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(54) Title: MONOCLONAL ANTIGEN-BINDING PROTEINS TO INTRACELLULAR ONCOGENE PRODUCTS

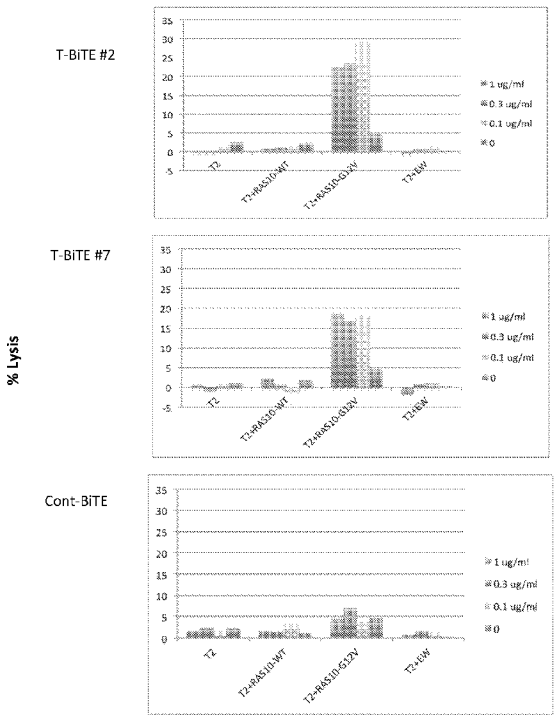

FIGURE 7

(57) Abstract: Antigen binding proteins specific for an HLA-A2 restricted Ras peptide are disclosed. The antigen binding proteins encompass antibodies in a variety of forms, including full-length antibodies, substantially intact antibodies, Fab fragments, F(ab')2 fragments, and single chain Fv fragments. Fusion proteins, such as scFv fusions with immunoglobulin or T-cell receptor domains, and bispecific antibodies incorporating the specificity of the antigen binding region for each peptide are also contemplated by the disclosure. Furthermore, immunoconjugates may include antibodies to which is linked a radioisotope, fluorescent or other detectable marker, cytotoxin, or other molecule are also encompassed by the disclosure. Among other things, immunoconjugates can be used for delivery of an agent to elicit a therapeutic effect or to facilitate an immune effector function.

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MONOCLONAL ANTIGEN-BINDING PROTEINS TO INTRACELLULAR ONCOGENE PRODUCTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/136,117, filed March 20, 2015, which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing, created on March 18, 2015; the file, in ASCII format, is designated 3314061AWO_ST25.txt and is 77.8 kilobytes in size. The file is hereby incorporated by reference in its entirety into the instant application.

BACKGROUND OF THE DISCLOSURE

Technical Field

[0003] The present disclosure relates generally to antigen-binding protein molecules involved in immune function. More particularly, the present disclosure relates to recombinant antibodies, chimeric antigen receptors and fragments or portions thereof with binding specificity for Ras proteins.

Background Information

[0004] Antibodies are increasingly being used as therapeutic agents to fight cancer, autoimmune disease and infection. Therapeutic antibodies have been exploited based on their multiple mechanisms of action, which include the following: 1) naked antibodies killing tumor cells directly by ADCC or CDC (e.g. trastuzumab), 2) blocking or stimulating a cell membrane molecule to induce cell death (e.g. cetuximab), 3) neutralizing a secreted moiety (e.g. bevacizumab), 4)

killing via an attached moiety such as a drug, toxin, radioisotope and 5) modulating the immune system via T cell effector functions.

[0005] In almost all cases, to generate a therapeutic benefit, antibodies have to possess certain properties including high affinity for their targeted antigen, minimal acute and long-term side effects, and in specific applications, high affinity for human Fc receptors (4). In addition, the targeted antigen has to be expressed in tumors but not on normal tissues (specificity or selectivity), consistently expressed in the specific tumor among patients and within patients (low heterogeneity), and should either be essential for the survival of the cancer cell or unlikely to be down regulated.

[0006] Ras is the most important oncogene in human cancers as it is mutated and involved in some of the most lethal cancers including cancers of the lung, pancreas, colon and rectum, among many others. Ras proteins are small GTPases that play a central role in transducing signals that regulate cell growth, differentiation and survival. All mammalian cells express 3 closely related Ras proteins, K-Ras, N-Ras and H-Ras, that promote oncogenesis when mutations occur at codons 12, 13 or 61. K-Ras mutations are far more frequently observed in cancer and are associated with >30% of all human cancers (up to 90% in pancreatic cancer) and are one of the first identified and the most common oncogenes found in human cancer. Because Ras is expressed in all normal cells, a safe and effective drug must be selective for the mutated Ras protein form alone. However, because the mutant Ras that is associated with cancers is so similar to the normal Ras protein found in all human cells, (the mutant differing by a single amino acid,) and because Ras's oncogenic function is not a mutated enzyme targetable by a small molecule in the traditional sense, it has been difficult to make a drug selective for Ras proteins. No drug for Ras is FDA approved for human use. Therefore, there is an important unmet need for such a drug to treat hundreds of thousands of patients with Ras associated cancers and leukemias.

[0007] Therapeutic monoclonal antibodies (mAbs) are highly specific and potent drugs, capable of initiating immunologic attack on tumor cells. Immune effector

functions of mAbs include antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP) and direct killing of the target cells. In addition, mAbs are highly versatile therapeutics. They can be conjugated to radioactive isotopes, toxins, or drugs, or carriers of such drugs, directly or by means of multi-step targeting, to specifically deliver more potent therapy to cancer cells. Furthermore, mAbs can also be engineered into chimeric antigen receptor (CAR) or bispecific T cell engager forms (T-BiTE), that bring powerful T cell cytotoxicity against the mAb-targeted cancer cells. Cytokines or other pro-inflammatory agents may be attached. All therapeutic mAbs currently marketed in the USA target extracellular or cell-surface molecules, while many important oncogenes and disease targets are intracellular.

[0008] But, unlike small molecule drugs that cross the cell membrane, mAbs cannot cross the membrane to access intracellular proteins like Ras and therefore, traditional antibody-based strategies targeting cell surface antigens are unavailable. Instead, immunotherapeutic approaches targeting Ras have been focused on generating T cell responses against the Ras-derived peptide epitopes presented on tumor cells by both MHC class I and class II. Though initial results suggest that Ras mutation-derived epitopes could be cancer-specific targets for T cell immunotherapy against a wide range of human cancers, peptide vaccines derived from Ras mutations have been evaluated in clinical trials in patients with pancreatic and other cancers, but clinical efficacy was not observed.

[0009] Accordingly, there remains a need for immunotherapeutics, including antibodies, which effectively target intracellular oncogenic proteins.

SUMMARY OF THE DISCLOSURE

[0010] The present disclosure is based on the identification of Ras-specific binding protein molecules, amino acid sequences of which can be used to

generate a variety of antigen-binding proteins, for example, an antibody specific for Ras or for Ras mutant peptide variants having a single amino acid substitution.

[0011] The present disclosure identifies and characterizes antigen-binding proteins, such as antibodies, that are able to target cytosolic/intracellular proteins, for example, the Ras oncoprotein. The disclosed antibodies target a peptide/MHC complex as it would typically appear on the surface of a cell following antigen processing of Ras protein and presentation by the cell. In that regard, the antibodies mimic T-cell receptors in that the antibodies have the ability to specifically recognize and bind to a peptide in an MHC-restricted fashion, that is, when the peptide is bound to an MHC antigen. The peptide/MHC complex recapitulates the antigen as it would typically appear on the surface of a cell following antigen processing and presentation of the Ras protein to a T-cell.

[0012] The antibodies disclosed specifically recognize and bind to a Ras peptide/HLA-A2 complex, particularly a Ras/HLA-A0201 complex. Examples of peptides that are recognized by the antigen-binding proteins of the disclosure as part of an HLA-peptide complex include, but are not limited to, those shown in Table 11, for example, a peptide with the amino acid sequence KLVVVGAVGV (Ras10-G12V; SEQ ID NO: 111)

[0013] In one aspect, therefore, the disclosure relates to an isolated antibody, or antigen-binding fragment/portion thereof, that binds to a peptide with the amino acid sequence, KLVVVGAVGV (SEQ ID NO: 111), when said peptide is bound to an MHC antigen, such as HLA-A2.

[0014] In another aspect, therefore, the disclosure relates to an recombinant antigen-binding protein or antigen-binding fragment/portion thereof comprising one of:

(A) an antigen binding region having the amino acid sequence of one of SEQ ID NOS: 81, 82, 83, 84, 85, 86, 87, or 88;

(B) an antigen binding region comprising a V_H and V_L, respectively, with amino acid sequences selected from SEQ ID NOs: 7 and 9; 17 and 19; 27 and 29; 37 and 39; 47 and 49; 57 and 59; 67 and 69; and 77 and 79; or

(C) an antigen binding region comprising:

(i) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively;

(ii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13, respectively;

(iii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively;

(iv) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33, respectively;

(v) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, respectively and (b) heavy chain CDRs (HC-

CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, respectively;

(vi) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 51, SEQ ID NO: 52, and SEQ ID NO: 53, respectively;

(vii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 64, SEQ ID NO: 65, and SEQ ID NO: 66, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63, respectively; or

(viii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 74, SEQ ID NO: 75, and SEQ ID NO: 76, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73, respectively.

[0015] In a related aspect, the disclosure relates to a recombinant antigen-binding protein or antigen-binding fragment thereof, wherein the antigen-binding protein is an antibody or chimeric antigen receptor (CAR) that specifically binds to a Ras peptide in conjunction with HLA2. The recombinant antibody is a full-length antibody, that is an intact or substantially intact antibody, a Fab fragment, a $F(ab')_2$ fragment or a single chain variable fragment (scFv), or comprises these elements.

[0016] In the recombinant antigen-binding protein, whether an antibody or CAR, the antigen-binding region specifically binds to an epitope of an HLA-2/Ras peptide complex.

[0017] The antigen binding proteins of the present disclosure demonstrated binding to a set of decamer and nonamer peptides containing the prevalent *ras* codon 12 mutations that are predicted minimal epitopes for HLA-A2. The decamers are based on amino acids 5-14 of *ras* wild-type, KLVVVGAGGV (SEQ ID NO: 110), while the nonamers correspond to amino acids 6-14 of *ras* wild-type, LVVVGAGGV (SEQ ID NO: 115).

[0018] Peptides that are recognized by the antigen-binding proteins of the disclosure as part of an HLA-Ras peptide complex include, but are not limited to, a 9 amino acid peptide with the amino acid sequence LVVVGAGGV (Ras9-WT, SEQ ID NO:115); and single amino acid substitutions thereof: LVVVGAVGV (Ras9-G12V, SEQ ID NO: 116); and LVVVGACGV (Ras9-G12C, SEQ ID NO: 117); and LVVVGADGV (Ras9-G12D, SEQ ID NO: 118) as well as a 10 amino acid peptide with the amino acid sequence KLVVVGAGGV (Ras10-WT, SEQ ID NO: 110); and single amino acid substitutions thereof: KLVVVGAVGV (Ras10-G12V SEQ ID NO: 111); KLVVVGACGV (Ras10-G12C SEQ ID NO: 112); and KLVVVGADGV (Ras10-G12D SEQ ID NO: 113) and KLVVVGASGV (R10-G12S SEQ ID NO: 114). In some embodiments, the peptide is recognized in association with an HLA antigen that is HLA-A2.

[0019] In yet another aspect, the recombinant antigen-binding protein of the disclosure is a scFv comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 81, 82, 83, 84, 85, 86, 87 and 88.

[0020] In some embodiments, the antigen-binding proteins or antigen-binding fragment/portion thereof binds to a peptide with the amino acid sequence of SEQ ID NO: 111 with an affinity in the range of 8.0 to10 nM, in some embodiments in the range of 8.5 to 9.5nM and in some embodiments in the range of 9.76 to 9.25 nM.

[0021] In a related aspect, the recombinant antigen-binding protein is a fusion protein comprising an antigen-binding region as disclosed in any of Tables 1-8 or a bispecific antibody, for example as shown in Table 10.

[0022] In another aspect, the disclosure relates to an immunoconjugate comprising a first component which is an antigen-binding protein, or antigen-binding fragment thereof as disclosed herein. The immunoconjugate comprises a second component that is a cytotoxin, a detectable label, a radioisotope, a therapeutic agent, a binding protein or a molecule having a second amino acid sequence. Where the second component is a binding protein or second antibody, the binding protein or second antibody has binding specificity for a target that is different from the HLA-peptide complex.

[0023] In a related aspect, the present disclosure relates to bispecific antibodies, including bispecific T-cell engaging antibodies comprising an antigen-binding protein or functional fragment thereof as described herein.

[0024] In another related aspect, the present disclosure relates to an antigen binding protein conjugated to a radionuclide for use in radioimmunotherapy (RIT) to deliver cytotoxic radiation to a target cell.

[0025] In a related aspect, the present disclosure relates to nucleic acids encoding the antigen-binding proteins of the disclosures, vectors/genetic constructs and cells comprising the nucleic acids that encode the antigen-binding proteins including CAR constructs and CAR T-cell antibodies comprising an antigen-binding protein or functional fragment introduced into a T cell as described herein.

[0026] In still other aspects, the disclosure relates to the use of an antigen-binding protein or antigen-binding fragment/portion thereof that binds specifically to an epitope within a variant of wild type Ras peptide, KLVVVGAGGV (SEQ ID NO: 110, amino acids 5-14) or LVVVGAGGV (SEQ ID NO: 115, amino acids 6-14) with a single amino acid substitution at position 12 for use in identifying and/or killing cells bearing a RAS mutant peptide that is displayed on the cell surface in conjunction with an MHC antigen such as HLA-A2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] **Figures 1A-D** show stabilization of HLA-A2 molecule by RAS G12-derived peptides. T2 cells (TAP^-, $HLA\text{-}A0201^+$) were incubated overnight at 37°C at 1 x 10^6 cells/ml in FCS-free RPMI medium supplemented with 10µg/ml human beta 2m (b2M, Sigma, St Louis, MO, USA) in the absence or presence of RAS10-WT (top panel), RAS10-G12V (middle panel), RAS10-G12C (lower panel) peptides (Figure 1**A**), RAS10-G12D (upper panel), or a control peptide derived from hepatitis B virus, HBV (lower panel) (Figure 1**B**). Binding of the peptides to HLA-A2 molecule was measured by staining T2 cells with mouse anti-HLA-A2 mAb conjugated to FITC. Red line shows BB7 staining on T2 cells alone. Blue, green and orange lines show BB7 staining on T2 cells pulsed with peptides at 50, 10 and 2 µg/ml, respectively. Light blue, purple and light brown lines show the isotype control (mouse IgG2b) staining on T2 cells pulsed with peptides at 50, 10 and 2 µg/ml, respectively. Dark brown line shows isotype staining on T2 cells alone. Similarly, binding of peptides to HLA-A2 was measured by BB7 staining on T2 cells pulsed with RAS9-WT (upper panel), RAS9-G12V (middle panel), RAS9-G12C (lower panel) (Figure 1**C**), RAS9-G12D upper panel) or control HBV (lower panel) (Figure 1**D**). Red line: T2 cells alone. Blue, green and orange lines: peptides at 50, 10 and 2 µg/ml, respectively. Isotype control did not show any binding to T2 cells and therefore, were not shown.

[0028] **Figures 2A** and **2B** show RAS-G12 mutant peptide induced-T cell response. T cells from healthy HLA-A0201 positive donors were stimulated with RAS10 peptides WT, G12V, G12C or G12D (shown on X axis) for 5 rounds (**A**). Similarly, T cells were stimulated with RAS9 peptides G12V, G12C or G12D for 3 (**B** upper) or 5 (**B** lower) rounds. The peptide-specific T cell response was measured by IFN-g ELISPOT assay when challenged with the individual peptides or controls shown in legends on right.

[0029] **Figures 3A-G** show the binding of mAbs to RAS10-G12V peptide/HLA A2 complex. RAS 10-WT, RAS10-G12V, G12-C or G12D peptides were pulsed onto T2 cells at 50µg/ml, (see **Figure 1** above). The binding of the mAb to the

peptide/HLA-A2 complex was measured by direct staining with mAbs conjugated to APC or by indirect staining with mAbs, followed by secondary goat anti-human IgG1/FITC mAb. The binding of the mAbs was measured by flow cytometry on a FACScalibur (Becton Dickenson) and analyzed with FlowJo 9.6.3 software. Simultaneously, the cells were stained with anti-HLA-A2 mAb, BB7.2, to measure the ability of the peptides to stabilize HLA-A2 molecule on the cell surface. (**A**) binding of the mAbs #2, 4 or 7 and isotype control on T2 cells pulsed with indicated peptides by indirect staining. (**B**) Direct staining with APC-conjugated mAbs, including BB7 and isotype control antibody. (**C**) Binding of the mAbs #2, 4 or 7 on T2 cells pulsed with alanine-substituted peptides at various positions as indicated. In this case alanine was substituted for the WT amino acid at the position indicated (positions #9-13 of the peptide) to probe the site of antibody binding. (**D**) Binding of the mAb #2 to RAS10-G12S or RAS10-A11G peptide at 10 or 1 μg/ml by indirect staining including BB7 and isotype control antibody. (**E**) Binding of the mAb#2 to various RAS10-G12-derived mutant peptides and CT and MTH peptides, in indirect staining. In this case alanine was substituted for the WT amino acid at the position indicated (positions #8 -13 of the peptide) to probe the site of antibody binding. (**F**) Antibody by the above peptides to T2 cells was measured. In this case alanine was substituted for the WT amino acid at the position indicated #8 -13 of the peptide) to confirm binding to HLA molecules. (**G**) T2 stabilization by the Ras 10 peptides was simultaneously measured by staining T2 cells with BB7 mAb. In this case alanine wsa substituted for the WT amino acid at the position indicated (positions #8 -13 of the peptide) to confirm binding to HLA molecules. Isotype controls showed no binding at all and therefore were not shown.

[0030] **Figure 4A** and **4B** show Mab binding to RAS 9-mer peptides/HLA A2 complex. RAS9WT or G12V peptides were pulsed onto T2 cells at 50μg/ml. The binding of the mAb to the peptide/HLA-A2 complex was measured by indirect (**A**) or indirect staining with mAbs conjugated to APC (**B**). Simultaneously, the cells were stained with anti-HLA-A2 mAb, BB7.2, to measure the relative binding of the peptides to HLA-A2 molecule.

[0031] **Figures 5A-C** show binding of the BiTE derived from the RAS mAbs to peptide/HLA-A2 complex and T cells. T2 cells were pulsed with RAS10-WT or RAS10-G12V peptides, and were stained with BiTEs at indicated concentrations, followed by secondary mouse anti-myc mAb/FITC (**A**). MFI: upper panel for RAS-G12V and lower panel for RAS10-WT. (**B** and **C**) Simultaneously, CD3 T cells purified from a healthy donor by negative immunomagnetic cell separation using a pan T cell isolation kit (Miltenyi Biotec) were stained with BiTEs #2, upper panel #4 lower panel (**B**) or #7 (**C**) at indicated concentrations and followed by secondary mouse anti-myc mAb/FITC.

[0032] **Figure 6** shows ADCC mediated by fresh PBMCs in the presence of the RAS mAbs. T2 cells pulsed with peptides (50 µg/ml, 2 hrs) were incubated with human PBMCs in the presence or absence of mAbs #2, 7 or isotype control at an E:T ratio of 50:1, for 4-5 hrs. The killing was measured by standard 51Cr-release assay. Each data point was the average of triplicate cultures.

[0033] **Figure 7** shows T-BiTE-mediated killing by T cells. T2 cells pulsed with peptides (50 µg/ml, 2 hrs) were incubated with purified human resting T cells in the presence or absence of T-BiTEs #2, 7 or isotype control at an E:T ratio of 30:1, for 4-5 hrs. T cell killing was measured by ^{51}Cr-release assay. Each data point was the average of triplicate cultures.

[0034] **Figure 8** shows the elution profile of peptide/HLA-A0201 complex. The unpurified sample was loaded and eluted for 1 column volume. The first peak, consisting of misfolded aggregates, eluted at approximately 110.63 mL after loading. The peak corresponding to the properly folded MHC complex was observed at 216.18 mL. Lastly, the peak consisting of free B2M was observed at 275.12 mL .

[0035] **Figure 9** shows the results of Ras phage antibody clones FACS binding assay. Clone #2 binds to K-Ras10 G12V (T2-014A2mut, light green line) and K-Ras9 G12V/HLAA0201 (T2-014A1mut, blue line) specifically, while doesn't

recognize empty T2 cells (T2-B2M, dark green line), or K-Ras WT peptide/HLA A0201 complexes (T2-0142WT, orange line and T2-0141WT, red line).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0036] All patents, publications, applications and other references cited herein are hereby incorporated in their entirety into the present application.

[0037] In practicing the present disclosure, many conventional techniques in molecular biology, microbiology, cell biology, biochemistry, and immunology are used, which are within the skill of the art. These techniques are described in greater detail in, for example, Molecular Cloning: a Laboratory Manual 3rd edition, J.F. Sambrook and D.W. Russell, ed. Cold Spring Harbor Laboratory Press 2001; Recombinant Antibodies for Immunotherapy, Melvyn Little, ed. Cambridge University Press 2009; "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Animal Cell Culture" (R. I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987, and periodic updates); "PCR: The Polymerase Chain Reaction", (Mullis et al., ed., 1994); "A Practical Guide to Molecular Cloning" (Perbal Bernard V., 1988); "Phage Display: A Laboratory Manual" (Barbas et al., 2001). The contents of these references and other references containing standard protocols, widely known to and relied upon by those of skill in the art, including manufacturers' instructions are hereby incorporated by reference as part of the present disclosure.

[0038] In the description that follows, certain conventions will be followed as regards the usage of terminology. Generally, terms used herein are intended to be interpreted consistently with the meaning of those terms as they are known to those of skill in the art.

[0039] An "antigen-binding protein" is a protein or polypeptide that comprises an antigen-binding region or antigen-binding portion, and has a strong affinity to another molecule to which it binds. Antigen-binding proteins encompass antibodies, antigen receptors and fusion proteins thereof. Antigen-binding proteins

of the disclosure can be made recombinantly using methods known to those of skill in the art.

[0040] "Antibody" and "antibodies" as those terms are known in the art refer to antigen binding proteins that arise in the context of the immune system. The term "antibody" as referred to herein includes whole, full length antibodies and any fragment thereof in which the "antigen-binding portion" or "antigen-binding region" is retained or single chains thereof. A naturally occurring "antibody" is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is, composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

[0041] The term "antigen-binding portion" or "antigen-binding region" of an antibody (or simply "antigen portion"), as used herein, refers to that region or portion of the antibody that confers antigen specificity; fragments of antigen-binding proteins, for example antibodies, therefore, includes one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., an HLA-peptide complex). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody.

Examples of antigen-binding fragments encompassed within the term "antibody fragments" of an antibody include a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH1 domains; a $F(ab)_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the V_H and CH1 domains; a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward et al., 1989 Nature 341:544-546), which consists of a V_H domain; and an recombinant complementarity determining region (CDR).

[0042] Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al., 1988 Science 242:423-426; and Huston et al., 1988 Proc. Natl. Acad. Sci. 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0043] A "recombinant antibody" or “recombinant antigen-binding protein” or "synthetic antibodies" are generally generated using recombinant technology or using peptide synthetic techniques known to those of skill in the art.

[0044] Normal Ras and mutated forms yield proteins that are intracellular and therefore, cannot be accessed by conventional monoclonal antibody (mAb) therapy. Therefore, immunotherapeutic approaches targeting Ras have been focused on generating T cell responses against the Ras-derived peptide epitopes presented on tumor cells by both MHC class I and class II. Various peptides with 9, 10, 13, 17 or 21 amino acids (aa) spanning Ras mutation G12V, G12D, G12R and G12C, in the context of HLA-A0201 or other HLA haplotypes have been shown to induce both CD4 and CD8 T cell responses (ref). A 9 aa Ras-G12V peptide, LVVVGAVGV (SEQ ID NO: 117) was shown to be able to generate

cytotoxic CD8 T cell clones that kill IFN-gamma pre-treated colon cancer cell line SW480 (HLA-A0201+ K-Ras-G12V mutation+) . Similarly, Ras G12VT mutation-derived peptides KLVVVGAVGV- (10aa, p5-14, SEQ ID NO: 113) and LVVVGAVGV- (9 aa, p6-14, SEQ ID NO: 117) peptides induced CD8 T cell responses from patients with pancreatic cancer to kill pancreatic cancer cell line PaTu (Ras-G12V) and also colon cancer cell line SW480 (Ras-G12VT), in the context of HLA-A0201. These results suggest that Ras mutation-derived epitopes could be cancer-specific targets for T cell immunotherapy against a wide range of human cancers. Accordingly, peptide vaccines-derived from Ras mutations have been evaluated in clinical trials in patients with pancreatic and other cancers, but clinical efficacy was not observed.

[0045] Monoclonal antibodies that mimic the specificity of TCRs (TCR-like) can bind cell-surface complexes specific to cells expressing an intracellular protein, yet retain favorable pharmacokinetics and effector functions that make mAbs powerful therapeutics. TCR-like antibodies are especially interesting in oncology, because many of the most important tumor-associated and oncogenic proteins are nuclear or cytoplasmic.

[0046] Ras mutation-derived epitopes represent truly tumor-specific antigens and their wide expression in human cancer cells make them attractive targets for immunotherapy using TCR-like mAbs. We describe several TCR-like mAbs specific for Ras mutations, specifically for K-Ras G12 mutations. Several of the mAbs recognize only the mutated sequence and not the normal sequence when in the context of human MHC, HLA-A0201. Some embodiments of the antibodies are capable of killing human cancer cells when the mutant epitope (Ras G12V/MHC) is on the cell surface, but not when the normal Ras peptide is on the surface.

[0047] The scFvs of the disclosure selected by phage display were initially tested for their ability to bind to peptide presented on the surface of HLA-positive cells. After T2 cells were incubated in the presence of peptide, the scFvs could selectively recognize them using flow cytometry.

[0048] In some embodiments, the antigen binding proteins of the disclosure include antibodies that have the scFv sequence fused to the 2nd and 3rd constant domains of the heavy chain ($CH_{2,3}$), forming the bottom third of the Fc region of a human immunoglobulin to yield a bivalent protein and fragments thereof, increasing the overall avidity and stability of the antibody. In addition, the Fc portion allows the direct conjugation of other molecules, including but not limited to fluorescent dyes, cytotoxins, radioisotopes etc. to the antibody for example, for use in antigen quantitation studies, to immobilize the antibody for affinity measurements using surface plasmon resonance (SPR), for targeted delivery of a therapeutic agent, to test for Fc-mediated cytotoxicity using CD16-expressing immune effector cells and many other applications.

[0049] The results presented here highlight the specificity, sensitivity and utility of the antigen binding proteins of the disclosure in targeting MHC-Ras oncoprotein complexes.

[0050] In one embodiment, therefore, the present disclosure relates to antigen-binding proteins and portions thereof, such as recombinant antibodies, that recognize a complex of a peptide/protein fragment derived from an intracellular protein, specifically Ras oncoprotein, and an MHC class I molecule, for example, as the complex might appear on the cell surface for recognition by a T-cell.

[0051] The molecules of the disclosure are based on the identification and selection of a single chain variable fragment (scFv) using phage display, the amino acid sequence of which confers the molecules' specificity for the MHC restricted peptide of interest and forms the basis of antigen binding proteins of the disclosure. The scFv, therefore, can be used to design a diverse array of "antibody" molecules, including, for example, full length antibodies, fragments thereof, such as Fab and $F(ab')_2$, minibodies, fusion proteins, including scFv-Fc fusions, multivalent antibodies, that is, antibodies that have more than one specificity for the same antigen or different antigens, for example, bispecific T-cell engaging antibodies (BiTE or T-BiTE), tribodies, etc. (see Cuesta et al., Multivalent antibodies: when design surpasses evolution. *Trends in Biotechnology*

28:355-362 2010). scFv may also be used to construct CARs which are introduced by various means known to the skilled artisan into living T cells to make cytotoxic CAR T cells.

[0052] In an embodiment in which the antigen-binding protein is a full length antibody, the heavy and light chains of an antibody of the disclosure may be full-length (e.g., an antibody can include at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains) or may include an antigen-binding portion (a Fab, $F(ab')_2$, Fv or a single chain Fv fragment ("scFv")). In other embodiments, the antibody heavy chain constant region is chosen from, e.g., IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE. In some embodiments, the immunoglobulin isotype is selected from IgG1, IgG2, IgG3, and IgG4, more particularly, IgG1 (e.g., human IgG1). The choice of antibody type will depend on the immune effector function that the antibody is designed to elicit.

[0053] In constructing a recombinant immunoglobulin, appropriate amino acid sequences for constant regions of various immunoglobulin isotypes and methods for the production of a wide array of antibodies are well known to those of skill in the art.

[0054] In some embodiments, the constant region of the antibody is altered, e.g., mutated, to modify the properties of the antibody (e.g., to increase or decrease one or more of: Fc receptor binding, antibody carbohydrate, for example glycosylation or fucosylation, the number of cysteine residues, effector cell function, or complement function).

[0055] In one embodiment, the antigen binding protein is an anti-RAS antigen-binding protein or fragment thereof having an antigen binding region that comprises the amino acid sequence of SEQ ID NO: 81 and specifically binds to KLVVVGAVGV (SEQ ID NO: 113)/HLA2. In other embodiments, the anti-RAS antigen-binding protein is a scFv, or scFv-Fc fusion protein, full length human IgG or fragment thereof with VH and VL regions or CDRs selected from Table 1.

Table 1

	Ab #1		
CDRs:	1	2	3
VH	GGTFSSYA (SEQ ID NO. 1)	IIPIFGKG (SEQ ID NO. 2)	ARHIPTFSFDY (SEQ ID NO. 3)
VL	SSNIGAGYD (SEQ ID NO: 4)	GNS (SEQ ID NO: 5)	QSYDSSLSGYV (SEQ ID NO. 6)
Full VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWM GGIIPIFGKGNYPQKFQGRVTITADESTGTAYMELSSLRSEDTAVYYCARHI PTFSFDYWGQGTLVTVSS (SEQ ID NO: 7)		
VH DNA	caggtgcagctggtgcagtctggggctgaggtgaagaagcctgggtcctcggtgaaggtctcctgcaag gcttctggaggcaccttcagcagctatgctatcagctgggtgcgacaggcccctggacaagggcttgagt ggatgggaggtatcatccctatctttggtaaaggaaactacccacagaagttccagggcagagtcacgat taccgcggacgaatctacgggcacagcctacatggagctgagcagcctgagatctgaggacacggcc gtgtattactgtgcgcgccatatcccgactttctctttcgattactggggtcaaggtactctggtgaccgtctcct ca (SEQ ID NO: 8)		
Full VL	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIY GNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGYV FGTGTKVTVLG (SEQ ID NO: 9)		
VL DNA	cagtctgtgttgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgcactg ggagcagctccaacatcggggcaggttatgatgtacactggtaccagcagcttccaggaacagccccc aaactcctcatctatggtaacagcaatcggccctcaggggtccctgaccgattctctggctccaagtctggc acctcagcctccctggccatcactgggctccaggctgaggatgaggctgattattactgccagtcctatgac agcagcctgagtggttatgtcttcggaactgggaccaaggtcaccgtcctaggt (SEQ ID NO: 10)		
scFv	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIY GNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGYV FGTGTKVTVLGSRGGGGSGGGGSGGGGSLEMAQVQLVQSGAEVKKPGS SVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGKGNYPQKFQG RVTITADESTGTAYMELSSLRSEDTAVYYCARHIPTFSFDYWGQGTLVTVS S (SEQ ID NO: 81)		
DNA (5' -3')	cagtctgtgttgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgcactg ggagcagctccaacatcggggcaggttatgatgtacactggtaccagcagcttccaggaacagccccc aaactcctcatctatggtaacagcaatcggccctcaggggtccctgaccgattctctggctccaagtctggc acctcagcctccctggccatcactgggctccaggctgaggatgaggctgattattactgccagtcctatgac agcagcctgagtggttatgtcttcggaactgggaccaaggtcaccgtcctaggtctagaggtggtggtggt agcggcggcggcggctctggtggtggtggatcccaggtgcagctggtgcagtctggggctgaggtgaag aagcctgggtcctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagctatgctatcagctg		

	ggtgcgacaggcccctggacaagggcttgagtggatgggaggtatcatccctatctttggtaaaggaaac tacccacagaagttccagggcagagtcacgattaccgcggacgaatctacgggcacagcctacatgga gctgagcagcctgagatctgaggacacggccgtgtattactgtgcgcgccatatcccgactttctctttcgat tactggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 89)

[0056] In one embodiment, the antigen binding protein is an anti-RAS antigen-binding protein or fragment thereof having an antigen binding region that comprises the amino acid sequence of SEQ ID NO: 82 and specifically binds to LVVVGAVGV (SEQ ID NO: 116)/HLA2 or KLVVVGAVGV (SEQ ID NO: 111)/HLA2. In other embodiments, the anti-RAS antigen-binding protein is a scFv, or scFv-Fc fusion protein or full length human IgG with VH and VL regions or CDRs selected from Table 2.

Table 2

	Ab #2		
CDRs:	1	2	3
VH	GGTFSSYT (SEQ ID NO. 11)	FIPISGTV (SEQ ID NO. 12)	ARPLDWTEDI (SEQ ID NO. 13)
VL	SSNIGAGYD (SEQ ID NO: 14)	GNS (SEQ ID NO: 15)	QSYDSSLSGSV (SEQ ID NO. 16)
Full VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYTINWVRQAPGQGLEWM GGFIPISGTVNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARP LDWTEDIWGQGTLVTVSS (SEQ ID NO: 17)		
VH DNA	caggtgcagctggtgcagtctggggctgaggtgaagaagcctgggtcctcggtgaaggtctcctgcaag gcttctggaggcaccttcagcagctatactatcaactgggtgcgacaggcccctggacaagggcttgag tggatgggagggttcatccctatctctggtacagtaaactacgcacagaagttccagggcagagtcacg attaccgcggacgaatccacgagcacagcctacatggaactgagcagcctgagatctgaggacactg ccgtgtattactgtgcgcgcccgctggactggactgaagatatctggggtcaaggtactctggtgaccgtc tcctca (SEQ ID NO: 18)		
Full VL	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLI YGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSG		

	SVFGTGTKVTVLG (SEQ ID NO: 19)
VL DNA	Cagtctgtgttgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgcact gggagcagctccaacatcggggcaggttatgatgtacactggtaccagcagcttccaggaacagcccc caaactcctcatctatggtaacagcaatcggccctcaggggtccctgaccgattctctggctccaagtctg gcacctcagcctccctggccatcactgggctccaggctgaggatgaggctgattattactgccagtcctat gacagcagcctgagtggttcagtcttcggaactgggaccaaggtcaccgtcctaggt (SEQ ID NO: 20)
scFv	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLI YGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSG SVFGTGTKVTVLGSRGGGGSGGGGSGGGGSLEMAVQLVQSGAEVKKP GSSVKVSCKASGGTFSSYTINWVRQAPGQGLEWMGGFIPISGTVNYAQK FQGRVTITADESTSTAYMELSSLRSEDTAVYYCARPLDWTEDIWGQGTLV TVSS (SEQ ID NO: 82)
DNA (5' -3')	cagtctgtgttgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgcact gggagcagctccaacatcggggcaggttatgatgtacactggtaccagcagcttccaggaacagcccc caaactcctcatctatggtaacagcaatcggccctcaggggtccctgaccgattctctggctccaagtctg gcacctcagcctccctggccatcactgggctccaggctgaggatgaggctgattattactgccagtcctat gacagcagcctgagtggttcagtcttcggaactgggaccaaggtcaccgtcctaggtctagaggtggtg gtggtagcggcggcggcggctctggtggtggtggatcccaggtgcagctggtgcagtctggggctgag gtgaagaagcctgggtcctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagctatacta tcaactgggtgcgacaggcccctggacaagggcttgagtggatgggagggttcatccctatctctggtac agtaaactacgcacagaagttccagggcagagtcacgattaccgcggacgaatccacgagcacagc ctacatggaactgagcagcctgagatctgaggacactgccgtgtattactgtgcgcgcccgctggactg gactgaagatatctggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 90)

[0057] In one embodiment, the antigen binding protein is an anti-RAS antigen-binding protein or fragment thereof having an antigen binding region that comprises the amino acid sequence of SEQ ID NO: 83 and specifically binds to KLVVVGAVGV (SEQ ID NO: 111)/HLA2. In other embodiments, the anti-RAS antigen-binding protein is a scFv, or scFv-Fc fusion protein or full length human IgG with VH and VL regions or CDRs selected from Table 3.

Table 3

	Ab #3		
CDRs:	1	2	3
VH	GYTFTAYY (SEQ ID NO. 21)	MNTNNGAT (SEQ ID NO. 22)	ARGDISQDFADV (SEQ ID NO. 23)
VL	SGSIASNY (SEQ ID NO: 24)	EDN (SEQ ID NO: 25)	QSYDDINHWV (SEQ ID NO. 26)
Full VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTAYYLHWLRQAPGQGLEWMGWMNTNNGATRYAQKFQDRVTMTRDTSINTAYMEMSGLSSDDTAMYYCARGDISQDFADVWGQGTLVTVSS (SEQ ID NO: 27)		
VH DNA	gaggtgcagctggtgcagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttctggatacaccttcaccgcctactatctgcactggctgcgacaggcccctggacaagggcttgagtggatgggatggatgaatactaacaatggtgccacaaggtatgcacagaaatttcaggacagggtcaccatgaccagggacacgtccattaacacagcctacatggagatgagcgggctgtcatctgacgacaccgccatgtattactgtgcgcgcggtgatatctctcaggacttcgctgatgttttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 28)		
Full VL	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTILIYEDNKRPSGVPDRFSGSIDSSSNSASLTISGLKTGDEADYYCQSYDDINHWVFGGGTKLTVLG (SEQ ID NO: 29)		
VL DNA	aattttatgctgactcagccccactctgtgtcggagtctccggggaagacggtaaccatctcctgcaccggcagcagtggcagcattgccagcaactatgtgcagtggtatcagcagcgcccgggcagtgcccccaccattctgatctatgaggataacaaaagaccctctggggtccctgatcggttctctggctccatcgacagctcctccaactctgcctccctcaccatctctggactgaagactggggacgaggctgactactactgtcagtcttatgatgacatcaatcattgggtgttcggcggagggaccaagctgaccgtcctaggt (SEQ ID NO: 30)		
scFv	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTILIYEDNKRPSGVPDRFSGSIDSSSNSASLTISGLKTGDEADYYCQSYDDINHWVFGGGTKLTVLGSRGGGGSGGGGSGGGGSLEMAEVQLVQSGAEVKKPGASVKVSCKASGYTFTAYYLHWLRQAPGQGLEWMGWMNTNNGATRYAQKFQDRVTMTRDTSINTAYMEMSGLSSDDTAMYYCARGDISQDFADVWGQGTLVTVSS (SEQ ID NO: 83)		
DNA (5' -3')	aattttatgctgactcagccccactctgtgtcggagtctccggggaagacggtaaccatctcctgcaccggcagcagtggcagcattgccagcaactatgtgcagtggtatcagcagcgcccgggcagtgcccccaccattctgatctatgaggataacaaaagaccctctggggtccctgatcggttctctggctccatcgacagctcctccaactctgcctccctcaccatctctggactgaagactggggacgaggctgactactactgtcagtcttat		

	gatgacatcaatcattgggtgttcggcggagggaccaagctgaccgtcctaggtctagaggtggtggtg gtagcggcggcggcggctctggtggtggtggatccgaggtgcagctggtgcagtctggggctgaggtg aagaagcctggggcctcagtgaaggtctcctgcaaggcttctggatacaccttcaccgcctactatctgc actggctgcgacaggcccctggacaagggcttgagtggatgggatggatgaatactaacaatggtgcc acaaggtatgcacagaaatttcaggacagggtcaccatgaccagggacacgtccattaacacagcct acatggagatgagcgggctgtcatctgacgacaccgccatgtattactgtgcgcgcggtgatatctctca ggacttcgctgatgtttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 91)

[0058] In one embodiment, the antigen binding protein is an anti-RAS antigen-binding protein or fragment thereof having an antigen binding region that comprises the amino acid sequence of SEQ ID NO: 84 and specifically binds to KLVVVGAVGV (SEQ ID NO: 111)/HLA2. In other embodiments, the anti-RAS antigen-binding protein is a scFv, or scFv-Fc fusion protein or full length human IgG with VH and VL regions or CDRs selected from Table 4.

Table 4

	Ab #4		
CDRs:	1	2	3
VH	GYTFTAYY (SEQ ID NO. 31)	MNTNNGAT (SEQ ID NO. 32)	ARGDISQDFADV (SEQ ID NO. 33)
VL	SGSIASNY (SEQ ID NO: 34)	EDN (SEQ ID NO: 35)	QSYDDINHWV (SEQ ID NO. 36)
Full VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTAYYLHWLRQAPGQGLEWM GWMNTNNGATRYAQKFQDRVTMTRDTSINTAYMEMSGLSSDDTAMYYC ARGDISQDFADVWGQGTLVTVSS (SEQ ID NO: 37)		
VH DNA	gaggtgcagctggtgcagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaa ggcttctggatacaccttcaccgcctactatctgcactggctgcgacaggcccctggacaagggcttgag tggatgggatggatgaatactaacaatggtgccacaaggtatgcacagaaatttcaggacagggtcac catgaccagggacacgtccattaacacagcctacatggagatgagcgggctgtcatctgacgacaccg ccatgtattactgtgcgcgcggtgatatctctcaggacttcgctgatgtttggggtcaaggtactctggtgac cgtctcctca (SEQ ID NO: 38)		

Full VL	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTILIYE DNKRPSGVPDRFSGSIDSSSNSASLTISGLKTGDEADYYCQSYDDINHWV FGGGTKLTVLG (SEQ ID NO: 39)
VL DNA	aattttatgctgactcagccccactctgtgtcggagtctccggggaagacggtaaccatctcctgcaccgg cagcagtggcagcattgccagcaactatgtgcagtggtatcagcagcgcccgggcagtgcccccacc attctgatctatgaggataacaaaagaccctctggggtccctgatcggttctctggctccatcgacagctcc tccaactctgcctccctcaccatctctggactgaagactggggacgaggctgactactactgtcagtcttat gatgacatcaatcattgggtgttcggcggagggaccaagctgaccgtcctaggt (SEQ ID NO: 40)
scFv	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTILIYE DNKRPSGVPDRFSGSIDSSSNSASLTISGLKTGDEADYYCQSYDDINHWV FGGGTKLTVLGSRGGGGSGGGGSGGGGSLEMAEVQLVQSGAEVKKPG ASVKVSCKASGYTFTAYYLHWLRQAPGQGLEWMGWMNTNNGATRYAQ KFQDRVTMTRDTSINTAYMEMSGLSSDDTAMYYCARGDISQDFADVWGQ GTLVTVSS (SEQ ID NO: 84)
DNA (5' -3')	aattttatgctgactcagccccactctgtgtcggagtctccggggaagacggtaaccatctcctgcaccgg cagcagtggcagcattgccagcaactatgtgcagtggtatcagcagcgcccgggcagtgcccccacc attctgatctatgaggataacaaaagaccctctggggtccctgatcggttctctggctccatcgacagctcc tccaactctgcctccctcaccatctctggactgaagactggggacgaggctgactactactgtcagtcttat gatgacatcaatcattgggtgttcggcggagggaccaagctgaccgtcctaggtctagaggtggtggtg gtagcggcggcggcggctctggtggtggtggatccgaggtgcagctggtgcagtctggggctgaggtg aagaagcctggggcctcagtgaaggtctcctgcaaggcttctggatacaccttcaccgcctactatctgc actggctgcgacaggcccctggacaagggcttgagtggatgggatggatgaatactaacaatggtgcc acaaggtatgcacagaaatttcaggacagggtcaccatgaccagggacacgtccattaacacagcct acatggagatgagcgggctgtcatctgacgacaccgccatgtattactgtgcgcgcggtgatatctctca ggacttcgctgatgtttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 92)

[0059] In one embodiment, the antigen binding protein is an anti-RAS antigen-binding protein or fragment thereof having an antigen binding region that comprises the amino acid sequence of SEQ ID NO: 85 and specifically binds to KLVVVGAVGV (SEQ ID NO: 111)/HLA2. In other embodiments, the anti-RAS antigen-binding protein is a scFv, or scFv-Fc fusion protein or full length human IgG with VH and VL regions or CDRs selected from Table 5.

Table 5

	Ab #5		
CDRs:	1	2	3
VH	GGSFSGYY (SEQ ID NO. 41)	VNHSGNT (SEQ ID NO. 42)	ARYFPPMIDV (SEQ ID NO. 43)
VL	SSNIENNY (SEQ ID NO: 44)	DNN (SEQ ID NO: 45)	GTWDSSLSAYV (SEQ ID NO. 46)
Full VH	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPGKGLEWIG EVNHSGNTNYNPSLKSRVTISLDTSKNQFSLKLNSVTAADTAVYYCARYFP PMIDVWGQGTLVTVSS (SEQ ID NO: 47)		
VH DNA	caggtgcagctacagcagtggggcgcaggactgttgaaaccttcggagaccctgtccctcacctgcgct gtctatggtgggtccttcagcggttactactggagctggatccgccagtccccagggaagggactggagt ggattggggaagtcaatcatagtggcaacaccaactacaacccgtccctcaagagtcgagtcaccatttc actagacacgtccaagaaccagttctccctgaaactgaactctgtgaccgccgccgacacggccgtgtat tactgtgcgcgctacttcccgccgatgatcgatgtttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 48)		
Full VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIENNYVSWYQQLPGTAPKLLIYD NNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAYVF GTGTKVTVLG (SEQ ID NO: 49)		
VL DNA	cagtctgtcgtgacgcagccgccctcagtgtctgcggccccaggacagaaggtcaccatctcctgctctg gaagcagctccaacattgagaataattatgtatcatggtaccagcagctcccaggaacagcccccaaac tcctcatttatgacaataataagcgaccctcagggattcctgaccgattctctggctccaagtctggcacgtc agccaccctgggcatcaccggactccagactggggacgaggccgattattactgcggaacatgggata gcagcctgagtgcctatgtcttcggaactgggaccaaggtcaccgtcctaggt (SEQ ID NO: 50)		
scFv	QSVVTQPPSVSAAPGQKVTISCSGSSSNIENNYVSWYQQLPGTAPKLLIYD NNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAYVF GTGTKVTVLGSRGGGGSGGGGSGGGGSLEMAQVQLQQWGAGLLKPSE TLSLTCAVYGGSFSGYYWSWIRQSPGKGLEWIGEVNHSGNTNYNPSLKS RVTISLDTSKNQFSLKLNSVTAADTAVYYCARYFPPMIDVWGQGTLVTVSS (SEQ ID NO: 85)		
DNA (5' -3')	cagtctgtcgtgacgcagccgccctcagtgtctgcggccccaggacagaaggtcaccatctcctgctctg gaagcagctccaacattgagaataattatgtatcatggtaccagcagctcccaggaacagcccccaaac tcctcatttatgacaataataagcgaccctcagggattcctgaccgattctctggctccaagtctggcacgtc agccaccctgggcatcaccggactccagactggggacgaggccgattattactgcggaacatgggata		

	gcagcctgagtgcctatgtcttcggaactgggaccaaggtcaccgtcctaggtctagaggtggtggtgga gcggcggcggcggctctggtggtggtggatcccaggtgcagctacagcagtggggcgcaggactgttg aaaccttcggagaccctgtccctcacctgcgctgtctatggtgggtccttcagcggttactactggagctgg atccgccagtccccagggaagggactggagtggattggggaagtcaatcatagtggcaacaccaacta caacccgtccctcaagagtcgagtcaccatttcactagacacgtccaagaaccagttctccctgaaactg aactctgtgaccgccgccgacacggccgtgtattactgtgcgcgctacttcccgccgatgatcgatgtttgg ggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 93)

[0060] In one embodiment, the antigen binding protein is an anti-RAS antigen-binding protein or fragment thereof having an antigen binding region that comprises the amino acid sequence of SEQ ID NO: 86 and specifically binds to KLVVVGAVGV (SEQ ID NO: 111)/HLA2. In other embodiments, the anti-RAS antigen-binding protein is a scFv, or scFv-Fc fusion protein or full length human IgG with VH and VL regions or CDRs selected from Table 6.

Table 6

	Ab #6		
CDRs:	1	2	3
VH	GGSISSSSYY (SEQ ID NO. 51)	INHSGST (SEQ ID NO. 52)	ARYSHHVDSGGYDV (SEQ ID NO. 53)
VL	SSNIGNNY (SEQ ID NO: 54)	DNN (SEQ ID NO: 55)	GTWDSSLSAVV (SEQ ID NO. 56)
Full VH	QLQLQESGPGLVKPSETLSLSCTVSGGSISSSSYYWGWIRQPPGKGLEWI GEINHSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARYS HHVDSGGYDVWGQGTLVTVSS (SEQ ID NO: 57)		
VH DNA	cagctgcagctgcaggagtcgggcccaggactggtgaagccttcggagaccctgtccctcagttgcact gtctctggtggctccatcagcagtagtagttactactggggctggatccgccagcccccagggaaggggc tggagtggattggggaaatcaatcatagtggaagcaccaactacaacccgtccctcaagagtcgagtca ccatatcagtagacacgtccaagaaccagttctccctgaagctgagttctgtgaccgccgcggacacggc cgtgtattactgtgcgcgctactctcatcatgttgactctggtggttacgatgtttggggtcaaggtactctggtg accgtctcctca (SEQ ID NO: 58)		
Full VL	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPRTAPRLLIYD NNKRPSGIPDRFSASKSGTSATLGITGLQTGDEADYYCGTWDSSLSAVVF		

	GGGTKLTVLG (SEQ ID NO: 59)
VL DNA	Cagtctgtcgtgacgcagccgccctcagtgtctgcggccccaggacagaaggtcaccatctcctgctctg gaagcagctccaacattgggaataattatgtatcctggtaccagcagctcccaagaacagcccccagac tcctcatttatgacaataataagcgaccctcagggattcctgaccgattctctgcctccaagtctggcacgtc agccaccctgggcatcaccggactccagactggggacgaggccgattattactgcggaacatgggata gcagcctgagtgctgtggtattcggcggagggaccaagctgaccgtcctaggt (SEQ ID NO: 60)
scFv	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPRTAPRLLIYD NNKRPSGIPDRFSASKSGTSATLGITGLQTGDEADYYCGTWDSSLSAVVF GGGTKLTVLGSRGGGGSGGGGSGGGGSLEMAQLQLQESGPGLVKPSET LSLSCTVSGGSISSSSYYWGWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR VTISVDTSKNQFSLKLSSVTAADTAVYYCARYSHHVDSGGYDVWGQGTLV TVSS (SEQ ID NO: 86)
DNA (5' -3')	gaagcagctccaacattgggaataattatgtatcctggtaccagcagctcccaagaacagcccccagac tcctcatttatgacaataataagcgaccctcagggattcctgaccgattctctgcctccaagtctggcacgtc agccaccctgggcatcaccggactccagactggggacgaggccgattattactgcggaacatgggata gcagcctgagtgctgtggtattcggcggagggaccaagctgaccgtcctaggtctagaggtggtggtggt agcggcggcggcggctctggtggtggtggatcccagctgcagctgcaggagtcgggcccaggactggt gaagccttcggagaccctgtccctcagttgcactgtctctggtggctccatcagcagtagtagttactactgg ggctggatccgccagcccccagggaaggggctggagtggattggggaaatcaatcatagtggaagca ccaactacaacccgtccctcaagagtcgagtcaccatatcagtagacacgtccaagaaccagttctccct gaagctgagttctgtgaccgccgcggacacggccgtgtattactgtgcgcgctactctcatcatgttgactct ggtggttacgatgtttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 94)

[0061] In one embodiment, the antigen binding protein is an anti-RAS antigen-binding protein or fragment thereof having an antigen binding region that comprises the amino acid sequence of SEQ ID NO: 87 and specifically binds to KLVVVGAVGV (SEQ ID NO: 111)/HLA2. In other embodiments, the anti-RAS antigen-binding protein is a scFv, or scFv-Fc fusion protein or full length human IgG with VH and VL regions or CDRs selected from Table 7.

Table 7

	Ab #7		
CDRs:	1	2	3
VH	GGTFSSYG (SEQ ID NO. 61)	IIPIFGTP (SEQ ID NO. 62)	ARSYYGYFDG (SEQ ID NO. 63)
VL	QDISNY (SEQ ID NO: 64)	DAS (SEQ ID NO: 65)	QQYKSYPLT (SEQ ID NO. 66)
Full VH	EVQLVESGAEVKEPGSSVKVSCKASGGTFSSYGISWIRQAPGQGLEWMG EIIPIFGTPNYAQKFQGRVTITADESTSTAYVELSSLRSDDTAVYYCARSYY GYFDGWGQGTLVTVSS (SEQ ID NO: 67)		
VH DNA	gaggtgcagctggtggagtctggggctgaggtgaaggagcctgggtcctcggtgaaggtctcctgcaa ggcttctggaggcaccttcagcagctatggtatcagctggattcgacaggcccctggacaagggcttga gtggatgggagagatcatccctatctttggtacaccaaactacgcgcagaagttccagggcagagtcac gattaccgcggacgaatccacgagcacagcctacgtggagctgagcagcctgagatctgacgacacg gccgtatattactgtgcgcgctcttactacggttacttcgatggttggggtcaaggtactctggtgaccgtctc ctca (SEQ ID NO: 68)		
Full VL	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDA SNLETGVPSRFSGSGSGTDFTFTISSLQPDDFATYYCQQYKSYPLTFGGG TKVEIKR (SEQ ID NO: 69)		
VL DNA	gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgcca ggcgagtcaggacattagcaactatttaaattggtatcagcagaaaccagggaaagcccctaagctcct gatctacgatgcatccaatttggaaacaggggtcccatcaaggttcagtggaagtggatctgggacaga ttttactttcaccatcagcagcctgcagcctgatgattttgcaacttattactgccaacagtataagagttacc cgctcactttcggcggagggaccaaggtggagatcaaacgt (SEQ ID NO: 70)		
scFv clone 45	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDA SNLETGVPSRFSGSGSGTDFTFTISSLQPDDFATYYCQQYKSYPLTFGGG TKVEIKRSRGGGGSGGGGSGGGGSLEMAEVQLVESGAEVKEPGSSVKV SCKASGGTFSSYGISWIRQAPGQGLEWMGEIIPIFGTPNYAQKFQGRVTIT ADESTSTAYVELSSLRSDDTAVYYCARSYYGYFDGWGQGTLVTVSS (SEQ ID NO: 87)		
DNA (5' -3')	gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgcca ggcgagtcaggacattagcaactatttaaattggtatcagcagaaaccagggaaagcccctaagctcct gatctacgatgcatccaatttggaaacaggggtcccatcaaggttcagtggaagtggatctgggacaga		

	ttttactttcaccatcagcagcctgcagcctgatgattttgcaacttattactgccaacagtataagagttacc cgctcactttcggcggagggaccaaggtggagatcaaacgtctagaggtggtggtggtagcggcggc ggcggctctggtggtggtggatccgaggtgcagctggtggagtctggggctgaggtgaaggagcctgg gtcctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagctatggtatcagctggattcgac aggcccctggacaagggcttgagtggatgggagagatcatccctatctttggtacaccaaactacgcgc agaagttccagggcagagtcacgattaccgcggacgaatccacgagcacagcctacgtggagctga gcagcctgagatctgacgacacggccgtatattactgtgcgcgctcttactacggttacttcgatggttggg gtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 95)

[0062] In one embodiment, the antigen binding protein is an anti-RAS antigen-binding protein or fragment thereof having an antigen binding region that comprises the amino acid sequence of SEQ ID NO: 88 and specifically binds to KLVVVGAVGV (SEQ ID NO: 111)/HLA2. In other embodiments, the anti-RAS antigen-binding protein is a scFv, or scFv-Fc fusion protein or full length human IgG with VH and VL regions or CDRs selected from Table 8.

Table 8

	Ab #8		
CDRs:	1	2	3
VH	GYTFTSYY (SEQ ID NO. 71)	INPSGGST (SEQ ID NO. 72)	ARSMYQYFLDS (SEQ ID NO. 73)
VL	SSNIGAGYD (SEQ ID NO: 74)	GNI (SEQ ID NO: 75)	QSYDSNLSG (SEQ ID NO. 76)
Full VH	EVQLVESGAEVKKPGASVKISCKASGYTFTSYYMHWVRQAPGQGLEWMG IINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARS MYQYFLDSWGQGTLVTVSS (SEQ ID NO: 77)		
VH DNA	gaggtgcagctggtggagtccggggctgaggtgaagaagcctggggcctcagtaaaaatttcctgcaag gcatctggatacaccttcaccagctactatatgcactgggtgcgacaggcccctggacaagggcttgagt ggatgggaataatcaaccctagtggtggtagcacaagctacgcacagaagttccagggcagagtcacc atgaccagggacacgtccacgagcacagtctacatggagctgagcagcctgagatctgaggacacgg ccgtatattactgtgcgcgctctatgtaccagtacttcctggattcttggggtcaaggtactctggtgaccgtct cctca (SEQ ID NO: 78)		

Full VL	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLI YGNINRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSNLSGY VFATGTKVTVLG (SEQ ID NO: 79)
VL DNA	cagtctgtcgtgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgcactg ggagcagctccaacatcggggcaggttatgatgtacactggtaccagcaacttccaggaacagccccc aaactcctcatctatggtaacatcaatcggccctcaggggtccctgaccgattctctggctccaagtctggc acctcagcctccctggccatcactgggctccaggctgaggatgaggctgattattactgccagtcctatgac agcaacctgagtggttatgtcttcgcaactgggaccaaggtcaccgtcctaggt (SEQ ID NO: 80)
scFv	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLI YGNINRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSNLSGY VFATGTKVTVLGSRGGGGSGGGGSGGGGSLEMAEVQLVESGAEVKKPG ASVKISCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQ GRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARSMYQYFLDSWGQGTLV TVSS (SEQ ID NO: 88)
DNA (5' -3')	cagtctgtcgtgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgcactg ggagcagctccaacatcggggcaggttatgatgtacactggtaccagcaacttccaggaacagccccc aaactcctcatctatggtaacatcaatcggccctcaggggtccctgaccgattctctggctccaagtctggc acctcagcctccctggccatcactgggctccaggctgaggatgaggctgattattactgccagtcctatgac agcaacctgagtggttatgtcttcgcaactgggaccaaggtcaccgtcctaggtctagaggtggtggtggt agcggcggcggcggctctggtggtggtggatccgaggtgcagctggtggagtccggggctgaggtgaa gaagcctggggcctcagtaaaaatttcctgcaaggcatctggatacaccttcaccagctactatatgcact gggtgcgacaggcccctggacaagggcttgagtggatgggaataatcaaccctagtggtggtagcaca agctacgcacagaagttccagggcagagtcaccatgaccagggacacgtccacgagcacagtctaca tggagctgagcagcctgagatctgaggacacggccgtatattactgtgcgcgctctatgtaccagtacttcc tggattcttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 96)

[0063] In some embodiments, antigen binding proteins of the disclosure comprise an antigen-binding region or portion having an amino acid sequence that is 100% identical to the amino acid sequences disclosed in Tables 1-8 above. In other embodiments, antigen binding proteins of the disclosure comprise an antigen-binding region or portion having an amino acid sequence that is 96-99.9% identical to the amino acid sequences disclosed in Tables 1-8 above. In still other embodiments, the antigen-binding proteins of the disclosure may comprise an antigen-binding region or portion having an amino acid sequence that is about

70%, 80%, 90%, or 95.9% identical to one of the sequences disclosed in Tables 1-8 above.

[0064] In one embodiment, the antigen binding protein is an anti-RAS antibody having an hIgG1 constant region, a human light chain (kappa) or human light chain (lambda) as shown in Table 9.

Table 9

Human heavy chain constant region and IgG1 Fc domain sequence	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK* (SEQ ID NO: 97)
DNA sequence	gtctcctcagcttccaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctc tgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtc gtggaactcaggcgccctgaccagcggcgtgcacaccttcccggccgtcctacagtcctcagg actctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatc tgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaggttgagcccaaatctt gtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtct tcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgt ggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtgg aggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggt cagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctc caacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccg agaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcag cctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgg gcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcct ctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgt gatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatg a (SEQ ID NO: 98)
Human light chain (kappa)	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC*(SEQ ID NO: 99)

DNA sequence	accgtggccgctccctccgtgttcatcttcccaccttccgacgagcagctgaagtccggcaccgc ttctgtcgtgtgcctgctgaacaacttctacccccgcgaggccaaggtgcagtggaaggtggac aacgccctgcagagcggcaactcccaggaatccgtgaccgagcaggactccaaggacagc acctactccctgtcctccaccctgaccctgtccaaggccgactacgagaagcacaaggtgtac gcctgcgaagtgacccaccagggcctgtctagccccgtgaccaagtctttcaaccggggcgag tgctag (SEQ ID NO: 100)
Human light chain (lambda)	QPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGS PVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHE GSTVEKTVAPTECS* (SEQ ID NO: 101)
DNA sequence	cagcctaaggccaaccctaccgtgaccctgttccccccatcctccgaggaactgcaggccaac aaggccaccctcgtgtgcctgatctccgacttctaccctggcgccgtgaccgtggcctggaagg ctgatggatctcctgtgaaggccggcgtggaaaccaccaagccctccaagcagtccaacaac aaatacgccgcctcctcctacctgtccctgaccctgagcagtggaagtcccaccggtcctaca gctgccaagtgacccacgagggctccaccgtggaaaagaccgtggctcctaccgagtgctcct ag (SEQ ID NO: 102)

[0065] In one embodiment, the antigen binding protein is an anti-RAS bispecific T-cell engaging antibody or BiTE having a Ras antibody light chain variable region, a first linker, and a Ras antibody heavy chain variable region. In certain embodiments, the BiTE antibody further comprises a second linker and an anti-CD3 scFv-His tag having sequences as shown in Table 10. Linkers used in generating BiTEs are generally glycine-rich and range in length from about 4 to 25 amino acids.

[0066] In some embodiments, a BiTE antibody of the present disclosure has an amino acid sequence that is 70%, 80%, 90%, 95% and in some cases, 99-100% identical to SEQ ID NO: 103.

Table 10

BiTE Antibody	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAP KLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSY DSSLSGSVFGTGTKVTVLGSRGGGGSGGGGSGGGGSLEMAQVQLV QSGAEVKKPGSSVKVSCKASGGTFSSYTINWVRQAPGQGLEWMGG FIPISGTVNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAR PLDWTEDIWGQGTLVTVSSGGGGSDVQLVQSGAEVKKPGASVKVSC KASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYTNYADSVKGRF TITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQGTTV TVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRA SQSVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGTDY SLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIKHHHHHH* (SEQ ID NO: 103)
Ras antibody light chain variable region	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAP KLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSY DSSLSGSVFGTGTKVTVLG (SEQ ID NO: 19)
Linker 1	SRGGGGSGGGGSGGGGSLEMA (SEQ ID NO: 104)
Linker 1 DNA	ctagaggtggtggtggtagcggcggcggcggctctggtggtggtggatcc (SEQ ID NO: 105
Ras antibody heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYTINWVRQAPGQGLE WMGGFIPISGTVNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVY YCARPLDWTEDIWGQGTLVTVSS

variable region	(SEQ ID NO: 17)
Linker 2 DNA	GGGGS (SEQ ID NO: 106)
DNA	ggcgggggaggatcc (SEQ ID NO: 107)
AntiCD3 scFv-his tag	DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGL EWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYMELSSLRSEDTAT YYCARYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD IVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPGKAPKRWIY DTSKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPL TFGGGTKVEIKHHHHHH* (SEQ ID NO: 108)
DNA	gacgtgcagctggtgcagagcggagctgaagtgaagaaacctggcgcctccgtgaaggtgtcct gcaaagctagcggctataccttcacccggtacaccatgcactgggtgcgccaggcacctggaca gggactggaatggatcggctacatcaaccctcccggggctacaccaactacgccgactctgtg aagggccggttcaccatcaccaccgataagtccaccagcaccgcttacatggaactgtcctccct gagatccgaggacaccgctacctactattgcgcccggtactacgacgaccactactgcctggact actggggacagggaaccacagtgaccgtgtcctctggcgagggcacctctactggatctggggg aagtggtggttctggcggcgctgacgacatcgtgctgacccagtctccagccaccctgtctctgag cccaggcgagagagctaccctgtcctgcagagcctcccagtccgtgtcctacatgaattggtatca gcagaagcctggcaaggcccctaagcggtggatctacgacacctccaaggtggcctctggcgtg ccagcccggttttccggatctggctctggcaccgactactccctgaccatcaacagcctggaagcc gaggacgctgccacctattactgccagcagtggtcctccaacccccctgacctttggaggcggcac caaggtggaaatcaagcaccaccatcatcaccactga (SEQ ID NO: 109)

EXAMPLES

Example 1: Epitope selection and peptide synthesis

[0067] For purposes of the present disclosure, K-Ras peptides are identified by amino acid position(s) relative to NCBI Reference Sequence NP_203524.1 of the NCBI protein database. Accordingly, the K-Ras peptides, LVVVGAGGV and KLVVVGAGGV correspond to amino acids 6-14 and 5-14, respectively, of the reference sequence.

[0068] In order to select HLA-A2-restricted epitopes derived from Ras codon 12-mutations with strong immunogenicity, the prediction scores of Ras mutation sequences were first screened using three online available databases (BIMAS, RANKPEP and SYFPEITHI). (Ras mutation sequences are described in Smith et al. *Oncogenic mutations in ras create HLA-A2.1 binding peptides but affect their extracellular antigen processing*. International Immunology Vol. 9(8), pp. 1085-1093, 1997).

[0069] Based on the predicted binding scores from all three databases, the following peptides were selected for testing to determine if the peptides were able to elicit epitope-specific T cell responses in HLA-A2-positive healthy donors.

Table 11

	SEQUENCE	
RAS10-WT	KLVVVGAGGV	SEQ ID NO: 110
RAS10-G12V	KLVVVGAVGV	SEQ ID NO: 111
RAS10-G12C	KLVVVGACGV	SEQ ID NO: 112
RAS10-G12D	KLVVVGADGV	SEQ ID NO: 113
RAS10-G12S	KLVVVGASGV	SEQ ID NO: 114

RAS9-WT	LVVVGAGGV	SEQ ID NO: 115
RAS9-G12V	LVVVGAVGV	SEQ ID NO: 116
RAS9-G12C	LVVVGACGV	SEQ ID NO: 117
RAS9-G12D	LVVVGADGV	SEQ ID NO: 118

[0070] All peptides were purchased and synthesized by Genemed Synthesis, Inc. (San Antonio, TX). Peptides were sterile with purity of 70% to 90%. The peptides were dissolved in DMSO and diluted in saline at 5 mg/mL and stored at -80 °C.

[0071] A human scFv antibody phage display library (10 x 10^{10} clones) constructed by Eureka Therapeutics was used for the selection of human mAbs specific to K-Ras G12V/HLA-A0201. In order to reduce the conformational change of MHC1 complex introduced by immobilizing the protein complex onto plastic surfaces, solution panning and cell panning were used in place of conventional plate panning. In solution panning, biotinylated antigens were first mixed with the human scFv phage library after extended washing with PBS buffer, and then antiben-scFv antibody phage complexes were pulled down by streptavidin-conjugated Dyna beads M-280 through a magnetic rack. The bound clones were then eluted and use to infect *E. coli* XL1-Blue. In cell panning, T2 cells loaded with Ras 10-G12V or Ras 9-G12V peptides were first mixed with the human scFv phage library. T2 cell is a TAP-deficient, HLA-A0201$^+$ lymphoblast cell line. To load peptide, T2 cells were pulsed with peptides (50 μg/ml) in serum free RPM!1640 medium, in the presence of 20 μg/ml β2 microglobulin overnight. After extended washing with PBS, peptide-loaded T2 cells with bound scFv antibody phage were spun down. The bound clones were then eluted and used to infect E. Coli XL1-Blue. The phage clones expressed in bacteria were then purified. The panning were performed for 3-4 rounds with either solution panning,

cell panning or a combination of solution and cell panning to enrich scFv phage clones that specifically bind to Ras10-G12V and/or Ras9-G12V/HLA-0201.

[0072] Table 12 is the summary of phage panning against K-Ras G12V/HLA-A0201. Eight independent pannings were carried out. Through FACS analysis, 122 positive clones were identified out of 436 clones screened. Out of 80 sequenced positive clones, 8 unique clones were found.

Table 12

	Beads solution panning	Cell panning	BBC panning	Total
Number of clones screened	62	312	62	436
Number of ELISA positive	4	93	25	122
Number sequenced	4	54	22	80
Number of unique clones	2	3	3	8

Example 2: T2 stabilization assay

[0073] The immunogenicity of MHC class I-restricted peptides requires the capacity to bind and stabilize MHC class I molecules on the live cell surface. Moreover, the computer prediction has only up to 70% accuracy; so the next step was to seek a direct measurement of the strength of the interaction between the peptides and the HLA-A0201 molecules using a conventional binding and stabilization assay that uses antigen-transporting-deficient (TAP2 negative) HLA-A0201 human T2 cells. T2 cells lack TAP function and consequently are defective in properly loading class I molecules with antigenic peptides generated in the cytosol. The association of exogenously added peptides with thermolabile, empty

HLA-A0201 molecules stabilizes them and results in an increase in the level of surface HLA-A0201 recognizable by a specific anti-HLA-A0201 mAb such as BB7.2.

[0074] The T2 binding assay showed that Ras 10-mer peptides wild type (WT), G12V, G12C, G12D increased the HLA-A2 expression on T2 cells, when used at 50μg/ml (Fig. **1A** and **B**) and the best peptide to stabilize HLA-A2 expression was RasG12V, followed by RasG12D, and to a lower extent with RasG12-C and wild type. Ras 9-mer peptides showed better HLA-A2 stabilizations at all concentrations used, but no significant difference was shown among the peptides (Fig. 1C and D).

Example 3: Mutant Ras peptide-induced T cell responses

[0075] After informed consent on Memorial Sloan-Kettering Cancer Center Institutional Review Board approved protocols, peripheral blood mononuclear cells (PBMCs) from HLA-typed healthy donors were obtained by Ficoll density centrifugation. T cell stimulation followed the protocol described previously (Dao T. et al. *Identification of a human cyclin D1-derived peptide that induces human cytotoxic CD4 cells*. Plos One Vol. 4(9) e6730, 2009). Peptide-specific T cell responses were measured by IFN-g ELISPOT assay (Dao, T, Science Tr Med 2013).

[0076] To expand the peptide-specific T cell precursors, three to five *in vitro* stimulations were performed and the specific T cell response was measured by IFN-g production, when challenged with individual peptide. Ras-G12V peptide stimulation induced strong T cell response against Ras10-G12V but showed no cross-reactivity to the peptides Ras10-WT, G12C and G12D (Fig.2A). Similarly, Ras 9-G12V also induced strong T cell response to itself, but not other peptides. Five stimulations of T cells enhanced the response and showed more IFN-g spots. Interestingly, Ras 9-G12D peptide also induced peptide-specific response to itself after 5 rounds of stimulation (Fig. 2B).

[0077] Based on T cell data, TCR-like mAbs specific for the Ras10-G12V and WT peptides in the context of HLA-A0201 molecule were generated.

Example 4: Biotinylated peptide/HLA-A0201 complex

[0078] Biotinylated peptide/HLA-A0201 complex monomers were prepared according to standard protocols (John D. Altman and Mark M. Davis Current Protocols in Immunology (2003) 17.3.1-17.3.33). In brief, DNA of full-length human β2m was synthesized by Genewiz and cloned into vector pET-27b. The BirA substrate peptide (BSP) was added to the C-terminus of HLA A0201 extracellular domain (ECD). DNA of HLA-A0201 ECD-BSP was synthesized by Genewiz and cloned into vector pET-27b. The vectors expressing human β2m and HLA-A0201 ECD-BSP were transformed into E.Coli BL21 separately, and isolated as inclusion bodies from bacterial culture. Peptide ligands Ras10-G12V and Ras10-WT were refolded with human β2m and HLA A0201 ECD-BSP to form Ras-G12V/HLA A0201 and Ras10-WT/HLA A0201 complex monomers. Folded peptide/HLA A0201 monomers were concentrated by ultrafiltration and further purified through size-exclusion chromatography (**Figure 8**). HiPrep 26/60 Sephacryl S-300 HR was equilibrated with Hyclone Dulbecco's Phosphate Buffered Saline solution (Thermo Scientific, Cat No. SH3002802) for 1.5 column volumes. The unpurified sample was loaded and eluted for 1 column volume. The first peak, consisting of misfolded aggregates, eluted at approximately 110.63 mL after loading. The peak corresponding to the properly folded MHC complex was observed at 216.18 mL. Lastly, the peak consisting of free B2M was observed at 275.12 mL.

[0079] Purified peptide/HLA A0201 monomers were visualized through SDS-PAGE(figure E-2). In brief, 4μg of the protein was mixed with 2.5μL of the NuPAGE LDS Sample Buffer (Life Technologies, NP0008) and filled up to 10μL with deionized water. The sample was heated at 70°C for 10 minutes, and then loaded onto the gel. Gel electrophoresis was performed at 180V for 1 hour. Two major bands were observed on the gel. The 30KD band was HLA A0201, and the 10KD band was B2M.

[0080] Peptide/HLA A0201 monomers were biotinylated via BirA-mediated enzymatic reaction and subsequently purified by high-resolution anion-exchange chromatography. Biotinylated peptide/HLA A0201 monomers were stored in PBS at -80°C.

Example 5: Screening of phage ScFv specific for K-Ras G12V/HLA-A0201 complex

[0081] Positive phage clones were determined by flow cytometry using Ras G12V bound live T2 cells. In brief, the cells were first stained with purified scFv phage clones, and followed by staining with a mouse anti-M13 mAb, and finally the R-PE conjugated horse anti-mouse IgG from Vector Labs. Each step of the staining was done between 30 -60 minutes on ice and the cells were washed twice between each step of the staining. **Figure 8** is an example of K-Ras G12V/HLA A0201 specific phage clone binding to peptide-loaded T2 cells. Clone#2 binds to K-Ras10 G12V (T2-014A2mut, light green line) and K-Ras9 G12V/HLA A0201(T2-014A1mut, blue line) specifically, while doesn't recognize empty T2 cells (T2-B2M, dark green line), or K-Ras WT peptide/HLA A0201 complexes (T2-0142WT, orange line and T2-0141WT, red line).

Example 6: Engineering full length mAb using the selected scFv fragments

[0082] Full-length human IgG1 of the selected phage clones were produced in HEK293 and Chinese hamster ovary (CHO) cell lines, as described (Tomimatsu K, Matsumoto S, Yamashita M, Teruya K, Katakura Y, Kabayama S & Shirahat S. Production of human monoclonal antibodies against FceRIa by a method combining in vitro immunization with phage display. Biosci Biotechnol Biochem 2009; 73 (7) 1465-1469.). In brief, antibody variable regions were subcloned into mammalian expression vectors, with matching human lambda or kappa light chain constant region and human IgG1 constant region sequences. Molecular weight of the purified full length IgG antibodies were measured under both reducing and non-reducing conditions by electrophoresis. Examples of electrophoresis (SDS-PAGE) are shown in figure E-4. Lane 1, clone #2, reducing condition, lane 2,

clone #4, reducing condition, lane 3, clone #7, reducing condition, lane 6-7, non-reducing condition, clone #2, #4 and #7, respectively.

Example 7: Engineering bispecific T cell engager (T-BiTE)

[0083] The BiTE antibodies are single-chain bispecific antibodies comprising K-Ras G12V/HLA A0201 specific antibodies in the scFv format, at the N-terminal end and an anti-human CD3ε scFv mouse monoclonal antibody at the C-terminal end (Brischwein, K. et al. MT110: A novel bispecific single-chain antibody construct with high efficacy in eradicating established tumors. Molecular Immunology 43, 1129-1143 (2006)). The DNA fragments coding for the Ras scFv antibody and the anti-human CD3ε scFv antibody were synthesized by Genewiz and subcloned into Eureka's mammalian expression vector pGSN-Hyg using standard DNA technology. A hexhistamine tag is inserted downstream of the Ras BiTE antibodies at the C-terminal end for antibody purification and detection. Chinese hamster ovary (CHO) cells were transfected with the Ras BiTEs expression vector and stable expression was achieved by standard drug selection with methionine sulfoximine (MSX), a glutamine synthetase(GS)-based method (reference 2). CHO cell supernatants containing secreted Ras BiTE molecules were collected. Ras BiTE was purified using HisTrap HP column (GE healthcare) by FPLC AKTA system. Briefly, CHO cell culture was clarified and loaded to the column with low imidazole concentration (20 mM), and then an isocratic high imidazole concentration elution buffer (500 mM) was used to elute the bound Ras BiTE proteins. Molecular weight of the purified Ras BiTEs antibodies were measured under non-reducing conditions by electrophoresis (figure E-5). Lane 1-4, reducing condition, clone #2, #4,901 control hIgG1 antibody and #7, respectively.

Example 8: Characterization of full-length human IgG1 specific for Ras 10-G12V/HLA-A0201 complex

[0084] To determine whether mAb clones #2, 4 and 7 bind to cell surface peptide/HLA-A0201 complexes on live cells, flow cytometry was used to study

HLA-A0201 positive, TAP-deficient T2 cells loaded with peptides. T2 cells were incubated with the peptides (50 μg/ml) and β2 microglobulin (β2M) at 10 μg/ml in a serum-free medium over night, and the cells were harvested and washed. The cells were stained with mAbs or isotype control human IgG1, for 30 minutes and washed, followed by staining with a secondary goat $(Fab)_2$ anti-human IgG1 mAb conjugated to FITC. The mAbs were also conjugated to an allophycocyanin (APC) fluorophore to perform direct staining.

[0085] Fig. 3A showed that mAb#2 specifically bound to T2 cells pulsed with Ras10-G12V peptide and also to a lesser degree to Ras-G12C peptide, but not to Ras WT, Ras10-G12D or T2 alone, or control HLA-A0201-binding peptide EW (Dao, T. et al., *Identification of a human cyclin D1-derived peptide that induces human cytotoxic CD4 cells.* Plos One vol. 4(9) e6730, 2009. MAb #4 and 7 did not show significant binding to T2 pulsed with those Ras10 peptides.

[0086] The results were confirmed by measuring the binding by APC-conjugated mAbs in direct staining. Since APC conjugation greatly amplified the binding signal, #7 mAb was seen binding to T2 cells pulsed with Ras10-G12V and also to Ras10-WT peptide, to a lesser degree. However, mAb titration showed that mAb#2 has the strongest affinity for the Ras10-G12V/HLA-A0201 complex. In this experiment, the binding of the mAbs to a potentially cross-reactive normal peptide CT (not from Ras) was also tested. Only mAb#7 bound to it, as well as Ras WT, demonstrating that mAb#7 is a more promiscuous mAb than #2 or #4 (Fig. 3B upper). The binding specificity was not due to differences in the peptide binding to HLA-A2 molecule, as HLA-A2 expression level by peptide CT was similar to other peptides used in the same condition (lower panel).

Binding affinity of Ras human IgG1 mAbs

[0087] The binding affinity of Ras hIgG1 mAbs towards peptide-loaded MHC complex were determined using ForteBio Octet QK. Data are shown in Table 13. 5 μg/mL of biotinylated Ras peptide/HLA-A0201 complex was loaded onto the Streptavidin biosensor. The excess antigen was washed off first. The Ras mAbs

were then individually tested at 10 µg/mL for association and dissociation steps. Binding parameters were calculated using 1:1 binding site model, partial fit. Ras antibody #2 and #4 specifically recognize Ras G12V mutant peptide/HLA-A0201 complex, while Ras antibody #7 recognize both mutant and wild type Ras peptide in the context of HLA-A0201 molecule.

Table 13

Binding affinity measurement of Ras hIgG1 antibodies

Protein	Antigen	k_d [1/s]	Error in k_d	k_a [1/Ms]	k_D [nM]
#2	Ras G12V/HLA-A0201	7.17E-4	1.42E-4	8.07E4	8.88
#4	Ras G12V/HLA-A0201	3.63E-3	5.11E-5	3.96E5	9.19
#7	Ras G12V/HLA-A0201	7.46E-4	8.11E-5	8.50E4	8.77
#2	Ras wt/HLA-A0201	--	--	--	--
#4	Ras wt/HLA-A0201	--	--	--	--
#7	Ras wt/HLA-A0201	1.53E-3	3.35E-5	2.34E4	65.1

Example 9: Peptide epitope mapping

[0088] To investigate with more precision the epitope for mAb recognition, Ras10-G12V peptides were substituted with alanine at Ras protein positions 8, 9, 10, 12, and 13 and pulsed onto T2 cells and were tested for mAb binding. Alanine substitution at position 12 completely abrogated the binding of #2 mAb. Alanine substitution at position 9, 10 and 13 also reduced the binding of the #2 mAb. Mabs #4 and 7 showed a reduction in binding similar to #2 mAb, however, binding

of #7 mAb to the peptide containing an alanine substitution at position 12 was still detectable (Fig. 3C). Since Ras protein position 11 of the Ras10-G12V peptide was already alanine, this position was next substituted with glycine (Ras10-A11G). Binding of the #2 mAb to Ras10-G12S peptide was also tested. Mab#2 showed weak but positive binding to T2 pulsed with Ras10-G12S and reduced binding to Ras10-A11G peptide. The loss of binding was not due to the reduction of the peptide binding affinity to the HLA-A2 molecule, as Ras10-A11G showed the strongest binding in T2 stabilization assays (Fig. 3D). These results show that the valine at Ras protein position 12 of the Ras10-G12V peptide is important for Ras mAb recognition and mAb#2 is highly specific for Ras10-G12V mutation. mAb#2 binding specificity using alanine substituted peptides and possible cross-reactive non-Ras peptides CT and MTH was further confirmed. No binding for either control CT or MTH peptide was seen (Fig. 3E). Flow cytometry data shows the binding of mAb #2 for various peptides and to HLA-A2 (Fig. 3F). Alanine substitution at Ras position 8 showed toxicity to T2 cells as shown by reduced HLA-A2 expression (Fig. 3G) and no reliable data were generated.

[0089] In addition to 10-mer peptides, binding of the mAbs to Ras 9-mer G12V mutation-derived peptides was tested. mAb #2 bound to Ras 9-mer G12V. mAbs #4 and 7 did not bind to either wild type or G12V mutant peptide, as shown by both indirect (Fig. 4A) and direct staining (Fig. 4B) and T2 confirmation of peptide binding (Fig. 4B lower.)

Example 10: Characterization of T-BiTE constructs

[0090] mAb killing functions can be enhanced in multiple ways. As a strategy to bring T cell cytotoxicity to the targets, bi-specific T cell engager (T-BiTE) constructs of the mAb were also generated and binding to the target Ras peptides was tested on T2 cells and binding to resting purified T cells (effectors) were tested by T-BiTE followed by a secondary mAb, mouse anti-myc conjugated to FITC, as BiTE constructs were myc-tagged.

[0091] BiTEs retained their binding specificity and affinity, showing the best binding by #2 to Ras10-G12V peptide, followed by #7 and #4 mAbs. mAb#7 also showed binding to WT peptide (Fig. 5A). mAb #4 showed the strongest binding to CD3 T cells, followed by isotype control BITE, mAb #2 and #7 (Fig. 5B and C).

Example 11: ADCC-mediated killing

[0092] ADCC is considered to be one of the major effector mechanisms of therapeutic mAb in humans. Therefore, we next tested if the mAbs were able to mediate ADCC, using freshly isolated human PBMCs from healthy donor, in a standard 51Cr-release assay. (Fig 6) Both mAbs #2 and 7 were able to kill T2 cells pulsed with Ras10-G12V peptides at the indicated concentrations in the similar degree. No killing was seen against unpulsed T2 cells. mAb #7 also killed the T2 pulsed with Ras10-WT peptide, but no killing was seen by #2 mAb. These results were consistent with the binding data and further demonstrated that the #2 mAb is specific for the mutant Ras10-G12V peptide/HLA-A2 complex.

Example 12: Killing by T-BITE's

[0093] Next, whether T-BiTEs of Ras mAbs were able to mediate T cell killing against targets was tested. CD3 T cells were purified and cytotoxicity was measured by standard ^{51}Cr-release assay, in the presence or absence of T-BiTEs #2, #7 and isotype-derived control BiTE. No killing was seen against control unpulsed T2 cells or cells pulsed with control peptide EW. Both #2 and #7 BiTEs were able to mediate T cell killing against T2 cells pulsed with Ras10-G12V peptide at the indicated concentrations of BiTEs (Fig. 7). BiTE#2 did not kill cells pulsed with Ras WT showing specificity of killing for the cells with the Ras mutant on the surface. BiTE#7 did not show killing against Ras10-WT pulsed T2 cells, which might be due to lower binding affinity to the WT/HLA-A2 complex, compared to Ras10-G12V/HLA-A2 complex.

EQUIVALENTS

[0094] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the disclosure. The foregoing description and Examples detail certain embodiments of the disclosure and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear, the disclosure may be practiced in many ways and the disclosure should be construed in accordance with the appended claims and any equivalents thereof.

References

1. Smith et al. *Oncogenic mutations in ras create HLA-A2.1 binding peptides but affect their extracellular antigen processing*. International Immunology Vol. 9(8), pp. 1085-1093, 1997.

2. Dao T. et al. *Identification of a human cyclin D1-derived peptide that induces human cytotoxic CD4 cells*. Plos One Vol. 4(9) e6730, 2009.

3. Cuesta et al., Multivalent antibodies: when design surpasses evolution. *Trends in Biotechnology* 28:355-362 2010.

CLAIMS

1. An antigen-binding protein or antigen-binding fragment/portion thereof that binds specifically to an epitope within a variant of wild type Ras peptide, KLVVVGAGGV (SEQ ID NO: 110, amino acids 5-14) or LVVVGAGGV (SEQ ID NO: 115, amino acids 6-14) with a single amino acid substitution at position 12.

2. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 1, wherein the variant Ras peptide with a single amino acid substitution at position 12 is selected from the group consisting of KLVVVGAVGV (SEQ ID NO: 111) KLVVVGASGV (SEQ ID NO: 114) and LVVVGAVGV (SEQ ID NO: 116).

3. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 1 or claim 2 comprising at least one of:

(A) an antigen binding region having the amino acid sequence of one of SEQ ID NOS: 81, 82, 83, 84, 85, 86, 87, or 88;

(B) an antigen binding region comprising a V_H and a V_L respectively, with amino acid sequences selected from SEQ ID NOs: (i) 7 and 9; (ii) 17 and 19; (iii) 27 and 29; (iv) 37 and 39; (v) 47 and 49; (vi) 57 and 59; (vii) 67 and 69; or (viii) 77 and 79; or

(C) an antigen binding region comprising:

(i) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively;

(ii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 14,

SEQ ID NO: 15, and SEQ ID NO: 16, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13, respectively;

(iii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively;

(iv) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33, respectively;

(v) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, respectively;

(vi) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 51, SEQ ID NO: 52, and SEQ ID NO: 53, respectively;

(vii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 64, SEQ ID NO: 65, and SEQ ID NO: 66, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63, respectively;

(viii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 74, SEQ ID NO: 75, and SEQ ID NO: 76, respectively and (b) heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73, respectively; or

(D) an antigen binding region comprising a fragment or variant of at least one of the amino acid sequences recited in any of (A), (B), and (C) (i) to (C) (vii).

4. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 1, 2 or 3, wherein the antigen-binding protein is an antibody.

5. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 4, wherein the antibody comprises a human variable region framework region.

6. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 4, wherein the antibody is fully human.

7. The antigen-binding protein or antigen-binding fragment/portion thereof of any preceding claim, wherein the antigen-binding protein or antigen-binding fragment/portion thereof specifically binds to a Ras peptide bound to HLA-A2.

8. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 7, wherein the HLA-A2 is HLA-A0201.

9. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 7 or 8, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 111.

10. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 7 or 8, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 116.

11. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 7 or 8, wherein said antigen-binding protein or antigen-binding fragment/portion thereof binds to a peptide with the amino acid sequence of SEQ ID NO: 111 with an affinity in the range of 8.5 to 10 nM.

12. The antigen-binding protein or antigen-binding fragment/portion thereof of any one of claims 4-6, wherein the antibody is a full-length antibody, an intact antibody, a Fab fragment, a $F(ab')_2$ fragment, or a single chain variable fragment (scFv).

13. The antigen-binding protein or antigen-binding fragment/portion thereof of any preceding claim, wherein the antigen-binding protein is a chimeric antigen receptor (CAR).

14. The antigen-binding protein or antigen-binding fragment/portion thereof of any preceding claim, conjugated to a therapeutic agent.

15. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 14, wherein said therapeutic agent is a drug, toxin, radioisotope, protein, or peptide.

16. A nucleic acid that encodes an antigen-binding protein of any preceding claim.

17. A nucleic acid comprising:

(A) first and second nucleotide sequences selected from the group consisting of SEQ ID NOS: 8 and 10; 18 and 20; 28 and 30; 39 and 40; 49 and 50; 59 and 60; 68 and 70; 78 and 80, and fragments or variants thereof; or

(B) a nucleotide sequence selected from the group consisting of SEQ ID NOS: 91, 92, 93, 94, 95, 96, 97, 98, and fragments or variants thereof.

18. The nucleic acid of claim 17, wherein said first nucleotide sequence encodes a V_H region and said second nucleotide sequence encodes a V_L.

19. The nucleic acid of claim 17 or 18, wherein said nucleotide sequence encodes a scFv.

20. A fusion protein comprising an antigen-binding protein or antigen-binding fragment/portion thereof of any of claims 1-15.

21. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 12, wherein the antibody is a single-chain variable fragment (scFv) comprising the amino acid sequence of an antigen-binding protein selected from the group consisting of SEQ ID NOS: 81, 82, 83, 84, 85, 86, 87, 88, or a fragment or variant thereof.

22. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 12 or 21, wherein the antibody is a scFv comprising a V_H and a V_L linked by an amino acid spacer, wherein the V_H and V_L respectively comprise an amino acid sequence selected from the group consisting of SEQ ID NOS: (i) 7 and 9; (ii) 17 and 19; (iii) 27 and 29; (iv) 37 and 39; (v) 47 and 49; (vi) 57 and 59; (vii) 67 and 69; (viii) 77 and 79, or a fragment or variant thereof.

23. The antigen-binding protein or antigen-binding fragment/portion thereof of claim 12 or 21, wherein the antibody is a scFv comprising:

(i) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively;

(ii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13, respectively;

(iii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively;

(iv) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33, respectively;

(v) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, respectively;

(vi) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 51, SEQ ID NO: 52, and SEQ ID NO: 53, respectively;

(vii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 64, SEQ ID NO: 65, and SEQ ID NO: 66, respectively and heavy chain CDRs (HC-CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63, respectively;

(viii) light chain complementarity determining regions (LC-CDRs) LC-CDR1, LC-CDR2 and LC-CDR3, with amino acid sequences SEQ ID NO: 74, SEQ ID NO: 75, and SEQ ID NO: 76, respectively and heavy chain CDRs (HC-

CDRs) HC-CDR1, HC-CDR2 and HC-CDR3, with amino acid sequences SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73, respectively; or

(ix) LC-CDR1, LC-CDR2, LC-CDR3, HC-CDR1, HC-CDR2, and HC-CDR3 comprising fragments or variants of at least one set of the amino acid sequences recited in any of (i) to (viii).

24. An immunoconjugate comprising a first component which is an antigen-binding protein or antigen-binding fragment/portion thereof of any one of claims 1-15 and 20-23.

25. The immunoconjugate of claim 24, comprising a second component having a second amino acid sequence.

26. The immunoconjugate according to claim 24 or 25, further comprising a cytotoxin or radionuclide.

27. The immunoconjugate of claim 25, wherein the second component is a binding protein or antibody having a binding specificity for a target that is different from the binding specificity of the first component.

28. A bispecific antibody comprising first and second antigen-binding portions wherein said first antigen-binding portion is an antigen-binding protein of antigen-binding fragment/portion thereof of any one of claims 1-15 and 20-23.

29. The bispecific antibody of claim 28, wherein the bispecific antibody comprises a second antigen-binding portion that has a binding specificity for a target that is different from the binding specificity of the first antigen binding portion.

30. A pharmaceutical composition comprising any of the antigen-binding proteins or antigen-binding portions thereof of claims 1-15 and 20-23 and a pharmaceutically acceptable carrier.

31. A method for selectively killing a human cancer cell that displays a RasG12V/MHC epitope on its surface comprising contacting said cell with an antigen-binding protein or antigen-binding fragment /portion thereof comprising:

(A) an antigen binding region having the amino acid sequence of one of SEQ ID NOS: 81, 82, 83, 84, 85, 86, 87, 88, fragments or variants thereof, or a combination thereof; or

(B) an antigen binding region comprising a V_H and a V_L respectively, with amino acid sequences selected from SEQ ID NOs: (i) 7 and 9; (ii) 17 and 19; (iii) 27 and 29; (iv) 37 and 39; (v) 47 and 49; (vi) 57 and 59; vii) 67 and 69; (viii) 77 and 79; (ix) 87 and 89, fragments or variants thereof, or a combination thereof.

32. A vector comprising the nucleic acid of any one of claims 16-19.

33. A cell comprising the nucleic acid of any one of claims 16-19.

34. A pharmaceutical composition comprising a nucleic acid of any one of claims 16-19.

35. A bispecific antibody comprising a least one amino acid sequence of an antigen-binding protein or antigen-binding fragment/portion thereof of claim 3.

36. The bispecific antibody of claim 35, wherein said bispecific antibody has the amino acid sequence of SEQ ID NO: 103.

37. A pharmaceutical composition comprising the bispecific antibody of claim 35 or 36.

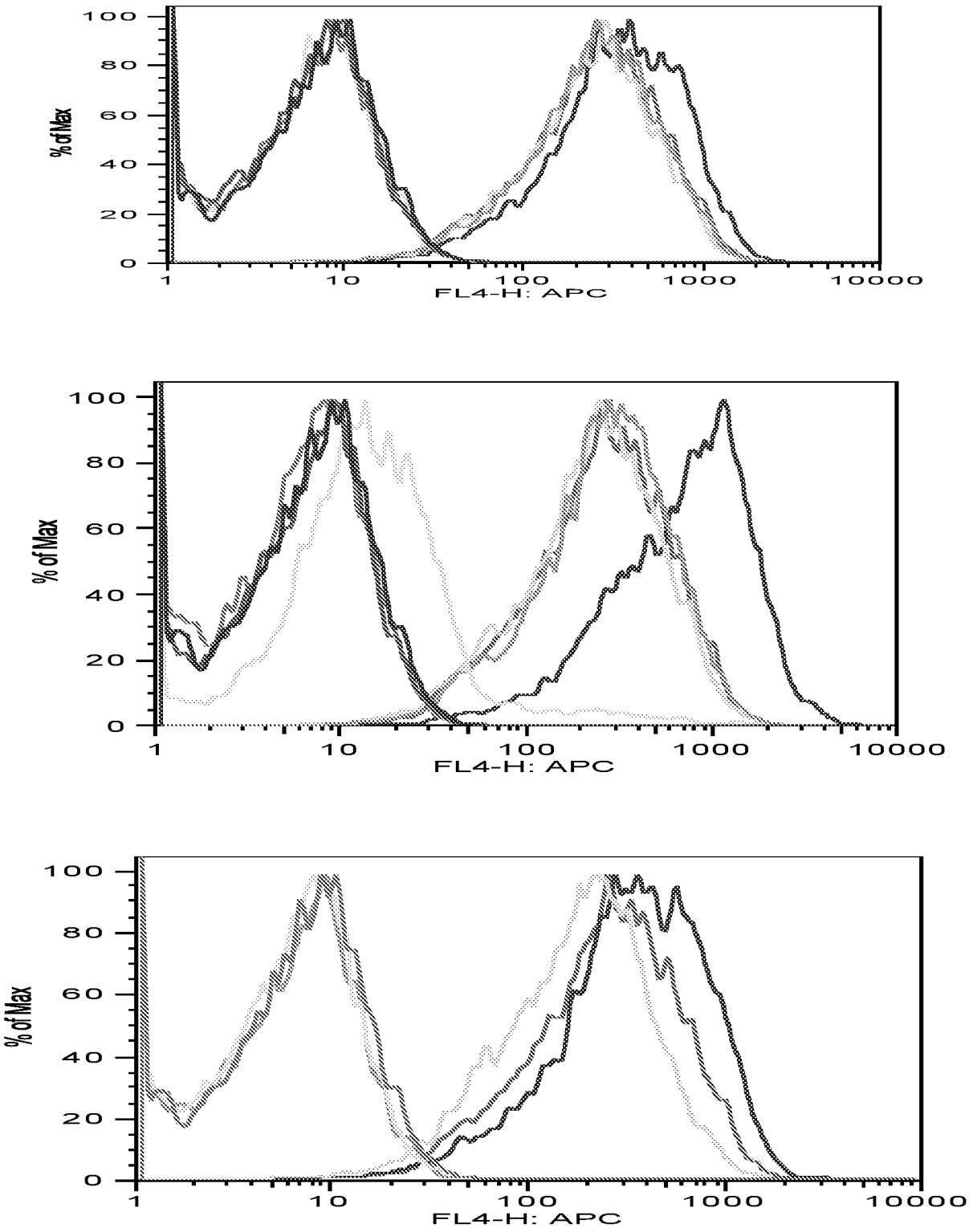


FIGURE 1A

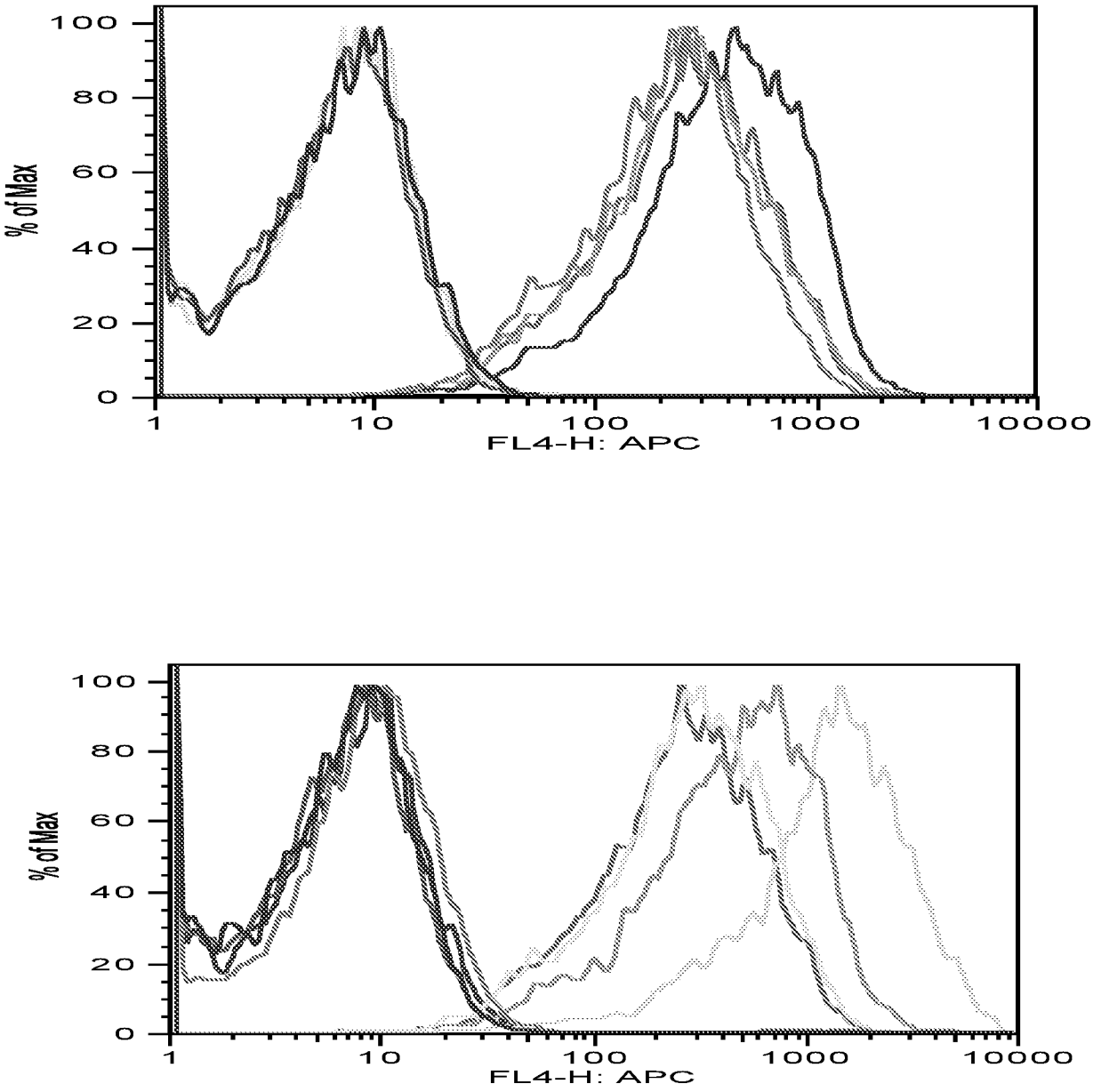

100
80
60
40
20
0
% of Max
1
10
100
1000
10000
FL4-H: APC
100
80
60
40
20
0
% of Max
1
10
100
1000
10000
FL4-H: APC

FIGURE 1B

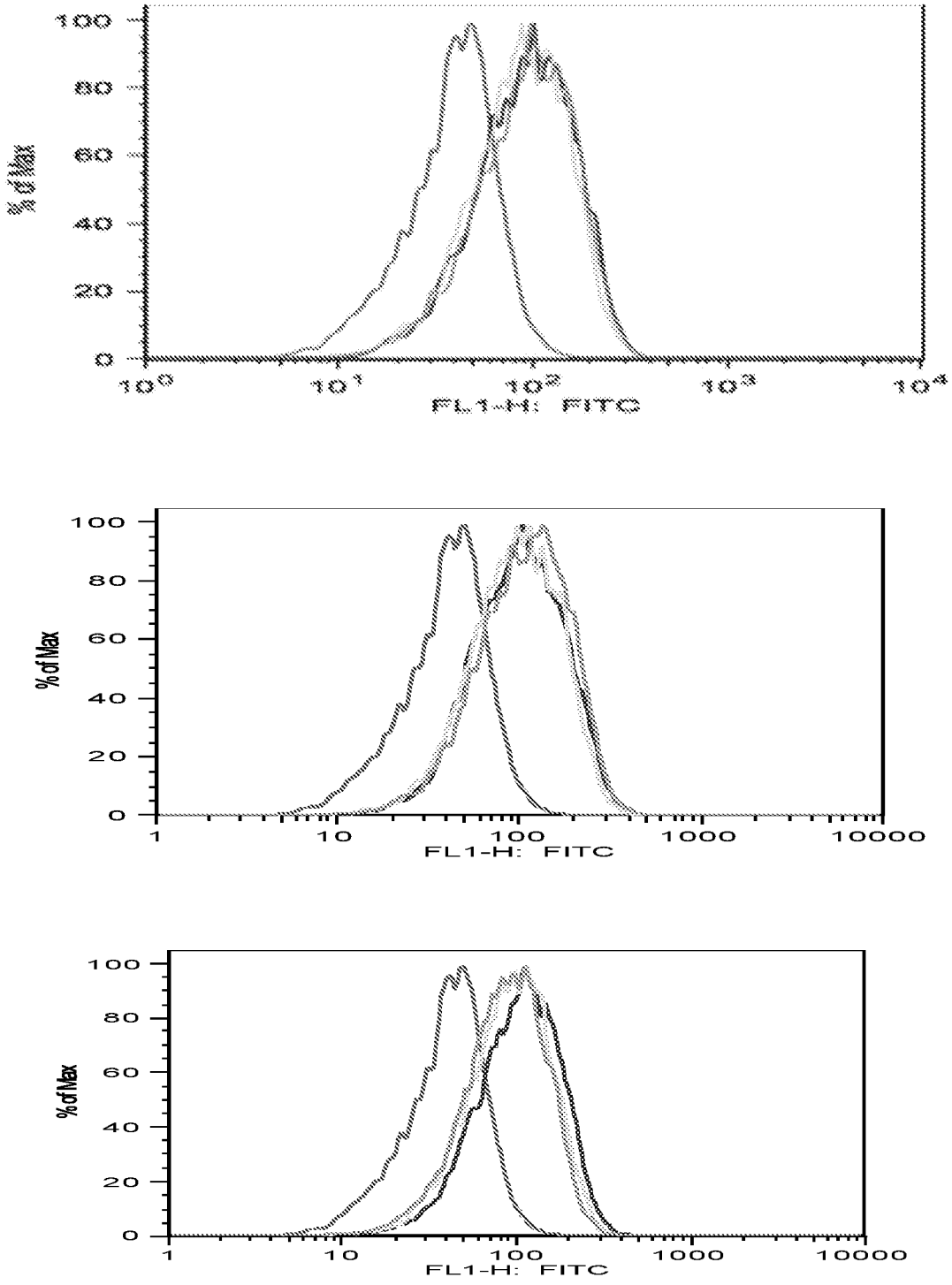

% of Max
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
FL1-H: FITC
% of Max
100
80
60
40
20
0
1
10
100
1000
10000
FL1-H: FITC
% of Max
100
80
60
40
20
0
1
10
100
1000
10000
FL1-H: FITC

FIGURE 1C

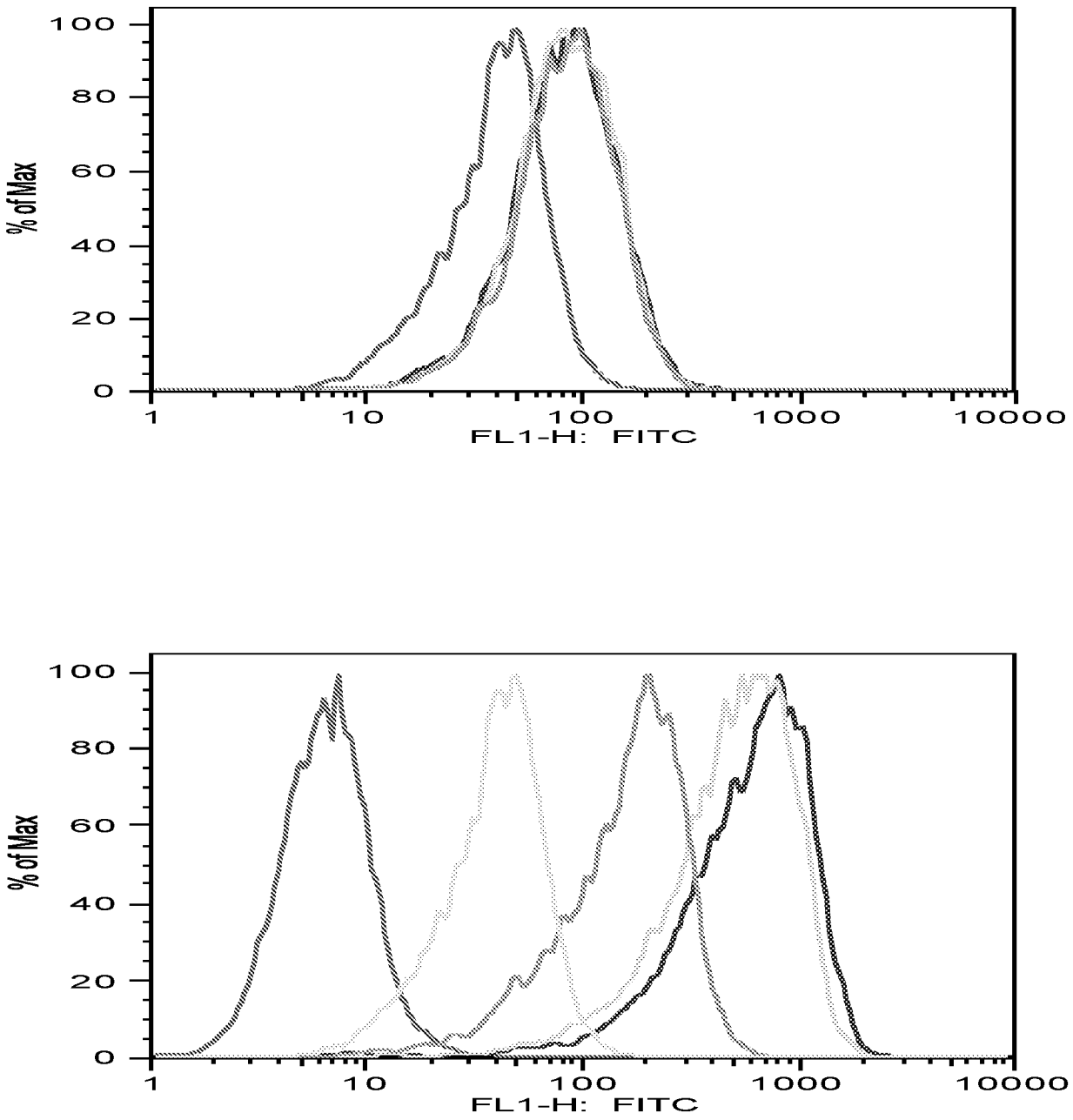

100
80
60
40
20
0
% of Max
1
10
100
1000
10000
FL1-H: FITC
100
80
60
40
20
0
% of Max
1
10
100
1000
10000
FL1-H: FITC

FIGURE 1D

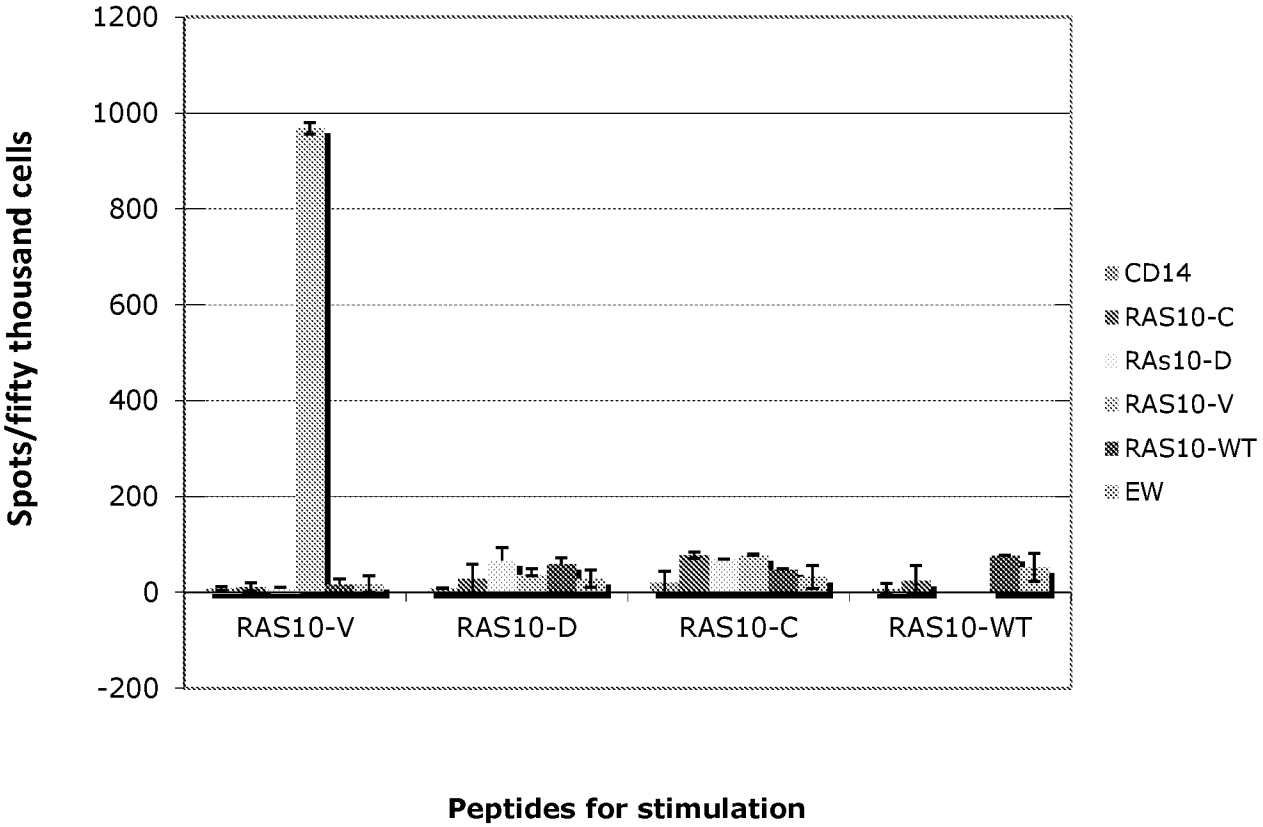
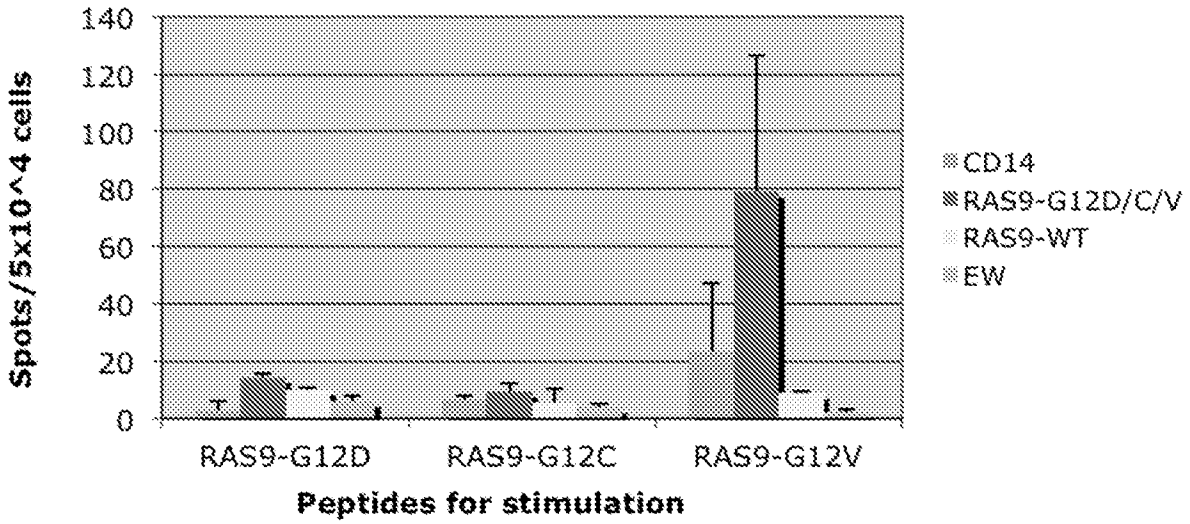

1200
1000
800
600
400
200
0
-200
Spots/fifty thousand cells
RAS10-V
RAS10-D
RAS10-C
RAS10-WT
CD14
RAS10-C
RAs10-D
RAS10-V
RAS10-WT
EW
Peptides for stimulation

FIGURE 2A


140
120
100
80
60
40
20
0
Spots/5x10^4 cells
RAS9-G12D
RAS9-G12C
RAS9-G12V
Peptides for stimulation
CD14
RAS9-G12D/C/V
RAS9-WT
EW

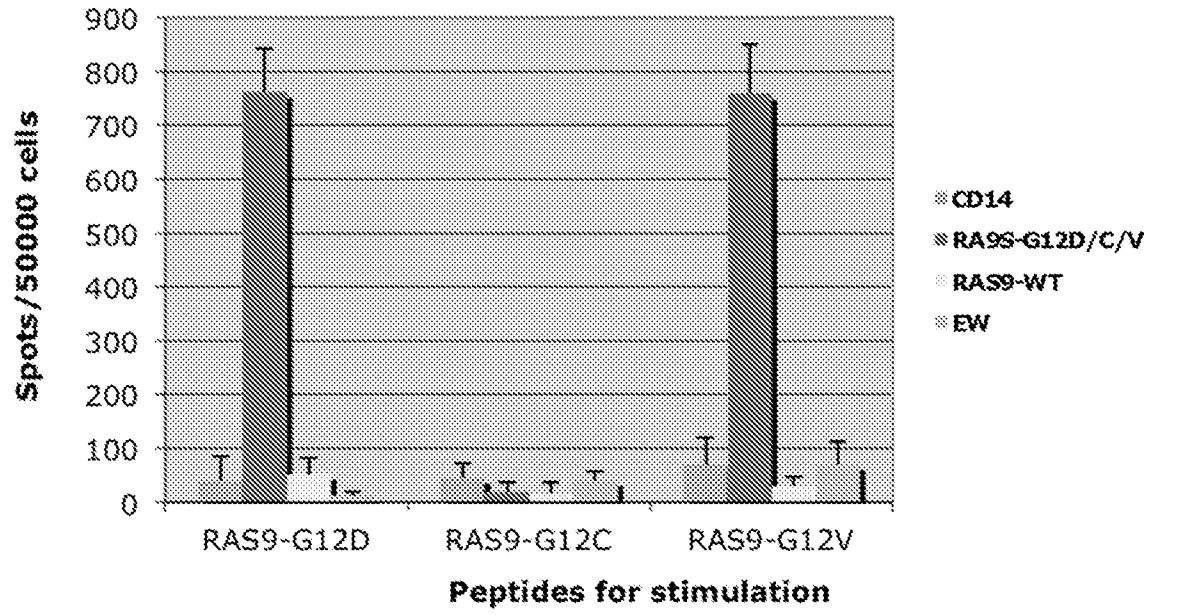

900
800
700
600
500
400
300
200
100
0
Spots/50000 cells
RAS9-G12D
RAS9-G12C
RAS9-G12V
Peptides for stimulation
CD14
RA9S-G12D/C/V
RAS9-WT
EW

FIGURE 2B

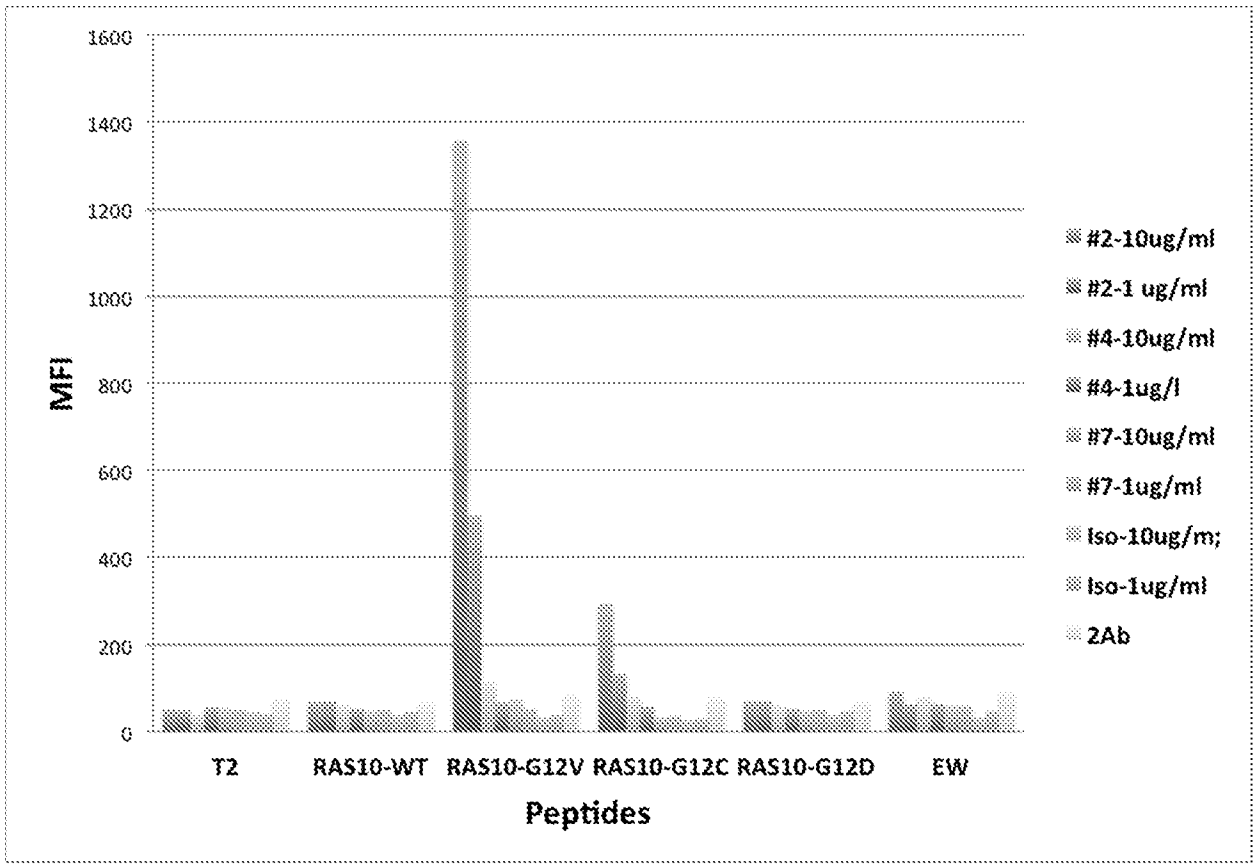
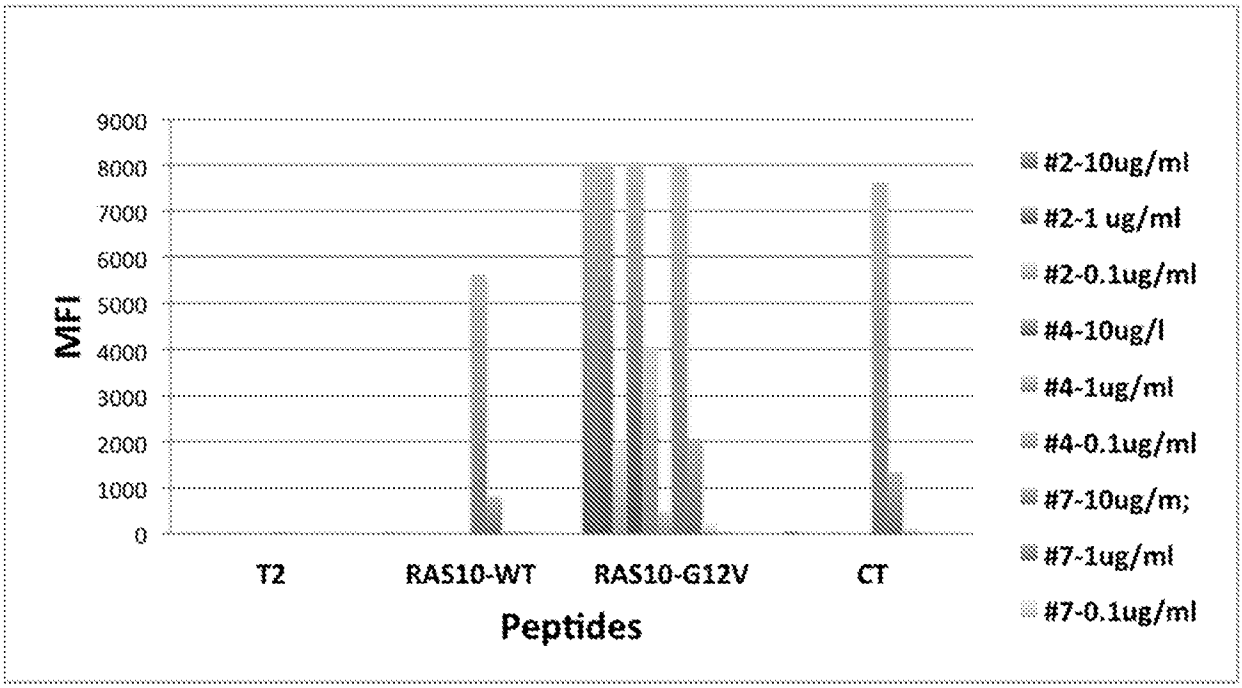

1600
1400
1200
1000
800
600
400
200
0
MFI
T2
RAS10-WT
RAS10-G12V
RAS10-G12C
RAS10-G12D
EW
Peptides
#2-10ug/ml
#2-1 ug/ml
#4-10ug/ml
#4-1ug/l
#7-10ug/ml
#7-1ug/ml
Iso-10ug/m;
Iso-1ug/ml
2Ab

FIGURE 3A


9000
8000
7000
6000
5000
4000
3000
2000
1000
0
MFI
T2
RAS10-WT
RAS10-G12V
CT
Peptides
#2-10ug/ml
#2-1 ug/ml
#2-0.1ug/ml
#4-10ug/l
#4-1ug/ml
#4-0.1ug/ml
#7-10ug/m;
#7-1ug/ml
#7-0.1ug/ml

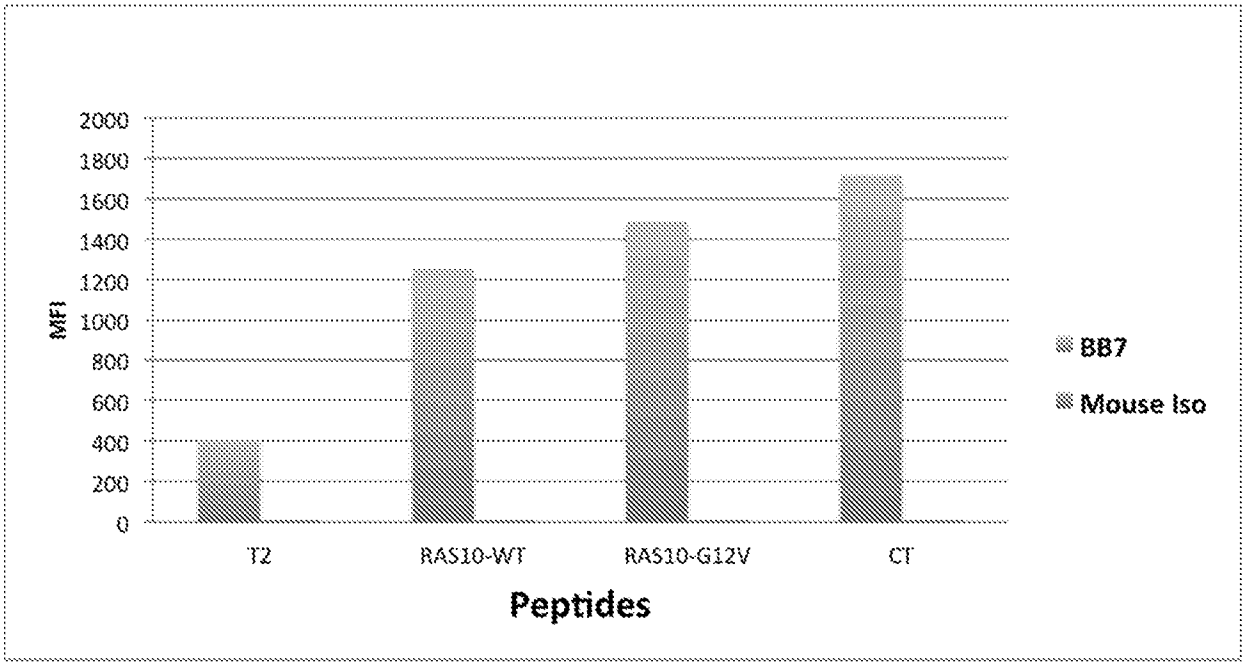

2000
1800
1600
1400
1200
1000
800
600
400
200
0
MFI
T2
RAS10-WT
RAS10-G12V
CT
Peptides
BB7
Mouse Iso

FIGURE 3B

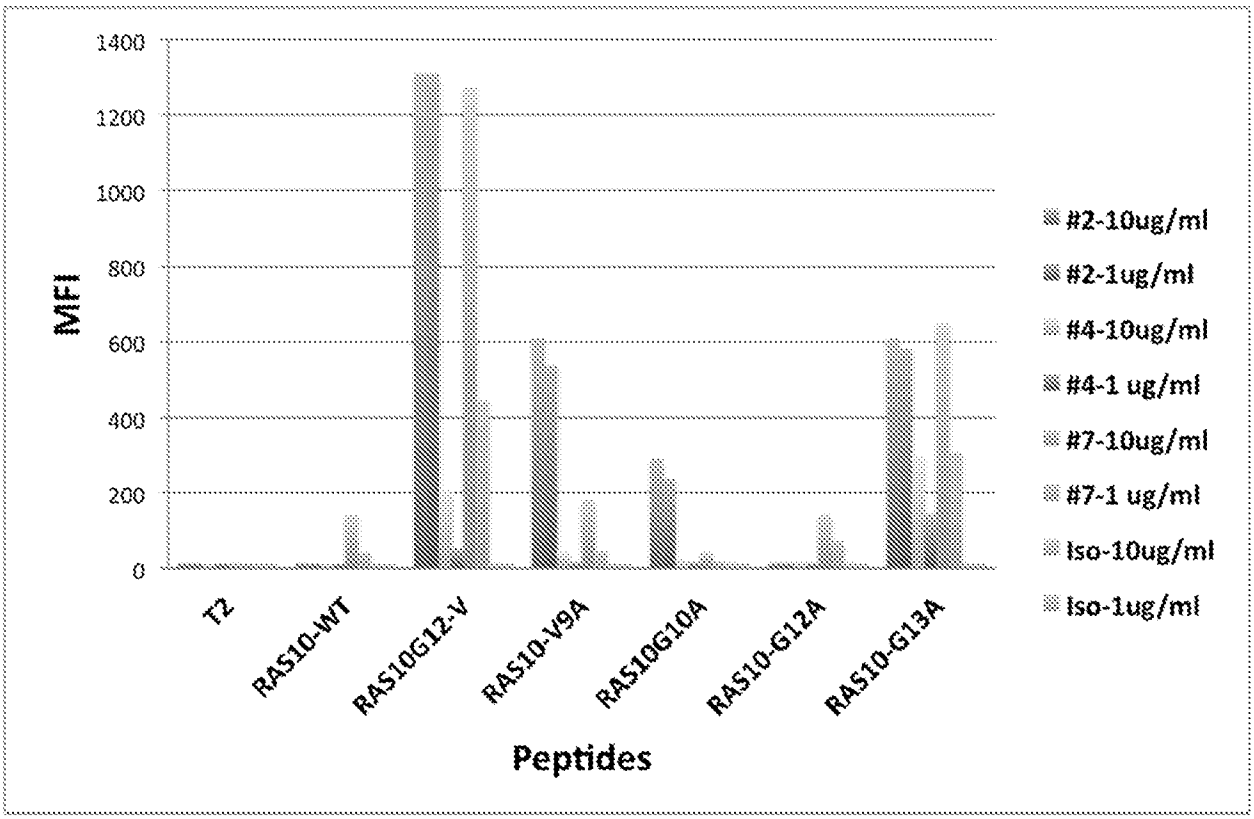
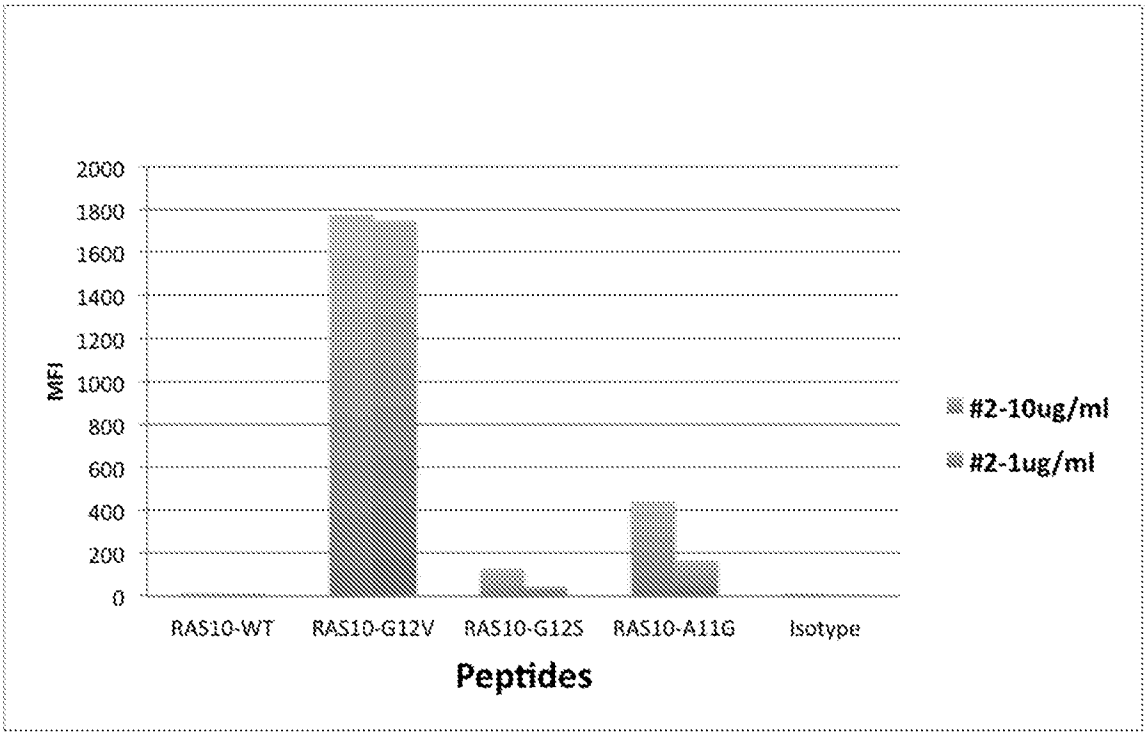

1400
1200
1000
800
600
400
200
0
MFI
T2
RAS10-WT
RAS10G12-V
RAS10-V9A
RAS10G10A
RAS10-G12A
RAS10-G13A
Peptides
#2-10ug/ml
#2-1ug/ml
#4-10ug/ml
#4-1 ug/ml
#7-10ug/ml
#7-1 ug/ml
Iso-10ug/ml
Iso-1ug/ml

FIGURE 3C


2000
1800
1600
1400
1200
1000
800
600
400
200
0
MFI
RAS10-WT
RAS10-G12V
RAS10-G12S
RAS10-A11G
Isotype
Peptides
#2-10ug/ml
#2-1ug/ml

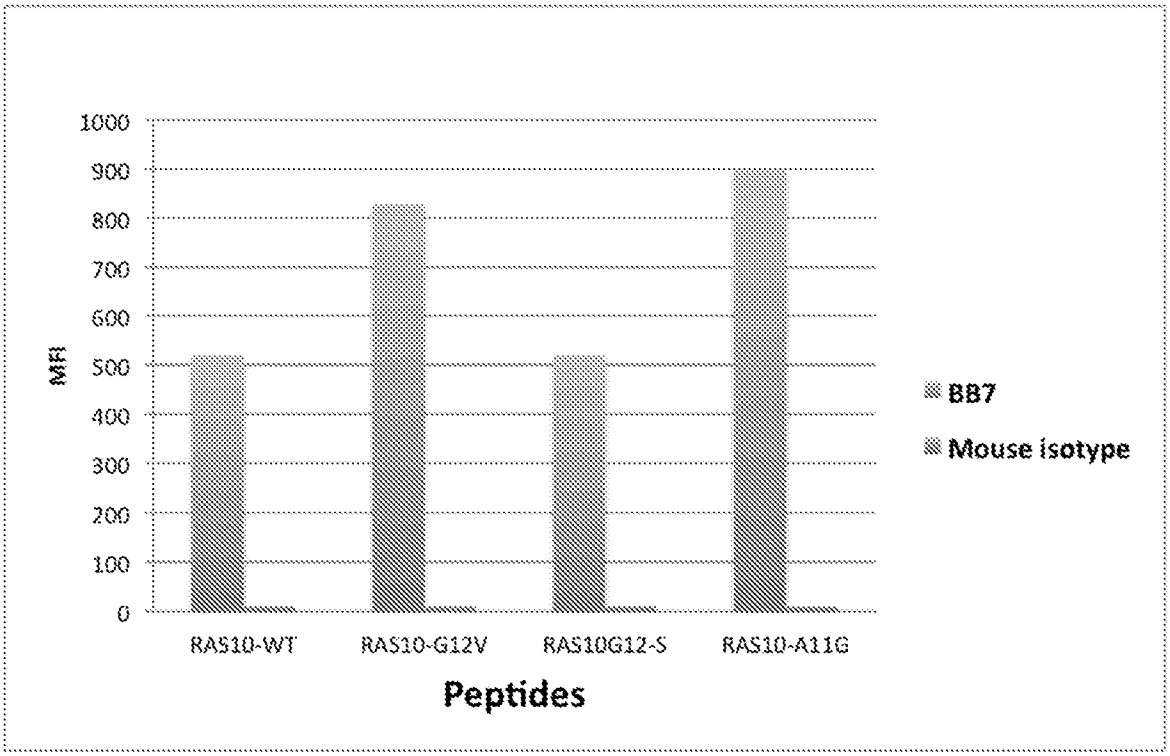

1000
900
800
700
600
500
400
300
200
100
0
MFI
RAS10-WT
RAS10-G12V
RAS10G12-S
RAS10-A11G
Peptides
BB7
Mouse isotype

FIGURE 3D

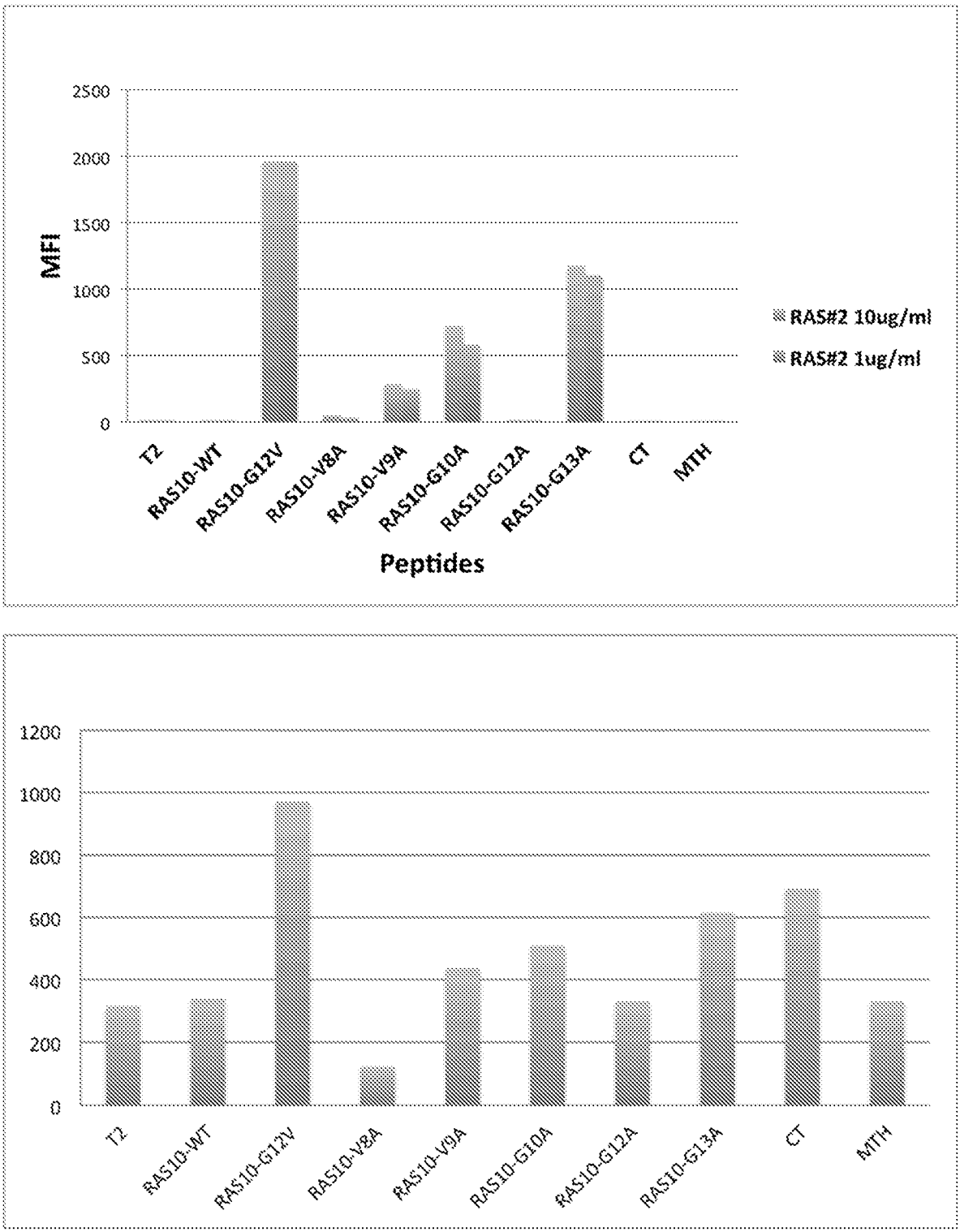
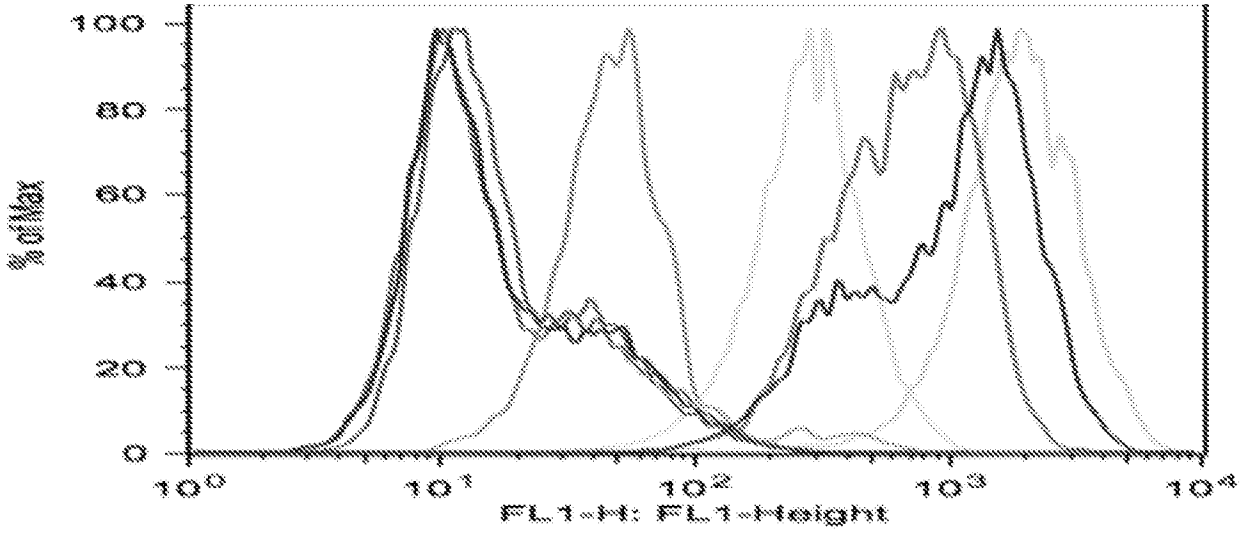

2500
2000
1500
1000
500
0
MFI
RAS#2 10ug/ml
RAS#2 1ug/ml
T2
RAS10-WT
RAS10-G12V
RAS10-V8A
RAS10-V9A
RAS10-G10A
RAS10-G12A
RAS10-G13A
CT
MTH
Peptides
1200
1000
800
600
400
200
0
T2
RAS10-WT
RAS10-G12V
RAS10-V8A
RAS10-V9A
RAS10-G10A
RAS10-G12A
RAS10-G13A
CT
MTH

FIGURE 3E



	Sample	Median:FL1-H
10-G13A	RAS#2 10ug-ml.003	1176
10-G12A	RAS#2 10ug-ml.003	14.3
10-G10A	RAS#2 10ug-ml.003	723
10-V9A	RAS#2 10ug-ml.003	286
10-V8A	RAS#2 10ug-ml.003	48.7
10-G12V	RAS#2 10ug-ml.003	1963
10-WT	RAS#2 10ug-ml.003	13.2
T2	RAS#2 10ug-ml.003	13.2

Sample	Median:FL1-H
RAS#2 1ug-ml.004	1104
RAS#2 1ug-ml.004	12.1
RAS#2 1ug-ml.004	583
RAS#2 1ug-ml.004	248
RAS#2 1ug-ml.004	30.8
RAS#2 1ug-ml.004	1963
RAS#2 1ug-ml.004	12.4
RAS#2 1ug-ml.004	12.5

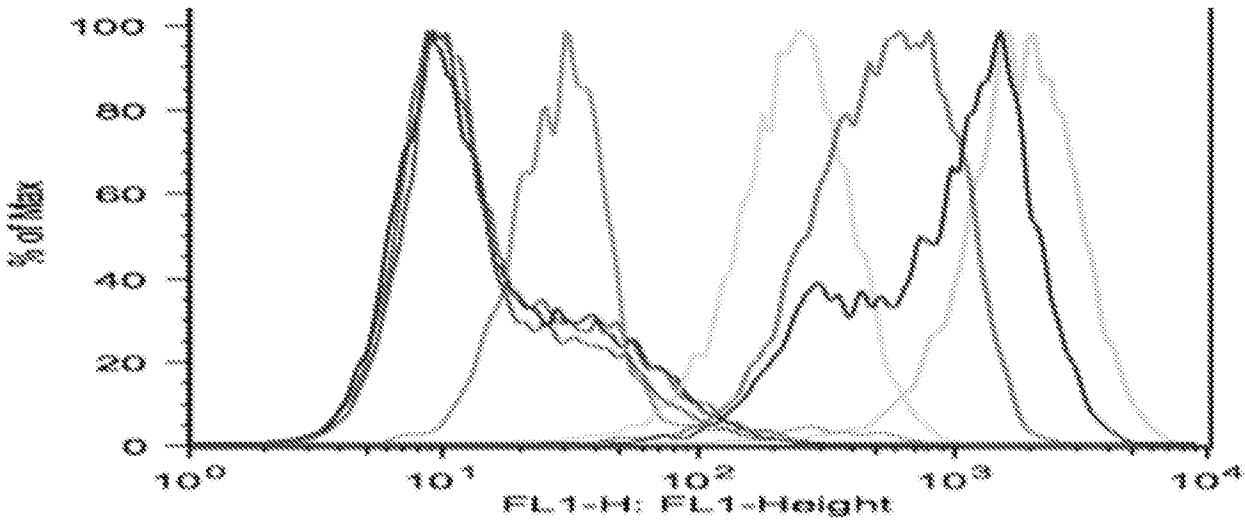
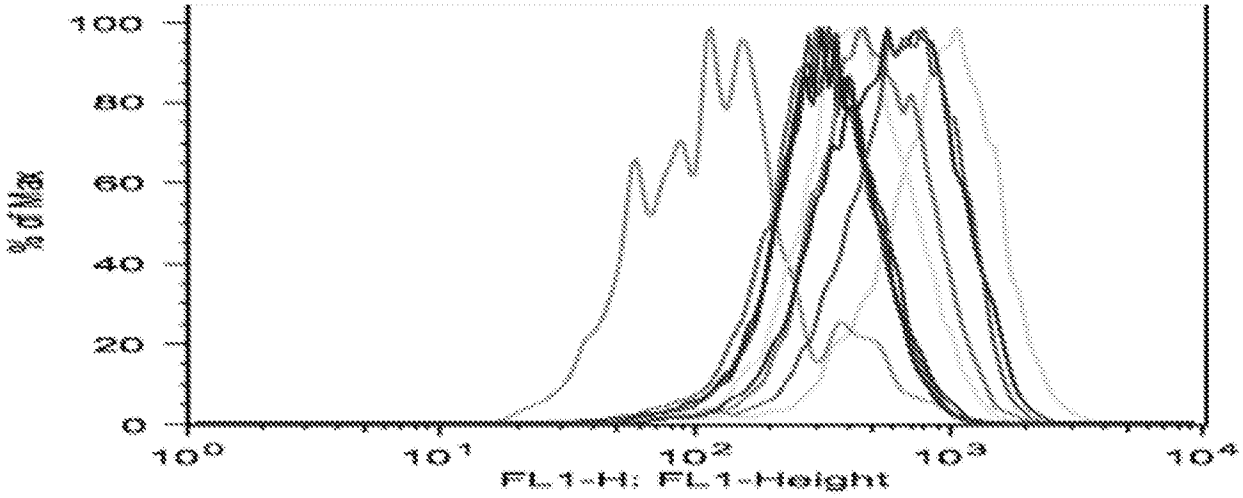
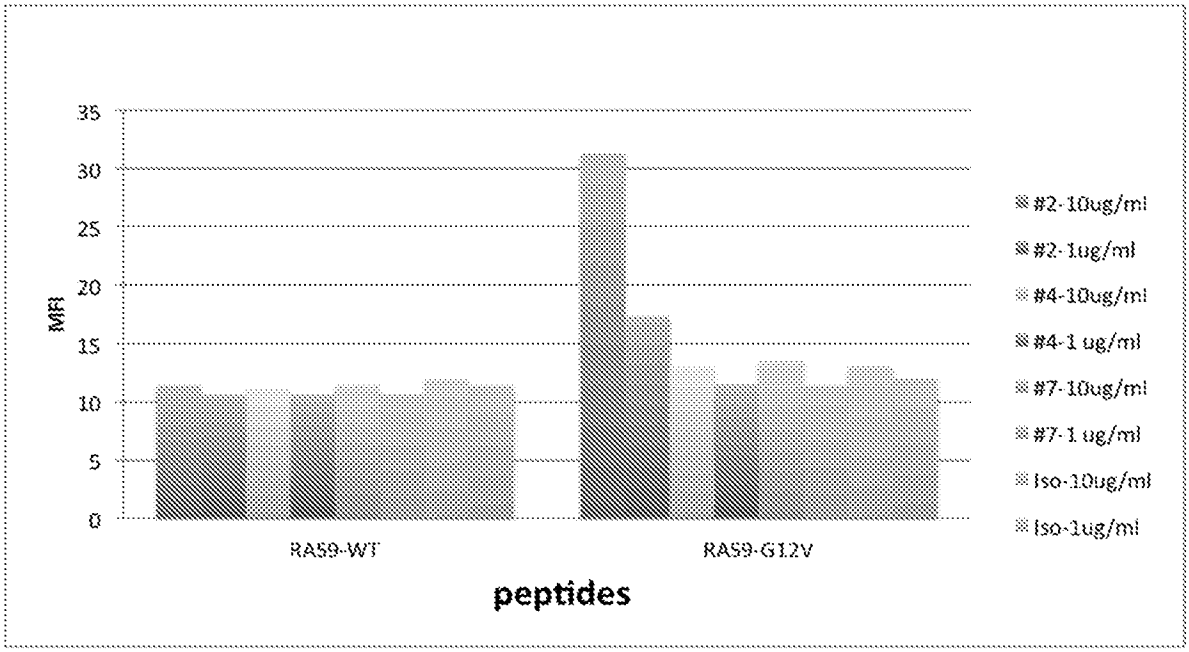


FIGURE 3F



	Sample	Median:FL1-H
MTH	BB7_F.011	331
CT	BB7_F.011	692
10-G13A	BB7_F.011	615
10-G12A	BB7_F.011	331
10-G10A	BB7_F.011	509
10-V9A	BB7_F.011	437
10-V8A	BB7_F.011	124
10-G12V	BB7_F.011	973
10-WT	BB7_F.011	340
T2	BB7_F.011	316

FIGURE 3G


35
30
25
20
15
10
5
0
MFI
RAS9-WT
RAS9-G12V
peptides
#2-10ug/ml
#2-1ug/ml
#4-10ug/ml
#4-1 ug/ml
#7-10ug/ml
#7-1 ug/ml
Iso-10ug/ml
Iso-1ug/ml

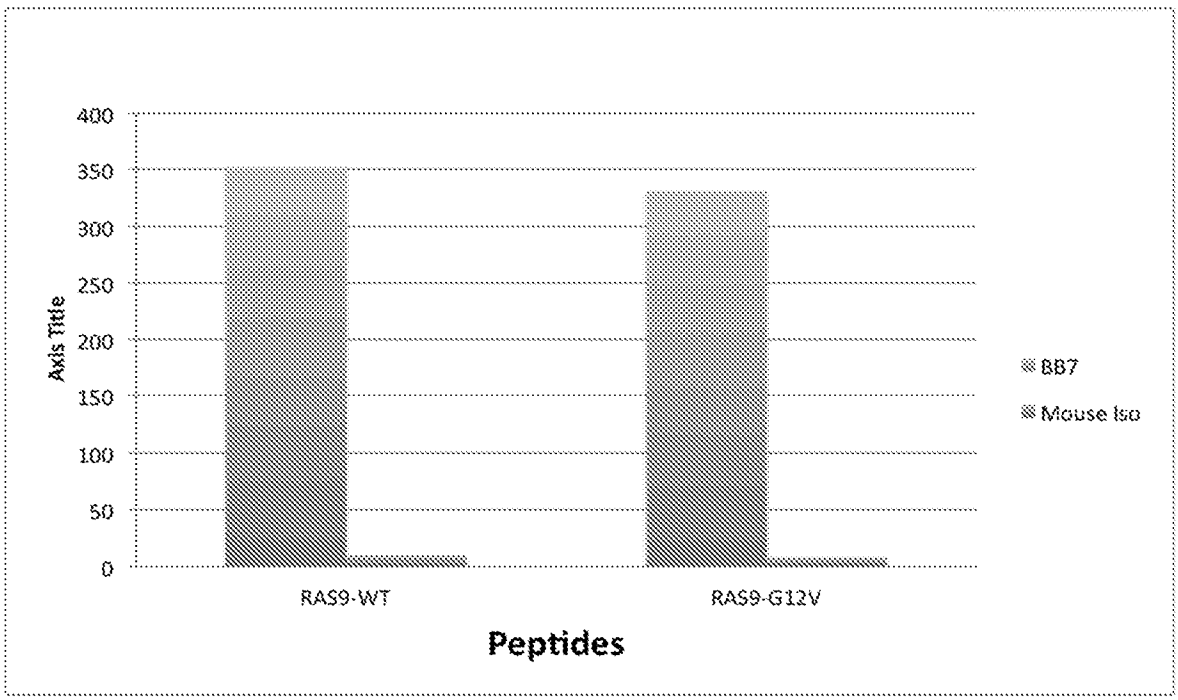
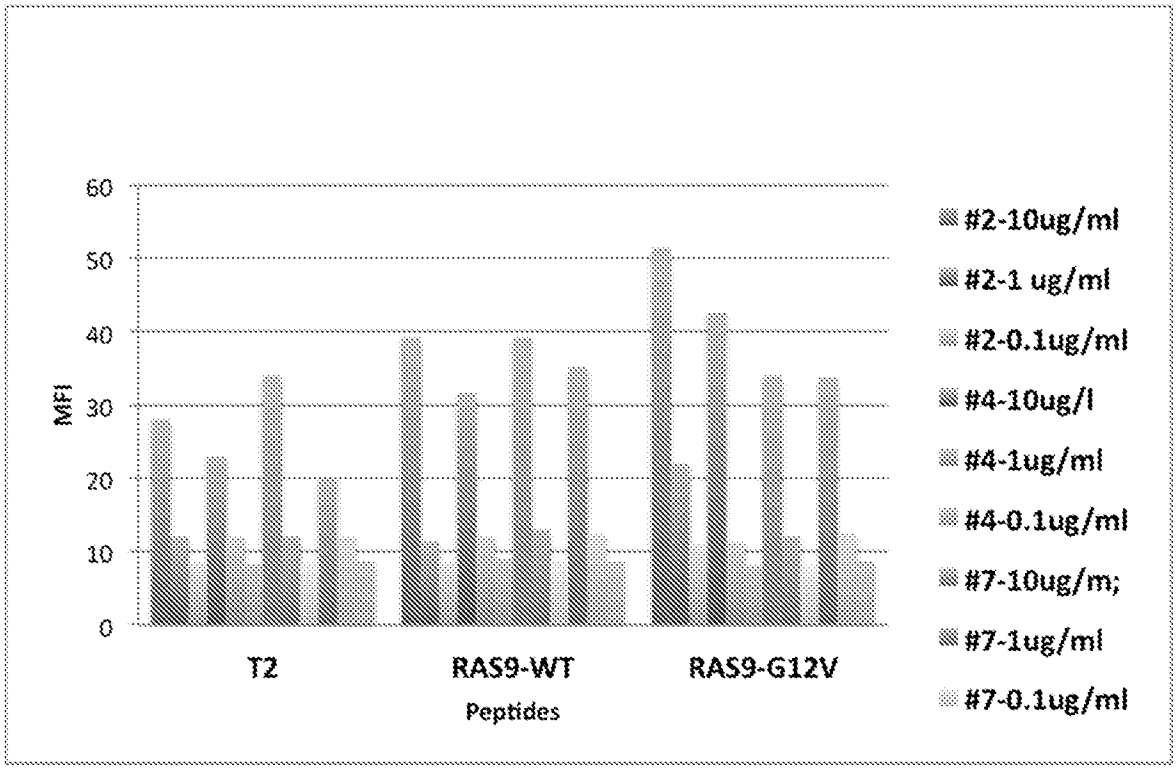

400
350
300
250
200
150
100
50
0
Axis Title
RAS9-WT
RAS9-G12V
Peptides
BB7
Mouse Iso

FIGURE 4A


60
50
40
30
20
10
0
MFI
T2
RAS9-WT
RAS9-G12V
Peptides
#2-10ug/ml
#2-1 ug/ml
#2-0.1ug/ml
#4-10ug/l
#4-1ug/ml
#4-0.1ug/ml
#7-10ug/m;
#7-1ug/ml
#7-0.1ug/ml

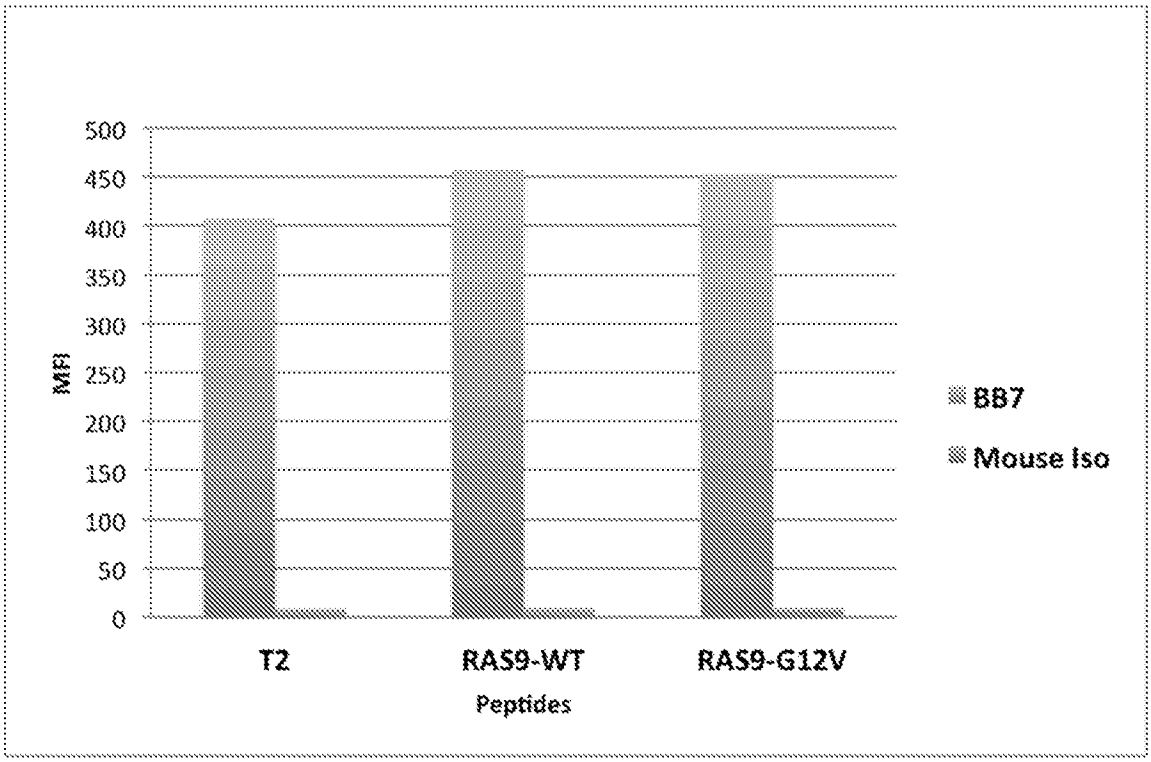
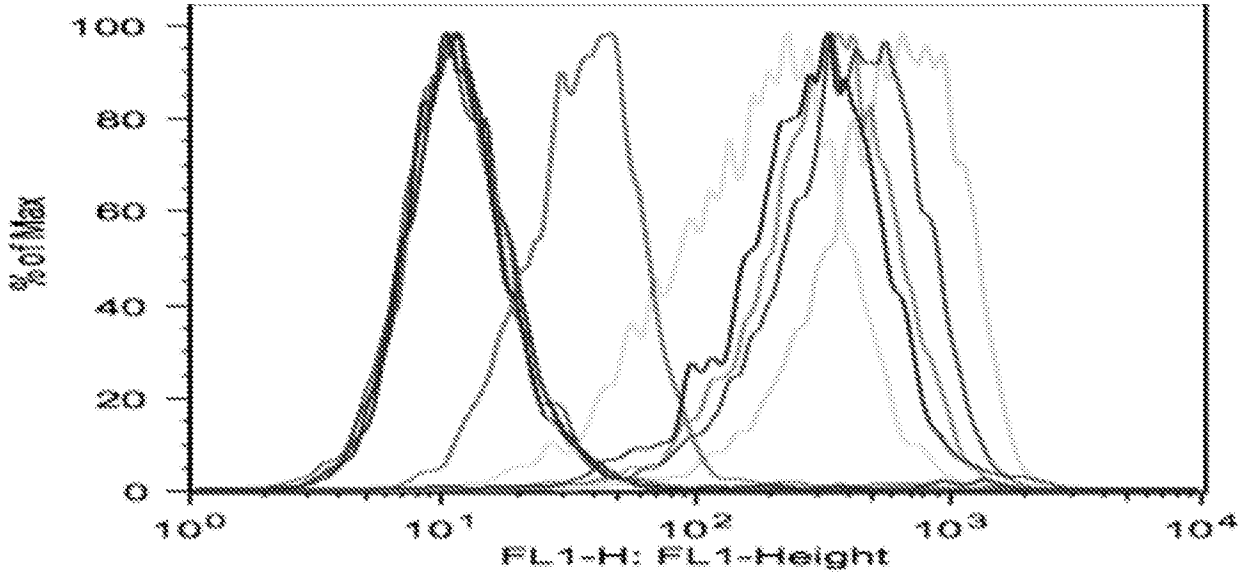

500
450
400
350
300
250
200
150
100
50
0
MFI
T2
RAS9-WT
RAS9-G12V
Peptides
BB7
Mouse Iso

FIGURE 4B



Sample	Median:FL1-H
IgG-BiTE 1ug-ml.010	11.2
IgG-BiTE 10ug-ml.009	11.4
RAS#7-BiTE 1ug-ml.008	292
RAS#7-BiTE 10ug-ml.007	414
RAS#4-BiTE 1ug-ml.006	36.8
RAS#4-BiTE 10ug-ml.005	183
RAS#2-BiTE 1ug-ml.004	337
RAS#2-BiTE 10ug-ml.003	610
2nd.002	11.7
uns.001	10.8

Sample	Median:FL1-H
IgG-BiTE 1ug-ml.010	10.7
IgG-BiTE 10ug-ml.009	11.2
RAS#7-BiTE 1ug-ml.008	30
RAS#7-BiTE 10ug-ml.007	97.3
RAS#4-BiTE 1ug-ml.006	11
RAS#4-BiTE 10ug-ml.005	10.9
RAS#2-BiTE 1ug-ml.004	11.1
RAS#2-BiTE 10ug-ml.003	10.4
2nd.002	11.1
uns.001	10.4

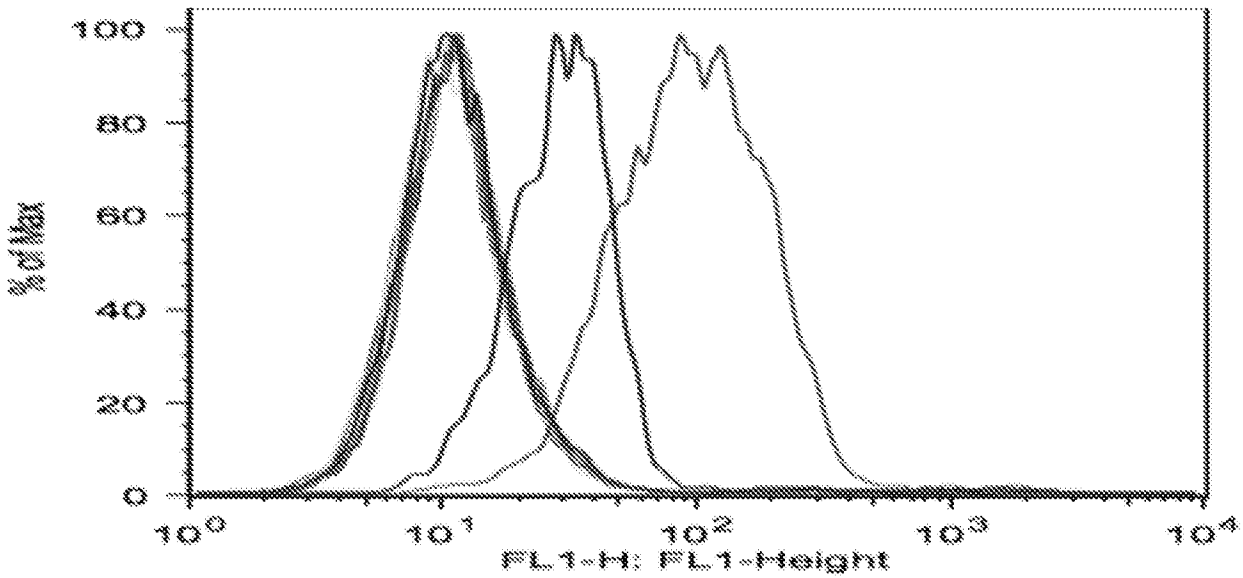
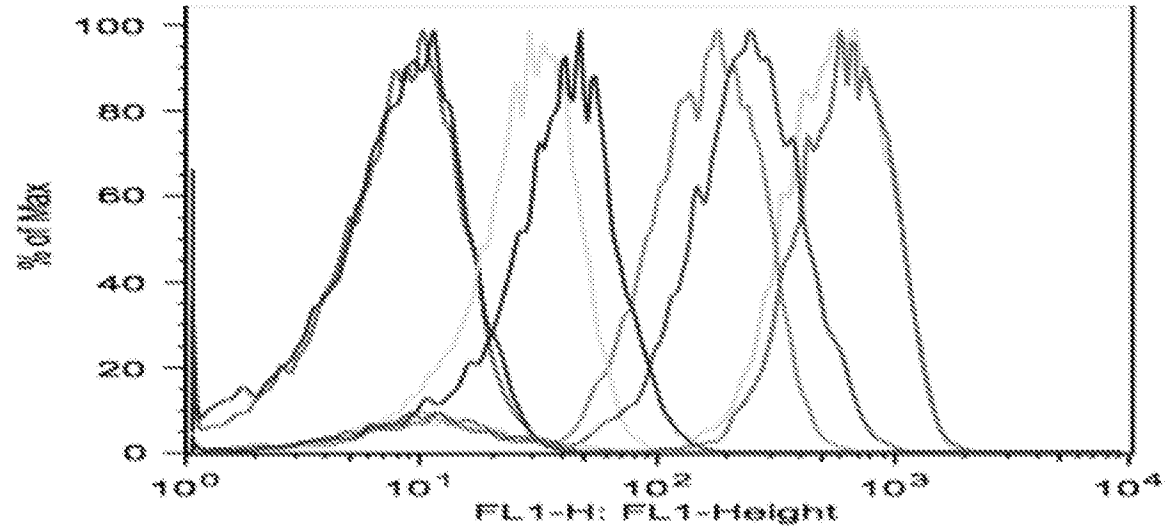


FIGURE 5A



Sample	Median:FL1-H
hIgG-BiTE 0.1ug-ml.014	39.6
hIgG-BiTE 1ug-ml.013	229
hIgG-BiTE 10ug-ml.012	804
RAS#2-BiTE 0.1ug-ml.005	28.4
RAS#2-BiTE 1ug-ml.004	155
RAS#2-BiTE 10ug-ml.003	557
GA6His_F 1;100.002	8.13
uns.001	8.13

Sample	Median:FL1-H
hIgG-BiTE 0.1ug-ml.014	39.6
hIgG-BiTE 1ug-ml.013	229
hIgG-BiTE 10ug-ml.012	804
RAS#4-BiTE 0.1ug-ml.008	114
RAS#4-BiTE 1ug-ml.007	583
RAS#4-BiTE 10ug-ml.006	1000
GA6His_F 1;100.002	8.13
uns.001	8.13

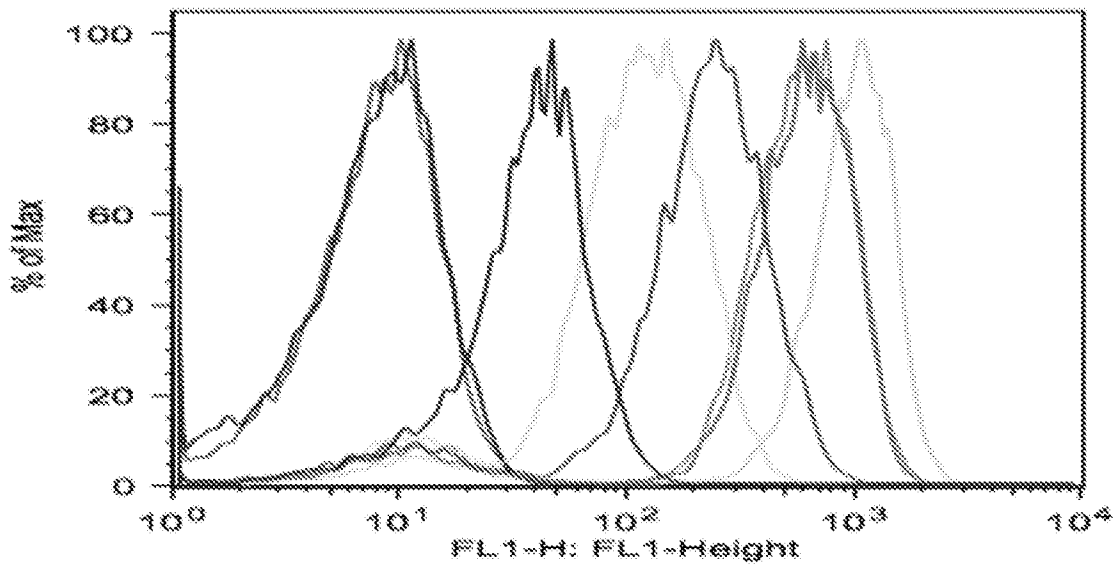
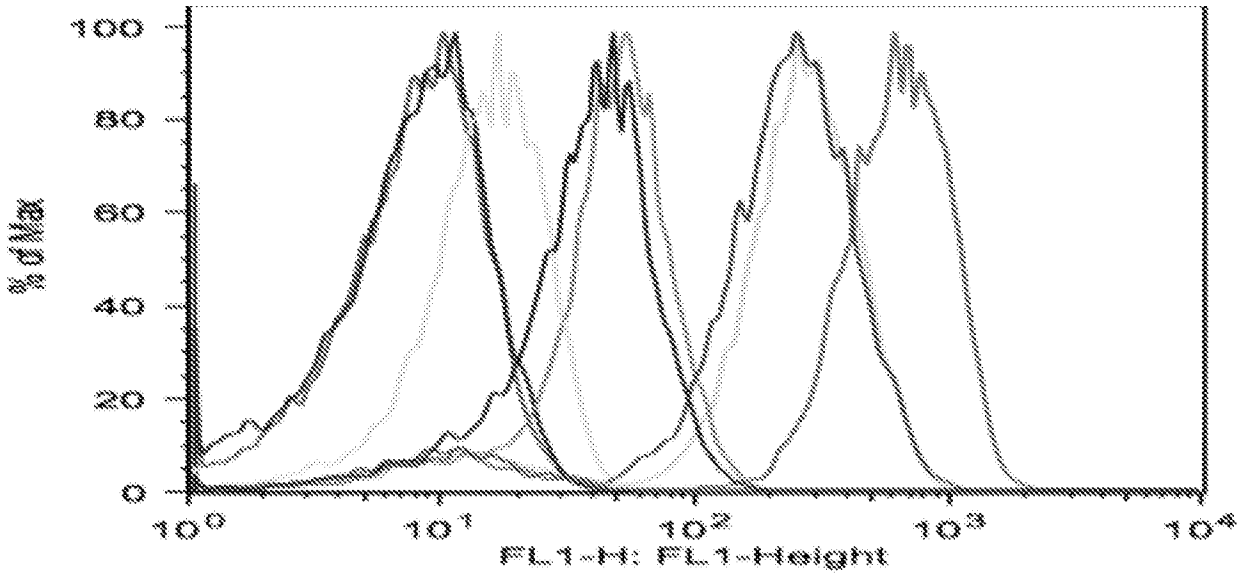


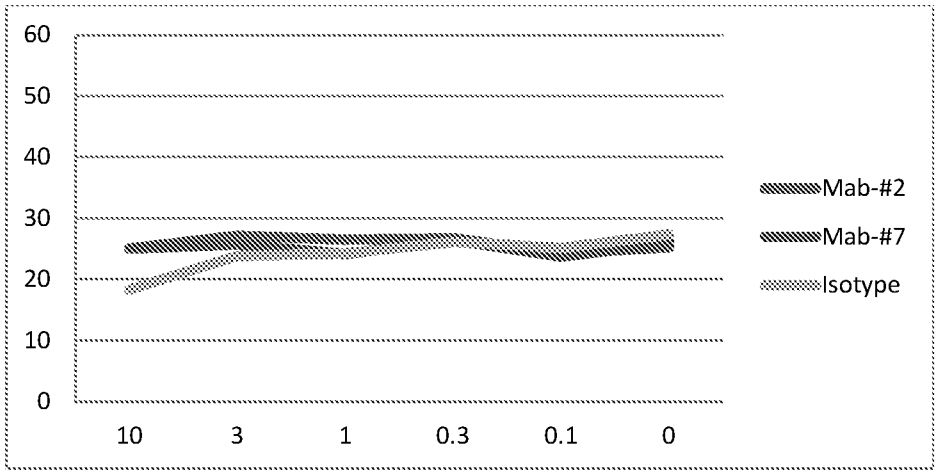
FIGURE 5B



Sample	Median:FL1-H
hIgG-BiTE 0.1ug-ml.014	39.6
hIgG-BiTE 1ug-ml.013	229
hIgG-BiTE 10ug-ml.012	604
RAS#7-BiTE 0.1ug-ml.011	15.4
RAS#7-BiTE 1ug-ml.010	48.7
RAS#7-BiTE 10ug-ml.009	255
GA6His_F 1;100.002	8.13
uns.001	8.13

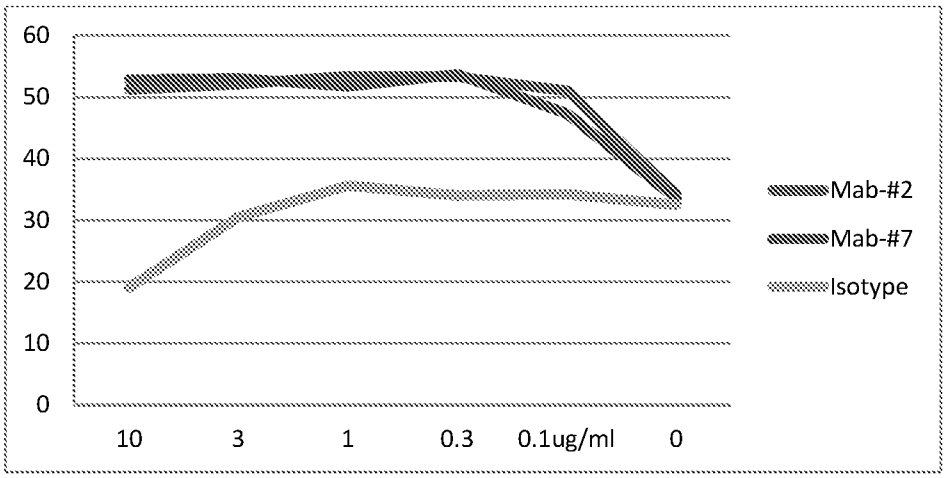
FIGURE 5C

T2



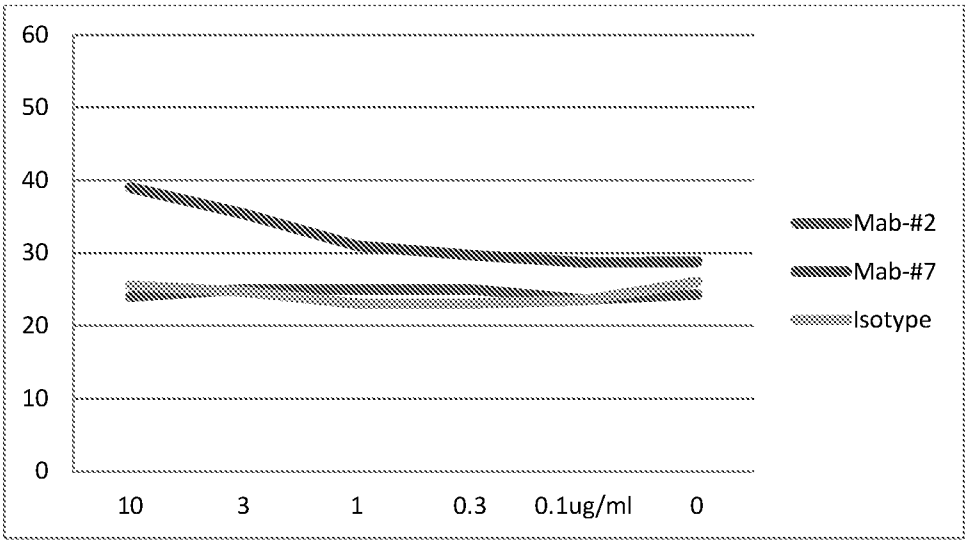
T2+

RAS10-G12V



T2+

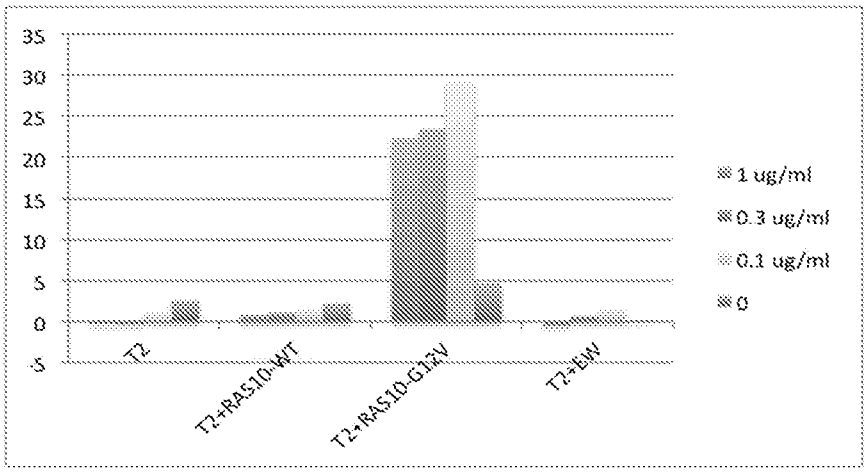
RAS10-WT



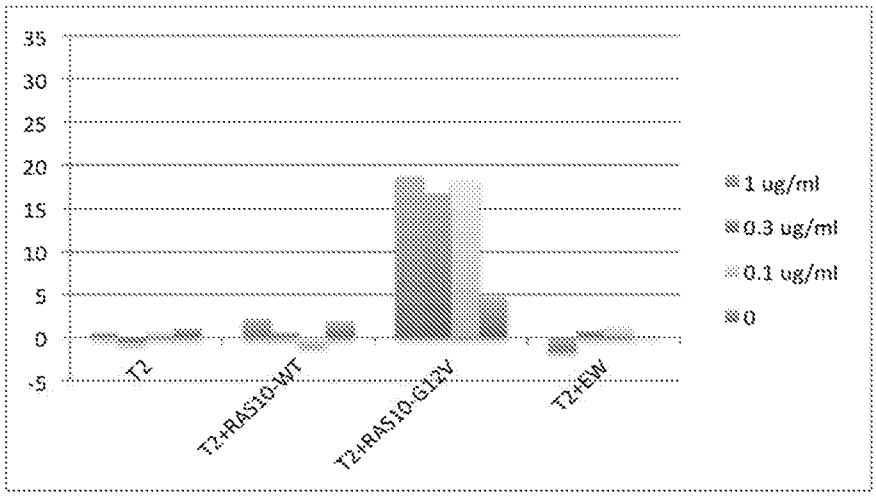
Mabs

FIGURE 6

T-BiTE #2



T-BiTE #7



% Lysis

Cont-BiTE

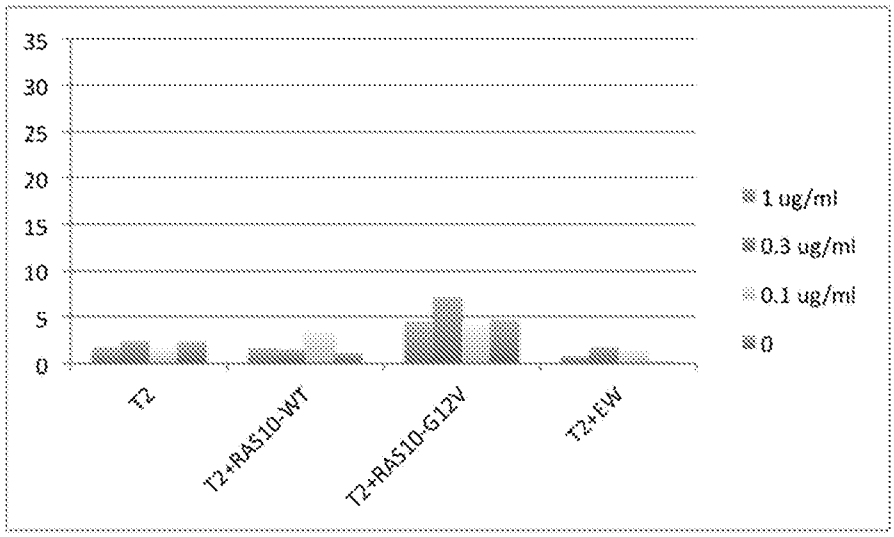


FIGURE 7

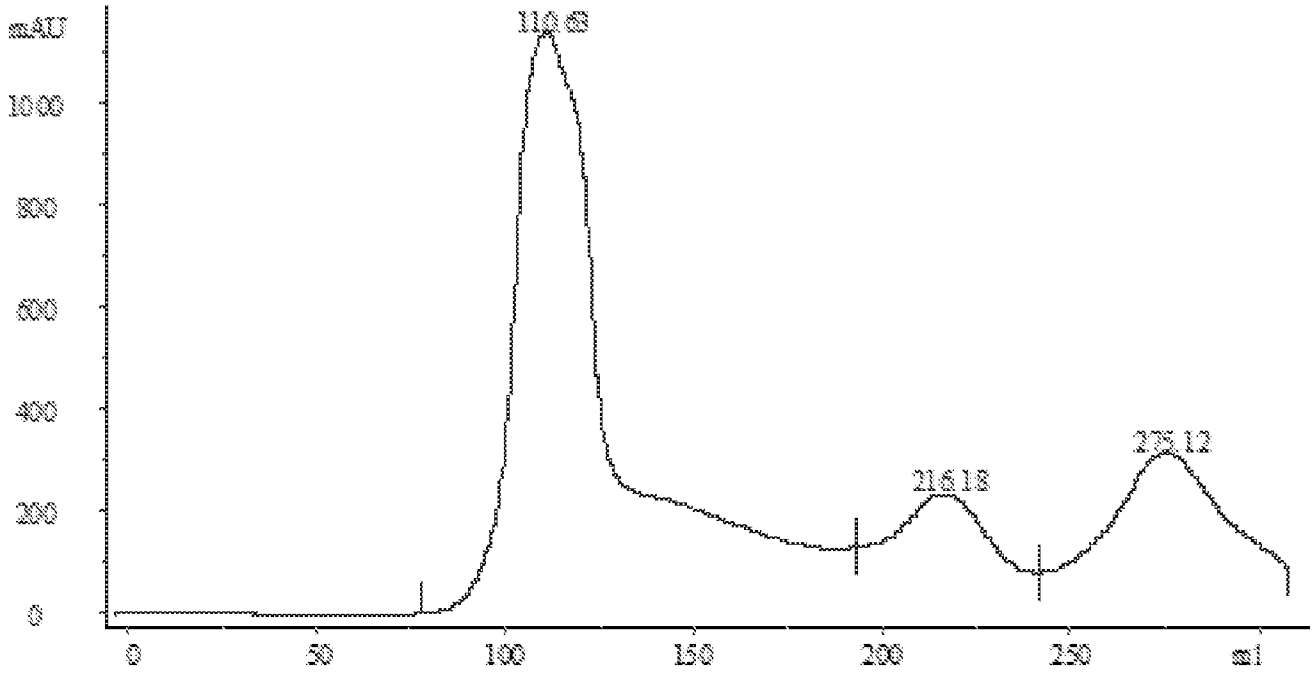
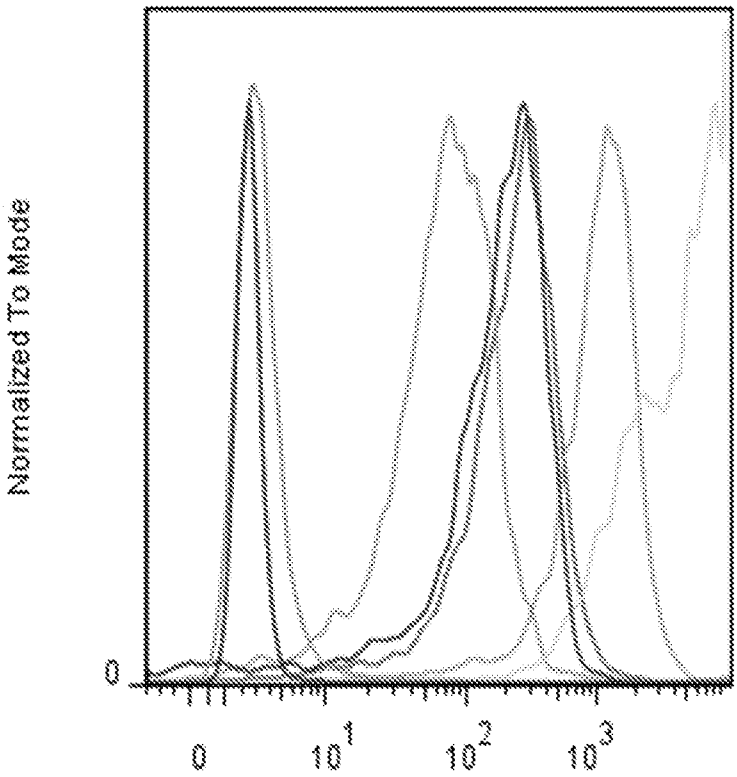

mAU
1000
800
600
400
200
0
110.65
216.18
275.12
0
50
100
150
200
250
ml

FIGURE 8



	Sample Name	Geom. Mean
	A01 T2-B2M.fcs	2.36
	A03 anti-M13 + PE anti-mouse IgG.fcs	3.26
	B02 T2-B2M +#2.fcs	129
	C02 T2-014A2mut + #2.fcs	3807
	D02 T2-014A2WT + #2.fcs	60.1
	E02 T2-014A1mut + #2.fcs	840
	F02 T2-014A1WT + #2.fcs	188

FIGURE 9