Dear editor:

Thanks for your reply.

Our responses are as follows:

For the Editorial comments:

1. Please note that the manuscript has been formatted to fit the journal standard.

2. Please revise the lines to avoid the issue of plagiarism: 151-153, 389-392.

Response:

We reword the contents as follows:

Line 151-153:

Original:

NOTE: One HA unit in the haemagglutinin titration is the minimum amount of virus that will cause complete agglutination of the red blood cells. The last well that shows complete agglutination is the well that contains one HA unit.

Editorial:

NOTE: HA units of the pseudoviruses is the highest dilution factor of the virus that can cause 100% hemagglutination of the red blood cells.

Line 389-392:

Original:

The IC50 (dashed line) is defined as the reciprocal dilutions that result in 50% inhibition. Data collected from three independent experiments are presented as mean ± SEM; error bars represent the standard error of the mean (SEM).

Editorial:

The IC_{50} is defined as the reciprocal dilutions of the neutralizing antibody that can 50% inhibition of the pseudovirus. Based on three independent experiments, the data are presented as mean ± SEM and the error bars mean the standard error of the mean (SEM).

Additionally, we reworded the contents in line 397

Line 397-398:

Original:

Data collected from three independent experiments are presented as mean ± SEM; error bars represent the standard error of the mean (SEM).

Editorial:

Based on three independent experiments, the data are presented as mean ± SEM and the error bars mean the standard error of the mean (SEM).

3. Since the protocol focuses on using immune sera for the assays, please consider including a brief section/step in the protocol describing how the animals were prepared for the injections, the volume of injections, route of delivery, the requirement of anesthesia, etc. based on the Supplementary figure 2.
Regarding animal treatment in the protocol, please add the following information to the text:
a) Please specify the euthanasia method if any. Please mention how animals are anesthetized and how proper anesthetization is confirmed
b) Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia.
c) For survival strategies, discuss post-surgical treatment of animals, including recovery conditions and

treatment for post-surgical pain.
d) Discuss maintenance of sterile conditions during survival surgery/injections.
e) Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.
f) Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.

Response：
We added the protocol "4 immune sera preparation" under the protocol "3 pseudovirus titration"
And renumbered the following protocols.

The contents of protocol "4 immune sera preparation" as follows:

4. Immune sera preparation

NOTE: The immune serum will be used for cell-related experiments, and the experimental operation should be carried out under aseptic conditions.

4.1 Prepare 12 eight-week-old female BALB/c mice. Divide equally the mice into two groups.

NOTE: one group was DDV group and the other was negative control group.

4.2 DDV group immunization: prime twice intramuscularly with 100 μg of codon-optimized DNA plasmid encoding A/Thailand/(KAN-1)/2004 (TH) HA protein at week 0 and week 3 and boost once intraperitoneally with 512 HAU TH virus-like particles (VLP) at week 6.

4.3. Control group immunization: prime twice intramuscularly with 100 μg empty vector plasmid DNA at week 0 and week 3 and boost once intraperitoneally with HIV-1 gag VLP at week 6.

4.4. Collect mouse blood 2 weeks after the last immunization.

NOTE: Blood was collected through submandibular vein and the mice will be immediately euthanized with CO_2. The mice were anesthetized with pentobarbital sodium (65 mg/kg) at the time of blood collection.

4.5 Keep the blood at room temperature for 2 h, then 4°C overnight. Centrifuge at 900 x *g* for 10 min at 4°C and collect the supernatant. Inactivate 56°C for 30 min.

4.6 Store immune sera in -80°C refrigerator for use.

In addition, we deleted the repeated contents in line 92, the changes as follows:
Line 91-95:
Original:
To obtain the immune sera used in assays, this protocol selected the HA protein originating from the TH strain as the immunogen to immunize mice. In the immune group, BALB/c mice were primed twice with DNA plasmid encoding H5 TH HA and boosted once with virus-like particles

(VLPs) from the same strain. While for the control group, BALB/c mice were primed twice with the DNA plasmid of the empty vector and boosted with HIV-1 gag alone.

Editorial:
To obtain the immune sera used in assays, this protocol selected the HA protein originating from the TH strain as the immunogen to immunize mice.

Besides, the product information of pentobarbital sodium is added to the material table.

The protocols' number changes as follows
Original:
1. Pseudovirus packaging with Calcium-phosphate transfection
2. Detection of the HA, NA and HIV-1 p24 protein expression of influenza pseudovirus
3. Pseudovirus titration
4. Pseudovirus neutralization (PN) assay.
5. Pseudovirus attachment assay
6. Assessment of viral entry

Editorial:
1. Pseudovirus packaging with Calcium-phosphate transfection
2. Detection of the HA, NA and HIV-1 p24 protein expression of influenza pseudovirus
3. Pseudovirus titration
4. immune sera preparation
5. Pseudovirus neutralization (PN) assay.
6. Pseudovirus attachment assay
7.Assessment of viral entry