

unknown. In humans, their presence correlates with milder disease and better outcome in influenza virus and respiratory syncytial virus infections. All these reasons justify renewed interest in using DVGs as antiviral therapy.

[0003] While DVGs have been described to exist for most viruses, DVGs were not considered to be relevant or predominant in many virus families. Generally, only a few DVGs have been described for any given virus. These previous reports relied on more classic methods of isolation, such as PCR amplification, that select for only one or two DVGs and bias towards the shortest or most abundant DVGs. These DVGs are not necessarily the best candidates in terms of ability to compete with wild type virus. A need thus exists for reproducible and rational methods to identify and generate the best DVG candidates to compete and interfere with wild type virus infection, that could be applied to any virus of interest, especially human pathogens. The inventions disclosed herein meet these and other needs.

[0004] Our combined experimental evolution and computational approach identifies thousands of different DVGs for any virus. First, our experimental approach generates all possible DVGs in a virus population. Expectedly, our list includes DVGs that resemble those identified by more classic methods. Second, our computational analysis allows us to predict which DVGs would work best as defective interfering particles, using temporal frequencies estimated from the sequence data. Using this combined approach, we have generated a list of top candidates for a wide range of viruses belonging to different virus families, of which up to 90% have confirmed capacity to interfere with wild type virus replication.

SUMMARY

[0004a] In one aspect, the present disclosure refers to a method for producing a defective interfering viral genome (DVG), the method comprising:

providing a first set of at least three replicate in vitro cell cultures, comprising a solid support and cells infected with a reference infectious virus at a high multiplicity of infection (MOI);

providing a second set of at least three replicate in vitro cell cultures, comprising a solid support and cells infected with the reference infectious virus at a low multiplicity of infection (MOI);

culturing the first and second sets of replicate *in vitro* cell cultures for at least 5 passages under mutagenic conditions;

collecting a plurality of DVG candidates from the medium of the first and second sets of *in vitro* replicate cell cultures;

deep sequencing the collected DVG candidates; and

selecting a DVG from the plurality of DVG candidates.

[0004b] In another aspect, the present disclosure refers to a DVG produced by the method as disclosed herein. In yet another aspect, the present disclosure refers to a DVG comprising or consisting of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1 (DG1 : EV71 DVG 293-390), SEQ ID NO: 2 (DG2 : EV71 DVG 294-404), SEQ ID NO: 3 (DG3 : EV71 DVG 1746-2895), SEQ ID NO: 4 (DG4 : EV71 DVG 1752-2894), SEQ ID NO: 5 (DG5 : EV71 DVG 1752-2896), SEQ ID NO: 6 (DG6 : EV71 DVG 1880-6487), SEQ ID NO: 7 (DG7 : EV71 DVG 1880-6488), SEQ ID NO: 8 (DG8 : EV71 DVG 3513-6516), SEQ ID NO: 9 (DG9 : EV71 DVG 5475-5634), SEQ ID NO: 10 (DG10: EV71 DVG 5610-6876), SEQ ID NO: 11 (DG11 : EV71 DVG 5610-6877), SEQ ID NO: 12 (DG12: EV71 DVG 5746-5821), SEQ ID NO: 13 (DG13 : EV71 DVG 6322-6356), SEQ ID NO: 14 (DG14: EV71 DVG 6322-6358), SEQ ID NO: 15 (DG15: EV71 DVG 6728-6779), SEQ ID NO: 16 (DG16: EV71 DVG 6966-7012), SEQ ID NO: 17 (DG17: EV71 DVG 6966-7014), SEQ ID NO: 18 (DG18: EV71 DVG 7098-7122), SEQ ID NO: 19 (DG19: EV71 DVG 7165-7200), SEQ ID NO: 20 (DG20: EV71 DVG 7165-7201), SEQ ID NO: 21 (DG21: EV71 DVG 7238-7292), SEQ ID NO: 22 (ZIKV DVG-A), SEQ ID NO: 23 (ZIKV DVG-B), SEQ ID NO: 24 (ZIKV DVG-C), SEQ ID NO: 25 (ZIKV DVG-D), SEQ ID NO: 26 (ZIKV DVG-E), SEQ ID NO: 27 (ZIKV DVG-F), SEQ ID NO: 28 (ZIKV DVG-G), SEQ ID NO: 29 (ZIKV DVG-H), SEQ ID NO: 30 (ZIKV DVG-I), SEQ ID NO: 31 (ZIKV DVG-J), SEQ ID NO: 32 (ZIKV DVG-K), SEQ ID NO: 33 (ZIKV DVG-L), SEQ ID NO: 34 (CHIKV DVG-IV1), SEQ ID NO: 35 (CHIKV DVG-IV2), SEQ ID NO: 36 (CHIKV DVG-IV3), SEQ ID NO: 37 (CHIKV DVG-IV4), SEQ ID NO: 38 (CHIKV DVG-IH1), SEQ ID NO: 39 (CHIKV DVG-IU1), SEQ ID NO: 40 (CHIKV DVG-CV1), SEQ ID NO: 41 (CHIKV DVG-CV2), SEQ ID NO: 42 (CHIKV DVG-CV3), SEQ ID NO: 43 (CHIKV DVG-CV4), SEQ ID NO: 44 (CHIKV DVG-CH1), SEQ ID NO: 45 (CHIKV DVG-CH2), SEQ ID NO: 46 (CHIKV DVG-CH3), SEQ ID NO: 47 (CHIKV DVG-CM1), SEQ ID NO: 48 (CHIKV DVG-CU1), SEQ ID NO: 49 (CHIKV DVG-CU2), SEQ ID NO: 50 (CHIKV DVG-CA1), SEQ ID NO: 51 (CHIKV DVG-CA2), SEQ ID NO: 52 (CHIKV DVG-CA3), SEQ ID NO: 53 (CHIKV DVG-CA4), SEQ ID NO: 55 (RV DVG A01a-TIP-01), SEQ ID NO: 56 (RV DVG A01a-TIP-02), SEQ ID NO: 57 (RV DVG A01a-TIP-03), SEQ ID NO: 58 (RV DVG A01a-TIP-04), SEQ ID NO: 59 (RV DVG A01a-TIP-05), SEQ ID NO: 60 (RV DVG A01a-TIP-06), SEQ ID NO: 62 (RV DVG A16-TIP-01), SEQ ID NO: 63 (RV DVG A16-TIP-02), SEQ ID NO: 64 (RV DVG A16-TIP-03), SEQ ID NO: 65 (RV DVG A16-TIP-04), SEQ ID NO: 66 (RV DVG A16-TIP-05), SEQ ID NO: 89 (RV DVG C15-TIP-01), SEQ ID NO: 90 (RV DVG C15-TIP-02), SEQ ID NO: 91 (RV DVG C15-TIP-03), SEQ ID NO: 92 (RV DVG C15-TIP-04), SEQ ID NO: 68 (RV DVG B14-TIP-01), SEQ ID NO: 69 (RV DVG B14-TIP-02), SEQ ID NO: 70 (RV DVG B14-TIP-03), SEQ ID NO: 71 (RV DVG B14-TIP-04), SEQ ID NO: 72 (RV DVG

B14-TIP-05), SEQ ID NO: 73 (RV DVG B14-TIP-06), SEQ ID NO: 74 (RV DVG B14-TIP-07), SEQ ID NO: 75 (RV DVG B14-TIP-08), SEQ ID NO: 76 (RV DVG B14-TIP-09), SEQ ID NO: 77 (RV DVG B14-TIP-10), SEQ ID NO: 78 (RV DVG B14-TIP-11), SEQ ID NO: 79 (RV DVG B14-TIP-12), SEQ ID NO: 80 (RV DVG B14-TIP-13), SEQ ID NO: 81 (RV DVG B14-TIP-14), SEQ ID NO: 82 (RV DVG B14-TIP-15), SEQ ID NO: 83 (RV DVG B14-TIP-16), SEQ ID NO: 84 (RV DVG B14-TIP-17), SEQ ID NO: 85 (RV DVG B14-TIP-18), SEQ ID NO: 86 (RV DVG B14-TIP-19), SEQ ID NO: 87 (RV DVG B14-TIP-20), SEQ ID NO: 93 (YFV DVG-A), SEQ ID NO: 94 (YFV DVG-B), SEQ ID NO: 95 (YFV DVG-C), SEQ ID NO: 96 (YFV DVG-D), SEQ ID NO: 97 (YFV DVG-E), SEQ ID NO: 98 (YFV DVG-F), SEQ ID NO: 99 (YFV DVG-G), SEQ ID NO: 100 (YFV DVG-H), SEQ ID NO: 101 (YFV DVG-I), SEQ ID NO: 102 (YFV DVG-J), SEQ ID NO: 103 (YFV DVG-K), SEQ ID NO: 104 (YFV DVG-L), SEQ ID NO: 105 (WNV DVG-1), SEQ ID NO: 106 (WNV DVG-2), SEQ ID NO: 107 (WNV DVG-3), SEQ ID NO: 108 (WNV DVG-4), SEQ ID NO: 109 (WNV DVG-5), SEQ ID NO: 110 (WNV DVG-6), SEQ ID NO: 111 (SARS-CoV-2 DVG 1), SEQ ID NO: 112 (SARS-CoV-2 DVG 2) and SEQ ID NO: 113 (SARS-CoV-2 DVG 3), or a nucleotide sequence having at least 70% identity, more preferably at least 80% identity; more preferably at least 90% identity, more preferably at least 91% identity, more preferably at least 92% identity, more preferably at least 93% identity, more preferably at least 94% identity, more preferably at least 95% identity, more preferably at least 96% identity, more preferably at least 97% identity, more preferably at least 98% identity, or more preferably at least 99% identity with one of these sequences.

[0004c] In one aspect, the present disclosure refers to a defective interfering particle comprising the DVG as disclosed herein. In another aspect, the present disclosure refers to a use of at least one DVG or defective interfering particle as disclosed herein in the manufacture of a medicament for treating a viral infection, wherein an efficient therapeutic amount of the medicament is to be administered to a subject. In yet another aspect, the present disclosure refers to a use of the defective interfering genome DVG or defective interfering particle as disclosed herein in the manufacture of a medicament for treating CHIKV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 34 (CHIKV DVG-IV1), SEQ ID NO: 35 (CHIKV DVG-IV2), SEQ ID NO: 36 (CHIKV DVG-IV3), SEQ ID NO: 37 (CHIKV DVG-IV4), SEQ ID NO: 38 (CHIKV DVG-IH1), SEQ ID NO: 39 (CHIKV DVG-IU1), SEQ ID NO: 40 (CHIKV DVG-CV1), SEQ ID NO: 41 (CHIKV DVG-CV2), SEQ ID NO: 42 (CHIKV DVG-CV3), SEQ ID NO: 43 (CHIKV DVG-CV4), SEQ ID NO: 44 (CHIKV DVG-CH1), SEQ ID NO: 45 (CHIKV DVG-CH2), SEQ ID NO: 46 (CHIKV DVG-CH3), SEQ ID NO: 47 (CHIKV DVG-CM1), SEQ ID NO: 48 (CHIKV DVG-CU1), SEQ ID NO: 49 (CHIKV DVG-CU2), SEQ ID NO: 50 (CHIKV DVG-CA1), SEQ ID NO: 51 (CHIKV DVG-CA2), SEQ ID NO: 52 (CHIKV DVG-CA3) and SEQ ID NO: 53 (CHIKV DVG-CA4). In one aspect, the present disclosure refers to a use of the defective interfering genome DVG or defective interfering particle as disclosed herein in the manufacture of a medicament for treating ZIKV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 22 (ZIKV DVG-A), SEQ ID NO: 23 (ZIKV DVG-

B), SEQ ID NO: 24 (ZIKV DVG-C), SEQ ID NO: 25 (ZIKV DVG-D), SEQ ID NO: 26 (ZIKV DVG-E), SEQ ID NO: 27 (ZIKV DVG-F), SEQ ID NO: 28 (ZIKV DVG-G), SEQ ID NO: 29 (ZIKV DVG-H), SEQ ID NO: 30 (ZIKV DVG-I), SEQ ID NO: 31 (ZIKV DVG-J), SEQ ID NO: 32 (ZIKV DVG-K) and SEQ ID NO: 33 (ZIKV DVG-L). In another aspect, the present disclosure refers to a use of the defective interfering genome DVG or defective interfering particle as disclosed herein in the manufacture of a medicament for treating EV71 infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1 (DG1 : EV71 DVG 293-390), SEQ ID NO: 2 (DG2 : EV71 DVG 294-404), SEQ ID NO: 3 (DG3 : EV71 DVG 1746-2895), SEQ ID NO: 4 (DG4 : EV71 DVG 1752-2894), SEQ ID NO: 5 (DG5 : EV71 DVG 1752-2896), SEQ ID NO: 6 (DG6 : EV71 DVG 1880-6487), SEQ ID NO: 7 (DG7 : EV71 DVG 1880-6488), SEQ ID NO: 8 (DG8 : EV71 DVG 3513-6516), SEQ ID NO: 9 (DG9 : EV71 DVG 5475-5634), SEQ ID NO: 10 (DG10: EV71 DVG 5610-6876), SEQ ID NO: 11 (DG11 : EV71 DVG 5610-6877), SEQ ID NO: 12 (DG12: EV71 DVG 5746-5821), SEQ ID NO: 13 (DG13 : EV71 DVG 6322-6356), SEQ ID NO: 14 (DG14: EV71 DVG 6322-6358), SEQ ID NO: 15 (DG15: EV71 DVG 6728-6779), SEQ ID NO: 16 (DG16: EV71 DVG 6966-7012), SEQ ID NO: 17 (DG17: EV71 DVG 6966-7014), SEQ ID NO: 18 (DG18: EV71 DVG 7098-7122), SEQ ID NO: 19 (DG19: EV71 DVG 7165-7200), SEQ ID NO: 20 (DG20: EV71 DVG 7165-7201) and SEQ ID NO: 21 (DG21: EV71 DVG 7238-7292). In yet another aspect, the present disclosure refers to a use of the defective interfering genome DVG or defective interfering particle as disclosed herein in the manufacture of a medicament for treating RV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 55 (RV DVG A01a-TIP-01), SEQ ID NO: 56 (RV DVG A01a-TIP-02), SEQ ID NO: 57 (RV DVG A01a-TIP-03), SEQ ID NO: 58 (RV DVG A01a-TIP-04), SEQ ID NO: 59 (RV DVG A01a-TIP-05), SEQ ID NO: 60 (RV DVG A01a-TIP-06), SEQ ID NO: 62 (RV DVG A16-TIP-01), SEQ ID NO: 63 (RV DVG A16-TIP-02), SEQ ID NO: 64 (RV DVG A16-TIP-03), SEQ ID NO: 65 (RV DVG A16-TIP-04), SEQ ID NO: 66 (RV DVG A16-TIP-05), SEQ ID NO: 89 (RV DVG C15-TIP-01), SEQ ID NO: 90 (RV DVG C15-TIP-02), SEQ ID NO: 91 (RV DVG C15-TIP-03), SEQ ID NO: 92 (RV DVG C15-TIP-04), SEQ ID NO: 68 (RV DVG B14-TIP-01), SEQ ID NO: 69 (RV DVG B14-TIP-02), SEQ ID NO: 70 (RV DVG B14-TIP-03), SEQ ID NO: 71 (RV DVG B14-TIP-04), SEQ ID NO: 72 (RV DVG B14-TIP-05), SEQ ID NO: 73 (RV DVG B14-TIP-06), SEQ ID NO: 74 (RV DVG B14-TIP-07), SEQ ID NO: 75 (RV DVG B14-TIP-08), SEQ ID NO: 76 (RV DVG B14-TIP-09), SEQ ID NO: 77 (RV DVG B14-TIP-10), SEQ ID NO: 78 (RV DVG B14-TIP-11), SEQ ID NO: 79 (RV DVG B14-TIP-12), SEQ ID NO: 80 (RV DVG B14-TIP-13), SEQ ID NO: 81 (RV DVG B14-TIP-14), SEQ ID NO: 82 (RV DVG B14-TIP-15), SEQ ID NO: 83 (RV DVG B14-TIP-16), SEQ ID NO: 84 (RV DVG B14-TIP-17), SEQ ID NO: 85 (RV DVG B14-TIP-18), SEQ ID NO: 86 (RV DVG B14-TIP-19) and SEQ ID NO: 87 (RV DVG B14-TIP-20). In one aspect, the present disclosure refers to a use of the defective interfering genome DVG or defective interfering particle as disclosed herein in the manufacture of a medicament for treating YFV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 93 (YFV

DVG-A), SEQ ID NO: 94 (YFV DVG-B), SEQ ID NO: 95 (YFV DVG-C), SEQ ID NO: 96 (YFV DVG-D), SEQ ID NO: 97 (YFV DVG-E), SEQ ID NO: 98 (YFV DVG-F), SEQ ID NO: 99 (YFV DVG-G), SEQ ID NO: 100 (YFV DVG-H), SEQ ID NO: 101 (YFV DVG-I), SEQ ID NO: 102 (YFV DVG-J), SEQ ID NO: 103 (YFV DVG-K) and SEQ ID NO: 104 (YFV DVG-L). In another aspect, the present disclosure refers to a use of the defective interfering genome DVG or defective interfering as disclosed herein in the manufacture of a medicament for treating WNV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO:105 (WNV DVG-1), SEQ ID NO: 106 (WNV DVG-2), SEQ ID NO: 107 (WNV DVG-3), SEQ ID NO: 108 (WNV DVG-4), SEQ ID NO: 109 (WNV DVG-5) and SEQ ID NO: 110 (WNV DVG-6). In yet another aspect, the present disclosure refers to a use of the defective interfering genome DVG or defective interfering particle as disclosed herein in the manufacture of a medicament for treating CV infection to a subject, in particular a SARS-CoV-2 infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 111 (SARS-CoV-2 DVG_1), SEQ ID NO: 112 (SARS-CoV-2 DVG_2) and SEQ ID NO: 113 (SARS-CoV-2 DVG_3).

[0004d] In one aspect, the present disclosure refers to a pharmaceutical, immunogenic, or therapeutic composition, comprising at least one DVG as disclosed herein, and a pharmaceutically acceptable carrier. In another aspect, the present disclosure refers to a pharmaceutical, immunogenic, or therapeutic composition, comprising at least one defective interfering particle as disclosed herein, and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a vaccine comprising the composition as disclosed herein.

[0004e] In one aspect, the present disclosure refers to a use of a DVG, or a defective interfering particle as disclosed herein, in the manufacture of a medicament for treating a patient infected with a target virus of the DVG or defective interfering particle. In another aspect, the present disclosure refers to a use of a DVG, or a defective interfering particle as disclosed herein, in the manufacture of a medicament for treating a patient infected with CHIKV. In yet another aspect, the present disclosure refers to a use of a DVG, or a defective interfering particle as disclosed herein, in the manufacture of a medicament for treating a patient infected with ZIKV. In one aspect, the present disclosure refers to a DVG, or a defective interfering particle as disclosed herein, in the manufacture of a medicament for treating a patient infected with EV71. In another aspect, the present disclosure refers to a use of a DVG, or a defective interfering particle as disclosed herein, in the manufacture of a medicament for treating a patient infected with RV. In yet another aspect, the present disclosure refers to a use of a DVG, or a defective interfering particle as disclosed herein, in the manufacture of a medicament for treating a patient infected with YFV. In one aspect, the present disclosure refers to a DVG, or a defective interfering particle as disclosed herein, in the manufacture of a medicament for treating a patient infected with WNV. In another aspect, the present disclosure refers to a use of a DVG, or a defective interfering particle as described herein, in the manufacture of a medicament for treating a patient infected with CV, in particular SARS-CoV-2.

[0004f] In one aspect, the present disclosure refers to a polynucleotide comprising or consisting of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1 (DG1 : EV71 DVG 293-390), SEQ ID NO: 2 (DG2 : EV71 DVG 294-404), SEQ ID NO: 3 (DG3 : EV71 DVG 1746-2895), SEQ ID NO: 4 (DG4 : EV71 DVG 1752-2894), SEQ ID NO: 5 (DG5 : EV71 DVG 1752-2896), SEQ ID NO: 6 (DG6 : EV71 DVG 1880-6487), SEQ ID NO: 7 (DG7 : EV71 DVG 1880-6488), SEQ ID NO: 8 (DG8 : EV71 DVG 3513-6516), SEQ ID NO: 9 (DG9 : EV71 DVG 5475-5634), SEQ ID NO: 10 (DG10: EV71 DVG 5610-6876), SEQ ID NO: 11 (DG11 : EV71 DVG 5610-6877), SEQ ID NO: 12 (DG12: EV71 DVG 5746-5821), SEQ ID NO: 13 (DG13 : EV71 DVG 6322-6356), SEQ ID NO: 14 (DG14: EV71 DVG 6322-6358), SEQ ID NO: 15 (DG15: EV71 DVG 6728-6779), SEQ ID NO: 16 (DG16: EV71 DVG 6966-7012), SEQ ID NO: 17 (DG17: EV71 DVG 6966-7014), SEQ ID NO: 18 (DG18: EV71 DVG 7098-7122), SEQ ID NO: 19 (DG19: EV71 DVG 7165-7200), SEQ ID NO: 20 (DG20: EV71 DVG 7165-7201), SEQ ID NO: 21 (DG21: EV71 DVG 7238-7292), SEQ ID NO: 22 (ZIKV DVG-A), SEQ ID NO: 23 (ZIKV DVG-B), SEQ ID NO: 24 (ZIKV DVG-C), SEQ ID NO: 25 (ZIKV DVG-D), SEQ ID NO: 26 (ZIKV DVG-E), SEQ ID NO: 27 (ZIKV DVG-F), SEQ ID NO: 28 (ZIKV DVG-G), SEQ ID NO: 29 (ZIKV DVG-H), SEQ ID NO: 30 (ZIKV DVG-I), SEQ ID NO: 31 (ZIKV DVG-J), SEQ ID NO: 32 (ZIKV DVG-K), SEQ ID NO: 33 (ZIKV DVG-L), SEQ ID NO: 34 (CHIKV DVG-IV1), SEQ ID NO: 35 (CHIKV DVG-IV2), SEQ ID NO: 36 (CHIKV DVG-IV3), SEQ ID NO: 37 (CHIKV DVG-IV4), SEQ ID NO: 38 (CHIKV DVG-IH1), SEQ ID NO: 39 (CHIKV DVG-IU1), SEQ ID NO: 40 (CHIKV DVG-CV1), SEQ ID NO: 41 (CHIKV DVG-CV2), SEQ ID NO: 42 (CHIKV DVG-CV3), SEQ ID NO: 43 (CHIKV DVG-CV4), SEQ ID NO: 44 (CHIKV DVG-CH1), SEQ ID NO: 45 (CHIKV DVG-CH2), SEQ ID NO: 46 (CHIKV DVG-CH3), SEQ ID NO: 47 (CHIKV DVG-CM1), SEQ ID NO: 48 (CHIKV DVG-CU1), SEQ ID NO: 49 (CHIKV DVG-CU2), SEQ ID NO: 50 (CHIKV DVG-CA1), SEQ ID NO: 51 (CHIKV DVG-CA2), SEQ ID NO: 52 (CHIKV DVG-CA3), SEQ ID NO: 53 (CHIKV DVG-CA4), SEQ ID NO: 55 (RV DVG A01a-TIP-01), SEQ ID NO: 56 (RV DVG A01a-TIP-02), SEQ ID NO: 57 (RV DVG A01a-TIP-03), SEQ ID NO: 58 (RV DVG A01a-TIP-04), SEQ ID NO: 59 (RV DVG A01a-TIP-05), SEQ ID NO: 60 (RV DVG A01a-TIP-06), SEQ ID NO: 62 (RV DVG A16-TIP-01), SEQ ID NO: 63 (RV DVG A16-TIP-02), SEQ ID NO: 64 (RV DVG A16-TIP-03), SEQ ID NO: 65 (RV DVG A16-TIP-04), SEQ ID NO: 66 (RV DVG A16-TIP-05), SEQ ID NO: 89 (RV DVG C15-TIP-01), SEQ ID NO: 90 (RV DVG C15-TIP-02), SEQ ID NO: 91 (RV DVG C15-TIP-03), SEQ ID NO: 92 (RV DVG C15-TIP-04), SEQ ID NO: 68 (RV DVG B14-TIP-01), SEQ ID NO: 69 (RV DVG B14-TIP-02), SEQ ID NO: 70 (RV DVG B14-TIP-03), SEQ ID NO: 71 (RV DVG B14-TIP-04), SEQ ID NO: 72 (RV DVG B14-TIP-05), SEQ ID NO: 73 (RV DVG B14-TIP-06), SEQ ID NO: 74 (RV DVG B14-TIP-07), SEQ ID NO: 75 (RV DVG B14-TIP-08), SEQ ID NO: 76 (RV DVG B14-TIP-09), SEQ ID NO: 77 (RV DVG B14-TIP-10), SEQ ID NO: 78 (RV DVG B14-TIP-11), SEQ ID NO: 79 (RV DVG B14-TIP-12), SEQ ID NO: 80 (RV DVG B14-TIP-13), SEQ ID NO: 81 (RV DVG B14-TIP-14), SEQ ID NO: 82 (RV DVG B14-TIP-15), SEQ ID NO: 83 (RV DVG B14-TIP-16), SEQ ID NO: 84 (RV DVG B14-TIP-17), SEQ ID NO: 85 (RV DVG B14-TIP-18), SEQ ID NO: 86 (RV DVG B14-TIP-19), SEQ ID NO: 87 (RV DVG B14-TIP-20), SEQ ID NO: 93 (YFV DVG-A), SEQ ID NO: 94 (YFV DVG-B), SEQ ID NO: 95 (YFV DVG-C), SEQ ID NO: 96 (YFV DVG-D), SEQ ID

NO: 97 (YFV DVG-E), SEQ ID NO: 98 (YFV DVG-F), SEQ ID NO: 99 (YFV DVG-G), SEQ ID NO: 100 (YFV DVG-H), SEQ ID NO: 101 (YFV DVG-I), SEQ ID NO: 102 (YFV DVG-J), SEQ ID NO: 103 (YFV DVG-K), SEQ ID NO: 104 (YFV DVG-L), SEQ ID NO: 105 (WNV DVG-1), SEQ ID NO: 106 (WNV DVG-2), SEQ ID NO: 107 (WNV DVG-3), SEQ ID NO: 108 (WNV DVG-4), SEQ ID NO: 109 (WNV DVG-5), SEQ ID NO: 110 (WNV DVG-6), SEQ ID NO: 111 (SARS-CoV-2 DVG 1), SEQ ID NO: 112 (SARS-CoV-2 DVG 2) and SEQ ID NO: 113 (SARS-CoV-2 DVG 3), or a polynucleotide having at least 70% identity, more preferably at least 80% identity ; more preferably at least 90% identity, more preferably at least 91% identity, more preferably at least 92% identity, more preferably at least 93% identity, more preferably at least 94% identity, more preferably at least 95% identity, more preferably at least 96% identity, more preferably at least 97% identity, more preferably at least 98% identity, or more preferably at least 99% identity with one of these sequences. In another aspect, the present disclosure refers to an expression vector or a plasmid comprising the polynucleotide as disclosed herein. In yet another aspect, the present disclosure refers to a cell line producing the DVG as disclosed herein.

DESCRIPTION

[0005] This disclosure provides methods of generating DVGs and also provides new DVGs generated by the methods. DVGs generated from chikungunya virus (CHIKV), Zika virus (ZIKV), enterovirus 71 (EV71), West Nile virus (WNV), yellow fever virus (YFV) and rhinovirus (RV), and coronavirus (CV) (for example, the recently identified novel coronavirus named SARS-CoV-2, 2019-nCov or COVID-19) are provided.

CLAIMS:

1. A method for producing a defective interfering viral genome (DVG), the method comprising:

providing a first set of at least three replicate *in vitro* cell cultures, comprising a solid support and cells infected with a reference infectious virus at a high multiplicity of infection (MOI);

providing a second set of at least three replicate *in vitro* cell cultures, comprising a solid support and cells infected with the reference infectious virus at a low multiplicity of infection (MOI);

culturing the first and second sets of replicate *in vitro* cell cultures for at least 5 passages under mutagenic conditions;

collecting a plurality of DVG candidates from the medium of the first and second sets of *in vitro* replicate cell cultures;

deep sequencing the collected DVG candidates; and

selecting a DVG from the plurality of DVG candidates.

2. The method of claim 1, further comprising:

providing a third set of at least three replicate *in vitro* cell cultures, comprising a solid support and cells infected with a reference infectious virus at a high multiplicity of infection (MOI); and/or

providing a fourth set of at least three replicate *in vitro* cell cultures, comprising a solid support and cells infected with the reference infectious virus at a low multiplicity of infection (MOI); and

culturing the third and/or fourth sets of replicate *in vitro* cell cultures for at least 5 passages under non-mutagenic conditions.

3. The method of claim 1 or claim 2, wherein selecting a DVG from the plurality of DVG candidates comprises:

comparing the sequences of the DVG candidates with the sequence of the genome of the reference infectious virus;

identifying at least one DVG candidate having a genome comprising at least one splicing event where the splicing event is a deletion or a rearrangement;

determining the relative frequency of at least one DVG candidate having a genome with at least one splicing event among the population of sequenced DVG candidate genomes; and,

selecting at least one DVG for which:

a) at least one splicing event is more abundant at high MOI cultures than at low MOI cultures; and/or

b) at least one splicing event appears in at least 2, 3, 4, 5, 6, 7, 8 or 9 different cell lines; and/or,

c) at least one splicing event is found in at least 3 of at least 12, 24 or 36 independent replicates, after at least 5, 10, 15 or 20 passages; and/or,

d) at least one splicing event is a deletion event and the nucleotide size of the deletion is at least 40, 42, 50, 100, 150, 200, 400, 600 or 1000 nucleotides; and/or,

e) an open reading frame in the DVG is maintained; and/or,

f) the structural domains and functions necessary for viral replication, are maintained in the DVG; and/or

g) the DVG comprises at least one mutation that reduces the self-replicative capacity of the reference infectious virus.

4. The method according to any one of claims 1 to 3, wherein the DVG is characterized by an in vitro inhibitory activity of at least 50%, 60%, 70%, 80% or 90% against the reference infectious virus.

5. The method according to any one of claims 1 to 4, wherein the reference infectious virus has a mutator phenotype.

6. The method according to any one of claims 1 to 5, wherein the reference infectious virus is a Chikungunya virus (CHIKV), Zika virus (ZIKV), Enterovirus 71 (EV71), Rhinovirus (RV), Yellow Fever virus (YFV), West Nile Virus (WNV) or Coronavirus (CV).

7. The method according to any one of claims 1 to 5, wherein the reference infectious virus is selected from the group consisting of: CHIKV Indian Ocean lineage (GenBank accession no. AM258994), CHIKV Caribbean strain (GenBank accession no. LN898104.1), ZIKV African strain MR766 (KY989511.1), EV71 Sep006 (GenBank: KX197462.1), RV-A01a (NC_038311.1), RV-A16 (L24917.1), RV-B14 (L05355.1), RV-C15 (GU219984.1), West Nile virus Israel 1998 and YFV strain, Yellow Fever Virus Asibi (YF-Asibi) strain, Yellow Fever Virus vaccine (YF-17D) strain, and Coronavirus SARS-CoV-2 strain.

8. The method according to any one of claims 1 to 7, wherein the cells are a mammalian cell line selected from the group consisting of: a cell line derived from African green monkey kidney cells (optionally Vero, optionally Vero-E6 cell line), a cell line derived from human muscle (optionally RD cell line), a cell line derived from baby hamster kidneys (optionally BHK cell line), a cell line derived from human embryonic kidney cells (optionally HEK293T cell line), a cell line derived from liver carcinoma cells (optionally Huh7 cell line), and a cell line derived from cervical adenocarcinoma (optionally H1-HeLa, optionally HeLa-E8 cell line).

9. The method according any one of claims 1 to 7, wherein the cells are a mosquito a cell line selected from the group consisting of: a cell line derived from Aedes aegypti (optionally Aag2 cell line), a cell derived from Aedes albopictus (optionally C6/36 cell line) and a cell line derived from Aedes albopictus (optionally U4.4 cell line).

10. The method according to any one of claims 1 to 9, wherein the low MOI for infection is from 0.001 to 0.1 PFU/cell, in particular from 0.01 to 0.1 PFU/cell.

11. The method according to any one of claims 1 to 10, wherein the high MOI for infection is from 1 PFU/cell to 100 PFU/cell, in particular from 5 to 50 PFU/cell or 5 to 20 PFU/cell.

12. A DVG produced by the method of any one of claims 1 to 11.

13. A DVG comprising or consisting of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1 (DG1 : EV71 DVG 293-390), SEQ ID NO: 2 (DG2 : EV71 DVG 294-404), SEQ ID NO: 3 (DG3 : EV71 DVG 1746-2895), SEQ ID NO: 4 (DG4 : EV71 DVG 1752-2894), SEQ ID NO: 5 (DG5 : EV71 DVG 1752-2896), SEQ ID NO: 6 (DG6 : EV71 DVG 1880-6487), SEQ ID NO: 7 (DG7 : EV71 DVG 1880-6488), SEQ ID NO: 8 (DG8 : EV71 DVG 3513-6516), SEQ ID NO: 9 (DG9 : EV71 DVG 5475-5634), SEQ ID NO: 10 (DG10: EV71 DVG 5610-6876), SEQ ID NO: 11 (DG11 : EV71 DVG 5610-6877), SEQ ID NO: 12 (DG12: EV71 DVG 5746-5821), SEQ ID NO: 13 (DG13 : EV71 DVG 6322-6356), SEQ ID NO: 14 (DG14: EV71 DVG 6322-6358), SEQ ID NO: 15 (DG15: EV71 DVG 6728-6779), SEQ ID NO: 16 (DG16: EV71 DVG 6966-7012), SEQ ID NO: 17 (DG17: EV71 DVG 6966-7014), SEQ ID NO: 18 (DG18: EV71 DVG 7098-7122), SEQ ID NO: 19 (DG19: EV71 DVG 7165-7200), SEQ ID NO: 20 (DG20: EV71 DVG 7165-7201), SEQ ID NO: 21 (DG21: EV71 DVG 7238-7292), SEQ ID NO: 22 (ZIKV DVG-A), SEQ ID NO: 23 (ZIKV DVG-B), SEQ ID NO: 24 (ZIKV DVG-C), SEQ ID NO: 25 (ZIKV DVG-D), SEQ ID NO: 26 (ZIKV DVG-E), SEQ ID NO: 27 (ZIKV DVG-F), SEQ ID NO: 28 (ZIKV DVG-G), SEQ ID NO: 29 (ZIKV DVG-H), SEQ ID NO: 30 (ZIKV DVG-I), SEQ ID NO: 31 (ZIKV DVG-J), SEQ ID NO: 32 (ZIKV DVG-K), SEQ ID NO: 33 (ZIKV DVG-L), SEQ ID NO: 34 (CHIKV DVG-IV1), SEQ ID NO: 35 (CHIKV DVG-IV2), SEQ ID NO: 36 (CHIKV DVG-IV3), SEQ ID NO: 37 (CHIKV DVG-IV4), SEQ ID NO: 38 (CHIKV DVG-IH1), SEQ ID NO: 39 (CHIKV DVG-IU1), SEQ ID NO: 40 (CHIKV DVG-CV1), SEQ ID NO: 41 (CHIKV DVG-CV2), SEQ ID NO: 42 (CHIKV DVG-CV3), SEQ ID NO: 43 (CHIKV DVG-CV4), SEQ ID NO: 44 (CHIKV DVG-CH1), SEQ ID NO: 45 (CHIKV DVG-CH2), SEQ ID NO: 46 (CHIKV DVG-CH3), SEQ ID NO: 47 (CHIKV DVG-CM1), SEQ ID NO: 48 (CHIKV DVG-CU1), SEQ ID NO: 49 (CHIKV DVG-CU2), SEQ ID NO: 50 (CHIKV DVG-CA1), SEQ ID NO: 51 (CHIKV DVG-CA2), SEQ ID NO: 52 (CHIKV DVG-CA3), SEQ ID NO: 53 (CHIKV DVG-CA4), SEQ ID NO: 55 (RV DVG A01a-TIP-01), SEQ ID NO: 56 (RV DVG A01a-TIP-02), SEQ ID NO: 57 (RV DVG A01a-TIP-03), SEQ ID NO: 58 (RV DVG A01a-TIP-04), SEQ ID NO: 59 (RV DVG A01a-TIP-05), SEQ ID NO: 60 (RV DVG A01a-TIP-06), SEQ ID NO: 62 (RV DVG A16-TIP-01), SEQ ID NO: 63 (RV DVG A16-TIP-02), SEQ ID NO: 64 (RV DVG A16-TIP-03), SEQ ID NO: 65 (RV DVG A16-TIP-04), SEQ ID NO: 66 (RV DVG A16-TIP-05), SEQ ID NO: 89 (RV DVG C15-TIP-01), SEQ ID NO: 90 (RV DVG C15-TIP-02), SEQ ID NO: 91 (RV DVG C15-TIP-03), SEQ ID NO: 92 (RV DVG C15-TIP-04), SEQ ID NO: 68 (RV DVG B14-TIP-01),

SEQ ID NO: 69 (RV DVG B14-TIP-02), SEQ ID NO: 70 (RV DVG B14-TIP-03), SEQ ID NO: 71 (RV DVG B14-TIP-04), SEQ ID NO: 72 (RV DVG B14-TIP-05), SEQ ID NO: 73 (RV DVG B14-TIP-06), SEQ ID NO: 74 (RV DVG B14-TIP-07), SEQ ID NO: 75 (RV DVG B14-TIP-08), SEQ ID NO: 76 (RV DVG B14-TIP-09), SEQ ID NO: 77 (RV DVG B14-TIP-10), SEQ ID NO: 78 (RV DVG B14-TIP-11), SEQ ID NO: 79 (RV DVG B14-TIP-12), SEQ ID NO: 80 (RV DVG B14-TIP-13), SEQ ID NO: 81 (RV DVG B14-TIP-14), SEQ ID NO: 82 (RV DVG B14-TIP-15), SEQ ID NO: 83 (RV DVG B14-TIP-16), SEQ ID NO: 84 (RV DVG B14-TIP-17), SEQ ID NO: 85 (RV DVG B14-TIP-18), SEQ ID NO: 86 (RV DVG B14-TIP-19), SEQ ID NO: 87 (RV DVG B14-TIP-20), SEQ ID NO: 93 (YFV DVG-A), SEQ ID NO: 94 (YFV DVG-B), SEQ ID NO: 95 (YFV DVG-C), SEQ ID NO: 96 (YFV DVG-D), SEQ ID NO: 97 (YFV DVG-E), SEQ ID NO: 98 (YFV DVG-F), SEQ ID NO: 99 (YFV DVG-G), SEQ ID NO: 100 (YFV DVG-H), SEQ ID NO: 101 (YFV DVG-I), SEQ ID NO: 102 (YFV DVG-J), SEQ ID NO: 103 (YFV DVG-K), SEQ ID NO: 104 (YFV DVG-L), SEQ ID NO: 105 (WNV DVG-1), SEQ ID NO: 106 (WNV DVG-2), SEQ ID NO: 107 (WNV DVG-3), SEQ ID NO: 108 (WNV DVG-4), SEQ ID NO: 109 (WNV DVG-5), SEQ ID NO: 110 (WNV DVG-6), SEQ ID NO: 111 (SARS-CoV-2 DVG_1), SEQ ID NO: 112 (SARS-CoV-2 DVG_2) and SEQ ID NO: 113 (SARS-CoV-2 DVG_3), or a nucleotide sequence having at least 70% identity, more preferably at least 80% identity; more preferably at least 90% identity, more preferably at least 91% identity, more preferably at least 92% identity, more preferably at least 93% identity, more preferably at least 94% identity, more preferably at least 95% identity, more preferably at least 96% identity, more preferably at least 97% identity, more preferably at least 98% identity, or more preferably at least 99% identity with one of these sequences.

14. A defective interfering particle comprising the DVG of claim 12 or claim 13.

15. Use of at least one DVG or defective interfering particle according to any one of claims 12 to 14 in the manufacture of a medicament for treating ~~A method of treating a viral infection in a subject, wherein an efficient therapeutic amount of the medicament is to be administered to a subject comprising administering an efficient therapeutic amount of at least one DVG or defective interfering particle according to any one of claims 12 to 14.~~

16. The ~~method-use~~ according to claim 15, wherein the DVG is to be administered as a naked RNA.

17. The ~~method-use~~ according to claim 15, wherein at least one DVG is a defective interfering CHIKV genome.

18. The ~~method-use~~ according to claim 17, wherein at least one defective interfering CHIKV genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 34 (CHIKV DVG-IV1), SEQ ID NO: 35 (CHIKV DVG-IV2), SEQ ID NO: 36 (CHIKV DVG-IV3), SEQ ID NO: 37 (CHIKV DVG-IV4), SEQ ID NO: 38 (CHIKV DVG-IH1), SEQ ID NO: 39 (CHIKV DVG-IU1), SEQ ID NO: 40 (CHIKV DVG-CV1), SEQ ID NO: 41 (CHIKV DVG-CV2), SEQ ID NO: 42 (CHIKV DVG-CV3), SEQ ID NO: 43 (CHIKV DVG-CV4), SEQ ID NO: 44 (CHIKV DVG-CH1), SEQ ID NO: 45 (CHIKV DVG-CH2), SEQ ID NO: 46 (CHIKV DVG-CH3), SEQ ID NO: 47 (CHIKV DVG-CM1), SEQ ID NO: 48 (CHIKV DVG-CU1), SEQ ID NO: 49 (CHIKV DVG-CU2), SEQ ID NO: 50 (CHIKV DVG-CA1), SEQ ID NO: 51 (CHIKV DVG-CA2), SEQ ID NO: 52 (CHIKV DVG-CA3) and SEQ ID NO: 53 (CHIKV DVG-CA4).

19. The ~~method-use~~ according to claim 15, wherein at least one defective interfering genome is a defective interfering ZIKV genome.

20. The ~~method-use~~ according to claim 19, wherein at least one interfering ZIKV genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 22 (ZIKV DVG-A), SEQ ID NO: 23 (ZIKV DVG-B), SEQ ID NO: 24 (ZIKV DVG-C), SEQ ID NO: 25 (ZIKV DVG-D), SEQ ID NO: 26 (ZIKV DVG-E), SEQ ID NO: 27 (ZIKV DVG-F), SEQ ID NO: 28 (ZIKV DVG-G), SEQ ID NO: 29 (ZIKV DVG-H), SEQ ID NO: 30 (ZIKV DVG-I), SEQ ID NO: 31 (ZIKV DVG-J), SEQ ID NO: 32 (ZIKV DVG-K) and SEQ ID NO: 33 (ZIKV DVG-L), in particular SEQ ID NO: 32 (ZIKV DVG-K).

21. The ~~method-use~~ according to claim 15, wherein at least one defective interfering genome is a defective interfering EV71 genome.

22. The ~~method-use~~ according to claim 21, wherein at least one defective interfering EV71 genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1 (DG1 : EV71 DVG 293-390), SEQ ID NO: 2 (DG2 : EV71 DVG 294-404), SEQ ID NO: 3 (DG3 : EV71 DVG 1746-2895), SEQ ID NO: 4 (DG4 : EV71 DVG 1752-2894), SEQ ID NO: 5 (DG5 : EV71 DVG 1752-2896), SEQ ID NO: 6 (DG6 : EV71 DVG 1880-6487), SEQ ID NO: 7 (DG7 : EV71 DVG 1880-6488), SEQ ID NO: 8 (DG8 : EV71 DVG 3513-6516), SEQ ID NO: 9 (DG9 : EV71 DVG 5475-5634), SEQ ID NO: 10 (DG10: EV71 DVG 5610-6876), SEQ ID NO: 11 (DG11 : EV71 DVG 5610-6877), SEQ ID NO: 12 (DG12: EV71 DVG 5746-5821), SEQ ID NO: 13 (DG13 : EV71 DVG 6322-6356), SEQ ID NO: 14 (DG14: EV71 DVG 6322-6358), SEQ ID NO: 15 (DG15: EV71 DVG 6728-6779), SEQ ID NO: 16 (DG16: EV71 DVG 6966-7012), SEQ ID NO: 17 (DG17: EV71 DVG 6966-7014), SEQ ID NO: 18 (DG18: EV71 DVG 7098-7122), SEQ ID NO: 19 (DG19: EV71 DVG 7165-7200), SEQ ID NO: 20 (DG20: EV71 DVG 7165-7201) and SEQ ID NO: 21 (DG21: EV71 DVG 7238-7292).

23. The ~~method-use~~ according to claim 15, wherein at least one defective interfering genome is a defective interfering RV genome.

24. The ~~method-use~~ according to claim 23, wherein at least one defective interfering RV genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 55 (RV DVG A01a-TIP-01), SEQ ID NO: 56 (RV DVG A01a-TIP-02), SEQ ID NO: 57 (RV DVG A01a-TIP-03), SEQ ID NO: 58 (RV DVG A01a-TIP-04), SEQ ID NO: 59 (RV DVG A01a-TIP-05), SEQ ID NO: 60 (RV DVG A01a-TIP-06), SEQ ID NO: 62 (RV DVG A16-TIP-01), SEQ ID NO: 63 (RV DVG A16-TIP-02), SEQ ID NO: 64 (RV DVG A16-TIP-03), SEQ ID NO: 65 (RV DVG A16-TIP-04), SEQ ID NO: 66 (RV DVG A16-TIP-05), SEQ ID NO: 89 (RV DVG C15-TIP-01), SEQ ID NO: 90 (RV DVG C15-TIP-02), SEQ ID NO: 91 (RV DVG C15-TIP-03), SEQ ID NO: 92 (RV DVG C15-TIP-04), SEQ ID NO: 68 (RV DVG B14-TIP-01), SEQ ID NO: 69 (RV DVG B14-TIP-02), SEQ ID NO: 70 (RV DVG B14-TIP-03), SEQ ID NO: 71 (RV DVG B14-TIP-04), SEQ ID NO: 72 (RV DVG B14-TIP-05), SEQ ID NO: 73 (RV DVG B14-TIP-06), SEQ ID NO: 74 (RV DVG B14-TIP-07), SEQ ID NO: 75 (RV DVG B14-TIP-08), SEQ ID NO: 76 (RV DVG B14-TIP-09), SEQ ID NO: 77 (RV DVG B14-TIP-10), SEQ ID NO:

78 (RV DVG B14-TIP-11), SEQ ID NO: 79 (RV DVG B14-TIP-12), SEQ ID NO: 80 (RV DVG B14-TIP-13), SEQ ID NO: 81 (RV DVG B14-TIP-14), SEQ ID NO: 82 (RV DVG B14-TIP-15), SEQ ID NO: 83 (RV DVG B14-TIP-16), SEQ ID NO: 84 (RV DVG B14-TIP-17), SEQ ID NO: 85 (RV DVG B14-TIP-18), SEQ ID NO: 86 (RV DVG B14-TIP-19) and SEQ ID NO: 87 (RV DVG B14-TIP-20).

25. The ~~method~~use according to claim 15, wherein the at least one defective interfering genome is a defective interfering YFV genome.

26. The ~~method~~use according to claim 25, wherein the at least one defective interfering YFV genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 93 (YFV DVG-A), SEQ ID NO: 94 (YFV DVG-B), SEQ ID NO: 95 (YFV DVG-C), SEQ ID NO: 96 (YFV DVG-D), SEQ ID NO: 97 (YFV DVG-E), SEQ ID NO: 98 (YFV DVG-F), SEQ ID NO: 99 (YFV DVG-G), SEQ ID NO: 100 (YFV DVG-H), SEQ ID NO: 101 (YFV DVG-I), SEQ ID NO: 102 (YFV DVG-J), SEQ ID NO: 103 (YFV DVG-K) and SEQ ID NO: 104 (YFV DVG-L).

27. The ~~method~~use according to claim 15, wherein the at least one defective interfering genome is a defective interfering WNV genome.

28. The ~~method~~use according to claim 27, wherein the at least one defective interfering WNV genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 105 (WNV DVG-1), SEQ ID NO: 106 (WNV DVG-2), SEQ ID NO: 107 (WNV DVG-3), SEQ ID NO: 108 (WNV DVG-4), SEQ ID NO: 109 (WNV DVG-5) and SEQ ID NO: 110 (WNV DVG-6).

29. The ~~method~~use according to claim 15, wherein the at least one defective interfering genome is a defective interfering CV genome.

30. The ~~method~~use according to claim 29, wherein the at least one defective interfering CV genome comprises or consists of a nucleotide sequence selected from the group consisting of:

SEQ ID NO: 111 (SARS-CoV-2 DVG_1), SEQ ID NO: 112 (SARS-CoV-2 DVG_2) and SEQ ID NO: 113 (SARS-CoV-2 DVG_3).

31. Use of the~~The~~ defective interfering genome DVG or defective interfering particle according to any one of claims 12 to 14 in the manufacture of a medicament for treating~~for use in a method of treatment of~~ CHIKV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 34 (CHIKV DVG-IV1), SEQ ID NO: 35 (CHIKV DVG-IV2), SEQ ID NO: 36 (CHIKV DVG-IV3), SEQ ID NO: 37 (CHIKV DVG-IV4), SEQ ID NO: 38 (CHIKV DVG-IH1), SEQ ID NO: 39 (CHIKV DVG-IU1), SEQ ID NO: 40 (CHIKV DVG-CV1), SEQ ID NO: 41 (CHIKV DVG-CV2), SEQ ID NO: 42 (CHIKV DVG-CV3), SEQ ID NO: 43 (CHIKV DVG-CV4), SEQ ID NO: 44 (CHIKV DVG-CH1), SEQ ID NO: 45 (CHIKV DVG-CH2), SEQ ID NO: 46 (CHIKV DVG-CH3), SEQ ID NO: 47 (CHIKV DVG-CM1), SEQ ID NO: 48 (CHIKV DVG-CU1), SEQ ID NO: 49 (CHIKV DVG-CU2), SEQ ID NO: 50 (CHIKV DVG-CA1), SEQ ID NO: 51 (CHIKV DVG-CA2), SEQ ID NO: 52 (CHIKV DVG-CA3) and SEQ ID NO: 53 (CHIKV DVG-CA4).

32. Use of the~~The~~ defective interfering genome DVG or defective interfering particle according to any one of claims 12 to 14 in the manufacture of a medicament for treating~~for use in a method of treatment of~~ ZIKV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 22 (ZIKV DVG-A), SEQ ID NO: 23 (ZIKV DVG-B), SEQ ID NO: 24 (ZIKV DVG-C), SEQ ID NO: 25 (ZIKV DVG-D), SEQ ID NO: 26 (ZIKV DVG-E), SEQ ID NO: 27 (ZIKV DVG-F), SEQ ID NO: 28 (ZIKV DVG-G), SEQ ID NO: 29 (ZIKV DVG-H), SEQ ID NO: 30 (ZIKV DVG-I), SEQ ID NO: 31 (ZIKV DVG-J), SEQ ID NO: 32 (ZIKV DVG-K) and SEQ ID NO: 33 (ZIKV DVG-L).

33. Use of the~~The~~ defective interfering genome DVG or defective interfering particle according to any one of claims 12 to 14 in the manufacture of a medicament for treating~~for use in a method of treatment of~~ EV71 infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ

ID NO: 1 (DG1 : EV71 DVG 293-390), SEQ ID NO: 2 (DG2 : EV71 DVG 294-404), SEQ ID NO: 3 (DG3 : EV71 DVG 1746-2895), SEQ ID NO: 4 (DG4 : EV71 DVG 1752-2894), SEQ ID NO: 5 (DG5 : EV71 DVG 1752-2896), SEQ ID NO: 6 (DG6 : EV71 DVG 1880-6487), SEQ ID NO: 7 (DG7 : EV71 DVG 1880-6488), SEQ ID NO: 8 (DG8 : EV71 DVG 3513-6516), SEQ ID NO: 9 (DG9 : EV71 DVG 5475-5634), SEQ ID NO: 10 (DG10: EV71 DVG 5610-6876), SEQ ID NO: 11 (DG11 : EV71 DVG 5610-6877), SEQ ID NO: 12 (DG12: EV71 DVG 5746-5821), SEQ ID NO: 13 (DG13 : EV71 DVG 6322-6356), SEQ ID NO: 14 (DG14: EV71 DVG 6322-6358), SEQ ID NO: 15 (DG15: EV71 DVG 6728-6779), SEQ ID NO: 16 (DG16: EV71 DVG 6966-7012), SEQ ID NO: 17 (DG17: EV71 DVG 6966-7014), SEQ ID NO: 18 (DG18: EV71 DVG 7098-7122), SEQ ID NO: 19 (DG19: EV71 DVG 7165-7200), SEQ ID NO: 20 (DG20: EV71 DVG 7165-7201) and SEQ ID NO: 21 (DG21: EV71 DVG 7238-7292).

34. Use of theThe defective interfering genome DVG or defective interfering particle according to any one of claims 12 to 14 in the manufacture of a medicament for treating~~for use in a method of treatment of~~ RV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 55 (RV DVG A01a-TIP-01), SEQ ID NO: 56 (RV DVG A01a-TIP-02), SEQ ID NO: 57 (RV DVG A01a-TIP-03), SEQ ID NO: 58 (RV DVG A01a-TIP-04), SEQ ID NO: 59 (RV DVG A01a-TIP-05), SEQ ID NO: 60 (RV DVG A01a-TIP-06), SEQ ID NO: 62 (RV DVG A16-TIP-01), SEQ ID NO: 63 (RV DVG A16-TIP-02), SEQ ID NO: 64 (RV DVG A16-TIP-03), SEQ ID NO: 65 (RV DVG A16-TIP-04), SEQ ID NO: 66 (RV DVG A16-TIP-05), SEQ ID NO: 89 (RV DVG C15-TIP-01), SEQ ID NO: 90 (RV DVG C15-TIP-02), SEQ ID NO: 91 (RV DVG C15-TIP-03), SEQ ID NO: 92 (RV DVG C15-TIP-04), SEQ ID NO: 68 (RV DVG B14-TIP-01), SEQ ID NO: 69 (RV DVG B14-TIP-02), SEQ ID NO: 70 (RV DVG B14-TIP-03), SEQ ID NO: 71 (RV DVG B14-TIP-04), SEQ ID NO: 72 (RV DVG B14-TIP-05), SEQ ID NO: 73 (RV DVG B14-TIP-06), SEQ ID NO: 74 (RV DVG B14-TIP-07), SEQ ID NO: 75 (RV DVG B14-TIP-08), SEQ ID NO: 76 (RV DVG B14-TIP-09), SEQ ID NO: 77 (RV DVG B14-TIP-10), SEQ ID NO: 78 (RV DVG B14-TIP-11), SEQ ID NO: 79 (RV DVG B14-TIP-12), SEQ ID NO: 80 (RV DVG B14-TIP-13), SEQ ID NO: 81 (RV DVG B14-TIP-14), SEQ ID NO: 82 (RV DVG B14-TIP-15), SEQ ID NO: 83 (RV DVG B14-TIP-16), SEQ ID NO: 84 (RV DVG B14-TIP-17), SEQ ID NO:

85 (RV DVG B14-TIP-18), SEQ ID NO: 86 (RV DVG B14-TIP-19) and SEQ ID NO: 87 (RV DVG B14-TIP-20).

35. Use of the~~The~~ defective interfering genome DVG or defective interfering particle according to any one of claims 12 to 14 in the manufacture of a medicament for treating for use in a method of treatment of YFV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 93 (YFV DVG-A), SEQ ID NO: 94 (YFV DVG-B), SEQ ID NO: 95 (YFV DVG-C), SEQ ID NO: 96 (YFV DVG-D), SEQ ID NO: 97 (YFV DVG-E), SEQ ID NO: 98 (YFV DVG-F), SEQ ID NO: 99 (YFV DVG-G), SEQ ID NO: 100 (YFV DVG-H), SEQ ID NO: 101 (YFV DVG-I), SEQ ID NO: 102 (YFV DVG-J), SEQ ID NO: 103 (YFV DVG-K) and SEQ ID NO: 104 (YFV DVG-L).

36. Use of the~~The~~ defective interfering genome DVG or defective interfering particle according to any one of claims 12 to 14 in the manufacture of a medicament for treating for use in a method of treatment of WNV infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 105 (WNV DVG-1), SEQ ID NO: 106 (WNV DVG-2), SEQ ID NO: 107 (WNV DVG-3), SEQ ID NO: 108 (WNV DVG-4), SEQ ID NO: 109 (WNV DVG-5) and SEQ ID NO: 110 (WNV DVG-6).

37. Use of the~~The~~ defective interfering genome DVG or defective interfering particle according to any one of claims 12 to 14 in the manufacture of a medicament for treating for use in a method of treatment of CV infection to a subject, in particular a SARS-CoV-2 infection to a subject, in particular wherein the defective interfering genome comprises or consists of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 111 (SARS-CoV-2 DVG_1), SEQ ID NO: 112 (SARS-CoV-2 DVG_2) and SEQ ID NO: 113 (SARS-CoV-2 DVG_3).

38. A pharmaceutical, immunogenic, or therapeutic composition, comprising at least one DVG according to claim 12 or claim 13, and a pharmaceutically acceptable carrier.

39. A pharmaceutical, immunogenic, or therapeutic composition, comprising at least one defective interfering particle according to claim 14, and a pharmaceutically acceptable carrier.

40. A vaccine comprising the composition according to claim 38 or 39.

41. Use of a DVG according to claim 12 or 13, or a defective interfering particle according to claim 14, in the manufacture of a medicament for treating ~~for the preparation of a drug for the treatment of~~ a patient infected with a target virus of the DVG or defective interfering particle.

42. Use of a DVG according to claim 12 or 13, or a defective interfering particle according to claim 14, in the manufacture of a medicament for treating ~~for the preparation of a drug for the treatment of~~ a patient infected with CHIKV.

43. Use of a DVG according to claim 12 or 13, or a defective interfering particle according to claim 14, in the manufacture of a medicament for treating ~~for the preparation of a drug for the treatment of~~ a patient infected with ZIKV.

44. Use of a DVG according to claim 12 or 13, or a defective interfering particle according to claim 14, in the manufacture of a medicament for treating ~~for the preparation of a drug for the treatment of~~ a patient infected with EV71.

45. Use of a DVG according to claim 12 or 13, or a defective interfering particle according to claim 14, in the manufacture of a medicament for treating ~~for the preparation of a drug for the treatment of~~ a patient infected with RV.

46. Use of a DVG according to claim 12 or 13, or a defective interfering particle according to claim 14, in the manufacture of a medicament for treating ~~for the preparation of a drug for the treatment of~~ a patient infected with YFV.

47. Use of a DVG according to claim 12 or 13, or a defective interfering particle according to claim 14, in the manufacture of a medicament for treating ~~for the preparation of a drug for the treatment of a patient infected with WNV.~~

48. Use of a DVG according to claim 12 or 13, or a defective interfering particle according to claim 14, in the manufacture of a medicament for treating ~~for the preparation of a drug for the treatment of a patient infected with CV, in particular SARS-CoV-2.~~

49. A polynucleotide comprising or consisting of a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1 (DG1 : EV71 DVG 293-390), SEQ ID NO: 2 (DG2 : EV71 DVG 294-404), SEQ ID NO: 3 (DG3 : EV71 DVG 1746-2895), SEQ ID NO: 4 (DG4 : EV71 DVG 1752-2894), SEQ ID NO: 5 (DG5 : EV71 DVG 1752-2896), SEQ ID NO: 6 (DG6 : EV71 DVG 1880-6487), SEQ ID NO: 7 (DG7 : EV71 DVG 1880-6488), SEQ ID NO: 8 (DG8 : EV71 DVG 3513-6516), SEQ ID NO: 9 (DG9 : EV71 DVG 5475-5634), SEQ ID NO: 10 (DG10: EV71 DVG 5610-6876), SEQ ID NO: 11 (DG11 : EV71 DVG 5610-6877), SEQ ID NO: 12 (DG12: EV71 DVG 5746-5821), SEQ ID NO: 13 (DG13 : EV71 DVG 6322-6356), SEQ ID NO: 14 (DG14: EV71 DVG 6322-6358), SEQ ID NO: 15 (DG15: EV71 DVG 6728-6779), SEQ ID NO: 16 (DG16: EV71 DVG 6966-7012), SEQ ID NO: 17 (DG17: EV71 DVG 6966-7014), SEQ ID NO: 18 (DG18: EV71 DVG 7098-7122), SEQ ID NO: 19 (DG19: EV71 DVG 7165-7200), SEQ ID NO: 20 (DG20: EV71 DVG 7165-7201), SEQ ID NO: 21 (DG21: EV71 DVG 7238-7292), SEQ ID NO: 22 (ZIKV DVG-A), SEQ ID NO: 23 (ZIKV DVG-B), SEQ ID NO: 24 (ZIKV DVG-C), SEQ ID NO: 25 (ZIKV DVG-D), SEQ ID NO: 26 (ZIKV DVG-E), SEQ ID NO: 27 (ZIKV DVG-F), SEQ ID NO: 28 (ZIKV DVG-G), SEQ ID NO: 29 (ZIKV DVG-H), SEQ ID NO: 30 (ZIKV DVG-I), SEQ ID NO: 31 (ZIKV DVG-J), SEQ ID NO: 32 (ZIKV DVG-K), SEQ ID NO: 33 (ZIKV DVG-L), SEQ ID NO: 34 (CHIKV DVG-IV1), SEQ ID NO: 35 (CHIKV DVG-IV2), SEQ ID NO: 36 (CHIKV DVG-IV3), SEQ ID NO: 37 (CHIKV DVG-IV4), SEQ ID NO: 38 (CHIKV DVG-IH1), SEQ ID NO: 39 (CHIKV DVG-IU1), SEQ ID NO: 40 (CHIKV DVG-CV1), SEQ ID NO: 41 (CHIKV DVG-CV2), SEQ ID NO: 42 (CHIKV DVG-CV3), SEQ ID NO: 43 (CHIKV DVG-CV4), SEQ ID NO: 44 (CHIKV DVG-CH1), SEQ ID NO: 45 (CHIKV DVG-CH2), SEQ ID NO: 46 (CHIKV DVG-CH3), SEQ ID NO: 47 (CHIKV DVG-CM1), SEQ ID NO: 48 (CHIKV DVG-CU1), SEQ ID NO: 49 (CHIKV DVG-CU2), SEQ ID NO: 50 (CHIKV DVG-

CA1), SEQ ID NO: 51 (CHIKV DVG-CA2), SEQ ID NO: 52 (CHIKV DVG-CA3), SEQ ID NO: 53 (CHIKV DVG-CA4), SEQ ID NO: 55 (RV DVG A01a-TIP-01), SEQ ID NO: 56 (RV DVG A01a-TIP-02), SEQ ID NO: 57 (RV DVG A01a-TIP-03), SEQ ID NO: 58 (RV DVG A01a-TIP-04), SEQ ID NO: 59 (RV DVG A01a-TIP-05), SEQ ID NO: 60 (RV DVG A01a-TIP-06), SEQ ID NO: 62 (RV DVG A16-TIP-01), SEQ ID NO: 63 (RV DVG A16-TIP-02), SEQ ID NO: 64 (RV DVG A16-TIP-03), SEQ ID NO: 65 (RV DVG A16-TIP-04), SEQ ID NO: 66 (RV DVG A16-TIP-05), SEQ ID NO: 89 (RV DVG C15-TIP-01), SEQ ID NO: 90 (RV DVG C15-TIP-02), SEQ ID NO: 91 (RV DVG C15-TIP-03), SEQ ID NO: 92 (RV DVG C15-TIP-04), SEQ ID NO: 68 (RV DVG B14-TIP-01), SEQ ID NO: 69 (RV DVG B14-TIP-02), SEQ ID NO: 70 (RV DVG B14-TIP-03), SEQ ID NO: 71 (RV DVG B14-TIP-04), SEQ ID NO: 72 (RV DVG B14-TIP-05), SEQ ID NO: 73 (RV DVG B14-TIP-06), SEQ ID NO: 74 (RV DVG B14-TIP-07), SEQ ID NO: 75 (RV DVG B14-TIP-08), SEQ ID NO: 76 (RV DVG B14-TIP-09), SEQ ID NO: 77 (RV DVG B14-TIP-10), SEQ ID NO: 78 (RV DVG B14-TIP-11), SEQ ID NO: 79 (RV DVG B14-TIP-12), SEQ ID NO: 80 (RV DVG B14-TIP-13), SEQ ID NO: 81 (RV DVG B14-TIP-14), SEQ ID NO: 82 (RV DVG B14-TIP-15), SEQ ID NO: 83 (RV DVG B14-TIP-16), SEQ ID NO: 84 (RV DVG B14-TIP-17), SEQ ID NO: 85 (RV DVG B14-TIP-18), SEQ ID NO: 86 (RV DVG B14-TIP-19), SEQ ID NO: 87 (RV DVG B14-TIP-20), SEQ ID NO: 93 (YFV DVG-A), SEQ ID NO: 94 (YFV DVG-B), SEQ ID NO: 95 (YFV DVG-C), SEQ ID NO: 96 (YFV DVG-D), SEQ ID NO: 97 (YFV DVG-E), SEQ ID NO: 98 (YFV DVG-F), SEQ ID NO: 99 (YFV DVG-G), SEQ ID NO: 100 (YFV DVG-H), SEQ ID NO: 101 (YFV DVG-I), SEQ ID NO: 102 (YFV DVG-J), SEQ ID NO: 103 (YFV DVG-K), SEQ ID NO: 104 (YFV DVG-L), SEQ ID NO: 105 (WNV DVG-1), SEQ ID NO: 106 (WNV DVG-2), SEQ ID NO: 107 (WNV DVG-3), SEQ ID NO: 108 (WNV DVG-4), SEQ ID NO: 109 (WNV DVG-5), SEQ ID NO: 110 (WNV DVG-6), SEQ ID NO: 111 (SARS-CoV-2 DVG_1), SEQ ID NO: 112 (SARS-CoV-2 DVG_2) and SEQ ID NO: 113 (SARS-CoV-2 DVG_3), or a polynucleotide having at least 70% identity, more preferably at least 80% identity ; more preferably at least 90% identity, more preferably at least 91% identity, more preferably at least 92% identity, more preferably at least 93% identity, more preferably at least 94% identity, more preferably at least 95% identity, more preferably at least 96% identity, more preferably at least 97% identity, more preferably at least 98% identity, or more preferably at least 99% identity with one of these sequences.-

50. An expression vector or a plasmid comprising the polynucleotide according to claim 49.

51. A cell line producing the DVG according to claim 12 or 13.