Table S1. Oligonucleotides used in this study

Pol Construct	Forward primer	Reverse primer
Plasmid (pBS)		
PolΔN43	5'-TAAGACACTAGTAT GAACTTTTACAACCCC-3'	5'-AATGAAGCTTTCAT GCTAGAGTATCAA-3'
PolΔN52	5'-TAAGATACTAGTATG GTCGGGACGCAA-3'	5'-AATGAAGCTTTCAT GCTAGAGTATCAA-3'
PolΔN67	5'-TAAGACACTAGTAT GTACTATAGCGAATGC-3'	5'-AATGAAGCTTTCAT GCTAGAGTATCAA-3'
PolΔN141	5'-TAGACACTAGTATG AACCCCACCGTCACC-3'	5'-AATGAAGCTTTCAT GCTAGAGTATCAA-3'
PolA_6	5'-CCCCCGCCTTGTTTGAG GCAAAACGCCGCCAACCCCGCCG CCGCCCCAGTCGGGACG-3'	5'-CGTCCCGACTGGGGCGG CGGCGGGGTTGGCGGCGTTTTGCC TCAAACAAGGCGGGGG-3'
	5'-CAAAACGCCGCCGCCG CCGCCGCCGCCC-3'	5'-GGGCGGCGGCGGCGGC GGCGGCGTTTTG-3'
PolΔC1216	5'-GAAGACACTAGTATG TTTTCCGGTGG-3'	5'-AATGAAGCTTTCAG CCGGCACC-3'
BAC		
*pol*ΔN43	5'-TCCCCCCTCTTTAGGGGT TCGGGTGGGAACAACCGCGATGA ACTTTTACAACCCCTACCTTAGGG ATAACAGGGTAATCGATTT-3'	5'-GTTGCGTCCCGACTGGG GCGAGGTAGGGGTTGTAAAAGTTC ATCGCGGTTGTTCCCACCCGCCAG TGTTACAACCAATTAACC-3'
*pol*ΔN52	5'-TCCCCCCTCTTTAGGGGT TCGGGTGGGAACAACCGCGATGG TCGGGACGCAACAGAAGCCTAGG GATAACAGGGTAATCGATTT-3'	5'-GGCGCTGGGTTGGCCCGG TCGGCTTCTGTTGCGTCCCGACCA TCGCGGTTGTTCCCACCCGCCAGT GTTACAACCAATTAACC-3'
*pol*ΔN52R	5'-GCTATAGGGACCCCCGC CTTGTTTGAGGCAAAACTTTTACA ACCCCTAGGGATAACAGGGTAAT CGATTT-3'	5'-GCTATAGGGTCCCGCCAG TGTTACAACCAATTAACC-3'
	5'-TCCCCCCTCTTTAGGGGT TCGGGTGGGAACAACCGCGATGT TTTCCGGTGGCGG-3'	5'-GGCGCTGGGTTGGCCCGG TCGGCTTCTGTTGCGTCCCGACTG GGGCGAGGTAGG-3'
polA_6	5'-CGGAGCCGGCCGGGGAC CCCCGCCTTGTTTGAGGCAAAAC GCCGCCGCCGCCGCCGCCCCC AGTCGGGACGCAACATAGGGATA ACAGGGTAATCGATTT-3'	5'-GGGTTGGCCCGGTCGGCT TCTGTTGCGTCCCGACTGGGGCGG CGGCGGCGGCGGCGGCGTTTTGCC TCAAACAAGGCGGCCAGTGTTACA ACCAATTAACC-3'
polA_6R	5'-CGGAGCCGGCCGGGGAC CCCCGCCTTGTTTGAGGCAAAAC TTTTACAACCCCTACCTCGCCCCA GTCGGGACGCAACATAGGGATAA CAGGGTAATCGATTT-3'	5'-GGGTTGGCCCGGTCGGCT TCTGTTGCGTCCCGACTGGGGCGA GGTAGGGGTTGTAAAAGGTTTTGC CTCAAACAAGGCGGCCAGTGTTAC AACCAATTAACC-3'
*pol*ΔN141	5'-TCCCCCCTCTTTAGGGGT TCGGGTGGGAACAACCGCGATGA ACCCCACCGTCACCGTCTTTAGG GATAACAGGGTAATCGATTT-3'	5'-CCAGGATGTCGTACACG TGAAAGACGGTGACGGTGGGGTT CATCGCGGTTGTTCCCACCCGCCA GTGTTACAACCAATTAACC-3'

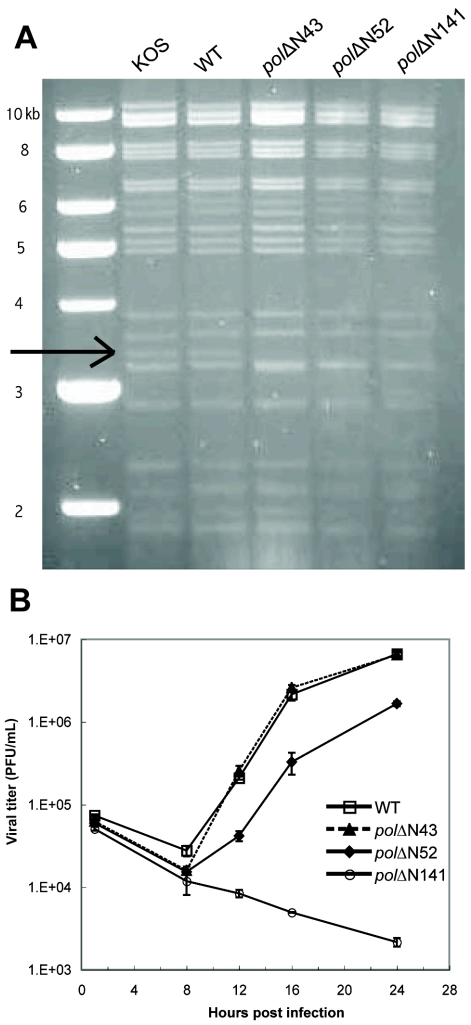

Figure S1. Growth kinetics of *pol* mutant viruses. (A) Restriction fragment polymorphism analysis. Purified viral DNA from KOS and reconstituted BAC-derived viruses was digested with BamHI and electrophoresed on a 0.8% agarose gel. The arrow indicates a 3.3kb band in the KOS and WT lanes that undergoes a mobility shift respective to the deletion present at the 5' end of the HSV-1 *pol* gene in each mutant virus. (B) Single cycle growth assay. Vero cells were infected with the indicated BAC-derived viruses at MOI=10 and whole cell lysate was harvested at the indicated time points. Lysate was thawed and sonicated prior to titration on polB3 cells. Viral yield is reported as PFU/mL with each data point representing the mean ± SD of triplicate samples.

Table S2. Primers for real-time PCR assays

Gene	Type	Name	Sequence
tk	forward[a,c]	tk-1	5'-CTTAACAGCGTCAACAGCGTGCCG -3'
	reverse[a,d]	tk-rev	5'-CCAAAGAGGTGCGGGAGTTT-3'
α-galactosyltransferase	forward[b,e]	vero-1	5'-ACACCCTAGGCCAGTCAGTG-3'
	reverse[b,e]	vero-2	5'-TGCATGCTGCTGACTCTTTC-3'

[a] For real-time PCR of viral DNA
[b] For real-time PCR of cellular DNA
[c] MF Kramer and DM Coen, J Virol 1995
[d] JM Pesola et al., J Virol 2005
[e] JM Pesola and DM Coen, unpublished data

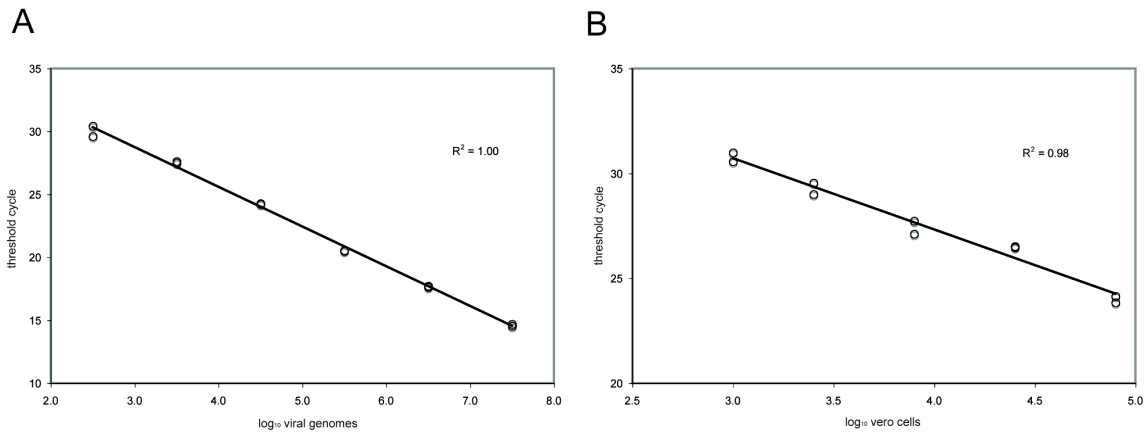

Figure S2. Standard curves for real-time PCR assays. This figure shows representative standard curves that were generated from real-time PCR assays and used to quantify the level of viral DNA copies harvested from infected cell lysates. For each graph, the individual values represent the threshold cycle from duplicate wells for each indicated standard sample. (A) Viral DNA standards. Ten-fold serial dilutions of purified viral DNA were spiked into mock-infected Vero lysate. (B) Cellular DNA standards. Standards were generated from three-fold serial dilutions of mock-infected Vero lysate and were used to normalize the number of viral genome copies detected in each real-time PCR reaction. Viral and cellular DNA samples were processed alongside experimental samples in order to account for the efficiency of DNA isolation from mock- and HSV- infected cell lysates. For all runs R^2 values were ≥0.98, indicating the reproducibility and quality of these quantitative assays.