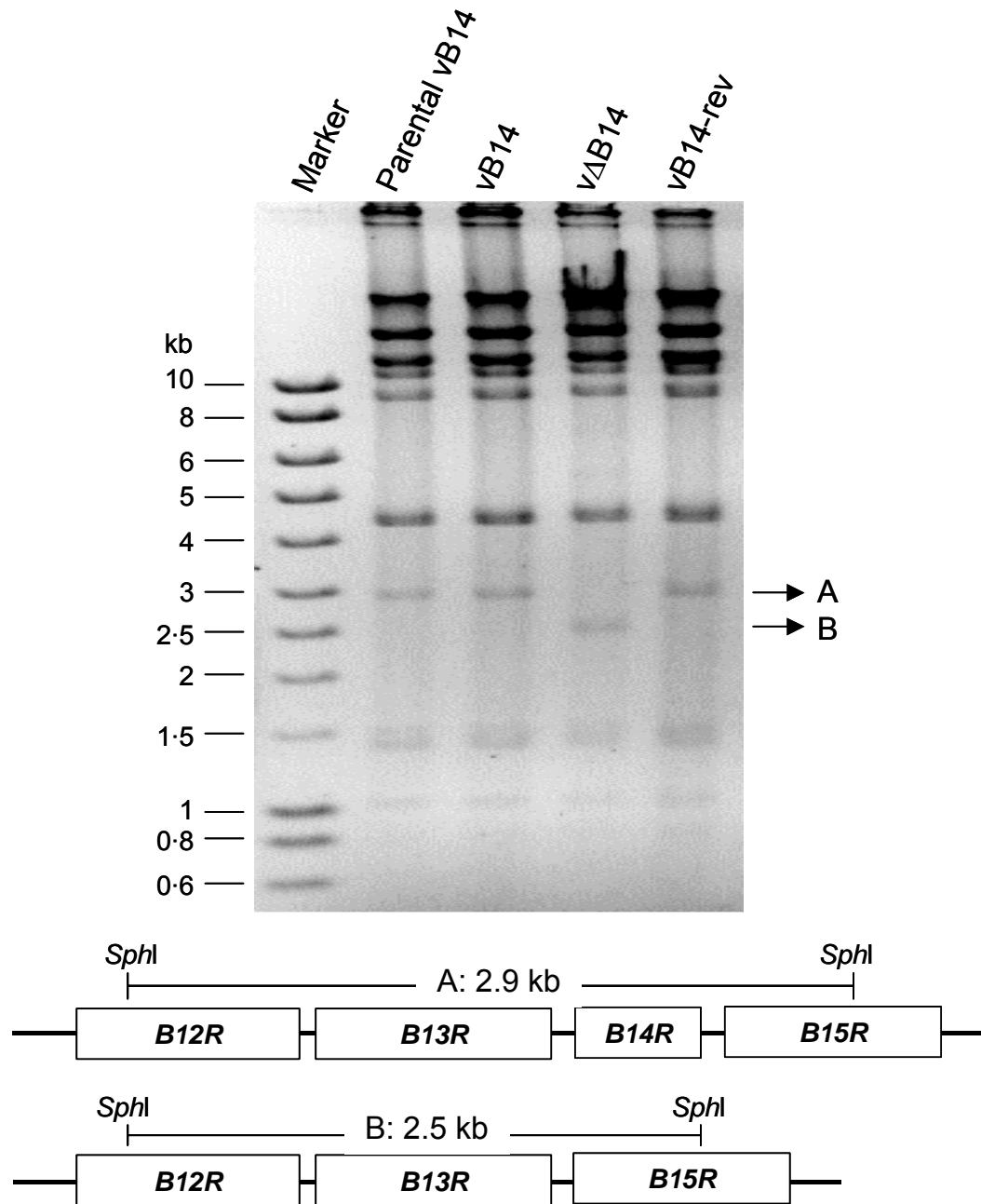


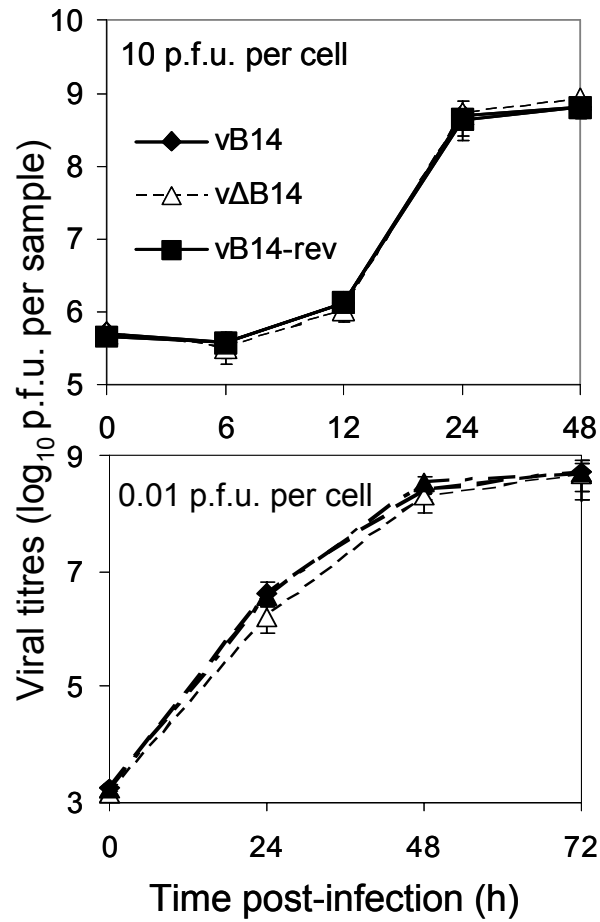
Supplementary Fig. S1. Analysis of recombinant VACV genomes by PCR. Virus DNA was extracted from cells infected with the indicated viruses or mock infected and used as template for PCR using L-FA and R-FB primers (Methods) flanking the *B14R* gene locus. PCR products were analysed by agarose gel electrophoresis. Arrows indicate the size of DNA fragments expected for the wild-type or deleted *B14R* gene alleles.

Chen, R. A.-J., Jacobs, N. and Smith, G. L. (2006). Vaccinia virus strain Western Reserve protein B14 is an intracellular virulence factor. *J Gen Virol* **87**.

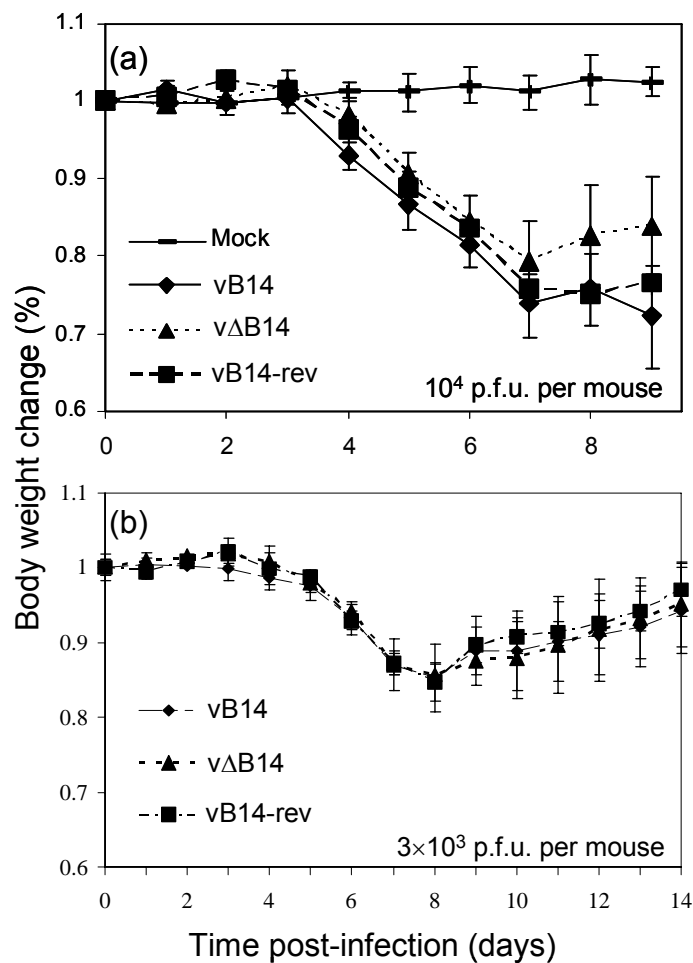


Supplementary Fig. S2. Characterization of the VACV genomes. DNA was extracted from purified intracellular mature virus for the indicated viruses and 10 μ g of each DNA was digested by *SphI* and the resulting DNA fragments were resolved on a 0.8 % agarose gel containing ethidium bromide. Arrows indicate the position of DNA fragments A (2.9 kb) and B (2.5 kb) containing the wild-type or deleted *B14R* alleles, respectively. A diagrammatic representation of the genome around the *B14R* gene is shown with the *SphI* cleavage sites marked. The parental virus was the initial viral stock to generate recombinant viruses.

Chen, R. A.-J., Jacobs, N. and Smith, G. L. (2006). Vaccinia virus strain Western Reserve protein B14 is an intracellular virulence factor. *J Gen Virol* **87**.



Supplementary Fig. S3. Growth kinetics of vΔB14. CV-1 cells were infected at either 10 (a) or 0.01 (b) p.f.u. per cell and aliquots of infected cells were collected at the indicated times p.i. Cells were frozen and thawed three times, sonicated and the virus infectivity was titrated in duplicate on BS-C-1 cell monolayers. Data are presented as the mean \log_{10} p.f.u. \pm SD.



Supplementary Fig. S4. The B14 protein did not affect virus virulence in a murine i.n. model under the conditions tested. (a) Mice were infected intranasally with 10^4 ($n=3$) (a) or 3×10^3 ($n=5$) (b) p.f.u. of vB14, vΔB14 or vB14-rev. The body weight of each mouse was monitored daily. The mean weight of each group of animals on each day is expressed as a percentage of the mean weight of the same group of animals on day zero. Data are presented as the mean weight \pm SD.