CLAIMS

1. An antigen-binding polypeptide capable of binding adeno-associated virus serotype 9 (AAV9), the polypeptide comprising a single-domain antibody (sdAb) variant having complementarity determining regions CDR1, CDR2 and CDR3, wherein

- CDR1 comprises an amino acid sequence selected from the group of sequences defined by SEQ ID NO: 1:

  $X_1X_2X_3SX_5X_6TMX_9$ (SEQ ID NO: 1)

  wherein, independently,

  X_1 is R, L or S, preferably R or L;

  X_2 is T or R, preferably T;

  X_3 is L or F, preferably L;

  X_5 is D, N or E, preferably D;

  X_6 is Y, N or F, preferably Y or F;

  X_9 is G or A, preferably G;

- CDR2 comprises a sequence selected from the group of sequences defined by defined by SEQ ID NO: 2:

  $X_1X_2SWSGX_7X_8TX_{10}$ (SEQ ID NO: 2)

  wherein, independently,

  X_1 is A, S, L, V or I, preferably A;

  X_2 is I or V, preferably I;

  X_7 is A or S, preferably A;

  X_8 is Y or F, preferably Y;

  X_{10} is K, F or Y;

and

- CDR3 comprises a sequence selected from the group of sequences defined by defined by SEQ ID NO: 3:

  $X_1X_2TX_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}$ (SEQ ID NO: 3)

  wherein, independently,

  X_1 is G or A, preferably G;

  X_2 is P or S, preferably P;

  X_4 is G or P, preferably G;

  X_5 is L, T or P, preferably L or P;

X_6 is L or I, preferably L;

X_7 is S, T or A, preferably S or A;

X_8 is K, R, N or Q;

X_9 is K, H or R, preferably K or R;

X_{10} is A, S or T, preferably A or T;

X_{11} is T, S, P or A, preferably T;

X_{12} is P, A, T or S, preferably P;

X_{13} is A, P, R or G, preferably A or P;

X_{14} is D, N, E or Q, preferably D or E;

X_{15} is Y or F, preferably Y;

X_{16} is D, N, E or Q, preferably D; and

X_{17} is Y, F or R, preferably Y or F.

2. The antigen-binding polypeptide according to claim 1, wherein the antigen-binding polypeptide selectively binds AAV9.

3. The antigen-binding polypeptide according to any one of the preceding claims, wherein the sdAb variant comprises framework regions (FWRs) and complementary determining regions (CDRs) in the following order from N-terminus to C-terminus: [FWR1]-[CDR1]-[FWR2]-[CDR2]-[FWR3]-[CDR3]-[FWR4].

4. The antigen-binding polypeptide according to claim 3, wherein at least one of said framework regions comprises at least one an amino acid substitution relative to SEQ ID NO: 4 and wherein the antigen-binding polypeptide exhibits improved alkaline stability compared to SEQ ID NO: 4.

5. The antigen-binding polypeptide according to claims 3 or 4, wherein each of FWR1, FWR2 and FWR3 has an amino acid sequence having at least 70% identity to a corresponding framework region of an sdAb selected from the group consisting of SEQ ID NOs: 91 and 92.

6. The antigen-binding polypeptide according to any one of the preceding claims, wherein in SEQ ID NO: 1, X_1 is R, X_2 is T, and X_3 is L.

7. The antigen-binding polypeptide according to any one of the preceding claims, wherein in SEQ ID NO: 1, X_6 is F and X_9 is G or A.

8. The antigen-binding polypeptide according to any one of the preceding claims, wherein in SEQ ID NO: 2 X_2 is I.

9. The antigen-binding polypeptide according to any one of the preceding claims, wherein in SEQ ID NO: 2 X_7 is A, X_8 is Y, and/or X_{10} is K.

10. The antigen-binding polypeptide according to any one of the preceding claims wherein SEQ ID NO: 3 X_5 is L or P and/or X_{10} is T.

11. The antigen-binding polypeptide according to claim 1 or 3, wherein CDR1 is selected from SEQ ID NOs: 9-17, such as selected from SEQ ID NO: 13, 14 16 and 17; and/or CDR2 is selected from SEQ ID NOs: 21-31, such as selected from SEQ ID NO: 26, 28, 29 and 31; and/or CDR3 is selected from SEQ ID NOs: 34-72 and 173, such as selected from SEQ ID NO: 36, 37, 39, 40, 42-46, 48-50, 55, 56, 62, 64, 68,70-72 and 173.

12. The antigen-binding polypeptide according to claim 3, wherein the individual FWR are selected from SEQ ID NO: 149-151 (FWR1), SEQ ID NO: 152-154 (FWR2), SEQ ID NO: 155-157 (FWR3) and SEQ ID NO: 8 (FWR4), and sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 %, identity thereto.

13. The antigen-binding polypeptide according to any one of the preceding claims, wherein the sdAb variant has

a) an amino acid sequence selected from SEQ ID NOs: 75-93, 95-98, 101, 102, 105-107, 109-124, 126-146 and 173, such as selected from SEQ ID NOs: SEQ ID NO: 92, 97, 109, 116, 131, 140-146 and 173; or

b) an amino acid sequences having at least 90 % identity, such as at least 95 % identity, to a sequence as defined in a) provided that the CDRs are as defined in claim 1.

14. The antigen-binding polypeptide according to any preceding claim, wherein the antigen-binding polypeptide comprises a sequence selected from SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 97, SEQ ID NO: 131, SEQ ID NO: 173 and SEQ ID NO: 177-182, and sequences having 95 % or more, such as 96 %, 97 %, 98 %, 99 %, identity thereto.

15. A multimeric polypeptide comprising at least two moieties, each moiety comprising a single-domain antibody variant as defined in any one of the preceding claims, said moieties optionally being joined by a peptide linker.

16. A fusion protein comprising at least one antigen-binding polypeptide according to any one of claims 1-14 or a multimeric polypeptide according to claim 15, and a further polypeptide moiety.

17. The fusion protein according to claim 16, wherein the further polypeptide moiety comprises an amino acid sequence having least at least 80 % identity, such as at least 85 % or at least 90 % identity to SEQ ID NO: 161 or 162.

18. The fusion protein according to claim 16 or 17, comprising the following structure

$([A\text{-}L1]_m\text{-}[Z\text{-}L2]_n)_p$

wherein

A represents an antigen-binding polypeptide as defined in any one of claim 1-14,

L1 for each occurrence may be present or absent, and where present, represents a linker or spacer,

Z represents a further polypeptide moiety optionally as defined in claim 17,

L2 for each occurrence may be present or absent, and where present, represents a linker or spacer,

m represents an integer of from 1 to 4,

n represents an integer of from 1 to 4, or when m≥2, n represents 0 or an integer of from 1 to 4,

and

p represents an integer of from 1 to 4.

19. A separation matrix comprising the antigen-binding polypeptide of any one of claims 1-14, the multimeric polypeptide of claim 15 or the fusion protein of any one of claims 16-18 coupled to a support material.

20. The separation matrix according to claim 19, wherein the support material is selected from a fibrous membrane, a particle or a bead, a fiber, a filter, a sheet, a porous monolith, a chip, a plate, and a well.

21. The separation matrix according to claim 19 or 20, wherein the support material is a chromatography matrix.

22. The separation matrix according to claim 21, wherein the support material is a chromatography matrix selected from the group consisting of a fibrous matrix, a membrane, a filter and a porous monolith.

23. The separation matrix according to claim 21, wherein the support material comprises beads comprising agarose, or a derivative thereof.

24. A method of chromatographic separation of AAV9 viral particles, comprising the steps of

(a) providing a separation matrix according to any one of claims 19-22,

(b) contacting the separation matrix with a liquid sample comprising said AAV9 viral particles under conditions allowing the antigen-binding polypeptide to bind to the AAV9 viral particles,

(c) optionally washing the separation matrix, and

(d) eluting the bound AAV9 viral particles from the separation matrix.

25. The method of claim 24, further comprising:

(e) cleaning the separation matrix material with a cleaning liquid.

26. The method of claim 25, wherein step (e) comprises cleaning the separation matrix with an alkaline cleaning liquid, wherein the cleaning liquid preferably comprises from 0.05 to 0.5 M of NaOH.

27. The method of any one of claims 25-26, wherein steps (a)-(e) are repeated at least 10 times, such as at least 20 times.

28. The method of any one of claims 25-27 wherein after 10 cycles of contact with cleaning liquid the polypeptide retains at least 50 %, such as at least 60 %, at least 70 %, at least 80 % or at least 90 % of its initial target entity binding capacity.

29. The method of any one of claims 25-28, wherein, after 10 cycles of contact with alkaline cleaning liquid, the polypeptide retains at least 60 %, at least 70 %, at least 80 %, at least 90 %, such as at least 95 % of the target entity binding capacity of the 2nd cycle.