Supplemental Information

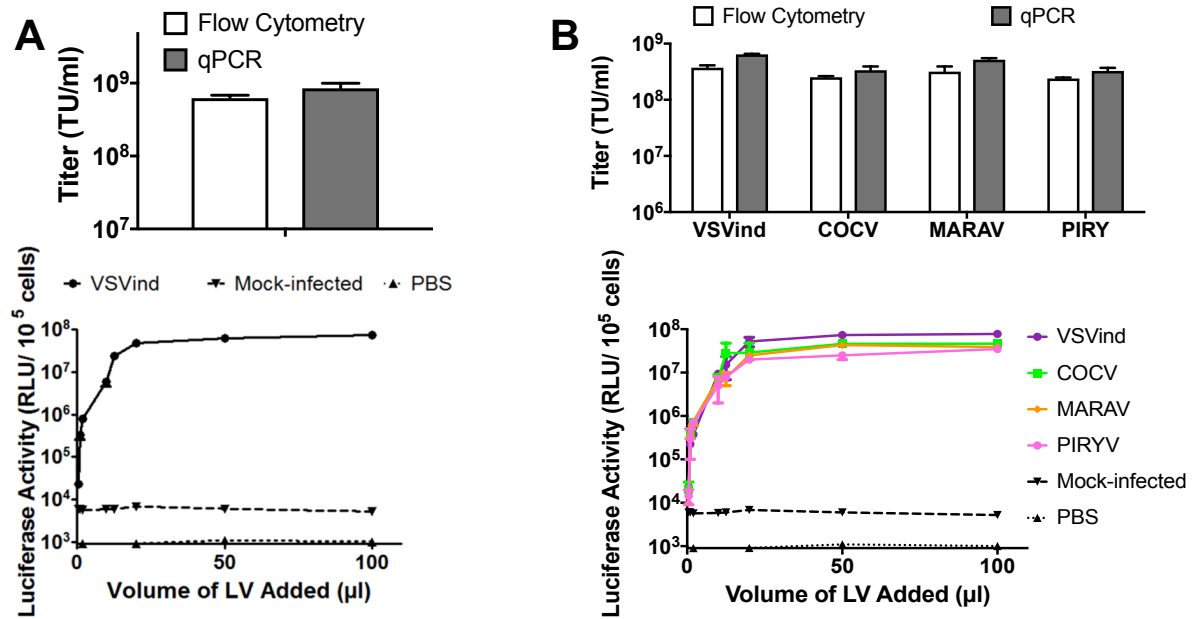# Use of Heterologous Vesiculovirus G Proteins

Circumvents the Humoral Anti-envelope Immunity

in Lentivector-Based *In Vivo* Gene Delivery

Altar M. Munis, Giada Mattiuzzo, Emma M. Bentley, Mary K. Collins, James E. Eyles, and Yasuhiro Takeuchi

SUPPLEMENTARY FIGURES

Supplementary Figure S1: Titers and Luciferase Activity of the LVs Used. qPCR and flow cytometry-based titers and luciferase activity of the vectors used in the **(A)** initial VSVind.G-LV challenge and **(B)** VesG-LV challenge studies.

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VSVind/1-511    1  --MKCLLYLAFLFIGVNCKFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPKSHKAIQADGWM  74
MARAV/1-512     1  --MLRLFLFCFLALGAHSKFTIVFPHHQKGNWKNVPSTYHYCPSSSDQNWHNDLTGVSLHVKIPKSHKAIQADGWM  74
COCV/1-512      1  -MNFLLLTFIVLPLCSHAKFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHNDLLGITMKVKMPKTHKAIQADGWM  75
PIRYV/1-529     1  MDLFPILVVVLMTDTVLGKFQIVFPDQNELEWRPVVGDSRHCPQSSEMQFDGSRSQTILTGKAPVGITPSKSDGFI  76

VSVind/1-511   75  CHASKWVTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQVTPHHV 150
MARAV/1-512    75  CHAAKWVTTCDFRWYGPKYITHSIHSMSPTLEQCKTSIEQTKQGVWINPGFPPQSCGYATVTDAEVVVVQATPHHV 150
COCV/1-512     76  CHAAKWITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSVAVVVQATPHHV 151
PIRYV/1-529    77  CHAAKWVTTCDFRWYGPKYITHSIHHLRPTTSDCETALQRYKDGSLINLGFPPESCGYATVTDSEAMLVQVTPHHV 152

VSVind/1-511  151  LVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSEDGELSSLGKEGTGFRSNYF 226
MARAV/1-512   151  LVDEYTGEWIDSQLVGGKCSKEVCQTVHNSTVWHADYKITGLCESNLASVDITFFSEDGQKTSLGKPNTGFRSNHF 226
COCV/1-512    152  LVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSEDGKKESIGKPNTGYRSNYF 227
PIRYV/1-529   153  GVDDYRGHWIDPLFPGGECSTNFCDTVHNSSVWIPKSQKTDICAQSFKNIKMTASY--PSEGALVSDRFAFHSAYH 226

VSVind/1-511  227  AYETGGKACKMQYCKHWGVRLPSGVWFEMADKDL----FAAARFPECPEGSSISAPSQTSVDVSLIQDVERILDYS 298
MARAV/1-512   227  AYESGEKACRMQYCTQWGIRLPSGVWFELVDKDL----FQAAKLPECPRGSSISAPSQTSVDVSLIQDVERILDYS 298
COCV/1-512    228  AYEKGDKVCKMNYCKHAGVRLPSGVWFEFVDQDV----YAAAKLPECPVGATISAPTQTSVDVSLILDVERILDYS 299
PIRYV/1-529   227  PNMPGSTVCIMDFCEQKGLRFTNGEWMGLNVEQSIREKKISAIFPNCVAGTEIRATLESEGARTLTWETQRMLDYS 302

VSVind/1-511  299  LCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIRVDIAAPILSRMVGMIS-GTTTERELW 373
MARAV/1-512   299  LCQETWSKIRAKLPVSPVDLSYLAPKNPGSGPAFTIINGTLKYFETRYIRVDISNPIIPHMVGTMS-GTTTERELW 373
COCV/1-512    300  LCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIRIDIDNPIISKMVGKIS-GSQTERELW 374
PIRYV/1-529   303  LCQNTWDKVSRKEPLSPLDLSYLSPRAPGKGMAYTVINGTLHSAHAKYIRTWIDYGEMKEIKGGRGEYSKAPELLW 378

VSVind/1-511  374  DDWAPYEDVEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESLFFGDTGLSKN 449
MARAV/1-512   374  NDWYPYEDVEIGPNGVLKTPTGFKFPLYMIGHGMLDSDLHKSSQAQVFEHPHAKDAASQLPDDETLFFGDTGLSKN 449
COCV/1-512    375  TEWFPYEGVEIGPNGIILKTPTGYKFPLFMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKN 450
PIRYV/1-529   379  SQWFDFGPFKIGPNGLLHTGKTFKFPLYLIGAGIIDEDLHELDEAAPIDHPQMPDAKSVLPEDEEIFFGDTGVSKN 454

VSVind/1-511  450  PIELVEGWFSSWKSSIASFFFIIGLIIGLFLVLRVGIHLCIKLKHTKKRQIYTDIEMN---------RLGK---- 511
MARAV/1-512   450  PVELVEGWFSSWKSTLASFFLIIGLGVALIFIIRIIVAIRYKYKGRKTQKIYNDVEMS---------RLGNK--- 512
COCV/1-512    451  PVELIEGWFSSWKSTVVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMS---------RFRK---- 512
PIRYV/1-529   455  PIELIQGWFSNWRESVMAIVGIVLLIVVTFLAIKTVRVLNCLWRPRKKRIVRQEVDVESRLNHFEMRGFPEYVKR 529
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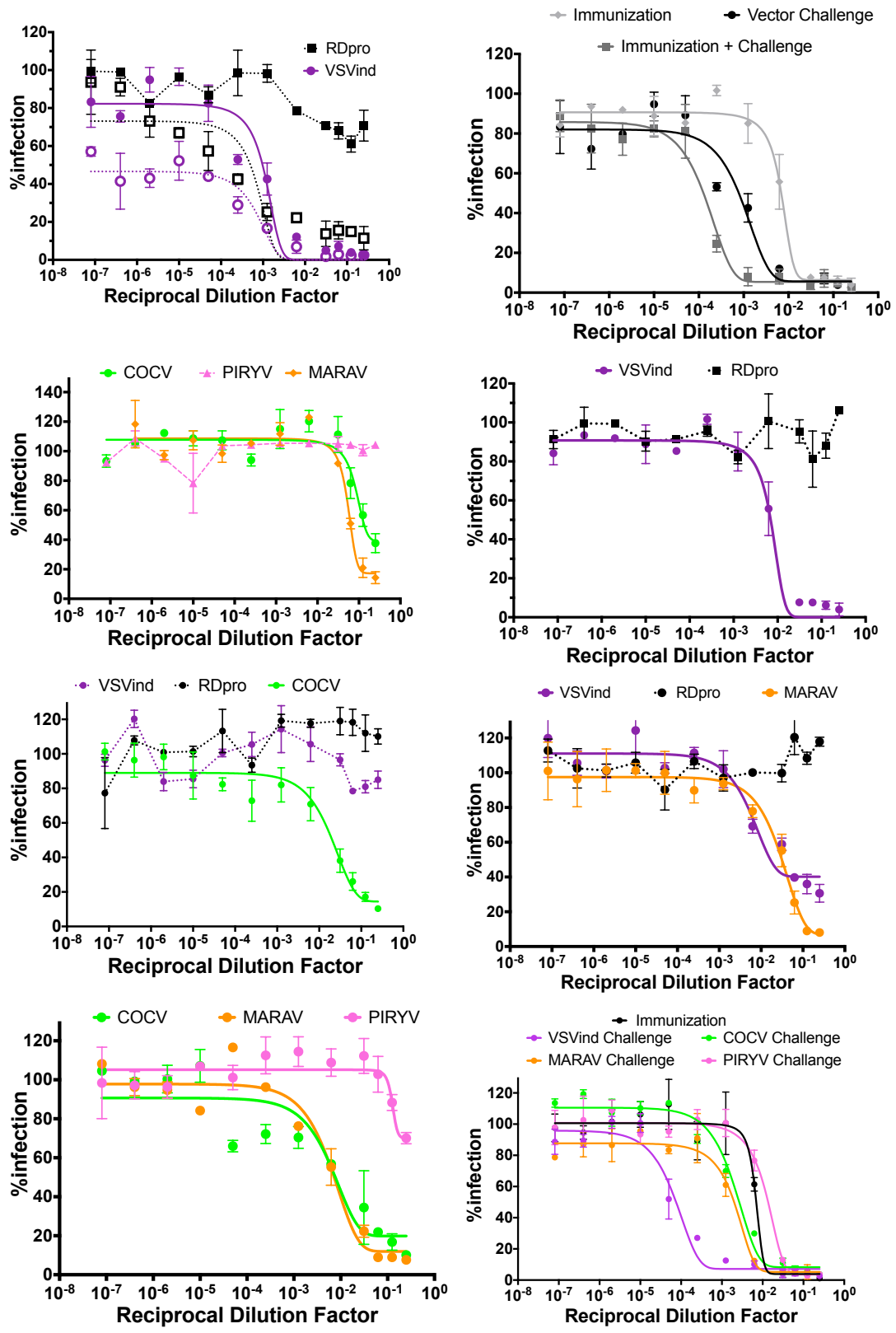
Supplementary Figure S2: Multiple Amino Acid Sequence Alignment of the G proteins of Vesiculoviruses. The sequences of vesiculoviruses (VSVind, UniProt: P03522; MARAV, UniProt: F8SPF4; COCV, UniProt: O56677; PIRYV, UniProt: Q85213) were aligned using ClustalOmega online multiple sequence alignment tool (EMBL-EPI), and the alignments were visualized using JalView software.[1] Dashed lines represent gaps introduced to maximize matching of amino acid residues. Blue shading indicates percent identity; dark blue: 80-100%, medium blue: 60-80% light blue: 40-60%, and no colour indicating <40% identity.

Supplementary Figure S3: Fitted Curves of the Neutralizations Assays Used to Calculate IC50 Values.

REFERENCES

1.   Waterhouse, AM, Procter, JB, Martin, DMA, Clamp, M, and Barton, GJ (2009).
        Jalview Version 2-a multiple sequence alignment editor and analysis workbench.
        Bioinformatics **25**: 1189-1191.