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Evaluating the influences of glycosylation on the antigenicity and immunogenicity of Ebola virus glycoprotein

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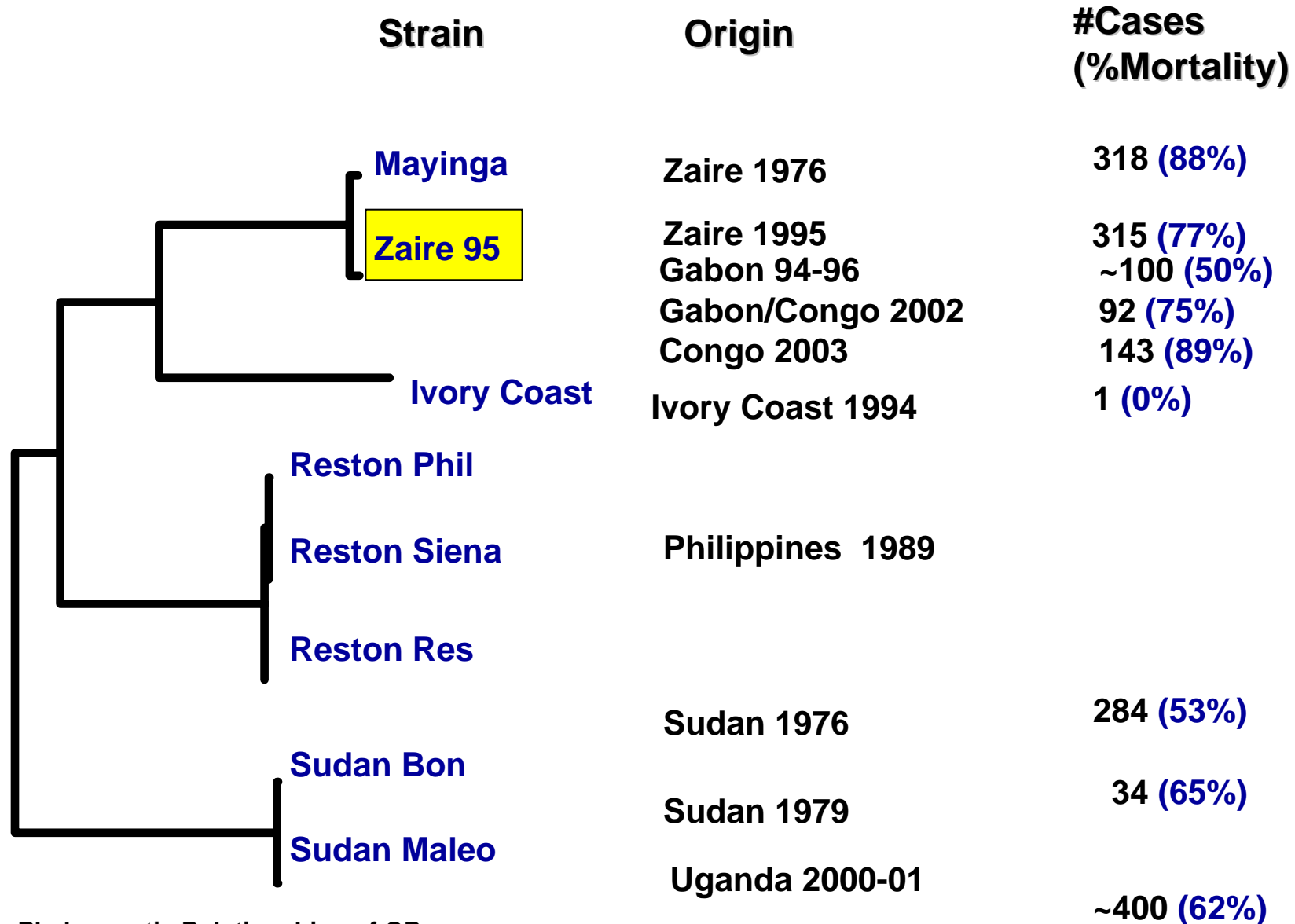
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Ebola Virus

- **Causative agent of severe hemorrhagic fever**
- **Identified in Zaire in 1976**
- **Sporadic outbreaks in Africa**
- **High mortality rate - 50-88%**
- **Animal reservoir unknown**
- **Lack of preventative vaccine or effective antiviral treatments**
- **Potential bioterrorism agent**

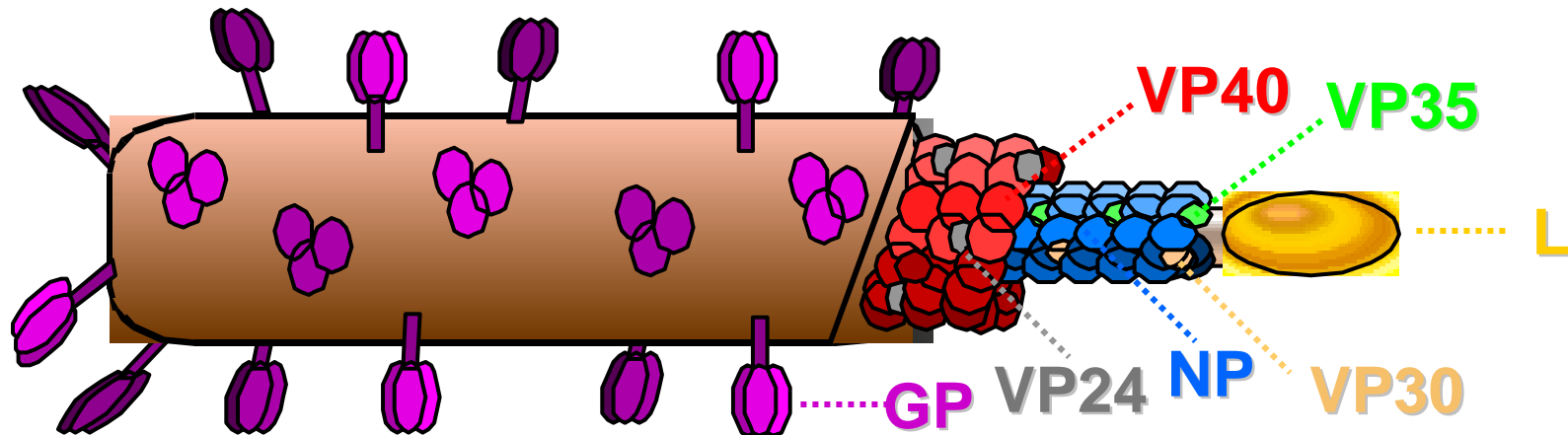


Ebola Virus Strains



Phylogenetic Relationships of GP gene sequence

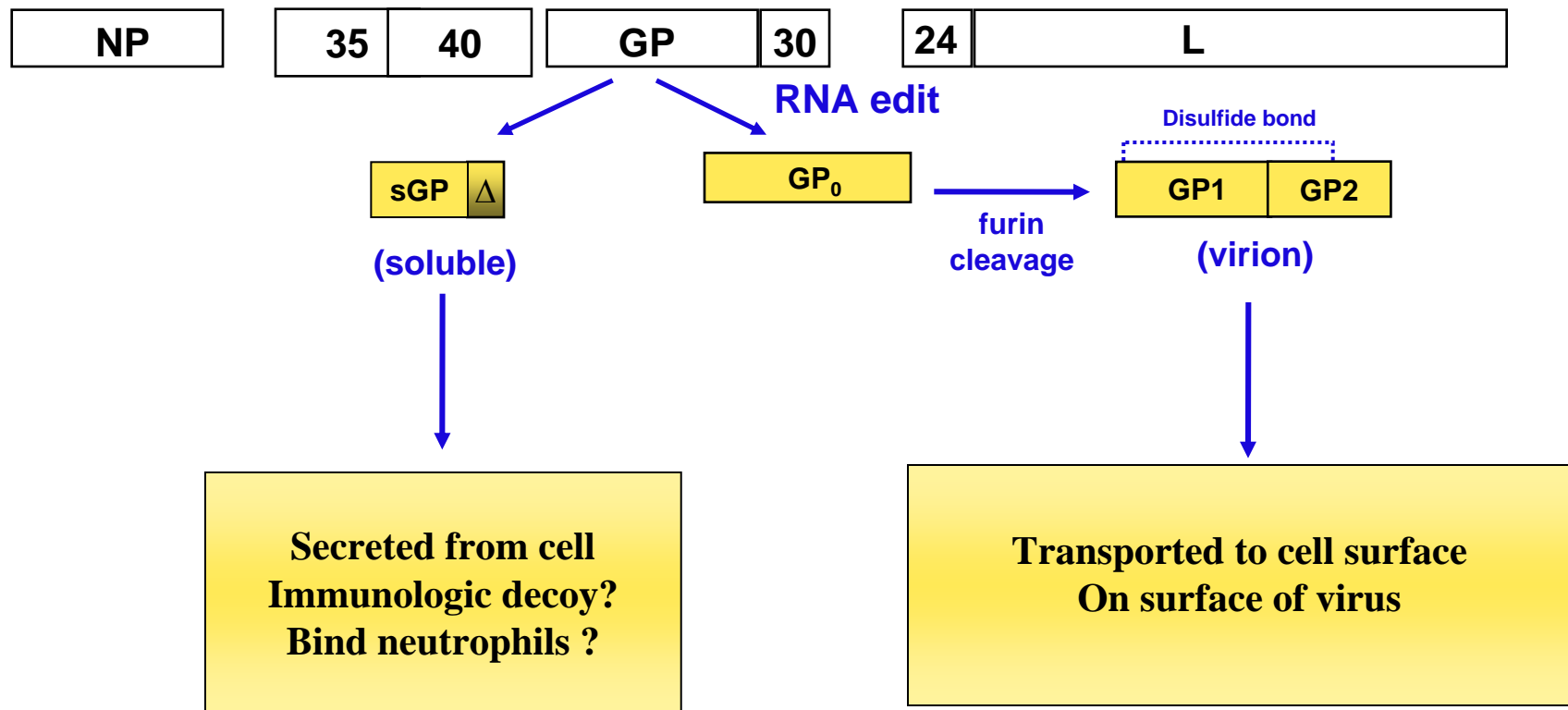
Ebola virus Structure

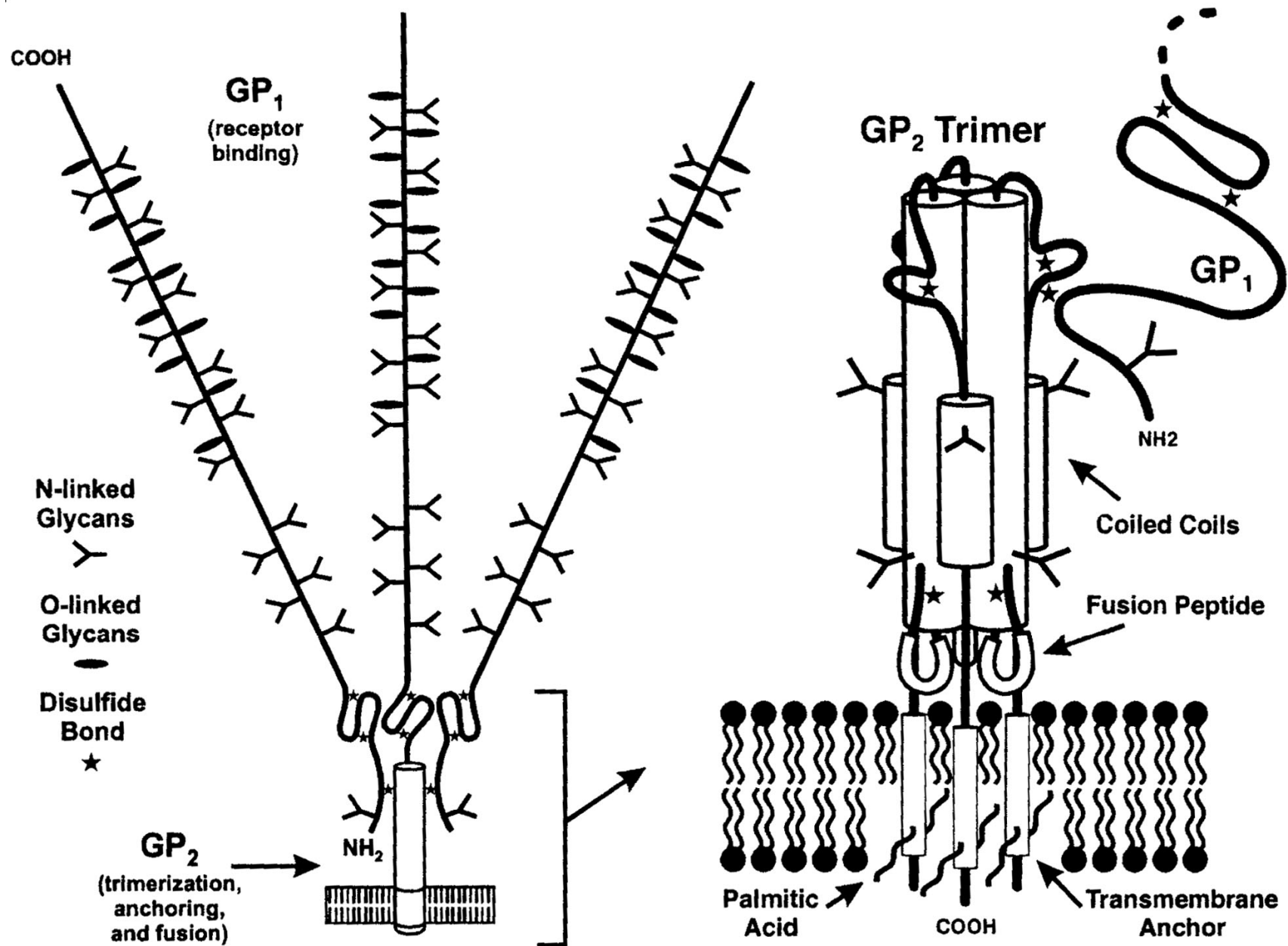


GP	Transmembrane Glycoprotein
VP40	Matrix Protein
VP24	Matrix Protein (Minor)
NP	Nucleoprotein
VP35	Phosphoprotein (Transcription Factor)
VP30	Ribonucleoprotein Associated (Minor)
L	RNA-Dependent RNA Polymerase



Synthesis of Ebola virus glycoproteins





From Fields Virology 3rd ed p. 1287

N-linked glycosylation

- Co-translational modification
- Eukaryotic cells in the endoplasmic reticulum
- Occurs at a triplet amino acid sequence (SEQUON):

Asn-X-Ser/Thr

- Not all sequons are glycosylated
- Influences protein folding and transport
- Changes in viral glycoproteins can lead to altered immunogenicity (HIV, CAEV, influenza, rabies) and virulence (influenza, NDV, MHV, PERV)



Ebola GP: N-linked Glycosylation sites

- Zaire GP - 17 potential sites
- Other strains:
 - Reston - 15
 - Sudan - 12
 - Ivory Coast - 12
- Conserved sites - 5 sites in GP1, 2 in GP2. Those in GP2 are the only sites identical at all 4 amino acid positions.



N-linked mutation set 1

N-linked glycosylation sites around *known* protective epitopes

- Set of 5 monoclonal antibodies
- Protect mice against EBOV infection
- “Protective” epitopes mapped for 3 of the antibodies
- Groups 1 and 2 have overlapping epitope in GP1
- N-linked glycosylation sites FLANK either side of this epitope

QUESTION:

Do glycans at either or both of these sites
shield or *enhance* this epitope?



N-linked mutation set 2

N-linked glycosylation sites in *known* immunogenic regions

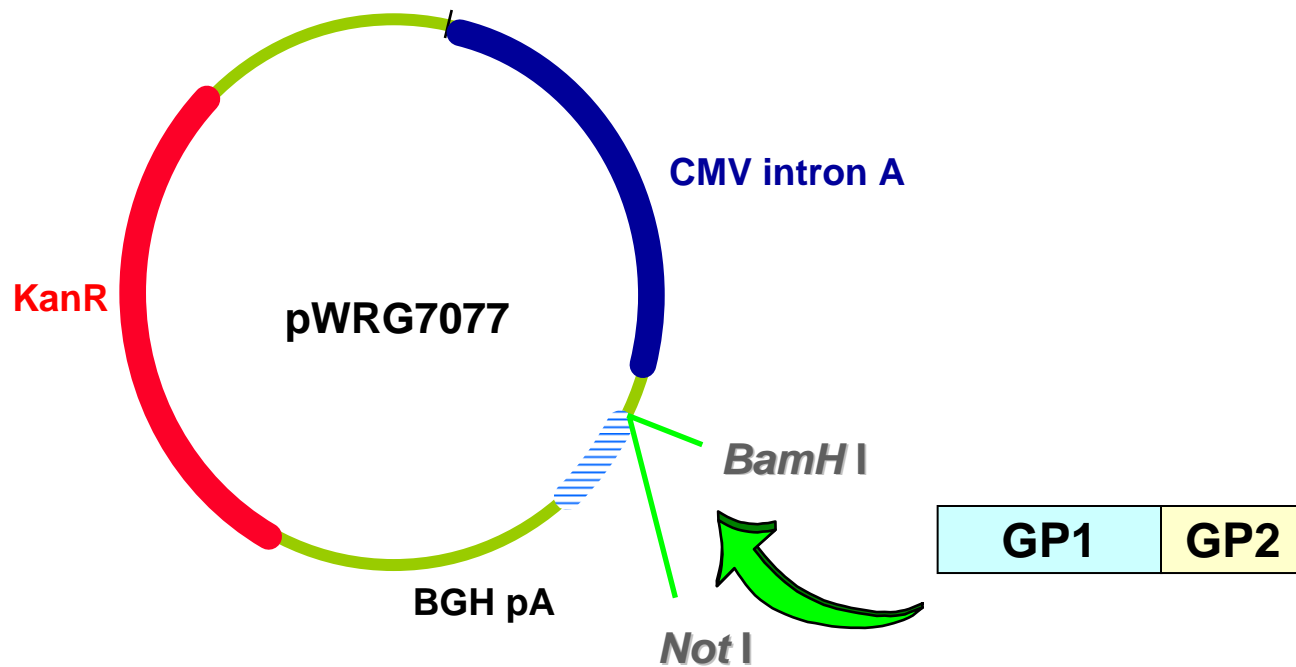
- GP2 shown to be protective for EBOV and MBGV
- Only two sites for N-linked glycosylation in GP2
- Highly conserved among different strains/subtypes of EBOV

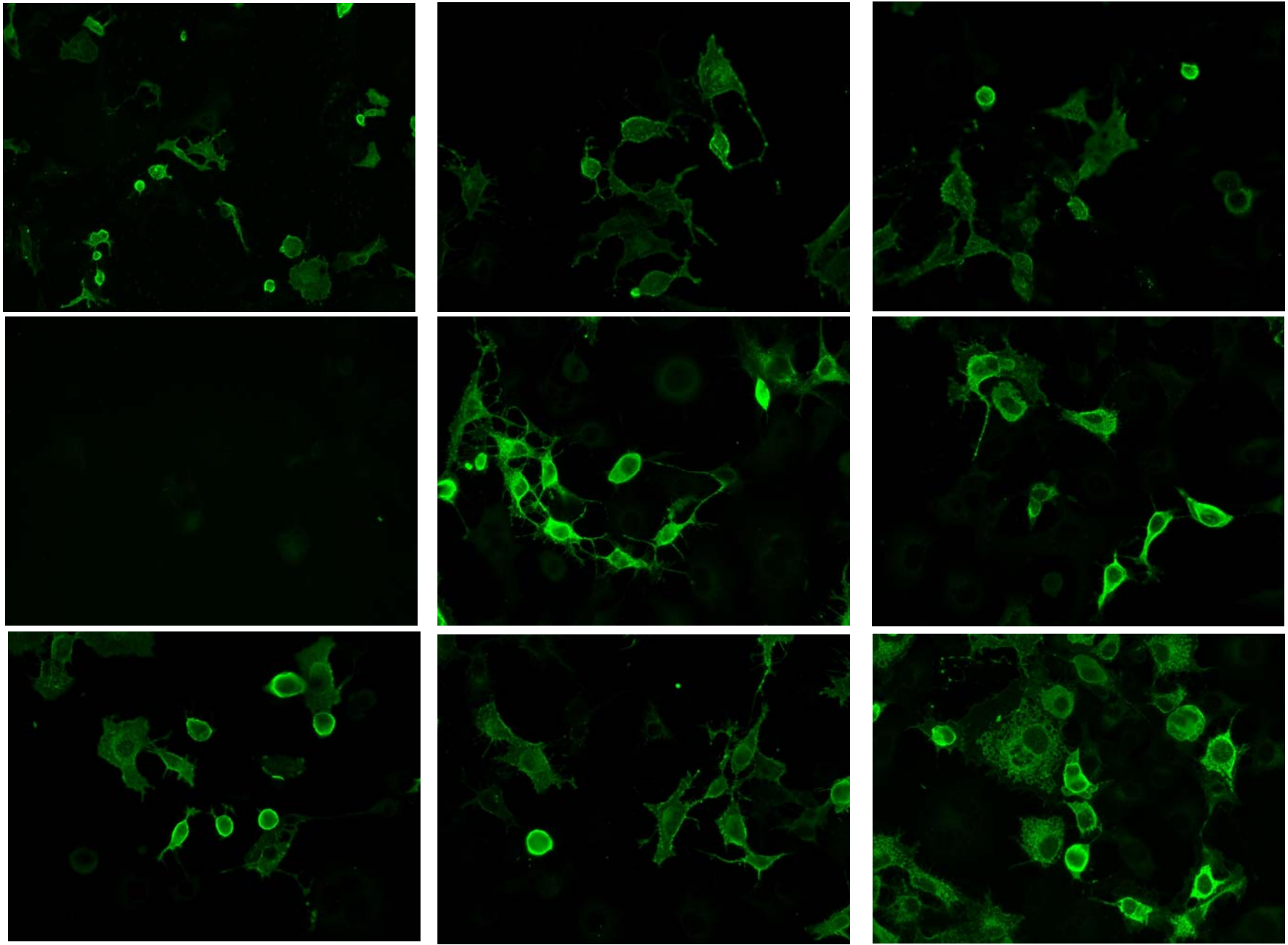
QUESTION:
Do glycans in GP2 affect
the immunogenicity of this region?



MGVTGILQLPRDRFKRTSFFLWVILFQRTFSIPLGVIHNSTLQVSDV
DKLVCRDKLSSTNQLRSVGLNLEGNGVATDVPSATKRWGFRSGV
PPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRY
VHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVAF
LILPQAKKDFSSHPLREPVNATEDPSSGYSTTIRYQATGFGTNET
EYLFEVDNLTYVQLESRFTPQFLLQLNETIYTS GKRSNTTGKLIWKV
NPEIDTTIGEWAFWETKKNLTRKIRSEELSFSAVSNRAKNISGQSPA
RTSSDPGTNTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLG
TISTSPQPPTTKPGPDNSTHNTPVYKLDISEATQVEQHRRRTDNDS
TASDTPPATAAGPLKAENTNTSKGTDLLDPATTTSPQNHSETAGN
NNTHHODTGEESASSGKLGITNTIAGVAGLITGGRRRREAVNA
QPKCNPNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYTEGLMHNQD
GLFCGLRQLANETQALQLFLRATTELRTFSILNRKAIDFLLQRWGG
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GWRQWIPAGIGVTGVIIAVIALFCICKFVF.

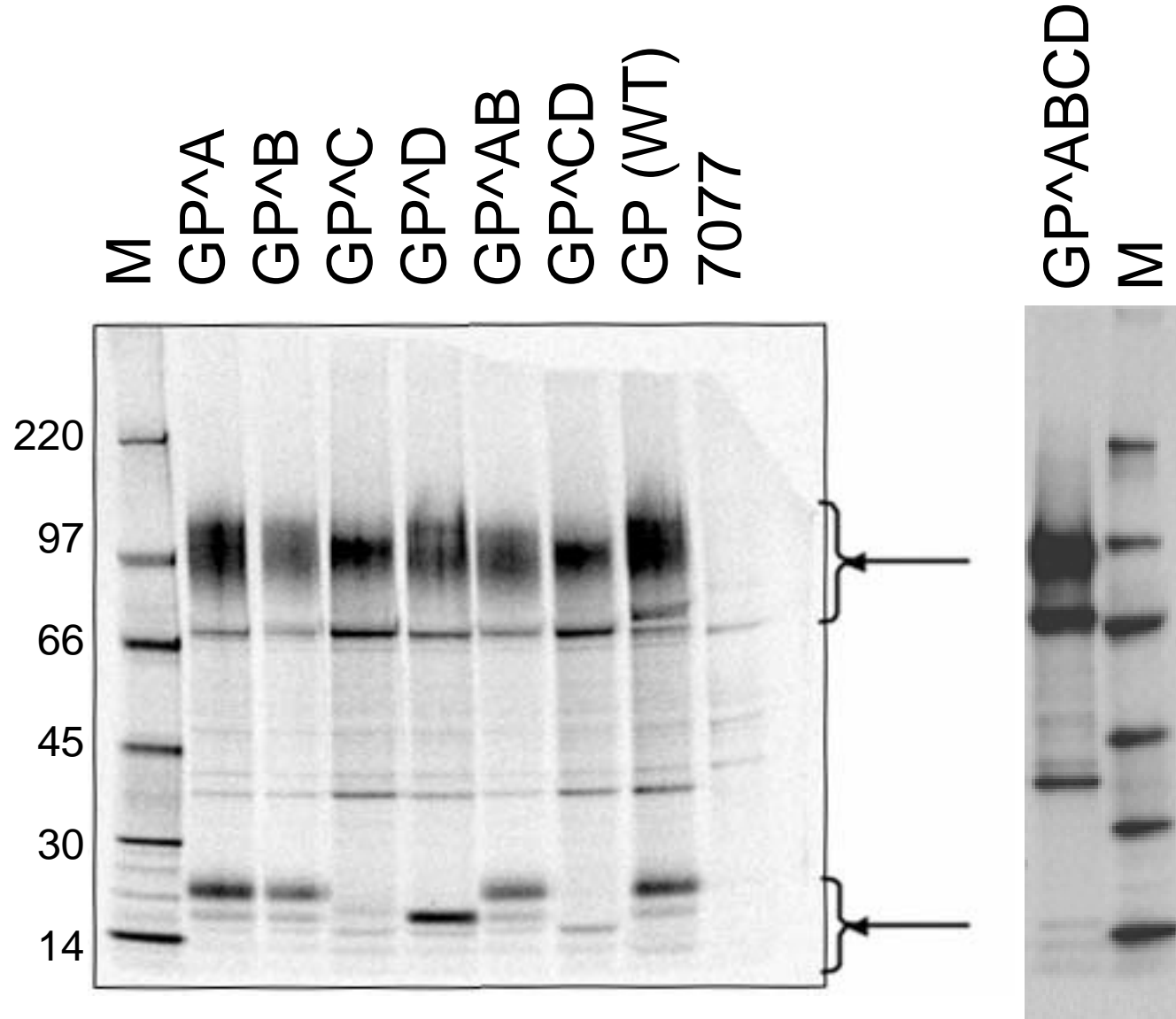
DNA vaccines for EBOV





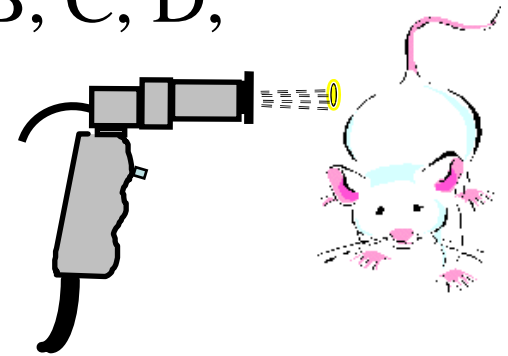
IFA-Fixed Cells

In vitro expression of EBO-Z GP N-linked glycosylation mutants



DNA Vaccination

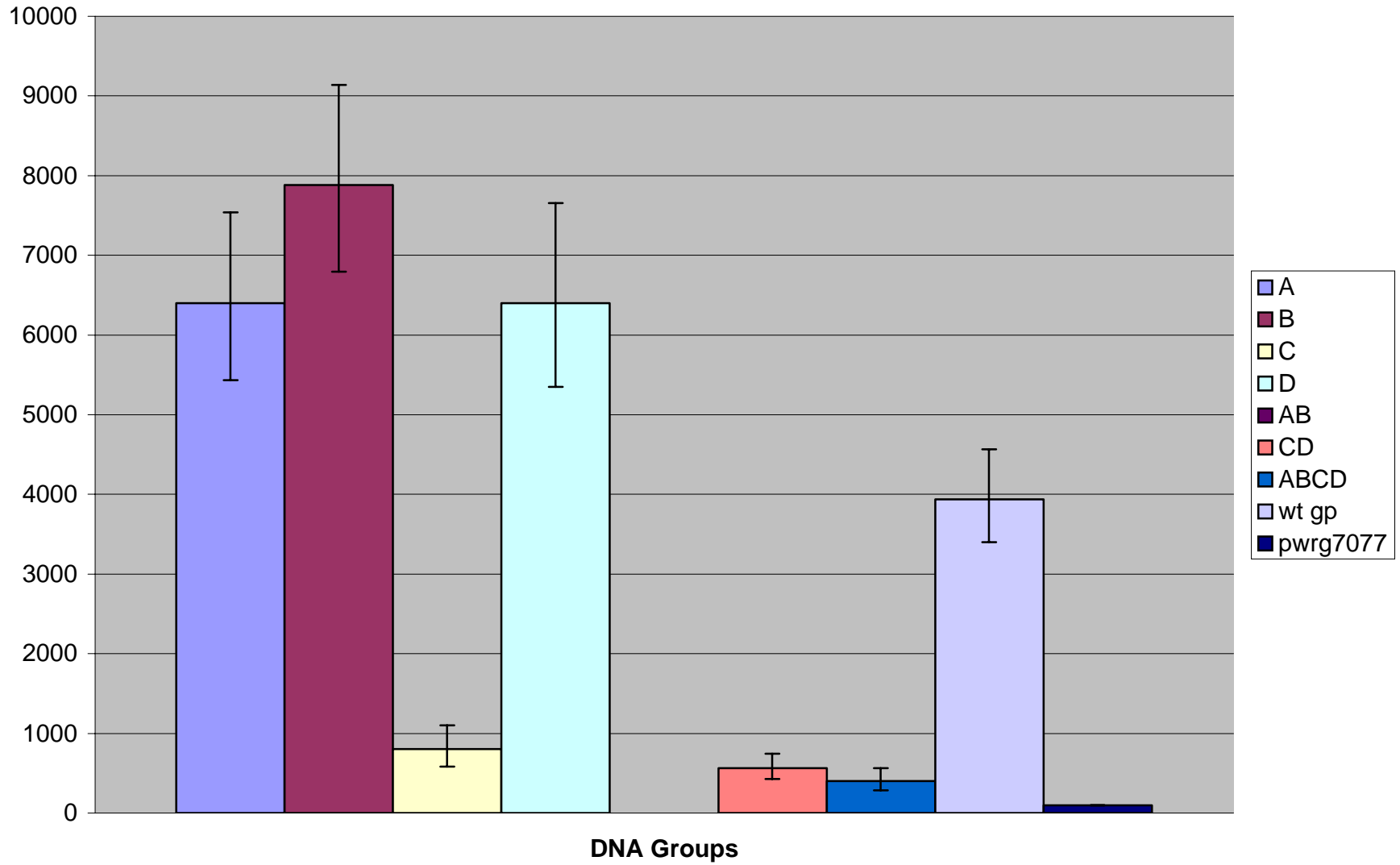
- 9 plasmids: GP wt, pWRG7077, A, B, C, D, AB, CD, ABCD
- 4-6 wk balb/c female mice
- 3 gene-gun vaccinations, 4 wk apart
- Challenge - with mouse-adapted Ebola virus
- ELISA- test sera using irradiated ZEBOV and pools of overlapping peptides as antigens
- ELISPOTs - stimulate with pools of overlapping peptides



Survival Data

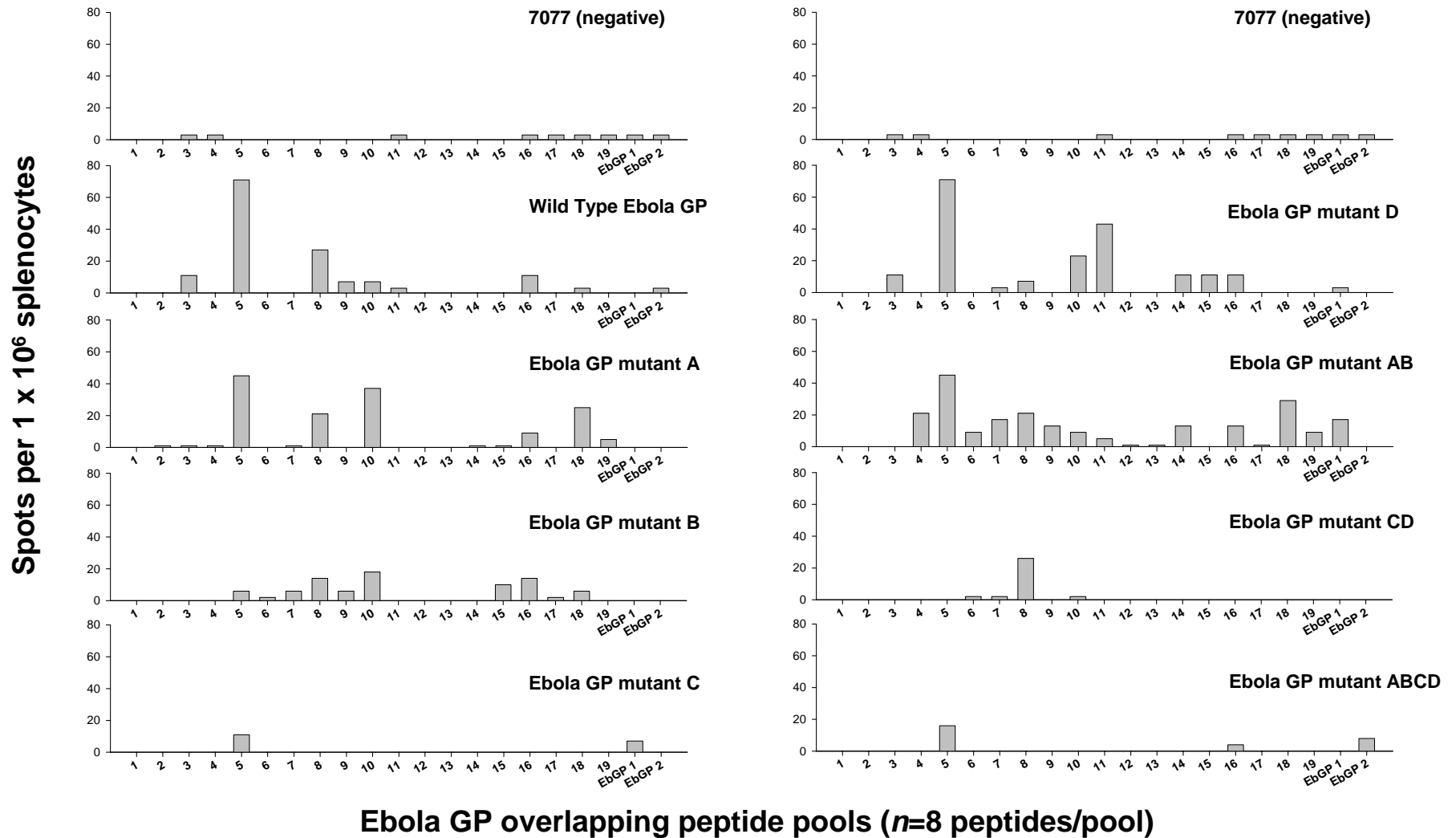
pWRG7077	0/7 (0%)	0/10 (0%)
GP (wt)	7/7 (100%)	10/10 (100%)
GPmutA	7/7 (100%)	10/10 (100%)
GPmutB	7/7 (100%)	9/9 (100%)
GPmutC	3/6 (50%)	6/10 (60%)
GPmutD	7/7 (100%)	9/9 (100%)
GPmutAB	ND	10/10 (100%)
GPmutCD	7/7 (100%)	7/10 (70%)
GPmutABCD	4/6 (67%)	8/10 (80%)

USAMRIID 1: Third Vaccination



Virus-specific T-cell responses in mice vaccinated with DNA vectors expressing the Ebola GP

Deletion of N-linked glycosylation sites alters T cell specificities and frequencies.



Summary

- All N-linked mutants express when transfected into mammalian cells, as shown by IFA and RIPA. Those containing the “C” mutation at position 586 (C, CD, ABCD) demonstrate a normal level of GP1 but a reduced amount of GP2 by RIP assay.
- Vaccination with wild-type GP or with mutants A, B, D and AB protected mice from challenge with mouse-adapted Ebola Zaire. Mutant C and ABCD afforded partial protection in both sets of experiments while CD was completely protective in the first experiment but only partially protective in the second experiment.
- ELISPOT results showed a decrease in breadth and intensity of T-cell responses from mice vaccinated with mutants C, CD and ABCD. Mutants A, B, D and AB had similar responses to wild type
- ELISA titers were decreased in mutants C, CD and ABCD compared to wild type and elevated slightly in mutants A, B, and D.



Future Directions

- Assess GP2 expression of C-containing mutants by IFA, RIPA and Western Blot
- Peptide ELISA
- Repeat of original 9 groups to test conflicting results (in progress)
- Other mutants: O-linked glycosylation mutants, new N-linked mutants



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Lab Animal Usage

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

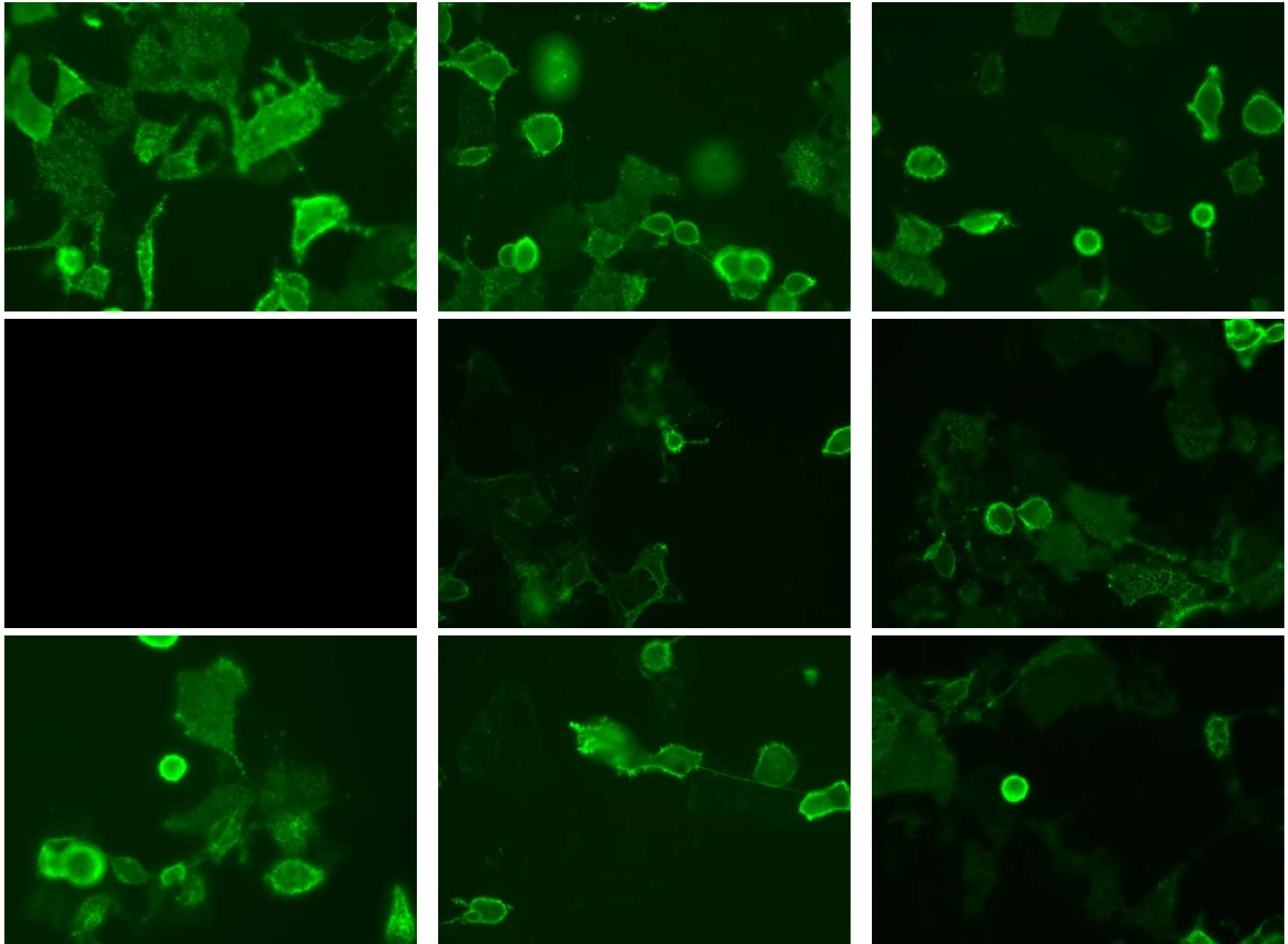
Disclaimer

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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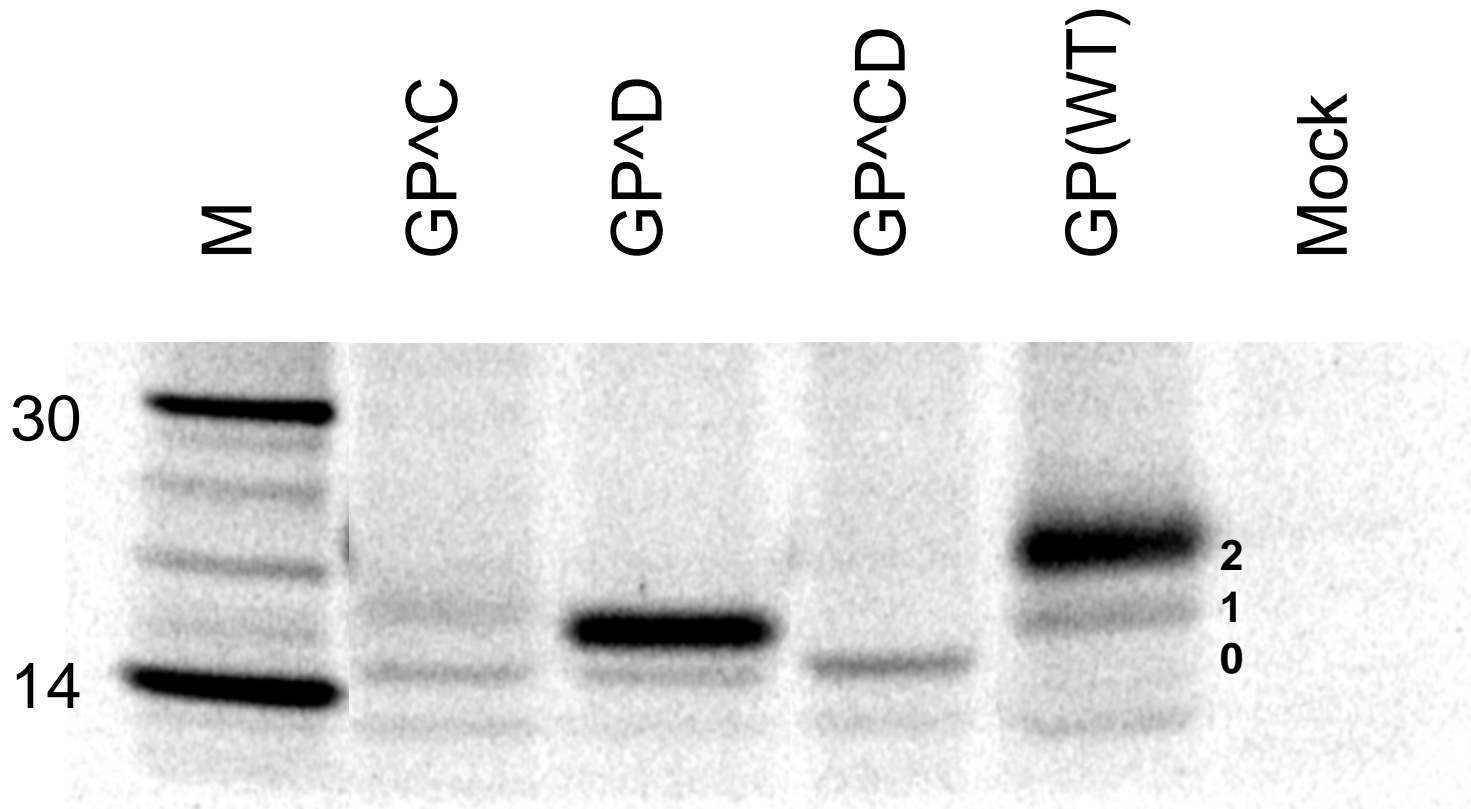
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IFA Live Cells

In vitro expression of ZEBO GP N-linked glycosylation mutants



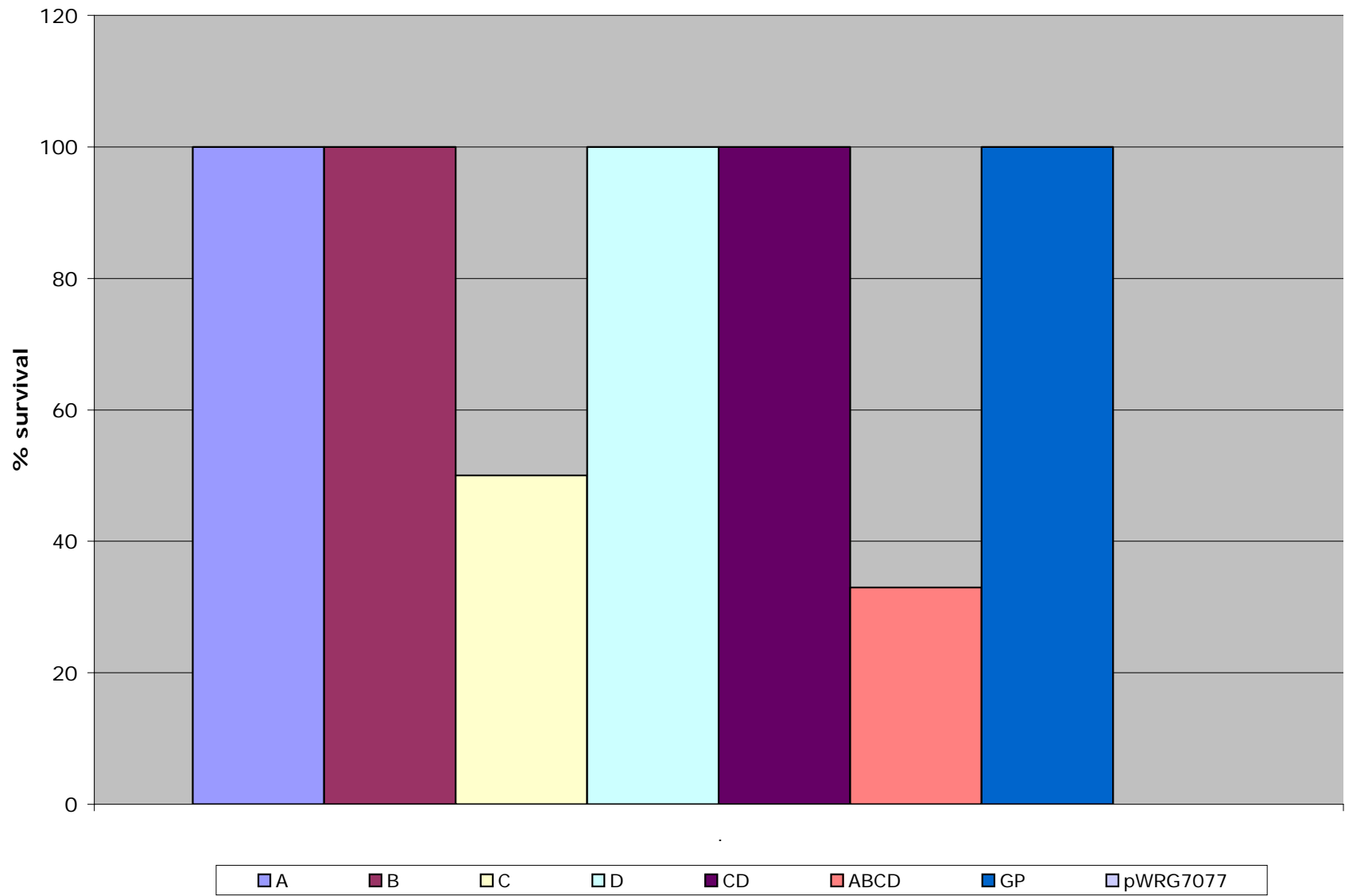
EAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGPAAEGIY
 TEGLMHNQDGLFCGLRQLAN**NET**TQALQLFLRATTELRTFSIL
 NRKAIDFLLQRWGGTCHILGPDC CIEPHDWTK**NIT**DKIDQIIH
 DFVDKTLPDQGDNDNWW TGW RQ WIPAGIGVTGVIIAIALF
CICKFVF.

Ebola Virus Vaccines

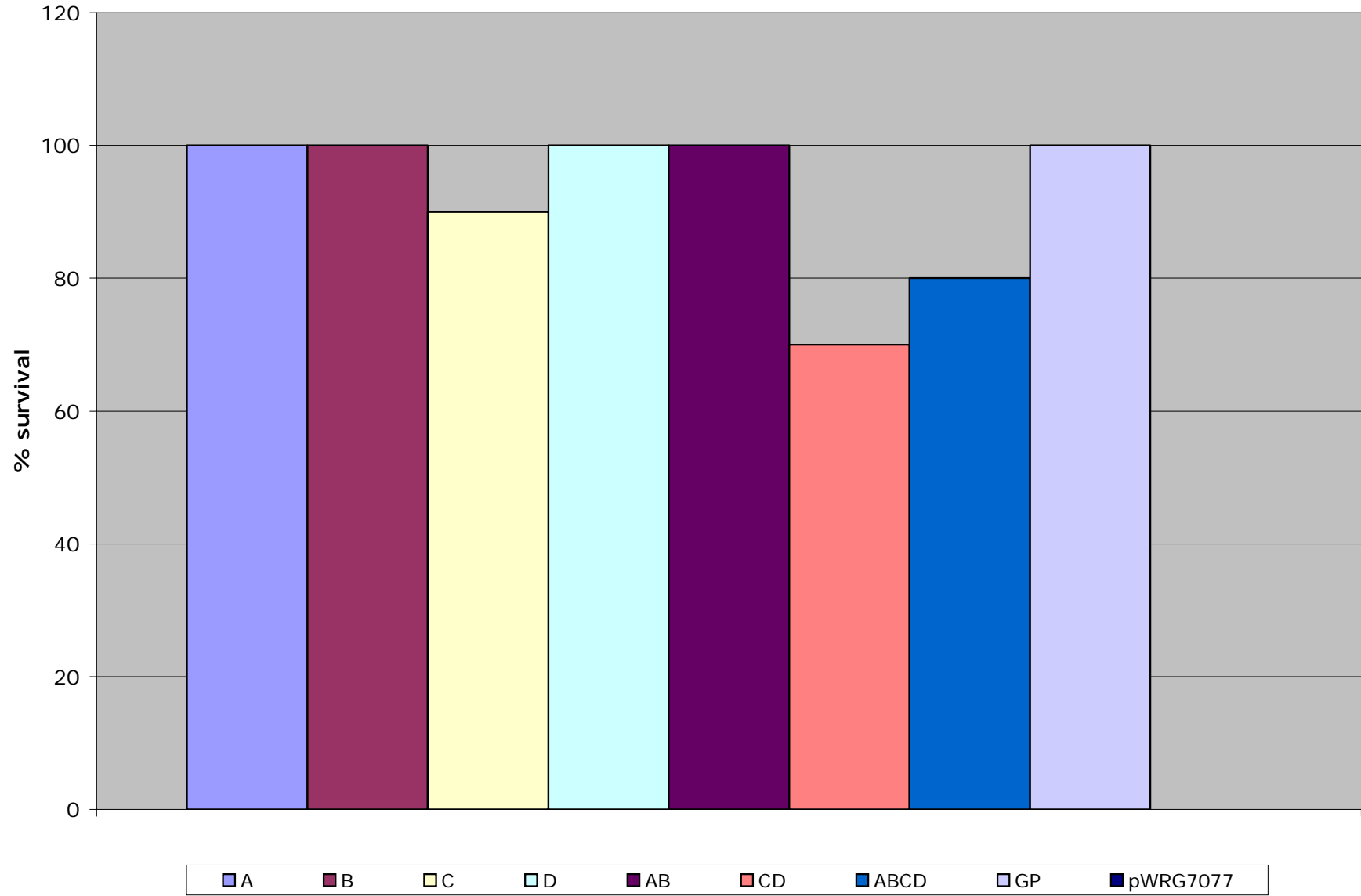
- GP has been tested as a vaccine candidate in the DNA, VEE replicon, VLP, recombinant VSV and adenovirus vector systems.
- GP protects mice from challenge in all of these systems.
- Both the humoral and cellular immune responses play important roles in protection.



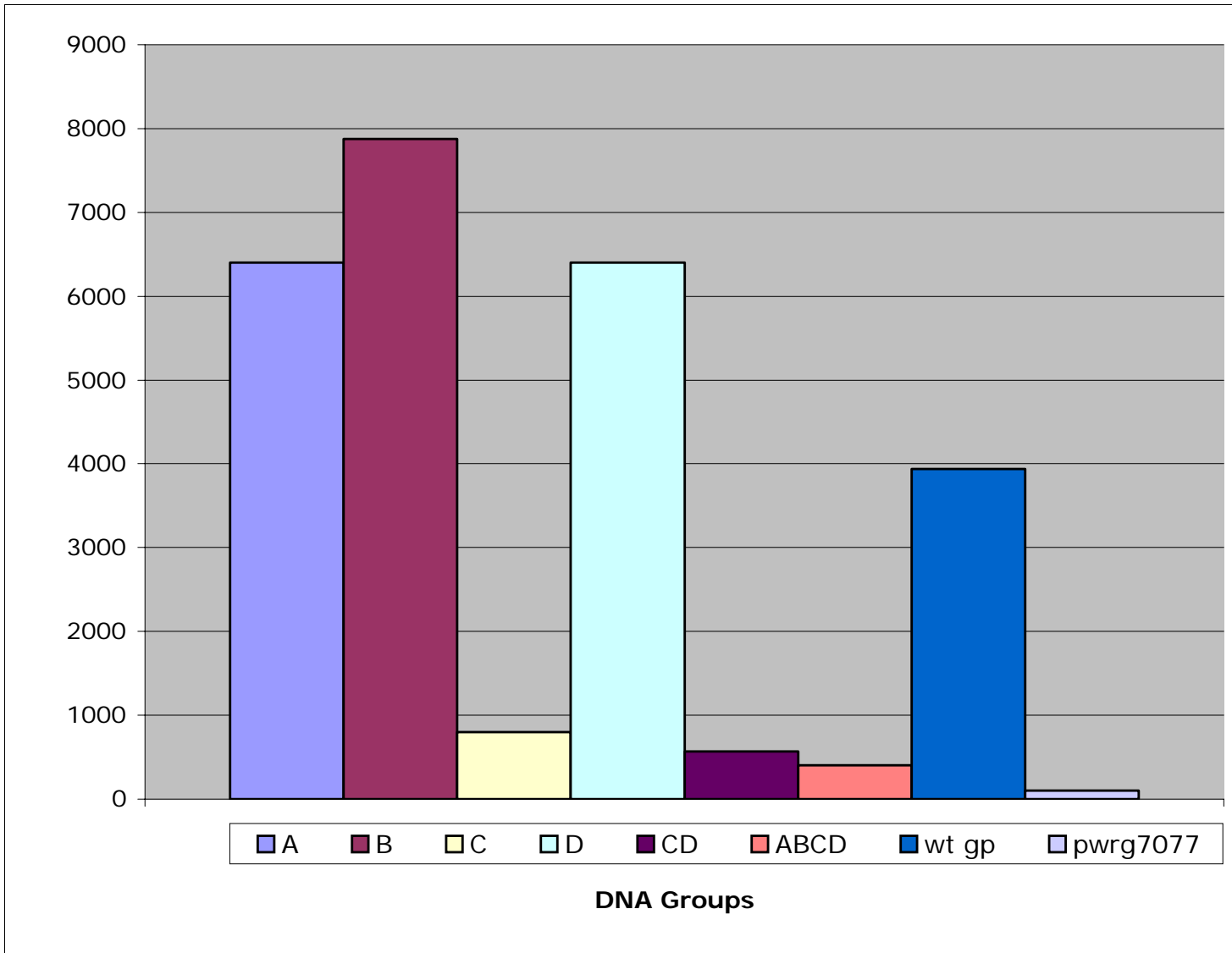
Survival Data Exp. 1



Survival Data Exp. 2



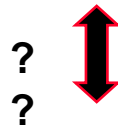
ELISA results



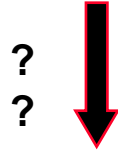
Ebola Virus



Unknown
reservoir

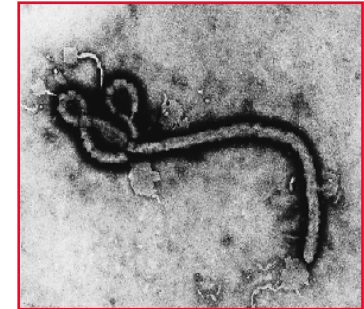


Monkey



Human

Fever, Malaise, Diarrhea, Hemorrhage



Filoviridae
enveloped, filamentous
ssRNA, (-) >19 kilobases



Ebola Virus Glycoprotein (GP)

- 676 amino acids , approx. 140 kDa
- Post-translation cleavage by furin at AA 501, forms GP1 and GP2 domains
- GP2 has a transmembrane domain and a small intracellular domain
- GP1 remains linked to GP2 by disulfide bonding
- Glycosylation accounts for about half of the molecular weight

