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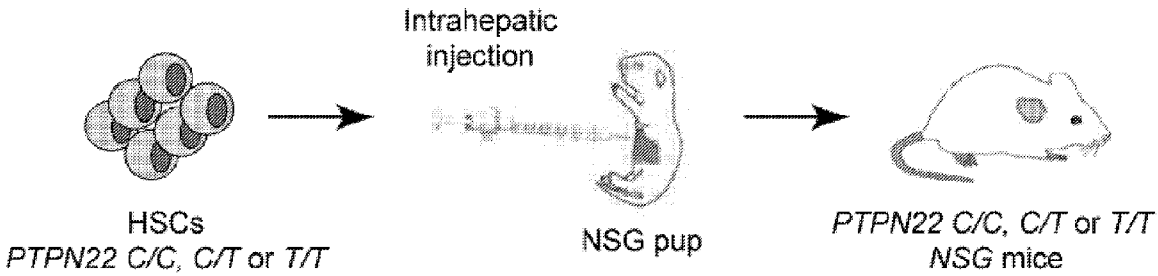

Figure 1A

(57) Abstract: The present invention provides compositions and methods for inhibiting PTPN22 for restoring human central B-cell tolerance or for treating or preventing an autoimmune disease or disorder.

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TITLE OF THE INVENTION

COMPOSITIONS AND METHODS FOR INHIBITING PTPN22

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is entitled to priority to U.S. Provisional Application No. 62/342,250, filed May 27, 2016, which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

A role for B cells in autoimmune disease is now established both in mouse models as well as in humans by successful treatment of rheumatoid arthritis and by showing efficacy at delaying other autoimmune diseases with anti-CD20 monoclonal antibodies that eliminate B cells. However, B cell depletion is a severe insult to the immune system that may be harmful for patients. In addition, patients often relapse after anti-B cell therapy several months later coinciding with the reappearance of B cells in the blood of these subjects. These newly generated B cells likely include many autoreactive clones because patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and type 1 diabetes (T1D) display abnormal early B-cell tolerance checkpoints resulting in a failure to remove developing autoreactive B cells. Hence, the efficacy of anti-B cell therapy may be limited because it may not fix the intrinsic tolerance mechanisms defective in autoimmune diseases, such as RA, SLE and T1D.

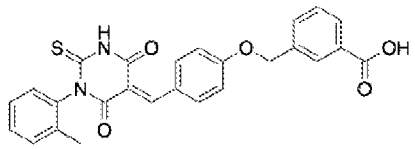
Rituximab, an anti-CD20 monoclonal antibody that eliminates B cells, has shown efficacy in T1D, RA and multiple sclerosis (MS), and exposes a role for B cells in promoting autoimmunity (Pescovitz et al., 2009, NEJM 361:2143-52; Edwards et al., 2004, NEJM 350:2572-81; Hauser et al., 2008, NEJM 358:676-88). However, anti-B cell therapy does not reset early B cell tolerance checkpoints defective in T1D likely because these impaired autoreactive B cell counterselection steps may be primary to the development of this autoimmune disease (Chamberlain et al., 2015, J Clin Invest 126:282-7). Indeed, asymptomatic individuals carrying the PTPN22 T allele display elevated frequencies of autoreactive B cells in their blood similar to those in T1D, RA and SLE patients (Menard et al., 2011, J Clin Invest 121:3635-44).

There is thus a need in the art for restoring human central B-cell tolerance and for prevention or treatment of autoimmunity. The present invention addresses this unmet need in the art.

SUMMARY OF THE INVENTION

In one aspect, the invention provides compositions and methods for treating or preventing an abnormal early B-cell tolerance checkpoint. In one embodiment, the composition of the invention comprises an inhibitor of PTPN22.

In one embodiment, the inhibitor of PTPN22 is at least one selected from the group consisting of a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, an antisense nucleic acid molecule.

In one embodiment, the inhibitor of PTPN22 is a small molecule chemical compound. For example, in one embodiment, the inhibitor of PTPN22 is  , a derivative thereof, or a salt thereof.

In one embodiment, the inhibitor of PTPN22 is a nucleic acid. For example, in one embodiment, the inhibitor of PTPN22 is a nucleic acid comprising a nucleoctide sequence selected from SEQ ID NO:1 and SEQ ID NO:2.

In one embodiment, the abnormal early B-cell tolerance checkpoint is associated with an autoimmune disease or disorder. For example, in one embodiment, the autoimmune disease or disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, Sjögren's syndrome, autoimmune thyroiditis, myasthenia gravis, and pemphigus.

In one aspect, the invention provides a method for treating or preventing an autoimmune disease or disorder. The invention also provides a method for restoring human central B-cell tolerance in a subject. In one embodiment, the method comprises administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof.

In one embodiment, the inhibitor of PTPN22 is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, an antisense nucleic acid molecule.

In one embodiment, the autoimmune disease or disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus.

In one embodiment, the subject fails to properly remove developing autoreactive B cells. In one embodiment, the subject has a 1858T PTPN22 polymorphism on at least one allele. In one embodiment, the subject is human.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of exemplary embodiments of the invention, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

Figure 1, comprising Figure 1A through Figure 1D, depicts results of experiments demonstrating defective central B-cell tolerance in humanized mouse engrafted with HSCs carrying PTPN22 T allele(s). Figure 1A depicts a schematic diagram depicting the generation of humanized mice. CD34+ hematopoietic stem cells (HSCs) carrying or not PTPN22 T allele(s) were injected in the liver of 3-day-old recipient NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice. Figure 1B depicts representative flow cytometry analysis of the frequency of human CD45+, CD3+ and CD19+ cells in the blood of the indicated recipient mice. The summary of blood engraftment from NSG mice transplanted with PTPN22 C/C, C/T or T/T HSCs is represented. Each dot represents an individual mouse and bars indicate mean values. Figure 1C depicts the frequencies of polyreactive new emigrant B cells from different types of humanized mice transplanted with indicated HSCs were determined and compared to those of healthy donors carrying or not PTPN22 T allele(s). Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area)

and non-reactive (open area) clones is summarized in pie charts, with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each diamond represents an individual and each dot a mouse. Averages are shown with a bar. Figure 1D depicts the frequencies of HEp-2 reactive new emigrant B cells from different types of humanized mice transplanted with indicated HSCs were determined and compared to those of healthy donors carrying or not PTPN22 T allele(s). Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts, with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each diamond represents an individual and each dot a mouse. Averages are shown with a bar.

Figure 2, comprising Figure 2A through Figure 2D depicts results of experiments demonstrating new migrant B cells isolated from NSG mice engrafted with PTPN22 C/T or T/T HSCs display normal IgH repertoire. Figure 2A depicts VH gene-usage frequencies in new emigrant B cells are represented for 7 NSG mice engrafted with PTPN22 C/C HSCs and 6 NSG mice engrafted with PTPN22 C/T or T/T HSCs. Sequences from 226 NSG PTPN22 C/C and 203 NSG PTPN22 C/T or T/T single transitional B cells were pooled. Figure 2B depicts D gene-usage frequencies in new emigrant B cells are represented for 7 NSG mice engrafted with PTPN22 C/C HSCs and 6 NSG mice engrafted with PTPN22 C/T or T/T HSCs. Sequences from 226 NSG PTPN22 C/C and 203 NSG PTPN22 C/T or T/T single transitional B cells were pooled. Figure 2C depicts JH gene-usage frequencies in new emigrant B cells are represented for 7 NSG mice engrafted with PTPN22 C/C HSCs and 6 NSG mice engrafted with PTPN22 C/T or T/T HSCs. Sequences from 226 NSG PTPN22 C/C and 203 NSG PTPN22 C/T or T/T single transitional B cells were pooled. Figure 2D depicts reading frame (RF) usages for the D6-6 and D3-22 genes are compared between new emigrant/transitional B cells from 7 NSG mice engrafted with PTPN22 C/C HSCs and 6 NSG mice engrafted with PTPN22 C/T or T/T HSCs.

Figure 3, comprising Figure 3A through Figure 3C, depicts results from experiments demonstrating defective central B-cell tolerance in humanized mouse engrafted with HSCs carrying PTPN22 T allele(s). Figure 3A depicts the frequencies of polyreactive new emigrant B cells from different types of humanized mice transplanted

with indicated HSCs were determined and compared to those of healthy donors carrying or not PTPN22 T allele(s). Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. Figure 3B depicts the frequencies of HEp-2 reactive new emigrant B cells from different types of humanized mice transplanted with indicated HSCs were determined and compared to those of healthy donors carrying or not PTPN22 T allele(s). Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. Figure 3C depicts the frequencies of antinuclear new emigrant B cells are compared between mouse engrafted with HSCs carrying or not the PTPN22 T allele.

Figure 4, comprising Figure 4A through Figure 4C, depicts results from experiments demonstrating PTPN22 620W overexpression interferes with central B cell tolerance. Figure 4A depicts a schematic of the experimental design. Humanized mice were generated with CD34+ HSCs transduced with lentiviruses allowing the expression of different variants of PTPN22 before being injected in the liver of 3-day-old recipient NSG mice. Figure 4B depicts the frequencies of polyreactive new emigrant B cells from sorted GFP+ fractions expressing 620W PTPN22, 620R PTPN22 or 263Q PTPN22 were determined and compared to those of GFP- new emigrant B cells. Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts, with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each symbols represents a mouse overexpressing 620W PTPN22 (green dots), 620R PTPN22 (green squares) or 263Q PTPN22 (green triangles) and averages are shown with a bar. Figure 4C depicts the frequencies of HEp-2 reactive new emigrant B cells from sorted GFP+ fractions expressing 620W PTPN22, 620R PTPN22 or 263Q PTPN22 were determined and compared to those of GFP- new emigrant B cells. Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts, with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each symbol represents a

mouse overexpressing 620W PTPN22 (green dots), 620R PTPN22 (green squares) or 263Q PTPN22 (green triangles) and averages are shown with a bar.

Figure 5, comprising Figure 5A and Figure 5B, depicts results from experiments demonstrating overexpression of PTPN22 variants in NSG mice. Figure 5A depicts western blot analysis of PTPN22 protein expression in Ramos B cell transduced with lentiviruses allowing the overexpression of PTPN22 620W variant. β-actin is used for normalization of protein expression. Figure 5B depicts representative flow cytometry analysis of CD19+ cells isolated from the spleen of NSG mice engrafted with HSCs transduced with a GFP-tagged lentivirus expressing 620W PTPN22, 620R PTPN22 and 263Q PTPN22. CD19+ were stained with anti-hCD19, anti-IgM and anti-hCD10 antibodies. The frequencies of GFP- and GFP+ shRNA+ new emigrant B cells are shown.

Figure 6, comprising Figure 6A through Figure 6D, depicts results from experiments demonstrating 620W PTPN22 overexpression interferes with the central B cell tolerance checkpoint. Figure 6A depicts the frequencies of polyreactive new emigrant B cells from sorted GFP+ fractions expressing 620W PTPN22, 620R PTPN22 or 263Q PTPN22 were determined and compared to those of GFP- new emigrant B cells. Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. Figure 6B depicts the frequencies of HEp-2 reactive new emigrant B cells from sorted GFP+ fractions expressing 620W PTPN22, 620R PTPN22 or 263Q PTPN22 were determined and compared to those of GFP- new emigrant B cells. Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. Figure 6C depicts the frequencies of antinuclear new emigrant B cells are compared between GFP- and GFP+ few emigrant B cells expressing 620W PTPN22, 620R PTPN22 or 263Q PTPN22. Figure 6D depicts autoreactive antibodies from GFP+ 620W PTPN22 expressing new emigrant B cells show various patterns of anti-nuclear HEp-2 staining. Original magnification, ×40.

Figure 7, comprising Figure 7A through Figure 7C depicts results of experiments showing inhibition of PTPN22 enzymatic activity resets central B cell

tolerance. Figure 7A depicts a schematic diagram depicting the PTPN22 inhibitor treatment strategy. NSG mice generated with CD34+ HSCs carrying PTPN22 T allele(s) were injected twice daily with 0.75 mg or 0.15 mg of PTPN22 inhibitor for one week. Figure 7B depicts the frequencies of polyreactive new emigrant B cells from NSG mice carrying PTPN22 T allele(s) and treated with the PTPN22 inhibitor were determined and compared to those of non-treated NSG mice. Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each dot represents an untreated mouse and full and half-filled diamonds mice treated with either 0.75 or 0.15 mg of LTV-1 PTPN22 inhibitor, respectively. Averages are shown with a bar. Figure 7C depicts the frequencies of HEp-2 reactive new emigrant B cells from NSG mice carrying PTPN22 T allele(s) and treated with the PTPN22 inhibitor were determined and compared to those of non-treated NSG mice. Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each dot represents an untreated mouse and full and half-filled diamonds mice treated with either 0.75 or 0.15 mg of LTV-1 PTPN22 inhibitor, respectively. Averages are shown with a bar.

Figure 8 depicts frequencies of anti-nuclear new emigrant B cells in PTPN22 C/T or T/T NSG mice treated with the LTV-1 PTPN22 inhibitor. Anti-nuclear frequencies are compared between the PTPN22 C/T or T/T NSG mice treated or not with 0.75mg or 0.15mg of LTV-1 PTPN22 inhibitor. Each symbol represents a mouse and horizontal bars denote means.

Figure 9, comprising Figure 9A through Figure 9E, depicts results from experiments demonstrating inhibition of PTPN22 expression during B cell development resets central B cell tolerance. Figure 9A depicts a schematic of the experimental design. CD34+ HSCs carrying PTPN22 T allele(s) were transduced with lentiviruses allowing the expression of PTPN22 shRNA before injection in the liver of 3 day-old NSG mice. Figure 9B depicts representative flow cytometry analysis of CD19+ cells isolated from the spleen of NSG mouse engrafted with PTPN22 C/T HSCs transduced with a GFP-

tagged lentivirus expressing PTPN22 specific shRNA. CD19+ B cells were stained with anti-hCD19, anti-IgM and anti-hCD10 antibodies. The frequencies of GFP- and GFP+ shRNA+ new emigrant B cells are shown. Figure 9C depicts PTPN22 protein expression in GFP- and GFP+ shRNA+ hCD19+ cells isolated from the spleen of NSG mice; β-actin is used for normalization of protein expression. Percentage of knock-down is indicated. Figure 9D depicts results of experiments demonstrating that B-cell intrinsic PTPN22 expression is required for central B-cell tolerance. The frequencies of polyreactive new emigrant B cells from sorted GFP+ fractions expressing PTPN22 shRNA were determined and compared to those of GFP- new emigrant B cells. Dotted line shows positive control. For each B cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each symbol represents a mouse and the average is shown with a bar. Figure 9E depicts results of experiments demonstrating that B-cell intrinsic PTPN22 expression is required for central B-cell tolerance. The frequencies of HEp-2 reactive new emigrant B cells from sorted GFP+ fractions expressing PTPN22 shRNA were determined and compared to those of GFP- new emigrant B cells. Dotted line shows positive control. For each B cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each symbol represents a mouse and the average is shown with a bar.

Figure 10, comprising Figure 10A through Figure 10D, depicts results from experiments demonstrating inhibition of PTPN22 expression during B cell development resets central B cell tolerance. Figure 10A depicts flow cytometry analysis of Ramos B cells transduction efficiency. Ramos B cells were transduced with three different GFP-tagged shRNA PTPN22 expressing lentiviruses and probed for GFP expression. Right panel shows PTPN22 protein expression in GFP- and GFP+ shRNA+ Ramos B cells. β-actin is used for normalization of protein expression. Percentage of knock-down is indicated. Figure 10B depicts the frequencies of polyreactive new emigrant B cells from sorted GFP- fractions were determined. Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive

(open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. Figure 10C depicts the frequencies of HEp-2 reactive new emigrant B cells from sorted GFP- fractions were determined. Dotted line shows positive control. For each B-cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. Figure 10D depicts the frequencies of antinuclear new emigrant B cells are compared between GFP- and GFP+ shRNA+ new emigrant B cells.

Figure 11 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #1.

Figure 12 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #2.

Figure 13 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #3.

Figure 14 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #4.

Figure 15 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #5.

Figure 16 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #6.

Figure 17 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #7.

Figure 18 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #8.

Figure 19 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #9.

Figure 20 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #10.

Figure 21 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #11 GFP.

Figure 22 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #11 GFP+ PTPN22 620W expression.

Figure 23 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #12 GFP.

Figure 24 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #12 GFP+ PTPN22 620W expression.

Figure 25 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #13 GFP.

Figure 26 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #13 GFP+ PTPN22 620W expression.

Figure 27 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #14 GFP+ PTPN22 WT expression.

Figure 28 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #15 GFP+ PTPN22 WT expression.

Figure 29 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #16 GFP+ PTPN22 263Q expression.

Figure 30 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #17 GFP+ PTPN22 263Q expression.

Figure 31 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #18 treated with 0.75 mg of LTV-1 PTPN22 inhibitor.

Figure 32 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #19 treated with 0.75 mg of LTV-1 PTPN22 inhibitor.

Figure 33 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #20 treated with 0.15 mg of LTV-1 PTPN22 inhibitor.

Figure 34 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #21 GFP.

Figure 35 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #21 GFP+ shRNA PTPN22.

Figure 36 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #22 GFP.

Figure 37 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #22 GFP+ PTPN22 shRNA.

Figure 38 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #23 GFP.

Figure 39 depicts results from experiments demonstrating the repertoire and reactivity of antibodies from new emigrant B cells of Mouse #23 GFP+ PTPN22 shRNA.

Figure 40, comprising Figure 40A through Figure 40D, depicts experimental results demonstrating that inhibition of PTPN22 diminishes the activation of Lyn and SHIP1 and augments calcium flux in B cells. Figure 40A depicts the phosphorylation of SHIP1, Lyn and ERK1/2 in total cell lysates of Ramos B cells treated or not with the PTPN22 inhibitor LTV-1 (5 μg/mL) for the indicated times. The cells were subjected to immunoblot analysis of P-LYN P-SHIP1, P-ERK1/2, and β-ACTIN. Figure 40B depicts flow cytometry analysis of calcium flux of Ramos B cells treated or not with the PTPN22 inhibitor LTV-1 (5 μg/mL) for the indicated times followed by stimulation with anti-IgM F(ab')2 at the indicated concentrations. Figure 40C depicts flow cytometry analysis of calcium flux of splenocyte cells treated or not with LTV-1 (0.75 mg) twice daily for 7 days and stimulated with anti-IgM F(ab')2 (25 μg/mL). Figure 40D depicts flow cytometry analysis of calcium flux of splenocyte expressing PTPN22 shRNA and stimulated with anti-IgM F(ab')2 (25 μg/mL).

Figure 41, comprising Figure 41A through Figure 41C, depicts experimental results demonstrating that PTPN22 enzymatic inhibition restores peripheral B cell tolerance. Figure 41A depicts a schematic diagram depicting the LTV-1 PTPN22 inhibitor treatment strategy. A NSG + thymus mouse generated with $CD34^+$ HSCs and thymic graft carrying the 1858T PTPN22 allele was injected twice daily with 0.75 mg of PTPN22 inhibitor for four weeks. Figure 41B depicts the frequencies of HEp-2 reactive mature naive B cells from NSG + thymus mice treated or not with the PTPN22 inhibitor were determined. Dotted line shows positive control. For each B cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each symbol represents either a subject or a humanized mouse. Averages are shown with a bar. Statistically significant differences are indicated ****$P \leq 0.0001$, ***$P \leq 0.001$. Figure 41C depicts the frequencies of polyreactive mature naive B cells from NSG + thymus mice treated or not with the PTPN22 inhibitor were determined. Dotted line shows positive control. For each B cell fraction, the frequency of reactive (filled area) and non-reactive (open area) clones is summarized in pie charts with the total number of clones tested indicated in the center. In summarized reactivity panels on the right, each symbol represents either a subject or a humanized mouse. Averages are shown with a bar. Statistically significant differences are indicated ****$P \leq 0.0001$, ***$P \leq 0.001$.

DETAILED DESCRIPTION

The present invention relates to compositions and methods for restoring human central B-cell tolerance in a subject. In certain instances, the subject is one who fails to properly remove developing autoreactive B cells. For example, in one embodiment, the subject has an autoimmune disease. In certain instances, the compositions and methods described herein relate to inhibiting protein tyrosine phosphatase non-receptor type 22 (PTPN22).

In one embodiment, the composition of the present invention comprises an inhibitor of PTPN22. For example, in one embodiment, the inhibitor of PTPN22 inhibits the expression, activity, or both of PTPN22. In one embodiment, PTPN22 of the subject

comprises a nucleotide change (cytidine to thymidine) at residue 1858 that results in an amino acid substitution from arginine to tryptophan at position 620 of the PTPN22.

In one embodiment, the method of the present invention comprises restoring human central B-cell tolerance in a subject. In another embodiment, the method of the present invention comprises treating or preventing an autoimmune disease. For example, in some embodiments, the method of the present invention comprises treating or preventing type 1 diabetes (T1D), rheumatoid arthritis (RA), multiple sclerosis (MS), or systemic lupus erythematosus (SLE). In one embodiment, the method comprises administering to a subject an effective amount of a composition comprising an inhibitor of PTPN22.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, and nucleic acid chemistry and hybridization are those well-known and commonly employed in the art.

Standard techniques are used for nucleic acid and peptide synthesis. The techniques and procedures are generally performed according to conventional methods in the art and various general references (e.g., Sambrook and Russell, 2012, Molecular Cloning, A Laboratory Approach, Cold Spring Harbor Press, Cold Spring Harbor, NY, and Ausubel et al., 2002, Current Protocols in Molecular Biology, John Wiley & Sons, NY), which are provided throughout this document.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

"About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20%, ±10%, ±5%, ±1%, or ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

The term "abnormal" when used in the context of organisms, tissues, cells or components thereof, refers to those organisms, tissues, cells or components thereof that differ in at least one observable or detectable characteristic (e.g., age, treatment, time of day, etc.) from those organisms, tissues, cells or components thereof that display the "normal" (expected) respective characteristic. Characteristics which are normal or expected for one cell or tissue type, might be abnormal for a different cell or tissue type.

"Antisense" refers particularly to the nucleic acid sequence of the non-coding strand of a double stranded DNA molecule encoding a protein, or to a sequence which is substantially homologous to the non-coding strand. As defined herein, an antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a protein. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule. The antisense sequence may be complementary to regulatory sequences specified on the coding strand of a DNA molecule encoding a protein, which regulatory sequences control expression of the coding sequences.

A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

A disease or disorder is "alleviated" if the severity of a sign or symptom of the disease or disorder, the frequency with which such a sign or symptom is experienced by a patient, or both, is reduced.

An "effective amount" or "therapeutically effective amount" of a compound is that amount of a compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of a compound, composition, vector, or delivery system of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention can, for example, be affixed to a container which contains the identified compound, composition, vector, or delivery system of the invention or be shipped together with a container which contains the identified compound, composition, vector, or delivery system. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

The terms "patient," "subject," "individual," and the like are used interchangeably herein, and refer to any animal, or cells thereof whether in vitro or in vivo, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs or symptoms of a disease or disorder, for the purpose of diminishing or eliminating those signs or symptoms.

As used herein, "treating a disease or disorder" means reducing the severity and/or frequency with which a sign or symptom of the disease or disorder is experienced by a patient.

By the term "specifically binds," as used herein with respect to an antibody, is meant an antibody which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody that specifically binds to an antigen from one species may also bind to that antigen from one or more species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. In another example, an antibody that specifically binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as specific.

In some instances, the terms "specific binding" or "specifically binding," can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

A "coding region" of a gene consists of the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene.

A "coding region" of a mRNA molecule also consists of the nucleotide residues of the mRNA molecule which are matched with an anti-codon region of a transfer RNA molecule during translation of the mRNA molecule or which encode a stop codon. The coding region may thus include nucleotide residues comprising codons for

amino acid residues which are not present in the mature protein encoded by the mRNA molecule (e.g., amino acid residues in a protein export signal sequence).

"Complementary" as used herein to refer to a nucleic acid, refers to the broad concept of sequence complementarity between regions of two nucleic acid strands or between two regions of the same nucleic acid strand. It is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds ("base pairing") with a residue of a second nucleic acid region which is antiparallel to the first region if the residue is thymine or uracil. Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is antiparallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. More preferably, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

The term "DNA" as used herein is defined as deoxyribonucleic acid.

The term "expression" as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

The term "expression vector" as used herein refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules, siRNA, ribozymes, and the like. Expression vectors can contain a variety of control sequences, which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operatively linked coding sequence in a particular host organism. In addition to control sequences that govern

transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well.

The term "fusion polypeptide" refers to a chimeric protein containing a protein of interest (e.g., luciferase) joined to a heterologous sequence (e.g., a non-luciferase amino acid or protein).

The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). Homology is often measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group. University of Wisconsin Biotechnology Center. 1710 University Avenue. Madison, Wis. 53705). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, insertions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

"Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in its normal context in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural context is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

The term "isolated" when used in relation to a nucleic acid, as in "isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one contaminant with which it is ordinarily associated in its source. Thus, an isolated nucleic acid is present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids (e.g., DNA and RNA) are found in the state they exist in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences (e.g., a specific mRNA sequence encoding a specific protein), are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. However, isolated nucleic acid includes, by way of example, such

nucleic acid in cells ordinarily expressing that nucleic acid where the nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid or oligonucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid or oligonucleotide is to be utilized to express a protein, the oligonucleotide contains at a minimum, the sense or coding strand (i.e., the oligonucleotide may be single-stranded), but may contain both the sense and anti-sense strands (i.e., the oligonucleotide may be double-stranded).

The term "isolated" when used in relation to a polypeptide, as in "isolated protein" or "isolated polypeptide" refers to a polypeptide that is identified and separated from at least one contaminant with which it is ordinarily associated in its source. Thus, an isolated polypeptide is present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated polypeptides (e.g., proteins and enzymes) are found in the state they exist in nature.

By "nucleic acid" is meant any nucleic acid, whether composed of deoxyribonucleosides or ribonucleosides, and whether composed of phosphodiester linkages or modified linkages such as phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate or sulfone linkages, and combinations of such linkages. The term nucleic acid also specifically includes nucleic acids composed of bases other than the five biologically occurring bases (adenine, guanine, thymine, cytosine and uracil). The term "nucleic acid" typically refers to large polynucleotides.

Conventional notation is used herein to describe polynucleotide sequences: the left-hand end of a single-stranded polynucleotide sequence is the 5'-end; the left-hand direction of a double-stranded polynucleotide sequence is referred to as the 5'-direction.

The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the DNA strand which are

located 5' to a reference point on the DNA are referred to as "upstream sequences"; sequences on the DNA strand which are 3' to a reference point on the DNA are referred to as "downstream sequences."

By "expression cassette" is meant a nucleic acid molecule comprising a coding sequence operably linked to promoter/regulatory sequences necessary for transcription and, optionally, translation of the coding sequence.

The term "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of sequences encoding amino acids in such a manner that a functional (e.g., enzymatically active, capable of binding to a binding partner, capable of inhibiting, etc.) protein or polypeptide is produced.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a n inducible manner.

An "inducible" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced substantially only when an inducer which corresponds to the promoter is present.

A "constitutive" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term "polynucleotide" as used herein is defined as a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric "nucleotides." The monomeric nucleotides can be hydrolyzed into

nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR, and the like, and by synthetic means.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

As used herein, a "peptidomimetic" is a compound containing non-peptidic structural elements that is capable of mimicking the biological action of a parent peptide. A peptidomimetic may or may not comprise peptide bonds.

The term "RNA" as used herein is defined as ribonucleic acid.

"Recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. An amplified or assembled recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell.

A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

The term "recombinant polypeptide" as used herein is defined as a polypeptide produced by using recombinant DNA methods.

As used herein, "conjugated" refers to covalent attachment of one molecule to a second molecule.

As used herein, the term "transdominant negative mutant gene" refers to a gene encoding a polypeptide or protein product that prevents other copies of the same gene or gene product, which have not been mutated (i.e., which have the wild-type sequence) from functioning properly (e.g., by inhibiting wild type protein function). The product of a transdominant negative mutant gene is referred to herein as "dominant negative" or "DN" (e.g., a dominant negative protein, or a DN protein).

The phrase "inhibit," as used herein, means to reduce a molecule, a reaction, an interaction, a gene, an mRNA, and/or a protein's expression, stability, function or activity by a measurable amount or to prevent entirely. Inhibitors are compounds that, e.g., bind to, partially or totally block stimulation, decrease, prevent, delay activation, inactivate, desensitize, or down regulate a protein, a gene, and an mRNA stability, expression, function and activity, e.g., antagonists.

"Variant" as the term is used herein, is a nucleic acid sequence or a peptide sequence that differs in sequence from a reference nucleic acid sequence or peptide sequence respectively, but retains essential biological properties of the reference molecule. Changes in the sequence of a nucleic acid variant may not alter the amino acid sequence of a peptide encoded by the reference nucleic acid, or may result in amino acid substitutions, additions, deletions, fusions and truncations. Changes in the sequence of peptide variants are typically limited or conservative, so that the sequences of the reference peptide and the variant are closely similar overall and, in many regions, identical. A variant and reference peptide can differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A variant of a nucleic acid or peptide can be a naturally occurring such as an allelic variant, or can be a variant that is not known to occur naturally. Non-naturally occurring variants of nucleic acids and peptides may be made by mutagenesis techniques or by direct synthesis.

A "vector" is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, and the like.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

The present invention relates to compositions and methods for treating or preventing an autoimmune disease or for restoring human central B-cell tolerance in a subject. The present invention is based upon the finding that that the PTPN22 T allele interferes with the establishment of central B cell tolerance using NOD-scid-common gamma chain (γc) knockout (NSG) mice engrafted with human hematopoietic stem cells (HSCs) expressing this allele. In contrast, the inhibition of either PTPN22 enzymatic activity or its expression by RNA interference restored defective central B cell tolerance in this model. Thus, the present invention relates to compositions and method to inhibit PTPN22 in order to restore human central B-cell tolerance.

In one embodiment, the composition of the present invention comprises an inhibitor of PTPN22. In one embodiment, the composition comprises an inhibitor PTPN22 expression. For example, in one embodiment, the composition comprises an isolated nucleic acid (e.g., siRNA, miRNA, ribozyme, antisense RNA, etc.) that reduces the expression level of PTPN22 in a cell.

In one embodiment, the composition comprises an inhibitor of PTPN22 activity. For example, in one embodiment, the composition comprises a nucleic acid, peptide, antibody, small molecule, antagonist, aptamer, or peptidomimetic that reduces the activity of PTPN22.

In one embodiment, the present invention provides a method for restoring human central B-cell tolerance in a subject. In one embodiment, the method comprises administering to a subject an effective amount of a composition comprising an inhibitor of PTPN22.

In another embodiment, the present invention provides a method for treating or preventing autoimmune disease in a subject. In one embodiment, the method comprises administering to a subject an effective amount of a composition comprising an inhibitor of PTPN22. In one embodiment, the autoimmune disease is T1D, RA, MS, or SLE. In another embodiment, the subject has at an 1858T PTPN22 polymorphism on at least one allele.

Inhibitors

In one embodiment, the present invention provides a composition for treating or preventing a disease or disorder associated with abnormal early B-cell tolerance checkpoints in a subject. In certain embodiments, the composition inhibits the expression, activity, or both of PTPN22 in the subject.

In one embodiment, the composition of the invention comprises an inhibitor of PTPN22. An inhibitor of PTPN22 is any compound, molecule, or agent that reduces, inhibits, or prevents the function of PTPN22. For example, an inhibitor of PTPN22 is any compound, molecule, or agent that reduces PTPN22 expression, activity, or both. In one embodiment, an inhibitor of PTPN22 comprises a nucleic acid, a peptide,

a small molecule, a siRNA, a ribozyme, an antisense nucleic acid, an antagonist, an aptamer, a peptidomimetic, or any combination thereof.

Small molecule inhibitors

In various embodiments, the inhibitor is a small molecule. When the inhibitor is a small molecule, a small molecule may be obtained using standard methods known to the skilled artisan. Such methods include chemical organic synthesis or biological means. Biological means include purification from a biological source, recombinant synthesis and in vitro translation systems, using methods well known in the art. In one embodiment, a small molecule inhibitor of the invention comprises an organic molecule, inorganic molecule, biomolecule, synthetic molecule, and the like.

Combinatorial libraries of molecularly diverse chemical compounds potentially useful in treating a variety of diseases and conditions are well known in the art as are method of making the libraries. The method may use a variety of techniques well-known to the skilled artisan including solid phase synthesis, solution methods, parallel synthesis of single compounds, synthesis of chemical mixtures, rigid core structures, flexible linear sequences, deconvolution strategies, tagging techniques, and generating unbiased molecular landscapes for lead discovery vs. biased structures for lead development.

In a general method for small library synthesis, an activated core molecule is condensed with a number of building blocks, resulting in a combinatorial library of covalently linked, core-building block ensembles. The shape and rigidity of the core determines the orientation of the building blocks in shape space. The libraries can be biased by changing the core, linkage, or building blocks to target a characterized biological structure ("focused libraries") or synthesized with less structural bias using flexible cores.

The small molecule and small molecule compounds described herein may be present as salts even if salts are not depicted and it is understood that the invention embraces all salts and solvates of the inhibitors depicted here, as well as the non-salt and non-solvate form of the inhibitors, as is well understood by the skilled artisan. In some

embodiments, the salts of the inhibitors of the invention are pharmaceutically acceptable salts.

Where tautomeric forms may be present for any of the inhibitors described herein, each and every tautomeric form is intended to be included in the present invention, even though only one or some of the tautomeric forms may be explicitly depicted. For example, when a 2-hydroxypyridyl moiety is depicted, the corresponding 2-pyridone tautomer is also intended.

The invention also includes any or all of the stereochemical forms, including any enantiomeric or diasteriomeric forms of the inhibitors described. The recitation of the structure or name herein is intended to embrace all possible stereoisomers of inhibitors depicted. All forms of the inhibitors are also embraced by the invention, such as crystalline or non-crystalline forms of the inhibitors. Compositions comprising an inhibitor of the invention are also intended, such as a composition of substantially pure inhibitor, including a specific stereochemical form thereof, or a composition comprising mixtures of inhibitors of the invention in any ratio, including two or more stereochemical forms, such as in a racemic or non-racemic mixture.

In one embodiment, the small molecule inhibitor of the invention comprises an analog or derivative of an inhibitor described herein.

In one embodiment, the small molecules described herein are candidates for derivatization. As such, in certain instances, the analogs of the small molecules described herein that have modulated potency, selectivity, and solubility are included herein and provide useful leads for drug discovery and drug development. Thus, in certain instances, during optimization new analogs are designed considering issues of drug delivery, metabolism, novelty, and safety.

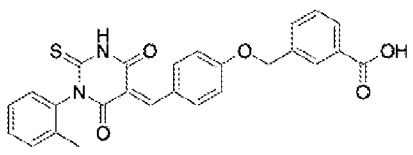
In some instances, small molecule inhibitors described herein are derivatized/analoged as is well known in the art of combinatorial and medicinal chemistry. The analogs or derivatives can be prepared by adding and/or substituting functional groups at various locations. As such, the small molecules described herein can be converted into derivatives/analogs using well known chemical synthesis procedures. For example, all of the hydrogen atoms or substituents can be selectively modified to generate new analogs. Also, the linking atoms or groups can be modified into longer or

shorter linkers with carbon backbones or hetero atoms. Also, the ring groups can be changed so as to have a different number of atoms in the ring and/or to include hetero atoms. Moreover, aromatics can be converted to cyclic rings, and vice versa. For example, the rings may be from 5-7 atoms, and may be homocycles or heterocycles.

As used herein, the term "analog," "analogue," or "derivative" is meant to refer to a chemical compound or molecule made from a parent compound or molecule by one or more chemical reactions. As such, an analog can be a structure having a structure similar to that of the small molecule inhibitors described herein or can be based on a scaffold of a small molecule inhibitor described herein, but differing from it in respect to certain components or structural makeup, which may have a similar or opposite action metabolically. An analog or derivative of any of a small molecule inhibitor in accordance with the present invention can be used to treat an autoimmune disease or disorder.

In one embodiment, the small molecule inhibitors described herein can independently be derivatized/analoged by modifying hydrogen groups independently from each other into other substituents. That is, each atom on each molecule can be independently modified with respect to the other atoms on the same molecule. Any traditional modification for producing a derivative/analog can be used. For example, the atoms and substituents can be independently comprised of hydrogen, an alkyl, aliphatic, straight chain aliphatic, aliphatic having a chain hetero atom, branched aliphatic, substituted aliphatic, cyclic aliphatic, heterocyclic aliphatic having one or more hetero atoms, aromatic, heteroaromatic, polyaromatic, polyamino acids, peptides, polypeptides, combinations thereof, halogens, halo-substituted aliphatics, and the like. Additionally, any ring group on a compound can be derivatized to increase and/or decrease ring size as well as change the backbone atoms to carbon atoms or hetero atoms.

In one embodiment, the small molecule inhibitor is



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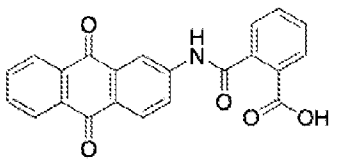

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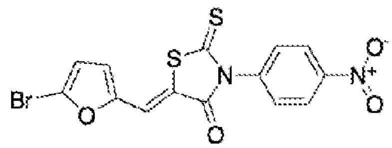
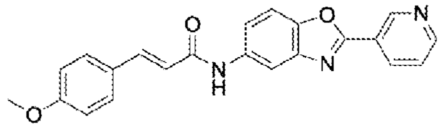
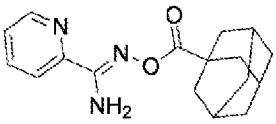
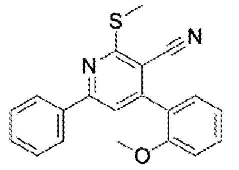
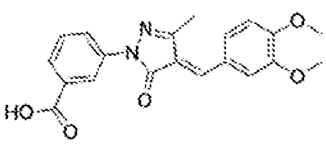
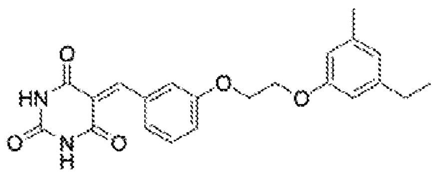
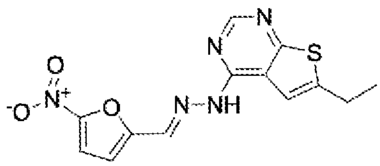
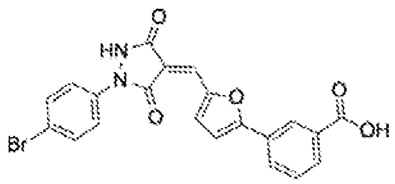
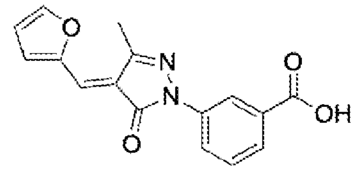
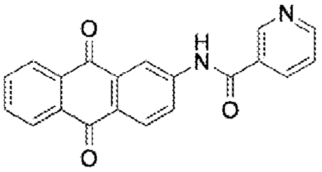
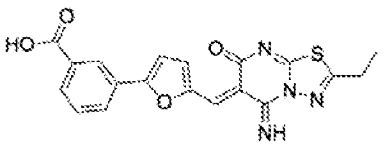
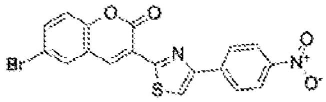
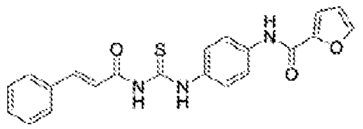
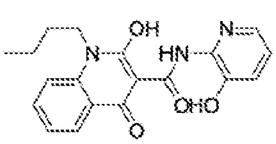
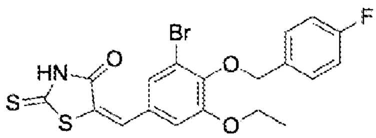
 ,  ,

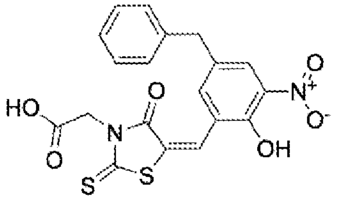
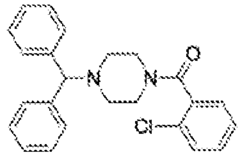
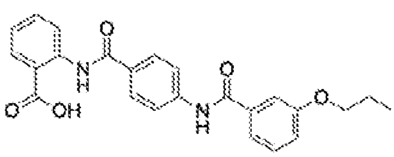
 ,  ,  ,

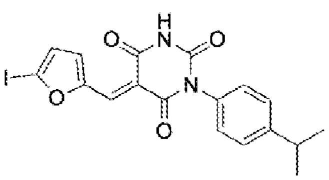
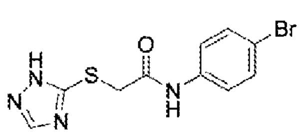
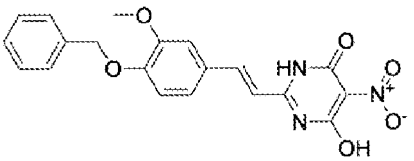
 ,  ,

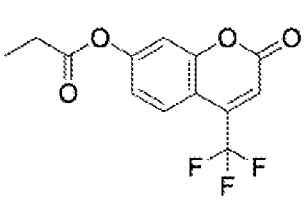
 ,  ,

 ,  ,  ,

 ,  ,  ,

 ,  ,  ,

 ,  ,  ,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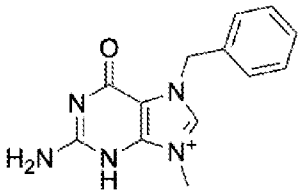
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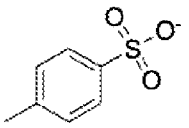
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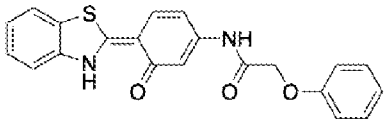
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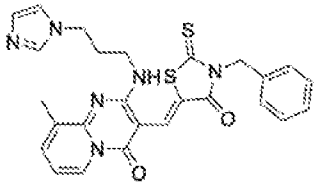
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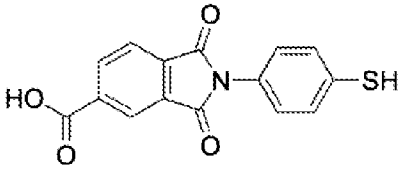
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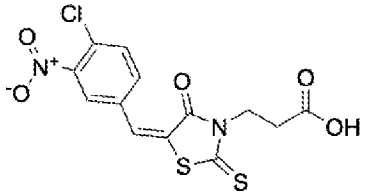
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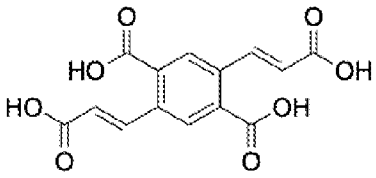
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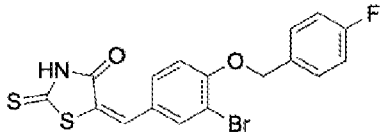
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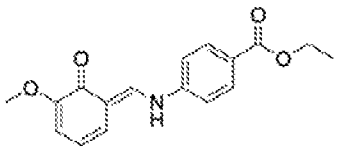
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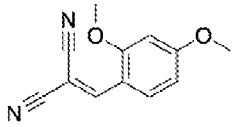
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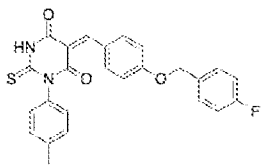
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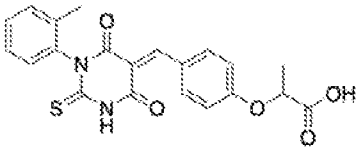
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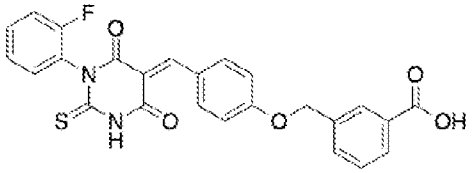
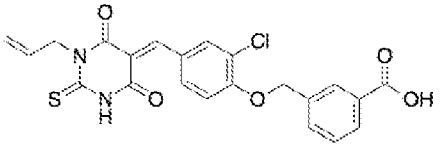
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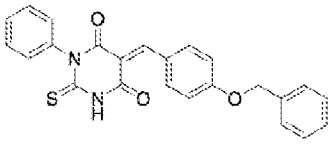
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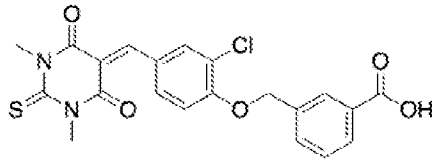
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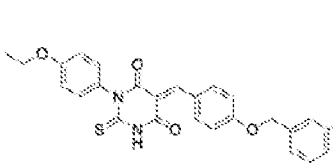
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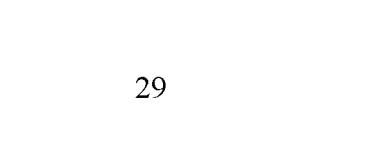
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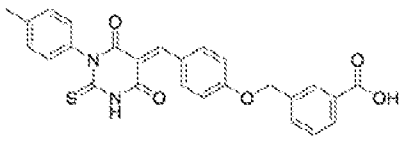
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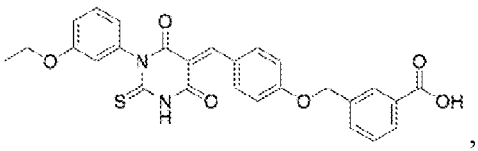
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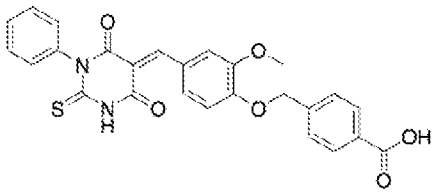
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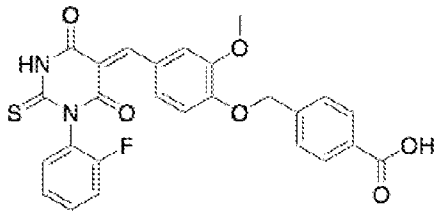
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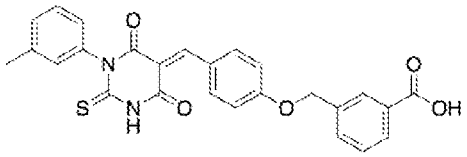
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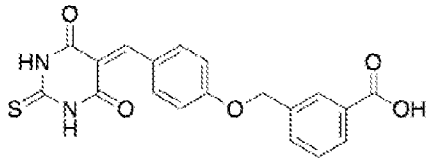
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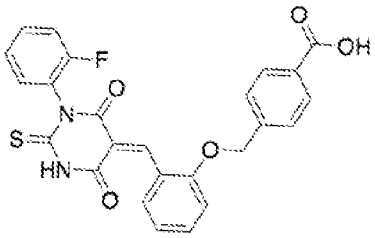
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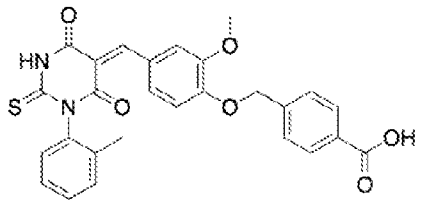
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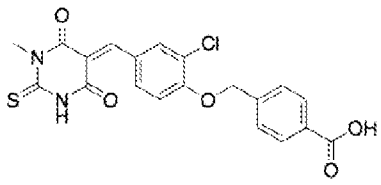
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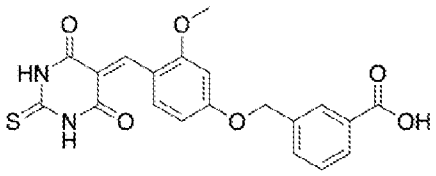
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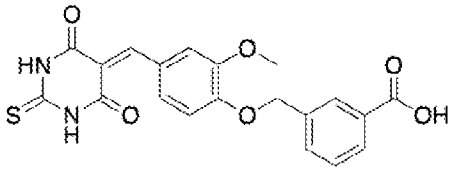

,derivatives thereof and salts thereof.

Nucleic acid inhibitors

In other related aspects, the invention includes an isolated nucleic acid. In some instances, the inhibitor is an siRNA, miRNA, or antisense molecule, which inhibits PTPN22. In one embodiment, the nucleic acid comprises a promoter/regulatory sequence such that the nucleic acid is preferably capable of directing expression of the nucleic acid. Thus, the invention encompasses expression vectors and methods for the introduction of

exogenous DNA into cells with concomitant expression of the exogenous DNA in the cells such as those described, for example, in Sambrook et al. (2012, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York) and as described elsewhere herein.

In another aspect of the invention, PTPN22, can be inhibited by way of inactivating and/or sequestering PTPN22. As such, inhibiting the activity of PTPN22 can be accomplished by using a transdominant negative mutant.

In one embodiment, siRNA is used to decrease the level of PTPN22 protein. RNA interference (RNAi) is a phenomenon in which the introduction of double-stranded RNA (dsRNA) into a diverse range of organisms and cell types causes degradation of the complementary mRNA. In the cell, long dsRNAs are cleaved into short 21-25 nucleotide small interfering RNAs, or siRNAs, by a ribonuclease known as Dicer. The siRNAs subsequently assemble with protein components into an RNA-induced silencing complex (RISC), unwinding in the process. Activated RISC then binds to complementary transcript by base pairing interactions between the siRNA antisense strand and the mRNA. The bound mRNA is cleaved and sequence specific degradation of mRNA results in gene silencing. See, for example, U.S. Patent No. 6,506,559; Fire et al., 1998, Nature 391(19):306-311; Timmons et al., 1998, Nature 395:854; Montgomery et al., 1998, TIG 14 (7):255-258; David R. Engelke, Ed., RNA Interference (RNAi) Nuts & Bolts of RNAi Technology, DNA Press, Eagleville, PA (2003); and Gregory J. Hannon, Ed., RNAi A Guide to Gene Silencing, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (2003). Soutschek et al. (2004, Nature 432:173-178) describe a chemical modification to siRNAs that aids in intravenous systemic delivery. Optimizing siRNAs involves consideration of overall G/C content, C/T content at the termini, Tm and the nucleotide content of the 3' overhang. See, for instance, Schwartz et al., 2003, Cell, 115:199-208 and Khvorova et al., 2003, Cell 115:209-216. Therefore, the present invention also includes methods of decreasing levels of PTPN22 using RNAi technology.

In another aspect, the invention includes a vector comprising an siRNA or antisense polynucleotide. Preferably, the siRNA or antisense polynucleotide is capable of inhibiting the expression of a target polypeptide, wherein the target polypeptide is

PTPN22. The incorporation of a desired polynucleotide into a vector and the choice of vectors is well-known in the art as described in, for example, Sambrook et al. (2012), and in Ausubel et al. (1997), and elsewhere herein.

In certain embodiments, the expression vectors described herein encode a short hairpin RNA (shRNA) inhibitor. shRNA inhibitors are well known in the art and are directed against the mRNA of a target, thereby decreasing the expression of the target. In certain embodiments, the encoded shRNA is expressed by a cell, and is then processed into siRNA. For example, in certain instances, the cell possesses native enzymes (e.g., dicer) that cleaves the shRNA to form siRNA.

In some embodiments, the shRNA inhibitors comprise a sequence of CTAGTGCTCTTGGTGTATATT (SEQ ID NO:1) or AAGAATCCACCTGACTTCC (SEQ ID NO:2).

The siRNA, shRNA, or antisense polynucleotide can be cloned into a number of types of vectors as described elsewhere herein. For expression of the siRNA or antisense polynucleotide, at least one module in each promoter functions to position the start site for RNA synthesis.

In order to assess the expression of the siRNA, shRNA, or antisense polynucleotide, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected using a viral vector. In other embodiments, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers are known in the art and include, for example, antibiotic-resistance genes, such as neomycin resistance and the like.

Therefore, in another aspect, the invention relates to a vector, comprising the nucleotide sequence of the invention or the construct of the invention. The choice of the vector will depend on the host cell in which it is to be subsequently introduced. In a particular embodiment, the vector of the invention is an expression vector. Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. In specific embodiments, the expression vector is selected from the group consisting of a viral

vector, a bacterial vector and a mammalian cell vector. Prokaryote- and/or eukaryote-vector based systems can be employed for use with the present invention to produce polynucleotides, or their cognate polypeptides. Many such systems are commercially and widely available.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2012), and in Ausubel et al. (1997), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193.

By way of illustration, the vector in which the nucleic acid sequence is introduced can be a plasmid, which is or is not integrated in the genome of a host cell when it is introduced in the cell. Illustrative, non-limiting examples of vectors in which the nucleotide sequence of the invention or the gene construct of the invention can be inserted include a tet-on inducible vector for expression in eukaryote cells.

The vector may be obtained by conventional methods known by persons skilled in the art (Sambrook et al., 2012). In a particular embodiment, the vector is a vector useful for transforming animal cells.

In one embodiment, the recombinant expression vectors may also contain nucleic acid molecules, which encode a peptide or peptidomimetic inhibitor of invention, described elsewhere herein.

A promoter may be one naturally associated with a gene or polynucleotide sequence, as may be obtained by isolating the 5′ non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as “endogenous.” Similarly, an enhancer may be one naturally associated with a polynucleotide sequence, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding polynucleotide segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally

associated with a polynucleotide sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a polynucleotide sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," i.e., containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR, in connection with the compositions disclosed herein (U.S. Patent 4,683,202, U.S. Patent 5,928,906). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type, organelle, and organism chosen for expression. Those of skill in the art of molecular biology generally know how to use promoters, enhancers, and cell type combinations for protein expression, for example, see Sambrook et al. (2012). The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and/or peptides. The promoter may be heterologous or endogenous.

The recombinant expression vectors may also contain a selectable marker gene, which facilitates the selection of transformed or transfected host cells. Suitable selectable marker genes are genes encoding proteins such as G418 and hygromycin, which confer resistance to certain drugs, β-galactosidase, chloramphenicol acetyltransferase, firefly luciferase, or an immunoglobulin or portion thereof such as the Fc portion of an immunoglobulin preferably IgG. The selectable markers may be introduced on a separate vector from the nucleic acid of interest.

Following the generation of the siRNA polynucleotide, a skilled artisan will understand that the siRNA polynucleotide will have certain characteristics that can

be modified to improve the siRNA as a therapeutic compound. Therefore, the siRNA polynucleotide may be further designed to resist degradation by modifying it to include phosphorothioate, or other linkages, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and the like (see, e.g., Agrwal et al., 1987, Tetrahedron Lett. 28:3539-3542; Stec et al., 1985 Tetrahedron Lett. 26:2191-2194; Moody et al., 1989 Nucleic Acids Res. 12:4769-4782; Eckstein, 1989 Trends Biol. Sci. 14:97-100; Stein, In: Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression, Cohen, ed., Macmillan Press, London, pp. 97-117 (1989)).

Any polynucleotide may be further modified to increase its stability in vivo. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiester linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine, and wybutosine and the like, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine, and uridine.

In one embodiment of the invention, an antisense nucleic acid sequence, which is expressed by a plasmid vector is used to inhibit PTPN22 protein expression. The antisense expressing vector is used to transfect a mammalian cell or the mammal itself, thereby causing reduced endogenous expression of PTPN22.

Antisense molecules and their use for inhibiting gene expression are well known in the art (see, e.g., Cohen, 1989, In: Oligodeoxyribonucleotides, Antisense Inhibitors of Gene Expression, CRC Press). Antisense nucleic acids are DNA or RNA molecules that are complementary, as that term is defined elsewhere herein, to at least a portion of a specific mRNA molecule (Weintraub, 1990, Scientific American 262:40). In the cell, antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule thereby inhibiting the translation of genes.

The use of antisense methods to inhibit the translation of genes is known in the art, and is described, for example, in Marcus-Sakura (1988, Anal. Biochem. 172:289). Such antisense molecules may be provided to the cell via genetic expression using DNA encoding the antisense molecule as taught by Inoue, 1993, U.S. Patent No. 5,190,931.

Alternatively, antisense molecules of the invention may be made synthetically and then provided to the cell. Antisense oligomers of between about 10 to about 30, and more preferably about 15 nucleotides, are preferred, since they are easily synthesized and introduced into a target cell. Synthetic antisense molecules contemplated by the invention include oligonucleotide derivatives known in the art which have improved biological activity compared to unmodified oligonucleotides (see U.S. Patent No. 5,023,243).

In one embodiment of the invention, a ribozyme is used to inhibit PTPN22 protein expression. Ribozymes useful for inhibiting the expression of a target molecule may be designed by incorporating target sequences into the basic ribozyme structure, which are complementary, for example, to the mRNA sequence encoding PTPN22. Ribozymes targeting PTPN22, may be synthesized using commercially available reagents (Applied Biosystems, Inc., Foster City, CA) or they may be genetically expressed from DNA encoding them.

In one embodiment, the inhibitor of PTPN22 may comprise one or more components of a CRISPR-Cas system. CRISPR methodologies employ a nuclease, CRISPR-associated (Cas), that complexes with small RNAs as guides (gRNAs) to cleave DNA in a sequence-specific manner upstream of the protospacer adjacent motif (PAM) in any genomic location. CRISPR may use separate guide RNAs known as the crRNA and tracrRNA. These two separate RNAs have been combined into a single RNA to enable site-specific mammalian genome cutting through the design of a short guide RNA. Cas and guide RNA (gRNA) may be synthesized by known methods. Cas/guide-RNA (gRNA) uses a non-specific DNA cleavage protein Cas, and an RNA oligo to hybridize to target and recruit the Cas/gRNA complex. In one embodiment, a guide RNA (gRNA) targeted to a gene encoding PTPN22, and a CRISPR-associated (Cas) peptide form a complex to induce mutations within the targeted gene. In one embodiment, the inhibitor comprises a gRNA or a nucleic acid molecule encoding a gRNA. In one embodiment, the inhibitor comprises a Cas peptide or a nucleic acid molecule encoding a Cas peptide.

Polypeptide inhibitors

In other related aspects, the invention includes an isolated peptide

inhibitor that inhibits PTPN22. For example, in one embodiment, the peptide inhibitor of the invention inhibits PTPN22 directly by binding to PTPN22 thereby preventing the normal functional activity of PTPN22. In another embodiment, the peptide inhibitor of the invention inhibits PTPN22 by competing with endogenous PTPN22. In yet another embodiment, the peptide inhibitor of the invention inhibits the activity of PTPN22 by acting as a transdominant negative mutant.

The variants of the polypeptides according to the present invention may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, (ii) one in which there are one or more modified amino acid residues, e.g., residues that are modified by the attachment of substituent groups, (iii) one in which the polypeptide is an alternative splice variant of the polypeptide of the present invention, (iv) fragments of the polypeptides and/or (v) one in which the polypeptide is fused with another polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification (for example, His-tag) or for detection (for example, Sv5 epitope tag). The fragments include polypeptides generated via proteolytic cleavage (including multi-site proteolysis) of an original sequence. Variants may be post-translationally, or chemically modified. Such variants are deemed to be within the scope of those skilled in the art from the teaching herein.

Antibody inhibitors

The invention also contemplates an inhibitor of PTPN22 comprising an antibody, or antibody fragment, specific for PTPN22. That is, the antibody can inhibit PTPN22 to provide a beneficial effect. In some embodiments, the antibody specifically binds PTPN22.

The antibodies may be intact monoclonal or polyclonal antibodies, and immunologically active fragments (e.g., a Fab or $(Fab)_2$ fragment), an antibody heavy chain, an antibody light chain, humanized antibodies, a genetically engineered single chain F_V molecule (Ladner et al, U.S. Pat. No. 4,946,778), or a chimeric antibody, for example, an antibody which contains the binding specificity of a murine antibody, but in

which the remaining portions are of human origin. Antibodies including monoclonal and polyclonal antibodies, humanized antibodies, fragments and chimeras, may be prepared using methods known to those skilled in the art.

The antibody may comprise a heavy chain and a light chain complementarity determining region ("CDR") set, respectively interposed between a heavy chain and a light chain framework ("FR") set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. The CDR set may contain three hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as "CDR1," "CDR2," and "CDR3," respectively. An antigen-binding site, therefore, may include six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

The antibody can be an immunoglobulin (Ig). The Ig can be, for example, IgA, IgM, IgD, IgE, and IgG. The immunoglobulin can include the heavy chain polypeptide and the light chain polypeptide. The heavy chain polypeptide of the immunoglobulin can include a VH region, a CH1 region, a hinge region, a CH2 region, and a CH3 region. The light chain polypeptide of the immunoglobulin can include a VL region and CL region.

The antibody can be a polyclonal or monoclonal antibody. The antibody can be a chimeric antibody, a single chain antibody, an affinity matured antibody, a human antibody, a humanized antibody, or a fully human antibody. The humanized antibody can be an antibody from a non-human species that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule.

The antibody can be a bispecific antibody. The bispecific antibody can bind or react with two antigens, for example, two of the antigens described below in more detail. The bispecific antibody can be comprised of fragments of two of the antibodies described herein, thereby allowing the bispecific antibody to bind or react with two desired target molecules, which may include the antigen, which is described below in more detail, a ligand, including a ligand for a receptor, a receptor, including a ligand-binding site on the receptor, a ligand-receptor complex, and a marker. Bispecific antibodies can comprise a first antigen-binding site that specifically binds to a first target and a second antigen-

binding site that specifically binds to a second target, with particularly advantageous properties such as producibility, stability, binding affinity, biological activity, specific targeting of certain T cells, targeting efficiency and reduced toxicity. In some instances, there are bispecific antibodies, wherein the bispecific antibody binds to the first target with high affinity and to the second target with low affinity. In other instances, there are bispecific antibodies, wherein the bispecific antibody binds to the first target with low affinity and to the second target with high affinity. In other instances, there are bispecific antibodies, wherein the bispecific antibody binds to the first target with a desired affinity and to the second target with a desired affinity.

Antibodies can be prepared using intact polypeptides or fragments containing an immunizing antigen of interest. The polypeptide or oligopeptide used to immunize an animal may be obtained from the translation of RNA or synthesized chemically and can be conjugated to a carrier protein, if desired. Suitable carriers that may be chemically coupled to peptides include bovine serum albumin and thyroglobulin, keyhole limpet hemocyanin. The coupled polypeptide may then be used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

Combinations

In one embodiment, the composition of the present invention comprises a combination of PTPN22 inhibitors described herein. In certain embodiments, a composition comprising a combination of inhibitors described herein has an additive effect, wherein the overall effect of the combination is approximately equal to the sum of the effects of each individual inhibitor. In other embodiments, a composition comprising a combination of inhibitors described herein has a synergistic effect, wherein the overall effect of the combination is greater than the sum of the effects of each individual inhibitor.

In some embodiments, the composition of the present invention comprises a combination of a PTPN22 inhibitor and second therapeutic agent. For example, in one embodiment the second therapeutic agents include, but are not limited to, a diabetes therapeutic, a rheumatoid arthritis therapeutic, a multiple sclerosis therapeutic, a systemic lupus erythematosus therapeutic and anti-inflammation therapeutics. In some

embodiments, therapeutic agents include Rituximab, Anti-BAFF therapies, and anti-TNF therapies.

In some embodiments, the second therapeutic is a diabetes therapeutic. Exemplary diabetes therapeutics include, but are not limited to, non-sulfonylurea secretagogues, insulin, insulin analogs, exendin-4 polypeptides, beta 3 adrenoceptor agonists, PPAR agonists, dipeptidyl peptidase IV inhibitors, statins and statin-containing combinations, inhibitors of cholesterol uptake and/or bile acid re-absorption, LDL-cholesterol antagonists, cholesteryl ester transfer protein antagonists, endothelin receptor antagonists, growth hormone antagonists, insulin sensitizers, amylin mimetics or agonists, cannabinoid receptor antagonists, glucagon-like peptide-1 agonists, melanocortins, melanin-concentrating hormone receptor agonists, SNRIs, a fibroblast growth factor 21 (FGF21) mimetic, a fibroblast growth factor receptor 1c (FGFR1c) agonist, an inhibitor of advanced glycation end product formation, such as, but not limited to, aminoguanidine, and protein tyrosine phosphatase inhibitors.

In some embodiments, the second therapeutic is a rheumatoid arthritis therapeutic. Exemplary rheumatoid arthritis therapeutics include, but are not limited to, Nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, methotrexate, leflunomide, hydroxychloroquine, sulfasalazine, abatacept, adalimumab, anakinra, certolizumab, etanercept, golimumab, infliximab, rituximab, tocilizumab and tofacitinib.

In some embodiments, the second therapeutic is a multiple sclerosis therapeutic or treatment. Exemplary multiple sclerosis therapeutics include, but are not limited to, corticosteroids, plasmapheresis, ocrelizumab, β-interferons, Glatiramer acetate, Dimethyl fumarate, Fingolimod, Teriflunomide, Natalizumab, Alemtuzumab, Mitoxantrone, baclofen, and tizanidine.

In some embodiments, the second therapeutic is a systemic lupus erythematosus therapeutic. Exemplary systemic lupus erythematosus therapeutics include, but are not limited to, glucocorticoid, prednisone, hydroxychloroquine, methotrexate and azathioprine.

A composition comprising a combination of inhibitors comprises individual inhibitors in any suitable ratio. For example, in one embodiment, the composition comprises a 1:1 ratio of two individual inhibitors. However, the combination

is not limited to any particular ratio. Rather any ratio that is shown to be effective is encompassed.

Therapeutic Methods

The present invention also provides methods of restoring central B-cell tolerance in a subject. For example, in one embodiment, the method of the invention increases calcium flux, and BCR signaling. In one embodiment, the method of the invention decreases the phosphorylation of LYN and SHIP1.

In certain embodiments, the method is used to treat or prevent a disease or disorder in a subject associated with abnormal early B-cell tolerance checkpoints. In one embodiment, the invention also provides methods of treating or preventing autoimmune disease in a subject. Exemplary autoimmune diseases include, but are not limited to, type 1 diabetes, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, Sjögren's syndrome, autoimmune thyroiditis, myasthenia gravis, and pemphigus.

In one embodiment, the subject fails to properly remove developing autoreactive B cells. In another embodiment, the subject has a 1858T PTPN22 polymorphism on at least one allele. In yet another embodiment, the subject is human.

It will be appreciated by one of skill in the art, when armed with the present disclosure including the methods detailed herein, that the invention is not limited to treatment of autoimmune disease that is already established. Particularly, the disease or disorder need not have manifested to the point of detriment to the subject; indeed, the disease or disorder need not be detected in a subject before treatment is administered. That is, significant signs or symptoms of autoimmune disease do not have to occur before the present invention may provide benefit. Therefore, the present invention includes a method for preventing autoimmune disease, in that a composition, as discussed previously elsewhere herein, can be administered to a subject prior to the onset of autoimmune disease, thereby preventing autoimmune disease.

One of skill in the art, when armed with the disclosure herein, would appreciate that the prevention of an autoimmune disease or disorder, encompasses administering to a subject a composition as a preventative measure against the

development of, or progression of autoimmune disease. As more fully discussed elsewhere herein, methods of modulating the level or activity of a gene, or gene product, encompass a wide plethora of techniques for modulating not only the level and activity of polypeptide gene products, but also for modulating expression of a nucleic acid, including either transcription, translation, or both.

The invention encompasses administration of an inhibitor of PTPN22, or a combination thereof. To practice the methods of the invention; the skilled artisan would understand, based on the disclosure provided herein, how to formulate and administer the appropriate modulator composition to a subject. The present invention is not limited to any particular method of administration or treatment regimen.

In one embodiment, the method comprises administering to the subject in need an effective amount of a composition that reduces or inhibits the expression or activity of PTPN22.

One of skill in the art will appreciate that the inhibitors of the invention can be administered singly or in any combination. Further, the inhibitors of the invention can be administered singly or in any combination in a temporal sense, in that they may be administered concurrently, or before, and/or after each other. One of ordinary skill in the art will appreciate, based on the disclosure provided herein, that the inhibitor compositions of the invention can be used to prevent or to treat an autoimmune disease or disorder, and that an inhibitor composition can be used alone or in any combination with another modulator to affect a therapeutic result. In various embodiments, any of the inhibitor compositions of the invention described herein can be administered alone or in combination with other modulators of other molecules associated with autoimmune diseases.

In one embodiment, the invention includes a method comprising administering a combination of inhibitors described herein. In certain embodiments, the method has an additive effect, wherein the overall effect of the administering a combination of inhibitors is approximately equal to the sum of the effects of administering each individual inhibitor. In other embodiments, the method has a synergistic effect, wherein the overall effect of administering a combination of inhibitors is greater than the sum of the effects of administering each individual inhibitor.

The method comprises administering a combination of inhibitors in any suitable ratio. For example, in one embodiment, the method comprises administering two individual inhibitors at a 1:1 ratio. However, the method is not limited to any particular ratio. Rather any ratio that is shown to be effective is encompassed.

Pharmaceutical Compositions and Formulations

The invention also encompasses the use of pharmaceutical compositions of the invention or salts thereof to practice the methods of the invention. Such a pharmaceutical composition may consist of at least one modulator composition of the invention or a salt thereof in a form suitable for administration to a subject, or the pharmaceutical composition may comprise at least one modulator composition of the invention or a salt thereof, and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The compound or conjugate of the invention may be present in the pharmaceutical composition in the form of a physiologically acceptable salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

In an embodiment, the pharmaceutical compositions useful for practicing the methods of the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In another embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 500 mg/kg/day.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

Pharmaceutical compositions that are useful in the methods of the invention may be suitably developed for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. A composition useful within the methods of the invention may be directly administered to

the skin, vagina or any other tissue of a mammal. Other contemplated formulations include liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations. The route(s) of administration will be readily apparent to the skilled artisan and will depend upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human subject being treated, and the like.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

As used herein, a "unit dose" is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage. The unit dosage form may be for a single daily dose or one of multiple daily doses (e.g., about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions that are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist may design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

In one embodiment, the compositions of the invention are formulated using one or more pharmaceutically acceptable excipients or carriers. In one embodiment, the pharmaceutical compositions of the invention comprise a therapeutically effective amount of a compound or conjugate of the invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers that are useful, include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey).

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate or gelatin. In one embodiment, the pharmaceutically acceptable carrier is not DMSO alone.

Formulations may be employed in admixtures with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, vaginal, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, e.g., other analgesic agents.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" that may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed. (1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA), which is incorporated herein by reference.

The composition of the invention may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of exposure to contaminants in the environment. Examples of preservatives useful in accordance with the invention included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. A particularly preferred preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

The composition preferably includes an anti-oxidant and a chelating agent that inhibits the degradation of the compound. Preferred antioxidants for some compounds are BHT, BHA, alpha-tocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. Preferably, the chelating agent is present in an amount of from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (e.g. disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition that may be detrimental to the shelf life of the formulation. While BHT and disodium edetate are the particularly preferred antioxidant and chelating agent respectively for some compounds, other suitable and equivalent

antioxidants and chelating agents may be substituted therefore as would be known to those skilled in the art.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water, and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin, and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para- hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. As used herein, an “oily” liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water. Liquid solutions of

the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water, and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e., such as with a physiologically degradable material), and methods of absorbing an

aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the subject either prior to or after a diagnosis of disease. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a subject, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to prevent or treat disease. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the activity of the particular compound employed; the time of administration; the rate of excretion of the compound; the duration of the treatment; other drugs, compounds or materials used in combination with the compound; the state of the disease or disorder, age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors well-known in the medical arts. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is from about 1 and 5,000 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

The compound may be administered to a subject as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day

administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject.

A medical doctor, e.g., physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of a disease in a subject.

In one embodiment, the compositions of the invention are administered to the subject in dosages that range from one to five times per day or more. In another embodiment, the compositions of the invention are administered to the subject in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It will be readily apparent to one skilled in the art that the frequency of administration of the various combination compositions of

the invention will vary from subject to subject depending on many factors including, but not limited to, age, disease or disorder to be treated, gender, overall health, and other factors. Thus, the invention should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any subject will be determined by the attending physical taking all other factors about the subject into account.

Compounds of the invention for administration may be in the range of from about 1 mg to about 10,000 mg, about 20 mg to about 9,500 mg, about 40 mg to about 9,000 mg, about 75 mg to about 8,500 mg, about 150 mg to about 7,500 mg, about 200 mg to about 7,000 mg, about 3050 mg to about 6,000 mg, about 500 mg to about 5,000 mg, about 750 mg to about 4,000 mg, about 1 mg to about 3,000 mg, about 10 mg to about 2,500 mg, about 20 mg to about 2,000 mg, about 25 mg to about 1,500 mg, about 50 mg to about 1,000 mg, about 75 mg to about 900 mg, about 100 mg to about 800 mg, about 250 mg to about 750 mg, about 300 mg to about 600 mg, about 400 mg to about 500 mg, and any and all whole or partial increments there between.

In some embodiments, the dose of a compound of the invention is from about 1 mg and about 2,500 mg. In some embodiments, a dose of a compound of the invention used in compositions described herein is less than about 10,000 mg, or less than about 8,000 mg, or less than about 6,000 mg, or less than about 5,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second compound (i.e., a drug used for treating the same or another disease as that treated by the compositions of the invention) as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

In one embodiment, the present invention is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound or conjugate of the invention, alone or in combination with a second pharmaceutical agent; and instructions for using the compound or conjugate to treat, prevent, or reduce one or more symptoms of a disease in a subject.

The term "container" includes any receptacle for holding the pharmaceutical composition. For example, in one embodiment, the container is the packaging that contains the pharmaceutical composition. In other embodiments, the container is not the packaging that contains the pharmaceutical composition, i.e., the container is a receptacle, such as a box or vial that contains the packaged pharmaceutical composition or unpackaged pharmaceutical composition and the instructions for use of the pharmaceutical composition. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the pharmaceutical composition may be contained on the packaging containing the pharmaceutical composition, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that the instructions may contain information pertaining to the compound's ability to perform its intended function, e.g., treating or preventing a disease in a subject, or delivering an imaging or diagnostic agent to a subject.

Routes of administration of any of the compositions of the invention include oral, nasal, parenteral, sublingual, transdermal, transmucosal (e.g., sublingual, lingual, (trans)buccal, and (intra)nasal,), intravesical, intraduodenal, intragastrical, rectal, intra-peritoneal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, or administration.

Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the

formulations and compositions that would be useful in the present invention are not limited to the particular formulations and compositions that are described herein.

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1: PTPN22 inhibition resets defective human central B cell tolerance

The data presented herein examines if inhibiting PTPN22 favors the elimination of autoreactive B cells. It is demonstrated herein that the PTPN22 T allele interferes with the establishment of central B cell tolerance using NOD-scid-common gamma chain (γc) knockout (NSG) mice engrafted with human hematopoietic stem cells (HSCs) expressing this allele. In contrast, the inhibition of either PTPN22 enzymatic activity or its expression by RNA interference restored defective central B cell tolerance in this model. Thus, PTPN22 blockade may represent a novel therapeutic strategy for the prevention or treatment of autoimmunity.

The materials and methods employed in these experiments are now described.

Human progenitor cell isolation and injection in NSG mice

Human $CD34^+$ cells were purified from fetal liver samples by density gradient centrifugation followed by positive immunomagnetic selection with anti-human CD34 microbeads (Miltenyi). Newborn NSG mice (within first 3 days of life) were sublethally irradiated (X-ray irradiation with X-RAD 320 irradiator at 180 cGy) and 100,000-150,000 $CD34^+$ cells in 20 μL of PBS were injected into the liver with a 22-gauge needle (Hamilton Company). Mice were used for experiments 10–12 weeks after transplantation. NSG mice treated with the PTPN22 inhibitor were injected twice daily i.p. with 0.75 mg or 0.15 mg of PTPN22 inhibitor for a week.

PTPN22 overexpression and silencing and $CD34^+$ HSCs transduction

The pTRIP-Ubi-GFP lentiviral vector has been used for overexpression of PTPN22 variants and short hairpin RNA (shRNA) delivery. Vector constructions have been described previously (Cantaert et al., 2015, Immunity 43:884-95; Ruer-Laventie et al., 2015, Immun Inflamm Dis 3:265-79). The following sequence are used for human PTPN22 targeting: shRNA1 5'-CTAGTGCTCTTGGTGTATATT-3' (SEQ ID NO: 1), shRNA2 5'-CTGTTGCCAACATCCTCTA-3'(SEQ ID NO: 3), or shRNA3 5'-AAGAATCCACCTGACTTCC-3'(SEQ ID NO: 2). Lentiviral particles were produced by transient transfection of 293T cells, as previously described (Schickel et al., 2012, EMBO Mol Med 4:1261-75). Viruses were then used to transduce $CD34^+$ HSCs in the presence of protamine sulfate (Sigma).

Single-cell sorting

B cells were enriched from splenocytes using magnetic separation with CD19 microbeads (Miltenyi Biotech) and stained with CD19-Pacific Blue, CD10-PE-Cy7, CD21-APC and IgM-biotin (all from Biolegend) prior to purification. Single $CD19^+CD10^+CD21^{low}GFP^-$ or GFP^+ new emigrant B cells were sorted on a FACSAria (BD Biosciences) into 96-well PCR plates and immediately frozen on dry ice.

cDNA synthesis, Ig genes amplification, antibody production, and purification

RNA from single cells was reverse-transcribed in the original 96 well plate in 12.5 µl reactions containing 100U of Superscript II RT (Gibco BRL) for 45 min at 42°C. RT-PCR reactions, primer sequences, cloning strategy, expression vectors, antibody expression and purification were as described (Tiller et al., 2008, J Immunol Methods 329:112-24).

ELISAs and immunofluorescence assays

Antibody reactivity analysis was performed as described previously with the highly polyreactive ED38 antibody as positive control for HEp-2 reactivity and polyreactivity (Wardemann, 2003, Science 301:1374-7). Antibodies were considered polyreactive when they recognized all 3 distinct antigens: dsDNA, insulin and LPS. For indirect immunofluorescence assays, HEp-2 cell-coated slides (Bion Enterprises Ltd.) were incubated in a moist chamber at room temperature with purified recombinant antibodies at 50-100 µg/mL according to the manufacturer's instructions. FITC-conjugated goat anti-human IgG was used as detection reagent.

Flow cytometry

The following monoclonal antibodies against human antigens were used: anti-CD10 (HI10a), anti-CD19 (HIB19), anti-CD27 (O323), anti-CD45 (HI30) (all from Biolegend) and anti-CD21 (B-ly4) and anti-IgM (G20-127; from BD Biosciences). Cells were acquired with a LSR II (BD Biosciences) and analyzed with FlowJo software.

Immunoblot

Total cell lysates were separated by SDS page, transferred to PVDF membranes, probed with mouse anti-PTPN22 (Invitrogen) and detected by chemiluminescence (Amersham ECL Prime Western Blotting detection Reagent) using a GBox documentation system (Syngene). For quantification, blots were stripped with stripping buffer (Pierce) and reprobed with a mouse anti-β-Actin antibody (Sigma-Aldrich).

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 5.0; GraphPad, San Diego, CA). Data are reported as mean ± standard deviation. Differences between groups of research subjects were analyzed for statistical significance with unpaired two-tailed Student's t-tests. A P-value of ≤ 0.05 was considered significant.

The results of the experiments are now described.

To further study the impact of PTPN22 variants on central B cell tolerance NOD-scid-common gamma chain (γc) knockout (NSG) immunodeficient mice were engrafted with $CD34^+$ hematopoietic stem cells (HSCs) isolated from human fetuses carrying or not PTPN22 T allele(s) (Shultz et al., 2005, J Immunol 174:6477-89; Rongvaux et al., 2014, Nat Biotech 32:364-72; Kalscheuer et al., 2012, Sci Transl Med 4:125ra30)

(Figure 1A and Table 1). Humanized NSG mice displayed high frequencies of $CD45^+$ human cells detected by flow cytometry around three months post-engraftment with HSCs regardless of the presence of PTPN22 T allele(s) (Figure 1B). Ratios between human $CD19^+$ B and $CD3^+$ T lymphocytes were also similar in NSG mice transplanted with PTPN22 C/C, PTPN22 C/T or PTPN22 T/T HSCs, demonstrating that the PTPN22 T allele does not affect either B- or T-cell development (Figure 1B). Pooled immunoglobulin heavy-chain (IgH) sequence analyses from new emigrant B cells of PTPN22 C/T or T/T NSG mice revealed no consistent differences in IgH variable (VH), diversity (D), or joining (J) gene usage compared to PTPN22 C/C NSG mice (Figures 2A, 2B and 2C). However, in contrast to new emigrant B cells of PTPN22 C/C NSG mice, the presence of a PTPN22 T allele favored the usage of different D reading frames encoding hydrophobic residues known to favor self-reactivity and which correlated with an abnormal central B cell tolerance checkpoint (Corbett et al., 1997, J Mol Biol 270:587-97; Ng et al., 2004, J Exp Med 200:927-34; Meyers et al., 2011, PNAS 108:11554-9) (Figure 2D). The analyses of antibody reactivity revealed that frequencies of polyreactive clones in splenic $CD19^+CD27^-CD10^+IgM^{hi}CD21^{lo}$ new emigrant/transitional B cells from NSG mice transplanted with PTPN22 C/C HSCs isolated from seven distinct fetuses were low and similar to those of new

emigrant/transitional B cells isolated from the blood of PTPN22 C/C healthy donors (Figures 1C, 3A, 11-17). The low frequencies of HEp-2 reactive new emigrant/transitional B cells and the virtual absence of anti-nuclear clones in this B cell compartment reveals that central B cell tolerance is established normally in humanized mice in the absence of the PTPN22 T allele (Figures 1D, 3B and 3C). In contrast, new emigrant/transitional B cells isolated from the spleen of NSG mice engrafted with PTPN22 C/T or T/T HSCs contained many autoreactive clones expressing polyreactive and HEp-2 reactive antibodies with similar frequencies to those observed in healthy donors carrying PTPN22 T allele(s) (Menard et al., 2011, J Clin Invest 121:3635-44) (Figures 1C, 1D, 3A, 3B and 18-20). Indirect immunofluorescence assays with HEp-2 cell–coated slides revealed that the proportions of anti-nuclear clones in NSG mice engrafted with PTPN22 C/T or T/T HSCs new emigrant B cells were increased but failed to reach significance (Figure 3C). These data demonstrate that the presence of the PTPN22 T allele in HSCs results in defective central B cell tolerance and the release of large numbers of autoreactive B cells from the bone marrow.

Table 1: Fetal donor characteristics

Donor #	PTPN22 genotype	Age (days)	Gender
1	C/C	105	Female
2	C/C	115	Female
3	C/C	108	Male
4	C/C	110	Female
5	C/C	120	Male
6	C/C	137	Female
7	C/C	112	Female
8	C/T	122	Female
9	C/T	125	Female
10	T/T	105	Female
11	C/C	111	Female
12	C/C	108	Female

C/C: homozygote for the PTPN22 C allele in position 1858
C/T: heterozygote for the PTPN22 T allele in position 1858
T/T: homozygote for the PTPN22 T allele in position 1858

To determine whether B-cell intrinsic expression of 620W PTPN22 phosphatases is sufficient to interfere with the removal of developing autoreactive immature B cells in the bone marrow, PTPN22 C/C HSCs were transduced with

lentiviruses expressing green fluorescent proteins (GFP) and the 620W PTPN22 autoimmunity-favoring variant, the common 620R or the 263Q loss-of-function PTPN22 enzyme (Figure 4A). Human $CD19^+$ B cells developed in NSG mice engrafted with HSCs transduced or not with the different lentiviruses, revealing that lentiviral transduction did not alter HSC engraftment or B cell development (Figures 5A and 5B). The presence of 620W PTPN22 alters the counterselection of developing autoreactive B cells as GFP^+ new emigrant/transitional B cells expressing this variant contained many autoreactive clones producing polyreactive antibodies (Figures 4B, 5A, and 21-26). High proportions of HEp-2 reactive and anti-nuclear GFP^+ new emigrant/transitional B cells corroborated this defective central B cell tolerance checkpoint (Figures 4C, 6B, 6C, and 6D). In contrast, GFP^- B cell counterparts that developed in the same NSG mice rarely expressed polyreactive antibodies and displayed low frequencies of HEp-2 reactive and anti-nuclear clones revealing that these B cells were properly selected in the absence of 620W PTPN22 expression (Figures 4B, 4C, 6A, 6B, 6C, and 6D). In addition, GFP^+ new emigrant/transitional B cells expressing either 620R PTPN22 or the loss-of-function 263Q PTPN22 variant displayed normal proportions of polyreactive, HEp-2 reactive and anti-nuclear clones demonstrating normal central B cell tolerance (Figures 4B, 4C, 6A, 6B, 6C, 6D, and 27-30). Regardless of how the 620W amino acid replacement alters PTPN22 function, our data demonstrate that B-cell intrinsic 620W PTPN22 expression is sufficient to interfere with the removal of developing autoreactive B cells and the establishment of human central B cell tolerance.

PTPN22 enzymatic activity can be inhibited in T cells in vitro by the LTV-1 specific inhibitor (Vang et al., 2012, Nat Chem Biol 8:437-46). To assess the impact of the inhibition of 620W PTPN22 enzymatic activity on central B cell tolerance, PTPN22 C/T or T/T engrafted NSG mice were injected about 3 months post transplant with 0.75 mg of LTV-1 compound twice daily for a week and determined the frequency of autoreactive new emigrant/transitional B cells (Figure 7A). LTV-1 treatment significantly reduced the frequencies of polyreactive new emigrant/transitional B cells in PTPN22 C/T or T/T transplanted mice, similar to those in NSG mice engrafted with HSCs that did not carry the PTPN22 T allele (Figures 7B and 31-33). In addition,

PTPN22 inhibition by LTV-1 also normalized the frequencies of HEp-2 reactive new emigrant/transitional B cells in PTPN22 C/T or T/T engrafted mice (Figure 7C) and anti-nuclear clone frequencies remained very low (Figure 8). Similar results were obtained with 0.15 mg of LTV-1 injections, revealing a large range for effective PTPN22 inhibition by this compound (Fig. 3B, C). Hence, inhibition of 620W PTPN22 enzymatic activity resets central B cell tolerance that is normally impaired by the presence of the PTPN22 T allele.

Although central B cell tolerance appears to be mainly regulated by B-cell intrinsic pathways involving B-cell receptor (BCR) and potentially Toll-like receptor (TLR) signaling (Meffre, 2011, Ann N Y Acad Sci 1246:1-10), this checkpoint might be restored via B-cell extrinsic pathways normalized by 620W PTPN22 inhibition. In addition, the LTV-1 PTPN22 inhibitor may also non-specifically alter the function of other phosphatases. To determine if specific B-cell intrinsic PTPN22 blockade is responsible for the correction of central tolerance, a strategy to inhibit the expression of PTPN22 in developing B cells using RNA interference was developed (Cantaert et al., 2015, Immunity 43:884-95). NSG mice were engrafted with PTPN22 C/T or T/T HSCs transduced with a GFP-tagged lentivirus expressing PTPN22 specific shRNA (Fig 9A). Two PTPN22 specific shRNA, shRNA#1 and #3, were identified that could inhibit about 80% of PTPN22 expression detected by western blot using human RAMOS B cell line and chose shRNA#1 for all further experiments (Figure 10A). A high proportion of GFP^+ human B cells expressing PTPN22 shRNA#1 developed in NSG mice, revealing that transduced HSCs retained engraftment and B cell development capacities (Figure 9B). In addition, GFP expression correlated with more than 90% decrease of PTPN22 expression in developing B cells (Figure 9C). Blocking PTPN22 expression in GFP^+ PTPN22 C/T or T/T new emigrant/transitional B cells significantly reduced the production of polyreactive and HEp-2 reactive clones compared to GFP^- counterparts that often expressed autoreactive antibodies (Figures 9D, 9E, 10B, 10C, and 34-39). In addition, it was previously shown using control shRNA lentiviruses that HSC transduction per se does not interfere with the counterselection of autoreactive B cells (Cantaert et al., 2015, Immunity 43:884-95). Altogether, these data demonstrate that the inhibition of PTPN22

expression in developing B cells can induce efficient removal of autoreactive clones and therefore restore central B cell tolerance that is otherwise impaired when the 620W PTPN22 variant is expressed.

In conclusion, the PTPN22 T allele is responsible for the production of autoreactive B cells that escape central tolerance (Menard et al., 2011, J Clin Invest 121:3635-44). These observations may explain why the PTPN22 T allele confers high risk to develop many autoimmune diseases as it induces central B cell tolerance defects observed in patients with T1D, RA and SLE (Chamberlain et al., 2015, J Clin Invest 126:282-7; Samuels, 2005, J Exp Med 201:1659-67; Yurasov, 2006, J Exp Med 201:703-11). Increased frequencies of autoreactive B cells may increase the probability to present self-antigens and initiate autoimmunity. In addition, the data presented herein shows that central B cell tolerance could likely be reset in PTPN22 C/T or T/T subjects by inhibiting PTPN22 enzymatic activity or expression. These data are in agreement with previous studies that have demonstrated that the 620W PTPN22 variant requires its enzymatic activity to mediate alternative outcomes but it remains to be determined how the 620W mutation modifies its function (Dai et al., 2013, J Clin Invest 123:2024-36). In addition, PTPN22 inhibition might also reset the T cell receptor (TCR) signaling threshold altered by 620W PTPN22 variants (Rieck et al., 2007, J Immunol 179:4704-10; Salmond et a., 2014, Nat Immunol 15:875-83) and therefore modify the TCR repertoire of both T effector and regulatory T cells selected in the thymus of PTPN22 T carriers. In conclusion, PTPN22 is a major regulator of human central B cell tolerance; its inhibition can normalize the elimination of developing autoreactive B cells and may thereby thwart the development of autoimmunity.

Example 2: PTPN22 inhibition restores proper BCR signaling regulating central B cell tolerance

The data presented herein demonstrates how PTPN22 inhibition may affect B cell responses, and specifically BCR function, and restores central B cell tolerance. Phosphorylation kinetics of LYN and SHIP1, an important regulator of calcium flux, and ERK in RAMOS cells after BCR triggering with or without PTPN22

enzymatic inhibition by LTV-1 suggest that PTPN22 inhibition decreases the phosphorylation of LYN at position Y396 and SHIP1 at position Y2010, which activates these molecules (Figure 40A). However, downstream ERK phosphorylation was not affected by PTPN22 inhibition (Figure 40A). Since PTPN22 inhibition enhanced TCR signaling in Jurkat cell line, it was investigated if PTPN22 blockade may also increase BCR signaling and calcium flux induced by BCR triggering was measured in RAMOS cells in the presence or not of LTV-1. Calcium flux was increased by PTPN22 inhibition, especially at lower concentration of BCR triggering agent (Figure 40B). Similarly, calcium flux was enhanced in B cells isolated from humanized mice treated with LTV-1 compared to non-treated counterparts (Figure 40C). Decreased SHIP1 phosphorylation after PTPN22 inhibition shown in Figure 40A likely contributes to this phenotype since SHIP1 normally mediates calcium flux downregulation. In addition, GFP^+ B cells isolated from a humanized NSG mouse in which PTPN22 production is inhibited by GFP-tagged lentivirus expressing PTPN22 specific shRNA also displayed increased calcium flux after BCR triggering compared to GFP^- counterparts that express PTPN22 (Figure 40D). Thus, while not wishing to be bound to any particular theory, PTPN22 blockade may restore central B cell tolerance by increasing BCR signaling and the deletion of immature B cells binding self-antigens in the bone marrow.

Example 3: PTPN22 blockage prevents the accumulation of autoreactive B cells in the periphery

The data presented herein demonstrates the peripheral B cell tolerance checkpoint in NSG mouse engrafted with fetal HSCs and autologous thymic tissue both carrying the 1858T PTPN22 allele. Autoreactive clones accumulated in the mature naïve B cell compartment in the spleen of this mouse, a situation that resembles that of asymptomatic 1858T PTPN22 carrier subjects who display elevated proportions of autoreactive mature naïve B cells in their blood (Figure 41). PTPN22 inhibition by LTV-1 treatment for a month effectively restored this impaired peripheral B cell tolerance checkpoint (Figure 41). Hence, these data show that NSG mice + Thymus when engrafted with fetal tissues carrying the PTPN22 T allele, represent a good model for the

accumulation of autoreactive B cells in the periphery induced by the presence of the 1858T PTPN22 allele and its correction by PTPN22 blockade.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

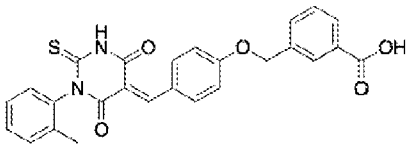
CLAIMS

What is claimed is:

1. A composition for treating or preventing an abnormal early B-cell tolerance checkpoint comprising an inhibitor of PTPN22.

2. The composition of claim 1, wherein the inhibitor of PTPN22 is at least one selected from the group consisting of a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, an antisense nucleic acid molecule.

3. The composition of claim 2, wherein the inhibitor of PTPN22 is a small molecule chemical compound.

4. The composition of claim 3, wherein the small molecule chemical compound is  , a derivative thereof, or a salt thereof.

5. The composition of claim 2, wherein the inhibitor of PTPN22 is a nucleic acid.

6. The composition of claim 5, wherein the nucleic acid comprises a nucleotide sequence selected from SEQ ID NO:1 and SEQ ID NO:2.

7. The composition of claim 1, wherein the abnormal early B-cell tolerance checkpoint is associated with an autoimmune disease or disorder.

8. The composition of claim 7, wherein the autoimmune disease or disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis,

multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, Sjögren's syndrome, autoimmune thyroiditis, myasthenia gravis, and pemphigus.

9. A method for treating or preventing an autoimmune disease or disorder the method comprising administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof.

10. The method of claim 9, wherein the inhibitor of PTPN22 is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, an antisense nucleic acid molecule.

11. The method of claim 9, wherein the autoimmune disease or disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus.

12. The method of claim 9 wherein the subject has a 1858T PTPN22 polymorphism on at least one allele.

13. The method of claim 9, wherein the subject is human.

14. A method for restoring human central B-cell tolerance in a subject the method comprising administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof.

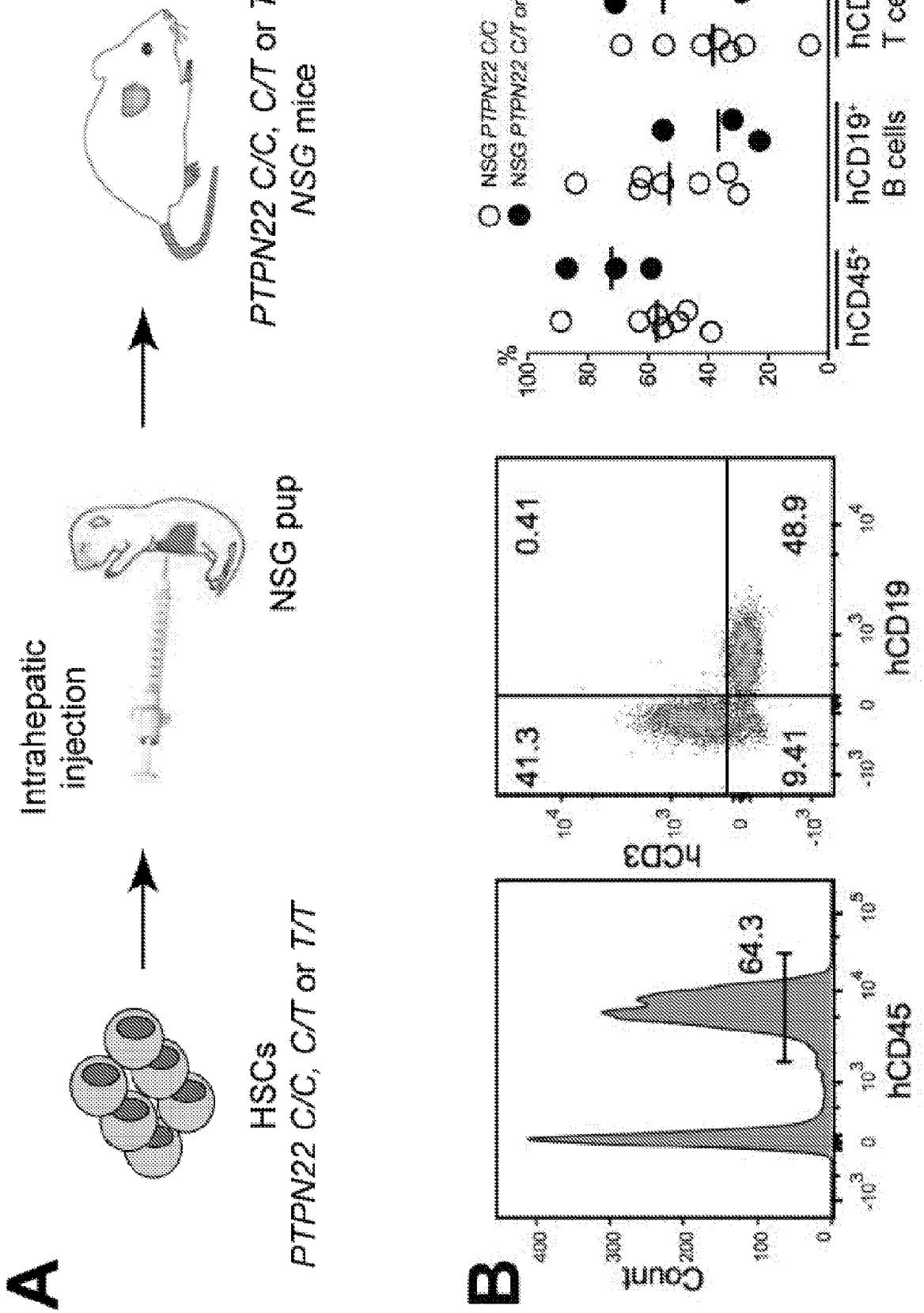
15. The method of claim 14, wherein the inhibitor of PTPN22 is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, an antisense nucleic acid molecule.

16. The method of claim 15, wherein the autoimmune disease or disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus.

17. The method of claim 14, wherein the subject fails to properly remove developing autoreactive B cells.

18. The method of claim 14 wherein the subject has a 1858T PTPN22 polymorphism on at least one allele.

19. The method of claim 14, wherein the subject is human.



Figures 1A – 1B

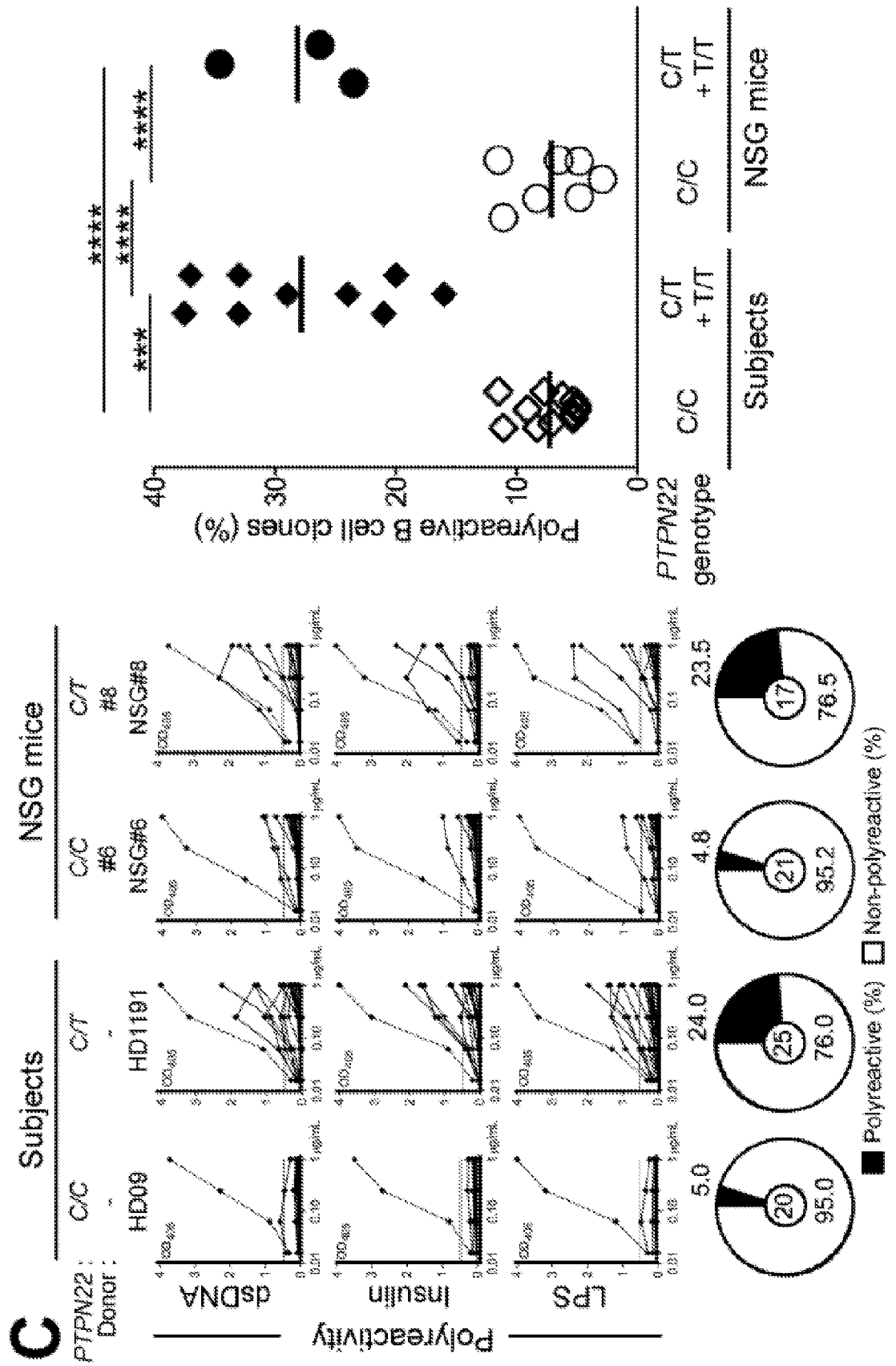

C
Subjects
NSG mice
PTPN22 :
Donor :
C/C
C/T
HD09
HD1101
C/C #6 NSG#6
C/T #8 NSG#8
dsDNA
Insulin
LPS
Polyreactivity
5.0
24.0
4.8
23.5
20
95.0
25
76.0
21
95.2
17
76.5
Polyreactive (%)
Non-polyreactive (%)
Polyreactive B cell clones (%)
PTPN22 genotype
C/C
C/T + T/T
Subjects
NSG mice
***
****

Figure 1C

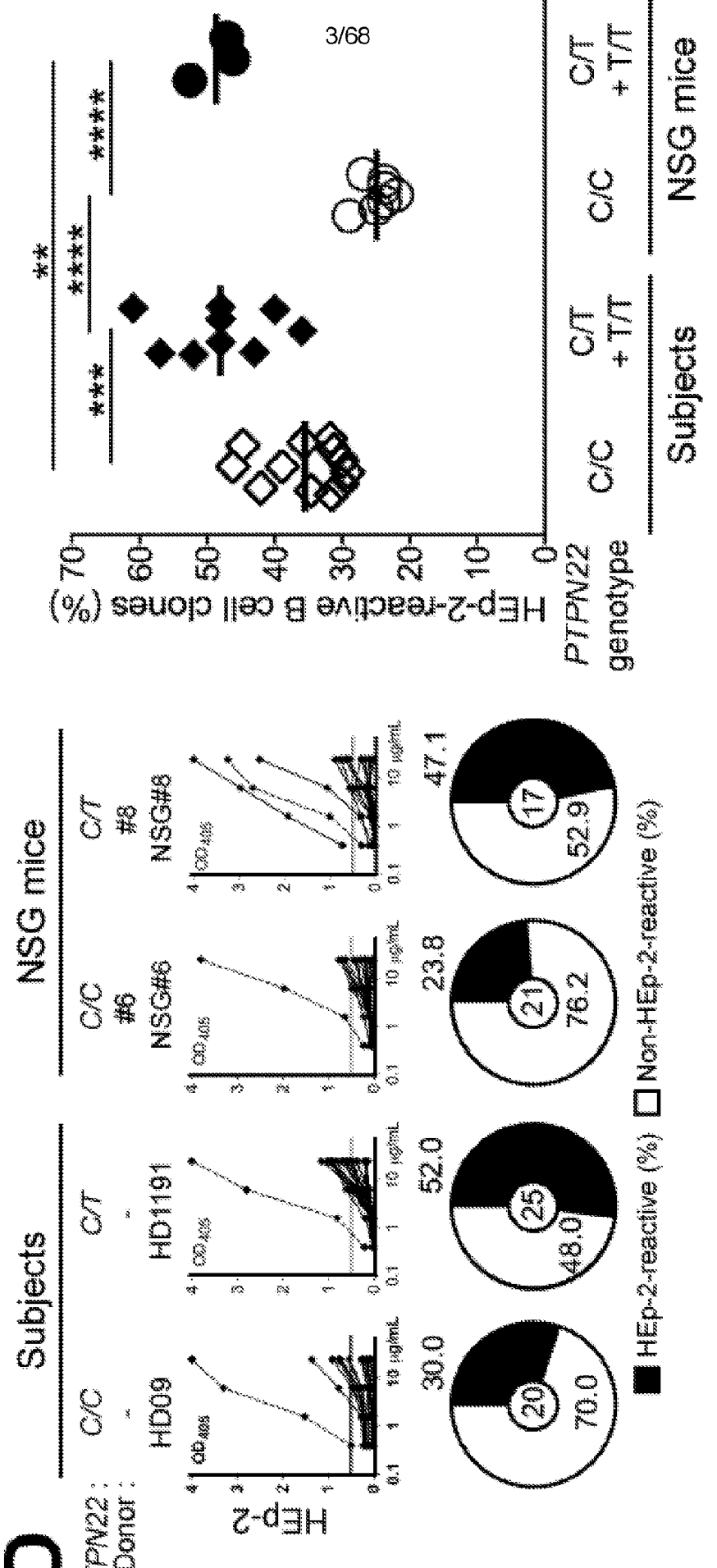
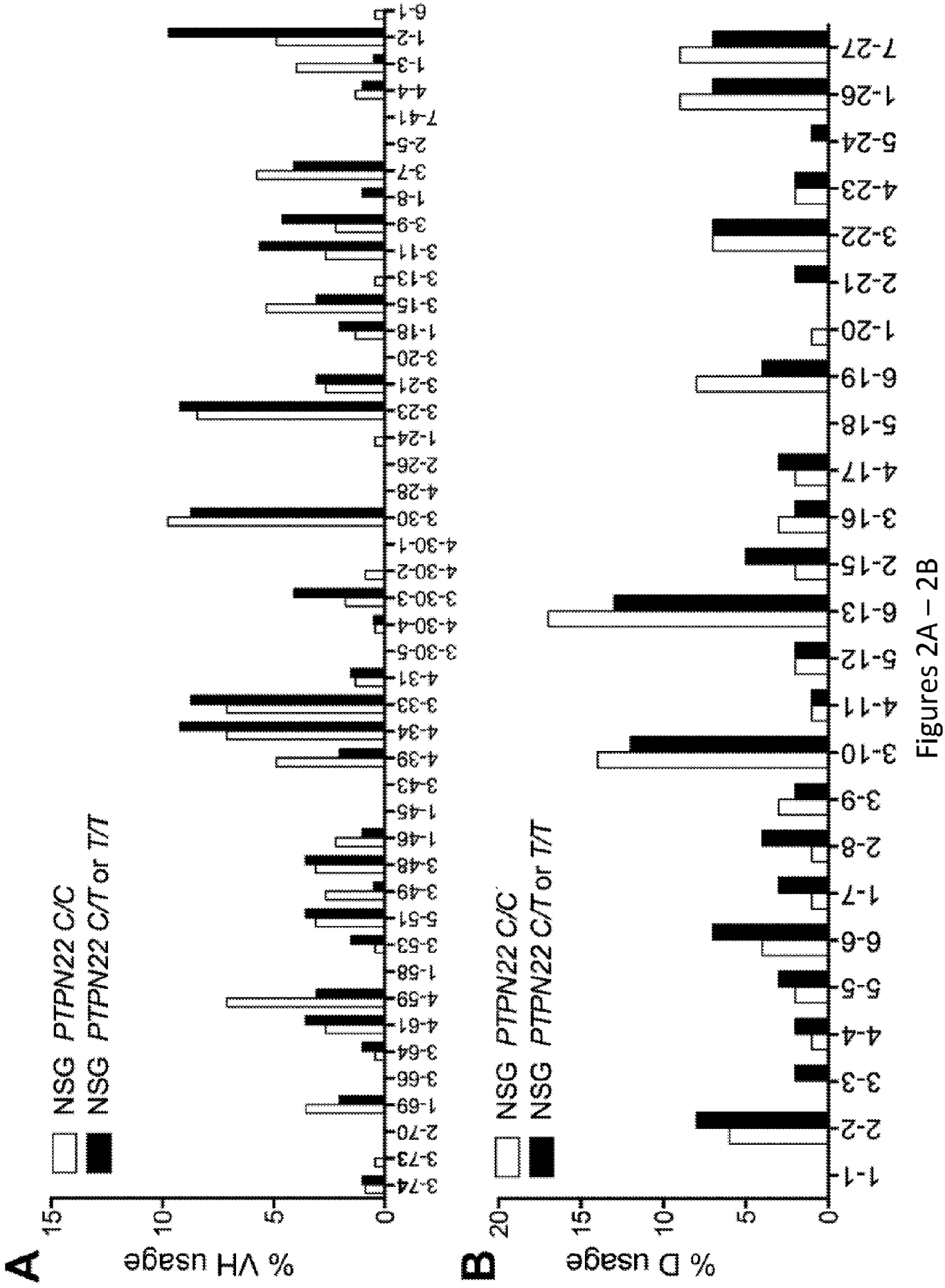
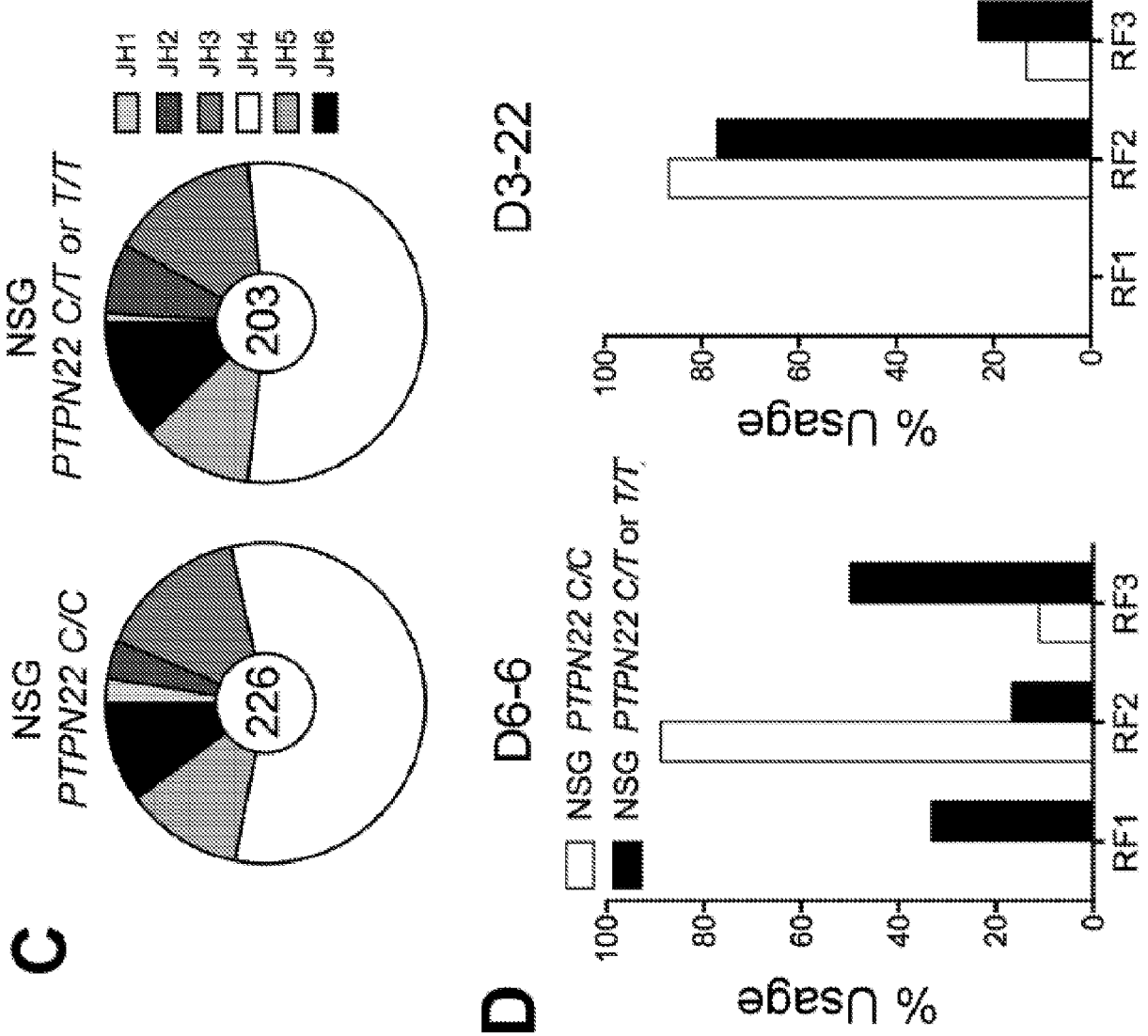

D
Subjects
NSG mice
PTPN22 :
Donor :
C/C
-
HD09
C/T
-
HD1191
C/C
#6
NSG#6
C/T
#8
NSG#8
HEp-2
HEp-2-reactive B cell clones (%)
PTPN22 genotype
C/C
C/T + T/T
Subjects
NSG mice
30.0
70.0
20
52.0
48.0
25
23.8
76.2
21
47.1
52.9
17
HEp-2-reactive (%)
Non-HEp-2-reactive (%)

Figure 1D



Figures 2A – 2B



Figures 2C – 2D

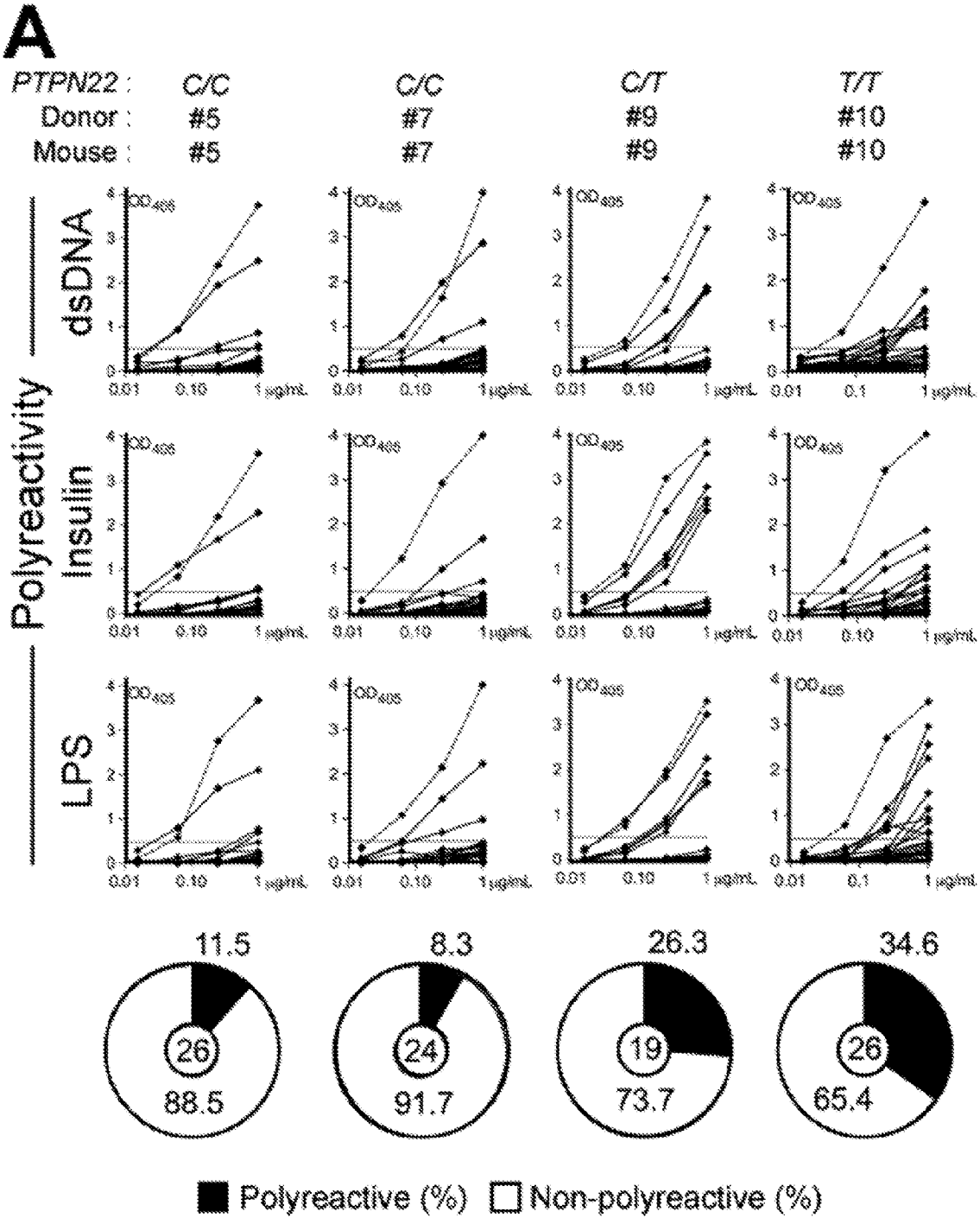
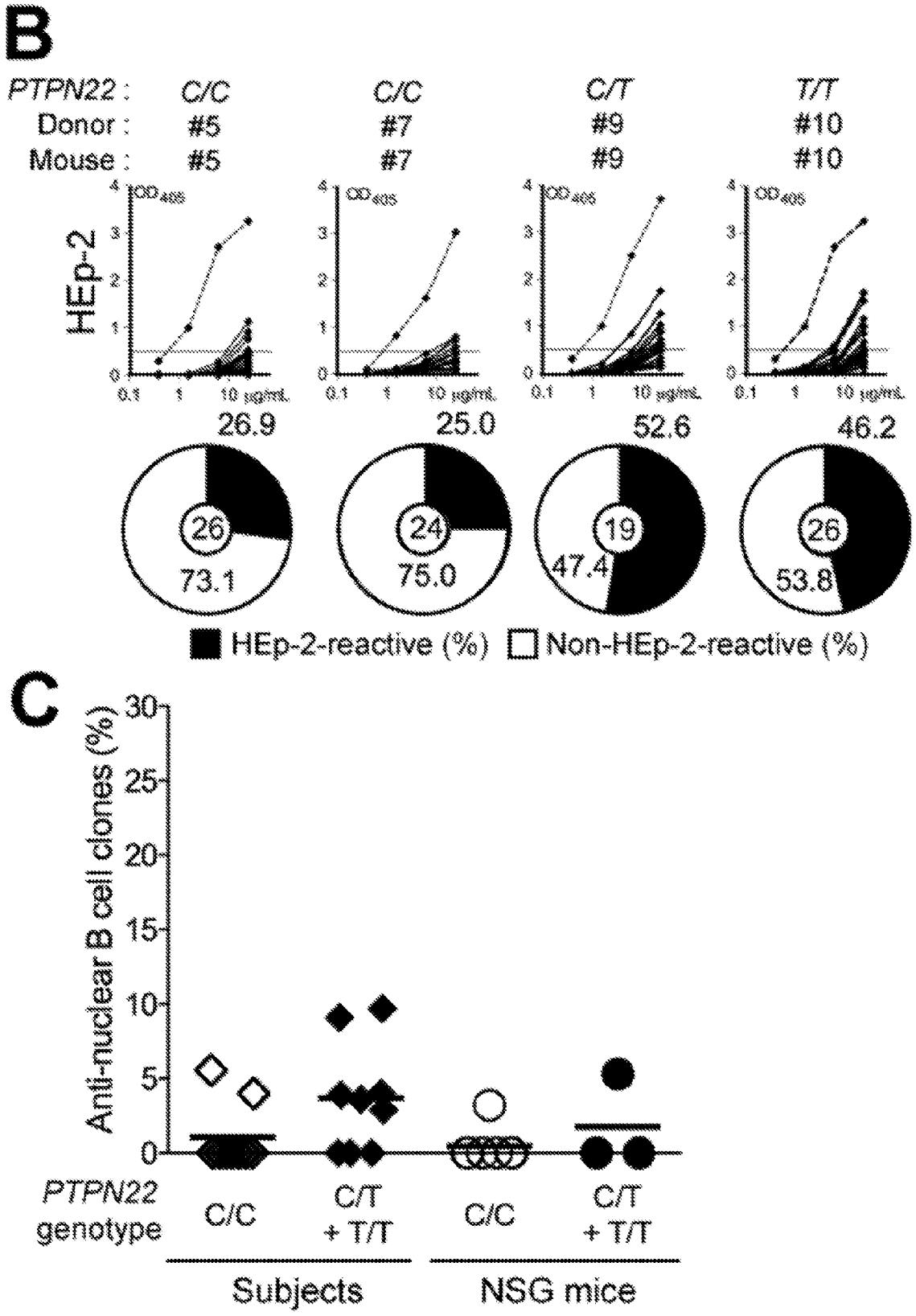

A
PTPN22 : C/C C/C C/T T/T
Donor : #5 #7 #9 #10
Mouse : #5 #7 #9 #10
Polyreactivity
dsDNA
Insulin
LPS
OD405
0.01
0.10
0.1
1 μg/mL
11.5
8.3
26.3
34.6
26
24
19
26
88.5
91.7
73.7
65.4
Polyreactive (%)
Non-polyreactive (%)

Figure 3A



Figures 3B – 3C

A
PTPN22 620W
PTPN22 620R
PTPN22 263Q
5'LTR
Ubiquitin
GFP
T2A
1858T PTPN22
1858C PTPN22
778G PTPN22
3'LTR
Transduction of HSCs with lentiviral vectors
HSCs
Intrahepatic injection
NSG pup

Figure 4A

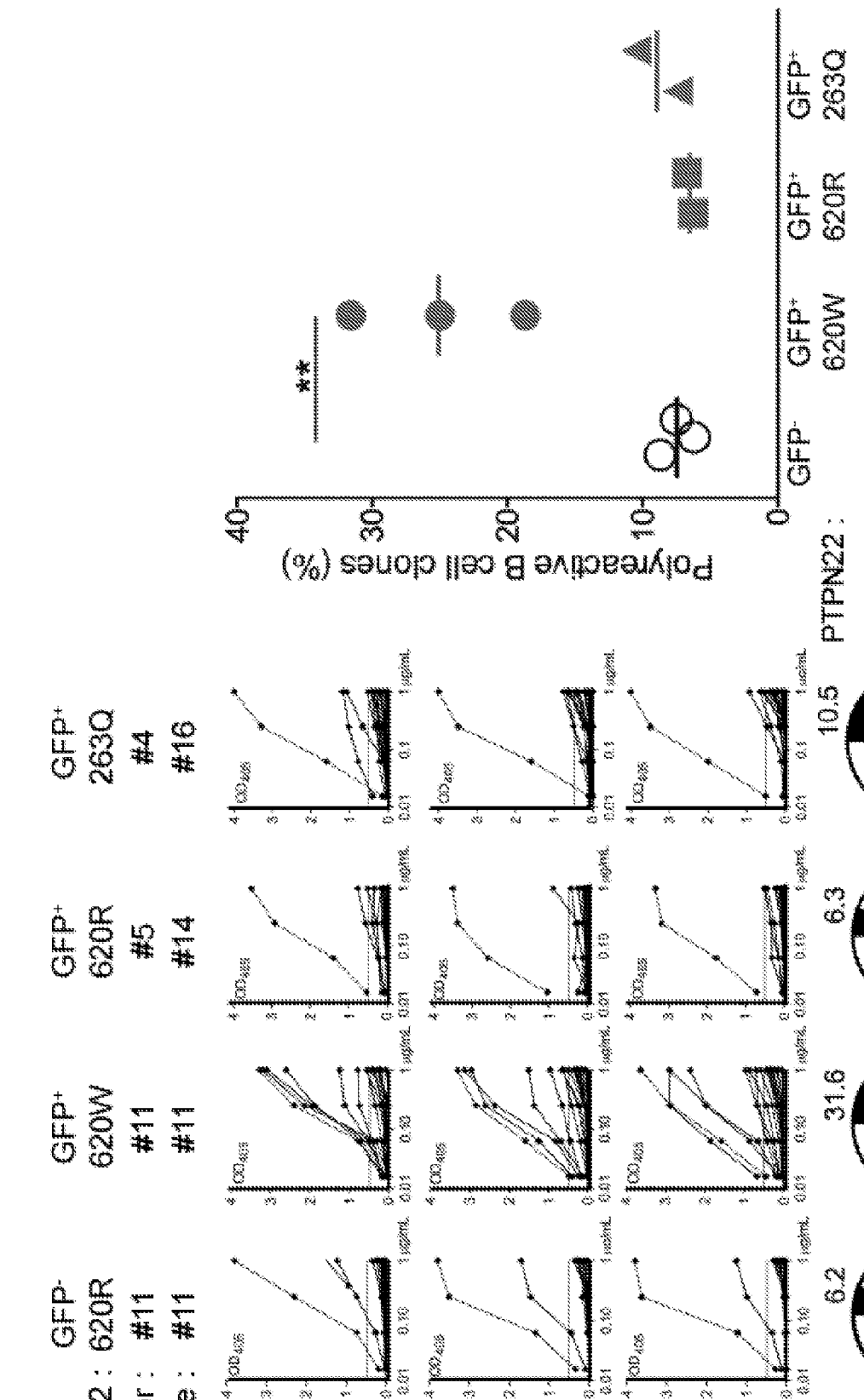

B
GFP-
PTPN22 : 620R
Donor : #11
Mouse : #11
GFP+
620W
#11
#11
GFP+
620R
#5
#14
GFP+
263Q
#4
#16
Polyreactivity
dsDNA
Insulin
LPS
Polyreactive B cell clones (%)
40
30
20
10
0
**
GFP-
GFP+
620W
GFP+
620R
GFP+
263Q
PTPN22 :
6.2
95.8
31.6
68.4
6.3
93.7
10.5
89.5
Polyreactive (%)
Non-polyreactive (%)

Figure 4B

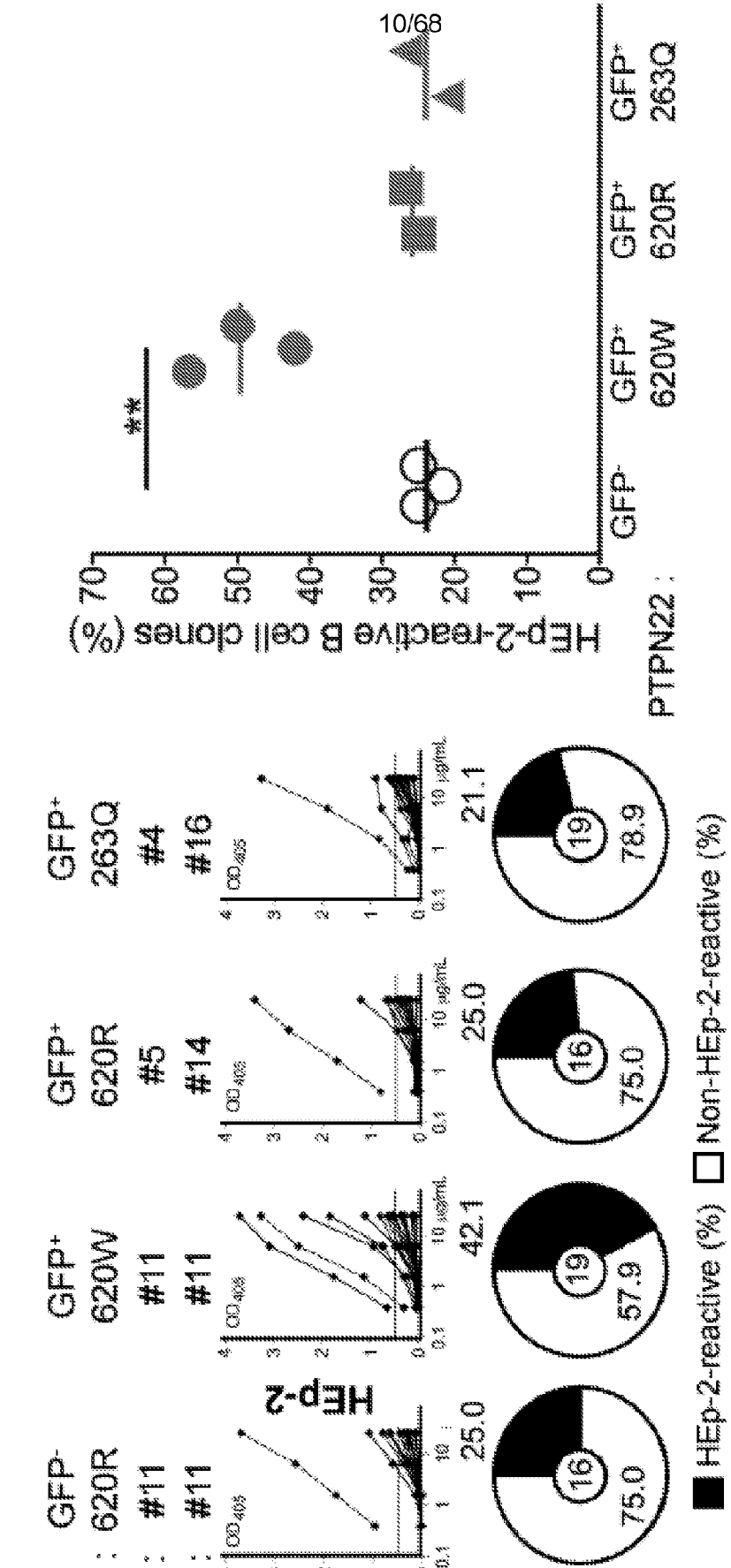
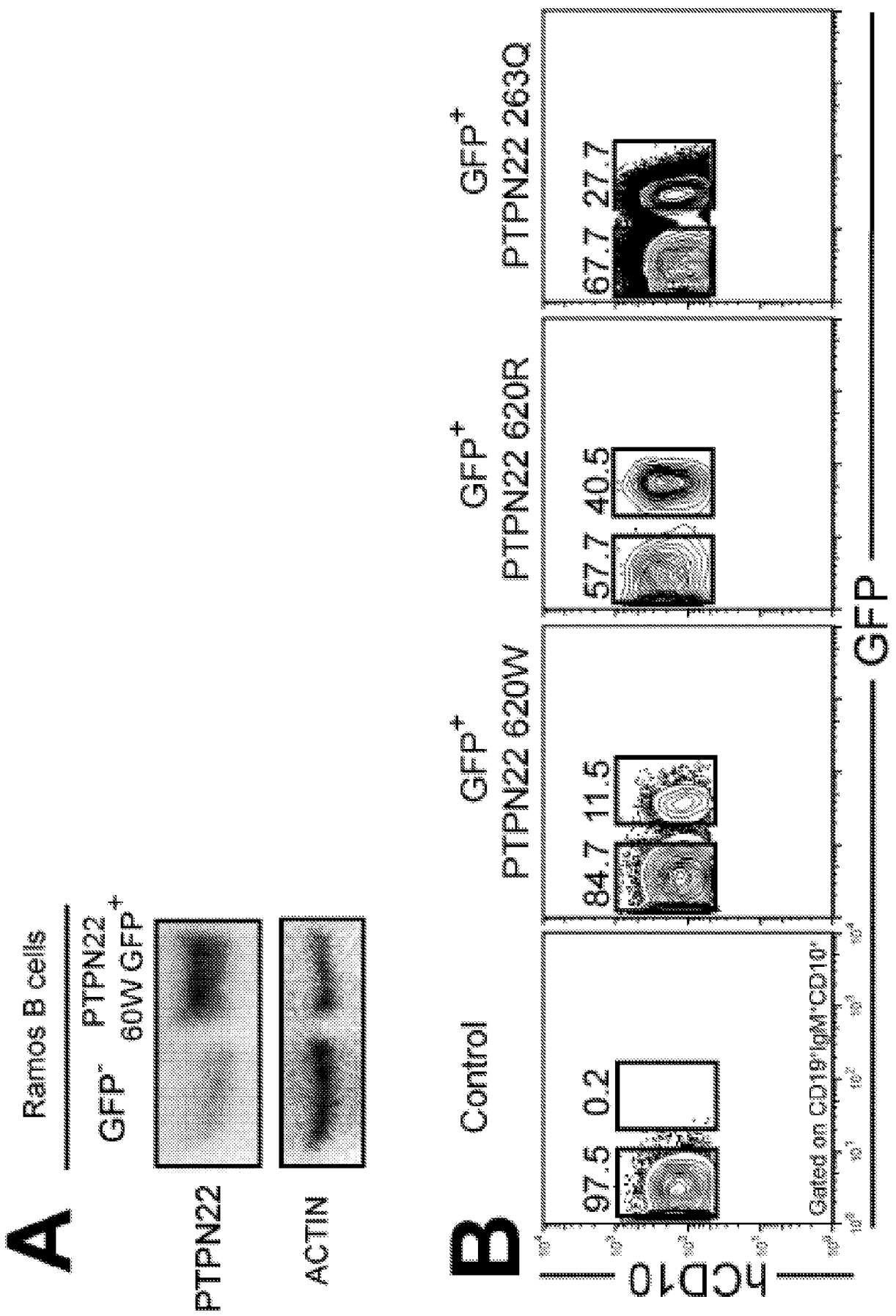

C
PTPN22 :
Donor :
Mouse :
GFP-
620R
#11
#11
HEp-2
GFP+
620W
#11
#11
GFP+
620R
#5
#14
GFP+
263Q
#4
#16
25.0
42.1
25.0
21.1
16
75.0
19
57.9
16
75.0
19
78.9
HEp-2-reactive (%)
Non-HEp-2-reactive (%)
HEp-2-reactive B cell clones (%)
**
GFP-
GFP+
620W
GFP+
620R
GFP+
263Q
PTPN22 :

Figure 4C



Figures 5A – 5B

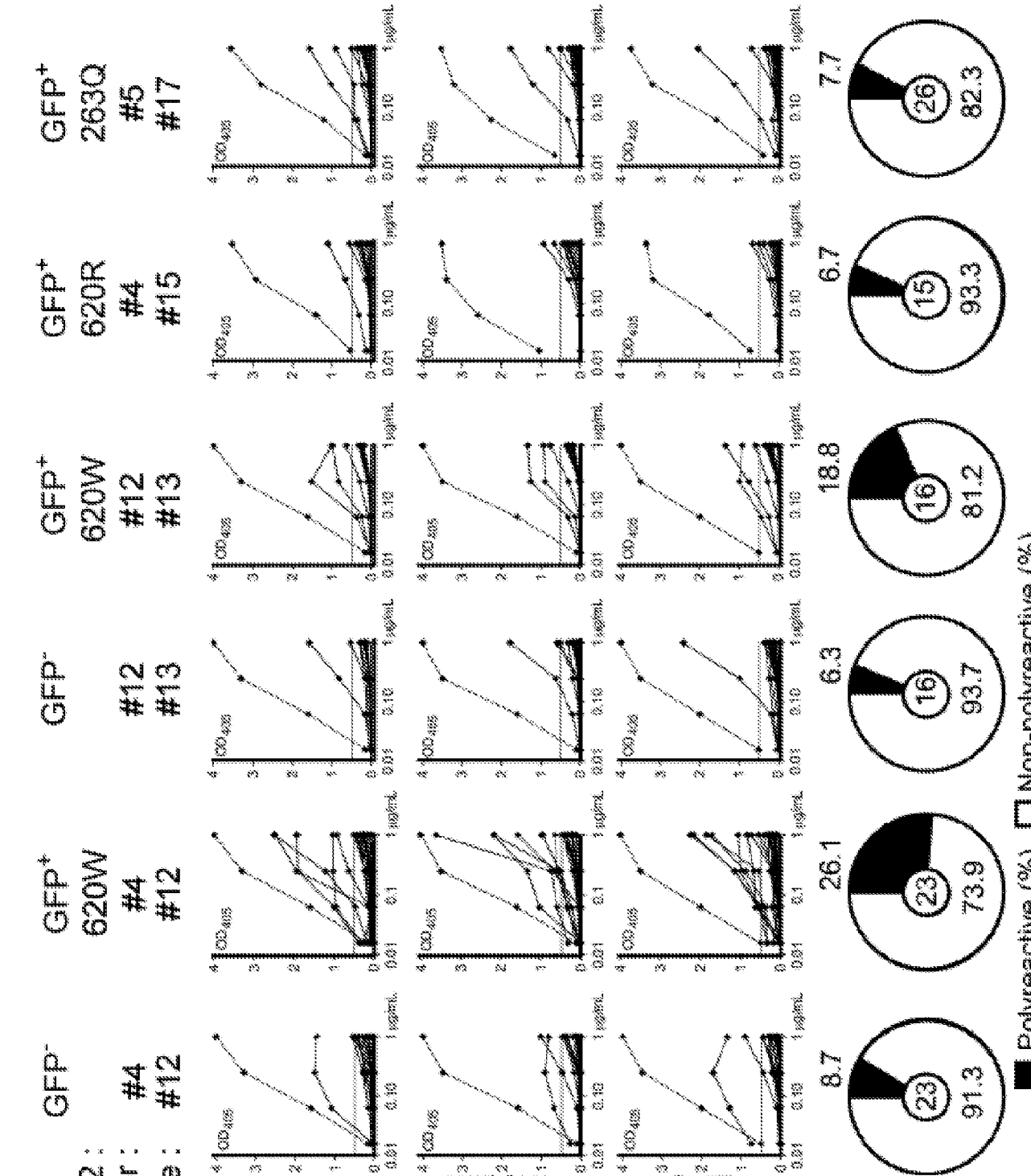

A
PTPN22 :
Donor :
Mouse :
GFP-
#4
#12
GFP+
620W
#4
#12
GFP-
#12
#13
GFP+
620W
#12
#13
GFP+
620R
#4
#15
GFP+
263Q
#5
#17
Polyreactivity
dsDNA
Insulin
LPS
OD405
ug/mL
8.7
91.3
26.1
73.9
6.3
93.7
18.8
81.2
6.7
93.3
7.7
82.3
Polyreactive (%)
Non-polyreactive (%)

Figure 6A

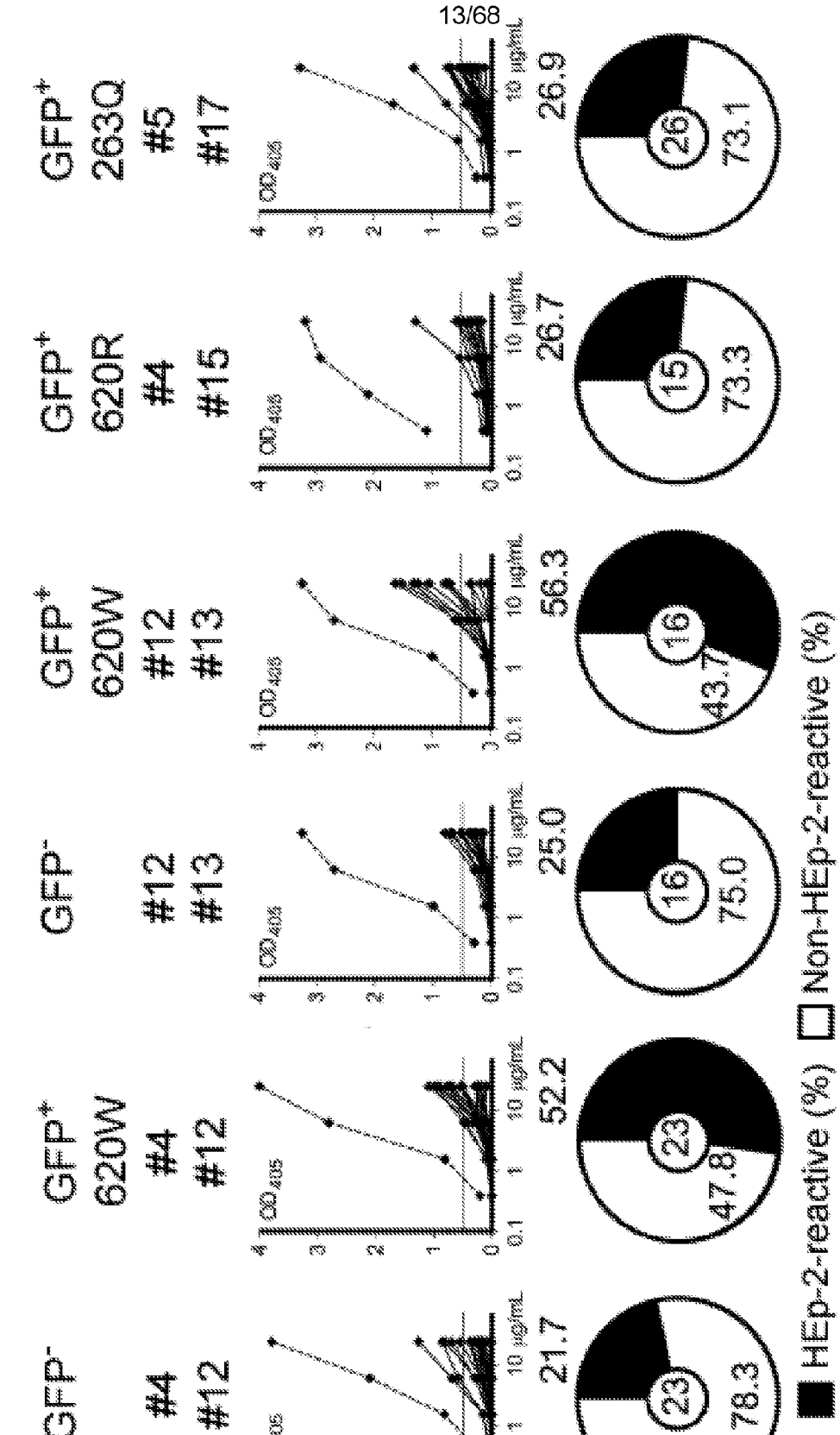
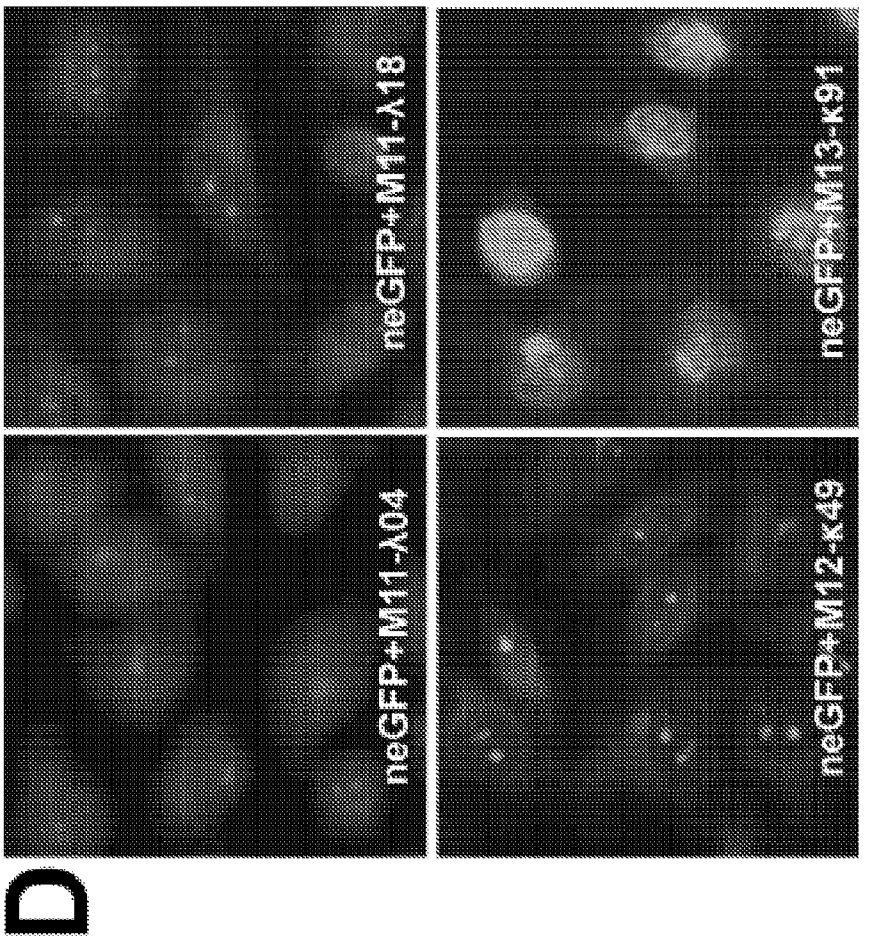
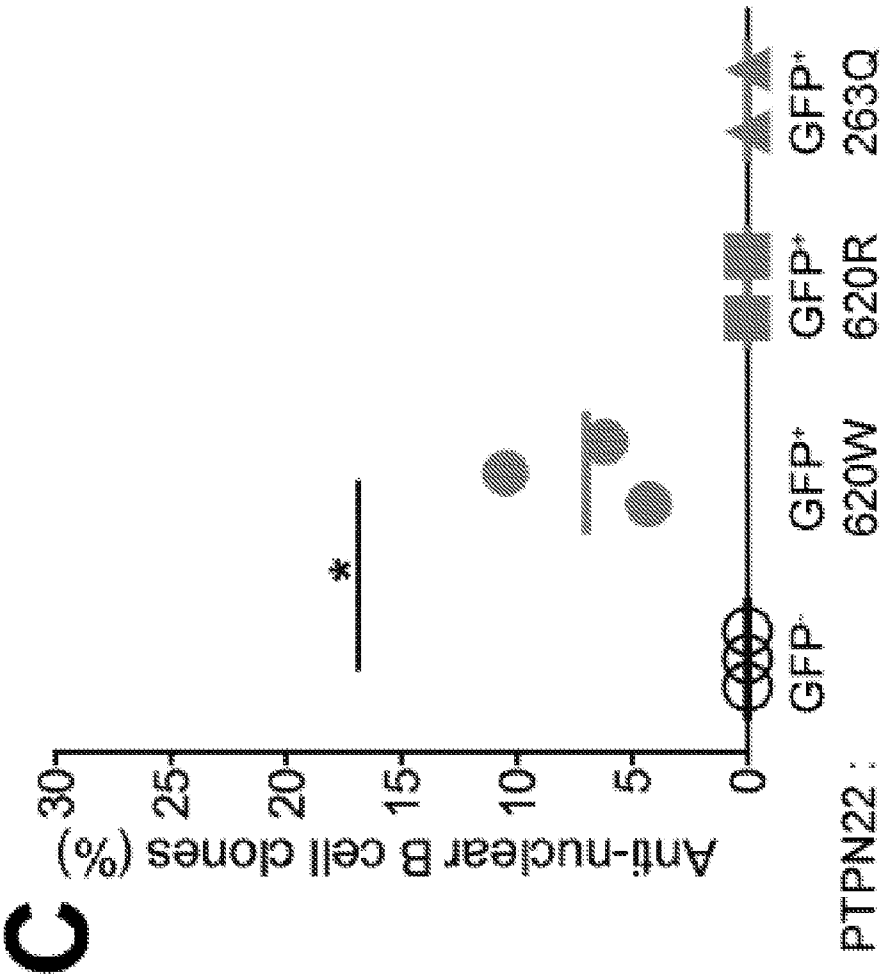

B
PTPN22 :
Donor :
Mouse :
GFP-
GFP+
620W
620R
263Q
#4
#12
#5
#15
#17
HEp-2
OD405
0.1
1
10 µg/mL
21.7
78.3
52.2
47.8
25.0
75.0
56.3
43.7
26.7
73.3
26.9
73.1
23
16
15
26
HEp-2-reactive (%)
Non-HEp-2-reactive (%)

Figure 6B





Figures 6C – 6D

A
PTPN22 C/T or T/T
NSG mice
i.p. injection 0.75 mg or 0.15 mg
LTV-1 PTPN22 inhibitor twice daily
for a week
Single cell sort of splenic
new emigrant B cells

Figure 7A

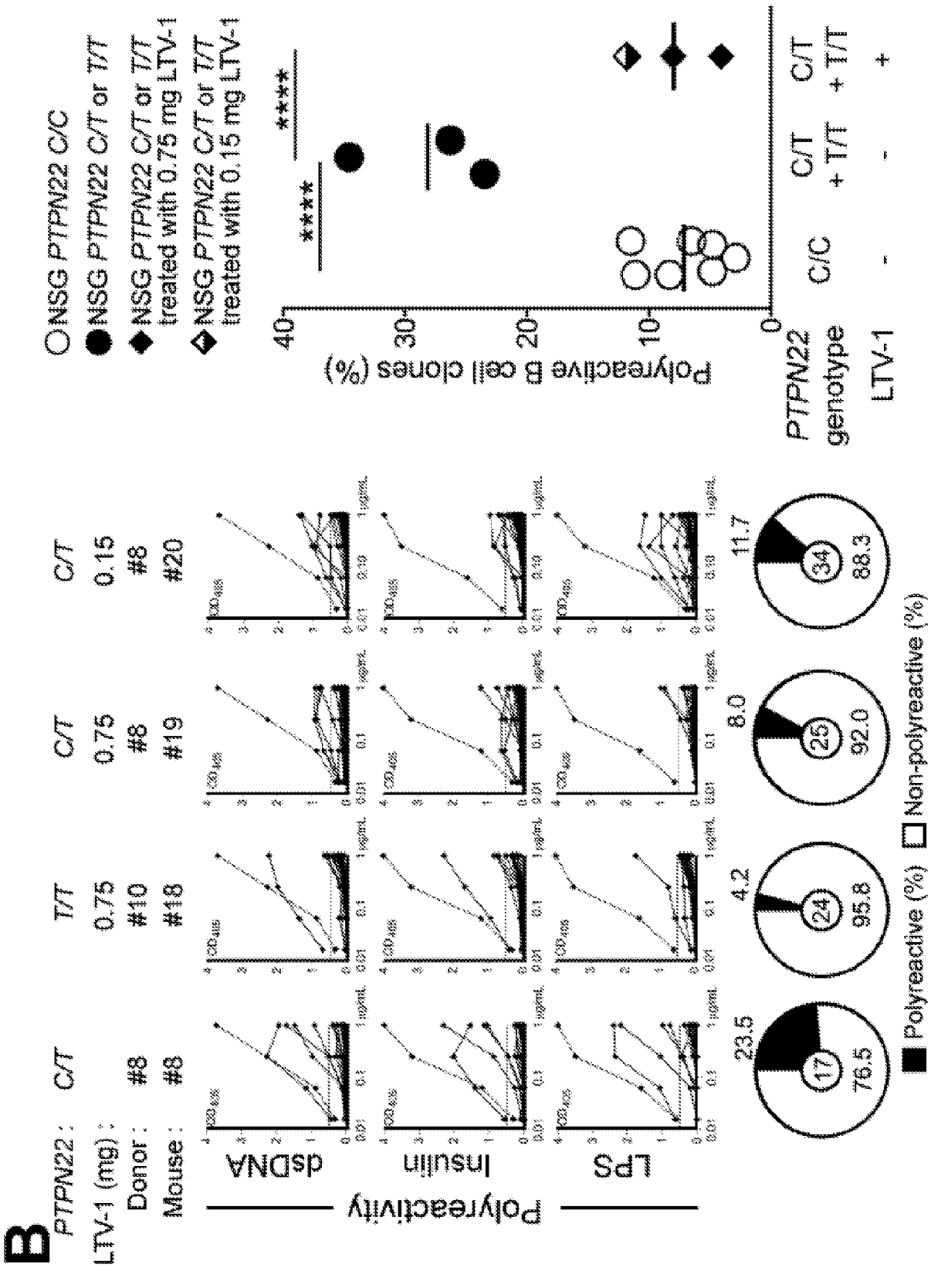

B
PTPN22 : C/T T/T C/T C/T
LTV-1 (mg) : 0.75 0.75 0.15
Donor : #8 #10 #8 #8
Mouse : #8 #18 #19 #20
Polyreactivity
dsDNA
Insulin
LPS
Polyreactive (%)
Non-polyreactive (%)
23.5
76.5
4.2
95.8
8.0
92.0
11.7
88.3
NSG PTPN22 C/C
NSG PTPN22 C/T or T/T
NSG PTPN22 C/T or T/T treated with 0.75 mg LTV-1
NSG PTPN22 C/T or T/T treated with 0.15 mg LTV-1
Polyreactive B cell clones (%)
****
****
PTPN22 genotype
C/C
C/T + T/T
C/T + T/T
LTV-1
-
-
+

Figure 7B

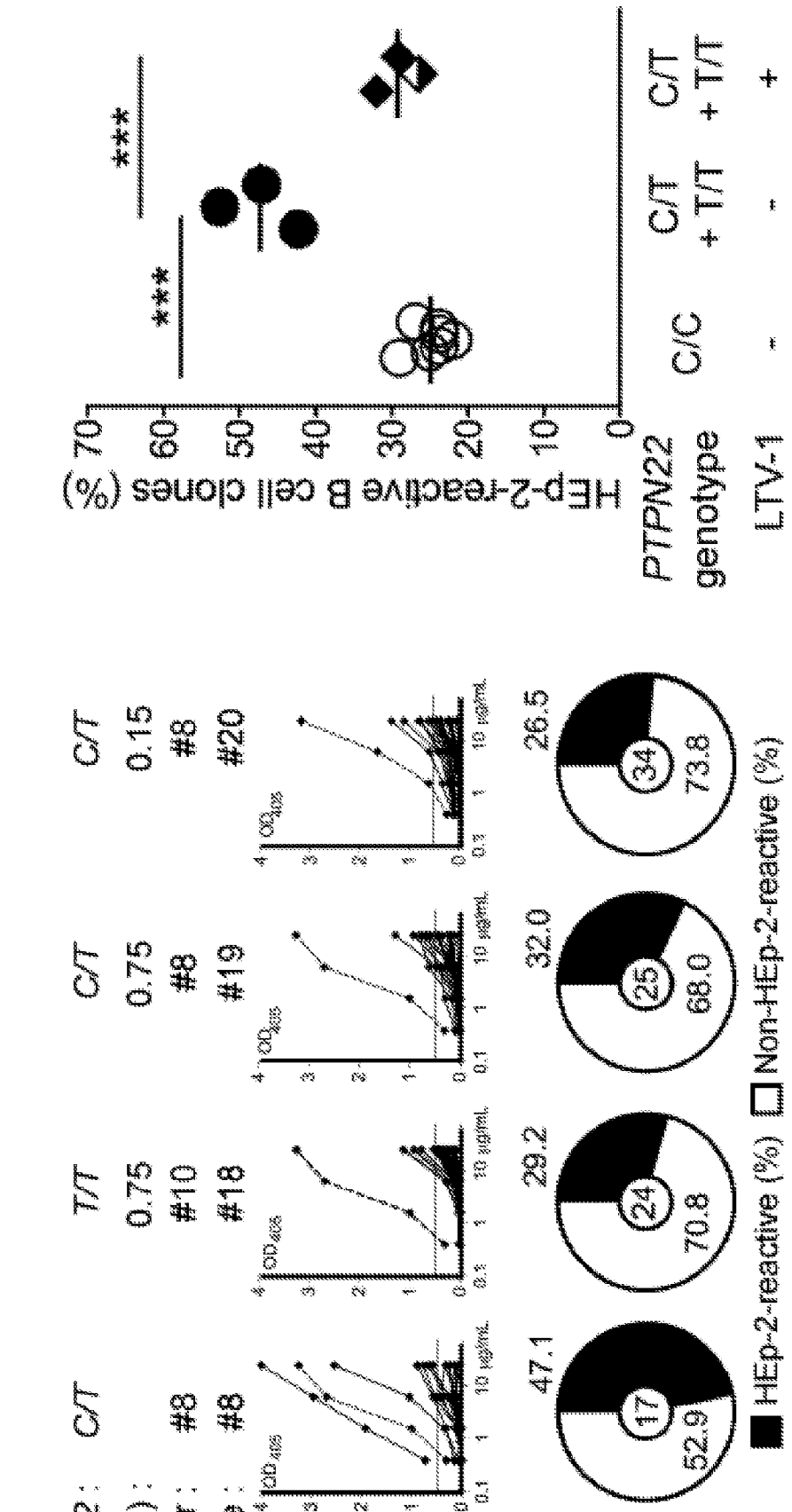

C
PTPN22 : C/T T/T C/T C/T
LTV-1 (mg) : 0.75 0.75 0.15
Donor : #8 #10 #8 #8
Mouse : #8 #18 #19 #20
HEp-2
OD405
0.1 1 10 µg/mL
47.1
52.9
17
29.2
70.8
24
32.0
68.0
25
26.5
73.8
34
HEp-2-reactive (%)
Non-HEp-2-reactive (%)
HEp-2-reactive B cell clones (%)
70 60 50 40 30 20 10 0
***
***
PTPN22 genotype C/C C/T + T/T C/T + T/T
LTV-1 - - +

Figure 7C

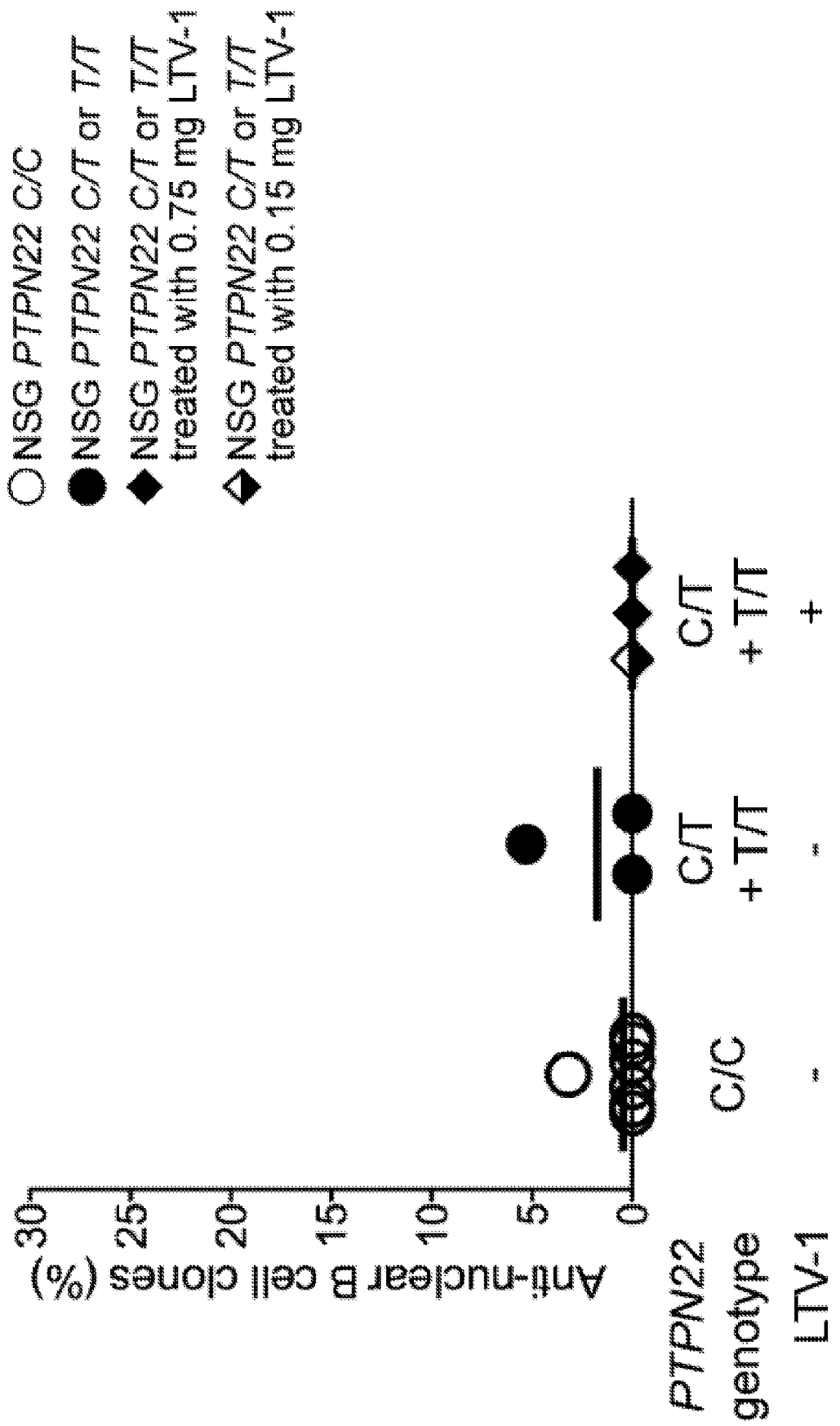

NSG PTPN22 C/C
NSG PTPN22 C/T or T/T
NSG PTPN22 C/T or T/T treated with 0.75 mg LTV-1
NSG PTPN22 C/T or T/T treated with 0.15 mg LTV-1
Anti-nuclear B cell clones (%)
30
25
20
15
10
5
0
PTPN22 genotype
C/C
C/T + T/T
C/T + T/T
LTV-1
-
+
+

Figure 8

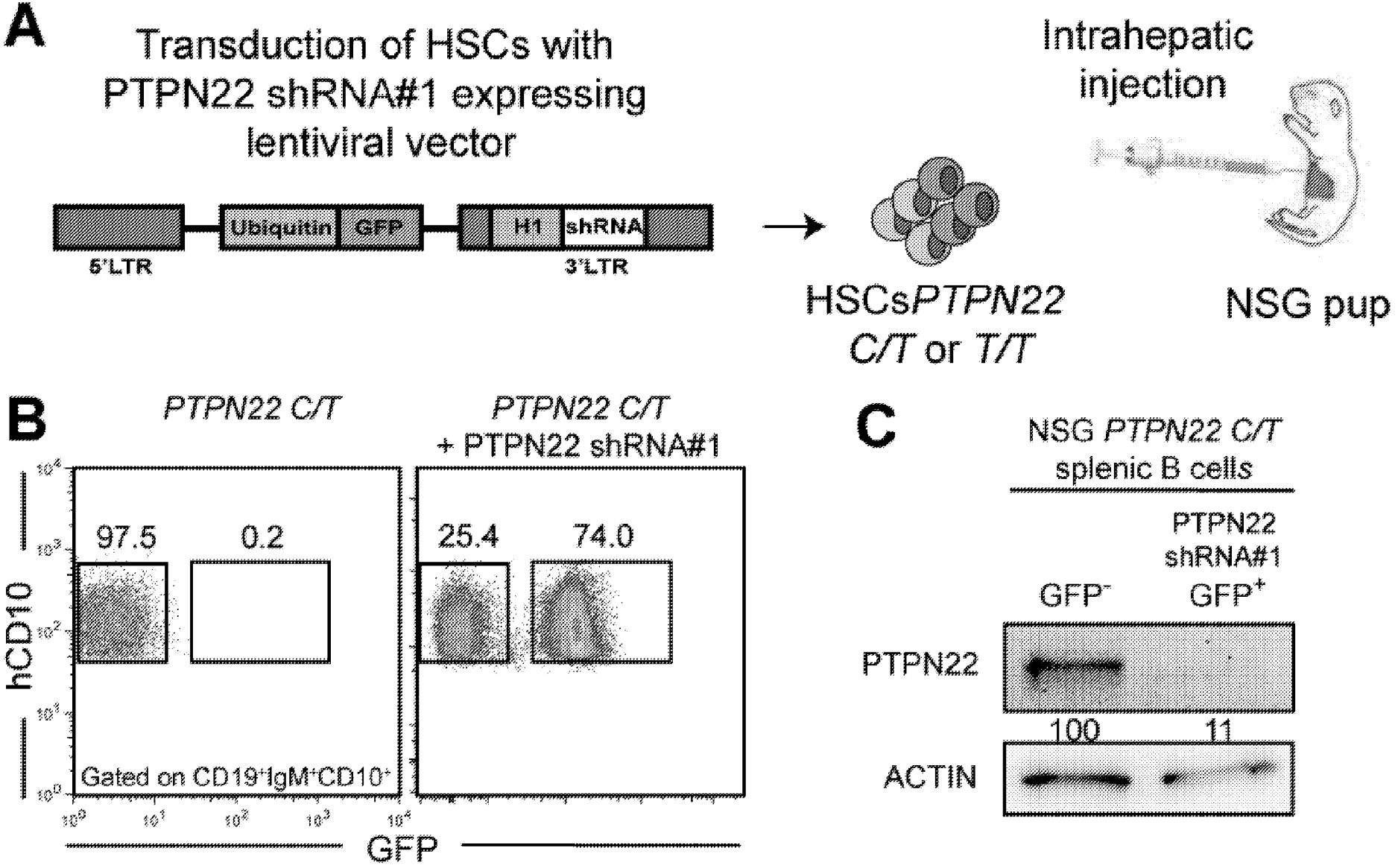
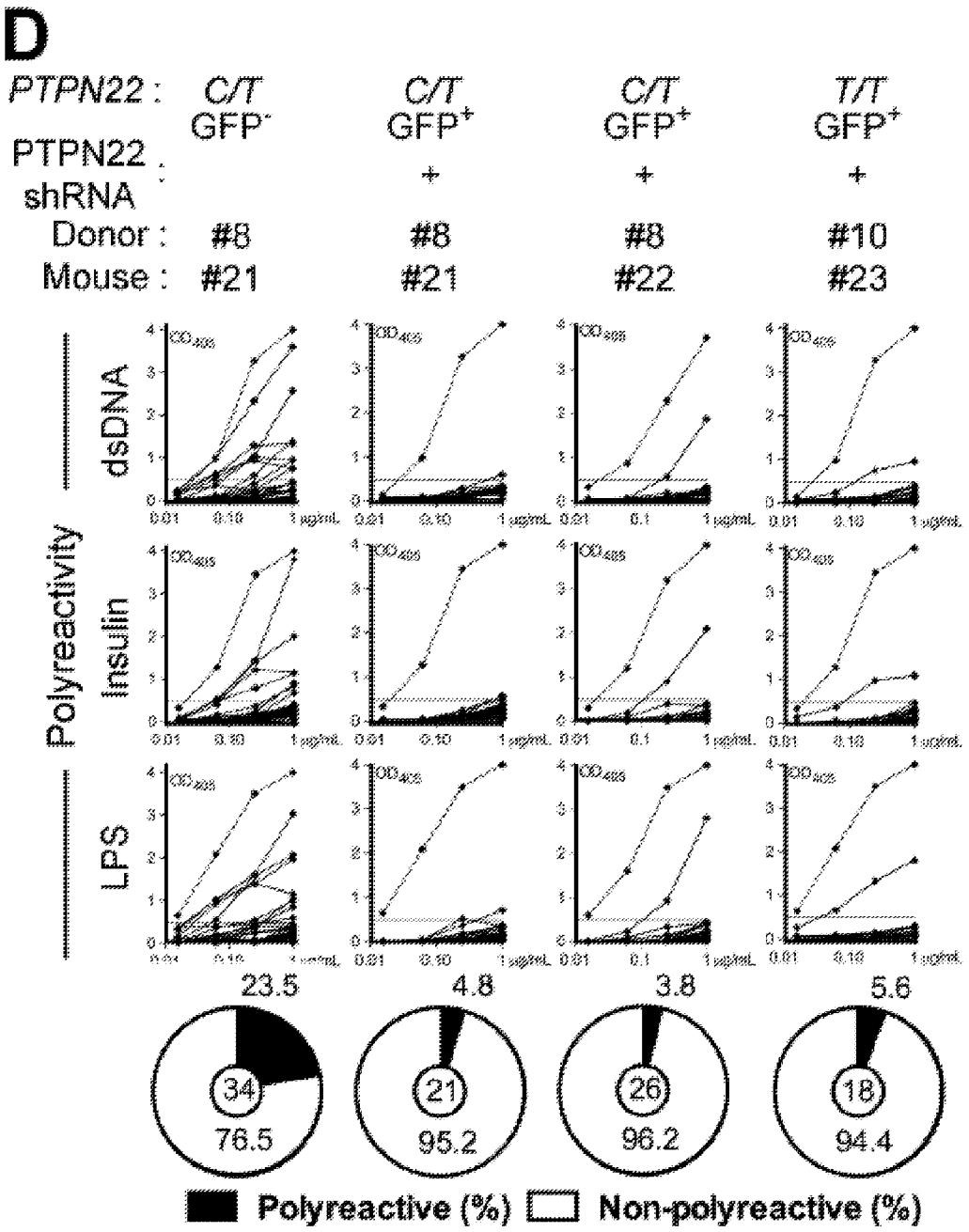

A
Transduction of HSCs with PTPN22 shRNA#1 expressing lentiviral vector
5'LTR
Ubiquitin
GFP
H1
shRNA
3'LTR
HSCsPTPN22 C/T or T/T
Intrahepatic injection
NSG pup
B
PTPN22 C/T
PTPN22 C/T + PTPN22 shRNA#1
97.5
0.2
25.4
74.0
hCD10
Gated on CD19+IgM+CD10+
GFP
C
NSG PTPN22 C/T splenic B cells
PTPN22 shRNA#1
GFP-
GFP+
PTPN22
100
11
ACTIN

Figure 9A – 9C


D
PTPN22 : C/T GFP- C/T GFP+ C/T GFP+ T/T GFP+
PTPN22 shRNA : + + +
Donor : #8 #8 #8 #10
Mouse : #21 #21 #22 #23
Polyreactivity
dsDNA
Insulin
LPS
23.5
4.8
3.8
5.6
34
76.5
21
95.2
26
96.2
18
94.4
Polyreactive (%)
Non-polyreactive (%)

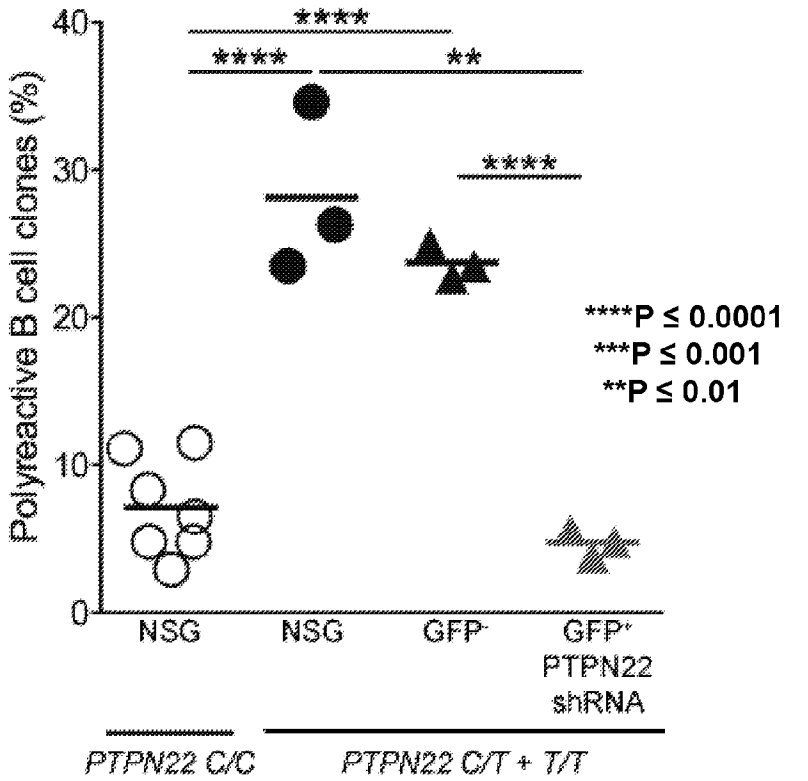

Polyreactive B cell clones (%)
****
****
**
****
****P ≤ 0.0001
***P ≤ 0.001
**P ≤ 0.01
NSG
NSG
GFP-
GFP+ PTPN22 shRNA
PTPN22 C/C
PTPN22 C/T + T/T

Figure 9D

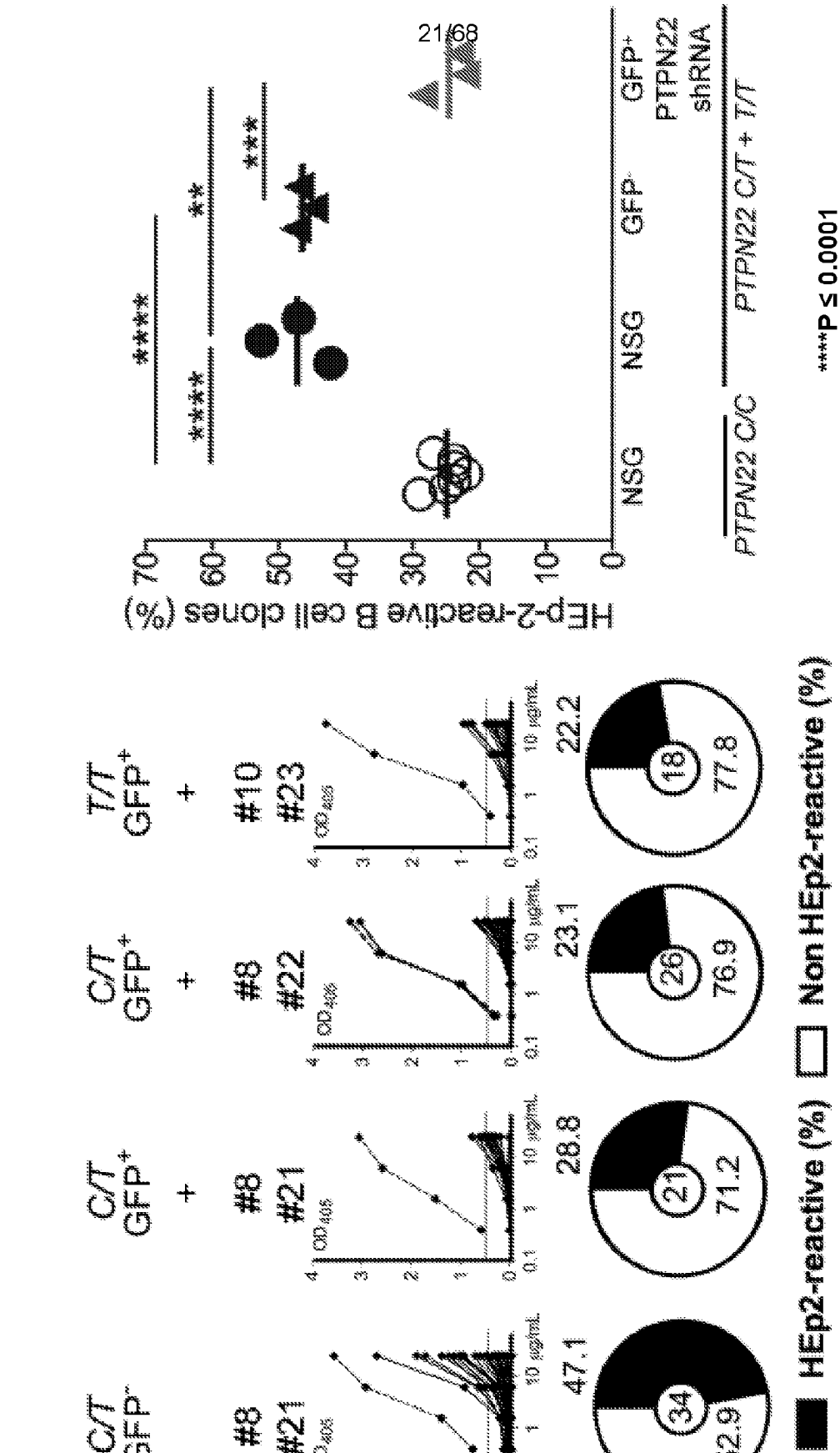

E
PTPN22 : C/T C/T C/T T/T
PTPN22 shRNA : - + + +
Donor : #8 #8 #8 #10
Mouse : #21 #21 #22 #23
GFP- GFP+ GFP+ GFP+
HEp-2
OD405
0.1 1 10 µg/mL
52.9 34 47.1
71.2 21 28.8
76.9 26 23.1
77.8 18 22.2
HEp2-reactive (%)
Non HEp2-reactive (%)
HEp-2-reactive B cell clones (%)
0 10 20 30 40 50 60 70
NSG NSG GFP- GFP+ PTPN22 shRNA
PTPN22 C/C
PTPN22 C/T + T/T
****
****
**
***
****P ≤ 0.0001
***P ≤ 0.001
**P ≤ 0.01

Figure 9E

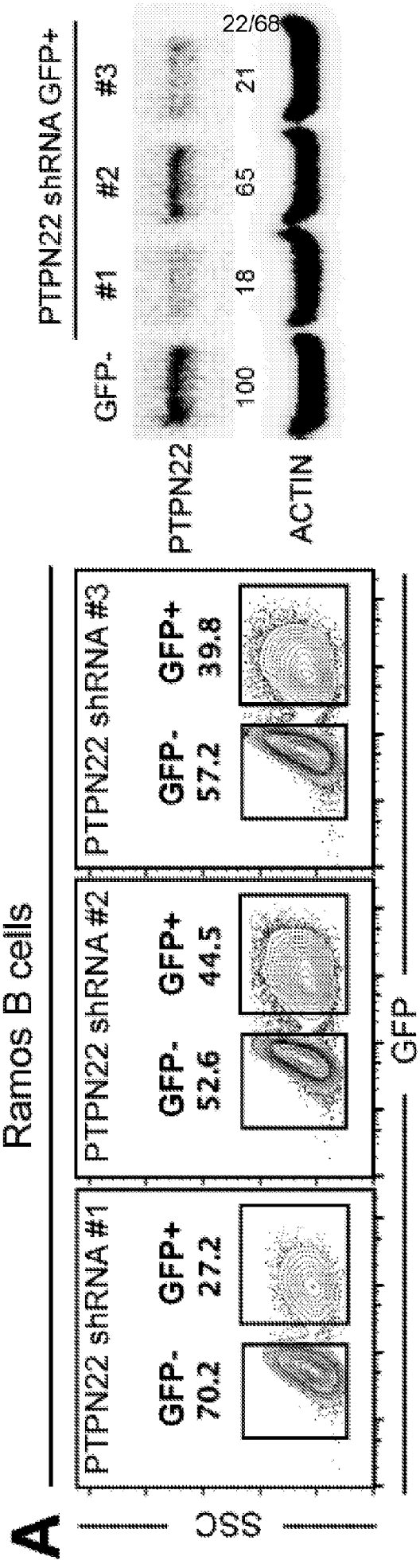

A
Ramos B cells
SSC
GFP
PTPN22 shRNA #1
GFP- 70.2
GFP+ 27.2
PTPN22 shRNA #2
GFP- 52.6
GFP+ 44.5
PTPN22 shRNA #3
GFP- 57.2
GFP+ 39.8
PTPN22 shRNA GFP+
GFP-
#1
#2
#3
PTPN22
ACTIN
100
18
65
21
22/68

Figure 10A

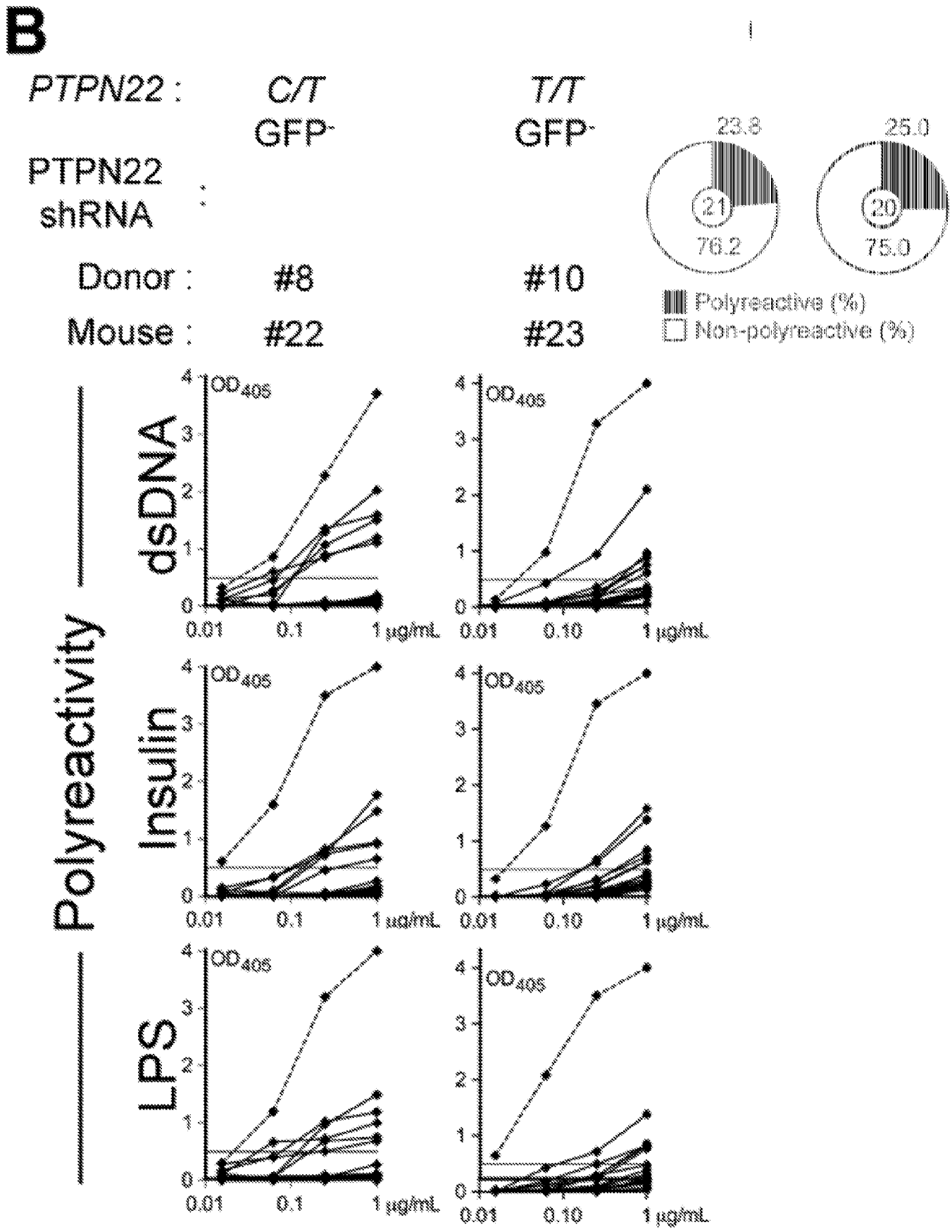
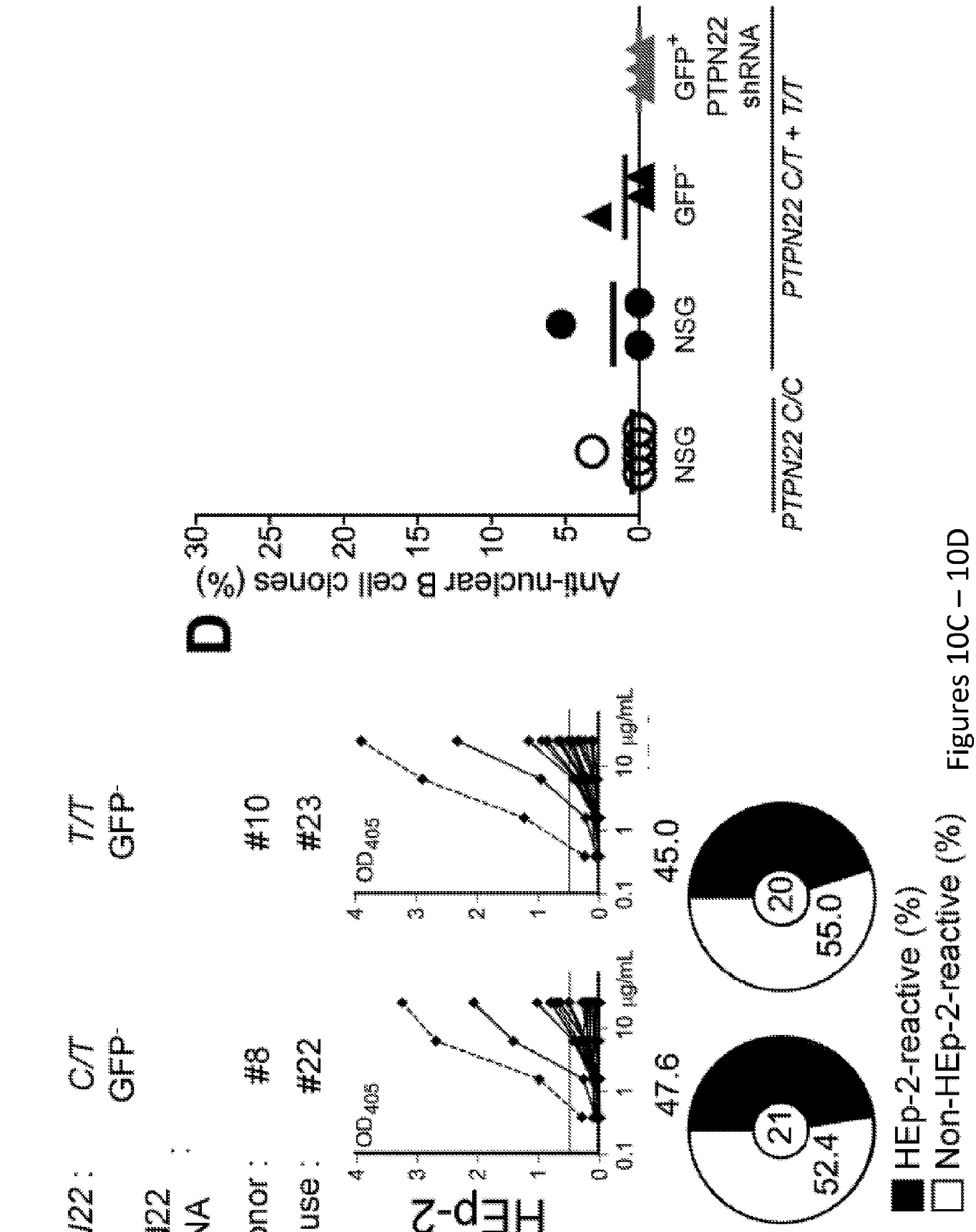

B
PTPN22 :
C/T
T/T
GFP-
GFP-
PTPN22 shRNA
Donor :
#8
#10
Mouse :
#22
#23
23.8
21
76.2
25.0
20
75.0
Polyreactive (%)
Non-polyreactive (%)
Polyreactivity
dsDNA
Insulin
LPS
OD405
0.01
0.1
0.10
1 µg/mL

Figure 10B



Figures 10C – 10D

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #1

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM1-K2	1-3	1-7	2	4	DLELYYFDY	4	9	3-20	1	QQYGSSPT	43	8	-	-	-
neM1-K5	4-30-2	3-10	2	4	ISGSYYNY	5	8	1-39	1	QQSYSTPWT	44	9	-	+	-
neM1-K8	4-59	3-9	2	4	SPPFDWIYYFDY	6	12	3-20	1	QQYGSSPWT	45	9	-	-	-
neM1-K9	3-73	4-23	2	4	RYGGNYYFDY	7	10	3-11	4	QQRSNWPLT	46	9	-	+	-
neM1-K1	3-15	1-26	1	3	QTEWELDAFDI	8	11	3-20	4	QQYGSSPPLT	47	10	-	-	-
neM1-K11	3-74	3-10	2	6	DPGKGYYGSGSYYYYYGMDV	9	20	1-6	2	LQDYNYPYT	48	9	-	-	-
neM1-K16	4-30-2	/	/	3	TNEPNAFDI	10	9	2-28	4	MQALQTPLT	49	9	-	-	-
neM1-K22	4-4	3-22	2	3	DRADYYDSSGYYYAFDI	11	17	1-17	4	LQHNSYPPT	50	9	-	-	-
neM1-K23	3-7	1-26	2	6	NRPPGAINYYGMDV	12	14	3-11	1	QQRSNWPWT	51	9	-	-	-
neM1-K25	4-34	1-26	3	4	GGGATEY	13	7	1-17	1	LQHNSYLWT	52	9	-	-	-
neM1-K32	4-59	/	/	3	THPQSDAFDI	14	10	2-30	4	MQGTHWPLT	53	9	-	+	-
neM1-K33#	3-7	3-10	3	3	ILGGITMVRGAEDAFDI	15	17	3-20	1	QQYGSSPRT	54	9			
neM1-K37	3-30	3-10	1	4	EKGWFGELEGLAIDY	16	15	3-15	1	QQYNNWPQT	55	9	-	-	-
neM1-K38	4-34	7-27	2	2	GLTGDSGTDWYFDL	17	14	1-39	4	QQSYSTPLT	56	9	-	-	-
neM1-K4	4-39	6-13	2	4	HPYSSSFDY	18	9	3-20	2	QQYGSSYT	57	8	-	-	-
neM1-K42	3-33	3-10	2	6	DPRLGSGSYYYYGMDV	19	16	1-39	3	QQSYSTPFT	58	9	-	+	-
neM1-K43#	4-30-4	7-27	2	4	NKLNWGLDY	20	9	1-5	1	QQYNSYSPWT	59	10			
neM1-K45	4-4	7-27	2	5	GWGFGNWFDP	21	10	3-20	2	QQYGSSPPYT	60	10	-	-	-
neM1-K46	4-59	/	/	4	SFSRLASFDY	22	10	3-20	1	QQYGSSLGT	61	9	-	-	-
neM1-K48	4-59	/	/	4	VGGRGGDFDY	23	10	1-33	3	QQYDNLPRVT	62	10	-	-	-

RF, reading frame: # antibody failed to be expressed

- non reactive; +, reactive

c, diffuse cytoplasmic staining; N, nuclear staining, F, cytoplasmic fibers; V, vesicles

Figure 11

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM1-L1	3-30	6-13	2	4	SGSSWYYFDY	24	10	2-14	2	SSYTSSSTV	63	9	-	-	-
neM1-L3	1-69	/	/	4	VMAVYYFDY	25	9	1-40	1	QSYDSSLSGYV	64	11	+	+	-
neM1-L6	3-30-3	3-16	2	4	GPSRGGEVFDY	26	11	3-21	3	QVWDSSSDQNWV	65	12	-	-	-
neM1-L7	5-51	3-22	2	4	LRIPDYDSSGYYFDY	27	15	2-8	3	SSYAGSNNLV	66	10	-	-	-
neM1-L12	4-4	/	/	4	GSIPSDYYFDY	28	11	3-25	2	QSADSSGTYPV	67	11	-	-	-
neM1-L13#	1-69	6-13	2	5	TIYSSSWYWFDP	29	12	5-37	2	MIWPSNAYVV	68	10			
neM1-L14	3-30	3-22	2	2	DYYDSSGYYYWYFDL	30	15	1-44	2	AAWDDSLNGPV	69	11	-	+	-
neM1-L24	3-13	6-13	2	3	GGRDSSWYIAFDI	31	13	2-23	1	CSYAGSSTYV	70	10	-	+	-
neM1-L26	1-3	7-27	2	5	GQTTNLGMGPNWFDP	32	15	1-40	2	QSYDSSLSGVV	71	11	-	-	-
neM1-L27	3-23	2-15	2	3	DPLSRYCSGGSCYSGAFDI	33	19	1-44	2	AAWDDSLNGHVV	72	12	-	-	-
neM1-L28	1-18	6-19	3	4	GIAVAGYFDY	34	10	2-8	2	SSYAGSNNLV	66	10	-	-	-
neM1-L3	3-7	/	/	4	DRLGSFDY	35	8	1-47	3	AAWDDSLSGWV	73	11			
neM1-L31	3-33	3-9	2	4	DAALRYFDWLLDY	36	13	2-14	1	SSYTSSSTLV	74	10	-	-	-
neM1-L34	3-7	/	/	4	DLVGIRATDY	37	10	1-51	1	GTWDSSLSAYV	75	11	-	+	-
neM1-L35	3-30	6-13	3	1	DVNAAAGNRAYFQH	38	14	3-21	1	QVWDSSSDHYV	76	11	-	-	-
neM1-L36	3-30	1-26	2	4	DGDSGSYFDY	39	10	2-23	2	CSYAGSSTYVV	70	11	-	-	-
neM1-L39	3-30-3	7-27	1	3	VCSPELGQWIDI	40	12	3-21	2	QVWDSSSDHVV	77	11	-	+	-
neM1-L41	3-15	3-16	2	3	DWGTRAFDI	41	9	1-51	3	GTWDSSLSAGV	78	11	-	-	-
neM1-H4	3-64	6-19	3	4	GIVAVAGNLDY	42	11				--				

RF, reading frame: # antibody failed to be expressed
- non reactive; +, reactive
c, diffuse cytoplasmic staining; N, nuclear staining, F, cytoplasmic fibers; V, vesicles

Figure 11 (cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #2

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM2- K04#	3-33	3-16	1	3	PRPRGEPLLNWSWAFDI	79	17	1-5	1	QHYNPYSRT	115	9			
neM2- K08	3-33	1-26	3	4	AEEGVGGSYFDY	80	12	2-24	1	MQATQFPWT	116	9	-	-	-
neM2- K10	4-31	3-22	2	3	NPAREYYYDSSGYYGYAFDI	81	20	4-1	2	QQYYSTPFMYT	117	11	-	-	-
neM2- K12	3-11	2-2	3	4	ARERGVVVPAAIVGDY	82	16	4-1	2	QQYYSAPQT	118	9	-	-	-
neM2- K13	4-34	2-8	2	4	GQQDNAPHTPLYY	83	13	2-28	2	MQALQTPT	119	8	-	-	-
neM2- K14	3-9	6-6	2	3	GTHTSSSGAFDI	84	12	4-1	4	QQYYSTPLT	120	9	-	-	-
neM2- K15	3-7	2-2	3	3	VSHIVVVPAAIRGGDAFDI	85	19	4-1	3	QQYYSTPCT	121	9	+	+	-
neM2- K16	3-15	1-26	3	4	DPVGACVFDY	86	10	3-20	4	QQYGSSPLT	122	9	-	-	-
neM2- K18	3-23	3-22	2	3	TVDYYDSSGYYYSAFDI	87	17	3-20	3	QQYGSSPPT	123	9	-	-	-
neM2- K24	3-21	2-8	2	4	GGDDSRSPNGGYCLDY	88	16	2-29	2	MQGIHLRYT	124	9	-	-	-
neM2- K25	3-11	1-26	3	4	EGPGIVGVHHPYFDY	89	15	1-39	1	QQSYSTPWT	44	9	-	-	-
neM2- K26	1-2	3-3	3	5	GSIGVGGSLYGNWFDP	90	16	1-16	4	QQYNSYPLT	125	9	-	-	-
neM2- K27	4-39	7-27	3	4	RGLGIVHYFDY	91	11	3-11	4	QQRINWLT	126	8	-	-	-
neM2- K29	1-2	6-13	3	3	LWGGIKPGIAAAGTAFDI	92	18	3-20	1	QQYGSSPRT	54	9	-	+	-
neM2- K30	1-46	4-23	2	4	ARLAEHYQRYGGNSGPFDY	93	19	3-20	1	QQYGSSPGT	127	9	-	+	N
neM2- K35#	4-34	3-10	3	5	RGKLTMDRGNWFDP	94	14	2-28	2	MQALQTPT	119	8			
neM2- K38	3-49	3-10	2	4	DHLIGSGSYFDY	95	12	3-20	2	QQYGSSPQT	128	9	-	+	-
neM2- K41	3-49	6-13	3	4	DPLEGKSIAAAGPWDFDY	96	18	2-30	2	MQGTHWPPYT	129	10	-	+	-

RF, reading frame: # antibody failed to be expressed
- non reactive; +, reactive
c, diffuse cytoplasmic staining; N, nuclear staining, F, cytoplasmic fibers; V, vesicles

Figure 12

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM2- K48#						--		1-39	2	QQSYSTPYT	130	9			
neM2- L01	3-48	6-19	3	4	GIAVAGESVDY	97	11	1-44	3	AWDDSLNGPV	131	10	-	-	-
neM2- L2	1-2	1-26	2	4	LRDSGSSYFDY	98	11	2-18	2	SSYTSSSNVV	132	10	-	-	-
neM2- L04	3-33	3-16	1	3	PRPRGEPLLNWSWAFDI	79	17	2-8	2	SSYAGSNNLV	66	10	-	-	-
neM2- L05#	3-53	6-13	2	4	AGYSSSWSAFDY	99	12	1-51	2	TWDSSLSAVV	133	10			
neM2- L07	1-46	6-13	3	6	DKAAAGTDYYYGMDV	100	15	6-57	2	QSYDSSNHVV	134	10	-	-	-
neM2- L09	3-9	6-13	2	5	DRAGYSSSDWFDP	101	13	1-40	2	QSYDSSLSGSV	135	11	-	-	-
neM2- L17	3-33	6-13	3	4	DTAAAGLQSRYYFDY	102	15	2-11	1	CSYAGSYTYV	136	10	-	-	-
neM2- L19	3-30	4-17	2	4	DPCSDYGDSYFDY	103	13	1-40	2	YDSSLSGSVV	137	10	+	+	-
neM2- L22	3-9	6-13	3	4	DMAAAGPYYFDY	104	12	2-14	2	SSYTSSSTFGV	138	11	-	-	-
neM2- L23	3-15	6-19	3	4	VHARIAVAARNDY	105	13	2-14	2	SSFTSITYVV	139	10	-	-	-
neM2- L28	4-59	5-24	2	1	DGGDGYKYGYFQH	106	13	1-44	3	AWDDSLNGVV	140	10	-	-	-
neM2- L31	3-23	1-26	3	4	VWGATTMGGHYFDY	107	14	2-14	2	SSYTSSSTFVV	141	11	-	-	-
neM2- L33	4-34	3-10	3	5	RGKLTMDRGNWFDP	94	14	2-23	1	AGSSTFHYV	142	9	-	+	-
neM2- L40	3-7	6-19	3	4	DVRIAVAGFDY	108	11	3-1	2	QAWDSSTVV	143	9	-	+	-
neM2- L42	3-11	7-27	1	2	RQLGLSIEYWYFDL	109	14	9-49	3	GADHGSGSNFVWV	144	13	-	+	-
neM2- L44	3-9	6-19	2	5	ALGRYSSGWTGWFDP	110	15	1-51	3	GTWDSSLSAPWV	145	12	-	-	-
neM2- L47#	3-23	1-26	2	2	GGGSPWGWYFDL	111	12	2-8	1	SSYAGSNNFYV	146	11			
neM2- L32#						--		2-8	1	SSYAGSNNYV	--	10			
neM2- H37	1-69	2-2	2	4	ASHHRNTYCSSTSCFDY	112	17				--				
neM2- H46	3-23	1-26	3	4	VWGATTMGGHYFDY	107	14				--				
neM2- H06	4-34	6-19	2	4	GGMVDSSGWYYFDY	113	14				--				
neM2- H20	3-33	3-22	2	4	DISLYYYDSSGYSAGVFDY	114	19				--				

RF, reading frame: # antibody failed to be expressed

- non reactive; +, reactive

c, diffuse cytoplasmic staining; N, nuclear staining, F, cytoplasmic fibers; V, vesicles

Figure 12 (cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #3

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM3 K02	4-39	/	/	2	PRGRYWYFDL	147	10	1-33	2	QQHDNLPYT	180	9	-	-	-
neM3 K03	4-34	6-19	2	4	GLGVSGWFDY	148	10	3-20	3	QQYGSSPFT	181	9	-	-	-
neM3 K08	5-a	/	/	6	HQARPYYYYGMDV	149	13	3-20	2	QQYGSSPKT	182	9	-	-	-
neM3 K09	4-59	7-27	3	3	VSGDAFDI	150	8	1-9	4	QQLNSYPLT	183	9	-	-	-
neM3 K11	1-69	7-27	2	5	ANWGSYNWFDP	151	11	3-11	4	QQRSNWPT	184	8	-	-	-
neM3 K15	4-59	7-27	2	2	DLSWGPYWYFDL	152	12	3-15	3	QQYNNWPFT	185	9	-	-	-
neM3 K16	4-39	6-13	2	3	QPGQYSSSWYAFDI	153	14	1-33	3	QQYDNLPIFT	186	10	-	-	-
neM3 K18	1-18	1-7	3	4	DRWAGITGTTPFDY	154	14	3-15	3	QQYNNWPPFT	187	10	-	-	-
neM3 K21	3-23	5-12	2	4	YSGYDFTYYFDY	155	12	1-8	3	QQYYSFPFT	188	9	-	+	-
neM3 K22	4-61	6-19	2	4	RDSAYSSGFDY	156	11	2-28	4	MQALQTPLT	49	9	-	-	-
neM3 K27	4-39	/	/	4	AVFIDY	157	6	3-15	2	QQYNNWPYT	189	9	+	+	-
neM3 K30	3-23	7-27	1	4	DPPELGMGESGFDY	158	14	1-5	1	QQYNSYSRT	190	9	-	-	-
neM3 K32	4-61	4-4	2	4	DLGSNYDY	159	8	3-10	1	QQYGSSPQT	128	9	-	-	-
neM3 K33	3-23	6-13	2	4	DPRSSWYFDY	160	10	1-39	3	QQSYSTPFT	58	9	-	-	-
neM3 K34	3-30	6-13	3	2	DRIAAAGTWYFDL	161	13	1-33	4	QQYDNLPPLT	191	10	-	-	-
neM3 K37	3-33	/	/	4	GAGYYFDY	162	8	3-11	1	QQRSNWRGT	192	9	-	+	-
neM3 K38	3-30	7-27	2	4	DSSAHEPSWGYFDY	163	14	3-15	4	QQYNNWPLT	193	9	-	-	-
neM3 K40	1-3	6-13	2	4	DLGADSSSWTFDY	164	13	3-15	2	QQYNNWPMYT	194	10	-	+	-
neM3 K41	3-30	/	/	5	GHPKGNWFDP	165	10	3-20	2	QQYGSSRYT	195	9	-	+	-
neM3 K42#	4-59	3-10	1	4	APRFGELYFDY	166	11	3-15	1	QQYNNWWT	196	8			
neM3 K46#	1-69	1-26	3	4	AVGATVDFDY	167	10	3-11	4	QQRSNWLT	197	8			

Figure 13

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM3 L4#	4-59	5-5	2	4	QPGYSYGFDY	168	10	2-11	2	CSYAGSYVV	198	9			
neM3 L12#	3-11	1-26	1	5	DGWELYHWFDP	169	11	2-23	2	CSYAGRV	199	7			
neM3 L17#	4-59	3-22	2	4	GRDTTKASYYDSSGYYY	170	17	3-21	1	QVWDSSSDHYV	76	11			
neM3 L19	1-24	1-14	2	3	VLYPGNAFDI	171	10	1-47	1	AAWDDSLSGYV	200	11	-	-	-
neM3 L24	4-39	1-26	2	4	NSGSYYFDY	172	9	2-23	1	CSYAGSSTYV	70	10	-	-	-
neM3 L25#	1-69	1-26	2	4	DSSAYSGSYYYFDY	173	14	1-51	2	GTWDSSLSAVV	201	11			
neM3 L31#	5-51	6-13	2	4	RGSSSWGYFDY	174	11	1-51	1	GTWDSSLSV	202	9			
neM3 L7						--		9-49	1	GADHGSGSNFVYV	203	13			
neM3 L10						--		3-21	2	QVWDSSSDHVV	77	11			
neM3 L29						--		3-1	1	QAWDSSTYV	204	9			
neM3 L36						--		3-25	1	QSADSSGTYGV	205	11			
neM3 L43						--		2-23	2	CSYAGSSTLV	206	10			
neM3 K13	4-31	6-13	3	6	DLAGTNYYYGMDV	175	13				--				
neM3 L20	1-69	6-19	2	1	HYSSRAEYFQH	176	11				--				
neM3 L26	5-51	4-4	2	4	RRDYSNYDY	177	9				--				
neM3 L45	5-51	3-10	2	6	ANGSGSYYNYYYGMDV	178	16				--				
neM3 K47	3-33	4-4	2	4	GTAHSNYSGFDY	179	12				--				

RF, reading frame: # antibody failed to be expressed

- non reactive; +, reactive

c, diffuse cytoplasmic staining; N, nuclear staining, F, cytoplasmic fibers; V, vesicles

Figure 13 (cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #4

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM4-K49	4-59	3-10	2	5	QGVTSAYNWFDP	207	12	1-39	2	QQSYSTPRYT	230	10	-	-	-
neM4-K61	1-2	/	/	4	DGPAGVGVGFDY	208	12	1-39	4	RQSYSTPRLT	231	10	-	-	-
neM4-K62	1-2	1-20	3	3	GFSASTGTTTDAFDI	209	15	3-11	1	QQRSNWPPWT	232	10	-	+	-
neM4-K66#	3-49	3-9	1	5	DFQPWGYFDWPLNWFDP	210	17	3-20	1	QQYGSSPRT	54	9			
neM4-K72	3-7	6-13	3	6	VRRGGAAAGIVDWYYYYGMDV	211	21	1-39	4	QQSYSTPLT	56	9	-	-	-
neM4-K74	1-46	6-6	2	4	RRYSSSFDY	212	9	3-20	1	QQYGSSPT	43	8		-	-
neM4-K75	1-2	2-2	3	5	ANIVVVPAAMYNWFDP	213	16	1-9	4	QQLNSYPLT	183	9	-	+	-
neM4-K77	3-30	6-13	3	4	VRGGIAAAGFLAGENGSFDY	214	20	2-28	3	MQALLTQFT	233	9	-	-	-
neM4-K79	3-30	3-9	2	5	DWADYDILTGSQSGPYWFDP	215	20	1-8	1	QQYYSYPQT	234	9	-	-	-
neM4-K81#	1-3	4-11	3	5	NPTTITTIIWFNP	216	13	1D-8	4	QQYYSFPLT	235	9			
neM4-K83	3-21	6-6	3	4	VPGSIAARPNLFDY	217	14	1-17	2	LQHNSYPVT	236	9	+	+	-
neM4-K85	4-61	3-22	2	6	GPGYYYDSSGPYYGMDV	218	17	1-9	4	QQLNSYPPT	237	9	-	-	-
neM4-K88	3-23	2-2	2	2	VGCSSTSCYEDFDL	219	14	1-39	2	QQSYSTPYT	130	9	-	-	-
neM4-K90	3-48	/	/	4	DRNSVDY	220	7	3-15	1	QQYNNWPPWT	238	10	-	-	-
neM4-L52	3-33	6-6	2	4	DPDLRYSSSSSFDY	221	14	2-23	1	CSYAGSSTPNYV	239	13	-	-	-
neM4-L53	3-30	/	/	6	LHAPNLYYMDV	222	11	1-51	3	GTWDSSLSAGV	78	11	-	-	-
neM4-L54	3-30	2-2	2	4	DCLGTWRYCSSTSCSVGADY	223	20	1-44	2	AAWDDSLNGVV	240	11	+	+	-
neM4-L55	3-30	6-13	3	5	DKGSAAADNWFDP	224	13	3-21	1	QVWDSSSDHYV	76	11	-	-	-
neM4-L58	4-59	6-19	1	4	MKRLGIDY	225	9	3-21	1	QVWDSSSDHYV	76	11	-	-	-
neM4-L63	3-30	6-13	3	6	SYEGIAADKNYYYYGMDV	226	19	6-57	2	QSYDSSNQV	241	9	-	-	-
neM4-L67	3-21	2-2	3	5	APRGVPAANWFDP	227	13	2-14	3	SSYTRSSTLV	242	10	-	-	-
neM4-L76#	4-34	2-2	3	3	ACERDIVVVPAVNGAFDI	228	18	2-14	2	SSYTSSSTRV	243	10			
neM4-L78#	3-23	2-15	3	3	DLNKTSLAAAGDRDAFDI	229	18	1-51	2	GTWDSSLSAGV	78	11			
neM4-L89#	3-48	/	/	4	DRNSVDY	220	7	1-51	2	GTWDSSLSAGVV	244	12			

RF, reading frame: # antibody failed to be expressed

- non reactive; +, reactive

c, diffuse cytoplasmic staining; N, nuclear staining, F, cytoplasmic fibers; V, vesicles

Figure 14

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #5

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM5-H08	3-74	/	/	3	EGSLNHDAFDI	245	11	3-15	1	QQYNNWPPWT	238	10	-	-	-
neM5-H12	1-2	6-19	3	4	GSTENRIAVAGRPGVGADY	246	19	3-20	4	QQYGSSPMQS	277	10	-	-	-
neM5-H17	3-33	3-10	2	4	GPSSGVPFAFDY	247	12	1-5	1	QQYNSYSRT	190	9	-	-	-
neM5-H19	4-39	1-26	2	4	RWTGGSYGY	248	9	1-39	3	QQSYSTPFT	58	9	-	-	-
neM5-H21#	5-10	5-5	2	4	LVREERYSYGLTNRDDY	249	17	3-20	2	QQYGSSPPNT	278	10			
neM5-H34	3-33	6-13	2	5	VDNSSSPYNWFDP	250	13	1-39	3	QQSYSTPFT	58	9	-	-	-
neM5-H35	4-61	4-23	3	2	RTVVGKKGDWYFDL	251	14	3-20	4	QQYGSSLT	279	8	-	-	-
neM5-H37	3-15	2-8	2	6	EAYCTGGVCYTGVLGYYYYYMDV	252	23	1-39	1	QQSYSTPTRGMT	281	12	+	+	-
neM5-H38	3-33	1-7	3	4	DTGITGTFDY	253	10	1-17	2	LQHNSYPYT	282	9	-	-	-
neM5-H45	4-59	3-22	2	4	GRAQDSSGPNPLDY	254	14	3-11	4	QQRSNWPLT	46	9	-	-	-
neM5-H46	3-48	3-9	2	4	DLHDYDILTGYYIFDY	255	16	1-39	1	QQSYSTPWT	44	9	-	-	-
neM5-H01	4-34	6-6	2	6	GFKWSGRNSSSSLNYYYYMDV	256	21	3-25	2	QSADSSGTYVV	283	11	-	+	-
neM5-H03	4-34	7-27	1	4	GPRLGTARKPFDY	257	13	9-49	3	GADHGSGSNFVWV	144	13	+	+	-
neM5-H04	3-30	7-27	1	4	LGSDYKPFDY	258	10	1-51	1	GTWDSSLSAYV	75	11	-	-	-
neM5-H05	6-19	/	/	4	VEGLSVVGLPY	259	11	1-47	2	AWDDSLSGVV	284	10	-	-	-
neM5-H06#	3-30-3	3-10	2	6	DRGSGSYLTYYYYYGMDV	260	18	2-14	1	SSYTSSSTYV	285	10			
neM5-H07	3-23	/	/	4	DELPPIDDESERFDY	261	15	1-44	2	AAWDDSLNGVV	240	11	-	+	-
neM5-H09	3-48	5-12	2	4	SKTDVADHLPRYSGYDPGNY	262	20	1-44	3	AAWDDSLNGWV	286	11	-	-	-
neM5-H11	4-34	4-17	2	4	APGDSTRMVDY	263	11	1-44	2	AAWDDSLNGRV	287	11	-	-	-
neM5-H13#	5-51	2-2	2	6	WYANLGYCSSTSCYTAGMDV	264	20	2-11	1	CSYAGSSYV	288	9			
neM5-H15	3-21	6-6	2	4	DLDEYSSSVPFDY	265	13	2-8	1	SSYAGSNNYV	289	10	-	-	-
neM5-H23#	4-31	2-15	3	3	QIVVVVDAIRDAFDI	266	15	1-47	2	AAWDDSLSGVV	290	11			
neM5-H24#	3-15	6-13	2	3	SDSSSWGAFDI	267	11	1-40	2	QSYDSSLSGSV	135	11			
neM5-H25	3-15	3-16	2	4	DVWGSYRYLDY	268	11	1-44	3	AAWDDSLNGPV	69	11	-	-	-
neM5-H26	3-15	1-20	3	4	GWITGTSPDFDY	269	12	2-14	1	SSYTSSSTLYV	291	11	-	-	-
neM5-H27	4-34	4-17	2	4	GDLHGDLDY	270	9	6-57	2	QSYDSNVV	292	8	-	-	-
neM5-H28	1-2	6-19	2	6	GGSGWSPPNYYYYMDV	271	16	2-23	2	CSYAGSSTYVV	70	11	-	+	-
neM5-H31	3-30-3	2-2	3	4	DPVVVPAAMWGTFDY	272	15	3-1	1	QAWDSSTNYV	293	10	-	-	-
neM5-H41	3-23	/	/	3	GAPRGAFDI	273	9	3-21	3	QVWDSSSDHRV	294	11	-	+	-
neM5-H43	1-46	3-16	2	5	ERDYVWGSYRYTGPWFDP	274	18	2-11	1	CSYAGSYTYV	136	10	+	+	-
neM5-H48	3-7	6-13	3	4	GRYGAIAAAGTVLDY	275	15	2-23	2	CSYAGSSSVV	295	10	-	-	-
neM5-H33	3-49	2-15	3	4	QDIDIVVVVAATPRGIDY	276	18				--				

Figure 15

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #6

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM6-K1	5-51	6-13	3	5	GIAAAGTWFDP	296	11	3-15	4	QQYNNWPPLT	330	10	-	-	-
neM6-K2	1-3	3-22	2	4	DPFRRFGGYYDSSGYYDY	297	18	3-15	2	QQYNNWPPYT	331	10	-	-	-
neM6-K3	6-1	6-13	3	5	GIAAAGTPYWFDP	298	13	1-39	1	QQSYSTRTF	332	9	-	-	-
neM6-K4	3-15	4-23	3	4	GAAVVTPY	299	8	3-20	1	QQYGSSPWT	45	9	-	-	-
neM6-K13	3-23	3-22	2	6	EKDYYDSSGRTSNYYYYGMDV	300	21	1-39	3	QQSYSTPFT	58	9	-	-	-
neM6-K17	4-34	7-27	2	6	RMTANWDYYYYGMDV	301	15	3-20	4	QQYGSSPLT	122	9	-	-	-
neM6-K18	3-7	1-1	2	5	DAKYNWNDGRS WFDP	302	16	1D-8	2	QQYYSFPYT	333	9	-	-	-
neM6-K22	1-3	6-13	3	3	DSSIAAAGTSDAFDI	303	15	1-5	2	QQYNSYSYT	334	9	-	-	-
neM6-K29#	1-3	4-11	3	5	DRTTVTTINWFDP	304	13	1-17	4	LQHNSYPLT	335	9			
neM6-K37#	3-21	3-22	3	4	SKINSPVTMIVVGAKGFDY	305	19	3-15	3	QQYNNWPFT	185	9			
neM6-K38	4-34	3-16	2	6	VRMLGDYDYVHYYYGMDV	306	18	3-11	2	QQRSNWPRYT	336	10	-	+	-
neM6-K39	3-7	/	/	4	DTPAGRSY	307	8	1-17	2	LQHNSYPYT	282	9	-	+	-
neM6-K41	3-49	6-6	2	4	PHSSSSDYFDY	308	11	1D-8	3	QQYYSFPPFT	337	10	-	-	-
neM6-K12#						--		1-5	1	QQYNSYSTFG	338	10			
neM6-K15#						--		3-15	3	QQYNNWPFT	185	9			
neM6-λ6	3-30	2-2	3	3	VLTVVPAADDAFDI	309	14	3-21	2	QVWDSSSDHVV	77	11	-	-	-
neM6-λ8	4-30	5-5	3	4	IIVDTIFDY	310	9	2-23	2	CSYAGSSTLV	206	10	-	+	-
neM6-λ11	3-11	/	/	4	DPKGIGY	311	8	1-51	2	GTWDSSLSAVV	201	11	-	-	-
neM6-λ16#	1-69	7-27	3	2	DRTQLGNTGTYWYFDL	312	17	2-11	3	CSYAGSYTWV	339	10			
neM6-λ20	1-3	6-19	2	6	ERVRRLAVAGPYYYYYGMDV	313	20	1-40	1	QSYDSSLSGYV	64	11	+	+	-
neM6-λ24	3-23	6-13	2	6	KGSSSQYYYYYYGMDV	314	16	2-14	2	SSYTSSSTVV	340	10	-	-	-
neM6-λ25	3-30	6-13	2	6	YGYSSSWYYYYYGMDV	315	16	2-8	2	SSYAGSNNLV	66	10	-	-	-
neM6-λ27	3-9	3-10	2	4	ALYYYGSGSYFDY	316	13	2-14	2	SSYTSSSTHVV	341	11	-	+	-
neM6-λ32	4-30	3-10	3	4	LRARGVIDY	317	9	3-1	3	QAWDSSTAV	342	10	-	-	-

Figure 16

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM6-λ33	3-15	6-13	2	3	GGSSSSWFDAFDI	318	13	2-8	1	SSYAGSNNYV	289	10	-	-	-
neM6-λ36	1-2	6-6	2	3	NSSSSAFDI	319	9	2-23	3	CSYAGSST	343	8	-	-	-
neM6-λ19						--		3-10	2	YSTDSSGNHRV	344	11			
neM6-λ28						--		3-21	1	QVWDSSSDHYV	76	11			
neM6-λ31						--		1-40	3	QSYDSSLSGWV	345	11			
neM6-λ26						--		1-47	3	AAWDDSLSGWV	73	11			
neM6-H34	4-30	2-15	2	6	YCSGGSCYGMDV	3	12				--				
neM6-H36	1-2	6-6	2	3	NSSSSAFDI	319	9				--				
neM6-H40	3-23	/	/	4	SFSGGDYDY	320	9				--				
neM6-H43	4-59	3-9	2	4	HPGTYDILTGYYFDY	321	15				--				
neM6-H10	4-39	3-10	3	4	RRVVRGVMIFDY	322	12				--				
neM6-H14	3-15	3-10	2	4	DDYYGSGSPYY	323	11				--				
neM6-H35	3-7	4-23	2	5	DNRDHDYGGNSPWFDP	324	16				--				
neM6-H45	3-23	3-10	2	5	DKEMYYYGSGSYYWFDP	325	17				--				
neM6-H46	3-30	3-10	1	4	EDDPLWFGDY	326	10				--				
neM6-H48	4-39	1-26	1	4	QFAGWELRKQYKNFDY	327	16				--				
neM6-H9	3-48	3-10	2	4	LISGSYYFDY	328	11				--				
neM6-H42	4-61	6-13	2	4	GAGYSSSWYYFDY	329	13				--				
neM6-H40	3-23	/	/	4	SFSGGDYDY	320	9				--				

Figure 16 (cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #7

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM7-κ1	1-18	7-27	2	3	GWAGNWGLGSDAFDI	346	15	1-5	1	QQYNSYWT	374	8			
neM7-κ2	3-21	3-9	2	4	CDILTGYYLFDY	347	12	3-11	4	QQRSNWPLT	46	9	-	+	-
neM7-κ3	3-30	6-13	3	4	SIAAAVHYFDY	348	11	3-15	2	QQYNNRPPYP	375	10	-	+	-
neM7-κ4	3-48	7-27	2	4	GRGGNWGSTQPGYYFDY	349	17	1-8	2	QQYYSFPYT	333	9	-	-	-
neM7-κ5	1-2	/	/	3	GPIVAWADAFDI	350	12	3-20	2	QQYGSSPYT	376	9	-	-	-
neM7-κ7	3-15	3-10	1	4	VNPSFGDQDFDY	351	12	1-8	3	QQYYSYPFT	377	9	-	-	-
neM7-κ9	3-33	5-5	1	4	GRGWIQLWTDLYYFDY	352	16	1-5	2	QQYNSYSPYT	378	10	+	+	-
neM7-κ11	3-7	3-10	1	1	DYRGGFGELLYFQH	353	14	3-11	2	QQRSNWPPYT	379	10	-	-	-
neM7-κ18	3-33	6-13	3	4	VKGIAAAGSFDY	354	12	1-8	2	QQYYSYPYT	380	9	-	+	C
neM7-κ22	4-38	3-10	2	1	PQKRYYYGSGSYDEYFQH	355	18	3-11	4	QQRSNSLT	381	8	-	+	-
neM7-κ25	3-11	1-26	2	4	GGRSGSYFDY	356	10	3-20	1	QQYGSSPPGT	382	10	-	-	-
neM7-κ26	4-38	6-19	3	4	HAAVAGTPYYFDY	357	13	3-15	1	QQYNNWPPTCT	383	11	-	-	-
neM7-κ28	1-3	3-10	2	4	GGSGSYYNLFDY	358	12	3-15	4	QQYNNWPPLT	330	10	-	-	-
neM7-κ31	4-59	3-10	3	3	VAMVRGVISAFDI	359	13	3-20	2	QQYGSSPPY	384	9	-	-	-
neM7-κ33#	3-49	3-22	2	6	DRYYYDSSGYYYYYYYMDV	360	19	1-8	2	QQYYSFPRT	385	9			
neM7-κ34	4-38	/	/	4	RGKYYFDY	361	8	3-15	2	QQYNNWPPYT	331	10	-	+	-
neM7-κ37	4-38	4-17	2	4	NYGDYVGFDY	362	10	1-12	4	QQANSFPPLT	386	10	-	-	-
neM7-κ38#	4-39	5-12	2	4	HIGGAGYSGPFDY	363	13	3-20	4	QQYGSFLT	387	8			
neM7-κ42	4-61	3-10	2	4	DSDYYGSGSYFDY	364	13	3-11	5	QQRSNWPLT	46	9	-	-	-
neM7-λ10	1-46	2-2	2	5	RYCSSTSCWFDP	365	12	6-57	2	QSYDSSNQV	241	9	-	-	-
neM7-λ14	3-30	3-10	2	3	GGRKYYGSGSYYDLDAFDI	366	19	2-14	3	SSYTSSSTWV	388	10	-	-	-
neM7-λ19	4-34	/	/	4	LGLKGLGPYFDY	367	12	2-23	3	CSYAGSSTKV	389	10	-	-	-
neM7-λ20	4-38	2-2	2	5	RYCSSTSCYVNWFDP	368	15	1-40	3	QSYDSSLSGSV	135	11	-	-	-
neM7-λ21	3-30	6-19	2	4	SYSSGWFDY	369	9	2-14	3	SSYTSSSTYWV	390	11	-	-	-
neM7-λ24	4-59	1-26	1	3	RSEWEPEGVDAFDI	370	14	3-21	2	QVWDSSDHVV	391	10	-	-	-
neM7-λ29#	4-34	3-10	2	4	NPYYYGSGRPPFDY	371	14	1-47		SLSGPDGTGTKVTVL	392	28			
neM7-λ32	3-7	3-10	2	4	VGYGSGSYFDY	372	11	3-10	3	YSTDSSGNQGV	393	11	-	-	-
neM7-λ38	4-39	5-12	2	4	HIGGAGYSGPFDY	363	13	1-47	2	AAWDDSLSGVV	290	11	-	-	-
neM7-λ41#	5-51	6-13	2	4	HRDHSSSWDTLGYFDY	373	16	2-23	3	CSYAGSSTWV	394	10			

Figure 17

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #8

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM8-K01	4-34	3-10	1	4	GQLHVLLWFGELLSSHIFDY	395	20	2-28	4	MQALQTPLT	49	9	+	+	-
neM8-K02	3-23	3-22	2	4	RSDYDSSGYPISVDY	396	15	1-16	3	QQYNSYPFT	420	9	-	-	-
neM8-K10	4-4	3-10	2	6	VGDPKDYYYGSGGGNYYYYGMDV	397	23	3-20	3	QQYGSSPPVFT	421	11	-	-	-
neM8-K13#	5-51	5-12	3	6	HVVATTNLASYYYYGMDV	398	18	3-15	2	QQYNNWPLYT	422	10			
neM8-K15#	4-59	2-8	2	2	STGYYWYFDL	399	10	3-11	3	QQRSNWPFT	423	9			
neM8-K18#	3-30	4-23	3	6	VFSRSVVTYYYYYGMDV	400	17	2-28	1	MQALQTPPT	424	9			
neM8-K20	3-30-3	3-10	2	5	YYGSGSLSD	401	9	3-20	3	QQYGSSPFT	181	9	-	-	-
neM8-K25	3-11	5-5	3	4	ETTRGDTAMVTPCNY	402	15	1-12	3	QQANSFPFT	425	9	+	+	-
neM8-K26#	1-46	3-9	2	3	DLCRGGGYDILTHDAFDI	403	18	3-20	2	QQYGSSPLYT	426	10			
neM8-K27#	4-34	2-2	2	6	DRYCSSTSCYSYYYYGMDV	404	19	3-20	5	QQYGSSPPIT	427	10			
neM8-K29	4-34	4-4	2	4	VAKEDDYSKLKVYYFDY	405	17	1-8	1	QQYYSFPWT	428	9	-	+	-
neM8-K35	3-30-3	/	/	6	VSTAGMDV	406	8	3-20	3	QQYGSSPHT	429	9	-	-	-
neM8-K41	4-34	/	/	4	ADFIDY	407	6	3-11	3	QQRSTWVT	430	8	-	-	-
neM8-K42#	5-51	6-13	3	2	HRIAAAGTWYFDL	408	13	1-5	1	QQYNSYSQT	431	9			
neM8-K43	3-64	/	/	6	RMTGMDV	409	7	1-17	3	LQHNSYPFT	432	9	-	+	-
neM8-K44	3-48	4-17	2	5	DPPPYGDYDGWFDP	410	14	1-8	4	QQYYSYPPL	433	9	-	-	-
neM8-K45	4-34	6-13	2	4	GLSSSPYYFDY	411	11	3-20	4	QQYGSSLT	279	8	-	-	-
neM8-K47	3-23	/	/	4	DFLYYFDY	412	8	3-15	2	QQYNNWPPYT	331	10	-	-	-
neM8-K48#	3-30	2-15	2	4	IGYCSGGSCYSQGDYFDY	413	18	2-30	5	MQGTHWPIT	434	9			
neM8-L07	1-3	/	/	4	EEGY	414	4	3-25	1	QSADSSGTYV	435	0	-	+	-
neM8-L19#	5-51	5-5	2	4	PRRGTAMAFGYSYGPYYFDY	415	20	2-14	3	SSYTSSSTQV	436	0			
neM8-L21	4-34	4-17	2	4	SPYGDSLPIDY	416	11	2-8	2	SSYAGSNNLV	66	0	-	-	-
neM8-L23#	1-69	2-15	2	6	DGYCSGGSCYAPYYYYGMDV	417	20	7-43	2	LLYYGGAVV	437	0			
neM8-L30	3-7	3-22	3	4	DQSVTMIVVVIWPEYYFDY	418	19	1-40	3	QSYDSSLSGSV	135	11	+	+	-
neM8-L38	3-11	1-7	2	6	ASGWNYYYYGMDV	419	13	1-47	3	AWDDSLSGPV	438	0	+	+	-
neM8-L42	5-51	6-13	3	2	HRIAAAGTWYFDL	408	13	2-14	2	SSYTSSSTLV	74	10	-	+	-
neM8-L09						--		2-14	1	SSYTSSSTYV	285	10			

Figure 18

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #9

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM9-K01	4-61	2-21	2	4	VGCGGDCPLDY	439	11	3-15	4	QQYNNWPPLT	330	10	-	+	-
neM9-K07#	3-48	3-10	2	6	LKAVRGVYYYYMDV	440	14	1-39	1	QQSYSTPPWT	463	10			
neM9-K08	3-23	2-2	2	3	DQGGYCSSTSCPDAFDI	441	17	1-16	2	QQYNSYPYT	464	9	-	-	-
neM9-K13	3-30-3	6-6	3	4	DGADTGSIASSSGYFDY	442	17	3-20	4	QQYGSSPLT	122	9	-	-	-
neM9-K15	3-30	/	/	2	ATGDRGNWYFDL	443	12	3-20	4	QQYGSSPPLT	47	10	+	+	-
neM9-K17	3-33	3-10	2	4	GVSEYGSGSYYFDY	444	14	3-20	4	QQYGSSSPLT	465	10	+	+	-
neM9-K22	3-49	2-8	2	4	DPTLLYCTNGVCHSFDY	445	17	3-20	1	QQYGSSPRT	54	9	+	+	-
neM9-K26	3-48	6-19	1	4	ESTTQWLDLDY	446	11	1-16	4	QQNSYPLT	466	8	-	+	-
neM9-K30	1-69	7-27	3	3	GGTGAQGFAFDI	447	12	1-17	4	LQHNSYPLT	335	9	+	+	-
neM9-K31	4-34	/	/	4	GDRHYFDY	448	8	1-39	1	QQSYSTPPWT	463	10	-	-	-
neM9-K32	3-11	7-27	1	4	QPLGKGLDFD	449	10	1-17	1	LQHNSYPWT	467	9	-	-	-
neM9-K34	4-59	6-13	3	4	GGIAAAGSLGHGY	450	13	3-20	1	QQYGSSPWT	468	9	-	+	-
neM9-K35	3-30-3	3-22	2	4	EASLPYDSSGRVGEATDY	451	18	3-20	1	QQYGSSPTWT	469	10	-	-	-
neM9-K39	3-30-3	/	/	3	DCVSGAFDI	452	9	3-15	4	QQYNNWPPLT	330	10	-	-	-
neM9-K42	4-34	6-6	2	4	GNPTPTYSSSYYFDY	453	15	2-28	2	MQALQTPQT	470	9	-	-	-
neM9-K43	3-21	1-26	2	4	NSGSYHLFDY	454	10	2-30	4	MQGTHWPPLT	471	10	-	-	-
neM9-K44#	3-23	7-27	2	4	DRDWGFIDY	455	9	3-20	1	QQYGSSPTWT	469	10			
neM9-L06	4-34	/	/	4	PLGFDY	456	6	1-51	3	GTWDSSLSAV	472	10	-	+	-
neM9-L11	3-33	6-13	3	4	PLAAAGLY	457	8	1-51	2	GTWDSSLSVV	473	10	+	+	N
neM9-L12#	3-11	1-26	2	3	DHGYSGSYYAFDI	458	13	1-44	2	AAWDDSLNGVV	240	11			
neM9-L24	3-15	7-27	3	4	DPPLSRTGGSDY	459	12	2-14	2	SSYTSSSTLNVV	474	12	-	-	-
neM9-L29	3-33	6-13	3	4	PLAAAGLY	457	8	3-1	2	QAWDSSTAHVV	475	11	-	+	-
neM9-L45	3-30	7-27	1	4	VALSPAAELGMRPFDY	460	16	2-14	2	SSYTSSSTLVV	74	11			
neM9-L48	4-38-2	2-8	3	4	SCLSQMVYAFDY	461	12	1-47	3	AAWDDSLSGRV	476	9			
neM9-H38	1-18	/	/	2	DQDPRPSYFDL	462	11				--				

Figure 19

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #10

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM10-K03#	4-34	7-27	1	2	ASLGNDRNWYFDL	477	13	1-37	4	QRTYNAP	514	7			
neM10-K04#	5-51	6-13	3	6	LPGIAAAGASYYYYYYMDV	478	19	3-20	4	QQYGSSPLT	122	9			
neM10-K07	3-15	/	/	3	DRWGAFDI	479	8	1-8	4	QQYYSYPLT	515	9	-	-	-
neM10-K11	3-9	6-13	2	4	GYSSSWYFDY	480	10	1-8	4	QQYYSYHGT	516	9	-	-	-
neM10-K14	3-21	7-27	2	3	HWGSGAFDI	481	9	3-11	3	QQRSNWPPFT	517	10	-	-	-
neM10-H17	3-15	6-19	2	4	DFPYSTYYFDY	482	11	1-8	2	QQYYSYPYS	518	9	+	+	-
neM10-K19	1-2	7-27	3	4	DTGTGAGDRFDY	483	12	3-20	4	QQYGSSRLT	519	9	-	-	-
neM10-K20	4-34	7-27	1	2	GGVQLGMVKGWYFDL	484	15	1-8	4	QQYYSYPLT	515	9	+	+	-
neM10-K21	1-2	1-7	2	4	ASYNWNYYFDY	485	11	3-11	1	QQRSTWPPWT	520	10	+	+	-
neM10-K23	4-39	6-6	2	5	RSSSYWFDP	486	9	1-39	3	QQSYSTPFT	58	9	-	+	-
neM10-K24	3-7	2-2	2	1	GKDCSSTSCYLSEYFQH	487	17	1-12	1	QQANSFPQT	521	9	-	-	-
neM10-K25	3-33	/	/	4	DSNGGSYYFDY	488	11	1-27	2	QKYNSAPRD	522	9	+	-	-
neM10-K26	3-33	6-13	1	3	GSSMSDPQLVSTDAFDI	489	17	2-30	4	MQGTHWPLT	53	9	+	-	-
neM10-K31	4-31	5-5	3	4	EWRNTAMVDY	490	10	1-39	2	QQSYSTPPYT	523	10	-	-	-
neM10-K32	3-53	1-26	2	4	ASRASSGSYYFDY	491	13	3-20	4	QQYGSSPPLT	47	10	-	+	-
neM10-K35	3-23	6-13	3	4	DQSFKAAAGPIDY	492	13	1D-8	1	QQYYSFPRT	385	9	-	+	-
neM10-K37	3-23	6-13	2	4	DRLGGYSSSWYFDY	493	14	1D-12	2	QQANSFPPYT	524	10	-	-	-
neM10-K39#	1-18	7-27	3	4	ITGDHHYFDY	494	10	1D-8	4	QQYYSFPLT	235	9			
neM10-K42	4-34	3-10	2	4	SRYYGSGSYYYYFDY	495	15	1-8	2	QQYYSYPYT	380	9	+	+	-

Figure 20

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM10-K43	3-23	/	/	4	RPGAGDY	496	7	1-39	4	QQSYSTPLT	56	9	+	+	-
neM10-K45	3-30-3	7-27	1	4	DRGQLGIFDY	497	10	3-15	2	QQYNNWPYS	525	9	-	-	-
neM10-K47	5-51	2-21	2	4	LVDFSGVGSGYCGGDCYFDY	498	20	3-15	4	QQYNNWPPLT	330	10	-	-	-
neM10-L01#	1-2	/	/	6	DLVGRQTEYYYYYGMDV	499	17	1-47	2	AAWDDSLSGVV	290	11			
neM10-L09	4-61	2-15	2	3	RYCSGGSCYSAFDI	500	14	3-1	2	QAWDSSTAHVV	475	11	-	-	-
neM10-L12	4-59	3-10	2	4	TAYGSGSYYIDY	501	12	3-25	1	QSADSSGTYV	435	10	+	+	-
neM10-L16	3-11	2-8	2	4	EATNGVCFDY	502	10	1-44	2	AAWDDSLNGPV	69	11	-	-	-
neM10-L18#	4-59	6-13	2	4	DRSWGSNYFDY	503	11	1-47	2	AAWDDSLSGPV	526	11			
neM10-L29	3-30-3	3-10	3	3	VMVRGVNAFDI	504	11	1-40	2	QSYDSSLSGVV	71	11	-	+	-
neM10-L30	3-23	4-17	2	4	ERTSYGDYFDY	505	11	2-11	3	CSYAGSYTWV	339	10	-	+	-
neM10-L34	3-7	1-26	2	4	DSGSYYYFDY	506	10	2-23	1	CSYAGSSTYV	70	10	+	+	-
neM10-L38	3-11	3-22	2	3	WDYYDSSGYYAFDI	507	14	2-18	2	SSYTSSSTLV	74	10	-	-	-
neM10-H02	3-23	1-7	3	3	IITGENAFDI	508	10				--				
neM10-H06	4-34	6-6	1	4	EGQLVGDYFDY	509	11				--				
neM10-H17	3-15	6-19	2	4	DFPYSTYYFDY	482	11				--				
neM10-H27	3-48	3-10	2	4	TYYYGSGSRYYFDY	510	14				--				
neM10-H36	4-38-2	6-13	1	2	GGEQQLVSWYFDL	511	13				--				
neM10-H40	1-2	6-13	2	4	GGSSSWYYFDY	512	11				--				
neM10-H41	1-2	/	/	4	ERWGDY	513	6				--				

Figure 20 (cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #11 GFP-

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP-M11-K53	4-4	6-19	2	4	VLSSGHFDY	527	9	1-8	4	QQYYSYPLT	515	9	-	-	-
neGFP-M11-K58#	4-59	1-26	2	3	EEEGGSYAFDI	528	11	1-33	3	QQYDNLP	545	7			
neGFP-M11-K61	4-34	2-2	2	4	RYCSSTSCYYFDY	529	13	3-15	3	QQYNNWPFT	185	9	-	-	-
neGFP-M11-K63	4-39	3-22	2	5	RDLNYYDSSGYYSWFDP	530	17	1-39	1	QQSYSTPPWT	463	10	-	-	-
neGFP-M11-K67#	3-30-3	6-13	3	1	DPQRIAAAGPFQH	531	13	1-5	1	QQYNSYSRT	190	9			
neGFP-M11-K68	3-48	/	/	2	DSQLGRGYFDL	532	11	3-15	1	QQYNNWPPWT	238	10	-	-	-
neGFP-M11-K73	4-4	4-11	2	4	GSSNRNTNDY	533	10	3-20	2	QQYGSSYS	546	8	-	-	-
neGFP-M11-K78	4-34	/	/	4	GVAANRLRSPGLDY	534	14	3-20	4	QQYGSSPPT	123	9	-	+	-
neGFP-M11-K80	4-34	6-19	2	4	PYSSGWYFDY	535	10	3-11	1	QQRSNWLYT	547	9	-	-	-
neGFP-M11-K64						--		1-17	2	LQHNSYPQYS	548	10			
neGFP-M11-K75						--		3-20	2	QQYGSSPPYS	549	10			
neGFP-M11-L59	4-34	1-26	2	4	HLPVRYSGSYPSSDY	536	15	1-36	1	AAWDDSLSGSYV	550	12	-	-	-
neGFP-M11-L62	4-61	7-27	2	4	IRPTTNWGFTKYYFDY	537	16	2-14	2	SSYTSSSTLV	74	10	+	+	-
neGFP-M11-L66	3-11	/	/	4	GVAGLGY	538	7	2-14	1	SSYTSSSTYV	285	10	-	+	-
neGFP-M11-L70	3-7	/	/	3	AAFDI	539	5	1-51	1	GTWDSSLSAYV	75	11	-	+	-
neGFP-M11-L76	4-34	4-17	2	3	VPHYGDYHDAFDI	540	13	1-47	3	AAWDDSLSGRV	476	11	-	-	-
neGFP-M11-L77	3-11	3-10	3	3	VKANRGAEDAFDI	541	13	2-14	3	SSYTSSSTWV	388	10	-	-	-
neGFP-M11-L81	4-59	3-22	2	3	SHASSGYYAFDI	542	12	2-23	1	CSYAGSSTYV	70	10	-	-	-
neGFP-M11-L91	4-39	3-10	2	4	LIRVFDYYGSGSAFDY	543	16	1-44	2	AAWDDSLNGHVV	72	12	-	-	-
neGFP-M11-L94	3-30-3	/	/	4	DPPLFDY	544	7	2-14	3	SSYTSSSTLV	74	10	-	-	-

Figure 21

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #11 GFP+ PTPN22 620W expression

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M11-K7	3-23D	6-13	3	4	DRGIAAAGTFDY	551	12	1-39	2	QQSYSTRYS	569	9	-	-	-
neGFP+M11-K8	3-7	3-3	3	6	GRFTADYYYYMDV	552	13	3-15	3	QQYNNWPT	570	8	-	-	-
neGFP+M11-K10	4-39	3-3	1	4	AEGDLLEWLSIGCFDY	553	16	1-27	4	QKYNSALLT	571	9	-	+	-
neGFP+M11-K19	3-74	7-27	/	3	AEYFLGDWHHDAFDI	554	15	1-12	3	QQANSFPFT	425	9	-	-	-
neGFP+M11-K24	3-23D	6-13	3	4	DRGIAAAGTFDY	551	12	1-39	1	QQSYSTPWT	44	9	-	-	-
neGFP+M11-K25	4-61	2-21	3	4	ATVVVTAQYYFDY	555	13	1-39	2	QQSYSTPYT	130	9	+	+	-
neGFP+M11-K29	3-23D	6-19	2	3	RGWGHAFDI	556	9	1-33	2	QQYDNLPPRGTA	572	12	+	-	-
neGFP+M11-K38	1-2	6-13	2	4	HSWYYFDY	557	8	3-11	1	QQRSNWWT	573	8	+	+	-
neGFP+M11-K47	3-30	2-21	2	3	KSPPYCGGDCYSRDDAFDI	558	19	3-20	5	QQYGSSVT	574	8	-	-	-
neGFP+M11-L2	3-21	2-15	1	4	DERGIGWYFDY	559	11	2-23	1	CSYAGSSTYV	70	10	-	+	-
neGFP+M11-L4	1-2	6-6	3	3	VRGIAARRPDAFDI	560	14	3-21	3	QVWDSSSDHPV	575	11	-	-	N
neGFP+M11-L6	3-11	4-17	2	4	RDYGDYGRGGPRWYFDY	561	17	3-21	1	QVWDSSSDHYV	76	11	-	-	-
neGFP+M11-L12	3-33	4-11	2	4	NDYSNYGAFDY	562	11	1-40	3	QSYDSSLSGWV	345	11	-	-	-
neGFP+M11-L13	3-21	3-22	3	3	GRVTMIVVDAFDI	563	13	1-51	2	GTWDSSLSAVV	201	11	-	+	-
neGFP+M11-L14	1-69	6-6	3	5	EGASIAARPYNWFDP	564	15	1-47	3	AAWDDSLSGWV	73	11	+	+	-
neGFP+M11-L18	3-73	3-22	2	4	SPGYYDSSGYYGY	565	13	2-11	3	CSYAGSYTWV	339	10	+	+	N
neGFP+M11-L32	3-30	6-6	1	6	GEQLSDFDYYYYGMDV	566	16	3-1	1	QAWDSSTAYV	576	10	-	-	-
neGFP+M11-L44	4-30	3-3	3	4	ERITIFGVAGSFDY	567	14	2-23	2	CSYAGSSTLV	206	10	+	+	-
neGFP+M11-L48	3-33	7-27	3	4	EGTITGPGYFDY	568	14	3-25	3	QSADSSGTYWV	577	11	-	-	-

Figure 22

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #12 GFP-

Ig	HEAVY CHAIN								LIGHT CHAIN				REACTIVITY			
	VH	D	RF	JH	(-)	CDR3 (aa)	SEQ ID NO	(+)	Length	Vκ	Jκ	CDR3(aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP-M12-k02	3-53	4-11	3	5	1	GWSTVTNWFDP	578	0	11	1-39	3	QQSYSTPFT	58	9	+	+	-
neGFP-M12-k07	3-33	6-13	1	4	3	PALHEQQLDYYFDY	579	1	14	3-15	1	QQYNNWPRWT	606	10	-	-	-
neGFP-M12-k16	1-3	6-13	3	5	2	DCGIAAAGNWFDP	580	0	13	1-8	3	QQYYSYPFT	377	9	-	+	-
neGFP-M12-k19#	1-69	7-27	3	4	2	VVETGVENY	581	0	9	3-11	1	QQRRNWPPWT	607	10			
neGFP-M12-k20	4-34	/	/	3	1	GRIPFDI	582	1	7	3-20	4	QQYGSPPGT	608	9	-	-	-
neGFP-M12-k24	1-3	/	/	4	1	AGMAFDY	583	0	7	1-5	1	QQYNSYSWT	609	9	-	+	-
neGFP-M12-k25	4-39	7-27	3	4	1	PGTGTNIDY	584	0	9	3-15	1	QQYNNWPRT	610	9	+	+	-
neGFP-M12-k26	4-34	7-27	1	4	2	SRHARKELGLLDY	585	3	13	1-5	2	QQYNSYST	611	8	-	-	-
neGFP-M12-k29	4-34	7-27	3	3	3	ARTRTGHRDDAFDI	586	4	14	4-1	1	QQYYSTPWT	612	9	-	-	-
neGFP-M12-k30	4-61	3-9	2	3	2	LGGFLTGYYNDAFDI	587	0	15	1-5	2	QQYNSYPYT	464	9	-	-	-
neGFP-M12-k32	6-1	2-21	2	4	3	DGYCGGDCYSVDY	588	0	13	2-30	1	MQGTHWPWT	613	9	-	-	-
neGFP-M12-k33	3-15	4-17	2	4	3	VGEDPRDY	589	1	8	3-20	2	QQYGSSPPYT	60	10	-	-	-
neGFP-M12-k37	1-18	7-27	1	6	2	DLGMVGMDV	590	0	9	3-20	1	QQYGSSRT	614	8	-	-	-
neGFP-M12-k40	6-1	7-27	2	4	2	GRGPPAGDFDY	591	1	11	2-30	1	MQGTHWPWT	613	9	-	-	-
neGFP-M12-k44	4-34	5-5	2	3	1	VQSGYSYGYAFDI	592	0	13	3-20	4	QQYGSSPPT	123	9	-	-	-
neGFP-M12-k45#	4-31	6-13	3	4	2	DHGIAAAGTFDY	593	1	12	1-39	1	QQSYSTPRT	615	9			
neGFP-M12-k46	4-61	4-23	2	4	2	DYGGNSVGYFDY	594	0	12	3-20	1	QQYGSSPWT	468	9	-	-	-
neGFP-M12-L04	3-11	3-22	2	4	3	GRDYYDSSGYYFDY	595	1	14	2-18	2	SSYTSSSTLV	74	10	-	-	-
neGFP-M12-L05	3-11	1-7	3	5	3	DWGDITGTNWFDP	596	0	13	2-23	2	CSYAGSSTFVV	616	11	-	-	-
neGFP-M12-L10#	3-48	3-10	2	4	3	DKPFDYYGSGSYYNYFDY	597	1	18	2-8	2	SSYAGSNNLV	66	10			
neGFP-M12-L12#	1-3	1-26	3	3	2	SKLTPDAFDI	598	1	10	2-14	3	SSYTSSSTWV	388	10			
neGFP-M12-L14	3-15	3-3	3	4	2	DPGITIFGVVIDY	599	0	13	2-14	3	SSYTSSSTLV	74	10	-	-	-
neGFP-M12-L15	1-2	6-6	2	2	3	DLKREAGWYFDL	600	2	12	3-1	2	QAWDSSTVV	143	9	-	+	-
neGFP-M12-L18	3-33	6-13	2	4	1	TGGYSSSWYFDY	601	0	12	2-14	2	SSYTSSSTPYVV	617	12	-	-	-
neGFP-M12-L28#	3-33	6-13	3	3	4	DRRIAAADDAFDI	602	2	13	1-44	1	AAWDDSLNGPYV	618	12			
neGFP-M12-L39	1-69	6-19	2	4	2	DHSSGWYYFDY	603		11	3-1	1	QAWDSSTNYV	293	10	-	-	-
neGFP-M12-L42	5-51	7-27	2	2	2	IPGDWYFDL	604	0	9	2-11	3	CSYAGSYTWV	339	10	-	-	-
neGFP-M12-L47	3-23	7-27	1	4	1	EELGIDY	605	0	7	1-47	3	AAWDDSLSGWV	73	11	-	-	-

Figure 23

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #12 GFP+ PTPN22 620W expression

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M12-K49	4-38-2	/	/	4	DGRTFFDY	619	8	3-11	2	QQRSNWPPYT	379	10	+	+	N
neGFP+M12-K50	4-34	6-13	2	3	TGYSSSWYRAFDI	620	13	3-15	3	QQYNNWPPGGP	645	11	+	+	-
neGFP+M12-K53	3-33	/	/	6	DYYYYGMDV	621	9	3-20	3	QQYGSSPT	43	8	-	+	-
neGFP+M12-K55	4-39	7-27	3	2	AELGGGYWYFDL	622	12	1-5	1	QQYNSYWT	374	8	+	+	-
neGFP+M12-K56	1-69	/	/	5	VGPRRDWFDP	623	10	3-11	4	QQRSNWLT	197	8	+	-	-
neGFP+M12-K58	1-69	/	/	4	RGIGGLDY	624	8	1-5	2	DYNYPYT	646	7	-	-	-
neGFP+M12-K60	3-23	/	/	4	VGKAPDEKLTHYYFDY	625	16	1-5	4	QQYNSYSLT	647	9	-	+	-
neGFP+M12-K63	4-4	7-27	3	3	SVLLPGDQRHDAFDI	626	15	2-30	1	MQGTHWPPTVRT	648	12	-	+	-
neGFP+M12-K65	1-69	1-26	2	4	VSGSYYYFDY	627	10	3-20	2	QQYGSSPRYT	649	10	+	+	-
neGFP+M12-K68	4-59	/	/	2	DGDWYFDL	628	8	1-NL1	3	QQYYSTPFT	650	9	-	+	-
neGFP+M12-K70	3-33	/	/	4	DLGRFDY	629	7	1-39	2	QQSYSTPQYT	651	10	-	+	-
neGFP+M12-K71	3-48	7-27	3	4	GATGDSFDY	630	9	1D-8	1	QQYYSFPWT	428	9	-	-	-
neGFP+M12-K81	3-21	/	/	4	DSWPSYYFDY	631	10	3-15	3	QQYNNWPFT	185	9	-	+	-
neGFP+M12-K91	4-39	/	/	3	HSTEKKNDAFDI	632	12	2-28	4	MQALQTPLS	652	9	-	+	-
neGFP+M12-K92#	1-69	5-5	2	5	YLLGYSYGYWFDP	633	13	3-20	4	QQYGSSPLT	122	9			
neGFP+M12-K95	3-64D	7-27	3	3	DRQLTGDLHDAFDI	634	14	1-5	1	QQYNSYWGT	653	9	-	-	-
neGFP+M12-L57	4-39	/	/	4	LGADRGWYFDY	635	11	3-21	3	QVWDSSSDHWV	654	11	-	+	-
neGFP+M12-L61#	3-30	3-10	2	4	ERLQSYYGSGSYYFDY	636	16	2-11	3	CSYAGSYTWV	339	10			
neGFP+M12-L69#	4-34	2-2	2	1	AGLGYCSSTSCYAEYFQH	637	18	2-23	7	CSYAGSSTGHAV	655	12			
neGFP+M12-L80	1-69	6-6	2	5	NDSSNWFDP	638	9	2-23	1	CSYAGSYV	656	8	-	-	-
neGFP+M12-L82	4-34	4-11	3	4	GGRMTTVTDYFDY	639	13	1-44	3	AAWDDSLNGWV	286	11	-	-	-
neGFP+M12-L83	3-53	3-16	3	2	DQFGDRTLHFDL	640	12	3-21	2	QVWDSSSDHPV	575	11	-	-	-
neGFP+M12-L84#	4-34	6-13	3	4	RQTAAAFDY	641	9	2-23	1	CSYAGSSTYV	70	10			
neGFP+M12-L85	4-39	7-27	3	2	RVLGIGRYFDL	642	11	3-21	1	QVWDSSSDLYV	657	11	-	-	-
neGFP+M12-L86	3-49	7-27	3	4	DLETGDLGFDY	643	11	2-23	2	CSYAGSSTVV	658	10	-	-	-
neGFP+M12-L92					see kappa	--		2-14	2	SSYTSSSTVV	340	10	+	-	-
neGFP+M12-L96	4-61	6-6	2	5	EGFHERWDSSSSNWFDP	644	17	2-23	3	CSYAGSSTWV	394	10	-	-	-

Figure 24

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #13 GFP-

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP-M13-K03	3-15	2-15	2	6	DPPYCSGGSCYYYGMDV	659	17	2-30	1	MQGTHWPRT	679	9	-	-	-
neGFP-M13-K04	3-15	/	/	4	DNSNYFDY	660	8	2-24	2	MQATQFPYT	680	9	-	-	-
neGFP-M13-K06#	4-34	6-6	2	4	GGEYSSSYFDY	661	11	1-9	3	QQLNSYPT	681	8			
neGFP-M13-K11	1-3	/	/	3	VGTGNDAFDI	662	10	3-15	1	QQYNNWPPWT	238	10	-	+	-
neGFP-M13-K13	4-61	6-6	1	4	VVEGQLVDY	663	9	3-15	2	QQYNNWPLYT	422	10	+	+	-
neGFP-M13-K16	3-20	/	/	4	RWKGYYFDY	664	9	3-11	3	QQRSNWPPFT	517	10	-	+	-
neGFP-M13-K18	3-13	6-6	2	2	GEYSSSDWYFDL	665	12	1-17	2	LQHNSYPHT	682	9	-	-	-
neGFP-M13-K20	3-15	1-26	2	4	GWRVFSGSYYFDY	666	13	1-9	3	QQLNSYPFT	683	9	-	-	-
neGFP-M13-K25	4-4	/	/	4	DGFQYYYYYY	667	10	3-20	3	QQYGSSPFT	181	9	-	-	-
neGFP-M13-K27	3-11	1-26	3	4	DGVGATDY	668	8	1-5	2	QQYNSYSYT	334	9	-	-	-
neGFP-M13-K32#	3-7	1-1	1	2	VQLGIVRWYFDL	669	12	3-20	3	QQYGSSPHNVVTEFT	684	15			
neGFP-M13-K39#	4-59	6-19	2	4	GGSGWYYFDY	670	10	3-11	4	QQRSNWPPLT	685	10			
neGFP-M13-K40	3-30	1-26	2	4	DGHSGSYFDY	671	10	1-17	2	LQHNSYPYT	282	9	-	-	-
neGFP-M13-L02	3-33	5-24	2	4	DWDGYNDY	672	8	1-47	1	AAWDDSLSGYV	200	11	-	-	-
neGFP-M13-L09	5-51	7-27	3	4	HEDSGLTGDHYFDY	673	14	2-14	1	SSYTSSSTLV	74	10	-	-	-
neGFP-M13-L14#	3-30	7-27	3	2	QSRLGTWYFDL	674	11	2-14	2	SSYTSSSTLV	74	10			
neGFP-M13-L17	3-33	6-13	3	4	EESIAAAGTDY	675	11	2-23	2	CSYAGSSTFVV	616	11	-	-	-
neGFP-M13-L28	3-7	6-13	2	4	DPGYSSSWFDY	676	11	2-23	2	CSYAGSHVV	686	9	-	-	-
neGFP-M13-L30	3-15	6-13	3	4	AIAAAGNY	677	8	2-23	3	CSYAGSSTWV	394	10	-	-	-
neGFP-M13-L46	3-30	4-11	2	4	ARHDYSNYFDY	678	11	2-14	1	SSYTSSSTYV	285	10	-	+	-

Figure 25

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #13 GFP+ PTPN22 620W expression

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M13-K49	4-39	6-6	2	4	RGYSSSFDY	687	9	1-5	1	QQYNSYSWT	609	9	-	+	-
neGFP+M13-K54#	3-11	1-26	2	4	EAQSGSYYY	688	9	3-15	1	QQYNNWPRT	610	9			
neGFP+M13-K56	3-11	3-10	2	2	REGSGSYYNWYFDL	689	14	1-39	2	QQSYSTPYT	130	9	-	-	-
neGFP+M13-K59	3-7	3-22	2	3	DRGYDSSGYYSDAFDI	690	16	1-NL1	2	QQYYSTPRT	709	9	-	+	-
neGFP+M13-K60	1-46	7-27	2	3	QNWGDAFDI	691	9	1-17	3	LQHNSYPFT	432	9	+	-	-
neGFP+M13-K62#	2-15	2-15	2	4	GSCSGGSCYFDY	692	12	4-1	2	QQYYSTPPT	710	9			
neGFP+M13-K64#	3-30	7-27	3	6	DRTGDLHYYYGMDV	693	14	3-20	2	QQYGSSPYT	376	9			
neGFP+M13-K66	3-23	3-10	1	5	DEGFGENNWFDP	694	12	1-39	2	QQSYSTPYT	130	9	+	-	-
neGFP+M13-K74#	4-4	7-27	2	4	RNWGVSGY	695	8	1D-8	4	QQYYSFPLT	235	9			
neGFP+M13-K77	4-4	5-5	1	3	VPGIQLWLKGAFDI	696	14	3-20	1	QQYGSSRT	614	8	-	-	-
neGFP+M13-K90	3-33	/	/	4	APPVLGGENYYFDY	697	14	3-20	4	QQYGSSPLT	122	9	-	+	-
neGFP+M13-K91	4-31	7-27	3	4	GVVTGDFDY	698	9	1-16	5	QQYNSYPIT	711	9	-	-	N
neGFP+M13-L50#	3-33	6-6	2	2	ARDSSSSWYFDL	699	12	6-57	7	QPYDSSNHAV	712	10			
neGFP+M13-L52	3-15	1-26	2	2	NHYSGSYNYWYFDL	700	14	1-51	1	GTWDSSLSAYV	75	11	-	-	-
neGFP+M13-L53	3-30-3	6-6	3	2	ERGRTIAARYFDL	701	13	2-14	1	SSYTSSSTLV	74	10	-	+	-
neGFP+M13-L61	3-23	/	/	4	DLILVLA	702	7	2-23	3	CSYAGSRV	713	8	-	+	-
neGFP+M13-L63	4-39	4-11	2	2	PPWFDDYSNYWYFDL	703	15	3-1	2	QAWDSSTAV	342	9	+	+	-
neGFP+M13-L65	3-53	/	/	2	GLRDGAAWYFDL	704	12	2-23	3	CSYAGSSTWV	394	10	-	-	-
neGFP+M13-L72#	3-53	3-10	3	2	ASWPGVWYFDL	705	11	2-23	3	CSYAGSYTWA	714	10			
neGFP+M13-L73	5-10-1	6-13	3	1	LVRIAAAGTEYFQH	706	14	2-14	3	SSYTSSSTLV	74	10	-	+	-
neGFP+M13-L88	4-34	4-17	2	2	GNDYGDYWYFDL	707	12	2-23	3	CSYAGSHWV	715	9	-	+	-
neGFP+M13-L93	4-23	4-23	2	6	GSGVYGGNYYYYGMDV	708	16	2-14	3	SSYTSSSTGWV	716	11	-	+	-

Figure 26

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #14 GFP+ PTPN22 WT expression

Ig	HEAVY CHAIN									LIGHT CHAIN					REACTIVITY		
	VH	D	RF	JH	(-)	CDR3 (aa)	SEQ ID NO	(+)	Length	Vκ	Jκ	CDR3(aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M14-K12	4-34	3-22	3	6	1	LVVIKSYYYYGMDV	717	1	14	1-9	4	QQLNSYPLT	183	9	-	+	-
neGFP+M14-K20	3-48	3-22	2	2	4	DGTDYYDSSGYYWYFDL	718	0	17	3-20	4	QQYGSSPLT	122	9	-	+	-
neGFP+M14-K21	3-21	4-17	2	4	2	ANYGDYFDY	719	0	9	3-15	4	QQYNNWPLT	193	9	-	-	-
neGFP+M14-K27	3-23	3-10	2	4	1	LIVREMATLYGSGSYQNYY	720	1	19	1-5	4	QQYNSYPLT	125	9	-	-	-
neGFP+M14-K29	1-69	6-13	2	5	1	NGVYSSSWYWFDP	721	0	13	4-1	2	QQYYSTPPYT	740	10	+	-	-
neGFP+M14-K30	3-30	4-17	2	4	3	DVYGDYFDY	722	0	9	1-39	2	QQSYSTPYT	130	9	-	-	-
neGFP+M14-K33	1-3	1-26	2	4	2	DLGVSGSYYFDY	723	0	12	3-20	4	QQYGSSLT	279	8	-	-	-
neGFP+M14-L01	3-15	6-19	3	4	1	GTHYFDY	724	1	7	3-27	2	YSAADNNLV	741	9	-	-	-
neGFP+M14-L02	3-30-3	6-13	3	4	2	EKAGTHYFDY	725	2	10	2-18	1	SSYTSSSTYV	285	10	-	+	-
neGFP+M14-L07	3-30	1-26	2	4	3	DDSGSYLIDY	726	0	10	1-44	3	AAWDDSLNGL	742	10	-	-	-
neGFP+M14-L08#	3-30-3	6-13	3	6	1	NVAAAGTYPSAVFYYYYGMDV	727	0	21	3-25	1	QSADSSGTYV	435	10			
neGFP+M14-L09#	3-7	3-22	2	4	1	GHSSGYYHYFDY	728	2	12	2-14	2	SSYTSSSTGV	743	10			
neGFP+M14-L11	4-4	4-17	2	4	5	GTDDYGDNRERNYFDY	729	2	16	2-14	2	SSYTSSSTP	744	9	-	-	-
neGFP+M14-L13	4-59	7-27	3	4	2	QGKITGEGNFDY	730	1	12	2-18	2	SSYTSSSTLVV	74	11	-	-	-
neGFP+M14-L14	4-39	3-22	2	5	3	HERYYYDSSGYYNWFDP	731	2	17	1-51	3	GTWDSSLSVNWV	745	12	-	-	-
neGFP+M14-L24#	1-3	3-3	2	4	3	TDTVRTDYDFWSGYSY	732	1	16	2-14	2	SSYTSSSVV	746	9			
neGFP+M14-L25#	1-3	2-15	2	4	2	NSIDCSGGSCYFDY	733	0	14	1-40	1	QSYDSSLSGSV	135	11			
neGFP+M14-L31	3-30-3	6-13	3	4	2	EKAGTHYFDY	725	2	10	2-18	2	SSYTSSSTFE	747	10	-	-	-
neGFP+M14-L39	4-34	2-2	2	4	1	RLHRGSTSCYDY	734	3	12	2-14	2	SSYTSSSTVV	340	10	-	+	-
neGFP+M14-L40	3-23	6-6	2	4	2	RLLTSSCDSGFDY	735	1	13	1-40	1	QSYDSSLSGFYV	748	12	-	-	-
neGFP+M14-L45#	4-34	6-13	3	3	3	GIAAADDAFDI	736	0	11	2-14	3	SSYTSSSWV	749	9			
neGFP+M14-H16	4-61	/	/	4	1	ATKLLSFDY	737	1	9				--				
neGFP+M14-L18	3-21	2-2	3	4	3	DQSPSHDIVVVPAAIYFDY	738	1	19				--				
neGFP+M14-H48	1-46	3-16	1	4	4	DHHYEGLRLGELSLFDY	739	3	17				--				

Figure 27

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #15 GFP+ PTPN22 WT expression

Ig	HEAVY CHAIN								LIGHT CHAIN				REACTIVITY			
	VH	D	RF	JH	(-)	CDR3 (aa)	SEQ ID NO	(+)	Length	Vκ	Jκ	CDR3(aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M15-K49	3-33	6-13	2	3	1	STRTYSSSWYAFDI	750	1	14	1-33	5	QQYDNLPLSFKVT	770	13	-	+	-
neGFP+M15-K51	3-30-3	6-6	2	3	2	RRIHSYSSLDAFDI	751	3	14	3-20	1	QQYGSSRT	614	8	+	+	-
neGFP+M15-K52	3-33	3-16	2	4	2	EAVWGFDY	752	0	8	1-6	1	LQDYNYPWT	771	9	-	-	-
neGFP+M15-K59	4-34	4-17	2	4	2	GLYGDYFDY	753	0	9	1-8	1	QQYYSYPRT	772	9	-	-	-
neGFP+M15-K60	3-72	7-27	3	3	1	AGLTGVGAFDI	754	0	11	3-20	1	QQYGSSRT	614	8	-	-	-
neGFP+M15-K67	3-74	3-3	1	4	3	DEGYLRFDY	755	1	9	1-17	4	LQHNSYPLT	335	9	-	-	-
neGFP+M15-K77	4-34	3-9	2	4	2	AGYYDILTGYYYFDY	756	0	15	3-20	4	QQYGSSPAKLT	773	11	-	+	-
neGFP+M15-K79#	3-30	3-3	2	6	3	DHDFWSGYYYYYGMDV	757	1	16	1-12	4	QQANSFPLT	774	9			
neGFP+M15-K80	3-33	6-6	2	4	2	DPYSSSYYFDY	758	0	11	1-39	4	QQSYSTPLT	56	9	-	-	-
neGFP+M15-K83	3-43	4-17	2	4	3	AIDYGDYYFDY	759	0	11	1-5	2	QQYNSYSYT	334	9	-	-	-
neGFP+M15-K86	4-61	3-22	2	4	1	GGRQRGSGYFDY	760	2	12	3-20	4	QQYGSSPFT	181	9	-	+	-
neGFP+M15-K94	4-39	3-22	2	4	3	HQIDSRSTYYYDSSGYYFDY	761	2	20	3-20	3	QQYGSSPT	43	8	-	-	-
neGFP+M15-L54#	1-46	1-1	1	4	2	DLQLGRYYFDY	762	1	11	2-23	1	CSYAGSSTSYV	775	11			
neGFP+M15-L55	1-2	6-6	2	4	2	SPDSSSMSKLPYFDY	763	1	15	2-23	1	CSYAGSSTV	776	9	-	-	-
neGFP+M15-L57	1-2	7-27	2	3	4	DLREANWDAFDI	764	1	12	2-23	1	CSYAGSSTV	776	9	-	-	-
neGFP+M15-L66	4-61	6-6	2	3	1	YSSSSFFAFDI	765	0	11	1-51	1	GTWDSSLSAHV	777	11	-	-	-
neGFP+M15-L89	1-8	2-2	2	6	1	GYCSSTSCYYYYYMDV	766	0	16	1-44	3	AAWDDSLNGPV	69	11	-	-	-
neGFP+M15-H62	4-31	7-27	3	3	3	DRRGDLGAFDI	767	2	11				--				
neGFP+M15-H69	3-15	4-23	2	3	1	NYGGNKNAFDI	768	1	11				--				
neGFP+M15-H93	4-61	6-6	3	4	1	SIAARPVDY	769	1	9				--				

Figure 28

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #16 GFP+ PTPN22 263Q expression

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M16-K2	3-30	1-26	2	4	TDHSGSYFDY	778	10	1-5	2	QQYNSYSMYT	807	10	-	-	-
neGFP+M16-K9	3-11	7-27	2	3	SETNWGDDAFDI	779	12	3-11	3	QQRSNWPFT	423	9	-	-	-
neGFP+M16-K10	4-34	7-27	3	4	EARTGAFFDY	780	10	1-5	2	QQYNNYSYS	808	9	-	+	-
neGFP+M16-K19	3-33	3-3	2	3	DYNFWSGYYTGDAFDI	781	16	2-28	4	MQALQTPLT	49	9	-	+	-
neGFP+M16-K21	3-30	7-27	2	4	HEDPNWGQPLDY	782	12	1-39	4	QQSYSTPLT	56	9	-	-	-
neGFP+M16-K24	4-34	4-17	2	4	GVFDDPLDYGDYFDY	783	15	1-17	1	LQHNSYPRT	809	9	-	-	-
neGFP+M16-K32	3-30	4-17	2	4	GLGYGDYVSLYFDY	784	14	4-1	2	QQYYSTAYT	810	9	-	-	-
neGFP+M16-K33#	4-59	5-5	3	4	AQEAMVIFDY	785	10	1D-8	4	QQYYSFPLT	235	9			
neGFP+M16-K37#	4-34	3-10	3	4	APITMVRGVPIYFDY	786	15	3-11	5	QQRSNWIT	811	8			
neGFP+M16-K41	4-34	2-2	2	3	LPCSSTSCYAFDI	787	13	3-11	4	QQRSTWPPT	812	9	-	-	-
neGFP+M16-K42#	3-33	/	/	3	DGAPGTGAFDI	788	11	1-39	1	QQSYSTPWT	44	9			
neGFP+M16-K44	4-61	6-13	2	2	DQRYSSSWYWYFDL	789	14	1-39	3	QQSYSTPFT	58	9	+	-	-
neGFP+M16-K45#	3-33	1-1	3	4	EPTGTTGTFDY	790	11	3-20	2	QQYGSSPPYT	60	10			
neGFP+M16-L1#	3-15	/	/	4	GCGADGY	791	7	2-14	3	SSYTSSGWV	813	9			
neGFP+M16-L8	4-34	4-17	2	4	TFYGDYFDY	792	9	2-14	3	SSYTSSSTWV	388	10	-	-	-
neGFP+M16-L15	3-30	6-19	2	4	ADSSGWYYFDY	793	11	1-47	3	AAWDDSLSGQV	814	11	-	-	-
neGFP+M16-L16	6-1	5-5	1	4	GQLWFDY	794	7	2-14	1	SSYTSSSTKV	815	10	-	-	-
neGFP+M16-L20	3-15	2-21	3	1	AGVVVTAIRAEYFQH	795	15	3-1	2	QAWDSSTAVV	816	10	-	-	-
neGFP+M16-L26	3-23	/	/	4	DRRYYFDY	796	8	1-40	3	QSYDSSLSGWV	345	11	-	+	-
neGFP+M16-L27	3-7	6-19	2	4	TSSGWYFDY	797	9	2-11	1	CSYAGSYTFV	817	10	-	-	-
neGFP+M16-L29	4-39	/	/	4	SARDFDY	798	7	1-47	3	AAWDDSLSGWV	73	11	+	+	-
neGFP+M16-L31	3-74	6-6	2	4	DRIGDSSSYYFDY	799	13	3-1	2	QAWDSSTVV	143	9	-	-	-
neGFP+M16-L36	4-59	/	/	3	PGPGHDAFDI	800	10	2-23	3	CSYAGYWV	818	8	-	-	-
neGFP+M16-L37#	4-34	3-10	3	4	APITMVRGVPIYFDY	786	15	1-47	2	AAWDDSLSGVV	290	11			
neGFP+M16-L40	4-34	6-6	3	3	GKAARAFDI	801	9	3-1	2	QAWDSSTVV	143	9	-	-	-
neGFP+M16-L43#	4-59	6-19	3	3	DGGIAVADAFDI	802	12	3-10	3	YSTDSSGNHRV	344	11			
neGFP+M16-K23	3-15	6-13	2	4	DLREYSSSWRFDY	803	13				--				
neGFP+M16-K35	4-34	1-26	2	4	GLGSYYFDY	804	9				--				
neGFP+M16-L38	4-59	3-9	2	3	AHYDILTGYYAFDI	805	14				--				
neGFP+M16-L39	1-46	6-13	2	4	DRSLPYSSSWYYFDY	806	15				--				

Figure 29

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #17 GFP+ PTPN22 263Q expression

Ig	HEAVY CHAIN									LIGHT CHAIN					REACTIVITY		
	VH	D	RF	JH	(-)	CDR3 (aa)	SEQ ID NO	(+)	Length	Vκ	Jκ	CDR3(aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M17-K1	3-30-3	1-7	2	4	1	VPASNWNYFDY	819	0	11	1D-8	2	QQYYSFPYT	333	9	-	-	-
neGFP+M17-K4	3-23D	7-27	2	4	2	SDWGYFDY	820	0	8	3-11	1	QQRSTWPRT	844	9	-	-	-
neGFP+M17-K10	3-30-3	6-13	3	3	2	WRAAADAFDI	821	1	10	1-17	4	LQHNSYPLT	335	9	-	-	-
neGFP+M17-K14	4-34	7-27	2	2	1	LKKANWGSGTHWYFDL	822	3	16	3-11	3	QQRSNWPLT	46	9	+	+	-
neGFP+M17-K15	3-30	6-13	2	4	2	DVSSSWYFDY	823	0	10	1D-8	2	QQYYSFPYT	333	9	-	-	-
neGFP+M17-K32	4-34	6-6	3	6	1	RPPSIAARYYYYYMDV	824	2	16	3-11	4	QQRSNWLT	197	8	+	+	-
neGFP+M17-K48#	3-30-3	6-6	2	4	2	GTEYSSSYYFDY	825	0	12	1D-8	1	QQYYSFPWT	428	9			
neGFP+M17-K55	3-33	7-27	3	4	3	DRPTGDLDY	826	1	9	3-11	4	QQRSNWPPLT	685	10	-	+	-
neGFP+M17-K72	3-30	6-19	2	4	2	DGAYSSGWYYFDY	827	0	13	1D-8	2	QQYYSFPYT	333	9	-	-	-
neGFP+M17-K78	4-39	6-19	3	4	1	ILTAGVDY	828	0	8	1-39	1	QQSYSTPPT	845	9	-	-	-
neGFP+M17-K80	3-30	6-13	2	4	2	DVSSSWYFDY	823	0	10	1D-8	2	QQYYSFPYT	333	9	-	-	-
neGFP+M17-K83	3-30-3	1-7	2	4	1	VPASNWNYFDY	819	0	11	1D-8	2	QQYYSFPYT	333	9	-	-	-
neGFP+M17-K89	3-30-3	6-6	2	4	2	GTEYSSSYYFDY	825	0	12	1D-8	1	QQYYSFPWT	428	9	-	-	-
neGFP+M17-K90	3-15	6-13	2	4	2	DHVYSSSWYFDY	829	1	12	3-15	3	QQYNNWLPFT	846	10	-	-	-
neGFP+M17-L3	4-34	3-10	3	2	1	GIRAYWYFDL	830	1	10	2-14	1	SSYTSSSTKV	815	10	-	-	-
neGFP+M17-L6	1-2	3-3	3	4	0	IFGVVIYY	831	0	8	2-14	3	SSYTSSSTWV	388	10	-	+	-
neGFP+M17-L7	3-23	6-6	2	1	2	DDRFKYSSSSYFQH	832	3	14	2-14	1	SSYTSSSTLYV	291	11	-	-	-
neGFP+M17-L16	1-2	3-3	3	4	0	IFGVVIYY	831	0	8	2-14	3	SSYTSSSTWV	388	10	-	+	-
neGFP+M17-L19	1-2	3-10	1	4	3	EPWFGEFYFDY	833	0	11	2-14	3	SSYTSSSTRV	243	10	-	-	-
neGFP+M17-L25	3-30-3	1-26	3	4	2	DQGVGRFDY	834	1	9	1-40	2	QSYDSSLSGSV	135	11	-	-	-
neGFP+M17-L38	3-23D	6-13	3	4	1	KVPAAGIFDY	835	1	10	5-37	1	MIWPSNAYV	847	9	-	-	-
neGFP+M17-L39	3-48	5-24	2	4	2	DRRGTGFDY	836	2	9	2-11	1	CSYAGSYTYV	136	10	-	-	-
neGFP+M17-L40	3-7	/	/	4	1	ISRAFDY	837	1	7	2-11	3	CSYAGSYTWV	339	10	-	+	-
neGFP+M17-L53	1-2	3-3	3	4	0	IFGVVIYY	831	0	8	2-14	3	SSYTSSSTWV	388	10	-	+	-
neGFP+M17-L63#	1-69	5-5	2	4	2	VGPYSYGYDYSNYYFDY	838	0	17	2-14	3	SSYTSSSTLGWV	848	12			
neGFP+M17-L66	3-7	4-11	2	4	2	DHPQYYFDY	839	1	9	1-44	3	AAWDDSLNGWV	286	11	-	-	-
neGFP+M17-L71	3-33	7-27	2	4	1	ANWGFYYFDY	840	0	10	1-44	3	AAWDDSLNGWV	286	11	-	-	-
neGFP+M17-L81	1-2	3-10	2	4	2	GDYGSGSYYNDY	841	0	12	7-46	3	LLSYSGAWV	849	9	-	-	-
neGFP+M17-L94#	1-69	4-17	2	5	2	HGDYWFDP	842	1	8	1-47	3	AAWDDSLSGRV	476	11			
neGFP+M17-L95#	4-61	6-6	2	3	2	AYSSSSGDAFDI	843	0	12	2-11	2	CSYAGSYHVV	850	10			

Figure 30

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #18 treated with 0.75 mg of LTV-1 PTPN22 inhibitor

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM18-K13	3-23	2-21	3	6	DRRIVVVTAINYYYYYMDV	851	19	1-39	1	QQSYSTRT	884	8	+	+	-
neM18-K15	3-23	3-10	2	4	EGLGDYFDY	852	9	1-5	1	QQYNSYSRT	190	9	-	-	-
neM18-K19	1-3	7-27	1	4	GELGPFDY	853	8	1-16	4	QQYNSYPLT	125	9	-	+	-
neM18-K20	3-74	/	/	3	GPRSFAFDI	854	9	3-15	2	QQYNNWPPYS	885	10	-	-	-
neM18-K21	3-23	5-12	2	4	YSGYDFTYYFDY	155	12	1-5	1	QQYNSYSRT	190	9	-	+	-
neM18-K25#	3-23	3-22	2	4	GVGSGYYYFDY	855	11	1-9	5	QQLNSYLSIT	886	10			
neM18-K27#	1-24	4-17	2	5	DLLPSRYGDYNWFDP	856	15	2-10	1	MQGTHWWT	887	8			
neM18-K29	3-30	7-27	3	4	ERTGAFDY	857	8	3-20	4	QQYATSLT	888	8	-	-	-
neM18-K30#	4-59	2-2	2	2	STTNAVYWYFDL	858	12	1-8	5	QQYYSYPIT	889	9			
neM18-K33	4-34	6-13	2	4	GHLKSSYSSSWYSY	859	14	2-24	1	MQATQFPWT	116	9	-	-	-
neM18-K34	3-9	1-7	3	4	DTRGTGTTYYFDY	860	13	3-20	4	QQYGSSLLT	890	9	-	-	-
neM18-K35	3-23	6-13	3	4	APASHIAAAGRIYFDY	861	16	3-20	1	QQYGSSPWT	468	9	-	-	-
neM18-K37	3-7	/	/	4	TGPYYFDY	862	8	1-39	2	QQSYSTPYT	130	9	-	+	-
neM18-K39	3-13	6-19	2	2	QVRGWYFDL	863	9	3-15	3	QQYNNWPFT	185	9	-	+	-
neM18-K44	3-48	1-26	2	4	DLGSYYDY	864	8	1-17	1	LQHQTYPRT	891	9	-	+	-
neM18-K45	4-34	/	/	2	PLTADYWYFDL	865	11	3-20	1	QQYGSSPV	892	8	-	-	-
neM18-K47#	3-13	/	/	3	AGTGAFDI	866	8	3-11	4	QQRSTWPLT	893	9			

Figure 31

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM18-L02	3-30-3	1-26	2	3	DYQLGGSYYDAFDI	867	14	2-23	2	CSYAGSSTLV	206	10	-	-	-
neM18-L03	4-38-2	2-21	2	4	SPAYDCGGDCRFDY	868	14	3-25	3	QSADSSGTWV	894	10	-	-	-
neM18-L04	4-30-4	6-13	3	5	AGTAAAGTGWFDP	869	13	3-25	3	QSADSSGTWV	894	10	-	-	-
neM18-L16	3-23	3-10	2	4	DQGYYYGSGSYLDY	870	14	2-23	3	CSYAGSRV	713	8	-	-	-
neM18-L17	4-39	7-27	2	3	QANWGSEEAFDI	871	12	1-47	2	AAWDDSLSGVV	290	11	-	-	-
neM18-L18#	1-18	4-17	2	5	GYGDYWFDP	872	9	2-23	2	CSYAGSSTHVV	895	11			
neM18-L23	3-30	/	/	4	EWVY	873	4	1-44	2	AAWDDSLNGVV	240	11	-	-	-
neM18-L28#	3-30	7-27	3	4	SVTGGLPMHY	874	10	5-37	3	MIWASNAWV	896	9			
neM18-L31	3-15	3-16	2	5	GVYYDYV	875	7	3-1	2	QAWDSSTVV	143	9	-	-	-
neM18-L32	3-33	1-26	1	4	NGRWELPTSRFNYFDY	876	16	3-21	3	QVWDSSSDHWV	654	11	-	+	-
neM18-L36	1-2	/	/	4	SKRTGEGDY	877	9	3-21	2	QVWDSSSDHPV	575	11	-	-	-
neM18-L38	3-30	7-27	3	4	DGAPTGDPILDY	878	12	2-14	2	SSYTSSSTLV	74	10	-	-	-
neM18-L43#	3-23	1-26	3	1	VIAEGATKYFQH	879	12	3-1	1	QAWDSSTGV	897	9			
neM18-L46	1-2	7-27	2	3	ISPNWGSGAFDI	880	12	2-23	2	CSYAGSSTFVV	616	11	-	-	-
neM18-L48#	3-21	3-22	2	5	DFSPLYDSSGYYFDP	881	15	2-23	2	CSYAGSSTYVV	70	11			
neM18-K05#	3-30	7-27	3	2	RTGDHWYFDL	882	10				--				
neM18-K40	1-69	1-26	2	5	AIRRYSGSYYWFDP	883	14				--				

Figure 31 (Cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #19 treated with 0.75 mg of LTV-1 PTPN22 inhibitor

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM19-K50	1-69	6-6	3	4	DFFRSIAARPSWGPFDY	898	17	3-20	2	QQYGSSPYT	376	9	+	+	-
neM19-K53	4-34	4-4	3	5	GSCGQTTENWFDP	899	13	3-20	2	QQYGSSPMYT	933	10	-	-	-
neM19-K55	3-11	2-2	2	6	GGYCSSTSCFNYGMDV	900	16	3-20	2	QQYGSSPPYT	60	10	-	-	-
neM19-K62	4-31	3-22	2	4	VFWTPRYDSSGYYYFDY	901	17	3-20	4	QQYGSSLT	279	8	-	-	-
neM19-K63	3-64	2-21	2	5	DRAYCGGDCYSGTGGFDP	902	18	4-1	2	QQYYSTPYT	934	9	-	-	-
neM19-K64	4-59	3-10	2	5	DSVYYYGSGSYYNEGSTLPKYNWFDP	903	26	3-15	1	QQYNNWPQT	55	9	-	+	-
neM19-K66#	4-39	3-22	3	4	LLFRLTMIVVVTGGIDY	904	17	3-20	3	QQYGSSPFY	935	9			
neM19-K68	3-23	1-7	3	5	EPPGTTSPSLTNWFDP	905	16	3-20	4	QQYGSSPLT	122	9	-	-	-
neM19-K73	3-21	4-17	2	5	DPFIYDRNDYGDYGWFDP	906	18	3-11	2	QQRSTWPYT	936	9	-	+	-
neM19-K74	3-66	5-24	3	6	VGVEMATTGRCYYGMDV	907	17	3-15	4	QQYNNWPPLT	330	10	-	+	-
neM19-K76#	3-23	3-10	1	6	CRASSGFGELGDYYGMDV	908	18	1-17	1	LQHNSYPWT	467	9			
neM19-K78	3-30-3	4-17	3	4	GPVTNGYYFDY	909	11	3-20	1	QQYGSSPQT	128	9	-	-	-
neM19-K85#	3-21	3-22	2	4	DQGYYDSSGYYGFDY	910	15	3-20	1	QQYGSSPRT	54	9			
neM19-K87	4-59	1-26	3	4	ARLGATFDY	911	9	1-17	1	LQHNSYPWT	467	9	-	-	-
neM19-K88	4-30-2	2-2	3	5	AGGALIPAAIDWFDP	912	15	1-39	3	QQSYSTPFT	58	9	-	-	-
neM19-K90	3-7	2-15	2	3	DHISGGSCYSAFDI	913	14	3-11	5	QQRSNWPIT	937	9	-	+	-
neM19-K92	3-74	3-3	3	4	GTIFGVVYSDY	914	11	3-15	2	QQYNNWPYA	938	9	+	+	-
neM19-K93#	1-69	/	/	4	PLNRSGFDY	915	9	3-15	1	QQYNNWPRT	610	9			
neM19-K79						--		1-5	1	QQYNSYST	611	8			

Figure 32

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM19-L56	4-61	3-22	2	4	MEGSYYYDSSGYPFFDY	916	17	1-51	3	GTWDSSLSAEV	939	11	-	-	-
neM19-L58	3-23	5-5	1	4	VPATTWIQLWGLDY	917	14	2-14	1	SSYTSSSTLV	74	10	-	-	-
neM19-L60	3-11	4-23	2	3	YGGNGGGLVGAFDI	918	14	2-14	3	SSYTSSSTLV	74	10	-	-	-
neM19-L67	1-3	3-10	2	5	GLRVGYVSGSYYNDWFDP	919	18	1-44	3	AAWDDSLNGWV	286	11	-	-	-
neM19-L69	1-18	3-10	3	4	DGREELNSRITMVRGPFDY	920	19	2-14	3	SSYTSSSTWV	388	10	-	-	-
neM19-L77	3-21	2-15	2	5	VPPLGYCSGGSCLNWFDP	921	18	5-37	3	MIWPSNAWV	940	9	-	+	-
neM19-L80	3-66	1-26	2	4	EIGDSGSYYFDY	922	12	1-47	3	AWDDSLSGPS	941	10	-	-	-
neM19-L81#	3-53	3-3	3	5	FGVVKAGNWFDP	923	12	1-44	3	AAWDDSLNGRV	287	11			
neM19-L83#	3-33	1-20	2	6	GGQMNLNDGPVLYYYYGMDV	924	20	2-23	1	CSYAGSSTNYV	942	11			
neM19-L84#	4-31	3-22	2	4	VDTYYYDSSGYSRYYFDY	925	18	2-23	3	AGSSTLVV	943	8			
neM19-L89	3-15	2-15	2	4	VSPKINCSGGSCFITHFDY	926	19	1-40	1	QSYDSSLSGPYV	944	12	-	-	-
neM19-L94	3-15	3-10	2	5	DLGDPRVEDWFDP	927	13	2-23	1	CSYAGSSTYV	70	10	-	-	-
neM19-L95	3-74	2-22	2	4	ALPYCSSTSCYQYYFDY	928	17	2-23	1	AGSSTYV	945	7	-	+	-
neM19-L96	3-23	4-17	2	4	DQVSYGDYRGYYFDY	929	15	2-11	3	CSYAGSYTV	946	9	-	-	-
neM19-L71						--		1-47	2	AAWDDSLSGHVV	947	12			
neM19-L82						--		1-44	3	AAWDDSLNGWV	286	12			
neM19-K52	3-7	5-5	3	4	DQVDTAMVLDY	930	11				--				
neM19-H59	4-39	3-22	2	4	LTYYYDSSGYYYFDY	931	15				--				
neM19-H72	1-69	6-19	2	4	DQDSYSSGWVGLDY	932	14				--				

Figure 32 (Cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #20 treated with 0.15 mg of LTV-1 PTPN22 inhibitor

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM20-K02	4-61	3-9	2	4	AHYDILTGYWFDY	948	13	2-30	1	MQGTHWPPT	985	9	+	+	-
neM20-K05	3-30	3-22	2	4	SGDSSGYYFDY	949	11	3-15	4	QQYNNWPLT	193	9	-	-	-
neM20-K06	3-30-3	6-13	2	4	TAGYSSSWFDY	950	11	1-16	3	QQYNSYPFT	420	9	-	-	-
neM20-K07	3-74	2-2	3	4	DIVVVPAATPGVSGFDY	951	17	1-27	3	QKYNSAPFT	986	9	-	-	-
neM20-K10	1-8	1-26	1	3	GPSVVMWELSSDAFDI	952	16	3-15	2	QQYNNWPLYT	422	10	-	-	-
neM20-K11	4-59	6-19	2	5	EWAGYSSGWSDYNWFDP	953	17	1-17	1	LQHNSYPRT	809	9	-	-	-
neM20-K13	4-31	7-27	3	2	SAGDWGWYFDL	954	11	3-20	2	QQYGSSPLYT	426	10	-	+	-
neM20-K14	4-34	4-11	2	4	VGDYSNYPADY	955	11	3-11	4	QQRSNWPPLT	685	10	-	-	-
neM20-K22	3-23	6-13	2	4	TYSSSWLYIDY	956	11	3-15	1	QQYNNWPPVWT	987	11	+	+	-
neM20-K23	3-21	6-13	2	5	DLVGYSSSWNWFDP	957	14	1-NL1	2	QQYYSTPYT	934	9	+	-	-
neM20-K27	3-23	3-22	2	4	DLYYYDSSGYPLGGGFDY	958	18	3-20	2	QQYGSSPYT	376	9	-	-	-
neM20-K32	4-59	7-27	3	4	GTKQELGKYYFDY	959	13	3-15	1	QQYNNWT	988	7	-	-	-
neM20-K34	3-23	6-6	1	4	AGQLEPHYFDY	960	11	1-5	1	QQYNSYSRT	190	9	-	-	-
neM20-K36	1-8	3-22	2	4	GLSYYDSRGDYFDY	961	14	3-20	1	QQYGSSPGT	127	9	-	-	-
neM20-K37	3-48	1-26	3	4	NNGATTWRADY	962	11	3-20	5	QQYGSSPIT	989	9	-	-	-
neM20-K39	3-23	2-15	2	3	DWGDNCSGGSCYSNKVPDAFDI	963	22	1-5	1	QQYNSYRT	990	8	-	-	-
neM20-K41	3-66	4-17	2	4	NEGYGDYLYYFDY	964	13	3-11	3	QQRSNCT	991	7	-	+	-
neM20-K45	3-72	1-26	3	5	ERVAIGIVGATTARGGQQYNWFDP	965	24	3-11	3	QQRSNWPT	184	8	-	+	-

Figure 33

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neM20-K47#	3-53	3-22	2	4	QLLNYYDSSGYYYY	966	14	3-20	2	QQYGSSPLVT	992	10			
neM20-K48	4-34	/	/	4	HGSGTYQHFDY	967	11	3-11	2	QQRSNWYT	993	8	-	-	-
neM20-L01	4-39	3-22	2	5	HFPAIGDSSGYVGAYNWFDP	968	20	8-61	3	VLYMGSGVWV	994	10	-	-	-
neM20-L08	1-2	6-13	3	3	EPNLAAAGTKKNAFDI	969	16	2-14	1	SSYTSSSTLV	74	10	-	-	-
neM20-L12	4-39	1-26	3	5	HFEVGAHRFDP	970	11	3-1	2	QAWDSSTYVV	204	10	-	-	-
neM20-L15	3-23	6-13	3	6	DGRGIAAAGTPYYYGMDV	971	18	3-1	1	QAWDSSTYV	204	9	-	-	-
neM20-L16	3-48	7-27	3	3	DLTGDRGNAFDI	972	12	3-21	3	QVWDSSSDHWV	654	11	-	-	-
neM20-L17	3-9	2-21	3	3	DFGAIVVVTDAFDI	973	14	2-11	2	CSYAGSYTVV	995	10	-	-	-
neM20-L18	3-33	5-5	3	6	GASDTAMVTYYYYGMDV	974	17	2-14	1	SSYTSSSTLV	74	10	-	+	-
neM20-L19	3-73	6-19	3	3	HAPPPIAVAGTVAFDI	975	16	1-44	3	AAWDDSLNGWV	286	11	-	-	-
neM20-L24	3-15	3-3	3	4	EVWSGYTIFGVVIY	976	14	2-14	3	SSYTSSSTWV	388	10	-	+	-
neM20-L26	3-21	6-19	2	5	TGYSSGWNWFDP	977	12	2-11	1	CSYAGSYTYV	136	10	-	-	-
neM20-L28	4-61	3-10	2	5	FGSSPHHSGSAVDWFDP	978	17	1-36	2	AAWDDSLNGVV	240	11	-	-	-
neM20-L29	3-23	3-22	2	3	DRVDYYYDSSGYPTDAFDI	979	19	1-44	2	AAWDDSLNVV	996	10	-	+	-
neM20-L31	3-15	/	/	4	DPPGKGTQSFDY	980	12	2-14	3	SSYTSSSTWV	388	10	-	-	-
neM20-L42	3-30	6-13	3	2	AGGPLVRYAAAGYWYFDL	981	18	2-23	1	CSYAGSSTYV	70	10	+	+	-
neM20-L43	4-34	3-10	3	5	RGTMVRGVNVGWFDP	982	15	2-11	3	CSYAGSYTWV	339	10	-	-	-
neM20-H25	3-66	5-5	2	4	DLGYSYGYTDY	983	11				--				
neM20-H30	3-49	3-9	2	4	DQYDILTGFDY	984	11				--				

Figure 33 (Cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #21 GFP-

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP-M21-K2	3-30	4-23	2	3	GASPYGGNSGAFDI	997	14	1-5	2	QQYNSYST	611	8	+	-	-
neGFP-M21-K11	3-15	1-7	2	2	GPNYPNQSRYFDL	998	13	3-15	2	QQYNNWPPYT	331	10	+	+	-
neGFP-M21-K12	1-69	2-15	2	4	KTDGGGGSSSHFDY	999	14	3-15	3	QQYNNWPPSST	1033	11	-	-	-
neGFP-M21-K13	3-30	3-22	3	3	DPTSVVVMSDAFDI	1000	14	3-15	1	QQYNNWLWT	1034	9	+	+	-
neGFP-M21-K14	3-23	6-19	1	1	DTVGGWLGKYFQH	1001	13	1-39	4	QQSYSTPLT	56	9	+	+	-
neGFP-M21-K17	4-39	1-26	2	4	RSGSNRGLGYFDY	1002	13	3-15	1	QQYNNWPPWT	238	10	+	+	-
neGFP-M21-K18	3-7	4-23	3	2	TNTVVGYWYFDL	1003	12	1-12	1	QQANSFPWT	1035	9	+	+	-
neGFP-M21-K19	3-23	2-21	2	4	DRGGDYFDY	1004	9	1-5	2	QQYNSYSHT	1036	9	-	-	-
neGFP-M21-K20	1-2	6-6	3	4	PGSIAARVLDY	1005	11	4-1	2	QQYYSTPYT	934	9	-	+	C
neGFP-M21-K23	4-39	6-13	3	4	HRGIAAAGDY	1006	10	1-39	1	QQSYSTPWT	44	9	-	+	-
neGFP-M21-K24	4-39	2-15	3	5	HPIQDIVVVVAANWFDP	1007	17	3-11	3	QQRNLVT	1037	7	-	-	-
neGFP-M21-K25#	4-61	3-22	2	6	DRYYDSSGYSDYYYGMDV	1008	18	3-20	2	QQYGSSPYT	376	9			
neGFP-M21-K29	3-23	6-19	3	4	DPGGSIAVAGDY	1009	12	3-11	4	QQRSNWLT	197	8	-	-	-
neGFP-M21-K30	4-4	4-17	2	6	VGWEDDYGDQGGRYYYYGMDV	1010	21	2-28	4	MQALQTLT	1038	8	-	-	-
neGFP-M21-K33	4-31	4-17	3	2	GAMTTVTTGGPAAGYFDL	1011	18	1-39	1	QQSYSTPGP	1039	9	-	+	-
neGFP-M21-K34	1-18	5-24	2	4	AGRRDGYNYYFDY	1012	13	3-15	2	QQYNNWPYT	189	9	-	+	-
neGFP-M21-K39	3-64	3-9	2	5	DPHYDILTGYSHNWFDP	1013	17	2-28	2	MQALQTPYT	1040	9	-	-	-
neGFP-M21-K40#	3-74	5-24	3	6	DRVEMATIYYYYYGMDV	1014	17	2-28	1	MQALQTPRT	1041	9			
neGFP-M21-K42	3-33	3-22	2	4	DAHYYDSSGYPPAYYFDY	1015	18	1-5	1	QQYNSYPWT	1042	9	+	+	-

Figure 34

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP-M21-K45	4-61	4-23	3	4	EGVVTKFPDY	1016	10	1-17	1	LQHNSYPWT	467	9	-	-	-
neGFP-M21-K46	4-61	2-2	2	4	WGVSNSCSSTSCYSSRLYYFDY	1017	22	1-17	3	LQHNSYLFT	1043	9	-	+	-
neGFP-M21-K47	3-11	/	/	4	DTGHTGDY	1018	8	1-39	2	QQSYSTPRT	615	9	-	+	-
neGFP-M21-K48	3-30	2-2	2	3	GYCSSTSCYRGAFDI	1019	15	3-20	3	QQYGSSPFT	181	9	-	+	-
neGFP-M21-L1	3-33	2-8	2	4	GNVGYCTNGVCSSISFDY	1020	18	1-51	1	GTWDSSLSAHYV	1044	12	-	+	-
neGFP-M21-L3	4-61	5-5	2	5	EFKRGYSYGYWWFDP	1021	15	1-51	3	GTWDSSLSAGV	78	11	+	+	N
neGFP-M21-L4	3-23	3-3	3	4	STIFGVVITPFDY	1022	13	2-11	3	CSYAGSVV	1045	8	-	-	-
neGFP-M21-L5	3-11	4-4	3	5	VGGLRPTVTTGYNWFDP	1023	17	2-14	3	SSYTSSSTWV	388	10	-	-	-
neGFP-M21-L6	3-30	4-17	2	4	PKADYGDYTPAQYYFDY	1024	17	2-11	2	CSYAGSYHVV	850	10	-	-	-
neGFP-M21-L8	3-9	/	/	6	VTHPLNYYYGMDV	1025	13	1-47	3	AAWDDSLSGPV	526	11	-	-	-
neGFP-M21-L9	3-30	3-10	1	4	DRGRFGEGYFDY	1026	12	1-51	3	GTWDSSLSAWV	1046	11	-	-	-
neGFP-M21-L10	3-30	3-3	2	4	DSFPRFWSGYCSFDY	1027	15	1-44	1	AAWDDSHYV	1047	9	-	+	-
neGFP-M21-L16	3-74	2-2	1	6	DRVVPHRGTRQLNGMDV	1028	17	2-11	2	CSYAGSYTLV	1048	10	-	-	-
neGFP-M21-L22	3-11	3-9	2	5	DRTPTYDILTGQLNWFDP	1029	18	2-8	2	SSYAGSNNVV	1049	10	-	-	-
neGFP-M21-L27	3-23	2-2	2	4	SLNCSSTSWYLPRVGFDY	1030	18	2-14	3	SSYTSSSIWV	1050	10	-	-	-
neGFP-M21-L38	3-21	6-6	3	4	VVAARWDIYYFDY	1031	13	2-14	2	SSYTSSSIPVV	1051	11	-	-	-
neGFP-M21-L43	3-33	4-4	2	5	GSEKNDYSNYMNWFDP	1032	16	1-51	3	GTWDSSLSAGV	78	11	-	-	-

Figure 34 (Cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #21 GFP+ shRNA PTPN22

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M21-K49	3-15	6-19	3	4	DRYVAVAGTVDY	1052	12	3-11	3	QQRSNLFT	1079	8	-	-	-
neGFP+M21-K58	4-4	6-6	2	4	AGPYSSSYYFDY	1053	12	1-17	1	LQHNSYPWT	467	9	-	+	-
neGFP+M21-K65#	1-46	2-15	1	4	TLRGWCPDY	1054	9	1-16	2	QQYNSYPHT	1080	9			
neGFP+M21-K67#	3-48	4-4	2	4	ADDYSNYFDY	1055	10	1-33	4	QQYDNLPLT	1081	9			
neGFP+M21-K68#	3-30	2-8	2	6	DGANGARLGGHYYYYYGMDV	1056	20	3-11	5	QQRSNWPPIT	1082	10			
neGFP+M21-K71	3-11	2-2	2	6	DHTARGACSSTSCYIYYYGMDV	1057	22	1-17	2	LQHNSYPYT	282	9	-	+	-
neGFP+M21-K74#	3-33	6-19	1	3	DYFFGQWLAAGDAFDI	1058	16	2-30	3	MQGTHWPRVT	1083	10			
neGFP+M21-K77	3-7	6-13	3	4	GVAAGIFGY	1059	9	3-20	4	QQYGSSPLT	122	9	+	+	-
neGFP+M21-K86	4-61	2-21	3	1	TYFVVVMEAEYFQH	1060	14	3-11	4	QQRRTWSLT	1084	9	-	-	-
neGFP+M21-K87	3-30	2-15	3	4	DGGRGVVAATLLYFDY	1061	16	1-8	4	QQYYSFPLT	235	9	-	+	-
neGFP+M21-K90	3-21	6-6	2	6	DLSEYSSSSSFFRYYGMDV	1062	19	1-39	3	QQSYSTPQT	1085	9	-	+	-
neGFP+M21-L51	3-30	6-19	2	4	DGRYSSGWSYFDY	1063	13	1-40	2	QSYDSSLSGSV	135	11	-	+	-
neGFP+M21-L52#	3-23	3-9	2	6	EPPPSYDILTGSNYYYYGMDV	1064	21	1-44	3	AAWDDSLNGWV	286	11			
neGFP+M21-L60	4-61	2-2	2	5	VSGCSSTSCFGGWFDP	1065	16	2-23	3	RSYAGSSTWV	1086	10	-	-	-
neGFP+M21-L62	3-23	3-22	2	3	VDTETDDSSGYAFDI	1066	15	1-47	2	AAWDDSLSGVV	290	11	-	-	-
neGFP+M21-L64	3-23	2-15	2	3	DRCSGGSCPDAFDI	1067	14	2-23	1	CSYAGSSTLYV	1087	11	-	-	-
neGFP+M21-L69	4-59	4-17	2	2	DRNGDYPWYFDL	1068	12	1-40	2	QSYDSSLSVV	1088	10	-	-	-
neGFP+M21-L75	1-18	4-23	3	4	GAVVTPGYFDY	1069	11	2-14	1	SSYTSSKV	1089	8	-	-	-
neGFP+M21-L79	3-30	2-21	2	4	SYCGGDCYFDY	1070	11	1-40	1	QSYDSSLSGYV	64	11	-	-	-
neGFP+M21-L81#	1-69	5-24	2	4	PSGDGYNYLEY	1071	11	2-14	3	SSYTSSSTWV	388	10			
neGFP+M21-L82	3-20	3-22	2	4	VQSNPLYYYDSSGYYYDY	1072	18	2-14	1	SSYTSSNYV	1090	9	-	-	-
neGFP+M21-L83	1-81	2-2	2	3	VEPPTRVAHCSSTSCYYLGAFDI	1073	23	2-14	1	SSYTSSSYV	1091	9	-	-	-
neGFP+M21-L84	4-34	4-17	2	3	GLYGDYVDAFDI	1074	12	1-44	3	AAWDDSLNGWV	286	11	-	-	-
neGFP+M21-L85	4-39	3-10	2	6	HGVHLPPYGSGSYYYYYGMDV	1075	21	1-40	3	QSYDSSLSGWV	345	11	-	-	-
neGFP+M21-L89	1-3	6-13	3	4	GKIAAAGFDY	1076	10	3-21	2	QVWDSSSDHPV	575	11	-	-	-
neGFP+M21-L91	1-46	4-4	3	4	TTTVIYYFDY	1077	10	2-11	1	CSYAGSYTYV	136	10	-	-	-
neGFP+M21-L96	3-48	4-17	2	4	GKYQEPYYGDYFPTRGFDY	1078	19	1-51	1	GTWDSSLSAYV	75	11	-	-	-

Figure 35

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #22 GFP-

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP-M22-K01	3-23	1-26	3	5	RKGATGDWFDP	1092	11	1-8	2	QQYYSYPYT	380	9	+	+	-
neGFP-M22-K02#	4-34	3-22	2	4	SDDSSGYYRSKAFDY	1093	15	3-11	1	QQRSTWPPWT	520	10			
neGFP-M22-K09	3-15	2-15	2	6	APSGYCSGGSCYYYYGMDV	1094	19	2-28	2	MQALQTPYT	1040	9	-	+	-
neGFP-M22-K16#	1-2	3-10	2	6	LYYYGSGSYSQYYYYGMDV	1095	19	3-11	4	QQRSTWPPLT	1129	10			
neGFP-M22-H25	4-59	2-8	1	2	STGYYWYFDL	399	10	4-1	1	QQYYDTPRA	1130	9	-	-	-
neGFP-M22-K29#	1-2	2-2	3	4	ESDIVVVPAASRASRYYDY	1096	19	3-15	2	QQYNNWPSYT	1131	10			
neGFP-M22-K35	3-33	3-22	2	4	VSRDYYDSSGYYGPFADY	1097	18	1-12	4	QQANSFPLT	774	9	-	+	-
neGFP-M22-K40#	3-53	5-12	3	5	DSGGVATWGQGTLVTVSSARTSQDP	1098	25	1-8	1	QQYYSYPRT	772	9			
neGFP-M22-K41	3-30	3-10	3	5	DRRRITMVRGVTNWFDP	1099	17	2-28	1	MQALQTPWT	1132	9	+	+	-
neGFP-M22-K43#	3-9	3-10	2	2	DMRMHYGSGGYWYFDL	1100	16	3-15	2	QQYNNWPPYT	331	10			
neGFP-M22-K44#	1-2	6-6	1	4	ALIWQLATSPLDY	1101	13	1-5	1	QQYNSYSQWT	1133	10			
neGFP-M22-K47#	4-34	3-9	2	5	EREPYYDISNWFDP	1102	14	3-11	4	QQRSTWPLT	893	9			
neGFP-M22-K22						--		1-39	2	QQSYSTPYT	130	9			
neGFP-M22-L03	3-9	3-10	2	3	GRVYDILTIGSGSLYAFDI	1103	19	2-14	2	SSYTSSSTLV	74	10	-	-	-
neGFP-M22-L04	3-30	2-15	2	5	PYCSGGSCYSVGSRALYNWFDP	1104	22	2-14	1	SSYTSSSTLYV	291	11	-	-	-
neGFP-M22-L05	1-2	2-2	3	6	EAVVPARSGMDV	1105	12	2-11	2	CSYAGSYTVV	995	10	-	-	-
neGFP-M22-L06#	1-18	5-12	2	4	DPGSGYDGNDY	1106	11	3-25	3	QSADSSGTWV	894	10			
neGFP-M22-L11	3-9	/	/	4	DRQMPQGIGAQGFDY	1107	15	3-1	2	QAWDSSTVV	143	9	-	+	-
neGFP-M22-L12#	3-23	6-13	2	6	HSGYSSSWPNVMDV	1108	14	3-21	2	QVWDSSSDHVV	77	11			
neGFP-M22-L13	3-33	6-13	2	3	GTRQDSSSWWGHAFDI	1109	16	1-51	1	GTWDSSLSAYV	75	11	-	-	-
neGFP-M22-L14#	1-69	6-13	2	5	EGGVSSSSWPYNWFDP	1110	16	1-40	3	QSYDSSLSGWV	345	11			
neGFP-M22-L18#	3-9	6-13	3	4	DGAAAGLRY	1111	9	1-44	3	AAWDDSLNGWV	286	11			
neGFP-M22-L19#	3-33	6-6	1	6	DRTQQLVPYYYYGMDV	1112	16	2-8	2	SSYAGSNNLV	66	10			

Figure 36

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP-M22-L20	1-2	3-10	1	4	DLLRFGELWDY	1113	11	1-47	1	AAWDDSLSGPV	526	11	-	+	C
neGFP-M22-L21	3-9	2-15	2	4	DVTNGRDCSGGSCYSFDY	1114	18	1-44	2	AAWDDSLNGVV	240	11	-	-	-
neGFP-M22-L23	3-48	2-2	2	2	DTHCSSTSCYSDWYFDL	1115	17	2-23	3	CSYAGSST	343	8	-	-	-
neGFP-M22-L24	1-2	2-2	3	5	EGDIVVVPAAHGWFDP	1116	16	2-23	1	AGSSTYNYV	1134	9	-	-	-
neGFP-M22-L25	4-59	2-8	1	2	STGYYWYFDL	399	10	3-1	2	QAWDSSTVV	143	9	-	-	-
neGFP-M22-L26	4-34	6-13	2	1	PGYSSSWYYFQH	1117	12	2-14	3	SSYTSSSTL	1135	9	+	+	-
neGFP-M22-L27	3-11	3-16	3	5	GTLETYDLYPRLGRGLYNWFDP	1118	22	1-47	3	AAWDDSLSGWV	73	11	+	+	-
neGFP-M22-L28	3-33	3-10	2	4	YYYGSGSYLGLRALHFDY	1119	18	2-11	2	CSYAGSYTLVV	1135	11	-	+	-
neGFP-M22-L30	3-21	2-21	3	6	DRVVVVIAEGLGYYYYGMDV	1120	20	2-14	3	SSYTSSSRV	1136	9	-	-	-
neGFP-M22-L31	3-30	3-10	1	3	GLKFGAVRSAFDI	1121	13	1-51	3	GTWDSSLSTNWV	1137	12	+	+	-
neGFP-M22-L39#	4-34	6-13	2	3	GCRNSSSWYVEKSDAFDI	1122	18	1-44	2	AAWDDSLNGHVV	72	12			
neGFP-M22-L46	3-23	3-22	2	6	PPYYYDSSGYYLYYGMDV	1123	18	1-40	2	QSYDSSLSGVV	71	11	-	-	-
neGFP-M22-L48#	3-33	5-5	2	3	GRAWHSYGDDAFDI	1124	14	1-51	1	GTWDSSLSAYV	75	11			
neGFP-M22-L10						--		1-40	3	QSYDSSLSGWV	345	11			
neGFP-M22-L38						--		3-1	2	QAWDSSTVV	143	9			
neGFP-M22-L08	7-4	2-2	1	5	GSEYQLLRGWFDP	1125	13				--				
neGFP-M22-H17	3-30	1-26	3	6	VLPVGAGHYYYGMDV	1126	15				--				
neGFP-M22-H29	1-2	2-2	3	4	ESDIVVVPAASRASRYYDY	1096	19				--				
neGFP-M22-H34	5-51	1-7	2	4	LWGLYNWNYFDY	1127	12				--				
neGFP-M22-H35	3-33	3-22	2	4	VSRDYYDSSGYYGPFADY	1097	18				--				
neGFP-M22-H41	3-30	3-10	3	5	DRRRITMVRGVTNWFDP	1099	17				--				
neGFP-M22-H43	3-9	3-10	2	2	DMRMHYGSGGYWYFDL	1100	16				--				
neGFP-M22-H44	1-2	6-6	1	4	ALIWQLATSPLDY	1101	13				--				
neGFP-M22-H47	4-34	3-9	2	5	EREPYYDISNWFDP	1102	14				--				

Figure 36 (Cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #22 GFP+ PTPN22 shRNA

Ig	HEAVY CHAIN								LIGHT CHAIN					REACTIVITY		
	VH	D	RF	JH	(-)	CDR3 (aa)	SEQ ID NO	(+)	Length	Vκ	Jκ	CDR3(aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M22-K49	3-30-3	3-10	2	3	2	GAYYGSGSYYKDAFDI	1138	1	16	1-17	4	LQHNSYPLT	335	9	-	+	-
neGFP+M22-K52	3-11	6-6	2	4	2	AGSDSSSSAVFDY	1139	0	13	1-33	2	QQYDNLP	545	7	-	-	-
neGFP+M22-K54#	1-18	1-26	2	6	2	DPRVPGGSYYYYGMDV	1140	1	16	1-16	4	QQYNSYPLT	125	9			
neGFP+M22-K55	3-21	/	/	4	1	EPAPLGVAGGY	1141	0	11	1-17	3	LQHNSYPFT	432	9	-	+	-
neGFP+M22-K56	3-11	3-3	2	3	3	LPFLDLFWSGYYADAFDI	1142	0	18	3-15	4	QQYNNWPLT	193	9	-	+	-
neGFP+M22-K63	3-7	/	/	4	3	VQDNRAPDFDY	1143	1	11	1-17	1	LQHNSYPWT	467	9	-	+	-
neGFP+M22-H65	1-2	6-13	2	4	1	AGGGSSSWYYFDY	1144	0	13	3-20	5	QQYGSSPIT	989	9	-	-	-
neGFP+M22-K68	3-7	6-13	2	4	1	SKYSSSWYGDY	1145	1	11	3-11	1	QQRSNWW	1176	7	-	+	-
neGFP+M22-K72	4-34	6-13	3	6	1	GNRLIAAAGSYYYYGMDV	1146	1	18	1-39	4	QQSYSTLT	1177	8	-	-	-
neGFP+M22-K79	3-23	3-10	2	4	1	VTNYGSGSYYNGFDY	1147	0	15	1-39	1	QQSYSTWT	1178	8	-	-	-
neGFP+M22-H80	3-30-3	6-13	3	1	2	DLSDSIAAAGVGYFQH	1148	1	16	3-20	3	QQYGSSPT	43	8	-	-	-
neGFP+M22-K83	3-74	4-4	3	4	1	LLTVTSYFDY	1149	0	10	1-13	5	QQLNSYPPIT	1179	10	-	-	-
neGFP+M22-K84	4-59	6-13	2	4	1	GPYSSSWFSIDY	1150	0	12	1-39	2	QQSYSTPYT	130	9	-	-	-
neGFP+M22-K85	3-15	1-26	2	6	2	DRVGGSYYYYYGMDV	1151	1	15	1-39	5	QQSYSTPPIT	1180	10	-	-	-
neGFP+M22-K89	1-2	1-26	1	4	2	GQWELDY	1152	0	7	3-20	4	QQYGSSLT	279	8	-	-	-
neGFP+M22-K92	3-33	7-27	2	4	2	DRNWGFDY	1153	1	8	3-20	2	QQYGSSPYT	376	9	-	-	-
neGFP+M22-K94#	1-2	/	/	4	1	GLLGLFDY	1154	0	8	2-30	2	MQGTHWPYT	1181	9			
neGFP+M22-K95#	4-39	3-10	1	4	2	LGFGELSHDY	1155	1	10	1-39	5	QQSYSTPPIT	1180	10			
neGFP+M22-K51							--			3-11	4	QQRSNWPPVT	1182	10			
neGFP+M22-K75							--			2-30	1	MQGTHWPPT	985	9			
neGFP+M22-L50	3-33	5-5	2	2	2	DSMGQSTGYSYGYPYWYFDL	1156	0	20	1-40	2	QSYDSSLSGHVV	1183	12	-	-	-

Figure 37

Ig	HEAVY CHAIN								LIGHT CHAIN					REACTIVITY		
	VH	D	RF	JH	(-)	CDR3 (aa)	SEQ ID NO	(+)	Length	Vκ	Jκ	CDR3(aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M22-L53	3-30	6-19	2	3	3	CYSSGWDDAFDI	1157	0	12	1-47	3	AWDDSLSGWV	1184	10	-	-	-
neGFP+M22-L60#	7-4-1	3-10	3	5	1	PSITMVRGVIIFNWFDP	1158	1	17	1-40	2	QSYDSSLSGDVV	1185	12			
neGFP+M22-L61	1-2	6-19	3	4	0	SNFQYSYGYY	1159	0	10	2-8	1	SSYAGSNNYV	289	10	+	+	-
neGFP+M22-L62	1-2	6-13	2	4	3	DRVKDGGSSWPDY	1160	2	13	2-14	2	SSYTSSSTLV	74	10	-	-	-
neGFP+M22-L64	3-7	1-7	3	5	2	ETGITGTTGWFDP	1161	0	13	3-1	1	QAWDSSTAYV	576	10	-	-	-
neGFP+M22-L66	4-59	6-13	1	6	1	VLSGGQQHPSYYYYYGMDV	1162	1	19	1-51	1	GTWDSSLSARYV	1186	12	-	-	-
neGFP+M22-L70	3-48	/	/	4	1	GMLFLGWYYFDY	1163	0	12	2-11	2	CSYAGSYTLV	1048	10	-	-	-
neGFP+M22-L73	3-53	3-22	2	3	2	SSGYRHDAFDI	1164	2	11	2-23	2	CSYAGSSTHVV	895	11	-	-	-
neGFP+M22-L77	3-11	2-15	2	2	2	VGSWVDYWYFDL	1165	0	12	1-44	3	AAWDDSLNGWV	286	11	-	-	-
neGFP+M22-L81#	3-15	7-27	2	4	3	GPPNWGEQRQDDY	1166	1	13	2-14	2	SSYTSSSTVV	340	10			
neGFP+M22-L82	3-9	/	/	3	2	DSPASGLAFDI	1167	0	11	2-14	1	SSYTSSSTYV	285	10	-	-	
neGFP+M22-L96	3-48	3-3	2	4	1	GASGSGQIGGFDY	1168	0	13	6-57	3	QSYDSSNHWV	1187	10	-	-	
neGFP+M22-H58	4-34	3-22	2	3	2	HYYYDSSGYYARLNAFDI	1169	2	18				--				
neGFP+M22-H59	4-34	6-19	3	4	1	VSLHIAVAGTGPFDY	1170	1	15				--				
neGFP+M22-K69	1-2	7-27	3	3	4	EGTGDPDAFDI	1171	0	11				--				
neGFP+M22-H71	4-34	2-8	2	3	1	GLVVRCTNGVCYNHAFDI	1172	2	18				--				
neGFP+M22-H74	3-30-3	3-9	2	4	3	DQYYDILTGSAVPFDY	1173	0	16				--				
neGFP+M22-H76	4-30-2	/	/	2	2	VGGDRYWYFDL	1174	1	11				--				
neGFP+M22-H80	3-30-3	6-13	3	1	2	DLSDSIAAAGVGYFQH	1148	1	16				--				
neGFP+M22-H88	3-15	1-26	2	6	2	DRVGGSYYYYYGMDV	1151	1	15				--				
neGFP+M22-H91	3-33	2-21	2	6	3	DGQYCGGDCYSPYYYYYGMDV	1175	0	21				--				

Figure 37 (Cont'd)

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #23 GFP-

Ig	Heavy Chain							Light Chain					Reactivity		
	VH	D	RF	JH	CDR3 (aa)	SEQ ID NO	Length	Vκ	Jκ	CDR3 (aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP-M23-K51	4-31	5-5	2	4	EMGMAAGYSYGFDY	1188	14	1-17	3	LQHNSYPFT	432	9	+	-	-
neGFP-M23-K56	3-53	/	/	4	DRGEFDY	1189	7	3-15	2	QQYNNWPLYS	1218	10	+	+	-
neGFP-M23-K57	3-48	1-7	3	4	GRGITGTNYFDY	1190	12	3-20	3	QQYGSSPPFT	1219	10	-	-	-
neGFP-M23-K58	3-33	6-6	3	4	DSSVVAARFDY	1191	11	1-39	3	QQSYSTPFT	58	9	-	+	-
neGFP-M23-K59	3-33	7-27	3	4	EGTGFDY	1192	7	1-5	4	QQYNSYSPLT	1220	10	+	+	-
neGFP-M23-K61	1-2	3-10	2	5	DLREYKGDWFDP	1193	12	3-20	4	QQYGSSPPLT	47	10			
neGFP-M23-K63	3-7	3-10	2	4	DGAYYYGSGSSTFDY	1194	15	3-20	3	QQYGSSLFT	1221	9	-	-	-
neGFP-M23-K64	3-7	3-22	2	4	GRRNFSRGDYYDSSGYYTTPFDY	1195	23	1-5	2	QQYNSYSPNS	1222	10	-	-	-
neGFP-M23-K65	3-30	2-8	2	3	DKTGASRLGYCTNGVCPDAFDI	1196	22	3-15	2	QQYNNWPPYS	885	10	-	+	-
neGFP-M23-K67	3-21	6-19	2	3	DSGYSSGWSHDAFDI	1197	15	3-20	1	QQYGSSPRT	54	9	-	+	-
neGFP-M23-K68	1-46	6-13	3	4	DWPGAAAGLDY	1198	11	3-20	4	QQYGSSPPLT	47	10	-	-	-
neGFP-M23-K71	4-34	3-22	3	3	GLFITMIVVGDAFDI	1199	15	2-24	2	MQATQFPRT	1223	9			
neGFP-M23-K72	1-2	2-2	3	4	DKIVVVPAAMGGNYFDY	1200	17	1-8	3	QQYYSYPLFT	1224	10	+	+	-
neGFP-M23-K75	3-48	1-26	1	3	ERWELRTDAFDI	1201	12	3-15	2	QQYNNWPPYS	885	10	-	+	-
neGFP-M23-K76	3-7	2-15	3	3	DGAGVVVVAATLLDDAFDI	1202	19	3-20	4	MQGTHWPLT	53	9	-	-	-
neGFP-M23-K84	3-30	6-6	3	4	ISSIAALPDY	1203	10	1-17	4	LQHNSYPLT	335	9			
neGFP-M23-L54	3-7	3-16	2	3	KNWGGCYAFDI	1204	11	2-14	3	SSYTSSSTGV	743	10	-	+	-
neGFP-M23-L60	4-30-4	3-16	1	3	LNGLGAFDI	1205	9	1-47	3	AAWDDSLSGWV	73	11	-	-	-
neGFP-M23-L79	3-9	3-3	1	4	DIRFLEWFDY	1206	10	3-21	1	QVWDSSSDHYV	76	11	-	-	-
neGFP-M23-L80	1-2	1-26	1	4	DTAGGELLPYYFDY	1207	14	2-23	2	CSYAGSSTLV	206	10	-	-	-
neGFP-M23-L83	5-10	4-11	1	4	LLVGRGLQKHYYFDY	1208	15	2-14	2	SSYTSSSTVV	340	10	+	+	-
neGFP-M23-L89	1-8	6-13	3	4	GRAAGGIDY	1209	9	8-61	3	VLYMGSGIWV	1225	10	-	-	-
neGFP-M23-L90	3-11	3-10	3	5	IITMVRGVIITYNWFDP	1210	17	2-23	2	CSYAGSSTFVV	616	11	-	-	-
neGFP-M23-L94	1-8	7-27	2	4	GNWDGGLFHY	1211	10	1-51	2	GTWDSSLSAVV	201	11			
neGFP-M23-H50	3-21	6-19	2	2	PGYSSGWDYWYFDL	1212	14				--				
neGFP-M23-H52	4-61	1-26	3	3	EDIVGAIRRAFDI	1213	13				--				
neGFP-M23-H55	1-2	1-26	2	3	DGGSYSAFDI	1214	10				--				
neGFP-M23-H70	3-30-3	6-6	3	4	DLSPNLIAARDAIDY	1215	15				--				
neGFP-M23-H73	3-33	/	/	3	GLGRATSYGDAFDI	1216	14				--				
neGFP-M23-H78	3-23	/	/	4	DRPVDY	1217	6				--				
neGFP-M23-H80	1-2	1-26	1	4	DTAGGELLPYYFDY	1207	14				--				

Figure 38

Repertoire and reactivity of antibodies from new emigrant B cells of Mouse #23 GFP+ PTPN22 shRNA

Ig	HEAVY CHAIN									LIGHT CHAIN					REACTIVITY		
	VH	D	RF	JH	(-)	CDR3 (aa)	SEQ ID NO	(+)	Length	Vκ	Jκ	CDR3(aa)	SEQ ID NO	Length	Poly	HEp-2	Staining
neGFP+M23-K1	3-30-3	6-19	2	3	2	GRQVGGGWKDAFDI	1226	2	14	3-20	4	QQYGSSPPLT	47	10	-	+	-
neGFP+M23-K4	4-34	6-19	2	4	2	GLWSEGVYYFDY	1227	0	12	2D-29	4	MQSIQLPLT	1247	9	-	-	-
neGFP+M23-K5	1-69	1-26	3	3	1	HGGSQPGIVGATLAFDI	1228	1	17	3-15	4	QQYNNWPLT	193	9	+	+	-
neGFP+M23-K6	4-34	4-17	3	5	1	GTGTVTTNWFDP	1229	0	12	3-20	2	KRYGNSPYT	1248	9	-	-	-
neGFP+M23-K8	3-15	/	/	6	2	DRLHYYYYYMDV	1230	2	13	3-20	3	QQYGSSPFT	181	9	-	+	-
neGFP+M23-K11	4-34	/	/	4	1	GEGVLGY	1231	0	7	1-8	1	QQYYSYPRT	772	9	-	+	-
neGFP+M23-K15	3-30	5-24	3	4	3	SQFLREMATIRNLTTDDY	1232	2	19	1-16	2	QQYNSYPRT	1249	9	-	-	-
neGFP+M23-K22	3-33	6-13	3	4	2	EGAAAGTSYYFDY	1233	0	13	3-11	3	QQRSTWPPFT	1250	10	-	-	-
neGFP+M23-K28	3-15	7-27	1	4	2	DGLGIRGVADY	1234	1	11	1-39	3	QQSYSTPFT	58	9	-	-	-
neGFP+M23-K29	5-10-1	4-11	3	4	1	HGSRPVTTYDY	1235	2	11	1-9	2	QQLNSYPYT	1251	9	-	-	-
neGFP+M23-K31	3-15	/	/	3	1	SGTGAFDI	1236	0	8	1D-33	3	QQYDNLPPLFT	1252	11	-	-	-
neGFP+M23-K34#	3-30-3	1-26	1	1	2	VFLGSVRELLAEYFQH	1237	2	16	3-11	4	QQRSNWPRLT	1253	10			
neGFP+M23-K38#	3-21	7-27	1	3	2	AWGSDAFDI	1238	0	10	3-20	3	QQYGSSPPFT	1219	10			
neGFP+M23-L22						See Kappa	1239			2-14	2	SSYTSSSTVV	340	10	-	-	-
neGFP+M23-L23	3-33	3-3		3	2	TISDAFDI	1240	0	8	2-14	2	SSYTSSSTLV	74	10	-	-	-
neGFP+M23-L25	4-4	1-26	2	4	3	DEGHSGSYFDY	1241	1	11	3-9	2	QVWDSSTVV	1254	9	-	-	-
neGFP+M23-L26	3-21	/	/	3	3	DPGDAFDI	1242	0	8	1-44	3	AAWDDSLNVWV	1255	11	-	-	-
neGFP+M23-L27	3-7	/	/	4	1	KRGVFDY	1243	2	7	2-14	2	SSYTSSSTVV	340	10	-	-	-
neGFP+M23-L35	1-2	6-6	2	6	3	DSPIEYSSSSGPYYYYYYGMDV	1244	0	22	2-8	1	SSYAGSNNYV	289	10	-	-	-
neGFP+M23-L40	3-33	1-26	2	4	3	EGGDSGSYYFDY	1245	0	12	3-25	3	QSADSSGTYWV	577	11	-	-	-
neGFP+M23-L44	3-30	3-22	3	4	1	ASARIVVVITNVDY	1246	1	14				--				

Figure 39

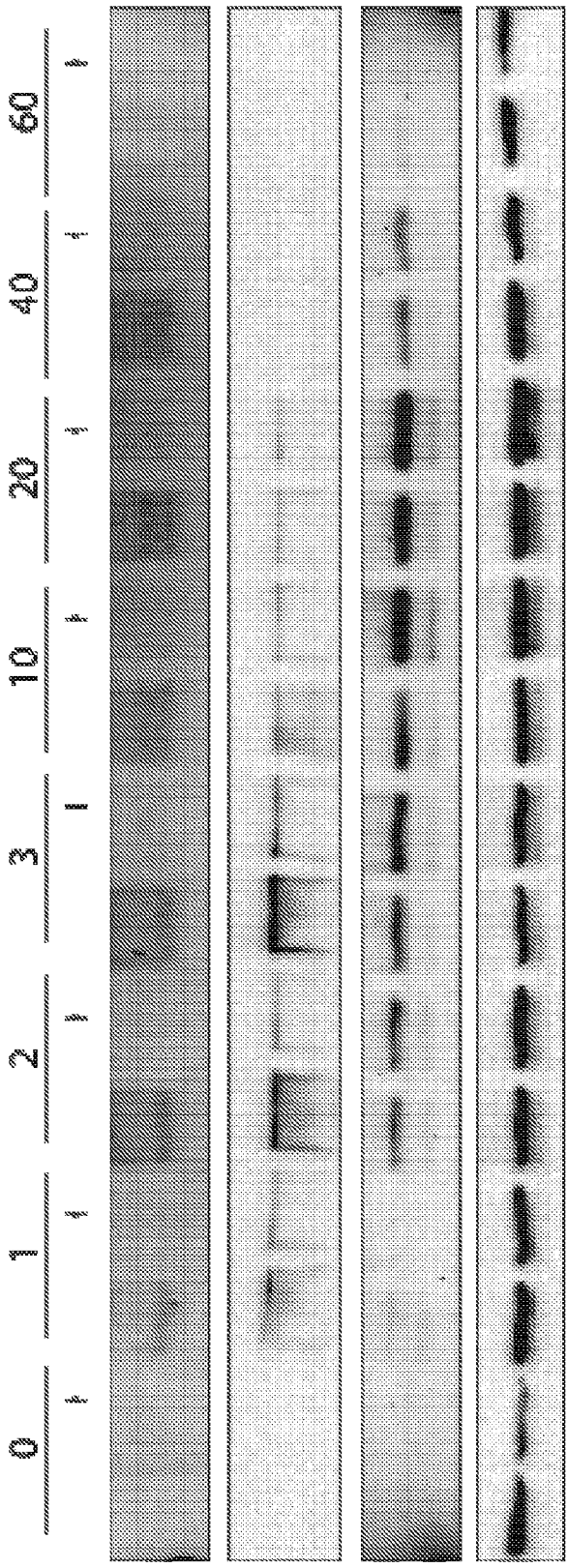
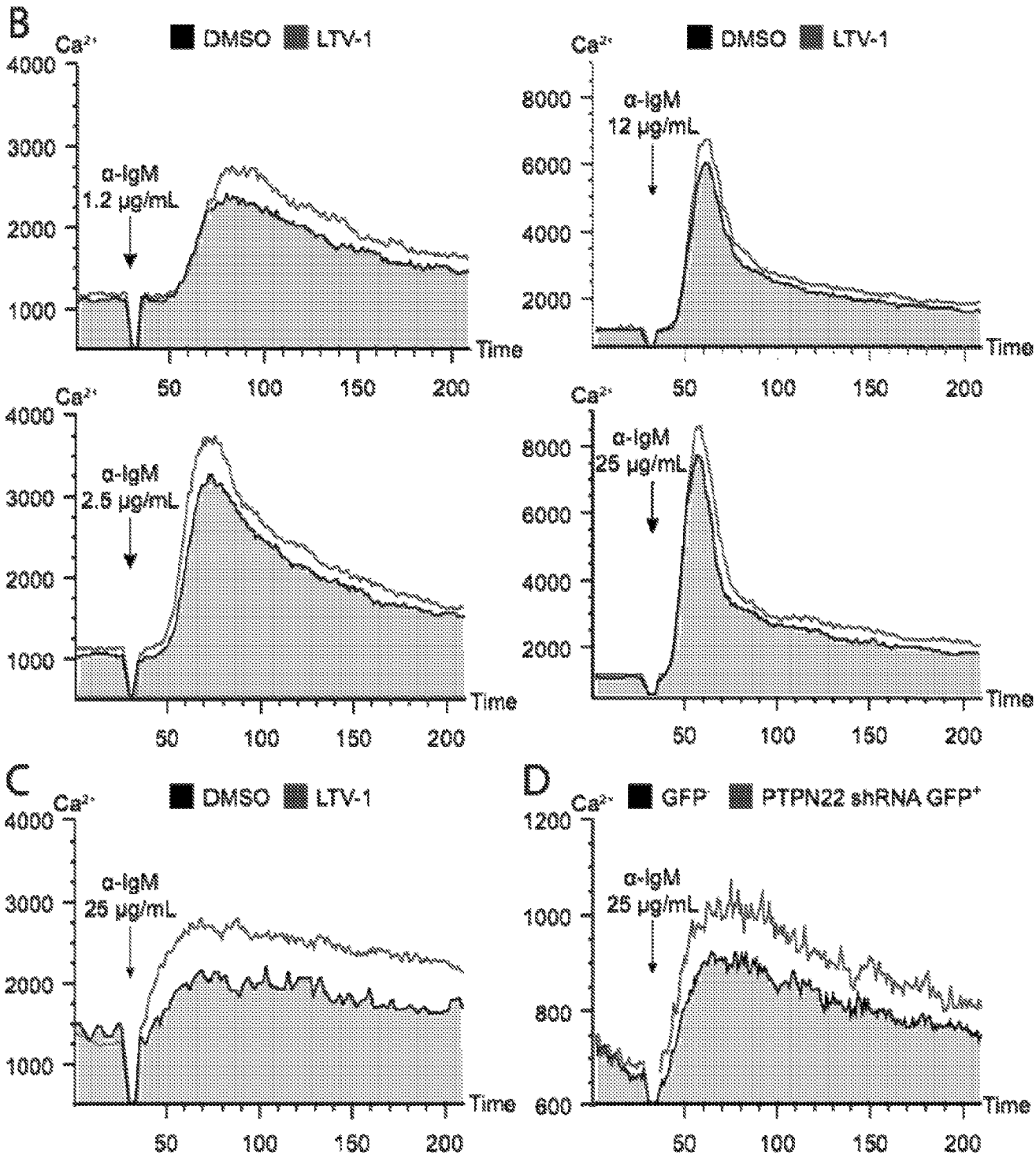
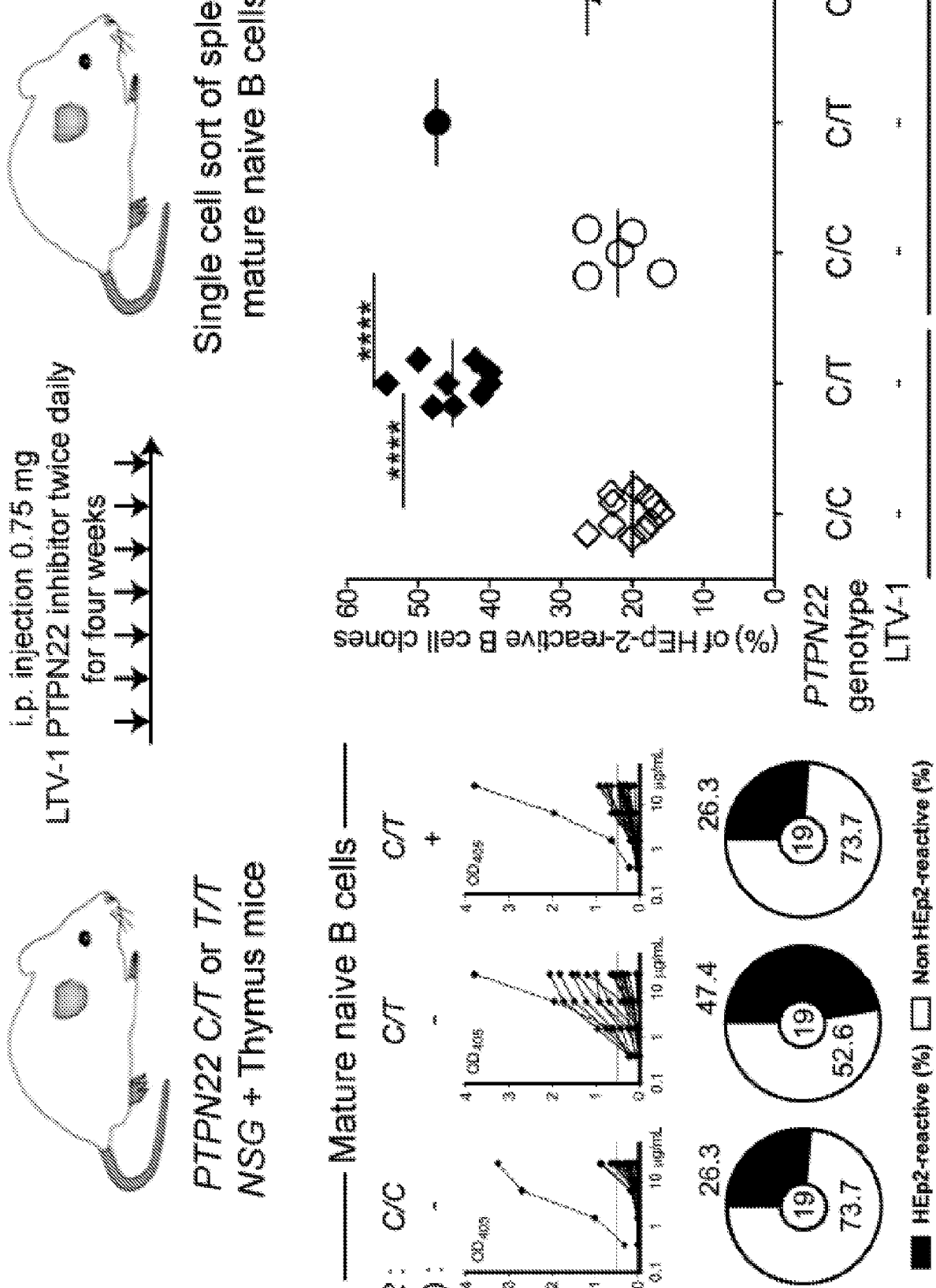

A
t [min]
0
1
2
3
10
20
40
60
LTV-1
P-LYN Y396
P-SHIP1 Y1020
P-ERK1/2 T202/Y204
β-ACTIN

Figure 40A



Figures 40B – 40D



Figures 41A – 41B

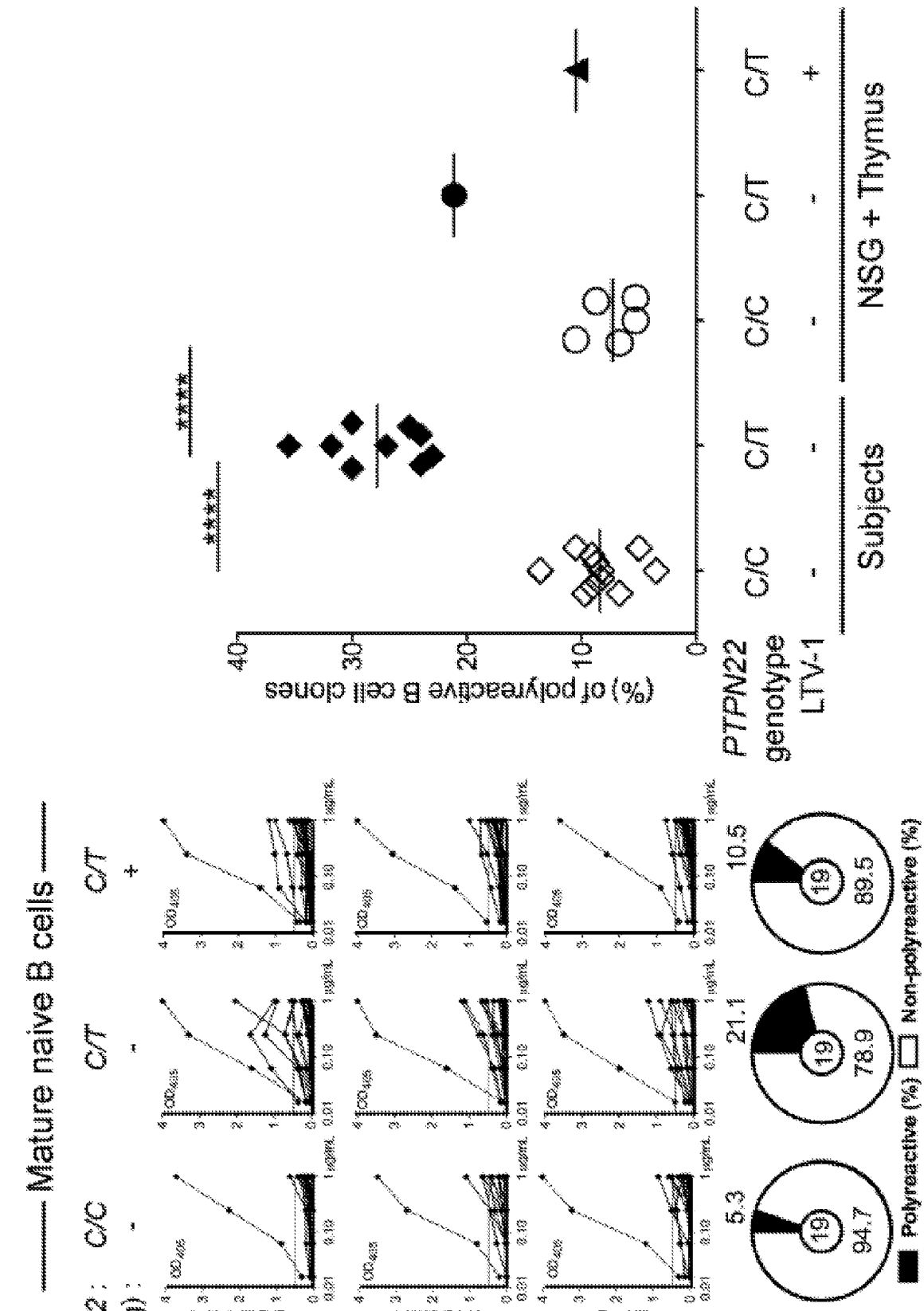

C
Mature naive B cells
PTPN22 : C/C C/T C/T
LTV-1 (mg) : - - +
Polyreactivity
dsDNA
Insulin
LPS
1 ug/mL
0.01
0.10
1
5.3
94.7
21.1
78.9
10.5
89.5
19
Polyreactive (%)
Non-polyreactive (%)
(%) of polyreactive B cell clones
40
30
20
10
0
****
****
PTPN22 genotype
LTV-1
C/C
C/T
C/C
C/T
C/T
-
-
-
-
+
Subjects
NSG + Thymus

Figure 41C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/34720

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/00, 39/00, 31/21, 31/215, 31/235, 31/24, 31/245, 31/33, 31/395, 31/495, 31/515 (2017.0

CPC - A61K 38/00, 38/005, 39/00, 39/0005, 39/0008, 31/21, 31/215, 31/235, 31/24, 31/245, 31/33, 31/395, 31/495, 31/515

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.
X --- Y	US 2010/0210836 (BOTTINI, N et al.) 19 August 2010; paragraph [0005], [0007]-[0008], [0017], [0038]-[0039]	1-3, 7-12, 13 ---------- 4-5, 14-19
Y	(MENARD, L et al.) The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. The Journal of Clinical Investigation. September 2011, Epub 3 June 2011, Vol. 121, No. 9; pages 3635-3644; Page 3642, 1st column, 1st paragraph - 2nd column, 1st paragraph: DOI: 10.1172/JCI45790	1-3, 7-8, 14-19
Y	(HE, R et al.) Small molecule tools for functional interrogation of protein tyrosine phosphatases. Federation of European Biomedical Societies Author Manuscript. January 2013, Epub 01 January 2014, Vol. 280, No. 2; pages 731-750; page 7, 2nd paragraph, compound 18, Figure 4: DOI: 10.1111/j.1742-4658.2012.09718.x.	4
Y	US 2014/0275220 A1 (THUM, T et al.) 18 September 2014; paragraph [0018], claim 10	5
A	US 2005/0221354 A1 (MOUNTS, WM) 6 October 2005; paragraph [0083], Attachment F. SEQ. ID NO: 81,344	6

☐ 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

"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
3 October 2017 (03.10.2017)	18 OCT 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/34720

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.:
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:
-***-Please see supplemental page-***-

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.:
Groups II, Claims 1-10; SEQ ID NO: 1 (shRNA inhibitor)

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/US17/34720

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

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 examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-19; and SEQ ID NO: 1 (shRNA inhibitor) are directed toward compositions and methods for inhibiting PTPN22 for restoring human central B-cell tolerance or for treating or preventing an autoimmune disease or disorder.

The PTPN22 inhibitor will be searched to the extent that it encompasses SEQ ID NO:1 (first exemplary shRNA inhibitor). Applicant is invited to elect additional shRNA inhibitor sequence(s), with specified SEQ ID NO: for each, to be searched. Additional shRNA inhibitor sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-5, 6 (in-part), and 7-19 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (shRNA inhibitor). Applicants must specify the claims that encompass any additionally elected shRNA inhibitor sequence(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/examined. An exemplary election would be a shRNA inhibitor encompassing SEQ ID NO: 2 (first exemplary elected shRNA inhibitor).

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

Groups I+ share the technical features including: a composition for treating or preventing an abnormal early B-cell tolerance checkpoint comprising an inhibitor of PTPN22; a method for treating or preventing an autoimmune disease or disorder the method comprising administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof; a method for restoring human central B-cell tolerance in a subject the method comprising administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof.

However, these shared technical features are previously shared by US 2010/0210836 A1 to Bottini, et al. (hereinafter 'Bottini') as further evidenced by the publication entitled 'The PTPN22 Allele Encoding an R620W Variant Interferes with the Removal of Developing Autoreactive B Cells in Humans' by Menard, et al. (hereinafter 'Menard').

Bottini discloses treating or preventing an abnormal early B-cell tolerance checkpoint (administering a small molecule inhibitor of PTPN22 (preventing an abnormal early B-cell tolerance checkpoint); paragraphs [0007], [0038]; as further indicated by the Menard Evidentiary reference small molecules inhibiting PTPN22 enzyme activity may reset the threshold for counterselection of developing autoreactive B cells by increasing BCR signaling in individuals carrying PTPN22 risk allele thereby preventing an abnormal early B-cell tolerance checkpoint; abstract; page 3642, first column, second paragraph) comprising an inhibitor of PTPN22 (injection or oral administration an inhibitor of PTPN22 (composition); paragraphs [0007], [0038]); a method for treating or preventing an autoimmune disease or disorder (a method for treating or preventing an autoimmune disease or disorder; abstract; paragraphs [0007], [0039]) the method comprising administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof (the method comprising administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof; paragraphs [0007], [0038]); a method for restoring human central B-cell tolerance in a subject (administering a small molecule inhibitor of PTPN22 (a method for restoring human central B-cell tolerance in a subject); paragraphs [0007], [0038]; as further indicated by the Menard Evidentiary reference small molecules inhibiting PTPN22 enzyme activity may reset the threshold for counterselection of developing autoreactive B cells by increasing BCR signaling in individuals carrying PTPN22 risk allele thereby restoring human central B-cell tolerance in a subject; abstract; page 3642, first column, second paragraph) the method comprising administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof (the method comprising injecting or orally administering a composition comprising an inhibitor of PTPN22 to a subject in need thereof; paragraphs [0007], [0038]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Bottini reference, unity of invention is lacking.