

ANTIBODIES AND USES THEREOF

CROSS REFERENCE

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 62/861,214, filed on June 13, 2019, the content of which is hereby
5 incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 12, 2020, is named AT-021_02_SL_ST25.txt and is 26,590
10 bytes in size.

TECHNICAL FIELD

[0003] The present disclosure relates to antigen binding molecules, including, but not limited to, antibodies, which specifically bind gene editing proteins (e.g., TALEN proteins comprising a FokI, also referred to as Fok-1 or Fok1, catalytic domain) and
15 polynucleotides encoding the same, and methods of use.

BACKGROUND

[0004] Genome editing tools such as Transcription activator-like effector nucleases (TALEN[®]), Zinc-finger nucleases (ZFNs), and RNA-guided FokI Nucleases (RFNs) are powerful tools that enable the precise revision, insertion or deletion of genes. TALENs,
20 ZFNs, and RFNs can bind to and cleave at targeted sites in a genome. The resulting cleaved DNA is repaired via the endogenous cell DNA repair mechanism of non-homologous end joining (NHEJ), or via homology-directed repair (HDR) when a repair template is provided. TALENs use a TAL effector DNA-binding domain fused to a DNA cleavage domain (e.g., FokI). ZFNs are composed of a restriction endonuclease cleavage or catalytic domain, such
25 as FokI, linked to engineered Cys₂His₂ zinc-finger, DNA-binding polypeptides. RFNs are composed of a FokI nuclease fused to catalytically inactive form of Cas9. For efficient genome editing in mammals (e.g., humans, dogs, cattle, non-human primates, etc.), it is envisioned that TALENs, ZFNs and RFNs will be expressed at high level. Due to the risk of sustained nuclease activity and off-target effects, as well as potential immunogenicity, there
30 is a need in the art for antibodies that recognize genome editing tools (e.g., anti-TALEN

and/or anti-FokI antibodies) capable of binding nuclease proteins in order to evaluate the safety and efficacy of various gene editing therapies.

SUMMARY

[0005] The present disclosure provides, among other things, antibodies for the detection of FokI catalytic domains and full-length TALEN proteins in a sample. As described herein, including in the Examples below, the present disclosure provides anti-TALEN antibodies which specifically bind TALEN and FokI proteins. In some examples anti-TALEN antibodies of the present disclosure can be used to evaluate gene editing therapies and processes.

[0006] In one example, the instant disclosure provides an anti-TALEN antibody comprising a variable heavy chain amino acid sequence that is at least 85% identical to SEQ ID NO: 3 and a variable light chain amino acid sequence that is at least 85% identical to SEQ ID NO: 7. In one example the anti-TALEN antibody is humanized. In various examples, the antibody is selected from the group consisting of an antibody, an scFv, a Fab, a Fab', a Fv, a F(ab')₂, a dAb, a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, an IgE antibody, an IgD antibody, an IgM antibody, an IgG1 antibody, an IgG1 antibody having at least one mutation in the hinge region, an IgG2 antibody an IgG2 antibody having at least one mutation in the hinge region, an IgG3 antibody, an IgG3 antibody having at least one mutation in the hinge region, an IgG4 antibody, an IgG4 antibody having at least one mutation in the hinge region, an antibody comprising at least one non-naturally occurring amino acid, and any combination thereof.

[0006a] In one aspect, the present disclosure refers to an antibody specific for FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO:1, said antibody comprising a variable heavy chain region (VH) CDR1, CDR2 and CDR3 and a variable light chain region (VL) CDR1, CDR2, and CDR3, wherein the antibody comprises:

- (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO:12 or 14;
- a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;
- a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
- a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
- a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 23; and
- a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26; or
- (b) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 13 or 14;
- a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16 or 19;
- a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;

a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
 a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 25; and
 a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26; or
 (c) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;
 a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;
 a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
 a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
 a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and
 a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28; or
 (d) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;
 a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 17 or 20;
 a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
 a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
 a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and
 a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27; or
 (e) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;
 a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;
 a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
 a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
 a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and
 a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[0007] In specific examples, the antibody comprises a variable heavy chain (HC) selected from the group consisting of SEQ ID NOs: 3-6, and in further examples the anti-TALEN antibody comprises a heavy chain CDR1 selected from the group consisting of SEQ ID NOs: 12-14, and/or a heavy chain CDR2 selected from the group consisting of SEQ ID NOs: 15-20, and/or a heavy chain CDR3 comprising SEQ ID NO: 21. In specific examples, an anti-TALEN antibody comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, each CDR comprising an amino acid sequence shown in Table 1c. In another example, the instant disclosure provides an anti-TALEN antibody comprising a VH amino acid sequence that is at least about 90%, at least about 95%, at least about 96%, at least about

97%, at least about 98%, at least about 99%, or about 100% identical to a VH of an antigen binding molecule provided herein.

[0008] In other examples an anti-TALEN antibody comprises a light chain variable region (VL) sequence selected from the group consisting of SEQ ID NOs: 7-11. In specific examples, an anti-TALEN antibody comprises a light chain CDR1 comprising SEQ ID NO: 22, and/or a light chain CDR2 selected from the group consisting of SEQ ID NO: 23-25, and/or a light chain CDR3 selected from the group consisting of SEQ ID NOs: 26-28. Further provided is an anti-TALEN antibody wherein the light chain comprises a light chain CDR1, a light chain CDR2 and a light chain CDR3, each CDR comprising an amino acid sequence in Table 1d. In another example, the instant disclosure provides an anti-TALEN antibody, comprising a VL amino acid sequence that is at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a VL of an antigen binding molecule provided herein.

[0009] In another example, provided herein is an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO:12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 23; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26 and in a further example, an anti-TALEN antibody comprises (a) a VH comprising the amino acid sequence of SEQ ID NO: 3; and (b) a VL comprising the amino acid sequence of SEQ ID NO: 7.

[0009a] In another example, the instant disclosure provides an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO:12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 23; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

[0010] In another example provided herein is an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27, and in a further example an anti-TALEN antibody comprises (a) a VH comprising the amino acid sequence of SEQ ID NO: 3; and (b) a VL comprising the amino acid sequence of SEQ ID NO: 8.

[0010a] In another example, the instant disclosure provides an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[0011] In a further example, provided herein is an anti-TALEN antibody comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 13; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26, and in a further example the anti-TALEN antibody comprises (a) a VH comprising the amino acid sequence of SEQ ID NO: 4; and (b) a VL comprising the amino acid sequence of SEQ ID NO: 9.

[0011a] In another example, the instant disclosure provides an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 13; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

[0012] In still a further example, provided herein is an anti-TALEN antibody comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28, and in a further example the anti-TALEN antibody comprises (a) a VH comprising the amino acid sequence of SEQ ID NO: 5; and (b) a VL comprising the amino acid sequence of SEQ ID NO: 10.

[0012a] In another example, the instant disclosure provides an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

[0013] Also provided is an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 17; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27, and in a further example the anti-TALEN antibody of claim 24, comprising (a) a VH comprising the amino acid sequence of SEQ ID NO: 6; and (b) a VL comprising the amino acid sequence of SEQ ID NO: 11.

[0013a] In another example, the instant disclosure provides an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 17; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[0013b] In another example, the instant disclosure provides an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[0013c] In another example, the instant disclosure provides an anti-TALEN antibody, comprising (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

In another aspect, the instant disclosure provides an antibody specific for FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1, said antibody comprising: (a) a VH comprising the amino acid sequence of SEQ ID NO: 3 and a VL comprising the amino acid sequence of SEQ ID NO: 7; or (b) a VH comprising the amino acid sequence of SEQ ID NO: 3 and a VL comprising the amino acid sequence of SEQ ID NO: 8; or (c) a VH comprising the amino acid sequence of SEQ ID NO: 4 and a VL comprising the amino acid sequence of SEQ ID NO: 9; or (d) a VH comprising the amino acid sequence of SEQ ID NO: 5 and a VL comprising the amino acid sequence of SEQ ID NO: 10; or (e) a VH comprising the amino acid sequence of SEQ ID NO: 6 and a VL comprising the amino acid sequence of SEQ ID NO: 11.

[0014] In various examples the anti-TALEN antibody further comprises a detectable label, and in some examples the detectable label is selected from the group consisting of a fluorescent label, a photochromic compound, a proteinaceous fluorescent label, a magnetic label, a radiolabel, and a hapten. When the detectable label is a fluorescent label, the fluorescent label can be selected from the group consisting of an Atto

dye, an Alexafluor dye, quantum dots, Hydroxycoumarin, Aminocouramin, Methoxycoumarin, Cascade Blue, Pacific Blue, Pacific Orange, Lucifer Yellow, NBD, R-Phycoerythrin (PE), PE-Cy5 conjugates, PE-Cy7 conjugates, Red 613, PerCP, TruRed, FluorX, Fluorescein, BODIPY-FL, Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7, TRITC, X-Rhodamine, Lissamine Rhocamine B, Texas Red, Allophycocyanin (APC), APC-Cy7 conjugates, Indo-1, Fluo-3, Fluo-4, DCFH, DHR, SNARF, GFP (Y66H mutation), GFP (Y66F mutation), EBFP, EBFP2, Azurite, GFPuv, T-Sapphire, Cerulean, mCFP, mTurquoise2, ECFP, CyPet, GFP (Y66W mutation), mKeima-Red, TagCFP, AmCyan1, mTFP1, GFP (S65A mutation), Midorishi Cyan, Wild Type GFP, GFP (S65C mutation), TurboGFP, TagGFP, GFP (S65L mutation), Emerald, GFP (S65T mutation), EGFP, Azami Green, ZsGreen1, TagYFP, EYFP, Topaz, Venus, mCitrine, YPet, TurboYFP, ZsYellow1, Kusabira Orange, mOrange, Allophycocyanin (APC), mKO, TurboRFP, tdTomato, TagRFP, DsRed monomer, DsRed2 ("RFP"), mStrawberry, TurboFP602, AsRed2, mRFP1, J-Red, R-phycoerythrin (RPE), B-phycoerythrin (BPE), mCherry, HcRed1, Katusha, P3, Peridinin Chlorophyll (PerCP), mKate (TagFP635), TurboFP635, mPlum, and mRaspberry. In specific examples the fluorescent label is R-Phycoerythrin (PE) or Allophycocyanin (APC)

[0015] Also provided is a composition comprising an anti-TALEN antibody provided herein and an acceptable carrier or vehicle, e.g., a pharmaceutically acceptable carrier or vehicle.

[0015a] In another aspect, the instant disclosure provides a composition comprising the antibody as disclosed herein and a pharmaceutically acceptable carrier or vehicle.

[0016] Polynucleotides encoding the heavy chain of an anti-TALEN antibody disclosed herein are also provided. Polynucleotide encoding the light chain of an anti-TALEN antibody disclosed herein are also provided. Vectors comprising one or both of the heavy and light chain encoding polynucleotides are also provided. Further, a cell comprising one or both of the vectors is provided, and the cell can comprise a cell selected from the group consisting of a CHO cell, a Sp2/0 cell, a rabbit cell and an *E. coli* cell. A method of making an anti-TALEN antibody disclosed herein is provided and comprises incubating a cell comprising one or both of the vectors described above under suitable conditions.

[0016a] In another aspect, the instant disclosure provides a polynucleotide encoding the antibody as disclosed herein. In another example, the instant disclosure provides a polynucleotide encoding the light chain of an anti-TALEN antibody as disclosed herein. In another aspect, the instant disclosure provides a vector comprising the polynucleotide as disclosed herein. In another aspect, the instant disclosure provides a cell comprising the polynucleotide as disclosed herein. In another aspect, the instant disclosure provides a cell comprising one or both of the vectors as disclosed herein. In another aspect, the instant disclosure provides a method of making an antibody specific for FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1 comprising incubating the cell as disclosed herein under suitable conditions.

[0017] In yet a further example a method of determining the presence or absence of a TALEN protein in a sample is provided and in one example comprises contacting the sample with an anti-TALEN antibody conjugated to a detectable label and

determining the presence or absence of the TALEN protein in the sample; in some examples the anti-TALEN antibody comprises an antibody provided herein, or a humanized form thereof. Additionally, the sample can be a tissue sample, a blood sample, a formalin-fixed sample, a tissue grown *ex vivo* or cell culture media.

[0017a] In another aspect, the instant disclosure provides a method of determining the presence or absence of FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1 in a sample, comprising contacting the sample with the antibody as disclosed herein conjugated to a detectable label and determining the presence or absence of the FokI nuclease catalytic domain in the sample.

In another aspect, the instant disclosure provides a method of determining the presence or absence of FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1 in a sample, comprising contacting the sample with the antibody as disclosed herein and determining the presence or absence of the FokI nuclease catalytic domain in the sample.

In another aspect, the instant disclosure provides a method of determining the presence or absence of FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1 in a sample, comprising contacting the sample with the composition as disclosed herein and determining the presence or absence of the FokI nuclease catalytic domain in the sample.

[0018] Various examples of the present disclosure are described in detail in the following sections. The use of sections is not meant to limit the present disclosure. Each section can apply to any example of the present disclosure. In this application, the use of “or” means “and/or” unless stated otherwise. All references cited herein are incorporated by reference in their entirety. 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 the present disclosure belongs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The drawings provided in the present disclosure are for illustration purposes only and not for limitation.

[0020] Figure 1 depicts a Western blot analysis of hybridoma supernatants from fusion #2299 (Numbers 13-16 noted in the figure) and #2296 (Numbers 3, 6, 7, 8, and 11 noted in the figure).

DEFINITIONS

[0021] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in the arts of genetic engineering, cell culture, molecular genetics, nucleic acid chemistry and biochemistry).

[0022] In order for the present disclosure to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the present disclosure.

[0023] **Affinity:** As is known in the art, “affinity” refers to a measure of the tightness that a particular ligand binds to its partner. Affinities can be measured in different ways. In some embodiments, affinity is measured by a quantitative assay. In some embodiments, binding partner concentration and/or ligand concentration can be varied. In

CLAIMS

What is claimed is:

1. An antibody specific for FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO:1, said antibody comprising a variable heavy chain region (VH) CDR1, CDR2 and CDR3 and a variable light chain region (VL) CDR1, CDR2, and CDR3, wherein the antibody comprises:
 - (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO:12 or 14;
a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;
a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 23; and
a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26; or
 - (b) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 13 or 14;
a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16 or 19;
a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 25; and
a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26; or
 - (c) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;
a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;
a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and
a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28; or
 - (d) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;
a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 17 or 20;
a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and
a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27; or
 - (e) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;
a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;
a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;

a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and
a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27.

- 5 2. The antibody of claim 1, comprising
- (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO:12 or 14;
 - (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;
 - (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
 - (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
 - 10 (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 23; and
 - (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26.
3. The antibody of claim 2, comprising
- (a) a VH comprising the amino acid sequence of SEQ ID NO: 3; and
 - (b) a VL comprising the amino acid sequence of SEQ ID NO: 7.
- 15
4. The antibody of claim 1, comprising
- (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 13 or 14;
 - (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16 or 19;
 - (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
 - 20 (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
 - (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 25; and
 - (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 26.
5. The antibody of claim 4, comprising
- (a) a VH comprising the amino acid sequence of SEQ ID NO: 4; and
 - 25 (b) a VL comprising the amino acid sequence of SEQ ID NO: 9.
6. The antibody of claim 1, comprising
- (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;
 - (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;
 - (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;
 - 30 (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;
 - (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and

(f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

7. The antibody of claim 6, comprising

(a) a VH comprising the amino acid sequence of SEQ ID NO: 5; and

(b) a VL comprising the amino acid sequence of SEQ ID NO: 10.

5 8. The antibody of claim 1, comprising

(a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;

(b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 17 or 20;

(c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;

(d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;

10 (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and

(f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27.

9. The antibody of claim 8, comprising

(a) a VH comprising the amino acid sequence of SEQ ID NO: 6; and

(b) a VL comprising the amino acid sequence of SEQ ID NO: 11.

15 10. The antibody of claim 1, comprising

(a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 12 or 14;

(b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 15 or 18;

(c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 21;

(d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 22;

20 (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 24; and

(f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 27.

11. The antibody of claim 10, comprising

(a) a VH comprising the amino acid sequence of SEQ ID NO: 3; and

25 (b) a VL comprising the amino acid sequence of SEQ ID NO: 8.

12. The antibody of claim 1, wherein

(a) the VH comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 3 and the VL comprises an amino acid sequence that is at least 96%

30 identical to SEQ ID NO: 7; or

- (b) the VH comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 3 and the VL comprises an amino acid sequence that is at least 96% identical to SEQ ID NO: 8; or
- 5 (c) the VH comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 4 and the VL comprises an amino acid sequence that is at least 96% identical to SEQ ID NO: 9; or
- (d) the VH comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 5 and the VL comprises an amino acid sequence that is at least 96% identical to SEQ ID NO: 10; or
- 10 (e) the VH comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 6 and the VL comprises an amino acid sequence that is at least 96% identical to SEQ ID NO: 11.

13. An antibody specific for FokI nuclease catalytic domain having the amino acid
15 sequence of SEQ ID NO: 1, said antibody comprising:

- (a) a VH comprising the amino acid sequence of SEQ ID NO: 3 and a VL comprising the amino acid sequence of SEQ ID NO: 7; or
- (b) a VH comprising the amino acid sequence of SEQ ID NO: 3 and a VL comprising the amino acid sequence of SEQ ID NO: 8; or
- 20 (c) a VH comprising the amino acid sequence of SEQ ID NO: 4 and a VL comprising the amino acid sequence of SEQ ID NO: 9; or
- (d) a VH comprising the amino acid sequence of SEQ ID NO: 5 and a VL comprising the amino acid sequence of SEQ ID NO: 10; or
- (e) a VH comprising the amino acid sequence of SEQ ID NO: 6 and a VL
25 comprising the amino acid sequence of SEQ ID NO: 11.

14. The antibody of any of claims 1-13, wherein the isolated antibody further comprises a detectable label.

30 15. The antibody of claim 14, wherein the detectable label is selected from the group consisting of a fluorescent label, a photochromic compound, a proteinaceous fluorescent label, a magnetic label, a radiolabel, and a hapten.

16. The antibody of claim 15, wherein the fluorescent label is selected from the group consisting of an Atto dye, an Alexafluor dye, quantum dots, Hydroxycoumarin, Aminocouramin, Methoxycoumarin, Cascade Blue, Pacific Blue, Pacific Orange, Lucifer Yellow, NBD, R-Phycoerythrin (PE), PE-Cy5 conjugates, PE-Cy7 conjugates, Red 613, PerCP, TruRed, FluorX, Fluorescein, BODIPY-FL, Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7, TRITC, X-Rhodamine, Lissamine Rhocamine B, Texas Red, Allophycocyanin (APC), APC-Cy7 conjugates, Indo-1, Fluo-3, Fluo-4, DCFH, DHR, SNARF, GFP (Y66H mutation), GFP (Y66F mutation), EBFP, EBFP2, Azurite, GFPuv, T-Sapphire, Cerulean, mCFP, mTurquoise2, ECFP, CyPet, GFP (Y66W mutation), mKeima-Red, TagCFP, AmCyan1, mTFP1, GFP (S65A mutation), Midorishi Cyan, Wild Type GFP, GFP (S65C mutation), TurboGFP, TagGFP, GFP (S65L mutation), Emerald, GFP (S65T mutation), EGFP, Azami Green, ZsGreen1, TagYFP, EYFP, Topaz, Venus, mCitrine, YPet, TurboYFP, ZsYellow1, Kusabira Orange, mOrange, Allophycocyanin (APC), mKO, TurboRFP, tdTomato, TagRFP, DsRed monomer, DsRed2 ("RFP"), mStrawberry, TurboFP602, AsRed2, mRFP1, J-Red, R-phycoerythrin (RPE), B-phycoerythrin (BPE), mCherry, HcRed1, Katusha, P3, Peridinin Chlorophyll (PerCP), mKate (TagFP635), TurboFP635, mPlum, and mRaspberry.
17. The antibody of claim 16, wherein the fluorescent label is R-Phycoerythrin (PE) or Allophycocyanin (APC).
18. A composition comprising the antibody of any one of claims 1-17 and a pharmaceutically acceptable carrier or vehicle.
19. A polynucleotide encoding the antibody of any one of claims 1-13.
20. A vector comprising the polynucleotide of claim 19.
21. A cell comprising the polynucleotide of claim 19.
22. A cell comprising the vector of claim 21.

23. The cell of claim 22, wherein the cell comprises a cell selected from the group consisting of a CHO cell, a Sp2/0 cell, a rabbit cell and an *E. coli* cell.
- 5 24. A method of making an antibody specific for FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1 comprising incubating the cell of any one of claims 21-23 under suitable conditions.
- 10 25. A method of determining the presence or absence of FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1 in a sample, comprising contacting the sample with the antibody of any one of claims 1-13 conjugated to a detectable label and determining the presence or absence of the FokI nuclease catalytic domain in the sample.
- 15 26. A method of determining the presence or absence of FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1 in a sample, comprising contacting the sample with the antibody of any one of claims 14-17 and determining the presence or absence of the FokI nuclease catalytic domain in the sample.
- 20 27. A method of determining the presence or absence of FokI nuclease catalytic domain having the amino acid sequence of SEQ ID NO: 1 in a sample, comprising contacting the sample with the composition of claim 18 and determining the presence or absence of the FokI nuclease catalytic domain in the sample.
- 25 28. The method of any one of claims 25-27, wherein the sample is a tissue sample, a blood sample, a formalin-fixed sample, a tissue grown *ex vivo* or cell culture media.