

Cellular Permissivity Factor for Viruses, and Uses Thereof

FIELD OF THE INVENTION

The present invention provides methods and compositions related to the generation of host cells permissive for virus growth for viruses of the family

5 *Asfarviridae* and *Arteriviridae*.

BACKGROUND OF THE INVENTION

Asfarviridae

Asfarviridae is a family of icosohedral enveloped viruses whose genome consists of a single molecule of linear double-stranded DNA of about 150000–190000 nucleotides
10 long. The name of the family is derived from African Swine Fever And Related Viruses. African Swine Fever Virus (ASFV) is the type species of the *Asfivirus* genus and is the sole member of the family. Recently, porcine CD163 polypeptide has been surmised by implication to be the cellular receptor for African swine fever virus (ASFV) (Sanchez-Torres et al., 2003)

15 *Arteriviridae*

Viruses of the family of *Arteriviridae* includes equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV) and simian hemorrhagic fever virus (SHFV). The Arterivirus having the greatest economic importance is Porcine Reproductive and Respiratory Syndrome Virus (PRRSV).

20 PRRSV

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most economically important diseases of swine. The syndrome appeared almost simultaneously in North American and in Western Europe in the late 1980s, and has since spread to become endemic in the major swine producing nations of Europe,
25 Asia, and the Americas. The etiologic agent of PRRS is a virus that has been designated PRRS virus or PRRSV. For both European and North American PRRS, the disease is characterized by reproductive failure in sows and gilts (late term abortions, still births, and mummies), high mortality among nursery pigs, and respiratory disease in swine of all ages. The disease has been the subject of recent
30 reviews (Mengeling and Lager, 2000; Murtaugh et al., 2002; Nodelijk, 2002; Plagemann, 2003).

In the pig, PRRSV infection is limited to a subset of cells of the monocyte/macrophage lineage. Fully differentiated porcine alveolar macrophage (PAM) cells are the primary target cells for viral replication (Duan et al., 1997a; Duan

et al., 1997b). Immortalization of PAM cells is technically challenging, and when successful, has resulted in cell lines that are not permissive for PRRS virus growth (Weingartl et al., 2002). PRRS virions are specifically bound by macrophages and internalized in clathrin-coated pits by endocytosis. Release from endocytic vesicles requires acidic pH (Nauwynck et al., 1999). Initial binding of virions is mediated by interaction of the viral matrix protein with heparin sulfate glycosaminoglycans (Delputte et al., 2002). Internalization can be facilitated by a 210 or 220 kDa membrane glycoprotein, as incubation of PAM cells with monoclonal antibodies to this polypeptide block infection with PRRS virus (Duan et al., 1998; Wissink et al., 2003). The 210 kDa glycoprotein has recently been identified as sialoadhesin, a member of siglec family of sialic acid binding immunoglobulin-like lectins (Pensaert et al., 2003). Transfection of the non-permissive PK-15 (porcine kidney) cell line with porcine sialoadhesin conferred the ability to internalize PRRSV particles, but there remained an apparent block at the uncoating stage, as virions entered into cellular vesicles but did not undergo nucleocapsid disintegration and vesicle membrane fusion. Viral genes were not expressed, and the transfected PK-15 cells were not rendered permissive for the PRRS virus (Vanderheijden et al., 2003). To our knowledge, transfection with sialoadhesin has not been shown to be sufficient to convert any PRRSV non-permissive cell line to a PRRSV-permissive phenotype.

Apart from primary porcine cells of the monocyte/macrophage lineage, the only other cell type known to be permissive for the growth of PRRSV in cell culture is the immortalized monkey kidney cell line MA-104 (Chladek et al., 1998) and derivatives such as MARC-145 (Kim et al., 1993) and CL-2621. It is not known why this one particular cell line is permissive, yet other mammalian cell lines are not. The PRRS virus binds specifically to a number of different cell types, but does not initiate infection (Kreutz, 1998; Therrien et al., 2000). In MARC-145 cells, the internalization of the virus by endocytosis and subsequent uncoating in low pH vesicles seems to mimic similar events in PAM cells (Kreutz and Ackermann, 1996). However, a number of monoclonal antibodies that bind to porcine sialoadhesin fail to detect a homologous protein on the surface of MARC-145 cells (Duan et al., 1998; Wissink et al., 2003), suggesting that MARC-145 cells may use a divergent member of the same protein family or a different receptor altogether.

Current PRRSV vaccines are propagated on simian cell lines, which is a potentially dangerous activity. The use of simian cell lines for vaccine production has the potential to introduce primate viruses of significance into swine lines intended for xenotransplant purposes. Because swine are being increasingly explored as a source of xenotransplanted organs for humans, the introduction of primate cell lines to swine populations may ultimately pose a risk to humans receiving xenotransplanted organs. Thus, it would be prudent to avoid the use of simian cell lines in swine vaccine preparations. It would be therefore desirable to identify or generate non-simian cells or cell lines capable of supporting PRRSV replication. Towards this goal, it is essential to identify the gene product(s) which may be responsible for conferring the permissivity for PRRSV replication as seen in certain simian cells lines as well as PAM cells. Once such gene products are identified, non-permissive cells might be rendered permissive by transfection of the essential gene into them, thereby affording a wider array of production lines for a vaccine.

One lab has reported that the tetraspanin protein CD151 from MARC-145 cells, when transfected into non-permissive BHK-21 cells, confers permissivity to the PRRS virus (Kapil and Shanmukhappa, 2003; Shanmukhappa and Kapil, 2001). This observation has yet to be confirmed by an independent lab. We describe here an unrelated polypeptide, which when transfected into non-permissive cells, confers permissivity to the PRRS virus.

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SUMMARY OF THE INVENTION

The invention includes a method of facilitating infection of one or more cells
5 by a virus that is selected from the group consisting of *Arteriviridae* and *Asfarviridae*,
which comprises the step of directing increased expression of a CD163 polypeptide
within said cell. In a preferred embodiment the CD163 is membrane bound. In one
embodiment the virus is selected from the group consisting of *Arteriviridae*. In a
preferred embodiment the virus is PRRSV. In another embodiment said virus is
10 equine arteritis virus (EAV). In yet another embodiment said virus is African Swine
Fever virus (ASFV).

Increased expression of a CD163 polypeptide may be accomplished by
methods such as introduction of exogenous nucleic acids encoding CD163
polypeptides such methods include but are not limited to transfection, electroporation
15 and fusion with a carrier of a polynucleotide comprising a polynucleotide encoding a
CD163 polypeptide. Increased expression may also be accomplished by induction of
expression of endogenous CD163 by chemical treatment.

The method may render previously non PRRSV-permissive cells PRRS
permissive. The method may also include rendering one or more cells that previously
20 did not express a CD163 polypeptide into cells that are induced to express a CD 163
polypeptide.

The cells in a preferred embodiment are animal cells. They may be vertebrate
or invertebrate cells. The cells may be mammalian. The cells or cell line may be an
insect cell line. The cells may be BHK21 cells. The cells may be derived from
25 porcine kidney cells. The cells or cell line may be derived from feline kidney cells.
The cells or cell line may be but are not limited to BHK-21, NLST-1, NLFK-1, Vero
or RL cells. The PRRSV may be or the European or North American genotype.

As noted above, increased expression of a CD163 polypeptide may be
accomplished by methods which include but are not limited to: transfection,
30 electroporation and fusion with a carrier of a polynucleotide comprising a
polynucleotide encoding a CD163 polypeptide. Any CD163 polypeptides are
contemplated. Those containing a transmembrane region are preferred. Exemplary

CD163 polypeptides are selected from the group consisting of the polynucleotides listed below.

One such polynucleotide comprises a polynucleotide encoding a polypeptide having at least 70% 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%,
5 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, 99%, identity with SEQ ID NO: 2.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 2 by no more than 20 conservative amino acid substitutions.

10 One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 2 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 2.

15 One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO:1

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 99% identity to a polypeptide set forth in SEQ ID NO: 14

One such polynucleotide comprises a polynucleotide encoding a polypeptide
20 differing from SEQ ID NO: 14 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 14 by no more than 10 conservative amino acid substitutions.

25 One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 14.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 13.

One such polynucleotide comprises a polynucleotide encoding a polypeptide
30 differing from SEQ ID NO: 24 by no more than 2 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 24.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 23.

One such polynucleotide comprises a polynucleotide encoding a polypeptide having at least 96% 97% 98%, or 99%, identity to a polypeptide set forth in SEQ ID
5 NO: 27.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 27 by no more than 20 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide
10 differing from SEQ ID NO: 27 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 27.

One such polynucleotide comprises a polynucleotide with the sequence set
15 forth in SEQ ID NO: 26.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 32

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 32 by no more than 15 conservative amino acid
20 substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 32 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide
25 comprising SEQ ID NO: 32.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 31.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ
30 ID NO: 34

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 34 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 34 by no more than 10 conservative amino acid substitutions.

5 One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 34.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 33.

10 One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 36

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 36 by no more than 15 conservative amino acid substitutions.

15 One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 36 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 36.

20 One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 35.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 42

25 One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 42 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 42 by no more than 10 conservative amino acid substitutions.

30 One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 42.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 41.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 44

One such polynucleotide comprises a polynucleotide encoding a polypeptide
5 differing from SEQ ID NO: 44 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 44 by no more than 10 conservative amino acid substitutions.

10 One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 44.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 43

One such polynucleotide comprises a polynucleotide encoding a polypeptide
15 that has at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 46

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 46 by no more than 15 conservative amino acid substitutions.

20 One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 46 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 46.

25 One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 45.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 48

30 One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 48 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 48 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 48.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 47.

The method of facilitating infection described above may further comprise the step of producing a culture of virus.

The invention further comprises the culture isolated by the method described above.

Any of the methods described above may further comprise the step of producing a PRRS or other viral vaccine. The vaccine may be killed or live attenuated.

The invention also comprises a cell or cell line wherein the ability of one or more cells to be infected by a virus selected from the group consisting of *Arteriviridae* and *Asfarviridae* has been modified by directing increased expression of a CD163 polypeptide within said cells.

In a preferred embodiment the CD163 polypeptide comprises a transmembrane region. In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

The cell or cell line of the invention may have been previously PRRSV non-permissive and is rendered PRRSV permissive by directing increased expression of a CD163 polypeptide within said cell or cell line.

The cell or cell line of the invention includes cells or cell lines that did not express a CD163 polypeptide and is induced to express a CD 163 polypeptide.

The cells in a preferred embodiment are animal cells. They may be vertebrate or invertebrate cells. The cells may be mammalian. The cell or cell line may be an insect cell or cell line. The cells may be BHK21 cells. The cells may be derived from porcine kidney cells. The cell or cell line may be derived from feline kidney cells.

The cells may be, but are not limited to, BHK-21, NLST-1, NLFK-1, Vero or RL cells. The PRRSV may be North American or European.

The invention includes a method for measuring the propensity of a test cell or cell line to allow infection by a virus selected from the group consisting of

5 *Arteriviridae* and *Asfarviridae* comprising:

- a) providing a sample containing nucleic acids from the test cell or cell line;
- b) determining the amount of polynucleotide encoding a CD163 polypeptide or its complement in said sample;

wherein an increased amount of polynucleotide encoding a CD163 polypeptide
10 relative to a control sample derived from a control cell or cell line known not to support the growth of said virus indicates a propensity of the test cell or cell line to support the replication of said virus.

In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment
15 said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

The amount of polynucleotide encoding a CD163 polypeptide may be determined by hybridization.

The amount of polynucleotide encoding a CD163 polypeptide may be
20 determined by PCR.

The invention also includes a method for measuring the propensity of a test cell or cell line to allow infection by a virus selected from the group consisting of *Arteriviridae* and *Asfarviridae* comprising:

- (a) providing a sample containing polypeptides from the test cell or cell line;
 - 25 (b) determining the amount of CD163 polypeptide in said sample;
- wherein an increased amount of a CD163 polypeptide relative to a control sample derived from a control cell or cell line known not to support the growth of said virus indicates a propensity of the test cell or cell line to support the replication of said virus.

30 In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

In one embodiment the determining is accomplished by contacting a CD163 polypeptide with an antibody specific for the CD163 polypeptide, under conditions wherein the antibody binds the CD163 polypeptide.

The invention includes a method for measuring the propensity of a pig to be infected by a virus selected from the group consisting of *Arteriviridae* and *Asfarviridae* comprising:

- a) providing a sample containing nucleic acids from the pig to be tested;
- b) determining the amount of polynucleotide encoding a CD163 polypeptide or its complement in said sample;

wherein an increased amount of polynucleotide encoding a CD163 polypeptide relative to a control sample derived from a pig known to be resistant to said virus infection indicates a propensity of the pig to be tested to be infected by said virus.

In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

In one embodiment the determining is accomplished by hybridization. In another embodiment the determining is accomplished by PCR.

The invention also includes a method for measuring the propensity of a pig to be infected by a virus selected from the group consisting of *Arteriviridae* and *Asfarviridae* comprising:

- (a) providing a sample containing polypeptides from pig to be tested;
- (b) determining the amount of CD163 polypeptide in said sample;

wherein an increased amount of a CD163 polypeptide relative to a control sample derived from a pig known to be resistant to said virus infection indicates a propensity of the pig to be tested to be infected by said virus.

In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

In one embodiment the determining is accomplished by contacting a CD163 polypeptide with an antibody specific for the CD163 polypeptide, under conditions wherein the antibody binds the CD163 polypeptide.

The invention also includes an isolated polypeptide wherein the polypeptide is selected from the group consisting of the polypeptides described below.

Therefore the invention also includes an isolated polypeptide having at least 70% 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%,
5 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, 99%, identity with SEQ ID NO: 2.

One such polypeptide is a polypeptide differing from SEQ ID NO: 2 by no more than 20 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 2 by no
10 more than 10 conservative amino acid substitutions.

One such polypeptide comprises SEQ ID NO: 2.

Therefore the invention also includes an isolated polypeptide having at least 99%, identity to a polypeptide set forth in SEQ ID NO: 14

One such polypeptide is a polypeptide differing from SEQ ID NO: 14 by no
15 more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 14 by no more than 10 conservative amino acid substitutions.

One such polypeptide comprises SEQ ID NO: 14.

Therefore the invention also includes an isolated polypeptide differing from
20 SEQ ID NO: 24 by no more than 2 conservative amino acid substitutions.

One such polypeptide comprises SEQ ID NO: 24.

Therefore the invention also includes an isolated polypeptide having at least 96% 97% 98%, or 99%, identity to a polypeptide set forth in SEQ ID NO: 27.

One such polypeptide is a polypeptide differing from SEQ ID NO: 27 by no
25 more than 20 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 27 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 27.

Therefore the invention also includes an isolated polypeptide having at least
30 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 32

One such polypeptide is a polypeptide differing from SEQ ID NO: 32 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 32 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 32.

Therefore the invention also includes an isolated polypeptide having at least
5 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 34

One such polypeptide is a polypeptide differing from SEQ ID NO: 34 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 34 by no more than 10 conservative amino acid substitutions.

10 One such polypeptide is a polypeptide comprising SEQ ID NO: 34.

Therefore the invention also includes an isolated polypeptide having at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 36

One such polypeptide is a polypeptide differing from SEQ ID NO: 36 by no more than 15 conservative amino acid substitutions.

15 One such polypeptide is a polypeptide differing from SEQ ID NO: 36 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 36.

The invention also includes an isolated polypeptide having at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 38

20 One such polypeptide is a polypeptide differing from SEQ ID NO: 38 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 38 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 40

25 Therefore the invention also includes an isolated polypeptide having at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 40

One such polypeptide is a polypeptide differing from SEQ ID NO: 40 by no more than 15 conservative amino acid substitutions.

30 One such polypeptide is a polypeptide differing from SEQ ID NO: 40 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 40.

Therefore the invention also includes an isolated polypeptide having at 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 42

One such polypeptide is a polypeptide differing from SEQ ID NO: 42 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 42 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 42.

Therefore the invention also includes an isolated polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 44

One such polypeptide is a polypeptide differing from SEQ ID NO: 44 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 44 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 44.

Therefore the invention also includes an isolated polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 46

One such polypeptide is a polypeptide differing from SEQ ID NO: 46 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 46 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 46.

Therefore the invention also includes an isolated polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 48

One such polypeptide is a polypeptide differing from SEQ ID NO: 48 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 48 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 48.

The invention also includes an isolated CD163 polynucleotide wherein said polynucleotide is selected from the group consisting of the polynucleotides enumerated below.

Therefore the invention also includes an isolated polynucleotide comprising:

- 5 (a) a polynucleotide sequence set forth in SEQ ID NOs: 1 or 5
- (b) a polynucleotide that encodes a polypeptide that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 2
- 10 (c) a polynucleotide encoding a polypeptide of SEQ ID: 2,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NOs: 12 or 13
- (b) a polynucleotide that encodes a polypeptide that has at least 99%, identity and or
- 15 similarity to a polypeptide set forth in SEQ ID NO: 14
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 14,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NOs: 22 or 23
- 20 (b) a polynucleotide encoding a polypeptide of SEQ ID: 24,
- (c) a polynucleotide which is the complement of any of (a) or (b).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NOs: 25 or 26
- (b) a polynucleotide that encodes a polypeptide that has at least 96% 97% 98%, or
- 25 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 27
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 27,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 30 or 31
- 30 (b) a polynucleotide that encodes a polypeptide that has at least 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 32
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 32,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 33
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%,, identity and or similarity to a polypeptide set forth in SEQ ID NO: 34
- 5 (c) a polynucleotide encoding a polypeptide of SEQ ID: 34,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 35
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or
- 10 99%,, identity and or similarity to a polypeptide set forth in SEQ ID NO: 36
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 36,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 37
- 15 (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%,, identity and or similarity to a polypeptide set forth in SEQ ID NO: 38
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 38,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- 20 (a) polynucleotide sequence set forth in SEQ ID NOs: 39
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%,, identity and or similarity to a polypeptide set forth in SEQ ID NO: 40
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 40,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

25 Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 41
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%,, identity and or similarity to a polypeptide set forth in SEQ ID NO: 42
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 42,
- 30 (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 43

(b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 44

(c) a polynucleotide encoding a polypeptide of SEQ ID: 44,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

5 Therefore the invention also includes an isolated polynucleotide comprising:

(a) polynucleotide sequence set forth in SEQ ID NOs: 45

(b) a polynucleotide that encodes a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 46

10 (c) a polynucleotide encoding a polypeptide of SEQ ID: 46,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

(a) polynucleotide sequence set forth in SEQ ID NOs: 47

(b) a polynucleotide that encodes a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 48

15 (c) a polynucleotide encoding a polypeptide of SEQ ID: 49,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes a CD163 polypeptide in which the transmembrane region is deleted.

20 Therefore the invention also includes a polynucleotide encoding a CD163 polypeptide in which the transmembrane region is deleted.

In addition to the foregoing, the invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations specifically mentioned above.

Brief Description of the Sequence Listings

SEQ ID NO: 1—cDNA sequence encoding porcine susCD163v1

SEQ ID NO: 2—predicted amino acid sequence of porcine susCD163v1

SEQ ID NO: 3-- cDNA sequence, Genbank accession number AJ311716

30 SEQ ID NO:4---predicted amino acid sequence derived from Genbank accession number AJ311716

SEQ ID NO: 5—cDNA sequence of susCD163v1 containing flanking (non-coding) sequence.

- SEQ ID NO: 6-11**---primer sequences
- SEQ ID NO:12** ---cDNA sequence encoding porcine susCD163v2 containing flanking (non-coding) sequence.
- SEQ ID NO:13** --cDNA sequence encoding porcine susCD163v2
- 5 **SEQ ID NO:14** ---predicted amino acid sequence of porcine susCD163v2
- SEQ ID NO:15-16**---primer sequences
- SEQ ID NO:17**---cDNA sequence encoding human CD163v2 containing flanking (non-coding) sequence.
- SEQ ID NO:18**--- cDNA sequence encoding human CD163v2
- 10 **SEQ ID NO:19**--- predicted amino acid sequence of human CD163v2
- SEQ ID NO:20-21**---primer sequences
- SEQ ID NO:22**---cDNA sequence encoding murine CD163v2 containing flanking (non-coding) sequence.
- SEQ ID NO:23**--- cDNA sequence encoding murine CD163v2
- 15 **SEQ ID NO:24**--- predicted amino acid sequence of murine CD163v2
- SEQ ID NO:25**---cDNA sequence encoding murine CD163v3 containing flanking (non-coding) sequence.
- SEQ ID NO:26**--- cDNA sequence encoding murine CD163v3
- SEQ ID NO:27**--- predicted amino acid sequence of murine CD163v3
- 20 **SEQ ID NO:28-29**---primer sequences
- SEQ ID NO:30**---cDNA sequence encoding MARC-145 CD163v3 containing flanking (non-coding) sequence.
- SEQ ID NO:31**--- cDNA sequence encoding MARC-145 CD163v3
- SEQ ID NO:32**--- predicted amino acid sequence of MARC-145 CD163v3
- 25 **SEQ ID NO:33**--cDNA sequence encoding Vero cell CD163v2 transcript
- SEQ ID NO:34**-- predicted amino acid sequence of Vero cell CD163v2
- SEQ ID NO:35**-- cDNA sequence encoding Vero cell CD163v3 transcript
- SEQ ID NO:36**-- predicted amino acid sequence of Vero cell CD163v3
- SEQ ID NO:37**-- cDNA sequence encoding Vero cell CD163v4 transcript
- 30 **SEQ ID NO:38**-- predicted amino acid sequence of Vero cell CD163v4
- SEQ ID NO:39**-- cDNA sequence encoding Vero cell CD163v5 transcript
- SEQ ID NO:40**-- predicted amino acid sequence of Vero cell CD163v5
- SEQ ID NO:41**-- cDNA sequence encoding Vero cell CD163v6 transcript

SEQ ID NO:42-- predicted amino acid sequence of Vero cell CD163v6

SEQ ID NO:43-- cDNA sequence encoding Vero cell CD163v7 transcript

SEQ ID NO:44-- predicted amino acid sequence of Vero cell CD163v7

SEQ ID NO:45-- cDNA sequence encoding canine CD163v2 transcript.

5 **SEQ ID NO:46--** predicted amino acid sequence of canine CD163v2

SEQ ID NO:47-- cDNA sequence encoding canine CD163v3 transcript.

SEQ ID NO:48-- predicted amino acid sequence of canine CD163v3

Brief Description of the Drawings

10 **Figure 1** Schematic comparison of susCD163v1 with AJ311716

Figure 2 Amino acid Sequence alignment of susCD163v1 (SEQ ID NO: 2) with AJ311716 (SEQ ID NO: 4)

Figure 3 Nucleotide Sequence alignment of susCD163v1 with AJ311716

Figure 4 Generation of DNA fragments and ligation to place CD163 directly behind the RSV promoter. Plasmids were digested with either *Dra*III or *Drd*I, followed by a blunting reaction with Klenow enzyme. After clean up, the plasmids were digested with *Not*I. Gel purification yielded DNA fragments that were subsequently ligated utilizing the cohesive *Not*I termini. Promoters from RSV (pRSV) and SV40 (pSV40) are indicated with arrows.

20 **Figure 5** Map of pCDNA3.1 Directional V5/His/TOPO cloning vector

Figure 6 Three BHK/CMV/v1 cell lines, #3, #5, and #12 and a non-permissive BHK cell line were infected with PRRSV isolate P129 and stained with SDOW17-FITC.

Panel A shows a non-permissive BHK21 cell clone. Panel B shows BHK/CMV/v1 clone #3. Panel C shows BHK/CMV/v1 clone #5. Panel D shows BHK/CMV/v1

25 clone #12.

Figure 7 Three BHK/RSV/v1 cell lines, #2, #3, and #4 were infected with PRRSV isolate P129 and stained with SDOW17-FITC. Panel A shows BHK/RSV/v1 clone #2. Panel B shows BHK/RSV/v1 clone #3. Panel C shows BHK/RSV/v1 clone #4.

Figure 8 Feline kidney cell lines stably expressing porcine CD163v1, showing PRRSV plaques. Cell lines NLFK-CMV-susCD163v1-G4F and NLFK-CMV-susCD163v1-G4L, both at passage 4, were infected with the P129 isolate of North American PRRSV and incubated for 6 days. Monolayers were fixed with 80% acetone and stained with monoclonal antibody SDOW17-FITC. Phase contrast

microscopy (right) shows localized regions of viral CPE (plaques), while FA detection (left) shows co-localized viral nucleocapsid antigen.

Figure 9 Four FK/RSV/v1 cell lines, #1, #2, #3, and #4 were infected with PRRSV isolate P129 and stained with monoclonal antibody SDOW17-FITC. Panel A shows
5 FK/RSVv1 #1 cell clone. Panel B shows FK/RSV/v1 clone #2. Panel C shows FK/RSV/v1 clone #3. Panel D shows FK/RSV/v1 #4.

Figure 10 PK-CMV-susCD163v1-A10 cells at passage 19, infected with PRRSV isolate P129. Left: The monolayer was fixed with 80% acetone and stained with
10 FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for PRRSV nucleocapsid. Right: The same well under bright field illumination, showing cell distribution.

Figure 11 BHK-CMVScript-susCD163v2-A9 at passage 17 infected with PRRSV isolate P129. The monolayer was fixed with 80% acetone and stained with FITC-
15 conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for PRRSV nucleocapsid.

Figure 12 Three representative examples of the BHK/RSV/v2 cell lines. The cells were infected with PRRSV isolate P129 and subsequently stained with SDOW17-FITC. Panel A shows cell line BHK/RSV/v2 #1, panel B shows cell line
BHK/RSV/v2 #34, and panel C shows cell line BHK/RSV/v2 #47.

Figure 13 FK-cDNA3.1D-humCD163v2-A6 at passage 15 infected with PRRSV isolate P129. The monolayer was then fixed with 80% acetone and stained with FITC-
20 conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for PRRSV nucleocapsid.

Figure 14 The amount of progeny PRRSV produced by four recombinant cell lines stably expressing susCD163v1, and by MARC-145 cells, was determined in a growth
25 curve experiment using the NVSL 94-3 isolate of PRRSV. Samples harvested at 12-hour intervals were titrated on MARC-145 cell monolayers.

Figure 15 Flow cytometry analysis of PRRSV infection in the presence of CD163 specific antibody. BHK-21 cells expressing MARC-145 CD163 from transient
30 transfection were incubated with either CD163 specific antibody or normal goat IgG (NGS) and infected with a GFP-expressing PRRSV. Each data point represents the results of triplicate wells.

Figure 16 Flow cytometry analysis of PRRSV infection in the presence of CD163 specific antibody. NLFK cells stably expressing human CD163 were incubated with either CD163 specific antibody or normal goat IgG (NGS) and infected with a GFP expressing PRRSV. At 24 hours post infection the percentage of GFP expressing infected cells was determined. Each data point represents the result from a single well of cells

Figure 17. Graphical depiction of six alternative splicing variants of CD163 mRNA recovered from Vero cells. The six variants differ in the presence or absence of three exons, designated E6, E105, and E83. Exons E6 and E105 have lengths that are multiples of three, and therefore do not result in a change in reading frame when absent. In contrast, the absence of E83 results in a shifted reading frame and an alternative amino acid sequence at the carboxy terminus of the protein (indicated by a hatched pattern in the figure). The hydrophobic transmembrane (TM) region is encoded within E105.

Figure 18. PK-RSVScript-susCD163v2 #9 cells infected with PRRSV isolate P129. Undiluted supernatant from PRRSV isolate P201 infected PAMs was used to infect PK-RSVScript-susCD163v2 #9 cells. After two days of incubation the cells were fixed and stained with monoclonal antibody SDOW17 as described in Example 11.

Figure 19. FK-RSVScript-susCD163v2 #51 cells infected with PRRSV isolate P129. Undiluted supernatant from PRRSV isolate P201 infected PAMs was used to infect FK-RSVScript-susCD163v2 #51 cells. Two days post infection the cells were acetone fixed and stained with monoclonal antibody SDOW17 as described in Example 11.

Figure 20. Infection of PK-RSVScript-susCD163v2 clone #9 cells with PRRSV isolate P201. Panel A shows a monolayer of cells infected with PRRSV P201 at passage 1, twenty-four hours post infection. Panel B shows a monolayer of cells 2 days post infection with cell free supernatant PRRSV P201 at passage 10.

Figure 21. NLFK parent cells and one subclone of FK-cDNA3.1D-humCD163v2-A6 were examined for the CD163 expression. Cells were fixed in 80% acetone and reacted with Goat anti-human CD163 (R&D System at 1:200) for one hour following by washing with PBS. For visualization, donkey anti-Goat IgG conjugated with FITC (Bioscience Inc at 1:100) were used. No specific fluorescence was detected in the

NLFK parent cells as shown in Figure 21A. The majority of the FK.A6.A2 subclone showed good fluorescent staining indicating the presence of CD163 (Figure 21B).

DETAILED DESCRIPTION OF THE INVENTION

5 General Definitions

Cells and cell lines can be either virus permissive” or “virus non-permissive”. For example, a cell or cell line that is virus permissive is capable of allowing virus infection, subsequent replication and virus production. A cell or cell line that is virus non-permissive is incapable of allowing virus infection, subsequent replication and
10 virus production. A cell line that is already somewhat permissive may be rendered more permissive by the methods of the invention.

Arteriviridae refers to a family of enveloped, positive-stranded RNA viruses belonging to the order *Nidovirales*. The family includes lactate dehydrogenase-elevating virus (LDV) of mice, equine arteritis virus (EAV), simian hemorrhagic fever
15 virus (SHFV), and PRRSV.

Asfarviridae is a family of icosohedral, enveloped viruses whose genomes consist of single molecules of linear double-stranded DNA about 150000–190000 nucleotides long. The name of the family is derived from African Swine Fever And Related Viruses. African Swine Fever Virus (ASFV) is the type species of the
20 Asfivirus genus and is the sole member of the family.

The term “PRRSV” or PRRS virus refers to both European and North American PRRS virus genotypes. Within each genotype, isolates typically share 85% or higher nucleotide identity. Between genotypes, however, the level of sequence identity is only about 60%.

25 The PRRS virus is a member of the family *Arteriviridae*. The genome of the arteriviruses is single-stranded RNA of positive polarity between 12 and 16 kb in length, capped at the 5’ end and polyadenylated at the 3’ end. Over two-thirds of the genome is dedicated to open reading frames (ORFs) 1a and 1b, which encode the non-structural functions of the virus. ORF1b is an extension of ORF1a, and is the result of
30 a ribosomal frameshift. ORFs 1a and 1b are translated directly from the genomic RNA. These large polypeptide products are cleaved by viral proteases to yield 12 or 13 discrete smaller peptides. The remaining ORFs, which encode viral structural proteins, are expressed from a series of 3’ co-terminal subgenomic RNAs (sgRNAs).

The sgRNAs are produced by discontinuous transcription of negative-stranded RNA, such that a common 5' leader sequence becomes fused to each transcript. The major structural proteins are the nucleocapsid (N, encoded by ORF7), the matrix protein (M, encoded by ORF6), and the major envelope glycoprotein (GP5, encoded by ORF5).

5 The remaining proteins, GP4 (ORF4), GP3 (ORF3), GP2 (ORF2a), and E (ORF2b) are minor structural components of the virion, whose functions have not yet been elucidated. The molecular biology of PRRSV has been the subject of recent review articles (Dea et al., 2000; Meulenberg, 2000; Snijder and Meulenberg, 2001).

As used herein, the term "CD163 polypeptide" means a protein encoded by a mammalian CD163 gene, including allelic variants containing conservative or non-conservative changes. A cDNA sequence that encodes a porcine CD163 polypeptide has been reported (Genbank accession number AJ311716). A murine CD163 polypeptide has also been reported (Genbank access number AF274883), as well as multiple human variants, exemplified by Genbank access numbers AAH51281 and CAA80543. We report herein polynucleotides that encode porcine, human, murine, canine, and african green monkey CD163 polypeptides and which comprise the sequences set forth in SEQ ID NO: 1, 5, 12 13, 17, 18, 22, 23, 25, 26, 30, 31 33, 35, 37, 39, 41, 42, 43, 45 and 47. A "CD163 polypeptide" is a member of the scavenger receptor cysteine-rich (SRCR) family of transmembrane glycoproteins, and is thought to be expressed exclusively on monocytes and macrophages. One identified role of CD163 is to inhibit oxidative tissue damage following hemolysis by consuming hemoglobin:haptoglobin complexes by endocytosis. The subsequent release of interleukin-10 and synthesis of heme oxygenase-1 results in antiinflammatory and cytoprotective effects (Philippidis et al., 2004; Graversen et al., 2002). The human CD163 gene spans 35 kb on chromosome 12, and consists of 17 exons and 16 introns. A number of isoforms of the CD163 polypeptide, including membrane bound, cytoplasmic and secreted types, are known to be generated by alternative splicing (Ritter et al., 1999). Isoforms that comprise a transmembrane domain are particularly preferred.

30 A transmembrane domain is characterized by a polypeptide segment of a larger sequence that is exposed on both sides of a membrane. The cytoplasmic and extracellular domains are separated by at least one membrane-spanning segment that traverses the hydrophobic environment of the lipid bilayer. The membrane- spanning

segment is composed of amino acid residues with nonpolar side chains, usually in the form of an alpha helix. Segments that contain about 20-30 hydrophobic residues are long enough to span a membrane as an alpha helix, and they can often be identified by means of a hydropathy plot. The predicted transmembrane domain of SEQ ID NO:2 and 14 are indicated by bolding in the specification. To determine whether other CD163 sequences contain a similar sequence feature is easily determined by inspection of the sequence or hydropathy plots. SEQ ID NOS: 37-40 are representative of variant CD163 proteins which do not contain a transmembrane domain and their encoding nucleic acids.

As used hereinafter, "polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, RNA that is mixture of single- and double-stranded regions, and hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

As used hereinafter, "polypeptide" refers to any peptide or protein comprising amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which

are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated
5 that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by
10 synthetic methods. Modifications or modified forms include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of
15 covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination
20 (see, for instance, *Proteins-Structure and Molecular Properties*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; Wold, F., *Post-translational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *Postranslational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and
25 nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan et al., "Protein Synthesis: Post-translational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:4842).

As used hereinafter, "isolated" means altered by the hand of man from the natural state. If an "isolated" composition or substance occurs in nature, it has been
30 changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Therefore "isolated" as

used herein and as understood in the art, whether referring to “isolated” polynucleotides or polypeptides, is taken to mean separated from the original cellular environment in which the polypeptide or nucleic acid is normally found. As used herein therefore, by way of example only, a transgenic animal or a recombinant cell line constructed with a polynucleotide of the invention makes use of the “isolated” nucleic acid. Specifically excluded from the definition of isolated polynucleotides of the invention are entire isolated chromosomes from native host cells from which the polynucleotide was originally derived.

In the disclosure to follow we will often make use of the term “identity” or similarity as applied to the amino acid sequences of polypeptides. Percent amino acid sequence “identity” with respect to polypeptides is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the target sequences after aligning both sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percent sequence identity is determined by conventional methods. For example, BLASTP 2.2.6 [Tatusova TA and TL Madden, “BLAST 2 sequences- a new tool for comparing protein and nucleotide sequences.” (1999) FEMS Microbiol Lett. 174:247-250.]

Briefly, as noted above, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 0.1, and the “blosum62” scoring matrix of Henikoff and Henikoff (Proc. Nat. Acad. Sci. USA 89:10915-10919. 1992).

The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{[\text{length of the longer sequence} + \text{number of gaps introduced into the longer sequence to align the two sequences}]} \times 100$$

Percent sequence “similarity” (often referred to as “homology”) with respect to a polypeptide of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the target sequences after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity (as described above), and also considering any conservative substitutions as part of the sequence identity.

Total number of identical matches and conservative substitutions x
 100
 [length of the longer sequence + number of gaps introduced into
 the longer sequence to align the two sequences]

5

Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties.

10 Exemplary conservative substitutions are set out in Table 1, 2, and 3 below.

Table 1
Conservative Substitutions I

15	SIDE CHAIN	
	CHARACTERISTIC	AMINO ACID
	Aliphatic	
	Non-polar	G A P I L V
20	Polar - uncharged	C S T M N Q
	Polar - charged	D E K R
	Aromatic	H F W Y
25	Other	N Q D E

Alternatively, conservative amino acids can be grouped as described in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77] as set out in Table 2, immediately below

30

Table 2
Conservative Substitutions II

<u>SIDE CHAIN</u>		
5	<u>CHARACTERISTIC</u>	<u>AMINO ACID</u>
	Non-polar (hydrophobic)	
	A. Aliphatic:	A L I V P
	B. Aromatic:	F W
10	C. Sulfur-containing:	M
	D. Borderline:	G
	Uncharged-polar	
	A. Hydroxyl:	S T Y
	B. Amides:	N Q
15	C. Sulfhydryl:	C
	D. Borderline:	G
	Positively Charged (Basic):	K R H
	Negatively Charged (Acidic):	D E

20 As still another alternative, exemplary conservative substitutions are set out in Table 3, immediately below.

Table 3
Conservative Substitutions III

<u>Original Residue</u>		<u>Exemplary Substitution</u>
25	Ala (A)	Val, Leu, Ile
	Arg (R)	Lys, Gln, Asn
	Asn (N)	Gln, His, Lys, Arg
	Asp (D)	Glu
30	Cys (C)	Ser
	Gln (Q)	Asn
	Glu (E)	Asp

	His (H)	Asn, Gln, Lys, Arg
	Ile (I)	Leu, Val, Met, Ala, Phe,
	Leu (L)	Ile, Val, Met, Ala, Phe
	Lys (K)	Arg, Gln, Asn
5	Met (M)	Leu, Phe, Ile
	Phe (F)	Leu, Val, Ile, Ala
	Pro (P)	Gly
	Ser (S)	Thr
	Thr (T)	Ser
10	Trp (W)	Tyr
	Tyr (Y)	Trp, Phe, Thr, Ser
	Val (V)	Ile, Leu, Met, Phe, Ala

Methods Directed to the Production of Virus and Host Cells of the Invention

15 The invention provides a method of modifying production a virus that is a member of the family *Arteriviridae* and *Asfarviridae* in a cell comprising the step of directing said cell to express a CD163 polypeptide. This may include rendering a virus non-permissive cell into a virus permissive cell, or may involve rendering a cell more permissive to the virus.

20 In one embodiment, the virus that is a member of the family *Arteriviridae* or *Asfarviridae* is selected from the group consisting of LDV of mice, equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), PRRSV of swine and ASFV of swine.

In a preferred embodiment the virus is PRRSV.

25 The invention further provides a method of preparing a culture of a virus that is a member of the family *Arteriviridae* or *Asfarviridae* comprising the steps of: providing a cell line; directing said cell line to express a CD163 polypeptide; infecting said cell line with virus; and causing said cell line to produce viral progeny.

30 In one embodiment, the virus that is a member of the family *Arteriviridae* is selected from the group consisting of LDV of mice, equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), PRRSV of swine and ASFV of swine.

In a preferred embodiment the virus is PRRSV.

All of the above methods utilize cells and cell lines expressing a CD163 polypeptide. CD163 may be facilitated or increased by methods that involve the introduction of exogenous nucleic acid into the cell. Such a cell may comprise a polynucleotide or vector in a manner that permits expression of an encoded CD163 polypeptide.

Polynucleotides that encode CD163 may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein-coding region, or in a viral vector. Methods for introducing exogenous nucleic acid into the host cell well known and routinely practiced in the art include transformation, transfection, electroporation, nuclear injection, or fusion with carriers such as liposomes, micelles, ghost cells, and protoplasts. Host cell systems of the invention include invertebrate and vertebrate cells systems. Hosts may include, but are not limited to, the following: insect cells, porcine kidney (PK) cells, feline kidney (FK) cells, swine testicular (ST) cells, African green monkey kidney cells (MA-104, MARC-145, VERO, and COS cells), Chinese hamster ovary (CHO) cells, baby hamster kidney cells, human 293 cells, and murine 3T3 fibroblasts. Insect host cell culture systems may also be used for the expression of CD163 polypeptides. In another embodiment, the CD163 polypeptides are expressed using a drosophila expression system.

The choice of a suitable expression vector for expression of the CD163 polypeptides will of course depend upon the specific host cell to be used, and is within the skill of the ordinary artisan. Examples of suitable expression vectors include pSport and pcDNA3 (Invitrogen), pCMV-Script (Stratagene), and pSVL (Pharmacia Biotech). Expression vectors for use in mammalian host cells may include transcriptional and translational control sequences derived from viral genomes. Commonly used promoter sequences and modifier sequences which may be used in the present invention include, but are not limited to, those derived from human cytomegalovirus (CMV), Rous sarcoma virus (RSV), Adenovirus 2, Polyoma virus, and Simian virus 40 (SV40). Methods for the construction of mammalian expression vectors are disclosed, for example, in Okayama and Berg (*Mol. Cell. Biol.* 3:280 (1983)); Cosman *et al.* (*Mol. Immunol.* 23:935 (1986)); Cosman *et al.* (*Nature* 312:768 (1984)); EP-A-0367566; and WO 91/18982.

Because CD163 sequences are known to exist in cells from various species, the endogenous gene may be modified to permit, or increase, expression of the CD163 polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased expression by replacing, in whole or in part, the naturally occurring CD163 promoter with all or part of a heterologous promoter, so that the cells express CD163 polypeptide at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to endogenous CD163 encoding sequences. [See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955.] It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., *ada*, *dhfr*, and the multifunctional *cad* gene, which encodes for carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the CD163 coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the CD163 coding sequences in the cells.

CD163 expression may also be induced by chemical treatment. Phorbol esters, especially phorbol myristyl acetate (PMA), activate one or more isozymes of the ubiquitous membrane receptor, protein kinase C (PKC) and are particularly preferred means of increasing CD163 expression. Other methods of intracellular calcium mobilization are also contemplated.

Vaccine Production

The methods described above may be used to produce any virus that is a member of the family *Arteriviridae* or *Asfarviridae* for the purpose of vaccine production or diagnostics.

In one embodiment the virus that is a member of the family *Arteriviridae* is selected from the group consisting of LDV of mice, equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), and PRRSV of swine.

In a preferred embodiment the virus is PRRSV.

Vaccine Production

The methods described above may be used to produce virus for the purpose of vaccine production or diagnostics.

Killed (inactivated) or live vaccines can be produced. Therefore, to make a live vaccine, a viral isolate, or an attenuated or mutated variant thereof, is grown in cell culture. The virus is harvested according to methods well known in the art. The virus may then be concentrated, frozen, and stored at -70°C , or freeze-dried and stored
5 at 4°C . Prior to vaccination the virus is mixed at an appropriate dosage, (which is from about 10^3 to 10^8 tissue culture infectious doses per ml ($\text{TCID}_{50}/\text{ml}$)), with a pharmaceutically acceptable carrier such as a saline solution, and optionally an adjuvant.

The vaccine produced might also comprise an inactivated or killed vaccine
10 comprising a virus grown by the methods of the invention. The inactivated vaccine is made by methods well known in the art. For example, once the virus is propagated to high titers, it would be readily apparent by those skilled in the art that the virus antigenic mass could be obtained by methods well known in the art. For example, the virus antigenic mass may be obtained by dilution, concentration, or extraction. All of
15 these methods have been employed to obtain appropriate viral antigenic mass to produce vaccines. The virus is then inactivated by treatment with formalin, betapropriolactone (BPL), binary ethyleneimine (BEI), or other methods known to those skilled in the art. The inactivated virus is then mixed with a pharmaceutically acceptable carrier such as a saline solution, and optionally an adjuvant. Examples of
20 adjuvants include, but not limited to, aluminum hydroxide, oil-in-water and water-in-oil emulsions, AMPHIGEN, saponins such as QuilA, and polypeptide adjuvants including interleukins, interferons, and other cytokines.

Inactivation by formalin is performed by mixing the viral suspension with 37% formaldehyde to a final formaldehyde concentration of 0.05%. The virus-
25 formaldehyde mixture is mixed by constant stirring for approximately 24 hours at room temperature. The inactivated virus mixture is then tested for residual live virus by assaying for growth on a suitable cell line.

Inactivation by BEI is performed by mixing the viral suspension of the present invention with 0.1 M BEI (2-bromo-ethylamine in 0.175 N NaOH) to a final BEI
30 concentration of 1 mM. The virus-BEI mixture is mixed by constant stirring for approximately 48 hours at room temperature, followed by the addition of 1.0 M sodium thiosulfate to a final concentration of 0.1 mM. Mixing is continued for an

additional two hours. The inactivated virus mixture is tested for residual live virus by assaying for growth on a suitable cell line.

Virus permissive cells that have been directed to express CD163 can also be used to quantify live virus. Two common methods, which are well known to those skilled in the art, are the plaque assay and the limiting dilution assay.

CD163-expressing cell lines of the present invention can be used to grow virus for the purpose of producing viral antigen for diagnostic kits. For example lysates from infected cells (with optional purification of viral particles or extraction of selected viral proteins) may be coated on ELISA plates in order to detect and quantify antibodies to the virus in swine sera.

Live or inactivated virus grown in CD163-expressing cells can be used after optional separation of the viral proteins to immunize animals in order to generate polyclonal, monospecific or monoclonal antibodies. These in turn can be used as the basis of diagnostic assays for the detection and quantification of virus in swine serum and other biological samples.

Assays of the Invention

The invention provides methods for determining the propensity of an animal to be infected by a virus that is a member of the family *Arteriviridae* or *Asfarviridae* or of a cell line to support the replication of a virus that is a member of the family *Arteriviridae* or *Asfarviridae*. Samples from either source are obtained and assayed for expression of CD163. The level of CD163 gene expression can be compared with levels of controls known not to support replication of the virus.

In the case of an animal, samples can be any sample comprising sample nucleic acid molecules or proteins, and obtained from any bodily tissue expressing CD163, including, but not limited to, alveolar macrophages, cultured cells, biopsies, or other tissue preparations. The level of expression can be assessed at either or both the level of messenger RNA or protein produced. In a preferred embodiment the member of the virus of the family *Arteriviridae* or *Asfarviridae* is selected from the group consisting of LDV of mice, equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), PRRSV of swine, and ASFV of swine.

Nucleic Acid based Assays

Methods of determining CD163 levels may be nucleic acid based as noted above. CD163-derived nucleic acids may be in solution or on a solid support. In

some embodiments, they may be employed as array elements in microarrays alone or in combination with other array element molecules. Nucleic acid based methods generally require the isolation of DNA or RNA from the sample and subsequent hybridization or PCR amplification using specific primers derived from any known CD163 encoding sequences in the art or those specifically disclosed as SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45, and 47. DNA or RNA can be isolated from the sample according to any of a number of methods well known to those of skill in the art. For example, methods of purification of nucleic acids are described in Tijssen, P. (1993) *Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation*, Elsevier, New York, N.Y. In one preferred embodiment, total RNA is isolated using the TRIZOL total RNA isolation reagent (Life Technologies, Inc., Gaithersburg Md.) and mRNA is isolated using oligo d(T) column chromatography or glass beads. When sample nucleic acid molecules are amplified it is desirable to amplify the sample nucleic acid molecules and maintain the relative abundances of the original sample, including low abundance transcripts. RNA can be amplified in vitro, in situ, or in vivo (See Eberwine U.S. Pat. No. 5,514,545).

It is also advantageous to include controls within the sample to assure that amplification and labeling procedures do not change the true distribution of nucleic acid molecules in a sample. For this purpose, a sample is spiked with an amount of a control nucleic acid molecule predetermined to be detectable upon hybridization to its complementary arrayed nucleic acid molecule and the composition of nucleic acid molecules includes reference nucleic acid molecules which specifically hybridize with the control arrayed nucleic acid molecules. After hybridization and processing, the hybridization signals obtained should reflect accurately the amounts of control arrayed nucleic acid molecules added to the sample.

Prior to hybridization, it may be desirable to fragment the sample nucleic acid molecules. Fragmentation improves hybridization by minimizing secondary structure and cross-hybridization to other sample nucleic acid molecules in the sample or noncomplementary nucleic acid molecules. Fragmentation can be performed by mechanical or chemical means.

Labeling

The sample nucleic acid molecules or probes may be labeled with one or more

labeling moieties to allow for detection of hybridized arrayed/sample nucleic acid molecule complexes. The labeling moieties can include compositions that can be detected by spectroscopic, photochemical, biochemical, bioelectronic, immunochemical, electrical, optical, or chemical means. The labeling moieties include radioisotopes, such as (32)P, (33)P or (35)S, chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectroscopic markers, such as fluorescent markers and dyes, magnetic labels, linked enzymes, mass spectrometry tags, spin labels, electron transfer donors and acceptors, and the like. Preferred fluorescent markers include Cy3 and Cy5 fluorophores (Amersham Pharmacia Biotech, Piscataway N.J.).

Hybridization

The nucleic acid molecule sequence of SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45 and 47 or other CD163 encoding sequences in the art and fragments thereof can be used in various hybridization technologies for various purposes. Hybridization probes may be designed or derived from any mammalian CD163 sequence but may make use of those sequences disclosed in SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45 and 47. Such probes may be made from a highly specific region or from a conserved motif, and used in protocols to quantify CD163 message, allelic variants, or related sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from any mammalian CD163 sequence known in the art or from those sequences disclosed herein as SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30 or from genomic sequences including promoters, enhancers, and introns of the mammalian gene. Hybridization or PCR probes may be produced using oligolabeling, nick translation, end-labeling, or PCR amplification in the presence of the labeled nucleotide. A vector containing the nucleic acid sequence may be used to produce an mRNA probe in vitro by addition of an RNA polymerase and labeled nucleic acid molecules. These procedures may be conducted using commercially available kits such as those provided by Amersham Pharmacia Biotech.

The stringency of hybridization is determined by the G+C content of the probe, salt concentration, and temperature. In particular, stringency can be increased by reducing the concentration of salt or raising the hybridization temperature. In solutions used for some membrane-based hybridizations, additions of an organic solvent such as

formamide allows the reaction to occur at a lower temperature. Hybridization can be performed at low stringency with buffers, such as 5xSSC with 1% sodium dodecyl sulfate (SDS) at 60°C., which permits the formation of a hybridization complex between nucleotide sequences that contain some mismatches. Subsequent washes are performed at higher stringency with buffers such as 0.2xSSC with 0.1% SDS at either 45°C (medium stringency) or 68°C (high stringency). At high stringency, hybridization complexes will remain stable only where the nucleic acid sequences are almost completely complementary. In some membrane-based hybridizations, preferably 35% or most preferably 50%, formamide can be added to the hybridization solution to reduce the temperature at which hybridization is performed, and background signals can be reduced by the use of other detergents such as Sarkosyl or Triton X-100 and a blocking agent such as salmon sperm DNA. Selection of components and conditions for hybridization are well known to those skilled in the art and are reviewed in Ausubel (supra) and Sambrook et al. (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview N.Y.

Exemplary highly stringent hybridization conditions are as follows: hybridization at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1M NaCl, 10% Dextran sulfate, and washing twice for 30 minutes at 60°C in a wash solution comprising 0.1 X SSC and 1% SDS. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel, et al. (Eds.), *Protocols in Molecular Biology*, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, et al., (Eds.), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

Hybridization specificity can be evaluated by comparing the hybridization of specificity-control nucleic acid molecules to specificity-control sample nucleic acid molecules that are added to a sample in a known amount. The specificity-control arrayed nucleic acid molecules may have one or more sequence mismatches compared with the corresponding arrayed nucleic acid molecules. In this manner, it is possible to determine whether only complementary arrayed nucleic acid molecules are

hybridizing to the sample nucleic acid molecules or whether mismatched hybrid duplexes are forming is determined.

Hybridization reactions can be performed in absolute or differential hybridization formats. In the absolute hybridization format, nucleic acid molecules from one sample are hybridized to the molecules in a microarray format and signals detected after hybridization complex formation correlate to nucleic acid molecule levels in a sample. In the differential hybridization format, the differential expression of a set of genes in two biological samples is analyzed. For differential hybridization, nucleic acid molecules from both biological samples are prepared and labeled with different labeling moieties. A mixture of the two labeled nucleic acid molecules is added to a microarray. The microarray is then examined under conditions in which the emissions from the two different labels are individually detectable. Molecules in the microarray that are hybridized to substantially equal numbers of nucleic acid molecules derived from both biological samples give a distinct combined fluorescence (Shalon et al.; PCT publication WO95/35505). In a preferred embodiment, the labels are fluorescent markers with distinguishable emission spectra, such as Cy3 and Cy5 fluorophores.

After hybridization, the microarray is washed to remove nonhybridized nucleic acid molecules and complex formation between the hybridizable array elements and the nucleic acid molecules is detected. Methods for detecting complex formation are well known to those skilled in the art. In a preferred embodiment, the nucleic acid molecules are labeled with a fluorescent label and measurement of levels and patterns of fluorescence indicative of complex formation is accomplished by fluorescence microscopy, preferably confocal fluorescence microscopy.

In a differential hybridization experiment, nucleic acid molecules from two or more different biological samples are labeled with two or more different fluorescent labels with different emission wavelengths. Fluorescent signals are detected separately with different photomultipliers set to detect specific wavelengths. The relative abundances/ expression levels of the nucleic acid molecules in two or more samples are obtained.

Typically, microarray fluorescence intensities can be normalized to take into account variations in hybridization intensities when more than one microarray is used under similar test conditions. In a preferred embodiment, individual arrayed-sample

nucleic acid molecule complex hybridization intensities are normalized using the intensities derived from internal normalization controls contained on each microarray.

Polypeptide Based Assays

The present invention provides methods and reagents for detecting and
5 quantifying CD163 polypeptides. These methods include analytical biochemical methods such as electrophoresis, mass spectroscopy, chromatographic methods and the like, or various immunological methods such as radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, western blotting, affinity capture mass spectrometry, biological activity, and others described
10 below and apparent to those of skill in the art upon review of this disclosure.

Immunoassays

The present invention also provides methods for detection of CD163 polypeptides employing one or more anti- CD163 antibody reagents (i.e., immunoassays). As used
herein, an immunoassay is an assay that utilizes an antibody (as broadly defined herein
15 and specifically includes fragments, chimeras and other binding agents) that specifically binds a CD163 polypeptide or epitope.

A number of well-established immunological binding assay formats suitable for the practice of the invention are known (see, e.g., U.S. Pat. Nos. 4,366,241; 4,376,110; 4,517,288; and 4,837,168). See, e.g., Methods in Cell Biology Volume 37:
20 Antibodies in Cell Biology, Asai, ed. Academic Press, Inc. New York (1993); Basic and Clinical Immunology 7th Edition, Stites & Terr, eds. (1991); Harlow and Lane, supra [e.g., Chapter 14], and Ausubel et al., supra, [e.g., Chapter 11]. Typically, immunological binding assays (or immunoassays) utilize a "capture agent" to specifically bind to and, often, immobilize the analyte to a solid phase. In one
25 embodiment, the capture agent is a moiety that specifically binds to a CD163 polypeptide or subsequence, such as an anti- CD163 antibody.

Usually the CD163 gene product being assayed is detected directly or indirectly using a detectable label. The particular label or detectable group used in the assay is usually not a critical aspect of the invention, so long as it does not
30 significantly interfere with the specific binding of the antibody or antibodies used in the assay. The label may be covalently attached to the capture agent (e.g., an anti- CD163 antibody), or may be attached to a third moiety, such as another antibody, that specifically binds to the CD163 polypeptide.

The present invention provides methods and reagents for competitive and noncompetitive immunoassays for detecting CD163 polypeptides. Noncompetitive immunoassays are assays in which the amount of captured analyte (in this case CD163) is directly measured. One such assay is a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the CD163 polypeptide. See, e.g., Maddox et al., 1983, J. Exp. Med., 158:1211 for background information. In one "sandwich" assay, the capture agent (e.g., an anti-CD163 antibody) is bound directly to a solid substrate where it is immobilized. These immobilized antibodies then capture any CD163 polypeptide present in the test sample. The CD163 polypeptide thus immobilized can then be labeled, i.e., by binding to a second anti-CD163 antibody bearing a label. Alternatively, the second CD163 antibody may lack a label, but be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second antibody alternatively can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

In competitive assays, the amount of CD163 polypeptide present in the sample is measured indirectly by measuring the amount of an added (exogenous) CD163 polypeptide displaced (or competed away) from a capture agent (e.g., CD163 antibody) by the CD163 polypeptide present in the sample. A hapten inhibition assay is another example of a competitive assay. In this assay CD163 polypeptide is immobilized on a solid substrate. A known amount of CD163 antibody is added to the sample, and the sample is then contacted with the immobilized CD163 polypeptide. In this case, the amount of anti-CD163 antibody bound to the immobilized CD163 polypeptide is inversely proportional to the amount of CD163 polypeptide present in the sample. The amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. In this aspect, detection may be direct, where the antibody is labeled, or indirect where the label is bound to a molecule that specifically binds to the antibody as described above.

Other Antibody-based Assay Formats

The invention also provides reagents and methods for detecting and quantifying the presence of CD163 polypeptide in the sample by using an immunoblot (Western blot)

format. Another immunoassay is the so- called "lateral flow chromatography." In a non-competitive version of lateral flow chromatography, a sample moves across a substrate by, e.g., capillary action, and encounters a mobile-labeled antibody that binds the analyte forming a conjugate. The conjugate then moves across the substrate and encounters an immobilized second antibody that binds the analyte. Thus, immobilized analyte is detected by detecting the labeled antibody. In a competitive version of lateral flow chromatography a labeled version of the analyte moves across the carrier and competes with unlabeled analyte for binding with the immobilized antibody. The greater the amount of the analyte in the sample, the less the binding by labeled analyte and, therefore, the weaker the signal. See, e.g., May et al., U.S. Pat. No. 5,622,871 and Rosenstein, U.S. Pat. No. 5,591,645.

Depending upon the assay, various components, including the antigen, target antibody, or anti-cathepsin S antibody, may be bound to a solid surface or support (i.e., a substrate, membrane, or filter paper). Many methods for immobilizing biomolecules to a variety of solid surfaces are known in the art. For instance, the solid surface may be a membrane (e.g., nitrocellulose), a microtiter dish (e.g., PVC, polypropylene, or polystyrene), a test tube (glass or plastic), a dipstick (e.g. glass, PVC, polypropylene, polystyrene, latex, and the like), a microcentrifuge tube, or a glass or plastic bead. The desired component may be covalently bound or noncovalently attached through nonspecific bonding.

A wide variety of organic and inorganic polymers, both natural and synthetic may be employed as the material for the solid surface. Illustrative polymers include polyethylene, polypropylene, poly (4- methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate) , rayon, nylon, poly(vinylbutyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and the like. Other materials which may be employed, include paper, glasses, ceramics, metals, metalloids, semiconductive materials, cements or the like. In addition, substances that form gels, such as proteins (e. g., gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides can be used. Polymers that form several aqueous phases, such as dextrans, polyalkylene glycols or surfactants, such as phospholipids, long chain (12-24 carbon atoms) alkyl ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes may be employed depending upon the nature of the system.

Mass Spectrometry

The mass of a molecule frequently can be used as an identifier of the molecule.

Therefore, methods of mass spectrometry can be used to identify a protein analyte.

Mass spectrometers can measure mass by determining the time required for an ionized
5 analyte to travel down a flight tube and to be detected by an ion detector. One method
of mass spectrometry for proteins is matrix-assisted laser desorption ionization mass
spectrometry ("MALDI"). In MALDI the analyte is mixed with an energy absorbing
matrix material that absorbs energy of the wavelength of a laser and placed on the
surface of a probe. Upon striking the matrix with the laser, the analyte is desorbed
10 from the probe surface, ionized, and detected by the ion detector. See, for example,
Hillenkamp et al., U.S. Pat. No. 5,118,937.

Other methods of mass spectrometry for proteins are described in Hutchens
and Yip, U.S. Pat. No. 5,719,060. In one such method referred to as Surfaces
Enhanced for Affinity Capture ("SEAC") a solid phase affinity reagent that binds the
15 analyte specifically or non-specifically, such as an antibody or a metal ion, is used to
separate the analyte from other materials in a sample. Then the captured analyte is
desorbed from the solid phase by, e.g., laser energy, ionized, and detected by the
detector.

Nucleic Acids of the Invention

20 The examples disclose our discovery of several novel CD163 polynucleotides.
The invention includes these novel CD163 polynucleotides. The present invention
provides several isolated novel polynucleotides (e.g., DNA sequences and RNA
transcripts, both sense and complementary antisense strands, both single and double-
stranded, including splice variants thereof, which encode novel CD163 polypeptides.
25 We report herein isolated novel polynucleotides which encode porcine, murine,
human, canine, and african green monkey CD163 polypeptides and which comprise
the sequences set forth in SEQ ID NO: 1, 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37,
39, 41, 43, 45, and 47.

It should be recognized that by disclosing SEQ ID NOs: 1, 5, 12 13, 22, 23, 25,
30 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 it provides one skilled in the art a
multitude of methods of obtaining these sequences. By way of example, it would be
possible to generate probes from the sequence disclosed in SEQ ID NOs: 1, 5, 12 13,
22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 and screen porcine, murine,

human, canine, and african green monkey cDNA or genomic libraries and thereby obtain the entire SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or its genomic equivalent. Sambrook, *et al.*, (Eds.), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989). Also by way of example, one skilled in the art would immediately recognize that given the sequence disclosed in SEQ ID NO: 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 it is then possible to generate the appropriate primers for PCR amplification to obtain the entire sequence represented by these sequences. (See e.g., PCR Technology, H. A. Erlich, ed., Stockton Press, New York, 1989; PCR Protocols: A Guide to Methods and Applications, M. A. Innis, David H. Gelfand, John J. Sninsky, and Thomas J. White, eds., Academic Press, Inc., New York, 1990.)

DNA polynucleotides of the invention include cDNA, and DNA that has been chemically synthesized in whole or in part and is also intended to include allelic variants thereof. Allelic variants are modified forms of a wild type gene sequence, the modification resulting from recombination during chromosomal segregation, or exposure to conditions which give rise to genetic mutation. Allelic variants, like wild type genes, are naturally occurring sequences (as opposed to non-naturally occurring variants which arise from in vitro manipulation).

DNA sequences encoding the novel CD163 polypeptides are set forth in SEQ ID NOs: 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47. The worker of skill in the art will readily appreciate that the DNA of the invention comprises a double stranded molecule, for example the molecule having the sequence set forth in SEQ ID NO: 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 along with the complementary molecule (the "non-coding strand" or "complement") having a sequence deducible from the sequence of SEQ ID NOs: 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 according to Watson-Crick base pairing rules for DNA. Also contemplated by the invention are other polynucleotides encoding for the porcine, murine and african green monkey CD163 polypeptides of SEQ ID NO: 2, 14, 24, 27 and 32, 34, 36, 38, 40, 42, 44, 46, 48 which differ in sequence from the polynucleotide of SEQ ID NOs: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45 and 47 by virtue of the well-known degeneracy of the universal genetic code, as is well known in the art. The

present invention, therefore, contemplates those other DNA and RNA molecules that, on expression, encode the polypeptides of SEQ ID NO: 2, 14, 24, 27 and 32. Having identified the amino acid residue sequence encoded the porcine CD163 polypeptide, and with the knowledge of all triplet codons for each particular amino acid residue, it is possible to describe all such encoding RNA and DNA sequences. DNA and RNA molecules other than those specifically disclosed herein characterized simply by a change in a codon for a particular amino acid, are, therefore, within the scope of this invention.

A table of amino acids and their representative abbreviations, symbols and codons is set forth below in the following Table 4.

Table 4

Amino acid	Abbrev.	Symbol	Codon(s)					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

As is well known in the art, codons constitute triplet sequences of nucleotides in mRNA and their corresponding cDNA molecules. Codons are characterized by the base uracil (U) when present in an mRNA molecule but are characterized by the base thymidine (T) when present in DNA. A simple change in a codon for the same amino acid residue within a polynucleotide will not change the sequence or structure of the encoded polypeptide. It is apparent that when a phrase stating that a particular 3 nucleotide sequence "encode(s)" any particular amino acid, the ordinarily skilled artisan would recognize that the table above provides a means of identifying the particular nucleotides at issue. By way of example, if a particular three-nucleotide

sequence encodes theonine the table above discloses that the possible triplet sequences are ACA, ACG, ACC and ACU (ACT if in DNA).

The invention includes therefore, an isolated polynucleotide comprising:

- (a) a susCD163v1 polynucleotide sequence set forth in SEQ ID NOs: 1, and 5
- 5 (b) a polynucleotide that encodes a polypeptide that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, or 99%, identity and/or similarity to a polypeptide set forth in SEQ ID NO: 2
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 2,
- 10 (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a susCD163v2 polynucleotide sequence set forth in SEQ ID NOs: 12 or 13
- (b) a polynucleotide that encodes a polypeptide that has at least 99%, identity and/or similarity to a polypeptide set forth in SEQ ID NO: 14
- 15 (c) a polynucleotide encoding a polypeptide of SEQ ID: 14,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a murine CD63v2 polynucleotide sequence set forth in SEQ ID NOs: 22 or 23
- (b) a polynucleotide encoding a polypeptide of SEQ ID: 24,
- 20 (c) a polynucleotide which is the complement of any of (a) or (b).

The invention also includes an isolated polynucleotide comprising:

- (a) a murine CD163v3 polynucleotide sequence set forth in SEQ ID NOs: 25 or 26
- (b) a polynucleotide that encodes a polypeptide that has at least 96% 97% 98%, or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 27
- 25 (c) a polynucleotide encoding a polypeptide of SEQ ID: 27,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) an african green monkey CD163v2 polynucleotide sequence set forth in SEQ ID NOs: 30 or 31
- 30 (b) a polynucleotide that encodes a polypeptide that has at least 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 32
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 32,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 33
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 34
- 5 (c) a polynucleotide encoding a polypeptide of SEQ ID: 34,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 35
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or
- 10 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 36
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 36,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 37
- 15 (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 38
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 38,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- 20 (a) a polynucleotide sequence set forth in SEQ ID NO: 39
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 40
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 40,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

25 The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 41
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 42
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 42,
- 30 (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 43

(b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 44

(c) a polynucleotide encoding a polypeptide of SEQ ID: 44,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

5 The invention also includes an isolated polynucleotide comprising:

(a) a polynucleotide sequence set forth in SEQ ID NO: 45

(b) a polynucleotide that encodes a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 46

10 (c) a polynucleotide encoding a polypeptide of SEQ ID: 46,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

 The invention also includes an isolated polynucleotide comprising:

(a) a polynucleotide sequence set forth in SEQ ID NO: 47

15 (b) a polynucleotide that encodes a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 48

(c) a polynucleotide encoding a polypeptide of SEQ ID: 49,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

20 The polynucleotide sequence information provided by the invention makes possible large-scale expression of the encoded polypeptide by techniques well known and routinely practiced in the art. Polynucleotides of the invention also permit identification and isolation of polynucleotides encoding related porcine CD163v1 polypeptides, such as human allelic variants and species homologs, by well-known techniques including Southern and/or Northern hybridization, and the polymerase
25 chain reaction (PCR).

 Knowledge of the sequence of a any of the CD163 sequences disclosed herein also makes possible through use of Southern hybridization or polymerase chain reaction (PCR), the identification of genomic DNA sequences encoding CD163 regulatory sequences, such as promoters, operators, enhancers, repressors, and the
30 like.

 As noted in the section above entitled "Assays of the Invention" polynucleotides of the invention are also useful in hybridization assays to detect the capacity of cells to express CD163, or to measure levels of CD163 expression.

Polynucleotides of the invention may also be the basis for diagnostic methods useful for determining the susceptibility of an animal to virus infection as described above.

The disclosure herein of the full-length polynucleotides encoding a CD163 polypeptide makes readily available to the worker of ordinary skill in the art fragments of the full length polynucleotide. The invention therefore provides unique fragments of the CD163 encoding polynucleotides comprising at least 15 through the length of the full-length sequence (including each and every integer value between) consecutive nucleotides of a polynucleotide encoding a CD163 disclosed herein. Because polynucleotides of the invention (including fragments) comprise sequences unique to the particular CD163-encoding polynucleotide sequence, they therefore would hybridize under highly stringent or moderately stringent conditions only (i.e., "specifically") to polynucleotides encoding the various CD163 polypeptides. Sequences unique to polynucleotides of the invention are recognizable through sequence comparison to other known polynucleotides, and can be identified through use of alignment programs routinely utilized in the art, e.g., those made available in public sequence databases. Such sequences also are recognizable from Southern hybridization analyses to determine the number of fragments of genomic DNA to which a polynucleotide will hybridize. Polynucleotides of the invention can be labeled in a manner that permits their detection, including radioactive, fluorescent, and enzymatic labeling.

One or more unique fragment polynucleotides (or other CD163 polynucleotides as discussed above) can be included in kits that are used to detect the presence of a polynucleotide encoding for CD163, or used to detect variations in a polynucleotide sequence encoding for CD163. Also made available by the invention are anti-sense polynucleotides that recognize and hybridize to polynucleotides encoding CD163. Full length and fragment anti-sense polynucleotides are provided. Fragment anti-sense molecules of the invention include (i) those that specifically recognize and hybridize to the CD163 variants disclosed herein (as determined by sequence comparison of DNA encoding CD163s to DNA encoding other known molecules). Identification of sequences unique to the novel CD163-encoding polynucleotides can be deduced through the use of any publicly available sequence database, and/or through the use of commercially available sequence comparison programs. The uniqueness of selected sequences in an entire genome can be further

verified by hybridization analyses. After identification of the desired sequences, isolation through restriction digestion or amplification using any of the various polymerase chain reaction techniques well known in the art can be performed. Antisense polynucleotides are particularly relevant to regulating expression of CD163 by those cells expressing CD163 mRNA.

Antisense nucleic acids (preferably 10 to 20 base pair oligonucleotides) capable of specifically binding to CD163 expression control sequences or CD163 RNA are introduced into cells (e.g., by a viral vector or colloidal dispersion system such as a liposome). The antisense nucleic acid binds to the porcine CD163 target nucleotide sequence in the cell and prevents transcription or translation of the target sequence. Phosphorothioate and methylphosphonate antisense oligonucleotides are specifically contemplated for therapeutic use by the invention. The antisense oligonucleotides may be further modified by poly-L-lysine, transferrin polylysine, or cholesterol moieties at their 5' end. Suppression of porcine CD163 expression at either the transcriptional or translational level is useful to generate cellular or animal models for diseases characterized by aberrant porcine CD163 expression or as a therapeutic modality.

As noted above in more detail, the nucleic acids of the invention include vectors comprising a polynucleotide of the invention. Such vectors are useful, e.g., for amplifying the polynucleotides in host cells to create useful quantities thereof. In other embodiments, the vector is an expression vector wherein the polynucleotide of the invention is operatively linked to a polynucleotide comprising an expression control sequence. Such vectors are useful for recombinant production of polypeptides of the invention.

Also as noted above the invention provides host cells that are transformed or transfected (stably or transiently) with polynucleotides of the invention or vectors of the invention. As stated above, such host cells are useful for the production of virus and the production of vaccines.

The invention also provides isolated CD163 polypeptides encoded by a novel polynucleotide of the invention.

Polypeptides of the Invention

The examples disclose our discovery of several novel CD163 polypeptides. The invention includes these novel CD163 polypeptide which are set forth in SEQ ID NOs: 2, 14, 19, 24, 27, 32, 34, 36, 38, 40, 42, 44, 46, and 48.

5 The invention includes therefore, an isolated polynucleotide comprising a susCD163v1 polypeptide with the sequence set forth in SEQ ID NO: 2.

 The invention also includes a polypeptide that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, or 99%,
10 identity and/or similarity to a polypeptide set forth in SEQ ID NO: 2.

 The invention includes therefore, an isolated polynucleotide comprising a susCD163v2 polypeptide with the sequence set forth in SEQ ID NO: 14.

 The invention also includes a polypeptide that has at least 99%, identity and or similarity to a sus CD163v2 polypeptide set forth in SEQ ID NO: 14.

15 The invention also includes a murine CD163v2 polypeptide having the sequence set forth in SEQ ID: 24.

 The invention also includes a murine CD163v3 polypeptide having the sequence set forth in SEQ ID: 27.

 The invention also includes at least a polypeptide having 96% 97% 98%, or
20 99%, identity and/or similarity to a polypeptide set forth in SEQ ID NO: 27.

 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 32.

 The invention also includes a polypeptide that has at least 98% or 99%, identity and/or similarity to a polypeptide set forth in SEQ ID NO: 32.

25 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 34.

 The invention also includes a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 34.

 The invention also includes a polypeptide having the sequence set forth in SEQ ID:
30 36.

 The invention also includes a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 36.

The invention also includes a polypeptide having the sequence set forth in SEQ ID: 38.

The invention also includes a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 39.

5 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 40.

The invention also includes a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 40.

The invention also includes a polypeptide having the sequence set forth in SEQ ID: 10 42.

The invention also includes a polypeptide that has at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 42.

The invention also includes a polypeptide having the sequence set forth in SEQ ID: 15 44.

The invention also includes a polypeptide that has at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 44.

The invention also includes a polypeptide having the sequence set forth in SEQ ID: 20 46.

The invention also includes a polypeptide that has at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 46.

The invention also includes a polypeptide having the sequence set forth in SEQ ID: 25 48.

The invention also includes a polypeptide that has at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 48.

30 Polypeptides of the invention may be isolated from natural cell sources or may be chemically synthesized, but are preferably produced by recombinant procedures involving host cells of the invention. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., glycosylation, truncation,

lipidation, and phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention. Glycosylated and non-glycosylated forms of the novel CD163 polypeptides are embraced.

Overexpression in eukaryotic and prokaryotic hosts as described above
5 facilitates the isolation of CD163 polypeptides. The invention therefore includes isolated CD163 polypeptides as set out in SEQ ID NOs: 2, 14, 19, 24, 27 32, 34, 36, 38, 40, 42, 44, 46, 48 and variants and conservative amino acid substitutions therein including labeled and tagged polypeptides.

The invention includes novel CD163 polypeptides that are "labeled". The
10 term "labeled" is used herein to refer to the conjugating or covalent bonding of any suitable detectable group, including enzymes (e.g., horseradish peroxidase, beta - glucuronidase, alkaline phosphatase, and beta-D-galactosidase), fluorescent labels (e.g., fluorescein, luciferase), and radiolabels (e.g., ^{14}C , ^{125}I , ^3H , ^{32}P , and ^{35}S) to the compound being labeled. Techniques for labeling various compounds, including
15 proteins, peptides, and antibodies, are well known. See, e.g., Morrison, Methods in Enzymology 32b, 103 (1974); Syvanen et al., J. Biol. Chem. 284, 3762 (1973); Bolton and Hunter, Biochem. J. 133, 529 (1973). The termed labeled may also encompass a polypeptide which has covalently attached an amino acid tag as discussed below.

20 In addition, the novel CD163 polypeptides of the invention may be indirectly labeled. This involves the covalent addition of a moiety to the polypeptide and subsequent coupling of the added moiety to a label or labeled compound that exhibits specific binding to the added moiety. Possibilities for indirect labeling include biotinylation of the peptide followed by binding to avidin coupled to one of the above
25 label groups. Another example would be incubating a radiolabeled antibody specific for a histidine tag with a CD163s polypeptide comprising a polyhistidine tag. The net effect is to bind the radioactive antibody to the polypeptide because of the considerable affinity of the antibody for the tag.

The invention also embraces variants (or analogs) of the novel CD163 protein.
30 In one example, insertion variants are provided wherein one or more amino acid residues supplement a novel CD163 amino acid sequence. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of the novel CD163 protein amino acid sequence. Insertional variants with additional

residues at either or both termini can include for example, fusion proteins and proteins including amino acid tags or labels. Insertion variants include novel CD163 polypeptides wherein one or more amino acid residues are added to a CD163 acid sequence, or to a biologically active fragment thereof.

5 Insertional variants therefore can also include fusion proteins wherein the amino and/or carboxy termini of the novel CD163 polypeptide is fused to another polypeptide. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the influenza HA tag polypeptide and its antibody
10 12CA5 [Field et al., *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., *Molecular and Cellular Biology*, 5:3610-3616 (1985)], and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., *Protein Engineering*, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag -peptide [Hopp et al.,
15 BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., *Science*, 255:192-194 (1992)]; an alpha -tubulin epitope peptide [Skinner et al., *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., *Proc. Natl. Acad. Sci. USA*, 87:6393-6397(1990)]. In addition, the CD163 polypeptide can be tagged with enzymatic proteins such as
20 peroxidase and alkaline phosphatase.

 In another aspect, the invention provides deletion variants wherein one or more amino acid residues in a novel CD163 polypeptide is removed. Deletions can be effected at one or both termini of the novel CD163 polypeptide, or with removal of one or more residues within the novel CD163 amino acid sequence. Deletion
25 variants, therefore, include all fragments of the novel CD163 polypeptide.

 CD163 polypeptides contain a transmembrane or membrane anchor region. It should be recognized that such transmembrane domains are useful when expressed in the context of a heterologous protein to aid in the targeting of the heterologous protein to membranes. It should also be recognized that it may be advantageous to delete
30 some transmembrane domains to enhance the purification or solubility of the protein. Transmembrane deleted variants of CD163 and polynucleotides encoding them are of potential value as antiviral therapeutics. Such variants are specifically disclosed here as SEQ ID NOs: 37-40.

The present invention also includes variants of the aforementioned polypeptides, that is, polypeptides that vary from the reference sequence by conservative amino acid substitutions,

Exemplary conservative substitutions are set out in Tables 1, 2 and 3 in the section above entitled "Definitions".

In those situations where it is preferable to partially or completely isolate the novel CD163 polypeptides, purification can be accomplished using standard methods well known to the skilled artisan. Such methods include, without limitation, separation by electrophoresis followed by electroelution, various types of chromatography (immunoaffinity, molecular sieve, and/or ion exchange), and/or high pressure liquid chromatography. In some cases, it may be preferable to use more than one of these methods for complete purification.

Purification of novel CD163 polypeptides can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (CD163 /hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, Conn.) or myc (Invitrogen, Carlsbad, Calif.) at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag or for the polypeptide directly (i.e., a monoclonal antibody specifically recognizing CD163). For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen Registered TM nickel columns) can be used for purification of CD163 /polyHis. (See for example, Ausubel et al., eds., Current Protocols in Molecular Biology, Section 10.11.8, John Wiley & Sons, New York [1993]).

Even if the novel CD163 polypeptide is prepared without a label or tag to facilitate purification, the novel CD163 of the invention may be purified by immunoaffinity chromatography. To accomplish this, antibodies specific for CD163 polypeptides must be prepared by means well known in the art.

Antibodies generated against the novel CD163 polypeptides of the invention can be obtained by administering the polypeptides or epitope-bearing fragments, analogues, or cells to an animal, preferably a non-human, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include

various techniques, such as those in Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor et al., *Immunology Today* 4: 72 (1983); Cole et al., pg. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985).

Where the novel CD163 polypeptides are prepared without a tag attached, and
5 no antibodies are available, other well-known procedures for purification can be used. Such procedures include, without limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of these techniques may be combined
10 to achieve increased purity.

It should be understood that the definition of polypeptides of the invention is intended to include polypeptides bearing modifications other than insertion, deletion, or substitution of amino acid residues. By way of example, the modifications may be covalent in nature, and include for example, chemical bonding with polymers, lipids,
15 other organic, and inorganic moieties.

Antibodies

Also comprehended by the present invention are antibodies (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and
20 complementary determining region (CDR)-grafted antibodies, including compounds which include CDR sequences which specifically recognize a polypeptide of the invention) specific for novel CD163 or fragments thereof.

The term "specific for," when used to describe antibodies of the invention, indicates that the variable regions of the antibodies of the invention recognize and
25 bind CD163s polypeptides exclusively (i.e., able to distinguish CD163s polypeptides from other known polypeptides by virtue of measurable differences in binding affinity, despite the possible existence of localized sequence identity, homology, or similarity between the novel CD163 and such polypeptides). It will be understood that specific antibodies may also interact with other proteins (for example, *S. aureus* protein A or
30 other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive

discussion of such assays, see Harlow et al. (Eds), *Antibodies A Laboratory Manual*; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), Chapter 6.

Antibodies that recognize and bind fragments of the CD163s polypeptides of the invention are also contemplated, provided that the antibodies are, first and foremost, specific for novel CD163 polypeptides. Antibodies of the invention can be produced using any method well known and routinely practiced in the art. Non-human antibodies may be humanized by any methods known in the art. In one method, the non-human CDRs are inserted into a human antibody or consensus antibody framework sequence. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity.

Antibodies of the invention are useful for, diagnostic purposes to detect or quantitate CD163s, as well as purification of CD163s. Kits comprising an antibody of the invention for any of the purposes described herein are also comprehended. In general, a kit of the invention also includes a control antigen for which the antibody is immunospecific

The present invention is further illustrated, but not limited, by the following examples.

Example 1: Transient transfection with porcine CD163 confers permissivity to PRRS virus infection to a non-permissive cell line. Total mRNA from primary porcine alveolar macrophage cells was used to construct a cDNA library in the plasmid pCMV-Sport6.1 (Invitrogen), with the cDNA cloned between the *EcoRV* and *NotI* sites. A member of this library, when isolated and transiently transfected into the BHK-21 (baby hamster kidney) cell line, conferred a PRRS-permissive phenotype. Cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% fetal bovine serum (FBS) in a 5% CO₂ atmosphere at 37°C. Cell cultures were transiently transfected using 10.0 uL of Lipofectamine 2000 (Invitrogen) and 2.0ug of plasmid. A duplicate monolayer was transfected with negative control plasmid pPAMB. This plasmid is pCMV-Sport6.1 lacking an insert. Transfection efficiency was monitored with a plasmid expressing green fluorescent protein (GFP). Approximately 24 hours post-transfection, monolayers were infected with either North American (isolate P129) or European (isolate 96V198) genotypes of PRRS virus. For detection of PRRS replication, the monolayers were fixed using 80% acetone approximately 24 hours post-infection and incubated for approximately 1 hour with

FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc.). This monoclonal antibody is specific for PRRS viral nucleocapsid expressed from open reading frame 7. A Nikon TE 300 inverted fluorescent microscope with a 10x objective was used to photograph a monolayer containing FITC positive cells and a negative control monolayer.

It was confirmed that transfected cells became permissive to both the North American (isolate P129) and European (isolate 96V198) genotypes of PRRSV. Expression of viral genes could be detected in many of the transfected BHK cells, and progeny virus was readily detectable in the supernatant. Control transfections using vector without insert or irrelevant plasmids did not confer permissivity.

Sequencing of the insert in the functional plasmid, using the Big Dye Terminator Version 1.0 Sequence Reaction kit (Applied Biosystems, Foster City, CA) and the Applied Biosystems 3730 DNA Analyzer (Applied Biosystems), revealed a gene that was highly homologous to the published porcine CD163 gene cDNA (Genbank accession number AJ311716). The cDNA we identified contained additional 5' and 3' untranslated regions relative to AJ311716, and the open reading frame differed in three ways: (1) a 738 bp internal deletion near the 5' end, (2) a 15 bp extension of the 5' end to an upstream ATG codon, and (3) sixteen nucleotide changes predicted to cause 10 amino acid changes. Nucleotide sequence identity between the sequences was 99.4%. Alignments of the newly discovered porcine CD163 sequence with the previously reported sequence AJ311716 are shown in figures 1 and 2. The novel porcine CD163 variant was designated "susCD163v1".

SEQUENCE			ID NO
gtaataatac aagaagattt aaatgggcat aaaaccttgg aatggacaaa ctcagaatgg	60		SEQ ID NO:5
tgctacatga aaactctgga tctgcagacc tgaaactgag agtggtagat ggagtcactg	120		
aatgttcagg aagattggaa gtgaaattcc aaggagaatg gggaacaatc tgtgatgatg	180		
gctgggatag tgatgatgcc gctgtggcat gtaagcaact gggatgtcca actgctgtca	240		
ctgccattgg tcgagttaac gccagtgagg gaactggaca catttggctt gacagtgttt	300		
cttgccatgg acacgagtct gctctctggc agtgtagaca ccatgaatgg ggaaagcatt	360		
attgcaatca taatgaagat gctggtgtga catgttctga tggatcagat ctggaactga	420		
gacttaaagg tggaggcagc cactgtgctg ggacagtgga ggtggaaatt cagaaactgg	480		
taggaaaagt gtgtgataga agctggggac tgaaagaagc tgatgtggtt tgcaggcagc	540		
tgggatgtgg atctgcactc aaaacatcat atcaagttaa ttccaaaacc aaggcaacaa	600		
acacatggct gtttgtaagc agctgtaatg gaaatgaaac ttctcttttg gactgcaaga	660		
attggcagtg ggggtggactt agttgtgatc actatgacga agccaaaatt acctgctcag	720		
cccacaggaa acccaggctg gttggagggg acattccctg ctctggtcgt gttgaagtac	780		

aacatggaga cacgtggggc accgtctctg attctgaact ctctctggag gcggccagcg	840	
tgctgtgcag ggaactacag tgcggcactg tggtttccct cctgggggga gctcactttg	900	
gagaaggaag tggacagatc tgggctgaag aattccagtg tgagggggcac gagtcccacc	960	
tttctactctg cccagtagca ccccgccctg acgggacatg tagccacagc agggacgtcg	1020	
gcgtagtctg ctcaagatac acacaaatcc gcttggtgaa tggcaagacc ccatgtgaag	1080	
gaagagtgga gctcaacatt cttgggtccct gggggtcctct ctgcaactct cactgggaca	1140	
tggaagatgc ccatgtttta tgccagcagc ttaaagtggg agttgccctt tctatcccgg	1200	
gaggagcacc ttttgggaaa ggaagtgagc aggtctggag gcacatgttt cactgcactg	1260	
ggactgagaa gcacatggga gattgttccg tctactgtct gggcgcatca ctctgttctt	1320	
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41 G T I C D D G W D S D D A A V A C K Q L		
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61 G C P T A V T A I G R V N A S E G T G H		
181 ggatgtccaactgctgtcactgccattggtcgagttaacgccagtggggaactggacac		
81 I W L D S V S C H G H E S A L W Q C R H		and 2

1 51	241 atttggcttgacagtgtttcttgccatggacacgagctctgctcttggcagtgtagacac 101 H E W G K H Y C N H N E D A G V T C S D 301 catgaatgggaaagcattattgcaatcataatgaagatgctggtgtgacatgttctgat 121 G S D L E L R L K G G G S H C A G T V E 361 ggatcagatctggaactgagacttaaaagtgaggcagccactgtgctgggacagtggag 141 V E I Q K L V G K V C D R S W G L K E A 421 gtggaaattcagaaactggtaggaaaagtgtgtgataagaagctggggactgaaagaagct 161 D V V C R Q L G C G S A L K T S Y Q V Y 481 gatgtgggttgcaggcagctgggatgtggatctgcactcaaaacatcatatcaagtttat 181 S K T K A T N T W L F V S S C N G N E T 541 tccaaaaccaaggcaacaacacatggctgtttgtgaagcagctgtaatggaaatgaaact 201 S L W D C K N W Q W G G L S C D H Y D E 601 tctctttgggactgcaagaattggcagtggtgggacttagttgtgatcactatgacgaa 221 A K I T C S A H R K P R L V G G D I P C 661 gccaaaattacctgctcagcccacaggaaacccaggtggtgggaggggacattccctgc 241 S G R V E V G H G D T W G T V C D S D F 721 tctggtcgtgtgaagtacaacatggagacagctggggcaccgtctgtgattctgacttc 261 S L E A A S V L C R E L Q C G T V V S L 781 tctctggagggcgccagctgctgtgcaggaactacagtgcggcactgtggtttccctc 281 L G G A H F G E G S G Q I W A E E F Q C 841 ctggggggagctcacttttgagaaggaagtggacagatctgggtgaagaattccagtg 301 E G H E S H L S L C P V A P R P D G T C 901 gaggggcagcagtgcccactttcactctgccagtagcaccccgcctgacgggacatgt 321 S H S R D V G V V C S R Y T Q I R L V N 961 agccacagcaggagcgtcggtgtagtctgctcaagatacacacaaatccgcttggtgaat 341 G K T P C E G R V E L N I L G S W G S L 1021 ggcaagacccccatgtgaaggaagagtgagctcaacattcttgggtcctgggggtccctc 361 C N S H W D M E D A H V L C Q Q L K C G 1081 tgcaactctcactgggacatggaagatgcccattgtttatgccagcagcttaaatgtgga 381 V A L S I P G G A P F G K G S E Q V W R 1141 gttgccctttctatcccgggaggagcaccttttgggaaaggaagtggagcaggtctggagg 401 H M F H C T G T E K H M G D C S V T A L 1201 cacatgtttcactgcaactgggactgagaagcacatgggagattgttccgtcactgctcg 421 G A S L C S S G Q V A S V I C S G N Q S 1261 ggcgcatcactctgttcttcagggaagtggcctctgtaattctgctcagggaaaccagagt 441 Q T L S P C N S S S S D P S S S I I S E 1321 cagacactatccccgtgcaattcatcctcggacccatcaagctctattatttcagaa 461 E N G V A C I G S G Q L R L V D G G G R 1381 gaaaatggtgttgctgcagtagggagtggtcaacttcgctggtcgatggaggtggtcgt 481 C A G R V E V Y H E G S W G T I C D D S 1441 tgtgctgggagtagaggtctatcatgagggctcctggggcaccatctgtgatgacagc 501 W D L N D A H V V C K Q L S C G W A I N 1501 tgggacctgaatgatgcccatgtggtgtgcaaacagctgagctgtggatgggccaat 521 A T G S A H F G E G T G P I W L D E I N 1561 gccactggttctgctcattttggggaaggaacagggccatttggctggatgagataaac 541 C N G K E S H I W Q C H S H G W G R H N 1621 tgtaatggaaaagaatctcatatttggcaatgccactcacatggttggggggcgccacaat 561 C R H K E D A G V I C S E F M S L R L I 1681 tgcaggcataaggaggatgcaggagtcctgctcgagttcatgtctctcagactgatc 581 S E N S R E T C A G R L E V F Y N G A W 1741 agtgaanaacagcagagagacctgtgcaggggcctggaagtgtttttacaacggagcttgg 601 G S V G K N S M S P A T V G V V C R Q L 1801 ggcagcgttggcaagaatagcatgtctccagccacagtgggggtggtatgcaggcagctg 621 G C A D R G D I S P A S S D K T V S R H 1861 ggctgtgcagacagaggggacatcagccctgcatcttcagacaagacagtgtccaggcac 641 M W V D N V Q C P K G P D T L W Q C P S 1921 atgtgggtggacaatgttcagtgctcctaaggacctgacaccctatggcagtgcccatca 661 S P W K K R L A S P S E E T W I T C A N 1981 tctccatggaagaagagactggccagccctcagaggagacatggatcacatgtccaac 681 K I R L Q E G N T N C S G R V E I W Y G 2041 aaaataagacttcaagaaggaaacactaatgttctggacgtgtggagatctggttacgga 701 G S W G T V C D D S W D L E D A Q V V C 2101 ggttctctggggcactgtgtgtgacgactcctgggaccttgaagatgctcaggtggtgtgc 721 R Q L G C G S A L E A G K E A A F G Q G 2161 cgacagctgggctgtggtcagcttggaggcaggaaaagagggccgcatcttggccagggg 741 T G P I W L N E V K C K G N E T S L W D 2221 actgggcccataatggctcaatgaagtgaagtgaagggaatgaaacctccttggggat 761 C P A R S W G H S D C G H K E D A A V T 2281 tgtcctgcccagatcctggggccacagtgactgtggacacaaggaggatgctgctgtgacg 781 C S E I A K S R E S L H A T G R S S F V 2341 tgttcagaaattgcaagagccgagaatccctacatgccacaggtcgctcatcttctgtt 801 A L A I F G V I L L A C L I A F L I W T 2401 gcacttgcaatcttgggtcattctgttggcctgtctcatcgattcctcatttggact 821 Q K R Q R Q R L S V F S G G E N S V H 2461 cagaagcgaagacagagggcagcggtctcagttttctcaggaggagagaattctgtccat 841 Q I Q Y R E M N S C L K A D E T D M L N 2521 caaattcaataccgggagatgaattcttgctgaaagcagatgaaacggatagctaaat 861 P S G D H S E V Q 2581 ccctcaggagaccactctgaagtacaa	SEQ ID
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201	SLWDCKNWQW	GGLSCDHYDE	AKITCSAHRK	PRLVGGDIPC	SGRVEVQHGD	
251	TWGTVCDSDF	SLEAASVLCR	ELQCGTVVSL	LGGAHFGEES	GQIWAEFQC	
301	EGHESHLSLC	PVAPRPDGTG	SHSRDVGVC	SRYTQIRLVN	GKTPCEGRVE	
351	LNILGSWGS	CNSHWMEDA	HVLCQQLKCG	VALSIPGGAP	FGKGSQVWR	
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451	SDPSSSIISE	ENGVAICGSG	QLRLVDGGGR	CAGRVEVYHE	GSWGTICDDS	
501	WDLNDAHVVC	KQLSCGWAIN	ATGSAHFEGE	TGPIWLDEIN	CNGKESHIWQ	
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651	GPDTLWQCP	SPWKKRLASP	SEETWITCAN	KIRLQEGNTN	CSGRVEIYWG	
701	GSWGTVCDDS	WDLEDAQVVC	RQLGCGSALE	AGKEAAGQGG	TGPIWLNEVK	
751	CKGNETSLWD	CPARSWGHS	CGHKEDAAVT	CSEIAKSRES	LHATGRSSFV	
801	ALAIFGVILL	ACLI AFLIWT	QKRRQRQLS	VFSGGENSVH	QIQYREMNCS	
851	LKADETMLN	PSGDHSEVQ				

Example 2: Construction of plasmid pCMVsusCD163v1

Construction of the plasmid pCMVsusCD163v1 was performed as follows. The functional clone identified in the primary porcine macrophage cDNA library as

5 conferring PRRSV permissivity served as template for PCR amplification of the CD163 insert, including the 5' and 3' untranslated regions, using the primers 5'DS-CD163 (SEQ ID NO:6) (5'-CGGAATTCCGCGGATGTAATAATACAAGAAGA-3') and 3'CD163 (SEQ ID NO:7) (5'CCGCTCGAGTAGTCCAGGTCTTCATCAAGGTATCTT-3'). Primer 5'DS-

10 CD163 incorporates a *Sac*II restriction site at the 5' end of the CD163 insert, while primer 3'CD163 incorporates an *Xho*I restriction site at the 3' end of the insert (underlined). Reactions containing 190ng of plasmid template were amplified using Platinum *Pfx* DNA polymerase (Invitrogen cat # 11708-013) following the manufacture's instructions. Reactions were heated to 94° for 2 minutes then cycled

15 35 times through 94° for 20 seconds, 55° for 30 seconds, and 68° for 3.5 minutes followed by a terminal extension at 72° for 7 minutes. The resulting PCR products were purified using the Qiaquick PCR purification kit (Qiagen cat # 28104), digested with restriction enzymes *Sac*II and *Xho*I, and the resulting fragments were gel purified using the Qiaquick Gel Extraction kit (Qiagen cat # 28704). The CD163 PCR

20 fragment was then ligated into the plasmid pCMV-Script (Stratagene cat # 212220) prepared to accept the digested PCR fragment by digestion with *Sac*II and *Xho*I followed by gel purification as described above. The ligated material was transformed into *E. coli* strain DH5α and recombinants were selected by growth in 50μg/ml kanamycin and identified by restriction analysis. The resulting plasmid,

25 "pCMVsusCD163v1", contains the internally deleted porcine CD163 insert described in Example 1 under the transcriptional control of the eukaryotic CMV promoter and

the neomycin/kanamycin resistance gene under the control of both eukaryotic and prokaryotic promoters.

Example 3: Construction of the pRSV-Script expression vector and pRSVsusCD163v1

5 The plasmid pRc/RSV (Invitrogen) was used as a template for PCR amplification of the RSV promoter. RSV promoter sequence was contained within nucleotides 209 through 604 of pRc/RSV. Forward primer PCIRSVLTR (SEQ ID NO:8) (5'-ACACTCGACATGTCGATGTACGGGCCAGATATACGCGT-3') and reverse primer VSRRTLSAC (SEQ ID NO: 9) (5'TTCCTTACAGAGCTCGAGGTGCACACCAATGTGGTGAA -3') were synthesized. Restriction endonuclease *Pci* I and *Sac* I recognition sites (underlined) were incorporated into the 5' and 3' primers, respectively, for future cloning. PCR was performed using the HotMaster Taq DNA Polymerase kit (Eppendorf) following the manufacturer's instructions. The reactions contained 0.9ng of pRc/RSV plasmid template and 0.3μM of each primer described above. The reactions were heated to 15 94° for 2 minutes then cycled 30 times through 94° for 20 seconds, 52° for 10 seconds, and 65° for 1 minute. The resulting PCR fragment was digested with restriction enzymes *Pci* I and *Sac* I, gel purified, and cloned into the plasmid pCMV-Script (Stratagene) that had been similarly digested to remove the CMV promoter sequence. The final construct placed the RSV promoter immediately upstream of the multiple cloning site, and was named "pRSV-Script".

The susCD163v1 insert was cloned behind the RSV promoter as follows. The susCD163v1 sequence was excised from plasmid pCMVsusCD163v1 by restriction digestion (*Kpn* I and *Sac* II) and gel purified. This fragment was ligated into pRSV-Script which had also been digested with the same enzymes and gel purified. The ligation mixture was transformed into DH5α *E. coli* and transformants selected using kanamycin at 50μg/ml. The clone contained the correct insert was designed "pRSVsusCD163v1".

Example 4: Cloning and characterization of a longer variant of porcine CD163 cDNA

30 Based on the porcine CD163v1 sequence, a forward primer 5'CD163NotIlong (SEQ ID NO:10) (5'CGGTCCGGAGCGGCCGCGATGTAATAATACAAGAAGATTAAATGG-3') and a reverse primer 3'CD163KpnI (SEQ ID NO:11)

(5'CGGTTGGTACCCAGCAATATTCTTTTTTATTTAATGCC-3') were designed using the Lasergene PrimerSelect program (DNASTAR Inc., Madison WI) for amplification of a full-length porcine CD163 gene. Restriction endonuclease sites for *Not* I and *Kpn* I (underlined) were included in 5' and 3' primers, respectively, to allow for convenient cloning. Total cellular RNA was prepared from primary alveolar macrophages (PAM) harvested from lung lavages of healthy pigs. RNA preparation was done using the RNeasy mini kit (Qiagen, Valencia, CA). RT-PCR reactions were prepared using the SuperScript one-step RT-PCR for Long Templates kit (Invitrogen, Carlsbad, CA) and RT-PCR parameters were set as follows: 50°C for 30 min, 94°C for 2 min, (94°C 30 sec, 55°C 30 sec and 68°C 4 min) for 35 cycles, 72°C for 10 min. PCR products were analyzed on 0.8% SeaKem GTG agarose gels. RT-PCR products of various sizes were cut from agarose gels and DNA was extracted using the GeneClean kit (QBiogene). These RT-PCR products were cloned into the pCR2.1-TOPO cloning vector (Invitrogen). Clones were analyzed by restriction enzyme digestion for the presence of an insert. Colonies containing inserts were sequenced using Big Dye Terminator Version 1.0 Sequence Reaction kit (Applied Biosystems, Foster City, CA) and Applied Biosystems 3730 DNA Analyzer (Applied Biosystems) to confirm sequence authenticity. Sequences were edited and assembled using the Lasergene EditSeq and SeqMan programs (DNASTAR Inc., Madison WI). One plasmid with a large insert was designated "pCRsusCD163v2" (pCR2.1 containing porcine CD163 variant 2 which we have designated SEQ ID NO:12). The coding sequence contained within SEQ ID NO:12 is reproduced below and is designated SEQ ID NO:13. Sequence analysis showed that this porcine CD163 encodes an amino acid sequence of 1115 amino acids which we have designated SEQ ID NO:14. When compared to the porcine CD163 sequence in GenBank (Accession No. AJ311716), our CD163v2 sequence is 98.9% identical at the amino acid level. CD163v2 also has an additional 5 amino acid residues at the extreme 5' end, extending the open reading frame to an in-frame upstream ATG initiation codon (as in the porcine CD163v1 sequence described in example 1). Porcine CD163 is 84.3% identical to human CD163 (GenBank Accession No. Z22968), and 73.7% identical to mouse CD163 (GenBank Accession No. AF274883) at the amino acid level. The predicted signal sequence and transmembrane region of SEQ ID NO:14 are indicated by underlining

and bolding respectively. To determine whether other CD163 sequences contain similar sequence features is easily determined by inspection of the sequence.

SEQUENCE			ID NO
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41 T S S L G G K D K E L R L T G G E N K C		
121 actagttctcttggaggaaaagacaaggagctgaggctaacgggtggtgaaaacaagtgc		
61 S G R V E V K V Q E E W G T V C N N G W		
181 tctggaagagtggaggtgaaagtgcaggaggagtgggaactgtgtgtaataatggctgg		
81 D M D V V S V V C R Q L G C P T A I K A		
241 gacatggatgtggtctctgttgtttaggcagctgggatgtccaactgctatcaaagcc		
101 T G W A N F S A G S G R I W M D H V S C		
301 actggatgggctaatttttagtgacaggttctggacgcatttggatggatcatgtttcttgt		
121 R G N E S A L W D C K H D G W G G K H N C		
361 cgagggaatgagtcagctctctgggactgcaaactgatggatgggaaagcataaactgt		
141 T H Q Q D A G V T C S D G S D L E M G L		
421 actcaccacaggatgctggagtaacctgctcagatggatctgatttagagatggggctg		
161 V N G G N R C L G R I E V K F Q G R W G		
481 gtgaatggaggaaaccggtgcttaggaagaatagaagtcaaatttcaaggacggtgggga		
181 T V C D D N F N I N H A S V V C K Q L E		
541 acagtgtgtgatgataacttcaacataaatcatgcttctgtggttgtgtaacaacttgaa		
201 C G S A V S F S G S A N F G E G S G P I		
601 tgtggaagtgtgtcagtttctctggttcagctaatttggagaaggttctggaccaatc		
221 W F D D L V C N G N E S A L W N C K H E		
661 tggtttgatcttctgtatgcaatggaaatgagtcagctctctggaactgcaaacatgaa		
241 G W G K H N C D H A E D A G V I C L N G		
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261 A D L K L R V V D G V T E C S G R L E V		
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281 K F Q G E W G T I C D D G W D S D D A A		
841 aaattccaaggagaatggggaacaatctgtgatgatggctgggatagtgatgatgccgct		
301 V A C K Q L G C P T A V T A I G R V N A		
901 gtggcatgtaagcaactgggatgtccaactgctgtcactgccatttggtcgagttaacgcc		
321 S E G T G H I W L D S V S C H G H E S A		
961 agtgagggaactggacacatttggcttgacagtgttcttggccatggacacgagctgct		

341	L W Q C R H H E W G K H Y C N H D E D A	
1021	ctctggcagtgtagacaccatgaatggggaaagcattattgcaatcatgatgaagtgct	
361	G V T C S D G S D L E L R L K G G G S H	
1081	gggtgtgacatgttctgatggatcagatctggaactgagacttaaagggtggaggcagccac	
381	C A G T V E V E I Q K L V G K V C D R S	
1141	tgtgctgggacagtggaggtggaaattcagaaactggtaggaaaagtgtgtgatagaagc	
401	W G L K E A D V V C R Q L G C G S A L K	
1201	tggggactgaaagaagctgatgtggtttgcaggcagctgggatgtggatctgcactcaaa	
421	T S Y Q V Y S K T K A T N T W L F V S S	
1261	acatcatatcaagttttattccaaaaccaaggcaacaacacatggctgtttgtaagcagc	
441	C N G N E T S L W D C K N W Q W G G L S	
1321	tgtaatggaaatgaaacttctctttgggactgcaagaattggcagtggtgggtggacttagt	
461	C D H Y D E A K I T C S A H R K P R L V	
1381	tgtgatcactatgacgaagccaaaattacctgctcagccacaggaaccagggtggtt	
481	G G D I P C S G R V E V Q H G D T W G T	
1441	ggaggggacattccctgctcgtgctgtgaagtacaacatggagacacgtggggcacc	
501	V C D S D F S L E A A S V L C R E L Q C	
1501	gtctgtgattctgacttctctctggaggcggccagcgtgctgtgcagggaactacagtg	
521	G T V V S L L G G A H F G E G S G Q I W	
1561	ggcactgtggtttccctcctggggggagctcactttggagaagggaagtggacagatctgg	
541	A E E F Q C E G H E S H L S L C P V A P	
1621	gctgaagaattccagtgaggggcagcagtgccacctttcactctgcccagtagcaccc	
561	R P D G T C S H S R D V G V V C S R Y T	
1681	cgccctgacgggacatgtagccacagcagggacgtcggtgtagtctgctcaagatacaca	
581	Q I R L V N G K T P C E G R V E L N I L	
1741	caaatcgcttggtgaatggcaagacccccatgtgaagggaagagtggagctcaacattctt	
601	G S W G S L C N S H W D M E D A H V L C	
1801	gggtcctgggggtccctctgcaactctcactgggacatggaagatgcccattgtttatgc	
621	Q Q L K C G V A L S I P G G A P F G K G	
1861	cagcagcttaaatgtggagttgcccccttatccgggaggagcaccttttgggaaagga	
641	S E Q V W R H M F H C T G T E K H M G D	
1921	agtggacaggtctggaggcacatgtttcactgcaactgggactgagaagcacatgggagat	
661	C S V T A L G A S L C S S G Q V A S V I	
1981	tgttccgtcactgctctgggcgcacactctgttcttcaggggcaagtggcctctgtaatc	
681	C S G N Q S Q T L S P C N S S S S D P S	
2041	tgtcagggaaccagagtcagacactatctccgtgcaattcatcatcctcgaccatca	
701	S S I I S E E N G V A C I G S G Q L R L	
2101	agctctattatttcagaagaaaatggtgtgctgcatagggagtggtcaacttcgcctg	
721	V D G G G R C A G R V E V Y H E G S W G	
2161	gtcgatggaggtggtgctgtgtgggagagtagaggtctatcatgagggtcctggggc	
741	T I C D D S W D L N D A H V V C K Q L S	
2221	accatctgtgatgacagctgggacctgaatgatgcccattgtggtgtgcaaacagctgagc	
761	C G W A I N A T G S A H F G E G T G P I	
2281	tgtggatggggcattaatgccactggttctgctcattttgggggaagggaacaggggccatt	
781	W L D E I N C N G K E S H I W Q C H S H	
2341	tggctggatgagataaaactgtaatggaaaagaatctcatatttggcaatgccactcacat	
801	G W G R H N C R H K E D A G V I C S E F	
2401	ggttggggggcgccacaattgcagggcataaggaggatgcaggagtcactgctcagagttc	
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841	F Y N G A W G S V G R N S M S P A T V G	
2521	ttttacaacggagcttggggcagcgttggcaggaatagcatgtctccagccacagtgagg	
861	V V C R Q L G C A D R G D I S P A S S D	
2581	gtggtatgcaggcagctgggctgtgcagacagaggggacatcagccctgcactcttcagac	
881	K T V S R H M W V D N V Q C P K G P D T	
2641	aagacagtgtccaggcacatgtgggtggacaattgttcagtgctcctaaaggacctgacaca	
901	L W Q C P S S P W K K R L A S P S E E T	
2701	ctatggcagtgcccatcatctccatggaagaagagactggccagccctcagaggagaca	
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2761	tggatcacatgtgccaacaaaataagacttcaagaaggaaactaattgttctggacgt	
941	V E I W Y G G S W G T V C D D S W D L E	
2821	gtggagatctggtacggaggttctctggggcactgtgtgtgacgactcctgggacctgaa	
961	D A Q V V C R Q L G C G S A L E A G K E	
2881	gatgctcaggtggtgtgccgacagctgggctgtggctcagcttggaggcaggaaaagag	
981	A A F G Q G T G P I W L N E V K C K G N	
2941	gccgcatttggccaggggactggggccatattggctcaatgaagtgaagtgaagggggaat	
1001	E T S L W D C P A R S W G H S D C G H K	
3001	gaaacctccttgtgggattgtcctgccagatcctggggccacagtgactgtggacacaag	
1021	E D A A V T C S E I A K S R E S L H A T	
3061	gaggatgctgctgtgacgtgctcagaaattgcaaagagccgagaatcctacatgccaca	
1041	G R S S F V A L A I F G V I L L A C L I	
3121	ggtcgtcatcttttgttgacttgcaatctttggggctcattctgttggcctgtctcatc	
1061	A F L I W T Q K R R Q R Q R L S V F S G	
3181	gcattcctcatttggactcagaagcgaagacagaggcagcggtctcagttttctcagga	
1081	G E N S V H Q I Q Y R E M N S C L K A D	
3241	ggagagaattctgtccatcaaatcaccgggagatgaattcttgctgaaagcagat	
1101	E T D M L N P S G D H S E V Q	
3301	gaaacggatatgctaataatccctcaggagaccactctgaagtacaa	
1	MDKLRMLHE NSGSADFRRC SAHLSSFTFA VVAVLSACLIV TSSLGGKDKE	
51	LRLTGGENKC SGRVEVKVQE EWGTVCNNGW DMDVVSVVCRLGCPATAIKA	SEQ ID

101	TGWANFSAGS	GRIWMDHVSC	RGNESALWDC	KHDGKGKHC	THQQDAGVTC	NO:14
151	SDGSDLEMGL	VNGGNRCLGR	IEVKFQGRWG	TVCCDDNFNIN	HASVVCQLE	
201	CGSAVSFSGS	ANFGECSGPI	WFDDLVCNGN	ESALWNCKHE	GWGKHNCDDHA	
251	EDAGVICLNG	ADLKLRVVDG	VTECSGRLEV	KFQGEWGTIC	DDGWDSDDA	
301	VACKQLGCPT	AVTAIGRVNA	SEGTGHIWLD	SVSCHGHESA	LWQCRHHEWG	
351	KHYCNHDEDA	GVTCSGSDL	ELRLKGGGSH	CAGTVEVEIQ	KLVGKVCDS	
401	WGLKEADVVC	RQLGCGSALK	TSYQVYSKTK	ATNTWLFVSS	CNGNETSLWD	
451	CKNWQWGLS	CDHYDEAKIT	CSAHRKPLRV	GGDIPCSGRV	EVQHGDWTGT	
501	VCDSDFSLEA	ASVLCRELQC	GTVVSLLGGA	HFEGSGQIWI	AEEFQCEGHE	
551	SHLSLCPVAP	RPDGTCSHSR	DVGVCSSRYT	QIRLVNGKTP	CEGRVELNIL	
601	GSWGSCLNSH	WDMEDAHVLC	QQLKCGVALS	IPGGAPFGKG	SEQVVRHMFH	
651	CTGTEKMGD	CSVTALGASL	CSSGQVASVI	CSGNQSQTLS	PCNSSSSDPS	
701	SSIISEENG	ACTGSGQLRL	VDGGGRCAGR	VEVYHEGWSG	TICDDSDWLN	
751	DAHVVCKQLS	CGWAINATGS	AHFEGGTGPI	WLDEINCNCK	ESHIWQCHSH	
801	GWGRHNCRHK	EDAGVICSEF	MSLRLLSENS	RETCAGRLEV	FYNGAWGSVG	
851	RNSMSPATVG	VVCRQLGCAD	RGDISPASSD	KTVSRHMWVD	NVQCPKGPDT	
901	LWQCPSPFWK	KRLASPSEET	WITCANKIRL	QEGNTNCSGR	VEIWWGGSWG	
951	TVCDDSWDLE	DAQVVCRLG	CGSALEAGKE	AAFGQGTGPI	WLNEVKCKGN	
1001	ETSLWDCPAR	SWGHSDCGHK	EDAAVTCSEI	AKSRESLHAT	GRSSFVALAI	
1051	FGVILLACLI	AFLIWTQKRR	QRQLSVFSG	GENSVHQIQY	REMNSCLKAD	
1101	ETDMLNPSGD	HSEVQ				

Sus CD163 v2 in pCRsusCD163v2 was liberated from pCR2.1 vector after restriction enzymes *Kpn* I and *Not* I digestion and gel purification. Recipient vector pCMV-script was also cut with the same restriction enzyme pair and allowed for directional cloning of susCD163v2 into the pCMV-script. After ligation of susCD163 v2 with pCMV-script, the ligated mixture was used to transform STBL 2 *E. coli* cells (Invitrogen). One transformant was found to contain the CD163 gene by restriction enzyme digestion analysis and was designed pCMV-script susCD163v2 clone#3.

Example 5: Preparation of a RSV promoter based expression system by direct ligation and transfection method.

A non-cloning based procedure to generate microgram quantities of linear DNA suitable for use in generating stable cell lines expressing CD163 from an RSV promoter was developed (figure 4). The procedure involves the isolation and ligation of two pieces of DNA, one containing the neomycin gene and RSV promoter cassette derived from pRSV-script, and the other containing the susCD163v2 coding sequence from pCMVsusCD163v2. Vector plasmid pRSV-Script was linearized with *Dra*III upstream of the neomycin gene, and bunted with the Klenow fragment of *E. coli* DNA polymerase. This plasmid was then digested with *Not*I immediately downstream of the RSV promoter. The pCMVsusCD163v2 clone was digested in the vector sequence downstream of the CD163 insert with *Dra*II, and blunted with Klenow fragment of DNA polymerase. The CD163 coding sequence was liberated from the vector with a *Not*I located immediately upstream of the CD163 coding sequence. For each plasmid digestion the appropriate fragments were purified from agarose gels. A large-scale ligation reaction was performed as follows. Approximately 20 µg of each

DNA fragment was incubated in a volume of 600 μ L with 15 units of T4 DNA ligase. The reaction was incubated at room temperature for 20 minutes, at which time an aliquot was removed and the reaction frozen on dry ice. Agarose gel analysis of the aliquot revealed that a significant amount of non-ligated DNA remained, so another
5 15 units of ligase was added and incubated for another 10 minutes at room temperature. Following ligation, a linear piece of DNA containing all of the appropriate elements was purified by agarose gel electrophoresis. Ligation of the two DNA fragments via the cohesive *Not* I termini resulted in the placement of the 5' sequences of the CD163 gene downstream of the RSV promoter, allowing for directed
10 expression of CD163 in mammalian cells. Once isolated, the purified DNA was used to transfect various mammalian cell lines.

Example 6: Cloning and characterization of human CD163 cDNA

Based on a known human CD163 cDNA sequence (GenBank Accession No. BC051281), a forward primer Hu5'Not (SEQ ID NO: 15)
15 (5'CACCGCGGCCGCGAAGTTATAAATCGCCACCATGAGCAAACTCAGAAT GG-3') and a reverse primer Hu3'Kpn (SEQ ID NO: 16)
(5'-TGCTCCGGTACCTAGTCCAGGTCTTCATCAAGGTATCTTA-3') were designed using the PrimerSelect program. Restriction sites for *Not*I and *Kpn*I (underlined) were incorporated into the 5' and 3' primers, respectively, to facilitate
20 cloning into expression vectors. The sequence CACC was added to the 5' end of the 5' primer to allow directional cloning into the pCDNA3.1D/V5/His/TOPO vector (Cat. No. K49001, Invitrogen, see figure 6). Human CD163 cDNAs were amplified from RNA extracted from the U937 cell line after stimulated with phorbol 12-myristate 13-acetate (100ng/ml) for 3 days. Total cellular RNA was prepared using
25 the RNeasy kit (Qiagen). RT-PCR reactions and sequencing methods were the same as described in Example 4. PCR products were separated on 0.8% SeaKem agarose gel and extracted from the gel using the GeneClean kit. PCR products were cloned directionally into the pCDNA3.1D/V5/His/TOPO vector following the manufacturer's instructions. Two clones with large inserts were sequenced. Sequencing and
30 sequence analysis methods were described in Example 4. A clone with a correct insert was designed "pCDNA3.1D-humCD163v2" and we have designated the sequence of the insert SEQ ID NO: 17

The CD163 open reading frame in pCDNA3.1D-humCD163v2 is 1121 residues in length (designated SEQ ID NO: 18 which encodes SEQ ID NO:19 disclosed below), and is 100% identical to Genbank Z22968 (a human CD163 cDNA of the same length). Our human CD163v2 sequence is also 100% identical to

5 Genbank BC051281 and Z22969 (splice variants of human CD163) except that 42 nonhomologous residues in the two Genbank sequences replace the seven carboxy-terminal residues of our sequence. This difference is due to the presence of an 83-nucleotide exon in BC051281 and Z22969, and the resulting frame shift at the 3' end of the exon. (Law, S.K., Micklem, K.J., Shaw, J.M., Zhang, X.P., Dong, Y., Willis,

10 A.C. and Mason, D.Y. (1993) A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. European Journal of Immunology 23 (9), 2320-2325).

SEQUENCE			ID NO
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 201 E C G S A V S F S G S S N F G E G S G P
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 221 I W F D D L I C N G N E S A L W N C K H
 661 atctggtttgatgatcttatatgaacggaaatgagtcagctctctggaactgcaaacat
 241 Q G W G K H N C D H A E D A G V I C S K
 721 caaggatgggaaagcataactgtgaccatgctgaggatgctggagtgatttgcctaaag
 261 G A D L S L R L V D G V T E C S G R L E
 781 ggagcagatctgagcctgagactggtagatggagtcactgaatgttcaggaagattagaa
 281 V R F Q G E W G T I C D D G W D S Y D A
 841 gtgagattccaaggggaatgggggacaatatgtgatgacggctgggacagttacgatgct
 301 A V A C K Q L G C P T A V T A I G R V N
 901 gctgtggcatgcaagcaactgggatgtccaactgccgtcacagcattggtcgagtaac
 321 A S K G F G H I W L D S V S C Q G H E P
 961 gccagtaagggatttggacacatctggcttgacagcgttctctgcccagggaacatgaacct
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 gctgtctggcaatgtaaacaccatgaatggggaagcattattgcaatcacaatgagat
 361 A G V T C S D G S D L E L R L R G G G S
 1081 gctggcgtgacatgttctgatggatcagatctggagctaagacttagaggtggaggcagc
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 cgctgtgctgggacagttgaggtggagattcagagactgttagggaaggtgtgtgacaga
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 ggctggggactgaaagaagctgatgtggtttgaggcagctgggatgtgactctgactc
 421 K T S Y Q V Y S K I Q A T N T W L F L S
 1261 aaaacatcttatcaagtgtactccaaatccaggcaacaaacacatggctgtttctaagt
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 agctgtaacggaaatgaaacttctcttgggactgcaagaactggcaatggggtggactt
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 acctgtgatcactatgaagaagccaaatctgctcagcccacaggaacccagactg
 481 V G G D I P C S G R V E V K H G D T W G
 1441 gttggaggggacattccctgttctggacgtgttgaagtgaagcatggtgacacgtggggc
 501 S I C D S D F S L E A A S V L C R E L Q
 1501 tccatctgtgattcggacttctcttgggaagctgccagcgttctatgcagggaattacag
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 tgtggacagttgtctctatctctgggggagctcactttggagagggaaatggacagatc
 541 W A E E F Q C E G H E S H L S L C P V A
 1621 tgggctgaagaattccagtggtgagggacatgagtcacatcttctactctgcccagtagca
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 ccccgccagaaaggaacttgtagccacagcagggatgttgagtagtctgctcaagatac
 581 T E I R L V N G K T P C E G R V E L K T
 1741 acagaaattcgcttggatgaatggcaagaccccggtgtgagggcagagtgagctcaaaacg
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 661 D C P V T A L G A S L C P S E Q V A S V
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 681 I C S G N Q Q T L S S C N S S L G P
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 2101 acaaggcctaccattccagaagaaagtgtgtggcctgcatagagagtggtcaacttcgc
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 2161 ctggtaaatggaggaggtcgctgtgtgggagagtagagatctatcatgagggtcctgg
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 ggcaccatctgtgatgacagctgggacctgagtgatgccacgtggttgcagacagctg
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 2341 atctggctggatgagatgaaatgcaatggaaaagaatcccgatttggcagtgccattca
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 821 F M S L R L T S E A S R E A C A G R L E
 2461 ttcattgtctctgagactgaccagtgagccagcagagaggcctgtgagggcgtctggaa
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 2581 ggtgtggtgtgcaggcagctgggctgtgcagacaaagggaaaatcaaccctgcatttta
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 2641 gacaaggccatgtccattcccatgtgggtggacaatgttcagtggtccaaaaggacctgac
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 2701 acgctgtggcagtgcccattcatctccatgggagaagagactggccagcccctcgaggag
 921 T W I T C D N K I R L Q E G P T S C S G
 2761 acctggatcacatgtgacaacaagataagacttcaggaaggacccacttctgttctgga
 941 R V E I W H G G S W G T V C D D S W D L
 2821 cgtgtggagatctggcatggaggttccctggggacagtggtgtgatgactcttgggacttg

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<pre> 1 MSKLRMVLLE DSGSADFRRH FVNLSPTTIT VVLLLSACFV TSSLGGTDKE 51 LRLVDGENKC SGRVEVKVQE EWGTVCNNGW SMEAVSVICN QLGCPAIIKA 101 PGWANSSAGS GRIWMDHVSC RGNESALWDC KHDGWGKHSN CTHQQDAGVT 151 CSDGSNLEMR LTRGGNMCSG RIEIKFQGRW GTVCDNFNI DHASVICRQL 201 ECGSAVSFSG SSNFGECSGP IWFDLLICNG NESALWNCKH QGWGKHNCBH 251 AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSYDA 301 AVACKQLGCP TAVTAIGRVN ASKGFQHIWL DSVSCQGHEP AVWQCKHHEW 351 GKHYCNHNEH AGVTCSDGSD LELRLRGGS RCAGTVEVEI QRLLGKVCDR 401 GWGLKEADV CRQLGCGSAL KTSYQVYSKI QATNTWLFLS SCNGNETSLW 451 DCKNWQWGL TCDHYEEAKI TCSAHREPRL VGGDIPCSGR VEVKHGDTWG 501 SICSDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEGNGQI WAEFFQCEGH 551 ESHLSLCPVA PRPEGTCSSHS RDVGVCSSRY TEIRLVNGKT PCEGRVELKT 601 LGAWGSLCNS HWDIEDAHLV CQQLKCGVAL STPGGARFGK GNGQIWRHMF 651 HCTGTBQHMG DCPVTALGAS LCPSEQVASV ICSGNQSQT LSSCNSSSLGP 701 TRPTTPEESA VACIESGQLR LVNNGGRCAG RVEIYHEGSW GTICDDSWDL 751 SDAHVVCRQL GCGEAINATG SAHFGEGTGP IWLDEMKNK KESRIWQCHS 801 HGWQQNCRH KEDAGVICSE FMSLRLTSEA SREACAGRL VFYNGAWGTV 851 GKSSMSETTV GVVCRQLGCA DKGKINPASI DKAMSI PMWV DNVQCPKGPD 901 TLWQCPSPW EKRLASPSEE TWITCDNKIR LQEGPSCSG RVEIWHGGSW 951 GTVCDSDWDL DDAQVVCQQL CGCPALKAFK EAEFGQGTGP IWLNEVKCKG 1001 NESSLWDPCA RRGHSECGH KEDAAVNCTD ISVQKTPQKA TTGRSSRQSS 1051 FIAVGILGVV LLAIFVALFF LTKRRQRQR LAVSSRGENT VHQIQYREMN 1101 SCLNADDLDL MNSSGGHSEP H </pre>	SEQ ID NO:19

Example 7: Cloning and characterization of murine CD163

Based on the murine CD163 sequence in GenBank (AF274883), a forward primer Mus-new5' (SEQ ID NO: 20) (5'-

- 5 CACCGCGGCCCGCCACACGGAGCCATCAAAATCATCAA-3') and a reverse primer Mus-new3' (SEQ ID NO:21) (5'-
- GGTACCGCGAACAAGCAAACCAATAGCAATATTGTTTAATTCCCTC-3') were designed using the PrimerSelect program. Restriction endonucleases sites for *NotI* and *KpnI* were included in 5' and 3' primers, respectively, to allow future cloning into other
- 10 expression vectors. Mouse peritoneal macrophages were harvested from mice 2 days after injecting thioglycollate medium into the peritoneal cavity. Total cellular RNA was prepared from peritoneal macrophages using the RNeasy kit. RT-PCR reactions and RT-PCR parameters were the same as described in Example 4, except the annealing
- 15 PCR product was purified on a 0.8% SeaKem agarose gel and directionally cloned into pCDNA3.1D/V5/His/TOPO according to the manufacturer's instructions. Several

clones with large inserts were identified for further analysis. A plasmid containing an insert (SEQ ID NO: 22) with a murine CD163 that encodes a protein of the same length as (1121 amino acids SEQ ID NO:24) and differs from Genbank AF274883 by only two amino acids (99.8% identity) was designated "pCDNA3.1D-murCD163v2".

- 5 Another plasmid, "pCDNA3.1D-murCD163v3 was generated which contained an insert (SEQ ID NO: 25) containing a murine CD163 coding sequence (SEQ ID NO: 26) which encodes a protein of 1159 amino acids in length (SEQ ID NO: 27). It differs from AF274883 by only 3 amino acids within the first 1107 residues (99.7% identity), but the sequences diverge completely beginning at residue 1108. This is due to an
- 10 insertion of 82 nucleotides in the cDNA, and a concomitant shift in reading frame downstream of the insertion. As a result, murine CD163v3 contains 52 amino acids at its carboxy-terminus that are not homologous to the 14 carboxy-terminal residues of murine CD163v2. These two alternative versions of "full length" murine CD163 are most likely splice variant of the same gene, as has been described for human CD163
- 15 (Law, S.K., Micklem, K.J., Shaw, J.M., Zhang, X.P., Dong, Y., Willis, A.C. and Mason, D.Y. (1993) A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. European Journal of Immunology 23 (9), 2320-2325).

SEQUENCE			ID NO
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	601 G A W G P L C S S H W D M E D A H V L C 1801 ggagcctgggggtccctctgcagttctcattgggacatggaagatgctcatgtcttatgt 621 Q Q L K C G V A Q S I P E G A H F G K G 1861 cagcagctgaagtgtgggttggcccaatctattccagaaggagcacattttgggaaagga 641 A G Q V W S H M F H C T G T E E H I G D 1921 gctgggtcaggtctggagtcacatgttccactgcactggaactgaggaacatataggagat 661 C L M T A L G A P T C S E G Q V A S V I 1981 tgcctcatgactgctctgggtgcccgcagctgttccgaaggacaggtggcctctgtcatc 681 C S G N Q S Q T L L P C S S L S P V Q T 2041 tgctcaggaaaccaatcccagacactattgccatgtagttcattgtctccagtcacaaca 701 T S S T I P K E S E V P C I A S G Q L R 2101 acaagctctacaattccaaaggagagtggaagttccctgcatagcaagtgccagcttcgc 721 L V G G G G R C A G R V E V Y H E G S W 2161 ttggtaggtggaggtgggtcgctgcgctggaagagtggaggtctaccacgagggctcttgg 741 G T V C D D N W D M T D A N V V C K Q L 2221 ggaccgtctgtgatgacaattgggatatgactgatgccaatgtggtgtgcaagcagctg 761 D C G V A I N A T G S A Y F G E G A G A 2281 gactgtggcgtggcaattaacgccactggctctgcttacttcggggaaggagcaggagct 781 I W L D E V I C T G K E S H I W Q C H S 2341 atctggctagacgaagtcatctgcactgggaaagagtctcatatttggcagtgccattca 801 H G W G R H N C R H K E D A G V I C S E 2401 catggctgggacgccataactgcaggcacaaagaagatgcaggtgttatctgctccgag 821 F M S L R L T N E A H K E N C T G R L E 2461 ttcattgtctctgaggtctgaccaacgaagcccacaaagaaaactgcacaggtcgcttgaa 841 V F Y N G T W G S I G S S N M S P T T V 2521 gtgtttttacatggtagctgggagctattggcagtagcaatgtctccaaccagctg 861 G V V C R Q L G C A D N G T V K P I P S 2581 ggggtggtgtgctgctgagctgggtgtgagacacgggactgtgaaaccatacttca 881 D K T P S R P M W V D R V Q C P K G V D 2641 gacaagacaccatccaggcccatgtgggttagatcgtgtgagtggtccaaaaggagttgac 901 T L W Q C P S S P W K Q R Q A S P S S Q 2701 actttgtggcagtgccctcgtcaccttggaaacagagacagggccagccctcctcccag 921 E S W I I C D N K I R L Q E G H T D C S 2761 gagtccctggatcatctgtgacaacaaaataagactccaggaaggccatacagactgttct 941 G R V E I W H K G S W G T V C D D S W D 2821 ggacgtgtggagatctggcacaaggttccctggggaacagtggtgtgatgactcctggat 961 L N D A K V V C K Q L G C G Q A V K A L 2881 cttaatgatgctaaaggtgtatgtaagcagttgggctgtggccaagctgtgaaggcacta 981 K E A A F G P G T G P I W L N E I K C R 2941 aaagaagcagcatttgggtccaggaactgggcccataatggctcaatgaaatgaatgtaga 1001 G N E S S L W D C P A K P W S H S D C G 3001 gggaatgagtcctccctgtgggattgtcctgccaacacgtggagtcacagcgactgtggg 1021 H K E D A S I Q C L P K M T S E S H H G 3061 cacaagaagatgcttccatccagtgctcccccataatgacttcagaatcacatcatggc 1041 T G H P T L T A L L V C G A I L L V L L 3121 acaggtcaccaccctcacggcactcttggttggtagccattctatttggtcctcctc 1061 I V F L L W T L K R R Q I Q R L T V S S 3181 attgtctcctcctgtggactctgaagcgacgacagattcagcgacttacagtttctcctca 1081 R G E V L I H Q V Q Y Q E M D S K A D D 3241 agaggagaggtcttgatcatcaagttcagtagcaagagatggattcaaaaggcgagatg 1101 L D L L K S S E N S N N S Y D F N D D G 3301 ctggacttgctgaaatcctcggaataattccaacaattcatatgattttaaagatgatgga 1121 L T S L S K Y L P I S G I K K G S F R G 3361 ctgacatctttgtctaaatatcttctctatttctggaattaaaaaggggtcattcagagggc 1141 T L R R K M I I Y N P L R L E F K K P 3421 aactgagaaggaaaatgataatttataatccactgaggttgagtttaagaagcct	
1 51 101 151 201 251 301 351 401 451 501 551 601 651 701 751 801 851 901 951 1001 1051 1101 1151	MGGHRMVLLG GAGSPGCKRF VHLGFFVAV SSLLSASAVT NAPGEMKKEL RLAGGENNCS GRVELKIHDK WGTVCNSGWS MNEVSVVCQQ LGCPSTIKAL GWANSSAGSG YIWMKVSC T GNESALWDCK HDGWGKHNC T HEKDAGVTCS DGSNLEMLRV NSAGHRCLGR VEIKFQGWG TVCDDNFSKD HASVICKQLG CGSAISFSGS AKLGAGSGPI WLDDLACNGN ESALWDCKHR GWGKHNCDAH EDVGVICLEG ADLSLRLVDG VSRCGRLEV RFQGEWGTVC DDNWDLRDAS VVKQLGCPT AISAIRVNA SEGSGQIWL NISCEGHEAT LWECKHQEWG KHYCHHREDA GVTCSGDADL ELRLVGGGSR CAGIVEVEIQ KLTGKMC SRG WTLADADVVC RQLGCGSALQ TQAKIYSKTG ATNTWLFPGS CNGNETTFWQ CKNWQWGLS CDNFEEAKVT CSGHREPLV GGEIPCSGRV EMKHGDVWGS VCDFDLSLEA ASVVCRELQC GTVVSILGGA HFGEHSGQIW GEEFQCSGDE SHLSLCSVAP PLDRCTHSR DVSVVC SRYI DIRLAGGESS CEGRVELKTL GAWGPLCSSH WDMEDAHVLC QQLKCGVAQS IPEGAHFGKG AGQVWSHMFH CTGTEEHIGD CLMTALGAPT CSEGQVASVI CSGNQSQTL PCSSLSPVQT TSSTIPKESE VPCIASGQLR LVGGGRCAG RVEVYHEGSW GTVCDNWDN TDANVCKQL DCGVAINATG SAYFGEAGAGA IWLDEVICTG KESHIWQCHS HGWGRHNCRH KEDAGVICSE FMSLRLTNEA HKENCTGRLE VFYNGTWGSI GSSNMSPTTV GVVCRLGCA DNGTVKPIPS DKTPSRPMWV DRVQCPKGV TLWQCPSSPW KQRQASPSQ ESWIICDNKI RLQEGHTDCS GRVELIWHKS WGTVCDDSWD LNDKVVCKQ LGCGQAVKAL KEAAGFGPTG PIWLNEIKCR GNESLWD C P AKPWSHSDCG HKEDASIQL PKMTSESHHG TGHPTLTALL VCGAILLVLL IVFLLWTLKR RQIQRLTVSS RGEVLIHQV YQEMDSKADD LDLLKSSENS NNSYDFNDDG LTSLSKYLPI SGIKKSFRG TLRRKMIYN PLRLEFKKP	ID NO:27

Example 8: Cloning and characterization of MARC-145 CD163

A forward primer 5'simianCD163 (SEQ ID NO: 28) (5'-
 CACCGGAATGAGCAAACCTCAGAATGG-3' based on human CD163) and a reverse
 5 primer HuCD163-3'Kpn (SEQ ID NO:29) (5'-
 TGCTCCGGTACCTAGTCCAGGTCTTCATCAAGGTATCTTA-3') were used to
 amplify CD163 cDNA from MARC-145 African Green Monkey kidney cells. Total
 cellular RNA was prepared from MARC-145 cells using the RNeasy kit. RT-PCR
 parameters were the same as described in Example 4. RT-PCR products were cloned
 10 directionally into the pCDNA3.1D/V5/His/TOPO vector according to the
 manufacturer's instruction. Several clones containing large inserts were analyzed.
 Clone #25 was designated "pCDNA3.1D-MARC-CD163v2". This novel CD163
 cDNA from MARC-145 cells is 1116 amino acids in length. When compared to the
 sequences in GenBank database, the MARC-145 CD163 amino acid sequence is 96.3%
 15 identical to human CD163 (Genbank Z22968), 84.7% identical to pig CD163 (Genbank
 AJ311716), and 73.9% identical to mouse CD163 (Genbank AF274883).

SEQUENCE			ID NO
atgagcaaac tcagaatggt gctacttgaa gactctggat ctgctgacgt cagaagacat	60	SEQ ID NO: 30	
tttgtcaact tgagtccttt cactattgct gtgggtcttac ttctccgtgc ctgttttgtc	120		
accagttctc ttggaggaac aaccaaggag ctgaggctag tggatggtga aaacaagtgt	180		
agtgggagag tggaagtga aatccaggag gagtggggaa cgggtgtgtaa taatggctgg	240		
agcatggaag cagtctctgt gatttgtaac cagctgggat gtccaactgc tatcaaagcc	300		
actggatggg ctaattccag tgcaggttct ggacgcattt ggatggatca tgtttcttgt	360		
cgtgggaatg agtcagctct ttgggactgc aaacatgatg gatggggaaa gcatagtaac	420		
tgtactcacc aacaagatgc tggagtgact tgctcagatg gatccgattt ggaaatgagg	480		
ctgacgaatg gagggaaatg gtgttctgga agaatagaga tcaaattcca aggacagtgg	540		
ggaacagtgt gtgatgataa cttcaacatc aatcatgcat ctgtggtttg taaacaactt	600		
gaatgtggaa gtgctgtcag tttctctggt tcagctaatt ttggagaagg ctctggacca	660		
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caaggatggg gaaagcataa ctgtgatcat gctgaggatg ctggagtgat ttgctcaaag	780		
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gtgagattcc aaggagaatg ggggacaata tgtgatgacg gctgggacag tcatgatgct	900		
gctgtggcat gcaagcaact gggatgtcca actgctatca ccgccattgg tcgagttaac	960		
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gcggtctggc aatgtaaaca ccatgaatgg ggaagcatt attgcaatca caatgaagat	1080		
gctggcgtaa catgttctga tggatcagat ctggagctaa gacttagagg tggaggcagc	1140		
cgtgtgctg ggacagttga ggtggagatt cagagactgt tagggaaggt gtgtgacaga	1200		
ggctggggac tgaaagaagc tgatgtgggt tgcaggcagc tgggatgtgg atctgcactc	1260		

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1 M S K L R M V L L E D S G S A D V R R H 1 atgagcaaacacagaatgggtgctacttgaagactctggatctgctgacgtcagaagacat 21 F V N L S P F T I A V V L L L R A C F V 61 tttgtcaacttgagtcccttcaactattgctgtggtcttacttctccgtgcctgtttgtc 41 T S S L G G T T K E L R L V D G E N K C 121 accagttctcttgagggaacaaccaaggagctgaggctagtggatgggtgaaaacaagtgt 61 S G R V E V K I Q E E W G T V C N N G W	SEQ ID NO: 31 and 32

181 agtgggagagtgggaagtgaataatccaggaggagtggggaacgggtgtgaataatggctgg
 81 S M E A V S V I C N Q L G C P T A I K A
 241 agcatggagcagtcctctgtgatttgaaccagctgggagtccaactgctatcaagcc
 101 T G W A N S S A G S G R I W M D H V S C
 301 actggatgggctaattccagtgacggttctggacgatttggatggatcatgtttctgt
 121 R G N E S A L W D C K H D G W G K H S N
 361 cgtgggaatgagtcagctctttgggactgcaaacatgatggatgggaaagcatagtaac
 141 C T H Q Q D A G V T C S D G S D L E M R
 421 tgtactaccaacaagatgctggagtacttgctcagatggatccgatttggaaatgagg
 161 L T N G G N M C S G R I E I K F Q G Q W
 481 ctgacgaatggagggaatatgttctggaagaatagagatcaaattccaaggacagtgg
 181 G T V C D D N F N I N H A S V V C K Q L
 541 ggaacagtggtgatgataacttcaacatcaatcatgcatctgtggttgaatacaactt
 201 E C G S A V S F S G S A N F G E G S G P
 601 gaattgtggaagtgtcagtttctctggttcagctaatttggagaaggctctggacca
 221 I W F D D L I C N G N E S A L W N C K H
 661 atctggtttgatgatcttatatgcaacggaatgagtcagctctctggaactgcaaacat
 241 Q G W G K H N C D H A E D A G V I C S K
 721 caaggatggggaagcataactgtgatcatgctgaggatgctggagtgttgcctcaaag
 261 G A D L S L R L V D G V T E C S G R L E
 781 ggagcagatctgagcctgagactggtagatggagtcactgaatgttcaggaagattagaa
 281 V R F Q G E W G T I C D D G W D S H D A
 841 gtgagattccaaggagaatgggggacaatatgtgatgacggctgggacagctcatgct
 301 A V A C K Q L G C P T A I T A I G R V N
 901 gctgtggcatgcaagcaactgggagtccaactgctatcacccgcatgtcgagttaac
 321 A S E G F G H I W L D S V S C Q G H E P
 961 gccagtggggtatttggacacatctggcttgacagtgttcttgcaggacatgaacct
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 gcggctcggcaatgtaaacacccatgaatggggaagcattattgcaatcacaatgaagat
 361 A G V T C S D G S D L E L R L R G G S
 1081 gctggcgtaacatgttctgatggatcagatctggagctaagacttagaggtggaggcagc
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 cgctgtgctgggacagttgaggtggagattcagagactgttagggaaggtgtgtgacaga
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 ggctggggactgaaagaagctgatgtggttgcaggcagctgggagtggatctgcactc
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 aaaacatcctatcaagtatactccaaaatccaggcaacaacatgtggtgtttctaagt
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 agctgtaacggaaatgaaacttctcttgggactgcaagaactggcaatggggtggactt
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 acctgtgatcactatgaagaagccaaaattacctgctcagccacaggaaccagactg
 481 V G G D I P C S G R V E V K H G D T W G
 1441 ttgggagagacattccctgttcttgacgcgttgagtgagtgagtgagtgagtgagtgag
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 tccgtctgtgattcggatttctctctggaagctgccagcgttctatgcagggaattacag
 521 C G T V V S I L G G A H F G E G N G Q I
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 1621 tgggctgaagaattccagtgtaggggacatgagtcctcctcactctgccagtagca
 561 P R P E G T C S H S R D V G V V C S R Y
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 1801 cttaatgcctggggtacccctctgcaactctcactgggacatagaagatgccacgttctt
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 tgccaacaacttaaatgtggagttgcccttctacccaggaggagcacatttggaaaa
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 1921 ggaatgggtcaggtctggaggcatatgttctactgcactgggactgagcagcacatggga
 661 D C P V T A L G A S L C P S G Q V A S V
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 681 I C S G N Q S Q T L S S C N S S S L G P
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 2101 acaaggcctaccattccagaagaaagtgtgtggcctgcatagagagtggcacttcgc
 721 L V N A G G G R C A G R V E I Y H E G S W
 2161 ttggttaattggagggtcgtgtgctgggagagttagagatttatcatgagggtcctgg
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 2221 ggcaccatctgtgatgacagctgggacctgagcagatgccacgtggtgtgacagagctg
 761 G C G E A I N A T G S A H F G E G T G P
 2281 ggctgtggagaggccattaatgccactggttctgctcatttggagaagggaacaggggcc
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 atctggctggatgagatgaaatgcaatggaaaagaatcccgatttggcagtgccattca
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 catggctgggggagcaaaactgcaggcacaaggaggatgcaggagtattctgctcagag
 821 F M S L R L T S E A S R E A C A G R L E
 2461 ttcatgtctctgagactgaccagtgaagccagcagagaggcctgtgcagggtcctagaa
 841 V F Y N G A W G S V G R S N M S E T T V
 2521 gttttttacaacggagcttggggcagtggttggcaggagtaacatgtctgaaaccactgtg
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 2581 ggtgtgtgtgcaggcagctgggctgtgcagacaaagggaatcaaccctgcattctta
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 2641 gacaaggccatgtccattcccatgtgggtggacaatgttcagtgctcaaaaggacctgac

901 T L W Q C P S S P W E K R L A R P S E E 2701 acgctgtggcagtgcccatcatctccatgggagaagagactggccaggccctcggaggag 921 T W I T C D N K M R L Q E G P T S C S G 2761 acctggatcacatgtgacaacaagatgagactacaagaaggacccacttcctgttctgga 941 R V E I W H G G S W G T V C D D S W D L 2821 cgtgtggagatctggcacggaggttcctgggggacagtgtgtgatgactcctgggacttg 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 aacgatgctcaggtggtgtgtcaacaactggctgtggtccagctttgaaagcattcaaa 981 E A E F G Q G T G P I W L N E V K C K G 2941 gaagcagagtttggtcaggggactggaccatattggctcaatgaagtgaagtgcagggg 1001 N E S S L W D C P A R R W G H S E C G H 3001 aatgagcttctcctgtgggattgtcctgccagacgctggggccacagtgaagtgtggacac 1021 K E D A A V N C T D I S T N K T P Q K A 3061 aaggaagagcgtgcagtgcaattgcacagatatttcaacgaacaaacccacaaaaagcc 1041 T T G Q S S L I A V G I L G V V L L V I 3121 acaacaggtcagtcacccattattgcagtcggaatccttggagttgttctcttggtcatt 1061 F V A L F L T Q K R R Q R Q R L T V S S 3181 ttcgtcgcatattcttgactcaaaagcgaagacagagacagcggttacagtttctcctca 1081 R G E N L V H Q I Q Y R E M N S C L N A 3241 agaggagagaacttagtccaccaaattcaataccgggagatgaattcttgctgaatgca 1101 D D L D L M N S S G G H S E A H 3301 gatgatctggacctaataatgaattcctcaggaggccattctgaggcacac	
1 MSKLRMVLL E DSGSADVRH FVNLSPTTIA VVLLLRACFV TSSLGGTTKE 51 LRLVDGENKC SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPATAKA 101 TGWANSSAGS GRIWMDHVSC RGNESALWDC KHDGWGKHSN CTHQQDAGVT 151 CSDGSDLEMR LTNGGNMCSG RIEIKFQGGW GTVCCDNFNI NHASVVCKQL 201 ECGSAVSFSG SANFGEESGP IWFDDLICNG NESALWNCKH QGWGKHNCNDH 251 AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA 301 AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQGHEP AVWQCKHHEW 351 GKHYCNHNEH AGVTCSDGSD LELRLRGSGS RCAGTVEVEI QRLLGKVCDR 401 GWGLKEADV CRQLGCGSAL KTSYQVYSKI QATNMWLFSL SCNGNETSLW 451 DCKNWQWGL TCDHYEEAKI TCSAHREPRL VGGDIPCSGR VEVKHGDTWG 501 SVCDSDFSLE AASVLCRELQ CGTVVVSILGG AHFGEENGQI WAEFFQCEGH 551 ESHLSLCPVA PRPEGTCSHS RDVGVCVCSRY TEIRLVNGKT PCEGRVELKT 601 LNAWGSLLCNS HWDIEDAHVL CQLKCGVAL STPGGAHFGK GNGQVWRHMF 651 HCTGTGEQHM DCPVTALGAS LCPSGQVASV ICSGNQSQTL SSCNSSSLGP 701 TRPTIPEESA VACIESGQLR LVNNGGRCAG RVEIYHEGSW GTICDDSDWL 751 SDAHVVCRLQ GCGEAINATG SAHFGEGTGP IWLDEMKNCG KESRIWQCHS 801 HGWQQQNCRH KEDAGVICSE FMSLRITSEA SREACAGRLE VFYNGAWGSV 851 GRSNMSETTV GVVCRQLGCA DKGKINPASL DKAMSI PMWV DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNKMRLQEGPTSCSG RVEIWHGGSW 951 GTVCDDSDWL NDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG 1001 NESSLWDCPA RRWGHSECGH KEDAAVNCTD ISTNKTPOKA TTGQSSLIIV 1051 GILGVVLLVI FVALFLTQKR RQRQLTVSS RGENLVHQIQ YREMNSCLNA 1101 DDLDMNSSG GHSEAH	SEQ ID NO: 32

Example 9. Cloning and characterization of simian CD163 from Vero cells.

- A forward primer 5'simianCD163 (SEQ ID NO: 28) (5'-
CACCGGAATGAGCAAACCTCAGAATGG-3' based on human CD163) and a reverse
primer HuCD163-3'Kpn (SEQ ID NO:29) (5'-
5 TGCTCCGGTACCTAGTCCAGGTCTTCATCAAGGTATCTTA-3') were used to
amplify CD163 cDNA from Vero cells. Total cellular RNA was prepared from Vero
cells using the RNeasy kit. RT-PCR parameters were the same as described in Example
4. RT-PCR products were cloned directionally into the pCDNA3.1D/V5/His/TOPO
vector according to the manufacturer's instruction. Eight clones containing large inserts
10 were sequenced, and six discreet splicing patterns were found. These patterns are
depicted graphically in Figure 17.

The six splicing variants differ in the presence or absence of three exons,
designated E6, E105, and E83. Omissions of E6 or E105 do not change the reading

frame, whereas omission of E83 does. Patterns similar to v2 and/or v3 were also seen in porcine, murine, human, and MARC-145 monkey cells. Patterns v4 and v5 lack the 105-nucleotide exon that encodes the hydrophobic transmembrane region. These cDNAs were unable to render BHK cells permissive to PRRSV infection in a transient transfection assay, probably because the CD163 is secreted rather than remaining membrane bound. Although CD163 molecules lacking a transmembrane region appear to be non-functional as cellular permissivity factors, it is possible that they may have utility either in direct virus neutralization (similar to neutralizing antibodies), or as an immunogen for the induction of anti-CD163 antibodies which would block viral infection in the host animal.

The longest splice variant, v7, contains all three of the exons E6, E105, and E83. This novel CD163 cDNA from Vero cells encodes a polypeptide that is 1153 amino acids in length. When compared to the sequences in Genbank database, the Vero CD163v7 amino acid sequence is 95.4% identical to human CD163 (Genbank Z22968), 83.7% identical to pig CD163 (Genbank AJ311716), and 72.1% identical to mouse CD163 (Genbank AF274883). The nucleotide and amino acid sequences of the six splice variants found in Vero cells are provided below (SEQ ID NOS:33-44).

SEQUENCE	ID NO
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACCTCAGAATGGTGCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTGTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGAAGTGAATAACCAAGGAGTGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTGTAAACAGCTGGGATGTCCAAGTCTATCAAAGCC 101 T G W A N S S A G S G R I W M D H V S C 301 ACTGGATGGGCTAATTCCAGTGCAGGTCTGGACGCATTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H S N 361 CGTGGGAATGAGTCAGCTCTTTGGGACTGCAACATGATGGATGGGGAAGCATAGTAAC 141 C T H Q Q D A G V T C S D G S D L E M R 421 TGTACTCACCACAAGATGCTGGAGTAAGTCTGCTCAGATGGATCCGATTGGAAATGAGG 161 L T N G G N M C S G R I E I K F Q G Q W 481 CTGACGAATGGAGGAATATGTGTTCTGGAAGAATAGAGATCAAATTCCAAGGACAGTGG 181 G T V C D D N F N I N H A S V V C K Q L 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAACAACCTT 201 E C G S A V S F S G S A N F G E G S G P 601 GAATGTGGAAGTGTCTGTCAGTTTCTCTGGTTCAGCTAATTTTGGAGAAGGCTCTGGACCA 221 I W F D D L I C N G N E S A L W N C K H 661 ATCTGGTTTGATGATCTTATATGCAACGGAATGAGTCTCTGGAAGTCAAACAT	SEQ ID NO:33 and 34

241 Q G W G K H N C D H A E D A G V I C S K
 721 CAAGGATGGGAAAAGCATAAAGTGTGATCATGCTGAGGATGCTGGAGTGATTGCTCAAAG
 261 G A D L S L R L V D G V T E C S G R L E
 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAAGGAAGATTAGAA
 281 V R F Q G E W G T I C D D G W D S H D A
 841 GTGAGATTCCAAGGAGAATGGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT
 301 A V A C K Q L G C P T A I T A I G R V N
 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCGCCATTGGTCGAGTTAAC
 321 A S E G F G H I W L D S V S C Q G H E P
 961 GCCAGTGAGGGATTGGACACATCTGGCTTGACAGTGTTTCTTGCCAGGGACATGAACCT
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAGCATTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S G L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGGTCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATTGAGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGACTGAAAGAAGCTGATGTGGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAAATCCAGGCAACAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAACGGAATGAAACTTCTCTTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCACAGGGAAACCCAGACTG
 481 V G G D I P C S G R V E V K H G D T W G
 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTCCGATTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
 541 W T E E F Q C E G H E S H L S L C P V A
 1621 TGGACTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTTCACTCTGCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCCAGCCAGAAAGGAACCTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCCTGTAAGTCTGCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S R N S S S L G P
 2041 ATTTGCTCAGGAAACCAGTCCCAAACACTGTCCTCGCGCAATTCATCATCTCTGGGCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCAGAAAGAAAGTGTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGGTGCTGTGCTGGGAGAGTAGAGATTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGCAGACAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S

2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGATCCCGCATTGTCAGTGCCATTCA 801 H G W G Q Q N C R H K E D A G V I C S E 2401 CATGGCTGGGGGAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG 821 F M S L R L T S E A S R E A C A G R L E 2461 TTCATGTCTCTGAGACTGACCAGTGAAGCCAGCAGAGAGGCTGTGCAGGGCGTCTAGAA 841 V F Y N G A W G S V G R S N M S E T T V 2521 GTTTTTCACACGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG 861 G V V C R Q L G C A D K G K I N S A S L 2581 GGTGTAGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAAATCAACTCTGCATCTTTA 881 D K A M S I P M W V D N V Q C P K G P D 2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTTCACTGTCCAAAAGGACCTGAC 901 T L W Q C P S S P W E K R L A R P S E E 2701 ACGCTGTGGCAGTGGCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG 921 T W I T C D N K M R L Q E G P T S C S G 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCTGTCTGGA 941 R V E I W H G G S W G T V C D D S W D L 2821 CGTGTGGAGATCTGGCAGGAGGTTCTTGGGGGACAGTGTGTGATGACTCTGGGACTTG 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGGCTGTGGTCCAGCTTTGAAAGCATTCAAA 981 E A E F G Q G T G P I W L N E V K C E G 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAGAGGG 1001 N E S S L W D C P A R R W G H S E C G H 3001 AATGAGTCTTCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC 1021 K E D A A V N C T D I S T R K T P Q K A 3061 AAGGAAGACGCTGCAGTGAATTGCACAGATATTTCAACGCGCAAAACCCACAAAAGCC 1041 T T G Q S S L I A V G I L G V V L L A I 3121 ACAACAGGTCACTCATCCCTTATTGCAGTCGGAATCCTTGGAGTGTCTTCTTGGCCATT 1061 F V A L F L T Q K R R Q R Q R L T V S S 3181 TTCGTCGCATTATTCTTGAAGTCAAAAGCGAAGACAGAGACAGCGGCTTACAGTTTCTCTCA 1081 R G E N L V H Q I Q Y R E M N S C L N A 3241 AGAGGAGAGAACTTAGTCCACCAAAATTCAATACCGGGAGATGAATTCTTGCCTGAATGCA 1101 D D L D L M N S S G G H S E A H 3301 GATGATCTGGACCTAATGAATTCTCTCAGGAGGCCATTCTGAGGCACAC	
1 MSKLRMVLE DSGSADVRRH FVNLSPTTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPTAIIA TGWANSSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQODAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGWQ 181 GTVCDNFNI NHASVVCQQL ECGSAVSFSG SANFGEESGP IWFDLLICNG NESALWNCKH 241 QGWGKHNCND AEDAGVICSK GADLSLRLLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA 301 AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQGHPE AVWQCKHHEW GKHYCNHNED 361 AGVTCSDGSG LELRLRGSGS RCAGTVEVEI QRLLGKVCDR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEEAKI TCSAHREPR 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEENGQI 541 WTEEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCVSRV TEIRLVNGKT PCEGRVELKT 601 LNAWGSLSNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTGEQHM 661 DCPVTALGAS LCPSGQVASV ICSGNQSQT LSSRNSSSLGP TRPTIPEESA VACIESGQLR 721 LVNNGGRCAG RVEIYHEGSW GTICDDSDWL SDAHVVCRQL GCGEAINATG SAHFGEETGP 781 IWLDEMCKNG KESRIWQCHS HGWGQONCRH KEDAGVICSE FMSLRILTSEA SREACAGRL 841 VFYNGAWGVS GRSNMSETTV GVVCRQLGCA DKGKINSASL DKAMSIPMW DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNKM LQEGPTSCSG RVEIWHGGSW GTVCDDSDWL 961 NDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCEG NESSLWDCA RRWGHSECGH 1021 KEDAAVNCTD ISTRKTPQKA TTGQSSLIIV GILGVLLAI FVALFLTQKR RQRQLTVSS 1081 RGENLVHQIQ YREMNSCLNA DDLMLNSSG GHSEAH	SEQ ID NO:34
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACTCAGAAATGGTGTCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAAGTGAATCCAGGAGAGTGGGAACGGTGTGTAATAATGGCTGG	SEQ ID NO:35 and 36

81 S M E A V S V I C N Q L G C P T A I K A
 241 AGCATGGAAGCAGTCTCTGTGATTGTAAACAGCTGGGATGTCCAAGTCTATCAAAGCC
 101 T G W A N S S A G S G R I W M D H V S C
 301 ACTGGATGGGCTAATTCAGTGCAGGTTCTGGACGCATTGGATGGATCATGTTTCTGT
 121 R G N E S A L W D C K H D G W G K H S N
 361 CGTGGGAATGAGTCAGCTCTTGGGACTGCAACATGATGGATGGGAAAGCATAGTAAC
 141 C T H Q Q D A G V T C S D G S D L E M R
 421 TGTACTACCAACAGATGCTGGAGTAAGTCTGCTCAGATGGATCCGATTGGAAATGAGG
 161 L T N G G N M C S G R I E I K F Q G Q W
 481 CTGACGAATGGAGGAATATGTGTCTGGAAGAATAGAGATCAAATCCAAGGACAGTGG
 181 G T V C D D N F N I N H A S V V C K Q L
 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAAACAATT
 201 E C G S A V S F S G S A N F G E G S G P
 601 GAATGTGGAAGTGTCTGTCAGTTTCTGTGTTTCTGCTAATTTGGAGAAGGCTCTGGACCA
 221 I W F D D L I C N G N E S A L W N C K H
 661 ATCTGGTTTGTATGATCTTATATGCAACGGAAATGAGTCAGCTCTCTGGAATGCAACAT
 241 Q G W G K H N C D H A E D A G V I C S K
 721 CAAGGATGGGAAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTGCTCAAAG
 261 G A D L S L R L V D G V T E C S G R L E
 781 GGAGCAGATCTGAGCTGAGACTGGTAGATGGAGTCACTGAATGTTGAGGAAGATTAGAA
 281 V R F Q G E W G T I C D D G W D S H D A
 841 GTGAGATTCCAAGGAGAATGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT
 301 A V A C K Q L G C P T A I T A I G R V N
 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCAGCATTGGTCGAGTTAAC
 321 A S E G F G H I W L D S V S C Q G H E P
 961 GCCAGTGAGGGATTGGACACATCTGGCTTGACAGTGTCTTGGCAGGGACATGACCTT
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAGCATTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S D L E L R L R G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGAGATTGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGACTGAAAGAAGCTGATGTGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCTTATCAAGTATACTCCAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAAACGGAATGAAACTTCTCTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAATACCTGCTCAGCCCACAGGGAACCCAGACTG
 481 V G G D I P C S G R V E V K H G D T W G
 1441 GTTGAGGAGACATTCCTGTCTGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTCGGATTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCTCTCTATCTGCGGGGAGCTCACTTGGAGAGGGAATGGACAGATC
 541 W T E E F Q C E G H E S H L S L C P V A
 1621 TGGACTGAAGAAATCCAGTGTGAGGGACATGAGTCCCATCTTCACTCTGCCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCC GCCAGAACTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAAACAATTAAATGTGGAGTTGCCCTTTTACCCAGGAGGACACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCTGTAACTGCTCTGGGTGCTTCACTATGTCTTTCAGGGCAAGTGGCCTGTGA
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 ATTTGCTCAGGAAACAGTCCCAAACACTGTCTCTGCAATTCACTCATCTCTGGGCCCA
 701 T R P E T I P E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCCAGAAGAAAGTGTGTGGCCTGCATAGAGAGTGGTCAACTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGTCTGTGCTGGGAGAGTAGAGATTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGCAGACAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 ATCTGGCTGGATGAGATGAATGCAATGGAAGAAATCCCGCATTGGCAGTGCCATTCA
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 CATGGCTGGGGGACAAAAGTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG
 821 F M S L R L T S E A S R E A C A G R L E
 2461 TTCATGTCTCTGAGACTGACCAGTGAAGCCAGCAGAGAGGCTGTGCAGGGCGTCTAGAA
 841 V F Y N G A W G S V G R S N M S E T T V
 2521 GTTTTTTACAACGGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG
 861 G V V C R Q L G C A D K G K I N S A S L
 2581 GGTGTAGTGTGACGAGCTGGGCTGTGACAGCAAAGGGAAAATCAACTCTGCATCTTTA
 881 D K A M S I P M W V D N V Q C P K G P D
 2641 GACAAGGCCATGTCCATTCCATGTGGGTGGACAATGTTCACTGTCCAAAGGACCTGAC
 901 T L W Q C P S S P W E K R L A R P S E E

2701 ACGCTGTGGCAGTCCCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG 921 T W I T C D N K M R L Q E G P T S C S G 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAGAAGGACCCACTTCTGTCTGGA 941 R V E I W H G G S W G T V C D D S W D L 2821 CGTGTGGAGATCTGGCAGGAGGTTCTTGGGGGACAGTGTGTGATGACTCCTGGGACTTG 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTGAAGCATTCAAA 981 E A E F G Q G T G P I W L N E V K C K G 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCATATGGCTCAATGAAGTGAAGTGCAAGGG 1001 N E S S L W D C P A R R W G H S E C G H 3001 AATGAGTCTTCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGTGTGGACAC 1021 K E D A A V N C T D I S T R K T P Q K A 3061 AAGGAAGACGCTGCAGTGAATTGCACAGATATTTCAACGCGCAAAACCCACAAAAAGCC 1041 T T G Q S S L I A V G I L G V V L L A I 3121 ACAACAGGTCAAGTCATCCCTTATTGCAGTCGGAATCCTTGGAGTTGTTCTTCTTGGCCATT 1061 F V A L F L T Q K R R Q R Q R L T V S S 3181 TTGCTCGCATTATCTTGAAGCGAAGACAGAGACAGCGGCTTACAGTTTCTCTCA 1081 R G E N L V H Q I Q Y R E M N S C L N A 3241 AGAGGAGAGAACCTTAGTCCACCAATTAATACCGGAGATGAATCTTGCCTGAATGCA 1101 D D L D L M N S S E N S N E S A D F N A 3301 GATGATCTGGACCTAATGAATTCCTCAGAAAATTCGAATGAGTCAGCTGATTTCAATGCT 1121 A E L I S V S K F L P I S G M E K E A I 3361 GCTGAACCTAATTTCTGTGTCTAAATTTCTTCTTATTTCTGGAATGAAAAGGAGGCCATT 1141 L R H T E K E N G N L 3421 CTGAGGCACACTGAAAAGGAAAATGGGAATTTA	
1 MSKLRMVLE DSGSADVRH FVNLSPTTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPAIIKA TGWANSSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGGW 181 GTVCDNFNI NHASVVCQQL ECGSAVSFSG SANFGEESGP IWFDLICNG NESALWNCKH 241 QGWGKHNCDE AEDAGVICSK GADLSRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA 301 AVACQQLGCP TAITAIGRVN ASEGFHIIWL DSVSCQGHPE AVWQCKHHEW GKHYCNHND 361 AGVTCSDGSD LELRLRGSGS RCAGTVEVEI QRLGKVCDR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEEAKI TCSAHREPR 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEENGQI 541 WTEEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCSTRY TEIRLVNGKT PCEGRVELKT 601 LNAWGSCLNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTQHM 661 DCPVTALGAS LCPSGQVASV ICSGNQSQTLL SSCNSSLGTP TRPTIPEESA VACIESGQLR 721 LVNGGGRACG RVEIYHEGSW GTICDDSWDL SDAHVVCRL GCGEAINATG SAHFEGGTGP 781 IWLDEMKNCG KESRIWQCHS HGWGQQNCRH KEDAGVICSE FMSLRLTSEA SREACAGRL 841 VFYNGAWGSV GRSNMSETTV GVVCRQLGCA DKGKINSASL DKAMSIPMWV DNVCQPKGPD 901 TLWQCPSSPW EKRLARPSEE TWITCDNKM LQEGPTSCSG RVEIWHGGSW GTVCDSDWDL 961 NDAQVVCQQL CGCPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWDPCA RRWGHSECGH 1021 KEDAAVNCTD ISTRKTPQKA TTGQSSLIIV GILGVLLAI FVALFLTQKR RQRQLTVSS 1081 RGENLVHQIQ YREMNSCLNA DDLDMNSSE NSNESADFNA AELISVSKFL PISGMEKEAI 1141 LRHTEKENGN L	SEQ ID NO:36
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACCTCAGAATGGTGCTACTTGAAGACTCTGGATCTGTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGAAGTGAAAATCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGAAGCAGTCTCTGTGATTGTGAACCAAGCTGGGATGTCCAAGTCTATCAAGCC 101 T G W A N S S A G S G R I W M D H V S C 301 ACTGGATGGGCTAATTCAGTGCAGGTTCTGGACGCATTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H S N 361 CGTGGGAATGAGTCAGTCTTTGGGACTGCAACATGATGGATGGGGAAGCATAGTAAC 141 C T H Q Q D A G V T C S D G S D L E M R 421 TGTAATCAACAAAGATGCTGGAGTAAGTGTCTCAGATGGATCCGATTGGAAATGAGG 161 L T N G G N M C S G R I E I K F Q G Q W 481 CTGACGAATGGAGGGAATATGTGTTCTGGAAGAATAGAGATCAAATTCAGGACAGTGG 181 G T V C D D N F N I N H A S V V C K Q L 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAAACAATT 201 E C G S A V S F S G S A N F G E G S G P 601 GAATGTGGAAGTGTGTGATGTTTCTGTGGTTCAGCTAATTTTGGAGAAGGCTCTGGACCA 221 I W F D D L I C N G N E S A L W N C K H 661 ATCTGGTTTGATGATCTTATATGCAACGGAATGAGTCAGCTCTCTGGAAGTCAACAT	SEQ ID NO:37 and 38

241 Q G W G K H N C D H A E D A G V I C S K
 721 CAAGGATGGGAAAGCATAAAGTGTGATCATGCTGAGGATGCTGGAGTGATTGCTCAAAG
 261 G A D L S L R L V D G V T E C S G R L E
 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAGGAAGATTAGAA
 281 V R F Q G E W G T I C D D G W D S H D A
 841 GTGAGATTCCAAGGAGAATGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT
 301 A V A C K Q L G C P T A I T A I G R V N
 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCGCCATTGGTCGAGTTAAC
 321 A S E G F G H I W L D S V S C Q G H E P
 961 GCCAGTGAGGGATTGGACACATCTGGCTTGACAGTGTCTTCCAGGGACATGAACCT
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAGCATTTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S D L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATTTCAGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGGACTGAAAGAAGCTGATGTGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAACGGAATGAAACTTCTCTTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCACAGGGAACCCAGACTG
 481 V G G D I P C S G R V E V K H G D T W G
 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTCGGATTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGAGCTCACTTTGGAGAGGGAATGGACAGATC
 541 W T E E F Q C E G H E S H L S L C P V A
 1621 TGGACTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTTCACTCTGCCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCCAGCCAGGAAGGAACCTTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCCTGTAAGTCTCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 ATTTGCTCAGGAAACAGTCCCAAACACTGTCCTCGTGCAATTCATCATCTCTGGGCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCAGAGAAAGTGCTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGGTGCTGTGTGCTGGGAGAGTAGAGATTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGCAGACAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S

2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAATCCCGCATTTGGCAGTGCCATTCA 801 H G W G Q Q N C R H K E D A G V I C S E 2401 CATGGCTGGGGGAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG 821 F M S L R L T S E A S R E A C A G R L E 2461 TTCATGTCTCTGAGACTGACCACTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA 841 V F Y N G A W G S V G R S N M S E T T V 2521 GTTTTTTACAACGAGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG 861 G V V C R Q L G C A D K G K I N S A S L 2581 GGTGTAGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAAATCAACTCTGCATCTTTA 881 D K A M S I P M W V D N V Q C P K G P D 2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTTCACTGTCCAAAAGGACCTGAC 901 T L W Q C P S S P W E K R L A R P S E E 2701 ACCTGTGGCAGTGCCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG 921 T W I T C D N K M R L Q E G P T S C S G 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCTGTCTGGA 941 R V E I W H G G S W G T V C D D S W D L 2821 CGTGTGGAGATCTGGCAGGAGGTTCTGGGGGACAGTGTGTGATGACTCTGGGACTTG 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA 981 E A E F G Q G T G P I W L N E V K C K G 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG 1001 N E S S L W D C P A R R W G H S E C G H 3001 AATGAGTCTTCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC 1021 K E D A A V N C T D I S T R K T P Q K A 3061 AAGGAAGACGCTGCGGTGAATTGCACAGATATTTCAACGCGCAAAACCCACAAAAAGCC 1041 T T V S S R G E N L V H Q I Q Y R E M N 3121 ACAACGTTTTCTCAAGAGGAGAGAACTTAGTCCACCAAATTCAATACCGGGAGATGAAT 1061 S C L N A D D L N L M N S S G G H S E A 3181 TCTTGCCTGAATGCAGATGATCTGAACCTAATGAATTCTCAGGAGGCCATTCTGAGGCA 1081 H * K G K W E F I T Q 3241 CACTGAAAAGGAAAATGGGAATTTATAACCCAG	
1 MSKLRLMVLLE DSGSADVRH FVNLSPFTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPAIIKA TGWANSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGGW 181 GTVCDNDFNI NHASVVCQQL ECGSAVSFSG SANFGEHSGP IWFDDLICNG NESALWNCKH 241 QGWGKHNCNH AEDAGVICS KADLSLRLVD GVTECSGRLE VRFOGEWGTI CDDGWDSDHA 301 AVACKQLGCP TAITAIGRVN ASEGFHGIWL DSVSCQGHPE AVWQCKHHEW GKHYCNHNE 361 AGVTCSDGSD LELRLRGGGS RCAGTVEVEI QRLLGKVCDR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEEAKI TCSAHREPR 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSVILGG AHFGEHNGQI 541 WTEEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCVSRV TEIRLVNGKT PCEGRVELKT 601 LNAWGLSCLNS HWDIEDAHVL CQQLKCGVAL STPGGAHFGK GNGQVVRHMF HCTGTGQHM 661 DCPVTALGAS LCPSGQVASV ICSGNQSQTL SSCNSSSLGP TRPTIPEESA VACIESGQLR 721 LVNGGGRCAG RVEIYHEGSW GTICDDSDWL SDAHVVCRQL CGGEAINATG SAHFGEHNGQI 781 IWLDEMKNCG KESRIWQCHS HGWGQONCRH KEDAGVICSE FMSLRLTSEA SREACAGRLE 841 VFYNGAWGSP GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNKM LQEGPTSCSG RVEIWHGGSW GTVCDSDWDL 961 NDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWDCA RRGHSECGH 1021 KEDAAVNCTD ISTRKTPQKA TTVSSRGENL VHQIYREMN SCLNADDLNL MNSSGGHSEA 1081 H	SEQ ID NO:38
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACTCaGAATGGTGTCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGTGTGGTCTTACTTCTCCGTGCCTGTTTTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAGTGAAAATCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTTGTAACCAGCTGGGATGTCCAACGCTATCAAAGCC	SEQ ID NO:39 and 40

101 T G W A N S S A G S G R I W M D H V S C
 301 ACTGGATGGGCTAATTCAGTGCAGGTTCTGGACGCATTGGATGGATCATGTTTCTTGT
 121 R G N E S A L W D C K H D G W G K H S N
 361 CGTGGGAATGAGTCAGCTCTTTGGGACTGCAAACATGATGGATGGGAAAGCATAGTAAC
 141 C T H Q Q D A G V T C S D G S D L E M R
 421 TGTACTCACCAACAAGATGCTGGAGTGACTTGCTCAGATGGATCCGATTTGGAAATGAGG
 161 L T N G G N M C S G R I E I K F Q G Q W
 481 CTGACGAATGGAGGGAATATGTGTTCTGGAAGAATAGAGATCAAATCCAAGGACAGTGG
 181 G T V C D D N F N V N H A S V V C K Q L
 541 GGAACAGTGTGTGATGATAACTTCAACGTCAATCATGCATCTGTGGTTTGTAAACAACCTT
 201 E C G S A V S F S G S A N F G E G S G P
 601 GAATGTGGAAGTCTGTCTAGTTTCTCTGGTTCAGCTAATTTTGGAGAAGGCTCTGGACCA
 221 I W F D D L I C N G N E S A L W N C K H
 661 ATCTGGTTTGATGATCTTATATGCAACGGAATGAGTCAGCTCTCTGGAAGTGCACAT
 241 Q G W G K H N C D H A E D A G V I C S K
 721 CAAGGATGGGGAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTGCTCAAAG
 261 G A D L S L R L V D G V T E C S G R L E
 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTGAGGAAGATTAGAA
 281 V R F Q G E W G T I C D D G W D S H D A
 841 GTGAGATTCCAAGGAGAATGGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT
 301 A V A C K Q L G C P T A I T A I G R V N
 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCGCCATTGGTCGAGTTAAC
 321 A S E G F G H I W L D S V S C Q G H E P
 961 GCCAGTGAGGGATTGGACACATCTGGCTTGACAGTGTCTTCCAGGGACATGAACCT
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAAGCATTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S D L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATCAGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGGACTGAAAGAAGCTGATGTGGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAAACGGAATGAACTTCTCTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCCCAGGGAACCCAGACTG
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 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
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 1501 TCCGTCTGTGATTCCGATTTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
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 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
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 1621 TGGGCTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTCACTCTGCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
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 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G

1921	GGAAATGGTTCAGGTCCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA	
661	D C P V T A L G A S L C P S G Q V A S V	
1981	GATTGTCTGTAACTGCTCTGGGTGCTTCACTATGTCTTCAGGGCAAGTGGCCTCTGTGA	
681	I C S G N Q S Q T L S S C N S S S L G P	
2041	ATTTGCTCAGGAAACCACTGCCAAACACTGTCCTCGTGCAATTCATCATCTCTGGGCCCA	
701	T R P T I P E E S A V A C I E S G Q L R	
2101	ACAAGGCCTACCATTCAGAAAGAAAGTGTGTGGCTGCATAGAGAGTGGTCAACTTCGC	
721	L V N G G G R C A G R V E I Y H E G S W	
2161	TTGGTAAATGGAGGAGTCTGTGTGGGAGAGTAGAGATTATCATGAGGGCTCTGTG	
741	G T I C D D S W D L S D A H V V C R Q L	
2221	GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGCAGACAGCTG	
761	G C G E A I N A T G S A H F G E G T G P	
2281	GGCTGTGGAGAGGCCATTAATGCCACTGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC	
781	I W L D E M K C N G K E S R I W Q C H S	
2341	ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAAATCCCGTATTTGGCAGTGCCATTCA	
801	H G W G Q Q N C R H K E D A G V I C S E	
2401	CATGGCTGGGGGAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG	
821	F M S L R L T S E A S R E A C A G R L E	
2461	TTCATGTCTCTGAGACTGACCAGTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA	
841	V F Y N G A W G S V G R S N M S E T T V	
2521	GTTTTTTTACAACGGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG	
861	G V V C R Q L G C A D K G K I N S A S L	
2581	GGTGTGGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAATCAACTCTGCATCTTTA	
881	D K A M S I P M W V D N V Q C P K G P D	
2641	GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTTCACTGTCCAAAAGGACCTGAC	
901	T L W Q C P S S P W E K R L A R P S E E	
2701	ACGCTGTGGCAGTGCCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG	
921	T W I T C D N K M R L Q E G P T S C S G	
2761	ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCTGTCTTGA	
941	R V E I W H G G S W G T V C D D S W D L	
2821	CGTGTGGAGATCTGGCACGGAGGTTCTGGGGGACAGTGTGTGATGACTCCTGGGACTTG	
961	N D A Q V V C Q Q L G C G P A L K A F K	
2881	AACGATGCTCAGGTGGTGTGTCAACAACCTTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA	
981	E A E F G Q G T G P I W L N E V K C K G	
2941	GAAGCAGAGTTTGGTCAAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG	
1001	N E S S L W D C P A R R W G H S E C G H	
3001	AATGAGTCTTCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC	
1021	K E D A A V N C T A Q K I S T H K T P Q	
3061	AAGGAAGACGCTGCAGTGAATTGCACAGCACAAAAAATTTCAACGCACAAAACCCACAA	
1041	K A T T V S S R G E N L V H Q I Q Y R E	
3121	AAAGCCACAACAGTTTCTCAAGAGGAGAGAAGCTTAGTCCACCAAATTAATACCGGGAG	
1061	M N S C L N A D D L D L M N S S G G H S	
3181	ATGAATTCTTGCTGAATGCAGATGATCTGGACCTAATGAATTCCTCAGGAGGCCATTCT	
1081	E A H * K G K W E F I T Q	
3241	GAGGCACACTGAAAAGGAAATGGGAATTTATAACCCAG	
1	MSKLRLVLE DSGSADVRH FVNLSPPTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC	SEQ ID NO:40
61	SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPTAIIA TGWANSSAGS GRIWMDHVSC	
121	RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGGW	
181	GTVCDDNFNV NHASVVCQQL ECGSAVSFSG SANFGBGSGP IWFDLLICNG NESALWNCKH	
241	QGWGKHNCBH AEDAGVICS KADLSRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA	
301	AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQGHPE AVWQCKHHEW GKHYCNHNE	
361	AGVTCSDGSD LELRLRGGGS RCAGTVEVEI QRLLGKVCDR GWGLKEADV CQRLGCGSAL	
421	KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEEAKI TCSAHREPL	
481	VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEGNGQI	
541	WAEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCVSRV TEIRLVNGKT PCEGRVELKT	
601	LNAWGLSLNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTQHM	
661	DCPVTALGAS LCPSGQVASV ICSGNQSQTL SSCNSSSLGP TRPTIPEESA VACIESGQLR	
721	LVNGGGRCAG RVEIYHEGSW GTICDDSDWL SDAHVVCRL GCGEAINATG SAHFEGGTGP	
781	IWLDEMKNCG KESRIWQCHS HGWGQNCRH KEDAGVICSE FMSLRILTSEA SREACAGRL	
841	VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP	

901 TLWQCPSSPW EKRLARPSEE TWITCDNMR LQEGPTSCSG RVEIWHGGSW GTVCDDSWDL 961 NDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWDPCA RRWGHSECGH 1021 KEDAAVNCTA QKISTHKTPQ KATTVSSRGE NLVHQIQYRE MNSCLNADDL DLMNSSGGHS 1081 EAH	
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACCTCAGAATGGTGTCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTGTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAACAACAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAGTGAAAATCCAGGAGGAGTGGGGAACGGTGTGAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTGTAAACAGCTGGGATGTCCAAGTCTATCAAAGCC 101 T G W A N S S A G S G R I W M D H V S C 301 ACTGGATGGGCTAATTCCAGTGCAGGTTCTGGACGCATTTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H S N 361 CGTGGGAATGAGTCAGCTCTTGGGACTGCAACATGATGGATGGGGAAGCATAGTAAC 141 C T H Q Q D A G V T C S D G S D L E M R 421 TGTAACCAACAAGATGCTGGAGTGACTTGCTCAGATGGATCCGATTTGGAATGAGG 161 L T N G G N M C S G R I E I K F Q G Q W 481 CTGACGAATGGAGGAATATGTGTTCTGGAAGAATAGAGATCAAATTCGAAGGACAGTGG 181 G T V C D D N F N I N H A S V V C K Q L 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAAACAACCTT 201 E C G S A V S F S G S A N F G E G S G P 601 GAATGTGGAAGTGTGTGAGTTTCTGTGTTCTAGCTAATTTGGAGAAGGCTCTGGACCA 221 I W F D D L I C N G N E S A L W N C K H 661 ATCTGTTTGTGATGATCTTATATGCAACGAAATGAGTCAGCTCTCTGGAAGTCAAACAT 241 Q G W G K H N C D H A E D A G V I C S K 721 CAAGGATGGGGAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTTGCTCAAAG 261 G A D L S L R L V D G V T E C S G R L E 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTCAAGGAAGATTAGAA 281 V R F Q G E W G T I C D D G W D S H D A 841 GTGAGATTCCAAGGAGAATGGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT 301 A V A C K Q L G C P T A I T A I G R V N 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCGCCATTGGTTCGAGTTAAC 321 A S E G F G H I W L D S V S C Q G H E P 961 GCCAGTGAGGGATTGGACACATCTGGCTTGACAGTGTCTTCCAGGGACATGAACCT 341 A V W Q C K H H E W G K H Y C N H N E D 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAAGCATTATTGCAATCACAATGAAGAT 361 A G V T C S D G S D L E L R L R G G G S 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC 381 R C A G T V E V E I Q R L L G K V C D R 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATTAGAGACTGTTAGGGAAGGTGTGTGACAGA 401 G W G L K E A D V V C R Q L G C G S A L 1201 GGCTGGGGACTGAAAGAAGCTGATGTGGTTTGCAGGCAGCTGGGATGTGGATCTGCACCTC 421 K T S Y Q V Y S K I Q A T N M W L F L S 1261 AAAACATCCTATCAAGTATACTCCAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT 441 S C N G N E T S L W D C K N W Q W G G L 1321 AGCTGTAAACGGAATGAAACTTCTCTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT 461 T C D H Y E E A K I T C S A H R E P R L 1381 ACCTGTGATCACTATGAAGAAGCCAAATACCTGCTCAGCCCCAGGGAACCCAGACTG 481 V G G D I P C S G R V E V K H G D T W G 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC 501 S V C D S D F S L E A A S V L C R E L Q	SEQ ID NO: 41 and 42

1501 TCCGTCCTGTGATTTCGATTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
 541 W A E E F Q C E G H E S H L S L C P V A
 1621 TGGGCTGAAGAATCCAGTGTGAGGGACATGAGTCCCATCTTTCACTCTGCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCCAGCCAGAAAGAACTTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTTAAATGTGGAGTTGCCCTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCTGTAACTGCTCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 ATTTGCTCAGGAAACAGTCCCAACACTGTCCTCGTGCAATTCATCATCTCTGGGCCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCAGAAAGTGTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGTCTGTGTGGGAGAGTAGAGATTTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTTGGTGTGCAGACAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAAATCCCGTATTGGCAGTGCCATTCA
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 CATGGCTGGGGGAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG
 821 F M S L R L T S E A S R E A C A G R L E
 2461 TTCATGTCTCTGAGACTGACAGTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA
 841 V F Y N G A W G S V G R S N M S E T T V
 2521 GTTTTTTACAACGGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG
 861 G V V C R Q L G C A D K G K I N S A S L
 2581 GGTGTGGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAATCAACTCTGCATCTTTA
 881 D K A M S I P M W V D N V Q C P K G P D
 2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTCACTGTCCAAAAGGACCTGAC
 901 T L W Q C P S S P W E K R L A R P S E E
 2701 ACGCTGTGGCAGTGGCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG
 921 T W I T C D N K M R L Q E G P T S C S G
 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCCTGTCTGGA
 941 R V E I W H G G S W G T V C D D S W D L
 2821 CGTGTGGAGATCTGGCACGGAGGTTCTGGGGACAGTGTGTGATGACTCCTGGGACTTG
 961 N D A Q V V C Q Q L G C G P A L K A F K
 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA
 981 E A E F G Q G T G P I W L N E V K C K G
 2941 GAAGCAGAGTTTGGTCAAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG
 1001 N E S S L W D C P A R R W G H S E C G H
 3001 AATGAGTCTTCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAAGTGTGGACAC
 1021 K E D A A V N C T A Q K I S T H K T P Q
 3061 AAGGAAGACGCTGCAGTGAATGCACAGCACAAAAAATTTCAACGCACAAAACCCACAA
 1041 K A T T G R S F L I A F G I L G V V L L
 3121 AAAGCCACAACAGGTCGGTCATTCTTATTGCATTTCGGAATCCTTGGAGTTGTCTCTTG

1061 A I F V A L F L T Q K R R Q R Q R L T V 3181 GCCATTTTCGTCGATTATTCTTGACTCAAAAGCGAAGACAGACAGCGGCTTACAGTT 1081 S S R G E N L V H Q I Q Y R E M N S C L 3241 TCCTCAAGAGGAGAGAAGTCTAGTCCACCAAAATTCAATACCGGGAGATGAATTCTTGCTCG 1101 N A D D L D L M N S S G G H S E A H 3301 AATGCAGATGATCTGGACCTAATGAATTCCTCAGGAGGCCATTCTGAGGCACAC	
1 MSKLRLVLE DSGSADVRH FVNLSPFTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPTAIIKA TGWANSSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGGW 181 GTVCDDNFNI NHASVVCQL ECGSAVSFSG SANFGEESGP IWFDLLICNG NESALWNCXH 241 QGWGKHNCBH AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA 301 AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQGHPE AVWQCKHHEW GKHYCNHNE 361 AGVTCSGSD LELRLRGGS RCAGTVEVEI QRLLGKVC DR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEEAKI TCSAHREPR 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEGNGQI 541 WAEFEQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCVSRV TEIRLVNGKT PCEGRVELKT 601 LNAWGLSCLNS HWDIEDAHL CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTQHM 661 DCPVTALGAS LCPSGQVASV ICSGNQSQTL SSCNSSSLGP TRPTIPEESA VACIESGQLR 721 LVNGGGRACG RVEIYHEGSW GTICDDSWDL SDAHVVCRQL GCCEAINATG SAHFGEGTGP 781 IWLDEMKNCG KESRIWQCHS HGWGQONCRH KEDAGVICSE FMSLRLTSEA SREACAGRLE 841 VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNKMRLQEGPTSCSG RVEIWHGGSW GTVCDSDWDL 961 NDAQVVCQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWDPCA RRWGHSECGH 1021 KEDAAVNCTA QKISTHKTPQ KATTGRSFLI AFGILGVLL AIFVALFLTQ KRRQRRLTV 1081 SSRGENLVHQ IQYREMNCL NADDLDLMNS SGGHSEAH	SEQ ID NO:42
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACTCAGAATGGTGCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCTGTTTGTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGAAGTGAATAATCCAGGAGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTGTAAACAGCTGGGATGTCCAATGCTATCAAAGCC 101 T G W A N S S A G S G R I W M D H V S C 301 ACTGGATGGGCTAATCCAGTGCAGGTTCTGGACGCAATTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H S N 361 CGTGGGAATGAGTCAGTCTCTTTGGGACTGCAACATGATGGATGGGGAAGCATAGTAAC 141 C T H Q Q D A G V T C S D G S D L E M R 421 TGTACTACCAACAAGATGCTGGAGTGACTTGCTCAGATGGATCCGATTGGAAATGAGG 161 L T N G G N M C S G R I E I K F Q G Q W 481 CTGACGAATGGAGGAATATGTGTTCTGGAAGAATAGAGATCAAATCCAAGGACAGTGG 181 G T V C D D N F N I N H A S V V C K Q L 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAAACAATT 201 E C G S A V S F S G S A N F G E G S G P 601 GAATGTGGAAGTGCTGTGCTAGTTTCTCTGGTTGAGCTAATTTTGGAGAAGGCTCTGGACCA 221 I W F D D L I C N G N E S A L W N C K H 661 ATCTGTTTGTGATGATCTTATATGCAACGGAAATGAGTCACTCTCTGGAAGTCAACAT 241 Q G W G K H N C D H A E D A G V I C S K 721 CAAGGATGGGGAAGCATAACTGTGATCATGTGAGGATGCTGGAGTGATTTGCTCAAAG 261 G A D L S L R L V D G V T E C S G R L E 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAAGAGATTAGAA 281 V R F Q G E W G T I C D D G W D S H D A 841 GTGAGATTCCAAGGAGAATGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT 301 A V A C K Q L G C P T A I T A I G R V N 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAATGCTATCACCGCCATTGGTCGAGTTAAC 321 A S E G F G H I W L D S V S C Q G H E P 961 GCCAGTGAGGATTTGGACACATCTGGCTTGACAGTGTTCCTTGCCAGGGACATGAACCT	SEQ ID NO:43 and 44

341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAAGCATTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S D L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATTGAGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGGACTGAAAGAAGCTGATGTGGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAAACGAAATGAAACTTCTCTTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCCACAGGGAACCCAGACTG
 481 V G G D I P C S G R V E V K H G D T W G
 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTCCGATTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCAGAGTCGTCTCTATCCTGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
 541 W A E E F Q C E G H E S H L S L C P V A
 1621 TGGGCTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTTCACTCTGCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCCCCCAGAAGGAACCTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACTTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCTGTAACTGCTCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 ATTTGCTCAGGAAACAGTCCCAAACACTGTCCTCGTGCAATTCATCATCTCTGGGCCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCCAGAAGAAAGTGTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGTTCGCTGTGCTGGGAGAGTAGAGATTTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGCAGACAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAAATCCCGTATTTGGCAGTGCCATTCA
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 CATGGCTGGGGCAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG
 821 F M S L R L T S E A S R E A C A G R L E
 2461 TTCATGTCTCTGAGACTGACCACTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA
 841 V F Y N G A W G S V G R S N M S E T T V
 2521 GTTTTTTACAACGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG
 861 G V V C R Q L G C A D K G K I N S A S L
 2581 GGTGTGGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAAATCAACTCTGCATCTTTA
 881 D K A M S I P M W V D N V Q C P K G P D

2641 GACAAGGCCATGTCCATTCCTCCATGTGGGTGGACAATGTTTCAGTGTCCAAAAGGACCTGAC 901 T L W Q C P S S P W E K R L A R P S E E 2701 ACGTGTGGCAGTGGCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG 921 T W I T C D N K M R L Q E G P T S C S G 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCCTGTTCTGGA 941 R V E I W H G G S W G T V C D D S W D L 2821 CGTGTGGAGATCTGGCACGGAGGTTCTGGGGGACAGTGTGTGATGACTCCTGGGACTTG 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA 981 E A E F G Q G T G P I W L N E V K C K G 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG 1001 N E S S L W D C P A R R W G H S E C G H 3001 AATGAGTCTTCCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC 1021 K E D A A V N C T A Q K I S T H K T P Q 3061 AAGGAAGACGCTGCAGTGAATTGCACAGCACAAAAAATTTCAACGCACAAAACCCACAA 1041 K A T T G Q S F L I A F G I L G V V L L 3121 AAAGCCACAACAGGTCAGTCATTCTTATTGTCATTCCGAATCCTTGGAGTTGTTCTCTTG 1061 A I F V A L F L T Q K R R Q R Q R L T V 3181 GCCATTTTCGTCGATTATTTCTTGACTCAAAAGCGAAGACAGAGACAGCGGCTTACAGTT 1081 S S R G E N L V H Q I Q Y R E M N S C L 3241 TCCTCAAGAGGAGAGAAGTCTAGTCCACCAAATTCATACCGGGAGATGAATTCTTGCTTG 1101 N A D D L D L M N S S E N S N E S A D F 3301 AATGCAGATGATCTGGACCTAATGAATTCCTCAGAAAATTCCAATGAGTCAGCTGATTTT 1121 N A A E L I S V S K F L P I S G M E K E 3361 AATGCTGCTGAACCTAATTTCTGTGTCTAAATTTCTTCTATTCTGGAATGAAAAGGAG 1141 A I L R H T E K E N G N L 3421 GCCATTCTGAGGCACACTGAAAAGGAAAATGGGAATTTA	
1 MSKLRMVLE DSGSADVRRH FVNLSPFTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPTAICA TGWANSSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGGW 181 GTVCDNDFNI NHASVVKQL ECGSAVSFSG SANFEGSGP IWFDLICNG NESALWNCKH 241 QGWGKHNCBH AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA 301 AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQGHPE AVWQCKHHEW GKHYCNHNE 361 AGVTCSDBGSD LELRLRGGS RCAGTVEVEI QRLLGKVCDR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEAKI TCSAHREPR 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEGNGQI 541 WAEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVVCSRY TEIRLVNGKT PCEGRVELKT 601 LNAWGLCNS HWDIEDAHVL CQQLKCGVAL STPGGAHFGK GNGQVVRHMF HCTGTEQHM 661 DCPVTALGAS LCPSGQVASV ICSGNQSQTLL SSCNSSSLGP TRPTIPEESA VACIESGQLR 721 LVNGGGRGAG RVEIYHEGSW GTICDDSDWL SDAHVVCRQL GCGEAINATG SAHFGEGTGP 781 IWLDEMKNCG KESRIWQCHS HGWGQNCRH KEDAGVICSE FMSLRILTSEA SREACAGRL 841 VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNMR LQEGPTSCSG RVEIWHGGSW GTVCDDSDWL 961 NDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWDCA RRWGHSECGH 1021 KEDAAVNCTA QKISTHKTPQ KATTGQSFLI AFGILGVLL AIFVALFLTQ KRRQRRLTV 1081 SSRGENLVHQ IQYREMNCSL NADDLDLMNS SENSNESADF NAELISVSK FLPISGMEKE 1141 AILRHTEKEN GNL	SEQ ID NO: 44

Example 10 Cloning and characterization of canine CD163 from DH82 cells.

A forward primer 5'simianCD163 (SEQ ID NO: 28) (5'-

- 5 CACCGGAATGAGCAAACCTCAGAATGG-3' based on human CD163) and a reverse primer HuCD163-3'Kpn (SEQ ID NO: 29) (5'-

GCTCCGGTACCTAGTCCAGGTCTTCA

TCAAGGTATCTTA-3') were used to amplify CD163 cDNA from DH82 cells. Total cellular RNA was prepared from DH82 cells using the RNeasy kit. RT-PCR

parameters were the same as described in Example 4. RT-PCR products were cloned directionally into the pCDNA3.1D/V5/His/TOPO vector according to the manufacturer's instruction. Several clones containing large inserts were analyzed. Several clones with large inserts were analyzed, and these fell into either the v2 or v3
5 splicing patterns seen in other species. The v2 variant is missing an 81-nucleotide exon (E81) relative to the v3 variant, which results in a reading frame shift and alternative carboxy terminal amino acid sequences. The canine CD163v2 cDNA from DH82 cells encodes a peptide of 1115 amino acids. When compared to the sequences in Genbank database, it is 83.9% identical to human CD163 (Genbank Z22968), 85.1% identical to
10 pig CD163 (Genbank AJ311716), and 74.3% identical to mouse CD163 (Genbank AF274883). The nucleotide and amino acid sequences of the two splice variants found in DH82 cells are provided below (SEQ ID NOS: 45-48).

SEQUENCE	ID NO
1 M S K L R M V P H G N S G S A D F R R C 1 ATGAGCAAACCTCAGAATGGTCCCACATGGAACTCTGGATCTGCTGACTTTAGAAGATGT 21 F A L L C P S A V A V V S I L S T C L M 61 TTGCCCCTCTTGTGTCCCTCTGCTGTGGCTGTGGTCTCCATTCTCAGTACCTGTTTGATG 41 T N S L G R A D K E M R L T D G E D N C 121 ACCAATTCTCTTGGGAGAGCAGATAAAGAGATGAGGCTAACGGATGGTGAAGACAATTGC 61 S G R V E V K V Q E E W G T V C N N G W 181 TCCGGGAGAGTGGAAGTGAAAGTCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 G M D E V S V I C R Q L G C P T A I K A 241 GGCATGGATGAAGTCTCTGTGATTGTCAGGCAGCTGGGATGTCCCACTGCTATCAAAGCC 101 A G W A N S R A G S G R I W M D H V S C 301 GCTGGATGGGCCAATTCCAGGGCAGGCTCTGGACGAATCTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H N C 361 CGAGGGAATGAATCTGCTCTCTGGGACTGCAAACATGATGGATGGGGAAGCACAACCTGC 141 S H Q Q D A G V T C S D G S S L E M R L 421 AGTCATCAACAGGATGCTGGAGTAACCTGTTTCTGATGGATCCAGTTTGGAGATGAGGTTG 161 M N G G N Q C S G R I E V K F Q G Q W G 481 ATGAACGCGGGAACCAAGTGTCTTGGCAGAATAGAAGTCAAGTTCCAGGGACAGTGGGGA 181 T V C D D N F N I D H A S V V C K Q L E 541 ACAGTGTGTGATGACAACTTCAACATAGATCATGCTTCTGTGGTTTGTAAACAGCTCGAA 201 C G S A V S F S G S A N F G E G S G P I 601 TGTGGAAGTGCTGTCTGAGTTTCTGCTGCTCAGCTAATTTTGGAGAAGGTTCTGGGCAATC 221 W F D D L V C S G N E S A L W N C K H E 661 TGGTTTGATGATCTTGTGTGTCAGTGGAATGAGTCAGCTCTCTGGAAGTCAAGCATGAA 241 G W G K H N C D H A E D V G V I C L D G 721 GGATGGGGAAGCATAACTGTGATCACGCTGAGGATGTTGGAGTGATTGCTTGGATGGA 261 A D L S L R L V D G V T E C S G R L E V 781 GCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAAGGAAGATTAGAAGTA 281 K F Q G E W G T V C D D G W D S N D A A 841 AAATTCCAAGGGAATGGGGACAGTGTGTGATGATGGCTGGGATAGTAATGATGCTGCT 301 V V C K Q L G C P T A V T A I G R V N A 901 GTGGTATGTAAACAACCTGGGATGCCCACTGCTGTACCGCCATTGGTCGAGTTAACGCC 321 S E G S G H I W L D N L S C Q G D E S A	SEQ ID NO: 45 and 46

961 AGTGAGGGAAGTGGACACATTTGGCTTGACAATCTTTCCTGCCAAGGAGACGAATCTGCT
 341 L W Q C R H H E W G K H Y C N H N E D A
 1021 CTCTGGCAGTGTAGACACCATGAATGGGGAAAGCATTATTGCAATCATAATGAAGATGCT
 361 G V T C S D G S D L E L R L V G G G S R
 1081 GGTGTGACATGTTCTGATGGATCAGACCTGGAGCTGAGACTTGTGCGTGGAGGCAGCCGC
 381 C A G T V E V E I Q K L L G K V C D R G
 1141 TGTGCTGGGACAGTGGAGGTTGAAATTCAGAACTGCTAGGGAAAGTATGTGATAGAGGC
 401 W G L K E A D V V C K Q L G C G S A L K
 1201 TGGGGACTGAAAGAAGCCGATGTGGTTTGAAGCAGTTGGGATGTGGATCTGCTCTCAA
 421 T S Y Q R Y S K V K A T N T W L F L S R
 1261 ACGTCCTATCAGCGTTATTCCAAAGTTAAGGCAACAAACACATGGCTGTTTAAAGCCGC
 441 C S G N E T S L W D C K N W Q W G G L S
 1321 TGTAAGTGGCAATGAAACTTCCCTTTGGGACTGCAAGAACTGGCAGTGGGGTGGACTGAGC
 461 C D H Y E E A K V T C S A H R E P R L V
 1381 TGTGATCACTATGAAGAAGCTAAAGTTACCTGCTCAGCCACAGGGAACCCAGACTAGTT
 481 G G D I P C S G R V E V K H G D T W G T
 1441 GGAGGAGATATCCCTGCTCTGGTCTGTGAAGTGAACATGGTGACACATGGGGCACC
 501 V C D S D F S L E A A S V L C R E L Q C
 1501 GTCTGTGATTCCGACTTCTCTTTGGAAGCTGCCAGTGTGCTGTGCAGAGAGTTACAGTGT
 521 G T V I S I L G G A H F G E G N G Q I W
 1561 GGCACAGTCATCTCCATCTAGGGGAGCTCACCTTTGGAGAAGGAAATGGACAGATCTGG
 541 A E E F Q C E G Q E S H L S L C S V A S
 1621 GCTGAAGAATCCAGTGTGAGGGGAGGAGTCCCATCTTTCACTCTGTTTCACTAGCCTCT
 561 R P D G T C S H S R D V G V V C S R Y T
 1681 CGCCAGATGGGACCTGTAGCCACAGCAGGGATGTTGGAGTCTGCTGCTCAAGATACAGC
 581 E I R L V N G Q S P C E G R V E L K I L
 1741 GAAATCCGCTTGGTGAATGGCCAGTCCCCGTGTGAAGGAAGAGTGGAGCTCAAGATACTT
 601 G N W G S L C N S H W D I E D A H V F C
 1801 GGGAAGTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCCATGTTTCTGT
 621 Q Q L K C G V A L S I P G G A H F G K G
 1861 CAGCAGCTCAAATGTGGAGTTGCCCTTCTATTCCGGGAGGAGCACATTTGGGAAAGGA
 641 S G Q I W R H M F H C T G T E Q H M G D
 1921 AGTGGTCAGATCTGGAGGCACATGTTTCACTGCACTGGGACTGAGCAGCACATGGGAGAT
 661 C P V T A L G A T L C S A G Q V A S V I
 1981 TGCCCTGTAAGTCTCTGGGCGCGACGCTGTGTCTGCTGGGCAAGTGGCCTCTGTAATC
 681 C S G N Q S Q T L S P C N S T S L D P T
 2041 TGCTCAGGAAATCAGAGCCAGACGCTATCCCCATGCAATTCAACATCTCTGGACCCAACA
 701 R S T T S E E S A V A C I A S G Q L R L
 2101 AGATCTACCACTTCGGAAGAAAGTGTGTTGCTTGTATTGCGAGTGGGCAACTTCGCTGT
 721 V N G G G R C A G R I E V Y H E G S W G
 2161 GTAAATGGAGGCGGTGCTGTGCTGGGAGAATAGAGGTCTACCATGAGGGCTCTGGGGC
 741 T I C D D S W D L S D A H V V C R Q L G
 2221 ACCATCTGTGATGACAGCTGGGACCTGAGTGATGCCCATGTGTTGTGCAGACAGCTGGGC
 761 C G V A I N A T G S A H F G E G T G P I
 2281 TGTGGAGTGGCCATTAATGCCACTGGCTCTGCTCATTTTGGGGAAGGAACAGGGCCATC
 781 W L D E V N C N G K E S H I W Q C R S H
 2341 TGGCTGGACGAGGTGAAGTGAATGGAAAGGAATCTCATATCTGGCAATGCCGCTCACAC
 801 G W G Q H N C R H K E D A G V I C S E F
 2401 GGCTGGGGGCAACAACTGCAGACATAAGGAGGATGCAGAGTTATCTGCTCAGAGTTC
 821 M S L R L I D E T S R D I C A G R L E V
 2461 ATGTCTCTCAGACTGATTGATGAAACCAGCAGAGACATCTGTGCAGGGCGTCTTGAAGTT
 841 F Y N G A W G S V G K S N M S A T T V E
 2521 TTTTACAATGGAGCTTGGGGCAGCGTTGGCAAGAGTAATATGTCTGCAACCACTGTGGAG
 861 V V C R Q L G C A D K G S I N P A S S D
 2581 GTGGTATGCAGGCAACTGGGTTGTGCAGACAAGGGGAGCATCAACCCTGCATCTTCAGAC

881 K P M S R H M W V D N V Q C P K G P D T 2641 AAGCCCATGTCCAGGCACATGTGGGTGGACAATGTCCAGTGTCCAAAAGGACCTGACACC 901 L W Q C P S S P W K Q R V A S S S E E T 2701 TTATGGCAGTGCCCATCTTCTCCATGGAAACAGAGAGTGGCCAGTCTTTCAGAGGAGACC 921 W I T C A N K I R L Q E G T S N C S G R 2761 TGGATCACATGTGCCAACAAGATAAGACTTCAAGAAGGAACCTCTAATTGTTCTGGACGT 941 V E L W H G G S W G T V C D D S W D L E 2821 GTGGAGCTCTGGCACGGAGGTTCTCTGGGGGACAGTGTGCGATGACTCTGGGACCTTGAA 961 D A Q V V C R Q L G C G P A L E A L K E 2881 GATGCACAAGTGGTGTGTGACAGCTGGGCTGTGGCCCAGCATTAGAAGCACTAAAAGAG 981 A A F G Q G T G P I W L N D V K C K G N 2941 GCAGCATTTGGTCAGGGGACTGGGCCTATATGGCTCAATGACGTGAAGTGCAAAGGGAAT 1001 E S S L W D C P A R P W G H S D C G H K 3001 GAGTCTTCCTTGTGGGATTGTCTGCTAGACCCTGGGGGCACAGTGAAGTGTGGCCACAAG 1021 E D A A V R C S E I A M A Q R S S N P R 3061 GAAGATGCTGCTGTGAGGTGCTCAGAAATTGCAATGGCCCAAAGATCATCAAATCCTAGA 1041 G H S S L V A L G I F G V I L L A F L I 3121 GGTCACATCCCTTGTTCATTGGGGATCTTTGGTGTCTTCTGCGCTTTCTCATC 1061 A L L L W T Q R R R Q Q Q R L T V S L R 3181 GCTCTCCTTGTGGACTCAAAGGCGAAGACAGCAACAGCGGCTTACAGTTTCTTGAGA 1081 G E N S V H Q I Q Y R E M N S S L K A D 3241 GGAGAGAATTCTGTCCACCAAATTCAATACCGGGAATGAATTCTTCCCTGAAAGCAGAT 1101 D L D V L T S S E D H F E V H 3301 GATCTGGACGTGCTGACTTCTCTCAGAAGACCATTTTGAGGTACAC	
1 MSLKRMVPHG NSGSADFRC FALLCPSAVA VVSILSTCLM TNSLGRADKE MRLTDGEDNC 61 SGRVEVKVQE EWGTVCNNGW GMDEVSVICR QLGCPATAIKA AGWANSRAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHNC SHQQDAGVTC SDGSSLEMRL MNGGNQCSGR IEVKFQGQWG 181 TVCDDNPNID HASVVCQLE CGSAVSFSGS ANFGEESGPI WFDDLVCNEN ESALWNCHE 241 GWGKHNCDAH EDVGVICLDG ADLSLRLVDG VTECSGRLEV KFQGEWGTVC DDGWDSDNDA 301 VVCKQLGCPT AVTAIGRVNA SEGSGHIWLD NLSCQGDSEA LWQCRHHEWG KHYCNHNEDA 361 GVTCSGSDSL ELRLVGGGSR CAGTVEVEIQ KLLGKVCDRG WGLKEADVVC KQLGCGSALK 421 TSYQRYSKVK ATNTWLFSLR CSGNETSLWD CKNWQWGLS CDHYEEAKVT CSAHREPRV 481 GGDIPCSGRV EVKHGDTWGT VCDSDFSLEA ASVLCRELQC GTVISILGGA HFGEGNGQIW 541 ABEFQCEGQE SHLSLCSVAS RPDGTCSHSR DVGTVCSRYT EIRLVNGQSP CEGRVELKIL 601 GNWGSLSNSH WDIEDAHVFC QQLKCGVALS IPGGAHFHKG SGQIWRHMFH CTGTEQHMGD 661 CPVTALGATL CSAGQVASVI CSGNQSQTLS PCNSTSLDPT RSTTSEESAV ACIAGGQLRL 721 VNGGGRCAGR IEVYHEGWSW TICDSDWDL S DAHVVCRLQ CGVAINATGS AHFGEETGPI 781 WLDEVNCGNK ESHIWQCRSH GWGQHNCRHK EDAGVICSEF MSLRLIDETS RDICAGRLEV 841 FYNGAWGSVG KSNMSATTVE VVCRQLGCAD KGSINPASSD KPMSRHMWVD NVQCPKGPDT 901 LWQCPSPFWK QRVASSEET WITCANKIRL QEGTSNCSGR VELWHGGSWG TVCDDSWDLE 961 DAQVVCRLQG CGPALEALKE AAFGQGTGPI WLNDVKCKGN ESSLWDCPAR PWGHSDCGHK 1021 EDAAVRCSEI AMAQRSSNPR GHSSLVALGI FGVILLAFIL ALLLWTQRRR QQQLRTVSLR 1081 GENSVHQIQY REMNSSLKAD DLDVLTSSD HFEVH	SEQ ID NO:46
1 M S K L R M V P H G N S G S A D F R R C 1 ATGAGCAAACCTCAGAATGGTCCCATGGAACCTCTGGATCTGCTGACTTTAGAAGATGT 21 F A L L C P S A V A V V S I L S T C L M 61 TTTGCCCTCTTGTGTCCCTCTGCTGTGGCTGTGGTCTCCATTCTCAGTACCTGTTTGATG 41 T N S L G R A D K E M R L T D G E D N C 121 ACCAATTCTCTTGGGAGAGCAGATAAAGAGATGAGGCTAACGGATGGTGAAGACAATTGC 61 S G R V E V K V Q E E W G T V C N N G W 181 TCCGGGAGAGTGAAGTGAAAGTCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 G M D E V S V I C R Q L G C P T A I K A 241 GGCATGGATGAAGTCTCTGTGATTGTCAGGCAGCTGGGATGTCCCACTGCTATCAAAGCC 101 A G W A N S R A G S G R I W M D H V S C 301 GCTGGATGGGCCAATTCAGGGCAGGCTCTGGACGAATCTGGATGGATCATGTTCTTGT 121 R G N E S A L W D C K H D G W G K H N C 361 CGAGGGAATGAATCTGCTCTCTGGGACTGCAACATGATGGATGGGGAAGCACAACTGC 141 S H Q Q D A G V T C S D G S S L E M R L	SEQ ID NO:47

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701 R S T T S E E S A V A C I A S G Q L R L	
2101 AGATCTACCACTTCGGAAGAAAGTGCTGTTGCTTGATTGCGAGTGGGCAACTTCGCCTG	
721 V N G G G R C A G R I E V Y H E G S W G	
2161 GTAAATGGAGGCGGTGCTGTGCTGGGAGAATAGAGGTCTACCATGAGGGCTCCTGGGGC	
741 T I C D D S W D L S D A H V V C R Q L G	
2221 ACCATCTGTGATGACAGCTGGGACCTGAGTGATGCCCATGTGGTGTGCAGACAGCTGGGC	
761 C G V A I N A T G S A H F G E G T G P I	
2281 TGTGGAGTGGCCATTAATGCCACTGGCTCTGCTCATTGTTGGGAAGGAACAGGGCCATC	
781 W L D E V N C N G K E S H I W Q C R S H	
2341 TGGCTGGACGAGGTGAAGTGAATGGAAGGAATCTCATATCTGGCAATGCCGCTCACAC	
801 G W G Q H N C R H K E D A G V I C S E F	
2401 GGCTGGGGGCAACACAAGTGCAGACATAAGGAGGATGCAGGAGTTATCTGCTCAGAGTTC	
821 M S L R L I D E T S R D I C A G R L E V	
2461 ATGTCTCTCAGACTGATTGATGAAACCAGCAGAGACATCTGTGCAGGGCGTCTTGAAGTT	
841 F Y N G A W G S V G K S N M S A T T V E	
2521 TTTTACAATGGAGCTTGGGGCAGCGTTGGCAAGAGTAATATGTCTGCAACCACTGTGGAG	
861 V V C R Q L G C A D K G S I N P A S S D	
2581 GTGGTATGCAGGCAACTGGGTTGTGCAGACAAGGGGAGCATCAACCCTGCATCTTCAGAC	
881 K P M S R H M W V D N V Q C P K G P D T	
2641 AAGCCCATGTCCAGGCACATGTGGGTGGACAATGTCCAGTGTCCAAAAGGACCTGACACC	
901 L W Q C P S S P W K Q R V A S S S E E T	
2701 TTATGGCAGTGGCCATCTTCTCCATGGAAACAGAGAGTGGCCAGTTCTTCAGAGGAGACC	
921 W I T C A N K I R L Q E G T S N C S G R	
2761 TGGATCACATGTGCCAACAAGATAAGACTTCAAGAAGGAACCTCTAATTGTTCTGGACGT	
941 V E L W H G G S W G T V C D D S W D L E	
2821 GTGGAGCTCTGGCACGGAGGTTCTGGGGGACAGTGTGCGATGACTCCTGGGACCTTGAA	
961 D A Q V V C R Q L G C G P A L E A L K E	
2881 GATGCACAAGTGGTGTGTCGACAGCTGGGCTGTGGCCCAGCATTAGAAGCACTAAAAGAG	
981 A A F G Q G T G P I W L N D V K C K G N	
2941 GCAGCATTTGGTCAGGGGACTGGGCCATATGGCTCAATGACGTGAAGTGCAAAGGGAAT	
1001 E S S L W D C P A R P W G H S D C G H K	
3001 GAGTCTTCTTGTGGGATTGTCTCTGCTAGACCTGGGGGACAGTGACTGTGGCCACAAG	
1021 E D A A V R C S E I A M A Q R S S N P R	
3061 GAAGATGCTGCTGTGAGGTGCTCAGAAATTGCAATGGCCCAAAGATCATCAAATCCTAGA	
1041 G H S S L V A L G I F G V I L L A F L I	
3121 GGTCACTCATCCCTTGTGTCATGGGGATCTTTGGTGTGATTCTTCTGGCCTTTCTCATC	
1061 A L L L W T Q R R R Q Q Q R L T V S L R	
3181 GCTCTCTCTTGTGGACTCAAAGGCGAAGACAGCAACAGCGGCTTACAGTTTCTTGTAGA	
1081 G E N S V H Q I Q Y R E M N S S L K A D	
3241 GGAGAGAATTCTGTCCACCAAATTCAATACCGGGAAATGAATTCTTCCCTGAAAGCAGAT	
1101 D L D V L T S S E Y P N E S D D F N D A	
3301 GATCTGGACGTGCTGACTTCTCAGAATATCCCAATGAGTCAGATGATTTTAATGATGCT	
1121 G L I S V S K S L P I S G	
3361 GGGCTAATTTCTGTGTCTAAATCTCTTCTTCTTCTGGA	
1 MSKLRMVPHG NSGSADFRR C FALLCP SAVA VVSILSTCLM TNSLGRADKE MRLTDGEDNC	SEQ ID NO: 48
61 SGRVEVKVQE EWGTVCNNGW GMDEVSVICR QLGCP TAIKA AGWANSRAGS GRIWMDHVSC	
121 RGNESALWDC KHDGWGKHNC SHQQDAGVTC SDGSSLEMLR MNGGNQCSGR IEVKFQGGQWG	
181 TVCDDNFNID HASVVCKQLE CGSAVSFSGS ANFEGSGPI WFDDLVCSGN ESALWNCKHE	
241 GWGKHNCDDHA EDVGVICLDG ADLSRLVDG VTECSGRLEV KFGQEWGTVC DDGWSNDAA	
301 VVCKQLGCPT AVTAIGRVNA SEGSGHIWLD NLSCQGDSEA LWQCRHHEWG KHYCNHNEDA	
361 GVTCSGSDSL ELRLVGGGSR CAGTVEVEIQ KLLGKVCDRG WGLKEADVVC KQLGCGSALK	
421 TSYQRYSKVK ATNTWLFSLR CSGNETSLWD CKNWQWGLS CDHYEEAKVT CSAHREPRIV	
481 GGDIPCSGRV EVKHGDTWGT VCDSDFSLEA ASVLCRELQC GTVISILGGA HFGEGNGQIW	
541 AEEFQCEGQE SHLSLCSVAS RPDGTCSHSR DVGVVCSRYT EIRLVNGQSP CEGRVELKIL	
601 GNWGSCLNSH WDIEDAHVFC QQLKCGVALS IPGGAHFHKG SGQIWRHMFH CTGTEQHMGD	
661 CPVTALGATL CSAGQVASVI CSGNQSTLS PCNSTSLDPT RSTTSEESAV ACIASGQLRL	
721 VNGGGRCAGR IEVYHEGWSW TICDSDWDL S DAHVVCRLG CGVAINATGS AHFEGGTGPI	
781 WLDEVNCGNK ESHIWQCRSH GWGQHNCRHK EDAGVICSEF MSLRLIDETS RDICAGRLEV	
841 FYNGAWGSVG KSNMSATTVE VVCRQLGCAD KGSINPASSD KPMSRHMWVD NVQCPKGPDT	

901	LWQCPSSPWK	QRVASSEET	WITCANKIRL	QEGTSNCSGR	VELWHGGSWG	TVCDDSWDLE
961	DAQVVCRLG	CGPALEALKE	AAFQQTGPI	WLNDVKCKGN	ESSLWDCPAR	PWGHSDCGHK
1021	EDAAVRCSEI	AMAQRSSNPR	GHSSLVALGI	FGVILLAFLI	ALLLWTQRRR	QQRLTVSLR
1081	GENSVHQIQY	REMNSSLKAD	DLDLVTSSEY	PNESDDFNDA	GLISVSKSLP	ISG

Example 11. Various cell lines are rendered permissive to North American PRRSV infection following transient transfection with pCMV-susCD163v1

5 Porcine Kidney (PK032495), Norden Labs Swine Testicular (NLST-1), Norden Labs Dog Kidney (NLDK-1) were obtained from Pfizer Inc. and were grown at 37° C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 5% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Cell lines Baby Hamster

10 Kidney (BHK21), Norden Labs Feline Kidney (NLFK-1), and Rabbit Lung (RL) were obtained from Pfizer Inc. and were grown at 37° C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Vero cells were obtained from Pfizer

15 Inc. and were grown at 37° C and 5% CO₂ in growth media consisting of Minimum Essential Medium Alpha (MEM, Pfizer Inc. formulation) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine and Gentamicin at 20 micrograms per mL. Cell culture wells (35 mm) containing approximately 1x10⁶ cells were transfected with 2 micrograms per well of plasmid pCMV-susCD163v1, in DMEM

20 without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. Cell line RL was transfected with 1.0 micrograms per well of plasmid pCMV-susCD163v1. A member of the PAM cell cDNA library without an insert, designated pPAMB (essentially and empty pSport plasmid vector), was used as a negative control plasmid. At 24 hours

25 post transfection wells were aspirated and washed twice with DMEM/5% FBS followed by infection with North American PRRSV isolate P129. Virus was allowed to adsorb in 0.5 ml growth media for a minimum of two hours, after which additional media was added to a final volume of 2.0ml and incubated overnight. The virus was then removed, wells washed twice with growth media, and fresh growth media added

30 (2.0 ml per well). A time zero sample of culture fluid was immediately taken in order to determine the background level of infectious virus from the inoculum. At a minimum of 48 hours post infection cultures were screened for permissivity by removing culture fluids in order to assay viable virus, and permissive cells in the

monolayer were detected by fluorescent antibody assay (FA). The FA was completed by fixing the monolayer with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for the PRRSV nucleocapsid protein. Viable virus was titrated by inoculating dilutions of culture fluids onto MARC-145 cells. Table 5 shows the results of virus infection by FA and the presence of progeny virus for each cell line tested.

Failure to detect progeny virus from some cell lines may be the result of low virus titer in the cell culture fluids, below the assay's limit of detection. Permissivity of Vero cells to PRRSV infection was augmented by the expression of susCD163v1. Compared to the time zero measurement of background virus, there was nearly a two-log increase in virus titers in Vero cells transfected with pCMV-susCD163v1, whereas there was less than a one-log in titer increase in cells transfected with negative control plasmid pPAMB. All cell lines except NLDK-1 were positive by FA for permissivity to North American PRRSV isolate P129 infection after transfection with pCMV-susCD163v1.

Table 5

Screening of various cell lines for permissivity to NA PRRSV isolate P129 following transient transfection with pCMV-susCD163v1 or pPAMB				
Transfected cell line	Fluorescent Antibody assay		Progeny virus produced	
	pCMV-susCD163v1	pPAMB	pCMV-susCD163v1	pPAMB
BHK21	+++	-	+++	-
PK032495	+	-	+	-
NKFK-1	+	-	+	-
NLST-1	+	-	-	-
NLDK-1	-	-	NT	NT
RL	+	-	-	-
Vero	++	+	++	+

+++ = Highly positive

++ = Moderately positive

20 + = Slightly positive

- = Not detectible

NT = Not tested

25 **Example 12. BHK21 cells are rendered permissive to European PRRSV infection following transient transfection with pCMV-susCD163v1**

Cell line Baby Hamster Kidney (BHK21) was obtained from Pfizer Inc. and grown at 37° C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium

(DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Cell culture wells (35 mm) containing approximately 1×10^6 cells were transfected with 2 micrograms per well of plasmid pCMV-susCD163v1, in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. At 24 hours post transfection wells were aspirated and washed twice with DMEM/5% FBS followed by infection with European PRRSV isolate 96V198. Virus was allowed to adsorb for a minimum of 2 hours. The virus was then removed, wells washed twice with growth media, and fresh growth media added (2.0 ml per well). A time zero sample of culture fluid was immediately taken in order to determine the background level of infectious virus from the inoculum. At a minimum of 48 hours post infection cultures were screened for permissivity by removing culture fluids in order to assay viable virus, and permissive cells in the monolayer were detected by fluorescent antibody assay (FA). The FA was completed by fixing the monolayer with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for the PRRSV nucleocapsid protein. Viable virus was titrated by inoculating dilutions of culture fluids onto MARC-145 cells. As a result of the transient transfection of BHK21 with pCMV-susCD163v1, cells were rendered permissive to European PRRSV isolate 96V198 infection and yielded progeny virus.

Example 13: CD163 genes from multiple animal species render BHK21 cells permissive to PRRS virus infection

BHK21 cells grown in DMEM (Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum, 1mM sodium pyruvate, and antibiotics, were used in transient transfection experiments. Before transfection cells were washed once with OptiMEM (Invitrogen) without serum or other additives. Lipofectamine 2000 (Invitrogen) was used in all transfection experiments according to the protocol provided by the manufacturer. The transfection mixture consisted of 10 microliters of Lipofectamine 2000 and 2-3 micrograms of DNA per 35 mm well. After overnight incubation, transfection medium was removed and cells were infected with PRRSV isolate P129. Infection was allowed to progress for 24-48 hours, when cells were fixed with 80% acetone and stained with monoclonal antibody SDOW17 conjugated

with FITC (Rural Technology Inc., Brookings, SD). Staining of the nucleocapsid protein was visualized under a fluorescence microscope.

Table 6. Transient transfection of BHK21 cells with various CD163 genes renders them permissive to PRRS virus infection

5

Table 6

Plasmid backbone	CD163 gene	PRRSV infection (FA)
pCMV-Script	Swine CD163v1	+++
pRSV-Script	Swine CD163v1	+++
pcDNA3.1D	Swine CD163v2	++
pcDNA3.1D	Human CD163v2	++
pcDNA3.1D	Mouse CD163v3	+
pcDNA3.1D	African green monkey (MARC-145 cell) CD163v2	+++
pcDNA3.1D	Vero cells CD163v7	+++
pcDNA3.1D	DH82 cell CD163v2	+++

+++ = Highly positive

++ = Moderately positive

+ = Slightly positive

10 **Example 14. Generation of PRRSV-permissive BHK21 stable cell lines using pCMV-susCD163v1**

BHK-21 cells were grown in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% fetal bovine serum, 1mM sodium pyruvate, and antibiotics. For transfection, cells were seeded at approximately 90% confluency in 6 well plates and incubated over night at 37°C in 5% CO₂. Cells were transfected with pCMV-susCD163v1 DNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. One day after transfection the cells were trypsinized and re-seeded in 96 well plates in a dilution series. To select for stable transfectants, the media was supplemented with 1 mg/ml Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) from this point forward. Medium was changed every 3-5 days. Plates were cultured until those wells with colonies derived from single cells reached confluency, at which point the plates were trypsinized and seeded into duplicate 96 well plates. One of the duplicate 96 well plates was infected with PRRSV isolate P129 and clones permissive to infection were identified by staining with FITC conjugated monoclonal antibody SDOW17. Positive clones were then expanded from the second duplicate plate. To ensure homogeneity the positive cultures were single-cell cloned by limiting dilution. At each cloning the subclones that displayed robust

25

growth and high PRRSV permissivity were chosen for expansion. Three clones designated BHK/CMV/v1 #3, BHK/CMV/v1 #5, and BHK/CMV/v1 #12 (Figure 6) were selected. These cell lines have maintained the permissive phenotype through 20 passages.

5 Example 15. Generation of PRRSV-permissive BHK21 stable cell lines using pRSV-susCD163v1

BHK-21 cells were cultured as described in Example 14. BHK-21 cells were transfected with pRSV-susCD163v1 using Lipofectamine 2000 as described in Example 14. Cloning of transfected cells and screening for permissive clones was
10 performed essentially as described in Example 14. From the original cloning 3 single cell clones were identified as permissive and were subsequently recloned two more times to ensure homogeneity and to attempt to isolate subclones of higher permissivity (see Figure 7). The resulting cell lines were named BHK/RSV/v1, #2, #3, and #4. All of these clones have maintained the permissive phenotype through the highest passage
15 tested (passage 11 for clone #2, passage 7 for clone #3, and passage 5 for clone #4).

Example 16. Generation of PRRSV-permissive feline kidney stable cell lines using pCMV-susCD163v1

Parental Norden Labs Feline Kidney (NLFK) cells were grown at 37 degrees C and 5% CO₂ in Dulbecco's Modified Eagle Medium (Invitrogen catalog number
20 11965-092) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, and antibiotics. Several 35 mm wells containing approximately 2×10^6 cells each were transfected with 4 micrograms per well of pCMV-susCD163v1, in OptiMEM, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were removed from the
25 substrate using Accutase (Innovative Cell Technologies, catalog number AT104) diluted in medium, and seeded into three 96-well plates at three densities (approximately 2×10^2 , 2×10^3 , and 2×10^4 cells per well). The cells were allowed to settle overnight at 37 degrees C before beginning selection of stable transformants. The next morning medium was replaced with 100 microliters / well fresh medium
30 containing 500 micrograms / ml Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) to select for cells expressing the neomycin resistance gene. Medium was changed every 2 or 3 days to maintain Geneticin potency. After 19 days of selection, the 96-well plate with the lowest initial cell density (approximately 200 cells / well) yielded 70 empty wells and 26 wells with one or more colonies of G418-resistant cells

(calculated number of resistant cells / well is 0.3, using the Poisson distribution).

These 26 wells were split into duplicate wells and allowed to settle overnight. One set of wells was infected with PRRSV isolate P129, incubated for 24 hours, then fixed with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for PRRSV nucleocapsid. Of the 26 clones, 8 contained some cells that were infected by PRRSV. One of these, designated "NLFK-CMV-susCD163v1-G4", was clearly more permissive than the others with nearly 100% of the cells staining positive for viral antigen.

By cell passage number 5, there was some evidence of phenotypic heterogeneity in the NLFK-CMV-susCD163v1-G4 cell line. Therefore, the cells were single-cell cloned by limiting dilution in G418-containing medium, starting with frozen stocks of NLFK-CMV-susCD163v1-G4 passage 4. Twelve such clones ("A" – "L") were expanded for study. Of these, clones NLFK-CMV-susCD163v1-G4F and NLFK-CMV-susCD163v1-G4L were notable for their ability to form discrete plaques (localized areas of CPE) when infected with PRRSV isolate P129 (see Figure 8).

Example 17. Generation of PRRSV-permissive feline kidney stable cell lines using pRSV-susCD163v1

Norden Labs Feline Kidney (NLFK) cells were grown at 37° C and 5% CO₂ in Minimal Essential Medium Alpha Medium (Invitrogen catalog number 12571-071) supplemented with 10% fetal bovine serum and antibiotics. NLFK cells were seeded in 6 well plates at approximately 90% confluency and allowed to attach overnight. The cells were then transfected with plasmid pRSV-susCD163v1 using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. After 24 hours the cells were cloned as described in Example 14. Screening for PRRSV permissive cell clones was performed as described in Example 14. Four clones were selected from the screening and were single cell cloned by limiting dilution two more times. Four clones named FK/RSV/v1 #1, FK/RSV/v1 #2, FK/RSV/v1 #3, and FK/RSV/v1 #4 were selected. These cell lines have maintained the PRRSV permissive phenotype through at least 8 passages (see Figure 9).

Example 18. Generation of PRRSV-permissive porcine kidney stable cell lines using pCMV-susCD163v1

Parental Porcine Kidney (PK032495) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's

Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 5% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1×10^6 cells each were transfected with 2 micrograms per well of plasmid pCMV-susCD163v1, in
5 DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS and removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104) and diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number
10 10131-027) at 1.0 milligram per ml and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and allowed to incubate until 100% confluency was achieved. One set of wells
15 was screened for PRRSV-permissivity by infecting with PRRSV isolate P129 for a minimum of 48 hours. Eleven clones were found to be permissive for PRRSV. One of these, designated "PK-CMV-susCD163v1-A10", clearly retained the permissive phenotype after numerous passages (see Figure 10).

Example 19. Generation of PRRSV-permissive BHK21 stable cell lines using pCMVScript-susCD163v2
20

Parental Baby Hamster Kidney (BHK21) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-
25 glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1×10^6 cells each were transfected with 2 micrograms per well of pCMVScript-susCD163v2, in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS and removed from the
30 substrate using Accutase (Innovative Cells Technologies, catalog number AT104) and diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 1.0 milligram per ml and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection.

Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for permissivity by infecting with PRRSV isolate P129 and incubating for a minimum of 48 hours. Three clones were found to be PRRSV-permissive, and one of these, designated "BHK-CMVScript-susCD163v2-A9", was chosen for further study (see Figure 11).

Example 20. Generation of PRRSV-permissive BHK-21 stable cell lines using pRSV-susCD163v2

BHK-21 cells were cultured as described in Example 14. BHK-21 cells were transfected with the ligated pRSV-susCD163v2 DNA construct described in Example 5 using Lipofectamine 2000 (Invitrogen) following manufacture's instructions. Subsequent cloning and selection of PRRSV permissive cell lines was performed as described in Example 14. Of 336 single cell clones screened, 129 were positive. Several of these cell clones have been passed up to 7 times and they have maintained the PRRSV permissive phenotype (see Figure 12). These cell lines have been named BHK/RSV/v2, followed by a numerical clone number.

Example 21. Generation of PRRSV-permissive porcine kidney stable cell lines using pCMVScript-susCD163v2

Parental Porcine Kidney (PK032495) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 5% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1x10⁶ cells each were transfected with 2 micrograms per well of pCMVScript-susCD163v2 in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS and removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104) and diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 1.0 milligram per ml and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells

containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for permissivity by infecting with PRRSV isolate P129 and incubating for a minimum 48 hours. One clone designated "PK-CMVScript-susCD163v2-D1" showed the PRRSV-permissive phenotype.

Example 22. Generation of PRRSV-permissive BHK21 stable cell lines using pcDNA3.1D-humCD163v2

Parental Baby Hamster Kidney (BHK21) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1x10⁶ cells each were transfected with 2 micrograms per well of pcDNA3.1D-humCD163v2, in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS and removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104) and diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 1.0 milligram per ml and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for permissivity by infecting with PRRSV isolate P129, incubated for a minimum 48 hours. Seven candidate clones were found to be PRRSV-permissive. There was some evidence of phenotypic heterogeneity in each of the seven candidate clones, likely because they were not clonal. Therefore, the candidate clones were single-cell cloned by limiting dilution in G418 containing medium. One single cell clone with clear PRRS-permissivity was obtained and designated BHK-cDNA3.1D-humCD163v2-H9.

Example 23. Generation of PRRSV-permissive feline kidney stable cell lines using pcDNA3.1D-humCD163v2

Parental Norden Labs Feline Kidney (NLFK) cells were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1x10⁶ cells each were transfected with 2 micrograms per well of pcDNA3.1D-humCD163v2 in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS, removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104), diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 500 micrograms per ml, and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for PRRSV-permissivity by infecting with PRRSV isolate P129 for a minimum 48 hours. Five clones were found to be permissive. One of these, designated "FK-cDNA3.1D-humCD163v2-A6", clearly displayed the permissive phenotype (see Figure 13).

NLFK parent cells and one subclone of FK-cDNA3.1D-humCD163v2-A6 were examined for the CD163 expression. Cells were fixed in 80% acetone and reacted with Goat anti-human CD163 (R&D System at 1:200) for one hour following by washing with PBS. For visualization, donkey anti-Goat IgG conjugated with FITC (Bioscience Inc at 1:100) were used. No specific fluorescence was detected in the NLFK parent cells as shown in Figure 21A. The majority of the FK.A6.A2 subclone showed good fluorescent staining indicating the presence of CD163 (Figure 21B).

Example 24. Generation of PRRSV-permissive porcine kidney stable cell lines using pcDNA3.1D-humCD163v2

Parental Porcine Kidney (PK032495) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 5% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1x10⁶ cells each

were transfected with 2 micrograms per well of pcDNA3.1D-humCD163v2, in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS, removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104), diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 1.0 milligram per ml, and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for PRRSV-permissivity by infecting with PRRSV isolate P129 for a minimum 48 hours. Two clones were found to be permissive. One of these, designated "PK-cDNA3.1D-humCD163v2-B11" clearly showed the PRRSV-permissive phenotype.

Example 25 Generation of PRRSV-permissive feline kidney stable cell line using ligated pRSV-Script MARC CD163v2

A non-cloning based procedure to generate microgram quantities of linear DNA suitable for use in generating stable cell lines expressing CD163 from an RSV promoter was developed (Figure 4). A similar process was adapted to place simian CD163v2 from MARC-145 cells behind the RSV promoter. The procedure involves the isolation and ligation of two pieces of DNA, one containing the neomycin gene and RSV promoter cassette derived from pRSV-script, and the other containing the MARC CD163v2 coding sequence from pCDNA3.1D MARC CD163v2. Vector plasmid pRSV-Script was linearized with *Hind* III and *Kpn* I. Plasmid was first digested with *Kpn* I and was bunted with the Klenow fragment of *E. coli* DNA polymerase. This plasmid was then digested with *Hind* III immediately downstream of the RSV promoter. The pCDNA3.1D MARC CD163v2 clone was digested in the vector sequence downstream of the CD163 insert with *EcoRV*, and *Hind* III upstream of CD163. The CD163 coding sequence was liberated from the vector. For each plasmid digestion the appropriate fragments were purified from agarose gels. A large-scale ligation reaction was performed as follows. Approximately 20 µg of each DNA fragment was incubated in a volume of 600 µL with 15 units of T4 DNA ligase. The reaction was incubated at room temperature for 1 hour. Following ligation, a linear

piece of DNA containing all of the appropriate elements was purified by agarose gel electrophoresis. Restriction enzyme digestion analysis was performed to confirm the authenticity of each ligated fragment. Ligation of the two DNA fragments via the cohesive *Hind* III termini resulted in the placement of the 5' sequences of the MARC CD163 gene downstream of the RSV promoter, allowing for directed expression of CD163 in mammalian cells. Once isolated, the purified DNA was used to transfect selected mammalian cell lines.

Norden Labs Feline Kidney (NLFK) cells were grown at 37° C and 5% CO₂ in DMEM supplemented with 5% fetal bovine serum and antibiotics. NLFK cells were seeded in 6 well plates at approximately 90% confluency and allowed to attach overnight. The cells were then transfected with ligated plasmid pRSV-MARC CD163v2 using Lipofectamine 2000 following the manufacturer's instructions. After 24 hours the cells were cloned as described in Example 12. Screening for PRRSV permissive cell clones was performed as described in Example 12. One clone was positive for PRRSV infection and designated NLFK-MARC CD163 D4. This D4 clone has maintained the PRRSV permissive phenotype through 9 passages.

Example 26. Growth kinetics of PRRSV isolate NVSL 94-3 in recombinant BHK-21 and NLFK cells stably expressing susCD163v1 from the CMV promoter.

The amounts of progeny virus produced by PRRSV-infected BHK-21 or NLFK cells stably engineered to express susCD163v1 were quantitated. Four cell lines expressing susCD163v1, BHK/CMV/susv1 #3, BHK/CMV/susv1 #5, BHK/CMV/susv1 #12, and FK/CMV/susv1 G4 were seeded at sub confluency in 6 well plates and, after overnight incubation, were infected with the NVSL 94-3 isolate of PRRSV. MARC-145 cells were included in the experiment for comparison. The cells were infected with virus at an m.o.i. of approximately 0.1. Virus was adsorbed for 60-90 minutes and was removed. Cells were washed three times with PBS to remove residual virus. One-milliliter aliquots were harvested from the cultures at 12-hour intervals starting immediately after infection and continued through 96 hrs. Fresh culture media was added to the cells at various time points to maintain a culture volume sufficient to prevent the cell monolayer from drying out. Culture supernatants were stored at -80° until all samples were collected. The amount of PRRSV present in the culture supernatants was determined by plaque assay on MARC-145 cells. Figure

14 shows that all CD163 expressing recombinant cell lines tested were able to produce progeny PRRSV.

Example 27. Blocking PRRSV infection with anti-CD163 antibody: Transiently transfected cells.

5 BHK-21 cells, seeded in 24 well plates, were transiently transfected with the plasmid pCDNA3.1D-MARC-CD163v2 described in example 8, using Lipofectamine 2000 as described in example 14. After overnight incubation to allow expression of CD163, a titration of goat polyclonal antibody specific for human CD163 (R&D Systems, cat # AF1607) in PBS was added to the cells in a volume of 100 μ l. As a
10 control, equivalent amounts of normal goat IgG (R&D Systems, cat # AB-108-C) were used. Following a one-hour incubation at 37°C, the monolayers were infected with approximately 1×10^7 pfu of a recombinant P129 strain of PRRSV that expresses GFP. The cell monolayers, with anti-CD163 antibody and PRRSV, were incubated at 37°C for one hour at which time the virus inoculum/antibody mixture was aspirated,
15 the cell monolayer washed once with PBS, and 1ml of growth medium added to the wells. The cells were incubated for 24 hours at 37°C to allow PRRSV directed GFP expression. For analysis, the cells were trypsinized, resuspended in 500 μ l of PBS and analyzed by flow cytometry to innumerate the PRRSV infected cells via GFP expression. For flow cytometry, uninfected BHK-21 cells were used to set the
20 baseline for fluorescence detection, and approximately 100,000 cells were analyzed from each subsequent sample. The results of this analysis, shown in Figure 15, show that the CD163 specific antibody was able to significantly reduce the number of infected cells when compared to cells incubated with normal goat IgG.

Example 28: Blocking PRRSV infection by anti CD163 antibody: Stably transfected cells.

25 The NLFK cells that stably express human CD163 (FK-cDNA3.1D-humCD163v2-A6), described in Example 23, were seeded into 24 wells plates. After allowing the cells to attach overnight, a titration of goat polyclonal antibody specific for human CD163 (R&D Systems, cat # AF1607) in PBS was added to the cells in a
30 volume of 100 μ l. As a control, equivalent amounts of normal goat IgG (R&D Systems, cat # AB-108-C) were used. Following a one-hour incubation at 37°C, the monolayers were infected with approximately 1×10^7 pfu of a recombinant P129 strain of PRRSV that expresses GFP. The cell monolayers, with anti-CD163 antibody and

PRRSV, were incubated at 37°C for one hour at which time the virus inoculum/antibody mixture was aspirated, the cell monolayer washed once with PBS, and 1ml of growth medium added to the wells. The cells were incubated for 24 hours at 37°C to allow PRRSV directed GFP expression. For analysis, the cells were

5 trypsinized, resuspended in 500µl of PBS, and analyzed by flow cytometry to innumerate the PRRSV infected cells via GFP expression. Approximately 100,000 cells were analyzed from each sample. The results of this analysis, shown in Figure 16, show that the CD163 specific antibody was able to significantly reduce the number of infected cells when compared to cells incubated with normal goat IgG.

10 **Example 29. Generation of PRRSV-permissive porcine kidney stable cell lines using pRSV-susCD163v2.**

Porcine kidney cells (PK032495) were cultured as described in Example 21. For transfection, cells were seeded in a 24 well plate at 80% confluency and allowed to recover overnight. Transfection of ligated pRSV-susCD163v2 DNA described in

15 Example 5 was performed using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. Subsequent cloning and selection of PRRSV permissive cells was performed essentially as described in Example 14. The initial cloning by limiting dilution failed to yield single cell derived clones, so 5 wells with PRRSV permissive cells were recloned by limiting dilution to yield clonal cell lines. 10 clones

20 were selected for further study and one of these clones, PK-RSVScript-susCD163v2 #9 showed the ability to support foci growth of PRRSV early after infection (see Figure 18).

Example 30. Generation of PRRSV-permissive feline kidney stable cell lines using pRSV-susCD163v2.

25 NLFK feline kidney cells were cultured as described in Example 17. For transfection, cells were seeded at approximately 80% of maximal density in 24 well plates. After overnight incubation the monolayers were transfected with ligation derived RSV/susCD163v2 (see example 5) using Lipofectamine following the manufacturer's instructions. Cloning of the transfected cells and selection of PRRSV

30 permissive cell clones was performed essentially as described in Example 14. Of the 67 cell clones tested for PRRSV permissivity, 20 were found to be positive. An example of the staining observed is shown in Figure 19.

Example 31. Passage of PRRSV isolate P201 in PK-RSVScript-susCD163v2 cells
Amplification of a PRRSV clinical isolate was performed as follows. Peripheral

35 alveolar macrophage (PAM) cells were seeded at 5.4E6 cells per 10cm² in a 6 well

dish using OptiMEM media supplemented with 2% FBS. After 6 hours the media was aspirated and a 2 ml aliquot of serum harvested from a PRRSV infected pig was added to the cells. Following a 90 minute adsorption, the serum inoculum was removed and replaced with OptiMEM. At approximately 40 post infection the
5 supernatant was harvested and clarified with a 10 minute centrifugation. The supernatant was directly used to infect PK-RSVScript-susCD163v2 clone #9 cells using a 6 hour adsorption. After removal of the inoculum the cells were refed with D-MEM. The P201 virus was serially passaged on the PK-RSVScript-susCD163v2 #9 cell line using alternating infected cell and cell free supernatant passes. We observed
10 that for efficient spread of the virus, the cells should be seeded at 50-70 % confluency the day before infection, using flasks of cells that were kept at sub-confluency. To follow the progression of infection, each passage was replicated in multiple wells of identically infected cells and at each day one of the wells was acetone fixed and stained with the FITC labeled monoclonal antibody SDOW17. If the percentage of
15 infected cells was not greater than 50% and significant progression of foci development over the prior days observations were not seen, the cells in an equivalent well were trypsinized and passed to multiple fresh wells. These infected cell passages were typically at a 1:4 split and sometimes included the addition of an equivalent number of cells from an uninfected culture. Alternatively, if the SDOW17 staining
20 revealed that the infected cell foci had spread sufficiently to account for greater than 50% of the total cells, cell free supernatant was harvested and used to infect multiple wells of freshly seeded cells (Figure20). After 11 passages the intervening cell passages were not necessary as the virus was able to grow to sufficient titer to allow consecutive cell free supernatant passaging of the virus.

25 **Example 32. Screening various CD163 cell lines for permissivity to various European and North American PRRSV isolates**

Various CD163 transgenic cell lines were assessed for permissivity to low passage European and North American PRRSV isolates (see Table 7). Transgenic CD163 cell lines as described in earlier examples included NLFK-MARC CD163 D4, PK-RSVScript-susCD163v2clone #9 and PK-CMV-susCD163v1-A10. Each CD163 cell
30 line along with cell lines MARC-145, parental feline kidney, parental porcine kidney cell lines (serving as controls) were planted onto 96-well tissue culture plates. Growth media was removed from monolayers and inoculated with 0.1 mL per well of each PRRSV isolate. At day 3 post infection, the plates were fixed with 80% acetone and

stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc.) which is specific for the nucleocapsid. Results of the fluorescent antibody (FA) assay are in Table 7. **Table 7**

FA results of screening of various CD163 cell lines for permissivity to European and North American PRRSV isolates						
CD163 Cell Line	PRRSV Isolate ^a					
	EU98V226	P129	P201	1151	94-3	IND5
NLFK-MARC CD163 D4	++	+	+++	+++	++	++++
PK-RSVScript-susCD163v2clone #9	+	+	++	+	+	++
PK-CMV-susCD163v1-A10	+	+	++	++	++	++
MARC-145	++	+	+++	+	++++	+++
Porcine Kidney (parental)	-	-	-	-	-	-
Feline Kidney (parental)	-	-	-	-	-	-

5

^a All PRRSV isolates are North American except EU98V226 is a European isolate.

Example 33. Phorbol 12-myristate 13-acetate (PMA) induction of CD163 renders human U937 cells permissive to PRRSV infection.

Human U937 cells obtained from ATCC (CRL-1593.2) were propagated in RPMI medium containing serum and additives according to ATCC specifications. These cells are known to express CD163 when activated by PMA treatment (Gronlund et al., 2000). U937 cells were seeded in duplicate in wells of a 6-well plate. One set of wells was treated with 100 ng/ml of PMA and the other set was left untreated. Three days after PMA stimulation, one well from each set was infected with the P129 isolate of PRRSV. The other well from each set was fixed and stained for expression of CD163 in an indirect immunofluorescent antibody assay using goat-anti human CD163 (R&D System) and donkey anti-goat IgG conjugated with FITC (BioDesign International).

Untreated U937 cells continued propagation to high density 3 days after initial planting. PMA-treated U937 cells stopped propagating, became enlarged, and attached to the surface of the culture wells. A small fraction of untreated U937 were positive for CD163 staining, whereas almost all PMA-treated U937 were positive for CD163 staining. In untreated U937 no PRRSV infected cells were observed. However, hundreds of PMA treated U937 cells became infected by PRRSV. This demonstrates that non-permissive cells can be rendered permissive for PRRSV infection following chemical induction of CD163 expression.

Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional
5 embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations that are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations that have not been described herein as critical are intended as aspects of
10 the invention.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

15 The entire disclosure of all publications cited herein are hereby incorporated by reference to the extent they are not inconsistent to the disclosure herein.

What is claimed is:

1. A method of facilitating infection of a vertebrate cell by PRRSV that comprises the step of
(a) directing increased expression of a CD163 polypeptide by said vertebrate cell.
5
2. The method of claim 1 that further comprises infecting said cell with PRRSV.
3. The method of claim 1 that further comprises producing a culture of PRRSV.
- 10 4. The method of claim 1 in which said cell is mammalian.
5. The method of claim 1 wherein said CD163 polypeptide comprises a transmembrane domain.
- 15 6. The method of claim 1 wherein said increased expression is accomplished by introduction of exogenous nucleic acid.
7. The method of claim 1 wherein said increased expression is accomplished by chemical treatment.
20
8. The method of claim 1 wherein said cell was PRRSV non-permissive and is rendered PRRSV permissive by step (a).
9. The method of claim 2 in which the PRRSV is of the European genotype.
25
10. The method of claim 2 in which the PRRSV is of the North American genotype.
11. The method of any of claims 2-10 further comprising the step of producing a PRRSV vaccine.
30
12. The method of claims 11 wherein the vaccine is killed.
13. The method of claim 11 wherein the vaccine is live attenuated.

14. A method for measuring the propensity of a test cell line to allow infection by PRRSV comprising:

- a) providing a sample containing nucleic acids from the test cell line;
- b) determining the amount of polynucleotide encoding a CD163 polypeptide or its complement in said sample;

wherein an increased amount of polynucleotide encoding a CD163 polypeptide relative to a control sample derived from a control cell line known not to support the growth of said PRRSV indicates a propensity of the test cell line to support the replication of said PRRSV.

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15 A method for measuring the propensity of a test cell line to allow infection by PRRSV comprising:

- (a) providing a sample containing polypeptides from the test cell line;
- (b) determining the amount of CD163 polypeptide in said sample;

15 wherein an increased amount of a CD163 polypeptide relative to a control sample derived from a control cell line known not to support the growth of said PRRSV indicates a propensity of the test cell line to support the replication of said PRRSV.