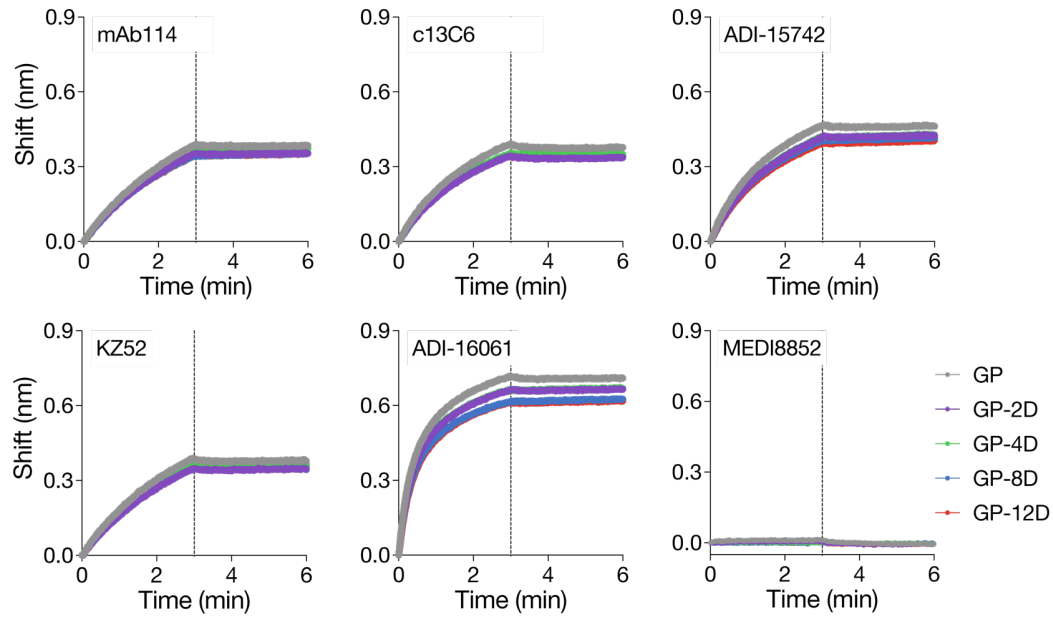
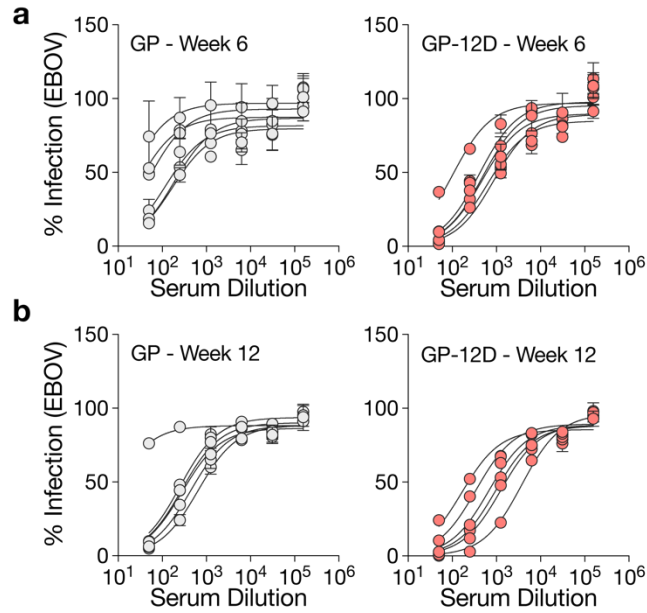


Vaccine design via antigen reorientation

In the format provided by the
authors and unedited

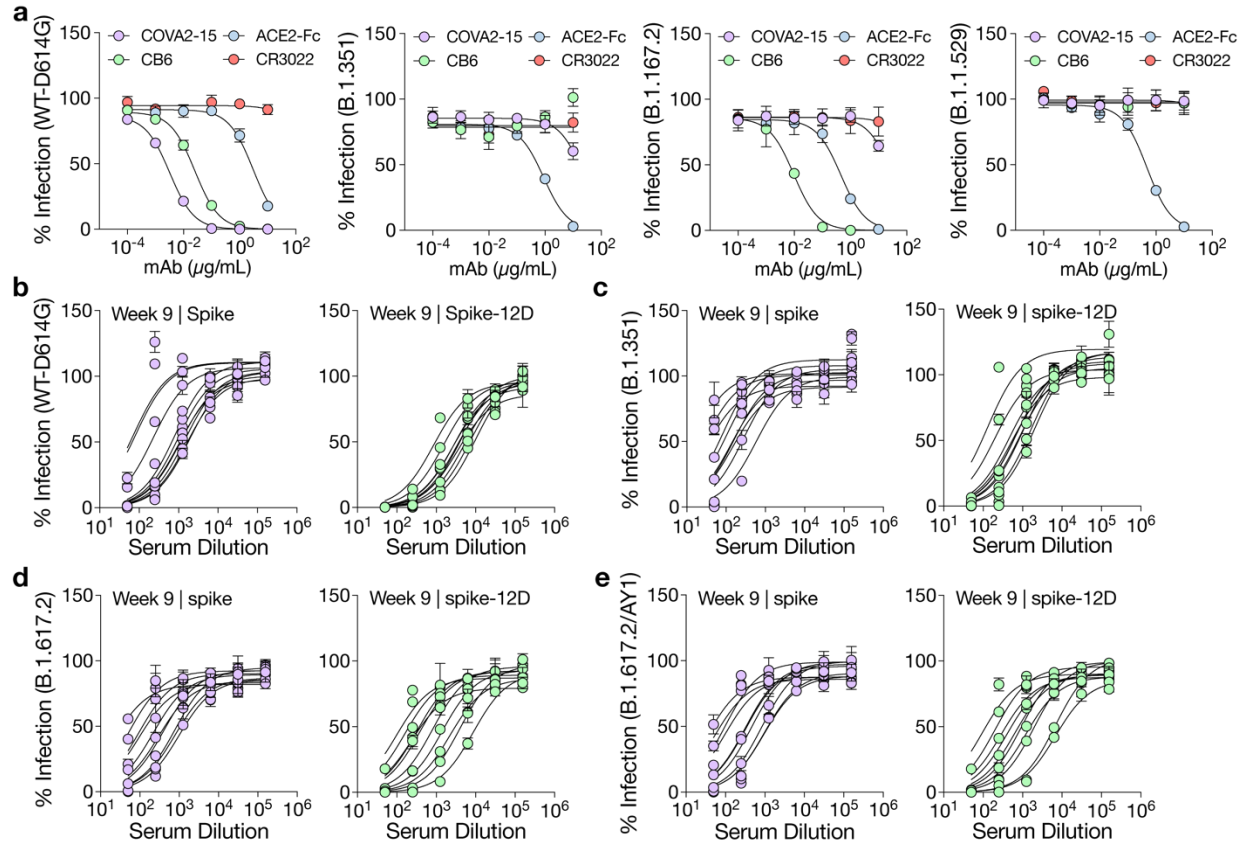


Supplementary Fig. 1 | Antigenicity of oligoD-modified GP. BLI binding profiles of wild-type or oligoD-modified GP with five GP-specific mAbs (mAb114, c13C6, ADI-15742, KZ52 and ADI-16061). An HA-specific mAb (MEDI8852) served as a negative control. Vertical dashed lines indicate the beginning of dissociation.



Supplementary Fig. 2 | Analysis of serological responses against GP or GP-12D.

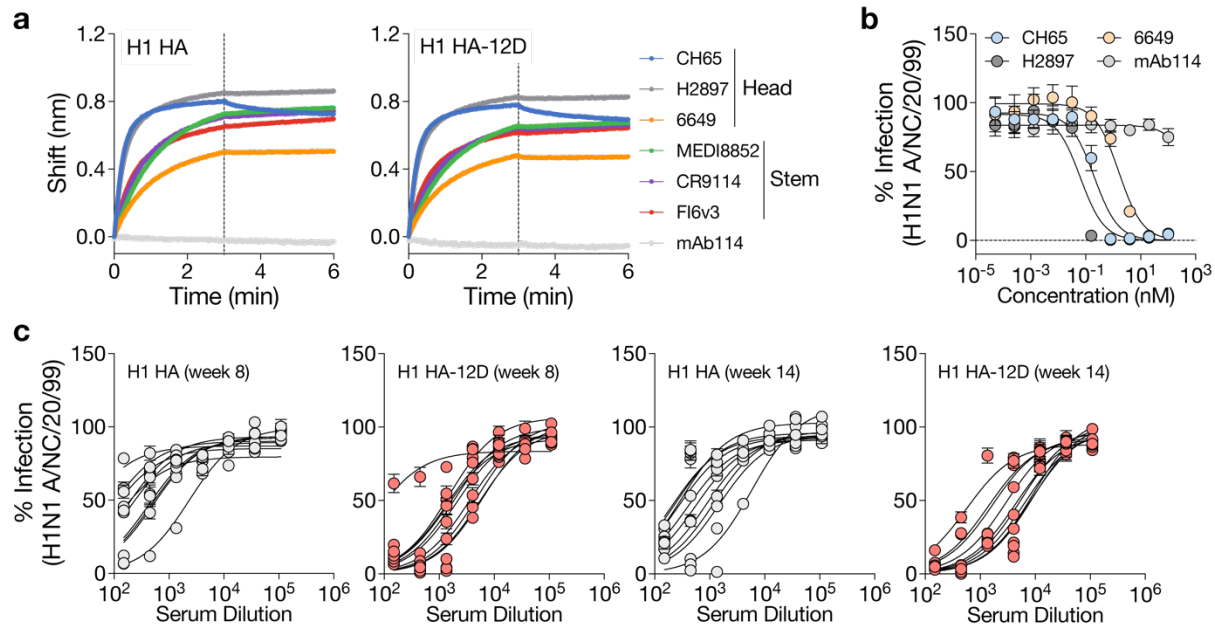
Neutralization of Ebola GP-pseudotyped lentiviruses by antisera collected at six (a) or 12 weeks (b) post-immunization ($n=6$ mice per group). Each curve is derived from a single mouse. Data are presented as mean $\pm$ s.d. of technical duplicates.



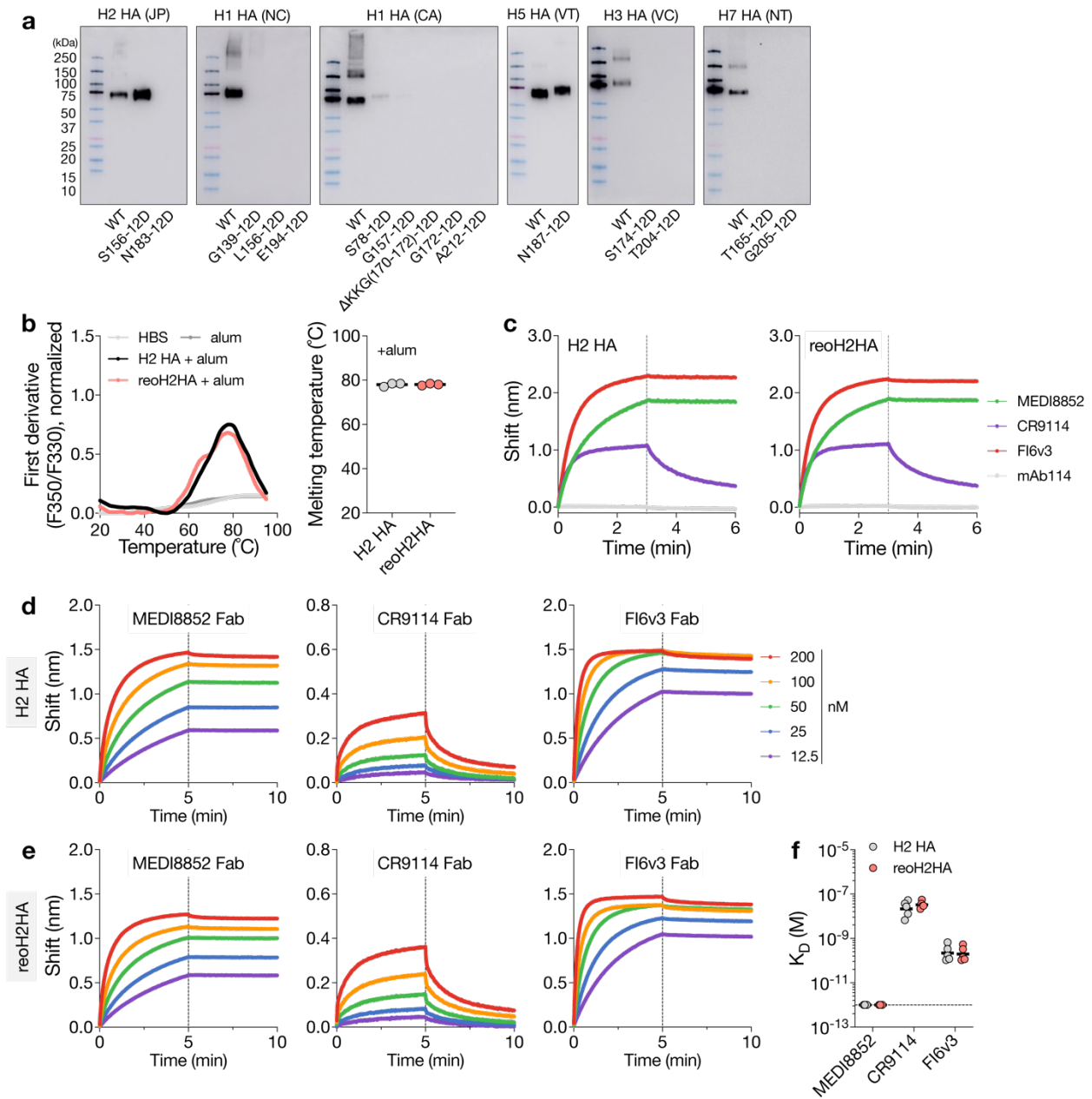
Supplementary Fig. 3 | Analysis of serological responses against spike or spike-12D. a,

Validation of the neutralization assay with ACE2-Fc and three Spike-specific mAbs (COVA-215, CB6 and CR3022) against spike-pseudotyped lentiviruses and three variants of concerns (B.1.351, B.1.167.2 and B.1.1.529). Data are presented as mean $\pm$ s.d. ($n=4$ technical replicates).

b-e, Serum neutralization of wild-type SARS-CoV-2 pseudoviruses (**b**) and variants of concern, including B.1.351 (**c**), B.1.617.2 (**d**) and B.1.617.2/AY1 (**e**) ($n=10$ mice per group). Data are presented as mean $\pm$ s.d. of technical duplicates in neutralization curves.

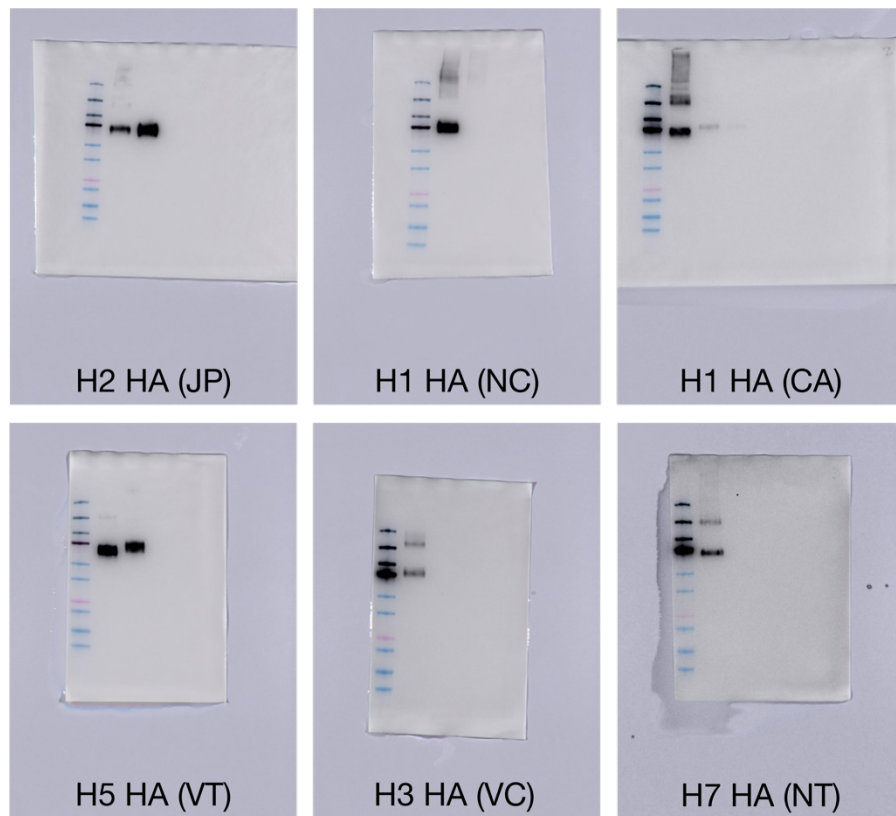


Supplementary Fig. 4 | Antigenicity of and serological analysis against H1 HA and H1 HA-12D. **a**, BLI binding profiles of wild-type or oligoD-modified H1 HA with three HA-head-directed mAbs (CH65, H2897 and 6649) and three HA-stem-directed mAbs (MEDI8852, CR9114 and FI6v3). A GP-specific mAb (mAb114) served as a negative control. Vertical dashed lines indicate the beginning of dissociation. **b**, Validation of the Influenza A virus (IAV) microneutralization assay with three H1 HA-specific mAbs (CH65, H2897 and 6649) against authentic A/New Caledonia/20/1999 (H1N1 A/NC/20/99) viruses. The dashed line indicates 100% viral neutralization by mAbs. Data are presented as mean $\pm$ s.d. ($n=4$ technical replicates). **c**, Neutralization of H1N1 A/NC/20/99 viruses by antisera collected at weeks eight or 14 post-immunization ($n=10$ mice per group). Each curve is derived from a single mouse. Data are presented as mean $\pm$ s.d. of technical duplicates in neutralization curves.

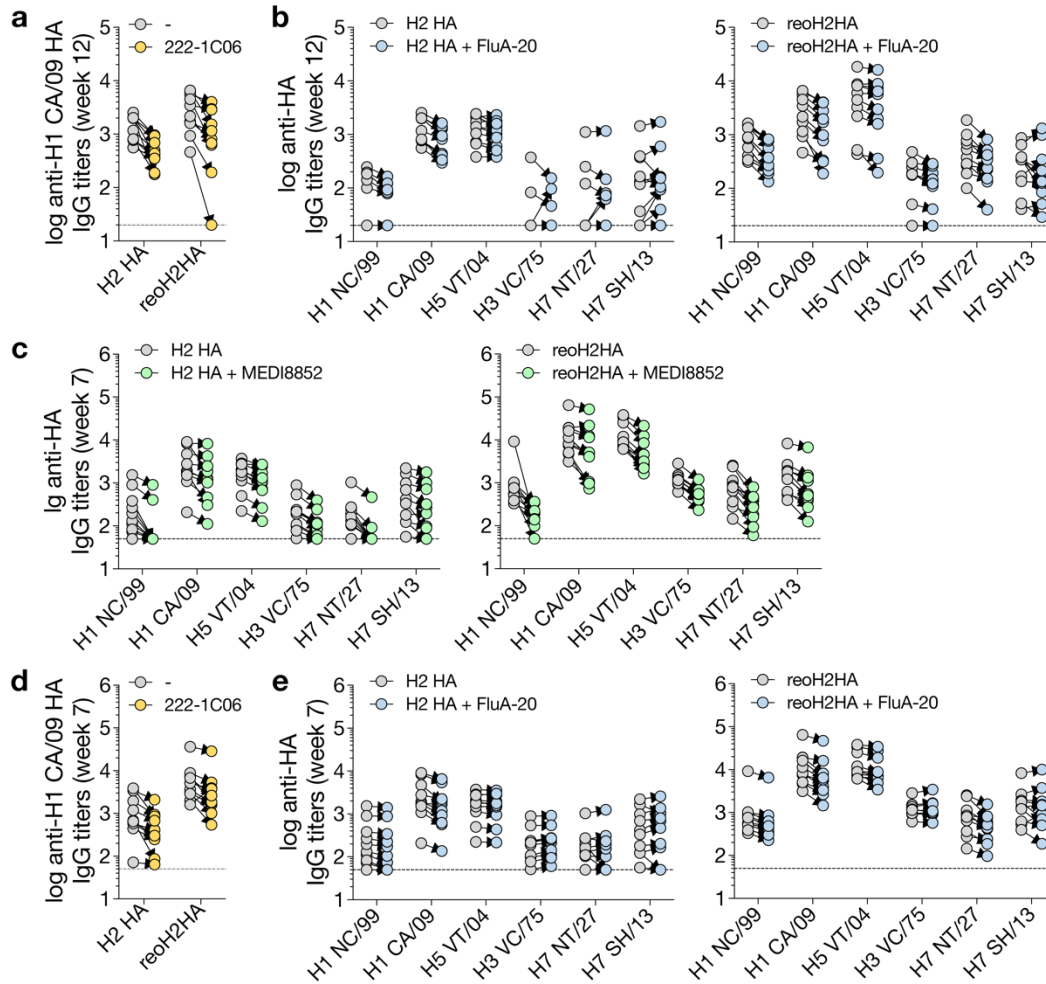


Supplementary Fig. 5 | OligoD insertion into HA-head. a, Screening of oligoD insertion to the head region of different HAs by Western-blot analysis. Blots were detected by a mouse anti-His Tag antibody. HA sequences were from H1 HA (NC – A/New Caledonia/20/99), H1 HA (CA – A/California/7/09), H2 HA (JP – A/Japan/305/1957), H5 HA (VT – A/Vietnam/1203/2004), H3 HA (VC – A/Victoria/3/1975) or H7 HA (NT – A/FPV/Dutch/1927). **b**, Thermal melting profiles and T_m of H2 HA and reoH2HA in the presence of alum (HA: alum = 1: 10, w/w). Data are

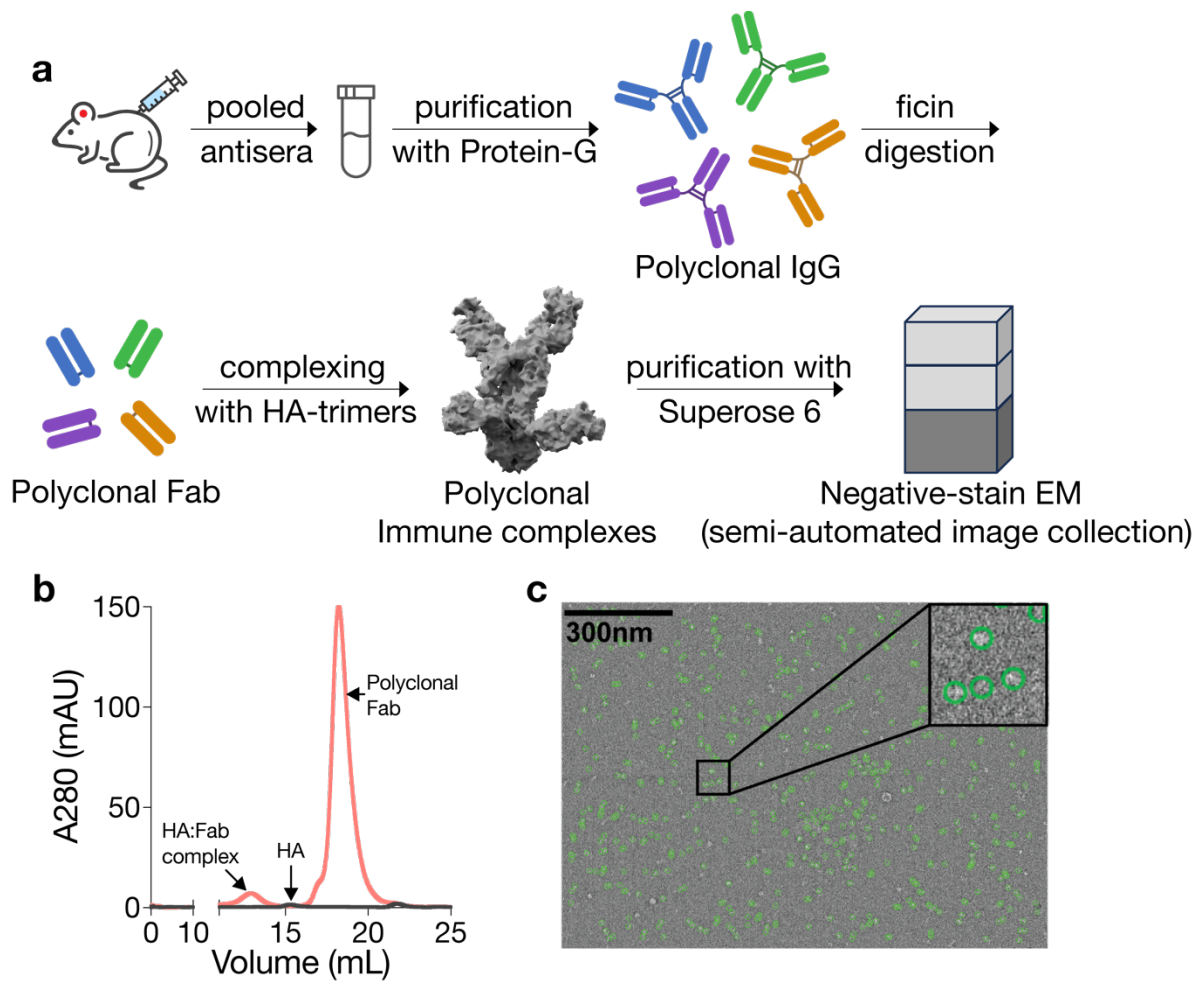
presented as mean $\pm$ s.d. in the dot plots ($n=3$ samples per group). **c**, BLI binding profiles of H2 HA or reoH2HA with three HA-stem-directed mAbs (MEDI8852, CR9114 and FI6v3). A GP-specific mAb (mAb114) served as a negative control. Vertical dashed lines indicate the beginning of dissociation. **d,e**, BLI binding profiles of H2 HA (**d**) or reoH2HA (**e**) with antigen-binding fragments (Fabs) of MEDI8852, CR9114 or FI6v3. Dashed lines indicate the beginning of dissociation. **f**, Binding affinities (K_D) of stem-directed antibodies to H2 HA or reoH2HA calculated based on individual curves in **d,e**. Data are presented as the geometric mean of the $\log_{10}$ -transformed K_D values. The dashed line indicates the limit of quantification (10^{-12} M $^{-1}$).



Source data for Supplementary Fig. 5a.



Supplementary Fig. 6 | Competition ELISA. **a,b**, Analysis with week-12 antisera from the immunization study in **Fig. 5e**. Serum binding titers to H1 HA (CA/09) in the presence of 222-1C06 (**a**), or to different group 1 and group 2 HAs in the presence of FluA-20 (**b**). Each circle represents a single mouse ($n=10$). **c-e**, Analysis with week-7 antisera from the immunization study in **Fig. 6a**. Serum binding titers to group 1 and 2 HAs in the presence of MEDI8852 (**c**), to H1 HA (CA/09) in the presence of a 222-1C06 (**d**), or to different group 1 and group 2 HAs in the presence of FluA-20 (**e**). Dashed lines indicate the limit of quantification. Data are presented as the geometric mean $\pm$ s.d. of the $\log_{10}$ -transformed values.



Supplementary Fig. 7 | Negative-stain electron microscopy polyclonal epitope mapping

(nsEMPEM). **a**, Workflow of nsEMPEM. Polyclonal IgGs were purified from pooled mouse antisera followed by ficin digestion to antigen-binding fragments (Fabs). Resulting Fabs were complexed with different HA-trimers and purified with size-exclusion chromatography.

Polyclonal immune complexes were then imaged in a semi-automated fashion by negative-stain electron microscopy. **b**, Purification of polyclonal immune complexes by size-exclusion chromatography.

c, A representative nsEM micrograph. Singles particles are highlighted in the top right box. Scale bar, 300 nm.

Supplementary Table 1 | Molecular weight calculation from SEC-MALS analysis.

Proteins	Theoretical Mw* ($\times 10^5$ Da)	Mw from SEC-MALS ($\times 10^5$ Da)
GP	1.7	$2.0 \pm (9.0\%)$
GP-12D	1.8	$2.1 \pm (7.6\%)$
spike	4.0	$4.1 \pm (9.4\%)$
spike-12D	4.0	$4.4 \pm (9.2\%)$
H1 HA	1.9	$1.9 \pm (7.7\%)$
H1 HA-12D	1.9	$1.9 \pm (9.5\%)$
H2 HA	1.9	$1.9 \pm (10.4\%)$
reoH2HA	1.9	$2.0 \pm (7.6\%)$
*Glycosylation was not accounted for in theoretical Mw calculation. Mw is calculated by the ASTRA software and reported as Mw $\pm$ (% s.d.).		

Supplementary Notes | Sequences of antigens

- All sequences are from N-terminus to C-terminus
- Signal peptides on the N-terminus are marked in grey

>GP

¹MGVTGILQLPRDRFKRTSFFLWVILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTN
QLRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNVEAGEWAENCYNLEIKKPDG
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LTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLT
RKIRSEELSFAGLITGGRRTRREAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGPAA
EGYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGG

TCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQWIPAGI-
MKQIEDKIEEILSKIYHIENEIARIKKLIGEVASSS-GLNDIFEAQKIEWHE-AHHHHHHHG

>GP-2D

¹MGVTGILQLPRDRFKRTSFFLWVILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTN
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TCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQWIPAGI-
MKQIEDKIEEILSKIYHIENEIARIKKLIGEVASSS-GLNDIFEAQKIEWHE-AHHHHHHHG-

GSDD

>GP-4D

¹MGVTGILQLPRDRFKRTSFFLWVILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTN
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GSDDDD

>GP-8D

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GSDDDDDDDD

>GP-12D

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MKQIEDKIEEILSKIYHIENEIARIKKLIGEVASSS-GLNDIFEAQKIEWHE-AHHHHHHG-
GSDDDDDDDDDDDD

>GP-R200-8D

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>GP-T294-8D

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>GP-T294-12D

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>GP-A309-8D

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>spike

¹MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
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AHHHHHHG

>spike-12D

¹MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
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AHHHHHHG-**GGSDDDDDDDDDDDDD**

>H1 HA

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>H1 HA-12D

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>H7 HA-12D

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KGKRTVDLGQCGLLTITGPPQCDQFLEFSADLIERREGSDVCFPGKFVNEEALRQILRE
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CFEIFHKCDDDCMASIRNNTYDHSKYREEAMQNRIQIDGS-
GYIPEAPRDGQAYVRKDGEWVLLSTFLGS-GLNDIFE AQKIEWHE-GHHHHHHH-
GSDDDDDDDDDDDDDD

>H2 HA

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GSYNNTSGEQMLIIWGVHHPNDETEQRTLYQNVGTYVSVGTSTLNKRSTPEIATRPKVN
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PLGAINTTLPFHNVHPLTIGECPRYVKSEKLVLATGLRNVPQIESRGLFGAIAAGFIEGGWQ
GMVDGWYGYHHSNDQGS GYAADKESTQKA FDGITNKVNSVIEKMNTQFEAVGKEFSN
LERRLENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRMQLRD
NVKELGNGCFEFYHKCDDECMNSVKNGTYDYPKYEEESKLN RNEGSGYIPEAPRDGQA
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>reoH2HA

MEKIVLLFAIVSLVKS¹RGDQICIGYHANNSTEKVDILERNVTVTTHAKDILEKTHNGKLC
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YLLSSVKHFEKVKILPKDRWTQHHTTTGGSRACAVSGNPSFFRNMVWLTKKGS **DDDDDD**
DDDDDDDYPVAKGSYNNTSGEQMLIIWGVHHPNDETEQRTLYQNVGTYVSVGTSTLN
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