# Supporting Information

# Induction of Enhanced Stem-directed Neutralizing Antibodies by HA2-16 Ferritin nanoparticles with H3 Influenza Virus Boost

*Qingyu Wang [a], Jiaojiao Nie [a], Zejinxuan Liu [a], Yaotian Chang [a], Yangang Wei [b], Xin Yao [a], Lulu Sun [a], Xiaoxi Liu [a], Qicheng Liu [c], Xinyu Liang [c], Xinran Zhang [c], Yong Zhang [a], Weiheng Su [a], Qi Zhao [d, e], Yaming Shan [a, f], Yingwu Wang [f], Xianbin Cheng [g, *], Yuhua Shi [a, *]*

[a] National Engineering Laboratory for AIDS Vaccine, School of Life Sciences, Jilin University, Changchun, Jilin 130012, China.

[b] Microthome Biotechnology Ltd., Zhongshan, Guangdong 528437, China

[c] High school attached to northeast normal university, Changchun, Jilin 130012, China.

[d] Faculty of Health Sciences, University of Macau, Taipa, Macau, China.

[e] MoE Frontiers Science Center for Precision Oncology, University of Macau, Taipa, Macau SAR, China.

[f] Key Laboratory for Molecular Enzymology and Engineering, The Ministry of Education, School of Life Sciences, Jilin University, Changchun, Jilin 130012, China.

[g] Department of Thyroid Surgery, The Second Hospital of Jilin University, Changchun, China

*Correspondence

X. B. Cheng, Department of Thyroid Surgery, The Second Hospital of Jilin University, Changchun, China

E-mail: chengxb19@mails.jlu.edu.cn

Y. H. Shi, School of Life Sciences, Jilin University, No. 2699 Qianjin Street, Changchun, Jilin 130012, China

E-mail: yhshi@jlu.edu.cn

KEYWORDS. H3 influenza virus, Hemagglutinin, Short linear epitope, Self-assembling, Nanoparticle, Ferritin

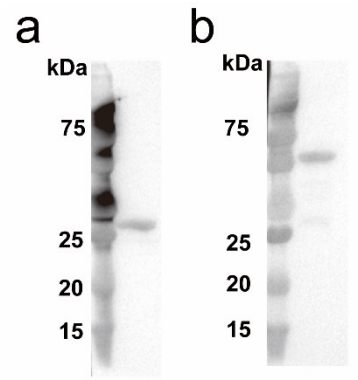


**Figure S1.** Determination of the integrity and stability of nanoparticles. Western blot analysis of (a) HA2-16-F using anti-HA2-16 peptide detection antibody and (b) HA2-F using anti-H3 influenza virus detection antibody.

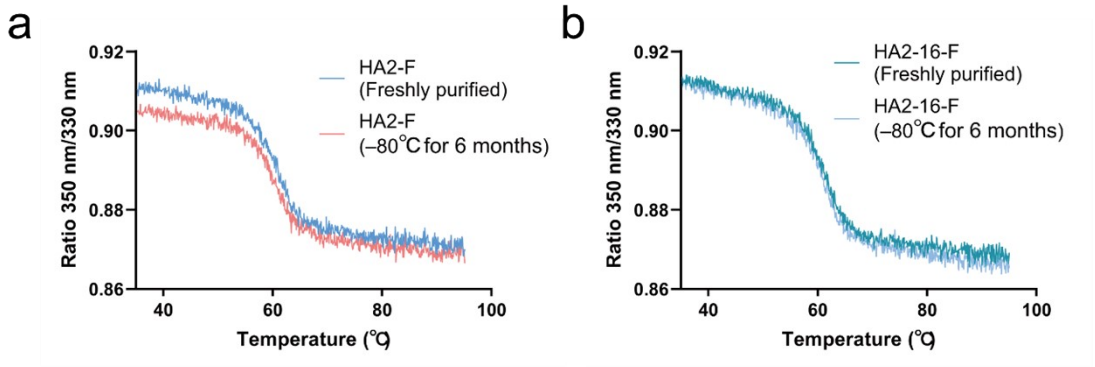


**Figure S2.** Unfolding profiles of freshly purified proteins and the proteins stored at -80°C for 6 months. (a) HA2-F, (b) HA2-16-F.

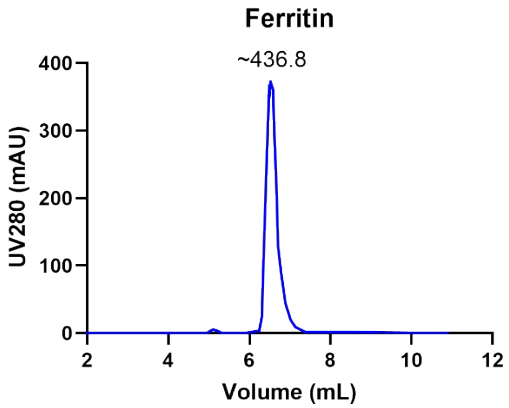


**Figure S3.** SEC analysis of ferritin nanoparticles. Calculated molecular weight using retention volumes of the ferritin nanoparticles was shown (~436.8 kDa).

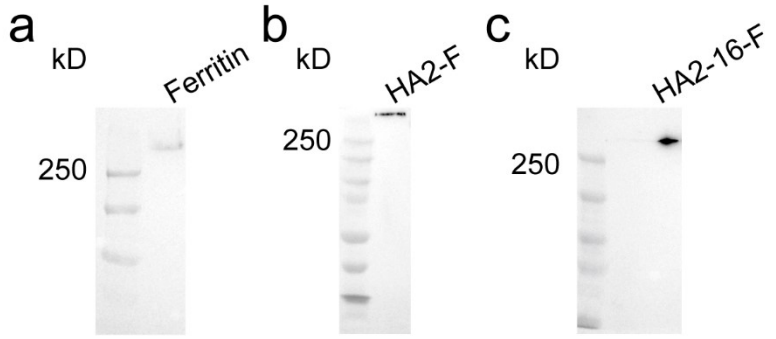


**Figure S4.** 8% Native-PAGE and western-blotting of purified NPs. (a) Native-PAGE of purified Ferritin NPs. (b) Native-PAGE of purified HA2-F NPs. (c) Native-PAGE of purified HA2-16-F NPs.

**pET-20b-HA2-Ferritin**

GIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFS
EVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHK
CDNACIGSIRNGTYDHDVYRDEALNNRFQIKEFGSGDIIKLLNEQVNKEMNSSNLYMSMSSWCYTHSLDGA
GLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDH
ATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKSGGGGSGGGGSGGGGSHH
HHHH

**pET-20b-HA2-16-Ferritin**

MDLWSYNAELLVALENQDIIKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLII
FLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVL
FKDILDKIELIGNENHGLYLADQYVKGIAKSRKSGGGGSGGGGSGGGGSHHHHHH

**pET-20b-Ferritin**

DIIKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPE
HKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLY
LADQYVKGIAKSRKSGGGGSGGGGSGGGGSHHHHHH

**Figure S5.** The amino acid sequences of pET-20b-HA2-Ferritin, pET-20b-HA2-16-Ferritin, and pET-20b-Ferritin, the linker information was marked in yellow.

## TABLE

**Table S1.** Serum HAI activity in the MF59 adjuvant immunization group

| H3N2 | Pre-immune | Mock | Ferritin | HA2-16-F | HA2-F |
|---|---|---|---|---|---|
| **A/Wisconsin/67/2005** | $2^3$ | $2^4$ | $2^4$ | $2^4$ | $2^4$ |
| **A/Hong Kong/4801/2014** | $2^3$ | $2^4$ | $2^4$ | $2^4$ | $2^4$ |