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Supplementary Materials for

Collective fusion activity determines neurotropism of an en bloc transmitted enveloped virus

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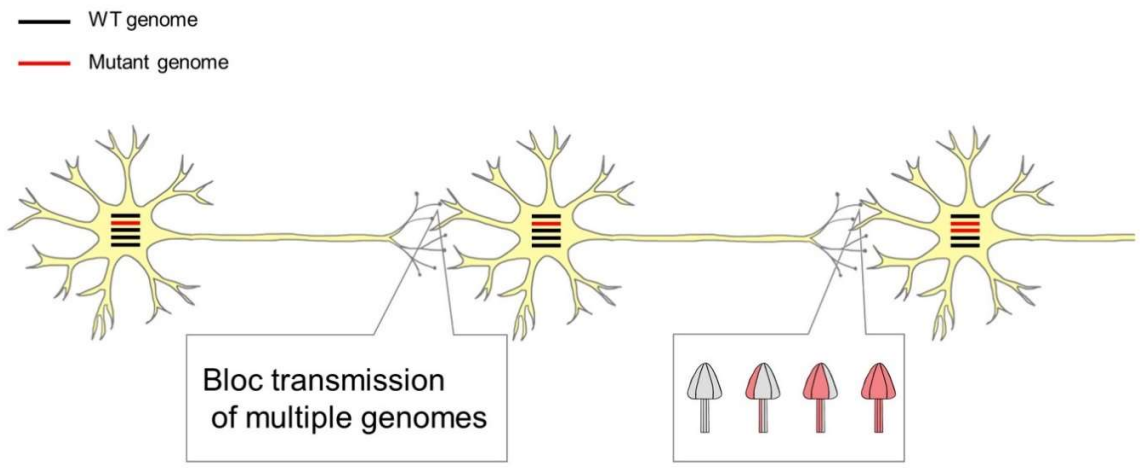

Fig. S1. En bloc transmission of MeV genomes between neurons. During persistence, the WT MeV acquires neuropathogenicity by hyperfusogenic F mutations which enable efficient spread between neurons through cell-cell membrane fusion. In this circumstance, the WT genomes and the emerged mutated genomes are likely to be cotransmitted to neighboring neurons, where different F proteins are expressed, forming heterooligomers.

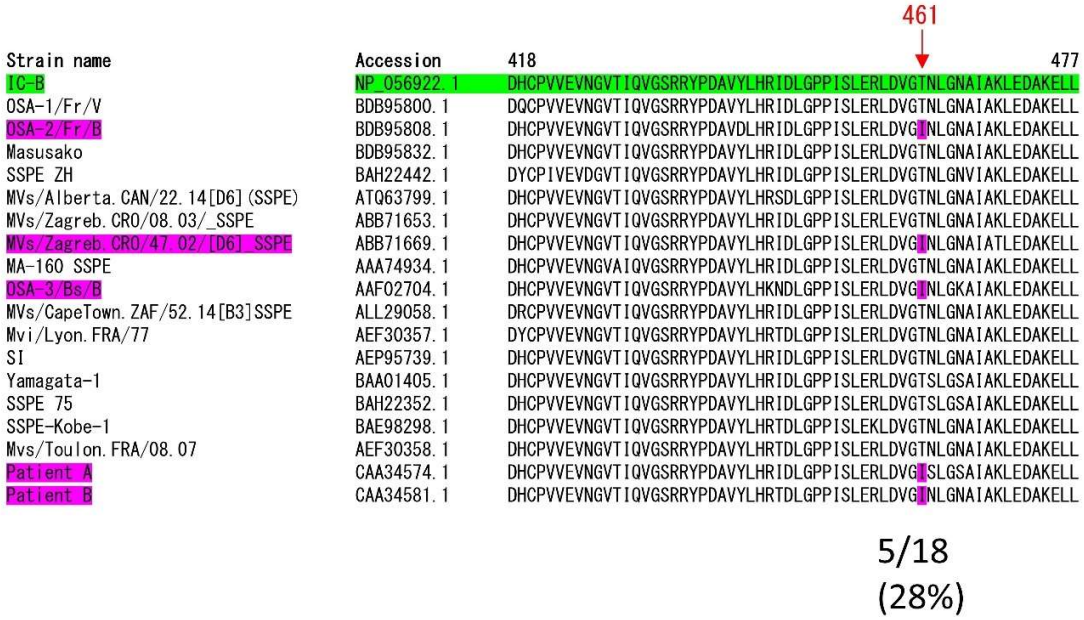

Fig. S2. The high percentage of SSPE-derived F proteins have the T461I substitution. The amino acid alignment of SSPE-derived F proteins (amino acid position 418-477) found in the protein database in NCBI is shown. Accession numbers of F proteins are obtained from the NCBI Reference Sequence for IC-B and GenBank for the other strains.

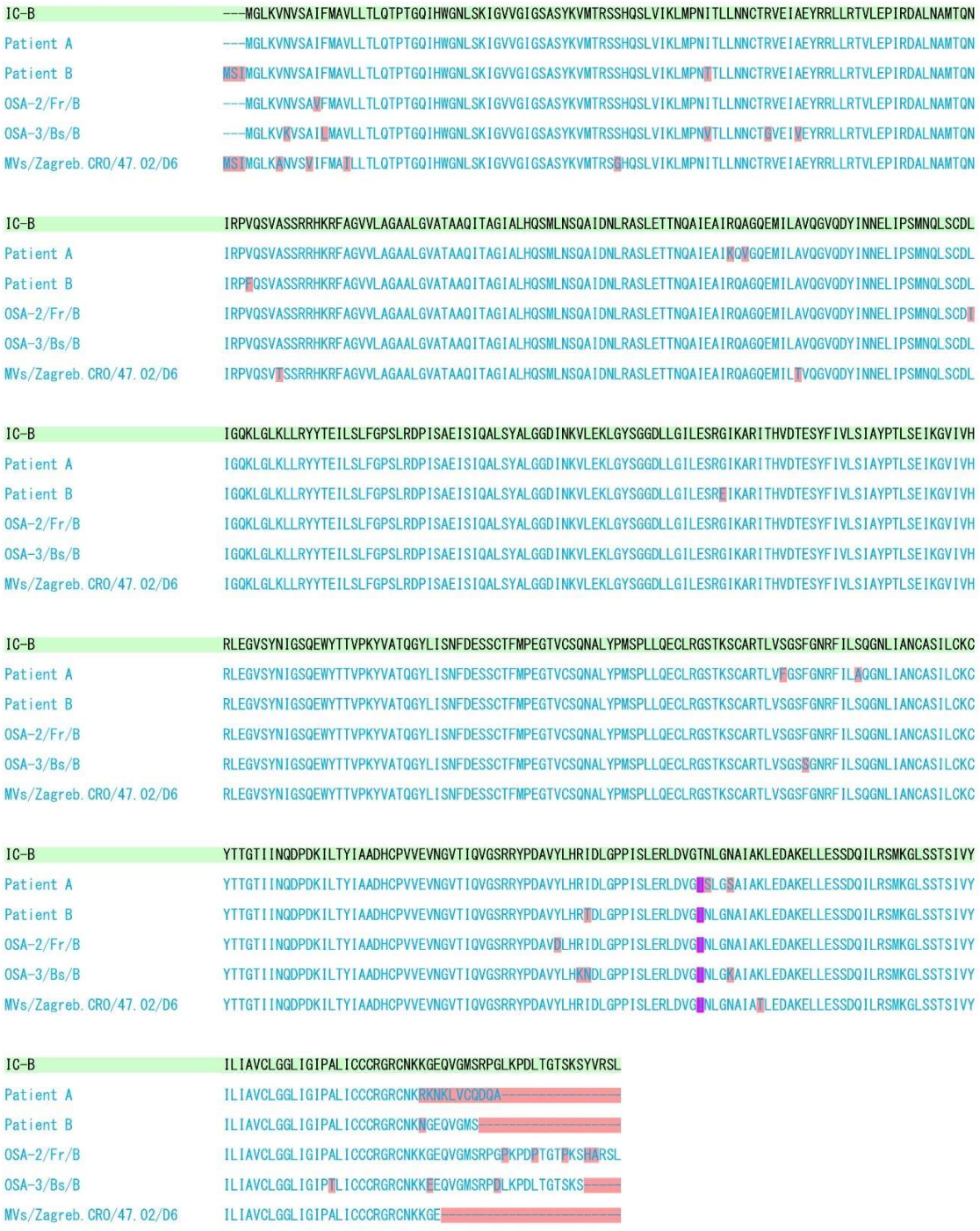

Fig. S3. Alignment of amino acid sequences of SSPE-derived MeV F Proteins possessing the T461I substitution, together with that of the IC-B strain (WT). The amino acid sequences of F proteins are from IC-B (the wild-type strain) (NP_056922.1, NCBI reference sequence), Patient A (CAA34574.1, GenBank), Patient B (CAA34581.1, GenBank), OSA-2/Fr/B (BDB95808.1, GenBank) , OSA-3/Bs/B (AAF02704.1, GenBank), and

MVs/Zagreb.CRO/47.02/[D6]_SSPE (ABB71669.1, GenBank). The T461I substitution is marked in purple, and the other substitutions are marked in red.

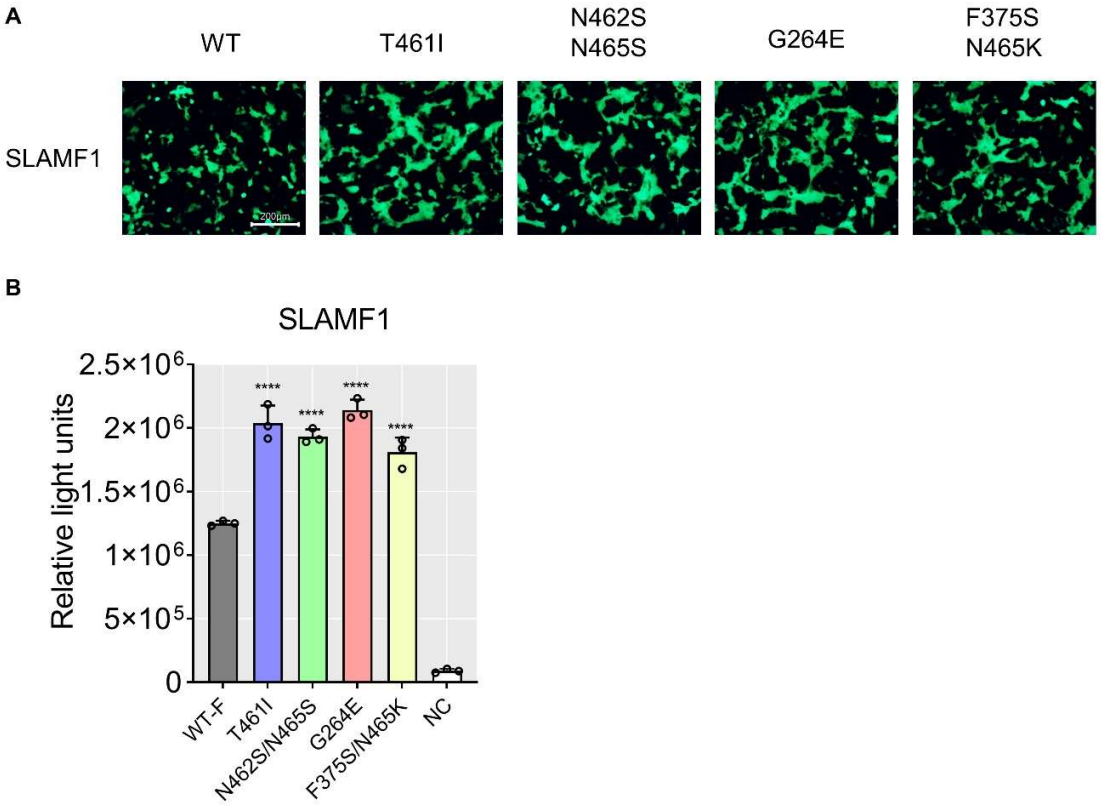

Fig. S4. Effect on the F fusogenicity of the N462S/N465S, G264E, and F375S/N465K mutations. (A) The WT H protein, MeV F (WT F, F(T461I), F(N462S/N465S), F(G264E), or F(F375S/N465K)), SLAMF1 and EGFP were expressed in 293FT cells. The cells were observed 24 hours after transfection under a fluorescence microscope. Scale bar = 200 μm. **(B)** The WT H protein, MeV F (F(T461I), F(N462S/N465S), F(G264E), or F(F375S/N465K), and SLAMF1 were expressed in mixed 293FT/DSP1 and 293FT/DSP2 cells. *Renilla* luciferase activity in the transfected cells was analyzed 24 hours after transfection. RLU, relative light units. Each data point represents one biological replicate (N = 3). Error bars indicate SDs. Significance of the difference in the luciferase activity (as compared with that obtained with the WT-F protein) was analyzed by one-way ANOVA: ****, $P < 0.0001$.

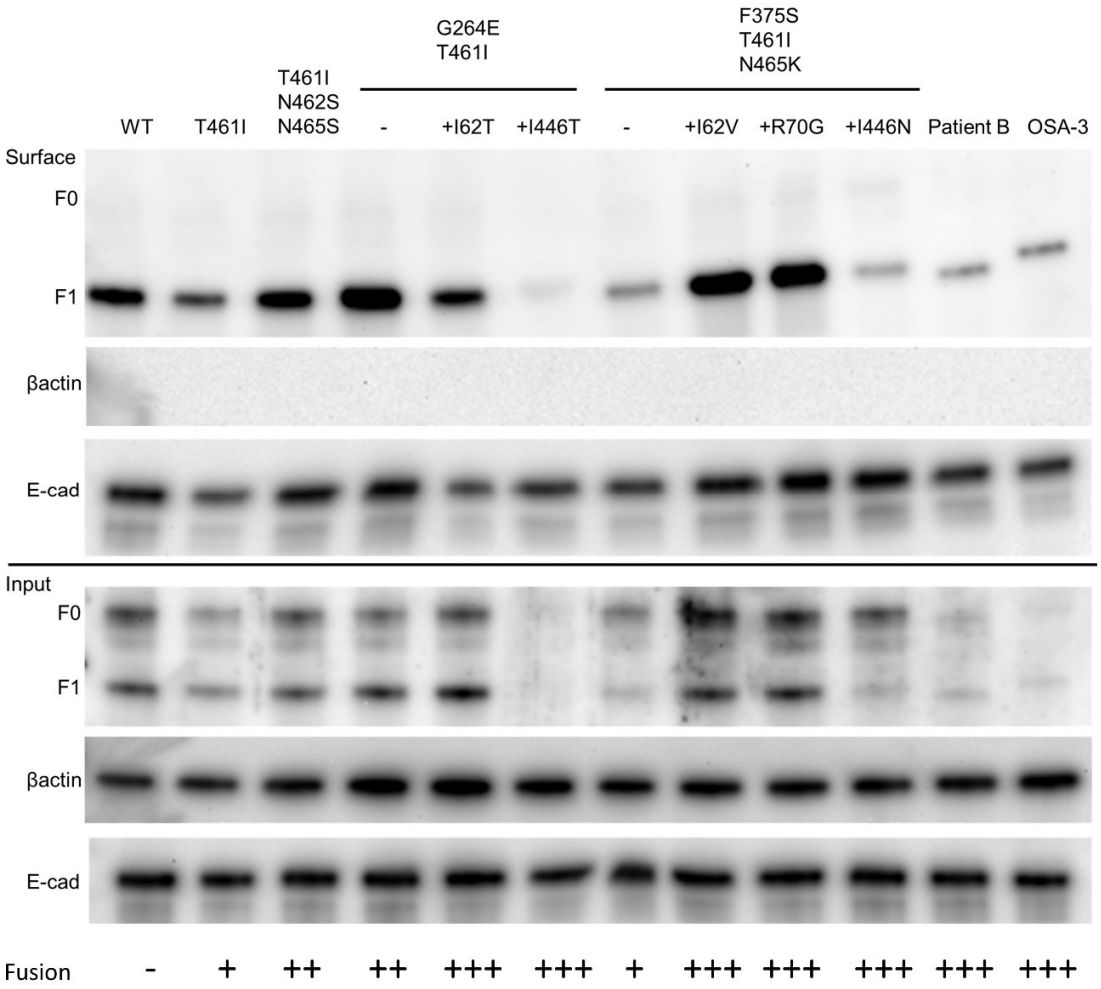

Fig. S5. Cell surface biotinylation assay with MeV F proteins used in this study. E-cadherin (a cell surface protein control) was expressed together with one of FLAG-tagged MeV F proteins in 293FT cells. Precipitates of biotinylated cell surface proteins (Surface) and cell lysates (Input) were examined by western blotting using anti FLAG (top), anti-beta-actin (middle), and anti-E-cadherin (bottom) antibodies. The levels of CADM1-dependent fusogenicities of the respective F proteins are also shown (- ~ +++).

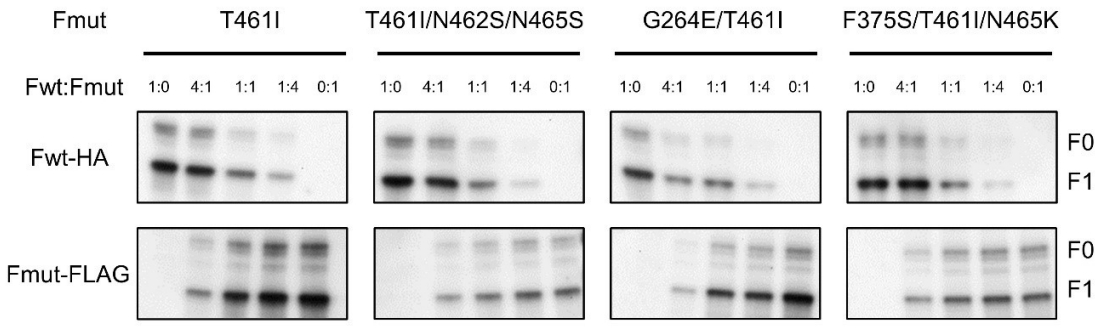

Fig. S6. Expression of the WT and mutant F proteins where the amounts of plasmids used for the transfection were changed. The WT H protein, the FLAG-tagged mutant MeV F protein (Fmut) (F(T461I), F(T461I/N462S/N465S), F(G264E/T461I), or F(F375S/T461I/N465K)), and CADM1 were expressed in mixed 293FT/DSP1 and 293FT/DSP2 cells with or without the HA-tagged WT F (Fwt) protein. The ratio of each mutant F protein to the WT F protein was 1:0, 4:1, 1:1, 1:4 or 0:1. The samples were analyzed by SDS-PAGE and Western blot analysis using anti-HA and anti-FLAG antibodies.

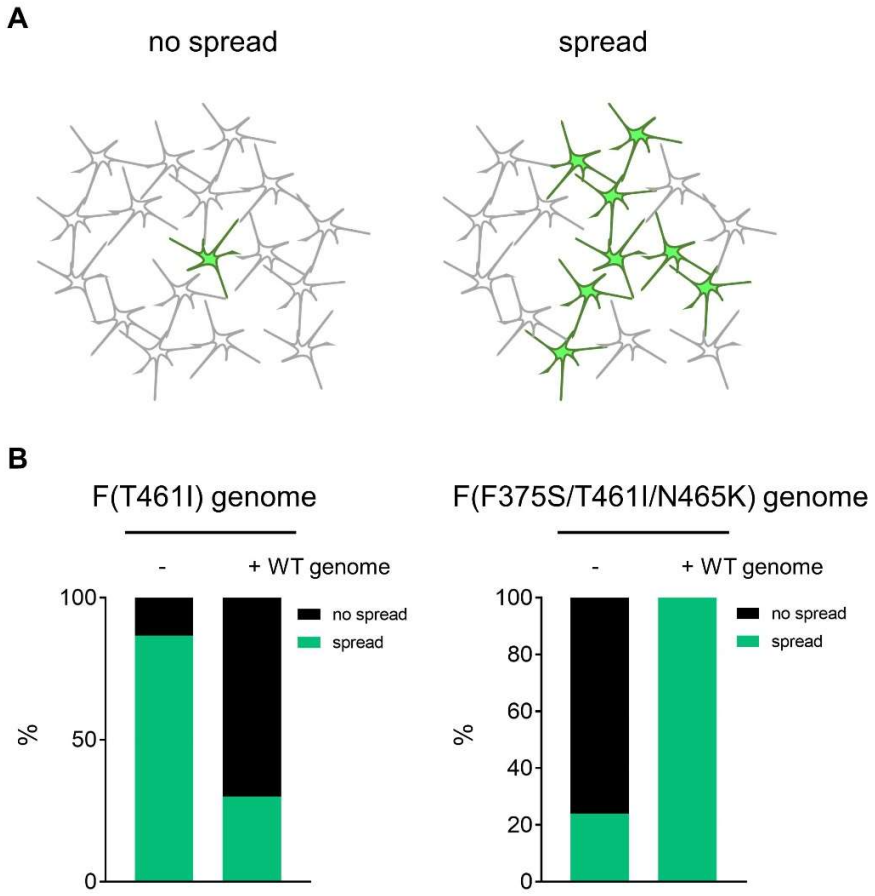

Fig. S7. The quantitative assessment of mouse primary neurons infected with mixed genome MeVs. (A) When mouse primary neurons infected with mixed genome viruses were observed by a fluorescence microscope, two types of infected spots were found. In some spots, only single cells were infected and the viruses did not spread to neighboring neurons (the "no spread" type). In others, the viruses spread to neighboring neurons, and multiple cells became infected around the firstly infected neuron (the "spread" type). Created with BioRender.com. **(B)** The percentages of the "no spread" and "spread" types of infected spots expressing the mutant viral genome alone (-) or both the mutant and WT viral genomes (+WT genome) when mouse primary neurons were infected with the mixed genome virus of MV323-mCherry (WT) and MV323-Venus-F(T461I) or that of MV323-mCherry (WT) and MV323-Venus-F(F375S/T461I/N465K). Because of the polyploidy nature of MeV, individual infectious particles may contain either or both of the mutant and WT viral genomes. Thus, each infected cell expressed the mutant viral genome, the WT viral genome or both.

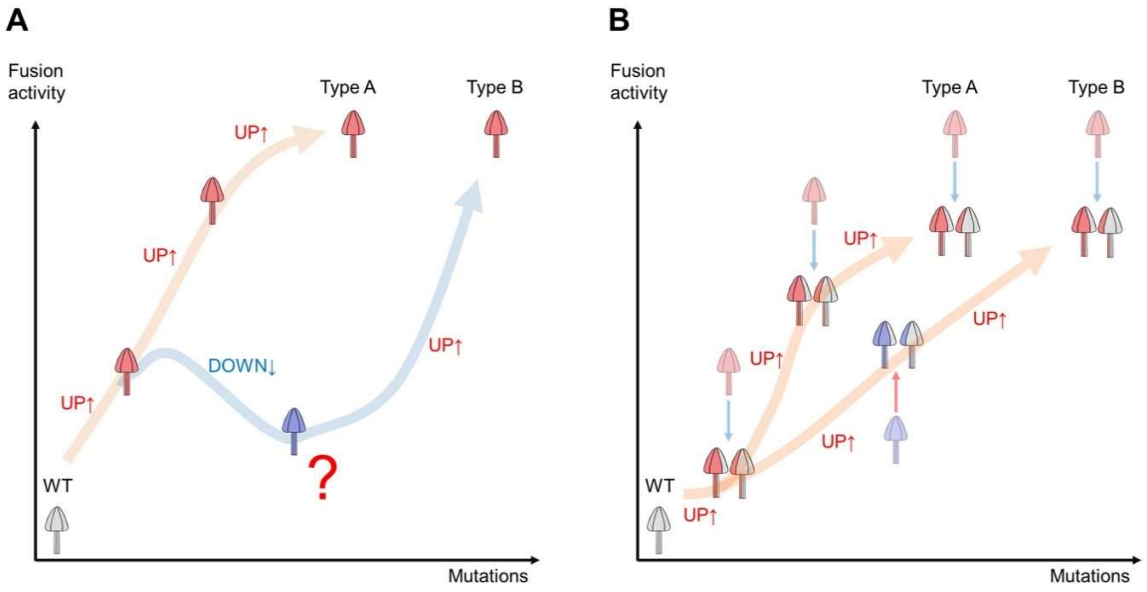

Fig. S8. Two types of MeV F evolutionary pathways to stronger neurotropism. (A) The cumulative mutations in the F gene generally increase (Type A), but sometimes decrease (Type B), the fusogenicity. **(B)** By the WT F protein coexpression, the fusogenicities of most mutant F proteins are downregulated, but those of some mutants are upregulated, resulting in step-by-step increase in the fusogenicity in both Type A and Type B.

Amino acid change in the F protein	Nucleotide change in the *F* gene
T461I	C to U
N462S	**A to G**
N465S	**A to G**
G264E	G to A
F375S	**U to C**
N465K	U to G
I62T	**U to C**
I446T	**U to C**
I62V	**A to G**
R70G	**A to G**
I446N	U to A

Table S1. Nucleotide changes in the *F* genes analyzed in this study.

A to G and U to C changes are indicated in bold.