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(54) Title: PHARMACEUTICAL COMPOSITIONS TARGETING IMMUNE-MEDIATED PROCESSES IN NEURODEGENERATIVE DISEASE

(57) Abstract: The present disclosure in various aspects provides methods for making pharmaceutical compositions for treating neurodegenerative diseases (e.g., demyelinating diseases), such as but not limited to multiple sclerosis, neuromyelitis optica, and transverse myelitis. The pharmaceutical compositions impact specific antibody-mediated processes involved in the biology of neurodegenerative disease. In certain aspects, the disclosure provides pharmaceutical compositions for treating neurodegenerative disease, which are based on inhibiting the action of pathologic antibodies, or alternatively providing antibodies to stimulate neuroprotection or repair processes.



DESCRIPTION

PHARMACEUTICAL COMPOSITIONS TARGETING IMMUNE-MEDIATED PROCESSES IN NEURODEGENERATIVE DISEASE

RELATED APPLICATIONS

5 The subject matter of this application is related in-part to PCT/US2014/064533,
which was published as WO 2015/070009 on May 14, 2015. The subject matter of this
application is also related in-part to U.S. Provisional Application Serial No.
62/289,736, filed February 1, 2016, the disclosures of PCT/US2014/064533 and U.S.
Provisional Application Serial No. 62/289,736 are hereby incorporated by reference in
10 their entireties. This application also claims benefit of priority to U.S. Provisional
Application Serial No. 62/336,409, filed May 13, 2016, the entire contents of which are
hereby incorporated by reference.

BACKGROUND

B cells are recognized to play a role in multiple sclerosis (MS) pathology in
15 addition to the well-accepted pathological role of T cells. B cells and antibodies are
present in both the cerebrospinal fluid (CSF) and the central nervous system (CNS) of
patients with MS and clinically isolated syndrome (CIS) patients, who are at high risk
of developing MS. Further, the most common form of MS lesion is characterized by
deposition of antibodies and complement (Lucchinetti *et al.*, 2000), and plasmapheresis
20 treatment of patients harboring these lesions leads to symptom improvement (Keegan *et al.*,
2005). In fact, elevated B cells in the CSF correlates with lesion activity on MRI
(Cepok *et al.*, 2005) and both increased intrathecal immunoglobulin synthesis
(Sellebjerg *et al.*, 2000) and complement activation (Sellebjerg *et al.*, 1998) are
associated with a more aggressive disease course. Collectively these findings implicate
25 a pathological role for antibodies in the pathoetiology of MS.

A biomarker for conversion from CIS to clinically definite MS (CDMS) in the
antibody genetics of V_H4-utilizing B cells in the CSF, termed the antibody gene
signature (AGS) has been described (Cameron *et al.*, 2009). Also see, US Patent
8,394,583, which is hereby incorporated by reference in its entirety. B cells isolated
30 from CNS lesions harbor the AGS (Ligocki *et al.*, 2010). This shared pattern of
somatic hypermutation at about 6 codons along the V_H4 gene implicates that the B cell

pools are recognizing a shared set of antigens in the MS disease state that are not recognized by B cells in healthy individuals. However, there has been no isolation, description, or characterization of antibodies that fit the AGS criteria, which would be valuable reagents for understanding the pathology of MS as well as for developing next
5 of class therapeutics for MS and related neurodegenerative diseases.

SUMMARY

The present disclosure in various aspects provides methods for making pharmaceutical compositions for treating neurodegenerative diseases, and in particular neuroimmunological diseases (*e.g.*, demyelinating diseases), such as but not limited to multiple sclerosis, autoimmune encephalitis, neuromyelitis optica, optic neuritis, and transverse myelitis. The pharmaceutical compositions impact specific immune-mediated processes involved in the biology of neurodegenerative disease. In certain aspects, the disclosure provides pharmaceutical compositions for treating neurodegenerative disease, which are based on inhibiting the action of pathologic antibodies, or alternatively stimulating neuroprotection or repair processes by administering therapeutic antibodies.

In one aspect, the disclosure provides a method for making a pharmaceutical composition for treating a neurodegenerative disease. The process comprises providing one or more VH4 antibodies having at least one or at least two mutations with respect to the germline sequence at codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89. These antibody-encoding sequences, which are isolated from clinically diagnosed MS patients as well as patients with initial CIS presentation, encode antibodies that bind to one or more antigens in human white and gray matter. In other aspects, the antibodies are derived from peripheral plasmablasts of neurodegenerative disease patients (*e.g.*, MS, neuromyelitis optica, or transverse myelitis), and these antibodies may also be VH4 antibodies. The antibodies from peripheral plasmablasts may or may not exhibit the VH4 mutational signature. The methods comprise selecting an active agent that reduces or inhibits the expression of the antibody(ies), or reduces or inhibits binding of the antibody(ies) to a cellular target or antigen in human gray or white matter. Active agents selected by this process are formulated as a pharmaceutical composition for human treatment.

Candidate active agents can be tested for their ability to competitively inhibit binding of the VH4 antibody or the antibody from peripheral plasmablast to its antigen or cellular target. Binding of the antibody to its target can be assessed *in vitro* or *in vivo*. Various types of candidate agents can be screened or evaluated in accordance with these aspects, and these include anti-idiotypic antibodies or antigen-binding portions thereof, an antibody or portion thereof with binding specificity for the same

antigen (*e.g.*, gray matter antigen), as well as peptide agents, aptamers, or small molecules, among others.

5 The effect of the active agent on neurodegenerative disease pathology can be evaluated in an animal model. For example, using a suitable animal model in which the animal expresses or is administered the target antibody in a manner that mimics a neurodegenerative disease (*e.g.*, the animal may form CNS lesions similar to a demyelination process), the impact of the active agent on the pathology may be evaluated. In some embodiments, the active agent reduces or inhibits demyelination, inflammatory infiltrates, and/or axonal injury.

10 The active agent that is selected is formulated for administration to patients, for example, for administration by subcutaneous, intravenous, intramuscular, or intrathecal administration, or other route. The active agent is formulated at an amount that will effectively reduce disease activity or expression of selected antibodies *in vivo*.

15 In some embodiments, the antibodies (either AGS+ VH4 antibodies, or antibodies derived from plasmablasts as described) are tested in one more animal models to evaluate whether the antibody positively impacts neuroprotection or repair.

 The disclosure in other aspects provides pharmaceutical compositions made through these methods, including protein, peptide, antibody, aptamer, small molecule, and oligonucleotide therapeutics.

20 Other aspects and embodiments will be apparent from the following detailed disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with
5 the detailed description of specific embodiments presented herein.

FIGS. 1A-1D show that AGS-enriched rhAbs bind to mouse brain. DAB images are shown at 20x magnification of the cortex and corpus callosum. The rhAbs in each row are grouped as follows: controls (FIG. 1A), CDMS (FIG. 1B), ON_{CIS} (FIG.
10 1C), and TM_{CIS} (FIG. 1D). The rhAb designation, patient type, and patient number are shown in the upper right corner of each image. Data are representative of three coronal sections per rhAb. Scale bar represents 100 μ m.

FIGS. 2A-2D show that AGS-enriched rhAbs bind to human multiple sclerosis gray matter (GM) brain tissue. DAB images are shown at 20x magnification of MS-GM. The rhAbs in each row are grouped as follows: controls (FIG. 2A), CDMS (FIG.
15 2B), ON_{CIS} (FIG. 2C), and TM_{CIS} (FIG. 2D). The rhAb designation, patient type, and patient number are shown in the upper right corner of each panel. Data are representative of three MS-GM sections per rhAb. Scale bar represents 100 μ m.

FIGS. 3A-3H show that IFC of AGS-enriched rhAbs target neuronal nuclei in
20 both mouse and human-GM brain tissue: AJL03, AJL10, and AJL15. Confocal images are shown at 63x magnification with the colocalization marker for NeuN (for neuronal nuclei) shown as red (Alexa Fluor 594). The primary rhAb is shown as green (Alexa Fluor 488) and nuclei are counterstained blue (DAPI). The images are shown as independent red and green channels above the overlay including DAPI. B1 (- SLE) and
25 G11 (+SLE) negative and positive controls respectively on mouse brain tissue are shown in FIGS. 3A and 3B. Mouse and human GM brain tissue IFC are shown for each rhAb labeled above the column: AJL03 (CDMS1) (FIGS. 3C, 3D), AJL10 (ON_{CIS}2) (FIGS. 3E, 3F), and AJL15 (TM_{CIS}5) (FIGS. 3G, 3H). Data are representative of six coronal sections per rhAb on mouse tissue and three sections per rhAb on GM tissue.
30 Scale bar represents 10 μ m.

FIGS. 4A-4H show that IFC of AGS-enriched rhAbs target astrocytes in both mouse and human GM brain tissue: WR12, WR13, and AJL01. Confocal images are shown at 63x magnification with the colocalization marker for GFAP (for astrocytes) shown as red (Alexa Fluor 594). The primary rhAb is shown as green (Alexa Fluor

488) and nuclei are counterstained blue (DAPI). The images are shown as independent red and green channels above the overlay including DAPI. B1 (- SLE) and G11 (+SLE) negative and positive controls respectively on mouse brain tissue are shown in FIGS. 4A and 4B. Mouse and human GM brain tissue IFC are shown for each rhAb labeled above the column: WR12 (ON_{CIS}2) (FIGS. 4C, 4D), WR13 (ON_{CIS}2) (FIGS. 4E, 4F), and AJL01 (TM_{CIS}4) (FIGS. 4G, 4H). Data are representative of six coronal sections per rhAb on mouse tissue and three sections per rhAb on MS-GM tissue. Scale bar represents 10 μ m.

FIGS. 5A-5L show that IFC of AGS-enriched rhAbs target both neuronal nuclei and astrocytes in both mouse and human GM brain tissue: AJL02, AJL07, WR10 and AJL19. Confocal images are shown at 63x magnification with the colocalization marker for NeuN (for neuronal nuclei) across the first row and GFAP (for astrocytes) across the second row shown as red (Alexa Fluor 594). The primary rhAb is shown as green (Alexa Fluor 488) and nuclei are counterstained blue (DAPI). The images are shown as independent red and green channels above the overlay including DAPI. The top two rows are NeuN and GFAP staining on mouse brain tissue with the bottom row showing human GM brain tissue with labels of the colocalization marker above the red panel. Each rhAb is labeled above the column: AJL02 (CDMS1) (FIGS. 5A-C), AJL07 (ON_{CIS}3) (FIGS. 5D-F), WR10 (TM_{CIS}4) (FIGS. 5G-I), and AJL19 (TM_{CIS}6) (FIGS. 5J-L). Data are representative of six coronal sections per rhAb on mouse tissue and three sections per rhAb on human MS-GM tissue. Scale bar represents 10 μ m.

FIGS. 6A-6C show AGS-enriched rhAbs bind to mouse brain. The remaining 22 rhAbs not in FIG. 1 are shown here. DAB images are shown at 20x magnification of the cortex and corpus callosum. The rhAbs in each section are grouped as follows: CDMS (FIG. 6A), ON_{CIS} (FIG. 6B), and TM_{CIS} (FIG. 6C). The rhAb designation, patient type, and patient number are shown in the upper right corner of each image. Data are representative of three coronal sections per rhAb. Scale bar represents 100 μ m.

FIGS. 7A-7D show AGS-enriched rhAb DAB staining on human multiple sclerosis white matter (MS-WM) brain. DAB images are shown at 20x magnification of MS-WM. The rhAbs in each row are grouped as follows: controls (FIG. 7A), CDMS (FIG. 7B), ON_{CIS} (FIG. 7C), and TM_{CIS} (FIG. 7D). The rhAb designation, patient type, and patient number are shown in the upper right corner of each panel. Data are representative of three MS-WM sections per rhAb. Scale bar represents 100 μ m.

FIGS. 8A-8D show AGS-enriched rhAb DAB staining on human brain MS plaque (MS-P). DAB images are shown at 20x magnification of MS-P. The rhAbs in each row are grouped as follows: controls (FIG. 8A), CDMS (FIG. 8B), ON_{CIS} (FIG. 8C), and TM_{CIS} (FIG. 8D). The rhAb designation, patient type, and patient number are shown in the upper right corner of each panel. Data are representative of three MS-P sections per rhAb. Scale bar represents 100 μ m.

FIGS. 9A-9D show AGS-enriched rhAb DAB staining on healthy human control white matter (HC-WM) brain tissue. DAB images are shown at 20x magnification of HC-WM. The rhAbs in each row are grouped as follows: controls (FIG. 9A), CDMS (FIG. 9B), ON_{CIS} (FIG. 9C), and TM_{CIS} (FIG. 9D). The rhAb designation, patient type, and patient number are shown in the upper right corner of each panel. Data are representative of three HC-WM sections per rhAb. Scale bar represents 100 μ m.

FIGS. 10A-10B show IFC of control rhAbs on human MS-GM brain: B1 (FIG. 10A) and G11 (FIG. 10B). Confocal images are shown at 63x magnification with the colocalization marker for NeuN (for neuronal nuclei) shown as red (Alexa Fluor 594), the primary rhAb as green (Alexa Fluor 488), and nuclei counterstained blue (DAPI). The images are shown as independent red and green channels above the overlay including DAPI with the rhAb label above each panel. Data are representative of three MS-GM sections per rhAb. Scale bar represents 10 μ m.

FIG. 11 shows the protein sequences for the antibody variable regions. Each consecutive pair of antibody sequences (heavy and light) constitute a single antibody. Underlined sequences are complementarity determining regions (CDRs).

FIG. 12 shows the nucleic acid sequences for the antibody variable regions. Each consecutive pair of antibody sequences (heavy and light) constitute a single antibody. Underlined sequences are restriction enzyme recognition sites.

FIGS. 13A-13F show IFC of AGS-enriched rhAbs targeting neuronal nuclei in both mouse and human GM brain tissue: AJL10 (ON_{CIS}2) and AJL07 (ON_{CIS}3). Confocal images are shown at 63x magnification for the mouse tissue (FIGS. 13A and 13C; FIGS. 13D and 13F) and 126x for the human tissue (FIGS. 13B and 13E) with the colocalization marker for NeuN (for neuronal nuclei) shown as red (Alexa Fluor 594). The primary rhAb is shown as green (Alexa Fluor 488) and nuclei are counterstained blue (DAPI). The images are shown as independent red and green channels above the overlay including DAPI. Data are representative of six coronal sections per rhAb on

mouse brain tissue and three sections per rhAb on human GM brain tissue. Scale bar represents 10 μ m.

FIGS. 14A-14F show IFC of AGS-enriched rhAbs target astrocytes in both mouse and human GM brain tissue: AJL01 (TM_{CIS}4) and WR13 (ON_{CIS}2). Confocal
5 images are shown at 63x magnification for the mouse tissue (FIGS. 14A and 14C; FIGS. 14D and 14F) and 126x for the human tissue (FIGS. 14B and 14E) with the colocalization marker for GFAP (for astrocytes) shown as red (Alexa Fluor 594). The primary rhAb is shown as green (Alexa Fluor 488) and nuclei are counterstained blue (DAPI). The images are shown as independent red and green channels above the
10 overlay including DAPI. Data are representative of six coronal sections per rhAb on mouse brain tissue and three sections per rhAb on human GM brain tissue. Scale bar represents 10 μ m.

FIGS. 15A-15F show that CDMS derived AGS-enriched rhAbs demonstrate reactivity to neuronal nuclei and astrocytes: AJL02 and AJL03 (CDMS1). Confocal
15 images are shown at 63x magnification for the mouse tissue (FIGS. 15A-B; FIGS. 15D-E) and 126x for the human tissue (FIGS. 15C and 15F) with the colocalization marker for NeuN (neuronal nuclei) or GFAP (for astrocytes) shown as red (Alexa Fluor 594). The primary rhAb is shown as green (Alexa Fluor 488) and nuclei are counterstained blue (DAPI). The images are shown as independent red and green channels above the
20 overlay including DAPI. Data are representative of six coronal sections per rhAb on mouse brain tissue and three sections per rhAb on human GM brain tissue. Scale bar represents 10 μ m.

FIGS. 16A-16L show that CIS derived AGS-enriched rhAbs demonstrate reactivity to neuronal nuclei and astrocytes: WR12 (ON_{CIS}2), WR10 (TM_{CIS}4), AJL15
25 (TM_{CIS}5), and AJL19 (TM_{CIS}6). Confocal images are shown at 63x magnification for the mouse tissue (FIGS. 16A, 16B, 16D, 16E, 16G, 16H, 16J, 16K) and 126x for the human tissue (FIGS. 16C, 16F, 16I, 16L) with the colocalization marker for NeuN (neuronal nuclei) or GFAP (for astrocytes) shown as red (Alexa Fluor 594). The primary rhAb is shown as green (Alexa Fluor 488) and nuclei are counterstained blue (DAPI). The images are shown as independent red and green channels above the
30 overlay including DAPI. Data are representative of six coronal sections per rhAb on mouse brain tissue and three sections per rhAb on human GM brain tissue. Scale bar represents 10 μ m.

FIGS. 17A-17D show that AGS-enriched rhAbs do not bind strongly to myelin components. (FIGS. 17A and B) Binding of 10 rhAbs to common myelin proteins MOG and MBP by ELISA. A dashed line represents the threshold for background signal, as observed with the negative control antibody B1. (FIG. 17C) Binding of 8 rhAbs to myelin-derived peptides demonstrate no reactivity compared to controls (EAEA and anti-MBP). (FIG. 17D) Binding of 10 rhAbs to HEK293 cells mock transfected (left column) or transfected with MOG (right column) demonstrated no reactivity compared to control (8-18C5).

FIGS. 18A-18M show AGS-enriched rhAbs demonstrate reactivity to neurons and astrocytes.

FIG. 19 illustrates a codon mutation decision tree.

FIGS. 20A-20D. Plasmablast expansion and genetics. (FIG. 20A) Representative flow cytometry gating for detecting plasmablasts in peripheral blood. (FIG. 20B) Percentage of plasmablasts (CD19⁺ CD27^{high}) in blood samples from patient groups as measured by flow cytometry. Levels are elevated for CIS-PTM patients as compared to healthy donors, much like that of NMO patients. (FIG. 20C) Single cell PCR of blood plasmablasts reveals an increase in usage of VH4 family genes in CIS-PTM patients, as compared to plasmablasts responding to influenza infection and total CD19⁺ cells from healthy donors. The increase is similar to what is seen in NMO plasmablasts and autologous memory B cells, although the latter does not reach significance. (FIG. 20D) Specific genes within the VH4 family listed as a percent of the total repertoire of genes. Only VH4-31 and VH4-34 genes are elevated in CIS-PTM patients over healthy donor total B cells or flu responding plasmablasts.

FIGS. 21A-21F. Detailed heavy chain plasmablast genetics. (FIG. 21A) Usage of specific heavy chain V gene segments in patient repertoires. Only VH4-31 and VH4-34 are elevated in CIS patient plasmablasts. (FIG. 21B) V gene family representation in the repertoire of each cell population. VH4 family genes are more highly represented in CIS and NMO patients, while VH6 family genes are depressed. VH1 family genes are over-represented in the flu responding plasmablasts. (FIG. 21C) J gene segment distribution in patient repertoires. Both CIS and NMO patients have a decreased usage of JH4 segments, while CIS patients have an increase in JH1 usage and NMO patients have an increase in JH6 usage. (FIG. 21D) The average heavy chain CDR3 length in each patient group. Flu responding plasmablasts have overall longer CDR3 regions. (FIG. 21E) The average charge of heavy chain CDR3s as calculated by the net number

of positive and negative amino acids per sequence. No differences were observed between the groups. (FIG. 21F) The abundance of isotypes found in each patient in the plasmablast and memory B cell populations. CIS patients are represented in black and NMO patients in gray.

5 **FIGS. 22A-22F.** Detailed heavy chain plasmablast genetics. (FIG. 22A) Usage of specific kappa chain V gene segments in patient repertoires. No kappa chains were over represented in the CIS patients as compared to the flu responding plasmablasts. (FIG. 22B) Usage of specific lambda chain V gene segments in patient repertoires. (FIG. 22C) Kappa family representation in patient repertoires. No significant
10 differences were observed. (FIG. 22D) Light chain J gene segment distribution in patient repertoires. CIS and NMO patients have a decrease in JK1 and JK4 usage and an increase in JK2 as compared to flu responding plasmablasts (CIS patients also displayed an increase in JK5 usage). (FIG. 22E) The average length of light chain CDR3 in patient repertoires. NMO patients have a slight decrease in length compared
15 to flu responding plasmablasts. (FIG. 22F) The average charge of the light chain CDR3 as calculated by the net number of positive and negative amino acids. The overall charge flips from positive to negative in the CIS and NMO patients.

FIGS. 23A-23B. Brain lysate reactivity of plasmblast antibodies by ELISA. (FIG. 23A) Brain ELISA results grouped by patient. Most of the plasmblast antibodies
20 did not recognize brain lysate antigens above the levels of healthy donor antibodies; however for most CIS patients two (33-50%) of the antibodies were highly reactive to brain lysate. (FIG. 23B) Results for all CIS rhAbs tested. Using healthy donor (HD) antibodies as a threshold for reactivity, 12 of the 38 CIS rhAbs and 2 of the 9 NMO rhAbs bound to brain lysate.

25 **FIGS. 24A-24C.** Comparison of VH4 and VH3 antibodies. (FIG. 24A) Brain ELISA results grouped by V gene family of rhAb. (FIG. 24B) Summary of ELISA results grouped by V gene family. All VH3 rhAbs cloned showed little reactivity of brain lysate, but many (40%) of the VH4 family rhAbs bound strongly to brain lysate. (FIG. 24C) V gene segments utilized by rhAbs that did and did not recognize brain
30 lysate. Only VH4 family genes recognized brain lysate above the level of healthy donor antibodies, but most VH4 family genes were represented in both the positive and negative group.

FIGS. 25A-25C. Other tissue lysate ELISAs (FIG. 25A) Kidney ELISA results grouped by patient. (FIG. 25B) Summary of kidney ELISA results grouped by patient.

A similar proportion of CIS rhAbs were reactive to kidney lysate and many that were reactive to brain were also reactive to kidney, however the overlap was not identical. (FIG. 25C) V gene segments of rhAbs that recognized kidney lysate versus those that did not. Both VH3 and VH4 antibodies recognized the kidney lysate, indicating that the repertoire of general autoreactivity is different than that of the brain reactive repertoire.

FIGS. 26A-26E. Plasmablast rhAbs react to neurons and glial cells in mouse brain. 20x images of rhAbs staining neurons and glia in the white (corpus callosum, left) and gray (cortex, right) matter of healthy mouse brain (FIG. 26A), stroke brain (FIG. 26B) and EAE brain (FIG. 26C); rhAbs that recognized neurons did so in a ring-like pattern around the nucleus and rhAbs that recognized glia bound to cell body processes (FIG. 26D). One quarter of the CIS rhAbs that were positive by ELISA recognized exclusively glia and the remaining three quarters recognized both glia and neurons (FIG. 26E).

FIGS. 27A-27C. Plasmablast rhAbs reactive to neurons and glial cells in stroke brain. (FIG. 27A) rhAb reactivity to stroke mouse corpus callosum brain tissue. (FIG. 27B) rhAb reactivity to stroke mouse cortex brain tissue. (FIG. 27C) 63x image of rhAb binding to stroke mouse brain. Two main patterns were observed with the rhAbs: neuronal body/cytoplasmic recognition in the cortex, and glial cell recognition in the corpus callosum.

FIGS. 28A-28B. Reactivity of more rhAbs with stroke mouse brain. (FIG. 28A) rhAb reactivity to stroke mouse corpus callosum. (FIG. 28B) rhAb reactivity to stroke mouse cortex.

FIGS. 29A-29B. Plasmablast rhAbs reactive to neurons and other cells. Figures show the representative rhAb binding to EAE and healthy mouse corpus callosum (FIG. 29A) and cortex (FIG. 29B). The pattern of rhAb recognition was similar in all three types of mouse brain tissue (FIGS. 27A-27C) and for these examples recognized primarily neuronal bodies, as shown by colocalization with NeuN. Isotype control antibody B1 did not bind EAE and healthy corpus callosum and cortex brain tissue.

FIGS. 30A-30B. rhAbs on EAE brain. Figures show representative images of additional rhAbs binding to the corpus callosum (FIG. 30A) and cortex (FIG. 30B) of EAE brain tissue. Similar patterns of recognition were observed in many of the rhAbs with neurons and glial cells being the primary targets.

FIGS. 31A-31B. Plasmablast rhAbs reactive to glial cells in CC. Figures show representative rhAb binding to EAE and healthy mouse corpus callosum (FIG. 31A)

and cortex (FIG. 31B). The pattern of rhAb recognition was similar in both tissues (FIGS. 27A-27C). The rhAbs shown here primarily colocalized with GFAP, indicating their binding to astrocytes. When no rhAb was incubated on EAE and healthy mouse brain, no flurochrome signal was detected.

5 **FIGS. 32A-32B.** rhAbs on healthy brain. Figures show representative images of additional rhAbs binding to the corpus callosum (FIG. 32A) and cortex (FIG. 32B) of healthy brain tissue. Similar patterns of recognition were observed in many of the rhAbs with neurons and glial cells being the primary targets.

10 **FIGS. 33A-33B.** rhAbs colocalization with oligodendrocytes. Figures show representative images of rhAb binding to EAE and healthy mouse corpus callosum (FIG. 33A) and cortex (FIG. 33B). rhAb colocalization with PDGFR indicates oligodendrocyte binding. From this panel of rhAbs, only CIS68 showed some colocalization with this marker.

15 **FIGS. 34A-34B:** Plasmablast rhAbs binding evaluation by histology. (FIG. 34A) Summary of all the CIS and NMO rhAbs tested for binding to mouse brain tissue. One third of the CIS rhAbs and one third of the NMO rhAbs displayed affinity for only glial cells while the remaining two thirds bound both glia and neurons. (FIG. 34B) Enumeration of positively stained cells in the cortex.

20 **FIGS. 35A-35B.** Reactivity to Sy5y by ICC. (FIG. 35A) Representative images from testing rhAb binding to SH-Sy5y neurons by ICC. B1 is an isotype negative control, and G11 is an anti-NMDAR positive control. Examples of each staining pattern are represented here with the remaining images in FIG. 36. CIS07 did not bind to the SH-Sy5y cells, NMO06 displayed nuclear recognition, CIS19 showed cytoplasmic and nuclear binding, and CIS42 had strong cytoplasmic recognition with
25 little binding to the nucleus. (FIG. 35B) Summary of ICC results. Most rhAbs derived from healthy controls did not recognize SH-Sy5y, while over half of the CIS rhAbs and a little less than a third of NMO rhAbs bound to this cell line. Cytoplasmic binding patterns were more common in the CIS rhAbs and nuclear binding patterns were more common in the NMO rhAbs.

30 **FIG. 36.** Reactiity to Sy5y by ICC. Figure shows all remaining images of rhAb binding to SH-Sy5y cells by ICC. R1, R2, and CSF10 served as additional negative and positive controls.

FIGS. 37A-37B. Reactivity to Hep2 by ICC. (FIG. 37A) All images of rhAb binding to Hep2 cells by ICC. (FIG. 37B) Summary of binding to Hep2 cells. None of

the healthy control rhAbs bound to Hep2 cells, but a small proportion of the CIS and NMO rhAbs did. 20% of the CIS and 10% of the NMO antibodies cross-reacted to Hep2 and SH-Sy5y, but the majority of the binding rhAbs were specific to SH-Sy5y.

FIGS. 38A-38B. Reactivity to Sy5y by flow cytometry. (FIG. 38A)
5 Representative histograms from testing rhAb binding to intracellular and extracellular portions of SH-Sy5y cells by flow cytometry. Examples of each binding pattern observed are presented, with the remaining plots in FIG. 39. B1 and G11 served as negative and positive controls respectively, HD10-42 and NM009 did not bind to the cell line, CIS09 and CIS35 only recognized the cell line intracellularly, and CIS48 and
10 CIS56 recognized molecules expressed by the SH-Sy5y cell line both inside (intracellular) and outside (extracellular) the cell. (FIG. 38B) Summary of the tested rhAbs that bound to SH-Sy5y cells. 77% of the tested CIS rhAbs and 38% of tested NMO rhAbs recognized the cell line by flow cytometry. Of the positive CIS rhAbs 52% bound the cells both intra- and extracellularly while 48% only bound intracellular
15 antigens. For the NMO rhAbs two thirds bound only intracellularly and one third bound both intracellular and extracellular targets. One third of the healthy donor rhAbs bound SH-Sy5y intracellularly, but none bound the exterior of the cells.

FIG. 39. Reactivity to Sy5y by flow cytometry. Figure shows histogram data with intracellular and extracellular rhAb recognition of SH-Sy5y cells by flow
20 cytometry.

FIGS. 40A-40B. Reactivity to C8-D1A by flow cytometry. (FIG. 40A)
Histogram data with intracellular and extracellular rhAb recognition of the astrocytic mouse cell line C8-D1A by flow cytometry. (FIG. 40B) Summary of rhAb recognition of C8-D1A astrocytes. Most healthy control rhAbs did not bind to this cell line, and
25 none bound extracellular targets. Two thirds of the CIS rhAbs and about 40% of the NMO rhAbs recognized C8-D1A. Of the positive CIS rhAbs, about half bound only intracellularly and half bound both inside and outside the cell. Two thirds of the positive NMO rhAbs bound only intracellularly, while the remaining rhAb bound antigens only on the exterior of the cell.

FIGS. 41A-41B. Reactivity to Neuro-2a by flow cytometry. (FIG. 41A)
30 Histogram data with intracellular and extracellular rhAb recognition of the mouse neuronal cell line Neuro-2a by flow cytometry. (FIG. 41B) Summary of rhAb recognition of Neuro-2a neurons. Most healthy control rhAbs did not bind to this cell line, except one that bound both the intracellular and extracellular portion. Two thirds

of the CIS rhAbs and three quarters of the NMO rhAbs recognized Neuro-2a. Of the positive CIS rhAbs, 60% bound only intracellularly and 40% both inside and outside the cell. Half of the positive NMO rhAbs bound only intracellularly, one quarter bound antigens only on the exterior of the cell, and one quarter bound antigens both inside and outside of the cells.

FIGS. 42A-42B. ELISAs with patient serum. Figures show brain lysate ELISA results with dilutions of plasma taken from 7 healthy donors responding to influenza vaccination, 7 NMO patients, and 16 CIS patients. (FIG. 42A) Absorbance data from plasma ELISAs grouped by patient category. (FIG. 42B) Summary of brain lysate ELISAs with plasma. 1 NMO and 9 CIS patient samples were two standard deviations above the mean of healthy plasma samples. The one positive NMO patient was the only NMO patient not on CellSept therapy, while all CIS patients were treatment naïve.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure in various aspects provides methods for making pharmaceutical compositions for treating neurodegenerative diseases (*e.g.*, demyelinating diseases), such as but not limited to autoimmune encephalitis, multiple sclerosis, and other related demyelinating diseases such as neuromyelitis optica, optic neuritis, and transverse myelitis. The pharmaceutical compositions impact specific immune-mediated processes involved in the biology of neurodegenerative disease. In certain aspects, the disclosure provides pharmaceutical compositions for treating neurodegenerative or neuroimmunological disease, which are based on inhibiting the action of pathologic antibodies, or alternatively providing therapeutic antibodies to stimulate neuroprotection or repair processes.

In one aspect, the disclosure provides a method for making a pharmaceutical composition for treating a neurodegenerative disease. The process comprises providing one or more VH4 antibodies having at least two mutations with respect to the germline sequence at codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89. These antibodies, which are isolated from clinically diagnosed MS patients as well as patients with initial CIS presentation, bind to one or more antigens in human gray matter. In other aspects, the antibodies are derived from peripheral plasmablasts of neurodegenerative disease patients (*e.g.*, MS, neuromyelitis optica, or transverse myelitis), and these antibodies may also be VH4 antibodies, and may or may not exhibit mutations at one or more codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89 in some embodiments. The methods comprise selecting an active agent that reduces or inhibits the expression of the antibody(ies), or reduces or inhibits binding of the antibody(ies) to the antigen in human gray matter and occasionally white matter. Active agents selected by this process are formulated as a pharmaceutical composition for treatment of patients.

It is demonstrated that AGS-enriched antibodies bind to targets within the CNS. A panel of 32 full-length recombinant human antibodies (rhAbs) were prepared from single CSF B cells whose antibody genes contained AGS-targeted mutations. Surveying B cells and antibodies within the CSF is relevant to CNS disease because there are shared B cell clones between the same MS patient's CSF and CNS (Obermeier *et al.*, 2011), between the meninges and CNS (Lovato *et al.*, 2011), and between the CSF and peripheral blood (Palanichamy *et al.*, 2014). This panel of 32 rhAbs came from a diverse set of patients including CDMS and two initial CIS

presentations (optic neuritis (ON_{CIS}) and transverse myelitis (TM_{CIS})). ON_{CIS} patients present with optic symptoms and lesions along the optic nerve, and TM_{CIS} patients exhibit sensory symptoms with lesions along short segments of the spinal cord. Regardless of either presentation of CIS, both patient types have CSF B cells pools
5 enriched for AGS and are at high risk of converting to CDMS.

CNS targeting of the rhAb panel was determined using immunohistochemistry on both mouse and human brain tissue. Surprisingly, the AGS-enriched B cells targeted gray matter (GM) rather than the anticipated myelin-rich white matter (WM), which has been extensively studied in the MS field (Lassmann *et al.*, 2007). GM
10 involvement in MS disease symptoms and advancement has been understudied even though the presence of cortical lesions correlates strongly with MS disease severity and progression as opposed to the more easily detected WM lesions (Bo *et al.*, 2007, Fisniku *et al.*, 2008 and Vercellino *et al.*, 2005). In fact, cortical GM demyelination is more extensive than WM (26.5% vs 6.5%) with the percentage of demyelination in the
15 cortex increasing with disability and disease length (Bo *et al.*, 2003). Immunofluorescence confirmed GM targeting of the rhAbs to astrocyte bodies and processes, and neuronal nuclei in both human and mouse brain tissue. This targeting pattern was observed with AGS-enriched rhAbs generated from both CIS presentations, ON_{CIS} and TM_{CIS}, as well as established CDMS. Thus, these MS derived antibodies
20 share a mutational pattern that targets GM, and suggest a previously unrecognized humoral immunity target for treating the pathology of multiple sclerosis and related neurodegenerative diseases.

The normal immune system has the ability to generate millions of antibodies with different antigen binding abilities. The diversity is brought about by the
25 complexities of constructing immunoglobulin molecules. These molecules consist of paired polypeptide chains (heavy and light) each containing a constant and a variable region. The structures of the variable regions of the heavy and light chains are specified by immunoglobulin V genes. The heavy chain variable region is derived from three gene segments known as VH, D and JH. In humans there are about 100
30 different VH segments, over 20 D segments and six JH segments. The light chain genes have only two segments, the VL and JL segments. Antibody diversity is the result of random combinations of V(D)J segments as well as somatic mutation.

The germline VH genes can be separated into at least six families (VH1 through VH6) based on DNA nucleotide sequence identity of the first 95 to 101 amino acids.

Members of the same family typically have 80% or more sequence identity, whereas members of different families have less than 70% identity. These families range in size from one VH6 gene to an estimated greater than 45 VH3 genes. In addition, many pseudogenes exist. It is estimated that the human VH repertoire is represented by approximately 50 functional VH segments with about an equal number of pseudogenes. The size of the VH locus is approximately 1100 kb. The VH4 family of genes contains 9 different members: 4-04, 4-28, 4-30, 4-31, 4-34, 4-39, 4-59, 4-61, and 4-B.

The VH4 antibody in accordance with the various aspects of the disclosure may have 2, 3, 4, 5, or 6 mutations with respect to the germline sequence at codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89. In some embodiments, the VH4 antibody has at least one mutation at residue 31B, 56 and/or 81, and one or more mutations at a position selected from 32, 40, 57, 60 and 89. Exemplary VH4 antibody amino acid sequences are shown in FIG. 11, and antibody nucleic acid sequences are shown in FIG. 12. The following table lists the VH4 antibody substitutions exemplified by the panel of 32 rhAbs:

TABLE 1 - ANTIBODIES WITH AGS SIGNATURE CHANGES

rhAb	# AGS	31B	40	56	57	81	89
AJL01	3			S→R --c; aga	T→A a--; gcc	K→N --g; aac	
AJL02	2		H→S ca-; tcc			K→R -a-; agg	
AJL03	2	S→R a--; cgt				K→N --g; aac	
AJL04	3		P→S c--; tcc	S→G a--; ggc	T→I -c-; atc		
AJL05	4	S→N -g-; aat			T→A a--; gcc	K→N --g; aat	V→F g-g; ttt
AJL06	3	G→N gg-; aat				K→R -ag; aga	V→I g-g; atc
AJL07	3			S→N -gc; aat	T→I -cc; att	K→N --g; aac	
AJL08	3			S→T -gc; act		K→N --g; aac	V→F g-g; ttt
AJL09	2		P→S c--; tcc			K→N --g; aac	
AJL10	4		P→S c--; tcc	S→T -gc; act		K→R -a-; agg	V→I g-g; ata
AJL11	3		P→S c--; tcc	S→N -g-; aac		K→M -a-; atg	
AJL12	3	S→D ag-; gat		S→N -g-; aac		K→N --g; aac	
AJL13	3			S→N	T→I	K→N	

				-gc; aat	-cc; att	--g; aac	
AJL14	3	S→N -g-; aat		S→Y ag-; tac			V→I g-g; atc
AJL15	4	S→N -g-; aat	P→S c--; tcc		T→D ac-; gac	K→R -a-; agg	
AJL16	3	S→P ag-; cct		S→H ag-; cac		K→R aa-; cgg	
AJL18	5	S→K -gt; aaa	H→L -a-; ctc	S→T -g-; acc		K→R -a-; agg	V→R gtg; cgc
AJL19	3			S→D agc; gat	T→A a--; gcc		V→L g-g; tta
AJL20	3			S→T -g-; acc		K→R -a-; agg	V→I g-g; ata
WR01	2				T→P a--; ccc	K→R -a-; agg	
WR02	2			S→G a--; ggc	T→A a-c; gcg		
WR03	3			S→G a--; ggc	T→A a--; gcc	K→N --g; aac	
WR04	3	S→G a-t; ggc	P→A c--; gcc			K→N --g; aat	
WR05	4	S→G a--; ggt		S→T -g-; acc	T→S a--; tcc	K→T -ag; aca	V→I g-g; att
WR06	3	S→A ag-; gct		S→N -g-; aac	T→K -cc; aaa		
WR07	4	S→T -g-; act	P→S c--; tcc	S→K -gc; aaa		K→N --g; aac	
WR08	2			S→T -g-; acc		K→N --g; aac	
WR09	2			S→T -g-; acc		K→N --g; aac	
WR10	2		P→S c--; tcc	S→G a--; ggc			
WR11	2		P→S c--; tcc	S→G a--; ggc			
WR12	2			S→T -g-; acc		K→R -a-; agg	
WR13	2			S→T -g-; acc		K→R -a-; agg	

In some embodiments, the VH4 antibody has at least three mutations with respect to the germline sequence at codon positions selected from 31B, 32, 40, 56, 57, 60, 81, and 89, and may have at least four mutations with respect to the germline sequence at codon positions selected from 31B, 32, 40, 56, 57, 60, 81, and 89. In some 5 embodiments, the VH4 antibody has one or more mutations with respect to the germline sequence at codon positions 31B, 56 and/or 81, and may further have mutations with respect to the germline sequence at one or more codon positions selected from codons 32, 40, 57, 60 and 89.

In some embodiments, the VH4 antibody has mutations at two or more of codons 56, 57, and 81 with respect to the germline sequence, or at two or more of codons 40, 56, 81, and 89 with respect to the germline sequence. In some embodiments, the VH4 antibody has mutations at one or more of codons 32, 40, 57, 60, and 89 with respect to the germline sequence. In some embodiments, the VH4 antibody has mutations at one or both of codon 40 and 81 with respect to the germline sequence.

While the identity of the amino acid substitutions are not limited, in some embodiments codon 31B may be R, N, D, P, K, G, A, or T, and in some embodiments is a charged amino acid, such as R, N, or D. In some embodiments, codon 40 may be S, L, or A. In some embodiments, codon 56 is R, G, N, T, Y, H, D, or K, and may be selected from N, T, and G. Codon 57 in various embodiments is A, I, D, S, or K, and may be selected from A and I in some embodiments. In various embodiments, codon 81 is N, R, or M. For example, codon 81 may be N or R. In some embodiments, codon 89 is F, I, R, or L. In some embodiments, codon 89 is a hydrophobic amino acid, such as F, I, or L. In various embodiments, the antibody contains a set of AGS codons shown in Table 1.

In some embodiments, the VH4 antibody has a T or R replacement at codon 56, optionally with the germline amino acid at codons 31B, 40, and 89. An antibody with this mutation has the potential to bind neurons and astrocytes. Antibody binding (*e.g.*, immunohistochemistry staining) patterns can be in part predicted from the codon signature, as illustrated in FIG. 19.

The antibody or fragment may have a heavy chain CDR or set of heavy chain CDRs selected from SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and light chain CDR or set of light chain CDRs selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62 and 64, respectively. Alternatively, the recombinant antibody or fragment may have a heavy chain variable region sequence selected from SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a light chain variable region sequence selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62 and 64, respectively.

In some embodiments, the VH4 germline is 4-04, 4-28, 4-30, 4-31, 4-34, 4-39, 4-59, 4-61, or 4-B.

In some embodiments, the antibody has a CDR sequence as above, but with from 1 to 5 amino acid substitutions (*e.g.*, 1, 2, 3, 4 or 5 substitutions) in the CDR sequence, while maintaining the identity at the AGS codons.

In still other embodiments, the antibody is derived from peripheral plasmablasts
5 from MS patients, or clinically isolated syndrome patients (CIS) at high risk to develop MS. The antibodies derived from peripheral plasmablasts may or may not meet the criteria of AGS antibodies.

Plasmablasts represent a large proportion (5-40%) of the B cell pool in the CSF of untreated MS patients. The plasmablast frequency in the CSF correlates with
10 increased inflammation as disclosed by MRI, and they are also elevated in the blood of patients experiencing their first clinical attack. When patients are left untreated, the frequency of plasmablasts continues to rise. Plasmablasts in the blood develop as a part of the antigen-stimulated memory B cell response to their cognate antigen, and after 2-3 weeks, apoptose or differentiate into long-lived plasma cells. Plasmablasts produce
15 large amounts of antibody and their autocrine production of IL-10 positively feeds back to promote the differentiation and expansion of IgG and IgM antibody secreting plasma cells. Thus the presence of plasmablasts indicates an active B cell response and represents the fraction of memory B cells and other B cell precursors that are currently responding to stimulus.

20 The antibody expressed by a B cell on its surface is the primary factor in issuing their development in the bone marrow and determining their activation response in the periphery. For example, in the periphery, the lack of cognate antigen recognition results in removal from the B cell pool while engagement of cognate antigen results in activation, clonal expansion and affinity maturation. Thus, there is a critical
25 relationship between the antibody expressed by these B cells, their cognate antigen and the B cell's potential to participate in an immune response and endure in the B cell pool.

Therefore, since patients display an expansion of plasmablasts at the time of their first attack of transverse myelitis, peripheral plasmablasts from these patients may be autoreactive. As shown herein, single peripheral plasmablasts were isolated from a
30 patient cohort, and the antibodies they express were cloned and investigated for reactivity to brain antigens. Peripheral plasmablasts from Neuromyelitis Optica (NMO) patients were used as a comparator population since NMO patients also present with transverse myelitis and possess pathogenic autoantibodies, but do not classically exhibit brain inflammation as indicated by MRI.

These studies show that antibodies expressed by plasmablasts from these early MS patients displayed high levels of reactivity for cellular and protein targets in the brain using a panel of methodology to verify binding. Remarkably, only those antibodies that utilized variable heavy chain family 4 (VH4) genes bound to brain antigens. In addition, CNS reactive antibodies were detected in blood plasma samples of all patients from whom single peripheral plasmablasts were isolated. This is the first evidence that peripheral plasmablasts from CIS patients displaying transverse myelitis symptoms express antibodies that bind to brain antigens, demonstrating their autoreactive nature.

10 The antibody in various embodiments is a human recombinant antibody. Antibodies may be produced by standard methods well known in the art (see, *e.g.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; U.S. Patent 4,196,265). For example, the VH4 antibodies can be prepared by co-transfection of cells with paired cloning vectors harboring IgK and IgH genes of the desired VH4 antibody. Recombinant antibodies can be harvested from transfected cell supernatants.

In some embodiments, the antibody further binds to mouse brain tissue, in addition to human gray matter. In some embodiments the VH4 antibody binds to astrocytes and neuronal nuclei in human gray matter, and optionally mouse gray matter.

In accordance with some aspects, the VH4 antibodies are used as a target for therapeutic agents that are useful for treating neurodegenerative disease (*e.g.*, a demyelinating disease), such as MS, NMO or TM. Candidate active agents can be tested for their ability to competitively inhibit binding of the VH4 antibody to its antigen. Binding of the VH4 antibody to its target can be assessed *in vitro* or *in vivo*, *e.g.*, using an animal model. For example, *in vitro* assays can make use of human or mouse brain tissue (*e.g.*, including by immunohistochemistry), fractionated portions of brain or CNS tissue, cell lines, or purified, semi-purified, or isolated gray matter antigen. Further, animal models can be created by administering the antibodies to an animal model, to create one or more symptoms relating to neurodegenerative disease (*e.g.*, a demyelinating disease). Reduction or inhibition of these symptoms can be determined upon administration of candidate agents to the model.

Various types of candidate agents can be screened in accordance with these aspects, and these include anti-idiotypic antibodies or antigen-binding portions thereof, an antibody or portion thereof with binding specificity for the same antigen (*e.g.*, GM antigen), as well as peptide agents, aptamers, or small molecules.

In some embodiments, the candidate agent is an anti-idiotypic antibody. The anti-idiotypic antibody binds to the idiotope of the VH4 antibody. The idiotype is the unique antigen binding site of the VH4 antibody. The combination of epitopes within the idiotype (i.e. the idiotopes) is unique for each antibody. Anti-idiotypic antibodies
5 can be generated to bind specifically to the hypervariable region of the selected antibody target.

In some embodiments, the candidate agent is an antibody or portion thereof with binding specificity for the same antigen (*e.g.*, gray matter antigen). For example, monoclonal antibody fragments can be prepared that compete for binding by the VH4
10 antibody, but lack immune effector functions (*e.g.*, Fc) that may be responsible for disease pathology. Immune effector functions can be altered by deletion of immunoglobulin domains or by mutation of one or more amino acids in the Fc region.

Fc receptors (FcRs) are key immune regulatory receptors connecting the antibody mediated (humoral) immune response to cellular effector functions. There are
15 receptors for all classes of immunoglobulins. For example, there are three classes of receptors for human IgG found on leukocytes: CD64 (FcγRI), CD32 (FcγRIIa, FcγRIIb and FcγRIIc) and CD16 (FcγRIIIa and FcγRIIIb). In antibody dependent cellular cytotoxicity (ADCC), FcRs on the surface of effector cells (natural killer cells, macrophages, monocytes and eosinophils) bind to the Fc region of an IgG which itself
20 is bound to a target cell. Upon binding, a signalling pathway is triggered which results in the secretion of various substances, such as lytic enzymes, perforin, granzymes and tumour necrosis factor, which mediate in the destruction of the target cell. The level of ADCC effector function varies for human IgG subtypes. Although this is dependent on the allotype and specific FcR, in simple terms, ADCC effector function is high for
25 human IgG1 and IgG3, and low for IgG2 and IgG4. Knowledge of the binding site has resulted in engineering efforts to modulate IgG effector functions.

Thus, the active agent can be an antibody fragment lacking an Fc. The antibody may be a F(ab')₂ or Fab, a single chain antibody, or single chain variable fragment (scFv). Other antibody or antigen-binding formats that can be employed include: a
30 single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein (cysteine knot protein, knottin), a DARPIn, a Tetranectin, an Affibody; a Transbody, an Anticalin, an AdNectin, an Affilin, a Microbody, a phylomer, a stradobody, a maxibody, an evibody, a fynomer, an armadillo repeat protein, a Kunitz domain, an

avimer, an atrimer, a probody, an immunobody, a triomab, a troybody, a pepbody, a vaccibody, a UniBody, a DuoBody. These other formats are described in US Patent Nos. or Patent Publication Nos. US 7,417,130, US 2004/132094, US 5,831,012, US 2004/023334, US 7,250,297, US 6,818,418, US 2004/209243, US 7,838,629, US 5 7,186,524, US 6,004,746, US 5,475,096, US 2004/146938, US 2004/157209, US 6,994,982, US 6,794,144, US 2010/239633, US 7,803,907, US 2010/119446, and/or US 7,166,697, the contents of which are hereby incorporated by reference in their entireties. See also, Storz MAb. 2011 May-Jun; 3(3): 310–317.

10 In some embodiments, the candidate agent is a peptide agent. The peptide agent in these embodiments may mimic the natural ligand by binding to the complementarity determining region (CDR), and may be identified by homology modeling and/or physical screening through use of antibody-antigen binding assays. Antibody variable regions may be modeled for docking studies using known processes, including homology models. Sircar *et al.*, “Rosetta Antibody: antibody variable region homology modeling server,” *Nucleic Acid Res.* 2009 Jul 1; 37(Web Server issue): W474–W479. 15 Antibody-antigen binding can be qualitatively or quantitatively assessed using any direct or competitive immunoassay. A peptide can be selected from a peptide library based on antibody-antigen binding, and then optimized for its binding affinity to the antibody target (*e.g.*, based on homology modeling and/or quantitative binding assay).

20 In some embodiments, the candidate agent is an aptamer. Aptamers are oligonucleotide or peptide molecules that bind to a specific target molecule. Aptamers can be identified by selecting them from a large random sequence pool. Aptamers can be classified as DNA, RNA, or XNA aptamers. They often consist of short strands of oligonucleotides. Nucleic acid aptamers can be engineered through repeated rounds of 25 *in vitro* selection (*e.g.*, SELEX, systematic evolution of ligands by exponential enrichment) to bind to the antibody target.

In some embodiments, the candidate agent is a small molecule. Small molecule drugs can mimic the natural antigen of the target antibody by binding to the complementarity determining region (CDR), and may be identified by homology modeling and/or physical screening through use of antibody-antigen binding assays. 30 Antibody variable regions may be modeled for docking studies using known processes, including homology models. Sircar *et al.*, “Rosetta Antibody: antibody variable region homology modeling server,” *Nucleic Acid Res.* 2009 Jul 1; 37(Web Server issue): W474–W479. Antibody-antigen binding can be qualitatively or quantitatively assessed

using any direct or competitive immunoassay. A small molecule hit or core can be selected from a small molecule library based on antibody binding, and then optimized for its binding affinity to the antibody target (*e.g.*, based on homology modeling and/or quantitative binding assay).

5 The effect of the active agent on neurodegenerative disease pathology can be evaluated in an animal model. For example, using a suitable animal model in which the animal expresses or is administered the target antibody in a manner that mimics a neurodegenerative disease (*e.g.*, the animal may form CNS lesions similar to a demyelination process), the impact of the active agent on the pathology may be
10 evaluated. In some embodiments, the active agent reduces or inhibits demyelination, inflammatory infiltrates, axonal injury or cell target function (*e.g.* metabolism).

 In still other embodiments, an oligonucleotide is designed to inhibit the expression of the antibody target. For example, the active agent can be an antisense oligonucleotide or siRNA. Generally, these molecules can be designed against the
15 CDR region of the target antibody, to specifically silence only this pathological immunity. For example, antisense oligonucleotides may be designed to hybridize to the CDR region of the antibody including at least two of the AGS codons, or alternatively, to any VH4-specific sequence (*e.g.*, of any VH4-specific germline sequence). Without limitation, exemplary VH4-specific regions and sequences are
20 disclosed in US 2014/0371103, which is hereby incorporated by reference. Antisense oligonucleotides are typically in the range of 8 to 30 nucleotides in length, and may contain one or more chemical modifications to impart stability and/or increase affinity for the target sequence. Exemplary chemical modifications include backbone modifications (*e.g.*, phosphorothioate or phosphorodiamidate morpholino), as well as 2'
25 modifications (*e.g.*, 2' O-methyl) and bridging modifications (*e.g.*, locked nucleic acid, or other 2' to 4' bridge), base modifications, and/or cap structures. Exemplary modifications are described in U.S. Patents 9,163,235 and 8,642,751, which are hereby incorporated by reference in their entireties. Candidate sequences can be tested in various *in vitro* systems (*e.g.*, cell culture), in which cells are transfected with
30 sequences to express the target antibody, and effects on expression of the antibody are evaluated upon delivery of the oligonucleotide to the cells. Candidate agents can also be tested *in vivo*, *e.g.*, in rodent models, where the rodents express antibodies comprising the target sequence.

The active agent is selected and formulated for administration to patients, for example, by subcutaneous, intravenous, intramuscular, oral, or intrathecal administration, or other route. In some embodiments, the active agent is delivered in an encapsulated form, either in micelles, liposomes, or nanoparticles, and which may be targeted to desired cells (*e.g.*, antibody expressing B cells). The active agent is formulated at an amount effective reduce activity or expression of target antibodies *in vivo*. In some embodiments, the pharmaceutical composition may be administered by intrathecal, intravenous, or subcutaneous injection at a frequency of at least once per week, or once per month. The pharmaceutical composition may be administered from 2 to 20 times per year in various embodiments.

In some embodiments, the antibodies (either AGS VH4 antibodies, or antibodies derived from plasmablasts as described) are tested in one more animal models to evaluate whether the antibody positively impacts neuroprotection or repair. Antibodies have been observed to positively impact MS lesions. See U.S. Patents 5,591,629 and 8,968,735, which are hereby incorporated by reference in their entirety.

Exemplary animal models include EAE (Experimental Autoimmune Encephalomyelitis) models that are reminiscent of monophasic, relapsing, progressive or relapsing to progressive forms of MS and toxicity models such as the cuprizone and lysolecithine models of demyelination that are not initiated by inflammatory components. A mild EAE model, in which sub-optimal doses of recombinant human MOG35-55 peptide are used to induce EAE resulting in scores within the 1-2 range on the EAE scale indicating mild CNS damage. Subsequent introduction of an anti-MOG antibody increases EAE scores. This model can be used to evaluate whether rhAbs contribute to CNS damage manifesting as increased EAE scores, decreased grip strength and loss of rotarod performance over control mice that receive a rhAb that does not bind mouse brain tissue.

For example, for the mild EAE model, C57BL/6 female mice (8-12 weeks of age) are immunized s.c. with 100 µg human MOG35-55 on day 0 and treated with 200 ng pertussis toxin i.p. on day 0 and 2. On day 17 post-immunization, mice with similar disease score range, mean and medium (10 mice/ group; mean disease score for each group of ~1) are divided into groups and injected i.v. with 200 µg of the anti-MOG rhAb as a positive control, 200 µg of a rhAb that has been documented with no binding properties to brain tissue as a negative control, and 200 µg of each individual rhAb documented to bind brain tissue as the experimental agents. Disease severity (as

measured by EAE scores), grip strength testing and rotarod testing is scored for an additional 13 days.

For the neurorepair model, a disease model using cuprizone may be employed. Cuprizone is a neurotoxin that causes profound demyelination in mice following 5 weeks of exposure. This model can be used to determine the neurorepair capability of certain rhAbs. CNS recovery as measured by remyelination occurs over the next two weeks once the mice are returned to a normal diet. For the cuprizone model, 6 to 8 week old C57BL/6 female mice are fed chow mixed with 0.2% cuprizone for 5 weeks to promote demyelination in the brain. The mice are then returned to a normal chow diet at which time CNS recovery as measured by remyelination occurs over the next two weeks.

For example, on Day 1 of the return to normal chow, 200 μ g of rhAb is injected intraperitoneally. This delivery system demonstrates efficient binding of human IgG1 to mouse brain. During this two-week period, the mice are subjected to grip strength testing and rotarod testing which have been demonstrated to improve during the recovery period. Y-maze and Novel Object tests are used to further evaluate behavior, as performance in these tests are also improved during the recovery period of cuprizone-treated mice.

Other measurements of CNS damage including demyelination may be evaluated, for example, using Luxol fast blue, enumeration of infiltrating lymphocytes (*e.g.*, using hematoxylin/eosin), and enumeration of injured axons (*e.g.*, using β -APP). Since astrocyte loss can lead to oligodendrocyte loss prior to detection of demyelination, the number of astrocytes using GFAP and the number of mature and precursor oligodendrocytes using Nogo-A and Olig2, respectively, can be enumerated. Detection of binding by the rhAbs to mouse CNS tissue may also be performed to confirm exposure of the CNS to the rhAbs.

Other useful models include the monophasic model (induction with MOG35-55 in C57Bl/6 mice) as reported by Sosa *et al.* (2013), and the RRMS to SPMS model (Biozzi ABH induced with syngeneic spinal cord homogenate), disclosed in Al-Izki *et al.* (2012) and Hampton *et al.* (2008).

Where antibodies are identified that positively impact neuroprotection or repair processes, an antibody can be formulated for delivery as a therapeutic agent.

In other aspects, the disclosure provides pharmaceutical compositions to stimulate neuroprotection or repair processes in neurodegenerative diseases, such as

MS. In some embodiments, the pharmaceutical composition comprises an effective amount of a VH4 antibody or antigen-binding portion thereof and a pharmaceutically acceptable carrier, the antibody or antigen-binding portion having at least two mutations selected from 31B, 32, 40, 56, 57, 60, 81, and 89, and which binds to an antigen in human gray matter and exhibit neuroprotection or repair. The antibody may exhibit neuroprotection in the EAE model, and/or neurorepair activity in cuprizone model. In some embodiments, the VH4 antibody has a set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and a set of light chain CDRs as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.

In still other embodiments, the VH4 antibody has a set of mutations selected from Table 1. For example, the VH4 antibody may have a set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a set of light chain CDRs as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.

In other aspects, the disclosure provides pharmaceutical compositions to block or reduce binding of pathologic antibodies. The compositions may comprise an anti-idiotypic antibody specific for a VH4 antibody having a set of mutations selected from Table 1. For example, the anti-idiotypic antibody may be specific for a VH4 antibody having a set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and a set of light chain CDRs as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.

In still other embodiments, the VH4 antibody has a set of mutations selected from Table 1, wherein the antibody lacks an Fc domain or has a derivatized Fc domain to alter one or more effector functions. For example, the VH4 antibody may have a set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a set of light chain CDRs as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62,

and 64, and which lacks an Fc domain or has a derivatized Fc domain to alter one or more effector functions.

In some embodiments, the VH4 antibody is a Single Chain Variable Fragment (scFv), which is a fusion of the variable regions of the heavy and light chains of immunoglobulins, linked together with a short (usually serine, glycine) linker. This chimeric molecule, also known as a single domain antibody, retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of a linker peptide. This modification usually leaves the specificity unaltered. Single domain or single chain variable fragments lack the constant Fc region found in complete antibody molecules.

Flexible linkers generally are comprised of helix- and turn-promoting amino acid residues such as alanine, serine and glycine. However, other residues can function as well.

In some embodiments, a single-chain antibody can be created by joining receptor light and heavy chains using a non-peptide linker or chemical unit. Generally, the light and heavy chains will be produced in distinct cells, purified, and subsequently linked together in an appropriate fashion (*i.e.*, the N-terminus of the heavy chain being attached to the C-terminus of the light chain via an appropriate chemical bridge).

Alternative antibody and antigen-binding platforms are described herein, which can be used for the pharmaceutical compositions in this aspect of the disclosure.

Antibody (or related) therapeutics can be formulated to contain from about 0.1 mg to about 100 mg of active agent per dose in some embodiments (*e.g.*, at a concentration of about 0.1 mg to about 100 mg per mL).

In other embodiments, the disclosure provides an oligonucleotide comprising a nucleotide sequence that is complementary to a nucleotide sequence encoding a VH4 heavy chain CDR sequence selected from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, or complementary to a nucleotide sequence encoding a VH4 light chain CDR sequence of any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64. For example, the oligonucleotide may have a nucleotide sequence that is complementary to an RNA encoding a VH4 CDR region comprising at least two AGS codons. For example, the nucleotide sequence may comprise a sequence that is complementary to a

sequence within one of SEQ ID NOS: 65-128. In some embodiments, the oligonucleotide is an antisense oligonucleotide or is a siRNA.

Antisense oligonucleotides are typically in the range of 8 to 30 nucleotides in length (*e.g.*, 12 to 20 nts in length), and may contain one or more chemical
5 modifications to impart stability and/or increase affinity for the target sequence. Exemplary chemical modifications include backbone modifications (*e.g.*, phosphorothioate or phosphorodiamidate morpholino), as well as 2' modifications (*e.g.*, 2' O-methyl) and bridging modifications (*e.g.*, locked nucleic acid, or other 2' to 4' bridge structure), base modifications, and/or cap structures. Exemplary modifications
10 are described in U.S. Patents 9,163,235 and 8,642,751, which are hereby incorporated by reference in their entirety.

RNA interference (RNAi) is a mechanism by which gene expression can be reduced or eliminated. siRNAs are designed so that they are specific and effective in suppressing the expression of the genes of interest. Methods of selecting the target
15 sequences, *i.e.*, those sequences present in the gene or genes of interest to which the siRNAs will guide the degradative machinery, are directed to avoiding sequences that may interfere with the siRNA's guide function while including sequences that are specific to the gene or genes. Typically, siRNA target sequences of about 21 to 23 nucleotides in length are most effective, but sequences in the range of 20-25 base pairs
20 in length can be used. This length reflects the lengths of digestion products resulting from the processing of much longer RNAs. Several further modifications to siRNA sequences may be employed to alter their stability or improve their effectiveness. For example, synthetic complementary 21-mer RNAs may have di-nucleotide overhangs (*i.e.*, 19 complementary nucleotides + 3' non-complementary dimers), which may
25 provide a greater level of suppression. These siRNAs use a sequence of two (2'-deoxy) thymidine nucleotides as the di-nucleotide overhangs.

RNA for use in siRNA may be chemically or enzymatically synthesized.

In some embodiments, the siRNA or antisense oligonucleotide targets and reduces the expression of VH4 antibodies generally. Nucleotide sequences that are
30 specific for VH4 antibodies over other families are described in U.S. Patent Publication 2014/0371103, which is hereby incorporated by reference in its entirety.

The structure, formulation, and delivery of siRNA therapeutics are described in U.S. Patent Publication 2014/0161894, U.S. Patent Publication 2014/0024699, U.S.

Patent Publication 2015/0197746, and U.S. Patent Publication 20160076040, which are hereby incorporated by reference in its entirety.

The present disclosure provides pharmaceutical compositions comprising antibody inhibitory substances. In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Other suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, saline, dextrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like.

The compositions can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

The formulations of the present disclosure may include classic pharmaceutical preparations. Administration of these compositions according to the present disclosure will be via any common route so long as the target tissue is available via that route. In some embodiments administration may be by intradermal, subcutaneous, intramuscular, intraperitoneal, intravenous, intrathecal injection, or other route.

In various embodiments, the active agents are used to treat a neurodegenerative disease, and in particular neuroimmunological or demyelinating diseases, such as MS, autoimmune encephalitis, neuromyelitis optica, optic neuritis, and transverse myelitis.

In some embodiments, the patient has clinically isolated syndrome (CIS). A clinically isolated syndrome (CIS) is a single monosymptomatic attack compatible with MS, such as optic neuritis, brain stem symptoms, and partial myelitis. Patients with CIS that experience a second clinical attack are generally considered to have clinically definite multiple sclerosis (CDMS). Over 80 percent of patients with CIS and MRI lesions go on to develop MS, while approximately 20 percent have a self-limited

process. Patients who experience a single clinical attack consistent with MS may have at least one lesion consistent with multiple sclerosis prior to the development of clinically definite multiple sclerosis. In various embodiments, the present methods are used to treat CIS so it does not develop into MS.

5 In various embodiments, the present methods are used to treat radiologically isolated syndrome (RIS). In RIS, incidental imaging findings suggest inflammatory demyelination in the absence of clinical signs or symptoms. In various embodiments, the present methods are used to treat RIS so it does not develop into MS.

10 In various embodiments, the active agents are used to treat benign multiple sclerosis; relapsing-remitting multiple sclerosis (RRMS); secondary progressive multiple sclerosis (SPMS); progressive relapsing multiple sclerosis (PRMS); and primary progressive multiple sclerosis (PPMS).

15 Benign multiple sclerosis is a retrospective diagnosis which is characterized by 1-2 exacerbations with complete recovery, no lasting disability and no disease progression for 10-15 years after the initial onset. Benign multiple sclerosis may, however, progress into other forms of multiple sclerosis. In various embodiments, the present methods are used to treat benign multiple sclerosis so it does not develop into MS.

20 Patients suffering from RRMS experience sporadic exacerbations or relapses, as well as periods of remission. Lesions and evidence of axonal loss may or may not be visible on MRI for patients with RRMS. In various embodiments, the present methods are used to treat RRMS. A clinical relapse, which may also be used herein as “relapse,” “confirmed relapse,” or “clinically defined relapse,” is the appearance of one or more new neurological abnormalities or the reappearance of one or more previously observed
25 neurological abnormalities.

 SPMS may evolve from RRMS. Patients afflicted with SPMS have relapses, a diminishing degree of recovery during remissions, less frequent remissions and more pronounced neurological deficits than RRMS patients. Enlarged ventricles, which are markers for atrophy of the corpus callosum, midline center and spinal cord, are visible
30 on MRI of patients with SPMS. In various embodiments, the present methods are used to treat RRMS so it does not develop into SPMS.

 PPMS is characterized by a steady progression of increasing neurological deficits without distinct attacks or remissions. Cerebral lesions, diffuse spinal cord damage and evidence of axonal loss are evident on the MRI of patients with PPMS.

PPMS has periods of acute exacerbations while proceeding along a course of increasing neurological deficits without remissions. Lesions are evident on MRI of patients suffering from PRMS. In various embodiments, the present methods are used to treat RRMS and/or SPMS so it does not develop into PPMS.

5 In some embodiments, the present methods are used to treat relapsing forms of MS. In some embodiments, the present methods are used to treat relapsing forms of MS to slow the accumulation of physical disability and/or reduce the frequency of clinical exacerbations, and, optionally, for patients who have experienced a first clinical episode and have MRI features consistent with MS. In some embodiments, the present
10 methods are used to treat worsening relapsing-remitting MS, progressive-relapsing MS or secondary-progressive MS to reduce neurologic disability and/or the frequency of clinical exacerbations. In some embodiments, the present methods can effectively reduce the frequency and/or severity of relapses.

EXAMPLES

15 The following examples are included to demonstrate preferred embodiments. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of embodiments, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present
20 disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1 – Selection of AGS Enriched rhAbs and Their Binding to Brain Tissues

Materials and Methods

25 **Patient Sample Acquisition and Processing.** CSF was obtained by lumbar puncture from patients recruited into the study in accordance with The University of Texas Southwestern Medical Center (UTSWMC) Institutional Review Board (IRB). This study includes patient samples as previously published by the inventor's group (Cameron *et al.*, 2009 and Ligocki *et al.*, 2013) containing patients with clinically
30 definite multiple sclerosis (CDMS), clinically isolated syndrome optic neuritis (ON_{CIS}), and clinically isolated syndrome transverse myelitis (TM_{CIS}). The samples were stained with fluorescently labeled antibodies and sorted for single CD19⁺ B cells through a

CD45⁺ lymphocyte gate as previously described into 96-well plates using either the BD FACSARIA flowcytometer (Becton Dickinson, San Jose, CA) or the MoFlo High-Performance Cell Sorter (Cytomation, Ft Collins, CO) (Ligocki *et al.*, 2013).

Single-cell PCR and genetic analysis of VH and Vκ genes. After the single
5 cell sort and cell lysis, either gDNA was amplified for the CDMS patient samples or
cDNA was generated for the ON_{CIS} and TM_{CIS} patient samples as previously described
(Ligocki *et al.*, 2013). Multi-plexed nested PCR was performed to amplify and the
Immunoglobulin (Ig) heavy chain and corresponding Ig kappa light chain from each
individually sorted CSF B cell. The products were purified, sequenced, catalogued, and
10 analyzed for gene and mutation characteristics (Ligocki *et al.*, 2013).

Germline rearrangements were identified using the IMGT/V-QUEST Ig
blasting tool (world-wide-web at imgt.org/IMGT_vquest/share/textes/). Antibody
variable heavy (V_H) and variable kappa (V_κ) sequences were analyzed and compiled
using a Perl program developed at UTSWMC (Ligocki *et al.*, 2010 and Ligocki *et al.*,
15 2013) using IMGT/V-QUEST as the initial source for sequence alignment.

Cloning of full-length recombinant human IgG antibodies (rhAbs).
Sequences from CDMS, ON_{CIS}, and TM_{CIS} patients were chosen as candidates for
cloning into full-length expression vectors based on their V_H genetics. The criteria
was: expressing a VH4 gene and have 2 or more of the 6 AGS codons mutated
20 (Cameron *et al.*, 2009; Ligocki *et al.*, 2010). 60% were also clonally expanded by
identifying another V_H sequence within the same patient with identical amino acids in
the CDR3 region. The corresponding V_κ sequence was amplified from the same well as
the V_H sequence to identify the antibody binding region of the single CSF B cell.
Sequence and patient details for each selection are shown in Table 5 and Table 6.
25 Additional rounds of PCR were done to add restriction enzyme sites to both the 5' and
3' ends of the original PCR products to allow for insertion into the expression vectors
using modifications of previously published primers (Yurasov *et al.*, 2005). Some
heavy and light chain rearrangement sequences were purchased from Integrated DNA
Technologies (IDT, IA, USA) for extraction into the expression vectors. Dr. Michel
30 Nussenzweig provided the backbone expression vectors for both chains. These vectors
and the procedure have been extensively described for the production of monoclonal
human IgG1 (Tiller *et al.*, 2008). Briefly, AgeI was used as the 5' restriction enzyme
site for both the V_H and V_κ inserts and plasmid backbone and SalI and BsiWI were

used as the 3' restriction enzyme site for the VH and VL respectively (NEB, MA, USA). After digestion, ligation of both the insert and the corresponding expression vector backbone was performed using T4 ligase (NEB). DH5 α cells were transformed with a plasmid and individual colonies from the plate were grown for miniprep (Qiagen, CA, USA). The vectors were sequenced in order to confirm that the insert matched the original patient heavy and light chain rearrangements captured by PCR and that the coding region remained in frame. Midiprep DNA (Qiagen) was used for transformation and production of rhAbs in culture. Sequences were validated after each growth.

Two control rhAbs were provided that were cloned from systemic lupus erythematosus (SLE) patient derived B cells. B1 has been shown to not bind to mouse brain and G11 has been shown to bind to NMDARs in the mouse brain as well as dsDNA (Zhang *et al.*, 2009). These two antibodies have been studied and published and were used as controls for the full-length IgG1 rhAb construct in all the experiments presented in this current study.

Production of monoclonal rhAbs. Human embryonic kidney fibroblast (HEK) 293T cells were grown to 50-80% confluency in a 10 cm dish in DMEM media supplemented with FCS (Gibco, Life Technologies). The cotransfection of paired cloning vectors corresponding to the IgK and the IgH of a rhAb were mixed (12.5 μ g total DNA) with JetPEI solution (Polyplus transfection) and added dropwise to the cells. The plates were incubated in a 5% CO₂ water-jacketed incubator (Nuair, MN, USA) at 37°C in 20 ml DMEM media supplemented with ultra-low IgG FCS media (Gibco). Supernatant was harvested and fresh media added on days 3, 5, 7, and 10. ELISAs were used to determine the yield and the concentration of the rhAbs produced in culture. Goat anti-human IgG Fc antibody (Santa Cruz, TX, USA) was used as the coating antibody and serially diluted samples were incubated for 2 hrs at room temperature. Plates were probed with goat anti-human IgG Fc HRP-conjugated antibody (Santa Cruz) for 1 hr and developed using tetramethylbenzidine (TMB) substrate solution (Ebioscience, CA, USA) and stopped with 1 M HCl. The plates were read at 450 nm using the Epoch Nano (Biotek, VT, USA). Standard curves and rhAb concentrations were interpolated using GraphPad Prism 6 (CA, USA). Supernatants were concentrated using the 10kDa MWCO Amicon Ultra centrifugal filter units (Millipore, MA, USA) following manufacturer's recommendations. A second ELISA was performed on the concentrated stocks of rhAbs and then aliquoted and stored at -80°C. Additionally, a

non-transfected cell culture supernatant was confirmed to not contain any IgG above ELISA detection. These concentrated rhAbs were used as primary antibodies for all mouse brain immunohistochemistry.

Biotinylation of monoclonal rhAbs. A set of ten AGS rhAbs and 2 control
5 rhAbs were purified by passing supernatant through a column with a bed of protein G sepharose beads followed by dialysis in PBS and DPBS (Life Technologies). Purity and yield were determined by SDS-page gel stained with coomassie blue and ELISA as described above. Each rhAb was biotinylated using 100 µg of column-purified product and following manufacturer's instructions for the Thermo Scientific EZ-Link Micro
10 NHS-PEG4-Biotinylation kit (Thermo Scientific, MA, USA). These biotinylated rhAbs were used as primary antibodies for all human brain immunohistochemistry.

Processing of frozen brain tissue. Mice were sacrificed 2-3 days post stroke induction as previously described (Stowe *et al.*, 2011) and perfused with 4% paraformaldehyde. The brains were extracted and preserved in 4% paraformaldehyde
15 for 48 hrs at 4°C followed by cryoprotection in sequential 15% and 30% sucrose solutions. Post-mortem human brain samples were provided by the Human Brain and Spinal Fluid Resource Center (UCLA, Los Angeles, CA). Three samples were used for the studies: white matter (WM) from a healthy control without neurological complications (HC), white matter plaque from a patient with clinically definite MS
20 (MS-P), normal appearing WM from the same MS patient (MS-WM), and normal appearing gray matter (MS-GM). Mean time to sampling from time of death was 16 hrs. Upon removing from -80°C, they were preserved similarly to mouse brains with 4% paraformaldehyde for 48 hrs at 4°C followed by cryoprotection in sequential 15% and 30% sucrose solutions. All tissues were embedded in O.C.T freezing compound and
25 stored at -20°C until cryosectioned. Tissue sections (12-16 µm) were cut and attached to charged glass slides using a cryostat (Thermo Scientific MICROM) and frozen at -20°C. Tissues were stained with cresyl violet to validate the integrity of the preservation of the tissue.

**Diaminobenzidine (DAB)-immunohistochemistry (IHC) staining of mouse
30 tissue.** Tissue sections were subjected to antigen retrieval for 2 min using low pH Antigen Unmasking Solution (Vector Laboratories, Burlingame, CA, USA). Endogenous biotin was blocked using 3% H₂O₂ solution for 5 min at room temperature and then washed. The sections were blocked with 3% normal goat serum in PBS for 10

min at room temperature, washed with PBS, and then were incubated overnight at 4°C with 1 µg rhAb (10 ng/µl) per brain slice. The next day, sections were washed and DAB staining was conducted following the manufacturer's instructions using a biotinylated secondary goat anti-human IgG Fc antibody (Vector Laboratories, Burlingame, CA, USA). The slides were dehydrated and cleared with sequential washes in increasing percentages of EtOH, from 70% to 100%, with two final washes in xylenes. Slides were mounted with a permount:xylene solution and imaged using a 40x brightfield lens on the NanoZoomer (Hamamatsu, Japan). Images were visualized using NDP.view software (Hamamatsu, Japan) and 20x images were exported for visualization and adjustments to brightness and contrast were done with ImageJ software (NIH, USA).

DAB-IHC staining of human tissue. Initial processing of the human brain tissue sections remained the same as the mouse tissue. After blocking with 3% normal goat serum in PBS for 10 min at room temperature, an additional blocking step was performed with BloxAll for 10 min at room temperature (Vector Laboratories, Burlingame, CA, USA). Tissues were incubated overnight at 4 °C with 1 µg biotinylated-rhAb (10 ng/µl) per brain slice. The next day, these biotinylated-rhAbs were detected without a secondary antibody and instead with ABC reagent alone (Vector Laboratories, Burlingame, CA, USA). Dehydration, clearing, mounting, and visualization of the human tissue followed the same procedure as the mouse tissue.

Immunofluorescence (IFC) staining of mouse tissue. Ten AGS rhAbs and 2 control rhAbs from the DAB panel were selected for further experiments using IFC (Table 5). Tissue sections were subjected to antigen retrieval for 2 min using low pH Antigen Unmasking Solution (Vector Laboratories, Burlingame, CA, USA). The sections were blocked with 1% normal goat serum and 1% Tween-20 in PBS for 1 hr at room temperature. Due to the presence of IgG deposits even in healthy brain and as an artifact of post-mortem tissue preparation, the set of 10 rhAbs and the 2 control rhAbs used in the mouse brain IFC were biotinylated to eliminate the need for a species specific secondary antibody. Most of the rhAbs were diluted in blocking solution. Pierce Immunostain Enhancer (Thermo Scientific) was used as the diluent for the primary rhAb incubation as well as the secondary Alexa Fluor488 for the following two rhAbs: AJL03, AJL15. Slides were washed with PBS, and then incubated overnight at 4°C with 1 µg rhAb (10 ng/µl) per brain slice. Next day, the sections were washed and

incubated for 1 hr at room temperature with the secondary antibody Alexa Fluor 488 goat anti-human IgG Fc (Life Technologies). Then a colocalization marker, either GFAP (Abcam) or NeuN (Chemicon) were used at 1:1000 and 1:100 dilutions respectively, was incubated for 1 hr at room temperature and then incubated for an additional hour with the appropriate secondary antibody Alexa Fluor 594 anti-rabbit IgG Fc for GFAP or Alexa Fluor 594 anti-mouse IgG Fc for NeuN detection (Life Technologies). Next, the stained tissue sections were incubated for three minutes with DAPI (1:1000) as a counterstain for nuclei (Life Technologies). The sections were washed and wet mounted with Fluoro-Gel (Electron Microscopy Diatome). Slides were viewed with a fluorescent Leica TCS SP5 confocal microscope (Leica microsystems) and viewed and adjusted in brightness and contrast using ImageJ software (NIH, USA).

IFC staining of human tissue. Initial processing of the human brain tissue sections remained the same as the mouse tissue above. After the initial blocking, endogenous biotin was blocked per manufacturer's instructions using the streptavidin-biotin blocking kit (Vector Laboratories, Burlingame, CA, USA). Pierce Immunostain Enhancer (Thermo Scientific) was used as the diluent for the primary rhAb incubation as well as the secondary Alexa Fluor 488 for all human tissue IFC. Slides were washed with PBS, and then incubated overnight at 4 °C with 2 µg rhAb (20 ng/µl) per brain slice. Next day, the sections were washed, and incubated for 1.5 hrs at room temperature with the secondary antibody Alexa Fluor 488 goat anti-streptavidin (Life Technologies). The colocalization with either GFAP or NeuN, DAPI counterstain, mounting and visualization followed the same procedure as the mouse brain tissue.

Results

Selection of AGS enriched rhAb. The inventor has previously shown that CIS patients at risk to convert and those with CDMS harbor B cells in the CSF with a unique mutational pattern in their VH4 repertoires termed the Antibody Gene Signature (AGS) (Cameron *et al.*, 2009 and Ligocki *et al.*, 2010). A shared pattern of increased replacement mutations at 6 specific codons within VH4 genes suggests selection and recognition of a shared set of antigens. Therefore, she sought to determine the biological significance of this mutational pattern by testing the binding ability of AGS-enriched antibodies to brain tissue. Using single cell sorting of CSF derived B cells from CDMS, ON_{CIS}, and TM_{CIS} patients, the inventor was able to determine the exact

antibody-binding variable regions from PCR amplified VHJH and VkJk sequences. Using a full-length recombinant human IgG1 antibody expression vector system, variable regions were cloned and expressed for further study. Only those B cells expressing a VH4 family gene with mutations in 2 or more of the 6 AGS codons were
5 considered for this analysis.

A total of 32 rhAbs were chosen for expression. The details of 10 rhAbs are shown in Table 5 and the remaining 22 in Table 6. Briefly, all rhAbs contained mutations at 2 or more of the 6 AGS codons, and the majority (66%) contained 3 or more AGS mutations. Additionally, 60% were also clonally expanded, suggesting an
10 antigen driven process. These rhAbs were cloned from 10 CSF patient repertoires: 9 rhAbs from 4 CDMS patients, 14 rhAbs from 3 ON_{CIS} patients, and 9 rhAbs from 3 TM_{CIS} patients. Two control rhAbs cloned from SLE patient B cells were provided by Dr. Betty Diamond as controls for the human IgG construct. The expected binding of this control set has been extensively published using mouse tissue. B1, the negative
15 control, does not react to brain, whereas G11, the positive control, reacts to NMDARs in the brain as well as dsDNA (Zhang *et al.*, 2009).

TABLE 5 – Patient, Gene and Staining Overview of the 10 rhAbs used for DAB and IFC

Patient #	Diagnosis	rhAb	V _H J _H ¹	V _K , J _K ²	Clone	# AGS ³	V _H J _H SHM ⁴	Mouse Liver ⁵	Mouse Stroke/EAE Brain ⁶	Human Fixed/Unfixed Brain ⁷	NeuN ⁸	GFAP ⁹
1	CDMS	AJL02	4-31; 4	1-39; 2	no	2	8.96%	-	+/+	+/+	+	+
		AJL03	4-39; 1	1-33; 2	no	2	7.96%	-	+/+	+/+	+	-
2	ONCS	AJL10	4-4; 6	2-28; 5	no	4	12.63%	+	+/+	+/+	+	-
		WR12	4-30; 4	2-28; 4	yes	2	12.44%	-	+/+	+/+	-	+
		WR13	4-30; 4	2-28; 2	yes	2	11.94%	-	+/+	+/+	-	+
3	ONCS	AJL07	4-59; 4	1-13; 2	yes	3	9.23%	-	+/+	+/+	+	+
4	TMCS	AJL01	4-34; 3	3-20; 5	yes	3	11.28%	-	+/+	+/+	-	+
		WR10	4-4; 6	3-20; 5	yes	2	4.55%	-	+/+	+/+	+	+
5	TMCS	AJL15	4-39; 5	2-29; 2	yes	4	7.46%	-	+/+	+/+	+	-
6	TMCS	AJL19	4-34; 3	3-20; 2	yes	3	8.21%	-	+/+	+/+	+	+

Abbreviations: CDMS: clinically definite multiple sclerosis, ONCS: clinically isolated syndrome - optic neuritis, TMCS: clinically isolated syndrome - transverse myelitis, rhAb: recombinant human antibody, VH: variable heavy chain, JH: variable heavy chain J segment, VK: variable kappa chain, JK: variable kappa chain J segment, AGS: antibody gene signature, V_HJ_H SHM: variable heavy chain rearrangement mutation frequency, EAE: experimental autoimmune encephalomyelitis, NeuN: neuronal nuclei, GFAP: glial fibrillary acid protein

¹ Variable heavy and J segment usage by the rhAb.
² Variable kappa and J segment usage by the rhAb.
³ Number of mutated AGS codons (6 total possible) in the V_HJ_H gene of the rhAb.
⁴ Somatic hypermutation frequency for the heavy chain rearrangement of the rhAb.
⁵ Positive (+) or negative (-) staining as determined by DAB staining on mouse liver tissue.
⁶ Positive (+) or negative (-) staining as determined by DAB staining on mouse post-stroke brain or EAE tissue.
⁷ Positive (+) or negative (-) staining as determined by DAB staining on human brain fixed with 4% paraformaldehyde or unfixed frozen brain. Positive (+) or negative (-) colocalization as determined by the mouse brain tissue IFC experiments.

Table 6 - Patient, gene, and staining overview of the 22 rhAbs used only for mouse brain tissue DAB. ¹				
Patient #	Diagnosis	rhAb	Clone	# AGS ²
2	ON _{CIS}	AJL11	no	3
3	ON _{CIS}	AJL06	no	3
		AJL08	yes	3
		AJL09	yes	2
		AJL13	yes	3
4	TM _{CIS}	WR11	yes	2
5	TM _{CIS}	AJL14	no	3
		AJL16	yes	3
6	TM _{CIS}	AJL18	yes	5
		AJL20	no	3
7	CDMS	WR01	yes	2
		WR02	yes	2
8	CDMS	WR03	no	3
		WR04	no	3
		WR05	no	4
		WR06	no	3
9	CDMS	WR07	no	4
10	ON _{CIS}	AJL04	yes	3
		AJL05	yes	4
		AJL12	no	3
		WR08	yes	2
		WR09	yes	2

Abbreviations: CDMS: clinically definite multiple sclerosis, ON_{CIS}: clinically isolated syndrome- optic neuritis, TM_{CIS}: clinically isolated syndrome- transverse myelitis, rhAb: recombinant human antibody, AGS: antibody gene signature

¹ See DAB images of the rhAbs listed here

² Number of mutated AGS codons (6 total possible) in the V_H4 gene of the rhAb.

AGS-enriched rhAbs bind to mouse brain tissue. The panel of 32 experimental AGS-enriched and clonally expanded rhAbs as well as the 2 control rhAbs was first tested for targeting to mouse brain tissue using DAB. This methodology offers sensitive detection of primary rhAb binding to the tissue. Due to previous work from other laboratories demonstrating a lack of binding of antibodies cloned from CDMS CSF B cells to normal brain tissue or WM (Owens *et al.*, 2009 and von Budingen *et al.*, 2008), the inventor chose to utilize brain tissue from a mouse model of transient stroke as a source of inflammation (Stowe *et al.*, 2011). This provided generalized non-antigen directed inflammation to minimize bias of any specific CNS antigen. The secondary antibody used for detection, goat anti-human IgG, did not react to mouse brain alone (FIG. 1A). As expected, the negative

construct control rhAb B1 also did not react to mouse brain, while the positive control rhAb G11 showed recognition to brain and thus validated the assay (FIG. 1A).

The full panel of 32 rhAbs was tested on mouse brain by DAB. There was a wide range of staining intensity. However, all but two of the 32 rhAbs, WR01 and WR11, displayed binding to brain tissue (FIGS. 1A-1D and FIGS. 6A-6C). There was no difference of rhAb staining patterns amongst the three patient groups used to derive the rhAbs. A common feature was that the binding appears cellular in the cortex and midbrain and was either absent or sparse along the heavily myelinated corpus callosum.

AGS-enriched rhAbs bind to human brain tissue. Four sources of human brain were used for the DAB staining experiments: MS normal appearing gray matter (MS-GM) (FIGS. 2A-2D), MS normal appearing white matter (MS-WM) (FIGS. 7A-7D), MS white matter plaque (MS-P) (FIGS. 8A-8D), and healthy control normal appearing (NA) WM (HC-WM) (FIGS. 9A-9D). Due to the rhAb staining pattern found in the mouse brain, the tissue source of most interest was the MS-GM. The two negative controls, ABC reagent alone and rhAb B1, did not bind to MS-GM whereas the positive control rhAb G11 bound MS-GM (FIG. 2A). The ten AGS-enriched rhAbs from 3 different disease types representing 6 different patients all showed binding to human MS-GM (FIGS. 2B-2D). As with the mouse brain DAB (FIGS. 1A-1D), the staining appeared to be cellular and exhibited similar staining patterns in the human brain DAB (FIGS. 2A-2D).

In contrast, the rhAbs demonstrated poor recognition to MS-WM (FIGS. 7A-7D). Plaque tissue from the MS patient showed evidence of damage, and the binding was diminished or absent in MS-P tissue (FIGS. 8A-8D). The rhAbs also had weak or no binding to HC-WM (FIGS. 9A-9D). A common feature shared by the rhAbs was that testing on all sources of WM tissue resulted in weaker staining patterns than was seen in GM tissue.

To confirm the paucity of rhAb reactivity to myelinated tracts, the inventor evaluated binding of the subset of rhAbs to myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG), two myelin components of considerable interest in MS.²¹ With the exception of AJL01, which was reactive to both MBP and MOG, all of the rhAbs tested negative to binding MBP and MOG by ELISA (FIGS. 17A-17B). In addition, none of the subset rhAbs reacted to MBP or MOG in a myelin array that has been previously described (FIG. 17C). Finally, none of these rhAbs reacted to MOG expressed on the cell surface of HeLa cells as assessed by flow cytometry (FIG. 17D).

AGS-enriched rhAbs target neurons and astrocytes in both mouse and human brain tissue. Due to the location and appearance of the DAB staining, the inventor

hypothesized that the rhAbs were binding to either of two major cell types in the brain, neurons and/or astrocytes. Therefore, IFC colocalization experiments were performed on 10 of the rhAbs from the initial cohort of 32. These 10 were chosen to sample all three disease subtypes and the same source of mouse brain tissue was utilized in these experiments. B1 and G11 were again used as negative and positive controls for the assay respectively. B1 did not recognize brain tissue (FIGS. 2A, 3A), but G11 did recognize mouse brain tissue (FIGS. 2B, 3B) as evidenced in both the NeuN and the GFAP colocalization experiments. Similar staining pattern of both B1 and G11 was confirmed in human MS-GM (FIGS. 10A, 10B).

Three rhAbs, one from each patient type, colocalized with neuronal nuclei identified by NeuN in both mouse and human brain. AJL03 from patient CDMS1 showed similar staining of neuronal nuclei in both species tissue type (FIGS. 3C, 3D). AJL10, from patient ONCIS2, showed colocalization with neuronal nuclei with the human targeting being very concentrated in the nucleus compared to a more diffuse staining pattern in mouse (FIGS. 3E, 3F). Additionally, there were areas of AJL10 binding that were independent of NeuN but were still associated with nuclei as marked by DAPI (FIG. 3E). AJL15 from patient TMCIS5 also colocalized with neuronal nuclei in both mouse and human tissue (FIGS. 3G, 3H). AGS-enriched B cells that recognize neuronal nuclei in the GM are found in all disease presentations and have conserved recognition between mouse and human species.

In order to see if targeting to astrocytes was present, the other major cell type in the GM in addition to neurons, IFC with GFAP was tested. WR12 and WR13 from the same patient, ONCIS2, colocalized with astrocytes and their processes in mouse tissue (FIGS. 4C, 4E). In human tissue, both rhAbs recognized GFAP-positive astrocyte bodies with additional vessel staining seen with WR12 (FIGS. 4D, 4F). AJL01 from patient TMCIS4 predominately colocalized with astrocytes with additional staining along the periventricular lining in mouse (FIG. 4G). AJL01 colocalized to astrocyte endfeet within a vessel in addition to the astrocyte bodies in MS-GM shown (FIG. 4H). In addition to AGS-enriched B cells that recognize neurons, this mutational pattern also imparts recognition to astrocytes.

Four AGS-enriched rhAbs from all 3 patient types displayed dual-cell targeting. AJL02 from patient CDMS1 predominantly colocalized with NeuN in a ring-like fashion within the nuclei (FIG. 5A) with minor colocalization with GFAP astrocyte processes (FIG. 5B). This ring-like staining pattern around neuronal nuclei was also seen in human tissue (FIG. 5C). AJL07 was cloned from patient ONCIS3, and displayed dual-cell targeting to both neurons and astrocytes in mouse brain (FIGS. 5D, 5E). In MS-GM AJL07 principally stained neuronal nuclei and also a vessel (FIG. 5F), keeping with the dual-cell targeting observed in

mouse. From patient TMCIS4, WR10 displayed dual-cell targeting with colocalization to both the edges of neuronal nuclei and astrocyte processes in mouse brain (FIGS. 5G, 5H). WR10 stained astrocyte bodies, endfeet around a vessel, as well as a neuron seen at the top of the panel in MS-GM (FIG. 5I). AJL19 from patient TMCIS6 reacted similarly to WR10 with dual-cell targeting as it colocalized with both neuronal nuclei and astrocytes (FIGS. 5J, 5K). Colocalization of AJL19 with neuronal nuclei was also seen in MS-GM (FIG. 5L).

Due to the focused cellular binding of these rhAbs by DAB, the inventor hypothesized that the rhAbs were binding to either neurons and/or astrocytes in the gray matter. Therefore,

IFC colocalization experiments were performed on the subset of rhAbs. In both mouse and human brain tissues, NeuN was utilized as a marker for neuronal nuclei and GFAP was utilized as a marker for astrocytes. FIGS. 13A-F and FIGS. 14A-F feature four of these rhAbs and the remaining six are summarized in FIGS. 15A-F (CDMS derived rhAbs) and FIGS. 16A-L (CIS derived rhAbs).

AJL10 is a rhAb cloned from patient ONCIS2 that had an AGS score of 6.07 and converted to MS 2.5 years after sampling. This antibody utilizes the VH4-4 gene paired with a JH6 segment and also uses a VK light chain. AJL10 has replacement SHM at 4 of the 6 AGS codons (40, 56, 81, and 89) with an overall high mutation frequency of 12.63% (Table 5). No additional clones of this B cell were detected in the patient's CSF. As shown in FIGS. 13A-F, AJL10 demonstrated co-localization with neuronal nuclei in both mouse (FIG. 13A), and human (FIG. 13B) gray matter. AJL10 did not cross-react with astrocytes as demonstrated by the lack of co-localization with the astrocyte specific antibody, GFAP (FIG. 13C). AJL07 is a rhAb cloned from a different CIS patient, ONCIS3, that had an AGS score of 10.68 and converted to MS 1 month after sampling. This antibody utilizes the VH4-59 gene paired with a JH4 segment and also uses a VK light chain. AJL07 has replacement SHM at 3 of the 6 AGS codons (56, 57, and 81) with an overall high mutation frequency of 9.23% (Table 5). Three additional clones of this B cell were detected in the patient's CSF. AJL07 co-localized with NeuN in both mouse and human gray matter (FIGS. 13D-E) and also cross-reacted with astrocytes as demonstrated by GFAP co-localization (FIG. 13F). Interestingly, AJL07 also bound to the vasculature in human gray matter (FIG. 13E).

Next, the inventor wanted to see if CIS patients presenting with TM would also display gray matter binding by their AGS-enriched rhAbs (FIGS. 14A-F). AJL01 is a rhAb cloned from patient TMCIS4 that had an AGS score of 17.90 and converted to MS 8 months after sampling. This antibody utilizes the VH4-34 gene paired with a JH3 segment and also uses a VK light chain. AJL01 has replacement SHM at 3 of the 6 AGS codons (56, 57, and

81) with an overall high mutation frequency of 11.28% (Table 5). This was detected as a four member clone within the patient's CSF. AJL01 colocalized to astrocyte endfeet within the blood-brain barrier in addition to the astrocyte bodies shown in both mouse and human GM (FIGS. 14A-B). AJL01 did not cross-react with neurons in mouse GM (FIG. 14C). Another
5 AGS-enriched rhAb, WR13, also bound to astrocytes. This rhAb was cloned from the same ONCIS2 patient as AJL10. This antibody utilizes the VH4-30 gene paired with a JH4 segment and also uses a VK light chain. WR13 has replacement SHM at 2 of the 6 AGS codons (56 and 81) with an overall mutation frequency of 11.94% (Table 5). Similar to AJL01 from a TM_{CIS} patient, WR13 from an ONCIS patient co-localized to astrocytes in both
10 mouse and human GM (FIGS. 14D-14E) and did not cross-react with neurons (FIG. 14F). A clonally related rhAb also detected in this patient's CSF, WR12, was tested and demonstrated the same staining pattern (FIGS. 16A-16L).

Of the 32 rhAbs that were evaluated for binding to brain tissue, 30 bound to brain tissue by DAB staining. Of those, 10 were evaluated for binding to neurons and astrocytes by
15 IFC. Of those 10, 4 bound to both neurons and astrocytes, 3 bound to neurons only and 3 bound to astrocytes only. Some of the results are shown in FIGS. 18A-18M. Further investigation of the 6 AGS codons revealed that all brain binding antibodies must have replacement mutations in at least two of the AGS codons. One of these mutations is typically an "R" or "N" replacement result at codon 81. Antibodies that do not have a mutation at
20 codons 31b, 40, or 89 but have a "T" or "R" replacement result at codon 56 bind astrocytes, but not neurons. Antibodies that do not meet the astrocyte binding criteria bind neurons, and also have an "S" replacement result at codon 40. Those antibodies that bind neurons and astrocytes do not have a mutation at codon 31b and no "R" replacement result at codon 56. This finding could have profound implications in the diagnostic and therapeutic treatment of
25 CNS disease, such as Multiple Sclerosis.

Tables 2-4 illustrate the sequenced heavy and light chains, and their staining patterns.

Table 2 - Heavy Chain Sequences of AGS Enriched CSF Antibodies

4-30 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
4-30 Kabat Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
	FR1																	
Germ. AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	Q	T
Germ. Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tca	cag	acc
WR12 AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	Q	T
WR12 Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tca	cag	acc
WR13 AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	Q	T
WR13 Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tca	cag	acc

4-30 Codon	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
4-30 Kabat	18	19	20	21	22	23	24	25	26	27	28	29	30			31	31a	31b	
Region	FR1								CDR1										
Germ. AA	L	S	L	T	C	T	V	S	G	G	S	I	S			S	G	D	
Germ. Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggg	ggc	tcc	atc	agc			agt	ggg	gat	
WR12 AA	L	S	L	T	C	T	V	S	G	G	S	V	S			S	N	D	
WR12 Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggc	ggc	tcc	gtc	agc			agt	ggg	gat	
WR13 AA	L	S	L	T	C	T	V	S	G	G	S	V	S			S	N	D	
WR13 Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggc	ggc	tcc	gtc	agc			agt	ggg	gat	

4-30 Codon	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
4-30 Kabat	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Region	CDR1									FR2								
Germ. AA	Y	Y	W	S	W	I	R	Q	P	P	G	K	G	L	E	W	I	G
Germ. Nuc	tac	tac	tgg	agt	tgg	atc	cgc	cag	ccc	cca	ggg	aag	ggc	ctg	gag	tgg	att	ggg
WR12 AA	Y	Y	W	S	W	I	R	Q	P	P	G	Q	G	L	E	W	I	G
WR12 Nuc	cac	tac	tgg	agt	tgg	atc	cgc	cag	ccc	cca	ggg	cag	ggc	ctg	gag	tgg	att	ggg
WR13 AA	Y	Y	W	S	W	I	R	Q	P	P	G	Q	G	L	E	W	I	G
WR13 Nuc	cac	tac	tgg	agt	tgg	atc	cgc	cag	ccc	cca	ggg	cag	ggc	ctg	gag	tgg	att	ggg

4-30 Codon	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	
4-30 Kabat	50	51	52	53	54				55	56	57	58	59	60	61	62	63	64	
Region	FR2	CDR2										FR3							
Germ. AA	Y	I	Y	Y	S				G	S	T	Y	Y	N	P	S	L	K	
Germ. Nuc	tac	atc	tat	tac	agt				ggg	agc	acc	tac	tac	aac	cgc	tcc	ctc	aag	
WR12 AA	Y	I	S	H	G				G	T	T	Y	Y	N	P	S	L	K	
WR12 Nuc	tac	atc	tat	cac	ggg				ggg	acc	acc	tac	tac	aac	cgc	tcc	ctc	aag	
WR13 AA	Y	I	S	H	G				G	T	T	Y	Y	N	P	S	L	K	
WR13 Nuc	tac	atc	tat	cac	ggg				ggg	acc	acc	tac	tac	aac	cgc	tcc	ctc	aag	

4-30 Codon	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
4-30 Kabat	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	
Region	FR3																	
Germ. AA	S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K	
Germ. Nuc	agt	cga	gtt	acc	ata	tca	gta	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	aag	
WR12 AA	S	R	V	T	M	S	I	D	T	S	I	N	Q	F	S	L	R	
WR12 Nuc	agt	cga	gtt	acc	atg	tgg	atc	gac	acg	tcc	acc	aac	cag	ttc	tcc	ctg	agg	
WR13 AA	S	R	V	T	M	S	I	D	T	S	I	N	Q	F	S	L	R	
WR13 Nuc	agt	cga	gtt	acc	atg	tgg	atc	gac	acg	tcc	acc	aac	cag	ttc	tcc	ctg	agg	

4-30 Codon	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106		
4-30 Kabat	82	82a	82b	82c	83	84	85	86	87	88	89	90	91	92	93	94		
Region	FR3														CDR3			
Germ. AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R	A	
Germ. Nuc	ctg	agc	tct	gtg	act	gcc	gca	gac	acg	gcc	gtg	tat	tac	tgt	gcc	aga	gca	
WR12 AA	V	I	S	V	R	A	A	D	M	A	V	Y	F	C	A	P	A	P
WR12 Nuc	gtg	acc	acc	gtg	cga	gcc	gca	gac	atg	gcc	gtc	tac	tac	tgt	gcc	agg	acc	cgg
WR13 AA	V	I	S	V	R	A	A	D	M	A	V	Y	F	C	A	P	A	P
WR13 Nuc	gtg	acc	acc	gtg	cga	gcc	gca	gac	atg	gcc	gtc	tac	tac	tgt	gcc	agg	acc	cgg

4-30 Codon																
4-30 Kabat Region																
	CDR3															
Germ. AA																
Germ. Nuc																
WR12 AA	A	P	I	T	T	F	G	M	V	T	P	V	P	Y	F	S

WR12 Nuc	gcc	cct	ata	acg	act	ttt	gga	atg	gtg	aca	cca	gtc	ccc	tac	ttt	cac	tcc
WR13 AA	A	P	I	T	T	F	G	M	V	T	P	V	P	Y	F	H	S
WR13 Nuc	gcc	cct	ata	acg	act	ttt	gga	atg	gtg	aca	cca	gtc	ccc	tac	ttt	cac	tcc

4-31 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
4-31 Kabat		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Region	FR1																	
Germ. AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	Q	T
Germ. Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tca	cag	acc
AJL02 AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	Q	T
AJL02 Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tca	cag	acc

4-31 Codon	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
4-31 Kabat	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33a	34a	35b
Region	FR1								CDR1									
Germ. AA	L	S	L	T	C	T	V	S	G	G	S	I	S			S	G	G
Germ. Nuc	ctg	tcc	ctc	acc	tgt	act	gtc	tct	ggg	ggc	tcc	atc	agc			agt	ggg	ggg
AJL02 AA	L	S	L	T	C	T	V	S	G	G	S	I	S			S	G	G
AJL02 Nuc	ctg	tcc	ctc	acc	tgt	act	gtc	tct	ggg	ggc	tcc	atc	agc			agt	ggg	ggg

4-31 Codon	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
4-31 Kabat	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Region	CDR1				FR2													
Germ. AA	Y	Y	W	S	W	I	R	Q	H	P	G	K	G	L	E	W	I	G
Germ. Nuc	tac	tac	tgg	agc	tgg	atc	cgc	cag	cac	cca	ggg	aag	ggc	ctg	gag	tgg	att	ggg
AJL02 AA	Y	Y	W	S	W	I	R	Q	S	P	G	K	G	L	E	W	I	G
AJL02 Nuc	tac	tac	tgg	agc	tgg	atc	cgc	cag	tcc	cca	ggg	aag	ggc	ctg	gag	tgg	att	ggg

4-31 Codon	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
4-31 Kabat	50	51	52	53	54				55	56	57	58	59	60	61	62	63	64
Region	FR2				CDR2								FR3					
Germ. AA	Y	I	Y	Y	S				G	S	T	Y	Y	N	P	S	L	K
Germ. Nuc	tac	atc	tat	tac	agt				ggg	agc	acc	tac	tac	aac	cgg	tcc	ctc	aag
AJL02 AA	N	V	Y	Y	S				G	S	T	Y	Y	T	P	S	L	S
AJL02 Nuc	aat	gtc	tat	tac	agt				gga	agc	acc	tac	tac	acc	cgg	tcc	ctc	aac

4-31 Codon	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
4-59 Kabat		65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
Region	FR3																	
Germ. AA		S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K
Germ. Nuc		agt	cga	gtt	acc	ata	tca	gta	gac	acg	tct	aag	aac	cag	ttc	tcc	ctg	aag
AJL02 AA		S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K
AJL02 Nuc		agc	cga	gtt	acc	ata	tca	gta	gac	acg	tct	aag	aac	cag	ttc	tcc	ctg	aag

4-31 Codon	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106		
4-31 Kabat	82	82a	82b	82c	83	84	85	86	87	88	89	90	91	92	93	94		
Region	FR3														CDR3			
Germ. AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R		
Germ. Nuc	ctg	agc	tct	gtg	act	gcc	gcg	gac	acg	gcc	gtg	tat	tac	tgt	gcg	aga		
AJL02 AA	L	S	N	V	T	V	A	D	T	A	V	Y	Y	C	A	R	G	R
AJL02 Nuc	ctg	agc	aat	gtg	act	gtc	gcg	gac	acg	gcc	gtg	tat	tac	tgt	gcg	aga	ggt	aga

4-31 Codon																		
4-31 Kabat																		
Region	CDR3																	
Germ. AA																		
Germ. Nuc																		
AJL02 AA	N	W	E	G	E	F	D	P	W	G	Q	G						
AJL02 Nuc	aat	tgg	gag	ggc	gaa	ttc	gac	ccc	tgg	ggc	caa	gga						

5

4-34 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
4-34 Kabat		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Region	FR1																	
Germ. AA	Q	V	Q	L	Q	Q	W	G	A		G	L	L	K	P	S	E	T
Germ. Nuc	cag	gtg	cag	cta	cag	cag	tgg	ggc	gca		gga	ctg	tgg	aag	cct	tgg	gag	acc

AJL01 AA	Q	V	Q	L	Q	Q	W	G	A		G	L	L	K	P	S	E	T
AJL01 Nuc	cag	gtg	cag	cta	cag	cag	tgg	ggc	gca		gga	ctg	tgg	aag	cct	tcg	gag	acc
AJL19 AA	Q	V	Q	L	Q	Q	W	G	A		G	L	L	K	P	S	E	T
AJL19 Nuc	cag	gtg	cag	cta	cag	cag	tgg	ggc	gca		gga	ctg	tgg	aag	cct	tcg	gag	acc

4-34 Codon	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
4-34 Kabat	18	19	20	21	22	23	24	25	26	27	28	29					30	31
Region	FR1								CDR1									
Germ. AA	L	S	L	T	C	A	V	Y	G	G	S	F					S	G
Germ. Nuc	ctg	tcc	ctc	acc	tgc	gct	gtc	tat	ggg	ggg	tcc	ttc					agt	ggt
AJL01 AA	L	S	L	T	C	A	V	Y	G	G	S	F					N	F
AJL01 Nuc	ctg	tcc	ctc	acc	tgc	gct	gtc	tat	ggg	ggg	tcc	ttc					aat	gaa
AJL19 AA	L	S	L	T	C	A	V	Y	G	G	S	F					S	G
AJL19 Nuc	ctg	tcc	ctc	acc	tgc	gct	gtc	tat	ggg	ggg	tcc	ttc					agt	ggt

4-34 Codon	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
4-34 Kabat	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Region	CDR1								FR2									
Germ. AA	Y	Y	W	S	W	I	R	Q	P	P	G	K	G	L	E	W	I	G
Germ. Nuc	tac	tac	tgg	agc	tgg	atc	cgc	cag	ccc	cca	ggg	aag	ggg	ctg	gag	tgg	att	ggg
AJL01 AA	F	Y	W	S	W	I	R	Q	P	A	R	K	G	L	E	W	I	G
AJL01 Nuc	tac	tac	tgg	agc	tgg	atc	cgc	cag	ccc	cca	ggg	aag	ggg	ctg	gag	tgg	att	ggg
AJL19 AA	Y	Y	W	S	W	I	R	Q	P	P	G	K	G	L	E	W	I	A
AJL19 Nuc	tac	tac	tgg	agc	tgg	atc	cgc	cag	ccc	cca	ggg	aag	ggg	ctg	gag	tgg	att	gaa

4-34 Codon	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
4-34 Kabat	50	51	52	53	54				55	56	57	58	59	60	61	62	63	64
Region	FR2								CDR2				FR3					
Germ. AA	E	I	N	H	S				G	S	T	N	Y	N	P	S	L	K
Germ. Nuc	gaa	atc	aat	cat	agt				gga	agc	acc	aac	tac	aac	cgc	tcc	ctc	aag
AJL01 AA	E	I	S	H	S				G	P	A	N	Y	N	P	S	L	K
AJL01 Nuc	gaa	atc	agt	cat	agt				gga	gga	gcc	aac	tac	aac	cgc	tcc	ctc	aag
AJL19 AA	E	I	N	H	S				G	P	A	N	Y	N	P	S	L	K
AJL19 Nuc	gaa	atc	aat	cat	agt				gga	gga	gcc	aac	tac	aac	cgc	tcc	ctc	aag

4-34 Codon	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
4-34 Kabat		65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
Region	FR3																	
Germ. AA		S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K
Germ. Nuc		agt	cga	gtc	acc	ata	tca	gta	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	aag
AJL01 AA		S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	N
AJL01 Nuc		agt	cga	gtc	acc	ata	tca	gta	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	aac
AJL19 AA		S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K
AJL19 Nuc		agt	cga	gtc	acc	ata	tca	gta	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	aag

4-34 Codon	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	
4-34 Kabat	82	82a	82b	82c	83	84	85	86	87	88	89	90	91	92	93	94	
Region	FR3														CDR3		
Germ. AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R	G
Germ. Nuc	ctg	agc	tct	gtg	acc	gcc	gcg	gac	acg	gct	gtg	tat	tac	tgt	gcg	aga	ggg
AJL01 AA	L	S	P	V	A	A	A	D	T	A	V	Y	Y	C	A	R	E
AJL01 Nuc	ctg	agc	cct	gtg	acc	gcc	gcg	gac	acg	gct	gtc	tat	tac	tgt	gcg	aga	ggg
AJL19 AA	M	S	S	V	T	N	A	D	T	A	L	Y	Y	C	A	T	G
AJL19 Nuc	atg	agc	tct	gtg	acc	gcc	gcg	gac	acg	gct	tta	tat	tac	tgt	gcg	aga	ggc

4-34 Codon																	
4-34 Kabat																	
Region	CDR3																
Germ. AA																	
Germ. Nuc																	
AJL01 AA	I	V	V	T	V	R	G	R	R	A	F	D	I				
AJL01 Nuc	ata	gtc	gtg	act	gtt	cgg	ggg	cgt	cgt	gct	ttt	gat	atc				
AJL19 AA	S	R	L	T	T	F	A	F	D	V							
AJL19 Nuc	tct	agg	tgg	act	aca	ttc	gct	ttt	gat	gtg							

4-39 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
4-39 Kabat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Region	FR1																	
Germ. AA	Q	L	Q	L	Q	E	S	G	P		G	L	V	K	P	S	E	T
Germ. Nuc	cag	ctg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tgg	gag	acc
AJL03 AA	Q	L	Q	L	Q	E	S	G	P		G	L	V	K	P	S	E	T
AJL03 Nuc	cag	ctg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tgg	gag	acc
AJL15 AA	Q	L	Q	L	Q	E	S	G	P		G	L	V	K	P	S	E	T
AJL15 Nuc	cag	ctg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tgg	gag	acc

4-39 Codon	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
4-39 Kabat	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33a	31a	31b
Region	FR1									CDR1								
Germ. AA	L	S	L	T	C	T	V	S	G	G	S	I	S			S	S	S
Germ. Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggg	ggc	tcc	atc	agc			agt	agt	agt
AJL03 AA	L	S	L	T	C	T	V	S	G	A	S	I	S			S	S	P
AJL03 Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggg	ggc	tcc	atc	agc			agt	agt	cgt
AJL15 AA	L	S	L	T	C	T	V	S	G	G	S	I	T			S	P	N
AJL15 Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggg	ggc	tcc	atc	acc			agt	acc	aat

4-39 Codon	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
4-39 Kabat	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Region	CDR1				FR2													
Germ. AA	Y	Y	W	G	W	I	R	Q	P	P	G	K	G	L	E	W	I	G
Germ. Nuc	tac	tac	tgg	ggc	tgg	atc	cgc	cag	ccc	cca	ggg	aag	ggg	ctg	gag	tgg	att	ggg
AJL03 AA	S	Y	W	G	W	I	R	Q	P	P	G	K	G	L	E	W	I	G
AJL03 Nuc	tcc	tac	tgg	ggc	tgg	atc	cgc	cag	ccc	cca	ggg	aag	ggg	ctg	gag	tgg	att	ggg
AJL15 AA	N	Y	W	G	W	I	R	Q	S	P	G	K	G	L	E	W	I	G
AJL15 Nuc	aac	tac	tgg	ggc	tgg	atc	cgc	cag	tcc	cca	ggg	aag	ggg	ctg	gag	tgg	att	ggg

4-39 Codon	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
4-39 Kabat	50	51	52	53	54				55	56	57	58	59	60	61	62	63	64
Region	FR2				CDR2							FR3						
Germ. AA	S	I	Y	Y	S				G	S	T	Y	Y	N	P	S	L	K
Germ. Nuc	agt	atc	tat	tat	agt				ggg	agc	acc	tac	tac	aac	cgg	tcc	ctc	aag
AJL03 AA	S	M	Y	Y	S				G	S	T	Y	Y	S	P	S	L	K
AJL03 Nuc	agt	atg	tat	tat	agt				ggg	agc	acc	tac	tac	aga	cgg	tcc	ctc	aag
AJL15 AA	S	L	Y	Y	T				G	S	T	Y	Y	N	P	S	L	K
AJL15 Nuc	agt	atc	tat	tat	agt				ggg	agc	acc	tac	tac	aac	cgg	tcc	ctc	aag

4-39 Codon	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
4-39 Kabat		65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
Region	FR3																	
Germ. AA		S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K
Germ. Nuc		agt	cga	gtc	acc	ata	tca	gta	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	aag
AJL03 AA		S	R	V	T	I	S	M	D	T	S	K	N	Q	F	S	L	N
AJL03 Nuc		agt	cga	gtc	acc	ata	tcc	atg	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	aac
AJL15 AA		S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	T
AJL15 Nuc		agt	cga	gtc	acc	ata	tcc	gta	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	agg

4-39 Codon	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106		
4-39 Kabat	82	82a	82b	82c	83	84	85	86	87	88	89	90	91	92	93	94		
Region	FR3												CDR3					
Germ. AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R	D	
Germ. Nuc	ctg	agc	tct	gtg	acc	gcc	gcg	gac	acg	gcc	gtg	tat	tac	tgt	gcg	aga	gat	
AJL03 AA	L	T	S	V	T	A	A	D	T	A	V	Y	F	C	A	R	T	S
AJL03 Nuc	ctg	acc	tct	gtg	acc	gcc	gcg	gac	acg	gcc	gtg	tat	acc	tgt	gcg	aga	cgt	tgg
AJL15 AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	V	R	V	N
AJL15 Nuc	ctg	acc	tct	gtg	acc	gcc	gcg	gac	acg	gcc	gtg	tat	acc	tgt	gcg	aga	cgt	aac

4-39 Codon																		
4-39 Kabat																		
Region	CDR3																	
Germ. AA																		
Germ. Nuc																		
AJL03 AA	N	P	G	T	A	N	K	L	R	L	G	E	F	S	P			
AJL03 Nuc	aac	ccc	gga	acg	gcg	aac	aaa	tgg	cgt	tgg	ggg	gag	ttt	tgg	ccc			
AJL15 AA	V	D	D	F	W	S	G	L	G	G	A	W	F	D	P			
AJL15 Nuc	gta	gac	gac	ttt	tgg	agt	ggg	tta	ggg	ggg	gcc	tgg	ttc	gac	ccc			

4-4 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
4-4 Kabat		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Region	FR1																	
Germ. AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	E	T
Germ. Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tgg	gag	acc
AJL10 AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	E	T
AJL10 Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tgg	gag	acc
WR10 AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	E	T
WR10 Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tgg	gag	acc

4-4 Codon	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
4-4 Kabat	18	19	20	21	22	23	24	25	26	27	28	29					30	31
Region	FR1								CDR1									
Germ. AA	L	S	L	T	C	T	V	S	G	G	S	I					S	S
Germ. Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggg	ggc	tcc	atc					agt	agt
AJL10 AA	L	S	L	T	C	T	V	S	G	G	A	V					S	N
AJL10 Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggg	ggc	gag	gtc					agt	agt
WR10 AA	L	S	L	T	C	A	V	S	G	G	S	I	S				N	N
WR10 Nuc	ctg	tcc	ctc	acc	tgc	gct	gtc	tct	ggg	ggc	tcc	atc	acc				agt	agt

4-4 Codon	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
4-4 Kabat	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Region	CDR1				FR2													
Germ. AA	Y	Y	W	S	W	I	R	Q	P	A	G	K	G	L	E	W	I	G
Germ. Nuc	tac	tac	tgg	agc	tgg	atc	cgg	cag	ccc	gcc	ggg	aag	gga	ctg	gag	tgg	att	ggg
AJL10 AA	Y	Y	W	S	W	I	R	Q	S	A	G	K	G	L	E	W	I	G
AJL10 Nuc	tac	tac	tgg	agc	tgg	atc	cgg	cag	tcc	gcc	ggg	aag	gga	ctg	gag	tgg	att	ggg
WR10 AA	K	W	W	N	W	V	R	Q	S	P	G	K	G	L	E	W	I	G
WR10 Nuc	aaa	tgg	tgg	aat	tgg	gtc	cgc	cag	tcc	acc	ggg	aag	ggg	ctg	gag	tgg	att	ggg

4-4 Codon	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
4-4 Kabat	50	51	52	53	54					55	56	57	58	59	60	61	62	63
Region	FR2				CDR2							FR3						
Germ. AA	R	I	Y	T	S				G	S	T	N	Y	N	P	S	L	K
Germ. Nuc	cgt	atc	tat	acc	agt				ggg	agc	acc	aac	tac	aac	ccc	tcc	ctc	aag
AJL10 AA	R	I	Y	I	N				G	T	T	Y	Y	N	P	S	L	R
AJL10 Nuc	cgt	atc	tat	acc	agt				gga	act	act	tac	tac	aac	ccc	tcc	ctc	agg
WR10 AA	E	I	Y	H	S				G	G	T	N	Y	N	P	S	L	K
WR10 Nuc	gaa	atc	tat	cat	agt				ggg	ggc	acc	aac	tac	aac	ccc	tcc	ctc	aag

4-4 Codon	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
4-4 Kabat		65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
Region	FR3																	
Germ. AA		S	R	V	T	M	S	V	D	T	S	K	N	Q	F	S	L	K
Germ. Nuc		agt	cga	gtc	acc	atg	tca	gta	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	aag
AJL10 AA		S	R	V	T	A	A	S	V	D	T	S	K	G	Q	F	S	L
AJL10 Nuc		agt	cga	gtc	acc	atg	tca	gta	gac	acg	tcc	aag	gag	cag	ttc	tcc	ctg	agg
WR10 AA		S	R	V	T	I	S	V	D	K	S	K	N	L	F	S	L	K
WR10 Nuc		agt	cga	gtc	acc	ata	acc	gta	gac	aag	tcc	aag	aac	ctg	ttc	tcc	ctg	aag

4-4 Codon	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106		
4-4 Kabat	82	82a	82b	82c	83	84	85	86	87	88	89	90	91	92	93	94		
Region	FR3														CDR3			
Germ. AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R		
Germ. Nuc	ctg	agc	tct	gtg	acc	gcc	ggc	gac	acg	gcc	gtg	tat	tac	tgt	ggc	aga		
AJL10 AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R	W	G
AJL10 Nuc	ctg	agc	tct	gtg	acc	gcc	ggc	gac	acg	gcc	ata	tat	tac	tgt	ggc	aga	tgg	ggt
WR10 AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	S	A	T
WR10 Nuc	ctg	agc	tct	gtg	acc	gcc	ggc	gac	acg	gcc	gtg	tat	tac	tgt	ggc	agt	ggc	act

4-4 Codon																		
4-4 Kabat																		
Region	CDR3																	
Germ. AA																		
Germ. Nuc																		
AJL10 AA	A	L	L	G	D	Y	Y	Y	G	L	D	V						

AJL10 Nuc	gcc	cta	ttg	ggc	gac	tac	tat	tac	ggt	ttg	gac	gtc					
WR10 AA	T	M	V	R	G	L	S	L	Y	Y	Y	G	L	D	V		
WR10 Nuc	act	atg	gtt	cgg	gga	ctg	agt	ctt	tac	tac	tac	ggt	ctg	gac	gtc		

4-59 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
4-59 Kabat		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Region	FR1																	
Germ. AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	E	T
Germ. Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tgg	gag	acc
AJL07 AA	Q	V	Q	L	Q	E	S	G	P		G	L	V	K	P	S	E	T
AJL07 Nuc	cag	gtg	cag	ctg	cag	gag	tgg	ggc	cca		gga	ctg	gtg	aag	cct	tgg	gag	acc

4-59 Codon	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
4-59 Kabat	18	19	20	21	22	23	24	25	26	27	28	29					30	31	
Region	FR1									CDR1									
Germ. AA	L	S	L	T	C	T	V	S	G	G	S	I					S	S	
Germ. Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggt	ggc	tcc	atc					agt	agt	
AJL07 AA	L	S	L	T	C	T	V	S	G	G	S	I					N	N	
AJL07 Nuc	ctg	tcc	ctc	acc	tgc	act	gtc	tct	ggt	ggc	tcc	atc					aat	aat	

4-59 Codon	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
4-59 Kabat	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Region	CDR1				FR2													
Germ. AA	Y	Y	W	S	W	I	R	Q	P	P	G	K	G	L	E	W	I	G
Germ. Nuc	tac	tac	tgg	agc	tgg	atc	cgg	cag	ccc	cca	ggg	aag	gga	ctg	gag	tgg	att	ggg
AJL07 AA	Y	Y	W	S	W	I	R	Q	P	P	G	K	G	L	E	W	I	G
AJL07 Nuc	tat	tac	tgg	agt	tgg	atc	cgg	cag	ccc	cca	ggg	aag	gga	ctg	gag	tgg	att	ggt

4-59 Codon	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
4-59 Kabat	50	51	52	53	54				55	56	57	58	59	60	61	62	63	64
Region	FR2				CDR2							FR3						
Germ. AA	Y	I	Y	Y	N				G	S	T	N	Y	N	P	S	L	K
Germ. Nuc	tat	atc	tat	tac	aat				ggg	agc	acc	aac	tac	aac	ccc	tcc	ctc	aag
AJL07 AA	Y	I	Y	Y	N				G	N	?	N	Y	N	P	S	L	K
AJL07 Nuc	tat	atc	tat	tac	aat				ggg	aat	att	aat	tac	aac	ccc	tcc	ctc	aag

4-59 Codon	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
4-59 Kabat		65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
Region	FR3																	
Germ. AA		S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K
Germ. Nuc		agt	cga	gtc	acc	ata	tca	gta	gac	acg	tcc	aag	aac	cag	ttc	tcc	ctg	aag
AJL07 AA		S	R	V	T	I	S	R	D	N	S	K	N	Q	F	S	L	N
AJL07 Nuc		agt	cga	gtc	acc	ata	tca	aga	gac	atg	tcc	aag	aac	cag	ttc	tcc	ctg	aac

4-59 Codon	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106		
4-59 Kabat	82	82a	82b	82c	83	84	85	86	87	88	89	90	91	92	93	94		
Region	FR3														CDR3			
Germ. AA	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R	E	
Germ. Nuc	ctg	agc	tct	gtg	acc	gct	gcg	gac	acg	gcc	gtg	tat	tac	tgt	gcg	aga	gaa	
AJL07 AA	L	R	S	V	T	A	A	D	T	A	V	Y	Y	C	G	I	G	Y
AJL07 Nuc	ctg	agg	tct	gtg	acc	gct	gcg	gac	acg	gcc	gtg	tat	tac	tgt	aga	att	gaa	tat

4-59 Codon																		
4-59 Kabat																		
Region	CDR3																	
Germ. AA																		
Germ. Nuc																		
AJL07 AA	S	A	V	A	A	G	T	V	D	Y								
AJL07 Nuc	agt	gcg	gtg	gca	gct	ggt	aca	gtt	gac	tac								

1-33 Codon	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38				
1-33 Kabat	20	21	22	23	24	25	26	27	28	29											30	31	32
Region	FR1							CDR1															
Germ. AA	T	I	T	C	Q	A	S	Q	D	I											S	N	Y
Germ. Nuc	acc	atc	act	tgc	cag	gag	agt	cag	gac	att											age	aac	lat
AJL03 AA	T	I	T	C	Q	A	S	Q	D	I											N	N	Y

1-39 Codon	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
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1-39 Kabat Region	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
Germ. AA	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q
Germ. Nuc	agt	ggc	agt	gga	tct	ggg	aca	gat	ttc	act	ctc	acc	atc	agg	agt	ctg	caa
AJL02 AA	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q
AJL02 Nuc	agt	ggc	agt	gga	tct	ggg	aca	gat	ttc	act	ctc	acc	atc	agg	agt	ctg	caa

1-39 Codon	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111
1-39 Kabat Region	80	81	82	83	84	85	86	87	88	89	90	91	92			
Germ. AA	P	E	D	F	A	T	Y	Y	C	Q	Q	S	Y	S	T	P
Germ. Nuc	cct	gaa	gat	ttt	gca	act	tac	tac	tgt	caa	cag	agt	tac	agt	acc	cct
AJL02 AA	P	E	D	F	A	T	Y	Y	C	Q	Q	S	Y	S	T	P
AJL02 Nuc	cct	gaa	gat	ttt	gca	act	tac	tac	tgt	cag	cag	agt	tac	agt	acc	cct

2-28 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
2-28 Kabat Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Germ. AA	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A
Germ. Nuc	gat	att	gtg	atg	act	cag	tct	cca	ctc	tcc	ctg	ccc	gtc	acc	cct	gga	gag	ccg	gcc
AJL10 AA	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A
AJL10 Nuc	gat	att	gtg	atg	act	cag	tct	cca	ctc	tcc	ctg	ccc	gtc	acc	cct	gga	gag	ccg	gcc
WR12 AA	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A
WR12 Nuc	gat	att	gtg	atg	act	cag	tct	cca	ctc	tcc	ctg	ccc	gtc	acc	cct	gga	gag	ccg	gcc
WR13 AA	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A
WR13 Nuc	gat	att	gtg	atg	act	cag	tct	cca	ctc	tcc	ctg	ccc	gtc	acc	cct	gga	gag	ccg	gcc

2-28 Codon	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
2-28 Kabat Region	20	21	22	23	24	25	26	27	27a	27b	27c	27d	27e		28	29	30	31	32
Germ. AA	S	I	S	C	R	S	S	Q	S	L	L	H	S		N	G	Y	N	Y
Germ. Nuc	tcc	atc	tcc	tgc	agg	tct	agt	cag	agc	ctc	ctg	cat	agt		aat	gga	tac	aac	tat
AJL10 AA	S	I	S	C	R	S	S	Q	S	L	L	H	S		N	E	Y	I	Y
AJL10 Nuc	tcc	atc	tcc	tgc	agg	tct	agt	cag	agc	ctc	ctg	cat	agt		aat	gaa	tac	aac	tat
WR12 AA	S	I	S	C	R	S	S	Q	S	L	L	H	S		N	G	Y	N	Y
WR12 Nuc	tcc	atc	tcc	tgc	agg	tct	agt	cag	agc	ctc	ctg	cat	agt		aat	gga	tac	aac	tat
WR13 AA	S	I	S	C	R	S	S	Q	S	L	L	H	S		N	G	Y	N	Y
WR13 Nuc	tcc	atc	tcc	tgc	agg	tct	agt	cag	agc	ctc	ctg	cat	agt		aat	gga	tac	aac	tat

2-28 Codon	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
2-28 Kabat Region	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
Germ. AA	L	D	W	Y	L	Q	K	P	G	Q	S	P	Q	L	L	I	Y	L	G
Germ. Nuc	ttg	gat	tgg	tac	ctg	cag	aag	cca	ggg	caa	tct	cca	caa	ctc	ctg	atc	tat	tig	ggc
AJL10 AA	L	D	W	Y	V	Q	K	P	G	Q	S	P	Q	L	L	I	F	L	A
AJL10 Nuc	tig	gat	tgg	tac	ctg	cag	aag	cca	ggg	caa	tct	cca	caa	ctc	ctg	atc	tat	tig	ggc
WR12 AA	L	S	W	Y	L	Q	K	P	G	Q	S	P	Q	L	L	I	F	S	S
WR12 Nuc	tig	gat	tgg	tac	ctg	cag	aag	cca	ggg	caa	tct	cca	caa	ctc	ctg	atc	tat	tig	ggc
WR13 AA	L	S	W	Y	L	Q	K	P	G	Q	S	P	Q	L	L	I	F	S	S
WR13 Nuc	tig	gat	tgg	tac	ctg	cag	aag	cca	ggg	caa	tct	cca	caa	ctc	ctg	atc	tat	tig	ggc

2-28 Codon	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76
2-28 Kabat Region								52	53	54	55	56	57	58	59		60	61	62
Germ. AA								S	N	R	A	S	G	V	P		D	R	F
Germ. Nuc								tct	aat	egg	gcc	tcc	ggg	gtc	cct		gac	agg	ttc
AJL10 AA								S	N	R	A	S	G	V	P		D	R	F
AJL10 Nuc								tct	aat	egg	gcc	tcc	ggg	gtc	cct		gac	agg	ttc
WR12 AA								S	I	R	A	S	G	V	P		D	R	F
WR12 Nuc								tct	aat	egg	gcc	tcc	ggg	gtc	cct		gac	agg	ttc
WR13 AA								S	I	R	A	S	G	V	P		D	R	F
WR13 Nuc								tct	aat	egg	gcc	tcc	ggg	gtc	cct		gac	agg	ttc

2-28 Codon	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
2-28 Kabat Region	63	64	65	66			67	68	69	70	71	72	73	74	75	76	77	78	79
Germ. AA	S	G	S	G			S	G	T	D	F	T	L	K	I	S	R	V	E

Germ. Nuc	agt	ggc	agt	gga		tca	ggc	aca	gat	ttt	aca	ctg	aaa	atc	agc	aga	gtg	gag
AJL10 AA	S	G	S	A		S	G	T	D	F	T	L	K	I	S	R	V	E
AJL10 Nuc	agt	ggc	agt	gga		tca	ggc	aca	gat	ttt	aca	ctg	aaa	atc	agc	aga	gtg	gag
WR12 AA	S	G	S	G		S	G	T	D	F	T	L	I	N	R	V	E	
WR12 Nuc	agt	ggc	agt	gga		tca	ggc	aca	gat	ttt	aca	ctg	aaa	atc	agc	aga	gtg	gag
WR13 AA	S	G	S	G		S	G	T	D	F	T	L	I	N	R	V	E	
WR13 Nuc	agt	ggc	agt	gga		tca	ggc	aca	gat	ttt	aca	ctg	aaa	atc	agc	aga	gtg	gag

2-28 Codon	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111		
2-28 Kabat	80	81	82	83	84	85	86	87	88	89	90	91	92					
	FR3									CDR3								
Germ. AA	A	E	D	V	G	V	Y	Y	C	M	Q	A	L	Q	T	P		
Germ. Nuc	gct	gag	gat	gtt	ggg	gtt	tat	tac	tgc	atg	caa	get	cta	caa	act	cct		
AJL10 AA	A	E	D	V	G	V	Y	Y	C	M	Q	A	L	E	A	P	W	T
AJL10 Nuc	gct	gag	gat	gtt	ggg	gtt	tat	tac	tgc	atg	caa	get	cta	aaa	get	cag	tgg	acg
WR12 AA	A	E	D	V	G	V	Y	Y	C	M	Q	A	L	Q	T	P	L	T
WR12 Nuc	gct	gag	gat	gtt	ggg	gtt	tat	tac	tgc	atg	caa	get	cta	caa	act	cag	ctc	act
WR13 AA	A	E	D	V	G	V	Y	Y	C	M	Q	A	L	Q	T	P	L	T
WR13 Nuc	gct	gag	gat	gtt	ggg	gtt	tat	tac	tgc	atg	caa	get	cta	caa	act	cag	ctc	act

2D-29 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
2D-29 Kabat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Region	FR1																		
Germ. AA	D	I	V	M	T	Q	T	P	L	S	L	S	V	T	P	G	Q	P	A
Germ. Nuc	gat	att	gtg	atg	acc	cag	act	cca	ctc	tct	ctg	tcc	gtc	acc	cct	gga	cag	cgg	gcc
AJL15 AA	D	I	V	M	T	Q	T	P	L	S	L	S	V	T	P	G	Q	P	A
AJL15 Nuc	gat	att	gtg	atg	acc	cag	act	cca	ctc	tct	ctg	tcc	gtc	acc	cct	gga	cag	cgg	gcc

2D-29 Codon	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	
2D-29 Kabat	20	21	22	23	24	25	26	27	27a	27b	27c	27d	27e		28	29	30	31	32	
Region	FR1										CDR1									
Germ. AA	S	I	S	C	K	S	S	Q	S	L	L	H	S		D	G	K	T	Y	
Germ. Nuc	tcg	atc	tcc	tgc	aag	tct	agt	cag	agc	ctc	ctg	cat	agt		gat	gga	aag	acc	tat	
AJL15 AA	S	I	S	C	K	S	S	Q	S	L	L	D	S		D	G	K	T	Y	
AJL15 Nuc	tcg	atc	tcc	tgc	aag	tct	agt	cag	agc	ctc	ctg	cat	agt		gat	gga	aag	acc	tat	

2D-29 Codon	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
2D-29 Kabat	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
Region	FR2																	CDR2	
Germ. AA	L	Y	W	Y	L	Q	K	P	G	Q	S	P	Q	L	L	I	Y	E	V
Germ. Nuc	ttg	tat	tgg	tac	ctg	cag	aag	cca	ggc	cag	tct	cca	cag	ctc	ctg	atc	tat	gaa	gtt
AJL15 AA	L	Y	W	Y	L	Q	K	P	G	Q	S	P	Q	S	L	I	Y	E	V
AJL15 Nuc	ttg	atc	tgg	tac	ctg	cag	aag	cca	ggc	cag	tct	cca	cag	tcc	ctg	atc	tat	gaa	gtt

2D-29 Codon	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	
2D-29 Kabat								52	53	54	55	56	57	58	59		60	61	62	
Region	CDR2								FR3											
Germ. AA								S	N	R	F	S	G	V	P		D	R	F	
Germ. Nuc								tcc	aac	cgg	ttc	tct	gga	gtg	cca		gat	agg	ttc	
AJL15 AA								S	K	R	F	S	G	V	P		D	R	F	
AJL15 Nuc								tcc	aac	cgg	ttc	tct	gga	gtg	cca		gat	agg	ttc	

2D-29 Codon	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
2D-29 Kabat	63	64	65	66			67	68	69	70	71	72	73	74	75	76	77	78	79
Region	FR3																		
Germ. AA	S	G	S	G			S	G	T	D	F	T	L	K	I	S	R	V	E
Germ. Nuc	agt	ggc	agc	ggg			tca	ggg	aca	gat	ttc	aca	ctg	aaa	atc	agc	cgg	gtg	gag
AJL15 AA	I	G	S	G			S	G	T	D	F	T	L	K	I	S	R	V	E
AJL15 Nuc	agt	ggc	agc	ggg			tca	ggg	aca	gat	ttc	aca	ctg	aaa	atc	agc	cgg	gtg	gag

2D-29 Codon	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111		
2D-29 Kabat	80	81	82	83	84	85	86	87	88	89	90	91	92					

Region	FR3									CDR3							
Germ. AA	A	E	D	V	G	V	Y	Y	C	M	Q	S	I	Q	L	P	
Germ. Nuc		ga															
AJL15 AA	A	E	D	V	G	L	Y	Y	C	M	Q	S	A	Q	L	P	Y T
AJL15 Nuc		ga															
	gct	g	gat	gtt	ggg	ctt	tat	tac	tgc	atg	caa	agt	aca	cag	ctt	ccg	tac act

3-20 Codon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
3-20 Kabat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Region	FR1																		
Germ. AA	E	I	V	L	T	Q	S	P	G	T	L	S	L	S	P	G	E	R	A
Germ. Nuc	gaa	att	gtg	tig	acg	cag	tct	cca	ggc	acc	ctg	tct	tig	tct	cca	ggg	gaa	aga	gcc
AJL01 AA	E	I	V	L	T	Q	S	P	G	T	L	S	L	S	P	G	E	R	A
AJL01 Nuc	gaa	att	gtg	tig	acg	cag	tct	cca	ggc	acc	ctg	tct	tig	tct	cca	ggg	gaa	aga	gcc
AJL19 AA	E	I	V	L	T	Q	S	P	G	T	L	S	L	S	P	G	E	R	A
AJL19 Nuc	gaa	att	gtg	tig	acg	cag	tct	cca	ggc	acc	ctg	tct	tig	tct	cca	ggg	gaa	aga	gcc
WR10 AA	E	I	V	L	T	Q	S	P	G	T	L	S	L	S	P	G	E	R	A
WR10 Nuc	gaa	att	gtg	tig	acg	cag	tct	cca	ggc	acc	ctg	tct	tig	tct	cca	ggg	gaa	aga	gcc

3-20 Codon	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38						
3-20 Kabat	20	21	22	23	24	25	26	27	27a	28	29														
Region	FR1							CDR1																	
Germ. AA	T	L	S	C	R	A	S	Q	S	V	S										S	S	Y		
Germ. Nuc	acc	ctc	tcc	tgc	agg	gcc	agt	cag	agt	gtt	agc										agc	agc	tac		
AJL01 AA	T	L	S	C	R	A	S	Q	S	L	L										G	S	F		
AJL01 Nuc	acc	ctc	tcc	tgc	agg	gcc	agt	cag	agt	ctt	acc										ggc	agc	ttc		
AJL19 AA	T	L	S	C	R	A	S	Q	S	V	S										S	G	S		
AJL19 Nuc	acc	ctc	tcc	tgc	agg	gcc	agt	cag	agt	gtt	agc										agc	gac	tcc		
WR10 AA	T	L	S	C	R	A	S	Q	S	L	L										G	S	F		
WR10 Nuc	acc	ctc	tcc	tgc	agg	gcc	agt	cag	agt	ctt	acc										ggc	agc	ttc		

3-20 Codon	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
3-20 Kabat	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
Region	FR2									CDR2									
Germ. AA	L	A	W	Y	Q	Q	K	P	G	Q	A	P	R	L	L	I	Y	G	A
Germ. Nuc	tta	gcc	tgg	tac	cag	cag	aaa	cct	ggc	cag	gct	ccc	agg	ctc	ctc	atc	tat	ggg	gca
AJL01 AA	L	A	W	Y	Q	Q	K	P	G	Q	A	P	R	L	L	I	Y	Y	Y
AJL01 Nuc	tta	gcc	tgg	tac	cag	cag	aaa	cct	ggc	cag	gct	ccc	agg	ctc	ctc	atc	tat	ggt	gca
AJL19 AA	L	A	W	Y	Q	Q	K	P	G	Q	A	P	R	L	L	I	Y	Y	Y
AJL19 Nuc	tta	gcc	tgg	tac	cag	cag	aaa	cct	ggc	cag	gct	ccc	agg	ctc	ctc	atc	tat	ggt	gca
WR10 AA	L	A	W	Y	Q	Q	K	P	G	Q	A	P	R	L	L	I	Y	Y	Y
WR10 Nuc	tta	gcc	tgg	tac	cag	cag	aaa	cct	ggc	cag	gct	ccc	agg	ctc	ctc	atc	tat	ggt	gca

3-20 Codon	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	
3-20 Kabat									53	54	55	56	57	58	59	73	60	61	62	
Region	CDR2								FR3											
Germ. AA									S	S	R	A	T	G	I	P		D	R	F
Germ. Nuc									tcc	agg	agg	ggc	act	ggc	atc	cca		gac	agg	ttc
AJL01 AA									S	N	R	A	S	G	I	P		D	R	F
AJL01 Nuc									tcc	agg	agg	ggc	act	ggc	atc	cca		gac	agg	ttc
AJL19 AA									S	T	R	A	A	G	I	P		D	R	F
AJL19 Nuc									tcc	agg	agg	ggc	act	ggc	atc	cca		gac	agg	ttc
WR10 AA									S	N	R	A	S	G	I	P		D	R	F
WR10 Nuc									tcc	agg	agg	ggc	act	ggc	atc	cca		gac	agg	ttc

3-20 Codon	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
3-20 Kabat	63	64	65	66					67	68	69	70	71	72	73	74	75	76	77
Region	FR3																		
Germ. AA	S	G	S	G					S	G	T	D	F	T	L	T	I	S	R
Germ. Nuc	agt	ggc	agt	ggg					tct	g	aca	gac	ttc	act	ctc	acc	atc	agg	ctg
AJL01 AA	S	G	G	G					F	G	T	D	F	T	L	T	I	S	R
AJL01 Nuc	agt	ggc	ggt	ggg					tct	g	aca	gac	ttc	act	ctc	acc	atc	agg	ctg
AJL19 AA	S	G	T	G					S	G	T	D	F	T	L	T	I	A	R
AJL19 Nuc	agt	ggc	act	ggg					tct	g	aca	gac	ttc	act	ctc	acc	atc	agg	ctg

WR10 AA	S	G	G	G			F	G	T	D	F	T	L	T	I	S	R	L	E
WR10 Nuc	agt	ggc	ggg	ggg			ttt	ggg	aca	gac	ttc	act	ctc	acc	atc	agc	aga	ctg	gag

3-20 Codon	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111			
3-20 Kabat	80	81	82	83	84	85	86	87	88	89	90	91	92						
Region					FR3									CDR3					
Germ. AA	P	E	D	F	A	V	Y	Y	C	Q	Q	Y	G	S	S	P			
Germ. Nuc	cct	gaa	gat	ttt	gca	gtg	tat	tac	tgt	cag	cag	tat	ggg	agc	tca	cct			
AJL01 AA	P	E	D	F	A	V	Y	Y	C	Q	Q	Y	G	S	S	P	I	T	
AJL01 Nuc	cct	gaa	gat	ttt	gca	gtg	tat	tac	tgt	cag	cag	tat	ggg	agc	tca	cct	atc	acc	
AJL19 AA	P	E	D	F	A	V	Y	Y	C	Q	H	Y	G	R	S	S	L	F	T
AJL19 Nuc	cct	gaa	gat	ttt	gca	gtg	tat	tac	tgt	cag	cac	tat	ggg	aga	tca	cct	cta	ttc	acc
WR10 AA	P	E	D	F	A	V	Y	Y	C	Q	Q	Y	G	S	S	P	I	T	
WR10 Nuc	cct	gaa	gat	ttt	gca	gtg	tat	tac	tgt	cag	cag	tat	ggg	agc	tca	cct	atc	acc	

Table 4 - AGS Mutations and Binding Patterns

	AGS Codon	36 (31b)						45 (40)						64 (56)					
		4-30	4-31	4-34	4-39	4-44	4-59	4-30	4-31	4-34	4-39	4-44	4-59	4-30	4-31	4-34	4-39	4-44	4-59
	AA	D	G	-	S	-	-	P	H	P	P	P	P	S	S	S	S	S	S
	Nuc.	gat	ggt	-	agt	-	-	ccc	cac	ccc	ccc	ccc	ccc	agc	agc	agc	agc	agc	agc
Dual Binding	AJL02	-	G	-	-	-	-	-	S	-	-	-	-	-	S	-	-	-	-
		-	ggt	-	-	-	-	-	ccc	-	-	-	-	-	agc	-	-	-	-
	AJL07	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	N
		-	-	-	-	-	-	-	-	-	-	-	ccc	-	-	-	-	-	aat
	AJL19	-	-	-	-	-	-	-	-	P	-	-	-	-	-	S	-	-	-
Astrocyte	WR10	-	-	-	-	-	-	-	-	ccc	-	-	-	-	-	gat	-	-	-
		-	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	G	-
		-	-	-	-	-	-	-	-	-	-	ccc	-	-	-	-	-	ggc	-
	AJL01	-	-	-	-	-	-	-	-	P	-	-	-	-	-	S	-	-	-
		-	-	-	-	-	-	-	-	ccc	-	-	-	-	-	aga	-	-	-
Neuron	WR12	D	-	-	-	-	-	P	-	-	-	-	-	T	-	-	-	-	-
		gat	-	-	-	-	-	ccc	-	-	-	-	-	acc	-	-	-	-	-
	WR13	D	-	-	-	-	-	P	-	-	-	-	-	T	-	-	-	-	-
		gat	-	-	-	-	-	ccc	-	-	-	-	-	acc	-	-	-	-	-
	AJL03	-	-	-	S	-	-	-	-	-	P	-	-	-	-	-	S	-	-
Dual Binding		-	-	-	agt	-	-	-	-	-	ccc	-	-	-	-	-	agc	-	-
	AJL10	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	T	-
		-	-	-	-	-	-	-	-	-	-	ccc	-	-	-	-	-	act	-
	AJL15	-	-	-	N	-	-	-	-	-	S	-	-	-	-	-	S	-	-
		-	-	-	aat	-	-	-	-	-	ccc	-	-	-	-	-	agc	-	-
	AGS Codon	65 (57)						90 (81)						101 (89)					
		4-30	4-31	4-34	4-39	4-44	4-59	4-30	4-31	4-34	4-39	4-44	4-59	4-30	4-31	4-34	4-39	4-44	4-59
	AA	T	T	T	T	T	T	K	K	K	K	K	K	V	V	V	V	V	V
	Nuc.	acc	acc	acc	acc	acc	acc	aag	aag	aag	aag	aag	aag	gtg	gtg	gtg	gtg	gtg	gtg
Dual Binding	AJL02	-	T	-	-	-	-	-	S	-	-	-	-	-	V	-	-	-	-
		-	acc	-	-	-	-	-	agg	-	-	-	-	-	gtc	-	-	-	-
	AJL07	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	V
		-	-	-	-	-	aat	-	-	-	-	-	aac	-	-	-	-	-	gtg
	AJL19	-	-	S	-	-	-	-	-	K	-	-	-	-	-	T	-	-	-
Astrocyte	WR10	-	-	gcc	-	-	-	-	-	aag	-	-	-	-	-	taa	-	-	-
		-	-	-	-	T	-	-	-	-	-	K	-	-	-	-	-	V	-
		-	-	-	-	acc	-	-	-	-	-	aag	-	-	-	-	-	gtg	-
	AJL01	-	-	A	-	-	-	-	-	N	-	-	-	-	-	V	-	-	-
		-	-	gcc	-	-	-	-	-	aac	-	-	-	-	-	gtc	-	-	-
Neuron	WR12	T	-	-	-	-	-	S	-	-	-	-	-	V	-	-	-	-	-
		acc	-	-	-	-	-	agg	-	-	-	-	-	gtc	-	-	-	-	-
	WR13	T	-	-	-	-	-	S	-	-	-	-	-	V	-	-	-	-	-
		acc	-	-	-	-	-	agg	-	-	-	-	-	gtc	-	-	-	-	-
	AJL03	-	-	-	T	-	-	-	-	-	N	-	-	-	-	-	V	-	-
Dual Binding		-	-	-	act	-	-	-	-	-	aac	-	-	-	-	-	gtg	-	-
	AJL10	-	-	-	-	T	-	-	-	-	-	S	-	-	-	-	-	T	-
		-	-	-	-	act	-	-	-	-	-	agg	-	-	-	-	-	ata	-
	AJL15	-	-	-	D	-	-	-	-	-	S	-	-	-	-	-	V	-	-
		-	-	-	gac	-	-	-	-	-	agg	-	-	-	-	-	gtg	-	-

It has been well-established in the literature that somatic hypermutations in the variable antibody gene regions enable antibodies to bind their cognate antigen with greater affinity. In fact, some studies have demonstrated that loss of particular mutations at certain codons in a single antibody gene can abrogate binding of the particular antibody to its cognate antigen. In the case of these AGS-enriched antibodies, the inventor describes, for the first time, a commonality of somatic hypermutation to particular replacement amino acids

among a panel of antibodies expressed by individual B cells with distinct CDR3 regions that bind neurons, astrocytes, both neurons and astrocytes or neither cell type. Thus, by following the Decision Tree based on the amino acid resulting from somatic hypermutation at particular codons, one can predict whether the antibody will bind neurons, astrocytes, both neurons and astrocytes or neither cell type (FIG. 19).

Example 2 - VH4+ Plasmablasts Demonstrate Autoreactive B Cell Expansion toward Brain Antigens in Early Multiple Sclerosis Patients

Materials and Methods

Patient Sample Processing. Treatment naïve clinically isolated syndrome (CIS) patients included in this study had partial transverse myelitis symptoms (PTM) and were at high risk for developing multiple sclerosis (details in Table 8). Neuromyelitis optica (NMO) patients had established disease and were either on Cellcept therapy or no therapy. Only the NMO patients not on immune modulatory therapy were included as comparators for immunoglobulin gene analysis and antibody cloning. Blood was collected from CIS-PTM and NMO patients according to the University of Texas Southwestern Medical Center (UTSWMC) institutional review board. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll separation and stained with fluorescent antibodies as previously described. Either memory B cells (CD19+CD27+) or plasmablasts (CD19+CD27hi) as defined by others were sorted individually into 96-well plates using the BD FACSAria flow cytometer (Becton Dickinson, San Jose, CA).

Single Cell Polymerase Chain Reaction and Immunoglobulin Gene Analysis. Individually sorted B cell subpopulations were flash frozen and lysed. Upon thawing, immunoglobulin mRNA was reverse transcribed and amplified with multiple rounds of PCR as previously described. Sanger sequencing was used at the UTSWMC sequencing core to generate the antibody variable domain reads. Sequences were analyzed using the IgBlast component of the VDJServer online repertoire analysis tool (<https://vdjserver.org/>). This output was filtered to remove any sequence which met at least one of the following criteria: out-of-frame junction, missing CYS or TRP/PHE to anchor CDR3, stop codon, out of frame sequence, missing CDR3 or truncated read length (sequence alignment starts after CDR1). Peter Lipsky at UTSWMC provided the data from healthy donor CD19+ cells and the antibody repertoire data from influenza responding plasmablasts was previously described.

Antibody Cloning and Production. Plasmablasts from CIS-PTM and NMO patients expressing highly mutated VH4 or VH3 heavy chains were selected for production. The

variable domains were synthesized (Integrated DNA Technologies, Corallville, IA) and bi-directionally cloned into an IgG1 backbone provided by Michel Nussenzweig at the Rockefeller University as previously described. The 6 IgG1 antibodies cloned from one healthy donor used as controls were previously also described. Betty Diamond at the Hofstra
5 Northwell School of Medicine provided the DNA for two control IgG1 antibodies, B1 and G11, which serve as isotype negative and positive controls, respectively. All recombinant human antibodies (rhAbs) were transiently transfected into HEK293T cells (ATCC, Manassas, VA) with the lipid transfection reagent JetPEI (PolyPlus Transfection, Illkirch, France). Supernatants from these cultures were collected on days 3, 5, 7 and 10, and antibody
10 was purified from these on a column of sepharose Protein G beads (Life Technologies, Grand Island, NY), dialyzed into dPBS (HyClone, Logan, UT), and concentrated to between 0.2 and 1.2 mg/ml. The exact concentration of the antibodies was measured by ELISA (Bethyl Laboratories, West Grove, PA).

Tissue and Antigen ELISAs. Mouse brain and kidney lysates were made as described elsewhere and the following protocol was adapted from this reference. Plates were
15 coated with 10 µg/ml of human brain or kidney lysate in bicarbonate buffer overnight at 4°C. The next day plates were blocked with 10% BSA in PBST for 2 hours at room temperature and primary antibodies were added at 20 µg/ml or patient serum was diluted to 1:100, 1:250, 1:500 and 1:1000 in 5% BSA and incubated overnight at 4°C. The following day 1 µg/ml of
20 biotinylated anti-human (eBioscience, San Diego, CA) in 3% BSA was incubated for 2 hours at room temperature, followed by 1 hour with a 1:2000 dilution of streptavidin-HRP (BD Pharmingen, San Jose, CA) in 3% BSA. Plates were developed for 30 seconds with TMB substrate and neutralized with 1 M HCl before reading at 450 nm with an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT). Thresholds for determining binding in this
25 human brain and kidney lysate ELISAs were set by averaging the absorbance values of control antibodies and adding two standard deviations.

Histology. Healthy and diseased (EAE or stroke) mouse brains were preserved and cryosectioned as described previously but stored at -80°C until use. Slides were briefly warmed, washed in PBS and antigens were uncovered by boiling for 2 minutes in unmasking
30 solution (Vector Labs, San Mateo, CA). Slides were blocked for 2 hours at room temperature with 1% goat serum in PBS with 0.1% Triton X. Primary rhAbs were added at 20 µg/ml in blocking buffer overnight. The next day goat anti-human AlexaFluor 488 (Life Technologies, Grand Island, NY) was incubated with the slides for 1 hour, followed by re-blocking for 2 hours. Primary antibodies for NeuN, GFAP (Abcam, Cambridge, MA), or PDGFR (Santa

Cruz Biotechnology, Dallas, TX) were incubated for 1 hour followed by 1 hour with anti-rabbit AlexaFluor 594 (Life Technologists, Grand Island, NY). Slides were stained with DAPI (Sigma Aldrich, St. Louis, MO) for 4 minutes at room temperature, sealed with VectaShield (Vector Labs, San Mateo, CA), and imaged on a Zeiss LSM780 upright confocal microscope (Zeiss, Oberkochen, Germany). Blinded experts in histology and pathology (co-authors RC, DR and AS) assessed staining of the rhAbs.

Immunocytochemistry. Hep2 immunocytochemistry (ICC) was performed with a Hep-2 Substrate Slide antinuclear antibody kit according to the manufacturers instructions (MBL International, Woburn, MA). For SH-Sy5y staining, glass coverslips were coated with laminin at 50 µg/ml in dPBS for 2 hours at 37°C, washed once with PBS and SH-Sy5y were plated overnight. The following day, cells were fixed with 4% PFA and permeabilized with 0.2% Triton X in 2 mg/ml BSA. Cells were blocked with 1% goat serum, 3% BSA and 0.1% Triton X for 2 hours at room temperature before adding primary rhAbs or commercial anti-Hsp70 (Abcam, Cambridge, MA) at 20 µg/ml overnight at 4°C. The next day, secondary anti-human AlexaFluor 488 or anti-rabbit AlexaFluor 594 (Life Technologies, Grand Island, NY) was added and the slides incubated for 1 hour at room temperature. Cells were then stained with DAPI (Sigma Aldrich, St. Louis, MO) for 4 minutes at room temperature. Coverslips were sealed onto a glass slide using VectaShield (Vector Labs, San Mateo, CA), and imaged on a Zeiss Axioscan slide scanner (Zeiss, Oberkochen, Germany).

IHC and ICC signal affinity verification. Antibody affinity for these cell types in tissue sections was validated using a lambda scan on the Ziess LSM780 Upright Confocal/Multiphoton microscope (Zeiss, Oberkochen, Germany). The image area is scanned at various wavelengths recording signal intensity so that points of interest can be selected and compared to background fluorescence. Only antibodies with a signal to noise ratio above 1.5 near the emission wavelength for AlexaFluor488 (519 nm) were considered positive (FIGS. 34A-34D). Many of the antibodies well exceeded this ratio, demonstrating high affinity binding.

Flow Cytometry. Cells were lifted with Accutase for 5 minutes at 37°C (Innovative Cell Technologies, San Diego, CA). Fc receptors were blocked with rat anti-mouse CD16 Fc block for 10 minutes at room temperature (BD Biosciences, Franklin Lakes, NJ). For intracellular staining, cells were fixed and permeabilized with BD fixation/perm solution for 30 minutes at room temperature (BD Biosciences, Franklin Lakes, NJ). 1 µg of rhAb was added to the intracellular stains and 5 µg of rhAb was added to the extracellular stains for 1 hour at room temperature. Secondary anti-human-APC (BD Pharmingen, San Jose, CA) or

anti-rabbit-FITC (BD Pharmigen, San Jose, CA) was incubated with the cells for 30 minutes at room temperature. Data was collected on a FACS Aria (Becton Dickinson, San Jose, CA) or LSR (Becton Dickinson, San Jose, CA) flow cytometer.

Results

5 **Expansion of peripheral PBs is common to patients experiencing transverse myelitis symptoms.** Previously, the inventor's laboratory demonstrated that peripheral plasmablasts are expanded in clinically isolated syndrome (CIS) patients experiencing their first partial transverse myelitis attack (CIS-PTM). Plasmablasts are also increased in Neuromyelitis Optica (NMO), a neurological disease where patients also experience
10 transverse myelitis symptoms, but without brain inflammation. To ascertain the extent of this increase, the inventor determined the frequency of CD19+CD27^{high} plasmablasts in PBMCs from healthy donors, CIS-PTM patients and NMO patients by flow cytometry (FIG. 20A). As expected, peripheral plasmablasts were present in CIS-PTM and NMO patients to a similar extent (2.10% vs 2.12%, $p=0.98$), and both were elevated over steady state production in
15 healthy donors (2.10% vs 0.71%, $p=0.005$) (FIG. 20B).

Expanded peripheral PBs in CIS-PTM patients over-utilize VH4 antibody genes. B cells create their unique antigen receptor by processes of immunoglobulin gene segment recombination, light and heavy chain pairing, and somatic hypermutation. Previous data from the inventors' laboratory demonstrate that B cells in the CSF of CIS-PTM patients at high
20 risk to convert to MS tend to over-utilize immunoglobulin heavy chain V-region subgroup 4 (VH4) gene segments. VH4 family genes are also associated with autoreactivity in MS patients and other autoimmune diseases such as systemic lupus erythematosus. To determine whether peripheral plasmablasts tend to utilize VH4 genes, the inventor isolated and sequenced the variable domain of immunoglobulins of individual peripheral plasmablasts
25 from CIS-PTM and NMO patients. Antibody repertoire data from these peripheral plasmablasts were compared to published control databases of healthy total peripheral B cells and flu-responding peripheral plasmablasts.

 Approximately 19% of peripheral plasmablasts from healthy donors responding to influenza infection utilize VH4 genes (FIG. 20C), which is a similar proportion to that of
30 healthy donor total peripheral B cells. In contrast, 32% of peripheral plasmablasts from CIS-PTM patients utilize VH4 genes, which was statistically higher than flu-responding peripheral plasmablasts ($p=0.04$) (FIG. 20C). Plasmablasts from NMO patients demonstrated a similar expansion of VH4 usage compared to CIS-PTM patients (33% v 32%, $p=0.88$).

When individual genes within the VH4 family are assessed, the frequency of VH4-34 was statistically elevated in plasmablasts from CIS-PTM patients compared to plasmablasts responding to influenza ($p=0.04$) (FIG. 20D). VH4-31 was statistically elevated in plasmablasts from CIS-PTM patients compared to healthy donor total peripheral B cells (3.25% vs 0.0%, $p=0.02$), but not plasmablasts responding to influenza ($p=0.05$). Normally these genes are selected against due to their natural propensity for autoreactivity. No other genes within the VH4 family were statistically elevated in CIS-PTM patients compared to controls as well as other V gene families. A more comprehensive antibody genetic analysis is provided in FIGS. 21A-21F and 22A-22E.

CIS-PTM peripheral plasmablasts bind strongly to brain antigens. The researchers next sought to examine whether antibodies expressed by the peripheral plasmablasts of CIS-PTM patients are autoreactive. To do this, the inventor generated recombinant human antibodies (rhAbs) from 38 peripheral plasmablasts isolated from 7 CIS-PTM patients. The researchers also generated 10 rhAbs from 4 NMO patients and 6 rhAbs from 1 healthy donor as controls. Of those CIS-PTM rhAbs from which the inventor could determine isotype, 85% were IgG+ and all had significant mutation accumulation that marked them as having undergone affinity maturation (Table 8).

The researchers first tested these recombinant human antibodies (rhAbs) for binding to brain antigens using a brain lysate ELISA. The six rhAbs from healthy donor peripheral B cells were used as negative controls and were not reactive in this assay (FIGS. 23A-23B). In contrast, 32% of the rhAbs (12 out of 38) from the CIS-PTM cohort recognized brain lysate by ELISA, and 22% of the rhAbs (2 out of 9) from the NMO cohort recognized brain lysate by ELISA. Of the 12 CIS-PTM rhAbs that demonstrated positive reactivity to brain lysate by ELISA, 7 of them also recognized kidney lysate, indicating autoreactivity to more ubiquitous antigens in some cases (FIGS. 24A-24C). Both of the NMO rhAbs that bound to brain lysate were reactive to kidney lysate (Table 8).

Next, the inventor binned the 38 rhAbs from the CIS-PTM patients by their use of VH4 or VH3 genes, and observed that the rhAbs reacting strongly to brain lysate utilized VH4 genes (FIG. 24A, 24B). None of the rhAbs utilizing VH3 genes reacted to brain lysate despite high mutation frequencies (FIG. 24A, 24B). Positive reactivity to brain lysate by ELISA was noted for rhAbs using 6 of the 9 VH4 genes, while rhAbs using one of the other 3 VH4 genes (VH4-4, 4-31, and 4-34) were not reactive in this assay (FIG. 24C). However it should be noted that the relative representation of antigens in the lysate might encourage false negatives.

CIS-PTM peripheral plasmablasts bind primarily to neurons and astrocytes.

Next, the 12 CIS-PTM rhAbs that demonstrated strong reactivity in the brain lysate ELISA were tested for binding to brain tissue by histology (FIGS. 26A-26E). NMO rhAbs (n=3) were included as comparators and two control antibodies from lupus patients were utilized in this part of the study (B1 as a negative control and G11 as a positive control). Healthy mouse brain was used to investigate binding to normal tissue, stroke mouse brain was used to test reactivity to generally inflamed tissue, and EAE brain was used to study reactivity to inflamed tissue in the context of an MS model (FIGS. 26A-26E). One negative control rhAb (B1) and two rhAbs (CIS19 and CIS07) from CIS-PTM patients are shown here as examples (FIGS. 26A-26E). All 12 CIS-PTM rhAbs that displayed strong binding by brain lysate ELISA also bound cellular targets in mouse brain tissue (FIGS. 26A-26E and FIGS. 27-33). Two structures were most commonly recognized: glial cells in the corpus callosum and neuronal bodies in the cortex. The CIS19 rhAb is presented in FIGS. 26A-26E as an example of binding to neuronal bodies, and CIS07 rhAb is presented in FIGS. 26A-26E as an example of binding to glial cells. Overall, the majority of highly mutated VH4 expressing CIS-PTM plasmablast rhAbs recognized both neurons and glial cells in multiple brain tissue types (Table 8, FIGS. 27-33), with a smaller portion recognizing only glial cells (FIG. 26E).

Of those rhAbs that recognized neuronal bodies, the majority recognized the cytoplasm in a ring-like structure around the nucleus as illustrated by CIS19 (FIGS. 26A-26D, top and left images). Of those rhAbs that recognized glial cells, the majority bound bodies and cell processes in the corpus callosum as illustrated by CIS07 rhAb (FIGS. 26A-26D, middle). In general there was a preference for astrocytes over oligodendrocytes, as the inventor rarely observed colocalization with the oligodendrocyte marker PDGFR (FIGS. 31A-31B and 33A-33B). One exception was CIS68 rhAb, which bound to both glial cell types (FIG. 33A-33B).

The researchers also included 6 additional CIS-PTM rhAbs that were negative by the brain lysate ELISA (FIGS. 23A-23B), but utilized VH4-31 or VH4-34 genes, which were over-represented in the heavy chain repertoire analysis (FIG. 20D and FIG. 21A). Notably, even though rhAbs expressing VH4-34 and VH4-31 antibody genes did not bind strongly by brain lysate ELISA, these rhAbs bound less abundant cell types in the brain such as glial cells in the corpus callosum (FIG. 37A).

Peripheral plasmablasts from CIS-PTM patients recognize cytoplasmic and nuclear targets on human neurons. The researchers used immunocytochemistry (ICC) to further investigate the binding of CIS-PTM peripheral plasmablast rhAbs to nuclear and

cytoplasmic targets on the human neuroblastoma cell line, SH-Sy5y (FIGS. 35A-35B). B1 is presented as a negative control, G11 is presented as a positive control, and 4 rhAbs are presented as examples in FIGS. 35A-35B. Each type of binding pattern is represented (negative, nuclear only, cytoplasmic only, nuclear and cytoplasmic) and G11 demonstrates both cytosolic and nuclear recognition of the cell line. Of the 38 CIS-PTM rhAbs, 21 of them (55%) bound SH-Sy5y, including 9 of the 12 CIS-PTM rhAbs that demonstrated strong binding by the brain lysate ELISA. The majority of these 21 CIS-PTM rhAbs bound to cytoplasmic targets, with some binding to both cytoplasmic and nuclear targets, and only one binding to nuclear targets alone. Of the 10 NMO rhAbs, 3 of them bound SH-Sy5y; 2 to nuclear targets alone and 1 to both cytoplasmic and nuclear targets. Only one of the HD rhAbs bound to cytoplasmic targets on SH-Sy5y. The rhAbs were additionally probed for binding to the Hep-2 cell line, a test commonly used to identify generic anti-nuclear antibodies in lupus patients. One NMO plasmablast rhAb and 20% of the CIS-PTM plasmablast rhAbs also recognized the Hep-2 cells, but most were not anti-nuclear in nature (FIG. 36).

CIS-PTM peripheral plasmablasts bind both intracellular and extracellular antigens. To further verify the cellular specificity observed by histological methods, the inventor used flow cytometry to determine whether the 38 CIS-PTM plasmablast rhAbs were binding intracellular and/or extracellular antigens. The human neuroblastoma line SH-Sy5y (FIG. 38A-38B and FIG. 39), mouse neuroblastoma line Neuro-2a (FIGS. 40A-40B), and mouse astrocytoma line C8-D1A (FIGS. 41A-41B) were all tested by this method. In FIGS. 38A-38B, B1 is presented as a negative control, G11 is presented as a positive control for intracellular targets, and 6 rhAbs are presented as examples of each type of binding pattern (negative, intracellular, or extracellular). With only 2 exceptions (CIS19 and CIS68), the rhAbs that recognized either neurons or glial cells by histology recognized their corresponding cell type by this method (Table 8). Not surprisingly some discrepancies exist between the recognition of mouse brain and human neuroblastoma cell lines by individual rhAbs (Table 8). The rhAbs demonstrated multiple patterns of recognition ranging from only intracellular (*i.e.*, CIS35, 99.4%) to primarily extracellular antigens (*i.e.*, CIS44, 98.7%) (FIGS. 38A-38B, FIG. 39, FIGS. 40A-40B and FIGS. 41A-41B). Of the 27 CIS-PTM rhAbs that recognized SH-Sy5y cells, 14 recognized both intracellular and extracellular antigens, while 13 recognized only intracellular antigens (FIG. 38B).

CIS-PTM plasmablast antibodies are well represented in the plasma pool. Even when expanded, plasmablasts are only a small portion of the peripheral B cell pool, and thus

may have a small impact on the ongoing auto-reactivity associated with MS. To assess whether this small expansion could lead to an observable increase in autoreactive antibodies in the plasma, the inventor tested plasma samples from 7 NMO patients, 8 healthy donors with plasmablasts responding to recent influenza vaccination, and 16 treatment naïve CIS-PTM patients for reactivity to brain lysate by ELISA (FIGS. 42A-42B), including all 7 of the CIS-PTM patients from which the inventor cloned rhAbs. Plasma samples from all 8 healthy donors and 6 of the 7 NMO patients were not reactive (FIG. 42A). Notably, the one NMO patient with high binding to brain lysate was treatment naïve while the remaining NMO patients were on Cellcept therapy (Table 8). Of the 16 CIS-PTM patients, 9 show plasma reactivity at least 2 standard deviations above the mean of healthy donors (FIG. 42B). Plasma from the 6 patients whose rhAbs were reactive to brain lysate also had higher antibody titers in the plasma towards brain lysate in comparison to plasma from healthy donors. The 1 patient who showed little rhAb reactivity also did not display plasma reactivity (FIG. 42A, Table 7).

TABLE 7 – Patient Information

	Patient	Gender	Age	Diagnosis at Draw	Current Diagnosis	Treatment at Draw	Current Treatment	Percent of Plasmablasts in Blood	Percent of Plasmablasts in CSF	Plasma ELISA Result
CIS Patients Included in Genetic Analysis	CIS924*	M	69	TM	TM	None	None	7.1	33.3	-
	CIS799*	F	28	CIS	RRMS	None	Avonex	5.74	13.3	+
	CIS991*	F	34	CIS	TM	None	steroids	3.3	15.4	+
	CIS111**	F	62	CIS	PPMS	None	Avonex	3.04	5.77	+
	CIS663*	F	32	TM	TM	None	None	2.85	10.8	-
	CIS431***	F	27	CIS	RRMS	None	Gilenya	2.07	35.9	+
	ATM4	M	24	CIS	RRMS	Prednisone	None	1.54	16.1	+
	CIS353***	F	58	CIS	RRMS	None	Copaxone	1.46	11.4	+
	CIS683***	F	39	CIS	RRMS	None	Tecfidera	0.82	26.6	+
CIS Patients Not Included in Genetic Analysis	CIS287**	F	45	TM	TM	None	Betaseron	6.58	37.9	-
	CIS873**	F	19	RRMS	RRMS	None	Avonex	2.98	26	-
	CIS527**	F	43	CIS	RRMS	None	Copaxone	1.81	14.1	+
	CIS699*	F	37	CIS	RRMS	None	Avonex	1.61	7.87	-
	CIS787**	M	33	CIS	RRMS	None	Copaxone	1.45	36.9	-
	CIS251**	F	53	TM	Sarcoidosis	None	None	1.43	2.57	-
	CIS942*	F	52	CIS	CIS	None	Copaxone	1.19	12.2	-
	CIS328**	M	32	CIS	RRMS	None	Avonex	0.64	2.41	-
	CIS371**	F	56	CIS	CIS	None	Copaxone	0.51	13.9	-
NMO Patients Included in Genetic Analysis	NMO.1	F	55	NMO	NMO	None	Cellcept	3.47	n/a	+
	NMO.2	F	36	NMO	NMO	None	None	0.22	n/a	-
	NMO.7	F	54	NMO	NMO	None	Cellcept	4.09	n/a	-
	NMO.8	F	64	NMO	NMO	None	Rituxan	2.43	n/a	-
NMO Patients Not Included in Genetic Analysis	NMO.3	F	39	NMO	NMO	Cellcept	Cellcept	1.05	n/a	-
	NMO.4	F	61	NMO	NMO	Cellcept	Cellcept	0.09	n/a	-
	NMO.5	F	41	NMO	NMO	Cellcept	Rituxan	1.08	n/a	-
	NMO.6	M	47	NMO	NMO	Cellcept	Rituxan	1.01	n/a	-
	NMO.9	F	53	NMO	NMO	Cellcept	Cellcept	1.34	n/a	-
	NMO.10	F	61	NMO	NMO	Cellcept	Cellcept	0.17	n/a	-
	NMO.31	F	46	NMO	NMO	AZT	Unknown	0	n/a	-
	NMO.33	M	38	NMO	NMO	Cellcept	Unknown	5.97	n/a	-
	NMO.70	F	45	NMO	NMO	AZT	Unknown	4.52	n/a	-
	NMO.250	F	47	NMO	NMO	Cellcept	Unknown	3.02	n/a	-
	NMO.625	M	36	NMO	NMO	AZT	Unknown	2.15	n/a	-
	NMO.740	F	29	NMO	NMO	AZT	Unknown	3.13	n/a	-
	NMO.745	F	50	NMO	NMO	Cellcept	Unknown	1.66	n/a	-

*CSF and peripheral B cells previously studied by flow cytometry

**CSF B cell previously studied by genetic analysis *Peripheral B cells previously studied by genetic analysis

TABLE 8 – Antibody Information

rh48	Patient	HC {V}	HC {I}	LC {V}	LC {I}	HC Mut	Isotype	Brain ELISA	Kidney ELISA	SySy ICC	Hsp2 ICC	Stroke Brain	EAE Brain	Healthy Brain	SySy FC	NIA FC	CDLA FC	MOG	MBP
CIS56	ATM4	H4-61	3	L5-45	7	26	ND	+	+	N+C	C	N+G	N+G	N+G	I+E	I		+	+
CIS57	ATM4	H4-59	5	K3-20	5	17	ND	+	+	N	C	N+G	N+G	N+G	I+E	I		+	+
CIS59	ATM4	H4-31	4	K2-24	2	15	IgG	-	-	C	-	-	-	N+G	I+E	I+E	I+E	-	-
CIS60	ATM4	H4-61	3	K2-30	2	15	IgM	-	-	-	-	-	-	N+G	-	I		-	-
CIS45	CIS111	H4-59	4	K1-5	2	31	IgG	+	-	C	-	N+G	N+G	N+G	-			+	+
CIS46	CIS111	H4-34	5	K1-39	2	22	IgG	-	-	C	-	N+G	G	N+G	I+E			-	-
CIS47	CIS111	H4-34	5	K4-1	5	21	IgG	-	-	-	-	-	N+G	G	I	I		-	-
CIS48	CIS111	H4-39	5	K1-12	2	20	ND	+	+	C	-	N+G	G	G	I+E	I		-	-
CIS49	CIS111	H4-34	5	K1-39	5	17	ND	-	-	N+C	-	-	-	-	I+E	I+E		-	-
CIS52	CIS111	H4-59	2	L1-44	7	36	ND	-	-	-	-	-	-	-	I			-	-
CIS05	CIS353	H4-39	4	K3-20	5	17	ND	-	-	-	-	-	-	-	-	-	-	-	-
CIS06	CIS353	H3-13	3	K4-1	3	25	IgG	-	-	-	-	-	-	-	I	-		-	-
CIS19	CIS353	H4-6	3	K1-5	2	28	IgG	+	-	C	-	N+G	N+G	N+G	-			+	+
CIS20	CIS353	H4-59	3	K1-33	2	18	IgG	-	+	-	-	-	-	-	I	-	I+E	-	-
CIS21	CIS353	H4-39	5	K3-20	5	18	ND	-	-	C	-	-	-	-	I	-	I+E	-	-
CIS22	CIS353	H4-4	1	K1-5	2	12	IgG	-	-	C	-	-	-	-	I	-	I+E	-	-
CIS23	CIS353	H3-21	4	K3-20	5	23	IgG	-	-	C	N+C	-	-	-	I	I	I+E	+	+
CIS26	CIS353	H4-6	3	L1-47	3	28	IgG	+	-	C	-	-	N+G	N+G	-			-	-
CIS09	CIS431	H4-4	4	K3-11	5	10	ND	-	-	C	-	-	-	-	I			-	-
CIS10	CIS431	H3-30	4	K1-5	4	32	ND	-	+	N	-	-	-	-	I+E			-	-
CIS35	CIS431	H4-59	4	L3-14	1	12	IgM	-	-	-	-	-	-	N+G	I			-	-
CIS42	CIS431	H4-30	4	L2-23	7	15	ND	+	+	C	C	N+G	N+G	N+G	I+E	I+E		+	+
CIS44	CIS431	H4-59	5	L2-8	3	20	ND	+	-	C	-	N+G	-	N+G	I+E			-	-
CIS07	CIS663	H4-59	5	K3-20	5	26	IgG	+	-	-	-	G	G	G	I			-	-
CIS08	CIS663	H3-23	5	K3-11	5	22	ND	-	-	-	-	-	-	-	-			-	-
CIS28	CIS663	H4-59	5	K3-11	5	8	IgG	+	+	N+C	N	N+G	N+G	N+G	I+E			+	+
CIS29	CIS663	H3-23	5	K1-5	2	20	IgG	-	-	-	-	-	-	-	I	I	I	-	-
CIS34	CIS663	H4-34	4	L3-10	2	5	IgG	-	-	N+C	N	N+G	N+G	N+G	-	I		+	+
CIS67	CIS799	H4-30	4	K2-30	2	17	ND	-	-	-	-	-	-	-	-	-	-	-	-
CIS68	CIS799	H4-38	4	K1-5	5	16	ND	+	+	N	N	N+G	N+G	N+G	I+E	I	-	+	+
CIS69	CIS799	H4-30	5	K1-27	5	11	ND	-	-	-	-	-	-	-	I+E	I+E	I	-	-
CIS70	CIS799	H3-30	4	K3-20	5	31	IgG	-	-	C	-	-	-	-	-	I+E	-	-	-
CIS76	CIS799	H4-39	2	L6-57	3	23	ND	+	+	-	-	N+G	N+G	N+G	I+E	-	-	+	-
CIS71	CIS924	H4-4	5	K1-9	2	19	IgG	-	-	C	-	-	-	-	I+E	I	I+E	-	+
CIS72	CIS924	H4-34	5	L2-14	2	13	ND	-	-	-	-	G	N+G	G	-	I	I	-	-
CIS73	CIS924	H4-31	4	K1-12	2	12	IgG	-	-	-	-	N+G	G	G	I	-	I	-	-
CIS74	CIS924	H3-30	4	K1-39	5	26	IgM	-	-	-	-	-	-	-	-	-	-	-	-
CIS75	CIS924	H3-23	5	K1-17	5	20	ND	-	-	-	-	-	-	-	-	I	I	-	-
NMO01	NMO.01	H4-30	5	L2-8	2	27	IgG	-	-	-	-	-	-	-	I	-	-	-	-
NMO02	NMO.01	H4-39	4	K1-5	2	7	IgM	-	-	C	-	-	-	-	I	I+E	I	-	-
NMO03	NMO.01	H4-39	4	L2-23	2	6	IgM	+	+	-	C	N+G	N+G	N+G	I+E	E	E	+	+
NMO04	NMO.01	H4-39	4	L3-13	2	1	IgM	-	-	-	-	-	-	-	-	-	-	-	-
NMO05	NMO.01	H3-7	3	K2-30	2	23	IgM	-	-	-	-	-	-	-	I	I	E	-	-
NMO06	NMO.02	H4-59	5	L6-57	3	9	IgG	+	+	N	-	N+G	N+G	N+G	-			+	+
NMO07	NMO.07	H4-4	5	K3-20	4	11	IgG	-	-	-	-	-	-	-	-	I	-	-	-
NMO08	NMO.07	H3-30	5	K1-28	2	12	IgG	-	-	N	-	-	-	-	-	I	-	-	-
NMO09	NMO.08	H4-31	4	K3-20	2	33	IgM	-	-	-	-	G	G	G	-	I	-	-	-
NMO15	NMO.08	H4-39	3	K3-15	3	15	ND	-	-	-	-	-	-	-	-	-	-	-	-

Bold = VH4 antibodies

Thin = VH3 antibodies

White = VK antibodies

Gray = VL antibodies

ND =

Not

Detect-

able

+ = Positive

- = Negative

N = Nuclear

C =

Cytoplasmic

N+C = Both

N+G = Neurons + Glia

G = Glia

I = Intracellular

E = Extracellular

I+E = Both

Discussion

Peripheral B cell pools are key components of MS pathogenesis because blocking their entrance to the CNS has a profound effect on disease (Stuve *et al.*, 2006). In this study the focus was on one particular subset of B cell, identified as a plasmablast by the upregulation of CD27 expression and simultaneous expression of CD38 and CD95 (Fink *et al.*, 2012; Jacobi *et al.*, 2010; Frolich *et al.*, 2010; Odendahl *et al.*, 2000; Avery *et al.*, 2005). Previously, the inventor demonstrated that the frequency of CD27-high plasmablasts in the CSF is elevated in patients experiencing their first attack of transverse myelitis (Ligocki *et al.*, 2013). Interestingly, the four patients in that study who later experienced additional MRI activity had elevated frequencies of CD27-high plasmablasts at the time of their initial event, which recapitulated findings by others that the frequency of plasmablasts correlates with parenchymal inflammatory disease activity as disclosed by MRI (Cepok *et al.*, 2005). In this example, the inventor extends those findings by demonstrating an expansion of plasmablasts in the blood of CIS-PTM patients compared to healthy donors. Interestingly, the frequency of peripheral plasmablasts in the current CIS-PTM cohort was similar to that of NMO patients, a CNS autoimmune disease in which patients also experience transverse myelitis symptoms, but without brain inflammation (Parratt *et al.*, 2010). Thus, it is possible that plasmablast expansion in the periphery is a feature shared among patients with CNS diseases who experience transverse myelitis symptoms independent of brain inflammation status. The events that drive CIS-PTM patients to MS rather than NMO remains unknown.

The antibody genetics of a B cell population can have profound impact on the understanding of disease, as the development and function of a B cell is dependent on the antibody it expresses (19, Brezinshcek *et al.*, 1997; Casellas *et al.*, 2001; Meffre *et al.*, 2000; Odendahl *et al.*, 2000; Wardemann *et al.*, 2003). In fact, antibody genetics studies have led to several key discoveries in MS that show expansion of particular genes, excessive receptor editing, dysregulation in B cell selection (Ligocki *et al.*, 2013; Krumbholz *et al.*, 2012; Monson *et al.*, 2005; Harp *et al.*, 2007; Cameron *et al.*, 2009; Owens *et al.*, 1998; Palanichamy *et al.*, 2014; von Budingen *et al.*, 2012; Owens *et al.*, 2003; Ritchie *et al.*, 2004; Qin *et al.*, 1998; Colombo *et al.*, 2000; Colombo *et al.*, 2003; Winges *et al.*, 2007; von Budingen *et al.*, 2008; von Budingen *et al.*, 2010; Bankoti *et al.*, 2014; Rounds *et al.*, 2014; Qin *et al.*, 2003; Haubold *et al.*, 2004), and even a mutational biomarker that identifies patients who will convert to MS with 86-92% accuracy (Rounds *et al.*, 2015). In this example, the inventor demonstrates that the variable heavy chain family 4 (VH4) genes are

used more extensively by peripheral plasmablasts from CIS-PTM and NMO patients in comparison to previously published generalized healthy donor B cell pool and healthy donor plasmablasts following flu vaccination. However, 7 of the 9 VH4 family genes were utilized at frequencies similar to these two control populations. This indicates that the VH4 family
5 itself is over-utilized in both CIS-PTM and NMO patient populations, rather than particular VH4 genes driving the over-utilization of the VH4 family. This data also suggests that VH4 expansion may be a generalized feature of patients with CNS diseases who experience transverse myelitis symptoms. Others have demonstrated VH4 family expansion in the CSF of MS patients (Bennett *et al.*, 2008; Owens *et al.*, 2007), which may suggest that VH4
10 expansion is an early and prolonged feature of particular CNS diseases. Interestingly, B cells from the cerebrospinal fluid of NMO patients are dominated by an expansion of VH2 genes, not VH4 genes (Bennett *et al.*, 2015).

In this study, the inventor demonstrates that the expansion of VH4 utilization in peripheral plasmablasts translates to an increase in autoreactivity toward brain antigens. The
15 inventor used a human brain lysate (hBL) ELISA to initially screen rhAbs cloned from plasmablasts for binding to brain targets, but soon after discovered that this assay often led to false negatives. Several rhAbs exhibited binding to neuron bodies and glial cell processes in the brain, but were not reactive in the hBL ELISA. However, it should be noted that the hBL preparation would consist mainly of cytosolic and easily soluble proteins, and much of it
20 would consist of myelin proteins, which represent as much as 30% of all proteins in the brain (Siegel *et al.*, 1999). Thus, non-myelin and hydrophobic protein targets are under-represented in the hBL antigen pool, diminishing the probability of identifying primary targets not associated with myelin. Furthermore, mild detergents in the buffer easily compromise the conformational integrity of proteins in ELISA platforms (von Budigen *et al.*, 2004; 2002).
25 Thus, the identification of brain-reactive rhAbs relied more heavily on their ability to bind cellular targets by either tissue histology or flow cytometric cell-based assays. Indeed, hBL ELISA positivity was noted for only about half of the CIS-PTM plasma samples from this cohort, despite clinical and immunological evidence of their CNS reactivity. For this reason, the inventor would agree that a multi-tiered pipeline for characterizing the CNS-reactive
30 potential of antibodies in any CNS disease setting with suspected autoimmune components involving humoral immunity is necessary as suggested by others (Zekeridou *et al.*, 2015).

When rhAb reactivity is considered in aggregate, it is interesting to note that whereas the CSF-derived rhAbs from these patients were largely reactive to neurons and astrocytes in the gray matter of the brain (Ligocki *et al.*, 2015), many of the rhAbs generated from the

peripheral plasmablasts were directed towards both gray and white matter targets. This data suggests that peripheral plasmablasts have a wider array of autoreactive specificities than CSF-derived B cells. This scenario is consistent with underlying dysregulation in tolerance of peripheral B cells in these CIS-PTM patients, and indeed others have demonstrated that there is a break in the peripheral tolerance checkpoint in MS patients (Tiller *et al.*, 2008). The exact mechanism of CNS-reactive effector B cell development in the blood is still unknown, and this break in tolerance could involve both B cell intrinsic and extrinsic mechanisms. The rapid return of memory B cells in the periphery following B cell depletion as a strong indicator of poor response to therapy further emphasizes the importance of studying the development of autoreactive B cells in the periphery.

During an exacerbation of MS, the blood brain barrier is often compromised, allowing increased exchange of antigen stimulated cells between the CNS and periphery (Minagar and Alexander, 2003; Holman *et al.*, 2011), including intracellular antigens whose ability to drive autoimmunity is only beginning to be understood (Lim *et al.*, 2006; Waldman and Madaio, 2005; Racanelli *et al.*, 2011; Yanase *et al.*, 1997; Jang *et al.*, 2009; Song *et al.*, 2008). There are many scenarios that may account for this discordance in antigen targets of peripheral and CSF-derived B cell subsets. For example, there may be an underlying open access to gray matter targets throughout the disease course, and access to white matter targets primarily during distinct points throughout the disease course. It is also possible that there is less reactivity to white matter targets at later stages of disease due to immune response exhaustion to those targets (Akbar and Henson, 2011; Ratts *et al.*, 2006). Alternatively, gray matter targets may be more immunogenic, considering the more extensive clonal expansion of CSF B cells that bind to these targets as compared to the peripheral plasmablasts. Interestingly, it has been demonstrated that antibodies targeting neurons from clonally expanded CSF B cells from MS patients mediate demyelination of axons *in vitro*, highlighting their potential to participate in the pathogenesis of disease (Blauth *et al.*, 2015). Delineating the pathway by which autoreactive plasmablasts develop, persist and mediate pathogenesis in MS patients will greatly improve the understanding of the disease, and is particularly important given that the frequency of plasmablasts increases the longer that CIS-PTM patients are left untreated (Ligocki *et al.*, 2013).

* * * * *

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the

compositions and methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the disclosure. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

- 5 U.S. Patent No. 5,475,096
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U.S. Patent No. 6,004,746
U.S. Patent No. 6,794,144
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CLAIMS

What is Claimed:

1. A process for making a pharmaceutical composition for treating a neurodegenerative or neuroimmunological disease, the process comprising:
 - 5 providing a VH4 antibody having at least one mutation with respect to the germline sequence at codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89, and which binds to an antigen in human gray or white matter;
selecting an active agent that reduces or inhibits the expression of said antibody, or reduces or inhibits binding of said antibody to said antigen; and
 - 10 formulating said active agent as a pharmaceutical composition.
2. The process of claim 1, wherein the VH4 antibody has 3, 4, 5, or 6 mutations with respect to the germline sequence at codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89.
- 15 3. The process of claim 1 or 2, wherein the VH4 antibody has mutations at two or more of codons 56, 57, and 81 with respect to the germline sequence, or at two or more of codons 40, 56, 81, and 89 with respect to the germline sequence.
- 20 4. The process of claim 1 or 2, wherein the VH4 antibody has mutations at one or more of codons 32, 40, 57, 60, and 89 with respect to the germline sequence.
5. The process of claim 2 or 3, wherein the VH4 antibody has mutations at one or both of codon 40 and 81 with respect to the germline sequence
- 25 6. The process of any one of claims 1 to 5, wherein the VH4 antibody has one or more of the following mutations:
codon 31B is R, N, D, P, K, G, A, or T, or is selected from R, N, and D;
codon 40 is S, L, or A;
30 codon 56 is selected from R, G, N, T, Y, H, D, and K, or is selected from N, T, and G;
codon 57 is A, I, D, S, or K, or is selected from A and I;
codon 81 is N, R, or M; and
codon 89 is selected from F, I, R, and L.

7. The process of claim 6, wherein the VH4 antibody has an R or N replacement at codon 81.
8. The process of claim 6, wherein the VH4 antibody has a T or R replacement at
5 codon 56, optionally with the germline amino acid at codons 31B, 40, and 89, and which optionally binds astrocytes.
9. The process of any one of claims 1 to 8, wherein the VH4 germline is 4-04, 4-28, 4-30, 4-31, 4-34, 4-39, 4-59, 4-61 or 4-B4.
10. The process of any one of claims 1 to 9, wherein the antibody has a set of mutations selected from Table 1.
11. The process of claim 1, wherein the VH4 antibody has a heavy chain CDR or
15 set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a light chain CDR or set of light chain CDRs as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.
12. The process of claim 1, wherein the VH4 antibody has a heavy chain variable
20 sequence as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a light chain variable sequence as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20,
25 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.
13. The process of any one of claims 1 to 12, wherein the VH4 antibody is a human recombinant antibody.
14. The process of any one of claims 1 to 13, wherein the antibody further binds to
30 mouse brain tissue.
15. The process of any one of claims 1 to 14, wherein the antibody binds to
astrocytes and neuronal nuclei in human gray and/or white matter, and optionally
35 mouse gray and/or white matter.

16. The process of claim 15, wherein the active agent is tested for its ability to competitively inhibit binding of said antibody to said antigen, optionally using human or mouse brain tissue.

5 17. The process of any one of claims 1 to 16, wherein the active agent comprises an anti-idiotypic antibody or antigen-binding portion thereof; an antibody or portion thereof with binding specificity for the same antigen; or a peptide, aptamer, or small molecule that is bound by said antibody at high affinity; or an oligonucleotide inhibiting the expression of said antibody.

10 18. The process of claim 17, wherein the active agent is an antibody fragment lacking an Fc.

15 19. The process of claim 17, wherein the antibody is a F(ab')₂ or Fab, a single chain antibody, or single chain variable fragment (scFv).

20. The process of claim 17, wherein the active agent is an antisense oligonucleotide or siRNA.

20 21. The process of any one of claims 1 to 20, wherein the active agent is formulated for administration by subcutaneous, intravenous, intramuscular, oral, or intrathecal administration.

25 22. The process of any one of claims 1 to 20, wherein the neurodegenerative disease is MS.

23. The process of any one of claims 1 to 22, wherein the antibody impacts demyelination, inflammatory infiltrates, axonal injury or cellular function in animal model of MS or demyelination.

30 24. A process for making a pharmaceutical composition for treating a neurodegenerative disease, the process comprising:

providing a VH4 antibody having at least two mutations selected from 31B, 32, 40, 56, 57, 60, 81, and 89, and which binds to an antigen in human gray matter and
35 exhibits neuroprotection or repair;

formulating an antibody that binds to said antigen as a pharmaceutical composition.

25. The process of claim 24, wherein the VH4 antibody has 3, 4, 5, or 6 mutations
5 with respect to the germline sequence at codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89.

26. The process of claim 24 or 25, wherein the VH4 antibody has mutations at two
or more of codons 56, 57, and 81 with respect to the germline sequence, or at two or
10 more of codons 40, 56, 81, and 89 with respect to the germline sequence.

27. The process of claim 24 or 25, wherein the VH4 antibody has mutations at one
or more of codons 32, 40, 57, 60, and 89 with respect to the germline sequence.

15 28. The process of claim 24 or 25, wherein the VH4 antibody has mutations at one
or both of codon 40 and 81 with respect to the germline sequence

29. The process of any one of claims 24 to 28, wherein the VH4 antibody has one
or more of the following mutations:

20 codon 31B is R, N, D, P, K, G, A, or T, or is selected from R, N, and D;
codon 40 is S, L, or A;
codon 56 is selected from R, G, N, T, Y, H, D, and K, or is selected from N, T,
or G;
codon 57 is A, I, D, S, or K, or is selected from A and I;
25 codon 81 is N, R, or M; and
codon 89 is selected from F, I, R, and L.

30. The process of claim 29, wherein the VH4 antibody has an R or N replacement
at codon 81.

30 31. The process of claim 29, wherein the VH4 antibody has a T or R replacement at
codon 56, optionally with the germline amino acid at codons 31B, 40, and 89, and
which optionally binds astrocytes.

35 32. The process of any one of claims 24 to 31, wherein the VH4 germline is 4-04,
4-28, 4-30, 4-31, 4-34, 4-39, 4-59, 4-61 or 4-B.

33. The process of any one of claims 24 to 32, wherein the antibody has a set of mutations selected from Table 1.

5 34. The process of claim 24, wherein the VH4 antibody has a heavy chain CDR or set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a light chain CDR or set of light chain CDRs as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50,
10 52, 54, 56, 58, 60, 62, and 64.

35. The process of claim 24, wherein the VH4 antibody has a heavy chain variable sequence as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a light
15 chain variable sequence as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.

36. The process of any one of claims 24 to 35, wherein the VH4 antibody is a human recombinant antibody.

20 37. The process of any one of claims 24 to 36, wherein the antibody further binds to mouse brain tissue.

38. The process of any one of claims 24 to 36, wherein the antibody binds to
25 astrocytes and neuronal nuclei in human gray and/or white matter, and optionally mouse gray and/or white matter.

39. The process of any one of claims 24 to 38, wherein the antibody that is formulated lacks an Fc, or has a derivatized Fc to alter one or more effector functions.

30 40. The process of any one of claims 24 to 38, wherein the antibody is a F(ab')₂ or Fab, a single chain antibody, or single chain variable fragment (scFv).

41. The method of any one of claims 24 to 40, wherein the antibody is tested for
35 neuroprotection in the EAE model, and/or tested for neurorepair activity in cuprizone or lysoecithan model.

42. The process of any one of claims 24 to 41, wherein the antibody is formulated for administration by subcutaneous, intravenous, intramuscular, or intrathecal administration.

5 43. The process of any one of claims B1 to B19, wherein the neurodegenerative disease is MS, neuromyelitis optica or transverse myelitis.

44. A process for making a pharmaceutical composition for treating a neurodegenerative disease, the process comprising:

10 providing peripheral plasmablasts from an MS patient or antibodies derived therefrom, and which bind to an antigen in human gray matter;

selecting an active agent that reduces or inhibits the expression of said antibodies, or reduces or inhibits binding of said antibody to said antigen; and

formulating said active agent as a pharmaceutical composition.

15

45. The process of claim 44, wherein the plasmablast produces a VH4 antibody having 3, 4, 5, or 6 mutations with respect to the germline sequence at codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89.

20 46. The process of claim 45, wherein the VH4 antibody has mutations at two or more of codons 56, 57, and 81 with respect to the germline sequence, or at two or more of codons 40, 56, 81, and 89 with respect to the germline sequence.

25 47. The process of claim 45 or 46, wherein the VH4 antibody has mutations at one or more of codons 32, 40, 57, 60, and 89 with respect to the germline sequence.

48. The process of claim 45 or 46, wherein the VH4 antibody has mutations at one or both of codon 40 and 81 with respect to the germline sequence.

30 49. The process of any one of claims 45 to 48, wherein the VH4 antibody has one or more of the following mutations:

codon 31B is R, N, D, P, K, G, A, or T, or is selected from R, N, and D;

codon 40 is S, L, or A;

codon 56 is selected from R, G, N, T, Y, H, D, and K, or is selected from N, T,

35 and G;

codon 57 is A, I, D, S, or K, or is selected from A and I;

codon 81 is N, R, or M; and
codon 89 is selected from F, I, R, and L.

50. The process of claim 49, wherein the VH4 antibody has an R or N replacement
5 at codon 81.

51. The process of claim 49, wherein the VH4 antibody has a T or R replacement at
codon 56, optionally with the germline amino acid at codons 31B, 40, and 89, and
which optionally binds astrocytes.

10 52. The process of any one of claims 45 to 51, wherein the VH4 germline is 4-04,
4-28, 4-30, 4-31, 4-34, 4-39, 4-59, 4-61 or 4-B4.

53. The process of any one of claims 45 to 52, wherein the antibody has a set of
15 mutations selected from Table 1.

54. The process of claim 45, wherein the VH4 antibody has a heavy chain CDR or
set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19,
21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63,
20 and/or a light chain CDR or set of light chain CDRs as in any one of SEQ ID NOS: 2,
4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50,
52, 54, 56, 58, 60, 62, and 64.

55. The process of claim 45, wherein the VH4 antibody has a heavy chain variable
25 sequence as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27,
29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a light
chain variable sequence as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20,
22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.

30 56. The process of any one of claims 44 to 55, wherein the plasmablast or antibody
further binds to mouse brain tissue.

57. The process of any one of claims 44 to 56, wherein the antibody binds to
astrocytes and neuronal nuclei in human gray and/or white matter, and optionally
35 mouse gray and/or white matter.

58. The process of claim 57, wherein the active agent is tested for its ability to competitively inhibit binding of said antibody to said antigen, optionally using human or mouse brain tissue.

5 59. The process of any one of claims 44 to 58, wherein the active agent is an anti-idiotypic antibody or antigen-binding portion thereof; an antibody or portion thereof with binding specificity for the same antigen; or a peptide, aptamer, or a small molecule that is bound by said antibody at high affinity; or an oligonucleotide inhibiting the expression of said antibody.

10 60. The process of claim 59, wherein the active agent is an antibody fragment lacking an Fc.

61. The process of claim 60, wherein the antibody is a F(ab')₂ or Fab, a single
15 chain antibody, or single chain variable fragment (scFv).

62. The process of claim 59, wherein the active agent is an antisense oligonucleotide or a siRNA.

20 63. The process of any one of claims 44 to 62, wherein the active agent is formulated for administration by subcutaneous, intravenous, intramuscular, oral, or intrathecal administration.

64. The process of any one of claims 44 to 63, wherein the neurodegenerative
25 disease is MS.

65. The process of any one of claims 44 to 64, wherein the antibody impacts demyelination, inflammatory infiltrates, axonal injury, or cell function in an animal model of MS.

30 66. A process for making a pharmaceutical composition for treating a neurodegenerative disease, the process comprising:

providing peripheral plasmablasts from MS patients or antibodies derived therefrom, and which bind to an antigen in human gray matter and exhibits
35 neuroprotection or repair;

formulating an antibody that binds to said antigen as a pharmaceutical composition.

67. The process of claim 66, wherein the plasmablast produces a VH4 antibody
5 having 3, 4, 5, or 6 mutations with respect to the germline sequence at codons selected from 31B, 32, 40, 56, 57, 60, 81, and 89.

68. The process of claim 66 or 67, wherein the antibody further binds to mouse brain tissue.

10

69. The process of any one of claims 66 or 67, wherein the antibody binds to astrocytes and neuronal nuclei in human gray matter, and optionally mouse gray matter.

15

70. The process of any one of claims 66 to 69, wherein the antibody that is formulated lacks an Fc, or has a derivatized Fc to alter one or more effector functions.

71. The process of any one of claims 66 to 70, wherein the antibody is a F(ab')₂ or Fab, a single chain antibody, or single chain variable fragment (scFv).

20

72. The method of any one of claims 66 to 71, wherein the antibody is tested for neuroprotection in the EAE model, and/or tested for neurorepair activity in cuprizone model.

25

73. The process of any one of claims 66 to 72, wherein the active agent is formulated for administration by subcutaneous, intravenous, intramuscular, or intrathecal administration.

30

74. The process of any one of claims 66 to 73, wherein the neurodegenerative disease is MS.

75. A method for treating a neurodegenerative disease, comprising, administering the pharmaceutical composition made by the process of any one of claims 1 to 74, to a patient having a neurodegenerative disease.

35

76. A pharmaceutical composition comprising an effective amount of a VH4 antibody or antigen-binding portion thereof and a pharmaceutically acceptable carrier, the antibody or antigen-binding portion having at least two mutations selected from

31B, 32, 40, 56, 57, 60, 81, and 89, and which binds to an antigen in human gray and/or white matter and exhibit neuroprotection or repair.

77. The method of claim 76, wherein the antibody exhibits neuroprotection in the
5 EAE model, and/or neurorepair activity in cuprizone model or lysolecithine model.

78. An anti-idiotypic antibody specific for a VH4 antibody having a set of mutations selected from Table 1.

10 79. An anti-idiotypic antibody specific for a VH4 antibody having a set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and a set of light chain CDRs as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.

15 80. A VH4 antibody having a set of mutations selected from Table 1, wherein the antibody lacks an Fc domain or has a derivatized Fc domain to alter one or more effector functions.

20 81. A VH4 antibody having a set of heavy chain CDRs as in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, and/or a set of light chain CDRs as in any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64, and which lacks an Fc domain or has a
25 derivatized Fc domain to alter one or more effector functions.

82. An oligonucleotide comprising a nucleotide sequence that is complementary to a nucleotide sequence encoding a VH4 heavy chain CDR sequence selected from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37,
30 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61 and 63, or complementary to a nucleotide sequence encoding a VH4 light chain CDR sequence of any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64.

35 83. The oligonucleotide of claim 82, wherein the nucleotide sequence is complementary to a sequence selected from SEQ ID NOS: 65-128.

84. The oligonucleotide of claim 83, wherein the oligonucleotide is an antisense oligonucleotide.
- 5 85. The oligonucleotide of claim 83, wherein the oligonucleotide is a siRNA.

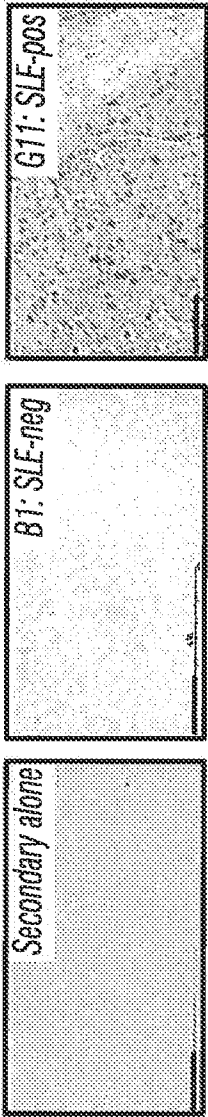


FIG. 1A

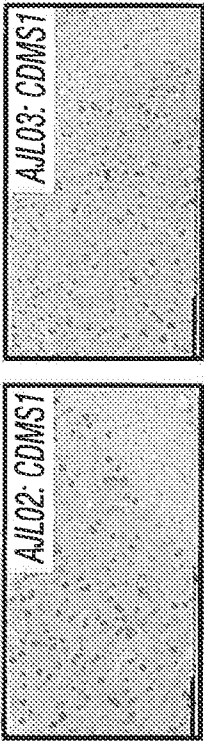


FIG. 1B

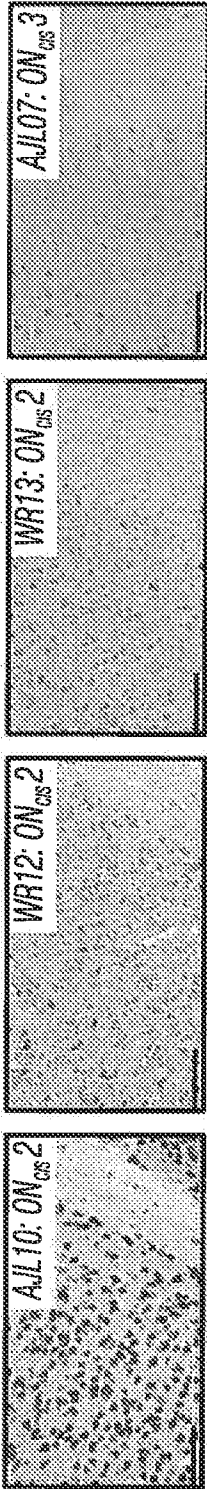


FIG. 1C

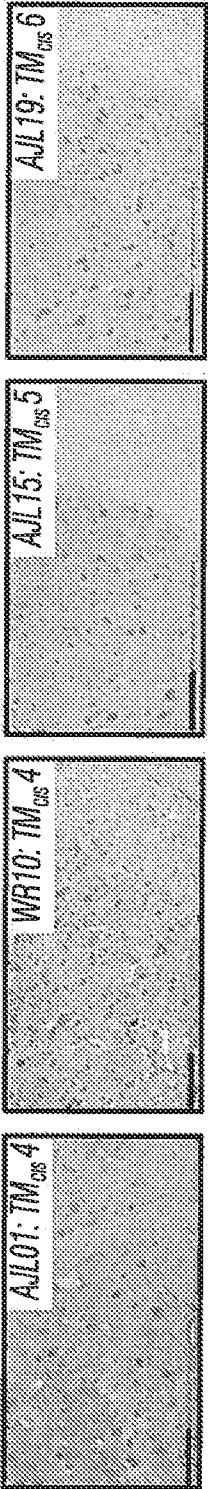
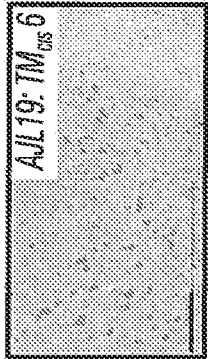


FIG. 1D



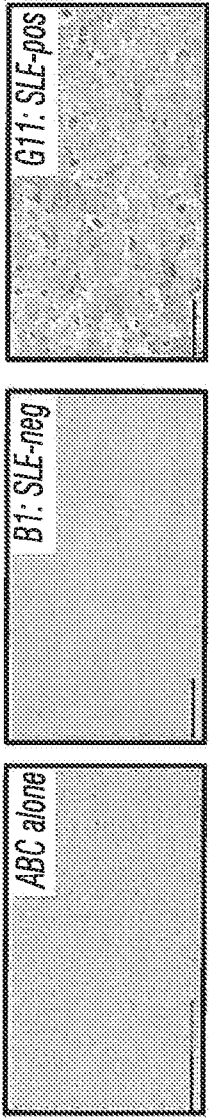


FIG. 2A

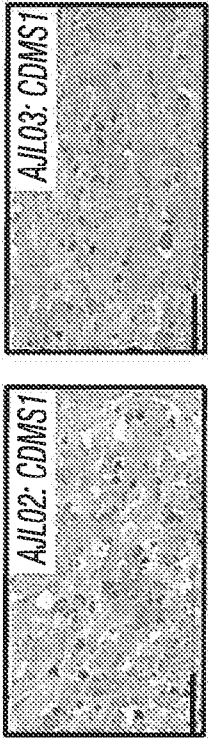


FIG. 2B

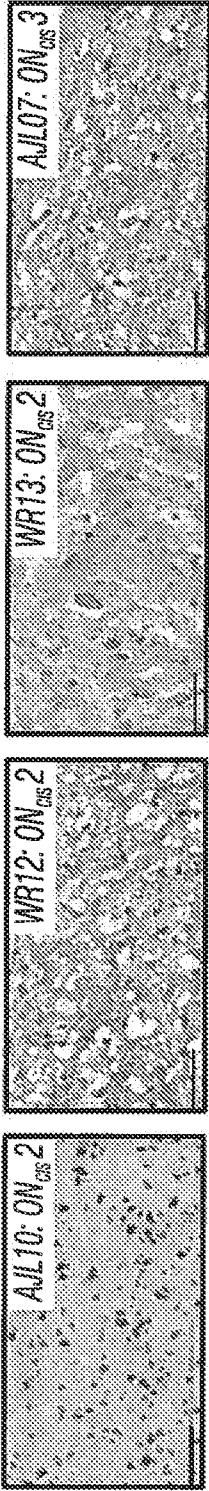


FIG. 2C

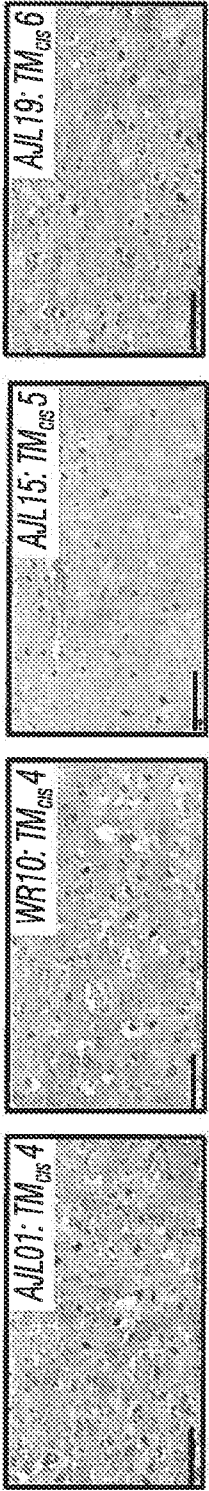


FIG. 2D

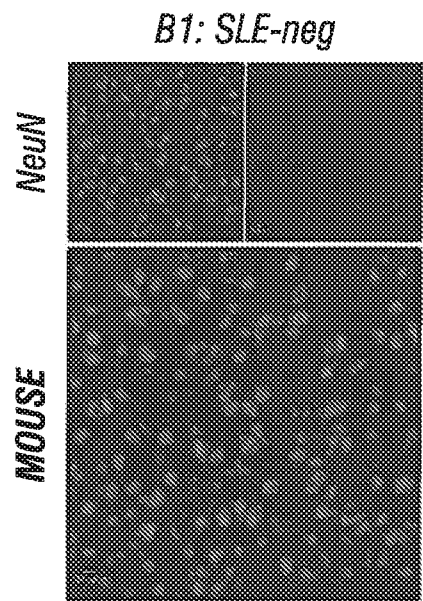


FIG. 3A

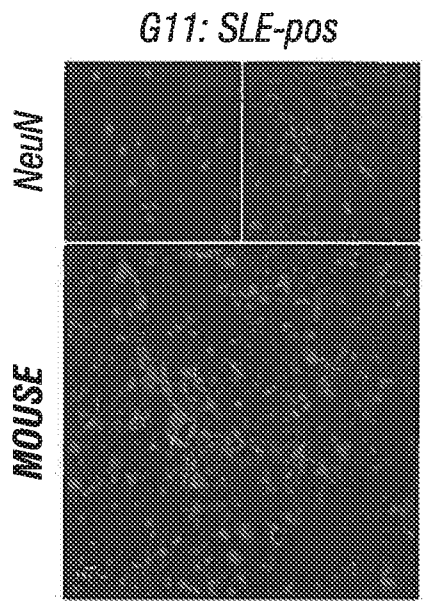


FIG. 3B

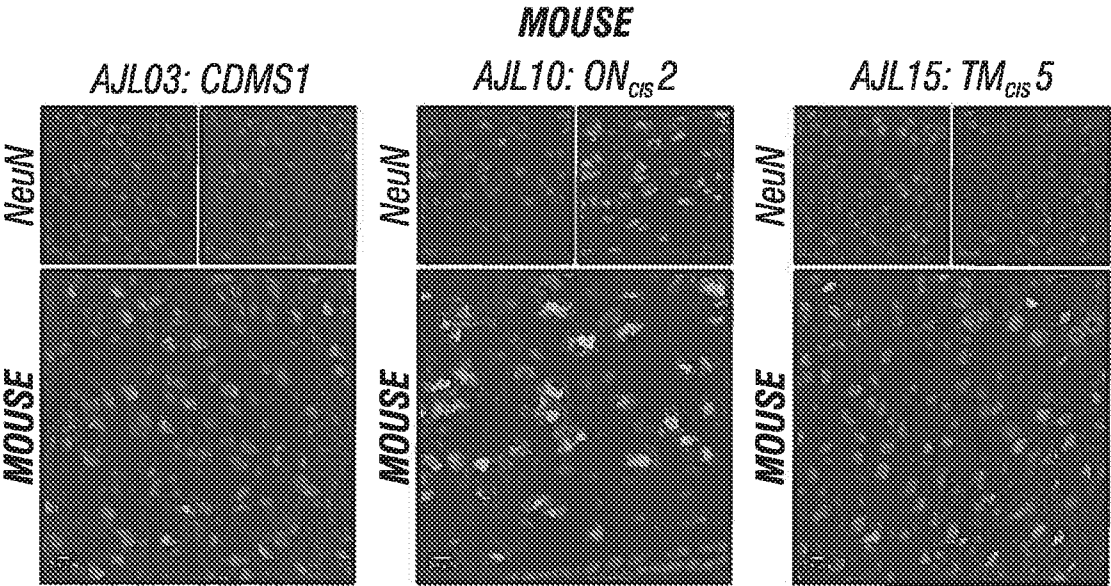


FIG. 3C

FIG. 3E

FIG. 3G

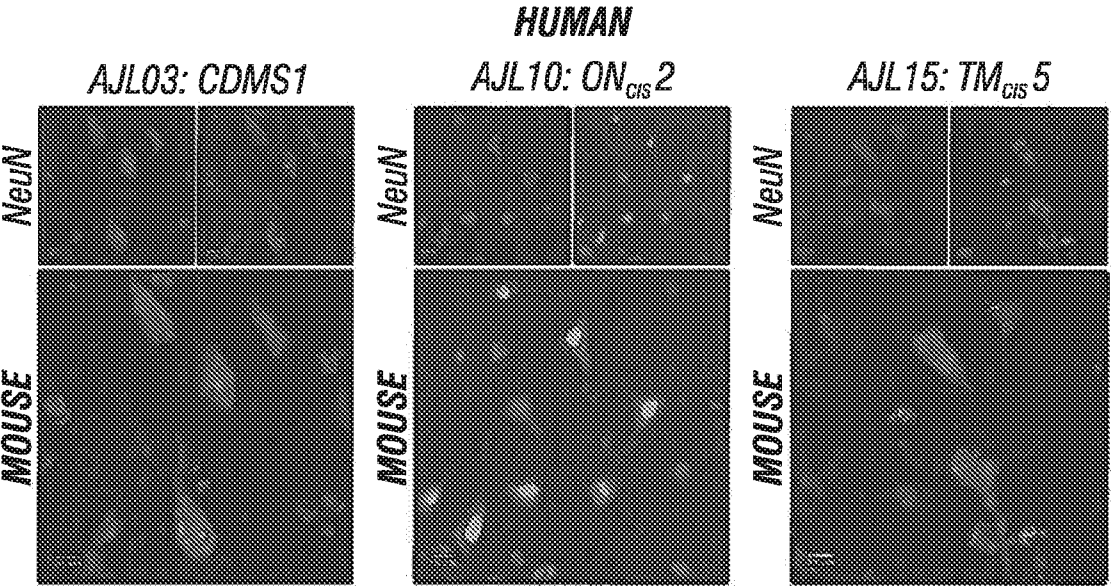


FIG. 3D

FIG. 3F

FIG. 3H

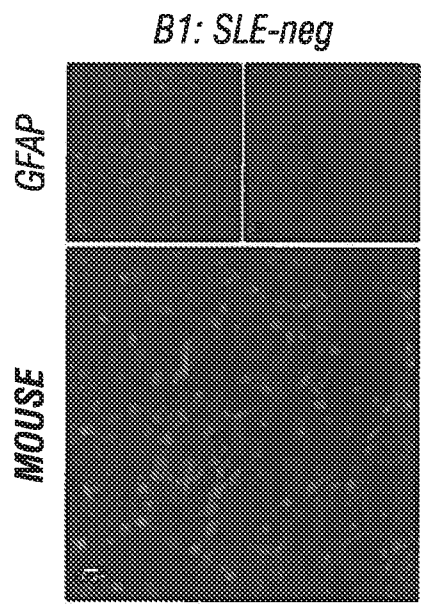


FIG. 4A

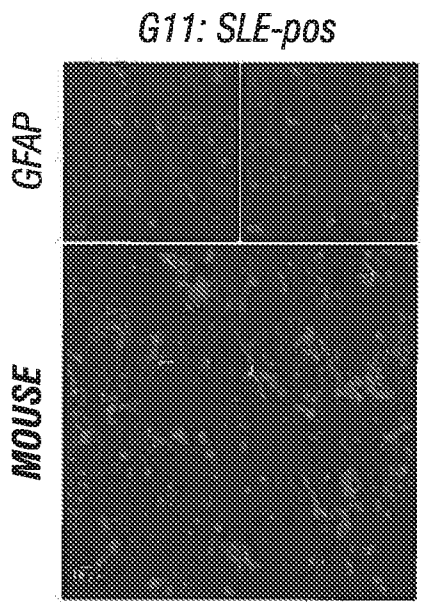
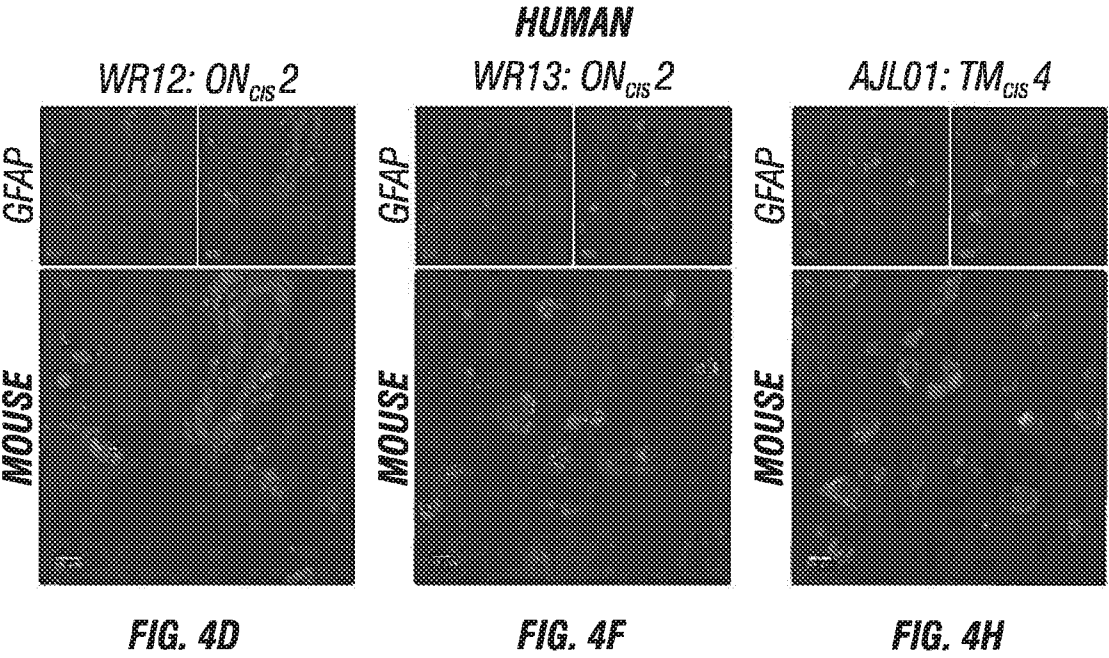
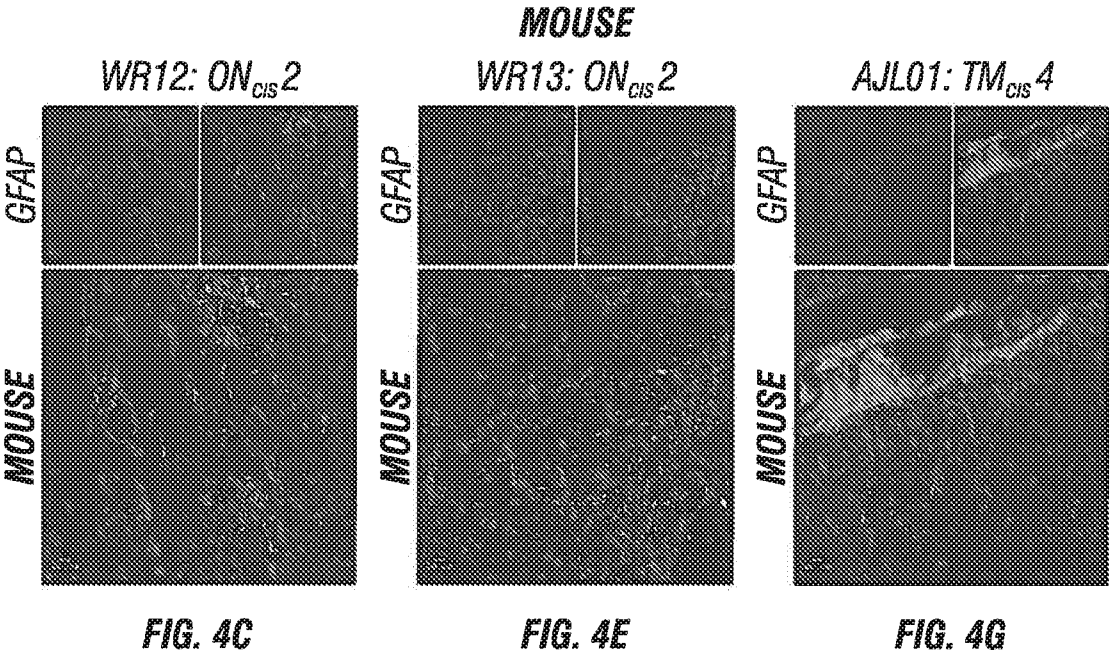


FIG. 4B



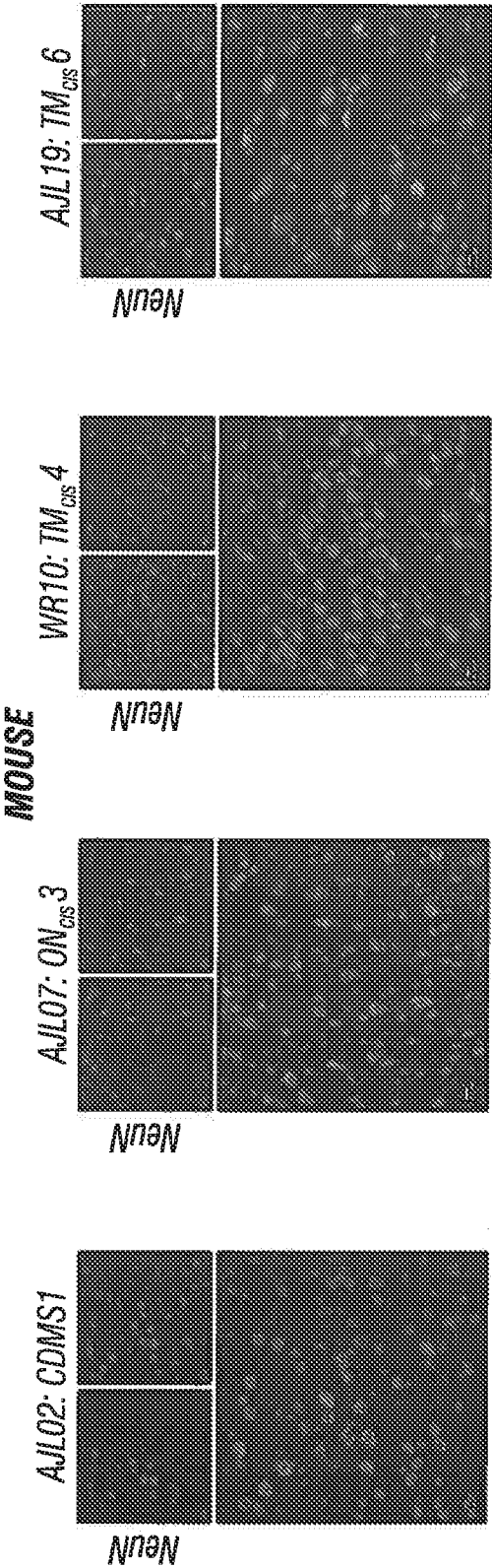


FIG. 5A

FIG. 5D

FIG. 5G

FIG. 5J

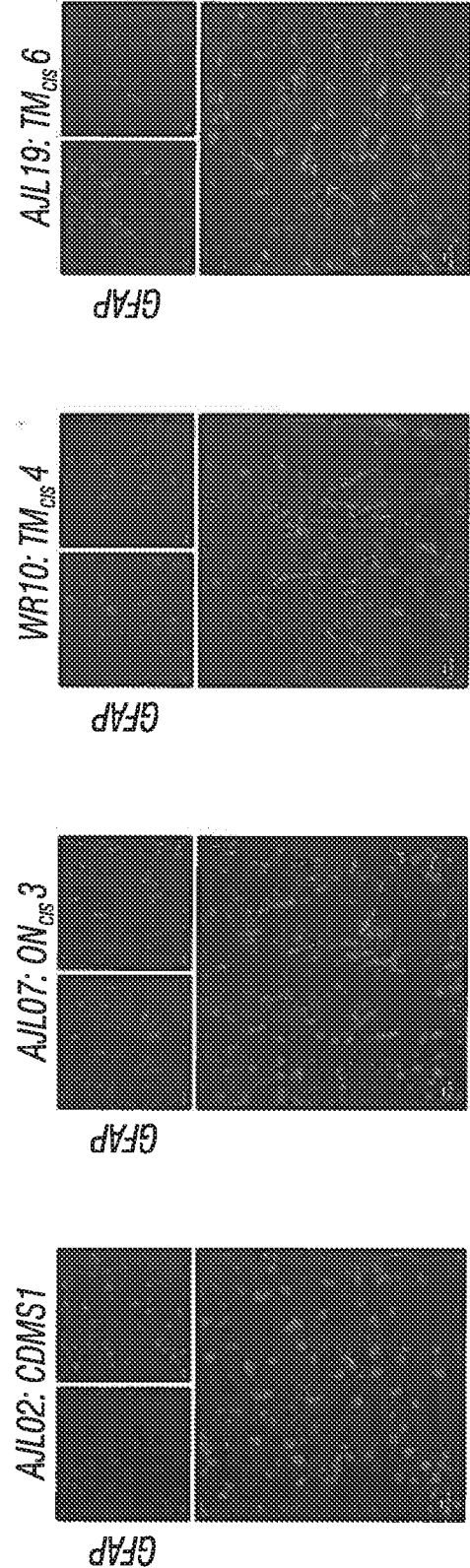


FIG. 5B

FIG. 5E

FIG. 5H

FIG. 5K

HUMAN

NeuN AJL02: CDMS1

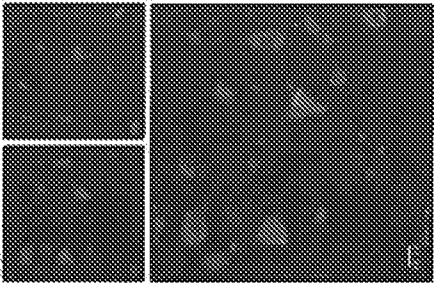


FIG. 5C

NeuN AJL07: ON_{CS} 3

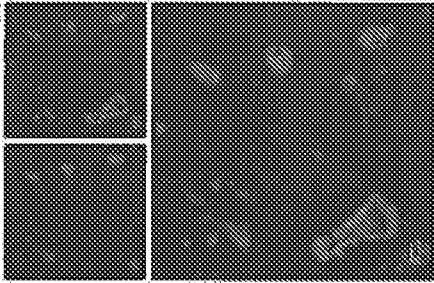


FIG. 5F

GFAP WR10: TM_{CS} 4

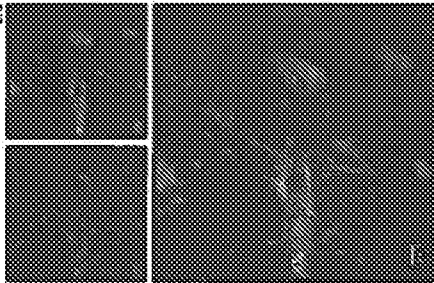


FIG. 5I

NeuN AJL19: TM_{CS} 6

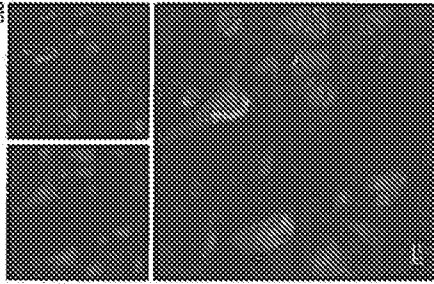


FIG. 5L

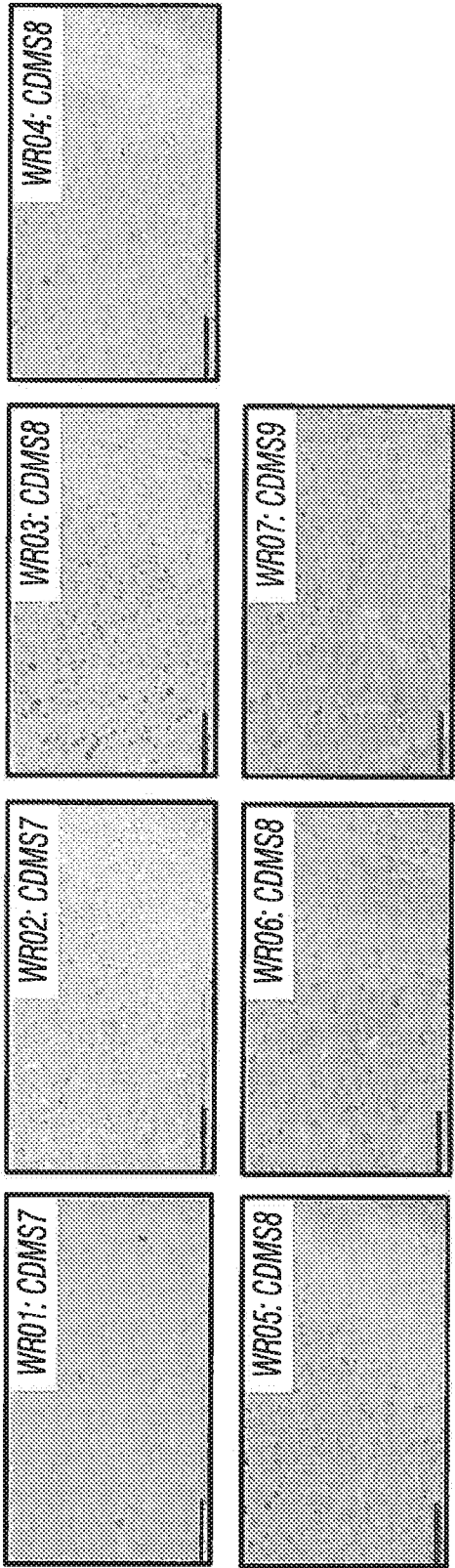


FIG. 6A

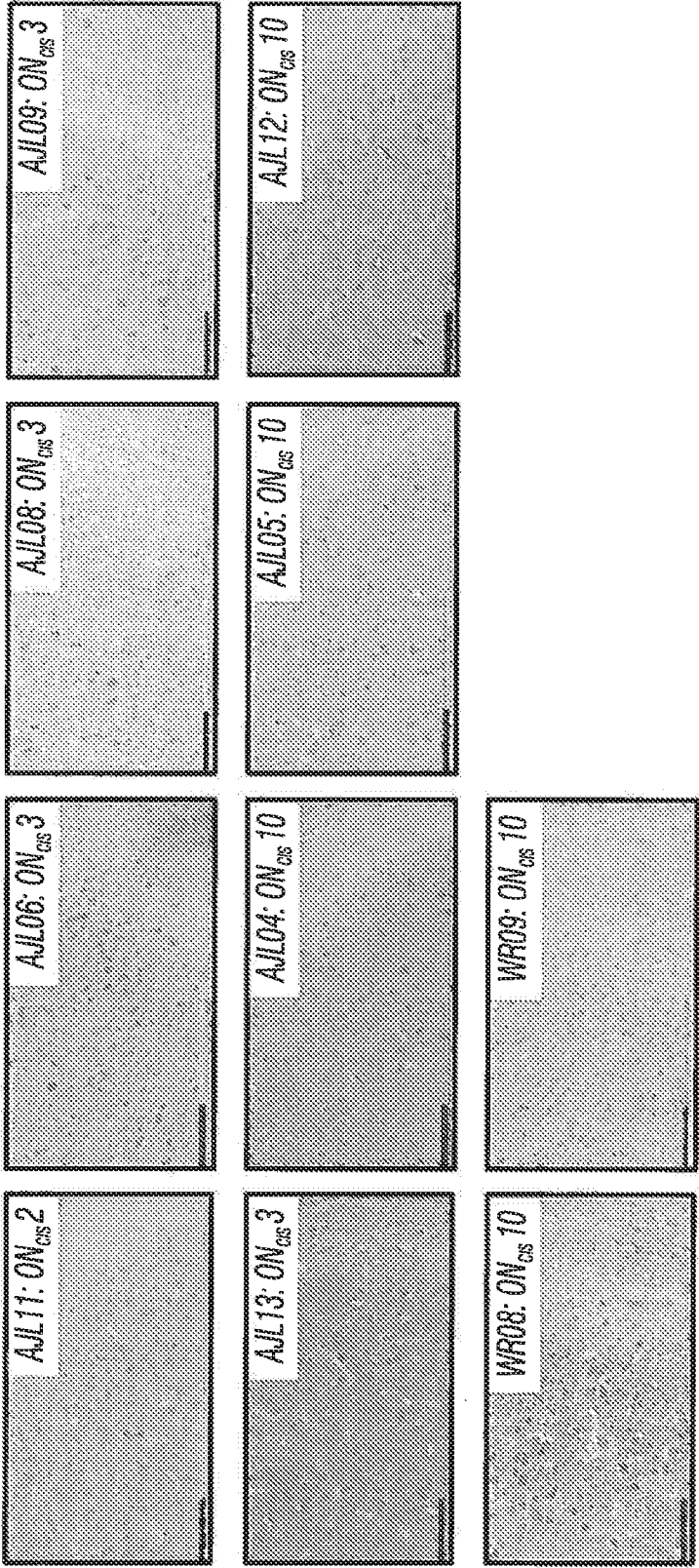


FIG. 6B

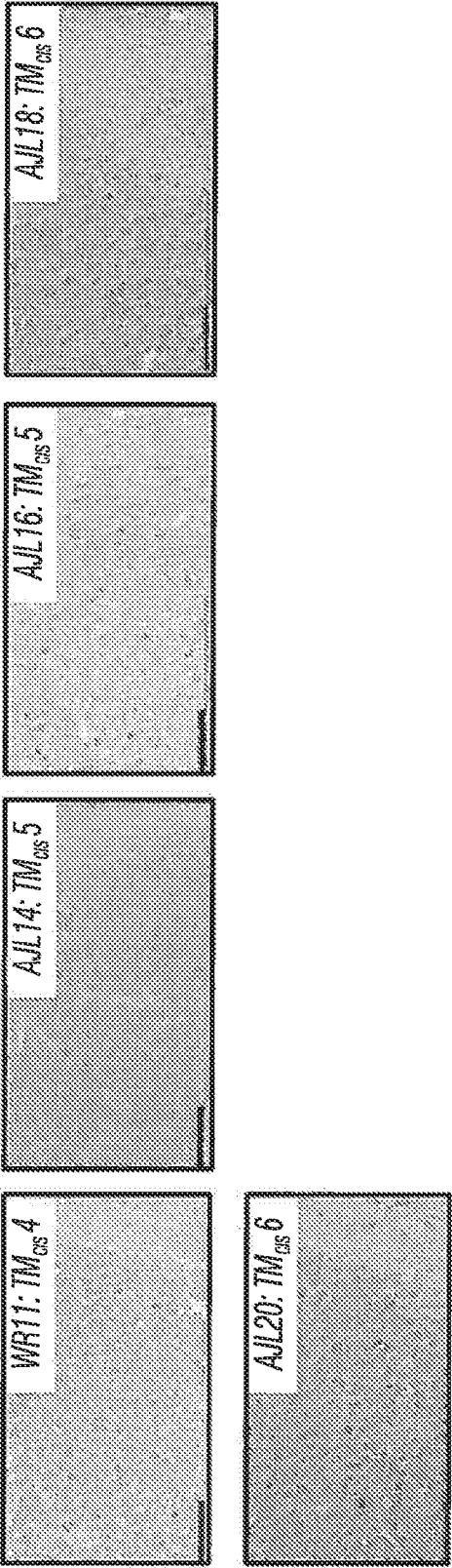


FIG. 6C

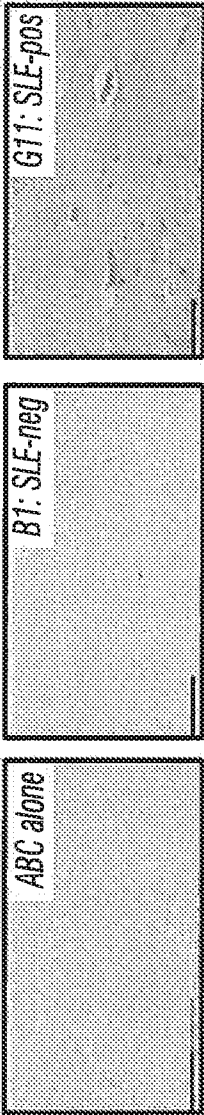


FIG. 7A

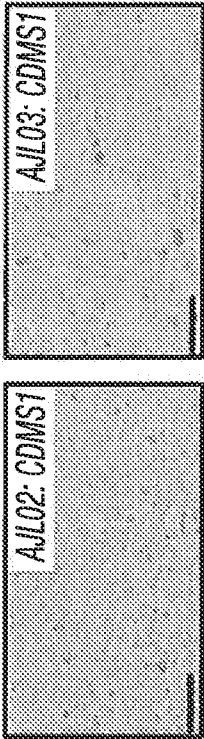


FIG. 7B

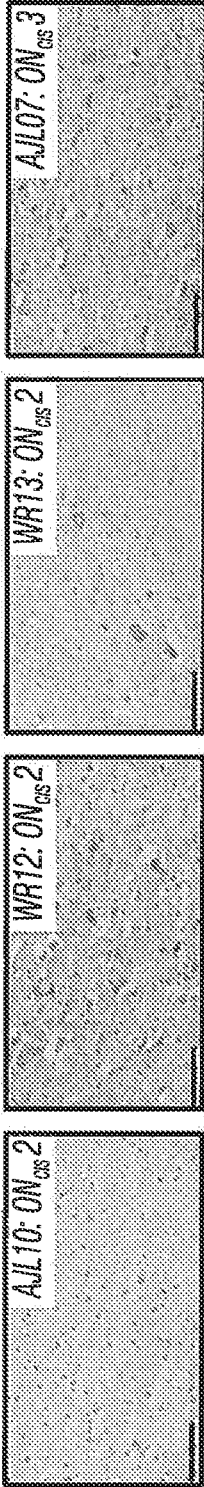


FIG. 7C

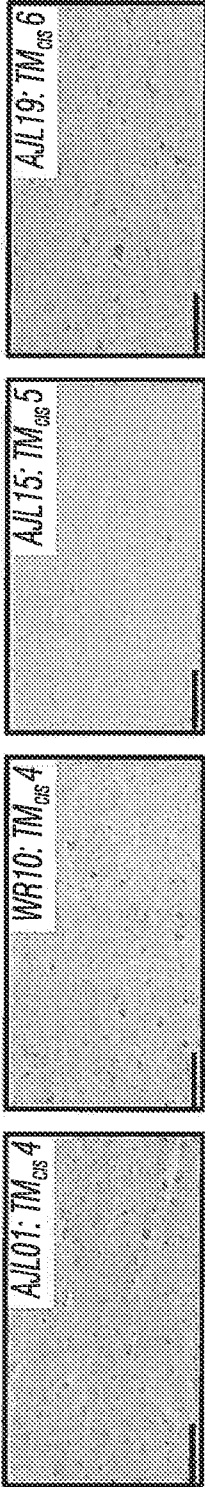


FIG. 7D

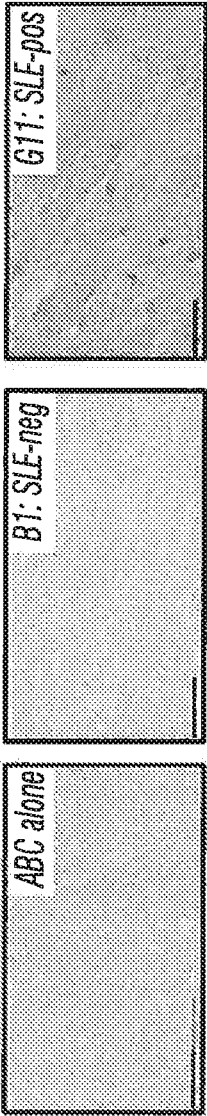


FIG. 8A

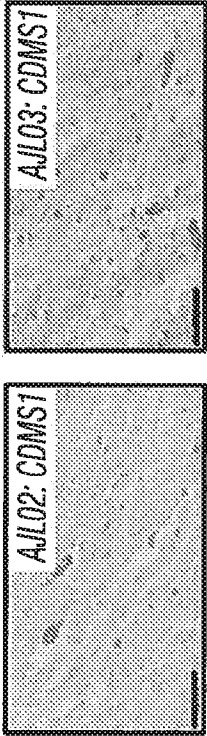


FIG. 8B

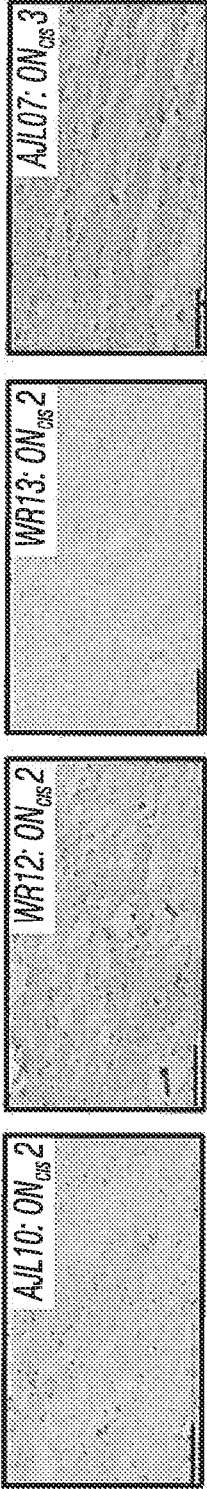


FIG. 8C

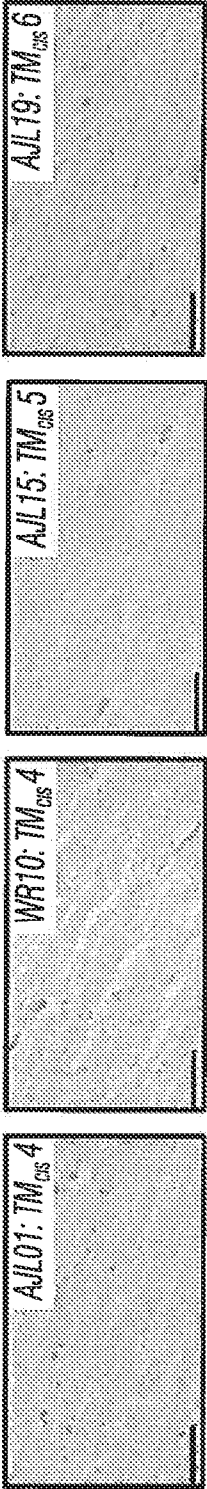


FIG. 8D

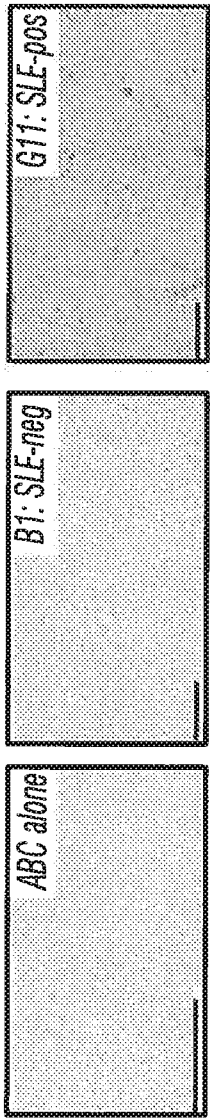


FIG. 9A

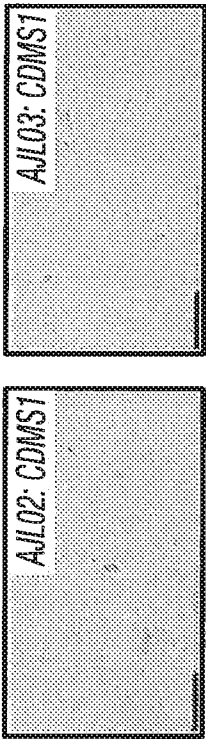


FIG. 9B

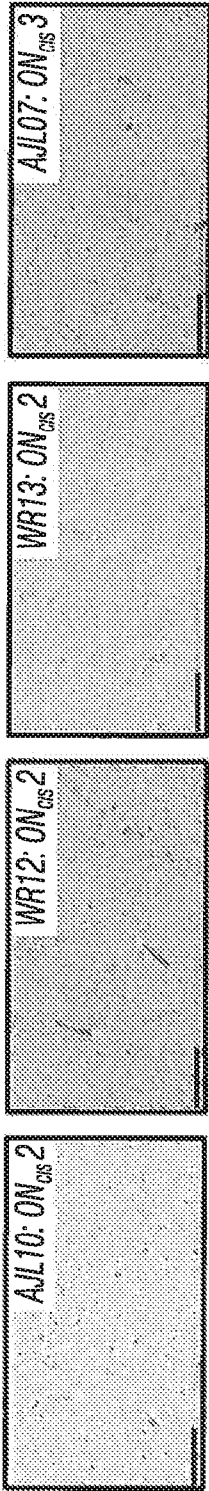


FIG. 9C

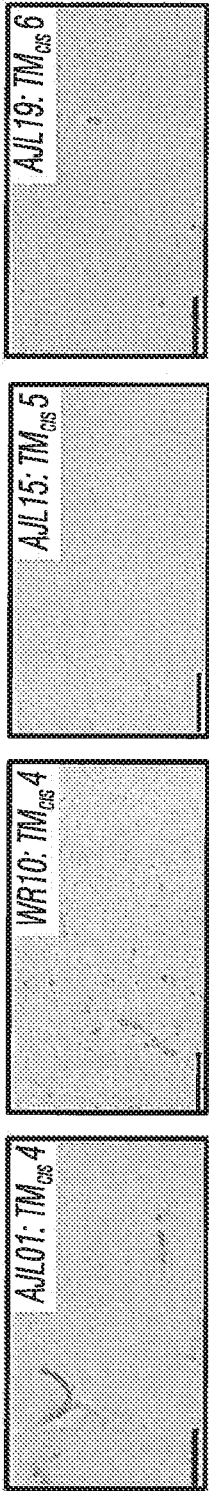
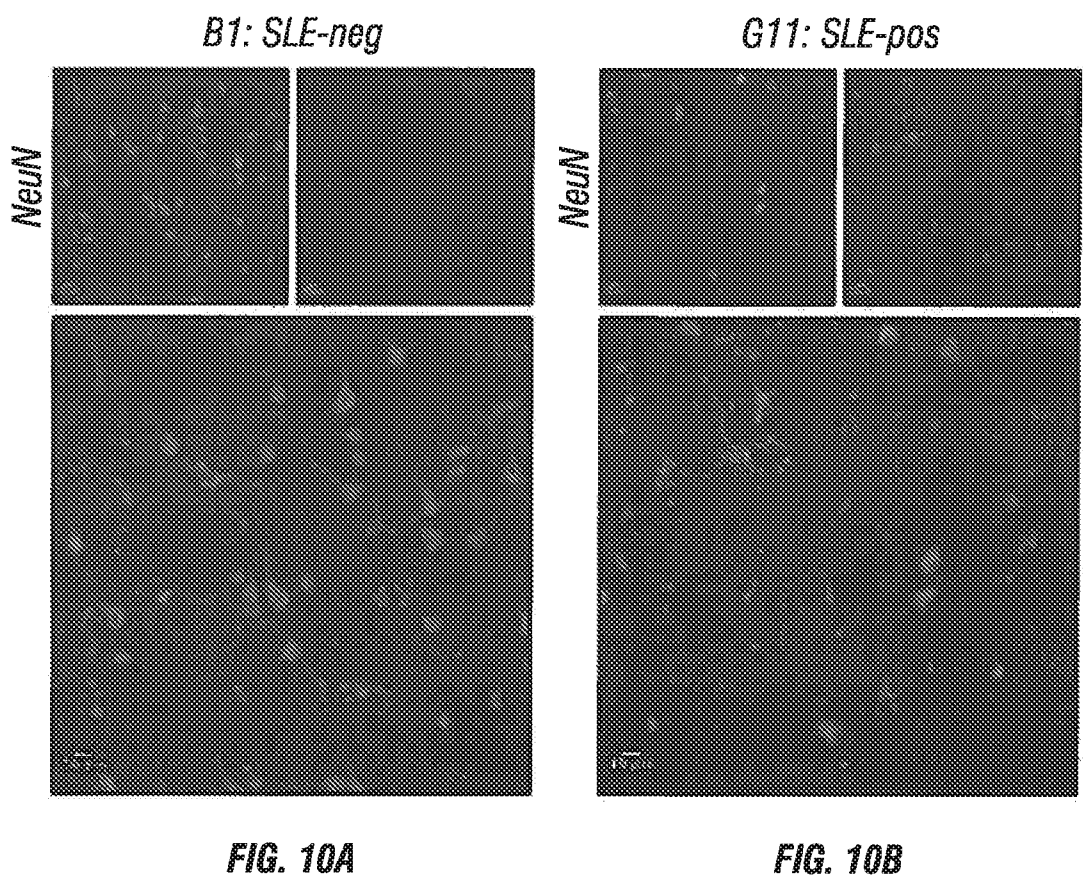


FIG. 9D



AIL01 IgG VH insert: *VH4-34:JH3* (SEQ ID NO: 1)
 TGVHSQVQLQQWGAGLLKPSETLSLTCAVYGGSFNEEYWSWIRQPARKGLEWIG~~EHSHS~~GRANYNPSLKSRVTLSDRSKNQFSLNLSPVAAADTAVYY
 CARREIVTVYRGRRAFDIWGQGTMTVTVSSAS

AIL01 IgK VK insert: *VK3-20:JK5* (SEQ ID NO: 2)
 TGVHSEIVLTQSPCTLSPGERAALSCRASOSLIGSELAWYQQKPGQAPRLLIYHTSNRASGIPDRFSGGGFTDFTLTISRLEPEDFAVYYCOQYDSSPIT
 FGQGTRLEIKRT

AIL02 IgG VH insert: *VH4-31:JH4* (SEQ ID NO: 3)
 TGVHSQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGHYW~~SWIRQSPGKGLEWIGNVVYSGSTYYTPSLDSRLTISLDT~~SKNQFSLRLSNVTVADTAVYY
 CARGRNWEGEEDPWGQGTMTVTVSSAS

AIL02 IgK VK insert: *VK1-39:JK2* (SEQ ID NO: 4)
 TGVHSDIQMTQSPSSLSASLGDRVTTTCRASQGISSSVNWFWQKPKKAPPELLIYAASTLQSGVPSRFSGSGSGTDFTLTVSSLPEDFATYYCQOSYSPRRT
 FGQGTKLEIKRT

FIG. 11

AIL03 IgG VH insert: VH4-39: JH1 (SEQ ID NO: 5)

TGVHSQQLQESGPGLVKPSSETLSLTCTVSGASISSRSYVWGWRQPPGKGLEWIGSMYQSGSTYYSPSLKSRVTISMDTSKNQFSLNLTSVTAADTAVYF
CARHSNPGTANKLRIGEFPWGQGTLLVTVSSAS

AIL03 IgK VK insert: VK1-33: JK2 (SEQ ID NO: 6)

TGVHSDIQMTQSPSSLSASVGDRTVITCOASQDINNLYLWFFQQPCKAPKLLIYDASKLOMGVPSRFSGSASCTDFTFTISSLPEDIGTYVCQQQYNLPY
TFGQGTKLEIKRT

AIL04 IgG VH insert: VH4-59: JH5 (SEQ ID NO: 7)

TGVHSQVLQESGPGLVKPSSETLSLTCTVSGDSIRSNEFWTWIRQSPGRGLEWIGYFSYSGGINYSPSLKSRVTISVDTSKNQFSLKLTSTVTAADAAYVYCA
RDPNGDYEYVNWVGQGTLLVTVSSAS

AIL04 IgK VK insert: VK1-16: JK5 (SEQ ID NO: 8)

TGVHSDIQMTQSPSSLSASVGDRTVITCRASQDIIHLYLAWFQQRPCKAPRSLIYSASTLQSGVPSKFSGSGGTFTLTISSLPEDSATYYCQQYKSYPIITFG
QGTRLEIKRT

FIG. 11 (cont.)

AIL05 IgG VH insert: VH4-39: JH5 (SEQ ID NO: 9)
 TGVHSQQLQESGPGLVKPSQTLTCTVSGGSRNSNYWZWIWQPPGKGLEWIGSGYSGSAYYHSSLKSRVSVSDTSKNQFSLNLTSTVTAADTAFYY
 CARRSYYYASGSHDYWGQGTLLVTVSSAS

AIL05 IgK VK insert: VK1-16: JK5 (SEQ ID NO: 10)
 TGVHSDIQMTQSPSSLSASVGDRTTITCRASODISSYLAWFQQKPGKAPKSLIYGASSLQSGVPSKFSKSGSGTDFTLTISGLQPEDFATYHCQQYRSEPIIF
 GQGTKLEIKRT

AIL06 IgG VH insert: VH4-30: JH4 (SEQ ID NO: 11)
 TGVHSQQLQESGGLVKPSQTLTCAVSGGSINTGNVFEWSWIRQPPGKGLEWIGYMERSTSTYYNPSTLKGRVTISGGTSLSQFSLRLDSVTAADTAIYY
 CARGRYYCGVNCHPFDSWGQGTLLVTVSSAS

AIL06 IgK VK insert: VK1-33: JK2 (SEQ ID NO: 12)
 TGVHSDIQMTQSPSSLSASVGDRTVITCQASQDISIYLNWVYQVKPGKAPKLLIYDASNLAQGVPSRFSKSGSGTDFSTISSLPQEDVAAYYCHQYDLSLPSF
 GQGTKLEIKRT

FIG. 11 (cont.)

AIL07 IgG VH insert: VH4-59: JH4 (SEQ ID NO: 13)

TGVHSQVLQESGPGLVKPSETLSLTCNVSGGSNNYYWSWIRQPPGKGLEWIGYIYNGNINYNPSLKSRVTISRDMSKNQFSLNRSVTAADTAVYYC
GIGYSAVAAGTVDYWGHTLVTVSSAS

AIL07 IgK VK insert: VK1-13: JK2 (SEQ ID NO: 14)

TGVHSAIQLTQSPSSLSASVGDRVTITCRASQGISSGLAWYQQEPGKAPKLLIYDASTILESGVPSRFSGSGSAIDFTLTISLQPEDFATYYCQQENTFPTTF
CQGTKLEIKRT

AIL08 IgG VH insert: VH4b: JH4 (SEQ ID NO: 15)

TGVHSQVLQESGPGLVKPSETLSLTCAVSGFSITSGYYWGWRQPPGKGLEWIGSYHTGTYYNPSLKSRVTISVDTSKNQFSLNNSVTAADTAFYYC
ARDPLFPGRNLLSVFDNWGGTLTVTVSSAS

AIL08 IgK VK insert: VK1-5: JK2 (SEQ ID NO: 16)

TGVHSDIQMTQSPSTLSASVGDRVTITCRASQGISITWLAWYQQKPGKAPRLLIYKASSLESGVPSRFSGSGTEFTLTISGLQPDDEFANYSQQYNIYPET
FGGTKLEIKRT

FIG. 11 (cont.)

AIL09 IgG VH insert: VH4-59: JH5 (SEQ ID NO: 17)

TGVHSQVLQESGPGLVKPSETLSLTCSVSGGSISSYYWGWRQSPGKLEICIGYIFESGTSYNPSLKSRVTISVDTAKNQISLNLTSVTAADTAVYFCARV
WGSSWYANWFDPWGQGTLVTVSSAS

AIL09 IgK VK insert: VK1-13: JK5 (SEQ ID NO: 18)

TGVHSAIQLTQSPSSLSASVGDVRVTTTCRASQGISSALAWYQHKPGKAPKLLIFDASTLAAGVPSRFSGSGGTDFLTITSSLPQEDFATYYCQQYNTYVLT
FGQGTRLEIKRT

AIL10 IgG VH insert: VH4-4: JH6 (SEQ ID NO: 19)

TGVHSQVLQESGPGLVKPSETLSLTCSVSGGCAVSNYYVWSWIRQSAGKGLEWLGRIYINGTTYNPSLSRVSMVDTSKGQFSLRLTSVTAADTAIYYC
ARWGALLGDYYYGLDYNWGQGTTVTVSSAS

AIL10 IgK VK insert: VK2-28: JK5 (SEQ ID NO: 20)

TGVHGDIVMTQSPSLPVTTPGEPASISCRSTQSLHHSNEYIVLDWYVQKPGQSPQLLIFLASNRASGVDPDRFSGSASGTDFTLKISRVEAEDVGVVYCMQA
LEAPWTFGQGTREIKRT

FIG. 11 (cont.)

AIL11 IgG VH insert: VH4-39: JH5 (SEQ ID NO: 21)

TGVHSQQLQESGPGLVKPSETLSLTCSVSGGSITSTSYWGWIRQSPGKGLEWIGSVYYSGNTEYNASLKSRVTISIDTSKYQFSLMLRSVTAADTAVYY
CARRHDWFEWEDPWGGGTLVTVSSAS

AIL11 IgK VK insert: VK3-20: JK2 (SEQ ID NO: 22)

TGVHSEIVLTQSPGTLSPGERVTLSCRASQSVSSSYLAWYQQKPGQAPRLLIYDSSSRATGIPDRFSGSGTDFTLTISRILDPEDFAVYYCQQYGSSPST
FGQGTKLEIKRT

AIL12 IgG VH insert: VH4-39: JH5 (SEQ ID NO: 23)

TGVHSQQLQESGPGLVKPSETLSLTCTVSGGSVTSSDYVWAWIRQPPGKGPFWIGSISNGNTYYSPSLKSRVSGDTSKKQFSLNLSSVTDADTAVYY
CTRHGHYYVSGGLGPWGGGTLVTVSSAS

AIL12 IgK VK insert: VK3-20: JK1 (SEQ ID NO: 24)

TGVHSEIVLTQSPGTLSPGERATLSCRASQSVGSYLAWYQQKPGQAPRLLIHGASSRATGTPDRFSGSGCTDFTLTISKLEPEDFALYYCQQYGPSP
WTFGQGTKVEIKRT

FIG. 11 (cont.)

AIL13 IgG.VH insert: VH4-59:JH4 (SEQ ID NO: 25)

TGVHSQVLQESGPGLVKPS~~ETLSLT~~CNVSGGSINNYVWSWIRQPPGKGLEWIGYIYNGNINYNP~~SL~~KSRVTISRDMSKNQFSLNLRSVTAADTAVYYC
GICYSAAAGTVDYWGQGT~~LV~~TVSSAS

AIL13 IgK.VK insert: VK2-30:JK5 (SEQ ID NO: 26)

TGVHGDVVM~~TQSP~~LSL~~PVT~~LGQPASISCRSSQSLVHRNGNTYLNWFQQRPGQSPRRLIYTVSKRGSV~~PD~~RFSGSGCTDFTLKISRVEAEDVGDYYCMQ
GTHWPWTSGQGT~~RL~~EIKRT

AIL14 IgG.VH insert: VH4-61:JH6 (SEQ ID NO: 27)

TGVHSQVLQESGPGLVKPSQ~~TL~~SLTCTVSGGSLSSVNYWNWIRQPA~~CK~~GLEWMGRIYASGYTTYNPSEQSRVTISLDPSKNQISLKVTS~~LT~~AA~~DT~~AIY
YCARHDLGHCSTSCYLSW~~FDA~~WGQGT~~TV~~TVSSAS

AIL14 IgK.VK insert: VK4-1:JK2 (SEQ ID NO: 28)

TGVHSDIVMTQSPDSLAVSLGERATINCRSSQTIEFSPNNNNHLAWYQKPCQPPRLIYWASTRESGV~~PD~~RFSGSGCTDFTLTISGLQAEDVAVYYCQ
QYYS~~LP~~YTFGQGT~~KL~~EIKRT

FIG. 11 (cont.)

AIL15 IgG VH insert: VH4-39: JH5 (SEQ ID NO: 29)

TGVHSQQLQESGPGLVKPSETLSLTCTVSGGSITSRNNYWGWIQSPGKGLEWIGSLYYTGSDYYNP~~SLKSRVTISVDTSKNQFSLRLSSVT~~AADTAVYY
CVRVNVDDEWSGLGGAWFDEPWGGT~~LVTVSSAS~~

AIL15 IgK VK insert: VK2D-29: JK2 (SEQ ID NO: 30)

TGVHGDIVMTQTPLSLSVTPGQPASISCKSSQSLDSDGKTHLYWYLOKPGQSPQSLIYEYSKR~~ESGVPDRFTGCGSGTDFTLKISRVEAEDVGLYYCMQ~~S
AQLPYTTFGQGT~~KL~~EIKRT

AIL16 IgG VH insert: VH4-39: JH5 (SEQ ID NO: 31)

TGVHSQQLQESGPGLVKPSETLSLTCTVSGGSISSPYWGWIRQPPGKGLEWIGSLYYSGHTYYNP~~SLKSRVTISVDTSKNQFSLRLTSVT~~AADTSVYYC
AKOTDDYGDYASRGWFDEPWGGT~~LVTVSSAS~~

AIL16 IgK VK insert: VK1-33: JK5 (SEQ ID NO: 32)

TGVHSDIQMTQSPSSLSASVGD~~RVFTTCQASHDISNVLNWYQQKPGKVPPELLIYDASNLKIGVPSRFSGSGT~~DFTFTISSLPEDIA~~TYTCQQYDNLPI~~
TFGQGT~~RL~~EIKRT

FIG. 11 (cont.)

AIL18 IgG VH insert: VH4-31: JH5 (SEQ ID NO: 33)

TGVHSQVLQESGPGLVKPSQTLSTCTVSGDSISSDKYYWTWIRQLPGKGLEWIGYISYSGTTVYNPSLKSRVSIISVDTSGNQFSLRLSSVTAADTARYY
 CARVYDVL~~SAYYNNMGSWVDPWGQGT~~LVTVSSAS

AIL18 IgK VK insert: VK1-39: JK1 (SEQ ID NO: 34)

TGVHSDIQMTQSPSSLPASVGD~~RVTTTCRASQSISSYL~~NWYQQKPGRAPKLLIYAASSLQSGVSSRFSGSGTDFTLTISSLPEDSATYYCQOQSYSTPWT
 FGQGTKVEIKRT

AIL19 IgG VH insert: VH4-34: JH3 (SEQ ID NO: 35)

TGVHSQVLQQWCGALLKPPETLSLTCAVFGGSLSGYYWSWIRQPPGKGP~~EWIAEINHSGDANVYNPSLKSRVTISVD~~TSKNQFSLKMSSVTVADTALYY
 CATQGSRLITFAFDVWGQGTMTVTVSSAS

AIL19 IgK VK insert: VK3-20: JK2 (SEQ ID NO: 36)

TGVHSEIVLTQSPGTL~~SLSPGERVTLSCRTSQSVSSDSLAWYQ~~KPGQTPRLIIYHTSTRAAGIPDRFSGTSGTDFTLTIARLEPEDFAVYVCQHYGRSSL
 FTFGQGTKLEIKRT

FIG. 11 (cont.)

AIL20 IgG VH insert: VH4-39: JH4 (SEQ ID NO: 37)

TGVHSQQLQESGPGLVKPSETLSLTCTVSGSGISGSSEYWGWRQPPGRGLEWIGTIYYRGITTYTPELSKSRVTISVDTSKNQFSLRLNSVTAADTAIYY
CASLPHYDEFWSGSVFFDYWGQGTLLVTVSSAS

AIL20 IgK VK insert: VK1-27: JK4 (SEQ ID NO: 38)

TGVHSDIQMTQSPSSLSASVGDRTTITCRASQGIANYLAWYQQKPGNIPKLLIYAASLTQSGVPSRFSGSGTDFALTISCLQPEDVAITYYCQKYNAPLI
FGGGTKVEIKRT

WR01 IgG VH insert: VH4-59: JH6 (SEQ ID NO: 39)

TGVHSQVLQESGPGLVKPSETLSLTCTVSGGSIDTYVWTWIRQPPGKGLEWIGIYISIGSPKYKPSLKSRVVMMSVDTSTNEFALRLSSVTAADTAVYYC
ARSSGEYVEHLEKVGQGTITVTVSSAS

WR01 IgK VK insert: VK3-20: JK2 (SEQ ID NO: 40)

TGVHSEIVLTQSPATLSLSPGERATLSCRASQIVSSSYLDWFQKPGQAPRLIYGASSRATGIPDRFSGSGCTDFTLTISRLEPEDFAVYYCQQQFATSPY
TFGQGTKLEIKRT

FIG. 11 (cont.)

WR02 IgG VH insert: VH4-30: JH6 (SEQ ID NO: 41)

TGVHSQVLQESGPGLVKPSQTLTLTCTVSGGSISSGDYHWSWIRQPGKGLEWIGNINYNGGAYHNPSLTNRVIMSVDTSKNHFSCLKLTSVTAADTAV
YYCARESQWLRYCAFGMDVWGQGTITVTVSSAS

WR02 IgK VK insert: VK3-20: JK2 (SEQ ID NO: 42)

TGVHSEIVLTQSPGTLSPGERATLSCRASQSVSSSHLVVYQQKAGQAPRLVIYGANRRASGTPDRFSGSGGTDFTLTISRLEPEDFAVYVCOQYGSSPY
TFGQGTKLEIKRT

WR03 IgG VH insert: VH4-4: JH3 (SEQ ID NO: 43)

TGVHSQVLQESGPGLVKPSGTLTLTCAVSGGSISSSYWWSWVRQPGKGLEWVGEIVHSGGANYSPLKSRVTISVDKSKNQFSLNLISVTAADTAVYF
CARSRMLYGADGGGAFDIWGQGTMTVTVSSAS

WR03 IgK VK insert: VK3-20: JK1 (SEQ ID NO: 44)

TGVHSEIVLTQSPGTLSPGERATLSCGASQSVSSSYLAWYQQKPGAPRLIYDASSRAIGIPDRFSGSGGTDFTLTISRLEPEDFAVYVCOQYGSSPQT
FGQGTKVEIKRT

FIG. 11 (cont.)

WR04 IgG VH insert: VH-39: JH4 (SEQ ID NO: 45)

TGVHSQQLQSGPGLVKPSETLSLTCTVSGGSISSGGSYWGWIHQAPGKGLEWIGSMYYSGSTFYNPVKSRVTISVDRSKEQFSLNLNAVTAADTAVY
YCVRRHRRSEPSDSWGQGTLLTVSSAS

WR04 IgK VK insert: VK3-15: JK2 (SEQ ID NO: 46)

TGVHSEIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGTSTRAIGIPARFSGSGSGTEFTLTISSLQSEDSAVYFCQQYNNWPL
YTFGGGTKVEIKRT

WR05 IgG VH insert: VH-61: JH1 (SEQ ID NO: 47)

TGVHSQVLQSGPGLVKPSETLSLTCSVSGGSVSSNGHFWISWIRLPPGKGLEWIGYVYNTGTSGYSPSLKSRVTISVDTSKNQFSLILRSVTAADTAIYY
CARGLTGNYPYSHWGQGTLLTVSSAS

WR05 IgK VK insert: VK1-5: JK4 (SEQ ID NO: 48)

TGVHSDIQMTQSPSSVSASVGDRTVITTCRASRGVSTWLAWYQQKPGCEAPKLLIYASSRLIEGGVPAHFSGSGSGTDFTLTISLQPEDFATYYCQQGNSEFL
ITFGGGTKVEIKRT

FIG. 11 (cont.)

WR06 IgG VH insert: VH4-39: JH3 (SEQ ID NO: 49)

TGVHSQLQLQESGPGLVKPSSETLSLTCTVSGGSVSSAYWWAWIRQPPGGLEWIGHIYYEGNKYYKSSLESRVTISLDASQNFSLKLTSTVTAADTALY
YCARVDITALAEFEWGGQTMVTVSSAS

WR06 IgK VK insert: VK1-5: JK3 (SEQ ID NO: 50)

TGVHSDIQMTQSPSSLSASVGDRVTITCRASQYVSSSLNWYQQKPGKAPTLIYLASNLRSGVPSRFGSGESGTDFTLTINSIQPEDVATYFCQQQSYSLPRI
FGPGTKVDIKRT

WR07 IgG VH insert: VH4-39: JH4 (SEQ ID NO: 51)

TGVHSQLQLQESGPGLVKPSSETLSLTCSVSGGSVSTTTYWGWIROSPGKGLEWIGSIYHSGKTYYNPSLKSRTISVDTSKNQFSLNLSSVTAADTAVVY
CARENSHHYDSSGYLGGEDYWGQGTLLTVSSAS

WR07 IgK VK insert: VK3-20: JK1 (SEQ ID NO: 52)

TGVHSEIVLTQSPGTLSSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLIYGASSRAIGIPDRFGSGSGTDFTLTISRLEPEDFAVYFCQHYGSPSTF
GGGTRKVEIKRT

FIG. 11 (cont.)

WR08 IgG VH insert: VH4-31:JH4 (SEQ ID NO: 53)
TGVHSQVQLQESGPGLVKPSQTLSTCTVSGGSISGGYYSWIRQHPGKGLDCIGYHYTGTTYNPSLKSLRTISVDTSKNQFSLNLTSTVAADTAVYYC
AREEYTTSSVDYWGRTLVTVSSAS

WR08 IgK VK insert: VK2-30:JK5 (SEQ ID NO: 54)
TGVHGDVVMQTQSPVLTGQPASISCRSSESLVSDGNTYLNWFHQRPQSPRLIYKVSNRDGVDPDRFSGSGGTDFTLKISRVEAEDVGIYYCMQ
ATHWLRITFGQGRLEIKRT

WR09 IgG VH insert: VH4-31:JH4 (SEQ ID NO: 55)
TGVHSQVQLQESGPGLVKPSQTLSTCTVSGGSISGGYYSWIRQHPGKGLGWIGYHYTGTTYNPSLKSLRVTSVDTSKNQFSLNLTSTVAADTAVYYC
AREEYTTSSVDYWGRTLVTVSSAS

WR09 IgK VK insert: VK3-20:JK2 (SEQ ID NO: 56)
TGVHSEIVLTQSPGTLSLSPGERATLSCRASQVSSGFLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFTLTISRLEPEDFAVYYCQQYESSPSR
YNFGQGTKLEIKRT

FIG. 11 (cont.)

WR10 IgG.VH insert: VH4-4: JH6 (SEQ ID NO: 57)

TGVHSQVQLQESGPGLVKPSGTLSTLTCAVSGGSISNNKWWNNWVRQSPGKGLEWICEIYHSGGTNYPNPSLKSRVTISVDKSKNLFSLKLSVTAADTAVY
YCASATTMVRGLSLYYGGLDVWGPGTTTVSSAS

WR10 IgK.VK insert: VK3-20: JK5 (SEQ ID NO: 58)

TGVHSEIVLTQSPGTLSPGERAALSCRASQSLIGSEFLAWYQQKPGQAPRLLIVHTSNRASGIPDRFSGGCGTDFTLTISRLEPEDFAVYYCQQYDSSPIT
FGQGTRLEIKRT

WR11 IgG.VH insert: VH4-4: JH6 (SEQ ID NO: 59)

TGVHSQVQLQESGPGLVKPSGTLSTLTCAVSGGSISNNKWWNNWVRQSPGKGLEWICEIYHSGGTNYPNPSLKSRVTISVDKSKNLFSLKLSVTAADTAVY
YCASATTMVRGLSLYYGGLDVWGQGTITVSSAS

WR11 IgK.VK insert: VK3-11: JK4 (SEQ ID NO: 60)

TGVHSEIVLTQSPGTLSPGERATLSCRASQSVSSEFLAWYQQKPGQAPRLLIYDASNRAIGIPARFSGSGGTDFLTISLLEPEDFAVYYCQQRTNWPSP
LTFGGGTKVEIKRT

FIG. 11 (cont.)

WR12 IgG VH insert: VH4-30: JH4 (SEQ ID NO: 61)

TGVHSQVQLQESGPGLVKPSQTLSTCTVSGDSVSSNDHYW~~SWIRQPPCQGLEWIGYISHGGTTYNP~~SLKSRVTMSIDTSTNQFSLRVTSVRAADMAY
YFCARAPAPITTEGMMVTPVPYEH~~SWGG~~TLTVTVSSAS

WR12 IgK VK insert: VK2-28: JK4 (SEQ ID NO: 62)

TGVHGDIVMTQSPLSLVSPGEPASISCRSSQSLHSNGYN~~YLSWYLQKPGQSPQLLIFSSSIRASGV~~PD~~RFSGSGGT~~DFTLTINRVEAEDVGVYYCMQAL
QTPLTFGGGKLEIKRT

WR13 IgG VH insert: VH4-30: JH4 (SEQ ID NO: 63)

TGVHSQVQLQESGPGLVKPSQTLSTCTVSGDSVSSNDHYW~~SWIRQPPCQGLEWIGYISHGGTTYNP~~SLKSRVTMSIDTSTNQFSLRVTSVRAADMAY
YFCARAPAPITTEGMMVTPVPYEH~~SWGG~~TLTVTVSSAS

WR13 IgK VK insert: VK2-28: JK2 (SEQ ID NO: 64)

TGVHGDIVMTQSPLSLVSPGESASISCRSSQSLHSNGYN~~YLSWYLQKPGQSPQLLIFSSSIRASGV~~PD~~RFSGSGGT~~DFTLTINRVEAEDVGVYYCMQAL
QTPLTFGGGKLEIKRT

FIG. 11 (cont.)

AJL01 IgG VH insert: VH4-34; JH3 (SEQ ID NO: 65)

ACCGGTGTACATTCCAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCCTCACCTGGCGTGTCTATGGTGGGTCTTCAAT
 GAATTCTACTGGAGCTGGATCCGTACGCCCGCACGGAAGGGCTGGAGTGGATTGGAGAAATCAGTCATAGCGGAAGAGCCAACTACAAACCCGTCCTCAAGA
 GTCGCGTCACCTGTCTGTAGACAGGTCCAAGAACCAAGTTCTCCCTGAACCTGAGCCCTGTGGCGCGCGGACACAGCTGTCTATTACTGTGCGCGACGGGAGA
 TAGTCGTAACCTGTTCCGGGGCGTCGTCTTTGATACTCTGGGGCCAAAGGGACAATGGTCAACCGTCTCTTCAGCGTCGAC

AJL01 IgK VK insert: VK3-20; JK5 (SEQ ID NO: 66)

ACCGGTGTACATTACAGAAATTGTGTGACGCAGTCTCCAGGCACCTGTCTTTGTCTCCAGGGGAAGAGCCGCCCTCTCCTGCAGGGCCAGTCAGAGTCTTATC
 GGCAGCTTCTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGGCTCTCATCTATCATACATCCAACAGGGCCTCTGGGCATCCCAGACAGGTTCAAGTGGC
 GGTGGGTTTGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTGCAGTTTATTACTGTCAACAGTATGATAGCTCACCGATCACCTTCGGCC
 AAGGGACACGACTGGAGATTAAACGTACG

AJL02 IgG VH insert: VH4-31; JH4 (SEQ ID NO: 67)

ACCGGTGTACATTCCAGGTGCAGCTGCAGGAGTGGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTCACCTGTACTGTCTCTGGTGGGTCCATCAGC
 AGTGGTGGTCACTACTGGAGCTGGATCCGCCAGTCCCGAGGGAAGGGCTGGAGTGGATTGGGAACGTCTATTATAGTGGGAAGCACTACTACACCCCGTCCCT
 CGACAGCCGACTTACCATATCATTAGACACGTTAAGAACCAAGTTCTCCCTGAGGCTGAGTAAATGTGACTGTGCGGGACACGGCCGTCTATTACTGTGCGAGAGG
 TAGAAATTGGAGGGCGAAATTCGACCCCTGGGGCCAAAGGAACCTGGTCAACCGTCTCCTCAGCGTCGAC

AJL02 IgK VK insert: VK1-39; JK2 (SEQ ID NO: 68)

ACCGGTGTACATTCTGACATCCAGATGACCCAGTCTCCGTCTCCCTGTCTGCGTCTTTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGCA
 GCTCTGTAAATTGGTTTCAGCAGAAACCGGGAAGCCCTGAACCTCTGATCTATGCTGCACTCCACTTTGCAAAGTGGGTCCCATCAAGATTCAAGTGGCAGTG
 GATCTGGGACAGATTTCACTCTCACCGTCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTGACGACAGATTACAGTCCCCCTCGAACTTTTGGCCAGGG
 GACCAAGCTGGAAATCAAACGTACG

FIG. 12

AJL03 IgG VH insert: VH4-39; JH1 (SEQ ID NO: 69)

ACCGGTGTACATTCCAGCTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACGTGCACCTGTCTGGTGCCTCCATCAGC
AGTAGTCGTTCTACTGGGGCTGGATCCGCCAGCCCCAGGGAAAGGGCTGGAGTGGATGTATCAAAAGTGGGAGCACCTTACTACAGTCCGTCCT
CAAGAGTCGAGTCACCATATCCATGGACACGTCCAAGAACCAAGTTCTCCCTAAACCTGACGTCTGTGACCGCGGACACGGCTGTGATTTTCTGTGCGAGACA
TTCGAACCCCGGAACGGCGAACAAATTGCGTTTGGGGAGTTTCGCCCTGGGGCCAGGGAACCTTGGTCACCGTCTCCTCAGCGTCGAC

AJL03 IgK VK insert: VK1-33; JK2 (SEQ ID NO: 70)

ACCGGTGTACATTCTGACATCCAGATGACCCAGTCTCCATCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCAGGCGAGTCAGGACATTAAACA
ACTATTTAAATTGGTTTCAGCAGCAACCCAGGGAAAGCCCCCTAAGCTGTGATCTACGATGCATCCAAATTGCAAAATGGGGGTCCCATCAAGGTTTCAGTGGAAAGTG
CATCTGGGACAGATTTTACTTTTACCATCAGCAGCCTGCAGCCTGAAGATAATTGSCACATATTACTGTCAACAGTATTAAATCTCCGTACACTTTTGGCCAGGGG
ACCAAGCTGGAGATCAACGTCG

AJL04 IgG VH insert: VH4-59; JH5 (SEQ ID NO: 71)

ACCGGTGTACATTCCCGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCACCTGTCTGGTGACTCCATCAGA
AGTAACTTCTGGACCTGGATCCGGCAGTCCCGGAGGAGGAGCTGGAGTGGATTGGGTATTTCTTACAGTGGGGGCATCAACTACAGCCCCCTCCCTCAAGAG
TCGGTCAACCATTTTCAGTGGACACATCCAAGAACCAGTTCTCCCTAAACTGACCTCTGTGACCGGGGAGCGGCCGTATATTACTGTGCGAGAGATCCCAA
CGGTGACTACGAAGTTAACTGGTGGGGCCAAGGAACCCCTGGTCACCGTCTCCTCAGCGTCGAC

AJL04 IgK VK insert: VK1-16; JK5 (SEQ ID NO: 72)

ACCGGTGTACATTCTGACATCCAGATGACCCAGTCTCCATCTTCACTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGTGGGCGAGTCAGGACATTATTA
TTTATTTAGCCTGGTTTCAGCAGAGACCCAGGGAAAGCCCCCTAGGTCCCTGATCTATTCTGCATCCACTTTCAGAGTGGGGTCCCATCAAAATTCAGCGGCAGTG
GATCTGGGACATATTTCACTCTCACCATCAGCAGCTGCAGCCTGAAGATTCTGCAACTTATTACTGCCAACAAATAAAAAGTTATCCCATCACCTTCGGGCCAAGG
GACACGACTGGAGATTAAACGTACG

FIG. 12 (cont.)

AJL05 IgG VH insert: VH4-39; JH5 (SEQ ID NO: 73)

ACCGGTGTACATCCAGCTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGAGACCCTGTCCCTCACCTGCACCTGTCTGGTGGCTCCATCAGA
AATAGTAATTACTACTGGGACTGGATCCGCCAGCCCCAGGAAAGGGCTGGAGTGGATTGGAGTGGCTATTATAGTGGGAGCGCTACTACCAATTCGTCCCT
CAAGAGTCGAGTCAGCATATCCGTAGACAGTCCAGAACCAAGTTTCCCTTAATCTGACCTCTGTGACCGCCGACAGACCGGCTTTTATTACTGTGCGAGACGT
TCCTATTATTATGCTTCGGGAGCCACGACTACTGGGGCCAGGGAACCCCTGGTCAACCCTCTCCCTCAGCGTCCGAC

AJL05 IgK VK insert: VK1-16; JK5 (SEQ ID NO: 74)

ACCGGTGTACATCTGACATCCAGATGACCCAGTCTCCATCCTCACTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGTGCGGCGAGTCAGGACATTAGCA
GTTACTTAGCCTGGTTTCAGCAGAAACCAGGGAAAGCCCCTAAGTCCCTGATCTATGGTGTCTCCAGTTGCAGAGTGGGTCCCATCAAAATTCAGCGGCAGTG
GATCTGGGACAGATTCACCTCAACCATCAGCGGCCTGCAGCCTGAAGATTTTGCAACTTATCACTGCCAACAAATACAGGAGTTTCCCTATCACTTTCGGCCCAAGG
GACACGACTGGAGATTAACGTACG

AJL06 IgG VH insert: VH4-30; JH4 (SEQ ID NO: 75)

ACCGGTGTACATCCAGCTGCAGCTGCAGGAGTCCGGCTCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTCACCTGGCTGTCTGTGGTGGCTCCATCAAC
ACTGGCAATTACTTCTGGAGCTGGATCCGGCAGCCACCAGGGAAGGGCTGGAGTGGATTGGGTACATGTTTCGAAGTACGAGTACCTACTACAACCCGTCCTCT
CAAGGTCGAGTCACCATTTTCAGGAGGCACGTCCTGAGCCAGTTCTCCCTGAGACTGGACTCTGTGACTGCCGCGGACACGGGCCATCTACTACTGTGCGAGAG
GACGTTATTATTGGGTGTTAATTGCCATCCCTTTGACTCTCGGGGCCAAGGAACCCCTGGTCAACGCTCTCCTCAGCGTCCGAC

AJL06 IgK VK insert: VK1-33; JK2 (SEQ ID NO: 76)

ACCGGTGTACATCTGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCATCATCACTTGCCAGGCGGAGCCAGACATTAGCA
TCTATTTAAATTGGTATCAGGTGAACCCAGGGAAGCCCCCTAACTCCTGATCTACGATGCTCCAAATTTGCAAGCAGGGGTCCCATCAAGGTTTCAGTGGGAAGTG
GATCAGGGACAGATTTTAGTTTCACCATCAGCAGCCTGCAGCCTGAAGATGTGGCAGCAATATTACTGTCAATCAGTATGATTTCTTACCGAGTTTTCGCGCAGGGGA
CCAAGCTGGAAATCAAAACGTACG

FIG. 12 (cont.)

AJL07 IgG VH insert: VH4-59; JH4 (SEQ ID NO: 77)

ACCGGTGTACATTCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGAGCCCTGCCTCACCTGCAATGTCTCTGGTGGCTCCATCAAC
AACTATTACTGGAGTTGGATCCGGCAGCCCCAGGGAAGGACTGGAGTGGTTATATCTATTACAAIGGGAATATTAATTACAACCCCTCCCTCAAGAGT
CGAGTCACCATATCAAGAGACATGTCCAAGAACCAGTTCTCCCTGAACCTCGGTCTGTGACCGCTGCGGACACGGCGGTATTACTGTGGAATTGGATATAGT
GCGGTGGCAGCTGGTACAGTTGACTACTGGGGCCATGGCACCCCTGGTCACCGTCTCCTCAGCGTCGAC

AJL07 IgK VK insert: VK1-13; JK2 (SEQ ID NO: 78)

ACCGGTGTACATTACGCCATCCAGTTGACCCAGTCTCCATCTCCCTGTCTGCATCTGTGGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGCA
GTGGTTTAGCCTGGTATCAGCAGGAACCAAGGAAAGCTCTAACTCCTGATCTATGATGCTCCACTTGGAAAGTGGGTCCCATCAAGATTCAAGTGGCAGTG
GATCTGCAATAGATTTCACTCTCACCATCAGCAGTCTGCAGCTGAAGATTTTGCAACTTATTACTGTCAACAGTTTAATACTTTCCCGTACACTTTTGGCCAGGGG
ACCAAGCTGGAGATCAAAACGTACG

AJL08 IgG VH insert: VH4b; JH4 (SEQ ID NO: 79)

ACCGGTGTACATTCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGAGCCCTGTCCCTCACCTGGCTGTCTCTGGTTCTCCATCACC
AGTGGTTACTACTGGGGCTGGATCCGGCAGCCCCCGGGAAGGGCTGGAGTGGATAGGGAGTATCTATCATACTGGGACTACCTACTACAACCCGTCCCTCAA
GAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAACCTAAACTCTGTGACCCCGCAGACACGGCCTTTTATTACTGTGCGAGAGATCCT
CTATTCGGGGGGAACCTACTCTCCGTTTTTGACAACTGGGGCCAGGGCACCCCTGGTCACCGTCTCCTCAGCGTCGAC

AJL08 IgK VK insert: VK1-5; JK2 (SEQ ID NO: 80)

ACCGGTGTACATTCTGACATCCAGATGACCCAGTCTCCTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCCAGTCAGAGTATTAGTA
CCTGGTTGGCCTGGTATCAGCAGAAACCAAGGAAAGCCCTAGGCTCCTGATCTATAAGGCATCTAGTTTAGAAAGTGGGTCCCATCACGTTTCAGCGGCAGT
GGATCTGGGACAGAAATCACTCTCACCATCAGCGGCCCTGCAGCCCTGATGACTTGCAAAATTATTCCTGCCAACAAATAATTTACCCGTTCACTTTTGGCCAGG
GGACCAAGCTGGAGATCAAAACGTACG

FIG. 12 (cont.)

AJL09 IgG VH insert: VH4-59: JH5 (SEQ ID NO: 81)

ACCGGTGTACATCCAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCAGTGTCTCTGGTGGCTCCATCAGT
AGTTACTACTGGGGCTGGATCCGGCAGTCCCCAGGGAAGGACTGGAGTGGCATCTATTTTCAGTGGGAGCACCAGCTACAACCCCTCCCTCAAGAG
TCGAGTCACCATATCAGTAGACACGGCCAAAGAACCATGATCTCCCTGAACCTGACCTCTGTGACCGCTGGGACACGGCCGTGTATTTTGTGCGAGAGTTTGGGG
CAGCAGCTGGTACGCTAACTGGTTCGACCCCTGGGGCCAGGGCACCCCTGGTACCGTCTCCTCAGCGTCGAC

AJL09 IgK VK insert: VK1-13: JK5 (SEQ ID NO: 82)

ACCGGTGTACATTCAGCCATCCAGTTGACCCAGTCTCCATCTCCCTGTCTGGTCTGGAGACAGATCACCATCATTGCCGGGCAAGTCAGGGCATTAGCA
GTGCCTTAGCCTGGTATCAGCACAAACCAGGGAGGCTCTAACTCCTGATCTTTGATGCTCCACTTTGGCAGCTGGGTCCCATCCAGGTTCAGCGGCAGTG
GATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAGCCTGAAGATTTTGTACTATTACTGTGACAGTATAATACTTACGTTCTCACTTTCGGCCAAAGGG
ACACGACTGGAGATTAAACGTACG

AJL10 IgG VH insert: VH4-4: JH6 (SEQ ID NO: 83)

ACCGGTGTACATCCAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCAGTGTCTCTGGTGGCGCGTCAG
TAATTACTACTGGAGTTGGATCCGGCAGTCCGCCGGAAGGAGCTGGAGTGGCTTGGCGGATCTATATCAATGGAACTATTACTACAACCCCTCCCTCAGGA
GCCGGGTCTCCATGTGACACGTCCAAGGGCCAGTTCCTCCTGAGTTGACCTCTGTGACCGCCGACACGGCCATATAATTATGTGCGAGATGGGGTG
CCCTATTGGGCGACTACTATTACGGTTTGGACGTCTGGGGCCAAAGGACCACGGTACCGTCTCCTCAGCGTCGAC

AJL10 IgK VK insert: VK2-28: JK5 (SEQ ID NO: 84)

ACCGGTGTACATGGGGATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATCTCTGCAGGTCTACTCAGAGCCTCCTAC
ACAGTAATGAATACATTTATTGTGATTGGTACGTGCAGAGGCAAGCCAGTCTCCACAACCTCTGATCTTTTGGCTTCTAATCGGGCCTCCGGGGTCCCTGACAG
GTTCAAGTGGCAGTGCATCAGGCACAGATTTACACTGAAATCAGCAGAGTGGAGGTGAGGATGTGGGGTTTATTACTGCAIGCAAGCTCTAGAAGCTCCGT
GGACGTTGGCCAAAGGACACGACTGGAGATTAAACGTACG

FIG. 12 (cont.)

AJL11 IgG VH insert: VH4-39; JH5 (SEQ ID NO: 85)

ACCGGTGTACATTCCAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCCTGTCCTCACCTGCTCTGTCTCTGTGGTCCATCACC
AGTACTTCTTATTACTGGGGGTGGATCCGCCAGTCCCGAGGGAAGGACTGGAGTGGATTGGCAGTGTCTATTACAGTGGGAACACCTTCTACAACGGTCCCTC
AAGATCGAGTCACCATATCCATAGACACGTCCAAGTACCACTTCTCCCTGATGCTGAGGTCTGTGACCCGCCGACACACGGCTGTGTATTACTGTGCGAGACGA
CATGATTGGTTCTGTTCGACCCCTGGGGCCAAGGAACCCCTGGTCAACCGTCTCCTCAGCGTCGAC

AJL11 IgK VK insert: VK3-20; JK2 (SEQ ID NO: 86)

ACCGGTGTACATTACAGAAATTGTGTTGACGCAGTCTCCAGGCACCTGTCTTGTCTCCAGGGGAAGAGTCAACCTCTCCTGCAGGGCCAGTCAGAGTGTAGC
AGCAGTACTTAGCCTGGTACCAAGCAAAACCTGGCCAGGCTCCAGGCTCCTCATCTATGATTCATCCAGCAGGGCCACTGGCATCCAGACAGGTTCAGTGGC
AGTGGGTCTGGACAGACTTCACTCTCACCATCAGCAGACTGGACCCCTGAAGATTTTGCCGTGTATTACTGTCAAGCATGTGGTAGCTCACCAGCACTTTTGGCC
AGGGGACCAAGCTGGAGATCAAAACGTACG

AJL12 IgG VH insert: VH4-39; JH5 (SEQ ID NO: 87)

ACCGGTGTACATTCCAGCTGCAGCTGCAGGAGTCGGGCCCCAGGACTGGTGAAGCCTTCGGAGACCCCTGTCCTCACCTGCACTGTCTCTGGTGGCTCCGTCAAC
AGTAGTGATTACTAGTGGCCCTGGATCCGCCAGCCCCAGGGGAAGGGCCGGAGTGGATCTCTAATAGTGGGAACACAGTATTACAGTCCGTCCCT
CAAGAGTCGAGTCTCCATATCGGGAGACACGTCCAAAGAGCAGTTCTCCCTGAACCTGAGCTCTGTGACCGACGACACGGCTGTGTATTACTGTACGAGAC
ACGGTCACTACGTTTCAGGGGGCTGGGCCCTGGGGCCAAAGAACCTGGTCAACCTGTCCTCAGCGTCGAC

AJL12 IgK VK insert: VK3-20; JK1 (SEQ ID NO: 88)

ACCGGTGTACATTACAGAAATTGTGTTGACGCAGTCTCCAGGCACCTGTCTTGTCTCCAGGGGAAGAGCAACCTCTCCTGCAGGGCCAGTCAGAGTGTGGC
AGTTACTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGGCTCCTCATCCACGGTGCATCCAGCAGGGCCACTGGCACCCAGACAGGTTCAGTGGC
AGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAAACTGGAGCCTGAAGATTTGCACTGTATTACTGTCAAGCAGTATGGTCCCTCACCTTGGACGTTTCGGCC
AAGGGACCAAGGTGGAAATCAAAACGTACG

FIG. 12 (cont.)

AJL13 IgG VH insert: VH4-59; JH4 (SEQ ID NO: 89)

ACCGGTGTACATTCCAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGAGCCCTGTCCTCACCTGCAATGTCTCTGGTGGCTCCATCAAC
AACATATTACTGGAGTTGGATCCGGCAGCCCCCAGGGAAGGACTGGAGTGGATTGGTTATAICTATTACAATGGGAATATTAAATTACAACCCCTCCCTCAAGAGT
CGAGTCACCATATCAAGAGACATGTCCAAGAACCAAGTTCTCCCTGAACCTGCGGTCTGTGACCGCTGGGACACGGCCGTGTATTACTGTGGAAATTGGATATAGT
GCGGTGGCAGCTGGTACAGTTGACTACTGGGGCCAGGGAACCCCTGGTCACCGTCTCTCTCAGCGTCCGAC

AJL13 IgK VK insert: VK2-30; JK5 (SEQ ID NO: 90)

ACCGGTGTACATGGGGATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCGTACCCCTTGGACAACCGGCCCTCCATCTCTGCAGGTCTAGTCAAAGCCTCGTAC
ACAGGAATGGAAACACCTACTTGAATTGGTTTCAGCAGAGGCCAATGCCAAGGCGCTAAATTTATACAGTTTCTAAGCGGGGCTCTGGGGTCCCAGAC
AGATTCAGCGGCAGTGGTTCAGGCACCTGATTTTCACACTGAAATCAGCAGGGTGGAGGCTGAAGATGTTGGGGAATACTGTCATGCAAGGTACACACTGGC
CTTGGACGTCCGGCCAAAGGGACACGACTGGAGATTAAACGTACG

AJL14 IgG VH insert: VH4-61; JH6 (SEQ ID NO: 91)

ACCGGTGTACATTCCAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCACAGACCCCTGTCCTCACCTGCACTGTCTCTGGTGGCTCCCTCAGT
AGTGTAAATTACTACTGGAACCTGGATCCGGCAGCCCGCGGGAAGGACTGGAGTGGATGGGGGTATCTATGCCAGCGGGTACACCACCTACAACCCGTCCTT
CCAGAGTCGAGTCACCATATCACTGGACCCGTCCAAGAACCAGATCTCCCTGAAGGTGACTTCTCTGACCGCGCAGACACGGCCAICTATTACTGTGCGAGACA
CGACCTTGGGCATTGTAGTAGTACGAGCTGTACCTCAGTTGGTTGACGCTGGGGCCAAAGGACCAAGGTCAACCGTCTCCTCAGCGTCCGAC

AJL14 IgK VK insert: VK4-1; JK2 (SEQ ID NO: 92)

ACCGGTGTACATTCCGGACATCGTGTGATGACCCAGTCTCCGAGCTCCCTGGCTGTGTCTCTGGGCGAGAGGGGCCACCATCAACTGCAGGTCCAGCCAGACTATTTTC
TTCAGCCCCCAACAATAACAACCACCTTAGCTTGGTACCAGCAAAAACAGGGCAGCCTCTAGGCTGCTCATTTACTGGGCATCTACCCGGGAATCCGGGGTCCCT
GACCGATTGAGTGGCAGCGGGTCTGGGACAGATTTTCACTCTCACCATCAGCGGCTGCAGGCTGAAGATGTGGCAGTTTATTACTGTCAGCAATATTATAGTCTT
CCCTACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAACGTACG

FIG. 12 (cont.)

AJL15 IgG VH insert: VH4-39; JH5 (SEQ ID NO: 93)

ACCGGTGTACATTCCAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCCTGTCCCTCACCTGCACTGTCTCTGGTGGCTCCATCACC
AGTAGGAATAACTACTGGGGCTGGATCCGCCAGTCCCCAGGGAAGGGCTGGAGTGGATTGGGAGTCTCTATTATCTGGGAGCGACTACTACAACCCGTCCTT
CAAGAGTCGAGTCACCATATCGGTAGACACATCGAAGAACCAATTCTCCCTGAGGCTGAGTTCGTGACCGCGGACACGGCCGTGTATTATTGTGTGACAGT
TAACGTAGACGACTTTTGGAGTGGTTAGGGGGGGCCCTGCTGACCCCTGGGGCCCAAGGAACCCCTGGTCAACCGTCTCCTCAGCGTCCGAC

AJL15 IgK VK insert: VK2D-29; JK2 (SEQ ID NO: 94)

ACCGGTGTACATGGGGATATTGTGATGACCCAGACTCCACTCTTTGTCCGTACCCCTGGACAGCCGGCCTCCATCTCCTGCAAACTAGTCAGAGCCTCCTGG
ATAGTGATGGAAGACCCATTGTGTACTGTACTGCTGACAGGCCAGGCTCCACAGTCCCTGATCTATGAAGTTTCTAAACGGTTCTCTGGAGTGCCAGATA
GGTTCACCTGGCAGCGGCTCAGGGACAGATTTACACTGAAAAATCAGCCGGGTGGAGGCTGAGGATGTTGGGCTTTATTACTGCATGCAAAAGTGACAGCTTCCG
TACACTTTTGGCCAGGGACCAAGCTGGAAATCAAACGTACG

AJL16 IgG VH insert: VH4-39; JH5 (SEQ ID NO: 95)

ACCGGTGTACATTCCAGCTGCAGCTGCAGGAGTCGGGCCCCAGGACTGGTGAAGCCTTCGGAGACCCCTGTCCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGC
AGTAGTCTTTACTACTGGGCTGGATCCGCCAGCCCCCAGGGAGGGCTGGAGTGGATTGGAGTATCTATTATAGTGGGCACACCTATTATAACCCCTCCCTC
AAGAGTCGAGTCACCATATCCGTTGACACGTCCAAGAACCAAGTTCTCCCTGCGGCTGACCTCTGTGACCCCGCAGACACGCTCTGTGTATTACTGTGCGAAACAG
ACCGATGACTACGGTGACTACGGTCCAGGGGCTGGTTGACCCCTGGGGCCCAAGGAACCCCTGGTCAACCGTCTCCTCAGCGTCCGAC

AJL16 IgK VK insert: VK1-33; JK5 (SEQ ID NO: 96)

ACCGGTGTACATTCTGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCTTCACCTGGCAGGGAGTCACGACATTAGCA
ACTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGTCCCTGAGCTCCTGATCTACGATGCATCCAATTTGAAAACAGGGGTCCCATCAAGGTTCAAGTGGAAAGTG
GATCTGGGACAGATTTACTTTACCATCAGCAGCTGCAGCCTGAAGATATTGCAACATATTACTGTCAACAGTATGATAATCTCCCTATCACCTTCGGCCCAAGG
GACACGACTGGAGATTAAACGTACG

FIG. 12 (cont.)

AJL18 IgG VH Insert: VH4-31; JH5 (SEQ ID NO: 97)

ACCGGTGTACATCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCTCACCTGCACTGTCTCTGGTGACTCCATCAGC
AGTGATAAATACTACTGGACCTGGATCCGCCAGCTCCAGGGAAGGCCCTGGAGTGAATTGGCTACATCTTACAGTGGGACCACCTACTACAATCCGTCCTC
AAGAGTCGAGTTCCATTTCAGTTGACACGTCGTGGGAACCAAGTTCTCCCTGAGGCTGAGCTGTGACTGCCGGGACACGGCCGCTATTACTGTGCGGAGTA
TATTACGATGTTTTGTCTCTATTATAATATGGGTCTCTGGGTAGACCCCTGGGCCAAGGAACCTGGTCACCGTCTCCTCAGCGTCGAC

AJL18 IgK VK Insert: VK1-39; JK1 (SEQ ID NO: 98)

ACCGGTGTACATCTGACATCCAGATGACCCAGTCTCCATCTCCCTGCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCA
GCTATTTAAATTGGTATCAGCAGAAACCAGGGAGAGCCCTAAGCTCCTGATCTATGCTGCATCCAGTTACAAAGTGGGTCTCATCAAGGTTCAAGGTCAGTGGCAGTG
GATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTCTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCGTGGACGTTCCGGCCAAGG
GACCAAGGTGGAGATCAAAACGTACG

AJL19 IgG VH Insert: VH4-34; JH3 (SEQ ID NO: 99)

ACCGGTGTACATCCAGGTGCAGTACAGCAGTGGGGCCAGGACTGTTGAAGCCTCCGGAGACCCTGTCCTCACCTGCGCTGTCTTGGTGGGTCCCTCAGT
GGTTACTACTGGAGTTGGATCCGCCAGCCCCCAGGGGAAGGGCGGAGTGGATTGCGGAATCAATCATAGTGGAGATGCCAACTACAACCCGTCCTCAAGA
GTCGAGTCACTATCTCAGTAGACACGTCCAAGAACCAGTTTCCCTGAAGATGAGTTCTGTGACCGTCGCAGACACGGCTTTATATTACTGTGCGGACTCAAGGCTC
TAGGTTGACTACATTCGCTTTTGATGTGTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAGCGTCGAC

AJL19 IgK VK Insert: VK3-20; JK2 (SEQ ID NO: 100)

ACCGGTGTACATTCAGAAATTGTGTGACGCAGTCTCCAGGGACCCTGTCTTGTCTCCAGGGGAAGAGTCACTCTCTGTCAGGACCAGTCAGAGTGTAGC
AGCGACTCTTAGCCTGTACCAAGCAGAGAAACCTGGCCAGACTCCAGGCTCCTCATTTATCATATCATCCACCAGGGCCGCTGGCATCCAGACAGGTTCAAGTGGC
ACTGGGTCTGGGACAGACTTCACCTCTCAACATCGCCAGACTGGAGCCTGAAGATTTGCAGTCTATTACTGTGACGACTATGGTGGGTATCCCTATTCACTTTG
GCCAGGGGACCAAGCTGGAAATCAAAACGTACG

FIG. 12 (cont.)

AJL20 IgG VH insert: VH4-39; JH4 (SEQ ID NO: 101)
 ACCGGTGTACATTCCAGCTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGAGCCCTGTCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGC
 GGTAGTCTTTCTACTGGGCTGGTCCGCCAGCCCCAGGGAGGGGCTGGAGTGGATGGCACTATCTATTATCGTGGGACCACCTATTACACCCCGTCTCTC
 AAGAGTCGAGTCACCATAATCCGTGGACACTCCAAGAACAGTTCTCCCTGAGGTGAACCTCTGTGACCGCCGACACAGGCTATATATTACTGTGCGAGCCTT
 CCCCACTACGATTTTGGAGTGGTCTGTCTTTCTTGACTACTGGGGCCAAGGAACCTGGTCACCCGTCTCTCAGCGTCGAC

AJL20 IgK VK insert: VK1-27; JK4 (SEQ ID NO: 102)
 ACCGGTGTACATTCTGACATCCAGATGACCCAGTCTCCATCTCCCTGTCTGCACTGTAGGAGACAGAGTACCATCACTTGCCGGGCGAGTCAGGGCATTGCCA
 ATTATTTAGCCTGTATCAGCAGAAACAGGGGAACATTCCTAAGCTCTGATCTATGCTGCATCCACTTTGCAATCAGGGGTCCCATCTCGGTTTCAGTGGCAGTGG
 ATCTGGAACAGATTTCCGCTCTCACCATCAGCTGCTACAGCTGAGGATGTTGCAACTTATTACTGTCAAAAGTATAACAGTGCCCCCTCTCACTTTCGGCGGAGGG
 ACCAAGGTGGAGATCAACGTACG

WR01 IgG VH insert: VH4-59; JH6 (SEQ ID NO: 103)
 ACCGGTGTACATTCCAGGTGTCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGAGCCCTGTCCCTCACGTGCACGTGTTTCTGGTGGCTCCATCGAT
 ACTTACTACTGGACCTGGATCCGGCAGCCCCCGGGGAAGGAGTGGAGTGGATATTTATTCCACTGGGAGCCCCAAGTACAAGCCCTCCCTCAAGAG
 TCGGGTCGTATGTAGTGGACACGTCCACGAACGAGTTCGCCCTGAGGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGATTACTGTGCGAGAAAGTTCGG
 GATTTACGTTGAACCTGGAAAGTGGGGCCAAGGACCGTCAACCGTCTCCTCAGCGTCGAC

WR01 IgK VK insert: VK3-20; JK2 (SEQ ID NO: 104)
 ACCGGTGTACATTCCAGAAATTGTGTGACGCACTCCAGCCACCCTGTCTTGTCTCCAGGGGAAGAGCCACTCTCTCTGCGAGGGCCAGTCAGACTGTGAGC
 AGCAGTACTTAGACTGGTTCAGCAGAAACCTGGCCAGGCTCCAGGCTCTCATCTATGTTGTCATCCAGCAGGGCCACTGGCATCCAGACAGGTTTCAGTGGC
 AGTGGGTCTGGGACAGACTTCACTTAACATATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGATTATTGTGTCAGCAGTTTGCTACTCTCACCGTACACTTTTGCC
 AGGGACCAAGCTGGAGATCAAAACGTACG

FIG. 12 (cont.)

WR02 IgG VH insert: VH4-30; JH6 (SEQ ID NO: 105)

ACCGGTGTACATTCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTCACCTGCACTGTCTCTGGTGGCTCCATCAGC
AGTGGTGATTACCACTGGAGTTGGATCCGCCAGCCCCAGGGAAGGCTGGAGTGATTGGGAACATCAATTAAATGGGGCGGTACCACAATCCGTCCT
CACGAATCGAGTTATCATGTCACTAGACACGTCCAAGAATCACTTCTCCCTGAAACTGACTCTGTGACTGCCGCAGACACGGCCGTGTTACTGTGCCAGAGA
GTCTCAGTGGCTGCGATACGGCGCTTCGGTATGGACGTCTGGGGCCAAGGGACACGGTCAACCGTCTCTCAGCGTCGAC

WR02 IgK VK insert: VK3-20; JK2 (SEQ ID NO: 106)

ACCGGTGTACATTACAGAAATTGTGTTGACGCAGTCTCCAGGACCCCTGCTTTGTCTCCAGGGGAAGAGCCACCTCTCTGCAAGGCCAGTCAGAGCGTTAGC
AGCAGCCACTTAGTCTGGTACCAGAGAAAGCTGGCCAGGCTCCAGGCTCGTCACTATGGTGCAACAGGAGGCTCTGGCACCCAGACAGGTTCAAGTGG
CAGTGGGTCTGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGACTTTGCAGTCTATTACTGTACGACGATATGGTAGCTGCGCGTACACTTTTGG
CCAGGGGACCAAGCTGGAGATCAAAACGTACG

WR03 IgG VH insert: VH4-4; JH3 (SEQ ID NO: 107)

ACCGGTGTACATTCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGGGACCCTGTCCTCACCTGGCGTGTCTCTGGTGGCTCCATCAGC
AGTAGTTACTGGTGGAGTTGGGTCCGCCAGCCCGGGAAGGGGTGGAGTGGGTGGAGAAATCTATCATAGTGGGGCGCCAACTACAGCCGTCCTCA
AGAGTCGAGTCACCATATCAGTGGACAAAGTCCAAGAACCACTTCTCCCTGAACCTGATCTCTGTGACCCCGCGGACACGGCCGTGATTTCTGTGCGAGATCAC
GGATGCTAGTGGGTGCTGATGGTGGAGGTGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAGCGTCGAC

WR03 IgK VK insert: VK3-20; JK1 (SEQ ID NO: 108)

ACCGGTGTACATTACAGAAATTGTGTTGACGCAGTCTCCAGGCACTCTCCAGGCACTGTCTTTGTCTCCAGGGGAAGAGCCACCTCTCTGCGGGGCCAGTCAGAGTGTAGC
AGCAGCTACTTAGCCTGGTACCAGAGAAACCTGGCTGGCGCCAGGCTCCTCATCTATGATGTCATCCAGCAGGGCCACTGGCATCCAGACAGGTTCAAGTGGC
AGTGGGTCTGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTCAGTGTATTACTGTACGAGTATGGTAGCTCACCTCAAAACGTTCCGGC
CAAGGGACCAAGGTGGAAATCAAAACGTACG

FIG. 12 (cont.)

WR04 IgG VH insert: VH4-39; JH4 (SEQ ID NO: 109)

ACCGGTGTACATTCCAGCTGCAGCTGCAGCAGTCGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCTCACCTGCACCTGTCTCTGGTGGCTCCATCAGC
AGTGGTGGCTCATACTGGGGCTGGATCCGCCAGGCCCCGGGAAGGCCCTGGAGTGGATTGGGAGTATGTATTATAGTGGGAGCACCTTCTACAACCCGTC
CAAGAGTCGAGTCACCATATCCGTAGACAGGTCCAAGGAGCAGTTCTCCCTCAATCTGAACGCTGTACCGCCGACACAGGCTGTGTATTACTGTGTGAGACA
TAGGAGATCGGAACCCAGCGACTCTCTGGGGCCAAGGAACCCCTGGTCAACCGTCTCCTCAGCGTCCGAC

WR04 IgK VK insert: VK3-15; JK2 (SEQ ID NO: 110)

ACCGGTGTACATTCAGAAATAGTGTGATGACGAGTCTCCAGCCACCCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCCTCTCTGCAGGGCCAGTCAGAGTGTAGC
AGCAACTTAGCCTGGTACCAGCAGAACTGGCCAGGCTCCAGGCTCCTCATCTATGGTATCATCCACAGGGCCACTGGCAATCCAGCCAGGTTCAAGTGGCAGT
GGGTCTGGGACAGAAATCACTCTCACCATCAGCAGCCTGCAGTCTGAAGATTCTGCAGTTTATTCTGTCAACAGTATAATAACTGGCCGCTGTACACTTTTGGCC
AGGGGACCAAGGTGGAGATCAAAACGTACG

WR05 IgG VH insert: VH4-61; JH1 (SEQ ID NO: 111)

ACCGGTGTACATTCCAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCTCTCACCTGCAGTGTCTCTGGTGGCTCCGTCAGC
AGCAATGGTCACTTCTGGAGCTGGATCCGGCTGCCCCAGGGGAAGGGAAGTGGCTATGTCTACAACTGGGACCTCCGGCTACAGCCCTCCCTC
AAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAAGTTCTCCCTGACACTGAGGTCTGTGACCGCTGCGGACACGGCCATTACTACTGCGCGAGAGGT
CTCACTGGGAACCTACCCGTCTCACTGGGGCCAAGGCACCCCTGGTCAACCGTCTCCTCAGCGTCCGAC

WR05 IgK VK insert: VK1-5; JK4 (SEQ ID NO: 112)

ACCGGTGTACATTCTGACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGTGGGGCGAGTCGGGGTGTAGT
ACCTGGTTAGCCTGGTATCAGCAGAAACCAAGGGGAAGCCCTAAGCTCCTGTATGTCTATCTCATCCGTTTAGAAGGTGGGTCCCAGCAAGTTCAAGCGGCAG
TGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACCTACTATTGTCAACAGGGTAACAGCTTCCCCCTCACTTTCGGGCGGA
GGGACCAAGGTGGAGATCAAAACGTACG

FIG. 12 (cont.)

WR06 IgG VH insert: VH4-39; JH3 (SEQ ID NO: 113)

ACCGGTGTACATCCAGCTGCAGCTGCAGGAGTCGGGCTCAGGACTGGTGAAGCCTTCGGAGAGCCCTGTCCCTCACCTGCACTGTCTCTGGTGGCTCCGTCAGT
AGTAGTGCTTACTGGTGGCTGGATCCGCCAGCCCCGGGGGAGGAGTGGAGTGGCCATATCTATTACTTTGGGAACAAATATTACAAGTCGTCCT
CGAGAGTCGAGTCACCATTTCACTAGACGCGTCCAGAACCAAGTTCTCCCTGAAGCTGACCTCCGTGACCGCCGAGACACGGCTCTGTATTACTGTGCGAGAGT
GGACACAGCTTGGCTTTGACTTCTGGGGCCCAAGGACAATGGTCACCGTCTCTCAGCGTCGAC

WR06 IgK VK insert: VK1-5; JK3 (SEQ ID NO: 114)

ACCGGTGTACATCTGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCAICTGTAGGAGACAGAGTCACCATCACTTGCCGGGGCAAGTCAGTATGTTAGTA
GTTCTTTGAATTGGTATCAGCAGAAACCAGGGAAAGCCCTACACTCTGATCTATCTTGGTCCAATTTGGGAAGTGGGGTCCCATCAAGGTTTCAGTGGCAGTG
AATCTGGACAGATTTCACTCTCACCATCAACAGTCTGCAGCCTGAAGATGTTGCAACTTACTTCTGTCAACAGAGTTATAGTCTCCCTCGCACCTTTCGGGCCCTGGG
ACCAAGTGGATATCAACGTCACG

WR07 IgG VH insert: VH4-39; JH4 (SEQ ID NO: 115)

ACCGGTGTACATCCAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCCTCGGAGAGCCCTGTCCCTCACCTGCTCTGTCTCTGGTGGCTCCGTCAGT
AGTACAACCTTACTACTGGGGTGGATCCGCCAGTCCCAGGGAAGGGCTGGAGTGGATTCGAGCATCTATCATAGTGGGAAAACCTACTACAACCCGTCCT
CAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAAGTTCTCCCTGAACCTGAGCTCTGTGACCCGCGGACACGGCCGTGTATTATTGTGCGAGAGA
GAATTCCTCATCATGATAGTAGTGGTTATTACTTAGGTGGATTGACTACTGGGGCCAGGGAACCTGGTCAACCGTCTCTCCTCAGCGTCGAC

WR07 IgK VK insert: VK3-20; JK1 (SEQ ID NO: 116)

ACCGGTGTACATTCAGAAATTGTGTGACGCACTCTCCAGGCACCTGTCTTGTCTCCAGGGGAAGAGCAACCTCTCCTGCAGGGCCAGTCAGAGTGTAGC
AGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGGCTCCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCAGACAGGTTTCAGTGGC
AGTGGGTCTGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTCGAGTATATTCTGCCAGCACTATGTTAGTCCCTCCACGTTTGGCCAG
GGGACCAAGGTGGAGATCAAAACGTACG

FIG. 12 (cont.)

WR08 IgG VH insert: VH4-31; JH4 (SEQ ID NO: 117)

ACCGGTGTACATTCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTACCTGCACTGTCTCTGGTGGCTCCATCAGC
AGTGGTTACTACTGGAGTTGGATCCGCCAGACCCAGGGAAGGCTGGATTGATTGGGTACATCCATTACACGGGGACCACCTACTACAACCCGTCCTGAA
GATCGACTTACCATATCAGTGGACACGTCTAAGAACAGTTCTCCCTGAACCTGACCTGTGACTGCCGGGACACGGCCGTCTATTATTGTGCGAGAGAAGA
ATATACAACCTCGTCAGTTGATTACTGGGGCCGGGGCACCCCTGGTCACCGTCTCCTCAGCGTCGAC

WR08 IgK VK insert: VK2-30; JK5 (SEQ ID NO: 118)

ACCGGTGTACATGGGGATTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTACCCTTGGACAGCCGCCCTCTATCTCTGCAGGCTAGTGAAGCCTCGTGT
CTGTTGATGGAAATACCTACTTGAATTGGTTTCACAGAGGCCAGGCAATCTCCAAGGCGCCTAAATTAAGGTTTCTAACCGGGACTCTGGGGTCCAGACA
GATTCAGCGGCAGTGGTCAAGCACTGATTTTCACACTGAAAATCAGCAGGGTGGAGCTGAGGATTTTGGGATTTATTACTGCATGCAAGCCACACACTGGCTT
CGGACGTCGGCCAAAGGACACGACTGGAGATTAAACGTACG

WR09 IgG VH insert: VH4-31; JH4 (SEQ ID NO: 119)

ACCGGTGTACATTCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTACCTGCACTGTCTCTGGTGGCTCCATCAGC
AGTGGTTACTACTGGAGTTGGATCCGCCAGCACCCAGGGGAAGGGCTGGAGTGGATTGGGTACATCCATTACACGGGGACCACCTACTACAACCCGTCCTGAA
GAGTCGAGTTACCATATCAGTGGACACGTCTAAGAACCAAGTTCTCCCTGAACCTGACCTCTGTGACTGCCGGGACACGGCCGTCTATTATTGTGCGAGAGAAGA
ATATACGACCTCGTCAGTTGATTACTGGGGCCGTGGCACCCCTGGTCACCGTCTCCTCAGCGTCGAC

WR09 IgK VK insert: VK3-20; JK2 (SEQ ID NO: 120)

ACCGGTGTACATTACAGAAATTGTGTGACGCAGTCTCCAGGCACCTGTCTTGTCTCCAGGGGAAGAGCCACCTCTCTGCAAGGGCCAGTCAGAGGGTTAGC
AGCGGCTTTTAGCCTGTACAGCAGAAACCTGGCCAGGCTCCAGGCTCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTTCAGTGGC
AGTGGCTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTGTCAGCAGTATGAAAGTTCAACCGTCACCGTACAAC
TTGGCCAGGGACCAAGCTGGAGATCAAAACGTACG

FIG. 12 (cont.)

WR10 IgG VH insert: VH4-4; JH6 (SEQ ID NO: 121)

ACCGGTGTACATTCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGGGACCCTGTCCCTCACCTGGCTGTCTCTGGTGCTCCATCAGC
AATAATAAGTGTGGAATTGGTCCGCCAGTCCCGAGGGAAGGGCTGGAGTGGATTGGGGAATCTATCATAGTGGGGCCACCAACTACAACCCGTCCCTCA
AGAGTCGAGTCAACATATCGGTAGACAAGTCCAAGAACCTGTTCTCCCTGAAGCTGAGCTCTGTACCGCGGGACAGGCCGTGTTACTGTGCGAGTGCG
ACTACTATGTTCCGGGACTGAGTCTTTACTACTACGGTCTGGACGTCTGGGGCCAGGGACCACGGTCACCGTCTCTCAGCGTCGAC

WR10 IgK VK insert: VK3-20; JK5 (SEQ ID NO: 122)

ACCGGTGTACATTCCAGAAATTGTGTGACGAGTCTCCAGGCACCTGTCTTGTCTCCAGGGGAAAGAGCCGCCCTCTCCTGCAGGGCCAGTCAGAGTCTTATC
GGCAGCTCTTAGCCTGTACCAAGCAGAAACCTGGCCAGGCTCCAGGCTCTCATCTATCATACATCCACAGGGCCTCTGGCATCCAGACAGGTTCAAGTGGC
GGTGGGTTTGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTTTATTACTGTCAACAGTATGATAGCTCACCGATCACCTTCGGCC
AAGGGACACGACTGGAGATTAAACGTACG

WR11 IgG VH insert: VH4-4; JH6 (SEQ ID NO: 123)

ACCGGTGTACATTCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCGGGGACCCTGTCCCTCACCTGGCTGTCTCTGGTGCTCCATCAGC
AATAATAAGTGTGGAATTGGTCCGCCAGTCCCGAGGGAAGGGCTGGAGTGGATTGGGGAATCTATCATAGTGGGGCCACCAACTACAACCCGTCCCTCA
AGAGTCGAGTCAACATATCGGTAGACAAGTCCAAGAACCTGTTCTCCCTGAAGCTGAGCTCTGTGACCGCGGGACAGGCCGTGTTACTGTGCGAGTGCG
ACTACTATGTTCCGGGACTGAGTCTTTACTACTACGGTCTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCTCAGCGTCGAC

WR11 IgK VK insert: VK3-11; JK4 (SEQ ID NO: 124)

ACCGGTGTACATTCCAGAAATTGTGTGACGAGTCTCCAGGCACCTGTCTTGTCTCAGGGGAAAGAGCCACCTCTCTGCAGGGCCAGTCAGAGTGTAGC
AGCTTCTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCAGACTCTCATCTATGATGTCATCCAACAGGGCCACTGGCATCCAGCCAGGTTCAAGTGGCAGT
GGGTCTGGGACAGATTTCACTCTCACCATCAGCAGCCTAGAGCCCTGAAGATTTTGCAGTTTATTACTGTGACGAGCGTACCAACTGGCCTCCCTCGCTCACTTTCCG
GCGGAGGGACCAAGGTGGAGATCAAAACGTACG

FIG. 12 (cont.)

WR12 IgG VH insert: VH4-30; JH4 (SEQ ID NO: 125)

ACCGGGTGATACATCCAGGTGCAGTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTCACCTGCACTGTCTCTGGCGACTCCGTCAGC
AGTAATGATCACTACTGGAGTTGGATCCGCCAGCCCCAGGGCAGGGCTGGAGTGGATTGGGTACATCTCTACGGTGGGACCACCTACTACAAACCCGTCCTC
AAGAGTCGAGTTACCATGTCGATCGACACGTCACGAACCAAGTTCTCCCTGAGGGTGACCTCCGTGCGAGCGCAGACATGGCCGTCTACTTTCTGTGCCAGGGCC
CCGGCCCTATAACGACTTTTGGAAATGGTGACACCAGTCCCTACTTTCACTCCTGGGGCCAAGGCACCCCTGGTCAACCGTCTCTCAGCGTCGAC

WR12 IgK VK insert: VK2-28; JK4 (SEQ ID NO: 126)

ACCGGGTATACATGGGGATATTGTGATGACTCAGTCTCCACTGTCCCTGCCGTGAGAGCCCTGAGAGCCGGCTCCATCTCTGCAGGTAGTCAGAGCCTCTCTCC
ATAGTAATGGATACAACATAATTGAGTTGGTACCTGCAGAGCCAGGGCAGTCTCCAACTCTGTGATCTTTTCGAGTTCTATTGGGGCCTCCGGGGTCCCTGACAG
GTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGACAAATCAACAGAGTGGAGGCTGAGGATGTTGGAGTTTATTACTGCATGCAGGCTCTACAAACTCCGCT
CACTTTCGGCGGAGGGACCAAGCTGGAAATCAAAACGTACG

WR13 IgG VH insert: VH4-30; JH4 (SEQ ID NO: 127)

ACCGGGTATACATCCAGGTGCAGTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTCACCTGCACTGTCTCTGGCGACTCCGTCAGC
AGTAATGATCACTACTGGAGTTGGATCCGCCAGCCCCAGGGCAGGGCTGGAGTGGATTGGGTACATCTCTACGGTGGGACCACCTACTACAAACCCGTCCTC
AAGAGTCGAGTTACCATGTCGATCGACACGTCACGAACCAAGTTCTCCCTGAGGGTGACCTCCGTGCGAGCCGCGAGACATGGCCGTCTACTTTCTGTGCCAGGGCC
CCGGCCCTATAACGACTTTTGGAAATGGTGACACCAGTCCCTACTTTCACTCCTGGGGCCAAGGCACCCCTGGTCAACCGTCTCTCAGCGTCGAC

WR13 IgK VK insert: VK2-28; JK2 (SEQ ID NO: 128)

ACCGGGTATACATGGGGATATTGTGATGACTCAGTCTCCACTGTCCCTGCCGTGAGAGTGGGCTCCATCTCTGCAGGTCTAGTCAGAGCCTCTCTCC
ATAGTAATGGATACAACATAATTGAGTTGGTACCTGCAGAAAGCCAGGGCAGTCTCCAACTCTGTGATCTTTTCGAGTTCTATTCCGGCCCTCCGGGGTCCCTGACAG
GTTCAGTGGCAGTGGATCAGGCACAGATTTTACCCTGACAATCAACAGAGTGGAGGCTGAGGATGTTGGAGTTTATTACTGCAATGCAGGCTCTACAAACTCCGCT
CACTTTCGGCCAGGGACCAAGCTGGAAATCAAAACGTACG

FIG. 12 (cont.)

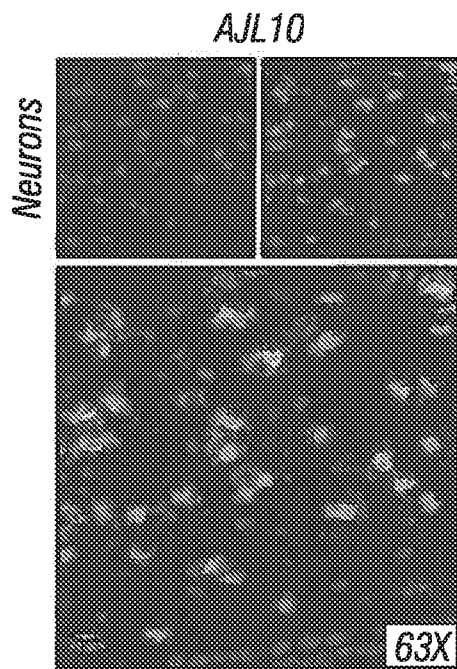


FIG. 13A

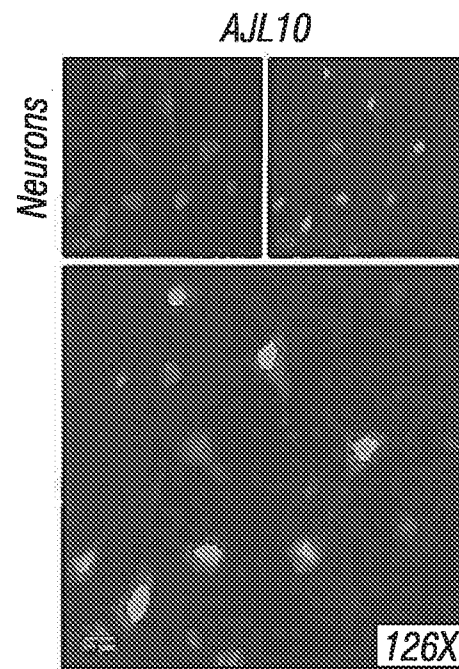


FIG. 13B

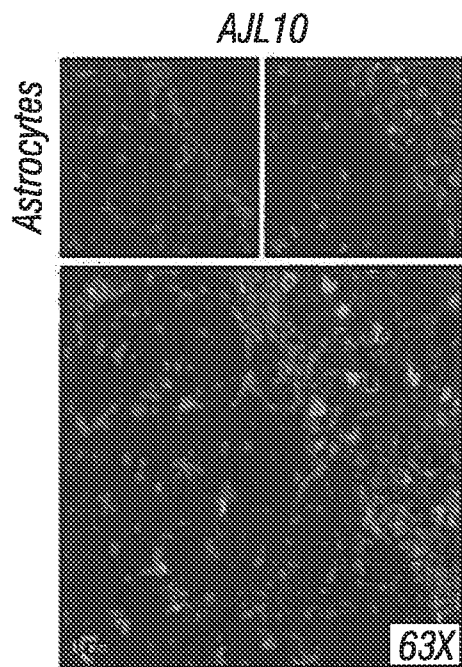


FIG. 13C

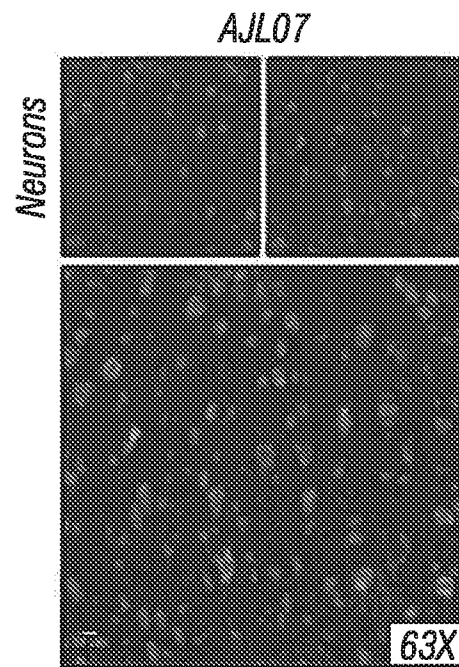


FIG. 13D

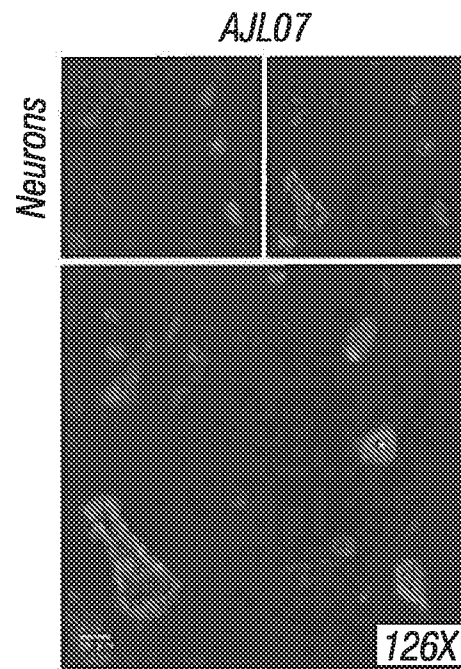


FIG. 13E

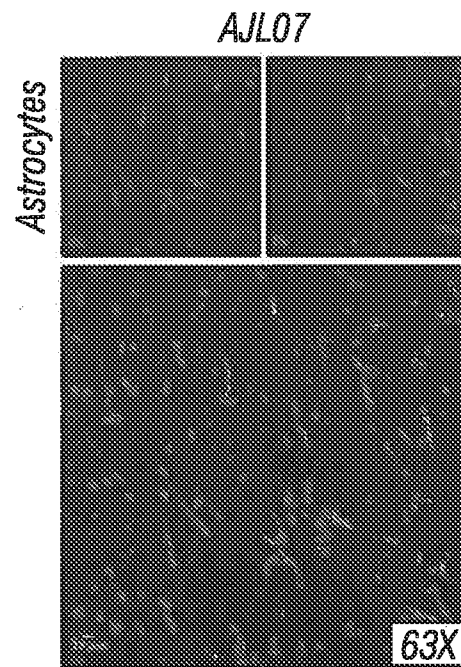


FIG. 13F

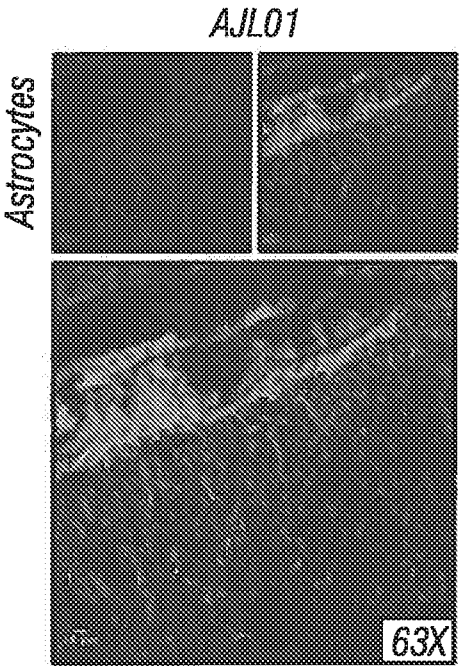


FIG. 14A

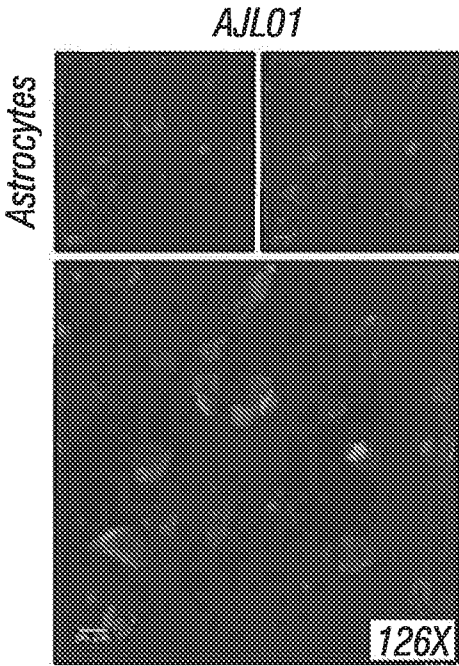


FIG. 14B

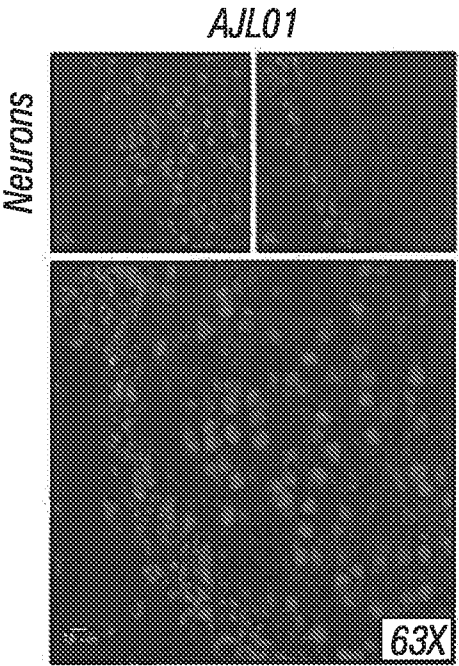


FIG. 14C

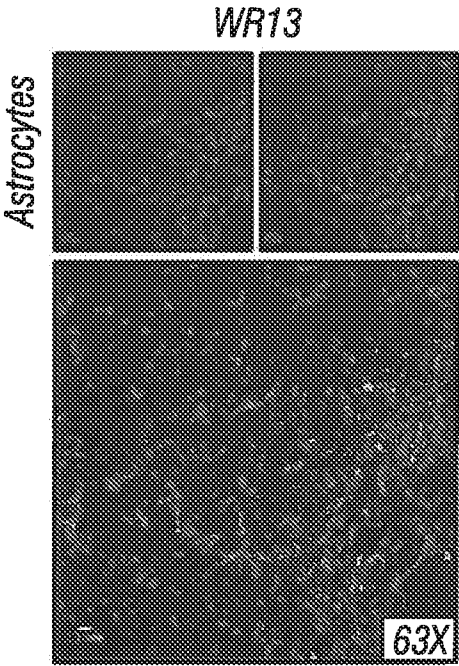


FIG. 14D

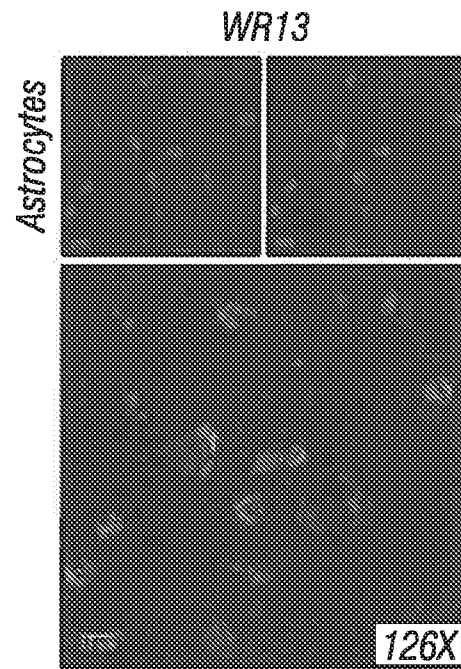


FIG. 14E

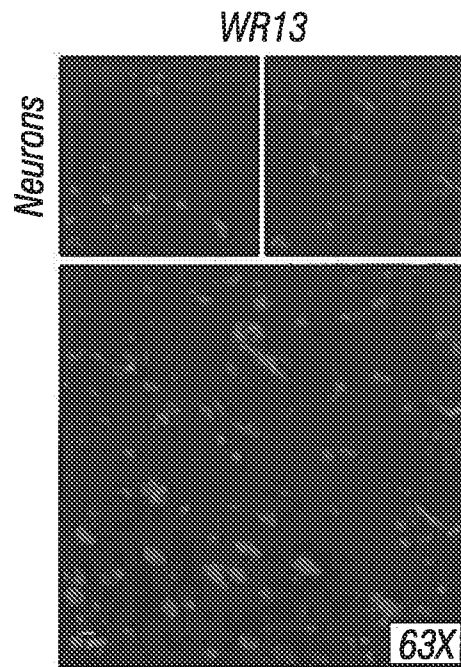


FIG. 14F

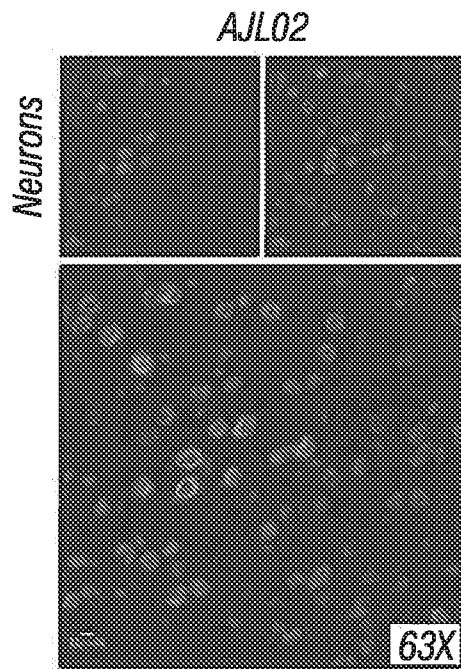


FIG. 15A

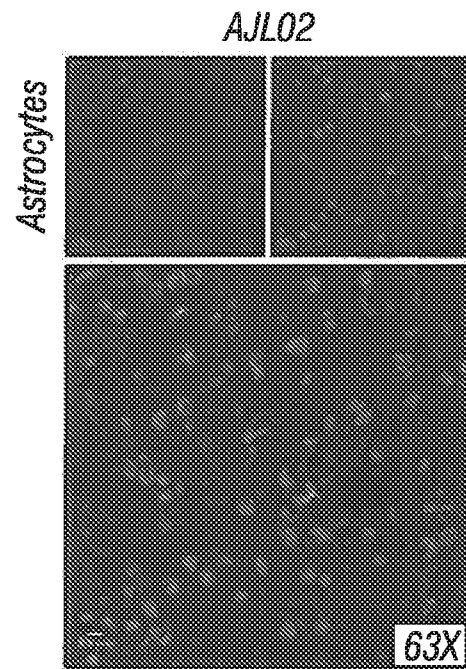


FIG. 15B

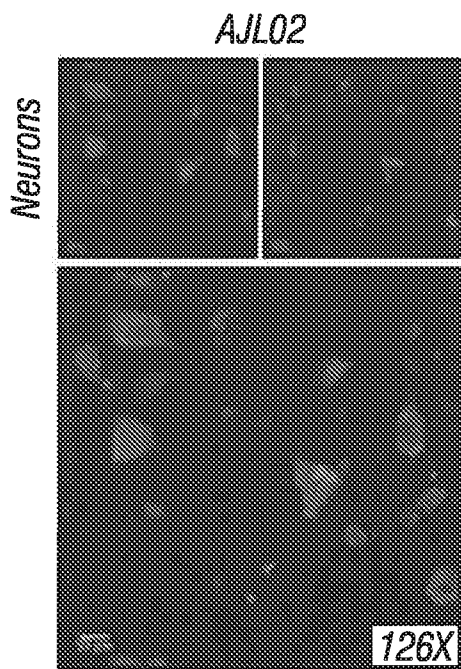


FIG. 15C

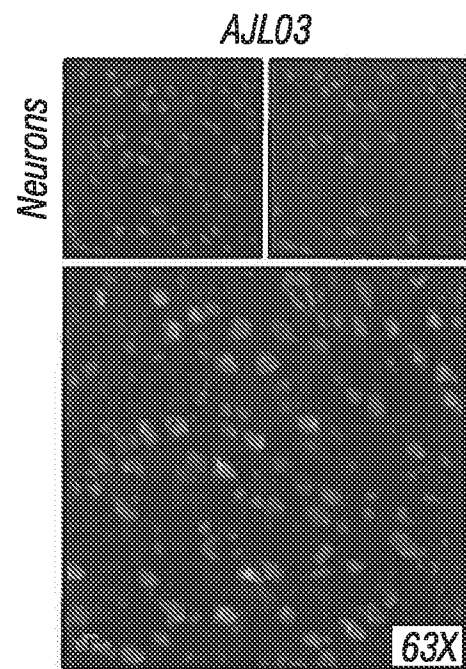


FIG. 15D

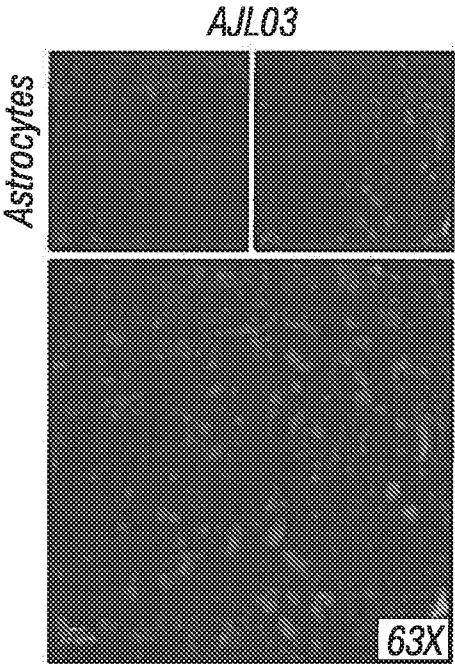


FIG. 15E

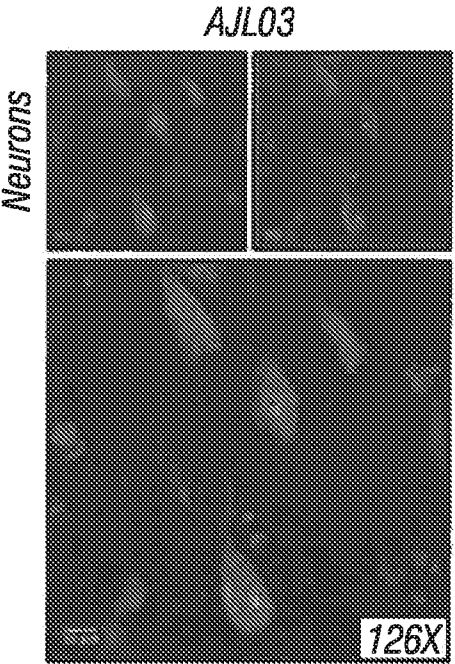


FIG. 15F

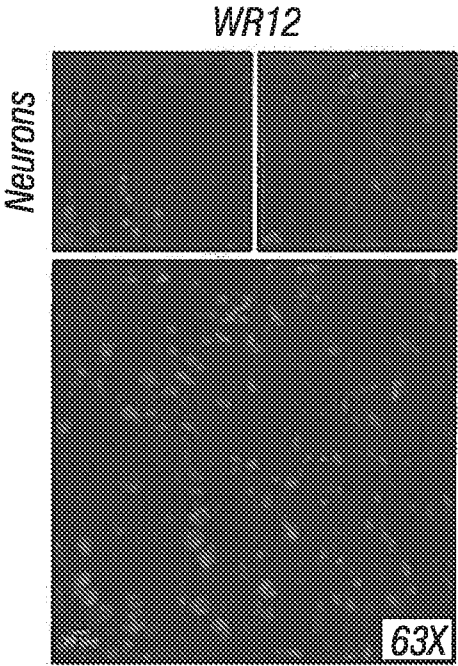


FIG. 16A

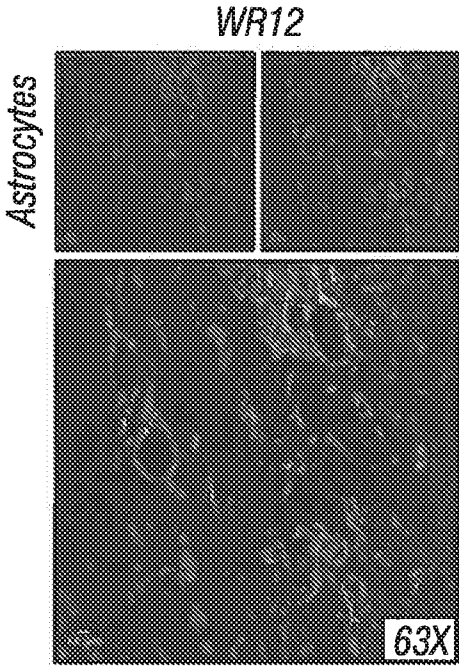


FIG. 16B

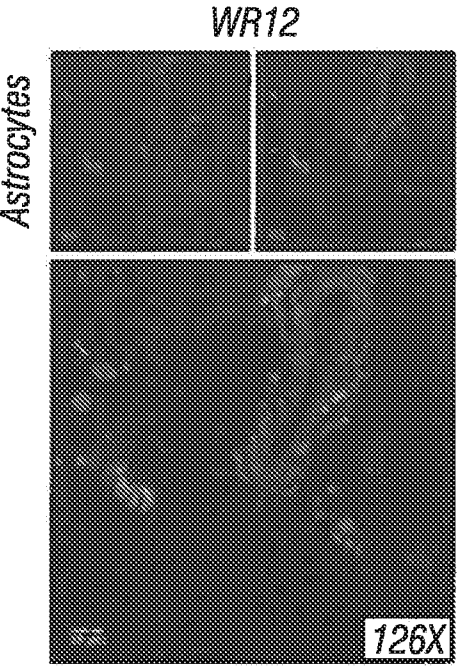


FIG. 16C

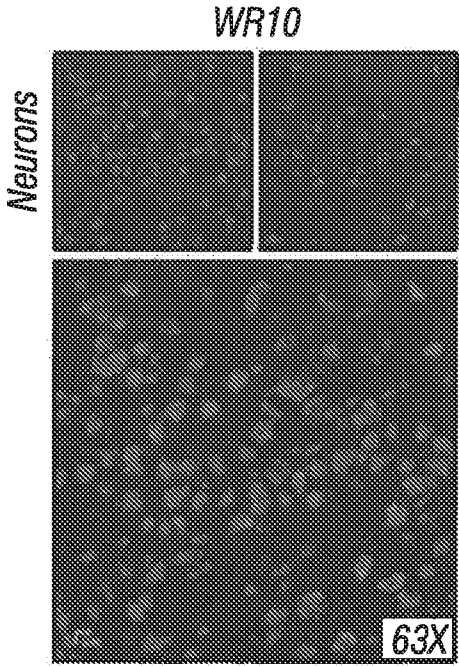


FIG. 16D

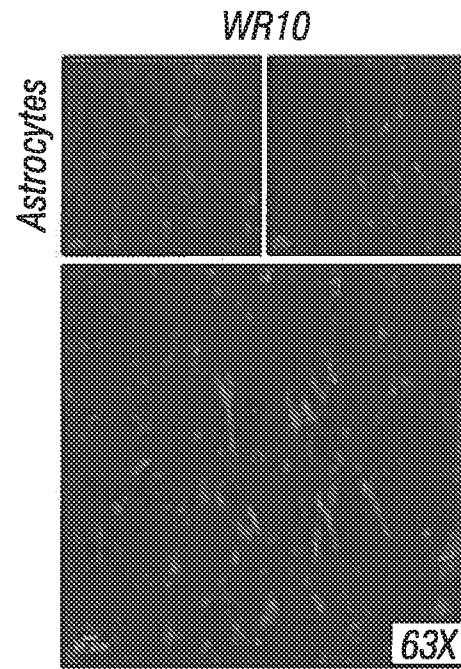


FIG. 16E

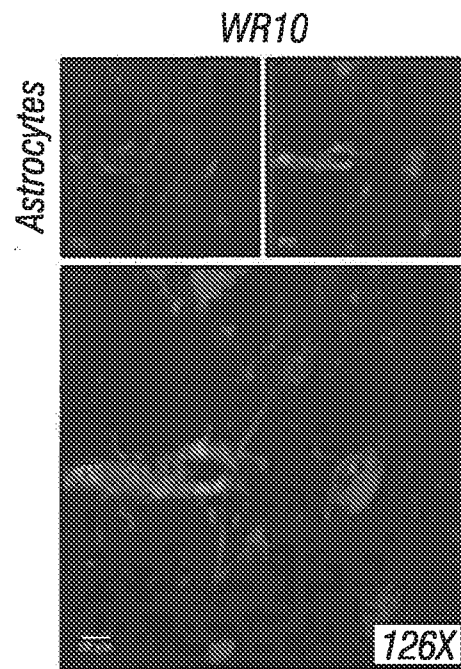


FIG. 16F

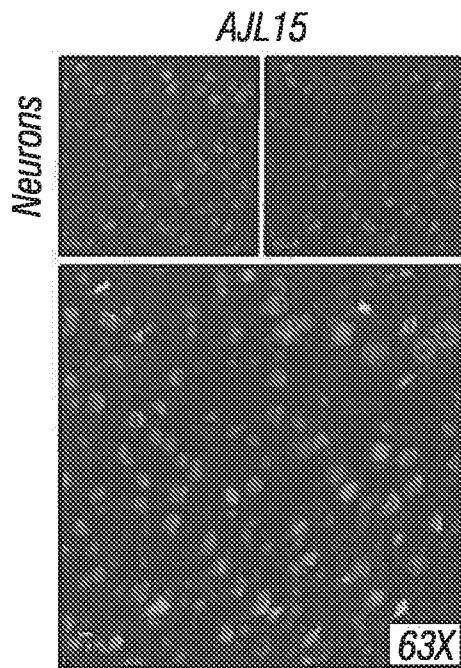


FIG. 16G

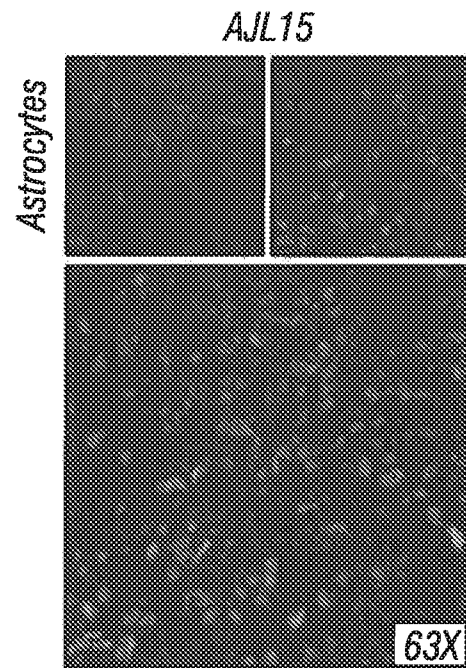


FIG. 16H

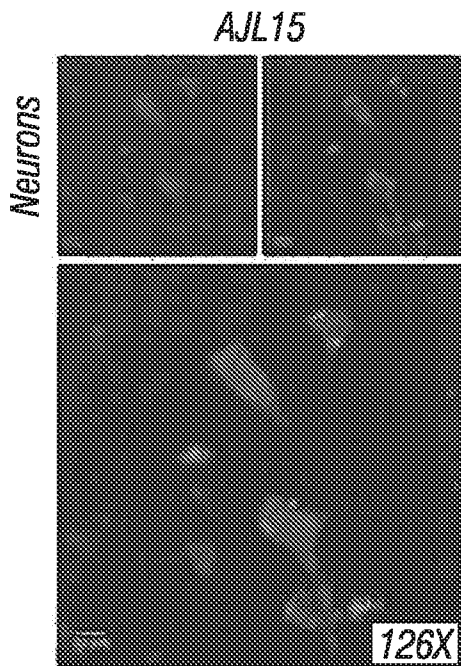


FIG. 16I

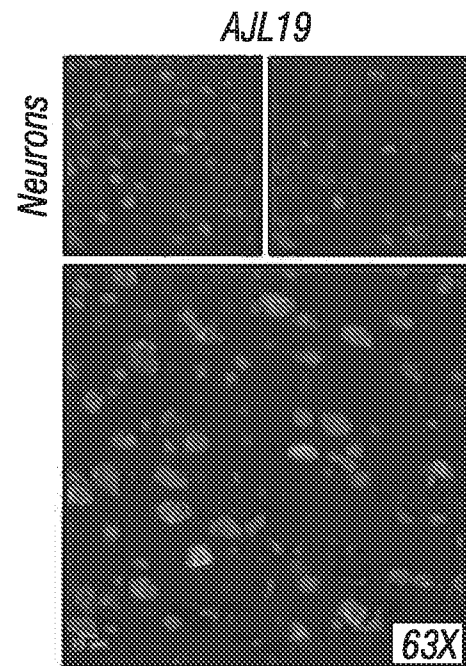


FIG. 16J

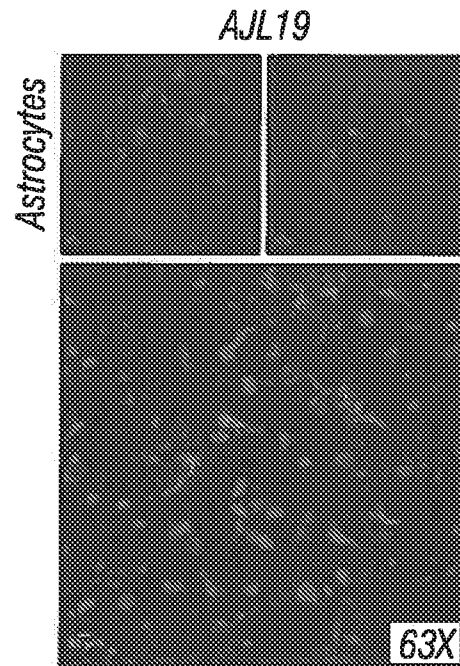


FIG. 16K

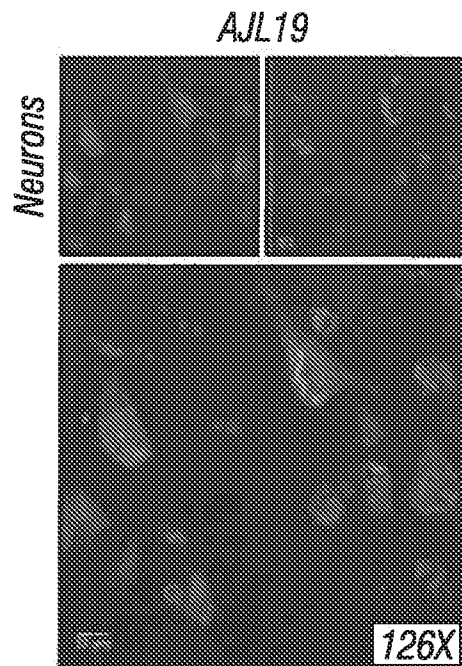


FIG. 16L

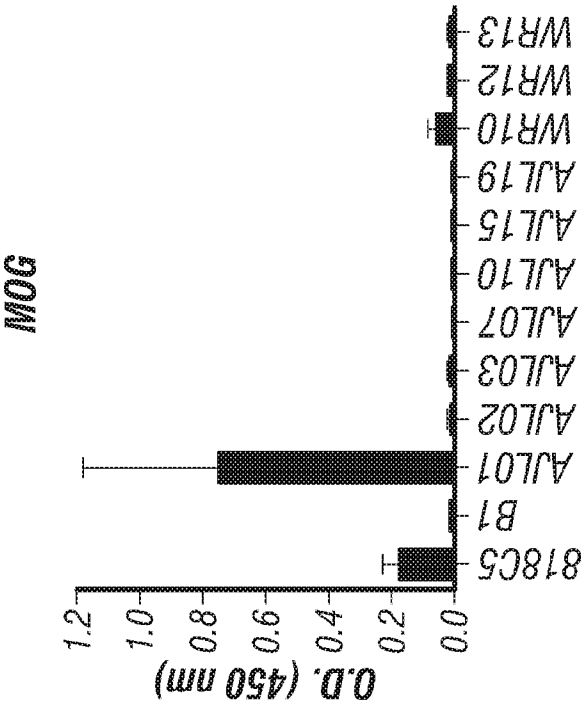


FIG. 17B

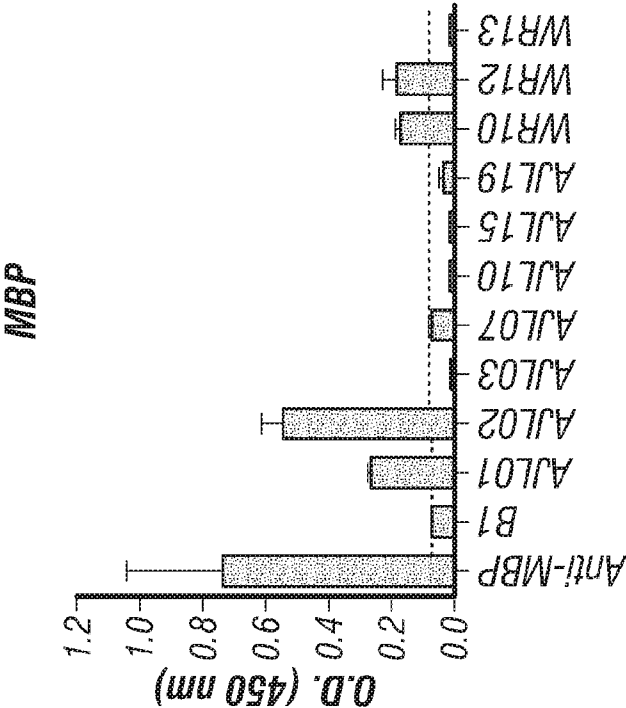


FIG. 17A

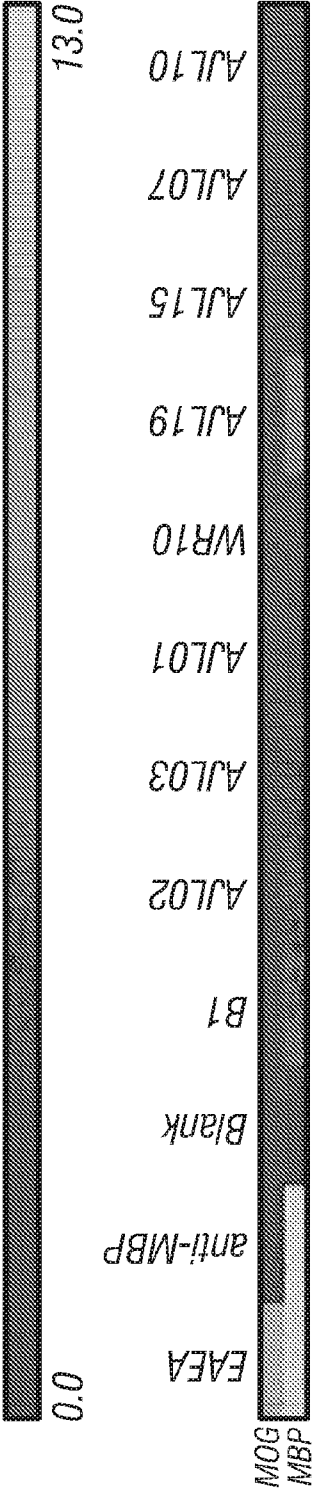
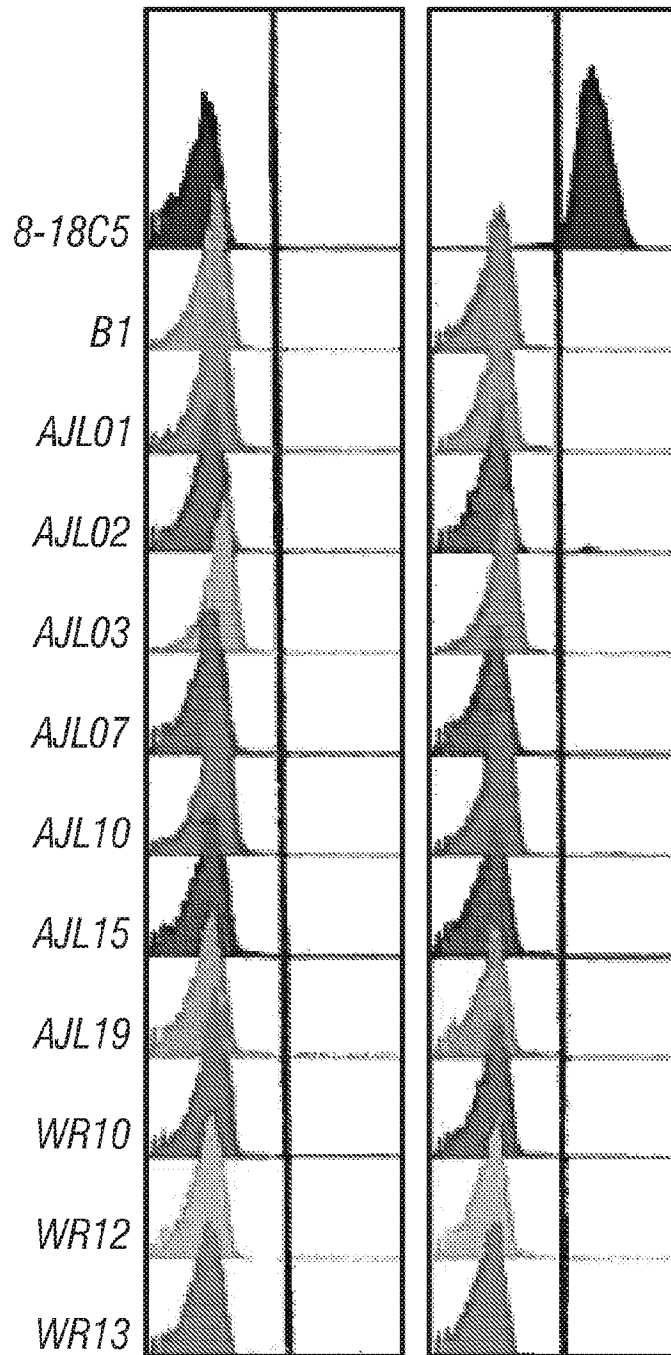


FIG. 17C

**FIG. 17D**

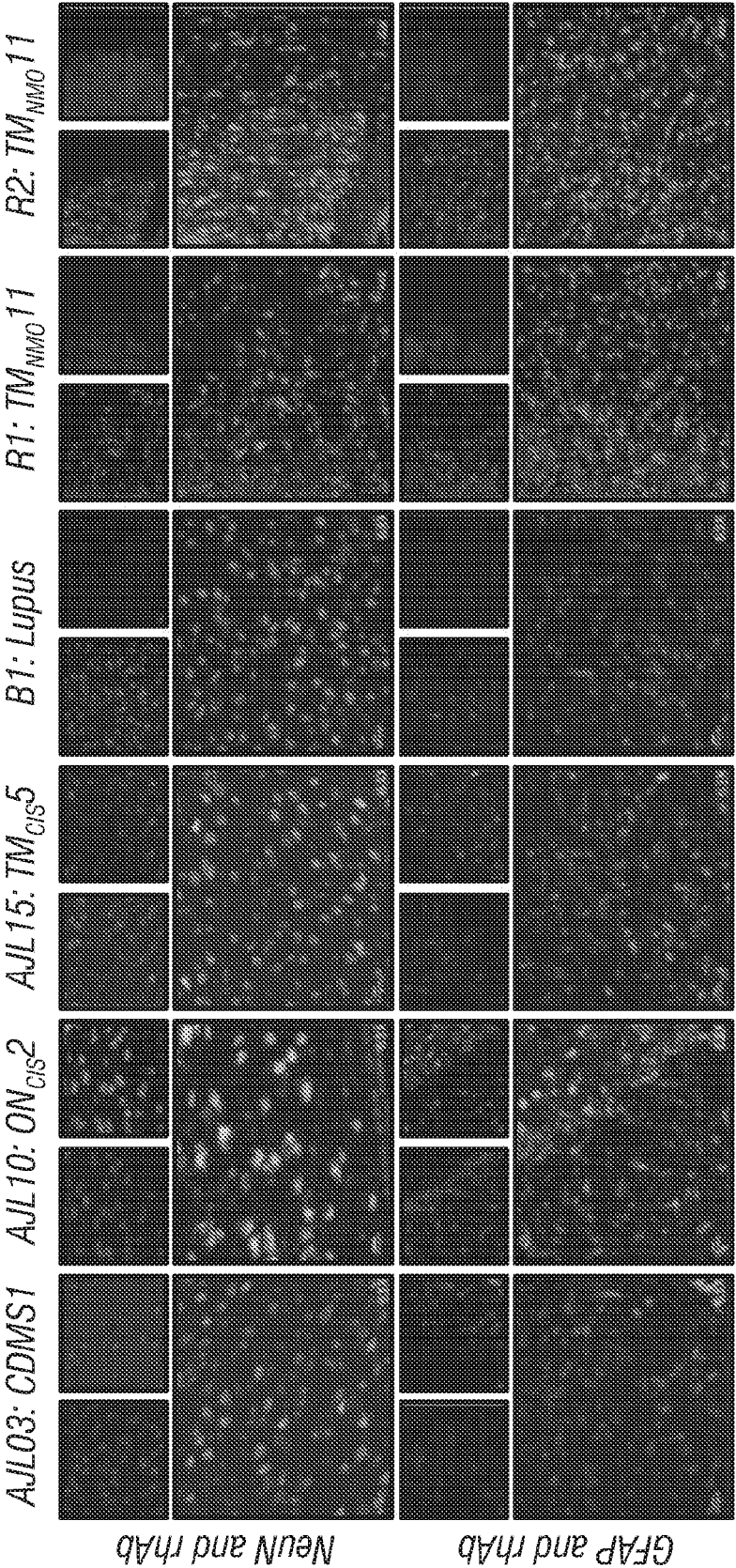


FIG. 18A

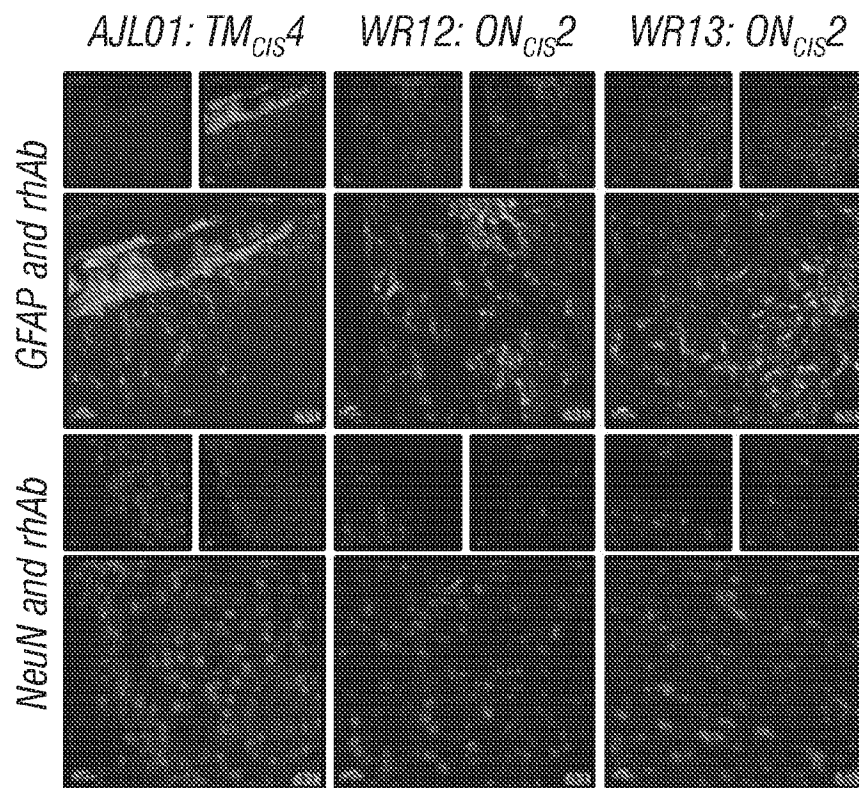
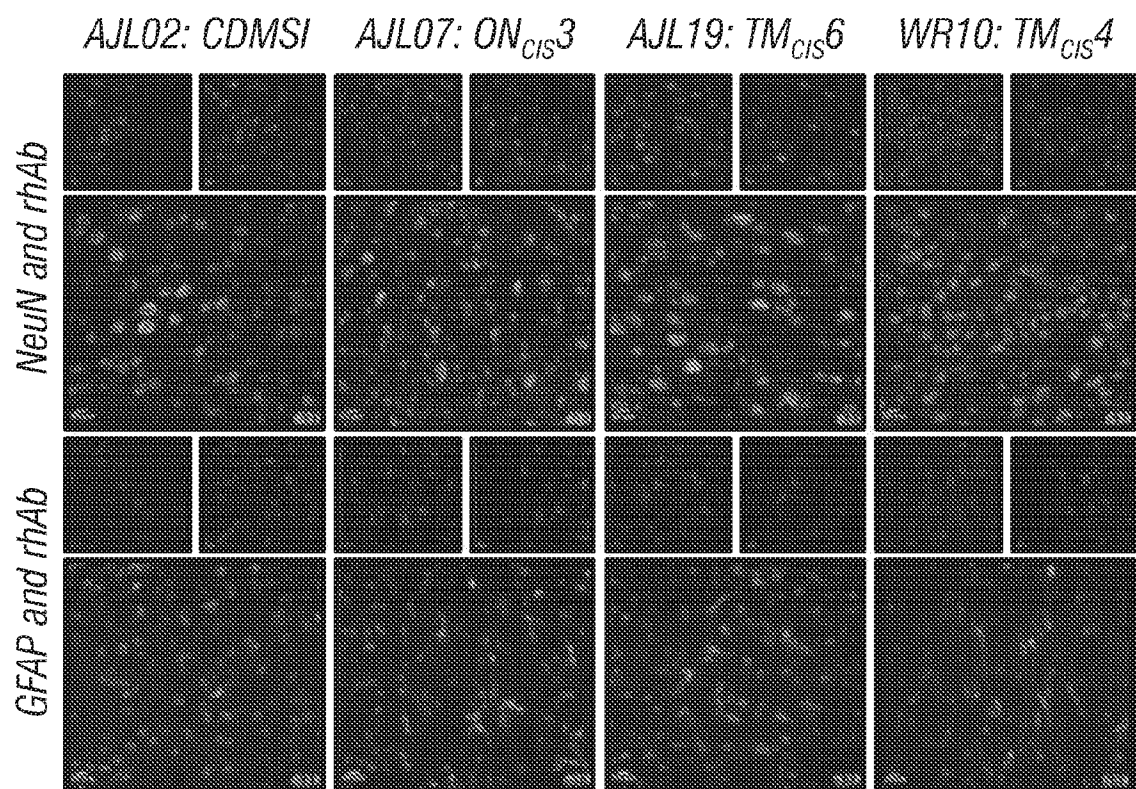
FIG. 18B

FIG. 18C

FIG. 18D

FIG. 18E

FIG. 18F

**FIG. 18G****FIG. 18H****FIG. 18I****FIG. 18J****FIG. 18K****FIG. 18L****FIG. 18M**

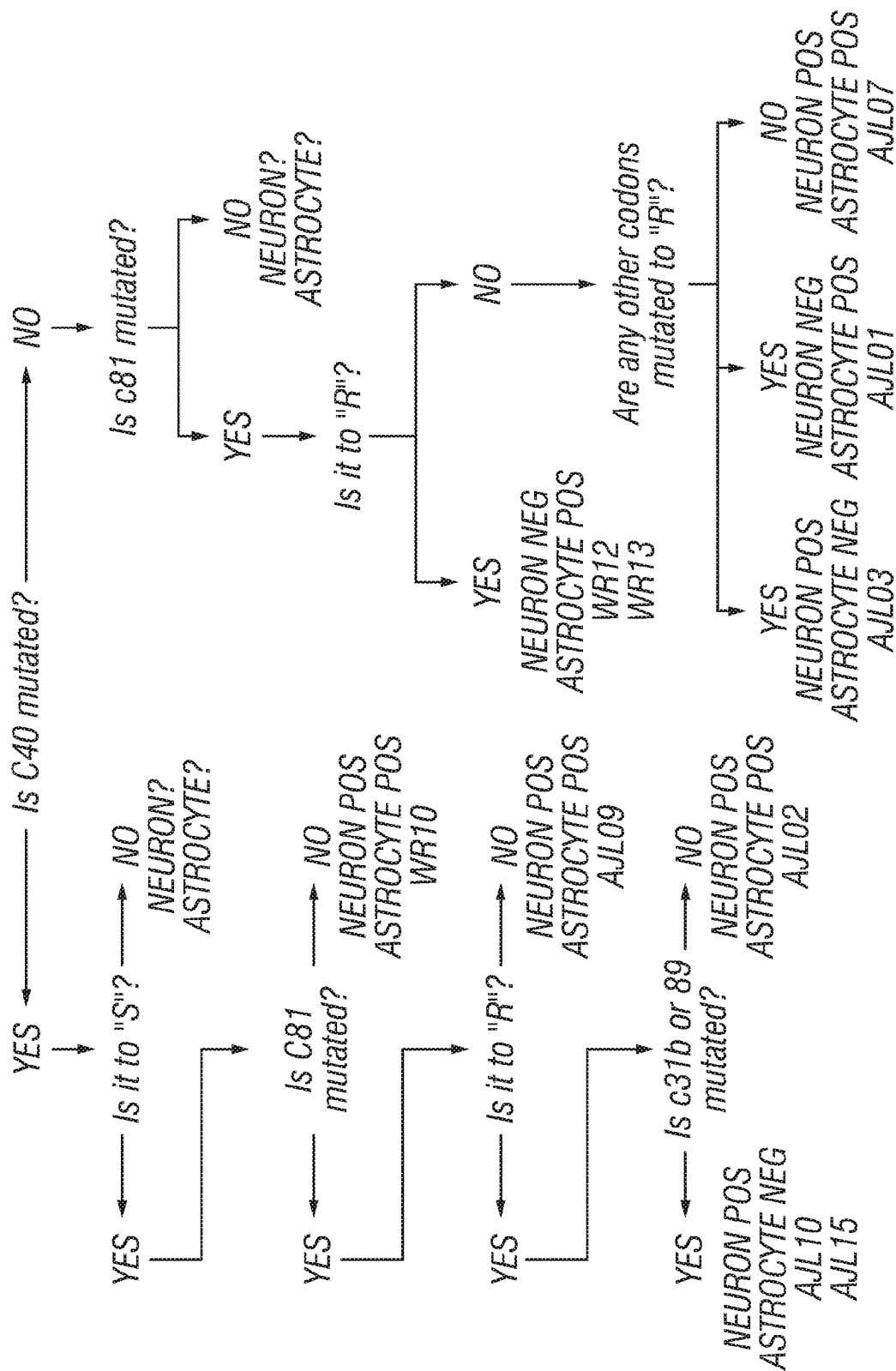


FIG. 19

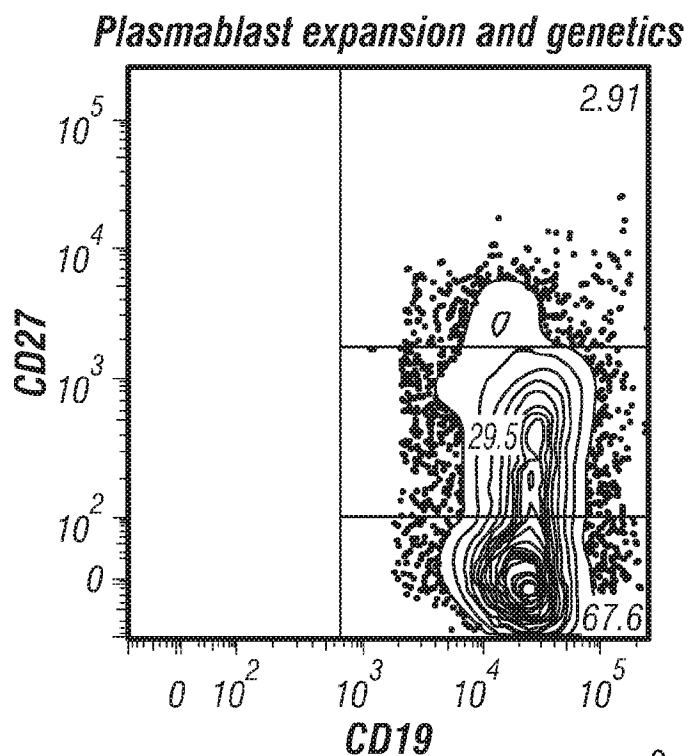


FIG. 20A

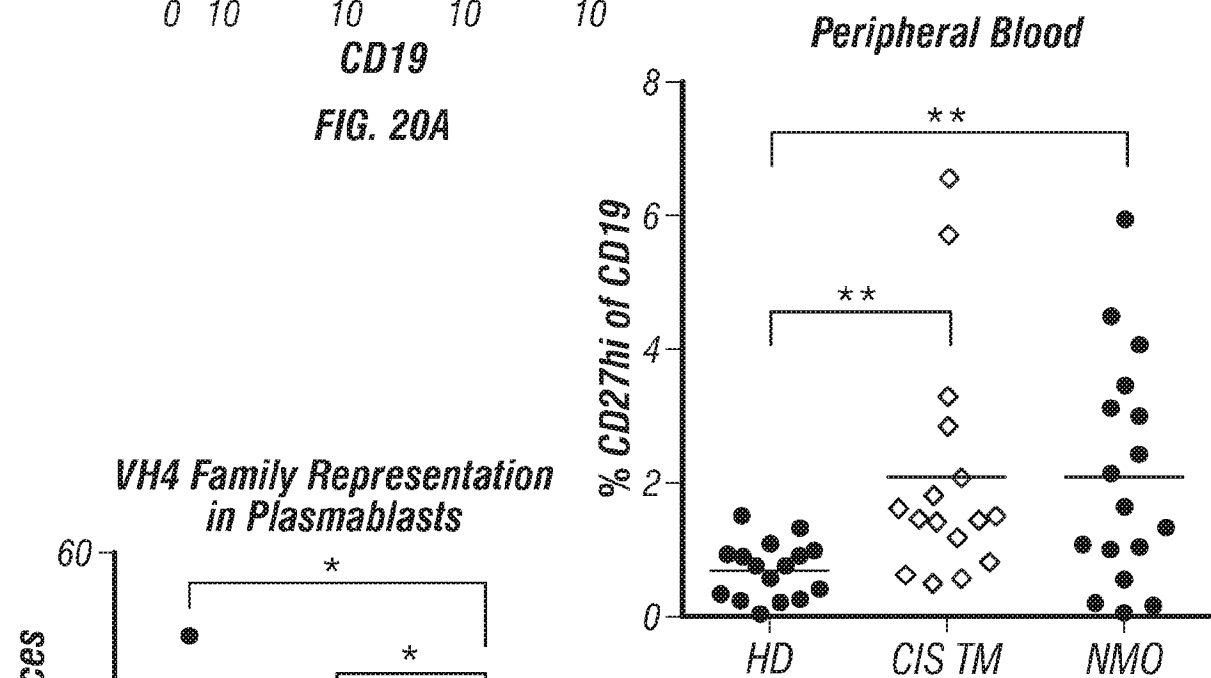


FIG. 20B

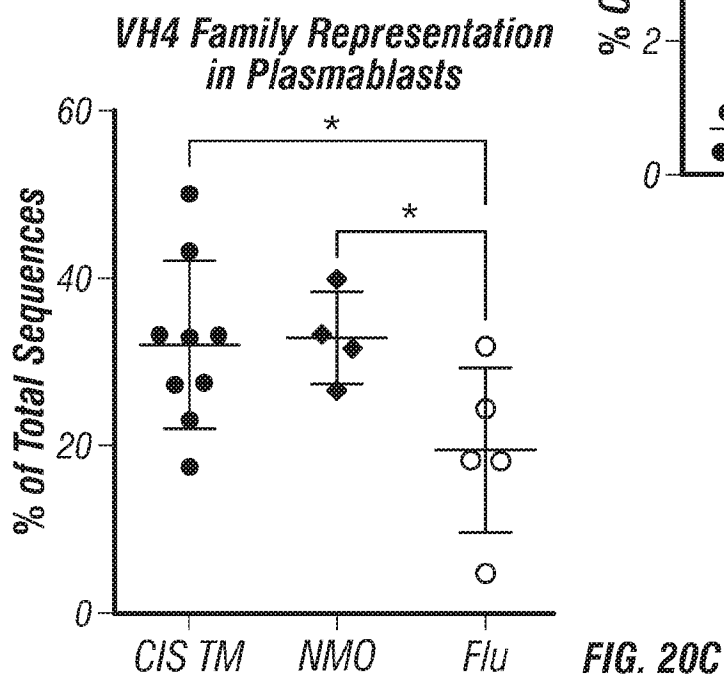


FIG. 20C

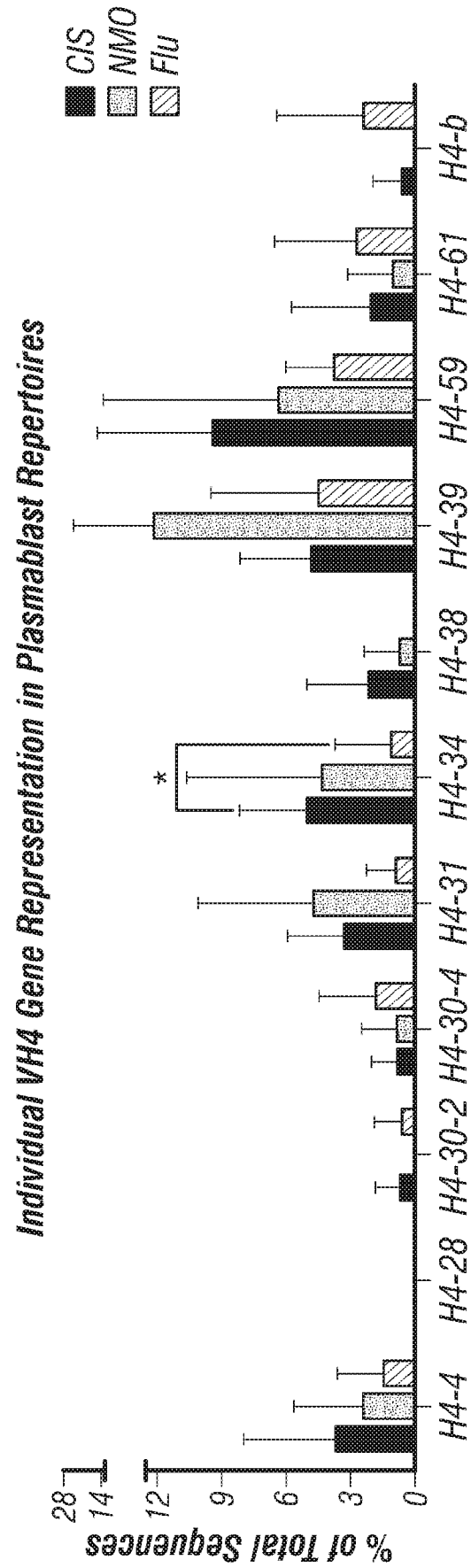


FIG. 20D

Detailed heavy chain plasmablast genetics
Heavy Chain V Gene Distribution

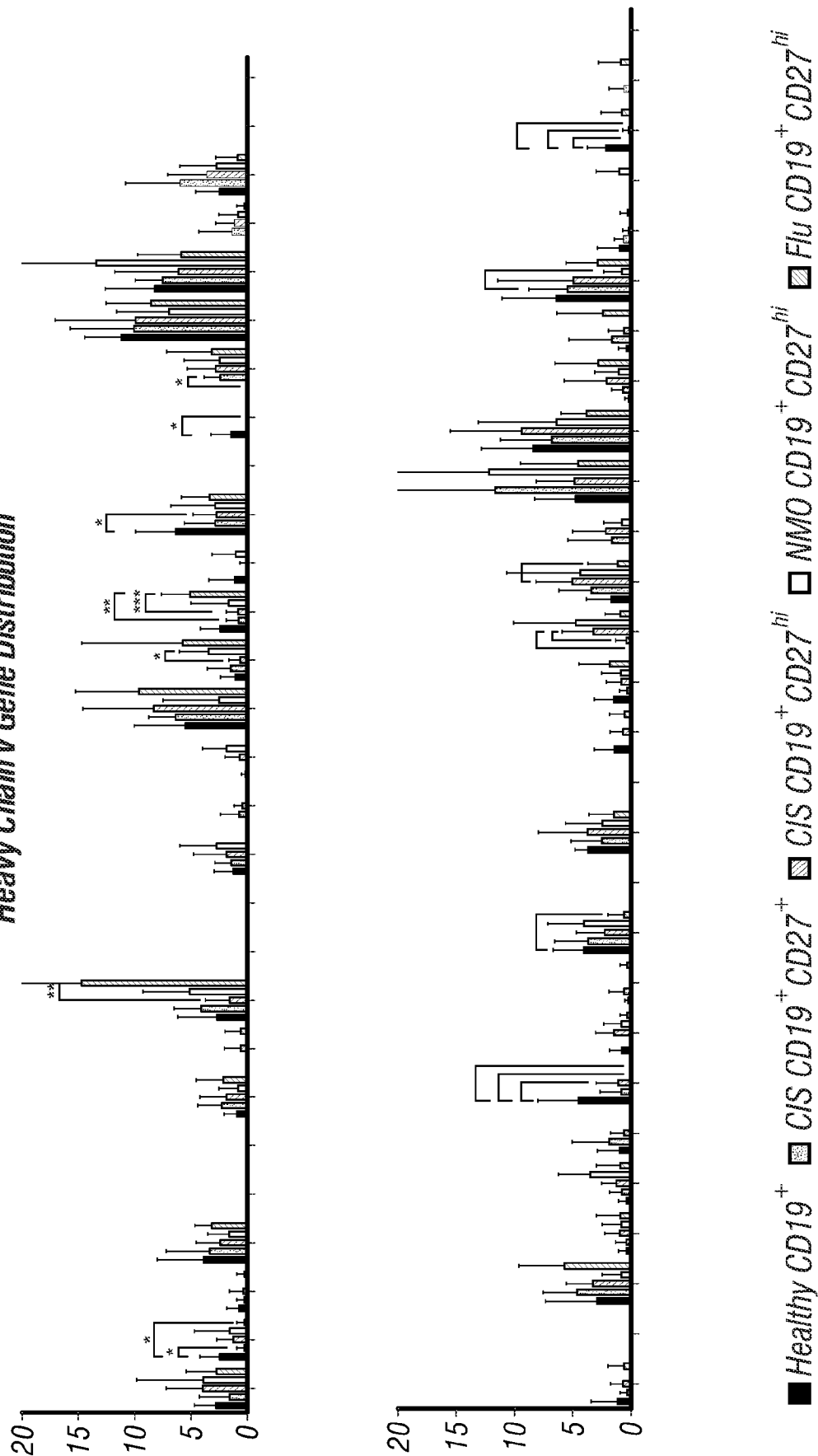


FIG. 21A

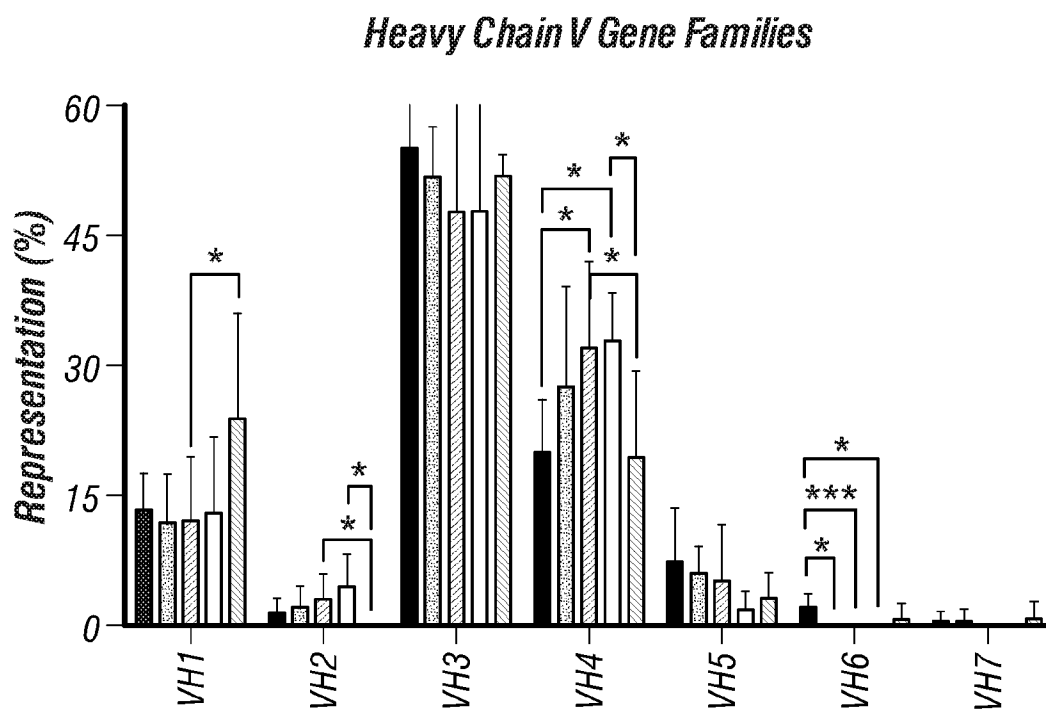


FIG. 21B

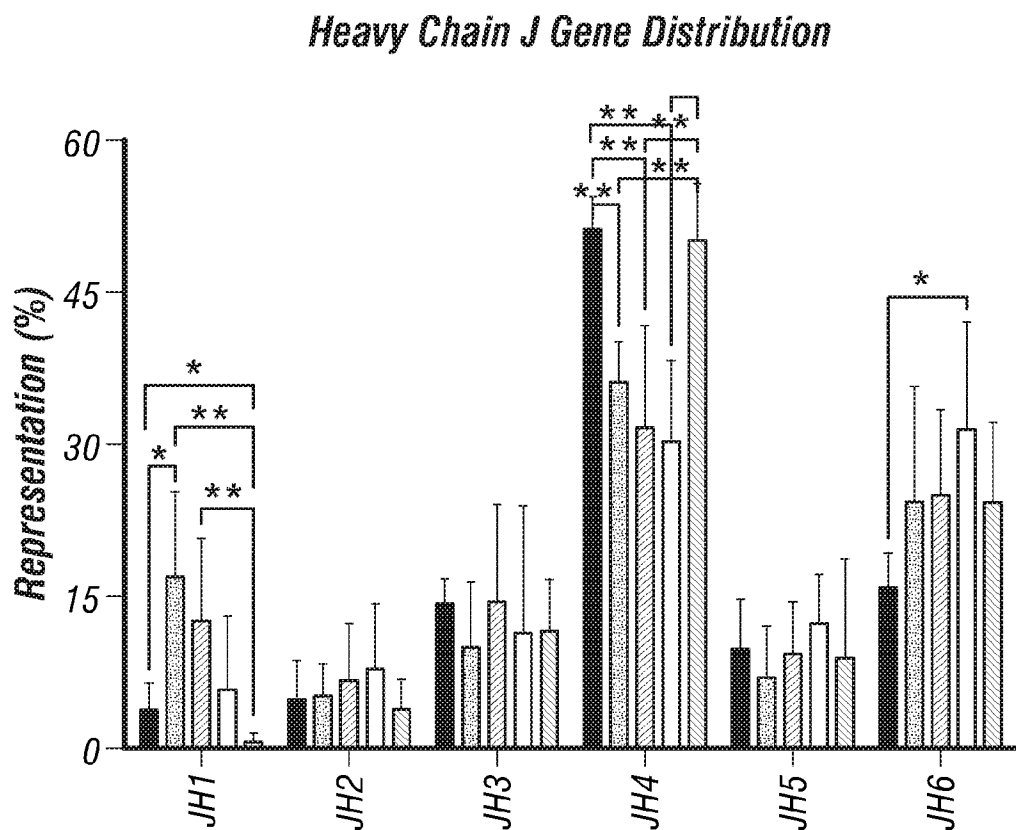
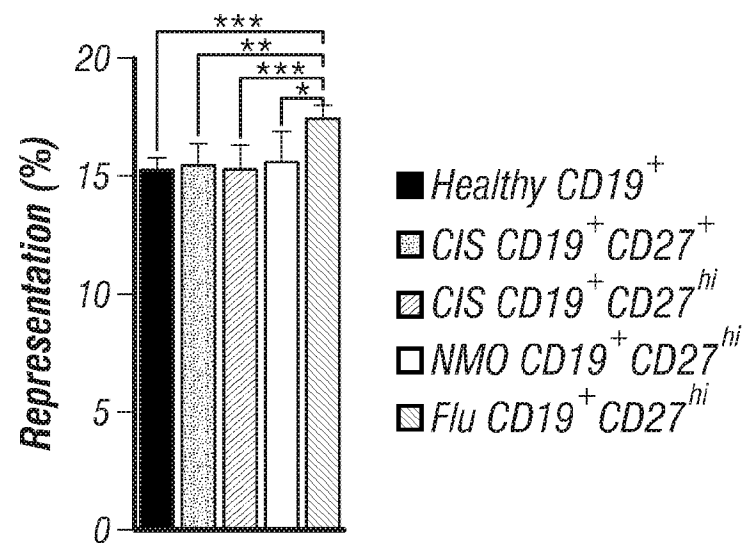
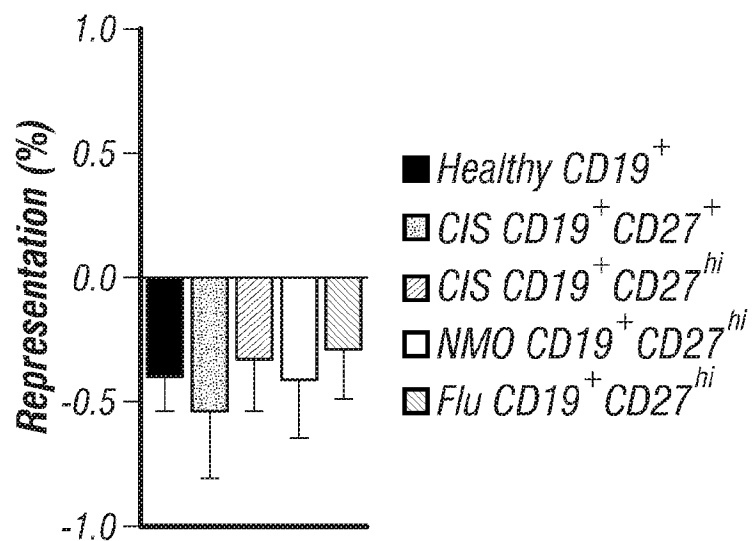


FIG. 21C

Heavy Chain CDR3 Length**FIG. 21D****Heavy Chain CDR3 Charge****FIG. 21E**

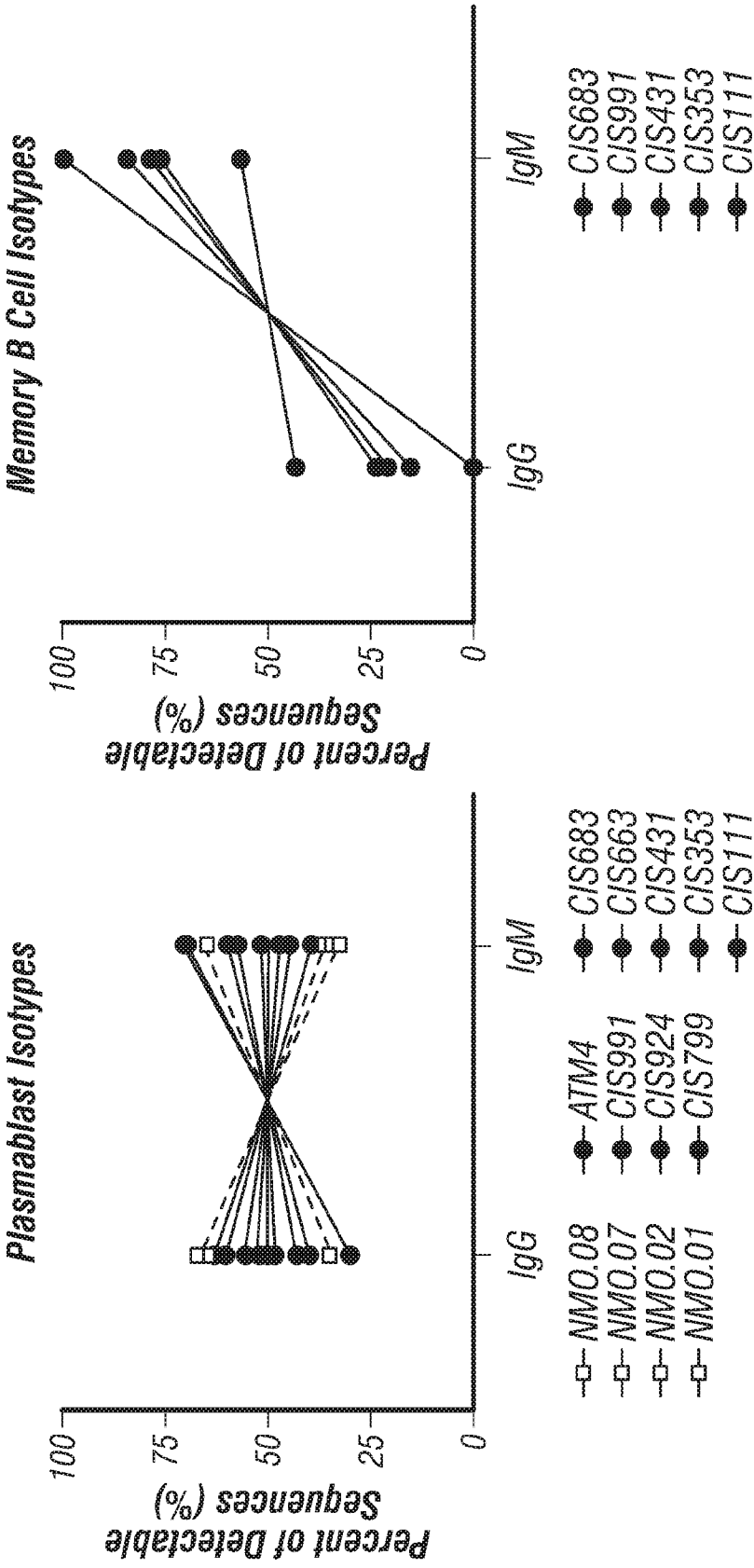


FIG. 21F

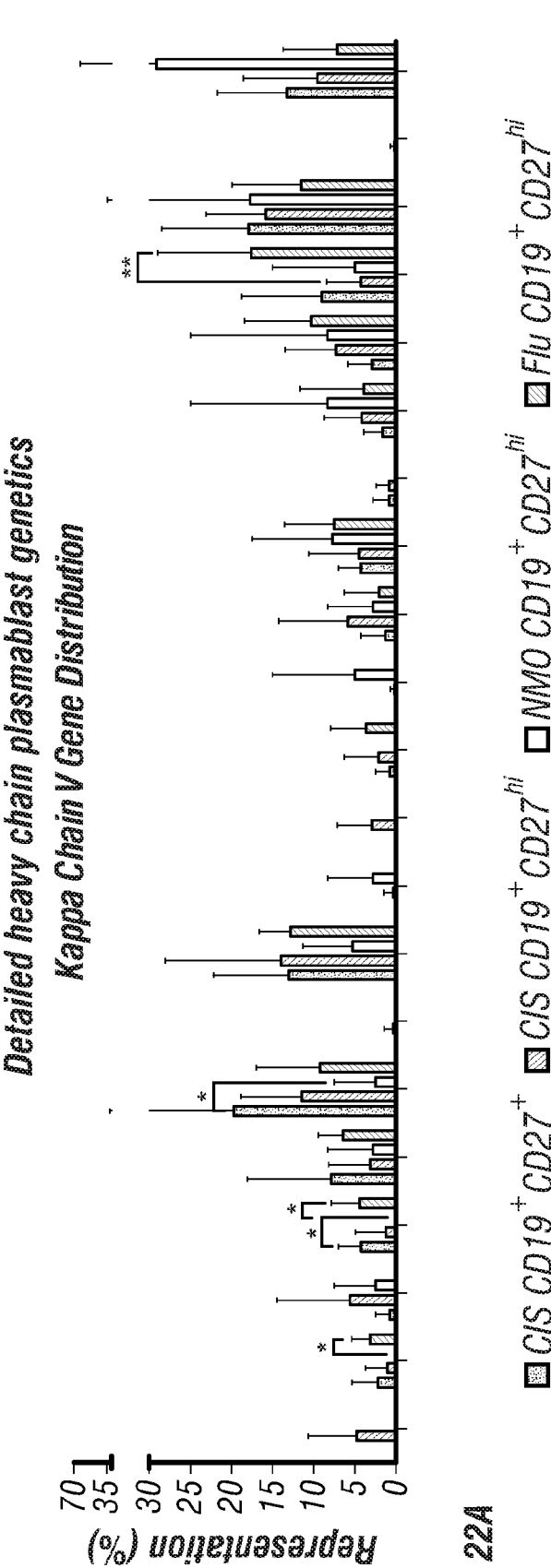


FIG. 22A

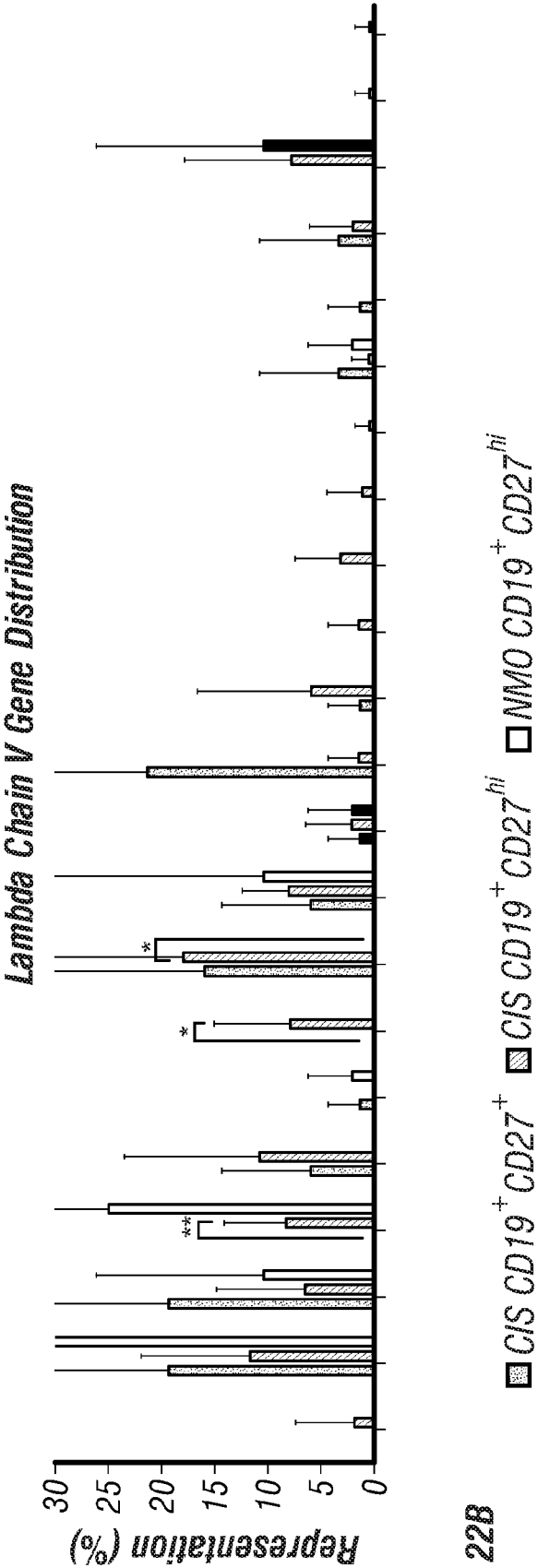
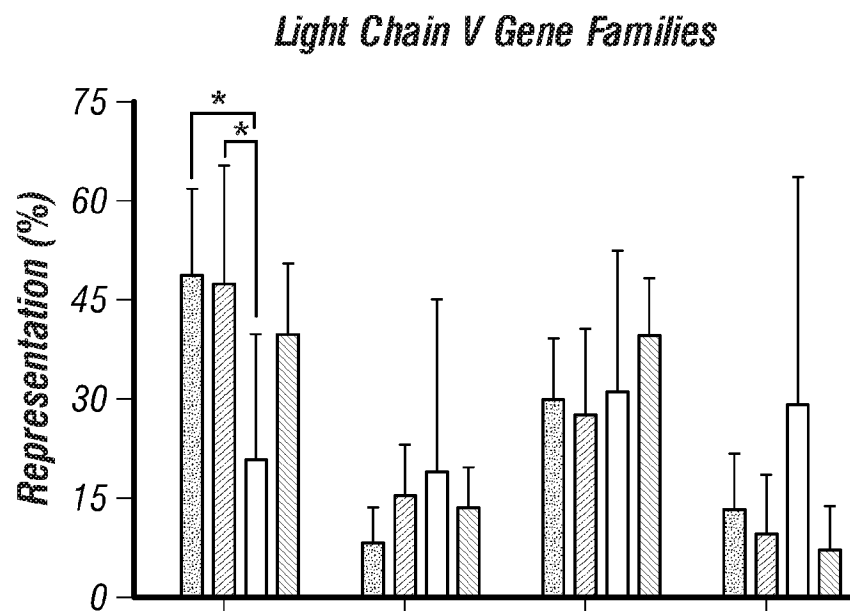
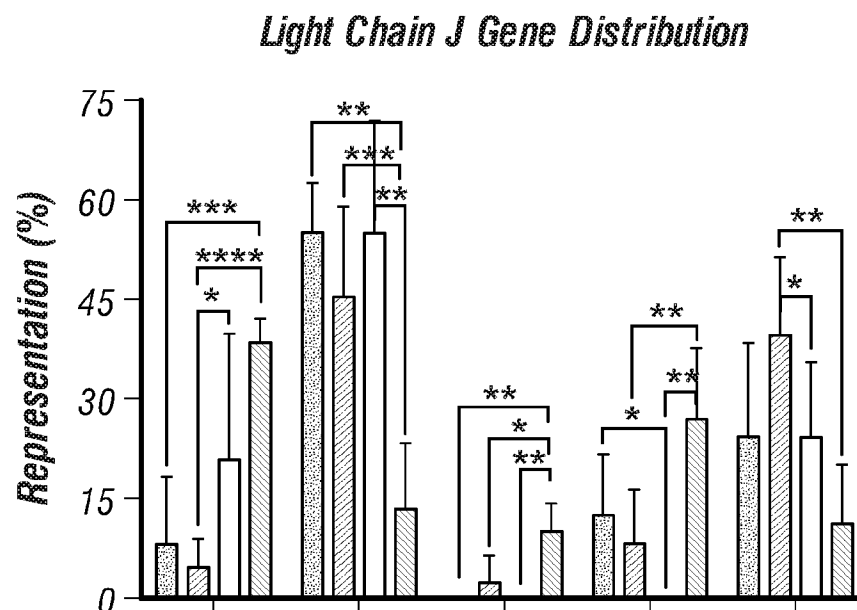
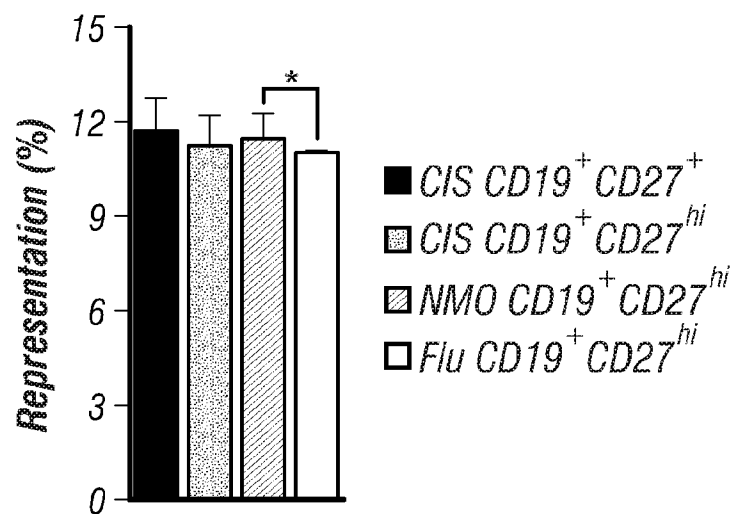
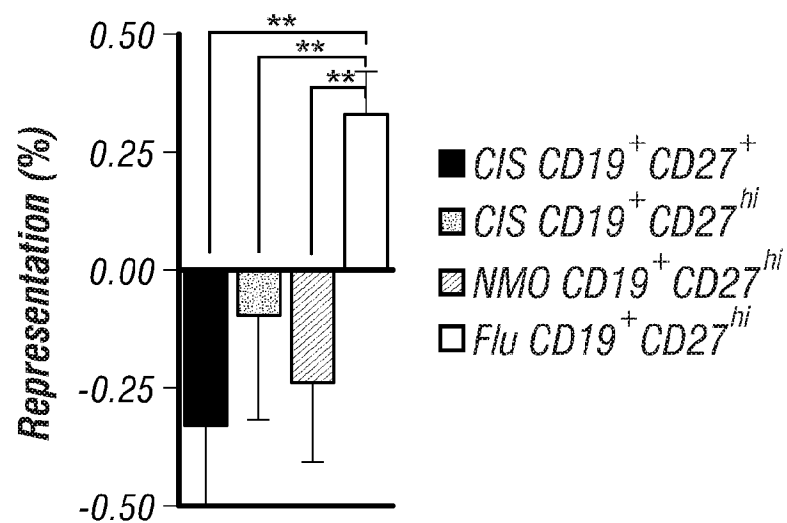


FIG. 22B

**FIG. 22C****FIG. 22D**

Light Chain CDR3 Length**FIG. 22E****Light Chain CDR3 Charge****FIG. 22F**

Brain lysate reactivity of plasmablast
antibodies by ELISA

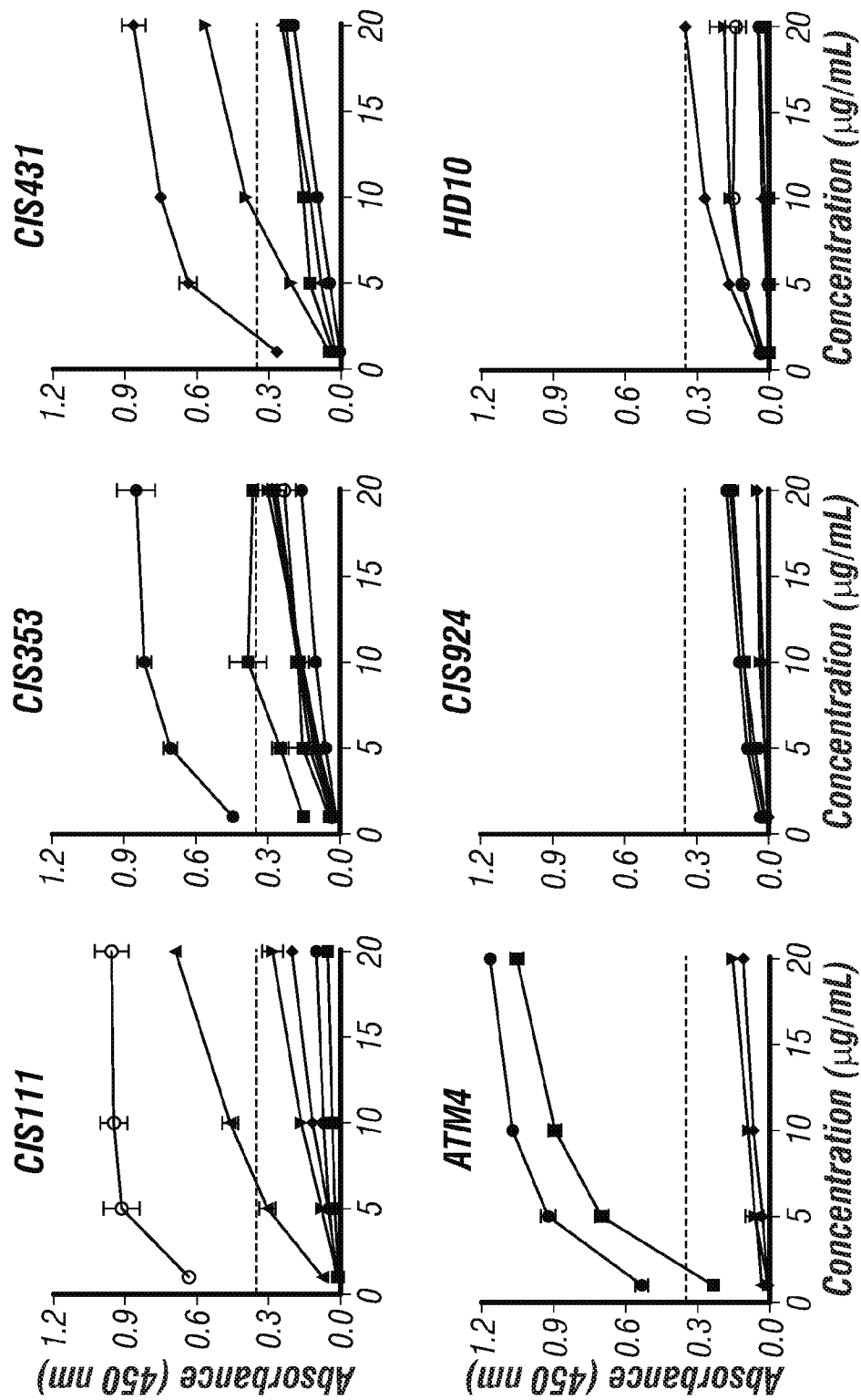


FIG. 23A

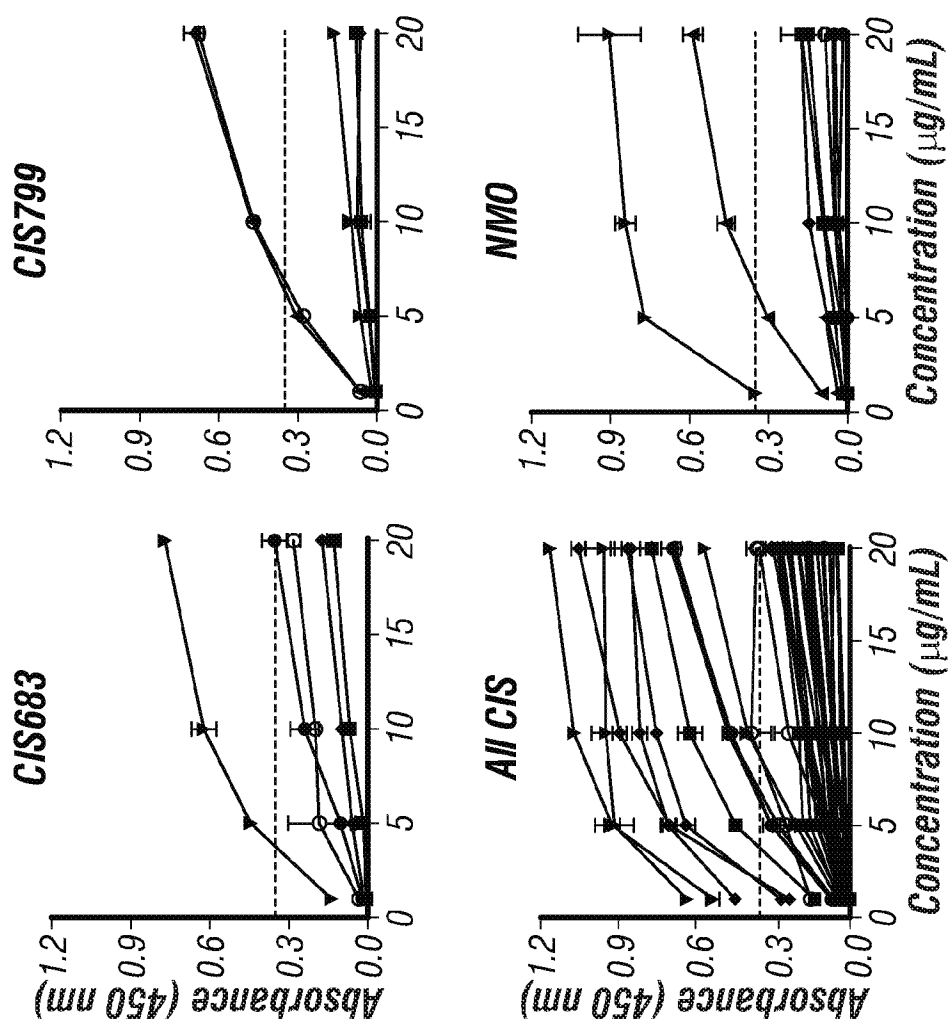


FIG. 23A
(Contd.)

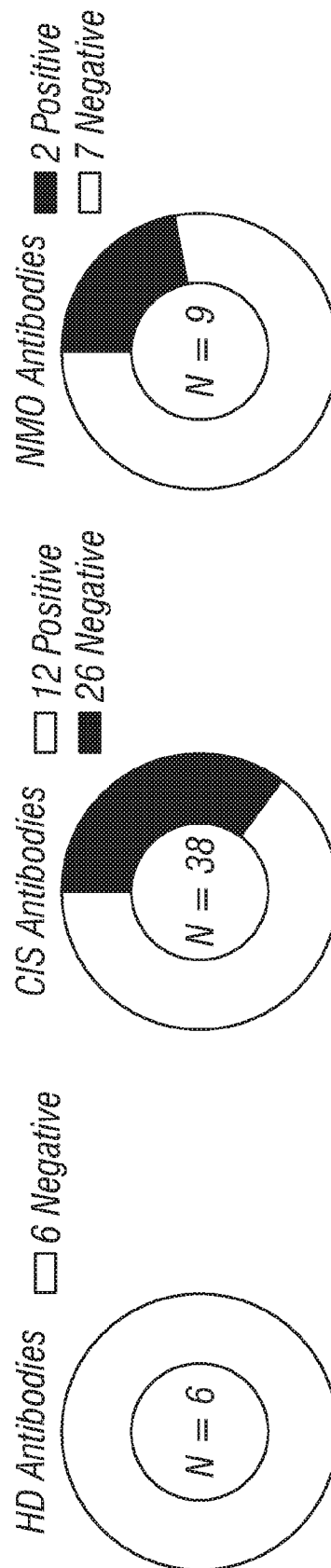


FIG. 23B

Comparison of VH4 and VH3 antibodies

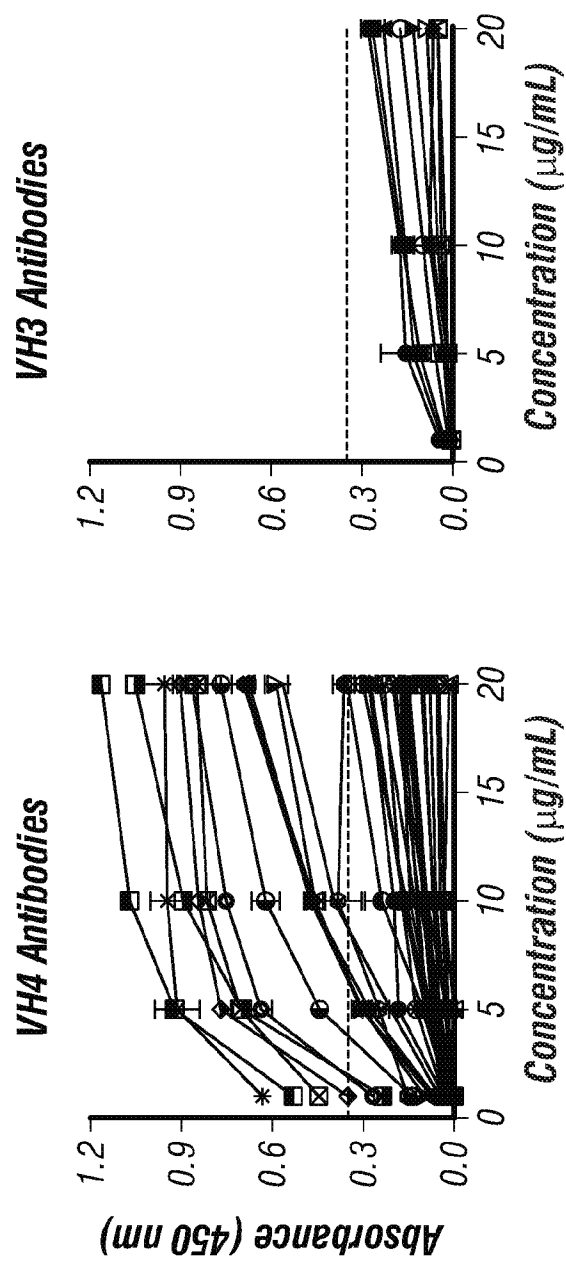


FIG. 24A

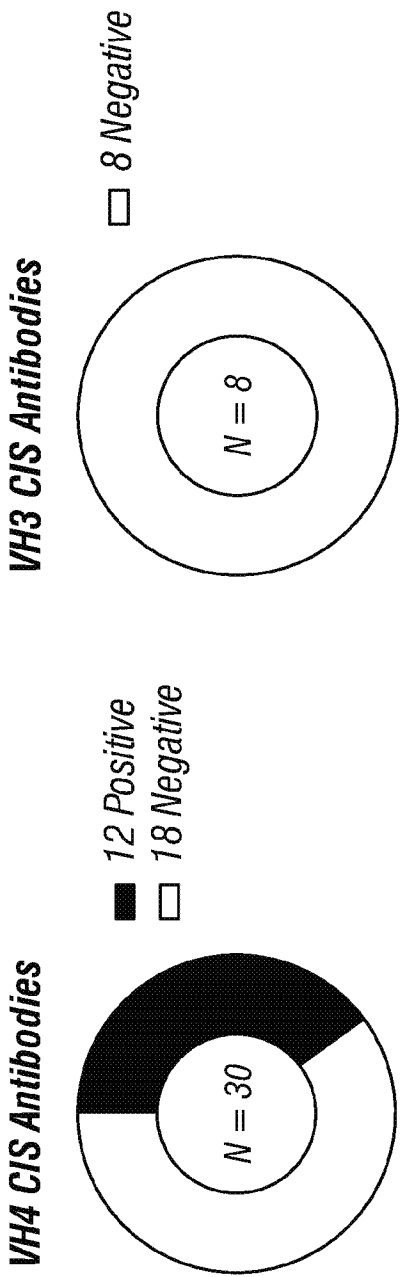


FIG. 24B

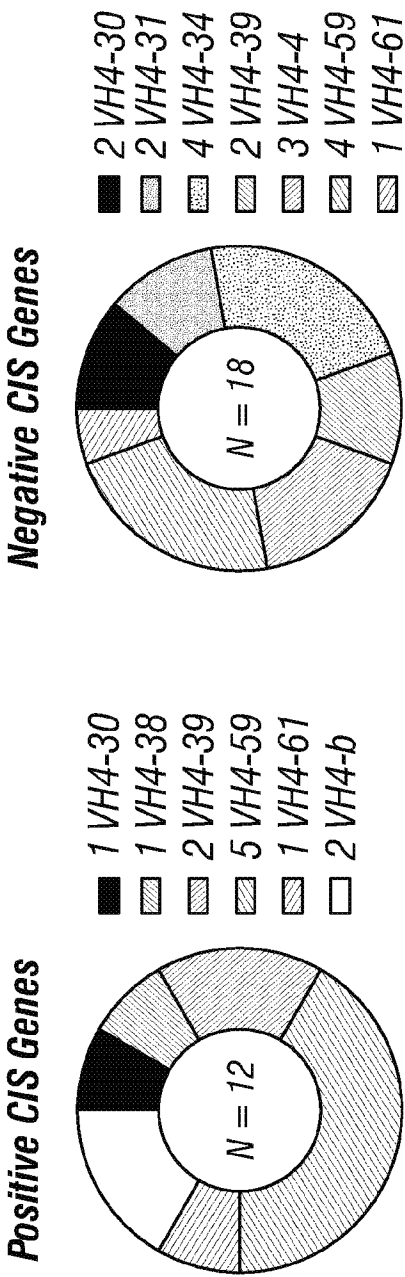
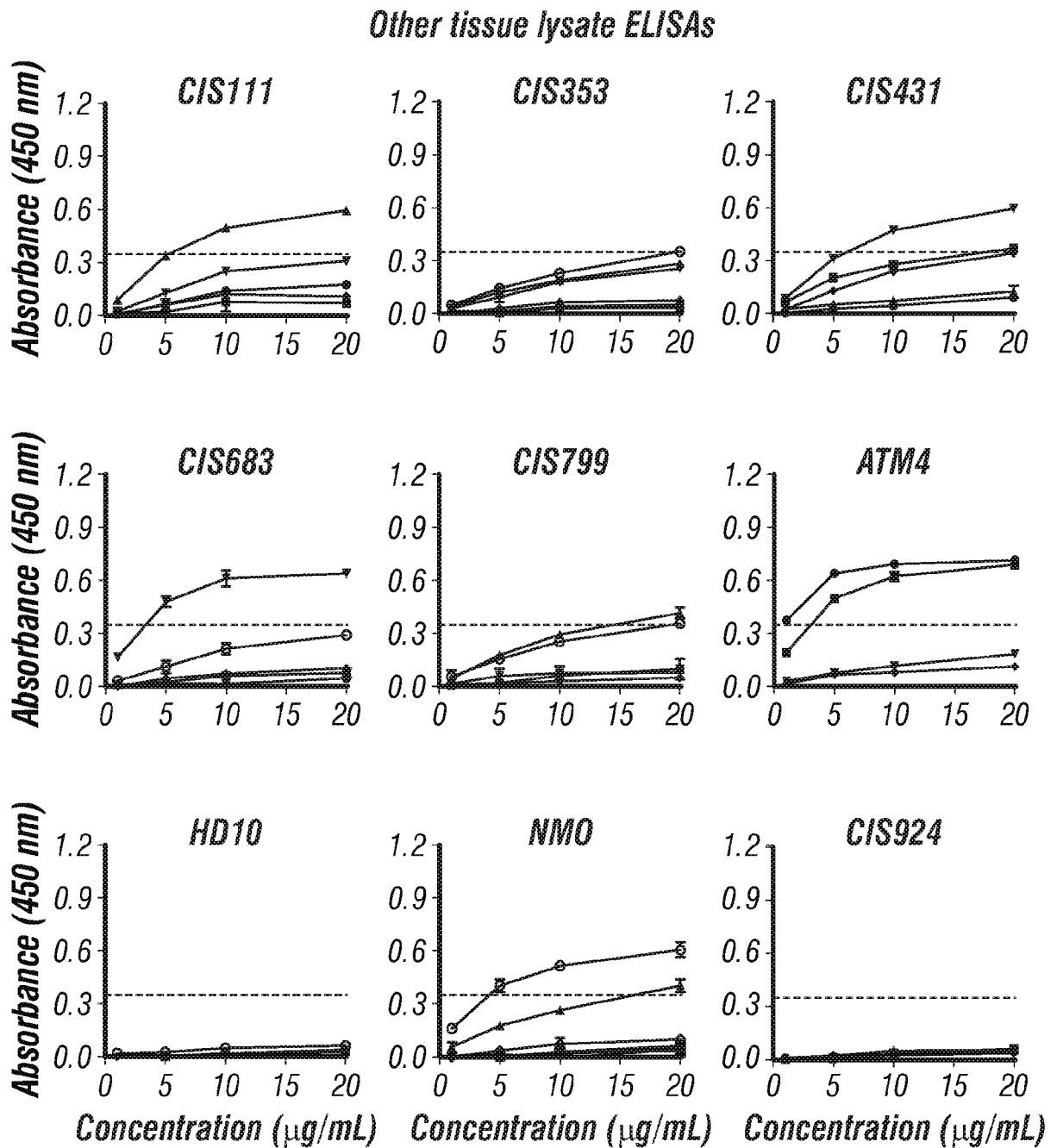


FIG. 24C

**FIG. 25A**

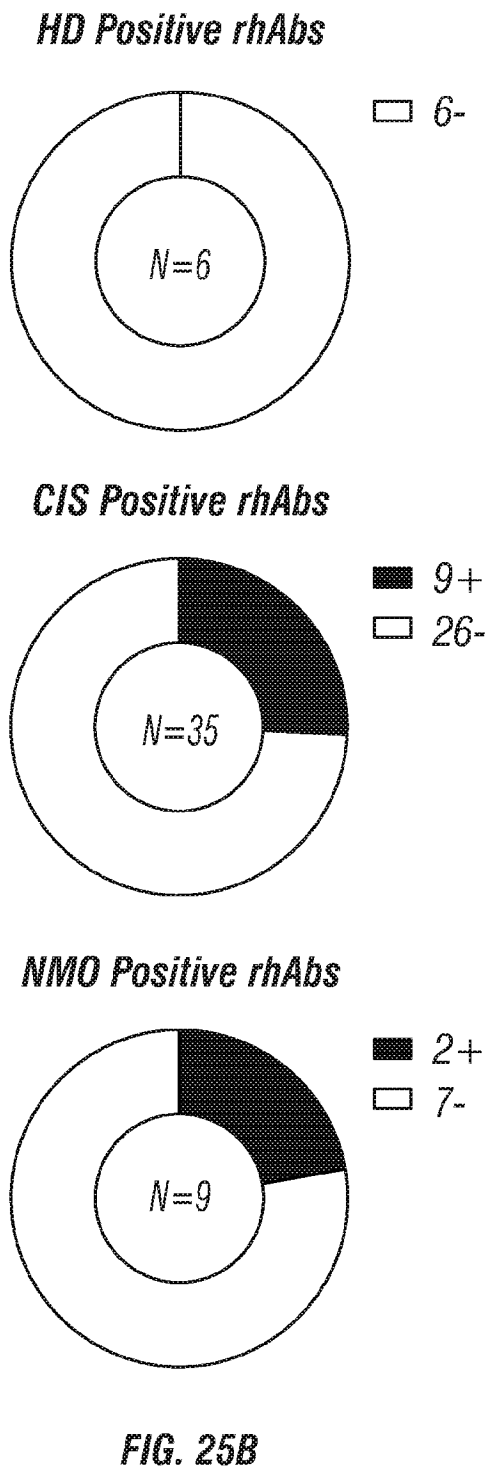
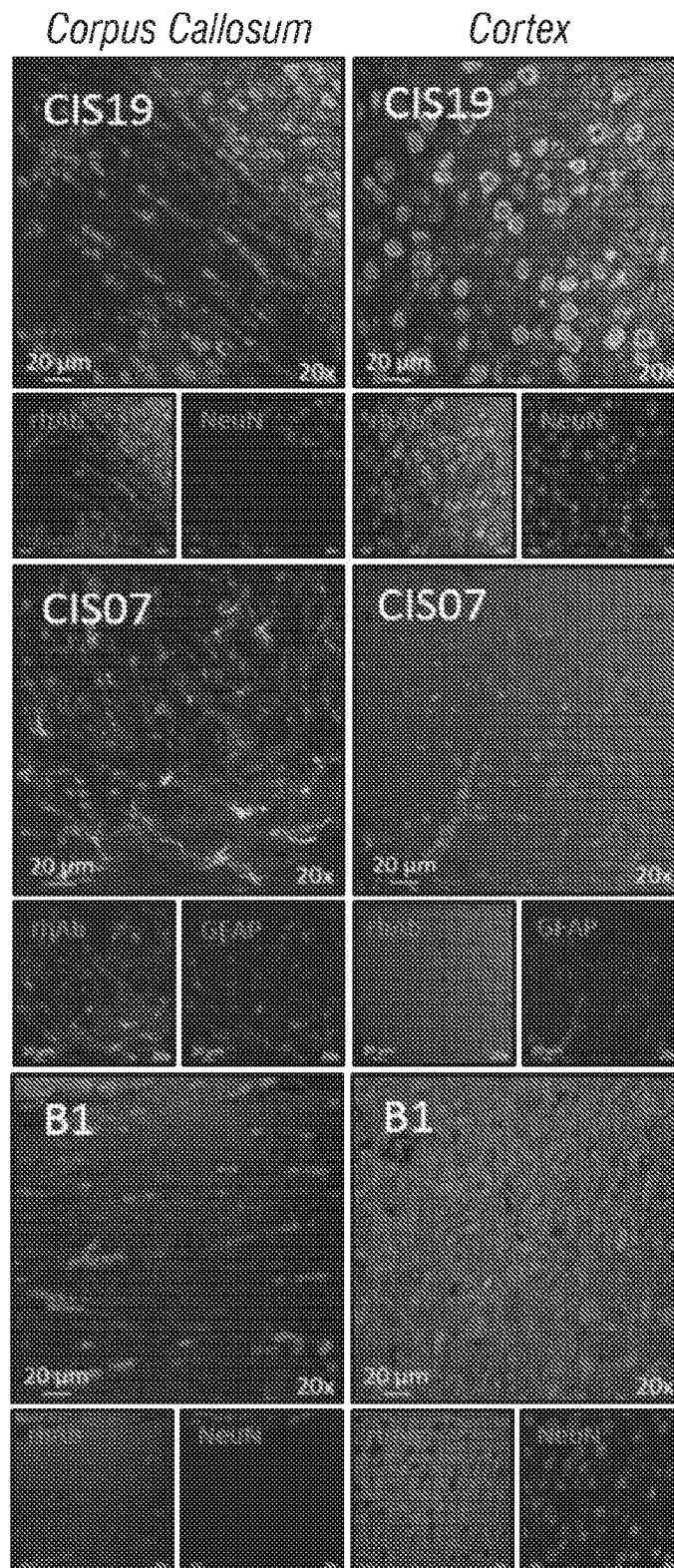
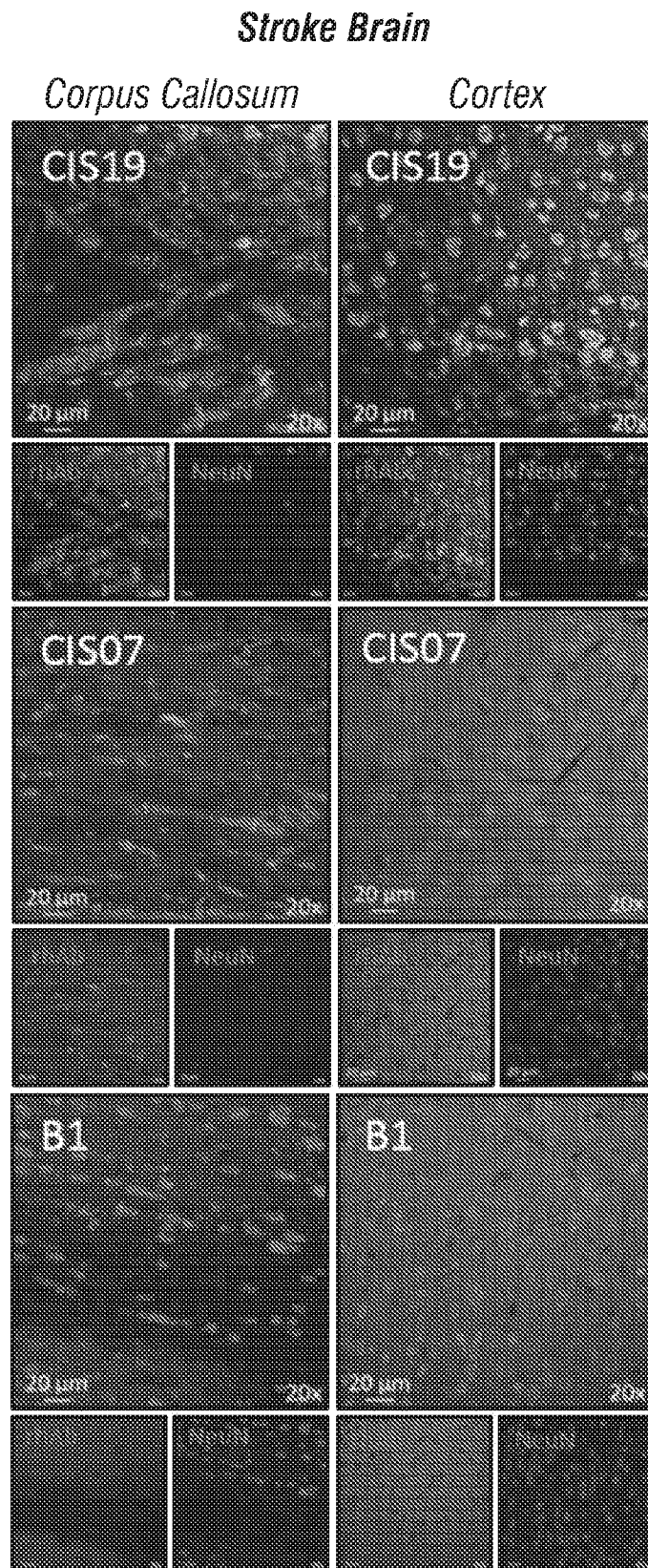
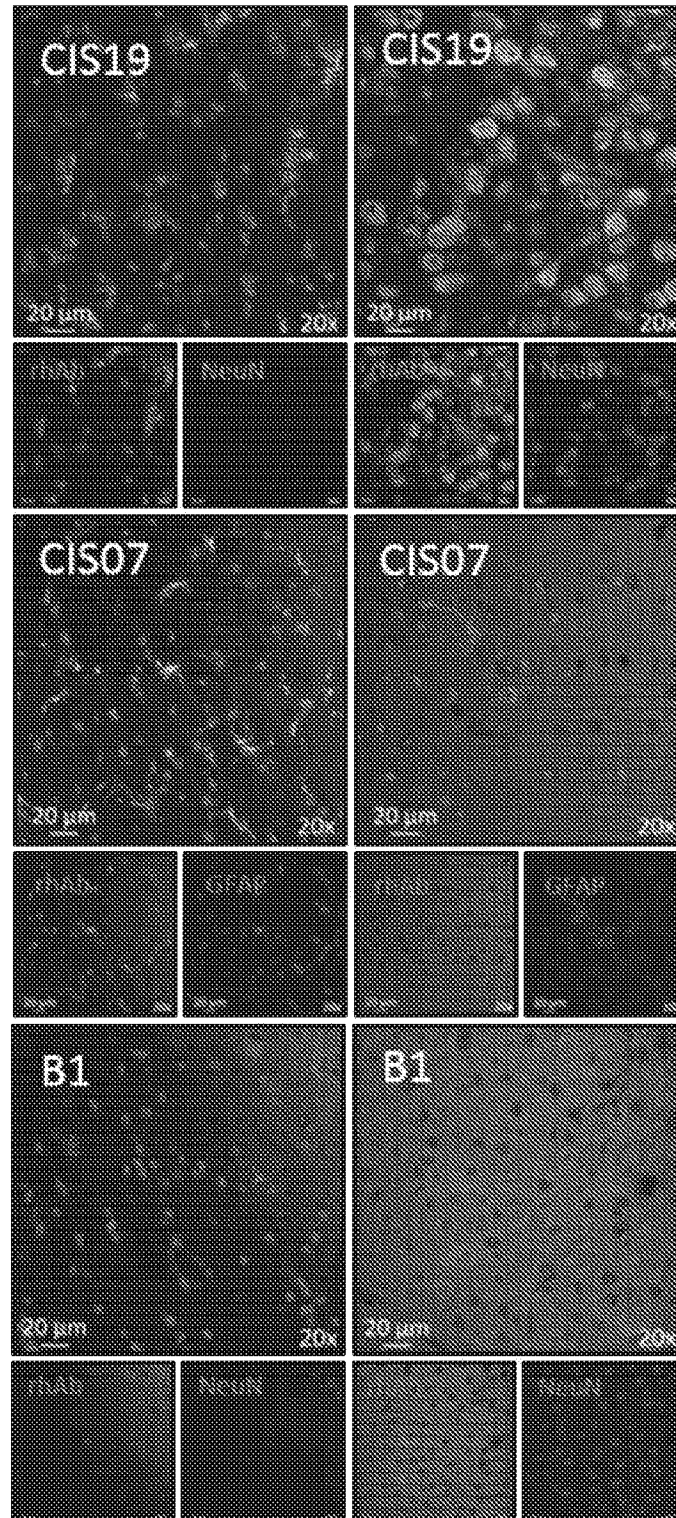


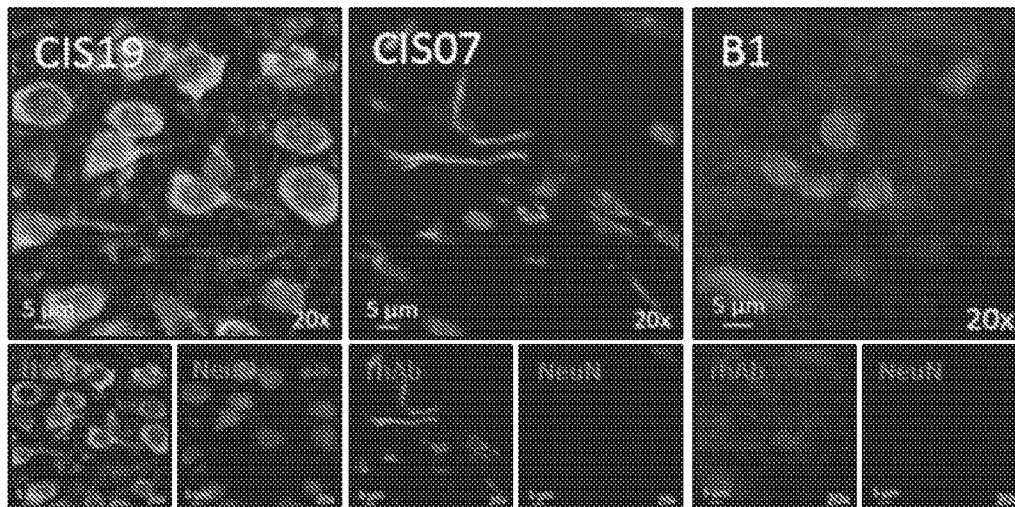
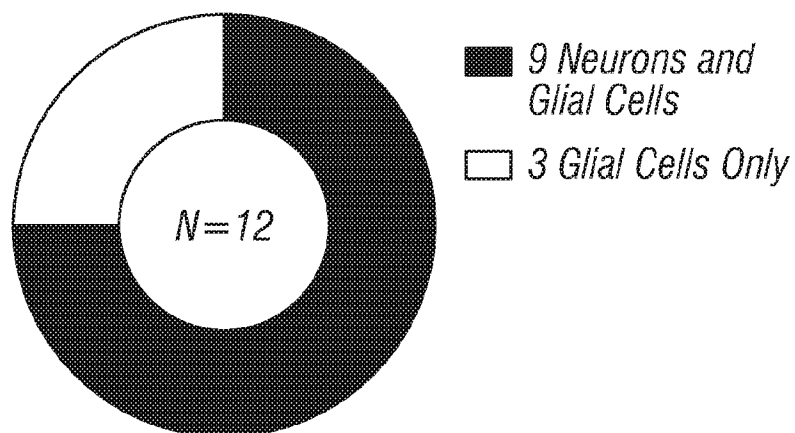


FIG. 25C

Plasmablast rhAbs react to neurons and glial cells in mouse brain***Healthy Brain******FIG. 26A***

**FIG. 26B**

EAE Brain*Corpus Callosum**Cortex****FIG. 26C***

**FIG. 26D****ELISA Positive CIS rhAbs****FIG. 26E**

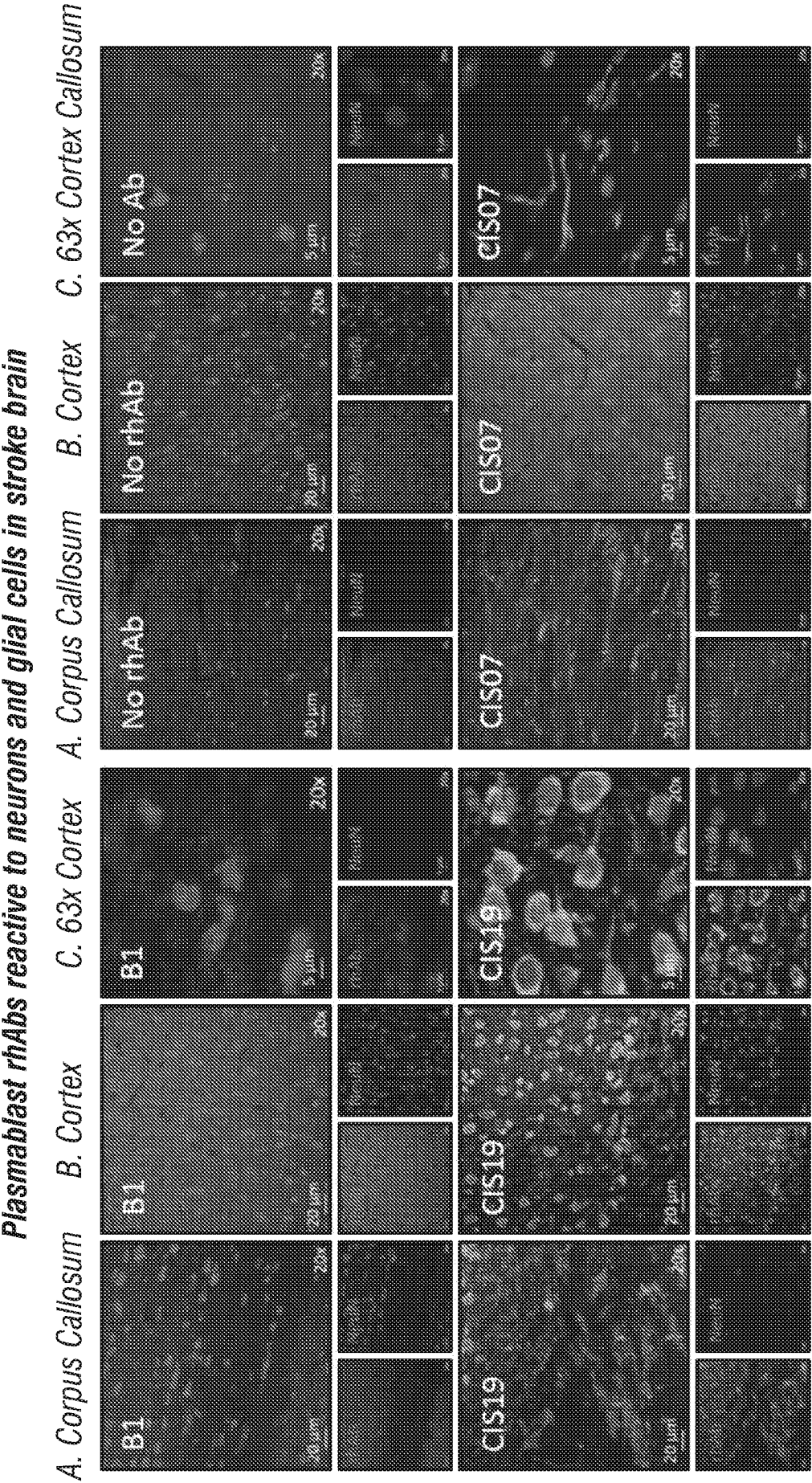


FIG. 27

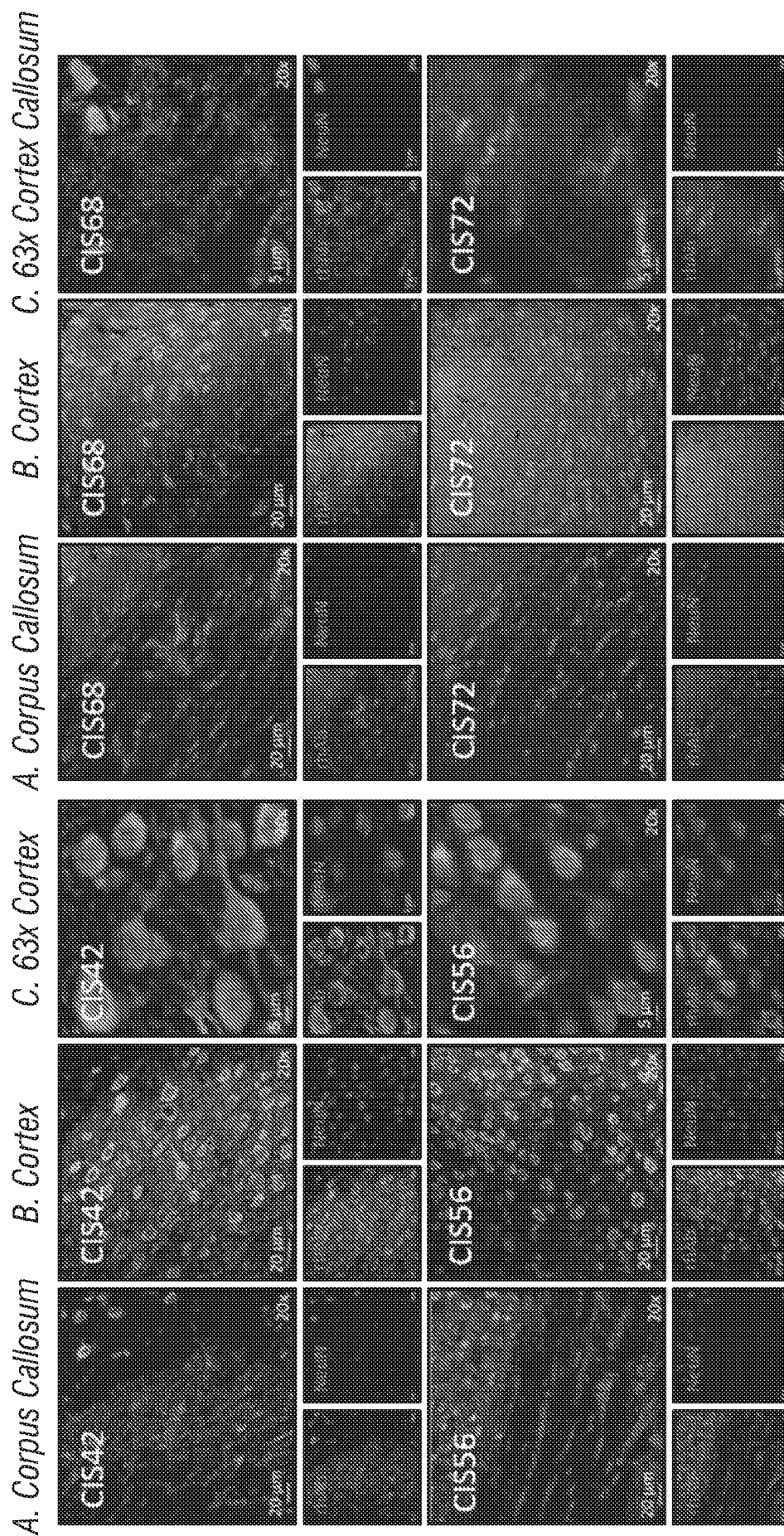


FIG. 27
(Cont'd.)

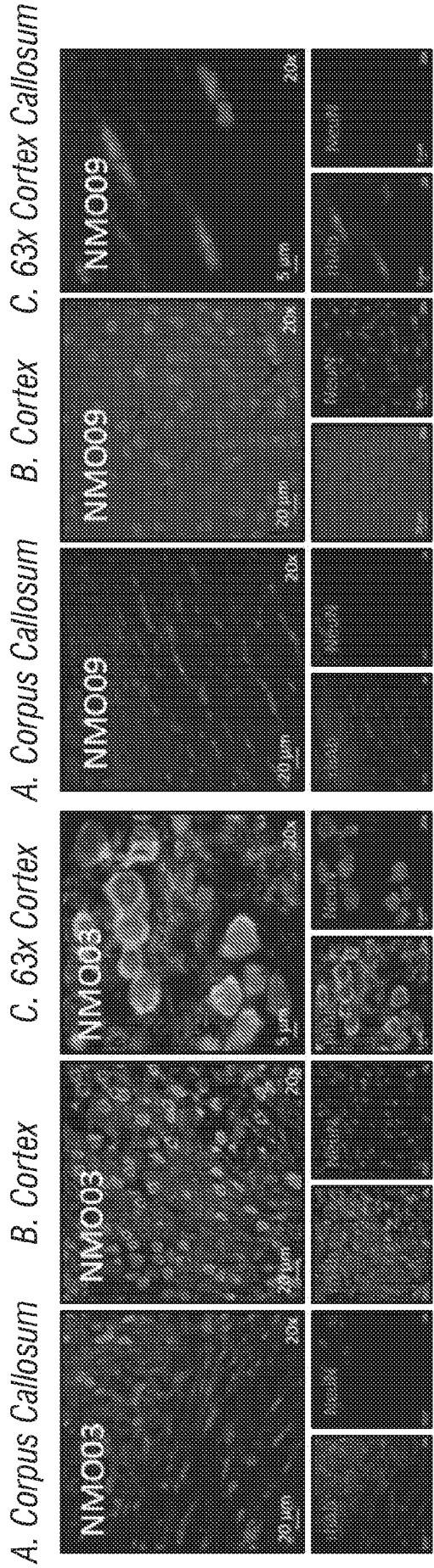


FIG. 27
(Cont'd.)

Reactivity of more rhAbs with stroke mouse brain

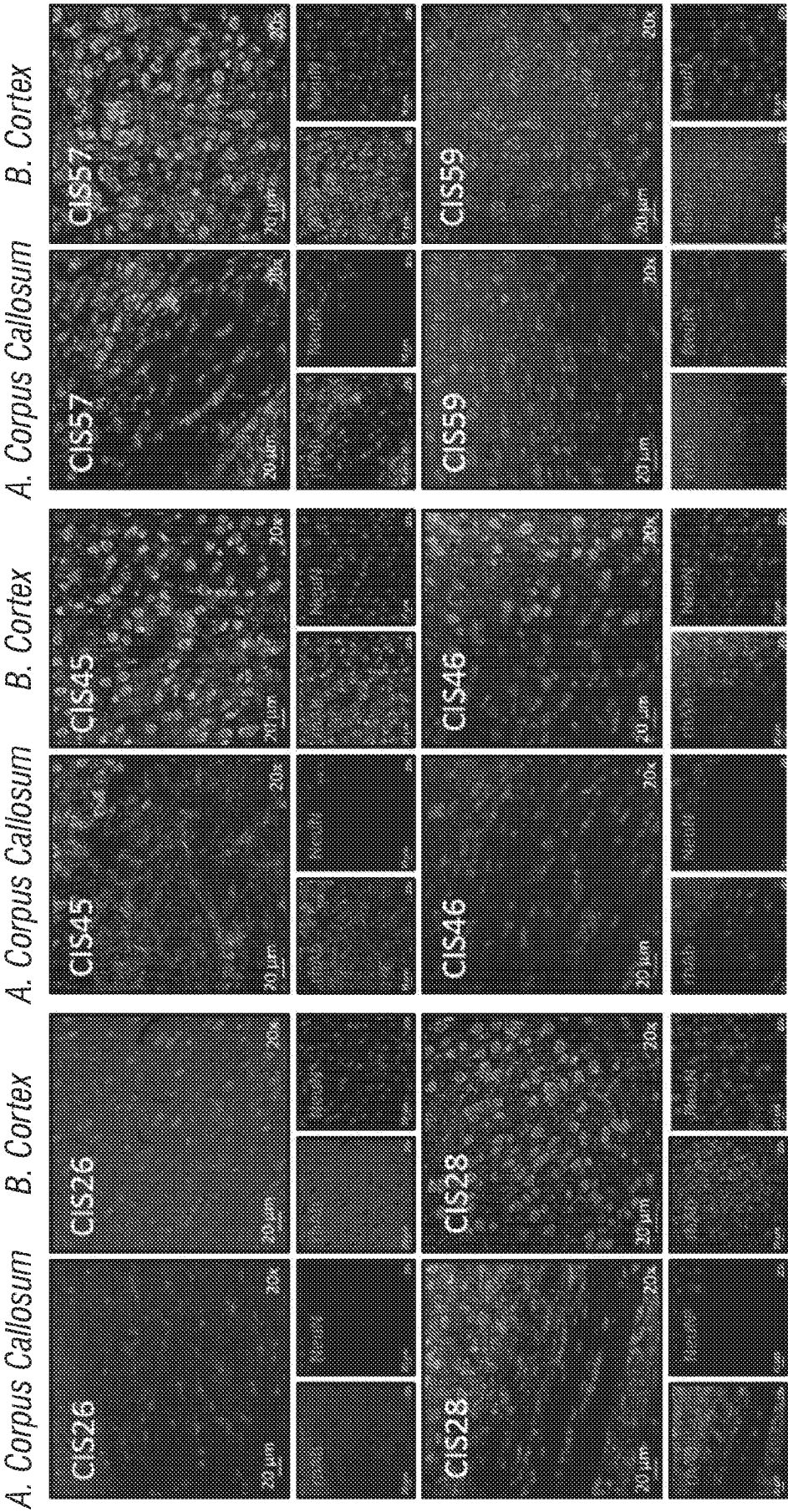


FIG. 28

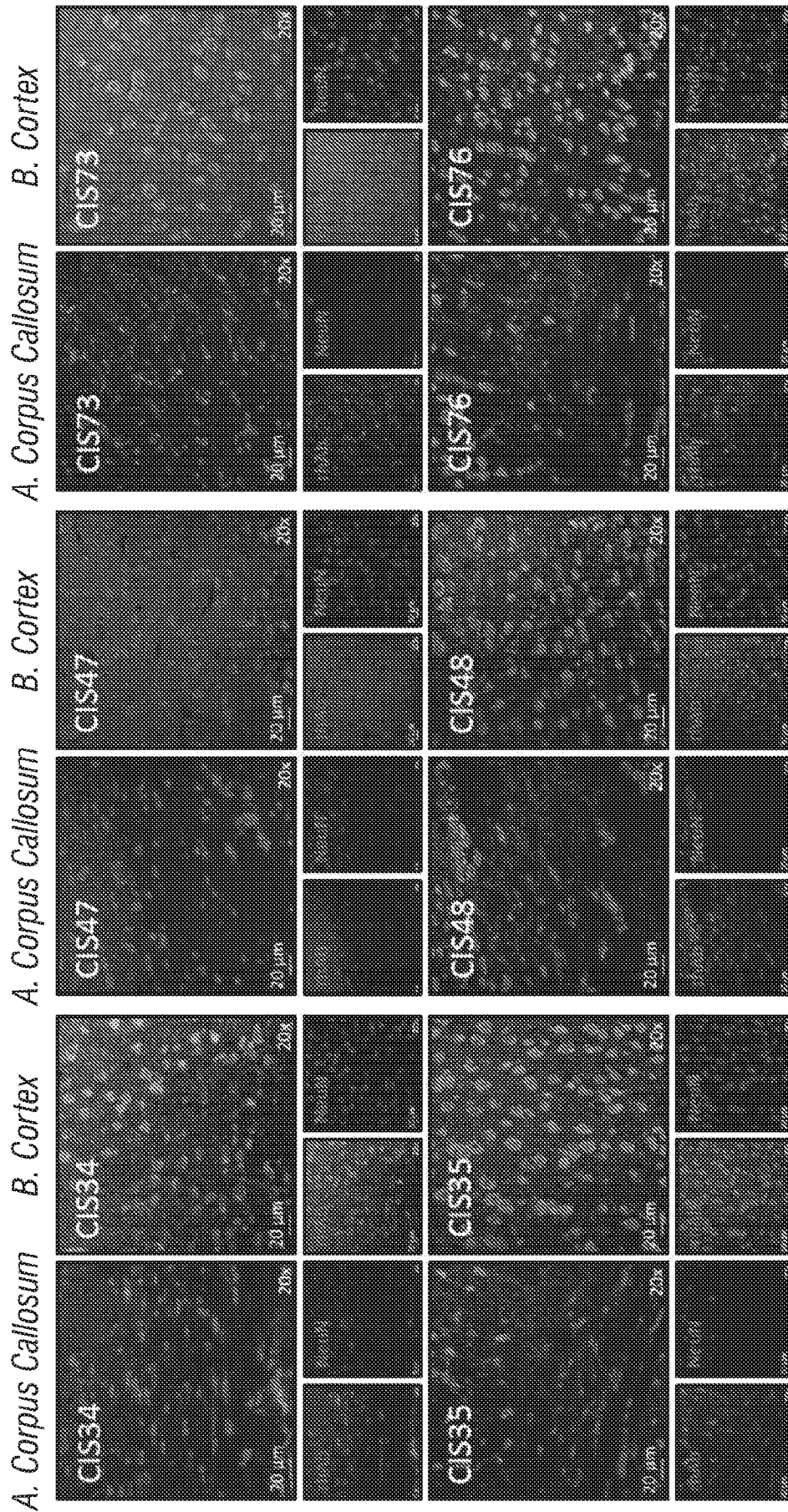


FIG. 28
(Cont'd.)

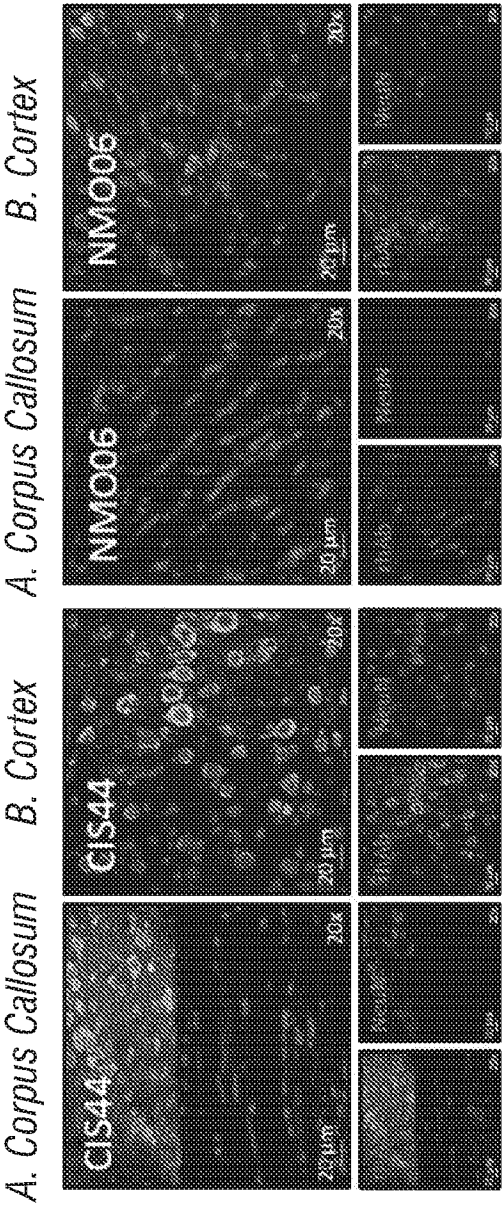


FIG. 28
(Cont'd.)

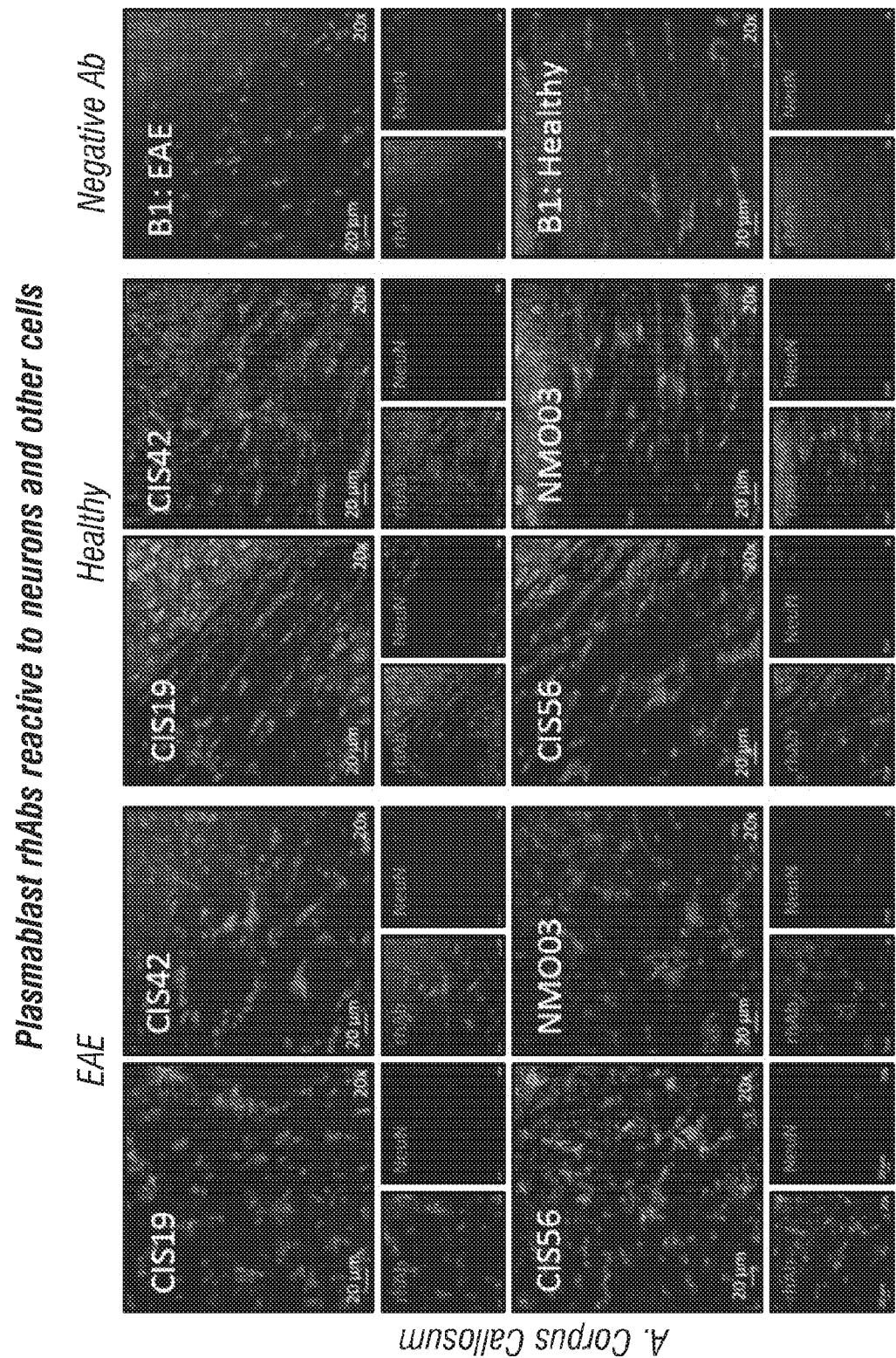


FIG. 29

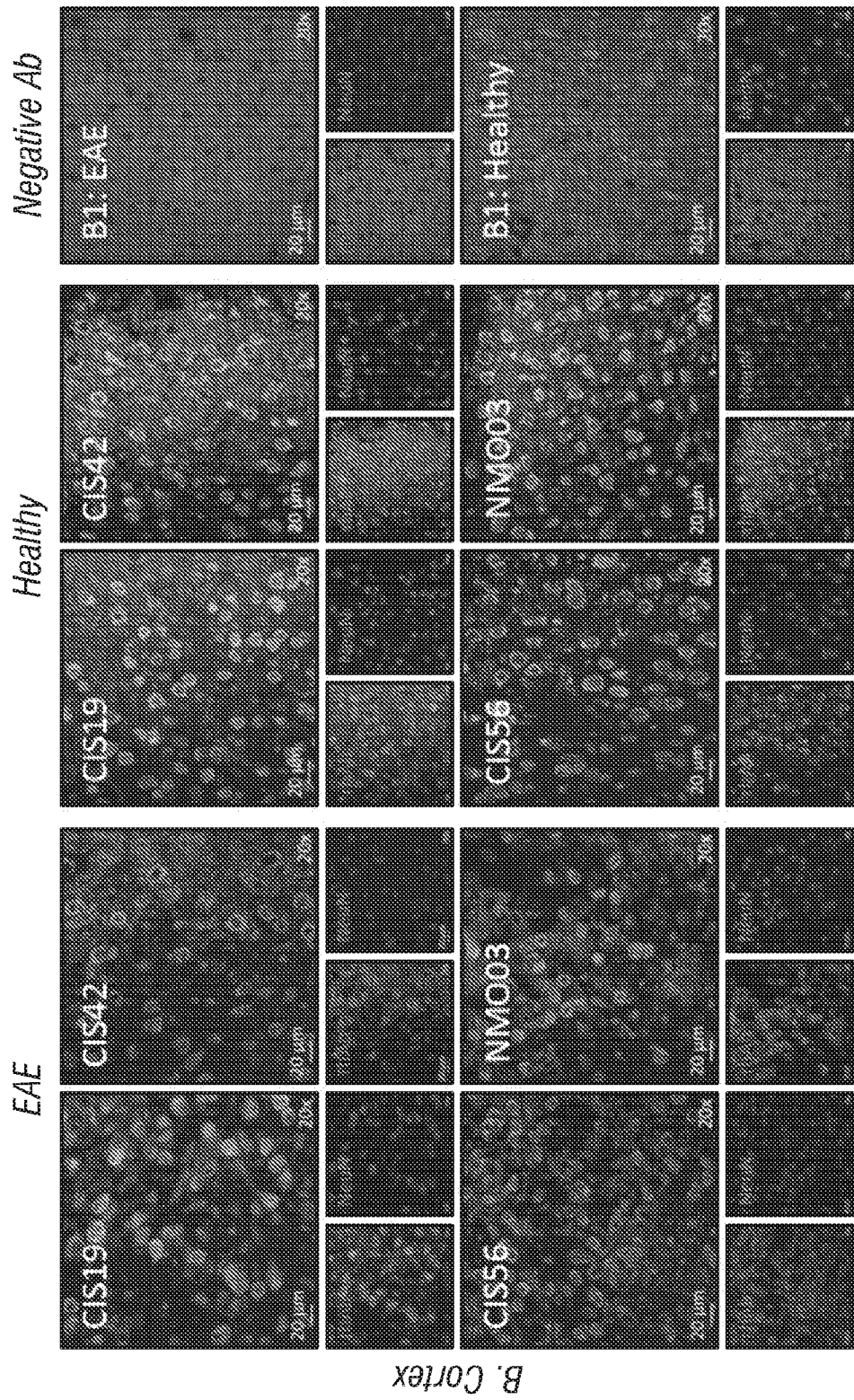


FIG. 29
(Cont'd.)

rhAbs on EAE brain

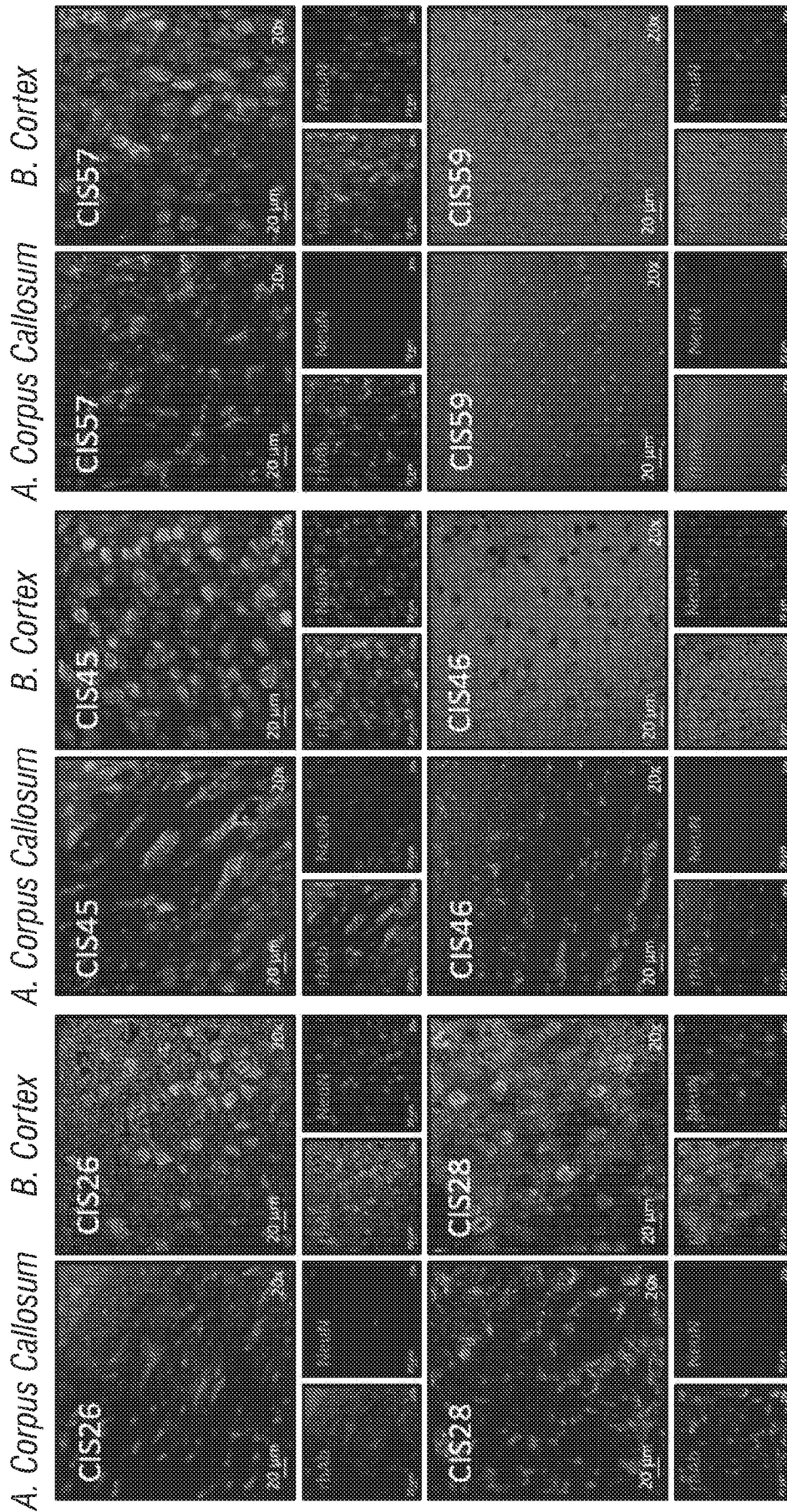


FIG. 30

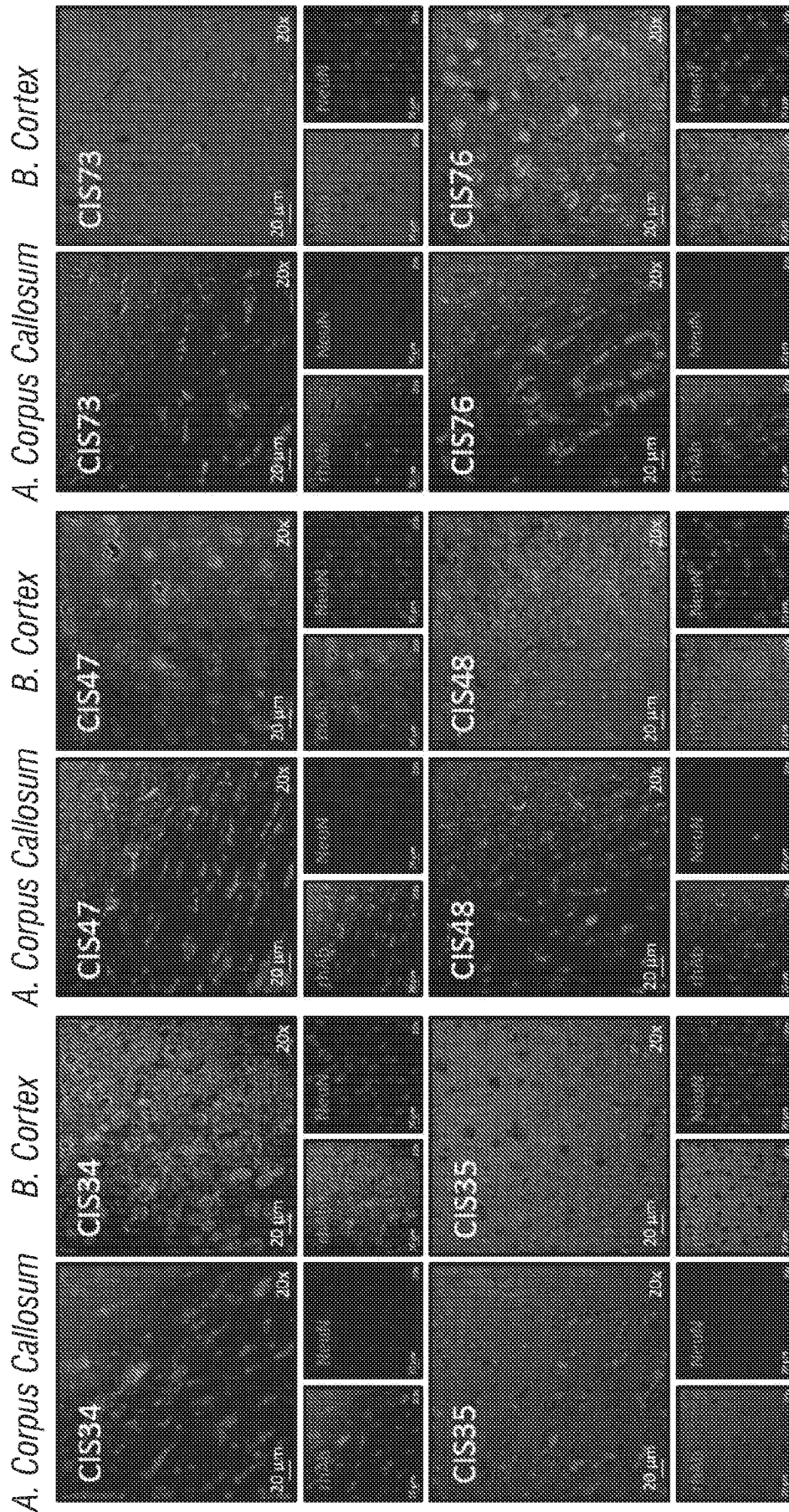


FIG. 30
(Cont'd.)

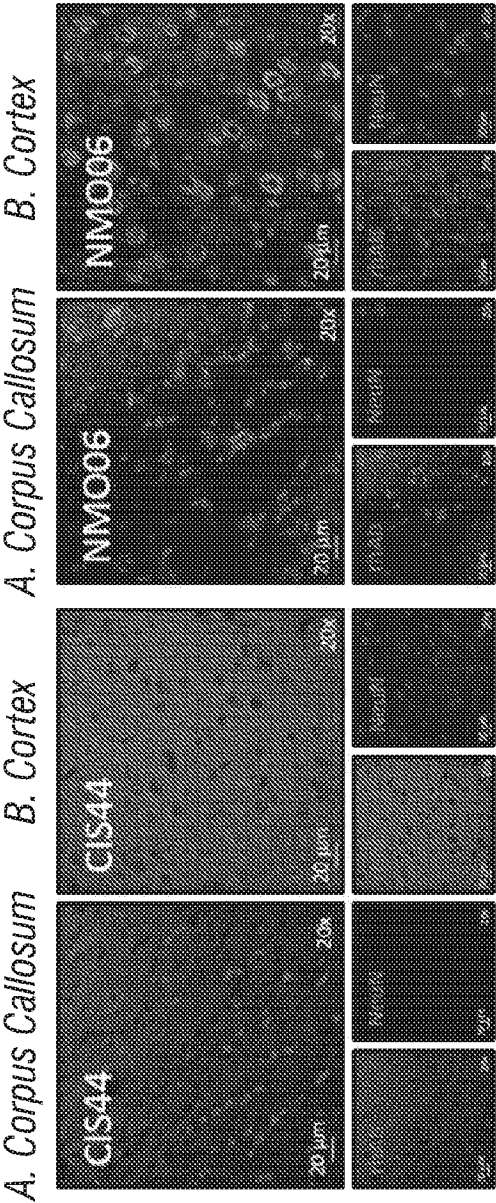


FIG. 30
(Cont'd.)

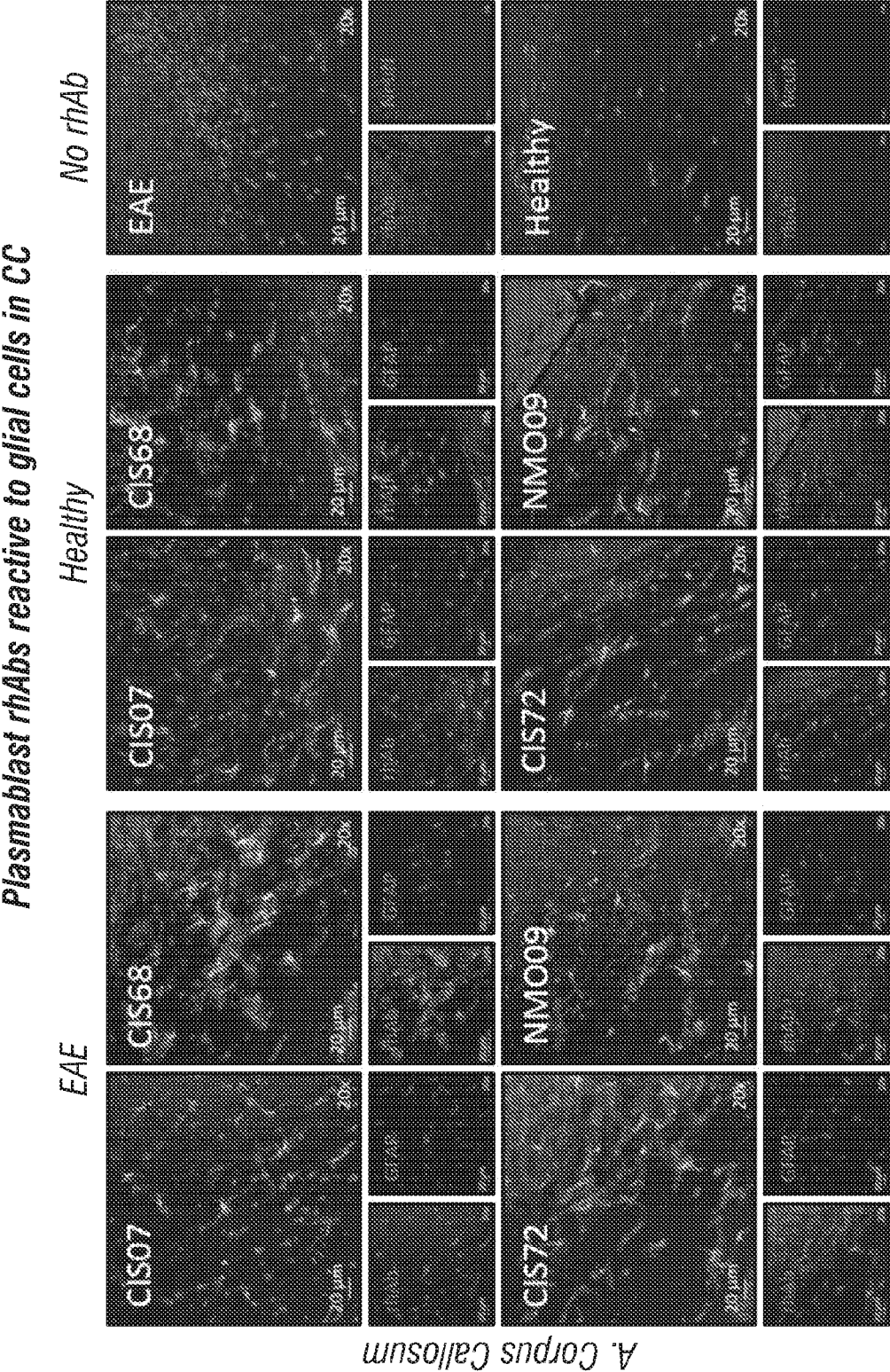


FIG. 31

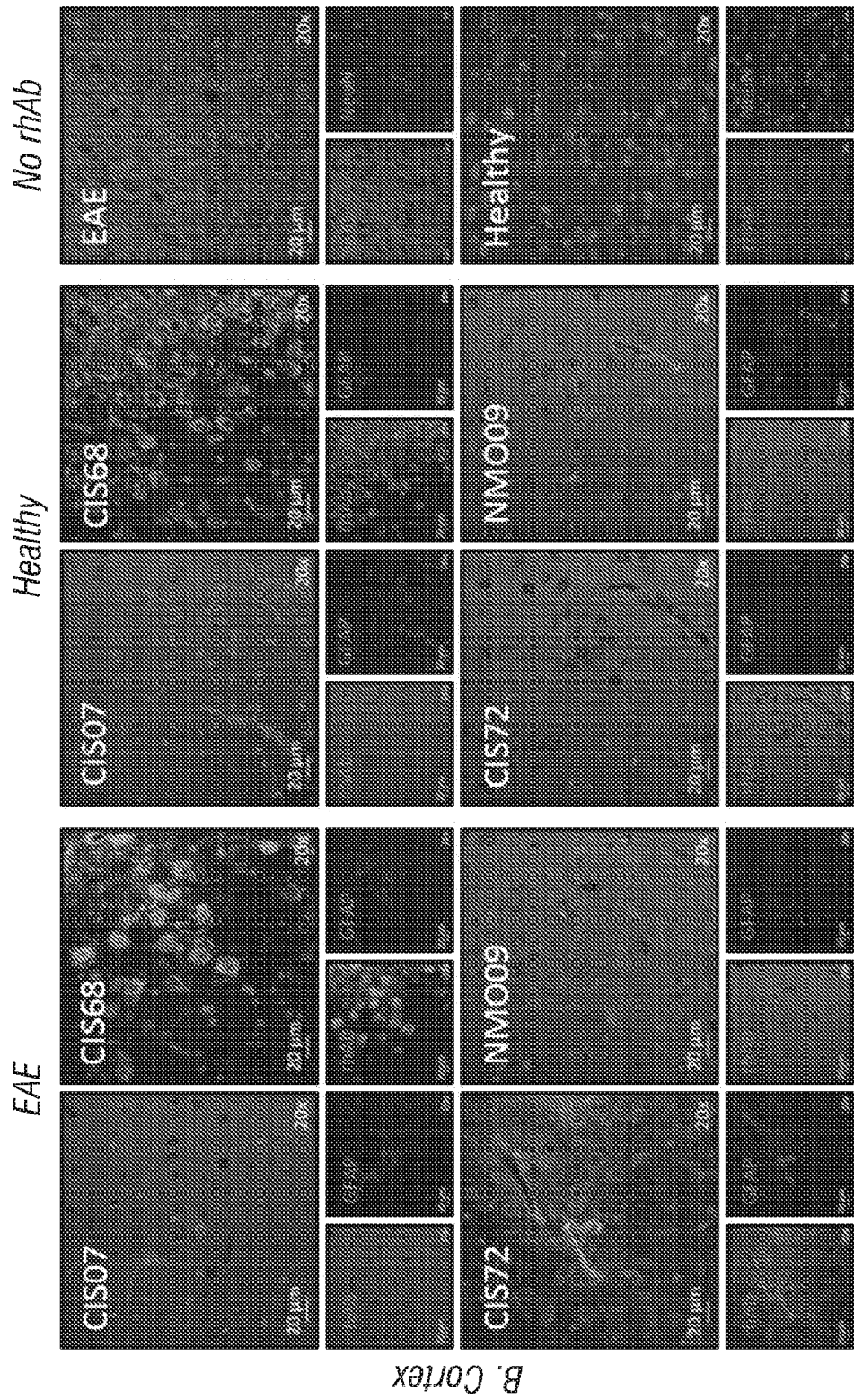


FIG. 31
(Cont'd.)

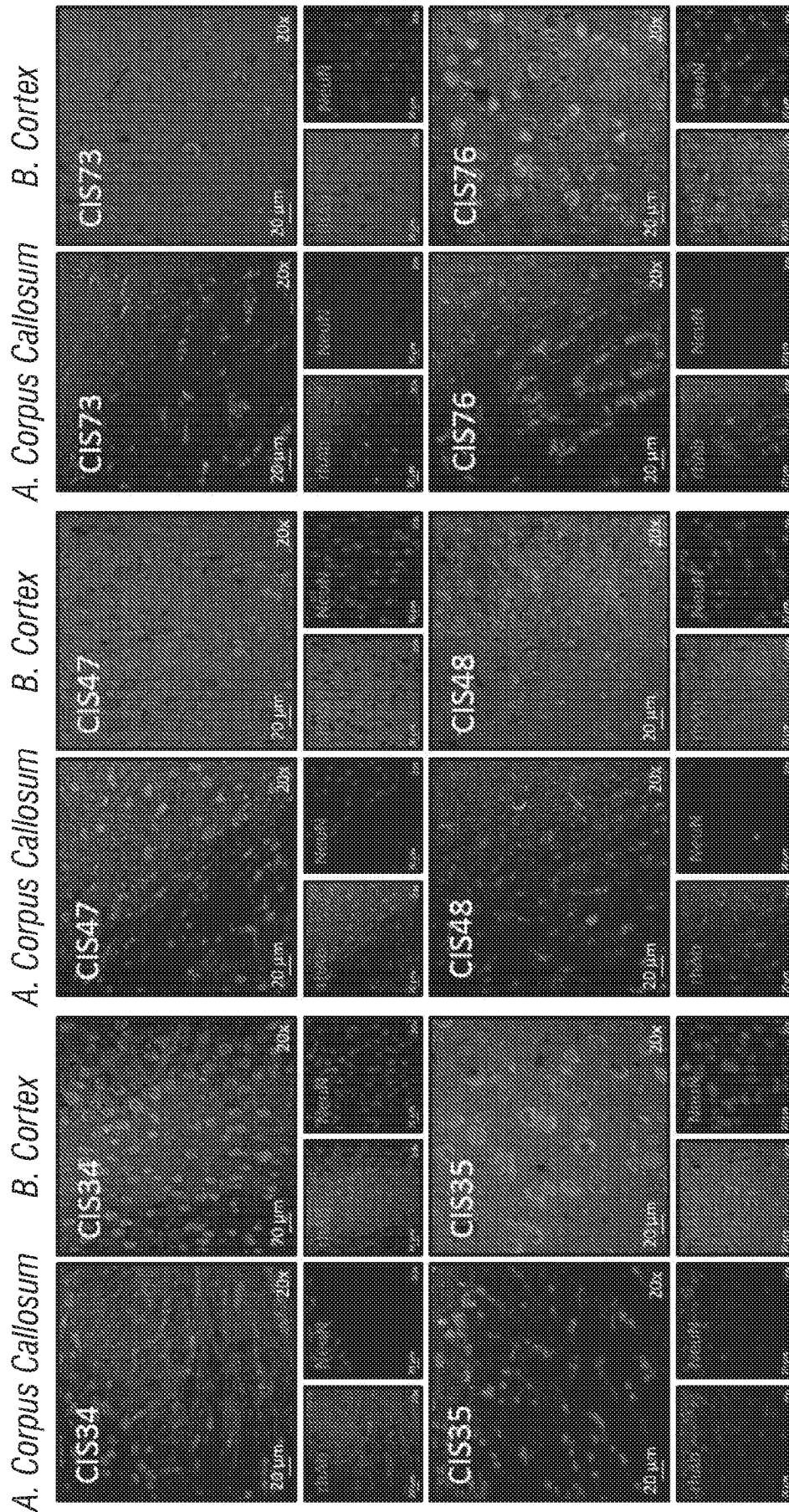


FIG. 32
(Cont'd.)

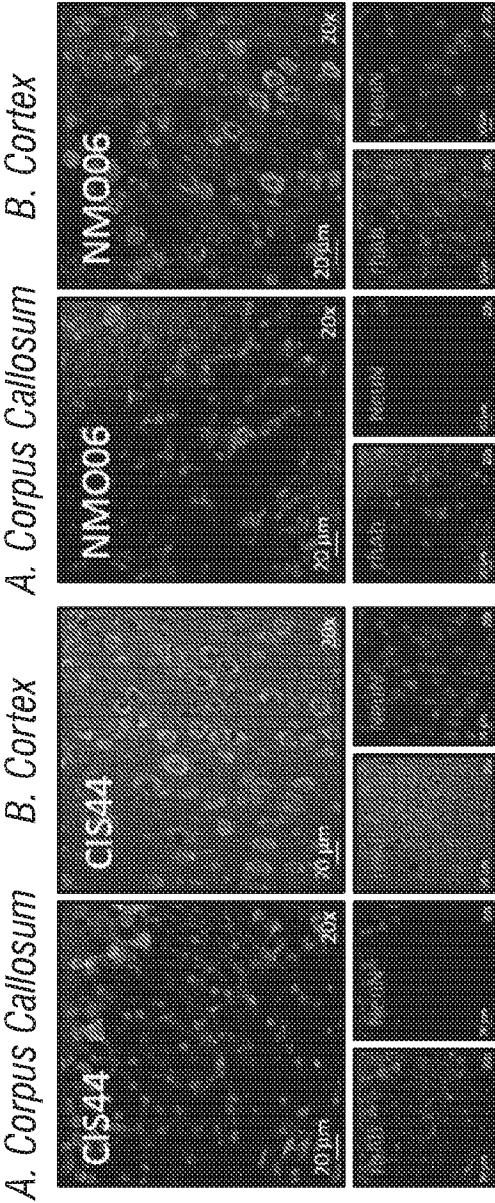


FIG. 32
(Cont'd.)

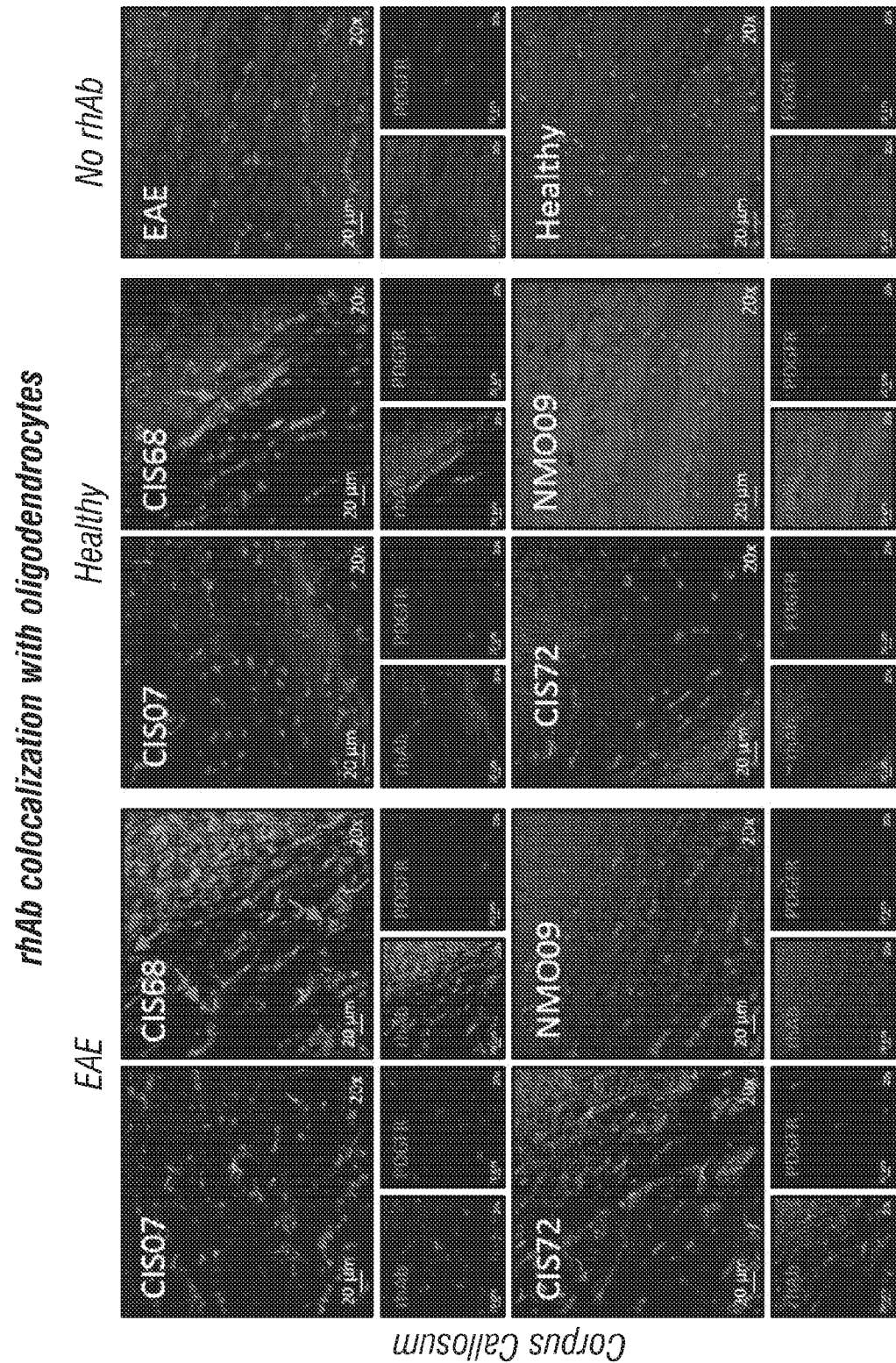


FIG. 33

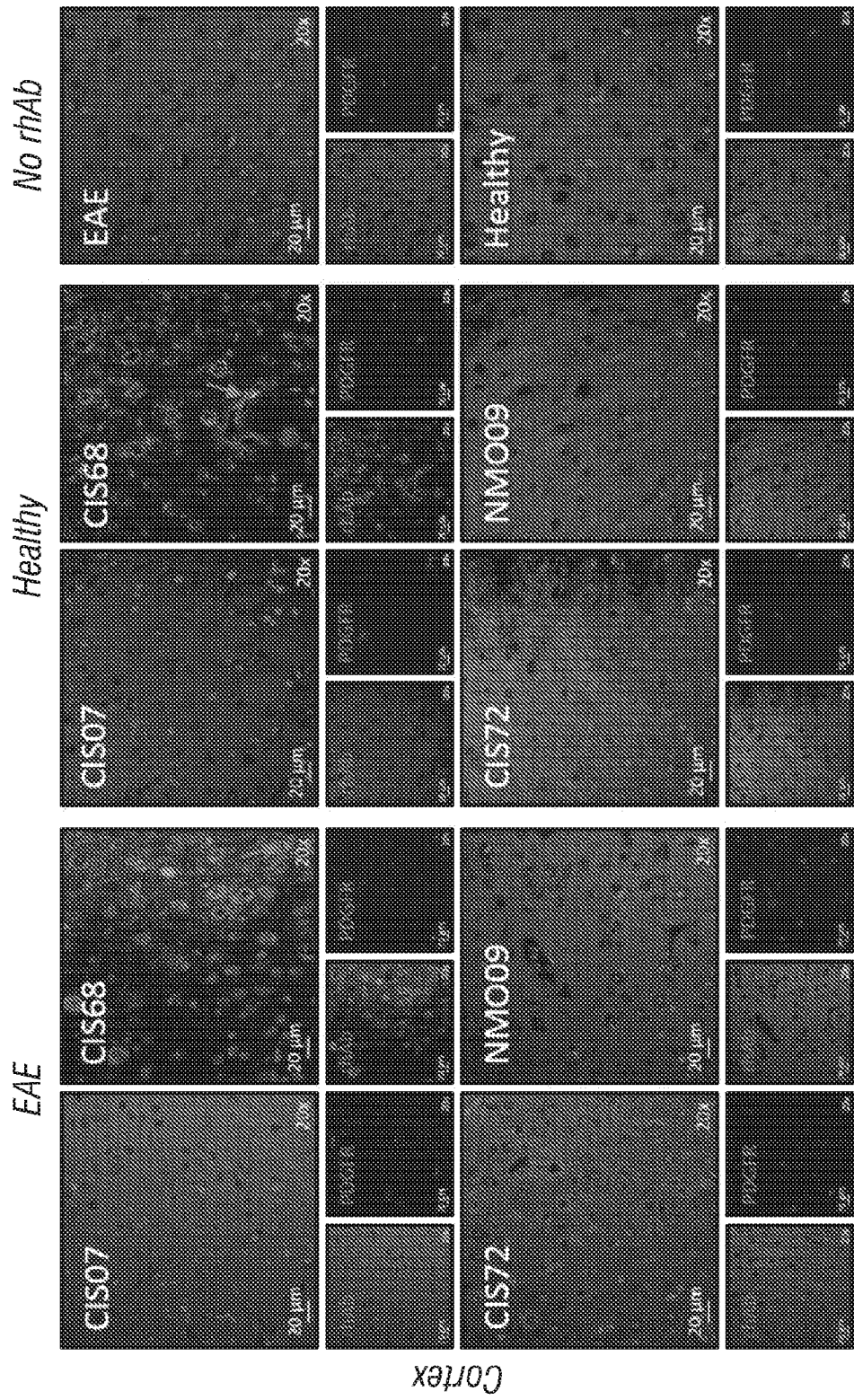
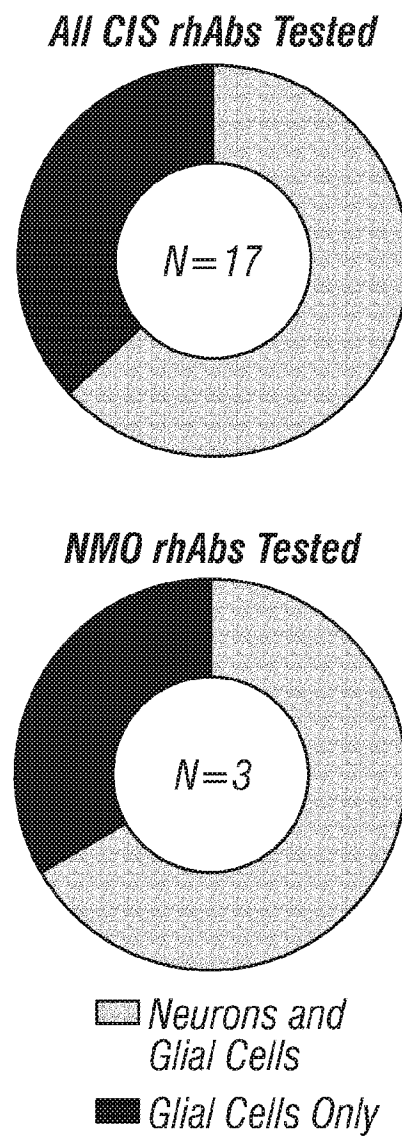


FIG. 33
(Cont'd.)

**FIG. 34A**

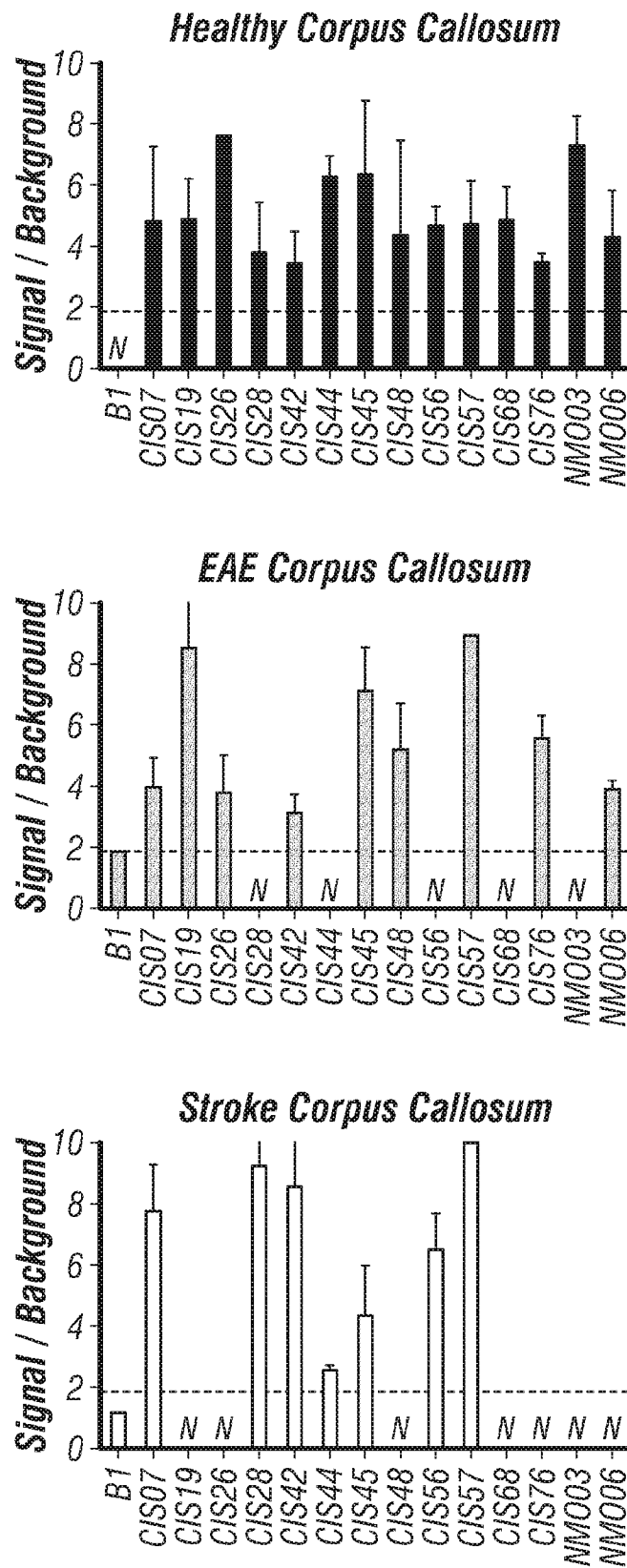


FIG. 34B

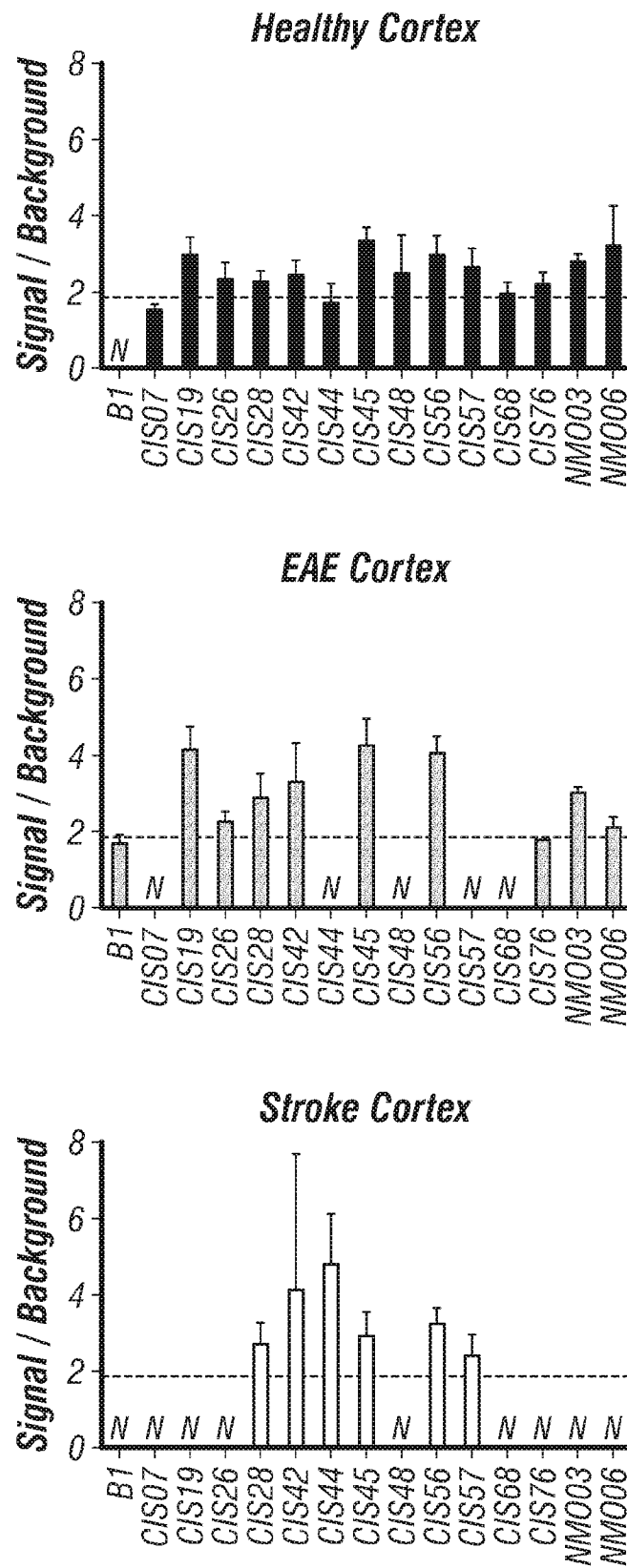
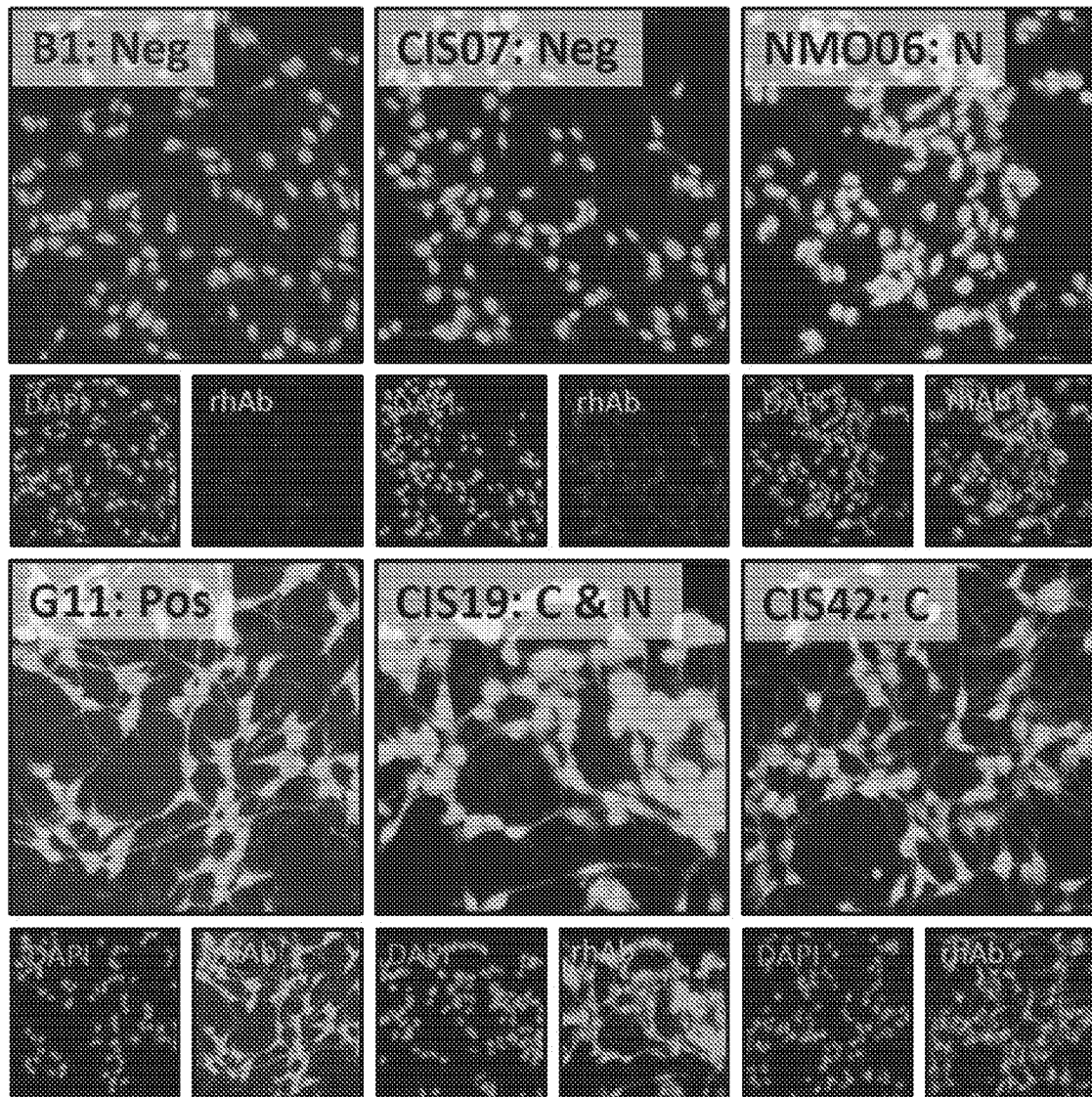


FIG. 34B
(Cont'd.)

Reactivity to Sy5y by ICC**FIG. 35A**

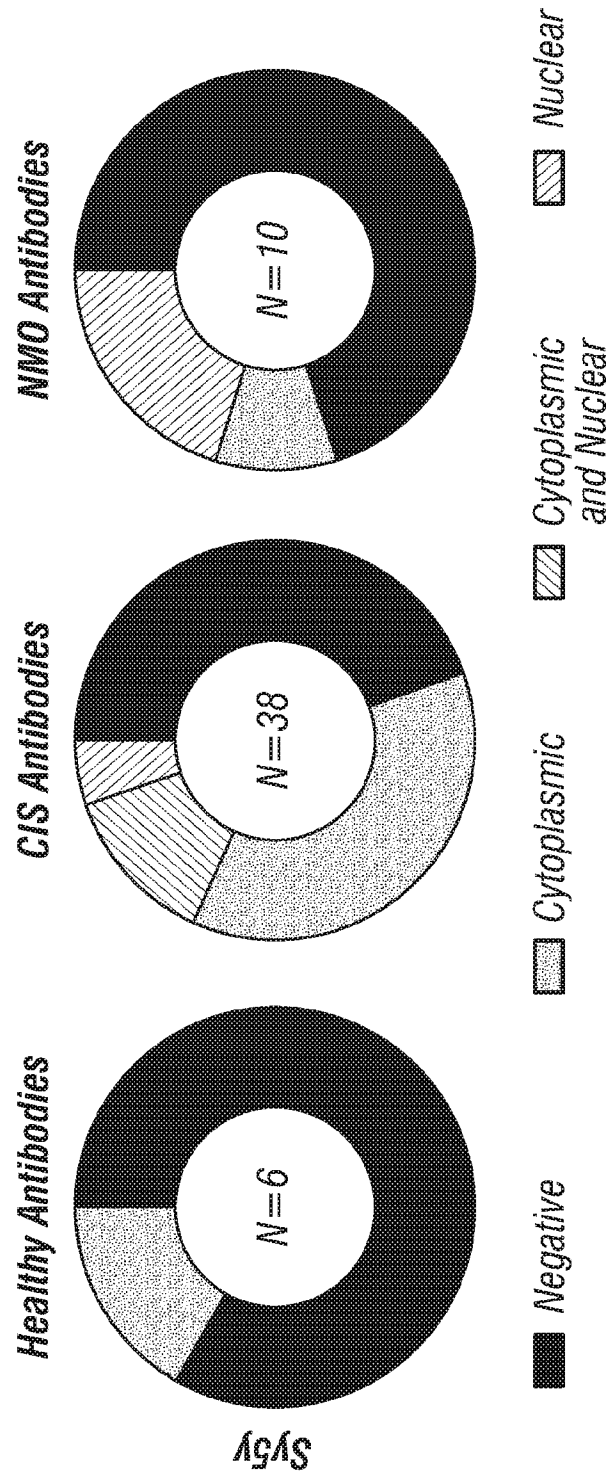


FIG. 35B

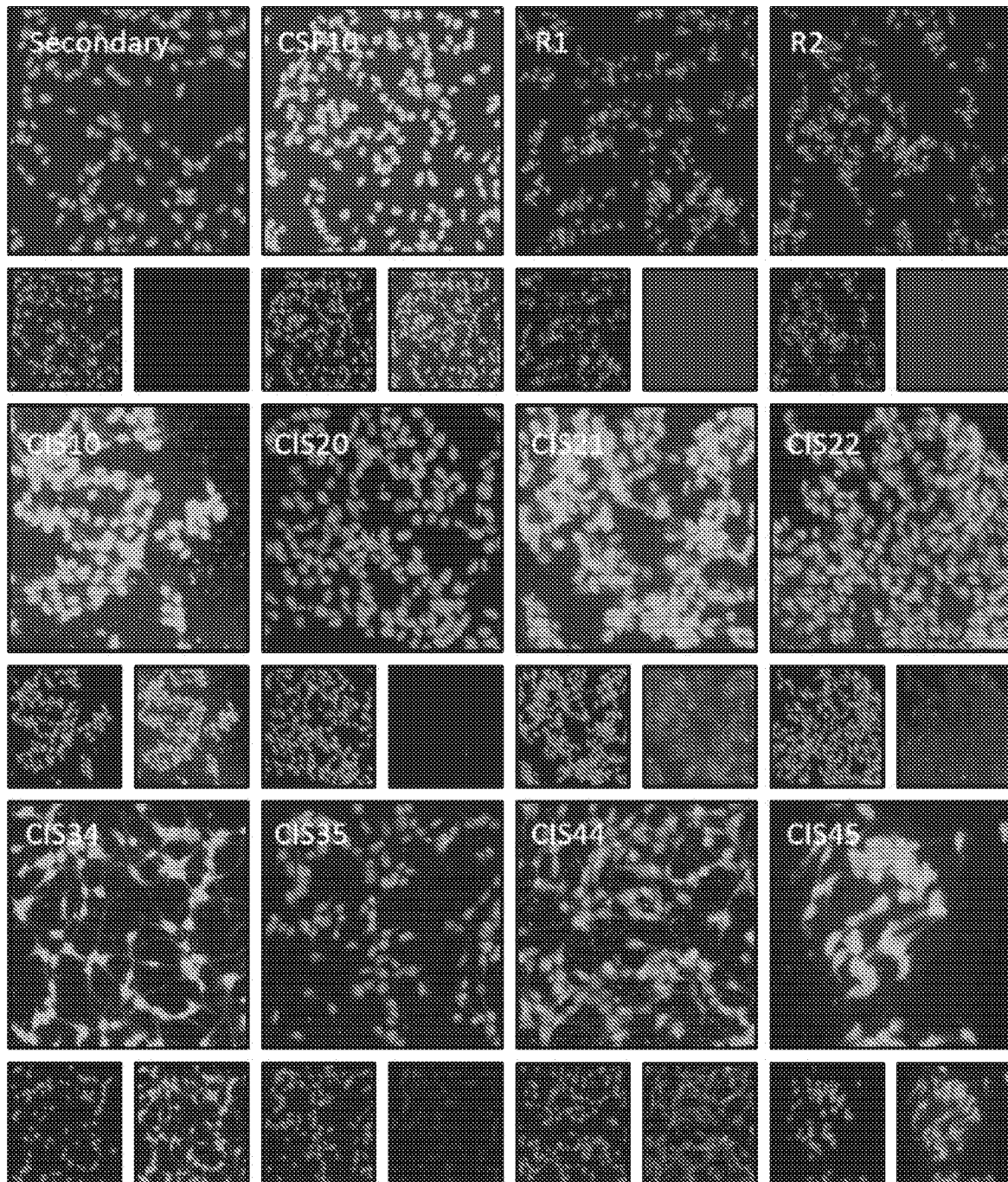


FIG. 36 *Reactivity to Sy5y by ICC*

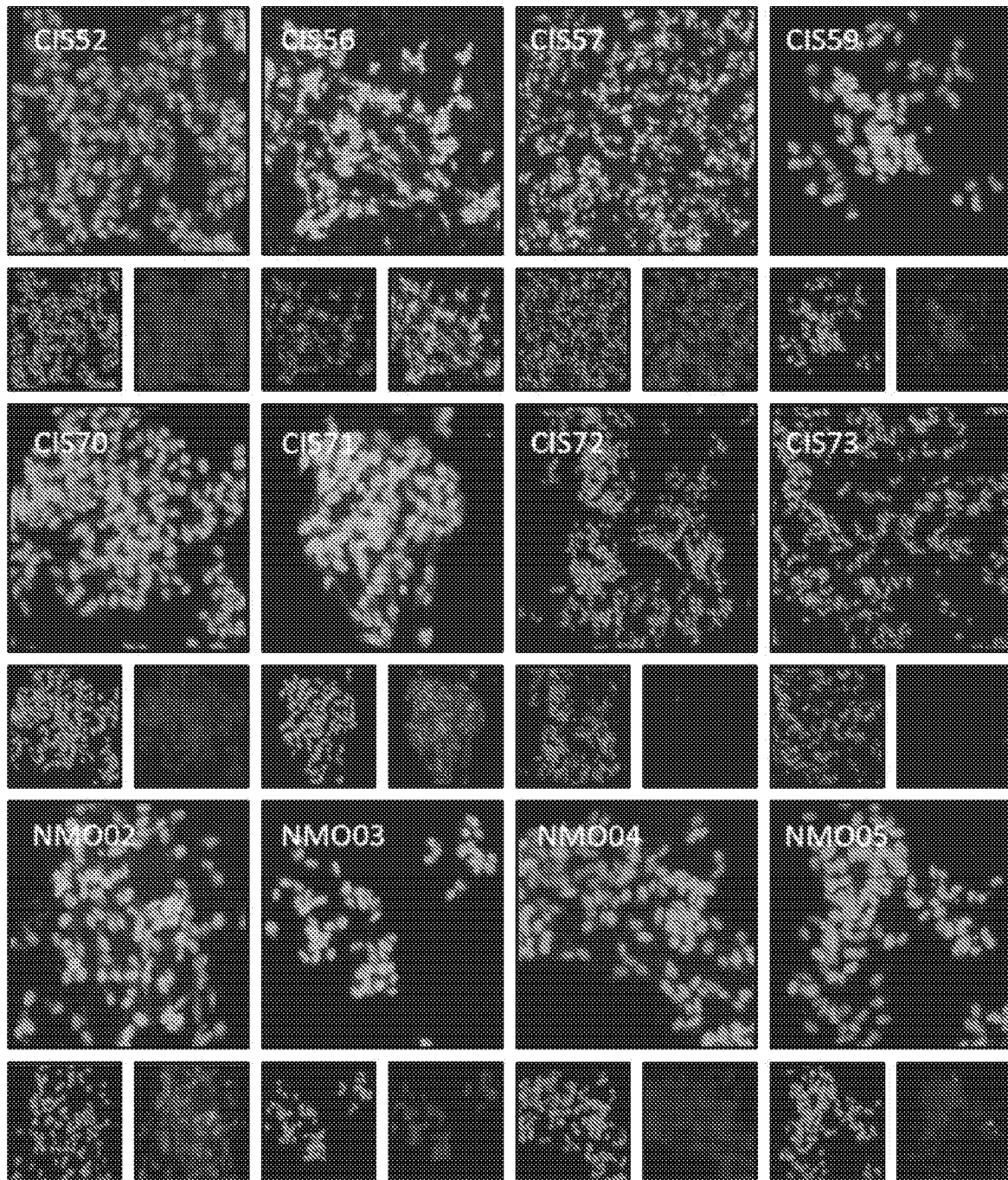
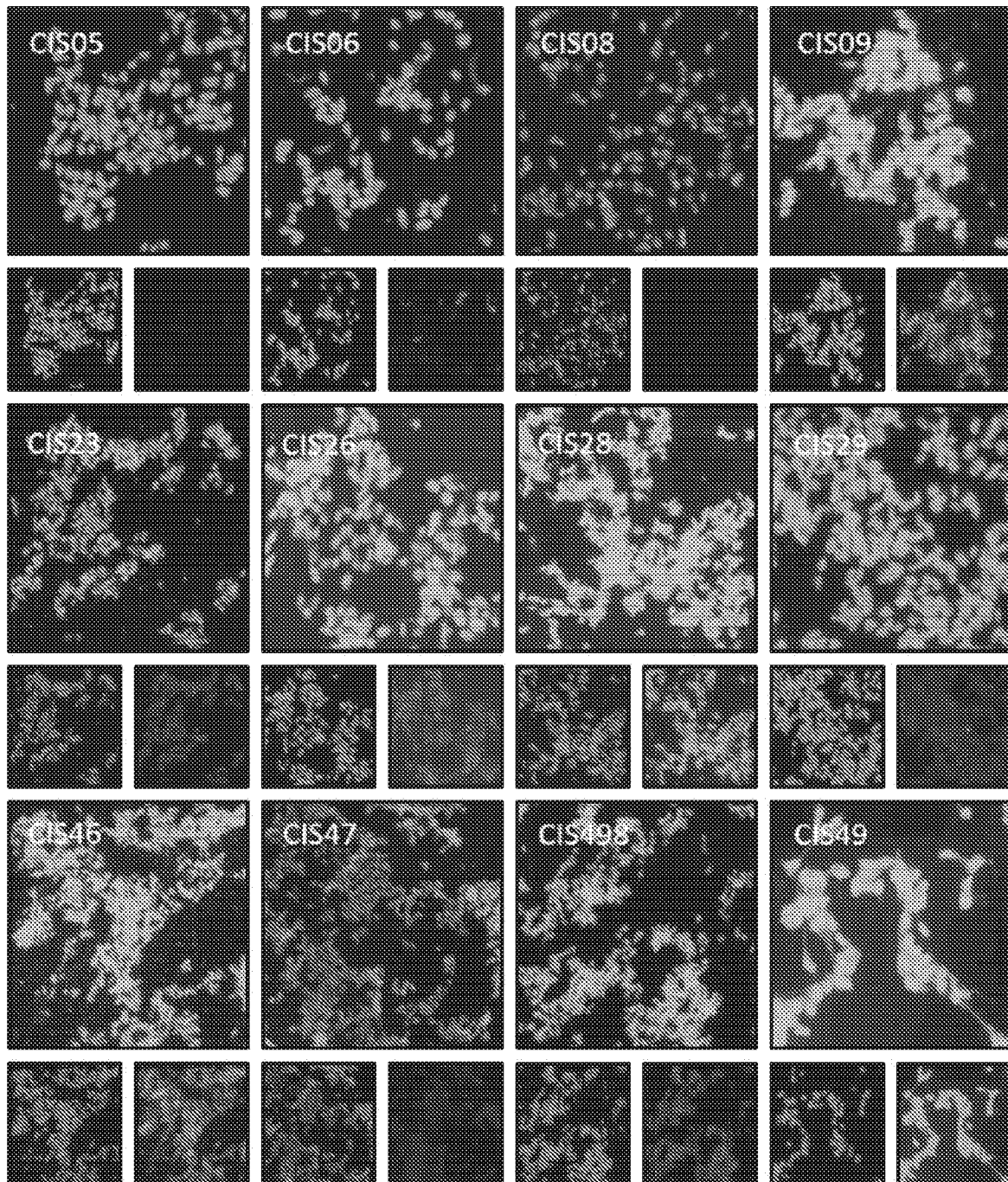


FIG. 36
(Cont'd.)

Reactivity to Sy5y by ICC



Reactivity to Sy5y by ICC

FIG. 36
(Cont'd.)

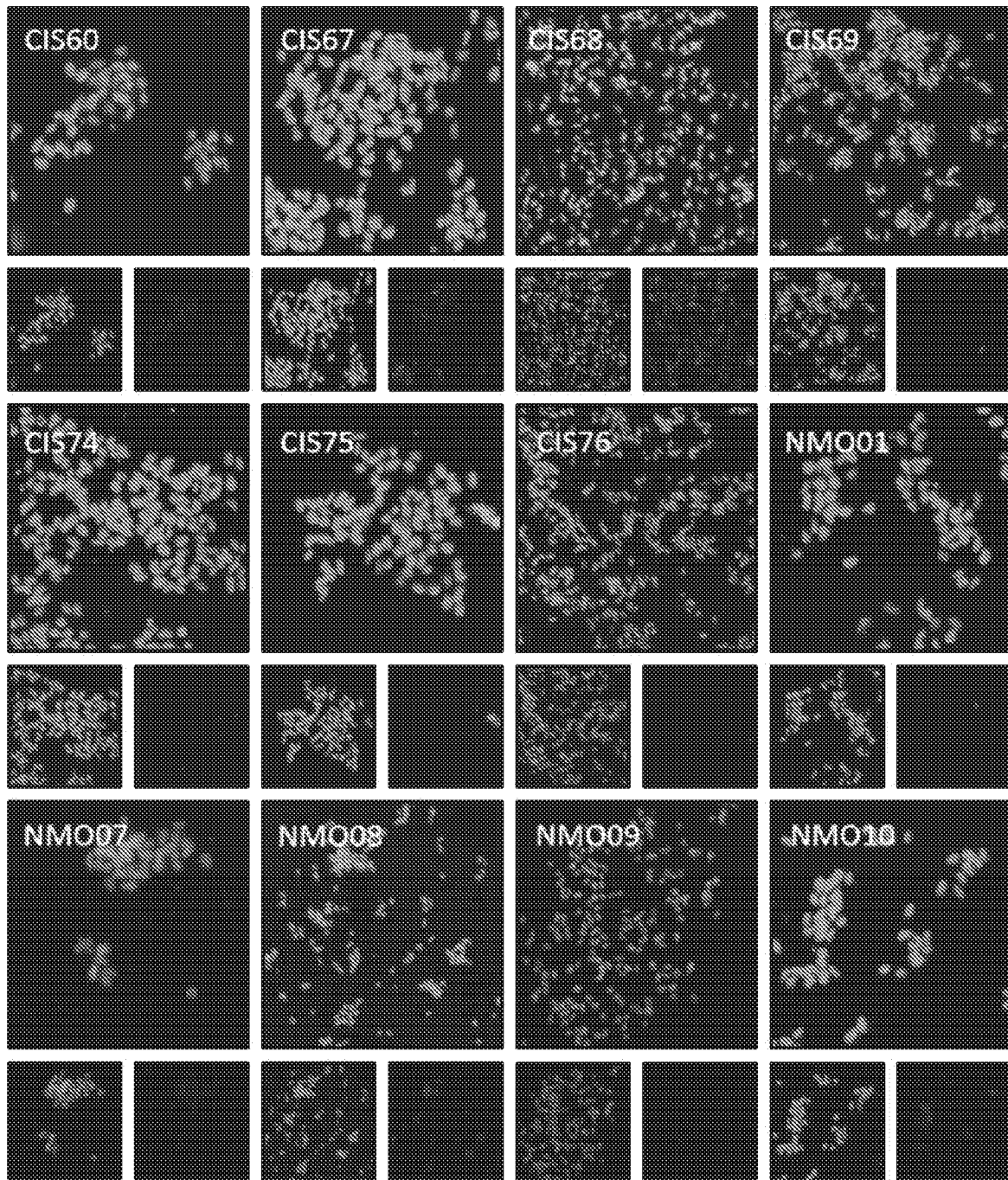


FIG. 36
(Cont'd.)

Reactivity to Sy5y by ICC

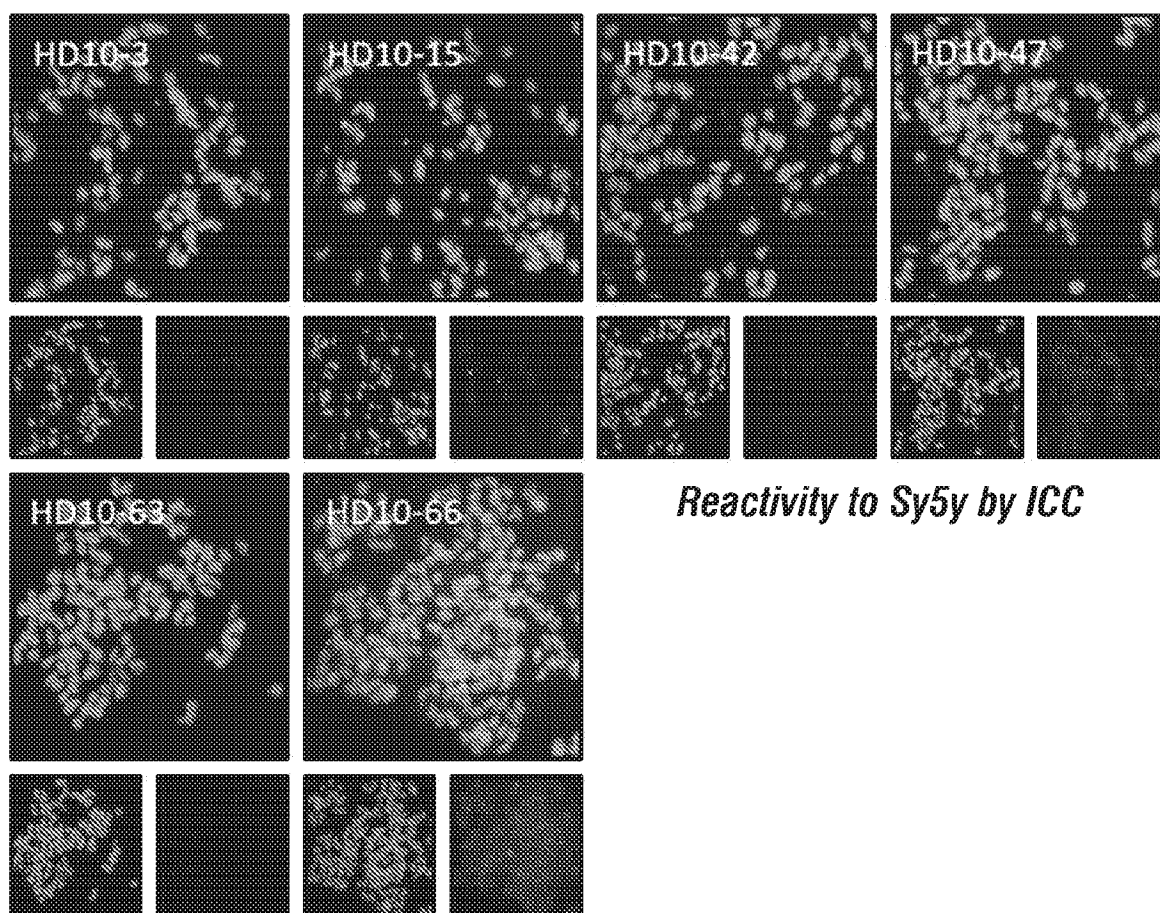
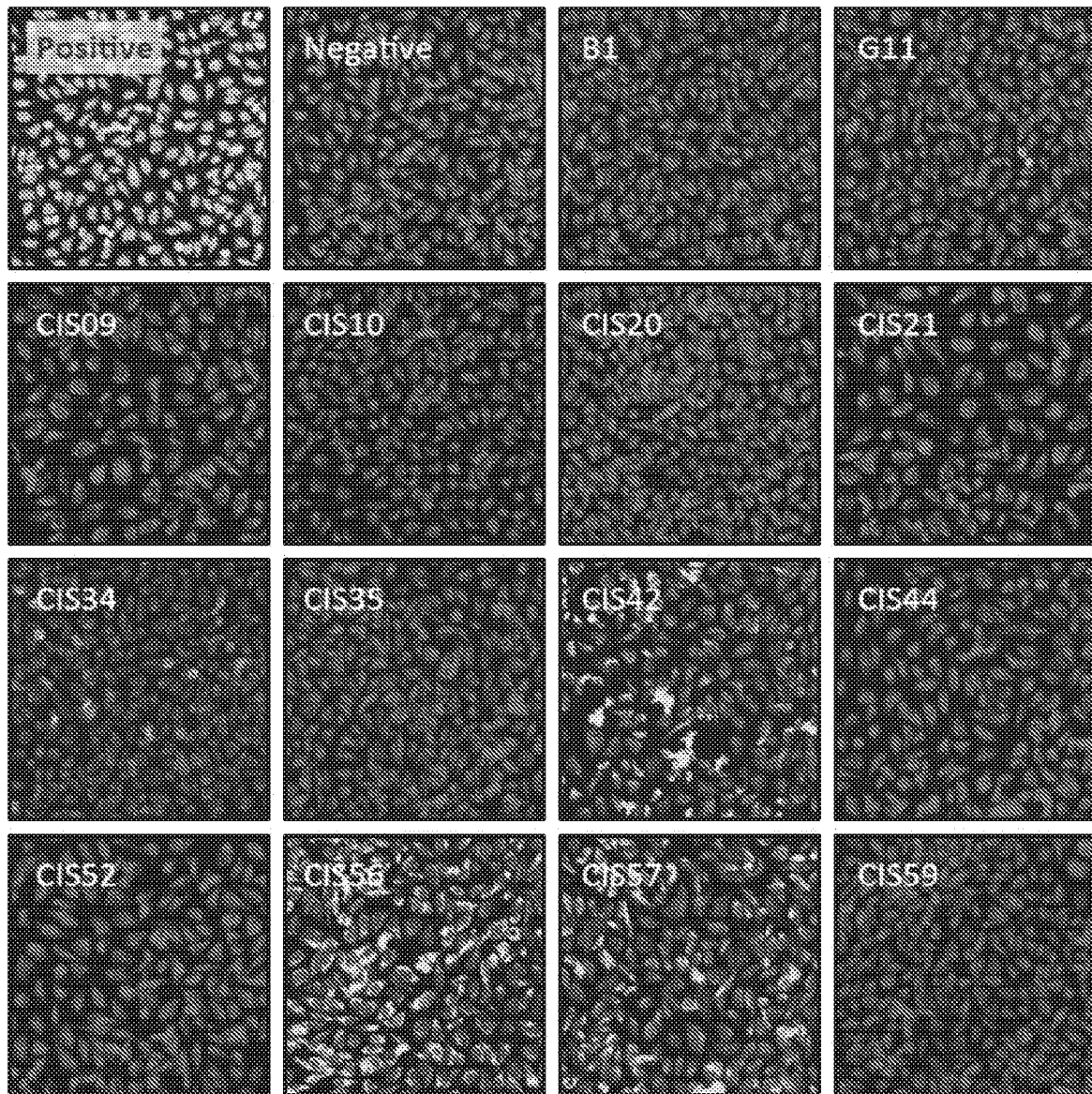


FIG. 36
(Cont'd.)

Reactivity to Hep2 by ICC**FIG. 37A**

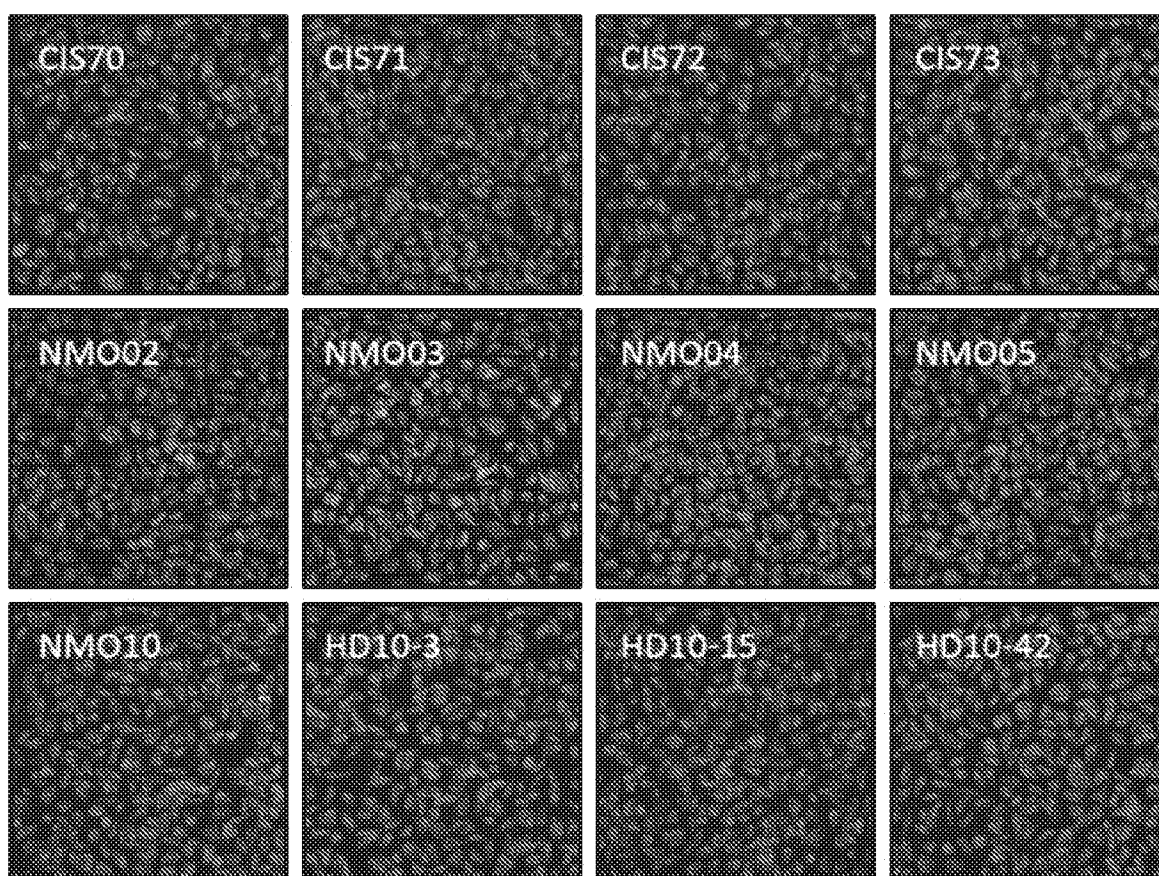


FIG. 37A
(Cont'd.)

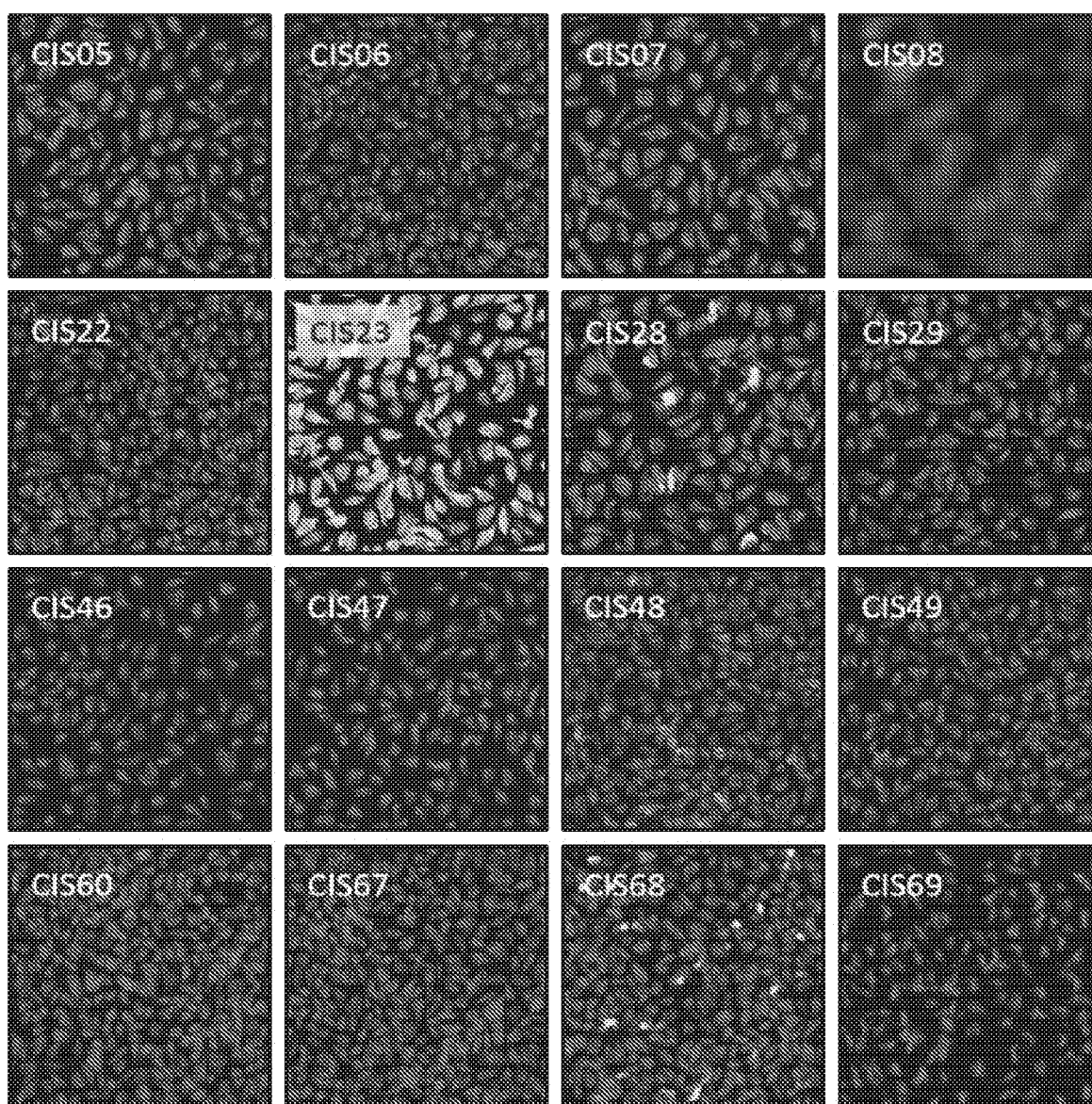


FIG. 37A
(Cont'd.)

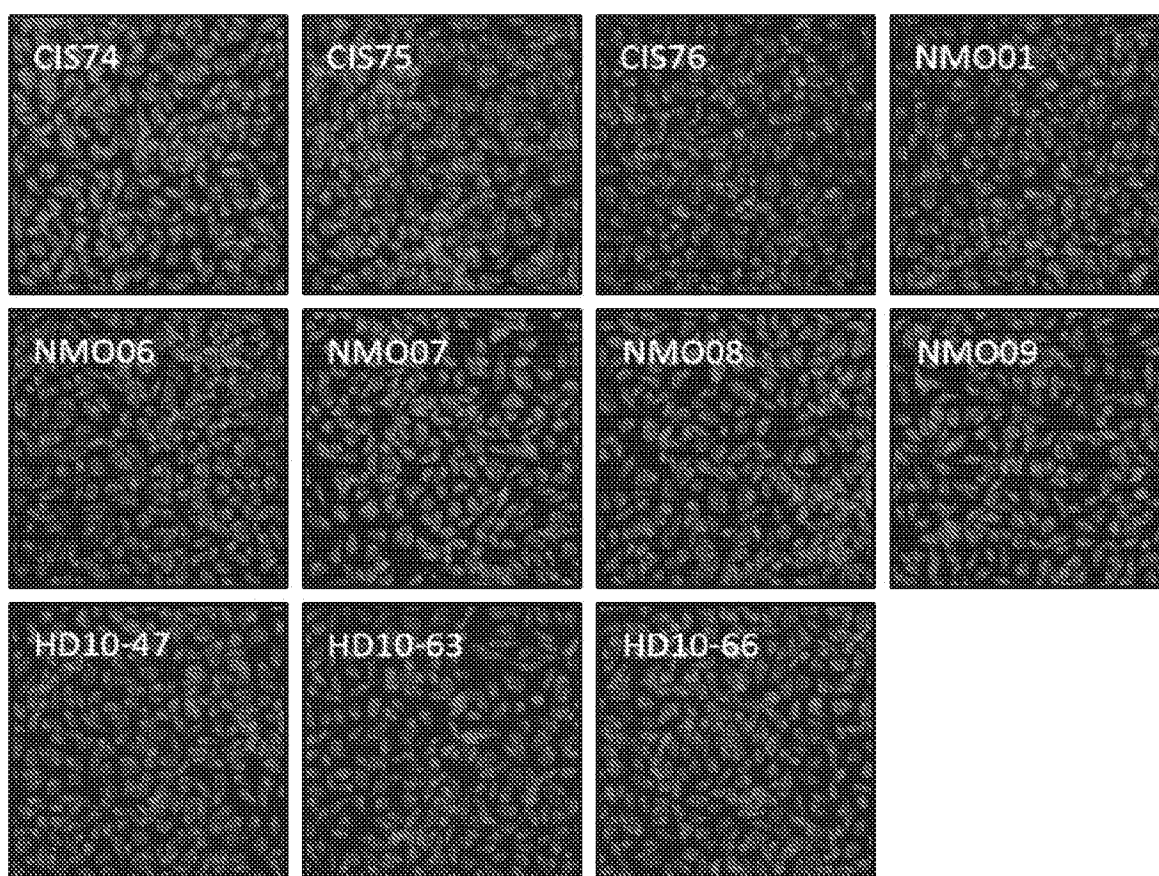


FIG. 37A
(Cont'd.)

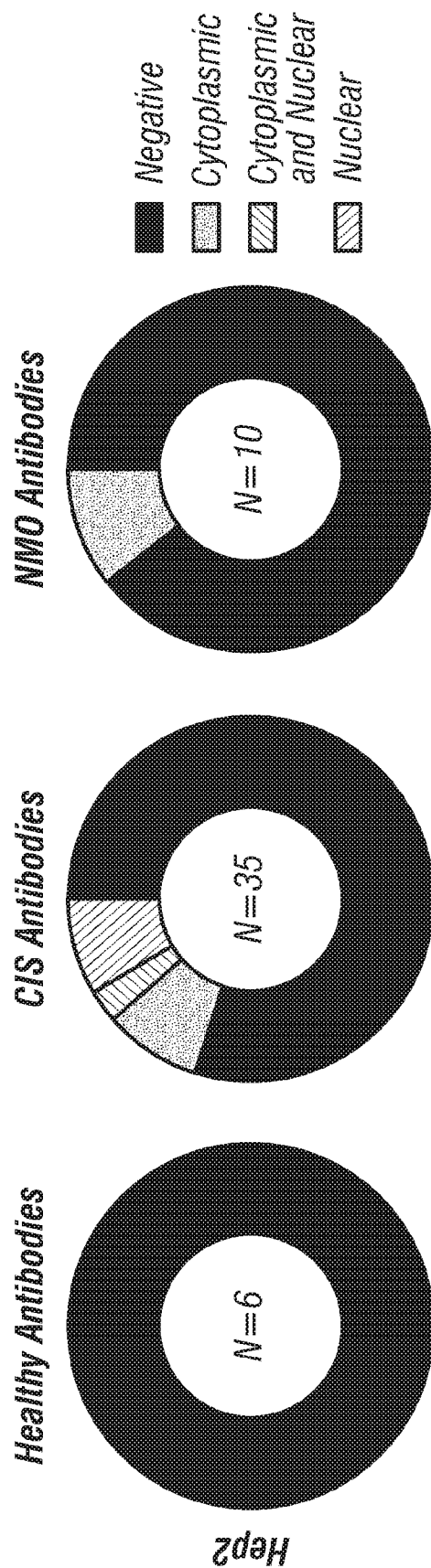


FIG. 37B

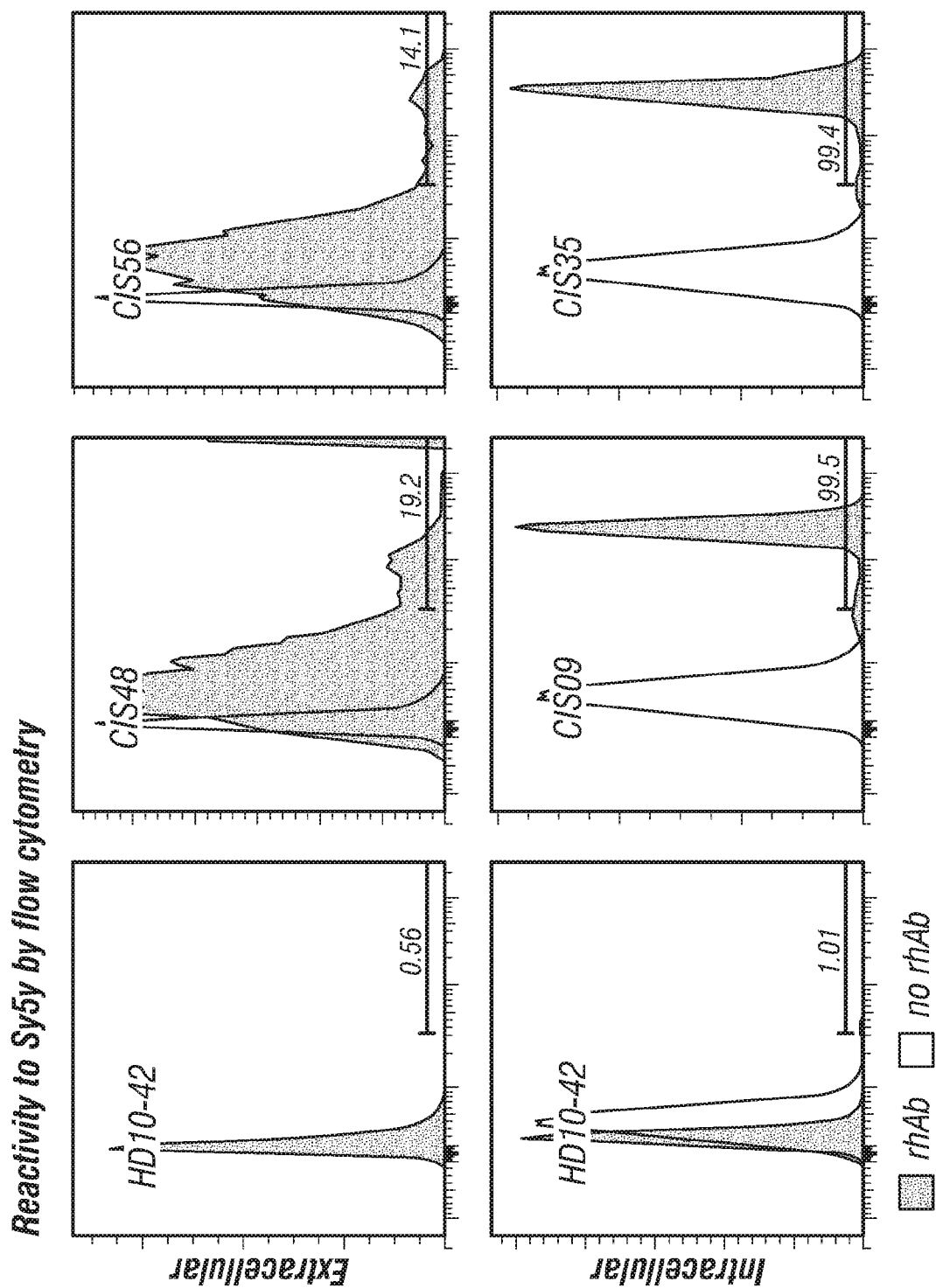


FIG. 38A

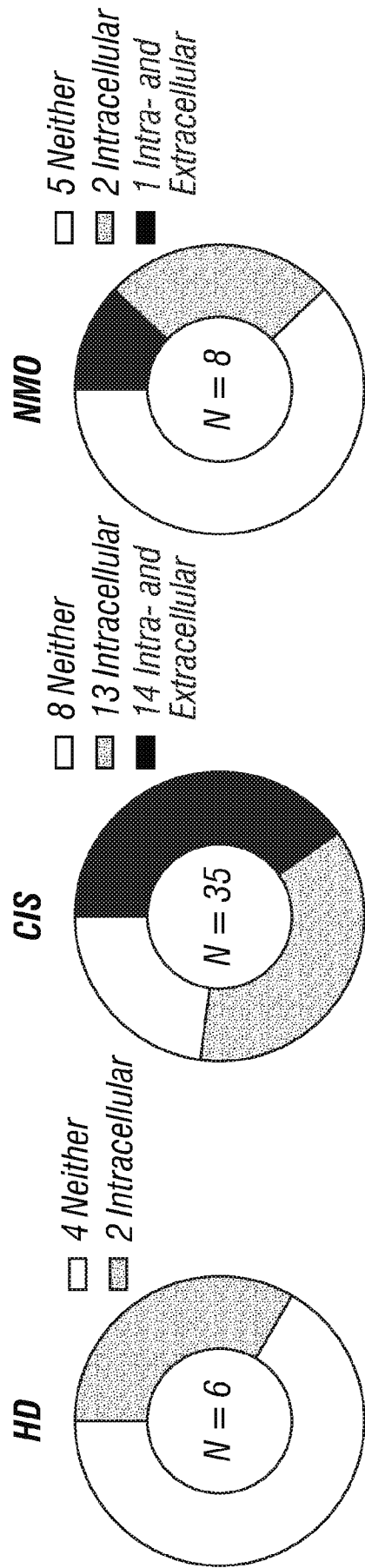
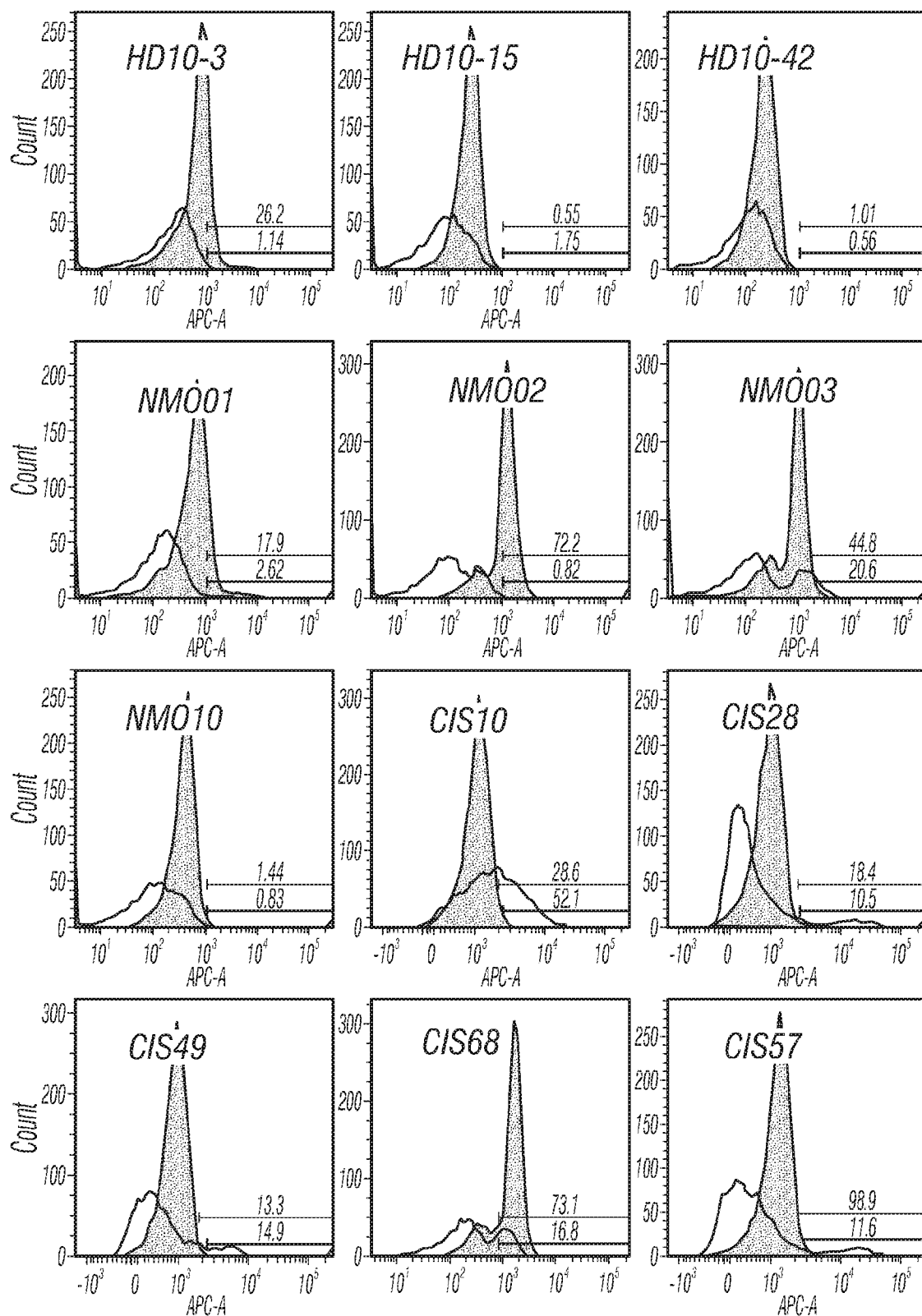


FIG. 38B

Reactivity to Sy5y by flow cytometry**FIG. 39**

■ Intracellular
□ Extracellular

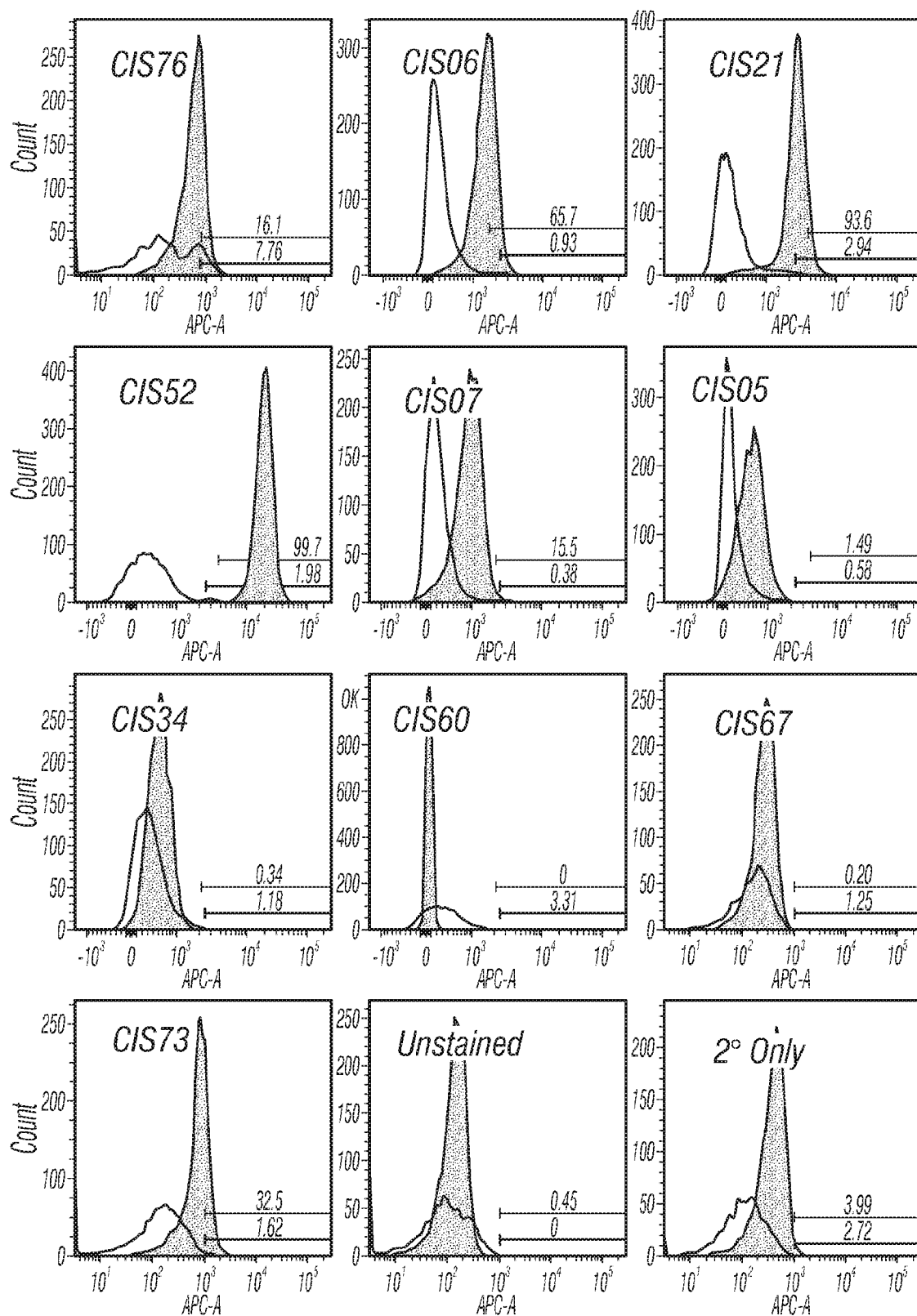


FIG. 39
(Cont'd.)

■ Intracellular
□ Extracellular

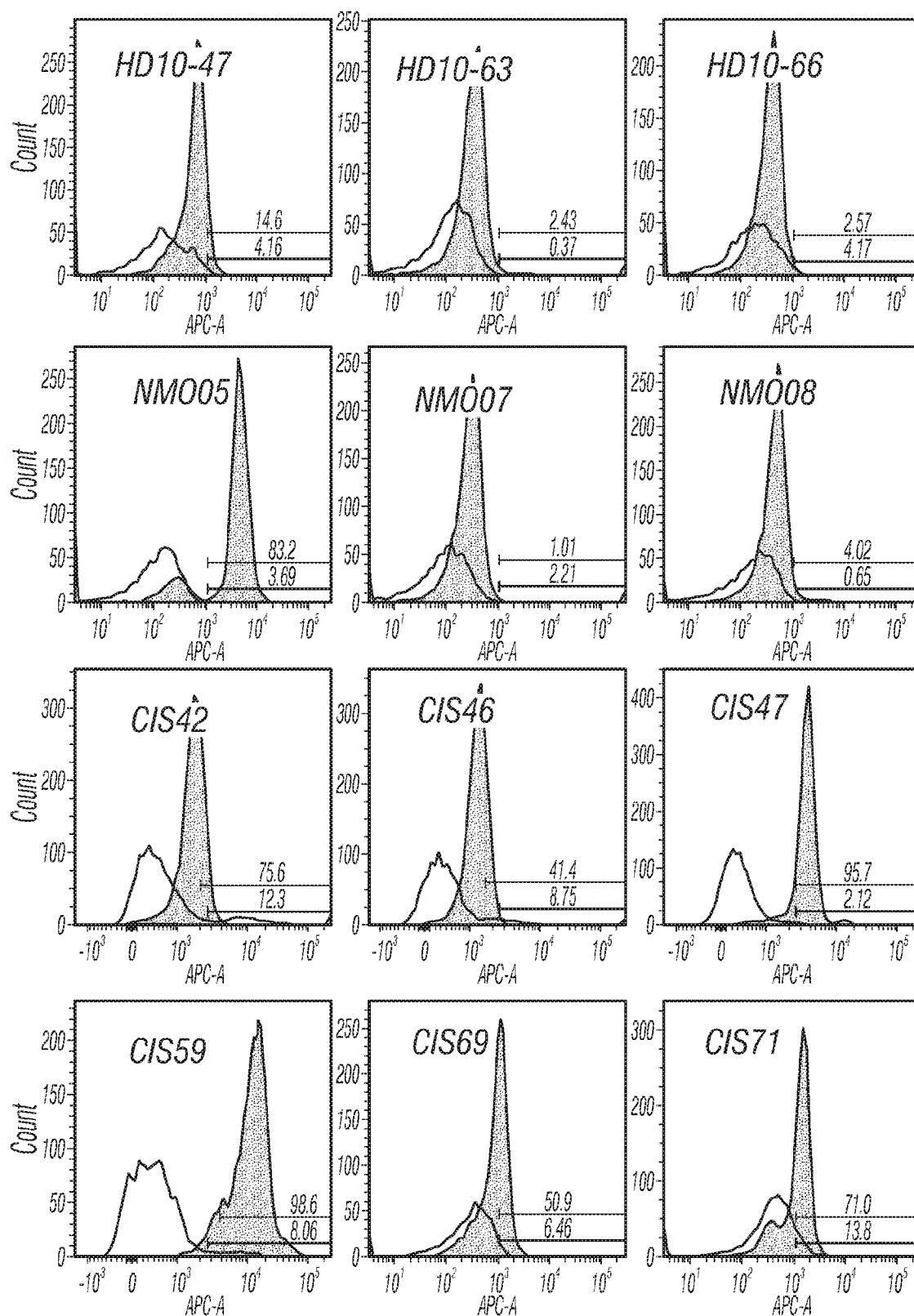
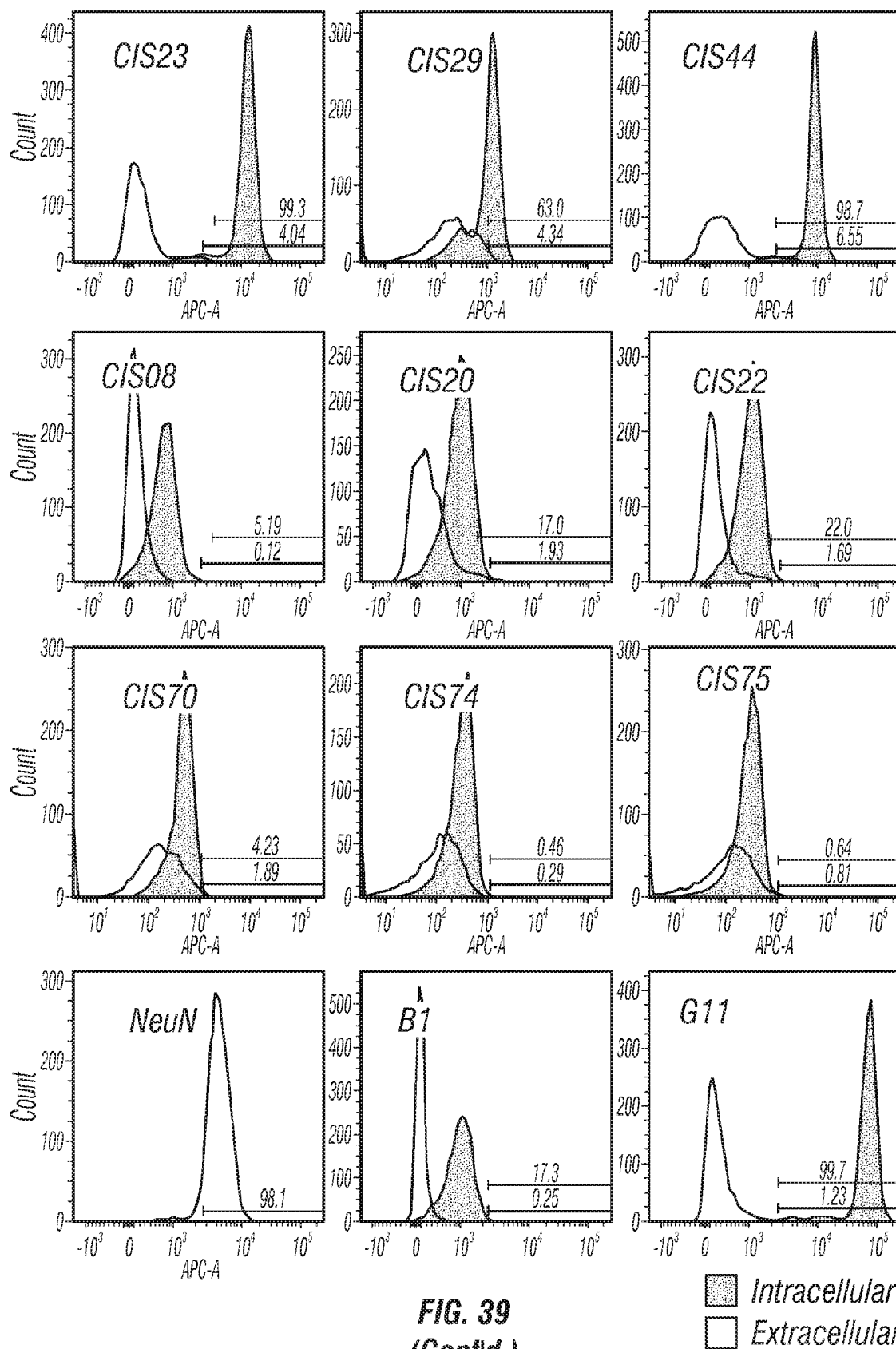


FIG. 39
(Cont'd.)

■ Intracellular
□ Extracellular



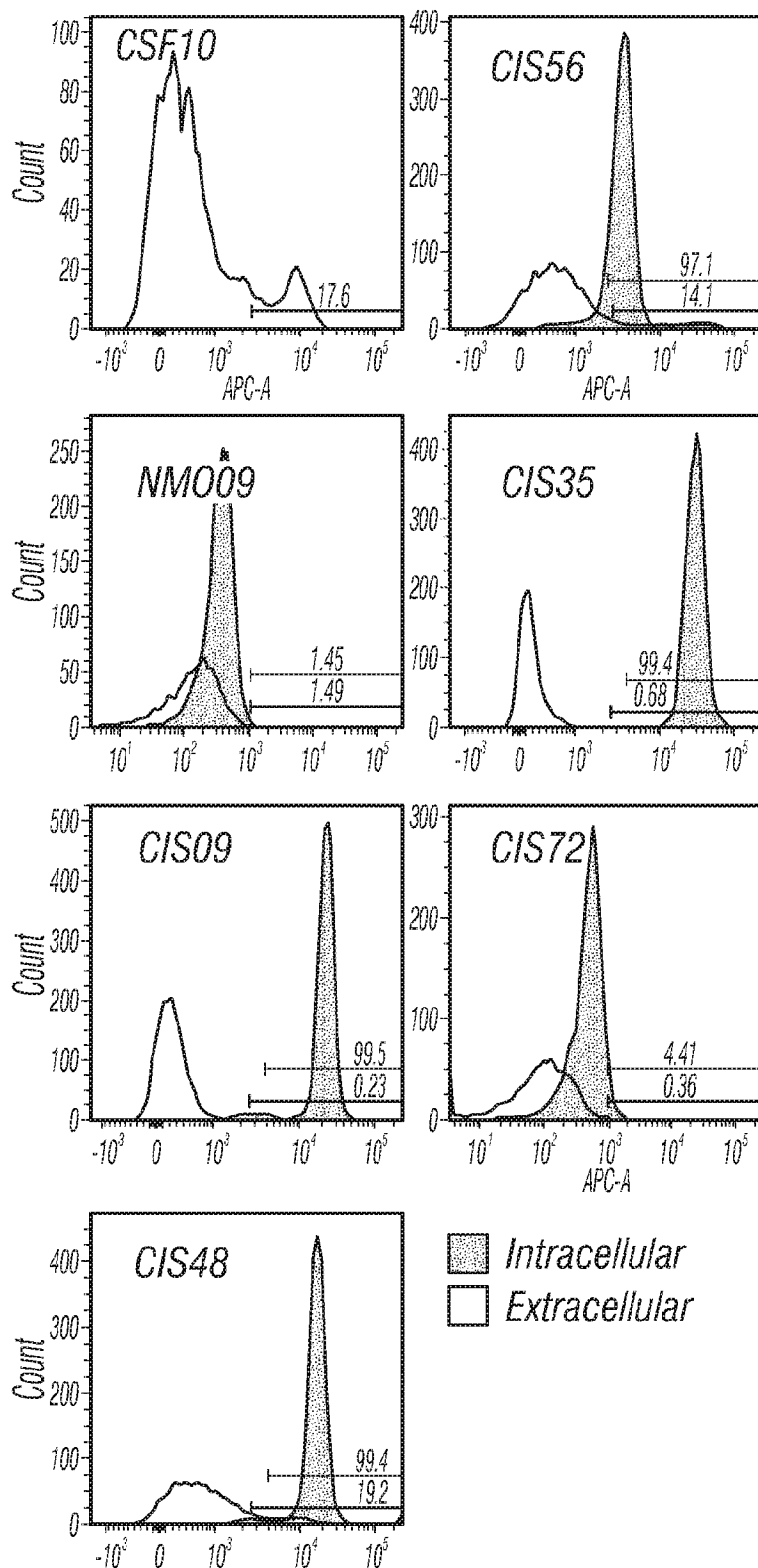
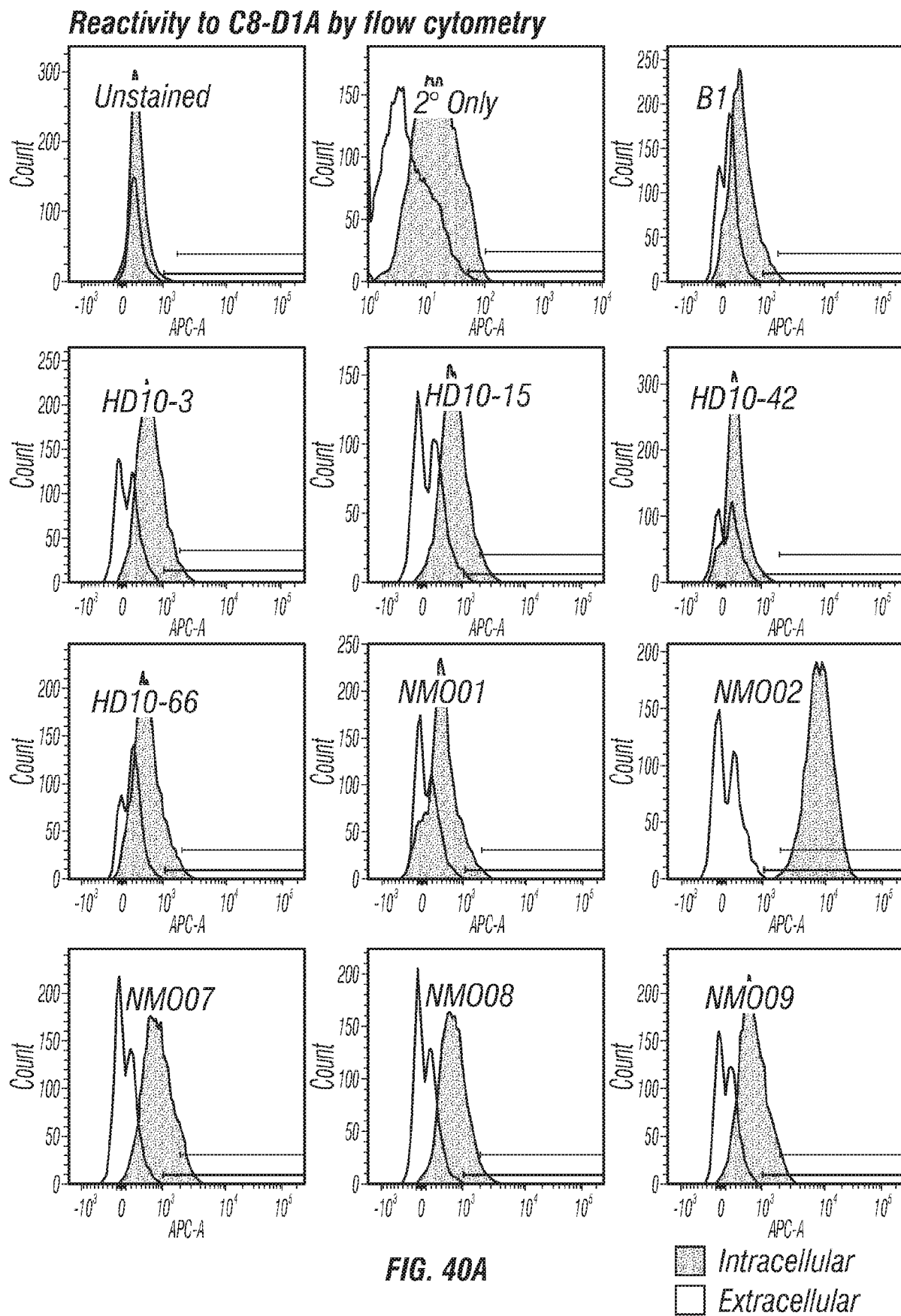


FIG. 39
(Cont'd.)



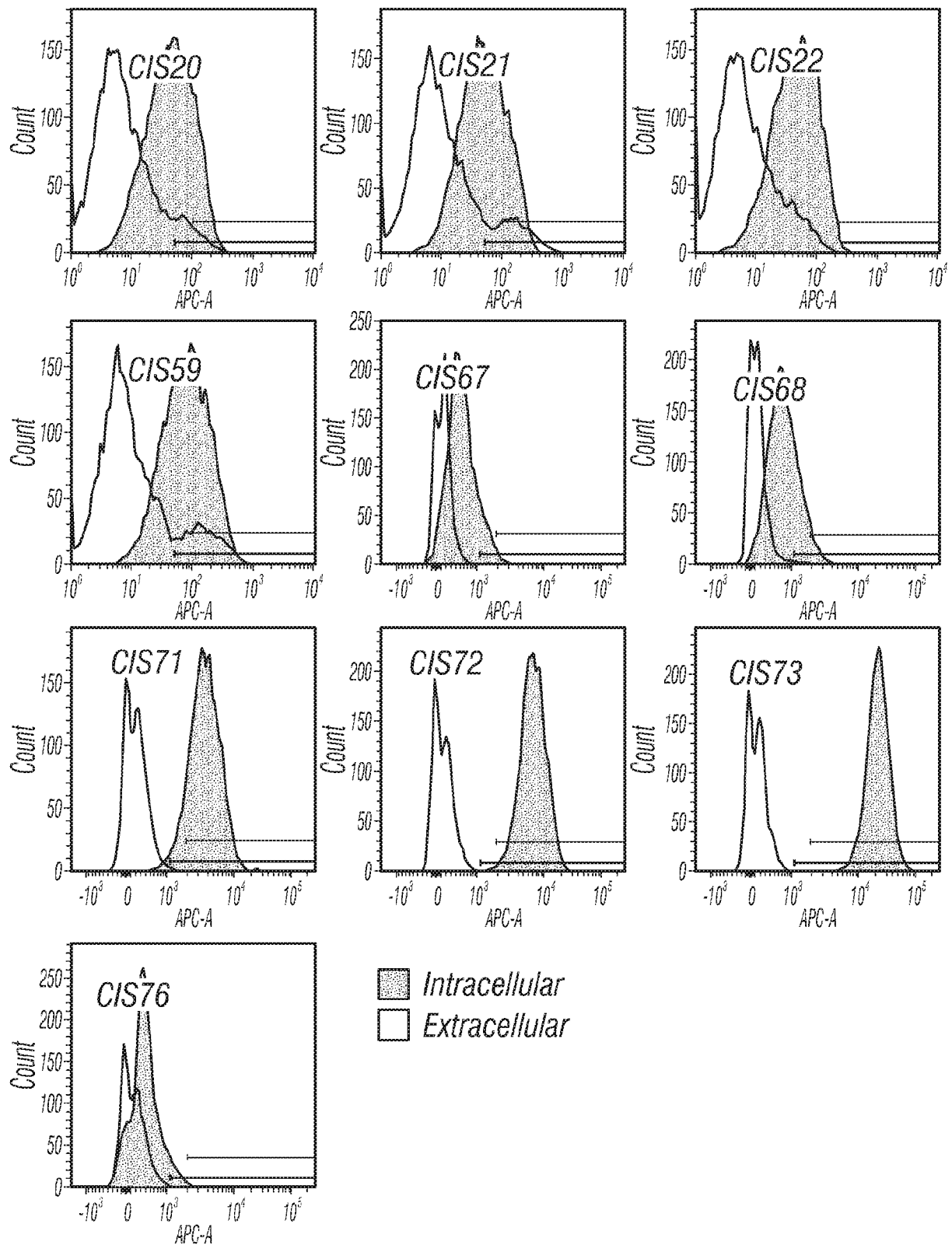


FIG. 40A
(Cont'd)

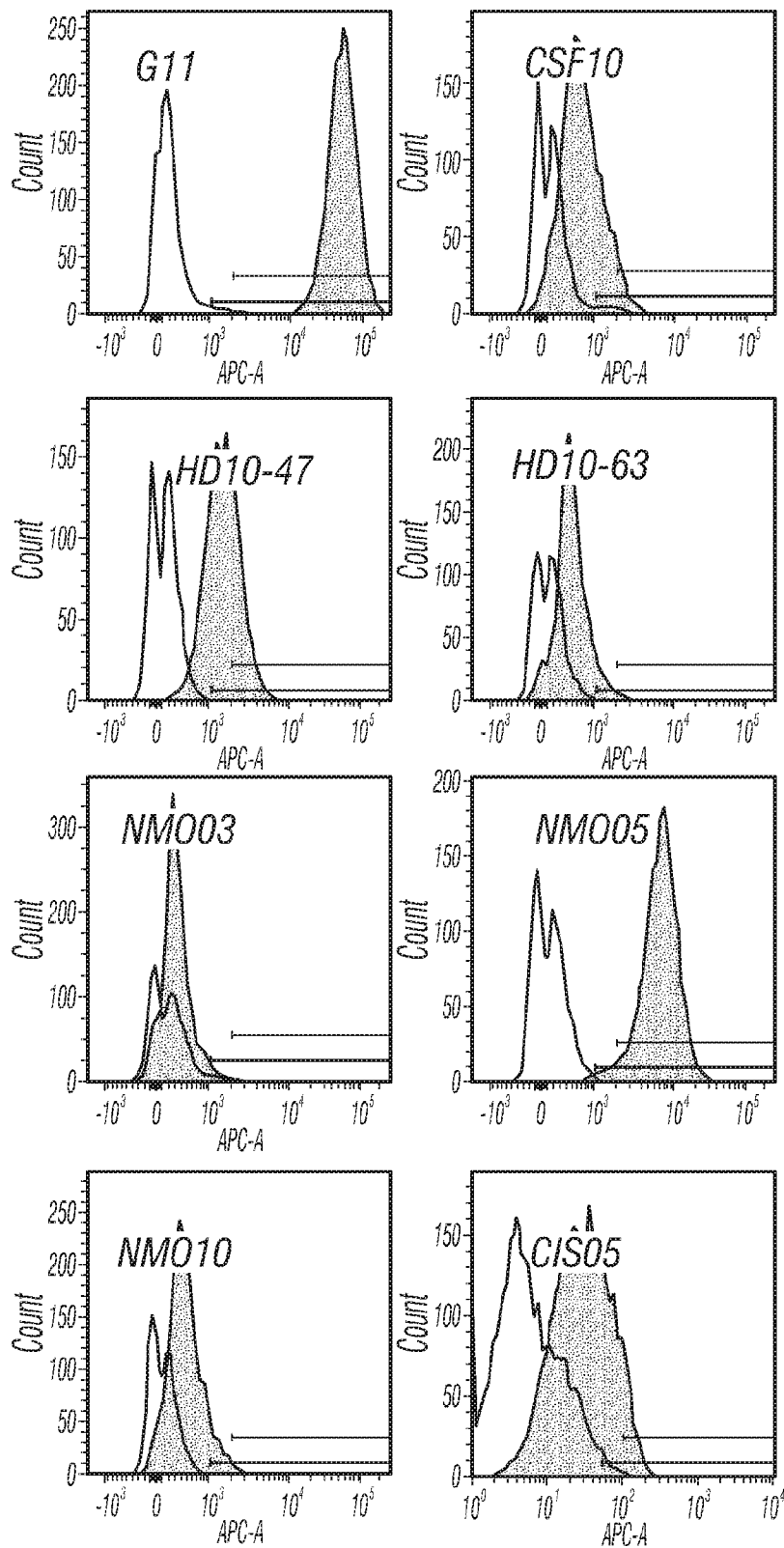


FIG. 40A
(Cont'd)

■ Intracellular
□ Extracellular

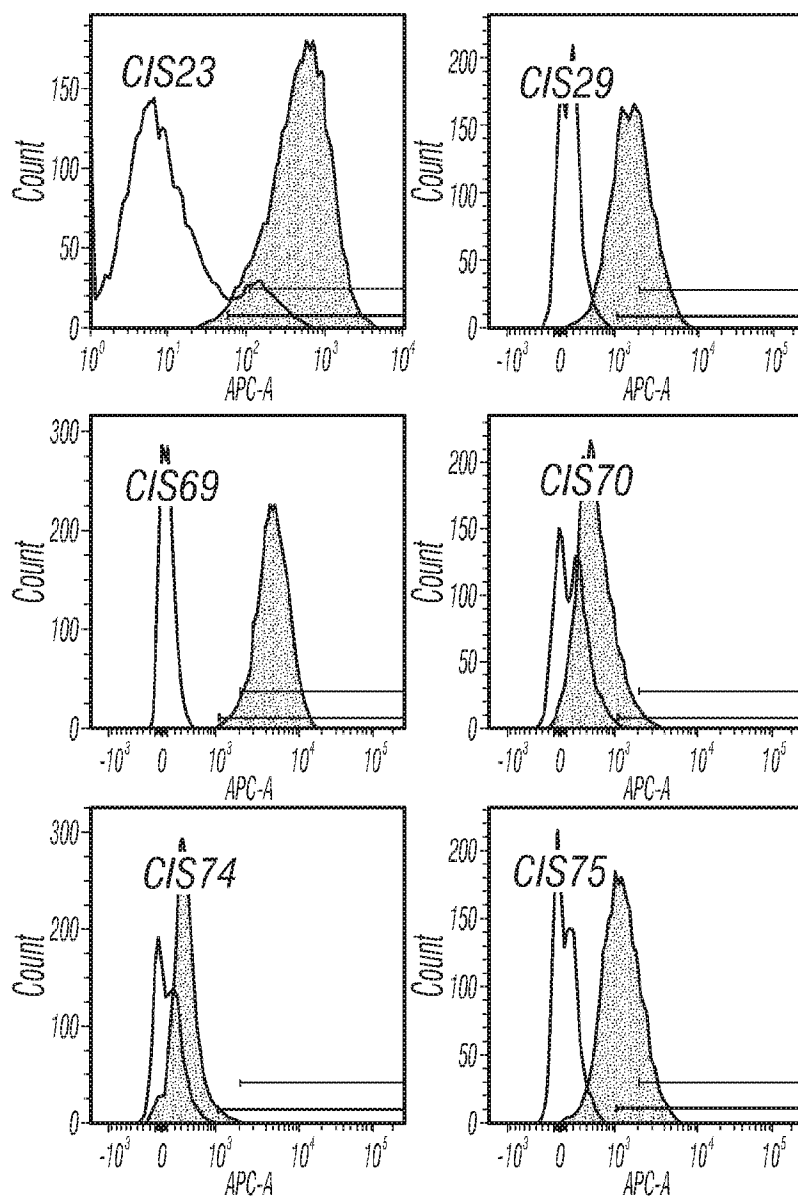


FIG. 40A
(Cont'd)

■ Intracellular
□ Extracellular

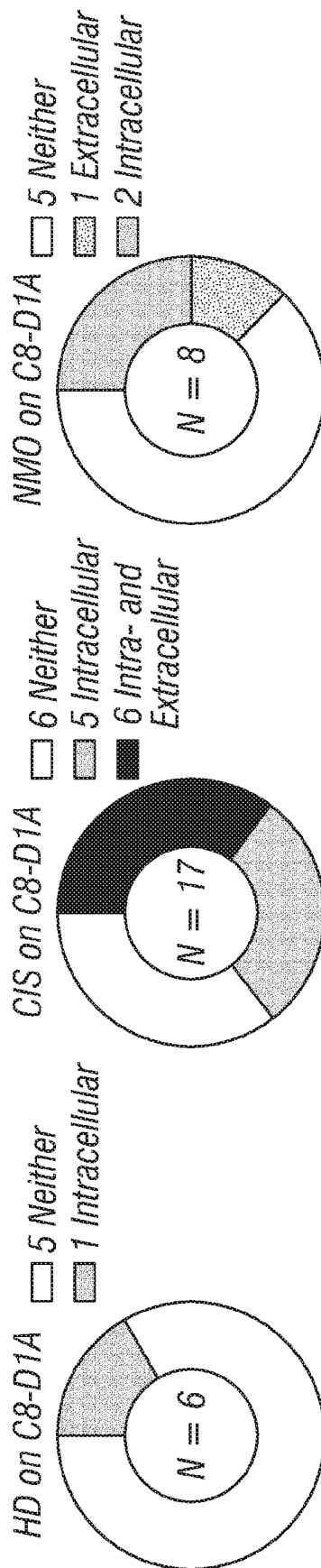
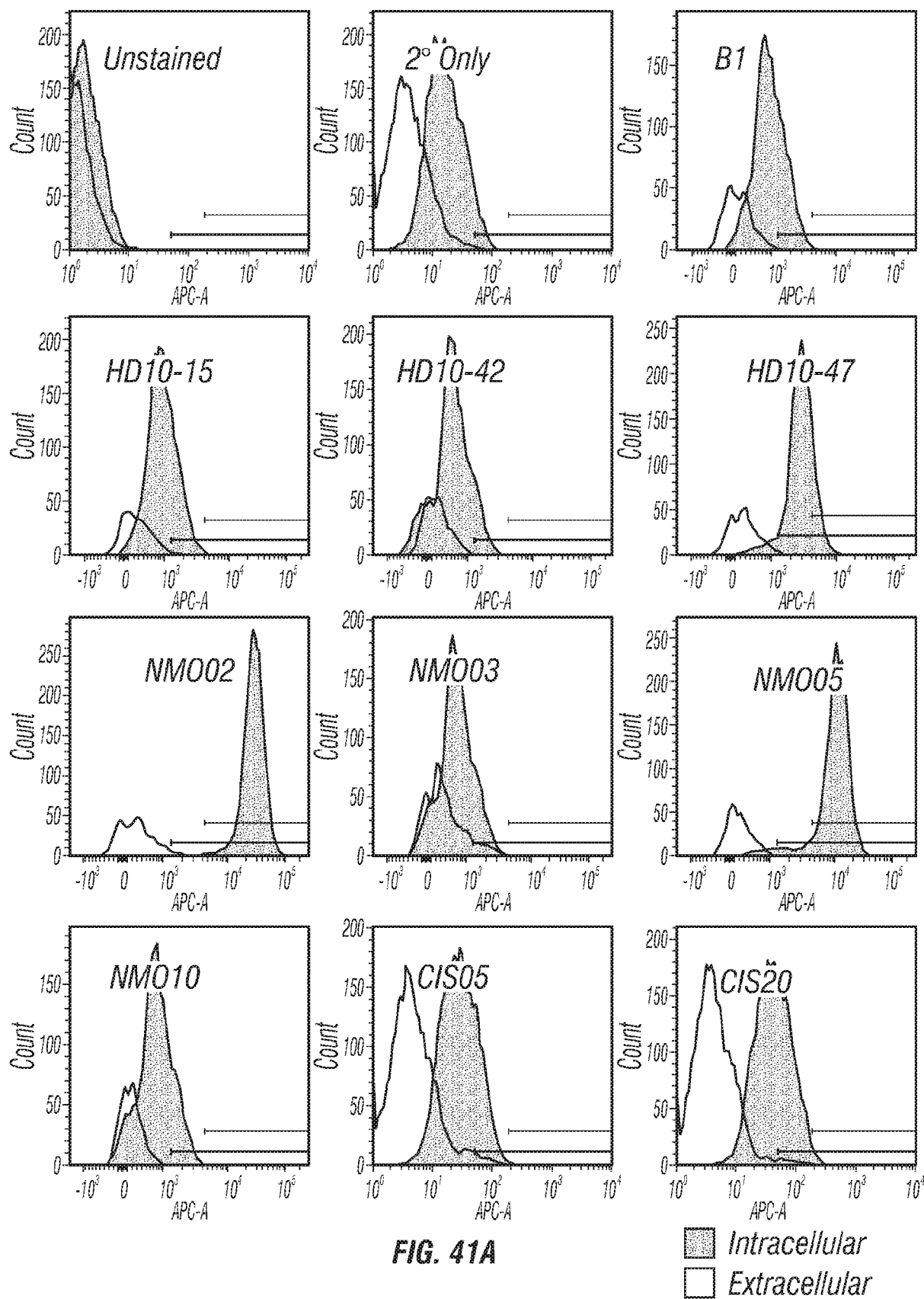


FIG. 40B

Reactivity to Neuro-2a by flow cytometry**FIG. 41A**

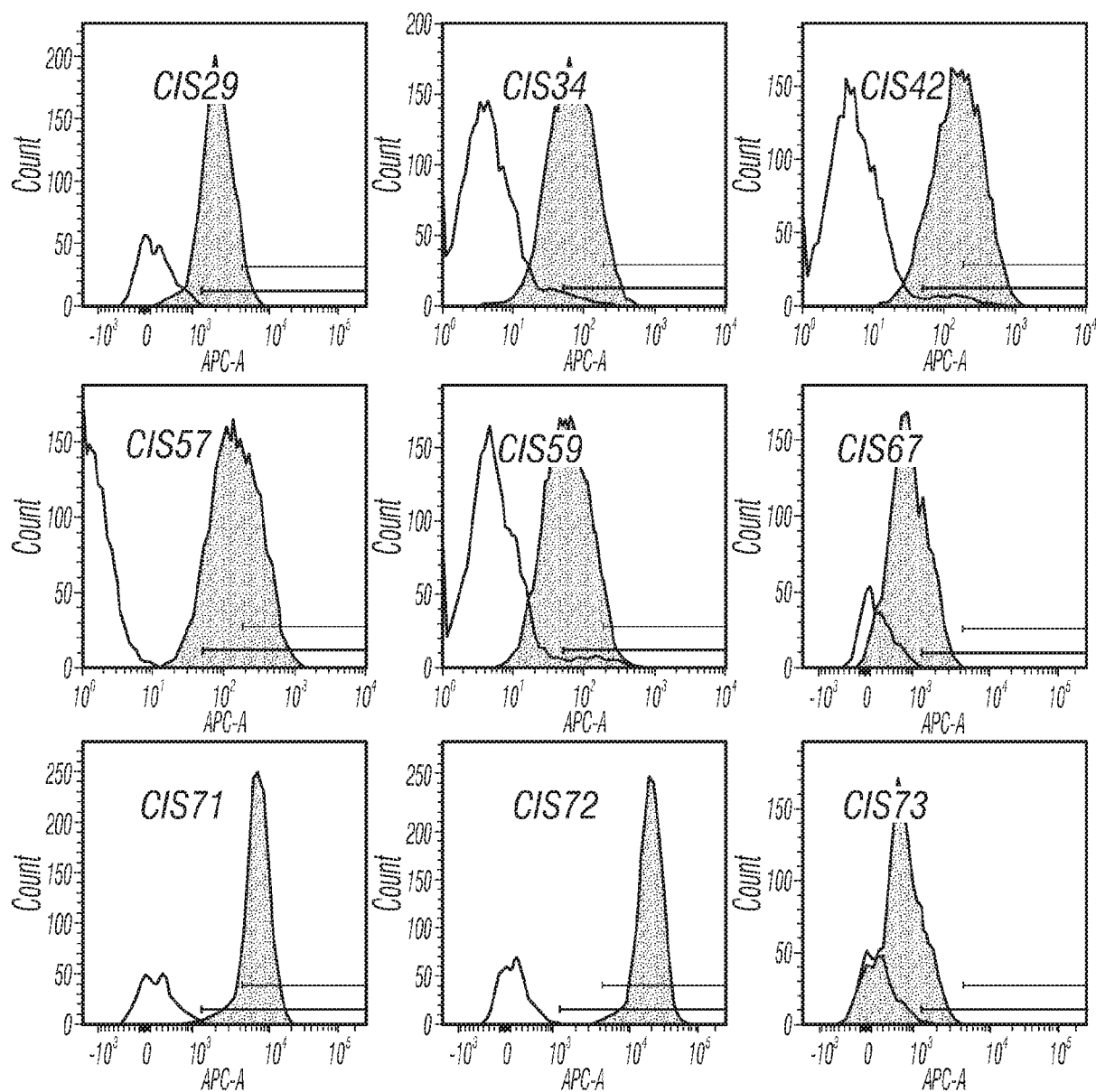


FIG. 41A
(Cont'd)

■ Intracellular
□ Extracellular

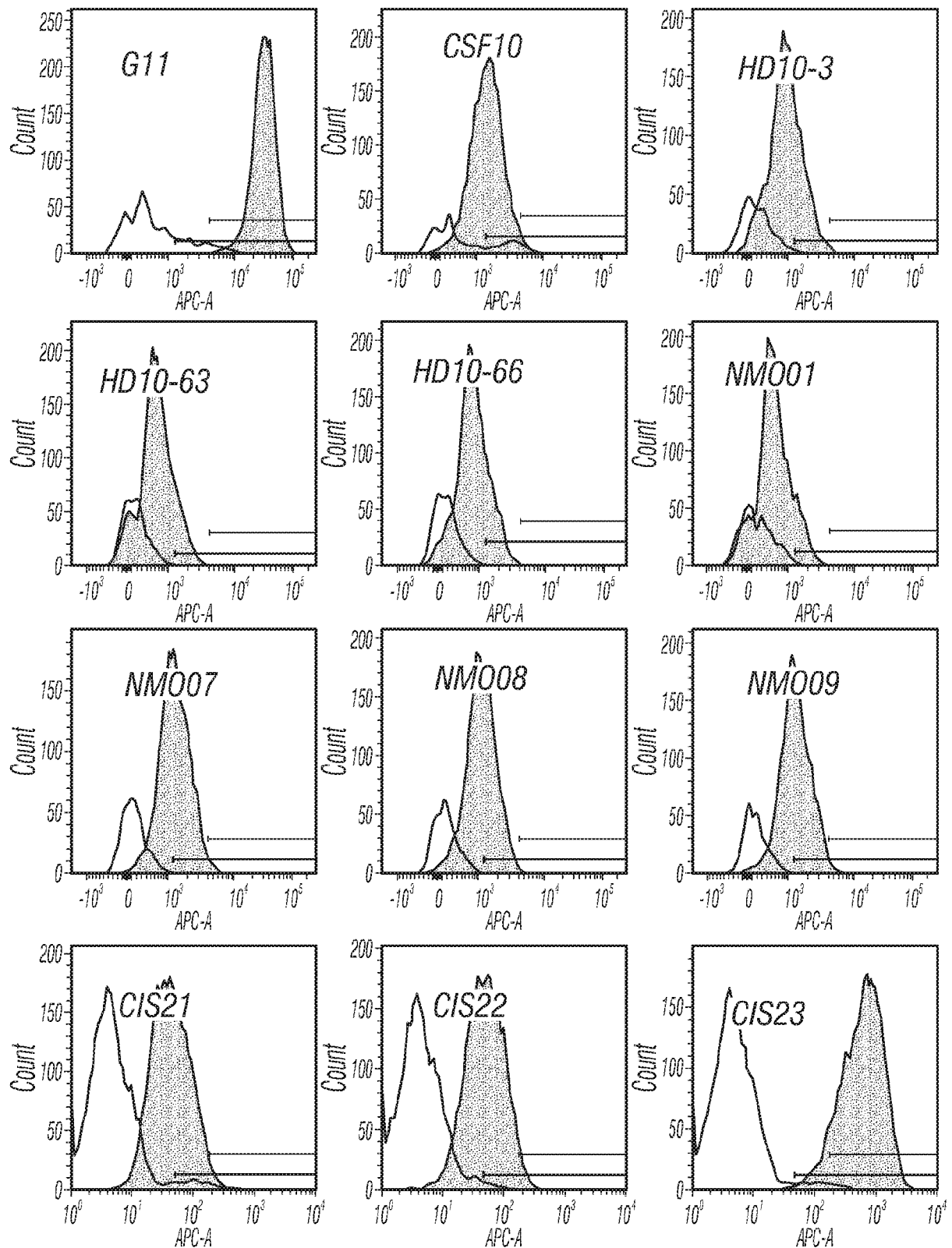


FIG. 41A
(Cont'd)

 Intracellular
 Extracellular

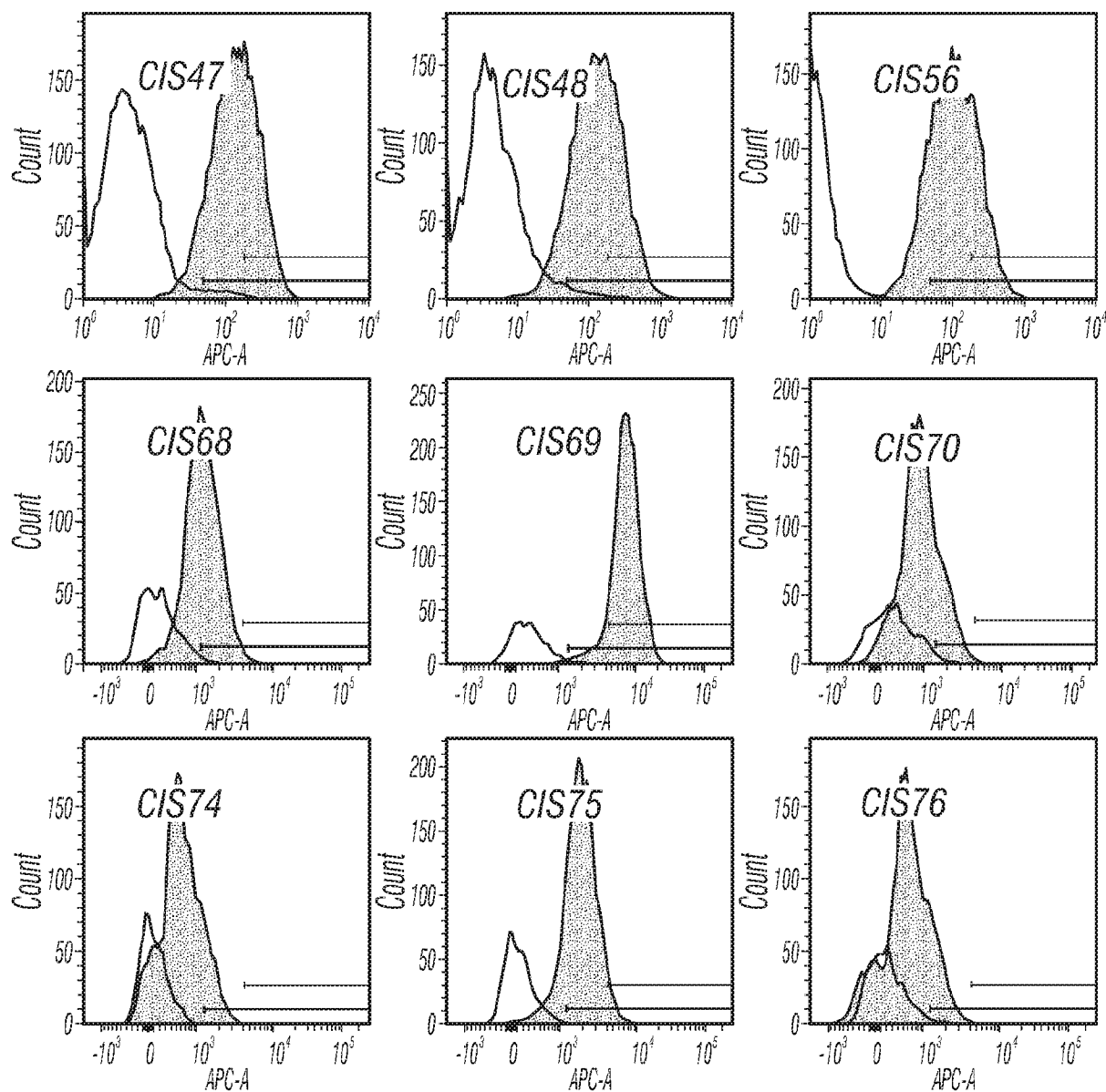


FIG. 41A
(Cont'd)

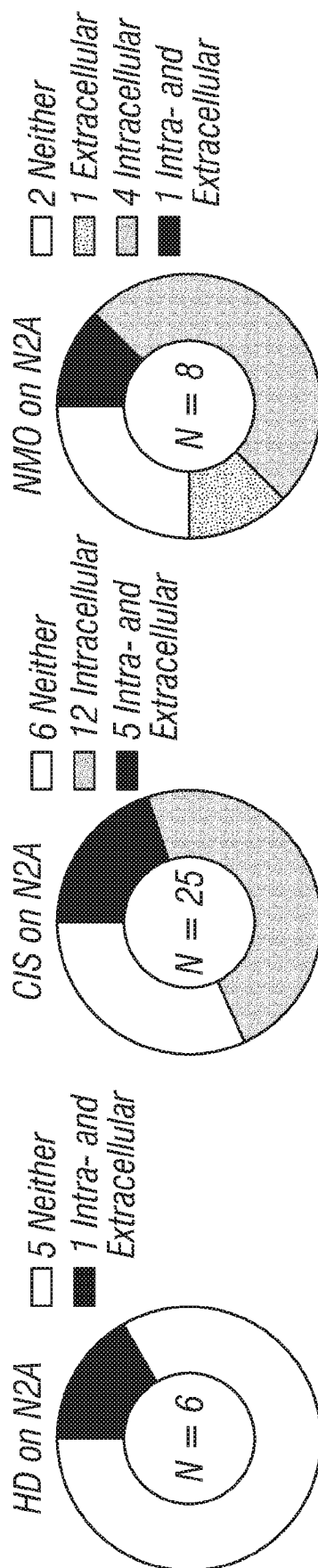


FIG. 41B

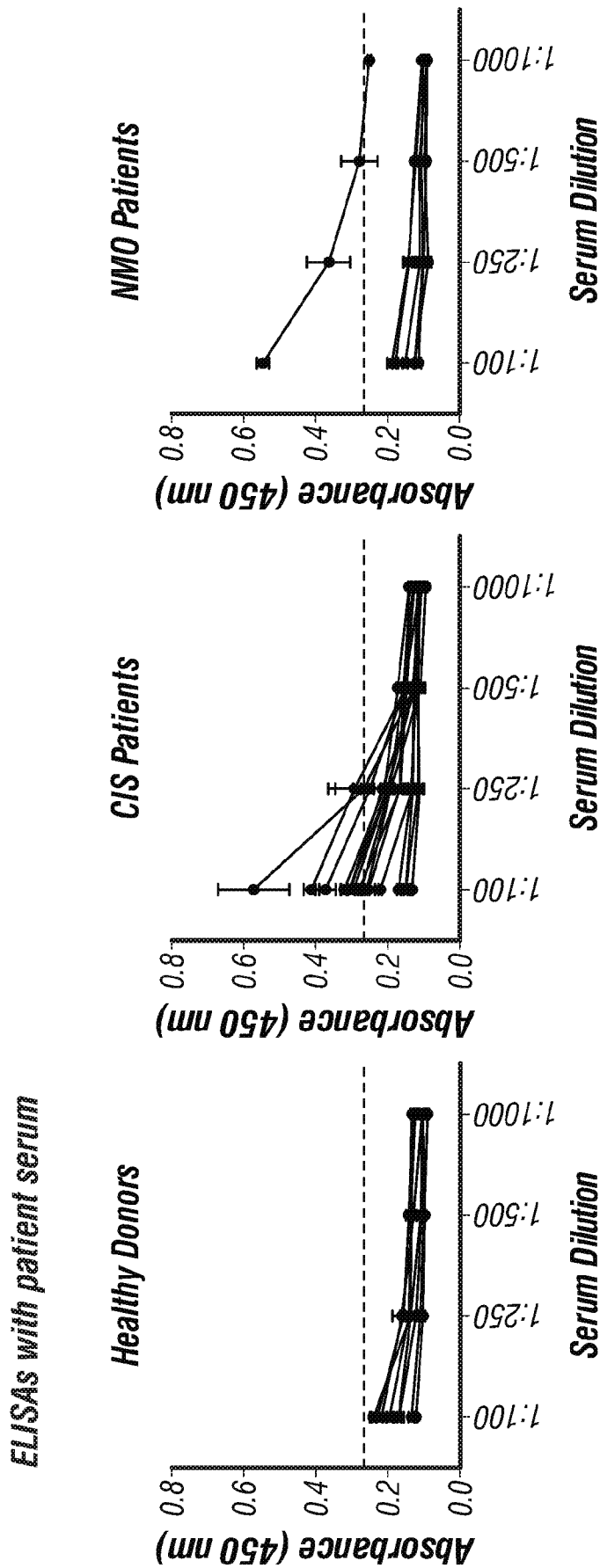


FIG. 42A

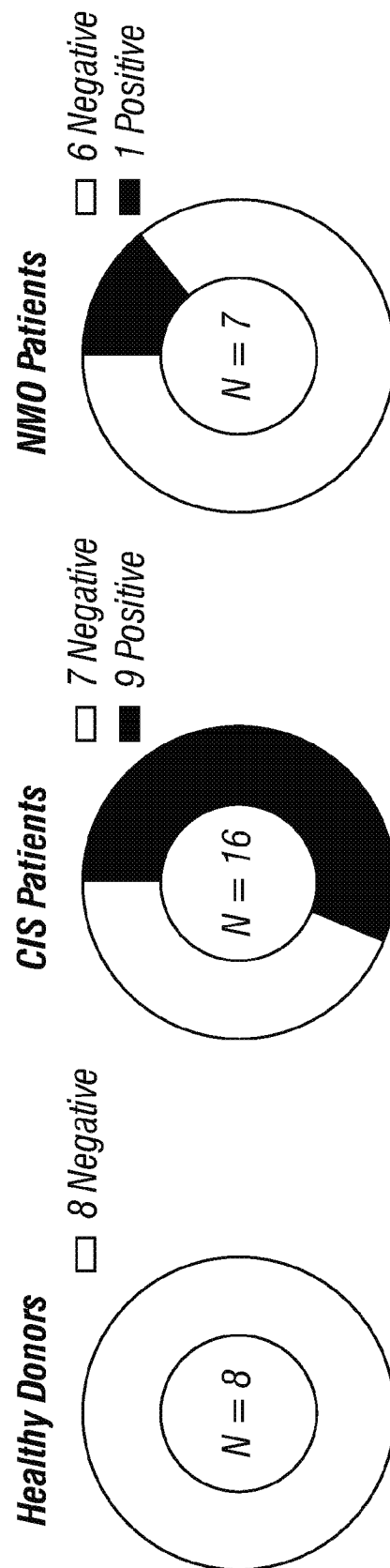


FIG. 42B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/32411

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395; C12N 15/113; G01N 33/53 (2017.01)

CPC - G01N 2800/285; A61K 39/39566; C07K 16/18, 2317/56, 2317/565; C12Q 2600/178

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	WO 2015/070009 A2 (The Board of Regents of the University of Texas System) 14 May 2015 (14.05.2015). Especially pg 1 ln 28-34, pg 2 ln 5-7, pg 4 ln 19-23, pg 57 ln 16-18, claims 1, 42, 43, SEQ ID NOs: 1-2	1-4, 11, 12, 24-28, 34, 35, 44-48, 54, 55, 66-69
A	US 2014/0371132 A1 (The Board of Regents of the University of Texas System) 18 December 2014 (14.12.2014). Especially claims 1-5, 16	1-4, 11, 12, 24-28, 34, 35, 44-48, 54, 55, 66-69

☐ 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

12 September 2017

Date of mailing of the international search report

06 OCT 2017

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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/32411

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*. 1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*. 1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore ver 6.4.1 SEQ ID NOs: 1, 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/32411

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.: 5-10, 13-23, 29-33, 36-43, 49-53, 56-65, 70-75
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:
-----Go to Extra Sheet for continuation-----

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.:
Claims 1-4, 11, 12, 24-28, 34, 35, 44-48, 54, 55, 66-69

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

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Continuation of Box 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.

Group I: Claims 1-4, 11, 12, 24-28, 34, 35, 44-48, 54, 55, 66-69, drawn to a process of making a pharmaceutical composition for treating a neurodegenerative or neuroimmunological disease.

Group II: Claims 76-77, 80-81, drawn to a pharmaceutical composition comprising an effective amount of a VH4 antibody which exhibit neuroprotection or repair.

Group III+: Claim 78-79, drawn to an anti-idiotypic antibody specific for a VH4 antibody. Group III+ will be searched upon payment of additional fee(s). The anti-idiotypic antibody specific for a VH4 antibody may be searched, for example, to the extent that the anti-idiotypic antibody is directed against a VH4 antibody having S56R, T57A, and K81N (the first antibody, AJL01, of Table 1) comprising a set of heavy chain CDRs in variable heavy chain sequence SEQ ID NO: 1; and a set of light chain CDRs in variable light chain sequence SEQ ID NO: 2 for an additional fee and election as such. It is believed that claims 78-79 read on this exemplary invention. Additional anti-idiotypic antibodies will be searched upon the payment of additional fees. Applicants must indicate, if applicable, which claims read on this 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 named invention to be searched/examined. An exemplary election would an anti-idiotypic antibody directed against a VH4 antibody having P40S, S56G, and T57I (Table 1, antibody, AJL04) comprising a set of heavy chain CDRs in variable heavy chain sequence SEQ ID NO: 7; and a set of light chain CDRs in variable light chain sequence SEQ ID NO: 8 (claims 78-79).

Group IV+: Claims 82-85, drawn to an oligonucleotide comprising a nucleotide sequence complementary to a nucleotide sequence encoding a VH4 CDRs. Group IV+ will be searched upon payment of additional fee(s). The oligonucleotide composition may be searched, for example, to the extent that it comprises a nucleotide sequence complementary to a VH4 heavy chain CDR sequence SEQ ID NO: 1 (corresponding to nucleotide sequence SEQ ID NO: 65). It is believed that claims 82-85 read on this exemplary invention. Additional oligonucleotides will be searched upon the payment of additional fees. Applicants must indicate, if applicable, which claims read on this 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 named invention to be searched/examined. An exemplary election would be an oligonucleotide that comprises a nucleotide sequence complementary to a VH4 heavy chain CDR sequence SEQ ID NO: 7 (corresponding to nucleotide sequence SEQ ID NO: 71) (Claims 82-85).

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

Special Technical Features:

The inventions of Group I have the special technical feature of a process of making a pharmaceutical composition for treating a neurodegenerative or neuroimmunological disease, not required by Groups II, III+, IV+.

The inventions of Group II have the special technical feature of a pharmaceutical composition comprising an effective amount of a VH4 antibody which exhibit neuroprotection or repair, not required by Groups I, III+, IV+.

The inventions of Group II have the special technical feature of a VH4 antibody that lacks an Fc domain, not required by Groups I, III+, and IV+.

The inventions of Group III+ have the special technical feature of an anti-idiotypic antibody specific for a VH4 antibody, not required by Groups I, II, IV+.

Among the inventions listed as Groups III+ are the specific VH4 antibodies with mutations and their polypeptide sequences. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among the named antibody mutants or their polypeptide sequences.

The inventions of Group IV+ have the special technical feature of an oligonucleotide comprising a nucleotide sequence complementary to a VH4 heavy chain CDR sequence, not required by Groups I, II, III+.

Among the inventions listed as Groups V+ are the specific oligonucleotide sequences complementary to a VH4 CDR sequences. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among the oligonucleotide sequences.

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INTERNATIONAL SEARCH REPORT

International application No.

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----continued from previous sheet----

Common Technical Feature:

1. Groups I, II, III+ share the common technical feature are VH4 antibodies having specific mutations that are capable of binding to human gray or white matter.
2. Groups I, II, III+ share the common technical features of VH4 antibodies having mutations and known and defined variable heavy chain and variable light chain sequences.
3. Group III+ inventions share the common technical feature of anti-idiotypic antibodies directed against VH4 antibodies.
4. Group IV+ inventions share the common technical features of an oligonucleotide comprising a nucleotide sequence complementary to a VH4 heavy chain CDR sequence.

However, said common technical features do not represent a contribution over the prior art, and is obvious over WO 2015/070009 A2 to The Board of Regents of the University of Texas System (hereinafter "Univ Texas").

As to common technical feature #1, Univ Texas teaches VH4 antibodies having specific mutations that are capable of binding to human gray or white matter (claim 1; 1. A recombinant antibody or antigen-binding fragment thereof, wherein the recombinant antibody or fragment binds to an antigen in human brain gray matter that is recognized by a VH4-comprising antibody having at least two mutations with respect to the germline sequence at codon positions selected from 31B, 32, 40, 56, 57, 60, 81, and 89).

As to common technical feature #2, Univ Texas teaches VH4 antibodies having mutations and known and defined variable heavy chain and variable light chain sequences (SEQ ID NO: 1; AA 1-130; 100% sequence identity to instant application SEQ ID NO: 1, which encodes rhVH4 monoclonal antibody AJL-01 variable heavy chain with mutations at positions 56, 57 and 81; SEQ ID NO: 2; AA 1-115; 100% sequence identity to instant application SEQ ID NO:2, which encodes rhVH4 monoclonal antibody variable light chain).

As to common technical feature #3, Univ Texas teaches anti-idiotypic antibodies directed against VH4 antibodies (claim 43; "The method of claim 42, wherein said agent comprises an anti-idiotypic antibody to said antibody, an antigen fragment that binds to said antibody, an siRNA that reduces said antibody's expression, or a non-Fe containing antibody that competes with said antibody"; pg 4 In 16-21: A method of treating multiple sclerosis (MS) or clinically isolated syndrome in a patient comprising administering to said subject an agent or subjecting said subject to a therapy that reduces the amount or function of an antibody having a sequence as defined above. The agent may comprise an anti-idiotypic antibody to said antibody, an antigen fragment that binds to said antibody, an siRNA that reduces said antibody's expression, or a non-Fe containing antibody that competes with said antibody.).

As to common technical feature #4, the common technical feature is obvious because Univ Texas teaches an siRNA directed against an antibody to treat multiple sclerosis (claim 43 and pg 4 In 16-21), where the nucleotide sequence of a VH4 antibody variable heavy chain comprising mutations was known (SEQ ID NO: 65; nt 1-392; 100% sequence identity to instant application nucleotide SEQ ID NO: 65, which encodes the variable heavy chain of VH4 monoclonal AJL-01). and the nucleotide sequence of a VH4 antibody variable light chain was also known (SEQ ID NO: 66; nt 1-345; 100% sequence identity to instant application nucleotide SEQ ID NO: 66, which encodes the variable light chain of VH4 monoclonal AJL-01). Thus, it would have been obvious to one of ordinary skill in the art to have prepared an antisense or a siRNA oligonucleotide directed at known VH4 CDR sequences.

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I, II, III+, IV+ lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning item 4: Claims 5-10, 13-23, 29-33, 36-43, 49-53, 56-65, 70-75 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).

Note, Claim 43 is drafted as: "The process of any one of claims B1 to B 19, wherein the neurodegenerative disease is....". In accord with claim 42 which depends on "any one of claims 24 to 41", claim 43 is reconstrued to depend on "any one of claims 24 to 42",

Note, Claim 77 is drafted as "the method of claim 76". Given that claim 76 discloses "a pharmaceutical composition", claim 77 is reconstrued to "the pharmaceutical composition of claim 76".