DNA-launched RNA replicon vaccines induce potent anti-Ebolavirus immune responses that can be further improved by a recombinant MVA boost

Running title: Replicon vaccines induce anti-Ebolavirus immune responses

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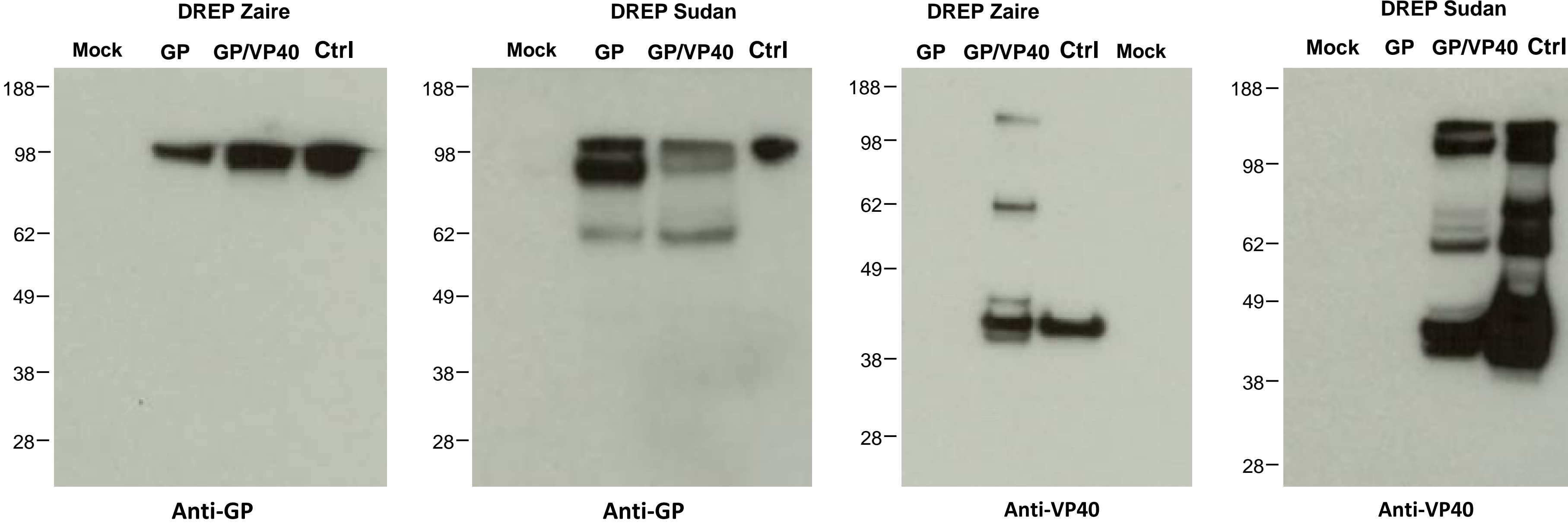
Supplementary figure 1.



Supplementary figure 1. Analysis of Ebolavirus GP and VP40 expression. Full-length (uncropped) blots presentation. BHK-21 cells were transfected using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. Cell lysates were harvested 24h later using ice-cold lysis buffer (20 mM HEPES pH 7.4; 110 mM potassium acetate; 2 mM magnesium chloride; 0.1% Tween 20; 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.5 M sodium chloride and Protease Inhibitors cocktail), and cell debris was removed by centrifugation at 4°C for 10 min at 14,000 rpm. Samples were then diluted in 4x Laemmli loading buffer (Bio-Rad), and run on a 12.5% SDS-PAGE before proteins were transferred onto a nitrocellulose membrane. Expression was detected using mouse anti-GP SUDV and EBOV antibodies (IBT Bioservices), mouse anti-VP40 SUDV antibody (IBT Bioservices), rabbit anti-VP40 EBOV antibody (IBT Bioservices) and appropriate secondary horseradish peroxidase (HRP) conjugated anti-Ig antibodies (Sigma). For the detection of HRP, Pierce ECL Western Blotting Substrate was used. Blots were then exposed to X-ray film and developed using AGFA Currix 60 processor after 30s exposure. Ebolavirus VLPs or recombinant Ebolavirus VP40 protein were used as positive controls, and non-transfected BHK-21 cells as negative control. No image processing (such as changing brightness and contrast) has been applied.