Ebolavirus-pp neutralization assays. Briefly, HEK293 T cells or TZM-bl cells at 1×10^4 were added to each well and the plate was incubated at 37°C for 48 h. After incubation, overlying media was removed, and cells were lysed. The firefly luciferase signal from infected cells was determined using Bright-Glo Luciferase Assay System (Promega) according to the manufacturer's instructions. Data were retrieved from a BioTek microplate reader with Gen 5 software, the average background luminescence from a series of uninfected wells was subtracted from each well, and neutralization curves were generated using GraphPad Prism 8.4.3, in which values from wells were compared against a well containing Ebolavirus-pp only. Lentiviral vectors pseudotyped with the murine leukemia virus (MLV) Env gene, termed MLV-pps, were produced in HEK293 T cells and included in the neutralization assays as a negative control. As non-NAb c13C6 exhibited enhanced MLV-pp infection, the MLV-pp assay was also used to detect the c13C6-like, ADE-causing antibody response in immunized animal samples.

[00131] Bulk sorting of EBOV GPΔmuc-specific mouse B cells: Spleens were harvested from immunized mice 15 days after the last immunization and cell suspension was prepared. Cells were stained as follows: dead cells were excluded by staining with Fixable Aqua Dead Cell Stain kit (Thermo Fisher L34957). Receptors FcγIII (CD16) and FcγII (CD32) were blocked by adding 20 µl of 2.4G2 mAb (BD Pharmigen N553142). Cells were then incubated with 10 µg/ml of biotinylated GPΔmuc, WT or UFOg^2. Briefly, GPΔmuc was generated by biotinylation of the individual Avi-tagged EBOV GPΔmuc using biotin ligase BirA according to the manufacturer's instructions (Avidity LLC). Biotin excess was removed by SEC on a HiLoad Superdex 200 16/600 column (GE Healthcare). In the SEC profile, the Avi-tagged GPΔmuc peak is centered at 14.5 ml, while a broader peak of biotin ligase can be found at 65-70 ml (WT) or 60-65 ml (UFOg^2). Cells and biotinylated proteins were incubated for 5 min at 4 °C, followed by the addition of 2.5 µl of anti-mouse IgG fluorescently labeled with FITC (Jackson ImmunoResearch 115-095-071) and incubated for 15 min at 4 °C. Finally, 5 µl of premium-grade allophycocyanin (APC)-labeled streptavidin were added to the cells and incubated for 15 min at 4 °C. In each step, cells were washed with DPBS and the sorting buffer was 0.5 ml FACS buffer. FITC$^+$ APC$^+$ GPΔmuc-specific B cells were sorted using BD FACSAria II into Eppendorf tube with 500 µl of FACS buffer.

[00132] Next-generation sequencing (NGS) and Bioinformatics Analysis of Mouse B cells: A 5'-rapid amplification of cDNA ends (RACE) protocol has been reported for unbiased sequencing of mouse B cell repertoires. Here, this protocol was applied to bulk-sorted, E2-specific mouse splenic B cells. Briefly, 5′-RACE cDNA was obtained from bulk-sorted splenic B cells of each mouse with SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (TaKaRa). The immunoglobulin PCRs were set up with Platinum *Taq* High-Fidelity DNA Polymerase (Life Technologies) in a total volume of 50 µl, with 5 µl of cDNA as template, 1 µl of 5′-RACE primer, and 1 µl of 10 µM reverse primer. The 5'-RACE primer contained a PGM/S5 P1 adaptor, while the reverse primer contained a PGM/S5 A adaptor. We adapted the mouse 3′-C_γ1-3/3′-C_μ inner primers and 3′-mC_κ outer primer as reverse primers for 5′-RACE PCR processing of heavy and light (κ) chains. A total of 25 cycles of PCR was performed and the expected PCR products (500-600 bp) were gel purified (Qiagen). NGS was performed on the Ion S5 GeneStudio system. Briefly, heavy and light (κ) chain libraries from the same mouse were quantitated using Qubit® 2.0 Fluorometer with Qubit® dsDNA HS Assay Kit, and then mixed using a ratio of 3:1 before being pooled with antibody libraries of other mice at an equal ratio for sequencing. Template preparation and (Ion 530) chip loading were performed on Ion Chef using the Ion 520/530 Ext Kit, followed by sequencing on the Ion S5 system with default settings. The mouse *Antibodyomics* pipeline was used to process the raw data and determine distributions for germline gene usage, somatic hypermutation (SHM), germline divergence, and H/KCDR3 loop length.

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[00133] The invention thus has been disclosed broadly and illustrated in reference to representative embodiments described above. It is understood that various modifications can be made to the present invention without departing from the spirit and scope thereof.

[00134] It is further noted that all publications, sequence accession numbers, patents and patent applications cited herein are hereby expressly incorporated by reference in their entirety and for all purposes as if each is individually so denoted. Definitions that are contained in text incorporated by reference are excluded to the extent that they contradict definitions in this disclosure.

WHAT IS CLAIMED IS:

1. An engineered Ebolavirus glycoprotein (GP) protein, comprising (a) substitution of residue W615 in heptad repeat 2 (HR2) with residue L, A, V, I, or F~~a smaller hydrophobic residue~~, and (b) one or more proline substitutions in heptad repeat 1 C segment ($HR1_C$); wherein the amino acid numbering is based on *Zaire* Ebolavirus GP sequence with UniProt ID Q05320 (SEQ ID NO:1).

2. The engineered Ebolavirus GP protein of claim 1, wherein the Ebolavirus is *Zaire Ebolavirus* (EBOV), *Sudan virus* (SUDV), *Taï forest virus* (TAFV), *Bundibugyo virus* (BDBV), or *Reston Ebolavirus* (RESTV).

~~**3.** The engineered Ebolavirus GP protein of claim 1, wherein residue W615 is replaced with L, A, V, I or F.~~

~~4.~~3. The engineered Ebolavirus GP protein of claim 1, wherein the proline substitutions comprise T577P or L579P.

~~5.~~4. The engineered Ebolavirus GP protein of claim 1, further comprising a truncation at the C-terminus of the membrane proximal external region (MPER).

~~6.~~5. The engineered Ebolavirus GP protein of claim ~~5~~4, further comprising (a) a deletion of the mucin-like domain (MLD) deleted from the GP1 subunit, and/or (b) a deletion of MPER from the GP2 subunit.

~~7.~~6. An engineered Ebolavirus glycoprotein (GP) protein, comprising:
(a) a truncated soluble GP of an Ebolavirus that has the mucin-like domain (MLD) deleted from the GP1 subunit and the membrane proximal external region (MPER) deleted from the GP2 subunit, and
(b) substitution of residue W615 in heptad repeat 2 (HR2) with residue L, A, V, I or F~~a smaller hydrophobic residue~~; wherein the amino acid numbering is based on *Zaire* Ebolavirus GP sequence corresponding to UniProt ID Q05320 (SEQ ID NO:1).

~~8.~~7. The engineered Ebolavirus GP protein of claim ~~7~~6, further comprising one or more modifications selected from (i) an extension of HR2 at the C terminus, (ii) one or more proline substitutions in heptad repeat 1 C segment ($HR1_C$), and (iii) one or more engineered inter-GP disulfide bonds.

~~9. The engineered Ebolavirus GP protein of claim 7, wherein the Ebolavirus is *Zaire Ebolavirus;* (EBOV), *Sudan virus* (SUDV), *Taï forest virus* (TAFV), *Bundibugyo virus* (BDBV), or *Reston Ebolavirus* (RESTV).~~

~~10.~~8. The engineered Ebolavirus GP protein of claim ~~7~~6, wherein the truncated soluble GP comprises SEQ ID NO:2 or SEQ ID NO:3, or a ~~conservatively modified or substantially identical variant thereof~~variant thereof with at least 99% sequence identity.

~~11. The engineered Ebolavirus GP protein of claim 7, further comprising a N-terminal leader sequence.~~

~~12. The engineered Ebolavirus GP protein of claim 11, wherein the leader sequence comprises SEQ ID NO:41 or a conservatively modified variant.~~

~~13. The engineered Ebolavirus GP protein of claim 7, wherein W615 is replaced with L, A, V, I or F.~~

~~14.~~9. The engineered Ebolavirus GP protein of claim ~~7~~6, comprising the sequence SEQ ID NO:4, ~~a conservatively modified variant or a substantially identical sequence thereof~~or a variant thereof with at least 99% sequence identity.

~~15.~~10. The engineered Ebolavirus GP protein of claim ~~7~~6, further comprising an extension of HR2 at the C-terminus.

~~16.~~11. The engineered Ebolavirus GP protein of claim ~~15~~10, wherein the HR2 extension comprises (a) extending HR2 C terminus with a N-terminal fragment of the adjacent membrane proximal external region (MPER) of the Ebolavirus glycoprotein or (b) replacing a HR2 C-terminal fragment with a longer leucine zipper motif.

~~17.~~12. The engineered Ebolavirus GP protein of claim ~~15~~10, wherein the HR2 extension comprises (a) extending the HR2 C terminus from residue 632 to residue 637 in MPER ~~(addition of residues KTLPD (SEQID NO:32))~~, (b) extending the HR2 C terminus from residue 632 to residues 643 in MPER ~~(addition of residues KTLPDQGDNDN (SEQ ID NO:33))~~, or (c) replacing residues 617-632 with a GCN4 leucine zipper sequence shown in SEQ ID NO:34~~)~~.

~~18.~~13. The engineered Ebolavirus GP protein of claim ~~15~~10, wherein the W615 substitution comprises W615L substitution or P612G/W615F double mutation.

~~19.~~14. The engineered Ebolavirus GP protein of claim ~~15~~10, comprising (a) W615L substitution and (b) extension of the HR2 C terminus from residue 632 to residue 637 in MPER of the Ebolavirus GP.

~~20.~~15. The engineered Ebolavirus GP protein of claim ~~18~~10, comprising (a) P612G/W615F double mutation in HR2 and (b) replacement of residues 617-632 with a GCN4 leucine zipper sequence shown in SEQ ID NO:34.

~~21.~~16. The engineered Ebolavirus GP protein of claim ~~15~~10, comprising an amino acid sequence as set forth in any one of SEQ ID NOs:5-8, ~~a conservatively modified variant or a substantially identical sequence thereof~~or a variant thereof with at least 99% sequence identity.

~~22.~~17. The engineered Ebolavirus GP protein of claim 10~~15~~, further comprising a C-terminal trimerization motif.

~~23.~~18. The engineered Ebolavirus GP protein of claim ~~22~~17, wherein the C-terminal trimerization motif comprises SEQ ID NO:29 or SEQ ID NO:30, or a variant thereof with at least 99% sequence identity ~~conservatively modified variant or a substantially identical sequence thereof~~.

~~24.~~19. The engineered Ebolavirus GP protein of claim ~~7~~6, further comprising one or more proline substitutions in the $HR1_C$ segment.

~~25. The engineered Ebolavirus GP protein of claim 24, wherein the proline substitution comprises T577P or L579P.~~

~~26. The engineered Ebolavirus GP protein claim 24, wherein W615 is replaced with L, A, V, I or F.~~

~~27.~~20. The engineered Ebolavirus GP protein of claim 19~~24~~, comprising an amino acid sequence as set forth in any one of SEQ ID NOs:9-17~~16~~ and 20, or a variant thereof with at least 99% sequence identity~~a conservatively modified variant or a substantially identical sequence thereof~~.

21. The engineered Ebolavirus GP protein of claim 19, comprising (a) W615L substitution, (b) proline substitution T577P or L579P, and (c) HR2 extension from residue 632 to residue 637 in MPER.

~~28.~~**22.** The engineered Ebolavirus GP protein of claim 19~~24~~, further comprising an extension of the HR2 C terminus.

~~29. The engineered Ebolavirus GP protein of claim 28, wherein the HR2 C terminus extension comprises extension from residue 632 to residue 637 in MPER.~~

~~30. The engineered Ebolavirus GP protein of claim 28, comprising an amino acid sequence as set forth in any one of SEQ ID NOs:17 and 20, a conservatively modified variant or a substantially identical sequence thereof.~~

~~31. The engineered Ebolavirus GP protein of claim 28, further comprising a C-terminal trimerization motif.~~

~~32. The engineered Ebolavirus GP protein of claim 31, wherein the C-terminal trimerization motif comprises SEQ ID NO:29 or SEQ ID NO:30, or a conservatively modified variant or a substantially identical sequence thereof.~~

~~33. The engineered Ebolavirus GP protein of claim 32, comprising (a) W615L substitution, (b) proline substitution T577P or L579P, and (c) HR2 extension from residue 632 to residue 637 in MPER.~~

~~34. The engineered Ebolavirus GP protein of claim 31, comprising an amino acid sequence as set forth in any one of SEQ ID NOs:18, 19, 21 and 22, a conservatively modified variant or a substantially identical sequence thereof.~~

~~35.~~**23.** The engineered Ebolavirus GP protein of claim ~~7~~6, further comprising an engineered disulfide bond between two neighboring GP protomers.

~~36.~~**24.** The engineered Ebolavirus GP protein of claim 23~~35~~, wherein the engineered disulfide bond is engineered between residues G91/A575 (SS2), F153/Y534 (SS1), T520/A575 (SS3), G157/I532 (SS4), D522/A575 (SS5) or K56/G599 (SS6).

~~37. The engineered Ebolavirus GP protein of claim 35, comprising an amino acid sequence as set forth in any one of SEQ ID NOs:23-28, a conservatively modified variant or a substantially identical sequence thereof.~~

~~38. A polynucleotide encoding the engineered Ebolavirus GP protein of claim 1 or claim 7.~~

~~39. A polynucleotide of claim 38, wherein the engineered Ebolavirus GP protein further comprises at the N-terminus a leader peptide sequence.~~

~~40.~~25. A pharmaceutical composition, comprising the engineered Ebolavirus GP protein of claim ~~6~~7, and a pharmaceutically acceptable carrier.

~~41.~~26. A vaccine composition, comprising an engineered Ebolavirus GP protein of claim ~~6~~7 that is displayed on the surface of a self-assembling nanoparticle.

~~42. The vaccine composition of claim 41, wherein C-terminus of the engineered Ebolavirus GP protein of claim 7 is fused to the N-terminus of subunit of the self-assembling nanoparticle.~~

~~43. The vaccine composition of claim 42, wherein C-terminus of the engineered Ebolavirus GP protein is fused to the subunit of the self-assembling nanoparticle via a linker sequence GGGGS (SEQ ID NO:43) or $(GGGGS)_2$ (SEQ ID NO:42).~~

~~44. The vaccine composition of claim 41, wherein the engineered Ebolavirus GP protein of claim 7 comprises an amino acid sequence as set forth in any one of SEQ ID NOs:4-28, a conservatively modified variant or a substantially identical sequence thereof.~~

~~45. The vaccine composition of claim 41, wherein subunit of the self-assembling nanoparticle comprises the polypeptide as shown in SEQ ID NO:36 (E2p), SEQ ID NO:37 (I3-01v9), or SEQ ID NO:38 (ferritin), a conservatively modified variant or a substantially identical sequence thereof.~~

~~46. The vaccine composition of claim 41, further comprising a locking domain (LD) that is fused to the C-terminus of the NP subunit.~~

~~47. The vaccine composition of claim 46, wherein the LD comprises SEQ ID NO:39 or 40, a conservatively modified variant or a substantially identical sequence thereof.~~

~~48. The vaccine composition of claim 46, further comprising a T-cell epitope that is fused to the C-terminus of the locking domain.~~

~~49. The vaccine composition of claim 48, wherein the T-cell epitope comprises SEQ ID NO:31, a conservatively modified variant or a substantially identical sequence thereof.~~

~~50.~~27. The vaccine composition of claim 26~~41~~, comprising (1) a polypeptide sequence containing from N terminus to C terminus (a) the engineered Ebolavirus GP protein, linker sequence G_4S (SEQ ID NO:43), nanoparticle sequence ferritin, (b) the engineered Ebolavirus GP protein, linker sequence G_4S (SEQ ID NO:43), nanoparticle sequence E2p, or (c) the engineered Ebolavirus GP protein, linker sequence $(G_4S)_2$ (SEQ ID NO:42), nanoparticle sequence I3-01v9; ~~or (2) a conservatively modified variant of the polypeptide sequence;~~ wherein the engineered Ebolavirus GP protein comprises W615L substitution, proline substitution T577P, and HR2 extension from residue 632 to residue 637 in MPER.

~~51.~~28. The vaccine composition of claim 27~~50~~, further comprising a locking domain and/or a T-cell epitope that is fused to the C-terminus of the nanoparticle subunit sequence.

~~52.~~29. The vaccine composition of claim 28~~51~~, comprising (1) a polypeptide sequence containing from N-terminus to C-terminus (a) the engineered Ebolavirus GP protein shown in SEQ ID NO:17, nanoparticle subunit sequence shown in SEQ ID NO:36 (E2p), locking domain shown in SEQ ID NO:39 (LD4), and T cell epitope shown in SEQ ID NO:31 (PADRE) or (b) the engineered Ebolavirus GP protein shown in SEQ ID NO:17, nanoparticle sequence shown in SEQ ID NO:37 (I3-01v9), locking domain shown in SEQ ID NO:40 (LD7), or (2) a ~~conservatively modified variant of the polypeptide sequence~~a variant of polypeptide sequence with at least 99% sequence identity.

30. A polynucleotide encoding the engineered Ebolavirus GP protein of claim 1 or claim 6.

31. A polynucleotide encoding the vaccine composition of claim 26.

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