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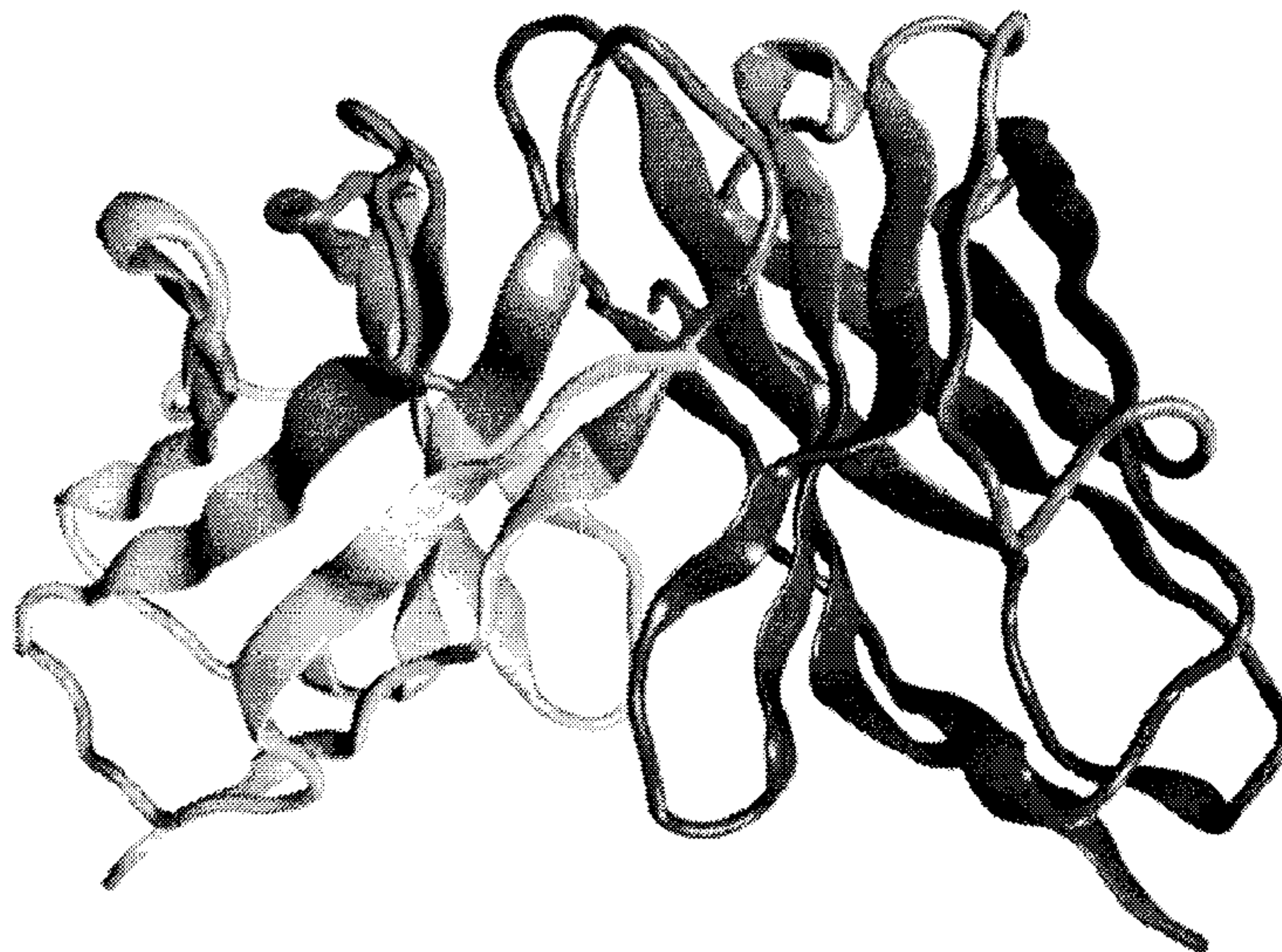
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(57) **Abrégé/Abstract:**

Described herein are antibodies that bind to alpha integrin 11. These antibodies have been modified to increase biophysical characteristics that predict ease of large-scale industrial production.

INTEGRIN ANTIBODIES AND USES THEREOF

ABSTRACT

Described herein are antibodies that bind to alpha integrin 11. These antibodies have been modified to increase biophysical characteristics that predict ease of large-scale industrial production.

INTEGRIN ANTIBODIES AND USES THEREOF

BACKGROUND

[0001] Integrin $\alpha 11$ is a collagen binding integrin that has been described to be overexpressed in cancer associated fibroblasts (CAFs) in a NSCLC model, where its upregulation induces extracellular matrix remodeling and provides a milieu for enhanced tumorigenesis. *See e.g.* Zhu et al. Integrin $\alpha 11$ regulates IGF2 expression in fibroblasts to enhance tumorigenicity of human non-small-cell lung cancer cells. *PNAS*. 2007; 104(28):11754-11759.

SUMMARY

[0002] Described herein are antibodies that bind human integrin alpha 11 (*ITAG11*). The antibodies possesses desirable biophysical properties that include a reduced propensity to aggregate, as illustrated by a low residence time during hydrophobic interaction chromatography (HIC). The antibodies described herein have been altered to reduce the propensity to aggregate, yet maintain binding affinity for *ITAG11*. In certain embodiments, at least one amino acid residue of a complementarity determining region (CDR) of the *ITAG11* binding antibodies described herein has been altered from a parental clone to reduce on column residence time during hydrophobic interaction chromatography (HIC). Thus, the antibodies described herein include variants of a certain parental clone that possess reduced on column residence time during HIC.

[0003] As more biotherapeutics are entering pharmaceutical pipelines, early-stage developability assessment and optimization strategies for biotherapeutics are needed. Thus, also, disclosed herein are methods of optimizing an amino acid sequence of an antibody for industrial scale production. Additionally disclosed herein are methods of identifying CDR residues to alter in order to optimize industrial scale production.

[0004] In certain aspects describe herein, is an antibody or antigen binding fragment thereof comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in any one of SEQ ID NOs: 1 to 97, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in any one of SEQ ID NOs: 98 to 194, wherein the recombinant antibody binds an integrin polypeptide. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment

thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a pharmaceutical composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

[0005] In certain aspects describe herein, is an antibody or antigen binding fragment thereof, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NOs: 43, 58, 59, 60, or 61, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a pharmaceutical composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method

comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

[0006] In certain aspects describe herein, is an antibody or antigen binding fragment thereof, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 43, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 140. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a pharmaceutical composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

[0007] In certain aspects describe herein, is an antibody or antigen binding fragment thereof, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 58, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 155. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or

deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a pharmaceutical composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

[0008] In certain aspects describe herein, is an antibody or antigen binding fragment thereof, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 459, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 156. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a pharmaceutical composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

[0009] In certain aspects describe herein, is an antibody or antigen binding fragment thereof, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 60, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 157. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a pharmaceutical composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

[0010] In certain aspects describe herein, is an antibody or antigen binding fragment thereof, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 61, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 158. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a pharmaceutical

composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

[0011] In certain aspects describe herein, is an antibody or antigen binding fragment thereof comprising a heavy chain CDR1 (CDR-H1) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a heavy chain CDR2 (CDR-H2) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a heavy chain CDR3 (CDR-H3) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a light chain CDR1 (CDR-L1) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, a light chain CDR2 (CDR-L2) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, and a light chain CDR3 (CDR-L3) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, wherein the recombinant antibody binds an integrin polypeptide. In certain embodiments, any one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, or CDR-L3 are identified by a numbering scheme selected from the list consisting of the Kabat numbering scheme, the Chothia numbering scheme, the IMGT numbering scheme, the contact numbering scheme, the Aho numbering scheme, and combinations thereof. In certain embodiments, two or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, or CDR-L3 are identified by at least two different numbering schemes selected from the list consisting of the Kabat numbering scheme, the Chothia numbering scheme, the IMGT numbering scheme, the contact numbering scheme, the Aho numbering scheme, and combinations thereof. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a

pharmaceutical composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

[0012] In certain aspects describe herein, is an antibody or antigen binding fragment thereof comprising a heavy chain CDR1 (CDR-H1) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60, or 61, a heavy chain CDR2 (CDR-H2) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60, or 61, a heavy chain CDR3 (CDR-H3) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60, or 61, a light chain CDR1 (CDR-L1) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, a light chain CDR2 (CDR-L2) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, and a light chain CDR3 (CDR-L3) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the recombinant antibody binds an integrin polypeptide. In certain embodiments, any one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, or CDR-L3 are identified by a numbering scheme selected from the list consisting of the Kabat numbering scheme, the Chothia numbering scheme, the IMGT numbering scheme, the contact numbering scheme, the Aho numbering scheme, and combinations thereof. In certain embodiments, two or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, or CDR-L3 are identified by at least two different numbering schemes selected from the list consisting of the Kabat numbering scheme, the Chothia numbering scheme, the IMGT numbering scheme, the contact numbering scheme, the Aho numbering scheme, and combinations thereof. In certain embodiments, the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody. In certain embodiments, the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains. In certain embodiments, the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized. In certain embodiments, the integrin polypeptide is an integrin alpha11 polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof is isolated

and purified. In certain embodiments, the antibody or antigen binding fragment thereof is part of a pharmaceutical composition comprising the antibody or antigen binding fragment thereof and pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the antibody or antigen binding fragment thereof of the pharmaceutical composition is formulated for intravenous administration. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in treating a cancer. In certain embodiments, the antibody or antigen binding fragment thereof or the pharmaceutical composition are for use in a method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of the pharmaceutical composition. In certain embodiments, the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The novel features described herein are set forth with particularity in the appended claims. A better understanding of the features and advantages of the features described herein will be obtained by reference to the following detailed description that sets forth illustrative examples, in which the principles of the features described herein are utilized, and the accompanying drawings of which:

[0014] **FIGS. 1A-1D** illustrate a homology model of the variable domains of the parental integrin binding antibody NB0268. The backbone of the Fv domains is depicted as ribbons with the CDRs highlighted (A) and protein patches are shown in (B); hydrophobic (green), positive (blue), negative (red). The log P hydrophobicity surface is shown in (C) where green is hydrophobic and purple is polar. In (D), the electrostatic surface displays delta negative regions in red and positive regions in blue.

[0015] **FIG 2** illustrates parental mAb residues selected for mutation. Atoms belonging to residues which contribute to a hydrophobic patch are indicated by the arrow. CDRs, as defined by the Kabat scheme, are dark grey in the sequence as is the corresponding 3D backbone ribbon.

[0016] **FIG. 3A** and **3B** illustrate a comparison of structural protein property descriptors to Hydrophobic interaction column-residence time (HIC RT) for 97 NB0268 variants tested (A). The HIC RT is the x-axis throughout, and its histogram is shown at the top. The Pearson correlation r^2 between the descriptor and the HIC RT is reported on each scatter plot. `hyd_sa` is the sum of the surface areas of all hydrophobic patches on the homology model. `cdr_hyd` is the same value but only calculated near the CDR regions. `avg_cdr_hyd` is the `cdr_hyd` value averaged over a LowModeMD generated sample of conformations. `avg_cdr_logp` is `avg_cdr_hyd` multiplied by the square of the

patch average log P hydrophobic potential. The parental integrin $\alpha 11$ mAb is indicated by the arrow throughout and antibodies that did not elute from the column were assigned an elution time of 30 minutes, representing the maximum value possible. **(B)** Comparison of protein QSPR model performance to sequence-based hydrophobic index for 97 mAb variants tested. `hyd_idx` is the Black & Mould hydrophobic index based on sequence alone. `avg_HI` is the hydrophobic imbalance descriptor, and `QSPR_2_Pred` is the 2-point protein QSPR model prediction.

[0017] FIG. 4A illustrates mutations that reduce hydrophobic patch surface area the parental mAb (top) compared to Y54H x Y102D (middle) and Y54D x Y102D (bottom). Y54H x Y102D is the fastest eluting variant due to the reduction in hydrophobic patch surface area. Y54D x Y102D is a false positive outlier in the HIC RT predictions which, despite having a similar surface profile to Y54H x T102D, does not elute, nor do several Y54D mutants. On the right side, 2D projections of the Fv domain surface patches are shown for each variant.

[0018] FIG. 4B illustrates mutations that reduce hydrophobic patch surface area the parental mAb (top) compared to Y102E x Y54H (bottom).

[0019] FIG. 5A-5C illustrate a comparison of structural protein property descriptors to HIC RT for 137 diverse clinical candidate mAbs **(A)**. The plots report the Pearson correlation r^2 between the descriptor and experimental HIC RT, with the top-most plot being a histogram of the HIC RT values. `hyd_idx` is the Black & Mould hydrophobic index based on sequence alone. `hyd_sa`, `avg_cdr_hyd`, and `avg_cdr_logp` are described in Fig. 3. **(B)** Comparison of protein QSPR model predictions of HIC RT for 137 diverse clinical candidate mAbs. `ASPmax` is the maximum average surface property (or hydrophobicity) descriptor and `QSPR_4_Pred` is a 4-point QSPR model fit to HIC RT for this series. **(C)** Comparison of structural protein property descriptors to inclusion body data available for 31 adnectin variants. The plots report the Pearson correlation r^2 between the descriptor and the experimental % IB, whose histogram appears as the top plot. `hyd_idx` is described above, `hyd_sa` is the sum of the surface areas of all hydrophobic patches on the homology model. `avg_hyd` is the same value but calculated as an average over a LowModeMD generated sample of conformations. `avg_logp` is `avg_hyd` multiplied by the square of the patch average log P hydrophobic potential.

[0020] FIG. 6A illustrates experimental HIC RT (top) of all mAb variants tested and their comparisons to hydrophobic surface area (middle top), CDR hydrophobic surface area (middle bottom), and `avg_CDR3_LogP` (bottom) protein property descriptors.

[0021] FIG. 6B illustrates experimental HIC RT (top) of all mAb variants tested and their correlation of experimental HIC RT with the Black & Mould sequence based hydrophobic index

(middle) and to the 2-point QSPR calculation derived from MOE 2016 (bottom).

DETAILED DESCRIPTION

[0022] Described herein, in a certain aspect, is an antibody or antigen binding fragment thereof comprising a heavy chain variable region comprising an amino acid sequence at least 80% identical to that set forth in in any one of SEQ ID NOs: 1 to 97, and a light chain variable region comprising an amino acid sequence at least 80% identical to that set forth in any one of SEQ ID NOs: 98 to 194, wherein the recombinant antibody binds an integrin polypeptide.

[0023] Described herein, in another aspect, is an antibody or antigen binding fragment thereof comprising a heavy chain CDR1 (CDR-H1) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a heavy chain CDR2 (CDR-H2) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a heavy chain CDR3 (CDR-H3) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a light chain CDR1 (CDR-L1) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, a light chain CDR2 (CDR-L2) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, and a light chain CDR3 (CDR-L3) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, wherein the recombinant antibody binds an integrin polypeptide.

Certain Definitions

[0024] In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the embodiments provided may be practiced without these details. Unless the context requires otherwise, throughout the specification and claims which follow, the word “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense, that is, as “including, but not limited to.” As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise. It should also be noted that the term “or” is generally employed in its sense including “and/or” unless the content clearly dictates otherwise. Further, headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed embodiments.

[0025] As used herein the term “about” refers to an amount that is near the stated amount by 10%.

[0026] As used herein the terms “individual,” “subject,” and “patient” are used interchangeably and include humans diagnosed with or suspected of being afflicted with a tumor, a cancer or other neoplasm.

Structural Aspects of the Antibodies Described Herein

[0027] Among the provided antibodies are monoclonal antibodies and antibody fragments. The antibodies include antibody-conjugates and molecules comprising the antibodies, such as chimeric molecules. Thus, an antibody includes, but is not limited to, full-length and native antibodies, as well as fragments and portion thereof retaining the binding specificities thereof, such as any specific binding portion thereof including those having any number of, immunoglobulin classes and/or isotypes (e.g., IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgD, IgE and IgM); and biologically relevant (antigen-binding) fragments or specific binding portions thereof, including but not limited to Fab, F(ab')₂, Fv, and scFv (single chain or related entity). A monoclonal antibody is generally one within a composition of substantially homogeneous antibodies; thus, any individual antibodies comprised within the monoclonal antibody composition are identical except for possible naturally occurring mutations that may be present in minor amounts. The monoclonal antibody can comprise a human IgG1 constant region. The monoclonal antibody can comprise a human IgG4 constant region.

[0028] The term “antibody” herein is used in the broadest sense and includes monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments thereof, including fragment antigen binding (Fab) fragments, F(ab')₂ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, single chain antibody fragments, including single chain variable fragments (sFv or scFv), and single domain antibodies (e.g., sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, e.g., bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term “antibody” should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD. The antibody can comprise a human IgG1 constant region. The antibody can comprise a human IgG4 constant region.

[0029] The terms “complementarity determining region,” and “CDR,” which are synonymous with “hypervariable region” or “HVR,” are known in the art to refer to non-contiguous sequences of

amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR-L3).

“Framework regions” and “FR” are known in the art to refer to the non-CDR portions of the variable regions of the heavy and light chains. In general, there are four FRs in each full-length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4). The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat numbering scheme”), Al-Lazikani et al., (1997) *JMB* 273,927-948 (“Chothia numbering scheme”), MacCallum et al., *J. Mol. Biol.* 262:732-745 (1996), “Antibody-antigen interactions: Contact analysis and binding site topography,” *J. Mol. Biol.* 262, 732-745.” (“contact numbering scheme”), Lefranc MP et al., “IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains,” *Dev Comp Immunol*, 2003 Jan;27(1):55-77 (“IMGT numbering scheme”), and Honegger A and Plückthun A, “Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool,” *J Mol Biol*, 2001 Jun 8;309(3):657-70, (“Aho numbering scheme”).

[0030] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, “30a,” and deletions appearing in some antibodies. The two schemes place certain insertions and deletions (“indels”) at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme.

[0031] The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (V_H and V_L , respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs (See e.g., Kindt et al. Kuby *Immunology*, 6th ed., W.H. Freeman and Co., page 91(2007)). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds

the antigen to screen a library of complementary V_L or V_H domains, respectively (See e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991)).

[0032] Among the provided antibodies are antibody fragments. An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv or sFv); and multispecific antibodies formed from antibody fragments. In particular embodiments, the antibodies are single-chain antibody fragments comprising a variable heavy chain region and/or a variable light chain region, such as scFvs.

[0033] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells. In some embodiments, the antibodies are recombinantly-produced fragments, such as fragments comprising arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, e.g., polypeptide linkers, and/or those that are not produced by enzyme digestion of a naturally-occurring intact antibody. In some aspects, the antibody fragments are scFvs.

[0034] A “humanized” antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of a non-human antibody refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0035] Among the provided antibodies are human antibodies. A “human antibody” is an antibody with an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human.

[0036] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of

the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. Human antibodies also may be derived from human antibody libraries, including phage display and cell-free libraries, containing antibody-encoding sequences derived from a human repertoire.

[0037] The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Polypeptides, including the provided antibodies and antibody chains and other peptides, e.g., linkers and binding peptides, may include amino acid residues including natural and/or non-natural amino acid residues. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. In some aspects, the polypeptides may contain modifications with respect to a native or natural sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0038] Percent (%) sequence identity with respect to a reference polypeptide sequence is the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are known for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Appropriate parameters for aligning sequences are able to be determined, including algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0039] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the %

amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y , where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0040] In some embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. A variant typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants can be naturally occurring or can be synthetically generated, for example, by modifying one or more of the above polypeptide sequences of the invention and evaluating one or more biological activities of the polypeptide as described herein and/or using any of a number of known techniques. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

[0041] In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for mutagenesis by substitution include the CDRs and FRs. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0042] In some embodiments, substitutions, insertions, or deletions may occur within one or more CDRs, wherein the substitutions, insertions, or deletions do not substantially reduce antibody binding to antigen. For example, conservative substitutions that do not substantially reduce binding affinity may be made in CDRs. Such alterations may be outside of CDR "hotspots". In some

embodiments of the variant V_H and V_L sequences, each CDR is unaltered.

[0043] Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity. Such alterations may be made in CDR encoding codons with a high mutation rate during somatic maturation (See e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and the resulting variant can be tested for binding affinity. Affinity maturation (e.g., using error-prone PCR, chain shuffling, randomization of CDRs, or oligonucleotide-directed mutagenesis) can be used to improve antibody affinity (See e.g., Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (2001)). CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling (See e.g., Cunningham and Wells *Science*, 244:1081-1085 (1989)). CDR-H3 and CDR-L3 in particular are often targeted. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0044] Amino acid sequence insertions and deletions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions and deletions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody. Examples of intrasequence insertion variants of the antibody molecules include an insertion of 3 amino acids in the light chain. Examples of terminal deletions include an antibody with a deletion of 7 or less amino acids at an end of the light chain.

[0045] In some embodiments, the antibodies are altered to increase or decrease their glycosylation (e.g., by altering the amino acid sequence such that one or more glycosylation sites are created or removed). A carbohydrate attached to an Fc region of an antibody may be altered. Native antibodies from mammalian cells typically comprise a branched, biantennary oligosaccharide attached by an N-linkage to Asn₂₉₇ of the CH2 domain of the Fc region (See e.g., Wright et al. *TIBTECH* 15:26-32 (1997)). The oligosaccharide can be various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, sialic acid, fucose attached to a GlcNAc in the stem of the biantennar oligosaccharide structure. Modifications of the oligosaccharide in an antibody can be made, for example, to create antibody variants with certain improved properties. Antibody glycosylation variants can have improved ADCC and/or CDC function. In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or

indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn₂₉₇, relative to the sum of all glycostructures attached to Asn₂₉₇ (See e.g., WO 08/077546). Asn₂₉₇ refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues; See e.g., Edelman et al. *Proc Natl Acad Sci U S A*. 1969 May; 63(1):78–85). However, Asn₂₉₇ may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants can have improved ADCC function (See e.g., Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); and Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004)). Cell lines, e.g., knockout cell lines and methods of their use can be used to produce defucosylated antibodies, e.g., Lec13 CHO cells deficient in protein fucosylation and alpha-1,6-fucosyltransferase gene (FUT8) knockout CHO cells (See e.g., Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006)). Other antibody glycosylation variants are also included (See e.g., U.S. Pat. No. 6,602,684).

[0046] In some embodiments, an antibody provided herein has a dissociation constant (K_D) of about 1 μ M, 100 nM, 50 nM, 40 nM, 30 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 0.5 nM, 0.1 nM, 0.05 nM, 0.01 nM, or 0.001 nM or less (e.g., 10^{-8} M or less, e.g., from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M) for the antibody target. K_D can be measured by any suitable assay. In certain embodiments, K_D can be measured using surface plasmon resonance assays (e.g., using a BIACORE®-2000 or a BIACORE®-3000).

[0047] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. An Fc region herein is a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. An Fc region includes native sequence Fc regions and variant Fc regions. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

[0048] In some embodiments, the antibodies of this disclosure are variants that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR)

binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 and 5,821,337. Alternatively, non-radioactive assays methods may be employed (*e.g.*, ACTI™ and CytoTox 96® non-radioactive cytotoxicity assays). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC), monocytes, macrophages, and Natural Killer (NK) cells.

[0049] Antibodies can have increased half-lives and improved binding to the neonatal Fc receptor (FcRn) (*See e.g.*, US 2005/0014934). Such antibodies can comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn, and include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434 according to the EU numbering system (*See e.g.*, U.S. Pat. No. 7,371,826). Other examples of Fc region variants are also contemplated (*See e.g.*, Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Pat. Nos. 5,648,260 and 5,624,821; and WO94/29351).

[0050] In some embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues. In some *embodiments*, the substituted residues occur at accessible sites of the antibody. Reactive thiol groups can be positioned at sites for conjugation to other moieties, such as drug moieties or linker drug moieties, to create an immunoconjugate. In some embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region.

[0051] In some embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known and available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(*n* vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or

unbranched. The number of polymers attached to the antibody may vary, and if two or more polymers are attached, they can be the same or different molecules.

[0052] This disclosure also provides for immunoconjugates comprising an anti-integrin antibodies described herein. An immunoconjugate is an antibody conjugated to one or more heterologous molecule(s). For example, an immunoconjugate can comprise an anti-integrin antibodies conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, protein domains, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes. In some embodiments, an immunoconjugate can comprise an anti-integrin antibodies, or fragment thereof (e.g., an scFv).

Specific Antibodies Described Herein

[0053] In certain embodiments, the antibodies and fragments thereof, described herein, are altered from a parental antibody to reduce the hydrophobic surface area of the variable region or one or more CDR regions. This reduction is reflected in a reduced residence time by hydrophobic interaction chromatography (HIC). Preferably, these modifications reduce HIC residence time and preserve or increase binding to human integrin alpha 11 when compared to the parental antibody. The parental antibody comprises CDRs listed below in **Table 1**:

Table 1				
SEQ ID NO:	CDR	Sequence	SEQ ID NO:	Alterable Sequence
195	CDR-H1	GFNLYSSYIH	201	GFNLXSSYIH
196	CDR-H2	SISSYSSYTYYADSVKG	202	SISSXSSXTYYADSVKG
197	CDR-H3	YWYYSAWALDY	203	XXXXSAWALDY
198	CDR-L1	VSSAVA	204	VSSAVA
199	CDR-L2	SASSLY	205	SXXXLX
200	CDR-L3	QQSAPYSSLIT	206	QQSAPXSSLIT
For SEQ ID NOs:201-206 residues given with an X are alterable from the parental sequence given in SEQ ID NOs 195 to 200.				

With regard to the integrin antibodies described herein, any of the CDR residues listed in **Table 1**

can be altered to reduce the hydrophobic surface area of a structural model of a parental antibody comprising one or more of these CDRs. In certain embodiments, the structural model comprises only the CDRs of the parental antibody, but in other embodiments the structural model can be based upon the amino acid sequence of the full parental variable regions given by SEQ ID NO:1 (V_H) and SEQ ID NO: 98 (V_L). In this embodiment, the constant regions are unimportant to determine the structure and residues that can be modified. In certain embodiments, only heavy chain CDRs are altered. In certain embodiments, any one or more of A51, S52, S53, Y55, and Y94 on the light chain (SEQ ID NO: 2; and Y30, Y54, Y57, W100, Y101, Y102 on the heavy chain (SEQ ID NO: 1) can be altered and replaced with a polar and charged amino acid, such as, Histidine, Asparagine, Serine, Threonine, Glutamine, or Glycine. In certain embodiments, Y102 (SEQ ID NO: 1) on the heavy chain can be altered and replaced with a polar and charged amino acid, such as, Histidine, Asparagine, Serine, Threonine, Glutamine, or Glycine. In certain embodiments, any one or more of S52, S53, or Y55 are not altered. In certain embodiments, any one or more of the residues denoted by an X in **Table 1** is Histidine, Asparagine, Serine, Threonine, Glutamine, or Glycine, while the remaining residues denoted by an X are identical to the parental. In certain embodiments, any two or more of the residues denoted by an X in **Table 1** is Histidine, Asparagine, Serine, Threonine, Glutamine, or Glycine, while the remaining residues denoted by an X are identical to the parental. In certain embodiments, any three or more of the residues denoted by an X in **Table 1** is Histidine, Asparagine, Serine, Threonine, Glutamine, or Glycine, while the remaining residues denoted by an X are identical to the parental.

[0054] Described herein are antibodies and antigen binding fragments thereof useful in human therapeutic and diagnostic applications. In certain embodiments, the antibody or antigen binding fragment thereof is recombinant. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 80% identical to that set forth in any one of SEQ ID NOs: 1 to 97, and a light chain variable region comprising an amino acid sequence at least 80% identical to that set forth in any one of SEQ ID NOs: 98 to 194, wherein the antibody binds an integrin polypeptide.

[0055] In certain embodiments, the antibody or antigen binding fragment thereof is recombinant. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 90% identical to that set

forth in in any one of SEQ ID NOs: 1 to 97, and a light chain variable region comprising an amino acid sequence at least 90% identical to that set forth in any one of SEQ ID NOs: 98 to 194, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 95% identical to that set forth in in any one of SEQ ID NOs: 1 to 97, and a light chain variable region comprising an amino acid sequence at least 95% identical to that set forth in any one of SEQ ID NOs: 98 to 194, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 98% identical to that set forth in in any one of SEQ ID NOs: 1 to 97, and a light chain variable region comprising an amino acid sequence at least 98% identical to that set forth in any one of SEQ ID NOs: 98 to 194, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence identical to that set forth in in any one of SEQ ID NOs: 1 to 97, and a light chain variable region comprising an amino acid sequence identical to that set forth in any one of SEQ ID NOs: 98 to 194, wherein the antibody binds an integrin polypeptide.

[0056] In certain embodiments, the antibody or antigen binding fragment thereof is recombinant. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 80% identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence at least 80% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 90% identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence at least 90% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an

amino acid sequence at least 95% identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence at least 95% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 98% identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence at least 98% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide.

[0057] In certain embodiments, the antibody or antigen binding fragment thereof is recombinant. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in in any one of SEQ ID NO: 43 and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in SEQ ID NO: 140, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in in any one of SEQ ID NO: 58 and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in SEQ ID NO: 155, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in in any one of SEQ ID NO: 59 and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in SEQ ID NO: 156, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 80%, at least

90%, or at least 95% identical to that set forth in in any one of SEQ ID NO: 60 and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in SEQ ID NO: 157, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in in any one of SEQ ID NO: 61 and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, or at least 95% identical to that set forth in SEQ ID NO: 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 90% identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence at least 90% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 95% identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence at least 95% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 98% identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence at least 98% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence identical to that set forth in in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, and a light chain variable region comprising an amino acid sequence identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the antibody binds an integrin polypeptide. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11.

[0058] Described herein are antibodies and antigen binding fragments thereof useful in human

therapeutic and diagnostic applications. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment is recombinant. In certain embodiments, the antibody comprises an antibody or antigen binding fragment thereof comprising a heavy chain CDR1 (CDR-H1) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a heavy chain CDR2 (CDR-H2) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a heavy chain CDR3 (CDR-H3) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a light chain CDR1 (CDR-L1) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, a light chain CDR2 (CDR-L2) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, and a light chain CDR3 (CDR-L3) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194.

[0059] Described herein are antibodies and antigen binding fragments thereof useful in human therapeutic and diagnostic applications. In certain embodiments, the antibody or antigen binding fragment thereof binds to human integrin alpha 11. In certain embodiments, the antibody or antigen binding fragment is recombinant. In certain embodiments, the antibody comprises an antibody or antigen binding fragment thereof comprising a heavy chain CDR1 (CDR-H1) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, a heavy chain CDR2 (CDR-H2) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, a heavy chain CDR3 (CDR-H3) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60 or 61, a light chain CDR1 (CDR-L1) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, a light chain CDR2 (CDR-L2) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, and a light chain CDR3 (CDR-L3) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158.

Therapeutic indications

[0060] In certain embodiments, the antibodies disclosed herein, are useful for treating tumors and cancers that express integrin alpha 11. In certain embodiments, an individual treated with the antibodies of this disclosure has been selected for treatment as having a integrin alpha 11 positive tumor/cancer. In certain embodiments, the cancer is lung cancer. In certain embodiments, the tumor is integrin alpha 11 positive or produces elevated levels of integrin alpha 11. In certain embodiments, integrin alpha 11 positivity is determined in comparison to a reference value or one or

more set pathological criteria. In certain embodiments, a integrin alpha 11 positive tumor expresses greater than 2-fold, 3- fold, 5-fold, 10-fold, 100-fold or more integrin alpha 11 than a non-transformed cell from which the tumor is derived. In certain embodiments, the tumor has acquired ectopic expression of integrin alpha 11. In certain embodiments, the tumor is a solid tumor. An integrin alpha 11 positive tumor can be determined histologically using, for example, immunohistochemistry with an anti- integrin alpha 11 antibody; or by commonly used molecular biology methods such as, for example, mRNA quantitation by real-time PCR or RNA-seq; or protein quantitation, for example, by western blot, flow cytometry, ELISA, or a homogenous protein quantitation assays (e.g., alphaLISA). In certain embodiments, the antibodies can be used to treat patients diagnosed with cancer. In certain embodiments, the cancer comprises one or more cancer stem cells or is one or more cancer stem cells.

[0061] In certain embodiments, disclosed herein, are antibodies useful for the treatment of a cancer or tumor. In certain embodiments, the cancer comprises breast, heart, lung, small intestine, colon, spleen, kidney, bladder, head, neck, ovarian, prostate, brain, pancreatic, skin, bone, bone marrow, blood, thymus, uterine, testicular and liver tumors. In certain embodiments, tumors which can be treated with the antibodies of the invention comprise solid tumors. In certain embodiments, tumors which can be treated with the antibodies of the invention comprise adenoma, adenocarcinoma, angiosarcoma, astrocytoma, epithelial carcinoma, germinoma, glioblastoma, glioma, hemangioendothelioma, hemangiosarcoma, hematoma, hepatoblastoma, leukemia, lymphoma, medulloblastoma, melanoma, neuroblastoma, osteosarcoma, retinoblastoma, rhabdomyosarcoma, sarcoma and/or teratoma. In certain embodiments, the tumor/cancer is selected from the group of acral lentiginous melanoma, actinic keratosis, adenocarcinoma, adenoid cystic carcinoma, adenomas, adenosarcoma, adenosquamous carcinoma, astrocytic tumors, Bartholin gland carcinoma, basal cell carcinoma, bronchial gland carcinoma, capillary carcinoid, carcinoma, carcinosarcoma, cholangiocarcinoma, chondrosarcoma, cystadenoma, endodermal sinus tumor, endometrial hyperplasia, endometrial stromal sarcoma, endometrioid adenocarcinoma, ependymal sarcoma, Swing's sarcoma, focal nodular hyperplasia, gastrinoma, germ line tumors, glioblastoma, glucagonoma, hemangioblastoma, hemangioendothelioma, hemangioma, hepatic adenoma, hepatic adenomatosis, hepatocellular carcinoma, insulinitis, intraepithelial neoplasia, intraepithelial squamous cell neoplasia, invasive squamous cell carcinoma, large cell carcinoma, liposarcoma, lung carcinoma, lymphoblastic leukemia, lymphocytic leukemia, leiomyosarcoma, melanoma, malignant melanoma, malignant mesothelial tumor, nerve sheath tumor, medulloblastoma, medulloepithelioma, mesothelioma, mucoepidermoid carcinoma, myeloid leukemia, neuroblastoma, neuroepithelial

adenocarcinoma, nodular melanoma, osteosarcoma, ovarian carcinoma, papillary serous adenocarcinoma, pituitary tumors, plasmacytoma, pseudosarcoma, prostate carcinoma, pulmonary blastoma, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, sarcoma, serous carcinoma, squamous cell carcinoma, small cell carcinoma, soft tissue carcinoma, somatostatin secreting tumor, squamous carcinoma, squamous cell carcinoma, undifferentiated carcinoma, uveal melanoma, verrucous carcinoma, vagina/vulva carcinoma, VIPoma, and Wilm's tumor. In certain embodiments, the tumor/cancer to be treated with one or more antibodies of the invention comprise brain cancer, head and neck cancer, colorectal carcinoma, acute myeloid leukemia, pre-B-cell acute lymphoblastic leukemia, bladder cancer, astrocytoma, preferably grade II, III or IV astrocytoma, glioblastoma, glioblastoma multiforme, small cell cancer, and non-small cell cancer, preferably non-small cell lung cancer, lung adenocarcinoma, metastatic melanoma, androgen-independent metastatic prostate cancer, androgen-dependent metastatic prostate cancer, prostate adenocarcinoma, and breast cancer, preferably breast ductal cancer, and/or breast carcinoma. In certain embodiments, the cancer treated with the antibodies of this disclosure comprises glioblastoma. In certain embodiments, the cancer treated with one or more antibodies of this disclosure comprises pancreatic cancer. In certain embodiments, the cancer treated with one or more antibodies of this disclosure comprises ovarian cancer. In certain embodiments, the cancer treated with one or more antibodies of this disclosure comprises or lung cancer.

Therapeutic methods

[0062] In certain embodiments, the antibodies can be administered by any route suitable for the administration of antibody-containing pharmaceutical compositions, such as, for example, subcutaneous, intraperitoneal, intravenous, intramuscular, intratumoral, or intracerebral, etc. In certain embodiments, the antibodies are administered intravenously. In certain embodiments, the antibodies are administered on a suitable dosage schedule, for example, weekly, twice weekly, monthly, twice monthly, etc. The antibodies can be administered in any therapeutically effective amount. In certain embodiments, the therapeutically acceptable amount is between about 0.1 mg/kg and about 50 mg/kg. In certain embodiments, the therapeutically acceptable amount is between about 1 mg/kg and about 40 mg/kg. In certain embodiments, the therapeutically acceptable amount is between about 5 mg/kg and about 30 mg/kg.

Additional therapeutic agents

[0063] In certain embodiments, the antibodies can be administered with or during treatment with an additional therapeutic agent. In certain embodiments, the therapeutic agent comprises a recombinant protein or monoclonal antibody. In certain embodiments, the recombinant protein or monoclonal antibody comprises Etaracizumab (Abegrin), Tacatuzumab tetraxetan, Bevacizumab (Avastin), Labetuzumab, Cetuximab (Erbix), Obinutuzumab (Gazyva), Trastuzumab (Herceptin), Clivatuzumab, Trastuzumab emtansine (Kadcyla), Ramucirumab, Rituximab (MabThera, Rituxan), Gemtuzumab ozogamicin (Mylotarg), Pertuzumab (Omnitarg), Girentuximab (Rencarex), or Nimotuzumab (Theracim, Theraloc). In certain embodiments, the monoclonal antibody comprises an immunomodulatory that targets a checkpoint inhibitor, for example PD-1 or CTLA-4. In certain embodiments, the immunomodulator comprises Nivolumab, Ipilimumab, Atezolizumab, or Pembrolizumab. In certain embodiments, the additional therapeutic agent is a chemotherapeutic agent. In certain embodiments, the chemotherapeutic agent is an alkylating agent (e.g., cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, or temozolomide), an anthracycline (e.g., doxorubicin, adriamycin, daunorubicin, epirubicin, or mitoxantrone), a cytoskeletal disruptor (e.g., paclitaxel or docetaxel), a histone deacetylase inhibitor (e.g., vorinostat or romidepsin), an inhibitor of topoisomerase (e.g., irinotecan, topotecan, amsacrine, etoposide, or teniposide), a kinase inhibitor (e.g., bortezomib, erlotinib, gefitinib, imatinib, vemurafenib, or vismodegib), a nucleoside analog or precursor analog (e.g., azacitidine, azathioprine, capecitabine, cytarabine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, or thioguanine), a peptide antibiotic (e.g., actinomycin or bleomycin), a platinum-based agent (e.g., cisplatin, oxaloplatin, or carboplatin), or a plant alkaloid (e.g., vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, or docetaxel). In some embodiments, the chemotherapeutic agent is a nucleoside analog. In some embodiments, the chemotherapeutic agent is gemcitabine. In certain embodiments, the additional therapeutic agent is radiation therapy.

Pharmaceutically acceptable excipients, carriers, and diluents

[0064] In certain embodiments, the antibodies of the current disclosure are administered suspended in a sterile solution. In certain embodiments, the solution comprises a physiologically appropriate salt concentration (e.g., NaCl). In certain embodiments, the solution comprises between about 0.6% and 1.2% NaCl. In certain embodiments, the solution comprises between about 0.7% and 1.1% NaCl. In certain embodiments, the solution comprises between about 0.8% and 1.0% NaCl. In

certain embodiments, a highly concentrated stock solution of antibody may be diluted in about 0.9% NaCl. In certain embodiments, the solution comprises about 0.9% NaCl. In certain embodiments, the solution further comprises one or more of: buffers, for example, acetate, citrate, histidine, succinate, phosphate, bicarbonate and hydroxymethylaminomethane (Tris); surfactants, for example, polysorbate 80 (Tween 80), polysorbate 20 (Tween 20), polysorbate and poloxamer 188; polyol/disaccharide/polysaccharides, for example, glucose, dextrose, mannose, mannitol, sorbitol, sucrose, trehalose, and dextran 40; amino acids, for example, histidine, glycine or arginine; antioxidants, for example, ascorbic acid, methionine; and chelating agents, for example, EGTA or EGTA. In certain embodiments, the antibodies of the current disclosure are shipped/stored lyophilized and reconstituted before administration. In certain embodiments, lyophilized antibody formulations comprise a bulking agent such as, mannitol, sorbitol, sucrose, trehalose, and dextran 40. In a certain embodiment, anti-integrin antibodies of this disclosure can be shipped and stored as a concentrated stock solution to be diluted at the treatment site of use.

Methods and Systems of Antibody Optimization

[0065] Disclosed herein, are methods of determining and optimizing production properties of antibodies such as hydrophobicity (e.g., tendency to aggregate). The method is based on homology modeling of an antibody's variable region or one or more CDRs. The advantage of this approach is that a crystal structure is not needed. In some embodiments, the method is performed on an antibody for which no crystal structure data is available. An antibody modeler constructs the model from an antibody with a known structure that is highly identical to the antibody to be optimized. In certain cases the percent identity of the V_H , V_L or at least one CDR modeled is equal to or greater than about 80%, 85%, 90%, or 95%. In certain embodiments, the constant region is disregarded for modeling purposes. In certain embodiments, there are no charged patches.

[0066] The production property optimized can be determined through a proxy measurement, such as a hydrophobic surface area of a plurality of amino acid residues of a V_H , V_L , or CDR. In certain embodiments, the plurality of amino acid residues is greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In certain embodiments, the plurality of amino acid residues is less than 40, 30, 25, 20, 15, or 10 residues. In certain embodiments, the plurality of amino acid residues are contiguous. Generally, a lower hydrophobic surface area is desirable. Thus, in certain embodiments, the method described herein reduces the hydrophobic surface area of a plurality of V_H or V_L amino acid residues (as measured in square Angstroms) by at least 20%, 30%,

40%, 50%, 60% or more. In certain embodiments, the method described herein reduces the hydrophobic surface area of a plurality of CDR amino acid residues (as measured in square Angstroms) by at least 20%, 30%, 40%, 50%, 60% or more. In certain embodiments, optimization of hydrophobic surface area does not reduce antibody binding to a target antigen.

[0067] Disclosed herein are methods of optimizing an amino acid sequence of an antibody for industrial scale production. In certain embodiments, the optimized antibody is a surface hydrophobicity minimized antibody. In certain embodiments, the method comprises: (a) constructing or receiving a model of a parental antibody that binds to a target; (b) determining the hydrophobic surface area of a plurality of amino acid residues of the parental antibody; (b) altering one or more of the plurality of amino acid residues of the parental antibody such that the hydrophobic surface area of the plurality of residues is reduced in a corresponding hydrophobic surface area minimized antibody. In certain embodiments, the method further comprises a step of functionally testing the affinity or functionality of the surface hydrophobicity minimized antibody. In certain embodiments, an affinity or functionality of the surface hydrophobicity minimized antibody is compared to an affinity or functionality of the parental antibody. In certain embodiments, the model is a homology model. In certain embodiments, the model is an X-ray crystal structure, an NMR structure, or a cryoelectron structure. In certain embodiments, the one or more amino acids that is altered is in a CDR region. In certain embodiments, the one or more amino acids that is altered is in a heavy chain CDR region. In certain embodiments, the one or more amino acids that is altered is a hydrophobic residue selected from Phenylalanine, Leucine, Alanine, Methionine, Isoleucine, Tryptophan, Valine or proline. In certain embodiments, the hydrophobic residue is altered to a Histidine, Asparagine, Serine, Threonine, Glutamine, or Glycine. In certain embodiments, one or more steps of the method is carried out by a computer system.

[0068] Additionally disclosed herein are methods of identifying CDR residues to alter in order to optimize industrial scale production. In certain embodiments, the optimized antibody is a surface hydrophobicity minimized antibody. In certain embodiments, the method comprises: (a) constructing or receiving a model of a parental antibody that binds to a target; (b) determining the hydrophobic surface area of a plurality of amino acid residues of the parental antibody; (c) altering one or more of the plurality of amino acid residues of the parental antibody such that the hydrophobic surface area of the plurality of residues is reduced in a corresponding hydrophobic surface area minimized antibody. In certain embodiments, the method further comprises a step of functionally testing the affinity or functionality of the surface hydrophobicity minimized antibody. In certain embodiments, an affinity or functionality of the surface hydrophobicity minimized antibody is compared to an

affinity or functionality of the parental antibody. In certain embodiments, the model is a homology model. In certain embodiments, the model is an X-ray crystal structure, an NMR structure, or a cryoelectron structure. In certain embodiments, the one or more amino acids that is altered is in a CDR region. In certain embodiments, the one or more amino acids that is altered is in a heavy chain CDR region. In certain embodiments, the one or more amino acids that is altered is a hydrophobic residue selected from Phenylalanine, Leucine, Alanine, Methionine, Isoleucine, Tryptophan, Valine or proline. In certain embodiments, the hydrophobic residue is altered to a Histidine, Asparagine, Serine, Threonine, Glutamine, or Glycine. In certain embodiments, one or more steps of the method is carried out by a computer system.

[0069] In certain aspects described herein is a computer-implemented system comprising: a computer comprising: at least one processor, a memory, an operating system configured to perform executable instructions, and a computer program including instructions executable by the at least one processor to create a hydrophobicity minimized antibody application, the application configured to perform the following: (a) construct or receive a model of a parental antibody that binds to a target; (b) determine a hydrophobic surface area of a plurality of amino acid residues of the parental antibody; and (c) return one or more alterations to the plurality of amino acid residues of the parental antibody such that the hydrophobic surface area of the plurality of residues is reduced in a corresponding hydrophobic surface area minimized antibody. In certain embodiments, the model is a homology model. In certain embodiments, the model is an X-ray crystal structure, an NMR structure, or a cryoelectron structure. In certain embodiments, the one or more amino acids that is altered is in a CDR region. In certain embodiments, the one or more amino acids that is altered is in a heavy chain CDR region. In certain embodiments, the one or more amino acids that is altered is a hydrophobic residue selected from Phenylalanine, Leucine, Alanine, Methionine, Isoleucine, Tryptophan, Valine or proline. In certain embodiments, the hydrophobic residue is altered to a Histidine, Asparagine, Serine, Threonine, Glutamine, or Glycine. In certain embodiments, the application returns a list of amino acid residues to be altered or specific hypothetical alterations ranked in order of alterations that reduce hydrophobic surface area to the greatest extent

[0070] The systems, and methods disclosed herein, in some cases, include a digital processing device, or use of the same. The digital processing device includes one or more hardware central processing units (CPUs) or general purpose graphics processing units (GPGPUs) that carry out the device's functions. The digital processing device further comprises an operating system configured to perform executable instructions. In some cases, the digital processing device is optionally connected to a computer network. In further cases, the digital processing device is optionally connected

to the Internet such that it accesses the World Wide Web. In some cases, the digital processing device is optionally connected to a cloud computing infrastructure. The digital processing device may be connected to an intranet and may be connected to a data storage device.

[0071] In accordance with the description herein, suitable digital processing devices include, by way of non-limiting examples, server computers, desktop computers, laptop computers, notebook computers, Internet appliances, tablet computers, and mobile smartphones. Those of skill in the art will recognize that many smartphones are suitable for use in the system described herein. Suitable tablet computers include those with booklet, slate, and convertible configurations, known to those of skill in the art.

[0072] The digital processing device includes an operating system configured to perform executable instructions. The operating system is, for example, software, including programs and data, which manages the device's hardware and provides services for execution of applications. Those of skill in the art will recognize that suitable server operating systems include, by way of non-limiting examples, FreeBSD, OpenBSD, NetBSD®, Linux, Apple® Mac OS X Server®, Oracle® Solaris®, Windows Server®, and Novell® NetWare®. Those of skill in the art will recognize that suitable personal computer operating systems include, by way of non-limiting examples, Microsoft® Windows®, Apple® Mac OS X®, UNIX®, and UNIX-like operating systems such as GNU/Linux®. In some embodiments, the operating system is provided by cloud computing. Those of skill in the art will also recognize that suitable mobile smart phone operating systems include, by way of non-limiting examples, Nokia® Symbian® OS, Apple® iOS®, Research In Motion® BlackBerry OS®, Google® Android®, Microsoft® Windows Phone® OS, Microsoft® Windows Mobile® OS, Linux®, and Palm® WebOS®.

[0073] The digital processing device includes a storage and/or memory device. The storage and/or memory device is one or more physical apparatuses used to store data or programs on a temporary or permanent basis. In some embodiments, the device is volatile memory and requires power to maintain stored information. In some embodiments, the device is non-volatile memory and retains stored information when the digital processing device is not powered. In further embodiments, the non-volatile memory comprises flash memory. In some embodiments, the non-volatile memory comprises dynamic random-access memory (DRAM). In some embodiments, the non-volatile memory comprises ferroelectric random access memory (FRAM). In some embodiments, the non-volatile memory comprises phase-change random access memory (PRAM). In other embodiments, the device is a storage device including, by way of non-limiting examples, CD-ROMs, DVDs, flash memory devices, magnetic disk drives, magnetic tapes drives, optical disk

drives, and cloud computing based storage. In further embodiments, the storage and/or memory device is a combination of devices such as those disclosed herein.

[0074] The digital processing device may include a display to send visual information to a user. In some embodiments, the display is a liquid crystal display (LCD). In further embodiments, the display is a thin film transistor liquid crystal display (TFT-LCD). In some embodiments, the display is an organic light emitting diode (OLED) display. In various further embodiments, on OLED display is a passive-matrix OLED (PMOLED) or active-matrix OLED (AMOLED) display. In some embodiments, the display is a plasma display. In other embodiments, the display is a video projector. In yet other embodiments, the display is a head-mounted display in communication with the digital processing device, such as a VR headset. In further embodiments, suitable VR headsets include, by way of non-limiting examples, HTC Vive, Oculus Rift, Samsung Gear VR, Microsoft HoloLens, Razer OSVR, FOVE VR, Zeiss VR One, Avegant Glyph, Freefly VR headset, and the like. In still further embodiments, the display is a combination of devices such as those disclosed herein.

[0075] The digital processing device may include an input device to receive information from a user. In some embodiments, the input device is a keyboard. In some embodiments, the input device is a pointing device including, by way of non-limiting examples, a mouse, trackball, track pad, joystick, game controller, or stylus. In some embodiments, the input device is a touch screen or a multi-touch screen. In other embodiments, the input device is a microphone to capture voice or other sound input. In other embodiments, the input device is a video camera or other sensor to capture motion or visual input. In further embodiments, the input device is a Kinect, Leap Motion, or the like. In still further embodiments, the input device is a combination of devices such as those disclosed herein.

[0076] The systems and methods disclosed herein, in some cases, include one or more non-transitory computer readable storage media encoded with a program including instructions executable by the operating system of an optionally networked digital processing device. In some cases, a computer readable storage medium is a tangible component of a digital processing device. In other cases, a computer readable storage medium is optionally removable from a digital processing device. In some embodiments, a computer readable storage medium includes, by way of non-limiting examples, CD-ROMs, DVDs, flash memory devices, solid state memory, magnetic disk drives, magnetic tape drives, optical disk drives, cloud computing systems and services, and the like. In some cases, the program and instructions are permanently, substantially permanently, semi-permanently, or non-transitorily encoded on the media.

[0077] The systems and methods disclosed herein, in some cases, include at least one computer

program, or use of the same. A computer program includes a sequence of instructions, executable in the digital processing device's CPU, written to perform a specified task. Computer readable instructions may be implemented as program modules, such as functions, objects, Application Programming Interfaces (APIs), data structures, and the like, that perform particular tasks or implement particular abstract data types. In light of the disclosure provided herein, those of skill in the art will recognize that a computer program may be written in various versions of various languages.

[0078] The functionality of the computer readable instructions may be combined or distributed as desired in various environments. In some embodiments, a computer program comprises one sequence of instructions. In some embodiments, a computer program comprises a plurality of sequences of instructions. In some embodiments, a computer program is provided from one location. In other embodiments, a computer program is provided from a plurality of locations. In various embodiments, a computer program includes one or more software modules. In various embodiments, a computer program includes, in part or in whole, one or more web applications, one or more mobile applications, one or more standalone applications, one or more web browser plug-ins, extensions, add-ins, or add-ons, or combinations thereof.

[0079] A computer program may include a web application. In light of the disclosure provided herein, those of skill in the art will recognize that a web application, in various embodiments, utilizes one or more software frameworks and one or more database systems. In some embodiments, a web application is created upon a software framework such as Microsoft® .NET or Ruby on Rails (RoR). In some embodiments, a web application utilizes one or more database systems including, by way of non-limiting examples, relational, non-relational, object oriented, associative, and XML database systems. In further embodiments, suitable relational database systems include, by way of non-limiting examples, Microsoft® SQL Server, MySQL™, and Oracle®. Those of skill in the art will also recognize that a web application, in various embodiments, is written in one or more versions of one or more languages. A web application may be written in one or more markup languages, presentation definition languages, client-side scripting languages, server-side coding languages, database query languages, or combinations thereof. In some embodiments, a web application is written to some extent in a markup language such as Hypertext Markup Language (HTML), Extensible Hypertext Markup Language (XHTML), or eXtensible Markup Language (XML). In some embodiments, a web application is written to some extent in a presentation definition language such as Cascading Style Sheets (CSS). In some embodiments, a web application is written to some extent in a client-side scripting language such as Asynchronous Javascript and XML (AJAX), Flash®

Actionscript, Javascript, or Silverlight®. In some embodiments, a web application is written to some extent in a server-side coding language such as Active Server Pages (ASP), ColdFusion®, Perl, Java™, JavaServer Pages (JSP), Hypertext Preprocessor (PHP), Python™, Ruby, Tcl, Smalltalk, WebDNA®, or Groovy. In some embodiments, a web application is written to some extent in a database query language such as Structured Query Language (SQL). In some embodiments, a web application integrates enterprise server products such as IBM® Lotus Domino®. In some embodiments, a web application includes a media player element. In various further embodiments, a media player element utilizes one or more of many suitable multimedia technologies including, by way of non-limiting examples, Adobe® Flash®, HTML 5, Apple® QuickTime®, Microsoft® Silverlight®, Java™, and Unity®.

EXAMPLES

[0080] The following illustrative examples are representative of embodiments of the compositions and methods described herein and are not meant to be limiting in any way.

Example 1 – Rational design of mutants for improved developability

[0081] The integrin $\alpha 11$ antibody NB0268 was identified by whole cell selections using an antibody phage library. The Fv region of NB0268 was modeled and its vdW surface was analyzed for hydrophobicity and electrostatics. This revealed hydrophobic regions on the CDRs and polar regions on the framework surface as highlighted in **FIG. 1**. Protein Patch Analysis revealed a number of regions of significant surface hydrophobicity, especially on the CDRs and a general lack of significant surface charged regions on the variable domain. When tested by HIC, NB0268 did not elute from the column during the 30 min method described herein. Thus, the high degree of CDR hydrophobicity identified by homology model analysis was confirmed empirically.

Phage Display

[0082] A Fab-phage from Library F was cycled through four rounds of binding selection using a parental CAF (cancer associated fibroblast) cell line as the background depleting step and an overexpressing CAF cell line as the target selection step. 10×10^6 cells were used for selections and were incubated for 2hrs at 4C in 1ml growth culture medium with a library of 3×10^{13} Fab-phage. Phage were eluted from cell-pellets using 0.1M hydrochloric acid, incubated for 10min at room temperature, and were subsequently neutralized with 11M Tris buffer. Cell debris was removed from phage eluate by high speed centrifugation.

Antibody homology model generation

[0083] A homology model of the variable region of the parental NB0268 mAb was created using the Antibody Modeler application in MOE 2016.0802. Using the method described by Maier & Labute. See Maier JKX, Labute P. Assessment of fully automated antibody homology modeling protocols in molecular operating environment. *Proteins*. 2014;82:1599–1610. A homology search was performed and 2R8S was used as the framework template for both chains. For the CDRs, 4KZE, 4XVU, 4XVU were used as templates for light chain CDR1, 2, and 3, respectively while 3PNW, 3S34, and 3AUV were used for the heavy chain CDR 1, 2, and 3, respectively. This chimeric template is 97 % identical to the parent for the V_L region and 90 % identical for V_H.

Example 2 – Rational design of mutants for improved developability

[0084] The binding and functional characteristics of integrin alpha 11 specific binding antibody NB0268 make it an attractive therapeutic candidate, but its significant hydrophobicity could lead to challenges in development, due to e.g. poor solubility and aggregation. Residues assumed to be contributing to CDR hydrophobicity (as determined by the homology model) were identified for site-directed mutagenesis. In total, 32 single mutations were generated by mutating A51, S52, S53, Y55, and Y94 on the light chain; and Y30, Y54, Y57, W100, Y101, Y102 on the heavy chain and replacing with mostly polar and charged amino acids, e.g. His, Asp, Ser, Thr, Gln, Gly (**FIG. 2** and **Table 1**).

Example 3-HIC and integrin binding results for the 97 variants tested

[0085] The 32 single mutants were analyzed for binding (FACS) and HIC RT (**Table 2**). The residues in CDR-H3 at the center of the largest hydrophobic patch were found to contribute significantly to the poor HIC RT of NB0268 as three out of five W100 mutants (His, Asp, Gln) eluted, all of the seven Y101 mutants eluted, and six out of seven Y102 mutants eluted. Mutations in the heavy chain positions Y30, Y54, Y57 did not improve HIC RT compared to the parent. The likely reason for this is that Y30 is on the edge of a smaller hydrophobic patch along with Y54, and Y57 is not part of a patch. Of note, most of the mutants showed maintained binding to integrin $\alpha 11$, with only 2/32 single mutants resulted in loss of binding.

[0086] The Y54S mutant increased the integrin $\alpha 11$ binding signal, therefore, hydrophobicity in this region is not a requirement for binding. Building from this, 57 Y54 double mutants were expressed, of which 24 were in positions Y30 x Y54 and 33 were in positions Y54 x Y102. For Y30

x Y54 double mutants, 16/57 variants (28%) eluted, representing a modest improvement after eliminating a relatively small hydrophobic patch. For the Y54 x Y102 double mutants, 29/34 variants (85%) eluted from the column, some with quite favorable elution times. This is exemplified by Y54H x Y102E with a RT of 21.2 min, which is the lowest of all variants tested, and the integrin $\alpha 11$ binding is maintained for this double variant. Also HC Tyrosine 102 seemed amendable to mutation as 35 of 41 were found to possess favorable characteristics. Overall, 62 of 86 variants made to reduce hydrophobic patch surface area were favorable.

[0087] On the light chain, the A51D and S53D variants eluted but with relatively high retention times (28.9 min and 29.1 min, respectively). The variant S52D did not elute, nor did Y55D. Furthermore, none of the Y94 mutants eluted. The light chain mutants either had relatively high HIC RT or did not elute, therefore the impact was small when mutating residues that were not part of a large hydrophobic patch and, overall, mutating residues that contribute to large surface hydrophobic patches was successful in reducing HIC retention times.

Binding Screens by Flow Cytometry

[0088] C2C12 and CAF cells were stably transfected to overexpress integrin $\alpha 11$ and their parental cell lines were used to establish background levels of expression (integrin $\alpha 11$ -negative). Briefly, cells were dissociated from flasks using TrypLE express reagent for 4min at 37C. Cells were resuspended in PBS + Ca/Mg and filtered to remove clumps before staining. Cells were then plated in 96 well V bottom plates at 300,000 c/ml and spun to remove any wash media. Antibodies were diluted to 100nM and added to the cells for 1hr at 4C. After 2x washes, secondary antibody was added to cells (Gt anti Hu-Fc Alexa 647, Jackson ImmunoResearch) and incubated for 30min at 4C. Cells were then washed 2x before being resuspended in 200uL FACS buffer for reading on Cytotflex (Beckman Coulter). Fold changes in MFI between C2C12 and CAF overexpressing cells relative to their parental MFI are listed in **Table 2**. Binding was considered to be 'lost' if it was below a 3-fold increase in signal on either C2C12 or CAF cells or considered to be 'increased' if it was greater than two times the parental fold shift on both C2C12 and CAF cells.

HPLC-HIC analyses

[0089] The HPLC-HIC analyses were performed with the Agilent 1290 Infinity LC system, including an Agilent 1290 Infinity Binary Pump, an Agilent 1290 Infinity Multisampler an Agilent 1290 Infinity, a Column Compartment, and an Agilent Infinity Diode Array Detector. To screen the mAbs hydrophobicity, 100 μ L media sample was injected onto a 7.5 $\times$ 75 mm, 10 μ m TSKgel Ether-

5PW from Tosoh equilibrated with 1.5M Ammonium sulfate and 0.1M Sodium Phosphate buffer, pH 6.5 (mobile phase A) at flow rate 1.0 mL/min. To elute the mAbs, a salt gradient was established from 100% mobile phase A (1.5M Ammonium sulfate and 0.1M Sodium Phosphate buffer, pH 6.5) to 100% mobile phase B (0.1M Sodium Phosphate buffer, pH 6.5) in 30 min. The column was re-equilibrated with mobile phase A for 10 min before the next injection.

Example 4-Structural protein property descriptor predictions of HIC RT

[0090] In order to further investigate the predictive ability of 3D protein properties for experimental hydrophobicity, we generated antibody homology models for all variants tested and calculated the total hydrophobic patch surface areas of the CDRs. We found that there is a trend where the higher the hydrophobic surface area of the CDRs, the higher the HIC RT as seen in **FIG. 3A**. More specifically, the higher the sum of the surface areas of hydrophobic patches, the higher the HIC RT, and if we only count the CDR regions and factor in the patch log P hydrophobic potential, the correlation increases. Finally, if a LowModeMDensemble is generated, and average descriptor values are obtained, the correlation increases. LowModeMD described in Labute P. LowModeMD—implicit low-mode velocity filtering applied to conformational search of macrocycles and protein loops. *J Chem Inf Model.* 2010;50:792–800. The best correlation observed for a single descriptor is avg_cdr_logp ($r^2 = 0.32$), which is the sum of the surface areas of hydrophobic patches at the CDRs multiplied by the square of the log P hydrophobicity of the patches averaged over a LowModeMD sample of conformations. In comparison, the Black & Mould sequence-based hydrophobic index (as described in Black SD, Mould DR. Development of hydrophobicity parameters to analyze proteins which bear post- or cotranslational modifications. *Anal Biochem.* 1991;193:72–82) has a Pearson correlation r^2 of 0.21 to the HIC RT as shown in **FIG. 3B**.

Property calculations on 3D models

[0091] Hydrophobic patches were identified with the Protein Patch Analyzer application in MOE 2016.0802. Patches consist of regions where a hydrophobic potential equal to or greater than that of a methyl group persists over surface area greater than 50 Å². The hydrophobic potential is determined using the SLogP method for each atom and mapping the result onto the surface. For averaged LowModeMD sample based patch surface area calculations, the framework backbone atoms were restrained to 0.25 Å of their modeled positions, the H3 loops were restrained to 2 Å and the other CDRs to 1 Å. Averaged patch surface area calculations were performed on 50 samples

for each structure using the Dynamic Property Sampling application within the Bio MOE extension of MOE 2016.0802 where the default Amber10:EHT forcefield and the reaction field solvation model was used. Other properties were calculated from the Protein Properties application in MOE 2016.0802 and the sequence-based hydrophobic index is calculated by a custom MOE implementation of the method. The protein QSPR calculations such as PLS fitting were performed with QSAR Model application in MOE 2016.0802 and the GA-MLR method within the AutoQSAR extension to MOE was used to identify PLS models.

Example 5-Protein QSPR model predictions of HIC retention times

[0092] In an effort to generate a more predictive model with a greater correlation to HIC RT, PLS regression QSPR models were generated and optimized for this system using a genetic algorithm to optimize multiple linear regression models (GA-MLR). This resulted in a 2-point QSPR ($r^2 = 0.47$, see Fig. 3B, and a leave-one-out cross validation correlation $x^2 = 0.44$) including avg_HI, which is the hydrophobic imbalance value as described by Salgado et al. (*J Chromatography A*. 2006;1107:110-119) and avg_hyd_1 which is the surface area of the largest hydrophobic patch averaged over the LowModeMD ensemble. The 2-point QSPR equation is shown below.

$$\text{HIC RT} = 4.29 + 3.94 * \text{avg_HI} + 0.05 * \text{avg_hyd_1}$$

(QSPR_2_Pred)

[0093] For the two measures of hydrophobicity, their coefficients are positive where the more hydrophobic, the higher the HIC RT, as one would expect from a biophysical perspective. Additional scatter matrix plots of descriptor correlations to each other and to experiments are available in the supplementary information.

[0094] The hydrophobic patch descriptors were generally able to assign high values to non-eluting antibodies and low values to fast-eluting variants, such as Y102D x Y54H (see **FIG. 4A**), and Y102E x Y54H (**FIG. 4B**). Twenty nine out of the 30 (97%) top scoring variants from the QSPR model had a reduced HIC RT compared to the parent, and many with binding affinity maintained. This model is useful for screening libraries of NB0268 mAb variants generated by experimental techniques, or by computational protein design for those with reduced HIC RT.

Example 6-Hydrophobicity descriptor HIC RT predictions for antibodies in clinical development

[0095] In order to test if the protein property descriptors calculated can be predictive for a broader range of antibodies, the 137 mAbs were modeled in clinical development with published

HIC RT measurements². Applying identical methods to model, sample, and calculate properties as used for the NB0268 variants, we find a similar trend where the more hydrophobic the CDR surface, the higher the HIC RT (see **FIG. 5A**). We found that the 3D descriptors were more predictive than the sequence based method, but that their correlations to experimental HIC RT are generally weaker compared to the NB0268 variants. This is to some extent expected since the 137 mAbs are clinical candidates and have likely been selected based on optimal solubility behavior. This is apparent in the HIC RT histogram at the top of **FIG. 5A**, compared to that of **FIG. 3A**. It is more difficult to rank order such a narrow range of data points for HIC RT, and the most predictive descriptor again was avg_cdr_logp, which is the sum of the surface areas of hydrophobic patches at the CDRs multiplied by the square of the log P hydrophobic potential of the patches averaged over a LowModeMD sample of conformations. **FIG 6A** shows correlation between experimental HIC and their comparisons to hydrophobic surface area (top middle), CDR hydrophobic surface area (bottom middle), and avg_CDR3_LogP (bottom) protein property descriptors. **FIG 6B** shows correlation between experimental HIC and the Black & Mould sequence based hydrophobic index (middle) and to the 2-point QSPR calculation derived from MOE 2016 (bottom).

Antibody homology model generation

[0096] For high-throughput antibody model generation of the datasets studied, the Bio MOE extension of MOE 2016.0802 was used to generate all models. For each, the best scoring Fv and CDR templates were used to build variable region models and these were grafted onto the Herceptin Fab structure 1N8Z and energy minimized with the Amber10:EHT forcefield in MOE 2016.0802 for Fab model completion. The first adnectin sequence was modeled with the Homology Model application in MOE 2016.0802 from the fibronectin template PDB 1FNF and the Automatic

[0097] A QSPR model was trained on this data using the GA-MLR method. This resulted in a 4-point QSPR ($r^2 = 0.41$, see **FIG. 5B**, and a leave-one-out cross validation correlation $x^2 = 0.36$) including ASPmax, which is the maximum average surface property value as described by Salgado et al.¹³, avg_cdr_logp (described previously), fvcharge which is the Fv regions's net charge and the pro_eccen, which stands for protein eccentricity, and is a measure of how spherical is the shape of the protein. The 4-point QSPR equation is shown below.

$$\text{HIC RT} = 9.34 + 8.36 * \text{ASPmax} + 0.10 * \text{avg_cdr_logp} + 2.77 * \text{fvcharge} - 20.77 * \text{pro_eccen} \quad (\text{QSPR_4_Pred})$$

[0098] For the two measures of hydrophobicity, their coefficients are positive where the more

hydrophobic, the higher the HIC RT, as one would expect from a biophysical perspective. This QSPR model, trained on diverse IgG1 candidates, can be applied to roughly predict HIC RT for future IgG1 mAbs and the predictive performance should be improved compared to single parameters alone.

Example 7-Performance of hydrophobicity descriptors on adnectin % IB prediction

[0099] There is an additional dataset available of 31 adnectin triple-mutant variant sequences, each having percent inclusion body data, a measure of insoluble protein aggregate formation. This data was modeled in similar fashion to the two previous examples and the % IB is correlated to the sequence-based Black & Mould hydrophobic index and also to the 3D hydrophobicity descriptors calculated (see Fig. 5C). The original publication reported that a sequence-based multi-parameter aggregation predictor, Zagg, achieved a correlation $r^2 = 0.61$ and this is similar to what we observe simply for sequence-based hydrophobic index. The avg_hyd is the hydrophobic patch surface area averaged over a LowModeMD generated ensemble of model conformations, and its correlation to % IB is $r^2 = 0.62$. In this case, a QSPR model was not trained because the single sequence and hydrophobic patch descriptors are adequately predictive.

[00100] Homology Model application within Bio MOE was used to generate the 31 adnectin variant models studied.

[00101] All patents, published applications, and papers disclosed herein are incorporated by reference in their entirety.

[00102] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

Sequence IDs described herein	
SEQ ID NO	Sequence
1	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSAWALDYWGQGTLVTVSS
2	EVQLVESGGGLVQPGGSLRLSCAASGFNLHSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSAWALDYWGQGTLVTVSS
3	EVQLVESGGGLVQPGGSLRLSCAASGFNLDSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSAWALDYWGQGTLVTVSS

4	EVQLVESGGGLVQPGGSLRLSCAASGFNLTSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
5	EVQLVESGGGLVQPGGSLRLSCAASGFNLQSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
6	EVQLVESGGGLVQPGGSLRLSCAASGFNLGSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
7	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSHSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
8	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSDSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
9	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSSSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
10	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSQSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
11	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSGSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
12	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSDTYYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
13	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSSTYYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
14	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSQTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
15	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYHYSSAWALDYWGQGTLVTVSS
16	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDYSSAWALDYWGQGTLVTVSS
17	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYSYSSAWALDYWGQGTLVTVSS
18	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYQYSSAWALDYWGQGTLVTVSS
19	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYGYSSAWALDYWGQGTLVTVSS
20	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWHYSSAWALDYWGQGTLVTVSS
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24	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWTYSSAWALDYWGQGTLVTVSS
25	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWQYSSAWALDYWGQGTLVTVSS
26	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWGYSAWALDYWGQGTLVTVSS

27	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYHSAWALDYWGQGTLVTVSS
28	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYDSAWALDYWGQGTLVTVSS
29	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYESAWALDYWGQGTLVTVSS
30	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
31	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYTSAWALDYWGQGTLVTVSS
32	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYQSAWALDYWGQGTLVTVSS
33	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYGS AWALDYWGQGTLVTVSS
34	EVQLVESGGGLVQPGGSLRLSCAASGFNLHSSYIHWVRQAPGKGLEWVASISSHSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
35	EVQLVESGGGLVQPGGSLRLSCAASGFNLHSSYIHWVRQAPGKGLEWVASISSDSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
36	EVQLVESGGGLVQPGGSLRLSCAASGFNLHSSYIHWVRQAPGKGLEWVASISSQSSYTYAD SVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
37	EVQLVESGGGLVQPGGSLRLSCAASGFNLHSSYIHWVRQAPGKGLEWVASISSGSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
38	EVQLVESGGGLVQPGGSLRLSCAASGFNLDSSYIHWVRQAPGKGLEWVASISSHSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
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41	EVQLVESGGGLVQPGGSLRLSCAASGFNLDSSYIHWVRQAPGKGLEWVASISSQSSYTYAD SVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
42	EVQLVESGGGLVQPGGSLRLSCAASGFNLDSSYIHWVRQAPGKGLEWVASISSGSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
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45	EVQLVESGGGLVQPGGSLRLSCAASGFNLTSSYIHWVRQAPGKGLEWVASISSSSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
46	EVQLVESGGGLVQPGGSLRLSCAASGFNLTSSYIHWVRQAPGKGLEWVASISSQSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
47	EVQLVESGGGLVQPGGSLRLSCAASGFNLTSSYIHWVRQAPGKGLEWVASISSGSSYTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
48	EVQLVESGGGLVQPGGSLRLSCAASGFNLQSSYIHWVRQAPGKGLEWVASISSHSSYTYAD SVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
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71	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYHSAWALDYWGQGTLTVSS
72	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYD SAWALDYWGQGTLTVSS

73	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSSSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYESAWALDYWGQGTLVTVSS
74	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSSSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
75	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSSSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYTSAWALDYWGQGTLVTVSS
76	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSSSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYQSAWALDYWGQGTLVTVSS
77	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSSSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYGSAWALDYWGQGTLVTVSS
78	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSQSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYHSAWALDYWGQGTLVTVSS
79	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSQSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYDSAWALDYWGQGTLVTVSS
80	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSQSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
81	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSQSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYTSAWALDYWGQGTLVTVSS
82	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSQSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYQSAWALDYWGQGTLVTVSS
83	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSQSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYGSAWALDYWGQGTLVTVSS
84	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSGSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYHSAWALDYWGQGTLVTVSS
85	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSGSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYDSAWALDYWGQGTLVTVSS
86	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSGSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYESAWALDYWGQGTLVTVSS
87	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSGSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
88	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSGSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYTSAWALDYWGQGTLVTVSS
89	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSGSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYQSAWALDYWGQGTLVTVSS
90	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSGSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYGSAWALDYWGQGTLVTVSS
91	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
92	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
93	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
94	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS
95	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSSAWALDYWGQGTLVTVSS

96	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSAWALDYWGQGTLLTVSS
97	EVQLVESGGGLVQPGGSLRLSCAASGFNLYSSYIHWVRQAPGKGLEWVASISSYSSYTTYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSAWALDYWGQGTLLTVSS
98	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
99	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
100	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
101	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
102	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
103	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
104	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
105	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
106	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
107	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
108	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
109	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
110	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
111	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
112	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
113	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
114	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
115	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
116	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
117	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
118	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK

188	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSDSSLYSGVPSRFSG SRSGTDFTLTISSLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
189	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSADSLYSGVPSRFS GSRSGTDFTLTISSLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
190	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASDLYSGVPSRFS GSRSGTDFTLTISSLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
191	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLDSGVPSRFS GSRSGTDFTLTISSLQPEDFATYYCQQSAPYSSLITFGQGGTKVEIK
192	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGTDFTLTISSLQPEDFATYYCQQSAPHSSLITFGQGGTKVEIK
193	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGTDFTLTISSLQPEDFATYYCQQSAPSSSLITFGQGGTKVEIK
194	DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSG SRSGTDFTLTISSLQPEDFATYYCQQSAPTSSLITFGQGGTKVEIK
195	GFNLYSSYIH
196	SISSYSSYTYADSVKG
197	YWYYSAWALDY
198	VSSAVA
199	SASSLY
200	QQSAPYSSLIT

Table 2.							
	Chain	Mutation	HIC Retention Time (min)	C2C12 Human Int a11 vs. C2C12 < 3-fold	C2C12 Ma11 vs. C2C12 < 1.5-fold	CAF a11 vs. CAF < 1.5-fold	Binding Assessment
NB0268		Parent	DNE				Maintained
NB0272	VH	Y30H	DNE	6.4	1.8	6.8	Maintained
NB0273	VH	Y30D	DNE	5.3	1.7	8.1	Maintained
NB0274	VH	Y30T	26.98	8.3	2.0	9.1	Maintained
NB0275	VH	Y30Q	DNE	7.0	1.9	7.3	Maintained
NB0276	VH	Y30G	DNE	9.2	2.1	9.1	Maintained
NB0277	VH	Y54H	28.05	8.7	2.0	8.4	Maintained
NB0278	VH	Y54D	DNE	10.0	1.7	8.7	Maintained
NB0279	VH	Y54S	DNE	44.5	7.5	16.5	Increased
NB0280	VH	Y54Q	DNE	8.5	1.9	8.3	Maintained
NB0281	VH	Y54G	28.45 (Low recovery)	7.7	1.6	8.9	Maintained
NB0282	VH	Y57D	DNE	7.2	1.6	8.5	Maintained
NB0283	VH	Y57S	DNE	13.3	2.4	7.6	Maintained
NB0284	VH	Y57Q	DNE	6.5	1.6	7.2	Maintained
NB0285	VH	W100H	25.87	7.1	2.1	8.2	Maintained
NB0286	VH	W100D	29.34	8.7	2.0	8.8	Maintained
NB0287	VH	W100S	DNE	6.4	2.1	8.2	Maintained
NB0288	VH	W100Q	26.31	7.9	1.9	8.9	Maintained
NB0289	VH	W100G	DNE	7.9	1.9	8.8	Maintained
NB0290	VH	Y101H	24.36	5.9	1.5	8.4	Maintained
NB0291	VH	Y101D	26.30	1.2	1.0	0.9	Loss
NB0292	VH	Y101E	26.98	1.5	1.0	1.2	Loss
NB0293	VH	Y101S	26.36	5.0	1.1	9.2	Maintained
NB0294	VH	Y101T	26.64	3.6	1.0	6.8	Maintained
NB0295	VH	Y101Q	26.11	5.5	1.1	9.7	Maintained
NB0296	VH	Y101G	26.53	4.0	0.1	5.6	Maintained
NB0297	VH	Y102H	27.59	7.6	2.1	9.0	Maintained
NB0298	VH	Y102D	26.58	10.1	1.8	12.1	Maintained
NB0299	VH	Y102E	26.14	10.2	2.0	11.2	Maintained
NB0300	VH	Y102S	26.56	9.5	2.8	5.5	Maintained
NB0301	VH	Y102T	27.75	6.3	2.0	4.3	Maintained
NB0302	VH	Y102Q	28.30	8.5	2.7	5.7	Maintained
NB0303	VH	Y102G	DNE	8.5	2.8	5.8	Maintained
NB0304	VH	Y30H Y54H	26.45	8.7	2.9	6.5	Maintained
NB0305	VH	Y30H Y54D	28.62	10.9	3.3	6.8	Maintained
NB0307	VH	Y30H Y54Q	28.54	10.4	3.2	6.8	Maintained
NB0308	VH	Y30H Y54G	29.11	11.3	3.4	7.3	Maintained
NB0309	VH	Y30D Y54H	28.28	9.9	3.2	7.7	Maintained
NB0310	VH	Y30D Y54D	29.10	10.6	2.0	9.9	Maintained
NB0311	VH	Y30D Y54S	DNE	9.7	3.0	8.6	Maintained
NB0312	VH	Y30D Y54Q	DNE	13.1	3.6	9.2	Maintained
NB0313	VH	Y30D Y54G	DNE	12.0	3.1	9.5	Maintained
NB0314	VH	Y30T Y54H	22.64	9.2	3.0	9.2	Maintained
NB0315	VH	Y30T Y54D	24.42	15.5	3.3	11.0	Maintained
NB0316	VH	Y30T Y54S	24.77	19.8	5.2	8.8	Maintained
NB0317	VH	Y30T Y54Q	24.17	19.9	5.4	7.0	Maintained
NB0318	VH	Y30T Y54G	24.12	19.4	4.6	7.5	Maintained
NB0319	VH	Y30Q Y54H	28.27	13.6	4.2	6.5	Maintained
NB0320	VH	Y30Q Y54D	DNE	12.4	3.4	7.3	Maintained
NB0321	VH	Y30Q Y54S	DNE	10.0	3.3	7.2	Maintained
NB0322	VH	Y30Q Y54Q	DNE	13.1	4.0	7.6	Maintained
NB0323	VH	Y30Q Y54G	DNE	14.4	4.0	7.4	Maintained
NB0324	VH	Y30G Y54H	25.96	12.1	3.8	4.5	Maintained
NB0325	VH	Y30G Y54D	27.92	17.2	3.4	8.6	Maintained
NB0326	VH	Y30G Y54S	DNE	15.2	4.3	6.7	Maintained
NB0327	VH	Y30G Y54Q	27.99	14.7	4.3	6.5	Maintained
NB0328	VH	Y30G Y54G	28.44	7.7	1.7	3.9	Maintained

NB0329	VH	Y54H Y102H	21.91	4.9	1.2	3.8	Maintained
NB0330	VH	Y54H Y102D	21.62	4.6	0.8	4.5	Maintained
NB0331	VH	Y54H Y102E	21.27	5.1	1.0	4.2	Maintained
NB0332	VH	Y54H Y102S	21.50	5.0	1.0	4.5	Maintained
NB0333	VH	Y54H Y102T	22.64	3.8	0.8	4.0	Maintained
NB0334	VH	Y54H Y102Q	22.65	6.5	1.5	3.9	Maintained
NB0335	VH	Y54H Y102G	25.14	8.6	1.7	4.0	Maintained
NB0336	VH	Y54D Y102H	24.81	8.4	1.0	2.9	Maintained
NB0337	VH	Y54D Y102D	DNE	1.7	0.8	1.0	Loss
NB0338	VH	Y54D Y102E	23.23	2.2	0.8	1.5	Loss
NB0339	VH	Y54D Y102S	23.75	3.0	0.8	2.1	Loss
NB0341	VH	Y54D Y102Q	25.28	3.1	1.0	2.1	Maintained
NB0342	VH	Y54D Y102G	27.96	3.5	0.9	2.8	Maintained
NB0343	VH	Y54S Y102H	26.66	4.8	1.3	2.7	Maintained
NB0344	VH	Y54S Y102D	25.85	9.3	1.3	2.8	Maintained
NB0345	VH	Y54S Y102E	25.41	25.6	2.3	6.2	Maintained
NB0346	VH	Y54S Y102S	25.70	15.1	2.0	4.3	Maintained
NB0347	VH	Y54S Y102T	26.71	9.2	1.2	3.0	Maintained
NB0348	VH	Y54S Y102Q	27.15	19.9	2.5	4.6	Maintained
NB0349	VH	Y54S Y102G	DNE	8.6	1.6	3.8	Maintained
NB0350	VH	Y54Q Y102H	24.40	7.2	1.5	3.3	Maintained
NB0351	VH	Y54Q Y102D	23.77	6.4	1.1	4.1	Maintained
NB0353	VH	Y54Q Y102S	23.65	12.1	1.3	3.0	Maintained
NB0354	VH	Y54Q Y102T	24.73	8.7	1.2	3.1	Maintained
NB0355	VH	Y54Q Y102Q	25.08	8.3	1.4	3.1	Maintained
NB0356	VH	Y54Q Y102G	DNE	8.6	1.5	2.5	Maintained
NB0357	VH	Y54G Y102H	25.47	18.3	2.3	3.9	Maintained
NB0358	VH	Y54G Y102D	24.59	5.4	1.2	2.2	Maintained
NB0359	VH	Y54G Y102E	24.17	6.5	1.0	3.4	Maintained
NB0360	VH	Y54G Y102S	24.51	4.3	2.5	3.5	Maintained
NB0361	VH	Y54G Y102T	25.51	2.7	1.8	2.9	Loss of
NB0362	VH	Y54G Y102Q	26.12	3.5	2.3	2.8	Maintained
NB0363	VH	Y54G Y102G	28.91	3.9	2.5	3.0	Maintained
NB0364	VL	A51D	28.94	13.2	6.0	4.2	Maintained
NB0365	VL	S52D	DNE	11.4	6.4	3.5	Maintained
NB0366	VL	S53D	29.10	8.9	5.1	3.5	Maintained
NB0367	VL	Y55D	DNE	21.1	10.9	3.7	Maintained
NB0368	VL	Y94H	DNE	8.0	4.8	3.4	Maintained
NB0371	VL	Y94S	DNE	11.3	4.1	5.1	Maintained
NB0372	VL	Y94T	DNE	10.6	4.9	4.2	Maintained

WHAT IS CLAIMED IS:

1. An antibody or antigen binding fragment thereof comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in any one of SEQ ID NOs: 1 to 97, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in any one of SEQ ID NOs: 98 to 194, wherein the recombinant antibody binds an integrin polypeptide.
2. An antibody or antigen binding fragment thereof of claim 1, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NOs: 43, 58, 59, 60, or 61, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158.
3. An antibody or antigen binding fragment thereof of claim 2, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 43, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 140.
4. An antibody or antigen binding fragment thereof of claim 2, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 58, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 155.
5. An antibody or antigen binding fragment thereof of claim 2, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 459, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 156.
6. An antibody or antigen binding fragment thereof of claim 2, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 60, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 157.
7. An antibody or antigen binding fragment thereof of claim 2, comprising a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 61, and a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or 100% identical to that set forth in SEQ ID NO: 158.
8. The antibody or antigen binding fragment thereof of any one of claims 1 to 7, wherein the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody.
9. The antibody or antigen binding fragment thereof of any one of claims 1 to 8, wherein the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains.
10. The antibody or antigen binding fragment thereof of any one of claims 1 to 9, wherein the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized.
11. The antibody or antigen binding fragment thereof of any one of claims 1 to 10, wherein the integrin polypeptide is an integrin alpha11 polypeptide.

12. The antibody or antigen binding fragment thereof of any one of claims 1 to 11, wherein the antibody or antigen binding fragment thereof is isolated and purified.
13. A pharmaceutical composition comprising the antibody or antigen binding fragment thereof of any one of claims 1 to 12, and pharmaceutically acceptable excipient, carrier, or diluent.
14. A formulation for intravenous administration comprising the antibody or antigen binding fragment thereof of any one of claims 1 to 12 or the pharmaceutical composition of claim 13.
15. The antibody or antigen binding fragment thereof of any one of claims 1 to 12, the pharmaceutical composition of claim 13, or the formulation of claim 15 for use in treating a cancer.
16. A method of treating a cancer and/or a tumor in subject, the method comprising administering to a subject the antibody or antigen binding fragment thereof of any one of claims 1 to 12, the pharmaceutical composition of claim 13, or the formulation of claim 15.
17. The method of claim 16, wherein the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.
18. An antibody or antigen binding fragment thereof comprising a heavy chain CDR1 (CDR-H1) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a heavy chain CDR2 (CDR-H2) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a heavy chain CDR3 (CDR-H3) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 1 to 97, a light chain CDR1 (CDR-L1) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, a light chain CDR2 (CDR-L2) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, and a light chain CDR3 (CDR-L3) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 98 to 194, wherein the recombinant antibody binds an integrin polypeptide.
19. The antibody or antigen binding fragment thereof of claim 18 comprising a heavy chain CDR1 (CDR-H1) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60, or 61, a heavy chain CDR2 (CDR-H2) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60, or 61, a heavy chain CDR3 (CDR-H3) amino acid sequence identified from a sequence set forth in any one of SEQ ID NOs: 43, 58, 59, 60, or 61, a light chain CDR1 (CDR-L1) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, a light chain CDR2 (CDR-L2) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, and a light chain CDR3 (CDR-L3) amino acid identified from a sequence set forth in any one of SEQ ID NOs: 140, 155, 156, 157, or 158, wherein the recombinant antibody binds an integrin polypeptide.
20. The antibody or antigen binding fragment of claim 18 or claim 19, wherein any one or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, or CDR-L3 are identified by a numbering scheme selected from the list consisting of the Kabat numbering scheme, the Chothia numbering scheme, the IMGT numbering scheme, the contact numbering scheme, the Aho numbering scheme, and combinations thereof.
21. The antibody or antigen binding fragment of claim 20, wherein two or more of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, or CDR-L3 are identified by at least two different numbering schemes selected from the list consisting of the Kabat numbering scheme, the Chothia numbering scheme, the IMGT numbering scheme, the contact numbering scheme, the Aho numbering scheme, and combinations thereof.
22. The antibody or antigen binding fragment thereof of any one of claims 18 to 21, wherein the antibody antigen binding fragment is a Fab, F(ab)₂, single-domain antibody, a single chain variable fragment (scFv), or a nanobody.

23. The antibody or antigen binding fragment thereof of any one of claims 18 to 22, wherein the antibody or antigen binding fragment thereof comprises two immunoglobulin heavy chains and two immunoglobulin light chains.
24. The antibody or antigen binding fragment thereof of any one of claims 18 to 23, wherein the antibody or antigen binding fragment thereof is chimeric, humanized, or deimmunized.
25. The antibody or antigen binding fragment thereof of any one of claims 18 to 24, wherein the integrin polypeptide is an integrin alpha11 polypeptide.
26. The antibody or antigen binding fragment thereof of any one of claims 18 to 25, wherein the antibody or antigen binding fragment thereof is isolated and purified.
27. A pharmaceutical composition comprising the antibody or antigen binding fragment thereof of any one of claims 18 to 26, and pharmaceutically acceptable excipient, carrier, or diluent.
28. A formulation for intravenous administration comprising the antibody or antigen binding fragment thereof of any one of claims 18 to 26 or the pharmaceutical composition of claim 27.
29. Use of the antibody or antigen binding fragment thereof of any one of claims 18 to 26, the pharmaceutical composition of claim 27, or the formulation of claim 28, for treating a cancer and/or a tumor.
30. A method of treating a cancer and/or a tumor in subject comprising administering to a subject the antibody or antigen binding fragment thereof of any one of claims 18 to 26, the pharmaceutical composition of claim 27, or the formulation of claim 28.
31. A method of making a treatment for cancer and/or a tumor comprising admixing the antibody or antigen binding fragment thereof of any one of claims 1 to 12 or any one of claims 18 to 26 and a pharmaceutically acceptable excipient, carrier, or diluent.
32. A polynucleotide encoding the antibody or antigen binding fragment thereof of any one of claims 1 to 12 or any one of claims 18 to 26.
33. The method of claim 30 or 31, or the use of claim 29, wherein the cancer comprises lung cancer, and/or the tumor comprises a solid tumor.

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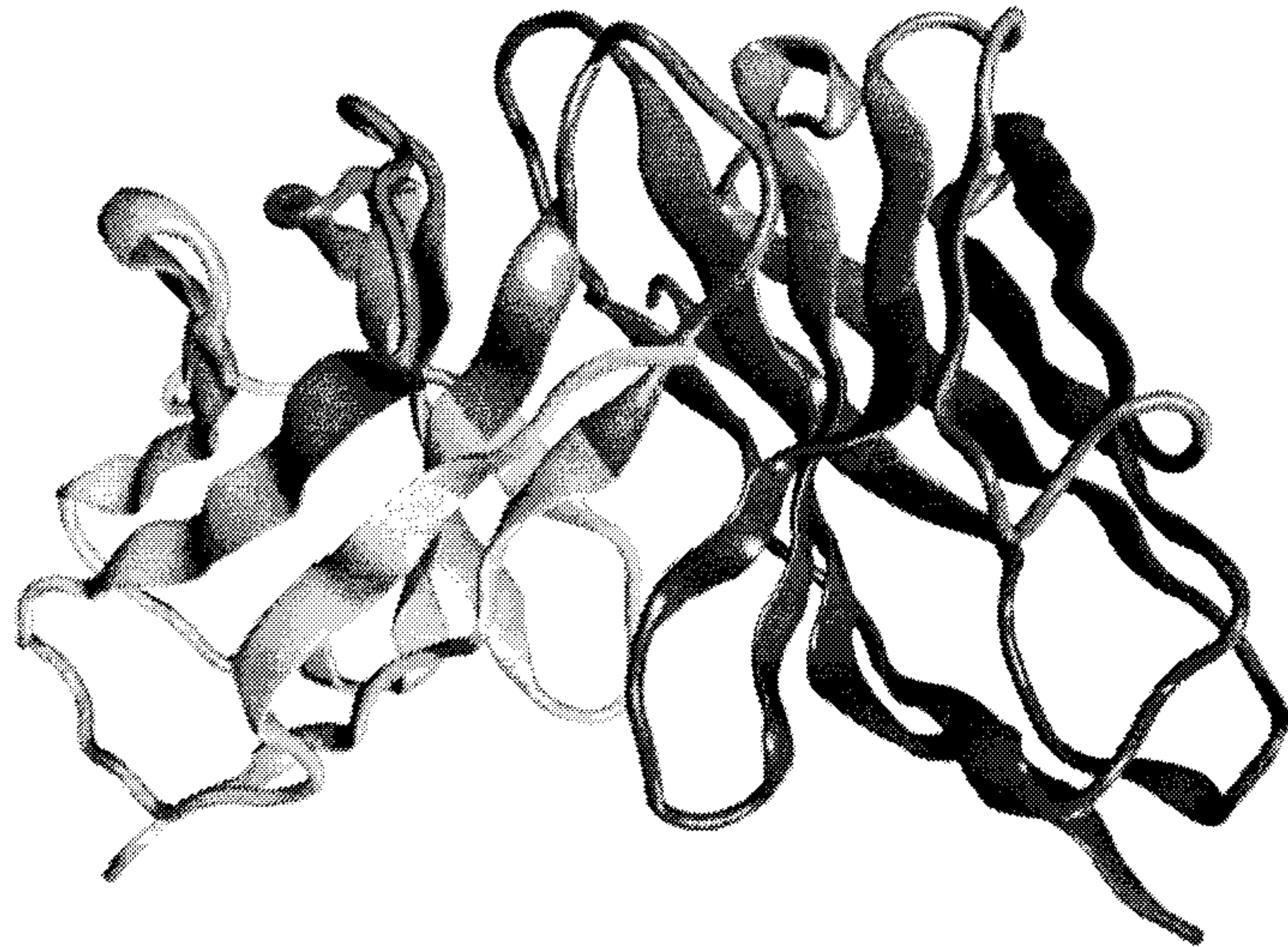


FIG. 1A

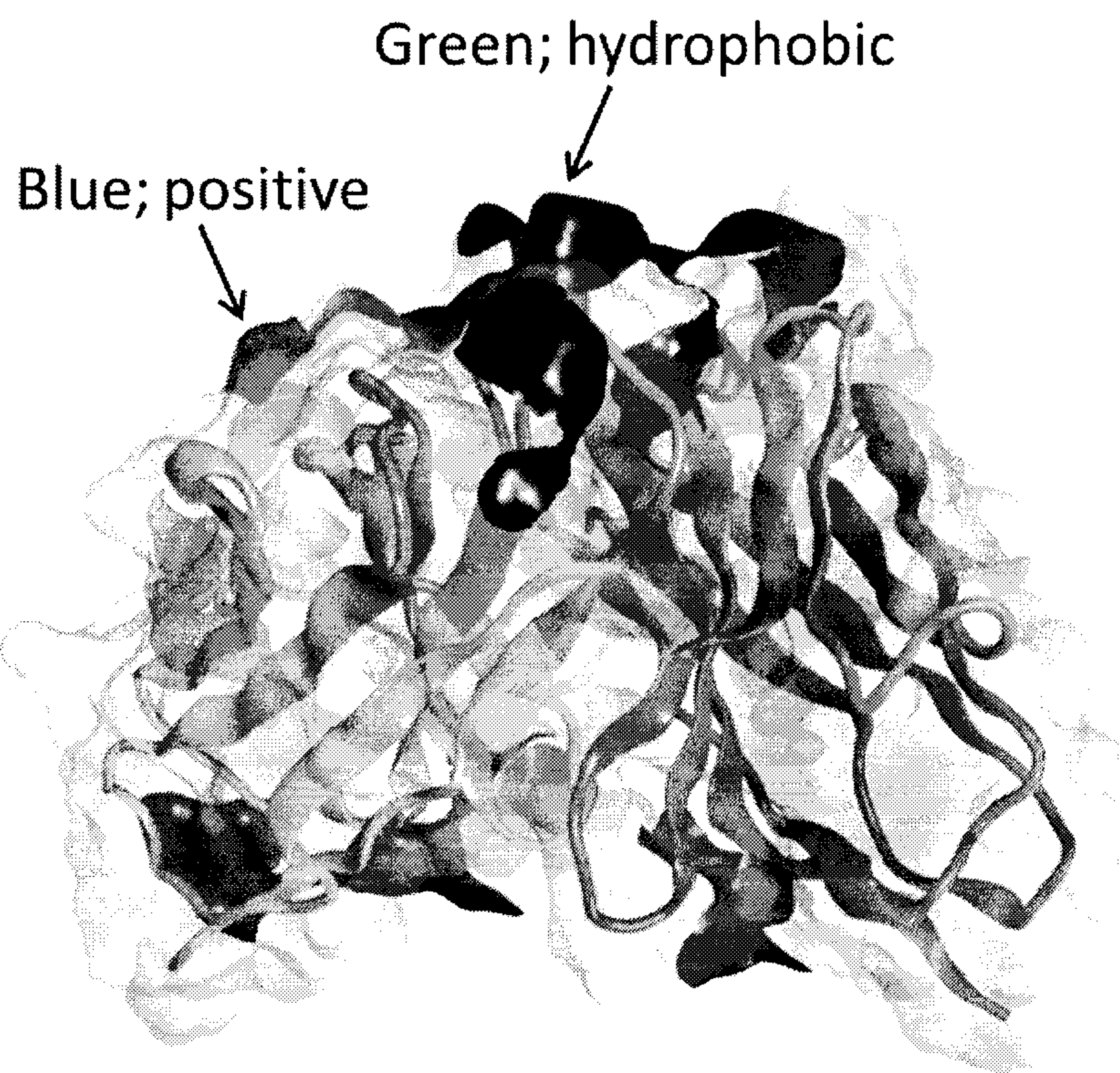


FIG. 1B

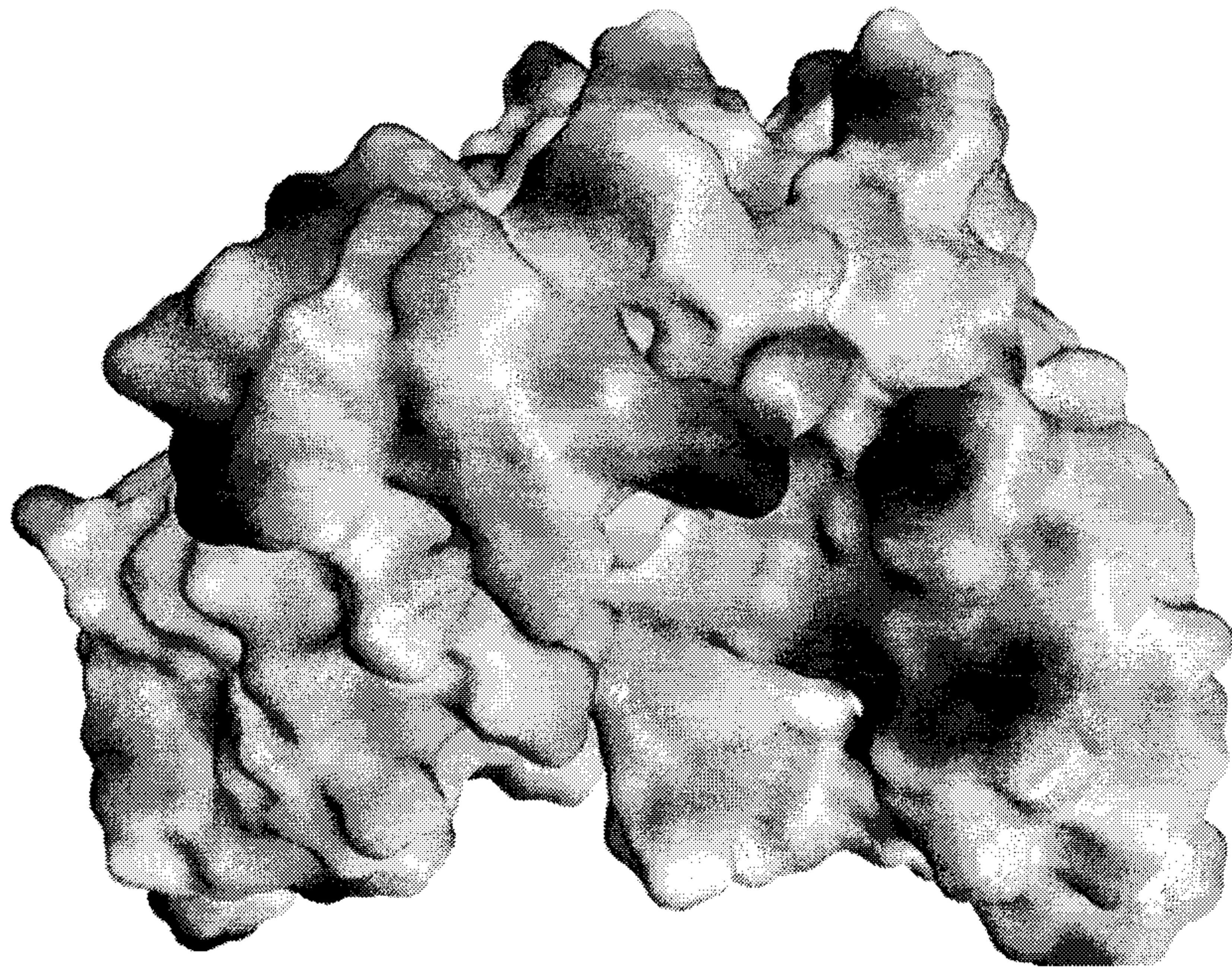


FIG. 1C

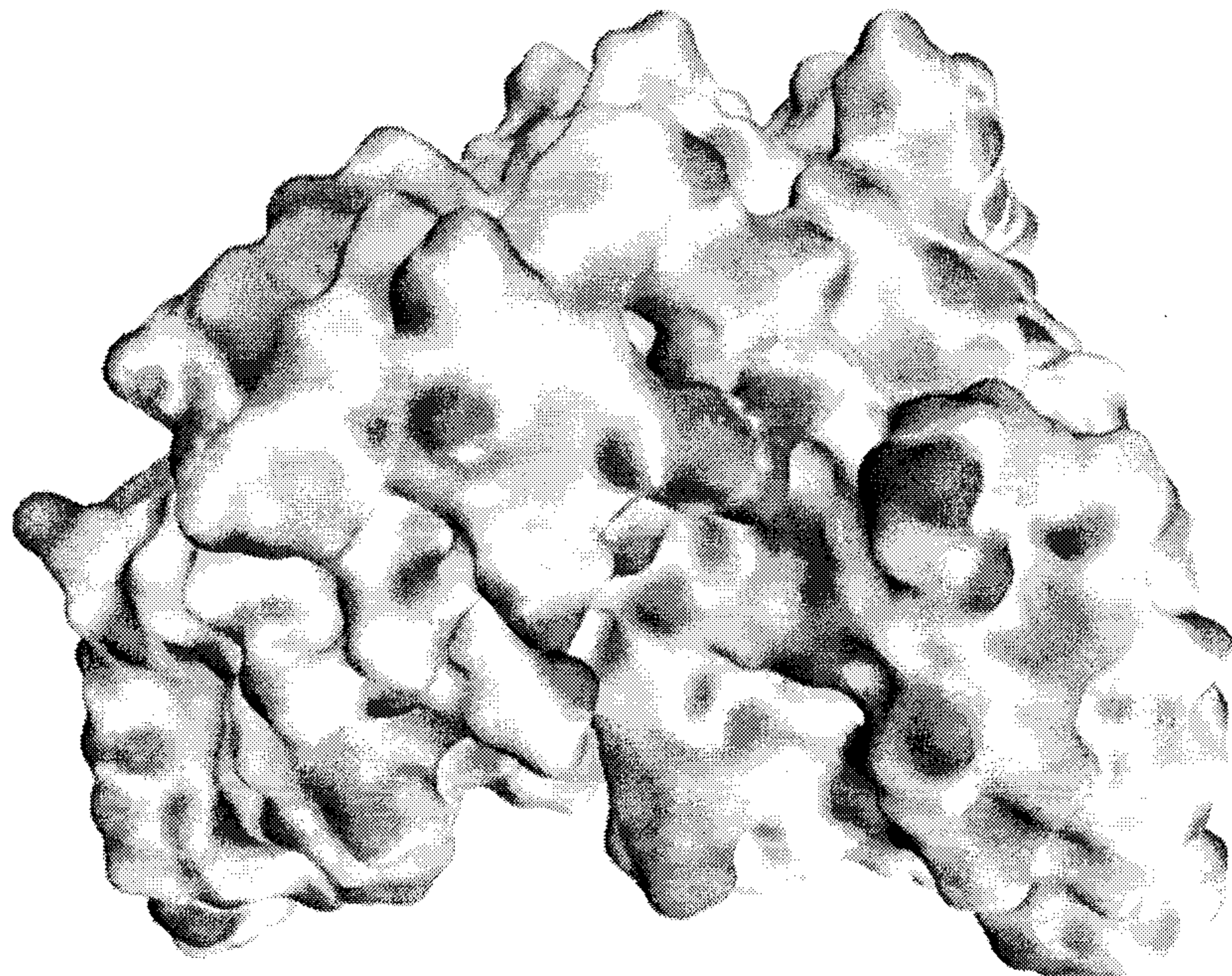
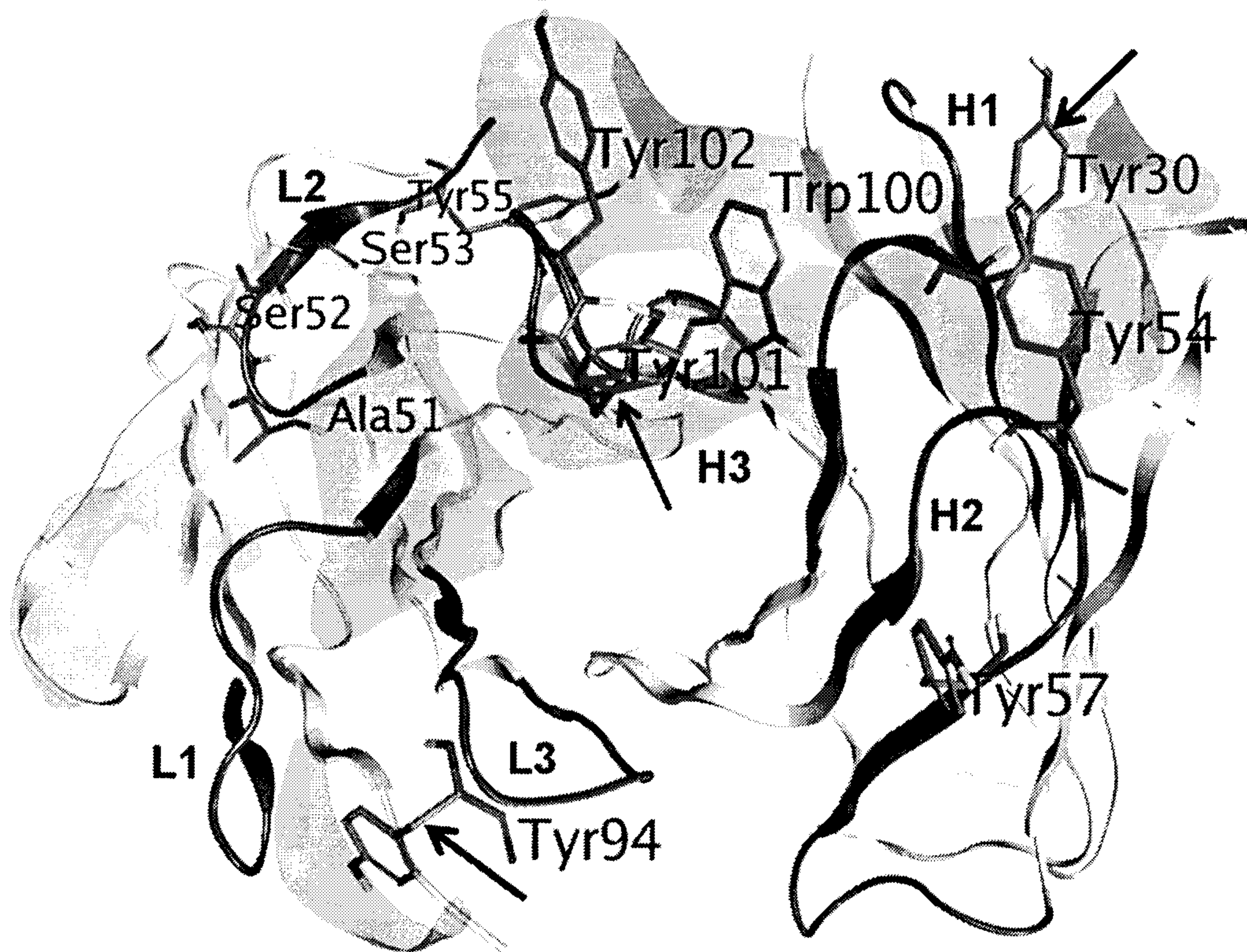


FIG. 1D



LC DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPS
 HC EVQLVESGGGLVQPGGSLRLSCAASGPNLYSSYIHWRQAPGKGLEWVASISSYSSYTY
 LC RFSGSRSGTDFTLTISLQPEDFATYYCQQSAPYSSLITFGQGTKVEIKR
 HC ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYWYSAWALDYWGQGLTVTVSS

FIG. 2

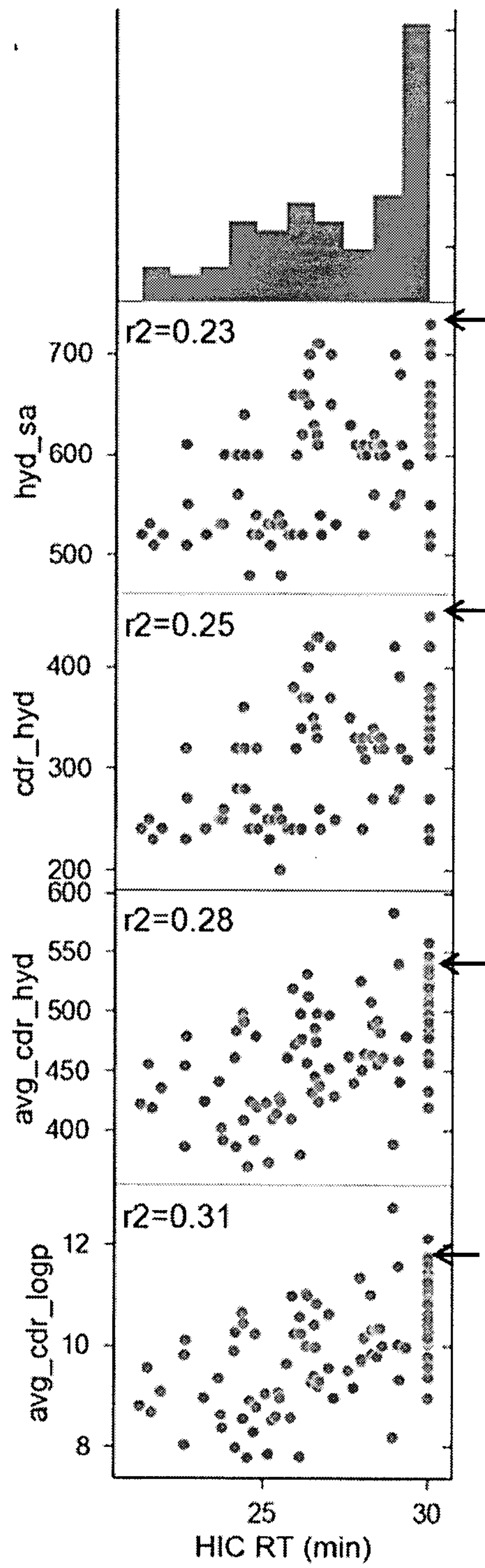


FIG. 3A

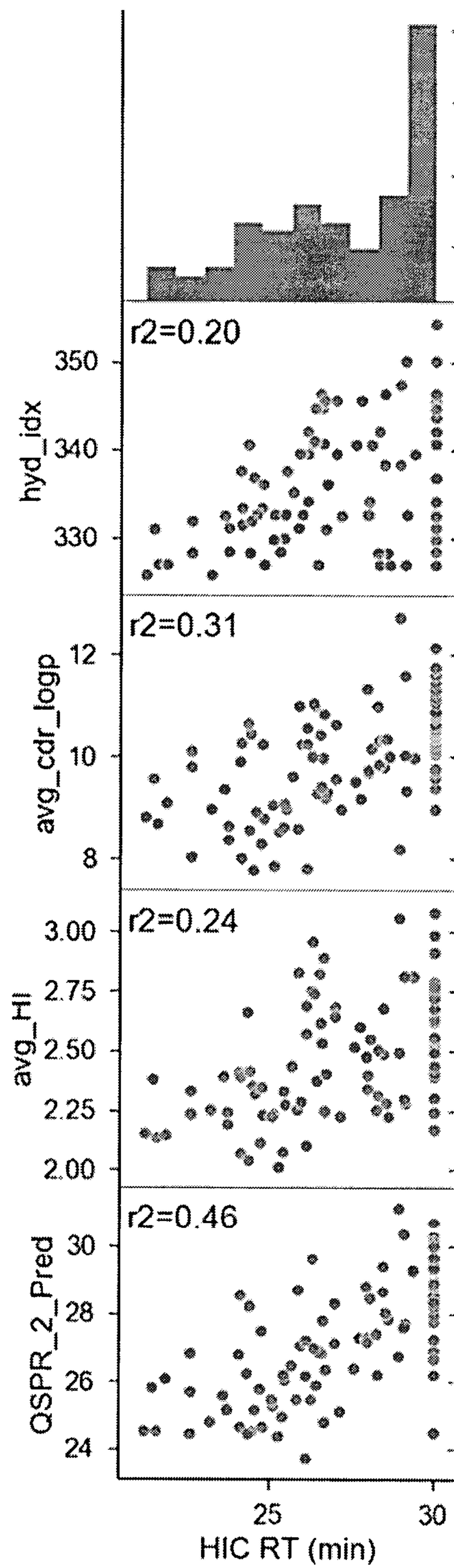


FIG. 3B

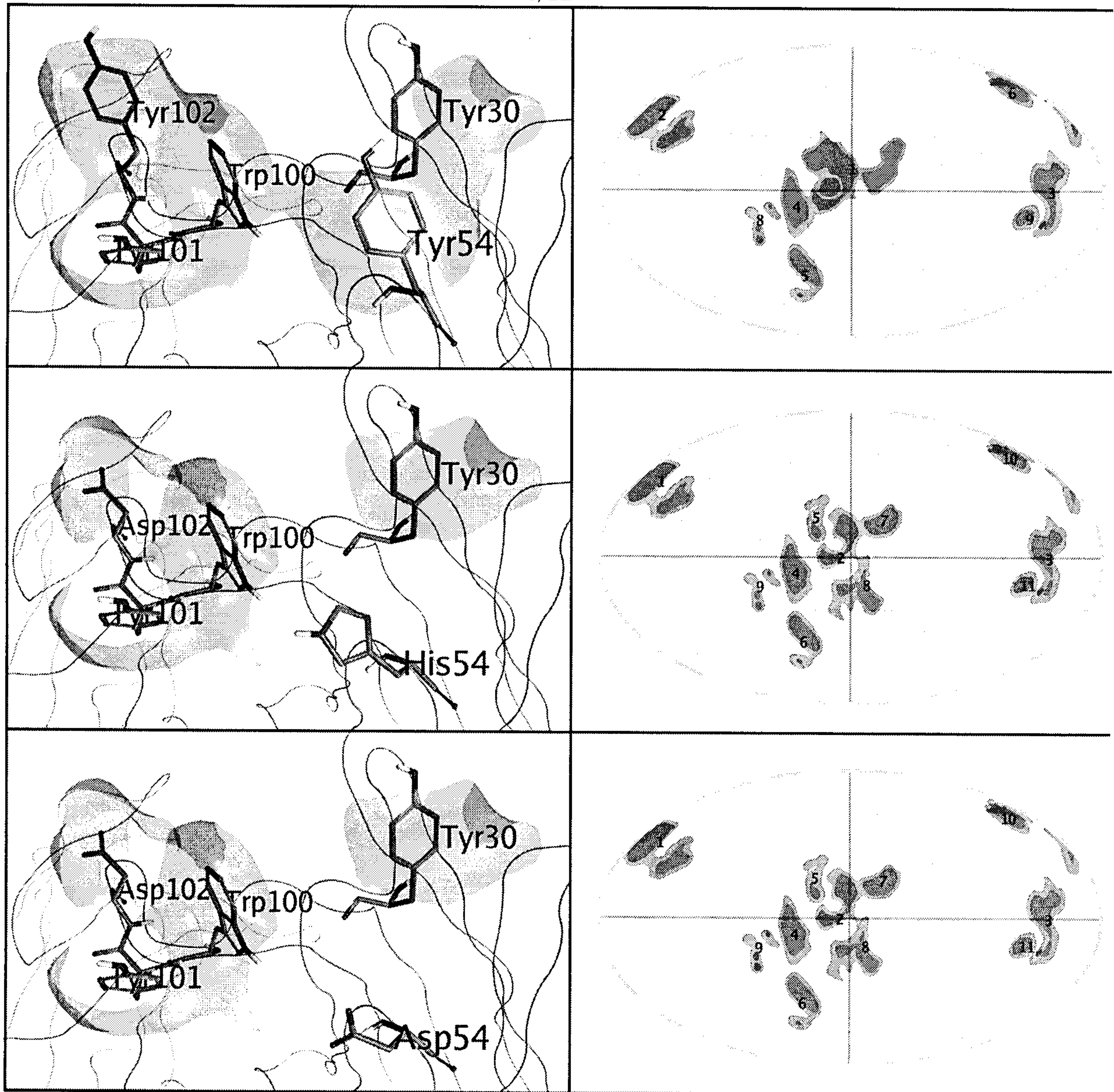
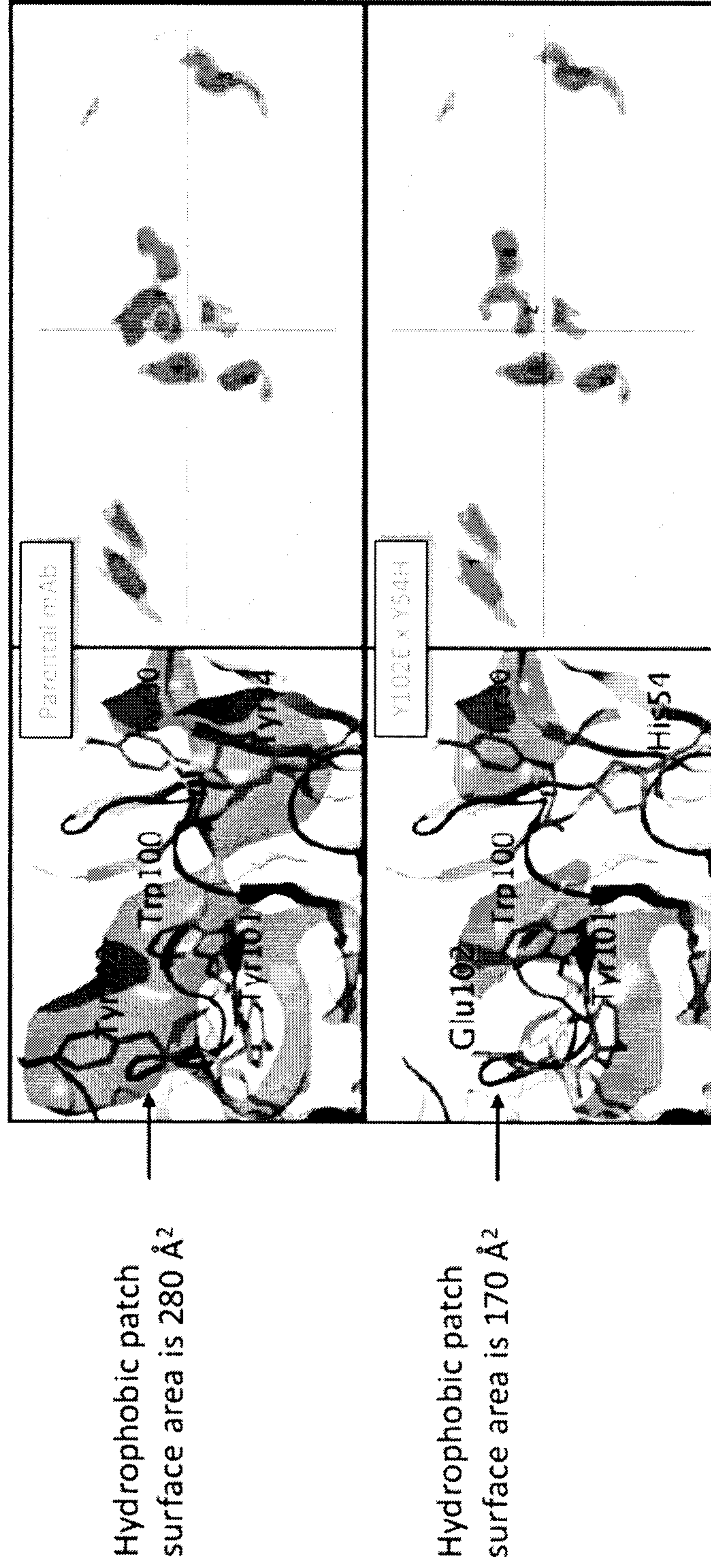


FIG. 4A

FIG. 4B



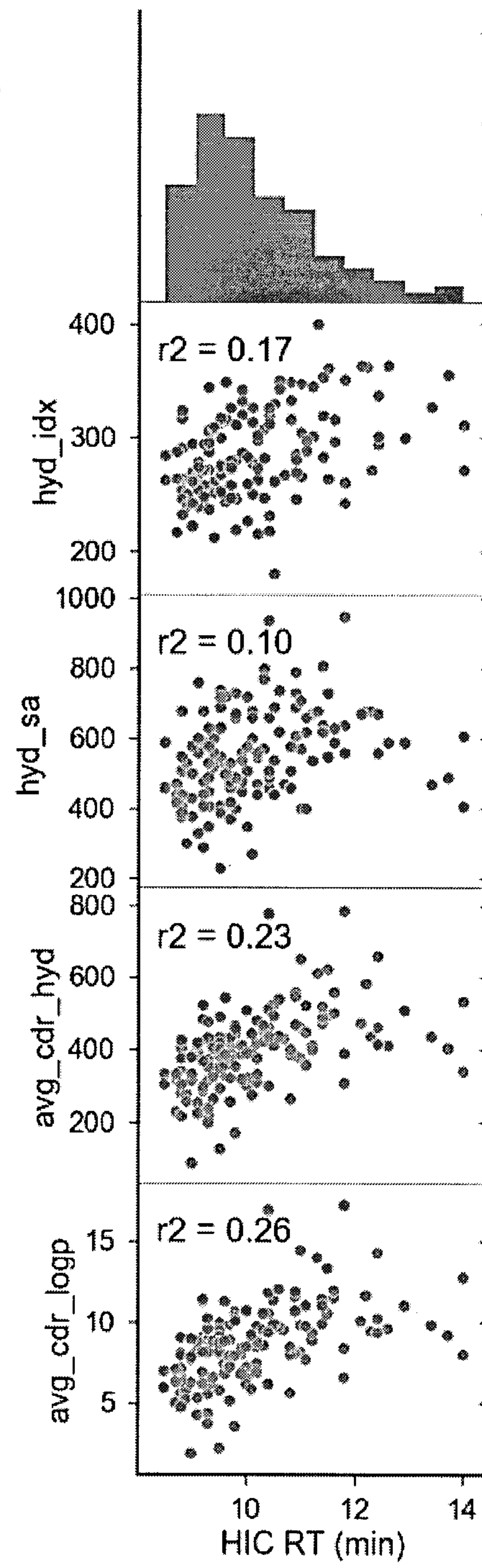
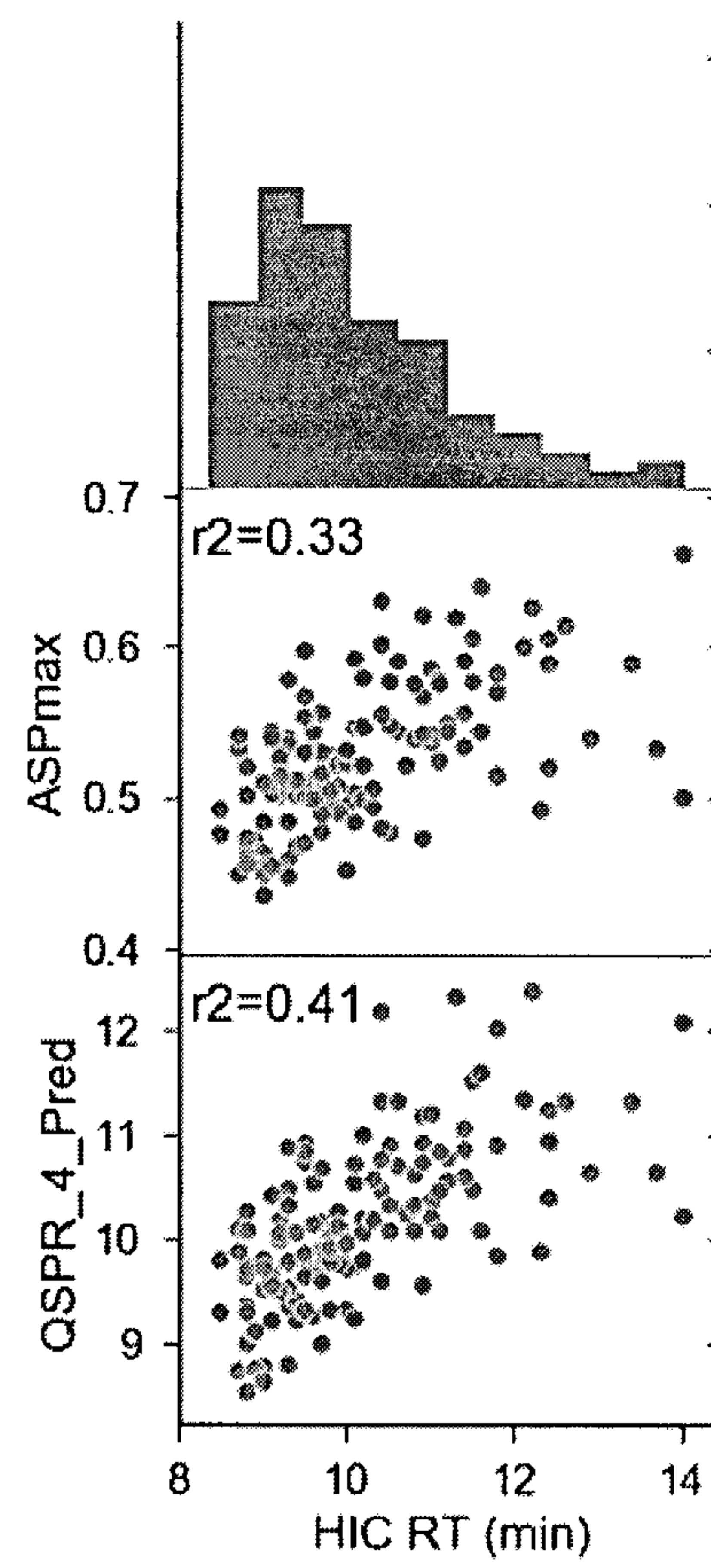


FIG. 5A

**FIG. 5B**

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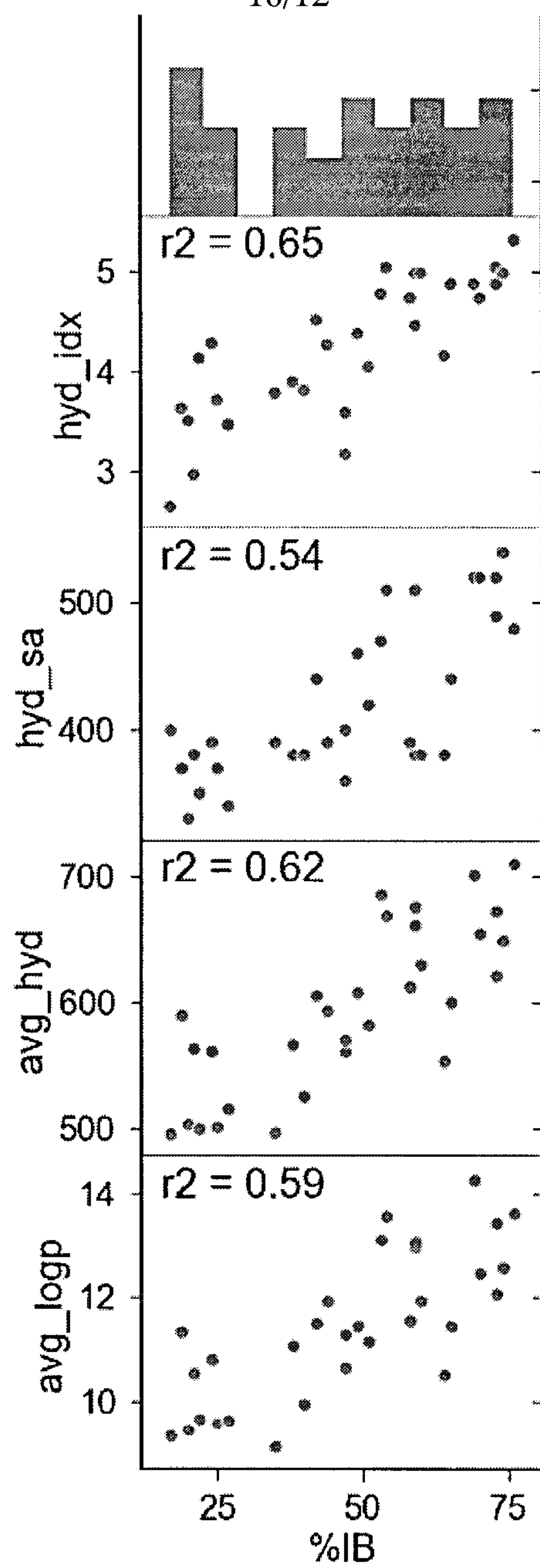


FIG. 5C

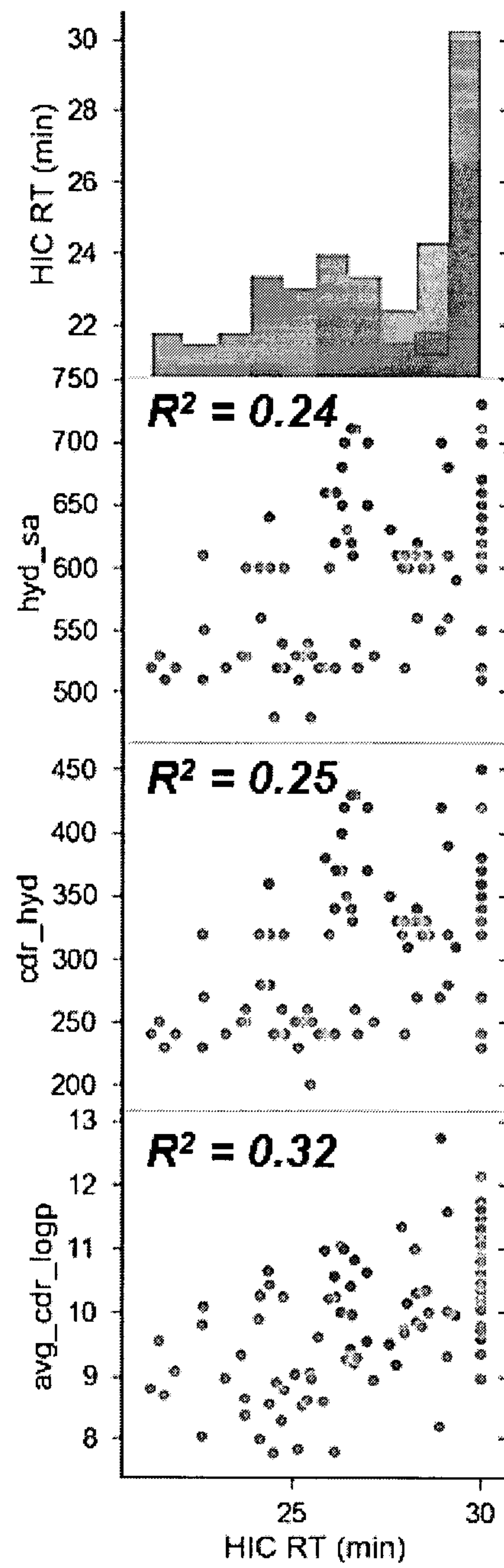


FIG. 6A

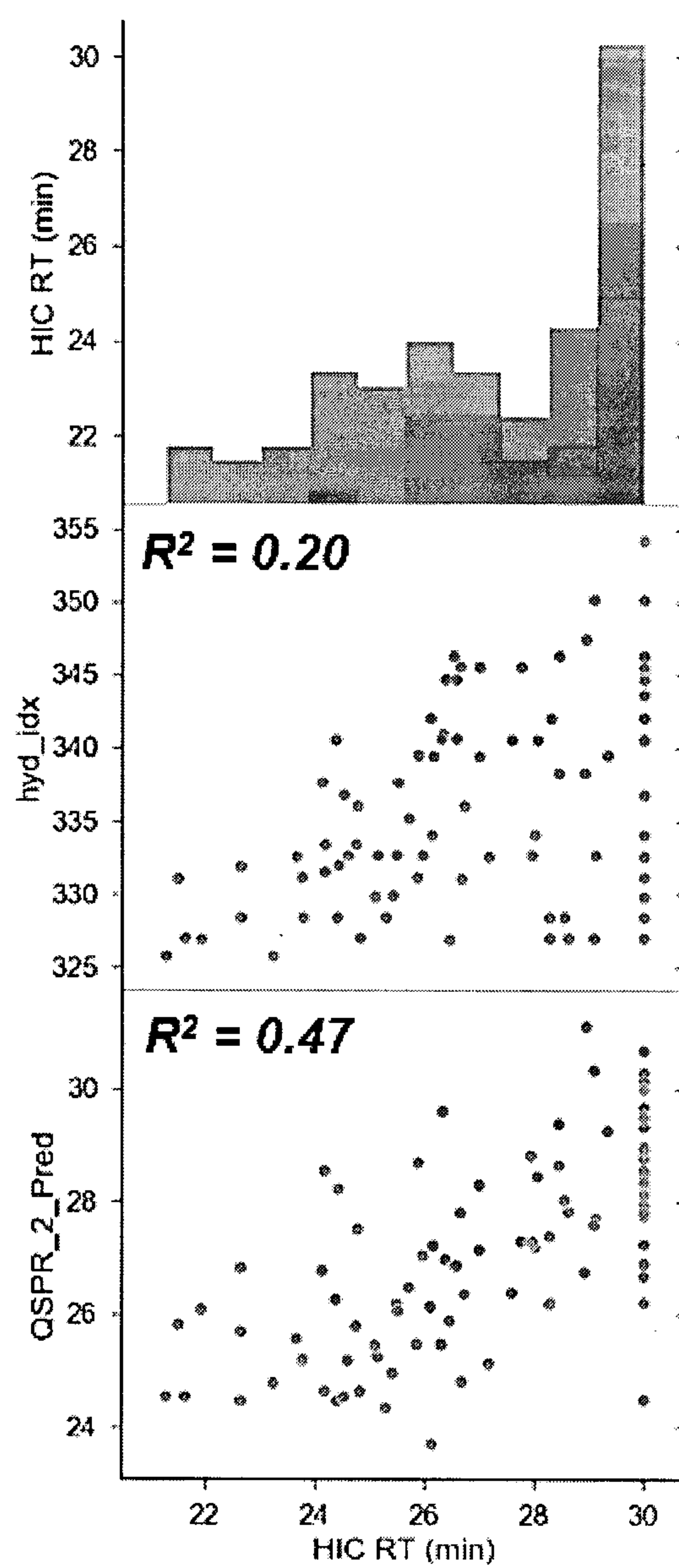


FIG. 6B