Supplementary Figures:

Figure S1: Glycosylation status of recombinant EBOV GP
SDS PAGE gel (10%) run under reducing conditions demonstrates that enzymatic deglycosylation of 1µg purified, recombinant GP (purified protein: lane 1) with PNGase F results in reduction in size of both GP_1 (top band) and GP_2 (bottom band) (lane 2: 1µg GP, PNGase treated). The GP_2 band becomes more defined due to expected reduction in structurally distinct subspecies. Lane 3: Further deglycosylation using the EDEGLY kit (Sigma, St. Louis, MO) which used debranching enzymes Sialidase A, β(1-4)-Galactosidase and β–N-Acetylglucosaminidase in addition to PNGase F did not result in further size reduction of the two GP-subunits. This suggests that no O-linked glycosylation of the polypeptide has occurred, the expected finding for expression in *Drosophila* S2 cells.

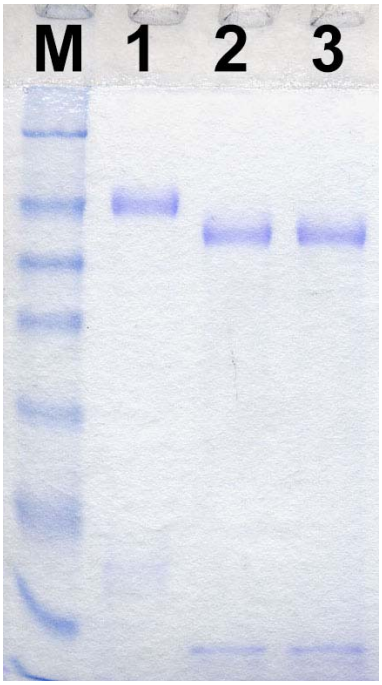


Figure S2: Glycosylation status of recombinant EBOV VP40

SDS PAGE gel (12%) run under reducing conditions demonstrates that enzymatic deglycosylation of 1µg purified, recombinant VP40 (purified protein: lane 1) with PNGase F results in reduction in size of recombinant EBOV VP40 (lane 2: 1 µg VP40, PNGase treated). Lane 3: Further deglycosylation using the EDEGLY kit (Sigma, St. Louis, MO) which used debranching enzymes Sialidase A, β(1-4)-Galactosidase and β–N-Acetylglucosaminidase in addition to PNGase F did not result in further size reduction of the VP40 protein. This suggests that no O-linked glycosylation of the polypeptide has occurred, the expected finding for expression in *Drosophila* S2 cells. While there are consistently two bands visible when analyzing recombinant VP40 on protein gels, it was confirmed that both bands reflect the same molecular weight (by MALDI-Tof analysis; Figure S3). This suggests that the bands result from different folding isoforms of the recombinant protein.

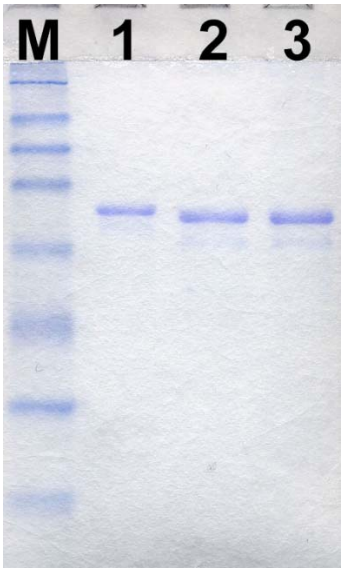


Figure S3: Mass spectrometry analysis of recombinant EBOV VP40
MALDI-Tof analysis reveals a major peak at 36.8 kDa consistent with the predicted molecular weight of polyprotein glycosylated on one internal glycosylation site (each glycosylation results in an addition of 1 kDa). As no second peak can be observed, the appearance of the second band on protein gels and Western blots appears to result from a portion of the protein being folded differently resulting in a difference in migration on a gel but no difference in actual molecular weight.

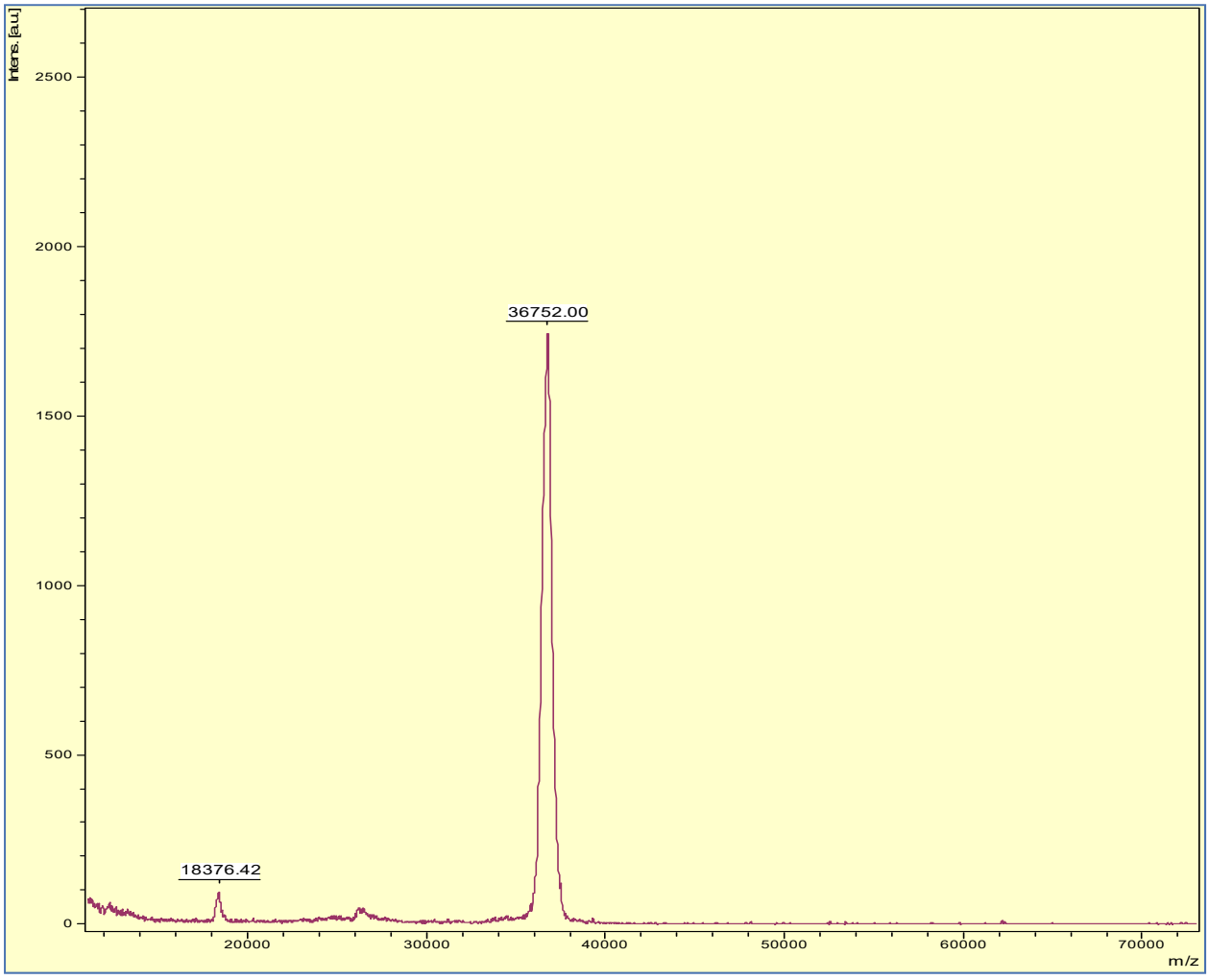


Figure S4: Mass spectrometry analysis of recombinant EBOV VP24
MALDI-Tof analysis reveals a major peak at 29.9 kDa with two smaller peaks at 28.9 and 30.9 kDa consistent with the predicted molecular weight of polyprotein glycosylated on one, two or three sites (each glycosylation results in an addition of 1 kDa).

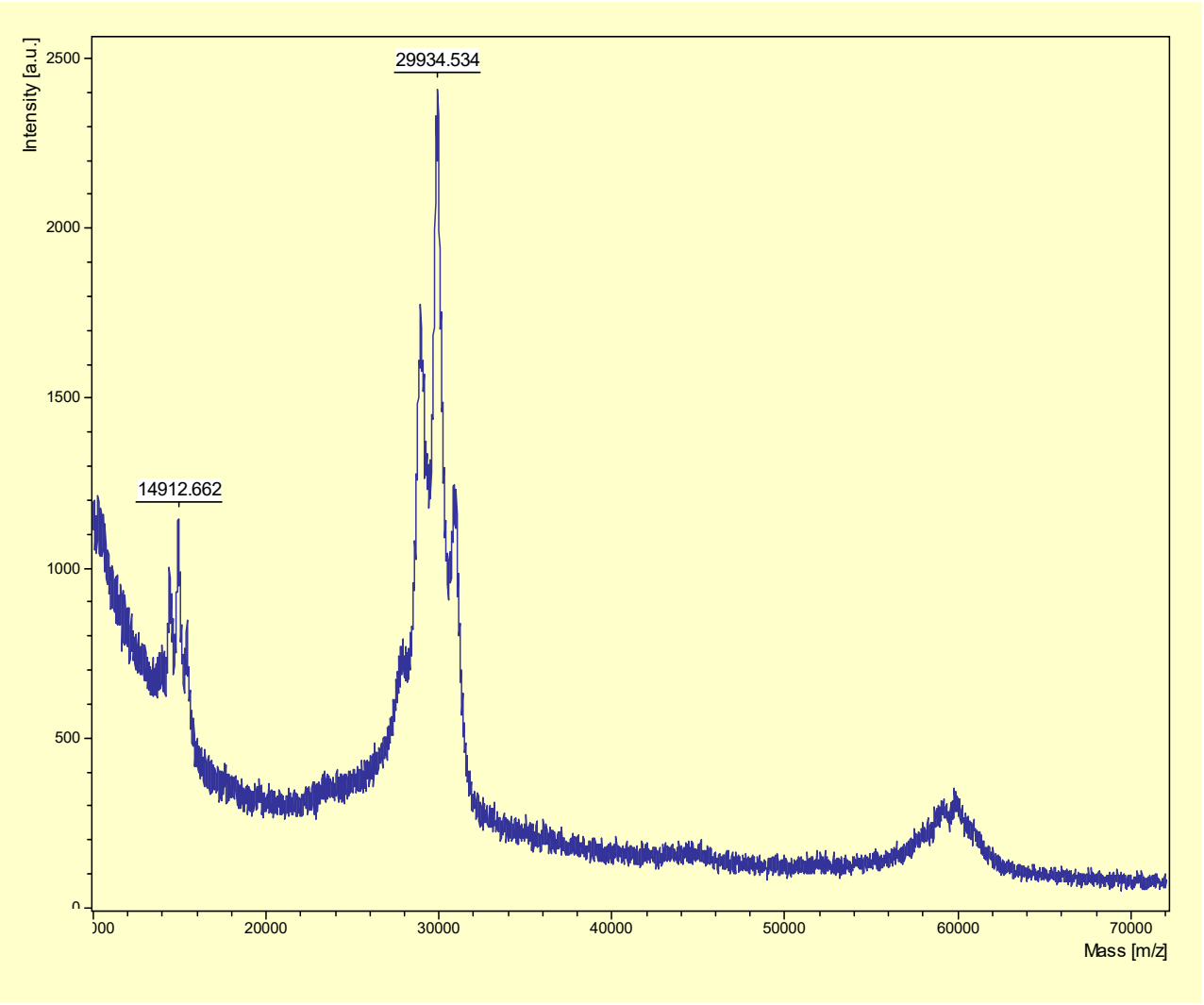
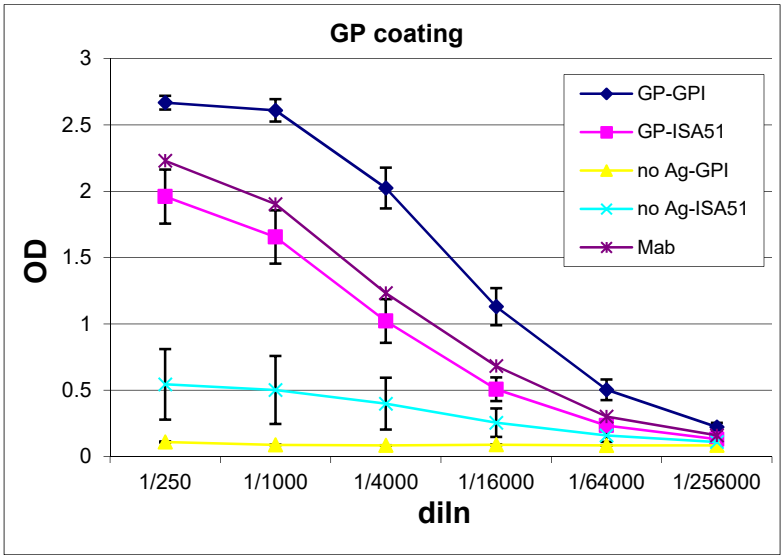
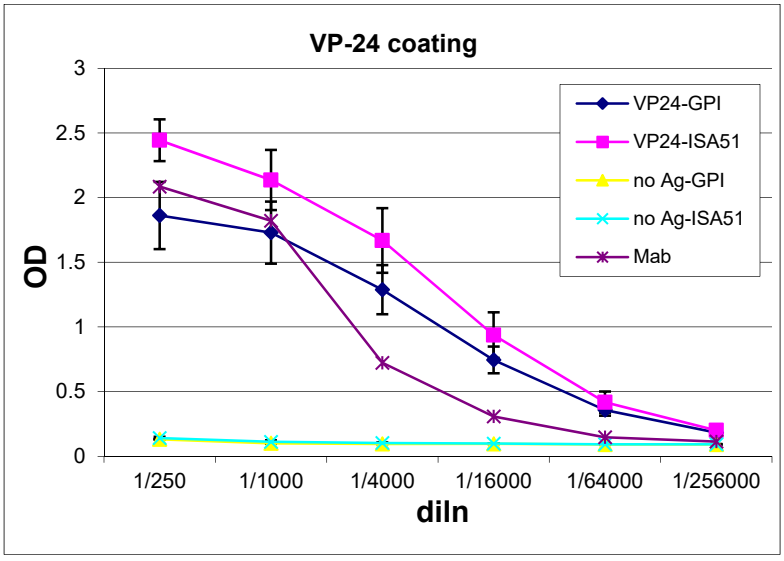


Figure S5. Antibody titration curves for mice immunized with GP, VP24, or VP40 with either GPI-0100 or ISA51 adjuvants, including control groups given adjuvant only
Panel A: ELISA plates coated with GP. Panel B: ELISA plates coated with VP24. Panel C: ELISA plates coated with VP40. Note negative control groups in all panels. Mab: antigen specific monoclonal antibodies used as positive controls.

Panel A: GP-specific IgG responses



Panel B: VP24-specific IgG responses



Panel C: VP40 specific IgG responses

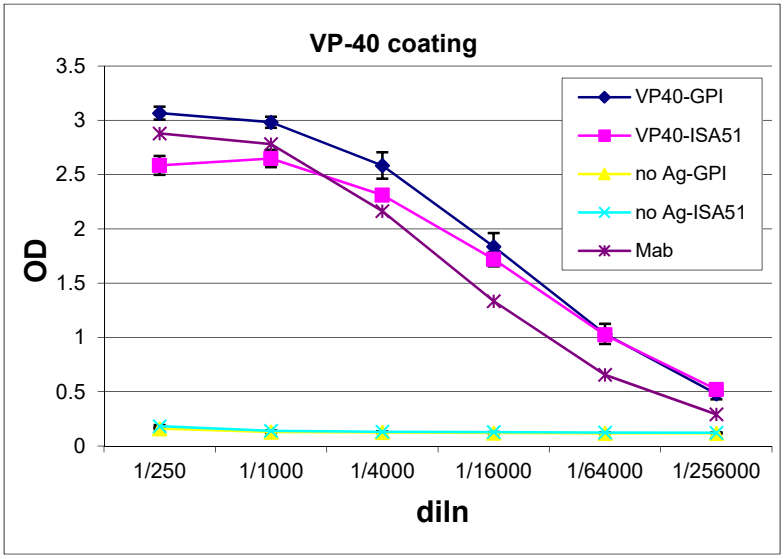
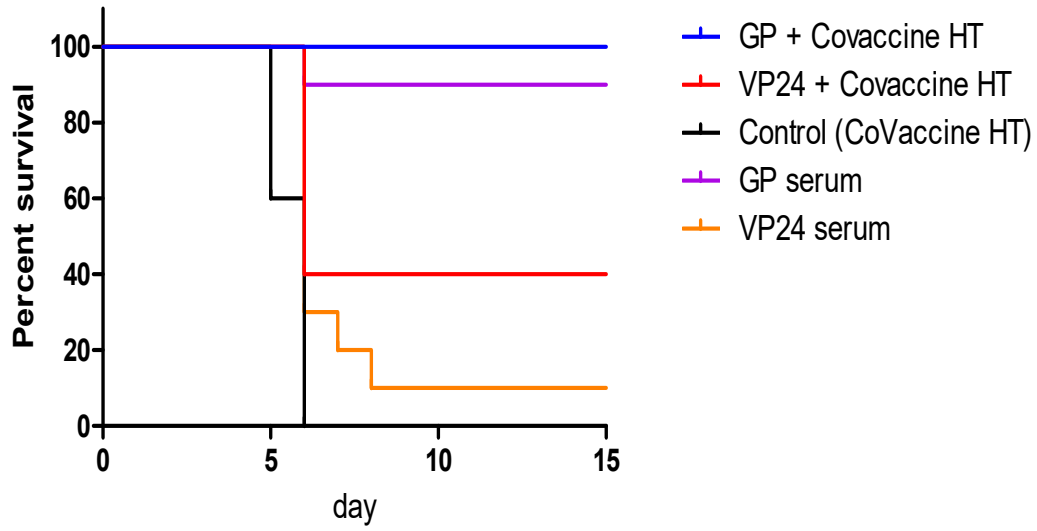


Figure S6. Kaplan-Meier survival plots of actively and passively immunized and challenged mice

Panel A: Mice actively immunized and passively immunized by serum transfer.

Panel B: Mice passively immunized by adoptive transfer of immune T cells.

A



B

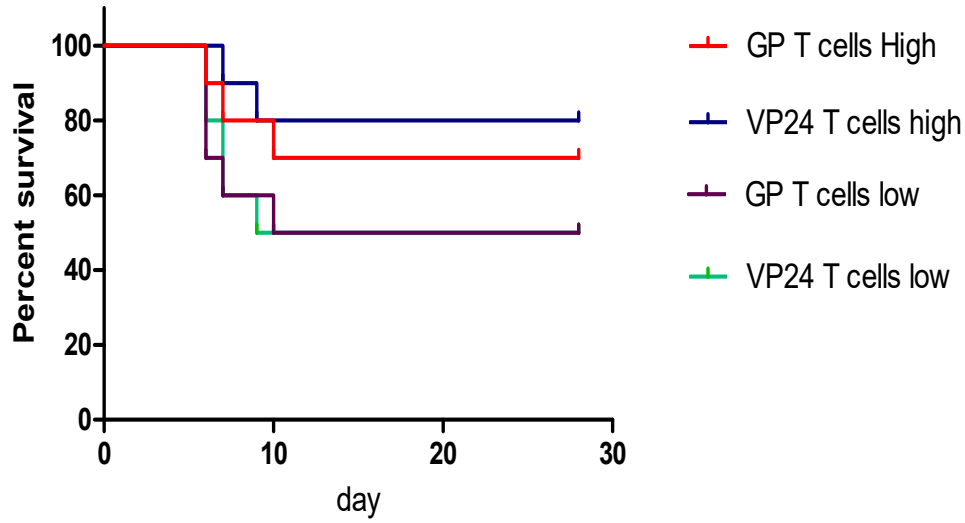


Figure S7. Weight change after challenge in actively or passively immunized mice and control mice