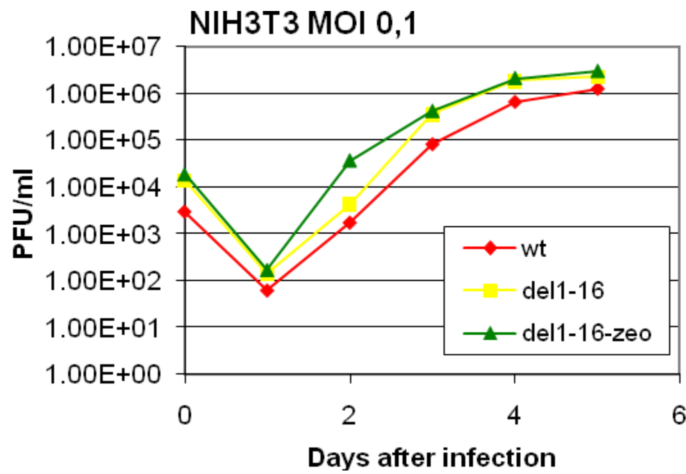
1 **Supplementary Table 1**

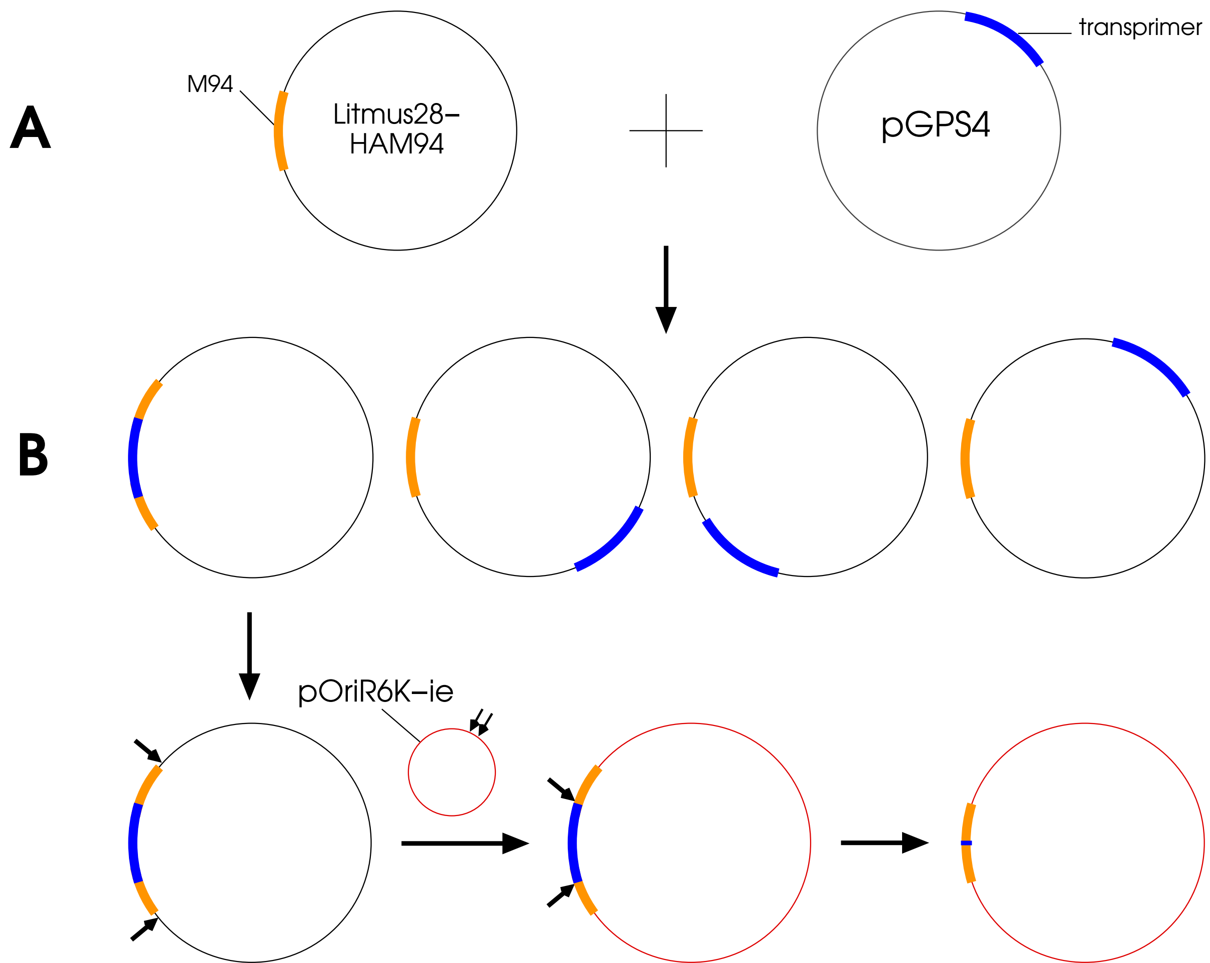
BAC	virus	description
pSM3fr-FRT	MCMV-FRT	Complete MCMV genome including FRT site between genes m16 and m17 (Bubic_Koszinowski-2004)
pSM3fr-Δ1-16-FRT	MCMVΔ1-16-FRT	MCMV genome deleted in genes m16 to m17 including FRT site between genes m16 and m17
pSM3fr-flox-ova-ΔM94	MCMVΔM94tTA	MCMV genome with m157 replaced by floxed ova-cassette and M94 replaced by tTA-cassette (Mohr_Sacher-2010)
pSM3fr-ΔM94-EPM94	MCMVΔM94-EPM94	MCMV genome with M94 replaced by kanamycin-cassette and ectopic insertion of M94 including its own promoter
pSM3fr-ΔM94-EHAM94	MCMVΔM94-EHAM94	MCMV genome with M94 replaced by kanamycin-cassette and ectopic insertion of N-terminal HA-tagged M94 under control of HCMV immediate early promoter enhancer (CMV-promoter)
pSM3fr-Δ1-16-RM94i7	MCMVΔ1-16-RM94i7	MCMV genome deleted in genes m01 to m16 and ectopic insertion of HA-M94 including 5 aa insertion at position 7 in the conditional expression cassette (Rupp_Koszinowski-2005) under control of the SVT-promoter
pSM3fr-Δ1-16-RM94i13	MCMVΔ1-16-RM94i13	MCMV genome deleted in genes m01 to m16 and ectopic insertion of HA-M94 including 5 aa insertion at position 13 in the conditional expression cassette (Rupp_Koszinowski-2005) under control of the SVT-promoter
pSM3fr-RM94i13	MCMV-RM94i13	MCMV genome with ectopic insertion of HA-M94 including 5 aa insertion at position 13 in the conditional expression cassette (Rupp_Koszinowski-2005) under control of the SVT-promoter
pSM3fr-Δ1-16-RHAM94	MCMVΔ1-16-RHAM94	MCMV genome deleted in genes m01 to m16 and ectopic insertion of HA-M94 in the conditional expression cassette (Rupp_Koszinowski-2005) under control of the SVT-promoter
pSM3fr-Δ1-16-EHAM94	MCMVΔ1-16-EHAM94	MCMV genome deleted in genes m01 to m16 and ectopic insertion of HA-M94 under control of the CMV-promoter

Supplementary Figure 1



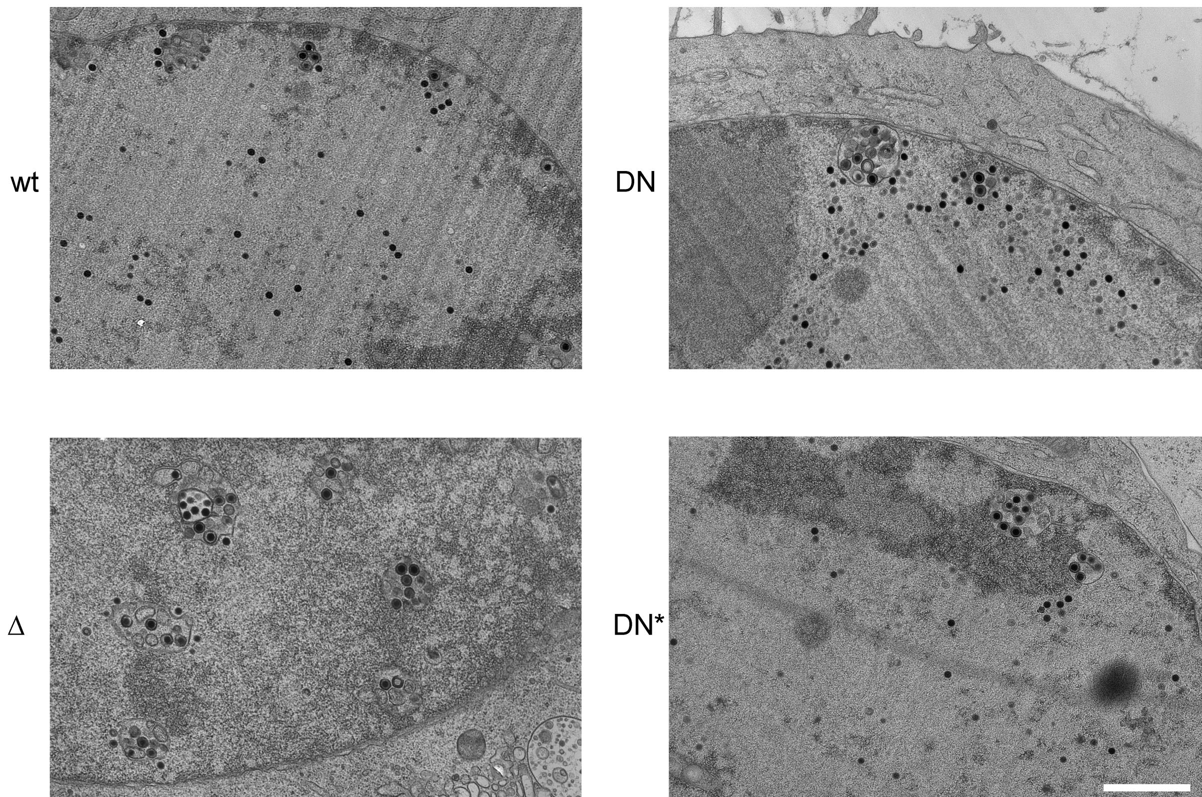
Growth kinetics of MCMV mutants deleted of the left terminal genome region. The release of infectious viral progeny at indicated timepoints was quantified under multistep growth conditions after infection of NIH/3T3 cells at a MOI of 0.1 with either wt BAC-derived wild type MCMV (wt), MCMVΔ1-16 (del1-16), or MCMVΔ1-16-zeo (del1-16-zeo). The latter was derived from pSMfr3-Δ1-16 BAC by the insertion of an empty pori6K-ie-zeo rescue vector. The results showed that there is no significant difference between the replication of wt MCMV and the deletion mutants lacking genes m1 to m16.

Supplementary Figure 2



Modified transposon mutagenesis. (A) The transprimer (blue) was excised from the donor vector pGPS4 and randomly inserted into the target vector Litmus28-HAM94, containing a HA-tagged M94 (orange). (B) The pool of mutants with random transprimer insertions in the HA-M94 gene was then subcloned into the expression vector pOriR6K-ie. Most of the transprimer sequence was subsequently removed by a digest with PmeI, leaving a 15 bp insertion.

Supplementary Figure 3



Ultra structural analysis of M94 mutants. NIH/3T3 cells were infected with MCMVΔ1-16-FRT (wt), MCMVΔM94tTA (Δ) and MCMVΔ1-16-RM94i13 in absence (DN) or presence (DN*) of Dox at a MOI of 1 followed by centrifugal enhancement. Cells were fixed 48 h after infection and prepared for electron microscopy by high-pressure freezing. The samples were freeze-substituted, plastic-embedded, thin-sectioned and analyzed by transmission electron microscopy. Representative examples are shown for the characteristic nuclear phenotypes. The scale bar represents 1 µm.