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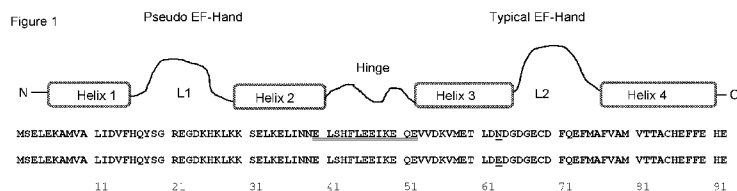
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(54) Title: ANTIBODIES TARGETING S100B AND METHODS OF USE



(57) Abstract: Novel antibodies that exhibit binding specificity for S100B are disclosed, as well as variants and fragments thereof that retain the binding specificity of the antibodies from which they are derived, collectively termed S100B binding agents. Methods of using these antibodies in the treatment and prevention of S100B-associated diseases are also provided. Methods of using these antibodies in the screening and diagnosis of subjects for S100B-associated diseases are further provided.

ANTIBODIES TARGETING S100B AND METHODS OF USE

SEQUENCE LISTING

[0001] A sequence listing in electronic (ASCII text file) format is filed with this application and incorporated herein by reference. The name of the ASCII text file is “2016_0245A_ST25.txt”; the file was created on March 11, 2016; the size of the file is 15 KB.

BACKGROUND

[0002] Ca^{2+} ions are important second messengers in all living cells [1]. Ca^{2+} -binding proteins, including members of the calmodulin/troponin/S100 superfamily, maintain the integrity of the Ca^{2+} signal and transmit it in a temporally and spatially coordinated manner [2]. S100s were discovered in 1965 [3], and as with other EF-hand containing proteins, S100s also transduce changes in $[\text{Ca}^{2+}]$ intracellular levels (i.e., $[\text{Ca}^{2+}]_i$) into cellular responses by binding Ca^{2+} , changing conformation, and then interacting with and modulating the activity of other proteins (target proteins).

[0003] S100 proteins are distinguished from other members of the calmodulin/troponin/S100 superfamily of EF-hand Ca^{2+} sensors by their 3D structure and highly conserved 14 amino acid Ca^{2+} binding loop [24]. Upon binding Ca^{2+} , S100 proteins undergo a conformational change which exposes a hydrophobic patch necessary for interacting with numerous intra- and extra-cellular protein targets and subsequent exertion of their biological effects [23]. S100 proteins regulate a large number of diverse cellular processes that include energy metabolism, cell proliferation, cytoskeletal organization, Ca^{2+} homeostasis, and signal transduction pathways.

[0004] The amino acid homology between the current family members ranges from approximately 20% to 55% [4]. Because of the extensive amino acid homology between S100B and S100A1, the first two family members identified, early models predicted that individual members were functionally redundant and essentially interchangeable. As the number of family members discovered has increased and differences in their cellular/subcellular localization, physical properties, and target proteins have expanded, additional models have arisen with specific S100 family members having unique biological functions [5–12, 20]. This multigenic family now contains at least twenty-one members (human) whose phylogenetic distribution is restricted to higher chordates. Oligomerization properties, affinities for divalent metal ions (Ca^{2+} ,

Zn^{2+} , Cu^{2+}), and posttranslational modifications also contribute to diversity among S100 family members [7, 12].

[0005] S100B is of particular interest. In addition to intracellular signaling, S100B is released into the extracellular space where it participates in local intercellular communication (autocrine and paracrine). It also enters the systemic circulation where it can coordinate biological events over long distances. The biological effects of extracellular S100B are concentration-dependent; nanomolar S100B levels beneficially promote neuronal survival while micromolar levels detrimentally promote apoptosis [25].

[0006] S100B lacks a signal peptide for secretion via the conventional Golgi-mediated pathway, and there is debate as to whether it is actively secreted from live cells or passively released. The short half-life (30 minutes) and renal clearance (2 hours) for S100B indicate that persistent elevations reflect active secretion or passive release [26]. S100B release/secretion is gender- and age-specific, and can be augmented by forskolin, lysophosphatidic acid, serotonin, glutamate, IL-6 β , metabolites, and the neurotoxic A β peptide [27-32]. Biochemical and cellular studies have identified a number of receptors for extracellular S100B including the advanced glycation end-products receptor (AGER or RAGE), toll-like receptor-4 (TLR4), FGF-receptor 1 (FGFR1), activated leukocyte adhesion molecule (ALCAM), and formyl peptide receptor like 1 (FPRL1) [33-38].

[0007] Aberrant S100B expression/activity is associated with numerous neurological disorders including normal cognitive aging, post-operative cognitive decline, cancers, traumatic brain injury, Parkinson's disease, Down's syndrome and Alzheimer's disease (AD) [13-23].

[0008] Given the numerous pathways and processes in which the protein is involved, the development of molecules that can act as agonists or antagonists of S100B, or simply bind the protein with specificity, is an important goal as such molecules could be useful in the treatment and/or prevention of diseases and aberrant conditions associated with the protein, and well as in the screening and diagnosis of subjects for such diseases and conditions. The present invention is directed to this and other important goals.

SUMMARY

[0009] Provided herein are novel antibodies that exhibit binding specificity for S100B as well as variants and fragments thereof that retain the binding specificity of the antibodies from which they are derived, collectively termed S100B binding agents. Methods of using these binding agents in the treatment and prevention of S100B-associated diseases are also provided. Methods of using these binding agents in the screening and diagnosis of subjects for S100B-associated diseases are further provided.

S100B Binding Agents

[0010] In a first embodiment, the present invention is directed to agents that exhibit binding specificity for the S100B polypeptide. Such S100B binding agents include antibodies and variants thereof that exhibit binding specificity for S100B, as well as fragments thereof that exhibit binding specificity for S100B.

[0011] In a first aspect of this embodiment, the invention is directed to antibodies having binding specificity for an epitope of S100B, wherein the epitope comprises the amino acid sequence set forth in SEQ ID NO:5. In a related aspect, the invention is directed to antibodies having binding specificity for S100B, wherein the antibody was raised against an epitope of S100B comprising the amino acid sequence set forth in SEQ ID NO:5.

[0012] Antibodies having binding specificity for S100B and S100B epitopes can be defined, in non-limiting aspects of the invention, based on their variable regions, i.e., as antibodies comprising one or more of (a) a V_L region comprising the amino acid sequence of SEQ ID NO:11, (b) a V_H region comprising the amino acid sequence of SEQ ID NO:9, (c) a V_L region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:11, and (d) a V_H region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:9.

[0013] In particular aspects, the invention is directed to antibodies having binding specificity for S100B comprising (a) a V_L region comprising the amino acid sequence of SEQ ID NO:11 and a V_H region comprising the amino acid sequence of SEQ ID NO:9; or (b) a V_L region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:11 and a V_H region comprising the amino acid sequence of SEQ ID NO:9; or (c) a V_L region comprising the amino acid sequence of SEQ ID NO:11 and a V_H region comprising an amino

acid sequence with at least about 95% sequence identity to SEQ ID NO:9; or (d) a V_L region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:11 and a V_H region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:9.

[0014] Antibodies having binding specificity for S100B and S100B epitopes can also be defined, in non-limiting aspects of the invention, based on their heavy and light chains, i.e., as antibodies comprising one or more of (a) a light chain comprising the amino acid sequence of SEQ ID NO:4, (b) a heavy chain comprising the amino acid sequence of SEQ ID NO:2, (c) a light chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:4, and (d) a heavy chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:2.

[0015] In particular aspects, the invention is directed to antibodies having binding specificity for S100B comprising (a) a light chain comprising the amino acid sequence of SEQ ID NO:4 and a heavy chain comprising the amino acid sequence of SEQ ID NO:2; or (b) a light chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:4 and a heavy chain comprising the amino acid sequence of SEQ ID NO:2; or (c) a light chain comprising the amino acid sequence of SEQ ID NO:4 and a heavy chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:2; or (d) a light chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:4 and a heavy chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:2.

[0016] In a specific aspect, the invention is directed to the 1C8 antibody, a S100B binding antibody comprising a light chain having the amino acid sequence set forth in SEQ ID NO:4 and a heavy chain having the amino acid sequence set forth in SEQ ID NO:2.

[0017] In particular aspects, the antibodies may be polyclonal or monoclonal antibodies, and the antibodies may be in the form of an antiserum comprising the antibodies. The antibodies may be isolated antibodies, purified antibodies, exogenous antibodies, endogenous antibodies, or a combination thereof. Further, the antibodies may be recombinant antibodies. The antibodies may be raised in humans, mice, rats, goats, rabbits, pigs, birds, or other animals. The antibodies may be raised against an epitope of S100B from human, mouse, rat, goat, rabbit, or pig S100B, or S100B from some other mammal.

[0018] The S100B binding agents include variants of each of the antibodies defined herein that retain the ability to bind S100B or an S100B epitope. The variants include, but are not limited to, humanized antibodies, chimeric antibodies, and fully human antibodies.

[0019] The S100B binding agents include fragments of the antibodies and variants defined herein that retain the ability to bind S100B or an S100B epitope. The fragments include, but are not limited to, Fab fragments, F(ab')₂ fragments, single chain Fv (scFv) antibodies, and fragments produced by an Fab expression library.

[0020] In antibodies comprising amino acid sequences with sequence identity to a particular sequence (e.g., 95% sequence identity to SEQ ID NO:2), the variation in sequence may be limited to the one or more of the framework regions (FRs) or limited to one or more of the complementarity determining regions (CDRs). The variations may also be found in one or more of the FRs and in one or more of the CDRs.

[0021] In each of the S100B binding agents of the invention, the antibodies, variants, and fragments exhibit at least 75% of the binding affinity for mouse S100B that is exhibited by the 1C8 antibody described herein.

[0022] The invention includes polynucleotides comprising nucleotide sequences encoding each the S100B binding agents provided in the various embodiments and aspects defined herein, as well as complementary strands thereof. The invention also includes cloning and expression vectors comprising the polynucleotides, and host cells comprising the polynucleotides and/or cloning and expression vectors. The invention further includes methods of producing the S100B binding agents defined herein, comprising culturing the host cells under conditions promoting expression of the binding agents encoded by the expression vectors, and recovering the binding agents from the cell cultures.

Pharmaceutical Formulations

[0023] In a second embodiment, the invention is directed to pharmaceutical formulations comprising one or more of the S100B binding agents defined herein and a pharmaceutically acceptable carrier or diluent. Thus, the pharmaceutical formulations include those comprising one or more of the antibodies defined herein and a pharmaceutically acceptable carrier; one or more the humanized variants defined herein and a pharmaceutically acceptable carrier; and one or more the S100B-binding fragments defined herein and a pharmaceutically acceptable carrier.

Kits, Cells Lines, and Agents

[0024] In a third embodiment, the invention is directed to kits comprising (a) one or more of the S100B binding agents as defined herein, and (b) a labeled secondary antibody recognizing the S100B binding agent(s) of (a). The S100B binding agents include one or more of the antibodies defined herein, one or more the humanized variants defined herein, and one or more the S100B-binding fragments defined herein.

[0025] In a fourth embodiment, the invention is directed to cell lines which produce one or more of the S100B binding agents defined herein. Thus, the cell lines of the invention include those which produce one or more of the antibodies defined herein, one or more the humanized variants defined herein, and one or more the S100B-binding fragments defined herein.

[0026] In a fifth embodiment, the invention is directed to diagnostic reagents for Alzheimer disease comprising one or more of the S100B binding agents defined herein. Thus, the diagnostic reagents of the invention include those comprising one or more of the antibodies defined herein, one or more the humanized variants defined herein, and one or more the S100B-binding fragments defined herein.

[0027] In a sixth embodiment, the invention is directed to therapeutic agents for a S100B-associated disease or condition comprising one or more of the S100B binding agents defined herein. Thus, the therapeutic agents of the invention include those comprising one or more of the antibodies defined herein, one or more the humanized variants defined herein, and one or more the S100B-binding fragments defined herein.

Methods of Treatment and Prevention

[0028] In a seventh embodiment, the invention is directed to methods of using S100B binding agents in the treatment or prevention of S100B-associated diseases or conditions in a subject. Such methods generally encompass administering one or more of the S100B binding agents defined herein, or a pharmaceutical formulation comprising one or more of the S100B binding agents, to a subject in need thereof. Such methods can be used to inhibit S100B activity and/or inhibit S100B binding, e.g. to a target protein or receptor. Such methods can be used to treat or prevent a symptom of a disease or condition associated with aberrant S100B activity.

Such methods can further be used to treat or prevent a disease or condition associated with aberrant S100B activity.

[0029] Thus, in one aspect of this embodiment, the invention is directed to a method of treating or preventing a S100B-associated disease or condition in a subject comprising administering a therapeutically-effective amount of one or more S100B binding agents to a subject in need thereof, thereby treating or preventing a S100B-associated disease or condition in the subject. The one or more S100B binding agents may be administered as a pharmaceutical formulation as defined herein.

[0030] S100B-associated diseases and conditions include, but are not limited to, aging, neurological disorders, acute injury, psychiatric and affective disorders, and cancer. Neurological disorders include, but are not limited to, Alzheimer's disease, Parkinson's disease, Down syndrome, COPD-induced cognitive decline, autoimmune-induced cognitive decline, post-operative cognitive decline, radiation-induced cognitive decline, vertigo and aging. Acute injuries include, but are not limited to traumatic brain injury, stroke/ischemia, and hypoxic ischemia at term. Psychiatric and affective disorders include, but are not limited to, disease-induced depression, mood disorders, suicide, schizophrenia, and delirium. Cancers include, but are not limited to, melanoma, astrocytoma, glioma, and brain cancer.

[0031] In certain aspects of the methods, the therapeutically-effective amount of the S100B binding agent is between 10 ug/kg and 100 mg/kg of the agent per body weight of the subject.

[0032] In certain aspects of these methods, the S100B binding agent is administered to the subject via intravenous, subcutaneous, or intranasal means, or other means.

Methods of Screening

[0033] In an eighth embodiment, the invention is directed to methods of using S100B binding agents in the screening for and/or quantifying of S100B in a biological sample. Such methods generally encompass using one or more of the S100B binding agents defined herein to detect S100B in a biological sample, such as a sample obtained from a subject.

[0034] In one aspect of this embodiment, the invention is directed to a method of screening a biological sample for the presence of S100B comprising contacting a biological sample with one or more of the S100B binding agents defined herein and detecting binding of S100B by the one or more S100B binding agents.

[0035] In another aspect of this embodiment, the invention is directed to a method of quantifying the amount of S100B in a biological sample comprising contacting a biological sample with one or more of the S100B binding agents defined herein, detecting binding of S100B by the one or more S100B binding agents, and quantifying the amount of S100B detected.

[0036] In each of the methods of this embodiment, the biological sample includes, but is not limited to, blood, serum, cerebrospinal fluid (CSF), tissue sample, tears, sweat, urine, lymph, tumor exudate, ascites fluid, and pleural fluids.

[0037] In each of the methods of this embodiment, binding of S100B by the one or more S100B binding agents is via an assay that includes, but is not limited to, an ELISA assay, an affinity column, a Western/immunoblot, immunohistochemistry, or a proximity ligation assay.

Methods of Diagnosis

[0038] In a ninth embodiment, the invention is directed to methods of using S100B binding agents in the diagnosing and/or monitoring of S100B-associated diseases or conditions in a subject. Such methods generally encompass using one or more of the S100B binding agents defined herein to quantifying the amount of S100B in a biological sample obtained from a subject and using the quantified amount of S100B to monitoring disease progression or to make a diagnosis of a S100B-associated disease or condition in the subject. Such methods include administering one or more of the S100B binding agents defined herein to a subject having a S100B-associated disease or condition, or suspected of having a S100B-associated disease or condition, and detecting binding of the one or more S100B binding agents to S100B in the subject.

[0039] In one aspect of this embodiment, the invention is directed to a method of monitoring a S100B-associated disease or condition in a subject comprising (a) contacting a biological sample obtained from a subject having a S100B-associated disease or condition with one or more of the S100B binding agents defined herein, (b) detecting binding of S100B by the one or more S100B binding agents, (c) quantifying the amount of S100B detected, (d) comparing the amount quantified in (c) with an amount quantified in a biological sample from the same subject at an earlier time point, thereby monitoring a S100B-associated disease or condition in the subject.

[0040] In another aspect of this embodiment, the invention is directed to a method of diagnosing a S100B-associated disease or condition in a subject comprising (a) contacting a biological sample obtained from a subject with one or more of the S100B binding agents defined herein, (b) detecting binding of S100B by the one or more S100B binding agents, (c) quantifying the amount of S100B detected, (d) comparing the amount quantified in (c) with one or more control amounts quantified in a biological sample obtained from a subject with a S100B-associated disease or condition, thereby diagnosing a S100B-associated disease or condition in the subject.

[0041] In further aspect of this embodiment, the invention is directed to a method of diagnosing a S100B-associated disease or condition in a subject comprising administering one or more of the S100B binding agents defined herein to a subject having a S100B-associated disease or condition, or suspected of having a S100B-associated disease or condition, and detecting binding of the one or more S100B binding agents to S100B in the subject.

[0042] In each of the relevant methods of this embodiment, the biological sample includes, but is not limited to, blood, serum, cerebrospinal fluid (CSF), tissue sample, tears, sweat, urine, lymph, tumor exudate, ascites fluid, and pleural fluids.

[0043] In each of the relevant methods of this embodiment, binding of S100B by the one or more S100B binding agents is via an assay that includes, but is not limited to, an ELISA assay, an affinity column, a Western/immunoblot, immunohistochemistry, a proximity ligation assay or *in vivo* imaging.

[0044] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described herein, which form the subject matter of the claims of the invention.

BRIEF DESCRIPTION OF DRAWINGS

[0045] **Figure 1.** Structure of S100B. The four helices, two loops (L1 and L2) and the hinge/linker domain are shown. The pseudo EF-hand comprises helices 1 and 2 and loop 1; the typical EF-hand comprises helices 3 and 4 and loop 2. The upper amino acid sequence is human S100B (SEQ ID NO:6). The lower amino acid sequence is mouse S100B (SEQ ID NO:7). The

epitope used in the production of antibodies (SEQ ID NO:5) is double underlined. The single amino acid change between human and mouse sequences is underlined.

[0046] Figures 2A-2D. S100B•TLR4 (Fig. 2A), S100B•FGFR1 (Fig. 2B) and S100B•RAGE (Fig. 2C), but not S100B•D2R (Fig. 2D), complexes are present in sporadic and familial human AD specimens. Representative micrographs of sporadic (olfactory cortex) and familial (cingulate gyrus) AD human specimens processed for proximity ligation assays using S100B (BL356) + TLR4, FGFR1, AGER or D2R primary antibodies (brown; solid arrows) and counterstained with hematoxylin to visualize nuclei (blue; dashed arrows). Bars = 50 μ m.

[0047] Figures 3A-3C. S100B•TLR4 (Fig. 3A), S100B•FGFR1 (Fig. 3B) and S100B•RAGE (Fig. 3C) complexes exhibit age-dependent increases in the PSAPP AD mouse model. Representative micrographs of parasagittal cortical sections from 6 and 12 month old PSAPP/S100B^{+/+} and PSAPP/S100B^{-/-} mice processed for proximity ligation assays using S100B (BL356) + TLR4, FGFR1 or AGER primary antibodies (brown; solid arrows) and counterstained with hematoxylin to visualize nuclei (blue; dashed arrows). Bars = 50 μ m.

[0048] Figures 4A-4C. Anti-S100B (BL356) antibody crosses the blood brain barrier and neutralizes S100B. Fig. 4A contains representative micrographs of sections from S100B^{+/+} or S100B^{-/-} mice stained with the anti-S100B (BL356) antibody (Bethyl Laboratories) (red) and counterstained with DAPI (blue) to visualize nuclei. Bars = 20 μ m. Fig. 4B contains *ex vivo* images of brain tissue from PSAPP mice 48 hours after receiving a single IV injection of IRDye® 800CW-labeled anti-S100B antibody (Bethyl Laboratories) and untreated mice (control). Fig. 4C contains representative micrographs of parasagittal cortical sections from treated and placebo animals stained with a commercially available anti-S100B antibody (BO Biosciences) (red) and DAPI (blue) to visualize S100B and nuclei, respectively. Bars = 100 μ m.

[0049] Figures 5A-5B. Neutralization of extracellular S100B blocks TLR4/NF- κ B signaling. Representative micrographs of parasagittal cortical sections from 7.5 month old PSAPP mice treated with an anti-S100B antibody (BL356) or isotype control-IgG/placebo and processed for proximity ligation assays using S100B + TLR4 primary antibodies (brown; solid arrows) and counterstained with hematoxylin to visualize nuclei (blue; dashed arrows) (Fig. 5A). Bars = 50 μ m. Fig. 5B contains representative micrographs of adjacent sections processed for immunohistochemistry using an NF- κ B primary antibody (red) and counterstained with DAPI to visualize nuclei (blue). Bars = 100 μ m.

[0050] Figures 6A-6B. Neutralization of extracellular S100B blocks FGFR/Akt signaling. Representative micrographs of parasagittal cortical sections from 7.5 month old PSAPP mice treated with an anti-S100B (BL356) antibody or isotype control-IgG/placebo and processed for proximity ligation assays using S100B + FGFR1 primary antibodies (brown; solid arrows) and counterstained with hematoxylin to visualize nuclei (blue; dashed arrows) (Fig. 6A). Fig. 6B contains representative micrographs of adjacent sections processed for immunohistochemistry using a PO4-Akt primary antibody (red) and counterstained with DAPI to visualize nuclei (blue). Bars = 50 μ m.

[0051] Figures 7A-7B. Neutralization of extracellular S100B reduces neuroinflammation. Representative micrographs of parasagittal cortical sections from 7.5 month old PSAPP mice receiving anti-S100B (BL356) antibody or an isotype control-IgG/placebo stained with the astrocytic marker GFAP (Fig. 7A) or the microglial marker Iba1 (Fig. 7B) and DAPI (blue) to visualize nuclei. Bars = 100 μ m. The histograms depict the mean GFAP or Iba1 burden + SEM in PSAPP mice receiving anti-S100B (white bars, n = 5) or an isotype control-IgG/placebo (grey bars, n = 4). Analysis by an independent samples t-test, asterisks denote $p < 0.05$ when compared to the placebo group.

[0052] Figure 8. Neutralization of extracellular S100B reduces amyloidosis. Representative micrographs of parasagittal cortical sections from 7.5 month old PSAPP mice receiving anti-S100B (BL356) antibody or isotype control-IgG/placebo stained with thioflavin S (green) to visualize plaques. Bars = 100 μ m. The histogram depicts the mean plaque load + SEM in PSAPP mice receiving anti-S100B (white bars, n = 5) or an isotype control-IgG/placebo (grey bars, n = 4). Analysis by an independent samples t-test, asterisks denote $p < 0.05$ when compared to the placebo group.

[0053] Figure 9. Neutralization of extracellular S100B reduces dystrophic neurites. Representative micrographs of parasagittal hippocampal sections from 7.5 month old PSAPP mice receiving anti-S100B (BL356) antibody or an isotype control-IgG/placebo stained with a PSD95 primary antibody (red) and DAPI (blue) to visualize dystrophic neurites and nuclei, respectively. Bars = 50 μ m.

[0054] Figure 10. Dose-response curves for engineered antibodies. A direct ELISA and serial dilutions ranging from 0.0025 to 83 micrograms were used to generate dose-response curves for engineered antibodies. All five antibodies exhibited greater sensitivity (concentration

for 10% binding) than the BL356 prototype. However, only three exhibited a lower EC₅₀/higher K_D (concentration for 50% binding) than the BL356 prototype.

[0055] Figure 11. *In vivo* pharmacodynamics/target engagement for engineered antibodies. Representative micrographs of brains sections from 6 month old PSAPP mice treated with a single dose of engineered antibody (BL356) or placebo/control for 7 days. Sections were processed for proximity ligation assays using S100B + TLR4 primary antibodies (brown; solid arrows) and counterstained with hematoxylin to visualize nuclei (blue; dashed arrows). Bars = 50 μ m.

[0056] Figure 12. muBL356(1C8) Detection. Indirect ELISAs were used to detect muBL356(1C8) in PBS and serum. The data are expressed as the mean percent binding $\pm$ the SEM (n=3). The shift in the dose response curve indicates that quantitative data can be obtained if control and experimental samples contain equivalent concentrations of serum.

[0057] Figure 13. Detection of muBL356(1C8)•S100B complexes. Indirect ELISAs were used to assess the ability of various capture antibodies (2 fold excess) to detect muBL356(1C8)•S100B complexes. The data are expressed as the mean relative binding (absorbance) $\pm$ the SEM (n=4). Only antibody #4 detected muBL356(1C8)•S100B complexes.

[0058] Figure 14A. The nucleotide sequence (SEQ ID NO:1) encoding the mouse 1C8 antibody heavy chain. The various domains encoded by the heavy chain sequence are indicated with underlining, ordered as shown. FR = framework region; CDR = complementarity determining region.

[0059] Figure 14B. The amino acid sequence (SEQ ID NO:2) of the mouse 1C8 antibody heavy chain. The various domains of the heavy chain are indicated with underlining, ordered as shown. FR = framework region 1; CDR = complementarity determining region.

[0060] Figure 14C. The nucleotide sequence (SEQ ID NO:8) encoding the mouse 1C8 antibody heavy chain variable region. The various domains encoded by the heavy chain variable region sequence are indicated with underlining, ordered as shown. FR = framework region; CDR = complementarity determining region.

[0061] Figure 14D. The amino acid sequence (SEQ ID NO:9) of the mouse 1C8 antibody heavy chain variable region. The various domains of the heavy chain variable region are indicated with underlining, ordered as shown. FR = framework region 1; CDR = complementarity determining region.

[0062] Figure 15A. The nucleotide sequence (SEQ ID NO:3) encoding the mouse 1C8 antibody light chain. The various domains encoded by the light chain sequence are indicated with underlining, ordered as shown. FR = framework region; CDR = complementarity determining region.

[0063] Figure 15B. The amino acid sequence (SEQ ID NO:4) of the mouse 1C8 antibody light chain. The various domains of the light chain are indicated with underlining, ordered as shown. FR = framework region; CDR = complementarity determining region.

[0064] Figure 15C. The nucleotide sequence (SEQ ID NO:10) encoding the mouse 1C8 antibody light chain variable region. The various domains encoded by the light chain variable region sequence are indicated with underlining, ordered as shown. FR = framework region; CDR = complementarity determining region.

[0065] Figure 15D. The amino acid sequence (SEQ ID NO:11) of the mouse 1C8 antibody light chain variable region. The various domains of the light chain variable region are indicated with underlining, ordered as shown. FR = framework region; CDR = complementarity determining region.

[0066] Figure 16. Neutralizing extracellular S100B antibodies halt primary melanoma tumorigenesis. Intralesional administration of an anti-S100B (BL356; prototype) antibody and candidate antibody 1C8 (35 $\mu\text{g}/100\text{mm}^3$) twice weekly for 25 days halted primary tumor growth in an immune competent BRAF wild-type mouse melanoma model. * denotes $p < 0.05$.

[0067] Figures 17A-17B. Inhibition of extracellular S100B reduces ERK/AKT activation. Immune competent BRAF wild-type animals received intralesional doses of candidate antibody 1C8 (35 $\mu\text{g}/100\text{mm}^3$) twice weekly. Twenty-five days later tissues were processed for PO_4 -ERK and PO_4 -AKT detection using fluorescence (Fig. 17A) or brightfield (Fig. 17B) microscopy.

[0068] Figures 18A-18B. S100B^{-/-} mice had improved retention memory function after TBI. Fig. 18A. TBI induced sensorimotor impairments at all time points when compared with sham-injured mice in the beam walk test (** $p < 0.001$, * $p < 0.01$, vs. sham). However, S100B^{-/-} mice failed to show any improvement in sensorimotor function. Analysis by repeated measures two-way ANOVA followed by Tukey's post-hoc. Fig. 18B. S100B^{-/-} mice showed significant improvement ($^+p < 0.05$, vs. S100B^{+/+}) in Discrimination Index (D.I.) in the Novel Object Recognition (NOR) task. Analysis by one-way ANOVA followed by Tukey's post-hoc test. (Mean $\pm$ S.E.M.; $n = 11-14/\text{group}$).

[0069] Figures 19A-19D. S100B^{-/-} mice had reduced lesion volume but no reduction in neuronal cell loss after TBI. Fig. 19A. Histological assessment using unbiased stereology showed that S100B^{+/+} mice developed a large lesion following TBI, whereas S100B^{-/-} mice showed a significant reduction in lesion size (* $p < 0.05$, vs., S100B^{+/+}). Analysis by one-tailed unpaired Student's t-test. Representative images of the cresyl violet-stained sections show the reduction in lesion volume observed in S100B^{-/-} samples. Stereological assessment of surviving neurons using unbiased stereological techniques was performed in the cortex (Fig. 19B), thalamus (Fig. 19C), and CA1 (Fig. 19D) sub-region of the hippocampus at 28 days after injury. TBI resulted in significant neuronal cell loss in the cortex (* $p < 0.05$, vs. sham), thalamus (* $p < 0.05$ or ** $p < 0.01$, vs. sham), and CA1 hippocampal sub-region (** $p < 0.01$, vs. sham). S100B^{-/-} mice did not show any improvement in neuronal survival in all the assessed brain regions. Analysis by one-way ANOVA followed by Tukey's post-hoc. (Mean $\pm$ S.E.M.; $n = 4-7$ /group).

[0070] Figures 20A-20B. S100B^{-/-} mice had reduced microglial activation in the injured cortex after TBI. Stereological assessment of microglial cell number and activation phenotype was performed in the cortex at 7 and 28 days after TBI. Fig. 20A. Representative images of the Iba-1 stained sections show morphological features of ramified, and hypertrophic and bushy (activated) microglia. Fig. 20B. S100B^{-/-} mice showed statistically significant increases in the numbers of ramified microglia on 28 days after injury (** $p < 0.01$, vs. sham; + $p < 0.05$, vs. S100B^{+/+}, # $p < 0.05$, vs S100B^{-/-} at 7 days), when compared with sham and S100B^{+/+} mice (at 28 days) and S100B^{-/-} mice at 7 days. TBI resulted in significant microglial activation at 7 days after injury (* $p < 0.05$, vs. sham) followed by sustained increases in numbers of activated microglia at 28 days (** $p < 0.001$, vs. sham), when compared to sham. S100B^{-/-} mice showed significant attenuation in numbers of activated microglia at 28 days (+ $p < 0.05$, vs. S100B^{+/+}), when compared to S100B^{+/+} mice. Analysis by oneway ANOVA followed by Tukey's post-hoc. (Mean $\pm$ S.E.M.; $n = 4-7$ /group).

[0071] Figures 21A-21B. Systemic administration of a neutralizing S100B antibody (BL356) improved functional outcomes following TBI. Fig. 21A. TBI induced significant sensorimotor impairments at all time points when compared with sham-injured mice in the beam walk test (** $p < 0.001$, vs. sham). Anti-S100B IgG-treated mice exhibited significant improvements in sensorimotor performance at 7, 14, 21 and 28 days (+ $p < 0.05$, vs. vehicle; ^ $p < 0.05$, vs. IgG control) after injury when compared with vehicle-treated and IgG control-

treated mice. Analysis by repeated measures two-way ANOVA followed by Tukey's post-hoc. Fig. 21B. TBI-induced deficits in retention memory ($p < 0.001$, vs. sham) in the NOR task were significantly attenuated by systemic administration of anti-S100B IgG ($^{++}p < 0.01$, vs. vehicle); data expressed as D.I. Analysis by one-way ANOVA followed by Tukey's post-hoc test. (Mean $\pm$ S.E.M.; $n = 8-14$ /injured group, $n = 6-7$ /sham group).

[0072] Figures 22A-22D. Systemic administration of a neutralizing S100B antibody (BL356) reduced lesion volume, improved neuronal survival and attenuated microglial activation in the cortex after TBI. Fig. 22A. Histological assessment using unbiased stereology showed that vehicle-treated mice developed a large lesion following TBI, whereas anti-S100B IgG treatment resulted in significant reduction in lesion size ($^{***}p < 0.001$, vs. vehicle). Systemic administration of IgG control significantly reduced the lesion volume ($^{**}p < 0.01$, vs. vehicle, $^{+}p < 0.05$, vs. anti-S100B IgG treatment). Analysis by one-way ANOVA followed by Tukey's post-hoc. (Mean $\pm$ S.E.M.; $n = 5-7$ /group). Fig. 22B. Representative images of the cresyl violet-stained sections show the reduction in lesion volume observed in anti-S100B IgG-treated samples. Fig. 22C.

Stereological assessment of surviving neurons was performed using unbiased stereological techniques in the cortex at 28 days after injury. TBI resulted in significant neuronal cell loss in the cortex ($^{**}p < 0.01$, vs. sham). Systemic administration of anti-S100B IgG significantly improved neuronal survival in the cortex ($^{+}p < 0.05$, vs. vehicle), when compared with vehicle-treated samples. Analysis by one-way ANOVA followed by Tukey's post-hoc. (Mean $\pm$ S.E.M.; $n = 5$ /group). Fig. 22D. Stereological assessment of microglial cell number and activation phenotype was performed in the cortex at 7 and 28 days after TBI. No statistically significant increases in the numbers of ramified microglia were observed on 7 and 28 days after injury. TBI resulted in significant and sustained increases in activated microglia at 7 days after injury followed by sustained increases at 28 days ($^{***}p < 0.001$, vs. sham). Systemic administration of anti-S100B IgG significantly attenuated microglial activation at 7 days after injury ($^{+++}p < 0.001$, vs. vehicle; $^{^^}p < 0.001$, vs. IgG control), when compared with vehicle-, and IgG control-treated samples. Furthermore, anti-S100B IgG continued to show significant reduction in numbers of activated microglia at 28 days post-injury ($^{+}p < 0.05$, vs. vehicle) when compared with vehicle-treated samples. Analysis by one-way ANOVA followed by Tukey's post-hoc. (Mean $\pm$ S.E.M.; $n = 5$ /group).

DETAILED DESCRIPTION

I. Definitions

[0073] Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found, for example, in Benjamin Lewin, *Genes VII*, published by Oxford University Press, 2000 (ISBN 019879276X); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Publishers, 1994 (ISBN 0632021829); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and other similar technical references.

[0074] As used herein, “a” or “an” may mean one or more. As used herein when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one. As used herein “another” may mean at least a second or more. Furthermore, unless otherwise required by context, singular terms include pluralities and plural terms include the singular.

[0075] As used herein, “about” refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term “about” generally refers to a range of numerical values (e.g., +/- 5-10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term “about” may include numerical values that are rounded to the nearest significant figure.

II. The Present Invention

[0076] Provided herein are novel antibodies that exhibit binding specificity for S100B as well as variants and fragments thereof that retain the binding specificity of the antibodies from which they are derived, collectively termed S100B binding agents. Such antibodies form the basis of the S100B binding agents of the present invention, along with variants of these antibodies, and fragments of the antibodies and variants. Particular antibodies disclosed herein include a novel anti-S100B monoclonal antibody (termed “1C8”), a polyclonal antibody (termed “BL356”), and other monoclonal antibodies that exhibit binding specificity for the S100B protein both *in vitro* and *in vivo*.

[0077] Methods of using these binding agents in the treatment and prevention of S100B-associated diseases are also provided. Methods of using these binding agents in the screening and diagnosis of subjects for S100B-associated diseases are further provided.

Antibodies

[0078] The antibodies of the present invention exhibit binding specificity for the S100B polypeptide. Such antibodies can be defined as those antibodies having binding specificity for an epitope of S100B, wherein the epitope comprises the amino acid sequence set forth in SEQ ID NO:5. Such antibodies can also be defined as those antibodies having binding specificity for S100B, wherein the antibody was raised against an epitope of S100B comprising the amino acid sequence set forth in SEQ ID NO:5.

[0079] Antibodies having binding specificity for S100B and S100B epitopes can be defined, in non-limiting aspects of the invention, based on their variable regions, i.e., as antibodies comprising one or more of (a) a V_L region comprising the amino acid sequence of SEQ ID NO:11, (b) a V_H region comprising the amino acid sequence of SEQ ID NO:9, or (c) both a V_L region comprising the amino acid sequence of SEQ ID NO:11 and a V_H region comprising the amino acid sequence of SEQ ID NO:9. The antibodies are not limited with respect to other characteristics. For example, the antibodies may be of any class, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD or IgE.

[0080] It will be readily understood by the skilled artisan that alterations can be made to the amino acid sequence of antibodies without affecting the binding activity, for example, alterations can be made to either or both the V_L and V_H regions, while maintaining the binding activity and/or binding specificity of the antibody. Thus, the present invention includes antibodies having one or more amino acid insertions, deletions and/or substitutions, that also retain binding specificity for S100B. In particular, the invention includes an antibody where the V_L region has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:11 and the V_H region comprises the amino acid sequence of SEQ ID NO:9. The invention also includes an antibody where the V_L region comprises the amino acid sequence of SEQ ID NO:11 and the V_H region has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth

in SEQ ID NO:9. The invention further includes an antibody where the V_L region has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:11 and the V_H region has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:9. In a non-limiting example, the invention includes an antibody where the V_L region has at least about 95% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:11, or the V_H region has at least about 95% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:9, or the V_L region has at least about 95% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:11 and the V_H region has at least about 95% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:9.

[0081] The isolated antibodies having binding specificity for S100B and S100B epitopes can also be defined, in non-limiting aspects of the invention, based on their heavy and light chains. In this aspect, the S100B binding antibodies comprise one or more of (a) a light chain comprising the amino acid sequence of SEQ ID NO:4, (b) a heavy chain comprising the amino acid sequence of SEQ ID NO:2, or (c) a light chain comprising the amino acid sequence of SEQ ID NO:4 and a heavy chain comprising the amino acid sequence of SEQ ID NO:2. The antibodies are not limited with respect to other characteristics. For example, the antibodies may be of any class, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD or IgE.

[0082] As suggested above, alterations can be made to the amino acid sequence of antibodies without affecting the binding activity, for example, alterations can be made to either or both the light and heavy chains, while maintaining the binding activity and/or binding specificity of the antibody. Thus, the present invention includes antibodies having one or more amino acid insertions, deletions and/or substitutions, that also retain binding specificity for S100B. In particular, the invention includes an antibody where the light chain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:4 and the heavy chain comprises the amino acid sequence of SEQ ID NO:2. The invention also includes an antibody where the light chain comprises the amino acid sequence of SEQ ID NO:4 and the heavy chain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%

sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:2. The invention further includes an antibody where the light chain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:4 and the heavy chain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:2. In non-limiting examples, the invention is directed to S100B binding antibodies comprising (a) a light chain comprising the amino acid sequence of SEQ ID NO:4 and a heavy chain comprising the amino acid sequence of SEQ ID NO:2; (b) a light chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:4 and a heavy chain comprising the amino acid sequence of SEQ ID NO:2; (c) a light chain comprising the amino acid sequence of SEQ ID NO:4 and a heavy chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:2; and (d) a light chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:4 and a heavy chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:2.

[0083] In a specific aspect, the invention is directed to the 1C8 antibody, a S100B binding antibody comprising a light chain having the amino acid sequence set forth in SEQ ID NO:4 and a heavy chain having the amino acid sequence set forth in SEQ ID NO:2.

[0084] The antibodies of the invention may be polyclonal or monoclonal antibodies, and the antibodies may be in the form of an antiserum comprising the antibodies. The antibodies may be isolated antibodies, purified antibodies, exogenous antibodies, endogenous antibodies, or a combination thereof. Further, the antibodies may be recombinant antibodies.

[0085] The S100B binding agents of the invention include variants of each of the antibodies defined herein that retain the ability to bind S100B. The term “variant” is intended to encompass modified versions of the antibodies, such as humanized antibodies and chimeric antibodies. It also encompasses fully human antibodies.

[0086] The S100B binding agents of the invention also include fragments of the antibodies and variants defined herein that retain the ability to bind S100B. The fragments included, but are not limited to, Fab fragments, F(ab')₂ fragments, single chain Fv (scFv) antibodies, and fragments produced by an Fab expression library, as well as bi-specific antibody and triple-specific antibodies.

[0087] Each of the variants and fragments of the invention exhibits at least 50% of the binding affinity for mouse S100B that is exhibited by the 1C8 antibody described herein, or at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more. The binding affinity can be determined using assays well known in the art, including ELISAs, Western/immunoblots, immunohistochemistry and proximity ligation assays. Antibodies may be produced in any species of animal, though preferably from a mammal such as a human, simian, mouse, rat, rabbit, guinea pig, horse, cow, sheep, goat, pig, dog or cat. For example, the antibodies can be human antibodies or humanized antibodies, or any antibody preparation suitable for administration to a human. For the production of the antibodies, the selected species of animal can be immunized by injection with one or more antigens, e.g., full-length S100B and/or one or more fragments thereof, a peptide comprising the linker peptide defined herein (ELSHFLEEIKEQE; SEQ ID NO:5), and a peptide consisting of the linker peptide defined herein (ELSHFLEEIKEQE; SEQ ID NO:5). The antigen may be an epitope of S100B derived from any mammalian species, including, but not limited to, human S100B, mouse S100B, rat S100B, goat S100B, rabbit S100B, pig S100B, or S100B from some other mammal.

[0088] The antigens may be administered in conjunction with one or more pharmaceutically acceptable adjuvants to increase the immunological response. Suitable adjuvants include, but are not limited to, Freund's Complete and Incomplete Adjuvant, Titermax, Oil in Water adjuvants, as well as aluminum compounds where antigens, normally peptides, are physically precipitated with hydrated insoluble salts of aluminum hydroxide or aluminum phosphate. Other adjuvants include liposome-type adjuvants comprising spheres having phospholipid bilayers that form an aqueous compartment containing the antigen and protect it from rapid degradation, and that provide a depot effect for sustained release. Surface active agents may also be used as adjuvants and include lipoteichoic acid of gram-positive organisms, lipid A, and TDM. Quil A and QS-21 (saponin-type adjuvants), monophosphoryl lipid A, and lipophilic MDP derivatives are suitable adjuvants that have hydrophilic and hydrophobic domains from which their surface-active properties arise. Compounds normally found in the body such as vitamin A and E, and lysolecithin may also be used as surface-active agents. Other classes of adjuvants include glycan analog, coenzyme Q, amphotericin B, dimethyldioctadecylammonium bromide (DDA), levamisole, and benzimidazole compounds. The immunostimulation provided by a surface active agent may also be accomplished by either developing a fusion protein with non-active portions

of the cholera toxin, exotoxin A, or the heat labile toxin from *E. coli*. Immunomodulation through the use of anti-IL-17, anti IFN- γ , anti-IL-12, IL-2, IL-10, or IL-4 may also be used to promote a strong Th2 or antibody mediated response to the immunogenic formulation.

[0089] Means for preparing antibodies are very well known in the art. The antibodies of the invention can be prepared using any known technique that provides for the production of antibody molecules. Suitable techniques include, but are not limited to, the hybridoma technique originally described by Koehler and Milstein (Nature 256:495-497 (1975)), the human B-cell hybridoma technique (Kosbor et al., Immunol Today 4:72 (1983); Cote et al., Proc Natl. Acad. Sci 80:2026-2030 (1983)), and the EBV-hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss Inc, New York N.Y., pp 77-96 (1985)). Each of these publications is herein incorporated by reference in its entirety. Additionally, antibodies can be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi et al., *Proc Natl. Acad. Sci. USA* 86: 3833-3837 (1989), and in Winter G. and Milstein C., *Nature* 349:293-299 (1991), both of which is herein incorporated by reference in its entirety.

[0090] Humanized antibodies are those antibodies where a human antibody has been engineered to contain non-human complementarity-determining regions (CDRs) derived from an antibody produced in a non-human host against a selected antigen. Means for producing humanized antibodies are well-known in the art and include Vaswani SK, and Hamilton RG, *Ann Allergy Asthma Immunol.* 81(2):105-15 (1998) and Kashmiri SV et al., *Methods* 36 (1):25-34 (2005), each of which is herein incorporated by reference in its entirety.

[0091] Chimeric antibodies are those where an antigen binding region (e.g., F(ab')₂ or hypervariable region) of a non-human antibody is transferred into the framework of a human antibody by recombinant DNA techniques. Techniques developed for the production of such antibodies include the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity. Such techniques are also well known and include: Morrison et al., *Proc Natl. Acad. Sci* 81:6851-6855 (1984); Neuberger et al., *Nature* 312:604-608(1984); Takeda et al., *Nature* 314:452-454(1985), each of which is herein incorporated by reference in its entirety.

[0092] Techniques for the production of single chain antibodies are described in U.S. Patent No. 4,946,778, incorporated herein by reference in its entirety.

[0093] Antibody fragments such as F(ab')₂ fragments can be produced by pepsin digestion of the antibody molecule, and Fab fragments can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse W. D. et al., Science 256:1275-1281 (1989), herein incorporated by reference in its entirety).

Polynucleotide, Expression Vectors, Host Cells and Method of Making

[0094] The invention includes polynucleotides comprising nucleotide sequences encoding each the binding agents provided herein, as well as complementary strands thereof.

[0095] The invention also includes vectors, such as cloning vectors and expression vectors, comprising the polynucleotides, and host cells comprising the polynucleotides and/or the vectors. Suitable expression vectors include, e.g., pcDNA3.1 and pSec-His. Suitable host cells include, e.g., Chinese hamster ovary cells (CHO cells) and human embryonic kidney cells 293 (HEK 293 cells).

[0096] The invention further includes methods of producing the binding agents defined herein, comprising culturing the host cells under conditions promoting expression of the binding agents encoded by the polynucleotides or the expression vectors, and recovering the binding agents from the cell cultures.

Kits, Cells Lines, and Agents

[0097] The invention includes kits comprising (a) one or more of the S100B binding agents as defined herein, and optionally (b) a labeled secondary antibody recognizing the S100B binding agent(s) of (a), and further optionally instructions for the use of the kit. The S100B binding agents include one or more of the antibodies defined herein, one or more the humanized variants defined herein, and one or more the S100B-binding fragments defined herein. The labeled secondary antibody may be any antibody that can bind the S100B binding agent and that is tagged with a detectable label.

[0098] The invention includes cell lines which produce one or more of the S100B binding agents defined herein. Thus, the cell lines of the invention include those which produce one or more of the antibodies defined herein, one or more the humanized variants defined herein, and one or more the S100B-binding fragments defined herein.

[0099] The invention includes diagnostic reagents for Alzheimer disease comprising one or more of the S100B binding agents defined herein. Thus, the diagnostic reagents of the invention include those comprising one or more of the antibodies defined herein, one or more the humanized variants defined herein, and one or more the S100B-binding fragments defined herein.

[00100] The invention includes therapeutic agents for a S100B-associated disease or condition comprising one or more of the S100B binding agents defined herein. Thus, the therapeutic agents of the invention include those comprising one or more of the antibodies defined herein, one or more the humanized variants defined herein, and one or more the S100B-binding fragments defined herein.

Pharmaceutical Formulations

[00101] The skilled artisan will understand that the S100B binding agents defined in the present invention can be used in a variety of applications, including methods of treating, preventing, screening for, or diagnosing S100B-associated diseases and conditions. Thus, the invention includes pharmaceutical formulations comprising the S100B binding agents and a pharmaceutically acceptable carrier or diluent (also termed antibody formulations herein).

[00102] Suitable examples of carriers and diluents are well known to those skilled in the art and include water, water-for-injection, saline, buffered saline, dextrose, glycerol, ethanol, propylene glycol, polysorbate 80 (Tween-80™), poly(ethylene)glycol 300 and 400 (PEG 300 and 400), PEGylated castor oil (e.g. Cremophor EL), poloxamer 407 and 188, hydrophilic and hydrophobic carriers, and combinations thereof. Hydrophobic carriers include, for example, fat emulsions, lipids, PEGylated phospholipids, polymer matrices, biocompatible polymers, lipospheres, vesicles, particles, and liposomes. The terms specifically exclude cell culture medium. The formulations may further comprise stabilizing agents, buffers, antioxidants and preservatives, tonicity agents, bulking agents, emulsifiers, suspending or viscosity agents, inert diluents, fillers, and combinations thereof.

[00103] The identity of the carriers and diluents will depend on the manner in which the S100B binding agents are being used and on the means used to administer pharmaceutical formulations comprising binding agents to a subject. For example, pharmaceutical formulations for intramuscular preparations can be prepared where the carrier is water-for-injection, 0.9%

saline, or 5% glucose solution. Pharmaceutical formulations may also be prepared as liquid or powdered atomized dispersions for delivery by inhalation. Such dispersion typically contain carriers common for atomized or aerosolized dispersions, such as buffered saline and/or other compounds well known to those of skill in the art. The delivery of the pharmaceutical formulations via inhalation has the effect of rapidly dispersing the immunogenic formulation to a large area of mucosal tissues as well as quick absorption by the blood for circulation. One example of a method of preparing an atomized dispersion is described in U.S. Patent No. 6,187,344, entitled, "Powdered Pharmaceutical Formulations Having Improved Dispersibility," which is hereby incorporated by reference in its entirety.

[00104] Additionally, the pharmaceutical formulations may be administered in a liquid form. The liquid can be for oral dosage, for ophthalmic or nasal dosage as drops, or for use as an enema or douche. When the pharmaceutical formulation is formulated as a liquid, the liquid can be either a solution or a suspension of the pharmaceutical formulation. There is a variety of suitable formulations for the solution or suspension of the pharmaceutical formulations that are well known to those of skill in the art, depending on the intended use thereof. Liquid formulations for oral administration prepared in water or other aqueous vehicles may contain various suspending agents such as methylcellulose, alginates, tragacanth, pectin, kelgin, carrageenan, acacia, polyvinylpyrrolidone, and polyvinyl alcohol. The liquid formulations may also include solutions, emulsions, syrups and elixirs containing, together with the active compound(s), wetting agents, sweeteners, and coloring and flavoring agents.

Methods of Treatment/Prevention

[00105] The S100B binding agents and pharmaceutical formulations defined herein can be used in the treatment or prevention of S100B-associated diseases or conditions in a subject. Such methods generally encompass administering one or more of the S100B binding agents defined herein, or a pharmaceutical formulation comprising one or more of the S100B binding agents, to a subject in need thereof. Such methods can be used to inhibit S100B activity, inhibit S100B binding, neutralize S100B activity, and/or neutralize S100B binding, e.g. to a binding partner, target protein and/or receptor. Such methods can be used to treat or prevent a symptom of a disease or condition associated with aberrant S100B activity. Such methods can further be used to treat or prevent a disease or condition associated with aberrant S100B activity (i.e., a S100B-

associated disease or condition). Thus, the S100B binding agents of the invention can have neutralizing activity. S100B binding agents having neutralizing activity, such as S100B neutralizing antibodies, can be used in the pharmaceutical formulations and methods of treatment and prevention described herein.

[00106] Methods of inhibiting S100B activity and/or inhibiting S100B binding, e.g. to a target protein or receptor, can be practiced *in vitro*, *in vivo* or *ex vivo*. Such methods include a method of inhibiting S100B activity in a subject comprising administering a therapeutically-effective amount of one or more S100B binding agents to a subject in need thereof, thereby inhibiting S100B activity in the subject. The one or more S100B binding agents may be administered as a pharmaceutical formulation as defined herein. Such methods also include a method of inhibiting S100B binding in a subject comprising administering a therapeutically-effective amount of one or more S100B binding agents to a subject in need thereof, thereby inhibiting S100B binding in the subject. The one or more S100B binding agents may be administered as a pharmaceutical formulation as defined herein. The method includes inhibiting S100B binding to a target protein, inhibiting S100B binding to a receptor, or both.

[00107] The methods of treatment and/or prevention include methods of treating or preventing a S100B-associated disease or condition in a subject comprising administering a therapeutically-effective amount of one or more S100B binding agents to a subject in need thereof, thereby treating or preventing a S100B-associated disease or condition in the subject. The one or more S100B binding agents may be administered as a pharmaceutical formulation as defined herein.

[00108] As used herein, the terms “treat”, “treating” and “treatment” have their ordinary and customary meanings, and include one or more of, ameliorating a symptom of a S100B-associated disease or condition; blocking or ameliorating a recurrence of a symptom of a S100B-associated disease or condition; decreasing in severity and/or frequency a symptom of a S100B-associated disease or condition; blocking progression of a S100B-associated disease or condition; and curing or resolving a S100B-associated disease or condition. Treatment means ameliorating, etc. by about 1% to about 100% versus a subject to which the treatment has not been administered. Preferably, the ameliorating, etc. is about 100%, about 99%, about 98%, about 97%, about 96%, about 95%, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, about 5% or about 1%. The treatment may begin prior to, concurrent with, or after the onset of clinical symptoms of the disease or condition. Thus, the subject may be

showing symptoms of a S100B-associated disease or condition, or merely diagnosed as having a S100B-associated disease or condition but so far free of symptoms of the disease or condition. The results of the treatment may be permanent or may continue for a period of days (such as 1, 2, 3, 4, 5, 6 or 7 days), weeks (such as 1, 2, 3 or 4 weeks) or months (such as 1, 2, 3, 4, 5, 6 or more months).

[00109] As used herein, the terms “prevent”, “preventing” and “prevention” have their ordinary and customary meanings, and include one or more of, stopping, averting, avoiding, or blocking the occurrence of a symptom of a S100B-associated disease or condition, the recurrence of a symptom of a S100B-associated disease or condition, or the development of a S100B-associated disease or condition. Prevention means stopping, etc. by at least about 95% versus a subject to which the prevention has not been administered. Preferably, the stopping is about 100%, about 99%, about 98%, about 97%, about 96% or about 95%. The course of therapy may begin prior to, concurrent with, or after the onset of clinical symptoms of the S100B-associated disease or condition. Thus, the subject may have a S100B-associated disease or condition or merely be susceptible to S100B-associated disease or condition. The results of the prevention may be permanent or may continue for a period of days (such as 1, 2, 3, 4, 5, 6 or 7 days), weeks (such as 1, 2, 3 or 4 weeks) or months (such as 1, 2, 3, 4, 5, 6 or more months).

[00110] In each of the methods of treatment and prevention of the present invention, the one or more S100B binding agents or pharmaceutical formulations are administered in a pharmaceutically acceptable form and in substantially non-toxic quantities. These agents and pharmaceutical formulations may be administered to a subject using different schedules, depending on the particular disease or condition being treated or prevented, and the severity thereof; the age and size of the subject; and the general health of the subject, to name only a few factors to be considered. In general, the agents and pharmaceutical formulations may be administered once, or twice, three times, four times, five times, six times or more, over a course of treatment or prevention. The timing between each dose in a dosing schedule may range between days, weeks, months, or years, and includes administered once every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more weeks. The same quantity of protein in the formulation may be administered in each dose of the dosing schedule, or the amounts in each dose may vary. The identity of the particular binding agents in the formulation may also vary or remain the same in each dose in a dosing schedule.

[00111] The amount of the S100B binding agent administered to a subject in a dose when the methods of the present invention are practiced will again vary. However, the amount administered to a subject in a dose will be sufficient to effect treatment or prevention. As an example, a therapeutically-effective amount of an S100B binding agent in a dose of a pharmaceutical formulation of the present invention is typically between about 0.1 ug to about 200 mg per kg of body weight of the subject to which the dose of the pharmaceutical formulation is being administered. Additional ranges of therapeutically-effective amounts of the S100B binding agents include, but are not limited to, about 10 ug/kg to 200 mg/kg; about 1 ug/kg to 200 mg/kg; about 100 ug/kg to 200 mg/kg; about 1 mg/kg to 200 mg/kg; about 10 mg/kg to 200 mg/kg; about 100 ug/kg to 100 mg/kg; about 1 mg/kg to 100 mg/kg; about 10 mg/kg to 100 mg/kg; about 1 mg/kg to 50 mg/kg; about 0.1 ug/kg to 50 mg/kg; about 1 ug/kg to 50 mg/kg; about 10 ug/kg to 50 mg/kg and about 100 ug/kg to 50 mg/kg.

[00112] Appropriate doses and dosing schedules can be readily determined by techniques well known to those of ordinary skill in the art without undue experimentation. Such a determination will be based, in part, on the tolerability and efficacy of a particular dose.

[00113] Administration of the pharmaceutical formulations may be via any of the means commonly known in the art of vaccine delivery. Such routes include intravenous, intraperitoneal, intramuscular, subcutaneous and intradermal routes of administration, as well as nasal application, by inhalation, ophthalmically, orally, rectally, vaginally, or by any other mode that results in the formulation contacting mucosal tissues.

[00114] S100B-associated diseases and conditions include, but are not limited to, aging, neurological disorders, acute injury, psychiatric and affective disorders, and cancer. Neurological disorders include, but are not limited to, Alzheimer's disease, Parkinson's disease, Down syndrome, COPD-induced cognitive decline, autoimmune-induced cognitive decline, post-operative cognitive decline, radiation-induced cognitive decline, vertigo, and aging. Acute injuries include, but are not limited to traumatic brain injury, stroke/ischemia, and hypoxic ischemia at term. Psychiatric and affective disorders include, but are not limited to, disease-induced depression, mood disorders, suicide, schizophrenia, and delirium. Cancers include, but are not limited to, melanoma, astrocytoma, glioma, and brain cancer.

[00115] S100B overexpression has been used as a diagnostic marker for primary and metastatic melanoma since the early 1980s [75-77]. It has been demonstrated that intracellular

S100B enhances tumor cell proliferation by binding to/promoting the degradation of the tumor suppressor p53, and subsequent MAPK activation [73, 74, 78, 79]. However, it is unlikely that the dramatic effects of S100B inhibition on tumor cell growth are limited to intracellular S100B [72, 80-82], as S100B is present in tumor exudates, ascites and pleural fluids from patients [83]. Serum S100B levels have been used as a prognostic indicator and to monitor response to treatment, including targeted therapies, in melanoma [84-85]. Extracellular S100B is a damage-associated molecular pattern molecule (DAMP) and regulates inflammatory responses in cancers [86-88] as well as fat [89], brain [46, 90, 91], gut [92] lung [93], and the immune system [94]. S100B released from tumor cells, T-cells, NK cells and macrophages [95-98], can activate tumor cells [99], monocytes [94, 100, 101] and macrophages [97, 98]. In melanoma cell lines, the metastatic switch is associated with an 8 fold increase in extracellular S100B [102] and extracellular S100B promotes invasion and metastasis in *in vitro* assays [103].

[00116] Each of the methods of treatment and prevention provided herein may be performed in conjunction with other types of therapy. For example, in methods of treating cancer via administration of one or more of the S100B binding agents of the invention, concurrent administration of traditional chemotherapeutics may occur.

[00117] As used herein, “subject” includes, but is not limited, to a human, a non-human primate, bird, horse, cow, goat, sheep, a companion animal, such as a dog, cat or rodent, or other mammal.

Methods of Screening and Diagnosis

[00118] The skilled artisan will appreciate that the S100B binding agents of the invention can be utilized in different capacities, including screening for the presence of S100B in a sample, determining the amount of S100B in a sample, monitoring progression of S100B-associated diseases or conditions in a subject, and diagnosing S100B-associated diseases or conditions in a subject. Each of these methods will generally utilize (i) a sample, such as a biological sample obtained from a subject, (ii) one or more of the S100B binding agents of the invention, such as the 1C8 antibody, where the S100B binding agent may be conjugated to a detectable label, and (iii) an assay or means for detecting binding between the S100B binding agent and S100B in the sample. The assay or means may further quantify the amount of S100B in the sample. The ability to quantify the amount of S100B will be important in determining whether there has been an

increase or decrease in the amount of S100B produced in a subject, relative to an earlier time point. Samples can be obtained from the same subject at different time points, such as before and after therapeutic intervention, and the disease or condition can be monitored in the subject. Similarly, the amount of S100B in a sample obtained from a subject can be measured, and the amount thus quantified can be compared to the amount present in a sample from the same or similar source from a subject known to have a particular S100B-associated disease or condition, thereby permitting diagnosis of a S100B-associated disease or condition. The binding of S100B by the one or more S100B binding agents may also be detected and/or quantified by direct *in vivo* imaging of a live subject. Whether based on the information regarding S100B levels alone, or in conjunction with other signs and symptoms of a S100B-associated disease or condition, a diagnosis regarding a particular S100B-associated disease or condition can be made.

[00119] Thus, the present invention is directed to methods of using S100B binding agents in the screening for and/or quantifying of S100B in a biological sample. Such methods generally encompass using one or more of the S100B binding agents defined herein to detect S100B in a biological sample, such as a sample obtained from a subject. In one aspect of this embodiment, the invention is directed to a method of screening a biological sample for the presence of S100B comprising contacting a biological sample with one or more of the S100B binding agents defined herein and detecting binding of S100B by the one or more S100B binding agents. In another aspect of this embodiment, the invention is directed to a method of quantifying the amount of S100B in a biological sample comprising contacting a biological sample with one or more of the S100B binding agents defined herein, detecting binding of S100B by the one or more S100B binding agents, and quantifying the amount of S100B detected. In some aspects of these methods, the S100B binding agent may be conjugated to a detectable label.

[00120] Methods of using the S100B binding agents in the diagnosing and/or monitoring of S100B-associated diseases or conditions in a subject generally encompass using one or more of the S100B binding agents defined herein to quantifying the amount of S100B in a biological sample obtained from a subject and using the quantified amount of S100B to monitoring disease progression or to make a diagnosis of a S100B-associated disease or condition in the subject. Alternatively, binding of S100B by the S100B binding agents can be directly imaged in the living subject.

[00121] In one aspect, the invention is directed to a method of monitoring a S100B-associated disease or condition in a subject comprising (a) contacting a biological sample obtained from a subject having a S100B-associated disease or condition with one or more of the S100B binding agents defined herein, (b) detecting binding of S100B by the one or more S100B binding agents, (c) quantifying the amount of S100B detected, (d) comparing the amount quantified in (c) with an amount quantified in a biological sample from the same subject at an earlier time point, thereby monitoring a S100B-associated disease or condition in the subject. The S100B binding agent may be conjugated to a detectable label.

[00122] In another aspect, the invention is directed to a method of diagnosing a S100B-associated disease or condition in a subject comprising (a) contacting a biological sample obtained from a subject with one or more of the S100B binding agents defined herein, (b) detecting binding of S100B by the one or more S100B binding agents, (c) quantifying the amount of S100B detected, (d) comparing the amount quantified in (c) with one or more control amounts quantified in a biological sample obtained from a subject with a S100B-associated disease or condition, thereby diagnosing a S100B-associated disease or condition in the subject. The S100B binding agent may be tagged with a detectable label.

[00123] In further aspect, the invention is directed to a method of diagnosing a S100B-associated disease or condition in a subject comprising administering one or more of the S100B binding agents defined herein to a subject having a S100B-associated disease or condition, or suspected of having a S100B-associated disease or condition, and detecting binding of the one or more S100B binding agents to S100B in the subject. The S100B binding agent may be conjugated to a detectable label.

[00124] As used herein, the term “biological sample” includes, but is not limited to blood, serum, cerebrospinal fluid (CSF), tissue sample, tears, sweat, urine, lymph, tumor exudate, ascites fluid, or pleural fluids.

[00125] The skilled artisan will understand that many of the vast array of molecules that can be conjugated to conventional antibodies may be conjugated to the S100B binding agents of the present invention. For example, the S100B binding agents can be conjugated to detectable labels such as an enzyme (e.g., peroxidase, alkaline phosphatase, glucose oxidase), a metal (e.g., gold for electron microscopy applications), a fluorescent marker (e.g., for immunofluorescence and flow cytometry applications, including CYE dyes, fluorescein isothiocyanate, rhodamine,

phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine), a fluorescence-emitting metals (e.g., ^{152}Eu), a radioactive marker (e.g., radioisotopes for diagnostic purposes, including ^3H , ^{131}I , ^{35}S , ^{14}C , and ^{125}I), a chemiluminescent marker (e.g., luminol, luciferin, isoluminol, therromatic acridinium ester, imidazole, acridinium salt and oxalate ester), and a protein tag (e.g., biotin, phycobiliprotein, c-Myc, HA, VSV-G, HSV, FLAG, V5, or HIS).

[00126] Suitable assays and means for detecting binding of S100B by the one or more S100B binding agents include, but are not limited to, ELISA assays, affinity columns, Western/immunoblots, immunohistochemistry, proximity ligation assays, and *in vivo* imaging.

[00127] Suitable assays and means for quantifying S100B bound by the one or more S100B binding agents include, but are not limited to, ELISA assays, Western/immunoblots, immunohistochemistry, proximity ligation assays, and *in vivo* imaging.

III. Examples

Example 1: Anti-S100B Polyclonal Antibody

[00128] Goats were immunized with a 13-amino acid portion of S100B corresponding to the hinge (linker) region of polypeptide (ELSHFLEEIKEQE; SEQ ID NO:5; see Figure 1) conjugated to KLH. The immune response was monitored by screening antiserum with ELISA. Standard procedures were used to prepare antisera, and affinity chromatography was used to isolate a polyclonal antibody with binding specificity for S100B. This antibody was termed “BL356”.

Example 2: Antibodies Targeting S100B-associated Neurological Disease/Disorders

[00129] S100B expression is elevated in familial as well as sporadic Alzheimer’s disease (AD), and the highest levels are observed in the most severely affected regions [13-15, 39]. S100B levels also increase in the precuneu and posterior cingulate gyrus during aging, two areas of the brain that exhibit early alterations during the course of AD [40]. The association of genetic polymorphisms in the *S100B* gene with increased risk of AD/dementia and correlation between serum/cerebrospinal fluid (CSF) S100B levels and cognitive function in some patient populations suggest that S100B signaling contributes to AD pathobiology [41-45]. Pharmacological and cell/molecular approaches were used to ascertain the contribution of extracellular S100B signaling to AD pathobiology.

[00130] PSAPP mice. The mouse PSAPP double transgenic line was generated by crossing the mouse Tg2576 line (“Swedish” APPK670N/M671L mutation) with the mouse 6.2 line (PS-1M146L) [49-51]. This mouse model mimics many facets of the human disease including A β plaque deposition, dystrophic neurites, glial activation, and memory deficits. The PSAPP line was maintained as previously described and all experimental animals were generated from PSAPP male X wildtype female (B6SJLF₁/J, strain 100012, Jackson Laboratories, Bar Harbor, ME) crosses [46,52]. Animals were housed at ambient temperature with a 12-hour light-dark cycle and provided standard mouse chow and water *ad libitum*. Genotyping was performed as previously described [51]. All procedures involving animals were approved by the Institutional Animal Care and Use Committee and comply with the *NIH Guide for the Care and Use of Laboratory Animals*.

[00131] Specimen acquisition. The BL356 mouse anti-S100B polyclonal antibody was used to neutralize extracellular S100B (generous gift of Bethyl Laboratory Inc., Montgomery, TX). Six month old PSAPP mice received IP injections (10 mg/kg) of BL356 anti-S100B or isotype IgG control (Bethyl Laboratory Inc.) on day 0, 14 and 28. On day 42, animals were euthanized, specimens harvested and processed as described below. For biodistribution studies, BL356 was labeled with the infrared dye IRDye® 800CW as described by the manufacturer (#928-38040, LI-COR Biosciences, Lincoln, NE). Animals were euthanized 48 hours after receiving a single IV injection of IRDye® labeled BL356 (2 mg/kg), and tissues were imaged immediately after harvesting using an IVIS Lumina XR (PerkinElmer, Waltham, MA) and Living Image software (PerkinElmer). Sections from human AD and control autopsy specimens were obtained from the Golden Brain Bank (Coatesville, PA). Archived specimens from PSAPP/S100B^{-/-} animals were used as negative controls [46].

[00132] Sample processing. Brains were removed from anesthetized animals and processed as previously described [46,52]. Briefly, specimens were rinsed in phosphate buffered saline (PBS), fixed in 4% (wt/vol) paraformaldehyde in PBS for 30 minutes and sliced into 2 mm sagittal sections. After a second 30 minute fixation in 4% (wt/vol) paraformaldehyde in PBS, slices were permeabilized (2mM MgCl₂, 0.01% (wt/vol) sodium deoxycholate, 0.02% (vol/vol) Nonidet P-40 in 100mM sodium phosphate buffer pH 7.5), post-fixed in 10% buffered formalin for 16 hours and embedded in paraffin. Five micron sagittal sections were mounted on glass slides for subsequent staining.

[00133] Proximity Ligation Assays. Slides were deparaffinized, rehydrated and processed for proximity ligation assays using a DUOLink II brightfield kit (#92012, OLINK, Uppsala, Sweden) as described by the manufacturer. Primary antibodies included a mouse monoclonal S100B antibody (1-50 dilution, #612376, BD Biosciences, San Jose, CA), rabbit polyclonal TLR4 antibody (1-150 dilution, #ab13556, Abcam, Cambridge, MA), goat polyclonal FGFR antibody (1-200 dilution, #SC-1884, Santa Cruz Biotechnology), rabbit polyclonal AGER antibody (1-20 dilution, #AP48164, ABGENT, San Diego, CA) and rabbit polyclonal D2DR antibody (1-400 dilution, #AB5084p, Millipore, Billerica, MA). Digital images (n=3-5 animals/patients) were captured on a Zeiss Axio Observer.Z1 using brightfield (20X objective) optics and Zen 2012 software (Carl Zeiss Group). To minimize variability, sections from experimental and control groups were processed concurrently.

[00134] Quantification of plaque load, astrocytosis and microgliosis. GFAP (astrocytosis) and Iba1 (microgliosis) staining was performed as previously described [46,52] using a rabbit polyclonal GFAP antibody (1-3000 dilution, #Z0334, Dako, Carpinteria, CA) and a rabbit polyclonal Iba1 antibody (1-150 dilution, #019-19741, Wako Chemicals US, Richmond, VA). Thioflavin S staining (T1892, Sigma, St. Louis, MO) was used to visualize plaques and performed as previously described [46,52]. To minimize variability, sections from experimental and control groups were processed simultaneously. For quantification, digital images were converted to gray scale and positive pixels quantified using Image J software (NIH Image, Bethesda, MD). Staining/load was defined as the % area, i.e. the area of positive pixels/total pixels X 100. The data were expressed as the mean $\pm$ SEM and an independent samples t-test (GraphPad Prism, La Jolla, CA) was used to determine the significance ($p < 0.05$) of measured differences between groups (n=4-6/group).

[00135] Immunofluorescence microscopy. Mouse monoclonal PO₄-Akt (Ser 473) (1-300 dilution, #4051, Cell Signaling Technology), rabbit polyclonal NF- κ B (1-150 dilution, #SC-109, Santa Cruz Biotechnology), and PSD95 (1-150 dilution, #3450, Cell Signaling Technology) were detected with an AP-polymer (Biocare Medical, Concord, CA) and Warp Red™ (Biocare Medical, Concord, CA) chromogen. Slides were mounted with ProLong Gold antifade reagent containing DAPI (P36935, Molecular Probes, Eugene, OR). Digital images (n=4 animals) were captured with a 10x/20x objective on a Zeiss Axio Observer.Z1 microscope equipped with Zen 2012 software (Carl Zeiss Group, Germany) using uniform hardware and software settings.

[00136] TLR4 and FGFR are primary extracellular S100B receptors in AD. Extracellular S100B (CSF/serum) is present in AD patients and biochemical/cellular studies have identified AGER/RAGE, TLR4, FGFR1 and DRD2 (dopamine receptor 2; D2R) as S100B receptors/target proteins. However, none of these protein-protein interactions have been verified *in vivo*. Proximity ligation assays and antibodies that recognize extracellular domains on putative S100 receptors were used to identify extracellular S100B receptors in sporadic and familial AD autopsy specimens. S100B•TLR4 and S100B•FGFR complexes were readily detectable in sporadic (olfactory cortex) and familial (cingulate gyrus) human AD specimens (Figure 2). Significantly fewer S100B•AGER complexes were present, and S100B•DRD2 (D2R) complexes were undetectable (Figures 2A-2D). The inability to detect some complexes was not due to technical issues since S100B•receptor complexes were readily discernible in adjacent sections and the suitability of all pairs of primary antibodies was verified by immunohistochemistry (data not shown). The overall reduction in staining intensity for S100B•receptor complexes in sporadic versus familial AD specimens may reflect differences in receptor levels (TLR4, FGFR and AGER), ligand levels (S100B, A β , HMGB, etc) or as of yet unidentified S100B receptors. This is the first direct demonstration of *in vivo* S100B receptor/target protein complexes and verification of TLR4, FGFR and AGER/RAGE as extracellular S100B receptors in sporadic and familial AD.

[00137] Because the recent failure of AD clinical trials has raised important issues regarding the ability of AD mouse models to mimic human AD pathobiology [53], proximity ligation assays were used to identify S100B receptor/target protein complexes in the PSAPP mouse model. S100B•TLR4, S100B•FGFR and S100B•RAGE complexes were detectable in the cortex (Figures 3A-3C) and hippocampus (data not shown) at 6 months of age, and the staining for all three complexes was increased at 12 months of age (Figures 3A-3C). S100B•TLR4, S100B•FGFR and S100B•RAGE complexes were also detected in age-matched controls, but the staining intensity was dramatically reduced (data not shown). S100B•DRD2 complexes were undetectable in control or PSAPP specimens at any age (data not shown). Genetic ablation of S100B abolished all staining (Figure 3). The age-dependent increase in the staining intensity/number of complexes may reflect differences in the levels of TLR4, FGFR and AGER, or their respective ligands (S100B, A β , HMGA, FGF, etc). Altogether, these data identify TLR4,

FGFR and AGER as extracellular S100B receptors in the human and mouse CNS and suggest that dysregulation of extracellular S100B signaling contributes to AD pathobiology.

[00138] Extracellular S100B activates TLR4/NF- κ B and FGFR/Akt signaling. Numerous studies have demonstrated that antibodies administered to AD mouse models via IP injection can cross the blood brain barrier and act in the CNS [48,54,55]. Therefore, the BL356 antibody, which is also an S100B neutralizing antibody, was used to ascertain the contribution of extracellular S100B to AD pathobiology in the PSAPP mouse model. This antibody was specific for S100B, entered the CNS and bound S100B/blocked S100B staining (Figures 4A-4C). Semi-weekly injections of this neutralizing antibody also reduced S100B•TLR4 and S100B•FGFR staining intensity/complexes in the brain when compared to a control isotype-IgG in 6 month old animals (Figures 5A-5B; Figures 6A-6B). Plaque associated staining for the transcription factor NF- κ B, a downstream target of TLR4, and PO₄-Akt, a downstream target of FGFR, were also reduced in anti-S100B treated mice when compared to isotype IgG/placebo controls (Figures 5A-5B; Figures 6A-6B). Collectively, these data confirm extracellular S100B activation of TLR4/NF- κ B and FGFR/Akt signaling in the PSAPP mouse model, and suggest that extracellular S100B signaling contributes to the phenotypic changes observed in AD mouse models in response to modulation of S100B expression.

[00139] Extracellular S100B signaling modulates neuroinflammation and amyloidosis.

To determine the contribution of extracellular S100B signaling to AD pathobiology, neuroinflammation and amyloidosis were quantified in PSAPP mice receiving the BL356 anti-S100B antibody or control IgG. Similar to chronic inhibition (genetic ablation) [46], acute neutralization of extracellular S100B had no effect on hippocampal GFAP burden (10.00 ± 0.95 and 12.09 ± 1.71 percent area), and reduced cortical GFAP burden (3.04 ± 0.73 and 9.70 ± 2.01 percent area) by 3 fold when compared to isotype-IgG/placebo treatment (Figures 7A-7B). This fold change is slightly greater than the previously reported 2-fold change in cortical GFAP burden in response to genetic ablation of S100B, suggesting that extracellular, not intracellular, S100B signaling modulates astrogliosis in AD. Acute inhibition of extracellular S100B was also as or more effective than genetic ablation in reducing microgliosis. BL356 anti-S100B treated animals exhibited a 2-fold decrease in hippocampal (1.31 ± 0.18 and 2.67 ± 0.75 percent area) and a 6-fold decrease in cortical (0.44 ± 0.19 and 2.78 ± 0.78) Iba1 burden when compared to isotype IgG treated animals (Figure 8). For comparison, genetic ablation has no effect on

hippocampal Iba1 burden and reduced cortical Iba1 burden by 2-fold [46]. The increased effectiveness of acute extracellular S100B inhibition was not attributable to neuroinflammatory changes in the control group as GFAP and Iba1 burden in the isotype-IgG control group was indistinguishable from historical data for age-matched untreated animals. The increased effectiveness of acute inhibition may be attributable to compensatory changes that occur in response to chronic inhibition (global genetic ablation) of S100B from conception. Regardless, these data demonstrate that extracellular S100B signaling modulates astrogliosis and microgliosis in the AD brain, and are consistent with cellular studies demonstrating toxic effects of exogenous S100B on astrocytes and microglia.

[00140] To determine if extracellular S100B signaling also contributed to amyloidosis, plaque load was quantified in PSAPP mice treated with BL356 anti-S100B or isotype IgG antibodies. Acute neutralization of extracellular S100B resulted in a 5-fold decrease in hippocampal (0.07 ± 0.03 and 0.32 ± 0.11 percent area) and an 8-fold decrease in cortical (0.06 ± 0.01 and 0.41 ± 0.10 percent area) plaque load when compared to isotype-IgG treated animals (Figure 8). These fold changes are larger than the 3-fold change in cortical and no change in hippocampal plaque load observed in response to genetic ablation [46]. As with neuroinflammation, the increased effectiveness of acute extracellular S100B inhibition may be attributable to compensatory changes that occur in response to chronic inhibition/genetic ablation of S100B from conception. Collectively, this is the first report of *in vivo* phenotypic effects in AD that are attributable to extracellular S100B signaling.

[00141] Extracellular S100B signaling modulates neuronal integrity. Structural degeneration in AD patients and mouse models is linked to cellular hyperexcitability, and S100B overexpressing mice exhibit reductions in dendritic density [56,57]. The post-synaptic marker PSD95 was used to determine if acute inhibition of extracellular S100B altered neuronal integrity [58]. As expected, PSAPP mice exhibited reduced dendritic and somatic PSD95 staining when compared to age-matched non-transgenic animals (Figure 9). Treatment of PSAPP mice with the BL356 anti-S100B antibody normalized the dendritic/somatic PSD95 staining pattern, while isotype IgG treatment had no effect. These data demonstrate that extracellular S100B signaling contributes to neuronal dysfunction in AD and are consistent with previous *in vivo* reports that inhibition of S100B improves network connectivity and cognitive function in animals [59,60].

[00142] This study demonstrates that extracellular S100B signaling exacerbates AD pathobiology and identifies TLR4, AGER/RAGE and FGFR as primary S100B receptors/target proteins in pre-clinical and clinical AD. The beneficial effects of inhibiting extracellular S100B may extend to other neurological disorders in which altered S100B levels/activity have been reported including normal cognitive aging, post-operative cognitive decline, cancers, traumatic brain injury, Parkinson's disease, Down's syndrome and Alzheimer's disease (AD) [13-23].

[00143] The results further demonstrate the involvement of S100B signaling in astrogliosis, microgliosis, amyloidosis and neuronal degeneration in the AD brain. Furthermore, extracellular, rather than intracellular, S100B is the primary signaling ligand as acute neutralization is as or more effective than genetic ablation [46]. These findings are consistent with previous reports that S100B antibodies delay neuronal injury in response to mechanical injury in cultured cells [61] and exogenous S100B increases astrogliosis in normal animals [47]. S100 antibodies also disrupt long-term memory in chicks [60], inhibit anxiety in rats [62] and have both beneficial and detrimental effects on neuronal responses that are stimuli-dependent [63]. The increase in neurite density reported here along with reports that S100B inhibition improves cognitive function in normal animals [59,60] suggest that extracellular S100B ablation/inhibition can improve cognitive performance.

[00144] Although, S100B is expressed in a variety of extracerebral tissues (adipose, bone/cartilage and skin), the limited information that is available indicates that changes in serum S100B levels in neurological diseases are primarily due to release from the brain [65]. In traumatic brain injury (TBI), release appears to occur via the glymphatic system [66]. Microglial activation is associated with increased blood brain barrier permeability and could also contribute to S100B release in neurological disorders [67,68]. While there are many unanswered questions regarding the mechanism(s) of S100B release from the CNS, it is evident that extracellular S100B is present in the peripheral circulation and contributes to myocyte scar formation via AGER/NF- κ B signaling [69] as well as skeletal muscle regeneration via AGER and FGF1R signaling [64].

Example 3: Anti-S100B Monoclonal Antibodies

[00145] *Mouse anti-S100B monoclonal antibodies and hybridomas.* Given the initial success of the polyclonal BL356 antibody, hybridomas were prepared that produce monoclonal

antibodies. Standard technology was used to generate stable mouse hybridoma cell lines from mice immunized with the same epitope used in the production of BL356, namely ELSHFLEEIKEQE (SEQ ID NO:5).

[00146] Supernatants from a panel of hybridomas, each of which expressed a single mouse monoclonal antibody with unique S100B binding sites, were tested for reactivity with S100B in single-point ELISA assays (maximal supernatant volume). Fifteen out of the 66 supernatants tested exhibited detectable reactivity with S100B and were further evaluated in multipoint (concentration) ELISAs. Five out of the 15 clones tested exhibited equivalent or improved affinity/ EC_{50} and sensitivity compared to BL356 (Figure 10). These five clones (8D11, 10C3, 2E1, 10A2 and 1C8) were selected for further evaluation and scale-up production.

[00147] For scale-up production, affinity purified antibodies were isolated from conditioned media (1 liter) of the hybridoma cell lines using Protein-G column chromatography (Paragon Bioservices, Baltimore, MD). Antibody-containing fractions were identified by gel electrophoresis and pooled. Bradford assays were used to determine protein concentrations and yield. Gel electrophoresis under denaturing and non-denaturing conditions was used to assess purity. EndoSafe assays were used to determine endotoxin levels. As shown in Table 1, all five clones met criteria for *in vivo* testing. However, clone 1C8 was deemed to be superior based on a 3-4 fold higher yield and low endotoxin levels (< 1.0 EU/mg). Because manufacturability is not predictive of effectiveness and sufficient quantities were available, all five clones were selected for *in vivo* testing.

Table 1: Scale-Up Production

Clone	Yield mg/L	Stability	Purity	Endotoxin EU/mg
<i>Criteria</i>	> 2.0	<i>Yes</i>	$> 90\%$	< 5.0
8D11	4.7	Yes	$> 90\%$	1.19
10C3	4.4	Yes	$> 90\%$	3.16
2E1	2.7	Yes	$> 90\%$	2.28
10A2	3.7	Yes	$> 90\%$	2.35
1C8	14.3	Yes	$> 90\%$	0.67

[00148] A single dose (10 mg/kg) of purified antibody was administered to 6 month old PSAPP mice (n=4/antibody) via IP injection. Body weight (semi-weekly) was used to assess systemic toxicity. Seven days later, animals were euthanized and visual inspection was used to evaluate gross tissue toxicity. The brain was removed, fixed and embedded in paraffin for further histological evaluation of necrosis, hemorrhage and edema. Proximity ligation assays (PLAs) were used to determine the effectiveness of the antibody in neutralizing S100B activation of the toll-like receptor 4 (TLR4). S100B•TLR4 complexes were detected as previously described [52] using a DUOLink II brightfield kit (#92012, OLINK, Uppsala, Sweden), one of the noted mouse S100B antibodies (1-50 dilution of #612377, BD Transduction Laboratories) and a rabbit TLR4 antibody (1-150 dilution of #ab13556, abcam). Digital images (n=3-5 animal) were captured on a Zeiss Axio Observer.D1 microscope using brightfield (20X objective) optics, Zen 2012 software (Carl Zeiss Group) and defined parameters. None of the antibodies tested exhibited acute gross or tissue toxicity. Only two out of the five antibodies tested, 8D11 and 1C8, exhibited target engagement, i.e. reduction in S100B•TLR4 complexes (Figure 11). Collectively, these data identified 8D11 and 1C8 as being safe and effective.

[00149] *Development of companion diagnostics.* ELISAs are the device/method of choice for companion diagnostics for complex biologics/ antibodies. ELISAs were developed that measure 1C8 levels, 1C8 activity (e.g., 1C8•S100B complexes), and anti-1C8 antibodies. Direct/indirect ELISAs used recombinant S100B protein [70] and a variety of capture and detection antibodies that included the BL356 prototype as well as clones from the S100B monoclonal antibody panel described above (e.g., 8D11 and 1C8). HRP-conjugated detection antibodies, detection reagents, and blocking reagents were obtained from Bethyl Laboratories (Montgomery, TX) and used in accordance with the manufacturer's recommendations. As shown in Figure 12, the dose-response curves for 1C8 (also termed “muBL356”) in presence of serum were shifted to the right when compared to curves for samples containing PBS only, indicating increased sensitivity with no change in dynamic range. 1C8•S100B complexes were undetectable with an indirect ELISA (capture antibody #1, Figure 13). To determine if these results were due to masking of the capture antibody binding site on S100B by 1C8, other S100B antibodies were screened for use as capture antibodies (Figure 13).

Example 4: 1C8 anti-S100B Monoclonal Antibody

[00150] In order to characterize the 1C8 mouse anti-S100B monoclonal antibody, total RNA was extracted from frozen 1C8 hybridoma cells and cDNA was synthesized from the RNA. PCR was then performed to amplify the variable regions (heavy and light chains) and constant regions of the antibody, which were then cloned into a standard cloning vector separately and sequenced.

[00151] In particular, total RNA was isolated from the hybridoma cells following the technical manual of TRIzol® Reagent (Ambion, Cat. No. 15596-026). The total RNA was analyzed by agarose gel electrophoresis. Total RNA was reverse transcribed into cDNA using isotype-specific anti-sense primers or universal primers following the technical manual of PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Cat. No. : 6110A). The antibody fragments of VH, VL, CH and CL were amplified according to the standard operating procedure of RACE of GenScript. Amplified antibody fragments were separately cloned into a standard cloning vector using standard molecular cloning procedures. Colony PCR screening was performed to identify clones with inserts of correct sizes. More than five single colonies with inserts of correct sizes were sequenced for each antibody fragment.

[00152] Five single colonies with correct VH, VL, CH and CL insert sizes as determined by agarose gel sizing (data not shown) were sequenced. The VH, VL, CH and CL genes of five different clones were found nearly identical. The consensus sequence is believed to be the sequence of the antibody produced by the hybridoma 1C8. The various antibody domains as they occur in the heavy and light chains are shown in Figures 14 and 15. In particular, Figs. 14A-14D provide the nucleic acid sequence encoding the 1C8 heavy chain (SEQ ID NO:1), the amino acid sequence of the 1C8 heavy chain (SEQ ID NO:2), the nucleic acid sequence encoding the 1C8 heavy chain variable region (SEQ ID NO:8), and the amino acid sequence of the 1C8 heavy chain variable region (SEQ ID NO:9), respectively. Figs. 15A-15D provide the nucleic acid sequence encoding the 1C8 light chain (SEQ ID NO:3), the amino acid sequence of the 1C8 light chain (SEQ ID NO:4), the nucleic acid sequence encoding the 1C8 light chain variable region (SEQ ID NO:10), and the amino acid sequence of the 1C8 light chain variable region (SEQ ID NO:11), respectively.

Example 5: Use of Anti-S100B Antibodies in Treating Cancer

[00153] Tumor and stromal cells within primary/metastatic melanomas of all genotypes/subclasses express high levels of S100B [23]. The presence of extracellular S100B in

clinical melanoma specimens and correlations between S100B levels and prognosis/treatment response in some melanoma patients suggest that antibodies having binding specificity for S100B may be a therapeutic agent in the treatment of cancer, such as melanoma and cancers associated with aberrant S100B expression.

[00154] As reported below, the S100B-binding BL356 and 1C8 antibodies halted primary melanoma tumor growth in an immune competent mouse model (Figure 16), suggesting that extracellular S100B inhibitors can be used alone or in combination with mainline therapies to safely and effectively reduce *in vivo* primary/metastatic melanoma tumorigenesis. The anti-S100B (BL356) antibody also reduced downstream ERK/AKT activation (Figure 17). This is the first *in vivo* demonstration of the role of extracellular S100B and mechanism of action in melanoma.

[00155] In the case of accessible tumors such as melanoma, drugs can be delivered directly to the tumor (intratumoral) via anti-S100B antibodies without optimization for systemic delivery and/or minimization of toxic effects on normal cells. In addition, intratumoral delivery can achieve significantly higher drug concentrations at the site of action than systemic delivery. The Tyr::RAS_{G12V}/INK4a/ARF $-/-$ mouse line is bigenic and contains two genomic mutations on an FVB background: a mutated H-ras_{G12V} transgene on the Y chromosome and inactivated INK4a/ARF alleles on chromosome 4. This model is not commercially available and a breeding colony is maintained that generates experimental animals as well as breeders for the individual Tyr::RAS_{G12V} and INK4a/ARF $-/-$ lines. Founders for both lines were obtained from the National Cancer Institute Mutant Mouse Resource (Frederick, MD). At 2–3 months of age, experimental Tyr::RAS_{G12V}/INK4a/ARF $-/-$ males develop spontaneous cutaneous melanomas in the pinna of the ears (30%), torso (23%), and tail (20%) without distant metastasis. The intratumoral *in vivo* screening protocol for BL356 and muBL356 (1C8) used a longitudinal design with a study period of 3–8 weeks and relative tumor proliferation rate (the tumor volume at a particular treatment interval/tumor volume at the time of treatment initiation) as the primary endpoint [72]. Intratumor administration of BL356 (prototype) and muBL356 (candidate) (35 micrograms/100mm³) twice weekly for 25 days halted tumor growth (data not shown).

[00156] Formalin-fixed specimens from this *in vivo* screening study and immunohistochemistry were used to assess the status of the two primary growth regulatory signaling pathways in melanoma biology. Significant reductions in ERK/MAPK and AKT

signaling as evidenced by decreased phospho-ERK and phospho-AKT were observed with BL356 treatment (Fig. 17A-17B).

[00157] Preliminary data documenting effectiveness and reduced ERK/AKT activity with intralesional administration for 21 days significantly reduces the risk that the candidate antibody is ineffective at safe doses. Alternatively, the absence of an effect on tumor volume would indicate that extracellular S100B inhibition is genotype/model or route of administration-dependent, and future studies would focus on spectrum of activity and optimization of pharmacokinetics.

Example 6: Use of Anti-S100B Antibodies in Treating Traumatic Brain Injury

[00158] Neuroinflammation following traumatic brain injury (TBI) is increasingly recognized as contributing to chronic tissue loss and neurological dysfunction. Circulating levels of S100B increase after TBI, and have been used as a biomarker. S100B is produced by activated astrocytes and can promote microglial activation; signaling by S100B through interaction with the multiligand Advanced Glycation End Product-specific Receptor (AGER) has been implicated in brain injury and microglial activation during chronic neurodegeneration. The effects of S100B inhibition were examined in a controlled cortical impact (CCI) model, using S100B knockout mice or administration of neutralizing S100B antibody (BL356).

[00159] As reported below, neutralizing S100B antibody significantly reduced TBI-induced lesion volume, improved retention memory function and attenuated microglial activation. The neutralizing antibody also significantly reduced sensorimotor deficits and improved neuronal survival in the cortex. The results strongly implicate S100B in TBI-induced neuroinflammation, cell loss and neurological dysfunction, thereby indicating that it is a potential therapeutic target for TBI.

[00160] Controlled cortical impact (CCI). A CCI-injury device [104, 105] was designed that consists of a microprocessor-controlled pneumatic impactor with a 3.5 mm diameter tip. Male mice were anesthetized with isoflurane (4% induction, 2% maintenance) evaporated in a gas mixture containing 70% N₂O and 30% O₂ and administered through a nose mask. The mouse was placed on a heated pad and a core body temperature was maintained at 37°C. A 10-mm midline incision was made over the skull, the skin and fascia were reflected, and a 4-mm craniotomy was made on the central aspect of the left parietal bone. Moderate injury was induced

using an impactor velocity of 6 m/s and deformation depth of 2 mm, as previously detailed [104, 105]. Sham animals underwent the same procedure as injured mice except for the impact. All experiments involving animals were approved by the Institutional Animal Care and Use Committee at the University of Maryland.

[00161] Genetic Intervention. All experiments used male S100B^{-/-} and S100B^{+/+} littermates (20-25g, 3 months old). Neutralizing anti-S100B Antibody Treatment: Male C57Bl/6 mice (20-25g, 3 months old) were randomized into six groups and administered 10 mg/kg anti-S100B (BL356, Bethyl Laboratories, Inc., Montgomery, TX) or an isotype matched IgG (Catalog #P50-1000, Bethyl Laboratories, Inc) antibody at 24 hours and 14 days post-injury, via the intra-peritoneal (IP) route, based on preliminary pharmacokinetic data.

[00162] Beam walk. Chronic motor recovery following TBI was assessed using a beam walk test as previously described [104, 105]. Mice were placed on one end of the beam and the number of foot faults of the right hindlimb was recorded over 50 steps. Mice were trained on the beam walk for three days prior to TBI and tested at 1, 7, 14, 21 and 28 days after injury.

[00163] Morris water maze. Spatial learning and working memory following TBI was assessed using the acquisition paradigm of Morris water maze (MWM) test on post-injury days 14, 15, 16 and 17, as described previously [104, 105]. Spatial learning and memory performance was assessed by determining the latency (seconds) to locate the sub-merged hidden platform with a 90 second limit per trial. Reference spatial memory was assessed by a probe trial, with a 60 second limit, on post-injury day 18. The time spent in the target quadrant was recorded.

[00164] Novel object recognition. Retention or intact memory was assessed by the novel object recognition (NOR) test on post-injury day 21. The apparatus consists of an open field (22.5 cm x 22.5 cm) with two adjacently-located imaginary circular zones, as previously designed [105]. The zones were equally spaced from the sides in the center of the square and designated as “old object” and “novel object” zones using the AnyMaze video tracking system. On post-injury day 20, all animals were placed in the open field for 5 minutes each without any objects present for habituation. Two 5 minute trials were performed on post-injury day 21: the first (training) trial with 2 old objects in both zones and the second (testing) phase with one old object and one novel object present in the respective zones of the open field. There was an inter-trial interval of 60 minutes. Retention memory was determined as the “Discrimination Index” (D.I.) for the second trial which was calculated using the following formula:

$$\% \text{ D.I.} = \frac{\text{Time spent in novel object zone} \times 100}{(\text{Time spent in old object zone} + \text{Time spent in novel object zone})}$$

[00165] Lesion volume. Sections were stained with cresyl violet (FD NeuroTechnologies, Baltimore, MD), dehydrated and mounted for analysis at 28 days post-TBI. Lesion volume was quantified based on the Cavalieri method of unbiased stereology using Stereoinvestigator software (MBF Biosciences, Williston, VT) [105]. The lesion volume was quantified by outlining the missing tissue on the injured hemisphere using the Cavalieri estimator with a grid spacing of 0.1 mm. Out of the total ninety-six 60 µm sections, every eighth section was analyzed beginning from a random start point.

[00166] Assessment of neuronal cell loss. The total number of surviving neurons was quantified in the cortex and thalamus using the optical fractionator method of unbiased stereology at 28 days post-TBI, as described previously [105]. The optical dissector had a size of 50 µm by 50 µm in the x and y-axis, respectively with a height of 10 µm and guard-zone of 4 µm from the top of the section. A grid spacing of 400 µm in the x-axis and 400 µm in the y-axis was used, resulting in an area fraction of one-sixty-fourth. To assess neuronal cell loss in the Cornu Ammonis (CA) 1, CA2, CA3 and dentate gyrus (DG) sub-regions of the hippocampus every fourth 60µm section between -1.22 mm and -2.54 mm from bregma was analyzed beginning from a random start point. The optical dissector had a size of 50 µm by 50 µm in the x and y-axis, respectively with a height of 10 µm and guardzone of 4 µm from the top of the section. For the CA1, CA2 and CA3 sub-regions a grid spacing of 75 µm in the x-axis and 100 µm in the y-axis was used, resulting in an area fraction of one-twelfth. For the DG sub-region, a grid spacing of 175 µm in the x-axis and 100 µm in the y-axis was used, resulting in an area fraction of one-twenty-eighth. The volume of the hippocampal subfield was measured using the Cavalieri estimator method with a grid spacing of 50 µm. The estimated number of surviving neurons in each field was divided by the volume of the region of interest to obtain the neuronal cellular density, expressed as counts/mm³.

[00167] Assessment of microglial activation. Sections were stained with Iba-1 (Wako Chemicals, Richmond, VA) and the activation status of the Iba-1 positive stained microglial cells was analyzed based on morphological features, as previously detailed [104]. Stereoinvestigator software was used to count cortical microglia in each of the three microglial morphological phenotypes (surveillant – ramified, and activated-hypertrophic and bushy) using the optical

fractionator method at 7 and 28 days post-TBI [106]. The sampled region was the ipsilateral cortex between -1.22mm and -2.54mm from bregma, and dorsal to a depth of 2.0mm from surface. Every fourth 60µm section was analyzed beginning from a random start point. The optical dissector had a size of 50µm by 50µm in the x and y-axis, respectively with a height of 10 µm and guard zone of 4µm from the top of the section. A grid spacing of 150 µm in the x-axis and 150 µm in the y-axis was used, resulting in an area fraction of one-ninth. The volume of the region of interest was measured using the Cavalieri estimator method with a grid spacing of 100 µm for the cortex. The estimated number of microglia in each morphological class was divided by the volume of the region of interest to obtain the cellular density expressed in counts/mm³.

[00168] Proximity Ligation Assays. Formalin fixed, paraffin embedded sections on glass slides collected from 7 day samples from TBI and sham groups were deparaffinized, rehydrated and processed for PLA using a DUOLink II brightfield kit (#92012, OLINK, Uppsala, Sweden) as previously described [52]. Primary antibodies included mouse anti-S100B (1-50 dilution of #612376, BD Transduction Laboratories) and rabbit anti-AGER (1- 20 dilution of #AP4861a, Abgent).

[00169] Statistical analysis. The number of animals per group for each assessment was based on prior studies using the CCI model [104-106] and satisfied power requirements. Quantitative data were expressed as mean ± standard errors of the mean (SEM) and in vitro data were expressed as mean ± standard deviation (SD). Functional data for beam walk and MWM acquisition task, respectively were analyzed by repeated measures two-way analysis of variance (ANOVA) to determine interactions between PIDs, injury/sham groups, and effect of pharmacological or genetic intervention, followed by Tukey's post-hoc test. The data for MWM probe trial were analyzed by repeated measures two-way ANOVA, and NOR task results were analyzed by one-way ANOVA followed by multiple pairwise comparisons using Tukey's post-hoc test. The lesion volume data were analyzed by unpaired Student's t test (genetic knockout study) or one-way ANOVA (anti-S100B IgG study). The stereological assessments of neuronal cell loss and microglial activation were analyzed by one-way ANOVA followed by Tukey's post hoc test. In vitro studies were analyzed by one-way ANOVA followed by Tukey's post hoc test. Data were analyzed using SigmaPlot 12 (Systat Software, San Jose, CA) or GraphPad Prism Version 4.0 for Windows (GraphPad Software, San Diego, CA). A p<0.05 was considered statistically significant.

[00170] Results

[00171] Genetic ablation of S100B provides neuroprotection.

[00172] Behavioral Outcomes. Functional assessment of fine motor coordination was performed at various time points after injury using a beam walk test (n=11-14/group). TBI induced sensorimotor impairments at all time points when compared with sham-injured mice (Fig. 18A; $p < 0.001$, vs. sham). However, S100B^{-/-} mice failed to show any improvement in sensorimotor function.

[00173] Spatial learning and reference memory were assessed using the acquisition phase and probe trial of the Morris water maze (MWM) test, respectively. TBI resulted in spatial learning impairments on post-injury days 16 and 17 ($p < 0.05$, vs. sham, data now shown); with no statistically significant differences between the two sham groups. S100B^{-/-} mice did not show improvements in cognitive performance in the acquisition phase as well as probe trial of the MWM test (data not shown). Retention or intact memory function was evaluated using the novel object recognition (NOR) task. The data were expressed as the discrimination index (D.I.) and statistically analyzed by one-way ANOVA followed by Tukey's post-hoc test (($F(3,32) = 7.308$; $p = 0.00007$)). S100B^{+/+} mice showed significant impairments in retention memory function (Fig. 18B; $p < 0.05$, vs. sham). In contrast, S100B^{-/-} mice showed a significant improvement in retention memory function, when compared to S100B^{+/+} mice (Fig 18B; $p < 0.05$, vs. S100B^{+/+}).

[00174] Neuropathological Changes. TBI-induced lesion volume (n=4-7/group) was estimated by unbiased stereological techniques. Histological assessment showed that S100B^{+/+} mice developed a large lesion following TBI (Fig. 19, $6.06 \pm 0.62 \text{ mm}^3$), whereas S100B^{-/-} mice showed a significant reduction in lesion size ($p < 0.05$, vs., S100B^{+/+}, $3.56 \pm 0.53 \text{ mm}^3$).

[00175] Stereological assessment of surviving neurons (n=4-7/group) was performed in the cortex (Fig. 19B), thalamus (Fig. 19C), and CA1 (Fig. 19D), CA2/3 and dentate gyrus (DG) (data not shown) sub-regions of the hippocampus at 28 days after injury. TBI resulted in significant neuronal cell loss in the cortex (Fig. 19B, $p < 0.05$, vs. sham), thalamus (Fig. 19C, $p < 0.01$ or 0.05 , vs. sham), and CA1 hippocampal sub-region (Fig. 19D, $p < 0.01$, vs. sham). Interestingly, S100B^{-/-} mice did not show any improvement in neuronal survival in all the assessed brain regions.

[00176] Brain injury results in a switch in microglial phenotype from a quiescent form displaying ramified cellular morphologies to more activated forms displaying hypertrophic or

bushy morphologies (Fig. 20A), with the latter being the most reactive. Stereological assessment of microglial cell number and activation phenotype (n=4-7/group) was performed in the cortex at 7 and 28 days after TBI. S100B^{-/-} mice ($37,071.59 \pm 5,518.46$ counts/mm³) showed statistically significant increases in the numbers of ramified microglia on 28 days after injury (Fig. 20B, $p < 0.01$, vs. sham; $p < 0.05$, vs. S100B^{+/+}), when compared with sham ($11,219.28 \pm 1,320.44$ counts/mm³), and injured S100B^{+/+} mice ($14,691.67 \pm 2,331.77$ counts/mm³). TBI resulted in significant microglial activation at 7 days after injury ($p < 0.05$, vs. sham, $41,202.63 \pm 7,785.17$ counts/mm³) followed by sustained increases in numbers of activated (hypertrophic and bushy) microglia at 28 days ($p < 0.001$, vs. sham, $70,259.36 \pm 8,821.79$ counts/mm³), when compared to sham (Fig. 20B). S100B^{-/-} mice showed significant attenuation in numbers of activated microglia at 28 days ($p < 0.05$, vs. S100B^{+/+}, $34,325.9 \pm 5,965.02$ counts/mm³), when compared to S100B^{+/+} mice.

[00177] Systemic treatment with a neutralizing S100B antibody provides neuroprotection.

[00178] Behavioral Outcomes. Functional assessment of fine motor co-ordination was performed at various time points after injury (n=8-14/injured group, n=6-7/sham group) using a beam walk test and the results for number of foot faults were statistically analyzed by two-way (injury/sham and treatment) repeated measures (measures/time) ANOVA followed by Tukey's post-hoc test. The interaction of "injury/sham X post-injury days" ($F(4,245)=19.939$, $p < 0.001$) and "injury/sham X treatment" ($F(2,245)=17.680$, $p < 0.001$) was statistically significant, and TBI induced significant sensorimotor impairments at all time points when compared with sham-injured mice (Fig. 21A; $p < 0.001$, vs. sham). Anti-S100B (BL356) IgG-treated mice exhibited significant improvements in sensorimotor performance at 7, 14, 21 and 28 days ($p < 0.05$, vs. vehicle and IgG control) after injury when compared with vehicle-treated and IgG control-treated mice.

[00179] Spatial learning and reference memory were assessed using the acquisition phase and probe trial of the Morris water maze (MWM) test, respectively. TBI resulted in spatial learning impairments on post-injury day 17 (data not shown; $p < 0.001$, vs. sham) in the acquisition phase of MWM test. In contrast, the data from the probe trial of the MWM test indicated that injured animals did not spend significantly greater amount of time in the target quadrant when compared with sham groups (data not shown) and there were no statistically significant differences

between the three injured groups. Anti-S100B IgG treated mice failed to show improvements in cognitive performance in the MWM test, when compared with vehicle- or IgG control-treated mice (data not shown).

[00180] Retention or intact memory was evaluated using the NOR test and statistically analyzed by one-way ANOVA followed by Tukey's post-hoc test ($F(5,49) = 0.8705$; $p < 0.0001$). TBI-induced cognitive deficits ($p < 0.001$, vs. sham) in the NOR task were significantly attenuated by systemic administration of anti-S100B IgG (Fig. 21B; $p < 0.01$, vs. vehicle).

[00181] Neuropathological Changes. TBI-induced lesion volume ($n=5-7/\text{group}$) was estimated by unbiased stereological techniques. Histological assessment showed that vehicle-treated mice developed a large lesion following TBI (Figs. 22A-22B, $10.74 \pm 0.62 \text{ mm}^3$), whereas anti-S100B IgG (BL356) treatment resulted in significant reduction in lesion size ($p < 0.001$, vs. vehicle; $p < 0.05$, vs. IgG control, $6.36 \pm 0.48 \text{ mm}^3$). Interestingly, systemic administration of IgG control significantly reduced the lesion volume ($p < 0.01$, vs. vehicle, $8.09 \pm 0.63 \text{ mm}^3$).

[00182] Stereological assessment of surviving neurons ($n=5/\text{group}$) was performed in the cortex (Fig. 22C), thalamus, and CA1, CA2/3 and dentate gyrus (DG) sub-regions of the hippocampus (data not shown) at 28 days after injury. TBI resulted in significant neuronal cell loss in the cortex (Fig. 22C, $p < 0.01$, vs. sham), thalamus (data not shown), and all hippocampal sub-regions (data not shown). Systemic administration of anti-S100B IgG significantly improved neuronal survival in the cortex (Fig. 22C; $p < 0.05$, vs. vehicle, $359,852 \pm 54,494.4 \text{ counts/mm}^3$), when compared with vehicle-treated samples ($173,830.3 \pm 40,864.82 \text{ counts/mm}^3$). However, anti-S100B IgG treatment did not attenuate TBI-induced neuronal cell loss in the thalamus as well as the hippocampus (data not shown).

[00183] Stereological assessment of microglial cell number and activation phenotype ($n=5/\text{group}$) was performed in the cortex at 7 and 28 days after TBI (Figs. 22D). No statistically significant increases in the numbers of ramified microglia were observed on 7 and 28 days after injury (Fig. 22D). TBI resulted in significant and sustained increases in activated microglia at 7 days after injury followed by sustained increases at 28 days (Fig. 22D; $p < 0.001$, vs. sham). Systemic administration of anti-S100B IgG significantly attenuated microglial activation at 7 days after injury ($p < 0.001$, vs. vehicle and IgG control, $28,571.64 \pm 5,702.16 \text{ counts/mm}^3$), when compared with vehicle- ($74,305.19 \pm 7,811.68 \text{ counts/mm}^3$), and IgG control-treated ($69,807.02$

$\pm 8,947.65$ counts/mm³) samples. Furthermore, anti-S100B IgG continued to show significant reduction in numbers of activated microglia at 28 days post-injury ($p < 0.05$, vs. vehicle, $28,727.6 \pm 3901.79$ counts/mm³) when compared with vehicle-treated ($53,348.67 \pm 4,536.09$ counts/mm³) samples.

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While the invention has been described with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various modifications may be made without departing from the spirit and scope of the invention. The scope of the appended claims is not to be limited to the specific embodiments described.

REFERENCES

[00184] All patents and publications mentioned in this specification are indicative of the level of skill of those skilled in the art to which the invention pertains. Each cited patent and publication is incorporated herein by reference in its entirety. All of the following references have been cited in this application:

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WHAT IS CLAIMED IS:

1. An isolated antibody having binding specificity for an epitope of S100B, wherein the epitope comprises the amino acid sequence set forth in SEQ ID NO:5.
2. An isolated antibody having binding specificity for S100B, wherein the antibody was raised against an epitope of S100B comprising the amino acid sequence set forth in SEQ ID NO:5.
3. The isolated antibody of claim 1 or 2, wherein the antibody comprises one or more:
 - (a) a V_L region comprising the amino acid sequence of SEQ ID NO:11,
 - (b) a V_H region comprising the amino acid sequence of SEQ ID NO:9,
 - (c) a V_L region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:11, and
 - (d) a V_H region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:9.
4. The isolated antibody of claim 1 or 2, wherein the antibody comprises a V_L region comprising the amino acid sequence of SEQ ID NO:11 and a V_H region comprising the amino acid sequence of SEQ ID NO:9.
5. The isolated antibody of claim 1 or 2, wherein the antibody comprises a V_L region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:11 and a V_H region comprising the amino acid sequence of SEQ ID NO:9.
6. The isolated antibody of claim 1 or 2, wherein the antibody comprises a V_L region comprising the amino acid sequence of SEQ ID NO:11 and a V_H region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:9.
7. The isolated antibody of claim 1 or 2, wherein the antibody comprises a V_L region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:11 and a V_H region comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:9.
8. The isolated antibody of claim 1 or 2, wherein the antibody comprises one or more:
 - (a) a light chain comprising the amino acid sequence of SEQ ID NO:4,

- (b) a heavy chain comprising the amino acid sequence of SEQ ID NO:2,
- (c) a light chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:4, and
- (d) a heavy chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:2.

9. The isolated antibody of claim 1 or 2, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:4 and a heavy chain comprising the amino acid sequence of SEQ ID NO:2.

10. The isolated antibody of claim 1 or 2, wherein the antibody comprises a light chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:4 and a heavy chain comprising the amino acid sequence of SEQ ID NO:2.

11. The isolated antibody of claim 1 or 2, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:4 and a heavy chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:2.

12. The isolated antibody of claim 1 or 2, wherein the antibody comprises a light chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:4 and a heavy chain comprising an amino acid sequence with at least about 95% sequence identity to SEQ ID NO:2.

13. The isolated antibody of any one of claims 1-12, wherein the antibody is a monoclonal antibody.

14. A humanized variant of an antibody of any one of claims 1-13.

15. An S100B-binding fragment of an antibody of any one of claims 1-14, wherein the fragment is a Fab fragment, F(ab')₂ fragment, or single chain Fv (scFv) fragment.

16. A pharmaceutical formulation comprising one or more of the isolated antibodies of any one of claims 1-13 and a pharmaceutically acceptable carrier.

17. A pharmaceutical formulation comprising one or more humanized variants of claim 14 and a pharmaceutically acceptable carrier.

18. A pharmaceutical formulation comprising one or more S100B-binding fragments of claim 15 and a pharmaceutically acceptable carrier.

19. A kit comprising (a) an isolated antibody of any one of claims 1-12, and (b) a labeled secondary antibody recognizing the isolated antibody of (a).

20. A diagnostic reagent for Alzheimer disease comprising an isolated antibody of any one of claims 1-12.

21. A cell line which produces an isolated antibody of any one of claims 1-12.

22. A cell line which produces a humanized variant of claim 14.

23. A cell line which produces a S100B-binding fragment of claim 15.

24. A therapeutic agent for a S100B-associated disease or condition comprising an isolated antibody of any one of claims 1-12.

25. A therapeutic agent for a S100B-associated disease or condition comprising a humanized variant of claim 14.

26. A therapeutic agent for a S100B-associated disease or condition comprising a S100B-binding fragment of claim 15.

27. A method of treating or preventing a S100B-associated disease or condition in a subject comprising administering a therapeutically-effective amount of pharmaceutical formulation of claim 16 to a subject in need thereof, thereby treating or preventing a S100B-associated disease or condition in the subject.

28. A method of treating or preventing a S100B-associated disease or condition in a subject comprising administering a therapeutically-effective amount of pharmaceutical formulation of claim 17 to a subject in need thereof, thereby treating or preventing a S100B-associated disease or condition in the subject.

29. A method of treating or preventing a S100B-associated disease or condition in a subject comprising administering a therapeutically-effective amount of pharmaceutical formulation of claim 18 to a subject in need thereof, thereby treating or preventing a S100B-associated disease or condition in the subject.

30. The method of any one of claims 27-29, wherein the S100B-associated disease or condition is selected from the group consisting of aging, neurological disorder, acute injury, psychiatric and affective disorder, and cancer.

31. The method of claim 30, wherein the neurological disorder is Alzheimer's disease, Parkinson's disease, Down syndrome, COPD-induced cognitive decline, autoimmune-induced cognitive decline, post-operative cognitive decline, radiation-induced cognitive decline, vertigo or aging.

32. The method of claim 30, wherein the acute injury is traumatic brain injury, stroke/ischemia, or hypoxic ischemia at term.

33. The method of claim 30, wherein the psychiatric and affective disorder is disease-induced depression, mood disorders, suicide, schizophrenia, or delirium.

34. The method of claim 30, wherein the cancer is melanoma, astrocytoma, glioma, or brain cancer.

35. A method of screening a biological sample for the presence of S100B comprising contacting a biological sample with one or more of the isolated antibodies of any one of claims 1-13 and detecting binding of S100B by the antibody or binding fragment.

36. A method of quantifying S100B in a biological sample comprising contacting a biological sample with one or more of the isolated antibodies of any one of claims 1-13, detecting binding of S100B by the antibody or binding fragment, and quantifying the amount of S100B detected.

37. A method of monitoring a S100B-associated disease or condition in a subject comprising (a) contacting a biological sample obtained from a subject having a S100B-associated disease or condition with one or more of the isolated antibodies of any one of claims 1-13, (b) detecting binding of S100B by the antibody or binding fragment, (c) quantifying the amount of S100B detected, (d) comparing the amount quantified in (c) with an amount quantified in a biological sample from the same subject at an earlier time point, thereby monitoring a S100B-associated disease or condition in the subject.

38. A method of diagnosing a S100B-associated disease or condition in a subject comprising (a) contacting a biological sample obtained from a subject with one or more of the isolated antibodies of any one of claims 1-13, (b) detecting binding of S100B by the antibody or binding fragment, (c) quantifying the amount of S100B detected, (d) comparing the amount quantified in (c) with one or more control amounts quantified in a biological sample obtained from a subject with a S100B-associated disease or condition, thereby diagnosing a S100B-associated disease or condition in the subject.

39. The method of claim 35, wherein the biological sample is selected from the group consisting of blood, serum, cerebrospinal fluid (CSF), tissue sample, tears, sweat, urine, lymph, tumor exudate, ascites fluid, and pleural fluids.

40. The method of claim 36, wherein the biological sample is selected from the group consisting of blood, serum, cerebrospinal fluid (CSF), tissue sample, tears, sweat, urine, lymph, tumor exudate, ascites fluid, and pleural fluids.

41. The method of claim 37, wherein the biological sample is selected from the group consisting of blood, serum, cerebrospinal fluid (CSF), tissue sample, tears, sweat, urine, lymph, tumor exudate, ascites fluid, and pleural fluids.

42. The method of claim 38, wherein the biological sample is selected from the group consisting of blood, serum, cerebrospinal fluid (CSF), tissue sample, tears, sweat, urine, lymph, tumor exudate, ascites fluid, and pleural fluids.

43. The method of claim 35, wherein the binding of S100B by the antibody is detected by an ELISA assay, an affinity column, a Western/immunoblot, immunohistochemistry, or a proximity ligation assay.

44. The method of claim 36, wherein the binding of S100B by the antibody is detected by an ELISA assay, an affinity column, a Western/immunoblot, immunohistochemistry, or a proximity ligation assay.

45. The method of claim 37, wherein the binding of S100B by the antibody is detected by an ELISA assay, an affinity column, a Western/immunoblot, immunohistochemistry, or a proximity ligation assay.

46. The method of claim 38, wherein the binding of S100B by the antibody is detected by an ELISA assay, an affinity column, a Western/immunoblot, immunohistochemistry, or a proximity ligation assay.

47. The method of claim 36, wherein the amount of S100B detected is quantified by an ELISA assay, a Western/immunoblot, immunohistochemistry, or a proximity ligation assay.

48. The method of claim 37, wherein the amount of S100B detected is quantified by an ELISA assay, a Western/immunoblot, immunohistochemistry, or a proximity ligation assay.

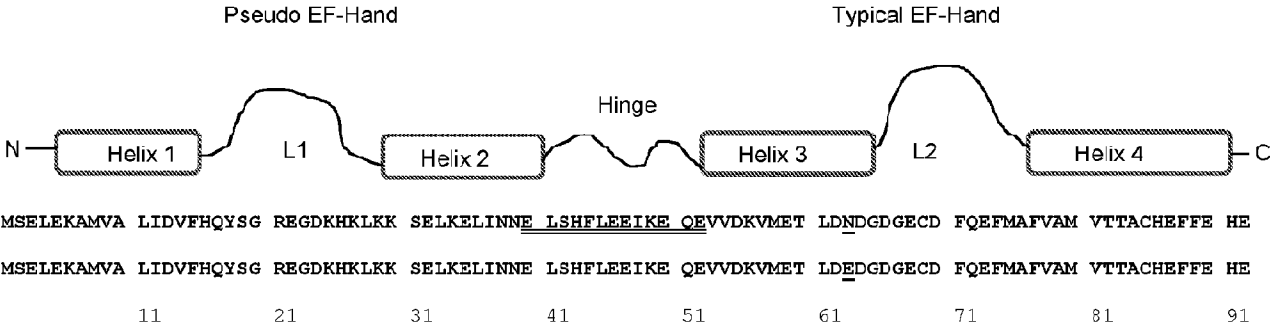
49. The method of claim 38, wherein the amount of S100B detected is quantified by an ELISA assay, a Western/immunoblot, immunohistochemistry, or a proximity ligation assay.

50. A method of diagnosing a S100B-associated disease or condition in a subject comprising administering one or more of the S100B binding agents defined herein to a subject having a S100B-associated disease or condition, or suspected of having a S100B-associated

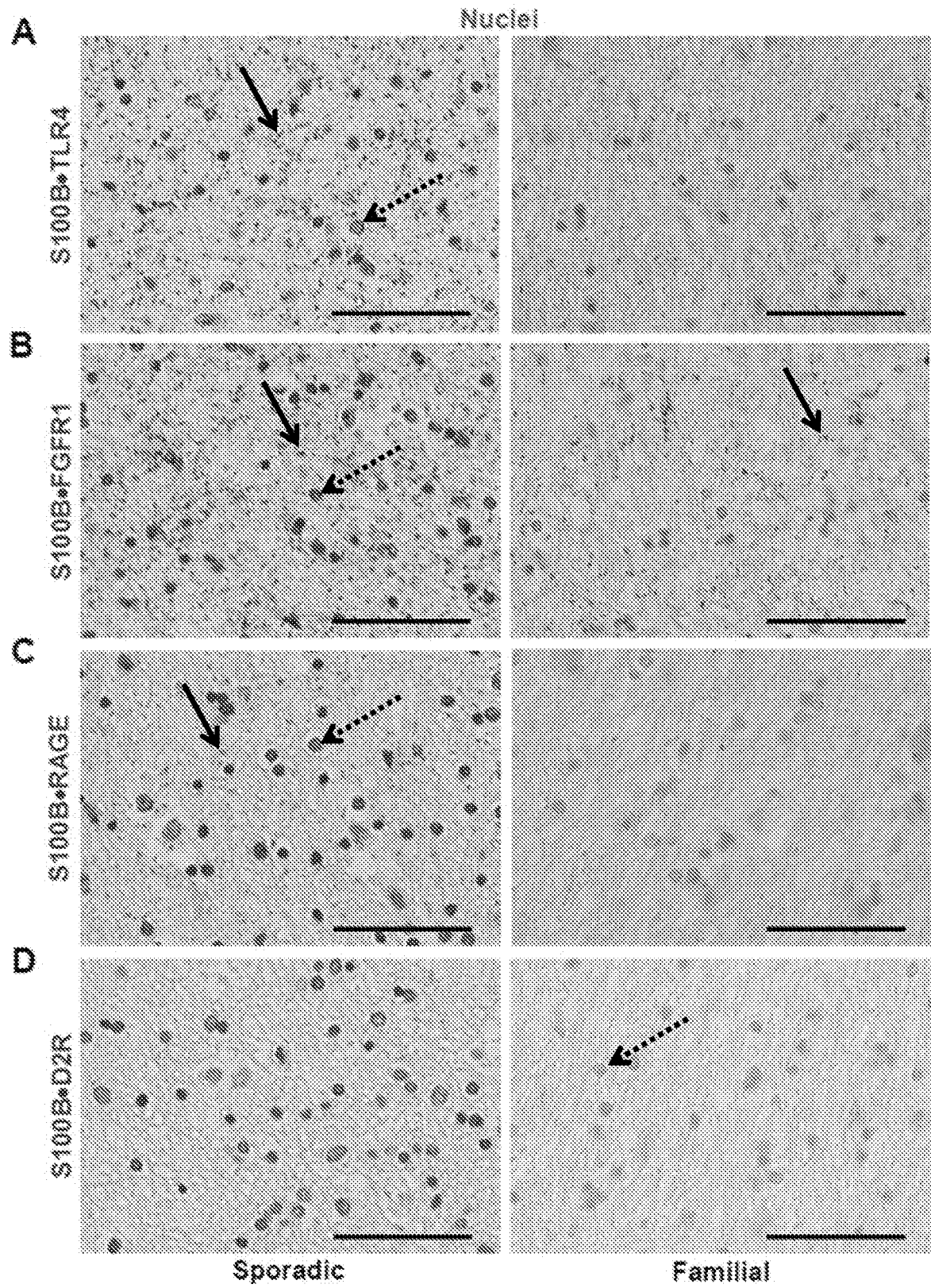
disease or condition, and detecting binding of the one or more S100B binding agents to S100B in the subject.

51. The method of claim 50, wherein binding of the one or more S100B binding agents to S100B is detecting by *in vivo* imaging.

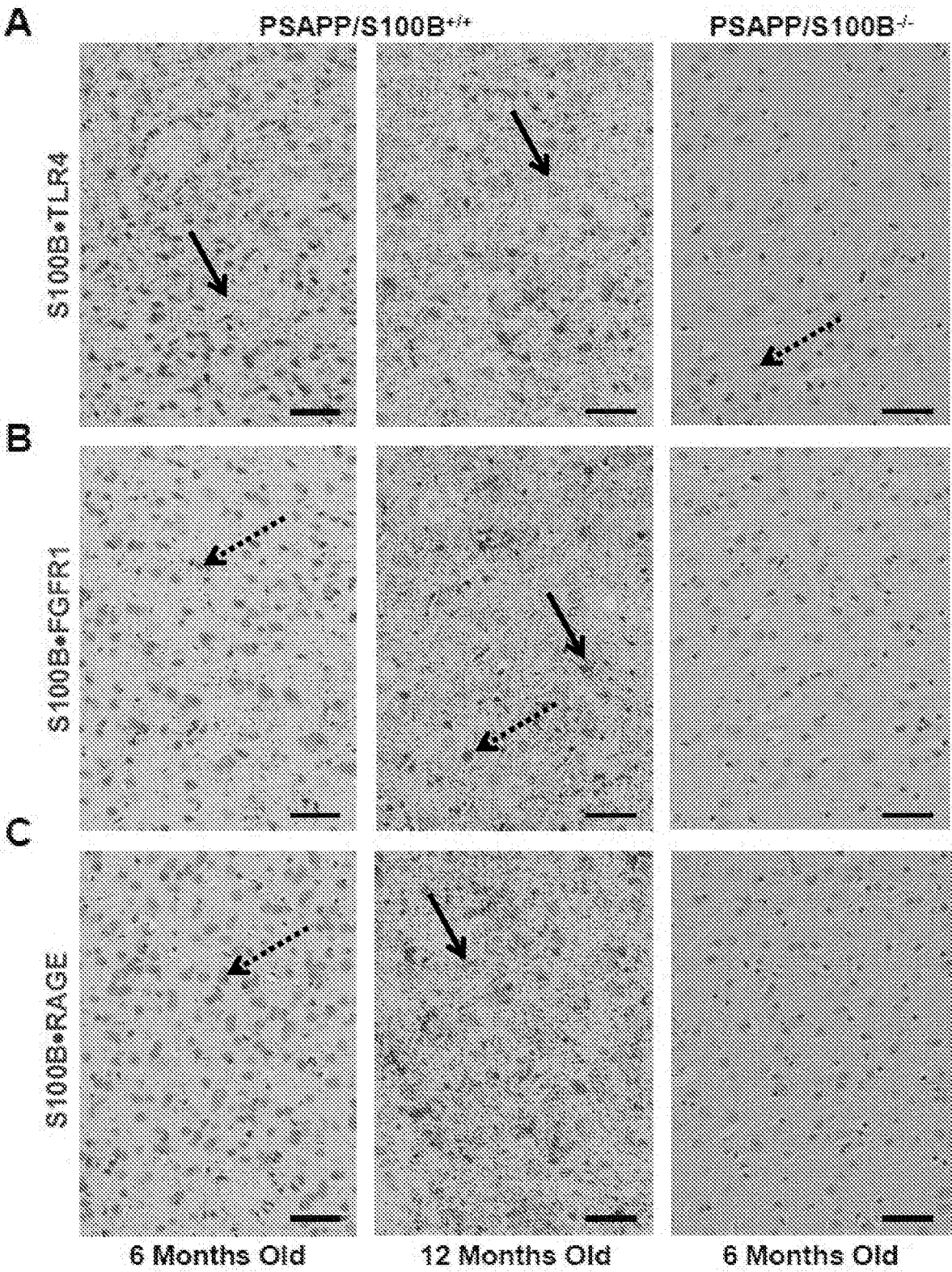
Figure 1



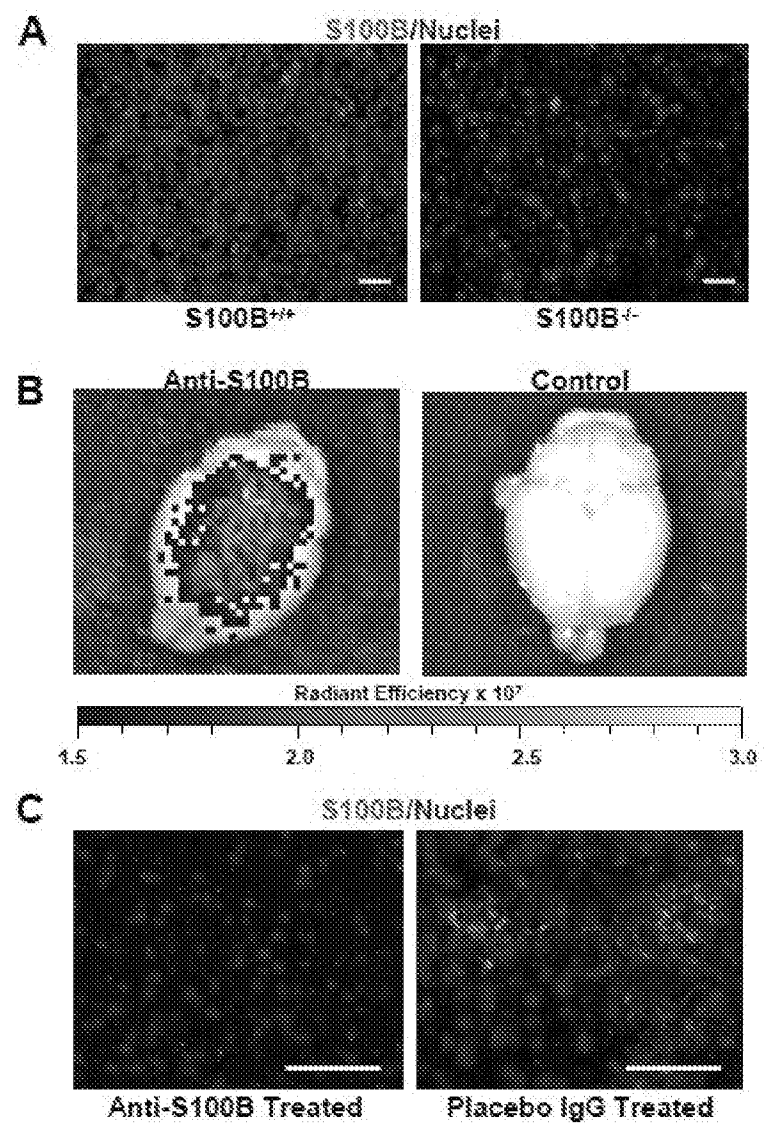
Figures 2A-2D



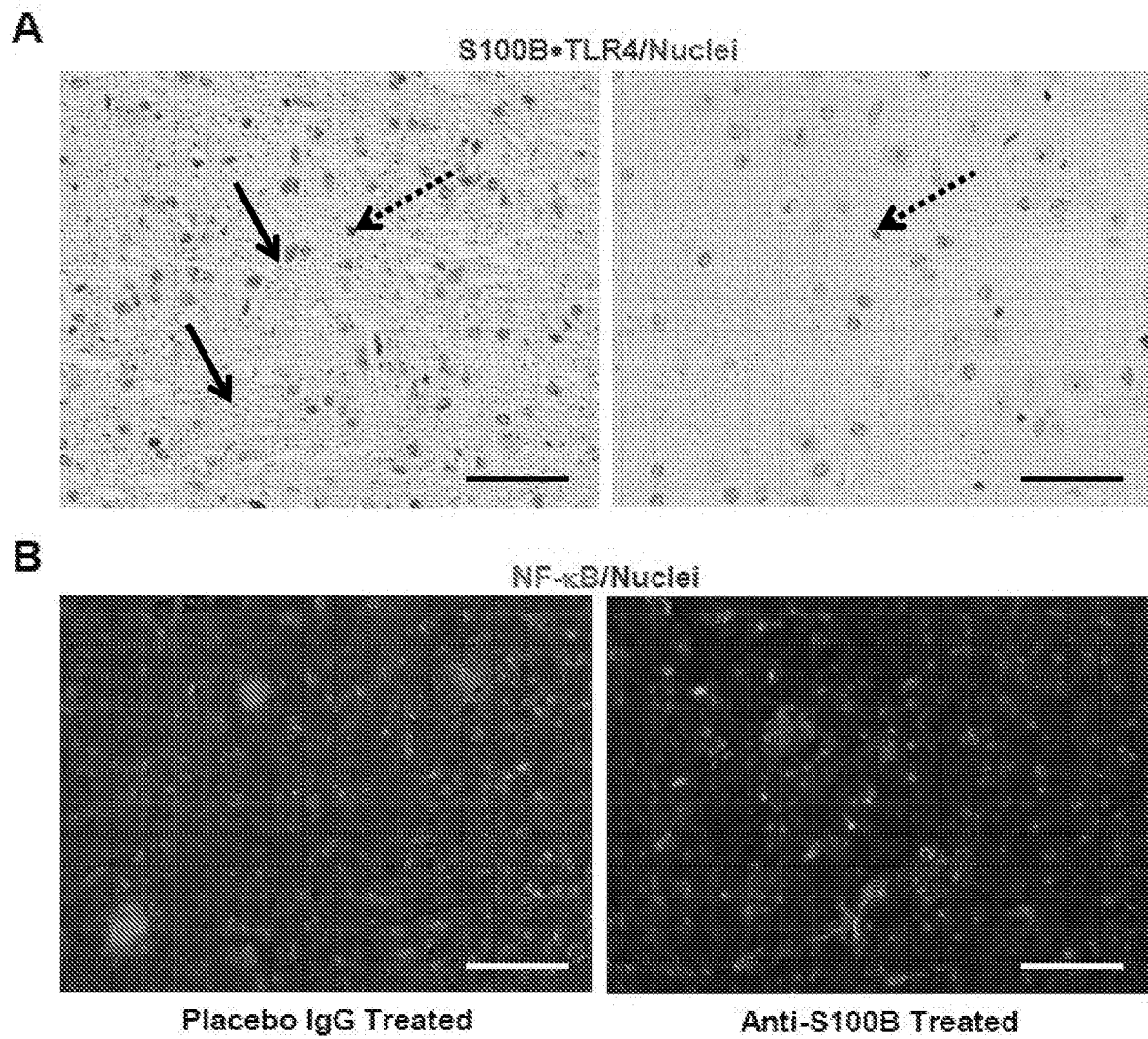
Figures 3A-3C



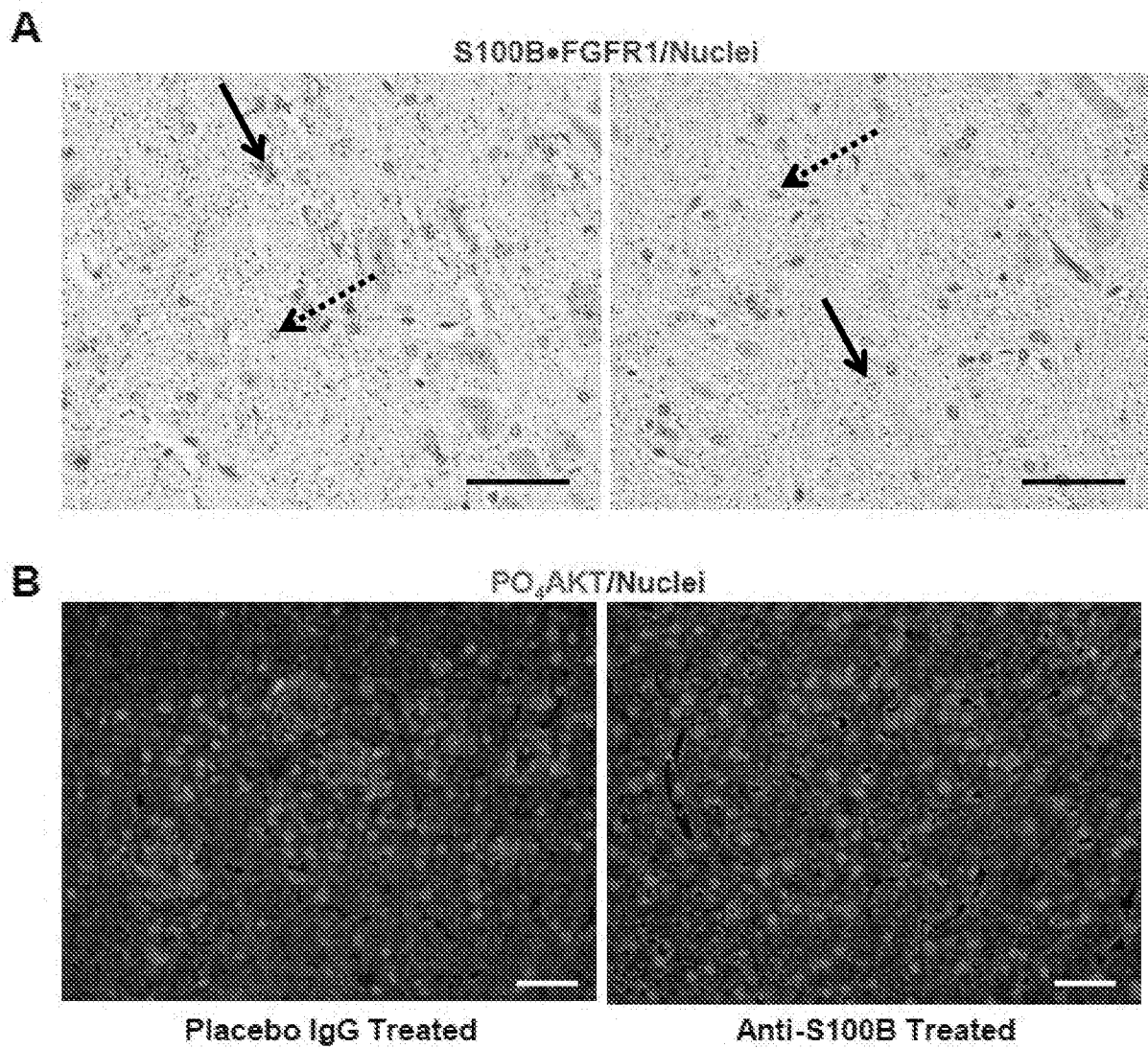
Figures 4A-4C



Figures 5A-5B



Figures 6A-6B



Figures 7A-7B

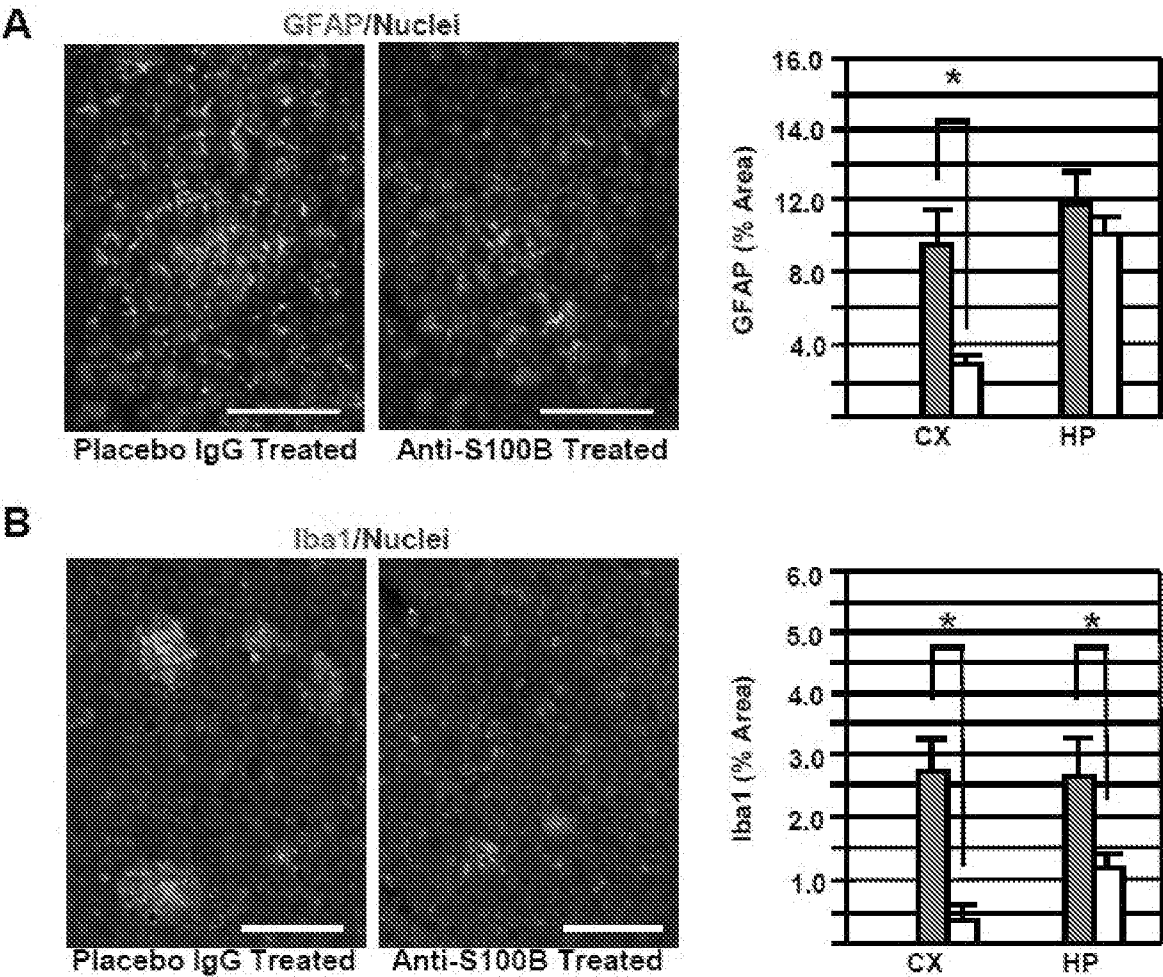


Figure 8

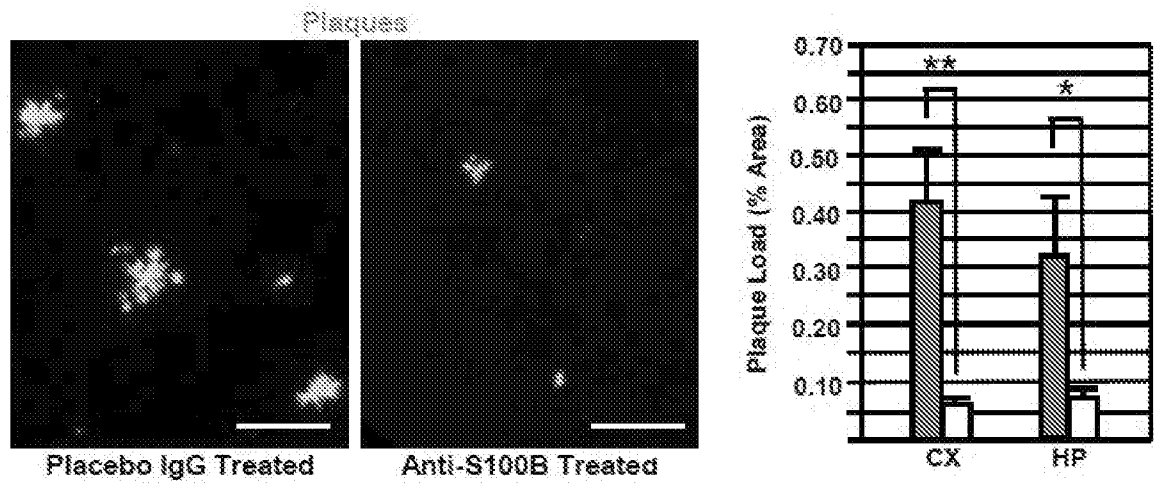


Figure 9

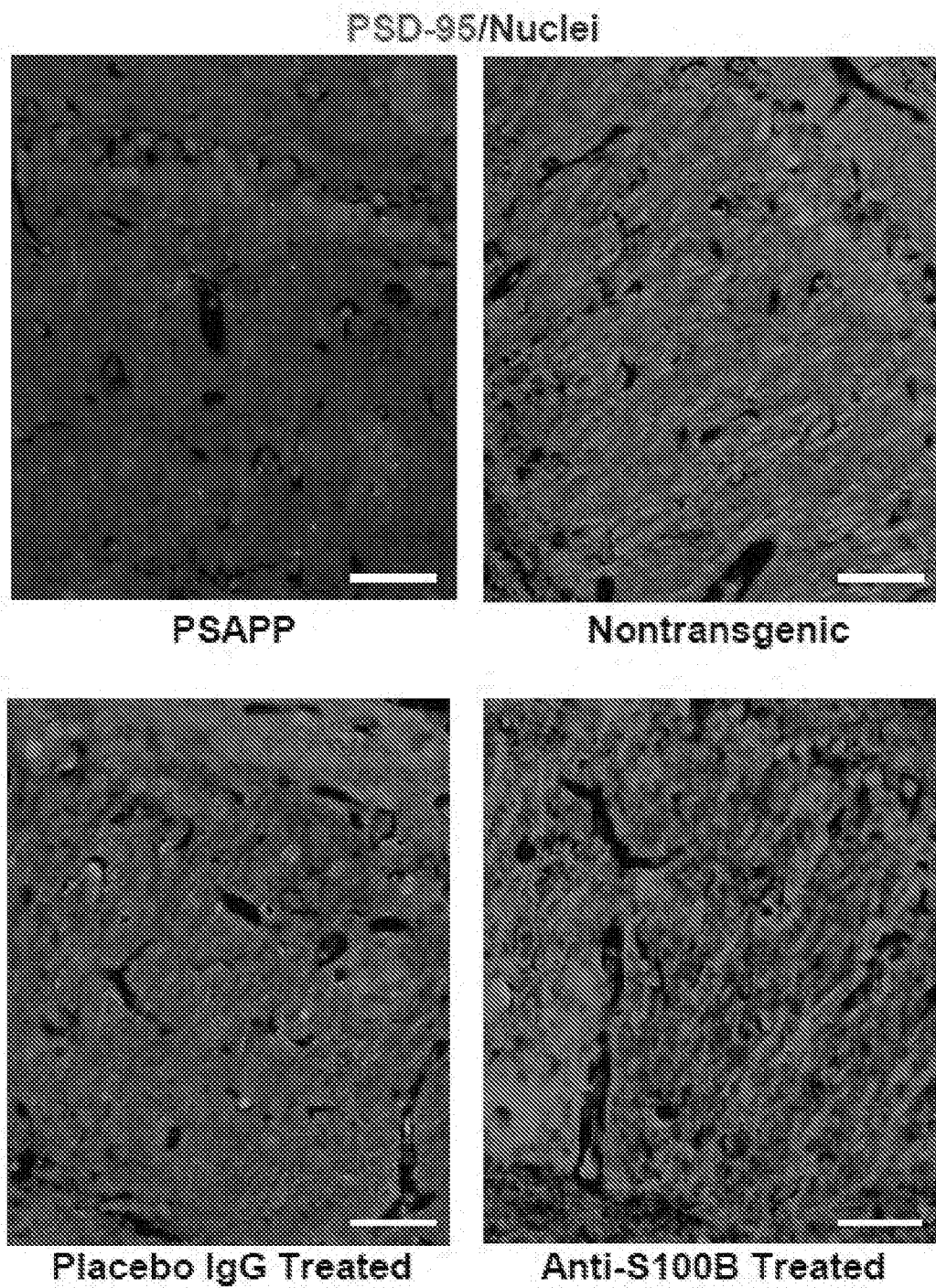


Figure 10

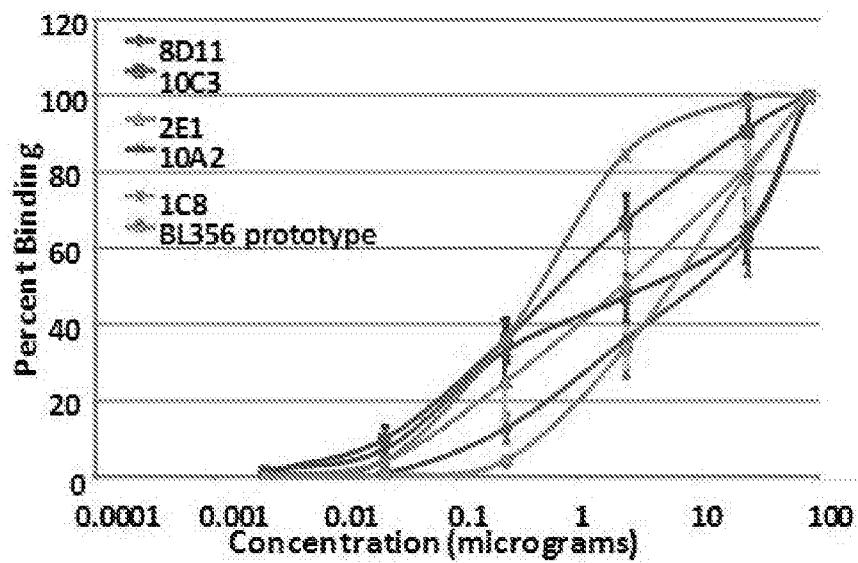


Figure 11

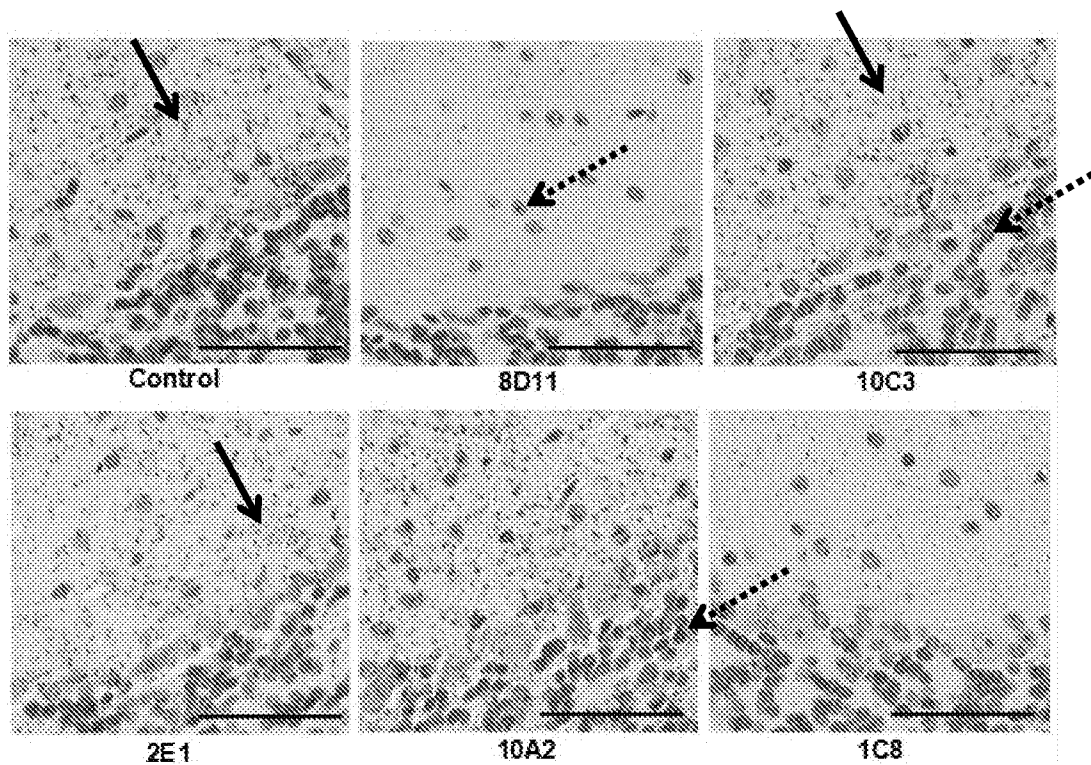


Figure 12

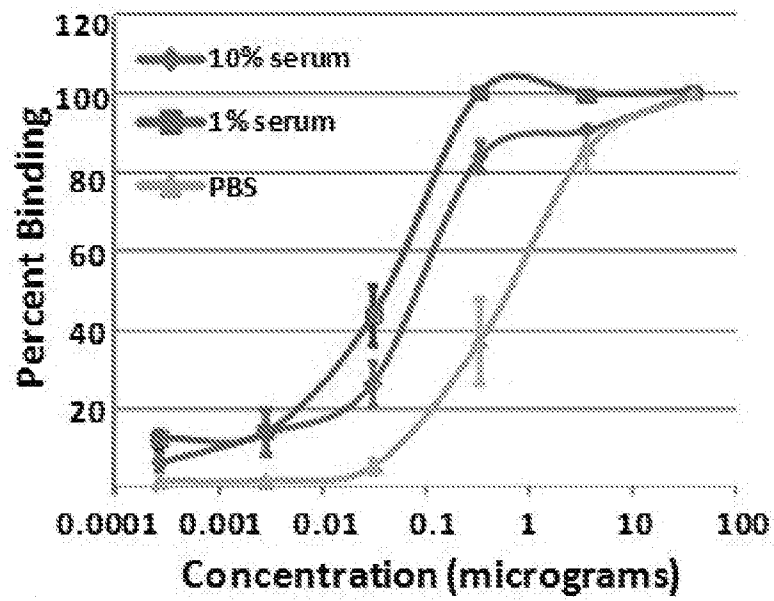


Figure 13

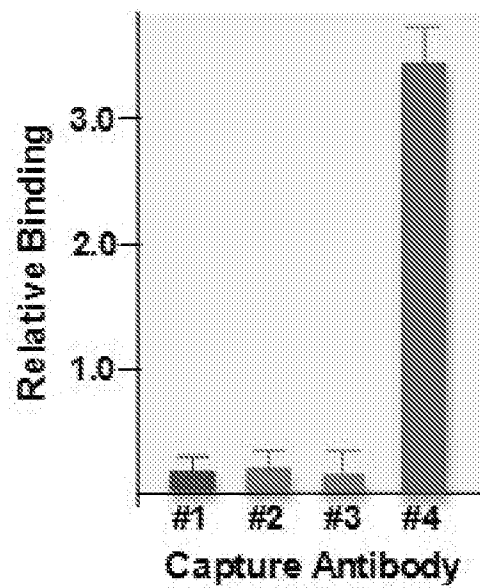


Figure 14A

1C8 heavy chain nucleotide sequence (SEQ ID NO:1)Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon

ATGGGATGGAGCTATATCATCCTCTTTTTGGTAGCAACAGCTACAGATGTCCACTCCCAGGTCCAAC
 TGCAGCAGCCTGGGGCTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGCTGTCTGCAAGGCTTCTGG
 CTACACCTTCACCAGGTATTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATT
 GCAGAGATTAATCCTACCAACGGTCGTAGTAACATAATGAGAAGTTCAAGAGCAAGGCCACACTGA
 CTGTAGACAGGTCCTCCAGCACAGCCTTCATGCAACTCAGCAGCCTGACATCTGACGACTCTGCGGT
 CTATTACTGTGCTCCAACAGGGACGGGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCT
 GCAGCCAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCCTGTGTGTGGAGATACAACTGGCTCCT
 CGGTGACTCTAGGATGCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCTGG
 ATCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACCCTCAGCAGC
 TCAGTGACTGTAACCTCGAGCACCTGGCCCAGCCAGTCCATCACCTGCAATGTGGCCCACCCGGCAA
 GCAGCACCAAGGTGGACAAGAAAATTGAGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAA
 ATGCCCAGCACCTAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTA
 CTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATG
 TCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGA
 TTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCCAGGACTGGATGAGTGGCAAG
 GAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCCA
 AAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACA
 GGTCACCTCTGACCTGCATGGTCACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAAC
 GGGAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGTTCTTACTTCATGT
 ACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCA
 CGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAATGA

Figure 14B

1C8 heavy chain amino acid sequence (SEQ ID NO:2)Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region

MGWSYIILFLVATATDVHSSQVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWVKQRPGQGLEW
 IAEINPTNGRSNYNEKFKSKATLTVDRSSSTAFMQLSSTLSDDSAVYYCAPTGTGFAYWGQGLVT
 VSAAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYT
 LSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFI FPPK
 IKDVLMI SLSPIVTCVVVDVSEDDPDVQISW FVNNEVHTAQ TQTHREDYNSTLRVVSALPIQH QD
 WMSGKEFKCKVNNKDL PAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVT LCMVTD FMPEDIY
 VEWTNNGKTELNYKNTEPVLDSDGSYFMY SKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRT
 PGK

Figure 14C

1C8 heavy chain variable region nucleotide sequence (SEQ ID NO:8)**FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4**

CAGGTCCAACTGCAGCAGCCTGGGGCTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGG
CTTCTGGCTACACCTTCACCAGGTATTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTG
GATTGCAGAGATTAATCCTACCAACGGTCGTAGTAACTACAATGAGAAGTTCAAGAGCAAGGCCACACTG
ACTGTAGACAGGTCCTCCAGCACAGCCTTCATGCAACTCAGCAGCCTGACATCTGACGACTCTGCGGTCT
ATTACTGTGCTCCAACAGGGACGGGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA

Figure 14D

1C8 heavy chain variable region amino acid sequence (SEQ ID NO:9)**FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4**

QVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWVKQRPGQGLEWIAEINPTNGRSNYNEKFKS
KATLTVDRSSSTAFMQLSSLTSDDSAVYYCAPTGTGFAYWGQGLVTVSA

Figure 15A

1C8 light chain nucleotide sequence (SEQ ID NO:3)Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon

ATGATGAGTCCTGCCCAGTTCCTGTTTCTGTTAGTGCTCTGGATTTCAGGAAACCAACGGTGATGTTG
 TGATGACCCAGACTCCACTCACTTTGTCTGGTTACCATTTGGACAACCAGCCTCTATCTCTTGCAAGTC
 AAGTCAGAGCCTCTTATATAGTAATGGAAAAACCTATTTGAATTGGTTATTACAGAGGCCAGGCCAG
 TCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTTCACTGGCA
 GTGGATCAGGAACAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTGAGGATTTGGGAGTTTATTA
 CTGCGTGCAAGGTACACATTTTCCGTGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCT
 GATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAG
 TCGTGTGCTTCTTGAACAACCTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGA
 ACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGC
 AGCACCCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACA
 AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG

Figure 15B

1C8 light chain amino acid sequence (SEQ ID NO:4)Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region

MMSPAQFLFLLVLWIQETNGDVVMTQTPLTSLVTIGQPASISCKSSQSLLYSNGKTYLNWLLQRPQG
 SPKRLIYLVS KLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVIYCVQGFTHFPWTFGGGTKLEIKRA
 DAAPT VSI FPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWTDQDSKDSTYSMS
 STLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

Figure 15C

1C8 light chain variable region nucleotide sequence (SEQ ID NO:10)FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

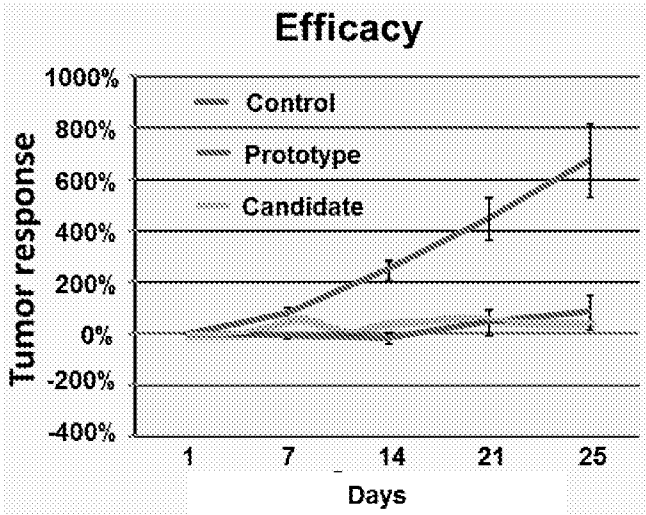
GATGTTGTGATGACCCAGACTCCACTCACTTTGTCTGGTTACCATTTGGACAACCAGCCTCTATCTCTTGCA
 AGTCAAGTCAGAGCCTCTTATATAGTAATGGAAAAACCTATTTGAATTGGTTATTACAGAGGCCAGGCCA
 GTCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTTCACTGGCAGT
 GGATCAGGAACAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTGAGGATTTGGGAGTTTATTACTGCG
 TGCAAGGTACACATTTTCCGTGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAA

Figure 15D

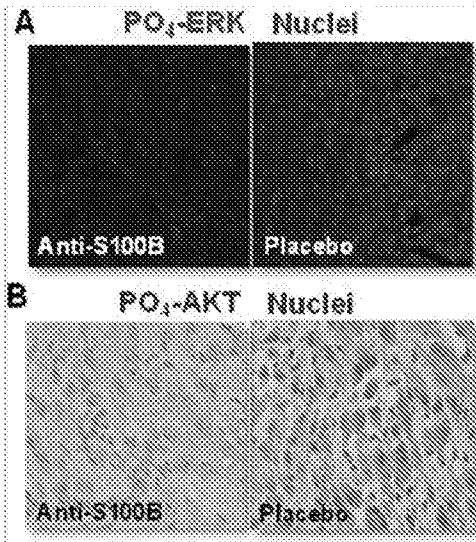
1C8 light chain variable region amino acid sequence (SEQ ID NO:11)FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

DVVMTQTPLTSLVTIGQPASISCKSSQSLLYSNGKTYLNWLLQRPQGSPKRLIYLVS KLDSGVPDRFTGS
 GSGTDFTLKISRVEAEDLGVIYCVQGFTHFPWTFGGGTKLEIK

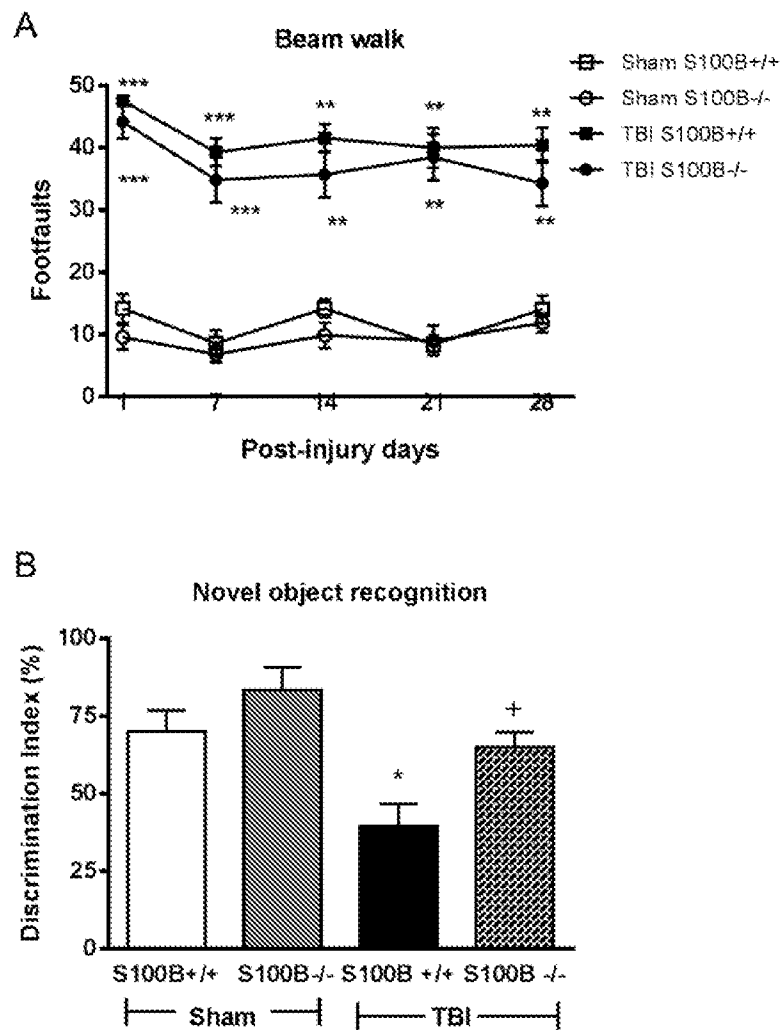
Figure 16



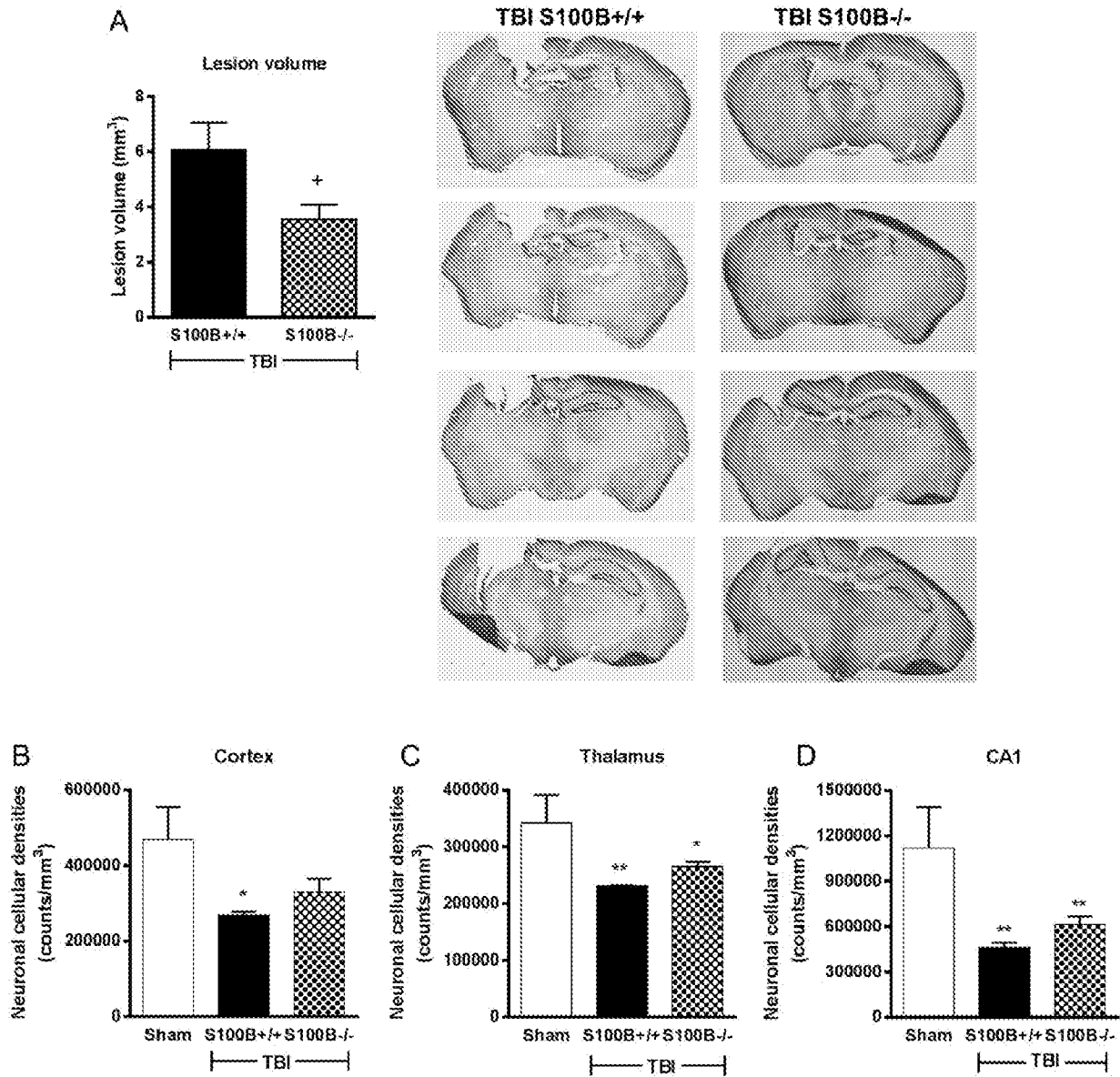
Figures 17A-17B



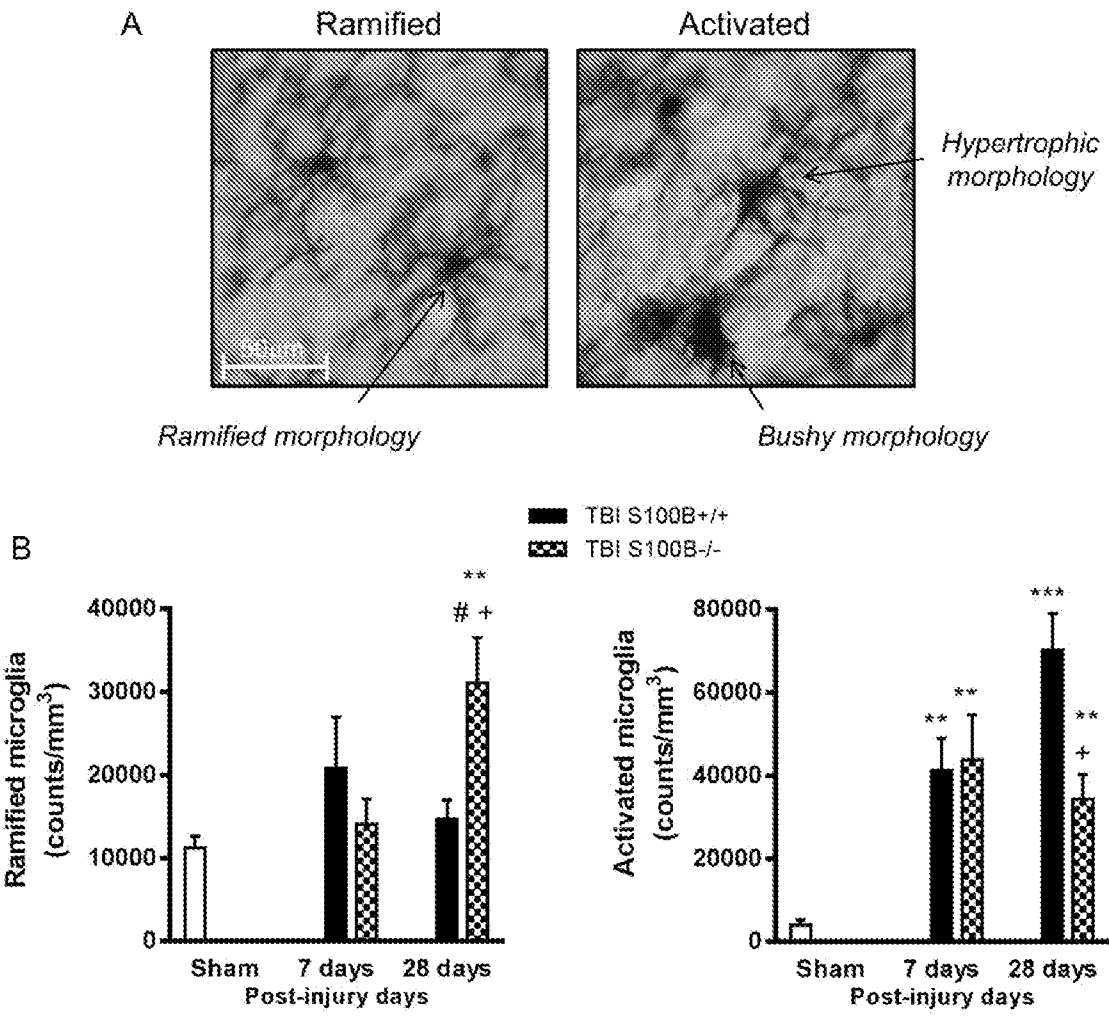
Figures 18A-18B



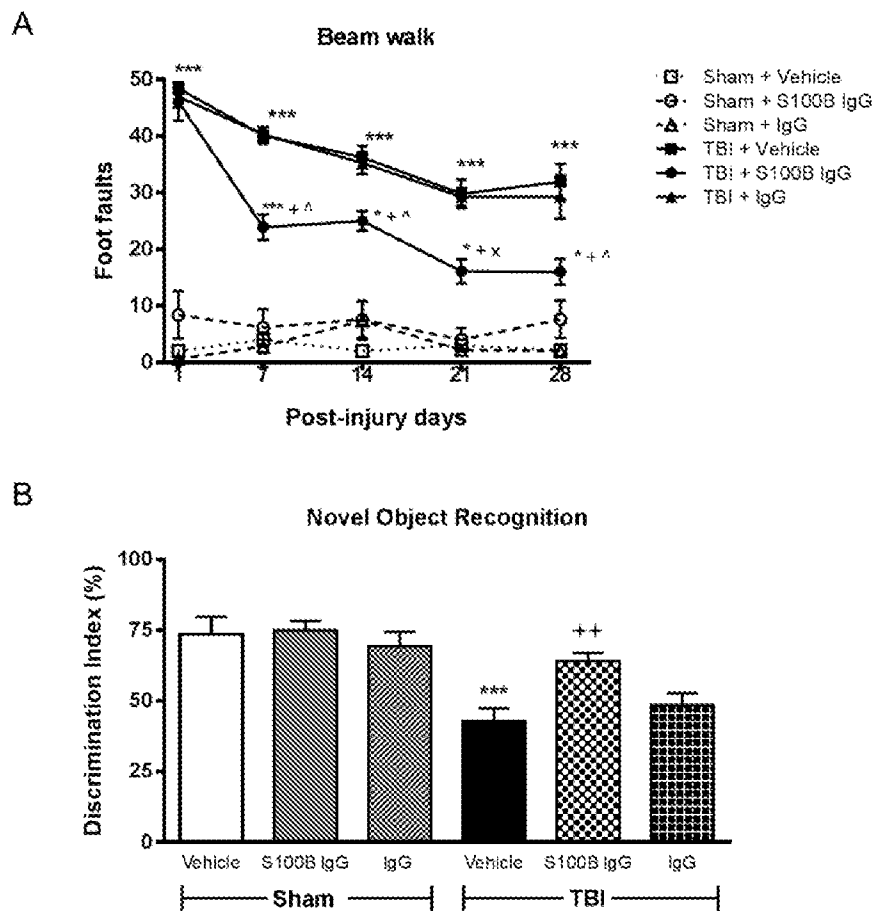
Figures 19A-19D



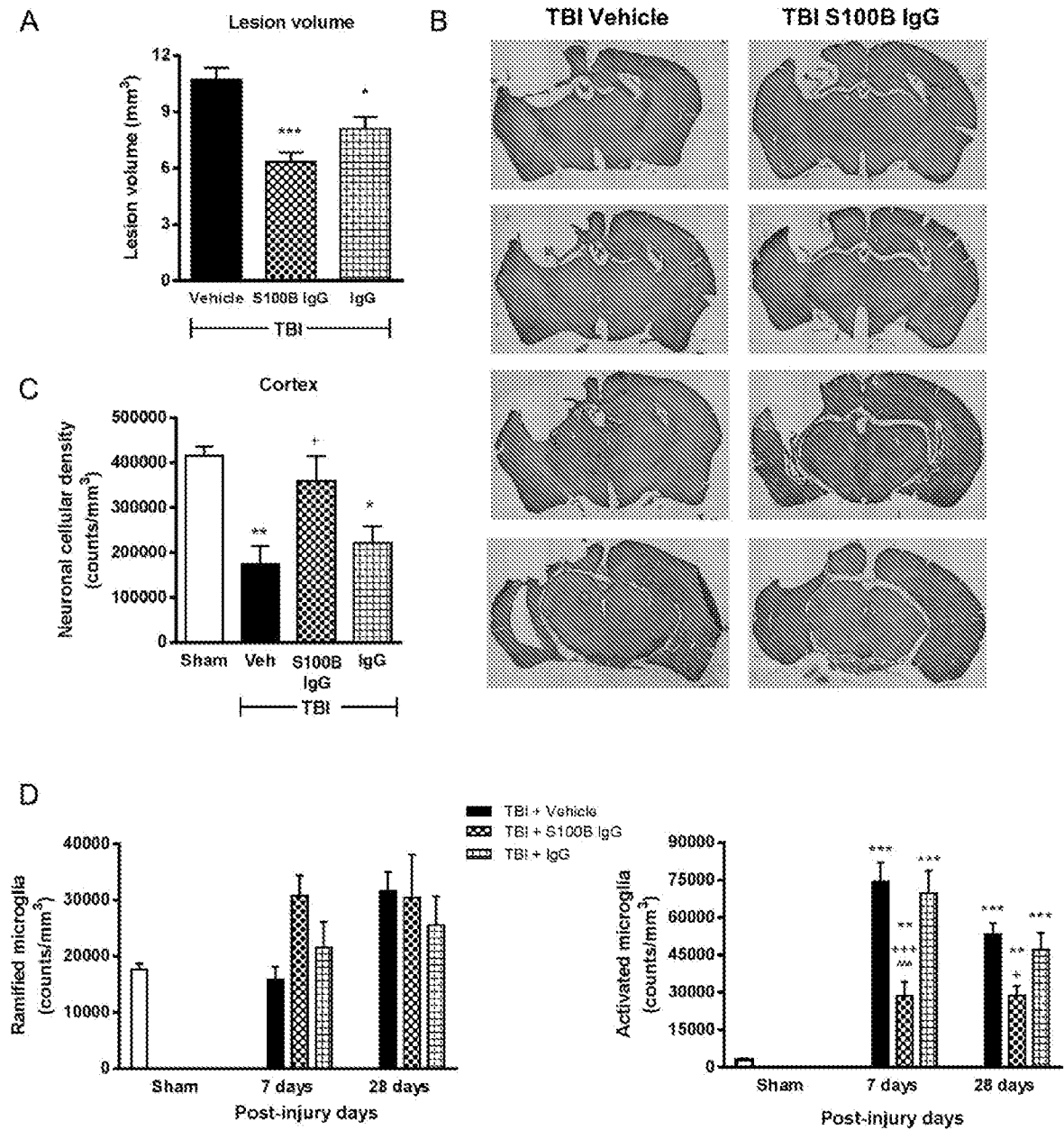
Figures 20A-20B



Figures 21A-21B



Figures 22A-22D



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/22104

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/53, 33/68; A61K 49/14, 49/16 (2016.01)

CPC - G01N 33/6896, 33/53; A61K 49/14, 49/16

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)

IPC(8): C07K 14/47; G01N 33/53, 33/68; A61K 49/14, 49/16 (2016.01)

CPC: C07K 14/4728; G01N 33/6896, 33/53; A61K 49/14, 49/16

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

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

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; PubMed; EBS CO Discovery Service; NCBI BLAST; The Lens PatSeq Finder; European Nucleotide Archive; 'administering', 'S100 calcium-binding protein B', 'S100B', 'Binding agent', 'Antibody', 'peptide', 'detect'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/194329 A1 (BANYAN BIOMARKERS, INC.) December 4, 2014; paragraphs [0010]-[0012], [0016], [0017], [0047], [0050], [0070]	1, 2, 50, 51
A	(SHLOMCHIK, M et al.) Anti-DNA Antibodies from Autoimmune Mice Arise by Clonal Expansion and Somatic Mutation. Journal of Experimental Medicine. 01 January 1990, Vol. 171, pages 265-267; GenPept supplement pages 1-2	3/1-2, 4/1-2, 5/1-2, 6/1-2, 7/1-2, 8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-2
A	US 8,858,949 B2 (YOKOSEKI, T et al.) October 14, 2014; column 2, lines 33-35; column 10, lines 62-64	3/1-2, 4/1-2, 5/1-2, 6/1-2, 7/1-2, 8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-2
A	WO 2010/119704 A1 (IMMUNAS PHARMA, INC.) October 21, 2010; paragraph [0012]	3/1-2, 4/1-2, 5/1-2, 6/1-2, 7/1-2, 8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-2
A	US 2011/0305699 A1 (CHANG, MS) December 15, 2011; column 9	8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-2
A	WO 2007/090807 A1 (UNIVERSITA DEGLI STUDI DI TORINO) August 16, 2007; page 22, lines 19-22	8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-2
A	EP 2127683 A1 (METHERESIS TRANSLATIONAL RESEARCH SA) December 2, 2009; paragraph [0040]	8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-2

☐ 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

23 May 2016 (23.05.2016)

Date of mailing of the international search report

10 JUN 2016

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/22104

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.: 13-49
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:

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.:

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.