

ANTI-ACVR2A ANTIBODIES AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims benefit of priority of International Patent Application No. PCT/CN2021/116485 filed on September 03, 2021, the disclosure of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] This application contains a computer readable Sequence Listing which has been submitted in XML file format with this application, the entire content of which is incorporated by reference herein in its entirety. The Sequence Listing XML file submitted with this application is entitled "14668-008-228_seqlist.xml", was created on August 27, 2022 and is 91,313 bytes in size.

1. FIELD

[0003] Provided herein are molecules capable of binding to ACVR2A, pharmaceutical compositions comprising same, and uses thereof.

2. BACKGROUND

[0004] Activin receptor type-2A (ACVR2A) is a receptor that mediates the functions of activins among other biological activities. Activins are dimeric growth and differentiation factors which belong to the transforming growth factor-beta (TGF-beta) superfamily of structurally related signaling proteins. Activins signal through a heteromeric complex of receptor serine kinases, including type I and type II receptors, and these receptors are all transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with serine/threonine specificity. Type I receptors are essential for signaling; and type II receptors are required for binding ligands and for phosphorylation of type I receptors. Although it has been shown that type II receptor ACVR2A is involved in various disease conditions, there is a need in the art for improved antibody therapies targeting ACVR2A.

3. SUMMARY

[0005] In one aspect, provided herein is an antibody or antigen binding fragment thereof that selectively binds ACVR2A. In some embodiments, the antibodies provided herein bind ACVR2A over ACVR2B. In some embodiments, the affinity of the antibody or antigen binding fragment provided herein to ACVR2A is at least 10 fold of that to ACVR2B. The disclosure provides methods of using the ACVR2A-binding proteins. In some embodiments,

the ACVR2A-binding proteins are capable of inhibiting or blocking the binding to ACVR2A to an ACVR2A ligands, such as Activin A, Activin B, GDF8 and GDF11. In some embodiments, the antibody or antigen binding fragment provided herein comprises:

- (i) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 1, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (ii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 3, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (iii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 4, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (iv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 5, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (v) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (vi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 8, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (vii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (viii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 10, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (ix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (x) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (xi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 13, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (xii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 14;
- (xiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 15;
- (xiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (xv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 16;

- (xvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 17;
- (xvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 18;
- (xviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 19;
- (xix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 20;
- (xx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 21;
- (xxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 22;
- (xxii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 23;
- (xxiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 24;
- (xxiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (xxv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 25;
- (xxvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 26;
- (xxvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 27;
- (xxviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 28;
- (xxix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 29;
- (xxx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 30;
- (xxxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 31;

- (xxxii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 32;
- (xxxiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 33;
- (xxxiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 34;
- (xxxv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 35;
- (xxxvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 36;
- (xxxvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 37;
- (xxxviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 38;
- (xxxix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 39;
- (xxxx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 40; or
- (xxxxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 41.

[0006] In some embodiments, the antibody or antigen binding fragment provided herein comprises:

- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 42, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (ii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

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- sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 67, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxiii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 68, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxiv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or
- (xxv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 69, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxvi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 70, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxvii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 71, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxviii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 72, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

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sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 77, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or (xxxvi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 78, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or (xxxvii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 79, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or (xxxviii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 69, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or (xxxix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 80, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or (xxxx) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 70, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or (xxxxi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 81, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47.

[0007] In some embodiments, the antibody or antigen binding fragment provided herein comprises:

- (i) a VH comprising the amino acid sequence of SEQ ID NO: 1, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (ii) a VH comprising the amino acid sequence of SEQ ID NO: 3, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (iii) a VH comprising the amino acid sequence of SEQ ID NO: 4, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (iv) a VH comprising the amino acid sequence of SEQ ID NO: 5, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (v) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (vi) a VH comprising the amino acid sequence of SEQ ID NO: 8, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (vii) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (viii) a VH comprising the amino acid sequence of SEQ ID NO: 10, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (ix) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (x) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (xi) a VH comprising the amino acid sequence of SEQ ID NO: 13, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (xii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 14;
- (xiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 15;
- (xiv) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (xv) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 16;

- (xvi) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 17;
- (xvii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 18;
- (xviii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 19;
- (xix) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 20;
- (xx) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 21;
- (xxi) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 22;
- (xxii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 23;
- (xxiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 24;
- (xxiv) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (xxv) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 25;
- (xxvi) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 26;
- (xxvii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 27;
- (xxviii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 28;
- (xxix) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 29;
- (xxx) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 30;
- (xxxi) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 31;

- (xxxii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 32;
- (xxxiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 33;
- (xxxiv) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 34;
- (xxxv) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 35;
- (xxxvi) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 36;
- (xxxvii) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 37;
- (xxxviii) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 38;
- (xxxix) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 39;
- (xxxx) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 40; or
- (xxxxi) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 41.

[0008] In some embodiments, the antibody provided herein is an IgG. In some embodiments, the antibody is a humanized antibody.

[0009] In some embodiments, the antibody or antigen binding fragment thereof is genetically fused or chemically conjugated to an agent.

[0010] In another aspect, provided herein is a nucleic acid molecule encoding the antibody or antigen binding fragment provided herein.

[0011] In another aspect, provided herein is a vector comprising the nucleic acid molecule encoding the antibody or antigen binding fragment provided herein.

[0012] In yet another aspect, provided herein is a host cell transformed with the vector encoding the antibody or antigen binding fragment provided herein.

[0013] In yet another aspect, provided herein is a composition comprising a therapeutically effective amount of the antibody or antigen binding fragment, the nucleic acid

molecule, or the vector encoding the antibody or antigen binding fragment provided herein, and a pharmaceutically acceptable excipient.

[0014] In yet another aspect, provided herein is a method of treating a disease or disorder in a subject, comprising administering to the subject the composition provided herein. In some embodiments, the method further comprises administering to the subject a second agent. In some embodiments, the disease or disorder is associated with ACVR2A. In some embodiments, the disease or disorder is associated with ACVR2A ligands, including Activins (such as Activin A, Activin B, Activin AB, Activin C, Activin AC and Activin E), Growth/differentiation factors (GDFs, such as GDF1, GDF3, GDF5, GDF6, GDF7, GDF8, GDF10 and GDF11), bone morphogenetic proteins (BMPs, such as BMP2, BMP4, BMP6, BMP7, BMP8a, BMP8b, BMP9 and BMP10).

[0015] The method of any one of claims 11-13, wherein the disease or disorder is associated with ACVR2A.

[0016] In some embodiments, the disease or disorder is a musculoskeletal disease or disorder. In some embodiments, the musculoskeletal disease or disorder is selected from a group consisting of anemia, muscle atrophy, spinal muscular atrophy and cancer cachexia. In some embodiments, the disease or disorder is an age-related condition selected from a group consisting of sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, frailty, memory loss, wrinkles, impaired kidney function, and age-related hearing loss. In some embodiments, the disease or disorder is a metabolic disorder selected from a group consisting of Type II Diabetes, Metabolic Syndrome, hyperglycemia, Nonalcoholic Steatohepatitis (NASH) and obesity. In some embodiments, the disease or disorder is selected from a group consisting of acute and/or chronic renal disease or failure, liver fibrosis or cirrhosis, lung fibrosis, pulmonary arterial hypertension, cancer, kidney fibrosis, Parkinson's Disease, Amyotrophic lateral sclerosis (ALS), brain atrophy, dementia and anemia, cachexia, sarcoma, bone-loss. In some embodiments, the disease or disorder is cancer, inducing ovarian cancer, breast cancer, esophageal cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, endometrial cancer and gastrointestinal cancer.

[0017] In yet another aspect, provided herein is a method for inhibiting or antagonizing ACVR2A in a cell, comprising contacting the cell with the composition provided herein.

4. BRIEF DESCRIPTION OF THE FIGURES

[0018] **FIGs. 1A-1D.** Activin A Competitive ELISA Assay of ACVR2A Hits. The anti-ACVR2A hits from CDRL1 and CDRL2 random mutational phage-display libraries bind ACVR2A and block interaction between Activin A and ACVR2A. FIG. 1A: Anti-ACVR2A Fab hits from CDRL1 mutational library. FIG. 1B: Anti-ACVR2A Fab hits from CDRL2 mutational library. FIG. 1C: Anti-ACVR2A IgG hits from CDRL1 and CDRL2 mutational library. FIG. 1D: Anti-ACVR2A IgG hits generated with CDR shuffling method.

[0019] **FIGs. 2A-2D.** Phospho-Smad Dependent Reporter Gene Assay of ACVR2A Hits. ACVR2B knockout stable cell line HEK293T-B1 were transfected with CAGA-12 luciferase reporter plasmid, luciferase signaling was tested after overnight incubation with IgG hits and Activin A. FIG. 2A: The hits J, 21047, 21155, 21169, 21341, 21343, 21366 and 275 inhibited Activin A induced phospho-Smad dependent signaling. FIGs. 2B and 2C: CDR shuffling IgG hits NGS-1, NGS-2, NGS-3, NGS-4, NGS-5, 6401, 6403, 3351, 3352, 3353, 3354 and 3355 inhibited Activin A induced phospho-Smad dependent signaling. FIG. 2D: The hits J, 275, 21155, 21169, 21341, 21343, 21366 and 6401 inhibited GDF8 induced phospho-Smad dependent signaling.

[0020] **FIG. 3.** Phospho-Smad3 inhibition by IgG hits in C2C12 Cells. The level of Smad3 phosphorylation in satellite cells C2C12 treated for 30min in presence of activin A (50 ng/ml) alone (PBS) and in combination with ACVR2A Ab hits was quantitated using the HTRF protocol. Hits J, 21155, 21169, 21341, 275 and 6401, decreased phospho-Smad3 level induced by Activin A.

[0021] **FIGs. 4A-4D.** ACVR2A Ab increased mouse body and muscle weight. Naive SCID mice were treated with ACVR2A Ab LA01 at 20 mg/kg once weekly for 28 days. FIG. 4A: Body weight change growth curve are expressed as means $\pm$ SEMs (n=8). Ab LA01 treatment increased body weight significantly. FIG. 4B: Tibialis anterior muscle weight was increased by Ab LA01 treatment. FIG. 4C: Inguinal adipose tissue weight change. FIG. 4D: Epididymal adipose tissue weight change. *, $P < 0.05$ versus the isotype control; **, $P < 0.01$ versus the control; ***, $P < 0.001$ versus the control (Student's t test).

[0022] **FIGs. 5A-5C.** ACVR2A Ab increased body and muscle weight in wild type mouse. C57BL/6 mice were treated with ACVR2A Ab LA01 at 10 mg/kg twice weekly for 6 weeks. FIG. 5A: Body weight change growth curve are expressed as means $\pm$ SEMs (n=8).

FIG. 5B: Ab LA01 increased tibialis anterior muscle weight. FIG. 5C: Ab LA01 increased gastrocnemius muscle weight change. *, $P < 0.05$ versus the isotype control; **, $P < 0.01$ versus the control; ***, $P < 0.001$ versus the control (Student's *t* test).

[0023] FIGs. 6A-6G. ACVR2A Ab promotes haemogenesis of red blood cells. C57BL/6 mice were treated with ACVR2A Ab LA01 and/or ACVR2B Trap ACE-536 at 10 mg/kg twice weekly for 2 weeks. After 7 days treatment, ACVR2B Trap ACE-536 single agent or combined with ACVR2A Ab LA01 increased red blood cells number (FIG. 6A), hemoglobin (FIG. 6B) and hematocrit (FIG. 6C). In contrast, Ab LA01 single treatment for 7 days had little effect on red blood maturation (FIGs. 6A, 6B and 6C). However, 7 days treatment by Ab LA01 increased reticulocyte numbers significantly. After 14 days treatment, Ab LA01 promoted haemogenesis similar with ACE-536, including red blood cells number (FIG. 6E), hemoglobin (FIG. 6F) and hematocrit (FIG. 6G). Ab LA01 and ACE-536 combination treatment was more efficacious in haemogenesis than single agent treatment (FIGs. 6E-6G). Means $\pm$ SEM are shown. *, $P < 0.05$ versus the isotype control; **, $P < 0.01$ versus the control; ***, $P < 0.001$ versus the control; ****, $P < 0.0001$ versus the control (One-way ANOVA test).

[0024] FIGs. 7A-7E. ACVR2A Ab attenuate CCl₄ induced liver fibrosis in mouse model. 6-8 weeks old of C57/BL6 mice was induced liver fibrosis using intraperitoneal injection of CCl₄, dissolved in a 1:10 ratio with corn oil, 1mL/kg, twice a week for 5 weeks. Mice were treated with ACVR2A Ab LA01, or Isotype control antibody subcutaneously at a dose of 20 mg/kg once a week for 5 weeks. Serum alanine aminotransferase (ALT) and (Aspartate aminotransferase) AST were analyzed after two weeks treatment of Ab LA01. At the end of study, Hematoxylin-eosin (HE) staining and Masson's trichrome staining of liver were performed to assess fibrosis, inflammation, hepatitis lesions, and analyzed with Knodell histology activity index (HAI) system. mRNA of collagen 3 (Col3) was analyzed with Real-time PCR assay. FIG. 7A: Ab LA01 increased body weights significantly compared to isotype control Ab, from day13 to day35. FIG. 7B: Two weeks treatment with Ab LA01 decreased serum ALT and AST level in CCl₄ induced liver fibrosis model. FIG. 7C: Liver Col3 expression was decreased after 5 weeks treatment with Ab LA01. FIG. 7D: Liver histological analysis. The liver fibrosis, portal inflammation and hepatitis lesions score showed Ab LA01 attenuate CCl₄ induced liver fibrosis and liver injury. FIG. 7E: Ab LA01 promoted erythropoiesis in CCl₄ induced liver fibrosis model. RBC number, hemoglobin concentrations and hematocrit values were increased after 24 days treatment with Ab LA01.

Means $\pm$ SEM are shown. *, $P < 0.05$ versus the isotype control; **, $P < 0.01$ versus the control; ***, $P < 0.001$ versus the control; ****, $P < 0.0001$ versus the control (One-way ANOVA test).

[0025] FIGs. 8A-8F. ACVR2A Ab attenuate liver fibrosis in HFD (High Fat Diet)-CCl₄ NASH model. 18-19 weeks old of diet-induced obese (DIO) C57/BL6 mice were fed HFD and treated with low dose CCl₄ (25% CCl₄, 0.5ml/kg) to induce NSAH. Mice were treated with ACVR2A Ab LA01 for 4 weeks, S.C, 4 or 20mg/kg, twice a week; the FXR agonist obeticholic acid (OCA) was used as positive control drug, 30 mpk p.o. q.d. FIG. 8A: ACVR2A Ab LA01 treatment increased mice body weight compared to isotype control group. FIGs. 8B, 8C: 20mg/kg LA01 treatment decreased liver fibrosis score and Sirius Red Staining signal. FIGs. 8D, 8E: The liver collagen gene expression (Col3a1) and liver α SMA were decreased in 20mg/kg LA01 treatment group. FIG. 8F: LA01 treatment increased liver weight. Means $\pm$ SEM are shown. *, $P < 0.05$ versus the isotype control; **, $P < 0.01$ versus the control; ***, $P < 0.001$ versus the control; (One-way ANOVA test).

[0026] FIGs.9A-9B. ACVR2A Ab LA01 inhibits CT26 tumor growth. Balb/C mice (n = 8 per treatment) subcutaneously inoculated with 0.5×10^6 viable CT26 cells (day-5) were treated with PBS, 10 mg/kg anti-PD-L1 antibody 10F.9G2 intraperitoneally (I.P), or 10 mg/kg 10F.9G2 (I.P) plus 10 mg/kg ACVR2A Ab LA01, subcutaneously (S.C) twice a week. FIG. 9A: Effect of LA01 on tumor growth. FIG. 9B: The individual tumor volume of each mouse on day14. The anti-PD-L1 antibody treatment alone had no efficacy, in contrast, combining ACVR2A Ab LA01 and PD-L1 antibody resulted in anti-tumor effect. Means $\pm$ SEM are shown. **, $P < 0.01$ (One-way ANOVA test).

[0027] FIG. 10. ACVR2A Ab LA01 inhibits LLC tumor growth in combination with PD-L1 Ab. C57/BL6 mice received an intramuscular (hind leg) inoculum of 5×10^5 LLC cells, were treated with PBS, 10 mg/kg PD-L1 Ab FAZ053, intravenous (I.V), twice a week, or PD-L1 Ab and ACVR2A Ab combination, 10 mg/kg FAZ053 I.V, plus 10 mg/kg ACVR2A Ab S.C, twice a week. PD-L1 Ab alone treatment had little effect on tumor growth (Group 2, T/C =0.62, $p=0.04$), combining ACVR2A Ab LA01 and PD-L1 antibody inhibited tumor growth significantly compared to control group (Group 3, T/C =0.41, $p<0.01$).

[0028] FIG.11A-11I. ACVR2A Ab LA01 inhibits LLC tumor growth in combination with carboplatin. C57/BL6 mice bearing LLC tumor were treated with vehicle control, carboplatin, or carboplatin and ACVR2A Ab combination. FIG. 11A: Combining ACVR2A Ab LA01 and carboplatin inhibited tumor growth more dramatically than carboplatin single

agent treatment. FIG. 11B: Individual tumor volume in each group, ACVR2A Ab LA01 combination enhanced the antitumor effect of carboplatin. FIGs. 11C, 11D and 11E: The CD8⁺ T effector cells (Teff), Foxp3⁺ T regulatory cells (Treg) percentage and the ratio of Teff to Treg in tumor of different groups. FIG. 11F: The ratio of Teff to Treg negatively correlated with tumor volume (R squared =0.30, p value =0.0054). FIGs. 11G, 11H and 11I: M1 phenotype macrophages, M2 phenotype macrophages and the ratio of M1/M2 in different groups. The ratio of M1/M2 increased significantly in combination group. Means $\pm$ SEM are shown. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001; ns, no significance, (Two-tailed t test).

[0029] FIGs. 12A-12F. ACVR2A Ab LA01 attenuated carboplatin induced anemia. The complete blood count result showed the decrease of white blood cells (WBC) (FIG. 12A): neutrophils (NEUT) (FIG. 12B) and platelets (PLT) (FIG. 12C) after carboplatin treatment, ACVR2A Ab treatment increased PLT numbers significantly. FIGs. 12D, 12E, and 12F: The red blood cells (RBC), hemoglobin (HGB) and hematocrit (HCT) change in different groups. *, P < 0.05; ***, P < 0.001; ****, P < 0.0001 (One-way ANOVA test).

[0030] FIGs. 13A-13D. ACVR2A Ab inhibited tumor growth in ovarian cancer CDX mouse model. FIG. 13A: TOV-21G cells secreted high level of Activin A in culture medium compared to other ovarian cancer cells. FIG. 13B: Effect of Ab LA01 on tumor growth. 6-8 week old female Balb/C Nude mice were inoculated with 3×10^6 viable TOV-21G cells in 0.1 ml PBS on the right flanks subcutaneously. ACVR2A Ab LA01 single agent treatment inhibited tumor growth significantly compared to control group (p<0.001). FIGs. 13C and 13D: Immunophenotyping analysis of tumor infiltrating lymphocytes (TIL). More lymphocytes were found infiltrated to tumors in LA01 Ab treatment group. The number of total NK cells (CD45⁺ CD3⁻ CD49⁺) as well as activated NK cells (CD69⁺ or NKG2D⁺) in tumor were elevated in LA01 Ab treatment compared to control group (p = 0.0108, p=0.0076 and p=0.0244, respectively) (FIG. 13C). Similarly, there were more macrophages in LA01 Ab treated tumor (p=0.00341) (FIG. 13D).

5. DETAILED DESCRIPTION

[0031] The present disclosure is based in part on the novel antibodies that bind to ACVR2A and superior properties thereof.

5.1. Definitions

[0032] Techniques and procedures described or referenced herein include those that are generally well understood and/or commonly employed using conventional methodology by

those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (3d ed. 2001); *Current Protocols in Molecular Biology* (Ausubel et al. eds., 2003); *Therapeutic Monoclonal Antibodies: From Bench to Clinic* (An ed. 2009); *Monoclonal Antibodies: Methods and Protocols* (Albitar ed. 2010); and *Antibody Engineering* Vols 1 and 2 (Kontermann and Dübel eds., 2d ed. 2010). Unless otherwise defined herein, technical and scientific terms used in the present description have the meanings that are commonly understood by those of ordinary skill in the art. For purposes of interpreting this specification, the following description of terms will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any description of a term set forth conflicts with any document incorporated herein by reference, the description of the term set forth below shall control.

[0033] The term “antibody,” “immunoglobulin,” or “Ig” is used interchangeably herein, and is used in the broadest sense and specifically covers, for example, monoclonal antibodies (including agonist, antagonist, neutralizing antibodies, full length or intact monoclonal antibodies), antibody compositions with polyepitopic or monoepitopic specificity, polyclonal or monovalent antibodies, multivalent antibodies, multispecific antibodies (*e.g.*, bispecific antibodies so long as they exhibit the desired biological activity), formed from at least two intact antibodies, single chain antibodies, and fragments thereof (*e.g.*, domain antibodies), as described below. An antibody can be human, humanized, chimeric and/or affinity matured, as well as an antibody from other species, for example, mouse, rabbit, llama, *etc.* The term “antibody” is intended to include a polypeptide product of B cells within the immunoglobulin class of polypeptides that is able to bind to a specific molecular antigen and is composed of two identical pairs of polypeptide chains, wherein each pair has one heavy chain (about 50-70 kDa) and one light chain (about 25 kDa), each amino-terminal portion of each chain includes a variable region of about 100 to about 130 or more amino acids, and each carboxy-terminal portion of each chain includes a constant region. *See, e.g.*, *Antibody Engineering* (Borrebaeck ed., 2d ed. 1995); and Kuby, *Immunology* (3d ed. 1997). Antibodies also include, but are not limited to, synthetic antibodies, recombinantly produced antibodies, antibodies including from Camelidae species (*e.g.*, llama or alpaca) or their humanized variants, intrabodies, anti-idiotypic (anti-Id) antibodies, and functional fragments (*e.g.*, antigen binding fragments) of any of the above, which refers to a portion of an antibody heavy or light chain polypeptide that retains some or all of the binding activity of the antibody from which the fragment was derived. Non-limiting examples of functional

fragments (*e.g.*, antigen binding fragments) include single-chain Fvs (scFv) (*e.g.*, including monospecific, bispecific, *etc.*), Fab fragments, F(ab') fragments, F(ab)₂ fragments, F(ab')₂ fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fv fragments, diabody, triabody, tetrabody, and minibody. In particular, antibodies provided herein include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, for example, antigen-binding domains or molecules that contain an antigen-binding site that binds to an antigen (*e.g.*, one or more CDRs of an antibody). Such antibody fragments can be found in, for example, Harlow and Lane, Antibodies: A Laboratory Manual (1989); Mol. Biology and Biotechnology: A Comprehensive Desk Reference (Myers ed., 1995); Huston *et al.*, 1993, Cell Biophysics 22:189-224; Plückthun and Skerra, 1989, Meth. Enzymol. 178:497-515; and Day, Advanced Immunochimistry (2d ed. 1990). The antibodies provided herein can be of any class (*e.g.*, IgG, IgE, IgM, IgD, and IgA) or any subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) of immunoglobulin molecule. Antibodies may be agonistic antibodies or antagonistic antibodies. Antibodies may be neither agonistic nor antagonistic.

[0034] An “antigen” is a structure to which an antibody can selectively bind. A target antigen may be a polypeptide, carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound. In some embodiments, the target antigen is a polypeptide. In certain embodiments, an antigen is associated with a cell, for example, is present on or in a cell.

[0035] An “intact” antibody is one comprising an antigen-binding site as well as a CL and at least heavy chain constant regions, CH1, CH2 and CH3. The constant regions may include human constant regions or amino acid sequence variants thereof. In certain embodiments, an intact antibody has one or more effector functions.

[0036] The terms “binds” or “binding” refer to an interaction between molecules including, for example, to form a complex. Interactions can be, for example, non-covalent interactions including hydrogen bonds, ionic bonds, hydrophobic interactions, and/or van der Waals interactions. A complex can also include the binding of two or more molecules held together by covalent or non-covalent bonds, interactions, or forces. The strength of the total non-covalent interactions between a single antigen-binding site on an antibody and a single epitope of a target molecule, such as an antigen, is the affinity of the antibody or functional fragment for that epitope. The ratio of dissociation rate (k_{off}) to association rate (k_{on}) of a binding molecule (*e.g.*, an antibody) to a monovalent antigen (k_{off}/k_{on}) is the dissociation constant K_D , which is inversely related to affinity. The lower the K_D value, the higher the

affinity of the antibody. The value of K_D varies for different complexes of antibody and antigen and depends on both k_{on} and k_{off} . The dissociation constant K_D for an antibody provided herein can be determined using any method provided herein or any other method well known to those skilled in the art. The affinity at one binding site does not always reflect the true strength of the interaction between an antibody and an antigen. When complex antigens containing multiple, repeating antigenic determinants, such as a polyvalent antigen, come in contact with antibodies containing multiple binding sites, the interaction of antibody with antigen at one site will increase the probability of a reaction at a second site. The strength of such multiple interactions between a multivalent antibody and antigen is called the avidity.

[0037] In connection with the binding molecules described herein terms such as “bind to,” “that specifically bind to,” and analogous terms are also used interchangeably herein and refer to binding molecules of antigen binding domains that specifically bind to an antigen, such as a polypeptide. A binding molecule or antigen binding domain that binds to or specifically binds to an antigen can be identified, for example, by immunoassays, Octet[®], Biacore[®], or other techniques known to those of skill in the art. In some embodiments, a binding molecule or antigen binding domain binds to or specifically binds to an antigen when it binds to an antigen with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as enzyme linked immunosorbent assay (ELISA). Typically, a specific or selective reaction will be at least twice background signal or noise and may be more than 10 times background. See, *e.g.*, Fundamental Immunology 332-36 (Paul ed., 2d ed. 1989) for a discussion regarding binding specificity. In certain embodiments, the extent of binding of a binding molecule or antigen binding domain to a “non-target” protein is less than about 10% of the binding of the binding molecule or antigen binding domain to its particular target antigen, for example, as determined by fluorescence activated cell sorting (FACS) analysis. A binding molecule or antigen binding domain that binds to an antigen includes one that is capable of binding the antigen with sufficient affinity such that the binding molecule is useful, for example, as a therapeutic and/or diagnostic agent in targeting the antigen. In certain embodiments, a binding molecule or antigen binding domain that binds to an antigen has a dissociation constant (K_D) of less than or equal to 1 μ M, 800 nM, 600 nM, 550 nM, 500 nM, 300 nM, 250 nM, 100 nM, 50 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, or 0.1

nM. In certain embodiments, a binding molecule or antigen binding domain binds to an epitope of an antigen that is conserved among the antigen from different species.

[0038] In certain embodiments, the binding molecules or antigen binding domains can comprise “chimeric” sequences in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (*see* U.S. Pat. No. 4,816,567; and Morrison *et al.*, 1984, Proc. Natl. Acad. Sci. USA 81:6851-55). Chimeric sequences may include humanized sequences.

[0039] In certain embodiments, the binding molecules or antigen binding domains can comprise portions of “humanized” forms of nonhuman (*e.g.*, camelid, murine, non-human primate) antibodies that include sequences from human immunoglobulins (*e.g.*, recipient antibody) in which the native CDR residues are replaced by residues from the corresponding CDR of a nonhuman species (*e.g.*, donor antibody) such as camelid, mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, one or more FR region residues of the human immunoglobulin sequences are replaced by corresponding nonhuman residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. A humanized antibody heavy or light chain can comprise substantially all of at least one or more variable regions, in which all or substantially all of the CDRs correspond to those of a nonhuman immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. In certain embodiments, the humanized antibody will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see*, Jones *et al.*, Nature 321:522-25 (1986); Riechmann *et al.*, Nature 332:323-29 (1988); Presta, Curr. Op. Struct. Biol. 2:593-96 (1992); Carter *et al.*, Proc. Natl. Acad. Sci. USA 89:4285-89 (1992); U.S. Pat. Nos: 6,800,738; 6,719,971; 6,639,055; 6,407,213; and 6,054,297.

[0040] In certain embodiments, the binding molecules or antigen binding domains can comprise portions of a “fully human antibody” or “human antibody,” wherein the terms are used interchangeably herein and refer to an antibody that comprises a human variable region and, for example, a human constant region. The binding molecules may comprise an

antibody sequence. In specific embodiments, the terms refer to an antibody that comprises a variable region and constant region of human origin. “Fully human” antibodies, in certain embodiments, can also encompass antibodies which bind polypeptides and are encoded by nucleic acid sequences which are naturally occurring somatic variants of human germline immunoglobulin nucleic acid sequence. The term “fully human antibody” includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences as described by Kabat *et al.* (See Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). A “human antibody” is one that possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries (Hoogenboom and Winter, *J. Mol. Biol.* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.* 222:581 (1991)) and yeast display libraries (Chao *et al.*, *Nature Protocols* 1: 755-68 (2006)). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, Monoclonal Antibodies and Cancer Therapy 77 (1985); Boerner *et al.*, *J. Immunol.* 147(1):86-95 (1991); and van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, mice (*see, e.g.*, Jakobovits, *Curr. Opin. Biotechnol.* 6(5):561-66 (1995); Brüggemann and Taussing, *Curr. Opin. Biotechnol.* 8(4):455-58 (1997); and U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA* 103:3557-62 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0041] In certain embodiments, the binding molecules or antigen binding domains can comprise portions of a “recombinant human antibody,” wherein the phrase includes human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal (*e.g.*, a mouse or cow) that is transgenic and/or transchromosomal for human immunoglobulin genes (*see, e.g.*, Taylor, L. D. *et al.*, *Nucl. Acids Res.* 20:6287-6295

(1992)) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies can have variable and constant regions derived from human germline immunoglobulin sequences (See Kabat, E. A. *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0042] In certain embodiments, the binding molecules or antigen binding domains can comprise a portion of a “monoclonal antibody,” wherein the term as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts or well-known post-translational modifications such as amino acid isomerization or deamidation, methionine oxidation or asparagine or glutamine deamidation, each monoclonal antibody will typically recognize a single epitope on the antigen. In specific embodiments, a “monoclonal antibody,” as used herein, is an antibody produced by a single hybridoma or other cell. The term “monoclonal” is not limited to any particular method for making the antibody. For example, the monoclonal antibodies useful in the present disclosure may be prepared by the hybridoma methodology first described by Kohler *et al.*, Nature 256:495 (1975), or may be made using recombinant DNA methods in bacterial or eukaryotic animal or plant cells (*see, e.g.*, U.S. Pat. No. 4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, Nature 352:624-28 (1991) and Marks *et al.*, J. Mol. Biol. 222:581-97 (1991), for example. Other methods for the preparation of clonal cell lines and of monoclonal antibodies expressed thereby are well known in the art. *See, e.g.*, Short Protocols in Molecular Biology (Ausubel *et al.* eds., 5th ed. 2002).

[0043] A typical 4-chain antibody unit is a heterotetrametric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one

covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (VH) followed by three constant domains (CH) for each of the α and γ chains and four CH domains for μ and ϵ isotypes. Each L chain has at the N-terminus, a variable domain (VL) followed by a constant domain (CL) at its other end. The VL is aligned with the VH, and the CL is aligned with the first constant domain of the heavy chain (CH1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a VH and VL together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, for example, Basic and Clinical Immunology 71 (Stites *et al.* eds., 8th ed. 1994); and Immunobiology (Janeway *et al.* eds., 5th ed. 2001).

[0044] The term “Fab” or “Fab region” refers to an antibody region that binds to antigens. A conventional IgG usually comprises two Fab regions, each residing on one of the two arms of the Y-shaped IgG structure. Each Fab region is typically composed of one variable region and one constant region of each of the heavy and the light chain. More specifically, the variable region and the constant region of the heavy chain in a Fab region are VH and CH1 regions, and the variable region and the constant region of the light chain in a Fab region are VL and CL regions. The VH, CH1, VL, and CL in a Fab region can be arranged in various ways to confer an antigen binding capability according to the present disclosure. For example, VH and CH1 regions can be on one polypeptide, and VL and CL regions can be on a separate polypeptide, similarly to a Fab region of a conventional IgG. Alternatively, VH, CH1, VL and CL regions can all be on the same polypeptide and oriented in different orders as described in more detail the sections below.

[0045] The term “variable region,” “variable domain,” “V region,” or “V domain” refers to a portion of the light or heavy chains of an antibody that is generally located at the amino-terminal of the light or heavy chain and has a length of about 120 to 130 amino acids in the heavy chain and about 100 to 110 amino acids in the light chain, and are used in the binding and specificity of each particular antibody for its particular antigen. The variable region of the heavy chain may be referred to as “VH.” The variable region of the light chain may be referred to as “VL.” The term “variable” refers to the fact that certain segments of the variable regions differ extensively in sequence among antibodies. The V region mediates antigen binding and defines specificity of a particular antibody for its particular antigen.

However, the variability is not evenly distributed across the 110-amino acid span of the variable regions. Instead, the V regions consist of less variable (*e.g.*, relatively invariant) stretches called framework regions (FRs) of about 15-30 amino acids separated by shorter regions of greater variability (*e.g.*, extreme variability) called “hypervariable regions” that are each about 9-12 amino acids long. The variable regions of heavy and light chains each comprise four FRs, largely adopting a β sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases form part of, the β sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see, e.g., Kabat et al., Sequences of Proteins of Immunological Interest* (5th ed. 1991)). The constant regions are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The variable regions differ extensively in sequence between different antibodies. In specific embodiments, the variable region is a human variable region.

[0046] The term “variable region residue numbering according to Kabat” or “amino acid position numbering as in Kabat”, and variations thereof, refer to the numbering system used for heavy chain variable regions or light chain variable regions of the compilation of antibodies in Kabat *et al., supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, an FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 and three inserted residues (*e.g.*, residues 82a, 82b, and 82c, *etc.* according to Kabat) after residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence. The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (*e.g., Kabat et al., supra*). The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (*e.g., the EU index reported in Kabat et al., supra*). The “EU index as in Kabat” refers to the residue numbering of the human IgG 1 EU antibody. Other numbering systems have been described, for example, by AbM, Chothia, Contact, IMGT, and AHon.

[0047] The term “heavy chain” when used in reference to an antibody refers to a polypeptide chain of about 50-70 kDa, wherein the amino-terminal portion includes a variable region of about 120 to 130 or more amino acids, and a carboxy-terminal portion includes a constant region. The constant region can be one of five distinct types, (*e.g.*, isotypes) referred to as alpha (α), delta (δ), epsilon (ϵ), gamma (γ), and mu (μ), based on the amino acid sequence of the heavy chain constant region. The distinct heavy chains differ in size: α , δ , and γ contain approximately 450 amino acids, while μ and ϵ contain approximately 550 amino acids. When combined with a light chain, these distinct types of heavy chains give rise to five well known classes (*e.g.*, isotypes) of antibodies, IgA, IgD, IgE, IgG, and IgM, respectively, including four subclasses of IgG, namely IgG1, IgG2, IgG3, and IgG4.

[0048] The term “light chain” when used in reference to an antibody refers to a polypeptide chain of about 25 kDa, wherein the amino-terminal portion includes a variable region of about 100 to about 110 or more amino acids, and a carboxy-terminal portion includes a constant region. The approximate length of a light chain is 211 to 217 amino acids. There are two distinct types, referred to as kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains.

[0049] As used herein, the terms “hypervariable region,” “HVR,” “Complementarity Determining Region,” and “CDR” are used interchangeably. A “CDR” refers to one of three hypervariable regions (H1, H2 or H3) within the non-framework region of the immunoglobulin (Ig or antibody) VH β -sheet framework, or one of three hypervariable regions (L1, L2 or L3) within the non-framework region of the antibody VL β -sheet framework. CDR1, CDR2 and CDR3 in VH domain are also referred to as HCDR1, HCDR2 and HCDR3, respectively. CDR1, CDR2 and CDR3 in VL domain are also referred to as LCDR1, LCDR2 and LCDR3, respectively. Accordingly, CDRs are variable region sequences interspersed within the framework region sequences.

[0050] CDR regions are well known to those skilled in the art and have been defined by well-known numbering systems. For example, the Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (*see, e.g.*, Kabat *et al.*, *supra*; Nick Deschacht et al., J Immunol 2010; 184:5696-5704). Chothia refers instead to the location of the structural loops (*see, e.g.*, Chothia and Lesk, J. Mol. Biol. 196:901-17 (1987)). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither

35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software (*see, e.g., Antibody Engineering* Vol. 2 (Kontermann and Dübel eds., 2d ed. 2010)). The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. Another universal numbering system that has been developed and widely adopted is ImMunoGeneTics (IMGT) Information System® (Lafranc *et al.*, Dev. Comp. Immunol. 27(1):55-77 (2003)). IMGT is an integrated information system specializing in immunoglobulins (IG), T-cell receptors (TCR), and major histocompatibility complex (MHC) of human and other vertebrates. Herein, the CDRs are referred to in terms of both the amino acid sequence and the location within the light or heavy chain. As the "location" of the CDRs within the structure of the immunoglobulin variable domain is conserved between species and present in structures called loops, by using numbering systems that align variable domain sequences according to structural features, CDR and framework residues are readily identified. This information can be used in grafting and replacement of CDR residues from immunoglobulins of one species into an acceptor framework from, typically, a human antibody. An additional numbering system (AHon) has been developed by Honegger and Plückthun, J. Mol. Biol. 309: 657-70 (2001). Correspondence between the numbering system, including, for example, the Kabat numbering and the IMGT unique numbering system, is well known to one skilled in the art (*see, e.g., Kabat, supra; Chothia and Lesk, supra; Martin, supra; Lefranc et al., supra*). The residues from each of these hypervariable regions or CDRs are exemplified in Table 1 below.

Table 1. Exemplary CDRs According to Various Numbering Systems

Loop	Kabat	AbM	Chothia	Contact	IMGT
CDR L1	L24--L34	L24--L34	L26--L32 or L24--L34	L30--L36	L27--L38
CDR L2	L50--L56	L50--L56	L50--L52 or L50--L56	L46--L55	L56--L65
CDR L3	L89--L97	L89--L97	L91--L96 or L89--L97	L89--L96	L105-L117
CDR H1	H31--H35B (Kabat Numbering)	H26--H35B	H26-- H32..34	H30--H35B	H27--H38
CDR H1	H31--H35 (Chothia Numbering)	H26--H35	H26--H32	H30--H35	

Loop	Kabat	AbM	Chothia	Contact	IMGT
CDR H2	H50--H65	H50--H58	H53--H55 or H52--H56	H47--H58	H56--H65
CDR H3	H95--H102	H95--H102	H96--H101 or H95-- H102	H93--H101	H105-H117

[0051] The boundaries of a given CDR may vary depending on the scheme used for identification. Thus, unless otherwise specified, the terms “CDR” and “complementary determining region” of a given antibody or region thereof, such as a variable region, as well as individual CDRs (*e.g.*, CDR-H1, CDR-H2) of the antibody or region thereof, should be understood to encompass the complementary determining region as defined by any of the known schemes described herein above. In some instances, the scheme for identification of a particular CDR or CDRs is specified, such as the CDR as defined by the IMGT, Kabat, Chothia, or Contact method. In other cases, the particular amino acid sequence of a CDR is given. It should be noted CDR regions may also be defined by a combination of various numbering systems, *e.g.*, a combination of Kabat and Chothia numbering systems, or a combination of Kabat and IMGT numbering systems. Therefore, the term such as “a CDR1 as set forth in a specific VH” includes any CDR1 as defined by the exemplary CDR numbering systems described above, but is not limited thereby. Once a variable region (*e.g.*, a VH or VL) is given, those skilled in the art would understand that CDRs within the region can be defined by different numbering systems or combinations thereof.

[0052] Hypervariable regions may comprise “extended hypervariable regions” as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 or 89-96 (L3) in the VL, and 26-35 or 26-35A (H1), 50-65 or 49-65 (H2), and 93-102, 94-102, or 95-102 (H3) in the VH.

[0053] The term “constant region” or “constant domain” refers to a carboxy terminal portion of the light and heavy chain which is not directly involved in binding of the antibody to antigen but exhibits various effector function, such as interaction with the Fc receptor. The term refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable region, which contains the antigen binding site. The constant region may contain the CH1, CH2, and CH3 regions of the heavy chain and the CL region of the light chain.

[0054] The term “framework” or “FR” refers to those variable region residues flanking the CDRs. FR residues are present, for example, in chimeric, humanized, human, domain

antibodies, diabodies, linear antibodies, and bispecific antibodies. FR residues are those variable domain residues other than the hypervariable region residues or CDR residues.

[0055] The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including, for example, native sequence Fc regions, recombinant Fc regions, and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is often defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. A “functional Fc region” possesses an “effector function” of a native sequence Fc region. Exemplary “effector functions” include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; downregulation of cell surface receptors (*e.g.*, B cell receptor), *etc.* Such effector functions generally require the Fc region to be combined with a binding region or binding domain (*e.g.*, an antibody variable region or domain) and can be assessed using various assays known to those skilled in the art. A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification (*e.g.*, substituting, addition, or deletion). In certain embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, or from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of a parent polypeptide. The variant Fc region herein can possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, or at least about 90% homology therewith, for example, at least about 95% homology therewith.

[0056] As used herein, an “epitope” is a term in the art and refers to a localized region of an antigen to which a binding molecule (*e.g.*, an antibody) can specifically bind. An epitope can be a linear epitope or a conformational, non-linear, or discontinuous epitope. In the case of a polypeptide antigen, for example, an epitope can be contiguous amino acids of the polypeptide (a “linear” epitope) or an epitope can comprise amino acids from two or more

non-contiguous regions of the polypeptide (a “conformational,” “non-linear” or “discontinuous” epitope). It will be appreciated by one of skill in the art that, in general, a linear epitope may or may not be dependent on secondary, tertiary, or quaternary structure. For example, in some embodiments, a binding molecule binds to a group of amino acids regardless of whether they are folded in a natural three dimensional protein structure. In other embodiments, a binding molecule requires amino acid residues making up the epitope to exhibit a particular conformation (*e.g.*, bend, twist, turn or fold) in order to recognize and bind the epitope.

[0057] “Percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or 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 within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0058] The term “specificity” refers to selective recognition of an antigen binding protein for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. The term “multispecific” as used herein denotes that an antigen binding protein has two or more antigen-binding sites of which at least two bind different antigens. “Bispecific” as used herein denotes that an antigen binding protein has two different antigen-binding specificities. The term “monospecific” antibody as used herein denotes an antigen binding protein that has one or more binding sites each of which bind the same antigen.

[0059] The term “valent” as used herein denotes the presence of a specified number of binding sites in an antigen binding protein. A natural antibody for example or a full length antibody has two binding sites and is bivalent. As such, the terms “trivalent”, “tetravalent”, “pentavalent” and “hexavalent” denote the presence of two binding site, three binding sites, four binding sites, five binding sites, and six binding sites, respectively, in an antigen binding protein.

[0060] The terms “polypeptide” and “peptide” and “protein” are used interchangeably herein and refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid, including but not limited to, unnatural amino acids, as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure may be based upon antibodies or other members of the immunoglobulin superfamily, in certain embodiments, a “polypeptide” can occur as a single chain or as two or more associated chains.

[0061] “Polynucleotide” or “nucleic acid,” as used interchangeably herein, refers to polymers of nucleotides of any length and includes DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. “Oligonucleotide,” as used herein, refers to short, generally single-stranded, synthetic polynucleotides that are generally, but not necessarily, fewer than about 200 nucleotides in length. The terms “oligonucleotide” and “polynucleotide” are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides. A cell that produces a binding molecule of the present disclosure may include a parent hybridoma cell, as well as bacterial and eukaryotic host cells into which nucleic acids encoding the antibodies have been introduced. Unless specified otherwise, the left-hand end of any single-stranded polynucleotide sequence disclosed herein is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 5' to the 5' end of the RNA transcript are referred to as “upstream sequences”; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 3' to the 3' end of the RNA transcript are referred to as “downstream sequences.”

[0062] An “isolated nucleic acid” is a nucleic acid, for example, an RNA, DNA, or a mixed nucleic acids, which is substantially separated from other genome DNA sequences as

well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. An “isolated” nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a specific embodiment, one or more nucleic acid molecules encoding an antibody as described herein are isolated or purified. The term embraces nucleic acid sequences that have been removed from their naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule may include isolated forms of the molecule. Specifically, an “isolated” nucleic acid molecule encoding an antibody described herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced.

[0063] Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0064] The term “control sequences” refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0065] As used herein, the term “operatively linked,” and similar phrases (*e.g.*, genetically fused), when used in reference to nucleic acids or amino acids, refer to the operational linkage of nucleic acid sequences or amino acid sequence, respectively, placed in functional relationships with each other. For example, an operatively linked promoter, enhancer elements, open reading frame, 5' and 3' UTR, and terminator sequences result in the accurate production of a nucleic acid molecule (*e.g.*, RNA). In some embodiments, operatively linked nucleic acid elements result in the transcription of an open reading frame and ultimately the production of a polypeptide (*i.e.*, expression of the open reading frame).

As another example, an operatively linked peptide is one in which the functional domains are placed with appropriate distance from each other to impart the intended function of each domain.

[0066] The term “vector” refers to a substance that is used to carry or include a nucleic acid sequence, including for example, a nucleic acid sequence encoding a binding molecule (*e.g.*, an antibody) as described herein, in order to introduce a nucleic acid sequence into a host cell. Vectors applicable for use include, for example, expression vectors, plasmids, phage vectors, viral vectors, episomes, and artificial chromosomes, which can include selection sequences or markers operable for stable integration into a host cell’s chromosome. Additionally, the vectors can include one or more selectable marker genes and appropriate expression control sequences. Selectable marker genes that can be included, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like, which are well known in the art. When two or more nucleic acid molecules are to be co-expressed (*e.g.*, both an antibody heavy and light chain or an antibody VH and VL), both nucleic acid molecules can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding nucleic acids can be operationally linked to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The introduction of nucleic acid molecules into a host cell can be confirmed using methods well known in the art. Such methods include, for example, nucleic acid analysis such as Northern blots or polymerase chain reaction (PCR) amplification of mRNA, immunoblotting for expression of gene products, or other suitable analytical methods to test the expression of an introduced nucleic acid sequence or its corresponding gene product. It is understood by those skilled in the art that the nucleic acid molecules are expressed in a sufficient amount to produce a desired product and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

[0067] The term “host” as used herein refers to an animal, such as a mammal (*e.g.*, a human).

[0068] The term “host cell” as used herein refers to a particular subject cell that may be transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid

molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0069] The term “transfected” or “transformed” or “transduced” as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[0070] The term “pharmaceutically acceptable” as used herein means being approved by a regulatory agency of the Federal or a state government, or listed in United States Pharmacopeia, European Pharmacopeia, or other generally recognized Pharmacopeia for use in animals, and more particularly in humans.

[0071] “Excipient” means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, solvent, or encapsulating material. Excipients include, for example, encapsulating materials or additives such as absorption accelerators, antioxidants, binders, buffers, carriers, coating agents, coloring agents, diluents, disintegrating agents, emulsifiers, extenders, fillers, flavoring agents, humectants, lubricants, perfumes, preservatives, propellants, releasing agents, sterilizing agents, sweeteners, solubilizers, wetting agents and mixtures thereof. The term “excipient” can also refer to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete or incomplete) or vehicle.

[0072] In some embodiments, excipients are pharmaceutically acceptable excipients. Examples of pharmaceutically acceptable excipients include buffers, such as phosphate, citrate, and other organic acids; antioxidants, including ascorbic acid; low molecular weight (*e.g.*, fewer than about 10 amino acid residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates, including glucose, mannose, or dextrans; chelating agents, such as EDTA; sugar alcohols, such as mannitol or sorbitol; salt-forming counterions, such as sodium; and/or nonionic surfactants, such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™. Other examples of pharmaceutically acceptable excipients are described in Remington and Gennaro, Remington's Pharmaceutical Sciences (18th ed. 1990).

[0073] In one embodiment, each component is “pharmaceutically acceptable” in the sense of being compatible with the other ingredients of a pharmaceutical formulation, and suitable for use in contact with the tissue or organ of humans and animals without excessive

toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. *See, e.g.*, Lippincott Williams & Wilkins: Philadelphia, PA, 2005; Handbook of Pharmaceutical Excipients, 6th ed.; Rowe et al., Eds.; The Pharmaceutical Press and the American Pharmaceutical Association: 2009; Handbook of Pharmaceutical Additives, 3rd ed.; Ash and Ash Eds.; Gower Publishing Company: 2007; Pharmaceutical Preformulation and Formulation, 2nd ed.; Gibson Ed.; CRC Press LLC: Boca Raton, FL, 2009. In some embodiments, pharmaceutically acceptable excipients are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. In some embodiments, a pharmaceutically acceptable excipient is an aqueous pH buffered solution.

[0074] In some embodiments, excipients are sterile liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water is an exemplary excipient when a composition (*e.g.*, a pharmaceutical composition) is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, particularly for injectable solutions. An excipient can also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations, and the like. Oral compositions, including formulations, can include standard excipients such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, *etc.*

[0075] Compositions, including pharmaceutical compounds, may contain a binding molecule (*e.g.*, an antibody), for example, in isolated or purified form, together with a suitable amount of excipients.

[0076] The term “effective amount” or “therapeutically effective amount” as used herein refers to the amount of an antibody or a therapeutic molecule comprising an agent and the antibody or pharmaceutical composition provided herein which is sufficient to result in the desired outcome.

[0077] The terms “subject” and “patient” may be used interchangeably. As used herein, in certain embodiments, a subject is a mammal, such as a non-primate or a primate (*e.g.*,

human). In specific embodiments, the subject is a human. In one embodiment, the subject is a mammal, *e.g.*, a human, diagnosed with a disease or disorder. In another embodiment, the subject is a mammal, *e.g.*, a human, at risk of developing a disease or disorder.

[0078] “Administer” or “administration” refers to the act of injecting or otherwise physically delivering a substance as it exists outside the body into a patient, such as by mucosal, intradermal, intravenous, intramuscular delivery, and/or any other method of physical delivery described herein or known in the art.

[0079] As used herein, the terms “treat,” “treatment” and “treating” refer to the reduction or amelioration of the progression, severity, and/or duration of a disease or condition resulting from the administration of one or more therapies. Treating may be determined by assessing whether there has been a decrease, alleviation and/or mitigation of one or more symptoms associated with the underlying disorder such that an improvement is observed with the patient, despite that the patient may still be afflicted with the underlying disorder. The term “treating” includes both managing and ameliorating the disease. The terms “manage,” “managing,” and “management” refer to the beneficial effects that a subject derives from a therapy which does not necessarily result in a cure of the disease.

[0080] The terms “prevent,” “preventing,” and “prevention” refer to reducing the likelihood of the onset (or recurrence) of a disease, disorder, condition, or associated symptom(s) (*e.g.*, diabetes or a cancer).

[0081] As used herein, “delaying” the development of cancer means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. A method that “delays” development of cancer is a method that reduces probability of disease development in a given time frame and/or reduces the extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of individuals. Cancer development can be detectable using standard methods, including, but not limited to, computerized axial tomography (CAT Scan), Magnetic Resonance Imaging (MRI), abdominal ultrasound, clotting tests, arteriography, or biopsy. Development may also refer to cancer progression that may be initially undetectable and includes occurrence, recurrence, and onset.

[0082] “ACVR2A associated disease or disorder” as used herein refers to a disease or disorder that comprises a cell or tissue in which ACVR2A is expressed or overexpressed. In some embodiments, ACVR2A associated disease or disorder comprises a cell on which ACVR2A is abnormally expressed. In other embodiments, ACVR2A associated disease or disorder comprises a cell in or on which ACVR2A is deficient in at least one of its activities.

[0083] “ACVR2A ligands associated disease or disorder” as used herein refers to a disease or disorder that comprises a cell or tissue in which one or several of ACVR2A ligands, including Activins (such as Activin A, Activin B, Activin AB, Activin C, Activin AC and Activin E), Growth/differentiation factors (GDFs, such as GDF1, GDF3, GDF5, GDF6, GDF7, GDF8, GDF10 and GDF11), bone morphogenetic proteins (BMPs, such as BMP2, BMP4, BMP6, BMP7, BMP8a, BMP8b, BMP9 and BMP10) is expressed or overexpressed. In some embodiments, ACVR2A ligands associated disease or disorder comprises a cell in which one or several of ACVR2A ligands abnormally expressed. In other embodiments, ACVR2A ligands associated disease or disorder comprises a cell in or in which one or several of ACVR2A ligands is deficient in at least one of its activities. In other embodiments, ACVR2A ligands associated disease or disorder comprises one or several of ACVR2A ligands protein level in serum, plasma or blood is abnormal.

[0084] The terms “about” and “approximately” mean within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, within 1%, or less of a given value or range.

[0085] As used in the present disclosure and claims, the singular forms “a”, “an” and “the” include plural forms unless the context clearly dictates otherwise.

[0086] It is understood that wherever embodiments are described herein with the term “comprising” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided. It is also understood that wherever embodiments are described herein with the phrase “consisting essentially of” otherwise analogous embodiments described in terms of “consisting of” are also provided.

[0087] The term “between” as used in a phrase as such “between A and B” or “between A-B” refers to a range including both A and B.

[0088] The term “and/or” as used in a phrase such as “A and/or B” herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following

embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

5.2. ACVR2A Binding Molecules

5.2.1. Antibodies that Bind to ACVR2A

[0089] In one aspect, provided herein are antibodies capable of binding to ACVR2A. ACVR2A is a receptor that mediates the functions of activins, which are members of the transforming growth factor-beta (TGF-beta) superfamily involved in diverse biological processes. ACVR2A is a transmembrane serine-threonine kinase receptor which mediates signaling by forming heterodimeric complexes with various combinations of type I and type II receptors and ligands in a cell-specific manner. The type II receptor is primarily involved in ligand-binding and includes an extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic serine-threonine kinase domain. Nucleic acid and amino acid sequences of ACVR2A are known (see GCID:GC02P147844, HGNC: 173, NCBI Entrez Gene: 92, Ensembl: ENSG00000121989, OMIM®: 102581, and UniProtKB/Swiss-Prot: P27037). In some embodiments, the antibodies provided herein bind to human ACVR2A. In some embodiments, the anti-ACVR2A antibody provided herein modulates one or more ACVR2A activities. In some embodiments, the anti-ACVR2A antibody provided herein is an antagonist antibody.

[0090] In one embodiment, the antibodies according to the disclosure are ACVR2A antagonists with no or low agonistic activity. In another embodiment, the antibody or functional fragment comprising an antigen-binding portion binds the target protein ACVR2A and decreases the binding of Activin A to ACVR2A to a basal level. In one aspect of this embodiment, the antibody or functional fragment reduces the amount of Activin A that binds to ACVR2A. In a further aspect of this embodiment, the antibody or functional fragment completely prevents Activin A from binding to ACVR2A. In a further embodiment, the antibody or functional fragment inhibits Smad activation. An antibody that inhibits one or more of these ACVR2A functional properties (e.g. biochemical, immunochemical, cellular, physiological or other biological activities, or the like) as determined according to methodologies known to the art and described herein, will be understood to relate to a statistically significant decrease in the particular activity relative to that seen in the absence of the antibody (e.g. or when a control antibody of irrelevant specificity is present). In some embodiments, an antibody that inhibits ACVR2A activity effects such a statistically significant decrease by at least 10% of the measured parameter, by at least 50%, 80% or 90%,

and in certain embodiments an antibody of the disclosure may inhibit greater than 95%, 98% or 99% of ACVR2A functional activity.

[0091] In one embodiment, the antibodies of the disclosure do not cross-react with an ACVR2A related protein, and more particularly do not cross-react with human ACVR2B (NP—001607.1, GI:4501897). In one embodiment, the antibodies of the disclosure in one embodiment bind to ACVR2A rather than ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 10-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 20-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 30-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 40-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 50-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 60-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 70-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 80-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 90-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with 100-fold greater affinity than they bind to ACVR2B. In one embodiment, the antibodies of the disclosure bind to ACVR2A with more than 100-fold greater affinity than they bind to ACVR2B, such as 500-fold or 1000-fold.

[0092] In some embodiments, the anti-ACVR2A antibody provided herein binds to ACVR2A (*e.g.*, human ACVR2A) with a dissociation constant (K_D) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (*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). A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present disclosure, including by RIA, for example, performed with the Fab version of an antibody of interest and its antigen (Chen et al., 1999, J. Mol Biol 293:865-81); by biolayer interferometry (BLI) or surface plasmon resonance (SPR) assays by Octet®, using, for example, an Octet®Red96 system, or by Biacore®, using, for example, a Biacore®TM-2000 or a Biacore®TM-3000. An “on-rate” or “rate of association” or “association rate” or “kon” may also be determined

with the same biolayer interferometry (BLI) or surface plasmon resonance (SPR) techniques described above using, for example, the Octet®Red96, the Biacore®TM-3000, or the Biacore®TM-8000 system.

[0093] In some embodiments, the anti-ACVR2A antibodies provided herein are those described in Section 7 below. Thus, in some embodiments, the antibody provided herein comprises one or more CDR sequences of any one of SEQ ID NOs: 1-41. CDR sequences can be determined according to well-known numbering systems. In some embodiments, the CDRs are according to IMGT numbering. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In other embodiments, the CDRs are according to Chothia numbering. In other embodiments, the CDRs are according to Contact numbering. In some embodiments, the anti-ACVR2A antibody is humanized. In some embodiments, the anti-ACVR2A antibody comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[0094] In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 1. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 3. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 4. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 5. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 7. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 8. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 9. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 10. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 11. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 12. In some embodiments, the anti-ACVR2A antibody provided herein comprises HCDR1, HCDR2, and HCDR3 as set forth in SEQ ID NO: 13. CDR sequences can be determined according to well-known numbering systems or a combination thereof. In some

embodiments, the CDRs are according to IMGT numbering. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In other embodiments, the CDRs are according to Chothia numbering. In other embodiments, the CDRs are according to Contact numbering.

[0095] In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 6. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 14. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 15. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 16. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 17. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 18. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 19. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 20. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 21. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 22. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 23. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 24. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 25. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 26. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 27. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 28. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 29. In some embodiments, the anti-ACVR2A antibody

provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 30. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 31. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 32. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 33. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 34. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 35. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 36. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 37. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 38. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 39. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 40. In some embodiments, the anti-ACVR2A antibody provided herein comprises LCDR1, LCDR2, and LCDR3 as set forth in SEQ ID NO: 41. CDR sequences can be determined according to well-known numbering systems or a combination thereof. In some embodiments, the CDRs are according to IMGT numbering. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In other embodiments, the CDRs are according to Chothia numbering. In other embodiments, the CDRs are according to Contact numbering.

[0096] In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 1, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 3, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 4, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and

an HCDR3 as set forth in SEQ ID NO: 5, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 8, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 10, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 13, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 14. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 15. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 16. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 17. In some embodiments, the antibody or antigen binding fragment provided

herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 18. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 19. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 20. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 21. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 22. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 23. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 24. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 25. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 26. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 27. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 28. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO:

29. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 30. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 31. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 32. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 33. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 34. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 35. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 36. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 37. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 38. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 39. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 40. In some embodiments, the antibody or antigen binding fragment provided herein comprises an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 41. CDR sequences can be determined according to well-known numbering systems or a combination thereof. In some embodiments, the CDRs

are according to IMGT numbering. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In other embodiments, the CDRs are according to Chothia numbering. In other embodiments, the CDRs are according to Contact numbering.

[0097] In other embodiments, provided herein is an antibody that binds to ACVR2A comprising an HCDR1 comprising an amino acid sequence having at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any of SEQ ID NOS: 42, 48, 49, 50, 54, 55, 56, 57, and 58; (ii) an HCDR2 comprising an amino acid sequence having at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any of SEQ ID NOS: 43 and 51, (iii) an HCDR3 comprising an amino acid sequence having at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 44; (iv) a LCDR1 comprising an amino acid sequence having at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any of SEQ ID NOS: 45, 52, 59, 60, 61, 62, 63, and 64; (v) a LCDR2 comprising an amino acid sequence having at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any of SEQ ID NOS: 46, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, and 82; and/or (vi) a LCDR3 comprising an amino acid sequence having at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to any of SEQ ID NOS: 47 and 53. In some embodiments, the anti-ACVR2A antibody is humanized. In some embodiments, the anti-ACVR2A antibody comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[0098] In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 42, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO:

44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 49, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 50, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 49, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 55, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of

SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 58, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 60, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of

SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 61, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 62, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 63, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 82, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 64, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of

SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 65, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 66, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 67, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 68, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 69, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of

SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 70, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 71, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 72, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 73, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 74, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 75, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of

SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 67, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 68, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 76, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 77, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 78, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 79, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of

SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 69, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 80, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 70, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47. In some specific embodiments, in the antibody or antigen binding fragment provided herein, the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 81, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47.

[0099] In some embodiments, the antibody further comprises one or more framework regions of SEQ ID NOs: 1-41. In some embodiments, the antibody provided herein is a humanized antibody. Framework regions described herein are determined based upon the boundaries of the CDR numbering system. In other words, if the CDRs are determined by, *e.g.*, Kabat, IMGT, or Chothia, then the framework regions are the amino acid residues surrounding the CDRs in the variable region in the format, from the N-terminus to C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. For example, FR1 is defined as the amino acid residues N-terminal to the CDR1 amino acid residues as defined by, *e.g.*, the Kabat numbering system, the IMGT numbering system, or the Chothia numbering system, FR2 is defined as the amino acid residues between CDR1 and CDR2 amino acid residues as defined by, *e.g.*, the Kabat numbering system, the IMGT numbering system, or the Chothia numbering system, FR3 is defined as the amino acid residues between CDR2 and CDR3 amino acid residues as defined by, *e.g.*, the Kabat numbering system, the IMGT numbering system, or the Chothia numbering system, and FR4 is defined as the amino acid residues C-

terminal to the CDR3 amino acid residues as defined by, *e.g.*, the Kabat numbering system, the IMGT numbering system, or the Chothia numbering system.

[00100] In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 1, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 3, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 4, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 5, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 8, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 10, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 13, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH

comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 15. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 18. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 19. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 21. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 24. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID

NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 28. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 29. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 30. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 31. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 32. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 33. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 34. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 36. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 38. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid

sequence of SEQ ID NO: 39. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 40. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 41.

[00101] In certain embodiments, an antibody described herein or an antigen binding fragment thereof comprises amino acid sequences with certain percent identity relative to any antibody provided herein, for example, those described in Section 7 below.

[00102] The determination of percent identity between two sequences (*e.g.*, amino acid sequences or nucleic acid sequences) can be accomplished using a mathematical algorithm. A non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. U.S.A.* 87:2264-2268 (1990), modified as in Karlin and Altschul, *Proc. Natl. Acad. Sci. U.S.A.* 90:5873-5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, *J. Mol. Biol.* 215:403 (1990). BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, word length=12 to obtain nucleotide sequences homologous to a nucleic acid molecules described herein. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, word length=3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997). Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov). Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, *CABIOS* 4:11-17 (1998). Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. The percent identity between two sequences can be determined using techniques similar to those described above, with or

[00103] In some embodiments, the antibody provided herein contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the anti-ACVR2A antibody comprising that sequence retains the ability to bind to ACVR2A. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in a reference amino acid sequence. In some embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (*i.e.*, in the FRs). Optionally, the anti-ACVR2A antibody provided herein includes post-translational modifications of a reference sequence.

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96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 5, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 8, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 9, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 10, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least

91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 11, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 13, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH

domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 18. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 19. In some embodiments, the

antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 20. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino

acid sequence of SEQ ID NO: 24. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 27. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7.

97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 29. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 11, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 30. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 31. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 32. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least

92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 33. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 9, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 34. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 11, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 35. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 11, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 36. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 11, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 37. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain

having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 38. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 39. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 12, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 40. In some embodiments, the antibody or antigen binding fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 9, and a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 41. In all the embodiments described above, the antibodies bind to ACVR2A.

[00105] In some embodiments, functional epitopes can be mapped, *e.g.*, by combinatorial alanine scanning, to identify amino acids in the ACVR2A protein that are necessary for interaction with anti-ACVR2A antibodies provided herein. In some embodiments, conformational and crystal structure of anti-ACVR2A antibody bound to ACVR2A may be employed to identify the epitopes. In some embodiments, the present disclosure provides an antibody that specifically binds to the same epitope as any of the anti-ACVR2A antibodies provided herein. For example, in some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 1, and a VL comprising the amino

acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 3, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 4, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 5, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 8, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 10, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 13, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL

comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 15. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 18. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 19. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 21. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL

comprising the amino acid sequence of SEQ ID NO: 24. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 28. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 29. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 30. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 31. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 32. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 33. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid

sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 34. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 36. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 38. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 39. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 40. In some embodiments, the antibody or antigen binding fragment provided herein binds to the same epitope as an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 41.

[00106] In some embodiments, provided herein is an anti-ACVR2A antibody, or antigen binding fragment thereof, that specifically binds to ACVR2A competitively with any one of the anti-ACVR2A antibodies described herein. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 1, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 3, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein

specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 4, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 5, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 8, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 10, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 13, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the antibody or antigen binding

fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 15. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 18. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 19. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 21. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments,

the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 24. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 28. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 29. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 30. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 31. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of

SEQ ID NO: 32. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 33. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 34. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 36. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 38. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 39. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 40. In some embodiments, the antibody or antigen binding fragment provided herein specifically binds to ACVR2A competitively with an anti-ACVR2A antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 41.

[00107] In some embodiments, provided herein is a ACVR2A binding protein comprising any one of the anti-ACVR2A antibodies described above. In some embodiments, the

ACVR2A binding protein is a monoclonal antibody, including a mouse, chimeric, humanized or human antibody. In some embodiments, the anti-ACVR2A antibody is an antibody fragment, *e.g.*, a scFv. In some embodiments, the ACVR2A binding protein is a fusion protein comprising the anti-ACVR2A antibody provided herein. In other embodiments, the ACVR2A binding protein is a multispecific antibody comprising the anti-ACVR2A antibody provided herein. Other exemplary ACVR2A binding molecules are described in more detail in the following sections.

[00108] In some embodiments, the anti-ACVR2A antibody or antigen binding protein according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 5.2.2 to 5.2.6 below.

5.2.2. Antibody Fragments

[00109] As used herein, the term “antibody” also includes various antibody fragments thereof. Antibodies provided herein include, but are not limited to, immunoglobulin molecules and immunologically active portions of immunoglobulin molecules. The immunoglobulin molecules provided herein can be of any class (*e.g.*, IgG, IgE, IgM, IgD, and IgA) or any subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) of immunoglobulin molecule. In some embodiments, the antibody is an IgG antibody. In some embodiments, the IgG antibody is an IgG1 antibody. In some embodiments, the IgG antibody is an IgG2, IgG3, or IgG4 antibody.

[00110] Variants and derivatives of antibodies include antibody functional fragments that retain the ability to bind to an antigen. Exemplary functional fragments include Fab fragments (*e.g.*, an antibody fragment that contains the antigen-binding domain and comprises a light chain and part of a heavy chain bridged by a disulfide bond); Fab' (*e.g.*, an antibody fragment containing a single antigen-binding domain comprising an Fab and an additional portion of the heavy chain through the hinge region); F(ab')₂ (*e.g.*, two Fab' molecules joined by interchain disulfide bonds in the hinge regions of the heavy chains; the Fab' molecules may be directed toward the same or different epitopes); a bispecific Fab (*e.g.*, a Fab molecule having two antigen binding domains, each of which may be directed to a different epitope); a single chain comprising a variable region, also known as, scFv (*e.g.*, the variable, antigen-binding determinative region of a single light and heavy chain of an antibody linked together by a chain of, *e.g.*, 10-25 amino acids); a disulfide-linked Fv, or dsFv (*e.g.*, the variable, antigen-binding determinative region of a single light and heavy chain of an antibody linked together by a disulfide bond); a camelized VH (*e.g.*, the variable,

antigen-binding determinative region of a single heavy chain of an antibody in which some amino acids at the VH interface are those found in the heavy chain of naturally occurring camel antibodies); a bispecific scFv (*e.g.*, an scFv or a dsFv molecule having two antigen-binding domains, each of which may be directed to a different epitope); a diabody (*e.g.*, a dimerized scFv formed when the VH domain of a first scFv assembles with the VL domain of a second scFv and the VL domain of the first scFv assembles with the VH domain of the second scFv; the two antigen-binding regions of the diabody may be directed towards the same or different epitopes); a triabody (*e.g.*, a trimerized scFv, formed in a manner similar to a diabody, but in which three antigen-binding domains are created in a single complex; the three antigen binding domains may be directed towards the same or different epitopes); and a tetrabody (*e.g.*, a tetramerized scFv, formed in a manner similar to a diabody, but in which four antigen-binding domains are created in a single complex; the four antigen binding domains may be directed towards the same or different epitopes).

[00111] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, 1992, J. Biochem. Biophys. Methods 24:107-17; and Brennan *et al.*, 1985, Science 229:81-83). However, these fragments can now be produced directly by recombinant host cells. For example, Fab, Fv, and scFv antibody fragments can all be expressed in and secreted from *E. coli* or yeast cells, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter *et al.*, 1992, Bio/Technology 10:163-67). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')₂ fragment with increased *in vivo* half-life comprising salvage receptor binding epitope residues are described in, for example, U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv) (see, *e.g.*, WO 93/16185; U.S. Pat. Nos. 5,571,894 and 5,587,458). Fv and scFv have intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during *in vivo* use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv (See, *e.g.*, Borrebaeck ed., *supra*). The antibody fragment may also be a "linear

antibody,” for example, as described in the references cited above. Such linear antibodies may be monospecific or multi-specific, such as bispecific.

5.2.3. Antibody Variants

[00112] In some embodiments, amino acid sequence modification(s) of the antibodies that bind to ACVR2A described herein are contemplated. For example, it may be desirable to optimize the binding affinity and/or other biological properties of the antibody, including but not limited to specificity, thermostability, expression level, effector functions, glycosylation, reduced immunogenicity, or solubility. Thus, in addition to the antibodies that bind to ACVR2A described herein, it is contemplated that variants of the antibodies that bind to ACVR2A described herein can be prepared. For example, antibody variants can be prepared by introducing appropriate nucleotide changes into the encoding DNA, and/or by synthesis of the desired antibody or polypeptide. Those skilled in the art who appreciate that amino acid changes may alter post-translational processes of the antibody.

Chemical Modifications

[00113] In some embodiments, the antibodies provided herein are chemically modified, for example, by the covalent attachment of any type of molecule to the antibody. The antibody derivatives may include antibodies that have been chemically modified, for example, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, or conjugation to one or more immunoglobulin domains (*e.g.*, Fc or a portion of an Fc). Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formulation, metabolic synthesis of tunicamycin, *etc.* Additionally, the antibody may contain one or more non-classical amino acids.

[00114] In some embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[00115] When the antibody provided herein is fused to an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally 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 may include various carbohydrates, *e.g.*, mannose, N-acetyl

glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in the binding molecules provided herein may be made in order to create variants with certain improved properties.

[00116] In other embodiments, when the antibody provided herein is fused to an Fc region, antibody variants provided herein may have a carbohydrate structure that lacks fucose attached (directly or indirectly) to said 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 Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); 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 may have improved ADCC function. *See, e.g.*, US Patent Publication Nos. US 2003/0157108 and US 2004/0093621. Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Patent Application No. US 2003/0157108; and WO 2004/056312, and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[00117] The binding molecules comprising an antibody provided herein are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region is bisected by GlcNAc. Such variants may have reduced fucosylation and/or improved ADCC function. Examples of such variants are described, *e.g.*,

in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such variants may have improved CDC function. Such variants are described, *e.g.*, in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

[00118] In molecules that comprise the present antibody and an Fc region, one or more amino acid modifications may be introduced into the Fc region, thereby generating an Fc region variant. 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.

[00119] In some embodiments, the present application contemplates variants that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the binding molecule *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 binding molecule 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. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see, for example*, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo, e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see, for example*, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie,

Blood 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[00120] Binding molecules with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[00121] Certain variants with improved or diminished binding to FcRs are described. (*See, e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[00122] In some embodiments, a variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues). In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[00123] Binding molecules with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those molecules comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants 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, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[00124] In some embodiments, it may be desirable to create cysteine engineered antibodies, 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. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to

other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein.

Substitutions, Deletions, or Insertions

[00125] Variations may be a substitution, deletion, or insertion of one or more codons encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the original antibody or polypeptide. Sites of interest for substitutional mutagenesis include the CDRs and FRs.

[00126] Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *e.g.*, conservative amino acid replacements. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule provided herein, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid substitutions. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. In certain embodiments, the substitution, deletion, or insertion includes fewer than 25 amino acid substitutions, fewer than 20 amino acid substitutions, fewer than 15 amino acid substitutions, fewer than 10 amino acid substitutions, fewer than 5 amino acid substitutions, fewer than 4 amino acid substitutions, fewer than 3 amino acid substitutions, or fewer than 2 amino acid substitutions relative to the original molecule. In a specific embodiment, the substitution is a conservative amino acid substitution made at one or more predicted non-essential amino acid residues. The variation allowed may be determined by systematically making insertions, deletions, or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the parental antibodies.

[00127] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing multiple residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue.

[00128] Antibodies generated by conservative amino acid substitutions are included in the present disclosure. In a conservative amino acid substitution, an amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. As described above, families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar

side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined. Conservative (*e.g.*, within an amino acid group with similar properties and/or side chains) substitutions may be made, so as to maintain or not significantly change the properties. Exemplary substitutions are shown in Table 2 below.

Table 2. Amino Acid Substitutions

Original Residue	Exemplary Substitutions	Original Residue	Exemplary Substitutions
Ala (A)	Val; Leu; Ile	Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Arg (R)	Lys; Gln; Asn	Lys (K)	Arg; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg	Met (M)	Leu; Phe; Ile
Asp (D)	Glu; Asn	Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Cys (C)	Ser; Ala	Pro (P)	Ala
Gln (Q)	Asn; Glu	Ser (S)	Thr
Glu (E)	Asp; Gln	Thr (T)	Val; Ser
Gly (G)	Ala	Trp (W)	Tyr; Phe
His (H)	Asn; Gln; Lys; Arg	Tyr (Y)	Trp; Phe; Thr; Ser
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[00129] Amino acids may be grouped according to similarities in the properties of their side chains (*see, e.g.*, Lehninger, Biochemistry 73-75 (2d ed. 1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); and (4) basic: Lys (K), Arg (R), His(H). Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala,

Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. For example, any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, for example, with another amino acid, such as alanine or serine, to improve the oxidative stability of the molecule and to prevent aberrant crosslinking. Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[00130] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[00131] Alterations (*e.g.*, substitutions) may be made in CDRs, *e.g.*, to improve antibody affinity. Such alterations may be made in CDR “hotspots,” *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant antibody or fragment thereof being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (*e.g.*, 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. More detailed description regarding affinity maturation is provided in the section below.

[00132] In some embodiments, substitutions, insertions, or deletions may occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. In some embodiments of the variant antibody sequences provided herein, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[00133] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells, *Science*, 244:1081-1085 (1989). In this method, a residue or group of target residues (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. 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.

[00134] Amino acid sequence insertions 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 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.

[00135] The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis (*see, e.g.*, Carter, *Biochem J.* 237:1-7 (1986); and Zoller *et al.*, *Nucl. Acids Res.* 10:6487-500 (1982)), cassette mutagenesis (*see, e.g.*, Wells *et al.*, *Gene* 34:315-23 (1985)), or other known techniques can be performed on the cloned DNA to produce the antibody variant DNA.

5.2.4. *In vitro* Affinity Maturation

[00136] In some embodiments, antibody variants having an improved property such as affinity, stability, or expression level as compared to a parent antibody may be prepared by *in vitro* affinity maturation. Like the natural prototype, *in vitro* affinity maturation is based on the principles of mutation and selection. Libraries of antibodies are displayed on the surface of an organism (*e.g.*, phage, bacteria, yeast, or mammalian cell) or in association (*e.g.*, covalently or non-covalently) with their encoding mRNA or DNA. Affinity selection of the displayed antibodies allows isolation of organisms or complexes carrying the genetic information encoding the antibodies. Two or three rounds of mutation and selection using display methods such as phage display usually results in antibody fragments with affinities in the low nanomolar range. Affinity matured antibodies can have nanomolar or even picomolar affinities for the target antigen.

[00137] Phage display is a widespread method for display and selection of antibodies. The antibodies are displayed on the surface of Fd or M13 bacteriophages as fusions to the bacteriophage coat protein. Selection involves exposure to antigen to allow phage-displayed antibodies to bind their targets, a process referred to as “panning.” Phage bound to antigen are recovered and used to infect bacteria to produce phage for further rounds of selection. For review, see, for example, Hoogenboom, *Methods. Mol. Biol.* 178:1-37 (2002); and Bradbury and Marks, *J. Immunol. Methods* 290:29-49 (2004).

[00138] In some embodiments, mammalian display systems may be used.

[00139] Diversity may also be introduced into the CDRs of the antibody libraries in a targeted manner or via random introduction. The former approach includes sequentially targeting all the CDRs of an antibody via a high or low level of mutagenesis or targeting isolated hot spots of somatic hypermutations (*see, e.g.*, Ho *et al.*, *J. Biol. Chem.* 280:607-17 (2005)) or residues suspected of affecting affinity on experimental basis or structural reasons. Diversity may also be introduced by replacement of regions that are naturally diverse via DNA shuffling or similar techniques (*see, e.g.*, Lu *et al.*, *J. Biol. Chem.* 278:43496-507 (2003); U.S. Pat. Nos. 5,565,332 and 6,989,250). Alternative techniques target hypervariable loops extending into framework-region residues (*see, e.g.*, Bond *et al.*, *J. Mol. Biol.* 348:699-709 (2005)) employ loop deletions and insertions in CDRs or use hybridization-based diversification (*see, e.g.*, U.S. Pat. Publication No. 2004/0005709). Additional methods of generating diversity in CDRs are disclosed, for example, in U.S. Pat. No. 7,985,840. Further methods that can be used to generate antibody libraries and/or antibody affinity maturation are disclosed, *e.g.*, in U.S. Patent Nos. 8,685,897 and 8,603,930, and U.S. Publ. Nos.

2014/0170705, 2014/0094392, 2012/0028301, 2011/0183855, and 2009/0075378, each of which are incorporated herein by reference.

[00140] Screening of the libraries can be accomplished by various techniques known in the art. For example, antibodies can be immobilized onto solid supports, columns, pins, or cellulose/poly (vinylidene fluoride) membranes/other filters, expressed on host cells affixed to adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads or used in any other method for panning display libraries.

[00141] For review of *in vitro* affinity maturation methods, see, *e.g.*, Hoogenboom, *Nature Biotechnology* 23:1105-16 (2005); Quiroz and Sinclair, *Revista Ingenieria Biomedica* 4:39-51 (2010); and references therein.

5.2.5. Modifications of Antibodies

[00142] Covalent modifications of antibodies are included within the scope of the present disclosure. Covalent modifications include reacting targeted amino acid residues of an antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the antibody. Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains (*see, e.g.*, Creighton, Proteins: Structure and Molecular Properties 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[00143] Other types of covalent modification of the antibody included within the scope of this present disclosure include altering the native glycosylation pattern of the antibody or polypeptide as described above (*see, e.g.*, Beck *et al.*, *Curr. Pharm. Biotechnol.* 9:482-501 (2008); and Walsh, *Drug Discov. Today* 15:773-80 (2010)), and linking the antibody to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth, for example, in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; or 4,179,337. The antibody that binds to ACVR2A of the disclosure may also be genetically fused or conjugated to one or more immunoglobulin constant regions or portions thereof (*e.g.*, Fc) to extend half-life and/or to impart known Fc-mediated effector functions.

[00144] The antibody that binds to ACVR2A of the present disclosure may also be modified to form chimeric molecules comprising the antibody that binds to ACVR2A fused

to another, heterologous polypeptide or amino acid sequence, for example, an epitope tag (*see, e.g.*, Terpe, Appl. Microbiol. Biotechnol. 60:523-33 (2003)) or the Fc region of an IgG molecule (*see, e.g.*, Aruffo, Antibody Fusion Proteins 221-42 (Chamow and Ashkenazi eds., 1999)).

[00145] Also provided herein are fusion proteins comprising the antibody that binds to ACVR2A of the disclosure and a heterologous polypeptide. In some embodiments, the heterologous polypeptide to which the antibody is genetically fused or chemically conjugated is useful for targeting the antibody to cells having cell surface-expressed ACVR2A.

[00146] Also provided herein are panels of antibodies that bind to an ACVR2A antigen. In specific embodiments, the panels of antibodies have different association rates, different dissociation rates, different affinities for an ACVR2A antigen, and/or different specificities for an ACVR2A antigen. In some embodiments, the panels comprise or consist of about 10 to about 1000 antibodies or more. Panels of antibodies can be used, for example, in 96-well or 384-well plates, for assays such as ELISAs.

5.2.6. Other Binding Molecules Comprising the Antibodies

[00147] In another aspect, provided herein is a binding molecule comprising an anti-ACVR2A antibody provided herein. In some embodiments, an antibody against ACVR2A provided herein is part of other binding molecules. Exemplary binding molecules of the present disclosure are described herein.

Fusion Protein

[00148] In various embodiments, the antibody provided herein can be genetically fused or chemically conjugated to another agent, for example, protein-based entities. The antibody may be chemically-conjugated to the agent, or otherwise non-covalently conjugated to the agent. The agent can be a peptide or antibody (or a fragment thereof).

[00149] Thus, in some embodiments, provided herein are antibodies that are recombinantly fused or chemically conjugated (covalent or non-covalent conjugations) to a heterologous protein or polypeptide (or fragment thereof, for example, to a polypeptide of about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 150, about 200, about 250, about 300, about 350, about 400, about 450 or about 500 amino acids, or over 500 amino acids) to generate fusion proteins, as well as uses thereof. In particular, provided herein are fusion proteins comprising an antigen binding fragment of the antibody provided herein (*e.g.*, CDR1, CDR2, and/or CDR3) and a heterologous protein, polypeptide, or peptide.

[00150] Moreover, antibodies provided herein can be fused to marker or “tag” sequences, such as a peptide, to facilitate purification. In specific embodiments, the marker or tag amino acid sequence is a hexa-histidine peptide, hemagglutinin (“HA”) tag, and “FLAG” tag.

[00151] Methods for fusing or conjugating moieties (including polypeptides) to antibodies are known (see, *e.g.*, Arnon et al., *Monoclonal Antibodies for Immunotargeting of Drugs in Cancer Therapy*, in *Monoclonal Antibodies and Cancer Therapy* 243-56 (Reisfeld et al. eds., 1985); Hellstrom et al., *Antibodies for Drug Delivery*, in *Controlled Drug Delivery* 623-53 (Robinson et al. eds., 2d ed. 1987); Thorpe, *Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review*, in *Monoclonal Antibodies: Biological and Clinical Applications* 475-506 (Pinchera et al. eds., 1985); Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy, in *Monoclonal Antibodies for Cancer Detection and Therapy* 303-16 (Baldwin et al. eds., 1985); Thorpe et al., *Immunol. Rev.* 62:119-58 (1982); U.S. Pat. Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,723,125; 5,783,181; 5,908,626; 5,844,095; and 5,112,946; EP 307,434; EP 367,166; EP 394,827; PCT publications WO 91/06570, WO 96/04388, WO 96/22024, WO 97/34631, and WO 99/04813; Ashkenazi et al., *Proc. Natl. Acad. Sci. USA*, 88: 10535-39 (1991); Traunecker et al., *Nature*, 331:84-86 (1988); Zheng et al., *J. Immunol.* 154:5590-600 (1995); and Vil et al., *Proc. Natl. Acad. Sci. USA* 89:11337-41 (1992)).

[00152] Fusion proteins may be generated, for example, through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as “DNA shuffling”). DNA shuffling may be employed to alter the activities of the antibodies as provided herein, including, for example, antibodies with higher affinities and lower dissociation rates (see, *e.g.*, U.S. Pat. Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458; Patten et al., *Curr. Opin. Biotechnol.* 8:724-33 (1997); Harayama, *Trends Biotechnol.* 16(2):76-82 (1998); Hansson et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques* 24(2):308-13 (1998)). Antibodies, or the encoded antibodies, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion, or other methods prior to recombination. A polynucleotide encoding an antibody provided herein may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[00153] In some embodiments, an antibody provided herein is conjugated to a second antibody to form an antibody heteroconjugate.

[00154] In various embodiments, the antibody is genetically fused to the agent. Genetic fusion may be accomplished by placing a linker (e.g., a polypeptide) between the antibody and the agent. The linker may be a flexible linker.

[00155] In various embodiments, the antibody is genetically conjugated to a therapeutic molecule, with a hinge region linking the antibody to the therapeutic molecule.

[00156] Also provided herein are methods for making the various fusion proteins provided herein. The various methods described in Section 5.4 may also be utilized to make the fusion proteins provided herein.

[00157] In a specific embodiment, the fusion protein provided herein is recombinantly expressed. Recombinant expression of a fusion protein provided herein may require construction of an expression vector containing a polynucleotide that encodes the protein or a fragment thereof. Once a polynucleotide encoding a protein provided herein or a fragment thereof has been obtained, the vector for the production of the molecule may be produced by recombinant DNA technology using techniques well-known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Also provided are replicable vectors comprising a nucleotide sequence encoding a fusion protein provided herein, or a fragment thereof, or a CDR, operably linked to a promoter.

[00158] The expression vector can be transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce a fusion protein provided herein. Thus, also provided herein are host cells containing a polynucleotide encoding a fusion protein provided herein or fragments thereof operably linked to a heterologous promoter.

[00159] A variety of host-expression vector systems may be utilized to express the fusion protein provided herein. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express a fusion protein provided herein *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors

containing coding sequences; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV, tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Bacterial cells such as *Escherichia coli*, or, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, can be used for the expression of a recombinant fusion protein. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies or variants thereof. In a specific embodiment, the expression of nucleotide sequences encoding the fusion proteins provided herein is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00160] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the fusion protein being expressed. For example, when a large quantity of such a fusion protein is to be produced, for the generation of pharmaceutical compositions of a fusion protein, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, EMBO 12:1791 (1983)), in which the coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00161] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region EI or E3) will result in a recombinant virus that is viable and capable of expressing the fusion protein in infected hosts (*e.g.*, see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, *etc.* (see, *e.g.*, Bittner *et al.*, Methods in Enzymol. 153:51-544 (1987)).

[00162] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT20 and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7030 and HsS78Bst cells.

[00163] For long-term, high-yield production of recombinant proteins, stable expression can be utilized. For example, cell lines which stably express the fusion proteins may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation

sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the fusion protein. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the binding molecule.

[00164] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, Cell 11:223 (1977)), hypoxanthineguanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy *et al.*, Cell 22:8-17 (1980)) genes can be employed in tk-, hgp^rt- or ap^rt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler *et al.*, Natl. Acad. Sci. USA 77:357 (1980); O'Hare *et al.*, Proc. Natl. Acad. Sci. USA 78:1527 (1981)); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIB TECH 11(5):155-2 15 (1993)); and *hygro*, which confers resistance to hygromycin (Santerre *et al.*, Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin *et al.*, J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[00165] The expression level of a fusion protein can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3 (Academic Press, New York, 1987)). When a marker in the vector system expressing a fusion protein is

amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the fusion protein gene, production of the fusion protein will also increase (Crouse *et al.*, Mol. Cell. Biol. 3:257 (1983)).

[00166] The host cell may be co-transfected with multiple expression vectors provided herein. The vectors may contain identical selectable markers which enable equal expression of respective encoding polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing multiple polypeptides. The coding sequences may comprise cDNA or genomic DNA.

[00167] Once a fusion protein provided herein has been produced by recombinant expression, it may be purified by any method known in the art for purification of a polypeptide (*e.g.*, an immunoglobulin molecule), for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, sizing column chromatography, and Kappa select affinity chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the fusion protein molecules provided herein can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

Immunoconjugates

[00168] In some embodiments, the present disclosure also provides immunoconjugates comprising any of the anti-ACVR2A antibodies described herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[00169] In some embodiments, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (*see* U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (*see* U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (*see* U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman *et al.*, *Cancer Res.* 53:3336-3342 (1993); and Lode *et al.*, *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (*see* Kratz *et al.*, *Current Med. Chem.* 13:477-523

(2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[00170] In some embodiments, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, croton, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[00171] In some embodiments, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[00172] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene

triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. *See* WO94/11026.

[00173] The linker may be a “cleavable linker” facilitating release of the conjugated agent in the cell, but non-cleavable linkers are also contemplated herein. Linkers for use in the conjugates of the present disclosure include, without limitation, acid labile linkers (*e.g.*, hydrazone linkers), disulfide-containing linkers, peptidase-sensitive linkers (*e.g.*, peptide linkers comprising amino acids, for example, valine and/or citrulline such as citrulline-valine or phenylalanine-lysine), photolabile linkers, dimethyl linkers, thioether linkers, or hydrophilic linkers designed to evade multidrug transporter-mediated resistance.

[00174] The immunoconjugates or ADCs herein contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (*e.g.*, from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

[00175] In other embodiments, antibodies provided herein are conjugated or recombinantly fused, *e.g.*, to a diagnostic molecule. Such diagnosis and detection can be accomplished, for example, by coupling the antibody to detectable substances including, but not limited to, various enzymes, such as, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidin/biotin or avidin/biotin; fluorescent materials, such as, but not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as, but not limited to, luciferase, luciferin, or aequorin; chemiluminescent material, such as, ^{225}Ac -emitting, Auger-emitting, β -emitting, an alpha-emitting or positron-emitting radioactive isotope.

5.3. Polynucleotides

[00176] In certain embodiments, the disclosure provides polynucleotides that encode the present antibodies that bind to ACVR2A and fusion proteins comprising the antibodies that bind to ACVR2A described herein. The polynucleotides of the disclosure can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or single-stranded, and if single stranded can be the coding strand

or non-coding (anti-sense) strand. In some embodiments, the polynucleotide is in the form of cDNA. In some embodiments, the polynucleotide is a synthetic polynucleotide.

[00177] The present disclosure further relates to variants of the polynucleotides described herein, wherein the variant encodes, for example, fragments, analogs, and/or derivatives of the antibody that binds ACVR2A of the disclosure. In certain embodiments, the present disclosure provides a polynucleotide comprising a polynucleotide having a nucleotide sequence at least about 75% identical, at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, and in some embodiments, at least about 96%, 97%, 98% or 99% identical to a polynucleotide encoding the antibody that binds ACVR2A of the disclosure. As used herein, the phrase “a polynucleotide having a nucleotide sequence at least, for example, 95% “identical” to a reference nucleotide sequence” is intended to mean that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence can include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence can be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence can be inserted into the reference sequence. These mutations of the reference sequence can occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[00178] The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some embodiments, a polynucleotide variant contains alterations which produce silent substitutions, additions, or deletions, but does not alter the properties or activities of the encoded polypeptide. In some embodiments, a polynucleotide variant comprises silent substitutions that results in no change to the amino acid sequence of the polypeptide (due to the degeneracy of the genetic code). Polynucleotide variants can be produced for a variety of reasons, for example, to optimize codon expression for a particular host (*i.e.*, change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*). In some embodiments, a polynucleotide variant comprises at least one silent mutation in a non-coding or a coding region of the sequence.

[00179] In some embodiments, a polynucleotide variant is produced to modulate or alter expression (or expression levels) of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to increase expression of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to decrease expression of the encoded polypeptide. In some embodiments, a polynucleotide variant has increased expression of the encoded polypeptide as compared to a parental polynucleotide sequence. In some embodiments, a polynucleotide variant has decreased expression of the encoded polypeptide as compared to a parental polynucleotide sequence.

[00180] Also provided are vectors comprising the nucleic acid molecules described herein. In an embodiment, the nucleic acid molecules can be incorporated into a recombinant expression vector. The present disclosure provides recombinant expression vectors comprising any of the nucleic acids of the disclosure. As used herein, the term “recombinant expression vector” means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors described herein are not naturally-occurring as a whole; however, parts of the vectors can be naturally-occurring. The described recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages. The non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

[00181] In an embodiment, the recombinant expression vector of the disclosure can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences, Glen Burnie, Md.), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.). Bacteriophage vectors, such as λ GT10, λ GT11, λ EMBL4, and λ NM1149, λ ZapII

(Stratagene) can be used. Examples of plant expression vectors include pBI01, pBI01.2, pBI121, pBI101.3, and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-C1, pMAM, and pMAMneo (Clontech). The recombinant expression vector may be a viral vector, *e.g.*, a retroviral vector, *e.g.*, a gamma retroviral vector.

[00182] In an embodiment, the recombinant expression vectors are prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *supra*, and Ausubel et al., *supra*. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, *e.g.*, from ColE1, SV40, 2 μ plasmid, λ , bovine papilloma virus, and the like.

[00183] The recombinant expression vector may comprise regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (*e.g.*, bacterium, plant, fungus, or animal) into which the vector is to be introduced, as appropriate, and taking into consideration whether the vector is DNA- or RNA-based.

[00184] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, *e.g.*, resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the described expression vectors include, for instance, neomycin/G418 resistance genes, histidinol x resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[00185] The recombinant expression vector can comprise a native or normative promoter operably linked to the nucleotide sequence of the disclosure. The selection of promoters, *e.g.*, strong, weak, tissue-specific, inducible and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, *e.g.*, a cytomegalovirus (CMV) promoter, an RSV promoter, an SV40 promoter, or a promoter found in the long-terminal repeat of the murine stem cell virus.

[00186] The recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

[00187] Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term “suicide gene” refers to a gene that causes the cell expressing

the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, *e.g.*, a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, and nitroreductase.

[00188] In certain embodiments, a polynucleotide is isolated. In certain embodiments, a polynucleotide is substantially pure.

[00189] Also provided are host cells comprising the nucleic acid molecules described herein. The host cell may be any cell that contains a heterologous nucleic acid. The heterologous nucleic acid can be a vector (*e.g.*, an expression vector). For example, a host cell can be a cell from any organism that is selected, modified, transformed, grown, used or manipulated in any way, for the production of a substance by the cell, for example the expression by the cell of a gene, a DNA or RNA sequence, a protein or an enzyme. An appropriate host may be determined. For example, the host cell may be selected based on the vector backbone and the desired result. By way of example, a plasmid or cosmid can be introduced into a prokaryote host cell for replication of several types of vectors. Bacterial cells such as, but not limited to DH5 α , JM109, and KCB, SURE[®] Competent Cells, and SOLOPACK Gold Cells, can be used as host cells for vector replication and/or expression. Additionally, bacterial cells such as *E. coli* LE392 could be used as host cells for phage viruses. Eukaryotic cells that can be used as host cells include, but are not limited to yeast (*e.g.*, YPH499, YPH500 and YPH501), insects and mammals. Examples of mammalian eukaryotic host cells for replication and/or expression of a vector include, but are not limited to, HeLa, NIH3T3, Jurkat, 293, COS, Saos, PC12, SP2/0 (American Type Culture Collection (ATCC), Manassas, VA, CRL-1581), NS0 (European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646) and Ag653 (ATCC CRL-1580) murine cell lines. An exemplary human myeloma cell line is U266 (ATCC CRL-TIB-196). Other useful cell lines include those derived from Chinese Hamster Ovary (CHO) cells such as CHO-K1SV (Lonza Biologics, Walkersville, MD), CHO-K1 (ATCC CRL-61) or DG44.

5.4. Preparation of Antibodies and Method of Making

[00190] Methods of preparing antibodies have been described. *See, e.g.*, Els Pardon et al, *Nature Protocol*, 9(3): 674 (2014). Antibodies (such as scFv fragments) may be obtained using methods known in the art such as by immunizing a *Camelid* species (such as camel or

llama) and obtaining hybridomas therefrom, or by cloning a library of antibodies using molecular biology techniques known in the art and subsequent selection by ELISA with individual clones of unselected libraries or by using phage display.

[00191] Antibodies provided herein may be produced by culturing cells transformed or transfected with a vector containing an antibody-encoding nucleic acids. Polynucleotide sequences encoding polypeptide components of the antibody of the present disclosure can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridomas cells or B cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in host cells. Many vectors that are available and known in the art can be used for the purpose of the present disclosure. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Host cells suitable for expressing antibodies of the present disclosure include prokaryotes such as Archaeobacteria and Eubacteria, including Gram-negative or Gram-positive organisms, eukaryotic microbes such as filamentous fungi or yeast, invertebrate cells such as insect or plant cells, and vertebrate cells such as mammalian host cell lines. Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Antibodies produced by the host cells are purified using standard protein purification methods as known in the art.

[00192] Methods for antibody production including vector construction, expression, and purification are further described in Plückthun *et al.*, Antibody Engineering: Producing antibodies in Escherichia coli: From PCR to fermentation 203-52 (McCafferty *et al.* eds., 1996); Kwong and Rader, *E. coli Expression and Purification of Fab Antibody Fragments*, in Current Protocols in Protein Science (2009); Tachibana and Takekoshi, *Production of Antibody Fab Fragments in Escherichia coli*, in Antibody Expression and Production (Al-Rubeai ed., 2011); and Therapeutic Monoclonal Antibodies: From Bench to Clinic (An ed., 2009).

[00193] It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare anti-ACVR2A antibodies. For instance, the appropriate amino acid sequence, or portions thereof, may be produced by direct peptide synthesis using

solid-phase techniques (*see, e.g.,* Stewart *et al.*, Solid-Phase Peptide Synthesis (1969); and Merrifield, J. Am. Chem. Soc. 85:2149-54 (1963)). *In vitro* protein synthesis may be performed using manual techniques or by automation. Various portions of the anti-ACVR2A antibody may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the desired anti-ACVR2A antibody. Alternatively, antibodies may be purified from cells or bodily fluids, such as milk, of a transgenic animal engineered to express the antibody, as disclosed, for example, in U.S. Pat. Nos. 5,545,807 and 5,827,690.

Polyclonal Antibodies

[00194] Polyclonal antibodies are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, *e.g.*, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R¹N=C=NR, where R and R¹ are independently lower alkyl groups. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

[00195] For example, the animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 µg or 5 µg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to fourteen days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitable to enhance the immune response.

Monoclonal Antibodies

[00196] Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translational modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Thus,

the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies.

[00197] For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567).

[00198] In a further embodiment, antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991). Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nucl. Acids Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[00199] The DNA also may be modified, for example, by substituting the coding sequence (U.S. Pat. No. 4,816,567; Morrison, *et al.*, *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such non-immunoglobulin polypeptides can be substituted to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[00200] Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

Recombinant Production in Prokaryotic Cells

[00201] Polynucleic acid sequences encoding the antibodies of the present disclosure can be obtained using standard recombinant techniques. Desired polynucleic acid sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the

purpose of the present disclosure. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to, an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

[00202] In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter *et al.*, U.S. Pat. No. 5,648,237.

[00203] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as GEM™-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as *E. coli* LE392.

[00204] The expression vector of the present application may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, *e.g.* the presence or absence of a nutrient or a change in temperature.

[00205] A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the present antibody by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the present application. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments,

heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

[00206] Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the -galactamase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trc promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleic acid sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target peptide (Siebenlist *et al.* *Cell* 20: 269 (1980)) using linkers or adaptors to supply any required restriction sites.

[00207] In one aspect, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (*i.e.* cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence can be substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP.

[00208] In some embodiments, the production of the antibodies according to the present disclosure can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. Certain host strains (*e.g.*, the *E. coli* trxB⁻ strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits.

[00209] Prokaryotic host cells suitable for expressing the antibodies of the present disclosure include Archaeobacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include *Escherichia* (*e.g.*, *E. coli*), *Bacilli* (*e.g.*, *B. subtilis*), Enterobacteria, *Pseudomonas* species (*e.g.*, *P. aeruginosa*), *Salmonella typhimurium*, *Serratia marcescans*, *Klebsiella*, *Proteus*, *Shigella*, *Rhizobia*, *Vitreoscilla*, or *Paracoccus*. In some embodiments, gram-negative cells are used. In one embodiment, *E. coli* cells are used as hosts. Examples of *E. coli* strains include strain W3110 (Bachmann, *Cellular and Molecular Biology*, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain

33D3 having genotype W3110 AfhuA (AtonA) ptr3 lac Iq lacL8 AompT A(nmpc-fepE) degP41 kan^R (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as *E. coli* 294 (ATCC 31,446), *E. coli* B, *E. coli* 1776 (ATCC 31,537) and *E. coli* RV308 (ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass *et al.*, *Proteins*, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, *E. coli*, *Serratia*, or *Salmonella* species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon.

[00210] Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

[00211] Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

[00212] Prokaryotic cells used to produce the antibodies of the present application are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

[00213] Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting

of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol. The prokaryotic host cells are cultured at suitable temperatures and pHs.

[00214] If an inducible promoter is used in the expression vector of the present application, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the present application, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. Preferably, the phosphate-limiting medium is the C.R.A.P medium (see, *e.g.*, Simmons *et al.*, *J. Immunol. Methods* 263:133-147 (2002)). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

[00215] The expressed antibodies of the present disclosure are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

[00216] Alternatively, protein production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. To improve the production yield and quality of the antibodies of the present disclosure, various fermentation conditions can be modified. For example, the chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen *et al.* *J Bio Chem* 274:19601-19605 (1999); U.S. Pat. No. 6,083,715; U.S. Pat. No. 6,027,888; Bothmann and Pluckthun, *J. Biol. Chem.* 275:17100-17105 (2000); Ramm and Pluckthun, *J. Biol. Chem.* 275:17106-17113 (2000); Arie *et al.*, *Mol. Microbiol.* 39:199-210 (2001).

[00217] To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present invention, as described in, for example, U.S. Pat. No. 5,264,365; U.S. Pat. No. 5,508,192; Hara *et al.*, *Microbial Drug Resistance*, 2:63-72 (1996). *E. coli* strains

deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins may be used as host cells in the expression system encoding the antibodies of the present application.

[00218] The antibodies produced herein can be further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75. Protein A immobilized on a solid phase for example can be used in some embodiments for immunoaffinity purification of binding molecules of the present disclosure. The solid phase to which Protein A is immobilized is preferably a column comprising a glass or silica surface, more preferably a controlled pore glass column or a silicic acid column. In some embodiments, the column has been coated with a reagent, such as glycerol, in an attempt to prevent nonspecific adherence of contaminants. The solid phase is then washed to remove contaminants non-specifically bound to the solid phase. Finally the antibodies of interest is recovered from the solid phase by elution.

Recombinant Production in Eukaryotic Cells

[00219] For eukaryotic expression, the vector components generally include, but are not limited to, one or more of the following, a signal sequence, an origin of replication, one or more marker genes, and enhancer element, a promoter, and a transcription termination sequence.

[00220] A vector for use in a eukaryotic host may also an insert that encodes a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such precursor region can be ligated in reading frame to DNA encoding the antibodies of the present application.

[00221] Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

[00222] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Selection genes may encode proteins that confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline; complement auxotrophic deficiencies; or supply critical nutrients not available from complex media.

[00223] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[00224] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up nucleic acid encoding the antibodies of the present application. For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An exemplary appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity. Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with the polypeptide encoding-DNA sequences, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' - phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic.

[00225] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid encoding the desired polypeptide sequences. Eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of the transcription of many genes may be included. The 3' end of most eukaryotic may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences may be inserted into eukaryotic expression vectors.

[00226] Polypeptide transcription from vectors in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter,

from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[00227] Transcription of a DNA encoding the antibodies of the present disclosure by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. *See also* Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the polypeptide encoding sequence, but is preferably located at a site 5' from the promoter.

[00228] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the polypeptide-encoding mRNA. One useful transcription termination component is the bovine growth hormone polyadenylation region.

[00229] Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather *et al.*,

Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[00230] Host cells can be transformed with the above-described expression or cloning vectors for antibodies production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[00231] The host cells used to produce the antibodies of the present application may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[00232] When using recombinant techniques, the antibodies can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[00233] The protein composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly (styrene-divinyl) benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered. Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography.

5.5. Pharmaceutical Compositions

[00234] In one aspect, the present disclosure further provides pharmaceutical compositions comprising at least one antibody or antigen binding fragment thereof of the present disclosure. In some embodiments, a pharmaceutical composition comprises therapeutically effective amount of an antibody or antigen binding fragment thereof provided herein and a pharmaceutically acceptable excipient.

[00235] Pharmaceutical compositions comprising an antibody or antigen binding fragment thereof are prepared for storage by mixing the fusion protein having the desired degree of purity with optional physiologically acceptable excipients (*see, e.g.*, Remington, Remington's Pharmaceutical Sciences (18th ed. 1980)) in the form of aqueous solutions or lyophilized or other dried forms.

[00236] The antibody or antigen binding fragment thereof of the present disclosure may be formulated in any suitable form for delivery to a target cell/tissue, *e.g.*, as microcapsules or macroemulsions (Remington, *supra*; Park *et al.*, 2005, *Molecules* 10:146-61; Malik *et al.*, 2007, *Curr. Drug. Deliv.* 4:141-51), as sustained release formulations (Putney and Burke, 1998, *Nature Biotechnol.* 16:153-57), or in liposomes (Maclean *et al.*, 1997, *Int. J. Oncol.* 11:325-32; Kontermann, 2006, *Curr. Opin. Mol. Ther.* 8:39-45).

[00237] An antibody or antigen binding fragment thereof provided herein can also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and

poly-(methylmethacrylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed, for example, in Remington, *supra*.

[00238] Various compositions and delivery systems are known and can be used with an antibody or antigen binding fragment thereof as described herein, including, but not limited to, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antigen binding fragment thereof, receptor-mediated endocytosis (*see, e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-32), construction of a nucleic acid as part of a retroviral or other vector, *etc.* In another embodiment, a composition can be provided as a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (*see, e.g.*, Langer, *supra*; Sefton, 1987, Crit. Ref. Biomed. Eng. 14:201-40; Buchwald *et al.*, 1980, Surgery 88:507-16; and Saudek *et al.*, 1989, N. Engl. J. Med. 321:569-74). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of a prophylactic or therapeutic agent (*e.g.*, an antibody or antigen binding fragment thereof as described herein) or a composition provided herein (*see, e.g.*, Medical Applications of Controlled Release (Langer and Wise eds., 1974); Controlled Drug Bioavailability, Drug Product Design and Performance (Smolen and Ball eds., 1984); Ranger and Peppas, 1983, J. Macromol. Sci. Rev. Macromol. Chem. 23:61-126; Levy *et al.*, 1985, Science 228:190-92; During *et al.*, 1989, Ann. Neurol. 25:351-56; Howard *et al.*, 1989, J. Neurosurg. 71:105-12; U.S. Pat. Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; and 5,128,326; PCT Publication Nos. WO 99/15154 and WO 99/20253).

Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In one embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable.

[00239] In yet another embodiment, a controlled or sustained release system can be placed in proximity of a particular target tissue, for example, the nasal passages or lungs, thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, Medical Applications of Controlled Release Vol. 2, 115-38 (1984)). Controlled release systems are discussed, for

example, by Langer, 1990, Science 249:1527-33. Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more antibody or antigen binding fragment thereof as described herein (*see, e.g.*, U.S. Pat. No. 4,526,938, PCT publication Nos. WO 91/05548 and WO 96/20698, Ning *et al.*, 1996, Radiotherapy & Oncology 39:179-89; Song *et al.*, 1995, PDA J. of Pharma. Sci. & Tech. 50:372-97; Cleek *et al.*, 1997, Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-54; and Lam *et al.*, 1997, Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-60).

5.6. Methods of Using the Antibodies

[00240] In one aspect, provided herein is a method of attenuating an activity of ACVR2A on a cell, comprising exposing the cell to an effective amount of an antibody or antigen binding fragment thereof provided herein.

[00241] In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 10%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 20%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 30%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 40%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 50%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 60%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 70%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 80%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 90%. In some embodiments, the antibody provided herein attenuates an ACVR2A activity by at least about 95%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) an ACVR2A activity by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) an ACVR2A activity by at least about 20% to about 65%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) an ACVR2A activity by at least about 30% to about 65%.

[00242] A non-limiting example of an ACVR2A activity is ACVR2A mediated signaling. Thus, in certain embodiments, provided herein is a method of attenuating (*e.g.*, partially attenuating) ACVR2A mediated signaling in a cell, comprising exposing the cell to an effective amount of an antibody or antigen binding fragment thereof provided herein.

[00243] In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 10%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 20%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 30%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 40%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 50%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 60%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 70%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 80%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 90%. In some embodiments, the antibody provided herein attenuates ACVR2A mediated signaling by at least about 95%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) ACVR2A mediated signaling by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) ACVR2A mediated signaling by at least about 20% to about 65%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) ACVR2A mediated signaling by at least about 30% to about 65%.

[00244] In some embodiments, the antibodies provided herein attenuate ACVR2A binding to its at least one of its ligands.

[00245] Another non-limiting example of an ACVR2A activity is binding to Activin (*e.g.*, Activin A). Thus, in certain embodiments, provided herein is a method of attenuating (*e.g.*, partially attenuating) the binding of ACVR2A to Activin (*e.g.*, Activin A), comprising exposing a cell to an effective amount of an antibody or antigen binding fragment thereof provided herein.

[00246] In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (*e.g.*, Activin A) by at least about 10%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (*e.g.*, Activin A) by at least about 20%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (*e.g.*, Activin A) by at least about 30%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (*e.g.*, Activin A) by at least about 40%. In some embodiments, the antibody provided herein

attenuates the binding of ACVR2A to Activin (e.g., Activin A) by at least about 50%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (e.g., Activin A) by at least about 60%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (e.g., Activin A) by at least about 70%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (e.g., Activin A) by at least about 80%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (e.g., Activin A) by at least about 90%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to Activin (e.g., Activin A) by at least about 95%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) the binding of ACVR2A to Activin (e.g., Activin A) by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) the binding of ACVR2A to Activin (e.g., Activin A) by at least about 20% to about 65%. In certain embodiments, the antibody described herein can attenuate (*e.g.*, partially attenuate) the binding of ACVR2A to Activin (e.g., Activin A) by at least about 30% to about 65%.

[00247] Yet another non-limiting example of an ACVR2A activity is signaling mediated by Activin (e.g., Activin A). Thus, in certain embodiments, provided herein is a method of attenuating (*e.g.*, partially attenuating) Activin (e.g., Activin A) mediated signaling in a cell, comprising exposing the cell to an effective amount of an antibody or antigen binding fragment thereof provided herein.

[00248] In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 10%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 20%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 30%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 40%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 50%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 60%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 70%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 80%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated

signaling by at least about 90%. In some embodiments, the antibody provided herein attenuates Activin (e.g., Activin A) mediated signaling by at least about 95%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) Activin (e.g., Activin A) mediated signaling by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) Activin (e.g., Activin A) mediated signaling by at least about 20% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) Activin (e.g., Activin A) mediated signaling by at least about 30% to about 65%.

[00249] Another non-limiting example of an ACVR2A activity is binding to GDF8. Thus, in certain embodiments, provided herein is a method of attenuating (e.g., partially attenuating) the binding of ACVR2A to GDF8, comprising exposing a cell to an effective amount of an antibody or antigen binding fragment thereof provided herein.

[00250] In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 10%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 20%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 30%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 40%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 50%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 60%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 70%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 80%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 90%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF8 by at least about 95%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) the binding of ACVR2A to GDF8 by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) the binding of ACVR2A to GDF8 by at least about 20% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) the binding of ACVR2A to GDF8 by at least about 30% to about 65%.

[00251] Yet another non-limiting example of an ACVR2A activity is signaling mediated by GDF8. Thus, in certain embodiments, provided herein is a method of attenuating (e.g.,

partially attenuating) GDF8 mediated signaling in a cell, comprising exposing the cell to an effective amount of an antibody or antigen binding fragment thereof provided herein.

[00252] In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 10%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 20%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 30%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 40%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 50%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 60%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 70%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 80%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 90%. In some embodiments, the antibody provided herein attenuates GDF8 mediated signaling by at least about 95%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) GDF8 mediated signaling by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) GDF8 mediated signaling by at least about 20% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) GDF8 mediated signaling by at least about 30% to about 65%.

[00253] Another non-limiting example of an ACVR2A activity is binding to GDF11. Thus, in certain embodiments, provided herein is a method of attenuating (e.g., partially attenuating) the binding of ACVR2A to GDF11, comprising exposing a cell to an effective amount of an antibody or antigen binding fragment thereof provided herein.

[00254] In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 10%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 20%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 30%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 40%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 50%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to

GDF11 by at least about 60%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 70%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 80%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 90%. In some embodiments, the antibody provided herein attenuates the binding of ACVR2A to GDF11 by at least about 95%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) the binding of ACVR2A to GDF11 by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) the binding of ACVR2A to GDF11 by at least about 20% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) the binding of ACVR2A to GDF11 by at least about 30% to about 65%.

[00255] Yet another non-limiting example of an ACVR2A activity is signaling mediated by GDF11. Thus, in certain embodiments, provided herein is a method of attenuating (e.g., partially attenuating) GDF11 mediated signaling in a cell, comprising exposing the cell to an effective amount of an antibody or antigen binding fragment thereof provided herein.

[00256] In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 10%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 20%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 30%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 40%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 50%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 60%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 70%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 80%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 90%. In some embodiments, the antibody provided herein attenuates GDF11 mediated signaling by at least about 95%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) GDF11 mediated signaling by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) GDF11 mediated signaling by at least about 20% to about 65%. In certain embodiments, the antibody described herein can

attenuate (e.g., partially attenuate) GDF11 mediated signaling by at least about 30% to about 65%.

[00257] In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 10%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 20%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 30%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 40%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 50%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 60%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 70%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 80%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 90%. In some embodiments, the antibody provided herein attenuates Smad activation or phosphorylation by at least about 95%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) Smad activation or phosphorylation by at least about 15% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) Smad activation or phosphorylation by at least about 20% to about 65%. In certain embodiments, the antibody described herein can attenuate (e.g., partially attenuate) Smad activation or phosphorylation by at least about 30% to about 65%.

[00258] In another aspect, provided herein is a method of treating a disease or disorder in a subject comprising administering to the subject an effective amount of an antibody or antigen binding fragment thereof provided herein. In one embodiment, the disease or disorder is an ACVR2A-mediated disease or disorder. In one embodiment, the disease or disorder is Activin (e.g., Activin A)-mediated disease or disorder. In some embodiments, the antibodies or antigen binding fragments provided herein can prevent the inhibition of muscle differentiation by the Smad-dependent pathway, leading to an increase in muscle mass and strength in a patient. Also provided herein is a method of treatment of a disease or disorder, wherein the subject is administered one or more therapeutic agents in combination with the antibody or antigen-binding fragment thereof provided herein.

[00259] The disclosure also relates to methods of using the antibodies provided herein to inhibit, i.e. antagonize, function of ACVR2A in order to inhibit Smad activation and thereby

regulate metabolism of different cells, for example, induce skeletal muscle differentiation, induce NK cells proliferation and activation, regulate dendritic cells (DCs) maturation, inhibit hepatic stellate cells activation, inhibit M2 polarization of macrophages, inhibit Treg cells activation; resulting in the treatment of a pathological disorder.

[00260] The pathological disorder may be a musculoskeletal disease or disorder, such as anemia, muscle atrophy, spinal muscular atrophy and cancer cachexia. There are many causes of muscle atrophy, including as a result of treatment with a glucocorticoid such as cortisol, dexamethasone, betamethasone, prednisone, methylprednisolone, or prednisolone. The muscle atrophy can also be a result of denervation due to nerve trauma or a result of degenerative, metabolic, or inflammatory neuropathy (e.g., Guillian-Barré syndrome, peripheral neuropathy, or exposure to environmental toxins or drugs).

[00261] The myopathy may be caused by a muscular dystrophy syndrome, such as Duchenne, Becker, myotonic, fascioscapulohumeral, Emery-Dreifuss, oculopharyngeal, scapulohumeral, limb girdle, Fukuyama, a congenital muscular dystrophy, or hereditary distal myopathy. The musculoskeletal disease can also be osteoporosis, a bone fracture, short stature, or dwarfism.

[00262] In addition, the muscle atrophy can be a result of an adult motor neuron disease, infantile spinal muscular atrophy, amyotrophic lateral sclerosis, juvenile spinal muscular atrophy, autoimmune motor neuropathy with multifocal conduction block, paralysis due to stroke or spinal cord injury, skeletal immobilization due to trauma, prolonged bed rest, voluntary inactivity, involuntary inactivity, metabolic stress or nutritional insufficiency, cancer, AIDS, fasting, a thyroid gland disorder, diabetes, benign congenital hypotonia, central core disease, burn injury, chronic obstructive pulmonary disease, liver diseases (examples such as fibrosis, cirrhosis), sepsis, renal failure, congestive heart failure, ageing, space travel or time spent in a zero gravity environment.

[00263] Examples of age-related conditions that may be treated by the present antibodies include, sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, frailty, memory loss, wrinkles, impaired kidney function, and age-related hearing loss; metabolic disorders, including Type II Diabetes, Metabolic Syndrome, hyperglycemia, Nonalcoholic Steatohepatitis (NASH) and obesity.

[00264] Other conditions that may be treated with the antibodies of the disclosure include acute and/or chronic renal disease or failure, liver fibrosis or cirrhosis, lung fibrosis, pulmonary arterial hypertension, kidney fibrosis; cancer such as ovarian cancers, breast cancer, esophageal cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, endometrial cancer and gastrointestinal cancers; Parkinson's Disease; conditions associated with neuronal death, such as Amyotrophic lateral sclerosis (ALS), brain atrophy, or dementia.. Further conditions include anemia, cachexia, cachexia associated with a rheumatoid arthritis and cachexia associated with cancer.

[00265] Based on reported evidence of a role of activins binding to ACVR2A amongst other receptors, in contributing to liver, kidney and pulmonary fibrosis and of a role for Activin A, GDF8, or ACVR2A in cancers, the antibodies of the disclosure may be used to treat liver, kidney, pulmonary fibrosis and cancers exemplified by but not restricted to sarcoma,, lung cancer, ovarian cancers, breast cancers, colorectal cancer, bone-loss inducing cancers, hepatocellular carcinomas, gastrointestinal cancers.

[00266] The prevention may be complete, e.g., the total absence of an age-related condition or metabolic disorder. The prevention may also be partial, such that the likelihood of the occurrence of the age-related condition or metabolic disorder in a subject is less likely to occur than had the subject not received an antibody of the present disclosure.

[00267] Methods of administration and dosing is described in more detail in Section 5.7 below.

[00268] In another aspect, provided herein is the use of the antibody or antigen binding fragment thereof provided herein in the manufacture of a medicament for treating a disease or disorder in a subject.

[00269] In another aspect, provided herein is the use of a pharmaceutical composition provided herein in the manufacture of a medicament for treating a disease or disorder in a subject.

[00270] In another aspect, provided herein is the use of an antibody or antigen binding fragment thereof provided herein in the manufacture of a medicament, wherein the medicament is for use in a method for detecting the presence of an ACVR2A in a biological sample, the method comprising contacting the biological sample with the antibody under conditions permissive for binding of the antibody to the ACVR2A protein, and detecting whether a complex is formed between the antibody and the ACVR2A protein.

[00271] In other aspects, the antibodies and fragments thereof of the present disclosure are useful for detecting the presence of an ACVR2A in a biological sample. The term “detecting” as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises bodily fluid, a cell, or a tissue. Diagnostic assays and methods are described in more detail in Section 5.9 below.

5.7. Methods of Administration and Dosing

[00272] In a specific embodiment, provided herein is a composition for use in the prevention and/or treatment of a disease or condition comprising an antibody or antigen binding fragment thereof provided herein. In one embodiment, provided herein is a composition for use in the prevention of a disease or condition, wherein the composition comprises an antibody or antigen binding fragment thereof provided herein. In one embodiment, provided herein is a composition for use in the treatment of a disease or condition, wherein the composition comprises an antibody or antigen binding fragment thereof provided herein. In some embodiments, the disease or condition is an ACVR2A-mediated disease. In some embodiments, the disease or condition is an ACVR2A ligand-mediated disease, ACVR2A ligands include Activins (such as Activin A, Activin B, Activin AB, Activin C, Activin AC and Activin E), Growth/differentiation factors (GDFs, such as GDF1, GDF3, GDF5, GDF6, GDF7, GDF8, GDF10 and GDF11), bone morphogenetic proteins (BMPs, such as BMP2, BMP4, BMP6, BMP7, BMP8a, BMP8b, BMP9 and BMP10). In some embodiments, the disease or disorder is associated with ACVR2A and or ACVR2A ligands, such as Activins (e.g., Activin A) GDFs or BMPs. In some embodiments, the disease or disorder is a pathological disorder including a musculoskeletal disease or disorder, such as anemia, muscle atrophy, spinal muscular atrophy and cancer cachexia. In other embodiments, the disease or disorder is age-related conditions including sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, frailty, memory loss, wrinkles, impaired kidney function, and age-related hearing loss; metabolic disorders, including Type II Diabetes, Metabolic Syndrome, hyperglycemia, NASH and obesity. In other embodiments, the disease or disorder is selected from a group consisting of acute and/or chronic renal disease or failure, liver fibrosis or cirrhosis, lung fibrosis, pulmonary arterial hypertension, kidney fibrosis; cancer such as ovarian cancers, breast cancer, esophageal

cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, endometrial cancer and gastrointestinal cancers; Parkinson's Disease; conditions associated with neuronal death, such as ALS, brain atrophy, or dementia. Further conditions include anemia, cachexia, cachexia associated with a rheumatoid arthritis and cachexia associated with cancer. In yet other embodiments, the disease or disorder is selected from a group consisting of liver, kidney, pulmonary fibrosis and cancers exemplified by but not restricted to sarcoma, lung cancer, ovarian cancers, breast cancers, colorectal cancer, bone-loss inducing cancers, hepatocellular carcinomas, gastrointestinal cancers. In certain embodiments, the subject is a subject in need thereof. In some embodiments, the subject has the disease or condition. In other embodiments, the subject is at risk of having the disease or condition. In some embodiments, the administration results in the prevention, management, treatment or amelioration of the disease or condition.

[00273] In one embodiment, provided herein is a composition for use in the prevention and/or treatment of a symptom of a disease or condition, wherein the composition comprises an antibody or antigen binding fragment thereof provided herein. In one embodiment, provided herein is a composition for use in the prevention of a symptom of a disease or condition, wherein the composition comprises an antibody or antigen binding fragment thereof provided herein. In one embodiment, provided herein is a composition for use in the treatment of a symptom of a disease or condition, wherein the composition comprises an antibody or antigen binding fragment thereof provided herein. In some embodiments, the disease or condition is an ACVR2A-mediated and or ACVR2A ligands, such as Activins (e.g., Activin A) GDFs or BMPs, mediated disease. In some embodiments, the disease or disorder is associated with ACVR2A. In some embodiments, the disease or disorder is a pathological disorder including a musculoskeletal disease or disorder, such as anemia, muscle atrophy, spinal muscular atrophy and cancer cachexia. In other embodiments, the disease or disorder is age-related conditions including sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, frailty, memory loss, wrinkles, impaired kidney function, and age-related hearing loss; metabolic disorders, including Type II Diabetes, Metabolic Syndrome, hyperglycemia, NASH and obesity. In other embodiments, the disease or disorder is selected from a group consisting of acute and/or chronic renal disease or failure, liver

fibrosis or cirrhosis, lung fibrosis, pulmonary arterial hypertension, kidney fibrosis; cancer such as ovarian cancers, breast cancer, esophageal cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, endometrial cancer and gastrointestinal cancers; Parkinson's Disease; conditions associated with neuronal death, such as ALS, brain atrophy, or dementia and anemia. Further conditions include cachexia, cachexia associated with a rheumatoid arthritis and cachexia associated with cancer. In yet other embodiments, the disease or disorder is selected from a group consisting of liver, kidney, pulmonary fibrosis and cancers exemplified by but not restricted to sarcoma, bone-loss inducing cancers, hepatocellular carcinomas, gastrointestinal cancers. In certain embodiments, the subject is a subject in need thereof. In some embodiments, the subject has the disease or condition. In other embodiments, the subject is at risk of having the disease or condition. In some embodiments, the administration results in the prevention or treatment of the symptom of the disease or condition.

[00274] In another embodiment, provided herein is a method of preventing and/or treating a disease or condition in a subject, comprising administering an effective amount of an antibody or antigen binding fragment thereof provided herein. In one embodiment, provided herein is a method of preventing a disease or condition in a subject, comprising administering an effective amount of an antibody or antigen binding fragment thereof provided herein. In one embodiment, provided herein is a method of treating a disease or condition in a subject, comprising administering an effective amount of an antibody or antigen binding fragment thereof provided herein. In some embodiments, the disease or condition is an ACVR2A-mediated disease. In some embodiments, the disease or condition is a ACVR2A ligands, such as Activins (e.g., Activin A) GDFs or BMPs -mediated disease. In some embodiments, the disease or disorder is associated with ACVR2A. In some embodiments, the disease or disorder is a pathological disorder including a musculoskeletal disease or disorder, such as anemia, muscle atrophy, spinal muscular atrophy and cancer cachexia. In other embodiments, the disease or disorder is age-related conditions including sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, frailty, memory loss, wrinkles, impaired kidney function, and age-related hearing loss; metabolic disorders, including Type II Diabetes, Metabolic Syndrome, hyperglycemia, and NASH and obesity. In other embodiments, the disease or

disorder is selected from a group consisting of acute and/or chronic renal disease or failure, liver fibrosis or cirrhosis, lung fibrosis, pulmonary arterial hypertension; cancer such as ovarian cancers, breast cancer, esophageal cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, endometrial cancer and gastrointestinal cancers; Parkinson's Disease; conditions associated with neuronal death, such as ALS, brain atrophy, or dementia. Further conditions include anemia, cachexia, cachexia associated with a rheumatoid arthritis and cachexia associated with cancer. In yet other embodiments, the disease or disorder is selected from a group consisting of liver, kidney, pulmonary fibrosis and cancers exemplified by but not restricted to sarcoma, bone-loss inducing cancers, hepatocellular carcinomas, gastrointestinal cancers. In certain embodiments, the subject is a subject in need thereof. In some embodiments, the subject has the disease or condition. In other embodiments, the subject is at risk of having the disease or condition. In some embodiments, the administration results in the prevention or treatment of the disease or condition.

[00275] In another embodiment, provided herein is a method of preventing and/or treating a symptom of a disease or condition in a subject, comprising administering an effective amount of an antibody or antigen binding fragment thereof provided herein. In one embodiment, provided herein is a method of preventing a symptom of a disease or condition in a subject, comprising administering an effective amount of an antibody or antigen binding fragment thereof provided herein. In one embodiment, provided herein is a method of treating a symptom of a disease or condition in a subject, comprising administering an effective amount of an antibody or antigen binding fragment thereof provided herein. In some embodiments, the disease or condition is an ACVR2A-mediated and/or ACVR2A ligands, such as Activins (e.g., Activin A) GDFs or BMPs, mediated disease. In some embodiments, the disease or disorder is associated with ACVR2A. In some embodiments, the disease or disorder is a pathological disorder including a musculoskeletal disease or disorder, such as anemia, muscle atrophy, spinal muscular atrophy and cancer cachexia. In other embodiments, the disease or disorder is age-related conditions including sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, frailty, memory loss, wrinkles, impaired kidney function, and age-related hearing loss; metabolic disorders,

including Type II Diabetes, Metabolic Syndrome, hyperglycemia, and NASH and obesity. In other embodiments, the disease or disorder is selected from a group consisting of acute and/or chronic renal disease or failure, liver fibrosis or cirrhosis, lung fibrosis, pulmonary arterial hypertension; cancer such as ovarian cancers, breast cancer, esophageal cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, endometrial cancer and gastrointestinal cancers Parkinson's Disease; conditions associated with neuronal death, such as ALS, brain atrophy, or dementia. Further conditions include anemia, cachexia, cachexia associated with a rheumatoid arthritis and cachexia associated with cancer. In yet other embodiments, the disease or disorder is selected from a group consisting of liver, kidney, pulmonary fibrosis and cancers exemplified by but not restricted to sarcoma, bone-loss inducing cancers, hepatocellular carcinomas, gastrointestinal cancers. In certain embodiments, the subject is a subject in need thereof. In some embodiments, the subject has the disease or condition. In other embodiments, the subject is at risk of having the disease or condition. In some embodiments, the administration results in the prevention or treatment of the symptom of the disease or condition.

[00276] Also provided herein are methods of preventing and/or treating a disease or condition by administering to a subject of an effective amount of an antibody or antigen binding fragment thereof provided herein, or pharmaceutical composition comprising an antibody or antigen binding fragment thereof provided herein. In one aspect, the antibody or antigen binding fragment thereof is substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side-effects). The subject administered a therapy can be a mammal such as non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats *etc.*) or a primate (*e.g.*, a monkey, such as a cynomolgus macaque monkey, or a human). In a one embodiment, the subject is a human. In another embodiment, the subject is a human with a disease or condition.

[00277] Various delivery systems are known and can be used to administer a prophylactic or therapeutic agent (*e.g.*, an antibody or antigen binding fragment thereof provided herein), including, but not limited to, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antigen binding fragment thereof, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, *etc.* Methods of administering a prophylactic or therapeutic agent (*e.g.*, an antibody or antigen binding fragment thereof provided herein), or pharmaceutical composition include, but are not limited

to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, a prophylactic or therapeutic agent (*e.g.*, an antibody or antigen binding fragment thereof provided herein), or a pharmaceutical composition is administered intranasally, intramuscularly, intravenously, or subcutaneously. The prophylactic or therapeutic agents, or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, intranasal mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entirety.

[00278] In a specific embodiment, it may be desirable to administer a prophylactic or therapeutic agent, or a pharmaceutical composition provided herein locally to the area in need of treatment. This may be achieved by, for example, and not by way of limitation, local infusion, by topical administration (*e.g.*, by intranasal spray), by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sikalastic membranes, or fibers. In some embodiments, when administering an antibody or antigen binding fragment thereof provided herein, care must be taken to use materials to which the antibody or antigen binding fragment thereof does not absorb.

[00279] In another embodiment, a prophylactic or therapeutic agent, or a composition provided herein can be delivered in a vesicle, in particular a liposome (see Langer, 1990, *Science* 249:1527-1533; Treat *et al.*, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).

[00280] In another embodiment, a prophylactic or therapeutic agent, or a composition provided herein can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:20; Buchwald *et al.*, 1980, *Surgery* 88:507; Saudek *et al.*, 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric

materials can be used to achieve controlled or sustained release of a prophylactic or therapeutic agent (*e.g.*, an antibody provided herein) or a composition provided herein (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy *et al.*, 1985, Science 228:190; During *et al.*, 1989, Ann. Neurol. 25:351; Howard *et al.*, 1989, J. Neurosurg. 71:105); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In an embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the therapeutic target, *i.e.*, the nasal passages or lungs, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)). Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more antibody or antigen binding fragment thereof provided herein. See, *e.g.*, U.S. Patent No. 4,526,938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning *et al.*, 1996, "Intratumoral Radioimmunotherapy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy & Oncology 39:179- 189, Song *et al.*, 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology 50:372-397, Cleek *et al.*, 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam *et al.*, 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in their entirety.

[00281] In a specific embodiment, where the composition provided herein is a nucleic acid encoding a prophylactic or therapeutic agent (*e.g.*, an antibody or antigen binding fragment thereof provided herein), the nucleic acid can be administered *in vivo* to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, *e.g.*, Joliot *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[00282] In a specific embodiment, a composition provided herein comprises one, two or more antibodies or antigen binding fragments thereof provided herein. In another embodiment, a composition provided herein comprises one, two or more antibodies or antigen binding fragments thereof provided herein and a prophylactic or therapeutic agent other than an antibody or antigen binding fragment thereof provided herein. In one embodiment, the agents are known to be useful for or have been or are currently used for the prevention, management, treatment and/or amelioration of a disease or condition. In addition to prophylactic or therapeutic agents, the compositions provided herein may also comprise an excipient.

[00283] The compositions provided herein include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, compositions that are suitable for administration to a subject or patient) that can be used in the preparation of unit dosage forms. In an embodiment, a composition provided herein is a pharmaceutical composition. Such compositions comprise a prophylactically or therapeutically effective amount of one or more prophylactic or therapeutic agents (*e.g.*, an antibody or antigen binding fragment thereof provided herein or other prophylactic or therapeutic agent), and a pharmaceutically acceptable excipient. The pharmaceutical compositions can be formulated to be suitable for the route of administration to a subject.

[00284] In a specific embodiment, the term “excipient” can also refer to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete or incomplete) or vehicle. Pharmaceutical excipients can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is

an exemplary excipient when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulation can include standard excipients such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, *etc.* Examples of suitable pharmaceutical excipients are described in Remington's Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA. Such compositions will contain a prophylactically or therapeutically effective amount of the antibody or antigen binding fragment thereof provided herein, such as in purified form, together with a suitable amount of excipient so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[00285] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Such compositions, however, may be administered by a route other than intravenous.

[00286] Generally, the ingredients of compositions provided herein are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00287] An antibody or antigen binding fragment thereof provided herein can be packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of antibody. In one embodiment, the antibody or antigen binding fragment thereof is supplied

as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. The lyophilized antibody or antigen binding fragment thereof can be stored at between 2 and 8°C in its original container and the antibody or antigen binding fragment thereof can be administered within 12 hours, such as within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, an antibody or antigen binding fragment thereof provided herein is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the antibody.

[00288] The compositions provided herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

[00289] The amount of a prophylactic or therapeutic agent (*e.g.*, an antibody or antigen binding fragment thereof provided herein), or a composition provided herein that will be effective in the prevention and/or treatment of a disease or condition can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of a disease or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances.

[00290] Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[00291] In certain embodiments, the route of administration for a dose of an antibody or antigen binding fragment thereof provided herein to a patient is intranasal, intramuscular, intravenous, subcutaneous, or a combination thereof, but other routes described herein are also acceptable. Each dose may or may not be administered by an identical route of administration. In some embodiments, an antibody or antigen binding fragment thereof provided herein may be administered via multiple routes of administration simultaneously or subsequently to other doses of the same or a different antibody or antigen binding fragment thereof provided herein.

[00292] In certain embodiments, the antibody or antigen binding fragment thereof provided herein are administered prophylactically or therapeutically to a subject. The antibody or antigen binding fragment thereof provided herein can be prophylactically or therapeutically administered to a subject so as to prevent, lessen or ameliorate a disease or symptom thereof.

5.8. Gene Therapy

[00293] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to a subject for use in a method provided herein, for example, to prevent, manage, treat and/or ameliorate an ACVR2A and/or ACVR2A ligand-mediated disease, disorder or condition, by way of gene therapy. Such therapy encompasses that performed by the administration to a subject of an expressed or expressible nucleic acid. In an embodiment, the nucleic acids produce their encoded antibody, and the antibody mediates a prophylactic or therapeutic effect.

[00294] Any of the methods for recombinant gene expression (or gene therapy) available in the art can be used.

[00295] For general review of the methods of gene therapy, see Goldspiel *et al.*, 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[00296] In a specific embodiment, a composition comprises nucleic acids encoding an antibody provided herein, the nucleic acids being part of an expression vector that expresses the antibody or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acids have promoters, such as heterologous promoters, operably linked to the antibody coding region, the promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller

and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra *et al.*, 1989, Nature 342:435-438).

[00297] Delivery of the nucleic acids into a subject can be either direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the subject. These two approaches are known, respectively, as *in vivo* or ex vivo gene therapy.

[00298] In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where the sequences are expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering the vector so that the sequences become intracellular, *e.g.*, by infection using defective or attenuated retroviral or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180; WO 92/22635; WO 92/20316; W093/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; and Zijlstra *et al.*, 1989, Nature 342:435-438).

[00299] In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody are used. For example, a retroviral vector can be used (see Miller *et al.*, 1993, Meth. Enzymol. 217:581-599). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy can be cloned into one or more vectors, which facilitates delivery of the gene into a subject. More

detail about retroviral vectors can be found in Boesen *et al.*, 1994, *Biotherapy* 6:291-302, which describes the use of a retroviral vector to deliver the MDR1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, 1994, *J. Clin. Invest.* 93:644-651; Klein *et al.*, 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

[00300] Adenoviruses are other viral vectors that can be used in the recombinant production of antibodies. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout *et al.*, 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, 1991, *Science* 252:431-434; Rosenfeld *et al.*, 1992, *Cell* 68:143-155; Mastrangeli *et al.*, 1993, *J. Clin. Invest.* 91:225-234; PCT Publication W094/12649; and Wang *et al.*, 1995, *Gene Therapy* 2:775-783. In a specific embodiment, adenovirus vectors are used.

[00301] Adeno-associated virus (AAV) can also be utilized (Walsh *et al.*, 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300; and U.S. Patent No. 5,436,146). In a specific embodiment, AAV vectors are used to express an anti-ACVR2A antibody as provided herein. In certain embodiments, the AAV comprises a nucleic acid encoding a VH domain. In other embodiments, the AAV comprises a nucleic acid encoding a VL domain. In certain embodiments, the AAV comprises a nucleic acid encoding a VH domain and a VL domain. In some embodiments of the methods provided herein, a subject is administered an AAV comprising a nucleic acid encoding a VH domain and an AAV comprising a nucleic acid encoding a VL domain. In other embodiments, a subject is administered an AAV comprising a nucleic acid encoding a VH domain and a VL domain. In certain embodiments, the VH and VL domains are over-expressed.

[00302] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated

transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

[00303] In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcellmediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen *et al.*, 1993, Meth. Enzymol. 217:618-644; Clin. Pharma. Ther. 29:69-92 (1985)) and can be used in accordance with the methods provided herein, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell, such as heritable and expressible by its cell progeny.

[00304] The resulting recombinant cells can be delivered to a subject by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) can be administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[00305] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[00306] In a specific embodiment, the cell used for gene therapy is autologous to the subject.

[00307] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any

stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the methods provided herein (see *e.g.*, PCT Publication WO 94/08598; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

[00308] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

5.9. Diagnostic Assays and Methods

[00309] Labeled antibodies and derivatives and analogs thereof, which immunospecifically bind to an ACVR2A antigen can be used for diagnostic purposes to detect, diagnose, or monitor an ACVR2A-mediated disease. Thus, provided herein are methods for the detection of an ACVR2A-mediated disease comprising: (a) assaying the expression of an ACVR2A antigen in cells or a tissue sample of a subject using one or more antibodies provided herein that immunospecifically bind to the ACVR2A antigen; and (b) comparing the level of the ACVR2A antigen with a control level, *e.g.*, levels in normal tissue samples (*e.g.*, from a patient not having an ACVR2A-mediated disease, or from the same patient before disease onset), whereby an increase in the assayed level of ACVR2A antigen compared to the control level of the ACVR2A antigen is indicative of an ACVR2A-mediated disease.

[00310] Also provided herein is a diagnostic assay for diagnosing an ACVR2A-mediated disease comprising: (a) assaying for the level of an ACVR2A antigen in cells or a tissue sample of an individual using one or more antibodies provided herein that immunospecifically bind to an ACVR2A antigen; and (b) comparing the level of the ACVR2A antigen with a control level, *e.g.*, levels in normal tissue samples, whereby an increase in the assayed ACVR2A antigen level compared to the control level of the ACVR2A antigen is indicative of an ACVR2A-mediated disease. In certain embodiments, provided herein is a method of treating an ACVR2A-mediated disease in a subject, comprising: (a) assaying for the level of an ACVR2A antigen in cells or a tissue sample of the subject using one or more antibodies provided herein that immunospecifically bind to an ACVR2A antigen; and (b) comparing the level of the ACVR2A antigen with a control level, *e.g.*, levels in normal tissue samples, whereby an increase in the assayed ACVR2A antigen level compared to the control level of the ACVR2A antigen is indicative of an ACVR2A-mediated

disease. In some embodiments, the method further comprises (c) administering an effective amount of an antibody provided herein to the subject identified as having the ACVR2A-mediated disease. A more definitive diagnosis of an ACVR2A-mediated disease may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the ACVR2A-mediated disease.

[00311] Antibodies provided herein can be used to assay ACVR2A antigen levels in a biological sample using classical immunohistological methods as described herein or as known to those of skill in the art (*e.g.*, see Jalkanen *et al.*, 1985, J. Cell. Biol. 101:976-985; and Jalkanen *et al.*, 1987, J. Cell. Biol. 105:3087-3096). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (¹²⁵I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (¹²¹In), and technetium (⁹⁹Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[00312] One aspect provided herein is the detection and diagnosis of an ACVR2A-mediated disease in a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled antibody that immunospecifically binds to an ACVR2A antigen; b) waiting for a time interval following the administering for permitting the labeled antibody to concentrate at sites in the subject where the ACVR2A antigen is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled antibody in the subject, such that detection of labeled antibody above the background level indicates that the subject has an ACVR2A-mediated disease.

Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[00313] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ⁹⁹Tc. The labeled antibody will then accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "Immunopharmacokinetics of Radiolabeled Antibodies

and Their Fragments.” (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B.A. Rhodes, eds., Masson Publishing Inc. (1982).

[00314] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled antibody to concentrate at sites in the subject and for unbound labeled antibody to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[00315] In one embodiment, monitoring of an ACVR2A-mediated disease is carried out by repeating the method for diagnosing the ACVR2A-mediated disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[00316] Presence of the labeled molecule can be detected in the subject using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods provided herein include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[00317] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston *et al.*, U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

5.10. Kits

[00318] Also provided herein are kits comprising an antibody (*e.g.*, an anti-ACVR2A antibody) provided herein, or a composition (*e.g.*, a pharmaceutical composition) thereof, packaged into suitable packaging material. A kit optionally includes a label or packaging insert including a description of the components or instructions for use *in vitro*, *in vivo*, or *ex vivo*, of the components therein.

[00319] The term “packaging material” refers to a physical structure housing the components of the kit. The packaging material can maintain the components sterile, and

can be made of material commonly used for such purposes (*e.g.*, paper, corrugated fiber, glass, plastic, foil, ampoules, vials, tubes, etc.).

[00320] Kits provided herein can include labels or inserts. Labels or inserts include “printed matter,” *e.g.*, paper or cardboard, separate or affixed to a component, a kit or packing material (*e.g.*, a box), or attached to, for example, an ampoule, tube, or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, such as a disk (*e.g.*, hard disk, card, memory disk), optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media, or memory type cards. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location, and date.

[00321] Kits provided herein can additionally include other components. Each component of the kit can be enclosed within an individual container, and all of the various containers can be within a single package. Kits can also be designed for cold storage. A kit can further be designed to contain antibodies provided herein, or cells that contain nucleic acids encoding the antibodies provided herein. The cells in the kit can be maintained under appropriate storage conditions until ready to use.

[00322] Also provided herein are panels of antibodies that immunospecifically bind to an ACVR2A antigen. In specific embodiments, provided herein are panels of antibodies having different association rate constants different dissociation rate constants, different affinities for ACVR2A antigen, and/or different specificities for an ACVR2A antigen. In certain embodiments, provided herein are panels of about 10, preferably about 25, about 50, about 75, about 100, about 125, about 150, about 175, about 200, about 250, about 300, about 350, about 400, about 450, about 500, about 550, about 600, about 650, about 700, about 750, about 800, about 850, about 900, about 950, or about 1000 antibodies or more. Panels of antibodies can be used, for example, in 96 well or 384 well plates, such as for assays such as ELISAs.

[00323] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described herein.

[00324] As used herein, numerical values are often presented in a range format throughout this document. The use of a range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention unless the context clearly indicates otherwise. Accordingly, the use of a range expressly includes all possible subranges, all individual numerical values within that range, and all numerical values or numerical ranges including integers within such ranges and fractions of the values or the integers within ranges unless the context clearly indicates otherwise. This construction applies regardless of the breadth of the range and in all contexts throughout this patent document. Thus, for example, reference to a range of 90-100% includes 91-99%, 92-98%, 93-95%, 91-98%, 91-97%, 91-96%, 91-95%, 91-94%, 91-93%, and so forth. Reference to a range of 90-100% also includes 91%, 92%, 93%, 94%, 95%, 95%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, etc., and so forth.

[00325] In addition, reference to a range of 1-3, 3-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130, 130-140, 140-150, 150-160, 160-170, 170-180, 180-190, 190-200, 200-225, 225-250 includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc. In a further example, reference to a range of 25-250, 250-500, 500-1,000, 1,000-2,500, 2,500-5,000, 5,000-25,000, 25,000-50,000 includes any numerical value or range within or encompassing such values, *e.g.*, 25, 26, 27, 28, 29...250, 251, 252, 253, 254...500, 501, 502, 503, 504..., etc.

[00326] As also used herein a series of ranges are disclosed throughout this document. The use of a series of ranges include combinations of the upper and lower ranges to provide another range. This construction applies regardless of the breadth of the range and in all contexts throughout this patent document. Thus, for example, reference to a series of ranges such as 5-10, 10-20, 20-30, 30-40, 40-50, 50-75, 75-100, 100-150, includes ranges such as 5-20, 5-30, 5-40, 5-50, 5-75, 5-100, 5-150, and 10-30, 10-40, 10-50, 10-75, 10-100, 10-150, and 20-40, 20-50, 20-75, 20-100, 20-150, and so forth.

6. EMBODIMENTS

[00327] The present disclosure includes the following non-limiting embodiments:

[00328] Embodiment 1. An antibody or antigen binding fragment thereof that binds ACVR2A, wherein the affinity of the antibody or antigen binding fragment to ACVR2A is at least 10 fold of that to ACVR2B, and wherein optionally the antibody or antigen binding fragment comprises:

- (i) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 1, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (ii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 3, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (iii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 4, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (iv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 5, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (v) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (vi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 8, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (vii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (viii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 10, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (ix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (x) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (xi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 13, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (xii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 14;
- (xiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 15;
- (xiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (xv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 16;
- (xvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 17;

- (xvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 18;
- (xviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 19;
- (xix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 20;
- (xx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 21;
- (xxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 22;
- (xxii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 23;
- (xxiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 24;
- (xxiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (xxv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 25;
- (xxvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 26;
- (xxvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 27;
- (xxviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 28;
- (xxix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 29;
- (xxx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 30;
- (xxxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 31;
- (xxxii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 32;

(xxxiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 33;

(xxxiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 34;

(xxxv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 35;

(xxxvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 36;

(xxxvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 37;

(xxxviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 38;

(xxxix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 39;

(xxxx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 40; or

(xxxxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 41.

[00329] Embodiment 2. The antibody or antigen binding fragment of embodiment 1, wherein

(i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 42, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(ii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(iii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 49, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO:

45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(iv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 50, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or

(v) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or

(vi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 49, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or

(vii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(viii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 55, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(ix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

62, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xvii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 63, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xviii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 82, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 64, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xx) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 65, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 66, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 67, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxiii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 68, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxiv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or

(xxv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 69, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxvi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 70, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxvii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 71, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxviii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 72, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO:

45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 73, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxx) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 74, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 75, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 67, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxiii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 68, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxiv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 76, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 77, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxvi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 78, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxvii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 79, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxviii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 69, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 80, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxx) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 70, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxxi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 81, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47.

[00330] Embodiment 3. The antibody or antigen binding fragment of embodiment 1 or 2, comprises:

- (i) a VH comprising the amino acid sequence of SEQ ID NO: 1, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (ii) a VH comprising the amino acid sequence of SEQ ID NO: 3, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (iii) a VH comprising the amino acid sequence of SEQ ID NO: 4, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (iv) a VH comprising the amino acid sequence of SEQ ID NO: 5, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (v) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (vi) a VH comprising the amino acid sequence of SEQ ID NO: 8, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (vii) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (viii) a VH comprising the amino acid sequence of SEQ ID NO: 10, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (ix) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (x) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (xi) a VH comprising the amino acid sequence of SEQ ID NO: 13, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (xii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 14;
- (xiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 15;
- (xiv) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (xv) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 16;
- (xvi) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 17;

- (xvii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 18;
- (xviii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 19;
- (xix) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 20;
- (xx) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 21;
- (xxi) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 22;
- (xxii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 23;
- (xxiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 24;
- (xxiv) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (xxv) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 25;
- (xxvi) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 26;
- (xxvii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 27;
- (xxviii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 28;
- (xxix) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 29;
- (xxx) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 30;
- (xxxi) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 31;
- (xxxii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 32;

- (xxxiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 33;
- (xxxiv) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 34;
- (xxxv) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 35;
- (xxxvi) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 36;
- (xxxvii) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 37;
- (xxxviii) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 38;
- (xxxix) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 39;
- (xxxx) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 40; or
- (xxxxi) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 41.

[00331] Embodiment 4. The antibody or antigen binding fragment of any one of embodiments 1-3, wherein the antibody is an IgG.

[00332] Embodiment 5. The antibody of any one of embodiments 1-4, wherein the antibody is a humanized antibody.

[00333] Embodiment 6. The antibody of any one of embodiments 1-5, wherein the antibody or antigen binding fragment thereof is genetically fused or chemically conjugated to an agent.

[00334] Embodiment 7. A nucleic acid molecule encoding the antibody or antigen binding fragment of any one of embodiments 1-6.

[00335] Embodiment 8. A vector comprising the nucleic acid molecule of embodiment 7.

[00336] Embodiment 9. A host cell transformed with the vector of embodiment 8.

[00337] Embodiment 10. A composition comprising a therapeutically effective amount of the antibody or antigen binding fragment of any one of embodiments 1-6, the nucleic acid

molecule of embodiment 7, or the vector of embodiment 8, and a pharmaceutically acceptable excipient.

[00338] Embodiment 11. A method of treating a disease or disorder in a subject, comprising administering to the subject the composition of embodiment 10.

[00339] Embodiment 12. The method of embodiment 11, wherein the disease or disorder is associated with ACVR2A.

[00340] Embodiment 13. The method of embodiment 11, wherein the disease or disorder is associated with ACVR2A ligands.

[00341] Embodiment 14. The method of embodiment 13, wherein the disease or disorder is associated with Activin A, GDF8 or GDF11.

[00342] Embodiment 15. The method of any one of embodiments 11-14, wherein the disease or disorder is a musculoskeletal disease or disorder.

[00343] Embodiment 16. The method of embodiment 15, wherein the musculoskeletal disease or disorder is selected from a group consisting of muscle atrophy, spinal muscular atrophy and cancer cachexia.

[00344] Embodiment 17. The method of any one of embodiments 11-14, wherein the disease or disorder is an age-related condition selected from a group consisting of sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, frailty, memory loss, wrinkles, impaired kidney function, and age-related hearing loss.

[00345] Embodiment 18. The method of any one of embodiments 11-14, wherein the disease or disorder is a metabolic disorder selected from a group consisting of Type II Diabetes, Metabolic Syndrome, hyperglycemia, NASH and obesity.

[00346] Embodiment 19. The method of any one of embodiments 11-14, wherein the disease or disorder is selected from a group consisting of acute and/or chronic renal disease or failure, liver fibrosis or cirrhosis, lung fibrosis, pulmonary arterial hypertension, kidney fibrosis, Parkinson's Disease, ALS, brain atrophy, dementia cachexia, bone-loss inducing cancers.

[00347] Embodiment 20. The method of any one of embodiments 11-14, wherein the disease or disorder is cancer selected from a group consisting of sarcoma, ovarian cancers, breast cancer, esophageal cancer, head and neck cancer, lung cancer, melanoma, multiple

myeloma, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, endometrial cancer and gastrointestinal cancers.

[00348] Embodiment 21. The method of any one of embodiments 11-14, wherein the disease or disorder is anemia.

[00349] Embodiment 22. The method of any one of embodiments 11 to 21, wherein the method further comprises administering to the subject a second agent.

[00350] Embodiment 23. The method of embodiment 22, wherein the second agent is an anti-ACVR2B antibody or an ACVR2B antagonist, wherein optionally the ACVR2B antagonist is Luspatercept.

[00351] Embodiment 24. The method of embodiment 22, wherein the second agent is an anti-PD-L1 antibody.

[00352] Embodiment 25. A method for inhibiting or antagonizing ACVR2A in a cell, comprising contacting the cell with the composition of embodiment 10.

[00353] For the sake of conciseness, certain abbreviations are used herein. One example is the single letter abbreviation to represent amino acid residues. The amino acids and their corresponding three letter and single letter abbreviations are as follows:

alanine	Ala	(A)
arginine	Arg	(R)
asparagine	Asn	(N)
aspartic acid	Asp	(D)
cysteine	Cys	(C)
glutamic acid	Glu	(E)
glutamine	Gln	(Q)
glycine	Gly	(G)
histidine	His	(H)
isoleucine	Ile	(I)
leucine	Leu	(L)
lysine	Lys	(K)
methionine	Met	(M)
phenylalanine	Phe	(F)
proline	Pro	(P)
serine	Ser	(S)

threonine	Thr	(T)
tryptophan	Trp	(W)
tyrosine	Tyr	(Y)
valine	Val	(V)

[00354] The invention is generally disclosed herein using affirmative language to describe the numerous embodiments. The invention also specifically includes embodiments in which particular subject matter is excluded, in full or in part, such as substances or materials, method steps and conditions, protocols, procedures, assays or analysis. Thus, even though the invention is generally not expressed herein in terms of what the invention does not include, aspects that are not expressly included in the invention are nevertheless disclosed herein.

[00355] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the following examples are intended to illustrate but not limit the scope of invention described in the claims.

7. EXAMPLES

[00356] The following is a description of various methods and materials used in the studies, and are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present disclosure, and are not intended to limit the scope of what the inventors regard as their disclosure nor are they intended to represent that the experiments below were performed and are all of the experiments that may be performed. It is to be understood that exemplary descriptions written in the present tense were not necessarily performed, but rather that the descriptions can be performed to generate the data and the like associated with the teachings of the present disclosure. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, percentages, *etc.*), but some experimental errors and deviations should be accounted for.

7.1. Example 1: Generation of Human Antibodies to ACVR2A

[00357] The objective of this study was to isolate human antibodies that bind to the ACVR2A target with high affinity and specificity, and block the interaction of ACVR2A with its ligands, such as Activin A, Activin B, GDF8, and GDF11. This was achieved by panning a human Fab fragment phage display library against a recombinant protein comprising the human ACVR2A extracellular domain (ECD) fused to the human IgG1 Fc

region, and screening the resulting antibodies for their ability to block the ACVR2A interaction with Activin A.

[00358] Antigen Preparation and Biotinylation. A recombinant protein comprising the human ACVR2A extracellular domain (ECD) fused to the human IgG1 Fc region was used for panning and screening. The Fc fusion protein ACVR2A-Fc was checked by SDS-PAGE analysis to confirm the molecular weight (MW). To facilitate phage panning in solution using streptavidin-coated magnetic beads, ACVR2A-Fc was biotinylated using EZ-link sulfo-NHS-LC-biotin (Thermo) biotinylation kits. Biotinylation reactions were performed following the manufacturer's protocol. Biotinylated-ACVR2A-Fc was checked by SDS-PAGE analysis, ELISA and Biotin Quantification Kit. Biotinylated- ACVR2A-Fc (100nM of mono-meric antigen) was tested by ELISA and 96-well Greiner plate was coated with biotin-antigen at 4°C overnight and blocked with 1% casein. The plate was washed with PBS between incubation and was detected by streptavidin-HRP (1:5000 dilutions in 1% casein) and the reading value showed at OD500 nm.

[00359] Phage libraries and library amplification. HDB169 and HDB323 libraries were constructed from the same group of donors with variable CDRH3 gene. They are productive human naive antibody libraries. Libraries were first amplified and induced to obtain Fab-displaying phage pools before each panning. Input-1 phages were obtained from amplification of 5×10^{10} per μl stock phages of each library. All the output phages of each round were also amplified to make input phages for the next round. To amplify library or input phages, 50 ml of XL1-blue cells were grown in 2YT medium containing 10 $\mu\text{g/ml}$ TET. When OD600 reach 0.5-0.8, E.coli was infected with phages and continue to grow at 37°C for 3 hours in the presence of 1 μM IPTG. Phage supernatant was collected by centrifugation and purified with PEG precipitation following standard protocol. The purified phages were suspended in 1 ml PBS and stored at 4°C. Amplified phages stored more than 4 weeks were discarded. Panning reactions were carried out in solution panning and immunotube panning.

[00360] Phage library solution panning against ACVR2A. The input library phages (around 5×10^{12} pfu in 1ml of 0.5% casein) were first incubated in casein-blocked 100 μL streptavidin-magnetic beads with biotin-human Fc protein for 15 min to deplete non-specific binders. The depleted library was then incubated with biotin-ACVR2A-FC for 2h, rolling up and down, followed by incubation with 100 μL casein blocked streptavidin-magnetic beads for 15 min. Unbound phages were removed by washing with PBST for 10-20 times. The bound phages were eluted with 400 μL of freshly prepared 100mM Triethylamine and

neutralized by addition of 200 μ L of 1M Tris-HCl, pH 6.4. The Output phage was kept on ice all the time.

[00361] Phage library immunotube panning against ACVR2A. Immunotube was coated with 1 ml antigen at 4°C overnight. The input library phages (around 5x10¹² pfu in 1ml of 1% BSA) were incubated in casein-coated immunotube and human Fc-coated immunotube, sequentially for 2h. The depleted library was then incubated in ACVR2A-Fc-coated immunotube for 2h, rolling up and down. Unbound phages were removed by washing with PBST for 10-20 times. The bound phages were eluted with 1 ml of freshly prepared 100mM Triethylamine and neutralized by addition of 0.5 ml of 1M Tris-HCl, pH 6.4. The Output phage was kept on ice all the time.

[00362] Determination of Phage Titer. 10 μ L of the initial phage library (input titer) or panning eluate (output titer) was serially diluted (10-fold) in PBS. A 90 μ L aliquot of each phage dilution was mixed with 500 μ L of TG1 E. coli cells grown to an optical density of 0.5 at 600 nm (OD 600 nm). Phage were allowed to infect the cells by stationary incubation for 30 mins, then shaking incubation (250 rpm) for 30 mins, all at 37°C. A 10 μ L aliquot of each infected cell culture was spotted on a 2YT agar plate supplemented with 2% glucose and 100 μ g/mL Ampicillin. Plates were incubated overnight at 30°C. Colonies growing from each 10 μ L spot were counted and used to calculate input and output titers.

[00363] Screening O3 phage pool by antigen specific filter lift. O3 phage was diluted and plated out (500-5000 pfu per plate) to grow at 37°C for 8h and captured by anti-kappa antibody-coated filter overnight at 22°C. Biotinylated ACVR2A-Fc (100nM) and NeutrAvidin-AP conjugate (1:1000 dilution) were applied to the filter to detect antigen binding anti-ACVR2A-Fc phages. Positive phage plaques were picked and eluted into 100 μ L of phage elution buffer.

[00364] PCR and DNA sequencing. Fab genes were amplified from antigen positive phages and sequenced at Genewiz Biotech Co. VL and VH sequences were analyzed to sort out unique hits and to determine the hit diversity.

[00365] Phage single-point ELISA. 96-well Greiner plate was coated with antigen at 4°C overnight and blocked with 1% casein. Amplified phages of antigen positive clones were first balanced in 0.1% casein for 1 hour and then incubated in the antigen-coated plate for 2 hours. The plate was washed with PBST between incubations. Antigen bound phages were detected by anti-M13-HRP (1:5000 dilutions in 1% casein). About 10-15 μ L eluted phages were used to infect 1 ml XL1 blue cells to make high titer phage samples (HT) for further analysis without

quantification. For maintain equal amount of phage, the HT phage was first grown in 15 ml XL1-blue cells and induced with IPTG for 3hours. Induced phages were then purified by PEG precipitation. The phage titer was tested before assay.

[00366] Antibody cloning, expression and purification. To verify kinetics of diverse antibody hits, antibody hits were produced through transient transfection of 293F cells and followed with purification for further experiments. VL and VH gene sequences of all hits were cloned into expression vectors pFUSE2ss-CLIg-hk (light chain, Invivogen, Cat No. pfuse2ss-hclk) and pFUSEss-CHlg-hG1 (heavy chain, Invivogen, Cat No. pfusess-hchg1). For each transfection sample, lipid-DNA complexes were prepared as follows: 15 µg of plasmid DNA were diluted in Opti-MEM® I to a total volume of 0.5 ml, mixing gently; 30 µl of 293fectin™ Reagent were diluted in Opti-MEM® I to a total volume of 0.5 ml, and mixed gently and incubated for 5 minutes at room temperature. After the 5 minute incubation, the diluted DNA were added to the diluted 293fectin™ Reagent to obtain a total volume of 1 ml, mixed gently and incubated for 30 minutes at room temperature to allow the DNA-293fectin™ complexes to form. 1 ml of DNA-293fectin™ complex were added to each shaker flask containing the cell suspension 1×10^6 cells/ml. The cells were incubated in a 37°C incubator with a humidified atmosphere of 8% CO₂ in air on an orbital shaker rotating at 125 rpm. On Day 4 post-transfection, the supernatant were harvested and then purified by pierce Protein A Plus Agarose and followed with buffer exchange with PBS. The amount of purified antibody was measured by OD280.

[00367] Binding affinity analysis via Biacore®3000. To measure the binding kinetics of diverse antibody hits, antibody hits were analyzed on Biacore®3000. After antibodies were expressed separately via 293F cells, the affinity and kinetics of antibody were analyzed by direct immobilization method at 25°C on a CM5 sensor chip using Biacore®3000 machine. The kinetics K_a (association rate) and K_d (dissociation rate) were calculated with concentration-dependent fits and the KD (equilibrium dissociation constant) was calculated with Biacore®3000 analysis software (BIAevaluation version 4.1). Approximately 100-800 resonance units (RU) of antigen ACVR2A-Fc, were immobilized separately on different channel of a CM5 chip with an activated amine group. After immobilization, antibodies were injected into the channels at 25°C in HBS-EP buffer, with a flow rate of 30 µl/ min, 1 min. When the data collection was finished in each cycle, the sensor surface was regenerated using 80 mM NaOH with a flow rate of 30 µl/ min, 15 sec and reused chip in next cycle. After

subtraction of reference surface and buffer injection, curve was locally fitted with BIAevaluation version 4.1 using a 1:1 Langmuir binding mode.

[00368] Total 24 anti-ACVR2A hits were discovered from panning 2 phage libraries (HDB169, HDB323) using solution and immunotube formats, including 169T-2, 169T-3, 169T-4, 169T-10, 169T-11, 169T-12, 169T-18, 169T-20, 169T-26, 169T-34, 169T-42, 169T-44, 169T-46, 169T-47, 169T-52, 169T-53, 169T-56, 169T-73, 169T-75, 169T-86, 169T-91, 169T-95, 323T-5, 323T-30. The antigen binding specificity was confirmed by SPE. All hits showed positive binding ability toward antigen. Fab genes were amplified from antigen positive binders. 24 VL and VH sequences anti-ACVR2A hits were analyzed to sort out unique hits and to determine the hit diversity.

7.2. Example 2: Affinity maturation of ACVR2A antibodies via mutational phage-display libraries

[00369] **1. Affinity maturation via kappa-chain-shuffling in combination with point mutations in CDRH1 and CDRH2.** A Fab phage-display library was constructed for affinity maturation. This library contained point mutation in CDRH1 and CDRH2 in combination with kappa-chain shuffling based on parental clone 169T-12. The library was panned against antigen ACVR2A-Fc using immunotube-panning protocol. Two rounds of panning were carried out. After two rounds of panning, proximately 15,000-20,000 output-3 (O3) phages were screened for binding to biotin-labeled antigens by the filter lift assay. Positive hits were then verified by phage Single Point ELISA (SPE), phage titration ELISA and followed by DNA sequencing.

[00370] HDB169-12 library was constructed based on hit 169T-12 obtained from panning described in Example 1. The library contains constant CDRH3 gene with point mutation on CDRH1 and CDRH2 plus kappa-chain shuffling. Library was first amplified and induced to obtain Fab-displaying phage pools before each panning. Input-1 phages were obtained from amplification of 5×10^{10} per μl stock phages. All the output phages of each round were also amplified to make input phages for the next round. To amplify library or input phages, 50 ml of XL1-blue cells were grown in 2YT medium containing 10 $\mu\text{g/ml}$ TET. When OD₆₀₀ reach 0.5-0.8, E.coli was infected with phages and continue to grow at 37°C for 3 hours in the presence of 1 μM IPTG. Phage supernatant was collected by centrifugation and purified with PEG precipitation following standard protocol. The purified phages were suspended in 1 ml PBS and stored at 4°C.

[00371] Total 119 anti-ACVR2A hits were discovered from phage library (HDB169-12) using immunotube format. The antigen binding specificity was confirmed by SPE. All hits showed different binding ability toward antigen. 49 selected hits were tested by phage titration ELISA and 15 hits were chosen for further experiments in following sections.

[00372] The affinity of anti-ACVR2AFab fragment was determined on Biacore®3000 as shown in **Table 3** below. The affinity of hit binding to antigen ACVR2A, was comparing with parental hit 169T-12. All the results showed that after affinity maturation, the affinity of hit number 335 and 231 have improvement. Those two hits were used for affinity maturation via CDRH1 saturated mutational phage-display libraries and affinity panning in following section.

Table 3. The affinity of anti-ACVR2A Fab fragment

Name	$K_a (M^{-1}s^{-1})$	$k_d (s^{-1})$	$K_D (M)$	$R_{max} (RU)$
Parental 169T-12	1.53E+03	7.83E-05	5.12E-08	119
3	1.07E+05	2.84E-04	2.64E-09	219
10	1.43E+04	2.96E-03	2.07E-07	243
19	1.44E+05	4.66E-04	3.24E-09	174
29	5.42E+04	7.56E-04	1.39E-08	237
139	5.42E+04	7.56E-04	1.39E-08	237
231	1.41E+05	5.41E-04	3.83E-09	217
239	5.20E+04	1.38E-04	2.65E-09	222
257	2.97E+02	4.08E-05	1.37E-07	321
264	8.31E+04	1.01E-03	1.21E-08	224
297	6.46E+03	1.08E-03	1.67E-07	170
309	7.49E+04	1.02E-04	1.37E-09	239
311	4.69E+02	3.88E-05	8.28E-08	224
321	1.84E+04	9.32E-04	5.06E-08	301
335	2.19E+05	1.26E-04	5.74E-10	97.6
336	2.24E+02	1.44E-05	6.41E-08	264

[00373] **2. Affinity maturation via CDRH1, CDRL1, CDRL2 random mutational phage-display libraries and affinity panning.** The 3 libraries contain random point mutations (except the canonical amino acid) on CDRH1, CDRL1 and CDRL2 respectively. The library was panned against antigen ACVR2A-Fc using immunotube-panning protocol, and cell panning with human ACVR2A overexpressed 293T cells 293T-2A. Affinity panning was carried out. After affinity panning, output-3 (O3) phages were screened for binding ability by Fab ELISA and followed by NGS DNA sequencing. Libraries were first amplified

and induced to obtain Fab-displaying phage pools before each panning. Input-1 phages were obtained from amplification of 2×10^9 pfu phages. All the output phages of each round were also amplified to make input phages for the next round. To amplify library or input phages, 100 mL of TG1 cells were grown in 2YT medium containing 10 $\mu\text{g/mL}$ Amp. When OD₆₀₀ reach 0.5-0.8, E.coli was infected with phages and continue to grow at 37°C over night in the presence of 1 μM IPTG. Phage supernatant was collected by centrifugation and purified with PEG precipitation following standard protocol. The purified phages were suspended in 1 mL PBS and stored at 4°C.

[00374] 2.1 Antibody and Fab fragment expression and purification. To verify kinetics of diverse antibody hits, hits were produced in Fab fragment forms in TG1, and followed with purification for further experiments. Fab fragment production from TG1. 3 mL 2YT with Ampicillin (Final conc. 100 $\mu\text{g/mL}$) and glucose (Final conc. 1%) addition were used for bacterial overnight culture at 37°C, 220rpm. To amplify culture, 200 mL of TG1 cells were grown in TB medium containing 200 μL Ampicillin (final conc. 100 $\mu\text{g/mL}$) and 500 μL 20% Glucose (final conc. 0.05%). When OD₆₀₀ reached 0.4-0.6, E.coli grew at 30°C for 16 hrs in the presence of 1mM IPTG to produce fab fragments. Cell pellet was collected by centrifugation and lysis by sonication. Supernatant was harvested and purified with Ni- NTA Sefinose (TM) Resin (Settled Resin) following standard protocol and followed with buffer exchange with PBS. The purified Fab fragments were suspended in 1 mL PBS and stored at 4°C. The amount of purified Fab fragments was measured by OD₂₈₀.

[00375] For antibody hits expression, VL and VH gene sequences of all hits were cloned into expression vectors pFUSE2ss-CLIg-hk (light chain, Invitrogen, Cat No. pfuse2ss-hclk) and pFUSEss-CHlg-hG1 (heavy chain, Invitrogen, Cat No. pfusess-hchg1). For each transfection sample, lipid-DNA complexes were prepared as follows: 15 μg of plasmid DNA were diluted in Opti-MEM® I to a total volume of 0.5 mL, mixing gently; 30 μL of 293fectin™ Reagent were diluted in Opti-MEM® I to a total volume of 0.5 mL, mixed gently and incubated for 5 minutes at room temperature. After the 5 minutes incubation, the diluted DNA were added to the diluted 293fectin™ Reagent to obtain a total volume of 1 mL, mixed gently, and incubated for 30 minutes at room temperature to allow the DNA-293fectin™ complexes to form. 1 mL of DNA-293fectin™ complex were added to each shaker flask containing the cell suspension 1×10^6 cells/ml. The cells were incubated in a 37°C incubator with a humidified atmosphere of 8% CO₂ in air on an orbital shaker rotating at 125 rpm. On

Day 4 post-transfection, the supernatant was harvested and then purified by Pierce Protein A Plus Agarose and followed with buffer exchange with PBS.

[00376] 2.2 Surface Plasmon resonance (SPR) measurements and affinity analysis by anti-human Fc capture format. After affinity panning, output-3 (O3) of CDRH1 library were screened for binding ability by Fab ELISA and followed by NGS DNA sequencing. Based on binding Elisa and sequence abundance from NGS data, 11 clones were selected for SPR analysis. Approximately 100-800 resonance units (RU) of antigen ACVR2A, was captured by anti-human Fc immobilized on a channel of a CM5 chip with an activated amine group. After immobilization, Fab antibodies were injected into the channels at 25°C in HBS-EP buffer, with a flow rate of 30 $\mu\text{L}/\text{min}$, 1 min. When the data collection was finished in each cycle, the sensor surface was regenerated using 80mM NaOH with a flow rate of 30 $\mu\text{L}/\text{min}$, 15 sec and reused chip in next cycle. After subtraction of reference surface and buffer injection, curve was locally fitted with BIA evaluation version 4.1 using a 1:1 Langmuir binding mode.

[00377] The affinity of selected 11 anti-ACVR2A monoclonal antibody clones were determined on Biacore®3000 and Biacore®8000 as shown in Table 4 below. Most of the clones showed high affinity to ACVR2A, and 4 clones E, G I, J were selected as parental clones for light chain mutation library construction.

Table 4. The affinity of anti-ACVR2A monoclonal antibody clones

Clone	1 st test (Biacore 3000)				2 nd test (Biacore 8000)			
	Ka(M ⁻¹ s ⁻¹)	kd(s ⁻¹)	R _{max} (RU)	K _D (M)	Ka(M ⁻¹ s ⁻¹)	kd(s ⁻¹)	R _{max} (RU)	K _D (M)
A	4.29E+05	2.27E-03	103	5.29E-09	4.81E+05	2.57E-03	91.6	5.35E-09
B	3.23E+05	2.20E-03	106	6.81E-09	4.28E+05	2.53E-03	85.1	5.91E-09
C	3.65E+04	4.16E-05	163	1.14E-09	9.03E+04	2.47E-04	93	3.08E-09
D	1.81E+05	2.32E-03	106	1.28E-08	2.73E+05	2.85E-03	99.7	1.04E-08
E	2.85E+05	1.90E-04	94	6.64E-10	3.07E+05	1.53E-04	87	5.01E-10

F	2.44E+04	2.88E-04	158	1.18E-08	2.25E+05	3.31E-04	94.4	1.47E-09
G	2.48E+05	1.52E-04	109	6.12E-10	6.37E+05	2.82E-04	88	4.43E-10
H	3.11E+05	6.98E-04	105	2.25E-09	6.51E+05	9.01E-04	103	1.38E-09
I	2.54E+05	4.68E-04	117	1.84E-09	3.74E+05	3.79E-04	72.9	1.01E-09
J	4.36E+05	4.41E-05	105	1.01E-10	1.39E+06	2.13E-04	86.2	1.53E-10
K	5.23E+05	2.56E-04	109	4.89E-10	1.22E+06	2.59E-04	80.5	2.12E-10

[00378] 2.3 Activin A Competitive ELISA. To select ACVR2A hits that could block the interaction between ACVR2A and its ligand Activin A, a competitive ELISA assay was set up. 30 μ L 4.17 nM ACVR2A mouse Fc fusion protein was coated in 384-well HB plate at 4°C overnight following blocking with 5% BSA. Anti-ACVR2A Fab fragment or antibodies with different concentrations were added to the plate and incubated on an orbital shaker at room temperature for 1 hours to allow binding of the antibodies. Then 16.7 nM activin A (R&D, 338-AC/CF) in blocking buffer was added to the plate, incubated at room temperature for 1 hours to reach equilibrium. After 5 times washing with TBST, 30 μ L of 20 nM goat anti-activin A polyclonal antibody (Abcam, ab7153) in blocking buffer was added into plate and incubated at room temperature for 1 hour. Last, 30 μ L of anti-goat IgG-HRP (Beyotime, A0181) in blocking buffer (1:500 dilution) was added to plate for another 30 min incubation following washing step. Luminescence signal was collected on Tecan infinite M1000 after adding 30ul ECL substrate.

[00379] FIGs. 1A-1D show examples of tested Anti-ACVR2A Fab fragments from CDRL1 (FIG. 1A) and CDRL2 (FIG. 1B) mutational libraries. The hits which showed smaller IC₅₀ than parental clone such as clone “J” in this competitive ELISA assay were chosen for IgG purification and further validation. As shown in FIG. 1C, most selected IgG blocked the interaction between ACVR2A and Activin A, and based on these results, more IgG were designed using CDR shuffling method. These designed and purified IgG 3351,

3352, 3353, 3354, 3355, 6401, 6402, 6403, NGS-1, NGS-2, NGS-3, NGS-4, NGS-5 showed comparable potency on Activin A blocking with the parental clone “J”, as shown in FIG. 1D.

[00380] 2.4 Phospho-Smad Dependent Reporter Gene Assay of ACVR2A Hits. To determine the capacity of anti-ACVR2A antibodies to inhibit Activin A-induced signaling, a reporter gene assay was developed using HEK293T-B1 cells and CAGA-12 luciferase reporter construction. Wild type HEK293T express ACVR2A and ACVR2B, both of them could initiate downstream signaling through SMAD-mediated signaling after binding to ligands such as Activins and GDFs. To minimize the effect of ACVR2B, a ACVR2B knockout stable cell line HEK293T-B1 generated with CRISPR/Cas9 technology was used for CAGA-12 luciferase transfection. The CAGA-12 luciferase reporter construct carries the luciferase gene downstream of a minimal promoter and multiple CAGA boxes which are specific for phosphorylated Smad-2 and Smad-3. Addition of purified Activin A (but also of GDF-11, activin or TGF β) induces Smad phosphorylation and thus binding to the CAGA-12 reporter and leads to luciferase gene expression.

[00381] At 90% confluency of HEK293T-B1 CAGA-12 luc cells, cells are detached as described and diluted in culture medium to a concentration of 2.5×10^5 cells/ml. Subsequently, 100 μ l cells per well are seeded into flat-bottomed 96-well plates and incubated at 37° C. and 5% CO₂ overnight. The next day, the antibodies (Fab or IgG) and the recombinant human ACVR2A/Fc, which served as the positive control, are diluted in PBS to the desired concentrations. 20 μ l of the antibody solutions are added to the seeded wells of the previous day and the cells cultivated for 1 hr to allow binding of the antibodies. Finally, 50 ng/ml Activin A (R&D, 338-AC/CF) or GDF8 (R&D, 788-G8) was added to the wells and the cells further cultivated overnight. The next morning, 120 μ l Bright-Glo luciferase reagent (Promega, E2610) was added to each well. After 2 min incubation time, the luminescence is read in a luminometer. The half maximal inhibitory concentration (IC₅₀ values) is calculated after full titration of the respective antibodies.

[00382] In according to Activin A Competitive ELISA result, the selected clones from CDRH1, CDRL1 and CDRL2 mutational library could inhibit Activin A induced phospho-Smad dependent reporter signaling with high potency. As shown in FIG. 2A, the hits J, 21047, 21155, 21169, 21341, 21343, 21366 and 275 inhibited Activin A induced p-Smad dependent signaling, with IC₅₀ from 2×10^{-9} M to 1×10^{-8} M. Similar with Activin A Competitive ELISA result, the designed IgG using CDR shuffling method, 3351, 3352, 3353,

3354, 3355, 6401, 6403, NGS-1, NGS-3, NGS-4, NGS-5 showed comparable or better potency with the hit “J” in Activin A induced phospho-Smad dependent reporter assay.

[00383] Besides Activin A, GDF8 (Myostatin) also binds to ACVR2A and induces Smad2/3 signaling. The HEK293T-B1 CAGA-12 luc cells was used here to test whether ACVR2A hits could block interaction between ACVR2A and GDF8. The results showed all of selected IgG hits inhibited GDF8 induced phospho-Smad dependent reporter signaling, including the hits J, 21047, 21155, 21169, 21341, 21343, 21366, 275 and 6401(FIG. 2D).

[00384] **2.5 Phospho-Smad3 HTRF Assay in C2C12 Cells.** Besides HEK293T cells, a mouse cell line derived from satellite cells was used here to test whether ACVR2A antibody hits could block Activin A induced endogenous phospho-Smad3 change. C2C12 cells (ATCC, CRL-1772) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium with 10% Fetal Bovine Serum (FBS). For phospho-Smad3 test, cells were plated in 384-well plate at a density of 1,000 cells per well, and incubated at 37°C and 5% CO₂ overnight. The next day, the antibodies were diluted in PBS to the desired concentrations. 10 µl of the antibody solutions were added to the seeded wells of the previous day and the cells cultivated for 1 hr to allow binding of the antibodies. Finally, 50 ng/ml Activin A was added to the wells and incubated for 30 min. Phospho-Smad3 level was detected with PHOSPHO-SMAD3 (SER423/425) HTRF KITS (Cisbio, 63ADK025PEG) following the manual instructions.

[00385] The phospho-Smad3 HTRF assay in C2C12 cells showed ACVR2A antibodies in this invention, including hits J, 21155, 21169, 21341, 275 and 6401, inhibited Activin A induced increase of phospho-Smad3 level.

[00386] In brief, we developed a series of ACVR2A antibodies that with high affinity to ACVR2A protein in this disclosure, these antibodies block the interaction between ACVR2A and its ligands, such as Activin A and GDF8, thereby block the Activin A and GDF8 induced signaling through ACVR2A. The property of some representative hits was summarized in Table 5, including affinity to ACVR2A, the IC₅₀ in Activin A competitive Elisa assay, Activin A or GDF8 induced luciferase assay.

Table 5. Summary of representative anti-ACVR2A antibodies

	Biacore			Competitive Elisa	HEK293T-B1 CAGA-Luc	
	ka (1/Ms)	kd (1/s)	KD (M)	Activin A	Activin A	GDF8
				IC ₅₀ (M)	IC ₅₀ (M)	IC ₅₀ (M)
J	2.53E+05	6.16E-05	2.44E-10	5.04E-09	5.12E-09	2.06E-09
275	3.66E+05	3.19E-04	8.72E-10	6.04E-09	4.91E-09	8.81E-10

21155	3.82E+05	8.07E-05	2.11E-10	5.13E-09	1.78E-09	6.51E-10
21169	3.59E+05	2.38E-04	6.63E-10	5.30E-09	2.66E-09	4.99E-10
21341	3.16E+05	5.44E-05	1.72E-10	4.83E-09	6.24E-09	1.60E-09
21343	1.05E+05	4.51E-05	4.30E-10	4.74E-09	9.07E-09	1.64E-09
21366	5.16E+04	7.91E-06	1.53E-10	4.15E-09	3.64E-09	1.65E-09
3352	1.61E+05	9.41E-05	5.84E-10	2.07E-09	2.28E-09	3.51E-10
6401	1.56E+05	1.35E-04	8.65E-10	1.95E-09	1.93E-09	2.16E-09
NGS-1	1.85E+05	1.18E-04	6.38E-10	1.86E-09	3.28E-09	2.42E-10

[00387] Variable region sequences of anti-ACVR2A antibodies are provided below in

Table 6. The CDRs sequences are provided in Table 7.

Table 6. V-regions of anti-ACVR2A antibodies

mAB	VH	VL
A	QVQLVQSGAEVKKPGSSVKVSC KASGGQFYHSRISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:1)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
B	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:3)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
C	QVQLVQSGAEVKKPGSSVKVSC KASGREFESLFISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:4)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
D	QVQLVQSGAEVKKPGSSVKVSC KASGQFYHSRISWVRQAPGQGL EWMGGIPIFGTANYAQKFQGR VTITADESTSTAYMELSSLRSED TAVYYCVKDLTWTNLGLDIWG QGMVTVSS (SEQ ID NO:5)	EIVMTQSPSSLSASVGDVTITCRA SQSIGRWLAWYQQKPGKAPRLLIY AASSLHSGVPSRFSGSGSGTHFTLT ISSLQPEDFATYFCQESYSNPYTFG PGTKLEIK (SEQ ID NO:6)
E	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQSIGRWLAWYQQKPGKAPRLLIY AASSLHSGVPSRFSGSGSGTHFTLT ISSLQPEDFATYFCQESYSNPYTFG PGTKLEIK (SEQ ID NO:6)
F	QVQLVQSGAEVKKPGSSVKVSC KASGREFESLFISWVRQAPGQG	EIVMTQSPSSLSASVGDVTITCRA SQSIGRWLAWYQQKPGKAPRLLIY

	LEWMGGLIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGTMTVTVSS (SEQ ID NO:8)	AASSLHSGVPSRFSGSGSGTHFTLT ISSLQPEDFATYFCQESYSNPYTFG PGTKLEIK (SEQ ID NO:6)
G	QVQLVQSGAEVKKPGSSVKVSC KASGRHFGSLQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGTMTVTVSS (SEQ ID NO:9)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
H	QVQLVQSGAEVKKPGSSVKVSC KASGLGFHNFQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGTMTVTVSS (SEQ ID NO:10)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
I	QVQLVQSGAEVKKPGSSVKVSC KASGESFEHFQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGTMTVTVSS (SEQ ID NO:11)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
J	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGTMTVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
K	QVQLVQSGAEVKKPGSSVKVSC KASGMPFQQSQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGTMTVTVSS (SEQ ID NO:13)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
275	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGTMTVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGPSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:14)
327	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGTMTVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQQQSTFLTWYQQKPGKAPRLLIY AASSLHSGVPSRFSGSGSGTHFTLT ISSLQPEDFATYFCQQSYSTPFTFGP GTKLEIK (SEQ ID NO:15)
258	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPIFGTANYAQKFQG	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL

	RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:2)
336	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLSISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGVSWWLPWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:16)
402	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQQQASPFLSWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:17)
509	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQQCSQQLQWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:18)
335	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDGVTITCRA SQSIGRWLAWYQQKPGKAPKLLIY GASSLQSGVPSRFSGSGSGTDFTLT ISSLQPEDFAIYYCQQSYSTPFTFG QGTKVEIK (SEQ ID NO:19)
366	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGASQWLKWYQQKPGKAPRLLI YAASSLHSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:20)
2046	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLSISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YGAGQLHSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:21)
2108	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLSISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YHARFLQSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:22)
3351	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE	EIVMTQSPSSLSASVGDVTITCRA SQGPSRWLAWYQQKPGKAPRLLI YGASPLSSGVPSRFSGSGSGTHFTL

	DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:23)
3352	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGPSRWLAWYQQKPGKAPRLLI YAAQGLSSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:24)
3353	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQSIGRWLAWYQQKPGKAPRLLIY AASSLHSGVPSRFSGSGSGTHFTLT ISSLQPEDFATYFCQESYSNPYTFG PGTKLEIK (SEQ ID NO:6)
6401	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLSISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGPSRWLAWYQQKPGKAPRLLI YSARALHSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:25)
6403	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLSISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGPSRWLAWYQQKPGKAPRLLI YWAAHLQSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:26)
NGS-1	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YSAASSLYSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:27)
NGS-3	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YSVASSLHGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:28)
NGS-5	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YSAASVCTGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:29)
21046	QVQLVQSGAEVKKPGSSVKVSC KASGESFEHFQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YSARGLRSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:30)

	DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:11)	
21047	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YRAKSLMSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:31)
21155	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YGASPLSSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:32)
21169	QVQLVQSGAEVKKPGSSVKVSC KASGFQFRTSLISWVRQAPGQG LEWMGGLIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:7)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAAQGLSSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:33)
21178	QVQLVQSGAEVKKPGSSVKVSC KASGRHFGSLQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:9)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAAEQLLSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:34)
21302	QVQLVQSGAEVKKPGSSVKVSC KASGESFEHFQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:11)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YAARRLHSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:35)
21328	QVQLVQSGAEVKKPGSSVKVSC KASGESFEHFQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:11)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YGARVLP SGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:36)
21335	QVQLVQSGAEVKKPGSSVKVSC KASGESFEHFQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:11)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YQASRLLSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:37)

21341 (LA01)	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLSISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YSARALHSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:38)
21343	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLSISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YNARSLFSGVPSRFSGSGSGTHFTL TISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:39)
21366	QVQLVQSGAEVKKPGSSVKVSC KASGPRFQDLSISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:12)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YWAAHLQSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:40)
21385	QVQLVQSGAEVKKPGSSVKVSC KASGRHFGSLQISWVRQAPGQG LEWMGGIPIYGTANYAQKFQG RVTITADESTSTAYMELSSLRSE DTAVYYCVKDLTWTNLGLDIW GQGMVTVSS (SEQ ID NO:9)	EIVMTQSPSSLSASVGDVTITCRA SQGVSRWLAWYQQKPGKAPRLLI YGANMLRSGVPSRFSGSGSGTHFT LTISSLQPEDFATYFCQQSYSTPFTF GPGTKLEIK (SEQ ID NO:41)

Table 7. CDR Amino Acid Sequence of anti-ACVR2A antibodies

mAB	H-CDR1	H-CDR2	H-CDR3	L-CDR1	L-CDR2	L-CDR3
A	GGQFYHS R (SEQ ID NO:42)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLH (SEQ ID NO:46)	QQSYST FT (SEQ ID NO:47)
B	GFQFRT L (SEQ ID NO:48)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLH (SEQ ID NO:46)	QQSYST FT (SEQ ID NO:47)
C	GRFESL F (SEQ ID NO:49)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLH (SEQ ID NO:46)	QQSYST FT (SEQ ID NO:47)
D	GQFYHS (SEQ ID NO:50)	LIPIFG (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQSI RWLA (SEQ ID NO:52)	AASSLH (SEQ ID NO:46)	QESYSN YT (SEQ ID NO:53)
E	GFQFRT L (SEQ ID NO:48)	LIPIFG (SEQ ID NO:51)	DLTWTN LGLDI	RASQSI RWLA	AASSLH (SEQ ID NO:46)	QESYSN YT (SEQ ID NO:53)

			(SEQ ID NO:44)	(SEQ ID NO:52)		
F	GREFESL F (SEQ ID NO:49)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQSIG RWLA (SEQ ID NO:52)	AASSLHS (SEQ ID NO:46)	QESYSNP YT (SEQ ID NO:53)
G	GRHFGSL Q (SEQ ID NO:54)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
H	GLGFHNF Q (SEQ ID NO:55)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
I	GESFEHF Q (SEQ ID NO:56)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
J	GPRFQDL S (SEQ ID NO:57)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
K	GMPFQQS Q (SEQ ID NO:58)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
275	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGPS RWLA (SEQ ID NO:59)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
327	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQQQS TFLT (SEQ ID NO:60)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
258	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
336	GPRFQDL S (SEQ ID NO:57)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV WWLP (SEQ ID NO:61)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)
402	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQQQ ASPFLS (SEQ ID NO:62)	AASSLHS (SEQ ID NO:46)	QSYSTP FT (SEQ ID NO:47)

509	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQQCS QQLQ (SEQ ID NO:63)	AASSLHS (SEQ ID NO:46)	QQSYSTP FT (SEQ ID NO:47)
335	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQSIG RWLA (SEQ ID NO:52)	<u>GASSLQS</u> (SEQ ID NO:82)	QQSYSTP FT (SEQ ID NO:47)
366	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGAS QWLK (SEQ ID NO:64)	AASSLHS (SEQ ID NO:46)	QQSYSTP FT (SEQ ID NO:47)
2046	GPRFQDL S (SEQ ID NO:57)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	GAGQLHS (SEQ ID NO:65)	QQSYSTP FT (SEQ ID NO:47)
2108	GPRFQDL S (SEQ ID NO:57)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	HARFLQS (SEQ ID NO:66)	QQSYSTP FT (SEQ ID NO:47)
3351	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQG RWLA (SEQ ID NO:59)	GASPLSS (SEQ ID NO:67)	QQSYSTP FT (SEQ ID NO:47)
3352	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQG RWLA (SEQ ID NO:59)	AAQGLSS (SEQ ID NO:68)	QQSYSTP FT (SEQ ID NO:47)
3353	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQSIG RWLA (SEQ ID NO:52)	AASSLHS (SEQ ID NO:46)	QESYSNP YT (SEQ ID NO:53)
6401	GPRFQDL S (SEQ ID NO:57)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQG RWLA (SEQ ID NO:59)	SARALHS (SEQ ID NO:69)	QQSYSTP FT (SEQ ID NO:47)
6403	GPRFQDL S (SEQ ID NO:57)	IPIYGT (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQG RWLA (SEQ ID NO:59)	WAAHLQ S (SEQ ID NO:70)	QQSYSTP FT (SEQ ID NO:47)
NGS-1	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASSLY (SEQ ID NO:71)	QQSYSTP FT (SEQ ID NO:47)
NGS-3	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	VASSLH (SEQ ID NO:72)	QQSYSTP FT (SEQ ID NO:47)

NGS-5	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AASVCT (SEQ ID NO:73)	QQSYSTP FT (SEQ ID NO:47)
21046	GESFEHF Q (SEQ ID NO:56)	IPIYGTA (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	SARGLRS GVP (SEQ ID NO:74)	QQSYSTP FT (SEQ ID NO:47)
21047	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	RAKSLMS (SEQ ID NO:75)	QQSYSTP FT (SEQ ID NO:47)
21155	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	GASPLSS (SEQ ID NO:67)	QQSYSTP FT (SEQ ID NO:47)
21169	GFQFRTS L (SEQ ID NO:48)	LIPIFGTA (SEQ ID NO:51)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AAQGLSS (SEQ ID NO:68)	QQSYSTP FT (SEQ ID NO:47)
21178	GRHFGSL Q (SEQ ID NO:54)	IPIYGTA (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AAEQLLS GVP (SEQ ID NO:76)	QQSYSTP FT (SEQ ID NO:47)
21302	GESFEHF Q (SEQ ID NO:56)	IPIYGTA (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	AARRLHS GVP (SEQ ID NO:77)	QQSYSTP FT (SEQ ID NO:47)
21328	GESFEHF Q (SEQ ID NO:56)	IPIYGTA (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	GARVLPS GVP (SEQ ID NO:78)	QQSYSTP FT (SEQ ID NO:47)
21335	GESFEHF Q (SEQ ID NO:56)	IPIYGTA (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	QASRLLS GVP (SEQ ID NO:79)	QQSYSTP FT (SEQ ID NO:47)
21341 (LA01)	GPRFQDL S (SEQ ID NO:57)	IPIYGTA (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	SARALHS (SEQ ID NO:69)	QQSYSTP FT (SEQ ID NO:47)
21343	GPRFQDL S (SEQ ID NO:57)	IPIYGTA (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV RWLA (SEQ ID NO:45)	NARSLFS (SEQ ID NO:80)	QQSYSTP FT (SEQ ID NO:47)

21366	GPRFQDL S (SEQ ID NO:57)	IPIYGT A (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV S RWLA (SEQ ID NO:45)	WAAHLQ S (SEQ ID NO:70)	QQSYST P FT (SEQ ID NO:47)
21385	GRHFGSL Q (SEQ ID NO:54)	IPIYGT A (SEQ ID NO:43)	DLTWTN LGLDI (SEQ ID NO:44)	RASQGV S RWLA (SEQ ID NO:45)	GANMLR SGVP (SEQ ID NO:81)	QQSYST P FT (SEQ ID NO:47)

7.3. Example 3: ACVR2A Ab increased mouse body and muscle weight

[00388] Activin A signaling antagonists, such as GDF8 antibody, Activin A antibody, ACVR2A and ACVR2B extracellular domain fusion proteins, were reported stimulated skeletal muscle hypertrophy. Here ACVR2A antibody of the invention and isotype control antibody were tested in different mouse models. For the study 1, male CB17-SCID mice of approximately 6-8 weeks of age were divided evenly according to body weight into 2 groups of 8 mice. Naive CB17-SCID mice were treated with ACVR2A Ab LA01 subcutaneously at a dose of 20 mg/kg once weekly for 28 days. Body weights were recorded twice a week. On day 28, mice were euthanized and total body weight for each mouse was measured. The tibialis anterior muscles and epididymal adipose tissue from each mouse were dissected and weighed.

[00389] For the study2, C57BL/6 mice of approximately 6-8 weeks of age were divided evenly according to body weight into 2 groups of 8 mice. Isotype control antibody or ACVR2A Ab LA01 with mouse IgG format, which linked LA01 Fab to the mouse IgG2a-LALA Fc domain, were administered at a dose of 10 mg/kg twice weekly for 6 weeks. Body weights were recorded twice a week. The tibialis anterior muscles and gastrocnemius muscles from each mouse were dissected and weighed at the end of study.

[00390] As shown in FIG. 4A, in the first study, ACVR2A Ab LA01 increased mice body weight significantly compared to isotype control group (Day7, 10, 14, $p<0.01$; Day 17, $p<0.001$, Day24, $p<0.01$; Day 28, $p<0.05$). Consistent with body weight change, ACVR2A Ab LA01 also increased muscle weight. The tibialis anterior muscle weight of LA01 treatment is 0.893% body weight, showed significant increase compared to the isotype control (0.726% body weight) treated mice (FIG. 4B, $p<0.05$).

[00391] Interestingly, the fat tissue weights were also changed significantly in ACVR2A Ab treated mice. There are two main types of fat in mammals, subcutaneous and visceral fat. Both subcutaneous and visceral fat, as well as being energy stores, also have endocrine

functions. They release hormones and proteins such as leptin, adiponectin, IL-6, TNF- α and angiotensin, which help regulate other organs and processes in our bodies. However, the hormones and proteins secreted by visceral fat are thought to be more pro-inflammatory than subcutaneous fat. Compared to subcutaneous fat, excess visceral fat is thought to be associated with a constellation of metabolic abnormalities, including insulin resistance, hyperinsulinemia, glucose intolerance, type 2 diabetes, high triglycerides, dyslipidemia, inflammation, and altered cytokine profile. In the first study, both subcutaneous and visceral fat were evaluated after antibodies treatment. The inguinal fat, which belonged to subcutaneous fat, had similar weights among different groups, control 0.54% vs treatment 0.43%, $p=0.13$, FIG. 4C. In contrast, the epididymal fat tissue, a kind of visceral fat, showed decreased tissue weight in ACVR2A Ab LA01 treatment group, control 1.098% vs treatment 0.668%, $p=0.002$, as shown FIG. 4D.

[00392] In the second study, C57BL/6 mice were treated with mouse IgG2A isoform of ACVR2A Ab LA01, the body weight increased significantly compared to isotype control group (FIG. 5A). Similar with study 1, ACVR2A Ab LA01 treatment alone induced hypertrophy in tibialis anterior (LA 01 406.5mg vs Ctrl 330.6mg, $p<0.05$; FIG. 5B) and gastrocnemius (LA 01 793.9mg vs Ctrl 570.5mg, $p<0.001$; FIG. 5C) muscle weights, compared to isotype control treatment.

7.4. Example 4: ACVR2A Ab promoted maturation of developing erythroblasts

[00393] Members of the TGF- β superfamily, such as BMPs, activins, GDFs have been studied as potential regulators of erythropoiesis. Both ACVR2A-Fc and ACVR2B-Fc showed promotion effect on erythroblast maturation. The mutant ACVR2B-Fc ACE-536 (Luspatercept), as an erythroid maturation agent, has been approved for the treatment of beta thalassemia and anemia patients with myelodysplastic syndromes. ACVR2A Ab LA01 demonstrated stimulating effect on erythroid maturation, it also had synergy effect with ACE-536.

[00394] In this study, female C57/BL6 mice of approximately 6-8 weeks of age were divided evenly according to body weight into 4 groups of 8 mice. Mice were treated with ACVR2A Ab LA01, ACE-536, LA01+ACE-536, or isotype control antibody, subcutaneously at a dose of 10 mg/kg twice a week for 14 days. Body weights were measured with an electronic scale every two days. To perform hematologic studies at day 7, the submandibular vein of mice was pierced by a sterile lancet, and 200 μ l of blood was collected in an EDTA-

coated microtainer tube. Terminal blood samples were taken from CO₂ euthanized mice via cardiac puncture. Complete blood counts were determined using an Advia 120 Automated Hematology Analyzer (Bayer).

[00395] After 7 days treatment, ACE-536 increased the red blood cells (RBC) number, hemoglobin concentrations and hematocrit values; but ACVR2A Ab LA01 alone didn't gave significant change on these RBC parameters, as shown in FIGs. 6A, 6B, and 6C. However, 7 days treatment of LA01 increased the number of reticulocytes significantly, as shown in FIG. 6D, indicating LA01 promote erythropoiesis through different mechanism with ACE-536. After 14 days treatment, both ACE-536 and ACVR2A Ab LA01 administration led to elevation of RBC parameters, including RBC number, hemoglobin concentrations and hematocrit values (FIGs. 6E, 6F, and 6G). The data on day 14 showed LA01+ ACE-536 combination gave more dramatic effect on elevation of RBC number, hemoglobin, and hematocrit, than LA01 or ACE-536 treatment alone (FIGs. 6E, 6F, and 6G). These results implicated both ACVR2A Ab LA01 and ACE-536 could stimulate erythropoiesis but act on different erythropoietic stages, they had synergistic effect on RBC production. This study showed the therapeutic potential of ACVR2A Ab LA01 on anemia treatment.

7.5. Example 5: ACVR2A Ab mitigated liver fibrosis in fibrosis and NASH mouse model

[00396] Hepatic fibrosis is a process that occurs when the liver is damaged, such damage can be the result of viral infections, exposure to chemicals, metabolic disorders cancer growth. The liver or serum Activin A was find associated with liver fibrosis stage. As a widely used mouse model, CCl₄-induced liver damage and fibrosis model was employed to evaluate whether effective inhibition of Activin A signaling with ACVR2A Ab treatment is effective in reducing liver fibrosis. For liver fibrosis setup, 6 weeks old of C57/BL6 mice was induced using intraperitoneal injection of corn oil or CCl₄, dissolved in a 1:10 ratio with corn oil, 1mL/kg, twice a week for 5 weeks. Mice were treated with ACVR2A Ab LA01, or Isotype control antibody subcutaneously at a dose of 20 mg/kg once a week for 5 weeks. Body weights were measured with an electronic scale twice a week. Serum alanine aminotransferase (ALT) and (Aspartate aminotransferase) AST were analyzed after two weeks treatment of Ab LA01. Mice were euthanized with CO₂ at the end of study, and two liver lobes were collected and fixed with 10% formalin for histological staining. Liver tissues were embedded in paraffin cut into 5- μ m-thick slices to visualize the cell structure. Hematoxylin-eosin (HE) staining and Masson's trichrome staining were then

performed to assess basic tissue structure and detect fibrosis, inflammation, and hepatitis lesions. The slides were analyzed with Knodell histology activity index (HAI) system by pathologists. Photos were taken under a Leica scanner.

[00397] To test fibrosis related gene expression, total RNA was extracted from liver tissues using RNeasy Mini Kit (Qiagen, 74104) follow kit's instruction. One microgram of RNA was reverse-transcribed using BeyoRT™ II First Strand cDNA Synthesis Kit (Beyotime, D7170L) with gDNA Eraser. Realtime PCR Amplification reactions were performed using a SYBR Green PCR Master Mix (Beyotime) on QuantStudio 1 (Applied Biosystems). The COL3 mRNA level was normalized to that of the GAPDH mRNA level.

[00398] Similar with other models, ACVR2A Ab LA01 treatment also increased body weight significantly (FIG. 7A) in this CCl₄ treated mouse model. The data showed that 2 weeks treatment with 20mg/kg ACVR2A Ab LA01 decreased serum ALT and AST level significantly compared to Isotype control group (FIG. 7B). The Realtime PCR data (FIG. 7C) showed COL3 mRNA level was reduced in Ab LA01 treated livers. Accordantly, the liver histological analysis by pathologists (FIG. 7D) showed that Ab LA01 treated attenuated liver fibrosis, portal inflammation, and chronic hepatitis lesions. These data indicate the therapeutic potential of ACVR2A Ab LA01 to treat liver fibrosis and live cirrhosis.

[00399] In addition, RBC number, hemoglobin concentrations and hematocrit values were found increased after 24 days treatment with ACVR2A Ab LA01 (FIG. 7E), consistent with the data in Example 4.

[00400] As NASH patients usually have higher serum Activin A level compared to healthy people, indicating Activin A is involved in NASH progression. In addition to fibrosis model, we also used mouse HFD (High Fat Diet)-CCl₄ NASH model to evaluate whether blocking Activin A signaling with ACVR2A Ab could reduce NASH. 18-19 weeks old of diet-induced obese (DIO) C57/BL6 mice were fed HFD and treated with low dose CCl₄ (25% CCl₄, 0.5ml/kg) to induce NASH. The mice were treated with ACVR2A Ab LA01 for 4 weeks, S.C, 4 or 20mg/kg, twice a week; the FXR agonist obeticholic acid (OCA) was used as positive control drug, 30 mpk p.o. q.d. Liver fibrosis was evaluated with similar method that used in CCl₄-induced liver fibrosis model. The data showed that ACVR2A Ab LA01 also decreased liver fibrosis in this HFD-CCl₄ NASH model. First, ACVR2A Ab LA01 treatment also increased mice body weight compared to isotype control group (FIG. 8A). Second, both liver fibrosis score analyzed by pathologist and Sirius Red Staining analyzed by software showed 20mg/kg LA01 treatment decreased liver fibrosis level (FIG. 8B, 8C). Third, the liver collagen gene expression (Col3a1) and liver

aSMA were decreased in 20mg/kg LA01 treatment group (FIG. 8D, 8E). Fourth, LA01 treatment increased liver weight, similar with that in CCl₄ induced fibrosis model (FIG. 8F).

[00401] In brief, ACVR2A Ab LA01 could decrease liver fibrosis and promote hepatocytes proliferation in CCl₄ induced fibrosis model and HFD-CCl₄ NASH model.

7.6. Example 6: ACVR2A Ab inhibited tumor growth in CT26 syngeneic mouse model

[00402] Activin A signaling was reported involved in tumor development and metastasis, in clinic, high serum activin A level associated with poor prognosis in many cancers, such as colorectal cancer, ovarian cancer, pancreatic cancer, head and neck cancer. 6-8 week old female Balb/C mice were inoculated with 0.5×10^6 viable CT26 cells in 0.1 ml PBS on the right flanks subcutaneously. About five days later, when tumors reached an average size of 20-50 mm³, mice were sorted into groups (N=8) so that the average tumor sizes of all groups were similar, and treatment by intraperitoneal (I.P) or subcutaneous (S.C) injections was initiated (Day 0). Group 1 received PBS control twice a week; Group 2 received 10 mg/kg of anti-PD-L1 antibody 10F.9G2, I.P, twice a week; Group 3 received 10 mg/kg of mouse PD-L1 antibody 10F.9G2, I.P, and ACVR2A Ab LA01, S.C, twice a week. Body weights were measured twice weekly. Tumor volumes were determined at different time points using the formula: tumor volume (mm³) = (length $\times$ width $\times$ width)/2. Any mice with tumors over 3000 mm³ were sacrificed following the institute's animal health protocol.

[00403] The tumor growth curve of 3 groups by the various treatments is shown in FIG. 9A, which showed the average tumor volumes of the mice; and FIG. 9B, which shows the individual tumor volume of the surviving mice. While the anti-PD-L1 antibody (Group 2, T/C = 0.938, p > 0.05, on Day 14) treatment alone had no efficacy in this model, combining ACVR2A Ab LA01 and PD-L1 antibody treatment resulted in anti-tumor effect (Group 3, T/C = 0.72, p < 0.01, on Day 14).

7.7. Example 7: ACVR2A Ab inhibited tumor growth in LLC syngeneic mouse model

[00404] It was reported Activin A antagonist soluble ACVR2B-Fc protein inhibited body weight loss in lewis lung carcinoma (LLC) mouse model. The effect of ACVR2A Ab on tumor growth was also tested in this model. C57/BL6 mice were received an intramuscular (hind leg) inoculum of 5×10^5 LLC cells obtained from exponential tumors. The LLC is a highly cachectic rapidly growing mouse tumor containing poorly differentiated cells, with a relatively short doubling time. The animals were divided in three groups: Group 1, vehicle

control; Group 2, PD-L1 Ab FAZ053, 10 mg/kg intravenous (I.V), twice a week; Group 3, PD-L1 Ab and ACVR2A Ab combination, 10 mg/kg FAZ053 I.V, plus 10 mg/kg ACVR2A Ab subcutaneous (S.C), twice a week. Body weights were measured twice weekly. At Day 14 after tumor transplantation, the animals were anesthetized with CO₂. The tumor was harvested from the hind leg, and its mass were evaluated. As shown in FIG. 10, PD-L1 Ab alone treatment had little effect on tumor growth (Group 2, T/C =0.62, p=0.04), combining ACVR2A Ab LA01 and PD-L1 antibody inhibited tumor growth significantly compared to control group (Group 3, T/C =0.41, p<0.01).

[00405] In a separate study, the anti-tumor effect was also tested in combination with carboplatin in LLC model. The animals were divided in three groups: Group 1, vehicle control; Group 2, carboplatin, 60 mg/kg intraperitoneal (I.P), once a week; Group 3, carboplatin and ACVR2A Ab combination, 60 mg/kg carboplatin I.P, plus 10 mg/kg ACVR2A Ab I.V, twice a week. The treatments were initiated on the day of randomization (Day 0) based on bodyweight, two days after tumor inoculation. Body weights and tumor volume were measured twice weekly. At Day 23, the animals were anesthetized with CO₂, terminal blood samples were taken for complete blood counts assay. The tumor was harvested from the hind leg for infiltrated lymphocytes analysis using a flow cytometry-based assay. Approximately 400-mg pieces of tumors were placed in cold FACS buffer (PBS with 3% FBS) and diced into 2-4mm pieces with sterile scissors. Samples were then homogenized in gentleMACS C Tubes using a GentleMACS Tissue Dissociator (Miltenyi Biotec, 130-096-427) and Tumor Dissociation Kit (Miltenyi BioTech, 130-096-730). Homogenates were filtered through a 40- μ m mesh filter strainer. To prepare cells for flow cytometry analysis, Fcy III/II receptor blocker (BD Biosciences) was added to each sample, and samples were subsequently incubated with fluorescent dye labeled antibodies. For T cells panel, samples were subsequently incubated with anti-CD45- BV785 (Biolegend, Cat. 103149), anti-CD3-BUV395 (BD, Cat.740268), CD4-BV421 (Biolegend, Cat.100438), CD8-PE-eFluor610 (eBiosciences61-0081-82) and FoxP3-PE (eBiosciences12-5773-82) antibodies. For macrophages panel, samples were subsequently incubated with CD11b-PE-Cy7 (Biolegend, Cat.101216), F4/80-BV510 (Biolegend, Cat.123135), I-A/I-E-AF700 (Biolegend, Cat.107622), CD206-Percp-cy5/5 (Biolegend, Cat.141716), Ly-6G-APC (Biolegend, Cat.127614), Ly-6C-BV711 (Biolegend, Cat.128037). After being washed, cells were incubated with APC-eFluor780 viability dye (eBiosciences, Cat.65-0865-14). Cells were gated by forward and side scatter (FSC, SSC), LIVE/DEAD viability, CD45, and lineage

expression markers. The MFI and number of tumor-infiltrating lymphocytes were determined for each sample and normalized to live CD45+ cells.

[00406] As shown in FIGs. 11A and 11B, carboplatin alone treatment inhibited tumor growth (Group 2, T/C =0.56, $p<0.001$), combining ACVR2A Ab LA01 and carboplatin inhibited tumor growth more dramatically compared to control group (Group 3, T/C =0.35, $p<0.0001$), indicating ACVR2A Ab LA01 increased anti-tumor effect of chemotherapy agent carboplatin. TIL analysis showed that carboplatin treatment increased both CD8+ T effector cells (Teff) and Foxp3+ T regulatory cells (Treg) percentage in tumor (FIGs. 11C and 11D), ACVR2A Ab LA01 combination treatment decreased Treg cells percentage significantly compared to carboplatin single treatment, thus increased the ratio of Teff to Treg (FIGs. 11D and 11E). As shown in FIG. 11F, the ratio of Teff to Treg negatively correlated with tumor volume ($R^2=0.30$, p value =0.0054), suggesting ACVR2A Ab could suppress immune suppression activity of Treg cells in tumor micro-environment. In addition, ACVR2A Ab also regulate macrophages in tumor, it increased M1 phenotype macrophages but decreased M2 macrophages percentage, thus the ratio of M1/M2 increased significantly in combination group (FIGs. 11G-11I). As result, the antitumor activity of macrophages was enhanced in ACVR2A Ab treatment group, because M1 phenotype macrophages are tumor-resistant due to intrinsic phagocytosis and enhanced antitumor inflammatory reactions, whereas M2 are endowed with a repertoire of tumor-promoting capabilities involving immuno-suppression, angiogenesis and neovascularization. These data indicated that ACVR2A Ab could inhibit tumor growth through boosting antitumor immunity in tumor micro-environment, such as increasing the ratio of Teff to Treg, and M1 phenotype to M2 phenotype macrophages.

[00407] It's well known that chemotherapy agents such as carboplatin could interfere with DNA repair process and kill cancer cells, however, they also show hematotoxicity in clinic, including anemia, neutropenia and thrombocytopenia. In this LLC model study, carboplatin treatment was also found decreased the count of white blood cells (WBC), neutrophils (NEUT) and platelets (PLT) significantly (FIGs. 12A-12C). Carboplatin showed a decrease trend of red blood cells (RBC), hemoglobin (HGB) and hematocrit (HCT) (FIGs. 12D-12F). ACVR2A Ab LA01 combination treatment had little effect on WBC and NEUT numbers, showed an increased trend on RBC, HGB and HCT parameters. Interestingly, the PLT number was increased dramatically in ACVR2A Ab combination treatment group (FIG. 12C). This data indicates that ACVR2A Ab LA01 is a potential therapeutic agent to attenuate anemia caused by chemotherapy.

7.8. Example 8: ACVR2A Ab inhibited tumor growth in ovarian cancer CDX mouse model

[00408] Overexpression of activin A was found in ovarian cancer cells and associated with shortened survival. TOV-21G is a clear cell ovarian cancer cell line, it was obtained from American Type Culture Collection (ATCC) and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum. 6-8 week old female Balb/C Nude mice were inoculated with 3×10^6 viable TOV-21G cells in 0.1 ml PBS on the right flanks subcutaneously. When tumors were palpable, length and width of tumors were measured through skin, and tumor volumes were calculated according to the formula $(\text{length} \times \text{width} \times \text{width})/2$. Seven days after cell inoculation, mice bearing tumors with acceptable morphology and size (mean volume of approximately 130 mm³) were randomized to groups containing 8 mice per group, so that the average tumor sizes of all groups were similar. The treatments were initiated on the day of randomization (Day 0). ACVR2A Ab LA01 was administered at 10 mg/kg s.c., twice weekly in a volume of 5 mL/kg. Body weight and tumor volume were measured two to three times per week. At the end of the experiment (Day 24), the mice were euthanized with CO₂. Tumors were collected for infiltrated lymphocytes analysis using a flow cytometry-based assay.

[00409] Approximately 200-mg pieces of tumors were placed in cold FACS buffer (PBS with 3% FBS) and diced into 2-4mm pieces with sterile scissors. Samples were then homogenized in gentleMACS C Tubes using a GentleMACS Tissue Dissociator (Miltenyi Biotec, 130-096-427) and Tumor Dissociation Kit (Miltenyi BioTech, 130-096-730). Homogenates were filtered through a 40- μ m mesh filter strainer. To prepare cells for flow cytometry analysis, Fc γ III/II receptor blocker (BD Biosciences) was added to each sample, and samples were subsequently incubated with fluorescent dye labeled antibodies. For NK cells panel, samples were subsequently incubated with anti-CD45-Alexa700, anti-CD49B-FITC, anti-NKG2D-APC, anti-CD3-PE-Cy7, anti-CD11b-BV510 and anti-CD69-PB antibodies (BD Biosciences). For macrophages panel, samples were subsequently incubated with anti-CD45-Alexa700, anti-CD11b-PE/BV510, anti-Gr-1-PE, anti-F4/80-BV421, anti-CD206-APC and anti-MHC-II-FITC antibodies. After being washed, cells were incubated with 7-AAD viability dye (BD Biosciences). Flow cytometry was performed on a FACS CytoFLEX Flow Cytometer (Beckman) instrument. Cells were gated by forward and side scatter (FSC, SSC), LIVE/DEAD-Near-IR (ThermoFisher, L10119) viability, CD45, and

lineage expression markers. The MFI and number of tumor-infiltrating lymphocytes were determined for each sample and normalized to sample weight.

[00410] As shown in FIG. 13A, TOV-21G cells secreted high level of Activin A in culture medium compared to other ovarian cancer cells. The *in vivo* study showed serum Activin A level increased dramatically after TOV-21G cells inoculation. The tumor secreted Activin A may dysfunction of lymphocytes, such as NK cells and macrophages. The result showed Activin A antagonist ACVR2A Ab LA01 single agent treatment inhibited tumor growth significantly compared to control group ($p < 0.01$) (FIG. 13B). The TIL analysis showed more lymphocytes infiltrated to tumors in LA01 Ab treatment group compared to control group, which contributed to tumor inhibitory effect. The number of total NK cells (CD45+ CD3-CD49+) as well as activated NK cells (CD69+ or NKG2D+) in tumor were elevated in LA01 Ab treatment compared to control group ($p = 0.0108$, $p = 0.0076$ and $p = 0.0244$, respectively, FIG. 13C). Similarly, there were more macrophages in LA01 Ab treated tumor ($p = 0.00341$, FIG. 13D), however, M1/M2 ratio did not showed significant difference ($p = 0.65$). These data showed LA01 Ab promoted lymphocyte infiltration to tumors and inhibited tumor growth.

What is claimed:

1. An antibody or antigen binding fragment thereof that binds ACVR2A, wherein the affinity of the antibody or antigen binding fragment to ACVR2A is at least 10 fold of that to ACVR2B, and wherein optionally the antibody or antigen binding fragment comprises:

- (i) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 1, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (ii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 3, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (iii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 4, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (iv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 5, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (v) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (vi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 8, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (vii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (viii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 10, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (ix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (x) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (xi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 13, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;
- (xii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 14;
- (xiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 15;
- (xiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 2;

- (xv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 16;
- (xvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 17;
- (xvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 18;
- (xviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 19;
- (xix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 20;
- (xx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 21;
- (xxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 22;
- (xxii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 23;
- (xxiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 24;
- (xxiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 6;
- (xxv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 25;
- (xxvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 26;
- (xxvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 27;
- (xxviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 28;
- (xxix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 29;
- (xxx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 30;

- (xxxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 31;
- (xxxii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 32;
- (xxxiii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 7, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 33;
- (xxxiv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 34;
- (xxxv) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 35;
- (xxxvi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 36;
- (xxxvii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 11, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 37;
- (xxxviii) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 38;
- (xxxix) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 39;
- (xxxx) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 12, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 40; or
- (xxxxi) an HCDR1, an HCDR2, and an HCDR3 as set forth in SEQ ID NO: 9, and a LCDR1, a LCDR2, and a LCDR3 as set forth in SEQ ID NO: 41.

2. The antibody or antigen binding fragment of claim 1, wherein
 - (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 42, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
 - (ii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises

the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

- (iii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 49, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (iv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 50, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or
- (v) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or
- (vi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 49, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or
- (vii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

- (viii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 55, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (ix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (x) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 58, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xiii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises

the amino acid sequence of SEQ ID NO: 60, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

- (xiv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 61, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xvi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 62, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xvii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 63, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xviii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 82, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

- (xix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 64, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xx) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 65, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 66, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 67, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxiii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 68, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxiv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises

the amino acid sequence of SEQ ID NO: 52, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 46, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 53, or

- (xxv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 69, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxvi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 59, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 70, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxvii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 71, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxviii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 72, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 73, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

- (xxx) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 74, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxxi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 75, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxxii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 67, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxxiii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 48, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 51, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 68, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxxiv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 76, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or
- (xxxv) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises

the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 77, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxvi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 78, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxvii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 56, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 79, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxviii) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 69, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxix) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 80, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxx) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 57, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 70, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47, or

(xxxxi) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 43, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 44, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 45, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 81, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 47.

3. The antibody or antigen binding fragment of claim 1 or 2, comprises:
- (i) a VH comprising the amino acid sequence of SEQ ID NO: 1, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
 - (ii) a VH comprising the amino acid sequence of SEQ ID NO: 3, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
 - (iii) a VH comprising the amino acid sequence of SEQ ID NO: 4, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
 - (iv) a VH comprising the amino acid sequence of SEQ ID NO: 5, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
 - (v) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
 - (vi) a VH comprising the amino acid sequence of SEQ ID NO: 8, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
 - (vii) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
 - (viii) a VH comprising the amino acid sequence of SEQ ID NO: 10, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
 - (ix) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
 - (x) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
 - (xi) a VH comprising the amino acid sequence of SEQ ID NO: 13, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
 - (xii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 14;

- (xiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 15;
- (xiv) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 2;
- (xv) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 16;
- (xvi) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 17;
- (xvii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 18;
- (xviii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 19;
- (xix) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 20;
- (xx) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 21;
- (xxi) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 22;
- (xxii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 23;
- (xxiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 24;
- (xxiv) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 6;
- (xxv) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 25;
- (xxvi) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 26;
- (xxvii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 27;
- (xxviii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 28;

- (xxix) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 29;
 - (xxx) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 30;
 - (xxxi) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 31;
 - (xxxii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 32;
 - (xxxiii) a VH comprising the amino acid sequence of SEQ ID NO: 7, and a VL comprising the amino acid sequence of SEQ ID NO: 33;
 - (xxxiv) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 34;
 - (xxxv) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 35;
 - (xxxvi) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 36;
 - (xxxvii) a VH comprising the amino acid sequence of SEQ ID NO: 11, and a VL comprising the amino acid sequence of SEQ ID NO: 37;
 - (xxxviii) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 38;
 - (xxxix) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 39;
 - (xxxx) a VH comprising the amino acid sequence of SEQ ID NO: 12, and a VL comprising the amino acid sequence of SEQ ID NO: 40; or
 - (xxxxi) a VH comprising the amino acid sequence of SEQ ID NO: 9, and a VL comprising the amino acid sequence of SEQ ID NO: 41.
4. The antibody or antigen binding fragment of any one of claims 1-3, wherein the antibody is an IgG.
5. The antibody of any one of claims 1-4, wherein the antibody is a humanized antibody.

6. The antibody of any one of claims 1-5, wherein the antibody or antigen binding fragment thereof is genetically fused or chemically conjugated to an agent.
7. A nucleic acid molecule encoding the antibody or antigen binding fragment of any one of claims 1-6.
8. A vector comprising the nucleic acid molecule of claim 7.
9. A host cell transformed with the vector of claim 8.
10. A composition comprising a therapeutically effective amount of the antibody or antigen binding fragment of any one of claims 1-6, the nucleic acid molecule of claim 7, or the vector of claim 8, and a pharmaceutically acceptable excipient.
11. A method of treating a disease or disorder in a subject, comprising administering to the subject the composition of claim 10.
12. The method of claim 11, wherein the disease or disorder is associated with ACVR2A.
13. The method of claim 11, wherein the disease or disorder is associated with ACVR2A ligands.
14. The method of claim 11, wherein the disease or disorder is associated with Activin A, GDF8 or GDF11.
15. The method of any one of claims 11-14, wherein the disease or disorder is a musculoskeletal disease or disorder.
16. The method of claim 15, wherein the musculoskeletal disease or disorder is selected from a group consisting of muscle atrophy, spinal muscular atrophy and cancer cachexia.
17. The method of any one of claims 11-14, wherein the disease or disorder is an age-related condition selected from a group consisting of sarcopenia, skin atrophy, muscle

wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, frailty, memory loss, wrinkles, impaired kidney function, and age-related hearing loss.

18. The method of any one of claims 11-14, wherein the disease or disorder is a metabolic disorder selected from a group consisting of Type II Diabetes, Metabolic Syndrome, hyperglycemia, NASH and obesity.

19. The method of any one of claims 11-14, wherein the disease or disorder is selected from a group consisting of acute and/or chronic renal disease or failure, liver fibrosis or cirrhosis, lung fibrosis, pulmonary arterial hypertension, kidney fibrosis, Parkinson's Disease, ALS, brain atrophy, dementia cachexia, bone-loss inducing cancers.

20. The method of any one of claims 11-14, wherein the disease or disorder is cancer selected from a group consisting of sarcoma, ovarian cancers, breast cancer, esophageal cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, endometrial cancer and gastrointestinal cancers.

21. The method of any one of claims 11-14, wherein the disease or disorder is anemia.

22. The method of any one of claims 11 to 21, wherein the method further comprises administering to the subject a second agent.

23. The method of claim 22, wherein the second agent is an ACVR2B antagonist, wherein optionally the ACVR2B antagonist is an ACVR2B extracellular domain fusion protein, Luspatercept.

24. The method of claim 22, wherein the second agent is an anti-PD-L1 antibody.

25. The method of claim 22, wherein the second agent is a chemotherapy agent such as carboplatin.
26. A method for inhibiting or antagonizing ACVR2A in a cell, comprising contacting the cell with the composition of claim 10.