activity. RNAi knock-down of *TMPRSS4* in Caco-2 cells resulted in reduced spread of the virus. In addition, TMPRSS4 was shown to be strongly upregulated in the lungs of mice infected with influenza (Böttcher *et al.* 2006 *J. Virol.* 80:9896; Böttcher *et al.* 2009 *Vaccine* 27: 6324; Böttcher-Friebershäusser *et al.* 2010 *J. Virol.* 84: 5604; Bertam *et al.* 2010 *J. Virol.* 84:10016; Bertam *et al.* 2010 *J. Virol.* 84:10016; Böttcher-Friebershäusser *et al.* 2011 *J. Virol.* 85: 1554; Bahgat *et al.* 2011 *Virol. J.* 8:27).

[0004] Development of an *in vivo* system, e.g., a rodent model of infection, is needed in order to identify and test compounds including antibodies that specifically target human type II transmembrane serine proteases for the treatment and prevention of viral infection and other diseases.

SUMMARY

[0005] The present disclosure encompasses the recognition that it is desirable to engineer rodent animals to provide *in vivo* systems for identifying and developing new therapeutics. For example, the present disclosure encompasses the recognition that rodents having a humanized *Tmprss* gene are desirable for use in identifying and developing therapeutics for the treatment and prevention of viral infections.

[0005a] In one aspect, the disclosure provides a rodent whose genome comprises a humanized *Tmprss* gene, wherein the humanized *Tmprss* gene

comprises a nucleotide sequence of an endogenous rodent *Tmprss* gene and a nucleotide sequence of a cognate human *TMPRSS* gene,

is located at an endogenous rodent *Tmprss* locus, and results from a replacement of a genomic sequence of the endogenous rodent *Tmprss* gene with said nucleotide sequence of the cognate human *TMPRSS* gene and is under control of the promoter of the endogenous rodent *Tmprss* gene,

and encodes a humanized Tmprss protein that comprises: (i) an ectodomain that has at least 90% sequence identity to the ectodomain of the human TMPRSS protein encoded by the cognate human *TMPRSS* gene and (ii) a cytoplasmic and transmembrane portion that has at

least 90% sequence identity to the cytoplasmic and transmembrane portion of the endogenous rodent Tmprss protein encoded by the endogenous rodent *Tmprss* gene.

[0005b] In another aspect, the disclosure provides a method of making a rodent having a humanized *Tmprss* gene, comprising:

(a) inserting a genomic fragment into an endogenous rodent *Tmprss* locus in a rodent embryonic stem cell, said genomic fragment comprising a nucleotide sequence of a cognate human *TMPRSS* gene, thereby forming a humanized *Tmprss* gene,

wherein the humanized *Tmprss* gene is under control of the promoter of the rodent *Tmprss* gene at the endogenous rodent *Tmprss* locus; and encodes a humanized Tmprss protein, and

wherein the humanized Tmprss protein comprises (i) an ectodomain that has at least 90% sequence identity to the ectodomain of the human TMPRSS protein encoded by the cognate human *TMPRSS* gene, and (ii) a cytoplasmic and transmembrane portion that has at least 90% sequence identity to the cytoplasmic and transmembrane portion of the rodent Tmprss protein encoded by the rodent *Tmprss* gene at said endogenous rodent *Tmprss* locus;

(b) obtaining a rodent embryonic stem cell comprising the humanized *Tmprss* gene of (a); and,

(c) generating a rodent using the rodent embryonic stem cell of (b).

[0005c] In another aspect, the disclosure provides a method of assessing the therapeutic efficacy of a compound in treating influenza A virus infection, comprising:

providing a rodent according to the disclosure;

administering an influenza A virus and a candidate compound to the rodent; and

monitoring the presence and severity of influenza A virus infection in the rodent to determine the therapeutic efficacy of the candidate compound.

[0005d] In another aspect, the disclosure provides an isolated cell or tissue of a rodent according to the disclosure, wherein the genome of the cell or tissue comprises the humanized *Tmprss* gene.

[0005e] In another aspect, the disclosure provides an isolated rodent embryonic stem (ES) cell comprising a humanized *Tmprss* gene, wherein the humanized *Tmprss* gene

comprises a nucleotide sequence of an endogenous rodent *Tmprss* gene and a nucleotide sequence of a cognate human *TMPRSS* gene,

is located at an endogenous rodent *Tmprss* locus, and results from a replacement of a genomic sequence of the endogenous rodent *Tmprss* gene with said nucleotide sequence of the cognate human *TMPRSS* gene and is under control of the promoter of the endogenous rodent *Tmprss* gene, and

encodes a humanized Tmprss protein that comprises: (i) an ectodomain that has at least 90% sequence identity to the ectodomain of the human TMPRSS protein encoded by the cognate human *TMPRSS* gene, and (ii) a cytoplasmic and transmembrane portion that has at least 90% sequence identity to the cytoplasmic and transmembrane portion of the endogenous rodent Tmprss protein encoded by the endogenous rodent *Tmprss* gene.

[0005f] In another aspect, the disclosure provides a rodent embryo comprising the rodent ES cell according to the disclosure.

[0005g] In another aspect, the disclosure provides a nucleic acid vector, comprising

a nucleic acid encoding the ectodomain of a human TMPRSS protein, flanked by a 5' nucleotide sequence and a 3' nucleotide sequence,

wherein the 5' nucleotide sequence and the 3' nucleotide sequence are homologous to genomic DNA sequences flanking a rodent genomic DNA at a rodent *Tmprss* locus encoding the ectodomain of a cognate rodent Tmprss protein, and are capable of mediating homologous recombination and replacement of the rodent genomic DNA with the nucleic acid encoding the ectodomain of a human TMPRSS protein to form a humanized *Tmprss* gene; and

wherein the rodent is mouse or rat.

[0006] In one example, the disclosure provides a rodent whose genome contains a humanized *Tmprss* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss* gene and a nucleotide sequence of a cognate human *TMPRSS* gene, wherein the

humanized *Tmprss* gene is under control of a 5' regulatory sequence(s), such as the promoter and/or enhancer(s), of the endogenous rodent *Tmprss* gene.

[0007] In some examples, the humanized *Tmprss* gene in rodents disclosed herein encodes a humanized Tmprss protein that contains an ectodomain substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical in sequence) to the ectodomain of a human TMPRSS protein. In some examples, the humanized Tmprss protein contains a cytoplasmic and transmembrane portion that is substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical in sequence) to the cytoplasmic and transmembrane portion of an endogenous rodent Tmprss protein.

[0008] In some examples, a rodent disclosed herein contains a humanized *Tmprss* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss* gene and a nucleotide sequence of a cognate human *TMPRSS* gene, wherein the nucleotide sequence of the cognate human *TMPRSS* gene encodes a polypeptide substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical in sequence) to the ectodomain of the human TMPRSS protein encoded by the cognate human *TMPRSS* gene. In some examples, a rodent disclosed herein contains a humanized *Tmprss* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss* gene and a nucleotide sequence of a cognate human *TMPRSS* gene, wherein the nucleotide sequence of the endogenous rodent *Tmprss* gene encodes a polypeptide substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical in sequence) to the cytoplasmic and transmembrane portion of the endogenous rodent Tmprss protein encoded by the endogenous rodent *Tmprss* gene.

[0009] In some examples, a rodent disclosed herein contains a humanized *Tmprss* gene located at an endogenous rodent *Tmprss* locus that results from a replacement of a contiguous genomic sequence of an endogenous rodent *Tmprss* gene with a contiguous genomic sequence of a cognate human *TMPRSS* gene. In specific examples, the contiguous genomic sequence of a cognate human *TMPRSS* gene being inserted includes exon sequences encoding an ectodomain substantially identical with the ectodomain of the human *TMPRSS* protein encoded by human *TMPRSS* gene. In some examples, the contiguous genomic sequence of a cognate human *TMPRSS* gene also includes the 3' UTR of the cognate human *TMPRSS* gene.

[0010] In some examples, a rodent disclosed herein is heterozygous for a humanized *Tmprss* gene at an endogenous rodent *Tmprss* locus. In other examples, a rodent is homozygous for a humanized *Tmprss* gene at an endogenous rodent *Tmprss* locus.

[0011] In further examples, a rodent contains two or more humanized *Tmprss* genes at different endogenous rodent *Tmprss* loci with each endogenous rodent *Tmprss* locus being humanized with a respective cognate human *TMPRSS* gene; for example, two or more of humanized *Tmprss2*, humanized *Tmprss4*, and humanized *Tmprss11d* genes.

[0012] In some examples, a rodent disclosed herein contains a humanized *Tmprss2* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss2* gene and a nucleotide sequence of a human *TMPRSS2* gene, wherein the humanized *Tmprss2* gene is under control of the promoter of the endogenous rodent *Tmprss2* gene.

[0013] In some examples, the humanized *Tmprss2* gene encodes a humanized Tmprss2 protein that contains an ectodomain substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical in sequence) with the ectodomain of the human TMPRSS2 protein encoded by the human *TMPRSS2* gene used in humanization. The human TMPRSS2 protein contains, in some examples, an amino acid sequence at least 85% identical (e.g., at least 90%, 95%, 98%, 99% or 100% identical) with the amino acid sequence as set forth in SEQ ID NO: 4. In some examples, a humanized Tmprss2 protein contains an ectodomain substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical) with the amino acid sequence composed of residues W106 to G492 or the C-terminal 387 amino acids of a human TMPRSS2 protein as set forth in, e.g., SEQ ID NO: 4. In some examples, the humanized *Tmprss2* gene encodes a humanized Tmprss2 protein that further contains a cytoplasmic and transmembrane portion that is substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical) with the cytoplasmic and transmembrane portion of the rodent Tmprss2 protein encoded by the endogenous rodent *Tmprss2* gene being humanized. An exemplary endogenous rodent Tmprss2 protein is set forth in SEQ ID NO: 2.

[0014] In some examples, a rodent contains a humanized *Tmprss2* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss2* gene and a nucleotide sequence of a human *TMPRSS2* gene, wherein the nucleotide sequence of the human *TMPRSS2* gene encodes an ectodomain substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical in sequence) with the ectodomain of the human TMPRSS2 protein encoded by the human *TMPRSS2* gene. In specific examples, the nucleotide sequence of a human *TMPRSS2* gene is a contiguous genomic sequence of a human *TMPRSS2* gene containing coding exon 4 through the stop codon in coding exon 13 of the human *TMPRSS2* gene. In particular examples, the contiguous genomic sequence of a human *TMPRSS2* gene further contains the 3' UTR of the human *TMPRSS2* gene. In some examples, the

nucleotide sequence of an endogenous rodent *Tmprss2* gene included in a humanized *Tmprss2* gene encodes a cytoplasmic and transmembrane portion that is substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical) with the cytoplasmic and transmembrane portion of the endogenous rodent Tmprss2 protein encoded by the endogenous rodent *Tmprss2* gene.

[0015] In particular examples, a humanized *Tmprss2* gene contains coding exons 1-2 of an endogenous rodent *Tmprss2* gene, and coding exon 4 through coding exon 13 of a human *TMPRSS2* gene, wherein the humanized *Tmprss2* gene encodes a humanized Tmprss2 protein that contains a cytoplasmic and transmembrane portion that is substantially identical with the cytoplasmic and transmembrane portion of the rodent Tmprss2 protein encoded by the endogenous rodent *Tmprss2* gene, and an ectodomain that is substantially identical with the ectodomain of the human TMPRSS2 protein encoded by the human *TMPRSS2* gene. The humanized *Tmprss2* gene contains an exon 3 that in some examples is coding exon 3 of a human *TMPRSS2* gene, and in other examples is coding exon 3 of an endogenous rodent *Tmprss2* gene. In some examples, the humanized *Tmprss2* gene contains an exon 3 that includes a 5' portion of coding exon 3 of an endogenous rodent *Tmprss2* gene and a 3' portion of coding exon 3 of a human *TMPRSS2* gene.

[0016] In some examples, a rodent disclosed herein contains a humanized *Tmprss4* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss4* gene and a nucleotide sequence of a human *TMPRSS4* gene, wherein the humanized *Tmprss4* gene is under control of the promoter of the endogenous rodent *Tmprss4* gene.

[0017] In some examples, the humanized *Tmprss4* gene encodes a humanized Tmprss4 protein that contains an ectodomain substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical in sequence) with the ectodomain of the human TMPRSS4 protein encoded by the human *TMPRSS4* gene used in humanization. The human TMPRSS4 protein contains, in some examples, an amino acid sequence at least 85% identical (e.g., at least 90%, 95%, 98%, 99% or 100% identical) with the amino acid sequence as set forth in SEQ ID NO: 11. In some examples, a humanized Tmprss4 protein contains an ectodomain substantially identical (e.g., at least 85%, 90%, 95%, 98%,

99% or 100% identical) with the amino acid sequence composed of residues K54 to L437 or the C-terminal 384 amino acids of a human TMPRSS4 protein as set forth in, e.g., SEQ ID NO: 11. In some examples, the humanized *Tmprss4* gene encodes a humanized Tmprss4 protein that further contains a cytoplasmic and transmembrane portion that is substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical) with the cytoplasmic and transmembrane portion of the rodent Tmprss4 protein encoded by the endogenous rodent *Tmprss4* gene being humanized. An exemplary endogenous rodent Tmprss4 protein is set forth in SEQ ID NO: 9.

[0018] In some examples, a rodent contains a humanized *Tmprss4* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss4* gene and a nucleotide sequence of a human *TMPRSS4* gene, wherein the nucleotide sequence of a human *TMPRSS4* gene encodes an ectodomain substantially identical with the ectodomain of the human TMPRSS4 protein encoded by the human *TMPRSS4* gene. In specific examples, the nucleotide sequence of a human *TMPRSS4* gene is a contiguous genomic sequence containing coding exon 4 through the stop codon in coding exon 13 of a human *TMPRSS4* gene. In some examples, the nucleotide sequence of an endogenous rodent *Tmprss4* gene included in a humanized *Tmprss4* gene encodes a cytoplasmic and transmembrane portion that is substantially identical with the cytoplasmic and transmembrane portion of the rodent Tmprss4 protein encoded by the endogenous rodent *Tmprss4* gene.

[0019] In particular examples, a humanized *Tmprss4* gene contains coding exon 1 through coding exon 3 of an endogenous rodent *Tmprss4* gene, and coding exon 4 through the stop codon in coding exon 13 of a human *TMPRSS4* gene.

[0020] In some examples, a rodent disclosed herein contains a humanized *Tmprss11d* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss11d* gene and a nucleotide sequence of a human *TMPRSS11D* gene, wherein the humanized *Tmprss11d* gene is under control of the promoter of the endogenous rodent *Tmprss11d* gene.

[0021] In some examples, the humanized *Tmprss11d* gene encodes a humanized Tmprss11d protein that contains an ectodomain substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical in sequence) with the ectodomain of the human

TMPRSS11D protein encoded by the human *TMPRSS11D* gene used in humanization. The human TMPRSS11D protein contains, in some examples, an amino acid sequence at least 85% identical (e.g., at least 90%, 95%, 98%, 99% or 100% identical) with the amino acid sequence as set forth in SEQ ID NO: 18. In some examples, a humanized Tmprss11d protein contains an ectodomain substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical) with the amino acid sequence composed of residues A42-I418 or the C-terminal 377 amino acids of a human TMPRSS11D protein as set forth in, e.g., SEQ ID NO: 18. In some examples, the humanized *Tmprss11d* gene encodes a humanized Tmprss11d protein that further contains a cytoplasmic and transmembrane portion that is substantially identical (e.g., at least 85%, 90%, 95%, 98%, 99% or 100% identical) with the cytoplasmic and transmembrane portion of the endogenous rodent Tmprss11d protein encoded by the endogenous rodent *Tmprss11d* gene being humanized. An exemplary endogenous rodent Tmprss11d protein is set forth in SEQ ID NO: 16.

[0022] In some examples, a rodent contains a humanized *Tmprss11d* gene that includes a nucleotide sequence of an endogenous rodent *Tmprss11d* gene and a nucleotide sequence of a human *TMPRSS11D* gene, wherein the nucleotide sequence of the human *TMPRSS11D* gene encodes an ectodomain substantially identical with the ectodomain of the human TMPRSS11D protein encoded by the human *TMPRSS11D* gene. In specific examples, the nucleotide sequence of a human *TMPRSS11d* gene is a contiguous genomic sequence containing coding exon 3 through the stop codon in coding exon 10 of a human *TMPRSS11D* gene. In particular examples, the contiguous genomic sequence of a human *TMPRSS11D* gene further contains the 3' UTR of the human *TMPRSS11D* gene. In some examples, the nucleotide sequence of an endogenous rodent *Tmprss11d* gene included in a humanized *Tmprss11d* gene encodes a cytoplasmic and transmembrane portion that is substantially identical with the cytoplasmic and transmembrane portion of the rodent Tmprss11d protein encoded by the endogenous rodent *Tmprss11d* gene.

[0023] In particular examples, a humanized *Tmprss11d* gene contains coding exons 1-2 of an endogenous rodent *Tmprss11d* gene, and coding exon 3 through coding exon 13 of a human *TMPRSS11D* gene.

[0024] In another example, the disclosure provides an isolated rodent cell or tissue whose genome contains a humanized *Tmprss* gene as described herein. In specific examples, the humanized *Tmprss* gene is selected from the group consisting of a humanized *Tmprss2* gene, a humanized *Tmprss4* gene, and a humanized *Tmprss11d* gene.

[0025] In still another example, the disclosure provides a rodent embryonic stem cell whose genome contains a humanized *Tmprss* gene as described herein. In specific examples, the humanized *Tmprss* gene is selected from the group consisting of a humanized *Tmprss2* gene, a humanized *Tmprss4* gene, and a humanized *Tmprss11d* gene.

[0026] In another example, a rodent embryo generated from the rodent embryonic stem cell disclosed herein is also provided.

[0027] In one example, the disclosure provides a nucleic acid vector suitable for use in humanizing an endogenous *Tmprss* gene in a rodent. In some examples, the nucleic acid vector includes a human *Tmprss* nucleic acid sequence (e.g., a human genomic DNA encoding the ectodomain of a human TMPRSS protein), flanked by a 5' homology arm and a 3' homology arm. The 5' and 3' homology arms are nucleic acid sequences that are placed at 5' and 3', respectively, to the human *Tmprss* nucleic acid sequence and are homologous to genomic DNA sequences at an endogenous *Tmprss* locus in a rodent that flank a rodent genomic DNA encoding the ectodomain of a cognate rodent Tmprss protein. Thus, the 5' and 3' homology arms are capable of mediating homologous recombination and replacement of the rodent genomic DNA encoding the ectodomain of the cognate rodent Tmprss protein with the human *Tmprss* nucleic acid sequence to form a humanized *Tmprss* gene as described herein.

[0028] In a further example, the disclosure is directed to a method of providing a rodent whose genome contains a humanized *Tmprss* gene. The method includes modifying the genome of a rodent to replace a genomic sequence of an endogenous rodent *Tmprss* gene with a genomic sequence of a cognate human *TMPRSS* gene to form a humanized *Tmprss* gene.

[0029] In some examples, the disclosure provides a method of making a rodent (such as a mouse or a rat) having a humanized *Tmprss* gene, the method including the steps of (a)

inserting a genomic fragment into an endogenous rodent *Tmprss* locus in a rodent embryonic stem cell, wherein the genomic fragment contains a nucleotide sequence of a cognate human *TMPRSS* gene, thereby forming a humanized *Tmprss* gene (such as those described herein); (b) obtaining a rodent embryonic stem cell comprising the humanized *Tmprss* gene of (a); and (c) creating a rodent using the rodent embryonic stem cell of (b).

[0030] In some examples, the humanized *Tmprss* gene is selected from the group consisting of a humanized *Tmprss2* gene, a humanized *Tmprss4* gene, and a humanized *Tmprss11d* gene. In various examples, the humanized *Tmprss* gene encodes a humanized Tmprss protein that contains an ectodomain substantially identical (e.g., at least 90%, 95%, 98%, 99% or 100% identical in sequence) to the ectodomain of the human TMPRSS protein encoded by the human *TMPRSS* gene used for humanization. In specific examples, the humanized Tmprss protein contains the ectodomain of a human TMPRSS protein selected from the group consisting of a human TMPRSS2 protein, a human TMPRSS4 protein, and a human TMPRSS11D protein. In specific examples, the humanized Tmprss protein further contains a cytoplasmic and transmembrane portion that is substantially identical with the cytoplasmic and transmembrane portion of the rodent Tmprss protein encoded by the endogenous rodent *Tmprss* gene being humanized.

[0031] In another example, the disclosure provides a method of using a rodent disclosed herein to assess the therapeutic efficacy of a compound (e.g., candidate inhibitors that specifically target a human TMPRSS protein) in treating influenza virus infection. The method can include the steps of providing a rodent described herein, administering an influenza virus and a candidate compound to the rodent; and monitoring the presence and severity of influenza virus infection in the rodent to determine the therapeutic efficacy of the drug candidate.

[0032] In some examples, the influenza virus is administered to the rodent before the compound. In other examples, the influenza virus is administered to the rodent after the compound.

[0033] In some examples, the candidate compound is an antibody or antigen-binding fragment thereof specific for a human TMPRSS protein. In specific examples, the

candidate compound is an antibody or antigen-binding fragment thereof specific for a human TMPRSS protein selected from the group consisting of a human TMPRSS2 protein, a human TMPRSS4 protein, and a human TMPRSS11D protein.

[0034] Other features, objects, and advantages of the present disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating examples of the present disclosure, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the disclosure will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The Drawings included herein, which are composed of the following Figures, are for illustration purposes only and not for limitation.

[0036] **Figures 1A-1D.** Exemplary strategy for humanization of mouse *Tmprss2*.

[0037] Figure 1A shows a diagram, not to scale, of the genomic organization of mouse *Tmprss2* and human *TMPRSS2* genes. Exons are represented by thin bars placed across the genomic sequences, with the first coding exon for both genes indicated by the start codon "ATG" above the exon, and the last coding exon indicated by the "Stop" codon above the exon. A mouse genomic fragment of about 25,291 bp to be deleted and a human genomic fragment of about 25,091 bp to be inserted are indicated. Locations of probes used in an assay described in Example 1 are indicated. TM: transmembrane domain; SRCR: scavenger receptor cysteine-rich like domain; LDLRa: low density lipoprotein receptor class A.

[0038] Figure 1B illustrates, not to scale, an exemplary modified BAC vector for humanization of an endogenous mouse *Tmprss2* gene, along with the junction sequences (SEQ ID NOS: 22, 23 and 24).

[0039] Figure 1C illustrates, not to scale, a humanized *Tmprss2* allele after the neomycin cassette has been deleted, along with the junction sequences (SEQ ID NOS: 22 and 25).

[0040] Figure 1D sets forth a sequence alignment of a human TMPRSS2 protein (SEQ ID NO: 4), a mouse Tmprss2 protein (SEQ ID NO: 2), and a humanized Tmprss2 protein ("7010 mutant pro") (SEQ ID NO: 7).

WHAT IS CLAIMED IS:

1. A rodent whose genome comprises a humanized *Tmprss* gene, wherein the humanized *Tmprss* gene

comprises a nucleotide sequence of an endogenous rodent *Tmprss* gene and a nucleotide sequence of a cognate human *TMPRSS* gene,

is located at an endogenous rodent *Tmprss* locus, and results from a replacement of a genomic sequence of the endogenous rodent *Tmprss* gene with said nucleotide sequence of the cognate human *TMPRSS* gene and is under control of the promoter of the endogenous rodent *Tmprss* gene,

and encodes a humanized Tmprss protein that comprises: (i) an ectodomain that has at least 90% sequence identity to the ectodomain of the human TMPRSS protein encoded by the cognate human *TMPRSS* gene and (ii) a cytoplasmic and transmembrane portion that has at least 90% sequence identity to the cytoplasmic and transmembrane portion of the endogenous rodent Tmprss protein encoded by the endogenous rodent *Tmprss* gene.

2. The rodent of claim 1, wherein the humanized *Tmprss* gene is a humanized *Tmprss2* gene, the endogenous rodent *Tmprss* gene is an endogenous rodent *Tmprss2* gene, and the cognate human *TMPRSS* gene is a human *TMPRSS2* gene.

3. The rodent of claim 2, wherein the nucleotide sequence of the human *TMPRSS2* gene comprises coding exon 4 through the stop codon in coding exon 13 of the human *TMPRSS2* gene. 4. The rodent of claim 2, wherein the humanized *Tmprss2* gene comprises

(i) coding exons 1-2 of the endogenous rodent *Tmprss2* gene,

(ii) an exon 3 that comprises a 5' portion of coding exon 3 of the endogenous rodent *Tmprss2* gene and a 3' portion of coding exon 3 of the human *TMPRSS2* gene, and

(iii) coding exon 4 through coding exon 13 of the human *TMPRSS2* gene, and

wherein the humanized *Tmprss2* gene encodes a humanized Tmprss2 protein that comprises a cytoplasmic and transmembrane portion that has at least 90% sequence identity

with the cytoplasmic and transmembrane portion of the rodent Tmprss2 protein encoded by said endogenous rodent *Tmprss2* gene, and an ectodomain that has at least 90% sequence identity with the ectodomain of the human TMPRSS2 protein encoded by said human *TMPRSS2* gene.

5. The rodent of claim 1, wherein the humanized *Tmprss* gene is a humanized *Tmprss4* gene, the endogenous rodent *Tmprss* gene is an endogenous rodent *Tmprss4* gene, and the cognate human *TMPRSS* gene is a human *TMPRSS4* gene.

6. The rodent of claim 5, wherein the nucleotide sequence of the human *TMPRSS4* gene comprises coding exon 4 through the stop codon in coding exon 13 of the human *TMPRSS4* gene.

7. The rodent of claim 5, wherein the humanized *Tmprss4* gene comprises coding exon 1 through coding exon 3 of the endogenous rodent *Tmprss4* gene, and coding exon 4 through the stop codon in coding exon 13 of the human *TMPRSS4* gene.

8. The rodent of claim 1, wherein the humanized *Tmprss* gene is a humanized *Tmprss11d* gene, the endogenous rodent *Tmprss* gene is an endogenous rodent *Tmprss11d* gene, and the cognate human *TMPRSS* gene is a human *TMPRSS11D* gene.

9. The rodent of claim 8, wherein the nucleotide sequence of the human *TMPRSS11D* gene comprises coding exon 3 through the stop codon in coding exon 10 of the human *TMPRSS11D* gene.

10. The rodent of claim 8, wherein the humanized *Tmprss11d* gene comprises coding exons 1-2 of the endogenous rodent *Tmprss11d* gene, and coding exons 3 through coding exon 10 of the human *TMPRSS11D* gene.

11. The rodent according to any one of the preceding claims, wherein the rodent is homozygous for the humanized *Tmprss* gene.

12. The rodent according to any one of claims 1-11, wherein the rodent is a mouse.

13. The rodent according to any one of claims 1-11, wherein the rodent is a rat.

14. A method of making a rodent having a humanized *Tmprss* gene, comprising:

(a) inserting a genomic fragment into an endogenous rodent *Tmprss* locus in a rodent embryonic stem cell, said genomic fragment comprising a nucleotide sequence of a cognate human *TMPRSS* gene, thereby forming a humanized *Tmprss* gene,

wherein the humanized *Tmprss* gene is under control of the promoter of the rodent *Tmprss* gene at the endogenous rodent *Tmprss* locus; and encodes a humanized Tmprss protein, and

wherein the humanized Tmprss protein comprises (i) an ectodomain that has at least 90% sequence identity to the ectodomain of the human TMPRSS protein encoded by the cognate human *TMPRSS* gene, and (ii) a cytoplasmic and transmembrane portion that has at least 90% sequence identity to the cytoplasmic and transmembrane portion of the rodent Tmprss protein encoded by the rodent *Tmprss* gene at said endogenous rodent *Tmprss* locus;

(b) obtaining a rodent embryonic stem cell comprising the humanized *Tmprss* gene of (a); and,

(c) generating a rodent using the rodent embryonic stem cell of (b).

15. The method of claim 14, wherein the humanized *Tmprss* gene is a humanized *Tmprss2* gene.

16. The method of claim 15, wherein the humanized *Tmprss2* gene comprises (i) coding exons 1-2 of the endogenous rodent *Tmprss2* gene, (ii) an exon 3 that comprises a 5' portion of coding exon 3 of the endogenous rodent *Tmprss2* gene and a 3' portion of coding exon 3 of the human *TMPRSS2* gene, and (iii) coding exon 4 through coding exon 13 of the human *TMPRSS2* gene.

17. The method of claim 14, wherein the humanized *Tmprss* gene is a humanized *Tmprss4* gene.

18. The method of claim 17, wherein the humanized *Tmprss4* gene comprises coding exon 1 through coding exon 3 of the endogenous rodent *Tmprss4* gene, and coding exon 4 through the stop codon in coding exon 13 of the human *TMPRSS4* gene.

19. The method of claim 14, wherein the humanized *Tmprss* gene is a humanized *Tmprss11d* gene.

20. The method of claim 19, wherein the humanized *Tmprss11d* gene comprises coding exons 1-2 of the endogenous rodent *Tmprss11d* gene, and coding exons 3 through coding exon 10 of the human *TMPRSS11D* gene.

21. The method according to any one of claims 14 to 20 , wherein the rodent is a mouse.

22. The method according to any one of claims 14 to 20 , wherein the rodent is a rat.

23. A method of assessing the therapeutic efficacy of a compound in treating influenza A virus infection, comprising:

providing a rodent according to any one of claims 1-11;

administering an influenza A virus and a candidate compound to the rodent; and

monitoring the presence and severity of influenza A virus infection in the rodent to determine the therapeutic efficacy of the candidate compound.

24. The method of claim 23, wherein the candidate compound is an antibody or antigen-binding fragment thereof specific for a human TMPRSS protein.

25. An isolated cell or tissue of a rodent according to any one of claims 1-11, wherein the genome of the cell or tissue comprises the humanized *Tmprss* gene.

26. An isolated rodent embryonic stem (ES) cell comprising a humanized *Tmprss* gene, wherein the humanized *Tmprss* gene

comprises a nucleotide sequence of an endogenous rodent *Tmprss* gene and a nucleotide sequence of a cognate human *TMPRSS* gene,

is located at an endogenous rodent *Tmprss* locus, and results from a replacement of a genomic sequence of the endogenous rodent *Tmprss* gene with said nucleotide sequence of the cognate human *TMPRSS* gene and is under control of the promoter of the endogenous rodent *Tmprss* gene, and

encodes a humanized Tmprss protein that comprises: (i) an ectodomain that has at least 90% sequence identity to the ectodomain of the human TMPRSS protein encoded by the cognate human *TMPRSS* gene, and (ii) a cytoplasmic and transmembrane portion that has at least 90% sequence identity to the cytoplasmic and transmembrane portion of the endogenous rodent Tmprss protein encoded by the endogenous rodent *Tmprss* gene.

27. The rodent ES cell of claim 26, wherein the humanized *Tmprss* gene is a humanized *Tmprss2* gene, the endogenous rodent *Tmprss* gene is an endogenous rodent *Tmprss2* gene, and the cognate human *TMPRSS* gene is a human *TMPRSS2* gene; and wherein the nucleotide sequence of the human *TMPRSS2* gene comprises coding exon 4 through the stop codon in coding exon 13 of the human *TMPRSS2* gene.

28. The rodent ES cell of claim 26, wherein the humanized *Tmprss* gene is a humanized *Tmprss4* gene, the endogenous rodent *Tmprss* gene is an endogenous rodent *Tmprss4* gene, and the cognate human *TMPRSS* gene is a human *TMPRSS4* gene; and wherein the nucleotide sequence of the human *TMPRSS4* gene comprises coding exon 4 through the stop codon in coding exon 13 of the human *TMPRSS4* gene.

29. The rodent ES cell of claim 26, wherein the humanized *Tmprss* gene is a humanized *Tmprss11d* gene, the endogenous rodent *Tmprss* gene is an endogenous rodent *Tmprss11d* gene, and the cognate human *TMPRSS* gene is a human *TMPRSS11D* gene; and wherein the nucleotide sequence of the human *TMPRSS11D* gene comprises coding exon 3 through the stop codon in coding exon 10 of the human *TMPRSS11D* gene.

30. The rodent ES cell according to any one of claims 26-29, wherein the rodent ES cell is homozygous for the humanized *Tmprss* gene.

31. The rodent ES cell according to any one of claims 26-30, wherein the rodent ES cell is a mouse ES cell.

32. The rodent ES cell according to any one of claims 26-30, wherein the rodent ES cell is a rat ES cell.

33. A rodent embryo comprising the rodent ES cell according to any one of claims 26-32.

34. A nucleic acid vector, comprising

a nucleic acid encoding the ectodomain of a human TMPRSS protein, flanked by a 5' nucleotide sequence and a 3' nucleotide sequence,

wherein the 5' nucleotide sequence and the 3' nucleotide sequence are homologous to genomic DNA sequences flanking a rodent genomic DNA at a rodent *Tmprss* locus encoding the ectodomain of a cognate rodent Tmprss protein, and are capable of mediating homologous recombination and replacement of the rodent genomic DNA with the nucleic acid encoding the ectodomain of a human TMPRSS protein to form a humanized *Tmprss* gene; and

wherein the rodent is mouse or rat.

35. The vector of claim 34, wherein the human TMPRSS protein is a human TMPRSS2 protein, the cognate rodent Tmprss protein is a rodent Tmprss2 protein, and the humanized *Tmprss* gene is a humanized *Tmprss*2 gene.

36. The vector of claim 35, wherein the nucleic acid comprises coding exon 4 through the stop codon in coding exon 13 of a human *TMPRSS2* gene.

37. The vector of claim 35, wherein the humanized *Tmprss2* gene comprises

(i) coding exons 1-2 of an endogenous rodent *Tmprss2* gene,

(ii) an exon 3 that comprises a 5' portion of coding exon 3 of the endogenous rodent *Tmprss2* gene and a 3' portion of coding exon 3 of a human *TMPRSS2* gene, and

(iii) coding exon 4 through coding exon 13 of the human *TMPRSS2* gene.

38. The vector of claim 34, wherein the human TMPRSS protein is a human TMPRSS4 protein, the cognate rodent Tmprss protein is a rodent Tmprss4 protein, and the humanized *Tmprss* gene is a humanized *Tmprss*4 gene.

39. The vector of claim 38, wherein the nucleic acid comprises coding exon 4 through the stop codon in coding exon 13 of a human *TMPRSS4* gene.

40. The vector of claim 38, wherein the humanized *Tmprss4* gene comprises coding exon 1 through coding exon 3 of an endogenous rodent *Tmprss4* gene, and coding exon 4 through the stop codon in coding exon 13 of a human *TMPRSS4* gene.

41. The vector of claim 34, wherein the human TMPRSS protein is a human TMPRSS11D protein, the cognate rodent Tmprss protein is a rodent Tmprss11d protein, and the humanized *Tmprss* gene is a humanized *Tmprss*11d gene.

42. The vector of claim 41, wherein the nucleic acid comprises coding exon 3 through the stop codon in coding exon 10 of a human *TMPRSS11D* gene.

43. The vector of claim 41, wherein the humanized *Tmprss11d* gene comprises coding exons 1-2 of an endogenous rodent *Tmprss11d* gene, and coding exons 3 through coding exon 10 of a human *TMPRSS11D* gene.

44. The vector according to any one of claims 34-43, wherein the rodent is mouse.

45. The vector according to any one of claims 34-43, wherein the rodent is rat.