Supplementary Information

Title: A Critical Domain of Ebolavirus Envelope Glycoprotein Determines Glycoform and Infectivity

Authors and Affiliations

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Supplementary Figure Legends

Supplementary Figure 1. Amino acid sequences of ZGP and RGP. Amino acid sequences of ZGP and RGP are shown. Red box: 33-50 amino acid residue, green box: mucin-like domain, orange line: border of GP1 and GP2.

Supplemental Figure 2. MALDI-TOF MS spectra of *N*-glycans released from VLPs. Representative MS spectra of *N*-glycans released from VLPs bearing **(a)** ZGP, **(b)** RGP, **(c)** Z33-50R, **(d)** Z33-186, **(e)** R33-50, or **(f)** R33-186 are shown. Magnified spectra between m/z=3800 and m/z=4200 are shown in the upper right window of each spectrum.

Supplementary Figure 3. Biosynthetic rate and intracellular localization of RGP and ZGP were not different. (a) Results of pulse chase experiments of [^{35}S]-labeled

RGP and [^{35}S]-labeled ZGP are shown. **(b)** Results of immunofluorescent staining of VLP-producing HEK293T cells. Red: GP (ZGP or RGP), Green: Golgi marker (GM130 or TGN46). Representative results are shown.

Supplementary Figure 4. Sialylation and fucosylation of *N*-glycans found in GPs were not different. Relative ratios of sialylated **(a)** or fucosylated **(b)** *N*-glycans are shown.

Supplementary Figure 5. Uncropped images of blots shown in the figures (Fig. 1c, 1d, 2b, 3b, 3d, 5a).

Supplementary Figure 6. GPs that lack 18 amino acid residues (33 through 50) is expressed on the VSV pseudotyped viruses and VLPs.

(a-b) Results of immunoblotting using VSV pseudotyped viruses or VLPs containing ZGPΔ33-50 or RGPΔ33-50 are shown. **(c)** Results of virus titration experiment of VSV pseudotyped ZGP, RGP, ZGPΔ33-50, and RGPΔ33-50 using Vero E6 cells. VSV pseudotyped with ZGP/RGP without 18 amino acids (N-terminal 33rd through 50th) did not show any infectivity to Vero E6 cells. (n=3)

Supplementary Materials and Methods

Pulse chase experiment. HEK293T cells (6×10^5) were seeded on a 6-well culture plate one day before transfection. Cells were transfected with pCAGGS-GP and pCAGGS-VP40 by using Trans-IT LT1 in the same way as in VLP production. The cells were washed with PBS 24 hours after transfection and subsequently cultured with DMEM-HG (10% FCS, without Met and Cys; Gibco) for 1 hour. Then the medium was changed to DMEM-HG (10% FCS, containing 150 μCi/mL of [^{35}S]-labeled Met and Cys) and cultured for 30 min. The medium was changed to DMED-HG (10% FCS) after washing with DMEM-HG (10% FCS) and cultured for 0, 1, 2, or 3 hours. Cells were collected after washing with PBS and solubilized with RIPA buffer containing protease inhibitor cocktail (Calbiochem) by shaking at 4°C for 2 hours. The solution was centrifuged at 21,500*g* for 15 min to remove cellular debris. The supernatant was collected, and the radioactivity of [^{35}S] in the supernatant was measured by a liquid scintillation counter (LS6000SC, BECKMAN). GPs were immunoprecipitated using mAb 42/3.7 from lysates containing equal amounts of [^{35}S] radioactivity. Immunoprecipitated GPs were separated on an 8% SDS-PAGE gel. The gel was then

fixed and stained with CBB (BioRad). The gel was dried (RapiDry mini; ATTO) and [^{35}S]-labeled GPs were detected with Typhoon FLA9000 (GE Healthcare).

Immunofluorescent staining. HEK293T cells (4×10^6) were seeded on a 10 cm culture dish one day before transfection. Cells were transfected with pCAGGS-GP and pCAGGS-VP40 by using Trans-IT LT1 in the same way as in VLP production. 24 hours after transfection, cells were washed with PBS three times and collected. Collected cells were fixed with 20 mM sodium phosphate buffer (pH 7.0) containing 4 (w/v) % paraformaldehyde at 4°C for 1 hour. Fixed cells were washed with 10 mM glycine in PBS, and permeabilized by 0.1 (w/v) % Triton X-100 in PBS (at room temperature for 5 min). The cells were blocked with 4 (w/v) % BSA in PBS at room temperature for 30 min, and incubated with anti-EBOV GP1 mAb 42/3.7 (10 μg/mL) and rabbit anti-GM130 (10 μg/mL; Abcam) or rabbit anti-TGN46 antibody (10 μg/mL; Abcam), followed by Alexa 568 goat anti-mouse IgG(H+L) (10 μg/mL; Molecular Probes) and Alexa 488 goat anti-rabbit IgG(H+L) (10 μg/mL; Molecular Probes) and DAPI (Boehringer Mannheim). Analysis was performed using a Leica TSC SP5 confocal microscope (Leica).

92 **Supplemental Table 1. Primer sequences that used to make chimeric GPs**

Chimeric GPs		Primer Sequences
Z311-462R	forward	CCGCTCGAGGTCTCGATAGAACTGTGAAAGACAACTCTTC
	reverse	CGGAATTCGGTCTCCAACACTCATCACCAAGATACC
Z297-462R	forward	CCGAATTCGGTCTCGAAAAGTTTTTTTTAGTTTCCCAGAAGGC
	reverse	CGGAATTCGGTCTCCAACACTCATCACCAAGATACC
Z260-462R	forward	CCGAATTCGGTCTCCGAAGTGTCTCATTCAGCTGGAGC
	reverse	CGGAATTCGGTCTCCAACACTCATCACCAAGATACC
Z187-462R	forward	CCGAATTCGGTCTCTCTGACAGTATCAGAAATGCAACGAC
	reverse	CGGAATTCGGTCTCCAACACTCATCACCAAGATACC
Z33-462R	forward	CGCGGTCTCGGCATGGAAAATGTTCTTTGGAAAAGG
	reverse	CGGAATTCGGTCTCCAACACTCATCACCAAGATACC
Z1-462R	forward	CCGCTCGAGGTCTCCCCATTGTGTTGTTGGATCCTC
	reverse	CGGAATTCGGTCTCCAACACTCATCACCAAGATACC
Z33-186R	forward	CGCGGTCTCCATGCCGCTTGGTATAGTG
	reverse	CGCGGTCTCCGGGGCAGAATTAAAAAAGCTACGACACC
Z33-50R	forward	CGCGGTCTCCATGCCGCTTGGTATAGTG
	reverse	CGCGGTCTCACTAGTTGATCAATTTCTGTTGCTTTG
R33-186Z	forward	CGCGGTCTCCATCCCACTTGGAGTCATC
	reverse	CGCGGTCTCTCTGACAGTATCAGAAATGCAACGACA
R33-50R	forward	CGCGGTCTCACTAGTTTGTCGTGACAAAC
	reverse	CGCGGTCTCGGCATGGAAAATGTTCTTTGGAAAAGG

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Figure S1

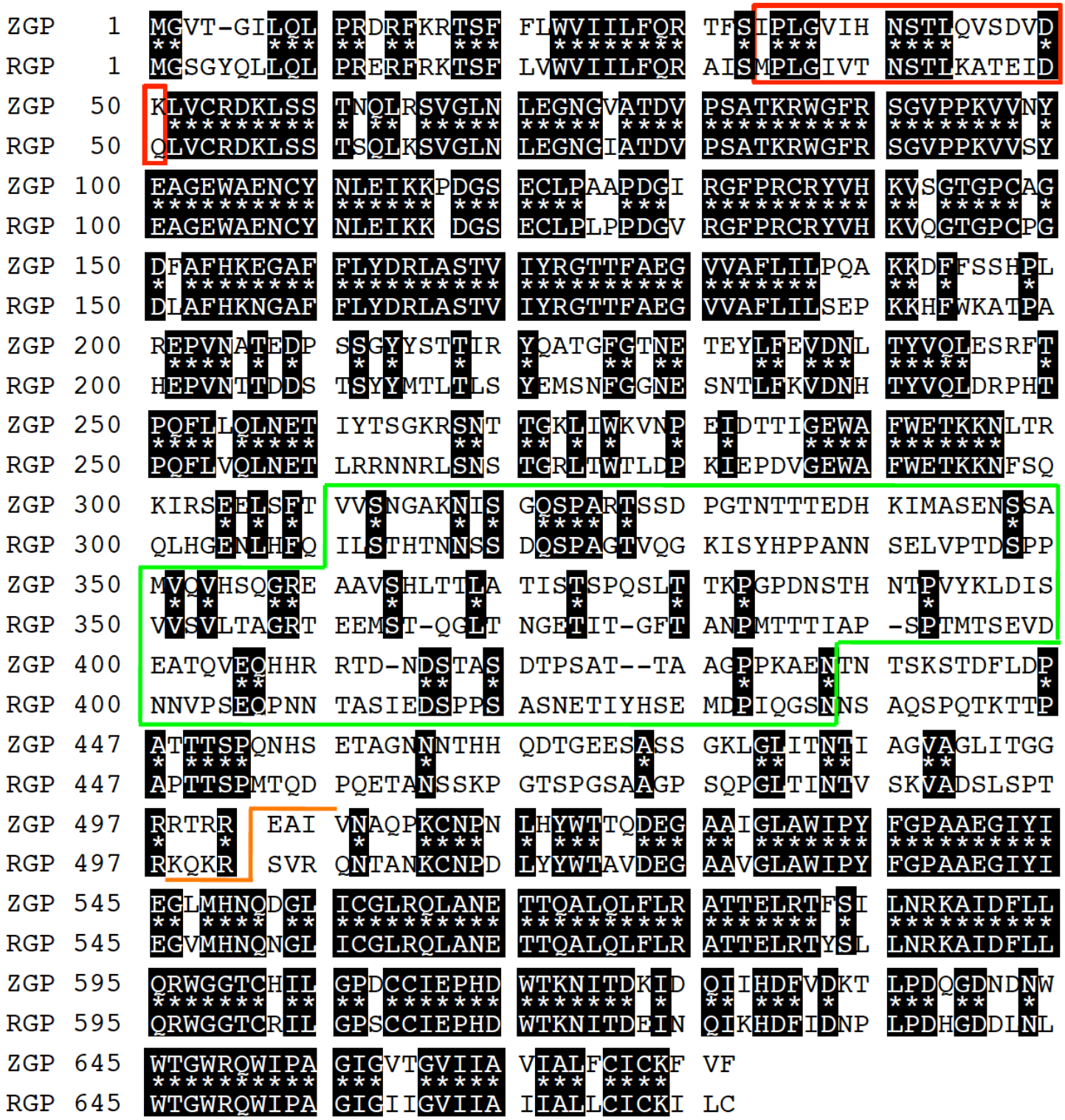

ZGP 1 MGVT-GILQL PRDRFKRTSF FLWVIILFQR TFSIPLGVIH NSTLQVSDVD
RGP 1 MGSGYQLLQL PRERFRKTSF LVWVIILFQR AISMPLGIVT NSTLKATEID
ZGP 50 KLVCRDKLSS TNQLRSVGLN LEGNGVATDV PSATKRWGFR SGVPPKVVNY
RGP 50 QLVCRDKLSS TSQLKSVGLN LEGNGIATDV PSATKRWGFR SGVPPKVVSY
ZGP 100 EAGEWAENCY NLEIKKPDGS ECLPAAPDGI RGFPRCRYVH KVSGTGPCAG
RGP 100 EAGEWAENCY NLEIKK DGS ECLPLPPDGV RGFPRCRYVH KVQGTGPCPG
ZGP 150 DFAFHKEGAF FLYDRLASTV IYRGTTFAEG VVAFLILPQA KKDFFSSHPL
RGP 150 DLAFHKNGAF FLYDRLASTV IYRGTTFAEG VVAFLILSEP KKHFWKATPA
ZGP 200 REPVNATEDP SSGYYSTTIR YQATGFGTNE TEYLFEVDNL TYVQLESRFT
RGP 200 HEPVNTTDDS TSYYMTLTLS YEMSNFGGNE SNTLFKVDNH TYVQLDRPHT
ZGP 250 PQFLLQLNET IYTSGKRSNT TGKLIWKVNP EIDTTIGEWA FWETKKNLTR
RGP 250 PQFLVQLNET LRRNNRLSNS TGRLIWTLDP KIEPDVGEWA FWETKKNFSQ
ZGP 300 KIRSEELSFT VVSNGAKNIS GQSPARTSSD PGTNTTTEDH KIMASENSSA
RGP 300 QLHGENLHFQ ILSTHTNNSS DQSPAGTVQG KISYHPPANN SELVPTDSPP
ZGP 350 MVQVHSQGRE AAVSHLTTLA TISTSPQSLT TKPGPDNSTH NTPVYKLDIS
RGP 350 VVSVLTAGRT EEMST-QGLT NGETIT-GFT ANPMTTTIAP -SPTMTSEVD
ZGP 400 EATQVEQHHR RTD-NDSTAS DTPSAT--TA AGPPKAENTN TSKSTDFLDP
RGP 400 NNVPSEQPNN TASIEDSPPS ASNETIYHSE MDPIQGSNNS AQSPQTKTTP
ZGP 447 ATTTSPQNHS ETAGNNNTHH QDTGEESASS GKLGLITNTI AGVAGLITGG
RGP 447 APTTSPMTQD PQETANSSKP GTSPGSAAGP SQPGLTINTV SKVADSLSPT
ZGP 497 RRTRR EAI VNAQPKCNPN LHYWTTQDEG AAIGLAWIPY FGPAAEGIYI
RGP 497 RKQKR SVR QNTANKCNPD LYYWTAVDEG AAVGLAWIPY FGPAAEGIYI
ZGP 545 EGLMHNQDGL ICGLRQLANE TTQALQLFLR ATTELRTFSI LNRKAIDFLL
RGP 545 EGVMHNQNGL ICGLRQLANE TTQALQLFLR ATTELRTYSL LNRKAIDFLL
ZGP 595 QRWGGTCHIL GPDCCIEPHD WTKNITDKID QIIHDFVDKT LPDQGDNDNW
RGP 595 QRWGGTCRIL GPSCCIEPHD WTKNITDEIN QIKHDFIDNP LPDHGDDLNL
ZGP 645 WTGWRQWIPA GIGVTGVIIA VIALFCICKF VF
RGP 645 WTGWRQWIPA GIGIIGVIIA IIALLCICKI LC

Figure S2

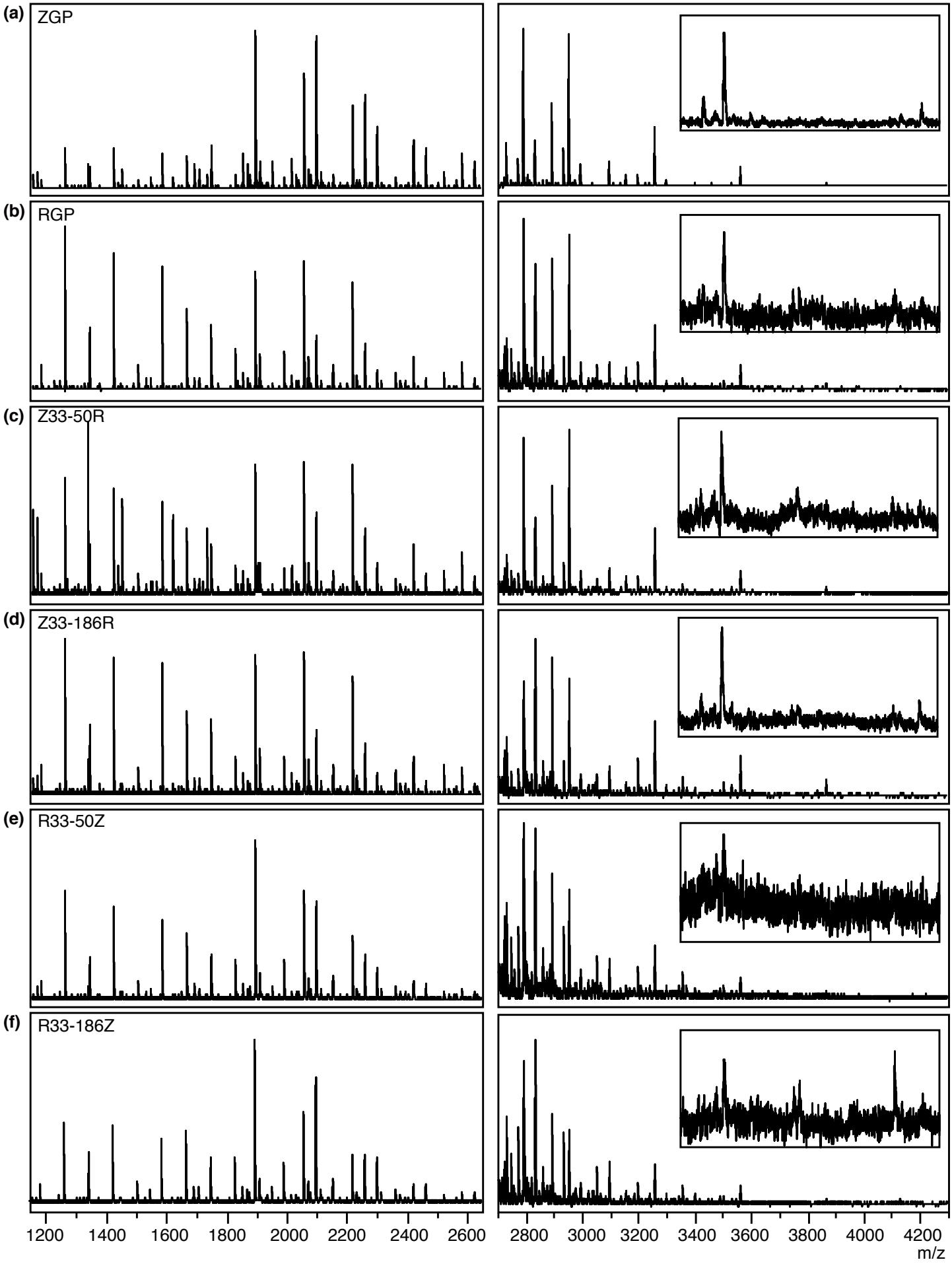

(a)
ZGP
(b)
RGP
(c)
Z33-50R
(d)
Z33-186R
(e)
R33-50Z
(f)
R33-186Z
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
4000
4200
m/z

Figure S3

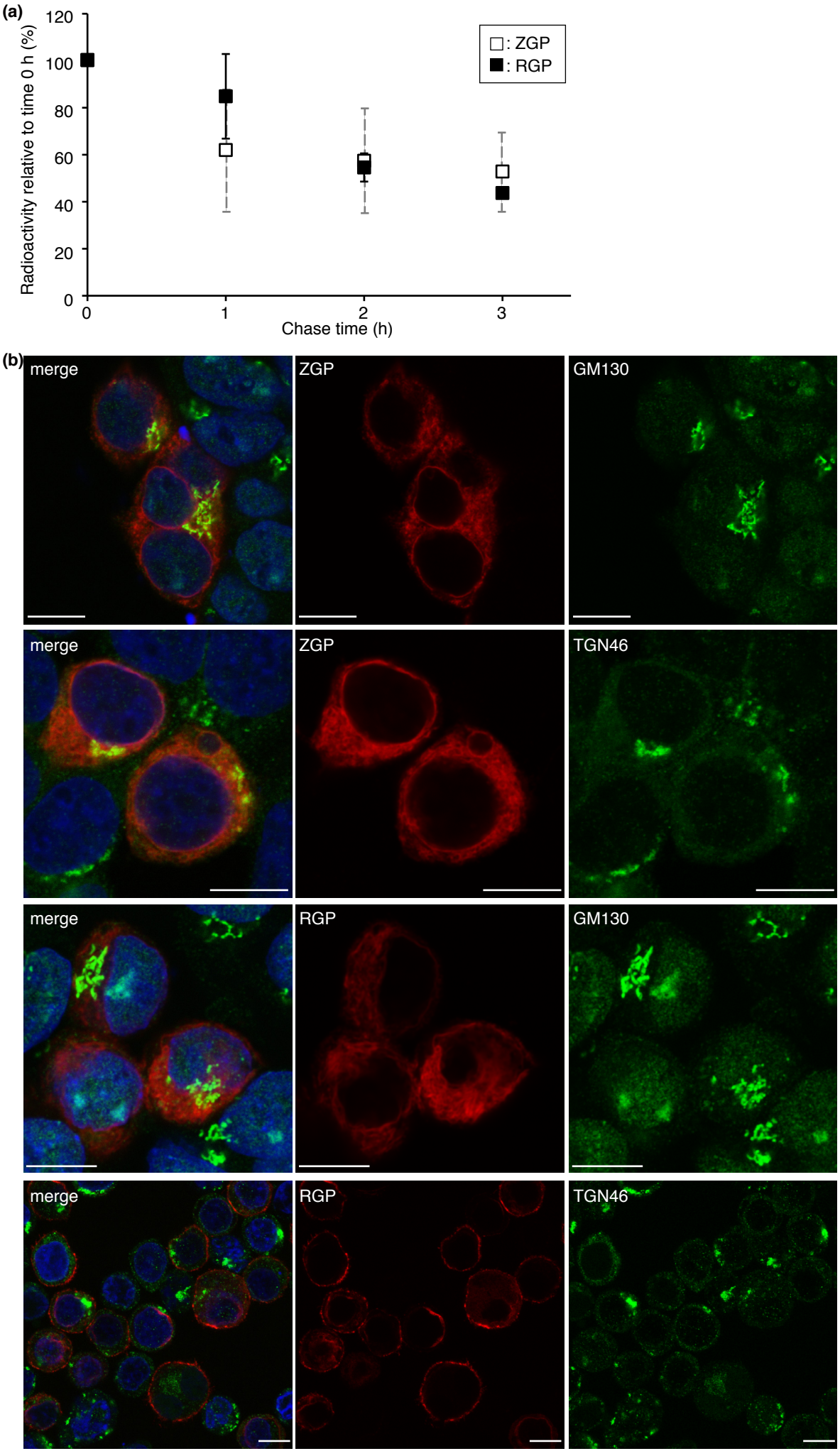

(a)
Radioactivity relative to time 0 h (%)
120
100
80
60
40
20
0
0
1
2
3
Chase time (h)
□: ZGP
■: RGP
(b)
merge
ZGP
GM130
merge
ZGP
TGN46
merge
RGP
GM130
merge
RGP
TGN46

Figure S4

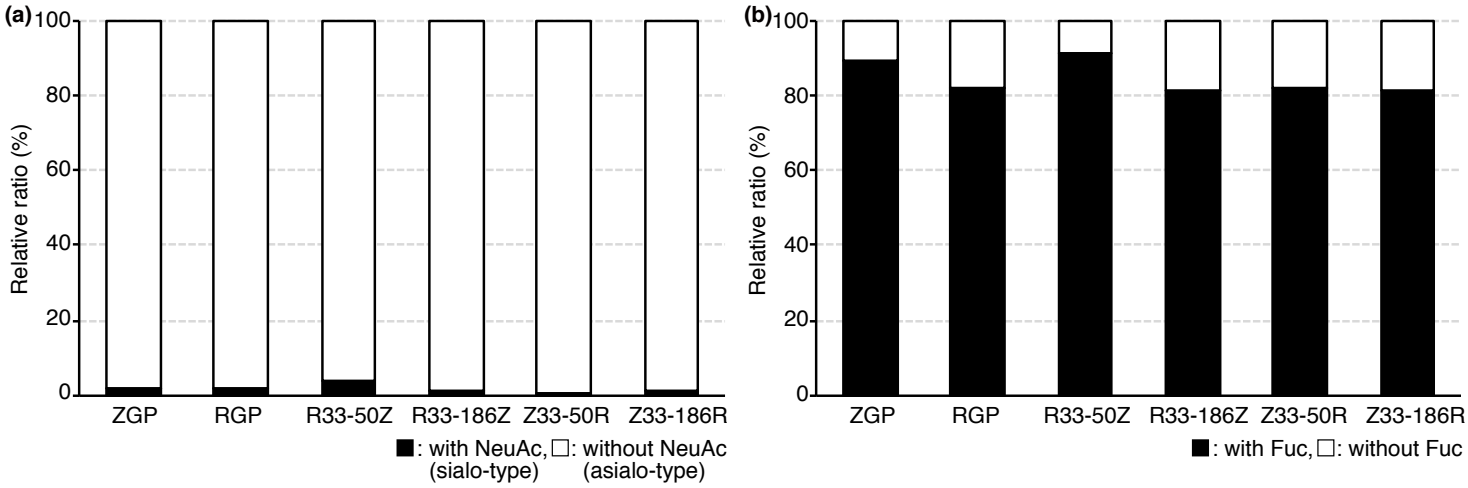

(a)
Relative ratio (%)
100
80
60
40
20
0
ZGP
RGP
R33-50Z
R33-186Z
Z33-50R
Z33-186R
■: with NeuAc, □: without NeuAc
(sialo-type) (asialo-type)
(b)
Relative ratio (%)
100
80
60
40
20
0
ZGP
RGP
R33-50Z
R33-186Z
Z33-50R
Z33-186R
■: with Fuc, □: without Fuc

Figure S5

Figure 1c

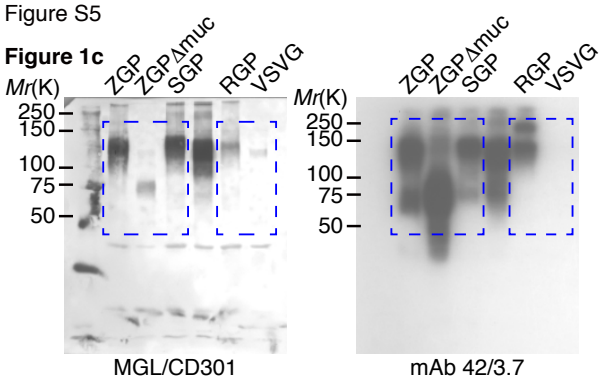


Figure 1d

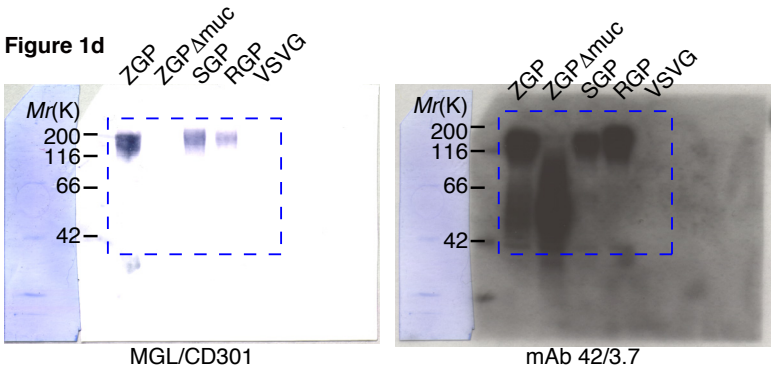


Figure 2b

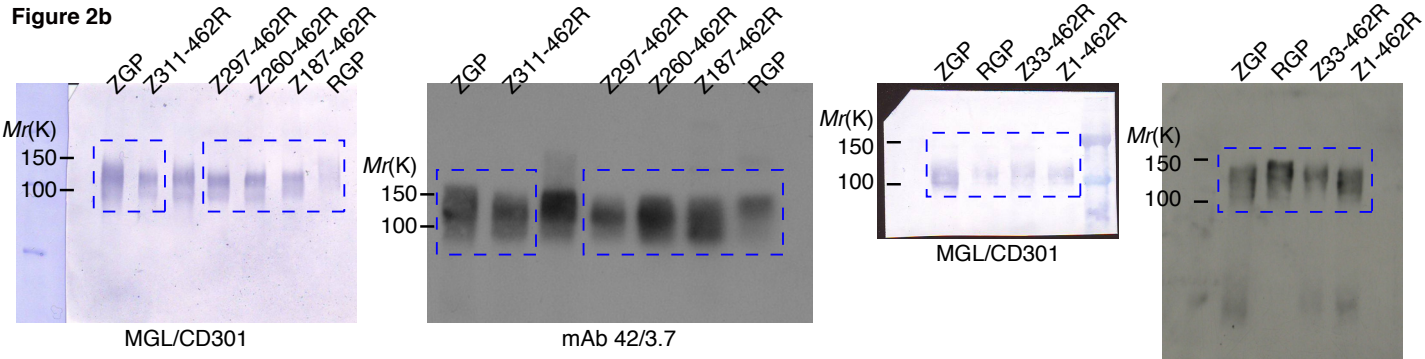


Figure 3b

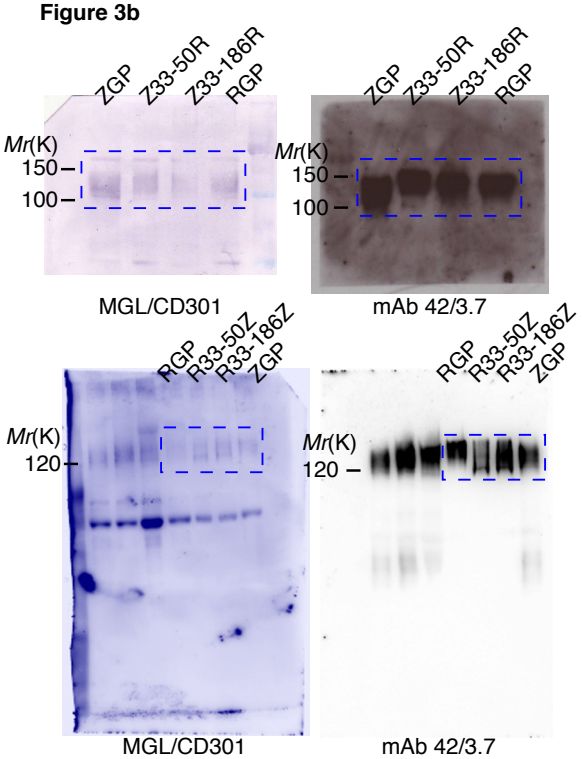


Figure 3d

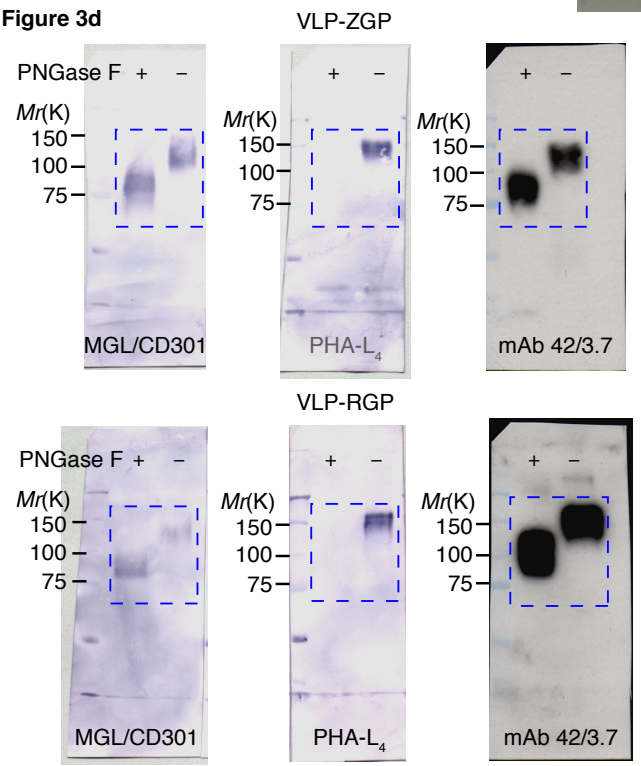


Figure 5a

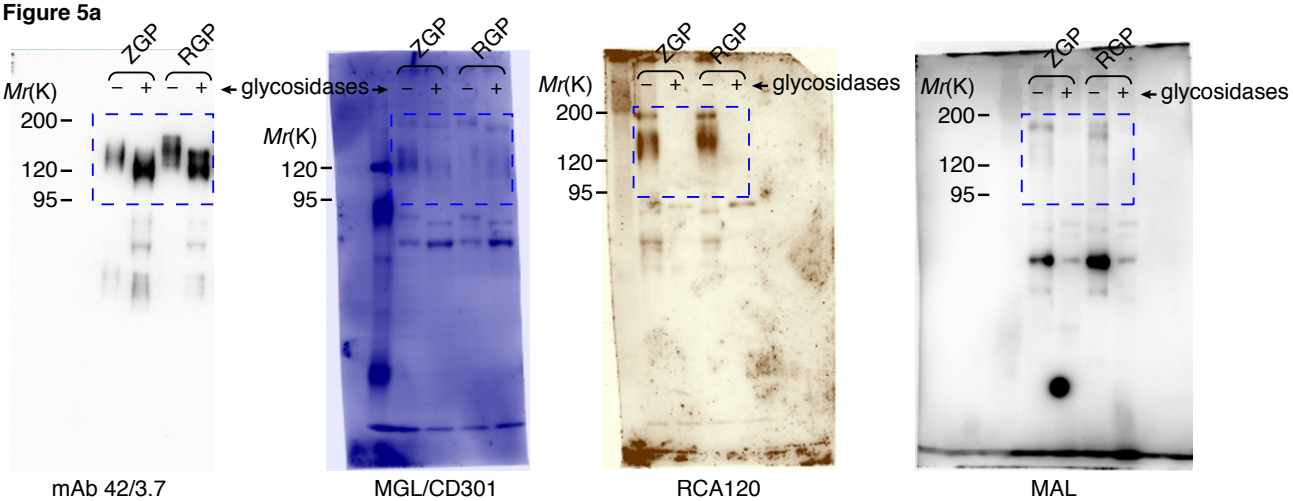
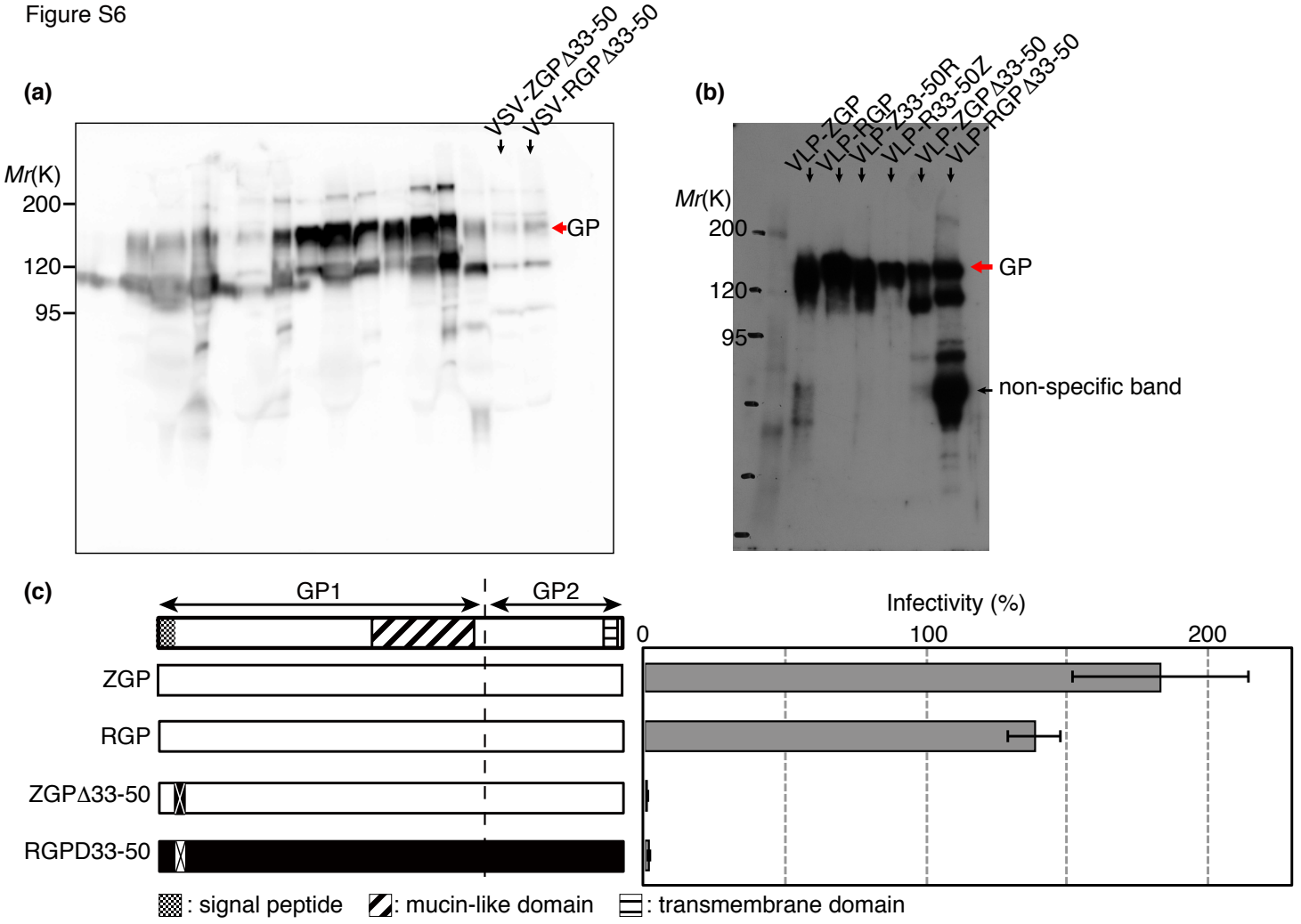


Figure S6


(a)
VSV-ZGPΔ33-50
VSV-RGPΔ33-50
Mr(K)
200
120
95
GP
(b)
VLP-ZGP
VLP-RGP
VLP-Z33-50R
VLP-R33-50Z
VLP-ZGPΔ33-50
VLP-RGPΔ33-50
Mr(K)
200
120
95
GP
non-specific band
(c)
GP1
GP2
ZGP
RGP
ZGPΔ33-50
RGPD33-50
Infectivity (%)
0
100
200
: signal peptide
: mucin-like domain
: transmembrane domain