The disclosure further relates to the use of the non-human mammal or an offspring thereof, or the tumor bearing non-human mammal, the animal model generated through the method as described herein in the development of a product related to an immunization processes of human cells, the manufacture of a human antibody, or the model system for a research in pharmacology, immunology, microbiology and medicine.

The disclosure also relates to the use of the non-human mammal or an offspring thereof, or the tumor bearing non-human mammal, the animal model generated through the method as described herein in the production and utilization of an animal experimental disease model of an immunization processes involving human cells, the study on a pathogen, or the development of a new diagnostic strategy and /or a therapeutic strategy.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DETAILED DESCRIPTION

Immunodeficient animals are an indispensable research tool for studying the mechanism of diseases, and methods of treating such diseases. They can easily accept xenogeneic cells or tissues due to their immunodeficiency, and have been widely used in the research. The commonly used immunodeficient animals include e.g., NOD-Prkdc^{scid} IL-2rγ^{null} mice, NOD-Rag 1$^{-/-}$-IL2rg$^{-/-}$ (NRG), Rag 2$^{-/-}$- IL2rg$^{-/-}$ (RG), NOD/SCID (NOD-Prkdc^{scid}), and NOD/SCID nude mice. Among them, NOD-Prkdc^{scid} IL-2rγ^{null} mice may be the best recipient mice for transplantation. Some of these mice are described in detail e.g., in Ito *et al*. "Current advances in

humanized mouse models." Cellular & molecular immunology 9.3 (2012): 208; and US20190320631A1, each of which is incorporated herein by reference in its entirety.

It has been well established that T cells recognize antigen in association with self MHC proteins but not in association with foreign MHC proteins: that is, T cells show MHC restriction. This restriction results from a process of positive selection during T cell development in the thymus. In this process, those immature T cells that will be capable of recognizing foreign peptides presented by self MHC proteins are selected to survive, while the remainder, which would be of no use to the animal, undergo apoptosis. Thus, MHC restriction is an acquired property of the immune system that emerges as T cells develop in the thymus. However, in these immunodeficient animals (e.g., NOD-Prkdc^{scid} IL-2rγ^{null}), the MHC of immunodeficient animals are still the endogenous MHC. After transplantation of human cells or tissues, human-derived immune cells cannot go through the restriction process mediated by human HLA in the animal's thymus, and cannot reflect the restriction of human MHC after immunotherapy or infection of pathogens. Thus, in some cases, the transplanted human T and B cells are not fully functionally mature in these immunodeficient animals.

This disclosure relates to genetically modified animals which express a human or chimeric (e.g., humanized) major histocompatibility complex (MHC) protein complex, and methods of use thereof. In some embodiments, the animal is an immunodeficient animal (e.g., with NOD-Prkdc^{scid} IL-2rγ^{null}, NOD-Rag 1$^{-/-}$-IL2rg$^{-/-}$ (NRG), Rag 2$^{-/-}$- IL2rg$^{-/-}$ (RG), or NOD/SCID (NOD-Prkdc^{scid}) background). The human or humanized MHC provides a better environment for the transplanted human immune cells (e.g., T cells and B cells) to mature, and particularly these animals provide a more actuate model for determining the efficacy of various therapies for human.

Major histocompatibility complex

The major histocompatibility complex (MHC) plays a key role in the defense mechanism of a body induced by T cell immune responses by presenting a cancer- or virus-derived antigen peptide. MHC is classified as class I or class II. Class I is expressed in all somatic cells except for germ cells and erythrocytes. MHC class I protein complex is composed of an α chain (alpha chain or heavy chain) and a smaller chain known as β2 microglobulin (B2M). The α chain is composed of α1 and α2 domains associated with the formation of an antigen peptide-holding

HLA-A nucleotide sequence (e.g., exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, or NM_001242758.1 (SEQ ID NO: 7)).

In some embodiments, the nucleic acid sequence has at least a portion (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 nucleotides, e.g., contiguous or non-contiguous nucleotides) that is the same as part of or the entire human HLA-A nucleotide sequence (e.g., exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, or NM_001242758.1 (SEQ ID NO: 7)).

In some embodiments, the amino acid sequence has at least a portion (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acid residues, e.g., contiguous or non-contiguous amino acid residues) that is different from part of or the entire mouse H2-D1 amino acid sequence (e.g., amino acids encoded by exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, and/or exon 8 of NM_010380.3 (SEQ ID NO: 5); or NP_034510.3 (SEQ ID NO: 6)).

In some embodiments, the amino acid sequence has at least a portion (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acid residues, e.g., contiguous or non-contiguous amino acid residues) that is the same as part of or the entire mouse H2-D1 amino acid sequence (e.g., amino acids encoded by exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, and/or exon 8 of NM_010380.3 (SEQ ID NO: 5); or NP_034510.3 (SEQ ID NO: 6)).

In some embodiments, the amino acid sequence has at least a portion (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acid residues, e.g., contiguous or non-contiguous amino acid residues) that is different from part of or the entire human HLA-A amino acid sequence (e.g., amino acids encoded by exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, and/or exon 8 of NM_001242758.1 (SEQ ID NO: ~~8~~7); NP_001229687.1 (SEQ ID NO: 8); or AAC24825.1 (SEQ ID NO: 59)).

In some embodiments, the amino acid sequence has at least a portion (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acid residues, e.g., contiguous or non-contiguous amino acid residues) that is the same as part of or the entire human HLA-A amino acid sequence (e.g., amino acids encoded by exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, and/or exon 8 of NM_001242758.1 (SEQ ID NO: 7~~8~~); NP_001229687.1 (SEQ ID NO: 8); or AAC24825.1 (SEQ ID NO: 59)).

from a human molecule where the humanized molecule is encoded by a nucleotide comprising a nucleic acid sequence that encodes the human molecule (or a portion thereof).

In some embodiments, the genetically-modified non-human animal does not express an endogenous B2M (e.g., mouse B2M). In some embodiments, the genetically-modified non-human animal does not express a functional endogenous B2M (e.g., mouse B2M). In some embodiments, the genetically-modified non-human animal does not express an endogenous MHC molecule (e.g., mouse H2-D1). In some embodiments, the genetically-modified non-human animal does not express a functional endogenous MHC molecule (e.g., mouse H2-D1) or a functional endogenous MHC protein complex.

In some embodiments, the genetically-modified non-human animal described herein is immunodeficient. In some embodiments, the animal has a NOD-Prkdc^{scid} IL-2rγ^{null}, NOD-Rag 1$^{-/-}$-IL2rg$^{-/-}$ (NRG), Rag 2$^{-/-}$- IL2rg$^{-/-}$ (RG), or NOD/SCID (NOD-Prkdc^{scid}) background.

In some embodiments , the genetically-modified non-human animal described herein (e.g., mouse) have a disrupted endogenous B2M gene. In some embodiments, the genetically-modified non-human animal described herein (e.g., mouse) expresses a dysfunctional endogenous B2M protein (e.g., mouse B2M). In some embodiments, the genetically-modified non-human animal described herein (e.g., mouse) have a disrupted endogenous MHC gene. In some embodiments, the genetically-modified non-human animal described herein (e.g., mouse) expresses a dysfunctional endogenous MHC molecule (e.g., mouse H2-D1) or a dysfunctional endogenous MHC protein complex.

As used herein, the term “leukocytes” or “white blood cells” include T cells (CD3+), B cells (CD19+), myeloid cells (CD33+), NK cells (CD56+), granulocytes (CD66b+), and monocytes (CD14+). All leukocytes have nuclei, which distinguishes them from the anucleated red blood cells (RBCs) and platelets. CD45, also known as leukocyte common antigen (LCA), is a cell surface marker for leukocytes. Lymphocyte is a subtype of leukocyte. Lymphocytes include natural killer (NK) cells (which function in cell-mediated, cytotoxic innate immunity), T cells, and B cells. Myeloid cell is a subtype of leukocyte. Myeloid cells include monocytes and granulocytes.

In some embodiments, the genetically-modified non-human animal is a mouse. In some embodiments, the genetically-modified non-human animal is a B-NDG mouse. Details of B-NDG mice can be found, e.g., in PCT/CN2018/079365; US10820580B2, each of which is

WHAT IS CLAIMED IS:

1. A genetically-modified non-human animal expressing a fusion protein comprising β2 microglobulin (B2M) and a human or humanized major histocompatibility complex (MHC) α chain.

2. The animal of claim 1, wherein the genome of the animal comprises at least one chromosome comprising a sequence encoding the fusion protein.

3. The animal of claim 1 or 2, wherein the fusion protein comprises a human or humanized B2M protein.

4. The animal of any one of claims 1-3, wherein the MHC α chain is a MHC class I α chain.

5. The animal of any one of claims 1-4, wherein the MHC α chain is a human HLA-A protein.

6. The animal of any one of claims 1-4, wherein the MHC α chain is a chimeric MHC α chain.

7. The animal of any one of claims 1-4, wherein the MHC α chain is a human HLA-A/mouse H2-D1 chimeric molecule.

8. The animal of claim 1, wherein the fusion protein comprises a human B2M protein and a chimeric MHC α chain comprising human HLA-A α1 and α2 domains.

9. The animal of claim 8, wherein the chimeric MHC α chain further comprises a mouse H2-D1 α3 domain.

10. The animal of claim 1, wherein the fusion protein comprises a human B2M protein and a human HLA-A protein.

11. The animal of any one of claims 2-10, wherein the sequence encoding the fusion protein is operably linked to an endogenous regulatory element (e.g., a promoter) at the endogenous β2 microglobulin (B2M) gene locus in the at least one chromosome.

12. The animal of any one of claims 2-10, wherein the sequence encoding the fusion protein is operably linked to an endogenous regulatory element (e.g., a promoter) at the endogenous MHC gene locus in the at least one chromosome.

13. The animal of any one of claims 2-10, wherein the animal is a mouse, and the sequence encoding the fusion protein is operably linked to an endogenous regulatory element at the mouse H2-D1 gene locus in the at least one chromosome.

14. The animal of any one of claims 5-13, wherein the human HLA-A is human HLA-A2.1.

15. The animal of any one of claims 5-13, wherein the human HLA-A is human HLA-A1*0101.

16. The animal of any one of claims 1-4, wherein the fusion protein comprises
(a) a human B2M; and
(b) a human HLA-A.

17. The animal of claim 16, wherein the human B2M and the human HLA-A are linked via a linker peptide sequence.

18. The animal of claim 16 or 17, wherein the human B2M comprises or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 4 or amino acids 21-119 of SEQ ID NO: 4.

19. The animal of any one of claims 16-18, wherein the human HLA-A is HLA-A2.1.

20. The animal of any one of claims 16-19, wherein the human HLA-A comprises or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 8, amino acids 25-365 of SEQ ID NO: 8, SEQ ID NO: 59, or amino acids 22-362 of SEQ ID NO: 59.

21. The animal of any one of claims 16-20, wherein the fusion protein comprises or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 62.

22. The animal of any one of claims 1-4, wherein the fusion protein comprises
(a) a human B2M; and
(b) a chimeric MHC α chain.

23. The animal of claim 22, wherein the human B2M and the chimeric MHC α chain are linked via a linker peptide sequence.

24. The animal of claim 22 or 23, wherein the human B2M comprises or consists of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 4 or amino acids 21-119 of SEQ ID NO: 4.

25. The animal of any one of claims 22-24, wherein the chimeric MHC α chain comprises human HLA-A α1 and α2 domains.

26. The animal of claim 25, wherein the chimeric MHC α chain further comprises a human HLA-A α3 domain.

27. The animal of claim 25, wherein the chimeric MHC α chain further comprises an endogenous MHC α3 domain and/or an endogenous MHC cytoplasmic region.

28. The animal of any one of claims 22-27, wherein the chimeric MHC α chain comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 8, amino acids 25-206 of SEQ ID NO: 8, SEQ ID NO: 59, or amino acids 22-203 of SEQ ID NO: 59.

29. The animal of any one of claims 22-28, wherein the chimeric MHC α chain comprises a α3 domain, a connecting peptide, a transmembrane region, and a cytoplasmic region of an endogenous MHC.

30. The animal of any one of claims 22-28, wherein the animal is a mouse, and the chimeric MHC α chain comprises a α3 domain, a connecting peptide, a transmembrane region, and a cytoplasmic region of mouse H2-D1.

31. The animal of any one of claims 22-30, wherein the chimeric MHC α chain comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to amino acids 207-362 of SEQ ID NO: 6.

32. The animal of any one of claims 22-31, wherein the chimeric MHC α chain comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 61 or SEQ ID NO: 63.

33. The animal of any one of claims 1-32, wherein the fusion protein further comprises a signal peptide of human HLA-A2.1 (e.g., at the N-terminus of the fusion protein).

34. The animal of claim 33, wherein the signal peptide comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to amino acids 1-21 of SEQ ID NO: 59.

35. The animal of any one of claims 1-34, wherein the animal is heterozygous with respect to the sequence encoding the fusion protein.

36. The animal of any one of claims 1-34, wherein the animal is homozygous with respect to the sequence encoding the fusion protein.

37. A genetically-modified, non-human animal whose genome comprises at least one chromosome comprising a sequence encoding a chimeric MHC α chain comprising a human HLA-A α1 domain, a human HLA-A α2 domain and an endogenous MHC α3 domain.

38. The animal of claim 37, wherein the sequence encoding the chimeric MHC α chain is operably linked to an endogenous regulatory element at the endogenous MHC α chain gene locus in the at least one chromosome.

39. The animal of claim 37 or 38, wherein the genome of the animal further comprises a sequence encoding a human B2M, wherein the human B2M and the chimeric MHC α chain can associate with each other, forming a functional MHC protein complex in the animal.

40. The animal of claim 39, wherein the sequence encoding the human B2M is operably linked to an endogenous regulatory element (e.g., a promoter) at the endogenous B2M gene locus.

41. The animal of any one of claims 37-40, wherein the animal is a mouse, and the sequence encoding the chimeric MHC α chain is operably linked to an endogenous regulatory element (e.g., a promoter) at the mouse H2-D1 gene locus.

42. The animal of any one of claims 37-41, wherein the human HLA-A is human HLA-A2.1.

43. A genetically-modified, non-human animal whose genome comprises at least one chromosome comprising a sequence encoding a human HLA-A.

44. The animal of claim 43, wherein the sequence encoding the human HLA-A is operably linked to an endogenous regulatory element at the endogenous MHC α chain gene locus in the at least one chromosome.

45. The animal of claim 43 or 44, wherein the genome of the animal further comprises a sequence encoding a human B2M, wherein the human B2M and the human HLA-A can associate with each other, forming a functional MHC protein complex in the animal.

46. The animal of claim 45, wherein the sequence encoding the human B2M is operably linked to an endogenous regulatory element (e.g., a promoter) at the endogenous B2M gene locus.

47. The animal of any one of claims 43-46, wherein the animal is a mouse, and the sequence encoding the human HLA-A is operably linked to an endogenous regulatory element (e.g., a promoter) at the mouse H2-D1 gene locus.

48. The animal of any one of claims 43-47, wherein the human HLA-A is human HLA-A2.1.

49. The animal of any one of claims 1-48, wherein the animal does not express endogenous B2M.

50. The animal of any one of claims 1-49, wherein the animal does not express endogenous MHC α chain.

51. The animal of any one of claims 1-50, wherein B2M and the MHC α chain can associate with each other, forming a functional MHC protein complex, wherein the protein complex can present a non-self antigen to the surface of one or more cells.

52. The animal of claim 51, wherein a human T cell (e.g., a cytotoxic T cell) can recognize the presented non-self antigen and initiate immune response.

53. The animal of claim 51, wherein an endogenous T cells (e.g., a cytotoxic T cell) can recognize the presented non-self antigen and initiate immune response.

54. The animal of any one claims 1-53, wherein the animal is a mammal, e.g., a monkey, a rodent or a mouse.

55. The animal of claim 54, wherein the animal is a mouse (e.g., with a C57BL/6 background).

56. The animal of any one of claims 1-55, wherein the animal is an immunodeficient mouse.

57. The animal of any one of claims 1-56, wherein the genome of the animal comprises a disruption in the animal's endogenous CD132 gene.

58. The animal of any one of claims 1-57, wherein the animal is a NOD/scid mouse, a NOD/scid nude mouse, or a B-NDG mouse.

59. The animal of any one of claims 1-58, wherein the animal further comprises a sequence encoding an additional human or chimeric protein.

60. The animal of claim 59, wherein the additional human or chimeric protein is programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), Lymphocyte Activating 3 (LAG-3), B And T Lymphocyte Associated (BTLA), Programmed Cell Death 1 Ligand 1 (PD-L1), CD27, CD28, SIRPα, CD47, THPO, CD137, CD154, T-Cell Immunoreceptor With Ig And ITIM Domains (TIGIT), T-cell Immunoglobulin and Mucin-Domain Containing-3 (TIM-3),

Glucocorticoid-Induced TNFR-Related Protein (GITR), Signal regulatory protein α(SIRPα) or TNF Receptor Superfamily Member 4 (OX40).

61. A method for making a genetically-modified, non-human animal, comprising:
replacing in at least one cell of the animal, at an endogenous B2M gene locus, a sequence encoding a region of endogenous B2M with a sequence encoding a human B2M or a sequence encoding a fusion protein comprising a human B2M and a human or humanized MHC α chain.

62. The method of claim 61, wherein the sequence encoding the region of endogenous B2M comprises all or a part of exon 1, exon 2, and exon 3 of endogenous B2M gene.

63. A method for making a genetically-modified, non-human animal, comprising:
replacing in at least one cell of the animal, at an endogenous MHC gene locus, a sequence encoding a region of endogenous MHC α chain with a sequence encoding a human MHC α chain or a sequence encoding a fusion protein comprising a human B2M and a human or humanized MHC α chain.

64. The method of claim 63, wherein the sequence encoding the region of endogenous MHC molecule comprises all or a part of exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, and exon 8 of endogenous MHC gene.

65. The method of claim 63, wherein the animal is mouse, and the sequence encoding the region of endogenous MHC comprises all or a part of exon 1, exon 2, exon 3 of mouse H2-D1 gene.

66. The method of any one of claims 61-65, wherein the sequence encoding the fusion protein comprises the following elements:
(a) exon 1, exon 2, and/or exon 3 of human B2M;
(b) an optional sequence encoding a linker peptide sequence; and
(c) exon 2 and/or exon 3 of human HLA-A2.1.

67. The method of claim 66, wherein the sequence encoding the fusion protein further comprises exon 4, exon 5, exon 6, exon 7, and/or exon 8 of the endogenous MHC molecule gene that is downstream of element (c).

68. The method of claim 66 or 67, wherein the animal is mouse, and the sequence encoding the fusion protein further comprises the 3' UTR of mouse H2-D1 gene.

69. The method of any one of claims 61-68, wherein the fusion protein comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

70. A method of determining effectiveness of an agent or a combination of agents for the treatment of cancer, comprising:
engrafting tumor cells to the animal of any one of claims 1-60, thereby forming one or more tumors in the animal;
administering the agent or the combination of agents to the animal; and
determining the inhibitory effects on the tumors.

71. The method of claim 70, wherein before engrafting the tumor cells to the animal, human peripheral blood cells (hPBMC) or human hematopoietic stem cells are injected to the animal.

72. The method of claim 70, wherein the tumor cells are from cancer cell lines.

73. The method of claim 70, wherein the tumor cells are from a tumor sample obtained from a human patient.

74. The method of claim 70, wherein the inhibitory effects are determined by measuring the tumor volume in the animal.

75. The method of claim 70, wherein the tumor cells are melanoma cells, lung cancer cells, primary lung carcinoma cells, non-small cell lung carcinoma (NSCLC) cells, small cell lung cancer (SCLC) cells, primary gastric carcinoma cells, bladder cancer cells, breast cancer cells, and/or prostate cancer cells.

76. A method of producing an animal comprising a human hemato-lymphoid system, the method comprising:
engrafting a population of cells comprising human hematopoietic cells or human peripheral blood cells into the animal of any one of claims 1-60.

77. The method of claim 76, wherein the human hemato-lymphoid system comprises human cells selected from the group consisting of hematopoietic stem cells, myeloid precursor cells, myeloid cells, dendritic cells, monocytes, granulocytes, neutrophils, mast cells, lymphocytes, and platelets.

78. The method of claim 76 or 77, further comprising: irradiating the animal prior to the engrafting.

79. A fusion protein comprising β2 microglobulin (B2M) and a human or humanized MHC α chain.

80. A nucleic acid encoding the fusion protein of claim 79.

81. A protein comprising an amino acid sequence, wherein the amino acid sequence is one of the following:
~~(f)~~(a) an amino acid sequence set forth in SEQ ID NO: 4, 8, 59, 61, 62, 63, or 64;
~~(g)~~(b) an amino acid sequence that is at least 90% identical to SEQ ID NO: 4, 8, 59, 61, 62, 63, or 64;

~~(h)~~(c) an amino acid sequence that is at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 4, 8, 59, 61, 62, 63, or 64;

~~(i)~~(d) an amino acid sequence that is different from the amino acid sequence set forth in SEQ ID NO: 4, 8, 59, 61, 62, 63, or 64 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid; and

~~(j)~~(e) an amino acid sequence that comprises a substitution, a deletion and /or insertion of one, two, three, four, five or more amino acids to the amino acid sequence set forth in SEQ ID NO: 4, 8, 59, 61, 62, 63, or 64.

82. A nucleic acid comprising a nucleotide sequence, wherein the nucleotide sequence is one of the following:

~~(e)~~(a) a sequence that encodes the protein of claim 81;

~~(f)~~(b) SEQ ID NO: 9, 10, 13, 14, 15, 16, 52, 54, or 65;

~~(g)~~(c) a sequence that is at least 90% identical to SEQ ID NO: 9, 10, 13, 14, 15, 16, 52, 54, or 65; and

~~(h)~~(d) a sequence that is at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 9, 10, 13, 14, 15, 16, 52, 54, or 65.

83. A cell comprising the protein of claim 81 and/or the nucleic acid of claim 82.

84. An animal comprising the protein of claim 81 and/or the nucleic acid of claim 82.