VIROLOGICA SINICA

Electronic Supplementary Material

Development of Improved Mumps Vaccine Candidates by Mutating Viral mRNA Cap Methyltransferase Sites in the Large Polymerase Protein

Xiaoqiang Hao[1] • Yilong Wang[2] • Mengying Zhu[1] • Dongming Zhou[2] • Rongxian Liu[1] • Bin Wang[3] • Yao-Wei Huang[3]✉ • Zhengyan Zhao[1,2]✉

1. Zhejiang University School of Medicine, Hangzhou 310058, China.
2. The Children's Hospital, Zhejiang University School of Medicine, Hangzhou 310052, China.
3. Institute of Preventive Veterinary Medicine and Key Laboratory of Animal Virology of Ministry of Agriculture, Department of Veterinary Medicine, Zhejiang University, Hangzhou 310058, China.

Supporting information to DOI: 10.1007/s12250-020-00326-y

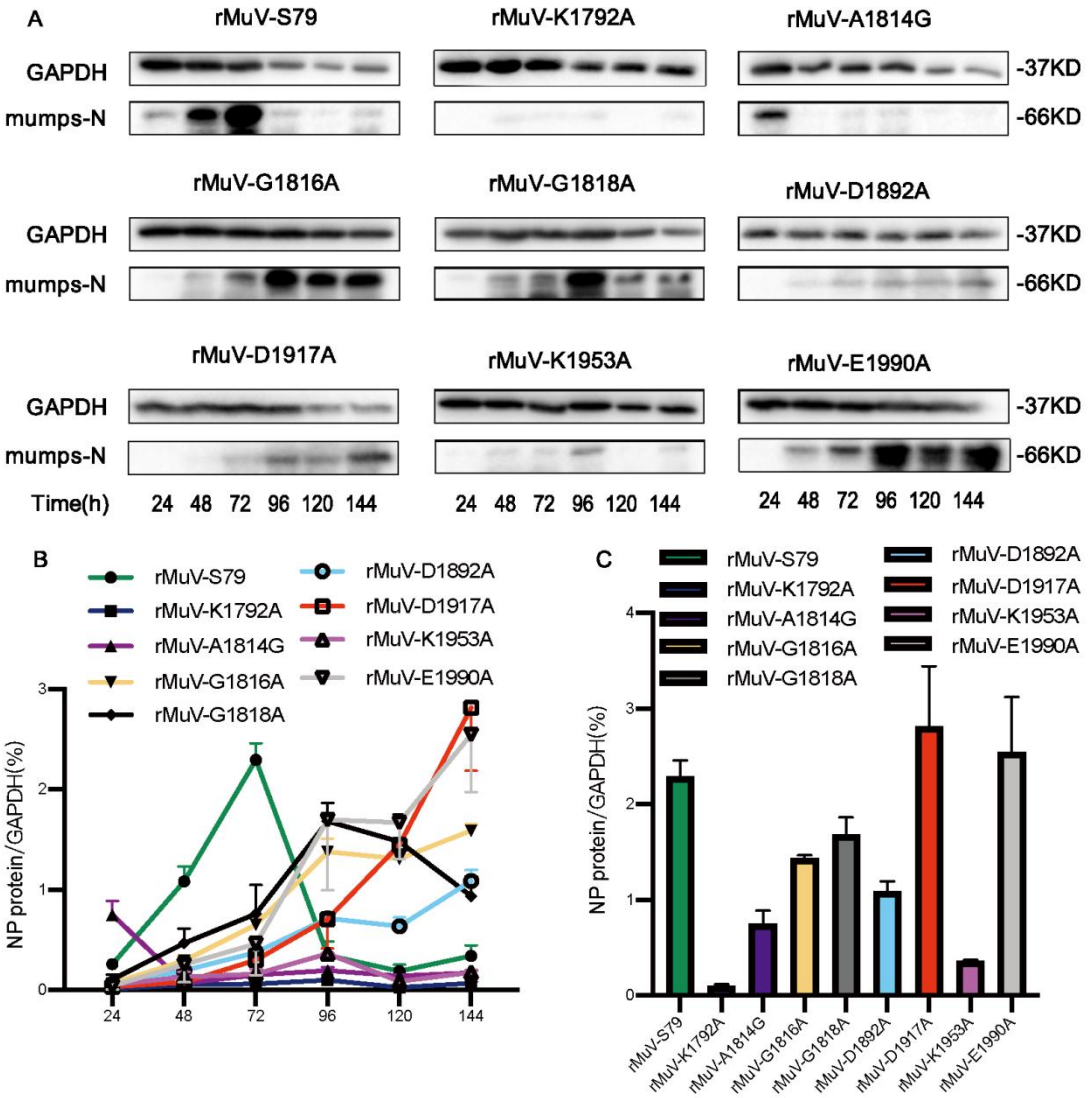

Fig. S1. NP antigen of mutant rMuVs in cells. **A** NP antigen of mutant rMuVs in Vero cells. Vero cells were infected by each recombinant rMuV at an MOI of 0.1. NP antigen and GAPDH were detected at 24, 48, 72, 96, 120, and 144 h after infection. **B** NP antigen expression curve of mutant rMuVs in Vero cells. Quantify the NP antigen of MuV through the intensity ratio of the NP protein band and the GAPDH protein band. **C** Top NP antigen expression of mutant rMuVs in Vero cells.

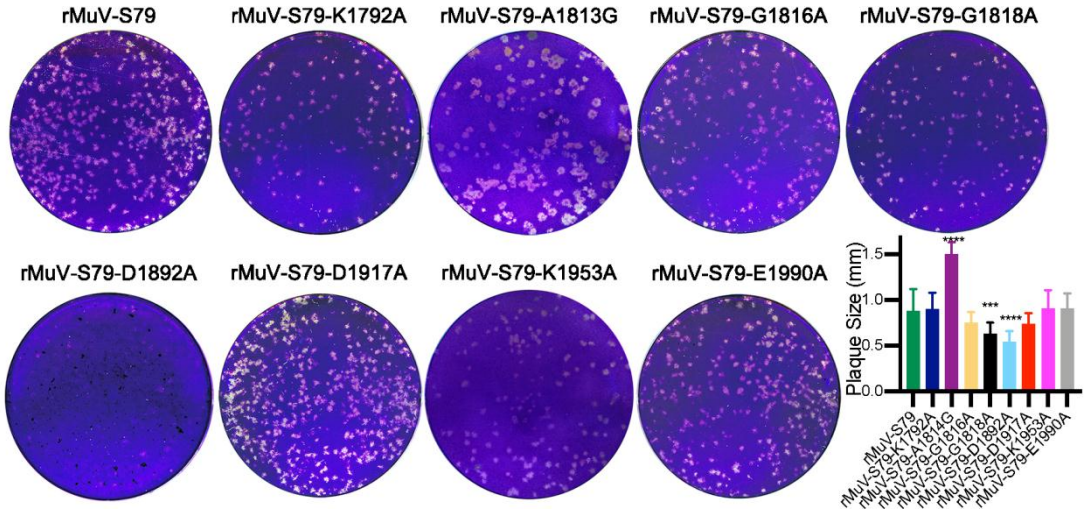

Fig. S2. Growth characteristics of unpurified rMuV mutants. Plaque morphology of unpurified rMuVs. A plaque assay was performed in Vero cells for each unisolated recombinant virus. After 7 days of incubation, plaques were fixed and stained with crystal violet. Comparison of plaque size of unpurified recombinant virus. Fifteen plaques of each unisolated recombinant virus were measured and the average plaque diameter was calculated. **P* <0.05; ***P* <0.01, ****P* <0.001, *****P* <0.0001.

Fig. S3. Sequence of recombinant MuVs carrying mutations. A 950 bp DNA fragment of CR VI derived from MuV L protein was amplified by a One-Step RT-PCR kit (Qiagen) according to the manufacturer's instructions using primers MuV-13722F and MuV-14671R. To confirm that the designed mutation was indeed present in the CR VI of the MuV L protein, the PCR product was sequenced using the primers described above.

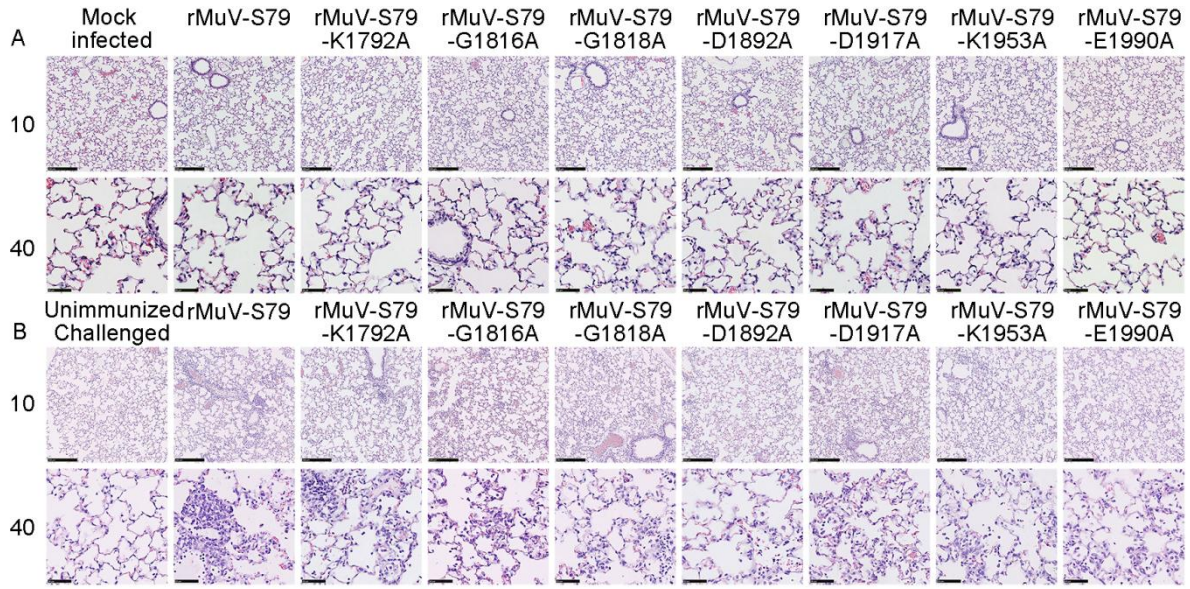

Fig. S4. Histologic examination of lungs of cotton rats infected with rMuV mutants. The right lung from each cotton rat (N=45) was stored in 4% (v/v) paraformaldehyde. The fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin (H&E) for examination of histological changes by light microscopy. **A** Lung tissues from replication study. Cotton rats were intranasally inoculated with 1.0×106 PFU of each rMuV mutant and were terminated at day 4 post-inoculation. A magnification of 10 or 40 was indicated in each panel. Scale bar: 50 μm. **B** Lung tissue from vaccine challenge study. Cotton rats were immunized intranasally with each rMuV mutant. At week 9 post-immunization, cotton rats were challenged with 1.0×107 PFU of a wild type MuV strain and were terminated at day 4 post-challenge. A magnification of 10 or 40 was indicated in each panel. Scale bar: 50 μm.

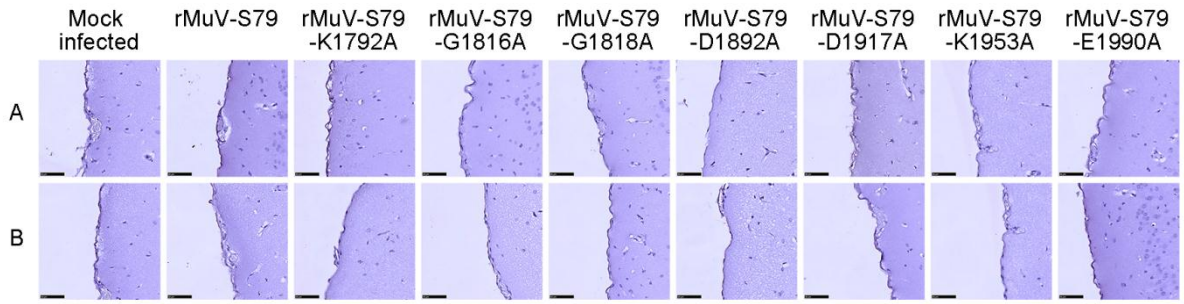

Fig. S5. Immunohistochemical (IHC) staining of brain of cotton rats infected with rMuV mutants. The right brain from each cotton rat (N=45) was fixed with 4% formaldehyde and embedded in paraffin, sectioned at 4 μm, and stained with monoclonal antibody against mumps N protein (Abcam, ab9880, 1/500 dilution in PBS) to determine the distribution of viral antigen. **A** Brain tissues from replication study. Cotton rats were intranasally inoculated with 1.0×106 PFU of each rMuV mutant and were terminated at day 4 post-inoculation. A magnification of 40× meninges was indicated in each panel. Scale bar: 50 μm. **B** Brain tissues from vaccine challenge study. Cotton rats were immunized intranasally with each rMuV mutant. At week 9 post-immunization, cotton rats were challenged with 1.0×107 PFU of a wild type MuV strain and were terminated at day 4 post-challenge. A magnification of 40× meninges was indicated in each panel. Scale bar: 50 μm.

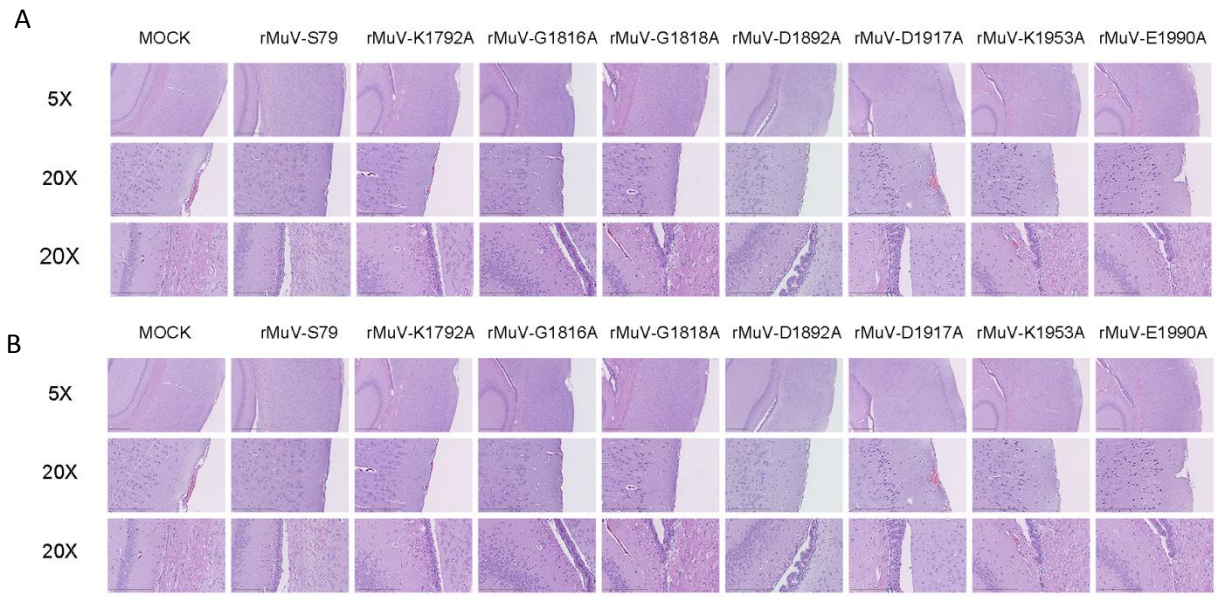

Fig. S6. Histologic examination of brain of cotton rats infected with rMuV mutants. The right brain from each cotton rat (N=45) was stored in 4% (v/v) paraformaldehyde. The fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin (H&E) for examination of histological changes by light microscopy. **A** Brain tissues from replication study. Cotton rats were intranasally inoculated with 1.0×106 PFU of each rMuV mutant and were terminated at day 4 post-inoculation. A magnification of 5×, 20× meninges, 20× ventricle was indicated in each panel. **B** Brain tissue from vaccine challenge study. Cotton rats were immunized intranasally with each rMuV mutant. At week 9 post-immunization, cotton rats were challenged with 1.0×107 PFU of a wild type MuV strain and were terminated at day 4 post-challenge. A magnification of 5×, 20× meninges, 20× ventricle was indicated in each panel.

Table S1. PCR primer sequences for point mutation and sequencing.

Primer	Sequence
rMuV-K1792A	F (forward primer): ATCCACCTCATGGTATgcaACAATCAGTGTTT R (reverse primer): AAACACTGATTGTtgcATACCATGAGGTGGAT
rMuV-A1814G	F (forward primer): GCCCATCTATACTTGggaGAGGGAAGTGGAGC R (reverse primer): GCTCCACTTCCCTCtccCAAGTATAGATGGGC
rMuV-G1816A	F (forward primer): CTATACTTGGCAGAGgcaAGTGGAGCCTCTAT R (reverse primer): ATAGAGGCTCCACTtgcCTCTGCCAAGTATAG
rMuV-G1818A	F (forward primer): TTGGCAGAGGGAAGTgcaGCCTCTATGTCACT R (reverse primer): AGTGACATAGAGGCtgcACTTCCCTCTGCCAA
rMuV-D1892A	F (forward primer): AACAGCGATATAACTgccTTAAGCACTAAAAC R (reverse primer): GTTTTAGTGCTTAAggcAGTTATATCGCTGTT
rMuV-D1917A	F (forward primer): GCATTAGTTCATGTGgctTTGGAAGGTGTCCC R (reverse primer): GGGACACCTTCCAAagcCACATGAACTAATGC
rMuV-K1953A	F (forward primer): CTTACTAATCTTGgcaGCTTCATGGGAACCCT R (reverse primer): AGGGTTCCCATGAAGCtgcCAAGATTAGTAAG
rMuV-E1990A	F (forward primer): GACCCGAATAATCACgcgGTTTACATAATAGC R (reverse primer): GCTATTATGTAAACcgcGTGATTATTCGGGTC
Sequencing primer	MuV-13722F: TGACGGCTGAGAATATGGAT MuV-14671R: GAATAGTCAGGCAGATCCAAC