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(54) Titre : COMPOSITIONS DE BIOMARQUEURS ET PROCEDES ASSOCIES
(54) Title: BIOMARKER COMPOSITIONS AND METHODS

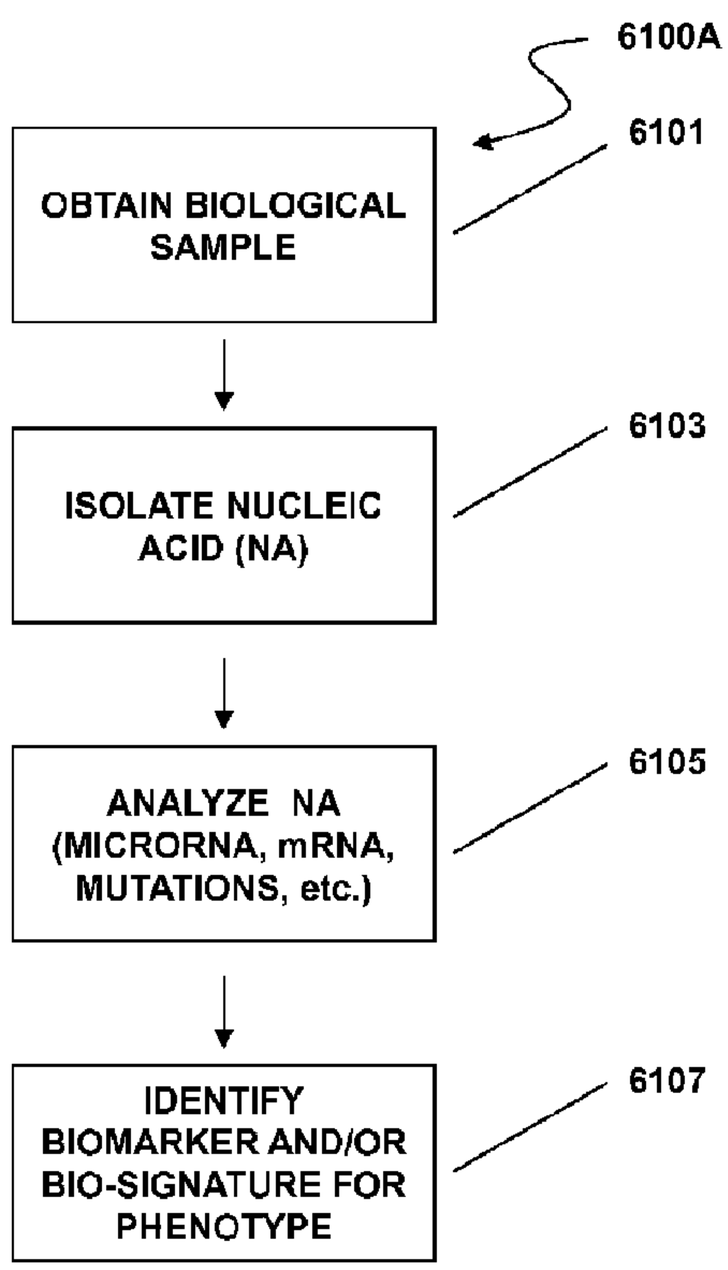


FIG. 1A

(57) Abrégé/Abstract:
Biomarkers can be assessed for diagnostic, therapy-related or prognostic methods to identify phenotypes, such as a condition or disease, or the stage or progression of a disease, select candidate treatment regimens for diseases, conditions, disease stages, and stages of a condition, and to determine treatment efficacy. Circulating biomarkers from a bodily fluid can be used in profiling of physiological states or determining phenotypes. These include nucleic acids, protein, and circulating structures such as vesicles, and nucleic acid-protein complexes.

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(54) Title: BIOMARKER COMPOSITIONS AND METHODS

(57) Abstract: Biomarkers can be assessed for diagnostic, therapy-related or prognostic methods to identify phenotypes, such as a condition or disease, or the stage or progression of a disease, select candidate treatment regimens for diseases, conditions, disease stages, and stages of a condition, and to determine treatment efficacy. Circulating biomarkers from a bodily fluid can be used in profiling of physiological states or determining phenotypes. These include nucleic acids, protein, and circulating structures such as vesicles, and nucleic acid-protein complexes.

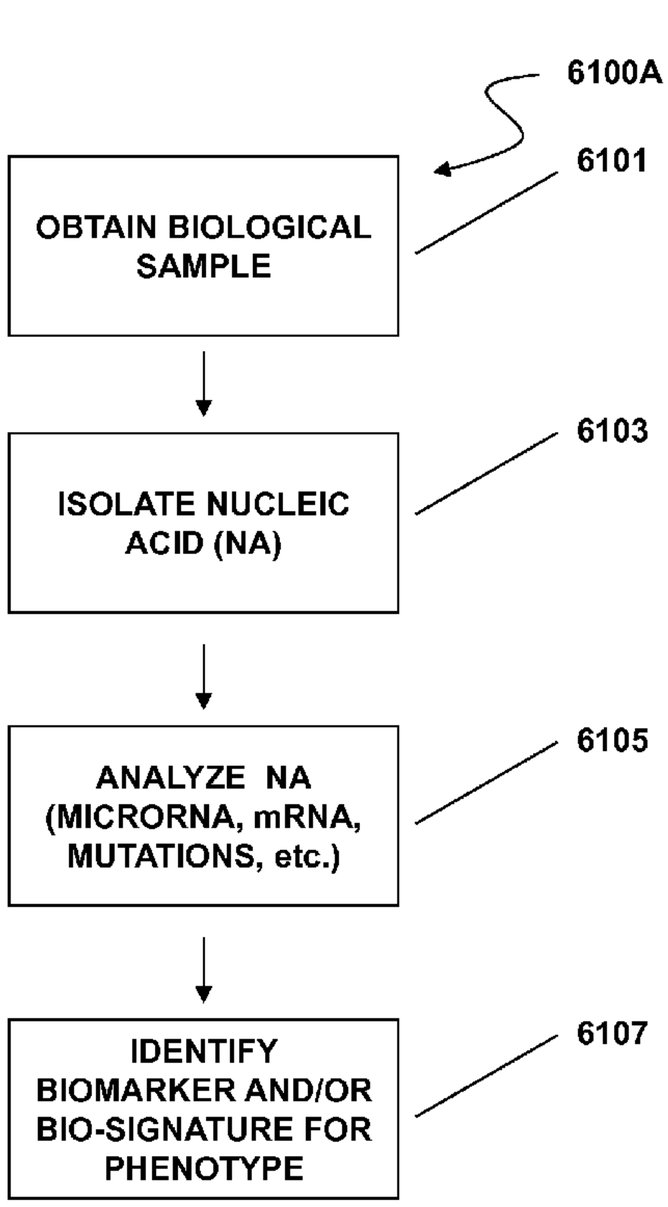


FIG. 1A

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BIOMARKER COMPOSITIONS AND METHODS

CROSS REFERENCE

[0001] This application claims the benefit of priority to U.S. Provisional Application Nos. 61/609,216, filed March 9, 2012; 61/619,816, filed April 3, 2012; and 61/729,986, filed November 26, 2012; which applications are incorporated herein by reference in their entirety.

BACKGROUND

[0002] Biomarkers for conditions and diseases such as cancer include biological molecules such as proteins, peptides, lipids, RNAs, DNA and variations and modifications thereof.

[0003] The identification of specific biomarkers, such as DNA, RNA and proteins, can provide biosignatures that are used for the diagnosis, prognosis, or theranosis of conditions or diseases. Biomarkers can be detected in bodily fluids, including circulating DNA, RNA, proteins, and vesicles. Circulating biomarkers include proteins such as PSA and CA125, and nucleic acids such as SEPT9 DNA and PCA3 messenger RNA (mRNA). Circulating biomarkers can be associated with circulating vesicles. Vesicles are membrane encapsulated structures that are shed from cells and have been found in a number of bodily fluids, including blood, plasma, serum, breast milk, ascites, bronchoalveolar lavage fluid and urine. Vesicles can take part in the communication between cells as transport vehicles for proteins, RNAs, DNAs, viruses, and prions. MicroRNAs are short RNAs that regulate the transcription and degradation of messenger RNAs. MicroRNAs have been found in bodily fluids and have been observed as a component within vesicles shed from tumor cells. The analysis of circulating biomarkers associated with diseases, including vesicles and/or microRNA, can aid in detection of disease or severity thereof, determining predisposition to a disease, as well as making treatment decisions.

[0004] Vesicles present in a biological sample provide a source of biomarkers, e.g., the markers are present within a vesicle (vesicle payload), or are present on the surface of a vesicle. Characteristics of vesicles (e.g., size, surface antigens, determination of cell-of-origin, payload) can also provide a diagnostic, prognostic or theranostic readout. There remains a need to identify biomarkers that can be used to detect and treat disease. microRNA, proteins and other biomarkers associated with vesicles as well as the characteristics of a vesicle can provide a diagnosis, prognosis, or theranosis.

[0005] The present invention provides methods and systems for characterizing a phenotype by detecting biomarkers that are indicative of disease or disease progress. The biomarkers can be circulating biomarkers including without limitation vesicle markers, protein, nucleic acids, mRNA, or and microRNA. The biomarkers can be nucleic acid-protein complexes.

SUMMARY

[0006] Disclosed herein are methods and compositions for characterizing a phenotype by analyzing circulating biomarkers, such as a vesicle, microRNA or protein present in a biological sample. Characterizing a phenotype for a subject or individual may include, but is not limited to, the diagnosis of a disease or condition, the prognosis of a disease or condition, the determination of a disease stage or a condition stage, a drug efficacy, a physiological condition, organ distress or organ rejection, disease or condition progression, therapy-related association to a disease or condition, or a specific physiological or biological state.

[0007] In an aspect, the invention provides a method of determining a KRAS nucleotide sequence in a biological sample that comprises one or more microvesicle, comprising: (a) contacting the biological sample with a binding agent to a microvesicle surface antigen; (b) isolating nucleic acids from the microvesicles that formed a complex with the binding agent to the microvesicle surface antigen in step (a); and (c) determining a v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) sequence within the nucleic acids isolated in step (b). The microvesicle surface antigen can be selected to isolate a desired vesicle population. For example, a general vesicle marker may facilitate isolation of a majority of microvesicles in a sample and also differentiate microvesicles from other cellular debris or the like, a tissue specific marker may facilitate isolation of microvesicles in a sample from a given tissue or cell-specific origin, and a disease marker can facilitate isolation of microvesicles representative of a certain disease, e.g., a cancer. A population of microvesicles can be isolated using a plurality of surface antigens, e.g., to isolate microvesicles indicative of a cancer from a given cancer lineage. The surface antigen can be selected from **Table 3**, **Table 4**, **Table 5**, **Table 7**, **Table 8**, or **Table 9** herein. For example, the surface antigen can be a cancer marker in **Table 5**, such as a marker for a lung cancer or a colorectal cancer. In an embodiment, the microvesicle surface antigen comprises Tissue factor, EpCam, B7H3, RAGE, carbonic anhydrase (CEA), CD66, TMEM211 and/or CD24. The surface antigen may comprise CD24. The invention also provides the isolated microvesicle population.

[0008] Multiple microvesicle surface antigens can be detected. For example, the method may further comprise contacting the biological sample with a binding agent to a general vesicle marker in step (a) and isolating the nucleic acids from microvesicles that also formed a complex with the binding agent to the general vesicle marker in step (b). In an embodiment, the general vesicle marker is selected from **Table 3**. The general vesicle marker can be a tetraspanin. The tetraspanin can be CD9, CD63 and/or CD81.

[0009] In one embodiment, the general vesicle marker comprises CD9, CD63 and/or CD81 and the microvesicle surface antigen comprises Tissue factor, EpCam, B7H3, RAGE, CEA, CD66, TMEM211 and/or CD24. For example, the general vesicle marker may comprise CD63 while the microvesicle surface antigen comprises CD24.

[0010] The KRAS sequence may be determined by pyrosequencing, chain-termination (e.g., dye-termination or Sanger sequencing), or Next Generation sequencing. The sequencing can be performed to determine whether the KRAS sequence comprises a mutation. The mutation can be an activating mutation. In an embodiment, the mutation comprises a 38G>A mutation in the nucleotide sequence. This mutation is also referred to as G13D. The G13D mutation results in an amino acid substitution at position 13 in KRAS, from a glycine (G) to an aspartic acid (D). Using similar terminology (i.e., nucleotide substitution (resulting amino acid substitution)), mutations in KRAS that may be detected include without limitation 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 of 34G>T (G12C), 34G>C (G12R), 34G>A (G12S), 35G>C (G12A), 35G>A (G12D), 35G>T (G12V), 37G>T (G13C), 37G>C (G13R), 37G>A (G13S), 38G>C (G13A), 38G>A (G13D), 38G>T (G13V), 181C>A (Q61K), 182A>T (Q61L), 182A>G (Q61R), 183A>C (Q61H), 183A>T (Q61H), 351A>C (K117N), 351A>T (K117N), 436G>C (A146P), 436G>A (A146T), and 437C>T (A146V).

[0011] The nucleic acids isolated in step (b) may comprise DNA or RNA, e.g., mRNA. In an embodiment, mRNAs are isolated from the microvesicle payload and an mRNA sequence is determined.

[0012] As described, the determined KRAS sequence may be used to provide a prognosis or a theranosis for a cancer. The theranosis comprises a therapy-related diagnosis or prognosis, e.g. the theranosis may comprise a prediction of whether a cancer is likely to respond or not respond to a chemotherapeutic agent. Accordingly, a treating physician or

other caregiver can use such information to help determine whether to treat or not treat a patient with the chemotherapeutic agent.

[0013] In embodiments, the chemotherapeutic agent comprises an epidermal growth factor receptor (EGFR) directed therapy. The epidermal growth factor receptor (EGFR) is an important player in cancer initiation and progression. KRAS plays a role as an effector molecule responsible for signal transduction from ligand-bound EGFR to the nucleus. Tumors carrying KRAS mutations are unlikely to respond to EGFR-targeted monoclonal antibodies or experience survival benefit from such treatment. EGFR directed therapy includes without limitation panitumumab, cetuximab, zalutumumab, nimotuzumab, matuzumab, gefitinib, erlotinib, and/or lapatinib.

[0014] Mutations in KRAS may also affect the efficacy of treatments directed to other molecular targets. In embodiments, the chemotherapeutic agent comprises a mammalian target of rapamycin (mTOR) directed therapy, a mitogen-activated or extracellular signal–regulated protein kinase kinase (MEK) directed therapy, and/or a v-raf murine sarcoma viral oncogene homolog B1 (BRAF) directed therapy. Such mTOR directed therapies include without limitation everolimus and/or temsirolimus.

[0015] The chemotherapeutic agent may comprise a cyclophosphamide or a combination of vincristine + carmustine (BCNU) + melphalan + cyclophosphamide + prednisone (VBMCP). These agents may be use to treat multiple myeloma (MM).

[0016] As described, a mutation in KRAS may be predictive that the cancer is less likely to respond to the chemotherapeutic agent. The cancer can be any appropriate cancer wherein KRAS may play a role in treatment selection. Accordingly, the cancer may include without limitation a solid tumor, a colorectal cancer (CRC), a pancreatic cancer, a non-small cell lung cancer (NSCLC), a bronchioloalveolar carcinoma (BAC) or adenocarcinoma (BAC subtype), a leukemia, or a multiple myeloma (MM).

[0017] In an aspect, the invention provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent to a cytokine receptor, wherein the cytokine receptor comprises one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, of 4-1BB, ALCAM, B7-1, BCMA, CD14, CD30, CD40 Ligand, CEACAM-1, DR6, Dtk, Endoglin, ErbB3, E-Selectin, Fas, Flt-3L, GITR, HVEM, ICAM-3, IL-1 R4, IL-1 RI, IL-10 Rbeta, IL-17R, IL-2Rgamma, IL-21R, LIMPII, Lipocalin-2, L-Selectin, LYVE-1, MICA, MICB, NRG1-beta1, PDGF Rbeta, PECAM-1, RAGE, TIM-1, TRAIL R3, Trappin-2, uPAR, VCAM-1, and XEDAR; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent to the cytokine receptor, thereby detecting the presence or level of the one or more microvesicle. In an embodiment, the cytokine receptor comprises one or more, e.g., 1, 2, or 3, of ErbB3, RAGE, and Trail R3. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can include, without limitation, a colorectal cancer or a late stage prostate cancer. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample,

thereby providing a diagnosis or prognosis of the colorectal cancer or late stage prostate cancer. The invention also provides the isolated one or more microvesicle.

[0018] In another aspect, the invention provides invention also provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more, e.g., 1, 2, 3 or 4, of IL-1 alpha, CA125, Filamin, and Amyloid A; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a colorectal cancer. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the colorectal cancer. The invention also provides the isolated one or more microvesicle.

[0019] In still another aspect, the invention provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more receptor, e.g., 1, 2, 3, 4, 5, 6, 7 or 8, of Involucrin, CD57, Prohibitin, Thrombospondin, Laminin B1/b1, Filamin, 14.3.3 gamma, 14.3.3 Pan; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a colorectal cancer and/or a colorectal adenoma. For example, the method can be used to compare levels of the one or more microvesicle between colorectal cancer and/or a colorectal adenoma samples, thereby providing a diagnosis or prognosis of the colorectal cancer and/or a colorectal adenoma, including distinguishing the samples as derived from cancer or adenoma. The invention also provides the isolated one or more microvesicle.

[0020] In another aspect, the invention provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more receptor, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, of Involucrin, Prohibitin, Laminin B1/b1, IL-3, Filamin, 14.3.3 gamma, 14.3.3 Pan, MMP-15 / MT2-MMP, hPL, Ubiquitin, and mRANKL; and (b) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to

characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a colorectal adenoma. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the colorectal adenoma. The invention also provides the isolated one or more microvesicle.

[0021] In still another aspect, the invention provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 or 76, of Prohibitin, CD57, Filamin, CD18, b-2-Microglobulin, IL-2, IL-3, CD16, p170, Keratin 19, Pds1, Glicentin, SRF (Serum Response Factor), E3-binding protein (ARM1), Collagen II, SRC1 (Steroid Receptor Coactivator-1) Ab-1, Caldesmon, GFAP, TRP75 / gp75, alpha-1-antichymotrypsin, Hepatic Nuclear Factor-3B, PLAP, Tyrosinase, NF kappa B / p50, Melanoma (gp100), Cyclin E, 6-Histidine, Mucin 3 (MUC3), TdT, CD21, XPA, Superoxide Dismutase, Glycogen Synthase Kinase 3b (GSK3b), CD54/ICAM-1, Thrombospondin, Gai1, CD79a mb-1, IL-1 beta, Cytochrome c, RAD1, bcl-X, CD50/ICAM-3, Neurofilament, Alkaline Phosphatase (AP), ER Ca+2 ATPase2, PCNA, F.VIII/VWF, SV40 Large T Antigen, Paxillin, Fascin, CD165, GRIP1, Cdk8, Nucleophosmin (NPM), alpha-1-antitrypsin, CD32/Fcg Receptor II, Keratin 8 (phospho-specific Ser73), DR5, CD46, TID-1, MHC II (HLA-DQ), Plasma Cell Marker, DR3, Calmodulin, AIF (Apoptosis Inducing Factor), DNA Polymerase Beta, Vitamin D Receptor (VDR), Bcl10 / CIPER / CLAP / mE10, Neuron Specific Enolase, CXCR4 / Fusin, Neurofilament (68kDa), PDGFR, beta, Growth Hormone (hGH), Mast Cell Chymase, Ret Oncoprotein, and Phosphotyrosine; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a brain cancer. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the brain cancer. The invention also provides the isolated one or more microvesicle.

[0022] In yet another aspect, the invention provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more, e.g., 1, 2, 3, 4, 5, 6, 7 or 8, of Caspase 5, Thrombospondin, Filamin, Ferritin, 14.3.3 gamma, 14.3.3 Pan, CD71 / Transferrin Receptor, and Prostate Apoptosis Response Protein-4; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle.

The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a melanoma. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the melanoma. The invention also provides the isolated one or more microvesicle.

[0023] In an aspect, the invention provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19, of 14.3.3 Pan, Filamin, 14.3.3 gamma, CD71 / Transferrin Receptor, CD30, Cdk5, CD138, Thymidine Phosphorylase, Ruv 5, Thrombospondin, CD1, Von Hippel-Lindau Protein, CD46, Rad51, Ferritin, c-Abl, Actin, Muscle Specific, LewisB; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a head and neck cancer. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the head and neck cancer. The invention also provides the isolated one or more microvesicle.

[0024] In any of the methods of the invention above, the biological sample may comprise a cell culture, such that the microvesicles are derived from cultered cells. The biological sample may also comprise a sample from a subject, e.g., a solid tumor sample or a bodily fluid from the subject. Appropriate bodily fluids comprise without limation peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, umbilical cord blood, or a derivative of any thereof.

[0025] In such embodiments, the method may comprise removal of one or more biological contaminant, e.g., an abundant protein, from the biological sample prior to or during the isolation of the one or more microvesicle. For example, abundant proteins may be removed prior to contacting the microvesicle with the binding agent, which may serve to enhance the binding efficiency, specificity and/or detection. When the biological sample comprises peripheral blood or a derivative thereof, e.g., serum or plasma, the abundant protein can be an abundant blood protein. Non-limiting examples one or more abundant protein that may be removed include one or more of albumin, IgG, transferrin, fibrinogen, fibrin, IgA, α2-Marcroglobulin, IgM, α1-Antitrypsin, complement C3, haptoglobulin, apolipoprotein A1,

A3 and B; α1-Acid Glycoprotein, ceruloplasmin, complement C4, C1q, IgD, prealbumin (transthyretin), plasminogen, a derivative of any thereof, and a combination thereof. Further examples of abundant proteins that may be removed comprise Albumin, Immunoglobulins, Fibrinogen, Prealbumin, Alpha 1 antitrypsin, Alpha 1 acid glycoprotein, Alpha 1 fetoprotein, Haptoglobin, Alpha 2 macroglobulin, Ceruloplasmin, Transferrin, complement proteins C3 and C4, Beta 2 microglobulin, Beta lipoprotein, Gamma globulin proteins, C-reactive protein (CRP), Lipoproteins (chylomicrons, VLDL, LDL, HDL), other globulins (types alpha, beta and gamma), Prothrombin, Mannose-binding lectin (MBL), a derivative of any thereof, and a combination thereof.

[0026] Various methodologies can be used to deplete abundant proteins from the biological sample. In some embodiments, the one or more abundant protein is depleted by immunoaffinity, precipitation, or a combination thereof. Commercially available columns can be used such described herein. Depleting the one or more abundant protein may also comprise contacting the biological sample with thromboplastin to precipitate fibrinogen.

[0027] In any of the methods of the invention above, the binding agent used to form a complex with the microvesicle can comprise any useful reagent, including without limitation a nucleic acid, DNA molecule, RNA molecule, antibody, antibody fragment, aptamer, peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, peptide, dendrimer, membrane protein labeling agent, chemical compound, or a combination thereof. Preferable binding agents include without limitation antibodies and/or aptamers. Other binding agents known in the art may be used as desired.

[0028] In an embodiment, the binding agent is tethered to a substrate. The binding agent may also comprise a label. When multiple binding agents are used, e.g., to identify microvesicles bearing a plurality of surface antigens, at least one binding agent can be tethered to a substrate and another binding agent can carry a label. This allows the label to identify microvesicles in complex with the tethered binding agent. In addition, multiple tethered binding agents can be used, e.g., in a series of columns, wells, or precipitations. For example, a vesicle population can be affinity selected using a binding agent to one surface antigen, eluted, and followed by another round of affinity selection. This may, e.g., allow selection of microvesicles bearing more than one surface antigen, or using different epitopes of a same antigen. Multiple labeled binding agents may be used as well. This may also allow identification of microvesicles bearing more than one surface antigen. Numerous examples of microvesicle antigens that may be used for affinity selection and/or detection are disclosed herein, e.g., in **Table 3**, **Table 4**, **Table 5**, **Table 7**, **Table 8**, or **Table 9** herein. The Examples provide further illustration of each of these applications.

[0029] As described herein, the one or more microvesicle may be subjected to size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, affinity capture, immunoassay, microfluidic separation, flow cytometry or combinations thereof. For example, a large microvesicle population can be isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, and/or nanomembrane ultrafiltration, then a subpopulation can be further isolated using immunoabsorbent capture, affinity purification, affinity capture, immunoassay and/or flow cytometry. Microvesicles may be at least partially identified or isolated by size. In an embodiment, the one or more microvesicle has a diameter between 10 nm and 2000 nm. For example, the one or more microvesicle may have a diameter between 20 nm and 200 nm. In other embodiments, microvesicles with a size greater than 800 nm, e.g., > 1000 nm, are interrogated.

[0030] Also as described herein, the methods of the invention can include detecting one or more payload biomarker within the one or more microvesicle. For example, the one or more payload biomarker may comprise one or more nucleic acid, peptide, protein, lipid, antigen, carbohydrate, and/or proteoglycan. The nucleic acid may be DNA, mRNA,

microRNA, snoRNA, snRNA, rRNA, tRNA, siRNA, hnRNA, or shRNA. In preferred embodiments, the one or more payload biomarker comprises microRNA and/or mRNA. The payload markers can be assessed as part of providing the theranosis.

[0031] The methods of the invention can be performed in vitro.

[0032] In another aspect, the invention provides a use of one or more reagent to carry out the method of any preceding claim. In a related aspect, the invention provides a kit comprising one or more reagent to carry out the method of the invention. The one or more reagent can be selected from the group consisting of one or more binding agent specific for a microvesicle surface antigen, a chromatography column, filtration units, membranes, flow reagents, a buffer, equipment to remove a highly abundant protein, one or more population of microvesicles, and a combination thereof. The one or more reagent can be a capture agent and/or a detector agent such as described herein. The kit can contain instructions for performing one or more steps of the methods of the invention.

[0033] In an aspect, the invention further provides isolated microvesicles comprising one or more of the biomarkers and biosignatures of the invention. In an embodiment, the isolated microvesicle is a CD24+ microvesicle comprising a mutated KRAS nucleic acid. The isolated microvesicle may further comprise a general vesicle marker, e.g., a marker in **Table 3** herein. The general vesicle marker may be a tetraspanin, e.g., CD9, CD63 and/or CD81. In an embodiment, the general vesicle marker comprises CD9. In another embodiment, the general vesicle marker comprises CD63. In still another embodiment, the general vesicle marker comprises CD81.

INCORPORATION BY REFERENCE

[0034] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] **FIG. 1A** depicts a method of identifying a biosignature comprising nucleic acid to characterize a phenotype. **FIG. 1B** depicts a method of identifying a biosignature of a vesicle or vesicle population to characterize a phenotype.

[0036] **FIGs. 2A-F** illustrate methods of characterizing a phenotype by assessing vesicle biosignatures. **FIG. 2A** is a schematic of a planar substrate coated with a capture antibody, which captures vesicles expressing that protein. The capture antibody is for a vesicle protein that is specific or not specific for vesicles derived from diseased cells ("disease vesicle"). The detection antibody binds to the captured vesicle and provides a fluorescent signal. The detection antibody can detect an antigen that is generally associated with vesicles, or is associated with a cell-of-origin or a disease, e.g., a cancer. **FIG. 2B** is a schematic of a bead coated with a capture antibody, which captures vesicles expressing that protein. The capture antibody is for a vesicle protein that is specific or not specific for vesicles derived from diseased cells ("disease vesicle"). The detection antibody binds to the captured vesicle and provides a fluorescent signal. The detection antibody can detect an antigen that is generally associated with vesicles, or is associated with a cell-of-origin or a disease, e.g., a cancer. **FIG. 2C** is an example of a screening scheme that can be performed by multiplexing using the beads as shown in **FIG. 2B**. **FIG. 2D** presents illustrative schemes for capturing and detecting vesicles to characterize a phenotype. **FIG. 2E** presents illustrative schemes for assessing vesicle payload to characterize a phenotype. **FIG. 2F** presents illustrative schemes for capturing and detecting vesicles and optionally assessing payload to characterize a phenotype.

[0037] **FIG. 3** illustrates a computer system that can be used in some exemplary embodiments of the invention.

[0038] **FIG. 4** illustrates a method of depicting results using a bead based method of detecting vesicles from a subject. The number of beads captured at a given intensity is an indication of how frequently a vesicle expresses the detection protein at that intensity. The more intense the signal for a given bead, the greater the expression of the detection protein. The figure shows a normalized graph obtained by combining normal patients into one curve and cancer patients into another, and using bio-statistical analysis to differentiate the curves. Data from each individual is normalized to account for variation in the number of beads read by the detection machine, added together, and then normalized again to account for the different number of samples in each population.

[0039] **FIG. 5** illustrates the capture of prostate cancer cells-derived vesicles from plasma with EpCam by assessing TMPRSS2-ERG expression. VCaP purified vesicles were spiked into normal plasma and then incubated with Dynal magnetic beads coated with either the EpCam or isotype control antibody. RNA was isolated directly from the Dynal beads. Equal volumes of RNA from each sample were used for RT-PCR and subsequent Taqman assays.

[0040] **FIG. 6** depicts a bar graph of miR-21 or miR-141 expression with CD9 bead capture. 1 ml of plasma from prostate cancer patients, 250 ng/ml of LNCaP, or normal purified vesicles were incubated with CD9 coated Dynal beads. The RNA was isolated from the beads and the bead supernatant. One sample (#6) was also uncaptured for comparison. microRNA expression was measured with qRT-PCR and the mean CT values for each sample compared. CD9 capture improves the detection of miR-21 and miR-141 in prostate cancer samples.

[0041] **FIG. 7A** illustrates separation and identification of vesicles using the MoFlo XDP. **FIG. 7B** illustrates FACS analysis of VCaP cells and exosomes stained with antibodies to CD9, B7H3, PCSA and PSMA. **FIG. 7C** illustrates different patterns of miR expression were obtained in flow sorted B7H3+ or PSMA+ vesicle populations as compared to overall vesicle population.

[0042] **FIGs. 8A-H** illustrates detecting vesicles in a sample wherein the presence or level of the desired vesicles are assessed using a microsphere platform. **FIG. 8A** represents a schematic of isolating vesicles from plasma using a column based filtering method, wherein the isolated vesicles are subsequently assessed using a microsphere platform. **FIG. 8B** represents a schematic of compression of a membrane of a vesicle due to high-speed centrifugation, such as ultracentrifugation. **FIG. 8C** represents a schematic of detecting vesicles bound to microspheres using laser detection. **FIG. 8D** represents an example of detecting prostate derived vesicles bound to a substrate. The microvesicles are captured with capture agents specific to PCSA, PSMA or B7H3 tethered to the substrate. The so-captured vesicles are labeled with fluorescently labeled detection agents specific to CD9, CD63 and CD81. **FIG. 8E** illustrates correlation of CD9 positive vesicles detected using a microsphere platform (Y-axis) or flow cytometry (X-axis). To calculate median fluorescence intensity (MFIs), vesicles were captured with anti-CD9 antibodies tethered to microspheres and detected using fluorescently labeled detection antibodies specific to CD9, CD63 and CD81. **FIG. 8F** illustrates correlation of PSMA, PCSA or B7H3 positive vesicles detected using a microsphere platform (Y-axis) or BCA protein assay (X-axis). To calculate MFIs, vesicles were captured with antibodies to B7H3, PSMA or PCSA tethered to microspheres and detected using fluorescently labeled detection antibodies specific to CD9, CD63 and CD81. **FIG. 8G** illustrates similar performance for detecting CD81 positive vesicles using a microsphere assay in a single-plex or multi-plex fashion. Vesicles were captured with anti-CD81 antibodies tethered to microspheres and detected using fluorescently labeled detection antibodies specific to CD9, CD63 and CD81. **FIG. 8H** illustrates similar performance for detecting B7H3, CD63, CD9 or EpCam positive vesicles using a microsphere assay in a single-plex or multi-plex fashion. Vesicles were captured with antibodies to B7H3, CD63, CD9 or EpCam tethered to microspheres and detected using fluorescently labeled detection antibodies specific to CD9, CD63 and CD81.

[0043] **FIG. 9A** illustrates the ability of a vesicle bio-signature to discriminate between normal prostate and PCa samples. Cancer markers included EpCam and B7H3. General vesicle markers included CD9, CD81 and CD63. Prostate specific markers included PCSA. PSMA can be used as well as PCSA. The test was found to be 98% sensitive and 95% specific for PCa vs normal samples. **FIG. 9B** illustrates mean fluorescence intensity (MFI) on the Y axis for vesicle markers of **FIG. 9A** in normal and prostate cancer patients.

[0044] **FIG. 10** is a schematic for a decision tree for a vesicle prostate cancer assay for determining whether a sample is positive for prostate cancer.

[0045] **FIG. 11** shows the results of a vesicle detection assay for prostate cancer following the decision tree versus detection using elevated PSA levels.

[0046] **FIGs. 12A-12E** illustrate the use of microRNA to identify false negatives from a vesicle-based diagnostic assay for prostate cancer. **FIG. 12A** illustrates a scheme for using miR analysis within vesicles to convert false negatives into true positives, thereby improving sensitivity. **FIG. 12B** illustrates a scheme for using miR analysis within vesicles to convert false positives into true negatives, thereby improving specificity. Normalized levels of miR-107 (**FIG. 12C**) and miR-141 (**FIG. 12D**) are shown on the Y axis for true positives (TP) called by the vesicle diagnostic assay, true negatives (TN) called by the vesicle diagnostic assay, false positives (FP) called by the vesicle diagnostic assay, and false negatives (FN) called by the vesicle diagnostic assay. miR-107 and miR-141 can be used in the schematic shown in **FIG. 12A** and **FIG. 12B**. **FIG. 12E** shows Taqman qRT-PCR verification of increased miR-107 in plasma cMVs of prostate cancer patients compared to patients without prostate cancer using a different sample cohort.

[0047] **FIGs. 13A-D** illustrate KRAS sequencing in a colorectal cancer (CRC) cell line and patient sample. Samples comprise genomic DNA obtained from the cell line (**FIG. 13B**) or from a tissue sample from the patient (**FIG. 13D**), or cDNA obtained from RNA payload within vesicles shed from the cell line (**FIG. 13A**) or from a plasma sample from the patient (**FIG. 13C**).

[0048] **FIGs. 14A-B** illustrate immunoprecipitation of microRNA from human plasma. **FIG. 14A** shows the mean quantity of miR-16 detected in various fractions of human plasma. “Beads” are the amount of miR-16 that co-immunoprecipitated using antibodies to Argonaute2 (Ago2), Apolipoprotein A1 (ApoA1), GW182, and an IgG control. “Dyna” refers to immunoprecipitation using Dynabead Protein G, whereas “Magna” refers to Magnabind Protein G beads. “Supernt” are the amount of miR-16 detected in the supernatant of the immunoprecipitation reactions. See Examples for details. **FIG. 14B** is the same as **FIG. 14A** except that miR-92a was detected.

[0049] **FIG. 15** illustrates flow sorting of complexes stained with PE labeled anti-PCSA antibodies and FITC labeled anti-Ago2 antibodies.

[0050] **FIGs. 16A-D** illustrate detection of microRNA in PCSA/Ago2 positive complexes in human plasma samples. The plasma samples were from subjects with prostate cancer (PrC) or normal controls (normal). **FIG. 16A** shows miR-22 copy number in total circulating microvesicle population from human plasma. **FIG. 16B** shows plasma-derived complexes were sorted using antibodies against PCSA and Argonaute 2 (Ago2). RNA was isolated and the copy number of miR-22 was determined in the population of PCSA/Ago2 double positive events. **FIG. 16C** shows the number of PCSA/Ago2 double positive events counted by flow cytometry for each plasma sample. **FIG. 16D** shows copy number of miR-22 divided by the total number of PCSA/Ago2 positive events for each plasma sample. This yields the copy number of miR-22 per PCSA/Ago2 double positive complex.

[0051] **FIGs. 17A-F** illustrate dot plots of raw background subtracted fluorescence values of selected mRNAs from microarray profiling of vesicle mRNA payload levels. In each plot, the Y axis shows raw background subtracted fluorescence values (Raw BGsub Florescence). The X axis shows dot plots for four normal control plasmas and four plasmas from prostate cancer patients. The mRNAs shown are A2ML1 (**FIG. 17A**), GABARAPL2 (**FIG. 17B**), PTMA (**FIG. 17C**), RABAC1 (**FIG. 17D**), SOX1 (**FIG. 17E**), and ETFB (**FIG. 17F**).

[0052] **FIG. 18** shows detection of a standard curve for a synthetic miR16 standard (10^6 – 10^1) and detection of miR16 in triplicate from a human plasma sample. As indicated by the legend, the data was taken from a Fluidigm Biomark (Fluidigm Corporation, South San Francisco, CA) using 48.48 Dynamic Array™ IFCs, 96.96 Dynamic Array™ IFCs, or with an ABI 7900HT Taqman assay (Applied Biosystems, Foster City, CA). All levels were determined under multiplex conditions.

[0053] **FIGs. 19A-G** show levels of alkaline phosphatase (intestinal) (**FIG. 19A**), CD-56 (**FIG. 19B**), CD-3 zeta (**FIG. 19C**), map1b (**FIG. 19D**), 14.3.3 pan (**FIG. 19E**), filamin (**FIG. 19F**), and thrombospondin (**FIG. 19G**) associated with microvesicles from plasma of normal (non-cancer) control individuals, breast cancer patients, brain cancer patients, lung cancer patients, colorectal cancer patients, colon adenoma patients, BPH patients (benign), inflamed prostate patients (inflammation), HGPIN patients, and prostate cancer patients, as indicated in the figures. Vesicles were concentrated then incubated with antibody arrays. Vesicles bound to antibodies to various proteins were fluorescently detected.

FIG. 20A illustrates a protein gel demonstrating removal of HSA and antibody heavy and light chains in the indicated samples. The columns in the gel are as follows: “Raw” (Plasma without any treatment); “Conc” (Plasma concentrated via nanomembrane filtration); “FTp” (Plasma flow through from treatment with Pierce Albumin and IgG Removal Kit, Thermo Fisher Scientific Inc., Rockford, IL USA); “FTv” (Plasma flow through from treatment with Vivapure® Anti-HSA/IgG Kit from Sartorius Stedim North America Inc., Edgewood, NY USA); “IgG” (IgG control); “M” (molecular weight marker). **FIG. 20B** shows an example using the protocol to detect microvesicles. The cMVs were detected using Anti-MMP7-FITC antibody conjugate (Millipore anti-MMP7 monoclonal antibody 7B2). The plot shows the frequency of events detected versus concentration of the detection antibody. **FIG. 20C** shows EpCam expression in human serum albumin (HSA) depleted plasma sample. The x-axis refers to concentration of EpCam+ vesicles in various aliquots. The Y axis illustrates median fluorescent intensity (MFI) detected in a microbead assay using PE labeled anti-EpCAM antibodies to detect the vesicles. “Isotype” refers to detection using PE anti-IgG antibodies as a control. **FIG. 20D** is similar to **FIG. 20C** except that PE-labeled anti-MMP7 antibodies were used to detect the microvesicles. Shown are samples that were pre-treated to remove HSA (“HSA depleted”) or not (“HSA non-depleted”). “iso” refers to the anti-IgG antibody controls. **FIG. 20E** illustrates detection of vesicles in plasma after treatment with thromboplastin to precipitate fibrinogen. The Y axis illustrates median fluorescent intensity (MFI) detected in a microbead assay using bead-conjugated anti-KLK2 to capture the vesicles and a PE labeled anti-EpCAM aptamer to detect the vesicles. The X-axis groups 4 plasma samples (cancer sample C1, cancer sample C2, benign sample B1, benign sample B2) into 6 experimental conditions, V1-V6. As indicated by the thromboplastin incubation time and concentration below the plot, the thromboplastin treatment stringency increased from V1-V6.

[0054] **FIG. 21** illustrates the use of an anti-EpCAM aptamer (Aptamer 4; SEQ ID NO. 1) to detect a microvesicle population. Vesicles in patient plasma samples were captured using bead-conjugated antibodies to the indicated microvesicle surface antigens. Fluorescently labeled Aptamer 4 was used as a detector in the microbead assay. The

figure shows average median fluorescence values (MFI values) for three prostate cancer (C1-C3) and three normal samples (N1-N3) in each plot. In each plot, the samples from left to right are ordered as: C1, C2, C3, N1, N2, N3.

[0055] **FIGs. 22A-H** shows detection of KRAS mutations using circulating microvesicles in human peripheral blood samples. **FIGs. 22A-F** show the detection of KRAS mutations in mRNA using pyrosequencing (**FIG. 22A**, **FIG. 22C**, **FIG. 22E**) and Sanger sequencing (**FIG. 22B**, **FIG. 22D**, **FIG. 22F**), as indicated. The input RNA was provided at different levels to test the detection limits of the assay. The input levels tested were 1.6 µg of cMV / ml plasma (**FIG. 22A** and **FIG. 22B**), 0.78 µg of cMV / ml plasma (**FIG. 22C** and **FIG. 22D**), and 0.39 µg of cMV / ml plasma (**FIG. 22E** and **FIG. 22F**), as indicated. The arrows in **FIGs. 22A-F** indicate where the c.38G>A mutation is visible. **FIG. 22G** shows CD24 positive-gated events analyzed for CD63 expression. **FIG. 22H** shows a pyrogram for pyrosequencing mRNA isolated from the CD24+/CD63+ events as shown in **FIG. 22G**. The pyrogram in **FIG. 22H** shows a mutation detected in exon 2 of KRAS, c.35G>C, p.Gly12Ala (indicated by the arrow).

[0056] **FIGs. 23A-C** shows levels of Trail R3 (**FIG. 23A**), RAGE (**FIG. 23B**) and ErbB3 (**FIG. 23C**) associated wtih microvesicles from plasma of normal (non-cancer) individuals, prostate cancer (PCa) patients and colorectal cancer (CRC) patients.

[0057] **FIG. 24** shows levels of tetraspanins associated with microvesicles from plasma of normal (non-cancer) control individuals, colon adenoma patients, and colorectal cancer (CRC) patients as detected using antibody arrays. Vesicles were concentrated then incubated with antibody arrays. Vesicles bound to antibodies to various proteins were fluorescently detected.

[0058] **FIGs. 25A-D** show levels of 14.3.3 gamma (**FIG. 25A**), 14.3.3 pan (**FIG. 25B**), thrombospondin (**FIG. 25C**) and filamin (**FIG. 25D**) associated wtih microvesicles from plasma of normal (non-cancer) control individuals, colon adenoma patients, colorectal cancer (CRC) patients, and other cancers as indicated in the figures. Vesicles were concentrated then incubated with antibody arrays. Vesicles bound to antibodies to various proteins were fluorescently detected.

[0059] **FIGs. 26A-D** graphically illustrate the observed fold-changes for 14.3.3 gamma (**FIG. 26A**), 14.3.3, Pan (**FIG. 26B**), CD71 / Transferrin Receptor (**FIG. 26C**) and Ferritin (**FIG. 26D**) associated wtih microvesicles from plasma of normal (non-cancer) control individuals and melanoma patients. Vesicles were concentrated then incubated with antibody arrays. Vesicles bound to antibodies to various proteins were fluorescently detected.

[0060] **FIGs. 27A-E** graphically illustrate the observed fold-changes for 14.3.3 gamma (**FIG. 27A**), 14.3.3, Pan (**FIG. 27B**), filamin (**FIG. 27C**), CDK5 (**FIG. 27D**) and thymidine phosphorylase (**FIG. 27D**) associated wtih microvesicles from plasma of normal (non-cancer) control individuals and head and neck cancer patients. Vesicles were concentrated then incubated with antibody arrays. Vesicles bound to antibodies to various proteins were fluorescently detected.

DETAILED DESCRIPTION OF THE INVENTION

[0061] Disclosed herein are methods and systems for characterizing a phenotype of a biological sample, e.g., a sample from a cell culture, an organism, or a subject. The phenotype can be characterized by assessing one or more biomarkers. The biomarkers can be associated with a vesicle or vesicle population, either presented vesicle surface antigens or vesicle payload. As used herein, vesicle payload comprises entities encapsulated within a vesicle. Vesicle associated biomarkers can comprise both membrane bound and soluble biomarkers. The biomarkers can also be circulating biomarkers, such as nucleic acids (e.g., microRNA) or protein/polypeptide, or functional fragments thereof, assessed in a bodily fluid. Unless otherwise specified, the terms “purified” or “isolated” as used herein in reference to

vesicles or biomarker components mean partial or complete purification or isolation of such components from a cell or organism. Furthermore, unless otherwise specified, reference to vesicle isolation using a binding agent includes binding a vesicle with the binding agent whether or not such binding results in complete isolation of the vesicle apart from other biological entities in the starting material.

[0062] A method of characterizing a phenotype by analyzing a circulating biomarker, e.g., a nucleic acid biomarker, is depicted in scheme **6100A** of **FIG. 1A**, as a non-limiting illustrative example. In a first step **6101**, a biological sample is obtained, e.g., a bodily fluid, tissue sample or cell culture. Nucleic acids are isolated from the sample **6103**. The nucleic acid can be DNA or RNA, e.g., microRNA. Assessment of such nucleic acids can provide a biosignature for a phenotype. By sampling the nucleic acids associated with target phenotype (e.g., disease versus healthy, pre- and post-treatment), one or more nucleic acid markers that are indicative of the phenotype can be determined. Various aspects of the present invention are directed to biosignatures determined by assessing one or more nucleic acid molecules (e.g., microRNA) present in the sample **6105**, where the biosignature corresponds to a predetermined phenotype **6107**. **FIG. 1B** illustrates a scheme **6100B** of using vesicles to determine a biosignature and/or characterize a phenotype. In one example, a biological sample is obtained **6102**, and one or more vesicles of interest, e.g., all vesicles, or vesicles from a particular cell-of-origin and/or vesicles associated with a particular disease state, are isolated from the sample **6104**. The vesicles can be analyzed **6106** by characterizing surface antigens associated with the vesicles and/or determining the presence or levels of components present within the vesicles ("payload"). Unless specified otherwise, the term "antigen" as used herein refers generally to a biomarker that can be bound by a binding agent, whether the binding agent is an antibody, aptamer, lectin, or other binding agent for the biomarker and regardless of whether such biomarker illicits an immune response in a host. Vesicle payload including without limitation protein, including peptides and polypeptides, nucleic acids such as DNA and RNAs, lipids and/or carbohydrates. RNA payload includes messenger RNA (mRNA) and microRNA (also referred to herein as miRNA or miR). A phenotype is characterized based on the biosignature of the vesicles **6108**. In another illustrative method of the invention, schemes **6100A** and **6100B** are performed together to characterize a phenotype. In such a scheme, vesicles and nucleic acids, e.g., microRNA, are assessed, thereby characterizing the phenotype.

[0063] According to the methods of the invention, multiple biomarkers can be assessed sequentially or concurrently to characterize a phenotype. For example, a subpopulation of vesicles can be assessed by concurrently detecting two vesicle surface antigens, e.g., using binding agents to both capture and detect vesicles. In another example, a subpopulation of vesicles can be assessed by sequentially detecting a vesicle surface antigen, e.g., to capture vesicles, and then the captured vesicles can be assessed for payload such as mRNA, microRNA or soluble protein. In some embodiments, characterizing a phenotype comprises both the concurrent assessment of one or more biomarker and sequential assessment of one or more other biomarker. As a non-limiting example, a vesicle subpopulation that is detecting using binding agents to more than one surface antigen can be sorted, and then payload can be assessed, e.g., one or more miRs. One of skill will recognize that many variations of sequential or concurrent assessment of biomarkers can be used to characterize a phenotype.

[0064] In another related aspect, methods are provided herein for the discovery of biomarkers comprising assessing vesicle surface markers or payload markers in one sample and comparing the markers to another sample. Markers that distinguish between the samples can be used as biomarkers according to the invention. Such samples can be from a subject or group of subjects. For example, the groups can be, e.g., diseased versus normal (e.g., non-diseased), known responders and non-responders to a given treatment for a given disease or disorder. Biomarkers discovered to

distinguish the known responders and non-responders provide a biosignature of whether a subject is likely to respond to a treatment such as a therapeutic agent, e.g., a drug or biologic.

[0065] Following long-standing patent law convention, the terms "a", "an", and "the" refer to "one or more" when used in this application, including the claims. Thus, for example, reference to "a biomarker" includes a plurality of such biomarkers, and so forth.

[0066] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about". Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently disclosed subject matter.

[0067] As used herein, the term "about," e.g., when referring to a value or to an amount of mass, weight, time, volume, concentration or percentage is meant to encompass variations of in some embodiments ±20%, in some embodiments ± 10%, in some embodiments ±5%, in some embodiments ±1%, in some embodiments ±0.5%, and in some embodiments ±0.1 % from the specified amount, as such variations are appropriate to perform the disclosed methods. In embodiments, "about" refers to ± 10%.

Phenotypes

[0068] Disclosed herein are products and processes for characterizing a phenotype of an individual by analyzing a vesicle such as a membrane vesicle. A phenotype can be any observable characteristic or trait of a subject, such as a disease or condition, a disease stage or condition stage, susceptibility to a disease or condition, prognosis of a disease stage or condition, a physiological state, or response to therapeutics. A phenotype can result from a subject's gene expression as well as the influence of environmental factors and the interactions between the two, as well as from epigenetic modifications to nucleic acid sequences.

[0069] A phenotype in a subject can be characterized by obtaining a biological sample from a subject and analyzing one or more vesicles from the sample. For example, characterizing a phenotype for a subject or individual may include detecting a disease or condition (including pre-symptomatic early stage detecting), determining the prognosis, diagnosis, or theranosis of a disease or condition, or determining the stage or progression of a disease or condition. Characterizing a phenotype can also include identifying appropriate treatments or treatment efficacy for specific diseases, conditions, disease stages and condition stages, predictions and likelihood analysis of disease progression, particularly disease recurrence, metastatic spread or disease relapse. A phenotype can also be a clinically distinct type or subtype of a condition or disease, such as a cancer or tumor. Phenotype determination can also be a determination of a physiological condition, or an assessment of organ distress or organ rejection, such as post-transplantation. The products and processes described herein allow assessment of a subject on an individual basis, which can provide benefits of more efficient and economical decisions in treatment.

[0070] In an aspect, the invention relates to the analysis of a biological sample to identify a biosignature to predict whether a subject is likely to respond to a treatment for a disease or disorder. Characterizating a phenotype includes predicting the responder / non-responder status of the subject, wherein a responder responds to a treatment for a disease and a non-responder does not respond to the treatment. Vesicles can be analyzed in the subject and compared to vesicle analysis of previous subjects that were known to respond or not to a treatment. If the vesicle biosignature in a subject more closely aligns with that of previous subjects that were known to respond to the treatment, the subject can be characterized, or predicted, as a responder to the treatment. Similarly, if the vesicle biosignature in the subject more

closely aligns with that of previous subjects that did not respond to the treatment, the subject can be characterized, or predicted as a non-responder to the treatment. The treatment can be for any appropriate disease, disorder or other condition. The method can be used in any disease setting where a vesicle biosignature that correlates with responder / non-responder status is known.

[0071] The term "phenotype" as used herein can mean any trait or characteristic that is attributed to a vesicle biosignature that is identified using methods of the invention. For example, a phenotype can be the identification of a subject as likely to respond to a treatment, or more broadly, it can be a diagnostic, prognostic or theranostic determination based on a characterized biosignature for a sample obtained from a subject.

[0072] In some embodiments, the phenotype comprises a disease or condition such as those listed in **Table 1**. For example, the phenotype can comprise the presence of or likelihood of developing a tumor, neoplasm, or cancer. A cancer detected or assessed by products or processes described herein includes, but is not limited to, breast cancer, ovarian cancer, lung cancer, colon cancer, hyperplastic polyp, adenoma, colorectal cancer, high grade dysplasia, low grade dysplasia, prostatic hyperplasia, prostate cancer, melanoma, pancreatic cancer, brain cancer (such as a glioblastoma), hematological malignancy, hepatocellular carcinoma, cervical cancer, endometrial cancer, head and neck cancer, esophageal cancer, gastrointestinal stromal tumor (GIST), renal cell carcinoma (RCC) or gastric cancer. The colorectal cancer can be CRC Dukes B or Dukes C-D. The hematological malignancy can be B-Cell Chronic Lymphocytic Leukemia, B-Cell Lymphoma-DLBCL, B-Cell Lymphoma-DLBCL-germinal center-like, B-Cell Lymphoma-DLBCL-activated B-cell-like, and Burkitt's lymphoma.

[0073] The phenotype can be a premalignant condition, such as actinic keratosis, atrophic gastritis, leukoplakia, erythroplasia, Lymphomatoid Granulomatosis, preleukemia, fibrosis, cervical dysplasia, uterine cervical dysplasia, xeroderma pigmentosum, Barrett's Esophagus, colorectal polyp, or other abnormal tissue growth or lesion that is likely to develop into a malignant tumor. Transformative viral infections such as HIV and HPV also present phenotypes that can be assessed according to the invention.

[0074] The cancer characterized by the methods of the invention can comprise, without limitation, a carcinoma, a sarcoma, a lymphoma or leukemia, a germ cell tumor, a blastoma, or other cancers. Carcinomas include without limitation epithelial neoplasms, squamous cell neoplasms squamous cell carcinoma, basal cell neoplasms basal cell carcinoma, transitional cell papillomas and carcinomas, adenomas and adenocarcinomas (glands), adenoma, adenocarcinoma, linitis plastica insulinoma, glucagonoma, gastrinoma, vipoma, cholangiocarcinoma, hepatocellular carcinoma, adenoid cystic carcinoma, carcinoid tumor of appendix, prolactinoma, oncocytoma, hurthle cell adenoma, renal cell carcinoma, grawitz tumor, multiple endocrine adenomas, endometrioid adenoma, adnexal and skin appendage neoplasms, mucoepidermoid neoplasms, cystic, mucinous and serous neoplasms, cystadenoma, pseudomyxoma peritonei, ductal, lobular and medullary neoplasms, acinar cell neoplasms, complex epithelial neoplasms, warthin's tumor, thymoma, specialized gonadal neoplasms, sex cord stromal tumor, thecoma, granulosa cell tumor, arrhenoblastoma, sertoli leydig cell tumor, glomus tumors, paraganglioma, pheochromocytoma, glomus tumor, nevi and melanomas, melanocytic nevus, malignant melanoma, melanoma, nodular melanoma, dysplastic nevus, lentigo maligna melanoma, superficial spreading melanoma, and malignant acral lentiginous melanoma. Sarcoma includes without limitation Askin's tumor, botryodies, chondrosarcoma, Ewing's sarcoma, malignant hemangio endothelioma, malignant schwannoma, osteosarcoma, soft tissue sarcomas including: alveolar soft part sarcoma, angiosarcoma, cystosarcoma phyllodes, dermatofibrosarcoma, desmoid tumor, desmoplastic small round cell tumor, epithelioid sarcoma, extraskeletal chondrosarcoma, extraskeletal osteosarcoma, fibrosarcoma, hemangiopericytoma,

hemangiosarcoma, kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, lymphosarcoma, malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma, and synovialsarcoma. Lymphoma and leukemia include without limitation chronic lymphocytic leukemia/small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma (such as waldenström macroglobulinemia), splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, monoclonal immunoglobulin deposition diseases, heavy chain diseases, extranodal marginal zone B cell lymphoma, also called malt lymphoma, nodal marginal zone B cell lymphoma (nmzl), follicular lymphoma, mantle cell lymphoma, diffuse large B cell lymphoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, burkitt lymphoma/leukemia, T cell prolymphocytic leukemia, T cell large granular lymphocytic leukemia, aggressive NK cell leukemia, adult T cell leukemia/lymphoma, extranodal NK/T cell lymphoma, nasal type, enteropathy-type T cell lymphoma, hepatosplenic T cell lymphoma, blastic NK cell lymphoma, mycosis fungoides / sezary syndrome, primary cutaneous CD30-positive T cell lymphoproliferative disorders, primary cutaneous anaplastic large cell lymphoma, lymphomatoid papulosis, angioimmunoblastic T cell lymphoma, peripheral T cell lymphoma, unspecified, anaplastic large cell lymphoma, classical hodgkin lymphomas (nodular sclerosis, mixed cellularity, lymphocyte-rich, lymphocyte depleted or not depleted), and nodular lymphocyte-predominant hodgkin lymphoma. Germ cell tumors include without limitation germinoma, dysgerminoma, seminoma, nongerminomatous germ cell tumor, embryonal carcinoma, endodermal sinus turmor, choriocarcinoma, teratoma, polyembryoma, and gonadoblastoma. Blastoma includes without limitation nephroblastoma, medulloblastoma, and retinoblastoma. Other cancers include without limitation labial carcinoma, larynx carcinoma, hypopharynx carcinoma, tongue carcinoma, salivary gland carcinoma, gastric carcinoma, adenocarcinoma, thyroid cancer (medullary and papillary thyroid carcinoma), renal carcinoma, kidney parenchyma carcinoma, cervix carcinoma, uterine corpus carcinoma, endometrium carcinoma, chorion carcinoma, testis carcinoma, urinary carcinoma, melanoma, brain tumors such as glioblastoma, astrocytoma, meningioma, medulloblastoma and peripheral neuroectodermal tumors, gall bladder carcinoma, bronchial carcinoma, multiple myeloma, basalioma, teratoma, retinoblastoma, choroidea melanoma, seminoma, rhabdomyosarcoma, craniopharyngeoma, osteosarcoma, chondrosarcoma, myosarcoma, liposarcoma, fibrosarcoma, Ewing sarcoma, and plasmocytoma.

[0075] In a further embodiment, the cancer under analysis may be a lung cancer including non-small cell lung cancer and small cell lung cancer (including small cell carcinoma (oat cell cancer), mixed small cell/large cell carcinoma, and combined small cell carcinoma), colon cancer, breast cancer, prostate cancer, liver cancer, pancreas cancer, brain cancer, kidney cancer, ovarian cancer, stomach cancer, skin cancer, bone cancer, gastric cancer, breast cancer, pancreatic cancer, glioma, glioblastoma, hepatocellular carcinoma, papillary renal carcinoma, head and neck squamous cell carcinoma, leukemia, lymphoma, myeloma, or a solid tumor.

[0076] In embodiments, the cancer comprises an acute lymphoblastic leukemia; acute myeloid leukemia; adrenocortical carcinoma; AIDS-related cancers; AIDS-related lymphoma; anal cancer; appendix cancer; astrocytomas; atypical teratoid/rhabdoid tumor; basal cell carcinoma; bladder cancer; brain stem glioma; brain tumor (including brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma); breast cancer; bronchial tumors; Burkitt lymphoma; cancer of unknown primary site; carcinoid tumor; carcinoma of unknown primary site; central nervous system atypical teratoid/rhabdoid tumor; central nervous system embryonal tumors; cervical cancer; childhood cancers; chordoma; chronic lymphocytic leukemia; chronic myelogenous

leukemia; chronic myeloproliferative disorders; colon cancer; colorectal cancer; craniopharyngioma; cutaneous T-cell lymphoma; endocrine pancreas islet cell tumors; endometrial cancer; ependymoblastoma; ependymoma; esophageal cancer; esthesioneuroblastoma; Ewing sarcoma; extracranial germ cell tumor; extragonadal germ cell tumor; extrahepatic bile duct cancer; gallbladder cancer; gastric (stomach) cancer; gastrointestinal carcinoid tumor; gastrointestinal stromal cell tumor; gastrointestinal stromal tumor (GIST); gestational trophoblastic tumor; glioma; hairy cell leukemia; head and neck cancer; heart cancer; Hodgkin lymphoma; hypopharyngeal cancer; intraocular melanoma; islet cell tumors; Kaposi sarcoma; kidney cancer; Langerhans cell histiocytosis; laryngeal cancer; lip cancer; liver cancer; malignant fibrous histiocytoma bone cancer; medulloblastoma; medulloepithelioma; melanoma; Merkel cell carcinoma; Merkel cell skin carcinoma; mesothelioma; metastatic squamous neck cancer with occult primary; mouth cancer; multiple endocrine neoplasia syndromes; multiple myeloma; multiple myeloma/plasma cell neoplasm; mycosis fungoides; myelodysplastic syndromes; myeloproliferative neoplasms; nasal cavity cancer; nasopharyngeal cancer; neuroblastoma; Non-Hodgkin lymphoma; nonmelanoma skin cancer; non-small cell lung cancer; oral cancer; oral cavity cancer; oropharyngeal cancer; osteosarcoma; other brain and spinal cord tumors; ovarian cancer; ovarian epithelial cancer; ovarian germ cell tumor; ovarian low malignant potential tumor; pancreatic cancer; papillomatosis; paranasal sinus cancer; parathyroid cancer; pelvic cancer; penile cancer; pharyngeal cancer; pineal parenchymal tumors of intermediate differentiation; pineoblastoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; pleuropulmonary blastoma; primary central nervous system (CNS) lymphoma; primary hepatocellular liver cancer; prostate cancer; rectal cancer; renal cancer; renal cell (kidney) cancer; renal cell cancer; respiratory tract cancer; retinoblastoma; rhabdomyosarcoma; salivary gland cancer; Sézary syndrome; small cell lung cancer; small intestine cancer; soft tissue sarcoma; squamous cell carcinoma; squamous neck cancer; stomach (gastric) cancer; supratentorial primitive neuroectodermal tumors; T-cell lymphoma; testicular cancer; throat cancer; thymic carcinoma; thymoma; thyroid cancer; transitional cell cancer; transitional cell cancer of the renal pelvis and ureter; trophoblastic tumor; ureter cancer; urethral cancer; uterine cancer; uterine sarcoma; vaginal cancer; vulvar cancer; Waldenström macroglobulinemia; or Wilm's tumor. The methods of the invention can be used to characterize these and other cancers. Thus, characterizing a phenotype can be providing a diagnosis, prognosis or theranosis of one of the cancers disclosed herein.

[0077] The phenotype can also be an inflammatory disease, immune disease, or autoimmune disease. For example, the disease may be inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), pelvic inflammation, vasculitis, psoriasis, diabetes, autoimmune hepatitis, Multiple Sclerosis, Myasthenia Gravis, Type I diabetes, Rheumatoid Arthritis, Psoriasis, Systemic Lupus Erythematosis (SLE), Hashimoto's Thyroiditis, Grave's disease, Ankylosing Spondylitis Sjogrens Disease, CREST syndrome, Scleroderma, Rheumatic Disease, organ rejection, Primary Sclerosing Cholangitis, or sepsis.

[0078] The phenotype can also comprise a cardiovascular disease, such as atherosclerosis, congestive heart failure, vulnerable plaque, stroke, or ischemia. The cardiovascular disease or condition can be high blood pressure, stenosis, vessel occlusion or a thrombotic event.

[0079] The phenotype can also comprise a neurological disease, such as Multiple Sclerosis (MS), Parkinson's Disease (PD), Alzheimer's Disease (AD), schizophrenia, bipolar disorder, depression, autism, Prion Disease, Pick's disease, dementia, Huntington disease (HD), Down's syndrome, cerebrovascular disease, Rasmussen's encephalitis, viral meningitis, neurospsychiatric systemic lupus erythematosus (NPSLE), amyotrophic lateral sclerosis, Creutzfeldt-Jacob disease, Gerstmann-Straussler-Scheinker disease, transmissible spongiform encephalopathy, ischemic reperfusion

damage (e.g. stroke), brain trauma, microbial infection, or chronic fatigue syndrome. The phenotype may also be a condition such as fibromyalgia, chronic neuropathic pain, or peripheral neuropathic pain.

[0080] The phenotype may also comprise an infectious disease, such as a bacterial, viral or yeast infection. For example, the disease or condition may be Whipple's Disease, Prion Disease, cirrhosis, methicillin-resistant staphylococcus aureus, HIV, hepatitis, syphilis, meningitis, malaria, tuberculosis, or influenza. Viral proteins, such as HIV or HCV-like particles can be assessed in a vesicle, to characterize a viral condition.

[0081] The phenotype can also comprise a perinatal or pregnancy related condition (e.g. preeclampsia or preterm birth), metabolic disease or condition, such as a metabolic disease or condition associated with iron metabolism. For example, hepcidin can be assayed in a vesicle to characterize an iron deficiency. The metabolic disease or condition can also be diabetes, inflammation, or a perinatal condition.

[0082] The methods of the invention can be used to characterize these and other diseases and disorders that can be assessed via a candidate biosignature comprising one or a plurality of biomarkers. Thus, characterizing a phenotype can be providing a diagnosis, prognosis or theranosis of one of the diseases and disorders disclosed herein.

[0083] In various embodiments of the invention, a biosignature for any of the conditions or diseases disclosed herein can comprise one or more biomarkers in one of several different categories of markers, wherein the categories include one or more of: 1) disease specific biomarkers; 2) cell- or tissue-specific biomarkers; 3) vesicle-specific markers (e.g., general vesicle biomarkers); 4. angiogenesis-specific biomarkers; and 5) immunomodulatory biomarkers. Examples of such markers for use in methods and compositions of the invention are disclosed herein. Furthermore, a biomarker that is characterized to have a role in a particular disease or condition can be adapted for use as a target in compositions and methods of the invention. In further embodiments, such biomarkers can be all vesicle surface markers, or a combination of vesicle surface markers and vesicle payload markers (i.e., molecules enclosed by a vesicle). In addition, as noted herein, the biological sample assessed can be any biological fluid, or can comprise individual components present within such biological fluid (e.g., vesicles, nucleic acids, proteins, or complexes thereof).

Subject

[0084] One or more phenotypes of a subject can be determined by analyzing one or more vesicles, such as vesicles, in a biological sample obtained from the subject. A subject or patient can include, but is not limited to, mammals such as bovine, avian, canine, equine, feline, ovine, porcine, or primate animals (including humans and non-human primates). A subject can also include a mammal of importance due to being endangered, such as a Siberian tiger; or economic importance, such as an animal raised on a farm for consumption by humans, or an animal of social importance to humans, such as an animal kept as a pet or in a zoo. Examples of such animals include, but are not limited to, carnivores such as cats and dogs; swine including pigs, hogs and wild boars; ruminants or ungulates such as cattle, oxen, sheep, giraffes, deer, goats, bison, camels or horses. Also included are birds that are endangered or kept in zoos, as well as fowl and more particularly domesticated fowl, i.e. poultry, such as turkeys and chickens, ducks, geese, guinea fowl. Also included are domesticated swine and horses (including race horses). In addition, any animal species connected to commercial activities are also included such as those animals connected to agriculture and aquaculture and other activities in which disease monitoring, diagnosis, and therapy selection are routine practice in husbandry for economic productivity and/or safety of the food chain.

[0085] The subject can have a pre-existing disease or condition, such as cancer. Alternatively, the subject may not have any known pre-existing condition. The subject may also be non-responsive to an existing or past treatment, such as a treatment for cancer.

Samples

[0086] The biological sample obtained from the subject can be any bodily or biological fluid. For example, the biological sample can be any biological fluid including but not limited to peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen (including prostatic fluid), Cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates or other lavage fluids. A biological sample may also include the blastocyl cavity, umbilical cord blood, or maternal circulation which may be of fetal or maternal origin. The biological sample may also be a tissue sample or biopsy from which vesicles and other circulating biomarkers may be obtained. For example, cells from the sample can be cultured and vesicles isolated from the culture (see **Examples**). In various embodiments, biomarkers and/or biosignatures disclosed herein can be assessed directly from such biological samples (e.g., identification of presence or levels of nucleic acid or polypeptide biomarkers or functional fragments thereof) using various methods, such as extraction of nucleic acid molecules from blood, plasma, serum or any of the foregoing biological samples, use of protein or antibody arrays to identify polypeptide (or functional fragment) biomarker(s), as well as other array, sequencing, PCR and proteomic techniques known in the art for identification and assessment of nucleic acid and polypeptide molecules. In addition, one or more components present in such samples can be first isolated or enriched and further processed to assess the presence or levels of selected biomarkers, e.g., to assess a given biosignature. For example, microvesicles can be isolated from a sample prior to profiling the microvesicles for protein and/or nucleic acid biomarkers.

[0087] **Table 1** lists illustrative examples of diseases, conditions, or biological states and a corresponding list of biological samples from which vesicles may be analyzed.

Table 1: Examples of Biological Samples for Vesicle Analysis for Various Diseases, Conditions, or Biological States

Illustrative Disease, Condition or Biological State	Illustrative Biological Samples
Cancers/neoplasms affecting the following tissue types/bodily systems: breast, lung, ovarian, colon, rectal, prostate, pancreatic, brain, bone, connective tissue, glands, skin, lymph, nervous system, endocrine, germ cell, genitourinary, hematologic/blood, bone marrow, muscle, eye, esophageal, fat tissue, thyroid, pituitary, spinal cord, bile duct, heart, gall bladder, bladder, testes, cervical, endometrial, renal, ovarian, digestive/gastrointestinal, stomach, head and neck, liver, leukemia, respiratory/thorasic, cancers of unknown primary (CUP)	Blood, serum, plasma, cerebrospinal fluid (CSF), urine, sputum, ascites, synovial fluid, semen, nipple aspirates, saliva, bronchoalveolar lavage fluid, tears, oropharyngeal washes, feces, peritoneal fluids, pleural effusion, sweat, tears, aqueous humor, pericardial fluid, lymph, chyme, chyle, bile, stool water, amniotic fluid, breast milk, pancreatic juice, cerumen, Cowper's fluid or pre-ejaculatory fluid, female ejaculate, interstitial fluid, menses, mucus, pus, sebum, vaginal lubrication, vomit
Neurodegenerative/neurological disorders: Parkinson's disease, Alzheimer's Disease and multiple sclerosis, Schizophrenia, and bipolar disorder, spasticity disorders, epilepsy	Blood, serum, plasma, CSF, urine
Cardiovascular Disease: atherosclerosis, cardiomyopathy, endocarditis, vunerable plaques, infection	Blood, serum, plasma, CSF, urine
Stroke: ischemic, intracerebral hemorrhage, subarachnoid hemorrhage, transient ischemic attacks (TIA)	Blood, serum, plasma, CSF, urine
Pain disorders: peripheral neuropathic pain and chronic neuropathic pain, and fibromyalgia,	Blood, serum, plasma, CSF, urine
Autoimmune disease: systemic and localized diseases, rheumatic disease, Lupus, Sjogren's syndrome	Blood, serum, plasma, CSF, urine, synovial fluid

Digestive system abnormalities: Barrett's esophagus, irritable bowel syndrome, ulcerative colitis, Crohn's disease, Diverticulosis and Diverticulitis, Celiac Disease	Blood, serum, plasma, CSF, urine
Endocrine disorders: diabetes mellitus, various forms of Thyroiditis, adrenal disorders, pituitary disorders	Blood, serum, plasma, CSF, urine
Diseases and disorders of the skin: psoriasis	Blood, serum, plasma, CSF, urine, synovial fluid, tears
Urological disorders: benign prostatic hypertrophy (BPH), polycystic kidney disease, interstitial cystitis	Blood, serum, plasma, urine
Hepatic disease/injury: Cirrhosis, induced hepatotoxicity (due to exposure to natural or synthetic chemical sources)	Blood, serum, plasma, urine
Kidney disease/injury: acute, sub-acute, chronic conditions, Podocyte injury, focal segmental glomerulosclerosis	Blood, serum, plasma, urine
Endometriosis	Blood, serum, plasma, urine, vaginal fluids
Osteoporosis	Blood, serum, plasma, urine, synovial fluid
Pancreatitis	Blood, serum, plasma, urine, pancreatic juice
Asthma	Blood, serum, plasma, urine, sputum, bronchiolar lavage fluid
Allergies	Blood, serum, plasma, urine, sputum, bronchiolar lavage fluid
Prion-related diseases	Blood, serum, plasma, CSF, urine
Viral Infections: HIV/AIDS	Blood, serum, plasma, urine
Sepsis	Blood, serum, plasma, urine, tears, nasal lavage
Organ rejection/transplantation	Blood, serum, plasma, urine, various lavage fluids
Differentiating conditions: adenoma versus hyperplastic polyp, irritable bowel syndrome (IBS) versus normal, classifying Dukes stages A, B, C, and/or D of colon cancer, adenoma with low-grade hyperplasia versus high-grade hyperplasia, adenoma versus normal, colorectal cancer versus normal, IBS versus. ulcerative colitis (UC) versus Crohn's disease (CD),	Blood, serum, plasma, urine, sputum, feces, colonic lavage fluid
Pregnancy related physiological states, conditions, or affiliated diseases: genetic risk, adverse pregnancy outcomes	Maternal serum, plasma, amniotic fluid, cord blood

[0088] The methods of the invention can be used to characterize a phenotype using a blood sample or blood derivative. Blood derivatives include plasma and serum. Blood plasma is the liquid component of whole blood, and makes up approximately 55% of the total blood volume. It is composed primarily of water with small amounts of minerals, salts, ions, nutrients, and proteins in solution. In whole blood, red blood cells, leukocytes, and platelets are suspended within the plasma. Blood serum refers to blood plasma without fibrinogen or other clotting factors (i.e., whole blood minus both the cells and the clotting factors).

[0089] The biological sample may be obtained through a third party, such as a party not performing the analysis of the biomarkers, whether direct assessment of a biological sample or by profiling one or more vesicles obtained from the biological sample. For example, the sample may be obtained through a clinician, physician, or other health care manager of a subject from which the sample is derived. Alternatively, the biological sample may obtained by the same party analyzing the vesicle. In addition, biological samples be assayed, are archived (e.g., frozen) or ortherwise stored in under preservative conditions.

[0090] The volume of the biological sample used for biomarker analysis can be in the range of between 0.1-20 mL, such as less than about 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0.1 mL.

[0091] A sample of bodily fluid can be used as a sample for characterizing a phenotype. For example, biomarkers in the sample can be assessed to provide a diagnosis, prognosis and/or theranosis of a disease. The biomarkers can be circulating biomarkers, such as circulating proteins or nucleic acids. The biomarkers can also be associated with a vesicle or vesicle population. Methods of the invention can be applied to assess one or more vesicles, as well as one or more different vesicle populations that may be present in a biological sample or in a subject. Analysis of one or more biomarkers in a biological sample can be used to determine whether an additional biological sample should be obtained for analysis. For example, analysis of one or more vesicles in a sample of bodily fluid can aid in determining whether a tissue biopsy should be obtained.

[0092] A sample from a patient can be collected under conditions that preserve the circulating biomarkers and other entities of interest contained therein for subsequent analysis. In an embodiment, the samples are processed using one or more of CellSave Preservative Tubes (Veridex, North Raritan, NJ), PAXgene Blood DNA Tubes (QIAGEN GmbH, Germany), and RNAlater (QIAGEN GmbH, Germany).

[0093] CellSave Preservative Tubes (CellSave tubes) are sterile evacuated blood collection tubes. Each tube contains a solution that contains Na2EDTA and a cell preservative. The EDTA absorbs calcium ions, which can reduce or eliminate blood clotting. The preservative preserves the morphology and cell surface antigen expression of epithelial and other cells. The collection and processing can be performed as described in a protocol provided by the manufacturer. Each tube is evacuated to withdraw venous whole blood following standard phlebotomy procedures as known to those of skill in the art. CellSave tubes are disclosed in US Patent Numbers 5,466,574; 5,512,332; 5,597,531; 5,698,271; 5,985,153; 5,993,665; 6,120,856; 6,136,182; 6,365,362; 6,551,843; 6,620,627; 6,623,982; 6,645,731; 6,660,159; 6,790,366; 6,861,259; 6,890,426; 7,011,794; 7,282,350; 7,332,288; 5,849,517 and 5,459,073, each of which is incorporated by reference in its entirety herein.

[0094] The PAXgene Blood DNA Tube (PAXgene tube) is a plastic, evacuated tube for the collection of whole blood for the isolation of nucleic acids. The tubes can be used for blood collection, transport and storage of whole blood specimens and isolation of nucleic acids contained therein, e.g., DNA or RNA. Blood is collected under a standard phlebotomy protocol into an evacuated tube that contains an additive. The collection and processing can be performed as described in a protocol provided by the manufacturer. PAXgene tubes are disclosed in US Patent Nos. 5,906,744; 4,741,446; 4,991,104, each of which is incorporated by reference in its entirety herein.

[0095] The RNAlater RNA Stabilization Reagent (RNAlater) is used for immediate stabilization of RNA in tissues. RNA can be unstable in harvested samples. The aqueous RNAlater reagent permeates tissues and other biological samples, thereby stabilizing and protecting the RNA contained therein. Such protection helps ensure that downstream analyses reflect the expression profile of the RNA in the tissue or other sample. The samples are submerged in an appropriate volume of RNAlater reagent immediately after harvesting. The collection and processing can be performed as described in a protocol provided by the manufacturer. According to the manufacturer, the reagent preserves RNA for up to 1 day at 37°C, 7 days at 18–25°C, or 4 weeks at 2–8°C, allowing processing, transportation, storage, and shipping of samples without liquid nitrogen or dry ice. The samples can also be placed at –20°C or –80°C, e.g., for archival storage. The preserved samples can be used to analyze any type of RNA, including without limitation total RNA, mRNA, and microRNA. RNAlater can also be useful for collecting samples for DNA, RNA and protein analysis. RNAlater is disclosed in US Patent Nos. 5,346,994, each of which is incorporated by reference in its entirety herein.

[0096] Unless otherwise specified, the biological sample of the invention is understood to comprise a sample containing a separated, depleted, enriched, isolated, or otherwise processed derivative of another biological sample. As

a non-limiting example, a component of a patient sample or a cell culture can be isolated from the patient sample or the cell culture and resuspended in a buffer for further analysis. One of skill will appreciate that the derivative component suspended in the buffer is a biological sample that can be assessed according to the methods of the invention. The component can be any useful biological entity as disclosed herein or known in the art, including without limitation circulating biomarkers, vesicles, proteins, nucleic acids, lipids or carbohydrates. The biological sample can be the biological entity, including without limitation circulating biomarkers, vesicles, proteins, nucleic acids, lipids or carbohydrates.

Vesicles

[0097] Methods of the invention can include assessing one or more vesicles, including assessing vesicle populations. A vesicle, as used herein, is a membrane vesicle that is shed from cells. Vesicles or membrane vesicles include without limitation: circulating microvesicles (cMVs), microvesicle, exosome, nanovesicle, dexosome, bleb, blebby, prostasome, microparticle, intralumenal vesicle, membrane fragment, intralumenal endosomal vesicle, endosomal-like vesicle, exocytosis vehicle, endosome vesicle, endosomal vesicle, apoptotic body, multivesicular body, secretory vesicle, phospholipid vesicle, liposomal vesicle, argosome, texasome, secresome, tolerosome, melanosome, oncosome, or exocytosed vehicle. Furthermore, although vesicles may be produced by different cellular processes, the methods of the invention are not limited to or reliant on any one mechanism, insofar as such vesicles are present in a biological sample and are capable of being characterized by the methods disclosed herein. Unless otherwise specified, methods that make use of a species of vesicle can be applied to other types of vesicles. Vesicles comprise spherical structures with a lipid bilayer similar to cell membranes which surrounds an inner compartment which can contain soluble components, sometimes referred to as the payload. In some embodiments, the methods of the invention make use of exosomes, which are small secreted vesicles of about 40–100 nm in diameter. For a review of membrane vesicles, including types and characterizations, see *Thery et al., Nat Rev Immunol. 2009 Aug;9(8):581-93*. Some properties of different types of vesicles include those in **Table 2**:

Table 2: Vesicle Properties

Feature	Exosomes	Microvesicles	Ectosomes	Membrane particles	Exosome-like vesicles	Apoptotic vesicles
Size	50-100 nm	100-1,000 nm	50-200 nm	50-80 nm	20-50 nm	50-500 nm
Density in sucrose	1.13-1.19 g/ml			1.04-1.07 g/ml	1.1 g/ml	1.16-1.28 g/ml
EM appearance	Cup shape	Irregular shape, electron dense	Bilamellar round structures	Round	Irregular shape	Heterogeneous
Sedimentation	100,000 g	10,000 g	160,000-200,000 g	100,000-200,000 g	175,000 g	1,200 g, 10,000 g, 100,000 g
Lipid composition	Enriched in cholesterol, sphingomyelin and ceramide; contains lipid rafts; expose PPS	Expose PPS	Enriched in cholesterol and diacylglycerol; expose PPS		No lipid rafts	
Major protein markers	Tetraspanins (e.g., CD63, CD9), Alix, TSG101	Integrins, selectins and CD40 ligand	CR1 and proteolytic enzymes; no CD63	CD133; no CD63	TNFRI	Histones

Intracellular origin	Internal compartments (endosomes)	Plasma membrane	Plasma membrane	Plasma membrane		

Abbreviations: phosphatidylserine (PPS); electron microscopy (EM)

[0098] Vesicles include shed membrane bound particles, or "microparticles," that are derived from either the plasma membrane or an internal membrane. Vesicles can be released into the extracellular environment from cells. Cells releasing vesicles include without limitation cells that originate from, or are derived from, the ectoderm, endoderm, or mesoderm. The cells may have undergone genetic, environmental, and/or any other variations or alterations. For example, the cell can be tumor cells. A vesicle can reflect any changes in the source cell, and thereby reflect changes in the originating cells, e.g., cells having various genetic mutations. In one mechanism, a vesicle is generated intracellularly when a segment of the cell membrane spontaneously invaginates and is ultimately exocytosed (see for example, *Keller et al., Immunol. Lett. 107 (2): 102–8 (2006)*). Vesicles also include cell-derived structures bounded by a lipid bilayer membrane arising from both herniated evagination (blebbing) separation and sealing of portions of the plasma membrane or from the export of any intracellular membrane-bounded vesicular structure containing various membrane-associated proteins of tumor origin, including surface-bound molecules derived from the host circulation that bind selectively to the tumor-derived proteins together with molecules contained in the vesicle lumen, including but not limited to tumor-derived microRNAs or intracellular proteins. Blebs and blebbing are further described in *Charras et al., Nature Reviews Molecular and Cell Biology, Vol. 9, No. 11, p. 730-736 (2008)*. A vesicle shed into circulation or bodily fluids from tumor cells may be referred to as a "circulating tumor-derived vesicle." When such vesicle is an exosome, it may be referred to as a circulating-tumor derived exosome (CTE). In some instances, a vesicle can be derived from a specific cell of origin. CTE, as with a cell-of-origin specific vesicle, typically have one or more unique biomarkers that permit isolation of the CTE or cell-of-origin specific vesicle, e.g., from a bodily fluid and sometimes in a specific manner. For example, a cell or tissue specific markers are used to identify the cell of origin. Examples of such cell or tissue specific markers are disclosed herein and can further be accessed in the Tissue-specific Gene Expression and Regulation (TiGER) Database, available at bioinfo.wilmer.jhu.edu/tiger/; Liu et al. (2008) TiGER: a database for tissue-specific gene expression and regulation. BMC Bioinformatics. 9:271; TissueDistributionDBs, available at genome.dkfz-heidelberg.de/menu/tissue_db/index.html.

[0099] A vesicle can have a diameter of greater than about 10 nm, 20 nm, or 30 nm. A vesicle can have a diameter of greater than 40 nm, 50 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1500 nm, 2000 nm or greater than 10,000 nm. A vesicle can have a diameter of about 20-2000 nm, about 20-1500 nm, about 30-1000 nm, about 30-800 nm, about 30-200 nm, or about 30-100 nm. In some embodiments, the vesicle has a diameter of less than 10,000 nm, 2000 nm,1500 nm, 1000 nm, 800 nm, 500 nm, 200 nm, 100 nm, 50 nm, 40 nm, 30 nm, 20 nm or less than 10 nm. As used herein the term "about" in reference to a numerical value means that variations of 10% above or below the numerical value are within the range ascribed to the specified value. Typical sizes for various types of vesicles are shown in **Table 2**. Vesicles can be assessed to measure the diameter of a single vesicle or any number of vesicles. For example, the range of diameters of a vesicle population or an average diameter of a vesicle population can be determined. Vesicle diameter can be assessed using methods known in the art, e.g., imaging technologies such as electron microscopy. In an embodiment, a diameter of one or more vesicles is determined using optical particle detection. See, e.g., U.S. Patent 7,751,053, entitled "Optical Detection and Analysis of Particles" and issued July 6, 2010; and U.S. Patent 7,399,600, entitled "Optical Detection and Analysis of Particles" and issued July 15, 2010.

[00100] In some embodiments, vesicles are directly assayed from a biological sample without prior isolation, purification, or concentration from the biological sample. For example, the amount of vesicles in the sample can by itself provide a biosignature that provides a diagnostic, prognostic or theranostic determination. Alternatively, the vesicle in the sample may be isolated, captured, purified, or concentrated from a sample prior to analysis. As noted, isolation, capture or purification as used herein comprises partial isolation, partial capture or partial purification apart from other components in the sample. Vesicle isolation can be performed using various techniques as described herein, e.g., chromatography, filtration, centrifugation, flow cytometry, affinity capture (e.g., to a planar surface or bead), and/or using microfluidics.

[00101] Vesicles such as exosomes can be assessed to provide a phenotypic characterization by comparing vesicle characteristics to a reference. In some embodiments, surface antigens on a vesicle are assessed. The surface antigens can provide an indication of the anatomical origin and/or cellular of the vesicles and other phenotypic information, e.g., tumor status. For example, wherein vesicles found in a patient sample, e.g., a bodily fluid such as blood, serum or plasma, are assessed for surface antigens indicative of colorectal origin and the presence of cancer. The surface antigens may comprise any informative biological entity that can be detected on the vesicle membrane surface, including without limitation surface proteins, lipids, carbohydrates, and other membrane components. For example, positive detection of colon derived vesicles expressing tumor antigens can indicate that the patient has colorectal cancer. As such, methods of the invention can be used to characterize any disease or condition associated with an anatomical or cellular origin, by assessing, for example, disease-specific and cell-specific biomarkers of one or more vesicles obtained from a subject.

[00102] In another embodiment, one or more vesicle payloads are assessed to provide a phenotypic characterization. The payload with a vesicle comprises any informative biological entity that can be detected as encapsulated within the vesicle, including without limitation proteins and nucleic acids, e.g., genomic or cDNA, mRNA, or functional fragments thereof, as well as microRNAs (miRs). In addition, methods of the invention are directed to detecting vesicle surface antigens (in addition or exclusive to vesicle payload) to provide a phenotypic characterization. For example, vesicles can be characterized by using binding agents (e.g., antibodies or aptamers) that are specific to vesicle surface antigens, and the bound vesicles can be further assessed to identify one or more payload components disclosed therein. As described herein, the levels of vesicles with surface antigens of interest or with payload of interest can be compared to a reference to characterize a phenotype. For example, overexpression in a sample of cancer-related surface antigens or vesicle payload, e.g., a tumor associated mRNA or microRNA, as compared to a reference, can indicate the presence of cancer in the sample. The biomarkers assessed can be present or absent, increased or reduced based on the selection of the desired target sample and comparison of the target sample to the desired reference sample. Non-limiting examples of target samples include: disease; treated/not-treated; different time points, such as a in a longitudinal study; and non-limiting examples of reference sample: non-disease; normal; different time points; and sensitive or resistant to candidate treatment(s).

MicroRNA

[00103] Various biomarker molecules can be assessed in biological samples or vesicles obtained from such biological samples. MicroRNAs comprise one class biomarkers assessed via methods of the invention. MicroRNAs, also referred to herein as miRNAs or miRs, are short RNA strands approximately 21-23 nucleotides in length. MiRNAs are encoded by genes that are transcribed from DNA but are not translated into protein and thus comprise non-coding RNA. The miRs are processed from primary transcripts known as pri-miRNA to short stem-loop structures called pre-miRNA and

finally to the resulting single strand miRNA. The pre-miRNA typically forms a structure that folds back on itself in self-complementary regions. These structures are then processed by the nuclease Dicer in animals or DCL1 in plants. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules and can function to regulate translation of proteins. Identified sequences of miRNA can be accessed at publicly available databases, such as www.microRNA.org, www.mirbase.org, or www.mirz.unibas.ch/cgi/miRNA.cgi.

[00104] miRNAs are generally assigned a number according to the naming convention " mir-[number]." The number of a miRNA is assigned according to its order of discovery relative to previously identified miRNA species. For example, if the last published miRNA was mir-121, the next discovered miRNA will be named mir-122, etc. When a miRNA is discovered that is homologous to a known miRNA from a different organism, the name can be given an optional organism identifier, of the form [organism identifier]- mir-[number]. Identifiers include hsa for Homo sapiens and mmu for Mus Musculus. For example, a human homolog to mir-121 might be referred to as hsa-mir-121 whereas the mouse homolog can be referred to as mmu-mir-121 and the rat homolog can be referred to as rno-mir-121, etc.

[00105] Mature microRNA is commonly designated with the prefix "miR" whereas the gene or precursor miRNA is designated with the prefix "mir." For example, mir-121 is a precursor for miR-121. When differing miRNA genes or precursors are processed into identical mature miRNAs, the genes/precursors can be delineated by a numbered suffix. For example, mir-121-1 and mir-121-2 can refer to distinct genes or precursors that are processed into miR-121. Lettered suffixes are used to indicate closely related mature sequences. For example, mir-121a and mir-121b can be processed to closely related miRNAs miR-121a and miR-121b, respectively. In the context of the invention, any microRNA (miRNA or miR) designated herein with the prefix mir-* or miR-* is understood to encompass both the precursor and/or mature species, unless otherwise explicitly stated otherwise.

[00106] Sometimes it is observed that two mature miRNA sequences originate from the same precursor. When one of the sequences is more abundant that the other, a "*" suffix can be used to designate the less common variant. For example, miR-121 would be the predominant product whereas miR-121* is the less common variant found on the opposite arm of the precursor. If the predominant variant is not identified, the miRs can be distinguished by the suffix "5p" for the variant from the 5' arm of the precursor and the suffix "3p" for the variant from the 3' arm. For example, miR-121-5p originates from the 5' arm of the precursor whereas miR-121-3p originates from the 3' arm. Less commonly, the 5p and 3p variants are referred to as the sense ("s") and anti-sense ("as") forms, respectively. For example, miR-121-5p may be referred to as miR-121-s whereas miR-121-3p may be referred to as miR-121-as.

[00107] The above naming conventions have evolved over time and are general guidelines rather than absolute rules. For example, the let- and lin- families of miRNAs continue to be referred to by these monikers. The mir/miR convention for precursor/mature forms is also a guideline and context should be taken into account to determine which form is referred to. Further details of miR naming can be found at www.mirbase.org or Ambros et al., A uniform system for microRNA annotation, RNA **9**:277-279 (2003).

[00108] Plant miRNAs follow a different naming convention as described in Meyers et al., Plant Cell. 2008 20(12):3186-3190.

[00109] A number of miRNAs are involved in gene regulation, and miRNAs are part of a growing class of non-coding RNAs that is now recognized as a major tier of gene control. In some cases, miRNAs can interrupt translation by binding to regulatory sites embedded in the 3′-UTRs of their target mRNAs, leading to the repression of translation. Target recognition involves complementary base pairing of the target site with the miRNA's seed region (positions 2–8 at the miRNA's 5′ end), although the exact extent of seed complementarity is not precisely determined and can be

modified by 3′ pairing. In other cases, miRNAs function like small interfering RNAs (siRNA) and bind to perfectly complementary mRNA sequences to destroy the target transcript.

[00110] Characterization of a number of miRNAs indicates that they influence a variety of processes, including early development, cell proliferation and cell death, apoptosis and fat metabolism. For example, some miRNAs, such as lin-4, let-7, mir-14, mir-23, and bantam, have been shown to play critical roles in cell differentiation and tissue development. Others are believed to have similarly important roles because of their differential spatial and temporal expression patterns.

[00111] The miRNA database available at miRBase (www.mirbase.org) comprises a searchable database of published miRNA sequences and annotation. Further information about miRBase can be found in the following articles, each of which is incorporated by reference in its entirety herein: Griffiths-Jones et al., miRBase: tools for microRNA genomics. NAR 2008 36(Database Issue):D154-D158; Griffiths-Jones et al., miRBase: microRNA sequences, targets and gene nomenclature. NAR 2006 34(Database Issue):D140-D144; and Griffiths-Jones, S. The microRNA Registry. NAR 2004 32(Database Issue):D109-D111. Representative miRNAs contained in Release 16 of miRBase, made available September 2010.

[00112] As described herein, microRNAs are known to be involved in cancer and other diseases and can be assessed in order to characterize a phenotype in a sample. See, e.g., Ferracin et al., Micromarkers: miRNAs in cancer diagnosis and prognosis, Exp Rev Mol Diag, Apr 2010, Vol. 10, No. 3, Pages 297-308; Fabbri, miRNAs as molecular biomarkers of cancer, Exp Rev Mol Diag, May 2010, Vol. 10, No. 4, Pages 435-444. Techniques to isolate and characterize vesicles and miRs are disclosed herein and/or known to those of skill in the art. In addition to the methodology presented herein, additional methods can be found in U.S. Patent No. 7,888,035, entitled "METHODS FOR ASSESSING RNA PATTERNS" and issued February 15, 2011; and International Patent Application Nos. PCT/US2010/058461, entitled "METHODS AND SYSTEMS FOR ISOLATING, STORING, AND ANALYZING VESICLES" and filed November 30, 2010; and PCT/US2011/021160, entitled "DETECTION OF GASTROINTESTINAL DISORDERS" and filed January 13, 2011; each of which applications are incorporated by reference herein in their entirety.

Circulating Biomarkers

[00113] Circulating biomarkers include biomarkers that are detectable in body fluids, such as blood, plasma, serum. Examples of circulating cancer biomarkers include cardiac troponin T (cTnT), prostate specific antigen (PSA) for prostate cancer and CA125 for ovarian cancer. Circulating biomarkers according to the invention include any appropriate biomarker that can be detected in bodily fluid, including without limitation protein, nucleic acids, e.g., DNA, mRNA and microRNA, lipids, carbohydrates and metabolites. Circulating biomarkers can include biomarkers that are not associated with cells, such as biomarkers that are membrane associated, embedded in membrane fragments, part of a biological complex, or free in solution. In one embodiment, circulating biomarkers are biomarkers that are associated with one or more vesicles present in the biological fluid of a subject.

[00114] Circulating biomarkers have been identified for use in characterization of various phenotypes. See, e.g., Ahmed N, et al., Proteomic-based identification of haptoglobin-1 precursor as a novel circulating biomarker of ovarian cancer. Br. J. Cancer 2004; Mathelin et al., Circulating proteinic biomarkers and breast cancer, Gynecol Obstet Fertil. 2006 Jul-Aug;34(7-8):638-46. Epub 2006 Jul 28; Ye et al., Recent technical strategies to identify diagnostic biomarkers for ovarian cancer. Expert Rev Proteomics. 2007 Feb;4(1):121-31; Carney, Circulating oncoproteins HER2/neu, EGFR and CAIX (MN) as novel cancer biomarkers. Expert Rev Mol Diagn. 2007 May;7(3):309-19; Gagnon, Discovery and application of protein biomarkers for ovarian cancer, Curr Opin Obstet Gynecol. 2008 Feb;20(1):9-13; Pasterkamp et

al., Immune regulatory cells: circulating biomarker factories in cardiovascular disease. Clin Sci (Lond). 2008 Aug;115(4):129-31; Fabbri, miRNAs as molecular biomarkers of cancer, Exp Rev Mol Diag, May 2010, Vol. 10, No. 4, Pages 435-444; PCT Patent Publication WO/2007/088537; U.S. Patents 7,745,150 and 7,655,479; U.S. Patent Publications 20110008808, 20100330683, 20100248290, 20100222230, 20100203566, 20100173788, 20090291932, 20090239246, 20090226937, 20090111121, 20090004687, 20080261258, 20080213907, 20060003465, 20050124071, and 20040096915, each of which publication is incorporated herein by reference in its entirety.

Sample Processing

[00115] A vesicle or a population of vesicles may be isolated, purified, concentrated or otherwise enriched prior to and/or during analysis. Unless otherwise specified, the terms "purified," "isolated," or similar as used herein in reference to vesicles or biomarker components are intended to include partial or complete purification or isolation of such components from a cell or organism. Analysis of a vesicle can include quantitiating the amount one or more vesicle populations of a biological sample. For example, a heterogeneous population of vesicles can be quantitated, or a homogeneous population of vesicles, such as a population of vesicles with a particular biomarker profile, a particular biosignature, or derived from a particular cell type can be isolated from a heterogeneous population of vesicles and quantitated. Analysis of a vesicle can also include detecting, quantitatively or qualitatively, one or more particular biomarker profile or biosignature of a vesicle, as described herein.

[00116] A vesicle can be stored and archived, such as in a bio-fluid bank and retrieved for analysis as necessary. A vesicle may also be isolated from a biological sample that has been previously harvested and stored from a living or deceased subject. In addition, a vesicle may be isolated from a biological sample which has been collected as described in *King et al., Breast Cancer Res 7(5): 198-204 (2005)*. A vesicle can be isolated from an archived or stored sample. Alternatively, a vesicle may be isolated from a biological sample and analyzed without storing or archiving of the sample. Furthermore, a third party may obtain or store the biological sample, or obtain or store the vesicle for analysis.

[00117] An enriched population of vesicles can be obtained from a biological sample. For example, vesicles may be concentrated or isolated from a biological sample using size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof.

[00118] Size exclusion chromatography, such as gel permeation columns, centrifugation or density gradient centrifugation, and filtration methods can be used. For example, a vesicle can be isolated by differential centrifugation, anion exchange and/or gel permeation chromatography (for example, as described in US Patent Nos. 6,899,863 and 6,812,023), sucrose density gradients, organelle electrophoresis (for example, as described in U.S. Patent No. 7,198,923), magnetic activated cell sorting (MACS), or with a nanomembrane ultrafiltration concentrator. Various combinations of isolation or concentration methods can be used.

[00119] Highly abundant proteins, such as albumin and immunoglobulin in blood samples, may hinder isolation of vesicles from a biological sample. For example, a vesicle can be isolated from a biological sample using a system that uses multiple antibodies that are specific to the most abundant proteins found in a biological sample, such as blood. Such a system can remove up to several proteins at once, thus unveiling the lower abundance species such as cell-of-origin specific vesicles. This type of system can be used for isolation of vesicles from biological samples such as blood, cerebrospinal fluid or urine. The isolation of vesicles from a biological sample may also be enhanced by high abundant protein removal methods as described in *Chromy et al. J Proteome Res 2004; 3:1120-1127*. In another embodiment, the isolation of vesicles from a biological sample may also be enhanced by removing serum proteins using glycopeptide

capture as described in *Zhang et al, Mol Cell Proteomics 2005;4:144-155*. In addition, vesicles from a biological sample such as urine may be isolated by differential centrifugation followed by contact with antibodies directed to cytoplasmic or anti-cytoplasmic epitopes as described in *Pisitkun et al., Proc Natl Acad Sci U S A, 2004;101:13368-13373*.

[00120] Plasma contains a large variety of proteins including albumin, immunoglobulins, and clotting proteins such as fibrinogen. About 60% of plasma protein comprises the protein albumin (e.g., human serum albumin or HSA), which contributes to osmotic pressure of plasma to assist in the transport of lipids and steroid hormones. Globulins make up about 35% of plasma proteins and are used in the transport of ions, hormones and lipids assisting in immune function. About 4% of plasma protein comprises fibrinogen which is essential in the clotting of blood and can be converted into the insoluble protein fibrin. Other types of blood proteins include: Prealbumin, Alpha 1 antitrypsin, Alpha 1 acid glycoprotein, Alpha 1 fetoprotein, Haptoglobin, Alpha 2 macroglobulin, Ceruloplasmin, Transferrin, complement proteins C3 and C4, Beta 2 microglobulin, Beta lipoprotein, Gamma globulin proteins, C-reactive protein (CRP), Lipoproteins (chylomicrons, VLDL, LDL, HDL), other globulins (types alpha, beta and gamma), Prothrombin and Mannose-binding lectin (MBL). Any of these proteins, including classes of proteins, or derivatives thereof (such as fibrin which is derived from the cleavage of fibrinogen) can be selectively depleted from a biological sample prior to further analysis performed on the sample. Without being bound by theory, removal of such background proteins may facilitate more sensitive, accurate, or precise detection of the biomarkers of interest in the sample.

[00121] Abundant proteins in blood or blood derivatives (e.g., plasma or serum) include without limitation albumin, IgG, transferrin, fibrinogen, IgA, α_2-Macroglobulin, IgM, α_1-Antitrypsin, complement C3, haptoglobulin, apolipoprotein A1, apolipoprotein A3, apolipoprotein B, α_1-Acid Glycoprotein, ceruloplasmin, complement C4, C1q, IgD, prealbumin (transthyretin), and plasminogen. Such proteins can be depleted using commercially available columns and kits. Examples of such columns comprise the Multiple Affinity Removal System from Agilent Technologies (Santa Clara, CA). This system include various cartridges designed to deplete different protein profiles, including the following cartridges with performance characteristics according to the manufacturer: Human 14, which eliminates approximately 94% of total protein (albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein (orosomucoid), IgM, apolipoprotein AI, apolipoprotein AII, complement C3 and transthyretin); Human 7, which eliminates approximately 85 - 90% of total protein (albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, and fibrinogen); Human 6, which eliminates approximately 85 - 90% of total protein (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin); Human Albumin/IgG, which eliminates approximately 69% of total protein (albumin and IgG); and Human Albumin, which eliminates approximately 50-55% of total protein (albumin). The ProteoPrep® 20 Plasma Immunodepletion Kit from Sigma-Aldrich is intended to specifically remove the 20 most abundant proteins from human plasma or serum, which is about remove 97-98% of the total protein mass in plasma or serum (Sigma-Aldrich, St. Louis, MO). According to the manufacturer, the ProteoPrep® 20 removes: albumin, IgG, transferrin, fibrinogen, IgA, α_2- Macroglobulin, IgM, α_1- Antitrypsin, complement C3, haptoglobulin, apolipoprotein A1, A3 and B; α_1- Acid Glycoprotein, ceruloplasmin, complement C4, C1q; IgD, prealbumin, and plasminogen. Sigma-Aldrich also manufactures ProteoPrep® columns to remove albumin (HSA) and immunoglobulins (IgG). The ProteomeLab IgY-12 High Capacity Proteome Partitioning kits from Beckman Coulter (Fullerton, CA) are specifically designed to remove twelve highly abundant proteins (Albumin, IgG, Transferrin, Fibrinogen, IgA, α2-macroglobulin, IgM, α1-Antitrypsin, Haptoglobin, Orosomucoid, Apolipoprotein A-I, Apolipoprotein A-II) from the human biological fluids such as serum and plasma. Generally, such systems rely on immunodepletion to remove the

target proteins, e.g., using small ligands and/or full antibodies. The PureProteome™ Human Albumin/Immunoglobulin Depletion Kit from Millipore (EMD Millipore Corporation, Billerica, MA, USA) is a magnetic bead based kit that enables high depletion efficiency (typically >99%) of Albumin and all Immunoglobulins (i.e., IgG, IgA, IgM, IgE and IgD) from human serum or plasma samples. The ProteoExtract® Albumin/IgG Removal Kit, also from Millipore, is designed to deplete >80% of albumin and IgG from body fluid samples. Other similar protein depletion products include without limitation the following: Aurum™ Affi-Gels Blue mini kit (Bio-Rad, Hercules, CA, USA); Vivapure® anti-HSA/IgG kit (Sartorius Stedim Biotech, Goettingen, Germany), Qproteome albumin/IgG depletion kit (Qiagen, Hilden, Germany); Seppro® MIXED12-LC20 column (GenWay Biotech, San Diego, CA, USA); Abundant Serum Protein Depletion Kit (Norgen Biotek Corp., Ontario, Canada); GBC Human Albumin/IgG/Transferrin 3 in 1 Depletion Column/Kit (Good Biotech Corp., Taiwan). These systems and similar systems can be used to remove abundant proteins from a biological sample, thereby improving the ability to detect low abundance circulating biomarkers such as proteins and vesicles.

[00122] Thromboplastin is a plasma protein aiding blood coagulation through conversion of prothrombin to thrombin. Thrombin in turn acts as a serine protease that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalyzing many other coagulation-related reactions. Thus, thromboplastin is a protein that can be used to facilitate precipitation of fibrinogen/fibrin (blood clotting factors) out of plasma. In addition to or as an alternative to immunoaffinity protein removal, a blood sample can be treated with thromboplastin to deplete fibrinogen/fibrin. Thromboplastin removal can be performed in addition to or as an alternative to immunoaffinity protein removal as described above using methods known in the art. Precipitation of other proteins and/or other sample particulate can also improve detection of circulating biomarkers such as vesicles in a sample. For example, ammonium sulfate treatment as known in the art can be used to precipitate immunoglobulins and other highly abundant proteins.

[00123] In an embodiment, the invention provides a method of detecting a presence or level of one or more circulating biomarker such as a microvesicle in a biological sample, comprising: (a) providing a biological sample comprising or suspected to comprise the one or more circulating biomarker; (b) selectively depleting one or more abundant protein from the biological sample provided in step (a); (c) performing affinity selection of the one or more circulating biomarker from the sample depleted in step (b), thereby detecting the presence or level of one or more circulating biomarker. The biological sample may comprise a bodily fluid, e.g., peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, umbilical cord blood, or a derivative of any thereof. In some embodiments, the biological sample comprises peripheral blood, serum or plasma.

[00124] An abundant protein may comprise a protein in the sample that is present in the sample at a high enough concentration to potentially interfere with downstream processing or analysis. Typically, an abundant protein is not the target of any further analysis of the sample. The abundant protein may constitute at least 10^{-5}, 10^{-4}, 10^{-3}, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98 or at least 99% of the total protein mass in the sample. In some embodiments, the abundant protein is present at less than 10^{-5}% of the total protein mass in the sample, e.g., in the case of a rare target of interest. As described herein, in the case of blood or a derivative thereof, the

one or more abundant protein may comprise one or more of albumin, IgG, transferrin, fibrinogen, fibrin, IgA, α2-Marcroglobulin, IgM, α1-Antitrypsin, complement C3, haptoglobulin, apolipoprotein A1, A3 and B; α1-Acid Glycoprotein, ceruloplasmin, complement C4, C1q, IgD, prealbumin (transthyretin), plasminogen, a derivative of any thereof, and a combination thereof. The one or more abundant protein in blood or a blood derivative may also comprise one or more of Albumin, Immunoglobulins, Fibrinogen, Prealbumin, Alpha 1 antitrypsin, Alpha 1 acid glycoprotein, Alpha 1 fetoprotein, Haptoglobin, Alpha 2 macroglobulin, Ceruloplasmin, Transferrin, complement proteins C3 and C4, Beta 2 microglobulin, Beta lipoprotein, Gamma globulin proteins, C-reactive protein (CRP), Lipoproteins (chylomicrons, VLDL, LDL, HDL), other globulins (types alpha, beta and gamma), Prothrombin, Mannose-binding lectin (MBL), a derivative of any thereof, and a combination thereof.

[00125] In some embodiments, selectively depleting the one or more abundant protein comprises contacting the biological sample with thromboplastin to initiate precipitation of fibrin. The one or more abundant protein may also be depleted by immunoaffinity, precipitation, or a combination thereof. For example, the sample can be treated with thromboplastin to precipitate fibrin, and then the sample may be passed through a column to remove HSA, IgG, and other abundant proteins as desired.

[00126] "Selectively depleting" the one or more abundant protein comprises depleting the abundant protein from the sample at a higher percentage than depletion another entity in the sample, such as another protein or microvesicle, including a target of interest for downstream processing or analysis. Selectively depleting the one or more abundant protein may comprise depleting the abundant protein at a 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold, 10^4-fold, 10^5-fold, 10^6-fold, 10^7-fold, 10^8-fold, 10^9-fold, 10^{10}-fold, 10^{11}-fold, 10^{12}-fold, 10^{13}-fold, 10^{14}-fold, 10^{15}-fold, 10^{16}-fold, 10^{17}-fold, 10^{18}-fold, 10^{19}-fold, 10^{20}-fold, or higher rate than another entity in the sample, such as another protein or microvesicle, including a target of interest for downstream processing or analysis. In an embodiment, there is little to no observable depletion of the target of interest as compared to the depletion of the abundant protein. In some embodiments, selectively depleting the one or more abundant protein from the biological sample comprises depleting at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the one or more abundant protein.

[00127] Removal of highly abundant proteins and other non-desired entities can further be facilitated with a non-stringent size exclusion step. For example, the sample can be processed using a high molecular weight cutoff size exclusion step to preferentially enrich high molecular weight vesicles apart from lower molecular weight proteins and other entities. In some embodiments, a sample is processed with a column (e.g., a gel filtration column) or filter having a molecular weight cutoff (MWCO) of 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10000, or greater than 10000 kiloDaltons (kDa). In an embodiment, a 700 kDa filtration column is used. In such a step, the vesicles will be retained or flow more slowly than the column or filter than the lower molecular weight entities. Such columns and filters are known in the art.

[00128] Isolation or enrichment of a vesicle from a biological sample can also be enhanced by use of sonication (for example, by applying ultrasound), detergents, other membrane-activating agents, or any combination thereof. For example, ultrasonic energy can be applied to a potential tumor site, and without being bound by theory, release of

vesicles from a tissue can be increased, allowing an enriched population of vesicles that can be analyzed or assessed from a biological sample using one or more methods disclosed herein.

[00129] With methods of detecting circulating biomarkers as described here, e.g., antibody affinity isolation, the consistency of the results can be optimized as necessary using various concentration or isolation procedures. Such steps can include agitation such as shaking or vortexing, different isolation techniques such as polymer based isolation, e.g., with PEG, and concentration to different levels during filtration or other steps. It will be understood by those in the art that such treatments can be applied at various stages of testing the vesicle containing sample. In one embodiment, the sample itself, e.g., a bodily fluid such as plasma or serum, is vortexed. In some embodiments, the sample is vortexed after one or more sample treatment step, e.g., vesicle isolation, has occurred. Agitation can occur at some or all appropriate sample treatment steps as desired. Additives can be introduced at the various steps to improve the process, e.g., to control aggregation or degradation of the biomarkers of interest.

[00130] The results can also be optimized as desireable by treating the sample with various agents. Such agents include additives to control aggregation and/or additives to adjust pH or ionic strength. Additives that control aggregation include blocking agents such as bovine serum albumin (BSA), milk or StabilGuard® (a BSA-free blocking agent; Product code SG02, Surmodics, Eden Prairie, MN), chaotropic agents such as guanidium hydro chloride, and detergents or surfactants. Useful ionic detergents include sodium dodecyl sulfate (SDS, sodium lauryl sulfate (SLS)), sodium laureth sulfate (SLS, sodium lauryl ether sulfate (SLES)), ammonium lauryl sulfate (ALS), cetrimonium bromide, cetrimonium chloride, cetrimonium stearate, and the like. Useful non-ionic (zwitterionic) detergents include polyoxyethylene glycols, polysorbate 20 (also known as Tween 20), other polysorbates (e.g., 40, 60, 65, 80, etc), Triton-X (e.g., X100, X114), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), CHAPSO, deoxycholic acid, sodium deoxycholate, NP-40, glycosides, octyl-thio-glucosides, maltosides, and the like. In some embodiments, Pluronic F-68, a surfactant shown to reduce platelet aggregation, is used to treat samples containing vesicles during isolation and/or detection. F68 can be used from a 0.1% to 10% concentration, e.g., a 1%, 2.5% or 5% concentration. The pH and/or ionic strength of the solution can be adjusted with various acids, bases, buffers or salts, including without limitation sodium chloride (NaCl), phosphate-buffered saline (PBS), tris-buffered saline (TBS), sodium phosphate, potassium chloride, potassium phosphate, sodium citrate and saline-sodium citrate (SSC) buffer. In some embodiments, NaCl is added at a concentration of 0.1% to 10%, e.g., 1%, 2.5% or 5% final concentration. In some embodiments, Tween 20 is added to 0.005 to 2% concentration, e.g., 0.05%, 0.25% or 0.5 % final concentration. Blocking agents for use with the invention comprise inert proteins, e.g., milk proteins, non-fat dry milk protein, albumin, BSA, casein, or serum such as newborn calf serum (NBCS), goat serum, rabbit serum or salmon serum. The proteins can be added at a 0.1% to 10% concentration, e.g., 1%, 2%, 3%, 3.5%, 4%, 5%, 6%, 7%, 8%, 9% or 10% concentration. In some embodiments, BSA is added to 0.1% to 10% concentration, e.g., 1%, 2%, 3%, 3.5%, 4%, 5%, 6%, 7%, 8%, 9% or 10% concentration. In an embodiment, the sample is treated according to the methodology presented in U.S. Patent Application 11/632946, filed July 13, 2005, which application is incorporated herein by reference in its entirety. Commercially available blockers may be used, such as SuperBlock, StartingBlock, Protein-Free from Pierce (a division of Thermo Fisher Scientific, Rockford, IL). In some embodiments, SSC/detergent (e.g., 20X SSC with 0.5% Tween 20 or 0.1% Triton-X 100) is added to 0.1% to 10% concentration, e.g., at 1.0% or 5.0% concentration.

[00131] The methods of detecting vesicles and other circulating biomarkers can be optimized as desired with various combinations of protocols and treatments as described herein. A detection protocol can be optimized by various

combinations of agitation, isolation methods, and additives. In some embodiments, the patient sample is vortexed before and after isolation steps, and the sample is treated with blocking agents including BSA and/or F68. Such treatments may reduce the formation of large aggregates or protein or other biological debris and thus provide a more consistent detection reading.

Filtration and Ultrafiltration

[00132] A vesicle can be isolated from a biological sample by filtering a biological sample from a subject through a filtration module and collecting from the filtration module a retentate comprising the vesicle, thereby isolating the vesicle from the biological sample. The method can comprise filtering a biological sample from a subject through a filtration module comprising a filter; and collecting from the filtration module a retentate comprising the vesicle, thereby isolating the vesicle from the biological sample. In one embodiment, the filter retains molecules greater than about 100 kiloDaltons.

[00133] The method can further comprise determining a biosignature of the vesicle. The method can also further comprise applying the retentate to a plurality of substrates, wherein each substrate is coupled to one or more capture agents, and each subset of the plurality of substrates comprises a different capture agent or combination of capture agents than another subset of the plurality of substrates.

[00134] Also provided herein is a method of determining a biosignature of a vesicle in a sample comprising: filtering a biological sample from a subject with a disorder through a filtration module, collecting from the filtration module a retentate comprising one or more vesicles, and determining a biosignature of the one or more vesicles. In one embodiment, the filtration module comprises a filter that retains molecules greater than about 100 or 150 kiloDaltons.

[00135] The method disclosed herein can further comprise characterizing a phenotype in a subject by filtering a biological sample from a subject through a filtration module, collecting from the filtration module a retentate comprising one or more vesicles; detecting a biosignature of the one or more vesicles; and characterizing a phenotype in the subject based on the biosignature, wherein characterizing is with at least 70% sensitivity. In some embodiments, characterizing comprises determining an amount of one or more vesicle having the biosignature. Furthermore, the characterizing can be from about 80% to 100% sensitivity.

[00136] Also provided herein is a method for multiplex analysis of a plurality of vesicles. In some embodiments, the method comprises filtering a biological sample from a subject through a filtration module; collecting from the filtration module a retentate comprising the plurality of vesicles, applying the plurality of vesicles to a plurality of capture agents, wherein the plurality of capture agents is coupled to a plurality of substrates, and each subset of the plurality of substrates is differentially labeled from another subset of the plurality of substrates; capturing at least a subset of the plurality of vesicles; and determining a biosignature for at least a subset of the captured vesicles. In one embodiment, each substrate is coupled to one or more capture agents, and each subset of the plurality of substrates comprises a different capture agent or combination of capture agents as compared to another subset of the plurality of substrates. In some embodiments, at least a subset of the plurality of substrates is intrinsically labeled, such as comprising one or more labels. The substrate can be a particle or bead, or any combination thereof. In some embodiments, the filter retains molecules greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10000, or greater than 10000 kiloDaltons (kDa). In one embodiment, the filtration module comprises a filter that retains molecules greater than about 100 or 150 kiloDaltons. In one embodiment, the filtration module comprises a filter that retains molecules greater than about 9, 20, 100 or 150

kiloDaltons. In still another embodiment, the filtration module comprises a filter that retains molecules greater than about 7000 kDa.

[00137] In some embodiments, the method for multiplex analysis of a plurality of vesicles comprises filtering a biological sample from a subject through a filtration module, wherein the filtration module comprises a filter that retains molecules greater than about 100 kiloDaltons; collecting from the filtration module a retentate comprising the plurality of vesicles; applying the plurality of vesicles to a plurality of capture agents, wherein the plurality of capture agents is coupled to a microarray; capturing at least a subset of the plurality of vesicles on the microarray; and determining a biosignature for at least a subset of the captured vesicles. In some embodiments, the filter retains molecules greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10000, or greater than 10000 kiloDaltons (kDa). In one embodiment, the filtration module comprises a filter that retains molecules greater than about 100 or 150 kiloDaltons. In one embodiment, the filtration module comprises a filter that retains molecules greater than about 9, 20, 100 or 150 kiloDaltons. In still another embodiment, the filtration module comprises a filter that retains molecules greater than about 7000 kDa.

[00138] The biological sample can be clarified prior to isolation by filtration. Clarification comprises selective removal of cellular debris and other undesirable materials. For example, cellular debris and other components that may interfere with detection of the circulating biomarkers can be removed. The clarification can be by low-speed centrifugation, such as at about 5,000x g, 4,000x g, 3,000x g, 2,000x g, 1,000x g, or less. The supernatant, or clarified biological sample, containing the vesicle can then be collected and filtered to isolate the vesicle from the clarified biological sample. In some embodiments, the biological sample is not clarified prior to isolation of a vesicle by filtration.

[00139] In some embodiments, isolation of a vesicle from a sample does not use high-speed centrifugation, such as ultracentrifugation. For example, isolation may not require the use of centrifugal speeds, such as about 100,000x g or more. In some embodiments, isolation of a vesicle from a sample uses speeds of less than 50,000 x g, 40,000 x g, 30,000 x g, 20,000 x g, 15,000 x g, 12,000 x g, or 10,000 x g.

[00140] Any number of applicable filter configurations can be used to filter a sample of interest. In some embodiments, the filtration module used to isolate the circulating biomarkers from the biological sample is a fiber-based filtration cartridge. For example, the fiber can be a hollow polymeric fiber, such as a polypropylene hollow fiber. A biological sample can be introduced into the filtration module by pumping the sample fluid, such as a biological fluid as disclosed herein, into the module with a pump device, such as a peristaltic pump. The pump flow rate can vary, such as at about 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, or 10 mL/minute. The flow rate can be adjusted given the configuration, e.g., size and throughput, of the filtration module.

[00141] The filtration module can be a membrane filtration module. For example, the membrane filtration module can comprise a filter disc membrane, such as a hydrophilic polyvinylidene difluoride (PVDF) filter disc membrane housed in a stirred cell apparatus (e.g., comprising a magnetic stirrer). In some embodiments, the sample moves through the filter as a result of a pressure gradient established on either side of the filter membrane.

[00142] The filter can comprise a material having low hydrophobic absorptivity and/or high hydrophilic properties. For example, the filter can have an average pore size for vesicle retention and permeation of most proteins as well as a surface that is hydrophilic, thereby limiting protein adsorption. For example, the filter can comprise a material selected from the group consisting of polypropylene, PVDF, polyethylene, polyfluoroethylene, cellulose, secondary cellulose

acetate, polyvinylalcohol, and ethylenevinyl alcohol (EVAL®, Kuraray Co., Okayama, Japan). Additional materials that can be used in a filter include, but are not limited to, polysulfone and polyethersulfone.

[00143] The filtration module can have a filter that retains molecules greater than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, or 500 kiloDaltons (kDa), such as a filter that has a MWCO (molecular weight cut off) of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, or 500 kDa, respectively. In embodiments, the filtration module has a MWCO of 1000 kDa, 1500 kDa, 2000 kDa, 2500 kDa, 3000 kDa, 3500 kDa, 4000 kDa, 4500 kDa, 5000 kDa, 5500 kDa, 6000 kDa, 6500 kDa, 7000 kDa, 7500 kDa, 8000 kDa, 8500 kDa, 9000 kDa, 9500 kDa, 10000 kDa, or greater than 10000 kDa. Ultrafiltration membranes with a range of MWCO of 9 kDa, 20 kDa and/or 150 kDa can be used. In some embodiments, the filter within the filtration module has an average pore diameter of about 0.01 μm to about 0.15 μm, and in some embodiments from about 0.05 μm to about 0.12 μm. In some embodiments, the filter has an average pore diameter of about 0.06 μm, 0.07 μm, 0.08 μm, 0.09 μm, 0.1 μm, 0.11 μm or 0.2 μm.

[00144] The filtration module can be a commerically available column, such as a column typically used for concentrating proteins or for isolating proteins (e.g., ultrafiltration). Examples include, but are not limited to, columns from Millpore (Billerica, MA), such as Amicon® centrifugal filters, or from Pierce® (Rockford, IL), such as Pierce Concentrator filter devices. Useful columns from Pierce include disposable ultrafiltration centrifugal devices with a MWCO of 9 kDa, 20 kDa and/or 150 kDa. These concentrators consist of a high-performance regenerated cellulose membrane welded to a conical device. The filters can be as described in U.S. Patents 6,269,957 or 6,357,601, both of which applications are incorporated by reference in their entirety herein.

[00145] The retentate comprising the isolated vesicle can be collected from the filtration module. The retentate can be collected by flushing the retentate from the filter. Selection of a filter composition having hydrophilic surface properties, thereby limiting protein adsorption, can be used, without being bound by theory, for easier collection of the retentate and minimize use of harsh or time-consuming collection techniques.

[00146] The collected retentate can then be used subsequent analysis, such as assessing a biosignature of one or more vesicles in the retentate, as further described herein. The analysis can be directly performed on the collected retentate. Alternatively, the collected retentate can be further concentrated or purified, prior to analysis of one or more vesicles. For example, the retentate can be further concentrated or vesicles further isolated from the retentate using size exclusion chromatography, density gradient centrifugation, differential centrifugation, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof, such as described herein. In some embodiments, the retentate can undergo another step of filtration. Alternatively, prior to isolation of a vesicle using a filter, the vesicle is concentrated or isolated using size exclusion chromatography, density gradient centrifugation, differential centrifugation, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof

[00147] Combinations of filters can be used for concentrating and isolating biomarkers. For example, the biological sample may first be filtered through a filter having a porosity or pore size of between about 0.01 μm to about 2 μm, about 0.05 μm to about 1.5 μm, and then the sample is filtered. For example, prior to filtering a biological sample through a filtration module with a filter that retains molecules greater than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10000, or greater than 10000 kiloDaltons (kDa), such as a filter that has a MWCO (molecular weight cut off) of about

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10000, or greater than 10000 kDa, respectively, the biological sample may first be filtered through a filter having a porosity or pore size of between about 0.01 μm to about 2 μm, about 0.05 μm to about 1.5 μm, In some embodiments, the filter has a pore size of about 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 μm. The filter may be a syringe filter. Thus, in one embodiment, the method comprises filtering the biological sample through a filter, such as a syringe filter, wherein the syringe filter has a porosity of greater than about 1 μm, prior to filtering the sample through a filtration module comprising a filter that retains molecules greater than about 100 or 150 kiloDaltons. In an embodiment, the filter is 1.2 μM filter and the filtration is followed by passage of the sample through a 7 ml or 20 ml concentrator column with a 150 kDa cutoff. Multiple concentrator columns may be used, e.g., in series. For example, a 7000 MWCO filtration unit can be used before a 150 MWCO unit.

[00148] The filtration module can be a component of a microfluidic device. Microfluidic devices, which may also be referred to as "lab-on-a-chip" systems, biomedical micro-electro-mechanical systems (bioMEMs), or multicomponent integrated systems, can be used for isolating, and analyzing, vesicles. Such systems miniaturize and compartmentalize processes that allow for binding of vesicles, detection of biomarkers, and other processes, such as further described herein.

[00149] The filtration module and assessment can be as described in Grant, R., et al., A filtration-based protocol to isolate human Plasma Membrane-derived Vesicles and exosomes from blood plasma, *J Immunol Methods* (2011) **371**:143-51 (Epub 2011 Jun 30), which reference is incorporated herein by reference in its entirety.

[00150] A microfluidic device can also be used for isolation of a vesicle by comprising a filtration module. For example, a microfluidic device can use one more channels for isolating a vesicle from a biological sample based on size from a biological sample. A biological sample can be introduced into one or more microfluidic channels, which selectively allows the passage of vesicles. The microfluidic device can further comprise binding agents, or more than one filtration module to select vesicles based on a property of the vesicles, for example, size, shape, deformability, biomarker profile, or biosignature.

Precipitation

[00151] Vesicles can be isolated using a polymeric precipitation method. The method can be in combination with or in place of the other isolation methods described herein. In one embodiment, the sample containing the vesicles is contacted with a formulation of polyethylene glycol (PEG). The polymeric formulation is incubated with the vesicle containing sample then precipitated by centrifugation. The PEG can bind to the vesicles and can be treated to specifically capture vesicles by addition of a capture moiety, e.g., a pegylated-binding protein such as an antibody. One of skill will appreciate that other polymers in addition to PEG can be used, e.g., PEG derivatives including methoxypolyethylene glycols, poly (ethylene oxide), and various polymers of formula HO-CH_2-(CH_2-O-CH_2-)n-CH_2-OH having different molecular weights.

[00152] In some embodiments of the invention, the vesicles are concentrated from a sample using the polymer precipitation method and the isolated vesicles are further separated using another approach. The second step can be used to identify a subpopulation of vesicles, e.g., that display certain biomarkers. The second separation step can comprise size exclusion, a binding agent, an antibody capture step, microbeads, as described herein.

[00153] In an embodiment, vesicles are isolated according to the ExoQuick™ and ExoQuick-TC™ kits from System Biosciences, Mountain View, CA USA. These kits use a polymer-based precipitation method to pellet vesicles.

Similarly, the vesicles can be isolated using the Total Exosome Isolation (from Serum) or Total Exosome Isolation (from Cell Culture Media) kits from Invitrogen / Life Technologies (Carlsbad, CA USA). The Total Exosome Isolation reagent forces less-soluble components such as vesicles out of solution, allowing them to be collected by a short, low-speed centrifugation. The reagent is added to the biological sample, and the solution is incubated overnight at 2 °C to 8 °C. The precipitated vesicles are recovered by standard centrifugation.

Binding Agents

[00154] Binding agents (also referred to as binding reagents) include agents that are capable of binding a target biomarker. A binding agent can be specific for the target biomarker, meaning the agent is capable of binding a target biomarker. The target can be any useful biomarker disclosed herein, such as a biomarker on the vesicle surface. In some embodiments, the target is a single molecule, such as a single protein, so that the binding agent is specific to the single protein. In other embodiments, the target can be a group of molecules, such as a family or proteins having a similar epitope or moiety, so that the binding agent is specific to the family or group of proteins. The group of molecules can also be a class of molecules, such as protein, DNA or RNA. The binding agent can be a capture agent used to capture a vesicle by binding a component or biomarker of a vesicle. In some embodiments, a capture agent comprises an antibody or fragment thereof, or an aptamer, that binds to an antigen on a vesicle. The capture agent can be optionally coupled to a substrate and used to isolate a vesicle, as further described herein.

[00155] A binding agent is an agent that binds to a circulating biomarker, such as a vesicle or a component of a vesicle. The binding agent can be used as a capture agent and/or a detection agent. A capture agent can bind and capture a circulating biomarker, such as by binding a component or biomarker of a vesicle. For example, the capture agent can be a capture antibody or capture antigen that binds to an antigen on a vesicle. A detection agent can bind to a circulating biomarker thereby facilitating detection of the biomarker. For example, a capture agent comprising an antibody or aptamer that is sequestered to a substrate can be used to capture a vesicle in a sample, and a detection agent comprising an antibody or aptamer that carries a label can be used to detect the captured vesicle via detection of the detection agent's label. In some embodiments, a vesicle is assessed using capture and detection agents that recognize the same vesicle biomarkers. For example, a vesicle population can be captured using a tetraspanin such as by using an anti-CD9 antibody bound to a substrate, and the captured vesicles can be detected using a fluorescently labeled anti-CD9 antibody to label the captured vesicles. In other embodiments, a vesicle is assessed using capture and detection agents that recognize different vesicle biomarkers. For example, a vesicle population can be captured using a cell-specific marker such as by using an anti-PCSA antibody bound to a substrate, and the captured vesicles can be detected using a fluorescently labeled anti-CD9 antibody to label the captured vesicles. Similarly, the vesicle population can be captured using a general vesicle marker such as by using an anti-CD9 antibody bound to a substate, and the captured vesicles can be detected using a fluorescently labeled antibody to a cell-specific or disease specific marker to label the captured vesicles.

[00156] The biomarkers recognized by the binding agent are sometimes referred to herein as an antigen. Unless otherwise specified, antigen as used herein is meant to encompass any entity that is capable of being bound by a binding agent, regardless of the type of binding agent or the immunogenicity of the biomarker. The antigen further encompasses a functional fragment thereof. For example, an antigen can encompass a protein biomarker capable of being bound by a binding agent, including a fragment of the protein that is capable of being bound by a binding agent.

[00157] In one embodiment, a vesicle is captured using a capture agent that binds to a biomarker on a vesicle. The capture agent can be coupled to a substrate and used to isolate a vesicle, as further described herein. In one embodiment, a capture agent is used for affinity capture or isolation of a vesicle present in a substance or sample.

[00158] A binding agent can be used after a vesicle is concentrated or isolated from a biological sample. For example, a vesicle can first be isolated from a biological sample before a vesicle with a specific biosignature is isolated or detected. The vesicle with a specific biosignature can be isolated or detected using a binding agent for the biomarker. A vesicle with the specific biomarker can be isolated or detected from a heterogeneous population of vesicles. Alternatively, a binding agent may be used on a biological sample comprising vesicles without a prior isolation or concentration step. For example, a binding agent is used to isolate or detect a vesicle with a specific biosignature directly from a biological sample.

[00159] A binding agent can be a nucleic acid, protein, or other molecule that can bind to a component of a vesicle. The binding agent can comprise DNA, RNA, monoclonal antibodies, polyclonal antibodies, Fabs, Fab', single chain antibodies, synthetic antibodies, aptamers (DNA/RNA), peptoids, zDNA, peptide nucleic acids (PNAs), locked nucleic acids (LNAs), lectins, synthetic or naturally occurring chemical compounds (including but not limited to drugs, labeling reagents), dendrimers, or a combination thereof. For example, the binding agent can be a capture antibody. In embodiments of the invention, the binding agent comprises a membrane protein labeling agent. See, e.g., the membrane protein labeling agents disclosed in Alroy et al., US. Patent Publication US 2005/0158708. In an embodiment, vesicles are isolated or captured as described herein, and one or more membrane protein labeling agent is used to detect the vesicles.

[00160] In some instances, a single binding agent can be employed to isolate or detect a vesicle. In other instances, a combination of different binding agents may be employed to isolate or detect a vesicle. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 different binding agents may be used to isolate or detect a vesicle from a biological sample. Furthermore, the one or more different binding agents for a vesicle can form a biosignature of a vesicle, as further described below.

[00161] Different binding agents can also be used for multiplexing. For example, isolation or detection of more than one population of vesicles can be performed by isolating or detecting each vesicle population with a different binding agent. Different binding agents can be bound to different particles, wherein the different particles are labeled. In another embodiment, an array comprising different binding agents can be used for multiplex analysis, wherein the different binding agents are differentially labeled or can be ascertained based on the location of the binding agent on the array. Multiplexing can be accomplished up to the resolution capability of the labels or detection method, such as described below. The binding agents can be used to detect the vesicles, such as for detecting cell-of-origin specific vesicles. A binding agent or multiple binding agents can themselves form a binding agent profile that provides a biosignature for a vesicle. One or more binding agents can be selected from Fig. 2 of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein. For example, if a vesicle population is detected or isolated using two, three, four or more binding agents in a differential detection or isolation of a vesicle from a heterogeneous population of vesicles, the particular binding agent profile for the vesicle population provides a biosignature for the particular vesicle population. The vesicle can be detected using any number of binding agents in a multiplex fashion. Thus, the binding agent can also be used to form a biosignature for a vesicle. The biosignature can be used to characterize a phenotype.

[00162] The binding agent can be a lectin. Lectins are proteins that bind selectively to polysaccharides and glycoproteins and are widely distributed in plants and animals. For example, lectins such as those derived from Galanthus nivalis in the form of Galanthus nivalis agglutinin ("GNA"), Narcissus pseudonarcissus in the form of Narcissus pseudonarcissus agglutinin ("NPA") and the blue green algae Nostoc ellipsosporum called "cyanovirin" (*Boyd et al. Antimicrob Agents Chemother 41(7): 1521 1530, 1997; Hammar et al. Ann N Y Acad Sci 724: 166 169, 1994; Kaku et al. Arch Biochem Biophys 279(2): 298 304, 1990*) can be used to isolate a vesicle. These lectins can bind to glycoproteins having a high mannose content (*Chervenak et al. Biochemistry 34(16): 5685 5695, 1995*). High mannose glycoprotein refers to glycoproteins having mannose-mannose linkages in the form of α-1→3 or α-1→6 mannose-mannose linkages.

[00163] The binding agent can be an agent that binds one or more lectins. Lectin capture can be applied to the isolation of the biomarker cathepsin D since it is a glycosylated protein capable of binding the lectins Galanthus nivalis agglutinin (GNA) and concanavalin A (ConA).

[00164] Methods and devices for using lectins to capture vesicles are described in International Patent Applications PCT/US2010/058461, entitled "METHODS AND SYSTEMS FOR ISOLATING, STORING, AND ANALYZING VESICLES" and filed November 30, 2010; PCT/US2009/066626, entitled "AFFINITY CAPTURE OF CIRCULATING BIOMARKERS" and filed December 3, 2009; PCT/US2010/037467, entitled "METHODS AND MATERIALS FOR ISOLATING EXOSOMES" and filed June 4, 2010; and PCT/US2007/006101, entitled "EXTRACORPOREAL REMOVAL OF MICROVESICULAR PARTICLES" and filed March 9, 2007, each of which applications is incorporated by reference herein in its entirety.

[00165] The binding agent can be an antibody. For example, a vesicle may be isolated using one or more antibodies specific for one or more antigens present on the vesicle. For example, a vesicle can have CD63 on its surface, and an antibody, or capture antibody, for CD63 can be used to isolate the vesicle. Alternatively, a vesicle derived from a tumor cell can express EpCam, the vesicle can be isolated using an antibody for EpCam and CD63. Other antibodies for isolating vesicles can include an antibody, or capture antibody, to CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, or 5T4. Other antibodies for isolating vesicles can include an antibody, or capture antibody, to DR3, STEAP, epha2, TMEM211, MFG-E8, Tissue Factor (TF), unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, or TETS.

[00166] In some embodiments, the capture agent is an antibody to CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, PSCA, ICAM, STEAP, or EGFR. The capture agent can also be used to identify a biomarker of a vesicle. For example, a capture agent such as an antibody to CD9 would identify CD9 as a biomarker of the vesicle. In some embodiments, a plurality of capture agents can be used, such as in multiplex analysis. The plurality of captures agents can comprise binding agents to one or more of: CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, PSCA, ICAM, STEAP, and EGFR. In some embodiments, the plurality of capture agents comprise binding agents to CD9, CD63, CD81, PSMA, PCSA, B7H3, MFG-E8, and/or EpCam. In yet other embodiments, the plurality of capture agents comprises binding agents to CD9, CD63, CD81, PSMA, PCSA, B7H3, EpCam, PSCA, ICAM, STEAP, and/or EGFR. The plurality of capture agents comprises binding agents to TMEM211, MFG-E8, Tissue Factor (TF), and/or CD24.

[00167] The antibodies referenced herein can be immunoglobulin molecules or immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen and synthetic antibodies. The immunoglobulin molecules can be of any class (e.g., IgG, IgE, IgM, IgD or IgA) or subclass of immunoglobulin molecule. Antibodies include, but are not limited to, polyclonal, monoclonal, bispecific, synthetic,

humanized and chimeric antibodies, single chain antibodies, Fab fragments and $F(ab')_2$ fragments, Fv or Fv' portions, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, or epitope-binding fragments of any of the above. An antibody, or generally any molecule, "binds specifically" to an antigen (or other molecule) if the antibody binds preferentially to the antigen, and, e.g., has less than about 30%, 20%, 10%, 5% or 1% cross-reactivity with another molecule.

[00168] The binding agent can also be a polypeptide or peptide. Polypeptide is used in its broadest sense and may include a sequence of subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. The polypeptides may be naturally occurring, processed forms of naturally occurring polypeptides (such as by enzymatic digestion), chemically synthesized or recombinantly expressed. The polypeptides for use in the methods of the present invention may be chemically synthesized using standard techniques. The polypeptides may comprise D-amino acids (which are resistant to L- amino acid-specific proteases), a combination of D- and L-amino acids, β amino acids, or various other designer or non-naturally occurring amino acids (e.g., β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids, etc.) to convey special properties. Synthetic amino acids may include ornithine for lysine, and norleucine for leucine or isoleucine. In addition, the polypeptides can have peptidomimetic bonds, such as ester bonds, to prepare polypeptides with novel properties. For example, a polypeptide may be generated that incorporates a reduced peptide bond, i.e., R_1-CH_2-NH-R_2, where R_1 and R_2 are amino acid residues or sequences. A reduced peptide bond may be introduced as a dipeptide subunit. Such a polypeptide would be resistant to protease activity, and would possess an extended half- live in vivo. Polypeptides can also include peptoids (N-substituted glycines), in which the side chains are appended to nitrogen atoms along the molecule's backbone, rather than to the α-carbons, as in amino acids. Polypeptides and peptides are intended to be used interchangeably throughout this application, i.e. where the term peptide is used, it may also include polypeptides and where the term polypeptides is used, it may also include peptides. The term "protein" is also intended to be used interchangeably throughout this application with the terms "polypeptides" and "peptides" unless otherwise specified.

[00169] A vesicle may be isolated, captured or detected using a binding agent. The binding agent can be an agent that binds a vesicle "housekeeping protein," or general vesicle biomarker. The biomarker can be CD63, CD9, CD81, CD82, CD37, CD53, Rab-5b, Annexin V or MFG-E8. Tetraspanins, a family of membrane proteins with four transmembrane domains, can be used as general vesicle markers. The tetraspanins include CD151, CD53, CD37, CD82, CD81, CD9 and CD63. There have been over 30 tetraspanins identified in mammals, including the TSPAN1 (TSP-1), TSPAN2 (TSP-2), TSPAN3 (TSP-3), TSPAN4 (TSP-4, NAG-2), TSPAN5 (TSP-5), TSPAN6 (TSP-6), TSPAN7 (CD231, TALLA-1, A15), TSPAN8 (CO-029), TSPAN9 (NET-5), TSPAN10 (Oculospanin), TSPAN11 (CD151-like), TSPAN12 (NET-2), TSPAN13 (NET-6), TSPAN14, TSPAN15 (NET-7), TSPAN16 (TM4-B), TSPAN17, TSPAN18, TSPAN19, TSPAN20 (UP1b, UPK1B), TSPAN21 (UP1a, UPK1A), TSPAN22 (RDS, PRPH2), TSPAN23 (ROM1), TSPAN24 (CD151), TSPAN25 (CD53), TSPAN26 (CD37), TSPAN27 (CD82), TSPAN28 (CD81), TSPAN29 (CD9), TSPAN30 (CD63), TSPAN31 (SAS), TSPAN32 (TSSC6), TSPAN33, and TSPAN34. Other commonly observed vesicle markers include those listed in **Table 3**. Any of these proteins can be used as vesicle markers. Furthermore, any of the markers disclosed herein or in **Table 3** can be selected in identifying a candidate biosignature for a disease or condition, where the one or more selected biomarkers have a direct or indirect role or function in mechanisms involved in the disease or condition.

Table 3: Proteins Observed in Vesicles from Multiple Cell Types

Class	Protein
Antigen Presentation	MHC class I, MHC class II, Integrins, Alpha 4 beta 1, Alpha M beta 2, Beta 2

Immunoglobulin family	ICAM1/CD54, P-selection
Cell-surface peptidases	Dipeptidylpeptidase IV/CD26, Aminopeptidase n/CD13
Tetraspanins	TSPAN1 (TSP-1), TSPAN2 (TSP-2), TSPAN3 (TSP-3), TSPAN4 (TSP-4, NAG-2), TSPAN5 (TSP-5), TSPAN6 (TSP-6), TSPAN7 (CD231, TALLA-1, A15), TSPAN8 (CO-029), TSPAN9 (NET-5), TSPAN10 (Oculospanin), TSPAN11 (CD151-like), TSPAN12 (NET-2), TSPAN13 (NET-6), TSPAN14, TSPAN15 (NET-7), TSPAN16 (TM4-B), TSPAN17, TSPAN18, TSPAN19, TSPAN20 (UP1b, UPK1B), TSPAN21 (UP1a, UPK1A), TSPAN22 (RDS, PRPH2), TSPAN23 (ROM1), TSPAN24 (CD151), TSPAN25 (CD53), TSPAN26 (CD37), TSPAN27 (CD82), TSPAN28 (CD81), TSPAN29 (CD9), TSPAN30 (CD63), TSPAN31 (SAS), TSPAN32 (TSSC6), TSPAN33, and TSPAN34
Heat-shock proteins	Hsp70, Hsp84/90
Cytoskeletal proteins	Actin, Actin-binding proteins, Tubulin
Membrane transport and fusion	Annexin I, Annexin II, Annexin IV, Annexin V, Annexin VI, RAB7/RAP1B/RADGDI
Signal transduction	Gi2alpha/14-3-3, CBL/LCK
Abundant membrane proteins	CD63, GAPDH, CD9, CD81, ANXA2, ENO1, SDCBP, MSN, MFGE8, EZR, GK, ANXA1, LAMP2, DPP4, TSG101, HSPA1A, GDI2, CLTC, LAMP1, CD86, ANPEP, TFRC, SLC3A2, RDX, RAP1B, RAB5C, RAB5B, MYH9, ICAM1, FN1, RAB11B, PIGR, LGALS3, ITGB1, EHD1, CLIC1, ATP1A1, ARF1, RAP1A, P4HB, MUC1, KRT10, HLA-A, FLOT1, CD59, C1orf58, BASP1, TACSTD1, STOM
Other Transmembrane Proteins	Cadherins: CDH1, CDH2, CDH12, CDH3, Deomoglein, DSG1, DSG2, DSG3, DSG4, Desmocollin, DSC1, DSC2, DSC3, Protocadherins, PCDH1, PCDH10, PCDH11x, PCDH11y, PCDH12, FAT, FAT2, FAT4, PCDH15, PCDH17, PCDH18, PCDH19; PCDH20; PCDH7, PCDH8, PCDH9, PCDHA1, PCDHA10, PCDHA11, PCDHA12, PCDHA13, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHAC1, PCDHAC2, PCDHB1, PCDHB10, PCDHB11, PCDHB12, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB17, PCDHB18, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHB7, PCDHB8, PCDHB9, PCDHGA1, PCDHGA10, PCDHGA11, PCDHGA12, PCDHGA2; PCDHGA3, PCDHGA4, PCDHGA5, PCDHGA6, PCDHGA7, PCDHGA8, PCDHGA9, PCDHGB1, PCDHGB2, PCDHGB3, PCDHGB4, PCDHGB5, PCDHGB6, PCDHGB7, PCDHGC3, PCDHGC4, PCDHGC5, CDH9 (cadherin 9, type 2 (T1-cadherin)), CDH10 (cadherin 10, type 2 (T2-cadherin)), CDH5 (VE-cadherin (vascular endothelial)), CDH6 (K-cadherin (kidney)), CDH7 (cadherin 7, type 2), CDH8 (cadherin 8, type 2), CDH11 (OB-cadherin (osteoblast)), CDH13 (T-cadherin - H-cadherin (heart)), CDH15 (M-cadherin (myotubule)), CDH16 (KSP-cadherin), CDH17 (LI cadherin (liver-intestine)), CDH18 (cadherin 18, type 2), CDH19 (cadherin 19, type 2), CDH20 (cadherin 20, type 2), CDH23 (cadherin 23, (neurosensory epithelium)), CDH10, CDH11, CDH13, CDH15, CDH16, CDH17, CDH18, CDH19, CDH20, CDH22, CDH23, CDH24, CDH26, CDH28, CDH4, CDH5, CDH6, CDH7, CDH8, CDH9, CELSR1, CELSR2, CELSR3, CLSTN1, CLSTN2, CLSTN3, DCHS1, DCHS2, LOC389118, PCLKC, RESDA1, RET

[00170] The binding agent can also be an agent that binds to a vesicle derived from a specific cell type, such as a tumor cell (e.g. binding agent for Tissue factor, EpCam, B7H3, RAGE or CD24) or a specific cell-of-origin. The binding agent used to isolate or detect a vesicle can be a binding agent for an antigen selected from **Fig. 1** of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein. The binding agent for a vesicle can also be selected from those listed in **Fig. 2** of International Patent Application Serial No. PCT/US2011/031479. The binding agent can be for an antigen such as a tetraspanin, MFG-E8, Annexin V, 5T4, B7H3, caveolin, CD63, CD9, E-Cadherin, Tissue factor, MFG-E8, TMEM211, CD24, PSCA, PCSA, PSMA, Rab-5B, STEAP, TNFR1, CD81, EpCam, CD59,

CD81, ICAM, EGFR, or CD66. A binding agent for a platelet can be a glycoprotein such as GpIa-IIa, GpIIb-IIIa, GpIIIb, GpIb, or GpIX. A binding agent can be for an antigen comprisine one or more of CD9, Erb2, Erb4, CD81, Erb3, MUC16, CD63, DLL4, HLA-Drpe, B7H3, IFNAR, 5T4, PCSA, MICB, PSMA, MFG-E8, Muc1, PSA, Muc2, Unc93a, VEGFR2, EpCAM, VEGF A, TMPRSS2, RAGE, PSCA, CD40, Muc17, IL-17-RA, and CD80. For example, the binding agent can be one or more of CD9, CD63, CD81, B7H3, PCSA, MFG-E8, MUC2, EpCam, RAGE and Muc17. One or more binding agents, such as one or more binding agents for two or more of the antigens, can be used for isolating or detecting a vesicle. The binding agent used can be selected based on the desire of isolating or detecting a vesicle derived from a particular cell type or cell-of-origin specific vesicle.

[00171] A binding agent can also be linked directly or indirectly to a solid surface or substrate. A solid surface or substrate can be any physically separable solid to which a binding agent can be directly or indirectly attached including, but not limited to, surfaces provided by microarrays and wells, particles such as beads, columns, optical fibers, wipes, glass and modified or functionalized glass, quartz, mica, diazotized membranes (paper or nylon), polyformaldehyde, cellulose, cellulose acetate, paper, ceramics, metals, metalloids, semiconductive materials, quantum dots, coated beads or particles, other chromatographic materials, magnetic particles; plastics (including acrylics, polystyrene, copolymers of styrene or other materials, polypropylene, polyethylene, polybutylene, polyurethanes, polytetrafluoroethylene (PTFE, Teflon®), etc.), polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, ceramics, conducting polymers (including polymers such as polypyrole and polyindole); micro or nanostructured surfaces such as nucleic acid tiling arrays, nanotube, nanowire, or nanoparticulate decorated surfaces; or porous surfaces or gels such as methacrylates, acrylamides, sugar polymers, cellulose, silicates, or other fibrous or stranded polymers. In addition, as is known the art, the substrate may be coated using passive or chemically-derivatized coatings with any number of materials, including polymers, such as dextrans, acrylamides, gelatins or agarose. Such coatings can facilitate the use of the array with a biological sample.

[00172] For example, an antibody used to isolate a vesicle can be bound to a solid substrate such as a well, such as commercially available plates (e.g. from Nunc, Milan Italy). Each well can be coated with the antibody. In some embodiments, the antibody used to isolate a vesicle is bound to a solid substrate such as an array. The array can have a predetermined spatial arrangement of molecule interactions, binding islands, biomolecules, zones, domains or spatial arrangements of binding islands or binding agents deposited within discrete boundaries. Further, the term array may be used herein to refer to multiple arrays arranged on a surface, such as would be the case where a surface bore multiple copies of an array. Such surfaces bearing multiple arrays may also be referred to as multiple arrays or repeating arrays.

[00173] Arrays typically contain addressable moieties that can detect the presense of an entity, e.g., a vesicle in the sample via a binding event. An array may be referred to as a microarray. Arrays or microarrays include without limitation DNA microarrays, such as cDNA microarrays, oligonucleotide microarrays and SNP microarrays, microRNA arrays, protein microarrays, antibody microarrays, tissue microarrays, cellular microarrays (also called transfection microarrays), chemical compound microarrays, and carbohydrate arrays (glycoarrays). DNA arrays typically comprise addressable nucleotide sequences that can bind to sequences present in a sample. MicroRNA arrays, e.g., the MMChips array from the University of Louisville or commercial systems from Agilent, can be used to detect microRNAs. Protein microarrays can be used to identify protein–protein interactions, including without limitation identifying substrates of protein kinases, transcription factor protein-activation, or to identify the targets of biologically active small molecules. Protein arrays may comprise an array of different protein molecules, commonly antibodies, or nucleotide sequences that bind to proteins of interest. In a non-limiting example, a protein array can be used to detect

vesicles having certain proteins on their surface. Antibody arrays comprise antibodies spotted onto the protein chip that are used as capture molecules to detect proteins or other biological materials from a sample, e.g., from cell or tissue lysate solutions. For example, antibody arrays can be used to detect vesicle-associated biomarkers from bodily fluids, e.g., serum or urine. Tissue microarrays comprise separate tissue cores assembled in array fashion to allow multiplex histological analysis. Cellular microarrays, also called transfection microarrays, comprise various capture agents, such as antibodies, proteins, or lipids, which can interact with cells to facilitate their capture on addressable locations. Cellular arrays can also be used to capture vesicles due to the similarity between a vesicle and cellular membrane. Chemical compound microarrays comprise arrays of chemical compounds and can be used to detect protein or other biological materials that bind the compounds. Carbohydrate arrays (glycoarrays) comprise arrays of carbohydrates and can detect, e.g., protein that bind sugar moieties. One of skill will appreciate that similar technologies or improvements can be used according to the methods of the invention.

[00174] A binding agent can also be bound to particles such as beads or microspheres. For example, an antibody specific for a component of a vesicle can be bound to a particle, and the antibody-bound particle is used to isolate a vesicle from a biological sample. In some embodiments, the microspheres may be magnetic or fluorescently labeled. In addition, a binding agent for isolating vesicles can be a solid substrate itself. For example, latex beads, such as aldehyde/sulfate beads (Interfacial Dynamics, Portland, OR) can be used.

[00175] A binding agent bound to a magnetic bead can also be used to isolate a vesicle. For example, a biological sample such as serum from a patient can be collected for colon cancer screening. The sample can be incubated with anti-CCSA-3 (Colon Cancer–Specific Antigen) coupled to magnetic microbeads. A low-density microcolumn can be placed in the magnetic field of a MACS Separator and the column is then washed with a buffer solution such as Tris-buffered saline. The magnetic immune complexes can then be applied to the column and unbound, non-specific material can be discarded. The CCSA-3 selected vesicle can be recovered by removing the column from the separator and placing it on a collection tube. A buffer can be added to the column and the magnetically labeled vesicle can be released by applying the plunger supplied with the column. The isolated vesicle can be diluted in IgG elution buffer and the complex can then be centrifuged to separate the microbeads from the vesicle. The pelleted isolated cell-of-origin specific vesicle can be resuspended in buffer such as phosphate-buffered saline and quantitated. Alternatively, due to the strong adhesion force between the antibody captured cell-of-origin specific vesicle and the magnetic microbeads, a proteolytic enzyme such as trypsin can be used for the release of captured vesicles without the need for centrifugation. The proteolytic enzyme can be incubated with the antibody captured cell-of-origin specific vesicles for at least a time sufficient to release the vesicles.

[00176] A binding agent, such as an antibody, for isolating vesicles is preferably contacted with the biological sample comprising the vesicles of interest for at least a time sufficient for the binding agent to bind to a component of the vesicle. For example, an antibody may be contacted with a biological sample for various intervals ranging from seconds days, including but not limited to, about 10 minutes, 30 minutes, 1 hour, 3 hours, 5 hours, 7 hours, 10 hours, 15 hours, 1 day, 3 days, 7 days or 10 days.

[00177] A binding agent, such as an antibody specific to an antigen listed in **Fig. 1** of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein, or a binding agent listed in **Fig. 2** of International Patent Application Serial No. PCT/US2011/031479, can be labeled to facilitate detection. Appropriate labels include without limitation a magnetic label, a fluorescent moiety, an enzyme, a chemiluminescent probe, a metal particle, a non-metal

colloidal particle, a polymeric dye particle, a pigment molecule, a pigment particle, an electrochemically active species, semiconductor nanocrystal or other nanoparticles including quantum dots or gold particles, fluorophores, quantum dots, or radioactive labels. Protein labels include green fluorescent protein (GFP) and variants thereof (e.g., cyan fluorescent protein and yellow fluorescent protein); and luminescent proteins such as luciferase, as described below. Radioactive labels include without limitation radioisotopes (radionuclides), such as ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, or ^{213}Bi. Fluorescent labels include without limitation a rare earth chelate (e.g., europium chelate), rhodamine; fluorescein types including without limitation FITC, 5-carboxyfluorescein, 6-carboxy fluorescein; a rhodamine type including without limitation TAMRA; dansyl; Lissamine; cyanines; phycoerythrins; Texas Red; Cy3, Cy5, dapoxyl, NBD, Cascade Yellow, dansyl, PyMPO, pyrene, 7-diethylaminocoumarin-3-carboxylic acid and other coumarin derivatives, Marina Blue™, Pacific Blue™, Cascade Blue™, 2-anthracenesulfonyl, PyMPO, 3,4,9,10-perylene-tetracarboxylic acid, 2,7-difluorofluorescein (Oregon Green™ 488-X), 5-carboxyfluorescein, Texas Red™-X, Alexa Fluor 430, 5-carboxytetramethylrhodamine (5-TAMRA), 6-carboxytetramethylrhodamine (6-TAMRA), BODIPY FL, bimane, and Alexa Fluor 350, 405, 488, 500, 514, 532, 546, 555, 568, 594, 610, 633, 647, 660, 680, 700, and 750, and derivatives thereof, among many others. See, e.g., "The Handbook--A Guide to Fluorescent Probes and Labeling Technologies," Tenth Edition, available on the internet at probes (dot) invitrogen (dot) com/handbook. The fluorescent label can be one or more of FAM, dRHO, 5-FAM, 6FAM, dR6G, JOE, HEX, VIC, TET, dTAMRA, TAMRA, NED, dROX, PET, BHQ, Gold540 and LIZ.

[00178] A binding agent can be directly or indirectly labeled, e.g., the label is attached to the antibody through biotin-streptavidin. Alternatively, an antibody is not labeled, but is later contacted with a second antibody that is labeled after the first antibody is bound to an antigen of interest.

[00179] For example, various enzyme-substrate labels are available or disclosed (see for example, U.S. Pat. No. 4,275,149). The enzyme generally catalyzes a chemical alteration of a chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Examples of enzyme-substrate combinations include, but are not limited to, horseradish peroxidase (HRP) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethylbenzidine hydrochloride (TMB)); alkaline phosphatase (AP) with para-nitrophenyl phosphate as chromogenic substrate; and β-D-galactosidase (β-D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl- β-D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl-β-D-galactosidase.

[00180] Depending on the method of isolation or detection used, the binding agent may be linked to a solid surface or substrate, such as arrays, particles, wells and other substrates described above. Methods for direct chemical coupling of antibodies, to the cell surface are known in the art, and may include, for example, coupling using glutaraldehyde or maleimide activated antibodies. Methods for chemical coupling using multiple step procedures include biotinylation, coupling of trinitrophenol (TNP) or digoxigenin using for example succinimide esters of these compounds. Biotinylation can be accomplished by, for example, the use of D-biotinyl-N-hydroxysuccinimide. Succinimide groups

react effectively with amino groups at pH values above 7, and preferentially between about pH 8.0 and about pH 8.5. Biotinylation can be accomplished by, for example, treating the cells with dithiothreitol followed by the addition of biotin maleimide.

Particle-based Assays

[00181] As an alternative to planar arrays, assays using particles, such as bead based assays, are capable of use with a binding agent. For example, antibodies or aptamers are easily conjugated with commercially available beads. *See, e.g.,* Fan *et al.*, Illumina universal bead arrays. Methods Enzymol. 2006 410:57-73; Srinivas *et al.* Anal. Chem. 2011 Oct. 21, *Aptamer functionalized Microgel Particles for Protein Detection*; See also, review article on aptamers as therapeutic and diagnostic agents, Brody and Gold, Rev. Mol. Biotech. 2000, 74:5-13.

[00182] Multiparametric assays or other high throughput detection assays using bead coatings with cognate ligands and reporter molecules with specific activities consistent with high sensitivity automation can be used. In a bead based assay system, a binding agent for a biomarker or vesicle, such as a capture agent (e.g. capture antibody), can be immobilized on an addressable microsphere. Each binding agent for each individual binding assay can be coupled to a distinct type of microsphere (i.e., microbead) and the assay reaction takes place on the surface of the microsphere, such as depicted in **FIG. 2B**. A binding agent for a vesicle can be a capture antibody or aptamer coupled to a bead. Dyed microspheres with discrete fluorescence intensities are loaded separately with their appropriate binding agent or capture probes. The different bead sets carrying different binding agents can be pooled as necessary to generate custom bead arrays. Bead arrays are then incubated with the sample in a single reaction vessel to perform the assay. *See* **FIGs. 8C-D** for illustrative methods of detecting microvesicles using microbeads with antibody binding agents.

[00183] A bead substrate can provide a platform for attaching one or more binding agents, including aptamer(s) or antibodies. One of skill will appreciate that the illustrative schemes shown in **FIGs. 8C-D** can employ aptamers along with or instead of antibodies. For multiplexing, multiple different bead sets (e.g., those commercially available from Illumina, Inc., San Diego, CA, USA, or Luminex Corporation, Austin, TX, USA) can have different binding agents which are specific to different target molecules. Beads can also be used for different purposes, e.g., detection and/or isolation. For example, a bead can be conjugated to an aptamer used to detect the presence (quantitatively or qualitatively) of a given biomarker, or it can also be used to isolate a component present in a selected biological sample (e.g., cell, cell-fragment or vesicle comprising the target molecule to which the binding agent is configured to bind or associate). Various molecules of organic origin can be conjugated to a microbeads, e.g., polysterene beads, through use of commercially available kits. One of skill will appreciate that an assay can use multiple types of binding agents. For example, a bead may be conjugated to an aptamer which serves to bind and capture a biomarker, and a labeled antibody can be used to further detect the captured biomarker. Similarly, a bead may be conjugated to an antibody which serves to bind and capture a biomarker, and a labeled aptamer can be used to further detect the captured biomarker. Any such useful combination of binding agents are contemplated by the invention.

[00184] One or more binding agent can be used with any bead based substrate, including but not limited to magnetic capture method, fluorescence activated cell sorting (FACS) or laser cytometry. Magnetic capture methods can include, but are not limited to, the use of magnetically activated cell sorter (MACS) microbeads or magnetic columns. Examples of bead or particle based methods that can be used in the methods of the invention include the bead systems described in any of U.S. Patent Nos. 4,551,435, 4,795,698, 4,925,788, 5,108,933, 5,186,827, 5,200,084 or 5,158,871; 7,399,632; 8,124,015; 8,008,019; 7,955,802; 7,445,844; 7,274,316; 6,773,812; 6,623,526; 6,599,331; 6,057,107; 5,736,330; or International Patent Application Nos. PCT/US2012/42519; PCT/US1993/04145.

Flow Cytometry

[00185] Isolation or detection of a vesicle using a particle such as a bead or microsphere can also be performed using flow cytometry. Flow cytometry can be used for sorting microscopic particles suspended in a stream of fluid. As particles pass through they can be selectively charged and on their exit can be deflected into separate paths of flow. It is therefore possible to separate populations from an original mix, such as a biological sample, with a high degree of accuracy and speed. Flow cytometry allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of single cells flowing through an optical/electronic detection apparatus. A beam of light, usually laser light, of a single frequency (color) is directed onto a hydrodynamically focused stream of fluid. A number of detectors are aimed at the point where the stream passes through the light beam; one in line with the light beam (Forward Scatter or FSC) and several perpendicular to it (Side Scatter or SSC) and one or more fluorescent detectors.

[00186] Each suspended particle passing through the beam scatters the light in some way, and fluorescent chemicals in the particle may be excited into emitting light at a lower frequency than the light source. This combination of scattered and fluorescent light is picked up by the detectors, and by analyzing fluctuations in brightness at each detector (one for each fluorescent emission peak), it is possible to deduce various facts about the physical and chemical structure of each individual particle. FSC correlates with the cell size and SSC depends on the inner complexity of the particle, such as shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness. Some flow cytometers have eliminated the need for fluorescence and use only light scatter for measurement.

[00187] Flow cytometers can analyze several thousand particles every second in "real time" and can actively separate out and isolate particles having specified properties. They offer high-throughput automated quantification, and separation, of the set parameters for a high number of single cells during each analysis session. Flow cytomers can have multiple lasers and fluorescence detectors, allowing multiple labels to be used to more precisely specify a target population by their phenotype. Thus, a flow cytometer, such as a multicolor flow cytometer, can be used to detect one or more vesicles with multiple fluorescent labels or colors. In some embodiments, the flow cytometer can also sort or isolate different vesicle populations, such as by size or by different markers.

[00188] The flow cytometer may have one or more lasers, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more lasers. In some embodiments, the flow cytometer can detect more than one color or fluorescent label, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 different colors or fluorescent labels. For example, the flow cytometer can have at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 fluorescence detectors.

[00189] Examples of commercially available flow cytometers that can be used to detect or analyze one or more vesicles, to sort or separate different populations of vesicles, include, but are not limited to the MoFlo™ XDP Cell Sorter (Beckman Coulter, Brea, CA), MoFlo™ Legacy Cell Sorter (Beckman Coulter, Brea, CA), BD FACSAria™ Cell Sorter (BD Biosciences, San Jose, CA), BD™ LSRII (BD Biosciences, San Jose, CA), and BD FACSCalibur™ (BD Biosciences, San Jose, CA). Use of multicolor or multi-fluor cytometers can be used in multiplex analysis of vesicles, as further described below. In some embodiments, the flow cytometer can sort, and thereby collect or sort more than one population of vesicles based one or more characteristics. For example, two populations of vesicles differ in size, such that the vesicles within each population have a similar size range and can be differentially detected or sorted. In another embodiment, two different populations of vesicles are differentially labeled.

[00190] The data resulting from flow-cytometers can be plotted in 1 dimension to produce histograms or seen in 2 dimensions as dot plots or in 3 dimensions with newer software. The regions on these plots can be sequentially separated by a series of subset extractions which are termed gates. Specific gating protocols exist for diagnostic and

clinical purposes especially in relation to hematology. The plots are often made on logarithmic scales. Because different fluorescent dye's emission spectra overlap, signals at the detectors have to be compensated electronically as well as computationally. Fluorophores for labeling biomarkers may include those described in *Ormerod, Flow Cytometry 2nd ed., Springer-Verlag, New York (1999),* and in *Nida et al., Gynecologic Oncology 2005;4 889-894* which is incorporated herein by reference.

[00191] In various embodiments of the invention, flow cytometry is used to assess a microvesicle population in a biological sample. If desired, the microvesicle population can be sorted from other particles (e.g., cell debris, protein aggregates, etc) in a sample by labeling the vesicles using one or more general vesicle marker. The general vesicle marker can be a marker in **Table 3**. Commonly used vesicle markers include tetraspanins such as CD9, CD63 and/or CD81. Vesicles comprising one or more tetraspanin are sometimes refered to as "Tet+" herein to indicate that the vesicles are tetraspanin-positive. The sorted microvesicles can be further assessed using methodology described herein. E.g., surface antigens on the sorted microvesicles can be detected using flow or other methods. In some embodiments, payload within the sorted microvesicles is assessed. As an illustrative example, a population of microvesicles is contacted with a labeled binding agent to a surface antigen of interest, the contacted microvesicles are sorted using flow cytometry, and payload with the microvesicles is assessed. The payload may be polypeptides, nucleic acids (e.g., mRNA or microRNA) or other biological entities as desired. Such assessment is used to characterize a phenotype as described herein, e.g., to diagnose, prognose or theranose a cancer.

[00192] In an embodiment, flow sorting is used to distinguish microvesicle populations from other biological complexes. In a non-limiting example, Ago2+/Tet+ and Ago2+/Tet- particles are detected using flow methodology to separate Ago2+ vesicles from vesicle-free Ago2+ complexes, respectively.

Multiplexing

[00193] Multiplex experiments comprise experiments that can simultaneously measure multiple analytes in a single assay. Vesicles and associated biomarkers can be assessed in a multiplex fashion. Different binding agents can be used for multiplexing different circulating biomarkers, e.g., microRNA, protein, or vesicle populations. Different biomarkers, e.g., different vesicle populations, can be isolated or detected using different binding agents. Each population in a biological sample can be labeled with a different signaling label, such as a fluorophore, quantum dot, or radioactive label, such as described above. The label can be directly conjugated to a binding agent or indirectly used to detect a binding agent that binds a vesicle. The number of populations detected in a multiplexing assay is dependent on the resolution capability of the labels and the summation of signals, as more than two differentially labeled vesicle populations that bind two or more affinity elements can produce summed signals.

[00194] Multiplexing of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 different circulating biomarkers may be performed. For example, one population of vesicles specific to a cell-of-origin can be assayed along with a second population of vesicles specific to a different cell-of-origin, where each population is labeled with a different label. Alternatively, a population of vesicles with a particular biomarker or biosignature can be assayed along with a second population of vesicles with a different biomarker or biosignature. In some cases, hundreds or thousands of vesicles are assessed in a single assay.

[00195] In one embodiment, multiplex analysis is performed by applying a plurality of vesicles comprising more than one population of vesicles to a plurality of substrates, such as beads. Each bead is coupled to one or more capture agents. The plurality of beads is divided into subsets, where beads with the same capture agent or combination of capture agents form a subset of beads, such that each subset of beads has a different capture agent or combination of

capture agents than another subset of beads. The beads can then be used to capture vesicles that comprise a component that binds to the capture agent. The different subsets can be used to capture different populations of vesicles. The captured vesicles can then be analyzed by detecting one or more biomarkers.

[00196] Flow cytometry can be used in combination with a particle-based or bead based assay. Multiparametric immunoassays or other high throughput detection assays using bead coatings with cognate ligands and reporter molecules with specific activities consistent with high sensitivity automation can be used. For example, beads in each subset can be differentially labeled from another subset. In a particle based assay system, a binding agent or capture agent for a vesicle, such as a capture antibody, can be immobilized on addressable beads or microspheres. Each binding agent for each individual binding assay (such as an immunoassay when the binding agent is an antibody) can be coupled to a distinct type of microsphere (i.e., microbead) and the binding assay reaction takes place on the surface of the microspheres. Microspheres can be distinguished by different labels, for example, a microsphere with a specific capture agent would have a different signaling label as compared to another microsphere with a different capture agent. For example, microspheres can be dyed with discrete fluorescence intensities such that the fluorescence intensity of a microsphere with a specific binding agent is different than that of another microsphere with a different binding agent. Biomarkers bound by different capture agents can be differentially detected using different labels.

[00197] A microsphere can be labeled or dyed with at least 2 different labels or dyes. In some embodiments, the microsphere is labeled with at least 3, 4, 5, 6, 7, 8, 9, or 10 different labels. Different microspheres in a plurality of microspheres can have more than one label or dye, wherein various subsets of the microspheres have various ratios and combinations of the labels or dyes permitting detection of different microspheres with different binding agents. For example, the various ratios and combinations of labels and dyes can permit different fluorescent intensities. Alternatively, the various ratios and combinations maybe used to generate different detection patters to identify the binding agent. The microspheres can be labeled or dyed externally or may have intrinsic fluorescence or signaling labels. Beads can be loaded separately with their appropriate binding agents and thus, different vesicle populations can be isolated based on the different binding agents on the differentially labeled microspheres to which the different binding agents are coupled.

[00198] In another embodiment, multiplex analysis can be performed using a planar substrate, wherein the substrate comprises a plurality of capture agents. The plurality of capture agents can capture one or more populations of vesicles, and one or more biomarkers of the captured vesicles detected. The planar substrate can be a microarray or other substrate as further described herein.

Binding Agents

[00199] A vesicle may be isolated or detected using a binding agent for a novel component of a vesicle, such as an antibody for a novel antigen specific to a vesicle of interest. Novel antigens that are specific to a vesicle of interest may be isolated or identified using different test compounds of known composition bound to a substrate, such as an array or a plurality of particles, which can allow a large amount of chemical/structural space to be adequately sampled using only a small fraction of the space. The novel antigen identified can also serve as a biomarker for the vesicle. For example, a novel antigen identified for a cell-of-origin specific vesicle can be a useful biomarker.

[00200] The term "agent" or "reagent" as used in respect to contacting a sample can mean any entity designed to bind, hybridize, associate with or otherwise detect or facilitate detection of a target molecule, including target polypeptides, peptides, nucleic acid molecules, leptins, lipids, or any other biological entity that can be detected as described herein or as known in the art. Examples of such agents/reagents are well known in the art, and include but are not limited to

universal or specific nucleic acid primers, nucleic acid probes, antibodies, aptamers, peptoid, peptide nucleic acid, locked nucleic acid, lectin, dendrimer, chemical compound, or other entities described herein or known in the art.

[00201] A binding agent can be identified by screening either a homogeneous or heterogeneous vesicle population against test compounds. Since the composition of each test compound on the substrate surface is known, this constitutes a screen for affinity elements. For example, a test compound array comprises test compounds at specific locations on the substrate addressable locations, and can be used to identify one or more binding agents for a vesicle. The test compounds can all be unrelated or related based on minor variations of a core sequence or structure. The different test compounds may include variants of a given test compound (such as polypeptide isoforms), test compounds that are structurally or compositionally unrelated, or a combination thereof.

[00202] A test compound can be a peptoid, polysaccharide, organic compound, inorganic compound, polymer, lipids, nucleic acid, polypeptide, antibody, protein, polysaccharide, or other compound. The test compound can be natural or synthetic. The test compound can comprise or consist of linear or branched heteropolymeric compounds based on any of a number of linkages or combinations of linkages (e.g., amide, ester, ether, thiol, radical additions, metal coordination, etc.), dendritic structures, circular structures, cavity structures or other structures with multiple nearby sites of attachment that serve as scaffolds upon which specific additions are made. Thes test compound can be spotted on a substrate or synthesized in situ, using standard methods in the art. In addition, the test compound can be spotted or synthesized in situ in combinations in order to detect useful interactions, such as cooperative binding.

[00203] The test compound can be a polypeptide with known amino acid sequence, thus, detection of a test compound binding with a vesicle can lead to identification of a polypeptide of known amino sequence that can be used as a binding agent. For example, a homogenous population of vesicles can be applied to a spotted array on a slide containing between a few and 1,000,000 test polypeptides having a length of variable amino acids. The polypeptides can be attached to the surface through the C-terminus. The sequence of the polypeptides can be generated randomly from 19 amino acids, excluding cysteine. The binding reaction can include a non-specific competitor, such as excess bacterial proteins labeled with another dye such that the specificity ratio for each polypeptide binding target can be determined. The polypeptides with the highest specificity and binding can be selected. The identity of the polypeptide on each spot is known, and thus can be readily identified. Once the novel antigens specific to the homogeneous vesicle population, such as a cell-of-origin specific vesicle is identified, such cell-of-origin specific vesicles may subsequently be isolated using such antigens in methods described hereafter.

[00204] An array can also be used for identifying an antibody as a binding agent for a vesicle. Test antibodies can be attached to an array and screened against a heterogeneous population of vesicles to identify antibodies that can be used to isolate or identify a vesicle. A homogeneous population of vesicles such as cell-of-origin specific vesicles can also be screened with an antibody array. Other than identifying antibodies to isolate or detect a homogeneous population of vesicles, one or more protein biomarkers specific to the homogenous population can be identified. Commercially available platforms with test antibodies pre-selected or custom selection of test antibodies attached to the array can be used. For example, an antibody array from Full Moon Biosystems can be screened using prostate cancer cell derived vesicles identifying antibodies to Bcl-XL, ERCC1, Keratin 15, CD81/TAPA-1, CD9, Epithelial Specific Antigen (ESA), and Mast Cell Chymase as binding agents, and the proteins identified can be used as biomarkers for the vesicles. The biomarker can be present or absent, underexpressed or overexpressed, mutated, or modified in or on a vesicle and used in characterizing a condition.

[00205] An antibody or synthetic antibody to be used as a binding agent can also be identified through a peptide array. Another method is the use of synthetic antibody generation through antibody phage display. M13 bacteriophage libraries of antibodies (e.g. Fabs) are displayed on the surfaces of phage particles as fusions to a coat protein. Each phage particle displays a unique antibody and also encapsulates a vector that contains the encoding DNA. Highly diverse libraries can be constructed and represented as phage pools, which can be used in antibody selection for binding to immobilized antigens. Antigen-binding phages are retained by the immobilized antigen, and the nonbinding phages are removed by washing. The retained phage pool can be amplified by infection of an *Escherichia coli* host and the amplified pool can be used for additional rounds of selection to eventually obtain a population that is dominated by antigen-binding clones. At this stage, individual phase clones can be isolated and subjected to DNA sequencing to decode the sequences of the displayed antibodies. Through the use of phase display and other methods known in the art, high affinity designer antibodies for vesicles can be generated.

[00206] Bead-based assays can also be used to identify novel binding agents to isolate or detect a vesicle. A test antibody or peptide can be conjugated to a particle. For example, a bead can be conjugated to an antibody or peptide and used to detect and quantify the proteins expressed on the surface of a population of vesicles in order to discover and specifically select for novel antibodies that can target vesicles from specific tissue or tumor types. Any molecule of organic origin can be successfully conjugated to a polystyrene bead through use of a commercially available kit according to manufacturer's instructions. Each bead set can be colored a certain detectable wavelength and each can be linked to a known antibody or peptide which can be used to specifically measure which beads are linked to exosomal proteins matching the epitope of previously conjugated antibodies or peptides. The beads can be dyed with discrete fluorescence intensities such that each bead with a different intensity has a different binding agent as described above.

[00207] For example, a purified vesicle preparation can be diluted in assay buffer to an appropriate concentration according to empirically determined dynamic range of assay. A sufficient volume of coupled beads can be prepared and approximately 1 μl of the antibody-coupled beads can be aliqouted into a well and adjusted to a final volume of approximately 50 μl. Once the antibody-conjugated beads have been added to a vacuum compatible plate, the beads can be washed to ensure proper binding conditions. An appropriate volume of vesicle preparation can then be added to each well being tested and the mixture incubated, such as for 15-18 hours. A sufficient volume of detection antibodies using detection antibody diluent solution can be prepared and incubated with the mixture for 1 hour or for as long as necessary. The beads can then be washed before the addition of detection antibody (biotin expressing) mixture composed of streptavidin phycoereythin. The beads can then be washed and vacuum aspirated several times before analysis on a suspension array system using software provided with an instrument. The identity of antigens that can be used to selectively extract the vesicles can then be elucidated from the analysis.

[00208] Assays using imaging systems can be used to detect and quantify proteins expressed on the surface of a vesicle in order to discover and specifically select for and enrich vesicles from specific tissue, cell or tumor types. Antibodies, peptides or cells conjugated to multiple well multiplex carbon coated plates can be used. Simultaneous measurement of many analytes in a well can be achieved through the use of capture antibodies arrayed on the patterned carbon working surface. Analytes can then be detected with antibodies labeled with reagents in electrode wells with an enhanced electro-chemiluminescent plate. Any molecule of organic origin can be successfully conjugated to the carbon coated plate. Proteins expressed on the surface of vesicles can be identified from this assay and can be used as targets to specifically select for and enrich vesicles from specific tissue or tumor types.

[00209] The binding agent can also be an aptamer, which refers to nucleic acids that can bond molecules other than their complementary sequence. An aptamer typically contains 30-80 nucleic acids and can have a high affinity towards a certain target molecule (K_d's reported are between 10^{-11}-10^{-6}mole/1). An aptamer for a target can be identified using systematic evolution of ligands by exponential enrichment (SELEX) (*Tuerk & Gold, Science 249:505-510, 1990; Ellington & Szostak, Nature 346:818-822, 1990*), such as described in U.S. Pat. Nos. 5,270,163, 6,482, 594, 6,291, 184, 6,376, 190 and US 6,458, 539. A library of nucleic acids can be contacted with a target vesicle, and those nucleic acids specifically bound to the target are partitioned from the remainder of nucleic acids in the library which do not specifically bind the target. The partitioned nucleic acids are amplified to yield a ligand-enriched pool. Multiple cycles of binding, partitioning, and amplifying (i.e., selection) result in identification of one or more aptamers with the desired activity. Another method for identifying an aptamer to isolate vesicles is described in U.S. Pat. No. 6,376,190, which describes increasing or decreasing frequency of nucleic acids in a library by their binding to a chemically synthesized peptide. Modified methods, such as Laser SELEX or deSELEX as described in U.S. Patent Publication No. 20090264508 can also be used.

[00210] The term "specific" as used herein in regards to a binding agent can mean that an agent has a greater affinity for its target than other targets, typically with a much great affinity, but does not require that the binding agent is absolutely specific for its target.

Microfluidics

[00211] The methods for isolating or identifying vesicles can be used in combination with microfluidic devices. The methods of isolating or detecting a vesicle, such as described herien, can be performed using a microfluidic device. Microfluidic devices, which may also be referred to as "lab-on-a-chip" systems, biomedical micro-electro-mechanical systems (bioMEMs), or multicomponent integrated systems, can be used for isolating and analyzing a vesicle. Such systems miniaturize and compartmentalize processes that allow for binding of vesicles, detection of biosignatures, and other processes.

[00212] A microfluidic device can also be used for isolation of a vesicle through size differential or affinity selection. For example, a microfluidic device can use one more channels for isolating a vesicle from a biological sample based on size or by using one or more binding agents for isolating a vesicle from a biological sample. A biological sample can be introduced into one or more microfluidic channels, which selectively allows the passage of a vesicle. The selection can be based on a property of the vesicle, such as the size, shape, deformability, or biosignature of the vesicle.

[00213] In one embodiment, a heterogeneous population of vesicles can be introduced into a microfluidic device, and one or more different homogeneous populations of vesicles can be obtained. For example, different channels can have different size selections or binding agents to select for different vesicle populations. Thus, a microfluidic device can isolate a plurality of vesicles wherein at least a subset of the plurality of vesicles comprises a different biosignature from another subset of the plurality of vesicles. For example, the microfluidic device can isolate at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different subsets of vesicles, wherein each subset of vesicles comprises a different biosignature.

[00214] In some embodiments, the microfluidic device can comprise one or more channels that permit further enrichment or selection of a vesicle. A population of vesicles that has been enriched after passage through a first channel can be introduced into a second channel, which allows the passage of the desired vesicle or vesicle population to be further enriched, such as through one or more binding agents present in the second channel.

[00215] Array-based assays and bead-based assays can be used with microfluidic device. For example, the binding agent can be coupled to beads and the binding reaction between the beads and vesicle can be performed in a microfluidic device. Multiplexing can also be performed using a microfluidic device. Different compartments can comprise different binding agents for different populations of vesicles, where each population is of a different cell-of-origin specific vesicle population. In one embodiment, each population has a different biosignature. The hybridization reaction between the microsphere and vesicle can be performed in a microfluidic device and the reaction mixture can be delivered to a detection device. The detection device, such as a dual or multiple laser detection system can be part of the microfluidic system and can use a laser to identify each bead or microsphere by its color-coding, and another laser can detect the hybridization signal associated with each bead.

[00216] Any appropriate microfluidic device can be used in the methods of the invention. Examples of microfluidic devices that may be used, or adapted for use with vesicles, include but are not limited to those described in U.S. Pat. Nos. 7,591,936, 7,581,429, 7,579,136, 7,575,722, 7,568,399, 7,552,741, 7,544,506, 7,541,578, 7,518,726, 7,488,596, 7,485,214, 7,467,928, 7,452,713, 7,452,509, 7,449,096, 7,431,887, 7,422,725, 7,422,669, 7,419,822, 7,419,639, 7,413,709, 7,411,184, 7,402,229, 7,390,463, 7,381,471, 7,357,864, 7,351,592, 7,351,380, 7,338,637, 7,329,391, 7,323,140, 7,261,824, 7,258,837, 7,253,003, 7,238,324, 7,238,255, 7,233,865, 7,229,538, 7,201,881, 7,195,986, 7,189,581, 7,189,580, 7,189,368, 7,141,978, 7,138,062, 7,135,147, 7,125,711, 7,118,910, 7,118,661, 7,640,947, 7,666,361, 7,704,735; and International Patent Publication WO 2010/072410; each of which patents or applications are incorporated herein by reference in their entirety. Another example for use with methods disclosed herein is described in *Chen et al., "Microfluidic isolation and transcriptome analysis of serum vesicles," Lab on a Chip, Dec. 8, 2009 DOI: 10.1039/b916199f.*

[00217] Other microfluidic devices for use with the invention include devices comprising elastomeric layers, valves and pumps, including without limitation those disclosed in U.S. Patent Nos. 5,376,252, 6,408,878, 6,645,432, 6,719,868, 6,793,753, 6,899,137, 6,929,030, 7,040,338, 7,118,910, 7,144,616, 7,216,671, 7,250,128, 7,494,555, 7,501,245, 7,601,270, 7,691,333, 7,754,010, 7,837,946; U.S. Patent Application Nos. 2003/0061687, 2005/0084421, 2005/0112882, 2005/0129581, 2005/0145496, 2005/0201901, 2005/0214173, 2005/0252773, 2006/0006067; and EP Patent Nos. 0527905 and 1065378; each of which application is herein incorporated by reference. In some instances, much or all of the devices are composed of elastomeric material. Certain devices are designed to conduct thermal cycling reactions (e.g., PCR) with devices that include one or more elastomeric valves to regulate solution flow through the device. The devices can comprise arrays of reaction sites thereby allowing a plurality of reactions to be performed. Thus, the devices can be used to assess circulating microRNAs in a multiplex fashion, including microRNAs isolated from vesicles. In an embodiment, the microfluidic device comprises (a) a first plurality of flow channels formed in an elastomeric substrate; (b) a second plurality of flow channels formed in the elastomeric substrate that intersect the first plurality of flow channels to define an array of reaction sites, each reaction site located at an intersection of one of the first and second flow channels; (c) a plurality of isolation valves disposed along the first and second plurality of flow channels and spaced between the reaction sites that can be actuated to isolate a solution within each of the reaction sites from solutions at other reaction sites, wherein the isolation valves comprise one or more control channels that each overlay and intersect one or more of the flow channels; and (d) means for simultaneously actuating the valves for isolating the reaction sites from each other. Various modifications to the basic structure of the device are envisioned within the scope of the invention. MicroRNAs can be detected in each of the reaction sites by using PCR methods. For example, the method can comprise the steps of the steps of: (i) providing a microfluidic device, the microfluidic device

comprising: a first fluidic channel having a first end and a second end in fluid communication with each other through the channel; a plurality of flow channels, each flow channel terminating at a terminal wall; wherein each flow channel branches from and is in fluid communication with the first fluidic channel, wherein an aqueous fluid that enters one of the flow channels from the first fluidic channel can flow out of the flow channel only through the first fluidic channel; and, an inlet in fluid communication with the first fluidic channel, the inlet for introducing a sample fluid; wherein each flow channel is associated with a valve that when closed isolates one end of the flow channel from the first fluidic channel, whereby an isolated reaction site is formed between the valve and the terminal wall; a control channel; wherein each the valve is a deflectable membrane which is deflected into the flow channel associated with the valve when an actuating force is applied to the control channel, thereby closing the valve; and wherein when the actuating force is applied to the control channel a valve in each of the flow channels is closed, so as to produce the isolated reaction site in each flow channel; (ii) introducing the sample fluid into the inlet, the sample fluid filling the flow channels; (iii) actuating the valve to separate the sample fluid into the separate portions within the flow channels; (iv) amplifying the nucleic acid in the sample fluid; (v) analyzing the portions of the sample fluid to determine whether the amplifying produced the reaction. The sample fluid can contain an amplifiable nucleic acid target, e.g., a microRNA, and the conditions can be polymerase chain reaction (PCR) conditions, so that the reaction results in a PCR product being formed.

[00218] In an embodiment, the PCR used to detect microRNA is digital PCR, which is described by Brown, et al., U.S. Pat. No. 6,143,496, titled "Method of sampling, amplifying and quantifying segment of nucleic acid, polymerase chain reaction assembly having nanoliter-sized chambers and methods of filling chambers", and by Vogelstein, et al, U.S. Pat. No. 6,446,706, titled "Digital PCR", both of which are hereby incorporated by reference in their entirety. In digital PCR, a sample is partitioned so that individual nucleic acid molecules within the sample are localized and concentrated within many separate regions, such as the reaction sites of the microfluidic device described above. The partitioning of the sample allows one to count the molecules by estimating according to Poisson. As a result, each part will contain "0" or "1" molecules, or a negative or positive reaction, respectively. After PCR amplification, nucleic acids may be quantified by counting the regions that contain PCR end-product, positive reactions. In conventional PCR, starting copy number is proportional to the number of PCR amplification cycles. Digital PCR, however, is not dependent on the number of amplification cycles to determine the initial sample amount, eliminating the reliance on uncertain exponential data to quantify target nucleic acids and providing absolute quantification. Thus, the method can provide a sensitive approach to detecting microRNAs in a sample.

[00219] In one embodiment, a microfluidic device for isolating or detecting a vesicle comprises a channel of less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, of 60 mm in width, or between about 2-60, 3-50, 3-40, 3-30, 3-20, or 4-20 mm in width. The microchannel can have a depth of less than about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65 or 70 μm, or between about 10-70, 10-40, 15-35, or 20-30 μm. Furthermore, the microchannel can have a length of less than about 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 cm. The microfluidic device can have grooves on its ceiling that are less than about 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 6, 65, 70, 75, or 80 μm wide, or between about 40-80, 40-70, 40-60 or 45-55 μm wide. The grooves can be less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 μm deep, such as between about 1-50, 5-40, 5-30, 3-20 or 5-15 μm.

[00220] The microfluidic device can have one or more binding agents attached to a surface in a channel, or present in a channel. For example, the microchannel can have one or more capture agents, such as a capture agent for EpCam, CD9, PCSA, CD63, CD81, PSMA, B7H3, PSCA, ICAM, STEAP, and EGFR. In one embodiment, a microchannel surface is treated with avidin and a capture agent, such as an antibody, that is biotinylated can be injected into the channel to bind the avidin. In other embodiments, the capture agents are present in chambers or other components of a microfluidic device. The capture agents can also be attached to beads that can be manipulated to move through the microfluidic channels. In one embodiment, the capture agents are attached to magnetic beads. The beads can be manipulated using magnets.

[00221] A biological sample can be flowed into the microfluidic device, or a microchannel, at rates such as at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 μl per minute, such as between about 1-50, 5-40, 5-30, 3-20 or 5-15 μl per minute. One or more vesicles can be captured and directly detected in the microfluidic device. Alternatively, the captured vesicle may be released and exit the microfluidic device prior to analysis. In another embodiment, one or more captured vesicles are lysed in the microchannel and the lysate can be analyzed, e.g., to examine payload within the vesicles. Lysis buffer can be flowed through the channel and lyse the captured vesicles. For example, the lysis buffer can be flowed into the device or microchannel at rates such as at least about a, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 26, 27, 28, 29, 30, 35, 40, 45, or 50 μl per minute, such as between about 1-50, 5-40, 10-30, 5-30 or 10-35 μl per minute. The lysate can be collected and analyzed, such as performing RT-PCR, PCR, mass spectrometry, Western blotting, or other assays, to detect one or more biomarkers of the vesicle.

[00222] The various isolation and detection systems described herein can be used to isolate or detect circulating biomarkers such as vesicles that are informative for diagnosis, prognosis, disease stratification, theranosis, prediction of responder / non-responder status, disease monitoring, treatment monitoring and the like as related to such diseases and disorders. Combinations of the isolation techniques are within the scope of the invention. In a non-limiting example, a sample can be run through a chromatography column to isolate vesicles based on a property such as size of electrophoretic motility, and the vesicles can then be passed through a microfluidic device. Binding agents can be used before, during or after these steps.

Combined isolation methodology

[00223] One of skill will appreciate that various methods of sample treatment and isolating and concentrating circulating biomarkers such as vesicles can be combined as desired. For example, a biological sample can be treated to prevent aggregation, remove undesired particulate and/or deplete highly abundant proteins. The steps used can be chosen to optimize downstream analysis steps. Next, biomarkers such as vesicles can be isolated, e.g., by chromotography, centrifugation, density gradient, filtration, precipitation, or affinity techniques. Any number of the later steps can be combined, e.g., a sample could be subjected to one or more of chromotography, centrifugation, density gradient, filtration and precipitation in order to isolate or concentrate all or most microvesicles. In a subsequent step, affinity techniques, e.g., using binding agents to one or more target of interest, can be used to isolate or identify microvesicles carrying desired biomarker profiles. Microfluidic systems can be employed to perform some or all of these steps.

[00224] An exemplary isolation scheme for isolating and analysis of microvesicles includes the following: Plasma or serum collection -> highly abundant protein removal -> ultrafiltration -> nanomembrane concentration -> flow cytometry or particle-based assay.

[00225] Using the methods disclosed herein or known in the art, circulating biomarkers such as vesicles can be isolated or concentrated by at least about 2-fold, 3-fold, 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 12-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, 50-fold, 55-fold, 60-fold, 65-fold, 70-fold, 75-fold, 80-fold, 90-fold, 95-fold, 100-fold, 110-fold, 120-fold, 125-fold, 130-fold, 140-fold, 150-fold, 160-fold, 170-fold, 175-fold , 180-fold, 190-fold, 200-fold, 225-fold, 250-fold, 275-fold, 300-fold, 325-fold, 350-fold, 375-fold, 400-fold, 425-fold, 450-fold, 475-fold, 500-fold, 525-fold, 550-fold, 575-fold, 600-fold, 625-fold, 650-fold, 675-fold, 700-fold, 725-fold, 750-fold, 775-fold, 800-fold, 825-fold, 850-fold, 875-fold, 900-fold, 925-fold, 950-fold, 975-fold, 1000-fold, 1500-fold, 2000-fold, 2500-fold, 3000-fold, 4000-fold, 5000-fold, 6000-fold, 7000-fold, 8000-fold, 9000-fold, or at least 10,000-fold. In some embodiments, the vesicles are isolated or concentrated concentrated by at least 1 order of magnitude, 2 orders of magnitude, 3 orders of magnitude, 4 orders of magnitude, 5 orders of magnitude, 6 orders of magnitude, 7 orders of magnitude, 8 orders of magnitude, 9 orders of magnitude, or 10 orders of magnitude or more.

[00226] Once concentrated or isolated, the circulating biomarkers can be assessed, e.g., in order to characterize a phenotype as described herein. In some embodiments, the concentration or isolation steps themselves shed light on the phenotype of interest. For example, affinity methods can detect the presence or level of specific biomarkers of interest.

Cell and Disease-Specific Vesicles

[00227] The bindings agent disclosed herein can be used to isolate or detect a vesicle, such as a cell-of-origin vesicle or vesicle with a specific biosignature. The binding agent can be used to isolate or detect a heterogeneous population of vesicles from a sample or can be used to isolate or detect a homogeneous population of vesicles, such as cell-of-origin specific vesicles with specific biosignatures, from a heterogeneous population of vesicles.

[00228] A homogeneous population of vesicles, such as cell-of-origin specific vesicles, can be analyzed and used to characterize a phenotype for a subject. Cell-of-origin specific vesicles are esicles derived from specific cell types, which can include, but are not limited to, cells of a specific tissue, cells from a specific tumor of interest or a diseased tissue of interest, circulating tumor cells, or cells of maternal or fetal origin. The vesicles may be derived from tumor cells or lung, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colorectal, breast, prostate, brain, esophagus, liver, placenta, or fetal cells. The isolated vesicle can also be from a particular sample type, such as urinary vesicle.

[00229] A cell-of-origin specific vesicle from a biological sample can be isolated using one or more binding agents that are specific to a cell-of-origin. Vesicles for analysis of a disease or condition can be isolated using one or more binding agent specific for biomarkers for that disease or condition.

[00230] A vesicle can be concentrated prior to isolation or detection of a cell-of-origin specific vesicle, such as through centrifugation, chromatography, or filtration, as described above, to produce a heterogeneous population of vesicles prior to isolation of cell-of-origin specific vesicles. Alternatively, the vesicle is not concentrated, or the biological sample is not enriched for a vesicle, prior to isolation of a cell-of-origin vesicle.

[00231] **FIG. 1B** illustrates a flowchart which depicts one method **6100B** for isolating or identifying a cell-of-origin specific vesicle. First, a biological sample is obtained from a subject in step **6102**. The sample can be obtained from a third party or from the same party performing the analysis. Next, cell-of-origin specific vesicles are isolated from the biological sample in step **6104**. The isolated cell-of-origin specific vesicles are then analyzed in step **6106** and a biomarker or biosignature for a particular phenotype is identified in step **6108**. The method may be used for a number of phenotypes. In some embodiments, prior to step **6104,** vesicles are concentrated or isolated from a biological sample to produce a homogeneous population of vesicles. For example, a heterogeneous population of vesicles may be isolated

using centrifugation, chromatography, filtration, or other methods as described above, prior to use of one or more binding agents specific for isolating or identifying vesicles derived from specific cell types.

[00232] A cell-of-origin specific vesicle can be isolated from a biological sample of a subject by employing one or more binding agents that bind with high specificity to the cell-of-origin specific vesicle. In some instances, a single binding agent can be employed to isolate a cell-of-origin specific vesicle. In other instances, a combination of binding agents may be employed to isolate a cell-of-origin specific vesicle. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75, or 100 different binding agents may be used to isolate a cell-of-origin vesicle. Therefore, a vesicle population (e.g., vesicles having the same binding agent profile) can be identified by using a single or a plurality of binding agents.

[00233] One or more binding agents can be selected based on their specificity for a target antigen(s) that is specific to a cell-of-origin, e.g., a cell-of-origin that is related to a tumor, autoimmune disease, cardiovascular disease, neurological disease, infection or other disease or disorder. The cell-of-origin can be from a cell that is informative for a diagnosis, prognosis, disease stratification, theranosis, prediction of responder / non-responder status, disease monitoring, treatment monitoring and the like as related to such diseases and disorders. The cell-of-origin can also be from a cell useful to discover biomarkers for use thereto. Non-limiting examples of antigens which may be used singularly, or in combination, to isolate a cell-of-origin specific vesicle, disease specific vesicle, or tumor specific vesicle, are shown in **Fig. 1** of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein, and are also described herein. The antigen can comprise membrane bound antigens which are accessible to binding agents. The antigen can be a biomarker related to characterizing a phenotype.

[00234] One of skill will appreciate that any applicable antigen that can be used to isolate an informative vesicle is contemplated by the invention. Binding agents, e.g., antibodies, aptamers and lectins, can be chosen that recognize surface antigens and/or fragments thereof, as outlined herein. The binding agents can recognize antigens specific to the desired cell type or location and/or recognize biomarkers associated with the desired cells. The cells can be, e.g., tumor cells, other diseased cells, cells that serve as markers of disease such as activated immune cells, etc. One of skill will appreciate that binding agents for any cells of interest can be useful for isolating vesicles associated with those cells. One of skill will further appreciate that the binding agents disclosed herein can be used for detecting vesicles of interest. As a non-limiting example, a binding agent to a vesicle biomarker can be labeled directly or indirectly in order to detect vesicles bound by one of more of the same or different binding agents.

[00235] A number of targets for binding agents useful for binding to vesicles associated with cancer, autoimmune diseases, cardiovascular diseases, neurological diseases, infection or other disease or disorders are presented in **Table 4**. A vesicle derived from a cell associated with one of the listed disorders can be characterized using one of the antigens in the table. The binding agent, e.g., an antibody or aptamer, can recognize an epitope of the listed antigens, a fragment thereof, or binding agents can be used against any appropriate combination. Other antigens associated with the disease or disorder can be recognized as well in order to characterize the vesicle. One of skill will appreciate that any applicable antigen that can be used to assess an informative vesicle is contemplated by the invention for isolation, capture or detection in order to characterize a vesicle.

Table 4: Illustrative Antigens for Use in Characterizing Various Diseases and Disorders

Disease or disorder	Target
Breast cancer, e.g., glandular or stromal cells	BCA-225, hsp70, MART1, ER, VEGFA, Class III b-tubulin, HER2/neu (for Her2+ breast cancer), GPR30,

	ErbB4 (JM) isoform, MPR8, MISIIR
Breast cancer	CD9, MIS Rii, ER, CD63, MUC1, HER3, STAT3, VEGFA, BCA, CA125, CD24, EPCAM, ERB B4
Breast cancer	BCA-225, hsp70, MART1, ER, VEGFA, Class III b-tubulin, HER2/neu (e.g., for Her2+ breast cancer), GPR30, ErbB4 (JM) isoform, MPR8, MISIIR, CD9, EphA2, EGFR, B7H3, PSM, PCSA, CD63, STEAP, CD81, ICAM1, A33, DR3, CD66e, MFG-E8, TROP-2, Mammaglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, EpCam, neurokinin receptor-1 (NK-1 or NK-1R), NK-2, Pai-1, CD45, CD10, HER2/ERBB2, AGTR1, NPY1R, MUC1, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUC1 secreted), CA27.29 (MUC1 secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1, SPB, SPC, NSE, PGP9.5, a progesterone receptor (PR) or its isoform (PR(A) or PR(B)), P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, IC3b, mesothelin, SPA, AQP5, GPCR, hCEA-CAM, PTP IA-2, CABYR, TMEM211, ADAM28, UNC93A, MUC17, MUC2, IL10R-beta, BCMA, HVEM/TNFRSF14, Trappin-2 Elafin, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNF
Breast cancer	CD10, NPGP/NPFF2, HER2/ERBB2, AGTR1, NPY1R, neurokinin receptor-1 (NK-1 or NK-1R), NK-2, MUC1, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUC1 secreted), CA27.29 (MUC1 secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1
Breast cancer	SPB, SPC, NSE, PGP9.5, CD9, P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, EGFR, B7H3, IC3b, MUC1, mesothelin, SPA, PCSA, CD63, STEAP, AQP5, CD81, DR3, PSM, GPCR, EphA2, hCEA-CAM, PTP IA-2, CABYR, TMEM211, ADAM28, UNC93A, A33, CD24, CD10, NGAL, EpCam, MUC17, TROP-2, MUC2, IL10R-beta, BCMA, HVEM/TNFRSF14, Trappin-2 Elafin, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNFR
Breast cancer	BRCA, MUC-1, MUC 16, CD24, ErbB4, ErbB2 (HER2), ErbB3, HSP70, Mammaglobin, PR, PR(B), VEGFA
Ovarian Cancer	CA125, VEGFR2, HER2, MISIIR, VEGFA, CD24, c-reactive protein EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rab13, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8, HLA-DR, CD95
Lung Cancer	CYFRA21-1, TPA-M, TPS, CEA, SCC-Ag, XAGE-1b, HLA Class 1, TA-MUC1, KRAS, hENT1, kinin B1 receptor, kinin B2 receptor, TSC403, HTI56, DC-LAMP
Lung Cancer	SPB, SPC, PSP9.5, NDUFB7, gal3-b2c10, iC3b, MUC1, GPCR, CABYR and muc17
Colorectal Cancer	CEA, MUC2, GPA33, CEACAM5, ENFB1, CCSA-3, CCSA-4, ADAM10, CD44, NG2, ephrin B1, plakoglobin, galectin 4, RACK1, tetraspanin-8, FASL, A33, CEA, EGFR, dipeptidase 1, PTEN, Na(+)-dependent glucose transporter, UDP-glucuronosyltransferase 1A, TMEM211, CD24
Prostate Cancer	PSA, TMPRSS2, FASLG, TNFSF10, PSMA, NGEP, Il-7RI, CSCR4, CysLT1R, TRPM8, Kv1.3, TRPV6, TRPM8, PSGR, MISIIR, galectin-3, PCA3,

	TMPRSS2:ERG
Brain Cancer	PRMT8, BDNF, EGFR, DPPX, Elk, Densin-180, BAI2, BAI3
Blood Cancer (hematological malignancy)	CD44, CD58, CD31, CD11a, CD49d, GARP, BTS, Raftlin
Melanoma	DUSP1, TYRP1, SILV, MLANA, MCAM, CD63, Alix, hsp70, meosin, p120 catenin, PGRL, syntaxin binding protein 1 & 2, caveolin
Liver Cancer (hepatocellular carcinoma)	HBxAg, HBsAg, NLT
Cervical Cancer	MCT-1, MCT-2, MCT-4
Endometrial Cancer	Alpha V Beta 6 integrin
Psoriasis	flt-1, VPF receptors, kdr
Autoimmune Disease	Tim-2
Irritable Bowel Disease (IBD or Syndrome (IBS)	IL-16, IL-1beta, IL-12, TNF-alpha, interferon-gamma, IL-6, Rantes, II-12, MCP-1, 5HT
Diabetes, e.g., pancreatic cells	IL-6, CRP, RBP4
Barrett's Esophagus	p53, MUC1, MUC6
Fibromyalgia	neopterin, gp130
Benign Prostatic Hyperplasia (BPH)	KIA1, intact fibronectin
Multiple Sclerosis	B7, B7-2, CD-95 (fas), Apo-1/Fas
Parkinson's Disease	PARK2, ceruloplasmin, VDBP, tau, DJ-1
Rheumatic Disease	Citrulinated fibrin a-chain, CD5 antigen-like fibrinogen fragment D, CD5 antigen-like fibrinogen fragment B, TNF alpha
Alzheimer's Disease	APP695, APP751 or APP770, BACE1, cystatin C, amyloid β, T-tau, complement factor H, alpha-2-macroglobulin
Head and Neck Cancer	EGFR, EphB4, Ephrin B2
Gastrointestinal Stromal Tumor (GIST)	c-kit PDGFRA, NHE-3
Renal Cell Carcinoma	c PDGFRA, VEGF, HIF 1 alpha
Schizophrenia	ATP5B, ATP5H, ATP6V1B, DNM1
Peripheral Neuropathic Pain	OX42, ED9
Chronic Neuropathic Pain	chemokine receptor (CCR2/4)
Prion Disease	PrPSc, 14-3-3 zeta, S-100, AQP4
Stroke	S-100, neuron specific enolase, PARK7, NDKA, ApoC-I, ApoC-III, SAA or AT-III fragment, Lp-PLA2, hs-CRP
Cardiovascular Disease	FATP6
Esophageal Cancer	CaSR
Tuberculosis	antigen 60, HSP, Lipoarabinomannan, Sulfolipid, antigen of acylated trehalose family, DAT, TAT, Trehalose 6,6 – dimycolate (cord-factor) antigen
HIV	gp41, gp120
Autism	VIP, PACAP, CGRP, NT3
Asthma	YKL-40, S-nitrosothiols, SSCA2, PAI, amphiregulin, periostin
Lupus	TNFR
Cirrhosis	NLT, HBsAg
Influenza	hemagglutinin, neurominidase
Vulnerable Plaque	Alpha v. Beta 3 integrin, MMP9

[00236] The foregoing **Table 4**, as well as other biomarker lists disclosed here are illustrative, and Applicants contemplate incorporating various biomarkers disclosed across different disease states or conditions. For example, method of the invention may use various biomarkers across different diseases or conditions, where the biomarkers are useful for providing a diagnostic, prognostic or theranostic signature. In one embodiment, angiogenic, inflammatory or immune-associated antigens (or biomarkers) disclosed herein or know in the art can be used in methods of the invention to screen a biological sample in identification of a biosignature. Indeed, the flexibility of Applicants'

multiplex approach to assessing microvesicle populations facilitates assessing various markers (and in some instances overlapping markers) for different conditions or diseases whose etiology necessarily may share certain cellular and biological mechanisms, e.g., different cancers implicating biomarkers for angiogenesis, or immune response regulation or modulation. The combination of such overlapping biomarkers with tissue or cell-specific biomarkers, along with microvesicle-associated biomarkers provides a powerful series of tools for practicing the methods and compositions of the invention.

[00237] A cell-of-origin specific vesicle may be isolated using novel binding agents, using methods as described herein. Furthermore, a cell-of-origin specific vesicle can also be isolated from a biological sample using isolation methods based on cellular binding partners or binding agents of such vesicles. Such cellular binding partners can include but are not limited to peptides, proteins, RNA, DNA, apatmers, cells or serum-associated proteins that only bind to such vesicles when one or more specific biomarkers are present. Isolation or deteciton of a cell-of-origin specific vesicle can be carried out with a single binding partner or binding agent, or a combination of binding partners or binding agents whose singular application or combined application results in cell-of-origin specific isolation or detection. Non-limiting examples of such binding agents are provided in **Fig. 2** of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein. For example, a vesicle for characterizing breast cancer can be isolated with one or more binding agents including, but not limited to, estrogen, progesterone, trastuzumab, CCND1, MYC PNA, IGF-1 PNA, MYC PNA, SC4 aptamer (Ku), AII-7 aptamer (ERB2), Galectin -3, mucin-type O-glycans, L-PHA, Galectin-9, or any combination thereof.

[00238] A binding agent may also be used for isolating or detecting a cell-of-origin specific vesicle based on: i) the presence of antigens specific for cell-of-origin specific vesicles; ii) the absence of markers specific for cell-of-origin specific vesicles; or iii) expression levels of biomarkers specific for cell-of-origin specific vesicles. A heterogeneous population of vesicles can be applied to a surface coated with specific binding agents designed to rule out or identify the cell-of-origin characteristics of the vesicles. Various binding agents, such as antibodies, can be arrayed on a solid surface or substrate and the heterogeneous population of vesicles is allowed to contact the solid surface or substrate for a sufficient time to allow interactions to take place. Specific binding or non-binding to given antibody locations on the array surface or substrate can then serve to identify antigen specific characteristics of the vesicle population that are specific to a given cell-of-origin. That is, binding events can signal the presence of a vesicle having an antigen recognized by the bound antibody. Conversely, lack of binding events can signal the absence of vesicles having an antigen recognized by the bound antibody.

[00239] A cell-of-origin specific vesicle can be enriched or isolated using one or more binding agents using a magnetic capture method, fluorescence activated cell sorting (FACS) or laser cytometry as described above. Magnetic capture methods can include, but are not limited to, the use of magnetically activated cell sorter (MACS) microbeads or magnetic columns. Examples of immunoaffinity and magnetic particle methods that can be used are described in U.S. Patent Nos. 4,551,435, 4,795,698, 4,925,788, 5,108,933, 5,186,827, 5,200,084 or 5,158,871. A cell-of-origin specific vesicle can also be isolated following the general methods described in U.S. Patent No. 7,399,632, by using combination of antigens specific to a vesicle.

[00240] Any other appropriate method for isolating or otherwise enriching the cell-of-origin specific vesicles with respect to a biological sample may also be used in combination with the present invention. For example, size exclusion chromatography such as gel permeation columns, centrifugation or density gradient centrifugation, and filtration

methods can be used in combination with the antigen selection methods described herein. The cell-of-origin specific vesicles may also be isolated following the methods described in *Koga et al., Anticancer Research, 25:3703-3708 (2005), Taylor et al., Gynecologic Oncology, 110:13-21 (2008), Nanjee et al., Clin Chem, 2000;46:207-223* or U.S Patent No. 7,232,653.

[00241] Vesicles can be isolated and/or detected to provide diagnosis, prognosis, disease stratification, theranosis, prediction of responder / non-responder status, disease monitoring, treatment monitoring and the like. In one embodiment, vesicles are isolated from cells having a disease or disorder, e.g., cells derived from a tumor or malignant growth, a site of autoimmune disease, cardiovascular disease, neurological disease, or infection. In some embodiments, the isolated vesicles are derived from cells related to such diseases and disorders, e.g., immune cells that play a role in the etiology of the disease and whose analysis is informative for a diagnosis, prognosis, disease stratification, theranosis, prediction of responder / non-responder status, disease monitoring, treatment monitoring and the like as relates to such diseases and disorders. The vesicles are further useful to discover novel biomarkers. By identifying biomarkers associated with vesicles, isolated vesicles can be assessed for characterizing a phenotype as described herein.

[00242] In some embodiments, methods of the invention are directed to characterizing presence of a cancer or likelihood of a cancer occurring in an individual by assessing one or more microvesicle population present in a biological sample from an individual. Microvesicles can be isolated using one or more processes disclosed herein or practiced in the art.

[00243] Such microvesicles populations can each separately or collectively provide a disease phenotype characterization for the individual by comparing the biomarker profile, or biosignature, for the microvesicle population(s) with a reference sample to provide a diagnostic, prognostic or theranostic characterization for the test sample.

[00244] The vesicle population(s) can be assessed from various biological samples and bodily fluids such as disclosed herein.

Biomarker Assessment

[00245] In an aspect of the invention, a phenotype of a subject is characterized by analyzing a biological sample and determining the presence, level, amount, or concentration of one or more populations of circulating biomarkers in the sample, e.g., circulating vesicles, proteins or nucleic acids. In embodiments, characterization includes determining whether the circulating biomarkers in the sample are altered as compared to a reference, which can also be referred to a standard or a control. An alteration can include any measurable difference between the sample and the reference, including without limitation an absolute presence or absence, a quantitative level, a relative level compared to a reference, e.g., the level of all vesicles present, the level of a housekeeping marker, and/or the level of a spiked-in marker, an elevated level, a decreased level, overexpression, underexpression, differential expression, a mutation or other altered sequence, a modification (glycosylation, phosphorylation, epigenetic change) and the like. In some embodiments, circulating biomarkers are purified or concentrated from a sample prior to determining their amount. Unless otherwise specified, "purified" or "isolated" as used herein refer to partial or complete purification or isolation. In other embodiments, circulating biomarkers are directly assessed from a sample, without prior purification or concentration. Circulating vesicles can be cell-of-origin specific vesicles or vesicles with a specific biosignature. A biosignature includes specific pattern of biomarkers, e.g., patterns of biomarkers indicative of a phenotype that is desireable to detect, such as a disease phenotype. The biosignature can comprise one or more circulating biomarkers. A

biosignature can be used when characterizing a phenotype, such as a diagnosis, prognosis, theranosis, or prediction of responder / non-responder status. In some embodiments, the biosignature is used to determine a physiological or biological state, such as pregnancy or the stage of pregnancy. The biosignature can also be used to determine treatment efficacy, stage of a disease or condition, or progression of a disease or condition. For example, the amount of one or more vesicles can be proportional or inversely proportional to an increase in disease stage or progression. The detected amount of vesicles can also be used to monitor progression of a disease or condition or to monitor a subject's response to a treatment.

[00246] The circulating biomarkers can be evaluated by comparing the level of circulating biomarkers with a reference level or value. The reference value can be particular to physical or temporal endpoint. For example, the reference value can be from the same subject from whom a sample is assessed, or the reference value can be from a representative population of samples (e.g., samples from normal subjects not exhibiting a symptom of disease). Therefore, a reference value can provide a threshold measurement which is compared to a subject sample's readout for a biosignature assayed in a given sample. Such reference values may be set according to data pooled from groups of sample corresponding to a particular cohort, including but not limited to age (e.g., newborns, infants, adolescents, young, middle-aged adults, seniors and adults of varied ages), racial/ethnic groups, normal versus diseased subjects, smoker v. non-smoker, subject receiving therapy versus untreated subject, different time points of treatment for a particular individual or group of subjects similarly diagnosed or treated or combinations thereof. Furthermore, by determining a biosignature at different timepoints of treatment for a particular individual, the individual's response to the treatment or progression of a disease or condition for which the individual is being treated for, can be monitored.

[00247] A reference value may be based on samples assessed from the same subject so to provide individualized tracking. In some embodiments, frequent testing of a biosignature in samples from a subject provides better comparisons to the reference values previously established for that subject. Such time course measurements are used to allow a physician to more accurately assess the subject's disease stage or progression and therefore inform a better decision for treatment. In some cases, the variance of a biosignature is reduced when comparing a subject's own biosignature over time, thus allowing an individualized threshold to be defined for the subject, e.g., a threshold at which a diagnosis is made. Temporal intrasubject variation allows each individual to serve as their own longitudinal control for optimum analysis of disease or physiological state. As an illustrative example, consider that the level of vesicles derived from prostate cells is measured in a subject's blood over time. A spike in the level of prostate-derived vesicles in the subject's blood can indicate hyperproliferation of prostate cells, e.g., due to prostate cancer.

[00248] Reference values can be established for unaffected individuals (of varying ages, ethnic backgrounds and sexes) without a particular phenotype by determining the biosignature of interest in an unaffected individual. For example, a reference value for a reference population can be used as a baseline for detection of one or more circulating biomarker populations in a test subject. If a sample from a subject has a level or value that is similar to the reference, the subject can be identified to not have the disease, or of having a low likelihood of developing a disease.

[00249] Alternatively, reference values or levels can be established for individuals with a particular phenotype by determining the amount of one or more populations of vesicles in an individual with the phenotype. In addition, an index of values can be generated for a particular phenotype. For example, different disease stages can have different values, such as obtained from individuals with the different disease stages. A subject's value can be compared to the index and a diagnosis or prognosis of the disease can be determined, such as the disease stage or progression wherein the subject's levels most closely correlate with the index. In other embodiments, an index of values is generated for

therapeutic efficacies. For example, the level of vesicles of individuals with a particular disease can be generated and noted what treatments were effective for the individual. The levels can be used to generate values of which is a subject's value is compared, and a treatment or therapy can be selected for the individual, e.g., by predicting from the levels whether the subject is likely to be a responder or non-responder for a treatment.

[00250] In some embodiments, a reference value is determined for individuals unaffected with a particular cancer, by isolating or detecting circulating biomarkers with an antigen that specifically targets biomarkers for the particular cancer. As a non-limiting example, individuals with varying stages of colorectal cancer and noncancerous polyps can be surveyed using the same techniques described for unaffected individuals and the levels of circulating vesicles for each group can be determined. In some embodiments, the levels are defined as means ± standard deviations from at least two separate experiments, performed in at least duplicate or triplicate. Comparisons between these groups can be made using statistical tests to determine statistical significance of distinguishing biomarkers observed. In some embodiments, statistical significance is determined using a parametric statistical test. The parametric statistical test can comprise, without limitation, a fractional factorial design, analysis of variance (ANOVA), a t-test, least squares, a Pearson correlation, simple linear regression, nonlinear regression, multiple linear regression, or multiple nonlinear regression. Alternatively, the parametric statistical test can comprise a one-way analysis of variance, two-way analysis of variance, or repeated measures analysis of variance. In other embodiments, statistical significance is determined using a nonparametric statistical test. Examples include, but are not limited to, a Wilcoxon signed-rank test, a Mann-Whitney test, a Kruskal-Wallis test, a Friedman test, a Spearman ranked order correlation coefficient, a Kendall Tau analysis, and a nonparametric regression test. In some embodiments, statistical significance is determined at a p-value of less than 0.05, 0.01, 0.005, 0.001, 0.0005, or 0.0001. The p-values can also be corrected for multiple comparisons, e.g., using a Bonferroni correction, a modification thereof, or other technique known to those in the art, e.g., the Hochberg correction, Holm-Bonferroni correction, Šidák correction, Dunnett's correction or Tukey's multiple comparisons. In some embodiments, an ANOVA is followed by Tukey's correction for post-test comparing of the biomarkers from each population. A biosignature comprising more than one marker can be evaluated using multivariate modeling techniques to build a classifier using techniques described herein or known in the art.

[00251] Reference values can also be established for disease recurrence monitoring (or exacerbation phase in MS), for therapeutic response monitoring, or for predicting responder / non-responder status.

[00252] In some embodiments, a reference value for vesicles is determined using an artificial vesicle, also referred to herein as a synthetic vesicle. Methods for manufacturing artificial vesicles are known to those of skill in the art, e.g., using liposomes. Artificial vesicles can be manufactured using methods disclosed in US20060222654 and US4448765, which are incorporated herein by reference in its entirety. Artificial vesicles can be constructed with known markers to facilitate capture and/or detection. In some embodiments, artificial vesicles are spiked into a bodily sample prior to processing. The level of intact synthetic vesicle can be tracked during processing, e.g., using filtration or other isolation methods disclosed herein, to provide a control for the amount of vesicles in the initial versus processed sample. Similarly, artificial vesicles can be spiked into a sample before or after any processing steps. In some embodiments, artificial vesicles are used to calibrate equipment used for isolation and detection of vesicles.

[00253] Artificial vesicles can be produced and used a control to test the viability of an assay, such as a bead-based assay. The artificial vesicle can bind to both the beads and to the detection antibodies. Thus, the artificial vesicle contains the amino acid sequence/conformation that each of the antibodies binds. The artificial vesicle can comprise a purified protein or a synthetic peptide sequence to which the antibody binds. The artificial vesicle could be a bead, e.g.,

a polystyrene bead, that is capable of having biological molecules attached thereto. If the bead has an available carboxyl group, then the protein or peptide could be attached to the bead via an available amine group, such as using carbodiimide coupling.

[00254] In another embodiment, the artificial vesicle can be a polystyrene bead coated with avidin and a biotin is placed on the protein or peptide of choice either at the time of synthesis or via a biotin-maleimide chemistry. The proteins/peptides to be on the bead can be mixed together in ratio specific to the application the artificial vesicle is being used for, and then conjugated to the bead. These artificial vesicles can then serve as a link between the capture beads and the detection antibodies, thereby providing a control to show that the components of the assay are working properly.

[00255] The value can be a quantitative or qualitative value. The value can be a direct measurement of the level of vesicles (example, mass per volume), or an indirect measure, such as the amount of a specific biomarker. The value can be a quantitative, such as a numerical value. In other embodiments, the value is qualitiative, such as no vesicles, low level of vesicles, medium level, high level of vesicles, or variations thereof.

[00256] The reference value can be stored in a database and used as a reference for the diagnosis, prognosis, theranosis, disease stratification, disease monitoring, treatment monitoring or prediction of non-responder / responder status of a disease or condition based on the level or amount of circulating biomarkers, such as total amount of vesicles or microRNA, or the amount of a specific population of vesicles or microRNA, such as cell-of-origin specific vesicles or microRNA or microRNA from vesicles with a specific biosignature. In an illustrative example, consider a method of determining a diagnosis for a cancer. Vesicles or other circulating biomarkers from reference subjects with and without the cancer are assessed and stored in the database. The reference subjects provide biosignature indicative of the cancer or of another state, e.g., a healthy state. A sample from a test subject is then assayed and the microRNA biosignature is compared against those in the database. If the subject's biosignature correlates more closely with reference values indicative of cancer, a diagnosis of cancer may be made. Conversely, if the subject's biosignature correlates more closely with reference values indicative of a healthy state, the subject may be determined to not have the disease. One of skill will appreciate that this example is non-limiting and can be expanded for assessing other phenotypes, e.g., other diseases, prognosis, theranosis, disease stratification, disease monitoring, treatment monitoring or prediction of non-responder / responder status, and the like.

[00257] A biosignature for characterizing a phenotype can be determined by detecting circulating biomarkers such as vesicles, including biomarkers associate with vesicles such as surface antigens or payload. The payload, e.g., protein or species of RNA such as mRNA or microRNA, can be assessed within a vesicle. Alternately, the payload in a sample is analyzed to characterize the phenotype without isolating the payload from the vesicles. Many analytical techniques are available to assess vesicles. In some embodiments, vesicle levels are characterized using mass spectrometry, flow cytometry, immunocytochemical staining, Western blotting, electrophoresis, chromatography or x-ray crystallography in accordance with procedures known in the art. For example, vesicles can be characterized and quantitatively measured using flow cytometry as described in *Clayton et al., Journal of Immunological Methods 2001;163-174,* which is herein incorporated by reference in its entirety. Vesicle levels may be determined using binding agents as described above. For example, a binding agent to vesicles can be labeled and the label detected and used to determine the amount of vesicles in a sample. The binding agent can be bound to a substrate, such as arrays or particles, such as described above. Alternatively, the vesicles may be labeled directly.

[00258] Electrophoretic tags or eTags can be used to determine the amount of vesicles. eTags are small fluorescent molecules linked to nucleic acids or antibodies and are designed to bind one specific nucleic acid sequence or protein, respectively. After the eTag binds its target, an enzyme is used to cleave the bound eTag from the target. The signal generated from the released eTag, called a "reporter," is proportional to the amount of target nucleic acid or protein in the sample. The eTag reporters can be identified by capillary electrophoresis. The unique charge-to-mass ratio of each eTag reporter--that is, its electrical charge divided by its molecular weight--makes it show up as a specific peak on the capillary electrophoresis readout. Thus by targeting a specific biomarker of a vesicle with an eTag, the amount or level of vesicles can be determined.

[00259] The vesicle level can determined from a heterogeneous population of vesicles, such as the total population of vesicles in a sample. Alternatively, the vesicles level is determined from a homogenous population, or substantially homogenous population of vesicles, such as the level of specific cell-of-origin vesicles, such as vesicles from prostate cancer cells. In yet other embodiments, the level is determined for vesicles with a particular biomarker or combination of biomarkers, such as a biomarker specific for prostate cancer. Determining the level vesicles can be performed in conjunction with determining the biomarker or combination of biomarkers of a vesicle. Alternatively, determining the amount of vesicle may be performed prior to or subsequent to determining the biomarker or combination of biomarkers of the vesicles.

[00260] Determining the amount of vesicles can be assayed in a multiplexed manner. For example, determining the amount of more than one population of vesicles, such as different cell-of-origin specific vesicles with different biomarkers or combination of biomarkers, can be performed, such as those disclosed herein.

[00261] Performance of a diagnostic or related test is typically assessed using statistical measures. The performance of the characterization can be assessed by measuring sensitivity, specificity and related measures. For example, a level of circulating biomarkers of interest can be assayed to characterize a phenotype, such as detecting a disease. The sensitivity and specificity of the assay to detect the disease is determined.

[00262] A true positive is a subject with a characteristic, e.g., a disease or disorder, correctly identified as having the characteristic. A false positive is a subject without the characteristic that the test improperly identifies as having the characteristic. A true negative is a subject without the characteristic that the test correctly identifies as not having the characteristic. A false negative is a person with the characteristic that the test improperly identifies as not having the characteristic. The ability of the test to distinguish between these classes provides a measure of test performance.

[00263] The specificity of a test is defined as the number of true negatives divided by the number of actual negatives (i.e., sum of true negatives and false positives). Specificity is a measure of how many subjects are correctly identified as negatives. A specificity of 100% means that the test recognizes all actual negatives - for example, all healthy people will be recognized as healthy. A lower specificity indicates that more negatives will be determined as positive.

[00264] The sensitivity of a test is defined as the number of true positives divided by the number of actual positives (i.e., sum of true positives and false negatives). Sensitivity is a measure of how many subjects are correctly identified as positives. A sensitivity of 100% means that the test recognizes all actual positives - for example, all sick people will be recognized as sick. A lower sensitivity indicates that more positives will be missed by being determined as negative.

[00265] The accuracy of a test is defined as the number of true positives and true negatives divided by the sum of all true and false positives and all true and false negatives. It provides one number that combines sensitivity and specificity measurements.

[00266] Sensitivity, specificity and accuracy are determined at a particular discrimination threshold value. For example, a common threshold for prostate cancer (PCa) detection is 4 ng/mL of prostate specific antigen (PSA) in serum. A level of PSA equal to or above the threshold is considered positive for PCa and any level below is considered negative. As the threshold is varied, the sensitivity and specificity will also vary. For example, as the threshold for detecting cancer is increased, the specificity will increase because it is harder to call a subject positive, resulting in fewer false positives. At the same time, the sensitivity will decrease. A receiver operating characteristic curve (ROC curve) is a graphical plot of the true positive rate (i.e., sensitivity) versus the false positive rate (i.e., 1 − specificity) for a binary classifier system as its discrimination threshold is varied. The ROC curve shows how sensitivity and specificity change as the threshold is varied. The Area Under the Curve (AUC) of an ROC curve provides a summary value indicative of a test's performance over the entire range of thresholds. The AUC is equal to the probability that a classifier will rank a randomly chosen positive sample higher than a randomly chosen negative sample. An AUC of 0.5 indicates that the test has a 50% chance of proper ranking, which is equivalent to no discriminatory power (a coin flip also has a 50% chance of proper ranking). An AUC of 1.0 means that the test properly ranks (classifies) all subjects. The AUC is equivalent to the Wilcoxon test of ranks.

[00267] A biosignature according to the invention can be used to characterize a phenotype with at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70% sensitivity, such as with at least 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, or 87% sensitivity. In some embodiments, the phenotype is characterized with at least 87.1, 87.2, 87.3, 87.4, 87.5, 87.6, 87.7, 87.8, 87.9, 88.0, or 89% sensitivity, such as at least 90% sensitivity. The phenotype can be characterized with at least 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% sensitivity.

[00268] A biosignature according to the invention can be used to characterize a phenotype of a subject with at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, or 97% specificity, such as with at least 97.1, 97.2, 97.3, 97.4, 97.5, 97.6, 97.7, 97.8, 97.8, 97.9, 98.0, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99.0, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% specificity.

[00269] A biosignature according to the invention can be used to characterize a phenotype of a subject, e.g., based on a level of a circulating biomarker or other characteristic, with at least 50% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 55% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 60% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 65% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 70% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 75% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 80% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 85% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 86% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 87% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 88% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 89% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 90% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 91% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 92% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 93% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 94% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 95% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 96% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or

100% specificity; at least 97% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 98% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; at least 99% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity; or substantially 100% sensitivity and at least 60, 65, 70, 75, 80, 85, 90, 95, 99, or 100% specificity.

[00270] A biosignature according to the invention can be used to characterize a phenotype of a subject with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, or 97% accuracy, such as with at least 97.1, 97.2, 97.3, 97.4, 97.5, 97.6, 97.7, 97.8, 97.8, 97.9, 98.0, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99.0, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or 100% accuracy.

[00271] In some embodiments, a biosignature according to the invention is used to characterize a phenotype of a subject with an AUC of at least 0.60, 0.61, 0.62, 0.63, 0.64, 0.65, 0.66, 0.67, 0.68, 0.69, 0.70, 0.71, 0.72, 0.73, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.80, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, or 0.97, such as with at least 0.971, 0.972, 0.973, 0.974, 0.975, 0.976, 0.977, 0.978, 0.978, 0.979, 0.980, 0.981, 0.982, 0.983, 0.984, 0.985, 0.986, 0.987, 0.988, 0.989, 0.99, 0.991, 0.992, 0.993, 0.994, 0.995, 0.996, 0.997, 0.998, 0.999 or 1.00.

[00272] Furthermore, the confidence level for determining the specificity, sensitivity, accuracy or AUC, may be determined with at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% confidence.

[00273] Other related performance measures include positive and negative likelihood ratios [positive LR = sensitivity/(1-specificity); negative LR = (1-sensitivity)/specificity]. Such measures can also be used to gauge test performance according to the methods of the invention.

Classification

[00274] Biosignature according to the invention can be used to classify a sample. Techniques for discriminate analysis are known to those of skill in the art. For example, a sample can be classified as, or predicted to be, a responder or non-responder to a given treatment for a given disease or disorder. Many statistical classification techniques are known to those of skill in the art. In supervised learning approaches, a group of samples from two or more groups are analyzed with a statistical classification method. One or more biomarkers, e.g., a panel of biomarkers that forms a biosignature, can be discovered that can be used to build a classifier that differentiates between the two or more groups. A new sample can then be analyzed so that the classifier can associate the new with one of the two or more groups. Commonly used supervised classifiers include without limitation the neural network (multi-layer perceptron), support vector machines, k-nearest neighbors, Gaussian mixture model, Gaussian, naive Bayes, decision tree and radial basis function (RBF) classifiers. Linear classification methods include Fisher's linear discriminant, logistic regression, naive Bayes classifier, perceptron, and support vector machines (SVMs). Other classifiers for use with the invention include quadratic classifiers, k-nearest neighbor, boosting, decision trees, random forests, neural networks, pattern recognition, Bayesian networks and Hidden Markov models. One of skill will appreciate that these or other classifiers, including modifications or improvements of those disclosed herein or known in the art, are contemplated within the scope of the invention.

[00275] Multivariate models that can be used to evaluate a biosignature comprising a presence or level of one or more biomarker include the following:

[00276] Linear discriminant analysis (LDA)

[00277] LDA is a well understood classification method that performs well for cases where predictors follow a generally normal distribution. The method can serve as a benchmark for more complex methods.

[00278] Diagonal linear discriminant analysis (DLDA)

[00279] DLDA is version of discriminant analysis which assumes that predictors are independent, an assumption that may not hold true. However, when training data sets are too small to properly estimate covariances between predictors, well-fit DLDA model may consistently outperform more complex models.

[00280] Shrunken centroids discriminant analysis (SCDA)

[00281] This method is commonly known within the mRNA micorarray community as "PAM" (prediction analysis for microarrays). The method is similar to other for discriminate analysis methods but uses more robust (stabilized) estimates of variance.

[00282] Support vector machines (SVM)

[00283] SVMs are a popular variety of machine learning. SVMs often outperforming traditional statistical methods when predictors are not easily transformed to a multivariate normal distribution. The final SVM model can be expressed in much the same way as an LDA model.

[00284] Tree-based gradient boosting (GBM)

[00285] This method generates binary decision trees, using "boosting" to combine weakly performing trees in a weighted fashion to form a stronger ensemble.

[00286] Lasso (Lasso)

[00287] This approach fits a logistic regression model using "lasso" penalized maximum likelihood method. This approach tends to pick one representative marker from a set of highly correlated markers, returning zero values for coefficients of the remaining markers.

[00288] A classifier's performance can be estimated using a "training" set of sample to build a classifier and an independent "test" set of samples to test the model. Other techniques can be used in the art to estimate predictive performance, such as cross-validation methods. One round of cross-validation involves partitioning a sample of data into complementary subsets, performing the analysis on one subset (the training set), and validating the analysis on the other subset (the validation set or testing set). To reduce variability, multiple rounds of cross-validation can be performed using different partitions, and the validation results are averaged over the rounds. Common types of cross-validation include the following:

[00289] K-fold cross-validation

[00290] The sample group is partitioned into k-partitions. One partition is used as the test set and the remainder are used as the training set. The process is repeated k times (or k folds) using each of the partitions once as the test set. The performance of the classifier model is averaged over the iterations. 10-fold cross validation is common though other numbers can be selected depending on sample size, computational resources, and the like.

[00291] 2-fold cross-validation

[00292] This is the simplest version of k-fold validation wherein the data is split into two equal size groups and each group is used for alternate rounds of training and testing.

[00293] Leave-one-out cross-validation

[00294] In this approach, a single sample is withdrawn from the cohort for testing and the rest of the samples are used for training. If each sample is used once as the test sample, this approach is a form of k-folds cross validation where the number of iterations equals the number of samples.

[00295] Repeated random sub-sampling validation

[00296] In this approach, random subsets are drawn for the training and test set for each round of testing. As a result, each sample may not be used for both testing and training over the course of validation.

[00297] Classification using supervised methods is generally performed by the following methodology:

[00298] In order to solve a given problem of supervised learning (e.g. learning to distinguish between two biological states) one generally considers various steps:

[00299] 1. Gather a training set. These can include, for example, samples that are from a subject with or without a disease or disorder, subjects that are known to respond or not respond to a treatment, subjects whose disease progresses or does not progress, etc. The training samples are used to "train" the classifier.

[00300] 2. Determine the input "feature" representation of the learned function. The accuracy of the learned function depends on how the input object is represented. Typically, the input object is transformed into a feature vector, which contains a number of features that are descriptive of the object. The number of features should not be too large, because of the curse of dimensionality; but should be large enough to accurately predict the output. The features might include a set of biomarkers such as those described herein.

[00301] 3. Determine the structure of the learned function and corresponding learning algorithm. A learning algorithm is chosen, e.g., artificial neural networks, decision trees, Bayes classifiers or support vector machines. The learning algorithm is used to build the classifier.

[00302] 4. Build the classifier. The learning algorithm is run the gathered training set. Parameters of the learning algorithm may be adjusted by optimizing performance on a subset (called a validation set) of the training set, or via cross-validation. After parameter adjustment and learning, the performance of the algorithm may be measured on a test set of naive samples that is separate from the training set.

[00303] Once the classifier is determined as described above, it can be used to classify a sample, e.g., that of a subject who is being analyzed by the methods of the invention. As an example, a classifier can be built using data for levels of circulating biomarkers of interest in reference subjects with and without a disease as the training and test sets. Circulating biomarker levels found in a sample from a test subject are assessed and the classifier is used to classify the subject as with or without the disease. As another example, a classifier can be built using data for levels of vesicle biomarkers of interest in reference subjects that have been found to respond or not respond to certain diseases as the training and test sets. The vesicle biomarker levels found in a sample from a test subject are assessed and the classifier is used to classify the subject as with or without the disease.

[00304] Unsupervised learning approaches can also be used with the invention. Clustering is an unsupervised learning approach wherein a clustering algorithm correlates a series of samples without the use the labels. The most similar samples are sorted into "clusters." A new sample could be sorted into a cluster and thereby classified with other members that it most closely associates. Many clustering algorithms well known to those of skill in the art can be used with the invention, such as hierarchical clustering.

Biosignatures

[00305] A biosignature can be obtained according to the invention by assessing a vesicle population, including surface and payload vesicle associated biomarkers, and/or circulating biomarkers including microRNA and protein. A biosignature derived from a subject can be used to characterize a phenotype of the subject. A biosignature can further include the level of one or more additional biomarkers, e.g., circulating biomarkers or biomarkers associated with a vesicle of interest. A biosignature of a vesicle of interest can include particular antigens or biomarkers that are present

on the vesicle. The biosignature can also include one or more antigens or biomarkers that are carried as payload within the vesicle, including the microRNA under examination. The biosignature can comprise a combination of one or more antigens or biomarkers that are present on the vesicle with one or more biomarkers that are detected in the vesicle. The biosignature can further comprise other information about a vesicle aside from its biomarkers. Such information can include vesicle size, circulating half-life, metabolic half-life, and specific activity in vivo or in vitro. The biosignature can comprise the biomarkers or other characteristics used to build a classifier.

[00306] In some embodiments, the microRNA is detected directly in a biological sample. For example, RNA in a bodily fluid can be isolated using commercially available kits such as *mir*Vana kits (Applied Biosystems/ Ambion, Austin, TX), MagMAX™ RNA Isolation Kit (Applied Biosystems/ Ambion, Austin, TX), and QIAzol Lysis Reagent and RNeasy Midi Kit (Qiagen Inc., Valencia CA). Particular species of microRNAs can be determined using array or PCR techniques as described below.

[00307] In some embodiments, the microRNA payload with vesicles is assessed in order to characterize a phenotype. The vesicles can be purified or concentrated prior to determining the biosignature. For example, a cell-of-origin specific vesicle can be isolated and its biosignature determined. Alternatively, the biosignature of the vesicle can be directly assayed from a sample, without prior purification or concentration. The biosignature of the invention can be used to determine a diagnosis, prognosis, or theranosis of a disease or condition or similar measures described herein. A biosignature can also be used to determine treatment efficacy, stage of a disease or condition, or progression of a disease or condition, or responder / non-responder status. Furthermore, a biosignature may be used to determine a physiological state, such as pregnancy.

[00308] A characteristic of a vesicle in and of itself can be assessed to determine a biosignature. The characteristic can be used to diagnose, detect or determine a disease stage or progression, the therapeutic implications of a disease or condition, or characterize a physiological state. Such characteristics include without limitation the level or amount of vesicles, vesicle size, temporal evaluation of the variation in vesicle half-life, circulating vesicle half-life, metabolic half-life of a vesicle, or activity of a vesicle.

[00309] Biomarkers that can be included in a biosignature include one or more proteins or peptides (e.g., providing a protein signature), nucleic acids (e.g. RNA signature as described, or a DNA signature), lipids (e.g. lipid signature), or combinations thereof. In some embodiments, the biosignature can also comprise the type or amount of drug or drug metabolite present in a vesicle, (e.g., providing a drug signature), as such drug may be taken by a subject from which the biological sample is obtained, resulting in a vesicle carrying the drug or metabolites of the drug.

[00310] A biosignature can also include an expression level, presence, absence, mutation, variant, copy number variation, truncation, duplication, modification, or molecular association of one or more biomarkers. A genetic variant, or nucleotide variant, refers to changes or alterations to a gene or cDNA sequence at a particular locus, including, but not limited to, nucleotide base deletions, insertions, inversions, and substitutions in the coding and non-coding regions. Deletions may be of a single nucleotide base, a portion or a region of the nucleotide sequence of the gene, or of the entire gene sequence. Insertions may be of one or more nucleotide bases. The genetic variant may occur in transcriptional regulatory regions, untranslated regions of mRNA, exons, introns, or exon/intron junctions. The genetic variant may or may not result in stop codons, frame shifts, deletions of amino acids, altered gene transcript splice forms or altered amino acid sequence.

[00311] In an embodiment, nucleic acid biomarkers, including nucleic acid payload within a vesicle, is assessed for nucleotide variants. The nucleic acid biomarker may comprise one or more RNA species, e.g., mRNA, miRNA,

snoRNA, snRNA, rRNAs, tRNAs, siRNA, hnRNA, shRNA, enhancer RNA (eRNA), or a combination thereof. Similarly, DNA payload can be assessed to form a DNA signature.

[00312] An RNA signature or DNA signature can also include a mutational, epigenetic modification, or genetic variant analysis of the RNA or DNA present in the vesicle. Epigenetic modifications include patterns of DNA methylation. See, e.g., Lesche R. and Eckhardt F., DNA methylation markers: a versatile diagnostic tool for routine clinical use. Curr Opin Mol Ther. 2007 Jun;9(3):222-30, which is incorporated herein by reference in its entirety. Thus, a biomarker can be the methylation status of a segment of DNA.

[00313] A biosignature can comprise one or more miRNA signatures combined with one or more additional signatures including, but not limited to, an mRNA signature, DNA signature, protein signature, peptide signature, antigen signature, or any combination thereof. For example, the biosignature can comprise one or more miRNA biomarkers with one or more DNA biomarkers, one or more mRNA biomarkers, one or more snoRNA biomarkers, one or more protein biomarkers, one or more peptide biomarkers, one or more antigen biomarkers, one or more antigen biomarkers, one or more lipid biomarkers, or any combination thereof.

[00314] A biosignature can comprise a combination of one or more antigens or binding agents (such as ability to bind one or more binding agents), such as listed in **Figs. 1** and **2**, respectively, of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein, or those described elsewhere herein. The biosignature can further comprise one or more other biomarkers, such as, but not limited to, miRNA, DNA (e.g. single stranded DNA, complementary DNA, or noncoding DNA), or mRNA. The biosignature of a vesicle can comprise a combination of one or more antigens, such as shown in **Fig. 1** of International Patent Application Serial No. PCT/US2011/031479, one or more binding agents, such as shown in **Fig. 2** of International Patent Application Serial No. PCT/US2011/031479, and one or more biomarkers for a condition or disease, such as listed in **Figs. 3-60** of International Patent Application Serial No. PCT/US2011/031479. The biosignature can comprise one or more biomarkers, for example miRNA, with one or more antigens specific for a cancer cell (for example, as shown in **Fig. 1** of International Patent Application Serial No. PCT/US2011/031479).

[00315] In some embodiments, a vesicle used in the subject methods has a biosignature that is specific to the cell-of-origin and is used to derive disease-specific or biological state specific diagnostic, prognostic or therapy-related biosignatures representative of the cell-of-origin. In other embodiments, a vesicle has a biosignature that is specific to a given disease or physiological condition that is different from the biosignature of the cell-of-origin for use in the diagnosis, prognosis, staging, therapy-related determinations or physiological state characterization. Biosignatures can also comprise a combination of cell-of-origin specific and non-specific vesicles.

[00316] Biosignatures can be used to evaluate diagnostic criteria such as presence of disease, disease staging, disease monitoring, disease stratification, or surveillance for detection, metastasis or recurrence or progression of disease. A biosignature can also be used clinically in making decisions concerning treatment modalities including therapeutic intervention. A biosignature can further be used clinically to make treatment decisions, including whether to perform surgery or what treatment standards should be used along with surgery (e.g., either pre-surgery or post-surgery). As an illustrative example, a biosignature of circulating biomarkers that indicates an aggressive form of cancer may call for a more aggressive surgical procedure and/or more aggressive therapeutic regimen to treat the patient.

[00317] A biosignature can be used in therapy related diagnostics to provide tests useful to diagnose a disease or choose the correct treatment regimen, such as provide a theranosis. Theranostics includes diagnostic testing that

provides the ability to affect therapy or treatment of a diseased state. Theranostics testing provides a theranosis in a similar manner that diagnostics or prognostic testing provides a diagnosis or prognosis, respectively. As used herein, theranostics encompasses any desired form of therapy related testing, including predictive medicine, personalized medicine, integrated medicine, pharmacodiagnostics and Dx/Rx partnering. Therapy related tests can be used to predict and assess drug response in individual subjects, i.e., to provide personalized medicine. Predicting a drug response can be determining whether a subject is a likely responder or a likely non-responder to a candidate therapeutic agent, e.g., before the subject has been exposed or otherwise treated with the treatment. Assessing a drug response can be monitoring a response to a drug, e.g., monitoring the subject's improvement or lack thereof over a time course after initiating the treatment. Therapy related tests are useful to select a subject for treatment who is particularly likely to benefit from the treatment or to provide an early and objective indication of treatment efficacy in an individual subject. Thus, a biosignature as disclosed herein may indicate that treatment should be altered to select a more promising treatment, thereby avoiding the great expense of delaying beneficial treatment and avoiding the financial and morbidity costs of administering an ineffective drug(s).

[00318] Therapy related diagnostics are also useful in clinical diagnosis and management of a variety of diseases and disorders, which include, but are not limited to cardiovascular disease, cancer, infectious diseases, sepsis, neurological diseases, central nervous system related diseases, endovascular related diseases, and autoimmune related diseases. Therapy related diagnostics also aid in the prediction of drug toxicity, drug resistance or drug response. Therapy related tests may be developed in any suitable diagnostic testing format, which include, but are not limited to, e.g., immunohistochemical tests, clinical chemistry, immunoassay, cell-based technologies, nucleic acid tests or body imaging methods. Therapy related tests can further include but are not limited to, testing that aids in the determination of therapy, testing that monitors for therapeutic toxicity, or response to therapy testing. Thus, a biosignature can be used to predict or monitor a subject's response to a treatment. A biosignature can be determined at different time points for a subject after initiating, removing, or altering a particular treatment.

[00319] In some embodiments, a determination or prediction as to whether a subject is responding to a treatment is made based on a change in the amount of one or more components of a biosignature (i.e., the microRNA, vesicles and/or biomarkers of interest), an amount of one or more components of a particular biosignature, or the biosignature detected for the components. In another embodiment, a subject's condition is monitored by determining a biosignature at different time points. The progression, regression, or recurrence of a condition is determined. Response to therapy can also be measured over a time course. Thus, the invention provides a method of monitoring a status of a disease or other medical condition in a subject, comprising isolating or detecting a biosignature from a biological sample from the subject, detecting the overall amount of the components of a particular biosignature, or detecting the biosignature of one or more components (such as the presence, absence, or expression level of a biomarker). The biosignatures are used to monitor the status of the disease or condition.

[00320] One or more novel biosignatures of a vesicle can also be identified. For example, one or more vesicles can be isolated from a subject that responds to a drug treatment or treatment regimen and compared to a reference, such as another subject that does not respond to the drug treatment or treatment regimen. Differences between the biosignatures can be determined and used to identify other subjects as responders or non-responders to a particular drug or treatment regimen.

[00321] In some embodiments, a biosignature is used to determine whether a particular disease or condition is resistant to a drug. If a subject is drug resistant, a physician need not waste valuable time with such drug treatment. To obtain

early validation of a drug choice or treatment regimen, a biosignature is determined for a sample obtained from a subject. The biosignature is used to assess whether the particular subject's disease has the biomarker associated with drug resistance. Such a determination enables doctors to devote critical time as well as the patient's financial resources to effective treatments.

[00322] Moreover, biosignature may be used to assess whether a subject is afflicted with disease, is at risk for developing disease or to assess the stage or progression of the disease. For example, a biosignature can be used to assess whether a subject has prostate cancer, colon cancer, or other cancer as described herein. Futhermore, a biosignature can be used to determine a stage of a disease or condition, such as colon cancer.

[00323] Furthermore, determining the amount of vesicles, such a heterogeneous population of vesicles, and the amount of one or more homogeneous population of vesicles, such as a population of vesicles with the same biosignature, can be used to characterize a phenotype. For example, determination of the total amount of vesicles in a sample (i.e. not cell-type specific) and determining the presence of one or more different cell-of-origin specific vesicles can be used to characterize a phenotype. Threshold values, or reference values or amounts can be determined based on comparisons of normal subjects and subjects with the phenotype of interest, as further described below, and criteria based on the threshold or reference values determined. The different criteria can be used to characterize a phenotype.

[00324] One criterion can be based on the amount of a heterogeneous population of vesicles in a sample. In one embodiment, general vesicle markers, such as CD9, CD81, and CD63 can be used to determine the amount of vesicles in a sample. The expression level of CD9, CD81, CD63, or a combination thereof can be detected and if the level is greater than a threshold level, the criterion is met. In another embodiment, the criterion is met if if level of CD9, CD81, CD63, or a combination thereof is lower than a threshold value or reference value. In another embodiment, the criterion can be based on whether the amount of vesicles is higher than a threshold or reference value. Another criterion can be based on the amount of vesicles with a specific biosignature. If the amount of vesicles with the specific biosignature is lower than a threshold or reference value, the criterion is met. In another embodiment, if the amount of vesicles with the specific biosignature is higher than a threshold or reference value, the criterion is met. A criterion can also be based on the amount of vesicles derived from a particular cell type. If the amount is lower than a threshold or reference value, the criterion is met. In another embodiment, if the amount is higher than a threshold value, the criterion is met.

[00325] In a non-limiting example, consider that vesicles from prostate cells are determined by detecting the biomarker PCSA or PSCA, and that a criterion is met if the level of detected PCSA or PSCA is greater than a threshold level. The threshold can be the level of the same markers in a sample from a control cell line or control subject. Another criterion can be based on whether the amount of vesicles derived from a cancer cell or comprising one or more cancer specific biomarkers. For example, the biomarkers B7H3, EpCam, or both, can be determined and a criterion met if the level of detected B7H3 and/or EpCam is greater than a threshold level or within a pre-determined range. If the amount is lower, or higher, than a threshold or reference value, the criterion is met. A criterion can also be the reliability of the result, such as meeting a quality control measure or value. A detected amount of B7H3 and/or EpCam in a test sample that is above the amount of these markers in a control sample may indicate the presence of a cancer in the test sample.

[00326] As described, analysis of multiple markers can be combined to assess whether a criterion is met. In an illustrative example, a biosignature is used to assess whether a subject has prostate cancer by detecting one or more of the general vesicle markers CD9, CD63 and CD81; one or more prostate epithelial markers including PCSA or PSMA; and one or more cancer markers such as B7H3 and/or EpCam. Higher levels of the markers in a sample from a subject

than in a control individual without prostate cancer indicates the presence of the prostate cancer in the subject. In some embodiments, the multiple markers are assessed in a multiplex fashion.

[00327] One of skill will understand that such rules based on meeting criterion as described can be applied to any appropriate biomarker. For example, the criterion can be applied to vesicle characteristics such as amount of vesicles present, amount of vesicles with a particular biosignature present, amount of vesicle payload biomarkers present, amount of microRNA or other circulating biomarkers present, and the like. The ratios of appropriate biomarkers can be determined. As illustrative examples, the criterion could be a ratio of an vesicle surface protein to another vesicle surface protein, a ratio of an vesicle surface protein to a microRNA, a ratio of one vesicle population to another vesicle population, a ratio of one circulating biomarker to another circulating biomarker, etc.

[00328] A phenotype for a subject can be characterized based on meeting any number of useful criteria. In some embodiments, at least one criterion is used for each biomarker. In some embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 or at least 100 criteria are used. For example, for the characterizing of a cancer, a number of different criteria can be used when the subject is diagnosed with a cancer: 1) if the amount of microRNA in a sample from a subject is higher than a reference value; 2) if the amount of a microRNA within cell type specific vesicles (i.e. vesicles derived from a specific tissue or organ) is higher than a reference value; or 3) if the amount of microRNA within vesicles with one or more cancer specific biomarkers is higher than a reference value. Similar rules can apply if the amount of microRNA is less than or the same as the reference. The method can further include a quality control measure, such that the results are provided for the subject if the samples meet the quality control measure. In some embodiments, if the criteria are met but the quality control is questionable, the subject is reassessed.

[00329] In other embodiments, a single measure is determined for assessment of multiple biomarkers, and the measure is compared to a reference. For illustration, a test for prostate cancer might comprise multiplying the level of PSA against the level of miR-141 in a blood sample. The criterion is met if the product of the levels is above a threshold, indicating the presense of the cancer. As another illustration, a number of binding agents to general vesicle markers can carry the same label, e.g., the same fluorophore. The level of the detected label can be compared to a threshold.

[00330] Criterion can be applied to multiple types of biomarkers in addition to multiple biomarkers of the same type. For example, the levels of one or more circulating biomarkers (e.g., RNA, DNA, peptides), vesicles, mutations, etc, can be compared to a reference. Different components of a biosignature can have different criteria. As a non-limiting example, a biosignature used to diagnose a cancer can include overexpression of one miR species as compared to a reference and underexpression of a vesicle surface antigen as compared to another reference.

[00331] A biosignature can be determined by comparing the amount of vesicles, the structure of a vesicle, or any other informative characteristic of a vesicle. Vesicle structure can be assessed using transmission electron microscopy, see for example, *Hansen et al., Journal of Biomechanics 31, Supplement 1: 134-134(1) (1998),* or scanning electron microscopy. Various combinations of methods and techniques or analyzing one or more vesicles can be used to determine a phenotype for a subject.

[00332] A biosignature can include without limitation the presence or absence, copy number, expression level, or activity level of a biomarker. Other useful components of a biosignature include the presence of a mutation (e.g., mutations which affect activity of a transcription or translation product, such as substitution, deletion, or insertion mutations), variant, or post-translation modification of a biomarker. Post-translational modification of a protein biomarker include without limitation acylation, acetylation, phosphorylation, ubiquitination, deacetylation, alkylation, methylation, amidation, biotinylation, gamma-carboxylation, glutamylation, glycosylation, glycyation, hydroxylation,

covalent attachment of heme moiety, iodination, isoprenylation, lipoylation, prenylation, GPI anchor formation, myristoylation, farnesylation, geranylgeranylation, covalent attachment of nucleotides or derivatives thereof, ADP-ribosylation, flavin attachment, oxidation, palmitoylation, pegylation, covalent attachment of phosphatidylinositol, phosphopantetheinylation, polysialylation, pyroglutamate formation, racemization of proline by prolyl isomerase, tRNA-mediation addition of amino acids such as arginylation, sulfation, the addition of a sulfate group to a tyrosine, or selenoylation of the biomarker.

[00333] The methods described herein can be used to identify a biosignature that is associated with a disease, condition or physiological state. The biosignature can also be used to determine if a subject is afflicted with cancer or is at risk for developing cancer. A subject at risk of developing cancer can include those who may be predisposed or who have pre-symptomatic early stage disease.

[00334] A biosignature can also be used to provide a diagnostic or theranostic determination for other diseases including but not limited to autoimmune diseases, inflammatory bowel diseases, cardiovascular disease, neurological disorders such as Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, sepsis or pancreatitis or any disease, conditions or symptoms listed in **Figs. 3-58** of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein.

[00335] The biosignature can also be used to identify a given pregnancy state from the peripheral blood, umbilical cord blood, or amniotic fluid (e.g. miRNA signature specific to Downs Syndrome) or adverse pregnancy outcome such as pre-eclampsia, pre-term birth, premature rupture of membranes, intrauterine growth restriction or recurrent pregnancy loss. The biosignature can also be used to indicate the health of the mother, the fetus at all developmental stages, the pre-implantation embryo or a newborn.

[00336] A biosignature can be used for pre-symptomatic diagnosis. Furthermore, the biosignature can be used to detect disease, determine disease stage or progression, determine the recurrence of disease, identify treatment protocols, determine efficacy of treatment protocols or evaluate the physiological status of individuals related to age and environmental exposure.

[00337] Monitoring a biosignature of a vesicle can also be used to identify toxic exposures in a subject including, but not limited to, situations of early exposure or exposure to an unknown or unidentified toxic agent. Without being bound by any one specific theory for mechanism of action, vesicles can shed from damaged cells and in the process compartmentalize specific contents of the cell including both membrane components and engulfed cytoplasmic contents. Cells exposed to toxic agents/chemicals may increase vesicle shedding to expel toxic agents or metabolites thereof, thus resulting in increased vesicle levels. Thus, monitoring vesicle levels, vesicle biosignature, or both, allows assessment of an individual's response to potential toxic agent(s).

[00338] A vesicle and/or other biomarkers of the invention can be used to identify states of drug-induced toxicity or the organ injured, by detecting one or more specific antigen, binding agent, biomarker, or any combination thereof. The level of vesicles, changes in the biosignature of a vesicle, or both, can be used to monitor an individual for acute, chronic, or occupational exposures to any number of toxic agents including, but not limited to, drugs, antibiotics, industrial chemicals, toxic antibiotic metabolites, herbs, household chemicals, and chemicals produced by other organisms, either naturally occurring or synthetic in nature. In addition, a biosignature can be used to identify conditions or diseases, including cancers of unknown origin, also known as cancers of unknown primary (CUP).

[00339] A vesicle may be isolated from a biological sample as previously described to arrive at a heterogeneous population of vesicles. The heterogeneous population of vesicles can then be contacted with substrates coated with specific binding agents designed to rule out or identify antigen specific characteristics of the vesicle population that are specific to a given cell-of-origin. Further, as described above, the biosignature of a vesicle can correlate with the cancerous state of cells. Compounds that inhibit cancer in a subject may cause a change, e.g., a change in biosignature of a vesicle, which can be monitored by serial isolation of vesicles over time and treatment course. The level of vesicles or changes in the level of vesicles with a specific biosignature can be monitored.

[00340] In an aspect, characterizing a phenotype of a subject comprises a method of determining whether the subject is likely to respond or not respond to a therapy. The methods of the invention also include determining new biosignatures useful in predicting whether the subject is likely to respond or not. One or more subjects that respond to a therapy (responders) and one or more subjects that do not respond to the same therapy (non-responders) can have their vesicles interrogated. Interrogation can be performed to identify vesicle biosignatures that classify a subject as a responder or non-responder to the treatment of interest. In some aspects, the presence, quantity, and payload of a vesicle are assayed. The payload of a vesicle includes, for example, internal proteins, nucleic acids such as miRNA, lipids or carbohydrates.

[00341] The presence or absence of a biosignature in responders but not in the non-responders can be used for theranosis. A sample from responders may be analyzed for one or more of the following: amount of vesicles, amount of a unique subset or species of vesicles, biomarkers in such vesicles, biosignature of such vesicles, etc. In one instance, vesicles such as microvesicles or exosomes from responders and non-responders are analyzed for the presence and/or quantity of one or more miRNAs, such as miRNA 122, miR-548c-5p, miR-362-3p, miR-422a, miR-597, miR-429, miR-200a, and/or miR-200b. A difference in biosignatures between responders and non-responders can be used for theranosis. In another embodiment, vesicles are obtained from subjects having a disease or condition. Vesicles are also obtained from subjects free of such disease or condition. The vesicles from both groups of subjects are assayed for unique biosignatures that are associated with all subjects in that group but not in subjects from the other group. Such biosignatures or biomarkers can then used as a diagnostic for the presence or absence of the condition or disease, or to classify the subject as belonging on one of the groups (those with/without disease, aggressive/non-aggressive disease, responder/non-responder, etc).

[00342] In an aspect, characterizing a phenotype of a subject comprises a method of staging a disease. The methods of the invention also include determining new biosignatures useful in staging. In an illustrative example, vesicles are assayed from patients having a stage I cancer and patients having stage II or stage III of the same cancer. In some embodiments, vesicles are assayed in patients with metastatic disease. A difference in biosignatures or biomarkers between vesicles from each group of patient is identified (e.g., vesicles from stage III cancer may have an increased expression of one or more genes or miRNA's), thereby identifying a biosignature or biomarker that distinguishes different stages of a disease. Such biosignature can then be used to stage patients having the disease.

[00343] In some instances, a biosignature is determined by assaying vesicles from a subject over a period of time, e.g., daily, semiweekly, weekly, biweekly, semimonthly, monthly, bimonthly, semiquarterly, quarterly, semiyearly, biyearly or yearly. For example, the biosignatures in patients on a given therapy can be monitored over time to detect signatures indicative of responders or non-responders for the therapy. Similarly, patients with differing stages of disease or in differing stages of a clinical trial have a biosignature interrogated over time. The payload or physical attributes of the vesicles in each point in time can be compared. A temporal pattern can thus form a biosignature that can then be used for theranosis, diagnosis, prognosis, disease stratification, treatment monitoring, disease monitoring or making a

prediction of responder / non-responder status. As an illustrative example only, an increasing amount of a biomarker (e.g., miR 122) in vesicles over a time course is associated with metastatic cancer, as opposed to a stagnant amounts of the biomarker in vesicles over the time course that are associated with non-metastatic cancer. A time course may last over at least 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 6 weeks, 8 weeks, 2 months, 10 weeks, 12 weeks, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, one year, 18 months, 2 years, or at least 3 years.

[00344] The level of vesicles, level of vesicles with a specific biosignature, or a biosignature of a vesicle can also be used to assess the efficacy of a therapy for a condition. For example, the level of vesicles, level of vesicles with a specific biosignature, or a biosignature of a vesicle can be used to assess the efficacy of a cancer treatment, e.g., chemotherapy, radiation therapy, surgery, or any other therapeutic approach useful for inhibiting cancer in a subject. In addition, a biosignature can be used in a screening assay to identify candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) that have a modulatory effect on the biosignature of a vesicle. Compounds identified via such screening assays may be useful, for example, for modulating, e.g., inhibiting, ameliorating, treating, or preventing conditions or diseases.

[00345] For example, a biosignature for a vesicle can be obtained from a patient who is undergoing successful treatment for a particular cancer. Cells from a cancer patient not being treated with the same drug can be cultured and vesicles from the cultures obtained for determining biosignatures. The cells can be treated with test compounds and the biosignature of the vesicles from the cultures can be compared to the biosignature of the vesicles obtained from the patient undergoing successful treatment. The test compounds that results in biosignatures that are similar to those of the patient undergoing successful treatment can be selected for further studies.

[00346] The biosignature of a vesicle can also be used to monitor the influence of an agent (e.g., drug compounds) on the biosignature in clinical trials. Monitoring the level of vesicles, changes in the biosignature of a vesicle, or both, can also be used in a method of assessing the efficacy of a test compound, such as a test compound for inhibiting cancer cells.

[00347] In addition to diagnosing or confirming the presence of or risk for developing a disease, condition or a syndrome, the methods and compositions disclosed herein also provide a system for optimizing the treatment of a subject having such a disease, condition or syndrome. The level of vesicles, the biosignature of a vesicle, or both, can also be used to determine the effectiveness of a particular therapeutic intervention (pharmaceutical or non-pharmaceutical) and to alter the intervention to 1) reduce the risk of developing adverse outcomes, 2) enhance the effectiveness of the intervention or 3) identify resistant states. Thus, in addition to diagnosing or confirming the presence of or risk for developing a disease, condition or a syndrome, the methods and compositions disclosed herein also provide a system for optimizing the treatment of a subject having such a disease, condition or syndrome. For example, a therapy-related approach to treating a disease, condition or syndrome by integrating diagnostics and therapeutics to improve the real-time treatment of a subject can be determined by identifying the biosignature of a vesicle.

[00348] Tests that identify the level of vesicles, the biosignature of a vesicle, or both, can be used to identify which patients are most suited to a particular therapy, and provide feedback on how well a drug is working, so as to optimize treatment regimens. For example, in pregnancy-induced hypertension and associated conditions, therapy-related diagnostics can flexibly monitor changes in important parameters (e.g., cytokine and/or growth factor levels) over time, to optimize treatment.

[00349] Within the clinical trial setting of investigational agents as defined by the FDA, MDA, EMA, USDA, and EMEA, therapy-related diagnostics as determined by a biosignature disclosed herein, can provide key information to optimize trial design, monitor efficacy, and enhance drug safety. For instance, for trial design, therapy-related diagnostics can be used for patient stratification, determination of patient eligibility (inclusion/exclusion), creation of homogeneous treatment groups, and selection of patient samples that are optimized to a matched case control cohort. Such therapy-related diagnostic can therefore provide the means for patient efficacy enrichment, thereby minimizing the number of individuals needed for trial recruitment. For example, for efficacy, therapy-related diagnostics are useful for monitoring therapy and assessing efficacy criteria. Alternatively, for safety, therapy-related diagnostics can be used to prevent adverse drug reactions or avoid medication error and monitor compliance with the therapeutic regimen.

[00350] In some embodiments, the invention provides a method of identifying responder and non-responders to a treatment undergoing clinical trials, comprising detecting biosignatures comprising circulating biomarkers in subjects enrolled in the clinical trial, and identifying biosignatures that distinguish between responders and non-responders. In a further embodiment, the biosignatures are measured in a drug naive subject and used to predict whether the subject will be a responder or non-responder. The prediction can be based upon whether the biosignatures of the drug naive subject correlate more closely with the clinical trial subjects identified as responders, thereby predicting that the drug naive subject will be a responder. Conversely, if the biosignatures of the drug naive subject correlate more closely with the clinical trial subjects identified as non-responders, the methods of the invention can predict that the drug naive subject will be a non-responder. The prediction can therefore be used to stratify potential responders and non-responders to the treatment. In some embodiments, the prediction is used to guide a course of treatment, e.g., by helping treating physicians decide whether to administer the drug. In some embodiments, the prediction is used to guide selection of patients for enrollment in further clinical trials. In a non-limiting example, biosignatures that predict responder / non-responder status in Phase II trials can be used to select patients for a Phase III trial, thereby increasing the likelihood of response in the Phase III patient population. One of skill will appreciate that the method can be adapted to identify biosignatures to stratify subjects on criteria other than responder / non-responder status. In one embodiment, the criterion is treatment safety. Therefore the method is followed as above to identify subjects who are likely or not to have adverse events to the treatment. In a non-limiting example, biosignatures that predict safety profile in Phase II trials can be used to select patients for a Phase III trial, thereby increasing the treatment safety profile in the Phase III patient population.

[00351] Therefore, the level of vesicles, the biosignature of a vesicle, or both, can be used to monitor drug efficacy, determine response or resistance to a given drug, or both, thereby enhancing drug safety. For example, in colon cancer, vesicles are typically shed from colon cancer cells and can be isolated from the peripheral blood and used to isolate one or more biomarkers e.g., KRAS mRNA which can then be sequenced to detect KRAS mutations. In the case of mRNA biomarkers, the mRNA can be reverse transcribed into cDNA and sequenced (e.g., by Sanger sequencing, pyrosequencing, NextGen sequencing, RT-PCR assays) to determine if there are mutations present that confer resistance to a drug (e.g., cetuximab or panitumimab). In another example, vesicles that are specifically shed from lung cancer cells are isolated from a biological sample and used to isolate a lung cancer biomarker, e.g., EGFR mRNA. The EGFR mRNA is processed to cDNA and sequenced to determine if there are EGFR mutations present that show resistance or response to specific drugs or treatments for lung cancer.

[00352] One or more biosignatures can be grouped so that information obtained about the set of biosignatures in a particular group provides a reasonable basis for making a clinically relevant decision, such as but not limited to a diagnosis, prognosis, or management of treatment, such as treatment selection.

[00353] As with most diagnostic markers, it is often desirable to use the fewest number of markers sufficient to make a correct medical judgment. This prevents a delay in treatment pending further analysis as well inappropriate use of time and resources.

[00354] Also disclosed herein are methods of conducting retrospective analysis on samples (e.g., serum and tissue biobanks) for the purpose of correlating qualitative and quantitative properties, such as biosignatures of vesicles, with clinical outcomes in terms of disease state, disease stage, progression, prognosis; therapeutic efficacy or selection; or physiological conditions. Furthermore, methods and compositions disclosed herein are used for conducting prospective analysis on a sample (e.g., serum and/or tissue collected from individuals in a clinical trial) for the purpose of correlating qualitative and quantitative biosignatures of vesicleswith clinical outcomes in terms of disease state, disease stage, progression, prognosis; therapeutic efficacy or selection; or physiological conditions can also be performed. As used herein, a biosignature for a vesicle can be used to identify a cell-of-origin specific vesicle. Furthermore, a biosignature can be determined based on a surface marker profile of a vesicle or contents of a vesicle.

[00355] The biosignatures used to characterize a phenotype according to the invention can comprise multiple components (e.g., microRNA, vesicles or other biomarkers) or characteristics (e.g., vesicle size or morphology). The biosignatures can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, or 100 components or characteristics. A biosignature with more than one component or characteristic, such as at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, or 100 components, may provide higher sensitivity and/or specificity in characterizing a phenotype. In some embodiments, assessing a plurality of components or characteristics provides increased sensitivity and/or specificity as compared to assessing fewer components or characteristics. On the other hand, it is often desirable to use the fewest number of components or characteristics sufficient to make a correct medical judgment. Fewer markers can avoid statistical overfitting of a classifier and can prevent a delay in treatment pending further analysis as well inappropriate use of time and resources. Thus, the methods of the invention comprise determining an optimal number of components or characteristics.

[00356] A biosignature according to the invention can be used to characterize a phenotype with a sensitivity, specificity, accuracy, or similar performance metric as described above. The biosignatures can also be used to build a classifier to classify a sample as belonging to a group, such as belonging to a group having a disease or not, a group having an aggressive disease or not, or a group of responders or non-responders. In one embodiment, a classifier is used to determine whether a subject has an aggressive or non-aggressive cancer. In the illustrative case of prostate cancer, this can help a physician to determine whether to watch the cancer, i.e., prescribe "watchful waiting," or perform a prostatectomy. In another embodiment, a classifier is used to determine whether a breast cancer patient is likely to respond or not to tamoxifen, thereby helping the physician to determine whether or not to treat the patient with tamoxifen or another drug.

Biomarkers

[00357] A biosignature used to characterize a phenotype can comprise one or more biomarkers. The biomarker can be a circulating marker, a membrane associated marker, or a component present within a vesicle or on a vesicle's surface. These biomarkers include without limitation a nucleic acid (e.g. RNA (mRNA, miRNA, etc.) or DNA), protein, peptide, polypeptide, antigen, lipid, carbohydrate, or proteoglycan.

[00358] The biosignature can include the presence or absence, expression level, mutational state, genetic variant state, or any modification (such as epigenetic modification, or post-translation modification) of a biomarker disclosed herein (e.g., **Table 3**, **Table 4** or **Table 5**) or previously disclosed (e.g. any one or more biomarker listed in **Figs. 1**, **3-60** of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein). One of skill will recognize that methods of the invention can be adapted to assess one or more biomarkers disclosed herein for a disease or condition different than a disease that is conventionally associated with a given biomarker. For example, one or more biomarkers disclosed herein for condition *x* may readily be utilized in obtaining a biosignature for a different condition *y,* based on the teachings of the instant disclosure and methods of the invention. The expression level of a biomarker can be compared to a control or reference, to determine the overexpression or underexpression (or upregulation or downregulation) of a biomarker in a sample. In some embodiments, the control or reference level comprises the amount of a same biomarker, such as a miRNA, in a control sample from a subject that does not have or exhibit the condition or disease. In another embodiment, the control of reference levels comprises that of a housekeeping marker whose level is minimally affected, if at all, in different biological settings such as diseased versus non-diseased states. In yet another embodiment, the control or reference level comprises that of the level of the same marker in the same subject but in a sample taken at a different time point. Other types of controls are described herein.

[00359] Nucleic acid biomarkers include various RNA or DNA species. For example, the biomarker can be mRNA, microRNA (miRNA), small nucleolar RNAs (snoRNA), small nuclear RNAs (snRNA), ribosomal RNAs (rRNA), heterogeneous nuclear RNA (hnRNA), ribosomal RNAS (rRNA), siRNA, transfer RNAs (tRNA), or shRNA. The DNA can be double-stranded DNA, single stranded DNA, complementary DNA, or noncoding DNA. miRNAs are short ribonucleic acid (RNA) molecules which average about 22 nucleotides long. miRNAs act as post-transcriptional regulators that bind to complementary sequences in the three prime untranslated regions (3' UTRs) of target messenger RNA transcripts (mRNAs), which can result in gene silencing. One miRNA may act upon 1000s of mRNAs. miRNAs play multiple roles in negative regulation, e.g., transcript degradation and sequestering, translational suppression, and may also have a role in positive regulation, e.g., transcriptional and translational activation. By affecting gene regulation, miRNAs can influence many biologic processes. Different sets of expressed miRNAs are found in different cell types and tissues.

[00360] Biomarkers for use with the invention further include peptides, polypeptides, or proteins, which terms are used interchangeably throughout unless otherwise noted. In some embodiments, the protein biomarker comprises its modification state, truncations, mutations, expression level (such as overexpression or underexpression as compared to a reference level), and/or post-translational modifications, such as described above. In a non-limiting example, a biosignature for a disease can include a protein having a certain post-translational modification that is more prevalent in a sample associated with the disease than without.

[00361] A biosignature may include a number of the same type of biomarkers (e.g., two or more different microRNA or mRNA species) or one or more of different types of biomarkers (e.g. mRNAs, miRNAs, proteins, peptides, ligands, and antigens).

[00362] One or more biosignatures can comprise at least one biomarker selected from those listed in **Figs. 1, 3-60** of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein. A specific cell-of-origin biosignature may include one or more biomarkers. **Figs. 3-58** of International Patent Application Serial No.

PCT/US2011/031479 depict tables which lists a number of disease or condition specific biomarkers that can be derived and analyzed from a vesicle. The biomarker can also be CD24, midkine, hepcidin, TMPRSS2-ERG, PCA-3, PSA, EGFR, EGFRvIII, BRAF variant, MET, cKit, PDGFR, Wnt, beta-catenin, K-ras, H-ras, N-ras, Raf, N-myc, c-myc, IGFR, PI3K, Akt, BRCA1, BRCA2, PTEN, VEGFR-2, VEGFR-1, Tie-2, TEM-1, CD276, HER-2, HER-3, or HER-4. The biomarker can also be annexin V, CD63, Rab-5b, or caveolin, or a miRNA, such as let-7a; miR-15b; miR-16; miR-19b; miR-21; miR-26a; miR-27a; miR-92; miR-93; miR-320 or miR-20. The biomarker can also be of any gene or fragment thereof as disclosed in PCT Publication No. WO2009/100029, such as those listed in Tables 3-15 therein.

[00363] In another embodiment, a vesicle comprises a cell fragment or cellular debris derived from a rare cell, such as described in PCT Publication No. WO2006054991. One or more biomarkers, such as CD 146, CD 105, CD31, CD 133, CD 106, or a combination thereof, can be assessed for the vesicle. In one embodiment, a capture agent for the one or more biomarkers is used to isolate or detect a vesicle. In some embodiments, one or more of the biomarkers CD45, cytokeratin (CK) 8, CK18, CK19, CK20, CEA, EGFR, GUC, EpCAM, VEGF, TS, Muc-1, or a combination thereof is assessed for a vesicle. In one embodiment, a tumor-derived vesicle is CD45-, CK+ and comprises a nucleic acid, wherein the membrane vesicle has an absence of, or low expression or detection of CD45, has detectable expression of a cytokeratin (such as CK8, CK18, CK19, or CK20), and detectable expression of a nucleic acid.

[00364] Any number of useful biomarkers that can be assessed as part of a vesicle biosignature are disclosed throughout the application, including without limitation CD9, EphA2, EGFR, B7H3, PSM, PCSA, CD63, STEAP, CD81, ICAM1, A33, DR3, CD66e, MFG-E8, TROP-2, Mammaglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, EpCam, neurokinin receptor-1 (NK-1 or NK-1R), NK-2, Pai-1, CD45, CD10, HER2/ERBB2, AGTR1, NPY1R, MUC1, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUC1 secreted), CA27.29 (MUC1 secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1, SPB, SPC, NSE, PGP9.5, P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, IC3b, mesothelin, SPA, AQP5, GPCR, hCEA-CAM, PTP IA-2, CABYR, TMEM211, ADAM28, UNC93A, MUC17, MUC2, IL10R-beta, BCMA, HVEM/TNFRSF14, Trappin-2 Elafin, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNFR, or a combination thereof.

[00365] Other biomarkers useful for assessment in methods and compositions disclosed herein include those associated with conditions or physiological states as disclosed in U.S. Patent No. 6329179 and 7,625,573; U.S. Patent Publication Nos. 2002/106684, 2004/005596, 2005/0159378, 2005/0064470, 2006/116321, 2007/0161004, 2007/0077553, 2007/104738, 2007/0298118, 2007/0172900, 2008/0268429, 2010/0062450, 2007/0298118, 2009/0220944 and 2010/0196426; U.S. Patent Application Nos. 12/524,432, 12/524,398, 12/524,462; Canadian Patent CA 2453198; and International PCT Patent Publication Nos. WO1994022018, WO2001036601, WO2003063690, WO2003044166, WO2003076603, WO2005121369, WO2005118806, WO/2005/078124, WO2007126386, WO2007088537, WO2007103572, WO2009019215, WO2009021322, WO2009036236, WO2009100029, WO2009015357, WO2009155505, WO 2010/065968 and WO 2010/070276; each of which patent or application is incorporated herein by reference in their entirety. The biomarkers disclosed in these patents and applications, including vesicle biomarkers and microRNAs, can be assessed as part of a signature for characterizing a phenotype, such as providing a diagnosis, prognosis or theranosis of a cancer or other disease. Furthermore, the methods and techniques disclosed therein can be used to assess biomarkers, including vesicle biomarkers and microRNAs.

[00366] Another group of useful biomarkers for assessment in methods and compositions disclosed herein include those associated with cancer diagnostics, prognostics and theranostics as disclosed in US Patents 6,692,916, 6,960,439, 6,964,850, 7,074,586; U.S. Patent Application Nos. 11/159,376, 11/804,175, 12/594,128, 12/514,686, 12/514,775,

12/594,675, 12/594,911, 12/594,679, 12/741,787, 12/312,390; and International PCT Patent Application Nos. PCT/US2009/049935, PCT/US2009/063138, PCT/US2010/000037; each of which patent or application is incorporated herein by reference in their entirety. Usefule biomarkers further include those described in U.S. Patent Application Nos., 10/703,143 and US 10/701,391 for inflammatory disease; 11/529,010 for rheumatoid arthritis; 11/454,553 and 11/827,892 for multiple sclerosis; 11/897,160 for transplant rejection; 12/524,677 for lupus; PCT/US2009/048684 for osteoarthritis; 10/742,458 for infectious disease and sepsis; 12/520,675 for sepsis; each of which patent or application is incorporated herein by reference in their entirety. The biomarkers disclosed in these patents and applications, including mRNAs, can be assessed as part of a signature for characterizing a phenotype, such as providing a diagnosis, prognosis or theranosis of a cancer or other disease. Furthermore, the methods and techniques disclosed therein can be used to assess biomarkers, including vesicle biomarkers and microRNAs.

[00367] Still other biomarkers useful for assessment in methods and compositions disclosed herein include those associated with conditions or physiological states as disclosed in Wieczorek *et al.*, Isolation and characterization of an RNA-proteolipid complex associated with the malignant state in humans, Proc Natl Acad Sci U S A. 1985 May;82(10):3455-9; Wieczorek *et al.*, Diagnostic and prognostic value of RNA-proteolipid in sera of patients with malignant disorders following therapy: first clinical evaluation of a novel tumor marker, Cancer Res. 1987 Dec 1;47(23):6407-12; Escola *et al. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes*. J. Biol. Chem. (1998) **273**:20121-27; Pileri *et al. Binding of hepatitis C virus to CD81* Science, (1998) **282**:938-41); Kopreski *et al. Detection of Tumor Messenger RNA in the Serum of Patients with Malignant Melanoma*, Clin. Cancer Res. (1999) **5**:1961-1965; Carr *et al. Circulating Membrane Vesicles in Leukemic Blood,* Cancer Research, (1985) **45**:5944-51; Weichert *et al*. Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival. Clinical Cancer Research, 2005, 11:6574-81); Iorio *et al. MicroRNA gene expression deregulation in human breast cancer*. Cancer Res (2005) **65**:7065-70; Taylor *et al. Tumour-derived exosomes and their role in cancer-associated T-cell signaling defects* British J Cancer (2005) **92**:305-11; Valadi *et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells* Nature Cell Biol (2007) **9**:654-59; Taylor *et al. Pregnancy-associated exosomes and their modulation of T cell signaling* J Immunol (2006) **176**:1534–42; Koga *et al. Purification, characterization and biological significance of tumor-derived exosomes* Anticancer Res (2005) **25**:3703-08; Seligson *et al. Epithelial cell adhesion molecule (KSA) expression: pathobiology and its role as an independent predictor of survival in renal cell carcinoma* Clin Cancer Res (2004) **10**:2659-69; Clayton *et al.* (*Antigen-presenting cell exosomes are protected from complement-mediated lysis by expression of CD55 and CD59*. Eur J Immunol (2003) **33**:522-31); Simak *et al. Cell Membrane Microparticles in Blood and Blood Products: Potentially Pathogenic Agents and Diagnostic Markers* Trans Med Reviews (2006) **20**:1-26; Choi *et al. Proteomic analysis of microvesicles derived from human colorectal cancer cells* J Proteome Res (2007) **6**:4646-4655; Iero *et al. Tumour-released exosomes and their implications in cancer immunity* Cell Death Diff (2008) **15**:80-88; Baj-Krzyworzeka *et al. Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes* Cencer Immunol Immunother (2006) **55**:808-18; Admyre *et al. B cell-derived exosomes can present allergen peptides and activate allergen-specific T cells to proliferate and produce TH2-like cytokines* J Allergy Clin Immunol (2007) **120**:1418-1424; Aoki *et al. Identification and characterization of microvesicles secreted by 3T3-Ll adipocytes: redox- and hormone dependent induction of milk fat globule-epidermal growth factor 8-associated microvesicles* Endocrinol (2007) **148**:3850-3862; Baj-Krzyworzeka *et al. Tumour-derived microvesicles carry several surface determinants and*

mRNA of tumour cells and transfer some of these determinants to monocytes Cencer Immunol Immunother (2006) **55**:808-18; Skog *et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers* Nature Cell Biol (2008) **10**:1470-76; El-Hefnawy *et al. Characterization of amplifiable, circulating RNA in plasma and its potential as a tool for cancer diagnostics* Clin Chem (2004) **50**:564-573; Pisitkun *et al., Proc Natl Acad Sci U S A*, 2004; **101**:13368-13373; Mitchell *et al.*, Can urinary exosomes act as treatment response markers in Prostate Cancer?, Journal of Translational Medicine 2009, 7:4; Clayton *et al.*, Human Tumor-Derived Exosomes Selectively Impair Lymphocyte Responses to Interleukin-2, Cancer Res 2007; **67**: (15). August 1, 2007; Rabesandratana *et al. Decay-accelerating factor (CD55) and membrane inhibitor of reactive lysis (CD59) are released within exosomes during In vitro maturation of reticulocytes*. Blood **91**:2573-2580 (1998); Lamparski *et al. Production and characterization of clinical grade exosomes derived from dendritic cells*. J Immunol Methods **270**:211-226 (2002); Keller *et al. CD24 is a marker of exosomes secreted into urine and amniotic fluid*. Kidney Int'l **72**:1095-1102 (2007); Runz *et al. Malignant ascites-derived exosomes of ovarian carcinoma patients contain CD24 and EpCAM*. Gyn Oncol **107**:563–571 (2007); Redman *et al. Circulating microparticles in normal pregnancy and preeclampsia placenta*. **29**:73-77 (2008); Gutwein *et al. Cleavage of L 1 in exosomes and apoptotic membrane vesicles released from ovarian carcinoma cells*. Clin Cancer Res **11**:2492-2501 (2005); Kristiansen et al., CD24 is an independent prognostic marker of survival in nonsmall cell lung cancer patients, Brit J Cancer **88**:231- 236 (2003); Lim and Oh, The Role of CD24 in Various Human Epithelial Neoplasias, Pathol Res Pract **201**:479-86 (2005); Matutes et al., The Immunophenotype of Splenic Lymphoma with Villous Lymphocytes and its Relevance to the Differential Diagnosis With Other B-Cell Disorders, Blood **83**:1558-1562 (1994); Pirruccello and Lang, Differential Expression of CD24-Related Epitopes in Mycosis Fungoides/Sezary Syndrome: A Potential Marker for Circulating Sezary Cells, Blood **76**:2343-2347 (1990). The biomarkers disclosed in these publications, including vesicle biomarkers and microRNAs, can be assessed as part of a signature for characterizing a phenotype, such as providing a diagnosis, prognosis or theranosis of a cancer or other disease. Furthermore, the methods and techniques disclosed therein can be used to assess biomarkers, including vesicle biomarkers and microRNAs.

[00368] Still other biomarkers useful for assessment in methods and compositions disclosed herein include those associated with conditions or physiological states as disclosed in *Rajendran et al., Proc Natl Acad Sci U S A 2006;103:11172-11177, Taylor et al., Gynecol Oncol 2008;110:13-21, Zhou et al., Kidney Int 2008;74:613-621, Buning et al., Immunology 2008, Prado et al. J Immunol 2008;181:1519-1525, Vella et al. (2008) Vet Immunol Immunopathol 124(3-4): 385-93, Gould et al. (2003). Proc Natl Acad Sci U S A 100(19): 10592-7, Fang et al. (2007). PLoS Biol 5(6): e158, Chen, B. J. and R. A. Lamb (2008). Virology 372(2): 221-32, Bhatnagar, S. and J. S. Schorey (2007). J Biol Chem 282(35): 25779-89, Bhatnagar et al. (2007) Blood 110(9): 3234-44, Yuyama, et al. (2008). J Neurochem 105(1): 217-24, Gomes et al. (2007). Neurosci Lett 428(1): 43-6, Nagahama et al. (2003). Autoimmunity 36(3): 125-31, Taylor, D. D., S. Akyol, et al. (2006). J Immunol 176(3): 1534-42, Peche, et al. (2006). Am J Transplant 6(7): 1541-50, Iero, M., M. Valenti, et al. (2008). Cell Death and Differentiation 15: 80–88, Gesierich, S., I. Berezoversuskiy, et al. (2006), Cancer Res 66(14): 7083-94, Clayton, A., A. Turkes, et al. (2004). Faseb J 18(9): 977-9, Skriner., K. Adolph, et al. (2006). Arthritis Rheum 54(12): 3809-14, Brouwer, R., G. J. Pruijn, et al. (2001). Arthritis Res 3(2): 102-6, Kim, S. H., N. Bianco, et al. (2006). Mol Ther 13(2): 289-300, Evans, C. H., S. C. Ghivizzani, et al. (2000). Clin Orthop Relat Res (379 Suppl): S300-7, Zhang, H. G., C. Liu, et al. (2006). J Immunol 176(12): 7385-93, Van Niel, G., J. Mallegol, et al. (2004). Gut 52: 1690-1697, Fiasse, R. and O. Dewit (2007). Expert Opinion on Therapeutic Patents 17(12): 1423-1441(19)*. The biomarkers disclosed in these publications, including vesicle

biomarkers and microRNAs, can be assessed as part of a signature for characterizing a phenotype, such as providing a diagnosis, prognosis or theranosis of a cancer or other disease. Furthermore, the methods and techniques disclosed therein can be used to assess biomarkers, including vesicle biomarkers and microRNAs.

[00369] In another aspect, the invention provides a method of assessing a cancer comprising detecting a level of one or more circulating biomarkers in a sample from a subject selected from the group consisting of CD9, HSP70, Gal3, MIS, EGFR, ER, ICB3, CD63, B7H4, MUC1, DLL4, CD81, ERB3, VEGF, BCA225, BRCA, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD31, cMET, MUC2 or ERB4. CD9, HSP70, Gal3, MIS, EGFR, ER, ICB3, CD63, B7H4, MUC1, DLL4, CD81, ERB3, VEGF, BCA225, BRCA, BCA200, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD31, cMET, MUC2 or ERB4. In another embodiment, the one or more circulating biomarkers are selected from the group consisting of CD9, EphA2, EGFR, B7H3, PSMA, PCSA, CD63, STEAP, STEAP, CD81, B7H3, STEAP1, ICAM1 (CD54), PSMA, A33, DR3, CD66e, MFG-8e, EphA2, Hepsin, TMEM211, EphA2, TROP-2, EGFR, Mammoglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, NK-2, EpCam, NGAL, NK-1R, PSMA, 5T4, PAI-1, and CD45. In still another embodiment, the one or more circulating biomarkers are selected from the group consisting of CD9, MIS Rii, ER, CD63, MUC1, HER3, STAT3, VEGFA, BCA, CA125, CD24, EPCAM, and ERB B4. Any number of useful biomarkers can be assessed from these groups, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the one or more biomarkers are one or more of Gal3, BCA200, OPN and NCAM, e.g., Gal3 and BCA200, OPN and NCAM, or all four. Assessing the cancer may comprise diagnosing, prognosing or theranosing the cancer. The cancer can be a breast cancer. The markers can be associated with a vesicle or vesicle population. For example, the one or more circulating biomarker can be a vesicle surface antigen or vesicle payload. Vesicle surface antigens can further be used as capture antigens, detector antigens, or both.

[00370] The invention further provides a method for predicting a response to a therapeutic agent comprising detecting a level of one or more circulating biomarkers in a sample from a subject selected from the group consisting of CD9, HSP70, Gal3, MIS, EGFR, ER, ICB3, CD63, B7H4, MUC1, DLL4, CD81, ERB3, VEGF, BCA225, BRCA, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD31, cMET, MUC2 or ERB4. Biomarkers can also be selected from the group consisting of CD9, EphA2, EGFR, B7H3, PSMA, PCSA, CD63, STEAP, STEAP, CD81, B7H3, STEAP1, ICAM1 (CD54), PSMA, A33, DR3, CD66e, MFG-8e, EphA2, Hepsin, TMEM211, EphA2, TROP-2, EGFR, Mammoglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, NK-2, EpCam, NGAL, NK-1R, PSMA, 5T4, PAI-1, and CD45. In still another embodiment, the one or more circulating biomarkers are selected from the group consisting of CD9, MIS Rii, ER, CD63, MUC1, HER3, STAT3, VEGFA, BCA, CA125, CD24, EPCAM, and ERB B4. Any number of useful biomarkers can be assessed from these groups, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the one or more biomarkers are one or more of Gal3, BCA200, OPN and NCAM, e.g., Gal3 and BCA200, OPN and NCAM, or all four. The therapeutic agent can be a therapeutic agent for treating cancer. The cancer can be a breast cancer. The markers can be associated with a vesicle or vesicle population. For example, the one or more circulating biomarker can be a vesicle surface antigen or vesicle payload. Vesicle surface antigens can further be used as capture antigens, detector antigens, or both.

[00371] Various methods or platforms can be used to assess or detect biomarkers identified herein. Examples of such methods or platforms include but are not limited to using an antibody array, microbeads, or other method disclosed herein or known in the art. For example, a capture antibody or aptamer to the one or more biomarkers can be bound to the array or bead. The captured vesicles can then be detected using a detectable agent. In some embodiments, captured vesicles are detected using an agent, e.g., an antibody or aptamer, that recognizes general vesicle biomarkers that detect

the overall population of vesicles, such as a tetraspanin or MFG-E8. These can include tetraspanins such as CD9, CD63 and/or CD81. In other embodiments, the captured vesicles are detected using markers specific for vesicle origin, e.g., a type of tissue or organ. In some embodiments, the captured vesicles are detected using CD31, a marker for cells or vesicles of endothelial origin. As desired, the biomarkers used for capture can also be used for detection, and vice versa.

[00372] Methods of the invention can be used to assess various diseases or conditions, where biomarkers correspond to various such diseases or conditions. For example, methods of the invention are applied to assess one or more cancers, such as those disclosed herein, wherein a method comprises detecting a level of one or more circulating biomarker in a sample from a subject selected from the group consisting of 5T4 (trophoblast), ADAM10, AGER/RAGE, APC, APP (β-amyloid), ASPH (A-10), B7H3 (CD276), BACE1, BAI3, BRCA1, BDNF, BIRC2, C1GALT1, CA125 (MUC16), Calmodulin 1, CCL2 (MCP-1), CD9, CD10, CD127 (IL7R), CD174, CD24, CD44, CD63, CD81, CEA, CRMP-2, CXCR3, CXCR4, CXCR6, CYFRA 21, derlin 1, DLL4, DPP6, E-CAD, EpCaM, EphA2 (H-77), ER(1) ESR1 α, ER(2) ESR2 β, Erb B4, Erbb2, erb3 (Erb-B3), PA2G4, FRT (FLT1), Gal3, GPR30 (G-coupled ER1), HAP1, HER3, HSP-27, HSP70, IC3b, IL8, insig, junction plakoglobin, Keratin 15, KRAS, Mammaglobin, MART1, MCT2, MFGE8, MMP9, MRP8, Muc1, MUC17, MUC2, NCAM, NG2 (CSPG4), Ngal, NHE-3, NT5E (CD73), ODC1, OPG, OPN, p53, PARK7, PCSA, PGP9.5 (PARK5), PR(B), PSA, PSMA, RAGE, STXBP4, Survivin, TFF3 (secreted), TIMP1, TIMP2, TMEM211, TRAF4 (scaffolding), TRAIL-R2 (death Receptor 5), TrkB, Tsg 101, UNC93a, VEGF A, VEGFR2, YB-1, VEGFR1, GCDPF-15 (PIP), BigH3 (TGFb1-induced protein), 5HT2B (serotonin receptor 2B), BRCA2, BACE 1, CDH1-cadherin. The methods can comprise detecting protein, RNA or DNA of the specified target biomarker. The one or more marker can be assessed directly from a biological fluid, such as those fluids disclosed herein, or can be assessed for its association with a vesicle, e.g., as a vesicle surface antigen or as vesicle payload (e.g., soluble protein, mRNA or DNA). A particular biosignature determined using methods and compositions of the invention can comprise any number of useful biomarkers, e.g., a biosignature can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more different biomarkers (or in some cases different molecules of the same biomarkers, such protein and nucleic acid). Vesicle surface antigens can also be used as capture antigens, detector antigens, or both, as disclosed herein or in applications incorporated by reference.

[00373] Methods and compositions of the invention are applied to assess various aspects of a cancer, including identifying different informative aspects of a cancer, e.g., identifying a biosignature that is indicative of metastasis, angiogenesis, or classifying different stages, classes or subclasses of the same tumor or tumor lineage.

[00374] Furthermore, methods of the invention comprise determining if a disease or condition affects immunomodulation in a subject. For example, the one or more circulating biomarker for immunomodulation can be one or more of CD45, FasL, CTLA4, CD80 and CD83. The one or more circulating biomarker for metastatis can be one or more of Muc1, CD147, TIMP1, TIMP2, MMP7, and MMP9. The one or more circulating biomarker for angiogenesis can be one or more of HIF2a, Tie2, Ang1, DLL4 and VEGFR2. Any number of useful biomarkers can be assessed from the groups, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. The cancer can be a breast cancer. The markers can be associated with a vesicle or vesicle population. For example, the one or more circulating biomarker can be a vesicle surface antigen or vesicle payload. Vesicle surface antigens can further be used as capture antigens, detector antigens, or both.

[00375] A biosignature can comprise DLL4 or cMET. Delta-like 4 (DLL4) is a Notch-ligand and is up-regulated during angiogenesis. cMET (also referred to as c-Met, MET, or MNNG HOS Transforming gene) is a proto-oncogene that encodes a membrane receptor tyrosine kinase whose ligand is hepatocyte growth factor (HGF). The MET protein is

sometimes referred to as the hepatocyte growth factor receptor (HGFR). MET is normally expressed on epithelial cells, and improper activation can trigger tumor growth, angiogenesis and metastasis. DLL4 and cMET can be used as biomarkers to detect a vesicle population.

[00376] Biomarkers that can be derived and analyzed from a vesicle include miRNA (miR), miRNA*nonsense (miR*), and other RNAs (including, but not limited to, mRNA, preRNA, priRNA, hnRNA, snRNA, siRNA, shRNA). A miRNA biomarker can include not only its miRNA and microRNA* nonsense, but its precursor molecules: pri-microRNAs (pri-miRs) and pre-microRNAs (pre-miRs). The sequence of a miRNA can be obtained from publicly available databases such as http://www.mirbase.org/, http://www.microrna.org/, or any others available. Unless noted, the terms miR, miRNA and microRNA are used interchangeably throughout unless noted. In some embodiments, the methods of the invention comprise isolating vesicles, and assessing the miRNA payload within the isolated vesicles. The biomarker can also be a nucleic acid molecule (e.g. DNA), protein, or peptide. The presence or absence, expression level, mutations (for example genetic mutations, such as deletions, translocations, duplications, nucleotide or amino acid substitutions, and the like) can be determined for the biomarker. Any epigenetic modulation or copy number variation of a biomarker can also be analyzed.

[00377] The one or more biomarkers analyzed can be indicative of a particular tissue or cell of origin, disease, or physiological state. Furthermore, the presence, absence or expression level of one or more of the biomarkers described herein can be correlated to a phenotype of a subject, including a disease, condition, prognosis or drug efficacy. The specific biomarker and biosignature set forth below constitute non-inclusive examples for each of the diseases, condition comparisons, conditions, and/or physiological states. Furthermore, the one or more biomarker assessed for a phenotype can be a cell-of-origin specific vesicle.

[00378] The one or more miRNAs used to characterize a phenotype may be selected from those disclosed in PCT Publication No. WO2009/036236. For example, one or more miRNAs listed in Tables I-VI (Figures 6-11) therein can be used to characterize colon adenocarcinoma, colorectal cancer, prostate cancer, lung cancer, breast cancer, b- cell lymphoma, pancreatic cancer, diffuse large BCL cancer, CLL, bladder cancer, renal cancer, hypoxia-tumor, uterine leiomyomas, ovarian cancer, hepatitis C virus-associated hepatocellular carcinoma, ALL, Alzheimer's disease, myelofibrosis, myelofibrosis, polycythemia vera, thrombocythemia, HIV, or HIV-I latency, as further described herein.

[00379] The one or more miRNAs can be detected in a vesicle. The one or more miRNAs can be miR-223, miR-484, miR-191, miR-146a, miR-016, miR-026a, miR-222, miR-024, miR-126, and miR-32. One or more miRNAs can also be detected in PBMC. The one or more miRNAs can be miR-223, miR-150, miR-146b, miR-016, miR-484, miR-146a, miR-191, miR-026a, miR-019b, or miR-020a. The one or more miRNAs can be used to characterize a particular disease or condition. For example, for the disease bladder cancer, one or more miRNAs can be detected, such as miR-223, miR-26b, miR-221, miR-103-1, miR-185, miR-23b, miR-203, miR-17-5p, miR-23a, miR-205 or any combination thereof. The one or more miRNAs may be upregulated or overexpressed.

[00380] In some embodiments, the one or more miRNAs is used to characterize hypoxia-tumor. The one or more miRNA may be miR-23, miR-24, miR-26, miR-27, miR-103, miR-107, miR-181, miR-210, or miR-213, and may be upregulated. One or more miRNAs can also be used to characterize uterine leiomyomas. For example, the one or more miRNAs used to characterize a uterine leiomyoma may be a let-7 family member, miR-21, miR-23b, miR-29b, or miR-197. The miRNA can be upregulated.

[00381] Myelofibrosis can also be characterized by one or more miRNAs, such as miR-190, which can be upregulated; miR-31, miR-150 and miR-95, which can be downregulated, or any combination thereof. Furthermore, myelofibrosis,

polycythemia vera or thrombocythemia can also be characterized by detecting one or more miRNAs, such as, but not limited to, miR-34a, miR-342, miR-326, miR- 105, miR-149, miR- 147, or any combination thereof. The one or more miRNAs may be downregulated.

[00382] Other examples of phenotypes that can be characterized by assessing a vesicle for one or more biomarkers are futher described herein.

[00383] The one or more biomarkers can be detected using a probe. A probe can comprise an oligonucleotide, such as DNA or RNA, an aptamer, monoclonal antibody, polyclonal antibody, Fabs, Fab', single chain antibody, synthetic antibody, peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, synthetic or naturally occurring chemical compound (including but not limited to a drug or labeling reagent), dendrimer, or a combination thereof. The probe can be directly detected, for example by being directly labeled, or be indirectly detected, such as through a labeling reagent. The probe can selectively recognize a biomarker. For example, a probe that is an oligonucleotide can selectively hybridize to a miRNA biomarker.

[00384] In aspects, the invention provides for the diagnosis, theranosis, prognosis, disease stratification, disease staging, treatment monitoring or predicting responder / non-responder status of a disease or disorder in a subject. The invention comprises assessing vesicles from a subject, including assessing biomarkers present on the vesicles and/or assessing payload within the vesicles, such as protein, nucleic acid or other biological molecules. Any appropriate biomarker that can be assessed using a vesicle and that relates to a disease or disorder can be used the carry out the methods of the invention. Furthermore, any appropriate technique to assess a vesicle as described herein can be used. Exemplary biomarkers for specific diseases that can be assessed according to the methods of the invention include the biomarkers described in International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein.

[00385] Any of the types of biomarkers or specific biomarkers described herein can be assessed to identify a biosignature or to identify a candidate biosignature. Exemplary biomarkers include without limitation those in **Table 5**. The markers in the table can be used for capture and/or detection of vesicles for characterizing phenotypes as disclosed herein. In some cases, multiple capture and/or detectors are used to enhance the characterization. The markers can be detected as protein or as mRNA, which can be circulating freely or in a complex with other biological molecules. The markers can be detected as vesicle surface antigens or and vesicle payload. The "Illustrative Class" indicates indications for which the markers are known markers. Those of skill will appreciate that the markers can also be used in alternate settings in certain instances. For example, a marker which can be used to characterize one type disease may also be used to characterize another disease as appropriate. Consider a non-limiting example of a tumor marker which can be used as a biomarker for tumors from various lineages. The biomarker references in Table 5 are those commonly used in the art. Gene aliases and descriptions can be found using a variety of online databases, including GeneCards® (www.genecards.org), HUGO Gene Nomenclature (www.genenames.org), Entrez Gene (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene), UniProtKB/Swiss-Prot (www.uniprot.org), UniProtKB/TrEMBL (www.uniprot.org), OMIM (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), GeneLoc (genecards.weizmann.ac.il/geneloc/), and Ensembl (www.ensembl.org). Generally, gene symbols and names below correspond to those approved by HUGO, and protein names are those recommended by UniProtKB/Swiss-Prot. Common alternatives are provided as well. In some cases, biomarkers are referred to by Ensembl reference numbers, which are of the form "ENSG" followed by a number, e.g., E.005893 which corresponds to LAMP2. In Table 5, solely for sake of brevity, "E." is sometimes used to represent "E.." For example, "E.005893 represents "E.005893." Where a

protein name indicates a precursor, the mature protein is also implied. Throughout the application, gene and protein symbols may be used interchangeably and the meaning can be derived from context as necessary.

Table 5: Illustrative Vesicle Associated Biomarkers

Illustrative Class	Biomarkers
Drug associated targets and prognostic markers	ABCC1, ABCG2, ACE2, ADA, ADH1C, ADH4, AGT, AR, AREG, ASNS, BCL2, BCRP, BDCA1, beta III tubulin, BIRC5, B-RAF, BRCA1, BRCA2, CA2, caveolin, CD20, CD25, CD33, CD52, CDA, CDKN2A, CDKN1A, CDKN1B, CDK2, CDW52, CES2, CK 14, CK 17, CK 5/6, c-KIT, c-Met, c-Myc, COX-2, Cyclin D1, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, E-Cadherin, ECGF1, EGFR, EML4-ALK fusion, EPHA2, Epiregulin, ER, ERBR2, ERCC1, ERCC3, EREG, ESR1, FLT1, folate receptor, FOLR1, FOLR2, FSHB, FSHPRH1, FSHR, FYN, GART, GNA11, GNAQ, GNRH1, GNRHR1, GSTP1, HCK, HDAC1, hENT-1, Her2/Neu, HGF, HIF1A, HIG1, HSP90, HSP90AA1, HSPCA, IGF-1R, IGFRBP, IGFRBP3, IGFRBP4, IGFRBP5, IL13RA1, IL2RA, KDR, Ki67, KIT, K-RAS, LCK, LTB, Lymphotoxin Beta Receptor, LYN, MET, MGMT, MLH1, MMR, MRP1, MS4A1, MSH2, MSH5, Myc, NFKB1, NFKB2, NFKBIA, NRAS, ODC1, OGFR, p16, p21, p27, p53, p95, PARP-1, PDGFC, PDGFR, PDGFRA, PDGFRB, PGP, PGR, PI3K, POLA, POLA1, PPARG, PPARGC1, PR, PTEN, PTGS2, PTPN12, RAF1, RARA, ROS1, RRM1, RRM2, RRM2B, RXRB, RXRG, SIK2, SPARC, SRC, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, Survivin, TK1, TLE3, TNF, TOP1, TOP2A, TOP2B, TS, TUBB3, TXN, TXNRD1, TYMS, VDR, VEGF, VEGFA, VEGFC, VHL, YES1, ZAP70
Drug associated targets and prognostic markers	ABL1, STK11, FGFR2, ERBB4, SMARCB1, CDKN2A, CTNNB1, FGFR1, FLT3, NOTCH1, NPM1, SRC, SMAD4, FBXW7, PTEN, TP53, AKT1, ALK, APC, CDH1, C-Met, HRAS, IDH1, JAK2, MPL, PDGFRA, SMO, VHL, ATM, CSF1R, FGFR3, GNAS, ERBB2, HNF1A, JAK3, KDR, MLH1, PTPN11, RB1, RET, c-Kit, EGFR, PIK3CA, NRAS, GNA11, GNAQ, KRAS, BRAF
Drug associated targets and prognostic markers	ALK, AR, BRAF, cKIT, cMET, EGFR, ER, ERCC1, GNA11, HER2, IDH1, KRAS, MGMT, MGMT promoter methylation, NRAS, PDGFRA, Pgp, PIK3CA, PR, PTEN, ROS1, RRM1, SPARC, TLE3, TOP2A, TOPO1, TS, TUBB3, VHL
Cancer treatment associated markers	AR, AREG (Amphiregulin), BRAF, BRCA1, cKIT, cMET, EGFR, EGFR w/T790M, EML4-ALK, ER, ERBB3, ERBB4, ERCC1, EREG, GNA11, GNAQ, hENT-1, Her2, Her2 Exon 20 insert, IGF1R, Ki67, KRAS, MGMT, MGMT methylation, MSH2, MSI, NRAS, PGP (MDR1), PIK3CA, PR, PTEN, ROS1, ROS1 translocation, RRM1, SPARC, TLE3, TOPO1, TOPO2A, TS, TUBB3, VEGFR2
Cancer treatment associated markers	AR, AREG, BRAF, BRCA1, cKIT, cMET, EGFR, EGFR w/T790M, EML4-ALK, ER, ERBB3, ERBB4, ERCC1, EREG, GNA11, GNAQ, Her2, Her2 Exon 20 insert, IGFR1, Ki67, KRAS, MGMT-Me, MSH2, MSI, NRAS, PGP (MDR-1), PIK3CA, PR, PTEN, ROS1 translocation, RRM1, SPARC, TLE3, TOPO1, TOPO2A, TS, TUBB3, VEGFR2
Colon cancer treatment associated markers	AREG, BRAF, EGFR, EML4-ALK, ERCC1, EREG, KRAS, MSI, NRAS, PIK3CA, PTEN, TS, VEGFR2
Colon cancer treatment associated markers	AREG, BRAF, EGFR, EML4-ALK, ERCC1, EREG, KRAS, MSI, NRAS, PIK3CA, PTEN, TS, VEGFR2
Melanoma treatment associated markers	BRAF, cKIT, ERBB3, ERBB4, ERCC1, GNA11, GNAQ, MGMT, MGMT methylation, NRAS, PIK3CA, TUBB3, VEGFR2
Melanoma treatment associated markers	BRAF, cKIT, ERBB3, ERBB4, ERCC1, GNA11, GNAQ, MGMT-Me, NRAS, PIK3CA, TUBB3, VEGFR2
Ovarian cancer treatment associated markers	BRCA1, cMET, EML4-ALK, ER, ERBB3, ERCC1, hENT-1, HER2, IGF1R, PGP(MDR1), PIK3CA, PR, PTEN, RRM1, TLE3, TOPO1, TOPO2A, TS
Ovarian cancer treatment associated markers	BRCA1, cMET, EML4-ALK (translocation), ER, ERBB3, ERCC1, HER2, PIK3CA, PR, PTEN, RRM1, TLE3, TS
Breast cancer treatment associated markers	BRAF, BRCA1, EGFR, EGFR T790M, EML4-ALK, ER, ERBB3, ERCC1, HER2, Ki67, PGP (MDR1), PIK3CA, PR, PTEN, ROS1, ROS1 translocation, RRM1, TLE3, TOPO1, TOPO2A, TS

Breast cancer treatment associated markers	BRAF, BRCA1, EGFR w/T790M, EML4-ALK, ER, ERBB3, ERCC1, HER2, Ki67, KRAS, PIK3CA, PR, PTEN, ROS1 translocation, RRM1, TLE3, TOPO1, TOPO2A, TS
NSCLC cancer treatment associated markers	BRAF, BRCA1, cMET, EGFR, EGFR w/T790M, EML4-ALK, ERCC1, Her2 Exon 20 insert, KRAS, MSH2, PIK3CA, PTEN, ROS1 (trans), RRM1, TLE3, TS, VEGFR2
NSCLC cancer treatment associated markers	BRAF, cMET, EGFR, EGFR w/T790M, EML4-ALK, ERCC1, Her2 Exon 20 insert, KRAS, MSH2, PIK3CA, PTEN, ROS1 translocation, RRM1, TLE3, TS
Cancer/Angio	Erb 2, Erb 3, Erb 4, UNC93a, B7H3, MUC1, MUC2, MUC16, MUC17, 5T4, RAGE, VEGF A, VEGFR2, FLT1, DLL4, Epcam
Tissue (Breast)	BIG H3, GCDFP-15, PR(B), GPR 30, CYFRA 21, BRCA 1, BRCA 2, ESR 1, ESR2
Tissue (Prostate)	PSMA, PCSA, PSCA, PSA, TMPRSS2
Inflammation/Immune	MFG-E8, IFNAR, CD40, CD80, MICB, HLA-DRb, IL-17-Ra
Common vesicle markers	HSPA8, CD63, Actb, GAPDH, CD9, CD81, ANXA2, HSP90AA1, ENO1, YWHAZ, PDCD6IP, CFL1, SDCBP, PKN2, MSN, MFGE8, EZR, YWHAG, PGK1, EEF1A1, PPIA, GLC1F, GK, ANXA6, ANXA1, ALDOA, ACTG1, TPI1, LAMP2, HSP90AB1, DPP4, YWHAB, TSG101, PFN1, LDHB, HSPA1B, HSPA1A, GSTP1, GNAI2, GDI2, CLTC, ANXA5, YWHAQ, TUBA1A, THBS1, PRDX1, LDHA, LAMP1, CLU, CD86
Common vesicle membrane markers	CD63, GAPDH, CD9, CD81, ANXA2, ENO1, SDCBP, MSN, MFGE8, EZR, GK, ANXA1, LAMP2, DPP4, TSG101, HSPA1A, GDI2, CLTC, LAMP1, CD86, ANPEP, TFRC, SLC3A2, RDX, RAP1B, RAB5C, RAB5B, MYH9, ICAM1, FN1, RAB11B, PIGR, LGALS3, ITGB1, EHD1, CLIC1, ATP1A1, ARF1, RAP1A, P4HB, MUC1, KRT10, HLA-A, FLOT1, CD59, C1orf58, BASP1, TACSTD1, STOM
Common vesicle markers	MHC class I, MHC class II, Integrins, Alpha 4 beta 1, Alpha M beta 2, Beta 2, ICAM1/CD54, P-selection, Dipeptidylpeptidase IV/CD26, Aminopeptidase n/CD13, CD151, CD53, CD37, CD82, CD81, CD9, CD63, Hsp70, Hsp84/90 Actin, Actin-binding proteins, Tubulin, Annexin I, Annexin II, Annexin IV, Annexin V, Annexin VI, RAB7/RAP1B/RADGDI, Gi2alpha/14-3-3, CBL/LCK, CD63, GAPDH, CD9, CD81, ANXA2, ENO1, SDCBP, MSN, MFGE8, EZR, GK, ANXA1, LAMP2, DPP4, TSG101, HSPA1A, GDI2, CLTC, LAMP1, Cd86, ANPEP, TFRC, SLC3A2, RDX, RAP1B, RAB5C, RAB5B, MYH9, ICAM1, FN1, RAB11B, PIGR, LGALS3, ITGB1, EHD1, CLIC1, ATP1A1, ARF1, RAP1A, P4HB, MUC1, KRT10, HLA-A, FLOT1, CD59, C1orf58, BASP1, TACSTD1, STOM
Vesicle markers	A33, a33 n15, AFP, ALA, ALIX, ALP, AnnexinV, APC, ASCA, ASPH (246-260), ASPH (666-680), ASPH (A-10), ASPH (D01P), ASPH (D03), ASPH (G-20), ASPH (H-300), AURKA, AURKB, B7H3, B7H4, BCA-225, BCNP, BDNF, BRCA, CA125 (MUC16), CA-19-9, C-Bir, CD1.1, CD10, CD174 (Lewis y), CD24, CD44, CD46, CD59 (MEM-43), CD63, CD66e CEA, CD73, CD81, CD9, CDA, CDAC1 1a2, CEA, C-Erb2, C-erbB2, CRMP-2, CRP, CXCL12, CYFRA21-1, DLL4, DR3, EGFR, Epcam, EphA2, EphA2 (H-77), ER, ErbB4, EZH2, FASL, FRT, FRT c.f23, GDF15, GPCR, GPR30, Gro-alpha, HAP, HBD 1, HBD2, HER 3 (ErbB3), HSP, HSP70, hVEGFR2, iC3b, IL 6 Unc, IL-1B, IL6 Unc, IL6R, IL8, IL-8, INSIG-2, KLK2, L1CAM, LAMN, LDH, MACC-1, MAPK4, MART-1, MCP-1, M-CSF, MFG-E8, MIC1, MIF, MIS RII, MMG, MMP26, MMP7, MMP9, MS4A1, MUC1, MUC1 seq1, MUC1 seq11A, MUC17, MUC2, Ncam, NGAL, NPGP/NPFF2, OPG, OPN, p53, p53, PA2G4, PBP, PCSA, PDGFRB, PGP9.5, PIM1, PR (B), PRL, PSA, PSMA, PSME3, PTEN, R5-CD9 Tube 1, Reg IV, RUNX2, SCRN1, seprase, SERPINB3, SPARC, SPB, SPDEF, SRVN, STAT 3, STEAP1, TF (FL-295), TFF3, TGM2, TIMP-1, TIMP1, TIMP2, TMEM211, TMPRSS2, TNF-alpha, Trail-R2, Trail-R4, TrKB, TROP2, Tsg 101, TWEAK, UNC93A, VEGF A, YPSMA-1
Vesicle markers	NSE, TRIM29, CD63, CD151, ASPH, LAMP2, TSPAN1, SNAIL, CD45, CKS1, NSE, FSHR, OPN, FTH1, PGP9, ANNEXIN 1, SPD, CD81, EPCAM, PTH1R, CEA, CYTO 7, CCL2, SPA, KRAS, TWIST1, AURKB, MMP9, P27, MMP1, HLA, HIF, CEACAM, CENPH, BTUB, INTG b4, EGFR, NACC1, CYTO 18, NAP2, CYTO 19, ANNEXIN V, TGM2, ERB2, BRCA1, B7H3, SFTPC, PNT, NCAM, MS4A1, P53, INGA3, MUC2, SPA, OPN, CD63, CD9, MUC1, UNCR3, PAN ADH, HCG, TIMP, PSMA, GPCR, RACK1, PSCA, VEGF, BMP2, CD81, CRP, PRO GRP, B7H3, MUC1, M2PK, CD9, PCSA, PSMA
Vesicle markers	TFF3, MS4A1, EphA2, GAL3, EGFR, N-gal, PCSA, CD63, MUC1, TGM2, CD81, DR3, MACC-1, TrKB, CD24, TIMP-1, A33, CD66 CEA, PRL, MMP9, MMP7, TMEM211,

	SCRN1, TROP2, TWEAK, CDACC1, UNC93A, APC, C-Erb, CD10, BDNF, FRT, GPR30, P53, SPR, OPN, MUC2, GRO-1, tsg 101, GDF15
Vesicle markers	CD9, Erb2, Erb4, CD81, Erb3, MUC16, CD63, DLL4, HLA-Drpe, B7H3, IFNAR, 5T4, PCSA, MICB, PSMA, MFG-E8, Muc1, PSA, Muc2, Unc93a, VEGFR2, EpCAM, VEGF A, TMPRSS2, RAGE, PSCA, CD40, Muc17, IL-17-RA, CD80
Benign Prostate Hyperplasia (BPH)	BCMA, CEACAM-1, HVEM, IL-1 R4, IL-10 Rb, Trappin-2, p53, hsa-miR-329, hsa-miR-30a, hsa-miR-335, hsa-miR-152, hsa-miR-151-5p, hsa-miR-200a, hsa-miR-145, hsa-miR-29a, hsa-miR-106b, hsa-miR-595, hsa-miR-142-5p, hsa-miR-99a, hsa-miR-20b, hsa-miR-373, hsa-miR-502-5p, hsa-miR-29b, hsa-miR-142-3p, hsa-miR-663, hsa-miR-423-5p, hsa-miR-15a, hsa-miR-888, hsa-miR-361-3p, hsa-miR-365, hsa-miR-10b, hsa-miR-199a-3p, hsa-miR-181a, hsa-miR-19a, hsa-miR-125b, hsa-miR-760, hsa-miR-7a, hsa-miR-671-5p, hsa-miR-7c, hsa-miR-1979, hsa-miR-103
Metastatic Prostate Cancer	hsa-miR-100, hsa-miR-1236, hsa-miR-1296, hsa-miR-141, hsa-miR-146b-5p, hsa-miR-17*, hsa-miR-181a, hsa-miR-200b, hsa-miR-20a*, hsa-miR-23a*, hsa-miR-331-3p, hsa-miR-375, hsa-miR-452, hsa-miR-572, hsa-miR-574-3p, hsa-miR-577, hsa-miR-582-3p, hsa-miR-937, miR-10a, miR-134, miR-141, miR-200b, miR-30a, miR-32, miR-375, miR-495, miR-564, miR-570, miR-574-3p, miR-885-3p
Metastatic Prostate Cancer	hsa-miR-200b, hsa-miR-375, hsa-miR-141, hsa-miR-331-3p, hsa-miR-181a, hsa-miR-574-3p
Prostate Cancer	hsa-miR-574-3p, hsa-miR-141, hsa-miR-432, hsa-miR-326, hsa-miR-2110, hsa-miR-181a-2*, hsa-miR-107, hsa-miR-301a, hsa-miR-484, hsa-miR-625*
Metastatic Prostate Cancer	hsa-miR-582-3p, hsa-miR-20a*, hsa-miR-375, hsa-miR-200b, hsa-miR-379, hsa-miR-572, hsa-miR-513a-5p, hsa-miR-577, hsa-miR-23a*, hsa-miR-1236, hsa-miR-609, hsa-miR-17*, hsa-miR-130b, hsa-miR-619, hsa-miR-624*, hsa-miR-198
Metastatic Prostate Cancer	FOX01A, SOX9, CLNS1A, PTGDS, XPO1, LETMD1, RAD23B, ABCC3, APC, CHES1, EDNRA, FRZB, HSPG2, TMPRSS2_ETV1 fusion
Prostate Cancer	hsa-let-7b, hsa-miR-107, hsa-miR-1205, hsa-miR-1270, hsa-miR-130b, hsa-miR-141, hsa-miR-143, hsa-miR-148b*, hsa-miR-150, hsa-miR-154*, hsa-miR-181a*, hsa-miR-181a-2*, hsa-miR-18a*, hsa-miR-19b-1*, hsa-miR-204, hsa-miR-2110, hsa-miR-215, hsa-miR-217, hsa-miR-219-2-3p, hsa-miR-23b*, hsa-miR-299-5p, hsa-miR-301a, hsa-miR-301a, hsa-miR-326, hsa-miR-331-3p, hsa-miR-365*, hsa-miR-373*, hsa-miR-424, hsa-miR-424*, hsa-miR-432, hsa-miR-450a, hsa-miR-451, hsa-miR-484, hsa-miR-497, hsa-miR-517*, hsa-miR-517a, hsa-miR-518f, hsa-miR-574-3p, hsa-miR-595, hsa-miR-617, hsa-miR-625*, hsa-miR-628-5p, hsa-miR-629, hsa-miR-634, hsa-miR-769-5p, hsa-miR-93, hsa-miR-96
Prostate Cancer	CD9, PSMA, PCSA, CD63, CD81, B7H3, IL 6, OPG-13, IL6R, PA2G4, EZH2, RUNX2, SERPINB3, EpCam
Prostate Cancer	A33, a33 n15, AFP, ALA, ALIX, ALP, AnnexinV, APC, ASCA, ASPH (246-260), ASPH (666-680), ASPH (A-10), ASPH (D01P), ASPH (D03), ASPH (G-20), ASPH (H-300), AURKA, AURKB, B7H3, B7H4, BCA-225, BCNP, BDNF, BRCA, CA125 (MUC16), CA-19-9, C-Bir, CD1.1, CD10, CD174 (Lewis y), CD24, CD44, CD46, CD59 (MEM-43), CD63, CD66e CEA, CD73, CD81, CD9, CDA, CDAC1 1a2, CEA, C-Erb2, C-erbB2, CRMP-2, CRP, CXCL12, CYFRA21-1, DLL4, DR3, EGFR, Epcam, EphA2, EphA2 (H-77), ER, ErbB4, EZH2, FASL, FRT, FRT c.f23, GDF15, GPCR, GPR30, Gro-alpha, HAP, HBD 1, HBD2, HER 3 (ErbB3), HSP, HSP70, hVEGFR2, iC3b, IL 6 Unc, IL-1B, IL6 Unc, IL6R, IL8, IL-8, INSIG-2, KLK2, L1CAM, LAMN, LDH, MACC-1, MAPK4, MART-1, MCP-1, M-CSF, MFG-E8, MIC1, MIF, MIS RII, MMG, MMP26, MMP7, MMP9, MS4A1, MUC1, MUC1 seq1, MUC1 seq11A, MUC17, MUC2, Ncam, NGAL, NPGP/NPFF2, OPG, OPN, p53, p53, PA2G4, PBP, PCSA, PDGFRB, PGP9.5, PIM1, PR (B), PRL, PSA, PSMA, PSME3, PTEN, R5-CD9 Tube 1, Reg IV, RUNX2, SCRN1, seprase, SERPINB3, SPARC, SPB, SPDEF, SRVN, STAT 3, STEAP1, TF (FL-295), TFF3, TGM2, TIMP-1, TIMP1, TIMP2, TMEM211, TMPRSS2, TNF-alpha, Trail-R2, Trail-R4, TrKB, TROP2, Tsg 101, TWEAK, UNC93A, VEGF A, YPSMA-1
Prostate Cancer Vesicle Markers	5T4, ACTG1, ADAM10, ADAM15, ALDOA, ANXA2, ANXA6, APOA1, ATP1A1, BASP1, C1orf58, C20orf114, C8B, CAPZA1, CAV1, CD151, CD2AP, CD59, CD9, CD9, CFL1, CFP, CHMP4B, CLTC, COTL1, CTNND1, CTSB, CTSZ, CYCS, DPP4, EEF1A1, EHD1, ENO1, F11R, F2, F5, FAM125A, FNBP1L, FOLH1, GAPDH, GLB1, GPX3, HIST1H1C, HIST1H2AB, HSP90AB1, HSPA1B, HSPA8, IGSF8, ITGB1, ITIH3, JUP, LDHA, LDHB, LUM, LYZ, MFGE8, MGAM, MMP9, MYH2, MYL6B, NME1, NME2, PABPC1, PABPC4, PACSIN2, PCBP2, PDCD6IP, PRDX2, PSA, PSMA, PSMA1, PSMA2, PSMA4, PSMA6, PSMA7, PSMB1, PSMB2, PSMB3, PSMB4, PSMB5, PSMB6,

	PSMB8, PTGFRN, RPS27A, SDCBP, SERINC5, SH3GL1, SLC3A2, SMPDL3B, SNX9, TACSTD1, TCN2, THBS1, TPI1, TSG101, TUBB, VDAC2, VPS37B, YWHAG, YWHAQ, YWHAZ
Prostate Cancer Vesicle Markers	FLNA, DCRN, HER 3 (ErbB3), VCAN, CD9, GAL3, CDADC1, GM-CSF, EGFR, RANK, CSA, PSMA, ChickenIgY, B7H3, PCSA, CD63, CD3, MUC1, TGM2, CD81, S100-A4, MFG-E8, Integrin, NK-2R(C-21), PSA, CD24, TIMP-1, IL6 Unc, PBP, PIM1, CA-19-9, Trail-R4, MMP9, PRL, EphA2, TWEAK, NY-ESO-1, Mammaglobin, UNC93A, A33, AURKB, CD41, XAGE-1, SPDEF, AMACR, seprase/FAP, NGAL, CXCL12, FRT, CD66e CEA, SIM2 (C-15), C-Bir, STEAP, PSIP1/LEDGF, MUC17, hVEGFR2, ERG, MUC2, ADAM10, ASPH (A-10), CA125, Gro-alpha, Tsg 101, SSX2, Trail-R4
Prostate Cancer Vesicle Markers	NT5E (CD73), A33, ABL2, ADAM10, AFP, ALA, ALIX, ALPL, AMACR, Apo J/CLU, ASCA, ASPH (A-10), ASPH (D01P), AURKB, B7H3, B7H4, BCNP, BDNF, CA125 (MUC16), CA-19-9, C-Bir (Flagellin), CD10, CD151, CD24, CD3, CD41, CD44, CD46, CD59(MEM-43), CD63, CD66e CEA, CD81, CD9, CDA, CDADC1, C-erbB2, CRMP-2, CRP, CSA, CXCL12, CXCR3, CYFRA21-1, DCRN, DDX-1, DLL4, EGFR, EpCAM, EphA2, ERG, EZH2, FASL, FLNA, FRT, GAL3, GATA2, GM-CSF, Gro-alpha, HAP, HER3 (ErbB3), HSP70, HSPB1, hVEGFR2, iC3b, IL-1B, IL6 R, IL6 Unc, IL7 R alpha/CD127, IL8, INSIG-2, Integrin, KLK2, Label, LAMN, Mammaglobin, M-CSF, MFG-E8, MIF, MIS RII, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, Ncam, NDUFB7, NGAL, NK-2R(C-21), NY-ESO-1, p53, PBP, PCSA, PDGFRB, PIM1, PRL, PSA, PSIP1/LEDGF, PSMA, RAGE, RANK, Reg IV, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2 (C-15), SPARC, SPC, SPDEF, SPP1, SSX2, SSX4, STEAP, STEAP4, TFF3, TGM2, TIMP-1, TMEM211, Trail-R2, Trail-R4, TrKB (poly), Trop2, Tsg 101, TWEAK, UNC93A, VCAN, VEGF A, wnt-5a(C-16), XAGE, XAGE-1
Prostate Vesicle Markers	ADAM 9, ADAM10, AGR2, ALDOA, ALIX, ANXA1, ANXA2, ANXA4, ARF6, ATP1A3, B7H3, BCHE, BCL2L14 (Bcl G), BCNP1, BDKRB2, BDNFCAV1-Caveolin1, CCR2 (CC chemokine receptor 2, CD192), CCR5 (CC chemokine receptor 5), CCT2 (TCP1-beta), CD10, CD151, CD166/ALCAM, CD24, CD283/TLR3, CD41, CD46, CD49d (Integrin alpha 4, ITGA4), CD63, CD81, CD9, CD90/THY1, CDH1, CDH2, CDKN1A cyclin-dependent kinase inhibitor (p21), CGA gene (coding for the alpha subunit of glycoprotein hormones), CLDN3- Claudin3, COX2 (PTGS2), CSE1L (Cellular Apoptosis Susceptibility), CXCR3, Cytokeratin 18, Eag1 (KCNH1), EDIL3 (del-1), EDNRB - Endothelial Receptor Type B, EGFR, EpoR, EZH2 (enhancer of Zeste Homolog2), EZR, FABP5, Farnesyltransferase/geranylgeranyl diphosphate synthase 1 (GGPS1), Fatty acid synthase (FASN), FTL (light and heavy), GAL3, GDF15-Growth Differentiation Factor 15, GloI, GM-CSF, GSTP1, H3F3A, HGF (hepatocyte growth factor), hK2 / Kif2a, HSP90AA1, HSPA1A / HSP70-1, HSPB1, IGFBP-2, IGFBP-3, IL1alpha, IL-6, IQGAP1, ITGAL (Integrin alpha L chain), Ki67, KLK1, KLK10, KLK11, KLK12, KLK13, KLK14, KLK15, KLK4, KLK5, KLK6, KLK7, KLK8, KLK9, Lamp-2, LDH-A, LGALS3BP, LGALS8, MMP 1, MMP 2, MMP 25, MMP 3, MMP10, MMP-14/MT1-MMP, MMP7, MTA1nAnS, Nav1.7, NKX3-1, Notch1, NRP1 / CD304, PAP (ACPP), PGP, PhIP, PIP3 / BPNT1, PKM2, PKP1 (plakophilin1), PKP3 (plakophilin3), Plasma chromogranin-A (CgA), PRDX2, Prostate secretory protein (PSP94) / β-Microseminoprotein (MSP) / IGBF, PSAP, PSMA, PSMA1, PTENPTPN13/PTPL1, RPL19, seprase/FAPSET, SLC3A2 / CD98, SRVN, STEAP1, Syndecan / CD138, TGFB, TGM2, TIMP-1TLR4 (CD284), TLR9 (CD289), TMPRSS1 / hepsin, TMPRSS2, TNFR1, TNFα, Transferrin receptor/CD71/TRFR, Trop2 (TACSTD2), TWEAK uPA (urokinase plasminoge activator) degrades extracellular matrix, uPAR (uPA receptor) / CD87, VEGFR1, VEGFR2
Prostate vesicle membrane	ADAM 34, ADAM 9, AGR2, ALDOA, ANXA1, ANXA 11, ANXA4, ANXA 7, ANXA2, ARF6, ATP1A1, ATP1A2, ATP1A3, BCHE, BCL2L14 (Bcl G), BDKRB2, CA215, CAV1-Caveolin1, CCR2 (CC chemokine receptor 2, CD192), CCR5 (CC chemokine receptor 5), CCT2 (TCP1-beta), CD166/ALCAM, CD49b (Integrin alpha 2, ITGA4), CD90/THY1, CDH1, CDH2, CDKN1A cyclin-dependent kinase inhibitor (p21), CGA gene (coding for the alpha subunit of glycoprotein hormones), CHMP4B, CLDN3- Claudin3, CLSTN1 (Calsyntenin-1), COX2 (PTGS2), CSE1L (Cellular Apoptosis Susceptibility), Cytokeratin 18, Eag1 (KCNH1) (plasma membrane-K+-voltage gated channel), EDIL3 (del-1), EDNRB- Endothelial Receptor Type B, Endoglin/CD105, ENOX2 – Ecto-NOX disulphide Thiol exchanger 2, EPCA-2 Early prostate cancer antigen2, EpoR, EZH2 (enhancer of Zeste Homolog2), EZR, FABP5, Farnesyltransferase/geranylgeranyl diphosphate synthase 1 (GGPS1), Fatty acid synthase (FASN, plasma membrane protein), FTL (light and heavy),

	GDF15-Growth Differentiation Factor 15, GloI, GSTP1, H3F3A, HGF (hepatocyte growth factor), hK2 (KLK2), HSP90AA1, HSPA1A / HSP70-1, IGFBP-2, IGFBP-3, IL1alpha, IL-6, IQGAP1, ITGAL (Integrin alpha L chain), Ki67, KLK1, KLK10, KLK11, KLK12, KLK13, KLK14, KLK15, KLK4, KLK5, KLK6, KLK7, KLK8, KLK9, Lamp-2, LDH-A, LGALS3BP, LGALS8, MFAP5, MMP 1, MMP 2, MMP 24, MMP 25, MMP 3, MMP10, MMP-14/MT1-MMP, MTA1, nAnS, Nav1.7, NCAM2 – Neural cell Adhesion molecule 2, NGEP/D-TMPP/IPCA-5/ANO7, NKX3-1, Notch1, NRP1 / CD304, PGP, PAP (ACPP), PCA3- Prostate cancer antigen 3, Pdia3/ERp57, PhIP, phosphatidylethanolamine (PE), PIP3, PKP1 (plakophilin1), PKP3 (plakophilin3), Plasma chromogranin-A (CgA), PRDX2, Prostate secretory protein (PSP94) / β-Microseminoprotein (MSP) / IGBF, PSAP, PSMA1, PTEN, PTGFRN, PTPN13/PTPL1, PKM2, RPL19, SCA-1 / ATXN1, SERINC5/TPO1, SET, SLC3A2 / CD98, STEAP1, STEAP-3, SRVN, Syndecan / CD138, TGFB, Tissue Polypeptide Specific antigen TPS, TLR4 (CD284), TLR9 (CD289), TMPRSS1 / hepsin, TMPRSS2, TNFR1, TNFα, CD283/TLR3, Transferrin receptor/CD71/TRFR, uPA (urokinase plasminoge activator) , uPAR (uPA receptor) / CD87, VEGFR1, VEGFR2
Prostate Cancer Treatment	hsa-miR-1974, hsa-miR-27b, hsa-miR-103, hsa-miR-146a, hsa-miR-22, hsa-miR-382, hsa-miR-23a, hsa-miR-376c, hsa-miR-335, hsa-miR-142-5p, hsa-miR-221, hsa-miR-142-3p, hsa-miR-151-3p, hsa-miR-21, hsa-miR-16
Prostate Cancer	let-7d, miR-148a, miR-195, miR-25, miR-26b, miR-329, miR-376c, miR-574-3p, miR-888, miR-9, miR1204, miR-16-2*, miR-497, miR-588, miR-614, miR-765, miR92b*, miR-938, let-7f-2*, miR-300, miR-523, miR-525-5p, miR-1182, miR-1244, miR-520d-3p, miR-379, let-7b, miR-125a-3p, miR-1296, miR-134, miR-149, miR-150, miR-187, miR-32, miR-324-3p, miR-324-5p, miR-342-3p, miR-378, miR-378*, miR-384, miR-451, miR-455-3p, miR-485-3p, miR-487a, miR-490-3p, miR-502-5p, miR-548a-5p, miR-550, miR-562, miR-593, miR-593*, miR-595, miR-602, miR-603, miR-654-5p, miR-877*, miR-886-5p, miR-125a-5p, miR-140-3p, miR-192, miR-196a, miR-2110, miR-212, miR-222, miR-224*, miR-30b*, miR-499-3p, miR-505*
Prostate (PCSA+ cMVs)	miR-182, miR-663, miR-155, mirR-125a-5p, miR-548a-5p, miR-628-5p, miR-517*, miR-450a, miR-920, hsa-miR-619, miR-1913, miR-224*, miR-502-5p, miR-888, miR-376a, miR-542-5p, miR-30b*, miR-1179
Prostate Cancer	miR-183-96-182 cluster (miRs-183, 96 and 182), metal ion transporter such as hZIP1, SLC39A1, SLC39A2, SLC39A3, SLC39A4, SLC39A5, SLC39A6, SLC39A7, SLC39A8, SLC39A9, SLC39A10, SLC39A11, SLC39A12, SLC39A13, SLC39A14
Prostate Cancer	RAD23B, FBP1, TNFRSF1A, CCNG2, NOTCH3, ETV1, BID, SIM2, LETMD1, ANXA1, miR-519d, miR-647
Prostate Cancer	RAD23B, FBP1, TNFRSF1A, NOTCH3, ETV1, BID, SIM2, ANXA1, BCL2
Prostate Cancer	ANPEP, ABL1, PSCA, EFNA1, HSPB1, INMT, TRIP13
Prostate Cancer	E2F3, c-met, pRB, EZH2, e-cad, CAXII, CAIX, HIF-1α, Jagged, PIM-1, hepsin, RECK, Clusterin, MMP9, MTSP-1, MMP24, MMP15, IGFBP-2, IGFBP-3, E2F4, caveolin, EF-1A, Kallikrein 2, Kallikrein 3, PSGR
Prostate Cancer	A2ML1, BAX, C10orf47, C1orf162, CSDA, EIFC3, ETFB, GABARAPL2, GUK1, GZMH, HIST1H3B, HLA-A, HSP90AA1, NRGN, PRDX5, PTMA, RABAC1, RABAGAP1L, RPL22, SAP18, SEPW1, SOX1
Prostate Cancer	NY-ESO-1, SSX-2, SSX-4, XAGE-lb, AMACR, p90 autoantigen, LEDGF
Prostate Cancer	A33, ABL2, ADAM10, AFP, ALA, ALIX, ALPL, ApoJ/CLU, ASCA, ASPH(A-10), ASPH(D01P), AURKB, B7H3, B7H3, B7H4, BCNP, BDNF, CA125(MUC16), CA-19-9, C-Bir, CD10, CD151, CD24, CD41, CD44, CD46, CD59(MEM-43), CD63, CD63, CD66eCEA, CD81, CD81, CD9, CD9, CDA, CDADC1, CRMP-2, CRP, CXCL12, CXCR3, CYFRA21-1, DDX-1, DLL4, DLL4, EGFR, Epcam, EphA2, ErbB2, ERG, EZH2, FASL, FLNA, FRT, GAL3, GATA2, GM-CSF, Gro-alpha, HAP, HER3(ErbB3), HSP70, HSPB1, hVEGFR2, iC3b, IL-1B, IL6R, IL6Unc, IL7Ralpha/CD127, IL8, INSIG-2, Integrin, KLK2, LAMN, Mammoglobin, M-CSF, MFG-E8, MIF, MISRII, MMP7, MMP9, MUC1, Muc1, MUC17, MUC2, Ncam, NDUFB7, NGAL, NK-2R(C-21), NT5E (CD73), p53, PBP, PCSA, PCSA, PDGFRB, PIM1, PRL, PSA, PSA, PSMA, PSMA, RAGE, RANK, RegIV, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2(C-15), SPARC, SPC, SPDEF, SPP1, STEAP, STEAP4, TFF3, TGM2, TIMP-1, TMEM211, Trail-R2, Trail-R4, TrKB(poly), Trop2, Tsg101, TWEAK, UNC93A, VEGFA, wnt-5a(C-16)
Prostate Vesicles	CD9, CD63, CD81, PCSA, MUC2, MFG-E8
Prostate Cancer	miR-148a, miR-329, miR-9, miR-378*, miR-25, miR-614, miR-518c*, miR-378, miR-765, let-7f-2*, miR-574-3p, miR-497, miR-32, miR-379, miR-520g, miR-542-5p, miR-342-3p,

	miR-1206, miR-663, miR-222
Prostate Cancer	hsa-miR-877*, hsa-miR-593, hsa-miR-595, hsa-miR-300, hsa-miR-324-5p, hsa-miR-548a-5p, hsa-miR-329, hsa-miR-550, hsa-miR-886-5p, hsa-miR-603, hsa-miR-490-3p, hsa-miR-938, hsa-miR-149, hsa-miR-150, hsa-miR-1296, hsa-miR-384, hsa-miR-487a, hsa-miRPlus-C1089, hsa-miR-485-3p, hsa-miR-525-5p
Prostate Cancer	hsa-miR-451, hsa-miR-223, hsa-miR-593*, hsa-miR-1974, hsa-miR-486-5p, hsa-miR-19b, hsa-miR-320b, hsa-miR-92a, hsa-miR-21, hsa-miR-675*, hsa-miR-16, hsa-miR-876-5p, hsa-miR-144, hsa-miR-126, hsa-miR-137, hsa-miR-1913, hsa-miR-29b-1*, hsa-miR-15a, hsa-miR-93, hsa-miR-1266
Inflammatory Disease	miR-588, miR-1258, miR-16-2*, miR-938, miR-526b, miR-92b*, let-7d, miR-378*, miR-124, miR-376c, miR-26b, miR-1204, miR-574-3p, miR-195, miR-499-3p, miR-2110, miR-888
Prostate Cancer	A33, ADAM10, AMACR, ASPH (A-10), AURKB, B7H3, CA125, CA-19-9, C-Bir, CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADC1, CSA, CXCL12, DCRN, EGFR, EphA2, ERG, FLNA, FRT, GAL3, GM-CSF, Gro-alpha, HER 3 (ErbB3), hVEGFR2, IL6 Unc, Integrin, Mammaglobin, MFG-E8, MMP9, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, PBP, PCSA, PIM1, PRL, PSA, PSIP1/LEDGF, PSMA, RANK, S100-A4, seprase/FAP, SIM2 (C-15), SPDEF, SSX2, STEAP, TGM2, TIMP-1, Trail-R4, Tsg 101, TWEAK, UNC93A, VCAN, XAGE-1
Prostate Cancer	A33, ADAM10, ALIX, AMACR, ASCA, ASPH (A-10), AURKB, B7H3, BCNP, CA125, CA-19-9, C-Bir (Flagellin), CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADC1, CRP, CSA, CXCL12, CYFRA21-1, DCRN, EGFR, EpCAM, EphA2, ERG, FLNA, GAL3, GATA2, GM-CSF, Gro alpha, HER3 (ErbB3), HSP70, hVEGFR2, iC3b, IL-1B, IL6 Unc, IL8, Integrin, KLK2, Mammaglobin, MFG-E8, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, p53, PBP, PCSA, PIM1, PRL, PSA, PSMA, RANK, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2 (C-15), SPC, SPDEF, SSX2, SSX4, STEAP, TGM2, TIMP-1, TRAIL R2, Trail-R4, Tsg 101, TWEAK, VCAN, VEGF A, XAGE
Prostate Vesicles	EpCam, CD81, PCSA, MUC2, MFG-E8
Prostate Vesicles	CD9, CD63, CD81, MMP7, EpCAM
Prostate Cancer	let-7d, miR-148a, miR-195, miR-25, miR-26b, miR-329, miR-376c, miR-574-3p, miR-888, miR-9, miR1204, miR-16-2*, miR-497, miR-588, miR-614, miR-765, miR92b*, miR-938, let-7f-2*, miR-300, miR-523, miR-525-5p, miR-1182, miR-1244, miR-520d-3p, miR-379, let-7b, miR-125a-3p, miR-1296, miR-134, miR-149, miR-150, miR-187, miR-32, miR-324-3p, miR-324-5p, miR-342-3p, miR-378, miR-378*, miR-384, miR-451, miR-455-3p, miR-485-3p, miR-487a, miR-490-3p, miR-502-5p, miR-548a-5p, miR-550, miR-562, miR-593, miR-593*, miR-595, miR-602, miR-603, miR-654-5p, miR-877*, miR-886-5p, miR-125a-5p, miR-140-3p, miR-192, miR-196a, miR-2110, miR-212, miR-222, miR-224*, miR-30b*, miR-499-3p, miR-505*
Prostate Cancer	STAT3, EZH2, p53, MACC1, SPDEF, RUNX2, YB-1, AURKA, AURKB
Prostate Cancer (Ensembl ENSG identifiers)	E.001036, E.001497, E.001561, E.002330, E.003402, E.003756, E.004838, E.005471, E.005882, E.005893, E.006210, E.006453, E.006625, E.006695, E.006756, E.007264, E.007952, E.008118, E.008196, E.009694, E.009830, E.010244, E.010256, E.010278, E.010539, E.010810, E.011052, E.011114, E.011143, E.011304, E.011451, E.012061, E.012779, E.014216, E.014257, E.015133, E.015171, E.015479, E.015676, E.016402, E.018189, E.018699, E.020922, E.022976, E.023909, E.026508, E.026559, E.029363, E.029725, E.030582, E.033030, E.035141, E.036257, E.036448, E.038002, E.039068, E.039560, E.041353, E.044115, E.047410, E.047597, E.048544, E.048828, E.049239, E.049246, E.049883, E.051596, E.051620, E.052795, E.053108, E.054118, E.054938, E.056097, E.057252, E.057608, E.058729, E.059122, E.059378, E.059691, E.060339, E.060688, E.061794, E.061918, E.062485, E.063241, E.063244, E.064201, E.064489, E.064655, E.064886, E.065054, E.065057, E.065308, E.065427, E.065457, E.065485, E.065526, E.065548, E.065978, E.066455, E.066557, E.067248, E.067369, E.067704, E.068724, E.068885, E.069535, E.069712, E.069849, E.069869, E.069956, E.070501, E.070785, E.070814, E.071246, E.071626, E.071859, E.072042, E.072071, E.072110, E.072506, E.073050, E.073350, E.073584, E.073756, E.074047, E.074071, E.074964, E.075131, E.075239, E.075624, E.075651, E.075711, E.075856, E.075886, E.076043, E.076248, E.076554, E.076864, E.077097, E.077147, E.077312, E.077514, E.077522, E.078269, E.078295, E.078808, E.078902, E.079246, E.079313, E.079785, E.080572, E.080823, E.081087, E.081138, E.081181, E.081721, E.081842, E.082212, E.082258,

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Prostate Markers (Ensembl ENSG identifiers)	E.005893 (LAMP2), E.006756 (ARSD), E.010539 (ZNF200), E.014257 (ACPP), E.015133 (CCDC88C), E.018699 (TTC27), E.044115 (CTNNA1), E.048828 (FAM120A), E.051620 (HEBP2), E.056097 (ZFR), E.060339 (CCAR1), E.063241 (ISOC2), E.064489 (MEF2BNB-MEF2B), E.064886 (CHI3L2), E.066455 (GOLGA5), E.069535 (MAOB), E.072042 (RDH11), E.072071 (LPHN1), E.074047 (GLI2), E.076248 (UNG), E.076554 (TPD52), E.077147 (TM9SF3), E.077312 (SNRPA), E.081842 (PCDHA6), E.086717 (PPEF1), E.088888 (MAVS), E.088930 (XRN2), E.089902 (RCOR1), E.090612 (ZNF268), E.092199 (HNRNPC), E.095380 (NANS), E.099783 (HNRNPM), E.100191 (SLC5A4), E.100216 (TOMM22), E.100242 (SUN2), E.100284 (TOM1), E.100401 (RANGAP1), E.100412 (ACO2), E.100836 (PABPN1), E.102054 (RBBP7), E.102103 (PQBP1), E.103599 (IQCH), E.103978 (TMEM87A), E.104177 (MYEF2), E.104228 (TRIM35), E.105428 (ZNRF4), E.105518 (TMEM205), E.106603 (C7orf44; COA1), E.108405 (P2RX1), E.111057 (KRT18), E.111218 (PRMT8), E.112081 (SRSF3), E.112144 (ICK), E.113013 (HSPA9), E.113368 (LMNB1), E.115221 (ITGB6), E.116096 (SPR), E.116754 (SRSF11), E.118197 (DDX59), E.118898 (PPL), E.119121 (TRPM6), E.119711 (ALDH6A1), E.120656 (TAF12), E.121671 (CRY2), E.121774 (KHDRBS1), E.122126 (OCRL), E.122566 (HNRNPA2B1), E.123901 (GPR83), E.124562 (SNRPC), E.124788 (ATXN1), E.124795 (DEK), E.125246 (CLYBL), E.126883 (NUP214), E.127616 (SMARCA4), E.127884 (ECHS1), E.128050 (PAICS), E.129351 (ILF3), E.129757 (CDKN1C), E.130338 (TULP4), E.130612 (CYP2G1P), E.131351 (HAUS8), E.131467 (PSME3), E.133315 (MACROD1), E.134452 (FBXO18), E.134851 (TMEM165), E.135940 (COX5B), E.136169 (SETDB2), E.136807 (CDK9), E.137727 (ARHGAP20), E.138031 (ADCY3), E.138050 (THUMPD2), E.138069 (RAB1A), E.138594 (TMOD3), E.138760

	(SCARB2), E.138796 (HADH), E.139613 (SMARCC2), E.139684 (ESD), E.140263 (SORD), E.140350 (ANP32A), E.140632 (GLYR1), E.142765 (SYTL1), E.143621 (ILF2), E.143933 (CALM2), E.144410 (CPO), E.147127 (RAB41), E.151304 (SRFBP1), E.151806 (GUF1), E.152207 (CYSLTR2), E.152234 (ATP5A1), E.152291 (TGOLN2), E.154723 (ATP5J), E.156467 (UQCRB), E.159387 (IRX6), E.159761 (C16orf86), E.161813 (LARP4), E.162613 (FUBP1), E.162694 (EXTL2), E.165264 (NDUFB6), E.167113 (COQ4), E.167513 (CDT1), E.167772 (ANGPTL4), E.167978 (SRRM2), E.168916 (ZNF608), E.169763 (PRYP3), E.169789 (PRY), E.169807 (PRY2), E.170017 (ALCAM), E.170144 (HNRNPA3), E.170310 (STX8), E.170954 (ZNF415), E.170959 (DCDC5), E.171302 (CANT1), E.171643 (S100Z), E.172283 (PRYP4), E.172590 (MRPL52), E.172867 (KRT2), E.173366 (TLR9), E.173599 (PC), E.177595 (PIDD), E.178473 (UCN3), E.179981 (TSHZ1), E.181163 (NPM1), E.182319 (Tyrosine-protein kinase SgK223), E.182795 (C1orf116), E.182944 (EWSR1), E.183092 (BEGAIN), E.183098 (GPC6), E.184254 (ALDH1A3), E.185619 (PCGF3), E.186889 (TMEM17), E.187837 (HIST1H1C), E.188771 (C11orf34), E.189060 (H1F0), E.196419 (XRCC6), E.196436 (NPIPL2), E.196504 (PRPF40A), E.196796, E.196993, E.197451 (HNRNPAB), E.197746 (PSAP), E.198131 (ZNF544), E.198156, E.198732 (SMOC1), E.198793 (MTOR), E.039068 (CDH1), E.173230 (GOLGB1), E.124193 (SRSF6), E.140497 (SCAMP2), E.168393 (DTYMK), E.184708 (EIF4ENIF1), E.124164 (VAPB), E.125753 (VASP), E.118260 (CREB1), E.135052 (GOLM1), E.010244 (ZNF207), E.010278 (CD9), E.047597 (XK), E.049246 (PER3), E.069849 (ATP1B3), E.072506 (HSD17B10), E.081138 (CDH7), E.099785 (MARCH2), E.104331 (IMPAD1), E.104812 (GYS1), E.120868 (APAF1), E.123908 (EIF2C2), E.125492 (BARHL1), E.127328 (RAB3IP), E.127329 (PTPRB), E.129514 (FOXA1), E.129657 (SEC14L1), E.129990 (SYT5), E.132881 (RSG1), E.136521 (NDUFB5), E.138347 (MYPN), E.141429 (GALNT1), E.144566 (RAB5A), E.151715 (TMEM45B), E.152582 (SPEF2), E.154957 (ZNF18), E.162385 (MAGOH), E.165410 (CFL2), E.168298 (HIST1H1E), E.169418 (NPR1), E.178187 (ZNF454), E.178741 (COX5A), E.179115 (FARSA), E.182732 (RGS6), E.183431 (SF3A3), E.185049 (WHSC2), E.196236 (XPNPEP3), E.197217 (ENTPD4), E.197813, E.203301, E.116833 (NR5A2), E.121057 (AKAP1), E.005471 (ABCB4), E.071859 (FAM50A), E.084234 (APLP2), E.101222 (SPEF1), E.103175 (WFDC1), E.103449 (SALL1), E.104805 (NUCB1), E.105514 (RAB3D), E.107816 (LZTS2), E.108375 (RNF43), E.109790 (KLHL5), E.112039 (FANCE), E.112715 (VEGFA), E.121690 (DEPDC7), E.125352 (RNF113A), E.134548 (C12orf39), E.136152 (COG3), E.143816 (WNT9A), E.147130 (ZMYM3), E.148396 (SEC16A), E.151092 (NGLY1), E.151779 (NBAS), E.155508 (CNOT8), E.163755 (HPS3), E.166526 (ZNF3), E.172733 (PURG), E.176371 (ZSCAN2), E.177674 (AGTRAP), E.181773 (GPR3), E.183048 (SLC25A10; MRPL12 SLC25A10), E.186376 (ZNF75D), E.187323 (DCC), E.198712 (MT-CO2), E.203908 (C6orf221; KHDC3L), E.001497 (LAS1L), E.009694 (ODZ1), E.080572 (CXorf41; PIH1D3), E.083093 (PALB2), E.089048 (ESF1), E.100065 (CARD10), E.100739 (BDKRB1), E.102904 (TSNAXIP1), E.104824 (HNRNPL), E.107404 (DVL1), E.110066 (SUV420H1), E.120328 (PCDHB12), E.121903 (ZSCAN20), E.122025 (FLT3), E.136930 (PSMB7), E.142025 (DMRTC2), E.144136 (SLC20A1), E.146535 (GNA12), E.147140 (NONO), E.153391 (INO80C), E.164919 (COX6C), E.171540 (OTP), E.177951 (BET1L), E.179796 (LRRC3B), E.197479 (PCDHB11), E.198804 (MT-CO1), E.086205 (FOLH1), E.100632 (ERH), E.100796 (SMEK1), E.104760 (FGL1), E.114302 (PRKAR2A), E.130299 (GTPBP3), E.133961 (NUMB), E.144485 (HES6), E.167085 (PHB), E.167635 (ZNF146), E.177239 (MAN1B1), E.184481 (FOXO4), E.188171 (ZNF626), E.189221 (MAOA), E.157637 (SLC38A10), E.100883 (SRP54), E.105618 (PRPF31), E.119421 (NDUFA8), E.170837 (GPR27), E.168148 (HIST3H3), E.135525 (MAP7), E.174996 (KLC2), E.018189 (RUFY3), E.183520 (UTP11L), E.173905 (GOLIM4), E.165280 (VCP), E.022976 (ZNF839), E.059691 (PET112), E.063244 (U2AF2), E.075651 (PLD1), E.089177 (KIF16B), E.089280 (FUS), E.094755 (GABRP), E.096060 (FKBP5), E.100023 (PPIL2), E.100359 (SGSM3), E.100612 (DHRS7), E.104131 (EIF3J), E.104419 (NDRG1), E.105409 (ATP1A3), E.107623 (GDF10), E.111335 (OAS2), E.113522 (RAD50), E.115053 (NCL), E.120837 (NFYB), E.122733 (KIAA1045), E.123178 (SPRYD7), E.124181 (PLCG1), E.126858 (RHOT1), E.128609 (NDUFA5), E.128683 (GAD1), E.130255 (RPL36), E.133874 (RNF122), E.135387 (CAPRIN1), E.135999 (EPC2), E.136383 (ALPK3), E.139405 (C12orf52), E.141012 (GALNS), E.143924 (EML4), E.144671 (SLC22A14), E.145741 (BTF3), E.145907 (G3BP1), E.149311 (ATM), E.153113 (CAST), E.157538

	(DSCR3), E.157992 (KRTCAP3), E.158901 (WFDC8), E.165259 (HDX), E.169410 (PTPN9), E.170421 (KRT8), E.171155 (C1GALT1C1), E.172831 (CES2), E.173726 (TOMM20), E.176515, E.177565 (TBL1XR1), E.177628 (GBA), E.179091 (CYC1), E.189091 (SF3B3), E.197299 (BLM), E.197872 (FAM49A), E.198205 (ZXDA), E.198455 (ZXDB), E.082212 (ME2), E.109956 (B3GAT1), E.169710 (FASN), E.011304 (PTBP1), E.057252 (SOAT1), E.059378 (PARP12), E.082258 (CCNT2), E.087301 (TXNDC16), E.100575 (TIMM9), E.101152 (DNAJC5), E.101812 (H2BFM), E.102384 (CENPI), E.108641 (B9D1), E.119138 (KLF9), E.119820 (YIPF4), E.125995 (ROMO1), E.132323 (ILKAP), E.134809 (TIMM10), E.134955 (SLC37A2), E.135476 (ESPL1), E.136527 (TRA2B), E.137776 (SLTM), E.139211 (AMIGO2), E.139428 (MMAB), E.139874 (SSTR1), E.143321 (HDGF), E.164244 (PRRC1), E.164270 (HTR4), E.165119 (HNRNPK), E.165637 (VDAC2), E.165661 (QSOX2), E.167258 (CDK12), E.167815 (PRDX2), E.168014 (C2CD3), E.168653 (NDUFS5), E.168769 (TET2), E.169242 (EFNA1), E.175334 (BANF1), E.175416 (CLTB), E.177156 (TALDO1), E.180035 (ZNF48), E.186591 (UBE2H), E.187097 (ENTPD5), E.188739 (RBM34), E.196497 (IPO4), E.197323 (TRIM33), E.197857 (ZNF44), E.197976 (AKAP17A), E.064201 (TSPAN32), E.088992 (TESC), E.092421 (SEMA6A), E.100601 (ALKBH1), E.101557 (USP14), E.103035 (PSMD7), E.106128 (GHRHR), E.115541 (HSPE1), E.121390 (PSPC1), E.124216 (SNAI1), E.130713 (EXOSC2), E.132002 (DNAJB1), E.139697 (SBNO1), E.140481 (CCDC33), E.143013 (LMO4), E.145020 (AMT), E.145990 (GFOD1), E.146070 (PLA2G7), E.164924 (YWHAZ), E.165807 (PPP1R36), E.167751 (KLK2), E.169213 (RAB3B), E.170906 (NDUFA3), E.172725 (CORO1B), E.174332 (GLIS1), E.181924 (CHCHD8), E.183128 (CALHM3), E.204560 (DHX16), E.204574 (ABCF1), E.146701 (MDH2), E.198366 (HIST1H3A), E.081181 (ARG2), E.185896 (LAMP1), E.077514 (POLD3), E.099800 (TIMM13), E.100299 (ARSA), E.105419 (MEIS3), E.108417 (KRT37), E.113739 (STC2), E.125868 (DSTN), E.145908 (ZNF300), E.168575 (SLC20A2), E.182271 (TMIGD1), E.197223 (C1D), E.186834 (HEXIM1), E.001561 (ENPP4), E.011451 (WIZ), E.053108 (FSTL4), E.064655 (EYA2), E.065308 (TRAM2), E.075131 (TIPIN), E.081087 (OSTM1), E.092020 (PPP2R3C), E.096384 (HSP90AB1), E.100348 (TXN2), E.100577 (GSTZ1), E.100802 (C14orf93), E.101365 (IDH3B), E.101654 (RNMT), E.103067 (ESRP2), E.104064 (GABPB1), E.104823 (ECH1), E.106565 (TMEM176B), E.108561 (C1QBP), E.115257 (PCSK4), E.116127 (ALMS1), E.117411 (B4GALT2), E.119335 (SET), E.120337 (TNFSF18), E.122033 (MTIF3), E.122507 (BBS9), E.122870 (BICC1), E.130177 (CDC16), E.130193 (C8orf55; THEM6), E.130413 (STK33), E.130770 (ATPIF1), E.133687 (TMTC1), E.136874 (STX17), E.137409 (MTCH1), E.139626 (ITGB7), E.141744 (PNMT), E.145888 (GLRA1), E.146067 (FAM193B), E.146433 (TMEM181), E.149480 (MTA2), E.152377 (SPOCK1), E.152763 (WDR78), E.156976 (EIF4A2), E.157827 (FMNL2), E.158485 (CD1B), E.158863 (FAM160B2), E.161202 (DVL3), E.161714 (PLCD3), E.163064 (EN1), E.163468 (CCT3), E.164309 (CMYA5), E.164916 (FOXK1), E.165215 (CLDN3), E.167658 (EEF2), E.170549 (IRX1), E.171680 (PLEKHG5), E.178234 (GALNT11), E.179869 (ABCA13), E.179912 (R3HDM2), E.180869 (C1orf180), E.180979 (LRRC57), E.182872 (RBM10), E.183207 (RUVBL2), E.184113 (CLDN5), E.185972 (CCIN), E.189144 (ZNF573), E.197353 (LYPD2), E.197779 (ZNF81), E.198807 (PAX9), E.100442 (FKBP3), E.111790 (FGFR1OP2), E.136044 (APPL2), E.061794 (MRPS35), E.065427 (KARS), E.068885 (IFT80), E.104164 (PLDN; BLOC1S6), E.105127 (AKAP8), E.123066 (MED13L), E.124831 (LRRFIP1), E.125304 (TM9SF2), E.126934 (MAP2K2), E.130305 (NSUN5), E.135298 (BAI3), E.135900 (MRPL44), E.136371 (MTHFS), E.136574 (GATA4), E.140326 (CDAN1), E.141378 (PTRH2), E.141543 (EIF4A3), E.150961 (SEC24D), E.155368 (DBI), E.161649 (CD300LG), E.161692 (DBF4B), E.162437 (RAVER2), E.163257 (DCAF16), E.163576 (EFHB), E.163781 (TOPBP1), E.163913 (IFT122), E.164597 (COG5), E.165359 (DDX26B), E.165646 (SLC18A2), E.169592 (INO80E), E.169957 (ZNF768), E.171492 (LRRC8D), E.171793 (CTPS; CTPS1), E.171953 (ATPAF2), E.175182 (FAM131A), E.177354 (C10orf71), E.181610 (MRPS23), E.181873 (IBA57), E.187792 (ZNF70), E.187823 (ZCCHC16), E.196872 (C2orf55; KIAA1211L), E.198168 (SVIP), E.160633 (SAFB), E.177697 (CD151), E.181072 (CHRM2), E.012779 (ALOX5), E.065054 (SLC9A3R2), E.074071 (MRPS34), E.100815 (TRIP11), E.102030 (NAA10), E.106153 (CHCHD2), E.126814 (TRMT5), E.126952 (NXF5), E.136450 (SRSF1), E.136710 (CCDC115), E.137124 (ALDH1B1), E.143353 (LYPLAL1), E.162490 (C1orf187; DRAXIN), E.167799 (NUDT8), E.171490 (RSL1D1), E.173826 (KCNH6),

	E.173898 (SPTBN2), E.176900 (OR51T1), E.181513 (ACBD4), E.185554 (NXF2), E.185945 (NXF2B), E.108848 (LUC7L3), E.029363 (BCLAF1), E.038002 (AGA), E.108312 (UBTF), E.166341 (DCHS1), E.054118 (THRAP3), E.135679 (MDM2), E.166860 (ZBTB39), E.183684 (THOC4; ALYREF), E.004838 (ZMYND10), E.007264 (MATK), E.020922 (MRE11A), E.041353 (RAB27B), E.052795 (FNIP2), E.075711 (DLG1), E.087087 (SRRT), E.090060 (PAPOLA), E.095139 (ARCN1), E.099715 (PCDH11Y), E.100271 (TTLL1), E.101057 (MYBL2), E.101265 (RASSF2), E.101901 (ALG13), E.102290 (PCDH11X), E.103194 (USP10), E.106554 (CHCHD3), E.107833 (NPM3), E.110063 (DCPS), E.111540 (RAB5B), E.113448 (PDE4D), E.115339 (GALNT3), E.116254 (CHD5), E.117425 (PTCH2), E.117614 (SYF2), E.118181 (RPS25), E.118292 (C1orf54), E.119318 (RAD23B), E.121022 (COPS5), E.121104 (FAM117A), E.123427 (METTL21B), E.125676 (THOC2), E.132275 (RRP8), E.137513 (NARS2), E.138028 (CGREF1), E.139517 (LNX2), E.143614 (GATAD2B), E.143889 (HNRPLL), E.145833 (DDX46), E.147403 (RPL10), E.148158 (SNX30), E.151690 (MFSD6), E.153904 (DDAH1), E.154781 (C3orf19), E.156650 (KAT6B), E.158669 (AGPAT6), E.159363 (ATP13A2), E.163530 (DPPA2), E.164749 (HNF4G), E.165496 (RPL10L), E.165688 (PMPCA), E.165792 (METTL17), E.166598 (HSP90B1), E.168036 (CTNNB1), E.168746 (C20orf62), E.170381 (SEMA3E), E.171180 (OR2M4), E.171202 (TMEM126A), E.172594 (SMPDL3A), E.172653 (C17orf66), E.173540 (GMPPB), E.173585 (CCR9), E.173809 (TDRD12), E.175166 (PSMD2), E.177694 (NAALADL2), E.178026 (FAM211B; C22orf36), E.184363 (PKP3), E.187634 (SAMD11), E.203837 (PNLIPRP3), E.169122 (FAM110B), E.197969 (VPS13A), E.136068 (FLNB), E.075856 (SART3), E.081721 (DUSP12), E.102158 (MAGT1), E.102174 (PHEX), E.102316 (MAGED2), E.104723 (TUSC3), E.105939 (ZC3HAV1), E.108883 (EFTUD2), E.110328 (GALNTL4), E.111785 (RIC8B), E.113163 (COL4A3BP), E.115604 (IL18R1), E.117362 (APH1A), E.117480 (FAAH), E.124767 (GLO1), E.126267 (COX6B1), E.130175 (PRKCSH), E.135926 (TMBIM1), E.138674 (SEC31A), E.140451 (PIF1), E.143797 (MBOAT2), E.149646 (C20orf152), E.157064 (NMNAT2), E.160294 (MCM3AP), E.165084 (C8orf34), E.166946 (CCNDBP1), E.170348 (TMED10), E.170703 (TTLL6), E.175198 (PCCA), E.180287 (PLD5), E.183292 (MIR5096), E.187492 (CDHR4), E.188846 (RPL14), E.015479 (MATR3), E.100823 (APEX1), E.090615 (GOLGA3), E.086062 (B4GALT1), E.138385 (SSB), E.140265 (ZSCAN29), E.140932 (CMTM2), E.167969 (ECI1), E.135486 (HNRNPA1), E.137497 (NUMA1), E.181523 (SGSH), E.099956 (SMARCB1), E.049883 (PTCD2), E.082556 (OPRK1), E.090674 (MCOLN1), E.107164 (FUBP3), E.108582 (CPD), E.109758 (HGFAC), E.111605 (CPSF6), E.115239 (ASB3), E.121892 (PDS5A), E.125844 (RRBP1), E.130826 (DKC1), E.132481 (TRIM47), E.135390 (ATP5G2), E.136875 (PRPF4), E.138621 (PPCDC), E.145632 (PLK2), E.150051 (MKX), E.153140 (CETN3), E.154127 (UBASH3B), E.156194 (PPEF2), E.163825 (RTP3), E.164053 (ATRIP), E.164442 (CITED2), E.168066 (SF1), E.170430 (MGMT), E.175602 (CCDC85B), E.177752 (YIPF7), E.182512 (GLRX5), E.188186 (C7orf59), E.198721 (ECI2), E.204389 (HSPA1A), E.010256 (UQCRC1), E.076043 (REXO2), E.102362 (SYTL4), E.161939 (C17orf49), E.173039 (RELA), E.014216 (CAPN1), E.054938 (CHRDL2), E.065526 (SPEN), E.070501 (POLB), E.078808 (SDF4), E.083720 (OXCT1), E.100084 (HIRA), E.101246 (ARFRP1), E.102241 (HTATSF1), E.103591 (AAGAB), E.104626 (ERI1), E.105221 (AKT2), E.105402 (NAPA), E.105705 (SUGP1), E.106346 (USP42), E.108639 (SYNGR2), E.110107 (PRPF19), E.112473 (SLC39A7), E.113282 (CLINT1), E.115234 (SNX17), E.115561 (CHMP3), E.119906 (FAM178A), E.120733 (KDM3B), E.125375 (ATP5S), E.125798 (FOXA2), E.127415 (IDUA), E.129810 (SGOL1), E.132382 (MYBBP1A), E.133313 (CNDP2), E.134077 (THUMPD3), E.134248 (HBXIP), E.135597 (REPS1), E.137814 (HAUS2), E.138041 (SMEK2), E.140382 (HMG20A), E.143578 (CREB3L4), E.144224 (UBXN4), E.144306 (SCRN3), E.144741 (SLC25A26), E.145919 (BOD1), E.146281 (PM20D2), E.152359 (POC5), E.152409 (JMY), E.154889 (MPPE1), E.157551 (KCNJ15), E.157764 (BRAF), E.158987 (RAPGEF6), E.162069 (CCDC64B), E.162910 (MRPL55), E.163749 (CCDC158), E.164045 (CDC25A), E.164300 (SERINC5), E.165898 (ISCA2), E.167987 (VPS37C), E.168763 (CNNM3), E.170374 (SP7), E.171488 (LRRC8C), E.178381 (ZFAND2A), E.180998 (GPR137C), E.182318 (ZSCAN22), E.198040 (ZNF84), E.198216 (CACNA1E), E.198265 (HELZ), E.198586 (TLK1), E.203795 (FAM24A), E.204231 (RXRB), E.123992 (DNPEP), E.184634 (MED12), E.181885 (CLDN7), E.186660 (ZFP91), E.126777 (KTN1), E.080823 (MOK), E.101811 (CSTF2), E.124570 (SERPINB6), E.148835 (TAF5),

	E.158715 (SLC45A3), E.110955 (ATP5B), E.127022 (CANX), E.142208 (AKT1), E.128881 (TTBK2), E.147231 (CXorf57), E.006210 (CX3CL1), E.009830 (POMT2), E.011114 (BTBD7), E.065057 (NTHL1), E.068724 (TTC7A), E.073584 (SMARCE1), E.079785 (DDX1), E.084463 (WBP11), E.091140 (DLD), E.099821 (POLRMT), E.101126 (ADNP), E.104442 (ARMC1), E.105486 (LIG1), E.110921 (MVK), E.113441 (LNPEP), E.115758 (ODC1), E.116726 (PRAMEF12), E.119681 (LTBP2), E.136933 (RABEPK), E.137815 (RTF1), E.138095 (LRPPRC), E.138294 (MSMB), E.141873 (SLC39A3), E.142698 (C1orf94), E.143390 (RFX5), E.148488 (ST8SIA6), E.148737 (TCF7L2), E.151491 (EPS8), E.152422 (XRCC4), E.154832 (CXXC1), E.158321 (AUTS2), E.159147 (DONSON), E.160285 (LSS), E.160862 (AZGP1), E.160948 (VPS28), E.160972 (PPP1R16A), E.165934 (CPSF2), E.167604 (NFKBID), E.167766 (ZNF83), E.168803 (ADAL), E.169612 (FAM103A1), E.171262 (FAM98B), E.172893 (DHCR7), E.173889 (PHC3), E.176971 (FIBIN), E.177548 (RABEP2), E.179119 (SPTY2D1), E.184378 (ACTRT3), E.184508 (HDDC3), E.185043 (CIB1), E.186814 (ZSCAN30), E.186868 (MAPT), E.196812 (ZSCAN16), E.198563 (DDX39B), E.124529 (HIST1H4B), E.141002 (TCF25), E.174100 (MRPL45), E.109814 (UGDH), E.138756 (BMP2K), E.065457 (ADAT1), E.105948 (TTC26), E.109184 (DCUN1D4), E.125257 (ABCC4), E.126062 (TMEM115), E.142515 (KLK3), E.144381 (HSPD1), E.166710 (B2M), E.198824 (CHAMP1), E.078902 (TOLLIP), E.099331 (MYO9B), E.102710 (FAM48A), E.107485 (GATA3), E.120948 (TARDBP), E.187764 (SEMA4D), E.103855 (CD276), E.117751 (PPP1R8), E.173714 (WFIKKN2), E.172115 (CYCS), E.005882 (PDK2), E.007952 (NOX1), E.008118 (CAMK1G), E.012061 (ERCC1), E.015171 (ZMYND11), E.036257 (CUL3), E.057608 (GDI2), E.058729 (RIOK2), E.071246 (VASH1), E.073050 (XRCC1), E.073350 (LLGL2), E.079246 (XRCC5), E.085733 (CTTN), E.091542 (ALKBH5), E.091732 (ZC3HC1), E.092621 (PHGDH), E.099899 (TRMT2A), E.099917 (MED15), E.101439 (CST3), E.103479 (RBL2), E.104611 (SH2D4A), E.105281 (SLC1A5), E.106392 (C1GALT1), E.107104 (KANK1), E.107798 (LIPA), E.108296 (CWC25), E.109572 (CLCN3), E.112110 (MRPL18), E.113790 (EHHADH), E.115648 (MLPH), E.117308 (GALE), E.117335 (CD46), E.118513 (MYB), E.118640 (VAMP8), E.119321 (FKBP15), E.122705 (CLTA), E.123983 (ACSL3), E.124232 (RBPJL), E.125901 (MRPS26), E.127399 (LRRC61), E.127554 (GFER), E.128708 (HAT1), E.129355 (CDKN2D), E.130340 (SNX9), E.130935 (NOL11), E.131771 (PPP1R1B), E.133863 (TEX15), E.134207 (SYT6), E.136935 (GOLGA1), E.141425 (RPRD1A), E.143374 (TARS2), E.143771 (CNIH4), E.146966 (DENND2A), E.148672 (GLUD1), E.150593 (PDCD4), E.153936 (HS2ST1), E.154099 (DNAAF1), E.156006 (NAT2), E.156282 (CLDN17), E.158545 (ZC3H18), E.158604 (TMED4), E.158813 (EDA), E.159184 (HOXB13), E.161267 (BDH1), E.163492 (CCDC141), E.163629 (PTPN13), E.164163 (ABCE1), E.164520 (RAET1E), E.165138 (ANKS6), E.165923 (AGBL2), E.166484 (MAPK7), E.166747 (AP1G1), E.166971 (AKTIP), E.167744 (NTF4), E.168071 (CCDC88B), E.169087 (HSPBAP1), E.170396 (ZNF804A), E.170445 (HARS), E.170632 (ARMC10), E.170743 (SYT9), E.171428 (NAT1), E.172346 (CSDC2), E.173805 (HAP1), E.175175 (PPM1E), E.175203 (DCTN2), E.177542 (SLC25A22), E.177679 (SRRM3), E.178828 (RNF186), E.182013 (PNMAL1), E.182054 (IDH2), E.182890 (GLUD2), E.184156 (KCNQ3), E.184697 (CLDN6), E.184735 (DDX53), E.184840 (TMED9), E.185219 (ZNF445), E.186198 (SLC51B), E.186205 (MOSC1; MARC1), E.189143 (CLDN4), E.196700 (ZNF512B), E.196743 (GM2A), E.198087 (CD2AP), E.198951 (NAGA), E.204406 (MBD5), E.002330 (BAD), E.105404 (RABAC1), E.114127 (XRN1), E.117713 (ARID1A), E.123143 (PKN1), E.130764 (LRRC47), E.131773 (KHDRBS3), E.137806 (NDUFAF1), E.142864 (SERBP1), E.158747 (NBL1), E.175063 (UBE2C), E.178104 (PDE4DIP), E.186472 (PCLO), E.069956 (MAPK6), E.112941 (PAPD7), E.116604 (MEF2D), E.142875 (PRKACB), E.147133 (TAF1), E.157510 (AFAP1L1), E.006625 (GGCT), E.155980 (KIF5A), E.134444 (KIAA1468), E.107968 (MAP3K8), E.117592 (PRDX6), E.123154 (WDR83), E.135297 (MTO1), E.135829 (DHX9), E.149548 (CCDC15), E.152086 (TUBA3E), E.167553 (TUBA1C), E.169826 (CSGALNACT2), E.171121 (KCNMB3), E.198033 (TUBA3C), E.147724 (FAM135B), E.170854 (MINA), E.006695 (COX10), E.067369 (TP53BP1), E.089248 (ERP29), E.112096 (SOD2), E.138073 (PREB), E.146856 (AGBL3), E.159423 (ALDH4A1), E.171345 (KRT19), E.172345 (STARD5), E.111647 (UHRF1BP1L), E.117877 (CD3EAP), E.155714 (PDZD9), E.156603 (MED19), E.075886 (TUBA3D), E.167699 (GLOD4), E.121749 (TBC1D15), E.090861 (AARS), E.093010 (COMT), E.117676 (RPS6KA1), E.157502 (MUM1L1),

	E.159921 (GNE), E.169562 (GJB1), E.179776 (CDH5), E.071626 (DAZAP1), E.085224 (ATRX), E.116478 (HDAC1), E.117298 (ECE1), E.176171 (BNIP3), E.177425 (PAWR), E.179348 (GATA2), E.187840 (EIF4EBP1), E.033030 (ZCCHC8), E.049239 (H6PD), E.060688 (SNRNP40), E.075239 (ACAT1), E.095627 (TDRD1), E.109625 (CPZ), E.113719 (ERGIC1), E.126773 (C14orf135; PCNXL4), E.149218 (ENDOD1), E.162975 (KCNF1), E.183785 (TUBA8), E.198589 (LRBA), E.105379 (ETFB), E.011052 (NME2), E.011143 (MKS1), E.048544 (MRPS10), E.062485 (CS), E.114054 (PCCB), E.138587 (MNS1), E.155959 (VBP1), E.181222 (POLR2A), E.183723 (CMTM4), E.184661 (CDCA2), E.204316 (MRPL38), E.140694 (PARN), E.035141 (FAM136A), E.095485 (CWF19L1), E.115540 (MOB4), E.123595 (RAB9A), E.140678 (ITGAX), E.141258 (SGSM2), E.158941 (KIAA1967), E.169189 (NSMCE1), E.198431 (TXNRD1), E.016402 (IL20RA), E.112234 (FBXL4), E.125445 (MRPS7), E.128342 (LIF), E.164051 (CCDC51), E.175866 (BAIAP2), E.102780 (DGKH), E.203813 (HIST1H3H), E.198231 (DDX42), E.030582 (GRN), E.106049 (HIBADH), E.130810 (PPAN), E.132475 (H3F3B), E.158290 (CUL4B), E.166266 (CUL5), E.026559 (KCNG1), E.059122 (FLYWCH1), E.107897 (ACBD5), E.121068 (TBX2), E.125944 (HNRNPR), E.134308 (YWHAQ), E.137558 (PI15), E.137601 (NEK1), E.147548 (WHSC1L1), E.149182 (ARFGAP2), E.159658 (KIAA0494), E.165699 (TSC1), E.170927 (PKHD1), E.186575 (NF2), E.188021 (UBQLN2), E.167552 (TUBA1A), E.003756 (RBM5), E.134138 (MEIS2), E.008196 (TFAP2B), E.079313 (REXO1), E.089127 (OAS1), E.106078 (COBL), E.113645 (WWC1), E.116288 (PARK7), E.121940 (CLCC1), E.136280 (CCM2), E.141639 (MAPK4), E.147475 (ERLIN2), E.155660 (PDIA4), E.162298 (SYVN1), E.176978 (DPP7), E.176994 (SMCR8), E.178175 (ZNF366), E.196591 (HDAC2), E.127824 (TUBA4A), E.163932 (PRKCD), E.143375 (CGN), E.076864 (RAP1GAP), E.138772 (ANXA3), E.163041 (H3F3A), E.165813 (C10orf118), E.166337 (TAF10), E.178078 (STAP2), E.184007 (PTP4A2), E.167004 (PDIA3), E.039560 (RAI14), E.119636 (C14orf45), E.140374 (ETFA), E.143633 (C1orf131), E.144935 (TRPC1), E.156735 (BAG4), E.159348 (CYB5R1), E.170275 (CRTAP), E.172717 (FAM71D), E.172939 (OXSR1), E.176105 (YES1), E.078295 (ADCY2), E.119888 (EPCAM), E.141522 (ARHGDIA), E.184047 (DIABLO), E.109062 (SLC9A3R1), E.170037 (CNTROB), E.066557 (LRRC40), E.074964 (ARHGEF10L), E.078269 (SYNJ2), E.090013 (BLVRB), E.100142 (POLR2F), E.100399 (CHADL), E.104365 (IKBKB), E.111261 (MANSC1), E.111907 (TPD52L1), E.112578 (BYSL), E.121957 (GPSM2), E.122884 (P4HA1), E.124693 (HIST1H3B), E.126653 (NSRP1), E.130402 (ACTN4), E.138757 (G3BP2), E.150991 (UBC), E.164828 (SUN1), E.175216 (CKAP5), E.176155 (CCDC57), E.177459 (C8orf47), E.183856 (IQGAP3), E.185122 (HSF1), E.122952 (ZWINT), E.151093 (OXSM), E.067704 (IARS2), E.088899 (ProSAP-interacting protein 1), E.091483 (FH), E.114388 (NPRL2), E.114861 (FOXP1), E.135914 (HTR2B), E.197837 (HIST4H4), E.127720 (C12orf26; METTL25), E.123416 (TUBA1B), E.047410 (TPR), E.117748 (RPA2), E.133835 (HSD17B4), E.067248 (DHX29), E.121879 (PIK3CA), E.132589 (FLOT2), E.136750 (GAD2), E.160789 (LMNA), E.166329, E.170088 (TMEM192), E.175946 (KLHL38), E.178163 (ZNF518B), E.182217 (HIST2H4B), E.184470 (TXNRD2), E.110321 (EIF4G2), E.171861 (RNMTL1), E.065978 (YBX1), E.115738 (ID2), E.143294 (PRCC), E.158042 (MRPL17), E.169093 (ASMTL), E.090565 (RAB11FIP3), E.185591 (SP1), E.156304 (SCAF4), E.092978 (GPATCH2), E.100056 (DGCR14), E.100583 (SAMD15), E.105723 (GSK3A), E.107551 (RASSF4), E.107581 (EIF3A), E.107890 (ANKRD26), E.110104 (CCDC86), E.112584 (FAM120B), E.113580 (NR3C1), E.114491 (UMPS), E.137312 (FLOT1), E.137955 (RABGGTB), E.141994 (DUS3L), E.147044 (CASK), E.152818 (UTRN), E.180667 (YOD1), E.184916 (JAG2), E.196526 (AFAP1), E.198783 (ZNF830), E.108465 (CDK5RAP3), E.156515 (HK1), E.036448 (MYOM2), E.061918 (GUCY1B3), E.070785 (EIF2B3), E.116044 (NFE2L2), E.128311 (TST), E.131473 (ACLY), E.132716 (DCAF8), E.138363 (ATIC), E.166596 (WDR16), E.170027 (YWHAG), E.174021 (GNG5), E.203879 (GDI1), E.160049 (DFFA), E.010810 (FYN), E.051596 (THOC3), E.006453 (BAI1-associated protein 2-like 1), E.126945 (HNRNPH2), E.165695 (AK8), E.069869 (NEDD4), E.111801 (BTN3A3), E.112232 (KHDRBS2), E.128626 (MRPS12), E.129636 (ITFG1), E.137948 (BRDT), E.147257 (GPC3), E.155380 (SLC16A1), E.159692 (CTBP1), E.166833 (NAV2), E.172466 (ZNF24), E.175110 (MRPS22), E.176102 (CSTF3), E.179388 (EGR3), E.185359 (HGS), E.198001 (IRAK4), E.100603 (SNW1), E.162641 (AKNAD1), E.069712 (KIAA1107), E.073756 (PTGS2), E.077522 (ACTN2), E.101639 (CEP192), E.106633 (GCK), E.115241 (PPM1G), E.116649 (SRM), E.120370 (GORAB), E.124143

	(ARHGAP40), E.127948 (POR), E.129315 (CCNT1), E.132646 (PCNA), E.135740 (SLC9A5), E.151726 (ACSL1), E.154380 (ENAH), E.157103 (SLC6A1), E.163930 (BAP1), E.164488 (DACT2), E.164754 (RAD21), E.175220 (ARHGAP1), E.180318 (ALX1), E.181234 (TMEM132C), E.197081 (IGF2R), E.092871 (RFFL), E.163644 (PPM1K), E.171723 (GPHN), E.108953 (YWHAE), E.072110 (ACTN1), E.077097 (TOP2B), E.090889 (KIF4A), E.114331 (ACAP2), E.114867 (EIF4G1), E.117593 (DARS2), E.118523 (CTGF), E.120915 (EPHX2), E.134759 (ELP2), E.138061 (CYP1B1), E.140743 (CDR2), E.151247 (EIF4E), E.152942 (RAD17), E.160685 (ZBTB7B), E.163923 (RPL39L), E.167642 (SPINT2), E.167996 (FTH1), E.185736 (ADARB2), E.198841 (KTI12), E.185860 (C1orf110), E.160226 (C21orf2), E.070814 (TCOF1), E.124749 (COL21A1), E.154639 (CXADR), E.065485 (PDIA5), E.023909 (GCLM), E.100714 (MTHFD1), E.108387 (SEPT4), E.160867 (FGFR4), E.134684 (YARS), E.123080 (CDKN2C), E.065548 (ZC3H15), E.116455 (WDR77), E.117448 (AKR1A1), E.100393 (EP300), E.138160 (KIF11), E.166263 (STXBP4), E.173473 (SMARCC1), E.124942 (AHNAK), E.174842 (GLMN), E.180198 (RCC1), E.185499 (MUC1), E.143947 (RPS27A), E.170315 (UBB), E.003402 (CFLAR), E.137055 (PLAA), E.142606 (MMEL1), E.147697 (GSDMC), E.163110 (PDLIM5), E.135842 (FAM129A), E.160691 (SHC1), E.197157 (SND1), E.029725 (RABEP1), E.127946 (HIP1), E.001036 (FUCA2), E.109846 (CRYAB), E.183831 (ANKRD45), E.189283 (FHIT), E.092820 (EZR), E.104067 (TJP1), E.120159 (C9orf82; CAAP1), E.154864 (PIEZO2), E.196975 (ANXA4), E.105220 (GPI), E.127914 (AKAP9), E.135870 (RC3H1), E.026508 (CD44), E.089154 (GCN1L1), E.100311 (PDGFB), E.119383 (PPP2R4), E.075624 (ACTB), E.177409 (SAMD9L), E.177731 (FLII), E.015676 (NUDCD3), E.146457 (WTAP), E.178950 (GAK), E.167110 (GOLGA2)
Prostate vesicle	LAMP2, ACPP, CTNNA1, HEBP2, ISOC2, HNRNPC, HNRNPM, TOMM22, TOM1, ACO2, KRT18, HSPA9, LMNB1, SPR, PPL, ALDH6A1, HNRNPA2B1, ATXN1, SMARCA4, ECHS1, PAICS, ILF3, PSME3, COX5B, RAB1A, SCARB2, HADH, ESD, SORD, ILF2, CALM2, ATP5A1, TGOLN2, ANGPTL4, ALCAM, KRT2, PC, NPM1, C1orf116, GPC6, ALDH1A3, HIST1H1C, XRCC6, HNRNPAB, PSAP, CDH1, SCAMP2, VASP, CD9, ATP1B3, HSD17B10, APAF1, EIF2C2, RAB5A, CFL2, FARSA, XPNPEP3, ENTPD4, APLP2, NUCB1, RAB3D, VEGFA, HPS3, TSNAXIP1, HNRNPL, PSMB7, GNA12, NONO, FOLH1, PRKAR2A, PHB, HIST3H3, MAP7, VCP, U2AF2, FUS, FKBP5, NDRG1, ATP1A3, NCL, RPL36, KRT8, C1GALT1C1, FASN, PTBP1, TXNDC16, DNAJC5, SLC37A2, HNRNPK, VDAC2, PRDX2, TALDO1, USP14, PSMD7, HSPE1, DNAJB1, YWHAZ, RAB3B, CORO1B, MDH2, HIST1H3A, LAMP1, STC2, DSTN, SLC20A2, ENPP4, WIZ, HSP90AB1, IDH3B, ECH1, C1QBP, SET, TNFSF18, ITGB7, SPOCK1, EIF4A2, CCT3, CLDN3, EEF2, LRRC57, RUVBL2, CLDN5, APPL2, TM9SF2, EIF4A3, DBI, DBF4B, SVIP, CD151, ALOX5, SLC9A3R2, RAB27B, DLG1, ARCN1, CHCHD3, RAB5B, RPS25, RPL10, DDAH1, HSP90B1, CTNNB1, PSMD2, PKP3, FLNB, EFTUD2, GLO1, PRKCSH, TMBIM1, SEC31A, TMED10, RPL14, MATR3, APEX1, B4GALT1, HNRNPA1, CPD, HSPA1A, CAPN1, CHRDL2, SPEN, SDF4, NAPA, SYNGR2, CHMP3, CNDP2, CCDC64B, SERINC5, VPS37C, DNPEP, CLDN7, KTN1, SERPINB6, ATP5B, CANX, AKT1, TTBK2, DDX1, DLD, LNPEP, LTBP2, LRPPRC, EPS8, AZGP1, VPS28, DHCR7, CIB1, DDX39B, HIST1H4B, UGDH, HSPD1, B2M, TOLLIP, CD276, CYCS, CUL3, GDI2, LLGL2, XRCC5, CTTN, PHGDH, CST3, RBL2, SLC1A5, CD46, VAMP8, CLTA, ACSL3, MRPS26, SNX9, GLUD1, TMED4, PTPN13, AP1G1, SYT9, DCTN2, IDH2, GLUD2, TMED9, CLDN4, GM2A, CD2AP, MBD5, SERBP1, NBL1, PRKACB, GGCT, PRDX6, DHX9, TUBA3E, TUBA1C, TUBA3C, ERP29, SOD2, KRT19, TUBA3D, AARS, COMT, MUM1L1, CDH5, ECE1, ACAT1, ENDOD1, TUBA8, ETFB, NME2, CS, VBP1, RAB9A, TXNRD1, LIF, BAIAP2, HIST1H3H, GRN, HIBADH, H3F3B, CUL4B, HNRNPR, YWHAQ, PKHD1, TUBA1A, PARK7, ERLIN2, PDIA4, TUBA4A, PRKCD, ANXA3, H3F3A, PTP4A2, PDIA3, ETFA, CYB5R1, CRTAP, OXSR1, YES1, EPCAM, ARHGDIA, DIABLO, SLC9A3R1, BLVRB, P4HA1, HIST1H3B, ACTN4, UBC, FH, HIST4H4, TUBA1B, HSD17B4, PIK3CA, FLOT2, LMNA, TMEM192, HIST2H4B, YBX1, EIF3A, FLOT1, UTRN, HK1, ACLY, ATIC, YWHAG, GNG5, GDI1, HNRNPH2, NEDD4, BTN3A3, SLC16A1, HGS, ACTN2, SRM, PCNA, ACSL1, RAD21, ARHGAP1, IGF2R, YWHAE, ACTN1, EIF4G1, EPHX2, EIF4E, FTH1, CXADR, MTHFD1, AKR1A1, STXBP4, AHNAK, MUC1, RPS27A, UBB, PDLIM5, FAM129A, SND1, FUCA2, CRYAB, EZR, TJP1, ANXA4, GPI, AKAP9, CD44, GCN1L1, ACTB, FLII, NUDCD3

Ribonucleoprotein complexes & vesicles	GW182, Ago2, miR-let-7a, miR-16, miR-22, miR-148a, miR-451, miR-92a, CD9, CD63, CD81
Prostate Cancer vesicles	PCSA, Muc2, Adam10
Prostate Cancer vesicles	Alkaline Phosphatase (AP), CD63, MyoD1, Neuron Specific Enolase, MAP1B, CNPase, Prohibitin, CD45RO, Heat Shock Protein 27, Collagen II, Laminin B1/b1, Gai1, CDw75, bcl-XL, Laminin-s, Ferritin, CD21, ADP-ribosylation Factor (ARF-6)
Prostate Cancer vesicles	CD56/NCAM-1, Heat Shock Protein 27/hsp27, CD45RO, MAP1B, MyoD1, CD45/T200/LCA, CD3zeta, Laminin-s, bcl-XL, Rad18, Gai1, Thymidylate Synthase, Alkaline Phosphatase (AP), CD63, MMP-16 / MT3-MMP, Cyclin C, Neuron Specific Enolase, SIRP a1, Laminin B1/b1, Amyloid Beta (APP), SODD (Silencer of Death Domain), CDC37, Gab-1, E2F-2, CD6, Mast Cell Chymase, Gamma Glutamylcysteine Synthetase (GCS)
Prostate Cancer vesicles	EpCAM, MMP7, PCSA, BCNP, ADAM10, KLK2, SPDEF, CD81, MFGE8, IL-8
Prostate Cancer vesicles	EpCAM, KLK2, PBP, SPDEF, SSX2, SSX4
Prostate Cancer vesicles	ADAM-10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, SSX4
Androgen Receptor (AR) pathway members in cMVs	GTF2F1, CTNNB1, PTEN, APPL1, GAPDH, CDC37, PNRC1, AES, UXT, RAN, PA2G4, JUN, BAG1, UBE2I, HDAC1, COX5B, NCOR2, STUB1, HIPK3, PXN, NCOA4
EGFR1 pathway members in cMVs	RALBP1, SH3BGRL, RBBP7, REPS1, SNRPD2, CEBPB, APPL1, MAP3K3, EEF1A1, GRB2, RAC1, SNCA, MAP2K3, CEBPA, CDC42, SH3KBP1, CBL, PTPN6, YWHAB, FOXO1, JAK1, KRT8, RALGDS, SMAD2, VAV1, NDUFA13, PRKCB1, MYC, JUN, RFXANK, HDAC1, HIST3H3, PEBP1, PXN, TNIP1, PKN2
TNF-alpha pathway members in cMVs	BCL3, SMARCE1, RPS11, CDC37, RPL6, RPL8, PAPOLA, PSMC1, CASP3, AKT2, MAP3K7IP2, POLR2L, TRADD, SMARCA4, HIST3H3, GNB2L1, PSMD1, PEBP1, HSPB1, TNIP1, RPS13, ZFAND5, YWHAQ, COMMD1, COPS3, POLR1D, SMARCC2, MAP3K3, BIRC3, UBE2D2, HDAC2, CASP8, MCM7, PSMD7, YWHAG, NFKBIA, CAST, YWHAB, G3BP2, PSMD13, FBL, RELB, YWHAZ, SKP1, UBE2D3, PDCD2, HSP90AA1, HDAC1, KPNA2, RPL30, GTF2I, PFDN2
Colorectal cancer	CD9, EGFR, NGAL, CD81, STEAP, CD24, A33, CD66E, EPHA2, Ferritin, GPR30, GPR110, MMP9, OPN, p53, TMEM211, TROP2, TGM2, TIMP, EGFR, DR3, UNC93A, MUC17, EpCAM, MUC1, MUC2, TSG101, CD63, B7H3
Colorectal cancer	DR3, STEAP, epha2, TMEM211, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, TETS
Colorectal cancer	A33, AFP, ALIX, ALX4, ANCA, APC, ASCA, AURKA, AURKB, B7H3, BANK1, BCNP, BDNF, CA-19-9, CCSA-2, CCSA-3&4, CD10, CD24, CD44, CD63, CD66 CEA, CD66e CEA, CD81, CD9, CDA, C-Erb2, CRMP-2, CRP, CRTN, CXCL12, CYFRA21-1, DcR3, DLL4, DR3, EGFR, Epcam, EphA2, FASL, FRT, GAL3, GDF15, GPCR (GPR110), GPR30, GRO-1, HBD 1, HBD2, HNP1-3, IL-1B, IL8, IMP3, L1CAM, LAMN, MACC-1, MGC20553, MCP-1, M-CSF, MIC1, MIF, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, Ncam, NGAL, NNMT, OPN, p53, PCSA, PDGFRB, PRL, PSMA, PSME3, Reg IV, SCRN1, Sept-9, SPARC, SPON2, SPR, SRVN, TFF3, TGM2, TIMP-1, TMEM211, TNF-alpha, TPA, TPS, Trail-R2, Trail-R4, TrKB, TROP2, Tsg 101, TWEAK, UNC93A, VEGFA
Colorectal cancer	miR 92, miR 21, miR 9, miR 491
Colorectal cancer	miR-127-3p, miR-92a, miR-486-3p, miR-378
Colorectal cancer	TMEM211, MUC1, CD24 and/or GPR110 (GPCR 110)
Colorectal cancer	hsa-miR-376c, hsa-miR-215, hsa-miR-652, hsa-miR-582-5p, hsa-miR-324-5p, hsa-miR-1296, hsa-miR-28-5p, hsa-miR-190, hsa-miR-590-5p, hsa-miR-202, hsa-miR-195
Colorectal cancer vesicle markers	A26C1A, A26C1B, A2M, ACAA2, ACE, ACOT7, ACP1, ACTA1, ACTA2, ACTB, ACTBL2, ACTBL3, ACTC1, ACTG1, ACTG2, ACTN1, ACTN2, ACTN4, ACTR3, ADAM10, ADSL, AGR2, AGR3, AGRN, AHCY, AHNAK, AKR1B10, ALB, ALDH16A1, ALDH1A1, ALDOA, ANXA1, ANXA11, ANXA2, ANXA2P2, ANXA4, ANXA5, ANXA6, AP2A1, AP2A2, APOA1, ARF1, ARF3, ARF4, ARF5, ARF6, ARHGDIA, ARPC3, ARPC5L, ARRDC1, ARVCF, ASCC3L1, ASNS, ATP1A1, ATP1A2, ATP1A3, ATP1B1, ATP4A, ATP5A1, ATP5B, ATP5I, ATP5L, ATP5O, ATP6AP2, B2M,

	BAIAP2, BAIAP2L1, BRI3BP, BSG, BUB3, C1orf58, C5orf32, CAD, CALM1, CALM2, CALM3, CAND1, CANX, CAPZA1, CBR1, CBR3, CCT2, CCT3, CCT4, CCT5, CCT6A, CCT7, CCT8, CD44, CD46, CD55, CD59, CD63, CD81, CD82, CD9, CDC42, CDH1, CDH17, CEACAM5, CFL1, CFL2, CHMP1A, CHMP2A, CHMP4B, CKB, CLDN3, CLDN4, CLDN7, CLIC1, CLIC4, CLSTN1, CLTC, CLTCL1, CLU, COL12A1, COPB1, COPB2, CORO1C, COX4I1, COX5B, CRYZ, CSPG4, CSRP1, CST3, CTNNA1, CTNNB1, CTNND1, CTTN, CYFIP1, DCD, DERA, DIP2A, DIP2B, DIP2C, DMBT1, DPEP1, DPP4, DYNC1H1, EDIL3, EEF1A1, EEF1A2, EEF1AL3, EEF1G, EEF2, EFNB1, EGFR, EHD1, EHD4, EIF3EIP, EIF3I, EIF4A1, EIF4A2, ENO1, ENO2, ENO3, EPHA2, EPHA5, EPHB1, EPHB2, EPHB3, EPHB4, EPPK1, ESD, EZR, F11R, F5, F7, FAM125A, FAM125B, FAM129B, FASLG, FASN, FAT, FCGBP, FER1L3, FKBP1A, FLNA, FLNB, FLOT1, FLOT2, G6PD, GAPDH, GARS, GCN1L1, GDI2, GK, GMDS, GNA13, GNAI2, GNAI3, GNAS, GNB1, GNB2, GNB2L1, GNB3, GNB4, GNG12, GOLGA7, GPA33, GPI, GPRC5A, GSN, GSTP1, H2AFJ, HADHA, hCG_1757335, HEPH, HIST1H2AB, HIST1H2AE, HIST1H2AJ, HIST1H2AK, HIST1H4A, HIST1H4B, HIST1H4C, HIST1H4D, HIST1H4E, HIST1H4F, HIST1H4H, HIST1H4I, HIST1H4J, HIST1H4K, HIST1H4L, HIST2H2AC, HIST2H4A, HIST2H4B, HIST3H2A, HIST4H4, HLA-A, HLA-A29.1, HLA-B, HLA-C, HLA-E, HLA-H, HNRNPA2B1, HNRNPH2, HPCAL1, HRAS, HSD17B4, HSP90AA1, HSP90AA2, HSP90AA4P, HSP90AB1, HSP90AB2P, HSP90AB3P, HSP90B1, HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA4, HSPA5, HSPA6, HSPA7, HSPA8, HSPA9, HSPD1, HSPE1, HSPG2, HYOU1, IDH1, IFITM1, IFITM2, IFITM3, IGH@, IGHG1, IGHG2, IGHG3, IGHG4, IGHM, IGHV4-31, IGK@, IGKC, IGKV1-5, IGKV2-24, IGKV3-20, IGSF3, IGSF8, IQGAP1, IQGAP2, ITGA2, ITGA3, ITGA6, ITGAV, ITGB1, ITGB4, JUP, KIAA0174, KIAA1199, KPNB1, KRAS, KRT1, KRT10, KRT13, KRT14, KRT15, KRT16, KRT17, KRT18, KRT19, KRT2, KRT20, KRT24, KRT25, KRT27, KRT28, KRT3, KRT4, KRT5, KRT6A, KRT6B, KRT6C, KRT7, KRT75, KRT76, KRT77, KRT79, KRT8, KRT9, LAMA5, LAMP1, LDHA, LDHB, LFNG, LGALS3, LGALS3BP, LGALS4, LIMA1, LIN7A, LIN7C, LOC100128936, LOC100130553, LOC100133382, LOC100133739, LOC284889, LOC388524, LOC388720, LOC442497, LOC653269, LRP4, LRPPRC, LRSAM1, LSR, LYZ, MAN1A1, MAP4K4, MARCKS, MARCKSL1, METRNL, MFGE8, MICA, MIF, MINK1, MITD1, MMP7, MOBKL1A, MSN, MTCH2, MUC13, MYADM, MYH10, MYH11, MYH14, MYH9, MYL6, MYL6B, MYO1C, MYO1D, NARS, NCALD, NCSTN, NEDD4, NEDD4L, NME1, NME2, NOTCH1, NQO1, NRAS, P4HB, PCBP1, PCNA, PCSK9, PDCD6, PDCD6IP, PDIA3, PDXK, PEBP1, PFN1, PGK1, PHB, PHB2, PKM2, PLEC1, PLEKHB2, PLSCR3, PLXNA1, PLXNB2, PPIA, PPIB, PPP2R1A, PRDX1, PRDX2, PRDX3, PRDX5, PRDX6, PRKAR2A, PRKDC, PRSS23, PSMA2, PSMC6, PSMD11, PSMD3, PSME3, PTGFRN, PTPRF, PYGB, QPCT, QSOX1, RAB10, RAB11A, RAB11B, RAB13, RAB14, RAB15, RAB1A, RAB1B, RAB2A, RAB33B, RAB35, RAB43, RAB4B, RAB5A, RAB5B, RAB5C, RAB6A, RAB6B, RAB7A, RAB8A, RAB8B, RAC1, RAC3, RALA, RALB, RAN, RANP1, RAP1A, RAP1B, RAP2A, RAP2B, RAP2C, RDX, REG4, RHOA, RHOC, RHOG, ROCK2, RP11-631M21.2, RPL10A, RPL12, RPL6, RPL8, RPLP0, RPLP0-like, RPLP1, RPLP2, RPN1, RPS13, RPS14, RPS15A, RPS16, RPS18, RPS20, RPS21, RPS27A, RPS3, RPS4X, RPS4Y1, RPS4Y2, RPS7, RPS8, RPSA, RPSAP15, RRAS, RRAS2, RUVBL1, RUVBL2, S100A10, S100A11, S100A14, S100A16, S100A6, S100P, SDC1, SDC4, SDCBP, SDCBP2, SERINC1, SERINC5, SERPINA1, SERPINF1, SETD4, SFN, SLC12A2, SLC12A7, SLC16A1, SLC1A5, SLC25A4, SLC25A5, SLC25A6, SLC29A1, SLC2A1, SLC3A2, SLC44A1, SLC7A5, SLC9A3R1, SMPDL3B, SNAP23, SND1, SOD1, SORT1, SPTAN1, SPTBN1, SSBP1, SSR4, TACSTD1, TAGLN2, TBCA, TCEB1, TCP1, TF, TFRC, THBS1, TJP2, TKT, TMED2, TNFSF10, TNIK, TNKS1BP1, TNPO3, TOLLIP, TOMM22, TPI1, TPM1, TRAP1, TSG101, TSPAN1, TSPAN14, TSPAN15, TSPAN6, TSPAN8, TSTA3, TTYH3, TUBA1A, TUBA1B, TUBA1C, TUBA3C, TUBA3D, TUBA3E, TUBA4A, TUBA4B, TUBA8, TUBB, TUBB2A, TUBB2B, TUBB2C, TUBB3, TUBB4, TUBB4Q, TUBB6, TUFM, TXN, UBA1, UBA52, UBB, UBC, UBE2N, UBE2V2, UGDH, UQCRC2, VAMP1, VAMP3, VAMP8, VCP, VIL1, VPS25, VPS28, VPS35, VPS36, VPS37B, VPS37C, WDR1, YWHAB, YWHAE, YWHAG, YWHAH, YWHAQ, YWHAZ
Colorectal Cancer	hsa-miR-16, hsa-miR-25, hsa-miR-125b, hsa-miR-451, hsa-miR-200c, hsa-miR-140-3p, hsa-miR-658, hsa-miR-370, hsa-miR-1296, hsa-miR-636, hsa-miR-502-5p
Breast cancer	miR-21, miR-155, miR-206, miR-122a, miR-210, miR-21, miR-155, miR-206, miR-122a,

	miR-210, let-7, miR-10b, miR-125a, miR-125b, miR-145, miR-143, miR-145, miR-1b
Breast cancer	GAS5
Breast cancer	ER, PR, HER2, MUC1, EGFR, KRAS, B-Raf, CYP2D6, hsp70, MART-1, TRP, HER2, hsp70, MART-1, TRP, HER2, ER, PR, Class III b-tubulin, VEGFA, ETV6-NTRK3, BCA-225, hsp70, MART1, ER, VEGFA, Class III b-tubulin, HER2/neu (e.g., for Her2+ breast cancer), GPR30, ErbB4 (JM) isoform, MPR8, MISIIR, CD9, EphA2, EGFR, B7H3, PSM, PCSA, CD63, STEAP, CD81, ICAM1, A33, DR3, CD66e, MFG-E8, TROP-2, Mammaglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, EpCam, neurokinin receptor-1 (NK-1 or NK-1R), NK-2, Pai-1, CD45, CD10, HER2/ERBB2, AGTR1, NPY1R, MUC1, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUC1 secreted), CA27.29 (MUC1 secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1, SPB, SPC, NSE, PGP9.5, progesterone receptor (PR) or its isoform (PR(A) or PR(B)), P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, IC3b, mesothelin, SPA, AQP5, GPCR, hCEA-CAM, PTP IA-2, CABYR, TMEM211, ADAM28, UNC93A, MUC17, MUC2, IL10R-beta, BCMA, HVEM/TNFRSF14, Trappin-2, Elafin, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNFR
Breast cancer	CD9, MIS Rii, ER, CD63, MUC1, HER3, STAT3, VEGFA, BCA, CA125, CD24, EPCAM, ERB B4
Breast cancer	CD10, NPGP/NPFF2, HER2/ERBB2, AGTR1, NPY1R, neurokinin receptor-1 (NK-1 or NK-1R), NK-2, MUC1, ESA, CD133, GPR30, BCA225, CD24, CA15.3 (MUC1 secreted), CA27.29 (MUC1 secreted), NMDAR1, NMDAR2, MAGEA, CTAG1B, NY-ESO-1
Breast cancer	SPB, SPC, NSE, PGP9.5, CD9, P2RX7, NDUFB7, NSE, GAL3, osteopontin, CHI3L1, EGFR, B7H3, IC3b, MUC1, mesothelin, SPA, PCSA, CD63, STEAP, AQP5, CD81, DR3, PSM, GPCR, EphA2, hCEA-CAM, PTP IA-2, CABYR, TMEM211, ADAM28, UNC93A, A33, CD24, CD10, NGAL, EpCam, MUC17, TROP-2, MUC2, IL10R-beta, BCMA, HVEM/TNFRSF14, Trappin-2 Elafin, ST2/IL1 R4, TNFRF14, CEACAM1, TPA1, LAMP, WF, WH1000, PECAM, BSA, TNFR
Breast cancer	BRCA, MUC-1, MUC 16, CD24, ErbB4, ErbB2 (HER2), ErbB3, HSP70, Mammaglobin, PR, PR(B), VEGFA
Breast cancer	CD9, HSP70, Gal3, MIS, EGFR, ER, ICB3, CD63, B7H4, MUC1, DLL4, CD81, ERB3, VEGF, BCA225, BRCA, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD31, cMET, MUC2, ERBB4
Breast cancer	CD9, EphA2, EGFR, B7H3, PSMA, PCSA, CD63, STEAP, CD81, STEAP1, ICAM1 (CD54), PSMA, A33, DR3, CD66e, MFG-8e, TMEM211, TROP-2, EGFR, Mammoglobin, Hepsin, NPGP/NPFF2, PSCA, 5T4, NGAL, NK-2, EpCam, NK-1R, PSMA, 5T4, PAI-1, CD45
Breast cancer	PGP9.5, CD9, HSP70, gal3-b2c10, EGFR, iC3b, PSMA, PCSA, CD63, MUC1, DLL4, CD81, B7-H3, HER 3 (ErbB3), MART-1, PSA, VEGF A, TIMP-1, GPCR GPR110, EphA2, MMP9, mmp7, TMEM211, UNC93a, BRCA, CA125 (MUC16), Mammaglobin, CD174 (Lewis y), CD66e CEA, CD24 c.sn3, C-erbB2, CD10, NGAL, epcam, CEA (carcinoembryonic Antigen), GPR30, CYFRA21-1, OPN, MUC17, hVEGFR2, MUC2, NCAM, ASPH, ErbB4, SPB, SPC, CD9, MS4A1, EphA2, MIS RII, HER2 (ErbB2), ER, PR (B), MRP8, CD63, B7H4, TGM2, CD81, DR3, STAT 3, MACC-1, TrKB, IL 6 Unc, OPG - 13, IL6R, EZH2, SCRN1, TWEAK, SERPINB3, CDAC1, BCA-225, DR3, A33, NPGP/NPFF2, TIMP1, BDNF, FRT, Ferritin heavy chain, seprase, p53, LDH, HSP, ost, p53, CXCL12, HAP, CRP, Gro-alpha, Tsg 101, GDF15
Breast cancer	CD9, HSP70, Gal3, MIS (RII), EGFR, ER, ICB3, CD63, B7H4, MUC1, CD81, ERB3, MART1, STAT3, VEGF, BCA225, BRCA, CA125, CD174, CD24, ERB2, NGAL, GPR30, CYFRA21, CD31, cMET, MUC2, ERB4, TMEM211
Breast Cancer	5T4 (trophoblast), ADAM10, AGER/RAGE, APC, APP (β-amyloid), ASPH (A-10), B7H3 (CD276), BACE1, BAI3, BRCA1, BDNF, BIRC2, C1GALT1, CA125 (MUC16), Calmodulin 1, CCL2 (MCP-1), CD9, CD10, CD127 (IL7R), CD174, CD24, CD44, CD63, CD81, CEA, CRMP-2, CXCR3, CXCR4, CXCR6, CYFRA 21, derlin 1, DLL4, DPP6, E-CAD, EpCaM, EphA2 (H-77), ER(1) ESR1 α, ER(2) ESR2 β, Erb B4, Erbb2, erb3 (Erb-B3), PA2G4, FRT (FLT1), Gal3, GPR30 (G-coupled ER1), HAP1, HER3, HSP-27, HSP70, IC3b, IL8, insig, junction plakoglobin, Keratin 15, KRAS, Mammaglobin, MART1, MCT2, MFGE8, MMP9, MRP8, Muc1, MUC17, MUC2, NCAM, NG2 (CSPG4), Ngal, NHE-3, NT5E (CD73), ODC1, OPG, OPN, p53, PARK7, PCSA, PGP9.5 (PARK5), PR(B), PSA, PSMA, RAGE, STXBP4, Survivin, TFF3 (secreted), TIMP1, TIMP2, TMEM211, TRAF4 (scaffolding), TRAIL-R2 (death Receptor 5), TrkB, Tsg 101, UNC93a, VEGF A, VEGFR2,

	YB-1, VEGFR1, GCDPF-15 (PIP), BigH3 (TGFb1-induced protein), 5HT2B (serotonin receptor 2B), BRCA2, BACE 1, CDH1-cadherin
Breast Cancer	AK5.2, ATP6V1B1, CRABP1
Breast Cancer	DST.3, GATA3, KRT81
Breast Cancer	AK5.2, ATP6V1B1, CRABP1, DST.3, ELF5, GATA3, KRT81, LALBA, OXTR, RASL10A, SERHL, TFAP2A.1, TFAP2A.3, TFAP2C, VTCN1
Breast Cancer	TRAP; Renal Cell Carcinoma; Filamin; 14.3.3, Pan; Prohibitin; c-fos; Ang-2; GSTmu; Ang-1; FHIT; Rad51; Inhibin alpha; Cadherin-P; 14.3.3 gamma; p18INK4c; P504S; XRCC2; Caspase 5; CREB-Binding Protein; Estrogen Receptor; IL17; Claudin 2; Keratin 8; GAPDH; CD1; Keratin, LMW; Gamma Glutamylcysteine Synthetase(GCS)/Glutamate-cysteine Ligase; a-B-Crystallin; Pax-5; MMP-19; APC; IL-3; Keratin 8 (phospho-specific Ser73); TGF-beta 2; ITK; Oct-2/; DJ-1; B7-H2; Plasma Cell Marker; Rad18; Estriol; Chk1; Prolactin Receptor; Laminin Receptor; Histone H1; CD45RO; GnRH Receptor; IP10/CRG2; Actin, Muscle Specific; S100; Dystrophin; Tubulin-a; CD3zeta; CDC37; GABA a Receptor 1; MMP-7 (Matrilysin); Heregulin; Caspase 3; CD56/NCAM-1; Gastrin 1; SREBP-1 (Sterol Regulatory Element Binding Protein-1); MLH1; PGP9.5; Factor VIII Related Antigen; ADP-ribosylation Factor (ARF-6); MHC II (HLA-DR) Ia; Survivin; CD23; G-CSF; CD2; Calretinin; Neuron Specific Enolase; CD165; Calponin; CD95 / Fas; Urocortin; Heat Shock Protein 27/hsp27; Topo II beta; Insulin Receptor; Keratin 5/8; sm; Actin, skeletal muscle; CA19-9; GluR1; GRIP1; CD79a mb-1; TdT; HRP; CD94; CCK-8; Thymidine Phosphorylase; CD57; Alkaline Phosphatase (AP); CD59 / MACIF / MIRL / Protectin; GLUT-1; alpha-1-antitrypsin; Presenillin; Mucin 3 (MUC3); pS2; 14-3-3 beta; MMP-13 (Collagenase-3); Fli-1; mGluR5; Mast Cell Chymase; Laminin B1/b1; Neurofilament (160kDa); CNPase; Amylin Peptide; Gai1; CD6; alpha-1-antichymotrypsin; E2F-2; MyoD1
Ductal carcinoma in situ (DCIS)	Laminin B1/b1; E2F-2; TdT; Apolipoprotein D; Granulocyte; Alkaline Phosphatase (AP); Heat Shock Protein 27/hsp27; CD95 / Fas; pS2; Estriol; GLUT-1; Fibronectin; CD6; CCK-8; sm; Factor VIII Related Antigen; CD57; Plasminogen; CD71 / Transferrin Receptor; Keratin 5/8; Thymidine Phosphorylase; CD45/T200/LCA; Epithelial Specific Antigen; Macrophage; CD10; MyoD1; Gai1; bcl-XL; hPL; Caspase 3; Actin, skeletal muscle; IP10/CRG2; GnRH Receptor; p35nck5a; ADP-ribosylation Factor (ARF-6); Cdk4 ; alpha-1-antitrypsin; IL17; Neuron Specific Enolase; CD56/NCAM-1; Prolactin Receptor; Cdk7; CD79a mb-1; Collagen IV; CD94; Myeloid Specific Marker; Keratin 10; Pax-5; IgM (m-Heavy Chain); CD45RO; CA19-9; Mucin 2; Glucagon; Mast Cell Chymase; MLH1; CD1; CNPase; Parkin; MHC II (HLA-DR) Ia; B7-H2; Chk1; Lambda Light Chain; MHC II (HLA-DP and DR); Myogenin; MMP-7 (Matrilysin); Topo II beta; CD53; Keratin 19; Rad18; Ret Oncoprotein; MHC II (HLA-DP); E3-binding protein (ARM1); Progesterone Receptor; Keratin 8; IgG; IgA; Tubulin; Insulin Receptor Substrate-1; Keratin 15; DR3; IL-3; Keratin 10/13; Cyclin D3; MHC I (HLA25 and HLA-Aw32); Calmodulin; Neurofilament (160kDa)
Ductal carcinoma in situ (DCIS) v. other Breast cancer	Macrophage; Fibronectin; Granulocyte; Keratin 19; Cyclin D3; CD45/T200/LCA; EGFR; Thrombospondin; CD81/TAPA-1; Ruv C; Plasminogen; Collagen IV; Laminin B1/b1; CD10; TdT; Filamin; bcl-XL; 14.3.3 gamma; 14.3.3, Pan; p170; Apolipoprotein D; CD71 / Transferrin Receptor; FHIT
Lung cancer	Pgrmc1 (progesterone receptor membrane component 1)/sigma-2 receptor, STEAP, EZH2
Lung cancer	Prohibitin, CD23, Amylin Peptide, HRP, Rad51, Pax-5, Oct-3/, GLUT-1, PSCA, Thrombospondin, FHIT, a-B-Crystallin, LewisA, Vacular Endothelial Growth Factor(VEGF), Hepatocyte Factor Homologue-4, Flt-4, GluR6/7, Prostate Apoptosis Response Protein-4, GluR1, Fli-1, Urocortin, S100A4, 14-3-3 beta, P504S, HDAC1, PGP9.5, DJ-1, COX2, MMP-19, Actin, skeletal muscle, Claudin 3, Cadherin-P, Collagen IX, p27Kip1, Cathepsin D, CD30 (Reed-Sternberg Cell Marker), Ubiquitin, FSH-b, TrxR2, CCK-8, Cyclin C, CD138, TGF-beta 2, Adrenocorticotrophic Hormone, PPAR-gamma, Bcl-6, GLUT-3, IGF-I, mRANKL, Fas-ligand, Filamin, Calretinin, O ct-1, Parathyroid Hormone, Claudin 5, Claudin 4, Raf-1 (Phospho-specific), CDC14A Phosphatase, Mitochondria, APC, Gastrin 1, Ku (p80), Gai1, XPA, Maltose Binding Protein, Melanoma (gp100), Phosphotyrosine, Amyloid A, CXCR4 / Fusin, Hepatic Nuclear Factor-3B, Caspase 1, HPV 16-E7, Axonal Growth Cones, Lck, Ornithine Decarboxylase, Gamma Glutamylcysteine Synthetase(GCS)/Glutamate-cysteine Ligase, ERCC1, Calmodulin, Caspase 7 (Mch 3), CD137 (4-1BB), Nitric Oxide Synthase, brain (bNOS), E2F-2, IL-10R, L-Plastin, CD18, Vimentin, CD50/ICAM-3, Superoxide Dismutase, Adenovirus Type 5

	E1A, PHAS-I, Progesterone Receptor (phospho-specific) - Serine 294, MHC II (HLA-DQ), XPG, ER Ca+2 ATPase2, Laminin-s, E3-binding protein (ARM1), CD45RO, CD1, Cdk2, MMP-10 (Stromilysin-2), sm, Surfactant Protein B (Pro), Apolipoprotein D, CD46, Keratin 8 (phospho-specific Ser73), PCNA, PLAP, CD20, Syk, LH, Keratin 19, ADP-ribosylation Factor (ARF-6), Int-2 Oncoprotein, Luciferase, AIF (Apoptosis Inducing Factor), Grb2, bcl-X, CD16, Paxillin, MHC II (HLA-DP and DR), B-Cell, p21WAF1, MHC II (HLA-DR), Tyrosinase, E2F-1, Pds1, Calponin, Notch, CD26/DPP IV, SV40 Large T Antigen, Ku (p70/p80), Perforin, XPF, SIM Ag (SIMA-4D3), Cdk1/p34cdc2, Neuron Specific Enolase, b-2-Microglobulin, DNA Polymerase Beta, Thyroid Hormone Receptor, Human, Alkaline Phosphatase (AP), Plasma Cell Marker, Heat Shock Protein 70/hsp70, TRP75 / gp75, SRF (Serum Response Factor), Laminin B1/b1, Mast Cell Chymase, Caldesmon, CEA / CD66e, CD24, Retinoid X Receptor (hRXR), CD45/T200/LCA, Rabies Virus, Cytochrome c, DR3, bcl-XL, Fascin, CD71 / Transferrin Receptor
Lung Cancer	miR-497
Lung Cancer	Pgrmc1
Ovarian Cancer	CA-125, CA 19-9, c-reactive protein, CD95(also called Fas, Fas antigen, Fas receptor, FasR, TNFRSF6, APT1 or APO-1), FAP-1, miR-200 microRNAs, EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rab13, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8, HLA-DR. MiR-200 microRNAs (miR-200a, miR-200b, miR-200c), miR-141, miR-429, JNK, Jun
Prostate Cancer v normal	AQP2, BMP5, C16orf86, CXCL13, DST, ERCC1, GNAO1, KLHL5, MAP4K1, NELL2, PENK, PGF, POU3F1, PRSS21, SCML1, SEMG1, SMARCD3, SNAI2, TAF1C, TNNT3
Prostate Cancer v Breast Cancer	ADRB2, ARG2, C22orf32, CYorf14, EIF1AY, FEV, KLK2, KLK4, LRRC26, MAOA, NLGN4Y, PNPLA7, PVRL3, SIM2, SLC30A4, SLC45A3, STX19, TRIM36, TRPM8
Prostate Cancer v Colorectal Cancer	ADRB2, BAIAP2L2, C19orf33, CDX1, CEACAM6, EEF1A2, ERN2, FAM110B, FOXA2, KLK2, KLK4, LOC389816, LRRC26, MIPOL1, SLC45A3, SPDEF, TRIM31, TRIM36, ZNF613
Prostate Cancer v Lung Cancer	ASTN2, CAB39L, CRIP1, FAM110B, FEV, GSTP1, KLK2, KLK4, LOC389816, LRRC26, MUC1, PNPLA7, SIM2, SLC45A3, SPDEF, TRIM36, TRPV6, ZNF613
Prostate Cancer	miRs-26a+b, miR-15, miR-16, miR-195, miR-497, miR-424, miR-206, miR-342-5p, miR-186, miR-1271, miR-600, miR-216b, miR-519 family, miR-203
Integrins	ITGA1 (CD49a, VLA1), ITGA2 (CD49b, VLA2), ITGA3 (CD49c, VLA3), ITGA4 (CD49d, VLA4), ITGA5 (CD49e, VLA5), ITGA6 (CD49f, VLA6), ITGA7 (FLJ25220), ITGA8, ITGA9 (RLC), ITGA10, ITGA11 (HsT18964), ITGAD (CD11D, FLJ39841), ITGAE (CD103, HUMINAE), ITGAL (CD11a, LFA1A), ITGAM (CD11b, MAC-1), ITGAV (CD51, VNRA, MSK8), ITGAW, ITGAX (CD11c), ITGB1 (CD29, FNRB, MSK12, MDF20), ITGB2 (CD18, LFA-1, MAC-1, MFI7), ITGB3 (CD61, GP3A, GPIIIa), ITGB4 (CD104), ITGB5 (FLJ26658), ITGB6, ITGB7, ITGB8
Glycoprotein	GpIa-IIa, GpIIb-IIIa, GpIIIb, GpIb, GpIX
Transcription factors	STAT3, EZH2, p53, MACC1, SPDEF, RUNX2, YB-1
Kinases	AURKA, AURKB
Disease Markers	6Ckine, Adiponectin, Adrenocorticotropic Hormone, Agouti-Related Protein, Aldose Reductase, Alpha-1-Antichymotrypsin, Alpha-1-Antitrypsin, Alpha-1-Microglobulin, Alpha-2-Macroglobulin, Alpha-Fetoprotein, Amphiregulin, Angiogenin, Angiopoietin-2, Angiotensin-Converting Enzyme, Angiotensinogen, Annexin A1, Apolipoprotein A-I, Apolipoprotein A-II, Apolipoprotein A-IV, Apolipoprotein B, Apolipoprotein C-I, Apolipoprotein C-III, Apolipoprotein D, Apolipoprotein E, Apolipoprotein H, Apolipoprotein(a), AXL Receptor Tyrosine Kinase, B cell-activating Factor, B Lymphocyte Chemoattractant, Bcl-2-like protein 2, Beta-2-Microglobulin, Betacellulin, Bone Morphogenetic Protein 6, Brain-Derived Neurotrophic Factor, Calbindin, Calcitonin, Cancer Antigen 125, Cancer Antigen 15-3, Cancer Antigen 19-9, Cancer Antigen 72-4, Carcinoembryonic Antigen, Cathepsin D, CD 40 antigen, CD40 Ligand, CD5 Antigen-like, Cellular Fibronectin, Chemokine CC-4, Chromogranin-A, Ciliary Neurotrophic Factor, Clusterin, Collagen IV, Complement C3, Complement Factor H, Connective Tissue Growth Factor, Cortisol, C-Peptide, C-Reactive Protein, Creatine Kinase-MB, Cystatin-C, Endoglin, Endostatin, Endothelin-1, EN-RAGE, Eotaxin-1, Eotaxin-2, Eotaxin-3, Epidermal Growth Factor, Epiregulin, Epithelial cell adhesion molecule, Epithelial-Derived Neutrophil-

	Activating Protein 78, Erythropoietin, E-Selectin, Ezrin, Factor VII, Fas Ligand, FASLG Receptor, Fatty Acid-Binding Protein (adipocyte), Fatty Acid-Binding Protein (heart), Fatty Acid-Binding Protein (liver), Ferritin, Fetuin-A, Fibrinogen, Fibroblast Growth Factor 4, Fibroblast Growth Factor basic, Fibulin-1C, Follicle-Stimulating Hormone, Galectin-3, Gelsolin, Glucagon, Glucagon-like Peptide 1, Glucose-6-phosphate Isomerase, Glutamate-Cysteine Ligase Regulatory subunit, Glutathione S-Transferase alpha, Glutathione S-Transferase Mu 1, Granulocyte Colony-Stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Growth Hormone, Growth-Regulated alpha protein, Haptoglobin, HE4, Heat Shock Protein 60, Heparin-Binding EGF-Like Growth Factor, Hepatocyte Growth Factor, Hepatocyte Growth Factor Receptor, Hepsin, Human Chorionic Gonadotropin beta, Human Epidermal Growth Factor Receptor 2, Immunoglobulin A, Immunoglobulin E, Immunoglobulin M, Insulin, Insulin-like Growth Factor I, Insulin-like Growth Factor-Binding Protein 1, Insulin-like Growth Factor-Binding Protein 2, Insulin-like Growth Factor-Binding Protein 3, Insulin-like Growth Factor Binding Protein 4, Insulin-like Growth Factor Binding Protein 5, Insulin-like Growth Factor Binding Protein 6, Intercellular Adhesion Molecule 1, Interferon gamma, Interferon gamma Induced Protein 10, Interferon-inducible T-cell alpha chemoattractant, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-2, Interleukin-2 Receptor alpha, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-6 Receptor, Interleukin-6 Receptor subunit beta, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-11, Interleukin-12 Subunit p40, Interleukin-12 Subunit p70, Interleukin-13, Interleukin-15, Interleukin-16, Interleukin-25, Kallikrein 5, Kallikrein-7, Kidney Injury Molecule-1, Lactoylglutathione lyase, Latency-Associated Peptide of Transforming Growth Factor beta 1, Lectin-Like Oxidized LDL Receptor 1, Leptin, Luteinizing Hormone, Lymphotactin, Macrophage Colony-Stimulating Factor 1, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Macrophage Inflammatory Protein-3 alpha, Macrophage inflammatory protein 3 beta, Macrophage Migration Inhibitory Factor, Macrophage-Derived Chemokine, Macrophage-Stimulating Protein, Malondialdehyde-Modified Low-Density Lipoprotein, Maspin, Matrix Metalloproteinase-1, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-7, Matrix Metalloproteinase-9, Matrix Metalloproteinase-9, Matrix Metalloproteinase-10, Mesothelin, MHC class I chain-related protein A, Monocyte Chemotactic Protein 1, Monocyte Chemotactic Protein 2, Monocyte Chemotactic Protein 3, Monocyte Chemotactic Protein 4, Monokine Induced by Gamma Interferon, Myeloid Progenitor Inhibitory Factor 1, Myeloperoxidase, Myoglobin, Nerve Growth Factor beta, Neuronal Cell Adhesion Molecule, Neuron-Specific Enolase, Neuropilin-1, Neutrophil Gelatinase-Associated Lipocalin, NT-proBNP, Nucleoside diphosphate kinase B, Osteopontin, Osteoprotegerin, Pancreatic Polypeptide, Pepsinogen I, Peptide YY, Peroxiredoxin-4, Phosphoserine Aminotransferase, Placenta Growth Factor, Plasminogen Activator Inhibitor 1, Platelet-Derived Growth Factor BB, Pregnancy-Associated Plasma Protein A, Progesterone, Proinsulin (inc. Total or Intact), Prolactin, Prostasin, Prostate-Specific Antigen (inc. Free PSA), Prostatic Acid Phosphatase, Protein S100-A4, Protein S100-A6, Pulmonary and Activation-Regulated Chemokine, Receptor for advanced glycosylation end products, Receptor tyrosine-protein kinase erbB-3, Resistin, S100 calcium-binding protein B, Secretin, Serotransferrin, Serum Amyloid P-Component, Serum Glutamic Oxaloacetic Transaminase, Sex Hormone-Binding Globulin, Sortilin, Squamous Cell Carcinoma Antigen-1, Stem Cell Factor, Stromal cell-derived Factor-1, Superoxide Dismutase 1 (soluble), T Lymphocyte-Secreted Protein I-309, Tamm-Horsfall Urinary Glycoprotein, T-Cell-Specific Protein RANTES, Tenascin-C, Testosterone, Tetranectin, Thrombomodulin, Thrombopoietin, Thrombospondin-1, Thyroglobulin, Thyroid-Stimulating Hormone, Thyroxine-Binding Globulin, Tissue Factor, Tissue Inhibitor of Metalloproteinases 1, Tissue type Plasminogen activator, TNF-Related Apoptosis-Inducing Ligand Receptor 3, Transforming Growth Factor alpha, Transforming Growth Factor beta-3, Transthyretin, Trefoil Factor 3, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor Necrosis Factor Receptor I, Tumor necrosis Factor Receptor 2, Tyrosine kinase with Ig and EGF homology domains 2, Urokinase-type Plasminogen Activator, Urokinase-type plasminogen activator Receptor, Vascular Cell Adhesion Molecule-1, Vascular Endothelial Growth Factor, Vascular endothelial growth Factor B, Vascular Endothelial Growth Factor C, Vascular endothelial growth Factor D, Vascular Endothelial Growth Factor Receptor 1, Vascular Endothelial Growth Factor Receptor 2, Vascular endothelial growth Factor

	Receptor 3, Vitamin K-Dependent Protein S, Vitronectin, von Willebrand Factor, YKL-40
Disease Markers	Adiponectin, Adrenocorticotropic Hormone, Agouti-Related Protein, Alpha-1-Antichymotrypsin, Alpha-1-Antitrypsin, Alpha-1-Microglobulin, Alpha-2-Macroglobulin, Alpha-Fetoprotein, Amphiregulin, Angiopoietin-2, Angiotensin-Converting Enzyme, Angiotensinogen, Apolipoprotein A-I, Apolipoprotein A-II, Apolipoprotein A-IV, Apolipoprotein B, Apolipoprotein C-I, Apolipoprotein C-III, Apolipoprotein D, Apolipoprotein E, Apolipoprotein H, Apolipoprotein(a), AXL Receptor Tyrosine Kinase, B Lymphocyte Chemoattractant, Beta-2-Microglobulin, Betacellulin, Bone Morphogenetic Protein 6, Brain-Derived Neurotrophic Factor, Calbindin, Calcitonin, Cancer Antigen 125, Cancer Antigen 19-9, Carcinoembryonic Antigen, CD 40 antigen, CD40 Ligand, CD5 Antigen-like, Chemokine CC-4, Chromogranin-A, Ciliary Neurotrophic Factor, Clusterin, Complement C3, Complement Factor H, Connective Tissue Growth Factor, Cortisol, C-Peptide, C-Reactive Protein, Creatine Kinase-MB, Cystatin-C, Endothelin-1, EN-RAGE, Eotaxin-1, Eotaxin-3, Epidermal Growth Factor, Epiregulin, Epithelial-Derived Neutrophil-Activating Protein 78, Erythropoietin, E-Selectin, Factor VII, Fas Ligand, FASLG Receptor, Fatty Acid-Binding Protein (heart), Ferritin, Fetuin-A, Fibrinogen, Fibroblast Growth Factor 4, Fibroblast Growth Factor basic, Follicle-Stimulating Hormone, Glucagon, Glucagon-like Peptide 1, Glutathione S-Transferase alpha, Granulocyte Colony-Stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Growth Hormone, Growth-Regulated alpha protein, Haptoglobin, Heat Shock Protein 60, Heparin-Binding EGF-Like Growth Factor, Hepatocyte Growth Factor, Immunoglobulin A, Immunoglobulin E, Immunoglobulin M, Insulin, Insulin-like Growth Factor I, Insulin-like Growth Factor-Binding Protein 2, Intercellular Adhesion Molecule 1, Interferon gamma, Interferon gamma Induced Protein 10, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-6 Receptor, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-11, Interleukin-12 Subunit p40, Interleukin-12 Subunit p70, Interleukin-13, Interleukin-15, Interleukin-16, Interleukin-25, Kidney Injury Molecule-1, Lectin-Like Oxidized LDL Receptor 1, Leptin, Luteinizing Hormone, Lymphotactin, Macrophage Colony-Stimulating Factor 1, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Macrophage Inflammatory Protein-3 alpha, Macrophage Migration Inhibitory Factor, Macrophage-Derived Chemokine, Malondialdehyde-Modified Low-Density Lipoprotein, Matrix Metalloproteinase-1, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-7, Matrix Metalloproteinase-9, Matrix Metalloproteinase-9, Matrix Metalloproteinase-10, Monocyte Chemotactic Protein 1, Monocyte Chemotactic Protein 2, Monocyte Chemotactic Protein 3, Monocyte Chemotactic Protein 4, Monokine Induced by Gamma Interferon, Myeloid Progenitor Inhibitory Factor 1, Myeloperoxidase, Myoglobin, Nerve Growth Factor beta, Neuronal Cell Adhesion Molecule, Neutrophil Gelatinase-Associated Lipocalin, NT-proBNP, Osteopontin, Pancreatic Polypeptide, Peptide YY, Placenta Growth Factor, Plasminogen Activator Inhibitor 1, Platelet-Derived Growth Factor BB, Pregnancy-Associated Plasma Protein A, Progesterone, Proinsulin (inc. Intact or Total), Prolactin, Prostate-Specific Antigen (inc. Free PSA), Prostatic Acid Phosphatase, Pulmonary and Activation-Regulated Chemokine, Receptor for advanced glycosylation end products, Resistin, S100 calcium-binding protein B, Secretin, Serotransferrin, Serum Amyloid P-Component, Serum Glutamic Oxaloacetic Transaminase, Sex Hormone-Binding Globulin, Sortilin, Stem Cell Factor, Superoxide Dismutase 1 (soluble), T Lymphocyte-Secreted Protein I-309, Tamm-Horsfall Urinary Glycoprotein, T-Cell-Specific Protein RANTES, Tenascin-C, Testosterone, Thrombomodulin, Thrombopoietin, Thrombospondin-1, Thyroid-Stimulating Hormone, Thyroxine-Binding Globulin, Tissue Factor, Tissue Inhibitor of Metalloproteinases 1, TNF-Related Apoptosis-Inducing Ligand Receptor 3, Transforming Growth Factor alpha, Transforming Growth Factor beta-3, Transthyretin, Trefoil Factor 3, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor necrosis Factor Receptor 2, Vascular Cell Adhesion Molecule-1, Vascular Endothelial Growth Factor, Vitamin K-Dependent Protein S, Vitronectin, von Willebrand Factor
Oncology	6Ckine, Aldose Reductase, Alpha-Fetoprotein, Amphiregulin, Angiogenin, Annexin A1, B cell-activating Factor, B Lymphocyte Chemoattractant, Bcl-2-like protein 2, Betacellulin, Cancer Antigen 125, Cancer Antigen 15-3, Cancer Antigen 19-9, Cancer Antigen 72-4, Carcinoembryonic Antigen, Cathepsin D, Cellular Fibronectin, Collagen IV, Endoglin, Endostatin, Eotaxin-2, Epidermal Growth Factor, Epiregulin, Epithelial cell adhesion molecule, Ezrin, Fatty Acid-Binding Protein (adipocyte), Fatty Acid-Binding Protein (liver),

	Fibroblast Growth Factor basic, Fibulin-1C, Galectin-3, Gelsolin, Glucose-6-phosphate Isomerase, Glutamate-Cysteine Ligase Regulatory subunit, Glutathione S-Transferase Mu 1, HE4, Heparin-Binding EGF-Like Growth Factor, Hepatocyte Growth Factor, Hepatocyte Growth Factor Receptor, Hepsin, Human Chorionic Gonadotropin beta, Human Epidermal Growth Factor Receptor 2, Insulin-like Growth Factor-Binding Protein 1, Insulin-like Growth Factor-Binding Protein 2, Insulin-like Growth Factor-Binding Protein 3, Insulin-like Growth Factor Binding Protein 4, Insulin-like Growth Factor Binding Protein 5, Insulin-like Growth Factor Binding Protein 6, Interferon gamma Induced Protein 10, Interferon-inducible T-cell alpha chemoattractant, Interleukin-2 Receptor alpha, Interleukin-6, Interleukin-6 Receptor subunit beta, Kallikrein 5, Kallikrein-7, Lactoylglutathione lyase, Latency-Associated Peptide of Transforming Growth Factor beta 1, Leptin, Macrophage inflammatory protein 3 beta, Macrophage Migration Inhibitory Factor, Macrophage-Stimulating Protein, Maspin, Matrix Metalloproteinase-2, Mesothelin, MHC class I chain-related protein A, Monocyte Chemotactic Protein 1, Monokine Induced by Gamma Interferon, Neuron-Specific Enolase, Neuropilin-1, Neutrophil Gelatinase-Associated Lipocalin, Nucleoside diphosphate kinase B, Osteopontin, Osteoprotegerin, Pepsinogen I, Peroxiredoxin-4, Phosphoserine Aminotransferase, Placenta Growth Factor, Platelet-Derived Growth Factor BB, Prostasin, Protein S100-A4, Protein S100-A6, Receptor tyrosine-protein kinase erbB-3, Squamous Cell Carcinoma Antigen-1, Stromal cell-derived Factor-1, Tenascin-C, Tetranectin, Thyroglobulin, Tissue type Plasminogen activator, Transforming Growth Factor alpha, Tumor Necrosis Factor Receptor I, Tyrosine kinase with Ig and EGF homology domains 2, Urokinase-type Plasminogen Activator, Urokinase-type plasminogen activator Receptor, Vascular Endothelial Growth Factor, Vascular endothelial growth Factor B, Vascular Endothelial Growth Factor C, Vascular endothelial growth Factor D, Vascular Endothelial Growth Factor Receptor 1, Vascular Endothelial Growth Factor Receptor 2, Vascular endothelial growth Factor Receptor 3, YKL-40
Disease	Adiponectin, Alpha-1-Antitrypsin, Alpha-2-Macroglobulin, Alpha-Fetoprotein, Apolipoprotein A-I, Apolipoprotein C-III, Apolipoprotein H, Apolipoprotein(a), Beta-2-Microglobulin, Brain-Derived Neurotrophic Factor, Calcitonin, Cancer Antigen 125, Cancer Antigen 19-9, Carcinoembryonic Antigen, CD 40 antigen, CD40 Ligand, Complement C3, C-Reactive Protein, Creatine Kinase-MB, Endothelin-1, EN-RAGE, Eotaxin-1, Epidermal Growth Factor, Epithelial-Derived Neutrophil-Activating Protein 78, Erythropoietin, Factor VII, Fatty Acid-Binding Protein (heart), Ferritin, Fibrinogen, Fibroblast Growth Factor basic, Granulocyte Colony-Stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Growth Hormone, Haptoglobin, Immunoglobulin A, Immunoglobulin E, Immunoglobulin M, Insulin, Insulin-like Growth Factor I, Intercellular Adhesion Molecule 1, Interferon gamma, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-12 Subunit p40, Interleukin-12 Subunit p70, Interleukin-13, Interleukin-15, Interleukin-16, Leptin, Lymphotactin, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Macrophage-Derived Chemokine, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-9, Monocyte Chemotactic Protein 1, Myeloperoxidase, Myoglobin, Plasminogen Activator Inhibitor 1, Pregnancy-Associated Plasma Protein A, Prostate-Specific Antigen (inc. Free PSA), Prostatic Acid Phosphatase, Serum Amyloid P-Component, Serum Glutamic Oxaloacetic Transaminase, Sex Hormone-Binding Globulin, Stem Cell Factor, T-Cell-Specific Protein RANTES, Thrombopoietin, Thyroid-Stimulating Hormone, Thyroxine-Binding Globulin, Tissue Factor, Tissue Inhibitor of Metalloproteinases 1, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor Necrosis Factor Receptor 2, Vascular Cell Adhesion Molecule-1, Vascular Endothelial Growth Factor, von Willebrand Factor
Neurological	Alpha-1-Antitrypsin, Apolipoprotein A-I, Apolipoprotein A-II, Apolipoprotein B, Apolipoprotein C-I, Apolipoprotein H, Beta-2-Microglobulin, Betacellulin, Brain-Derived Neurotrophic Factor, Calbindin, Cancer Antigen 125, Carcinoembryonic Antigen, CD5 Antigen-like, Complement C3, Connective Tissue Growth Factor, Cortisol, Endothelin-1, Epidermal Growth Factor Receptor, Ferritin, Fetuin-A, Follicle-Stimulating Hormone, Haptoglobin, Immunoglobulin A, Immunoglobulin M, Intercellular Adhesion Molecule 1, Interleukin-6 Receptor, Interleukin-7, Interleukin-10, Interleukin-11, Interleukin-17, Kidney Injury Molecule-1, Luteinizing Hormone, Macrophage-Derived Chemokine, Macrophage Migration Inhibitory Factor, Macrophage Inflammatory Protein-1 alpha, Matrix

	Metalloproteinase-2, Monocyte Chemotactic Protein 2, Peptide YY, Prolactin, Prostatic Acid Phosphatase, Serotransferrin, Serum Amyloid P-Component, Sortilin, Testosterone, Thrombopoietin, Thyroid-Stimulating Hormone, Tissue Inhibitor of Metalloproteinases 1, TNF-Related Apoptosis-Inducing Ligand Receptor 3, Tumor necrosis Factor Receptor 2, Vascular Endothelial Growth Factor, Vitronectin
Cardiovascular	Adiponectin, Apolipoprotein A-I, Apolipoprotein B, Apolipoprotein C-III, Apolipoprotein D, Apolipoprotein E, Apolipoprotein H, Apolipoprotein(a), Clusterin, C-Reactive Protein, Cystatin-C, EN-RAGE, E-Selectin, Fatty Acid-Binding Protein (heart), Ferritin, Fibrinogen, Haptoglobin, Immunoglobulin M, Intercellular Adhesion Molecule 1, Interleukin-6, Interleukin-8, Lectin-Like Oxidized LDL Receptor 1, Leptin, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Malondialdehyde-Modified Low-Density Lipoprotein, Matrix Metalloproteinase-1, Matrix Metalloproteinase-10, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-7, Matrix Metalloproteinase-9, Monocyte Chemotactic Protein 1, Myeloperoxidase, Myoglobin, NT-proBNP, Osteopontin, Plasminogen Activator Inhibitor 1, P-Selectin, Receptor for advanced glycosylation end products, Serum Amyloid P-Component, Sex Hormone-Binding Globulin, T-Cell-Specific Protein RANTES, Thrombomodulin, Thyroxine-Binding Globulin, Tissue Inhibitor of Metalloproteinases 1, Tumor Necrosis Factor alpha, Tumor necrosis Factor Receptor 2, Vascular Cell Adhesion Molecule-1, von Willebrand Factor
Inflammatory	Alpha-1-Antitrypsin, Alpha-2-Macroglobulin, Beta-2-Microglobulin, Brain-Derived Neurotrophic Factor, Complement C3, C-Reactive Protein, Eotaxin-1, Factor VII, Ferritin, Fibrinogen, Granulocyte-Macrophage Colony-Stimulating Factor, Haptoglobin, Intercellular Adhesion Molecule 1, Interferon gamma, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-12 Subunit p40, Interleukin-12 Subunit p70, Interleukin-15, Interleukin-17, Interleukin-23, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Matrix Metalloproteinase-2, Matrix Metalloproteinase-3, Matrix Metalloproteinase-9, Monocyte Chemotactic Protein 1, Stem Cell Factor, T-Cell-Specific Protein RANTES, Tissue Inhibitor of Metalloproteinases 1, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor necrosis Factor Receptor 2, Vascular Cell Adhesion Molecule-1, Vascular Endothelial Growth Factor, Vitamin D-Binding Protein, von Willebrand Factor
Metabolic	Adiponectin, Adrenocorticotropic Hormone, Angiotensin-Converting Enzyme, Angiotensinogen, Complement C3 alpha des arg, Cortisol, Follicle-Stimulating Hormone, Galanin, Glucagon, Glucagon-like Peptide 1, Insulin, Insulin-like Growth Factor I, Leptin, Luteinizing Hormone, Pancreatic Polypeptide, Peptide YY, Progesterone, Prolactin, Resistin, Secretin, Testosterone
Kidney	Alpha-1-Microglobulin, Beta-2-Microglobulin, Calbindin, Clusterin, Connective Tissue Growth Factor, Creatinine, Cystatin-C, Glutathione S-Transferase alpha, Kidney Injury Molecule-1, Microalbumin, Neutrophil Gelatinase-Associated Lipocalin, Osteopontin, Tamm-Horsfall Urinary Glycoprotein, Tissue Inhibitor of Metalloproteinases 1, Trefoil Factor 3, Vascular Endothelial Growth Factor
Cytokines	Granulocyte-Macrophage Colony-Stimulating Factor, Interferon gamma, Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-7, Interleukin-8, Interleukin-10, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Matrix Metalloproteinase-2, Monocyte Chemotactic Protein 1, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Brain-Derived Neurotrophic Factor, Eotaxin-1, Intercellular Adhesion Molecule 1, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 Receptor antagonist, Interleukin-12 Subunit p40, Interleukin-12 Subunit p70, Interleukin-15, Interleukin-17, Interleukin-23, Matrix Metalloproteinase-3, Stem Cell Factor, Vascular Endothelial Growth Factor
Protein	14.3.3 gamma, 14.3.3 (Pan), 14-3-3 beta, 6-Histidine, a-B-Crystallin, Acinus, Actin beta, Actin (Muscle Specific), Actin (Pan), Actin (skeletal muscle), Activin Receptor Type II, Adenovirus, Adenovirus Fiber, Adenovirus Type 2 E1A, Adenovirus Type 5 E1A, ADP-ribosylation Factor (ARF-6), Adrenocorticotrophic Hormone, AIF (Apoptosis Inducing Factor), Alkaline Phosphatase (AP), Alpha Fetoprotein (AFP), Alpha Lactalbumin, alpha-1-antichymotrypsin, alpha-1-antitrypsin, Amphiregulin, Amylin Peptide, Amyloid A, Amyloid A4 Protein Precursor, Amyloid Beta (APP), Androgen Receptor, Ang-1, Ang-2, APC, APC11, APC2, Apolipoprotein D, A-Raf, ARC, Ask1 / MAPKKK5, ATM, Axonal Growth Cones, b Galactosidase, b-2-Microglobulin, B7-H2, BAG-1, Bak, Bax, B-Cell, B-cell

	Linker Protein (BLNK), Bcl10 / CIPER / CLAP / mE10, bcl-2a, Bcl-6, bcl-X, bcl-XL, Bim (BOD), Biotin, Bonzo / STRL33 / TYMSTR, Bovine Serum Albumin, BRCA2 (aa 1323-1346), BrdU, Bromodeoxyuridine (BrdU), CA125, CA19-9, c-Abl, Cadherin (Pan), Cadherin-E, Cadherin-P, Calcitonin, Calcium Pump ATPase, Caldesmon, Calmodulin, Calponin, Calretinin, Casein, Caspase 1, Caspase 2, Caspase 3, Caspase 5, Caspase 6 (Mch 2), Caspase 7 (Mch 3), Caspase 8 (FLICE), Caspase 9, Catenin alpha, Catenin beta, Catenin gamma, Cathepsin D, CCK-8, CD1, CD10, CD100/Leukocyte Semaphorin, CD105, CD106 / VCAM, CD115/c-fms/CSF-1R/M-CSFR, CD137 (4-1BB), CD138, CD14, CD15, CD155/PVR (Polio Virus Receptor), CD16, CD165, CD18, CD1a, CD1b, CD2, CD20, CD21, CD23, CD231, CD24, CD25/IL-2 Receptor a, CD26/DPP IV, CD29, CD30 (Reed-Sternberg Cell Marker), CD32/Fcg Receptor II, CD35/CR1, CD36GPIIIb/GPIV, CD3zeta, CD4, CD40, CD42b, CD43, CD45/T200/LCA, CD45RB, CD45RO, CD46, CD5, CD50/ICAM-3, CD53, CD54/ICAM-1, CD56/NCAM-1, CD57, CD59 / MACIF / MIRL / Protectin, CD6, CD61 / Platelet Glycoprotein IIIA, CD63, CD68, CD71 / Transferrin Receptor, CD79a mb-1, CD79b, CD8, CD81/TAPA-1, CD84, CD9, CD94, CD95 / Fas, CD98, CDC14A Phosphatase, CDC25C, CDC34, CDC37, CDC47, CDC6, cdh1, Cdk1/p34cdc2, Cdk2, Cdk3, Cdk4, Cdk5, Cdk7, Cdk8, CDw17, CDw60, CDw75, CDw78, CEA / CD66e, c-erbB-2/HER-2/neu Ab-1 (21N), c-erbB-4/HER-4, c-fos, Chk1, Chorionic Gonadotropin beta (hCG-beta), Chromogranin A, CIDE-A, CIDE-B, CITED1, c-jun, Clathrin, claudin 11, Claudin 2, Claudin 3, Claudin 4, Claudin 5, CLAUDIN 7, Claudin-1, CNPase, Collagen II, Collagen IV, Collagen IX, Collagen VII, Connexin 43, COX2, CREB, CREB-Binding Protein, Cryptococcus neoformans, c-Src, Cullin-1 (CUL-1), Cullin-2 (CUL-2), Cullin-3 (CUL-3), CXCR4 / Fusin, Cyclin B1, Cyclin C, Cyclin D1, Cyclin D3, Cyclin E, Cyclin E2, Cystic Fibrosis Transmembrane Regulator, Cytochrome c, D4-GDI, Daxx, DcR1, DcR2 / TRAIL-R4 / TRUNDD, Desmin, DFF40 (DNA Fragmentation Factor 40) / CAD, DFF45 / ICAD, DJ-1, DNA Ligase I, DNA Polymerase Beta, DNA Polymerase Gamma, DNA Primase (p49), DNA Primase (p58), DNA-PKcs, DP-2, DR3, DR5, Dysferlin, Dystrophin, E2F-1, E2F-2, E2F-3, E2F-4, E2F-5, E3-binding protein (ARM1), EGFR, EMA/CA15-3/MUC-1, Endostatin, Epithelial Membrane Antigen (EMA / CA15-3 / MUC-1), Epithelial Specific Antigen, ER beta, ER Ca+2 ATPase2, ERCC1, Erk1, ERK2, Estradiol, Estriol, Estrogen Receptor, Exo1, Ezrin/p81/80K/Cytovillin, F.VIII/VWF, Factor VIII Related Antigen, FADD (FAS-Associated death domain-containing protein), Fascin, Fas-ligand, Ferritin, FGF-1, FGF-2, FHIT, Fibrillin-1, Fibronectin, Filaggrin, Filamin, FITC, Fli-1, FLIP, Flk-1 / KDR / VEGFR2, Flt-1 / VEGFR1, Flt-4, Fra2, FSH, FSH-b, Fyn, Ga0, Gab-1, GABA a Receptor 1, GAD65, Gai1, Gamma Glutamyl Transferase (gGT), Gamma Glutamylcysteine Synthetase(GCS)/Glutamate-cysteine Ligase, GAPDH, Gastrin 1, GCDFP-15, G-CSF, GFAP, Glicentin, Glucagon, Glucose-Regulated Protein 94, GluR 2/3, GluR1, GluR4, GluR6/7, GLUT-1, GLUT-3, Glycogen Synthase Kinase 3b (GSK3b), Glycophorin A, GM-CSF, GnRH Receptor, Golgi Complex, Granulocyte, Granzyme B, Grb2, Green Fluorescent Protein (GFP), GRIP1, Growth Hormone (hGH), GSK-3, GST, GSTmu, H.Pylori, HDAC1, HDJ-2/DNAJ, Heat Shock Factor 1, Heat Shock Factor 2, Heat Shock Protein 27/hsp27, Heat Shock Protein 60/hsp60, Heat Shock Protein 70/hsp70, Heat Shock Protein 75/hsp75, Heat Shock Protein 90a/hsp86, Heat Shock Protein 90b/hsp84, Helicobacter pylori, Heparan Sulfate Proteoglycan, Hepatic Nuclear Factor-3B, Hepatocyte, Hepatocyte Factor Homologue-4, Hepatocyte Growth Factor, Heregulin, HIF-1a, Histone H1, hPL, HPV 16, HPV 16-E7, HRP, Human Sodium Iodide Symporter (hNIS), I-FLICE / CASPER, IFN gamma, IgA, IGF-1R, IGF-I, IgG, IgM (m-Heavy Chain), I-Kappa-B Kinase b (IKKb), IL-1 alpha, IL-1 beta, IL-10, IL-10R, IL17, IL-2, IL-3, IL-30, IL-4, IL-5, IL-6, IL-8, Inhibin alpha, Insulin, Insulin Receptor, Insulin Receptor Substrate-1, Int-2 Oncoprotein, Integrin beta5, Interferon-a(II), Interferon-g, Involucrin, IP10/CRG2, IPO-38 Proliferation Marker, IRAK, ITK, JNK Activating kinase (JKK1), Kappa Light Chain, Keratin 10, Keratin 10/13, Keratin 14, Keratin 15, Keratin 16, Keratin 18, Keratin 19, Keratin 20, Keratin 5/6/18, Keratin 5/8, Keratin 8, Keratin 8 (phospho-specific Ser73), Keratin 8/18, Keratin (LMW), Keratin (Multi), Keratin (Pan), Ki67, Ku (p70/p80), Ku (p80), L1 Cell Adhesion Molecule, Lambda Light Chain, Laminin B1/b1, Laminin B2/g1, Laminin Receptor, Laminin-s, Lck, Lck (p56lck), Leukotriene (C4, D4, E4), LewisA, LewisB, LH, L-Plastin, LRP / MVP, Luciferase, Macrophage, MADD, MAGE-1, Maltose Binding Protein, MAP1B, MAP2a,b, MART-1/Melan-A, Mast Cell Chymase, Mcl-1, MCM2, MCM5, MDM2, Medroxyprogesterone Acetate (MPA), Mek1, Mek2, Mek6, Mekk-1, Melanoma (gp100), mGluR1, mGluR5, MGMT, MHC I (HLA25 and HLA-

	Aw32), MHC I (HLA-A), MHC I (HLA-A,B,C), MHC I (HLA-B), MHC II (HLA-DP and DR), MHC II (HLA-DP), MHC II (HLA-DQ), MHC II (HLA-DR), MHC II (HLA-DR) Ia, Microphthalmia, Milk Fat Globule Membrane Protein, Mitochondria, MLH1, MMP-1 (Collagenase-I), MMP-10 (Stromilysin-2), MMP-11 (Stromelysin-3), MMP-13 (Collagenase-3), MMP-14 / MT1-MMP, MMP-15 / MT2-MMP, MMP-16 / MT3-MMP, MMP-19, MMP-2 (72kDa Collagenase IV), MMP-23, MMP-7 (Matrilysin), MMP-9 (92kDa Collagenase IV), Moesin, mRANKL, Muc-1, Mucin 2, Mucin 3 (MUC3), Mucin 5AC, MyD88, Myelin / Oligodendrocyte, Myeloid Specific Marker, Myeloperoxidase, MyoD1, Myogenin, Myoglobin, Myosin Smooth Muscle Heavy Chain, Nck, Negative Control for Mouse IgG1, Negative Control for Mouse IgG2a, Negative Control for Mouse IgG3, Negative Control for Mouse IgM, Negative Control for Rabbit IgG, Neurofilament, Neurofilament (160kDa), Neurofilament (200kDa), Neurofilament (68kDa), Neuron Specific Enolase, Neutrophil Elastase, NF kappa B / p50, NF kappa B / p65 (Rel A), NGF-Receptor (p75NGFR), brain Nitric Oxide Synthase (bNOS), endothelial Nitric Oxide Synthase (eNOS), nm23, NOS-i, NOS-u, Notch, Nucleophosmin (NPM), NuMA, O ct-1, Oct-2/, Oct-3/, Ornithine Decarboxylase, Osteopontin, p130, p130cas, p14ARF, p15INK4b, p16INK4a, p170, p170 / MDR-1, p18INK4c, p19ARF, p19Skp1, p21WAF1, p27Kip1, p300 / CBP, p35nck5a, P504S, p53, p57Kip2 Ab-7, p63 (p53 Family Member), p73, p73a, p73a/b, p95VAV, Parathyroid Hormone, Parathyroid Hormone Receptor Type 1, Parkin, PARP, PARP (Poly ADP-Ribose Polymerase), Pax-5, Paxillin, PCNA, PCTAIRE2, PDGF, PDGFR alpha, PDGFR beta, Pds1, Perforin, PGP9.5, PHAS-I, PHAS-II, Phospho-Ser/Thr/Tyr, Phosphotyrosine, PLAP, Plasma Cell Marker, Plasminogen, PLC gamma 1, PMP-22, Pneumocystis jiroveci, PPAR-gamma, PR3 (Proteinase 3), Presenillin, Progesterone, Progesterone Receptor, Progesterone Receptor (phospho-specific) - Serine 190, Progesterone Receptor (phospho-specific) - Serine 294, Prohibitin, Prolactin, Prolactin Receptor, Prostate Apoptosis Response Protein-4, Prostate Specific Acid Phosphatase, Prostate Specific Antigen, pS2, PSCA, Rabies Virus, RAD1, Rad51, Raf1, Raf-1 (Phospho-specific), RAIDD, Ras, Rad18, Renal Cell Carcinoma, Ret Oncoprotein, Retinoblastoma, Retinoblastoma (Rb) (Phospho-specific Serine608), Retinoic Acid Receptor (b), Retinoid X Receptor (hRXR), Retinol Binding Protein, Rhodopsin (Opsin), ROC, RPA/p32, RPA/p70, Ruv A, Ruv B, Ruv C, S100, S100A4, S100A6, SHP-1, SIM Ag (SIMA-4D3), SIRP a1, sm, SODD (Silencer of Death Domain), Somatostatin Receptor-I, SRC1 (Steroid Receptor Coactivator-1) Ab-1, SREBP-1 (Sterol Regulatory Element Binding Protein-1), SRF (Serum Response Factor), Stat-1, Stat3, Stat5, Stat5a, Stat5b, Stat6, Streptavidin, Superoxide Dismutase, Surfactant Protein A, Surfactant Protein B, Surfactant Protein B (Pro), Survivin, SV40 Large T Antigen, Syk, Synaptophysin, Synuclein, Synuclein beta, Synuclein pan, TACE (TNF-alpha converting enzyme) / ADAM17, TAG-72, tau, TdT, Tenascin, Testosterone, TGF beta 3, TGF-beta 2, Thomsen-Friedenreich Antigen, Thrombospondin, Thymidine Phosphorylase, Thymidylate Synthase, Thymine Glycols, Thyroglobulin, Thyroid Hormone Receptor beta, Thyroid Hormone Receptor, Thyroid Stimulating Hormone (TSH), TID-1, TIMP-1, TIMP-2, TNF alpha, TNFa, TNR-R2, Topo II beta, Topoisomerase IIa, Toxoplasma Gondii, TR2, TRADD, Transforming Growth Factor a, Transglutaminase II, TRAP, Tropomyosin, TRP75 / gp75, TrxR2, TTF-1, Tubulin, Tubulin-a, Tubulin-b, Tyrosinase, Ubiquitin, UCP3, uPA, Urocortin, Vacular Endothelial Growth Factor(VEGF), Vimentin, Vinculin, Vitamin D Receptor (VDR), von Hippel-Lindau Protein, Wnt-1, Xanthine Oxidase, XPA, XPF, XPG, XRCC1, XRCC2, ZAP-70, Zip kinase
Known Cancer Genes	ABL1, ABL2, ACSL3, AF15Q14, AF1Q, AF3p21, AF5q31, AKAP9, AKT1, AKT2, ALDH2, ALK, ALO17, APC, ARHGEF12, ARHH, ARID1A, ARID2, ARNT, ASPSCR1, ASXL1, ATF1, ATIC, ATM, ATRX, BAP1, BCL10, BCL11A, BCL11B, BCL2, BCL3, BCL5, BCL6, BCL7A, BCL9, BCOR, BCR, BHD, BIRC3, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRD3, BRD4, BRIP1, BTG1, BUB1B, C12orf9, C15orf21, C15orf55, C16orf75, CANT1, CARD11, CARS, CBFA2T1, CBFA2T3, CBFB, CBL, CBLB, CBLC, CCNB1IP1, CCND1, CCND2, CCND3, CCNE1, CD273, CD274, CD74, CD79A, CD79B, CDH1, CDH11, CDK12, CDK4, CDK6, CDKN2A, CDKN2a(p14), CDKN2C, CDX2, CEBPA, CEP1, CHCHD7, CHEK2, CHIC2, CHN1, CIC, CIITA, CLTC, CLTCL1, CMKOR1, COL1A1, COPEB, COX6C, CREB1, CREB3L1, CREB3L2, CREBBP, CRLF2, CRTC3, CTNNB1, CYLD, D10S170, DAXX, DDB2, DDIT3, DDX10, DDX5, DDX6, DEK, DICER1, DNMT3A, DUX4, EBF1, EGFR, EIF4A2, ELF4, ELK4, ELKS, ELL, ELN, EML4, EP300, EPS15, ERBB2, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ETV1, ETV4, ETV5, ETV6, EVI1, EWSR1, EXT1, EXT2, EZH2, FACL6, FAM22A, FAM22B,

	FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FBXO11, FBXW7, FCGR2B, FEV, FGFR1, FGFR1OP, FGFR2, FGFR3, FH, FHIT, FIP1L1, FLI1, FLJ27352, FLT3, FNBP1, FOXL2, FOXO1A, FOXO3A, FOXP1, FSTL3, FUBP1, FUS, FVT1, GAS7, GATA1, GATA2, GATA3, GMPS, GNA11, GNAQ, GNAS, GOLGA5, GOPC, GPC3, GPHN, GRAF, HCMOGT-1, HEAB, HERPUD1, HEY1, HIP1, HIST1H4I, HLF, HLXB9, HMGA1, HMGA2, HNRNPA2B1, HOOK3, HOXA11, HOXA13, HOXA9, HOXC11, HOXC13, HOXD11, HOXD13, HRAS, HRPT2, HSPCA, HSPCB, IDH1, IDH2, IGH@, IGK@, IGL@, IKZF1, IL2, IL21R, IL6ST, IL7R, IRF4, IRTA1, ITK, JAK1, JAK2, JAK3, JAZF1, JUN, KDM5A, KDM5C, KDM6A, KDR, KIAA1549, KIT, KLK2, KRAS, KTN1, LAF4, LASP1, LCK, LCP1, LCX, LHFP, LIFR, LMO1, LMO2, LPP, LYL1, MADH4, MAF, MAFB, MALT1, MAML2, MAP2K4, MDM2, MDM4, MDS1, MDS2, MECT1, MED12, MEN1, MET, MITF, MKL1, MLF1, MLH1, MLL, MLL2, MLL3, MLLT1, MLLT10, MLLT2, MLLT3, MLLT4, MLLT6, MLLT7, MN1, MPL, MSF, MSH2, MSH6, MSI2, MSN, MTCP1, MUC1, MUTYH, MYB, MYC, MYCL1, MYCN, MYD88, MYH11, MYH9, MYST4, NACA, NBS1, NCOA1, NCOA2, NCOA4, NDRG1, NF1, NF2, NFE2L2, NFIB, NFKB2, NIN, NKX2-1, NONO, NOTCH1, NOTCH2, NPM1, NR4A3, NRAS, NSD1, NTRK1, NTRK3, NUMA1, NUP214, NUP98, OLIG2, OMD, P2RY8, PAFAH1B2, PALB2, PAX3, PAX5, PAX7, PAX8, PBRM1, PBX1, PCM1, PCSK7, PDE4DIP, PDGFB, PDGFRA, PDGFRB, PER1, PHOX2B, PICALM, PIK3CA, PIK3R1, PIM1, PLAG1, PML, PMS1, PMS2, PMX1, PNUTL1, POU2AF1, POU5F1, PPARG, PPP2R1A, PRCC, PRDM1, PRDM16, PRF1, PRKAR1A, PRO1073, PSIP2, PTCH, PTEN, PTPN11, RAB5EP, RAD51L1, RAF1, RALGDS, RANBP17, RAP1GDS1, RARA, RB1, RBM15, RECQL4, REL, RET, ROS1, RPL22, RPN1, RUNDC2A, RUNX1, RUNXBP2, SBDS, SDH5, SDHB, SDHC, SDHD, SEPT6, SET, SETD2, SF3B1, SFPQ, SFRS3, SH3GL1, SIL, SLC45A3, SMARCA4, SMARCB1, SMO, SOCS1, SOX2, SRGAP3, SRSF2, SS18, SS18L1, SSH3BP1, SSX1, SSX2, SSX4, STK11, STL, SUFU, SUZ12, SYK, TAF15, TAL1, TAL2, TCEA1, TCF1, TCF12, TCF3, TCF7L2, TCL1A, TCL6, TET2, TFE3, TFEB, TFG, TFPT, TFRC, THRAP3, TIF1, TLX1, TLX3, TMPRSS2, TNFAIP3, TNFRSF14, TNFRSF17, TNFRSF6, TOP1, TP53, TPM3, TPM4, TPR, TRA@, TRB@, TRD@, TRIM27, TRIM33, TRIP11, TSC1, TSC2, TSHR, TTL, U2AF1, USP6, VHL, VTI1A, WAS, WHSC1, WHSC1L1, WIF1, WRN, WT1, WTX, XPA, XPC, XPO1, YWHAE, ZNF145, ZNF198, ZNF278, ZNF331, ZNF384, ZNF521, ZNF9, ZRSR2
Known Cancer Genes	AR, androgen receptor; ARPC1A, actin-related protein complex 2/3 subunit A; AURKA, Aurora kinase A; BAG4, BCl-2 associated anthogene 4; BCl2l2, BCl-2 like 2; BIRC2, Baculovirus IAP repeat containing protein 2; CACNA1E, calcium channel voltage dependent alpha-1E subunit; CCNE1, cyclin E1; CDK4, cyclin dependent kinase 4; CHD1L, chromodomain helicase DNA binding domain 1-like; CKS1B, CDC28 protein kinase 1B; COPS3, COP9 subunit 3; DCUN1D1, DCN1 domain containing protein 1; DYRK2, dual specificity tyrosine phosphorylation regulated kinase 2; EEF1A2, eukaryotic elongation transcription factor 1 alpha 2; EGFR, epidermal growth factor receptor; FADD, Fas-associated via death domain; FGFR1, fibroblast growth factor receptor 1, GATA6, GATA binding protein 6; GPC5, glypican 5; GRB7, growth factor receptor bound protein 7; MAP3K5, mitogen activated protein kinase kinase kinase 5; MED29, mediator complex subunit 5; MITF, microphthalmia associated transcription factor; MTDH, metadherin; NCOA3, nuclear receptor coactivator 3; NKX2-1, NK2 homeobox 1; PAK1, p21/CDC42/RAC1-activated kinase 1; PAX9, paired box gene 9; PIK3CA, phosphatidylinositol-3 kinase catalytic a; PLA2G10, phopholipase A2, group X; PPM1D, protein phosphatase magnesium-dependent 1D; PTK6, protein tyrosine kinase 6; PRKCI, protein kinase C iota; RPS6KB1, ribosomal protein s6 kinase 70kDa; SKP2, s-phase kinase associated protein; SMURF1, sMAD specific E3 ubiquitin protein ligase 1; SHH, sonic hedgehog homologue; STARD3, sTAR-related lipid transfer domain containing protein 3; YWHAQ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta isoform; ZNF217, zinc finger protein 217
Mitotic Related Cancer Genes	Aurora kinase A (AURKA); Aurora kinase B (AURKB); Baculoviral IAP repeat-containing 5, survivin (BIRC5); Budding uninhibited by benzimidazoles 1 homolog (BUB1); Budding uninhibited by benzimidazoles 1 homolog beta, BUBR1 (BUB1B); Budding uninhibited by benzimidazoles 3 homolog (BUB3); CDC28 protein kinase regulatory subunit 1B (CKS1B); CDC28 protein kinase regulatory subunit 2 (CKS2); Cell division cycle 2 (CDC2)/CDK1 Cell division cycle 20 homolog (CDC20); Cell division cycle-associated 8, borealin (CDCA8); Centromere protein F, mitosin (CENPF); Centrosomal protein 110 kDa

	(CEP110); Checkpoint with forkhead and ring finger domains (CHFR); Cyclin B1 (CCNB1); Cyclin B2 (CCNB2); Cytoskeleton-associated protein 5 (CKAP5/ch-TOG); Microtubule-associated protein RP/ EB family member 1. End-binding protein 1, EB1 (MAPRE1); Epithelial cell transforming sequence 2 oncogene (ECT2); Extra spindle poles like 1, separase (ESPL1); Forkhead box M1 (FOXM1); H2A histone family, member X (H2AFX); Kinesin family member 4A (KIF4A); Kinetochore-associated 1 (KNTC1/ROD); Kinetochore-associated 2; highly expressed in cancer 1 (KNTC2/HEC1); Large tumor suppressor, homolog 1 (LATS1); Large tumor suppressor, homolog 2 (LATS2); Mitotic arrest deficient-like 1; MAD1 (MAD1L1); Mitotic arrest deficient-like 2; MAD2 (MAD2L1); Mps1 protein kinase (TTK); Never in mitosis gene a-related kinase 2 (NEK2); Ninein, GSK3b interacting protein (NIN); Non-SMC condensin I complex, subunit D2 (NCAPD2/CNAP1); Non-SMC condensin I complex, subunit H (NACPH/CAPH); Nuclear mitotic apparatus protein 1 (NUMA1); Nucleophosmin (nucleolar phosphoprotein B23, numatrin); (NPM1); Nucleoporin (NUP98); Pericentriolar material 1 (PCM1); Pituitary tumor-transforming 1, securin (PTTG1); Polo-like kinase 1 (PLK1); Polo-like kinase 4 (PLK4/SAK); Protein (peptidylprolyl cis/trans isomerase) NIMA-interacting 1 (PIN1); Protein regulator of cytokinesis 1 (PRC1); RAD21 homolog (RAD21); Ras association (RalGDS/AF-6); domain family 1 (RASSF1); Stromal antigen 1 (STAG1); Synuclein-c, breast cancer-specific protein 1 (SNCG, BCSG1); Targeting protein for Xklp2 (TPX2); Transforming, acidic coiled-coil containing protein 3 (TACC3); Ubiquitin-conjugating enzyme E2C (UBE2C); Ubiquitin-conjugating enzyme E2I (UBE2I/UBC9); ZW10 interactor, (ZWINT); ZW10, kinetochore-associated homolog (ZW10); Zwilch, kinetochore-associated homolog (ZWILCH)
Ribonucleoprotein complexes	Argonaute family member, Ago1, Ago2, Ago3, Ago4, GW182 (TNRC6A), TNRC6B, TNRC6C, HNRNPA2B1, HNRPAB, ILF2, NCL (Nucleolin), NPM1 (Nucleophosmin), RPL10A, RPL5, RPLP1, RPS12, RPS19, SNRPG, TROVE2, apolipoprotein, apolipoprotein A, apo A-I, apo A-II, apo A-IV, apo A-V, apolipoprotein B, apo B48, apo B100, apolipoprotein C, apo C-I, apo C-II, apo C-III, apo C-IV, apolipoprotein D (ApoD), apolipoprotein E (ApoE), apolipoprotein H (ApoH), apolipoprotein L, APOL1, APOL2, APOL3, APOL4, APOL5, APOL6, APOLD1
Cytokine Receptors	4-1BB, ALCAM, B7-1, BCMA, CD14, CD30, CD40 Ligand, CEACAM-1, DR6, Dtk, Endoglin, ErbB3, E-Selectin, Fas, Flt-3L, GITR, HVEM, ICAM-3, IL-1 R4, IL-1 RI, IL-10 Rbeta, IL-17R, IL-2Rgamma, IL-21R, LIMPII, Lipocalin-2, L-Selectin, LYVE-1, MICA, MICB, NRG1-beta1, PDGF Rbeta, PECAM-1, RAGE, TIM-1, TRAIL R3, Trappin-2, uPAR, VCAM-1, XEDAR
Prostate and colorectal cancer vesicles	ErbB3, RAGE, Trail R3
Colorectal cancer vesicles	IL-1 alpha, CA125, Filamin, Amyloid A
Colorectal cancer v adenoma vesicles	Involucrin, CD57, Prohibitin, Thrombospondin, Laminin B1/b1, Filamin, 14.3.3 gamma, 14.3.3 Pan
Colorectal adenoma vesicles	Involucrin, Prohibitin, Laminin B1/b1, IL-3, Filamin, 14.3.3 gamma, 14.3.3 Pan, MMP-15 / MT2-MMP, hPL, Ubiquitin, and mRANKL
Brain cancer vesicles	Prohibitin, CD57, Filamin, CD18, b-2-Microglobulin, IL-2, IL-3, CD16, p170, Keratin 19, Pds1, Glicentin, SRF (Serum Response Factor), E3-binding protein (ARM1), Collagen II, SRC1 (Steroid Receptor Coactivator-1) Ab-1, Caldesmon, GFAP, TRP75 / gp75, alpha-1-antichymotrypsin, Hepatic Nuclear Factor-3B, PLAP, Tyrosinase, NF kappa B / p50, Melanoma (gp100), Cyclin E, 6-Histidine, Mucin 3 (MUC3), TdT, CD21, XPA, Superoxide Dismutase, Glycogen Synthase Kinase 3b (GSK3b), CD54/ICAM-1, Thrombospondin, Gai1, CD79a mb-1, IL-1 beta, Cytochrome c, RAD1, bcl-X, CD50/ICAM-3, Neurofilament, Alkaline Phosphatase (AP), ER Ca+2 ATPase2, PCNA, F.VIII/VWF, SV40 Large T Antigen, Paxillin, Fascin, CD165, GRIP1, Cdk8, Nucleophosmin (NPM), alpha-1-antitrypsin, CD32/Fcg Receptor II, Keratin 8 (phospho-specific Ser73), DR5, CD46, TID-1, MHC II (HLA-DQ), Plasma Cell Marker, DR3, Calmodulin, AIF (Apoptosis Inducing Factor), DNA Polymerase Beta, Vitamin D Receptor (VDR), Bcl10 / CIPER / CLAP / mE10, Neuron Specific Enolase, CXCR4 / Fusin, Neurofilament (68kDa), PDGFR, beta, Growth Hormone (hGH), Mast Cell Chymase, Ret Oncoprotein, and Phosphotyrosine
Melanoma vesicles	Caspase 5, Thrombospondin, Filamin, Ferritin, 14.3.3 gamma, 14.3.3 Pan, CD71 / Transferrin Receptor, and Prostate Apoptosis Response Protein-4

Head and neck cancer vesicles	14.3.3 Pan, Filamin, 14.3.3 gamma, CD71 / Transferrin Receptor, CD30, Cdk5, CD138, Thymidine Phosphorylase, Ruv 5, Thrombospondin, CD1, Von Hippel-Lindau Protein, CD46, Rad51, Ferritin, c-Abl, Actin, Muscle Specific, LewisB
Membrane proteins	carbonic anhydrase IX, B7, CCCL19, CCCL21, CSAp, HER-2/neu, BrE3, CD1, CD1a, CD2, CD3, CD4, CD5, CD8, CD11A, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD29, CD30, CD32b, CD33, CD37, CD38, CD40, CD40L, CD44, CD45, CD46, CD52, CD54, CD55, CD59, CD64, CD67, CD70, CD74, CD79a, CD80, CD83, CD95, CD126, CD133, CD138, CD147, CD154, CEACAM5, CEACAM-6, alpha-fetoprotein (AFP), VEGF, ED-B fibronectin, EGP-1, EGP-2, EGF receptor (ErbB1), ErbB2, ErbB3, Factor H, FHL-1, Flt-3, folate receptor, Ga 733,GROB, HMGB-1, hypoxia inducible factor (HIF), HM1.24, HER-2/neu, insulin-like growth factor (ILGF), IFN-γ, IFN-α, IL-β, IL-2R, IL-4R, IL-6R, IL-13R, IL-15R, IL-17R, IL-18R, IL-2, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, IL-25, IP-10, IGF-1R, Ia, HM1.24, gangliosides, HCG, HLA-DR, CD66a-d, MAGE, mCRP, MCP-1, MIP-1A, MIP-1B, macrophage migration-inhibitory factor (MIF), MUC1, MUC2, MUC3, MUC4, MUC5, placental growth factor (P1GF), PSA (prostate-specific antigen), PSMA, PSMA dimer, PAM4 antigen, NCA-95, NCA-90, A3, A33, Ep-CAM, KS-1, Le(y), mesothelin, S100, tenascin, TAC, Tn antigen, Thomas-Friedenreich antigens, tumor necrosis antigens, tumor angiogenesis antigens, TNF-α, TRAIL receptor (R1 and R2), VEGFR, RANTES, T101, cancer stem cell antigens, complement factors C3, C3a, C3b, C5a, C5
Cluster of Differentiation (CD) proteins	CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD12w, CD13, CD14, CD15, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD61, CD62E, CD62L, CD62P, CD63, CD68, CD69, CD71, CD72, CD73, CD74, CD80, CD81, CD82, CD83, CD86, CD87, CD88, CD89, CD90, CD91, CD95, CD96, CD100, CD103, CD105, CD106, CD107, CD107a, CD107b, CD109, CD117, CD120, CD127, CD133, CD134, CD135, CD138, CD141, CD142, CD143, CD144, CD147, CD151, CD152, CD154, CD156, CD158, CD163, CD165, CD166, CD168, CD184, CDw186, CD195, CD197, CD209, CD202a, CD220, CD221, CD235a, CD271, CD303, CD304, CD309, CD326
Interleukin (IL) proteins	IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 or CXCL8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-35, IL-36
IL receptors	CD121a/IL1R1, CD121b/IL1R2, CD25/IL2RA, CD122/IL2RB, CD132/IL2RG, CD123/IL3RA, CD131/IL3RB, CD124/IL4R, CD132/IL2RG, CD125/IL5RA, CD131/IL3RB, CD126/IL6RA, CD130/IR6RB, CD127/IL7RA, CD132/IL2RG, CXCR1/IL8RA, CXCR2/IL8RB/CD128, CD129/IL9R, CD210/IL10RA, CDW210B/IL10RB, IL11RA, CD212/IL12RB1, IR12RB2, IL13R, IL15RA, CD4, CDw217/IL17RA, IL17RB, CDw218a/IL18R1, IL20R, IL20R, IL21R, IL22R, IL23R, IL20R, LY6E, IL20R1, IL27RA, IL28R, IL31RA
Mucin (MUC) proteins	MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC12, MUC13, MUC15, MUC16, MUC17, MUC19, and MUC20
Tumor markers	Alphafetoprotein (AFP), Carcinoembryonic antigen (CEA), CA-125, MUC-1, Epithelial tumor antigen (ETA), Tyrosinase, Melanoma-associated antigen (MAGE), p53
Tumor markers	Alpha fetoprotein (AFP), CA15-3, CA27-29, CA19-9, CA-125, Calretinin, Carcinoembryonic antigen, CD34, CD99, CD117, Chromogranin, Cytokeratin (various types), Desmin, Epithelial membrane protein (EMA), Factor VIII, CD31 FL1, Glial fibrillary acidic protein (GFAP), Gross cystic disease fluid protein (GCDFP-15), HMB-45, Human chorionic gonadotropin (hCG), immunoglobulin, inhibin, keratin (various types), PTPRC (CD45), lymphocyte marker (various types, MART-1 (Melan-A), Myo D1, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase (PLAP), prostate-specific antigen, S100 protein, smooth muscle actin (SMA), synaptophysin, thyroglobulin, thyroid transcription factor-1, Tumor M2-PK, vimentin
Cell adhesion molecule (CAMs)	Immunoglobulin superfamily CAMs (IgSF CAMs), N-CAM (Myelin protein zero), ICAM (1, 5), VCAM-1, PE-CAM, L1-CAM, Nectin (PVRL1, PVRL2, PVRL3), Integrins, LFA-1 (CD11a+CD18), Integrin alphaXbeta2 (CD11c+CD18), Macrophage-1 antigen (CD11b+CD18), VLA-4 (CD49d+CD29), Glycoprotein IIb/IIIa (ITGA2B+ITGB3), Cadherins, CDH1, CDH2, CDH3, Desmosomal, Desmoglein (DSG1, DSG2, DSG3, DSG4),

	Desmocollin (DSC1, DSC2, DSC3), Protocadherin , PCDH1, T-cadherin, CDH4, CDH5, CDH6, CDH8, CDH11, CDH12, CDH15, CDH16, CDH17, CDH9, CDH10, Selectins, E-selectin, L-selectin, P-selectin, Lymphocyte homing receptor: CD44, L-selectin, integrin (VLA-4, LFA-1), Carcinoembryonic antigen (CEA), CD22, CD24, CD44, CD146, CD164
Annexins	ANXA1; ANXA10; ANXA11; ANXA13; ANXA2; ANXA3; ANXA4; ANXA5; ANXA6; ANXA7; ANXA8; ANXA8L1; ANXA8L2; ANXA9

[00386] The instant disclosure provides various biomarkers that can be assessed in determining a biosignature for a given test sample, and which include assessment of polypeptides and/or nucleic acid biomarkers associated with various cancers, as well as the state of the cancer (e.g., metastatic v. non-metastatic).

[00387] In one example, a test sample can be assessed for a cancer by determining the presence or level of one or more biomarker including but not limited to CA-125, CA 19-9, and c-reactive protein. The cancer can be a cancer of the reproductive tract, e.g., an ovarian cancer. The one or more biomarker can further comprise one or more biomarkers, e.g., 1, 2, 3,4,5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more biomarkers, comprising one or more of CD95, FAP-1, miR-200 microRNAs, EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rab13, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8 and HLA-DR. MiR-200 microRNAs (i.e., the miR-200 microRNA family) comprises miR-200a, miR-200b, miR-200c, miR-141 and miR-429. Such assessment can include determining the presence or levels of proteins, nucleic acids, or both for each of the biomarkers disclosed herein.

[00388] CD95 (also called Fas, Fas antigen, Fas receptor, FasR, TNFRSF6, APT1 or APO-1) is a prototypical death receptor that regulates tissue homeostasis mainly in the immune system through the induction of apoptosis. During cancer progression, CD95 is frequently downregulated and the cells are rendered apoptosis resistant, thereby implicating loss of CD95 as part of a mechanism for tumour evasion. The tumorigenic activity of CD95 is mediated by a pathway involving JNK and Jun. FAP-1 (also referred to as Fas-associated phosphatase 1, protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase), PTPN13) is a member of the protein tyrosine phosphatase (PTP) family. FAP-1 has been reported to interact with, and dephosphorylate, CD95, thereby implicating a role in Fas mediated programmed cell death. MiR-200 family members can regulate CD95 and FAP-1. See Schickel et al. miR-200c regulates induction of apoptosis through CD95 by targeting FAP-1. Mol. Cell., 38, 908-915 (2010), which publication is incorporated by reference in its entirety herein.

[00389] Methods of the invention disclosed herein can use CD95 and/or FAP-1 characterization or profiling for microvesicle populations present in a biological sample to determine the presence of or predisposition to cancer, including without limitation any of the cancers disclosed herein. Methods of the invention comprising multiplexed analysis for multiple biomarkers use CD95 and/or FAP-1 biomarker characterization, along with other biomarkers disclosed herein, including but not limited to miR-200 microRNAs (e.g., miR-200c). In an embodiment, a biological test sample from an individual is assessed to determine the presence and level of CD95 and/or FAP-1 protein, or a presence or level of a CD95+ and/or FAP-1+ circulating microvesicle ("cMV") population, and the presence or levels are compared to a reference (e.g., samples from non-disease or normal, pre-treatment, or different treatment timepoints). This comparison is used to characterize the test sample. For example, comparison of the presence or levels of CD95 protein, FAP-1 protein, CD95+ cMVs and/or FAP-1+ cMVs in the test sample and reference are used to determine a disease phenotype or predict a response/non-response to treatment. In related embodiments, the cMV population is further assessed to determine a presence or level of miR-200 microRNAs, which are predetermined in a

training set of reference samples to be indicative of disease or other prognostic, theranostic or diagnostic readout. Increased levels of FAP-1 in the test sample as compared to a non-cancer reference may indicate the presence of a cancer, or the presence of a more aggressive cancer. Decreased levels of CD95 or miR200 family members such as miR-200c as compared to a non-cancer reference may indicate the presence of a cancer, or the presence of a more aggressive cancer. The cMV population to be assessed can be isolated through immunoprecipitation, flow cytometry, or other isolation methodology disclosed herein or known in the art.

[00390] In a related aspect, the invention provides a method of characterizing a cancer comprising detecting a level of one or more biomarker, e.g., 1, 2, 3,4,5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 biomarkers, selected from the group consisting of A2ML1, BAX, C10orf47, C1orf162, CSDA, EIFC3, ETFB, GABARAPL2, GUK1, GZMH, HIST1H3B, HLA-A, HSP90AA1, NRGN, PRDX5, PTMA, RABAC1, RABAGAP1L, RPL22, SAP18, SEPW1, SOX1, and a combination thereof. The one or more biomarker can comprise PTMA (prothymosin, alpha), a member of the pro/parathymosin family which is cleaved into Thymosin alpha-1 and has a role in immune modulation. Thymosin alpha-1 is approved in at least 35 countries for the treatment of Hepatitis B and C, and it is also approved for inclusion with vaccines to boost the immune response in the treatment of other diseases. In an embodiment, the biomarkers comprise mRNA. The mRNAs can be isolated from vesicles that have been isolated as described herein. In some embodiments, a total vesicle population in a sample is isolated, e.g., by filtration or centrifugation. The vesicles can also by isolated by affinity, e.g., using a binding agent to a general vesicle biomarker, a disease biomarker or a cell-specific biomarker.The levels of the biomarkers can be compared to a control such as a sample without cancer, wherein a change between the levels of the biomarkers versus the control is used to characterize the cancer. The cancer can be a prostate cancer.

[00391] In an embodiment, the cancer assessed by the invention comprises prostate cancer and microRNAs (miRs) are used to differentiate between metastatic versus non-metastatic prostate cancer. Prostate cancer staging is a process of categorizing the risk of cancer spread beyond the prostate. Such spread is related to the probability of being cured with local therapies such as surgery or radiation. The information considered in such prognostic classification is based on clinical and pathological factors, including physical examination, imaging studies, blood tests and/or biopsy examination.

[00392] The most common scheme used to stage prostate cancer is promulgated by the American Joint Committee on Cancer, and is referred to as the TNM system. The TNM system evaluates the size of the tumor, the extent of involved lymph nodes, metastasis and also takes into account cancer grade. As with many other cancers, the cancers are often grouped by stage, e.g., stages I–IV). Generally, Stage I disease is cancer that is found incidentally in a small part of the sample when prostate tissue was removed for other reasons, such as benign prostatic hypertrophy, and the cells closely resemble normal cells and the gland feels normal to the examining finger. In Stage II more of the prostate is involved and a lump can be felt within the gland. In Stage III, the tumor has spread through the prostatic capsule and the lump can be felt on the surface of the gland. In Stage IV disease, the tumor has invaded nearby structures, or has spread to lymph nodes or other organs.

[00393] The Whitmore-Jewett stage is another staging scheme that is now used less often. The Gleason Grading System is based on cellular content and tissue architecture from biopsies, which provides an estimate of the destructive potential and ultimate prognosis of the disease.

[00394] The TNM tumor classification system can be used to describe the extent of cancer in a subject's body. T describes the size of the tumor and whether it has invaded nearby tissue, N describes regional lymph nodes that are

involved, and M describes distant metastasis. TNM is maintained by the International Union Against Cancer (UICC) and is used by the American Joint Committee on Cancer (AJCC) and the International Federation of Gynecology and Obstetrics (FIGO). Those of skill in the art understand that not all tumors have TNM classifications such as, e.g., brain tumors. Generally, T (a,is,(0), 1-4) is measured as the size or direct extent of the primary tumor. N (0-3) refers to the degree of spread to regional lymph nodes: N0 means that tumor cells are absent from regional lymph nodes, N1 means that tumor cells spread to the closest or small numbers of regional lymph nodes, N2 means that tumor cells spread to an extent between N1 and N3; N3 means that tumor cells spread to most distant or numerous regional lymph nodes. M (0/1) refers to the presence of metastasis: MX means that distant metastasis was not assessed; M0 means that no distant metastasis are present; M1 means that metastasis has occurred to distant organs (beyond regional lymph nodes). M1 can be further delineated as follows: M1a indicates that the cancer has spread to lymph nodes beyond the regional ones; M1b indicates that the cancer has spread to bone; and M1c indicates that the cancer has spread to other sites (regardless of bone involvement). Other parameters may also be assessed. G (1-4) refers to the grade of cancer cells (i.e., they are low grade if they appear similar to normal cells, and high grade if they appear poorly differentiated). R (0/1/2) refers to the completeness of an operation (i.e., resection-boundaries free of cancer cells or not). L (0/1) refers to invasion into lymphatic vessels. V (0/1) refers to invasion into vein. C (1-4) refers to a modifier of the certainty (quality) of V.

[00395] Prostate tumors are often assessed using the Gleason scoring system. The Gleason scoring system is based on microscopic tumor patterns assessed by a pathologist while interpreting a biopsy specimen. When prostate cancer is present in the biopsy, the Gleason score is based upon the degree of loss of the normal glandular tissue architecture (i.e. shape, size and differentiation of the glands). The classic Gleason scoring system has five basic tissue patterns that are technically referred to as tumor "grades." The microscopic determination of this loss of normal glandular structure caused by the cancer is represented by a grade, a number ranging from 1 to 5, with 5 being the worst grade. Grade 1 is typically where the cancerous prostate closely resembles normal prostate tissue. The glands are small, well-formed, and closely packed. At Grade 2 the tissue still has well-formed glands, but they are larger and have more tissue between them, whereas at Grade 3 the tissue still has recognizable glands, but the cells are darker. At high magnification, some of these cells in a Grade 3 sample have left the glands and are beginning to invade the surrounding tissue. Grade 4 samples have tissue with few recognizable glands and many cells are invading the surrounding tissue. For Grade 5 samples, the tissue does not have recognizable glands, and are often sheets of cells throughout the surrounding tissue.

[00396] miRs that distinguish metastatic and non-metastatic prostate cancer can be overexpressed in metastatic samples versus non-metastatic. Alternately, miRs that distinguish metastatic and non-metastatic prostate cancer can be overexpressed in non-metastatic samples versus metastatic. Useful miRs for distinguishing metastatic prostate cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7 or 8, miRs selected from the group consisting of miR-495, miR-10a, miR-30a, miR-570, miR-32, miR-885-3p, miR-564, and miR-134. In another embodiment, miRs for distinguishing metastatic prostate cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7, 8, 9, 10, 11, 12, 13 or 14, miRs selected from the group consisting of hsa-miR-375, hsa-miR-452, hsa-miR-200b, hsa-miR-146b-5p, hsa-miR-1296, hsa-miR-17*, hsa-miR-100, hsa-miR-574-3p, hsa-miR-20a*, hsa-miR-572, hsa-miR-1236, hsa-miR-181a, hsa-miR-937, and hsa-miR-23a*. In still another embodiment, useful miRs for distinguishing metastatic prostate cancer include, e.g., 1, 2, 3,4,5, 6, 7, 8 or 9, miRs selected from the group consisting of hsa-miR-200b, hsa-miR-375, hsa-miR-582-3p, hsa-miR-17*, hsa-miR-1296, hsa-miR-20a*, hsa-miR-100, hsa-miR-452, and hsa-miR-577. The miRs for distinguishing metastatic prostate cancer can be one or more, e.g., 1, 2, 3 or 4, miRs selected from the group consisting of miR-141, miR-375, miR-200b and miR-574-3p.

[00397] In an aspect, microRNAs (miRs) are used to differentiate between cancer and non-cancer samples. Vesicles derived from patient samples can be analyzed for miR payload contained within the vesicles. The sample can be a bodily fluid, including semen, urine, blood, serum or plasma. The sample can also comprise a tissue or biopsy sample. A number of different methodologies are available for detecting miRs. In some embodiments, arrays of miR panels are use to simultaneously query the expression of multiple miRs. The Exiqon mIRCURY LNA microRNA PCR system panel (Exiqon, Inc., Woburn, MA) can be used for such purposes. miRs that distinguish cancer can be overexpressed in cancer versus control samples. Alternately, miRs that distinguish cancer can be overexpressed in cancer samples versus controls. Useful miRs for distinguishing cancer from non-cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7, 8, 9, 10, 11, 12 or 13, miRs selected from the group consisting of hsa-miR-574-3p, hsa-miR-331-3p, hsa-miR-326, hsa-miR-181a-2*, hsa-miR-130b, hsa-miR-301a, hsa-miR-141, hsa-miR-432, hsa-miR-107, hsa-miR-628-5p, hsa-miR-625*, hsa-miR-497, and hsa-miR-484. In another embodiment, useful miRs for distinguishing cancer from non-cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7, 8, 9 or 10, miRs selected from the group consisting of hsa-miR-574-3p, hsa-miR-141, hsa-miR-331-3p, hsa-miR-432, hsa-miR-326, hsa-miR-2110, hsa-miR-107, hsa-miR-130b, hsa-miR-301a, and hsa-miR-625*. In still another embodiment, the useful miRs for distinguishing cancer from non-cancer include one or more, e.g., 1, 2, 3,4,5, 6, 7, 8 or 9, miRs selected from the group consisting of hsa-miR-107, hsa-miR-326, hsa-miR-432, hsa-miR-574-3p, hsa-miR-625*, hsa-miR-2110, hsa-miR-301a, hsa-miR-141 or hsa-miR-373*. The cancer can comprise those cancers listed above. In an exemplary embodiment, the cancer is a prostate cancer and the microRNAs (miRs) are used to differentiate between prostate cancer and non-cancer samples.

[00398] The method contemplates assessing combinations of circulating biomarkers. For example, multiple markers from antibody arrays and miR analysis can be used to distinguish prostate cancer from normal, BPH and PCa, or metastatic versus non-metastatic disease. In this manner, improved sensitivity, specificity, and/or accuracy can be obtained. In some embodiments, the levels of one or more, e.g., 1, 2, 3,4,5 or 6, miRs selected from the group consisting of hsa-miR-432, hsa-miR-143, hsa-miR-424, hsa-miR-204, hsa-miR-581f and hsa-miR-451 are detected in a patient sample to assess the presence of prostate cancer. Any of these miRs can be elevated in patients with PCa but having serum PSA < 4.0 ng/ml. In an embodiment, the invention provides a method of assessing a prostate cancer, comprising determining a level of one or more, e.g., 1, 2, 3,4,5 or 6, miRs selected from the group consisting of hsa-miR-432, hsa-miR-143, hsa-miR-424, hsa-miR-204, hsa-miR-581f and hsa-miR-451 in a sample from a subject. The sample can be a bodily fluid, e.g., blood, plasma or serum. The miRs can be isolated in vesicles isolated from the sample. The subject can have a PSA level less than some threshold, such as 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, or 6.0 ng/ml in a blood sample. Higher levels of the miRs than in a reference sample can indicate the presence of PCa in the sample. In some embodiments, the reference comprises a level of the one or more miRs in control samples from subjects without PCa. In some embodiments, the reference comprises a level of the one or more miRs in control samples from subject with PCa and PSA levels ≥ some threshold, such as 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, or 6.0 ng/ml. The threshold can be 4.0 ng/ml.

[00399] In some embodiments of the invention, vesicles in patient samples are assessed to provide a diagnostic, prognostic or theranostic readout. Vesicle analysis of patient samples includes the detection of vesicle surface biomarkers, e.g., surface antigens, and/or vesicle payload, e.g., mRNAs and microRNAs, as described herein. Methods for analysis of vesicles are presented in PCT Patent Application PCT/US09/06095, entitled "METHODS AND SYSTEMS OF USING EXOSOMES FOR DETERMINING PHENOTYPES" and filed November 12, 2009; U.S.

Provisional Patent Application 61/362,674, entitled "METHODS AND SYSTEMS OF USING VESICLES FOR DETERMINING PHENOTYPES" and filed July 7, 2010; and U.S. Provisional Patent Application 61/393,823, entitled "DETECTION OF GI CANCERS" and filed October 15, 2010, which applications are incorporated by reference herein in their entirety.

[00400] In one aspect, the invention includes a method of identifying a bio-signature of one or more vesicles in a biological sample from said subject, wherein the bio-signature comprises analysis of vesicle surface antigens and vesicle payload. The surface antigens can comprise surface proteins and the vesicle payload can comprise microRNA. For example, vesicles can be captured using binding agents that recognize vesicle surface antigens, and the microRNA inside these captured vesicles can be assessed. Accordingly, the bio-signature may comprise the surface antigens used for capture as well as the microRNA inside the vesicles. The bio-signature can be used for diagnostic, prognostic or theranostic purposes. For example, the bio-signature can be a signature that identifies cancer, identifies aggressive or metastatic cancer, or identifies a cancer that is likely to respond to a candidate therapeutic agent.

[00401] As an illustrative example, consider a method of capturing vesicles in a sample using an antibody to B7H3 and then assessing the levels of miR-141 within the captured vesicles. In this example, the bio-signature comprises the level of miR-141 within exosomes displaying B7H3 on their surface. Depending on the levels of B7H3+ vesicles in the sample as well as the levels of miR-141 within the sample, the bio-signature may indicate that the sample comprises a cancer, comprises an aggressive cancer, is likely to respond to a certain treatment or chemotherapeutic agent, etc.

[00402] In one embodiment, the method of assessing cancer in a subject comprises: identifying a bio-signature of one or more vesicles in a biological sample from said subject, comprising: determining a level of one or more general vesicles protein biomarkers; determining a level of one or more cell-specific protein biomarkers; determining a level of one or more disease-specific protein biomarkers; and determining the level of one or more microRNA biomarkers in the vesicles, wherein said characterizing comprises comparing said levels of biomarkers in said sample to a reference to determine whether said subject may be predisposed to or afflicted with cancer. The protein biomarkers can be detected in a multiplex fashion in a single assay. The microRNA biomarkers can also be detected in a multiplex fashion in a single assay. In some cases, the cell-specific and disease-specific biomarker may overlap, e.g., one biomarker may serve to identify a cancer from a particular cellular origin. The biological sample can be a bodily fluid, such as blood, serum or plasma.

[00403] In an example, the method of the invention comprises a diagnostic test for prostate cancer comprising isolating vesicles from a blood sample from a patient to detect vesicles indicative of the presence or absence of prostate cancer. The blood can be serum or plasma. The vesicles are isolated by capture with "capture antibodies" that recognize specific vesicle surface antigens. The surface antigens for the prostate cancer diagnostic assay include the tetraspanins CD9, CD63 and CD81, which are generally present on vesicles in the blood and therefore act as general vesicle biomarkers, the prostate specific biomarkers PSMA and PCSA, and the cancer specific biomarker B7H3. In some cases, EpCam is used as a cancer specific biomarker as well or instead of B7H3. The capture antibodies can be tethered to a substrate. In an embodiment, the substrate comprises fluorescently labeled beads, wherein the beads are differentially labeled for each capture antibody. As desired, the payload of the detected vesicles can be assessed in order to characterize the cancer.

[00404] As described above, the biomarkers of the invention can be assessed to identify a biosignature. In an aspect, the invention provides a method comprising: determining a presence or level of one or more biomarker in a biological sample, wherein the one or more biomarker comprises one or more biomarker selected from **Table 5**; and identifying a

biosignature comprising the presence or level of the one or more biomarker. In some embodiments, the method further comprises comparing the biosignature to a reference biosignature, wherein the comparison is used to characterize a cancer, including the cancers disclosed herein or known in the art. The reference biosignature can be from a subject without the cancer. The reference biosignature can also be from the subject, e.g., from normal adjacent tissue or from a sample taken at another point in time. Various ways of characterizing a cancer are disclosed herein. For example, characterizing the cancer may comprise identifying the presence or risk of the cancer in a subject, or identifying the cancer in a subject as metastatic or aggressive. The comparing step comprises determining whether the biosignature is altered relative to the reference biosignature, thereby providing a prognostic, diagnostic or theranostic characterization for the cancer. The biological sample comprises a bodily fluid, including without limitation the bodily fluids disclosed herein. For example, the bodily fluid may comprise urine, blood or a blood derivative.

[00405] The one or more biomarker can be one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more, selected from the group consisting of miR-22, let7a, miR-141, miR-182, miR-663, miR-155, mirR-125a-5p, miR-548a-5p, miR-628-5p, miR-517*, miR-450a, miR-920, hsa-miR-619, miR-1913, miR-224*, miR-502-5p, miR-888, miR-376a, miR-542-5p, miR-30b*, miR-1179, and a combination thereof. In an embodiment, the one or more biomarker is selected from the group consisting of miR-22, let7a, miR-141, miR-920, miR-450a, and a combination thereof. The one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more, may be a messenger RNA (mRNA) selected from the group consisting of the genes in the Examples herein, and a combination thereof. For example, the one or more biomarker may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more messenger RNA (mRNA) selected from the group consisting of A2ML1, BAX, C10orf47, C1orf162, CSDA, EIFC3, ETFB, GABARAPL2, GUK1, GZMH, HIST1H3B, HLA-A, HSP90AA1, NRGN, PRDX5, PTMA, RABAC1, RABAGAP1L, RPL22, SAP18, SEPW1, SOX1, and a combination thereof. The one or more biomarker may comprise 1, 2, 3, 4, 5, or 6 messenger RNA (mRNA) selected from the group consisting of A2ML1, GABARAPL2, PTMA, RABAC1, SOX1, EFTB, and a combination thereof. The one or more biomarker may be isolated as payload of a population of microvesicles. The population can be a total population of microvesicles from the sample or a specific population, such as a PCSA+ population. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer.

[00406] In an embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of CA-125, CA 19-9, c-reactive protein, CD95, FAP-1, EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rab13, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8, HLA-DR, a miR200 microRNA, miR-200c, and a combination thereof. The one or more biomarker may comprise 1, 2, 3, 4 or 5 biomarker selected from the group consisting of CA-125, CA 19-9, c-reactive protein, CD95, FAP-1, and a combination thereof. The one or more biomarker may be isolated directly from sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, the method is used to assess an ovarian cancer. For example, the method can be used to distinguish a sample comprising ovarian cancer from a sample without ovarian cancer. Altenarately, the method can be used to distinguish amongst ovarian cancer having different stage or prognosis.

[00407] In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of hsa-miR-574-3p, hsa-miR-141, hsa-miR-432, hsa-miR-326, hsa-miR-2110, hsa-miR-181a-2*, hsa-miR-107, hsa-miR-301a, hsa-miR-484, hsa-miR-625*, and a combination thereof. The method can

be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. In still another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of hsa-miR-582-3p, hsa-miR-20a*, hsa-miR-375, hsa-miR-200b, hsa-miR-379, hsa-miR-572, hsa-miR-513a-5p, hsa-miR-577, hsa-miR-23a*, hsa-miR-1236, hsa-miR-609, hsa-miR-17*, hsa-miR-130b, hsa-miR-619, hsa-miR-624*, hsa-miR-198, and a combination thereof. For example, the method can be used to distinguish a sample comprising metastatic prostate cancer from a sample with non-metastatic prostate cancer. The one or more biomarker may be isolated as payload of a population of microvesicles.

[00408] The one or more biomarker may be miR-497. The method can be used to assess a lung cancer. For example, the method can be used to distinguish a lung cancer sample from a non-cancer sample. The one or more biomarker may be isolated as payload of a population of microvesicles.

[00409] The one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, may comprise a messenger RNA (mRNA) selected from the group consisting of AQP2, BMP5, C16orf86, CXCL13, DST, ERCC1, GNAO1, KLHL5, MAP4K1, NELL2, PENK, PGF, POU3F1, PRSS21, SCML1, SEMG1, SMARCD3, SNAI2, TAF1C, TNNT3, and a combination thereof. The mRNA may be isolated from microvesicles. The method can be used to characterize a prostate cancer, such as distinguish a prostate cancer sample from a normal sample without cancer. In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, comprises a messenger RNA (mRNA) selected from the group consisting of ADRB2, ARG2, C22orf32, CYorf14, EIF1AY, FEV, KLK2, KLK4, LRRC26, MAOA, NLGN4Y, PNPLA7, PVRL3, SIM2, SLC30A4, SLC45A3, STX19, TRIM36, TRPM8, and a combination thereof. The mRNA may be isolated from microvesicles. The method can be used to characterize a prostate cancer, such as distinguish a prostate cancer sample from a sample having another cancer, e.g., a breast cancer. In still another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, comprises a messenger RNA (mRNA) selected from the group consisting of ADRB2, BAIAP2L2, C19orf33, CDX1, CEACAM6, EEF1A2, ERN2, FAM110B, FOXA2, KLK2, KLK4, LOC389816, LRRC26, MIPOL1, SLC45A3, SPDEF, TRIM31, TRIM36, ZNF613, and a combination thereof. The mRNA may be isolated from microvesicles. The method can be used to characterize a prostate cancer, such as distinguish a prostate cancer sample from a sample having another cancer, e.g., a colorectal cancer. In yet another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, comprises a messenger RNA (mRNA) selected from the group consisting of ASTN2, CAB39L, CRIP1, FAM110B, FEV, GSTP1, KLK2, KLK4, LOC389816, LRRC26, MUC1, PNPLA7, SIM2, SLC45A3, SPDEF, TRIM36, TRPV6, ZNF613, and a combination thereof. The mRNA may be isolated from microvesicles. The method can be used to characterize a prostate cancer, such as distinguish a prostate cancer sample from a sample having another cancer, e.g., a lung cancer. The one or more biomarker can also be a microRNA that regulates one or more of the mRNAs used to characterize a prostate cancer. For example, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, may comprise a microRNA selected from the group consisting of miRs-26a+b, miR-15, miR-16, miR-195, miR-497, miR-424, miR-206, miR-342-5p, miR-186, miR-1271, miR-600, miR-216b, miR-519 family, miR-203, and a combination thereof. The microRNA can be assessed as payload of a microvesicle population.

[00410] In still another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more biomarkers, is selected from the group consisting of A33, ABL2, ADAM10, AFP, ALA, ALIX, ALPL, ApoJ/CLU, ASCA, ASPH(A-10), ASPH(D01P), AURKB, B7H3, B7H3, B7H4, BCNP, BDNF, CA125(MUC16), CA-19-9, C-Bir,

CD10, CD151, CD24, CD41, CD44, CD46, CD59(MEM-43), CD63, CD63, CD66eCEA, CD81, CD81, CD9, CD9, CDA, CDADC1, CRMP-2, CRP, CXCL12, CXCR3, CYFRA21-1, DDX-1, DLL4, DLL4, EGFR, Epcam, EphA2, ErbB2, ERG, EZH2, FASL, FLNA, FRT, GAL3, GATA2, GM-CSF, Gro-alpha, HAP, HER3(ErbB3), HSP70, HSPB1, hVEGFR2, iC3b, IL-1B, IL6R, IL6Unc, IL7Ralpha/CD127, IL8, INSIG-2, Integrin, KLK2, LAMN, Mammoglobin, M-CSF, MFG-E8, MIF, MISRII, MMP7, MMP9, MUC1, Muc1, MUC17, MUC2, Ncam, NDUFB7, NGAL, NK-2R(C-21), NT5E (CD73), p53, PBP, PCSA, PCSA, PDGFRB, PIM1, PRL, PSA, PSA, PSMA, PSMA, RAGE, RANK, RegIV, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2(C-15), SPARC, SPC, SPDEF, SPP1, STEAP, STEAP4, TFF3, TGM2, TIMP-1, TMEM211, Trail-R2, Trail-R4, TrKB(poly), Trop2, Tsg101, TWEAK, UNC93A, VEGFA, wnt-5a(C-16), and a combination thereof. The one or more biomarker may be detected directly in a sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, a binding agent to the one or more biomarker is used to capture a microvesicle population. The captured microvesicle population can be detected using another binding agent, e.g., a labeled binding agent to a general vesicle marker such as one or more protein in **Table 3**, or a cell-of-origin or or cancer-specific biomarker such as in **Table 4** or **Table 5**. In an embodiment, the antigen used for detection comprises one or more of CD9, CD63, CD81, PCSA, MUC2, and MFG-E8. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. Altenarately, the method is used to distinguish amongst prostate cancers having different stage or prognosis.

[00411] In a related embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more biomarkers, is selected from the group consisting of A33, ADAM10, AMACR, ASPH (A-10), AURKB, B7H3, CA125, CA-19-9, C-Bir, CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADC1, CSA, CXCL12, DCRN, EGFR, EphA2, ERG, FLNA, FRT, GAL3, GM-CSF, Gro-alpha, HER 3 (ErbB3), hVEGFR2, IL6 Unc, Integrin, Mammaglobin, MFG-E8, MMP9, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, PBP, PCSA, PIM1, PRL, PSA, PSIP1/LEDGF, PSMA, RANK, S100-A4, seprase/FAP, SIM2 (C-15), SPDEF, SSX2, STEAP, TGM2, TIMP-1, Trail-R4, Tsg 101, TWEAK, UNC93A, VCAN, XAGE-1, and a combination thereof. The one or more biomarker may be detected directly in a sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, a binding agent to the one or more biomarker is used to capture a microvesicle population. The captured microvesicle population can be detected using another binding agent, e.g., a labeled binding agent to a general vesicle marker such as one or more protein in **Table 3**, or a cell-of-origin or or cancer-specific biomarker such as in **Table 4** or **Table 5**. In an embodiment, the antigen used for detection comprises one or more of EpCAM, CD81, PCSA, MUC2 and MFG-E8. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. Altenarately, the method is used to distinguish amongst prostate cancers having different stage or prognosis.

[00412] In another related embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or more biomarkers, is selected from the group consisting of A33, ADAM10, ALIX, AMACR, ASCA, ASPH (A-10), AURKB, B7H3, BCNP, CA125, CA-19-9, C-Bir (Flagellin), CD24, CD3, CD41, CD63, CD66e CEA, CD81, CD9, CDADC1, CRP, CSA, CXCL12, CYFRA21-1, DCRN, EGFR, EpCAM, EphA2, ERG, FLNA, GAL3, GATA2, GM-CSF, Gro alpha, HER3 (ErbB3), HSP70, hVEGFR2, iC3b, IL-1B, IL6 Unc, IL8, Integrin, KLK2, Mammaglobin, MFG-E8, MMP7, MMP9, MS4A1, MUC1, MUC17, MUC2, NGAL, NK-2R(C-21), NY-ESO-1, p53, PBP, PCSA, PIM1, PRL, PSA, PSMA, RANK, RUNX2, S100-A4, seprase/FAP, SERPINB3, SIM2 (C-15), SPC, SPDEF, SSX2, SSX4, STEAP, TGM2, TIMP-1, TRAIL R2, Trail-R4, Tsg 101, TWEAK, VCAN, VEGF A, XAGE, and a combination thereof. The

one or more biomarker may be detected directly in a sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, a binding agent to the one or more biomarker is used to capture a microvesicle population. The captured microvesicle population can be detected using another binding agent, e.g., a labeled binding agent to a general vesicle marker such as one or more protein in **Table 3**, or a cell-of-origin or or cancer-specific biomarker such as in **Table 4** or **Table 5**. In an embodiment, the antigen used for detection comprises one or more of EpCAM, CD81, PCSA, MUC2 and MFG-E8. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. Altenarately, the method is used to distinguish amongst prostate cancers having different stage or prognosis.

[00413] In still another related embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 biomarkers, is selected from the group consisting of ADAM-10, BCNP, CD9, EGFR, EpCam, IL1B, KLK2, MMP7, p53, PBP, PCSA, SERPINB3, SPDEF, SSX2, SSX4, and a combination thereof. The one or more biomarker may be detected directly in a sample, or as surface antigens or payload of a population of microvesicles. In an embodiment, a binding agent to the one or more biomarker is used to capture a microvesicle population. The captured microvesicle population can be detected using another binding agent, e.g., a labeled binding agent to a general vesicle marker such as one or more protein in **Table 3**, or a cell-of-origin or or cancer-specific biomarker such as in **Table 4** or **Table 5**. In an embodiment, the antigen used for detection comprises one or more of EpCAM, KLK2, PBP, SPDEF, SSX2, SSX4. In a non-limiting example, consider that the detector binding agent is a binding agent to EpCam, e.g., an antibody or aptamer to EpCam, wherein the antibody or aptamer is optionally labeled to facilitate detection thereof. In such case, the one or more biomarker comprises one or more pair of biomarkers selected from the group consisting of EpCam – ADAM-10, EpCam – BCNP, EpCam – CD9, EpCam – EGFR, EpCam – EpCam, EpCam – IL1B, EpCam – KLK2, EpCam – MMP7, EpCam – p53, EpCam – PBP, EpCam – PCSA, EpCam – SERPINB3, EpCam – SPDEF, EpCam – SSX2, EpCam – SSX4, and a combination thereof. In an embodiment, the method is used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. Altenarately, the method is used to distinguish amongst prostate cancers having different stage or prognosis.

[00414] In one embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of miR-148a, miR-329, miR-9, miR-378*, miR-25, miR-614, miR-518c*, miR-378, miR-765, let-7f-2*, miR-574-3p, miR-497, miR-32, miR-379, miR-520g, miR-542-5p, miR-342-3p, miR-1206, miR-663, miR-222, and a combination thereof. In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of hsa-miR-877*, hsa-miR-593, hsa-miR-595, hsa-miR-300, hsa-miR-324-5p, hsa-miR-548a-5p, hsa-miR-329, hsa-miR-550, hsa-miR-886-5p, hsa-miR-603, hsa-miR-490-3p, hsa-miR-938, hsa-miR-149, hsa-miR-150, hsa-miR-1296, hsa-miR-384, hsa-miR-487a, hsa-miRPlus-C1089, hsa-miR-485-3p, hsa-miR-525-5p, and a combination thereof. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. In still another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of miR-588, miR-1258, miR-16-2*, miR-938, miR-526b, miR-92b*, let-7d, miR-378*, miR-124, miR-376c, miR-26b, miR-1204, miR-574-3p, miR-195, miR-499-3p, miR-2110, miR-888, and a combination thereof. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample with inflammatory prostate disease. The one or more biomarker may be isolated as payload of a population of microvesicles.

[00415] In one embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of let-7d, miR-148a, miR-195, miR-25, miR-26b, miR-329, miR-376c, miR-574-3p, miR-888, miR-9, miR1204, miR-16-2*, miR-497, miR-588, miR-614, miR-765, miR92b*, miR-938, let-7f-2*, miR-300, miR-523, miR-525-5p, miR-1182, miR-1244, miR-520d-3p, miR-379, let-7b, miR-125a-3p, miR-1296, miR-134, miR-149, miR-150, miR-187, miR-32, miR-324-3p, miR-324-5p, miR-342-3p, miR-378, miR-378*, miR-384, miR-451, miR-455-3p, miR-485-3p, miR-487a, miR-490-3p, miR-502-5p, miR-548a-5p, miR-550, miR-562, miR-593, miR-593*, miR-595, miR-602, miR-603, miR-654-5p, miR-877*, miR-886-5p, miR-125a-5p, miR-140-3p, miR-192, miR-196a, miR-2110, miR-212, miR-222, miR-224*, miR-30b*, miR-499-3p, miR-505*, and a combination thereof. In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of hsa-miR-451, hsa-miR-223, hsa-miR-593*, hsa-miR-1974, hsa-miR-486-5p, hsa-miR-19b, hsa-miR-320b, hsa-miR-92a, hsa-miR-21, hsa-miR-675*, hsa-miR-16, hsa-miR-876-5p, hsa-miR-144, hsa-miR-126, hsa-miR-137, hsa-miR-1913, hsa-miR-29b-1*, hsa-miR-15a, hsa-miR-93, hsa-miR-1266, and a combination thereof. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. The one or more biomarker may be isolated as payload of a population of microvesicles. The population can comprise PCSA+ microvesicles. In an embodiment, the population consists of PCSA+ microvesicles. In one embodiment, a population of PCSA+ vesicles is isolated and microRNA within the isolated vesicles are assessed using methods as described herein or known in the art. Elevated levels of miR-1974 in a test sample as compared to a control sample (e.g., non-cancer sample) are indicative of a prostate cancer in the test sample. Similarly, decreased levels of miR-320b in a test sample as compared to a control sample (e.g., non-cancer sample) can indicate the presence of a prostate cancer in the test sample.

[00416] The one or more biomarker can comprise EpCAM and MMP7. The biomarkers may be isolated from microvesicles. In an embodiment, EpCAM+ / MMP7+ microvesicles are detected in a sample, such as blood or a blood derivative. In a non-limiting example, the EpCAM+ / MMP7+ microvesicles are identified by EpCAM and MMP7 binding agents using methods as described herein, e.g., using flow cytometry. As described, vesicles in a biological sample can be identified by flow sorting using general vesicle markers, e.g., the marker in **Table 3** such as tetraspanins including CD9, CD63 and/or CD81. The levels of the EpCAM+ / MMP7+ microvesicles can be used to characterize a cancer, such as distinguish a cancer sample from a normal sample without cancer. In one embodiment, lower levels of MMP7 in EpCAM+ vesicles as compared to a non-cancer control sample indicate the presense of cancer. As EpCAM and MMP7 comprise cancer markers, one of skill will appreciate that the method can be used to assess various cancers in a sample. In an embodiment, the cancer comprises prostate cancer.

[00417] In another embodiment, the one or more biomarker comprises a transcription factor. The transcription factor can be one or more, e.g., 2, 3, 4, 5, 6, 7, 8, 9 or 10 of c-Myc, AEBP1, HNF4a, STAT3, EZH2, p53, MACC1, SPDEF, RUNX2 and YB-1. In another embodiment, the one or more biomarker may also comprise a kinase. The kinase can be one or more of AURKA and AURKB. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a sample without prostate cancer. The one or more biomarker may be isolated as payload of a population of microvesicles. In an embodiment, elevated levels of the transcription factors and/or kinases in the microvesicle population as compared to normal controls indicate the presence of a cancer. As these are cancer-related transcription factors, one of skill will appreciate that any appropriate cancer can be assessed using the method. In an embodiment, the cancer comprises a prostate cancer or a breast cancer.

[00418] The one or more biomarker can comprise PCSA, Muc2 and Adam10. The biomarkers may be isolated from microvesicles. In an embodiment, PCSA+ / Muc2+ / Adam10+ microvesicles are detected in a sample, such as blood or a blood derivative. In a non-limiting example, the PCSA+ / Muc2+ / Adam10+ microvesicles are identified by PCSA, Muc2 and Adam10 binding agents using methods as described herein, e.g., using flow cytometry. As described, vesicles in a biological sample can be identified by flow sorting using general vesicle markers, e.g., the marker in **Table 3** such as tetraspanins including CD9, CD63 and/or CD81. The levels of the PCSA+ / Muc2+ / Adam10+ microvesicles can be used to characterize a cancer, such as distinguish a cancer sample from a normal sample without cancer. In one embodiment, elevated levels of PCSA+ / Muc2+ / Adam10+ vesicles as compared to a non-cancer control sample indicate the presense of cancer. In an embodiment, the cancer comprises prostate cancer.

[00419] In one embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting Alkaline Phosphatase (AP), CD63, MyoD1, Neuron Specific Enolase, MAP1B, CNPase, Prohibitin, CD45RO, Heat Shock Protein 27, Collagen II, Laminin B1/b1, Gai1, CDw75, bcl-XL, Laminin-s, Ferritin, CD21, ADP-ribosylation Factor (ARF-6). In another embodiment, the one or more biomarker, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more biomarkers, is selected from the group consisting of CD56/NCAM-1, Heat Shock Protein 27/hsp27, CD45RO, MAP1B, MyoD1, CD45/T200/LCA, CD3zeta, Laminin-s, bcl-XL, Rad18, Gai1, Thymidylate Synthase, Alkaline Phosphatase (AP), CD63, MMP-16 / MT3-MMP, Cyclin C, Neuron Specific Enolase, SIRP a1, Laminin B1/b1, Amyloid Beta (APP), SODD (Silencer of Death Domain), CDC37, Gab-1, E2F-2, CD6, Mast Cell Chymase, Gamma Glutamylcysteine Synthetase (GCS), and a combination thereof. The one or more biomarker can comprise protein. The one or more biomarker may be isolated as payload of a population of microvesicles. The method can be used to assess a prostate cancer. For example, the method can be used to distinguish a sample comprising prostate cancer from a control sample without prostate cancer. The control sample can be a sample from a non-diseased state, a non-malignant prostate condition, or it can be a sample indicative of another type of cancer or related disorder, such as a breast cancer, brain cancer, lung cancer, colorectal cancer or colorectal adenoma. In an embodiment, elevated levels of Alkaline Phosphatase (AP) as compared to the control indicate the presence of prostate cancer. Similarly, elevated levels of CD56 (NCAM) as compared to the control can indicate the presence of prostate cancer. In an embodiment, elevated levels of CD-3 zeta as compared to the control indicate the presence of prostate cancer. In anther embodiment, elevated levels of Map1b as compared to the control can indicate the presence of prostate cancer. Conversely, elevated levels of 14.3.3 and/or filamin may indicate a colorectal cancer and not prostate cancer or other cancers or prostate disorders. Similarly, elevated levels of thrombospondin may indicate a colorectal or lung cancer and not prostate cancer or other cancers or prostate disorders.

[00420] In one embodiment, the one or more biomarker comprises MMP7. The one or more biomarker can comprise protein. The one or more biomarker may be a surface antigen or payload of a population of microvesicles. The method can be used to assess a cancer. One of skill will appreciate that any appropriate cancer can be assessed using the method as MMP7 is a known cancer marker. In an embodiment, the cancer comprises a prostate cancer.

[00421] In an aspect, the invention provides a method of identifying a biosignature by assessing biomarker complexes. In an aspect, the method comprises isolating one or more nucleic acid-protein complex from a biological sample; determining a presence or level of one or more nucleic acid biomarker with the one or more nucleic acid-protein complex; and identifying a biosignature comprising the presence or level of the one or more nucleic acid biomarker. In some embodiments, the biosignature may also comprise the presence or level of one or more protein or other component of the complex. The nucleic acid-protein complex may be isolated from the biological sample using

methodology disclosed herein or known in the art. For example, the complex may be isolated by affinity selection such as by immunoprecipitation, column chromatography or flow cytometry, using a binding agent to a component of the complex. Binding agents can be as described herein, e.g., an antibody or aptamer to a protein component of the complex. In some embodiments, the method further comprises comparing the biosignature to a reference biosignature, wherein the comparison is used to characterize a cancer, including the cancers disclosed herein or known in the art. The reference biosignature can be from a subject without the cancer. The reference biosignature can also be from the subject, e.g., from normal adjacent tissue or from a sample taken at another point in time. Various ways of characterizing a cancer are disclosed herein. For example, characterizing the cancer may comprise identifying the presence or risk of the cancer in a subject, or identifying the cancer in a subject as metastatic or aggressive. The comparing step comprises determining whether the biosignature is altered relative to the reference biosignature, thereby providing a prognostic, diagnostic or theranostic characterization for the cancer. The biological sample comprises a bodily fluid, including without limitation the bodily fluids disclosed herein. For example, the bodily fluid may comprise urine, blood or a blood derivative.

[00422] In an embodiment, the nucleic acid-protein complex comprises one or more protein, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more proteins, selected from the group consisting of one or more Argonaute family member, Ago1, Ago2, Ago3, Ago4, GW182 (TNRC6A), TNRC6B, TNRC6C, HNRNPA2B1, HNRPAB, ILF2, NCL (Nucleolin), NPM1 (Nucleophosmin), RPL10A, RPL5, RPLP1, RPS12, RPS19, SNRPG, TROVE2, apolipoprotein, apolipoprotein A, apo A-I, apo A-II, apo A-IV, apo A-V, apolipoprotein B, apo B48, apo B100, apolipoprotein C, apo C-I, apo C-II, apo C-III, apo C-IV, apolipoprotein D (ApoD), apolipoprotein E (ApoE), apolipoprotein H (ApoH), apolipoprotein L, APOL1, APOL2, APOL3, APOL4, APOL5, APOL6, APOLD1, and a combination thereof. For example, the nucleic acid-protein complex may comprise one or more protein selected from the group consisting of one or more Argonaute family member, Ago1, Ago2, Ago3, Ago4, GW182 (TNRC6A), and a combination thereof. The nucleic acid-protein complex comprises one or more protein selected from the group consisting of Ago2, Apolipoprotein I, GW182 (TNRC6A), and a combination thereof.

[00423] In embodiments, the one or more nucleic acid in the nucleic acid-protein complex comprises one or more microRNA. For example, the one or more microRNA, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50 or more microRNA, can be a microRNA in **Table 5**. The one or more microRNA may comprise one or more microRNA, e.g., 1, 2, 3, 4, 5 or 6 microRNA, selected from the group consisting of miR-22, miR-16, miR-148a, miR-92a, miR-451, let7a, and a combination thereof. The one or more microRNA may be assessed in order to characterize, e.g., diagnose, prognose or theranose, a cancer including without limitation a prostate cancer.

[00424] In an embodiment, the nucleic acid-protein complex comprises one or more protein selected from the group consisting of Ago2, Apolipoprotein I, GW182 (TNRC6A), and a combination thereof; and the one or more microRNA comprises one or more microRNA selected from the group consisting of miR-16 and miR-92a, and a combination thereof. The one or more microRNA may be assessed in order to characterize a prostate cancer.

[00425] The invention further provides a method of determining a biosignature comprising detecting nucleic acids in microvesicle population of interest. The vesicle population can be a whole population in a biological sample, or a subpopulation such as a subpopulation having certain surface antigens. The method comprises detecting one or more protein biomarker in a microvesicle population from a biological sample; determining a presence or level of one or more one or more nucleic acid biomarker associated with the detected microvesicle population; and identifying a biosignature comprising the presence or level of the one or more nucleic acid. Techniques for detecting microvesicle

populations, detecting proteins, and assessing nucleic acids can be disclosed herein or as known in the art. For example, the microvesicles can be isolated by affinity selection against the one or more protein, and nucleic acid can be isolated from the selected microvesicles. The level of the one or more one or more nucleic acid biomarker can be normalized to the level of the one or more protein biomarker or to the level of the microvesicle population. In some embodiments, the method further comprises comparing the biosignature to a reference biosignature, wherein the comparison is used to characterize a cancer, including the cancers disclosed herein or known in the art. The reference biosignature can be from a subject without the cancer. The reference biosignature can also be from the subject, e.g., from normal adjacent tissue or from a sample taken at another point in time. Various ways of characterizing a cancer are disclosed herein. For example, characterizing the cancer may comprise identifying the presence or risk of the cancer in a subject, or identifying the cancer in a subject as metastatic or aggressive. The comparing step comprises determining whether the biosignature is altered relative to the reference biosignature, thereby providing a prognostic, diagnostic or theranostic characterization for the cancer. The biological sample comprises a bodily fluid, including without limitation the bodily fluids disclosed herein. For example, the bodily fluid may comprise urine, blood or a blood derivative.

[00426] The proteins used for detecting one or more protein biomarker in a microvesicle population may comprise one or more biomarker disclosed herein, such as in **Table 3**, **Table 4**, **Table 5**, **Table 8**, **Table 9** or **Table 10**. For example, the one or more protein can be selected from the group consisting of PCSA, Ago2, CD9 and a combination thereof. For example, the one or more protein can be PCSA, Ago2, CD9, PCSA and Ago2, PCSA and CD9, Ago2 and CD9, or all of PCSA, Ago2 and CD9. Another general vesicle marker such as in **Table 3**, e.g., a tetraspanin such as CD63 or CD81 can be substituted for or used in addition to CD9. Such multiple biomarkers can be used to identify a microvesicle population having a certain origin. E.g., PCSA can identify prostate-derived vesicles while CD9 identifies vesicles apart from cellular debris. PCSA, PSMA, PSCA, KLK2 or PBP (prostate binding protein) can be used as a biomarker to characterize a prostate cancer.

[00427] The one or more nucleic acid biomarker may comprise one or more nucleic acid disclosed herein, such as in **Table 5**. In an embodiment, the one or more nucleic acid comprises one or more microRNA. For example, the one or more microRNA can be selected from 1, 2, 3, 4, 5 or 6 of miR-22, miR-16, miR-148a, miR-92a, miR-451, and let7a. In an embodiment, the one or more protein biomarker comprises PCSA and Ago2; and the one or more nucleic acid biomarker comprises miR-22. In another embodiment, the one or more protein biomarker comprises PCSA and/or CD9; and the one or more nucleic acid biomarker comprises miR-22. The method can be used to characterize a cancer such as a prostate cancer, e.g., to distinguish a cancer sample from a non- cancer sample.

[00428] In other embodiments, the one or more nucleic acid comprises mRNA. mRNA can be assessed as payload within microvesicles. For example, the one or more nucleic acid biomarker comprises a messenger RNA (mRNA) selected from **Table 5**. The mRNA may also be selected from any of **Table 18**, **Table 19**, or **Table 20**. In some embodiments, the one or more protein biomarker comprises PCSA; and the one or more nucleic acid biomarker comprises a messenger RNA (mRNA) selected from any of **Table 18**, **Table 19**, or **Table 20**. The method can be used to characterize a cancer such as a prostate cancer, e.g., to distinguish a cancer sample from a non- cancer sample.

[00429] The level of the one or more one or more nucleic acid biomarker can be normalized to the level of the one or more protein biomarker. In an embodiment, the biosignature comprises a score calculated from a ratio of the level of the one or more protein biomarker and one or more nucleic acid biomarker. For example, the level of the nucleic acids can be divided by the level of the proteins.

[00430] The score can be calculated from multiple proteins and multiple nucleic acids. In an embodiment, the one or more protein biomarker comprises PCSA and PSMA and the one or more nucleic acid biomarker comprises miR-22 and let7a. The method is used to characterize a prostate cancer, e.g., to distinguish a prostate cancer sample from a non-prostate cancer sample. The score may comprise taking the sum of: a) a first multiple of the level of miR-22 payload in the microvesicle subpopulation divided by the level of PCSA protein associated with the microvesicle subpopulation; b) a second multiple of the level of let7a payload in the microvesicle subpopulation divided by the level of PCSA protein associated with the microvesicle subpopulation; and c) a third multiple of the level of PSMA protein associated with the microvesicle subpopulation. The first, second and third multiples can be chosen to maximize the ability of the method to distinguish the prostate cancer. For example, the multiple can be about 0.0001, 0.001, 0.01, 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 100, 1000 or 10000. In an embodiment, the first multiple is 10, the second multiple is 10, and the third multiple is 1. The score can be an average of the sum as:

Score = *Average*(10*miR22/PCSA MFI, 10*let-7a/PCSA MFI, PSMA MFI)

[00431] One of skill will appreciate that calculating the score may comprise a monotonic transformation of the sum. A similar scoring equation can be developed for other biomarkers in other settings, such as using alternate biomarkers to characterize other cancers.

[00432] By selecting a proper reference sample for comparison, the biosignatures identified can provide a diagnostic readout (e.g., reference sample is normal or non-disease), prognostic (e.g., reference sample is for poor or good disease outcome, aggressiveness or the like), or theranostic (e.g., reference sample is from a cohort responsive or non-responsive to selected treatment).

[00433] Additional biomarkers that can be used in the methods of the invention include those disclosed in International Patent Application PCT/US2012/025741, filed February 17, 2012; International Patent Application PCT/US2011/048327, filed August 18, 2011; International Patent Application PCT/ US2011/026750, filed March 1, 2011; and International Patent Application PCT/US2011/031479, filed April 6, 2011; each of which is incorporated by reference herein in its entirety.

[00434] ***Gene Fusions***

[00435] The one or more biomarkers assessed of vesicle, can be a gene fusion. A fusion gene is a hybrid gene created by the juxtaposition of two previously separate genes. This can occur by chromosomal translocation or inversion, deletion or via trans-splicing. The resulting fusion gene can cause abnormal temporal and spatial expression of genes, such as leading to abnormal expression ofcell growth factors, angiogenesis factors, tumor promoters or other factors contributing to the neoplastic transformation of the cell and the creation of a tumor. Such fusion genes can be oncogenic due to the juxtaposition of: 1) a strong promoter region of one gene next to the coding region of a cell growth factor, tumor promoter or other gene promoting oncogenesis leading to elevated gene expression, or 2) due to the fusion of coding regions of two different genes, giving rise to a chimeric gene and thus a chimeric protein with abnormal activity.

[00436] An example of a fusion gene is BCR-ABL, a characteristic molecular aberration in ~90% of chronic myelogenous leukemia (CML) and in a subset of acute leukemias (*Kurzrock et al., Annals of Internal Medicine 2003; 138(10):819-830*). The BCR-ABL results from a translocation between chromosomes 9 and 22. The translocation brings together the 5' region of the BCR gene and the 3' region of ABL1, generating a chimeric BCR-ABL1 gene, which encodes a protein with constitutively active tyrosine kinase activity (Mittleman et al., Nature *Reviews Cancer* 2007; 7(4):233-245). The aberrant tyrosine kinase activity leads to de-regulated cell signaling, cell growth and cell

survival, apoptosis resistance and growth factor independence, all of which contribute to the pathophysiology of leukemia (Kurzrock et al., *Annals of Internal Medicine* 2003; 138(10):819-830).

[00437] Another fusion gene is IGH-MYC, a defining feature of ~80% of Burkitt's lymphoma (Ferry et al. *Oncologist* 2006; 11(4):375-83). The causal event for this is a translocation between chromosomes 8 and 14, bringing the c-Myc oncogene adjacent to the strong promoter of the immunoglobin heavy chain gene, causing *c-myc* overexpression (Mittleman et al., *Nature Reviews Cancer* 2007; 7(4):233-245). The c-*myc* rearrangement is a pivotal event in lymphomagenesis as it results in a perpetually proliferative state. It has wide ranging effects on progression through the cell cycle, cellular differentiation, apoptosis, and cell adhesion (Ferry et al. *Oncologist* 2006; 11(4):375-83).

[00438] A number of recurrent fusion genes have been catalogued in the Mittleman database (cgap.nci.nih.gov/Chromosomes/Mitelman) and can be assessed in a vesicle, and used to characterize a phenotype. The gene fusion can be used to characterize a hematological malignancy or epithelial tumor. For example, TMPRSS2-ERG, TMPRSS2-ETV and SLC45A3-ELK4 fusions can be detected and used to characterize prostate cancer; and ETV6-NTRK3 and ODZ4-NRG1 for breast cancer.

[00439] Furthermore, assessing the presence or absence, or expression level of a fusion gene can be used to diagnosis a phenotype such as a cancer as well as a monitoring a therapeutic response to selecting a treatment. For example, the presence of the BCR-ABL fusion gene is a characteristic not only for the diagnosis of CML, but is also the target of the Novartis drug Imatinib mesylate (Gleevec), a receptor tyrosine kinase inhibitor, for the treatment of CML. Imatinib treatment has led to molecular responses (disappearance of BCR-ABL+ blood cells) and improved progression-free survival in BCR-ABL+ CML patients (*Kantarjian et al., Clinical Cancer Research 2007; 13(4):1089-1097*).

[00440] Assessing a vesicle for the presence, absence, or expression level of a gene fusion can be of by assessing a heterogeneous population of vesicles for the presence, absence, or expression level of a gene fusion. Alternatively, the vesicle that is assessed can be derived from a specific cell type, such as cell-of-origin specific vesicle, as described above. Illustrative examples of use of fusions that can be assessed to characterize a phenotype include those described in International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein.

[00441] ***Gene-Associated MiRNA Biomarkers***

[00442] Illustrative examples of use of miRNA biomarkers known to interact with certain transcripts and that can be assessed to characterize a phenotype include those described in International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein.

Biomarker Detection

[00443] A biosignature can be detected qualitatively or quantitatively by detecting a presence, level or concentration of a circulating biomarker, e.g., a microRNA, protein, vesicle or other biomarker, as disclosed herein. These biosignature components can be detected using a number of techniques known to those of skill in the art. For example, a biomarker can be detected by microarray analysis, polymerase chain reaction (PCR) (including PCR-based methods such as real time polymerase chain reaction (RT-PCR), quantitative real time polymerase chain reaction (Q-PCR/qPCR) and the like), hybridization with allele-specific probes, enzymatic mutation detection, ligation chain reaction (LCR), oligonucleotide ligation assay (OLA), flow-cytometric heteroduplex analysis, chemical cleavage of mismatches, mass spectrometry, nucleic acid sequencing, single strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), restriction fragment polymorphisms, serial

analysis of gene expression (SAGE), or combinations thereof. A biomarker, such as a nucleic acid, can be amplified prior to detection. A biomarker can also be detected by immunoassay, immunoblot, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA; EIA), radioimmunoassay (RIA), flow cytometry, or electron microscopy (EM).

[00444] Biosignatures can be detected using capture agents and detection agents, as described herein. A capture agent can comprise an antibody, aptamer or other entity which recognizes a biomarker and can be used for capturing the biomarker. Biomarkers that can be captured include circulating biomarkers, e.g., a protein, nucleic acid, lipid or biological complex in solution in a bodily fluid. Similarly, the capture agent can be used for capturing a vesicle. A detection agent can comprise an antibody or other entity which recognizes a biomarker and can be used for detecting the biomarker vesicle, or which recognizes a vesicle and is useful for detecting a vesicle. In some embodiments, the detection agent is labeled and the label is detected, thereby detecting the biomarker or vesicle. The detection agent can be a binding agent, e.g., an antibody or aptamer. In other embodiments, the detection agent comprises a small molecule such as a membrane protein labeling agent. See, e.g., the membrane protein labeling agents disclosed in Alroy et al., US. Patent Publication US 2005/0158708. In an embodiment, vesicles are isolated or captured as described herein, and one or more membrane protein labeling agent is used to detect the vesicles. In many cases, the antigen or other vesicle-moiety that is recognized by the capture and detection agents are interchangeable. As a non-limiting example, consider a vesicle having a cell-of-origin specific antigen on its surface and a cancer-specific antigen on its surface. In one instance, the vesicle can be captured using an antibody to the cell-of-origin specific antigen, e.g., by tethering the capture antibody to a substrate, and then the vesicle is detected using an antibody to the cancer-specific antigen, e.g., by labeling the detection antibody with a fluorescent dye and detecting the fluorescent radiation emitted by the dye. In another instance, the vesicle can be captured using an antibody to the cancer specific antigen, e.g., by tethering the capture antibody to a substrate, and then the vesicle is detected using an antibody to the cell-of-origin specific antigen, e.g., by labeling the detection antibody with a fluorescent dye and detecting the fluorescent radiation emitted by the dye.

[00445] In some embodiments, a same biomarker is recognized by both a capture agent and a detection agent. This scheme can be used depending on the setting. In one embodiment, the biomarker is sufficient to detect a vesicle of interest, e.g., to capture cell-of-origin specific vesicles. In other embodiments, the biomarker is multifunctional, e.g., having both cell-of-origin specific and cancer specific properties. The biomarker can be used in concert with other biomarkers for capture and detection as well.

[00446] One method of detecting a biomarker comprises purifying or isolating a heterogeneous population of vesicles from a biological sample, as described above, and performing a sandwich assay. A vesicle in the population can be captured with a capture agent. The capture agent can be a capture antibody, such as a primary antibody. The capture antibody can be bound to a substrate, for example an array, well, or particle. The captured or bound vesicle can be detected with a detection agent, such as a detection antibody. For example, the detection antibody can be for an antigen of the vesicle. The detection antibody can be directly labeled and detected. Alternatively, the detection agent can be indirectly labeled and detected, such as through an enzyme linked secondary antibody that can react with the detection agent. A detection reagent or detection substrate can be added and the reaction detected, such as described in PCT Publication No. WO2009092386. In an illustrative example wherein the capture agent binds Rab-5b and the detection agent binds or detects CD63 or caveolin-1, the capture agent can be an anti-Rab 5b antibody and the detection agent can be an anti-CD63 or anti-caveolin-1 antibody. In some embodiments, the capture agent binds CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, or 5T4. For example, the capture agent can be an

antibody to CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, or 5T4. The capture agent can also be an antibody to MFG-E8, Annexin V, Tissue Factor, DR3, STEAP, epha2, TMEM211, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, or TETS. The detection agent can be an agent that binds or detects CD63, CD9, CD81, B7H3, or EpCam, such as a detection antibody or aptamer to CD63, CD9, CD81, B7H3, or EpCam. Various combinations of capture and/or detection agents can be used in concert. In an embodiment, the capture agents comprise PCSA, PSMA, B7H3 and optionally EpCam, and the detection agents comprise one or more general vesicle biomarker, e.g., a tetraspanin such as CD9, CD63 and CD81. In another embodiment, the capture agents comprise TMEM211 and CD24, and the detection agents comprise one or more tetraspanin such as CD9, CD63 and CD81. In another embodiment, the capture agents comprise CD66 and EpCam, and the detection agents comprise one or more tetraspanin such as CD9, CD63 and CD81. The capture agent and/or detection agent can be to an antigen comprising one or more of CD9, Erb2, Erb4, CD81, Erb3, MUC16, CD63, DLL4, HLA-Drpe, B7H3, IFNAR, 5T4, PCSA, MICB, PSMA, MFG-E8, Muc1, PSA, Muc2, Unc93a, VEGFR2, EpCAM, VEGF A, TMPRSS2, RAGE*, PSCA, CD40, Muc17, IL-17-RA, and CD80. For example, capture agent and/or detection agent can be to one or more of CD9, CD63, CD81, B7H3, PCSA, MFG-E8, MUC2, EpCam, RAGE and Muc17. Increasing numbers of such tetraspanins and/or other general vesicle markers can improve the detection signal in some cases. Proteins or other circulating biomarkers can also be detected using sandwich approaches. The captured vesicles can be collected and used to analyze the payload contained therein, e.g., mRNA, microRNAs, DNA and soluble protein.

[00447] In some embodiments, the capture agent binds or targets EpCam, B7H3, RAGE or CD24, and the one or more biomarkers detected on the vesicle are CD9 and/or CD63. In one embodiment, the capture agent binds or targets EpCam, and the one or more biomarkers detected on the vesicle are CD9, EpCam and/or CD81. The single capture agent can be selected from CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, or 5T4. The single capture agent can also be an antibody to DR3, STEAP, epha2, TMEM211, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, MFG-E8, TF, Annexin V or TETS. In some embodiments, the single capture agent is selected from PCSA, PSMA, B7H3, CD81, CD9 and CD63.

[00448] In other embodiments, the capture agent targets PCSA, and the one or more biomarkers detected on the captured vesicle are B7H3 and/or PSMA. In other embodiments, the capture agent targets PSMA, and the one or more biomarkers detected on the captured vesicle are B7H3 and/or PCSA. In other embodiments, the capture agent targets B7H3, and the one or more biomarkers detected on the captured vesicle are PSMA and/or PCSA. In yet other embodiments, the capture agent targets CD63 and the one or more biomarkers detected on the vesicle are CD81, CD83, CD9 and/or CD63. The different capture agent and biomarker combinations disclosed herein can be used to characterize a phenotype, such as detecting, diagnosing or prognosing a disease, e.g., a cancer. In some embodiments, vesicles are analyzed to characterize prostate cancer using a capture agent targeting EpCam and detection of CD9 and CD63; a capture agent targeting PCSA and detection of B7H3 and PSMA; or a capture agent of CD63 and detection of CD81. In other embodiments, vesicles are used to characterize colon cancer using capture agent targeting CD63 and detection of CD63, or a capture agent targeting CD9 coupled with detection of CD63. One of skill will appreciate that targets of capture agents and detection agents can be used interchangeably. In an illustrative example, consider a capture agent targeting PCSA and detection agents targeting B7H3 and PSMA. Because all of these markers are useful for detecting PCa derived vesicles, B7H3 or PSMA could be targeted by the capture agent and PCSA could be recognized by a detection agent. For example, in some embodiments, the detection agent targets PCSA, and one or more biomarkers used to capture the vesicle comprise B7H3 and/or PSMA. In other embodiments, the detection agent

targets PSMA, and the one or more biomarkers used to capture the vesicle comprise B7H3 and/or PCSA. In other embodiments, the detection agent targets B7H3, and the one or more biomarkers used to capture the vesicle comprise PSMA and/or PCSA. In some embodiments, the invention provides a method of detecting prostate cancer cells in bodily fluid using capture agents and/or detection agents to PSMA, B7H3 and/or PCSA. The bodily fluid can comprise blood, including serum or plasma. The bodily fluid can comprise ejaculate or sperm. In further embodiments, the methods of detecting prostate cancer further use capture agents and/or detection agents to CD81, CD83, CD9 and/or CD63. The method further provides a method of characterizing a GI disorder, comprising capturing vesicles with one or more of DR3, STEAP, epha2, TMEM211, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, and TETS, and detecting the captured vesicles with one or more general vesicle antigen, such as CD81, CD63 and/or CD9. Additional agents can improve the test performance, e.g., improving test accuracy or AUC, either by providing additional biological discriminatory power and/or by reducing experimental noise.

[00449] Techniques of detecting biomarkers for use with the invention include the use of a planar substrate such as an array (e.g., biochip or microarray), with molecules immobilized to the substrate as capture agents that facilitate the detection of a particular biosignature. The array can be provided as part of a kit for assaying one or more biomarkers or vesicles. A molecule that identifies the biomarkers described above and shown in **Figs. 1 or 3-60** of International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein, can be included in an array for detection and diagnosis of diseases including presymptomatic diseases. In some embodiments, an array comprises a custom array comprising biomolecules selected to specifically identify biomarkers of interest. Customized arrays can be modified to detect biomarkers that increase statistical performance, e.g., additional biomolecules that identifies a biosignature which lead to improved cross-validated error rates in multivariate prediction models (e.g., logistic regression, discriminant analysis, or regression tree models). In some embodiments, customized array(s) are constructed to study the biology of a disease, condition or syndrome and profile biosignatures in defined physiological states. Markers for inclusion on the customized array be chosen based upon statistical criteria, e.g., having a desired level of statistical significance in differentiating between phenotypes or physiological states. In some embodiments, standard significance of p-value = 0.05 is chosen to exclude or include biomolecules on the microarray. The p-values can be corrected for multiple comparisons. As an illustrative example, nucleic acids extracted from samples from a subject with or without a disease can be hybridized to a high density microarray that binds to thousands of gene sequences. Nucleic acids whose levels are significantly different between the samples with or without the disease can be selected as biomarkers to distinguish samples as having the disease or not. A customized array can be constructed to detect the selected biomarkers. In some embodiments, customized arrays comprise low density microarrays, which refer to arrays with lower number of addressable binding agents, e.g., tens or hundreds instead of thousands. Low density arrays can be formed on a substrate. In some embodiments, customizable low density arrays use PCR amplification in plate wells, e.g., TaqMan® Gene Expression Assays (Applied Biosystems by Life Technologies Corporation, Carlsbad, CA).

[00450] A planar array generally contains addressable locations (e.g., pads, addresses, or micro-locations) of biomolecules in an array format. The size of the array will depend on the composition and end use of the array. Arrays can be made containing from 2 different molecules to many thousands. Generally, the array comprises from two to as many as 100,000 or more molecules, depending on the end use of the array and the method of manufacture. A microarray for use with the invention comprises at least one biomolecule that identifies or captures a biomarker present in a biosignature of interest, e.g., a microRNA or other biomolecule or vesicle that makes up the biosignature. In some

arrays, multiple substrates are used, either of different or identical compositions. Accordingly, planar arrays may comprise a plurality of smaller substrates.

[00451] The present invention can make use of many types of arrays for detecting a biomarker, e.g., a biomarker associated with a biosignature of interest. Useful arrays or microarrays include without limitation DNA microarrays, such as cDNA microarrays, oligonucleotide microarrays and SNP microarrays, microRNA arrays, protein microarrays, antibody microarrays, tissue microarrays, cellular microarrays (also called transfection microarrays), chemical compound microarrays, and carbohydrate arrays (glycoarrays). These arrays are described in more detail above. In some embodiments, microarrays comprise biochips that provide high-density immobilized arrays of recognition molecules (e.g., antibodies), where biomarker binding is monitored indirectly (e.g., via fluorescence). **FIG. 2A** shows an illustrative configuration in which capture antibodies against a vesicle antigen of interest are tethered to a surface. The captured vesicles are then detected using detector antibodies against the same or different vesicle antigens of interest. The capture antibodies can be substituted with tethered aptamers as available and desirable. Fluorescent detectors are shown. Other detectors can be used similarly, e.g., enzymatic reaction, detectable nanoparticles, radiolabels, and the like. In other embodiments, an array comprises a format that involves the capture of proteins by biochemical or intermolecular interaction, coupled with detection by mass spectrometry (MS). The vesicles can be eluted from the surface and the payload therein, e.g., microRNA, can be analyzed.

[00452] An array or microarray that can be used to detect one or more biomarkers of a biosignature can be made according to the methods described in U.S. Pat. Nos. 6,329,209; 6,365,418; 6,406,921; 6,475,808; and 6,475,809, and U.S. Patent Application Ser. No. 10/884,269, each of which is herein incorporated by reference in its entirety. Custom arrays to detect specific selections of sets of biomarkers described herein can be made using the methods described in these patents. Commercially available microarrays can also be used to carry out the methods of the invention, including without limitation those from Affymetrix (Santa Clara, CA), Illumina (San Diego, CA), Agilent (Santa Clara, CA), Exiqon (Denmark), or Invitrogen (Carlsbad, CA). Custom and/or commercial arrays include arrays for detection proteins, nucleic acids, and other biological molecules and entities (e.g., cells, vesicles, virii) as described herein.

[00453] In some embodiments, molecules to be immobilized on an array comprise proteins or peptides. One or more types of proteins may be immobilized on a surface. In certain embodiments, the proteins are immobilized using methods and materials that minimize the denaturing of the proteins, that minimize alterations in the activity of the proteins, or that minimize interactions between the protein and the surface on which they are immobilized.

[00454] Array surfaces useful may be of any desired shape, form, or size. Non-limiting examples of surfaces include chips, continuous surfaces, curved surfaces, flexible surfaces, films, plates, sheets, or tubes. Surfaces can have areas ranging from approximately a square micron to approximately 500 cm^2. The area, length, and width of surfaces may be varied according to the requirements of the assay to be performed. Considerations may include, for example, ease of handling, limitations of the material(s) of which the surface is formed, requirements of detection systems, requirements of deposition systems (e.g., arrayers), or the like.

[00455] In certain embodiments, it is desirable to employ a physical means for separating groups or arrays of binding islands or immobilized biomolecules: such physical separation facilitates exposure of different groups or arrays to different solutions of interest. Therefore, in certain embodiments, arrays are situated within microwell plates having any number of wells. In such embodiments, the bottoms of the wells may serve as surfaces for the formation of arrays, or arrays may be formed on other surfaces and then placed into wells. In certain embodiments, such as where a surface without wells is used, binding islands may be formed or molecules may be immobilized on a surface and a gasket

having holes spatially arranged so that they correspond to the islands or biomolecules may be placed on the surface. Such a gasket is preferably liquid tight. A gasket may be placed on a surface at any time during the process of making the array and may be removed if separation of groups or arrays is no longer necessary.

[00456] In some embodiments, the immobilized molecules can bind to one or more biomarkers or vesicles present in a biological sample contacting the immobilized molecules. In some embodiments, the immobilized molecules modify or are modified by molecules present in the one or more vesicles contacting the immobilized molecules. Contacting the sample typically comprises overlaying the sample upon the array.

[00457] Modifications or binding of molecules in solution or immobilized on an array can be detected using detection techniques known in the art. Examples of such techniques include immunological techniques such as competitive binding assays and sandwich assays; fluorescence detection using instruments such as confocal scanners, confocal microscopes, or CCD-based systems and techniques such as fluorescence, fluorescence polarization (FP), fluorescence resonant energy transfer (FRET), total internal reflection fluorescence (TIRF), fluorescence correlation spectroscopy (FCS); colorimetric/spectrometric techniques; surface plasmon resonance, by which changes in mass of materials adsorbed at surfaces are measured; techniques using radioisotopes, including conventional radioisotope binding and scintillation proximity assays (SPA); mass spectroscopy, such as matrix-assisted laser desorption/ionization mass spectroscopy (MALDI) and MALDI-time of flight (TOF) mass spectroscopy; ellipsometry, which is an optical method of measuring thickness of protein films; quartz crystal microbalance (QCM), a very sensitive method for measuring mass of materials adsorbing to surfaces; scanning probe microscopies, such as atomic force microscopy (AFM), scanning force microscopy (SFM) or scanning electron microscopy (SEM); and techniques such as electrochemical, impedance, acoustic, microwave, and IR/Raman detection. See, e.g., *Mere L, et al.,"Miniaturized FRET assays and microfluidics: key components for ultra-high-throughput screening," Drug Discovery Today 4(8):363-369 (1999),* and references cited therein*; Lakowicz J R, Principles of Fluorescence Spectroscopy, 2nd Edition, Plenum Press (1999), or Jain KK: Integrative Omics, Pharmacoproteomics, and Human Body Fluids. In: Thongboonkerd V, ed., ed. Proteomics of Human Body Fluids: Principles, Methods and Applications. Volume 1: Totowa, N.J.: Humana Press, 2007*, each of which is herein incorporated by reference in its entirety.

[00458] Microarray technology can be combined with mass spectroscopy (MS) analysis and other tools. Electrospray interface to a mass spectrometer can be integrated with a capillary in a microfluidics device. For example, one commercially available system contains eTag reporters that are fluorescent labels with unique and well-defined electrophoretic mobilities; each label is coupled to biological or chemical probes via cleavable linkages. The distinct mobility address of each eTag reporter allows mixtures of these tags to be rapidly deconvoluted and quantitated by capillary electrophoresis. This system allows concurrent gene expression, protein expression, and protein function analyses from the same sample *Jain KK: Integrative Omics, Pharmacoproteomics, and Human Body Fluids. In: Thongboonkerd V, ed., ed. Proteomics of Human Body Fluids: Principles, Methods and Applications. Volume 1: Totowa, N.J.: Humana Press, 2007, which* is herein incorporated by reference in its entirety.

[00459] A biochip can include components for a microfluidic or nanofluidic assay. A microfluidic device can be used for isolating or analyzing biomarkers, such as determining a biosignature. Microfluidic systems allow for the miniaturization and compartmentalization of one or more processes for isolating, capturing or detecting a vesicle, detecting a microRNA, detecting a circulating biomarker, detecting a biosignature, and other processes. The microfluidic devices can use one or more detection reagents in at least one aspect of the system, and such a detection reagent can be used to detect one or more biomarkers. In one embodiment, the device detects a biomarker on an

isolated or bound vesicle. Various probes, antibodies, proteins, or other binding agents can be used to detect a biomarker within the microfluidic system. The detection agents may be immobilized in different compartments of the microfluidic device or be entered into a hybridization or detection reaction through various channels of the device.

[00460] A vesicle in a microfluidic device can be lysed and its contents detected within the microfluidic device, such as proteins or nucleic acids, e.g., DNA or RNA such as miRNA or mRNA. The nucleic acid may be amplified prior to detection, or directly detected, within the microfluidic device. Thus microfluidic system can also be used for multiplexing detection of various biomarkers. In an embodiment, vesicles are captured within the microfluidic device, the captured vesicles are lysed, and a biosignature of microRNA from the vesicle payload is determined. The biosignature can further comprise the capture agent used to capture the vesicle.

[00461] Novel nanofabrication techniques are opening up the possibilities for biosensing applications that rely on fabrication of high-density, precision arrays, e.g., nucleotide-based chips and protein arrays otherwise know as heterogeneous nanoarrays. Nanofluidics allows a further reduction in the quantity of fluid analyte in a microchip to nanoliter levels, and the chips used here are referred to as nanochips. (See, e.g., *Unger M et al., Biotechniques 1999; 27(5):1008-14, Kartalov EP et al., Biotechniques 2006; 40(1):85-90*, each of which are herein incorporated by reference in their entireties.) Commercially available nanochips currently provide simple one step assays such as total cholesterol, total protein or glucose assays that can be run by combining sample and reagents, mixing and monitoring of the reaction. Gel-free analytical approaches based on liquid chromatography (LC) and nanoLC separations (*Cutillas et al. Proteomics, 2005;5:101-112 and Cutillas et al., Mol Cell Proteomics 2005;4:1038-1051*, each of which is herein incorporated by reference in its entirety) can be used in combination with the nanochips.

[00462] An array suitable for identifying a disease, condition, syndrome or physiological status can be included in a kit. A kit can include, as non-limiting examples, one or more reagents useful for preparing molecules for immobilization onto binding islands or areas of an array, reagents useful for detecting binding of a vesicle to immobilized molecules, and instructions for use.

[00463] Further provided herein is a rapid detection device that facilitates the detection of a particular biosignature in a biological sample. The device can integrate biological sample preparation with polymerase chain reaction (PCR) on a chip. The device can facilitate the detection of a particular biosignature of a vesicle in a biological sample, and an example is provided as described in *Pipper et al., Angewandte Chemie, 47(21), p. 3900-3904 (2008)*, which is herein incorporated by reference in its entirety. A biosignature can be incorporated using micro-/nano-electrochemical system (MEMS/NEMS) sensors and oral fluid for diagnostic applications as described in *Li et al., Adv Dent Res 18(1): 3-5 (2005),* which is herein incorporated by reference in its entirety.

[00464] As an alternative to planar arrays, assays using particles, such as bead based assays as described herein, can be used in combination with flow cytometry. Multiparametric assays or other high throughput detection assays using bead coatings with cognate ligands and reporter molecules with specific activities consistent with high sensitivity automation can be used. In a bead based assay system, a binding agent for a biomarker or vesicle, such as a capture agent (e.g. capture antibody), can be immobilized on an addressable microsphere. Each binding agent for each individual binding assay can be coupled to a distinct type of microsphere (i.e., microbead) and the assay reaction takes place on the surface of the microsphere, such as depicted in **FIG. 2B**. A binding agent for a vesicle can be a capture antibody coupled to a bead. Dyed microspheres with discrete fluorescence intensities are loaded separately with their appropriate binding agent or capture probes. The different bead sets carrying different binding agents can be pooled as necessary to generate custom bead arrays. Bead arrays are then incubated with the sample in a single reaction vessel to perform the

assay. Examples of microfluidic devices that may be used, or adapted for use with the invention, include but are not limited to those described herein.

[00465] Product formation of the biomarker with an immobilized capture molecule or binding agent can be detected with a fluorescence based reporter system (see for example, **FIG. 2A-B**). The biomarker can either be labeled directly by a fluorophore or detected by a second fluorescently labeled capture biomolecule. The signal intensities derived from captured biomarkers can be measured in a flow cytometer. The flow cytometer can first identify each microsphere by its individual color code. For example, distinct beads can be dyed with discrete fluorescence intensities such that each bead with a different intensity has a different binding agent. The beads can be labeled or dyed with at least 2 different labels or dyes. In some embodiments, the beads are labeled with at least 3, 4, 5, 6, 7, 8, 9, or 10 different labels. The beads with more than one label or dye can also have various ratios and combinations of the labels or dyes. The beads can be labeled or dyed externally or may have intrinsic fluorescence or signaling labels.

[00466] The amount of captured biomarkers on each individual bead can be measured by the second color fluorescence specific for the bound target. This allows multiplexed quantitation of multiple targets from a single sample within the same experiment. Sensitivity, reliability and accuracy are compared or can be improved to standard microtiter ELISA procedures. An advantage of a bead-based system is the individual coupling of the capture biomolecule or binding agent for a vesicle to distinct microspheres provides multiplexing capabilities. For example, as depicted in **FIG. 2C**, a combination of 5 different biomarkers to be detected (detected by antibodies to antigens such as CD63, CD9, CD81, B7H3, and EpCam) and 20 biomarkers for which to capture a vesicle, (using capture antibodies, such as antibodies to CD9, PSCA, TNFR, CD63, B7H3, MFG-E8, EpCam, Rab, CD81, STEAP, PCSA, PSMA, 5T4, and/or CD24) can result in approximately 100 combinations to be detected. As shown in **FIG. 2C** as "EpCam 2x," "CD63 2X," multiple antibodies to a single target can be used to probe detection against various epitopes. In another example, multiplex analysis comprises capturing a vesicle using a binding agent to CD24 and detecting the captured vesicle using a binding agent for CD9, CD63, and/or CD81. The captured vesicles can be detected using a detection agent such as an antibody. The detection agents can be labeled directly or indirectly, as described herein.

[00467] Multiplexing of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 different biomarkers may be performed. For example, an assay of a heterogeneous population of vesicles can be performed with a plurality of particles that are differentially labeled. There can be at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 differentially labeled particles. The particles may be externally labeled, such as with a tag, or they may be intrinsically labeled. Each differentially labeled particle can be coupled to a capture agent, such as a binding agent, for a vesicle, resulting in capture of a vesicle. The multiple capture agents can be selected to characterize a phenotype of interest, including capture agents against general vesicle biomarkers, cell-of-origin specific biomarkers, and disease biomarkers. One or more biomarkers of the captured vesicle can then be detected by a plurality of binding agents. The binding agent can be directly labeled to facilitate detection. Alternatively, the binding agent is labeled by a secondary agent. For example, the binding agent may be an antibody for a biomarker on the vesicle. The binding agent is linked to biotin. A secondary agent comprises streptavidin linked to a reporter and can be added to detect the biomarker. In some embodiments, the captured vesicle is assayed for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75 or 100 different biomarkers. For example, multiple detectors, i.e., detection of multiple biomarkers of a captured vesicle or population of vesicles, can increase the signal obtained, permitted increased sensitivity, specificity, or both, and the use of smaller amounts of samples. For example, detection with more than one general vesicle marker can improve the signal as compared to using a lesser number of detection

markers, such as a single marker. To illustrate, detection of vesicles with labeled binding agents to two or three of CD9, CD63 and CD81 can improve the signal compared to detection with any one of the tetraspanins individually.

[00468] An immunoassay based method or sandwich assay can also be used to detect a biomarker of a vesicle. An example includes ELISA. A binding agent or capture agent can be bound to a well. For example an antibody to an antigen of a vesicle can be attached to a well. A biomarker on the captured vesicle can be detected based on the methods described herein. **FIG. 2A** shows an illustrative schematic for a sandwich-type of immunoassay. The capture antibody can be against a vesicle antigen of interest, e.g., a general vesicle biomarker, a cell-of-origin marker, or a disease marker. In the figure, the captured vesicles are detected using fluorescently labeled antibodies against vesicle antigens of interest. Multiple capture antibodies can be used, e.g., in distinguishable addresses on an array or different wells of an immunoassay plate. The detection antibodies can be against the same antigen as the capture antibody, or can be directed against other markers. The capture antibodies can be substituted with alternate binding agents, such as tethered aptamers or lectins, and/or the detector antibodies can be similarly substituted, e.g., with detectable (e.g., labeled) aptamers, lectins or other binding proteins or entities. In an embodiment, one or more capture agents to a general vesicle biomarker, a cell-of-origin marker, and/or a disease marker are used along with detection agents against general vesicle biomarker, such as tetraspanin molecules including without limitation one or more of CD9, CD63 and CD81.

[00469] **FIG. 2D** presents an illustrative schematic for analyzing vesicles according to the methods of the invention. Capture agents are used to capture vesicles, detectors are used to detect the captured vesicles, and the level or presence of the captured and detected antibodies is used to characterize a phenotype. Capture agents, detectors and characterizing phenotypes can be any of those described herein. For example, capture agents include antibodies or aptamers tethered to a substrate that recognize a vesicle antigen of interest, detectors include labeled antibodies or aptamers to a vesicle antigen of interest, and characterizing a phenotype includes a diagnosis, prognosis, or theranosis of a disease. In the scheme shown in **FIG. 2D i)**, a population of vesicles is captured with one or more capture agents against general vesicle biomarkers (**6300**). The captured vesicles are then labeled with detectors against cell-of-origin biomarkers (**6301**) and/or disease specific biomarkers (**6302**). If only cell-of-origin detectors are used (**6301**), the biosignature used to characterize the phenotype (**6303**) can include the general vesicle markers (**6300**) and the cell-of-origin biomarkers (**6301**). If only disease detectors are used (**6302**), the biosignature used to characterize the phenotype (**6303**) can include the general vesicle markers (**6300**) and the disease biomarkers (**6302**). Alternately, detectors are used to detect both cell-of-origin biomarkers (**6301**) and disease specific biomarkers (**6302**). In this case, the biosignature used to characterize the phenotype (**6303**) can include the general vesicle markers (**6300**), the cell-of-origin biomarkers (**6301**) and the disease biomarkers (**6302**). The biomarkers combinations are selected to characterize the phenotype of interest and can be selected from the biomarkers and phenotypes described herein.

[00470] In the scheme shown in **FIG. 2D ii)**, a population of vesicles is captured with one or more capture agents against cell-of-origin biomarkers (**6310**) and/or disease biomarkers (**6311**). The captured vesicles are then detected using detectors against general vesicle biomarkers (**6312**). If only cell-of-origin capture agents are used (**6310**), the biosignature used to characterize the phenotype (**6313**) can include the cell-of-origin biomarkers (**6310**) and the general vesicle markers (**6312**). If only disease biomarker capture agents are used (**6311**), the biosignature used to characterize the phenotype (**6313**) can include the disease biomarkers (**6311**) and the general vesicle biomarkers (**6312**). Alternately, capture agents to one or more cell-of-origin biomarkers (**6310**) and one or more disease specific biomarkers (**6311**) are used to capture vesicles. In this case, the biosignature used to characterize the phenotype (**6313**) can include the cell-of-

origin biomarkers (**6310**), the disease biomarkers (**6311**), and the general vesicle markers (**6313**). The biomarkers combinations are selected to characterize the phenotype of interest and can be selected from the biomarkers and phenotypes described herein.

[00471] Biomarkers comprising vesicle payload can be analyzed to characterize a phenotype. Payload comprises the biological entities contained within a vesicle membrane. These entities include without limitation nucleic acids, e.g., mRNA, microRNA, or DNA fragments; protein, e.g., soluble and membrane associated proteins; carbohydrates; lipids; metabolites; and various small molecules, e.g., hormones. The payload can be part of the cellular milieu that is encapsulated as a vesicle is formed in the cellular environment. In some embodiments of the invention, the payload is analyzed in addition to detecting vesicle surface antigens. Specific populations of vesicles can be captured as described above then the payload in the captured vesicles can be used to characterize a phenotype. For example, vesicles captured on a substrate can be further isolated to assess the payload therein. Alternately, the vesicles in a sample are detected and sorted without capture. The vesicles so detected can be further isolated to assess the payload therein. In an embodiment, vesicle populations are sorted by flow cytometry and the payload in the sorted vesicles is analyzed. In the scheme shown in **FIG. 2E iii)**, a population of vesicles is captured and/or detected (**6320**) using one or more of cell-of-origin biomarkers (**6320**), disease biomarkers (**6321**), and general vesicle markers (**6322**). The vesicles can also be detected using one or more of angiogenic or immunomodulatory biomarkers.The payload of the isolated vesicles is assessed (**6323**). A biosignature detected within the payload can be used to characterize a phenotype (**6324**). In a non-limiting example, a vesicle population can be analyzed in a plasma sample from a patient using antibodies against one or more vesicle antigens of interest. The antibodies can be capture antibodies which are tethered to a substrate to isolate a desired vesicle population. Alternately, the antibodies can be directly labeled and the labeled vesicles isolated by sorting with flow cytometry. The presence or level of microRNA or mRNA extracted from the isolated vesicle population can be used to detect a biosignature. The biosignature is then used to diagnose, prognose or theranose the patient.

[00472] In other embodiments, vesicle payload is analyzed in a vesicle population without first capturing or detected subpopulations of vesicles. For example, vesicles can be generally isolated from a sample using centrifugation, filtration, chromatography, or other techniques as described herein. The payload of the isolated vesicles can be analyzed thereafter to detect a biosignature and characterize a phenotype. In the scheme shown in **FIG. 2E iv)**, a population of vesicles is isolated (**6330**) and the payload of the isolated vesicles is assessed (**6331**). A biosignature detected within the payload can be used to characterize a phenotype (**6332**). In a non-limiting example, a vesicle population is isolated from a plasma sample from a patient using size exclusion and membrane filtration. The presence or level of microRNA or mRNA extracted from the vesicle population is used to detect a biosignature. The biosignature is then used to diagnose, prognose or theranose the patient.

[00473] Another illustrative scheme for characterizing a phenotype is shown in **FIG. 2F v)**. One or more vesicle of interest is captured and detected using a combination of cell-of-origin biomarkers (**6340**) and disease biomarkers (**6341**). For example, the vesicles of interest can be captured using a cell-of-origin (**6340**) biomarker and detected using a disease-specific (**6341**) biomarker. Similarly, the vesicles of interest can be captured using a disease-specific (**6341**) biomarker and detected using a cell-of-origin (**6340**) biomarker. If appropriate, the vesicle of interest can be captured and detected using only cell-of-origin (**6340**) biomarkers or only disease-specific (**6341**) biomarkers. In this case, the same biomarker could be used for capture and detection (e.g., anti-EpCAM capture and anti-EpCAM detector, or anti-PCSA capture and anti-PCSA detector, etc.), or different biomarkers from the same class can be used for capture and

detection (e.g., anti-EpCAM capture and anti-B7H3 detector, or anti-PCSA capture and anti-PSMA detector, etc.). The phenotype can be characterized based on the detected vesicles. Optionally, payload (**6342**) in the vesicles of interest can be assessed in order to characterize the phenotype.

[00474] The methods of characterizing a phenotype can employ a combination of techniques to assess a vesicle population in a sample of interest. In an embodiment, the sample is split into various aliquots and each is analyzed separately. For example, protein content of one or more aliquot is determined and microRNA content of one or more other aliquot is determined. The protein content and microRNA content can be combined to characterize a phenotype. In another embodiment, vesicles of interest are isolated and the payload therein is assessed. For example, a population of vesicles with a given surface marker can be isolated by affinity isolation such as flow cytometry, immunoprecipitation, or other immunocapture technique using a binding agent to the surface marker of interest. The isolated vesicles can then be assessed for biomarkers such as surface content or payload. The biomarker profile of vesicles having the given surface marker can be used to characterize a phenotype. As a non-limiting example, a PCSA+ capture agent can be used to isolate a prostate specific vesicle population. Levels of surface antigens such as PCSA itself, PSMA, B7H3, or EpCam can be assessed from the PCSA+ vesicles. Levels of payload in the PCSA+ can also be assessed, e.g., microRNA or mRNA content. A biosignature can be constructed from a combination of the markers in the PCSA+ vesicle population.

[00475] In an embodiment, the invention provides a method of isolating a microvesicle population and assessing the microRNA with the isolated microvesicles. The microvesicle can be bound in a microtiter plate well that has been coated with a binding agent to a general vesicle biomarker, a cell-of-origin vesicle biomarker, or a disease-specific vesicle biomarker. As desired, vesicles in the wells can be detected using one or more detector agent to a general vesicle biomarker, a cell-of-origin vesicle biomarker, or a disease-specific vesicle biomarker. RNA can be isolated from microvesicles in wells that comprise the vesicles of interest. MicroRNA or miRNA content derived from the microvesicles are then detected. The presence or levels of the vesicle markers and RNA markers can be used to construct a biosignature as described herein. The biosignature can be used to characterize a phenotype of interest.

[00476] In another embodiment, contaminants are removed from a biological sample and the remaining vesicles are assessed for surface content and/or payload. For example, a column can be constructed comprising binding agents to contaminating proteins, vesicles, or other entities in the biological sample. The flow through will thereby be enriched in the circulating biomarkers or circulating microvesicles of interest. In a non-limiting example, a column is constructed to remove microvesicles derived from blood cells. The column can be used to enrich microvesicles in a blood sample that are derived from non-blood cell origin. The enrichment scheme can be used to remove protein aggregates, nucleic acids in solution, etc. One of skill will appreciate that this enrichment can be used with other vesicle or biomarkers methodology presented herein to assess vesicle or biomarkers or interest. To continue the non-limiting example, the flow through that has been depleted in vesicles from blood cells can then be analyzed via a positive selection for vesicles of interest using affinity techniques or the like.

[00477] A peptide or protein biomarker can be analyzed by mass spectrometry or flow cytometry. Proteomic analysis of a vesicle may be carried out by immunocytochemical staining, Western blotting, electrophoresis, SDS-PAGE, chromatography, x-ray crystallography or other protein analysis techniques in accordance with procedures well known in the art. In other embodiments, the protein biosignature of a vesicle may be analyzed using 2 D differential gel electrophoresis as described in, *Chromy et al. J Proteome Res, 2004;3:1120-1127,* which is herein incorporated by reference in its entirety, or with liquid chromatography mass spectrometry as described in *Zhang et al. Mol Cell*

Proteomics, 2005;4:144-155, which is herein incorporated by reference in its entirety. A vesicle may be subjected to activity-based protein profiling described for example, in *Berger et al., Am J Pharmacogenomics, 2004;4:371-381*, which is in incorporated by reference in its entirety. In other embodiments, a vesicle may be profiled using nanospray liquid chromatography-tandem mass spectrometry as described in *Pisitkun et al., Proc Natl Acad Sci U S A, 2004; 101:13368-13373,* which is herein incorporated by reference in its entirety. In another embodiment, the vesicle may be profiled using tandem mass spectrometry (MS) such as liquid chromatography/MS/MS (LC-MS/MS) using for example a LTQ and LTQ-FT ion trap mass spectrometer. Protein identification can be determined and relative quantitation can be assessed by comparing spectral counts as described in *Smalley et al., J Proteome Res, 2008;7:2088-2096*, which is herein incorporated by reference in its entirety.

[00478] The expression of circulating protein biomarkers or protein payload within a vesicle can also be identified. The latter analysis can optionally follow the isolation of specific vesicles using capture agents to capture populations of interest. In an embodiment, immunocytochemical staining is used to analyze protein expression. The sample can be resuspended in buffer, centrifuged at 100 x g for example, for 3 minutes using a cytocentrifuge on adhesive slides in preparation for immunocytochemical staining. The cytospins can be air-dried overnight and stored at -80°C until staining. Slides can then be fixed and blocked with serum-free blocking reagent. The slides can then be incubated with a specific antibody to detect the expression of a protein of interest. In some embodiments, the vesicles are not purified, isolated or concentrated prior to protein expression analysis.

[00479] Biosignatures comprising vesicle payload can be characterized by analysis of a metabolite marker or metabolite within the vesicle. Various metabolite-oriented approaches have been described such as metabolite target analyses, metabolite profiling, or metabolic fingerprinting, see for example, *Denkert et al., Molecular Cancer 2008; 7: 4598-4617, Ellis et al., Analyst 2006; 8: 875-885, Kuhn et al., Clinical Cancer Research 2007; 24: 7401-7406, Fiehn O., Comp Funct Genomics 2001;2:155-168, Fancy et al., Rapid Commun Mass Spectrom 20(15): 2271-80 (2006), Lindon et al., Pharm Res, 23(6): 1075-88 (2006), Holmes et al., Anal Chem. 2007 Apr 1;79(7):2629-40. Epub 2007 Feb 27. Erratum in: Anal Chem. 2008 Aug 1;80(15):6142-3, Stanley et al., Anal Biochem. 2005 Aug 15;343(2):195-202., Lehtimäki et al., J Biol Chem. 2003 Nov 14;278(46):45915-23*, each of which is herein incorporated by reference in its entirety.

[00480] Peptides can be analyzed by systems described in *Jain KK: Integrative Omics, Pharmacoproteomics, and Human Body Fluids. In: Thongboonkerd V, ed., ed. Proteomics of Human Body Fluids: Principles, Methods and Applications. Volume 1: Totowa, N.J.: Humana Press, 2007,* which is herein incorporated by reference in its entirety. This system can generate sensitive molecular fingerprints of proteins present in a body fluid as well as in vesicles. Commercial applications which include the use of chromatography/mass spectroscopy and reference libraries of all stable metabolites in the human body, for example Paradigm Genetic's Human Metabolome Project, may be used to determine a metabolite biosignature. Other methods for analyzing a metabolic profile can include methods and devices described in U.S. Patent No. 6,683,455 (Metabometrix), U.S. Patent Application Publication Nos. 20070003965 and 20070004044 (Biocrates Life Science), each of which is herein incorporated by reference in its entirety. Other proteomic profiling techniques are described in *Kennedy, Toxicol Lett 120:379-384 (2001), Berven et al., Curr Pharm Biotechnol 7(3): 147-58 (2006), Conrads et al., Expert Rev Proteomics 2(5): 693-703, Decramer et al., World J Urol 25(5): 457-65 (2007), Decramer et al., Mol Cell Proteomics 7(10): 1850-62 (2008), Decramer et al., Contrib Nephrol, 160: 127-41 (2008), Diamandis, J Proteome Res 5(9): 2079-82 (2006), Immler et al., Proteomics 6(10): 2947-58 (2006), Khan et al., J Proteome Res 5(10): 2824-38 (2006), Kumar et al., Biomarkers 11(5): 385-405 (2006), Noble et*

al., Breast Cancer Res Treat 104(2): 191-6 (2007), Omenn, Dis Markers 20(3): 131-4 (2004), Powell et al., Expert Rev Proteomics 3(1): 63-74 (2006), Rai et al., Arch Pathol Lab Med, 126(12): 1518-26 (2002), Ramstrom et al., Proteomics, 3(2): 184-90 (2003), Tammen et al., Breast Cancer Res Treat, 79(1): 83-93 (2003), Theodorescu et al., Lancet Oncol, 7(3): 230-40 (2006), or Zurbig et al., Electrophoresis, 27(11): 2111-25 (2006).

[00481] For analysis of mRNAs, miRNAs or other small RNAs, the total RNA can be isolated using any known methods for isolating nucleic acids such as methods described in U.S. Patent Application Publication No. 2008132694, which is herein incorporated by reference in its entirety. These include, but are not limited to, kits for performing membrane based RNA purification, which are commercially available. Generally, kits are available for the small-scale (30 mg or less) preparation of RNA from cells and tissues, for the medium scale (250 mg tissue) preparation of RNA from cells and tissues, and for the large scale (1 g maximum) preparation of RNA from cells and tissues. Other commercially available kits for effective isolation of small RNA-containing total RNA are available. Such methods can be used to isolate nucleic acids from vesicles.

[00482] Alternatively, RNA can be isolated using the method described in U.S. Patent No. 7,267,950, which is herein incorporated by reference in its entirety. U.S. Patent No. 7,267,950 describes a method of extracting RNA from biological systems (cells, cell fragments, organelles, tissues, organs, or organisms) in which a solution containing RNA is contacted with a substrate to which RNA can bind and RNA is withdrawn from the substrate by applying negative pressure. Alternatively, RNA may be isolated using the method described in U.S. Patent Application No. 20050059024, which is herein incorporated by reference in its entirety, which describes the isolation of small RNA molecules. Other methods are described in U.S. Patent Application No. 20050208510, 20050277121, 20070238118, each of which is incorporated by reference in its entirety.

[00483] In one embodiment, mRNA expression analysis can be carried out on mRNAs from a vesicle isolated from a sample. In some embodiments, the vesicle is a cell-of-origin specific vesicle. An expression pattern generated from a vesicle can be indicative of a given disease state, disease stage, therapy related signature, or physiological condition.

[00484] In one embodiment, once the total RNA has been isolated, cDNA can be synthesized and either qRT-PCR assays (e.g. Applied Biosystem's Taqman® assays) for specific mRNA targets can be performed according to manufacturer's protocol, or an expression microarray can be performed to look at highly multiplexed sets of expression markers in one experiment. Methods for establishing gene expression profiles include determining the amount of RNA that is produced by a gene that can code for a protein or peptide. This can be accomplished by quantitative reverse transcriptase PCR (qRT-PCR), competitive RT-PCR, real time RT-PCR, differential display RT-PCR, Northern Blot analysis or other related tests. While it is possible to conduct these techniques using individual PCR reactions, it is also possible to amplify complementary DNA (cDNA) or complementary RNA (cRNA) produced from mRNA and analyze it via microarray.

[00485] The level of a miRNA product in a sample can be measured using any appropriate technique that is suitable for detecting mRNA expression levels in a biological sample, including but not limited to Northern blot analysis, RT-PCR, qRT-PCR, in situ hybridization or microarray analysis. For example, using gene specific primers and target cDNA, qRT-PCR enables sensitive and quantitative miRNA measurements of either a small number of target miRNAs (via singleplex and multiplex analysis) or the platform can be adopted to conduct high throughput measurements using 96-well or 384-well plate formats. See for example, *Ross JS et al, Oncologist. 2008 May;13(5):477-93,* which is herein incorporated by reference in its entirety. A number of different array configurations and methods for microarray production are known to those of skill in the art and are described in U.S. patents such as: U.S. Pat. Nos. 5,445,934;

5,532,128; 5,556,752; 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,436,327; 5,472,672; 5,527,681; 5,529,756; 5,545,531; 5,554,501; 5,561,071; 5,571,639; 5,593,839; 5,599,695; 5,624,711; 5,658,734; or 5,700,637; each of which is herein incorporated by reference in its entirety. Other methods of profiling miRNAs are described in *Taylor et al., Gynecol Oncol. 2008 Jul;110(1):13-21, Gilad et al, PLoS ONE. 2008 Sep 5;3(9):e3148, Lee et al., Annu Rev Pathol. 2008 Sep 25 and Mitchell et al, Proc Natl Acad Sci U S A. 2008 Jul 29;105(30):10513-8, Shen R et al, BMC Genomics. 2004 Dec 14;5(1):94, Mina L et al, Breast Cancer Res Treat. 2007 Jun;103(2):197-208, Zhang L et al, Proc Natl Acad Sci U S A. 2008 May 13;105(19):7004-9, Ross JS et al, Oncologist. 2008 May;13(5):477-93, Schetter AJ et al, JAMA. 2008 Jan 30;299(4):425-36, Staudt LM, N Engl J Med 2003;348:1777-85, Mulligan G et al, Blood. 2007 Apr 15;109(8):3177-88. Epub 2006 Dec 21, McLendon R et al, Nature. 2008 Oct 23;455(7216):1061-8,* and U.S. Patent Nos. 5,538,848, 5,723,591, 5,876,930, 6,030,787, 6,258,569, and 5,804,375, each of which is herein incorporated by reference. In some embodiments, arrays of microRNA panels are use to simultaneously query the expression of multiple miRs. The Exiqon mIRCURY LNA microRNA PCR system panel (Exiqon, Inc., Woburn, MA) or the TaqMan® MicroRNA Assays and Arrays systems from Applied Biosystems (Foster City, CA) can be used for such purposes.

[00486] Microarray technology allows for the measurement of the steady-state mRNA or miRNA levels of thousands of transcripts or miRNAs simultaneously thereby presenting a powerful tool for identifying effects such as the onset, arrest, or modulation of uncontrolled cell proliferation. Two microarray technologies, such as cDNA arrays and oligonucleotide arrays can be used. The product of these analyses are typically measurements of the intensity of the signal received from a labeled probe used to detect a cDNA sequence from the sample that hybridizes to a nucleic acid sequence at a known location on the microarray. Typically, the intensity of the signal is proportional to the quantity of cDNA, and thus mRNA or miRNA, expressed in the sample cells. A large number of such techniques are available and useful. Methods for determining gene expression can be found in U.S. Pat. No. 6,271,002 to Linsley, et al.; U.S. Pat. No. 6,218,122 to Friend, et al.; U.S. Pat. No. 6,218,114 to Peck et al.; or U.S. Pat. No. 6,004,755 to Wang, et al., each of which is herein incorporated by reference in its entirety.

[00487] Analysis of an expression level can be conducted by comparing such intensities. This can be performed by generating a ratio matrix of the expression intensities of genes in a test sample versus those in a control sample. The control sample may be used as a reference, and different references to account for age, ethnicity and sex may be used. Different references can be used for different conditions or diseases, as well as different stages of diseases or conditions, as well as for determining therapeutic efficacy.

[00488] For instance, the gene expression intensities of mRNA or miRNAs derived from a diseased tissue, including those isolated from vesicles, can be compared with the expression intensities of the same entities in normal tissue of the same type (e.g., diseased breast tissue sample versus normal breast tissue sample). A ratio of these expression intensities indicates the fold-change in gene expression between the test and control samples. Alternatively, if vesicles are not normally present in from normal tissues (e.g. breast) then absolute quantitation methods, as is known in the art, can be used to define the number of miRNA molecules present without the requirement of miRNA or mRNA isolated from vesicles derived from normal tissue.

[00489] Gene expression profiles can also be displayed in a number of ways. A common method is to arrange raw fluorescence intensities or ratio matrix into a graphical dendogram where columns indicate test samples and rows indicate genes. The data is arranged so genes that have similar expression profiles are proximal to each other. The expression ratio for each gene is visualized as a color. For example, a ratio less than one (indicating down-regulation)

may appear in the blue portion of the spectrum while a ratio greater than one (indicating up-regulation) may appear as a color in the red portion of the spectrum. Commercially available computer software programs are available to display such data.

[00490] mRNAs or miRNAs that are considered differentially expressed can be either over expressed or under expressed in patients with a disease relative to disease free individuals. Over and under expression are relative terms meaning that a detectable difference (beyond the contribution of noise in the system used to measure it) is found in the amount of expression of the mRNAs or miRNAs relative to some baseline. In this case, the baseline is the measured mRNA/miRNA expression of a non-diseased individual. The mRNA/miRNA of interest in the diseased cells can then be either over or under expressed relative to the baseline level using the same measurement method. Diseased, in this context, refers to an alteration of the state of a body that interrupts or disturbs, or has the potential to disturb, proper performance of bodily functions as occurs with the uncontrolled proliferation of cells. Someone is diagnosed with a disease when some aspect of that person's genotype or phenotype is consistent with the presence of the disease. However, the act of conducting a diagnosis or prognosis includes the determination of disease/status issues such as determining the likelihood of relapse or metastasis and therapy monitoring. In therapy monitoring, clinical judgments are made regarding the effect of a given course of therapy by comparing the expression of genes over time to determine whether the mRNA/miRNA expression profiles have changed or are changing to patterns more consistent with normal tissue.

[00491] Levels of over and under expression are distinguished based on fold changes of the intensity measurements of hybridized microarray probes. A 2X difference is preferred for making such distinctions or a p-value less than 0.05. That is, before an mRNA/miRNA is the to be differentially expressed in diseased/relapsing versus normal/non-relapsing cells, the diseased cell is found to yield at least 2 times more, or 2 times less intensity than the normal cells. The greater the fold difference, the more preferred is use of the gene as a diagnostic or prognostic tool. mRNA/miRNAs selected for the expression profiles of the instant invention have expression levels that result in the generation of a signal that is distinguishable from those of the normal or non-modulated genes by an amount that exceeds background using clinical laboratory instrumentation.

[00492] Statistical values can be used to confidently distinguish modulated from non-modulated mRNA/miRNA and noise. Statistical tests find the mRNA/miRNA most significantly different between diverse groups of samples. The Student's t-test is an example of a robust statistical test that can be used to find significant differences between two groups. The lower the p-value, the more compelling the evidence that the gene shows a difference between the different groups. Nevertheless, since microarrays measure more than one mRNA/miRNA at a time, tens of thousands of statistical tests may be performed at one time. Because of this, one is unlikely to see small p-values just by chance and adjustments for this using a Sidak correction as well as a randomization/permutation experiment can be made. A p-value less than 0.05 by the t-test is evidence that the gene is significantly different. More compelling evidence is a p-value less then 0.05 after the Sidak correction is factored in. For a large number of samples in each group, a p-value less than 0.05 after the randomization/permutation test is the most compelling evidence of a significant difference.

[00493] In one embodiment, a method of generating a posterior probability score to enable diagnostic, prognostic, therapy-related, or physiological state specific biosignature scores can be arrived at by obtaining circulating biomarker expression data from a statistically significant number of patients; applying linear discrimination analysis to the data to obtain selected biomarkers; and applying weighted expression levels to the selected biomarkers with discriminate

function factor to obtain a prediction model that can be applied as a posterior probability score. Other analytical tools can also be used to answer the same question such as, logistic regression and neural network approaches.

[00494] For instance, the following can be used for linear discriminant analysis:

where,

$I(p_s i_d)$ = The log base 2 intensity of the probe set enclosed in parenthesis. d(cp) = The discriminant function for the disease positive class $d(C_N)$ = The discriminant function for the disease negative class

$P_{(CP)}$ = The posterior p-value for the disease positive class

$P_{(CN)}$ = The posterior p-value for the disease negative class

[00495] Numerous other well-known methods of pattern recognition are available. The following references provide some examples: *Weighted Voting: Golub et al. (1999); Support Vector Machines: Su et al. (2001); and Ramaswamy et al. (2001); K-nearest Neighbors: Ramaswamy (2001); and Correlation Coefficients: van 't Veer et al. (2002),* all of which are herein incorporated by reference in their entireties.

[00496] A biosignature portfolio, further described below, can be established such that the combination of biomarkers in the portfolio exhibit improved sensitivity and specificity relative to individual biomarkers or randomly selected combinations of biomarkers. In one embodiment, the sensitivity of the biosignature portfolio can be reflected in the fold differences, for example, exhibited by a transcript's expression in the diseased state relative to the normal state. Specificity can be reflected in statistical measurements of the correlation of the signaling of transcript expression with the condition of interest. For example, standard deviation can be a used as such a measurement. In considering a group of biomarkers for inclusion in a biosignature portfolio, a small standard deviation in expression measurements correlates with greater specificity. Other measurements of variation such as correlation coefficients can also be used in this capacity.

[00497] Another parameter that can be used to select mRNA/miRNA that generate a signal that is greater than that of the non-modulated mRNA/miRNA or noise is the use of a measurement of absolute signal difference. The signal generated by the modulated mRNA/miRNA expression is at least 20% different than those of the normal or non-modulated gene (on an absolute basis). It is even more preferred that such mRNA/miRNA produce expression patterns that are at least 30% different than those of normal or non-modulated mRNA/miRNA.

[00498] MiRNA can also be detected and measured by amplification from a biological sample and measured using methods described in U.S. Patent No. 7,250,496, U.S. Application Publication Nos. 20070292878, 20070042380 or 20050222399 and references cited therein, each of which is herein incorporated by reference in its entirety. The microRNA can be assessed as in U.S. Patent No. 7,888,035, entitled "METHODS FOR ASSESSING RNA PATTERNS," issued February 15, 2011, which application is incorporated by reference herein in its entirety.

[00499] The levels of microRNA can be normalized using various techniques known to those of skill in the art. For example, relative quantification of miRNA expression can be performed using the $2^{-\Delta\Delta CT}$ method (Applied Biosystems User Bulletin N°2). The levels of microRNA can also be normalized to housekeeping nucleic acids, such as housekeeping mRNAs, microRNA or snoRNA. Further methods for normalizing miRNA levels that can be used with the invention are described further in Vasilescu, MicroRNA fingerprints identify miR-150 as a plasma prognostic marker in patients with sepsis. PLoS One. 2009 Oct 12;4(10):e7405; and Peltier and Latham, Normalization of microRNA expression levels in quantitative RT-PCR assays: identification of suitable reference RNA targets in normal and cancerous human solid tissues. RNA. 2008 May;14(5):844-52. Epub 2008 Mar 28; each of which reference is herein incorporated by reference in its entirety.

[00500] Peptide nucleic acids (PNAs) which are a new class of synthetic nucleic acid analogs in which the phosphate–sugar polynucleotide backbone is replaced by a flexible pseudo-peptide polymer may be used in analysis of a biosignature. PNAs are capable of hybridizing with high affinity and specificity to complementary RNA and DNA sequences and are highly resistant to degradation by nucleases and proteinases. Peptide nucleic acids (PNAs) are an attractive new class of probes with applications in cytogenetics for the rapid in situ identification of human chromosomes and the detection of copy number variation (CNV). Multicolor peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH) protocols have been described for the identification of several human CNV-related disorders and infectious diseases. PNAs can also be used as molecular diagnostic tools to non-invasively measure oncogene mRNAs with tumor targeted radionuclide-PNA-peptide chimeras. Methods of using PNAs are described further in *Pellestor F et al, Curr Pharm Des. 2008;14(24):2439-44, Tian X et al, Ann N Y Acad Sci. 2005 Nov;1059:106-44, Paulasova P and Pellestor F, Annales de Génétique, 47 (2004) 349–358, Stender H. Expert Rev Mol Diagn. 2003 Sep;3(5):649-55. Review, Vigneault et al., Nature Methods, 5(9), 777 – 779 (2008),* each reference is herein incorporated by reference in its entirety. These methods can be used to screen the genetic materials isolated from a vesicle. When applying these techniques to a cell-of-origin specific vesicle, they can be used to identify a given molecular signal that directly pertains to the cell of origin.

[00501] Mutational analysis may be carried out for mRNAs and DNA, including those that are identified from a vesicle. For mutational analysis of a target or biomarker that is of RNA origin, the RNA (mRNA, miRNA or other) can be reverse transcribed into cDNA and subsequently sequenced or assayed, such as for known SNPs (by Taqman SNP assays, for example) or single nucleotide mutations, as well as using sequencing to look for insertions or deletions to determine mutations present in the cell-of-origin. Multiplexed ligation dependent probe amplification (MLPA) could alternatively be used for the purpose of identifying CNV in small and specific areas of interest. For example, once the total RNA has been obtained from isolated colon cancer-specific vesicles, cDNA can be synthesized and primers specific for exons 2 and 3 of the KRAS gene can be used to amplify these two exons containing codons 12, 13 and 61 of the KRAS gene. The same primers used for PCR amplification can be used for Big Dye Terminator sequence analysis on the ABI 3730 to identify mutations in exons 2 and 3 of KRAS. Mutations in these codons are known to confer resistance to drugs such as Cetuximab and Panitumimab. Methods of conducting mutational analysis are described in *Maheswaran S et al, July 2, 2008 (10.1056/NEJMoa0800668) and Orita, M et al, PNAS 1989, (86): 2766-70,* each of which is herein incorporated by reference in its entirety.

[00502] Other methods of conducting mutational analysis include miRNA sequencing. Applications for identifying and profiling miRNAs can be done by cloning techniques and the use of capillary DNA sequencing or "next-generation" sequencing technologies. The new sequencing technologies currently available allow the identification of low-abundance miRNAs or those exhibiting modest expression differences between samples, which may not be detected by hybridization-based methods. Such new sequencing technologies include the massively parallel signature sequencing (MPSS) methodology described in *Nakano et al. 2006, Nucleic Acids Res. 2006;34:D731–D735. doi: 10.1093/nar/gkj077,* the Roche/454 platform described in *Margulies et al. 2005, Nature. 2005;437:376–380* or the Illumina sequencing platform described in *Berezikov et al. Nat. Genet. 2006b;38:1375–1377*, each of which is incorporated by reference in its entirety.

[00503] Additional methods to determine a biosignature includes assaying a biomarker by allele-specific PCR, which includes specific primers to amplify and discriminate between two alleles of a gene simultaneously, single-strand conformation polymorphism (SSCP), which involves the electrophoretic separation of single-stranded nucleic acids

based on subtle differences in sequence, and DNA and RNA aptamers. DNA and RNA aptamers are short oligonucleotide sequences that can be selected from random pools based on their ability to bind a particular molecule with high affinity. Methods of using aptamers are described in Ulrich H et al, Comb Chem High Throughput Screen. 2006 Sep;9(8):619-32, Ferreira CS et al, Anal Bioanal Chem. 2008 Feb;390(4):1039-50, Ferreira CS et al, Tumour Biol. 2006;27(6):289-301, each of which is herein incorporated by reference in its entirety.

[00504] Biomarkers can also be detected using fluorescence in situ hybridization (FISH). Methods of using FISH to detect and localize specific DNA sequences, localize specific mRNAs within tissue samples or identify chromosomal abnormalities are described in *Shaffer DR et al, Clin Cancer Res. 2007 Apr 1;13(7):2023-9, Cappuzo F et al, Journal of Thoracic Oncology, Volume 2, Number 5, May 2007, Moroni M et al, Lancet Oncol. 2005 May;6(5):279-86*, each of which is herein incorporated by reference in its entirety.

[00505] An illustrative schematic for analyzing a population of vesicles for their payload is presented in **FIG. 2E**. In an embodiment, the methods of the invention include characterizing a phenotype by capturing vesicles (**6330**) and determining a level of microRNA species contained therein (**6331**), thereby characterizing the phenotype (**6332**).

[00506] A biosignature comprising a circulating biomarker or vesicle can comprise a binding agent thereto. The binding agent can be a DNA, RNA, aptamer, monoclonal antibody, polyclonal antibody, Fabs, Fab', single chain antibody, synthetic antibody, aptamer (DNA/RNA), peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, synthetic or naturally occurring chemical compounds (including but not limited to drugs and labeling reagents).

[00507] A binding agent can used to isolate or detect a vesicle by binding to a component of the vesicle, as described above. The binding agent can be used to detect a vesicle, such as for detecting a cell-of-origin specific vesicle. A binding agent or multiple binding agents can themselves form a binding agent profile that provides a biosignature for a vesicle. For example, if a vesicle population is detected or isolated using two, three, four or more binding agents in a differential detection or isolation of a vesicle from a heterogeneous population of vesicles, the particular binding agent profile for the vesicle population provides a biosignature for the particular vesicle population.

[00508] As an illustrative example, a vesicle for characterizing a cancer can be detected with one or more binding agents including, but not limited to, PSA, PSMA, PCSA, PSCA, B7H3, EpCam, TMPRSS2, mAB 5D4, XPSM-A9, XPSM-A10, Galectin-3, E-selectin, Galectin-1, or E4 (IgG2a kappa), or any combination thereof.

[00509] The binding agent can also be for a general vesicle biomarker, such as a "housekeeping protein" or antigen. The biomarker can be CD9, CD63, or CD81. For example, the binding agent can be an antibody for CD9, CD63, or CD81. The binding agent can also be for other proteins, such as for tissue specific or cancer specific vesicles. The binding agent can be for PCSA, PSMA, EpCam, B7H3, or STEAP. The binding agent can be for DR3, STEAP, epha2, TMEM211, MFG-E8, Annexin V, TF, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, or TETS. For example, the binding agent can be an antibody or aptamer for PCSA, PSMA, EpCam, B7H3, DR3, STEAP, epha2, TMEM211, MFG-E8, Annexin V, TF, unc93A, A33, CD24, NGAL, EpCam, MUC17, TROP2, or TETS.

[00510] Various proteins are not typically distributed evenly or uniformly on a vesicle shell. Vesicle-specific proteins are typically more common, while cancer-specific proteins are less common. In some embodiments, capture of a vesicle is accomplished using a more common, less cancer-specific protein, such as one or more housekeeping proteins or antigen or general vesicle antigen (e.g., a tetraspanin), and one or more cancer-specific biomarkers and/or one or more cell-of-origin specific biomarkers is used in the detection phase. In another embodiment, one or more cancer-specific biomarkers and/or one or more cell-of-origin specific biomarkers are used for capture, and one or more

housekeeping proteins or antigen or general vesicle antigen (e.g., a tetraspanin) is used for detection. In embodiments, the same biomarker is used for both capture and detection. Different binding agents for the same biomarker can be used, such as antibodies or aptamers that bind different epitopes of an antigen.

[00511] Additional cellular binding partners or binding agents may be identified by any conventional methods known in the art, or as described herein, and may additionally be used as a diagnostic, prognostic or therapy-related marker. For example, vesicles can be detected using one or more binding agent listed in **Table 3**, **Table 4** or **Table 5** herein. For example, the binding agent can also be for a general vesicle biomarker, such as a "housekeeping protein" or antigen. The general vesicle biomarker can be CD9, CD63, or CD81, or other biomarker in **Table 3**. The binding agent can also be for other proteins, such as for cell of origin specific or cancer specific vesicles. As a non-limiting example, in the case of prostate cancer, the binding agent can be for PCSA, PSMA, EpCam, B7H3, RAGE or STEAP. The binding agent can be for a biomarker in **Table 4** or **Table** 5. For example, the binding agent can be an antibody or aptamer for PCSA, PSMA, EpCam, B7H3, RAGE, STEAP or other biomarker in **Table 4** or **Table** 5.

[00512] Various proteins may not be distributed evenly or uniformly on a vesicle surface. For example, vesicle-specific proteins are typically more common, while cancer-specific proteins are less common. In some embodiments, capture of a vesicle is accomplished using a more common, less cancer-specific protein, such as a housekeeping protein or antigen, and cancer-specific proteins is used in the detection phase. Depending on the sensitivity of the detection system, the opposite method can also be used wherein a large vesicle population is captured using a binding agent to a general vesicle marker and then cell-specific vesicles are detected with detection agents specific to a sub-population of interest.

[00513] Furthermore, additional cellular binding partners or binding agents may be identified by any conventional methods known in the art, or as described herein, and may additionally be used as a diagnostic, prognostic or therapy-related marker.

Biosignatures for Cancer

[00514] As described herein, biosignatures comprising circulating biomarkers can be used to characterize a cancer. The biomarkers can be selected from those disclosed herein. For example, a non-exclusive list of biomarkers that can be used as part of a biosignature are listed in **Table 5**. The biosignature can be used to characterize a cancer, e.g., for prostate, GI, or ovarian cancer. In some embodiments, the circulating biomarkers are associated with a vesicle or with a population of vesicles. For example, circulating biomarkers associated with vesicles can be used to capture and/or to detect a vesicle or a vesicle population.

[00515] It will be appreciated that the biomarkers presented herein, e.g., in **Table 5**, may be useful in biosignatures for other diseases, e.g., other proliferative disorders and cancers of other cellular or tissue origins. For example, transformation in various cell types can be due to common events, e.g., mutation in p53 or other tumor suppressor. A biosignature comprising cell-of-origin biomarkers and cancer biomarkers can be used to further assess the nature of the cancer. Biomarkers for metastatic cancer may be used with cell-of-origin biomarkers to assess a metastatic cancer. Such biomarkers for use with the invention include those in Dawood, Novel biomarkers of metastatic cancer, Exp Rev Mol Diag July 2010, Vol. 10, No. 5, Pages 581-590, which publication is incorporated herein by reference in its entirety.

[00516] For example, a biosignature comprising one or more of miR-378, miR-127-3p, miR-92a, and miR-486-3p can be used to characterize colorectal cancer. The presence of KRAS mutations can be associated with miR expression levels. See, e.g., Mosakhani et al., MicroRNA profiling differentiates colorectal cancer according to KRAS status.

Genes Chromosomes Cancer. 2011 Sep 15. doi: 10.1002/gcc.20925, which publication is incorporated herein by reference in its entirety. For example, KRAS mutations can be associated with up-regulation miR-127-3p, miR-92a, and miR-486-3p and down-regulation of miR-378. Somatic KRAS mutations are found at high rates in various disorders, including without limitation leukemias, colon cancer, pancreatic cancer and lung cancer. KRAS mutations are predictive of poor response to panitumumab and cetuximab therapy. A KRAS+ phenotype is also associated with poor response to anti-EGFR therapies such as erlotinib and/or gefitinib. Thus, in an embodiment, levels of miRs correlated with KRAS status are used as part of a biosignature to provide a theranosis for cancers, e.g., metastatic colorectal cancer or lung cancer.

[00517] As another example, Pgrmc1 can be elevated in lung cancer tissue compared to normal tissue and in the plasma of lung cancer patients compared to non-cancer patients. See, e.g., Mir et al., Elevated Pgrmc1 (progesterone receptor membrane component 1)/sigma-2 receptor levels in lung tumors and plasma from lung cancer patients. Int J Cancer. 2011 Sep 14. doi: 10.1002/ijc.26432, which publication is incorporated herein by reference in its entirety. In an embodiment, a presense or level of circulating Pgrmc1 is assessed in a patient sample in order to characterize a cancer. The cancer can be a lung cancer, including without limitation a squamous cell lung cancer (SCLC) or a lung adenocarcinoma. Elevated levels of Pgrmc1 compared to a control can indicate the presense of the cancer. The sample can be a tissue sample or a bodily fluid, e.g, sputum, peripheral blood, or a blood derivative. In an embodiment, the Pgrmc1 is associated with a population of vesicles.

[00518] The biosignatures of the invention may comprise markers that are upregulated, downregulated, or have no change, depending on the reference. Solely for illustration, if the reference is a normal sample, the biosignature may indicate that the subject is normal if the subject's biosignature is not changed compared to the reference. Alternately, the biosignature may comprise a mutated nucleic acid or amino acid sequence so that the levels of the components in the biosignature are the same between a normal reference and a diseased sample. In another case, the reference can be a cancer sample, such that the subject's biosignature indicates cancer if the subject's biosignature is substantially similar to the reference. The biosignature of the subject can comprise components that are both upregulated and downregulated compared to the reference. Solely for illustration, if the reference is a normal sample, a cancer biosignature can comprise both upregulated oncogenes and downregulated tumor suppressors. Vesicle markers can also be differentially expressed in various settings. For example, tetraspanins may be overexpressed in cancer vesicles compared to non-cancer vesicles, whereas MFG-E8 can be overexpressed in non-cancer vesicles as compared to cancer vesicles.

[00519] In an embodiment, the biosignature comprises one or more cytokine receptor, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, of 4-1BB, ALCAM, B7-1, BCMA, CD14, CD30, CD40 Ligand, CEACAM-1, DR6, Dtk, Endoglin, ErbB3, E-Selectin, Fas, Flt-3L, GITR, HVEM, ICAM-3, IL-1 R4, IL-1 RI, IL-10 Rbeta, IL-17R, IL-2Rgamma, IL-21R, LIMPII, Lipocalin-2, L-Selectin, LYVE-1, MICA, MICB, NRG1-beta1, PDGF Rbeta, PECAM-1, RAGE, TIM-1, TRAIL R3, Trappin-2, uPAR, VCAM-1, and XEDAR. The invention provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent to a cytokine receptor, wherein the cytokine receptor comprises one or more, e.g., 1, 2 or 3, of ErbB3, RAGE, and Trail R3; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent to the cytokine receptor, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The

biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can include, without limitation, a colorectal cancer or a late stage prostate cancer. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the colorectal cancer or late stage prostate cancer.

[00520] The invention also provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more, e.g., 1, 2, 3 or 4, of IL-1 alpha, CA125, Filamin, and Amyloid A; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a colorectal cancer. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the colorectal cancer.

[00521] The invention also provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more receptor, e.g., 1, 2, 3, 4, 5, 6, 7 or 8, of Involucrin, CD57, Prohibitin, Thrombospondin, Laminin B1/b1, Filamin, 14.3.3 gamma, 14.3.3 Pan; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a colorectal cancer and/or a colorectal adenoma. For example, the method can be used to compare levels of the one or more microvesicle between colorectal cancer and/or a colorectal adenoma samples, thereby providing a diagnosis or prognosis of the colorectal cancer and/or a colorectal adenoma, including distinguishing the samples as derived from cancer or adenoma.

[00522] The invention also provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more receptor, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, of Involucrin, Prohibitin, Laminin B1/b1, IL-3, Filamin, 14.3.3 gamma, 14.3.3 Pan, MMP-15 / MT2-MMP, hPL,

Ubiquitin, and mRANKL; and (b) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a colorectal adenoma. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the colorectal adenoma.

[00523] The invention also provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75 or 76, of Prohibitin, CD57, Filamin, CD18, b-2-Microglobulin, IL-2, IL-3, CD16, p170, Keratin 19, Pds1, Glicentin, SRF (Serum Response Factor), E3-binding protein (ARM1), Collagen II, SRC1 (Steroid Receptor Coactivator-1) Ab-1, Caldesmon, GFAP, TRP75 / gp75, alpha-1-antichymotrypsin, Hepatic Nuclear Factor-3B, PLAP, Tyrosinase, NF kappa B / p50, Melanoma (gp100), Cyclin E, 6-Histidine, Mucin 3 (MUC3), TdT, CD21, XPA, Superoxide Dismutase, Glycogen Synthase Kinase 3b (GSK3b), CD54/ICAM-1, Thrombospondin, Gai1, CD79a mb-1, IL-1 beta, Cytochrome c, RAD1, bcl-X, CD50/ICAM-3, Neurofilament, Alkaline Phosphatase (AP), ER Ca+2 ATPase2, PCNA, F.VIII/VWF, SV40 Large T Antigen, Paxillin, Fascin, CD165, GRIP1, Cdk8, Nucleophosmin (NPM), alpha-1-antitrypsin, CD32/Fcg Receptor II, Keratin 8 (phospho-specific Ser73), DR5, CD46, TID-1, MHC II (HLA-DQ), Plasma Cell Marker, DR3, Calmodulin, AIF (Apoptosis Inducing Factor), DNA Polymerase Beta, Vitamin D Receptor (VDR), Bcl10 / CIPER / CLAP / mE10, Neuron Specific Enolase, CXCR4 / Fusin, Neurofilament (68kDa), PDGFR, beta, Growth Hormone (hGH), Mast Cell Chymase, Ret Oncoprotein, and Phosphotyrosine; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a brain cancer. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the brain cancer.

[00524] The invention also provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more, e.g., 1, 2, 3, 4, 5, 6, 7 or 8, of Caspase 5, Thrombospondin, Filamin, Ferritin, 14.3.3 gamma, 14.3.3 Pan, CD71 / Transferrin Receptor, and Prostate Apoptosis Response Protein-4; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex

with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a melanoma. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the melanoma.

[00525] The invention also provides a method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising: (a) isolating a population of microvesicles from a biological sample; (b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19, of 14.3.3 Pan, Filamin, 14.3.3 gamma, CD71 / Transferrin Receptor, CD30, Cdk5, CD138, Thymidine Phosphorylase, Ruv 5, Thrombospondin, CD1, Von Hippel-Lindau Protein, CD46, Rad51, Ferritin, c-Abl, Actin, Muscle Specific, LewisB; and (c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle. The one or more microvesicle can be detected to provide a biosignature. The biosignature may be used to characterize a malignancy. As described herein, the presence or level of the one or more microvesicle can be compared to a reference in order to characterize the malignancy. Further as described herein, the characterizing can include without limitation providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive. The malignancy can be a head and neck cancer. For example, the method can be used to compare levels of the one or more microvesicle to a non-cancer control sample, thereby providing a diagnosis or prognosis of the head and neck cancer.

Theranosis

[00526] As disclosed herein, methods are disclosed for characterizing a phenotype for a subject by assessing one or more biomarkers, including vesicle biomarkers and/or circulating biomarkers. The biomarkers can be assessed using methods for multiplexed analysis of vesicle biomarkers disclosed herein. Characterizing a phenotype can include providing a theranosis for a subject, such as determining if a subject is predicted to respond to a treatment or is predicted to be non-responsive to a treatment. A subject that responds to a treatment can be termed a responder whereas a subject that does not respond can be termed a non-responder. A subject suffering from a condition can be considered to be a responder for a treatment based on, but not limited to, an improvement of one or more symptoms of the condition; a decrease in one or more side effects of an existing treatment; an increased improvement, or rate of improvement, in one or more symptoms as compared to a previous or other treatment; or prolonged survival as compared to without treatment or a previous or other treatment. For example, a subject suffering from a condition can be considered to be a responder to a treatment based on the beneficial or desired clinical results including, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment or if receiving a different treatment.

[00527] The systems and methods disclosed herein can be used to select a candidate treatment for a subject in need thereof. Selection of a therapy can be based on one or more characteristics of a vesicle, such as the biosignature of a vesicle, the amount of vesicles, or both. Vesicle typing or profiling, such as the identification of the biosignature of a vesicle, the amount of vesicles, or both, can be used to identify one or more candidate therapeutic agents for an individual suffering from a condition. For example, vesicle profiling can be used to determine if a subject is a non-responder or responder to a particular therapeutic, such as a cancer therapeutic if the subject is suffering from a cancer.

[00528] Vesicle profiling can be used to provide a diagnosis or prognosis for a subject, and a therapy can be selected based on the diagnosis or prognosis. Alternatively, therapy selection can be directly based on a subject's vesicle profile. Furthermore, a subject's vesicle profile can be used to follow the evolution of a disease, to evaluate the efficacy of a medication, adapt an existing treatment for a subject suffering from a disease or condition, or select a new treatment for a subject suffering from a disease or condition.

[00529] A subject's response to a treatment can be assessed using biomarkers, including vesicles, microRNA, and other circulating biomarkers. In one embodiment, a subject is determined, classified, or identified as a non-responder or responder based on the subject's vesicle profile assessed prior to any treatment. During pretreatment, a subject can be classifed as a non-responder or responder, thereby reducing unnecessary treatment options, and avoidance of possible side effects from ineffective therapeutics. Furthermore, the subject can be identified as a responder to a particular treatment, and thus vesicle profiling can be used to prolong survival of a subject, improve the subject's symptoms or condition, or both, by providing personalized treatment options. Thus, a subject suffering from a condition can have a biosignature generated from vesicles and other circulating biomarkers using one or more systems and methods disclosed herein, and the profile can then be used to determine whether a subject is a likely non-responder or responder to a particular treatment for the condition. Based on use of the biosignature to predict whether the subject is a non-responder or responder to the initially contemplated treatment, a particular treatment contemplated for treating the subject's condition can be selected for the subject, or another potentially more optimal treatment can be selected.

[00530] In one embodiment, a subject suffering from a condition is currently being treated with a therapeutic. A sample can be obtained from the subject before treatment and at one or more timepoints during treatment. A biosignature including vesicles or other biomarkers from the samples can be assessed and used to determine the subject's response to the drug, such as based on a change in the biosignature over time. If the subject is not responding to the treatment, e.g., the biosignature does not indicate that the patient is responding, the subject can be classified as being non-responsive to the treatment, or a non-responder. Similarly, one or more biomarkers associated with a worsening condition may be detected such that the biosignature is indicative of patient's failure to respond favorably to the treatment. In another example, one or more biomarkers associated with the condition remain the same despite treatment, indicating that the condition is not improving. Thus, based on the biosignature, a treatment regimen for the subject can be changed or adapted, including selection of a different therapeutic.

[00531] Alternatively, the subject can be determined to be responding to the treatment, and the subject can be classified as being responsive to the treatment, or a responder. For example, one or more biomarkers associated with an improvement in the condition or disorder may be detected. In another example, one or more biomarkers associated with the condition changes, thus indicating an improvement. Thus, the existing treatment can be continued. In another embodiment, even when there is an indiciation of improvement, the existing treatment may be adapted or changed if the biosignature indicates that another line of treatment may be more effective. The existing treatment may be combined with another therapeutic, the dosage of the current therapeutic may be increased, or a different candidate

treatment or therapeutic may be selected. Criteria for selecting the different candidate treatment can depend on the setting. In one embodiment, the candidate treatment may have been known to be effective for subjects with success on the existing treatment. In another embodiment, the candidate treatment may have been known to be effective for other subjects with a similar biosignature.

[00532] In some embodiments, the subject is undergoing a second, third or more line of treatment, such as cancer treatment. A biosignature according to the invention can be determined for the subject prior to a second, third or more line of treatment, to determine whether a subject would be a responder or non-resonder to the second, third or more line of treatment. In another embodiment, a biosignature is determined for the subject during the second, third or more line of treatment, to determine if the subject is responding to the second, third or more line of treatment.

[00533] The methods and systems described herein for assessing one or more vesicles can be used to determine if a subject suffering from a condition is responsive to a treatment, and thus can be used to select a treatment that improves one or more symptoms of the condition; decreases one or more side effects of an existing treatment; increases the improvement, or rate of improvement, in one or more symptoms as compared to a previous or other treatment; or prolongs survival as compared to without treatment or a previous or other treatment. Thus, the methods described herein can be used to prolong survival of a subject by providing personalized treatment options, and/or may reduce unnecessary treatment options and unnecessary side effects for a subject.

[00534] The prolonged survival can be an increased progression-free survival (PFS), which denotes the chances of staying free of disease progression for an individual or a group of individuals suffering from a disease, e.g., a cancer, after initiating a course of treatment. It can refer to the percentage of individuals in the group whose disease is likely to remain stable (e.g., not show signs of progression) after a specified duration of time. Progression-free survival rates are an indication of the effectiveness of a particular treatment. In other embodiments, the prolonged survival is disease-free survival (DFS), which denotes the chances of staying free of disease after initiating a particular treatment for an individual or a group of individuals suffering from a cancer. It can refer to the percentage of individuals in the group who are likely to be free of disease after a specified duration of time. Disease-free survival rates are an indication of the effectiveness of a particular treatment. Two treatment strategies can be compared on the basis of the disease-free survival that is achieved in similar groups of patients. Disease-free survival is often used with the term overall survival when cancer survival is described.

[00535] The candidate treatment selected by vesicle profiling as described herein can be compared to a non-vesicle profiling selected treatment by comparing the progression free survival (PFS) using therapy selected by vesicle profiling (period B) with PFS for the most recent therapy on which the subject has just progressed (period A). In one setting, a PFSB/PFSA ratio ≥ 1.3 is used to indicate that the vesicle profiling selected therapy provides benefit for subject *(*see for example, *Robert Temple, Clinical measurement in drug evaluation. Edited by Wu Ningano and G.T. Thicker John Wiley and Sons Ltd. 1995; Von Hoff, D.D. Clin Can Res. 4: 1079, 1999: Dhani et al. Clin Cancer Res. 15: 118-123, 2009).*

[00536] Other methods of comparing the treatment selected by vesicle profiling can be compared to a non-vesicle profiling selected treatment by determine response rate (RECIST) and percent of subjects without progression or death at 4 months. The term "about" as used in the context of a numerical value for PFS means a variation of +/- ten percent (10%) relative to the numerical value. The PFS from a treatment selected by vesicle profiling can be extended by at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at least 90% as compared to a non- vesicle profiling selected treatment. In some embodiments, the PFS from a treatment selected by vesicle profiling can be extended by at

least 100%, 150%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or at least about 1000% as compared to a non-vesicle profiling selected treatment. In yet other embodiments, the PFS ratio (PFS on vesicle profiling selected therapy or new treatment / PFS on prior therapy or treatment) is at least about 1.3. In yet other embodiments, the PFS ratio is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0. In yet other embodiments, the PFS ratio is at least about 3, 4, 5, 6, 7, 8, 9 or 10.

[00537] Similarly, the DFS can be compared in subjects whose treatment is selected with or without determining a biosignature according to the invention. The DFS from a treatment selected by vesicle profiling can be extended by at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at least 90% as compared to a non- vesicle profiling selected treatment. In some embodiments, the DFS from a treatment selected by vesicle profiling can be extended by at least 100%, 150%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or at least about 1000% as compared to a non-vesicle profiling selected treatment. In yet other embodiments, the DFS ratio (DFS on vesicle profiling selected therapy or new treatment / DFS on prior therapy or treatment) is at least about 1.3. In yet other embodiments, the DFS ratio is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0. In yet other embodiments, the DFS ratio is at least about 3, 4, 5, 6, 7, 8, 9 or 10.

[00538] In some embodiments, the candidate treatment selected by microvescile profiling does not increase the PFS ratio or the DFS ratio in the subject; nevertheless vesicle profiling provides subject benefit. For example, in some embodiments no known treatment is available for the subject. In such cases, vesicle profiling provides a method to identify a candidate treatment where none is currently identified. The vesicle profiling may extend PFS, DFS or lifespan by at least 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 24 months or 2 years. The vesicle profiling may extend PFS, DFS or lifespan by at least 2 ½ years, 3 years, 4 years, 5 years, or more. In some embodiments, the methods of the invention improve outcome so that subject is in remission.

[00539] The effectiveness of a treatment can be monitored by other measures. A complete response (CR) comprises a complete disappearance of the disease: no disease is evident on examination, scans or other tests. A partial response (PR) refers to some disease remaining in the body, but there has been a decrease in size or number of the lesions by 30% or more. Stable disease (SD) refers to a disease that has remained relatively unchanged in size and number of lesions. Generally, less than a 50% decrease or a slight increase in size would be described as stable disease. Progressive disease (PD) means that the disease has increased in size or number on treatment. In some embodiments, vesicle profiling according to the invention results in a complete response or partial response. In some embodiments, the methods of the invention result in stable disease. In some embodiments, the invention is able to achieve stable disease where non-vesicle profiling results in progressive disease.

[00540] The theranosis based on a biosignature of the invention can be for a phenotype including without limitation those listed herein. Characterizing a phenotype includes determining a theranosis for a subject, such as predicting whether a subject is likely to respond to a treatment ("responder") or be non-responsive to a treatment ("non-responder"). As used herein, identifying a subject as a "responder" to a treatment or as a "non-responder" to the treatment comprises identifying the subject as either likely to respond to the treatment or likely to not respond to the treatment, respectively, and does not require determining a definitive prediction of the subject's response. One or more vesicles, or populations of vesicles, obtained from subject are used to determine if a subject is a non-responder or

responder to a particular therapeutic, by assessing biomarkers disclosed herein, e.g., those listed in **Table 6**. Detection of a high or low expression level of a biomarker, or a mutation of a biomarker, can be used to select a candidate treatment, such as a pharmaceutical intervention, for a subject with a condtion. **Table 6** contains illustrative conditions and pharmaceutical interventions for those conditions. The table lists biomarkers that affect the efficacy of the intervention. The biomarkers can be assessed using the methods of the invention, e.g., as circulating biomarkers or in association with a vesicle.

Table 6: Examples of Biomarkers and Pharmaceutical Intervention for a Condition

Condition	Pharmaceutial intervention	Biomarker
Peripheral Arterial Disease	Atorvastatin, Simvastatin, Rosuvastatin, Pravastatin, Fluvastatin, Lovastatin	C-reactive protein(CRP), serum Amylyoid A (SAA), interleukin-6, intracellular adhesion molecule (ICAM), vascular adhesion molecule (VCAM), CD40L, fibrinogen, fibrin D-dimer, fibrinopeptide A, von Willibrand factor, tissue plasminogen activator antigen (t-PA), factor VII, prothrombin fragment 1, oxidized low density lipoprotein (oxLDL), lipoprotein A
Non-Small Cell Lung Cancer	Erlotinib, Carboplatin, Paclitaxel, Gefitinib	EGFR, excision repair cross-complementation group 1 (ERCC1), p53, Ras, p27, class III beta tubulin, breast cancer gene 1 (BRCA1), breast cancer gene 1 (BRCA2), ribonucleotide reductase messenger 1 (RRM1)
Colorectal Cancer	Panitumumab, Cetuximab	K-ras
Breast Cancer	Trastuzumab, Anthracyclines, Taxane, Methotrexate, fluorouracil	HER2, toposiomerase II alpha, estrogen receptor, progesterone receptor
Alzheimer's Disease	Donepezil, Galantamine, Memantine, Rivastigmine, Tacrine	beta-amyloid protein, amyloid precursor protein (APP), APP670/671, APP693, APP692, APP715, APP716, APP717, APP723, presenilin 1, presenilin 2, cerebrospinal fluid amyloid beta protein 42 (CSF-Abeta42), cerebrospinal fluid amyloid beta protein 40 (CSF-Abeta40), F2 isoprostane, 4-hydroxynonenal, F4 neuroprostane, acrolein
Arrhythmia	Disopyramide, Flecainide, Lidocaine, Mexiletine, Moricizine, Procainamide, Propafenone, Quinidine, Tocainide, Acebutolol, Atenolol, Betaxolol, Bisoprolol, Carvedilol, Esmolol, Metoprolol, Nadolol, Propranolol, Sotalol, Timolol, Amiodarone, Azimilide, Bepridil, Dofetilide, Ibutilide, Tedisamil, Diltiazem, Verapamil, Azimilide, Dronedarone, Amiodarone, PM101, ATI-2042, Tedisamil, Nifekalant, Ambasilide, Ersentilide, Trecetilide, Almokalant, D-sotalol, BRL-32872, HMR1556, L768673, Vernakalant, AZD70009, AVE0118, S9947, NIP-141/142, XEN-D0101/2, Ranolazine, Pilsicainide, JTV519, Rotigaptide, GAP-134	SERCA, AAP, Connexin 40, Connexin 43, ATP-sensitive potassium channel, Kv1.5 channel, acetylcholine-activated posassium channel

Rheumatoid arthritis	Methotrexate, infliximab, adalimumab, etanercept, sulfasalazine	677CC/1298AA MTHFR, 677CT/1298AC MTHFR, 677CT MTHFR, G80AA RFC-1, 3435TT MDR1 (ABCB1), 3435TT ABCB1, AMPD1/ATIC/ITPA, IL1-RN3, HLA-DRB103, CRP, HLA-D4, HLA DRB-1, anti-citrulline epitope containing peptides, anti-A1/RA33, Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), SAA (serum amyloid-associated protein), rheumatoid factor, IL-1, TNF, IL-6, IL-8, IL-1Ra, Hyaluronic acid, Aggrecan, Glc-Gal-PYD, osteoprotegerin, RNAKL, carilage oligomeric matrix protein (COMP), calprotectin
Arterial Fibrillation	warfarin, aspirin, anticoagulants, heparin, ximelagatran	F1.2, TAT, FPA, beta-throboglobulin, platelet factor 4, soluble P-selectin, IL-6, CRP
HIV Infection	Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Saquinavir, Ritonavir, Indinavir, Nevirane, Nelfinavir, Delavirdine, Stavudine, Efavirenz, Etravirine, Enfuvirtide, Darunavir, Abacavir, Amprenavir, Lonavir/Ritonavirc, Tenofovir, Tipranavir	HIV p24 antigen, TNF-alpha, TNFR-II, CD3, CD14, CD25, CD27, Fas, FasL, beta2 microglobulin, neopterin, HIV RNA, HLA-B *5701
Cardiovascular Disease	lisinopril, candesartan, enalapril	ACE inhibitor, angiotensin

[00541] ***Cancer***

[00542] Vesicle biosignatures can be used in the theranosis of a cancer, such as identifying whether a subject suffering from cancer is a likely responder or non-responder to a particular cancer treatment. The subject methods can be used to theranose cancers including those listed herein, e.g., in the "Phenotype" section above. These include without limitation lung cancer, non-small cell lung cancerm small cell lung cancer (including small cell carcinoma (oat cell cancer), mixed small cell/large cell carcinoma, and combined small cell carcinoma), colon cancer, breast cancer, prostate cancer, liver cancer, pancreatic cancer, brain cancer, kidney cancer, ovarian cancer, stomach cancer, melanoma, bone cancer, gastric cancer, breast cancer, glioma, gliobastoma, hepatocellular carcinoma, papillary renal carcinoma, head and neck squamous cell carcinoma, leukemia, lymphoma, myeloma, or other solid tumors.

[00543] A biosignature of circulating biomarkers, including markers associated with vesicle, in a sample from a subject suffering from a cancer can be used select a candidate treatment for the subject. The biosignature can be determined according to the methods of the invention presented herein. In some embodiments, the candidate treatment comprises a standard of care for the cancer. The biosignature can be used to determine if a subject is a non-responder or responder to a particular treatment or standard of care. The treatment can be a cancer treatment such as radiation, surgery, chemotherapy or a combination thereof. The cancer treatment can be a therapeutic such as anti-cancer agents and chemotherapeutic regimens. Cancer treatments for use with the methods of the invention include without limitation those listed in **Table 7**:

Table 7: Cancer Treatments

	Treatment or Agent
Cancer therapies	Radiation, Surgery, Chemotherapy, Biologic therapy, Neo-adjuvant therapy, Adjuvant therapy, Palliative therapy, Watchful waiting

Anti-cancer agents (chemotherapies and biologics)	13-cis-Retinoic Acid, 2-CdA, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 5-FU, 6-Mercaptopurine, 6-MP, 6-TG, 6-Thioguanine, Abraxane, Accutane®, Actinomycin-D, Adriamycin®, Adrucil®, Afinitor®, Agrylin®, Ala-Cort®, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ®, Alkeran®, All-transretinoic Acid, Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron®, Anastrozole, Arabinosylcytosine, Ara-C, Aranesp®, Aredia®, Arimidex®, Aromasin®, Arranon®, Arsenic Trioxide, Asparaginase, ATRA, Avastin®, Azacitidine, BCG, BCNU, Bendamustine, Bevacizumab, Bexarotene, BEXXAR®, Bicalutamide, BiCNU, Blenoxane®, Bleomycin, Bortezomib, Busulfan, Busulfex®, C225, Calcium Leucovorin, Campath®, Camptosar®, Camptothecin-11, Capecitabine, Carac™, Carboplatin, Carmustine, Carmustine Wafer, Casodex®, CC-5013, CCI-779, CCNU, CDDP, CeeNU, Cerubidine®, Cetuximab, Chlorambucil, Cisplatin, Citrovorum Factor, Cladribine, Cortisone, Cosmegen®, CPT-11, Cyclophosphamide, Cytadren®, Cytarabine, Cytarabine Liposomal, Cytosar-U®, Cytoxan®, Dacarbazine, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib, Daunomycin Daunorubicin, Daunorubicin Hydrochloride, Daunorubicin Liposomal, DaunoXome®, Decadron, Decitabine, Delta-Cortef®, Deltasone®, Denileukin, Diftitox, DepoCyt™, Dexamethasone, Dexamethasone Acetate Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex Docetaxel, Doxil®, Doxorubicin, Doxorubicin Liposomal, Droxia™, DTIC, DTIC-Dome®, Duralone®, Efudex®, Eligard™, Ellence™, Eloxatin™, Elspar®, Emcyt®, Epirubicin, Epoetin Alfa, Erbitux, Erlotinib, Erwinia L-asparaginase, Estramustine, Ethyol Etopophos®, Etoposide, Etoposide Phosphate, Eulexin®, Everolimus, Evista®, Exemestane, Fareston®, Faslodex®, Femara®, Filgrastim, Floxuridine, Fludara®, Fludarabine, Fluoroplex®, Fluorouracil, Fluorouracil (cream), Fluoxymesterone, Flutamide, Folinic Acid, FUDR®, Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gemzar, Gleevec™, Gliadel® Wafer, GM-CSF, Goserelin, Granulocyte - Colony Stimulating Factor, Granulocyte Macrophage Colony Stimulating Factor, Halotestin®, Herceptin®, Hexadrol, Hexalen®, Hexamethylmelamine, HMM, Hycamtin®, Hydrea®, Hydrocort Acetate®, Hydrocortisone, Hydrocortisone Sodium Phosphate, Hydrocortisone Sodium Succinate, Hydrocortone Phosphate, Hydroxyurea, Ibritumomab, Ibritumomab, Tiuxetan, Idamycin®, Idarubicin, Ifex®, IFN-alpha, Ifosfamide, IL-11, IL-2, Imatinib mesylate, Imidazole Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG Conjugate), Interleukin-2, Interleukin-11, Intron A® (interferon alfa-2b), Iressa®, Irinotecan, Isotretinoin, Ixabepilone, Ixempra™, Kidrolase (t), Lanacort®, Lapatinib, L-asparaginase, LCR, Lenalidomide, Letrozole, Leucovorin, Leukeran, Leukine™, Leuprolide, Leurocristine, Leustatin™, Liposomal Ara-C Liquid Pred®, Lomustine, L-PAM, L-Sarcolysin, Lupron®, Lupron Depot®, Matulane®, Maxidex, Mechlorethamine, Mechlorethamine Hydrochloride, Medralone®, Medrol®, Megace®, Megestrol, Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex™, Methotrexate, Methotrexate Sodium, Methylprednisolone, Meticorten®, Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol®, MTC, MTX, Mustargen®, Mustine, Mutamycin®, Myleran®, Mylocel™, Mylotarg®, Navelbine®, Nelarabine, Neosar®, Neulasta™, Neumega®, Neupogen®, Nexavar®, Nilandron®, Nilutamide, Nipent®, Nitrogen Mustard, Novaldex®, Novantrone®, Octreotide, Octreotide acetate, Oncospar®, Oncovin®, Ontak®, Onxal™, Oprevelkin, Orapred®, Orasone®, Oxaliplatin, Paclitaxel, Paclitaxel Protein-bound, Pamidronate, Panitumumab, Panretin®, Paraplatin®, Pediapred®, PEG Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON™, PEG-L-asparaginase, PEMETREXED, Pentostatin, Phenylalanine Mustard, Platinol®, Platinol-AQ®, Prednisolone, Prednisone, Prelone®, Procarbazine, PROCRIT®, Proleukin®, Prolifeprospan 20 with Carmustine Implant, Purinethol®, Raloxifene, Revlimid®, Rheumatrex®, Rituxan®, Rituximab, Roferon-A® (Interferon Alfa-2a), Rubex®, Rubidomycin hydrochloride, Sandostatin®, Sandostatin LAR®, Sargramostim, Solu-Cortef®, Solu-Medrol®, Sorafenib, SPRYCEL™, STI-571, Streptozocin, SU11248, Sunitinib, Sutent®, Tamoxifen, Tarceva®, Targretin®, Taxol®, Taxotere®, Temodar®, Temozolomide, Temsirolimus, Teniposide, TESPA, Thalidomide, Thalomid®, TheraCys®, Thioguanine, Thioguanine Tabloid®, Thiophosphoamide, Thioplex®, Thiotepa, TICE®, Toposar®, Topotecan, Toremifene, Torisel®, Tositumomab, Trastuzumab, Treanda®, Tretinoin, Trexall™, Trisenox®, TSPA, TYKERB®, VCR, Vectibix™, Velban®, Velcade®, VePesid®, Vesanoid®,

	Viadur™, Vidaza®, Vinblastine, Vinblastine Sulfate, Vincasar Pfs®, Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VM-26, Vorinostat, VP-16, Vumon®, Xeloda®, Zanosar®, Zevalin™, Zinecard®, Zoladex®, Zoledronic acid, Zolinza, Zometa®
Combination Therapies	CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); CVP (cyclophosphamide, vincristine, and prednisone); RCVP (Rituximab+CVP); RCHOP (Rituximab+CHOP); RICE (Rituximab+ifosamide, carboplatin, etoposide); RDHAP, (Rituximab+dexamethasone, cytarabine, cisplatin); RESHAP (Rituximab+etoposide, methylprednisolone, cytarabine, cisplatin); combination treatment with vincristine, prednisone, and anthracycline, with or without asparaginase; combination treatment with daunorubicin, vincristine, prednisone, and asparaginase; combination treatment with teniposide and Ara-C (cytarabine); combination treatment with methotrexate and leucovorin; combination treatment with bleomycin, doxorubicin, etoposide, mechlorethamine, prednisone, vinblastine, and vincristine; FOLFOX4 regimen (oxaliplatin, leucovorin, and fluorouracil [5-FU]); FOLFIRI regimen (Irinotecan Hydrochloride, Fluorouracil, and Leucovorin Calcium); Levamisole regimen (5-FU and levamisole); NCCTG regimen (5-FU and low-dose leucovorin); NSABP regimen (5-FU and high-dose leucovorin); XAD (Xelox (Capecitabine + Oxaliplatin) + Bevacizumab + Dasatinib); FOLFOX/Bevacizumab/Hydroxychloroquine; German AIO regimen (folic acid, 5-FU, and irinotecan); Douillard regimen (folic acid, 5-FU, and irinotecan); CAPOX regimen (Capecitabine, oxaliplatin); FOLFOX6 regimen (oxaliplatin, leucovorin, and 5-FU); FOLFIRI regimen (folic acid, 5-FU, and irinotecan); FUFOX regimen (oxaliplatin, leucovorin, and 5-FU); FUOX regimen (oxaliplatin and 5-FU); IFL regimen (irinotecan, 5-FU, and leucovorin); XELOX regimen (capecitabine oxaliplatin); KHAD-L (ketoconazole, hydrocortisone, dutasteride and lapatinib);
Biologics	anti-CD52 antibodies (e.g., Alemtuzumab), anti-CD20 antibodies (e.g., Rituximab), anti-CD40 antibodies (e.g., SGN40)
Classes of Treatments	Anthracyclines and related substances, Anti-androgens, Anti-estrogens, Antigrowth hormones (e.g., Somatostatin analogs), Combination therapy (e.g., vincristine, bcnu, melphalan, cyclophosphamide, prednisone (VBMCP)), DNA methyltransferase inhibitors, Endocrine therapy - Enzyme inhibitor, Endocrine therapy - other hormone antagonists and related agents, Folic acid analogs (e.g., methotrexate), Folic acid analogs (e.g., pemetrexed), Gonadotropin releasing hormone analogs, Gonadotropin-releasing hormones, Monoclonal antibodies (EGFR-Targeted - e.g., panitumumab, cetuximab), Monoclonal antibodies (Her2-Targeted - e.g., trastuzumab), Monoclonal antibodies (Multi-Targeted - e.g., alemtuzumab), Other alkylating agents, Antineoplastic agents (e.g., asparaginase, ATRA, bexarotene, celecoxib, gemcitabine, hydroxyurea, irinotecan, topotecan, pentostatin), Cytotoxic antibiotics, Platinum compounds, Podophyllotoxin derivatives (e.g., etoposide), Progestogens, Protein kinase inhibitors (EGFR-Targeted), Protein kinase inhibitors (Her2 targeted therapy - e.g., lapatinib), Pyrimidine analogs (e.g., cytarabine), Pyrimidine analogs (e.g., fluoropyrimidines), Salicylic acid and derivatives (e.g., aspirin), Src-family protein tyrosine kinase inhibitors (e.g., dasatinib), Taxanes (e.g., nab-paclitaxel), Vinca Alkaloids and analogs, Vitamin D and analogs, Monoclonal antibodies (Multi-Targeted - e.g., bevacizumab), Protein kinase inhibitors (e.g., imatinib, sorafenib, sunitinib)
Prostate Cancer Treatments	Watchful waiting (i.e., monitor without treatment); Surgery (e.g., Pelvic lymphadenectomy, Radical prostatectomy, Transurethral resection of the prostate (TURP); Orchiectomy); Radiation therapy (e.g., external-beam radiation therapy (EBRT), Proton beam radiation; implantation of radioisotopes (i.e., iodine I 125, palladium, and iridium)); Hormone therapy (e.g., Luteinizing hormone-releasing hormone agonists such as leuprolide, goserelin, buserelin or ozarelix; Antiandrogens such as flutamide, 2-hydroxyflutamide, bicalutamide, megestrol acetate, nilutamide, ketoconazole, aminoglutethimide; calcitriol, gonadotropin-releasing hormone (GnRH), estrogens (DES, chlorotrianisene, ethinyl estradiol, conjugated estrogens USP, and DES-diphosphate), triptorelin, finasteride, cyproterone acetate, ASP3550); Cryosurgery/cryotherapy; Chemotherapy and Biologic therapy (dutasteride, zoledronate, azacitidine, docetaxel, prednisolone, celecoxib, atorvastatin, AMT2003, soy protein, LHRH agonist, PD-103, pomegranate extract, soy extract, taxotere, I-125, zoledronic acid, dasatinib, vitamin C, vitamin D, vitamin D3, vitamin E, gemcitabine, cisplatin, lenalidomide, prednisone, degarelix, OGX-011, OGX-427, MDV3100, tasquinimod, cabazitaxel, TOOKAD®, lanreotide, PROSTVAC, GM-CSF, lenalidomide, samarium

	Sm-153 lexidronam, N-Methyl-D-Aspartate (NMDA)-Receptor Antagonist, sorafenib, sorafenib tosylate, mitoxantrone, ABI-008, hydrocortisone, panobinostat, soy-tomato extract, KHAD-L, TOK-001, cixutumumab, temsirolimus, ixabepilone, TAK-700, TAK-448, TRC105, cyclophosphamide, lenalidomide, MLN8237, GDC-0449, Alpharadin®, ARN-509, PX-866, ISIS EIF4E Rx, AEZS-108, 131I-F16SIP Monoclonal Antibody, anti-OX40 antibody, Muscadine Plus, ODM-201, BBI608, ZD4054, erlotinib, rIL-2, epirubicin, estramustine phosphate, HuJ591-GS monoclonal (177Lu-J591), abraxane, IVIG, fermented wheat germ nutriment (FWGE), 153Sm-EDTMP, estramustine, mitoxantrone, vinblastine, carboplatin, paclitaxel, pazopanib, cytarabine, testosterone replacement, Zoledronic Acid, Strontium Chloride Sr 89, paricalcitol, satraplatin, RAD001 (everolimus), valproic acid, tea extract, Hamsa-1, hydroxychloroquine, sipuleucel-T, selenomethionine, selenium, lycopene, sunitinib, vandetanib, IMC-A12 antibody, monoclonal antibody IMC-3G3, ixabepilone, diindolylmethane, metformin, efavirenz, dasatinib, nilutamide, abiraterone, cabozantinib (XL184), isoflavines, cinacalcet hydrochloride, SB939, LY2523355, KX2-391, olaparib, genestein, digoxin, RO4929097, ipilimumab, bafetinib, cediranib maleate, MK2206, phenelzine sulfate, triptorelin pamoate, saracatinib, STA-9090, tesetaxel, pasireotide, afatinib, GTx 758, lonafarnib, satraplatin, radiolabeled antibody 7E11, FP253/fludarabine, Coxsackie A21 (CVA21) virus, ARRY-380, ARRY-382, anti-PSMA designer T cells, pemetrexed disodium, bortezomib, MDX-1106, white button mushroom extract, SU011248, MLN9708, BMTP-11, ABT-888, CX-4945, 4SC-205, temozolomide, MGAH22, vinorelbine ditartrate, Sodium Selenite, vorinostat, Ad-REIC/Dkk-3, ASG-5ME, IMF-001, PROHIBITIN-TP01, DSTP3086S, ridaforolimus, MK-2206, MK-0752, polyunsaturated fatty acids, I-125, statins, cholecalciferol, omega-3 fatty acids, raloxifene, etoposide, POMELLA™ extract, Lucrin depot); Cancer vaccines (e.g., DNA vaccines, peptide vaccines, dendritic cell vaccines, PEP223, PSA/TRICOM, PROSTVAC-V/TRICOM, PROSTVAC-F/TRICOM, PSA vaccine, TroVax®, GI-6207, PSMA and TARP Peptide Vaccine); Ultrasound; Proton beam radiation
Colorectal Cancer Treatments	Primary Surgical Therapy (e.g., local excision; resection and anastomosis of primary lesion and removal of surrounding lymph nodes); Adjuvant Therapy (e.g., fluorouracil (5-FU), capecitabine, leucovorin, oxaliplatin, erlotinib, irinotecan, aspirin, mitomycin C, suntinib, cetuximab, bevacizumab, pegfilgrastim, panitumumab, ramucirumab, curcumin, celecoxib, FOLFOX4 regimen, FOLFOX6 regimen, FOLFIRI regimen, FUFOX regimen, FUOX regimen, IFL regimen, XELOX regimen, 5-FU and levamisole regimens, German AIO regimen, CAPOX regimen, Douillard regimen, XAD, RAD001 (everolimus), ARQ 197, BMS-908662, JI-101, hydroxychloroquine (HCQ), Yttrium Microspheres, EZN-2208, CS-7017, IMC-1121B, IMC-18F1, docetaxel, lonafarnib, Maytansinoid DM4-Conjugated Humanized Monoclonal Antibody huC242, paclitaxel, ARRY-380, ARRY-382, IMO-2055, MDX1105-01, CX-4945, Pazopanib, Ixabepilone, OSI-906, NPC-1C Chimeric Monoclonal Antibody, brivanib, Poly-ADP Ribose (PARP) Inhibitor, RO4929097, Anti-cancer vaccine, CEA vaccine, cyclophosphamide, yttrium Y 90 DOTA anti-CEA monoclonal antibody M5A, MEHD7945A, ABT-806, ABT-888, MEDI-565, LY2801653, AZD6244, PRI-724, BKM120, tivozanib, floxuridine, dexamethosone, NKTR-102, perifosine, regorafenib, EP0906, Celebrex, PHY906, KRN330, imatinib mesylate, azacitidine, entinostat, PX-866, ABX-EGF, BAY 43-9006, ESO-1 Lymphocytes and Aldesleukin, LBH589, olaparib, fostamatinib, PD 0332991, STA-9090, cholecalciferol, GI-4000, IL-12, AMG 706, temsirolimus, dulanermin, bortezomib, ursodiol, ridaforolimus, veliparib, NK012, Dalotuzumab, MK-2206, MK-0752, lenalidomide, REOLYSIN®, AUY922, PRI-724, BKM120, avastin, dasatinib); Adjuvant Radiation Therapy (particularly for rectal cancer)

[00544] As shown in **Table 7**, cancer treatments include various surgical and therapeutic treatments. Anti-cancer agents include drugs such as small molecules and biologicals. The methods of the invention can be used to identify a biosignature comprising circulating biomarkers that can then be used for theranostic purposes such as monitoring a treatment efficacy, classifying a subject as a responder or non-responder to a treatment, or selecting a candidate therapeutic agent. The invention can be used to provide a theranosis for any cancer treatments, including without

limitation thernosis involving the cancer treatments in **Table 7**, **Table 8**, or **Table 9**. Cancer therapies that can be identified as candidate treatments by the methods of the invention include without limitation the chemotherapeutic agents listed in **Table 7**, **Table 8**, or **Table 9** and any appropriate combinations thereof. In one embodiment, the treatments are specific for a specific type of cancer, such as the treatments listed for prostate cancer, colorectal cancer, breast cancer and lung cancer in **Table 7**. In other embodiments, the treatments are specific for a tumor regardless of its origin but that displays a certain biosignature, such as a biosignature comprising a marker listed in **Table 8**, **Table 9**, or **Table 10**.

[00545] The invention provides methods of monitoring a cancer treatment comprising identifying a series of biosignatures in a subject over a time course, such as before and after a treatment, or over time after the treatment. The biosignatures are compared to a reference to determine the efficacy of the treatment. In an embodiment, the treatment is selected from **Table 7**, **Table 8**, or **Table 9**, such as radiation, surgery, chemotherapy, biologic therapy, neo-adjuvant therapy, adjuvant therapy, or watchful waiting. The reference can be from another individual or group of individuals or from the same subject. For example, a subject with a biosignature indicative of a cancer pre-treatment may have a biosignature indicative of a healthy state after a successful treatment. Conversely, the subject may have a biosignature indicative of cancer after an unsuccessful treatment. The biosignatures can be compared over time to determine whether the subject's biosignatures indicate an improvement, worsening of the condition, or no change. Additional treatments may be called for if the cancer is worsening or there is no change over time. For example, hormone therapy may be used in addition to surgery or radiation therapy to treat more aggressive prostate cancers. One or more of the following miRs can be used in a biosignature for monitoring an efficacy of prostate cancer treatment: hsa-miR-1974, hsa-miR-27b, hsa-miR-103, hsa-miR-146a, hsa-miR-22, hsa-miR-382, hsa-miR-23a, hsa-miR-376c, hsa-miR-335, hsa-miR-142-5p, hsa-miR-221, hsa-miR-142-3p, hsa-miR-151-3p, hsa-miR-21, hsa-miR-16. One or more miRs listed in the following publication can be used in a biosignature for monitoring treatment of a cancer of the GI tract: Albulescu et al., Tissular and soluble miRNAs for diagnostic and therapy improvement in digestive tract cancers, Exp Rev Mol Diag, **11**:1, 101-120.

[00546] In some embodiments, the invention provides a method of identifying a biosignature in a sample from a subject in order to select a candidate therapeutic. For example, the biosignature may indicate that a drug-associated target is mutated or differentially expressed, thereby indicating that the subject is likely to respond or not respond to certain treatments. The candidate treatments can be chosen from the anti-cancer agents or classes of therapeutic agents identified in **Table 7**, **Table 8**, or **Table 9**. In some embodiments, the candidate treatments identified according to the subject methods are chosen from at least the groups of treatments consisting of 5-fluorouracil, abarelix, alemtuzumab, aminoglutethimide, anastrozole, asparaginase, aspirin, ATRA, azacitidine, bevacizumab, bexarotene, bicalutamide, calcitriol, capecitabine, carboplatin, celecoxib, cetuximab, chemotherapy, cholecalciferol, cisplatin, cytarabine, dasatinib, daunorubicin, decitabine, doxorubicin, epirubicin, erlotinib, etoposide, exemestane, flutamide, fulvestrant, gefitinib, gemcitabine, gonadorelin, goserelin, hydroxyurea, imatinib, irinotecan, lapatinib, letrozole, leuprolide, liposomal-doxorubicin, medroxyprogesterone, megestrol, megestrol acetate, methotrexate, mitomycin, nab-paclitaxel, octreotide, oxaliplatin, paclitaxel, panitumumab, pegaspargase, pemetrexed, pentostatin, sorafenib, sunitinib, tamoxifen, taxanes, temozolomide, toremifene, trastuzumab, VBMCP, and vincristine.

[00547] Similar to selecting a candidate treatment, the invention also provides a method of determining whether to treat a cancer at all. For example, prostate cancer can be a non-aggressive disease that is unlikely to substantially harm the subject. Radiation therapy with androgen ablation (hormone reduction) is the standard method of treating locally

advanced prostate cancer. Morbidities of hormone therapy include impotence, hot flashes, and loss of libido. In addition, a treatment such as prostatectomy can have morbidities such as impotence or incontinence. Therefore, the invention provides biosignatures that indicate aggressiveness or a progression (e.g., stage or grade) of the cancer. A non-aggressive cancer or localized cancer might not require immediate treatment but rather be watched, e.g., "watchful waiting" of a prostate cancer. Whereas an aggressive or advanced stage lesion would require a concomitantly more aggressive treatment regimen.

[00548] Examples of biomarkers that can be detected, and treatment agents that can be selected or possibly avoided are listed in **Table 8**. For example, a biosignature is identified for a subject with a prostate cancer, wherein the biosignature comprises levels of androgen receptor (AR). Overexpression or overproduction of AR, such as high levels of mRNA levels or protein levels in a vesicle, provides an identification of candidate treatments for the subject. Such treatments include agents for treating the subject such as Bicalutamide, Flutamide, Leuprolide, or Goserelin. The subject is accordingly identified as a responder to Bicalutamide, Flutamide, Leuprolide, or Goserelin. In another illustrative example, BCRP mRNA, protein, or both is detected at high levels in a vesicle from a subject suffering from NSCLC. The subject may then be classified as a non-responder to the agents Cisplatin and Carboplatin, or the agents are considered to be less effective than other agents for treating NSCLC in the subject and not selected for use in treating the subject. Any of the following biomarkers can be assessed in a vesicle obtained from a subject, and the biomarker can be in the form including but not limited to one or more of a nucleic acid, polypeptide, peptide or peptide mimetic. In yet another illustrative example, a mutation in one or more of KRAS, BRAF, PIK3CA, and/or c–kit can be used to select a candidate treatment. For example, a mutation in KRAS or BRAF in a patient may indicate that cetuximab and/or panitumumab are likely to be less effective in treating the patient. Illustrative cancer lineages are indicated in the table as having known associations with the indicated agents. The lineages

Table 8: Examples of Biomarkers, Lineage and Agents

Biomarker	Cancer Lineage	Possibly Less Effective Agents	Possible Agents to Consider
AR (high expression)	Prostate		Bicalutamide, Flutamide, Leuprolide, Goserelin
AR (high expression)	default		Bicaluamide, Flutamide, Leuprolide, Goserelin
BCRP (high expression)	Non-small cell lung cancer (NSCLC)	Cisplatin, Carboplatin	
BCRP (low expression)	Non-small cell lung cancer (NSCLC)		Cisplatin, Carboplatin
BCRP (high expression)	default	Cisplatin, Carboplatin	
BCRP (low expression)	default		Cisplatin, Carboplatin
BRAF V600E (mutation positive)	Colorectal	Cetuximab, Panitumumab	
BRAF V600E (mutation negative)	Colorectal		Cetuximab, Panitumumab
BRAF V600E (mutation positive)	All other	Cetuximab, Panitumumab	
BRAF V600E (mutation negative)	All other		Cetuximab, Panitumumab
BRAF V600E (mutation positive)	default	Cetuximab, Panitumumab	
BRAF V600E (mutation negative)	default		Cetuximab, Panitumumab
CD52 (high	Leukemia		Alemtuzumab

expression)			
CD52 (low expression)	Leukemia	Alemtuzumab	
CD52 (high expression)	default (Hematologic malignancies only)		Alemtuzumab
CD52 (low expression)	default (Hematologic malignancies only)	Alemtuzumab	
c-kit	Uveal Melanoma		
c-kit (high expression)	Gastrointestinal Stromal Tumors [GIST]		Imatinib
c-kit (high expression)	Extrahepatic Bile Duct Tumors		Imatinib
c-kit (high expression)	Acute myeloid leukemia (AML)		Imatinib
c-kit (high expression)	default		Imatinib
EGFR (high copy number)	Head and neck squamous cell carcinoma (HNSCC)		Erlotinib, Gefitinib
EGFR	Head and neck squamous cell carcinoma (HNSCC)	Erlotinib, Gefitinib	
EGFR (high copy number)	Non-small cell lung cancer (NSCLC)		Erlotinib, Gefitinib
EGFR (low copy number)	Non-small cell lung cancer (NSCLC)	Erlotinib, Gefitinib	
EGFR (high copy number)	default		Cetuxumab, Panitumumab, Erlotinib, Gefitinib
EGFR (low copy number)	default	Cetuxumab, Panitumumab, Erlotinib, Gefitinib	
ER (high expression)	Breast	Ixabepilone	Tamoxifen-based treatment, aromatase inhibitors (anastrazole, letrozole)
ER (low expression)	Breast		Ixabepilone
ER (high expression)	Ovarian		Tamoxifen-based treatment, aromatase inhibitors (anastrazole, letrozole)
ER (high expression)	default		Tamoxifen-based treatment, aromatase inhibitors (anastrazole, letrozole)
ERCC1 (high expression)	Non-small cell lung cancer (NSCLC)	Carboplatin, Cisplatin	
ERCC1 (low expression)	Non-small cell lung cancer (NSCLC)		Carboplatin, Cisplatin
ERCC1 (high expression)	Small Cell Lung Cancer (SCLC)	Carboplatin, Cisplatin	
ERCC1 (low expression)	Small Cell Lung Cancer (SCLC)		Carboplatin, Cisplatin
ERCC1 (high expression)	Gastric	Oxaliplatin	
ERCC1 (low expression)	Gastric		Oxaliplatin
ERCC1 (high expression)	default	Carboplatin, Cisplatin, Oxaliplatin	
ERCC1 (low expression)	default		Carboplatin, Cisplatin, Oxaliplatin
HER-2 (high expression)	Breast		Lapatinib, Trastuzumab
HER-2 (high expression)	default		Lapatinib, Trastuzumab
KRAS (mutation positive)	Colorectal cancer	Cetuximab, Panitumumab	

KRAS (mutation negative)	Colorectal cancer		Cetuximab, Panitumumab
KRAS (mutation positive)	Non-small cell lung cancer (NSCLC)	Erlotinib, Gefitinib	
KRAS (mutation negative)	Non-small cell lung cancer (NSCLC)		Erlotinib, Gefitinib
KRAS (mutation positive)	Bronchioloalveolar carcinoma (BAC) or adenocarcinoma (BAC subtype)	Erlotinib	
KRAS (mutation negative)	Bronchioloalveolar carcinoma (BAC) or adenocarcinoma (BAC subtype)		Erlotinib
KRAS (mutation positive)	Multiple myeloma	VBMCP/Cyclophosphamide	
KRAS (mutation negative)	Multiple myeloma		VBMCP/Cyclophosphamide
KRAS (mutation positive)	default	Cetuximab, Panitumumab	
KRAS (mutation negative)	default		Cetuximab, panitumumab
KRAS (mutation positive)	default	Cetuximab, Erlotinib, Panitumumab, Gefitinib	
KRAS (mutation negative)	default		Cetuximab, Erlotinib, Panitumumab, Gefitinib
MGMT (high expression)	Pituitary tumors, oligodendroglioma	Temozolomide	
MGMT (low expression)	Pituitary tumors, oligodendroglioma		Temozolomide
MGMT (high expression)	Neuroendocrine tumors	Temozolomide	
MGMT (low expression)	Neuroendocrine tumors		Temozolomide
MGMT (high expression)	default	Temozolomide	
MGMT (low expression)	default		Temozolomide
MRP1 (high expression)	Breast	Cyclophosphamide	
MRP1 (low expression)	Breast		Cyclophosphamide
MRP1 (high expression)	Small Cell Lung Cancer (SCLC)	Etoposide	
MRP1 (low expression)	Small Cell Lung Cancer (SCLC)		Etoposide
MRP1 (high expression)	Nodal Diffuse Large B-Cell Lymphoma	Cyclophosphamide/Vincristine	
MRP1 (low expression)	Nodal Diffuse Large B-Cell Lymphoma		Cyclophosphamide/Vincristine
MRP1 (high expression)	default	Cyclophosphamide, Etoposide, Vincristine	
MRP1 (low expression)	default		Cyclophosphamide, Etoposide, Vincristine
PDGFRA (high expression)	Malignant Solitary Fibrous Tumor of the Pleura (MSFT)		Imatinib
PDGFRA (high expression)	Gastrointestinal stromal tumor (GIST)		Imatinib

PDGFRA (high expression)	Default		Imatinib
p-glycoprotein (high expression)	Acute myeloid leukemia (AML)	Etoposide	
p-glycoprotein (low expression)	Acute myeloid leukemia (AML)		Etoposide
p-glycoprotein (high expression)	Diffuse Large B-cell Lymphoma (DLBCL)	Doxorubicin	
p-glycoprotein (low expression)	Diffuse Large B-cell Lymphoma (DLBCL)		Doxorubicin
p-glycoprotein (high expression)	Lung	Etoposide	
p-glycoprotein (low expression)	Lung		Etoposide
p-glycoprotein (high expression)	Breast	Doxorubicin	
p-glycoprotein (low expression)	Breast		Doxorubicin
p-glycoprotein (high expression)	Ovarian	Paclitaxel	
p-glycoprotein (low expression)	Ovarian		Paclitaxel
p-glycoprotein (high expression)	Head and neck squamous cell carcinoma (HNSCC)	Vincristine	
p-glycoprotein (low expression)	Head and neck squamous cell carcinoma (HNSCC)		Vincristine
p-glycoprotein (high expression)	default	Vincristine, Etoposide, Doxorubicin, Paclitaxel	
p-glycoprotein (low expression)	default		Vincristine, Etoposide, Doxorubicin, Paclitaxel
PR (high expression)	Breast	Chemoendocrine therapy	Tamoxifen, Anastrazole, Letrozole
PR (low expression)	default	Chemoendocrine therapy	Tamoxifen, Anastrazole, Letrozole
PTEN (high expression)	Breast		Trastuzumab
PTEN (low expression)	Breast	Trastuzumab	
PTEN (high expression)	Non-small cell Lung Cancer (NSCLC)		Gefitinib
PTEN (low expression)	Non-small cell Lung Cancer (NSCLC)	Gefitinib	
PTEN (high expression)	Colorectal		Cetuximab, Panitumumab
PTEN (low expression)	Colorectal	Cetuximab, Panitumumab	
PTEN (high expression)	Glioblastoma		Erlotinib, Gefitinib
PTEN (low expression)	Glioblastoma	Erlotinib, Gefitinib	
PTEN (high expression)	default		Cetuximab, Panitumumab, Erlotinib, Gefitinib and Trastuzumab
PTEN (low expression)	default	Cetuximab, Panitumumab, Erlotinib, Gefitinib and Trastuzumab	
RRM1 (high experssion)	Non-small cell lung cancer (NSCLC)	Gemcitabine	
RRM1 (low	Non-small cell lung cancer		Gemcitabine

expression)	(NSCLC)		
RRM1 (high experssion)	Pancreas	Gemcitabine	
RRM1 (low expression)	Pancreas		Gemcitabine
RRM1 (high experssion)	default	Gemcitabine	
RRM1 (low expression)	default		Gemcitabine
SPARC (high expression)	Breast		nab-paclitaxel
SPARC (high expression)	default		nab-paclitaxel
TS (high expression)	Colorectal	fluoropyrimidines	
TS (low expression)	Colorectal		fluoropyrimidines
TS (high expression)	Pancreas	fluoropyrimidines	
TS (low expression)	Pancreas		fluoropyrimidines
TS (high expression)	Head and Neck Cancer	fluoropyrimidines	
TS (low expression)	Head and Neck Cancer		fluoropyrimidines
TS (high expression)	Gastric	fluoropyrimidines	
TS (low expression)	Gastric		fluoropyrimidines
TS (high expression)	Non-small cell lung cancer (NSCLC)	fluoropyrimidines	
TS (low expression)	Non-small cell lung cancer (NSCLC)		fluoropyrimidines
TS (high expression)	Liver	fluoropyrimidines	
TS (low expression)	Liver		fluoropyrimidines
TS (high expression)	default	fluoropyrimidines	
TS (low expression)	default		fluoropyrimidines
TOPO1 (high expression)	Colorectal		Irinotecan
TOPO1 (low expression)	Colorectal	Irinotecan	
TOPO1 (high expression)	Ovarian		Irinotecan
TOPO1 (low expression)	Ovarian	Irinotecan	
TOPO1 (high expression)	default		Irinotecan
TOPO1 (low expression)	default	Irinotecan	
TopoIIa (high epxression)	Breast		Doxorubicin, liposomal-Doxorubicin, Epirubicin
TopoIIa (low expression)	Breast	Doxorubicin, liposomal-Doxorubicin, Epirubicin	
TopoIIa (high epxression)	default		Doxorubicin, liposomal-Doxorubicin, Epirubicin
TopoIIa (low expression)	default	Doxorubicin, liposomal-Doxorubicin, Epirubicin	

[00549] Other examples of biomarkers that can be detected and the treatment agents that can be selected or possibly avoided based on the biomarker signatures are listed in **Table 9**. For example, for a subject suffering from cancer, detecting overexpression of ADA in vesicles from a subject is used to classify the subject as a responder to pentostatin, or pentostatin identified as an agent to use for treating the subject. In another example, for a subject suffering from cancer, detecting overexpression of BCRP in vesicles from the subject is used to classify the subject as a non-responder

to cisplatin, carboplatin, irinotecan, and topotecan, meaning that cisplatin, carboplatin, irinotecan, and topotecan are identified as agents that are suboptimal for treating the subject.

Table 9: Examples of Biomarkers, Agents and Resistance

Gene Name	Expression Status	Candidate Agent(s)	Possible Resistance
ADA	Overexpressed	pentostatin	
ADA	Underexpressed		cytarabine
AR	Overexpressed	abarelix, bicalutamide, flutamide, gonadorelin, goserelin, leuprolide	
ASNS	Underexpressed	asparaginase, pegaspargase	
BCRP (ABCG2)	Overexpressed		cisplatin, carboplatin, irinotecan, topotecan
BRAF	Mutated		panitumumab, cetuximmab
BRCA1	Underexpressed	mitomycin	
BRCA2	Underexpressed	mitomycin	
CD52	Overexpressed	alemtuzumab	
CDA	Overexpressed		cytarabine
c-erbB2	High levels of phosphorylation in epithelial cells	Trastuzumab, c-erbB2 kinase inhibitor, lapatinib	
CES2	Overexpressed	irinotecan	
c-kit	Overexpressed	sorafenib, sunitinib, imatinib	
COX-2	Overexpressed	celecoxib	
DCK	Overexpressed	gemcitabine	cytarabine
DHFR	Underexpressed	methotrexate, pemetrexed	
DHFR	Overexpressed		methotrexate
DNMT1	Overexpressed	azacitidine, decitabine	
DNMT3A	Overexpressed	azacitidine, decitabine	
DNMT3B	Overexpressed	azacitidine, decitabine	
EGFR	Overexpressed	erlotinib, gefitinib, cetuximab, panitumumab	
EML4-ALK	Overexpressed (present)	petrexmed, crizotinib	
EPHA2	Overexpressed	dasatinib	
ER	Overexpressed	anastrazole, exemestane, fulvestrant, letrozole, megestrol, tamoxifen, medroxyprogesterone, toremifene, aminoglutethimide	
ERCC1	Overexpressed		carboplatin, cisplatin, oxaliplatin
GART	Underexpressed	pemetrexed	
GRN (PCDGF, PGRN)	Overexpressed		anti-oestrogen therapy, tamoxifen, faslodex, letrozole, herceptin in Her-2 overexpressing cells, doxorubicin
HER-2 (ERBB2)	Overexpressed	trastuzumab, lapatinib	
HIF-1α	Overexpressed	sorafenib, sunitinib, bevacizumab	
IκB-α	Overexpressed	bortezomib	
IGFBP3	Underexpressed	letrozole	
IGFBP4	Overexpressed	letrozole	
IGFBP5	Underexpressed	letrozole	
Ki67	Underexpressed	tamoxifen + chemotherapy	

KRAS	Mutated		panitumumab, cetuximab
MET	Overexpressed		gefitinib, erlotinib
MGMT	Underexpressed	temozolomide	
MGMT	Overexpressed		temozolomide
MRP1 (ABCC1)	Overexpressed		etoposide, paclitaxel, docetaxel, vinblastine, vinorelbine, topotecan, teniposide
P-gp (ABCB1)	Overexpressed		doxorubicin, etoposide, epirubicin, paclitaxel, docetaxel, vinblastine, vinorelbine, topotecan, teniposide, liposomal doxorubicin
PDGFR-α	Overexpressed	sorafenib, sunitinib, imatinib	
PDGFR-β	Overexpressed	sorafenib, sunitinib, imatinib	
PIK3CA/PI3K	Mutation		cetuximab, panitumumab, trastuzumab
PR	Overexpressed	exemestane, fulvestrant, gonadorelin, goserelin, medroxyprogesterone, megestrol, tamoxifen, toremifene	
PTEN	Underexpressed		cetuximab, panitumumab, trastuzumab
RARA	Overexpressed	ATRA	
RRM1	Underexpressed	gemcitabine, hydroxyurea	
RRM2	Underexpressed	gemcitabine, hydroxyurea	
RRM2B	Underexpressed	gemcitabine, hydroxyurea	
RXR-α	Overexpressed	bexarotene	
RXR-β	Overexpressed	bexarotene	
SPARC	Overexpressed	nab-paclitaxel	
SRC	Overexpressed	dasatinib	
SSTR2	Overexpressed	octreotide	
SSTR5	Overexpressed	octreotide	
TLE3			
TOPO I	Overexpressed	irinotecan, topotecan	
TOPO IIα	Overexpressed	doxorubicin, epirubicin, liposomal- doxorubicin	
TOPO IIβ	Overexpressed	doxorubicin, epirubicin, liposomal- doxorubicin	
TS	Underexpressed	capecitabine, 5-fluorouracil, pemetrexed	
TS	Overexpressed		capecitabine, 5-fluorouracil
TUBB3	Overexpressed		paclitaxel, docetaxel
VDR	Overexpressed	calcitriol, cholecalciferol	
VEGFR1 (Flt1)	Overexpressed	sorafenib, sunitinib, bevacizumab	
VEGFR2	Overexpressed	sorafenib, sunitinib, bevacizumab	
VHL	Underexpressed	sorafenib, sunitinib	

[00550] Further drug associations and rules that are used in embodiments of the invention are found in U.S. Patent Application 12/658,770, filed February 12, 2010; and International PCT Patent Applications PCT/US2010/000407, filed February 11, 2010; PCT/US2010/54366, filed October 27, 2010; PCT/US2011/067527, filed December 28, 2011; and PCT/US2012/041393, filed June 7, 2012, all of which applications are incorporated by reference herein in their entirety. See, e.g., "Table 4: Rules Summary for Treatment Selection" of PCT/US2010/54366; "Table 5: Rules Summary for Treatment Selection" of PCT/US2011/067527; and Tables 7-12 of PCT/US2012/041393.

[00551] Any drug-associated target can be part of a biosignature for providing a theranosis. A "druggable target" comprising a target that can be modulated with a therapeutic agent such as a small molecule or biologic, is a candidate for inclusion in the biosignature of the invention. Drug-associated targets also include biomarkers that can confer resistance to a treatment, such as shown in **Table 8** and **Table 9**. The biosignature can be based on either the gene, e.g., DNA sequence, and/or gene product, e.g., mRNA or protein, or the drug-associated target. Such nucleic acid and/or polypeptide can be profiled as applicable as to presence or absence, level or amount, activity, mutation, sequence, haplotype, rearrangement, copy number, or other measurable characteristic. The gene or gene product can be associated with a vesicle population, e.g., as a vesicle surface marker or as vesicle payload. In an embodiment, the invention provides a method of theranosing a cancer, comprising identifying a biosignature that comprises a presence or level of one or more drug-associated target, and selecting a candidate therapeutic based on the biosignature. The drug-associated target can be a circulating biomarker, a vesicle, or a vesicle associated biomarker. Because drug-associated targets can be independent of the tissue or cell-of-origin, biosignatures comprising drug-associated targets can be used to provide a theranosis for any proliferative disease, such as cancers from various anatomical origins, including cancers of unknown origin such as CUPS.

[00552] The drug-associated targets assessed using the methods of the invention comprise without limitation ABCC1, ABCG2, ACE2, ADA, ADH1C, ADH4, AGT, AR, AREG, ASNS, BCL2, BCRP, BDCA1, beta III tubulin, BIRC5, B-RAF, BRCA1, BRCA2, CA2, caveolin, CD20, CD25, CD33, CD52, CDA, CDKN2A, CDKN1A, CDKN1B, CDK2, CDW52, CES2, CK 14, CK 17, CK 5/6, c-KIT, c-Met, c-Myc, COX-2, Cyclin D1, DCK, DHFR, DNMT1, DNMT3A, DNMT3B, E-Cadherin, ECGF1, EGFR, EML4-ALK fusion, EPHA2, Epiregulin, ER, ERBR2, ERCC1, ERCC3, EREG, ESR1, FLT1, folate receptor, FOLR1, FOLR2, FSHB, FSHPRH1, FSHR, FYN, GART, GNA11, GNAQ, GNRH1, GNRHR1, GSTP1, HCK, HDAC1, hENT-1, Her2/Neu, HGF, HIF1A, HIG1, HSP90, HSP90AA1, HSPCA, IGF-1R, IGFRBP, IGFRBP3, IGFRBP4, IGFRBP5, IL13RA1, IL2RA, KDR, Ki67, KIT, K-RAS, LCK, LTB, Lymphotoxin Beta Receptor, LYN, MET, MGMT, MLH1, MMR, MRP1, MS4A1, MSH2, MSH5, Myc, NFKB1, NFKB2, NFKBIA, NRAS, ODC1, OGFR, p16, p21, p27, p53, p95, PARP-1, PDGFC, PDGFR, PDGFRA, PDGFRB, PGP, PGR, PI3K, POLA, POLA1, PPARG, PPARGC1, PR, PTEN, PTGS2, PTPN12, RAF1, RARA, ROS1, RRM1, RRM2, RRM2B, RXRB, RXRG, SIK2, SPARC, SRC, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, Survivin, TK1, TLE3, TNF, TOP1, TOP2A, TOP2B, TS, TUBB3, TXN, TXNRD1, TYMS, VDR, VEGF, VEGFA, VEGFC, VHL, YES1, ZAP70, or any combination thereof. A biosignature including one or combination of these markers can be used to characterize a phenotype according to the invention, such as providing a theranosis. These markers are known to play a role in the efficacy of various chemotherapeutic agents against proliferative diseases. Accordingly, the markers can be assessed to select a candidate treatment for the cancer independent of the origin or type of cancer. In an embodiment, the invention provides a method of selecting a candidate therapeutic for a cancer, comprising identifying a biosignature comprising a level or presence of one or more drug associated target, and selecting the candidate

therapeutic based on its predicted efficacy for a patient with the biosignature. The one or more drug-associated target can be one of the targets listed above, or in **Table 8**, **Table 9**, or **Table 10**. In some embodiments, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 35, 40, 45, or at least 50 of the one or more drug-associated targets are assessed. The one or more drug-associated target can be associated with a vesicle, e.g., as a vesicle surface marker or as vesicle payload as either nucleic acid (e.g., DNA, mRNA) or protein. In some embodiments, the presence or level of a microRNA known to interact with the one or more drug-associated target is assessed, wherein a high level of microRNA known to suppress the one or more drug-associated target can indicate a lower expression of the one or more drug-associated target and thus a lower likelihood of response to a treatment against the drug-associated target. The one or more drug-associated target can be circulating biomarkers. The one or more drug-associated target can be assessed in a tissue sample. The predicted efficacy can be determined by comparing the presence or level of the one or more drug-associated target to a reference value, wherein a higher level that the reference indicates that the subject is a likely responder. The predicted efficacy can be determined using a classifier algorithm, wherein the classifier was trained by comparing the biosignature of the one or more drug-associated target in subjects that are known to be responders or non-responders to the candidate treatment. Molecular associations of the one or more drug-associated target with appropriate candidate targets are displayed in **Table 8**, **Table 9**, or **Table 10** herein and U.S. Patent Application 12/658,770, filed February 12, 2010; International PCT Patent Application PCT/US2010/000407, filed February 11, 2010; International PCT Patent Application PCT/US2010/54366, filed October 27, 2010; International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011; International Patent Application Serial No. PCT/US2011/067527, entitled "MOLECULAR PROFILING OF CANCER" and filed December 28, 2011; and U.S. Provisional Patent Application 61/427,788, filed December 28, 2010; all of which applications are incorporated by reference herein in their entirety.

[00553] Table 11 of International Patent Application Serial No. PCT/US2011/031479, provides a listing of gene and corresponding protein symbols and names of many of the theranostic targets that are analyzed according to the methods of the invention. As understood by those of skill in the art, genes and proteins have developed a number of alternative names in the scientific literature. Thus, the listing in Table 11 of PCT/US2011/031479 and Table 2 of PCT/US2011/067527 comprise illustrative but not exhaustive compilations. A further listing of gene aliases and descriptions can be found using a variety of online databases, including GeneCards® (www.genecards.org), HUGO Gene Nomenclature (www.genenames.org), Entrez Gene (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene), UniProtKB/Swiss-Prot (www.uniprot.org), UniProtKB/TrEMBL (www.uniprot.org), OMIM (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), GeneLoc (genecards.weizmann.ac.il/geneloc/), and Ensembl (www.ensembl.org). Generally, gene symbols and names below correspond to those approved by HUGO, and protein names are those recommended by UniProtKB/Swiss-Prot. Common alternatives are provided as well. Where a protein name indicates a precursor, the mature protein is also implied. Throughout the application, gene and protein symbols may be used interchangeably and the meaning can be derived from context as necessary.

[00554] As an illustration, a treatment can be selected for a subject suffering from Non-Small Cell Lung Cancer. One or more biomarkers, such as, but not limited to, EGFR, excision repair cross-complementation group 1 (ERCC1), p53, Ras, p27, class III beta tubulin, breast cancer gene 1 (BRCA1), breast cancer gene 1 (BRCA2), and ribonucleotide reductase messenger 1 (RRM1), can be assessed from a vesicle from the subject. Based on one or more characteristics of the one or more biomarkers, the subject can be determined to be a responder or non-responder for a treatment, such as, but not limited to, Erlotinib, Carboplatin, Paclitaxel, Gefitinib, or a combination thereof.

[00555] In another embodiment, a treatment can be selected for a subject suffering from Colorectal Cancer, and a biomarker, such as, but not limited to, K-ras, can be assessed from a vesicle from the subject. Based on one or more characteristics of the one or more biomarkers, the subject can be determined to be a responder or non-responder for a treatment, such as, but not limited to, Panitumumab, Cetuximab, or a combination thereof.

[00556] In another embodiment, a treatment can be selected for a subject suffering from Breast Cancer. One or more biomarkers, such as, but not limited to, HER2, toposiomerase II α, estrogen receptor, and progesterone receptor, can be assessed from a vesicle from the subject. Based on one or more characteristics of the one or more biomarkers, the subject can be determined to be a responder or non-responder for a treatment, such as, but not limited to, trastuzumab, anthracyclines, taxane, methotrexate, fluorouracil, or a combination thereof.

[00557] As described, the biosignature used to theranose a cancer can comprise analysis of one or more biomarker, which can be a protein or nucleic acid, including a mRNA or a microRNA. The biomarker can be detected in a bodily fluid and/or can be detected associated with a vesicle, e.g., as a vesicle antigen or as vesicle payload. In an illustrative example, the biosignature is used to identify a patient as a responder or non-responder to a tyrosine kinase inhibitor. The biomarkers can be one or more of those described in WO/2010/121238, entitled "METHODS AND KITS TO PREDICT THERAPEUTIC OUTCOME OF TYROSINE KINASE INHIBITORS" and filed April 19, 2010; or WO/2009/105223, entitled "SYSTEMS AND METHODS OF CANCER STAGING AND TREATMENT" and filed February 19, 2009; both of which applications are incorporated herein by reference in their entirety.

[00558] In an aspect, the present invention provides a method of determining whether a subject is likely to respond or not to a tyrosine kinase inhibitor, the method comprising identifying one or more biomarker in a vesicle population in a sample from the subject, wherein differential expression of the one or more biomarker in the sample as compared to a reference indicates that the subject is a responder or non-responder to the tyrosine kinase inhibitor. In an embodiment, the one or more biomarker comprises miR-497, wherein reduced expression of miR-497 indicates that the subject is a responder (i.e., sensitive to the tyrosine kinase inhibitor). In another embodiment, the one or more biomarker comprises onr or more of miR-21, miR-23a, miR-23b, and miR-29b, wherein upregulation of the microRNA indicates that the subject is a likely non-responder (i.e., resistant to the tyrosine kinase inhibitor). In some embodiments, the one or more biomarker comprises onr or more of hsa-miR-029a, hsa-let-7d, hsa-miR-100, hsa-miR-1260, hsa-miR-025, hsa-let-7i, hsa-miR-146a, hsa-miR-594-Pre, hsa-miR-024, FGFR1, MET, RAB25, EGFR, KIT and VEGFR2. In another embodiment, the one or more biomarker comprises FGF1, HOXC10 or LHFP, wherein higher expression of the biomarker indicates that the subject is a non-responder (i.e., resistant to the tyrosine kinase inhibitor). The method can be used to determine the sensitivity of a cancer to the tyrosine kinase inhibitor, e.g., a non-small cell lung cancer cell, kidney cancer or GIST. The tyrosine kinase inhibitor can be erlotinib, vandetanib, sunitinib and/or sorafenib, or other inhibitors that operate by a similar mechanism of action. A tyrosine kinase inhibitor includes any agent that inhibits the action of one or more tyrosine kinases in a specific or non-specific fashion. Tyrosine kinase inhibitors include small molecules, antibodies, peptides, or any appropriate entity that directly, indirectly, allosterically, or in any other way inhibits tyrosine residue phosphorylation. Specific examples of tyrosine kinase inhibitors include N-(trifluoromethylphenyl)-5 -methylisoxazol-4-carboxamide, 3 - [(2,4-dimethylpyrrol-5 - yl)methylidenyl)indolin-2-one, 17-(allylamino)- 17-demethoxygeldanamycin, 4-(3-chloro-4- fluorophenylamino)-7-methoxy-6-[3-(4-morpholinyl)propoxyl]q- uinazoline, N-(3- ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine, BIBX1382, 2,3,9,10,11,12- hexahydro- 10-(hydroxymethyl)- 10-hydroxy-9-methyl-9, 12-epox- y- lH-döndolo[1,2,3-fg:3',2', 1 '-kl]pyrrolo[3,4-i][l,6]benzodiazocin-l-one, SH268, genistein, STI571, CEP2563, 4-(3- chlorophenylamino)-5,6-

dimethyl-7H-pyrrolo[2,3-d]pyrimidinemethane sulfonate, 4-(3-bromo- 4-hydroxyphenyl)amino-6,7-dimethoxyquinazoline, 4-(4'-hydroxyphenyl)amino-6,7- dimethoxyquinazoline, SU6668, STI571A, N-4-chlorophenyl-4-(4-pyridylmethyl)-l- phthalazinamine, N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro- 1,2- dihydro-2-oxo-3H-indol- 3-ylidine)methyl]-2,4-dimethyl-lH-pyrrole-3-carboxamide (commonly known as sunitinib), A- [A- [[4-chloro-3 (trifluoromethyl)phenyl] carbamoylamino] phenoxy] -N-methyl-pyridine-2- carboxamide (commonly known as sorafenib), EMD121974, and N-(3-ethynylphenyl)-6, 7- bis(2-methoxyethoxy)quinazolin-4-amine (commonly known as erlotinib). In some embodiments, the tyrosine kinase inhibitor has inhibitory activity upon the epidermal growth factor receptor (EGFR), VEGFR, PDGFR beta, and/or FLT3.

[00559] Thus, a treatment can be selected for the subject suffering from a cancer, based on a biosignature identified by the methods of the invention. Accordingly, the biosignature can comprise a presence or level of a circulating biomarker, including a microRNA, a vesicle, or any useful vesicle associated biomarker.

[00560] Biomarkers that can be used for theranosis of other diseases using the methods of the invention, including cardiovascular disease, neurological diseases and disorders, immune diseases and disorders and infectious disease, are described in International Patent Application Serial No. PCT/US2011/031479, entitled "Circulating Biomarkers for Disease" and filed April 6, 2011, which application is incorporated by reference in its entirety herein.

[00561] ***Mutation associated theranostics***

[00562] Mutational or sequence analysis can be performed using any number of techniques described herein or known in the art, including without limitation sequencing (e.g., Sanger, Next Generation, pyrosequencing), PCR, variants of PCR such as RT-PCR, fragment analysis, and the like. **Table 10** describes a number of genes bearing mutations that have been identified in various cancer lineages. In an aspect, the invention provides a theranostic method comprising isolating a microvesicle population using methods as described herein, isolating nucleic acids from the isolated microvesicle population (i.e., the nucleic acids comprise microvesicle payload), and determining a sequence of a nucleic acid that may affect a drug efficacy. The microvesicle population may comprise all microvesicles isolated from a biological sample, e.g., using filtration or centrifugation methods to isolate microvesicles from a tissue sample or bodily fluid such as blood. The microvesicle population may also comprise a subpopulation, e.g., isolated using a binding agent to one or more surface antigen. These techniques can be combined as desired. Such methodology and useful surface antigens are described in further detail herein. The nucleic acids can be mRNAs. In one embodiment, the nucleic acid sequences are assessed using Next Generation sequencing methods, e.g., using a HiSeq/TruSeq system offered by Illumina Corporation (Austin, TX) or an Ion Torrent system from Life Technologies (Carlsbad, CA). In another embodiment, the nucleic acid sequences are assessed using pyrosequencing. One of skill will appreciate that the profiling may be used to identify candidate treatments for cancer lineages other than those described in **Table 10**. Clinical trials in the table can be found at www.clinicaltrials.gov using the indicated identifiers.

Table 10: Exemplary Mutated Genes and Gene Products and Related Therapies

Biomarker	Description
ABL1	Most CML patients have a chromosomal abnormality due to a fusion between Abelson (Abl) tyrosine kinase gene at chromosome 9 and break point cluster (Bcr) gene at chromosome 22 resulting in constitutive activation of the Bcr-Abl fusion gene. Imatinib is a Bcr-Abl tyrosine kinase inhibitor commonly used in treating CML patients. Mutations in the ABL1 gene are common in imatinib resistant CML patients which occur in 30-90% of the patients. However, more than 50 different point mutations in the ABL1 kinase domain may be inhibited by the second generation kinase inhibitors, dasatinib, bosutinib and nilotinib. The gatekeeper mutation, T315I that causes resistance to all currently approved TKIs accounts for about 15% of the mutations found in patients with imatinib resistance.

	BCR-ABL1 mutation analysis is recommended to help facilitate selection of appropriate therapy for patients with CML after treatment with imatinib fails. Agents that target this biomarker are in clinical trials, e.g.: NCT01528085.
STK11	STK11, also known as LKB1, is a serine/threonine kinase. It is thought to be a tumor suppressor gene which acts by interacting with p53 and CDC42. It modulates the activity of AMP-activated protein kinase, causes inhibition of mTOR, regulates cell polarity, inhibits the cell cycle, and activates p53. Somatic mutations in STK11are associated with a history of smoking and KRAS mutation in NSCLC patients. The frequency of STK11 mutation in lung adenocarcinomas ranges from 7%-30%. STK11 loss may play a role in development of metastatic disease in lung cancer patients. Mutations of this gene also drive progression of HPV-induced dysplasia to invasive, cervical cancer and hence STK11 status may be exploited clinically to predict the likelihood of disease recurrence. Agents that target STK11are in clinical trials, e.g.: NCT01578551. In addition, germline mutations in STK11 are associated with Peutz-Jeghers syndrome which is characterized by early onset hamartomatous gastro-intestinal polyps and increased risk of breast, colon, gastric and ovarian cancer.
FGFR2	FGFR2 is a receptor for fibroblast growth factor. Activation of FGFR2 through mutation and amplification has been noted in a number of cancers. Somatic mutations of the FGFR2 tyrosine kinase have been observed in endometrial carcinoma, lung squamous cell carcinoma, cervical carcinoma, and melanoma. In the endometrioid histology of endometrial cancer, the frequency of FGFR2 mutation is 16% and the mutation is associated with shorter disease free survival in patients diagnosed with early stage disease. Loss of function FGFR2 mutations occur in about 8% melanomas and contribute to melanoma pathogenesis. Functional polymorphisms in the FGFR2 promoter are associated with breast cancer susceptibility. Agents that target FGFR2 are in clinical trials, e.g.: NCT01379534. In addition, germline mutations in FGFR2 are associated with numerous medical conditions that include congenital craniofacial malformation disorders, Apert syndrome and the related Pfeiffer and Crouzon syndromes.
ERBB4	ERBB4 is a member of the Erbb receptor family known to play a pivotal role in cell-cell signaling and signal transduction regulating cell growth and development. The most commonly affected signaling pathways are the PI3K-Akt and MAP kinase pathways. Erbb4 was found to be somatically mutated in 19% of melanomas and Erbb4 mutations may confer “oncogene addiction” on melanoma cells. Erbb4 mutations have also been observed in various other cancer types, including, gastric carcinomas (1.7%), colorectal carcinomas (0.68–2.9%), non-small cell lung cancer (2.3–4.7%) and breast carcinomas (1.1%), however, their biological impact is not uniform or consistent across these cancers. Agents that target ERBB4 are in clinical trials, e.g.: NCT0126408.
SMARCB1	SMARCB1 also known as SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1, is a tumor suppressor gene implicated in cell growth and development. Loss of expression of SMARCB1 has been observed in tumors including epithelioid sarcoma, renal medullary carcinoma, undifferentiated pediatric sarcomas, and a subset of hepatoblastomas. In addition, germline mutation in SMARCB1 causes about 20% of all rhabdoid tumors which makes it important for clinicians to facilitate genetic testing and refer families for genetic counseling. Germline SMARCB1 mutations have also been identified as the pathogenic cause of a subset of schwannomas and meningiomas.
CDKN2A	CDKN2A or cyclin-dependent kinase inhibitor 2A is a tumor suppressor gene that encodes two cell cycle regulatory proteins p16INK4A and p14ARF. As upstream regulators of the retinoblastoma (RB) and p53 signaling pathways, CDKN2A controls the induction of cell cycle arrest in damaged cells that allows for repair of DNA. Loss of CDKN2A through whole-gene deletion, point mutation, or promoter methylation leads to disruption of these regulatory proteins and consequently dysregulation of growth control. Somatic CDKN2A mutations are documented to occur in squamous cell lung cancers, head and neck cancer, colorectal cancer, chronic myelogenous leukemia and malignant pleural mesothelioma. Currently, there are agents that target downstream of CDKN2A such as CDK4/6 inhibitors which function by restoring the cell’s ability to induce cell cycle arrest. CDK4/6 inhibitors are in clinical trials for advanced solid tumors, including LEE011 (NCT01237236) and PD0332991 (NCT01522989, NCT01536743, NCT01037790). In addition, germline CDKN2A mutations are associated with melanoma-pancreatic

	carcinoma syndrome, which increases the risk for familial malignant melanoma and pancreatic cancer.
CTNNB1	CTNNB1 or cadherin-associated protein, beta 1, encodes for β-catenin, a central mediator of the Wnt signaling pathway which regulates cell growth, migration, differentiation and apoptosis. Mutations in CTNNB1 (often occurring in exon 3) avert the breakdown of β-catenin, which allows the protein to accumulate resulting in persistent transactivation of target genes including c-myc and cyclin-D1. Somatic CTNNB1 mutations account for 1-4% of colorectal cancers, 2-3% of melanomas, 25-38% of endometrioid ovarian cancers, 84-87% of sporadic desmoid tumors, as well as the pediatric cancers, hepatoblastoma, medulloblastoma and Wilms' tumors. Compounds that suppress the Wnt/β-catenin pathway are available in clinical trials including PRI-724 for advanced solid tumors (NCT01302405) and LGK974 for melanoma and lobular breast cancer.
FGFR1	FGFR1, or fibroblast growth factor receptor 1, encodes for FGFR1 which is important for cell division, regulation of cell maturation, formation of blood vessels, wound healing and embryonic development. Somatic activating mutations have been documented in melanoma, glioblastoma, and lung tumors. Other aberrations of FGFR1 including protein overexpression and gene amplification are common in breast cancer, squamous cell lung cancer, colorectal cancer, and, to some extent in adenocarcinoma of the lung. Recently, it has been shown that osteosarcoma and advanced solid tumors that exhibit FGFR1 amplification are sensitive to the pan-FGFR inhibitor, NVP-BGJ398. Other FGFR1-targeted agents under clinical investigation include dovitinib (NCT01440959). In addition, germline, gain-of-function mutations in FGFR1 result in developmental disorders including Kallmann syndrome and Pfeiffer syndrome.
FLT3	FLT3, or Fms-like tyrosine kinase 3 receptor, is a member of class III receptor tyrosine kinase family, which includes PDGFRA/B and KIT. Signaling through FLT3 ligand-receptor complex regulates hematopoiesis, specifically lymphocyte development. The FLT3 internal tandem duplication (FLT3-ITD) is the most common genetic lesion in acute myeloid leukemia (AML), occurring in 25% of cases. FLT3 mutations are as common in solid tumors but have been documented in breast cancer. Several small molecule multikinase inhibitors targeting the RTK-III family are in clinical trials, including phase II trials for crenolanib in AML (NCT01657682), famitinib for nasopharyngeal carcinoma (NCT01462474), dovitinib for GIST (NCT01440959), and phase I trial for PLX108-01 in solid tumors (NCT01004861).
NOTCH1	NOTCH1, or notch homolog 1, translocation-associated, encodes a member of the Notch signaling network, an evolutionary conserved pathway that regulates developmental processes by regulating interactions between physically adjacent cells. Notch signaling modulates interplay between tumor cells, stromal matrix, endothelial cells and immune cells, and mutations in NOTCH1 play a central role in disruption of microenvironmental communication, potentially leading to cancer progression. Due to the dual, bi-directional signaling of NOTCH1, activating mutations have been found in ALL and CLL, however loss of function mutations in NOTCH1 are prevalent in 11-15% of HNSCC. NOTCH1 mutations have also been found in 2% of glioblastomas, ~1% of ovarian cancers, 10% lung adenocarcinomas, 8% of squamous cell lung cancers and 5% of breast cancers. Notch pathway-directed therapy approaches differ depending on whether the tumor harbors gain or loss of function mutations, thus are classified as Notch pathway inhibitors or activators, respectively. Notch pathway modulators are being investigated in clinical trials, including MK0752 for advanced solid tumors (NCT01295632) and panobinostat (LBH589) for various refractory hematologic malignancies and many types of solid tumors including thyroid cancer (NCT01013597) and melanoma (NCT01065467).
NPM1	NPM1, or nucleophosmin, is a nucleolar phosphoprotein belonging to a family of nuclear chaperones with proliferative and growth-suppressive roles. In several hematological malignancies, the NPM locus is lost or translocated, leading to expression of oncogenic proteins. NPM1 is mutated in one-third of patients with adult AML and leads to aberrant localization in the cytoplasm leading to activation of downstream pathways including JAK/STAT, RAS/ERK, and PI3K, leading to cell proliferation, survival and cytoskeletal rearrangements. In addition, the most common translocation in anaplastic large cell lymphoma (ALCL) is the NPM-ALK translocation which leads to expression of an oncogenic fusion protein with constitutive kinase activity. AML cells with mutant NPM are more sensitive to some chemotherapeutic agents including daunorubicin and camptothecin. ALK-targeted therapies such as crizotinib are under clinical investigation for ALK-NPM

	positive ALCL (NCT00939770).
SRC	SRC, or c-Src is a non-receptor tyrosine kinase, plays a critical role in cellular growth, proliferation, adhesion and angiogenesis. Normally maintained in a repressed state by intramolecular interactions involving the SH2 and SH3 domains, Src mutation prevents these restrictive intramolecular interactions, conferring a constitutively active state. Mutations are found in 12% of colon cancers (especially those metastatic to the liver) and 1-2% of endometrial cancers. Agents that target SRC are in clinical trials, e.g.: dasatinib for treatment of GIST (NCT01643278), endometrial cancer (NCT01440998), and other solid tumors (NCT01445509); saracatinib (AZD0530) for breast (NCT01216176) and pancreatic (NCT00735917) cancers; and bosutinib (SKI-606) for glioblastoma (NCT01331291).
SMAD4	SMAD4, or mothers against decapentaplegic homolog 4, is one of eight proteins in the SMAD family, whose members are involved in multiple signaling pathways and are key modulators of the transcriptional responses to the transforming growth factor-β (TGFβ) receptor kinase complex. SMAD4 resides on chromosome 18q21, one of the most frequently deleted chromosomal regions in colorectal cancer. Smad4 stabilizes Smad DNA-binding complexes and also recruits transcriptional coactivators such as histone acetyltransferases to regulatory elements. Dysregulation of SMAD4 may occur late in tumor development, and can occur through mutations of the MH1 domain which inhibits the DNA-binding function, thus dysregulating TGFβR signaling. Mutated (inactivated) SMAD4 is found in 50% of pancreatic cancers and 10-35% of colorectal cancers. Studies have shown that preservation of SMAD4 through retention of the 18q21 region, leads to clinical benefit from 5-fluorouracil-based therapy. In addition, various clinical trials investigating agents which target the TGFβR signaling axis are available including PF-03446962 for advanced solid tumors including NCT00557856. In addition, germline mutations in SMAD4 are associated with juvenile polyposis (JP) and combined syndrome of JP and hereditary hemorrhagic teleangiectasia (JP-HHT).
FBXW7	FBXW7, or E3 ligase F-box and WD repeat domain containing 7, also known as Cdc4, encodes three protein isoforms which constitute a component of the ubiquitin-proteasome complex. Mutation of FBXW7 occurs in hotspots and disrupts the recognition of and binding with substrates which inhibits the proper targeting of proteins for degradation (e.g. Cyclin E, c-Myc, SREBP1, c-Jun, Notch-1 and mTOR). Mutation frequencies identified in cholangiocarcinomas, T-ALL, and carcinomas of endometrium, colon and stomach are 35%, 31%, 9%, 9%, and 6%, respectively. Therapeutic strategies comprise targeting an oncoprotein downstream of FBXW7, such as mTOR or c-Myc. Tumor cells with mutated FBXW7 are particularly sensitive to rapamycin treatment, indicating FBXW7 loss (mutation) can be a predictive biomarker for treatment with inhibitors of the mTOR pathway.
PTEN	PTEN, or phosphatase and tensin homolog, is a tumor suppressor gene that prevents cells from proliferating. PTEN is an important mediator in signaling downstream of EGFR, and loss of PTEN gene function/expression due to gene mutations or allele loss is associated with reduced benefit to EGFR-targeted monoclonal antibodies. Mutation in PTEN is found in 5-14% of colorectal cancer and 7% of breast cancer. PTEN mutation is generally related to loss of function of the encoded phosphatase, and an upregulation of the PIK3CA/AKT pathway. The role of PTEN loss in response to PIK3CA and mTOR inhibitors has been evaluated in some clinical studies. Agents that target PTEN and/or its downstream or upstream effectors are in clinical trials, including the following: NCT01430572, NCT01306045. In addition, germline PTEN mutations associate with Cowden disease and Bannayan-Riley-Ruvalcaba syndrome. These dominantly inherited disorders belong to a family of hamartomatous polyposis syndromes which feature multiple tumor-like growths (hamartomas) accompanied by an increased risk of breast carcinoma, follicular carcinoma of the thyroid, glioma, prostate and endometrial cancer. Trichilemmoma, a benign, multifocal neoplasm of the skin is also associated with PTEN germline mutations.
TP53	TP53, or p53, plays a central role in modulating response to cellular stress through transcriptional regulation of genes involved in cell-cycle arrest, DNA repair, apoptosis, and senescence. Inactivation of the p53 pathway is essential for the formation of the majority of human tumors. Mutation in p53 (TP53) remains one of the most commonly described genetic events in human neoplasia, estimated to occur in 30-50% of all cancers with the highest mutation rates occurring in head and neck squamous cell carcinoma and colorectal cancer. Generally, presence of a disruptive p53 mutation is associated with a poor

	prognosis in all types of cancers, and diminished sensitivity to radiation and chemotherapy. Agents are in clinical trials which target p53's downstream or upstream effectors. Utility may depend on the p53 status. For p53 mutated patients, Chk1 inhibitors in advanced cancer (NCT01115790) and Wee1 inhibitors in refractory ovarian cancer (NCT01164995) are being investigated. For p53 wildtype patients with sarcoma, mdm2 inhibitors (NCT01605526) are being investigated. In addition, germline p53 mutations are associated with the Li-Fraumeni syndrome (LFS) which may lead to early-onset of several forms of cancer currently known to occur in the syndrome, including sarcomas of the bone and soft tissues, carcinomas of the breast and adrenal cortex (hereditary adrenocortical carcinoma), brain tumors and acute leukemias.
AKT1	AKT1 gene (v-akt murine thymoma viral oncogene homologue 1) encodes a serine/threonine kinase which is a pivotal mediator of the PI3K-related signaling pathway, affecting cell survival, proliferation and invasion. Dysregulated AKT activity is a frequent genetic defect implicated in tumorigenesis and has been indicated to be detrimental to hematopoiesis. Activating mutation E17K has been described in breast (2-4%), endometrial (2-4%), bladder cancers (3%), NSCLC (1%), squamous cell carcinoma of the lung (5%) and ovarian cancer (2%). This mutation in the pleckstrin homology domain facilitates the recruitment of AKT to the plasma membrane and subsequent activation by altering phosphoinositide binding. A mosaic activating mutation E17K has also been suggested to be the cause of Proteus syndrome. Mutation E49K has been found in bladder cancer, which enhances AKT activation and shows transforming activity in cell lines. Agents targeting AKT1 are in clinical trials, e.g., the AKT inhibitor MK-2206 is in trials for patients carrying AKT mutations (see NCT01277757, NCT01425879).
ALK	APC, or adenomatous polyposis coli, is a key tumor suppressor gene that encodes for a large multi-domain protein. This protein exerts its tumor suppressor function in the Wnt/β-catenin cascade mainly by controlling the degradation of β-catenin, the central activator of transcription in the Wnt signaling pathway. The Wnt signaling pathway mediates important cellular functions including intercellular adhesion, stabilization of the cytoskeleton, and cell cycle regulation and apoptosis, and it is important in embryonic development and oncogenesis. Mutation in APC results in a truncated protein product with abnormal function, lacking the domains involved in β-catenin degradation. Somatic mutation in the APC gene can be detected in the majority of colorectal tumors (80%) and it is an early event in colorectal tumorigenesis. APC wild type patients have shown better disease control rate in the metastatic setting when treated with oxaliplatin, while when treated with fluoropyrimidine regimens, APC wild type patients experience more hematological toxicities. APC mutation has also been identified in oral squamous cell carcinoma, gastric cancer as well as hepatoblastoma and may contribute to cancer formation. Agents that target this gene and/or its downstream or upstream effectors are in clinical trials, e.g.: NCT01198743. In addition, germline mutation in APC causes familial adenomatous polyposis, which is an autosomal dominant inherited disease that will inevitably develop to colorectal cancer if left untreated. COX-2 inhibitors including celecoxib may reduce the recurrence of adenomas and incidence of advanced adenomas in individuals with an increased risk of CRC. Turcot syndrome and Gardner's syndrome have also been associated with germline APC defects. Germline mutations of the APC have also been associated with an increased risk of developing desmoid disease, papillary thyroid carcinoma and hepatoblastoma.
APC	APC, or adenomatous polyposis coli, is a key tumor suppressor gene that encodes for a large multi-domain protein. This protein exerts its tumor suppressor function in the Wnt/β-catenin cascade mainly by controlling the degradation of β-catenin, the central activator of transcription in the Wnt signaling pathway. Wnt signaling pathway mediates important cellular functions including intercellular adhesion, stabilization of the cytoskeleton and cell cycle regulation and apoptosis, and is important in embryonic development and oncogenesis. Mutation in APC results in a truncated protein product with abnormal function, lacking the domains involved in β -catenin degradation. Germline mutation is APC causes familial adenomatous polyposis, which is an autosomal dominant inherited disease that will inevitably develop to colorectal cancer if left untreated. Somatic mutation in APC gene can be detected in the majority of colorectal tumors (~80%) and is an early event in colorectal tumorigenesis. APC mutation has been identified in about 12.5% of oral squamous cell carcinoma and may contribute to the genesis of the cancer. Chemoprevention studies in preclinical models show APC deficient pre-malignant cells

	respond to a combination of TRAIL (tumor necrosis factor-related apoptosis-inducing ligand, or Apo2L) and RAc (9-cis-retinyl acetate) in vitro without normal cells being affected.
CDH1	CDH1 (epithelial cadherin/E-cad) encodes a transmembrane calcium dependent cell adhesion glycoprotein that plays a major role in epithelial architecture, cell adhesion and cell invasion. Loss of function of CDH1 contributes to cancer progression by increasing proliferation, invasion, and/or metastasis. Various somatic mutations in CDH1 have been identified in diffuse gastric, lobular breast, endometrial and ovarian carcinomas; the resultant loss of function of E-cad can contribute to tumor growth and progression. In addition, germline mutations in CDH1 cause hereditary diffuse gastric cancer and colorectal cancer; affected women are predisposed to lobular breast cancer with a risk of about 50%. CDH1 mutation carriers have an estimated cumulative risk of gastric cancer of 67% for men and 83% for women, by age of 80 years.
C-Met	C-Met is a proto-oncogene that encodes the tyrosine kinase receptor of hepatocyte growth factor (HGF) or scatter factor (SF). c-Met mutation causes aberrant MET signaling in various cancer types including renal papillary, hepatocellular, head and neck squamous, gastric carcinomas and non-small cell lung cancer. Activating point mutations of MET kinase domain can cause cancer of various types, and may also decrease endocytosis and/or degradation of the receptor, resulting in enhanced tumor growth and metastasis. Mutations in the juxtamembrane domain (exon 14, 15) results in the constitutive activation and show enhanced tumorigenicity. c-MET inhibitors are in clinical trials for patients carrying MET mutations, e.g.: NCT01121575, NCT00813384. Germline mutations in c-MET have been associated with hereditary papillary renal cell carcinoma.
HRAS	HRAS (homologous to the oncogene of the Harvey rat sarcoma virus), together with KRAS and NRAS, belong to the superfamily of RAS GTPase. RAS protein activates RAS-MEK-ERK/MAPK kinase cascade and controls intracellular signaling pathways involved in fundamental cellular processes such as proliferation, differentiation, and apoptosis. Mutant Ras proteins are persistently GTP-bound and active, causing severe dysregulation of the effector signaling. HRAS mutations have been identified in cancers from the urinary tract (10%-40%), skin (6%) and thyroid (4%) and they account for 3% of all RAS mutations identified in cancer. RAS mutations (especially HRAS mutations) occur (5%) in cutaneous squamous cell carcinomas and keratoacanthomas that develop in patients treated with BRAF inhibitor vemurafenib, likely due to the paradoxical activation of the MAPK pathway. Agents that target HRAS and/or its downstream or upstream effectors are in clinical trials, e.g.: NCT01306045. In addition, germline mutation in HRAS has been associated with Costello syndrome, a genetic disorder that is characterized by delayed development and mental retardation and distinctive facial features and heart abnormalities.
IDH1	IDH1 encodes for isocitrate dehydrogenase in cytoplasm and is found to be mutated in ~5% of primary gliomas and 60-90% of secondary gliomas, as well as in 12-18% of patients with acute myeloid leukemia. Mutated IDH1 results in impaired catalytic function of the enzyme, thus altering normal physiology of cellular respiration and metabolism. Furthermore, this mutation results in tumorigenesis. In gliomas, IDH1 mutations are associated with lower-grade astrocytomas and oligodendrogliomas (grade II/III). IDH gene mutations are associated with markedly better survival in patients diagnosed with malignant astrocytoma; and clinical data support a more aggressive surgery for IDH1 mutated patients because these individuals may be able to achieve long-term survival. In contrast, IDH1 mutation is associated with a worse prognosis in AML. In low-grade glioma patients receiving temozolomide before anaplastic transformation, IDH mutations (IDH1 and IDH2) have been shown to predict response to temozolomide. Agents that target IDH and/or its downstream or upstream effectors are in clinical trials, e.g.: NCT01534845.
JAK2	JAK2 or Janus kinase 2 is a part of the JAK/STAT pathway which mediates multiple cellular responses to cytokines and growth factors including proliferation and cell survival. It is also essential for numerous developmental and homeostatic processes, including hematopoiesis and immune cell development. Mutations in the JAK2 kinase domain result in constitutive activation of the kinase and the development of chronic myeloproliferative neoplasms such as polycythemia vera (95%), essential thrombocythemia (50%) and myelofibrosis (50%). JAK2 mutations were also found in BCR-ABL1-negative acute lymphoblastic leukemia patients and the mutated patients show a poor outcome. Agents

	that target JAK2 and/or its downstream or upstream effectors are in clinical trials for patients carrying JAK2 mutations, e.g.: NCT00668421, NCT01038856. In addition, germline mutations in JAK2 have been associated with myeloproliferative neoplasms and thrombocythemia.
MPL	MPL or myeloproliferative leukemia gene encodes the thrombopoietin receptor, which is the main humoral regulator of thrombopoiesis in humans. MPL mutations cause constitutive activation of JAK-STAT signaling and have been detected in 5-7% of patients with primary myelofibrosis (PMF) and 1% of those with essential thrombocythemia (ET). In addition, germline mutations in MPL (S505N) have been associated with familial thrombocythemia.
PDGFRA	PDGFRA is the alpha subunit of platelet-derived growth factor receptor, a surface tyrosine kinase receptor, which can activate multiple signaling pathways including PIK3CA/AKT, RAS/MAPK and JAK/STAT. PDGFRA mutations are found in 5-8% of gastrointestinal stromal tumor cases, and in 40-50% of KIT wild type GISTs. Gain of function PDGFRA mutations confer imatinib sensitivity, while substitution mutation in exon 18 (D842V) shows resistance to the drug. A PDGFRA mutation in the extracellular domain was shown to identify a subgroup of DIPG (diffuse intrinsic pontine glioma) patients with significantly worse outcome PDGFRA inhibitors (e.g., crenolanib, pazopanib) are in clinical trials for patients carrying PDGFRA mutations, e.g.: NCT01243346, NCT01524848, NCT01478373. In addition, germline mutations in PDGFRA have been associated with Familial gastrointestinal stromal tumors and Hypereosinophillic Syndrome (HES).
SMO	SMO (smoothened) is a G protein-coupled receptor which plays an important role in the Hedgehog signaling pathway. It is a key regulator of cell growth and differentiation during development, and is important in epithelial and mesenchymal interaction in many tissues during embryogenesis. Dysregulation of the Hedgehog pathway is found in cancers including basal cell carcinomas (12%) and medulloblastoma (1%). A gain-of-function mutation in SMO results in constitutive activation of hedgehog pathway signaling, contributing to the genesis of basal cell carcinoma. SMO mutations have been associated with the resistance to SMO antagonist GDC-0449 in medulloblastoma patients. SMO mutation may also contribute to resistance to SMO antagonist LDE225 in BCC. SMO antagonists are in clinical trials, e.g.: NCT01529450.
VHL	VHL or von Hippel-Lindau gene encodes for tumor suppressor protein pVHL, which polyubiquitylates hypoxia-inducible factor in an oxygen dependent manner. Absence of pVHL causes stabilization of HIF and expression of its target genes, many of which are important in regulating angiogenesis, cell growth and cell survival. VHL somatic mutation has been seen in 20-70% of patients with sporadic clear cell renal cell carcinoma (ccRCC) and the mutation may imply a poor prognosis, adverse pathological features, and increased tumor grade or lymph-node involvement. Renal cell cancer patients with a 'loss of function' mutation in VHL show a higher response rate to therapy (bevacizumab or sorafenib) than is seen in patients with wild type VHL. Agents which target VHL and/or its downstream or upstream effectors are in clinical trials, e.g.: NCT01538238. In addition, germline mutations in VHL cause von Hippel-Lindau syndrome, associated with clear-cell renal-cell carcinomas, central nervous system hemangioblastomas, pheochromocytomas and pancreatic tumors.
ATM	ATM, or ataxia telangiectasia mutated, is activated by DNA double-strand breaks and DNA replication stress. It encodes a protein kinase that acts as a tumor suppressor and regulates various biomarkers involved in DNA repair, e.g., p53, BRCA1, CHK2, RAD17, RAD9, and NBS1. ATM is associated with hematologic malignancies, and somatic mutations have also been found in colon (18.2%), head and neck (14.3%), and prostate (11.9%) cancers. Inactivating ATM mutations may make patients more susceptible to PARP inhibitors. Agents that target ATM and/or its downstream or upstream effectors are in clinical trials, e.g.: NCT01311713. In addition, germline mutations in ATM are associated with ataxia-telangiectasia (also known as Louis-Bar syndrome) and a predisposition to malignancy.
CSF1R	CSF1R or colony stimulating factor 1 receptor gene encodes a transmembrane tyrosine kinase, a member of the CSF1/PDGF receptor family. CSF1R mediates the cytokine (CSF-1) responsible for macrophage production, differentiation, and function. Mutations of this gene are associated with hematologic malignancies, as well as cancers of the liver (21.4%), colon (12.5%), prostate (3.3%), endometrium (2.4%), and ovary (2.4%). Patients with CSF1R mutations may respond to imatinib. Agents that target CSF1R and/or its

	downstream or upstream effectors are in clinical trials, e.g.: NCT01346358, NCT01440959. In addition, germline mutations in CSF1R are associated with diffuse leukoencephalopathy, a rapidly progressive neurodegenerative disorder.
FGFR3	FGFR3 or fibroblast growth factor receptor type 3 gene encodes a member of the FGFR tyrosine kinase family, which include FGFR1, 2, 3, and 4. Dysregulation of FGFR3 has been implicated in activating the RAS-ERK pathway. FGFR3 has been found in various malignancies, including bladder cancer and multiple myeloma. Somatic mutations of this gene have also been found in skin (25.8%), head and neck (20.0%), and testicular (4.3%) cancers. Studies indicate FGFR3 and PIK3CA mutations occur together. FGFR3 mutations could serve as a strong prognostic indicator of a low recurrence rate in bladder cancer. Agents that target FGFR3 and/or its downstream or upstream effectors are in clinical trials, e.g.: NCT01004224. In addition, germline mutations in FGFR3 are associated with achondroplasia, hypochondroplasia, and Muenke syndrome, disorders involving but not limited to craniosynostosis and shortened extremities. FGFR3 is also associated with Crouzon syndrome with acanthosis nigricans.
GNAS	GNAS (or GNAS complex locus) encodes a stimulatory G protein alpha-subunit. These guanine nucleotide binding proteins (G proteins) are a family of heterotrimeric proteins which couple seven-transmembrane domain receptors to intracellular cascades. Stimulatory G-protein alpha-subunit transmits hormonal and growth factor signals to effector proteins and is involved in the activation of adenylate cyclases. Mutations of GNAS gene at codons 201 or 227 lead to constitutive cAMP signaling. GNAS somatic mutations have been found in pituitary (27.9%), pancreatic (19.2%), ovarian (11.4%), adrenal gland (6.2%), and colon (6.0%) cancers. SNPs in GNAS1 are a predictive marker for tumor response in cisplatin/fluorouracil-based radiochemotherapy in esophageal cancer. In addition, germline mutations of GNAS have been shown to be the cause of McCune-Albright syndrome (MAS), a disorder marked by endocrine, dermatologic, and bone abnormalities. GNAS is usually found as a mosaic mutation in patients. Loss of function mutations are associated with pseudohypoparathyroidism and pseudopseudohypoparathyroidism.
ERBB2	ERBB2 (HER2) or v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. This gene binds to other ligand-bound EGF receptor family members to form a heterodimer and enhances kinase-mediated activation of downstream signaling pathways, leading to cell proliferation. The most common mechanism for activation of HER2 is gene amplification, seen in approximately 15% of breast cancers. Somatic mutations have been found in colon (3.8%), endometrium (3.7%), prostate (3.0%), ovarian (2.5%), breast (1.7%) gastric (1.9%) cancers and 2-4% of lung adenocarcinomas. HER2 activated patients may respond to trastuzumab, afatinib, or lapatinib. Agents that target HER2 are in clinical trials, e.g.: NCT01306045.
HNF1A	HNF1A, or hepatocyte nuclear factor 1 homeobox A, encodes a transcription factor that is highly expressed in the liver, found on chromosome 12. It regulates a large number of genes, including those for albumin, alpha1-antitrypsin, and fibrinogen. HNF1A has been associated with an increased risk of pancreatic cancer. HNF1A somatic mutations are found in liver (30.1%), colon (14.5%), endometrium (11.1%), and ovarian (2.5%) cancers. In addition, germline mutations of HNF1A are associated with maturity-onset diabetes of the young type 3.
JAK3	JAK3 or Janus activated kinase 3 is an intracellular tyrosine kinase involved in cytokine signaling, while interacting with members of the STAT family. Like JAK1, JAK2, and TYK2, JAK3 is a member of the JAK family of kinases. When activated, kinase enzymes phosphorylate one or more signal transducer and activator of transcription (STAT) factors, which translocate to the cell nucleus and regulate the expression of genes associated with survival and proliferation. JAK3 signaling is related to T cell development and proliferation. This biomarker is found in malignancies like head and neck (20.8%) colon (7.2%), prostate (4.8%), ovary (3.5%), breast (1.7%), lung (1.2%), and stomach (0.6%) cancer. In addition, germline mutations of JAK3 are associated with severe, combined immunodeficiency disease (SCID).
KDR	KDR (VEGFR2) or Kinase insert domain receptor gene, also known as vascular endothelial growth factor receptor-2 (VEGFR2), is involved with angiogenesis and is expressed on

	almost all endothelial cells. VEGF ligands bind to KDR, which leads to receptor dimerization and signal transduction. Somatic mutations in KDR have been observed in angiosarcoma (10.0%), and colon (12.7%), skin (12.7%), gastric (5.3%), lung (3.2%), renal (2.3%), and ovarian (1.9%) cancers. VEGFR antagonists that are FDA-approved or in clinical trials include bevacizumab, regorafenib, pazopanib, and vandetanib. Additional agents that target KDR and/or its downstream or upstream effectors are in clinical trials, e.g.: NCT01068587.
MLH1	MLH1 or mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) gene encodes a mismatch repair (MMR) protein which repairs DNA mismatches that occur during replication. Although the frequency is higher in colon cancer (10.4%), MLH1 somatic mutations have been found in esophageal (6.4%), ovarian (5.4%), urinary tract (5.3%), pancreatic (5.2%), and prostate (4.7%) cancers. Germline mutations of MLH1 are associated with Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC). Patients with Lynch syndrome are at increased risk for various malignancies, including intestinal, gynecologic, and upper urinary tract cancers and in its variant, Muir-Torre syndrome, with sebaceous tumors.
PTPN11	PTPN11, or tyrosine-protein phosphatase non-receptor type 11, is a proto-oncogene that encodes a signaling molecule, Shp-2, which regulates various cell functions like mitogenic activation and transcription regulation. PTPN11 gain-of-function somatic mutations have been found to induce hyperactivation of the Akt and MAPK networks. Because of this hyperactivation, Ras effectors such as Mek and PI3K are targets for candidate therapies in those with PTPN11 gain-of-function mutations. PTPN11 somatic mutations are found in hematologic and lymphoid malignancies (8%), gastric (2.4%), colon (2%), ovarian (1.7%), and soft tissue (1.6%) cancers. In addition, germline mutations of PTPN11 are associated with Noonan syndrome, which itself is associated with juvenile myelomonocytic leukemia (JMML). PTPN11 is also associated with LEOPARD syndrome, which is associated with neuroblastoma and myeloid leukemia.
RB1	RB1, or retinoblastoma-1, is a tumor suppressor gene whose protein regulates the cell cycle by interacting with various transcription factors, including the E2F family (which controls the expression of genes involved in the transition of cell cycle checkpoints). RB1 mutations have also been detected in ocular and other malignancies, such as ovarian (10.4%), bladder (41.3%), prostate (8.2%), breast (6.1%), brain (5.6%), colon (5.3%), and renal (1.5%) cancers. RB1 status, along with other mitotic checkpoints, has been associated with the prognosis of GIST patients. In addition, germline mutations of RB1 are associated with the pediatric tumor, retinoblastoma. Inherited retinoblastoma is usually bilateral. Patients with a history of retinoblastoma are at increased risk for secondary malignancies.
RET	RET or rearranged during transfection gene, located on chromosome 10, activates cell signaling pathways involved in proliferation and cell survival. RET mutations are mostly found in papillary thyroid cancers and medullary thyroid cancers (MTC), but RET fusions have also been found in 1% of lung adenocarcinomas. A 10-year study notes that medullary thyroid cancer patients with somatic mutations of RET correlate with a poor prognosis. Approximately 50% of patients with sporadic MTC have somatic RET mutations; 85% of these involve the M918T mutation, which is associated with a higher response rate to vandetanib in comparison to M918T negative patients. Agents that target RET are in clinical trials, e.g.: NCT00514046, NCT01582191. Germline activating mutations of RET are associated with multiple endocrine neoplasia type 2 (MEN2), which is characterized by the presence of medullary thyroid carcinoma, bilateral pheochromocytoma, and primary hyperparathyroidism. Germline inactivating mutations of RET are associated with Hirschsprung's disease.
c-Kit	c-Kit is a cytokine receptor expressed on the surface of hematopoietic stem cells as well as other cell types. This receptor binds to stem cell factor (SCF, a cell growth factor). As c-Kit is a receptor tyrosine kinase, ligand binding causes receptor dimerization and initiates a phosphorylation cascade resulting in changes in gene expression. These changes affect proliferation, apoptosis, chemotaxis and adhesion. C-KIT mutation has been identified in various cancer types including gastrointestinal stromal tumors (GIST) (up to 85%) and melanoma (7%). c-Kit is inhibited by multi-targeted agents including imatinib, sunitinib and sorafenib. Agents which target c-KIT and/or its downstream or upstream effectors are also in clinical trials for patients carrying c-KIT mutation, e.g.: NCT01028222,

	NCT01092728. In addition, germline mutations in c-KIT have been associated with multiple gastrointestinal stromal tumors (GIST) and Piebald trait.
EGFR	EGFR or epidermal growth factor receptor, is a transmembrane receptor tyrosine kinase belonging to the ErbB family of receptors. Upon ligand binding, the activated receptor triggers a series of intracellular pathways (Ras/MAPK, PI3K/Akt, JAK-STAT) that result in cell proliferation, migration and adhesion. Dysregulation of EGFR through mutation leads to ligand-independent activation and constitutive kinase activity, which results in uncontrolled growth and proliferation of many human cancers. EGFR mutations have been observed in 20-25% of non-small cell lung cancer (NSCLC), 10% of endometrial and peritoneal cancers. Somatic gain-of-function EGFR mutations, including in-frame deletions in exon 19 or point mutations in exon 21, confer sensitivity to first-generation EGFR-targeted tyrosine kinase inhibitors, whereas the secondary mutation, T790M in exon 20, confers resistance to tyrosine kinase inhibitors. New agents and combination therapies that include EGFR TKIs are in clinical trials for primary treatment of EGFR-mutated patients, including second-generation tyrosine kinase inhibitors such as icotinib (NCT01665417) for NSCLC or afatinib for advanced solid tumors (NCT00809133) and lung neoplasms (NCT01466660). In addition, new therapies and combination therapies are being explored for patients that have progressed on EGFR-targeted agents including afatinib (NCT01647711) for NSCLC. Germline mutations and polymorphisms of EGFR have been associated with familial lung adeocarcinomas.
PIK3CA	PIK3CA or phosphoinositide-3-kinase catalytic alpha polypeptide encodes a protein in the PI3 kinase pathway. This pathway is an active target for drug development. PIK3CA somatic mutations have been found in breast (26.1%), endometrial (23.3%), urinary tract (19.3%), colon (13.0%), and ovarian (10.8%) cancers. Somatic mosaic activating mutations in PIK3CA may cause CLOVES syndrome. PIK3CA mutations have been associated with benefit from mTOR inhibitors (e.g., everolimus, temsirolimus). Breast cancer patients with activation of the PI3K pathway due to PTEN loss or PIK3CA mutation/amplification may have a shorter survival following trastuzumab treatment. PIK3CA mutated (exon 20) colorectal cancer patients are less likely to respond to EGFR targeted monoclonal antibody therapy. Agents that target PIK3CA are in clinical trials, e.g.: NCT00877773, NCT01277757, NCT01219699, NCT01501604.
NRAS	NRAS is an oncogene and a member of the (GTPase) ras family, which includes KRAS and HRAS. This biomarker has been detected in multiple cancers including melanoma (15%), colorectal cancer (4%), AML (10%) and bladder cancer (2%). Acquired mutations in NRAS may be associated with resistance to vemurafenib in melanoma patients. In colorectal cancer patients NRAS mutation is associated with resistance to EGFR-targeted monoclonal antibodies. Agents which target this gene and/or its downstream or upstream effectors are in clinical trials, e.g.: NCT01306045, NCT01320085 In addition, germline mutations in NRAS have been associated with Noonan syndrome, autoimmune lymphoproliferative syndrome and juvenile myelomonocytic leukemia.
GNA11	GNA11 is a proto-oncogene that belongs to the Gq family of the G alpha family of G protein coupled receptors. Known downstream signaling partners of GNA11 are phospholipase C beta and RhoA and activation of GNA11 induces MAPK activity. Over half of uveal melanoma patients lacking a mutation in GNAQ exhibit somatic mutations in GNA11. Agents that target GNA11 are in clinical trials, e.g.: NCT01587352, NCT01390818, NCT01143402.
GNAQ	GNAQ encodes the Gq alpha subunit of G proteins. G proteins are a family of heterotrimeric proteins coupling seven-transmembrane domain receptors. Oncogenic mutations in GNAQ result in a loss of intrinsic GTPase activity, resulting in a constitutively active Galpha subunit. This results in increased signaling through the MAPK pathway. Somatic mutations in GNAQ have been found in 50% of primary uveal melanoma patients and up to 28% of uveal melanoma metastases. Agents that target GNAQ are in clinical trials, e.g.: NCT01587352, NCT01390818, NCT01143402.
KRAS	KRAS, or V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, encodes a signaling intermediate involved in many signaling cascades including the EGFR pathway. KRAS somatic mutations have been found in pancreatic (57.4%), colon (34.9%), lung (16.0%), biliary tract (28.2%), and endometrial (14.6%) cancers. Mutations at activating hotspots are associated with resistance to EGFR tyrosine kinase inhibitors (e.g., erlotinib, gefitinib) and

	monoclonal antibodies (e.g., cetuximab, panitumumab). Agents that target KRAS are in clinical trials, e.g.: NCT01248247, NCT01229150. In addition, germline mutations of KRAS (V14I, T58I, and D153V amino acid substitutions) are associated with Noonan syndrome.
BRAF	BRAF encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERK signaling pathway initiated by EGFR activation, which affects cell division, differentiation, and secretion. BRAF somatic mutations have been found in melanoma (43%), thyroid (39%), biliary tree (14%), colon (12%), and ovarian tumors (12%). Patients with mutated BRAF genes have a reduced likelihood of response to EGFR targeted monoclonal antibodies, such as cetuximab. A BRAF enzyme inhibitor, vemurafenib, was approved by FDA to treat unresectable or metastatic melanoma patients harboring BRAF V600E mutations. Agents that target BRAF are also in clinical trials, e.g.: NCT01543698, NCT01352273, NCT01709292. In addition, BRAF inherited mutations are associated with Noonan/Cardio-Facio-Cutaneous (CFC) syndrome, syndromes associated with short stature, distinct facial features, and potential heart/skeletal abnormalities.

[00563] In an aspect, the invention provides a theranosis for a cancer which comprises mutational analysis of a panel of nucleic acids isolated from a microvesicle population, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 35, 40, 45 or at least 50 genes. As described herein, the mutational analysis can be used to identify a candidate agent that is likely to benefit the cancer patient. The mutational analysis can also be used to identify a candidate agent that is not likely to benefit the cancer patient. A report can be generated that describes results of the mutational analysis. The report may include a summary of the mutational analysis for the genes assessed. The report may also provide a linkage of the mutational analysis with the predicted efficacy of various treatments based on the mutational analysis. The report may also comprise one or more clinical trials associated with one or more identified mutation in the patient.

[00564] The mutational analysis may be performed for one or more gene in **Table 10**. For example, the mutational analysis may be performed for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or at least 50 genes genes in **Table 10**. In an embodiment, the mutational analysis is performed for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45 of ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CSF1R, CTNNB1, EGFR, FLT3, GNA11, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, VHL. For example, the mutational analysis may be performed for ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CSF1R, CTNNB1, EGFR, FLT3, GNA11, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR (VEGFR2), KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, and VHL.

[00565] In an embodiment, the mutational analysis is performed for the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) gene. The KRAS gene encodes a protein that is a member of the small GTPase superfamily and is a signaling intermediate involved in various signaling cascades including the EGFR pathway. Once activated, KRAS recruits and activates proteins necessary for the propagation of growth factor and other receptor signals, such as c-Raf and PI 3-kinase.

[00566] A single amino acid substitution in KRAS from a single nucleotide substitution can be responsible for an activating mutation. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. Somatic KRAS mutations are found at in various cancers, e.g., leukemias, colon cancer, pancreatic cancer and lung cancer. Mutations

at activating hotspots are associated with resistance to EGFR tyrosine kinase inhibitors (erlotinib, gefitinib) and monoclonal antibodies (cetuximab, panitumumab).

[00567] In an aspect, the invention provides a method of determining a KRAS nucleotide sequence in a biological sample that comprises one or more microvesicle, comprising: (a) contacting the biological sample with a binding agent to a microvesicle surface antigen; (b) isolating nucleic acids from the microvesicles that formed a complex with the binding agent to the microvesicle surface antigen in step (a); and (c) determining a v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) sequence within the nucleic acids isolated in step (b). The microvesicle surface antigen can be selected to isolate a desired vesicle population. For example, a general vesicle marker may facilitate isolation of a majority of microvesicles in a sample and also differentiate microvesicles from other cellular debris or the like, a tissue specific marker may facilitate isolation of microvesicles in a sample from a given tissue or cell-specific origin, and a disease marker can facilitate isolation of microvesicles representative of a certain disease, e.g., a cancer. A population of microvesicles can be isolated using a plurality of surface antigens, e.g., to isolate microvesicles indicative of a cancer from a given cancer lineage. The surface antigen can be selected from **Table 3**, **Table 4** or **Table 5** herein. In an embodiment, the microvesicle surface antigen comprises Tissue factor, EpCam, B7H3, RAGE and/or CD24. The surface antigen may comprise CD24.

[00568] Multiple microvesicle surface antigens can be detected. For example, the method may further comprise contacting the biological sample with a binding agent to a general vesicle marker in step (a) and isolating the nucleic acids from microvesicles that also formed a complex with the binding agent to the general vesicle marker in step (b). In an embodiment, the general vesicle marker is selected from **Table 3**. The general vesicle marker can be a tetraspanin. The tetraspanin can be CD9, CD63 and/or CD81.

[00569] The KRAS sequence may be determined by pyrosequencing, chain-termination (e.g., dye-termination or Sanger sequencing), or Next Generation sequencing. The sequencing can be performed to determine whether the KRAS sequence comprises a mutation. The mutation can be an activating mutation. In an embodiment, the mutation comprises a 38G>A mutation in the nucleotide sequence. This mutation is also referred to as G13D. The G13D mutation results in an amino acid substitution at position 13 in KRAS, from a glycine (G) to an aspartic acid (D). Using similar terminology (i.e., nucleotide substitution (resulting amino acid substitution)), mutations in KRAS that may be detected include without limitation 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 of 34G>T (G12C), 34G>C (G12R), 34G>A (G12S), 35G>C (G12A), 35G>A (G12D), 35G>T (G12V), 37G>T (G13C), 37G>C (G13R), 37G>A (G13S), 38G>C (G13A), 38G>A (G13D), 38G>T (G13V), 181C>A (Q61K), 182A>T (Q61L), 182A>G (Q61R), 183A>C (Q61H), 183A>T (Q61H), 351A>C (K117N), 351A>T (K117N), 436G>C (A146P), 436G>A (A146T), and 437C>T (A146V).

[00570] The nucleic acids isolated in step (b) may comprise DNA or RNA, e.g., mRNA. In an embodiment, mRNAs are isolated from the microvesicle payload and an mRNA sequence is determined.

[00571] As described, the determined KRAS sequence may be used to provide a prognosis or a theranosis for a cancer. The theranosis comprises a therapy-related diagnosis or prognosis, e.g. the theranosis may comprise a prediction of whether a cancer is likely to respond or not respond to a chemotherapeutic agent. Accordingly, a treating physician or other caregiver can use such information to help determine whether to treat or not treat a patient with the chemotherapeutic agent.

[00572] In embodiments, the chemotherapeutic agent comprises an epidermal growth factor receptor (EGFR) directed therapy. The epidermal growth factor receptor (EGFR) is an important player in cancer initiation and progression.

KRAS plays a role as an effector molecule responsible for signal transduction from ligand-bound EGFR to the nucleus. Tumors carrying KRAS mutations are unlikely to respond to EGFR-targeted monoclonal antibodies or experience survival benefit from such treatment. EGFR directed therapy includes without limitation panitumumab, cetuximab, zalutumumab, nimotuzumab, matuzumab, gefitinib, erlotinib, and/or lapatinib.

[00573] Mutations in KRAS may also affect the efficacy of treatments directed to other molecular targets. In embodiments, the chemotherapeutic agent comprises a mammalian target of rapamycin (mTOR) directed therapy, a mitogen-activated or extracellular signal–regulated protein kinase kinase (MEK) directed therapy, and/or a v-raf murine sarcoma viral oncogene homolog B1 (BRAF) directed therapy. Such mTOR directed therapies include without limitation everolimus and/or temsirolimus.

[00574] The chemotherapeutic agent may comprise a cyclophosphamide or a combination of vincristine + carmustine (BCNU) + melphalan + cyclophosphamide + prednisone (VBMCP). These agents may be use to treat multiple myeloma (MM).

[00575] As described, a mutation in KRAS may be predictive that the cancer is less likely to respond to the chemotherapeutic agent. The cancer can be any appropriate cancer wherein KRAS may play a role in treatment selection. Accordingly, the cancer may include without limitation a solid tumor, a colorectal cancer (CRC), a pancreatic cancer, a non-small cell lung cancer (NSCLC), a bronchioloalveolar carcinoma (BAC) or adenocarcinoma (BAC subtype), a leukemia, or a multiple myeloma (MM).

[00576] The biological sample may comprise a cell culture, such that the microvesicles are derived from cultered cells. The biological sample may also comprise a sample from a subject, e.g., a solid tumor sample or a bodily fluid from the subject. Appropriate bodily fluids comprise without limation peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, umbilical cord blood, or a derivative of any thereof.

[00577] In some embodiments, the biological sample comprises peripheral blood or a derivative thereof, e.g., serum or plasma. In such embodiments, the method may comprise removal of one or more abundant protein, e.g., an abundant blood protein, from the biological sample prior to or during the isolation of the one or more microvesicle. For example, abundant proteins may be removed prior to contacting the microvesicle with the binding agent. Non-limiting examples one or more abundant protein that may be removed include one or more of albumin, IgG, transferrin, fibrinogen, fibrin, IgA, α2-Marcroglobulin, IgM, α1-Antitrypsin, complement C3, haptoglobulin, apolipoprotein A1, A3 and B; α1-Acid Glycoprotein, ceruloplasmin, complement C4, C1q, IgD, prealbumin (transthyretin), plasminogen, a derivative of any thereof, and a combination thereof. Further examples of abundant proteins that may be removed comprise Albumin, Immunoglobulins, Fibrinogen, Prealbumin, Alpha 1 antitrypsin, Alpha 1 acid glycoprotein, Alpha 1 fetoprotein, Haptoglobin, Alpha 2 macroglobulin, Ceruloplasmin, Transferrin, complement proteins C3 and C4, Beta 2 microglobulin, Beta lipoprotein, Gamma globulin proteins, C-reactive protein (CRP), Lipoproteins (chylomicrons, VLDL, LDL, HDL), other globulins (types alpha, beta and gamma), Prothrombin, Mannose-binding lectin (MBL), a derivative of any thereof, and a combination thereof.

[00578] Various methodologies can be used to deplete abundant proteins from the biological sample. In some embodiments, the one or more abundant protein is depleted by immunoaffinity, precipitation, or a combination thereof. Commercially available columns can be used such described herein. Depleting the one or more abundant protein may also comprise contacting the biological sample with thromboplastin to precipitate fibrinogen.

[00579] The binding agent used to form a complex with the microvesicle can comprise any useful reagent, including without limitation a nucleic acid, DNA molecule, RNA molecule, antibody, antibody fragment, aptamer, peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, peptide, dendrimer, membrane protein labeling agent, chemical compound, or a combination thereof. Preferable binding agents include without limitation antibodies and/or aptamers.

[00580] In an embodiment, the binding agent is tethered to a substrate. The binding agent may also comprise a label. When multiple binding agents are used, e.g., to identify microvesicles bearing a plurality of surface antigens, at least one binding agent can be tethered to a substrate and another binding agent can carry a label. This allows the label to identify microvesicles in complex with the tethered binding agent. In addition, multiple tethered binding agents can be used, e.g., in a series of columns, wells, or precipitations. Multiple labeled binding agents may be used as well. The Examples provide illustration of each of these applications.

[00581] As described herein, the one or more microvesicle may be subjected to size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, affinity capture, immunoassay, microfluidic separation, flow cytometry or combinations thereof. For example, a large microvesicle population can be isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, and/or nanomembrane ultrafiltration, then a subpopulation can be further isolated using immunoabsorbent capture, affinity purification, affinity capture, immunoassay and/or flow cytometry. Microvesicles may be at least partially identified or isolated by size. In an embodiment, the one or more microvesicle has a diameter between 10 nm and 2000 nm. For example, the one or more microvesicle may have a diameter between 20 nm and 200 nm. In other embodiments, microvesicles with a size greater than 800 nm, e.g., > 1000 nm, are interrogated.

[00582] Also as described herein, the method can include detecting one or more payload biomarker within the one or more microvesicle. For example, the one or more payload biomarker may comprise one or more nucleic acid, peptide, protein, lipid, antigen, carbohydrate, and/or proteoglycan. The nucleic acid may be DNA, mRNA, microRNA, snoRNA, snRNA, rRNA, tRNA, siRNA, hnRNA, or shRNA. In preferred embodiments, the one or more payload biomarker comprises microRNA and/or mRNA. The payload markers can be assessed as part of providing the theranosis.

Detection System and Kits

[00583] Also provided is a detection system configured to determine one or more biosignatures for a vesicle. The detection system can be used to detect a heterogeneous population of vesicles or one or more homogeneous population of vesicles. The detection system can be configured to detect a plurality of vesicles, wherein at least a subset of the plurality of vesicles comprises a different biosignature from another subset of the plurality of vesicles. The detection system detect at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different subsets of vesicles, wherein each subset of vesicles comprises a different biosignature. For example, a detection system, such as using one or more methods, processes, and compositions disclosed herein, can be used to detect at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different populations of vesicles.

[00584] The detection system can be configured to assess at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 1000, 2500, 5000, 7500, 10,000, 100,000, 150,000, 200,000, 250,000, 300,000, 350,000, 400,000, 450,000, 500,000, 750,000, or 1,000,000 different biomarkers for one or more vesicles. In some embodiments, the one or more biomarkers are selected from any of **Table 3**, **Table 4**, or **Table 5**, or as disclosed herein. The detection system can be configured to assess a specific population of vesicles, such as vesicles from a specific cell-of-origin, or to assess a plurality of specific populations of vesicles, wherein each population of vesicles has a specific biosignature.

[00585] The detection system can be a low density detection system or a high density detection system. For example, a low density detection system can detect up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different vesicle populations, whereas a high density detection system can detect at least about 15, 20, 25, 50, or 100 different vesicle populations In another embodiment, a low density detection system can detect up to about 100, 200, 300, 400, or 500 different biomarkers, whereas a high density detection system can detect at least about 750, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9,000, 10,000, 15,000, 20,000, 25,000, 50,000, or 100,000 different biomarkers. In yet another embodiment, a low density detection system can detect up to about 100, 200, 300, 400, or 500 different biosignatures or biomarker combinations, whereas a high density detection system can detect at least about 750, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9,000, 10,000, 15,000, 20,000, 25,000, 50,000, or 100,000 biosignatures or biomarker combinations.

[00586] The detection system can comprise a probe that selectively hybridizes to a vesicle. The detection system can comprise a plurality of probes to detect a vesicle. In some embodiments, a plurality of probes is used to detect the amount of vesicles in a heterogeneous population of vesicles. In yet other embodiments, a plurality of probes is used to detect a homogeneous population of vesicles. A plurality of probes can be used to isolate or detect at least two different subsets of vesicles, wherein each subset of vesicles comprises a different biosignature.

[00587] A detection system, such as using one or more methods, processes, and compositions disclosed herein, can comprise a plurality of probes configured to detect, or isolate, such as using one or more methods, processes, and compositions disclosed herein at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different subsets of vesicles, wherein each subset of vesicles comprises a different biosignature.

[00588] For example, a detection system can comprise a plurality of probes configured to detect at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100 different populations of vesicles. The detection system can comprise a plurality of probes configured to selectively hybridize to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 1000, 2500, 5000, 7500, 10,000, 100,000, 150,000, 200,000, 250,000, 300,000, 350,000, 400,000, 450,000, 500,000, 750,000, or 1,000,000 different biomarkers for one or more vesicles. In some embodiments, the one or more biomarkers are selected from any of **Table 3**, **Table 4**, or **Table 5**, or as disclosed herein. The plurality of probes can be configured to assess a specific population of vesicles, such as vesicles from a specific cell-of-origin, or to assess a plurality of specific populations of vesicles, wherein each population of vesicles has a specific biosignature.

[00589] The detection system can be a low density detection system or a high density detection system comprising probes to detect vesicles. For example, a low density detection system can comprise probes to detect up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different vesicle populations, whereas a high density detection system can comprise probes to detect at least about 15, 20, 25, 50, or 100 different vesicle populations. In another embodiment, a low density detection system can comprise probes to detect up to about 100, 200, 300, 400, or 500 different biomarkers, whereas a high density detection system can comprise probes to detect at least about 750, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9,000, 10,000, 15,000, 20,000, 25,000, 50,000, or 100,000 different biomarkers. In yet another embodiment, a low

density detection system can comprise probes to detect up to about 100, 200, 300, 400, or 500 different biosignatures or biomarker combinations, whereas a high density detection system can comprise probes to detect at least about 750, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9,000, 10,000, 15,000, 20,000, 25,000, 50,000, or 100,000 biosignatures or biomarker combinations.

[00590] The probes can be specific for detecting a specific vesicle population, for example a vesicle with a particular biosignature, and as described above. A plurality of probes for detecting prostate specific vesicles is also provided. A plurality of probes can comprise probes for detecting one or more of the biomarkers in **Table 3**, **Table 4**, or **Table 5**. The plurality of probes can also comprise one or more probes for detecting one or more of the biomarkers in **Table 3**, **Table 4**, or **Table 5**.

[00591] A plurality of probes for detecting one or more miRNAs of a vesicle can comprise probes for detecting one or more of the following miRNAs: miR-9, miR-629, miR-141, miR-671-3p, miR-491, miR-182, miR-125a-3p, miR-324-5p, miR-148b, and miR-222. In another embodiment, the plurality of probes comprises one or more probes for detecting EpCam, CD9, PCSA, CD63, CD81, PSMA, B7H3, PSCA, ICAM, STEAP, and EGFR. In some embodiments, the plurality of probes comprises one or more probes for detecting EpCam, CD9, PCSA, CD63, CD81, PSMA, and B7H3. In other embodiments, the plurality of probes comprises one or more probes for detecting EpCam, CD9, PCSA, CD63, CD81, PSMA, B7H3, PSCA, ICAM, STEAP, and EGFR. In yet another embodiment, a subset of the plurality of probes are capture agents for one or more of EpCam, CD9, PCSA, CD63, CD81, PSMA, B7H3, PSCA, ICAM, STEAP, and EGFR, and another subset are probes for detecting one or more of CD9, CD63, and CD81. A plurality of probes can also comprises one or more probes for detecting r miR-92a-2*, miR-147, miR-574-5p, or a combination thereof. A plurality of probes can also comprise one or more probes for detecting miR-548c-5p, miR-362-3p, miR-422a, miR-597, miR-429, miR-200a, miR-200b or a combination thereof. A plurality of probes can also comprise one or more probes for detecting EpCam, CK, and CD45. In some embodiments, the one or more probes may be capture agents. In another embodiment, the probes may be detection agents. In yet another embodiment, the plurality of probes comprises capture and detection agents.

[00592] The probes, such as capture agents, may be attached to a solid substrate, such as an array or bead. Alternatively, the probes, such as detection agents, are not attached. The detection system may be an array based system, a sequencing system, a PCR-based system, or a bead-based system, such as described above. The detection system can also be a microfluidic device as described above.

[00593] The detection system may be part of a kit. Alternatively, the kit may comprise the one or more probe sets or plurality of probes, as described herein. The kit may comprise probes for detecting a vesicle or a plurality of vesicles, such as vesicles in a heterogeneous population. The kit may comprise probes for detecting a homogeneous population of vesicles. For example, the kit may comprise probes for detecting a population of specific cell-of-origin vesicles, or vesicles with the same specific biosignature.

[00594] In a related aspect, the invention provides a kit comprising one or more reagent to carry out the method of the invention. The one or more reagent can be selected from the group consisting of one or more binding agent specific for a microvesicle surface antigen, a chromatography column, filtration units, membranes, flow reagents, a buffer, equipment to remove a highly abundant protein, one or more population of microvesicles, and a combination thereof. The one or more reagent can be a capture agent and/or a detector agent such as described herein. The kit can contain instructions for performing one or more steps of the methods of the invention.

Computer Systems

[00595] A vesicle can be assayed for molecular features, for example, by determining an amount, presence or absence of one or more biomarkers. The data generated can be used to produce a biosignature, which can be stored and analyzed by a computer system, such as shown in **FIG. 3**. The assaying or correlating of the biosignature with one or more phenotypes can also be performed by computer systems, such as by using computer executable logic.

[00596] A computer system, such as shown in **FIG. 3,** can be used to transmit data and results following analysis. Accordingly, **FIG. 3** is a block diagram showing a representative example logic device through which results from a vesicle can be analyzed and the analysis reported or generated. **FIG. 3** shows a computer system (or digital device) **800** to receive and store data generated from a vesicle, analyze of the data to generate one or more biosignatures, and produce a report of the one or more biosignatures or phenotype characterization. The computer system can also perform comparisons and analyses of biosignatures generated, and transmit the results. Alternatively, the computer system can receive raw data of vesicle analysis, such as through transmission of the data over a network, and perform the analysis.

[00597] The computer system **800** may be understood as a logical apparatus that can read instructions from media **811** and/or network port **805**, which can optionally be connected to server **809** having fixed media **812**. The system shown in **FIG. 3** includes CPU **801**, disk drives **803**, optional input devices such as keyboard **815** and/or mouse **816** and optional monitor **807**. Data communication can be achieved through the indicated communication medium to a server **809** at a local or a remote location. The communication medium can include any means of transmitting and/or receiving data. For example, the communication medium can be a network connection, a wireless connection or an internet connection. Such a connection can provide for communication over the World Wide Web. It is envisioned that data relating to the present invention can be transmitted over such networks or connections for reception and/or review by a party **822**. The receiving party **822** can be but is not limited to an individual, a health care provider or a health care manager. Thus, the information and data on a test result can be produced anywhere in the world and transmitted to a different location. For example, when an assay is conducted in a differing building, city, state, country, continent or offshore, the information and data on a test result may be generated and cast in a transmittable form as described above. The test result in a transmittable form thus can be imported into the U.S. to receiving party **822**. Accordingly, the present invention also encompasses a method for producing a transmittable form of information on the diagnosis of one or more samples from an individual. The method comprises the steps of (1) determining a diagnosis, prognosis, theranosis or the like from the samples according to methods of the invention; and (2) embodying the result of the determining step into a transmittable form. The transmittable form is the product of the production method. In one embodiment, a computer-readable medium includes a medium suitable for transmission of a result of an analysis of a biological sample, such as biosignatures. The medium can include a result regarding a vesicle, such as a biosignature of a subject, wherein such a result is derived using the methods described herein.

EXAMPLES

Example 1: Purification of Vesicles From Prostate Cancer Cell Lines

[00598] Prostate cancer cell lines are cultured for 3-4 days in culture media containing 20% FBS (fetal bovine serum) and 1% P/S/G. The cells are then pre-spun for 10 minutes at 400x g at 4˚C. The supernatant is kept and centrifuged for 20 minutes at 2000 x g at 4. The supernatant containing vesicles can be concentrated using a Millipore Centricon Plus-70 (Cat # UFC710008 Fisher).

[00599] The Centricon is pre washed with 30mls of PBS at 1000 x g for 3 minutes at room temperature. Next, 15- 70 mls of the pre-spun cell culture supernatant is poured into the Concentrate Cup and is centrifuged in a Swing Bucket Adapter (Fisher Cat # 75-008-144) for 30 minutes at 1000 x g at room temperature.

[00600] The flow through in the Collection Cup is poured off. The volume in the Concentrate Cup is brought back up to 60mls with any additional supernatant. The Concentrate Cup is centrifuged for 30 minutes at 1000 x g at room temperature to concentrate the cell supernatant.

[00601] The Concentrate Cup is washed by adding 70mls of PBS and centrifuged for 30-60 minutes at 1000 x g until approximately 2 mls remains. The vesicles are removed from the filter by inverting the concentrate into the small sample cup and centrifuge for 1 minute at 4˚C. The volume is brought up to 25 mls with PBS. The vesicles are now concentrated and are added to a 30% Sucrose Cushion.

[00602] To make a cushion, 4 mls of Tris/30%Sucrose/D2O solution (30g protease-free sucrose, 2.4g Tris base, 50ml D2O, adjust pH to 7.4 with 10N NCL drops, adjust volume to 100mls with D2O, sterilize by passing thru a 0.22-um filter) is loaded to the bottom of a 30ml V bottom thin walled Ultracentrifuge tube. The diluted 25 mls of concentrated vesicles is gently added above the sucrose cushion without disturbing the interface and is centrifuged for 75 minutes at 100,000 x g at 4˚C. The ~25mls above the sucrose cushion is carefully removed with a 10ml pipet and the ~3.5mls of vesicles is collected with a fine tip transfer pipet (SAMCO 233) and transferred to a fresh ultracentrifuge tube, where 30 mls PBS is added. The tube is centrifuged for 70 minutes at 100,000 x g at 4˚C. The supernatant is poured off carefully. The pellet is resuspended in 200ul PBS and can be stored at 4˚C or used for assays. A BCA assay (1:2) can be used to determine protein content and Western blotting or electron micrography can be used to determine vesicle purification.

Example 2: Purification of Vesicles from VCaP and 22Rv1

[00603] Vesicles from Vertebral-Cancer of the Prostate (VCaP) and 22Rv1, a human prostate carcinoma cell line, derived from a human prostatic carcinoma xenograft (CWR22R) were collected by ultracentrifugation by first diluting plasma with an equal volume of PBS (1 ml). The diluted fluid was transferred to a 15 ml falcon tube and centrifuged 30 minutes at 2000 x g 4°C. The supernatant (~2 mls) was transferred to an ultracentrifuge tube 5.0 ml PA thinwall tube (Sorvall # 03127) and centrifuged at 12,000 x g, 4°C for 45 minutes.

[00604] The supernatant (~2 mls) was transferred to a new 5.0 ml ultracentrifuge tubes and filled to maximum volume with addition of 2.5 mls PBS and centrifuged for 90 minutes at 110,000 x g at 4°C. The supernatant was poured off without disturbing the pellet and the pellet resuspended with 1 ml PBS. The tube was filled to maximum volume with addition of 4.5 ml of PBS and centrifuged at 110,000 x g, 4°C for 70 minutes.

[00605] The supernatant was poured off without disturbing the pellet and an additional 1 ml of PBS was added to wash the pellet. The volume was increased to maximum volume with the addition of 4.5 mls of PBS and centrifuged at 110,000 x g for 70 minutes at 4°C. The supernatant was removed with P-1000 pipette until ~ 100 µl of PBS was in the bottom of the tube. The ~ 90 µl remaining was removed with P-200 pipette and the pellet collected with the ~10 µl of PBS remaining by gently pipetting using a P-20 pipette into the microcentrifuge tube. The residual pellet was washed from the bottom of a dry tube with an additional 5 µl of fresh PBS and collected into microcentrifuge tube and suspended in phosphate buffered saline (PBS) to a concentration of 500 µg/ml.

Example 3: Plasma Collection and Vesicle Purification

[00606] Blood is collected via standard veinpuncture in a 7ml K2-EDTA tube. The sample is spun at 400g for 10 minutes in a 4°C centrifuge to separate plasma from blood cells (SORVALL Legend RT+ centrifuge). The supernatant

(plasma) is transferred by careful pipetting to 15ml Falcon centrifuge tubes. The plasma is spun at 2,000g for 20 minutes and the supernatant is collected.

[00607] For storage, approximately 1ml of the plasma (supernatant) is aliquoted to a cryovials, placed in dry ice to freeze them and stored in -80°C. Before vesicle purification, if samples were stored at -80°C, samples are thawed in a cold water bath for 5 minutes. The samples are mixed end over end by hand to dissipate insoluble material.

[00608] In a first prespin, the plasma is diluted with an equal volume of PBS (example, approximately 2 ml of plasma is diluted with 2 ml of PBS). The diluted fluid is transferred to a 15 ml Falcon tube and centrifuged for 30 minutes at 2000 x g at 4°C.

[00609] For a second prespin, the supernatant (approximately 4 mls) is carefully transferred to a 50 ml Falcon tube and centrifuged at 12,000 x g at 4°C for 45 minutes in a Sorval.

[00610] In the isolation step, the supernatant (approximately 2 mls) is carefully transferred to a 5.0 ml ultracentrifuge PA thinwall tube (Sorvall # 03127) using a P1000 pipette and filled to maximum volume with an additional 0.5 mls of PBS. The tube is centrifuged for 90 minutes at 110,000 x g at 4°C.

[00611] In the first wash, the supernatant is poured off without disturbing the pellet. The pellet is resuspended or washed with 1 ml PBS and the tube is filled to maximum volume with an additional 4.5 ml of PBS. The tube is centrifuged at 110,000 x g at 4°C for 70 minutes. A second wash is performed by repeating the same steps.

[00612] The vesicles are collected by removing the supernatant with P-1000 pipette until approximately 100 µl of PBS is in the bottom of the tube. Approximately 90 µl of the PBS is removed and discarded with P-200 pipette. The pellet and remaining PBS is collected by gentle pipetting using a P-20 pipette. The residual pellet is washed from the bottom of the dry tube with an additional 5 µl of fresh PBS and collected into a microcentrifuge tube.

Example 4: Analysis of Vesicles Using Antibody-Coupled Microspheres and Directly Conjugated Antibodies

[00613] This example demonstrates the use of particles coupled to an antibody, where the antibody captures the vesicles. See, e.g., **FIG. 2B**. An antibody, the detector antibody, is directly coupled to a label, and is used to detect a biomarker on the captured vesicle.

[00614] First, an antibody-coupled microsphere set is selected (Luminex, Austin, TX). The microsphere set can comprise various antibodies, and thus allows multiplexing. The microspheres are resuspended by vortex and sonication for approximately 20 seconds. A Working Microsphere Mixture is prepared by diluting the coupled microsphere stocks to a final concentration of 100 microspheres of each set/µL in Startblock (Pierce (37538)). 50 µL of Working Microsphere Mixture is used for each well. Either PBS-1% BSA or PBS-BN (PBS, 1% BSA, 0.05% Azide, pH 7.4) may be used as Assay Buffer.

[00615] A 1.2 µm Millipore filter plate is pre-wet with 100 µl/well of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and aspirated by vacuum manifold. An aliquot of 50 µl of the Working Microsphere Mixture is dispensed into the appropriate wells of the filter plate (Millipore Multiscreen HTS (MSBVN1250)). A 50 µl aliquot of standard or sample is dispensed into to the appropriate wells. The filter plate is covered and incubated for 60 minutes at room temperature on a plate shaker. The plate is covered with a sealer, placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that the speed is set to 550 for the duration of the incubation.

[00616] The supernatant is aspirated by vacuum manifold (less than 5 inches Hg in all aspiration steps). Each well is washed twice with 100 µl of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirated by vacuum manifold. The microspheres are resuspended in 50 µL of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))). The phycoerythrin (PE) conjugated detection antibody is diluted to 4 µg/mL (or appropriate

concentration) in PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))). (Note: 50 µL of diluted detection antibody is required for each reaction.) A 50 µl aliquot of the diluted detection antibody is added to each well. The filter plate is covered and incubated for 60 minutes at room temperature on a plate shaker. The filter plate is covered with a sealer, placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that the speed is set to 550 for the duration of the incubation. The supernatant is aspirated by vacuum manifold. The wells are washed twice with 100 µl of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and aspirated by vacuum manifold. The microspheres are resuspended in 100 µl of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))). The microspheres are analyzed on a Luminex analyzer according to the system manual.

Example 5: Analysis of Vesicles Using Antibody-Coupled Microspheres and Biotinylated Antibody

[00617] This example demonstrates the use of particles coupled to an antibody, where the antibody captures the vesicles. An antibody, the detector antibody, is biotinylated. A label coupled to streptavidin is used to detect the biomarker.

[00618] First, the appropriate antibody-coupled microsphere set is selected (Luminex, Austin, TX). The microspheres are resuspended by vortex and sonication for approximately 20 seconds. A Working Microsphere Mixture is prepared by diluting the coupled microsphere stocks to a final concentration of 50 microspheres of each set/µL in Startblock (Pierce (37538)). (Note: 50 µl of Working Microsphere Mixture is required for each well.) Beads in Start Block should be blocked for 30 minutes and no more than 1 hour.

[00619] A 1.2 µm Millipore filter plate is pre-wet with 100 µl /well of PBS-1% BSA + Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirated by vacuum manifold. A 50 µl aliquot of the Working Microsphere Mixture is dispensed into the appropriate wells of the filter plate (Millipore Multiscreen HTS (MSBVN1250)). A 50 µl aliquot of standard or sample is dispensed to the appropriate wells. The filter plate is covered with a seal and is incubated for 60 minutes at room temperature on a plate shaker. The covered filter plate is placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that, the speed is set to 550 for the duration of the incubation.

[00620] The supernatant is aspirated by a vacuum manifold (less than 5 inches Hg in all aspiration steps). Aspiration can be done with the Pall vacuum manifold. The valve is place in the full off position when the plate is placed on the manifold. To aspirate slowly, the valve is opened to draw the fluid from the wells, which takes approximately 3 seconds for the 100 µl of sample and beads to be fully aspirated from the well. Once the sample drains, the purge button on the manifold is pressed to release residual vacuum pressure from the plate.

[00621] Each well is washed twice with 100 µl of PBS-1% BSA + Azide (PBS-BN)(Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirates by vacuum manifold. The microspheres are resuspended in 50 µl of PBS-1% BSA+ Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032)))

[00622] The biotinylated detection antibody is diluted to 4 µg/mL in PBS-1% BSA + Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))). (Note: 50 µl of diluted detection antibody is required for each reaction.) A 50 µl aliquot of the diluted detection antibody is added to each well.

[00623] The filter plate is covered with a sealer and is incubated for 60 minutes at room temperature on a plate shaker. The plate is placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that, the speed is set to 550 for the duration of the incubation.

[00624] The supernatant is aspirated by vacuum manifold. Aspiration can be done with the Pall vacuum manifold. The valve is place in the full off position when the plate is placed on the manifold. To aspirate slowly, the valve is opened

to draw the fluid from the wells, which takes approximately 3 seconds for the 100 ul of sample and beads to be fully aspirated from the well. Once all of the sample is drained, the purge button on the manifold is pressed to release residual vacuum pressure from the plate.

[00625] Each well is washed twice with 100 µl of PBS-1% BSA + Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirated by vacuum manifold. The microspheres are resuspended in 50 µl of PBS-1% BSA (Sigma (P3688-10PAK + 0.05% NaAzide (S8032))).

[00626] The streptavidin-R-phycoerythrin reporter (Molecular Probes 1 mg/ml) is diluted to 4 µg/mL in PBS-1% BSA+ Azide (PBS-BN). 50 µl of diluted streptavidin-R-phycoerythrin was used for each reaction. A 50 µl aliquot of the diluted streptavidin-R-phycoerythrin is added to each well.

[00627] The filter plate is covered with a sealer and is incubated for 60 minutes at room temperature on a plate shaker. The plate is placed on the orbital shaker and set to 900 for 15-30 seconds to re-suspend the beads. Following that, the speed is set to 550 for the duration of the incubation.

[00628] The supernatant is aspirated by vacuum manifold. Aspiration can be done with the Pall vacuum manifold. The valve is place in the full off position when the plate is placed on the manifold. To aspirate slowly, the valve is opened to draw the fluid from the wells, which takes approximately 3 seconds for the 100 ul of sample and beads to be fully aspirated from the well. Once all of the sample is drained, the purge button on the manifold is pressed to release residual vacuum pressure from the plate.

[00629] Each well is washed twice with 100 µl of PBS-1% BSA + Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and is aspirated by vacuum manifold. The microspheres are resuspended in 100 µl of PBS-1% BSA+ Azide (PBS-BN)((Sigma (P3688-10PAK + 0.05% NaAzide (S8032))) and analyzed on the Luminex analyzer according to the system manual.

Example 6: Vesicle Concentration from Plasma

[00630] Supplies and Equipment: Pall life sciences Acrodisc, 25mm syringe filter w/1.2 um, Versapor membrane (sterile) Part number: 4190; Pierce concentrators 7 ml/150 K MWCO (molecular weight cut off), Part number: 89922; BD syringe filter, 10 ml, Part number: 305482; Sorvall Legend RT Plus Series Benchtop Centrifuge w 15 ml swinging bucket rotor; PBS, pH 7.4, Sigma cat#P3813-10PAK prepared in Sterile Molecular grade water; Co-polymer 1.7ml microfuge tubes, USA Scientific, cat#1415-2500. Water used for reagents is Sterile Filtered Molecular grade water (Sigma, cat#W4502). Handling of patient plasma is done in a biosafety hood.

Procedure:

1. Filter procedure for plasma samples

1.1. Remove plasma samples from -80°C (-65°C to -85°C) freezer
1.2. Thaw samples in room temperature water (10-15 minutes).
1.3. Prepare syringe and filter by removing the number necessary from their casing.
1.4. Pull plunger to draw 4mL of sterile molecular grade water into the syringe. Attach a 1.2µm filter to the syringe tip and pass contents through the filter onto the 7 ml/150 K MWCO Pierce column.
1.5. Cap the columns and place in the swing bucket centrifuge at spin at 1000xg in Sorvall Legend RT plus centrifuge for 4 minutes at 20°C (16°C-24°C).
1.6. While spinning, disassemble the filter from syringe. Then remove plunger from syringe.
1.7. Discard flow through from the tube and gently tap column on paper towels to remove any residual water.
1.8. Measure and record starting volumes for all plasma samples. Samples with a volume less than 900µl may not be processed.

1.9. Place open syringe and filter on open Pierce column. Fill open end of syringe with 5.2mL of 1X PBS and pipette mix plasma into PBS three to four times.

1.10. Replace the plunger of the syringe and slowly depress the plunger until the contents of the syringe have passed through the filter onto the Pierce column. Contents should pass through the filter drop wise.

2. Microvesicle concentration centrifugation protocol

2.1. Spin 7 ml/150 K MWCO Pierce columns at 2000xg at 20ºC (16°C-24°C) for 60 minutes or until volume is reduced to 250-300µL. If needed, spin for additional 15 minutes increments to reach required volume.

2.2. At the conclusion of the spin, pipette mix on the column 15x (avoid creating bubbles) and withdraw volume (300µL or less) and transfer to a new 1.7mL co-polymer tube.

2.3. The final volume of the plasma concentrate is dependent on the initial volume of plasma. Plasma is concentrated to 300ul if the original plasma volume is 1ml. If the original volume of plasma is less than 1ml, then the volume of concentrate should be consistent with that ratio. For example, if the original volume is 900ul, then the volume of concentrate is 270ul. The equation to follow is: x=(y/1000)*300, where x is the final volume of concentrate and y is the initial volume of plasma.

2.4. Record the sample volume and add 1X PBS to the sample to make the final sample volume.

2.5. Store concentrated microvesicle sample at 4ºC (2°C to 8°C).

Calculations:

1. Final volume of concentrated plasma sample
x=(y/1000)*300, where x is the final volume of concentrate and y is the initial volume of plasma.

Example 7: Capture of Vesicles Using Magnetic Beads

[00631] Vesicles isolated as described in **Example 2** are used. Approximately 40 µl of the vesicles are incubated with approximately 5 µg (~50 µl) of EpCam antibody coated Dynal beads (Invitrogen, Carlsbad, CA) and 50 µl of Starting Block. The vesicles and beads are incubated with shaking for 2 hours at 45°C in a shaking incubator. The tube containing the Dynal beads is placed on the magnetic separator for 1 minute and the supernatant removed. The beads are washed twice and the supernatant removed each time. Wash beads twice, discarding the supernatant each time.

Example 8: Detection of mRNA Transcripts in Vesicles

[00632] RNA from the bead-bound vesicles of **Example 7** was isolated using the Qiagen miRneasy™ kit, (Cat. No. 217061), according to the manufacturer's instructions.

[00633] The vesicles are homogenized in QIAzol™ Lysis Reagent (Qiagen Cat. No. 79306). After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper, aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions for all RNA molecules from 18 nucleotides (nt) upwards. The sample is then applied to the RNeasy™ Mini spin column, where the total RNA binds to the membrane and phenol and other contaminants are efficiently washed away. High quality RNA is then eluted in RNase-free water.

[00634] RNA from the VCAP bead captured vesicles was measured with the Taqman TMPRSS:ERG fusion transcript assay (*Kirsten D. Mertz et al. Neoplasia. 2007 March; 9(3): 200–206.*). RNA from the 22Rv1 bead captured vesicles was measured with the Taqman SPINK1 transcript assay (*Scott A. Tomlins et al. Cancer Cell 2008 June 13(6):519-528*). The GAPDH transcript (control transcript) was also measured for both sets of vesicle RNA.

[00635] Higher CT values indicate lower transcript expression. One change in cycle threshold (CT) is equivalent to a 2 fold change, 3 CT difference to a 4 fold change, and so forth, which can be calculated with the following: $2^{\wedge CT1-CT2.}$

This experiment shows a difference in CT of the expression of the fusion transcript TMPRSS:ERG and the equivalent captured with the IgG2 negative control bead (**FIG. 5**). The same comparison of the SPINK1 transcript in 22RV1 vesicles showed a CT difference of 6.14 for a fold change of 70.5. Results with GAPDH were similar (not shown).

Example 9: Obtaining Serum Samples from Subjects

[00636] Blood is collected from subjects (both healthy subjects and subjects with cancer) in EDTA tubes, citrate tubes or in a 10 ml Vacutainer SST plus Blood Collection Tube (BD367985 or BD366643, BD Biosciences). Blood is processed for plasma isolation within 2 h of collection.

[00637] Samples are allowed to sit at room temperature for a minimum of 30 min and a max of 2 h. Separation of the clot is accomplished by centrifugation at 1,000–1,300 x *g* at 4°C for 15–20 min. The serum is removed and dispensed in aliquots of 500 µl into 500 to 750 µl cryotubes. Specimens are stored at -80°C.

[00638] At a given sitting, the amount of blood drawn can range from ~20 to ~90 ml. Blood from several EDTA tubes is pooled and transferred to RNase/DNase-free 50-ml conical tubes (Greiner), and centrifuged at 1,200 x *g* at room temperature in a Hettich Rotanta 460R benchtop centrifuge for 10 min. Plasma is transferred to a fresh tube, leaving behind a fixed height of 0.5 cm plasma supernatant above the pellet to avoid disturbing the pellet. Plasma is aliquoted, with inversion to mix between each aliquot, and stored at -80°C.

Example 10: RNA Isolation From Human Plasma and Serum Samples

[00639] Four hundred µl of human plasma or serum is thawed on ice and lysed with an equal volume of 2X Denaturing Solution (Ambion). RNA is isolated using the *mir*Vana PARIS kit following the manufacturer's protocol for liquid samples (Ambion), modified such that samples are extracted twice with an equal volume of acid-phenol chloroform (as supplied by the Ambion kit). RNA is eluted with 105 µl of Ambion elution solution according to the manufacturer's protocol. The average volume of eluate recovered from each column is about 80 µl.

[00640] A scaled-up version of the *mir*Vana PARIS (Ambion) protocol is also used: 10 ml of plasma is thawed on ice, two 5-ml aliquots are transferred to 50-ml tubes, diluted with an equal volume of *mir*Vana PARIS 2X Denaturing Solution, mixed thoroughly by vortexing for 30 s and incubated on ice for 5 min. An equal volume (10 ml) of acid/phenol/chloroform (Ambion) is then added to each aliquot. The resulting solutions are vortexed for 1 min and spun for 5 min at 8,000 rpm, 20°C in a JA17 rotor. The acid/phenol/chloroform extraction is repeated three times. The resulting aqueous volume is mixed thoroughly with 1.25 volumes of 100% molecular-grade ethanol and passed through a *mir*Vana PARIS column in sequential 700-µl aliquots. The column is washed following the manufacturer's protocol, and RNA is eluted in 105 µl of elution buffer (95°C). A total of 1.5 µl of the eluate is quantified by Nanodrop.

Example 11: Measurement of miRNA Levels in RNA from Plasma and Serum using qRT-PCR

[00641] A fixed volume of 1.67 µl of RNA solution from about ~80 µl -eluate from RNA isolation of a given sample is used as input into the reverse transcription (RT) reaction. For samples in which RNA is isolated from a 400- µl plasma or serum sample, for example, 1.67 µl of RNA solution represents the RNA corresponding to (1.67/80) X 400 = 8.3 µl plasma or serum. For generation of standard curves of chemically synthesized RNA oligonucleotides corresponding to known miRNAs, varying dilutions of each oligonucleotide are made in water such that the final input into the RT reaction has a volume of 1.67 µl. Input RNA is reverse transcribed using the TaqMan miRNA Reverse Transcription Kit and miRNA-specific stem-loop primers (Applied BioSystems) in a small-scale RT reaction comprised of 1.387 µl of H2O, 0.5 µl of 10X Reverse-Transcription Buffer, 0.063 µl of RNase-Inhibitor (20 units / µl), 0.05 µl of 100 mM dNTPs with dTTP, 0.33 µl of Multiscribe Reverse- Transcriptase, and 1.67 µl of input RNA; components other than the

DEMANDE OU BREVET VOLUMINEUX

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CLAIMS

WHAT IS CLAIMED IS:

1. A method of determining a KRAS nucleotide sequence in a biological sample that comprises one or more microvesicle, comprising:

(a) contacting the biological sample with a binding agent to a microvesicle surface antigen, wherein the microvesicle surface antigen is selected from **Table 3**, **Table 4**, **Table 5**, **Table 8**, **Table 9** or **Table 10**;

(b) isolating nucleic acids from the microvesicles that formed a complex with the binding agent to the microvesicle surface antigen in step (a); and

(c) determining a v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) sequence within the nucleic acids isolated in step (b).

2. The method of claim **1**, wherein the microvesicle surface antigen comprises Tissue factor, EpCam, B7H3, RAGE, CEA, CD66, TMEM211 and/or CD24.

3. The method of claim **1**, wherein the microvesicle surface antigen comprises CD24.

4. The method of claim **1**, further comprising contacting the biological sample with a binding agent to a general vesicle marker in step (a) and isolating the nucleic acids from microvesicles that also formed a complex with the binding agent to the general vesicle marker in step (b).

5. The method of claim **4**, wherein the general vesicle marker is selected from **Table 3**.

6. The method of claim **4**, wherein the general vesicle marker comprises a tetraspanin.

7. The method of claim **4**, wherein the general vesicle marker comprises CD9, CD63 and/or CD81.

8. The method of claim **4**, wherein the general vesicle marker comprises CD9, CD63 and/or CD81 and the microvesicle surface antigen comprises Tissue factor, EpCam, B7H3, RAGE, CEA, CD66, TMEM211 and/or CD24.

9. The method of claim **4**, wherein the general vesicle marker comprises CD63 and the microvesicle surface antigen comprises CD24.

10. The method of any preceding claim, wherein the KRAS sequence is determined by pyrosequencing, Sanger sequencing, or Next Generation sequencing.

11. The method of any preceding claim, wherein the determined KRAS sequence comprises a mutation.

12. The method of claim **11**, wherein the mutation comprises an activating mutation.

13. The method of claim **11**, wherein the mutation is selected from the group consisting of 34G>T (G12C), 34G>C (G12R), 34G>A (G12S), 35G>C (G12A), 35G>A (G12D), 35G>T (G12V), 37G>T (G13C), 37G>C (G13R), 37G>A (G13S), 38G>C (G13A), 38G>A (G13D), 38G>T (G13V), 181C>A (Q61K), 182A>T (Q61L), 182A>G (Q61R), 183A>C (Q61H), 183A>T (Q61H), 351A>C (K117N), 351A>T (K117N), 436G>C (A146P), 436G>A (A146T), 437C>T (A146V), and a combination thereof.

14. The method of claim **11**, wherein the mutation comprises a 38G>A (G13D) mutation.

15. The method of any preceding claim, wherein the nucleic acids isolated in step (b) comprise mRNA.

16. The method of any preceding claim, wherein the determined KRAS sequence is used to provide a prognosis or a theranosis for a cancer.

17. The method of claim **16**, wherein the theranosis comprises a prediction of whether a cancer is likely to respond or not respond to a chemotherapeutic agent.

18. The method of claim **17**, wherein the chemotherapeutic agent comprises an epidermal growth factor receptor (EGFR) directed therapy.

19. The method of claim **18**, wherein the EGFR directed therapy comprises panitumumab, cetuximab, zalutumumab, nimotuzumab, matuzumab, gefitinib, erlotinib, and/or lapatinib.

20. The method of claim **17**, wherein the chemotherapeutic agent comprises mammalian target of rapamycin (mTOR) directed therapy, mitogen-activated or extracellular signal–regulated protein kinase kinase (MEK) directed therapy and/or v-raf murine sarcoma viral oncogene homolog B1 (BRAF) directed therapy.

21. The method of claim **20**, wherein the mTOR directed therapy comprises everolimus and/or temsirolimus.

22. The method of claim **17**, wherein the chemotherapeutic agent comprises cyclophosphamide or a combination of vincristine + carmustine (BCNU) + melphalan + cyclophosphamide + prednisone (VBMCP).

23. The method of any of claims **17-22**, wherein a mutation in KRAS is predictive that the cancer is less likely to respond to the chemotherapeutic agent.

24. The method of any of claims **16-23**, wherein the cancer comprises a solid tumor, a colorectal cancer (CRC), a pancreatic cancer, a non-small cell lung cancer (NSCLC), a bronchioloalveolar carcinoma (BAC) or adenocarcinoma (BAC subtype), a leukemia, or a multiple myeloma (MM).

25. A method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising:

(a) isolating a population of microvesicles from a biological sample;

(b) contacting the isolated microvesicle population with a binding agent to a cytokine receptor, wherein the cytokine receptor comprises ErbB3, RAGE, and/or Trail R3; and

(c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent to the cytokine receptor, thereby detecting the presence or level of the one or more microvesicle.

26. A method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising:

(a) isolating a population of microvesicles from a biological sample;

(b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more of IL-1 alpha, CA125, Filamin, and Amyloid A; and

(c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle.

27. A method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising:

(a) isolating a population of microvesicles from a biological sample;

(b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more of Involucrin, CD57, Prohibitin, Thrombospondin, Laminin B1/b1, Filamin, 14.3.3 gamma, 14.3.3 Pan; and

(c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle.

28. A method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising:

(a) isolating a population of microvesicles from a biological sample;

(b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more of Involucrin, Prohibitin, Laminin B1/b1, IL-3, Filamin, 14.3.3 gamma, 14.3.3 Pan, MMP-15 / MT2-MMP, hPL, Ubiquitin, and mRANKL; and

(c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle.

29. A method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising:

(a) isolating a population of microvesicles from a biological sample;

(b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more of Prohibitin, CD57, Filamin, CD18, b-2-Microglobulin, IL-2, IL-3, CD16, p170, Keratin 19, Pds1, Glicentin, SRF (Serum Response Factor), E3-binding protein (ARM1), Collagen II, SRC1 (Steroid Receptor Coactivator-1) Ab-1, Caldesmon, GFAP, TRP75 / gp75, alpha-1-antichymotrypsin, Hepatic Nuclear Factor-3B, PLAP, Tyrosinase, NF kappa B / p50, Melanoma (gp100), Cyclin E, 6-Histidine, Mucin 3 (MUC3), TdT, CD21, XPA, Superoxide Dismutase, Glycogen Synthase Kinase 3b (GSK3b), CD54/ICAM-1, Thrombospondin, Gai1, CD79a mb-1, IL-1 beta, Cytochrome c, RAD1, bcl-X, CD50/ICAM-3, Neurofilament, Alkaline Phosphatase (AP), ER Ca+2 ATPase2, PCNA, F.VIII/VWF, SV40 Large T Antigen, Paxillin, Fascin, CD165, GRIP1, Cdk8, Nucleophosmin (NPM), alpha-1-antitrypsin, CD32/Fcg Receptor II, Keratin 8 (phospho-specific Ser73), DR5, CD46, TID-1, MHC II (HLA-DQ), Plasma Cell Marker, DR3, Calmodulin, AIF (Apoptosis Inducing Factor), DNA Polymerase Beta, Vitamin D Receptor (VDR), Bcl10 / CIPER / CLAP / mE10, Neuron Specific Enolase, CXCR4 / Fusin, Neurofilament (68kDa), PDGFR, beta, Growth Hormone (hGH), Mast Cell Chymase, Ret Oncoprotein, and Phosphotyrosine; and

(c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle.

30. A method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising:

(a) isolating a population of microvesicles from a biological sample;

(b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more of Caspase 5, Thrombospondin, Filamin, Ferritin, 14.3.3 gamma, 14.3.3 Pan, CD71 / Transferrin Receptor, and Prostate Apoptosis Response Protein-4; and

(c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle.

31. A method of detecting a presence or level of one or more microvesicle in a biological sample that comprises or is suspected to comprise the one or more microvesicle, comprising:

(a) isolating a population of microvesicles from a biological sample;

(b) contacting the isolated microvesicle population with a binding agent, wherein the binding agent is specific to one or more of 14.3.3 Pan, Filamin, 14.3.3 gamma, CD71 / Transferrin Receptor, CD30, Cdk5, CD138, Thymidine Phosphorylase, Ruv 5, Thrombospondin, CD1, Von Hippel-Lindau Protein, CD46, Rad51, Ferritin, c-Abl, Actin, Muscle Specific, LewisB; and

(c) detecting a presence or level of the isolated microvesicle population that forms a complex with the binding agent, thereby detecting the presence or level of the one or more microvesicle.

32. The method of any of claims **25-31**, wherein the detected presence or level the one or more microvesicle is used to characterize a malignancy.

33. The method of claim **32**, wherein the presence or level of the one or more microvesicle is compared to a reference in order to characterize the malignancy.

34. The method of claim **32**, wherein the characterizing comprises providing a prognostic, diagnostic or theranostic determination for the malignancy, identifying the presence or risk of the malignancy in a subject, or identifying the malignancy in a subject as metastatic or aggressive.

35. The method of any of claims **32-34** as depends from claim **25**, wherein the malignancy comprises a colorectal cancer or a late stage prostate cancer.

36. The method of any of claims **32-34** as depends from claim **26**, wherein the malignancy comprises a colorectal cancer.

37. The method of any of claims **32-34** as depends from claim **27**, wherein the malignancy comprises a colorectal adenoma.

38. The method of any of claims **32-34** as depends from claim **28**, wherein the malignancy comprises a colorectal cancer and/or a colorectal adenoma.

39. The method of any of claims **32-34** as depends from claim **29**, wherein the malignancy comprises a brain cancer.

40. The method of any of claims **32-34** as depends from claim **30**, wherein the malignancy comprises a melanoma.

41. The method of any of claims **32-34** as depends from claim **31**, wherein the malignancy comprises a head and neck cancer.

42. The method of any preceding claim, wherein the biological sample comprises a bodily fluid from a subject.

43. The method of claim **42**, wherein the bodily fluid comprises peripheral blood, sera, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, umbilical cord blood, or a derivative of any thereof.

44. The method of claim **42**, wherein the biological sample comprises peripheral blood, serum or plasma.

45. The method of claim **44**, further comprising selectively depleting one or more abundant protein from the biological sample prior to the contacting step.

46. The method of claim **45**, wherein the one or more abundant protein comprises one or more of albumin, IgG, transferrin, fibrinogen, fibrin, IgA, α_2-Marcroglobulin, IgM, α_1-Antitrypsin, complement C3, haptoglobulin, apolipoprotein A1, A3 and B; α_1-Acid Glycoprotein, ceruloplasmin, complement C4, C1q, IgD, prealbumin (transthyretin), plasminogen, a derivative of any thereof, and a combination thereof.

47. The method of claim **45**, wherein the one or more abundant protein comprises one or more of Albumin, Immunoglobulins, Fibrinogen, Prealbumin, Alpha 1 antitrypsin, Alpha 1 acid glycoprotein, Alpha 1 fetoprotein, Haptoglobin, Alpha 2 macroglobulin, Ceruloplasmin, Transferrin, complement proteins C3 and C4, Beta 2 microglobulin, Beta lipoprotein, Gamma globulin proteins, C-reactive protein (CRP), Lipoproteins (chylomicrons, VLDL, LDL, HDL), other globulins (types alpha, beta and gamma), Prothrombin, Mannose-binding lectin (MBL), a derivative of any thereof, and a combination thereof.

48. The method of claim **45**, wherein selectively depleting the one or more abundant protein comprises contacting the biological sample with thromboplastin to precipitate fibrinogen.

49. The method of claim **45**, wherein the one or more abundant protein is depleted by immunoaffinity, precipitation, or a combination thereof.

50. The method of claim **45**, wherein selectively depleting the one or more abundant protein from the biological sample comprises depleting at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the one or more abundant protein.

51. The method of any of claims **1-41**, wherein the biological sample comprises a cell culture sample.

52. The method of any preceding claim, wherein the binding agent comprises a nucleic acid, DNA molecule, RNA molecule, antibody, antibody fragment, aptamer, peptoid, zDNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), lectin, peptide, dendrimer, membrane protein labeling agent, chemical compound, or a combination thereof.

53. The method of any preceding claim, wherein the binding agent comprises an antibody and/or an aptamer.

54. The method of any preceding claim, wherein the binding agent is tethered to a substrate.

55. The method of any preceding claim, wherein the binding agent comprises a label.

56. The method of any preceding claim, wherein the one or more microvesicle has a diameter between 10 nm and 2000 nm.

57. The method of any preceding claim, wherein the one or more microvesicle has a diameter between 20 nm and 200 nm.

58. The method of any preceding claim, wherein the one or more microvesicle is subjected to size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, affinity capture, immunoassay, microfluidic separation, flow cytometry or combinations thereof.

59. The method of any preceding claim, further comprising detecting one or more payload biomarker within the one or more microvesicle.

60. The method of claim **59**, wherein the one or more payload biomarker comprises one or more nucleic acid, peptide, protein, lipid, antigen, carbohydrate, and/or proteoglycan.

61. The method of claim **60**, wherein the nucleic acid comprises one or more DNA, mRNA, microRNA, snoRNA, snRNA, rRNA, tRNA, siRNA, hnRNA, or shRNA.

62. The method of claim **59**, wherein the one or more payload biomarker comprises microRNA and/or mRNA.

63. The method of any preceding claim, wherein the method is performed in vitro.

64. Use of one or more reagent to carry out the method of any preceding claim.

65. A kit comprising one or more reagent to carry out the method of any of claims **1-63**.

66. The use of claim **64** or the kit of claim **65**, wherein the one or more reagent is selected from the group consisting of one or more binding agent specific for a microvesicle surface antigen, equipment to remove a highly abundant protein, one or more population of microvesicles, and a combination thereof.

67. An isolated CD24+ microvesicle comprising a mutated KRAS nucleic acid.

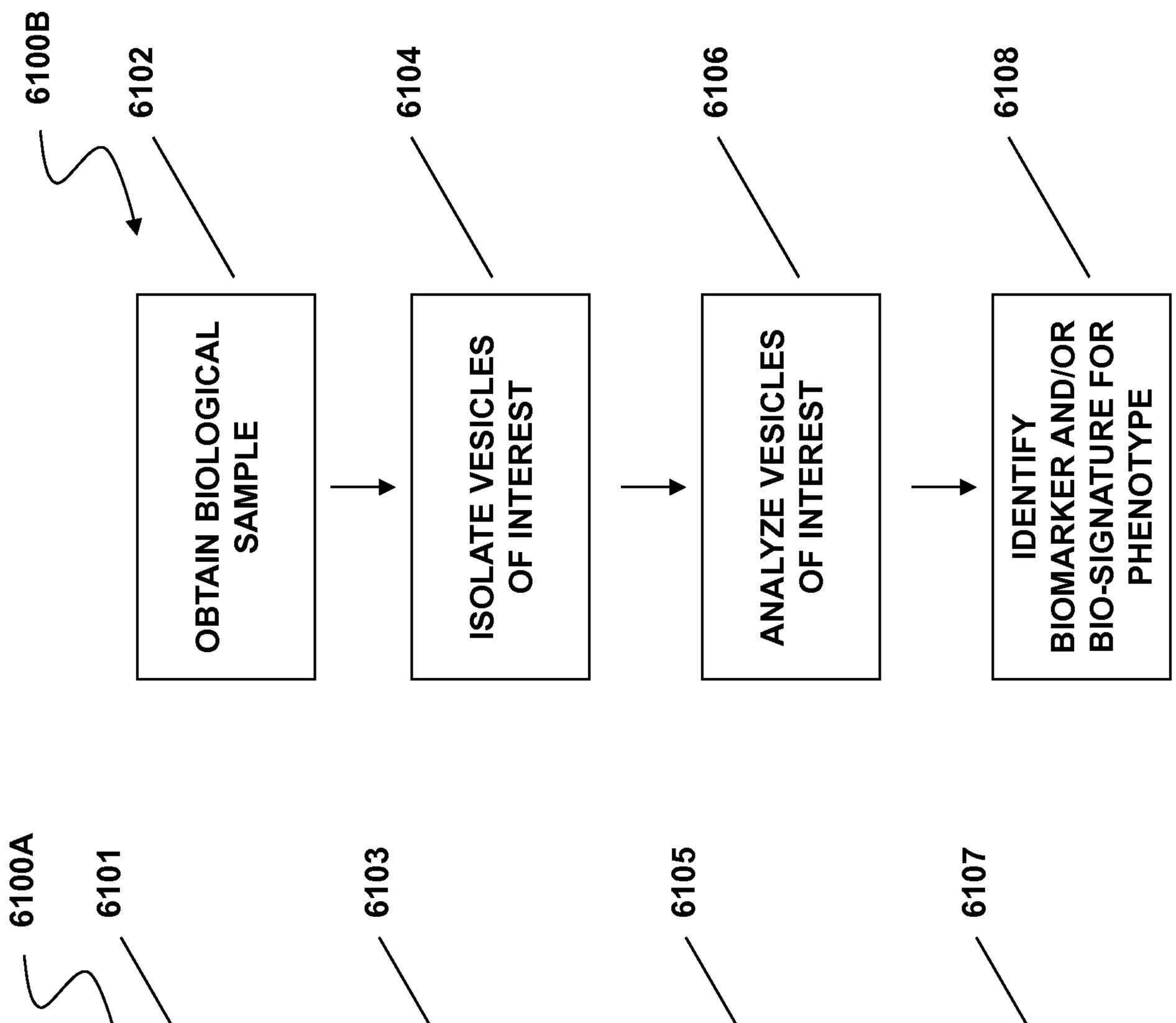

6100A
6101
OBTAIN BIOLOGICAL SAMPLE
6103
ISOLATE NUCLEIC ACID (NA)
6105
ANALYZE NA (MICRORNA, mRNA, MUTATIONS, etc.)
6107
IDENTIFY BIOMARKER AND/OR BIO-SIGNATURE FOR PHENOTYPE

FIG. 1A

6100B
6102
OBTAIN BIOLOGICAL SAMPLE
6104
ISOLATE VESICLES OF INTEREST
6106
ANALYZE VESICLES OF INTEREST
6108
IDENTIFY BIOMARKER AND/OR BIO-SIGNATURE FOR PHENOTYPE

FIG. 1B

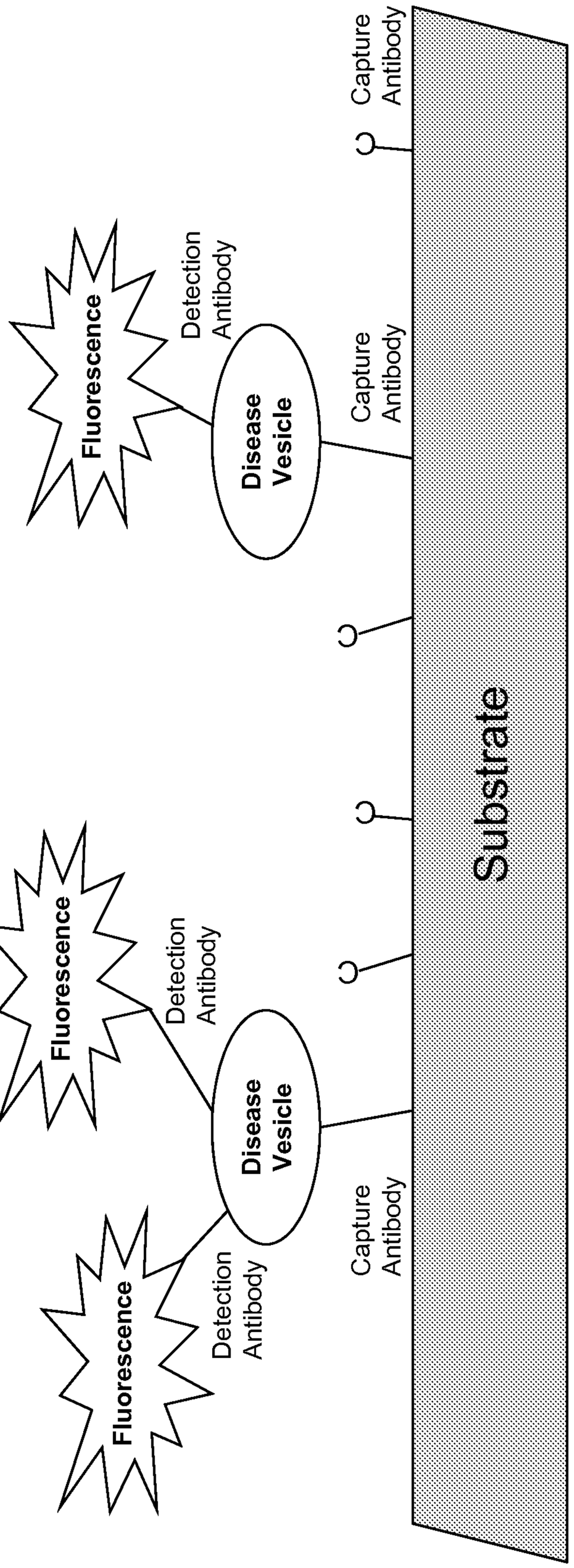

Capture Antibody
Substrate
Capture Antibody
Disease Vesicle
Detection Antibody
Fluorescence
Capture Antibody
Disease Vesicle
Detection Antibody
Fluorescence
Capture Antibody
Disease Vesicle
Detection Antibody
Fluorescence

FIG. 2A

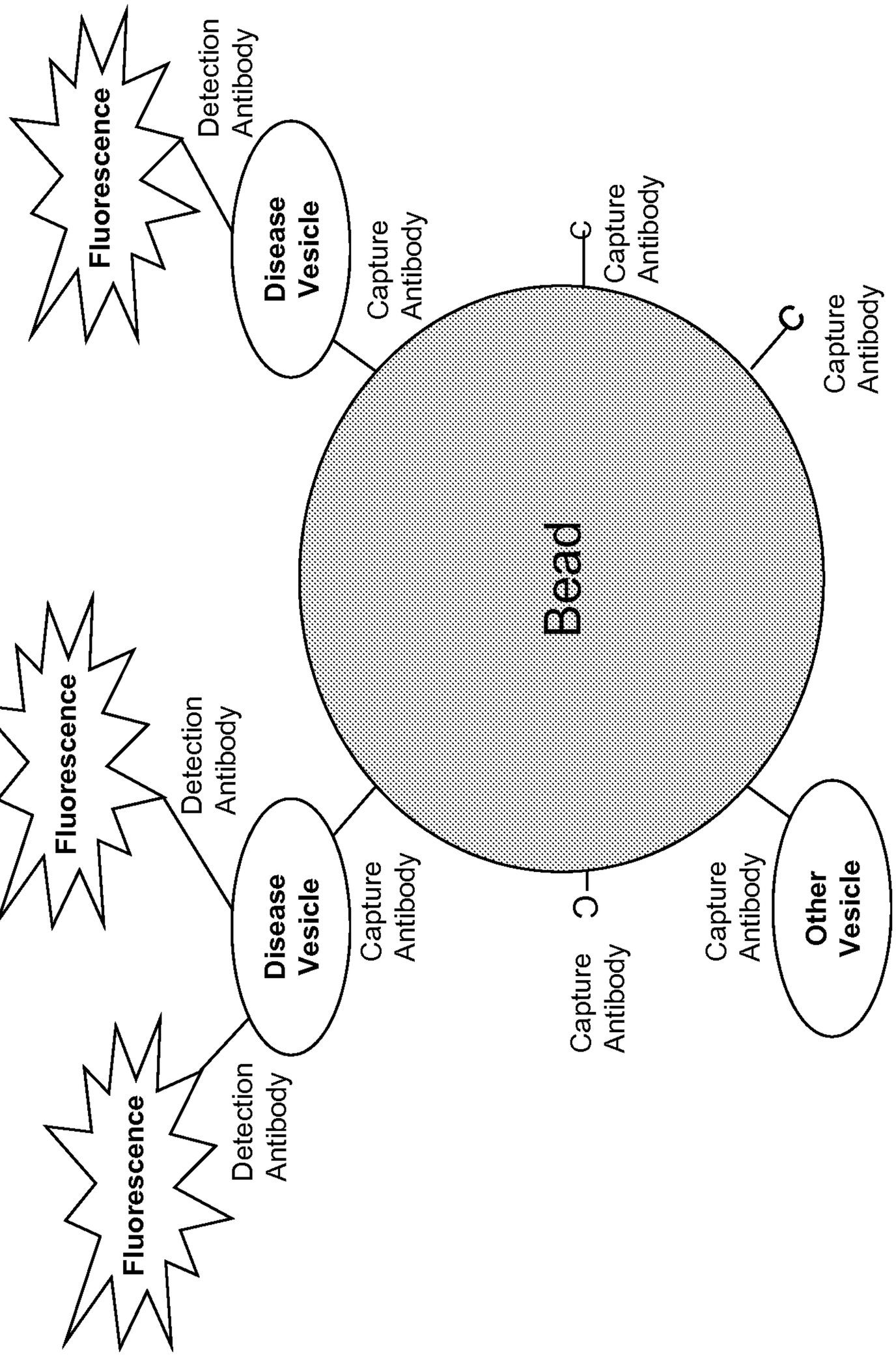

Fluorescence
Detection Antibody
Disease Vesicle
Capture Antibody
Capture Antibody
Capture Antibody
Bead
Fluorescence
Detection Antibody
Disease Vesicle
Capture Antibody
Capture Antibody
Capture Antibody
Other Vesicle
Fluorescence
Detection Antibody

FIG. 2B

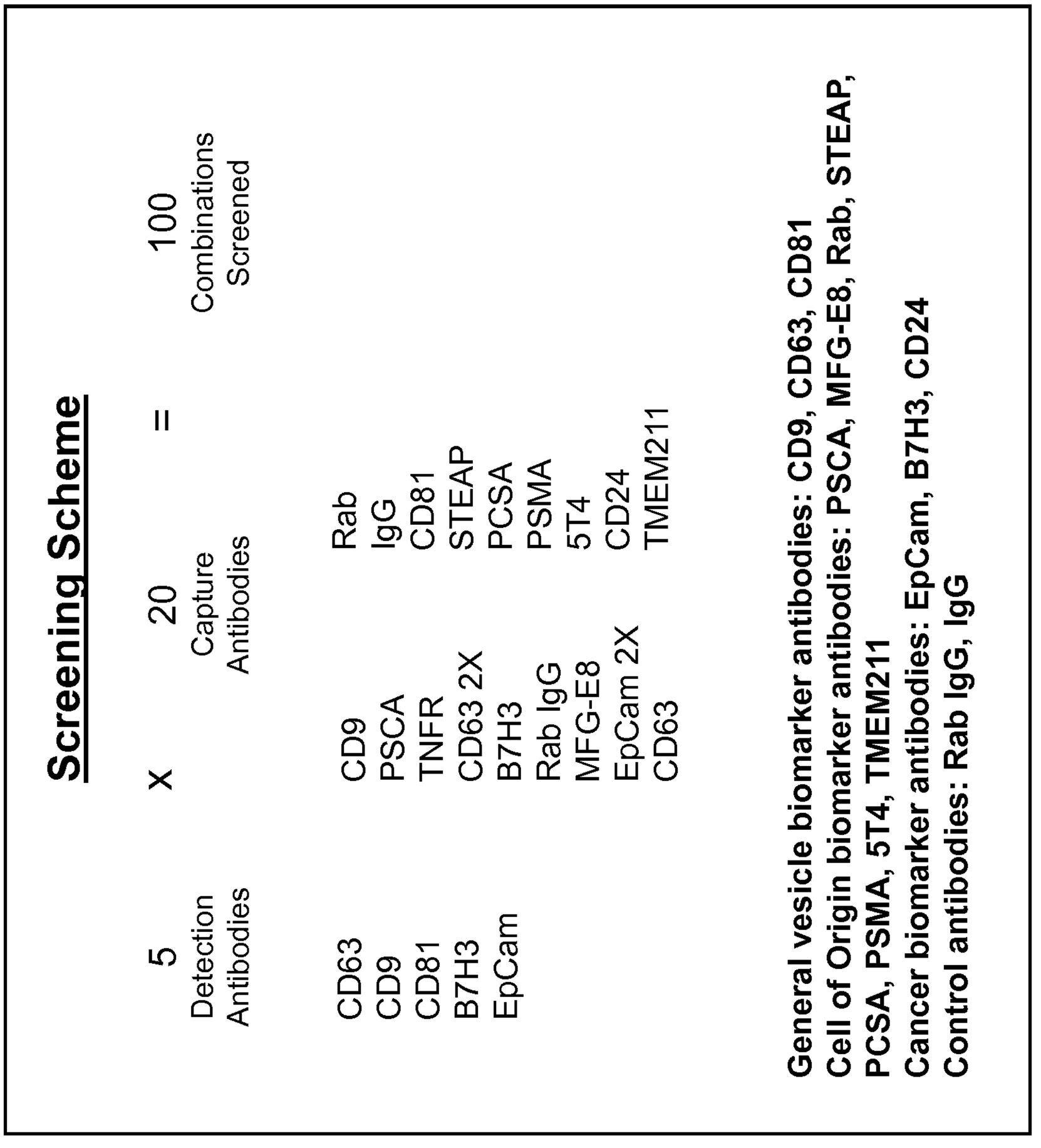

Screening Scheme
5 Detection Antibodies
X
20 Capture Antibodies
=
100 Combinations Screened
CD63
CD9
CD81
B7H3
EpCam
CD9
PSCA
TNFR
CD63 2X
B7H3
Rab IgG
MFG-E8
EpCam 2X
CD63
Rab
IgG
CD81
STEAP
PCSA
PSMA
5T4
CD24
TMEM211
General vesicle biomarker antibodies: CD9, CD63, CD81
Cell of Origin biomarker antibodies: PSCA, MFG-E8, Rab, STEAP, PCSA, PSMA, 5T4, TMEM211
Cancer biomarker antibodies: EpCam, B7H3, CD24
Control antibodies: Rab IgG, IgG

FIG. 2C

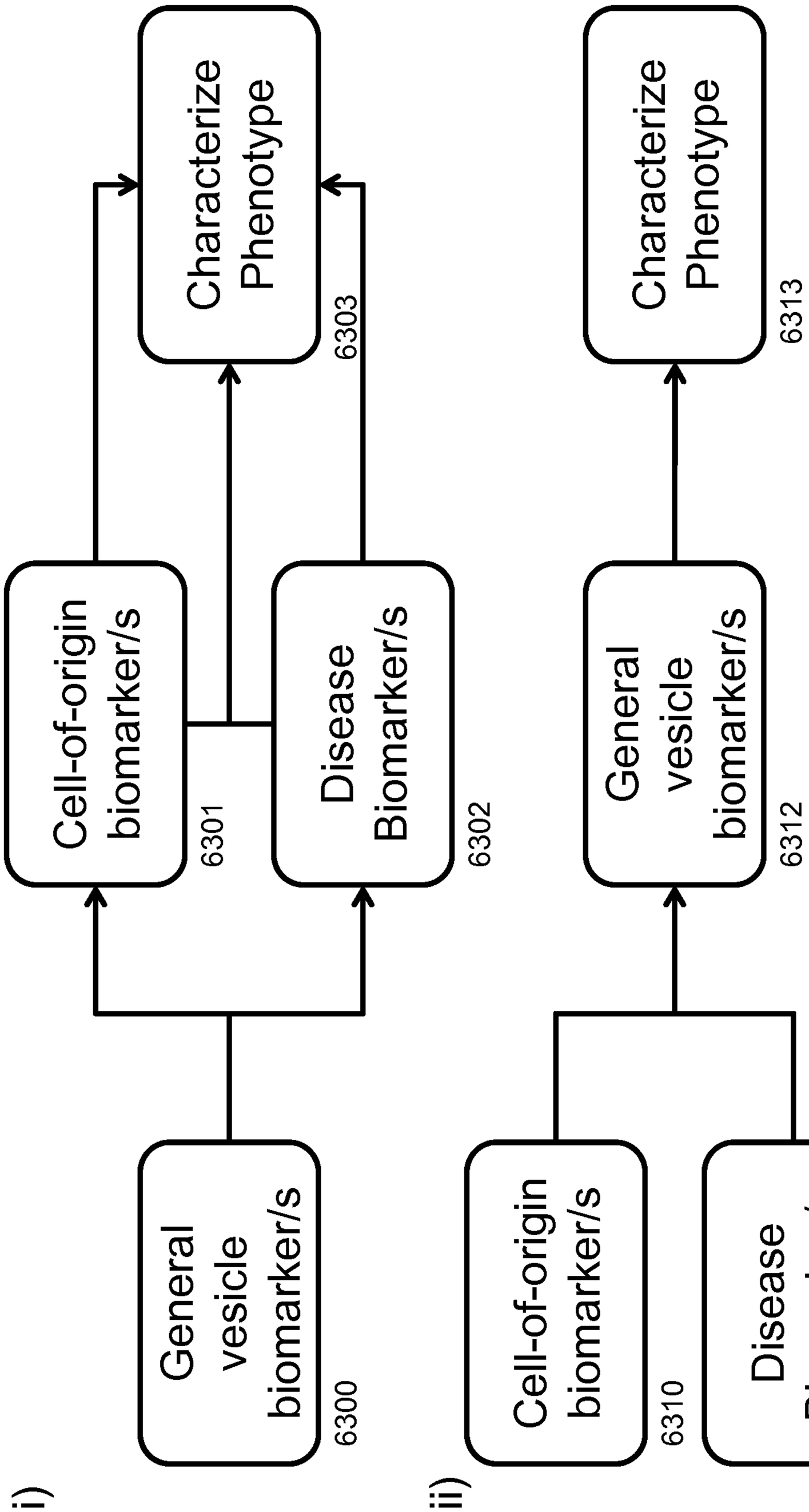

i)
General vesicle biomarker/s
6300
Cell-of-origin biomarker/s
6301
Disease Biomarker/s
6302
Characterize Phenotype
6303
ii)
Cell-of-origin biomarker/s
6310
Disease Biomarker/s
6311
General vesicle biomarker/s
6312
Characterize Phenotype
6313
Capture
Detectors

FIG. 2D

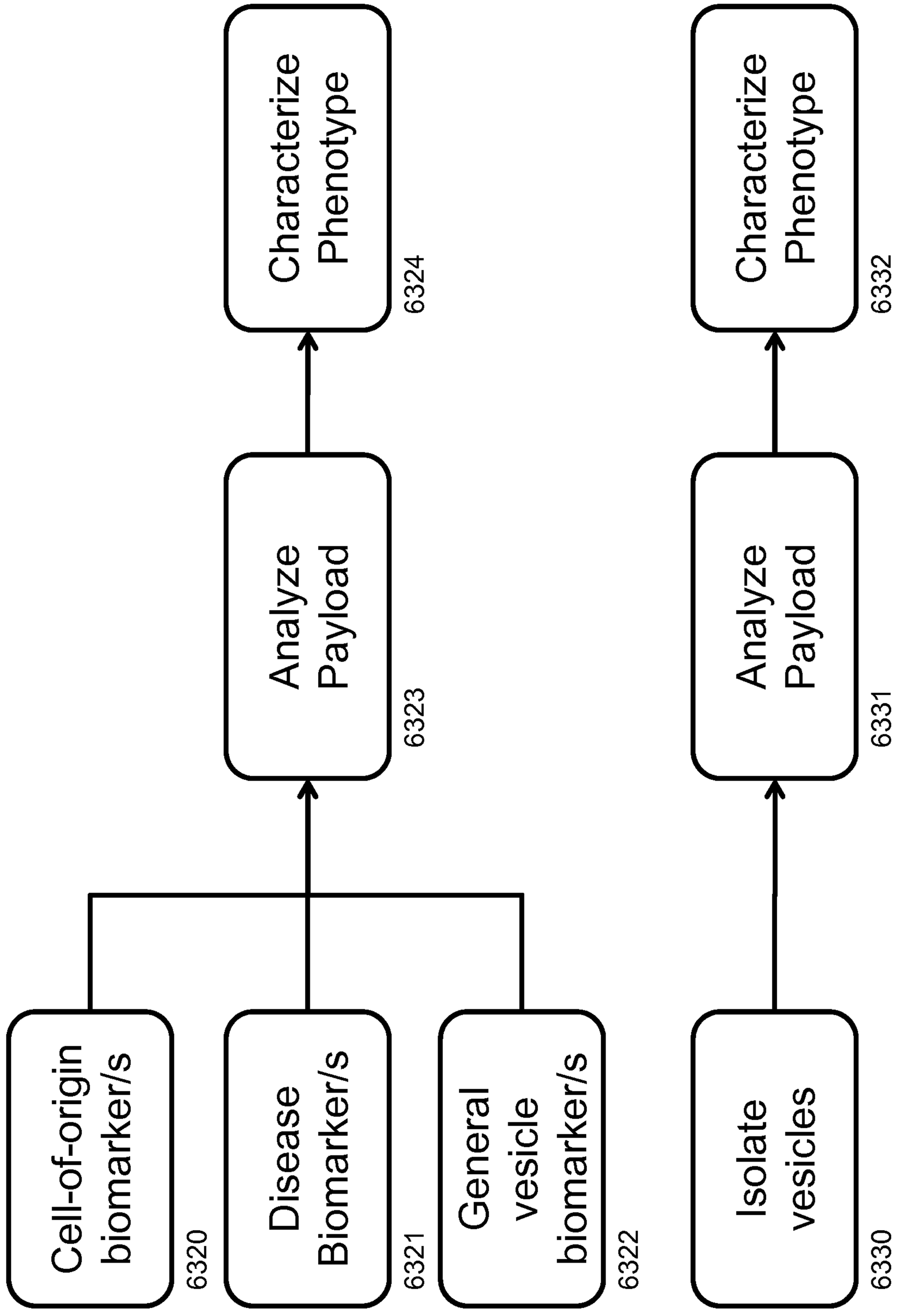

iii)
Cell-of-origin biomarker/s
6320
Disease Biomarker/s
6321
General vesicle biomarker/s
6322
Analyze Payload
6323
Characterize Phenotype
6324
iv)
Isolate vesicles
6330
Analyze Payload
6331
Characterize Phenotype
6332

FIG. 2E

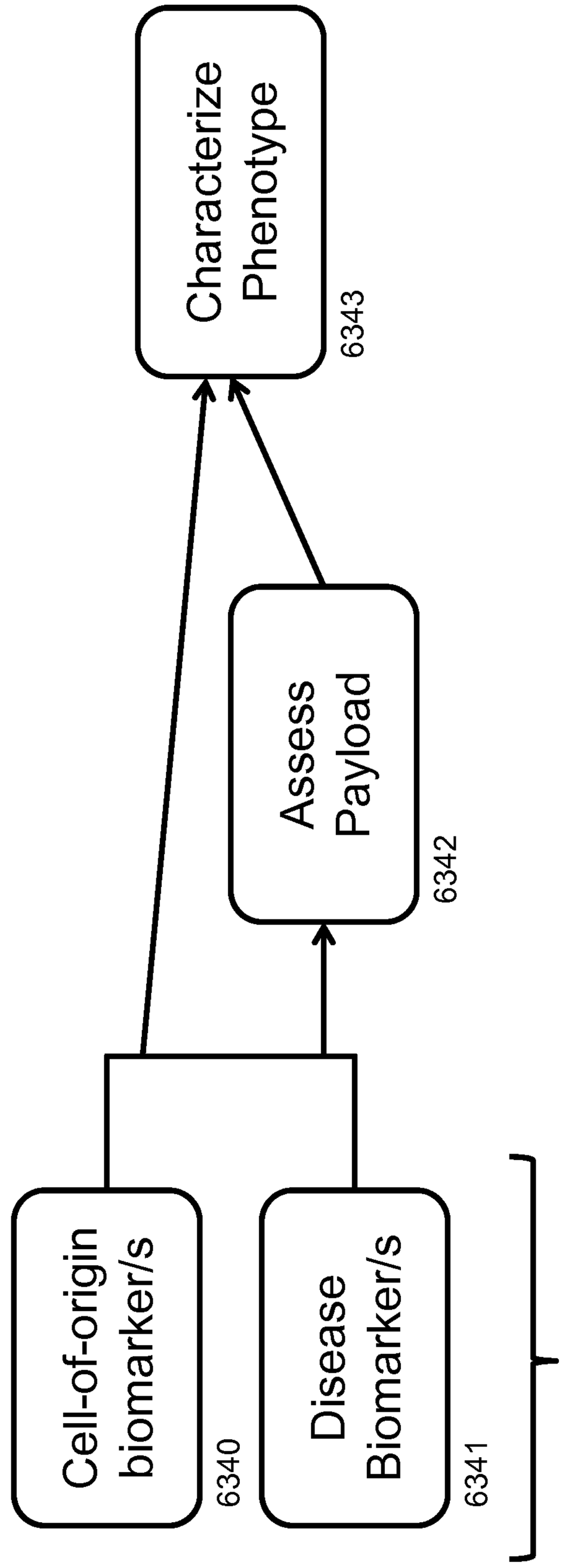

Capture & Detectors
Cell-of-origin biomarker/s
6340
Disease Biomarker/s
6341
v)
Assess Payload
6342
Characterize Phenotype
6343

FIG. 2F

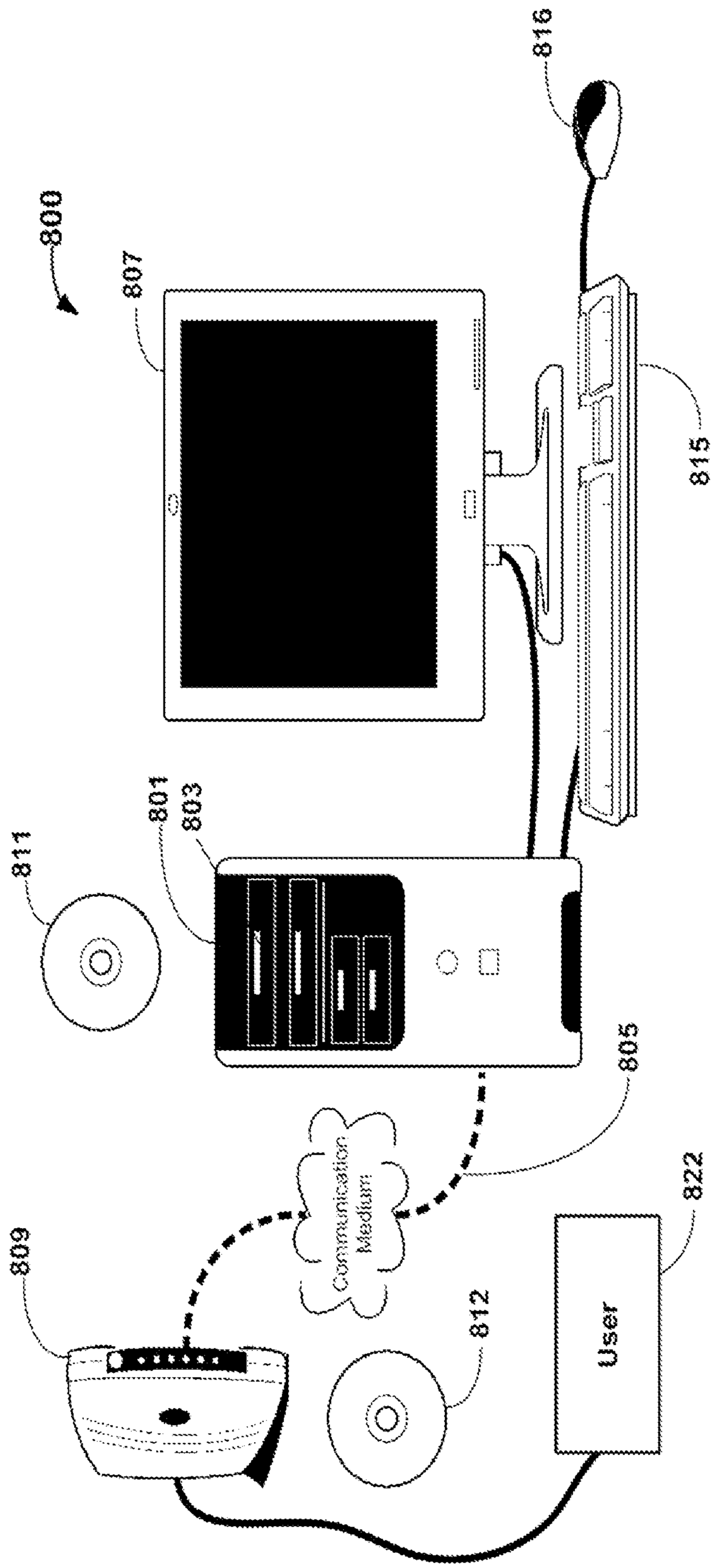

800
807
816
815
801
803
811
805
809
Communication Medium
812
822
User

FIG. 3

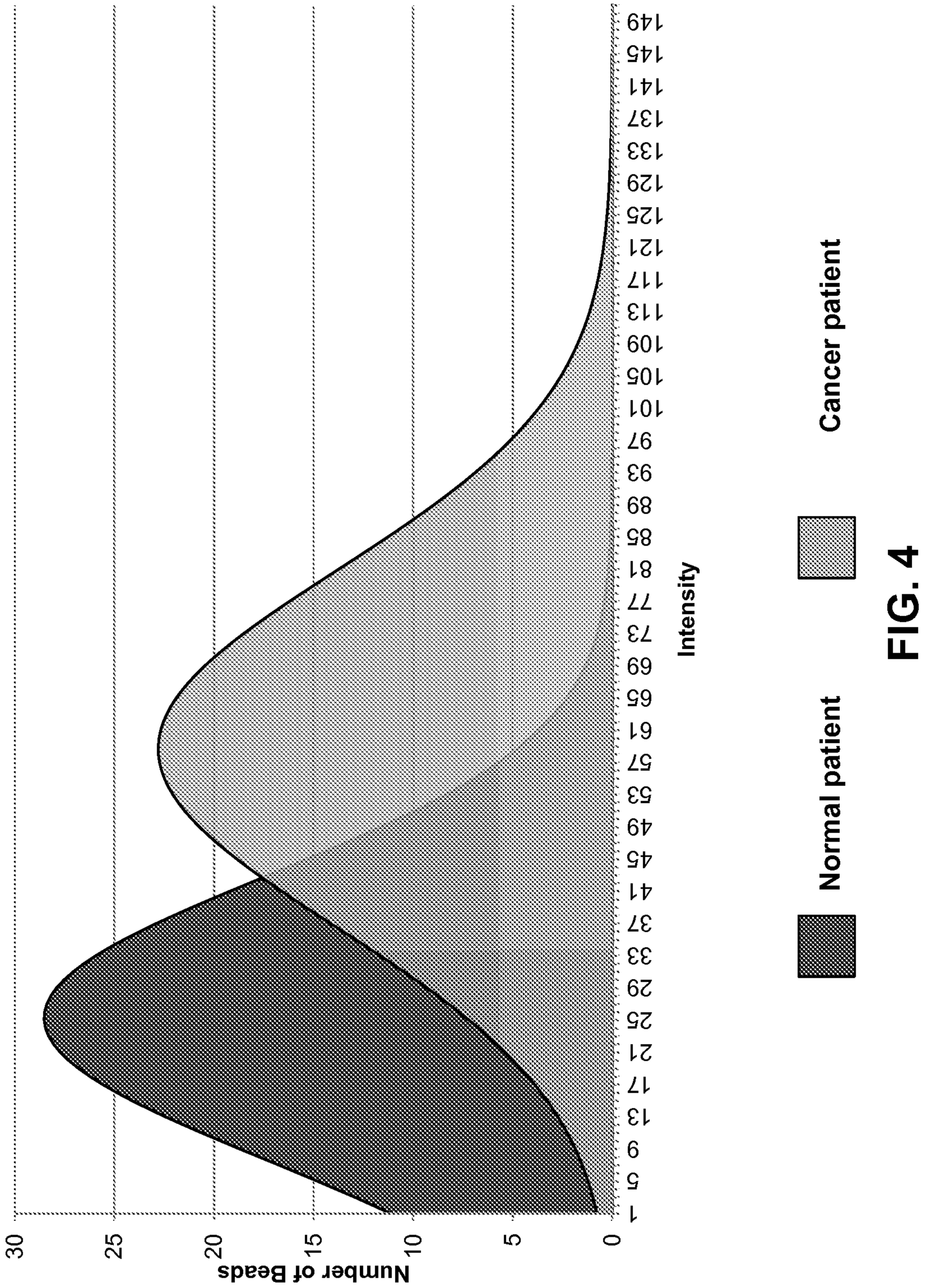

Cancer patient
Normal patient
Intensity
149
145
141
137
133
129
125
121
117
113
109
105
101
97
93
89
85
81
77
73
69
65
61
57
53
49
45
41
37
33
29
25
21
17
13
9
5
1
30
25
20
15
10
5
0
Number of Beads

FIG. 4

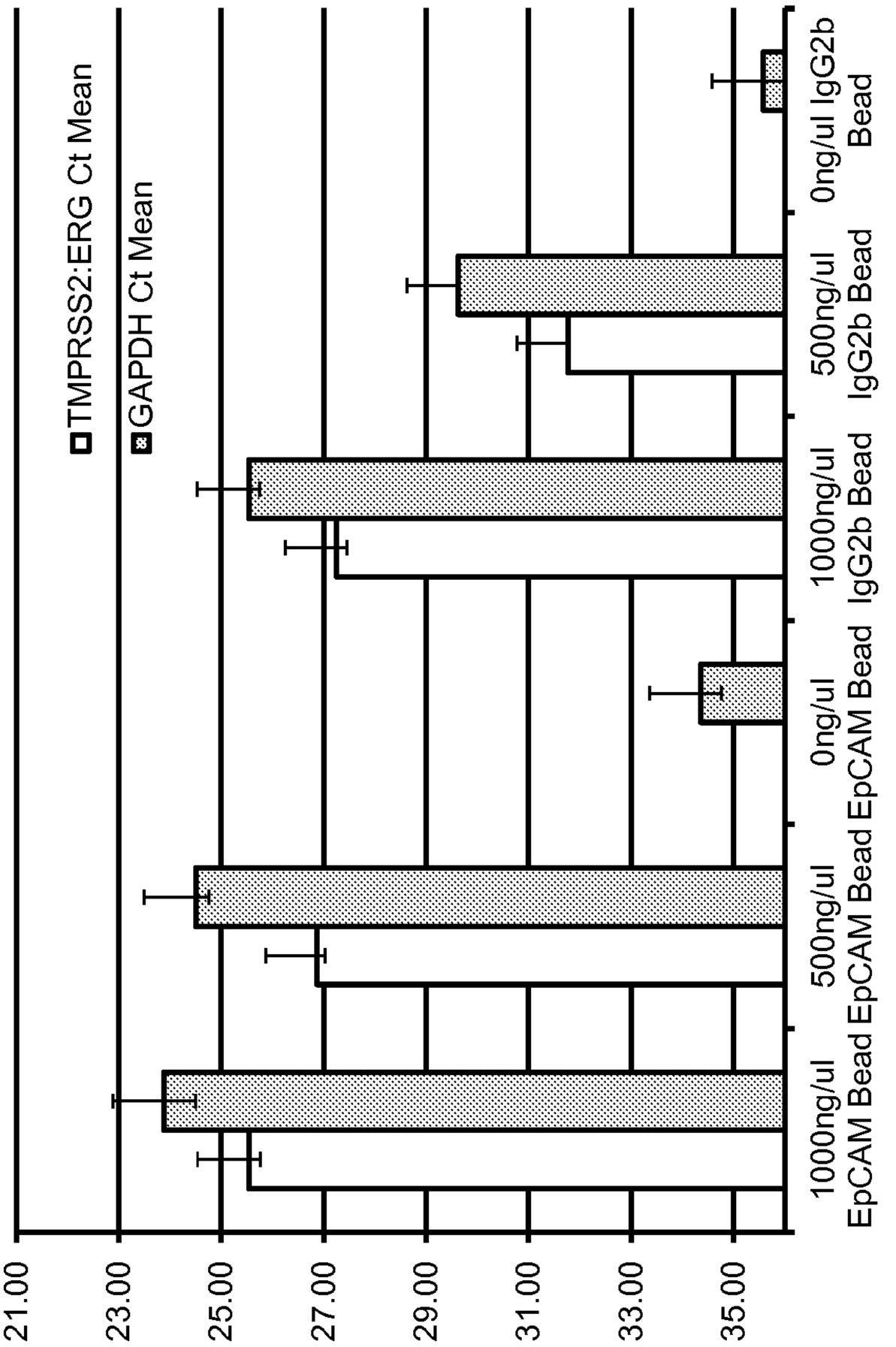
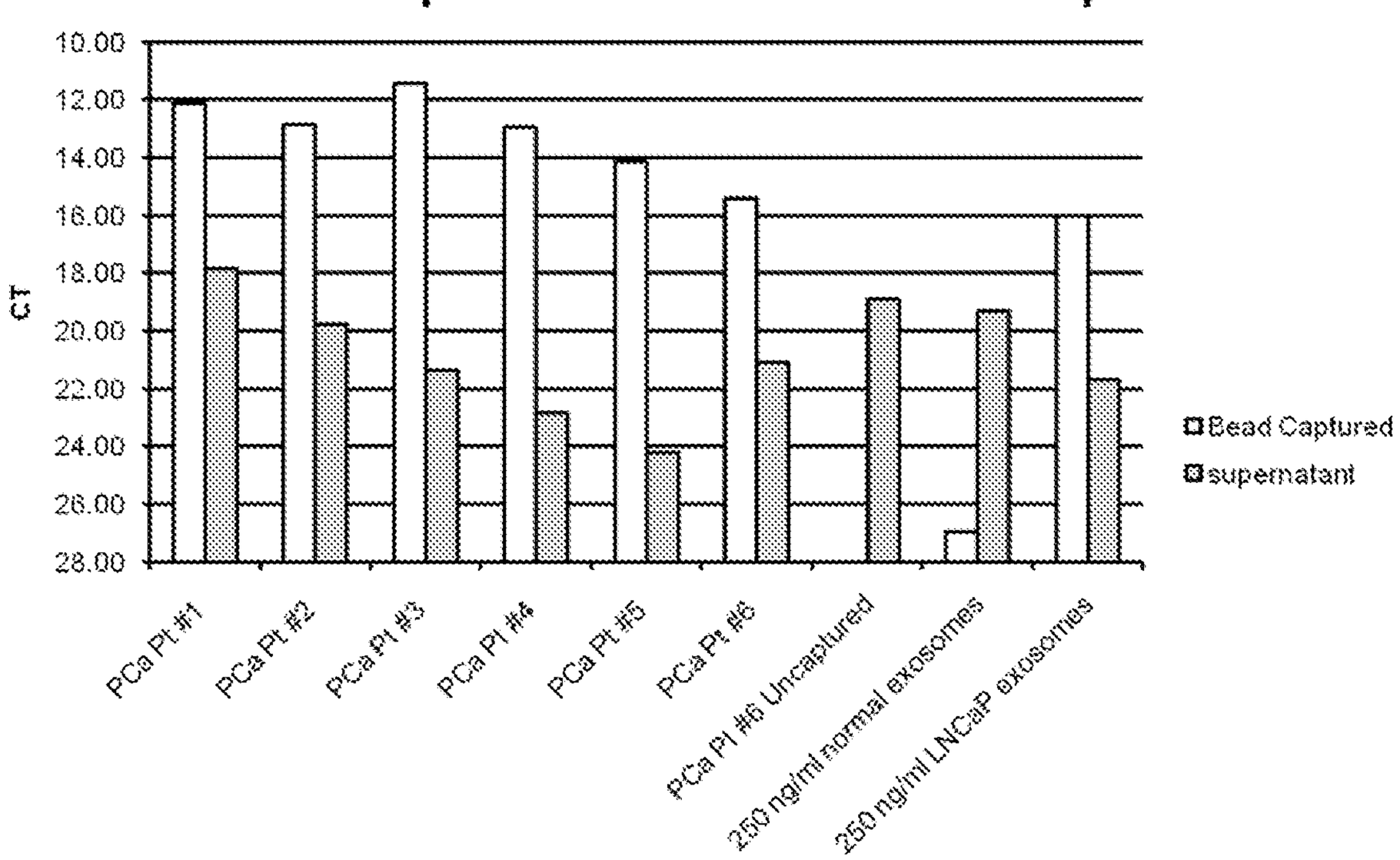

TMPRSS2:ERG Ct Mean
GAPDH Ct Mean
1000ng/ul EpCAM Bead
500ng/ul EpCAM Bead
0ng/ul EpCAM Bead
1000ng/ul IgG2b Bead
500ng/ul IgG2b Bead
0ng/ul IgG2b Bead
21.00
23.00
25.00
27.00
29.00
31.00
33.00
35.00
Ct

FIG. 5


miR 21 Expression with CD-9 Bead Capture
CT
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
PCa Pt #1
PCa Pt #2
PCa Pt #3
PCa Pt #4
PCa Pt #5
PCa Pt #6
PCa Pt #6 Uncaptured
250 ng/ml normal exosomes
250 ng/ml LNCaP exosomes
Bead Captured
supernatant

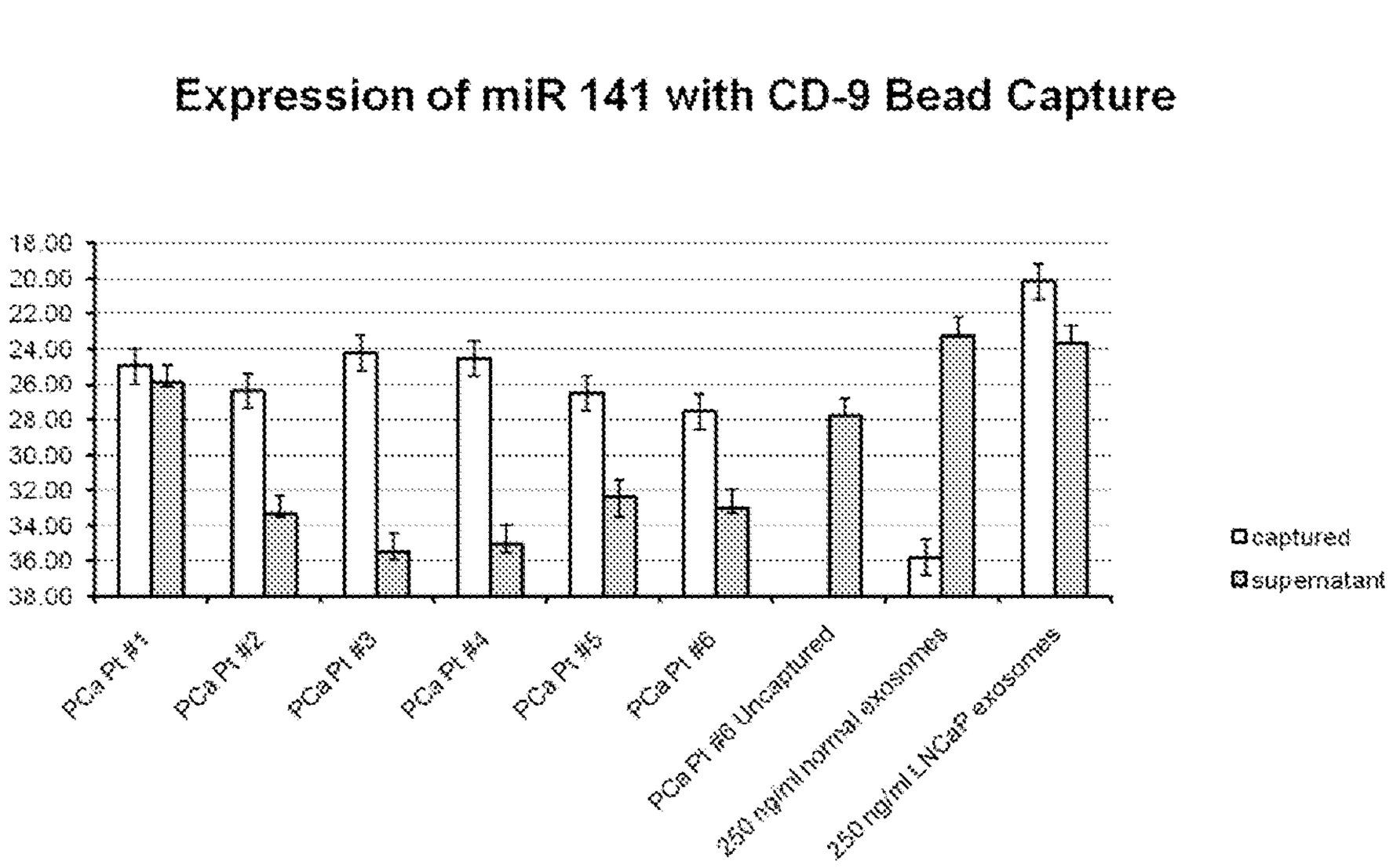

Expression of miR 141 with CD-9 Bead Capture
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
38.00
PCa Pt #1
PCa Pt #2
PCa Pt #3
PCa Pt #4
PCa Pt #5
PCa Pt #6
PCa Pt #6 Uncaptured
250 ng/ml normal exosomes
250 ng/ml LNCaP exosomes
captured
supernatant

FIG. 6

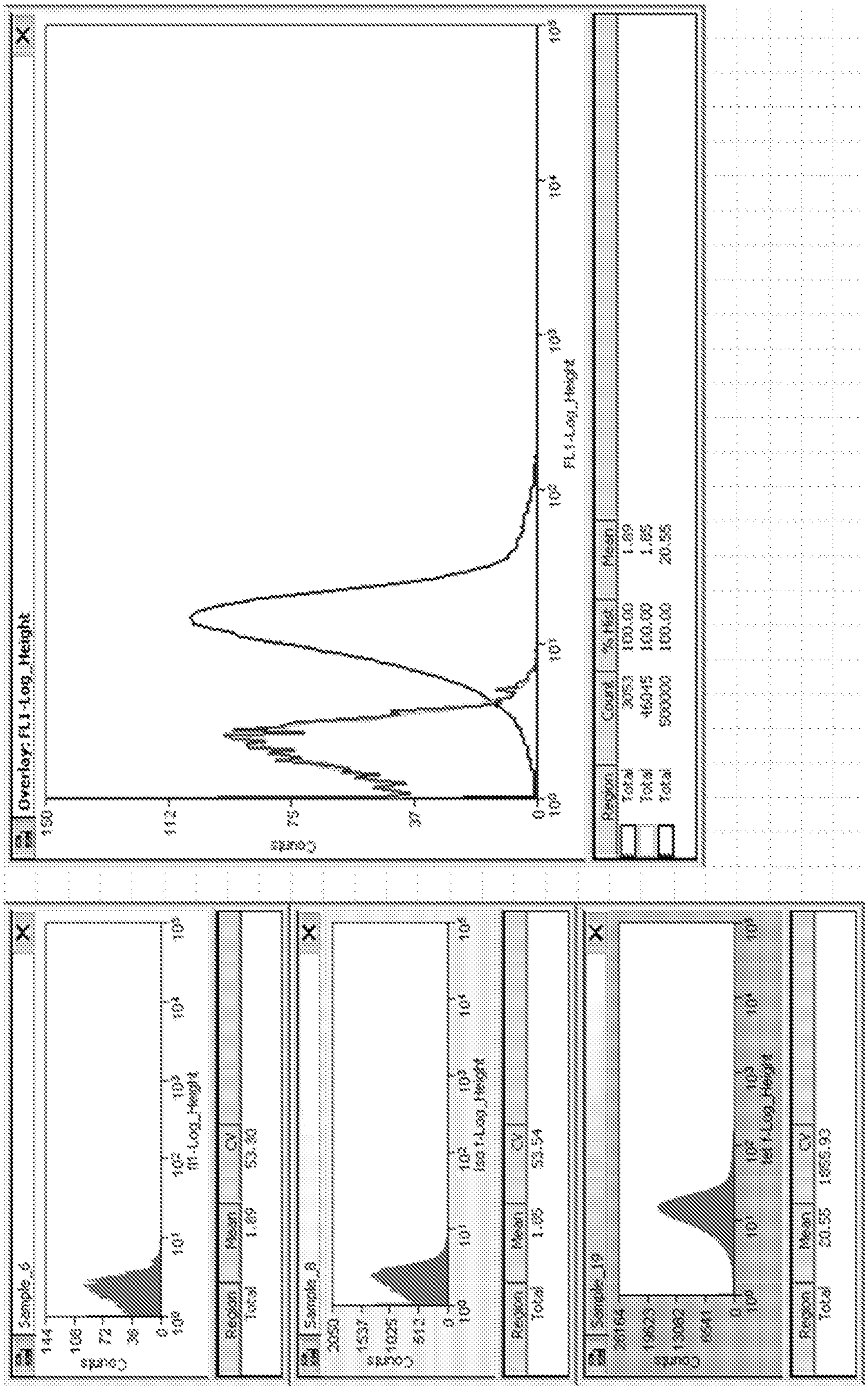


FIG. 7A

Markers	VCaP exosome phenotype	VCaP cell phenotype
CD9	92%	100%
B7H3	47%	100%
PCSA	17%	5%
PSMA	78%	75%

FIG. 7B

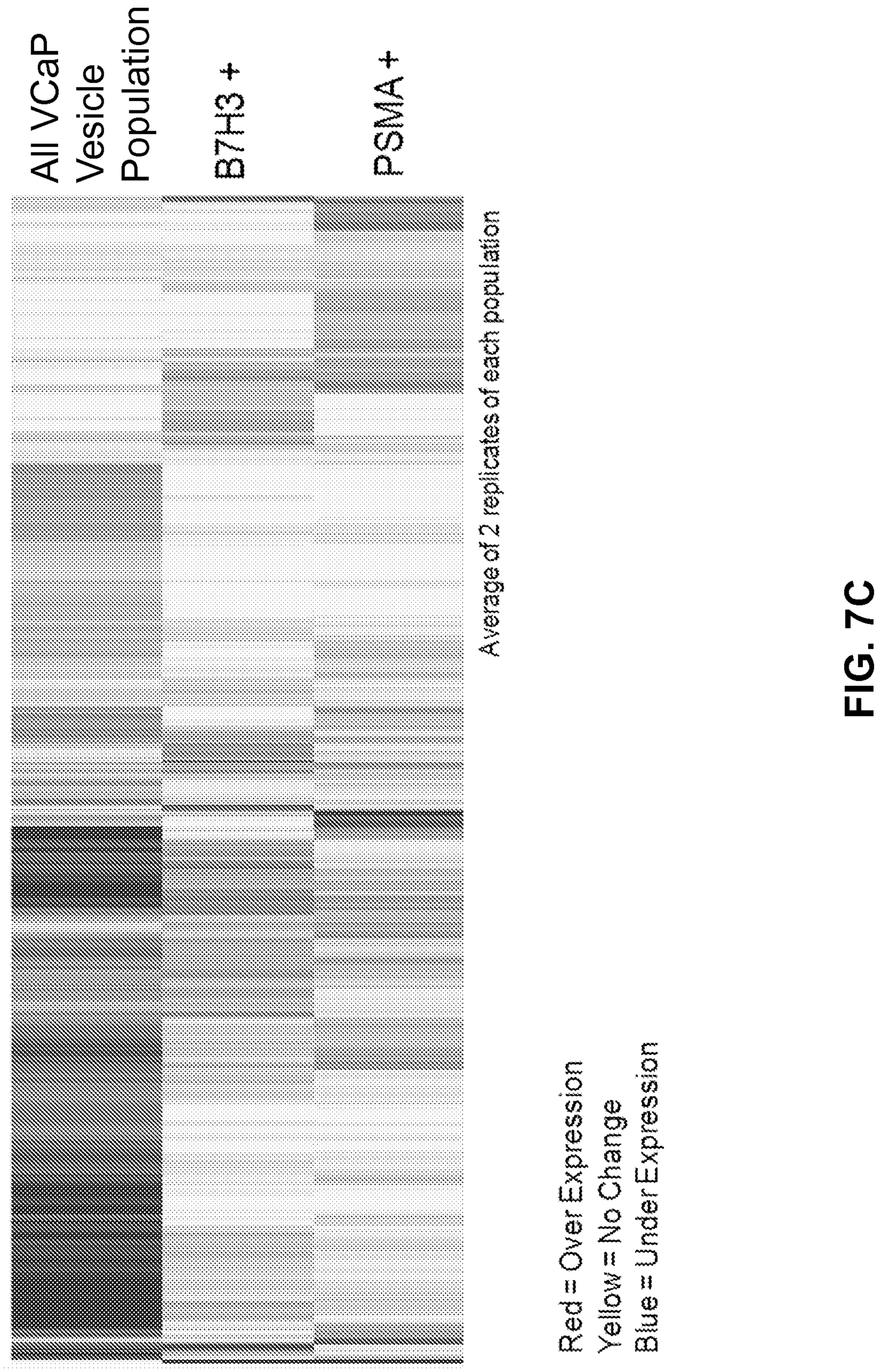

All VCaP Vesicle Population
B7H3 +
PSMA +
Average of 2 replicates of each population
Red = Over Expression
Yellow = No Change
Blue = Under Expression

FIG. 7C

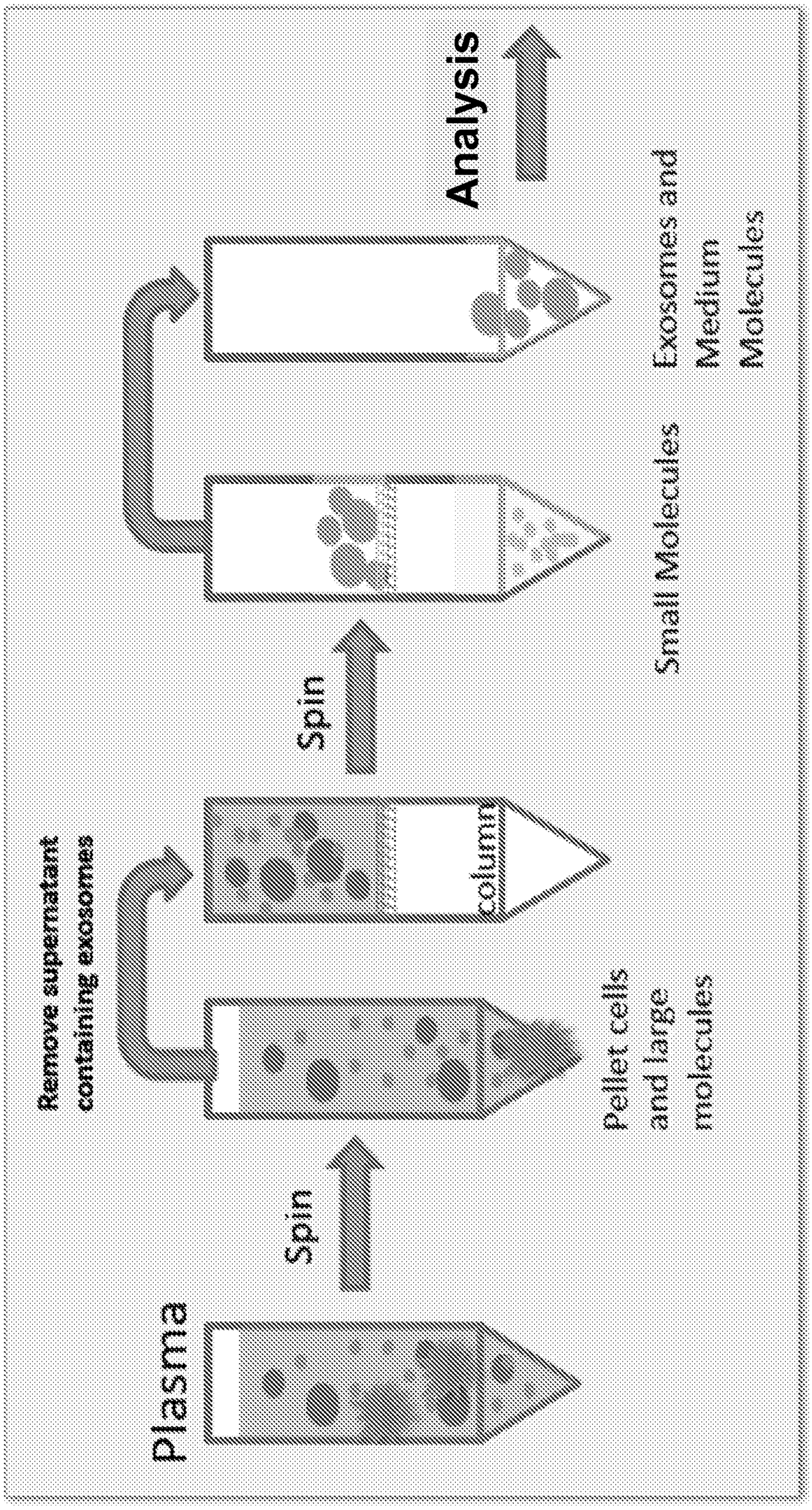

Analysis
Exosomes and
Medium
Molecules
Small Molecules
Spin
Remove supernatant
containing exosomes
Pellet cells
and large
molecules
Plasma

FIG. 8A

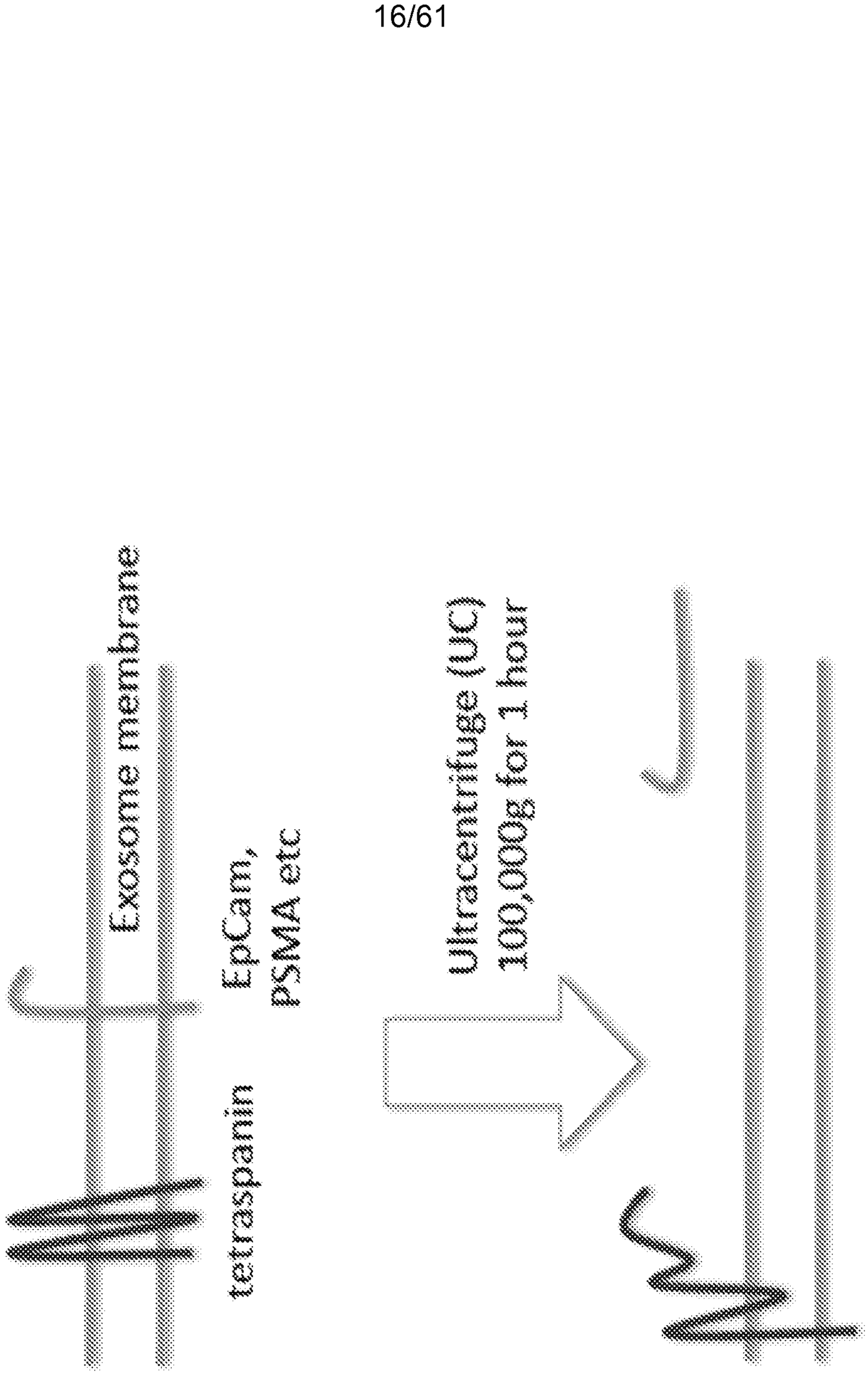

Exosome membrane
EpCam,
PSMA etc
tetraspamin
Ultracentrifuge (UC)
100,000g for 1 hour

FIG. 8B

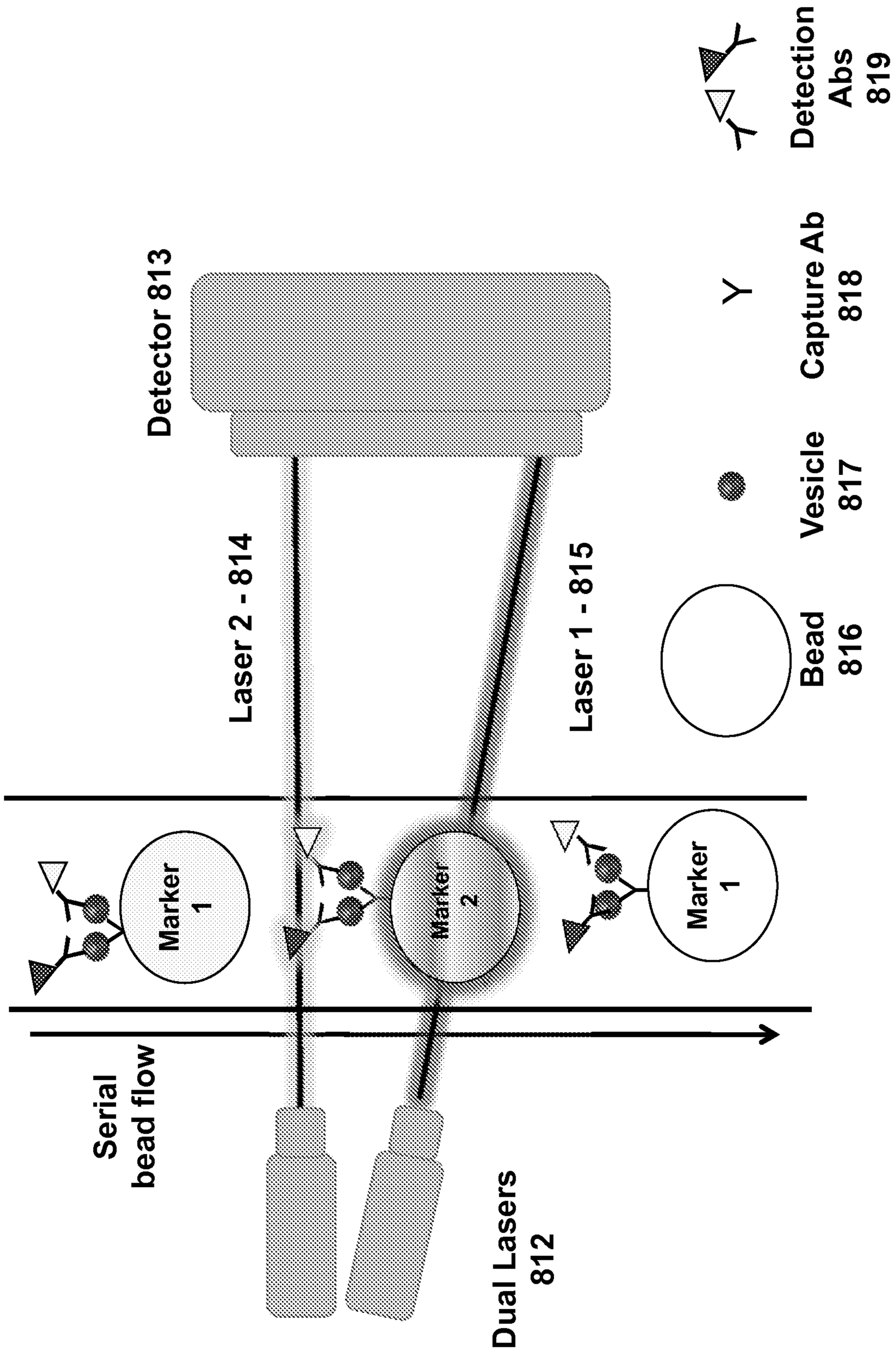

Detection Abs 819
Y
Capture Ab 818
Vesicle 817
Bead 816
Detector 813
Laser 2 - 814
Laser 1 - 815
Capillary 811
Marker 1
Marker 2
Marker 1
Serial bead flow
Dual Lasers 812

FIG. 8C

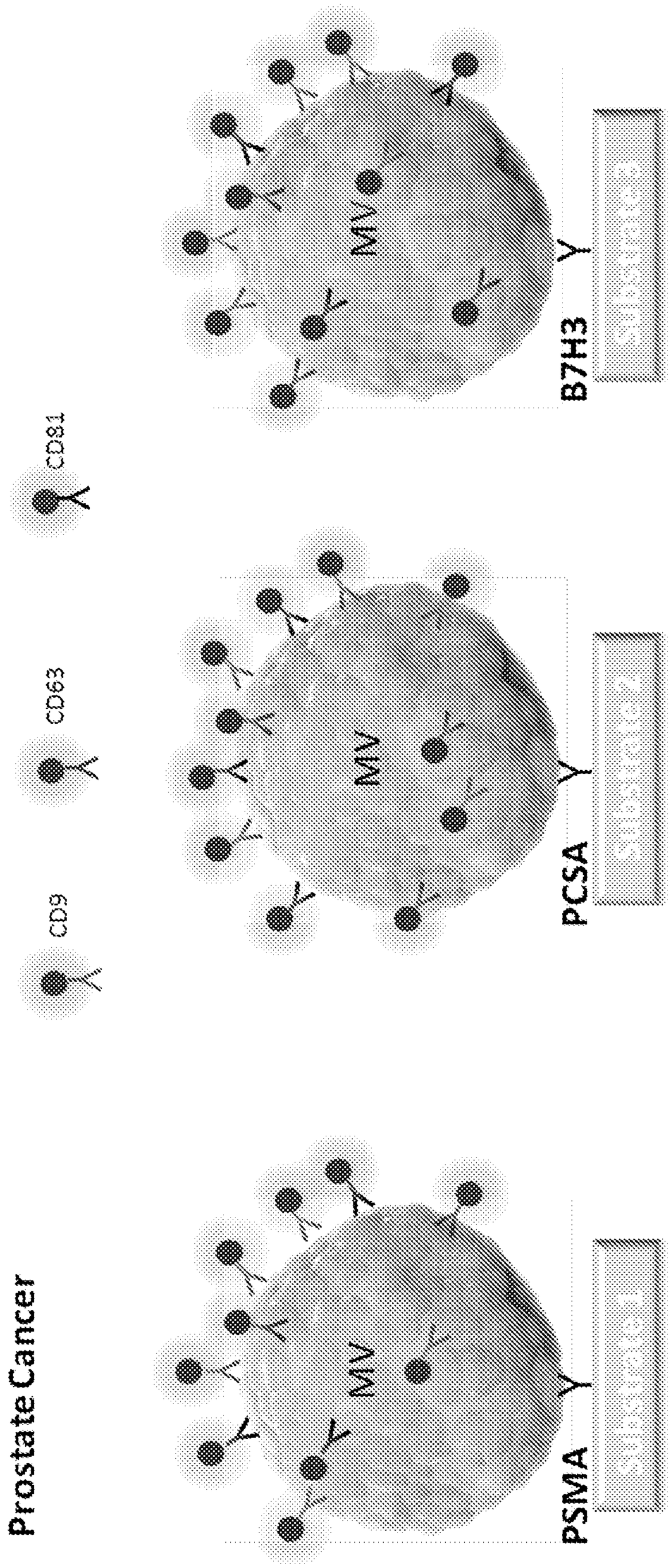

Prostate Cancer
CD9
CD63
CD81
MV
PSMA
Tissue-specific
Prostate Cancer
MV
PCSA
Tissue-specific
Prostate Cancer
MV
PSMA
Tissue-specific
MV
B7H3
Cancer-specific

FIG. 8D

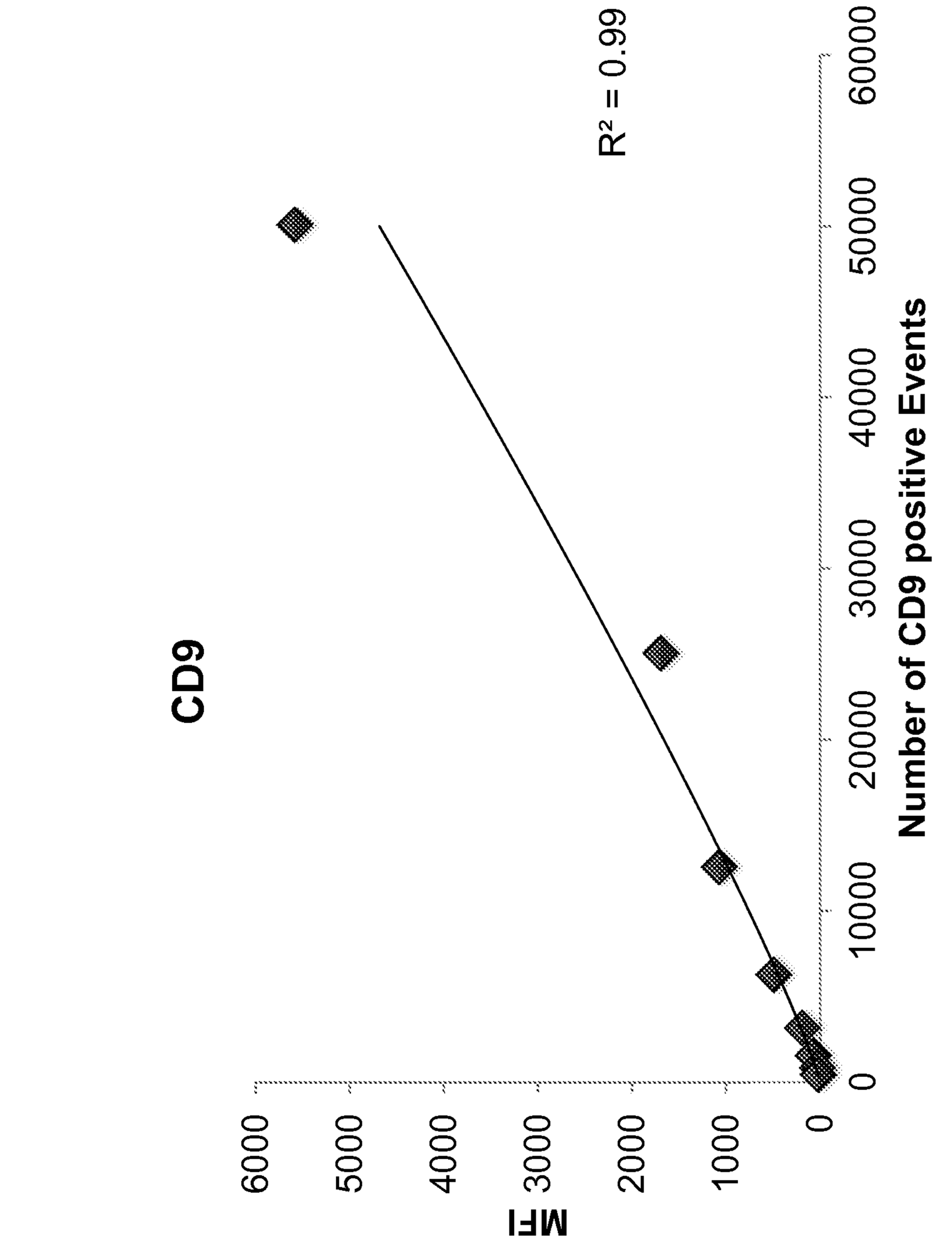

CD9
R² = 0.99
Number of CD9 positive Events
0
10000
20000
30000
40000
50000
60000
0
1000
2000
3000
4000
5000
6000

FIG. 8E

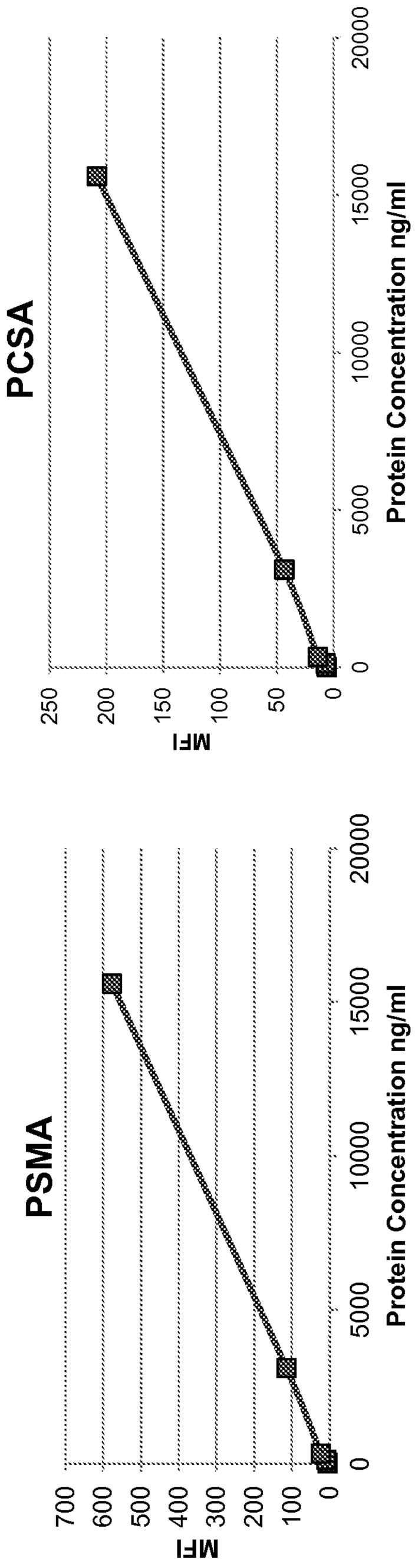

PCSA
MFI
250
200
150
100
50
0
0
5000
10000
15000
20000
Protein Concentration ng/ml
CD276 (B7H3)
MFI
700
600
500
400
300
200
100
0
0
5000
10000
15000
20000
Protein Concentration ng/ml
PSMA
MFI
700
600
500
400
300
200
100
0
0
5000
10000
15000
20000
Protein Concentration ng/ml

FIG. 8F

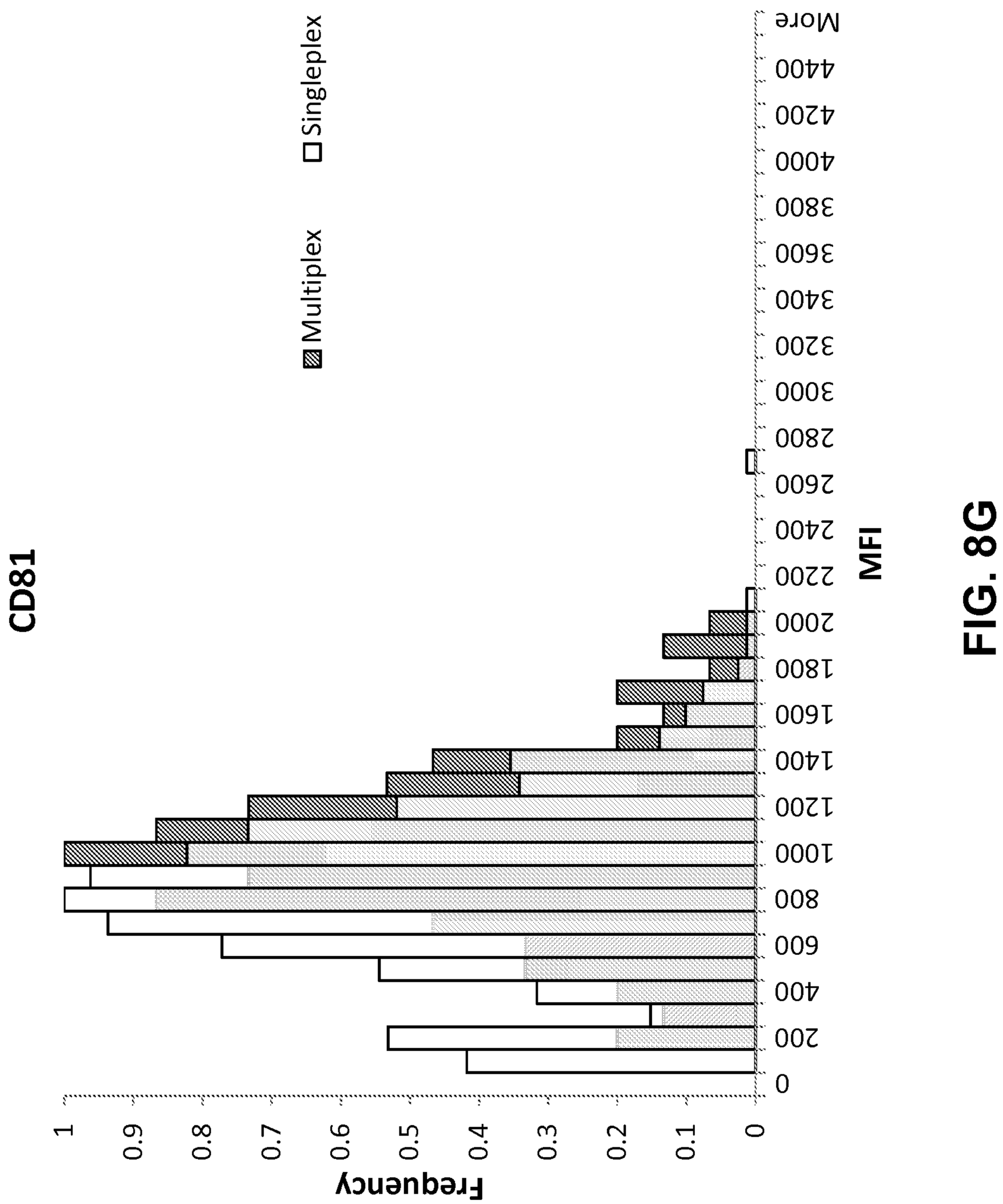

CD81
MFI
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
4000
4200
4400
More
Frequency
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Multiplex
Singleplex

FIG. 8G

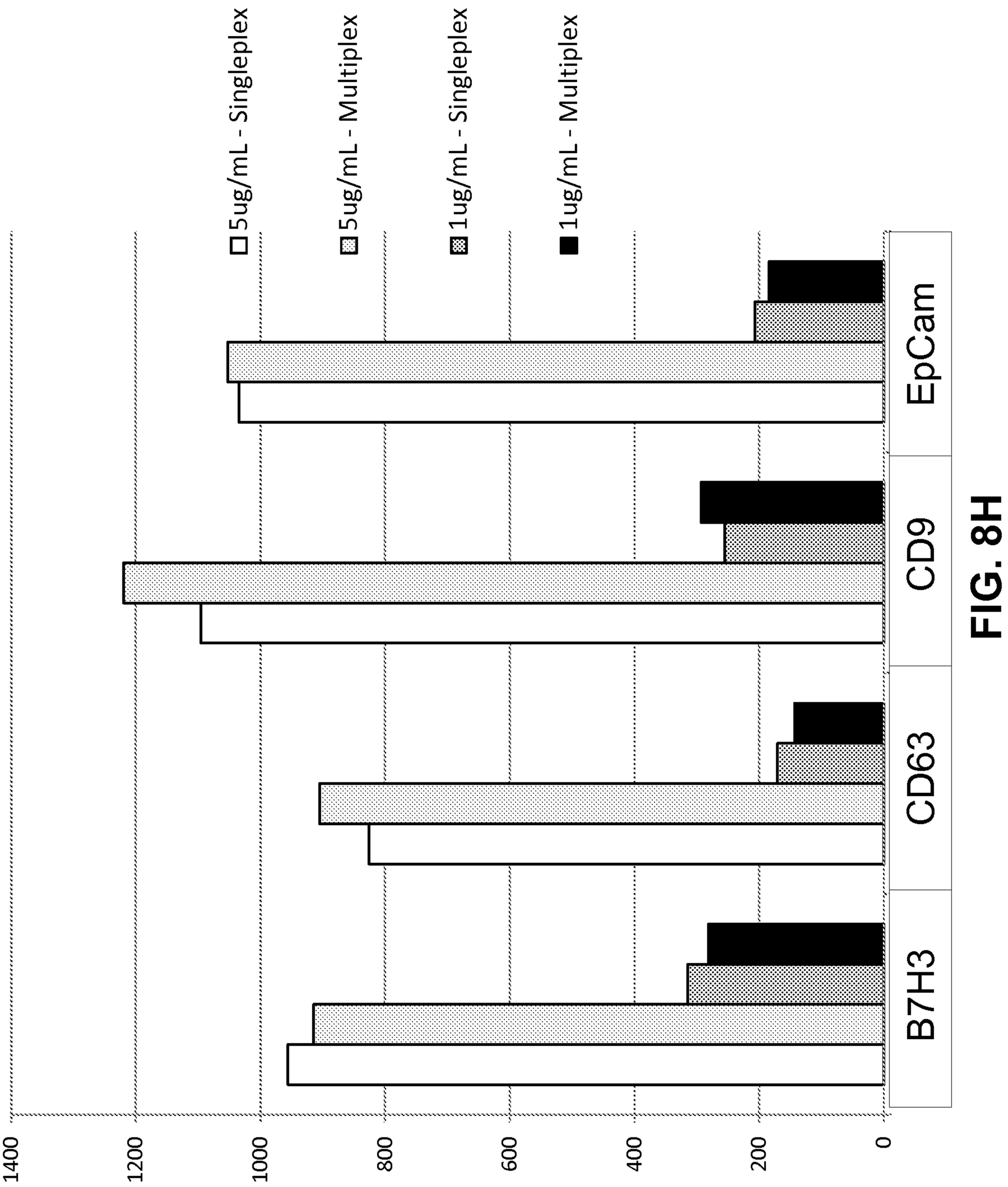

5ug/mL - Singleplex
5ug/mL - Multiplex
1ug/mL - Singleplex
1ug/mL - Multiplex
EpCam
CD9
CD63
B7H3
MFI
1400
1200
1000
800
600
400
200
0

FIG. 8H

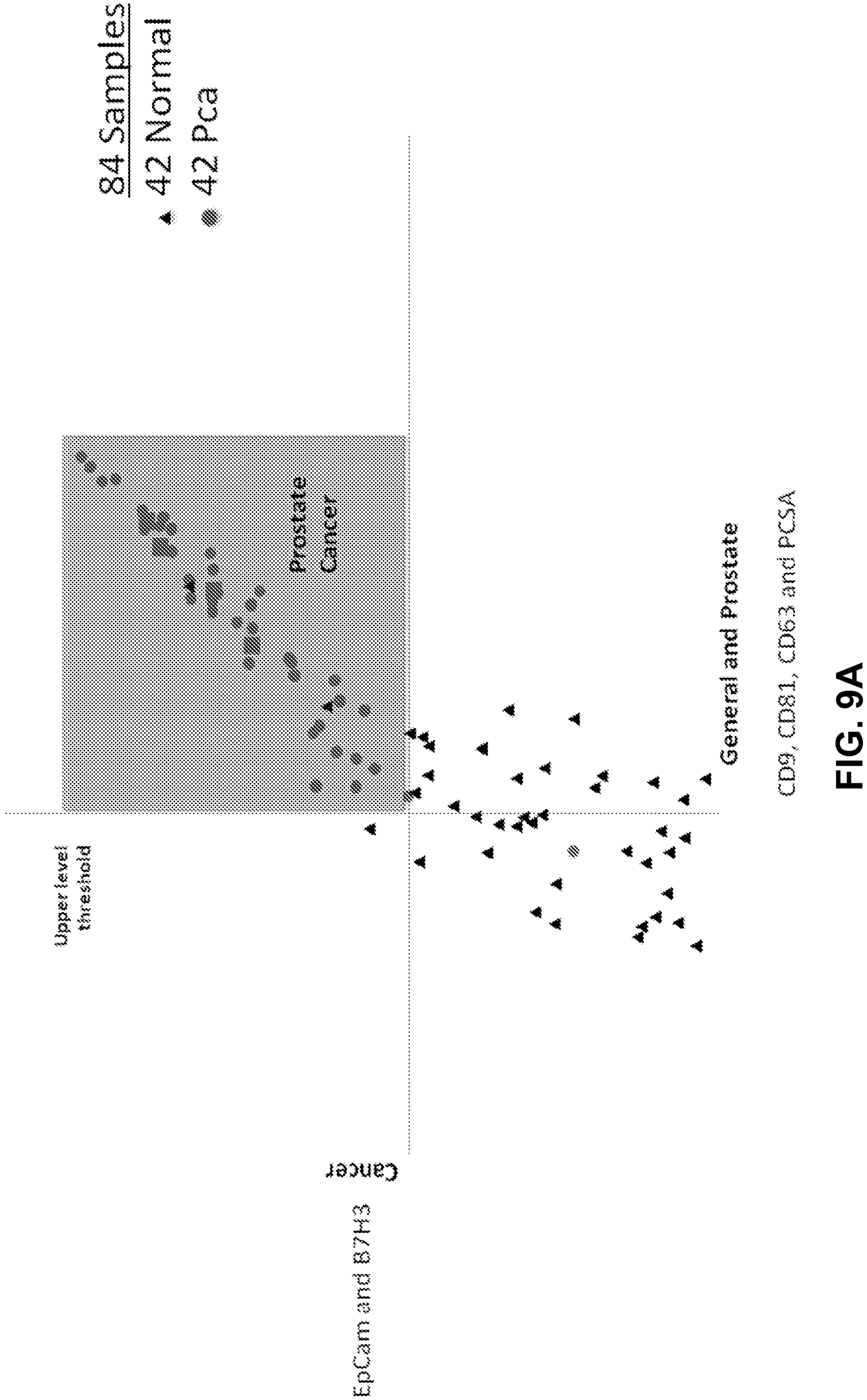

84 Samples
42 Normal
42 Pca
Prostate Cancer
Upper level threshold
General and Prostate
CD9, CD81, CD63 and PCSA
EpCam and B7H3
Cancer

FIG. 9A

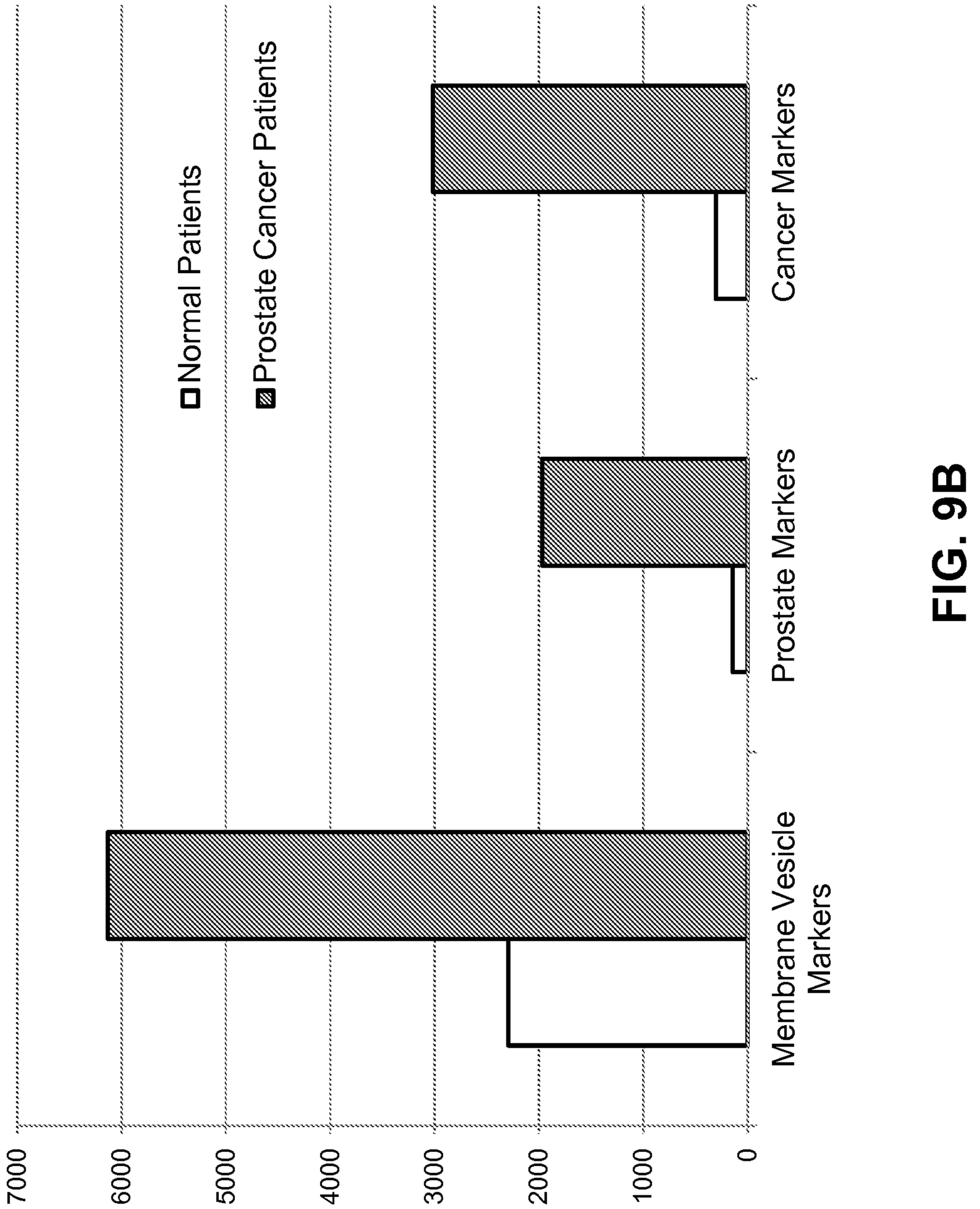

Normal Patients
Prostate Cancer Patients
Cancer Markers
Prostate Markers
Membrane Vesicle Markers
7000
6000
5000
4000
3000
2000
1000
0

FIG. 9B

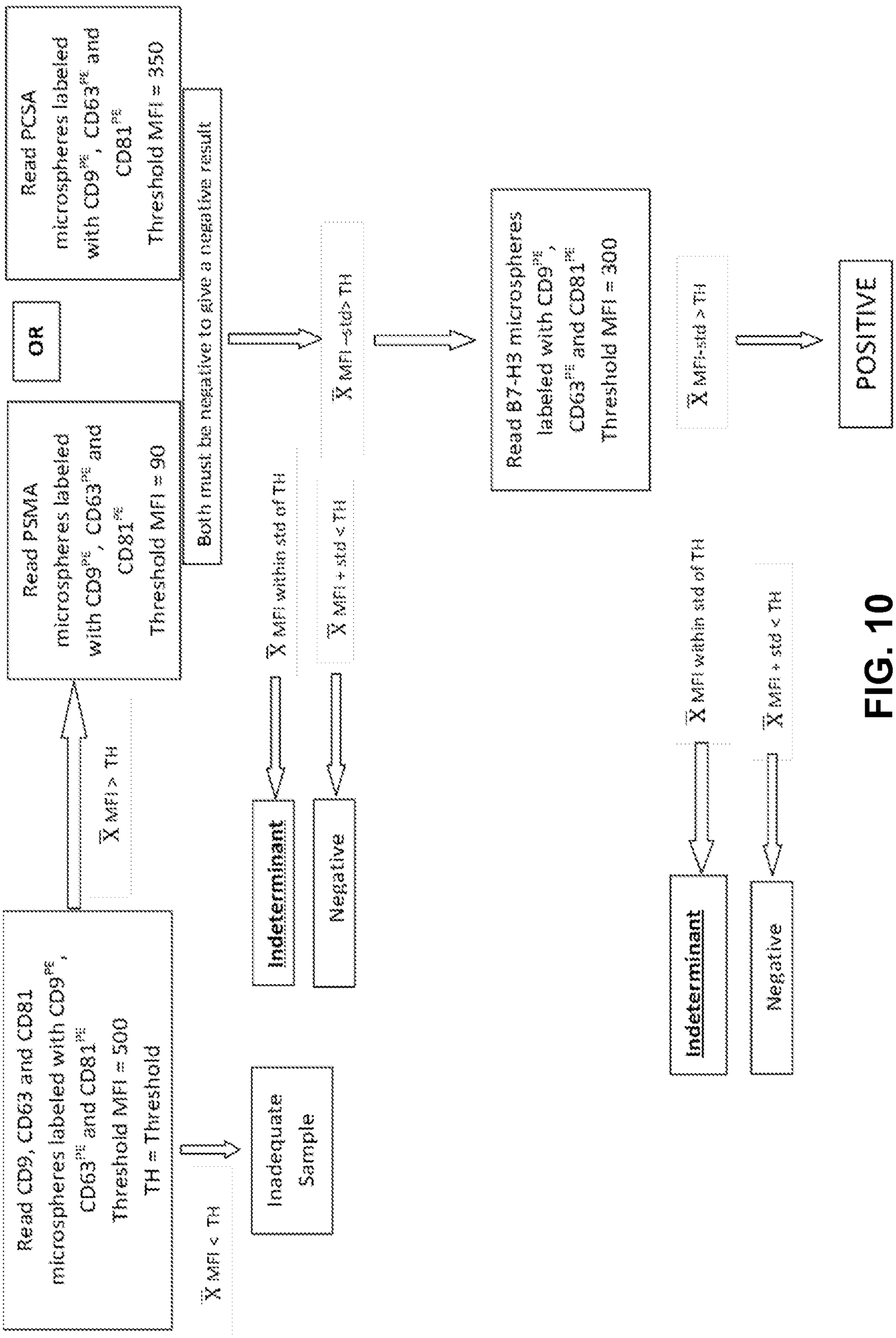

Read PSMA microspheres labeled with CD9PE, CD63PE and CD81PE
Threshold MFI = 90
X̄ MFI > TH
Read CD9, CD63 and CD81 microspheres labeled with CD9PE, CD63PE and CD81PE
Threshold MFI = 500
TH = Threshold
X̄ MFI < TH
Inadequate Sample
OR
Read PSMA microspheres labeled with CD9PE, CD63PE and CD81PE
Threshold MFI = 350
Both must be negative to give a negative result
X̄ MFI within std of TH
X̄ MFI < std < TH
X̄ MFI >std> TH
Read B7-H3 microspheres labeled with CD9PE, CD63PE and CD81PE
Threshold MFI = 300
X̄ MFI within std of TH
X̄ MFI < std < TH
X̄ MFI-std > TH
POSITIVE
Indeterminant
Negative
Indeterminant
Negative

FIG. 10

	Vesicle PCA Test	PSA
AUC	0.94	0.68

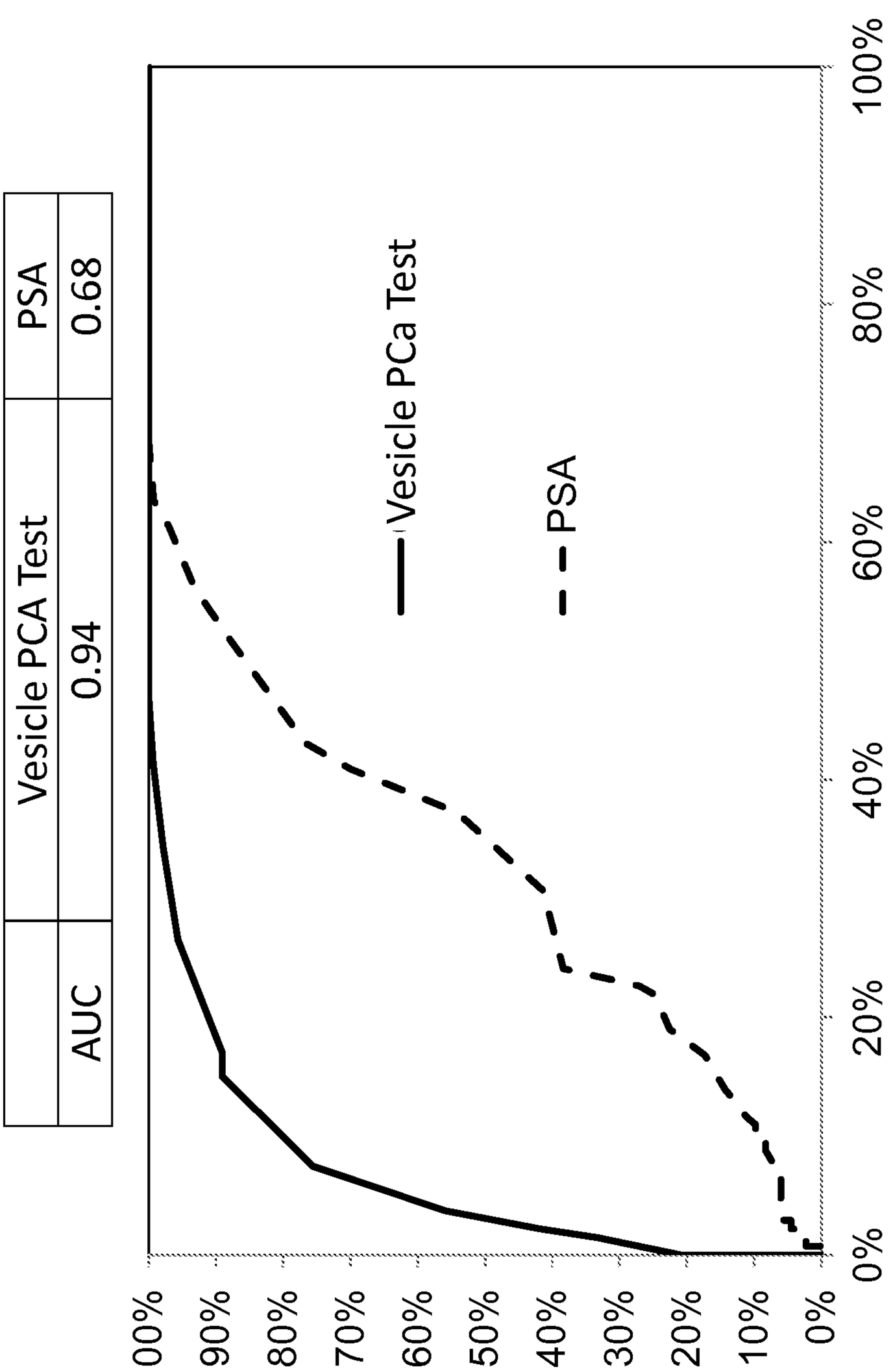
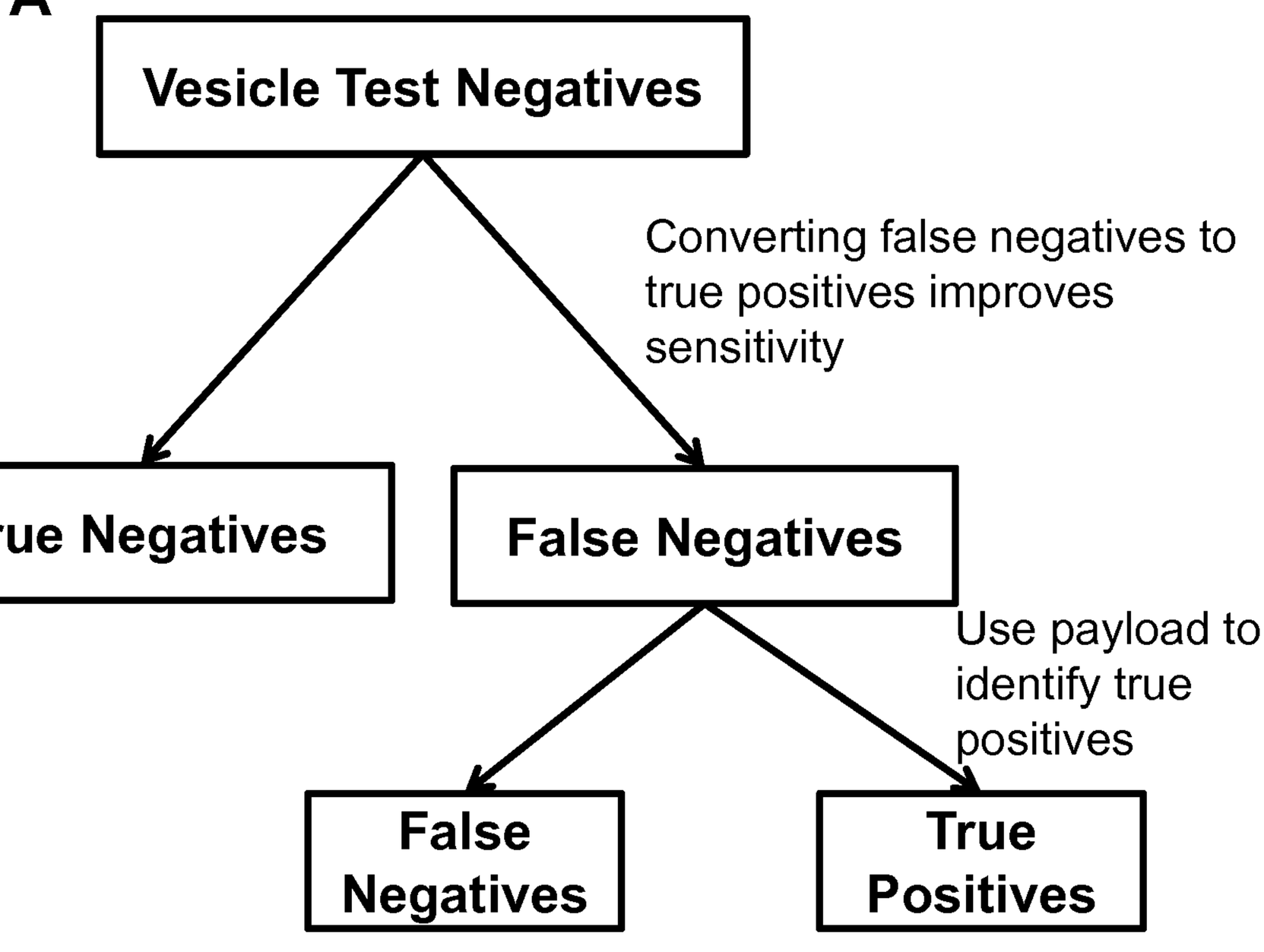
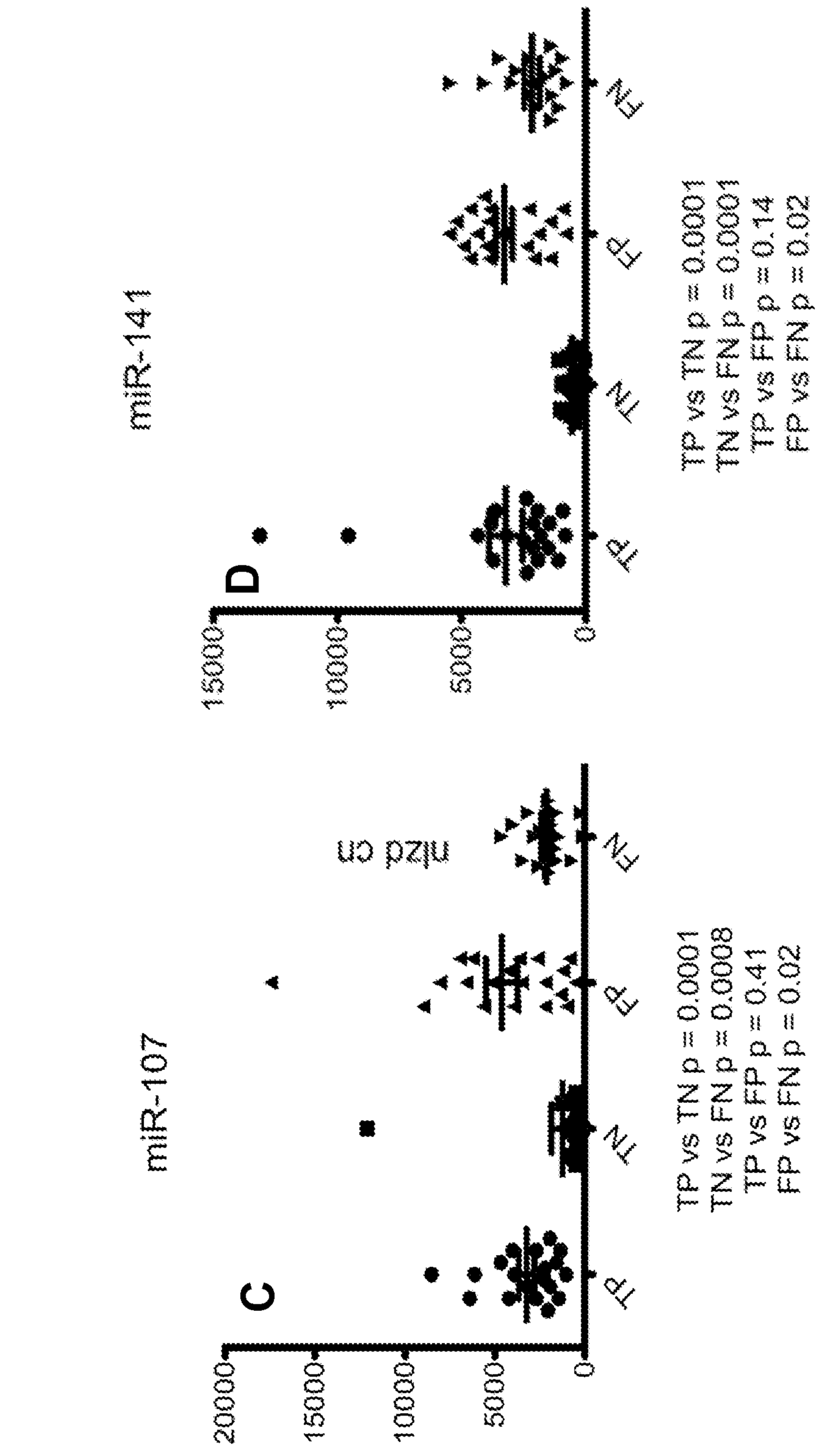


FIG. 11


A
Vesicle Test Negatives
Converting false negatives to true positives improves sensitivity
True Negatives
False Negatives
Use payload to identify true positives
False Negatives
True Positives
B
Vesicle Test Positives
Converting false positives to true negatives improves specificity
False Positives
True Positives
Use payload to identify true positives
True Negatives
• Mishandled samples
• Confounding disease
False Positives
• Undiagnosed disease

FIGs. 12A-B


D
miR-141
15000
10000
5000
0
TP
TN
FP
FN
TP vs TN p = 0.0001
TN vs FN p = 0.0001
TP vs FP p = 0.14
FP vs FN p = 0.02
C
miR-107
20000
15000
10000
5000
0
TP
TN
FP
FN
TP vs TN p = 0.0001
TN vs FN p = 0.0008
TP vs FP p = 0.41
FP vs FN p = 0.02
nlzd cn

FIGs. 12C-D

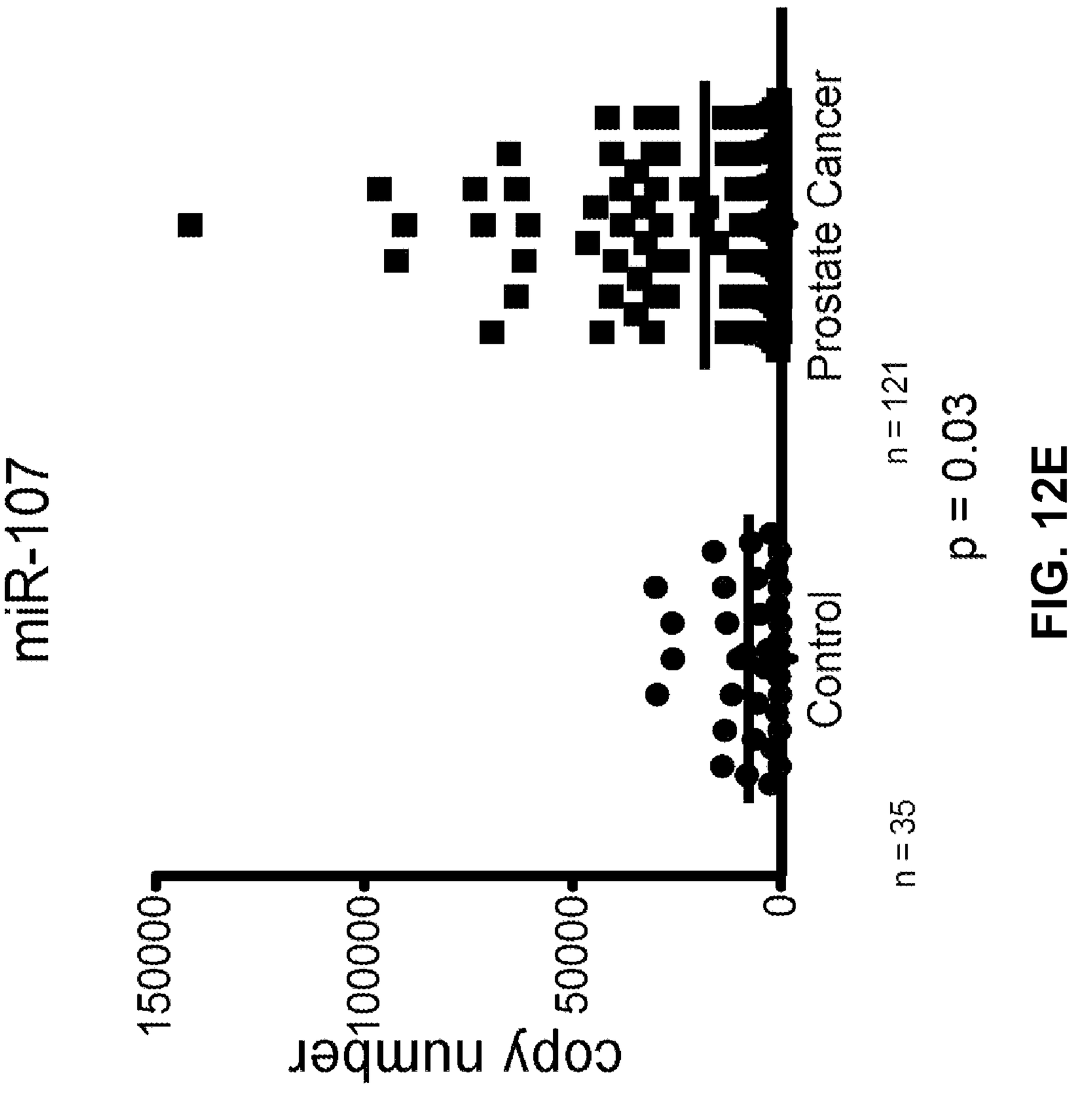
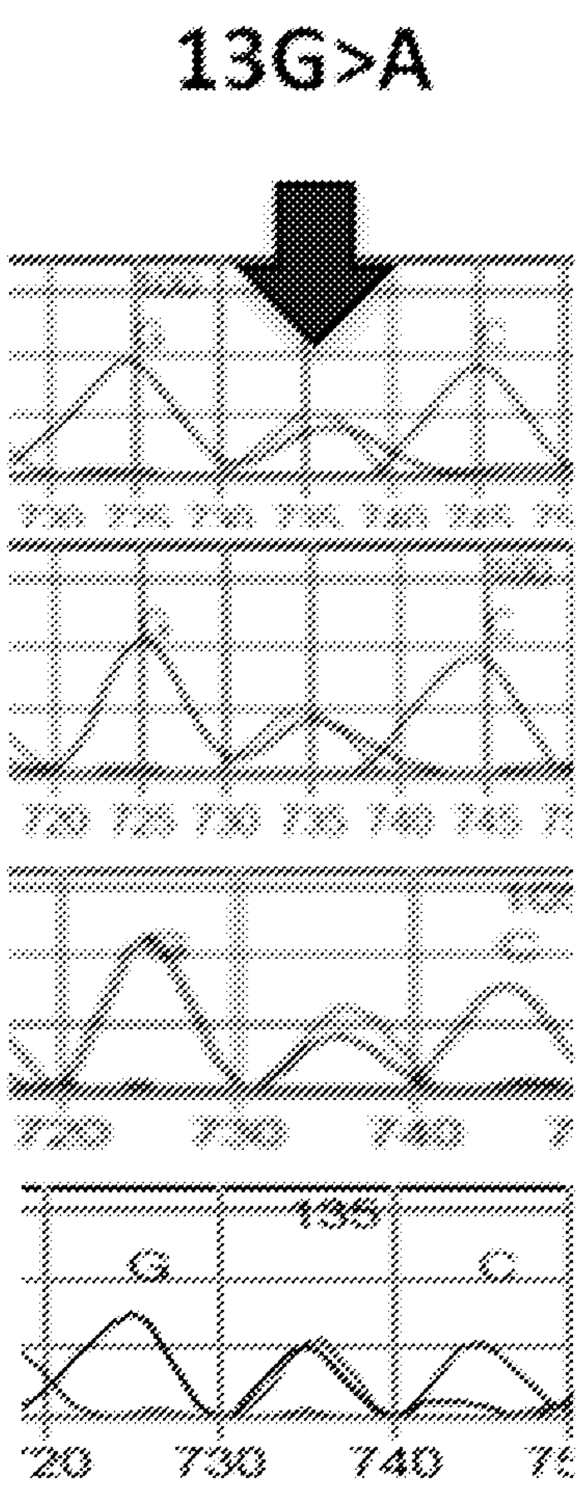

miR-107
copy number
150000
100000
50000
0
Control
n = 35
Prostate Cancer
n = 121
p = 0.03

FIG. 12E



A) CRC cell line cDNA from vesicles

B) CRC cell line cDNA from cells

C) Plasma-derived vesicle cDNA from CRC patient

D) FFPE DNA from CRC patient

FIG. 13A-D

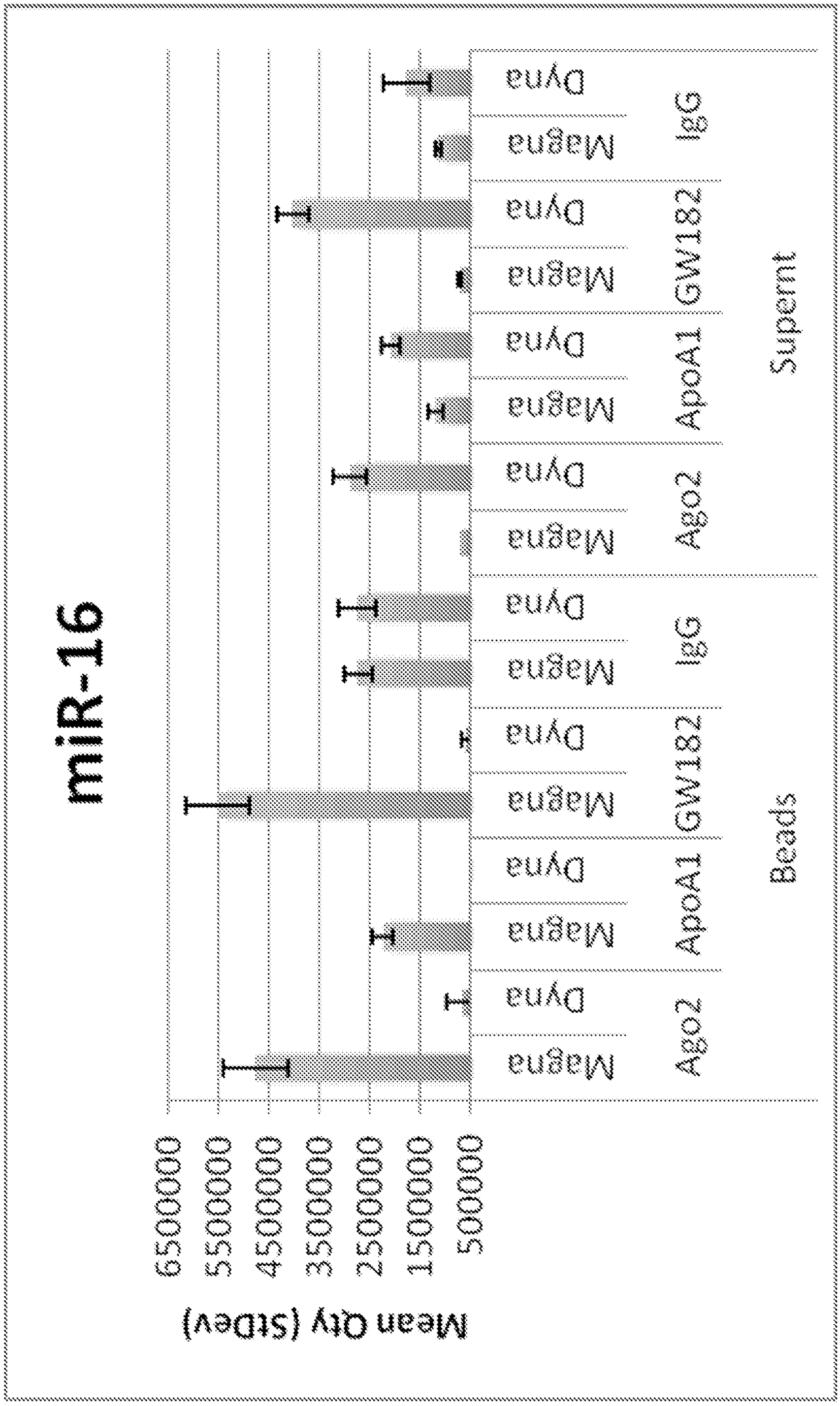

miR-16
Mean Qty (StDev)
6500000
5500000
4500000
3500000
2500000
1500000
500000
Beads
Supernt
Ago2
ApoA1
GW182
IgG
Dyna
Magna

FIG. 14A

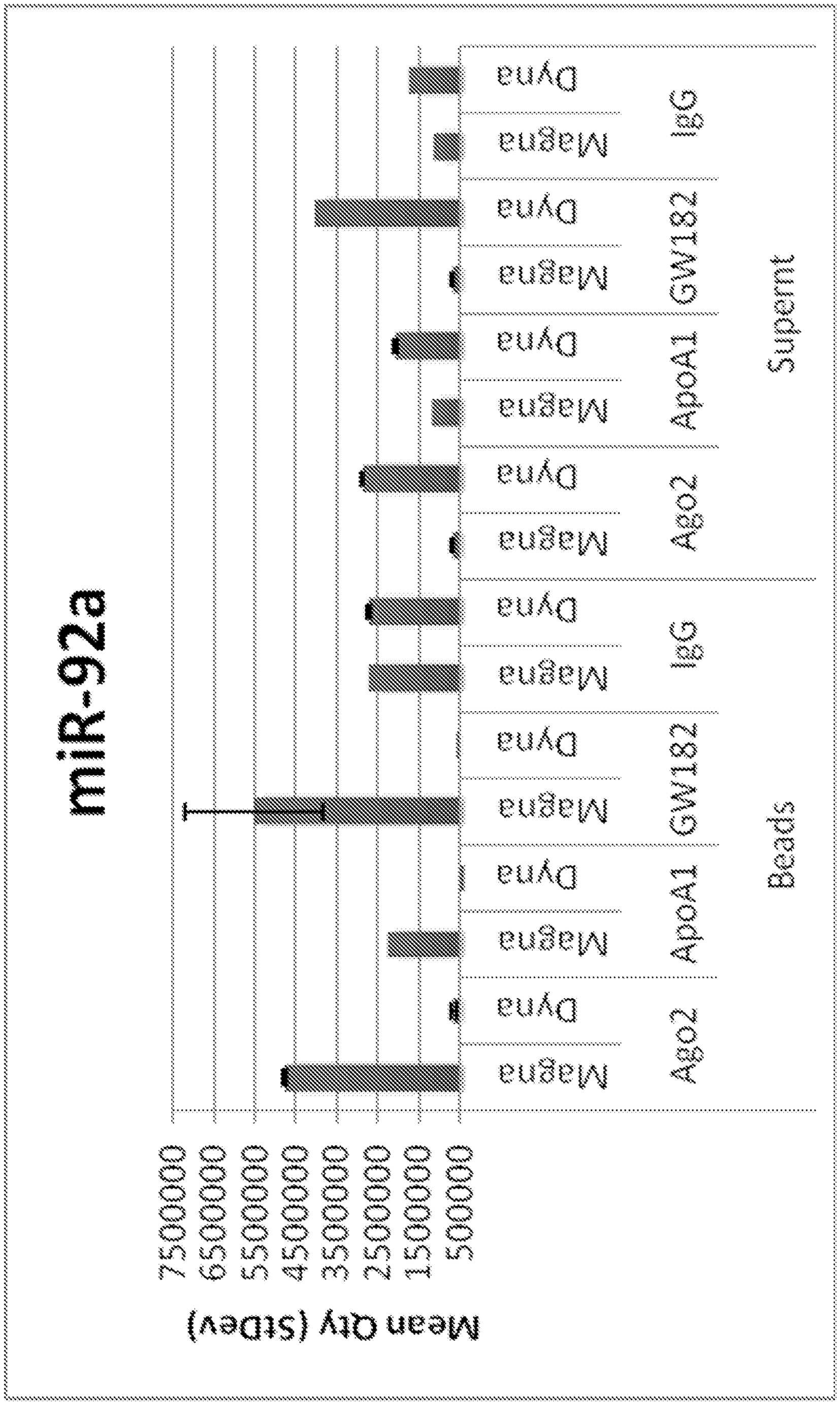

miR-92a
Mean Qty (stDev)
7500000
6500000
5500000
4500000
3500000
2500000
1500000
500000
Beads
Supernt
Dyna
Magna
Ago2
ApoA1
IgG
GW182

FIG. 14B

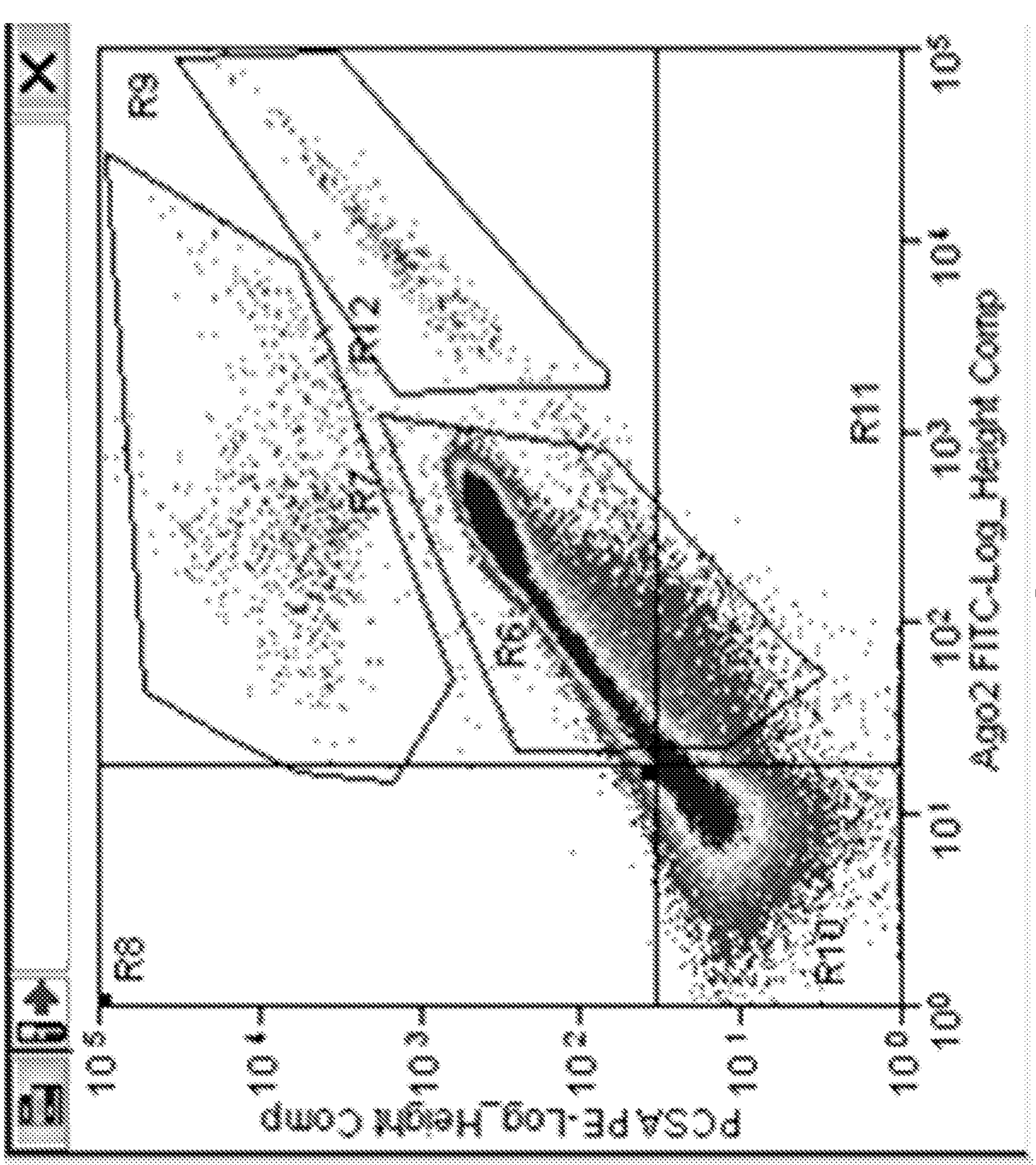

R9
R12
R7
R11
R6
R10
R8
10^5
10^4
10^3
10^2
10^1
10^0
PCS4 PE-Log Height Comp
Ag02 FITC-Log Height Comp

FIG. 15

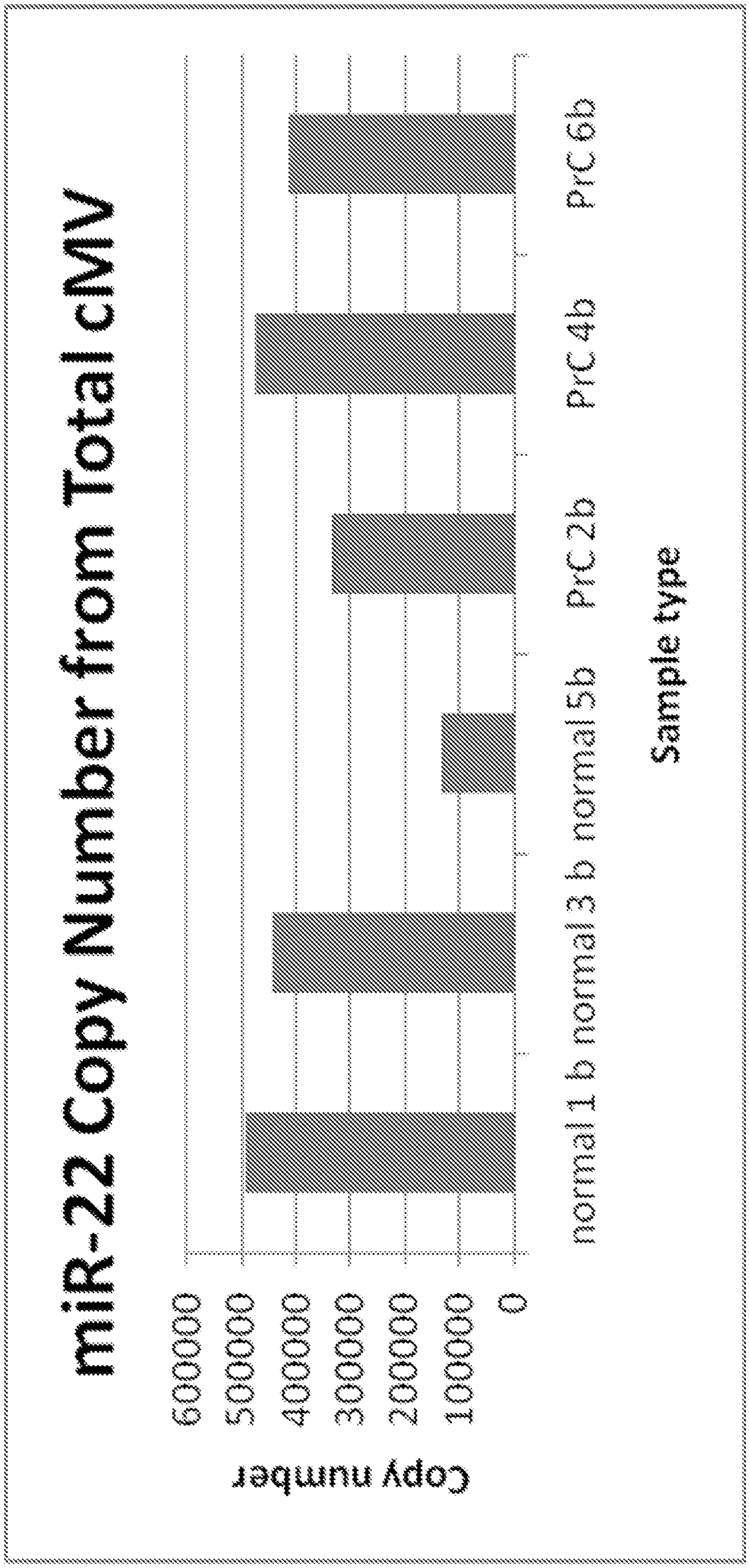

miR-22 Copy Number from Total cfRNA
Copy number
6000000
5000000
4000000
3000000
2000000
1000000
0
normal 1 b normal 3 b normal 5b PrC 2b PrC 4b PrC 6b
Sample type

FIG. 16A

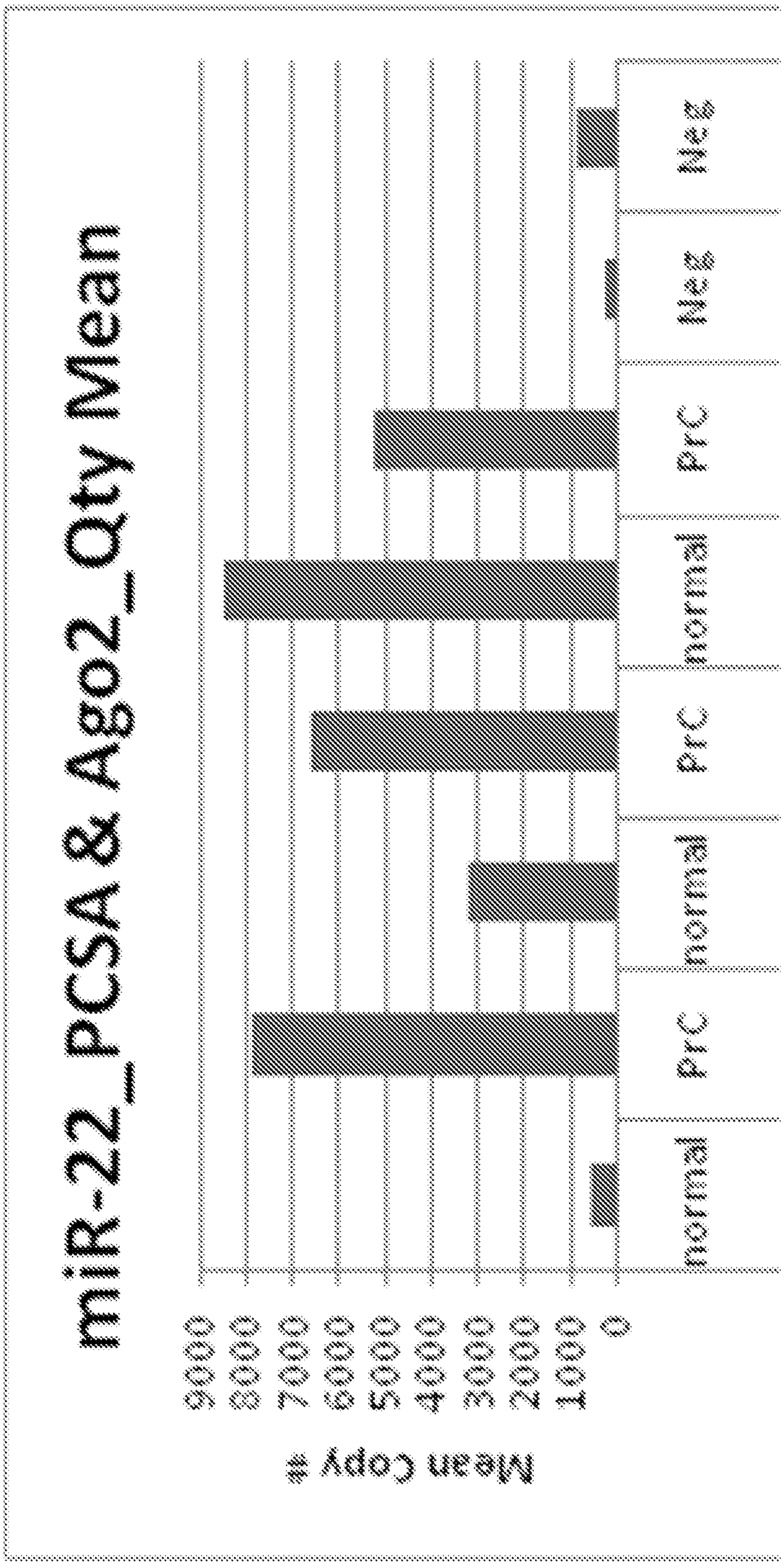

iR-22_PCSA & A80
02_Qty Mean
normal
PrC
Me2
normal
PrC
normal
PrC
normal
PrC
Mean copy #
0
1
2
3
4
5
6
7
8
9

FIG. 16B

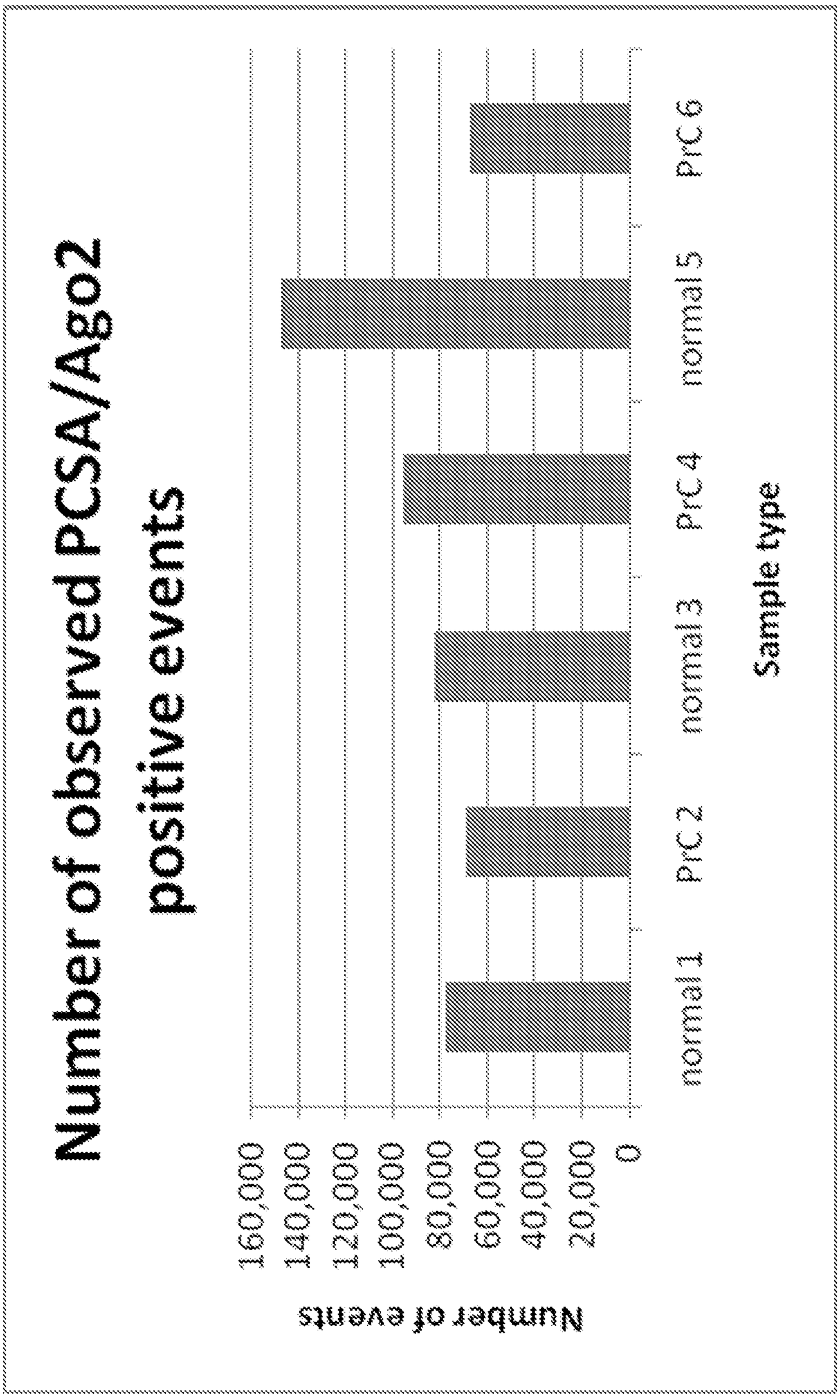

Number of observed PCSA/Ago2 positive events
Number of events
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
normal 1
PrC 2
normal 3
PrC 4
normal 5
Sample type

FIG. 16C

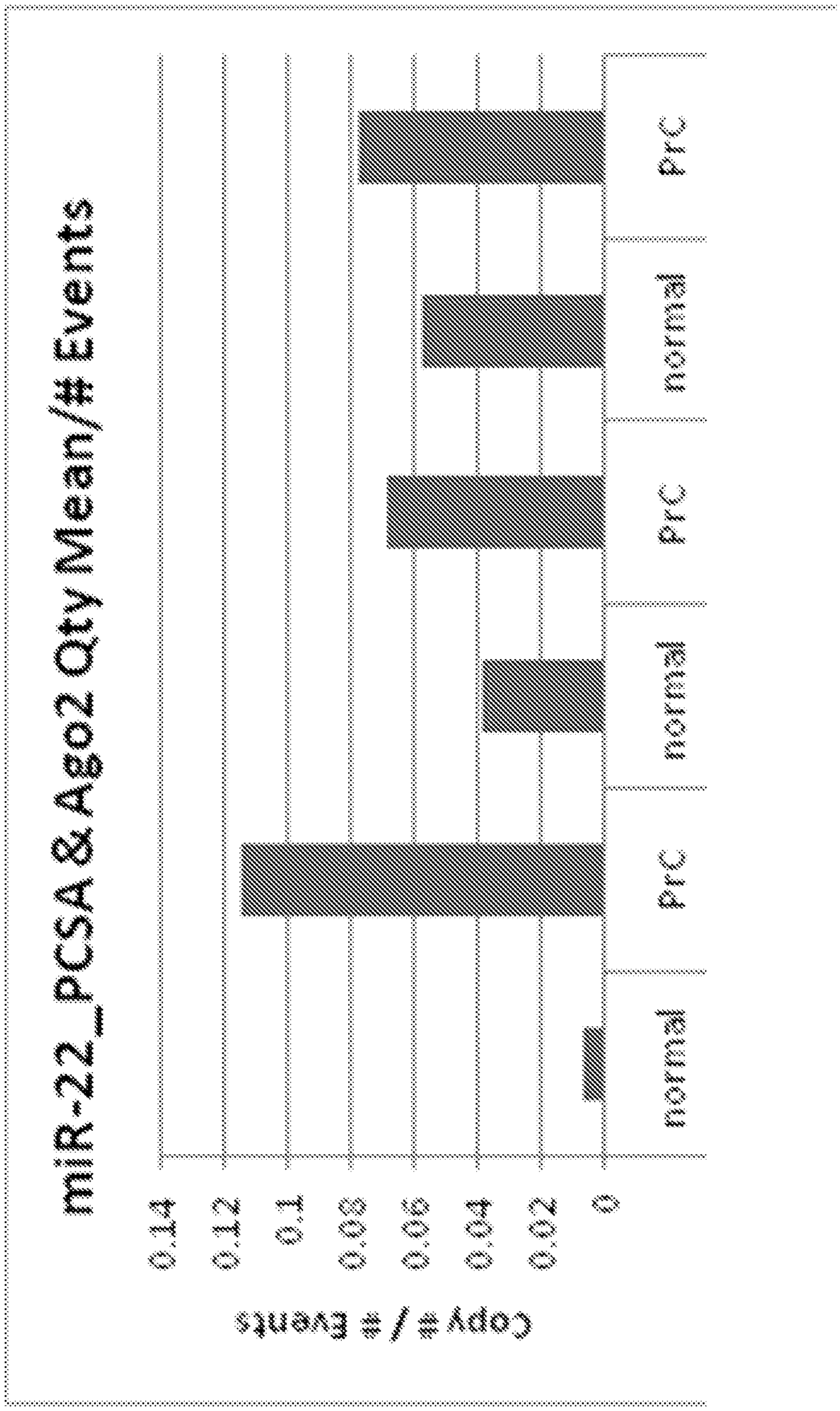
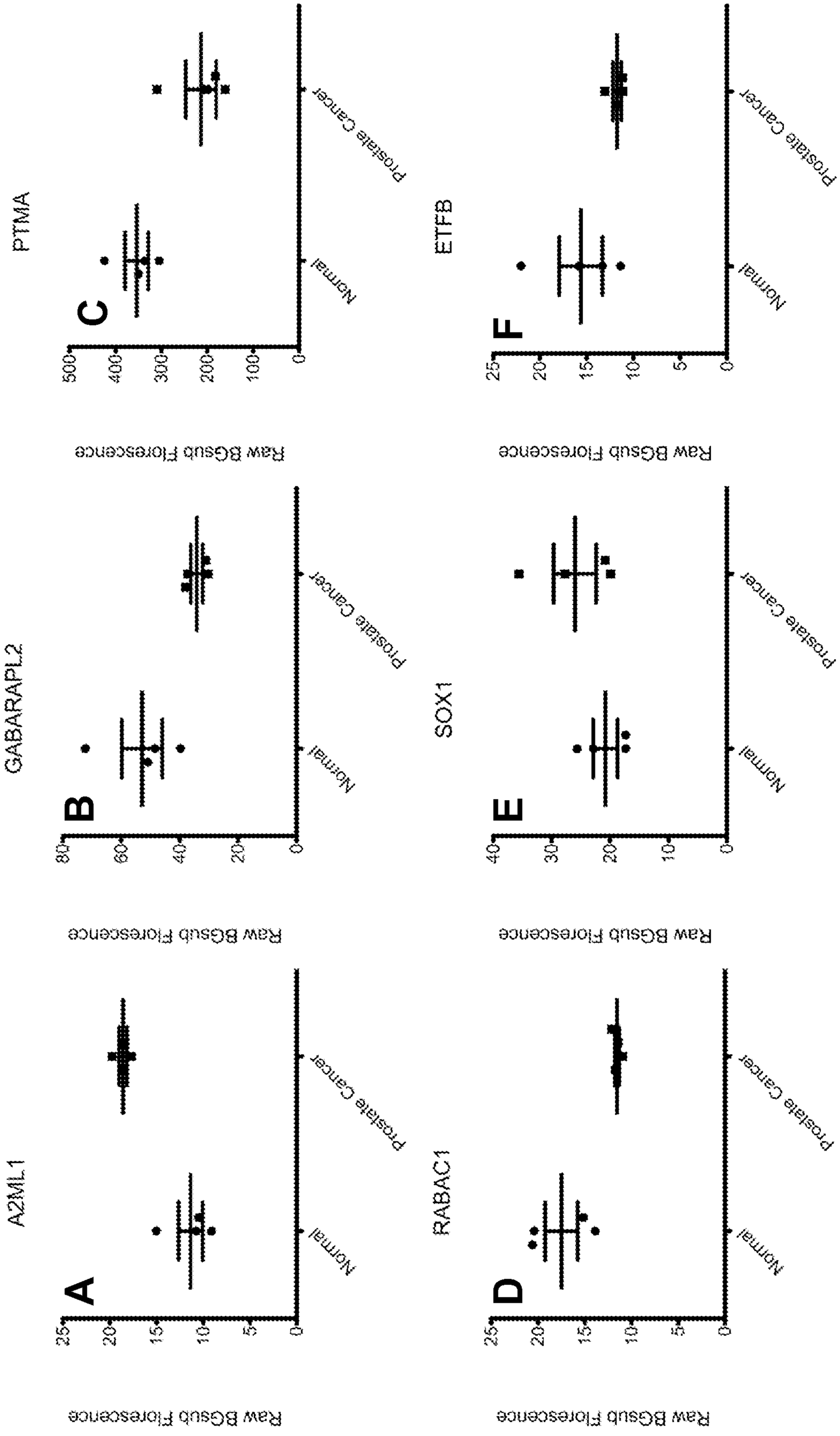

miR-22_PCSA & Age2 Qty Mean/# Events
copy # / # Events
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
normal
prC
normal
prC
normal
prC

FIG. 16D


A
A2ML1
Raw BGsub Fluorescence
Normal
Prostate Cancer
B
GABARAPL2
C
PTMA
D
RABAC1
E
SOX1
F
ETFB

FIGs. 17A-F

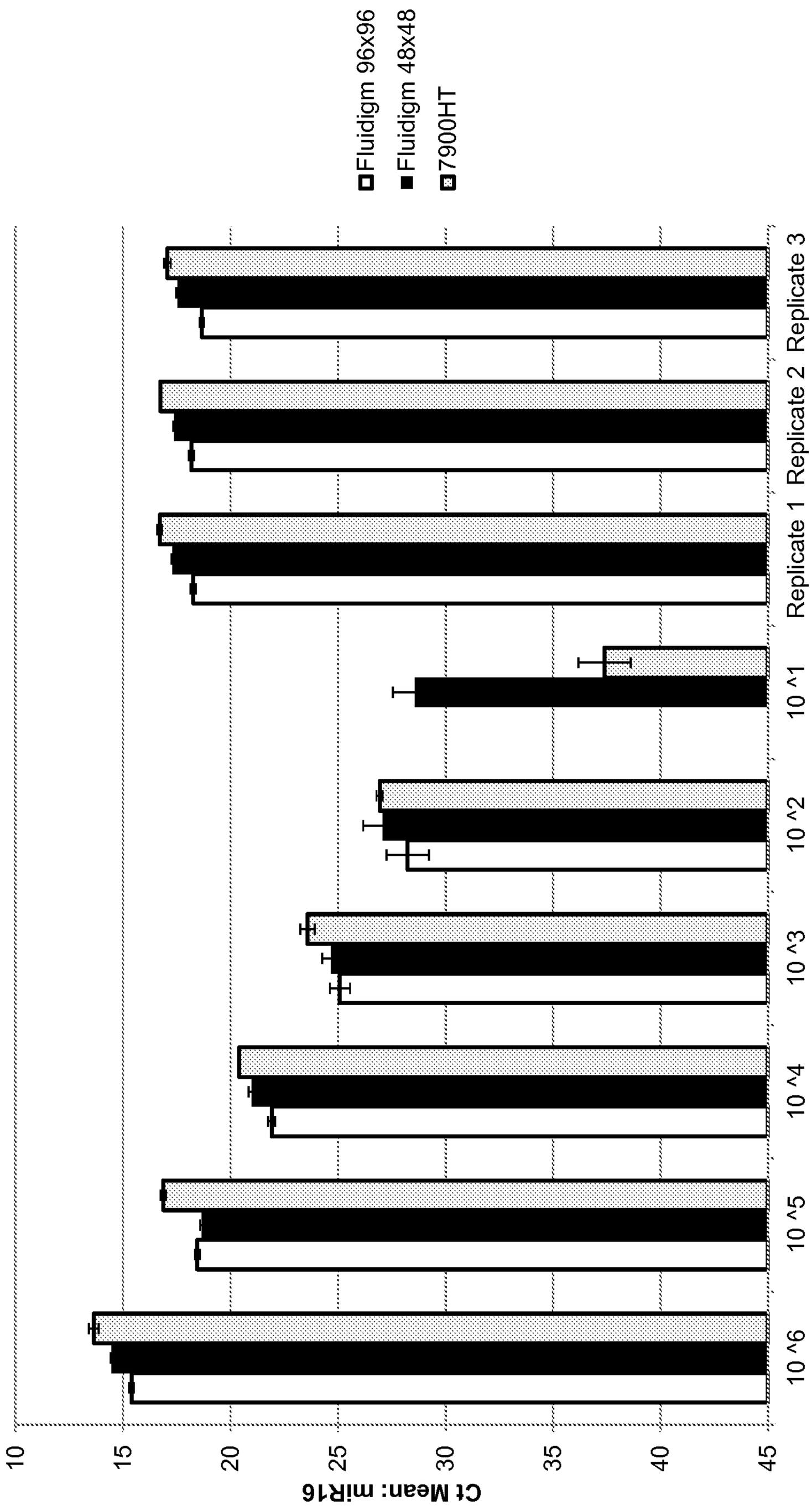

Fluidigm 96x96
Fluidigm 48x48
7900HT
10 ^1
Replicate 1
Replicate 2
Replicate 3
10 ^2
10 ^3
10 ^4
10 ^5
10 ^6
10
15
20
25
30
35
40
45
Ct Mean: miR16

FIG. 18

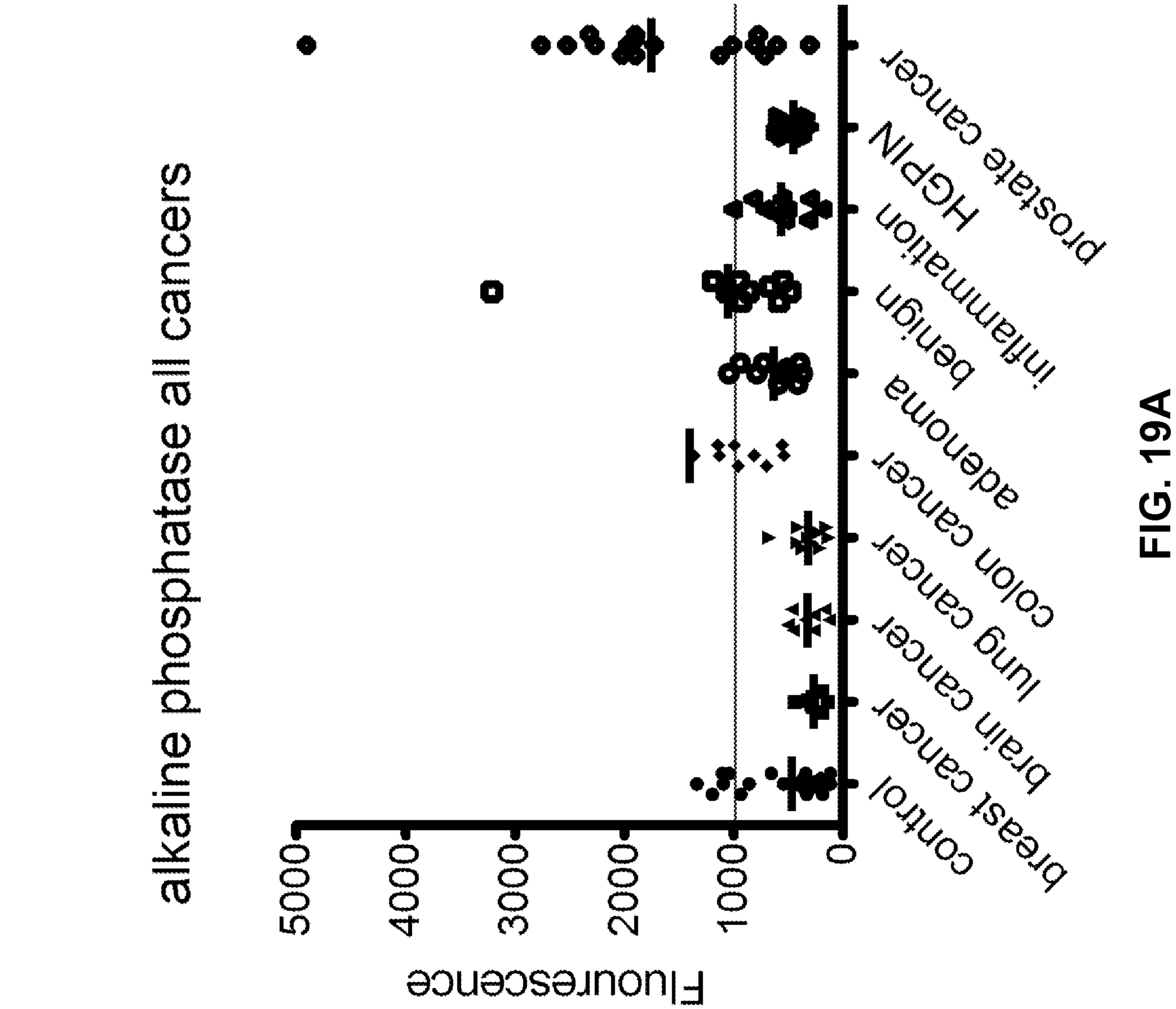

alkaline phosphatase activity in all cancers
Fluorescence
5000
4000
3000
2000
1000
0
control
breast cancer
brain cancer
lung cancer
colon cancer
adenoma
benign
inflammation
HGPIN
prostate cancer

FIG. 19A

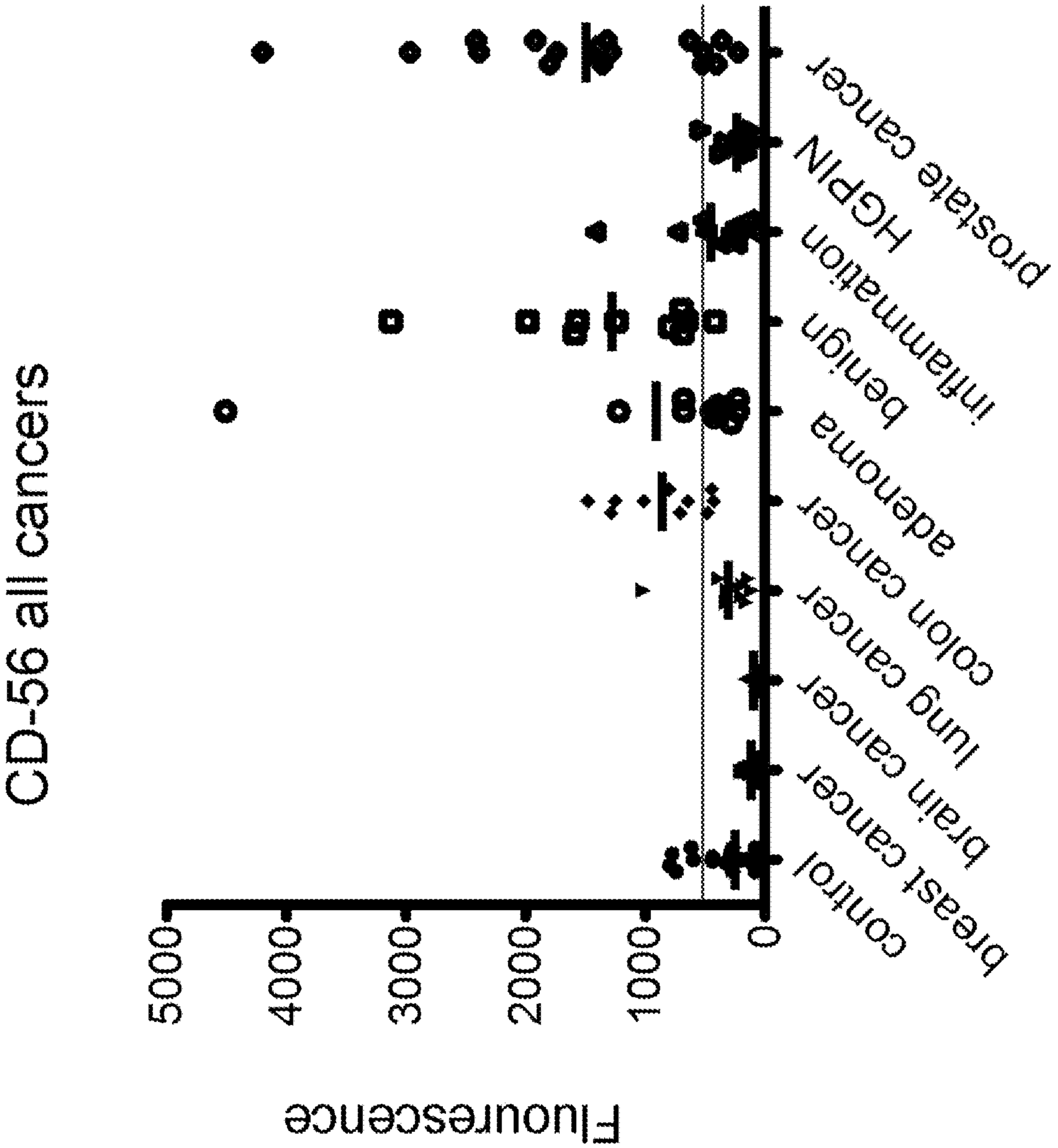
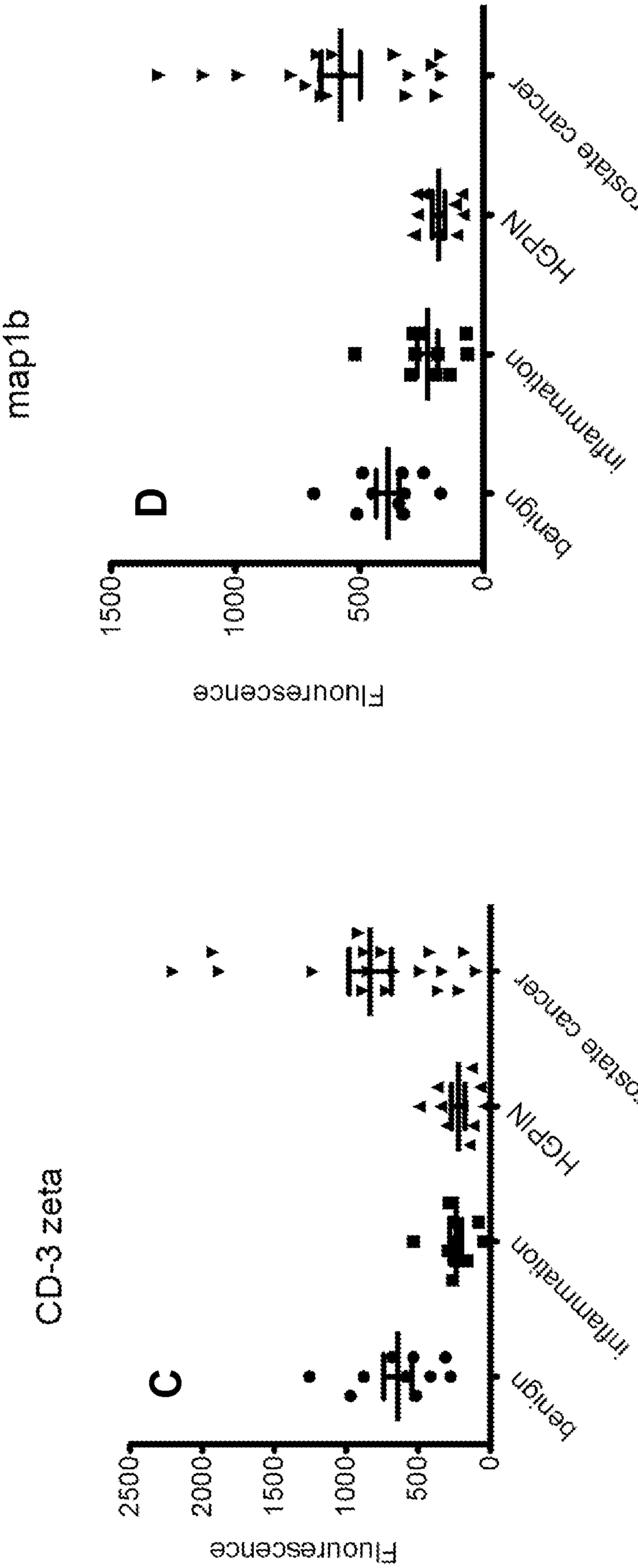

CD-56 all cancers
Fluorescence
5000
4000
3000
2000
1000
0
control
breast cancer
brain cancer
lung cancer
colon cancer
prostate cancer
HGPIN
inflammation
benign
adenoma

FIG. 19B


map1b
D
Fluorescence
1500
1000
500
0
benign
inflammation
HGPIN
prostate cancer
CD-3 zeta
C
Fluorescence
2500
2000
1500
1000
500
0
benign
inflammation
HGPIN
prostate cancer

FIGs. 1C-D

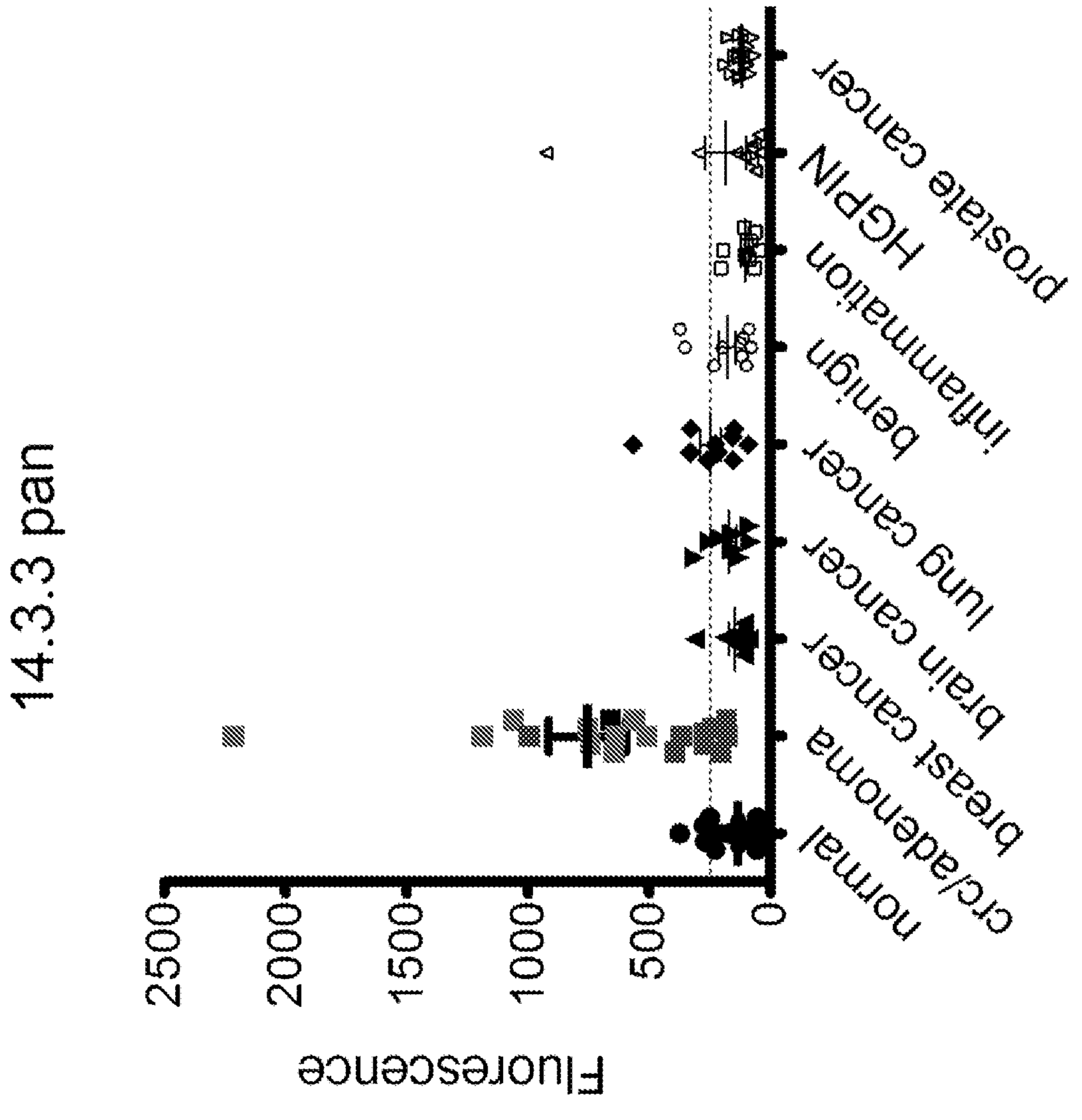

14.3.3 pan
Fluorescence
2500
2000
1500
1000
500
0
normal
crc/adenoma
breast cancer
brain cancer
lung cancer
benign
inflammation
HGPIN
prostate cancer

FIG. 19E

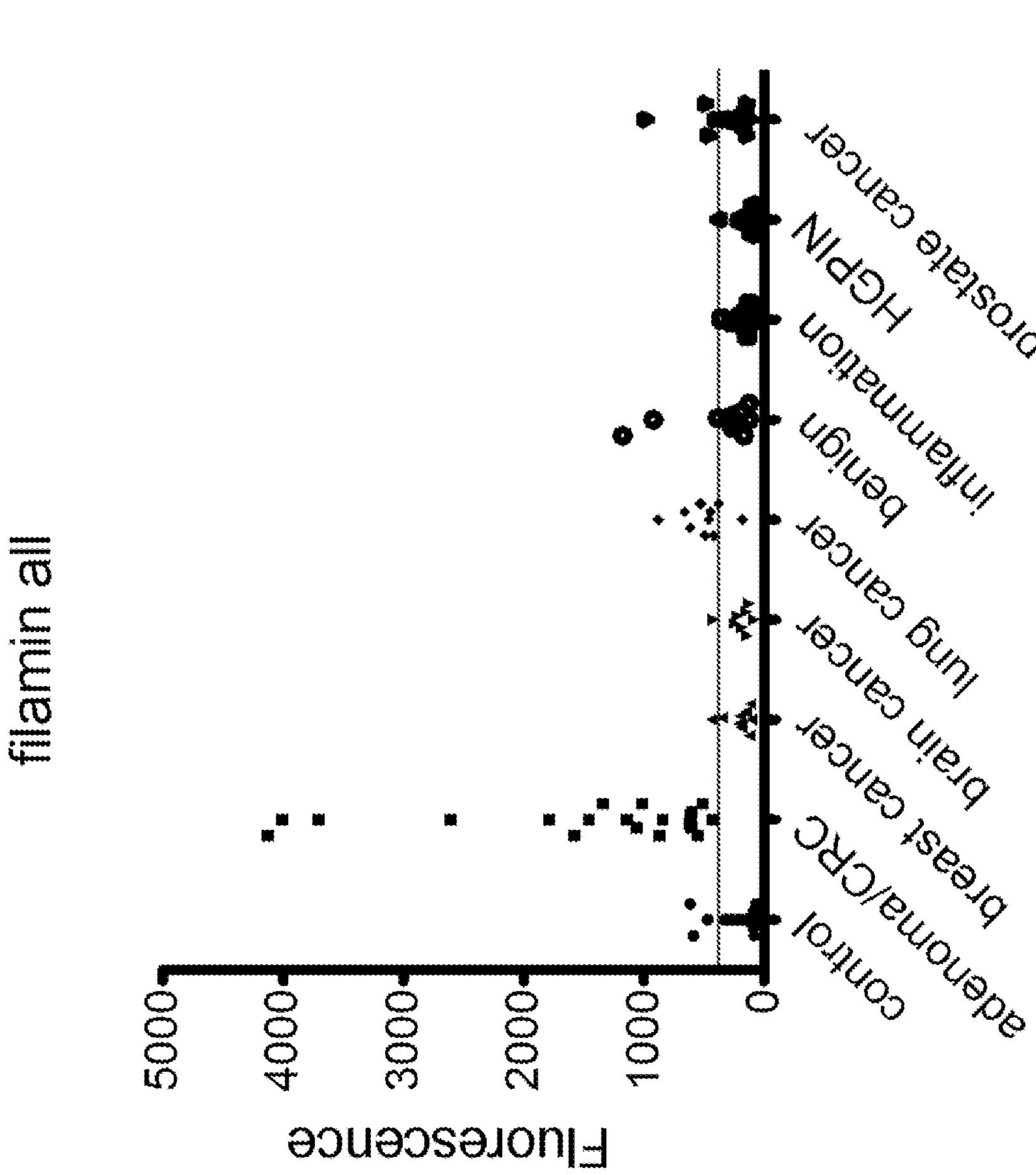
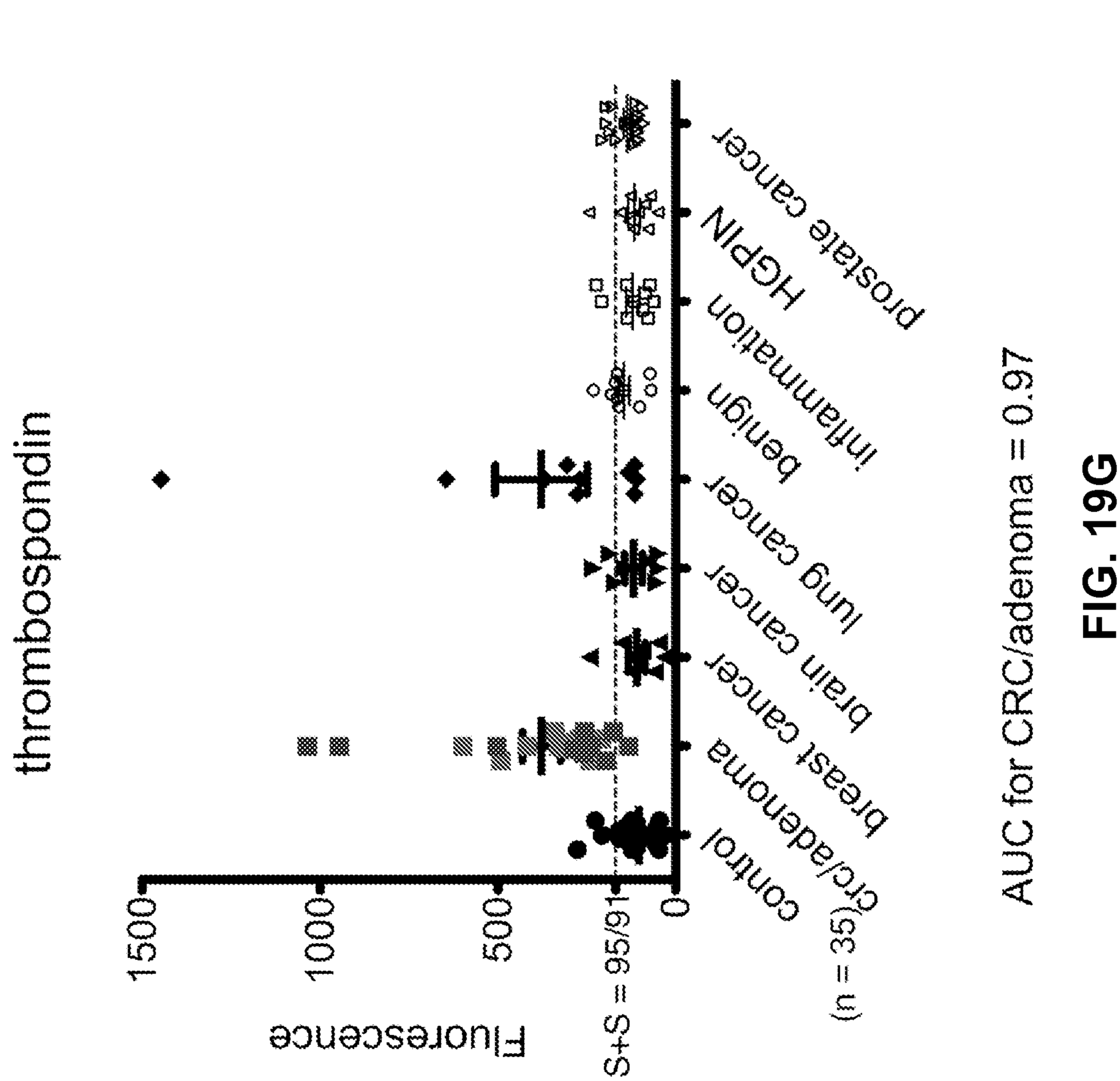

filamin A on all
Fluorescence
5000
4000
3000
2000
1000
0
control
adenoma/CRC
breast cancer
brain cancer
lung cancer
benign
inflammation
HGPIN
prostate cancer

FIG. 19F



AUC for CRC/adenoma = 0.67

FIG. 19G

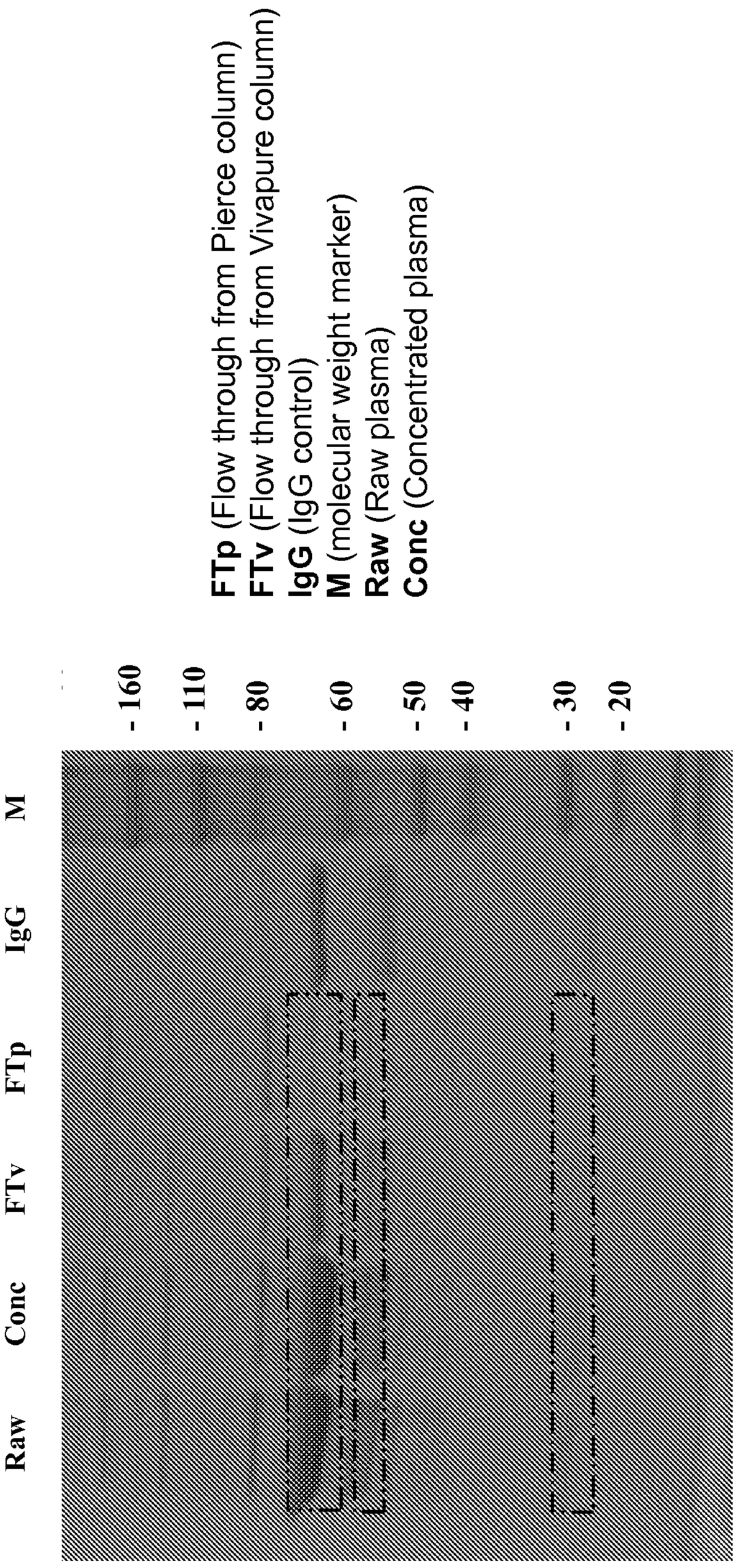

FTp (Flow through from Pierce column)
FTv (Flow through from Vivapure column)
IgG (IgG control)
M (molecular weight marker)
Raw (Raw plasma)
Conc (Concentrated plasma)
M
- 160
- 110
- 80
- 60
- 50
- 40
- 30
- 20
IgG
FTp
FTv
Conc
Raw
HSA -
H chain -
L chain -

FIG. 20A

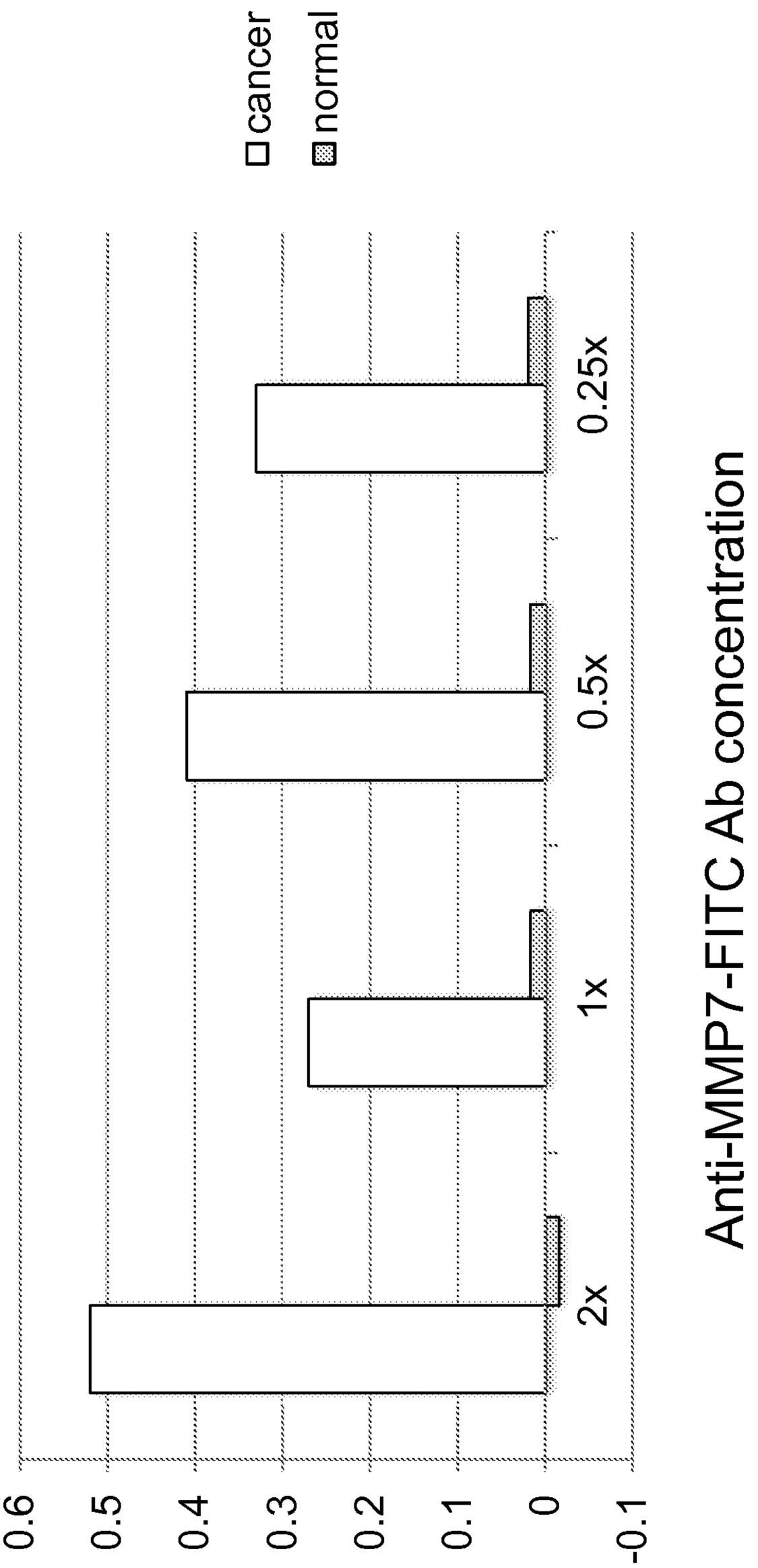

cancer
normal
0.25x
0.5x
1x
2x
Anti-MMP7-FITC Ab concentration
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
Frequency

FIG. 20B

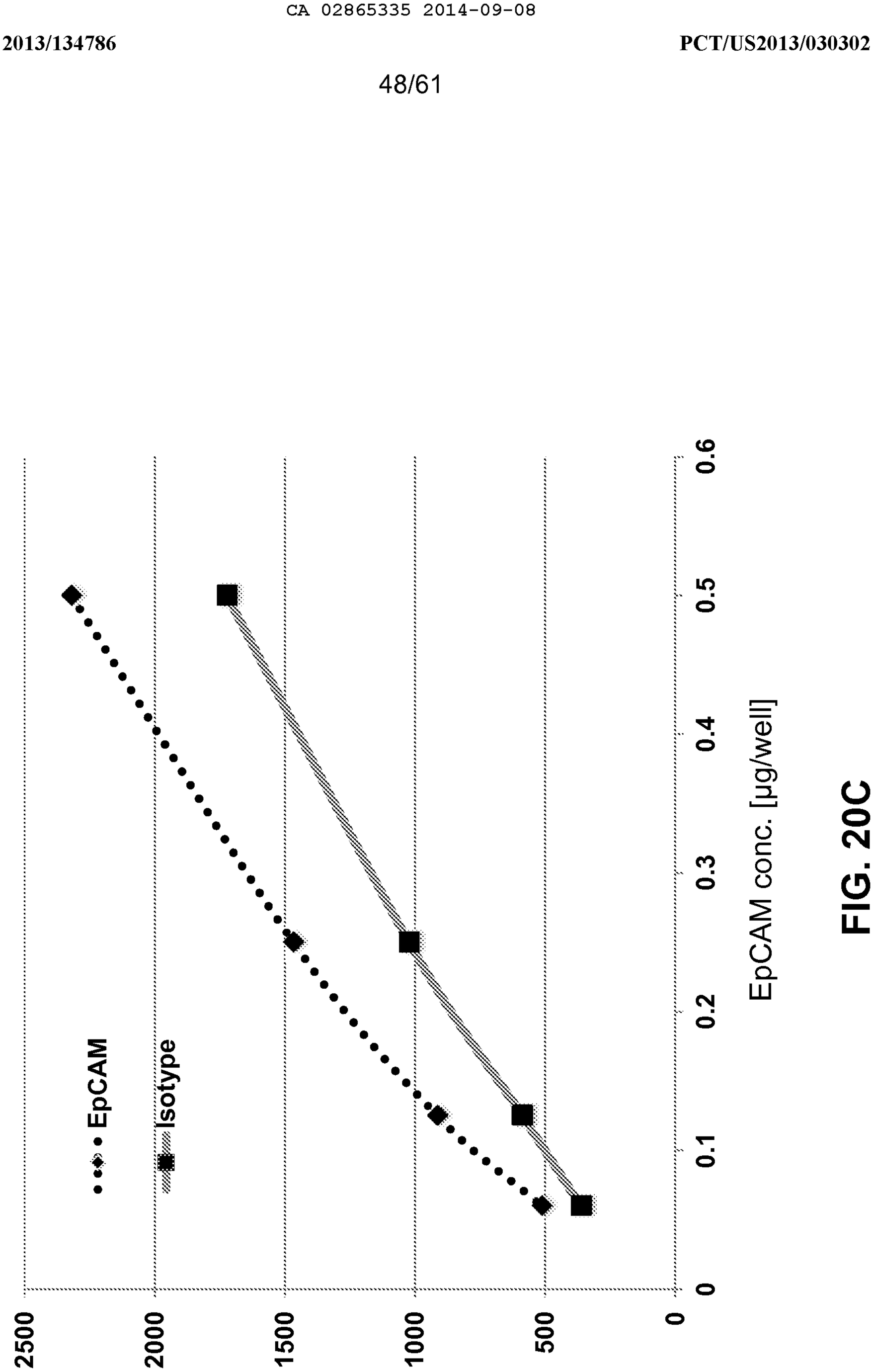

EpCAM
Isotype
MFI (EpCAM-PE sc)
2500
2000
1500
1000
500
0
EpCAM conc. [µg/well]
0
0.1
0.2
0.3
0.4
0.5
0.6

FIG. 20C

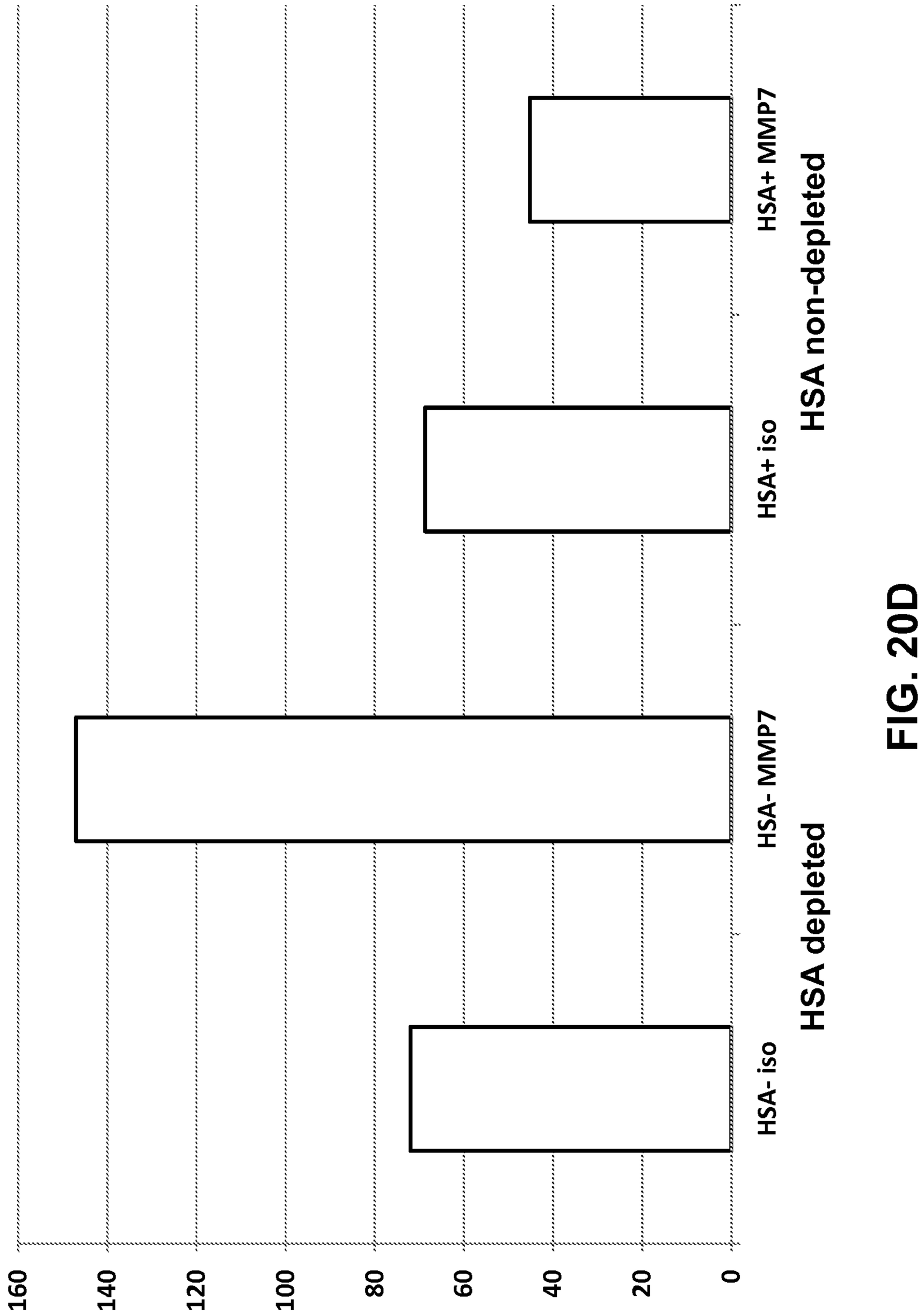

HSA depleted
HSA non-depleted
HSA- iso
HSA- MMP7
HSA+ iso
HSA+ MMP7
MMP7 expression (MFI)
160
140
120
100
80
60
40
20
0

FIG. 20D

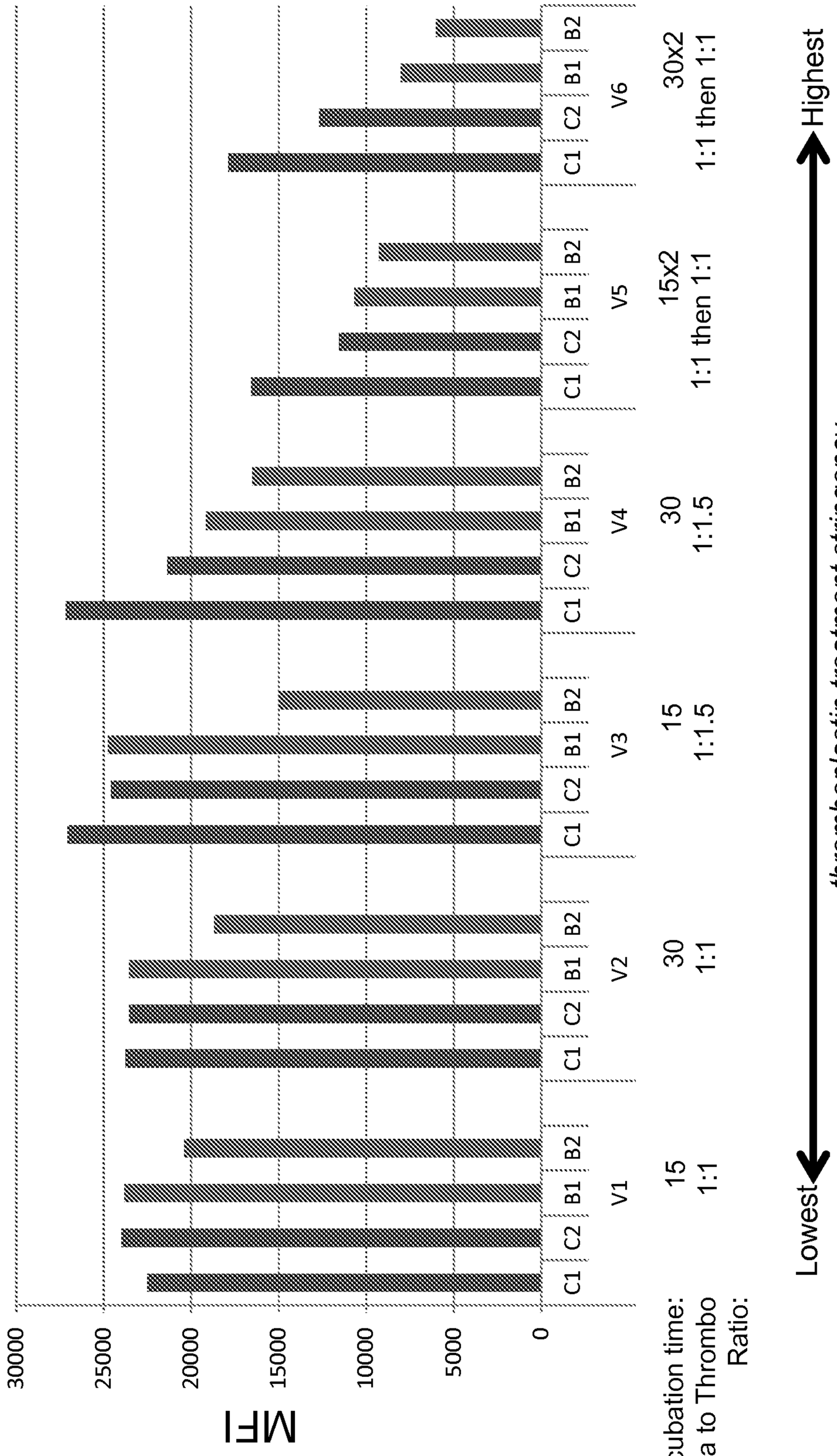

99-KLK2 (Novus)
MFI
30000
25000
20000
15000
10000
5000
0
C1
C2
B1
B2
V1
V2
V3
V4
V5
V6
15
1:1
30
1:1
15
1:1.5
30
1:1.5
15x2
1:1 then 1:1
30x2
1:1 then 1:1
Lowest
Highest
thromboplastin treatment stringency
Incubation time:
Plasma to Thrombo Ratio:

FIG. 20E

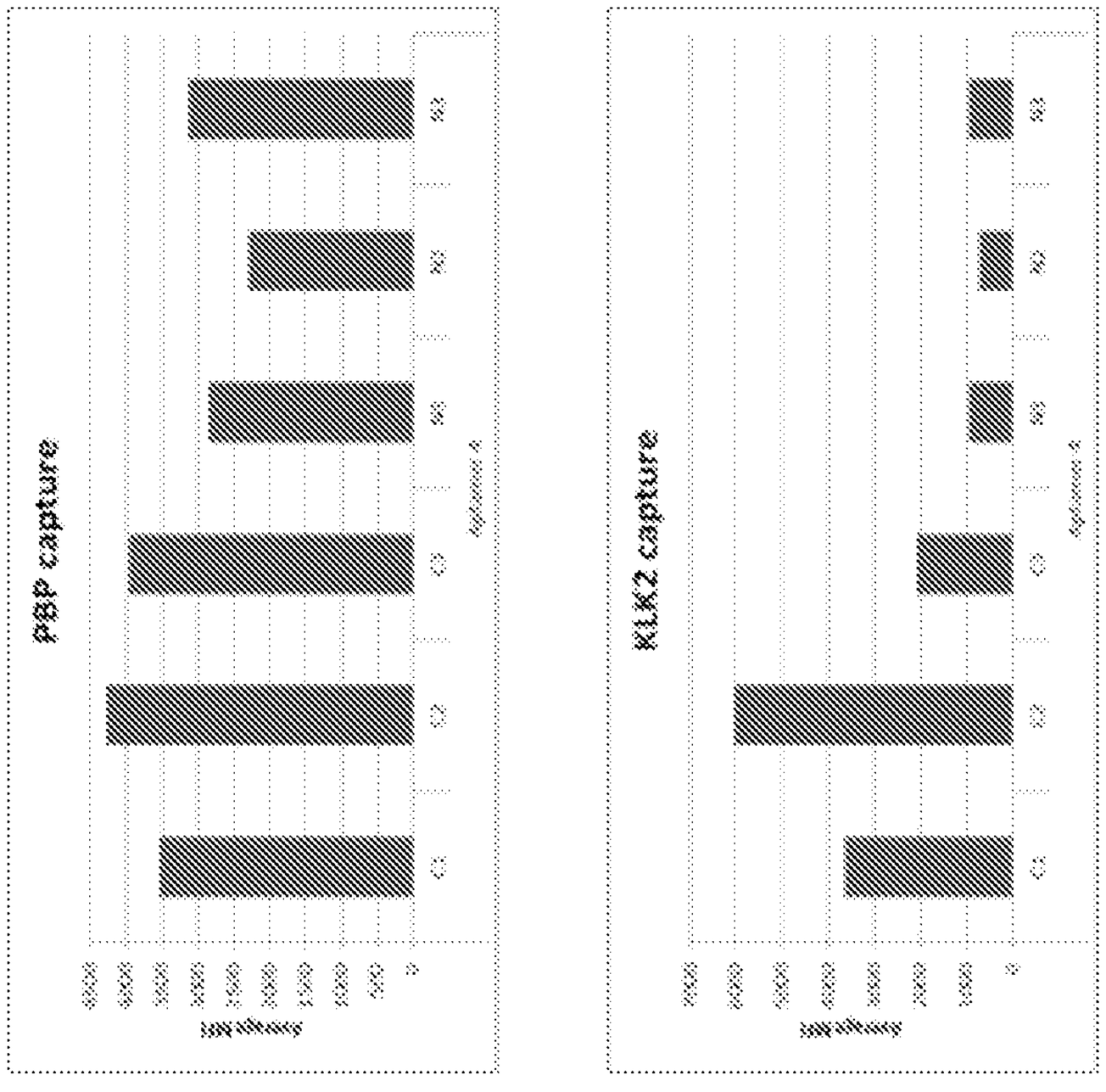
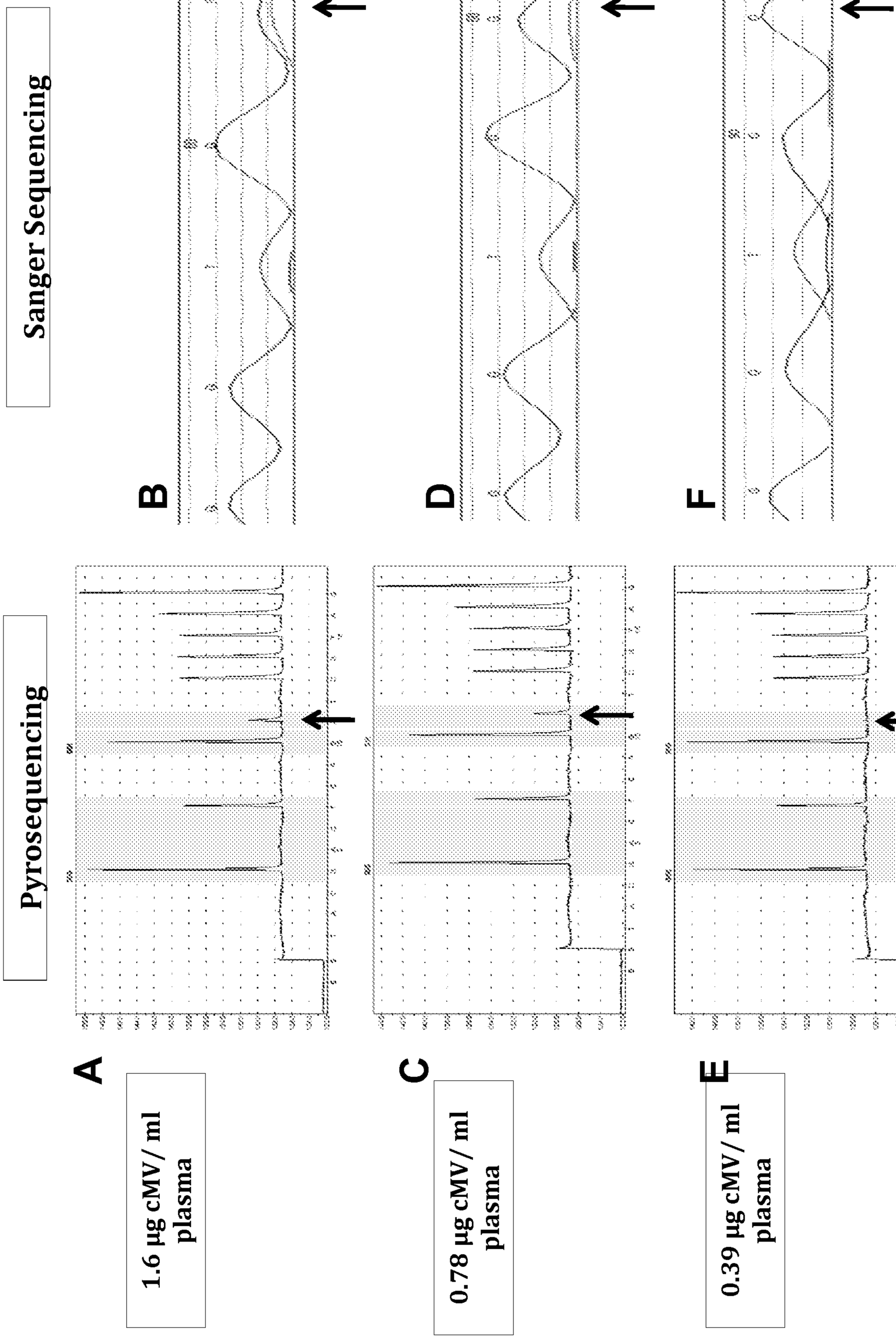
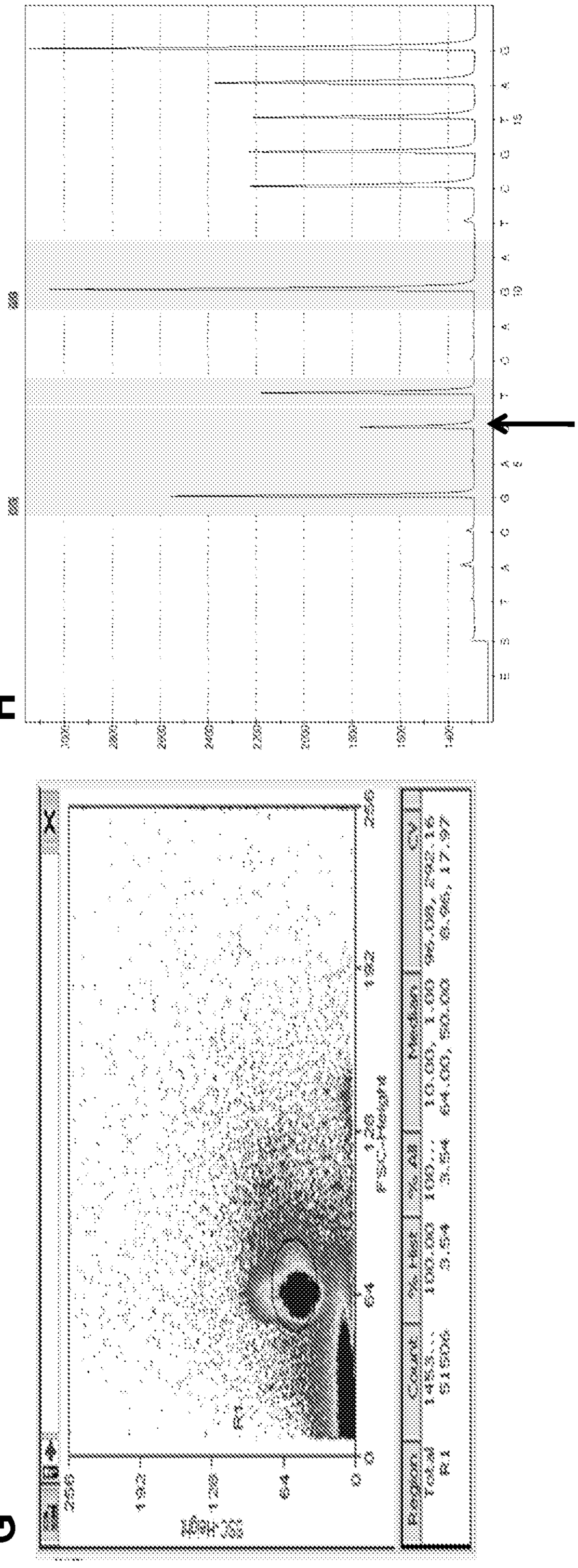

PSP capture
RLK2 capture
EGFR capture
EpCAM capture
Aptamer 4
Sample Set
Aptamer 4 (SEQ ID NO. 1)

FIG. 21

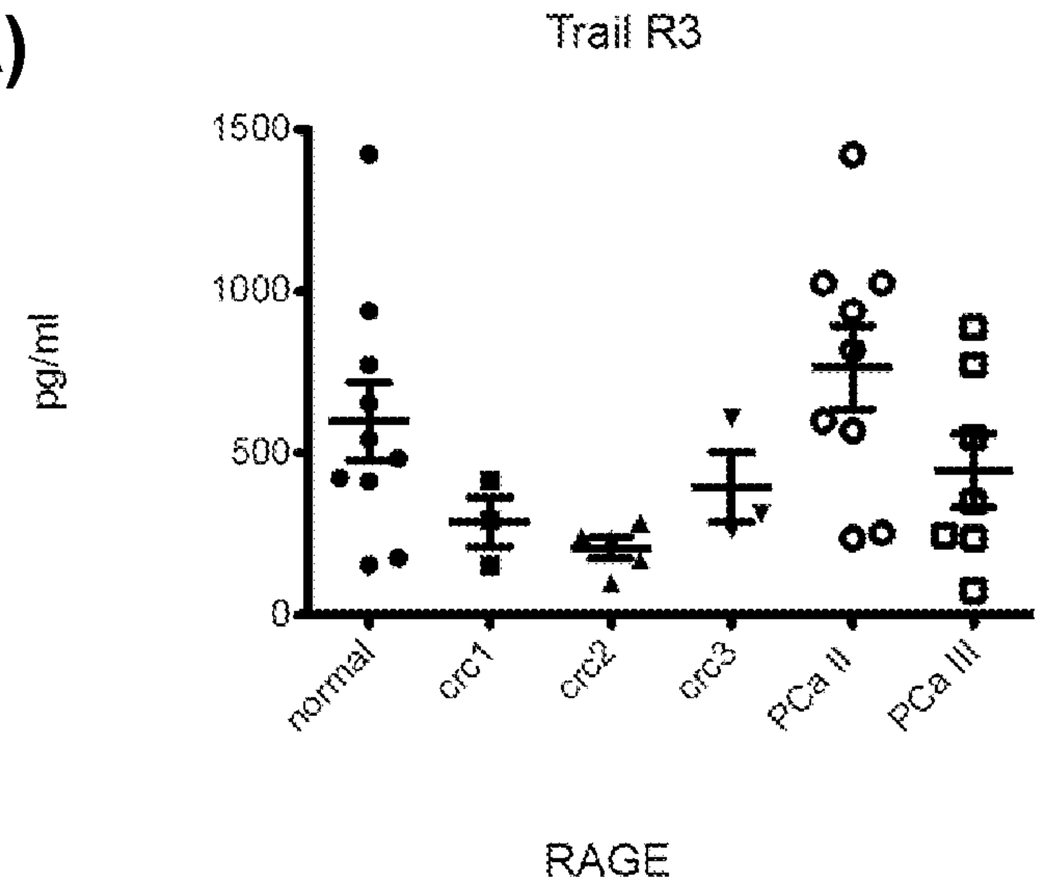

Sanger Sequencing
B
D
F
Pyrosequencing
A
1.6 µg cMV/ ml plasma
C
0.78 µg cMV/ ml plasma
E
0.39 µg cMV/ ml plasma

FIGs. 2A-F

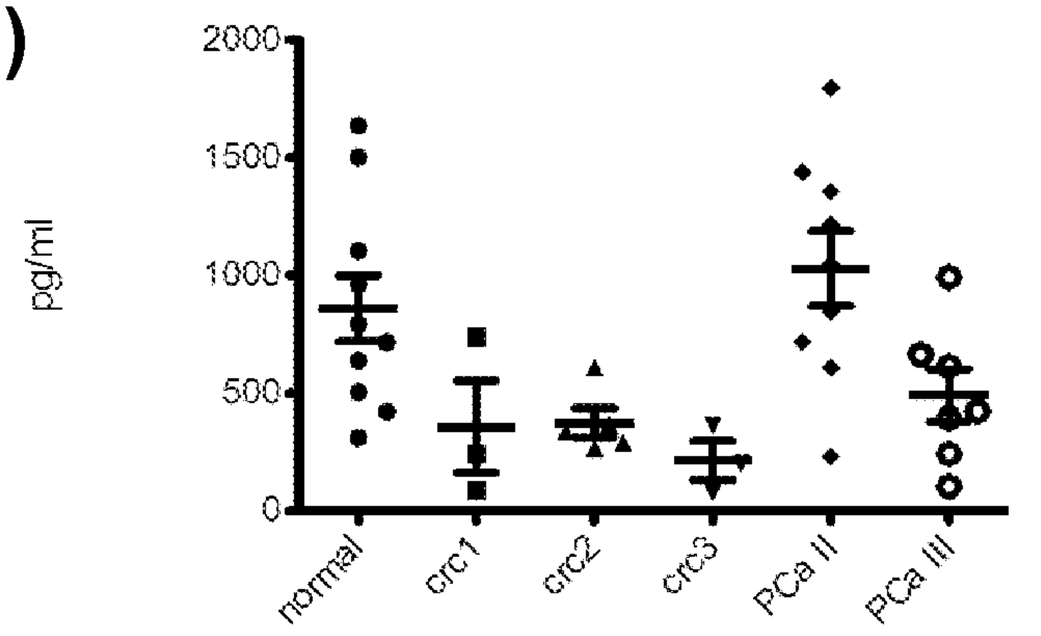


FIGs. 2G-H

A)


Trail R3
pg/ml
1500
1000
500
0
normal
crc1
crc2
crc3
PCa II
PCa III
RAGE

B)


pg/ml
2000
1500
1000
500
0
normal
crc1
crc2
crc3
PCa II
PCa III
ErbB3 all

C)

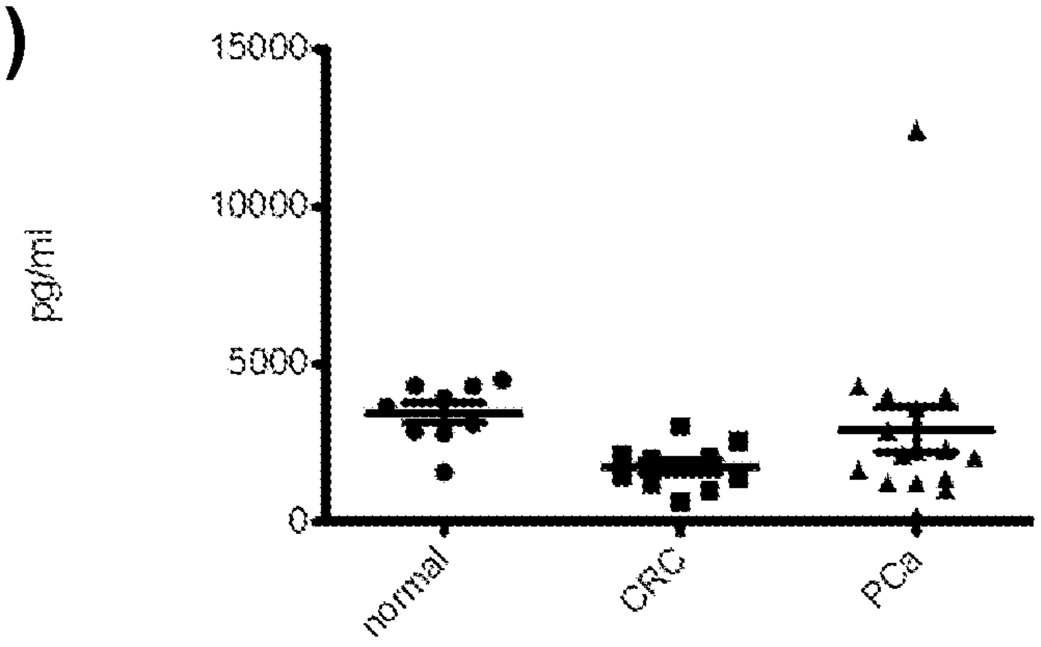

pg/ml
15000
10000
5000
0
normal
CRC
PCa

FIG. 23

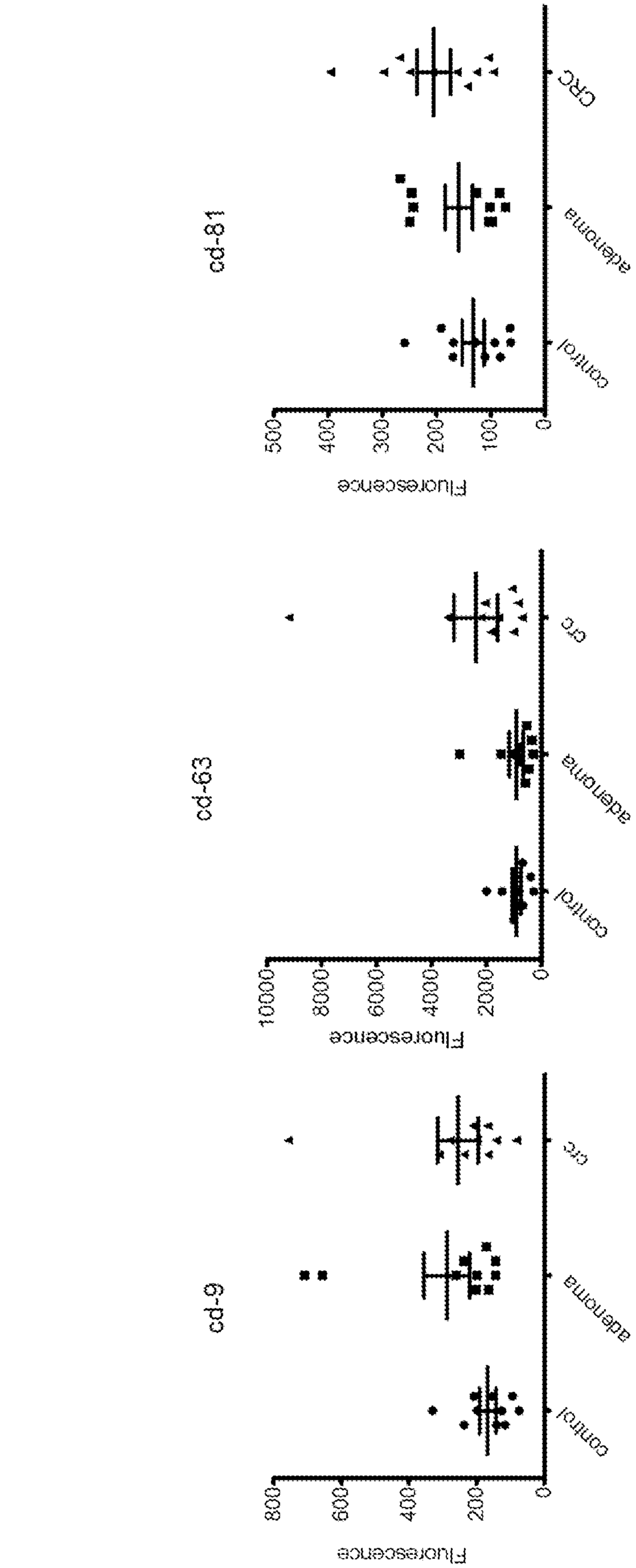
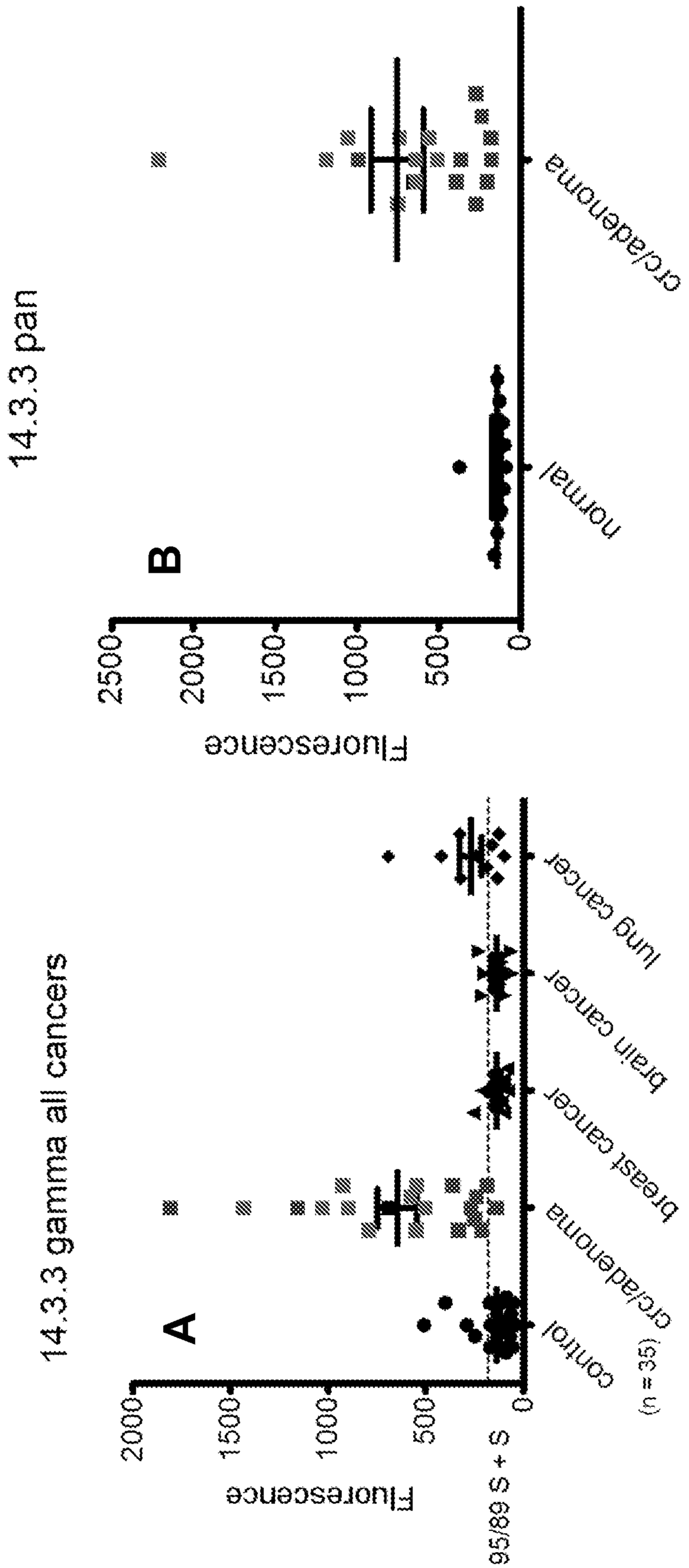
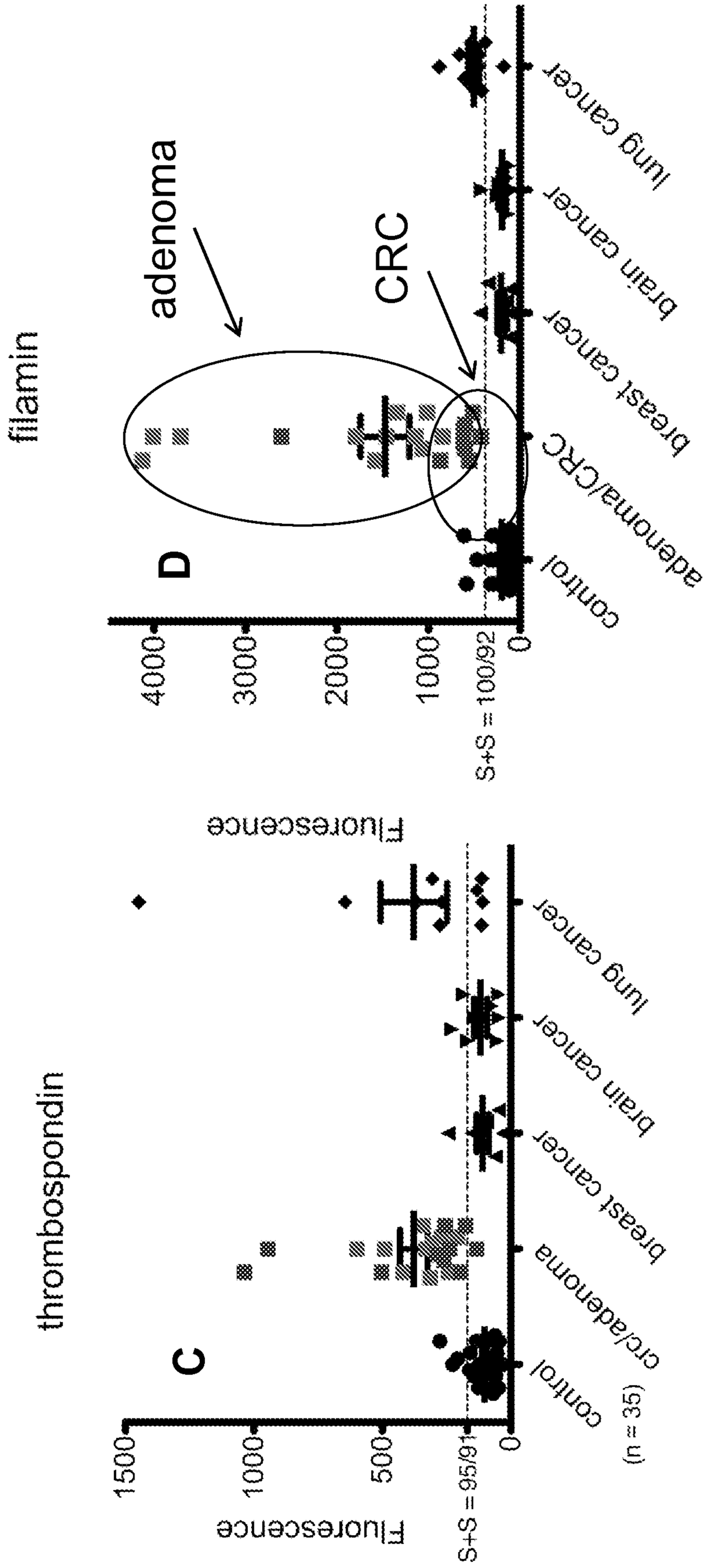

cd-81
Fluorescence
500
400
300
200
100
0
control
adenoma
CRC
cd-63
Fluorescence
10000
8000
6000
4000
2000
0
control
adenoma
cfc
cd-9
Fluorescence
800
600
400
200
0
control
adenoma
cfc

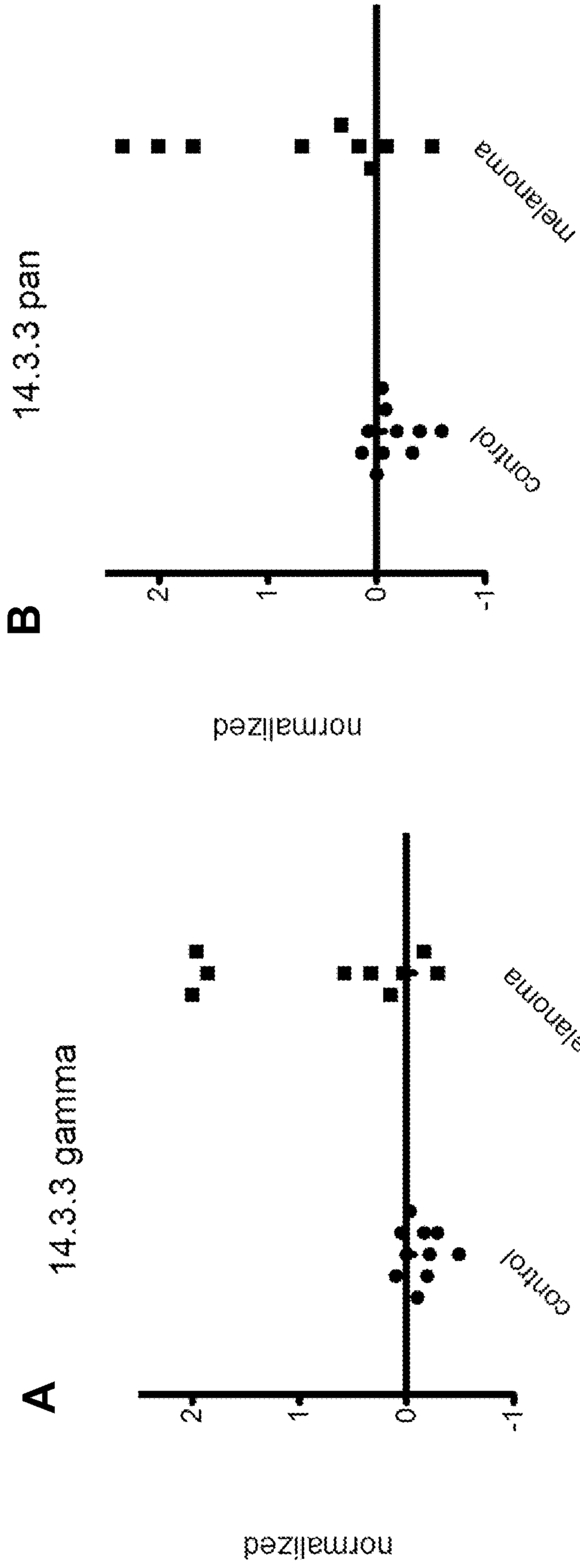
FIG. 24


A
14.3.3 gamma all cancers
Fluorescence
2000
1500
1000
500
0
95/89 S + S
control
adenoma (n = 35)
colorectal adenoma
breast cancer
brain cancer
lung cancer
AUC for CRC/Adenoma = 0.95
B
14.3.3 pan
Fluorescence
2500
2000
1500
1000
500
0
normal
adenoma
colorectal adenoma

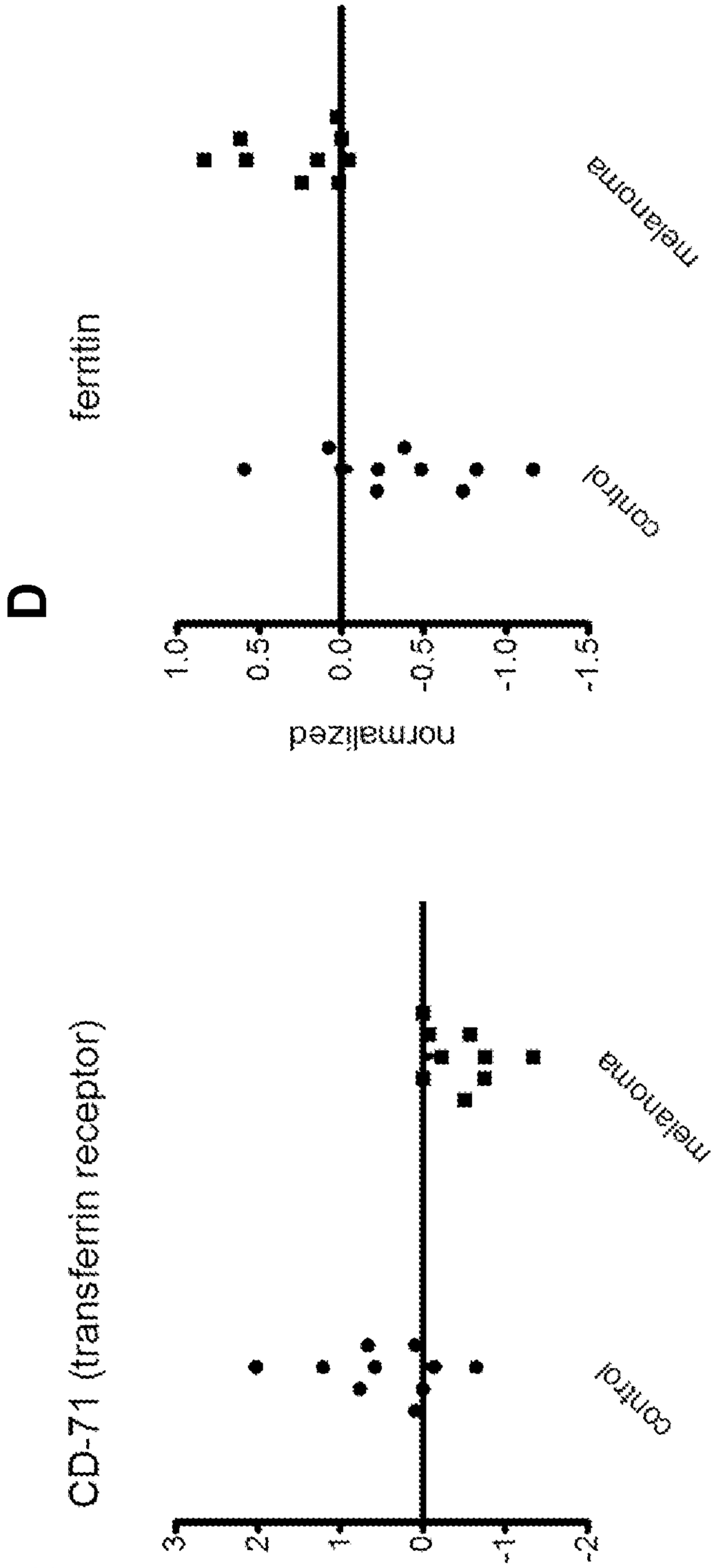
FIGs. 2A-B


filamin
D
adenoma
CRC
4000
3000
2000
1000
0
S+S = 100/92
control
adenoma/CRC
breast cancer
brain cancer
lung cancer
thrombospondin
C
Fluorescence
1500
1000
500
0
S+S = 95/91
control
adenoma
breast cancer
brain cancer
lung cancer
AUC for CRC/adenoma = 0.97
(n = 35)

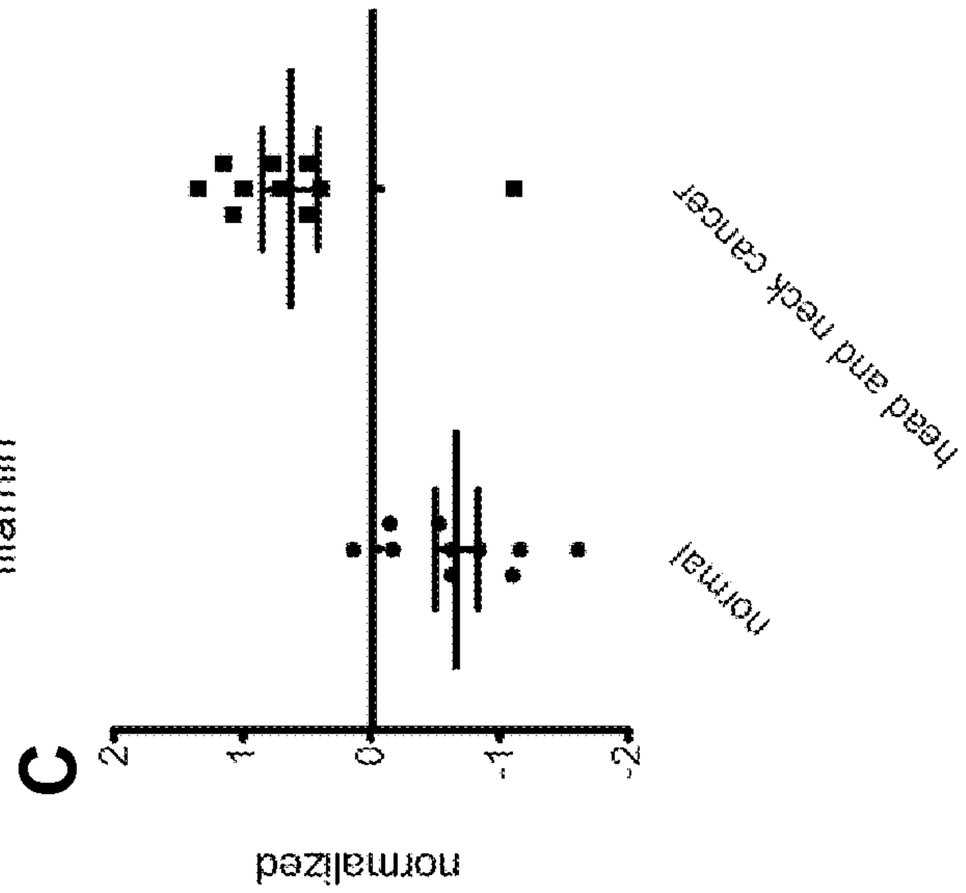
FIGs. 25C-D

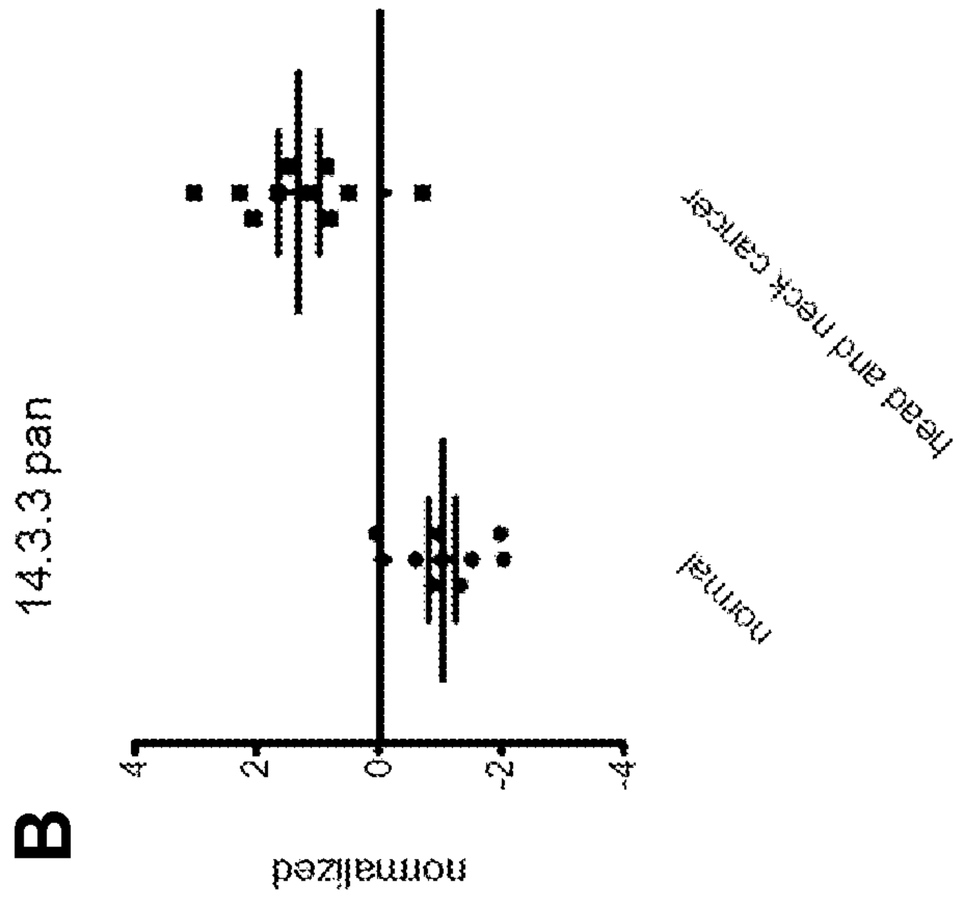

B
14.3.3 pan
melanoma
control
2
1
0
-1
normalized
A
14.3.3 gamma
melanoma
control
2
1
0
-1
normalized

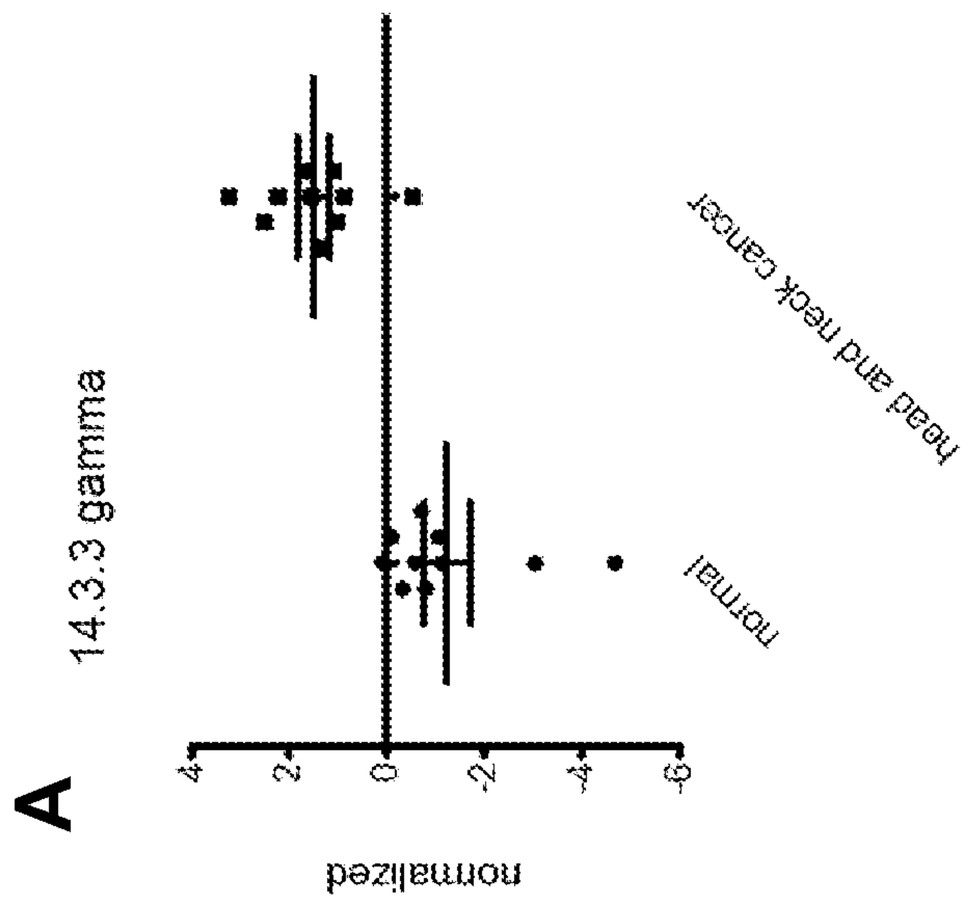
FIGs. 26A-B


D
ferritin
melanoma
control
1.0
0.5
0.0
-0.5
-1.0
-1.5
normalized
CD-71 (transferrin receptor)
melanoma
control
3
2
1
0
-1
-2
C
normalized

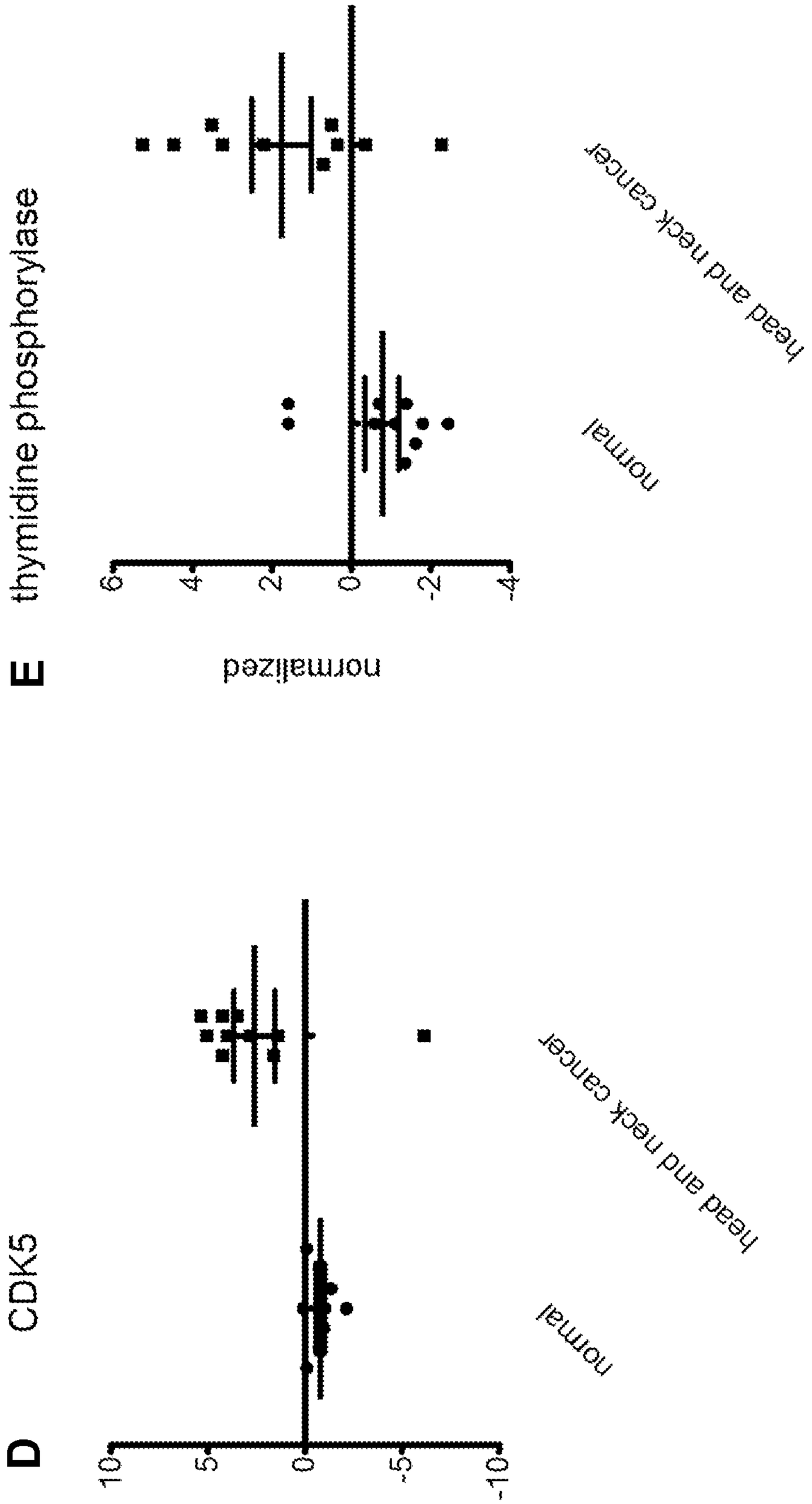
FIGs. 26C-D


C
filamin
normalized
2
1
0
-1
-2
normal
head and neck cancer


B
14.3.3 pan
normalized
4
2
0
-2
-4
normal
head and neck cancer


A
14.3.3 gamma
normalized
4
2
0
-2
-4
-6
normal
head and neck cancer

FIGs. 27A-C


D
CDK5
normalized
10
5
0
-5
-10
normal
head and neck cancer
E
thymidine phosphorylase
normalized
6
4
2
0
-2
-4
normal
head and neck cancer

FIGs. 27D-E

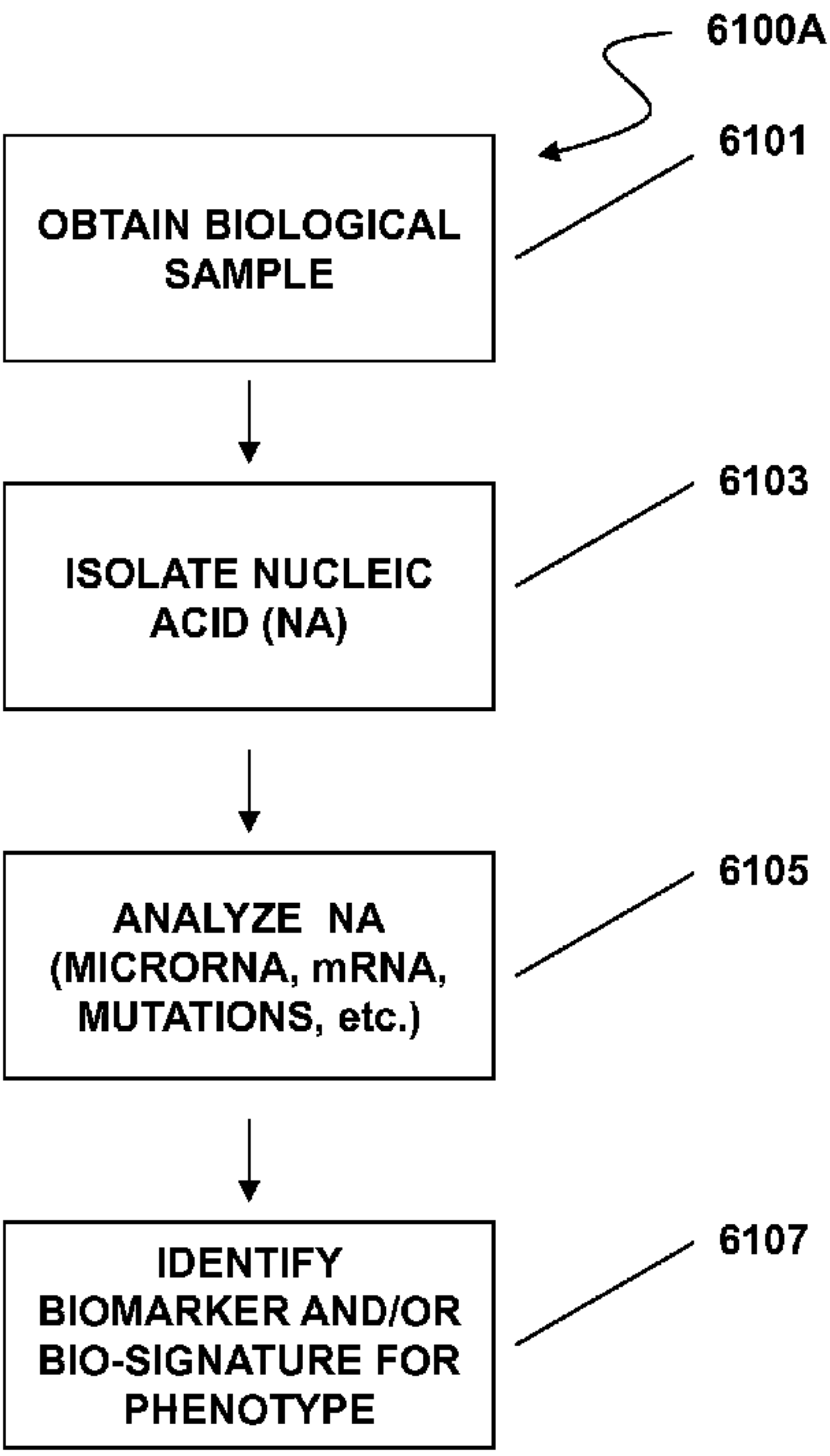

6100A
OBTAIN BIOLOGICAL SAMPLE
6101
ISOLATE NUCLEIC ACID (NA)
6103
ANALYZE NA (MICRORNA, mRNA, MUTATIONS, etc.)
6105
IDENTIFY BIOMARKER AND/OR BIO-SIGNATURE FOR PHENOTYPE
6107

FIG. 1A