Supplementary Methods

Generation of MVA vaccines

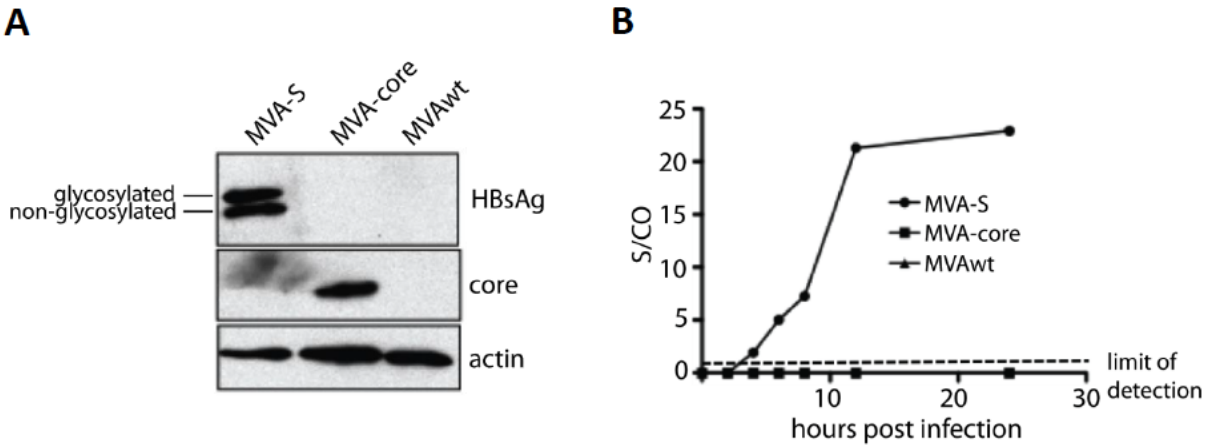
Recombinant MVA were generated by homologous recombination and host range selection as described previously [41]. The entire HBcAg (genotype D, subtype ayw) and HBsAg open reading frames (genotype D, subtype ayw or adw) were cloned into MVA transfer plasmids pIIIΔHR-PH5 or pIIIΔHR-P7.5, thereby placing the HBV proteins under the control of the early/late Vaccinia virus-specific promoters PH5 (HBcAg ayw /HBsAg ayw/HBsAg adw) or P7.5 (HBsAg ayw). After construction of each virus, gene expression, sequence of inserted DNA, and viral purity were verified. For generation of vaccine preparations, MVA were routinely propagated in CEF, purified by ultracentrifugation through sucrose, reconstituted in 1 mM Tris-HCL pH 9.0 and titrated following standard methodology [42].

Immunoblot

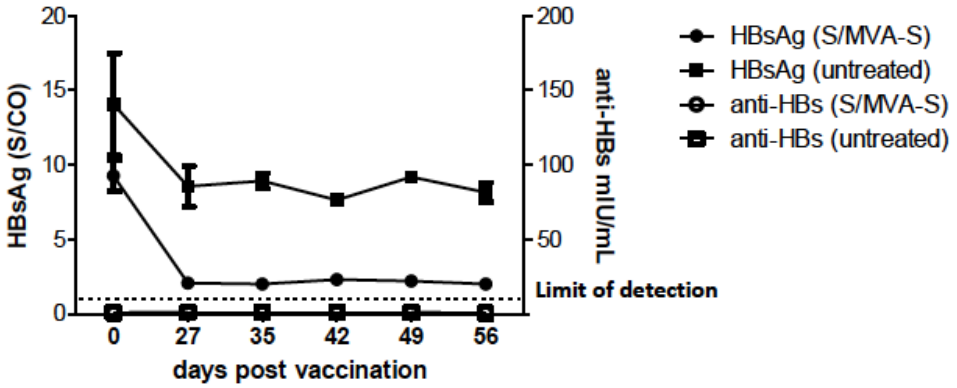
NIH-3T3 mouse fibroblasts (CRL-1658) were cultured in RPMI 1640 medium supplemented with 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were harvested in lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1% Nonidet P-40, 0.02% NaN3, and 100 µg/ml phenylmethylsulfonyl fluoride) 16 h post infection, dissolved on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and blotted onto a nitrocellulose membrane (0.45 µM; Bio-Rad, Munich, Germany). Membranes were incubated at 4°C with anti-HBc (antiserum H800; kindly provided by H. Schaller), anti-HBs (Murex HBsAg version 3; Abbott, Abbott Park, IL, USA) or anti-actin (Sigma, Munich, Germany) antibodies at 1:10000, 1:50 and 1:10000 dilutions, respectively. Horseradish peroxidase-labeled secondary mouse and rabbit antibodies (Dianova, Hamburg, Germany) were used at a 1:5000 dilution for 1 h at 21°C. Antibodies were diluted in phosphate-buffered saline containing 5% skim milk. Enhanced chemiluminescence was used as directed (Roche, Mannheim, Germany).

Secreted HBsAg in supernatant of cultured cells was determined using Abbott AxSYM HBsAg assay (Abbott Laboratories, Abbott Park, IL, USA).

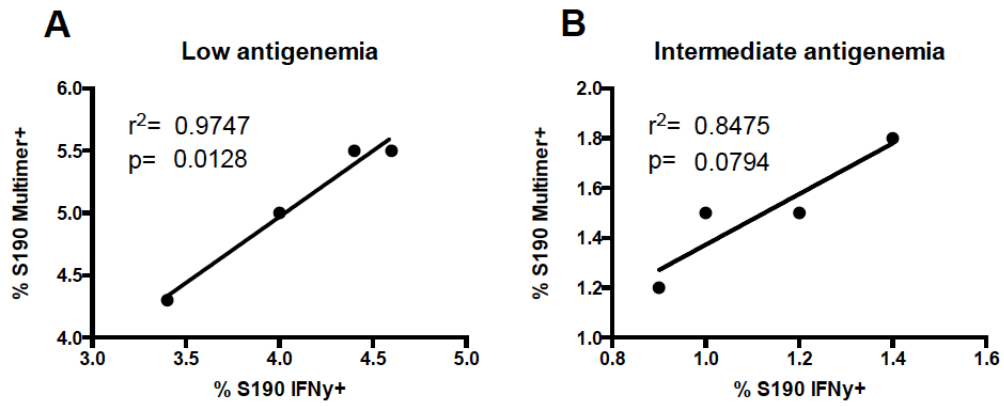
Supplementary figures



Supplementary Fig. 1: Expression of HBV antigens by MVA vectors. (A) and (B) Murine NIH-3T3 cells were infected with MVA-S, MVA-core or MVAwt (MOI of 10). 16 h post infection (A) total cellular lysates were analyzed for HBsAg and HBcAg expression by Western blot. (B) secreted HBsAg in the supernatant was determined by HBsAg-specific ELISA. S/CO: signal to cutoff.



Supplementary Fig. 2: Vaccination with CpG adjuvanted HBsAg. High-antigenemic HBVtg mice were immunized with 12 µg HBsAg containing CpG as adjuvant. On day 21, mice were boosted with MVA-S. On days 0, 27, 35, 42, 49 and 56 post prime immunization, sera were analyzed for levels of HBsAg and anti-HBs. S/CO: signal to cutoff.



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48 **Supplementary Fig. 3: Correlation of multimer and intracellular cytokine stainings of HBV**
49 **specific CD8+ T cells.** HBVtg mice were immunized with 12 µg HBsAg containing CpG as
50 adjuvant. On day 21, mice were boosted with MVA-S. HBV-specific T cell responses were
51 detected at day 28 by either S190 multimer staining or ICS after ex vivo restimulation with
52 peptide S190.
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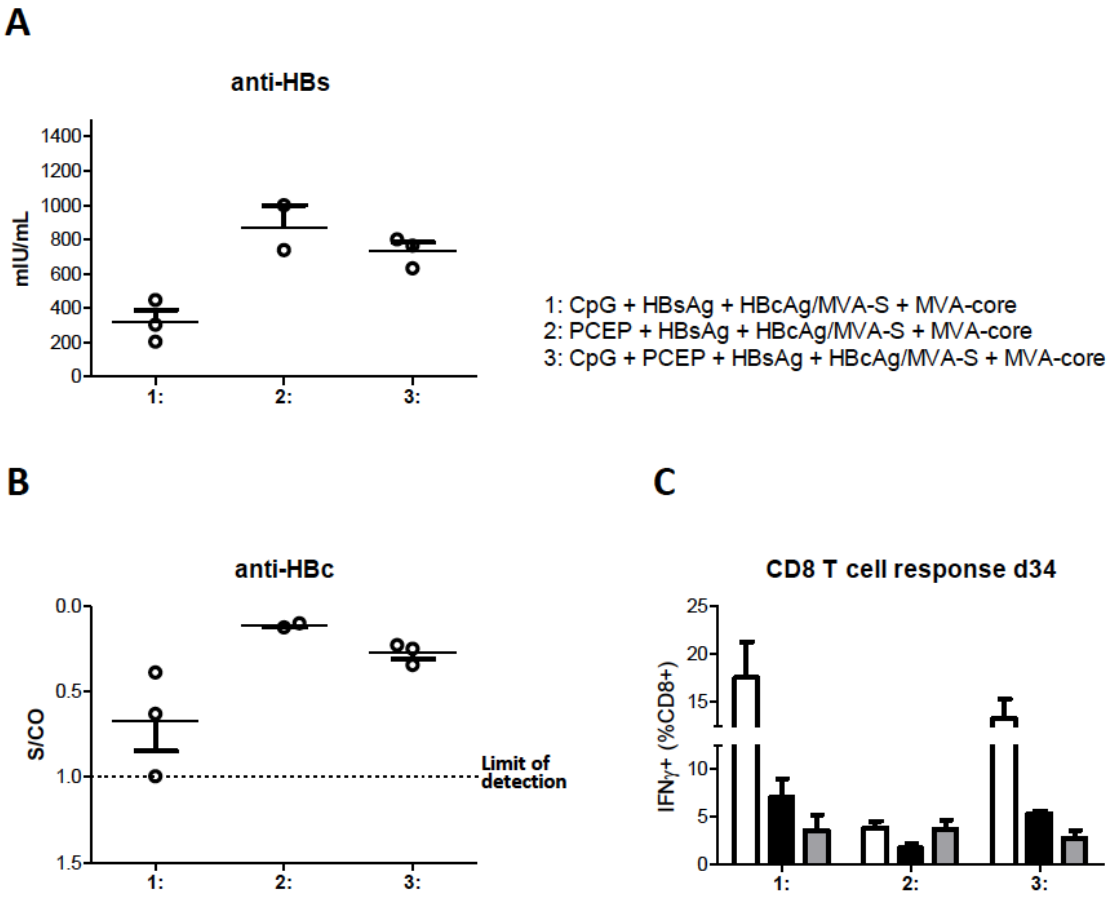

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56 **Supplementary Fig. 4: Comparison of adjuvants for protein-prime vaccination.** (A) to (C)
57 Wildtype mice were vaccinated with 16 µg HBsAg (subtype ayw) and 16 µg HBcAg (subtype

ayw) together with the indicated adjuvant(s) on days 0. On day 28, mice were boosted with MVA-PH5-S ($5x10^7$ i.u.; subtype ayw) and MVA-core ($5x10^7$ i.u.). On day 6 post boost sera were analyzed for presence of (A) anti-HBs and (B) anti-HBc. (C) On day 6 post boost splenocytes were analyzed by ICS after stimulation with HBsAg (S_{190} and S_{208adw})- or HBcAg (C_{93})-specific peptides. Bars show percentage (mean ± SEM) of CD8+ cells staining positive for IFNγ after background subtraction. i. u. infectious units

Reference :

[40] Staib C, Lowel M, Erfle V, Sutter G. Improved host range selection for recombinant modified vaccinia virus Ankara. Biotechniques 2003;34(4):694–6, 8, 700.

[41] Staib C, Drexler I, Sutter G. Construction and isolation of recombinant MVA. Methods Mol Biol 2004;269:77–100.