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(54) **Title:** TRANSGENIC MOUSE MODELS SUPPORTING INNATE IMMUNE FUNCTION

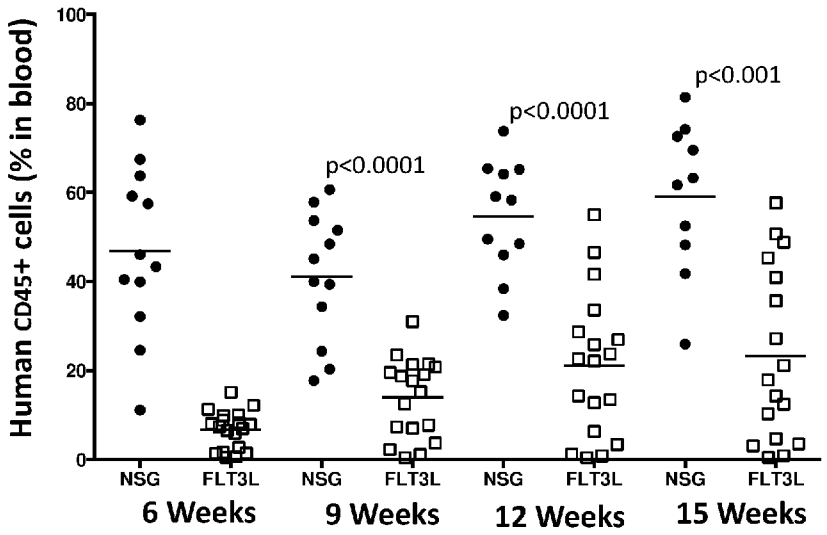


*FIG. 2*

(57) **Abstract:** Provided herein, in some aspects, is a NOD.Cg -Prkdc$^{scid}$ Il2rg$^{tm1wjl}$/SzJ (NOD scid gamma or NSG$^{TM}$) mouse comprising a nucleic acid encoding human FLT3L and an inactivated mouse Flt3 allele, methods of producing the mouse, and methods of using the mouse.

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# TRANSGENIC MOUSE MODELS SUPPORTING INNATE IMMUNE FUNCTION

## GOVERNMENT LICENSE RIGHTS

This invention was made with government support under grant number 1R01132963 awarded by National Institutes of Health. The government has certain rights in the invention.

## RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application number 62/805257, filed February 13, 2019, which is incorporated by reference herein in its entirety.

## BACKGROUND

Signaling through the fms-related tyrosine kinase 3 (FLT3) receptor supports survival, proliferation, and differentiation of hematopoietic progenitor cells and dendritic cells (DCs) (1, 2). Human DCs are necessary to present antigen to human T cells and are required for the development of a robust human immune response (4). Mature human DCs also produce interleukin 15 and other factors that support development of natural killer (NK) cells and other components of a human innate immune system (5).

## SUMMARY

Provided herein, in some embodiments, is an immunodeficient NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NSG™) mouse (a "NOD *scid* gamma" mouse), comprising a nucleic acid encoding human FLT3L (e.g., comprising a human *FLT3L* transgene) and an inactivated mouse *Flt3* allele. Although the NSG™ mouse supports human hematopoietic stem cell (HSC) engraftment, it exhibits impaired development of human HSCs into dendritic cell (DC) populations (3). To provide a mouse model that supports development of human HSCs into DC populations, a transgenic NSG™ mouse expressing human FLT3L (NSG™-Tg(Hu-FLT3L)) was generated. Unexpectedly, however, engraftment of human HSCs was significantly lower in the NSG™-Tg(Hu-FLT3L) mouse compared with the NSG™ control. In an effort to understand the phenotype observed in the NSG™-Tg(Hu-FLT3L) mouse, the endogenous mouse receptor for FLT3L – *Flt3* – was knocked out. Surprisingly, engraftment with human HSCs of this transgenic line, referred to herein as NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L), results in (1) significantly increased percentages of both human CD3$^+$ T cells and human CD33$^+$ myeloid cells, (2) increased percentages of human CD123$^+$ plasmacytoid dendritic cells, CD56$^+$ human natural killer (NK) cells, CD14$^+$ human monocyte macrophages, and CD11C$^+$ HLA-DR$^+$ human

myeloid dendritic cells, and (3) support of mucosal engraftment of human $CD45^+$ cells in the small intestines. Without being bound by theory, the decreased human HSC engraftment NSG™-Tg(Hu-FLT3L) may have been a consequence of human FLT3L activating, through the host mouse FLT3 receptor, the host mouse DCs and possibly other innate immune mouse components.

Thus, some aspects of the present disclosure provide a NSG™ mouse comprising a nucleic acid encoding human FLT3L and an inactivated mouse *Flt3* allele (NSG™ $Flt3^{null}$-Tg(Hu-FLT3L)). This mouse model supports development of HSCs into many different cell types of the human innate immune system, including dendritic cells.

In some embodiments, the mouse comprises a genomic modification that inactivates the mouse *Flt3* allele. The genomic modification, in some embodiments, is in at least one region of the mouse *Flt3* allele selected from coding regions, non-coding regions, and regulatory regions. In some embodiments, the genomic modification is in at least one coding region of the mouse *Flt3* allele. For example, the genomic modification may be in exon 6, exon 7, and/or exon 8. In some embodiments, the genomic modification is selected from genomic deletions, genomic insertions, genomic substitutions, and combinations thereof. For example, the genomic modification may be a genomic deletion. The mouse *Flt3* allele may comprise, for example, a genomic deletion of nucleotide sequences in exon 6, exon 7, and exon 8.

In some embodiments, the nucleic acid sequence of SEQ ID NO: 5 has been deleted from the mouse *Flt3* allele.

In some embodiments, the modified mouse *Flt3* allele comprises the nucleic acid sequence of SEQ ID NO: 6.

In some embodiments, the nucleic acid encoding human FLT3L comprises a human *FLT3L* transgene. In some embodiments, the human *FLT3L* transgene comprises a nucleic acid sequence of SEQ ID NO: 7. In some embodiments, the mouse expresses human FLT3L. The human FLT3L may be expressed, for example at a level of at least 10,000 pg/ml. In some embodiments, the human FLT3L is expressed at a level of 10,000 pg/ml to 30,000 pg/ml. For example, the human FLT3L may be expressed at a level of 15,000 +/- 1000 pg/mL to 17,000 +/- 100 pg/ml.

In some embodiments, the mouse expresses mouse FLT3L. In some embodiments, the mouse FLT3L is expressed at a level of at least 2,000 pg/ml. For example, the mouse FLT3L may be expressed at a level of 5,000 pg/ml to 10,000 pg/ml. In some embodiments, the mouse FLT3L is expressed at a level of 6,000 pg/ml to 8,000 ml.

In some embodiments, the mouse does not express a detectable level of mouse FLT3. In some embodiments, a detectable level of mouse FLT3 expressed by the mouse is less than 1,000 pg/ml.

In some embodiments, the mouse lacks a detectable number of $CD135^+$ multipotent progenitor cells.

In some embodiments, the mouse further comprises human $CD34^+$ hematopoietic stem cells. The human $CD34^+$ hematopoietic stem cells, in some embodiments, are from human umbilical cord blood, bone marrow, or mobilized peripheral blood.

In some embodiments, the mouse comprises a population of human $CD45^+$ cells. The population of human $CD45^+$ cells comprises, in some embodiments, human $CD45^+/CD3^+$ T cells and/or human $CD45^+/CD33^+$ myeloid cells.

In some embodiments, the population of human $CD45^+$ cells comprises an increased percentage of human $CD45^+/CD3^+$ T cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse. For example, the percentage of human $CD45^+/CD3^+$ T cells in the mouse may be increased by at least 25%, at least 50%, or at least 100%.

In some embodiments, the population of human $CD45^+$ cells comprises an increased percentage of human $CD45^+/CD33^+$ myeloid cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse. For example, the percentage of human $CD45^+/CD33^+$ myeloid cells in the mouse may be increased by at least 25%, at least 50%, or at least 100%.

In some embodiments, the mouse comprises an increased percentage of human $CD123^+$ plasmacytoid dendritic cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse. For example, the percentage of human $CD123^+$ plasmacytoid dendritic cells in the mouse may be increased by at least 25%, at least 50%, or at least 100%.

In some embodiments, the mouse comprises an increased percentage of human $CD56^+$ natural killer cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse. For example, the percentage of human $CD56^+$ natural killer cells in the mouse may be increased by at least 25%, at least 50%, or at least 100%.

In some embodiments, the mouse comprises an increased percentage of human $CD14^+$ monocyte macrophages, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse. For example, the percentage of human $CD14^+$ monocyte macrophages in the mouse may be increased by at least 25%, at least 50%, or at least 100%.

In some embodiments, the mouse comprises an increased percentage of human $CD11C^+$ $HLA\text{-}DR^+$ myeloid dendritic cells, relative to a NOD *scid* gamma control mouse or a NOD *scid*

gamma-Hu-FLT3L control mouse. For example, the percentage of human $CD11C^+$ $HLA\text{-}DR^+$ myeloid dendritic cells in the mouse is increased by at least 25%, at least 50%, or at least 100%.

In some embodiments, the mouse exhibits mucosal engraftment of human $CD45^+$ cells in the small intestines of the mouse.

Other aspects of the present disclosure provide a method comprising sublethally irradiating the mouse of any one of claims 1-23, and injecting the mouse with human CD34+ hematopoietic stem cells.

In some embodiments, the method further comprises administering to the mouse an agent of interest. In some embodiments, the method further comprises assessing an effect of the agent on human immune cells in the mouse.

In some embodiments, the method further comprises the human immune cells are selected from T cells, dendritic cells, natural killer cells, and macrophages.

Yet other aspects of the present disclosure provide a method comprising injecting a pronucleus of a NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NOD *scid* gamma) mouse with a nucleic acid encoding human FLT3L, producing a NSG Tg(Hu-FLT3L) mouse, and inactivating a mouse *Flt3* allele in the NSG Tg(Hu-FLT3L) mouse.

Further other aspects of the present disclosure provide a method comprising inactivating a mouse *Flt3* allele in a NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NOD *scid* gamma) mouse to produce a NSG *Flt3*$^{null}$ mouse, and injecting a pronucleus of the NSG *Flt3*$^{null}$ mouse with a nucleic acid encoding human FLT3L.

Still other aspects of the present disclosure provide a method comprising breeding female mice homozygous for *Prkdc*$^{scid}$, homozygous for *Il2rg*$^{tm1Wjl}$, homozygous for *Flt3*$^{null}$, and homozygous for a human *FLT3L* transgene with male mice homozygous for *Prkdc*$^{scid}$, hemizygous for the X-linked *Il2rg*$^{tm1Wjl}$, homozygous for *Flt3*$^{null}$, and homozygous for a human *FLT3L* transgene to produce progeny mice.

Further still, some aspects of the present disclosure provide a NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ cell comprising a nucleic acid encoding human FLT3L and an inactivated endogenous *Flt3* allele.

Yet other aspects of the present disclosure a transgenic rodent comprising a cell comprising a nucleic acid encoding human FLT3L and an inactivated endogenous *Flt3* allele. In some embodiments, the transgenic rodent is a transgenic mouse.

Some aspects of the present disclosure provide a gRNA targeting mouse *Flt3*, optionally wherein the gRNA targets exon 6 or exon 8 or mouse *Flt3*. In some embodiments, the gRNA comprises the sequence of SEQ ID NO: 1. In some embodiments, the gRNA comprises the sequence of SEQ ID NO: 2.

Also provided herein is a mouse oocyte comprising any one of the gRNAs described herein. In some embodiments, the mouse oocyte is fertilized.

Some aspects further provide a mouse oocyte comprising a first gRNA targeting exon 6 of mouse *Flt3* and a second gRNA targeting exon 8 of mouse *Flt3*, optionally wherein the mouse oocyte is fertilized.

A mouse oocyte, in some embodiments, further comprises Cas9 mRNA and/or Cas9 protein.

A mouse oocyte, in some embodiments, further comprises a human *FLT3L* transgene.

## BRIEF DESCRIPTION OF THE DRAWINGS

**FIG. 1** shows human FLT3L levels in sera from NSG™-transgenic mice expressing human FLT3L (NSG™-Tg(Hu-FLT3L) that are hemizygous (labeled as Het) or homozygous (labeled as hom) for human FLT3L from founder lines 7 and 8. Each bar represents values from 3-4 female or male mice at 5 to 15 weeks of age.

**FIG. 2** shows decreased levels of human hematopoietic stem cell (HSC) engraftment in homozygous NSG™-Tg(Hu-FLT3L) mice that are expressed as percentages of human $CD45^+$ cells in blood. Each data point represents the percentage of human $CD45^+$ cells in individual mice tested at 6-15 weeks post-engraftment. Both male and female mice were used.

**FIG. 3** shows the absence of mouse $CD135^+$ myeloid multipotential (MMP3) progenitor cells in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse bone marrow. Each bar represents values from three (3) female mice at ten (10) weeks of age.

**FIG. 4A** shows the levels of mouse FLT3L in NSG™*Flt3*$^{null}$-Tg(Hu-FLT3L) and NSG™ control mice. **FIG. 4B** shows the levels of human FLT3L in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) and NSG™ control mice.

**FIG. 5A** shows human $CD45^+$ T-cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC and $CD45^+$ T-cell development in NSG™ control mice. Eight (8) NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice and ten (10) NSG™ control mice were injected at 8-12 weeks of age. Both male and female mice were used. **FIG. 5B** shows an increased percentage of human $CD3^+$ T cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. **FIG. 5C** shows a decreased percentage of human $CD20^+$ B-cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. This may be due to an increase in the percentage of HSCs differentiating into innate immune system cells (*e.g.,* dendritic cells, natural killer cells, monocytes, macrophages, eosinophils, basophils, neutrophils) as opposed to adaptive immune system cells (*e.g.,* T cells and B cells). **FIG. 5D** shows increased percentage of human $CD33^+$

myeloid cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice.

**FIG. 6A** shows increased human CD123$^+$ plasmacytoid dendritic cell (DC) development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. Eight (8) NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice and ten (10) NSG™ control mice were injected at 8-12 weeks of age. Both male and female mice were used. **FIG. 6B** shows increased human CD56$^+$ natural killer cell (NK) development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. **FIG. 6C** shows increased human CD14$^+$ monocyte/macrophage cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. **FIG. 6D** shows increased human CD11c/HLA-DR$^+$ myeloid DC cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice.

**FIG. 7** shows the small intestine from a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse and a NSG™ control mouse showing mucosal engraftment with HSC. Eight (8) NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice and ten (10) NSG™ control mice were injected at 8-12 weeks of age. Both male and female mice were used.

**FIG. 8A** shows comparable human CD45$^+$ cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. **FIG. 8B** shows increased human CD33$^+$ myeloid cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. **FIG. 8C** shows increased human CD3$^+$ T cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. **FIG. 8D** shows increased human CD14$^+$ monocyte cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. **FIG. 8E** shows increased human CD11c$^+$ HLA-DR$^+$ myeloid dendritic cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice. **FIG. 8F** shows increased human CD56$^+$ natural killer (NK) cell development in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice engrafted with human HSC compared with NSG™ control mice.

## DETAILED DESCRIPTION

### NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) Mouse

The present disclosure provides a NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NSG™) mouse comprising a nucleic acid encoding human FLT3L and an inactivated mouse *Flt3* allele. This mouse is referred to herein as a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse.

The NSG™ mouse is an immunodeficient mouse that lack mature T cells, B cells, and natural killer (NK) cells, is deficient in multiple cytokine signaling pathways, and has many defects in innate immunity (see, e.g., Shultz LD et al. *Nat. Rev. Immunol.* 2007; 7 (2): 118–130; Shultz LD et al. 2005; *J. Immunol.* 174 (10): 6477–89; and Shultz LD et al. *J. Immunol.* 1995; 154 (1): 180–91). The NSG™ mouse, derived from the non-obese diabetic (NOD) mouse strain NOD/ShiLtJ (see, e.g., Makino S et al. *Jikken Dobutsu* 1980; 29 (1): 1–13), include the *Prkdc*$^{scid}$ mutation (also referred to as the "severe combined immunodeficiency" mutation or the "scid" mutation) and the *Il2rg*$^{tm1Wjl}$ targeted mutation. The *Prkdc*$^{scid}$ mutation is a loss-of-function mutation in the mouse homolog of the human *PRKDC* gene – this mutation essentially eliminates adaptive immunity (see, e.g., Greiner DL et al. 1998; *Stem Cells* 16 (3): 166–177; and Blunt T et al. 1995; *Cell* 80 (5): 813–23). The *Il2rg*$^{tm1Wjl}$ mutation is a null mutation in the gene encoding the interleukin 2 receptor gamma chain (IL2Rγ, homologous to *IL2RG* in humans), which blocks NK cell differentiation, thereby removing an obstacle that prevents the efficient engraftment of primary human cells (Shultz LD et al. 2005; Greiner et al. 1998; and Cao X. et al. *Immunity* 1995; 2 (3): 223–38). A loss-of-function mutation, as is known in the art, results in a gene product with little or no function. By comparison, a null mutation results in a gene product with no function. An inactivated allele may be a loss-of-function allele or a null allele.

The NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse provided herein comprises a nucleic acid encoding human FLT3L. FLT3L (e.g., NC_000019.10; chromosome:GRCh38:19:49473607: 49486831:1) is a cytokine and growth factor that stimulates the production of immune cells (*e.g.,* B cells and T cells) by binding and activating the FLT3 receptor (see, e.g., Klein O. et al. *Eur J Immunol.* 2013; 43(2): 533-539). FLT3L is important for the development of steady-state dendritic cells. In some embodiments, the nucleic acid encoding human FLT3L comprises a human *FLT3L* transgene. Surprisingly, the data described herein show that human FLT3L is capable of binding mouse FLT3, which, without being bound by theory, may activate innate mouse immunity. Thus, in some embodiments, the NSG™ mouse provided herein comprises a nucleic acid (e.g., DNA) encoding human FLT3L and an inactivated mouse *Flt3* allele.

A nucleic acid may be DNA, RNA, or a chimera of DNA and RNA. In some embodiments, a nucleic acid (e.g., DNA) encoding human FLT3L comprises a gene encoding FLT3L. A gene is a sequence of nucleotides (DNA or RNA) that encodes a molecule (e.g., a protein) having a function. A gene may be endogenous (occurring naturally in a host organism) or exogenous (transferred, naturally or through genetic engineering, to a host organism). An allele is one of two or more alternative forms of a gene that arise by mutation and are found at the same locus on a chromosome. A gene, in some embodiments, includes a promoter sequence, coding regions (e.g., exons), non-coding regions (e.g., introns), and regulatory regions (also

referred to as regulatory sequences). As is known in the art, a promoter sequence is a DNA sequence at which transcription of a gene begins. Promoter sequences are typically located directly upstream of (at the 5' end of) a transcription initiation site. An exon is a region of a gene that codes for amino acids. An intron (and other non-coding DNA) is a region of a gene that does not code for amino acids.

A mouse comprising a human gene is considered to comprise a human transgene. A transgene is a gene exogenous to a host organism. That is, a transgene is a gene that has been transferred, naturally or through genetic engineering, to a host organism. A transgene does not occur naturally in the host organism (the organism, e.g., mouse, comprising the transgene). In some embodiments, a mouse as provided herein, comprises a *FLT3L* transgene, such as a human *FLT3L* transgene. In some embodiments, the human *FLT3L* transgene is integrated into the mouse genome. In some embodiments, the human *FLT3L* transgene comprises the nucleic acid sequence of SEQ ID NO: 7.

An inactivated allele is an allele that does not produce a detectable level of a functional gene product (e.g., a functional protein). In some embodiments, an inactivated allele is not transcribed. In some embodiments, an inactivated allele does not encode a functional protein. Thus, a mouse comprising an inactivated mouse *Flt3* allele does not produce a detectable level of functional FLT3. In some embodiments, a mouse comprising an inactivated mouse *Flt3* allele does not produce any functional FLT3.

In some embodiments, a mouse (e.g., a NSG™ $Flt3^{null}$-Tg(Hu-FLT3L) mouse) comprises a genomic modification that inactivates the mouse *Flt3* allele. A modification, with respect to a nucleic acid, is any manipulation of the nucleic acid, relative to the corresponding wild-type nucleic acid (e.g., the naturally-occurring nucleic acid). A genomic modification is thus any manipulation of a nucleic acid in a genome, relative to the corresponding wild-type nucleic acid (e.g., the naturally-occurring nucleic acid) in the genome. Non-limiting examples of nucleic acid (e.g., genomic) modifications include deletions, insertions, "indels" (deletion and insertion), and substitutions (e.g., point mutations). In some embodiments, a deletion, insertion, indel, or other modification in a gene results in a frameshift mutation such that the gene no longer encodes a functional product (e.g. protein). Modifications also include chemical modifications, for example, chemical modifications of at least one nucleobase. Methods of nucleic acid modification, for example, those that result in gene inactivation, are known and include, without limitation, RNA interference, chemical modification, and gene editing (e.g., using recombinases or other programmable nuclease systems, e.g., CRISPR/Cas, TALENs, and/or ZFNs). In some embodiments, CRISPR/Cas gene editing is used to inactivate the mouse *Flt3* allele, as described elsewhere herein.

In some embodiments, a genomic modification (e.g., a deletion or an indel) is in a (at least one) region of the mouse *Flt3* allele selected from coding regions, non-coding regions, and regulatory regions. In some embodiments, the genomic modification (e.g., a deletion or an indel) is a coding region of the mouse *Flt3* allele. For example, the genomic modification (e.g., a deletion or an indel) may be in exon 6, exon 7, exon 8, or it may span exons 6-8 of the mouse *Flt3* allele. In some embodiments, the genomic modification is a genomic deletion. For example, the mouse *Flt3* allele may comprise a genomic deletion of nucleotide sequences in exon 6, exon 7, and exon 8. In some embodiments, the nucleotide sequence of SEQ ID NO: 5 has been deleted from an inactivated mouse *Flt3* allele. In some embodiments, an inactivated mouse *Flt3* allele comprises the nucleotide sequence of SEQ ID NO: 6.

A NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse provided herein, in some embodiments, expresses human FLT3L. In some embodiments, human FLT3L is expressed at a level of at least 5,000 pg/ml or at least 10,000 pg/ml. For example, human FLT3L may be expressed at a level of at least 5,000 pg/ml, 7,500 pg/ml, 10,000 pg/ml, 12,500 pg/ml, 15,000 pg/ml, 17,500 pg/ml, 20,000 pg/ml, 22,500 pg/ml, 25,000 pg/ml, 27,500 pg/ml, 30,000 pg/ml, 32,500 pg/ml, 35,000 pg/ml, 37,500 pg/ml, 40,000 pg/ml, 42,500 pg/ml, 45,000 pg/ml, 47,500 pg/ml, or 50,000 pg/ml. In some embodiments, human FLT3L is expressed at a level of 10,000 pg/ml to 30,000 pg/ml. In some embodiments, human FLT3L is expressed at a level of 15,000 +/- 1000 pg/mL to 17,000 +/- 100 pg/ml. Methods of detecting FLT3L protein expression are known and may be used as provided herein. For example, flow cytometry and/or an ELISA (enzyme-linked immunosorbent assay) using an anti-FLT3L antibody may be used to detect the level of human FLTL3 protein present in mouse tissue and/or blood.

In some embodiments, a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse may also expression mouse FLTL3. In some embodiments, mouse FLT3L is expressed at a level of at least 1,000 pg/ml or at least 2,000 pg/ml. For example, mouse FLT3L may be expressed at a level of 3,000 pg/ml, 4,000 pg/ml, 5,000 pg/ml, 6,000 pg/ml, 7,000 pg/ml, 8,000 pg/ml, 9,000 pg/ml, or 10,000 pg/ml. In some embodiments, mouse FLT3L is expressed at a level of 5,000 pg/ml to 10,000 pg/ml. In some embodiments, mouse FLT3L is expressed at a level of 6,000 pg/ml to 8,000 ml.

In some embodiments, a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse does not express a detectable level of mouse FLT3. A detectable level of mouse FLT3 is any level of FLT3 protein detected using a standard protein detection assay, such as flow cytometry and/or an ELISA. In some embodiments, a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse expresses an undetectable level or a low level of mouse FLT3. For example, a mouse may express less than 1,000 pg/ml mouse FLT3. In some embodiments, a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse expresses less than 500 pg/ml mouse FLT3 or less than 100 pg/ml mouse FLT3. The mouse FLT3 receptor is also

referred to as cluster of differentiation antigen CD135. Thus, in some embodiments, a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse does not comprise (there is an absence of) CD135$^+$ multipotent progenitor (MPP3) cells.

**Human Innate Immune System Model**

The NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse of the present disclosure, in some embodiments, is used to support engraftment of human CD34$^+$ HSCs and development of a human innate immune system. The human immune system includes the innate immune system and the adaptive immune system. The innate immune system is responsible for recruiting immune cells to sites of infection, activation of the complement cascade, the identification and removal of foreign substances from the body by leukocytes, activation of the adaptive immune system, and acting as a physical and chemical barrier to infectious agents.

In some embodiments, the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is sublethally irradiated (e.g., 100 – 300 cGy) to kill resident mouse HSCs, and then the irradiated mouse is engrafted with human CD34$^+$ HSCs (e.g., 50,000 to 200,000 HSCs) to initiate the development of a human innate immune system. Thus, in some embodiments, the mouse further comprises human CD34$^+$ HSCs. Human CD34$^+$ HSCs may be from any source including, but not limited to, human umbilical cord blood, mobilized peripheral blood, and bone marrow. In some embodiments, the human CD34$^+$ HSCs are from human umbilical cord blood.

The differentiation of human CD34$^+$ HSCs into divergent immune cells (*e.g.,* T cells, B cells, dendritic cells) is a complex process in which successive developmental steps are regulated by multiple cytokines. This process can be monitored through cell surface antigens, such as cluster of differentiation (CD) antigens. CD45, for example, is expressed on the surface of HSCs, macrophages, monocytes, T cells, B cells, natural killer cells, and dendritic cells, thus can be used as a marker indicative of engraftment. On T cells, CD45 regulates T cell receptor signaling, cell growth, and cell differentiation. In some embodiments, the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse comprises human CD45$^+$ cells. Unexpectedly, the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse exhibits mucosal engraftment of human CD45$^+$ cells in the small intestines. In some embodiments, the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse also exhibits engraftment of human CD45$^+$ cells to tissues in the lung, thymus, spleen, and/or lymph nodes.

As CD45+ cells mature, they begin to express additional biomarkers, indicative of the various developmental stages and differentiating cell types. Developing T cells, for example, also express CD3, CD4, and CD8. As another example, developing myeloid cells express CD33$^+$. The NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse of the present disclosure, advantageously,

comprises not only human CD45$^+$ cells but also double positive human CD45$^+$/CD3$^+$ T cells as well as double positive human CD45+/CD33+ myeloid cells.

Thus, in some embodiments, a population of human CD45$^+$ cells in a NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse comprises human CD45$^+$/CD3$^+$ T cells. In some embodiments, the population of human CD45$^+$ cells comprises an increased percentage of human CD45$^+$/CD3$^+$ T cells, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD45$^+$/CD3$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse is increased by at least 25%, relative to a NSG™ control mouse. For example, the percentage of human CD45$^+$/CD3$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse may be increased by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD45$^+$/CD3$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse is increased by at least 50%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD45$^+$/CD3$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse is increased by at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD45$^+$/CD3$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse is increased by 25%-100%, 25%-75%, 25%-50%, 50%-100%, 50%-75%, or 75%-100%, relative to a NSG™ control mouse.

In some embodiments, a population of human CD45$^+$ cells in a NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse comprises human CD45$^+$/CD33$^+$ T cells. In some embodiments, the population of human CD45$^+$ cells comprises an increased percentage of human CD45$^+$/CD33$^+$ T cells, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD45$^+$/CD33$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse is increased by at least 25%, relative to a NSG™ control mouse. For example, the percentage of human CD45$^+$/CD33$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse may be increased by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD45$^+$/CD33$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse is increased by at least 50%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD45$^+$/CD33$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse is increased by at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD45$^+$/CD33$^+$ T cells in the NSG™ *Flt3$^{null}$*Tg(Hu-FLT3L) mouse is increased by 25%-100%, 25%-75%, 25%-50%, 50%-100%, 50%-75%, or 75%-100%, relative to a NSG™ control mouse.

The NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse provided herein, surprisingly, is also capable of supporting engraftment of dendritic cells (e.g., plasmacytoid dendritic cells and myeloid dendritic cells), natural killer cells, and monocyte-derived macrophages (monocyte macrophages). Plasmacytoid dendritic cells (pDCs) secrete high levels of interferon alpha; myeloid dendritic cells (mDCs) secrete interleukin 12, interleukin 6, tumor necrosis factor, and chemokines; natural killer cells destroy damaged host cells, such as tumor cells and virus-infected cells; and macrophages consume substantial numbers of bacteria or other cells or microbes.

In some embodiments, a NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse comprises an increased percentage of human CD123$^+$ plasmacytoid dendritic cells, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD123$^+$ plasmacytoid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 25%, relative to a NSG™ control mouse. For example, the percentage of human CD123$^+$ plasmacytoid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse may be increased by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD123$^+$ plasmacytoid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 50%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD123$^+$ plasmacytoid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD123$^+$ plasmacytoid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by 25%-100%, 25%-75%, 25%-50%, 50%-100%, 50%-75%, or 75%-100%, relative to a NSG™ control mouse.

In some embodiments, a NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse comprises an increased percentage of human CD56+ natural killer cells, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD56+ natural killer cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 25%, relative to a NSG™ control mouse. For example, the percentage of human CD56+ natural killer cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse may be increased by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD56+ natural killer cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 50%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD56+ natural killer cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is

increased by at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD56+ natural killer cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by 25%-100%, 25%-75%, 25%-50%, 50%-100%, 50%-75%, or 75%-100%, relative to a NSG™ control mouse.

In some embodiments, a NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse comprises an increased percentage of human CD14$^+$ monocyte macrophages, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD14$^+$ monocyte macrophages in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 25%, relative to a NSG™ control mouse. For example, the percentage of human CD14$^+$ monocyte macrophages in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse may be increased by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD14$^+$ monocyte macrophages in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 50%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD14$^+$ monocyte macrophages in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD14$^+$ monocyte macrophages in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by 25%-100%, 25%-75%, 25%-50%, 50%-100%, 50%-75%, or 75%-100%, relative to a NSG™ control mouse.

In some embodiments, a NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse comprises an increased percentage of human CD11C$^+$ HLA-DR$^+$ myeloid dendritic cells, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD11C$^+$ HLA-DR$^+$ myeloid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 25%, relative to a NSG™ control mouse. For example, the percentage of human CD11C$^+$ HLA-DR$^+$ myeloid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse may be increased by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD11C$^+$ HLA-DR$^+$ myeloid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 50%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD11C$^+$ HLA-DR$^+$ myeloid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by at least 100%, relative to a NSG™ control mouse. In some embodiments, the percentage of human CD11C$^+$ HLA-DR$^+$ myeloid dendritic cells in the NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse is increased by 25%-100%, 25%-75%, 25%-50%, 50%-100%, 50%-75%, or 75%-100%, relative to a NSG™ control mouse.

**Methods of Producing Transgenic Animals**

Provided herein, in some aspects, are methods of producing a transgenic animal that expresses human FLT3L. A transgenic animal, herein, refers to an animal that has a foreign (exogenous) nucleic acid (e.g. transgene) inserted into (integrated into) its genome. In some embodiments, the transgenic animal is a transgenic rodent, such as a mouse or a rat. In some embodiments, the transgenic animal is a mouse. Methods of producing transgenic mice, for example, are well known. Three conventional methods used for the production of transgenic animals include DNA microinjection (Gordon and Ruddle, *Science* 1981: 214: 1244-124, incorporated herein by reference), embryonic stem cell-mediated gene transfer (Gossler et al., *Proc. Natl. Acad. Sci.* 1986; 83: 9065-9069, incorporated herein by reference) and retrovirus-mediated gene transfer (Jaenisch, *Proc. Natl. Acad. Sci.* 1976; 73: 1260-1264, incorporated herein by reference), any of which may be used as provided herein.

The nucleic acid encoding human FLT3L, in some embodiments, comprises a human *FLT3L* transgene that comprise a promoter (e.g., a constitutively active promoter) operably linked to a nucleotide sequence encoding human FLT3L. In some embodiments, the nucleic acid encoding human FLT3L used to produce a transgenic animal (e.g., mouse) is present on an vector, such as a plasmid, a bacterial artificial chromosome (BAC), or a yeast artificial chromosome (YAC), which is delivered, for example, to the pronucleus/nucleus of a fertilized embryo where the nucleic acid randomly integrates into the animal genome. In some embodiments, the nucleic acid (e.g., carried on a BAC) is delivered to a fertilized embryo of a NSG™ mouse to produce a NSG™ Tg(Hu-FLT3L) mouse. Following injection of the fertilized embryo, the fertilized embryo is transferred to a pseudopregnant female, which subsequently gives birth to offspring comprising the nucleic acid encoding human FLTL3. The presence or absence of the nucleic acid encoding human FLTL3 may be confirmed, for example, using any number of genotyping methods (e.g., sequencing and/or genomic PCR).

Also provided herein are methods of inactivating an endogenous *Flt3* allele. In some embodiments, an endogenous *Flt3* allele is inactivated in a transgenic animal. In some embodiments, the transgenic animal is a NSG™ Tg(Hu-FLT3L) mouse. Thus, in some embodiments, the method comprise inactivating a mouse *Flt3* allele in a NSG™ Tg(Hu-FLT3L) mouse to produce a NSG™ $Flt3^{null}$Tg(Hu-FLT3L) mouse. Methods of gene (allele) inactivation are known, any of which may be used as provided herein. In some embodiments, a gene/genome editing method is used. Engineered nuclease-based gene editing systems that may be used as provided herein include, for example, clustered regularly interspaced short palindromic repeat (CRISPR) systems, zinc-finger nucleases (ZFNs), and transcription activator-like effector

nucleases (TALENs). See, e.g., Carroll D *Genetics*. 2011; 188(4): 773–782; Joung JK et al. *Nat Rev Mol Cell Biol*. 2013; 14(1): 49–55; and Gaj T et al. *Trends Biotechnol.* 2013 Jul; 31(7): 397–405, each of which is incorporated by reference herein.

In some embodiments, a CRISPR system is used to inactivate an endogenous *Flt3* allele, for example, to produce a NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mouse. See, e.g., Harms DW et al., *Curr Protoc Hum Genet.* 2014; 83: 15.7.1–15.7.27; and Inui M et al., *Sci Rep*. 2014; 4: 5396, each of which are incorporated by reference herein). For example, Cas9 mRNA or protein and one or multiple guide RNAs (gRNAs) can be injected directly into mouse embryos to generate precise genomic edits into a *Flt3* gene. Mice that develop from these embryos are genotyped or sequenced to determine if they carry the desired mutation(s), and those that do are bred to confirm germline transmission.

The CRISPR/Cas system is a naturally occurring defense mechanism in prokaryotes that has been repurposed as a RNA-guided-DNA-targeting platform for gene editing. Engineered CRISPR systems contain two main components: a guide RNA (gRNA) and a CRISPR-associated endonuclease (e.g., Cas protein). The gRNA is a short synthetic RNA composed of a scaffold sequence for nuclease-binding and a user-defined nucleotide spacer (e.g., ~15-25 nucleotides, or ~20 nucleotides) that defines the genomic target to be modified. Thus, one can change the genomic target of the Cas protein by simply changing the target sequence present in the gRNA. In some embodiments, the CRISPR-associated endonuclease is selected from Cas9, Cpf1, C2c1, and C2c3. In some embodiments, the Cas nuclease is Cas9.

A guide RNA comprises at least a spacer sequence that hybridizes to (binds to) a target nucleic acid sequence (e.g., a region of the *Flt3* allele, such as the promoter sequence, a coding sequence, or a noncoding sequence), and a CRISPR repeat sequence that binds the endonuclease and guides the endonuclease to the target nucleic acid sequence. As is understood by the person of ordinary skill in the art, each gRNA is designed to include a spacer sequence complementary to its genomic target sequence (e.g., a region of the *Flt3* allele). See, e.g., Jinek et al., *Science*, 2012; 337: 816-821 and Deltcheva et al. *Nature*, 2100; 471: 602-607, each of which is incorporated by reference herein. In some embodiments, a gRNA used in the methods provided herein binds to exon 6 of a mouse *Flt3* allele. In some embodiments, a gRNA used in the methods provided herein binds to exon 7 of a mouse *Flt3* allele. In some embodiments, a gRNA used in the methods provided herein binds to exon 8 of a mouse *Flt3* allele. In some embodiments, multiple gRNAs are used to target multiple regions of the *Flt3* allele. In some embodiments, two gRNAs are used, one binding to exon 6 and one binding to exon 8 of the *Flt3* allele. In some embodiments, a gRNA of the present disclosure comprises the nucleotide

sequence of SEQ ID NO: 1. In some embodiments, a gRNA of the present disclosure comprises the nucleotide sequence of SEQ ID NO: 2.

**Methods of Use**

The mouse model provided herein may be used for any number of applications. For example, the NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse may be used to test how a particular agent (e.g., therapeutic agent) or medical procedure (e.g., tissue transplantation) affects the human innate immune system (e.g., human innate immune cell responses).

In some embodiments, the NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse is used to evaluate an effect of an agent on human innate immune system development. Thus, provided herein are methods that comprise administering an agent to the NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse, and evaluating an effect of the agent on human innate immune system development in the mouse. Effects of an agent may be evaluated, for example, by measuring a human innate immune cell (e.g., T cell and/or dendritic cell) response (e.g., cell death, cell signaling, cell proliferation, etc.). Non-limiting examples of agents include therapeutic agents, such as anti-cancer agents and anti-inflammatory agents, and prophylactic agents, such as immunogenic compositions (e.g., vaccines).

In other embodiments, the NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse is used to evaluate an immunotherapeutic response to a human tumor. Thus, provided herein are methods that comprise administering an agent to a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse that has a human tumor, and evaluating an effect of the agent on the human innate immune system and/or on the tumor in the mouse. Effects of an agent may be evaluated by measuring a human innate immune cell (e.g., T cell and/or dendritic cell) response and/or tumor cell response (e.g., cell death, cell signaling, cell proliferation, etc.). In some embodiments, the agent is an anti-cancer agent.

In yet other embodiments, the NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse is used to evaluate a human innate immune response to an infectious microorganism. Thus, provided herein are methods that comprise exposing the NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse to an infectious microorganism (e.g., bacteria and/or virus), and evaluating an effect of the infectious microorganism on the human innate immune response. Effects of an infectious microorganism may be evaluated by measuring a human innate immune cell (e.g., T cell and/or dendritic cell) response (e.g., cell death, cell signaling, cell proliferation, etc.). These methods may further comprise administering a drug or an anti-microbial agent (e.g., an anti-bacterial agent or an anti-viral agent) to the mouse, and evaluating an effect of the drug or anti-microbial agent on the infectious microorganism.

In still further embodiments, the NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse is used to evaluate a human immune response to tissue transplantation. Thus, provided herein are methods that comprise transplanting tissue (e.g., allogeneic tissue) to a NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mouse, and evaluating an effect of the transplanted tissue on the human innate immune response. Effects of a transplanted tissue may be evaluated by measuring a human innate immune cell (e.g., T cell and/or dendritic cell) response (e.g., cell death, cell signaling, cell proliferation, etc.) to the transplanted tissue.

## EXAMPLES

### Example 1: Production of NSG™-Tg(Hu-FLT3L) Mice

To support human DC development and innate immune function, a panel of transgenic NSG™ mouse lines expressing human FLT3 ligand (FLT3L) was produced. To produce these transgenic mouse lines, a 13.8 kilobase (kb) BamHI restriction fragment from the bacterial artificial chromosome (BAC) clone RP11-360G9 obtained from CHORI BACPAK was subcloned into pBluescript to eliminate other genes in the BAC. Purified, linearized DNA was then injected into the pronuclei of NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NSG™) fertilized eggs.

Eleven founders were identified by PCR. Of these founders, four founder lines (lines 3, 7, 8, and 10) had detectable levels of circulating human FLT3L when tested by enzyme linked immunosorbent assay (ELISA). These founders were mated with NSG non-transgenic mice. The transgenic offspring of this cross were tested by ELISA for circulating human FLT3L levels. The levels of FLT3L in transgenic NSG mice that were hemizygous or homozygous for the human FLT3L transgene (lines 7 and 8) ranged from 400 to 600 pg/mL (**FIG. 1**).

### Example 2: NSG™-Tg(Hu-FLT3L) Mice Exhibit Impaired Development of Human HSCs

Transgenic NSG™ mice expressing human FLT3L (NSG™-Tg(Hu-FLT3L)) were then engrafted with human cord blood CD34$^+$ HSCs. Cohorts of NSG™-Tg(Hu-FLT3L) mice and NSG™ non-transgenic control mice were sublethally irradiated (200cGy) and injected intravenously with 100,000 human umbilical cord blood HSCs. Flow cytometry of peripheral blood from engrafted mice was conducted at 6, 9, 12, and 15 weeks post-engraftment. Unexpectedly, the percentage of human CD45$^+$ cells was significantly lower in the NSG™-Tg(Hu-FLT3L) mice compared with NSG™ controls at all time points tested (**FIG. 2**).

### Example 3: Production of NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) Mice

To test whether the decreased human HSC engraftment in NSG™-Tg(Hu-FLT3L) mice is a consequence of human FLT3L activating the host mouse DCs and possibly other innate immune mouse components, the gene encoding the mouse FLT3 receptor (*Flt3*) was knocked out (to prevent human FLT3L from binding to the mouse FLT3 receptor and activating mouse innate immunity). Without being bound by theory, without the mouse FLT3 receptor, both the human FLT3L and mouse FLT3L would be available to bind to the human FLT3 receptor.

The mouse *Flt3* gene encoding the FLT3 receptor was knocked out directly in the NSG™-Tg(Hu-FLT3L) homozygous strain that expressed the human FLT3 ligand. To knock-out the mouse *Flt3* gene, two single guide RNA (sgRNAs, see Table 1 below) were designed to target exons 6 and 8, which should eliminate the immunoglobulin-like domain, even if the transcript is retained and a hypomorph results. The sgRNAs were designed using breaking Cas (6) and Zifit (7) software to minimize the likelihood of off-target cutting and were also checked against the human sequence to ensure the human *FLT3L* transgene would not be targeted. Both sgRNAs were designed as Tru-Guides ("truncated"), each with only 19 base recognition sites, in order to further reduce the likelihood of off-target cutting (8). The sgRNAs were prepared as described previously (9).

The screening for mouse *Flt3* knock-outs was performed using PCR primers (see **Table 1** below) that flanked the region of interest (*e.g.,* exons 6-8). The PCR primers yield a 1942 base pair (bp) wild-type amplicon, with a predicted size of 670 bp for the mutant dropout ("DO") allele. Following PCR, samples were screened by Sanger sequencing using the same primers. Three founders carrying knock-out alleles were identified by PCR and subsequently sequenced. Three knock-out lines were established (Lines 1, 2, and 4) by intercrossing N2 mice from the founder with the largest deletion. Knock-outs were typed by PCR, and ultimately, a single knock-out line was expanded, validated, and characterized (NPD.Cg-*Flt3*$^{em1Mvw}$*Prkdc*$^{scid}$*Il2rg*$^{tm1Wjl}$Tg(Hu-FLT3L)7Sz, abbreviated as NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) mice.

The modified *Flt3*$^{null}$ allele lost 1500 bp beginning before exon 6 and termination just before the end of exon 8. The deleted gene sequence is shown as SEQ ID NO: 5 (1500 base pairs deleted in the mouse *Flt3* receptor gene in NSG™ *Flt3*$^{null}$-Tg(Hu-FLT3L) mice). The resulting PCR product for the modified *Flt3*$^{null}$ allele is 442 bp. The sequence of this PCR product is shown as SEQ ID NO: 6 (PCR product resulting from targeted mouse *Flt3* gene deletion). Mouse FLT3 receptor is called cluster of differentiation antigen CD135. The NSG™ *Flt3*$^{null}$ Tg(Hu-FLT3L) mice were validated for lack of mouse FLT3 by flow cytometry of bone marrow dendritic cells by absence of CD135+ multipotent progenitor (MPP3) cells (**FIG. 3**).

**Table 1: Sequences used to generate and screen *Flt3* knock-out mice**

| Sequence | Name | SEQ ID NO: |
|---|---|---|
| 5'-GAACAGCUUGGUGCAUUCG | sgRNA-Exon 6 | 1 |
| 5'-GAUGGUCACCAACGCUGAC | sgRNA-Exon 8 | 2 |
| 5'-CCAACCTGAACTGTATGGAGATAG | PCR-Forward | 3 |
| 5'-CCACAGGGAAAGCCACTAAA | PCR-Reverse | 4 |

**Example 4: NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L) Mice Support Dendritic Cell Development**

ELISA assays of the sera of the NSG™ *Flt3*$^{null}$ Tg(Hu-FLT3L) mice expressing the human FLT3L transgene and lacking the mouse FLT3 receptor showed levels of the human FLT3L ranging from 15,175 +/- 1,137 pg/mL to 17,120 +/- 92.7 pg/mL. Sera from the NSG™ non-transgenic control mice showed no detectable FLT3L levels (**FIGS. 4A-4B**). The levels of mouse FLT3L ranged from 6,000 to 8,000 pg/mL in NSG™ *Flt3*$^{null}$Tg(Hu-FLT3L), while the level of mouse FLT3L in NSG™ control was ~ 1,000 pg/mL.

To determine the ability of the NSG™ *Flt3*$^{null}$ Tg(Hu-FLT3L) mice to support engraftment with human CD34+ HSC and development of a human immune system, groups of NSG™ *Flt3*$^{null}$ Tg(Hu-FLT3L) mice (n=8) and NSG™ control mice (n=10) at 8-12 weeks of age were sublethally irradiated (200cGy) and injected intravenously (IV) with 100,000 human umbilical cord blood CD34+ HSC. Flow cytometry analyses of the peripheral blood of the engrafted mice at 6, 9, 12, 15, and 18 weeks post-engraftment showed that the NSG™ *Flt3*$^{null}$ Tg(Hu-FLT3L) and NSG™ control mice had similar percentages of human CD45+ leukocytes. However, in the human CD45+ cell population, the NSG™ *Flt3*$^{null}$ Tg(Hu-FLT3L) mice had significantly increased percentages of both human CD3+ T cells and human CD33+ myeloid cells (**FIGS. 5A-5D and FIGS. 8A-8C**).

NSG™ *Flt3*$^{null}$ Tg(Hu-FLT3L) mice also showed increased percentages of human CD123+ plasmacytoid dendritic cells, CD56+ human natural killer (NK) cells, CD14+ human monocyte macrophages, and CD11C+ HLA-DR+ human myeloid dendritic cells (**FIGS. 6A-6D, 8D-8F**). Moreover, histochemical analyses of tissues from the engrafted mice unexpectedly showed mucosal engraftment of human CD45+ cells in the small intestines of NSG™ *Flt3*$^{null}$ Tg(Hu-FLT3L) mice (**FIG. 7**).

## SEQUENCES

ATCCCCCAGTGCTGGTGACCTTGAACACAGGGTCTGCCCTGAGCAGTGCTGACAGCCTGTTAGCTGTC
ATTTTGAAGCATTTCCTGAAGGAAACTTACCTTATCATTGCAGCTGTAAAGAAGAAGGCCCTGCTGTT
GTCAGAAAGGAGGAAAAGGTACTTCATGAGTTGTTCGGAACAGACATCAGATGCTGTGCTAGAAATG
CACTGGGCCGCGAATGCACCAAGCTGTTCACCATAGGTAATGGGGGACTGTCGGCTATGTGTCCTTAG
TGACGGTTCCTAAGGGACCGGTGATAGCTTCTGTGTGGGTAATCATTCCACTTCAGAAAGATGATGGA
AAGTTATACTGAAATCCGAATCTAGATCGAATAGTCCACATTCTGACATAAAAGCAATTGAACACACA
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ATTTGATTTGATTTGACTTAGGTGCTGGGCTCAAACCCAGGGACTCAGAGCATCTGCTTTGCCACTGA
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All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

The terms “about” and “substantially” preceding a numerical value mean ±10% of the recited numerical value.

Where a range of values is provided, each value between the upper and lower ends of the range are specifically contemplated and described herein.

What is claimed is:

## CLAIMS

1. A NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NOD *scid* gamma) mouse comprising
a nucleic acid encoding human FLT3L and
an inactivated mouse *Flt3* allele.

2. The mouse of claim 1, wherein the mouse comprises a genomic modification that inactivates the mouse *Flt3* allele.

3. The mouse of claim 2, wherein the genomic modification is in at least one region of the mouse *Flt3* allele selected from coding regions, non-coding regions, and regulatory regions.

4. The mouse of claim 3, wherein the genomic modification is in at least one coding region of the mouse *Flt3* allele.

5. The mouse of claim 4, wherein the genomic modification is in exon 6, exon 7, and/or exon 8.

6. The mouse of any one of claims 2-5, wherein the genomic modification is selected from genomic deletions, genomic insertions, genomic substitutions, and combinations thereof.

7. The mouse of claim 6, wherein the genomic modification is a genomic deletion.

8. The mouse of claim 7, wherein the mouse *Flt3* allele comprises a genomic deletion of nucleotide sequences in exon 6, exon 7, and exon 8.

9. The mouse of claim 8, wherein the nucleic acid sequence of SEQ ID NO: 5 has been deleted from the mouse *Flt3* allele.

10. The mouse of claim 9, wherein the modified mouse *Flt3* allele comprises the nucleic acid sequence of SEQ ID NO: 6.

11. The mouse of any one of claims 1-10, wherein the nucleic acid encoding human FLT3L comprises a human *FLT3L* transgene.

12. The mouse of claim 11, wherein the human *FLT3L* transgene comprises a nucleic acid sequence of SEQ ID NO: 7.

13. The mouse of any one of claims 1-12, wherein the mouse expresses human FLT3L.

14. The mouse of claim 13, wherein the human FLT3L is expressed at a level of at least 10,000 pg/ml.

15. The mouse of claim 14, wherein the human FLT3L is expressed at a level of 10,000 pg/ml to 30,000 pg/ml.

16. The mouse of claim 14 or 15, wherein the human FLT3L is expressed at a level of 15,000 +/- 1000 pg/mL to 17,000 +/- 100 pg/ml.

17. The mouse of any one of claims 1-16, wherein the mouse expresses mouse FLT3L.

18. The mouse of claim 17, wherein the mouse FLT3L is expressed at a level of at least 2,000 pg/ml.

19. The mouse of claim 18, wherein the mouse FLT3L is expressed at a level of 5,000 pg/ml to 10,000 pg/ml.

20. The mouse of claim 18, wherein the mouse FLT3L is expressed at a level of 6,000 pg/ml to 8,000 ml.

21. The mouse of any one of claims 1-20, wherein the mouse does not express a detectable level of mouse FLT3.

22. The mouse of any one of claims 1-21, wherein a detectable level of mouse FLT3 expressed by the mouse is less than 1,000 pg/ml.

23. The mouse of any one of claims 1-22, wherein the mouse lacks a detectable number of $CD135^+$ multipotent progenitor cells.

24. The mouse of any one of claims 1-23 further comprising human $CD34^+$ hematopoietic stem cells.

25. The mouse of claim 24, wherein the human $CD34^+$ hematopoietic stem cells are from human umbilical cord blood, bone marrow, or mobilized peripheral blood.

26. The mouse of any one of claims 1-25, wherein the mouse comprises a population of human $CD45^+$ cells.

27. The mouse of claim 26, wherein the population of human $CD45^+$ cells comprises human $CD45^+/CD3^+$ T cells and/or human $CD45^+/CD33^+$ myeloid cells.

28. The mouse of claim 27, wherein the population of human $CD45^+$ cells comprises an increased percentage of human $CD45^+/CD3^+$ T cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse.

29. The mouse of claim 28, wherein the percentage of human $CD45^+/CD3^+$ T cells in the mouse is increased by at least 25%, at least 50%, or at least 100%.

30. The mouse of any one of claims 27-29, wherein the population of human $CD45^+$ cells comprises an increased percentage of human $CD45^+/CD33^+$ myeloid cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse.

31. The mouse of claim 30, wherein the percentage of human $CD45^+/CD33^+$ myeloid cells in the mouse is increased by at least 25%, at least 50%, or at least 100%.

32. The mouse of any one of claims 1-31, wherein the mouse comprises an increased percentage of human $CD123^+$ plasmacytoid dendritic cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse.

33. The mouse of claim 32, wherein the percentage of human $CD123^+$ plasmacytoid dendritic cells in the mouse is increased by at least 25%, at least 50%, or at least 100%.

34. The mouse of any one of claims 1-33, wherein the mouse comprises an increased percentage of human $CD56^+$ natural killer cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse.

35. The mouse of claim 34, wherein the percentage of human $CD56^+$ natural killer cells in the mouse is increased by at least 25%, at least 50%, or at least 100%.

36. The mouse of any one of claims 1-35, wherein the mouse comprises an increased percentage of human $CD14^+$ monocyte macrophages, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse.

37. The mouse of claim 36, wherein the percentage of human $CD14^+$ monocyte macrophages in the mouse is increased by at least 25%, at least 50%, or at least 100%.

38. The mouse of any one of claims 1-37, wherein the mouse comprises an increased percentage of human $CD11C^+$ $HLA\text{-}DR^+$ myeloid dendritic cells, relative to a NOD *scid* gamma control mouse or a NOD *scid* gamma-Hu-FLT3L control mouse.

39. The mouse of claim 38, wherein the percentage of human $CD11C^+$ $HLA\text{-}DR^+$ myeloid dendritic cells in the mouse is increased by at least 25%, at least 50%, or at least 100%.

40. The mouse of any one of claims 1-39, wherein the mouse exhibits mucosal engraftment of human $CD45^+$ cells in the small intestines of the mouse.

41. A method comprising sublethally irradiating the mouse of any one of claims 1-23, and injecting the mouse with human $CD34^+$ hematopoietic stem cells.

42. The method of claim 41 further comprising administering to the mouse an agent of interest.

43. The method of claim 42 further comprising assessing an effect of the agent on human immune cells in the mouse.

44. The method of claim 43, wherein the human immune cells are selected from T cells, dendritic cells, natural killer cells, and macrophages.

45. A method comprising injecting a pronucleus of a NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NOD *scid* gamma) mouse with a nucleic acid encoding human FLT3L, producing a NSG Tg(Hu-FLT3L) mouse, and inactivating a mouse *Flt3* allele in the NSG Tg(Hu-FLT3L) mouse.

46. A method comprising inactivating a mouse *Flt3* allele in a NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NOD *scid* gamma) mouse to produce a NSG *Flt3*$^{null}$ mouse, and injecting a pronucleus of the NSG *Flt3*$^{null}$ mouse with a nucleic acid encoding human FLT3L.

47. A NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ cell comprising a nucleic acid encoding human FLT3L and an inactivated endogenous *Flt3* allele.

48. A transgenic rodent comprising the cell of claim 47, optionally wherein the transgenic rodent is a transgenic mouse.

49. A method comprising breeding female mice homozygous for *Prkdc*$^{scid}$, homozygous for *Il2rg*$^{tm1Wjl}$, homozygous for *Flt3*$^{null}$, and homozygous for a human *FLT3L* transgene with male mice homozygous for *Prkdc*$^{scid}$, hemizygous for the X-linked *Il2rg*$^{tm1Wjl}$, homozygous for *Flt3*$^{null}$, and homozygous for a human *FLT3L* transgene to produce progeny mice.

50. A gRNA targeting mouse *Flt3*, optionally wherein the gRNA targets exon 6 or exon 8 or mouse *Flt3*.

51. The gRNA of claim 50, wherein the gRNA comprises the sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

52. A mouse oocyte comprising the gRNA of claim 50 or 51, optionally wherein the mouse oocyte is fertilized.

53. A mouse oocyte comprising a first gRNA targeting exon 6 of mouse *Flt3* and a second gRNA targeting exon 8 of mouse *Flt3*, optionally wherein the mouse oocyte is fertilized.

54. The mouse oocyte of claim 52 or 53 further comprising Cas9 mRNA and/or Cas9 protein.

55. The mouse oocyte of any one of claims 52-54 further comprising a human *FLT3L* transgene.

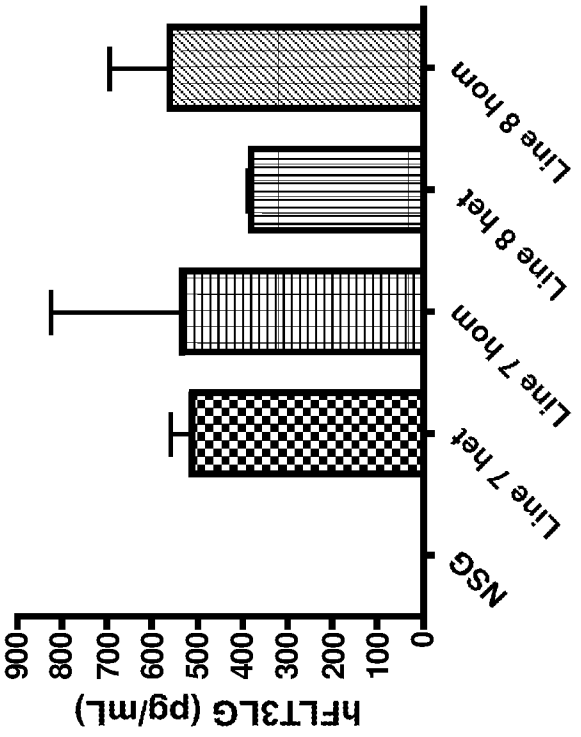

hFLT3LG (pg/mL)
900
800
700
600
500
400
300
200
100
0
NSG
Line 7 het
Line 7 hom
Line 8 het
Line 8 hom

*FIG. 1*

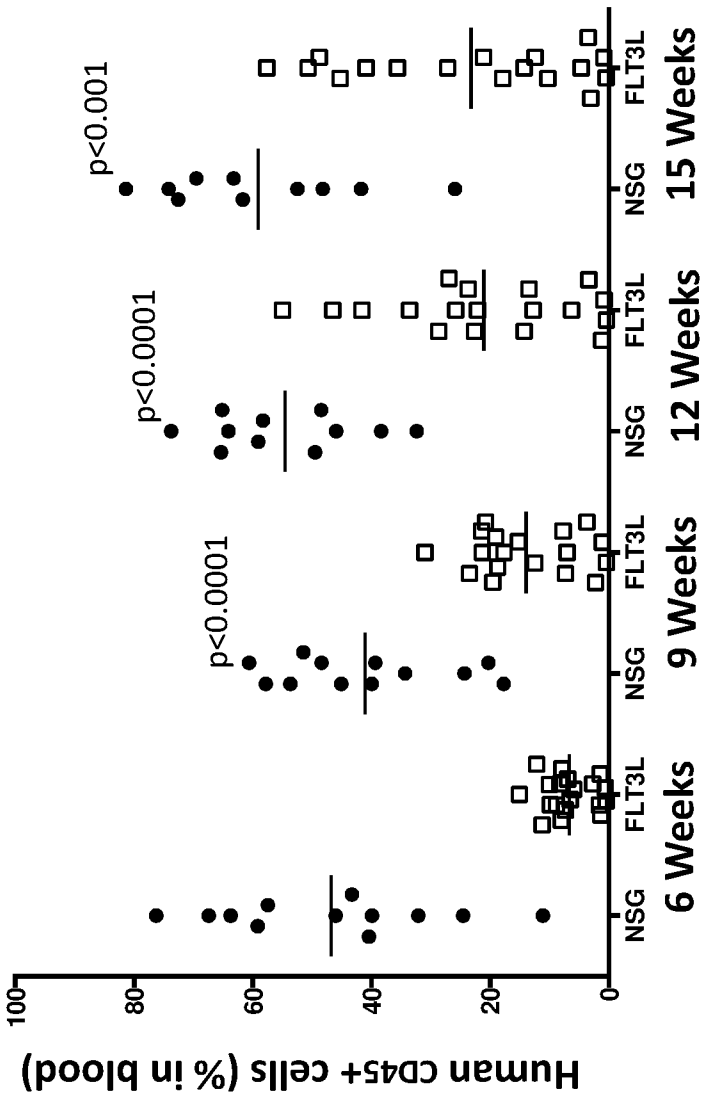

Human CD45+ cells (% in blood)
100
80
60
40
20
0
NSG
FLT3L
6 Weeks
p<0.0001
9 Weeks
p<0.0001
12 Weeks
p<0.001
15 Weeks

*FIG. 2*

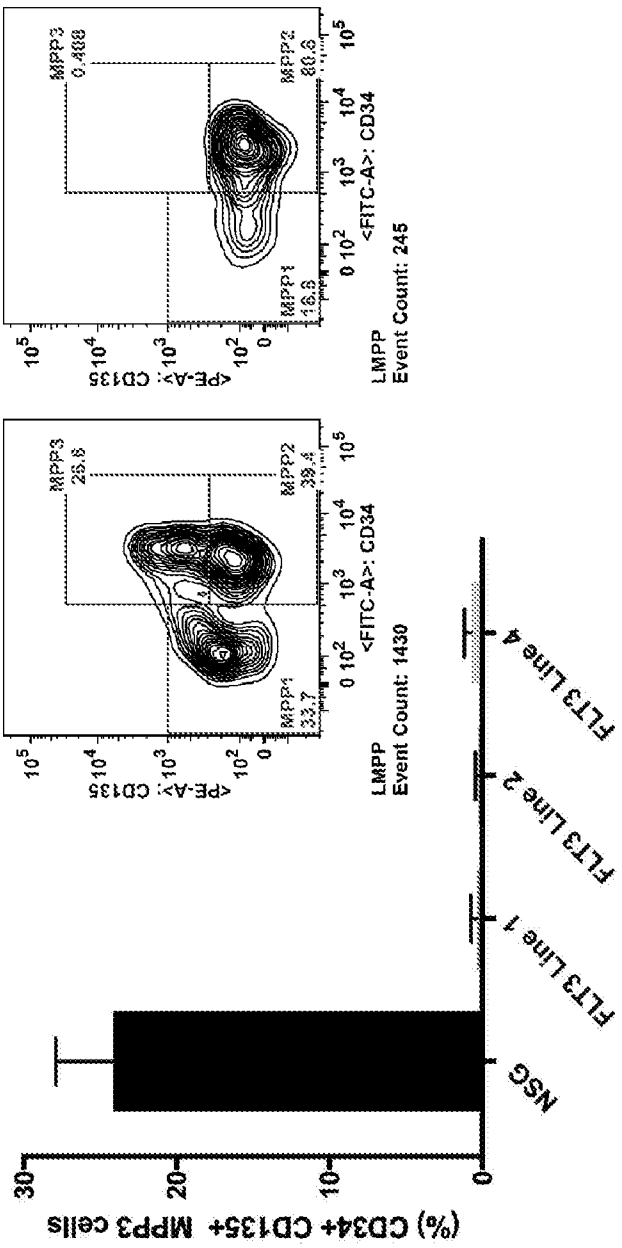
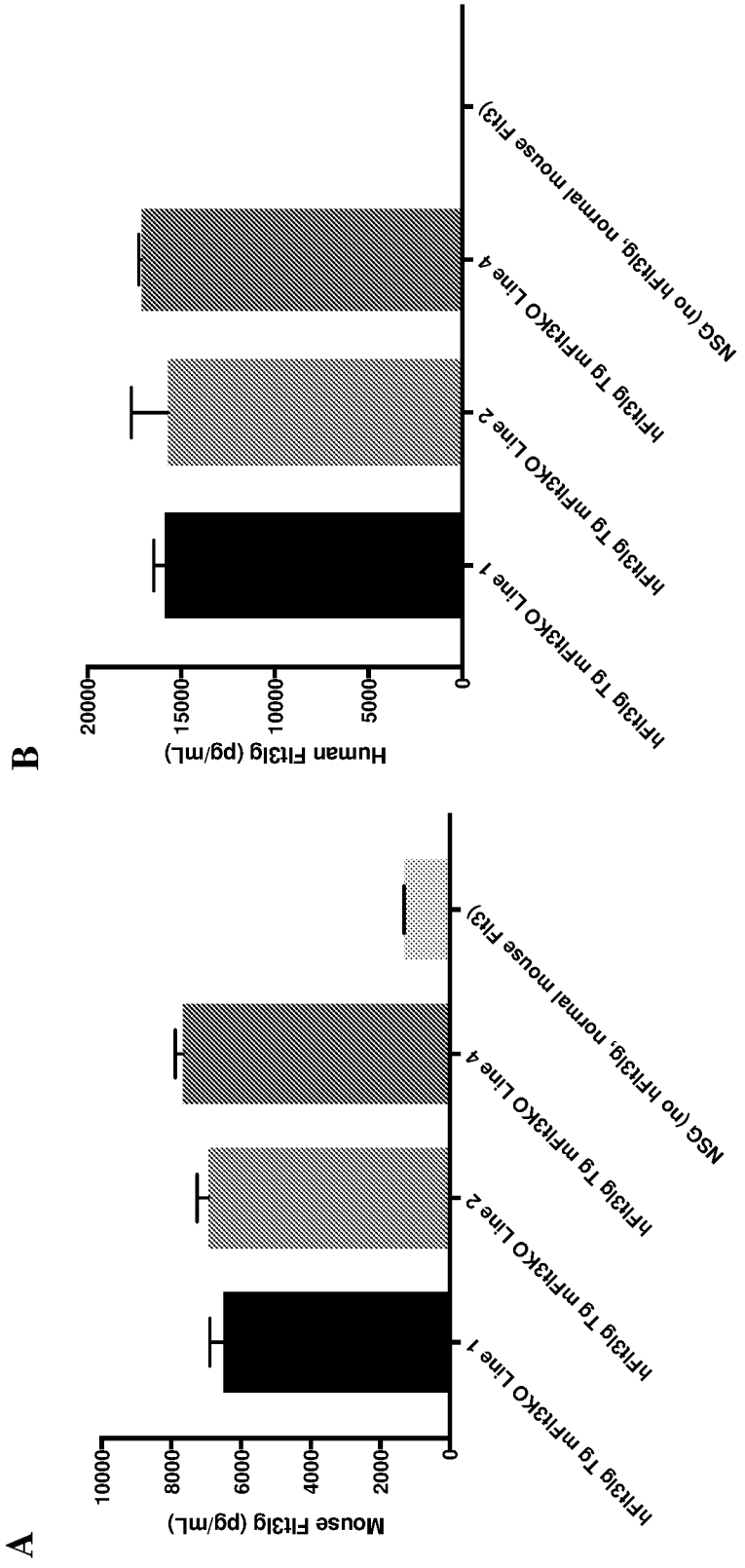
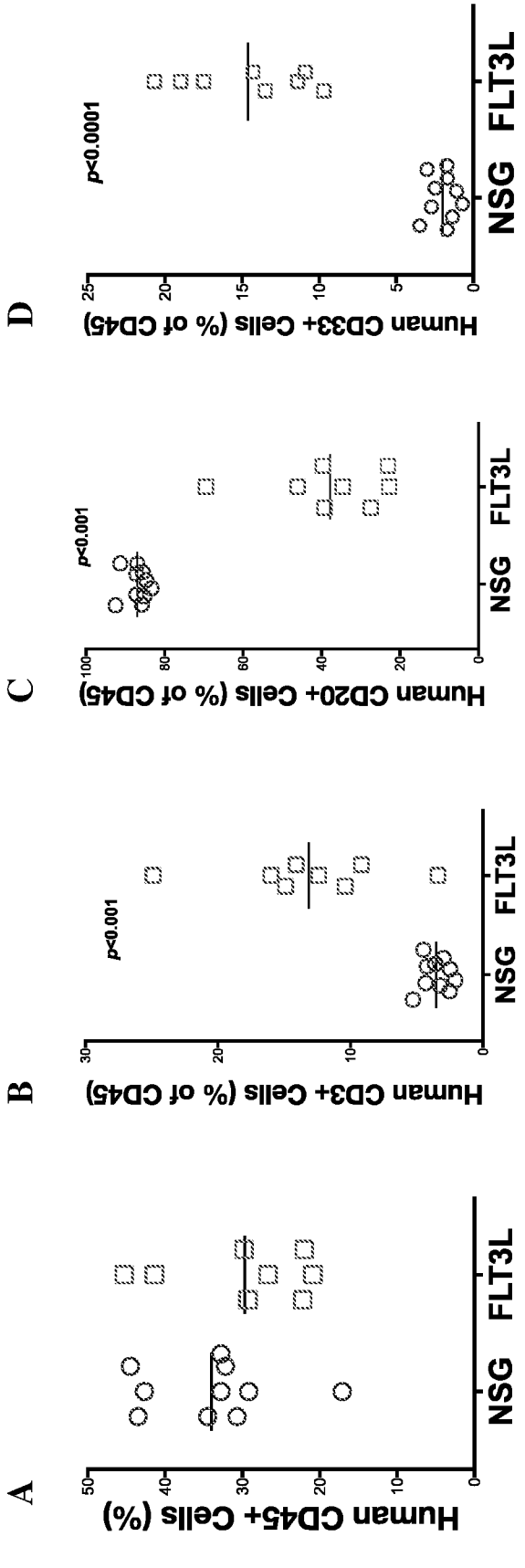

(%) CD34+ CD135+ MPP3 cells
30
20
10
0
NSG
FLT3 Line 1
FLT3 Line 2
FLT3 Line 4
LMPP
Event Count: 1430
<PE-A>: CD135
<FITC-A>: CD34
MPP3
25.6
MPP2
39.4
MPP1
33.7
LMPP
Event Count: 245
<PE-A>: CD135
<FITC-A>: CD34
MPP3
0.488
MPP2
80.6
MPP1
16.8

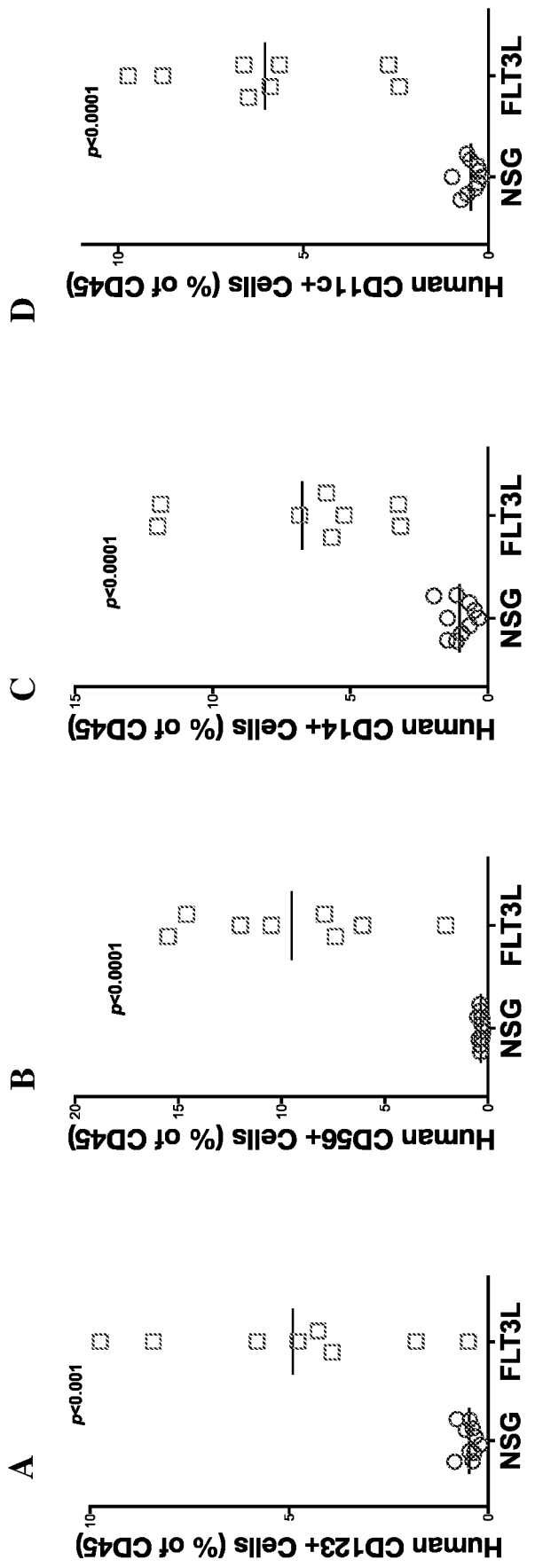
*FIG. 3*


A
Mouse Flt3lg (pg/mL)
0
2000
4000
6000
8000
10000
hFlt3lg Tg mFlt3KO Line 1
hFlt3lg Tg mFlt3KO Line 2
hFlt3lg Tg mFlt3KO Line 4
NSG (no hFlt3lg, normal mouse Flt3)
B
Human Flt3lg (pg/mL)
0
5000
10000
15000
20000
hFlt3lg Tg mFlt3KO Line 1
hFlt3lg Tg mFlt3KO Line 2
hFlt3lg Tg mFlt3KO Line 4
NSG (no hFlt3lg, normal mouse Flt3)

*FIGS. 4A-4B*


A
Human CD45+ Cells (%)
50
40
30
20
10
0
NSG
FLT3L
B
Human CD3+ Cells (% of CD45)
p<0.001
30
20
10
0
NSG
FLT3L
C
Human CD20+ Cells (% of CD45)
p<0.001
100
80
60
40
20
0
NSG
FLT3L
D
Human CD33+ Cells (% of CD45)
p<0.0001
25
20
15
10
5
0
NSG
FLT3L

*FIGS. 5A-5D*


A
Human CD123+ Cells (% of CD45)
p<0.001
NSG
FLT3L
B
Human CD56+ Cells (% of CD45)
p<0.0001
NSG
FLT3L
C
Human CD14+ Cells (% of CD45)
p<0.0001
NSG
FLT3L
D
Human CD11c+ Cells (% of CD45)
p<0.0001
NSG
FLT3L

*FIGS. 6A-6D*

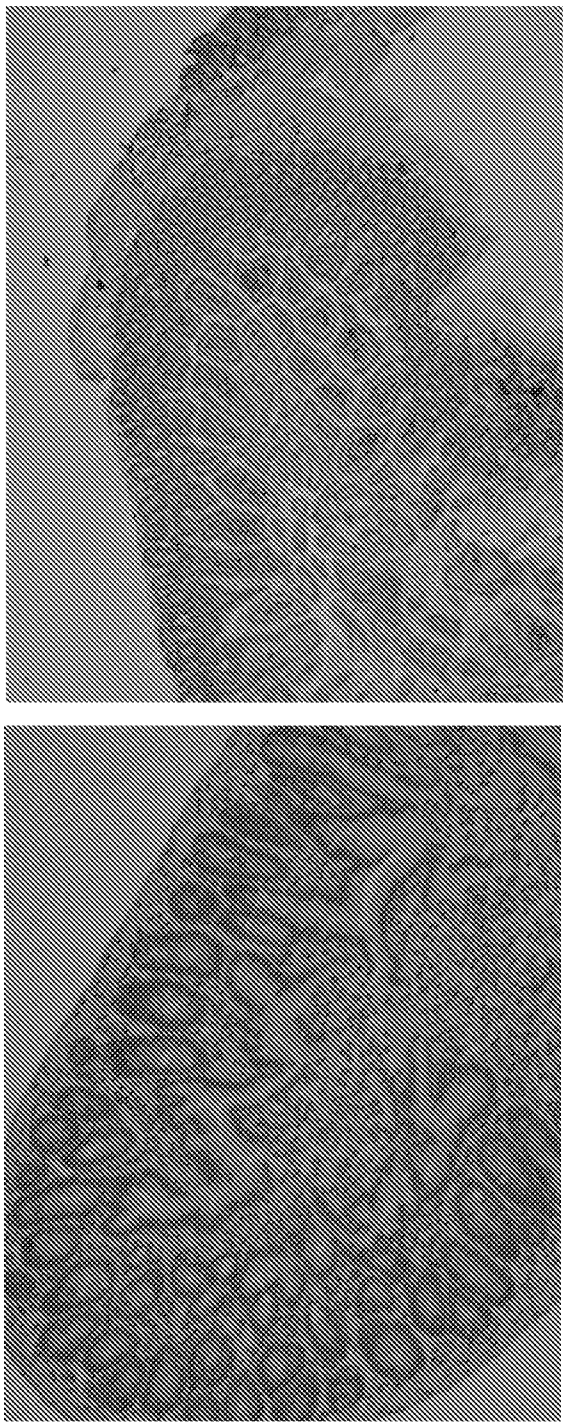



***FIG. 7***

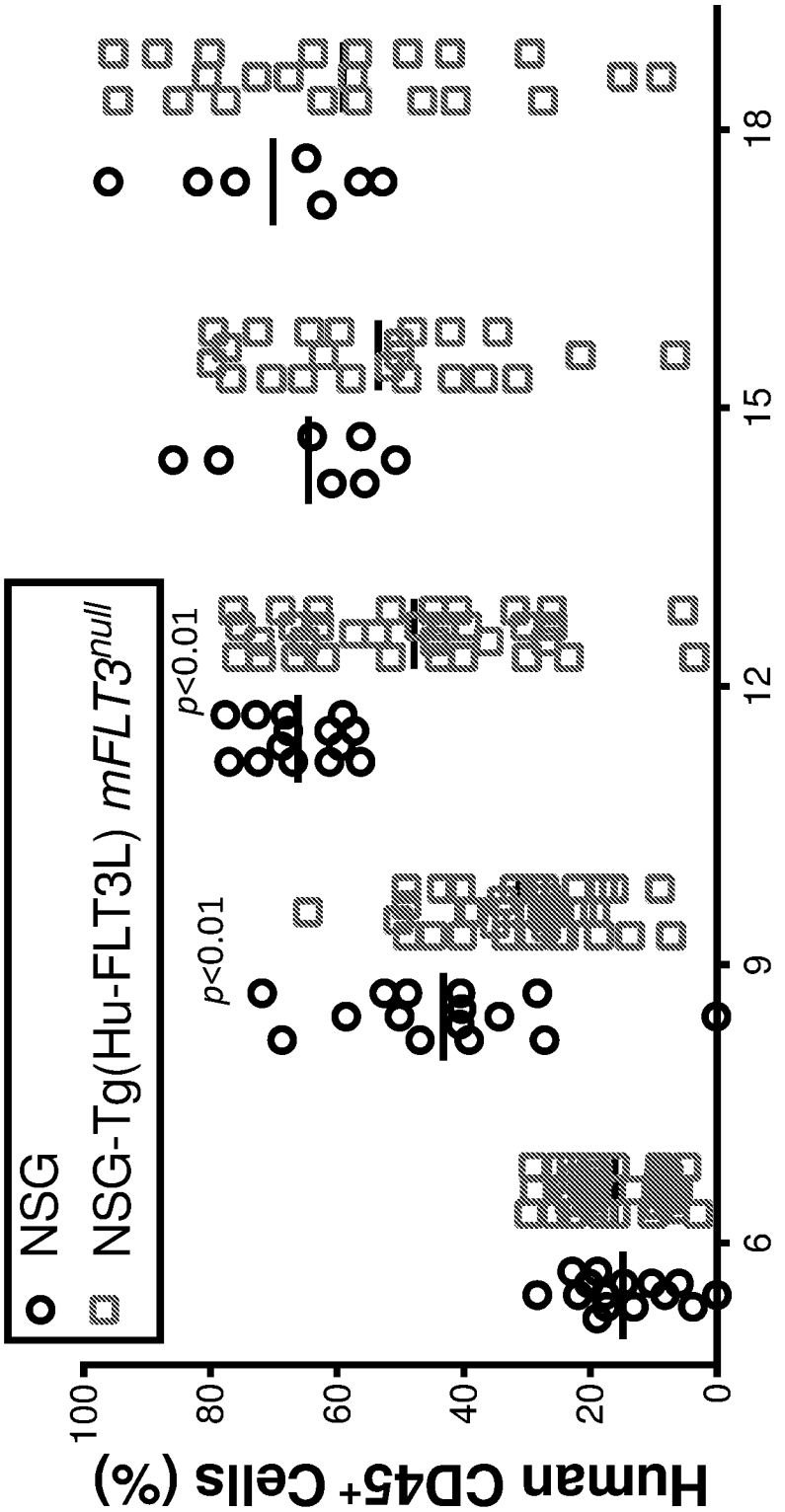

NSG
NSG-Tg(Hu-FLT3L) mFLT3null
p<0.01
p<0.01
Human CD45+ Cells (%)
0
20
40
60
80
100
6
9
12
15
18
Weeks Post CD34+ HSC-Engraftment

*FIG. 8A*

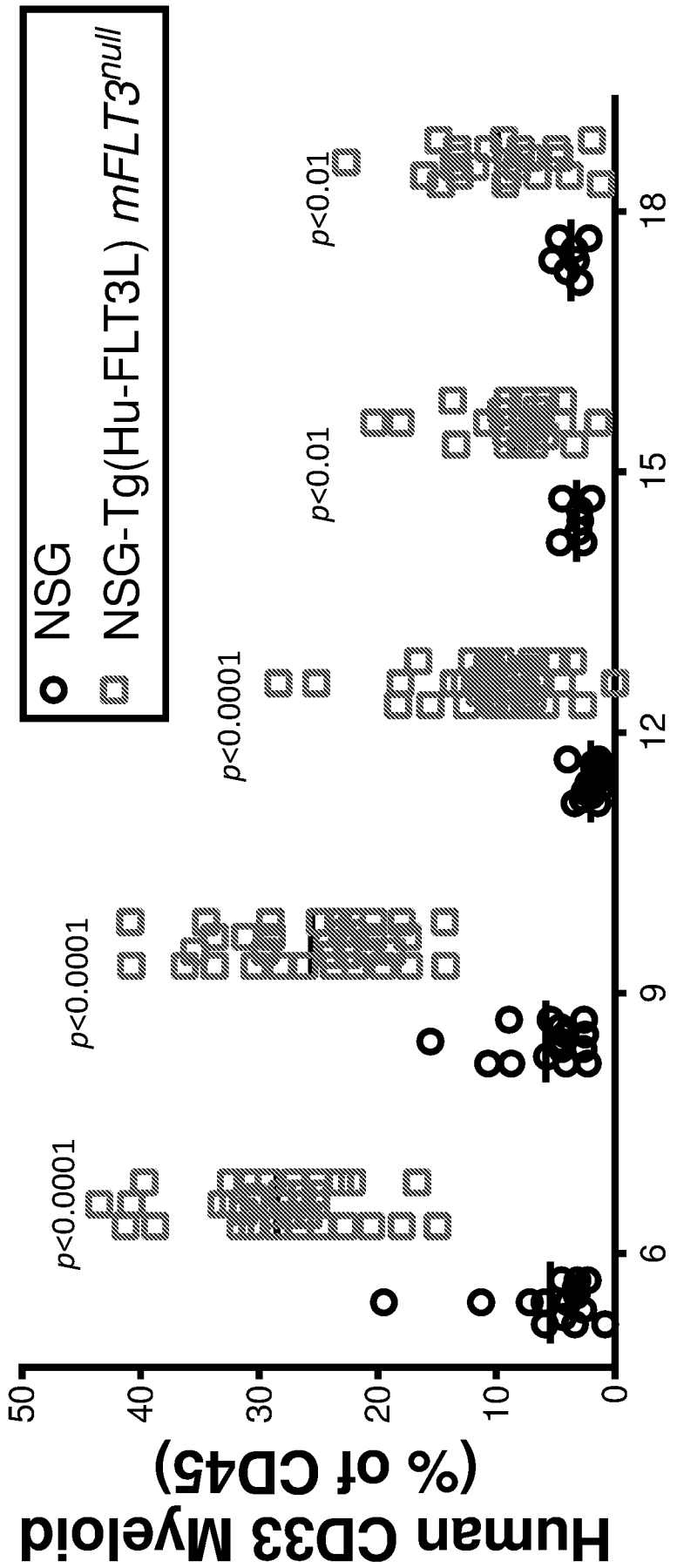

NSG
NSG-Tg(Hu-FLT3L) mFLT3null
Human CD33 Myeloid
(% of CD45)
0
10
20
30
40
50
6
9
12
15
18
Weeks Post CD34+ HSC-Engraftment
p<0.0001
p<0.0001
p<0.0001
p<0.01
p<0.01

*FIG. 8B*


Human CD3 T cells
(% of CD45)
70
60
50
40
30
20
10
0
6
9
12
15
18
Weeks Post CD34+ HSC-Engraftment
NSG
NSG-Tg(Hu-FLT3L) mFLT3null
p<0.001
p<0.01
p<0.01

*FIG. 8C*

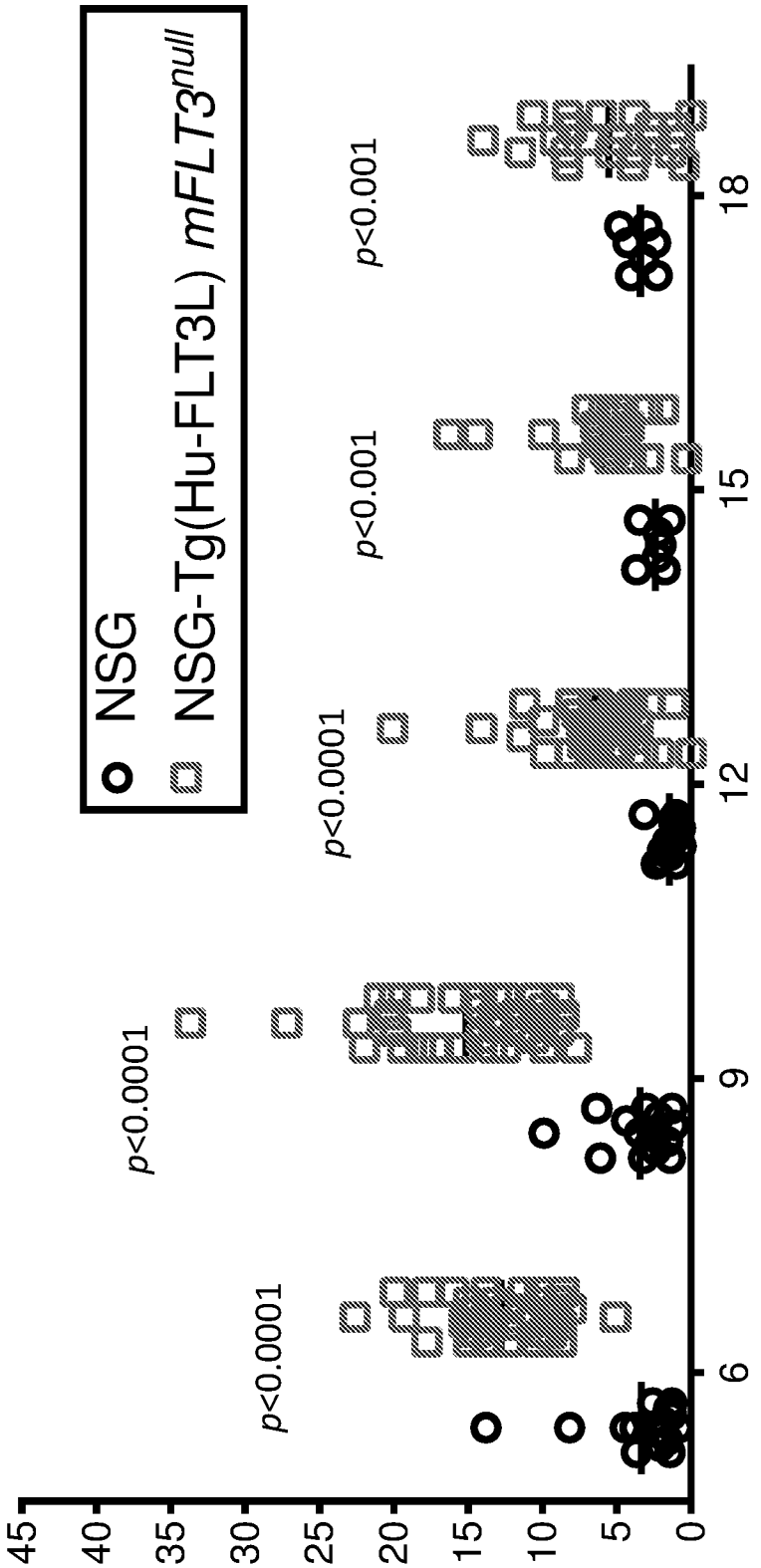

Human CD14 Monocytes
(% of CD45)
45
40
35
30
25
20
15
10
5
0
6
9
12
15
18
Weeks Post CD34+ HSC-Engraftment
NSG
NSG-Tg(Hu-FLT3L) mFLT3null
p<0.0001
p<0.0001
p<0.0001
p<0.001
p<0.001

*FIG. 8D*

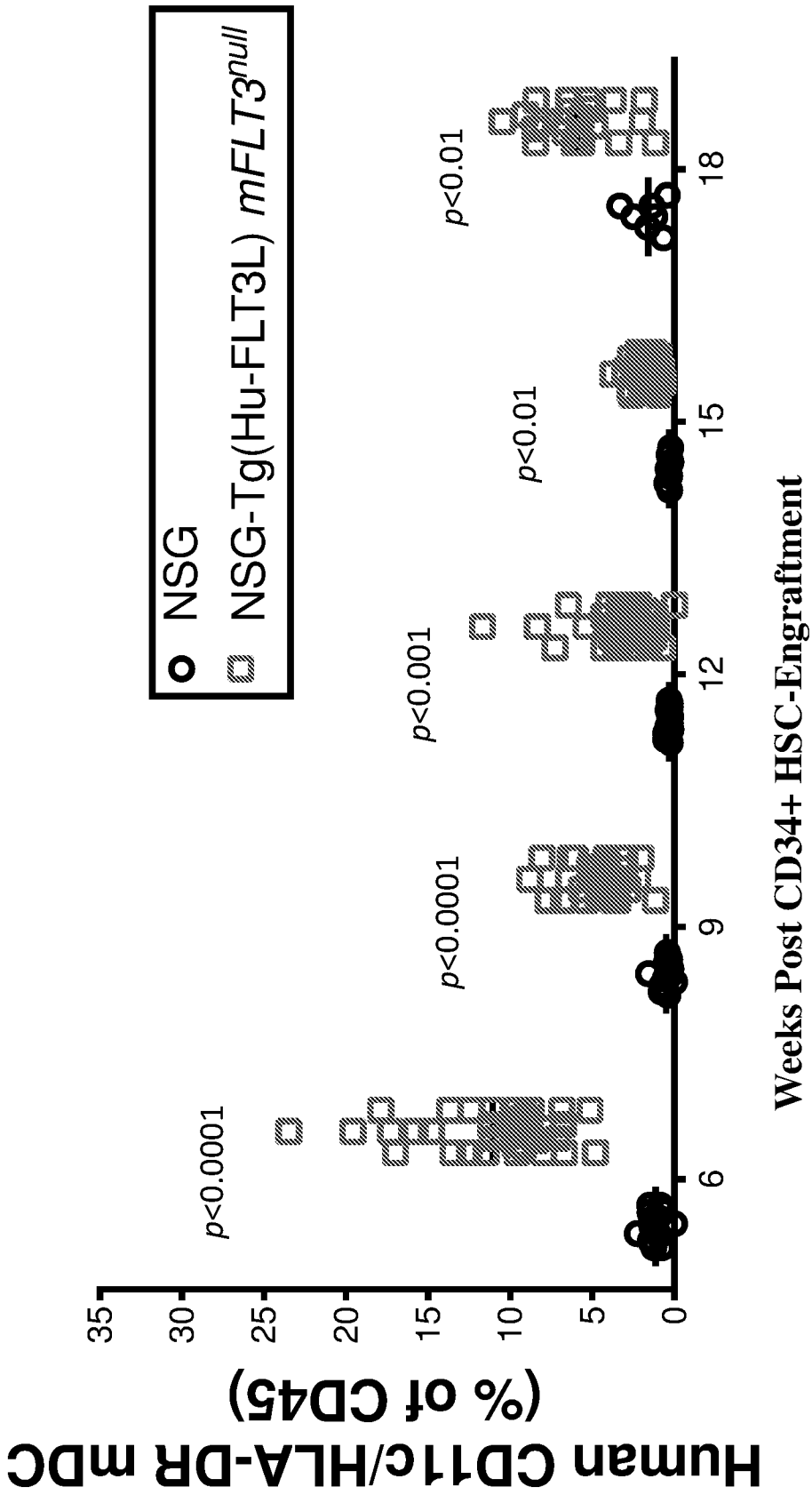

NSG
NSG-Tg(Hu-FLT3L) mFLT3null
p<0.0001
p<0.0001
p<0.001
p<0.01
p<0.01
Human CD11c/HLA-DR mDC
(% of CD45)
0
5
10
15
20
25
30
35
6
9
12
15
18
Weeks Post CD34+ HSC-Engraftment

*FIG. 8E*

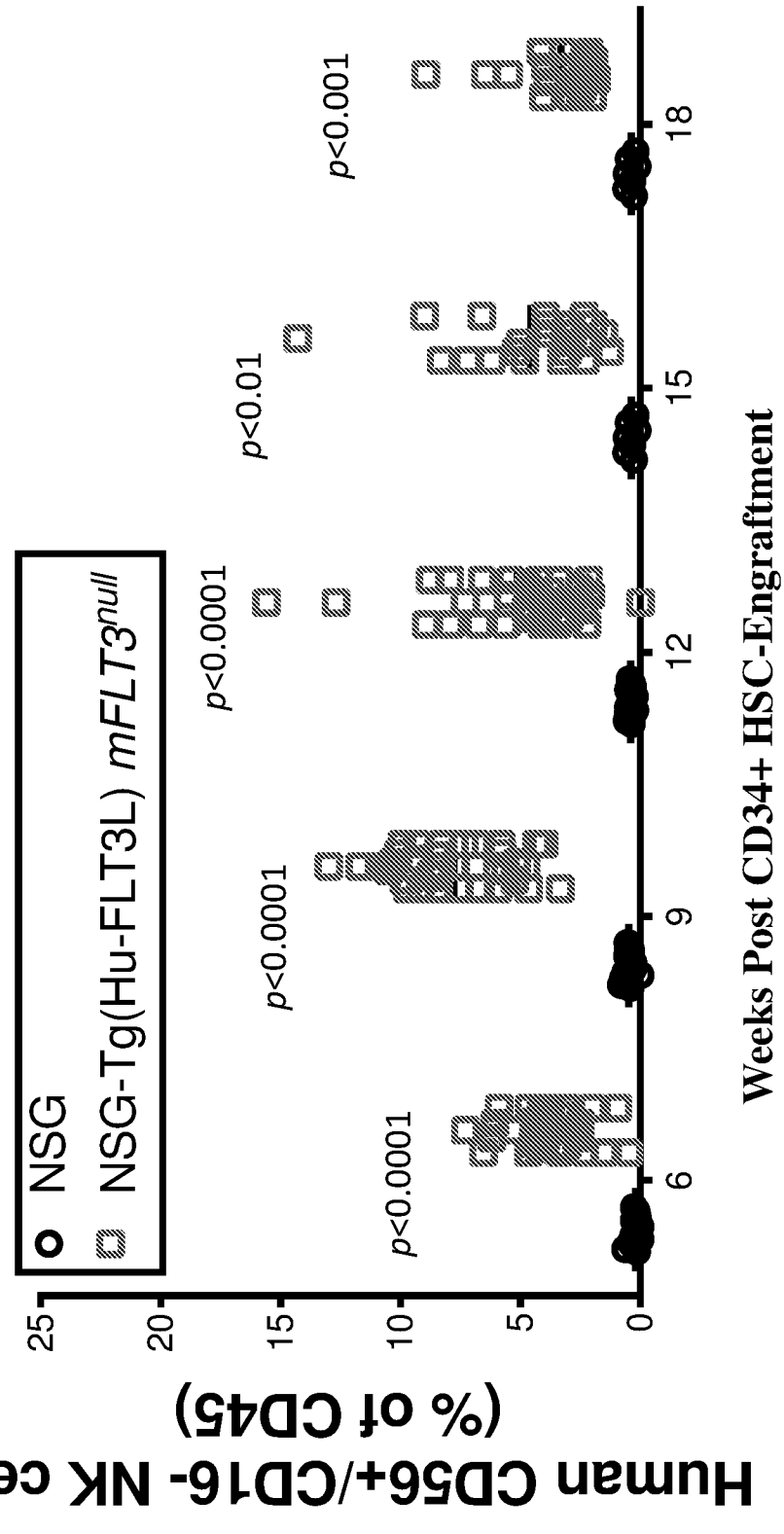

Human CD56+/CD16- NK cells
(% of CD45)
25
20
15
10
5
0
6
9
12
15
18
Weeks Post CD34+ HSC-Engraftment
NSG
NSG-Tg(Hu-FLT3L) mFLT3null
p<0.0001
p<0.0001
p<0.01
p<0.001

*FIG. 8F*

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/18033

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 11-44, 54, 55
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

-----please see continuation in extra sheet------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 47-48 limited to SEQ ID NO: 5

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/18033

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A01K 67/027, C07K 14/54 (2020.01)
CPC - A01K 67/0275, A61K 67/0276, C07K 14/52, C07K 14/705, A01K 2217/075

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y<br>---<br>A | US 2012/0157667 A1 (CHEN et al.) 21 June 2012 (21.06.2012) Especially Abstract, para [0046], [0049] | 1-4, (6-7)/(2-4), 47-48<br>---<br>5, (6-7)/5, 8, 9 |
| Y<br>---<br>A | DURAI et al. Altered compensatory cytokine signaling underlies the discrepancy between Flt3-/- and Flt3l-/-mice. Journal of Experimental Medicine, 7 May 2018, Vol 215, No 5, pp 1417-1435 Especially Abstract | 1-4, (6-7)/(2-4), 47-48<br>---<br>5, (6-7)/5, 8, 9 |
| Y<br>---<br>A | ZRIWIL et al. Direct role of FLT 3 in regulation of early lymphoid progenitors. British Journal of Haematology, November 2018, Vol 183, No 4, pp 588-600 Especially Abstract; pg 589, col 1, para 3-col 2, para 1 | 2-4, (6-7)/(2-4)<br>---<br>5, (6-7)/5, 8, 9 |
| A | WO 2017/205756 A1 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM et al.) 30 November 2017 (30.11.2017) Especially Abstract; pg 28, ln 4-10; Table 4 | 5, (6-7)/5, 8, 9 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 14 June 2020 | 06 JUL 2020 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer<br>Lee Young<br>Telephone No. PCT Helpdesk: 571-272-4300 |

**INTERNATIONAL SEARCH REPORT**
Information on patent family members

International application No.
PCT/US 20/18033

Continuation of Box No. III Observations where unity of invention is lacking

Groups I+: Claims 1-10, 47-48, 50-53, drawn to a NOD scid gamma mouse comprising a nucleic acid encoding human FLT3L and an inactivated mouse Flt3 allele. The composition will be searched to the extent that the inactivation mouse Flt3 allele encompasses deletion of SEQ ID NO: 5. It is believed that claims 1-9, 47-48, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 5. Additional inactivated mouse Flt3 allele(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected inactivated mouse Flt3 allele(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an inactivated mouse Flt3 allele comprising the gRNA of SEQ ID NO: 1 (Claims 1-8, 47-48, 50-53).

Group II: claims 45, 46, 49, drawn to a method comprising inactivating a mouse Flt3 allele in a NOD scid gamma mouse.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I+ include the special technical feature of a composition which differs from the special technical feature of a method, as disclosed by Group II.

No technical features are shared between the nucleic acid sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ and II were considered to share the technical features of including: an inactivated mouse Flt3 allele, these shared technical features are previously disclosed by US 2012/0157667 A1 to Chen et al. (hereinafter 'Chen') and the article "Altered compensatory cytokine signaling underlies the discrepancy between Flt3-/- and Flt3l-/- mice" by Durai et al. (hereinafter 'Durai') (J. Exp. Med. 2018 Vol. 215 No.5, 1417-1435).

Chen teaches (instant claim 1) a NOD.Cg-Prkdc(scid) Il2rg(tmlWjt)/SzJ (NOD scid gamma) mouse (Abstract - "Provided herein are methods of reconstituting functional human blood cell lineages in a non-human mammal comprising introducing human hematopoietic stem cells (HSCs) and nucleic acid encoding one or more human cytokines into an immunodeficient non-human mammal."; para [0049] - "the non-human mammal is an immunodeficient non-human mammal, that is, a non-human mammal that has one or more deficiencies in its immune system (e.g., NSG or NOD scid gamma (NOD.Cg-Prkdc(scid) Il2rg(tmlWjt)/SzJ mice) and, as a result, allow reconstitution of human blood cell lineages when human HSCs are introduced") comprising a nucleic acid encoding human FLT3L (para [0046] - "There are a variety of human cytokines that can be used in the methods of the invention. Examples of such human cytokines include interleukin-12 (IL-12), interleukin-15 (IL-15), Fms-related tyrosine kinase 3 ligand (Flt3L)"), but does not teach the mouse comprising an inactivated mouse Flt3 allele.
Durai teaches a method of administration exogenous Flt3L to a Flt3 knock-out mouse to determine the impact of Flt3 inactivation in the presence of Flt3 ligand (Abstract - "The receptor Flt3 and its ligand Flt3L are both critical for dendritic cell (DC) development, but DC deficiency is more severe in Flt3l-/- mice than in Flt3-/- mice. This has led to speculation that Flt3L binds to another receptor that also supports DC development. However, we found that Flt3L administration does not generate DCs in Flt3-/- mice, arguing against a second receptor."). It would have been obvious to one of ordinary skill in the art to have provided a NOD scid gamma mouse having an inactivated Flt3 allele to, for example, test the impact of human Flt3 ligand expression in a Flt3 knock out model.

Some inventions of Groups I+ share the special technical feature of a gRNA targeting Flt3, which is disclosed by the article "A CRISPR Dropout Screen Identifies Genetic Vulnerabilities and Therapeutic Targets in Acute Myeloid Leukemia" by Tzelepis et al. (hereinafter "Tzelepis") (Cell Reports 17, 1193-1205, October 18, 2016) (pg 1197, col 1, para 2, In addition, gRNAs against FLT3 and NRAS showed specific depletion in cell lines carrying activating mutations in these genes, whereas NPM1 was depleted in four of the five AML lines including OCI-AML3 (Figure 3E).).

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (continued):
Claims 11-44, 54, 55 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).