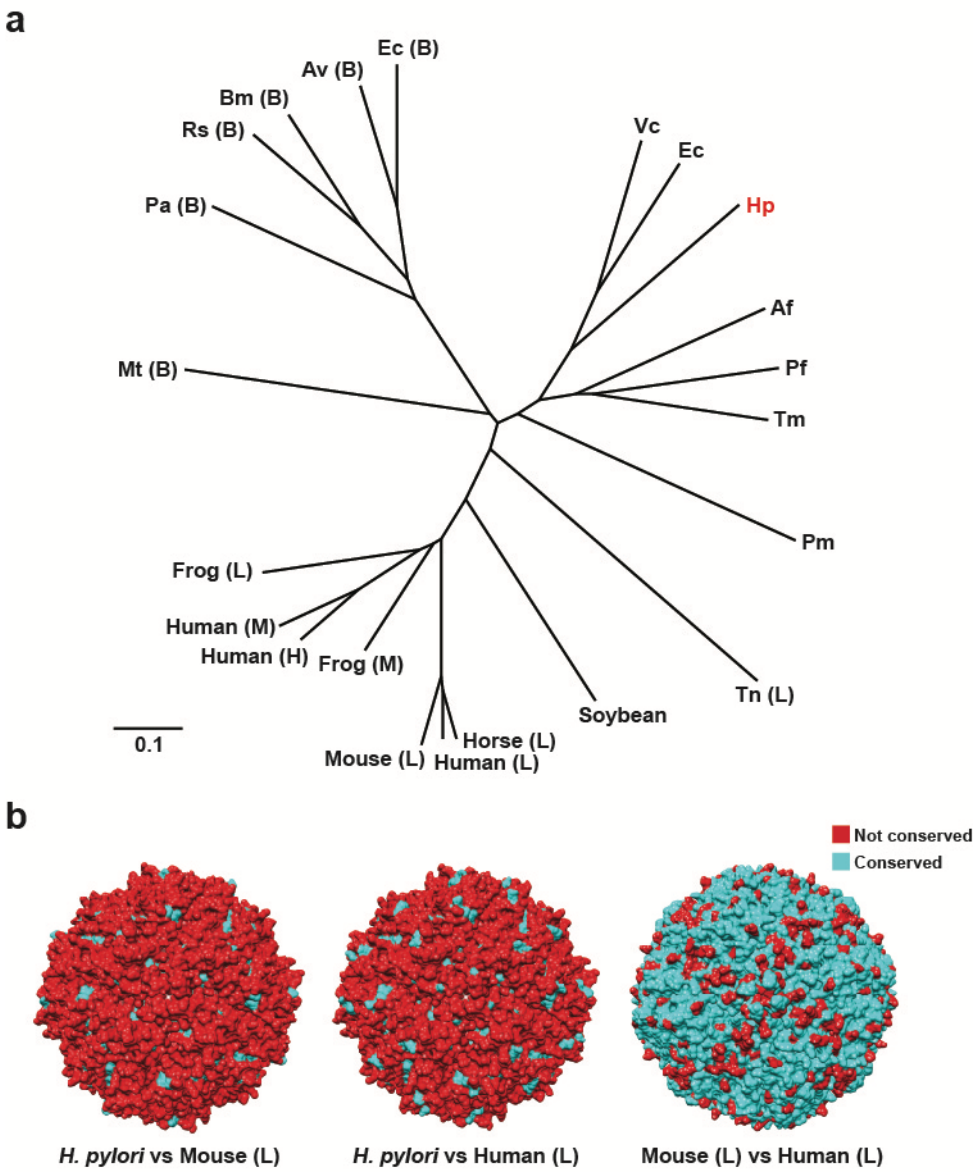
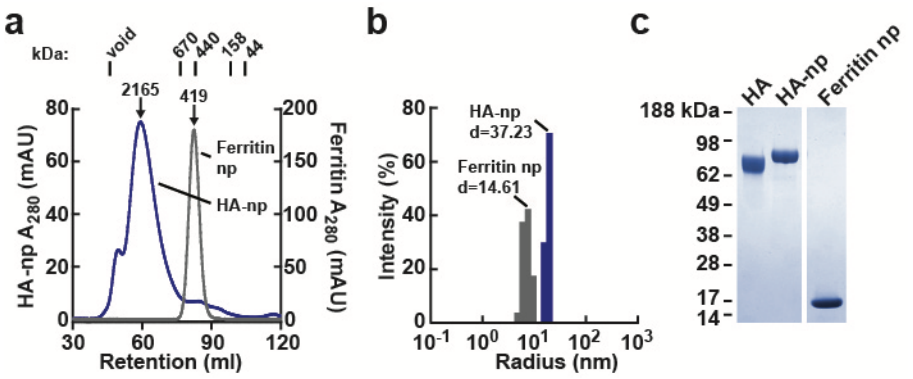
SUPPLEMENTARY FIGURES

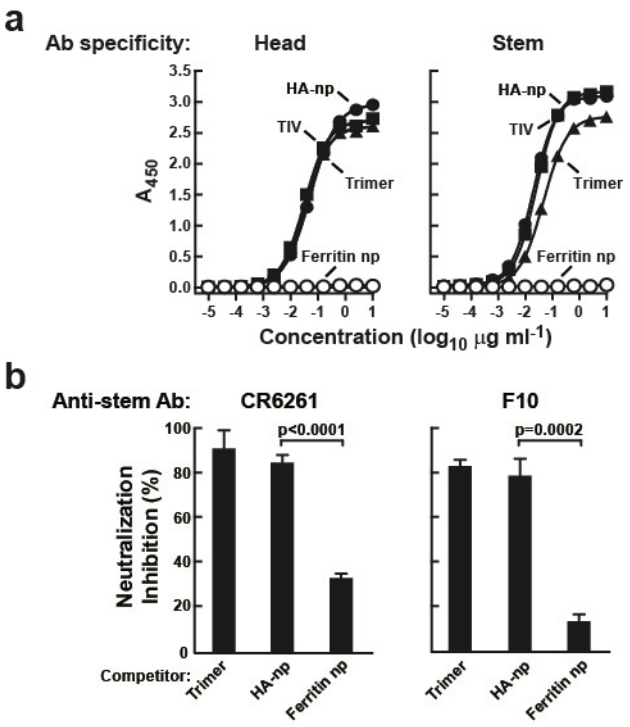


Supplementary Figure 1. Genetic and structural comparison of ferritins. a, Phylogenetic tree analysis of ferritins found in RSCB PDB. Twenty-two sequences contain 16 ferritins including Vc (*Vibrio cholerae*), Ec (*E. coli*), Hp (*H. pylori*), Af (*Archaeoglobus fulgidus*), Pf (*Pyrococcus furiosus*), Tm (*Thermatoga maritime*), Pm (*Pseudo-nitzschia multiseries*), Tn (L) (*Trichoplusia ni* light chain), Soybean (chloroplastic), Horse (L) (light chain), Human (L), (H) and (M) (light, heavy chains and mitochondrial, respectively), Mouse (L) (light chain), and Frog (M) and (L) (middle and lower subunits,

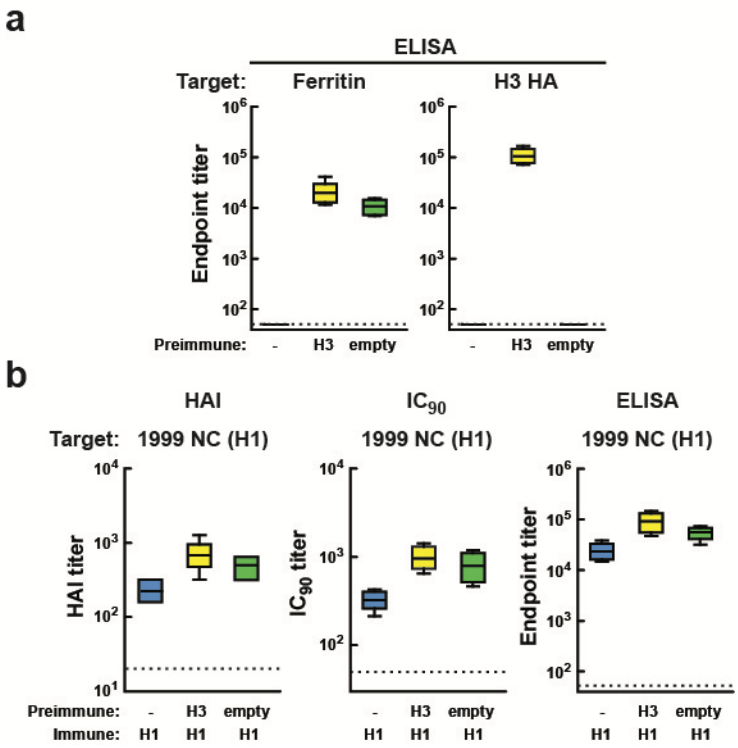
respectively), and 6 bacterioferritins (B) including Mt (B) (*Mycobacterium tuberculosis*), Pa (B) (*Pseudomonas aeruginosa*), Rs (B) (*Rhodabacter sphaeroides*), Bm (B) (*Brucella melitensis*), Av (B) (*Azobacter vinelandii*), and Ec (B). Protein sequences were aligned using Clustal W2 (www.ebi.ac.uk/Tools/msa/clustalw2) with Gonnet matrix and a phylogenetic tree was generated with the Phylodendron program (http://iubio.bio.indiana.edu/treeapp/treeprint-form.html) using the neighbor-joining method. **b,** Comparison of surface exposed residues between *H. pylori* and mouse (light chain) (left) or human (light chain) (middle), and mouse and human (light chains) (right). Conservation of surface exposed residues was rendered by UCSF Chimera using a protein sequence alignment generated by Clustal W2. Conserved and varied residues between the two ferritins were colored in cyan and red, respectively. Amino acid sequence identities of *H. pylori* nonheme ferritin to mouse ferritin heavy and light chains and human ferritin heavy and light chains are 23.6, 17.4, 24.1 and 18.9%, respectively. PDB files 3bve (*H. pylori*) (left and middle) and 1h96 (mouse light chain) (right) were used for surface rendering.



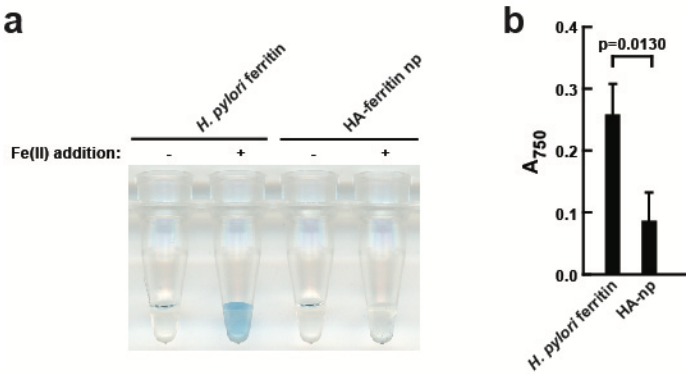
Supplementary Figure 2. Biochemical characterization of HA-nanoparticles. a, Size exclusion chromatography of ferritin and HA-nanoparticles (np). Molecular weights (kDa) of calibration standards are indicated. Calculated molecular weights using retention volumes of the ferritin and HA-nanoparticles were shown, and were within 10% of the predicted molecular weights (408 and 2,040 kDa, respectively). **b, c,** Dynamic light scattering and SDS-PAGE analyses of purified nanoparticles respectively. Mean diameters (d) are indicated. Purified HA trimer was used as a control.



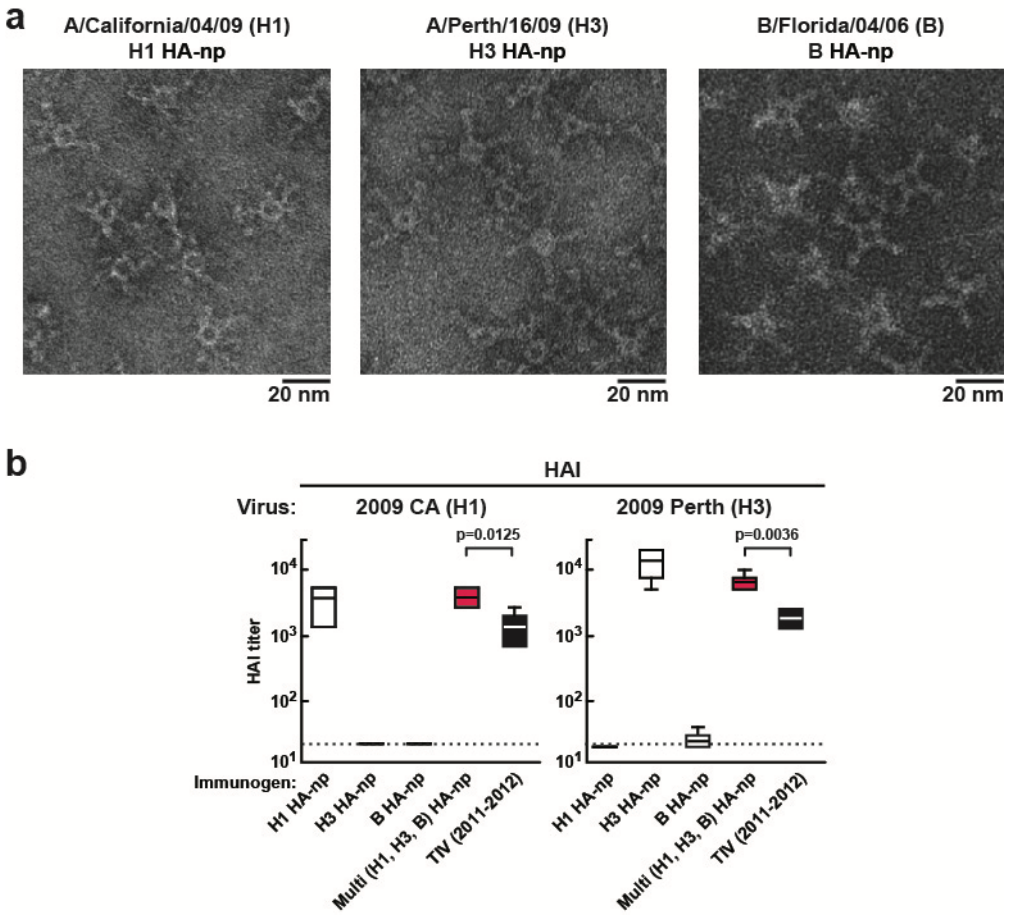
Supplementary Figure 3. Antigenic characterization of HA-nanoparticles. a, Binding of mAbs directed to the globular head and stem of HA was measured by ELISA. Molar equivalents (200 ng of H1 HA per well) of HA trimer (▲), TIV (■), HA-nanoparticles (●) or ferritin nanoparticles (equimolar to HA-nanoparticle) (○) were coated on the plates and wells were probed with anti-head and stem (CR6261) mAbs. **b,** Inhibition of anti-stem mAb-mediated neutralization of 1999 NC pseudotyped virus using HA trimer, HA-nanoparticles or ferritin nanoparticles as competitors. Inhibition of neutralization was plotted as percent inhibition with respect to control competitor (irrelevant HIV protein). The level of inhibition of CR6261 by empty ferritin nanoparticles appeared higher than that of F10. F10 has a much slower off-rate once it is bound to HA[8], and this may contribute to the lower background in this assay. Nonetheless, the difference between the inhibition mediated by HA- and empty nanoparticles was significant ($p<0.0001$). F10 (left) and CR6261 (right) were used at 3.125 and 25 μg ml^{-1}, respectively. Competitor proteins were added to the reactions at final concentration of 20 μg ml^{-1}.



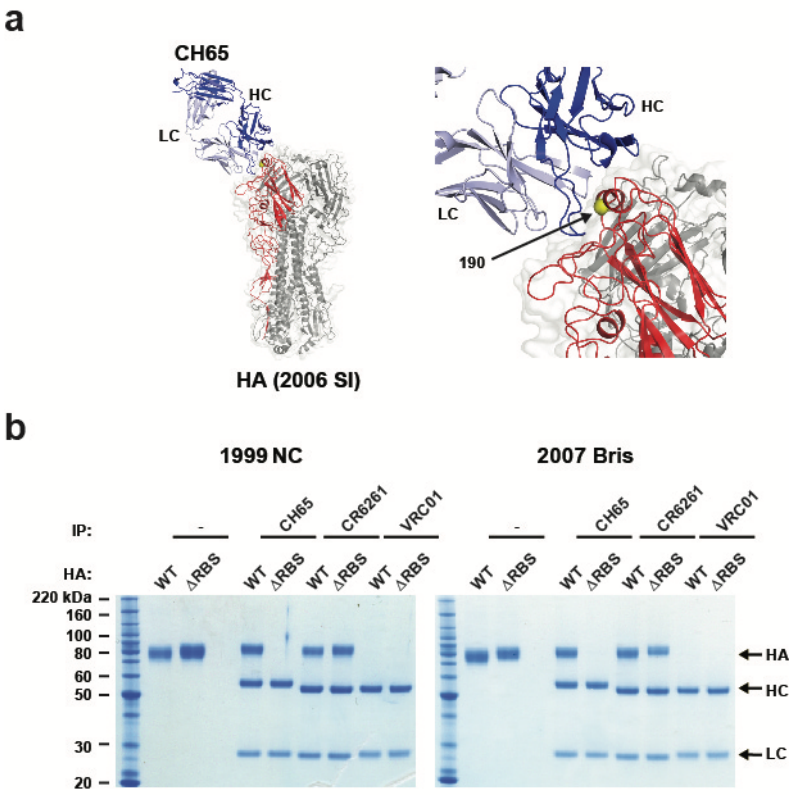
Supplementary Figure 4. Successive immunization of HA-nanoparticles in mice. Mice were pre-immunized with 1.67 μg (amount of HA) of 2009 Perth (H3) HA-nanoparticles or 0.57 μg (equimolar to HA-nanoparticle) of empty ferritin nanoparticles at week 0 and then immunized with 1.67 μg (amount of HA) of 1999 NC (H1) HA-nanoparticles at week 3. Ribi was used as an adjuvant. Another group of mice was immunized with 1999 NC (H1) HA-nanoparticles without pre-immunization of empty ferritin nanoparticles or H3 HA-nanoparticles. **a,** Ab responses to *H. pylori* ferritin (left) and 2009 Perth H3 HA (right). Immune sera collected 2 weeks after the immunization with H3 HA-nanoparticles (yellow) or empty ferritin nanoparticles (green) were analyzed by ELISA. **b,** Immune responses to 1999 NC (H1) after 1999 NC (H1) HA-nanoparticle immunization. HAI (left), IC_{90} neutralization (middle) and ELISA (right) ab titers were measured 2 weeks after immunization with 1999 NC (H1) HA-nanoparticles. The data are presented as box-and-whiskers plots with lines at the mean (n=5).



Supplementary Figure 5. Iron incorporation by ferritin and HA-nanoparticle derived from *H. pylori*. Empty *H. pylori* ferritin or HA-nanoparticle (2009 CA) (0.5 mg ml^{-1}) was dialyzed against ferrous ammonium sulfate solution (10 mM Tris, 1 mM ferrous ammonium sulfate, pH 7.5) for 2 hours at room temperature. The samples were then developed using an iron stain kit (Sigma) using the Prussian blue reaction (**a**), and absorbance was measured at 750 nm (**b**). IgG protein was used as an additional negative control and subtracted to determine background levels.



Supplementary Figure 6. Development of multispecific HA-nanoparticles. a, HA-nanoparticles consisting of HAs from 2009 CA (H1), 2009 Perth (H3) or 2006 FL (type B) were visualized by TEM. **b,** HAI titers against 2009 CA (H1N1) and 2009 Perth (H3N2) viruses in immunized mice. Mice were immunized with either a single H1, H3 or type B HA-nanoparticle, a mixture of all three HA-nanoparticles, or TIV (2011-2012 season). The data are presented as box-and-whiskers plots with lines at the mean (n=5).



Supplementary Figure 7. Characterization of ΔRBS HA probe. a, Crystal structure of HA (A/Solomon Islands/3/2006) complex with an anti-RBS mAb CH65 Fab (PDB: 3sm5)[6] (left). Close up view of CH65 contact area (right). The residue HA1 190 which has been mutated to be glycosylated in ΔRBS mutant is highlighted in a yellow sphere. The CH65 Fab-bound HA1 protomer is colored in red. **b,** Characterization of the soluble trimer of WT and ΔRBS HAs from 1999 NC and 2007 Bris. The WT and ΔRBS HA proteins were immunoprecipitated with anti-RBS (CH65), stem (CR6261) and control (VRC01) mAbs. Immune complexes were then dissolved in Lamini buffer and analyzed by SDS-PAGE. Antibody heavy and light chains are labeled as HC and LC, respectively.