

infectious particles are required to cause AGE, and they have high environmental stability, and shedding after infection lasts for weeks (8). However, it has been very difficult to develop effective anti-NoV drugs or vaccines for many years due to the lack of adequate cell lines for viral culture and successful animal models for drug and vaccine evaluation (9, 10). Therefore, there is a need in the art for novel compositions and methods to elicit immunity to NoV.

SUMMARY

[0005] In an example of the current disclosure, polynucleotides are provided. In some examples, the polynucleotides comprise: a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide. In some examples, the polynucleotide is operably linked to a promoter. In some examples, the promoter is a T7 promoter or a T3 promoter. In some examples, the promoter is a T7 promoter. In some examples, the polynucleotide encodes a positive sense viral transcript. In some examples, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about 90% identity to SEQ ID NO: 1. In some examples, the heterologous polynucleotide encodes a peptide or protein. In some examples, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some examples, the peptide or protein comprises an antigenic peptide or protein. In some examples, the peptide or protein comprises a microorganismal peptide or protein. In some examples, the peptide or protein comprises a bacterial peptide or protein. In some examples, the peptide or protein comprises a viral peptide or protein. In some examples, the peptide or protein comprises a norovirus (NoV) peptide or protein. In some examples, the NoV peptide or protein comprises NoV VP1 protein. In some examples, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some examples, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some examples, the SARS-CoV-2 protein or peptide is selected from an N protein, an S protein, or fragment of either an N protein or an S protein. In some examples, the SARS-CoV-2 protein is an S1 protein or a fragment thereof. In some examples, the peptide or protein comprises a cleavage site. In some examples, the cleavage site is a protease cleavage site. In some examples, the cleavage site is a thrombin cleavage site (SEQ ID NO: 28). In some examples, the cleavage site is a self-cleaving peptide sequence. In some examples, the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29). In some examples, the peptide or protein

comprises a linker. In some examples, the linker is a GAG flexible linker or a GSG flexible linker. In some examples, the heterologous polynucleotide is about 2.6 kb or less in length. In some examples, the heterologous polynucleotide is about 1.55 kb or less in length. In some examples, the heterologous polynucleotide is about 1.3 kb or less in length. In some examples, the peptide or protein is a glycoprotein. In some examples, the peptide or protein comprises one or more glycosylation site. In some examples, the heterologous polynucleotide encodes a reporter. In some examples, the reporter is selected from a fluorescent reporter, an enzyme, and an antigen tag. In some examples, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some examples, the polynucleotides encoding a protein selected from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some examples, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22.

[0005a] In one aspect, provided herein is a polynucleotide comprising:

a sequence encoding a rotavirus (RV) NSP3 protein; and

a heterologous polynucleotide, wherein the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3, and wherein the peptide or protein comprises a viral peptide or protein selected from one of SEQ ID NOs: 2-6, 8-11, 81-84, or a sequence with at least 90% identity to one of SEQ ID NOs: 2-6, 8-11, or 81-84.

[0005b] In another aspect, provided herein is an infectious particle comprising the polynucleotide described herein.

[0005c] In another aspect, provided herein is a pharmaceutical composition comprising the infectious particle described herein, optionally, further comprising a pharmaceutically acceptable carrier or excipient.

[0005d] In another aspect, provided herein is the use of the pharmaceutical composition described herein in the manufacture of a medicament for eliciting an immune response to one or more microorganism in a subject, wherein an effective amount of the pharmaceutical composition is to be administered to the subject to elicit an immune response to the one or more microorganism.

[0005e] In another aspect, provided herein is the use of the pharmaceutical composition described herein in the manufacture of a medicament for vaccinating a subject against one or more pathogens, wherein an effective amount of the pharmaceutical composition is to be administered to the subject to vaccinate said subject against the one or more pathogens.

[0005f] In another aspect, provided herein is a method of generating recombinant rotavirus (RV) *in vitro* comprising:

introducing a polynucleotide described herein into a cell; allowing the cell to express the polynucleotide; incubating the cells for a sufficient time to produce RV; and harvesting virus produced by the cells to generate RV *in vitro*, wherein the method further comprises introducing one or more additional polynucleotides into the cell before the allowing step, wherein the one or more additional polynucleotides comprise a sequence encoding an RV protein selected from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4, and NSP5, wherein each sequence encoding an RV protein is operably linked to a promoter, optionally wherein the one or more additional polynucleotides comprise a sequence encoding an RV protein selected from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP4, and NSP5.

[0005g] In another aspect, provided herein is a system, platform, or kit comprising:

- (a) the polynucleotide described herein; and
- (b) cells capable of expressing the polynucleotides of (a).

[0005h] In another aspect, provided herein is the pharmaceutical composition described herein for use in therapy.

[0006] In another example of the current disclosure, infectious particles are provided. In some examples, the infectious particles comprise a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide. In some examples, the polynucleotide is operably linked to a promoter. In some examples, the promoter is a T7 promoter or a T3 promoter. In some examples, the promoter is a T7 promoter. In some examples, the polynucleotide encodes a positive sense viral transcript. In some examples, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about 90% identity to SEQ ID NO: 1. In some examples, the heterologous polynucleotide encodes a peptide or protein. In some examples, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some examples, the peptide or protein comprises an antigenic peptide or protein. In some examples, the peptide or protein comprises a microorganismal peptide or protein. In some examples, the peptide or protein comprises a bacterial peptide or protein. In some examples, the peptide or protein comprises a viral peptide or protein. In some examples, the peptide or protein comprises a norovirus (NoV) peptide or protein. In some examples, the NoV peptide or protein comprises NoV VP1 protein. In some examples, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some examples, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some examples, the SARS-CoV-2 protein or peptide is selected from an N protein, an S protein, or fragment of either an N protein or an S protein. In some examples, the SARS-CoV-2 protein

is an S1 protein or a fragment thereof. In some examples, the peptide or protein comprises a cleavage site. In some examples, the cleavage site is a protease cleavage site. In some examples, the cleavage site is a thrombin cleavage site (SEQ ID NO: 28). In some examples, the cleavage site is a self-cleaving peptide sequence. In some examples, the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29). In some examples, the peptide or protein comprises a linker. In some examples, the linker is a GAG flexible linker or a GSG flexible linker. In some examples, the heterologous polynucleotide is about 2.6 kb or less in length. In some examples, the heterologous polynucleotide is about 1.55 kb or less in length. In some examples, the heterologous polynucleotide is about 1.3 kb or less in length. In some examples, the peptide or protein is a glycoprotein. In some examples, the peptide or protein comprises one or more glycosylation site. In some examples, the heterologous polynucleotide encodes a reporter. In some examples, the reporter is selected from a fluorescent reporter, an enzyme, and an antigen tag. In some examples, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some examples, the polynucleotides encoding a protein selected from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some examples, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22.

[0007] In some examples, the infectious particles are made by introducing a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide into a cell. In some examples, the polynucleotide is operably linked to a promoter. In some examples, the promoter is a T7 promoter or a T3 promoter. In some examples, the promoter is a T7 promoter. In some examples, the polynucleotide encodes a positive sense viral transcript. In some examples, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about 90% identity to SEQ ID NO: 1. In some examples, the heterologous polynucleotide encodes a peptide or protein. In some examples, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some examples, the peptide or protein comprises an antigenic peptide or protein. In some examples, the peptide or protein comprises a microorganismal peptide or protein. In some examples, the peptide or protein comprises a bacterial peptide or protein. In some examples, the peptide or protein comprises a viral peptide or protein. In some examples,

the peptide or protein comprises a norovirus (NoV) peptide or protein. In some examples, the NoV peptide or protein comprises NoV VP1 protein. In some examples, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some examples, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some examples, the SARS-CoV-2 protein or peptide is selected from an N protein, an S protein, or fragment of either an N protein or an S protein. In some examples, the SARS-CoV-2 protein is an S1 protein or a fragment thereof. In some examples, the peptide or protein comprises a cleavage site. In some examples, the cleavage site is a protease cleavage site. In some examples, the cleavage site is a thrombin cleavage site (SEQ ID NO: 28). In some examples, the cleavage site is a self-cleaving peptide sequence. In some examples, the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29). In some examples, the peptide or protein comprises a linker. In some examples, the linker is a GAG flexible linker or a GSG flexible linker. In some examples, the heterologous polynucleotide is about 2.6 kb or less in length. In some examples, the heterologous polynucleotide is about 1.55 kb or less in length. In some examples, the heterologous polynucleotide is about 1.3 kb or less in length. In some examples, the peptide or protein is a glycoprotein. In some examples, the peptide or protein comprises one or more glycosylation site. In some examples, the heterologous polynucleotide encodes a reporter. In some examples, the reporter is selected from a fluorescent reporter, an enzyme, and an antigen tag. In some examples, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some examples, the polynucleotides encoding a protein selected from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some examples, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22.

[0008] In another example of the current disclosure, pharmaceutical composition comprising an infectious particle comprising a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide, optionally, further comprising a pharmaceutically acceptable carrier or excipient. In some examples, the polynucleotide is operably linked to a promoter. In some examples, the promoter is a T7 promoter or a T3 promoter. In some examples, the promoter is a T7 promoter. In some examples, the polynucleotide encodes a positive sense viral transcript. In some examples, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about

90% identity to SEQ ID NO: 1. In some examples, the heterologous polynucleotide encodes a peptide or protein. In some examples, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some examples, the peptide or protein comprises an antigenic peptide or protein. In some examples, the peptide or protein comprises a microorganismal peptide or protein. In some examples, the peptide or protein comprises a bacterial peptide or protein. In some examples, the peptide or protein comprises a viral peptide or protein. In some examples, the peptide or protein comprises a norovirus (NoV) peptide or protein. In some examples, the NoV peptide or protein comprises NoV VP1 protein. In some examples, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some examples, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some examples, the SARS-CoV-2 protein or peptide is selected from an N protein, an S protein, or fragment of either an N protein or an S protein. In some examples, the SARS-CoV-2 protein is an S1 protein or a fragment thereof. In some examples, the peptide or protein comprises a cleavage site. In some examples, the cleavage site is a protease cleavage site. In some examples, the cleavage site is a thrombin cleavage site (SEQ ID NO: 28). In some examples, the cleavage site is a self-cleaving peptide sequence. In some examples, the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29). In some examples, the peptide or protein comprises a linker. In some examples, the linker is a GAG flexible linker or a GSG flexible linker. In some examples, the heterologous polynucleotide is about 2.6 kb or less in length. In some examples, the heterologous polynucleotide is about 1.55 kb or less in length. In some examples, the heterologous polynucleotide is about 1.3 kb or less in length. In some examples, the peptide or protein is a glycoprotein. In some examples, the peptide or protein comprises one or more glycosylation site. In some examples, the heterologous polynucleotide encodes a reporter. In some examples, the reporter is selected from a fluorescent reporter, an enzyme, and an antigen tag. In some examples, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some examples, the polynucleotides encoding a protein selected from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some examples, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22.

[0009] In some examples, the pharmaceutical compositions comprise an infectious particle made by introducing a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide into a cell. In some examples, the polynucleotide is operably linked to a promoter. In some examples, the promoter is a T7 promoter or a T3 promoter. In some examples, the promoter is a T7 promoter. In some examples, the polynucleotide encodes a positive sense viral transcript. In some examples, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about 90% identity to SEQ ID NO: 1. In some examples, the heterologous polynucleotide encodes a peptide or protein. In some examples, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some examples, the peptide or protein comprises an antigenic peptide or protein. In some examples, the peptide or protein comprises a microorganismal peptide or protein. In some examples, the peptide or protein comprises a bacterial peptide or protein. In some examples, the peptide or protein comprises a viral peptide or protein. In some examples, the peptide or protein comprises a norovirus (NoV) peptide or protein. In some examples, the NoV peptide or protein comprises NoV VP1 protein. In some examples, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some examples, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some examples, the SARS-CoV-2 protein or peptide is selected from an N protein, an S protein, or fragment of either an N protein or an S protein. In some examples, the SARS-CoV-2 protein is an S1 protein or a fragment thereof. In some examples, the peptide or protein comprises a cleavage site. In some examples, the cleavage site is a protease cleavage site. In some examples, the cleavage site is a thrombin cleavage site (SEQ ID NO: 28). In some examples, the cleavage site is a self-cleaving peptide sequence. In some examples, the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29). In some examples, the peptide or protein comprises a linker. In some examples, the linker is a GAG flexible linker or a GSG flexible linker. In some examples, the heterologous polynucleotide is about 2.6 kb or less in length. In some examples, the heterologous polynucleotide is about 1.55 kb or less in length. In some examples, the heterologous polynucleotide is about 1.3 kb or less in length. In some examples, the peptide or protein is a glycoprotein. In some examples, the peptide or protein comprises one or more glycosylation site. In some examples, the heterologous polynucleotide encodes a reporter. In some examples, the reporter is selected

from a fluorescent reporter, an enzyme, and an antigen tag. In some examples, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some examples, the polynucleotides encoding a protein selected from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some examples, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22. In some examples, the pharmaceutical compositions further comprise a pharmaceutically acceptable carrier.

[0010] In another example of the current disclosure, methods of eliciting an immune response to one or more microorganism in a subject are provided. In some examples, the methods comprise: administering an effective amount of a pharmaceutical composition comprising an infectious particle comprising a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide, optionally, further comprising a pharmaceutically acceptable carrier or excipient; to a subject to elicit an immune response to the one or more microorganism. In some examples, the pharmaceutical compositions comprise an infectious particle made by introducing a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide into a cell. In some examples, the polynucleotide is operably linked to a promoter. In some examples, the promoter is a T7 promoter or a T3 promoter. In some examples, the promoter is a T7 promoter. In some examples, the polynucleotide encodes a positive sense viral transcript. In some examples, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about 90% identity to SEQ ID NO: 1. In some examples, the heterologous polynucleotide encodes a peptide or protein. In some examples, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some examples, the peptide or protein comprises an antigenic peptide or protein. In some examples, the peptide or protein comprises a microorganismal peptide or protein. In some examples, the peptide or protein comprises a bacterial peptide or protein. In some examples, the peptide or protein comprises a viral peptide or protein. In some examples, the peptide or protein comprises a norovirus (NoV) peptide or protein. In some examples, the NoV peptide or protein comprises NoV VP1 protein. In some examples, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some examples, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some examples, the SARS-CoV-2 protein or peptide is selected from an N protein, an S protein, or

fragment of either an N protein or an S protein. In some examples, the SARS-CoV-2 protein is an S1 protein or a fragment thereof. In some examples, the peptide or protein comprises a cleavage site. In some examples, the cleavage site is a protease cleavage site. In some examples, the cleavage site is a thrombin cleavage site (SEQ ID NO: 28). In some examples, the cleavage site is a self-cleaving peptide sequence. In some examples, the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29). In some examples, the peptide or protein comprises a linker. In some examples, the linker is a GAG flexible linker or a GSG flexible linker. In some examples, the heterologous polynucleotide is about 2.6 kb or less in length. In some examples, the heterologous polynucleotide is about 1.55 kb or less in length. In some examples, the heterologous polynucleotide is about 1.3 kb or less in length. In some examples, the peptide or protein is a glycoprotein. In some examples, the peptide or protein comprises one or more glycosylation site. In some examples, the heterologous polynucleotide encodes a reporter. In some examples, the reporter is selected from a fluorescent reporter, an enzyme, and an antigen tag. In some examples, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some examples, the polynucleotides encoding a protein selected from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some examples, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22. In some examples, the pharmaceutical compositions further comprise a pharmaceutically acceptable carrier. In some examples, the one or more pathogens comprises NoV. In some examples, the one or more pathogens comprises RV and NoV. In some examples, the one or more pathogens comprises SARS-CoV-2. In some examples, the one or more pathogens comprises RV and SARS-CoV-2.

[0011] In another example of the current disclosure, methods of vaccinating a subject against one or more pathogens are provided. In some examples, the methods comprise: administering an effective amount of a pharmaceutical composition comprising an infectious particle comprising a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide, optionally, further comprising a pharmaceutically acceptable carrier or excipient to a subject to vaccinate a subject against the one or more pathogens. In some examples, the pharmaceutical compositions comprise an infectious particle made by introducing a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and

a heterologous polynucleotide into a cell. In some examples, the polynucleotide is operably linked to a promoter. In some examples, the promoter is a T7 promoter or a T3 promoter. In some examples, the promoter is a T7 promoter. In some examples, the polynucleotide encodes a positive sense viral transcript. In some examples, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about 90% identity to SEQ ID NO: 1. In some examples, the heterologous polynucleotide encodes a peptide or protein. In some examples, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some examples, the peptide or protein comprises an antigenic peptide or protein. In some examples, the peptide or protein comprises a microorganismal peptide or protein. In some examples, the peptide or protein comprises a bacterial peptide or protein. In some examples, the peptide or protein comprises a viral peptide or protein. In some examples, the peptide or protein comprises a norovirus (NoV) peptide or protein. In some examples, the NoV peptide or protein comprises NoV VP1 protein. In some examples, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some examples, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some examples, the SARS-CoV-2 protein or peptide is selected from an N protein, an S protein, or fragment of either an N protein or an S protein. In some examples, the SARS-CoV-2 protein is an S1 protein or a fragment thereof. In some examples, the peptide or protein comprises a cleavage site. In some examples, the cleavage site is a protease cleavage site. In some examples, the cleavage site is a thrombin cleavage site (SEQ ID NO: 28). In some examples, the cleavage site is a self-cleaving peptide sequence. In some examples, the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29). In some examples, the peptide or protein comprises a linker. In some examples, the linker is a GAG flexible linker or a GSG flexible linker. In some examples, the heterologous polynucleotide is about 2.6 kb or less in length. In some examples, the heterologous polynucleotide is about 1.55 kb or less in length. In some examples, the heterologous polynucleotide is about 1.3 kb or less in length. In some examples, the peptide or protein is a glycoprotein. In some examples, the peptide or protein comprises one or more glycosylation site. In some examples, the heterologous polynucleotide encodes a reporter. In some examples, the reporter is selected from a fluorescent reporter, an enzyme, and an antigen tag. In some examples, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some examples, the polynucleotides encoding a protein selected

from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some examples, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22. In some examples, the pharmaceutical compositions further comprise a pharmaceutically acceptable carrier. In some examples, the one or more pathogens comprises NoV. In some examples, the one or more pathogens comprises RV and NoV. In some examples, the one or more pathogens comprises SARS-CoV-2. In some examples, the one or more pathogens comprises RV and SARS-CoV-2. In some examples, the one or more pathogens comprises NoV. In some examples, the one or more pathogens comprises RV and NoV. In some examples, the one or more pathogens comprises SARS-CoV-2. In some examples, the one or more pathogens comprises RV and SARS-CoV-2.

[0012] In another example of the current disclosure, methods of generating recombinant rotavirus (RV) *in vitro* are provided. In some examples, the methods comprise: introducing a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide into a cell; allowing the cell to express the polynucleotide; incubating the cells for a sufficient time to produce RV; and harvesting virus produced by the cells to generate RV *in vitro*. In some examples, the polynucleotide is operably linked to a promoter. In some examples, the promoter is a T7 promoter or a T3 promoter. In some examples, the promoter is a T7 promoter. In some examples, the polynucleotide encodes a positive sense viral transcript. In some examples, the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least about 90% identity to SEQ ID NO: 1. In some examples, the heterologous polynucleotide encodes a peptide or protein. In some examples, the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3. In some examples, the peptide or protein comprises an antigenic peptide or protein. In some examples, the peptide or protein comprises a microorganismal peptide or protein. In some examples, the peptide or protein comprises a bacterial peptide or protein. In some examples, the peptide or protein comprises a viral peptide or protein. In some examples, the peptide or protein comprises a norovirus (NoV) peptide or protein. In some examples, the NoV peptide or protein comprises NoV VP1 protein. In some examples, the NoV peptide or protein is selected from SEQ ID NOs: 24, 26, or 80-84. In some examples, the peptide or protein comprises a SARS-CoV-2 protein or peptide. In some examples, the SARS-CoV-2

protein or peptide is selected from an N protein, an S protein, or fragment of either an N protein or an S protein. In some examples, the SARS-CoV-2 protein is an S1 protein or a fragment thereof. In some examples, the peptide or protein comprises a cleavage site. In some examples, the cleavage site is a protease cleavage site. In some examples, the cleavage site is a thrombin cleavage site (SEQ ID NO: 28). In some examples, the cleavage site is a self-cleaving peptide sequence. In some examples, the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29). In some examples, the peptide or protein comprises a linker. In some examples, the linker is a GAG flexible linker or a GSG flexible linker. In some examples, the heterologous polynucleotide is about 2.6 kb or less in length. In some examples, the heterologous polynucleotide is about 1.55 kb or less in length. In some examples, the heterologous polynucleotide is about 1.3 kb or less in length. In some examples, the peptide or protein is a glycoprotein. In some examples, the peptide or protein comprises one or more glycosylation site. In some examples, the heterologous polynucleotide encodes a reporter. In some examples, the reporter is selected from a fluorescent reporter, an enzyme, and an antigen tag. In some examples, the reporter is fused in-frame 3' to the sequence encoding the RV protein. In some examples, the polynucleotides encoding a protein selected from SEQ ID NOs: 2-11, or a sequence with at least about 90% identity to a sequence selected from SEQ ID NOs: 2-11. In some examples, the polynucleotides comprise any one of SEQ ID NOs: 13-22 or a sequence with at least about 90% identity to any one of SEQ ID NOs: 13-22. In some examples, the method further comprises introducing one or more additional polynucleotides into the cell before the allowing step, wherein the one or more additional polynucleotides comprise a sequence encoding an RV protein selected from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4, and NSP5, wherein each sequence encoding an RV protein is operably linked to a promoter. In some examples, the one or more additional polynucleotides comprise a sequence encoding an RV protein selected from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP4, and NSP5. In some examples, the one or more additional polynucleotides comprise a sequence encoding a capping enzyme operably linked to a promoter. In some examples, the capping enzyme is African swine fever virus capping enzyme. In some examples, the cell is selected from an MA-104 cell, a Vero cell, and a BHK-1 cell. In some examples, the cell expresses a heterologous RNA polymerase. In some examples, the heterologous RNA polymerase is selected from T7 RNA polymerase and T3 RNA polymerase. In

some examples, the cell is a BHK-1 cell comprising T7 RNA polymerase. In some examples, the method produces an RV protein fused to a glycoprotein when expressed in the cell.

[0013] In another example of the current disclosure, cells are provided. In some examples, the cells comprise a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide. In some examples, the cells comprise a infectious particle comprising a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide. In some examples, the cells comprise an infectious particle made by introducing a polynucleotide comprising a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide into a cell. In some examples, the cell is an MA-104 cell, a Vero cell or a BHK-1 cell. In some examples, the cell expresses a heterologous RNA polymerase. In some examples, the heterologous RNA polymerase is selected from T7 RNA polymerase and T3 RNA polymerase.

[0014] In another example of the current disclosure, systems, platforms, or kits for generating recombinant rotavirus (RV) are provided. In some examples, the systems, platforms, or kits comprise: (a) a polynucleotide comprising a sequence encoding a rotavirus (RV) NSP3 protein; and a heterologous polynucleotide; and (b) cells capable of expressing the polynucleotides of (a). In some examples, the systems, platforms, or kits further comprise one or more additional polynucleotides comprising a sequence encoding an RV protein selected from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4, and NSP5, wherein each sequence encoding an RV protein is operably linked to a promoter. In some examples, the one or more additional polynucleotides comprise at least one sequence encoding an RV protein selected from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP4, and NSP5. In some examples, the cells comprise a heterologous RNA polymerase and, optionally, comprising African swine fever virus capping enzyme. In some examples, the cells comprise a cells from a cell line selected from MA-104 cells, Vero cells, and BHK-1 cells. In some examples, the cells comprise BHK-1 cells comprising T7 RNA polymerase. In some examples, the cells comprise BHK-1 cells comprising T7 RNA polymerase, Vero cells, and MA-104 cells.

BRIEF DESCRIPTION OF THE FIGURES

CLAIMS

What is claimed is:

1. A polynucleotide comprising:
a sequence encoding a rotavirus (RV) NSP3 protein; and
a heterologous polynucleotide, wherein the heterologous polynucleotide encodes a peptide or protein and is in frame with the NSP3, and wherein the peptide or protein comprises a viral peptide or protein selected from one of SEQ ID NOs: 2-6, 8-11, 81-84, or a sequence with at least 90% identity to one of SEQ ID NOs: 2-6, 8-11, or 81-84.
2. The polynucleotide of claim 1, wherein the polynucleotide is operably linked to a promoter.
3. The polynucleotide of claim 1, wherein the sequence encoding an RV NSP3 is a sequence encoding SEQ ID NO: 1, or a sequence with at least 90% identity to SEQ ID NO: 1.
4. The polynucleotide of claim 1, wherein the polynucleotide comprises a sequence encoding a cleavage site between the sequence encoding the RV NSP3 and the heterologous polynucleotide.
5. The polynucleotide of claim 4, wherein the cleavage site is a protease cleavage site.
6. The polynucleotide of claim 4, wherein the cleavage site is a self-cleaving peptide sequence.
7. The polynucleotide of claim 6, wherein the self-cleaving peptide sequence is porcine teschovirus 2A element (SEQ ID NO: 29).

8. The polynucleotide of claim 1, wherein the heterologous polynucleotide is 2.6 kb or less in length.
9. The polynucleotide of claim 1, wherein the peptide or protein is a glycoprotein.
10. The polynucleotide of claim 1, wherein the peptide or protein comprises one or more glycosylation site.
11. The polynucleotide of claim 1, wherein the polynucleotide comprises any one of SEQ ID NOs: 13-22 or a sequence with at least 90% identity to any one of SEQ ID NOs: 13-17 or 19-22.
12. An infectious particle comprising the polynucleotide of any one of claims 1-11.
13. A pharmaceutical composition comprising the infectious particle of claim 12, optionally, further comprising a pharmaceutically acceptable carrier or excipient.
14. Use of the pharmaceutical composition of claim 13 in the manufacture of a medicament for eliciting an immune response to one or more microorganism in a subject, wherein an effective amount of the pharmaceutical composition is to be administered to the subject to elicit an immune response to the one or more microorganism.
15. Use of the pharmaceutical composition of claim 13 in the manufacture of a medicament for vaccinating a subject against one or more pathogens, wherein an effective amount of the pharmaceutical composition is to be administered to the subject to vaccinate said subject against the one or more pathogens.
16. A method of generating recombinant rotavirus (RV) *in vitro* comprising:
introducing the polynucleotide of any one of claims 1-12 into a cell; allowing the cell to express the polynucleotide; incubating the cells for a sufficient time to produce RV; and harvesting virus produced by the cells to generate RV *in vitro*, wherein the method further comprises introducing one or more additional polynucleotides into the cell before the allowing step, wherein the one or

more additional polynucleotides comprise a sequence encoding an RV protein selected from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4, and NSP5, wherein each sequence encoding an RV protein is operably linked to a promoter, optionally wherein the one or more additional polynucleotides comprise a sequence encoding an RV protein selected from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP4, and NSP5.

17. A system, platform, or kit comprising:
 - (a) the polynucleotide of any one of claims 1-12; and
 - (b) cells capable of expressing the polynucleotides of (a).
18. The pharmaceutical composition of claim 13 for use in therapy.