Protection against Marburg virus using a recombinant VSV-vaccine depends on T and B cell activation

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Supplementary figures



Figure S1. Lymphocyte frequency and MARV-specific T cell responses. Frequency of CD4 T cells (A), CD8 T cells (B) and CD20 B cells (C) in VSV-MARV vaccinated (positive control) animals (blue; n=4) and VSV-EBOV vaccinated (negative control) animals (red; n=3). (D) Frequency of MARV-specific T cells was measured using ELISPOT technology following stimulation of PBMCs collected after vaccination with overlapping peptide library covering MARV GP. Longitudinal changes were assessed using one-way repeated measures ANOVA test followed by Dunnett's multiple comparison post-test to determine differences between the time of vaccination (day 0) and subsequent days post vaccination. (E) Heatmap representing hierarchical clustering of samples and genes that were differentially expressed at 7 and/or 14 DPV. Range of colors is based on rlog transformation of read counts (red represents increased expression while blue represents decreased expression); each column represents 1 animal. (F) Violin plot of the log₁₀RPKM of DEGs 7 DPV that enriched to "immune system process" (as shown in Figure 2D) at 0 DPV, animal 3 at 7DPV (high responding animal), and animal 1 and 2 at 7 DPV (other responding animals).



Figure S2. ImmGen analysis of PBMCs 7 and 14 days post VSV-MARV vaccination. Heatmap showing expression profile of DEGs, with fold change > 2.5, detected 7 DPV (**A**) and 14 DPV (**B**) across various immune cell populations as predicted by ImmGen's MyGeneSet application. Red indicates high while blue indicates low likelihood of expression within indicated immune cell population.



Days post challenge

Figure S3. Plasma levels of cytokines, chemokines and growth factors. Plasma levels of IL-1 β , IL-6, IL-1Ra, MIP-1 α , TNF α , IFN- γ , IL-2, and FGF- β were measured using Luminex technology (VSV-EBOV n=3; VSV-MARV n=5). Longitudinal changes of cytokines were carried out using one-way repeated measures ANOVA test followed by Dunnett's multiple comparison post-test to determine differences between the time of challenge (day 0) and subsequent days post challenge; * denotes VSV-MARV animals, † denotes VSV-EBOV animals; * or † p < 0.05; †† p< 0.01; ††† p < 0.001.



Figure S4. Blood cell counts following challenge. (A, B) Counts of White blood cell (WBC) (VSV-EBOV n=4; VSV-MARV n=6) (A) and lymphocytes (LY) (VSV-EBOV n=4; VSV-MARV n=6) (B) throughout infection.



Figure S5. Principal component analysis post challenge. Principal component analysis of normalized transcript numbers in negative control animals and VSV-MARV vaccinated animals following MARV challenge. Each dot represents 1 biological replicate.