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MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG,
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RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY,

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(54) Title: ANTIGEN TARGETING TO PORCINE LANGERIN

FIGURE 1
2M3c6c-pg6h2-PEPV S Ag construct

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TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,
VN, ZA, ZM, ZW.

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE,

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- with sequence listing part of description (*Rule 5.2(a)*)

ANTIGEN TARGETING TO PORCINE LANGERIN

The present invention relates to an anti-porcine Langerin antibody. The invention also relates to antigens fused or covalently attached to the anti-porcine Langerin antibody.

- 5 The antigen/antibody chimeric molecule is used to immunize a porcine against a disease caused by an infectious pathogen. Methods of treating a porcine against a disease caused by an infectious pathogen are also provided.

- Infectious pathogens can cause great economic damage in the pork industry. Commercially available vaccines against pathogenic viruses and bacteria pose safety
10 concerns and/or lack complete efficacy. For example, vaccines based on modified live (i.e. attenuated) viruses (MLV) often show good efficacy, but a safety risk exists in that the MLV could mutate or recombine to revert to virulence. Inactivated (i.e. killed) virus vaccines are safer to use, but are rarely as effective as MLV vaccines. Inactivated viruses also may not induce effective cellular immunity, as do MLV. Subunit vaccines
15 containing isolated or recombinant viral antigens are the safest alternative, but may not effectively elicit protective antibody responses unless the antigens are modified and strong adjuvants are incorporated into the vaccines.

- One method of increasing the efficacy of subunit vaccines is to increase the efficiency by which the subunit antigens are taken up by antigen presenting cells (APC),
20 such as macrophages and dendritic cells (DC). In this method, modified antigens are generated in which the subunit antigen is linked to a binding protein recognizing a molecule expressed on APC. One example would be to link an antigen to an antibody which specifically recognizes a surface marker on an APC. An antibody recognizing porcine DC-SIGN has been described, in which an antigen linked to the antibody is
25 targeted to DC. Subramaniam et al., *Vaccine* 32: 6768-75 (2014). Another such APC surface marker could be Langerin, also called CD (cluster of differentiation) 207, which is expressed by DC. However, no specific and high affinity to porcine Langerin (pLangerin) has been identified.

- An anti-pLangerin antibody would be valuable to the development of safe and
30 effective subunit vaccines against porcine pathogens. Particularly advantageous would be a monoclonal antibody (mAb) that could be molecularly altered to create a single chain

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(sc) fusion protein that retained specificity and affinity for pLangerin. Antigens (Ag) derived from porcine pathogens could be linked to the sc fusion protein either recombinantly or chemically. The sc fusion protein-Ag complex could then be used to immunize porcines to protect the animals from infectious disease.

5 Accordingly, disclosed herein is an anti-pLangerin monoclonal antibody, 3B3, whose variable (V) regions can be expressed as a sc fusion protein. The 3B3 sc fusion protein can be recombinantly linked to another domain, such an immunoglobulin heavy chain constant fragment (Fc), to facilitate isolation of the chimeric molecule and provide a domain for additional modifications and linkages. The chimera can be further modified
10 by the addition of an antigen from a porcine pathogen. A representative antigen is the Spike antigen (S Ag) from Porcine Epidemic Diarrhea Virus (PEDV). The 3B3scFv-pIgG₂Fc2-PEDV S Ag chimera can be used to elicit immune responses in a porcine, such as viral neutralizing serum antibodies.

 The present invention provides for a nucleic acid comprising a sequence of SEQ
15 ID NO: 1, wherein the nucleic acid encodes an anti-porcine Langerin antibody heavy chain variable region. The antibody heavy chain can comprise the amino acid sequence of SEQ ID NO: 2. The nucleic acid can further comprise, without limitation, any of the following: a signal sequence, a sequence of an immunoglobulin light chain, a sequence of a light chain variable region, a sequence of an immunoglobulin heavy chain, and/or a
20 sequence of a heavy chain constant region (Fc).

 The present invention provides a nucleic acid comprising a sequence of SEQ ID NO: 1 and further comprising a sequence of SEQ ID NO: 3, wherein the nucleic acid encodes a porcine Langerin binding protein. The nucleic acid can encode immunoglobulin heavy chain regions and immunoglobulin light chain regions in separate
25 open reading frames. The nucleic acid can encode a single chain variable region fragment (scFv) in a porcine Langerin binding protein. The nucleic acid can further comprise, without limitation, a signal sequence and/or a flexible linker region sequence. The nucleic acid can comprise a sequence at least 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 99.5% identical to nucleotides 1-894 of SEQ
30 ID NO: 5. The nucleic acid can comprise a sequence identical to nucleotides 1-894 of SEQ ID NO: 5. The nucleic acid can comprise a sequence identical to at least nucleotides 67-444 and 514-894 of SEQ ID NO:5.

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The present invention provides for a nucleic acid comprising a sequence of SEQ ID NO: 1 and further comprising a sequence of SEQ ID NO: 3, wherein the nucleic acid encodes a single chain variable region fragment (scFv) of a porcine Langerin binding protein. The nucleic acid can further comprise an immunoglobulin heavy chain constant region fragment (Fc) sequence. The Fc sequence can be a sequence of a porcine immunoglobulin Fc. A nucleic acid comprising a sequence of SEQ ID NO: 1, SEQ ID NO: 3, and a porcine immunoglobulin Fc sequence can comprise a sequence at least 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 99.5% identical to SEQ ID NO: 5. For example and without limitation, variability can occur in a signal sequence, a sequence of a flexible linker region, a sequence of a short linker region, and/or a sequence of a porcine immunoglobulin Fc. Further, different codons may be substituted for those contained within SEQ ID NO: 5 without altering the peptide encoded by SEQ ID NO: 5.

The present invention provides for a nucleic acid comprising a sequence of SEQ ID NO: 1, SEQ ID NO: 3, and a porcine immunoglobulin Fc sequence, wherein the nucleic acid encodes a peptide having an amino acid sequence at least 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 99.5% identical to SEQ ID NO: 6. For example and without limitation, variability can occur in a signal sequence, a sequence of a flexible linker region, a sequence of a short linker region, and/or a sequence of a porcine immunoglobulin Fc. The present invention provides for a nucleic acid comprising a sequence of SEQ ID NO: 1, SEQ ID NO: 3, and a porcine immunoglobulin Fc sequence, wherein the nucleic acid encodes a peptide having an amino acid sequence identical to at least amino acids 23-148 and 172-298 of SEQ ID NO: 6.

The present invention provides for a nucleic acid comprising a sequence of SEQ ID NO: 1, a nucleic acid comprising a sequence of SEQ ID NO: 1 and SEQ ID NO: 3, and a nucleic acid comprising a sequence of SEQ ID NOs: 1 and 3 and a porcine immunoglobulin Fc sequence, wherein each nucleic acid can further comprise a sequence derived from a porcine pathogen. The porcine pathogen may be a virus or a bacterial strain. The porcine pathogen may be a virus selected from the group of a Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a Porcine Epidemic Diarrhea virus (PEDV), a Transmissible Gastroenteritis virus (TGEV), a Classical Swine Fever (SCF) virus, a swine influenza virus, a swine coronavirus, a porcine circovirus, a porcine

parvovirus, a swine pox virus, a swine adenovirus, a swine rabies virus, a swine herpes virus, and a swine picornavirus. The present invention provides for a nucleic acid comprising a sequence of SEQ ID NO: 1, a nucleic acid comprising a sequence of SEQ ID NO: 1 and SEQ ID NO: 3, and a nucleic acid comprising a sequence of SEQ ID NOs: 1 and 3 and a porcine immunoglobulin Fc sequence, wherein each nucleic acid can further comprise a sequence derived from a porcine pathogen, which is a Spike antigen sequence derived from the porcine pathogen PEDV.

The present invention provides for a nucleic acid comprising a sequence of SEQ ID NO: 1, SEQ ID NO: 3, a porcine immunoglobulin Fc sequence, and a sequence derived from a porcine pathogen, wherein the nucleic acid comprises a sequence at least 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 99.5% identical to SEQ ID NO: 7. For example and without limitation, variability can occur in a signal sequence, a sequence of a flexible linker region, a sequence of a short linker region, and/or a sequence of a porcine immunoglobulin Fc. A sequence derived from a porcine pathogen may vary among different strains of a given porcine pathogen. The present invention provides for a nucleic acid comprising a sequence of SEQ ID NO: 1, SEQ ID NO: 3, a porcine immunoglobulin Fc sequence, and a sequence derived from a porcine pathogen, wherein the nucleic acid comprises a sequence of SEQ ID NO: 7.

The present invention provides for a nucleic acid comprising a sequence of SEQ ID NO: 3, wherein the nucleic acid encodes a porcine Langerin binding protein. SEQ ID NO: 3 represents a nucleic acid sequence of a light chain variable region of a mouse anti-porcine Langerin antibody. The antibody can comprise the amino acid sequence of SEQ ID NO: 4. The nucleic acid can further comprise, without limitation, any one of a signal sequence, a sequence of an immunoglobulin light chain, a sequence of a light chain variable region, a sequence of an immunoglobulin heavy chain, and/or a sequence of a heavy chain constant region (Fc).

The present invention provides for the use of the nucleic acid comprising a sequence of SEQ ID NO: 1 in the manufacture of a medicament for treating a porcine for an infectious disease. The present invention provides for the use of the nucleic acid comprising a sequence of SEQ ID NO: 3 in the manufacture of a medicament for treating a porcine for an infectious disease. The present invention provides for the use of the nucleic acid comprising a sequence of SEQ ID NO: 1 and SEQ ID NO: 3 in the

manufacture of a medicament for treating a porcine for an infectious disease. The present invention provides for the use of the nucleic acid comprising a sequence of SEQ ID NOs: 1 and 3 and a porcine immunoglobulin Fc sequence in the manufacture of a medicament for treating a porcine for an infectious disease. The present invention provides for the use of the nucleic acid comprising a sequence of SEQ ID NO: 5 in the manufacture of a medicament for treating a porcine for an infectious disease. The present invention provides for the use of the nucleic acid comprising a sequence of nucleotides 1-894 of SEQ ID NO: 5 in the manufacture of a medicament for treating a porcine for an infectious disease. The present invention provides for the use of the nucleic acid comprising a sequence of nucleotides 67-444 and 514-894 of SEQ ID NO: 5 in the manufacture of a medicament for treating a porcine for an infectious disease. The present invention provides for the use of the nucleic acid comprising a sequence of SEQ ID NO: 1, SEQ ID NO: 3, a porcine immunoglobulin Fc sequence, and a sequence derived from a porcine pathogen in the manufacture of a medicament for treating a porcine for an infectious disease. The present invention provides for the use of the nucleic acid comprising a sequence of SEQ ID NO: 7 in the manufacture of a medicament for treating a porcine for an infectious disease.

The present invention provides the nucleic acid comprising a sequence of SEQ ID NO: 1 for use in therapy. The present invention provides the nucleic acid comprising a sequence of SEQ ID NO: 3 for use in therapy. The present invention provides the nucleic acid comprising a sequence of SEQ ID NO: 1 and SEQ ID NO: 3 for use in therapy. The present invention provides for the nucleic acid comprising a sequence of SEQ ID NOs: 1 and 3 and a porcine immunoglobulin Fc sequence for use in therapy. The present invention provides the nucleic acid comprising a sequence of SEQ ID NO: 5 for use in therapy. The present invention provides the nucleic acid comprising a sequence of nucleotides 1-894 of SEQ ID NO: 5 for use in therapy. The present invention provides the nucleic acid comprising a sequence of nucleotides 67-444 and 514-894 of SEQ ID NO: 5 for use in therapy. The present invention provides the nucleic acid comprising a sequence of SEQ ID NO: 1, SEQ ID NO: 3, a porcine immunoglobulin Fc sequence, and a sequence derived from a porcine pathogen for use in therapy. The present invention provides the nucleic acid comprising a sequence of SEQ ID NO: 7 for use in therapy.

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The present invention provides for a porcine Langerin binding protein comprising an amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4. The amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4 may be present on separate peptides or on a single peptide. The present invention provides for a porcine Langerin binding protein

5 comprising an amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, wherein the porcine Langerin binding protein is a single chain variable region fragment (scFv) fusion protein. The present invention provides for a porcine Langerin binding protein comprising an amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, or an scFv comprising an amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, wherein the

10 porcine Langerin binding protein or scFv further comprises at least one of a flexible linker region and an immunoglobulin heavy chain constant region fragment (Fc) peptide. The present invention provides for a porcine Langerin binding protein comprising an amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, or an scFv comprising an amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, wherein the porcine Langerin

15 binding protein or scFv further comprises both a flexible linker region and an immunoglobulin heavy chain constant region fragment (Fc) peptide. The present invention provides for a porcine Langerin binding protein comprising an amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, or an scFv comprising an amino acid sequence of SEQ ID NO: 2 and SEQ ID NO: 4, wherein the porcine Langerin binding

20 protein or scFv further comprises both a flexible linker region and an immunoglobulin heavy chain constant region fragment (Fc) peptide, and wherein the Fc peptide is a porcine Fc peptide.

The present invention provides for a porcine Langerin binding protein, wherein the porcine Langerin binding protein comprises an amino acid sequence at least 95%

25 identical, 96% identical, 97% identical, 98% identical, 99% identical, or 99.5% identical to SEQ ID NO: 6. The present invention provides for a porcine Langerin binding protein, wherein the porcine Langerin binding protein comprises an amino acid sequence of SEQ ID NO: 6. The present invention provides for a porcine Langerin binding scFv fusion protein, wherein the fusion protein comprises an amino acid sequence at least 95%

30 identical, 96% identical, 97% identical, 98% identical, 99% identical, or 99.5% identical to residues 23-298 of SEQ ID NO: 6. The present invention provides for a porcine Langerin binding scFv fusion protein, wherein the fusion protein comprises an amino acid

sequence of residues 23-298 of SEQ ID NO: 6. The present invention provides for a porcine Langerin binding protein or a porcine Langerin binding scFv fusion protein which further comprises an antigenic peptide from a porcine pathogen, wherein the porcine pathogen is a virus or a bacterial strain. The porcine pathogen may be a virus selected
5 from the group of a Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a Porcine Epidemic Diarrhea virus (PEDV), a Transmissible Gastroenteritis virus (TGEV), a Classical Swine Fever (SCF) virus, a swine influenza virus, a swine coronavirus, a porcine circovirus, a porcine parvovirus, a swine pox virus, a swine adenovirus, a swine rabies virus, a swine herpes virus, and a swine picornavirus. The present invention
10 provides for a porcine Langerin binding protein or a porcine Langerin binding scFv fusion protein which further comprises an antigenic peptide from a porcine pathogen, wherein the antigenic peptide from a porcine pathogen is a PEDV Spike antigen peptide.

The present invention provides for an immunogenic composition comprising any of the porcine Langerin binding proteins or porcine Langerin binding scFv fusion protein
15 constructs described herein. The immunogenic composition may further comprise at least one of an adjuvant, an excipient, a stabilizer, a solubilizer, and a diluent.

The present invention provides any of the porcine Langerin binding proteins or porcine Langerin binding scFv fusion protein constructs for use in therapy. The present invention provides an immunogenic composition comprising any of the porcine Langerin
20 binding proteins or porcine Langerin binding scFv fusion protein constructs for use in therapy.

The present invention provides for the use of any of the porcine Langerin binding proteins or porcine Langerin binding scFv fusion protein constructs in the manufacture of a medicament for treating a porcine for an infectious disease. The present invention
25 provides for the use of an immunogenic composition comprising any of the porcine Langerin binding proteins or porcine Langerin binding scFv fusion protein constructs in the manufacture of a medicament for treating a porcine for an infectious disease. The infectious disease of a porcine may be a disease caused by a porcine pathogen such as a virus or a bacterial strain. The porcine pathogen may be a virus selected from the group
30 of a Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a Porcine Epidemic Diarrhea virus (PEDV), a Transmissible Gastroenteritis virus (TGEV), a Classical Swine Fever (SCF) virus, a swine influenza virus, a swine coronavirus, a porcine circovirus, a

porcine parvovirus, a swine pox virus, a swine adenovirus, a swine rabies virus, a swine herpes virus, and a swine picornavirus.

The present invention provides any of the porcine Langerin binding proteins or porcine Langerin binding scFv fusion protein constructs for use in the treatment of a
5 porcine for an infectious disease. The present invention provides an immunogenic composition comprising any of the porcine Langerin binding proteins or porcine Langerin binding scFv fusion protein constructs for use in the treatment of a porcine for an infectious disease. The infectious disease of a porcine may be a disease caused by a porcine pathogen such as a virus or a bacterial strain. The porcine pathogen may be a
10 virus selected from the group of a Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a Porcine Epidemic Diarrhea virus (PEDV), a Transmissible Gastroenteritis virus (TGEV), a Classical Swine Fever (SCF) virus, a swine influenza virus, a swine coronavirus, a porcine circovirus, a porcine parvovirus, a swine pox virus, a swine adenovirus, a swine rabies virus, a swine herpes virus, and a swine picornavirus.

15 The present invention provides a method of treating a porcine for an infectious disease, the method comprising administering to a porcine a porcine Langerin binding protein or porcine Langerin binding scFv fusion protein which further comprises an antigenic peptide from a porcine pathogen. The infectious disease of a porcine may be a disease caused by a porcine pathogen such as a virus or a bacterial strain. The porcine
20 pathogen may be a virus selected from the group of a Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a Porcine Epidemic Diarrhea virus (PEDV), a Transmissible Gastroenteritis virus (TGEV), a Classical Swine Fever (SCF) virus, a swine influenza virus, a swine coronavirus, a porcine circovirus, a porcine parvovirus, a swine pox virus, a swine adenovirus, a swine rabies virus, a swine herpes virus, and a
25 swine picornavirus. The antigenic peptide from a porcine may be a PEDV S antigen peptide. Administering the porcine Langerin binding protein or porcine Langerin binding scFv fusion protein may be done prophylactically or therapeutically.

The present invention provides a method of treating a porcine for an infectious disease, the method comprising administering to a porcine an immunogenic composition
30 comprising a porcine Langerin binding protein or porcine Langerin binding scFv fusion protein which further comprises an antigenic peptide from a porcine pathogen. The infectious disease of a porcine may be a disease caused by a porcine pathogen such as a

virus or a bacterial strain. The porcine pathogen may be a virus selected from the group of a Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a Porcine Epidemic Diarrhea virus (PEDV), a Transmissible Gastroenteritis virus (TGEV), a Classical Swine Fever (SCF) virus, a swine influenza virus, a swine coronavirus, a porcine circovirus, a
 5 porcine parvovirus, a swine pox virus, a swine adenovirus, a swine rabies virus, a swine herpes virus, and a swine picornavirus. The antigenic peptide from a porcine may be a PEDV S antigen peptide. Administering immunogenic composition comprising a porcine Langerin binding protein or a porcine Langerin binding scFv fusion protein may be done prophylactically or therapeutically.

10 The present invention further provides for vectors, such as expression vectors, and host cells that comprise a nucleic acid as disclosed herein. Non-limiting examples of expression vectors are discussed in the illustrative Examples. Such vectors may comprise a sequence of SEQ ID NO: 1 and further a sequence of SEQ ID NO: 3, wherein the vector can express a porcine Langerin binding protein. The vector may comprise nucleic acid
 15 sequence encoding immunoglobulin heavy chain regions and immunoglobulin light chain regions in separate open reading frames. The vector may include a nucleic acid encoding a single chain variable region fragment (scFv) of a porcine Langerin binding protein. The vectors may include nucleic acid sequence that further comprise, without limitation, a signal sequence and/or a flexible linker region sequence. The vector can include a
 20 nucleic acid sequence comprising a sequence at least 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 99.5% identical to nucleotides 1-894 of SEQ ID NO: 5; a sequence identical to nucleotides 1-894 of SEQ ID NO: 5; or a sequence identical to at least nucleotides 67-444 and 514-894 of SEQ ID NO:5.

A host cell may be transformed with the expression vectors and nucleic acid
 25 sequences disclosed herein and may be a prokaryotic cell or eukaryotic cell. Examples of such host cells are known in the art and may include bacterial cells such as *E. coli*; animal cells, such as mammalian host cells CHO and COS; plant cells; yeast cells, such as *S. cerevisiae*; or fungal cells. Accordingly, the present invention provides host cells comprising the vectors and/or nucleic acids that are described herein.

30 **Figure 1.** Nucleotide sequence of a 3B3scFv-pIgG₂Fc2-PEDV S Ag fusion protein construct. The construct includes a signal sequence from the human interleukin-2

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(IL-2) gene, the 3B3 light chain variable (V) region (double underlined), a flexible linker region, the 3B3 heavy chain V region (underlined), a linker region, porcine Fc (bold underlined), a short linker region, and the PEDV Spike (S) antigen (dotted underlined). Boxed nucleotides in the porcine Fc portion represent base changes from GenBank

5 Accession No. AK405797.1.

Disclosed herein is a monoclonal antibody, 3B3, with high affinity for porcine Langerin. The nucleotide sequence encoding the heavy chain variable region of mAb 3B3 mAb is:

1 ATGGCAGCTG CCCAAAGTAT CCAAGCACAG ATCCAGTTGG TGCAGTCTGG ACCTGAGCTG
 10 61 AAGAAGCCTG GAGAGACAGT CAAGATCTCC TGCAAGGCTT CTGGTTATAC CTTACACAGAC
 121 TATTCAATGC ACTGGGTGAA GCAGGCTCCA GGAAAGGGTT TAAAGTGGAT GGGCTGGATA
 181 AACACTGAGA CTGGTGAGCC AACATATGCA GATGACTTCA AGGGACGGTT TGCCTTCTCT
 241 TTGGAAACCT CTGCCAGCAC TGCCTATTG CAGATCAACA ACCTCAAAAA TGAGGACACG
 301 GCTACATATT TCTGTGCTAG AGATATGGAC AACTGGGGTC AAGGAACCTC AGTCACCGTC
 15 361 TCCACAGCCA AAACGACACC C

(SEQ ID NO: 1). The amino acid sequence of the heavy chain variable region of mAb 3B3 is:

1 MAAQSIQIQ IQLVQSGPEL KKPGETVKIS CKASGYTFTD YSMHWVKQAP GKGLKWMGWI
 61 NTETGEPTYA DDFKGRFAFS LETSASTAYL QINNLIKNET ATYFCARDMD NWQGTSVTV
 20 121 STAKTTP

(SEQ ID NO: 2).

The nucleotide sequence of the light chain variable domain of mAb 3B3 comprises the sequence of:

1 ATGTTCTGGA TTCCTGCTTC CAGCAGTGAT GTTTTGATGA CCCAACTCC ACTCTCCCTA
 25 61 CCTGTCAGTC TTGGAGATCA AGCCTCCATC TCTTGAGAT CTAGTCAGAG CATTGTACAT
 121 AGTAGTGGA ACACCTTTTT AGAATGGTAC CTGCAGAAAC CAGGCCAGTC TCCAAAGCTC
 181 CTGATCTACA AAGTTTCCAA CCGATTTTCT GGGGTCCCAG ACAGGTTTCA TGCCACTGGA
 241 TCAGGGACAG ATTTACACT CAAGATCAGC AGAGTGGAGG CTGAGGATCT GGGAGTTTAT
 301 TACTGCTTTC AAGGTTTACA TGTTCCGTAC ACGTTCGGAG GGGGGACCAA GCTGGAAATA
 30 361 AAACGGGCTG ATGCTGCACC AACTGTA

(SEQ ID NO: 3). The amino acid sequence of the light chain variable region of mAb 3B3 is:

1 MFWIPASSSD VLMTQTPLSL PVSIGDQASI SCRSSQSIVH SSGNTFLEWY LQKPGQSPKL
 61 LIYKVSNNRFS GVPDRFSGTG SGTDFTLKIS RVEAEDLGVI YCFQGSHVPY TFGGGKLEI
 35 121 KRADAAPT

(SEQ ID NO: 4).

An antibody unit can have two heavy chains, and two light chains, each containing variable and constant domains. Antigen binding domains and fragments can be designed and generated based on portions of the heavy and light chain variable domain sequences. For example, a F(ab')₂ fragment can contain the light chains and the variable domain, first constant domain, and hinge region of the two heavy chains. A Fab fragment can contain a single light chain and the variable domain and first constant domain of a single heavy chain. A single chain containing a variable light chain domain in sequence with a variable heavy chain domain can also be formed, the result being a "scFv" fusion peptide. Accordingly, the disclosure provides for a porcine Langerin binding protein or a Langerin-binding portion thereof that comprises one or more antigen binding domains of the porcine Langerin antibodies disclosed herein. Determination of the Langerin binding activity can be assessed using any common binding assay that can detect, determine, and/or quantify the amount of binding between Langerin (e.g., porcine Langerin) and the Langerin-binding protein or binding portion thereof.

The 3B3 heavy and light chain variable domains can be linked in a scFv construct. The linking domain must be flexible and long enough to allow the variable peptide domains to pair in a functional conformation, but otherwise the amino acid sequence of the linker and/or linker domain can vary. The linker sequences of the Langerin binding proteins disclosed herein may vary in length depending on whether they are identified as a "short linker" or a sequence that is longer and provides a more "flexible linker" sequence. Non-limiting examples of short linker sequences are provided in the illustrative sequences and are generally from 1 to no more than 10 (e.g., 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) amino acid residues in length. Shorter linkers may be used to join domains and/or regions that are positioned near to one another after the molecule has assembled where tertiary interactions and orientation of the domains with respect to other domains is not necessary for desired function. In some embodiments the short linker is from 1 to 5 amino acids in length (e.g., 1, 2, 3, 4, or 5 amino acid residues). Other longer linker regions, including flexible linkers, generally comprise at least 8 amino acid residues and may comprise up to about 50 amino acid residues (e.g., 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, or approximately 50 residues in length. Portions of such linkers may be flexible, hydrophilic and have little or no

secondary structure of their own (linker portions or flexible linker portions). As illustrated herein, when multiple linkers are used to interconnect different domains/portions of the Langerin binding protein, the linkers may be the same or different (e.g., the same or different length and/or amino acid sequence). The linkers may facilitate formation of the tertiary structure of the Langerin binding protein and help to confer or modify Langerin binding activity or affinity. Non-limiting examples of linkers may comprise (Gly-Ser)_n residues wherein n is a positive integer (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10), with some Glu or Lys residues dispersed throughout to increase solubility. Other linker motifs and sequences may be used with the Langerin binding protein disclosed herein.

A 3B3 scFv fusion protein can also contain a leader sequence, such as a leader sequence which directs the extracellular secretion of the fusion protein. As one skilled in the art would appreciate, many different leader sequences (e.g., signal sequences) could be incorporated into a 3B3 scFv fusion protein. The leader sequence is usually cleaved from the remaining mature form of the fusion protein.

A 3B3 scFv fusion protein can further contain an additional domain. The additional domain can be useful in purification or isolation of the fusion protein, or can provide residues for chemical modification or linkages. For example, a heavy chain constant region fragment (Fc) can be added to a 3B3 scFv fusion protein to permit isolation of the fusion protein using the antibody-binding bacterial Protein A and/or Protein G. If the 3B3 scFv fusion protein is to be used to immunize a porcine, a porcine Fc can be used, which reduces the risk of a host immune response against the 3B3 scFv-Fc chimeric protein.

A 3B3 scFv fusion protein can further contain an antigen from a porcine pathogen. The pathogen can be a virus or a bacterium. For example, the antigen can be the Spike ("S") protein of Porcine Epidemic Diarrhea Virus (PEDV). Multiple antigens or T cell epitopes can be included in a single 3B3 scFv fusion protein. An antigen can be chemically linked to a 3B3 scFv fusion protein. Alternatively, a nucleotide sequence of an antigen can be attached to a nucleotide sequence of a 3B3 scFv fusion construct, such that a 3B3 scFv fusion protein and an antigen are present in a single polypeptide.

A 3B3 scFv fusion protein containing at least one antigen or antigenic epitope can be used to immunize a porcine. The 3B3 scFv portion of the construct would bind

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porcine Langerin expressed by porcine dendritic cells (DC). This binding should facilitate uptake of the chimeric protein by the DC. The chimeric protein would be processed and peptides presented to T lymphocytes in the context of major histocompatibility complex (MHC) class 1 proteins on the surface of the DC. An immunogenic composition containing a 3B3 scFv fusion protein having at least one antigen or antigenic epitope can also contain an adjuvant, such that the adjuvant activates the DC and increases stimulation of T cells by the DC.

An “antigen” is any molecule capable of being specifically detected by the immune system of an organism. Typically a viral antigen is a viral protein encoded by the viral genome or derived from products of the viral genome. The presence of viral antigens can be specifically detected by the surface antigen receptors of both host T lymphocytes and host B lymphocytes and by antibody molecules synthesized by host cells. An “epitope” is a peptide or peptide conformation recognized by an antigen-specific receptor expressed by lymphocytes.

A “fusion” or “chimeric” protein is a peptide which contains two or more amino acid sequences not naturally associated in a single sequence.

“Immunogenicity” refers to the ability of an antigen to elicit an immune response, said immune response comprising both antigen-specific responses and non-antigen-specific responses or innate immune responses. “Protective immunity” is an immune response which can reduce or prevent clinical symptoms when an immunized animal is challenged or exposed to a pathogenic virus strain. As one skilled in the art would appreciate, protective immunity may decline with time or increased age of the immunized animal. Protective immunity as used herein should be effective for at least four months, but preferably at least six months, from the latest date of immunization. Protective immunity may be elicited with a single dose of a vaccine. A second or further dose may be used to increase or prolong the protective immune response. For example, increasing the protective immune response in a breeding sow may result in an increased level of maternally derived antibody in piglets.

In contrast to an antigen, an “adjuvant” is a non-specific stimulator of an immune response. An adjuvant could stimulate the innate immune response by binding and activating a pattern recognition receptor (PRR). Such stimulators of PRRs could be, for

example, viral or bacterial nucleic acids, lipids from bacteria or parasites, or bacterial proteins or toxins, or any artificially-constructed mimic of such molecules. Adjuvants also include, without limitation: inorganic compounds that aggregate antigens to facilitate recognition by B lymphocytes or uptake by phagocytes, such as alum, aluminum
5 hydroxide, aluminum phosphate, calcium phosphate hydroxide or ammonium sulfate; oils; and detergents. Adjuvants could also be host mediators of immune signaling, such as, without limitation, cytokines, lymphokines, chemokines, interferons, anaphylatoxins, growth factors, differentiation factors, and adhesion molecules.

As used herein, an “immunogenic composition” is a composition that elicits an
10 immune response when administered to an animal. An immunogenic composition comprises at least one antigen and at least one pharmaceutically-acceptable excipient, stabilizer, solubilizer, or diluent. A description of pharmaceutically-acceptable excipients, stabilizers, solubilizers, or diluents can be found, for example, in “Remington: The Science and Practice of Pharmacy,” Lloyd V. Allen, ed., Pharmaceutical Press,
15 London, UK, 22nd edition, 2012. The antigen can be a whole virus, bacterium, or other pathogen, either live or inactivated. The antigen can also be isolated, purified, or partially purified antigenic molecule from a virus, bacterium, or other pathogen. The antigen can be a polypeptide, a polysaccharide, a nucleic acid, or a lipid.

As used herein, a “vaccine” is an immunogenic composition which confers
20 protection from, resistance to, prevention of, or treatment for a disease symptom when administered to an animal, wherein said symptom is caused by a pathogenic organism, for example a virus. A vaccine may include, without limitation, viral antigens or intact virions, either live or inactivated, in composition with pharmaceutically-acceptable adjuvants, excipients, stabilizers, solubilizers, or diluents.

25 As used herein, the terms “treating”, “to treat”, or “treatment”, include restraining, slowing, stopping, reducing, ameliorating, or reversing the progression or severity of an existing symptom, disorder, condition, or disease.

As used herein, the terms “preventing”, “to prevent”, or “prevention”, include
without limitation decreasing, reducing, or ameliorating the risk of a symptom, disorder,
30 condition, or disease, and protecting an animal from a symptom, disorder, condition, or disease. A prevention may be applied or administered prophylactically.

“Percent identity” can be determined by calculating the number of identical nucleotides or amino acids at the same relative positions in a nucleic acid or protein. Two or more sequences can be compared over the full length of one sequence or a fragment of a sequence. Calculation of percent identity includes determination of the optimal
5 alignment between two or more sequences. Alignment can take into account insertions and deletions (i.e. “gaps”) in each of the sequences to be tested, such as, without limitation, in the non-coding regions of nucleic acids and truncations or extensions of polypeptide sequences. Computer programs and algorithms such as the Basic Local Alignment Search Tool (BLAST) may be used to determine the percent identity. BLAST
10 one of the many resources provided by the U.S. National Center for Biotechnology Information. Because the genetic code is degenerate, and more than one codon can encode a given amino acid, coding regions of nucleic acids are considered identical if the nucleic acids encode identical polypeptides. Thus, percent identity could also be calculated based on the polypeptide encoded by the nucleic acid.

15 As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly indicates otherwise. Thus, for example, reference to “a V region” or “an antigen” and various hyphenated and unhyphenated forms is a reference to one or more such proteins and includes equivalents thereof known to those of ordinary skill in the art.

20 The following experimental examples are illustrative of anti-porcine Langerin antibodies, and the use of such antibodies to target antigens to porcine dendritic cells. It will be appreciated that other embodiments and uses will be apparent to those skilled in the art and that the invention is not limited to these specific illustrative examples or preferred embodiments.

25 EXAMPLE 1

The porcine Langerin extracellular domain is cloned as a mouse Fc fusion protein, expressed and purified from the supernatants of transiently-transfected 293T cell cultures, and used to generate mouse anti-pLangerin antibodies as follows.

30 The nucleic acid sequence encoding the porcine Langerin extracellular domain (228-1011 nucleotides, GenBank Accession No. NM_001129957) is PCR-amplified and

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cloned in pFUSE-mIgG_{2a}Fc2 and pFUSE-hIgG₁Fc2 expression vectors (InvivoGen). The pLangerin extracellular domain is expressed as mouse F_c fusion proteins in 293T cells by transient transfection using LIPOFECTAMINE® LTX (Thermo Fisher Scientific). 293T cells are maintained in Pro293aCDM (Conditioned Defined Media) (Lonza)

5 supplemented with 4% FBS. Transiently transfected 293T cells are maintained in Iscove's modified Dulbecco's Medium (IMDM) supplemented with 2% ultra-low IgG FBS (fetal bovine serum) (Gibco). The expressed proteins are purified from culture supernatants by Protein A paramagnetic beads (Millipore). BALB/c mice are immunized subcutaneously with purified pLangerin - mIgG_{2a} F_c proteins along with Freund's adjuvant (Sigma). A

10 panel of hybridoma cell lines was established by using the CLONACELL®-HY hybridoma kit (Stemcell Technologies). The monoclonal antibodies produced by the hybridomas are screened by an indirect ELISA in which the pLangerin- hIgG₁F_c and anti-mouse IgG-HRP (horseradish peroxidase) (Kirkegaard & Perry Laboratories) are used as coating antigen and secondary antibody, respectively. Monoclonal antibodies from the

15 hybridoma culture supernatants are purified by Protein A paramagnetic beads (Millipore) and isotyped using a rapid antibody isotyping kit (Pierce).

Two hybridoma cell lines specific to porcine Langerin as measured by the above ELISA have been established, and termed 3B3 and 5G7. The antibodies are also specific to porcine Langerin as measured by immunofluorescence assays on

20 wild-type Langerin transfected 293T cells. The nucleic acid that encodes wild-type Langerin (nucleotides 25-1014, GenBank Accession No. NM_001129957) is PCR-amplified and cloned in pFUSE-hIgG₁-Fc2 (Invivogen). CHO-K1 cells are transfected with pFUSE vector expressing porcine Langerin or CD137 (irrelevant gene control) using LIPOFECTAMINE® LTX (Thermo Fisher Scientific). CHO-K1 cells (ATCC) are

25 maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS. At 48 hours post transfection, cells are fixed with 3.2% of paraformaldehyde, permeabilized with Triton X-100, and blocked with 3% of bovine serum albumin (BSA). Cells are subsequently stained with the Langerin-specific antibodies (1 µg in 100 µL) in blocking buffer followed by incubation with fluorochrome-conjugated secondary

30 antibodies. DAPI (4',6-diamidino-2-phenylindole) is used to stain the cell nucleus, and cells are imaged in Nikon Eclipse TE 300 fluorescence microscope.

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Affinities of the two monoclonal antibodies (mAb) are determined by thiocyanate elution. The binding of the mAb to pLangerin- hIgG₁F_c in an ELISA is assessed in the presence of increasing concentrations of the chaotropic ammonium thiocyanate (Sigma-Aldrich) ion from 0 to 3.5 M, which disrupts binding of the antibody to the antigen.

- 5 Resistance to thiocyanate elution is a measure of affinity whereby an affinity index represents a 50% reduction in the binding in the absence of thiocyanate. The relative affinity of 3B3 mAb (affinity index > 3.5) has been found to be much higher than that of 5G7 mAb (affinity index $\approx$ 0.4).

- 10 The isotype of 3B3 antibody has been determined by an immunoglobulin isotyping kit (ThermoFisher Scientific) and is a mouse IgG1 with a kappa light chain.

EXAMPLE 2

- 15 The variable (V) domains of mAb 3B3 are amplified from DNA extracted from hybridoma cells. Primers for the amplification have been obtained from a mouse Ig-primer set (EMD Millipore). PCR products of mAb 3B3 variable domains are cloned in pCR-Blunt vector (Invitrogen) and the coding DNA sequences are verified by Sanger DNA sequencing (Genomics Research Laboratory, Biocomplexity Institute, Virginia Tech).

- 20 The polynucleotide sequence of the 3B3 mAb heavy chain variable (HCV) is:

```

1 ATGGCAGCTG CCCAAAGTAT CCAAGCACAG ATCCAGTTGG TGCAGTCTGG ACCTGAGCTG
61 AAGAAGCCTG GAGAGACAGT CAAGATCTCC TGCAAGGCTT CTGGTTATAC CTTACACAGAC
121 TATTCAATGC ACTGGGTGAA GCAGGCTCCA GGAAAGGGTT TAAAGTGGAT GGGCTGGATA
181 AACACTGAGA CTGGTGAGCC AACATATGCA GATGACTTCA AGGGACGGTT TGCCTTCTCT
25 241 TTGGAAACCT CTGCCAGCAC TGCCTATTTG CAGATCAACA ACCTCAAAAA TGAGGACACG
301 GCTACATATT TCTGTGCTAG AGATATGGAC AACTGGGGTC AAGGAACCTC AGTCACCGTC
361 TCCACAGCCA AAACGACACC C

```

(SEQ ID NO: 1). The amino acid sequence of the 3B3 heavy chain variable region is:

- ```

1 MAAQSIQAQ IQLVQSGPEL KKPGETVKIS CKASGYTFTD YSMHWVKQAP GKGLKWMGWI
30 61 NTETGEPTYA DDFKGRFAFS LETSASTAYL QINNLKNEDT ATYFCARDMD NWGQGTSVTV
121 STAKTTP

```

(SEQ ID NO: 2).

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The polynucleotide sequence 3B3 mAb light chain variable (LCV) domain is:

```

1 ATGTTCTGGA TTCCTGCTTC CAGCAGTGAT GTTTTGATGA CCCAAACTCC ACTCTCCCTA
61 CCTGTCAGTC TTGGAGATCA AGCCTCCATC TCTTGACAGAT CTAGTCAGAG CATTGTACAT
121 AGTAGTGGAA ACACCTTTTT AGAATGGTAC CTGCAGAAAC CAGGCCAGTC TCCAAAGCTC
5 181 CTGATCTACA AAGTTTCCAA CCGATTTTCT GGGGTCCCAG ACAGGTTTCAG TGGCACTGGA
241 TCAGGGACAG ATTTACACT CAAGATCAGC AGAGTGGAGG CTGAGGATCT GGGAGTTTAT
301 TACTGCTTTC AAGGTTTACA TGTTCCGTAC ACGTTTCGGAG GGGGGACCAA GCTGGAAATA
361 AAACGGGCTG ATGCTGCACC AACTGTA

```

(SEQ ID NO: 3). The amino acid sequence of the 3B3 light chain variable region is:

```

10 1 MFWIPASSSD VLMTQTPLSL PVS LGDQASI SCRSSQSIVH SSGNTFLEWY LQKPGQSPKL
61 LIYKVS NRFS GVPDRFSGTG SGTDFTLKIS RVEAEDLG VY YCFQGSHPY TFGGGTKLEI
121 KRADAAPTV

```

(SEQ ID NO: 4).

### EXAMPLE 3

15 The light and heavy chain V region domains of mAb 3B3 are genetically linked through a flexible linker to make a single-chain-variable-fragment (scFv). The human interleukin-2 (IL-2) signal sequence is also added to the 5' end of the light chain region.

Porcine F<sub>c</sub> cDNA (802-1495 nts, see for example NCBI: AK405797.1) is prepared from pig spleen total RNA using specific primers, cloned in pFUSE-hIgG<sub>1</sub>Fc2 by  
 20 replacing human F<sub>c</sub> and introducing an *XhoI* site at its c-terminus to achieve the vector named pFUSE-pIgG<sub>2</sub>Fc2).

The 3B3scFv is cloned in pFUSE-pIgG<sub>2</sub>Fc2 and pFUSE-hIgG<sub>1</sub>Fc2 (Invivogen) to make porcine and human F<sub>c</sub> fusion antibodies, respectively. The nucleotide sequence of the 3B3scFv- pIgG<sub>2</sub>Fc2 is as follows:

```

25 1 ATGTACAGGA TGCAACTCCT GTCTTGCAAT GCACTAAGTC TTGCACTTGT CACGAATTCTG
61 ATATCTATTCTGCTTCCAG CAGTGATGTT TTGATGACCC AAACCTCCACT CTCCCTACCT
121 GTCAGTCTTG GAGATCAAGC CTCCATCTCT TGCAGATCTA GTCAGAGCAT TGTACATAGT
181 AGTGGAACA CCTTTT TAGA ATGGTACCTG CAGAAACCAG GCCAGTCTCC AAAGCTCCTG
241 ATCTACAAAG TTTCCAACCG ATTTCTGGG GTCCCAGACA GGTTCAGTGG CACTGGATCA
30 301 GGGACAGATT TCACACTCAA GATCAGCAGA GTGGAGGCTG AGGATCTGGG AGTTTATTAC
361 TGCTTTCAAG GTTCACATGT TCCGTACAG TTCCGAGGGG GGACCAAGCT GGAAATAAAA
421 CGGGCTGATG CTGCACCAAC TGTATCCATC TTCCCACCAT CCAGTTCAGG TGGTGGCGGT
481 TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA TCGATGGCAG CTGCCCAAAG TATCCAAGCA
541 CAGATCCAGT TGGTGCAGTC TGGACCTGAG CTGAAGAAGC CTGGAGAGAC AGTCAAGATC

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601 TCCTGCAAGG CTTCTGGTTA TACCTTCACA GACTATTCAA TGCACTGGGT GAAGCAGGCT  
 661 CCAGGAAAAGG GTTTAAAGTG GATGGGCTGG ATAAACACTG AGACTGGTGA GCCAACATAT  
 721 GCAGATGACT TCAAGGGACG GTTTCCTTC TCTTTGAAA CCTCTGCCAG CACTGCCTAT  
 781 TTGCAGATCA ACAACCTCAA AAATGAGGAC ACGGCTACAT ATTTCTGTGC TAGAGATATG  
 5 841 GACAACTGGG GTCAAGGAAC CTCAGTCACC GTCTCCACAG CCAAACGAC ACCCCCATCT  
 901 GTCTATCCAC TGGCCCCTGG AGCCATGGTT AGATCTGGAA CAAAGACCAA ACCACCATGT  
 961 CCCATATGCC CAGCCTGTGA ATCGCCAGGG CCCTCGGTCT TCATCTTCCC TCCAAAACCC  
 1021 AAGGACACCC TCATGATCTC CCGGACACCC CAGGTCACGT GCGTGGTAGT TGATGTGAGC  
 1081 CAGGAGAACC CGGAGGTCCA GTTCTCCTGG TACGTGGACG GCGTAGAGGT GCACACGGCC  
 10 1141 CAGACGAGGC CAAAGGAGGA GCAGTTCAAC AGCACCTACC GCGTGGTCAG CGTCCTGCCC  
 1201 ATCCAGCACC AGGACTGGCT GAACGGGAAG GAGTTCAAGT GCAAGGTCAA CAACAAAGAC  
 1261 CTCCCAGCCC CCATCACAAG GATCATCTCC AAGGCCAAAG GGCAGACCCG GGAGCCGCAG  
 1321 GTGTACACCC TGCCCCCACC CACCGAGGAG CTGTCCAGGA GCAAAGTCAC GCTAACCTGC  
 1381 CTGGTCACTG GCTTCTACCC ACCTGACATC GATGTCGAGT GGCAAAGAAA CGGACAGCCG  
 15 1441 GAGCCAGAGG GCAATTACCG CACCACCCCG CCCCAGCAGG ACGTGGACGG GACCTACTTC  
 1501 CTGTACAGCA AGTTCTCGGT GGACAAGGCC AGCTGGCAGG GTGGAGGCAT ATTCCAGTGT  
 1561 GCGGTGATGC ACGAGGCTCT GCACAACCAC TACACCCAGA AGTCTATCTC CAAGACTCCG  
 1621 GGTAAATGA

- 20 (SEQ ID NO: 5). The 3B3scFv portion (including leader sequence) represents nucleotides 1-894 of SEQ ID NO: 5, which is followed by a linker region to the porcine Fc. The amino acid sequence of this chimeric peptide is as follows:

1 MYRMQLLSCL ALSLALVTNS ISIPASSSDV LMTQTPLSLP VSLGDQASIS CRSSQSIVHS  
 61 SGNTFLEWYL QKPGQSPKLL IYKVSNRFSG VPDRFSGTGS GTDFTLKISR VEAEDLGVYY  
 25 121 CFQGSHPVYT FGGGTKLEIK RADAAPT VSI FPPSSSGGGG SGGGSGGGG SMAAQSIQA  
 181 QIQLVQSGPE LKKPGETVKI SCKASGYFTF DYSMHWVKQA PGKGLKWMGW INTETGEPTY  
 241 ADDFKGRFAF SLETSASTAY LQINN LKNE D TATYFCARDM DNWGQGTSVT VSTAKTTPPS  
 301 VYPLAPGAMV RSGTKTKPPC PICPACESPG PSVFI FPPKP KDTLMIS RTP QVTCVVVDVS  
 361 QENPEVQFSW YVDGVEVHTA QTRPK EEQFN STYRVVSVLP IQHQDWLNGK EFKCKVNNKD  
 30 421 LPAPITRIIS KAKGQTR EPQ VYTLPPPT EE LSR SKVTLTC LVTGFYPPDI DVEWQRNGQP  
 481 EPEGNYR TTP PQQDV DGT YF LYSKF SVDKA SWQGGGIFQC AVMHEALHNH YTQKSISKTP  
 541 GKL

- (SEQ ID NO: 6). The first 298 amino acid residues represent the 3B3scFv peptide, including the human IL-2 leader peptide, which accounts for the first 22 amino acid  
 35 residues. Amino acids 23-148 of SEQ ID NO: 6 are derived from the 3B3 LCV domain, which in this construct is missing the first three amino acids of the LCV domain. Following the flexible linker region, amino acids 172-298 are derived from the 3B3 HCV

domain. Another short linker region joins the above sequences with the amino acids of a porcine immunoglobulin constant region (Fc), found at amino acids 313-543.

#### EXAMPLE 4

5 PEDV-CO/13 strain is propagated in Vero cells with Eagle minimum essential medium (MEM) supplemented with 0.02% yeast extract, 0.3% tryptose phosphate broth and 2 µg/mL trypsin. Uninfected Vero cells (ATCC) are maintained in MEM supplemented with 10% fetal bovine serum (FBS). The N-terminal domain (NTD) and S1 domain of the S protein (amino acids 21-737) of PEDV MN strain (GenBank  
10 Accession No. KF468752.1) are genetically combined with the carboxy terminus of porcine F<sub>c</sub> or 3B3scFv-porcine F<sub>c</sub> in pFUSE vectors. The UGA stop codon for the porcine Fc has been converted to a UCA codon for serine, and a short linker region of Gly-Leu-Glu-Gln is inserted (see Figure 1). The fusion proteins and chimeras to be used as vaccine antigens are produced in 293T cells by large-scale transient transfections using  
15 Polyethyleneimine-Max (Polysciences, Inc). The antigens are purified from culture supernatants with CAPTIVA PRIMAB Protein A agarose beads (Repligen), eluted with 0.2 M Glycine buffer (pH 2.5) and the pH is neutralized with 1 M Tris buffer to approximately 7.5. The molecular sizes of vaccine antigens are analyzed by SDS-PAGE and BIOSAFE Coomassie Blue (Biorad) staining. The stained gel is scanned by  
20 ODYSSEY CLx Infrared Imaging System (LI-COR). The vaccine antigens are quantified by QUICK START Bradford assay (Bio-rad). The standard curve is prepared using the QUICK START Gamma Globulin Standard Set (Biorad). Vaccine antigens are snap-frozen in liquid nitrogen and subsequently stored at -80 °C.

#### 25 EXAMPLE 5

A 3B3scFv-pIgG<sub>2</sub>Fc2-PEDV S Ag chimeric polypeptide can be used to stimulate PEDV-specific immune responses in porcines by targeting the S antigen (Ag) to dendritic cells. The 3B3scFv-pIgG<sub>2</sub>Fc2-PEDV S Ag chimeric polypeptide is encoded by SEQ ID NO: 7.

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A total of 31 four-weeks-old PEDV-negative piglets were divided into four treatment groups: an adjuvant control group (Group 1), a non-targeted group receiving the pIgG<sub>2</sub>Fc2-PEDV S Ag chimeric polypeptide (Group 2), a two DC-targeted groups receiving the 3B3scFv-pIgG<sub>2</sub>Fc2-PEDV S Ag chimeric polypeptide: one given a primary immunization transdermally (Group 3), and a second given a primary immunization intramuscularly (Group 4). Animals in Groups 2-4 received 200 µg of recombinant antigen mixed with 50 µg of cholera toxin (CT), while pigs in Group 1 received 50 µg of CT only. On day 28, all groups received a secondary or boosting injection intramuscularly. Blood was collected on days 0, 7, 21, 28, 35 and 42 for measurement of immune responses.

Antigen-specific T cell responses were analyzed in T cell populations by *in vitro* antigen stimulation of mononuclear cells isolated from peripheral blood followed by intracellular staining of IFN-γ and subsequent flow cytometry analysis. Peripheral blood mononuclear cells (PBMC) were isolated from blood taken on days 0, 7, 28 and 35. The PBMC were stimulated *in vitro* with 20µg/mL recombinant pIgG<sub>2</sub>Fc2-PEDV S Ag for 17h, and stained for intracellular IFN-γ. Flow cytometry analyses showed that the CD4<sup>+</sup>CD8<sup>+</sup> T cell subset was the only subset showing increased mean frequencies of IFN-γ producing cells in response to PEDV antigen. As early as 7 days after the primary vaccination in pigs, the antigen-specific CD4 T cell frequencies in Group 3 (0.969±0.201, p≤0.025) were significantly higher than those in the non-targeted group (0.395±0.158) and the Group 4 (0.483±0.067). The CD4 T cell priming were sustained in a proportion of pigs (2/8) in the Group 3 (p=0.027) up until 28 days after the primary vaccination. At day 7 after the second vaccination (i.e. on day 35), IFN-γ-specific CD4 T cell responses increased significantly in Group 3 (0.391±0.107, p=0.016) as compared to the non-targeted group (0.108±0.052). However, there was no significant difference in CD4 T cell responses observed between Groups 3 and 4. The IFN-γ responses were not found to be significantly elevated among CD4<sup>+</sup>CD8<sup>+</sup> T cells in any group.

The humoral immune response was measured to detect serum IgG and IgA levels on days 21, 28, 35, and 42. Antibody levels were determined by ELISA. ELISA plates (96-well) were coated overnight at 4 °C with 100 µL of PEDV S Ag-mouse IgG<sub>2a</sub> Fc

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antigen (2 µg/mL, diluted in 0.2 M Carbonate/Bicarbonate buffer, pH 9.4). Wells were washed thrice with PBS/Tween-20 (0.1%) and blocked with 150 µL of 5% non-fat milk in PBS/Tween for 2 hours at 37 °C. Test serum, positive and negative serum samples were diluted (1:400) in the blocking buffer. Diluted sera (100 µL) were added to each well in  
5 duplicates. The plates were incubated at 37 °C for 1 hour. Wells were washed thrice with PBS/Tween and then incubated 37 °C for 1 hour with 100 µL of secondary conjugates: horseradish peroxidase (HRP)-conjugated goat anti-pig IgG (H+L), human/mouse serum pre-adsorbed antiserum (Santa Cruz Biotech) (1 µg: 16000 µL) or HRP-conjugated goat anti-pig IgA antiserum (Bethyl) (1 µg: 2000 µL). Wells were washed thrice with  
10 PBS/Tween and incubated with 100 µL of Sure Blue substrate (KPL) for 5 minutes. The reaction was stopped promptly by adding 100 µL of 1N HCl per well. Absorbance was measured at 450 nm in microplate reader (Tecon or Promega GLOMAX). Sample-to-positive (S/P) ratio was calculated by the formula: (sample mean – negative control mean) / (positive control mean – negative control mean) where “mean” was the average  
15 of optical densities (OD) at 450 nm of duplicate samples.

The first vaccine dose induced detectable levels of antigen-specific serum IgG but not IgA by day 21 in all vaccinated groups, but not in the control group. However, serum IgG levels specific to PEDV antigen increased considerably upon the second vaccine dose. Particularly, at day 7 after the second administration (i.e. day 35), the mean IgG S/P  
20 ratio was significantly higher in Group 4 ( $1.372 \pm 0.039$ ,  $p=0.037$ ) than in Group 3 ( $1.230 \pm 0.059$ ). The higher serum IgG levels in Group 4 was sustained until day 35 after boosting (mean S/P ratio:  $1.337 \pm 0.052$ ,  $p=0.005$ ) as compared to Group 3 (mean S/P ratio:  $1.048 \pm 0.076$ ) at the same time point. In contrast, substantial IgA levels were detected only at days 35 in all vaccinated groups, but not in the control group; and the  
25 mean IgA S/P ratio was much higher in the Group 4 ( $0.346 \pm 0.118$ ,  $p=0.001$ ) than Group 3 ( $0.053 \pm 0.007$ ). The serum IgA responses were observed on 42, although at low levels. Interestingly, serum IgG responses were also observed at significant levels in pigs vaccinated with non-targeted PEDV antigen (Group 2), but there was no significant difference noticed in antibody levels between Group 2 and the DC-targeted Group 3.

The serum antibodies measured above were tested for the ability to neutralize PED virus in an *in vitro* infection assay. Vero cells were seeded in 96-well cell culture plates one day prior to actual test. Pig serum samples were heat-inactivated at 56°C for 30 minutes and serially diluted two-fold with MEM. PEDV was prepared at a concentration of 2,000 TCID<sub>50</sub>/mL in MEM and added to diluted sera at 1:1 ratio. The virus-serum mixtures were incubated at 37°C for 1 hour, and then transferred to Vero cell monolayers on 96-well cell culture plates (100 µL per well) which were previously washed with MEM once. Cells were incubated at 37°C for 1 hour and subsequently washed with MEM thrice. Maintenance medium containing trypsin (2 µg/mL) was added to cells and further incubated at 37°C for 5 days. Serum virus neutralization (SVN) titers were calculated as the reciprocals of the highest serum dilution resulting in complete inhibition of PEDV-induced cytopathic effect. Geometric mean was calculated for triplicates as well as groups.

Strong neutralizing antibody (NAb) responses were detected in all vaccinated groups (Groups 2-4) on day 35, with no significant difference in NAb levels between these groups, while the adjuvant control group did not show serum neutralizing activity. The mean PEDV-specific NAb titers ranged between 32 and 64 in the vaccinated groups irrespective of DC targeting. There was a strong positive correlation between PEDV-specific virus-neutralizing antibody levels and IgG levels in serum ( $r = 0.88$ ,  $p < 0.0001$ ), but there was no evidence of correlation between virus-neutralizing antibody levels and IgA levels in serum ( $r = 0.34$ ,  $p = 0.062$ )

## WHAT IS CLAIMED IS:

1. A nucleic acid comprising a sequence of SEQ ID NO: 1, wherein the nucleic acid encodes a porcine Langerin binding protein or a Langerin-binding portion thereof.  
5
2. The nucleic acid of Claim 1, wherein the nucleic acid encodes a binding protein comprising the amino acid sequence of SEQ ID NO: 2.
3. The nucleic acid of Claim 1, further comprising a sequence of SEQ ID NO: 3.
- 10 4. The nucleic acid of Claim 3, wherein the nucleic acid encodes a single chain variable region fragment (scFv) fusion protein.
5. The nucleic acid of Claim 3, further comprising a signal sequence and/or a flexible linker region sequence.
6. The nucleic acid Claim 5, wherein the nucleic acid comprises a sequence  
15 at least 95% identical to nucleotides 1-894 of SEQ ID NO:5.
7. The nucleic acid of Claim 5, further comprising an immunoglobulin heavy chain constant region fragment (Fc) sequence.
8. The nucleic acid of Claim 7, wherein the Fc sequence is a sequence of a porcine immunoglobulin Fc.
- 20 9. The nucleic acid of Claim 7, comprising a sequence at least 95% identical to SEQ ID NO: 5.
10. The nucleic acid of Claim 7, wherein the nucleic acid encodes a peptide having an amino acid sequence at least 95% identical to SEQ ID NO: 6.

11. The nucleic acid of any preceding claim, further comprising a sequence derived from a porcine pathogen; wherein the porcine pathogen is a virus or a bacterial strain.
- 5 12. The nucleic acid of Claim 10; wherein the porcine pathogen is a virus selected from the group of a Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a Porcine Epidemic Diarrhea virus (PEDV), a Transmissible Gastroenteritis virus (TGEV), a Classical Swine Fever (SCF) virus, a swine influenza virus, a swine coronavirus, a porcine circovirus, a porcine parvovirus, a swine pox virus, a swine adenovirus, a
- 10 swine rabies virus, a swine herpes virus, and a swine picornavirus.
13. The nucleic acid of Claim 10, wherein the sequence derived from a porcine pathogen is a PEDV Spike antigen sequence.
14. The nucleic acid of Claim 10, wherein the nucleic acid comprises a sequence at least 95% identical to SEQ ID NO: 7.
- 15 15. A nucleic acid comprising a sequence of SEQ ID NO: 3, wherein the nucleic acid encodes a porcine Langerin binding protein or a Langerin-binding portion thereof.
16. The nucleic acid of Claim 15, wherein the nucleic acid encodes a binding protein comprising the amino acid sequence of SEQ ID NO: 4.
- 20 17. Use of the nucleic acid according to any one of claims 1 to 16 in the manufacture of a medicament for treating a porcine for an infectious disease.
18. A protein comprising an amino acid sequence of SEQ ID NO:2 and SEQ ID NO: 4.

19. The protein of Claim 18, wherein the protein is a single chain variable region fragment (scFv) fusion protein.
20. The protein of any one of Claims 18 or 19, further comprising a flexible linker region.
- 5 21. The protein of any one of Claims 18-20, further comprising an immunoglobulin heavy chain constant region fragment (Fc) peptide.
22. The protein of Claim 21, wherein the Fc peptide is a porcine Fc peptide.
23. The protein of any one of Claims 18-22, wherein the protein comprises an amino acid sequence at least 95% identical to SEQ ID NO: 6; or at least
- 10 95% identical to residues 23-298 of SEQ ID NO: 6.
24. The protein of any of Claims 18-23, further comprising an antigenic peptide from a porcine pathogen; wherein the porcine pathogen is a virus or a bacterial strain.
25. The protein of Claim 24; wherein the porcine pathogen is a virus selected
- 15 from the group of a Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a Porcine Epidemic Diarrhea virus (PEDV), a Transmissible Gastroenteritis virus (TGEV), a Classical Swine Fever (SCF) virus, a swine influenza virus, a swine coronavirus, a porcine circovirus, a porcine parvovirus, a swine pox virus, a swine adenovirus, a swine rabies virus, a
- 20 swine herpes virus, and a swine picornavirus.
26. The protein of Claim 24, wherein the antigenic peptide from a porcine pathogen is a PEDV Spike antigen peptide.
27. An immunogenic composition comprising the protein of any one of Claims 18-26.

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28. The immunogenic composition of Claim 27, further comprising at least one of an adjuvant, an excipient, a stabilizer, a solubilizer, and a diluent.
29. The protein of any one of Claims 18-26 or the immunogenic composition of any one of Claims 27-28 for use in therapy.
- 5 30. Use of the protein of any one of Claims 18-26 or the immunogenic composition of any one of Claims 27-28 in the manufacture of a medicament for treating a porcine for an infectious disease.
31. A method of inducing an immune response in a porcine comprising administering to the subject at least one of
- 10 a. the protein of any one of Claims 18-26;  
b. the immunogenic composition of any one of Claims 27-28; or  
c. the nucleic acid of any one of Claims 1 to 16,  
in an amount effective to induce an immune response in the porcine against a porcine pathogen.
- 15 32. The method according to Claim 31 wherein the method induces a protective immune response against the porcine pathogen and the porcine is at risk of exposure to a porcine pathogen or at risk of developing an infectious disease caused by the porcine pathogen.
33. The method according to Claim 31 wherein the method induces an
- 20 immune response in a porcine that has been exposed to the porcine pathogen or has developed an infectious disease caused by the porcine pathogen.
34. The method according to any of Claims 31 to 33 wherein the porcine pathogen is a Porcine Epidemic Diarrhea Virus (PEDV).

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- 35. An expression vector comprising the nucleic acid of any one of Claims 1 to 16.
- 36. A host cell comprising the nucleic acid of any one of Claims 1 to 16, or the expression vector of claim 35.

Figure 1 (page 1/3)

## PEDV Spike (S) protein

1621 GGTAAATCAG GTCTCGAGCA AGATGTCACC AGGTGCTCAG CTAACACTAA TTTTAGGCGG  
 1681 TTCTTTTCAA AATTTAATGT TCAGGCGCCT GCAGTTGTTG TACTGGGCGG TTATCTACCT  
 1741 ATTGGTGAAA ACCAGGGTGT CAATTCAACT TGGTACTGTG CTGGCCAACA TCCAACGTCT  
 5 1801 AGTGGCGTTC ATGGTATCTT TGTTAGCCAT ATTAGAGGTG GTCATGGCTT TGAGATTGGC  
 1861 ATTTGCAAG AGCCTTTTGA CCCTAGTGGT TACCAGCTTT ATTTACATAA GGCTACTAAC  
 1921 GGTAACACTA ATGCTACTGC GCGACTGCGC ATTTGCCAGT TTCCTAGCAT TAAACATTG  
 1981 GGCCCCACTG CTAATAATGA TGTTACACA GGTGTAATT GCCTATTTAA CAAAGCCATC  
 2041 CCAGCTCATA TGAGTGAACA TAGTGTGTC GGCATAACAT GGGATAATGA TCGTGTCACT  
 10 2101 GTCTTTTCTG ACAAGATCTA TTATTTTAT TTTAAAAATG ATTGGTCCCG TGTTGCGACA  
 2161 AAGTGTTACA ACAGTGGAGG TTGTGCTATG CAATATGTTT ACGAACCAC CTATTACATG  
 2221 CTTAATGTTA CTAGTGCTGG TGAGGATGGT ATTTTATATC AACCTGTAC AGCTAATTGC  
 2281 ATTGGTTATG CTGCAATGT ATTTGCTACT GAGCCCAATG GCCACATACC AGAAGGTTTT  
 2341 AGTTTTAATA ATTGGTTTCT TTTGTCCAAT GATTCCACTT TGGTGATGG TAAGGTGGTT  
 15 2401 TCCAACCAAC CATTGTTGGT CAATTGTCTT TTGGCCATTC CTAAGATTTA TGGACTAGGC  
 2461 CAATTTTTCT CCTTTAATCA AACGATCGAT GGTGTTTGTA ATGGAGCTGC TGTGCAGCGT  
 2521 GCACCAGAGG CTCTGAGGTT TAATATTAAT GACACCTCTG TCATTCTTGC TGAAGGCTCA  
 2581 ATTGTACTTC ATACTGCTTT AGGAACAAAT TTTTCTTTTG TTTGCAGTAA TTCCTCAAAT  
 2641 CCTCACTTAG CCACCTTCGC CATACTTTG GGTGCTACCC AAGTACCCTA TTATTGTTTT  
 20 2701 CTTAAAGTGG ATACTTACAA CTCCACTGTT TATAAATTCT TGGCTGTTTT ACCTCCAACC  
 2761 GTCAGGGAAA TTGTCATCAC CAAGTATGGT GATGTTTATG TCAATGGGTT TGGATACTTG  
 2821 CATCTCGGTT TGTTGGATGC TGTCACAATT AATTTCACTG GTCATGGCAC TGACGATGAT  
 2881 GTTTCTGGTT TTTGGACCAT AGCATCGACT AATTTGTTG ATGCACTCAT CGAAGTTCAA  
 2941 GGAACCGCCA TTCAGCGTAT TCTTTATTGT GATGATCCTG TTAGCCAACCT CAAGTGTCT  
 25 3001 CAGGTIGCTT TTGACCTTGA CGATGGTTTT TACCATATTT CTTCTAGAAA CCTTCTGAGT  
 3061 CATGAACAGC CAATTTCTTT TGTTACTCTG CCATCATTTA ATGATCATTC TTTTGTTAAC  
 3121 ATTACTGTAT CTGCTTCCTT TGGTGGTCAT AGTGGTGCCA ACCTTATTGC ATCTGACACT  
 3181 ACTATCAATG GGTTTAGTTC TTTCTGTGTT GACACTAGAC AATTTACCAT TTCACTGTTT  
 3241 TATAACGTTA CAAACAGTTA TGGTTATGTG TCTAAATCAC AGGACAGTAA TTGCCCTTTC  
 30 3301 ACCTTGCAAT CTGTTAATGA TTACCTGTCT TTTAGCAAAT TTTGTGTTTC CACCAGCCTT  
 3361 TTGGCTAGTG CCTGTACCAT AGATCTTTTT GGTACCCTG AGTTTGGTAG TGGTGTTAAG  
 3421 TTTACGTCCC TTTACTTTCA ATTCACAAAG GGTGAGTTGA TTAGTGGCAC GCCTAAACCA  
 3481 CTTGAAGGTG TCACGGACGT TTCTTTTATG ACTCTGGATG TGTGTACCAA GTATACTATC  
 3541 TATGGCTTTA AAGGTGAGGG TATCATTACC CTTACAAATT CTAGCTTTTT GGCAGGTGTT  
 35 3601 TATTACACAT CTGATTCTGG ACAGTTGTTA GCCTTTAAGA ATGTCACTAG TGGTGCTGTT

Figure 1 continued (page 2/3)

3661 TATTCTGTTA CGCCATGTTT TTTTCAGAG CAGGCTGCAT ATGTTGATGA TGATATAGTG  
3721 GGTGTTATTT CTAGTTTGTC TAGCTCCACT TTTAACAGTA CTAGGGAGTT GCCTGGTTTC  
STOP  
3781 TTCTACCATT GA

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30 Figure 1 continued (page 3/3)

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2017/012251

A. CLASSIFICATION OF SUBJECT MATTER  
INV. C07K16/28  
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, Sequence Search, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                     | Relevant to claim No. |
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Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

30 March 2017

Date of mailing of the international search report

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International application No  
PCT/US2017/012251

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                     |                       |
|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
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Information on patent family members

International application No

PCT/US2017/012251

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