[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] 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.

[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 some embodiments, 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.

[009] In another aspect, an aptamer that binds GDF11 is provided, 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.

[010] In some embodiments, an aptamer that binds GDF11 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).

In some embodiments of the sequences above, if R is G, the first W may be A, and wherein if R is A, the first W may be C. In some embodiments, at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 n are 0. In some embodiments, each n is 0. In some embodiments, at least 1, at least 2, at least 3 n are 1. In some embodiments, at least one m is 0. In some embodiments, at least one m is 1.

[011] In some embodiments, an aptamer that binds GDF11 comprises the sequence 5'-CPPGMPPP-3' (SEQ ID NO: 114), wherein each P is independently, and for each

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

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

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

In some embodiments, 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.

[015] In some embodiments, the aptamer comprises a sequence selected from SEQ ID NOs: 12, 13, 15 to 109, and 117 to 140. In some embodiments, the aptamer comprises a sequence selected from SEQ ID NOs: 12, 15, 26, 105 to 109, and 142 to 150.

[016] In any of the embodiments described herein, the aptamer may consist 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. In any of the embodiments described herein, the aptamer may comprise a detectable label.

[017] In some embodiments, GDF11 is human GDF11 and GDF8 is human GDF8. In some embodiments, 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.

[018] In one aspect, a method of detecting GDF11 in a sample is provided, comprising contacting proteins from the sample with an aptamer described herein. In some embodiments, 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.

[019] In another aspect, a method of determining whether a sample comprises GDF11 is provided, comprising contacting proteins from the sample with an aptamer described herein. In some embodiments, the sample comprises GDF8. In some embodiments, the method comprises contacting the sample with the aptamer under stringent conditions. In some embodiments, the stringent conditions comprise 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.

[020] In some embodiments, the sample is a sample from a human. In some embodiments, the sample is selected from blood, serum, plasma, saliva, urine, and a tissue sample. In some embodiments, the tissue sample is selected from heart muscle tissue, skeletal muscle tissue, pancreatic tissue, cartilage tissue and neural tissue.

[021] In some embodiments, the proteins have been separated from at least one other component of the sample. In some embodiments, the proteins have not been separated from other components of the sample.

[022] In one aspect, compositions are provided comprising an aptamer described herein and proteins from a sample. In some embodiments, the sample comprises GDF8. In some embodiments, the composition comprises 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 some embodiments, the sample is a sample from a human. In some embodiments, the sample is selected from blood, serum, plasma, saliva, urine, and a tissue sample. In some embodiments, the tissue sample is selected from heart muscle tissue, skeletal muscle tissue, pancreatic tissue, cartilage tissue and neural tissue. In some embodiments, the proteins have been separated from at least one other component of the sample. In some embodiments, the proteins have not been separated from other components of the sample.

[023] In another embodiment, the aptamer is at least 25 nucleotides in length. In another embodiment, the aptamer is at least 30 nucleotides in length. In another embodiment, the aptamer is at least 40 nucleotides in length. In another embodiment, the aptamer is from about 40 to about 100 nucleotides in length (or from 40,41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 nucleotides in length).

[024] The present disclosure further provides a composition comprising an aptamer and a GDF11 protein, wherein the aptamer and the GDF11 protein are bound by a non-covalent interaction.

[025] The foregoing and other objects, features, and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[026] **FIG. 1** shows a comparison of the binding affinities of aptamer clone 12060-28_3 for human GDF11 protein and human myostatin (GDF8) protein in the presence or absence of a polyanionic inhibitor (Z-block). The x-axis shows the concentrations for the respective proteins and the y-axis shows the percent of the aptamer bound to the protein (1.0 is 100%).

[027] **FIG. 2** shows a comparison of the binding affinities of aptamer 12058-6_3 for human GDF11 protein and human myostatin (GDF8) protein in the presence or absence of a