Supplementary Information to:

Glycan shield of the ebolavirus envelope glycoprotein GP

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Supplementary Figures S1-S5

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EBOV-GP Q05320 BDBV-GP A0A510C2V9 TAFV-GP Q66810	MGVTGLLQLPRDFKRTSFFLWVIILFQRTFSIPLGVIHNTLQVSDUDKLVCRDKLSSTNQLRSVGLNLEONGVATDVPSATKRWGFRSGVPFKVVNYEAGEWAENCYNLDIKKADGSECLPAAPDGIRGFPRCKVYHKV WTSGLLQLPREKFRKTSFFUWVIILFHKVFSIPLGVVHNNTLQVSDIDKLVCRDKLSSTSQLKSVGLNLEONGVATDVPTAKKRWGFRSGVPFKVVNYEAGEWAENCYNLDIKKADGSECLPEAPEGVRGFPRCRVYHKV MGASGLLQLPREKFRKTSFFVWVIILFHKVFSIPLGVVHNNTLQVSDIDKFVCRDKLSSTSQLKSVGLNLEONGVATDVPTAKKRWGFRSGVPFKVVNYEAGEWAENCYNLDIKKADGSECLPEAPEGVRGFPRCRVYHKV
BOMV-GP Ã0A4D5SG72 RESTV-GP Q66799 GUDU GD Q66794	MILQVPEKRHQRTVLFIWLVILFQRAVSVPLGVIHNSTLQVSDIDKLVCHDKLTSTNQLRSIGLNLEGNGIATDVPSATKRWGFRAGVPFKVVGYEAGEWAENCYNLEIKKPDGSECLPMAPEGIRGFPRCRYVHKV MGSGVQLLQLPREFFRKTSFLVWVIILFQRAISWPLGIVTNSTLKATEIDQLVCRDKLSSTSDIKSVGINLEGNGIATDVPSATKRWGFRAGVPFKVVSYEAGEWAENCYNLEIKKSDGSECLPLPPDGVRGFPRCRYVHKV
SODV-GP 000014 LLOV-GP G8EFI4+G8EFI5 MARV-GP A0A0E3N843	MVPTYPYSSLLDWRPPPNTLPWILNLVVFYTIAWLPGGVSGIPLGLLGNNSITQUVDNVVCKEHLATTDOLQAIGLGLEGLGEHADLPTATKRWGFRSDVIPKIVGYTAGEWVENCYNLEITKKDGPPCLPSPPTGLLGYPRCKVHRA MVPTYPYSSLLDWRPPPNTLPWILNLVVFYTIAWLPGGVSGIPLGLLGNNSITQUVDNVVCKEHLATTDOLQAIGLGLEGLGEHADLPTATKRWGFRSDVIPKIVGYTAGEWVENCYNLEITKKDGPPC MKTTCLLISLILIQGVKTLPILEIASNIQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKVADSPLEASKRWAFRAGVPPKNVEYTEGEEAKTCYNISVTDPSGKSLLDPPTNIRDYPKCKTIHHI
EBOV-GP Q05320	* *:* *:**** :*:* *:* :*: *:* :*: *:*: *:*: *:*: *:*: *:*: *:*: *:*: *:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:* SGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATE-DPSSGYYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIG
BDBV-GP A0A510C2V9 TAFV-GP Q66810 BOMV-GP A0A4D5SG72	SGTG9CPEGYAFHKEGAFFLYDRLASTIIYRSTTFSEGVVAFLILPETKKDFFQSPPLHEPANMTT-DPSSYYHTVTLNYVDNFGTNMTNFLFQVDHLTYVQLERFTPQFLVULNETIYTNGRRSNTTGFLIWKVNPTVDTGVG SGTG9CPGGLAFHKEGAFFLYDRLASTIIYRGTFAEGVVAFLLPKARKDFFQSPPLHEPANMTT-DPSSYYHTVTINYVVDNFGTNTTEFLFQVDHLTYVQLEARFTPQFLVLLNETIYSDTGKLIWKVNPTVDTSMGF SGTGSCESGFAFHKEGAFFLYDRLASTIIYRGTFAEGVVAFILLPKAEKNFLQPPLGQFTNTTN-DPSSNYHSTLEVETTRFGTRRSAFWFKVDLTFVVDLESRFTPQFLVELNETIYIEGKRSNTTGKLIWKVNPTVDTGVG
RESTV-GP Q66799 SUDV-GP Q66814	QGTGPCPGDLAFHKNGAFFLYDRLASTVIYRGTTFAEGVVAFLILSEPKKHFWKATPAHEPVNTTD-DSTSYYMTLTLSYEMSNFGGNESNTLFKVDNHTYVQLDRPHTPOPLVQLNETLRRNNRLSNSTGRLTWTLDPKIEPDVGF QGTGPCFGDVAFHKDGAFFLYDRLASTVIYRGVNFAEGVIAFLILAKPKETFLQSPPIREAANYTE-NTSSYYATSYLEVEIENFGAQHSTTFKINNNTFVLLDRPHTP QFLFQLNDTIQLHQCLSNTTGKLIWTLDANINADIG
LLOV-GP G8EF14+G8EF15 MARV-GP A0A0E3N843	KUAGPEPEGENAFHERESFLIHEGASTVIIHEVIFEGIIAFLIVEADAYKLKAGLGIGFSHQAENUNPNNOFKITILDIDVNSPWNDNAFFFFKAREDTBALTUKIPPANLELVQEKLANLIGDQADPSKREIVAEVITLELG QGQNPHAQGIALHLWGAFFLYDRIASTTMYRGKVFTEGNIAAMIVNKTVHKMIFSRQGQQIRHMNLSSTNKYWTSENGTOTNDTGCFGTLQEYNSTKNQTCAPSKREPLPLPTAHPEVKLTSTSTDATKLNTTDPNSDDEDLTTSGSGSGG
EBOV-GP Q05320	:
TAFV-GP Q66810 BOMV-GP A0A4D5SG72	WAPWENKKNFUKTLSSEELSFVPVPETQNQVLDTTATVSPFISANNAGTDHKELVSEDSTPVVQMQNIKGKDIMPTTVEGVPTT
RESTV-GP Q66799 SUDV-GP Q66814 LLOV-GP G8FFT4 + G8FFT5	WAFWETKKNFSQQLHGENLHFQLLSTHTNNSSDQSPAGTVQGKISYHPPANNSELVPTDSPPVVSVLTAGRTEEMSTQGLTNGETITGFTANDMTTTIAPSPTMTS WAFWENKKNLSEQLRGEELSFETLSLNSTEDDDATSSRTTKGRISDRAT
MARV-GP A0A0E3N843	QEPUT SDAATKQGLESTMPPTPSPQPSTPQQGGNNTNHSQGVVTEPGKINTAQPSMPPHNTTISTNNTSKHNLSTPSVPIQNATNYNTGSTAPENEQTSAPSKTILLPTENPTTAK
EBOV-GP Q05320	*::* *: * *::***:** ATQVEQHHRRTDND ST ASDTPS-ATTAAGPPKAENTNTSKSTDFLDPATTSPQNHSETAG-NNNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAOPKCNPNLHYWTTODEGAAIGLAWIPYFGF
BDBV-GP A0A510C2V9 TAFV-GP Q66810 DOWN GD A0A4D55C72	ERNNTAHPETLANNPPONTTPS-TPPQOGERTSSHTTPSPRPVPTSTIHPTTRETHIPTTMTTSHDTDSNRPNPIDISEPTEPGPLTNTTRGAANLLTGSRRTRREITLRTQAKCNPNLHWWTTQDEGAAIGLAWIPYFGPA EDHSTTQPAKTTSQPTNSTEST-TLNPTSEPSSRCTQPSSPTVPNTTEEHAELGKTPTTLPEQHTAASAIPRAVHPDELSGPGFLTNTIRGVNLLTGSRRKRRDVTPNTQPKCNPNLHWWTALDEGAAIGLAWIPYFGPA GOADDIA DAADDIA
RESTV-GP Q66799 SUDV-GP Q66814	SQAEDITRADDES INSATE - DE VOGITVERVELE DE LI DIFEI QUE CENTA DE LA CARACINA DE LA CARACINA DE LA CARACINA DE LE VDNIVESQENNITASIEDSPFS-ASNETIYHSEMDFIQGSNNSAQSQUETTPAPTSPMTQDPQETANSKPGSAGGSQFGELINTVSKVADSLGPTRKQKRSVRQNTANKCNPDLYYWTAVDEQHNAAGIAWIPYFGP IGTGLSSQILSSSTMAPSPE-DOTSTYTFKL-PVMTTESSTFPNNSPGSTEAPTLITPENITIAVKTVMPQESTSNGLTSSVTGLIGSLGLRKKSRQVNTRATGKCNPNLHYWTADEQHNAAGIAWIPYFGP(
LLOV-GP G8EFI4+G8EFI5 MARV-GP A0A0E3N843	KSATELTPINHSQEIQLASVTNTPTPTTQSKSWTVDYNNTTPTMDPTILLTTPDTATIPDNSSDENATTSKRRRRQVNPVPPTTQQTSTSINTSHHPNMTTQLARHPSVQTRMQNPSCNPNLRYWTSREMSNAGGLAWIPWIGP SINSTKSPTTTVPNTTNKYSTSPSPTPNSTAQHLVYFRKKNILWRGDMFPFLDGLINAPIDFDPVPNTKTIFDESSSSGASAEEDQHASPNISLTLSYFPKVNENTAHSGENENDCDAELRIWSVQEDDLAAGLSWIPFFGPC
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BDBV-GP Q05320 BDBV-GP A0A510C2V9 TAFV-GP 066810	AEGIYIEGIMENQOGJICGLRQLANETTQALQIFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIHDFVDKIFDPDGDDDNWWTGWRQWDPAGIGITGVIAVIALFCICKFVF AEGIYTEGIMENQNGLICGLRQLANETTQALQIFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIHDFIDKPLPDQTDNDNWWTGWRQWVPAGIGITGVIAVIALFCICKFL AEGIYTEGIMENQNGLICGLRQLANETTQALQIFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIHDFIDKPLPDQTDNDNWWTGWRQWVPAGIGITGVIAVIALFCICKFL AEGIYTEGIMENQNGLICGLRQLANETTQALQIFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIHDFIDKPLPDQSDNDNWWTGWRQWVPAGIGITGVIIAVIALFCICKFL
BOMV-GP A0A4D5SG72 RESTV-GP Q66799	AEGIYTEGLMONONELICGLROLANETTOALOLFLRSTTELRTFSILNRKAIDFLLORWGGTCRILGPDCCIEPHDWTKNITDRIDQIIHDFVDKPLPDQSNNDNWWTGWROWIPAGIGVVGVIAAFIALICICKIIC AEGIYIEGVMHNQNGLICGLROLANETTOALOLFLRATTELRTYSLLNRKAIDFLLORWGGTCRILGPSCCIEPHDWTKNITDEINQIKHDFIDNPLPDHGDDLNLWTGWROWIPAGIGIIGVIAAFIALLCICKILC
SUDV-GP Q66814 LLOV-GP G8EFI4+G8EFI5 MARV-GP A0A0E3N843	AEGIYTEGLMINQNALVCGLRQLANETTQALQIFLRATTELRTYTLNRKAIDFLLRRWGGTCRILGPDCCIEPHDWIKNITDKINQIIHDFIDNPLPNQDNDDNWWTGWRQWIPATGASALGIILAIIALLCVCKLLC IEGGITDGIMEHQNTIVCQLRELANTTKALQIFLRATTELRTYSILNRHAIDFLLQRWGGTCRILGPNCCIEPHDWSANITAEINHIREDILNHHELQPSQDFSWWTGWQWIPATGASALGIILAILALLCLCRITR IEGLYTAGLIXNQNNLVCRLRRLANQTASLELLRVTTEERFFSLINRHAIDFLLARWGGTCKVLGPDCCIEPHDWSANITAEINHIREDILNHHELQPSQDFSWWTSDWQVLTNLGILLLSIAVLIALSCICRIFTKWIG

Supplementary Figure S1. Multiple sequence alignment of filovirus GP used for the analyses presented in main text (Figure 1). Corresponding UniProt IDs are provided in the sequence names.



Supplementary Figure S2. Overview of site-specific N-linked glycan processing in ebola virus GP Δ TM from HEK293 and S2 cells, versus full-length GP from HEK293 derived VLPs as determined by LC-MS/MS. The glycans were classified by HexNAc content as truncated, paucimannose, high-mannose, hybrid or complex. Shown is the average of a duplicate experiment.



Supplementary Figure S3. MS/MS spectra of glycopeptides containing sites N257 or N563 of full-length EBOV and BDBV GP from HEK293 derived VLPs with unprocessed high-mannose and hybrid glycans.



Supplementary Figure S4. MS/MS spectra of glycopeptides containing sites O-linked glycosylation outside the MLD and C-linked mannosylation at W288 in full-length EBOV and BDBV GP from HEK293 derived VLPs with unprocessed high-mannose and hybrid glycans.



Supplementary Figure S5. MS/MS spectrum of glycopeptide from HEK293-derived BDBV GP Δ TM with O-linked glycosylation in the Strep-tag of the recombinant soluble ectodomain.