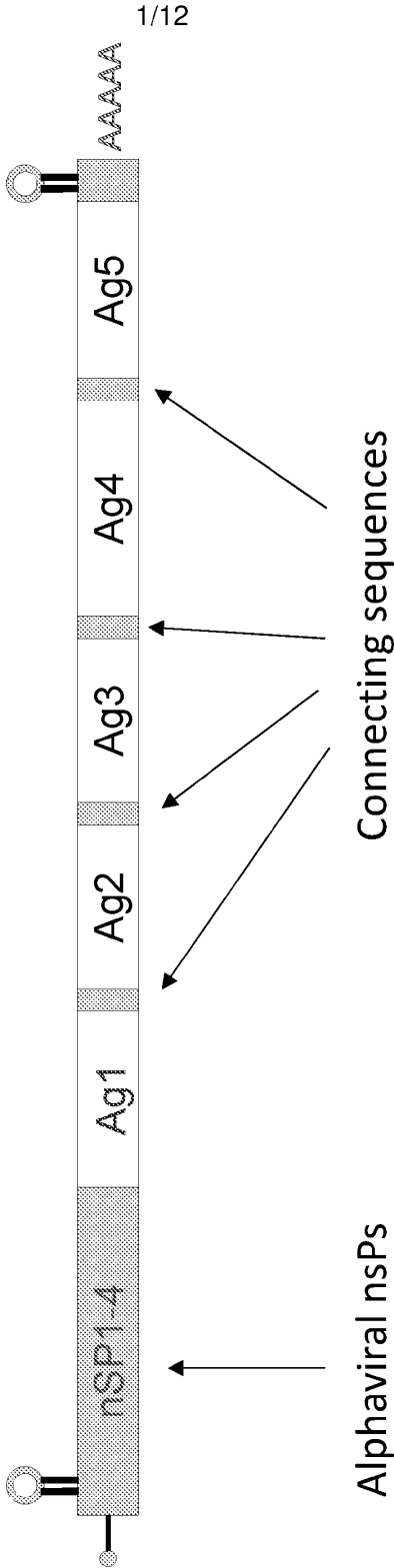


**Exemplary design of a multigenic EBV vaccine**



**FIG. 1**

Summary of srRNA constructs

| Construct Number of Antigens |   |                             | Antigen characteristics |      | Antigen cassette configuration |      |         |      |       |      |       |  |  |  |
|------------------------------|---|-----------------------------|-------------------------|------|--------------------------------|------|---------|------|-------|------|-------|--|--|--|
| Rep-674                      | 1 | BZLF1                       |                         |      |                                |      |         |      |       |      |       |  |  |  |
| Rep-675                      | 2 | gH, gL                      | gH(-TM) P2A gL          |      |                                |      |         |      |       |      |       |  |  |  |
| Rep-678                      | 2 | gH, gL                      | gL P2A gH               |      |                                |      |         |      |       |      |       |  |  |  |
| Rep-679                      | 1 | gp350                       |                         |      |                                |      |         |      |       |      |       |  |  |  |
| Rep-680                      | 1 | LMP2B                       |                         |      |                                |      |         |      |       |      |       |  |  |  |
| Rep-692                      | 5 | BZLF1, LMP2B, gH, gL, gp350 | BZLF1                   | IRES | gH(-TM)                        | P2A  | gL      | P2A  | gp350 | P2A  | LMP2B |  |  |  |
| Rep-693                      | 5 | BZLF1, LMP2B, gH, gL, gp350 | BZLF1                   | P2A  | gH(-TM)                        | P2A  | gL      | IRES | gp350 | P2A  | LMP2B |  |  |  |
| Rep-694                      | 5 | BZLF1, LMP2B, gH, gL, gp350 | BZLF1                   | P2A  | gH(-TM)                        | P2A  | gL      | P2A  | gp350 | IRES | LMP2B |  |  |  |
| Rep-695                      | 5 | BZLF1, LMP2B, gH, gL, gp350 | BZLF1                   | P2A  | gH(-TM)                        | P2A  | gL      | P2A  | gp350 | P2A  | LMP2B |  |  |  |
| Rep-696                      | 5 | BZLF1, LMP2B, gH, gL, gp350 | gH(-TM)                 | P2A  | gL                             | P2A  | gp350   | IRES | BZLF1 | P2A  | LMP2B |  |  |  |
| Rep-697                      | 5 | BZLF1, LMP2B, gH, gL, gp350 | gH(-TM)                 | P2A  | gL                             | P2A  | gp350   | IRES | LMP2B | P2A  | BZLF1 |  |  |  |
| Rep-698                      | 5 | BZLF1, LMP2B, gH, gL, gp350 | gH(-TM)                 | P2A  | gL                             | P2A  | gp350   | P2A  | LMP2B | IRES | BZLF1 |  |  |  |
| Rep-699                      | 5 | BZLF1, LMP2B, gH, gL, gp350 | gp350                   | P2A  | gH(-TM)                        | P2A  | gL      | P2A  | LMP2B | IRES | BZLF1 |  |  |  |
| Rep-728                      | 3 | gH, gL, gp350               | gH(-TM)                 | P2A  | gL                             | P2A  | gp350   |      |       |      |       |  |  |  |
| Rep-729                      | 3 | gH, gL, gp350               | gL                      | P2A  | gH(-TM)                        | P2A  | gp350   |      |       |      |       |  |  |  |
| Rep-730                      | 3 | gH, gL, gp350               | gp350                   | P2A  | gH(-TM)                        | P2A  | gL      |      |       |      |       |  |  |  |
| Rep-731                      | 3 | gH, gL, gp350               | gp350                   | P2A  | gL                             | P2A  | gH(-TM) |      |       |      |       |  |  |  |
| Rep-733                      | 3 | gH, gL, gp350               | gL                      | P2A  | gH(-TM)                        | IRES | gp350   |      |       |      |       |  |  |  |
| Rep-734                      | 3 | gH, gL, gp350               | gp350                   | IRES | gH(-TM)                        | P2A  | gL      |      |       |      |       |  |  |  |
| Rep-735                      | 3 | gH, gL, gp350               | gp350                   | IRES | gL                             | P2A  | gH(-TM) |      |       |      |       |  |  |  |
| Rep-736                      | 2 | BZLF1, LMP2B                | LMP2B                   | IRES | BZLF1                          |      |         |      |       |      |       |  |  |  |
| Rep-737                      | 2 | BZLF1, LMP2B                | BZLF1                   | IRES | LMP2B                          |      |         |      |       |      |       |  |  |  |

All gp350 genes listed here lack transmembrane domain (-TM)

FIG. 2

In vitro bioactivity of mono and multigenic srRNAs (measured %dsRNA)

Bioactivity of srRNA

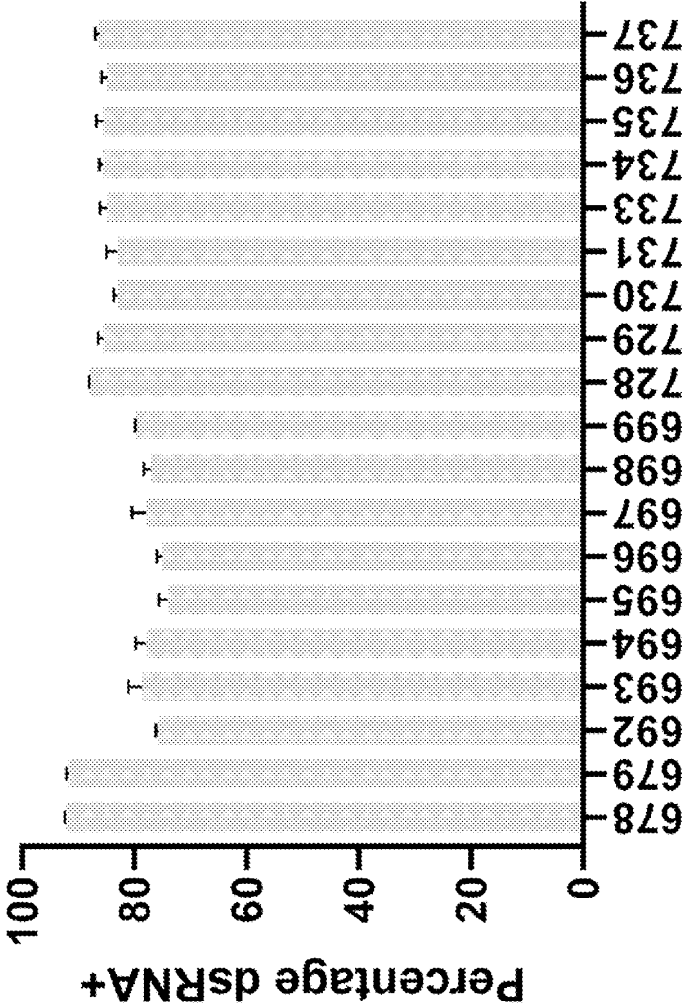
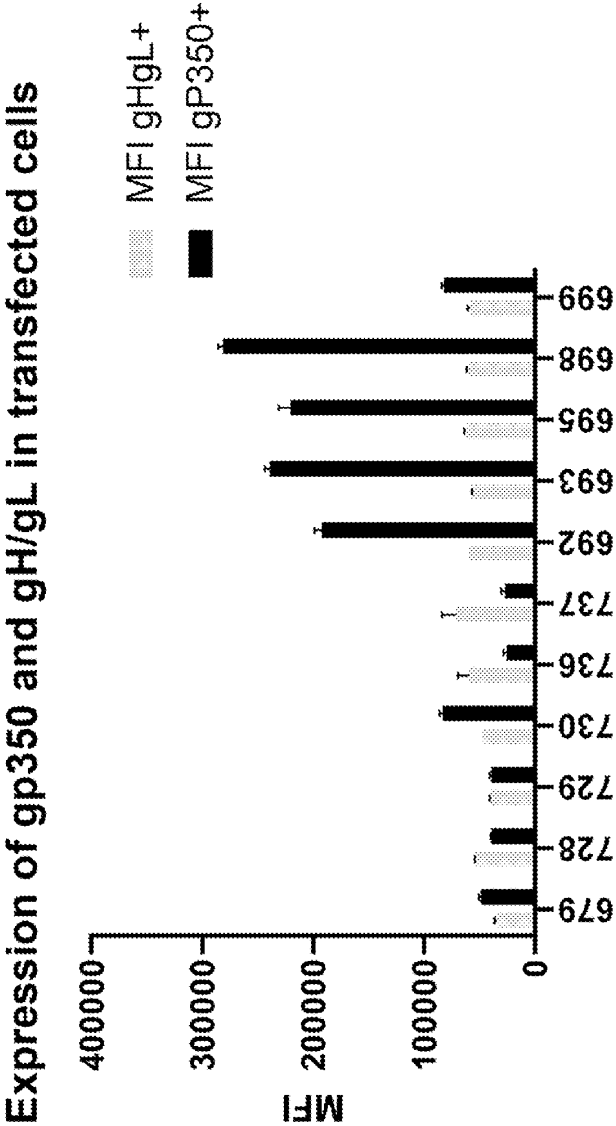


FIG. 3

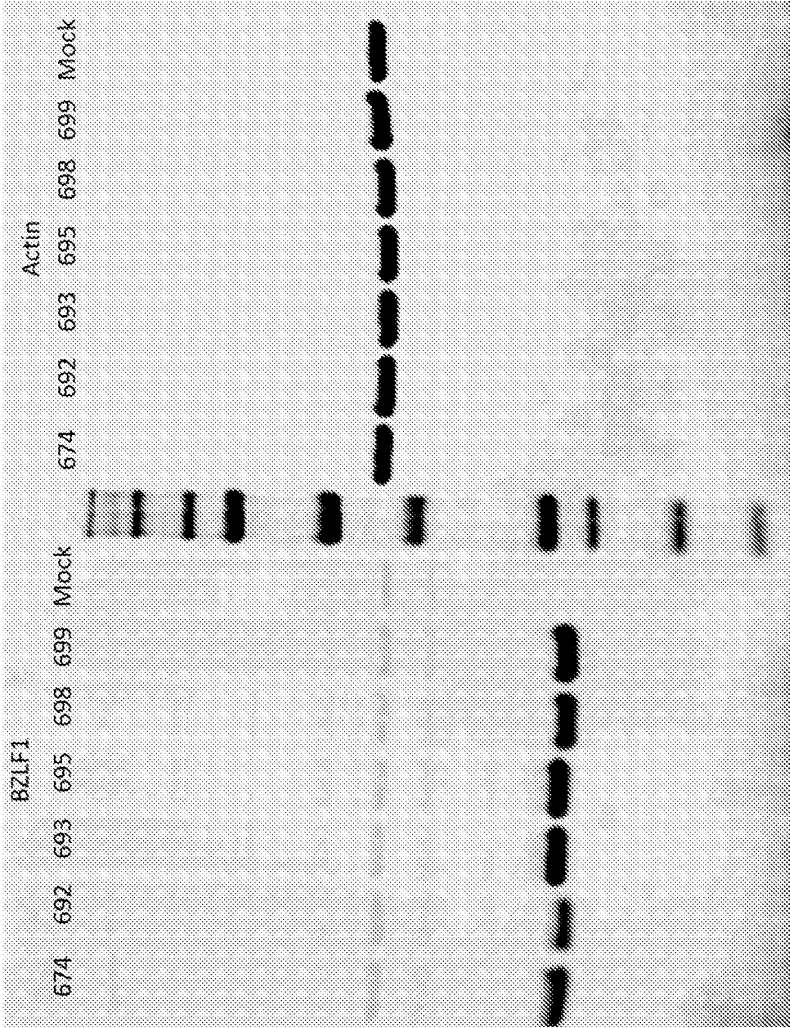
**In vitro expression of encoded EBV antigens of mono and multigenic srRNAs**



Immunostaining was performed for gp350 and gH and gL only due to reagent availability

**FIG. 4**

**In vitro expression of encoded EBV antigen BZLF1 of monogenic and pentagenic srRNAs by immunoblot**



**FIG. 5**

Antibody responses to gH/gL in mice

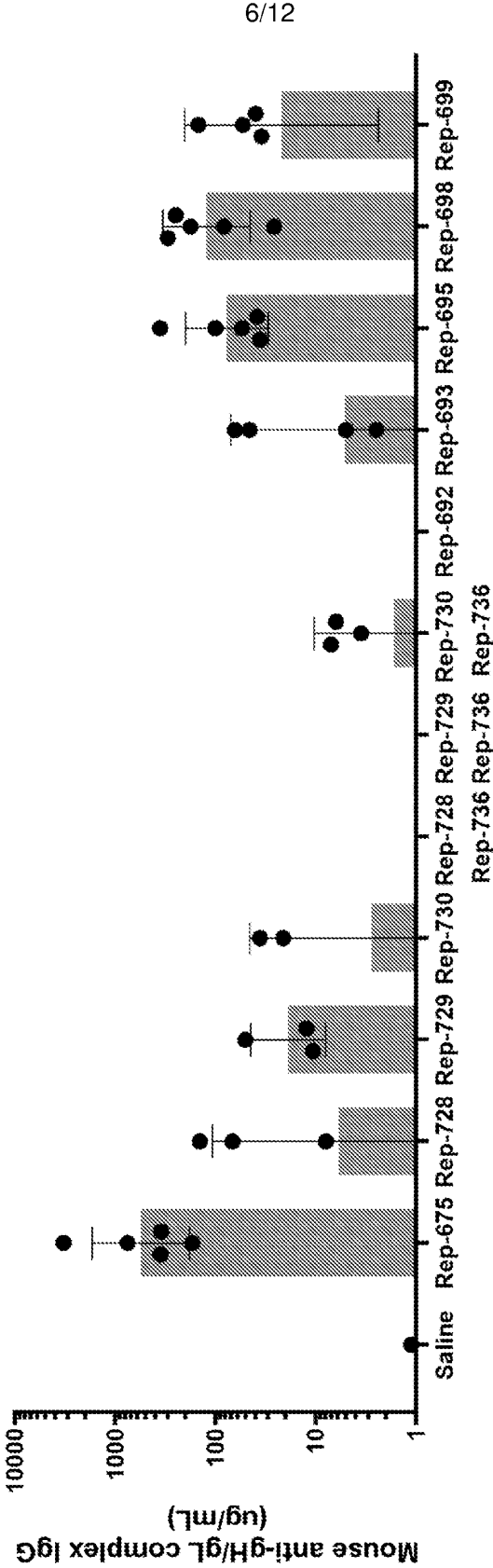
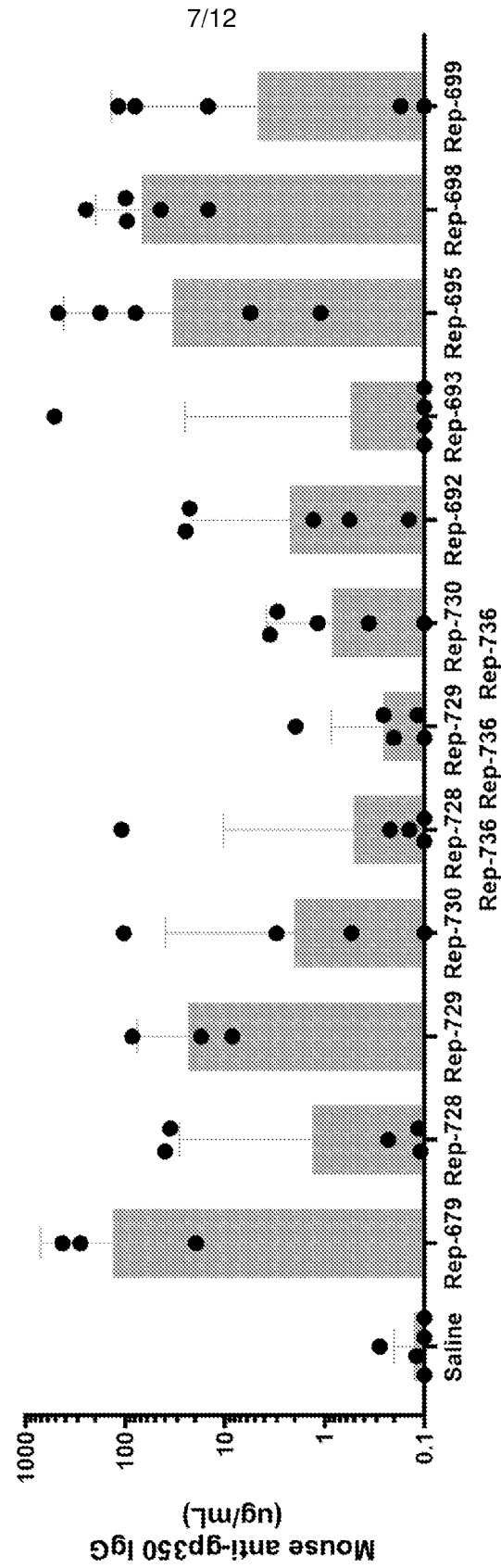


FIG. 6A

## Antibody responses to gp350 in mice



**FIG. 6B**

T cell responses to gH/gL, gp350, LMP2, BZLF1 in mice

gH/gL

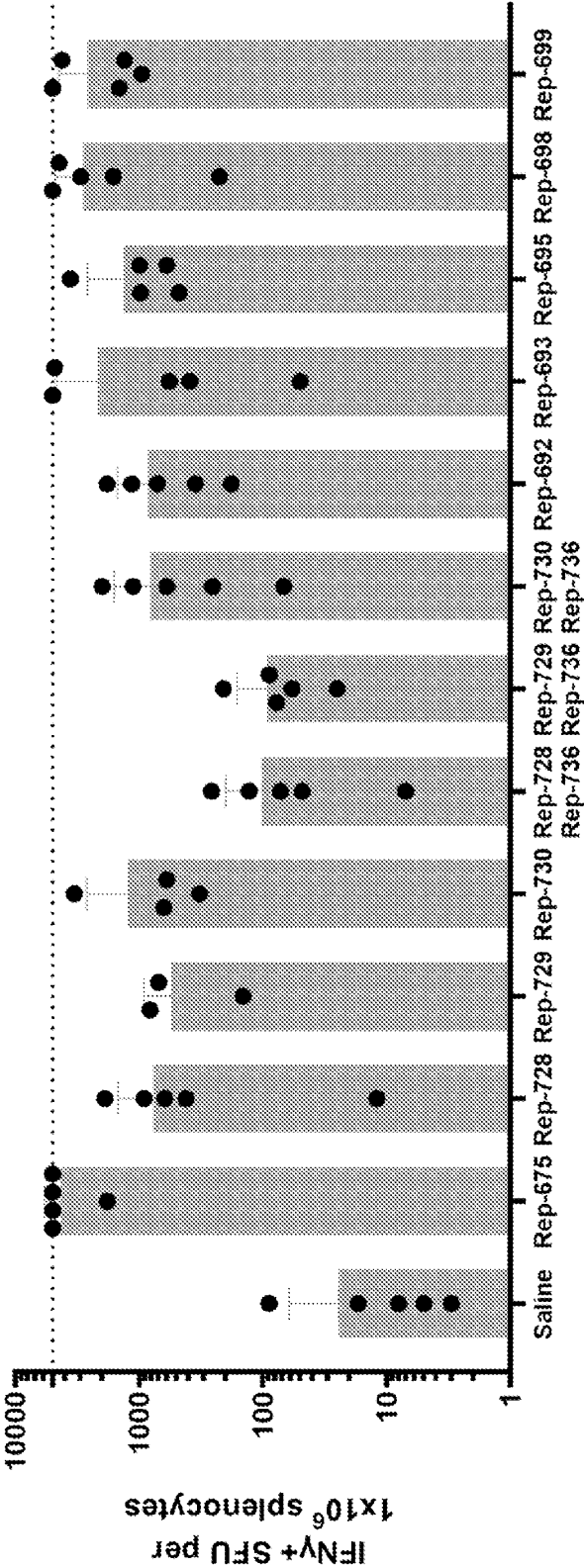


FIG. 7A

T cell responses to gH/gL, gp350, LMP2, BZLF1 in mice

gp350

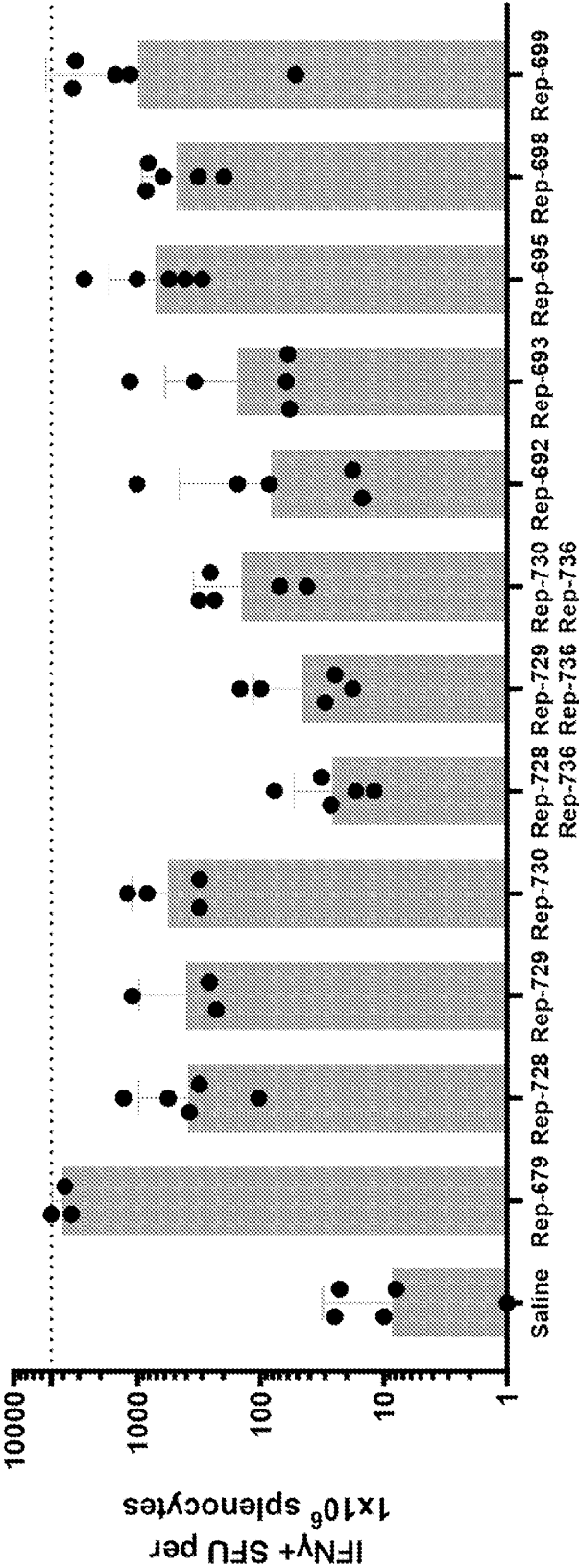


FIG. 7B

T cell responses to gH/gL, gp350, LMP2, BZLF1 in mice

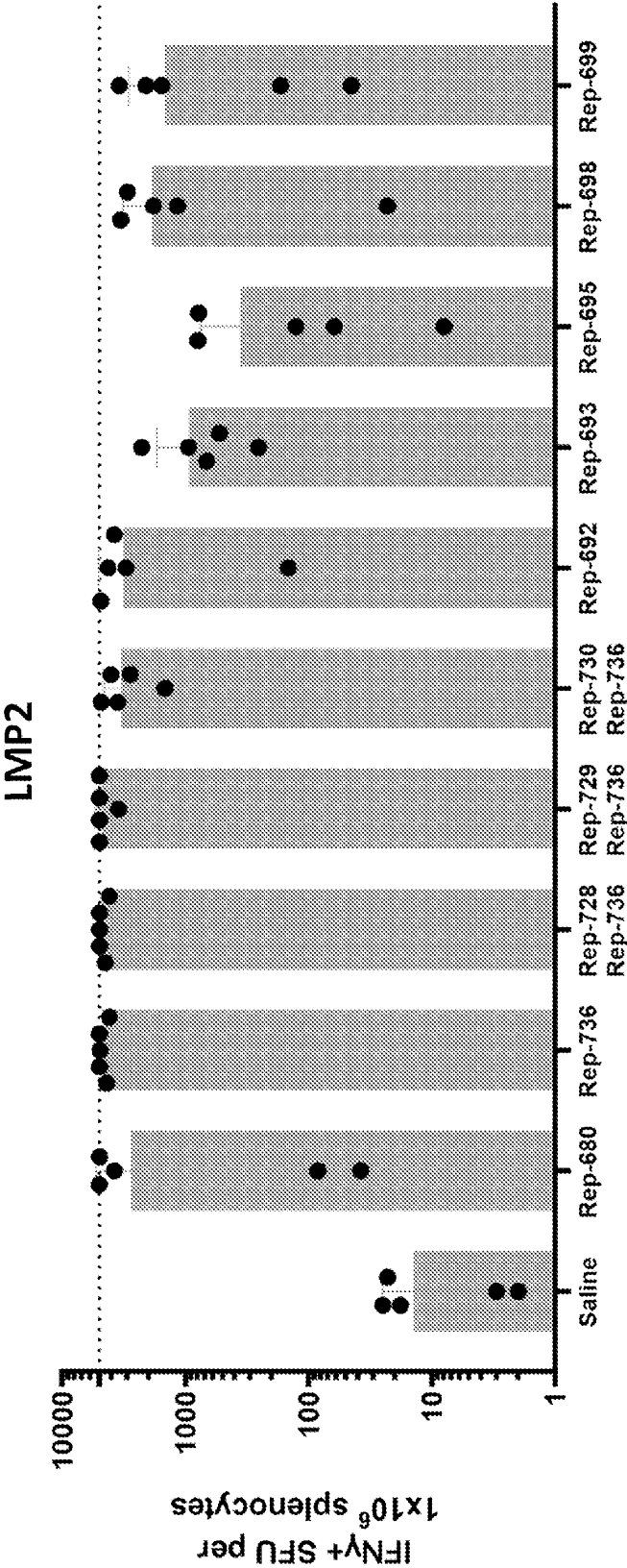
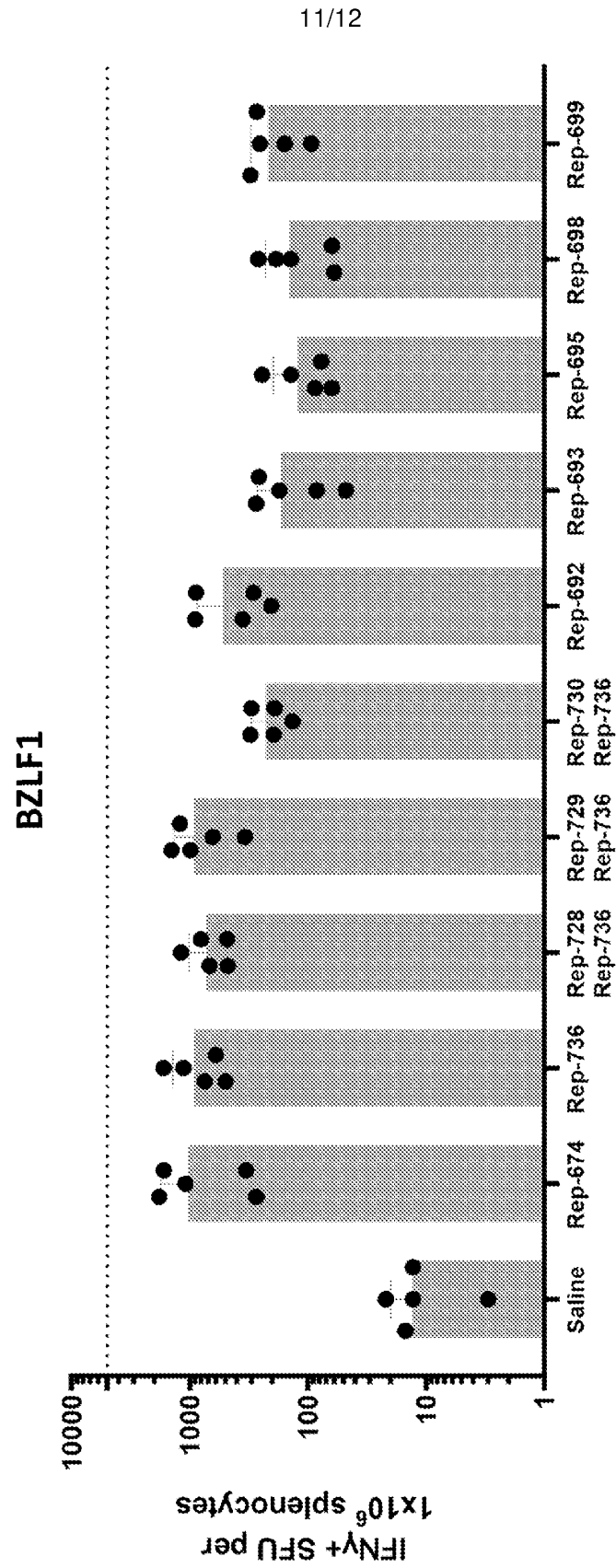


FIG. 7C

## T cell responses to gH/gL, gp350, LMP2, BZLF1 in mice



**FIG. 7D**

srRNA EBV vaccines neutralize viral entry into B cells

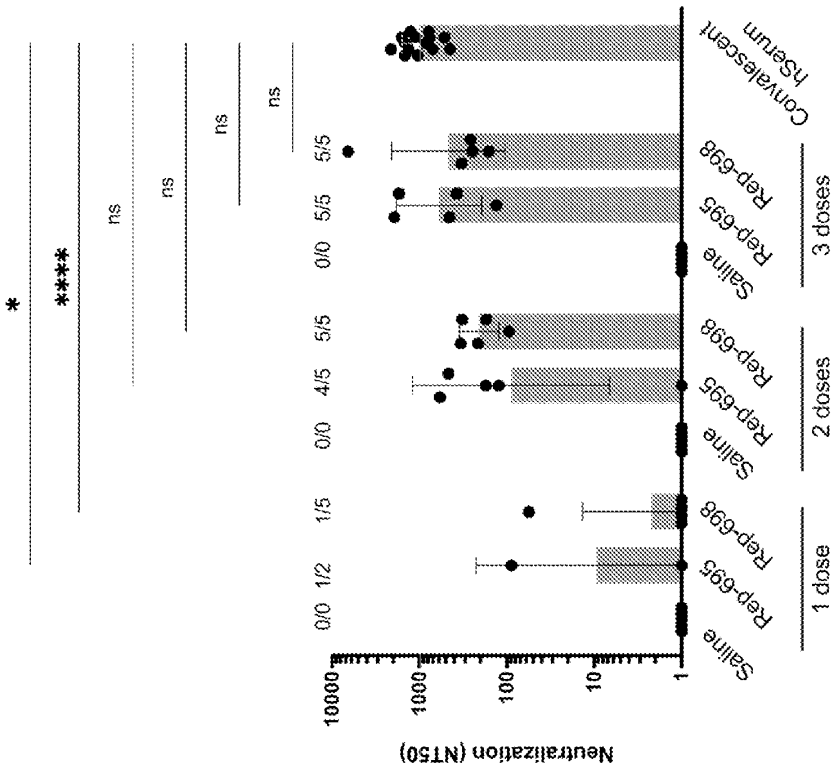


FIG. 8