[0007] The invention further provides compositions comprising an antibody or antigen-binding fragment or polypeptide or conjugate, as described herein, admixed with a biological sample, e.g., a biological sample obtained from a human. In exemplary aspects, the biological sample comprises human plasma, or a diluted fraction thereof, and/or human tissue, or cells thereof. In exemplary aspects, the biological sample comprises human plasma proteins, wherein at least one of the human plasma proteins is selected from the group consisting of Factor IX, Factor II, and Factor X, and variants thereof. Optionally, the composition comprises a detecting agent.

[0008] Such binding constructs provided herein are useful in, e.g., detection methods that allow for unambiguous or specific detection of FIX Padua in samples, e.g., clinical or preclinical samples comprising, e.g., wild-type FIX. Accordingly, provided herein are methods of detecting Factor IX Padua comprising the amino acid sequence of SEQ ID NO: 1 in a sample obtained from a subject. In exemplary embodiments, the method comprises (i) contacting the sample with a binding construct (e.g., an antibody, antigen-binding fragment, polypeptide or conjugate, as described herein) to form a complex, e.g., an immunocomplex, comprising the FIXp and the binding construct, and (ii) detecting the complex in the sample.

[0008a] According to a first aspect of the invention there is provided an antibody or antigen-binding fragment thereof that binds a Factor IX Padua comprising the amino acid sequence of SEQ ID NO: 1 and does not bind to a wild-type (WT) Factor IX, wherein antibody or antigen-binding fragment thereof comprises the amino acid sequences SSYAIS (SEQ ID NO: 6), GIVPAFGTANYAQKFQG (SEQ ID NO: 7), SWGVISFAY (SEQ ID NO: 8), RASQDISSYLN (SEQ ID NO: 9), AASNLQS (SEQ ID NO: 10), and MQYDSLPFTF (SEQ ID NO: 11).

[0008b] According to a second aspect of the invention there is provided an isolated polypeptide comprising each of SEQ ID NOs: 6-11.

[0008c] According to third aspect of the invention there is provided a conjugate comprising the antibody or antigen-binding fragment of the first aspect or the polypeptide of the second aspect, conjugated to a heterologous moiety.

[0008d] According to a fourth aspect of the invention there is provided an isolated

nucleic acid comprising a nucleotide sequence encoding the antibody or antigen-binding fragment of the first aspect, the polypeptide of any one of the second aspect, or a polypeptide portion of the conjugate of the third aspect.

[0008e] According to a fifth aspect of the invention there is provided a vector comprising the nucleic acid of the fourth aspect.

[0008f] According to sixth aspect of the invention there is provided a host cell comprising the nucleic acid of the fourth aspect or the vector of the fifth aspect.

[0008g] According to a seventh aspect of the invention there is a provided kit comprising the antibody or antigen-binding fragment of the first aspect, the polypeptide of the second aspect, the conjugate of the third aspect, the nucleic acid of the fourth aspect, the vector of the fifth aspect, and/or the host cell of the sixth aspect.

[0008h] According to an eighth aspect of the invention there is provided a composition comprising an antibody or antigen-binding fragment thereof according to the first aspect a polypeptide according to the second aspect or a conjugate according to the third aspect.

[0008i] According to a ninth aspect of the invention there is provided a composition comprising an antibody or antigen-binding fragment thereof according to the first aspect admixed with a biological sample obtained from a human comprising human plasma proteins, wherein at least one of the human plasma proteins is selected from the group consisting of Factor IX, or a variant thereof, Factor II, and Factor X.

[0008j] According to a tenth aspect of the invention there is provided use of the antibody or antigen-binding fragment of the first aspect, the polypeptide of the second aspect, the conjugate of the third aspect, the nucleic acid of the fourth aspect, the vector of the fifth aspect, the host cell of the sixth aspect, and/or the kit of the seventh aspect for detecting Factor IX Padua in a sample.

[0008k] According to an eleventh aspect of the invention there is provided a method of detecting Factor IX Padua in a sample obtained from a subject, comprising (i) contacting the sample with the antibody or antigen-binding fragment the first aspect, the

polypeptide of the second aspect, or the conjugate of the third aspect to form a complex comprising the Factor IX Padua and the antibody, antigen-binding fragment, polypeptide or conjugate, and (ii) detecting the complex in the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figure 1 represents a schematic of the amino acid sequences of WT Factor IX and of FIX Padua.

[0010] Figure 2 represents a graph of the binding signals of FIX Padua (left panel) or WT Factor IX (right panel) to Ni^{2+} plates coated with 5 µg/ml BC1 (blue bars closest to Z-axis (i.e., to the left)), 1 µg/ml BC1 (red bars right of the blue bars), 0.2 µg/ml BC1 (green bars adjacent to the red bars) or 0.04 µg/ml BC1.

[0011] Figure 3 represents a graph of the binding signals of BC1 (triangles), BC2 (diamonds), or BC3 (squares) coated on MaxiSorp plates to the indicated concentration of FIX Padua.

WHAT IS CLAIMED IS:

1. An antibody or antigen-binding fragment thereof that binds a Factor IX Padua comprising the amino acid sequence of SEQ ID NO: 1 and does not bind to a wild-type (WT) Factor IX, wherein antibody or antigen-binding fragment thereof comprises the amino acid sequences SSYAIS (SEQ ID NO: 6), GIVPAFGTANYAQKFQG (SEQ ID NO: 7), SWGVISFAY (SEQ ID NO: 8), RASQDISSYLN (SEQ ID NO: 9), AASNLQS (SEQ ID NO: 10), and MQYDSLPFTF (SEQ ID NO: 11).

2. The antibody or antigen-binding fragment of claim 1, which binds an epitope of SEQ ID NO: 1, wherein the epitope is a linear epitope within the amino acid sequence DRATCLLSTKFT (SEQ ID NO: 3) or a conformational epitope of the folded structure of the amino acid sequence LVDRATCLLSTKFTIYNNMFCAGFH (SEQ ID NO: 5).

3. The antibody or antigen-binding fragment of claim 2, wherein the folded structure comprises a disulfide bridge.

4. The antibody or antigen-binding fragment of any one of claims 1 to 3, which does not bind a wild-type (WT) Factor IX comprising the amino acid sequence (SEQ ID NO: 2).

5. The antibody or antigen-binding fragment of any one of claims 1 to 4, which does not bind to at least one of an amino acid sequence of DRATCLRSTKFT (SEQ ID NO: 14) or an amino acid sequence of LVDRATCLRSTKFTIYNNMFCAGFH (SEQ ID NO:15).

6. The antibody or antigen-binding fragment of any one of claims 1 to 5, wherein the antibody or antigen-binding fragment binds to the Factor IX Padua with a K_D of about 100 nM or less, or within a range of about 25 to about 75 nM, or within a range of about 50 nM to about 60 nM.

7. The antibody or antigen-binding fragment of any one of claims 1 to 6, wherein the antibody or antigen-binding fragment binds to the Factor IX Padua and does not bind to a WT Factor IX in a sample comprising human plasma.

8. The antibody or antigen-binding fragment of claim 7, wherein the sample comprises at least or about 5%, at least or about 10%, or at least or about 20% human plasma and the sample comprises at least or about 5 µg/mL WT Factor IX.

9. The antibody or antigen-binding fragment of any one of claims 1 to 8, wherein the antibody or antigen-binding fragment does not bind to at least one of a Factor II polypeptide or a Factor X polypeptide.

10. The antibody or antigen-binding fragment of any one of claims 1 to 9, which is a Fab or Fab2' antibody fragment.

11. The antibody or antigen-binding fragment of any one of claims 1 to 10, which is monospecific.

12. The antibody or antigen-binding fragment of any one of claims 1 to 11, which is fully human.

13. The antibody or antigen-binding fragment of any one of claims 1 to 12, which is bivalent.

14. The antibody or antigen-binding fragment of any one of claims 1 to 13, comprising dimerized Fab fragments or dimerized Fab mini antibody.

15. The antibody or antigen-binding fragment of any one of claims 1 to 14, which is a dimerized Fab fragment via a linker.

16. The antibody or antigen-binding fragment of any one of claims 1-15, comprising the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 27.

17. The antibody or antigen-binding fragment of any one of claims 1-16, comprising the amino acid sequences of SEQ ID NOs: 24 and 25 or the amino acid sequences of SEQ ID NOs: 26 and 27.

18. An isolated polypeptide comprising an amino acid sequence comprising each of SEQ ID NO: 6-11.

19. The isolated polypeptide of claim 18, wherein the polypeptide specifically recognizes Factor IX Padua without cross-reactivity to WT Factor IX.

20. The isolated polypeptide of claim 18 or 19, wherein one or more amino acids are present between each of SEQ ID NOs: 6-11.

21. The isolated polypeptide of any one of claims 18 to 20, wherein the polypeptide further comprises a FLAG tag comprising DYKDDDDK (SEQ ID NO: 12) and/or a hexa-His tag comprising HHHHHH (SEQ ID NO: 13).

22. The isolated polypeptide of claim 21, wherein the FLAG tag and/or the hexa-His tag are located at the C-terminal end of the polypeptide.

23. A conjugate comprising the antibody or antigen-binding fragment of any one of claims 1 to 17 or the polypeptide of any one of claims 18 to 22, conjugated to a heterologous moiety.

24. The conjugate of claim 23, wherein the heterologous moiety is selected from the group consisting of: a polymer, a carbohydrate, a lipid, a nucleic acid, an oligonucleotide, an amino acid, peptide, polypeptide, protein, and a detecting agent.

25. The conjugate of claim 23 or claim 24 wherein the antibody or antigen-binding fragment or polypeptide is conjugated to: (i) agarose, cellulose, dextran, polyacrylamide, latex, or controlled pore glass; (ii) a fluorophore, chromophore, radioisotope, enzymatic label, or biotin; or both (i) and (ii).

26. The conjugate of any one of claims 23 to 25, comprising a homodimer of the polypeptide of any one of claims 18 to 22.

27. The conjugate of claim 26, wherein the polypeptides of the dimer are linked via a helix-turn-helix structure.

28. An isolated nucleic acid comprising a nucleotide sequence encoding the antibody or antigen-binding fragment of any one of claims 1 to 17, the polypeptide of any one of claims 18 to 22, or a polypeptide portion of the conjugate of any one of claims 23 to 27.

29. A vector comprising the nucleic acid of claim 28.

30. A host cell comprising the nucleic acid of claim 28 or the vector of claim 29.

31. A kit comprising the antibody or antigen-binding fragment of any one of claims 1 to 17, the polypeptide of any one of claims 18 to 22, the conjugate of any one of claims 23 to 27, the nucleic acid of claim 28, the vector of claim 29, and/or the host cell of claim 30.

32. The kit of claim 31, further comprising a secondary antibody which binds to the antibody, antigen-binding fragment, polypeptide, or conjugate.

33. The kit of claim 31 or claim 32, further comprising a solid support.

34. The kit of claim 33, wherein the antibody, antigen-binding fragment, polypeptide or conjugate is pre-coated on the solid support.

35. The kit of claim 33 or claim 34, wherein the solid support is a polymer bead, a microtiter plate, a membrane, or a filter.

36. The kit of any one of claims 31 to 35, comprising a solid support pre-coated with a solution comprising about 100 ng or more, about 150 ng or more, about 200 ng or more, about 500 ng or more of the antigen binding fragment.

37. A composition comprising an antibody or antigen-binding fragment thereof according to any one of claims 1 to 17, a polypeptide according to any one of claims 18 to 22, or a conjugate according to any one of claims 23 to 27.

38. The composition of claim 37, wherein the composition is admixed with a biological sample obtained from a human comprising human plasma, or a diluted fraction thereof, and/or human tissue, or cells thereof.

39. The composition of claim 38, wherein the composition is admixed with a biological sample obtained from a human comprising human plasma proteins, wherein at least one of the human plasma proteins is selected from the group consisting of Factor IX or a Factor IX Padua, Factor II, and Factor X.

40. The composition of any one of claims 37 to 39, wherein the composition comprises a detecting agent.

41. Use of the antibody or antigen-binding fragment of any one of claims 1 to 17, the polypeptide of any one of claims 18 to 22, the conjugate of any one of claims 23 to 27, the nucleic acid of claim 28, the vector of claim 29, the host cell of claim 30, and/or the kit of any one of claims 31 to 36 for detecting Factor IX Padua in a sample.

42. A method of detecting Factor IX Padua in a sample obtained from a subject, comprising (i) contacting the sample with the antibody or antigen-binding fragment of any one of claims 1 to 17, the polypeptide of any one of claims 18 to 22, or the conjugate of any one of claims 23 to 27 to form a complex comprising the Factor IX Padua and the antibody, antigen-binding fragment, polypeptide or conjugate, and (ii) detecting the complex in the sample.

43. The method of claim 42, wherein the antibody or antigen-binding fragment or polypeptide is conjugated to a detecting agent and/or a solid support or wherein the conjugate comprises a detecting agent.

44. The method of claim 43, comprising contacting the sample with a secondary antibody comprising a detecting agent, wherein the secondary antibody binds to the antibody or antigen-binding fragment or polypeptide or conjugate.

45. The method of claim 43 or claim 44, wherein detecting the complex comprises detecting a signal of the detecting agent.

46. The method of claim 45, wherein the signal is an enzymatic activity, binding activity and/or chromogenic activity.

47. The method of any one of claims 42 to 46, wherein the sample is a blood sample, a serum sample, or a plasma sample.

48. The method of any one of claims 42 to 47, wherein the subject has been treated with a vector comprising a nucleotide sequence encoding Factor IX Padua.