[006] Both GDF11 and GDF8 (myostatin) play important roles in the course of development, and in the regulation of cell growth and differentiation in adult tissues. The ability to localize and/or measure these two proteins is important to further understand and distinguish their contributions in development and adult tissues (e.g., cardiac and skeletal muscle). However, due to their homology it is difficult to distinguish the presence and/or levels of these proteins with current protein binding reagents (e.g., antibodies). Thus, there is a need for protein binding reagents that are capable of distinguishing the proteins GDF11 and GDF8. The present disclosure meets such needs by providing aptamers having binding specificity to a GDF11 protein.

SUMMARY

[007] The present disclosure describes aptamers capable of binding to growth differentiation factor 11 (GDF11) protein. In some embodiment, aptamers that bind GDF11 with an equilibrium binding constant (K_d) of less than 100 nM are provided. In another embodiment, the K_d is from about 0.1 nM to about 100 nM (or from about 0.1 nM to about 50 nM, or from about 0.1 nM to about 10 nM, or from abou 0.5 nM to about 10 nM, or from about 0.5 nM to about 5 nM).

[007a] Disclosed is an aptamer that binds GDF11 with an affinity of less than 10 nM, wherein, under the same conditions, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11 or does not bind GDF8.

[008] In some embodiments, an aptamer that binds GDF11 with an affinity of less than 10 nM is provided. In some embodiments, the aptamer binds GDF11 with an affinity of less than 10 nM, and, under the same conditions, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11. In some embodiments, the aptamer does not bind GDF8. In some embodiments, the aptamer binds GDF8 with an affinity that is at least 20-fold weaker, or at least 30-fold weaker, or at least 50-fold weaker than the affinity for GDF11. In some embodiments, the aptamer binds GDF8 with an affinity greater than 50 nM, or greater than 100 nM, or greater than 150 nM, or greater than 200 nM, or greater than 250 nM, or greater than 300 nM. In some embodiments, the aptamer binds GDF11 with an affinity of less than 8 nM, or less than 7 nM, or less than 6 nM, or less than 5 nM, or less than 4 nM, or less than 3 nM, or less than 2 nM, or less than 1 nM. In some embodiments, affinity is determined using a binding assay comprising a polyanionic inhibitor. In some embodiments, the polyanionic inhibitor is selected from dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, and dNTPs. In one aspect, there is provided an aptamer that binds GDF11 with an affinity of less than 10 nM, wherein, under the same conditions, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11 or does not bind GDF8, wherein the aptamer comprises the sequence 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3'(SEQ ID NO:110) or 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 151), wherein:

each P is independently, and for each occurrence, a C-5 modified pyrimidine;

R is A or G;

LISTING OF CLAIMS

1. An aptamer that binds GDF11 with an affinity of less than 10 nM, wherein, under the same conditions, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11 or does not bind GDF8, wherein the aptamer comprises the sequence 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3'(SEQ ID NO:110) or 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 151), wherein:

each P is independently, and for each occurrence, a C-5 modified pyrimidine;

R is A or G;

each W is independently, and for each occurrence, A, T, or U;

each M is independently, and for each occurrence, A or C;

each S is independently, and for each occurrence, G or C;

each n is independently, and for each occurrence, 0 or 1; and

each m is independently, and for each occurrence, 0 or 1.

2. An aptamer that binds GDF11, wherein the aptamer comprises the sequence 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO:110) or 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 151), wherein:

each P is independently, and for each occurrence, a C-5 modified pyrimidine;

R is A or G;

each W is independently, and for each occurrence, A, T, or U;

each M is independently, and for each occurrence, A or C;

each S is independently, and for each occurrence, G or C;

each n is independently, and for each occurrence, 0 or 1; and

each m is independently, and for each occurrence, 0 or 1.

3. The aptamer of claim 1 or claim 2, wherein the aptamer comprises a sequence selected from:

a) 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 111);

b) 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 152);

c) 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 112);

d) 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 153);

e) 5'-$RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGC$-3' (SEQ ID NO: 113); and

f) $RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGS$-3' (SEQ ID NO: 154).

4. The aptamer of any one of claims 1 to 3, wherein if R is G, the first W is A, and wherein if R is A, the first W is C.

5. The aptamer of any one of claims 1 to 4, wherein at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 n are 0.

6. The aptamer of any one of claims 1 to 4, wherein each n is 0.

7. The aptamer of any one of claims 1 to 4, wherein at least 1, at least 2, at least 3 n are 1.

8. The aptamer of any one of claims 1 to 7, wherein at least 1 m is 0.

9. The aptamer of any one of claims 1 to 7, wherein at least 1 m is 1.

10. The aptamer of any one of the preceding claims, wherein the aptamer comprises the sequence 5'-CPPGMPPP-3' (SEQ ID NO: 114), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, and wherein M is C or A.

11. The aptamer of any one of the preceding claims, wherein the aptamer comprises the sequence 5'-PPPAGC-3'(SEQ ID NO: 115) or 5'-PPPAGG-3' (SEQ ID NO: 155), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine.

12. The aptamer of any one of the preceding claims, wherein the aptamer comprises the sequence 5'-CPPGMPPPN$_x$PPPAGC-3' (SEQ ID NO: 160) or 5'-CPPGMPPPN$_x$PPPAGG-3' (SEQ ID NO: 116), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, wherein x is 2, 3, 4, or 5, wherein M is C or A, and wherein each N is independently, and for each occurrence, selected from A, C, G, T, and U.

13. The aptamer of claim 9, wherein x is 3 or 4.

14. The aptamer of claim 12 or claim 13, wherein N_x comprises the sequence 5'-AAG-3' or 5'-ACG-3' or 5'-AGG-3'.

15. The aptamer of any one of the preceding claims, wherein the aptamer comprises the sequence 5'-NNCPPGRPPPAMGPPPAGS-3' (SEQ ID NO: 141), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, R is A or G; each N is independently, and for each occurrence, A, G, or C; M is A or C; and S is G or C.

16. The aptamer of any one of claims 1 to 15, wherein each P is independently, and for each occurrence, selected from:

5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU),

5-(N-benzylcarboxyamide)-2'-O-methyluridine,

5-(N-benzylcarboxyamide)-2'-fluorouridine,

5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PEdU),

5-(N-thiophenylmethylcarboxyamide)-2'-deoxyuridine (ThdU),

5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU),

5-(N-tyrosylcarboxyamide)-2'-deoxyuridine (TyrdU),

5-(N-3,4-methylenedioxybenzylcarboxyamide)-2'-deoxyuridine (MBndU),

5-(N-4-fluorobenzylcarboxyamide)-2'-deoxyuridine (FBndU),

5-(N-3-phenylpropylcarboxyamide)-2'-deoxyuridine (PPdU),

5-(N-imidizolylethylcarboxyamide)-2'-deoxyuridine (ImdU),

5-(N-isobutylcarboxyamide)-2'-O-methyluridine,

5-(N-isobutylcarboxyamide)-2'-fluorouridine,

5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU),

5-(N-*R*-threoninylcarboxyamide)-2'-deoxyuridine (ThrdU),

5-(N-tryptaminocarboxyamide)-2'-O-methyluridine,

5-(N-tryptaminocarboxyamide)-2'-fluorouridine,

5-(N-[1-(3-trimethylamonium) propyl]carboxyamide)-2'-deoxyuridine chloride,

5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU),

5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine),

5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU),

5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-1-naphthylethylcarboxyamide)-2'-deoxyuridine (NEdU),

5-(N-1-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylethylcarboxyamide)-2'-deoxyuridine (2NEdU),

5-(N-2-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-deoxyuridine (BFdU),

5-(N-3-benzofuranylethylcarboxyamide)-2'-O-methyluridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzothiophenylethylcarboxyamide)-2'-deoxyuridine (BTdU),

5-(N-3-benzothiophenylethylcarboxyamide)-2'-O-methyluridine, and

5-(N-3-benzothiophenylethylcarboxyamide)-2'-fluorouridine.

17. The aptamer of any one of claims 1 to 15, wherein each P is independently, and for each occurrence, selected from:

5-(N-1-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU),

5-(N-1-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU),

5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-1-naphthylethylcarboxyamide)-2'-deoxyuridine (NEdU),

5-(N-1-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylethylcarboxyamide)-2'-deoxyuridine (2NEdU),

5-(N-2-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-deoxyuridine (BFdU),

5-(N-3-benzofuranylethylcarboxyamide)-2'-O-methyluridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzothiophenylethylcarboxyamide)-2'-deoxyuridine (BTdU),

5-(N-3-benzothiophenylethylcarboxyamide)-2'-O-methyluridine, and

5-(N-3-benzothiophenylethylcarboxyamide)-2'-fluorouridine.

18. The aptamer of any one of claims 1 to 15, wherein at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 P are each independently selected from 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, and 5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine.

19. The aptamer of any one of claims 1 to 15, wherein each P is independently selected from 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, and 5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine.

20. The aptamer of any one of the preceding claims, wherein the aptamer comprises a sequence selected from SEQ ID NOs: 12, 13, 15 to 109, and 117 to 140.

21. The aptamer of any one of the preceding claims, wherein the aptamer comprises a sequence selected from SEQ ID NOs: 12, 15, 26, 105 to 109, and 142 to 150.

22. The aptamer of any one of the preceding claims, wherein the aptamer consists of 18 to 200 nucleotides, or 18 to 150 nucleotides, or 18 to 100 nucleotides, or 18 to 75 nucleotides, or 18 to 50 nucleotides, or 20 to 150 nucleotides, or 20 to 100 nucleotides, or 20 to 75 nucleotides, or 20 to 50 nucleotides, wherein each nucleotide may, independently, be a modified or unmodified nucleotide.

23. The aptamer of any one of the preceding claims, wherein the aptamer comprises a detectable label.

24. The aptamer of any one of the preceding claims, wherein GDF11 is human GDF11 and GDF8 is human GDF8.

25. The aptamer of any one of the preceding claims, wherein GDF11 is mature human GDF11 comprising the sequence of SEQ ID NO: 118 and GDF8 is mature human GDF8 comprising the sequence of SEQ ID NO: 119.

26. A method of detecting GDF11 in a sample, comprising contacting proteins from the sample with an aptamer of any one of claims 1 to 25.

27. The method of claim 26, wherein the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11 or does not bind GDF8.

28. A method of determining whether a sample comprises GDF11, comprising contacting proteins from the sample with an aptamer of any one of claims 1 to 25.

29. The method of claim 28, wherein the sample comprises GDF8.

30. The method of any one of claims 26 to 29, wherein the method comprises contacting the sample with the aptamer under stringent conditions.

31. The method of claim 30, wherein the stringent conditions comprise a polyanionic inhibitor.

32. The method of claim 31, wherein the polyanionic inhibitor is selected from dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, and dNTPs.

33. The method of any one of claims 26 to 32, wherein the sample is a sample from a human.

34. The method of claim 33, wherein the sample is selected from blood, serum, plasma, saliva, urine, and a tissue sample.

35. The method of claim 34, wherein the tissue sample is selected from heart muscle tissue, skeletal muscle tissue, pancreatic tissue, cartilage tissue and neural tissue.

36. The method of any one of claims 26 to 35, wherein the proteins have been separated from at least one other component of the sample.

37. The method of any one of claims 26 to 35, wherein the proteins have not been separated from other components of the sample.

38. A composition comprising an aptamer of any one of claims 1 to 25 and proteins from a sample.

39. The composition of claim 38, wherein the sample comprises GDF8.

40. The composition of claim 38 or claim 39, comprising a polyanionic inhibitor.

41. The composition of claim 40, wherein the polyanionic inhibitor is selected from dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, and dNTPs.

42. The composition of any one of claims 38 to 41, wherein the sample is a sample from a human.

43. The composition of claim 42, wherein the sample is selected from blood, serum, plasma, saliva, urine, and a tissue sample.

44. The composition of claim 43, wherein the tissue sample is selected from heart muscle tissue, skeletal muscle tissue, pancreatic tissue, cartilage tissue and neural tissue.

45. The composition of any one of claims 38 to 44, wherein the proteins have been separated from at least one other component of the sample.

46. The composition of any one of claims 38 to 44, wherein the proteins have not been separated from other components of the sample.