

## Supplemental Information for:

Nitschel *et al.*

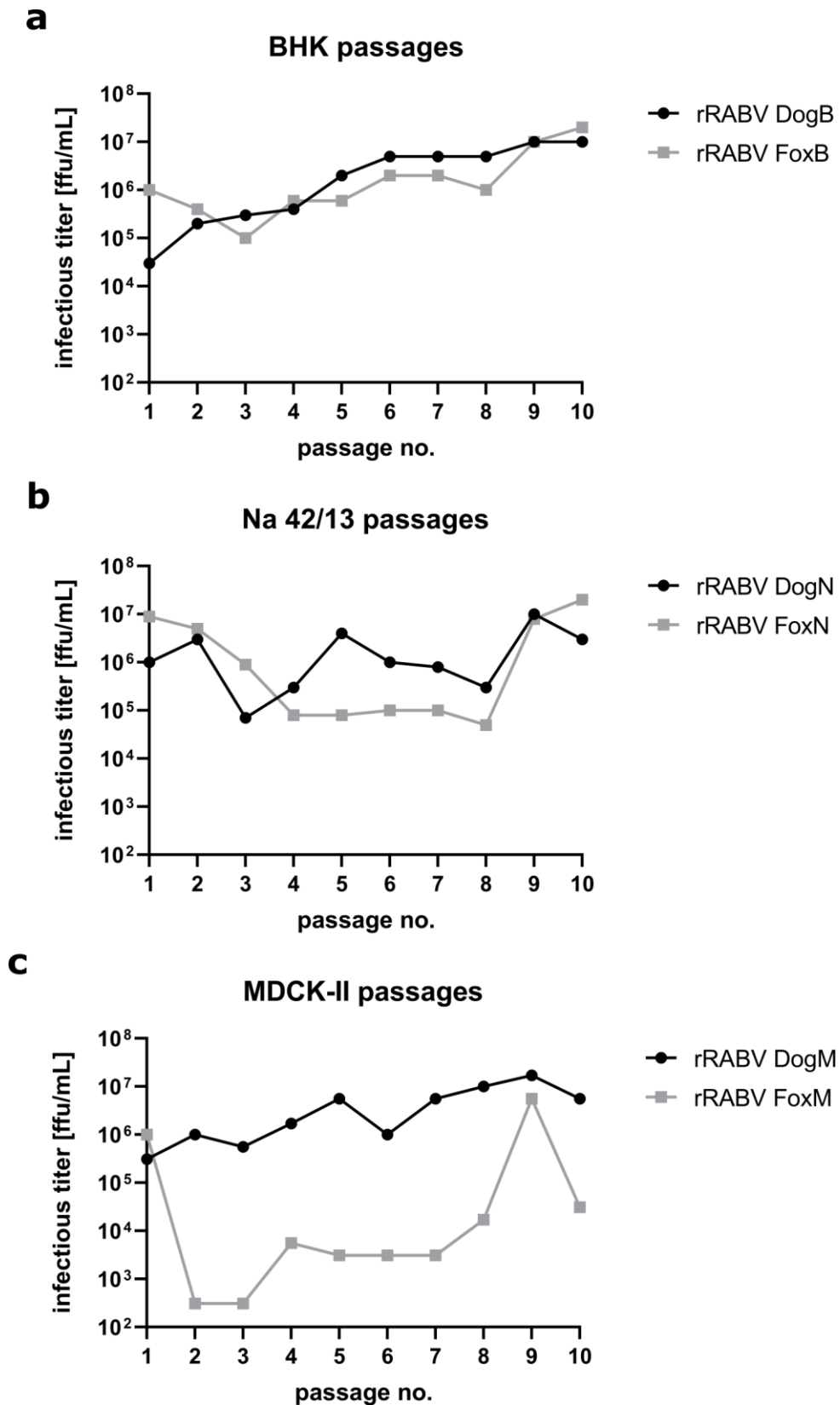
“Point Mutations in the Glycoprotein Ectodomain of Field Rabies Viruses Mediate Cell Culture Adaptation Through Improved Virus Release in a Host Cell-Dependent and -Independent Manner.”

**Table S1: PCR primers used for amplicon sequencing of P/M region.** The oligonucleotides used for the first round of PCR contain a gene-specific sequence region and an instrument-specific linker that is used as target for the sequencing platform specific second PCR.

1 <sup>st</sup> PCR: Gene-specific primers (instrument specific linker / gene-specific)	
Illumina_PM_fw	5'-CTTCCCTACACGACGCTCTTCCGATCTGGCCAATTCCAAGAAATTCCA-3'
Illumina_PM_rv	5'-GGAGTTCAGACGTGTGCTCTTCCGATCGGAAGCCACAGGTCATCGT-3'
2 <sup>nd</sup> PCR: Indexing primers (index / binding site to instrument specific linker)	
Dual Indexed Adapter 97 - P5	5'-AATGATACGGCGACCACCGAGATCTACACGACCTGTAACACTCTTCCCTACACGACGCTCTTCCGATCT-3'
Dual Indexed Adapter 97 - P7	5'-CAAGCAGAAGACGGCATACGAGATTATGCTGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-3'
Dual Indexed Adapter 110 - P5	5'-AATGATACGGCGACCACCGAGATCTACACATGTAACACTCTTCCCTACACGACGCTCTTCCGATCT-3'
Dual Indexed Adapter 110 - P7	5'-CAAGCAGAAGACGGCATACGAGATATAAGCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-3'
Dual Indexed Adapter 123 - P5	5'-AATGATACGGCGACCACCGAGATCTACACGTTTCAGAACACTCTTCCCTACACGACGCTCTTCCGATCT-3'
Dual Indexed Adapter 123 - P7	5'-CAAGCAGAAGACGGCATACGAGATGAGACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-3'
Dual Indexed Adapter 136 - P5	5'-AATGATACGGCGACCACCGAGATCTACACCACAGGATACACTCTTCCCTACACGACGCTCTTCCGATCT-3'
Dual Indexed Adapter 136 - P7	5'-CAAGCAGAAGACGGCATACGAGATCTATGAAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-3'
Dual Indexed Adapter 162 - P5	5'-AATGATACGGCGACCACCGAGATCTACACCGGTGGTAACACTCTTCCCTACACGACGCTCTTCCGATCT-3'
Dual Indexed Adapter 162 - P7	5'-CAAGCAGAAGACGGCATACGAGATTTTGCATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-3'





**Table S2: PCR primers used for amplicon sequencing of G-1 and G-2 region.** The oligonucleotides contain a gene-specific sequence region and an instrument-specific sequence.

Gene-specific primers (instrument specific / gene-specific)	
IonTorrent_G1_P1_fw	5'-CCTCTCTATGGGCAGTCGGTGATTGGTGGAAAGTGCTCAGGAA-3'
IonTorrent_G1_P1_rv	5'-CCTCTCTATGGGCAGTCGGTGATCCAAGTATCAGGAGGGCA-3'
IonTorrent_G1_A_IX44_fw	5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCCAGCGATGGTGGAAAGTGCTCAGGAA-3'
IonTorrent_G1_A_IX44_rv	5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCCAGCGATCCAAGTATCAGGAGGGCA-3'
IonTorrent_G1_A_IX45_fw	5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGAGCTTCCTCGATGGTGGAAAGTGCTCAGGAA-3'
IonTorrent_G1_A_IX45_rv	5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGAGCTTCCTCGATCCAAGTATCAGGAGGGCA-3'
IonTorrent_G2_P1_fw	5'-CCTCTCTATGGGCAGTCGGTGATCCCTCCTCCAGCAACATATG-3'
IonTorrent_G2_P1_rv	5'-CCTCTCTATGGGCAGTCGGTGATACCAGCCTTCACAGTCTAGT-3'
IonTorrent_G2_A_IX44_fw	5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCCAGCGATCCCTCCTCCAGCAACATATG-3'
IonTorrent_G2_A_IX44_rv	5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCCAGCGATACCAGCCTTCACAGTCTAGT-3'
IonTorrent_G2_A_IX45_fw	5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGAGCTTCCTCGATCCCTCCTCCAGCAACATATG-3'
IonTorrent_G2_A_IX45_rv	5'-CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGAGCTTCCTCGATACCAGCCTTCACAGTCTAGT-3'



**Figure S1: Infectious virus titers in the cell culture supernatant determined over the course of virus passaging on BHK (rRABV DogB), NA 42/13 (rRABV DogN) and MDCK-II (rRABV DogM) cells. Passage numbers are indicated by the x-axis.**

1 10 20 30 40 50 60  
 rRABV Dog K F P I Y T I P D K L G P W S P I D I H H L S C P N N L V V E D E G C T N L S G F S Y M E L K V G Y I S A I K V N G F T  
 rRABV Fox K F P I Y T I P D K L G P W S P I D I H H L S C P N N L V V E D E G C T N L S G F S Y M E L K V G Y I S A I K V N G F T  
 SAD L16 K F P I Y T I P D K L G P W S P I D I H H L S C P N N L V V E D E G C T N L S G F S Y M E L K V G Y I L A I K V N G F T  
 70 80 90 100 110 120  
 rRABV Dog C T G V V T E A E T Y T N F V G Y V T T T F K R K H F R P T P D A C R A A Y N W K M A G D P R Y E E S L H N P Y P D Y H  
 rRABV Fox C T G V V T E A E T Y T N F V G Y V T T T F K R K H F R P T P D A C R A A Y N W K M A G D P R Y E E S L H N P Y P D Y H  
 SAD L16 C T G V V T E A E T Y T N F V G Y V T T T F K R K H F R P T P D A C R A A Y N W K M A G D P R Y E E S L H N P Y P D Y R  
 130 140 150 160 170 180  
 rRABV Dog W L R T V K T T K E S L V I I S P S V A D L D P Y D K S L H S R V F P G G K C S G I T V S S T Y C S T N H D Y T I W M P  
 rRABV Fox W L R T V K T T K E S L V I I S P S V A D L D P Y D K S L H S R V F P G G K C S G I T V S S T Y C S T N H D Y T I W L P  
 SAD L16 W L R T V K T T K E S L V I I S P S V A D L D P Y D R S L H S R V F P S G K C S G V A V S S T Y C S T N H D Y T I W M P  
 190 200 210 220 230 240  
 rRABV Dog E N P R L G T S C D I F T N S R G K R A S K G S K T C G F V D E R G L Y K S L K G A C K L K L C G V L G L R L M D G T W  
 rRABV Fox E N P R L G T S C D I F T N S R G K R A S K G S K T C G F V D E R G L Y K S L K G A C K L K L C G V L G L R L M D G T W  
 SAD L16 E N P R L G T S C D I F T N S R G K R A S K G S K T C G F V D E R G L Y K S L K G A C K L K L C G V L G L R L M D G T W  
 250 260 270 280 290 300  
 rRABV Dog V A M Q T S D E T K W C P P D Q L V N L H D F R S D E I E H L V V E E L V K K R E E C L D A L E S I M T T K S V S F R R  
 rRABV Fox V A M Q T S D E T K W C P P D Q L V N L H D F R S D E I E H L V V E E L V K K R E E C L D A L E S I M T T K S V S F R R  
 SAD L16 V S M Q T S N E T K W C P P D K L V N L H D F R S D E I E H L V V E E L V K R E E C L D A L E S I M T T K S V S F R R  
 310 320 330 340 350 360  
 rRABV Dog L S H L R K L V P G F G K A Y T I F N K T L M E A D A H Y K S V R T W N E I I P S K G C L R V G G R C H P H V N G V F F  
 rRABV Fox L S H L R K L V P G F G K A Y T I F N K T L M E A D A H Y K S V R T W N E I I P S K G C L R V G G R C H P H V N G V F F  
 SAD L16 L S H L R K L V P G F G K A Y T I F N K T L M E A D A H Y K S V R T W N E I L P S K G C L R V G G R C H P H V N G V F F  
 370 380 390 400 410 420  
 rRABV Dog N G I I L G P D G H V L I P E M Q S S L L Q Q H M E L L E S S V I P L M H P L A D P S T V F K D G D E A E D F V E V H L  
 rRABV Fox N G I I L G P D G H V L I P E M Q S S L L Q Q H M E L L E S S V I P L M H P L A D P S T V F K D G D E A E D F V E V H L  
 SAD L16 N G I I L G P D G H V L I P E M Q S S L L Q Q H M E L L E S S V I P L M H P L A D P S T V F K D G D E A E D F V E V H L  
 430 440  
 rRABV Dog P D V H K Q V S G V D L G L P N W G K Y  
 rRABV Fox P D V H K Q V S G V D L G L P N W G K Y  
 SAD L16 P D V H N Q V S G V D L G L P N W G K Y

-  D247N in all P10 viruses
-  A400T / K425N in rRABV DogB-P10 or rRABV FoxB-P10
-  R264L / R346S / R350G / S379A in rRABV DogM-P10 or rRABV FoxM-P10
-  T187 in rRABV DogN-P10

**Figure S2: Comparison of the amino acid sequences of the rRABV Dog, rRABV Fox, and SAD L16 glycoprotein ectodomains.** The attenuated SAD L16 sequence differed at 15 position from the rRABV Dog and rRABV Fox G sequences. rRABV Dog and rRABV Fox differed from each other at 3 positions. Amino acid positions associated with cell culture adaptation in the P10 viruses are indicated by coloured rectangles. Coloured squares indicate differences between authentic field virus clones rRABV Dog, rRABV Fox and SAD L16.