SUPPLEMENTARY DATA

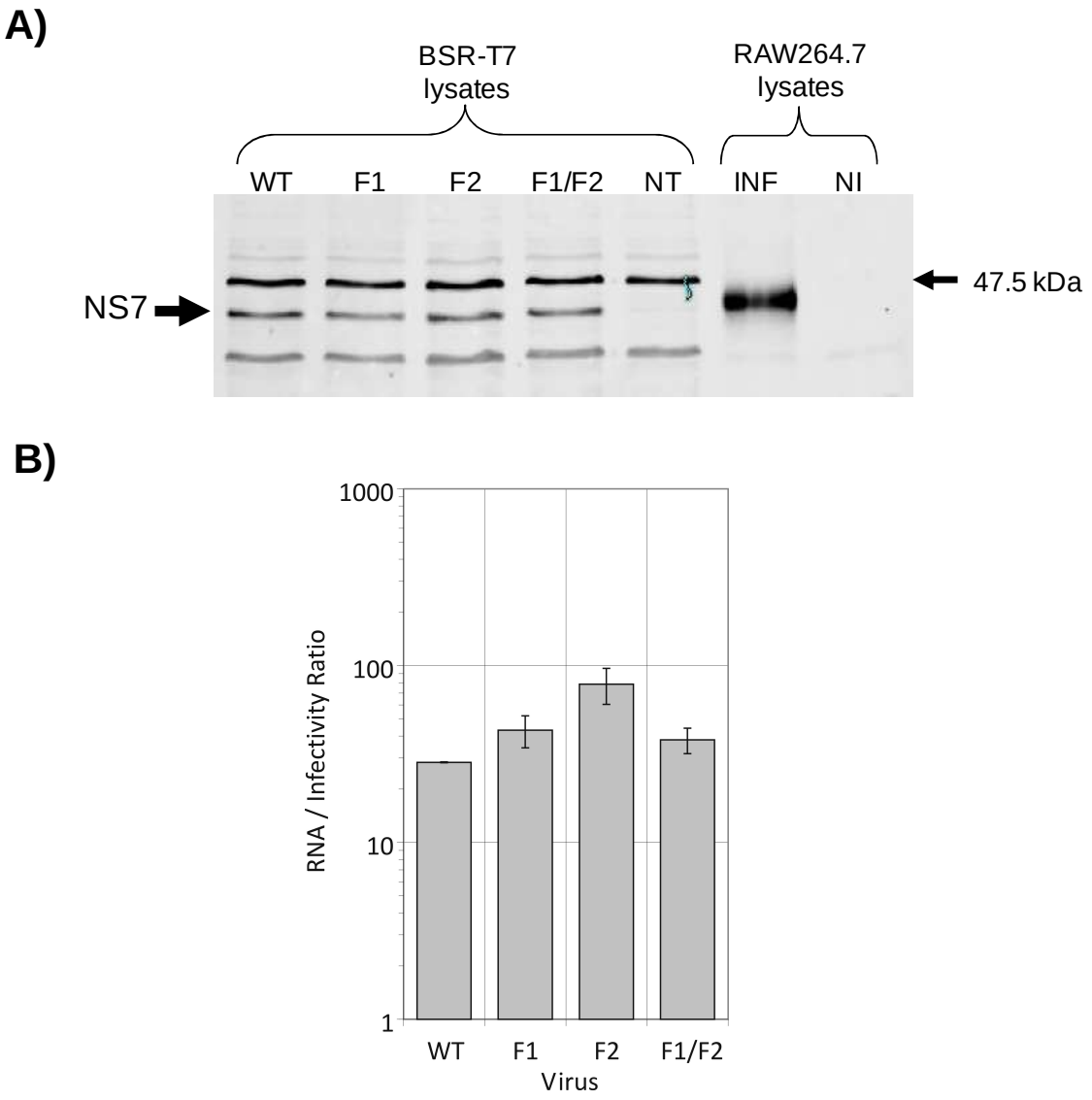
TABLE S1

PRIMERS USED FOR AMPLIFICATION OF MNV RNA SEQUENCES

Assay	Pos/Orient	Sequence (5'-3')
Region 1 PCR	1081F	GCATGGCCTTCGGTCTGACATC
	1121F	GCTTGCCTCCACCAACTCGG
	2500R	TGGTGGTAGATAGAGAAGGCTGT
	2650R	CGCCCTCGGCCCTTCTTGTTCTTACCCTTCTTGC
Region 2 PCR	2902F	GCAAGCCAATTGACTGGAATGTGGTTGGCCCC
	2961F	GACTACGGTGAGAAGATCAGCTTCG
	4128R	CGCGAATCATGGTGCCAAGGTCAGAGCC
	4155R	CACAGAAGGGGCCAAAAGCGCGAGC
Region 3 PCR	2342F	CATGGTTGAGAAAGTCAAGGAGC
	2412F	GCCCGGCTCCGCTGTTATTGC
	3099R	CGGTGCCCTTGGGTGGCGCCACATG
	3035R	CCATCCCGTGCCGAACTGTACGACG
Reverse transcription	7350R	AAAATGCATCTAAATACTACTAAAAGAAAAGAGC
Quantitative PCR	5380R	GAACCTCCATGTTCCCAACCCAGCCGGTGTACATGG
	5028S	CCGCAGGAACGCTCAGCAGT
	5177R	GCAAGAGCCGCGCCAGCCACGGGCTGAATGGGGACGGCCT
	5077R-Probe	CTGCGCCATCACTCAT
	18sS	GTAACCCGTTGAACCCCATTC
	18sR	ACCATCCAATCGGTAGTAGCG
	5275F	ACCCCAGGTGAAATACTGTTTG
	5452R	TGGGAAAATAGGGTGGTACAAG
Allele-specific PCR (Region 1)	2141F	TGCTGGCAAGGTTACCGCCTTC
	2650R	CGCCCTCGGCCCTTCTTGTTCTTACCCTTCTTGC
	2206F	CAGCGATTGGCTACAAGGCCATGGGCTGCACCTG
	2401R_F1	CGCCTGACGCAGTTCACCAGTG
Digest PCR (Region 1)	1081F	GCATGGCCTTCGGTCTGACATC
	1121F	GCTTGCCTCCACCAACTCGG
	1522R	GGCCTGCCAGAGACCATAATCAC
	2650R	CGCCCTCGGCCCTTCTTGTTCTTACCCTTCTTGC
Allele-specific PCR (Region 2)	2902F	GCAAGCCAATTGACTGGAATGTGGTTGGCCCC
	4155R	CACAGAAGGGGCCAAAAGCGCGAGC

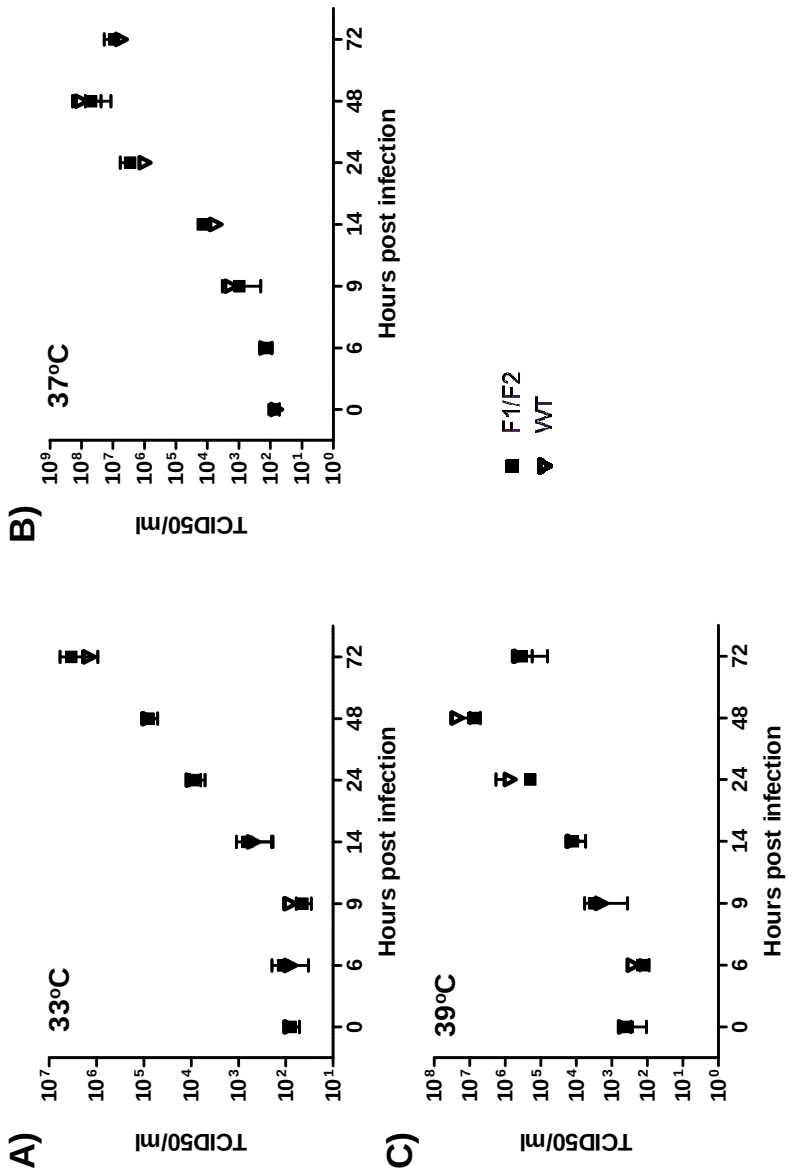
	3678F	GCTTACCTGGGYTCCAAGGATGAGAG
	3846R_F2	GGGCTCCAGCGTATTCTCAAGT
Digest PCR (region 2)	2902F	GCAAGCCAATTGACTGGAATGTGGTTGGCCCC
	3678F	GCTTACCTGGGYTCCAAGGATGAGAG
	4128R	CGCGAATCATGGTGCCAAGGTCAGAGCC
	4155R	CACAGAAGGGGCCAAAAGCGCGAGC

FIGURE S1



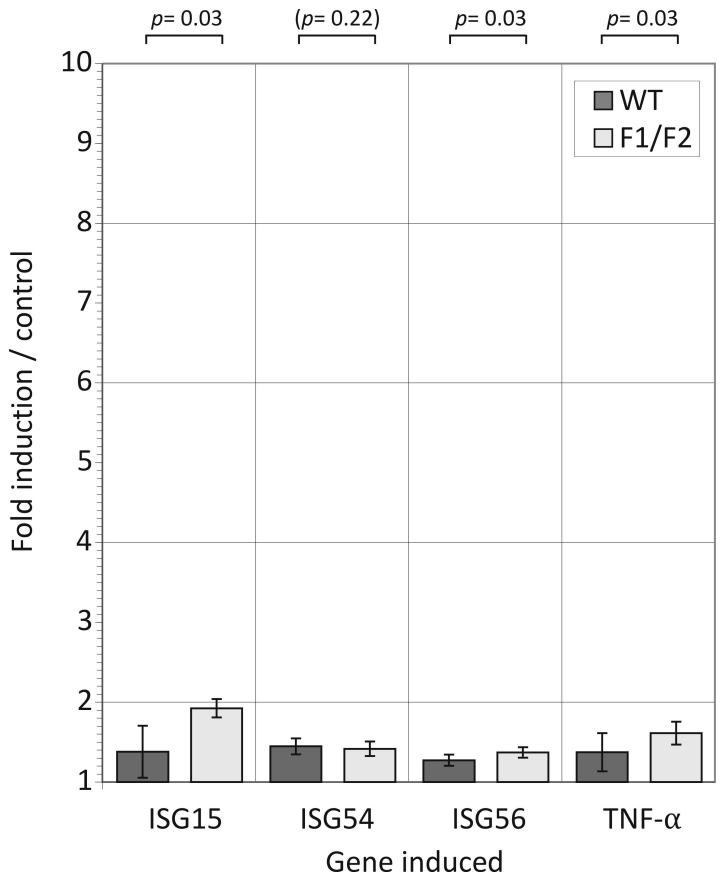
(A) Western blot detection of NS7 (viral RNA polymerase) expressed in BSRT-7 cells after transfection with WT and RNA-structure disrupted mutant cDNA. Infected and non-infected RAW264.7 lysates acted as an assay control. (B) Infectivity to RNA ratios was measured by extracting RNA from equal amounts of viral particles and quantifying viral copy numbers by qPCR.

FIGURE S2



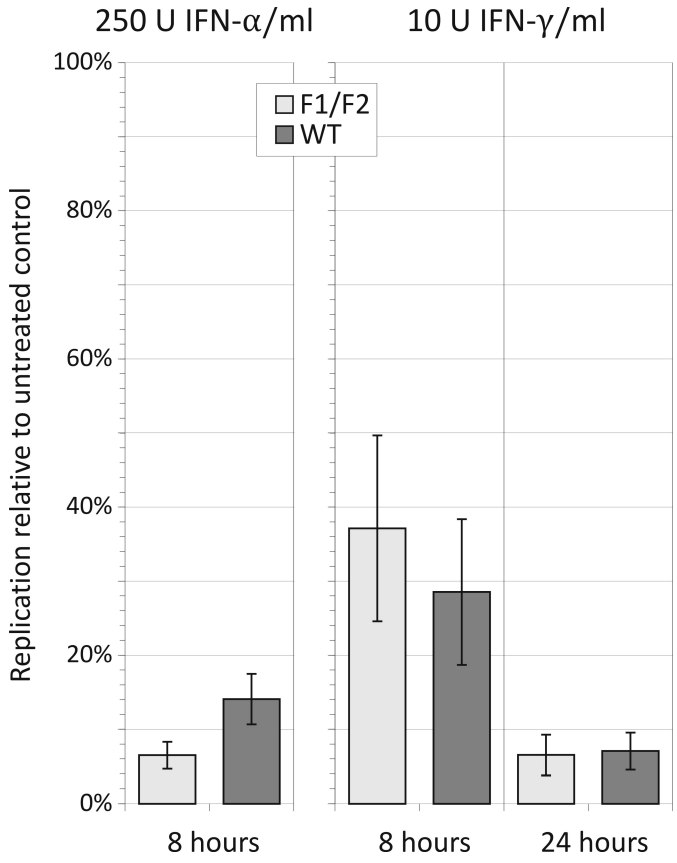
Influence of incubation temperature on the replicaion of WT and F1/F2 viruses. Multistep growth curves (m.o.i of 0.01) were performed in RAW264.7 cells at (A) 33^{o}C, (B) 37^{o}C and (C) 39^{o}C. Infectivity titres expressed as TCID50s / ml were determined at several time points post-infection..

FIGURE S3A



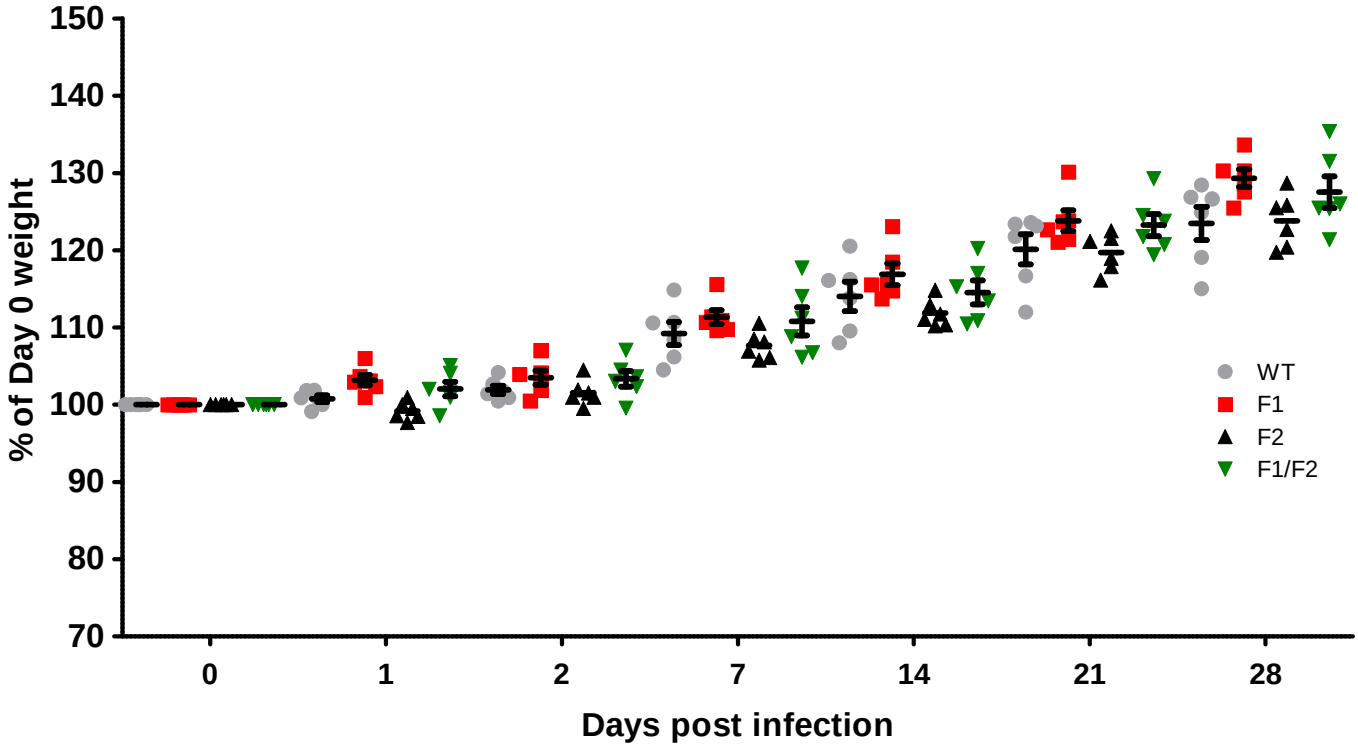
Low level induction of ISGs 15, 54 and 56 and TNF-α in RAW264.7 infected with MNV3 WT or F1/F2 mutants at an m.o.i. of 1 and assaying mRNA levels by qPCR at 8 hours. All assays were performed in triplicate (mean values and SEMs shown by bars and error bars).

FIGURE S3B



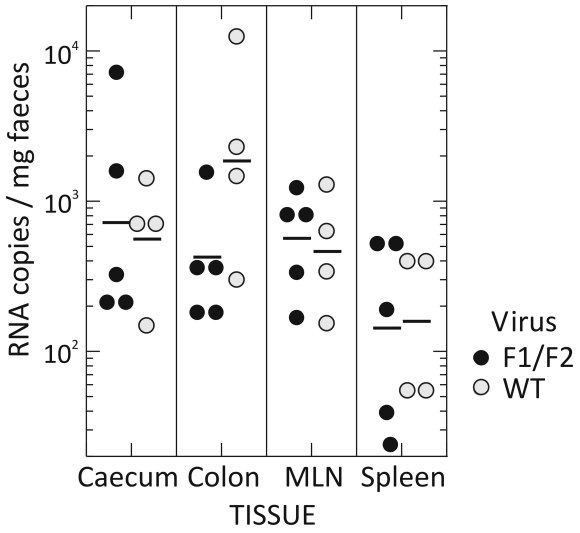
Sensitvity of WT and F1/F2 variants of MNV to IFN-α and IFN-γ shown as the proportion of RNA levels in IFN-treated cells compared to the untreated control. No significant differences between replication rates of WT and F1/F2 variants were detected (student T test, $p > 0.05$ for all comparisons)

FIGURE S4



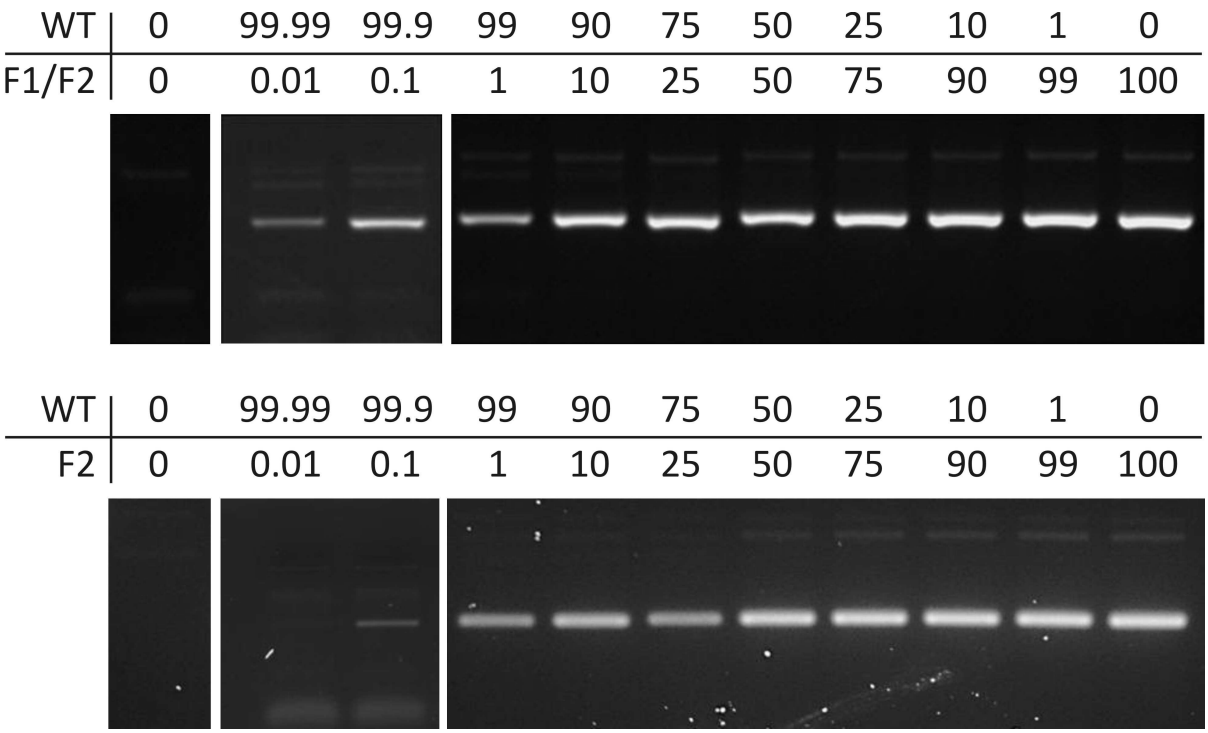
Change in weight of mice infected with F1, F2 and F1/F2 mutant viruses compared to those infected with WT virus. Bars show means and SEMs for weight distributions in each category.

FIGURE S5



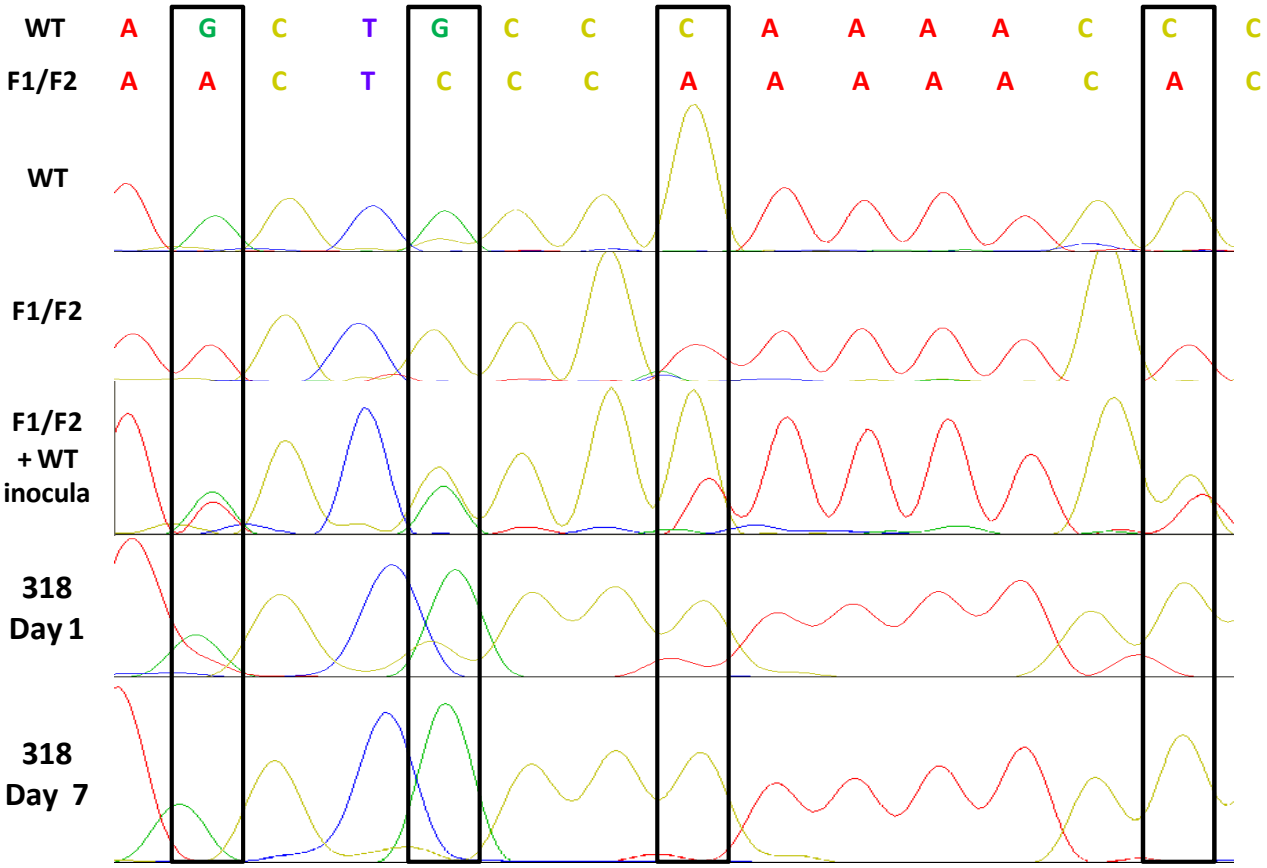
Viral loads in tissues collected 9 months post-infection with WT and F1/F2 mutant viruses. No significant differences in viral loads were detected in mice infected mutant and WT viruses in any tissue.

FIGURE S6



Sensitivity of mutant-specific PCR for different input proportions of WT and F1 or F2 mutant viruses (proportions indicated above gel images).

FIGURE S7



Representative chromatograms of Sanger sequencing reactions of regions of F1 variable in sequence between WT and mutant viruses (genome position 2368-2381; polymorphic sites shown in boxes). This compares sequence traces of WT and F1 sequences with the inoculum strain (50%/50% $TCID_{50}$ ratio of WT and F1/F2) and with viral RNA extracted from faecal samples of co-infected mice collected on days 1 and 7 post inoculation.

SEQUENCE SUPPLEMENT

Sequences of MNV recovered from mice infected with WT and F1/F2 mutants.

Region 1

>F1+2
GCCTCCACCAACTCGGCCCTCAAGGCCGCGCAGGAGATGGGGAAGTTTGC
GATTGAGGTTTTCAAGCAAATCATGGCCTGGATCTGGCCGTCCGAGGACC
CGGTACCCGCCCTGCTCTCAAACATGGAGCAGGCTGTCATCAAGAACGAG
TGCCAGCTCGAGAACCAACTCACCACCATGTTGAGGGACCGAAATGCAGG
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